Antioxidant Activity of Mung Bean Hulls

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ABSTRACT: The antioxidant activity of methanolic extracts of mung bean hulls was investigated. Extracts at a concentration of 100 ppm exhibited stronger antioxidant activity than 100 ppm dl-α-tocopherol (Toc) or 100 ppm butylated hydroxyanisole on the peroxidation of linoleic acid. Moreover, a synergistic effect was observed when 100 ppm of the extract was mixed with 100 ppm Toc. Also, the extracts showed good inhibitory activity in soybean oil oxidation, which was examined by peroxide value, thiobarbituric acid, and gas chromatography of oxidized fatty acid methyl esters. The extracts can reduce the formation of both primary and secondary oxidation products of soybean oil. The extracts had reducing power and scavenged 1,1-diphenyl-2-picrylhydrazyl radical, which may in part be responsible for their antioxidant activity. Based on the results obtained, mung bean hulls are a potential source of natural antioxidants owing to their marked antioxidant activity.
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KEY WORDS: Antioxidant activity, BHA, free radical, mung bean hulls, natural antioxidant, reducing power, scavenging effect, soybean oil, synergistic effect, tocopherol.

Antioxidants are used to preserve foods by retarding rancidity, discoloration, or deterioration due to autoxidation. Hence, the addition of antioxidants to foods has become increasingly common as a means of increasing the shelf life and improving the stability of lipids and lipid-containing foods. Synthetic antioxidants are widely used because they are effective and cheaper than natural types. However, the safety and toxicity of synthetic antioxidants have been important concerns (1). Natural alternatives for synthetic antioxidants have been studied over the last few decades. Antioxidative substances obtained from natural sources, such as oilseeds, grains, beans, vegetables, fruits, leaf waxes, bark, roots, spices and seaweeds, have been elucidated (2–6).

Recently, investigations of plant hulls that possess antioxidant activity have been reported. These plant hulls include those from rice (7,8), navy bean (9), and peanut (10). This research has shown that some antioxidative components may exist in the plant hulls. Seed hulls may play a defensive role, such as resistance to pests (11). Mung bean is a leguminous seed, and its hulls could be utilized as a valuable ingredient in various products. However, it remains unclear if mung bean hulls possess antioxidant activity. Thus, the objectives of this work were to investigate the antioxidant activity of mung bean hulls and to compare them with commercial antioxidants, such as butylated hydroxyanisole (BHA) and dl-α-tocopherol (Toc).

MATERIALS AND METHODS

Materials. Mung beans (*Phaseolus aureus*) were obtained from Tainan District Agriculture Improvement Station (Taiwan, Republic of China). After harvesting, the mung beans were shelled. Mung bean hulls were first ground in a mill (Tecator Cemotec 1090 sample mill, Hoganas, Sweden), then sealed in a plastic bottle, and finally stored at 4°C until used.

Extraction. Mung bean hulls (5 g) were extracted with 50 mL methanol in a shaking incubator at 25°C for 8 h. The extracts were filtered; the residue was reextracted under the same conditions, and the combined filtrates were evaporated to dryness *in vacuo* and weighed to determine the yield of soluble constituents.

Antioxidant activity determination. The antioxidant activities of the extracts were determined by the thiocyanate method (12) with 0.2 mL of extract, containing 50 to 500 ppm (wt/vol) dry filtrate. Each sample was added to a solution containing a mixture of linoleic acid (0.13 mL) in 99.0% ethanol (10 mL) and 0.2 M phosphate buffer (pH 7.0, 10 mL); and distilled water was added until the total volume reached 25 mL. The mixed solution was incubated in a conical flask at 40°C. At regular intervals, the extent of peroxidation was determined by the thiocyanate method, with 10 mL ethanol (75%), 0.2 mL of an aqueous solution of ammonium thiocyanate (30%), and 0.2 mL of ferrous chloride solution (20 mM in 3.5% HCl) being added sequentially. After stirring for 3 min, the absorbance of the mixture, measured at 500 nm, was indicated as the peroxide value (PV).

Soybean oil oxidation. Oxidation tests were conducted with refined, bleached, and deodorized soybean oil obtained from a commercial source (President Co., Tainan, Taiwan, Republic of China). Extracts (5 mg) were mixed with 50 g soybean oil that contained 190.0 ± 2.83 ppm Toc by high-per-

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formance liquid chromatography, after the oil sample had been treated with active carbon (E. Merck, Darmstadt, Germany) (13), and then stored at 60°C to accelerate the oxidation. The PV and thiobarbituric acid-reactive substances (TBARS) were determined by the iodometric titration procedure of the Association of Official Analytical Chemists' method Cd-8-53 (14) and by the method of Yen and Chen (15), respectively, at intervals during incubation.

For heating at 180°C, 5.0-mg samples of the extracts were mixed with 50 g soybean oil, treated with active carbon as previously described (13), and heated in air at 180°C for 2 h. Then oil samples were placed in vials, flushed with nitrogen, and frozen at −18°C for later measurement of fatty acid composition by the gas chromatography method. All treatments were carried out in open beakers (100 mL). All tests were replicated three times, and mean values are reported.

Fatty acid analysis. Fatty acids of soybean oil were methylated and analyzed in a Shimadzu GC-14B gas chromatograph (GC; Shimadzu Corp., Tokyo, Japan) with flame-ionization detector according to Peisker (16). Separation was accomplished with a 60 m × 0.252 mm i.d. capillary column (DB23; Supelco Inc., Bellefonte, PA) which was temperature-programmed from 170 to 200°C at 3°C/min. The injector and detector temperatures were 250°C. Flow rate of the hydrogen carrier gas was 1 mL/min with a split ratio of 10:1. After the solution was heated at 50°C for 10 min, 0.1 mL glacial acetic acid and 5 mL water were added immediately. The solution was extracted with *n*-hexane. The extracts were then dehydrated with anhydrous sodium sulfate and concentrated to a volume of 1 mL. A portion of the concentrate was injected (1.0 μL) into the GC. A low ratio of $C_{18:3}/C_{16:0}$ indicated a greater degree of oil deterioration.

Reducing power. The reducing power of extracts of mung bean hulls was measured according to the method of Oyaizu (17). The extract in 1.0 mL methanol was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$](2.5 mL, 1.0 %), and the mixture was then incubated at 50°C for 20 min. An aliquot (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 650 × *g* for 10 min. The upper layer of solution (2.5 mL) was mixed with water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The extracts (4.0 mL) were added to a methanolic solution (1 mL) of DPPH radical (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and left to stand for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Hitachi U-2000, Tokyo, Japan) (18). Values reported are means of results from triplicate determinations of two experiments.

Statistical analyses. Statistical analysis involved use of the Statistical Analysis System (19) software package. Analysis of variance (ANOVA) was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple-range tests.

RESULTS AND DISCUSSION

Figure 1 shows that the antioxidant activity of the extract during linoleic acid oxidation, determined by the thiocyanate method, increased with increasing concentration up to 100 ppm, and then no significant differences ($P > 0.05$) in antioxidant activity were shown with concentrations from 100 to 500 ppm, indicating that 100 ppm of extract exhibited strong antioxidant activity (97.6%). Dziezak (20) reported that antioxidant activity of Toc is concentration-dependent, and the most effective concentrations are in the range of 0.01–0.02%. However, Cillard *et al.* (21) noted that α-tocopherol showed a prooxidant effect as the ratio of α-tocopherol/linoleic acid was $\geq 5 \times 10^{-3}$. These results indicate that antioxidant activity is closely related to concentration.

The antioxidant activities of the extracts are compared with two commercial antioxidants, Toc and BHA, as determined by the thiocyanate method, in Figure 2. For all treatments there was a rise in absorbance for extracts stored at 40°C indicating linoleic acid oxidation. The control (without added antioxidants) had the highest PV of all treatments during storage at 40°C, indicating the highest intensity of oxidation. The significantly ($P < 0.05$) lower absorbances for different concentrations of the extracts and commercial antioxidants (compared to the control) indicate the greater antioxidant activity of the extracts. The extent of antioxidant activity followed the order of 200 ppm BHA > 200 ppm extract > 100 ppm extract = 200 ppm Toc > 100 ppm Toc > 100 ppm BHA after 12 d of storage, indicating that 100 ppm extracts had stronger antioxidant activity than 100 ppm BHA and 100–200 ppm Toc. Furthermore, the sample treated with 100 ppm extract combined with100 ppm Toc had the lowest rise in PV and exhibited stronger antioxidant activity, not only when compared with the sample treated with 100 ppm Toc

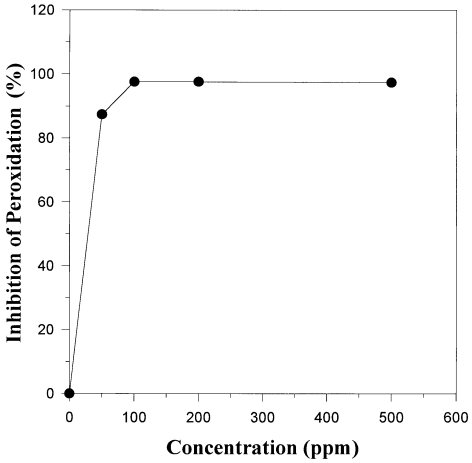

FIG. 1. Effect of different concentrations of a methanolic extract of mung bean hulls on inhibition of linoleic acid peroxidation. Antioxidant activity was determined by the thiocyanate method (Ref. 12), and the percentage inhibition of linoleic acid peroxidation, 100 − [(Abs. increase of sample/Abs. increase of control)], was calculated to express antioxidant activity.

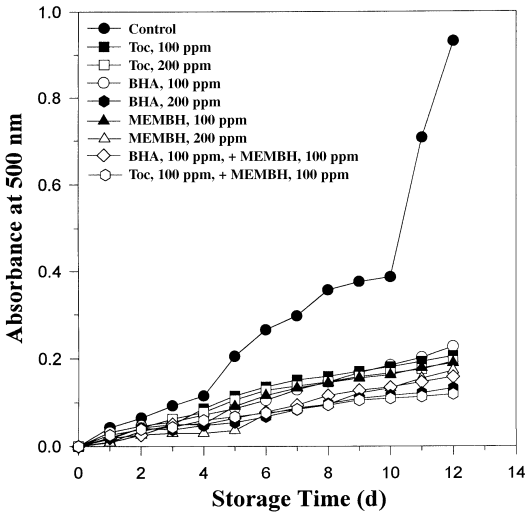

FIG. 2. Antioxidant activity of a methanolic extract of mung bean hulls (MEMBH), butylated hydroxyanisole (BHA), and α-tocopherol (Toc) on inhibition of linoleic acid peroxidation. The antioxidant activity was determined by the thiocyanate method (Ref. 12).

but also with 200 ppm Toc. These results indicate the synergistic effect of the extract on the inhibitory effect of Toc.

Because no significant differences ($P > 0.05$) in antioxidant activity occurred with concentrations between 100 and 500 ppm, 100 ppm of the extract was tested in soybean oil. Figure 3 shows the effects of the extract on oxidation of soybean oil stored at 60°C. The control sample, which reached a maximum PV of 181.0 ± 16.5 meq/kg at 8 d of testing, was oxidized to the highest extent. Samples treated with 100 ppm extracts, 100 ppm BHA, or 100 ppm Toc showed a gradual increase in PV, which reached a PV of 44.0 ± 3.1, 46.0 ± 2.6, and 53.0 ± 3.8 meq/kg, respectively, indicating that they exhibited more oxidative stability than the control during oxidation. The primary purpose of using antioxidants in lipids is to delay a significant accumulation of primary oxidative products and thus to improve oxidative stability (22). The PV of soybean oil that contained the extract was significantly ($P < 0.05$) lower than that of the control, which clearly showed the marked antioxidant effect of the extract. The primary products of lipid oxidation are hydroperoxides, which are generally referred to as peroxides. Therefore, the results from determining the concentration of peroxides for oxidized oil are a clear index of lipid peroxidation (23). However, measurement of peroxides only provides information about the initial oxidation potential of the oil (22). To elucidate the antioxidant effect of the extracts on other stages of lipid oxidation, it is necessary to measure whether the extracts possess an inhibitory effect during the later stages of peroxidation.

The effects of the extract and synthetic antioxidants on the TBARS values of soybean oil after accelerated oxidation at 60°C are shown in Figure 4. TBARS formation of the control increased with an increase in storage time. However, the values for the samples treated with the extract and antioxidants were significantly ($P < 0.05$) lower than those of the control. Among the additives used, 100 ppm extract lowered the content of TBARS significantly ($P < 0.05$) more than did 100 ppm BHA or 100 ppm Toc after 10 d of storage. TBARS measure the formation of secondary oxidation products, which may contribute to the off-flavor of oxidized oil (22). These results indicated that the extracts exhibited antioxidant activity not only in linoleic acid (Figs. 1 and 2) but also during soybean oil peroxidation (Figs. 3 and 4).

Changes in the $C_{18:3}/C_{16:0}$ ratio may provide a reliable measure of oil deterioration. The changes in relative percentages of fatty acids in soybean oil for all treatments before and after 2 h of heating at 180°C are shown in Table 1. No significant difference ($P > 0.05$) was found between the fresh oil and the oil containing the extract (100 ppm). In addition, there

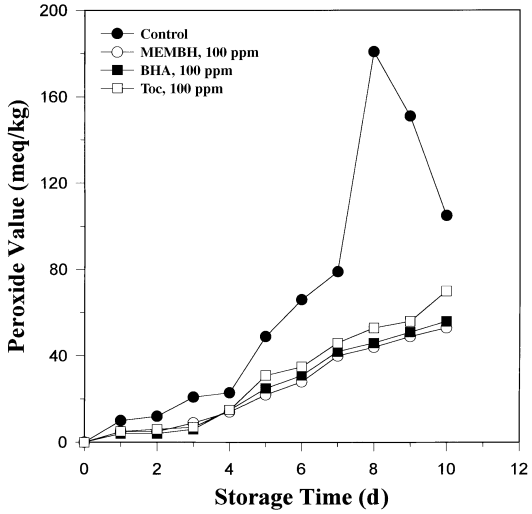

FIG. 3. Oxidation of soybean oil treated with a MEMBH, BHA, and Toc during storage at 60°C as measured by peroxide value (PV). See Figure 2 for other abbreviations.

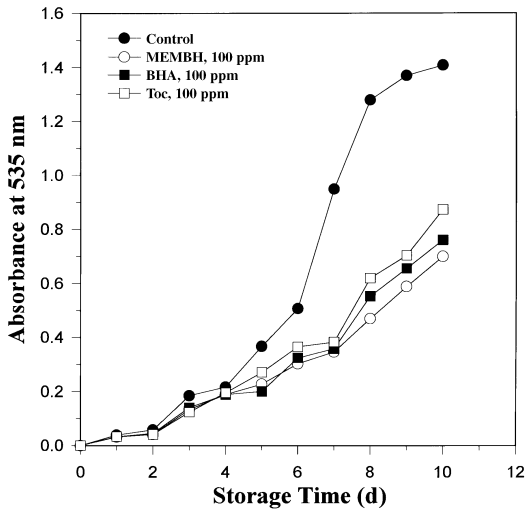

FIG. 4. Oxidation of soybean oil treated with a MEMBH,BHA, and Toc during storage at 60°C, measured as formation of thiobarbituric acid-reactive substances. See Figure 2 for abbreviations.

TABLE 1
Percentages of Fatty Acid Methyl Esters of Fresh Soybean Oil and Oil Heated at 180°C for 2 h with or without Treatment

Treatment[a]	16:0	18:0	18:1	18:2	18:3	18:3/16:0
			(mean ± SD)[b]			
Fresh oil (hour 0)	10.6 ± 0.02[b]	4.1 ± 0.01[b]	22.2 ± 0.02[d]	54.5 ± 0.01[bc]	8.1 ± 0.05[a]	0.8 ± 0.00[a]
Heated oil (hour 2)	11.6 ± 0.05[a]	4.1 ± 0.01[b]	22.7 ± 0.10[bc]	54.6 ± 0.19[b]	7.0 ± 0.32[b]	0.6 ± 0.03[b]
MEMBH	10.8 ± 0.04[b]	4.2 ± 0.02[ab]	22.6 ± 0.02[c]	54.2 ± 0.02[c]	7.9 ± 0.02[a]	0.7 ± 0.00[a]
BHA	11.3 ± 0.13[a]	4.1 ± 0.04[ab]	22.9 ± 0.01[ab]	55.0 ± 0.13[a]	6.6 ± 0.04[bc]	0.6 ± 0.01[b]
Toc	11.5 ± 0.17[a]	4.2 ± 0.11[a]	23.0 ± 0.16[a]	54.8 ± 0.04[ab]	6.5 ± 0.08[c]	0.6 ± 0.00[b]

[a]5.0 mg of extracts/antioxidants were mixed with 50 g soybean oil (the concentrations of methanol extracts of mung bean hulls (MEMBH), butylated hydroxyanisole (BHA), and α-tocopherol (Toc) 100 ppm, respectively, and heated in air at 180°C for 2 h, and then the fatty acid composition of oil was determined by gas chromatographic method.
[b]Means with different superscript letters in the column are significantly different ($P < 0.05$).

were significant differences ($P < 0.05$) between the control and the extract (100 ppm), indicating that the extract exhibited antioxidant activity in heated oil, but no significant difference ($P > 0.05$) was found between the control and Toc (100 ppm) and BHA (100 ppm). Furthermore, the higher ratios for treatments with the extract than for the control implied the soybean oil with the extract possessed a high resistance to oil oxidation.

As shown in Table 2, the reducing power (absorbance at 700 nm) of extract increased with an increased amount of extract. The equation of reducing power (y) and amounts of extracts (x) used is $y = 0.542625x + 0.013132$ ($r^2 = 0.97$, $P < 0.05$), indicating that the reducing power correlates well with amounts of extract. The extract at 0.8 mg exhibited a greater reducing power than did 0.05 mg ascorbic acid, which is a potent reducing agent (24). In general, the equation of antioxidant activity and reducing power is $y = 7.200999x + 89.43694$ ($r^2 = 0.87$, $P < 0.05$). This correlation analysis between antioxidant activity and reducing power showed a high positive correlation, indicating that reducing power in methanolic extracts of mung been hulls (MEMBH) was a contributor to antioxidant activity. These results are in good agreement with previous reports (25,26) where the antioxidant properties were shown to be concomitant with the development of reducing power. Thus, the extracts may contain reductones and react with free radicals to stabilize and terminate radical chain reactions.

The scavenging effect of the extract on the DPPH radical is shown in Figure 5. The activity of scavenging DPPH radical by the extract that we tested depended upon its concentration and correlated ($r^2 = 0.93$, $P < 0.05$) well with the extent of antioxidant activity. Hence, the antioxidant activity of the extract may be attributed to its hydrogen-donating ability (27). This result reveals that the extracts are free-radical inhibitors, possibly as primary antioxidants that react with free radicals.

Antioxidant activity of natural antioxidants has been shown to be involved with termination of free-radical reactions and reducing power (25–28). According to the data obtained, the marked inhibitory effect of MEMBH in both

TABLE 2
Reducing Power of Methanolic Extracts of Mung Bean Hulls

Amoung (mg)	Reducing power[a,b]
0.03	0.05 ± 0.000[j]
0.1	0.06 ± 0.005[i]
0.15	0.09 ± 0.003[h]
0.2	0.12 ± 0.000[g]
0.3	0.18 ± 0.003[f]
0.4	0.24 ± 0.011[e]
0.6	0.30 ± 0.002[d]
0.7	0.37 ± 0.001[c]
0.8	0.49 ± 0.003[a]
Ascorbic acid[c]	0.40 ± 0.03[b]

[a]The reducing power of extracts was determined by absorbance at 700 nm with a spectrophotometer.
[b]Means within a column with different superscript letters are not significantly different ($P > 0.05$).
[c]The amount of ascorbic acid was 0.05 mg.

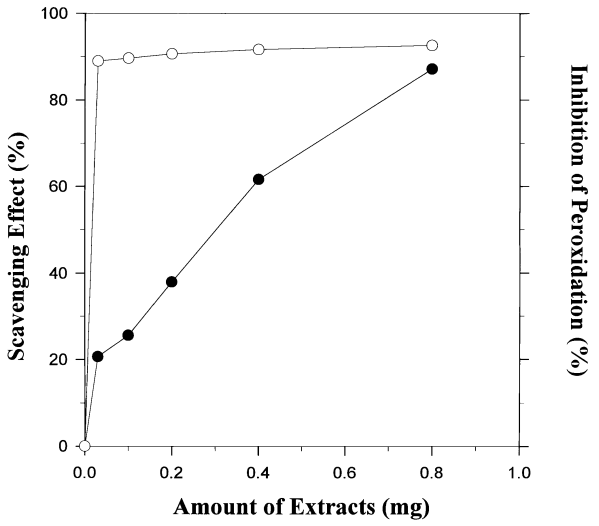

FIG. 5. Scavenging effect of the 1,1-diphenyl-2-picrylhydrazyl radical and antioxidant activity of different amounts of MEMBH. The antioxidant activity was determined by the thiocyanate method (Ref. 12), and the percentage inhibition of linoleic acid peroxidation is defined as in Figure 1. See Figure 2 for abbreviation; ●, scavenging effect; ○, inhibition of peroxidation.

linoleic acid and soybean oil peroxidation could in part be caused by its properties of scavenging free radicals and containing reductones. Further research on the isolation of the antioxidative components in mung bean hulls is in progress.

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