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Assaying Antioxidant Characteristics of Sesame Seed, Rice Bran, and Bene Hull Oils and their Unsaponifiable Matters by Using DPPH Radical-Scavenging Model System

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ABSTRACT

DPPH radical-scavenging activity of the bene hull oil (BHO), sesame oil (SEO), rice bran oil (RBO), the oils' methanolic (CH_3OH/H_2O, 80:20 v/v) or hexane extracts, and their unsaponifiable matters fraction were measured and compared with each other. As a radical-scavenger, SEO was significantly stronger than RBO and BHO, respectively. Unlike BHO and RBO, the unsaponifiable (USM) fraction of the SEO could not considerably scavenge the free radicals at the concentrations studied. The contribution of aqueous methanolic and hexane extracts of the SEO to the inhibition of the DPPH radicals were calculated to be about 75 and 25%, respectively. The aqueous methanolic extract of the RBO could not considerably scavenge the free radicals at the concentrations experimented and the inhibition activity belonged mainly to the hexane extract. The DPPH radical-scavenging active components of the BHO were approximately distributed equally (48 and 52%) between the two extracts.

Keywords: Antioxidative activity, Lipid oxidation, *Pistacia atlantica*, Solvent extraction.

INTRODUCTION

Lipid oxidation is a free radical process responsible for foodstuffs deterioration. The free radicals produced during lipid oxidation cause the loss of fatty acids and vitamins, formation of off-flavours, and production of highly reactive and toxic compounds. In living systems, they attack key biological molecules, leading to many degenerative disease conditions such as aging, membrane damage, heart disease, and cancer (Ramarathnam *et al.*, 1995). Free radicals can be inhibited by molecules which present antioxidant activity. In general, synthetic antioxidants are widely used to prevent the deteriorative effects of free radicals, however, their safety has been questioned (Buxiang and Fukuhara, 1997).

Sesame oil (SEO), with almost 85% unsaturated fatty acids, and rice bran oil (RBO) are considered to be two of the most oxidatively stable dietary oils. Lignan compounds along with γ-tocopherol (Figure 1-a) contribute to their higher stability against oxidation compared to other vegetable oils (Fukuda *et al.,* 1986a; Gertz *et al.*, 2000). Sesamin (Figure 1-b) and sesamolin (Figure 1-c) have been found to be the dominant components of lignans in SEO. It has been also reported that SEO contains small amounts of other kinds of lignans that are formed from sesamolin during oil processing. It has been shown that sesamolin decomposed to sesamol (Figure 1-e) and sesamin during high-temperature processes (Namiki *et al.,* 2002). Fukuda *et al.* (1986a) stated that the antioxidative activity of refined unroasted sesame oil is mainly attributed to a new lignan compound termed sesaminol (Figure 1-d), which was

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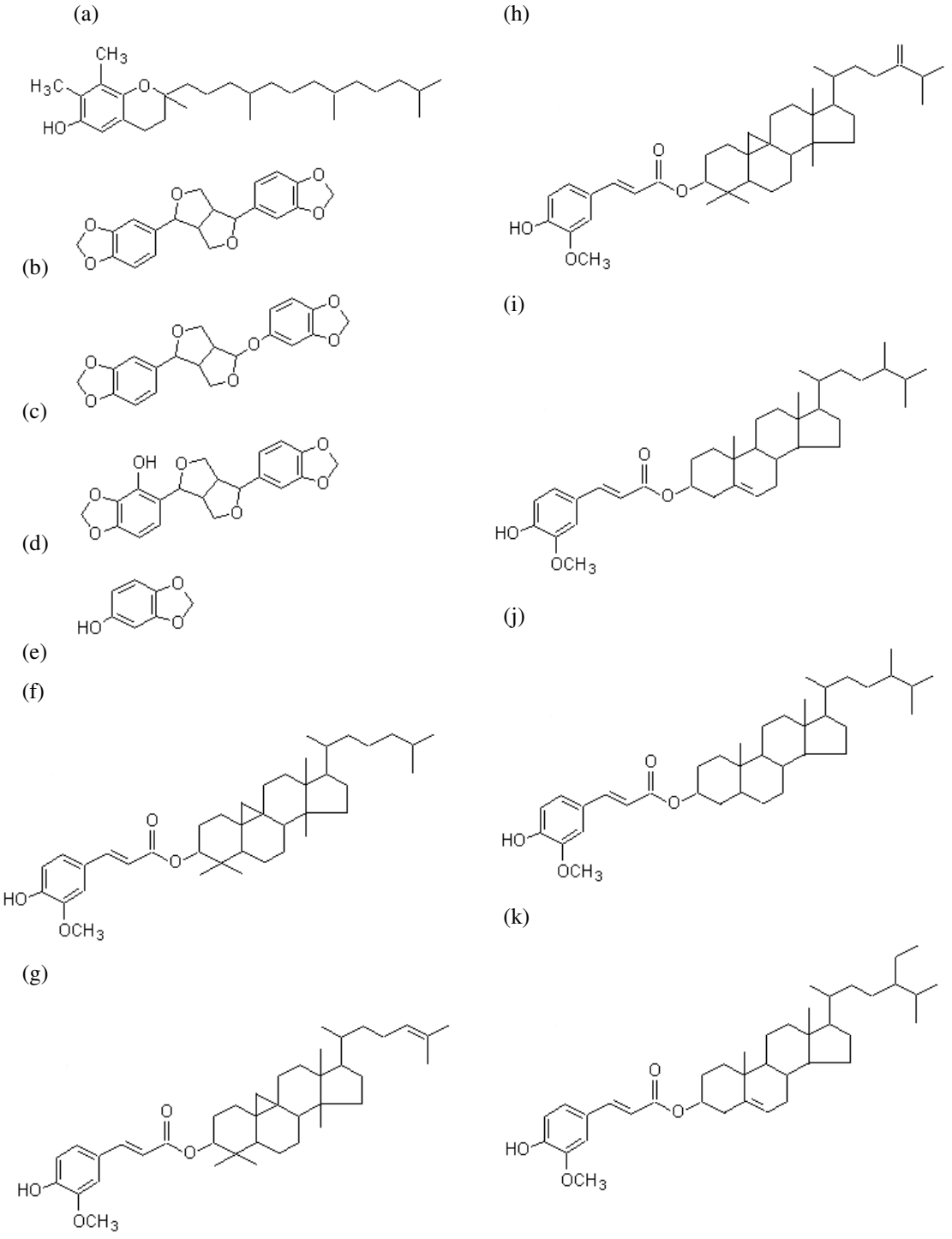

Figure 1. Chemical structure of a few natural antioxidative components: (a) γ-tocopherol; (b) Sesamin; (c) Sesamolin; (d) Sesaminol; (e) Sesamol; (f) Cycloartanylferulate; (g) Cycloartenylferulate; (h) 24-methylene-cycloartanylferulate; (i) Campesterylferulate; (j) Campestanylferulate, (k) β-sitosterylferulate.

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produced from sesamolin during the bleaching process with acid clay. Gamma-oryzanol, tocotrienols (the unsaturated homologues of tocopherols), and phytosterols are bioactive minor components known to be responsible for powerful antioxidative activity of RBO. Gamma-oryzanol comprises the whole group of ferulic acid esters of triterpenic alcohols and phytosterols. The six major components of γ-oryzanol in RBO have already been identified as cycloartanylferulate (Figure 1-f), cycloartenylferulate (Figure 1-g), 24-methylene-cycloartanylferulate (Figure 1-h), campesterylferulate (Figure 1-i), campestanylferulate (Figure 1-j), and β-sitosterylferulate (Figure 1k) (Stoggl *et al.,* 2005). RBO is regarded as a rich natural source of dietary phytosterols and tocotrienols compared to other vegetable oils (Hoed *et al.,* 2006).

Bene (*Pistacia atlantica* subsp. *mutica*) hull oil (BHO) as a new source of highly stable and antioxidative vegetable oils has been recently introduced to the world (Farhoosh *et al.*, 2009). Bene trees grow in large populations and cover an area more than 1,200,000 ha in Iran, mainly in the western, central and eastern parts of the country. Its fruits consist of ~24% dark green soft hull, which yield up to ~30% oil (Farhoosh and Tavakoli, 2008). Our previous findings showed that the antioxidant activity of BHO was higher than that of SEO and RBO during frying of sunflower oil (Sharif *et al.,* 2009). Also, the results from different oxidative tests indicated that the stability of sunflower oil improved considerably in the presence of the unsaponifiable matters extracted from the BHO (Farhoosh and Tavassoli Kafrani, 2010a, b).

Antioxidant activity in food and biological systems can be expressed in terms of radical scavenging ability during reaction with a relatively stable radical such as 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH radical assay can be employed easily and it is frequently used as a first approach for the evaluation of antioxidant capacity of a large variety of compounds both in pure form and in complex mixtures like herbs, spices, fruits, and seeds extracts. As mentioned above, the SEO, RBO, and BHO contain a variety of antioxidant components with different chemical structures. Hexane is an effective solvent to extract non-polar organic matter, while methanol is useful for extraction of slightly water soluble organic compounds with some polar functional groups. Moreover, the solvent system used for the separation of unsaponifiable matter (USM) fraction of vegetable oils extracts a series of components, which may act as oxidation inhibitors. This study was undertaken to examine and compare the DPPH radical-scavenging activity of the BHO, SEO, RBO, the oils' methanolic or hexane extracts, and their unsaponifiable matters fractions.

MATERIALS AND METHODS

Materials

The ripe fruits of bene were collected from the fields of Islamabad, Ilam province. The SEO and RBO with no added antioxidants were purchased from a local shop (about 3 months after harvest). The bene fruits and oil samples were stored at -18 ºC until use. Fatty acid methyl ester standards, and all chemicals and solvents used in this study were of analytical reagent grade and supplied by Merck and Sigma Chemical Companies.

Oil Extraction

After drying in the shade, the dark green soft hulls of bene fruits were separated. The hulls were ground in a laboratory grinder. The oil was extracted with n-hexane (1:4 w/v) by solvent extraction method for 48

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hours. The solvent was evaporated *in vacuo* at 40ºC to dryness.

USM Extraction

A mixture of 5 g of the oil sample and 50 ml 1N ethanolic KOH was saponified in a capped flask in an oven for 1 hour at 95ºC. After cooling, 100 ml of distilled water was added and mixed. The resulting solution was extracted two times with 100 ml diethyl ether. The upper organic layers were combined and washed twice with 75 ml distilled water, once with 100 ml 0.5N ethanolic KOH, and then 100 ml distilled water until neutrality. The organic layer was then separated and dried over anhydrous Na_2SO_4. After filtration of the solution, the solvent was evaporated to dryness under vacuum at 45ºC. To purify more effectively, the dry USM was dissolved in chloroform and then filtered and evaporated to dryness under vacuum at 45ºC. The residues were weighed to determine the yield USM extractions (Lozano *et al.,* 1993).

TLC Separation of the USM

A chloroform solution (5%) of the USM (50 mg plate^{-1}) was streaked using a thin-layer chromatography (TLC) applicator (CAMAG, Muttenz, Switzerland) along a line at 1 cm from the edges of a 20×20 cm plate coated with a 0.5-mm layer of silica gel (G), which had been activated for 15 minutes at 110ºC. The plate was developed in ascending direction for 15 cm with the solvent system n-hexane/diethyl ether (7:3 v/v). The developed plate was then dried with a hairdryer, and visualization of the chromatogram was carried out by spraying a saturated solution of $K_2Cr_2O_7$ in H_2SO_4 (80%) and then carbonating at 130ºC for 25 minutes. Fractions with the same R_f were carefully scraped from the plate and thoroughly extracted with chloroform; then, the extract was filtered through a 0.45-mm membrane filter (Millipore, HVLP) and evaporated to almost dryness *in vacuo* at 40ºC. The residue was weighed to determine the yield of each fraction (Frega and Lercker, 1985).

Peroxide Value (PV)

The PV was determined according to the thiocyanate method. The sample (0.01-0.30 g, depending on the extent of peroxidation) was mixed in a glass tube with 9.8-mL chloroform-methanol (7:3 v/v) on a vortex mixer for 2-4 s. Ammonium thiocyanate solution (50 µL, 30% w/v) was added and the sample vortexed for 2-4 seconds. Then, 50 µL of iron (II) chloride solution ([0.4 g barium chloride dihydrate dissolved in 50 mL H_2O]+[0.5 g $FeSO_4 \cdot 7H_2O$ dissolved in 50 mL H_2O]+2 mL 10M HCl, with the precipitate, barium sulfate, filtered off to produce a clear solution]) was added, and the sample was mixed on a vortex mixer for 2-4 seconds. After 5 minutes incubation at room temperature, the absorbance of the sample was read at 500 nm against a blank that contained all the reagents except the sample. The entire procedure was conducted in subdued light and completed within 10 min. All the PV analysis results were expressed as meq O_2 kg^{-1} oil (Shantha and Decker, 1994).

HPLC Analysis for Tocopherols

Total tocopherols (TT) content was determined according to the colorimetric method described by Wong *et al.* (1988). Tocopherol isomers in the oils was determined by using a high-performance liquid chromatograph (WATERS, Alliance system, USA) with a spherisorb column (25 cm×4 mm id, WATERS, USA) packed with silica (5 µm particle size) and a fluorescence detector operating at an excitation wavelength of 290 nm and an emission wavelength of 330 nm (ISO 9936, 1997). The mobile phase used was hexane/

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isopropanol (98.5:0.5 v/v) at a flow rate of 1 mL min^{-1}. Tocopherols in test samples were verified by comparison of retention times with those of reference standards.

Total Phenolics (TP) Content

A calibration curve of gallic acid in methanol was performed in the concentration range of 0.04-0.40 mg mL^{-1}. The solutions for the spectrophotometric analysis were performed as follows: in a 50 ml volume flask 1 mL of a standard solution of gallic acid, 6 mL of methanol, 2.5 mL of the Folin-Ciocalteau reagent, 5 mL of 7.5% Na_2CO_3 were added, reaching the final volume with deionized water. The solutions were stored overnight and the spectrophotometric analysis was performed at λ= 765 nm. The TP determination was performed as follows: 2.5 g of the oil samples were diluted with 2.5 mL of n-hexane and extracted three times by 5 minutes centrifugation (5000 rpm) with CH_3OH/H_2O (80:20 v/v) extract. The extract was added to 2.5 mL Folin-Ciocalteau reagent, 5 mL of Na_2CO_3 (7.5%), in a 50 mL volume flask reaching the final volume with deionized water. The samples were stored overnight and the spectrophotometric analysis was performed at λ= 765 nm (Capannesi *et al.,* 2000). To determine the DPPH radical-scavenging activity, the volume of aqueous methanolic and hexane extracts was adjusted to 10 mL with the corresponding solvents and were stored separately until analysis.

DPPH Radical-scavenging Assay

Various concentrations of toluene sample solutions (1 mL) were mixed with 1 mL of toluenic solution containing DPPH radicals (0.006% w/w). The mixture was shaken vigorously and left to stand for 60 minutes in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm (Ramadan *et al.,* 2003). The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the following equation:

$$\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100 \quad (1)$$

Where, A_S is the absorbance of the solution when the sample has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The sample concentration providing 50% of radical-scavenging activity (IC_{50}) was calculated by interpolation from the graph of *RSA* percentage against sample concentration. The antiradical power of the samples were calculated as $(1/IC_{50}) \times 100$. Alpha-tocopherol was used as standard.

Statistical Analysis

All experiments and measurements were carried out in triplicate, and data were subjected to analysis of variance (ANOVA) and regression analysis using the MSTAT-C and Excel software. Significant differences between means were determined by Duncan's multiple range tests. *P* values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The PV as an initial quality criterion of edible fats and oils was in a relatively acceptable level for the oils (quantities for the SEO, RBO, and BHO were 0.82, 2.45, and 2.65 meq O_2 kg^{-1} oil, respectively). Figure 2 shows the TT content, tocopherol composition and TP content of the SEO, RBO and BHO. The SEO showed the highest contents of TT and TP (980.7 and 1,140 mg kg^{-1}, respectively) among the three antioxidative oils. The RBO had a TT content significantly higher than that of the BHO (829.3 vs. 580.4 mg kg^{-1}), whereas its TP content was significantly lower (122.6 *vs.* 310.2 mg kg^{-1}). Tocopherols and phenolic compounds are particularly

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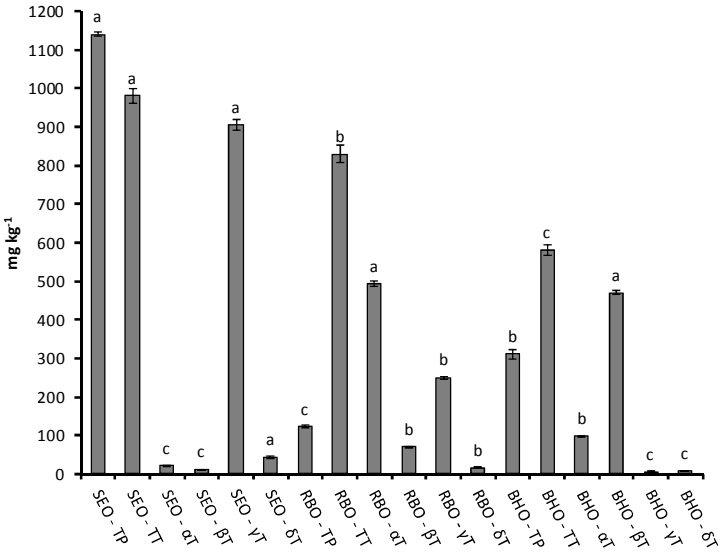

Figure 2. The contents of total tocopherols (TT), tocopherol composition and total phenolics (TP) of the sesame (SEO), rice bran (RBO) and bene hull (BHO) oils. The columns showing the similar components with the same lowercase letters are not significantly different at $P< 0.05$. Error bars indicate standard deviations.

important functional constituents of vegetable oils. Tocopherols have antioxidant properties and they are active as vitamin E, which makes them particularly important in terms of human health. Interest in phenolic compounds is related primarily to their antioxidant activity; nevertheless, they also show important biological activity *in vivo* and may be beneficial in combating diseases related to excessive oxygen radical formation exceeding the antioxidant defense capacity of the human body (Morello *et al.,* 2004). Gamma-tocopherol was the major tocopherol homologue in the SEO (904 mg kg^{-1}), followed by α- (21.1 mg kg^{-1}), β- (12.5 mg kg^{-1}), and δ-tocopherols (43.2 mg kg^{-1}) (Figure 2-a). Although γ-tocopherol has a lower vitamin E value in biological systems than α-tocopherol, it is a more potent antioxidant in oils (Burton and Traber,

Table 1. The composition (%w/w) of the unsaponifiable matters (USM) fraction of the sesame (SEO), rice bran (RBO) and bene hull (BHO) oils [a].

	R_f	SEO	RBO	BHO
USM (%w/w of oil)		2.42±0.53 b	4.36±0.85 a	4.12±0.75 a
Hydrocarbons	0.86	25.43±5.40 a	2.18±0.17 c	7.28±0.81 b
Carotenes	0.80	7.72±1.59 b	12.33±1.27 a	7.13±0.40 b
Tocopherols and tocotrienols	0.57	8.38±0.84 c	12.45±1.38 b	48.43±2.97 a
Linear and triterpenic alcohols (4,4'-dimethylsterols)	0.40	6.83±0.67 b	18.51±3.20 a	1.68±0.24 c
4-Methylsterols	0.33	14.82±2.57 a	10.44±2.35 ab	11.24±1.17 b
Desmethylsterols	0.23	27.65±3.67 b	36.29±6.31 a	6.23±0.17 c
Triterpenic dialcohols	0.13	5.78±0.45 b	6.55±1.56 b	9.16±0.93 a
Triterpenic dialcohol methyl esters	0.08	3.38 ± 0.61 b	1.25 ± 0.48 c	8.85 ± 0.76 a

[a] Mean±SD (standard deviation) within a row with the same lowercase letters are not significantly different at $P< 0.05$.

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1990). The RBO contained higher amounts of α- (492.7 mg kg^{-1}) and γ-tocopherols (250.5 mg kg^{-1}) than β- (70.4 mg kg^{-1}) and δ-isomers (15.7 mg kg^{-1}). Beta-tocopherol was found to be the major homologue (470 mg kg^{-1}) in the BHO, followed by α- (97.3 mg kg^{-1}), δ- (7.31 mg kg^{-1}), and γ-tocopherols (5.85 mg kg^{-1}).

The composition of the USM fraction of the SEO, RBO and BHO is shown in Table 1. The least statistically significant content of the USM was observed in the SEO (2.42%) and there was no significant difference between the USM contents of the RBO (4.36%) and BHO (4.12%). Vegetable oils typically contain 0.5–2.5% USM, but RBO and BHO have been reported to have exceptional amounts (Farhoosh and Tavassoli Kafrani 2010a). The major component of the USM fractions of the SEO and RBO was the sterol compounds (49.3 and 65.2%, respectively, *vs.* 19.2% of the BHO), whereas tocopherols and tocotrienols constituted 48.3% of the USM fraction of the BHO (*vs.* 8.4% of the SEO and 12.5% of the RBO). Tocotrienols are also active as vitamin E (Wang *et al.,* 2002) and have been shown to have up to 50 times more antioxidant potential than tocopherols (Serbinova *et al.,* 1991). The BHO had a content of triterpenic dialcohols (18%) significantly higher than those of the SEO (9.2%) and RBO (7.8%). Steroidal phytochemicals contained in vegetable oils have been reported to possess hypocholesterolemic effects and may also be potent antioxidants (Wang *et al.,* 2002). The USM fractions of the SEO, BHO, and RBO contained 25.4, 7.3, and 2.2% hydrocarbons. These compounds are mainly linear saturated chains of 15-33 carbon atoms; in food matrices, most of the hydrocarbons have an odd number of carbon atoms and are mainly constituted by squalene. It has been indicated that squalene has a potential to retard the degradation of unsaturated fatty acids in lipid systems heated at high temperatures (Malecka, 1991). The RBO had a significantly higher amount of carotenes (12.3%) than the SEO (7.7%) and BHO (7.1%). These compounds have been shown to protect lipids from free-radical autoxidation by reacting with peroxy radicals, thereby inhibiting propagation and promoting termination of the oxidation chain reaction. They are also effective quenchers of singlet oxygen during inhibition of photooxidation (Matsushita and Terao, 1980).

Figure 3 and Table 2 show the quantities

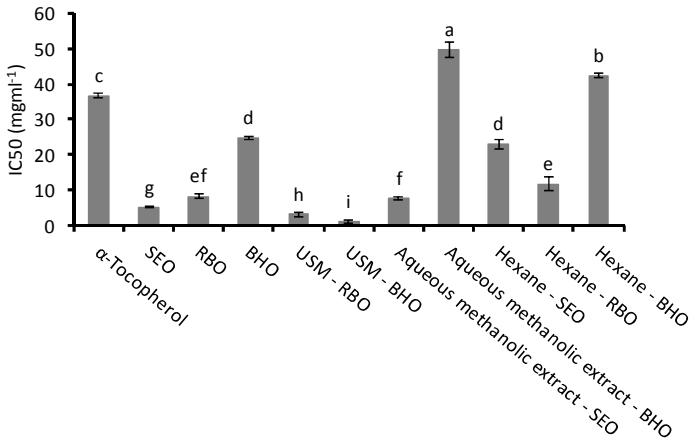

Figure 3. The sample concentration providing 50% of radical-scavenging activity (IC_{50}) of sesame (SEO), rice bran (RBO) and bene hull (BHO) oils, the oils' unsaponifiable matters (USM), and their CH_3OH/H_2O (80:20 v/v) and hexane extracts. Means±SD (standard deviation) with the same lowercase letters are not significantly different at $P< 0.05$.

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Table 2. The DPPH radical-scavenging activity of sesame (SEO), rice bran (RBO) and bene hull (BHO) oils, the oils' unsaponifiable matters (USM), and their CH_3OH/H_2O (80:20 v/v) and hexane extracts in terms of the indices calculated from the IC_{50} values.

	SEO			RBO			BHO		
	AP [a]	IF [b]	IP [c]	AP	IF	IP	AP	IF	IP
Oil	19.16	7.04	-	11.96	4.40	-	4.03	1.48	-
USM	-	-	-	31.45	11.56	-	101.01	37.14	-
CH_3OH/H_2O extract	12.94	4.76	75	-	-	-	2.01	0.74	48
Hexane extract	4.34	1.60	25	8.45	3.11	100	2.16	0.79	52

[a] Antiradical Power= $(1/IC_{50})\times100$; [b] Inhibition Factor= $AP_{sample}/AP_{\alpha\text{-tocopherol}}$ ($AP_{\alpha\text{-tocopherol}}$= 2.72), [c] Inhibition Percentage= $[IF_{extract}/(IF_{CH3OH/H2O}+IF_{hexane})]\times100$.

related to the DPPH radical-scavenging activity of the oils and their various fractions and extracts. As can be seen, all the antioxidative oils had the IC_{50} and antiradical power values significantly lower and higher, respectively, than those of α-tocopherol. The SEO behaved as a DPPH radical-scavenger significantly more powerful than the RBO and BHO, respectively. The antiradical power value of the SEO was 7.04 times that of α-tocopherol (inhibition factor), whereas this criterion for the RBO and BHO was 4.40 and 1.48, respectively. This can naturally be attributed to the content and more possibly to the composition of endogenous antioxidative components (e.g. tocols and phenolics contents) of the oils studied (Figure 2 and Table 1) and their synergistic effects. In the study on the vegetable oil blends with coconut oil, Bhatnagar *et al.* (2009) showed very good correlations between TT content and DPPH radical-scavenging activity. On the other hand, all lignan components of SEO (Figure 1) have been shown to possess DPPH radical-scavenging activity in the order sesamol> sesamin> sesamolin> sesaminol triglucoside> sesaminol diglucoside (Suja *et al.*, 2004). Furthermore, synergistic effects of tocopherols and lignan compounds on the lipid oxidation have been also reported (Fukuda *et al.,* 1986b; Gertz *et al.,* 2000). Regarding the RBO, published reports have documented that its γ-oryzanol has a higher DPPH radical-scavenging activity when compared to α- or γ-tocopherols or tocotrienols. Moreover, γ-oryzanol has been shown to have synergistic effects with tocopherols on the lipid oxidation (Chotimarkorn and Silalai, 2008).

Despite the fact that the SEO indicated the highest DPPH radical-scavenging activity among the oils studied, its USM fraction could not scavenge 50% of the free radicals at the tested concentrations and, therefore, no IC_{50} and antiradical power value were calculated for it (Table 2). This indicates that the chemical identity of DPPH radical-scavenging active components of the SEO do not allow them to effectively enter into the USM fraction; moreover, the USM fraction's components of the SEO had no characteristic DPPH radical-scavenging activity. On the contrary, the USM fraction of the BHO (Inhibition factor= 37.14) and that of RBO (Inhibition factor= 11.56) acted much better than that of the SEO. Considering that the antioxidant activity of the RBO is mainly due to its tocol compounds and terpenic alcohols (γ-oryzanol) concentrated in the USM fraction (Stoggl *et al.,* 2005), it can be expected that the very high inhibition factor of the USM fraction of the BHO may be related to its considerable content of these types of chemical compounds (Table 1).

The inhibition factors of the aqueous methanolic and hexane extracts of the SEO were calculated to be 4.76 and 1.60, respectively (Table 2). In other words, the contributions of its aqueous methanolic and hexane extracts from the inhibition of the DPPH radicals were about 75 and 25%, respectively. This indicates that the

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aqueous methanolic extract mainly contains lignan compounds, whereas the hexane extract probably consists of less hydrophilic components like tocopherols. The aqueous methanolic extract of the RBO could not scavenge 50% of the free radicals at the concentrations experimented and, therefore, no IC_{50} and antiradical power values were calculated for it. Its hexane extract, however, had an inhibition factor of 3.11, indicating the predominant presence of γ-oryzanol and tocol compounds of the RBO in this extract. It was interesting to find that the DPPH radical-scavenging active components of the BHO were approximately equally distributed (48 and 52%) between the two extracts. This revealed that the natural antioxidative compounds present in the BHO possessed both hydrophilic and hydrophobic identities. Future research in this regard should attempt to identify effective molecules which are highly soluble in both media.

CONCLUSIONS

The results of the present study indicated that the SEO, RBO, and BHO had significantly different DPPH radical-scavenging activities that were higher than that of α-tocopherol, respectively. The different activities were attributed to the presence of various contents and chemical compositions of their DPPH radical-scavenging active components, especially tocopherols and phenolic compounds. In practical point of view, it was interesting to find that the oils' active components enter the antioxidative fractions with different proportions. The DPPH radical-scavenging active components of the SEO were mainly hydrophilic and almost did not enter the USM fraction. On the contrary, RBO contained the antioxidative components which were almost completely hydrophobic and entered the USM fraction at great extent. The highest level of accumulation of DPPH radical-scavenging active components in the USM fraction was observed in the BHO, and their chemical nature was almost equally hydrophilic/hydrophobic.

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مشخصات آنتی‌اکسیدانی روغنها و مواد صابونی ناشونده کنجد، سبوس برنج و پوست بنه بر حسب سیستم مدل مهارکنندگی رادیکال DPPH

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چکیده

قدرت مهارکنندگی رادیکال DPPH روغن پوست بنه، روغن کنجد، روغن سبوس برنج، عصاره‌های متانولی (متانول/آب، ۸۰ به ۲۰ حجمی/حجمی) و هگزانی روغنهای مزبور، و فراکسیون مواد صابونی‌ناشونده آنها مورد اندازه‌گیری قرار گرفت و با یکدیگر مقایسه شد. قدرت مهار کنندگی رادیکال DPPH روغن کنجد به طور معنی‌دار و به ترتیب بیش از روغنهای سبوس برنج و پوسته بنه بود. به رغم روغنهای پوست بنه و سبوس برنج، غلظتهای مورد آزمایش فراکسیون صابونی‌ناشونده روغن کنجد نتوانست رادیکال مزبور را به طرز قابل ملاحظه‌ای مهار کند. سهم عصاره‌های متانولی و هگزانی روغن کنجد درخصوص مهار رادیکالهای DPPH به ترتیب ۷۵ و ۲۵ درصد محاسبه شد. غلظتهای مورد آزمایش عصاره متانولی روغن سبوس برنج قادر نبود رادیکالهای DPPH را به طور مشهودی مهار نماید و قدرت مهار کنندگی عمدتاً ناشی از عصاره هگزانی بود. اجزاء مهار کننده رادیکالهای DPPH در خصوص روغن پوسته بنه تقریباً به طور مساوی (۴۸ و ۵۲ درصد) بین دو عصاره توزیع شدند.

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