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Changes in antioxidant activity and components of methanolic extracts of peanut hulls irradiated with ultraviolet light

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The antioxidant activity and the variations of components in methanolic extracts of peanut hulls (MEPH) after UV irradiation were investigated. MEPH of varied periods of irradiation (0, 3 and 6 days), exhibited 96·0% inhibition of peroxidation of linoleic acid. The relative remaining ratios of luteolin in MEPH after irradiation for 3 and 6 days were 87·9% and 69·5%, respectively, and those of total phenolic compounds were 96·5% and 90·4%, respectively. MEPH irradiated for 0, 3 and 6 days exhibited a similarly marked antioxidant activity as a result of a large content of total phenolic compounds. MEPH of varied periods of irradiation always showed the same UV-vis spectra and Rf values on a TLC plate. Thus the antioxidant activity of MEPH was unaffected by UV irradiation.

INTRODUCTION

Natural sources of antioxidative activity include soy bean products, oat products, crude vegetable oils, amino acids, peptides, proteins, guaiac gum, nordihydroguaiaretic acid, flavonoids, spices, herbs and Maillard reaction products, etc. (Schuler, 1990). Many authors reported the antioxidative activity of hull extracts, such as navy bean hulls (Onyeneho & Hettiarachchy, 1991), rice hulls (Ramarathnam *et al.*, 1989) and peanut hulls (Duh *et al.*, 1992). Antioxidative components of these sources have been identified; however, there is no report of antioxidative properties being affected by ultraviolet irradiation. Some researchers have reported that ultraviolet irradiation not only initiates lipid peroxidation but leads to oxidation of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethyl protocatechuate (EP) and propyl gallate (PG) (Kurechi & Yamaguchi, 1980; Kurechi & Kato, 1980; Kurechi & Kunugi, 1983), indicating that UV irradiation may affect the functional properties of antioxidants. Although the factors affecting antioxidative activity of peanut hulls are reported (Yen & Duh, 1993), no attempt has been made to examine the effects of antioxidative activity of peanut hulls after ultraviolet irradiation. Thus, the objectives of this work were to investigate the effects of irradiation on antioxidative activity of peanut hulls and to examine the variation of components of MEPH after UV irradiation.

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MATERIALS AND METHODS

Materials

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 then ground into a fine powder in a mill (Tecator Cemotec 1090 Sample Mill, Hohanas, Sweden). The material which passed through an 80-mesh sieve was retained for use and sealed in a plastic bottle and then stored at 4°C until used.

Extraction procedure

Peanut hull powder (5·0 g) was extracted with 50 ml of 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 in a vacuum below 40°C on a rotary evaporator to a final volume of 5 ml.

UV irradiation

The irradiation of MEPH was examined according to the method of Kurechi and Kato (1980). Four millilitres of

MEPH were put into a beaker 40 mm in diameter and 55 mm deep. The solution was placed in a UV irradiation box (110 V, 60 Hz, CAMAG 29100 Muttenz-Schweiz) and irradiated continuously for 6 days. The distance between the beaker and the light source was 110 mm. The sample volume was adjusted at exactly 4 ml with methanol every 24 h. The temperature of the equipment was maintained at 25 ± 1°C.

Thin-layer chromatography

The methanolic extracts (0.4 ml) were streaked on a precoated silica gel plate (20 × 20 cm, F254, 0·25 mm, E. Merck) and developed with the solvent system benzene:ethyl formate:formic acid (70:25:5, v/v/v) (BEF). After drying, fractions on the plate were located by a thin-layer chromatography (TLC) Scanner (CAMAG Ltd, Muttenz, Switzerland) under short-wavelength (254 nm) ultraviolet radiation.

Determination of antioxidant activity

The antioxidant activity of methanolic extracts was determined according to the thiocyanate method (Osawa & Namiki, 1981). Methanolic extracts (0·2 ml) of each sample were added to a solution mixture of linoleic acid (0·13 ml) in 99% ethanol (10 ml) and 0·2 M phosphate buffer (pH 7·0, 10 ml), and the total volume was made up to 25 ml with distilled water. The mixed solution in a conical flask was incubated at 40°C and the degree of oxidation was measured by the thiocyanate method, with 10 ml of ethanol (75%), 0·2 ml of an aqueous solution of ammonium thiocyanate (30%), 0·2 ml sample solution, and 0·2 ml ferrous chloride solution (20 mM in 3·5% HCl) being added sequentially. After stirring for 3 min, the absorption values of the mixtures measured at 500 nm were indicated as the peroxide content. All test data are the average of three replicate analyses.

Quantitative analysis of luteolin

The luteolin in MEPH was determined by HPLC performed with a Hitachi Liquid Chromatograph (Hitachi Ltd., Tokyo, Japan), 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 programme 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 methanol 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. Peak areas were used for the calculation, and a regression analysis was used to quantify the content of luteolin in the peanut hulls. 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 content of the flask mixed thoroughly. 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 and then the mixture was allowed to stand for 30 min with intermittent shaking. The blue colour 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

Antioxidant activity of methanolic extracts of peanut hulls (MEPH) under irradiation with UV light for various periods is shown in Table 1. Methanolic extracts of peanut hulls irradiated for 0, 3 and 6 days exhibited an

Table 1. Antioxidant activity of methanolic extracts of peanut hulls under irradiation with UV light for various periods[a]

Days	Absorbance at 500 nm[b]	Antioxidant activity (%)[cd]
0	0·158 ± 0·003 A[e]	99·7 ± 0·16 A[e]
3	0·162 ± 0·004 A	96·5 ± 0·21 A
6	0·162 ± 0·005 A	96·5 ± 0·25 A

[a]Four millilitres of methanolic extracts of peanut hulls (MEPH) were placed in a UV irradiation box and irradiated for various periods. The temperature of the equipment was maintained at 25 ± 1°C.
[b]The absorption value at 500 nm of control before and after incubation was 0·090 and 1·859, respectively, and that of each sample before incubation was 0·10.
[c]The antioxidant activity of extract (9·6 mg) was determined by thiocyanate methods and the percent inhibition of linoleic acid peroxidation, 100 − [(Abs increase of sample/Abs increase of control) × 100] was calculated to express antioxidant activity.
[d]Values are mean ± SD of three replicate analyses.
[e]Means within a column with the same upper case letters are not significantly different ($P > 0·05$).

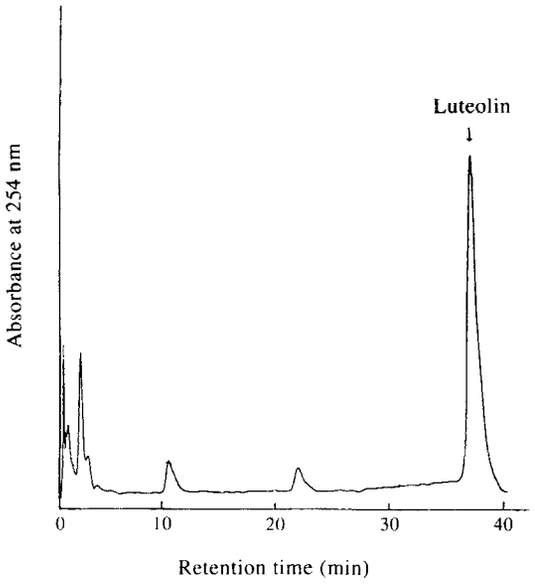

Fig. 1. HPLC chromatogram of methanolic extracts of peanut hulls. Conditions: column, LiChrospher 100 RP-18 reversed-phase (5 μm, 125 × 4 mm); eluent, A and B containing water : acetic acid (99 : 1, v/v) and methanol, gradient elution with 80% A and 20% B linearly to 60% A and 40% B in 35 min; flow rate, 1·6 ml/min; detector, ultraviolet 254 nm.

antioxidant activity of approximately 96·0% and no significant differences ($P > 0.05$) were found, indicating that the antioxidant activity of MEPH irradiated with UV rays in the model test was unaffected.

Luteolin has been identified as an antioxidative component (Das & Pereira, 1990; Duh *et al.*, 1992). As shown in Fig. 1, the retention time (36·8 min) of luteolin in MEPH was the same as that of an authentic sample, indicating that luteolin was not co-eluting with other components of the mixture. The amounts of luteolin and total phenolic compounds in MEPH irradiated with UV light for various periods are shown in Table 2. The amounts of luteolin decreased with increasing period of irradiation. Relative to the amount of luteolin of MEPH not irradiated, the remaining ratios of luteolin of MEPH irradiated with UV light for 3 and 6 days were 87·9% and 69·5%, respectively.

Table 2. Amounts of luteolin and total phenolic compounds in methanolic extracts of peanut hulls under irradiation with UV light for various periods[a]

Days	Luteolin[b] (mg/g) of hulls	Total phenolics[b] (mg/g) of hulls
0	1·74 ± 0·051 (100·0)[c]A[d]	7·80 ± 0·165 (100·0)[c]A[d]
3	1·53 ± 0·000 (87·9)B	7·53 ± 0·044 (96·5)B
6	1·21 ± 0·023 (69·5)C	7·05 ± 0·041 (90·4)C

[a]Four millilitres of methanolic extracts of peanut hulls (MEPH) were placed in a UV irradiation box and irradiated for various periods. The temperature of the equipment was maintained at 25 ± 1°C.
[b]Values are mean ± SD of three replicate analyses.
[c]Values in parentheses are percentages relative to control values (100%).
[d]Means within a column with the different upper case letters are significantly different ($P < 0.05$).

Kurechi and Kato (1980) reported that the amounts of butylated hydroxyanisole and butylated hydroxytoluene gradually decreased after a 10-day irradiation and about 65% of butylated hydroxyanisole and 20% of butylated hydroxytoluene remained. Phenolic compounds are evidently affected by ultraviolet radiation. The characterization of natural materials exhibiting antioxidant activity was well correlated with the content of phenolic compounds (Hayase & Kato, 1984). Gutfinger (1981) and Ramarathnam *et al.* (1986) discovered that phenolic compounds play an important role in stabilizing oxidation of the oils; Yen *et al.* (1993) reported that peanut hulls exhibited marked antioxidant activity as a result of their containing a considerable amount of phenolic compounds. Hence, the total phenolic compounds may retard fat rancidity and improve the stability of lipid peroxidation.

The amounts of total phenolic compounds of MEPH decreased with the period of irradiation with UV light. Relative to the total phenolic compounds of MEPH not irradiated, the remaining ratios of total phenolic compounds of MEPH irradiated with UV light for 3 and 6 days were 96·5% and 90·4%, respectively, indicating that the amounts of total phenolic compounds of

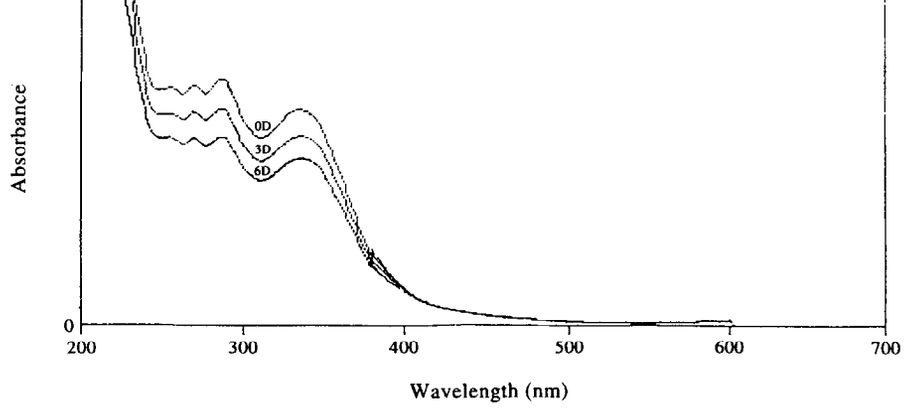

Fig. 2. Absorption spectra of methanolic extracts of peanut hulls under irradiation with UV light for 0, 3 and 6 days.

MEPH decreased somewhat as a result of irradiation with UV light under the conditions of testing. Yen *et al.* (1993) reported that peanut hulls with total phenolic compounds over 0·1671 mg/g of hulls showed strong antioxidant activity. Based on these data, MEPH irradiated with UV light for 6 days still retained 7·05 mg/g of hulls of total phenolic compounds; hence MEPH still exhibited strong antioxidant activity after irradiation with UV light.

Characteristic flavonoid components in MEPH were identified by UV spectrometry (Duh *et al.*, 1992). Hence, the variation of components of MEPH irradiated with UV light for various periods may be elucidated via UV-vis spectra. Figure 2 presents UV-vis spectra of MEPH irradiated with UV light. The spectral values of λ_{max} of MEPH irradiated with UV light for 0, 3 and 6 days are the same at 255, 269, 287 and 366 nm. Apparently, the absorption spectra of MEPH after irradiation with UV light for various periods remains unaltered; however, the absorbance of MEPH not irradiated was greater than that of MEPH irradiated for various periods. Therefore the degree of absorbance of MEPH decreased with period of irradiation, indicating that UV-vis spectra of MEPH irradiated for various periods remained unaltered except for decreased absorbance. This observation is consistent with a decrease of total phenolic compounds with increased period of irradiation.

Oxidation products of synthetic antioxidants were detected by TLC (Kurechi & Yamaguchi, 1980). Thus, the variations of components of MEPH irradiated with UV light for various periods can be examined via TLC. Figure 3 shows that the TLC profile of MEPH after irradiation with UV light for various periods remains unaltered. Kurechi and Yamaguchi (1980) reported that 2,2'-dihydroxy-3-tert-butyl-5-methoxy-5'-carboethoxydiphenyl ether was an oxidation product of an equimolar mixture of butylated hydroxyanisole and ethyl protocatechuate after irradiation with UV light for seven days. 3,3'5'-Tri-tert-butyl-5-methoxy-2,4'-dihydroxydiphenyl methane was an oxidation product of an equimolar mixture of butylated hydroxyanisole and butylated hydroxytoluene after irradiation with UV light for 15 days. Small amounts of total phenolic compounds of MEPH after irradiation were lost, but no oxidation product was observed on the TLC plate. This result may be attributed to MEPH being a complex mixture, unlike butylated hydroxyanisole or butylated hydroxytoluene, each of which is a known compound; thus the oxidation products derived from MEPH after irradiation may not be separated completely. Moreover, the loss of a tenth of the total phenolic compounds of MEPH after irradiation for 6 days was significantly small; therefore, the amounts of oxidation products on the TLC plate were too small to be observed if any oxidation products were produced.

Although irradiation of MEPH with UV light for 6 days afforded no oxidation product under our conditions of testing, the MEPH exhibited marked antioxidant activity which is attributed to the remaining (about 90%) total phenolic compounds paying an important role in the stability of lipid peroxidation. According to these results, the antioxidant activity of MEPH was unaffected by UV irradiation as a result of remaining high in total phenolic compounds. This may help to explain that MEPH is highly resistant to photoirradiation.

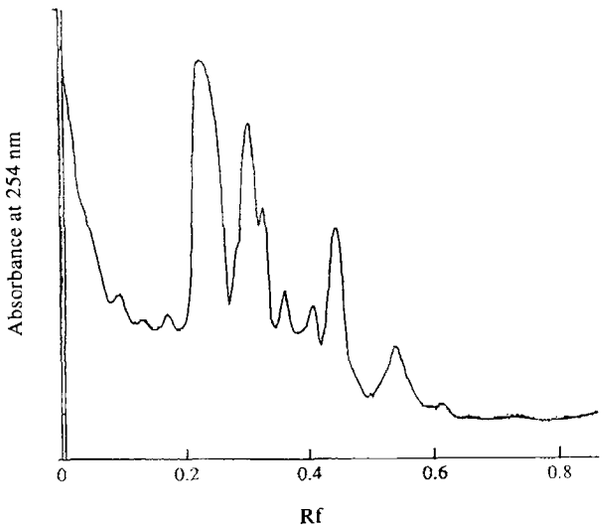

Fig. 3. TLC of photooxidation products of methanolic extracts of peanut hulls under irradiation with UV light for 0, 3 and 6 days. Solvent system = benzene:ethyl formate:formic acid (70:25:5, v/v/v).

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