Antioxidant Efficacy of Methanolic Extracts of Peanut Hulls in Soybean and Peanut Oils

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ABSTRACT: Antioxidant activity of methanolic extracts of peanut hulls (MEPH) was evaluated in soybean and peanut oils after accelerated oxidation at 60°C. Results showed that the oils with 0.12, 0.48, and 1.20% MEPH had significantly ($P < 0.05$) lower peroxide values and acid values than the control after storage at 60°C. Moreover, oils with 0.48 and 1.20% MEPH were significantly ($P < 0.05$) superior to 0.02% butylated hydroxyanisole (BHA) in reducing oxidation of both oils. Negative synergism was observed when 0.48 and 1.20% MEPH were mixed with 0.01% dl-α-tocopherol or 0.01% BHA in soybean oil compared to MEPH alone.
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KEY WORDS: Antioxidant, BHA, peanut hulls, peanut oil, soybean oil.

Lipid oxidation not only lowers quality and nutritional value of foods, it is also associated with aging, membrane damage, heart disease, and cancer (1). The addition of antioxidants is effective in retarding oxidation of fats. Labuza (2) noted that antioxidants can increase shelf life of foods by 15–200%. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butyl hydroquinone (TBHQ), are widely used in the food industry because they are effective and less expensive than natural antioxidants. Their safety, however, has been questioned. Ito *et al.* (3) reported BHA to be carcinogenic in animal experiments. In these circumstances, research on and development of safer natural antioxidants are therefore essential. Recently, many investigations have been carried out, and some antioxidative substances have been found in natural sources. For example, Sheabar and Neeman (4) investigated purified extracts from olives to obtain an effective antioxidant. Kikuzaki and Nakatani (5) found five antioxidative compounds in extracts from oregano leaves. Gallic acid and eugenol were reported by Kramer (6) as two major antioxidants in clove. Duh *et al.* (7) noted that luteolin was an antioxidant in peanut hulls.

Extracts from natural sources, such as oleoresin rosemary, have been shown to suppress lipid oxidation of chicken nuggets (8), beef steaks (9), or pork steaks (10). Antioxidant activity of methanolic extracts of peanut hulls (MEPH) in the oxidation of linoleic acid has been reported (7,11–13). Although the antioxidant efficacy of MEPH was superior to BHA in reducing oxidation of lard aged at 100°C (11), the antioxidant activity of MEPH in vegetable oils has not been investigated. Thus, the objective of this work was to examine the antioxidant efficacy of MEPH in soybean oil (SBO) and peanut oil (PO).

MATERIALS AND METHODS

Materials. Peanuts, Tainan no. 11, Spanish type, were obtained from the Tainan District Agriculture Improvement Station (Tainan, Taiwan, Republic of China). The peanuts were washed and hand-shelled. The hulls were freeze-dried and ground into a fine powder in a Tecator Cemotec 1090 Sample Mill, (Hoganas, Sweden). The material that passed through an 80-mesh sieve was retained, sealed in a plastic bottle, and stored at 4°C until used. Refined, bleached, and deodorized SBO and PO, with 950 ppm and 405 ppm total tocopherol content [determined by high-performance liquid chromatography (HPLC)], respectively, were obtained from commercial sources (Taichung, Taiwan).

Extraction. Peanut hull powder (5.0 g) was extracted with 50 mL methanol in a shaking incubator at 25°C for 24 h. The extract was filtered, and the residue was re-extracted under the same conditions. The extraction was repeated twice, and the combined filtrates were evaporated to 5 mL in a rotary evaporator at 40–42°C.

Oxidation. In an open 50-mL beaker, MEPH (2.4, 9.6, or 24.0 mg) was mixed with 2.0 g oil and sonicated in an ultrasonic cleaner (Branson 8210; Branson Ultrasonic Co., Danbury, CT) for 3 min. Each treatment was placed in an oven at 60°C. The peroxide value (PV) and acid value (AV) of each treatment were determined by AOCS Official Methods Cd 8-53 and Cd 3a-63, respectively (14), at intervals during storage. The percentage inhibition of oil oxidation, 100% − [(PV increase of sample/PV increase of control) × 100%] was calculated to represent antioxidant activity (15). All tests were run in triplicate, and analyses of all samples were run in duplicate and averaged.

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To study any synergistic effects, MEPH (9.6 or 24.0 mg) was mixed by ultrasonication with 2.0 g SBO that contained 0.01% dl-α-tocopherol or 0.01% BHA and aged at 60°C. The PV was determined by AOCS Official Method Cd 8-53 (14). The degree of synergistic effect was defined as the percentage of synergism (SYN%). SYN% was calculated as follows (16):

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

where *C* represents the oxidative stability of SBO without the addition of MEPH and an antioxidant (BHA or dl-α-tocopherol); *S*, *M*, and *A* represent oxidative stability values of SBO treated with a combined mixture (*S*) of MEPH and BHA or α-tocophenol, MEPH (*M*), and BHA or α-tocopherol (*A*), respectively. Oxidative stability of SBO was expressed as the time required to reach a PV of 70 meq/kg (17,18). Each treatment was run in duplicate and the results were averaged.

Tocopherol analysis. Tocopherol in oil was determined by HPLC performed with a Waters liquid chromatograph (Waters, Ltd., Milford, MA), consisting of a model 510 pump, a model 481 ultraviolet-visible detector set at 292 nm. A LiChrospher Si 60 column (5 µm, 250 × 4 mm, i.d., E. Merck, Darmstadt, Germany) was used for analysis. The mobile phase was *n*-hexane/isopropanol/ethanol (97.5:2:0.5, vol/vol/vol) at a flow rate 0.6 mL/min.

Statistical analyses. Statistical analyses were conducted with the Statistical Analysis System (19) software package of replicate test data. Analyses of variance were performed by ANOVA procedures. Significant differences ($P < 0.05$) between means were determined by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Determination of PV of oils oxidized at 100°C is unreliable because hydroperoxides decompose at elevated temperature (20); therefore, antioxidant efficacy of MEPH in SBO and PO oil was evaluated at 60°C.

SBO. SBO without MEPH or BHA reached a maximum PV of 680.4 meq/kg at 8 d of storage (Fig. 1). A significant difference ($P < 0.05$) in PV was found between the control and SBO containing MEPH or BHA, which slowed the rate of peroxide formation. The PV of SBO with 0.12, 0.48, or 1.2% MEPH and 0.02% (0.02%) BHA were 213.1, 55.7, 28.9, and 118.2 meq/kg, respectively. These samples had 68.7, 91.8, 95.8, and 82.6% inhibition of oxidation after 8 d of storage, compared with the control. Significant differences ($P < 0.05$) were found between these values. These results indicated that MEPH inhibited SBO oxidation. Furthermore, antioxidant activities of 0.48 and 1.2% MEPH were significantly more effective ($P < 0.05$) than that of 0.02% BHA.

Acid value, a measure of titratable acidity of fat to determine extent of oxidative deterioration (21), increases as a result of the formation of fatty acids by hydrolysis of triacylglycerols and by oxidative and thermal reactions. Handle *et al.* (21) reported that any acidic compounds in oils will contribute to the AV. The AV of SBO without MEPH rapidly developed after 8 d of storage at 60°C (Fig. 2). However, the AV of SBO with MEPH or BHA increased only slightly after 8 d

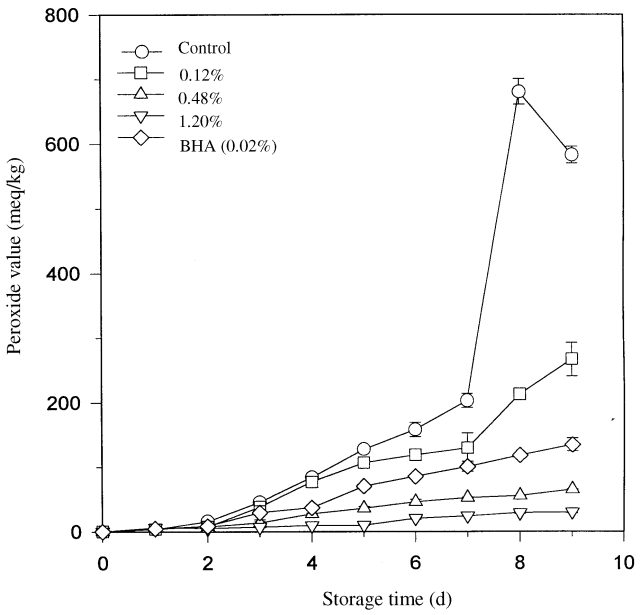

FIG. 1. Peroxide values of soybean oils with 0.12, 0.48, and 1.20% methanolic extracts from peanut hulls and 0.02% butylated hydroxyanisole (BHA) during storage at 60°C. Each point represents the mean ± SD of three experiments.

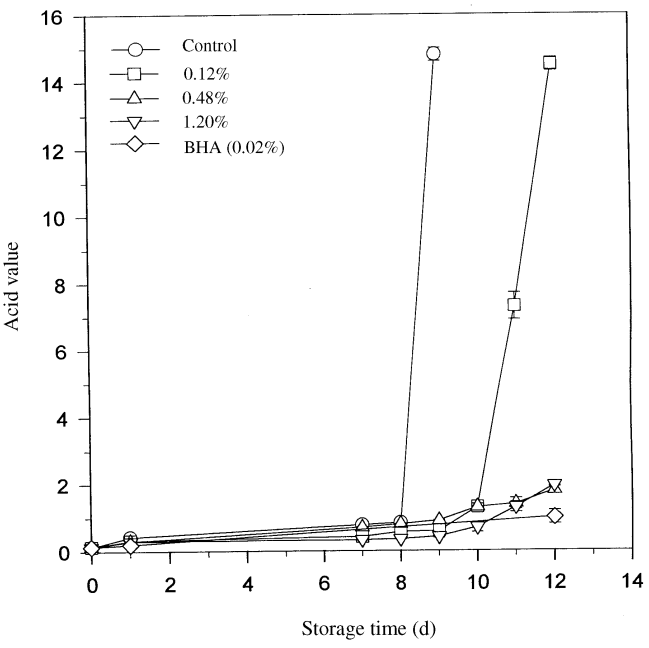

FIG. 2. Acid values of soybean oils with 0.12, 0.48, and 1.20% methanolic extracts from peanut hulls and 0.02% BHA during storage at 60°C. See Figure 1 for abbreviation. Each point represents the mean ± SD of three experiments.

of storage, indicating that both MEPH and BHA significantly ($P < 0.05$) reduced SBO oxidation compared to the control. Nawar (22) reported that hydroperoxides break down in several steps to yield a wide variety of decomposition products. In the later stages of oxidation, the rate of decomposition exceeds the rate of formation of oxidation products. In the present work, the SBO control reached a maximum PV at 8 d of storage (Fig. 1), after which the PV decreased. This result may explain why the AV of SBO without MEPH or BHA rapidly increased at 8 d (Fig. 2).

Peanut oil oxidation. No significant differences ($P > 0.05$) were found between the PO control and PO that contained both 0.12% MEPH and 0.02% BHA, which slowed the rate of peroxide formation slightly after 20 d of storage (Fig. 3). However, significant differences ($P < 0.05$) were found between these samples (control, 0.12% MEPH, and 0.02% BHA) and both 0.48 and 1.20% MEPH. PO without MEPH reached a maximum PV of 700.3 meq/kg after 34 d of storage. However, at this point, the PV of MEPH at 0.12, 0.48, or 1.2%, and BHA (0.02%) were 424.7, 114.7, 69.9, and 282.8 meq/kg, respectively. The antioxidant activities were calculated as 39.4, 83.6, 90.0, and 59.6%, respectively, and significant differences ($P < 0.05$) were found between these values, indicating that MEPH exhibited an inhibitory effect in PO oxidation. Moreover, 0.48 and 1.2% MEPH were significantly ($P < 0.05$) more effective than 0.02% BHA.

The AV of PO without MEPH or BHA rapidly increased at 35 d of storage, indicating decomposition of peroxides (Fig. 4). The AV of PO with 0.12, 0.48, or 1.2% MEPH or 0.02% BHA were significantly ($P < 0.05$) lower than that of the control at 35 d.

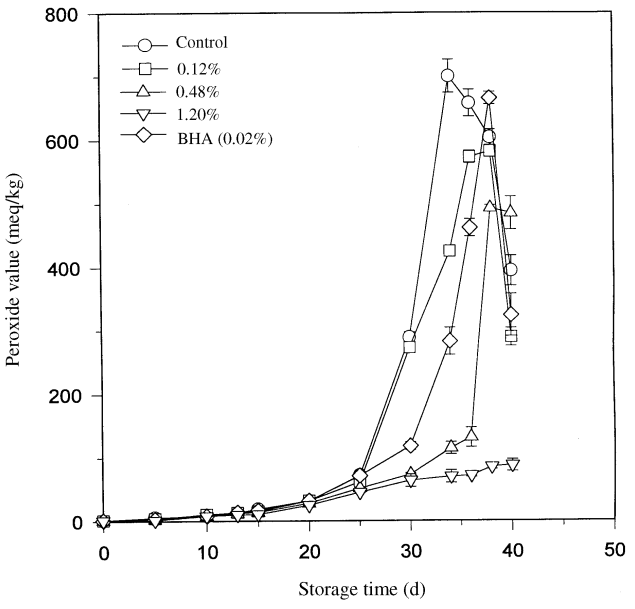

FIG. 3. Peroxide values of peanut oils with 0.12, 0.48, and 1.20% of methanolic extracts from peanut hulls and 0.02% BHA during storage at 60°C. See Figure 1 for abbreviation. Each point represents the mean ± SD of three experiments.

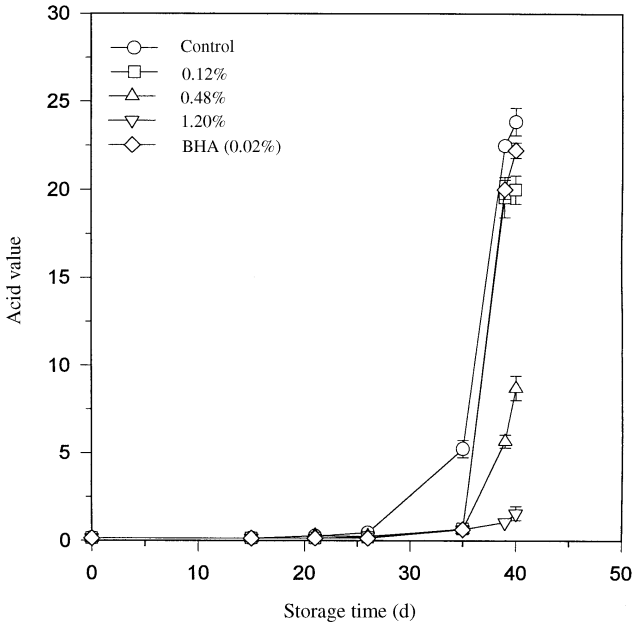

FIG. 4. Acid values of peanut oils with 0.12, 0.48 and 1.20% of methanolic extracts from peanut hulls and 0.02% BHA during storage at 60°C. See Figure 1 for abbreviation. Each point represents the mean ± SD of three experiments.

Synergistic effect of MEPH. Although a significant difference ($P < 0.05$) was found between the control and SBO with 0.01% dl-α-tocopherol (Table 1), the oxidative stability of dl-α-tocopherol was only slightly more effective than that of SBO. These results agreed with findings of Dziezak (23) and Sherwin (17) who reported that unsaturated vegetable oils with their inherent tocopherol contents do not benefit much from additional tocopherol.

In a previous report (11), no synergistic effects of ascorbic

TABLE 1
Effects of dl-α-Tocopherol, Butylated Hydroxyanisole (BHA), and/or Methanolic Extracts of Peanut Hulls (MEPH) on the Oxidative Stability of Soybean Oil

Chemical compounds	Oxidative stability (h)[a]	Synergism (%)
Control	107[b,i]	
dl-α-tocopherol (0.01%)	119[h]	
BHA (0.01%)	143[g]	
MEPH (0.48%)	194[d]	
MEPH (1.20%)	292[a]	
dl-α-tocopherol (0.01%) + MEPH (0.48%)	173[f]	−50.0[b,c]
BHA (0.01%) + MEPH (0.48%)	183[e]	−61.8[d]
dl-α-tocopherol (0.01%) + MEPH (1.20%)	247[c]	−40.7[b]
BHA (0.01%) + MEPH (1.20%)	266[b]	−39.0[a]

[a]Hours of storage at 60°C to obtain a peroxide value of 70 meq/kg for soybean oil.
[b]Means with different superscript letters in the column are significantly different ($P < 0.05$).

acid, citric acid, cysteine, or dl-α-tocopherol were observed on the inhibitory effect of MEPH in linoleic acid oxidation. However, the synergistic effect of MEPH and either α-tocopherol or BHA in edible oils has not been reported. Because PO had good oxidative stability, SBO was used as substrate to evaluate synergistic effects. Negative synergism was calculated for MEPH when mixed with 0.01% dl-α-tocopherol or 0.01% BHA (Table 1). Moreover, both 0.01% dl-α-tocopherol and 0.01% BHA decreased the efficacy of MEPH in the inhibition of SBO oxidation.

The results in this study have demonstrated that MEPH acts as an antioxidant in SBO and PO. However, toxicological tests are necessary before MEPH can be used in food products.

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REFERENCES

1. Cosgrove, J.P., D.F. Church, and W.A. Pryor, The Kinetics of the Autoxidation of Polyunsaturated Fatty Acids, *Lipids 22*:299–304 (1987).
2. Labuza, T.P., Kinetics of Lipid Oxidation in Foods, *CRC Crit. Rev. Food Technol. 2*:355–405 (1971).
3. Ito, N., A. Hagiwara, M. Shibata, T. Ogiso, and S. Fukushima, Induction of Squamous Cell Carcinoma in the Forestomach of F344 Rats Treated with Butylated Hydroxyanisole, *Gann 73*:332–334 (1982).
4. Sheabar, F.Z., and I. Neeman, Separation and Concentration of Natural Antioxidants from the Rape of Olives, *J. Am. Oil Chem. Soc. 65*:990–993 (1988).
5. Kikuzaki, H., and N. Nakatani, Structure of a New Antioxidative Phenolic Acid from Oregano, *Agric. Biol. Chem. 53*:519–524 (1989).
6. Kramer, R.E., Antioxidants in Clove. *J. Am. Oil Chem. Soc. 62*: 111–113 (1985).
7. Duh, P.D., D.B. Yeh, and G.C. Yen, Extraction and Identification of an Antioxidative Component from Peanut Hulls, *Ibid. 69*:814–818 (1992).
8. Lai, S.M., J.I. Gray, A.M. Booren, and R.L. Crackel, Effects of Oleoresin Rosemary, Tertiary Butylhydroquinone, and Sodium Tripolyphosphate on the Development of Oxidative Rancidity in Restructured Chicken Nuggets, *J. Food Sci. 56*:616–620 (1991).
9. Stoick, S.M., J.L. Gray, A.M. Booren, and D.J. Buckley, Oxidative Stability of Restructured Beef Steaks Processed with Oleoresin Rosemary, Tertiary Butylhydroquinone, and Sodium Tripolyphosphate. *Ibid. 56*:597–600 (1991).
10. Liu, H.F., A.M. Booren, J.I. Gray, and R.L. Crackel, Antioxidant Efficacy of Oleoresin Rosemary and Sodium Tripolyphosphate in Restructured Pork Steaks, *Ibid. 57*:803–806 (1992).
11. Yen, G.C., and P.D. Duh, Antioxidative Properties of Methanolic Extracts from Peanut Hulls, *J. Am. Oil Chem. Soc. 70*:383–386 (1993).
12. Yen, G.C., P.D. Duh, and C.L. Tsai, Relationship Between Antioxidant Activity and Maturity of Peanut Hulls, *J. Agric. Food Chem. 41*:67–70 (1993).
13. Yen, G.C., and P.D. Duh, Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free-Radical and Active-Oxygen Species, *Ibid. 42*:629–632 (1994).
14. *Official and Tentative Methods of the American Oil Chemists' Society*, Champaign, 1990, Method Cd 3A-63, Cd 8-53.
15. Rhee, K.S., and K.C. Rhee, Antioxidant Activity Increase in Heating Oilseed Protein Ingredients with Glucose, *J. Food Prot. 45*:452–454 (1982).
16. Bishov, S.J., and A.S. Henick, Antioxidant Effect of Protein Hydrolysates in a Freeze-Dried Model System, *J. Food Sci. 37*:873–875 (1972).
17. Sherwin, E.N. Antioxidants for Vegetable Oils, *J. Am. Oil Chem. Soc. 53*:430–436 (1976).
18. Buck, D.F., Antioxidants, in *Food Additive User's Handbook*, edited by J. Smith, Blackie Academic & Professional, New York, 1991, pp. 17–19.
19. SAS, *SAS User's Guide: Statistics*, SAS Institute, Cary, 1985.
20. Frankel, E.N., In Search of Better Methods to Evaluate Natural Antioxidants and Oxidative Stability in Food Lipids, *Trends Food Sci. Technol. 4*:220–225 (1993).
21. Handle, A.P., F.L. Hamouz, A.N. Dumper, and M.E. Knickrehm, Evaluation of Fluid Shortening for Deep-Fat Frying Under Institutional Conditions, *J. Foodservice Systems 3*:49–61 (1984).
22. Nawar, W.W., Lipids, in *Food Chemistry*, edited by O.R. Fennema, Marcel Dekker, Inc., New York, 1985, p. 182.
23. Dziezak, J.D., Preservatives: Antioxidants, the Ultimate Answer to Oxidation, *Food Technol. 40*:94–102 (1986).

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