Relationship between Antioxidant Activity and Maturity of Peanut Hulls

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The effect of varied maturity on the antioxidant activity of peanut hulls was investigated. Methanolic extracts of peanut hulls of varied maturity exhibited a similarly marked antioxidant activity, 92.9–94.8% inhibition of peroxidation of linoleic acid. The content of both luteolin and total phenolics increased significantly with maturity and seemed to show no correlation with antioxidant activity. However, the antioxidant activity remained constant after 1.671 mg/g of hulls of total phenolic content was reached. Total phenolics (1.671 mg/g of hulls) in peanut hulls seemed to be an initial point of maximum antioxidant activity. High total phenolic content in peanut hulls of varied maturity is associated with a high antioxidant activity and with an important role in the stability of lipid oxidation.

INTRODUCTION

Hundreds of materials, both synthetic and natural, have been developed as antioxidants to preserve food (Lea, 1952), but only butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butyl hydroquinone (TBHQ) are used in practice as synthetic antioxidants and tocopherols as natural ones. However, the most widely used antioxidants, BHA and BHT, are suspected to cause liver damage (Imida et al., 1983); therefore, synthetic chemicals as food additives are no longer used. Tocopherols are less effective than synthetic antioxidants and the manufacturing cost is high (Osawa and Namiki, 1981). In these circumstances, as a result of much research, some antioxidative substances have been found from natural sources (Pratt and Birac, 1979; Gutfinger, 1981; Wu et al., 1982; Kramer, 1985; Huang and Chang, 1986; Kikuzaki and Nakatani, 1989; Ramarathnam et al., 1989; Yen et al., 1990; Duve and White, 1991).

The peanut is a principal agricultural plant in the world. The antioxidative property of peanut kernels has been investigated by some researchers. For example, the flavonoid dihydroquercetin, extracted from peanut kernels, exhibited antioxidant activity (Pratt and Miller, 1984). The oxidative stability of peanut oil can be improved by heating the peanut kernels before they are pressed for oil (Huang et al., 1988; Yen et al., 1990). Moreover, methanolic extracts of peanut hulls were found to exhibit marked antioxidative activity, and an antioxidative component of methanolic extracts was identified as luteolin (Duh et al., 1992). Concentrations of flavonoids in plum tree were reported to change markedly during maturation and storage (Hillis and Swain, 1959). In addition, the color of the mesocarp layer of the peanut hulls changed as peanuts matured (Williams and Drexler, 1981; Daigle et al., 1988), and the flavonoid content in the hull could be associated with these color changes (Daigle et al., 1988). Thus, it is necessary to elucidate whether the antioxidant activity of methanolic extracts of peanut hulls is affected by maturation. Total phenolic compounds have been confirmed to retard fat rancidity and to improve the stability of lipid peroxidation (Gutfinger, 1981; Ramarathnam et al., 1986). Furthermore, the defense property of peanuts had been studied (Jackson, 1964; Mixon and Roger, 1973; Turner et al., 1975; Holley et al., 1985). Lindsey and Turner (1975) suggested that phenolic compounds in peanut may play an active role in protecting the embryo from fungal infection. Characteristics of peanut hulls are not only important in yield but also in general appearance and resistance to pests (Norden et al., 1982). In other words, such a defense system could occur in the peanut hulls. Although the relationship between maturity and flavonoid components of peanut hulls has been reported by Daigle et al. (1988), whether the antioxidant activity of methanolic extracts of peanut hulls is also affected by the total phenolic compounds remains unclear.

The purposes of this study were to investigate the relationships between antioxidant activity and both maturity and total phenolic compounds in peanut hulls and to elucidate the role that total phenolic compounds play in the stability of lipid peroxidation.

MATERIALS AND METHODS

Materials. In mid-March 1991, peanuts of Tainan select no. 11, Spanish type, were planted in a research plot at the Tainan District Agricultural Improvement Station, Tainan, Taiwan. To obtain peanuts at advancing stages of maturity, plants were harvested at 74, 84, 94, 104, 114, 124, 134, and 144 days after planting (DAP). At harvest time, the fields were dug by hand, and all pods of harvestable size were hand-picked and then washed. Approximately 8 kg of peanuts from random field and plot locations was used in each sample. The hand-shelled hulls were stored in a freezer below −40 °C until 144 DAP, and then the hulls were freeze-dried and ground into a fine powder in a mill (Tecator Cemotec 1090 sample mill). The material which passed through an 80-mesh sieve was retained for use, sealed in a plastic bottle, and stored at 4 °C until used.

Determination of Color Value. Color values, L, a, b, and ΔE, of peanut hull powder were measured with a color difference meter (80 color measuring system Nippon Denshoku Industrial Co. Ltd.) (Collins and Post, 1981); calibration was made against a white tile. Powder of peanut hulls was placed in a cuvette, and one reading is the average of triplicate samples.

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Table I. Moisture Content and Color Difference of Peanut Hull Powder of Different Maturities[a]

DAP	moisture,[b] %	L^b	a^b	b^b	ΔE
74	7.9 ± 0.25A	68.7 ± 0.02A	5.3 ± 0.13C	16.9 ± 0.04B	0.0 ± 0.00A
84	7.7 ± 0.48A	71.8 ± 0.13B	5.6 ± 0.02BC	18.8 ± 0.05A	3.7 ± 0.10B
94	7.1 ± 0.19A	62.6 ± 0.22C	6.6 ± 0.15A	16.9 ± 0.13B	6.2 ± 0.20C
104	7.8 ± 0.55A	60.8 ± 0.50D	5.9 ± 0.00B	16.0 ± 0.13C	7.7 ± 0.22D
114	7.9 ± 0.70A	59.8 ± 0.21E	5.4 ± 0.07C	14.5 ± 0.10D	9.2 ± 0.23E
124	7.3 ± 0.30A	55.6 ± 0.05F	5.6 ± 0.32BC	14.2 ± 0.20D	13.4 ± 0.10F
134	7.7 ± 0.27A	53.5 ± 0.18G	5.4 ± 0.50C	13.5 ± 0.57E	15.5 ± 0.05G
144	7.3 ± 0.57A	54.5 ± 0.14H	5.6 ± 0.33BC	13.8 ± 0.15E	14.5 ± 0.09H

[a] Values are mean ± standard deviation of three replicate analyses. [b] Means within a column with the same upper case letters are not significantly different at $P > 0.05$.

Extraction of Antioxidant Components from Peanut Hulls. The peanut hull powder (5 g) was extracted with methanol (50 mL) overnight in a shaking incubator at room temperature. The extracts were filtered with filter paper Toyo 5A, and the residue was extracted again under the same conditions. The combined filtrate was evaporated to dryness in vacuo.

Determination of Antioxidant Activity. The antioxidant activity of methanolic extracts was determined according to the thiocyanate method (Osawa and Namiki, 1981). Methanolic extracts (100 μL) were added to a solution mixture of linoleic acid–100% ethanol–0.2 M phosphate buffer (pH 7.0). The mixed solution in a conical flask was incubated at 40 ± 1 °C, and the peroxide value was determined by reading the absorbance at 500 nm after coloring with $FeCl_2$ and thiocyanate at intervals during incubation. All test data are the average of triplicate analyses.

Quantitative Analysis of Luteolin. The luteolin in peanut hulls was determined by HPLC performed with a Hitachi liquid chromatograph (Hitachi, Ltd., Tokyo) consisting of a Model L-6200 pump, a Rheodyne Model 7125 syringe loading sample injector, a Model L-4200 UV–vis detector set at 254 nm, and a Model D-2500 integrator. A LiChrospher 100 RP-18 reversed-phase column (5 μm, 125 × 4 mm i.d., E. Merck) was used for analysis. The volume injected was 10 μL. The elution solvents were A and B containing water–acetic acid (99:1 v/v) and methanol, respectively. The gradient elution program was set at 1.6 mL/min, starting with 80% A and 20% B linearly to 60% A and 40% B in 35 min.

To determine the luteolin in the methanolic extracts, an authentic sample of luteolin was prepared; dilutions were made so that the range of concentration correlated with the estimated content of luteolin in the samples. The content of luteolin in the methanolic extracts of peanut hulls was calculated from the standard curve of luteolin. Triplicate samples were run for each set.

Determination of Total Phenolic Compounds. The total phenolic compounds present in the peanut hulls was determined spectrophotometrically using Folin–Denis reagent (AOAC, 1984). The methanolic extracts (0.1 mL) of peanut hulls in a volumetric flask were diluted with glass-distilled water (75 mL). Folin–Denis reagent (5 mL) was added, and the contents of the flask were mixed throughly. After 3 min, Na_2CO_3 solution (10 mL, of concentration 10 g/100 mL) was added and finally quantified to 100 mL with glass-distilled water; the mixture was allowed to stand for 30 min with intermittent shaking. The blue color was measured with a spectrophotometer (Hitachi U-2000). The concentration of total phenolic compounds in the peanut hulls was determined by comparison with the absorbance of standard catechin at different concentrations.

Statistical Analysis. Statistical analysis involved use of the Statistical Analysis Systems (SAS, 1985) software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple-range tests.

RESULTS AND DISCUSSION

The yield of flour was 41% after the Spanish-type hulls were milled and passed through an 80-mesh sieve. The remaining material consisted of debris and siliceous material that could not be converted into flour. This result is in agreement with that of Collins and Post (1981), who reported that 42.2% flour of Runner-type hulls was obtained.

Table II. Effect of Amount of Luteolin and Total Phenolic Compounds in Methanolic Extracts of Peanut Hulls of Different Maturities on Inhibition of Linoleic Acid Peroxidation[a]

DAP	antioxidant activity,[b,c] %	luteolin,[c] mg/g of hulls	total phenolics,[c] mg/g of hulls
74	92.9 ± 1.80A	1.93 ± 0.108A	33.4 ± 0.230A
84	94.8 ± 2.20A	4.87 ± 0.174B	33.7 ± 0.098A
94	93.8 ± 0.60A	9.05 ± 0.094C	46.3 ± 0.167B
104	93.6 ± 0.90A	9.46 ± 0.310D	50.5 ± 0.432C
114	93.0 ± 1.10A	14.91 ± 0.112E	62.4 ± 0.087D
124	93.1 ± 1.30A	16.33 ± 0.051F	66.8 ± 0.112E
134	93.7 ± 0.08A	24.15 ± 0.033G	70.0 ± 0.672F
144	93.9 ± 0.90A	28.60 ± 0.071H	71.3 ± 0.277G

[a] Values are mean ± standard deviation of three replicate analyses. [b] Determined by thiocyanate method. [c] Means within a column with the same upper case letters are not significantly different at $P > 0.05$.

The amounts of moisture and values of color difference of peanut hulls from different days after planting (DAP) are presented in Table I. The range of moisture of hulls from different DAP is 7.1–7.9%; no difference ($P > 0.05$) in the moisture was found among the samples. The color changed with the maturity; hulls of maturity after 114 DAP produced a dark flour (lower Hunter L value and lower Hunter b value). The values of ΔE significantly differed ($P < 0.05$) with maturity. These color differences caused flour of the mature hulls to appear brown in comparison with flour from immature hulls. This result agrees with previous reports (Williams and Drexler, 1981; Daigle et al., 1988) that the color of the mesocarp layer of the peanut shell changes from orange to brown and finally to black. Moreover, color transitions of the mesocarp provide definitive criteria for pod maturity assessments throughout all stages of development (Williams and Drexler, 1981).

Table II shows the antioxidant activity of methanolic extracts and luteolin content of the hulls. Methanolic extracts of peanut hulls from different DAP, specifically 74, 84, 94, 104, 114, 124, 134, and 144 days, exhibited 92.9, 94.8, 93.8, 93.6, 93.0, 93.1, 93.7, and 93.9% of antioxidant activity on linoleic acid peroxidation, respectively, and no significant difference ($P > 0.05$) was found in antioxidant activity of peanut hulls from different DAP. Thus, the methanolic extracts of peanut hulls from different DAP exhibited a similar and strong antioxidant activity for peroxidation of linoleic acid in aqueous dispersion.

Luteolin, a flavonoid substance, occurs in numerous plants (Harborne, 1967) and possesses greater antioxidant activity than BHT in inhibiting malonaldehyde formation of palm oil (Das and Pereira, 1990). According to Table II, the luteolin content of hulls from various DAP differed significantly ($P < 0.05$). These data indicate that the luteolin content found in peanut hulls increased with the

Table III. Effect of Various Amounts of Total Phenolic Compounds in Methanolic Extracts of Peanut Hulls from 74 Days after Planting on Inhibition of Linoleic Acid Peroxidation[a]

total phenolics, mg/g of hulls	antioxidant activity,[b,c] %	total phenolics, mg/g of hulls	antioxidant activity,[b,c] %
3.3420	92.9 ± 0.60A	0.0134	81.9 ± 1.40D
1.6710	92.4 ± 0.80A	0.0067	58.0 ± 1.20E
0.6684	89.0 ± 1.10B	0.0033	41.2 ± 0.30F
0.3342	88.6 ± 0.50B	0.0017	16.5 ± 1.50G
0.0334	86.3 ± 0.60C		

[a] Values are mean ± standard deviation. [b] Determined by thiocyanate method. [c] Means within a column with the same upper case letters are not significantly different at $P > 0.05$.

maturity of the peanuts. This result agrees with that reported by Hillis and Swain (1959), that the concentration of flavonoid changes markedly during maturation, and that reported by Daigle et al. (1988), that luteolin is the dominant flavonoid in shells from mature peanuts.

Luteolin has been identified as an antioxidative component of methanolic extracts of peanut hulls and showed strong antioxidant activity on peroxidation of linoleic acid in aqueous dispersion (Duh et al., 1992). The luteolin concentration at 144 DAP was 15 times that at 74 DAP; however, the antioxidant activities of the two samples were the same. Thus, the results in the model tested showed no positive correlation between antioxidant activity and luteolin content in the hulls. Apparently, some factors seem to contribute to the prevention of lipid peroxidation other than luteolin.

Gutfinger (1981) discovered that a high polyphenol content was associated with high resistance to oxidation of the oils. Ramarathnam et al. (1986) reported a strong influence of the level of total phenolic constituents in rice hulls on the storage ability of rice seeds. Thus, the total phenolic compounds may retard fat rancidity and improve the stability of lipid peroxidation.

The amounts of total phenolic compounds determined in the hulls at the respective DAP indicate that all had total phenolic compounds ranging from 33.4 to 71.3 mg/g of hulls (Table II). After 84 DAP, the content of total phenolic compounds in hulls increased significantly ($P < 0.05$) with increasing maturity. However, the hulls having 33.4–71.3 mg/g of hulls of total phenolic compounds exhibited similar and strong antioxidant activities (92.9–94.8%). Furthermore, dilutions of methanolic extracts of peanut hulls from 74 DAP were made, and the antioxidant activity changed (Table III). For example, as total phenolic compounds in hulls were diluted to 0.0017 mg/g of hulls, the antioxidant activity decreased to 16.5%. However, the peanut hulls with total phenolic compounds over 1.671 mg/g of hulls showed strong antioxidant activity (>92.0%), and for that reason methanolic extracts of peanut hulls of different maturities exhibited similar antioxidant activities (Table II). In contrast, the antioxidant activity with content less than 1.671 mg/g of hulls of total phenolic compounds decreased significantly ($P < 0.05$) with the decreasing content of total phenolic compounds. Hence, the content of 1.671 mg/g of hulls of total phenolic compounds seems to be an initial point of maximum antioxidant activity (Figure 1). The data reported here demonstrate that the methanolic extracts of peanut hulls from different harvest periods exhibited similar and strong antioxidant activities on peroxidation of linoleic acid in aqueous dispersion due to high levels of total phenolic compounds.

The mechanism of aging in living systems is believed to involve reactions related to lipid peroxidation. Certain

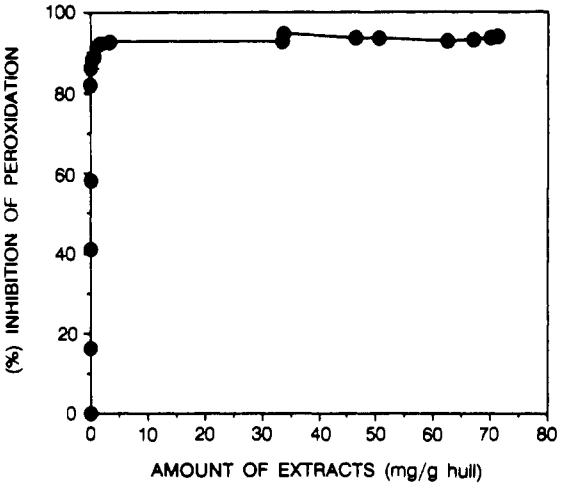

Figure 1. Effect of different amounts of total phenolic compounds in methanolic extracts of peanut hulls on inhibition of linoleic acid peroxidation.

inherent defense systems govern such reactions and facilitate the maintenance of vital physiological processes; the defense system must be located in the hulls (Ramarathnam et al., 1986). The data represented here demonstrated that the level of phenolic compounds found in peanut hulls changed as the peanuts matured. Therefore, there is a direct correlation between each maturity class and specific contents of total phenolic compounds of the hull. Methanolic extracts of peanut hulls of different maturities are sufficient for the preservation of lipid peroxidation due to high levels of total phenolic compounds in the hulls and may explain why the methanolic extracts of peanut hulls of different maturities showed similar and strong antioxidant activities. Further studies to investigate the antioxidant properties of methanolic extracts of peanut hulls and their application in foods are in progress.

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