Antioxidative Properties of Methanolic Extracts from Peanut Hulls

Gow-Chin Yen[a,*] and **Pin-Der Duh**[b]

[a]Department of Food Science, National Chung Hsing University, Taichung, Taiwan, Republic of China and [b]Department of Food Health, Chia-Nan Junior College of Pharmacy, Tainan, Taiwan, Republic of China

Antioxidative properties of methanolic extracts from peanut hulls (MEPH) have been investigated. MEPH had good thermal stability and showed an 85.2% inhibition of peroxidation of linoleic acid when heated at 185°C for 2 h. Only a slight decrease in antioxidative activity of MEPH occurred when the extract was stored at different temperatures (−20, 5 and 30°C) under air or nitrogen (30°C) for 70 d. Antioxidative activity of MEPH decreased with an increase of pH from 3 to 9. No synergistic effect of ascorbic acid, citric acid, cysteine or α-tocopherol was observed on the inhibitory effect of MEPH. The reducing power of MEPH increased with an increase in concentration and was significantly correlated ($r = 0.9793$, $P < 0.05$) to the extent of antioxidative activity. MEPH also showed good inhibitory activity in lard oxidation when compared with butylated hydroxyanisole.

KEY WORDS: Antioxidant, lard oxidation, peanut hulls, synergism.

Antioxidants play an important role in manufacturing, packaging and storage of fats and fatty foods. Numerous compounds have been evaluated as antioxidants in fats, oils and fatty foods (1–5). However, the most widely used antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene, are suspected to cause liver damage (6), and their continued use in foods is being questioned. Tocopherol, a natural antioxidant, is less effective than synthetic antioxidants and the manufacturing cost is high (7). Identification and development of safer, natural antioxidants is therefore essential.

Previous literature is replete with reports of extracts from natural sources that have demonstrated strong antioxidant activity. These extracts have been reported to be more effective in many instances than some major synthetic antioxidants (8–10). Sheabar and Neeman (11) investigated purified extracts from olives to obtain an effective antioxidant. Kikuzaki and Nakatani (12) found five antioxidative compounds extracted from the leaves of oregano. Gallic acid and eugenol were reported by Kramer (13) as the two major antioxidants in clove. A process for extracting antioxidants from rosemary, sage and parsley was previously patented by Nestle (14). Rosemary extract has since become commercialized after being approved by the Food and Drug Administration, Washington, D.C., for direct food application.

The antioxidant activity of methanolic extracts of peanut hulls (MEPH) has been demonstrated (15), and it showed activity equal to BHA and stronger than α-tocopherol. However, if an antioxidant such as MEPH is to be used in foods, its effectiveness will depend on various factors such as the pH of the food, storage temperature and the extent of thermal processing applied to the food. These factors have to be investigated to determine the feasibility of using MEPH as an antioxidant in food. Thus, the objectives of this work were to further examine the factors affecting the antioxidative activity of MEPH and to evaluate its effects on inhibition of lard oxidation.

*To whom correspondence should be addressed at Department of Food Science, National Chung Hsing University, 250 Kuokuang Road, Taichung, Taiwan, Republic of China.

MATERIALS AND METHODS

Material. Peanuts of Tainan no. 11, Spanish type, were obtained from Tainan District Agriculture Improvement Station, Taiwan, Republic of China. The peanuts were washed and hand-shelled. The hulls were freeze-dried and ground into a fine powder in a mill (Tecator Cemotec 1090 Sample Mill, Hoganas, Sweden). The material that passed through an 80-mesh sieve was retained for use, sealed in a plastic bottle and stored at 4°C until used.

Extraction procedure. Peanut hull powder (5.0 g) was extracted with 50 mL methanol overnight in a shaking incubator at room temperature. The extract was filtered, and the residue was re-extracted under the same conditions. The combined filtrate was evaporated to 5 mL in a rotary evaporator below 40°C. The concentration of the extract in solvent was 44.5 ± 1.98 mg/mL, which was the average of three replicate analyses.

Antioxidative activity determination. The MEPH was determined by the thiocyanate method (7) with 200 μL of extract for the assay. Each sample was added to a solution 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 the volume was made up to 2 mL with distilled water. 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 (7), with 10 mL of 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. Distilled water was used as the control.

Effect of pH. Both a citrate buffer (pH 3.0 and 5.0) and a phosphate buffer (pH 7.0 and 9.0) at strengths of 0.2 M were used to study the influence of pH on antioxidative activity of MEPH. Comparisons were always made with controls at the same pH and with the same buffer as in the sample. The antioxidative activity was determined according to the thiocyanate method (7). Each treatment of each replicate was run in duplicate, and the results were averaged.

Heat treatment. MEPH was evaporated first to remove the solvent. Samples of MEPH (3 mg) were individually placed in 10-mL beakers and heated in air at 185°C for 0, 10, 20, 30, 40, 50, 60, 90 or 120 min. The beakers were then cooled to room temperature, and their contents were dissolved in 0.3 mL methanol for measuring antioxidative activity. All tests and analyses were run in duplicate and averaged.

Storage tests. Samples of MEPH (9.6 mg) were individually placed in glass bottles (8 × 45 mm), sealed and incubated at −20, 5 or 30°C for 70 d. Other MEPH samples were placed in bottles, bubbled at 30°C with nitrogen for 3 min before tightly closing the caps and then incubated at 30°C for 70 d to study the influence of an

inert atmosphere on the stability of MEPH. In both storage tests, the samples were withdrawn at various times for measurement of the antioxidative activity. All tests were run in duplicate, and analyses of all samples were run in duplicate and averaged.

Synergistic effect. Chemical compounds, including ascorbic acid, citric acid, cysteine and α-tocopherol, were added at a fixed level of 0.2 or 0.5% of linoleic acid. Synergism of these compounds on the antioxidative activity of MEPH was determined in an aqueous system by the thiocyanate method (7). The percentage of synergism was calculated as follows (16):

$$\text{syn\%} = 100 \times [(S - C) - (M - C) - (A - C)]/(S - C) \quad [1]$$

where C represents the induction period of linoleic acid without the addition of a chemical compound; S, M and A represent induction periods of linoleic acid incorporated with a combined mixture of MEPH and one of the chemical compounds (S), MEPH (M) and one of the chemical compounds (A), respectively. Induction period was expressed as the time required to reach an absorbance of 0.3 (17). The synergistic effects of each treatment were run in duplicate and the results were averaged.

Determination of reducing power. The determination of reducing power was performed by the method reported by Evans *et al.* (18). MEPH (0.48, 1.2, 2.4, 3.6 or 4.8 mg) was 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. Five milliliters of 10% trichloroacetic acid was 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 the absorbance was measured at 700 nm. The reducing power tests were run in duplicate, and analyses of all samples were run in triplicate and averaged.

Application of MEPH to lard. Fresh rendered lard, free from additives and not chemically processed, was used as the substrate for oxidation studies. MEPH (2.4, 9.6 or 24 mg) was mixed with 2.0 g of lard and then incubated at 100 ± 2°C. The peroxide values (POV) were determined by the iodometric titration procedure of AOCS Method Cd 8-53 (19). The percent inhibition of lard oxidation, 100 − [(POV increase of sample/POV increase of control) × 100] was calculated to express antioxidative activity (20). All tests were run in triplicate, and analyses of all samples were run in duplicate and averaged.

Statistical analysis. Statistical analysis involved use of the Statistical Analysis System (21) software package on replicated test data. Significant differences between means were determined by Duncan's Multiple Range tests.

RESULTS AND DISCUSSION

Effect of heat treatment. The antioxidative activity, as determined by the thiocyanate method (7), of MEPH heated at 185°C for various times is shown in Figure 1. Heating for 20 min did not significantly ($P > 0.05$) reduce antioxidant potency. The activity was slightly reduced by heating for 30 min or longer. Little change was observed between 30 and 60 min of heating. MEPH exhibited 85.2% inhibition of peroxidation of linoleic acid when heated at 185°C for 120 min. Therefore, MEPH had good thermal stability in comparison with BHA, which was previously reported by Hamama and Nawar (22) to have half of the remaining antioxidant activity after heating at 185°C for 45 min. As a whole, the antioxidative activity of MEPH decreased with heating time. The loss of activity at such an elevated temperature (185°C) may result from evaporation of extracts as well as from chemical decomposition (22). Because the antioxidants in peanut hulls were somewhat thermally stable, they might be applicable to frying or thermal processing.

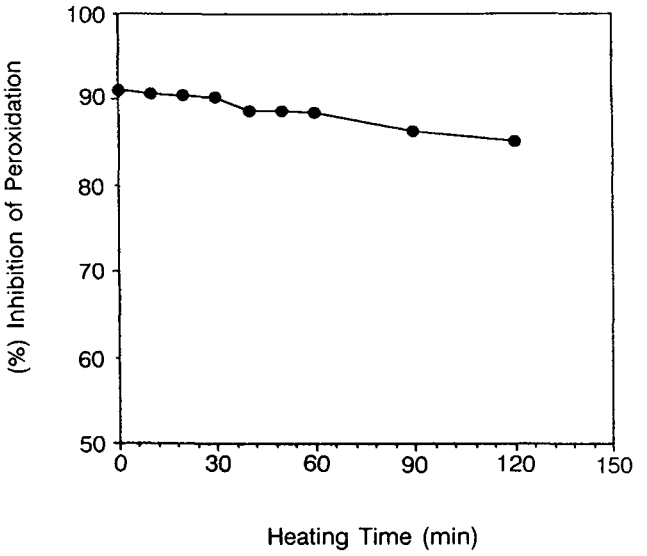

FIG. 1. Antioxidative activity of methanolic extracts from peanut hulls as a function of heating time at 185°C. The activity was determined by the thiocyanate method.

Storage test. Figure 2 shows the antioxidative activity of MEPH stored at −20, 5 or 30°C for 70 d under air. One treatment was stored at 30°C under nitrogen. A slight

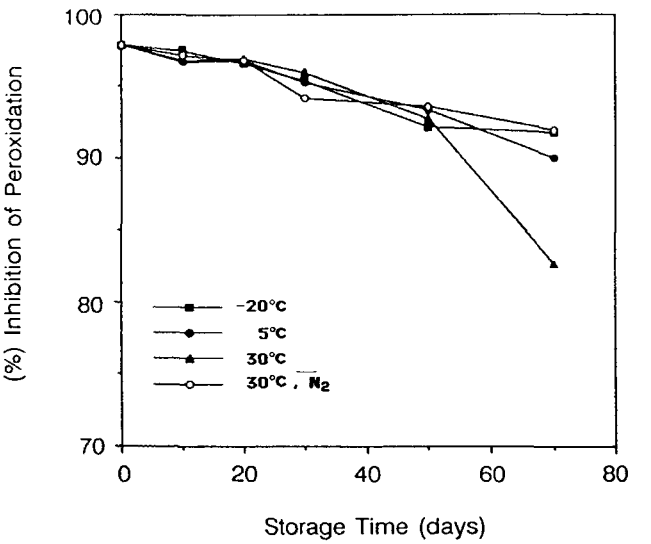

FIG. 2. Antioxidative activity of methanolic extracts from peanut hulls as a function of storage time. The activity was determined by the thiocyanate method.

loss of antioxidative activity was observed in the four treatments incubated between 10 and 30 d. No significant ($P > 0.05$) difference at 70-d storage was observed among samples stored at 5°C, at −20°C or at 30°C under nitrogen. The treatment stored in air at 30°C, however, had a significantly lower ($P < 0.05$) antioxidative activity at 70 d than did the other treatments, but even this treatment retained 82.6% of its antioxidative activity. This observation is in agreement with the findings of Sherwin (23) who showed that heat greatly accelerated oxidation, and it has been estimated that the rate of oxidation reactions doubles for approximately 15°C increase in temperature (24).

Effect of pH. The influence of pH on the stability of MEPH also was studied. The results are given in Figure 3, where the percent inhibition of peroxidation of linoleic acid is shown. The antioxidative activity of MEPH decreased with an increase of pH from 3 to 9, indicating an obviously strong dependence ($P < 0.05$) on the pH. The MEPH exhibited high antioxidant activity at neutral and acidic pH values but showed no effect at pH 9.

Synergistic effect. Synergistic effects of ascorbic acid, citric acid, cysteine or α-tocopherol on the antioxidative activity of MEPH were investigated in aqueous systems with a phosphate buffer (pH 7.0) by using the thiocyanate method (7). The extent of the combined effects is shown in Table 1. No synergism of ascorbic acid, citric acid, cysteine or α-tocopherol on the inhibitory effect of MEPH was observed.

In the current system, ascorbic acid added at a level of 0.2% (5.3×10^{-5} M) and 0.5% (1.3×10^{-4} M) based on linoleic acid (tests I and II, respectively) exhibited a prooxidative activity. These results are in agreement with the findings of Dziezak (25) who reported that ascorbic acid at low levels possibly elicited prooxidative effects. Singleton and Pattee (26) also reported that ascorbic acid has a prooxidative effect at concentrations below 2×10^{-2} M when applied to lipoxygenase in a peanut homogenate. In current work, however, ascorbic acid exhibited an antioxidative effect when the addition of the amount increased to 2% (data not shown).

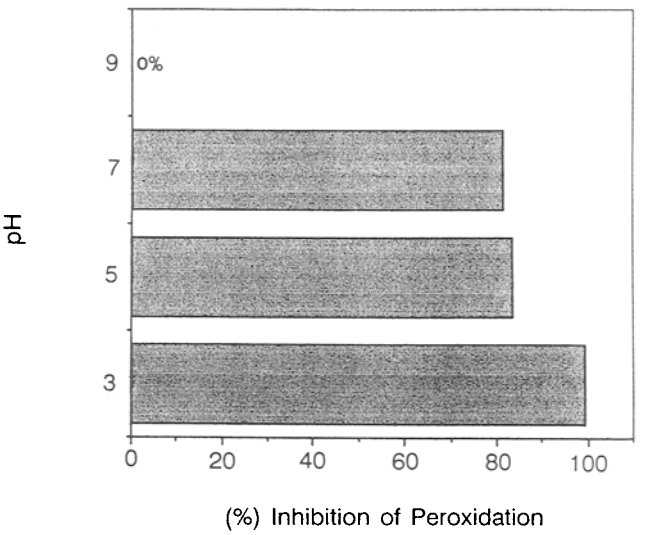

FIG. 3. Influence of pH on antioxidative activity of methanolic extracts from peanut hulls under citrate buffer (pH 3 and 5) and phosphate buffer (pH 7 and 9). The activity was determined by the thiocyanate method.

At a level of 0.2% of linoleic acid (a ratio of 0.0013 of mol α-tocopherol/mol linoleic acid), α-tocopherol exhibited weak antioxidative activity; but at a level of 0.5% of linoleic acid (a ratio of 0.0033 mol α-tocopherol/mol linoleic acid), α-tocopherol exhibited no antioxidative activity (Table 1). α-Tocopherol was reported by Cillard *et al.* (27) to be a prooxidant, especially when the ratio of mol α-tocopherol/mol linoleic acid was greater than 0.005. Although a synergistic effect exists when phenolic antioxidants are used together with certain acids (17), as given in Table 1, both citric acid and cysteine were not

TABLE 1

Synergistic Effect of Some Chemical Compounds on the Antioxidative Activity of Methanolic Extracts from Peanut Hulls (MEPH)

Chemical compounds	Test I[a]		Test II[a]	
	Induction period (days)	Synergism (%)	Induction period (days)	Synergism (%)
None	5a[b]		5a[b]	
MEPH	11b		11b	
Ascorbic acid (AA)	3c		3c	
Citric acid (CA)	11b		12d	
Cysteine (Cys)	7d		7e	
α-Tocopherol (Tol)	6e		5a	
MEPH + AA	9f	—[c]	9f	—
MEPH + CA	11b	—	11b	—
MEPH + Cys	12g	—	12d	—
MEPH + Tol	8h	—	5a	—

[a] 9.6 mg MEPH was added, and other chemical compounds were added at a level of 0.2 and 0.5% of linoleic acid for Tests I and II, respectively. Induction period means the days to reach an absorbance of 0.3 at 500 nm by using the thiocyanate method.

[b] Values in a column with the same letters are not significantly different ($P > 0.05$).

[c] Means no synergistic effect.

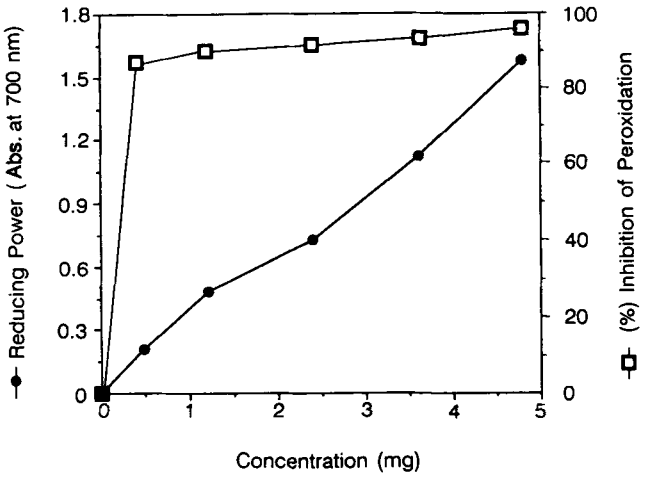

FIG. 4. Reducing power and antioxidative activity of different amounts of methanolic extracts from peanut hulls. Abs, absorbance.

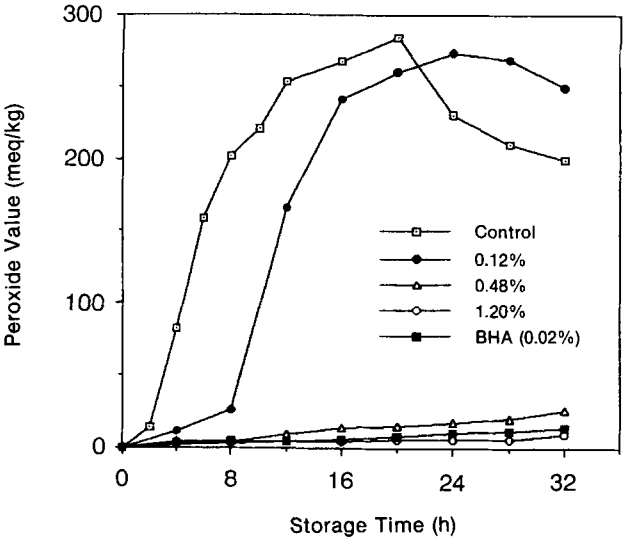

FIG. 5. Peroxide value of lard with different amounts of methanolic extracts from peanut hulls during heating at 100°C. BHA, butylated hydroxyanisole.

synergistic to the inhibitory effect of MEPH, even though they individually exhibited an antioxidative effect.

Effect of concentration. The effect of MEPH concentration on reducing power and antioxidative activity is shown in Figure 4. MEPH at 4.8 mg exhibited a greater reducing power than did 0.5 mg of ascorbic acid (data not shown). The reducing power of MEPH increased with an increase in concentration and correlated ($r = 0.9793$, $P < 0.05$) well with the extent of antioxidative activity. These results are in good agreement with previous reports (18, 28-30) where the antioxidative properties were shown to be concomitant with the development of reducing power.

Inhibition of lard oxidation. The antioxidative activity of MEPH at different concentrations in lard held at 100 ± 2°C for 32 h is shown in Figure 5. The effect of MEPH addition on POV development of lard was related to the amount of MEPH added. In the early stages, the autoxidation of lard without added MEPH was accompanied by a rapid increase of POV, which reached a maximum value of 284 meq/kg at 20 h of testing. A significant difference ($P < 0.05$) was found between the control and the lard containing MEPH (0.12%), which slowed the rate of peroxide formation slightly. The autoxidation of lard was, however, greatly inhibited in the presence of MEPH at concentrations of 0.48 and 1.20% of lard. Moreover, there was no significant difference ($P > 0.05$) between BHA (200 ppm) and MEPH (1.2%), but significant differences ($P < 0.05$) were found between MEPH (0.48%) and both BHA (200 ppm) and MEPH (1.20%) in inhibition of lard peroxidation. These results indicated that MEPH exhibited antioxidative activity not only in linoleic acid peroxidation (15) but also in lard peroxidation.

In previous work, an antioxidative component of MEPH was identified as luteolin (15). Further research on the mechanism of the antioxidative effects of MEPH is in progress. Testing must, however, be carried out prior to the application of MEPH to food products in order to prove that MEPH is toxicologically safe.

ACKNOWLEDGMENT

This research work was supported in part by the National Science Council, Republic of China, under grant no. NSC81-0204-B005-01.

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[Received August 11, 1992; accepted January 28, 1993]