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Peanut Roots as a Source of Resveratrol

R.-S. CHEN,[†] P.-L. WU,[‡] AND ROBIN Y.-Y. CHIOU*,[†]

Graduate Institute of Biotechnology, National Chiayi University, Chiayi, Taiwan, and Department of Chemistry, Cheng Kung University, Tainan, Taiwan, Republic of China

A potent antioxidant, resveratrol (3,4′,5-trihydroxystilbene), was extracted using 80% methanol from peanut roots (*Arachis hypogaea* L.), isolated with a solid-phase extraction column, purified by a semipreparative HPLC, and identified with ^{1}H NMR and MS. The highest and lowest resveratrol contents in the peanut roots of 2000 fall and 2001 spring crops were 1.330 and 0.130 mg/g and 0.063 and 0.015 mg/g, respectively. When the dehydrated peanut root powders of spring and fall crops were combined and cooked with pork-fat patties (1%, w/w) and the separated oils were stored at 60 °C for conjugated diene hydroperoxide (CDHP) determination, CDHP contents of the control oils increased after 3 days of storage, whereas the contents in the peanut root-treated oils of spring and fall crops did not increase after 9 and 15 days of storage, respectively. It is of merit to find that peanut roots, usually left in the field as agricultural waste, contain resveratrol and bear potent antioxidative activity.

KEYWORDS: Resveratrol; peanut; peanut root; 3,4′,5-trihydroxystilbene; antioxidant

INTRODUCTION

Extraction and characterization of endogenous phytochemicals with antioxidative and/or cancer chemopreventive activities have attracted extensive interest from individuals involved in biomedical research and development. Use of phytochemicals present in plant foods or feedstuff may have low risk of food intoxication or biological toxicity. Peanuts (*Arachis hypogaea* L.) are a source of natural antioxidants. Peanut shells contain three flavonoid antioxidants including 5,7-dihydroxychromone, eriodictyol, and luteolin (*1*−*3*). The flavonoid antioxidant, dihydroquercetin, was found in Spanish peanuts (*4*). Stilbene phytoalexins as *cis*- and *trans*-isomers of derivatives closely related to 3,4′,5-trihydroxy-4-isopentenylstilbene have been isolated from the extracts of germinating seeds or from stems of American peanut plants challenged with microorganisms (*5*−*7*). Ingham (*8*) isolated two related but distinct compounds from the fungus-infected hypocotyls of an African-grown cultivar of *A. hypogaea* L. and identified as cis- and *trans*-3,4′,5-trihydroxystilbene (resveratrol). Isopentenylstilbenes and resveratrols are stilbene phytoalexins elicited in the peanut seeds by wounding (by slicing) and incubating the seed slices in the dark. Their contents vary with genotype, water activity, soil temperature, and maturity (*9*−*13*).

In recent years, resveratrol in edible peanuts and commercial peanut commodities has been reported (*14*, *15*), and it has been indicated that peanut tissues during normal cultivation also produced stilbene phytoalexins. During cultivation, it is inevitable that peanut plants are frequently challenged by the surrounding microflora and environmental stress to induce formation of phytoalexins. It would be worthwhile to isolate bioactive phytochemicals from the disposed agricultural material after harvest, such as peanut vines or roots.

Resveratrol, a phytoalexin found in grapes and other plants, was found to act as an antioxidant and antimutagen (*16*). It not only inhibits the development of preneoplastic lesions in cultures of carcinogen-treated mouse mammary glands but also inhibits tumorigenesis in a mouse skin cancer model. In particular, it has been suggested to be responsible for health benefits of red wines rendering chemoprevention of coronary diseases. The search for other potent sources of resvertraol, in addition to grapes and wines, is necessary.

In this study, resveratrol has been isolated from peanut roots after harvest and identified. Investigation and evaluation of the resveratrol contents in the roots as affected by cultivar and crop season were done. The dehydrated peanut root powders were further subjected to cooking with pork-fat patties, and the separated oils were stored at 60 °C for antioxidative characterization.

MATERIALS AND METHODS

Peanut Roots. Peanuts of Tainan 9, Tainan 11, and Tainan 12 of 2000 fall and 2001 spring crops were grown in two plots in the experimental field of National Chiayi University with normal cultivation practice (*17*). After the mature pods had been dug and harvested, the roots were collected, cleaned with tap water, and dehydrated at 40−45 °C in a forced-air oven. Roots were pulverized and stored at −25 °C for analysis.

Resveratrol Extraction and Identification. The previously reported procedure for isoflavone extraction (*18*) was followed with a minor modification. For each extraction, 0.5 g of the dehydrated peanut root was transferred to a 10 mL centrifuge tube (Teflon centrifuge tube,

* Author to whom correspondence should be addressed (telephone 886-5-2857976; fax 886-5-2775524; e-mail rychiou@mail.ncyu.edu.tw).
[†] National Chiayi University.
[‡] Cheng Kung University.

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Nalgene 3110). The powder was homogenized with 5 mL of 80% (v/v) methanol using a polytron (Kinematica Polytron, 15000 rpm, 1 min with an aggregate probe). The probe was rinsed with an additional 2 mL of 80% methanol and pooled. The tubes were screw-capped, heated in a water bath at 70 °C, and shaken occasionally for 30 min. The tubes were centrifuged at 8000*g* at 20 °C for 15 min. The supernatants from 30 extraction tubes were pooled, membrane filtered (0.45 μM), and diluted with water to reach 20% (v/v) methanol. Aliquots (6 mL) of the solution were loaded onto solid-phase extraction (SPE) columns (sorbent mass = 500 mg, LiChrolut RP-18, Merck KgaA, Darmstadt, Germany) and eluted for cleanup with 3 mL of 28% (v/v) methanol through an extraction unit (LiChrolut extraction unit, Merck).

The absorbed resveratrol was eluted with 1 mL of 47.5% (v/v) methanol. The eluates were repeatedly injected into a semipreparative HPLC column (Thermal Hypersil ODS, Thermal Hypersil GmbH, Kleinostheim, Germany) with 2.4 mL of injection volume and separated with a gradient solvent system initiated with 20% methanol to 80% methanol in 16 min and held for an additional 2 min (Hitachi L-7100 pump, L-7420 UV−vis detector, and D-2500 Chromato-Integrator, Hitachi Co., Tokyo, Japan). The flow rate was 3 mL/min, and the active fractions were collected, pooled, and subjected to further separation for purification according to the same semi-HPLC procedure. The collected solutions were evaporated to dry white powder using a Speed-Vac drying system (VaCo I Sublimator, Zirbus Technology, Bad Grund, Germany).

The powders were identified as resveratrol by spectral analyses. ^{1}H NMR spectroscopy was measured in CD_3OD on a Bruker AMX-400 spectrometer. Chemical shifts were shown in δ values (parts per million) with tetramethylsilane as an internal reference. EIMS was taken on an ITD mass spectrometer by direct inlet system. Meanwhile, a sample was spiked with genuine resveratrol (Sigma Chemical Co., St. Louis, MO) and analyzed by HPLC to confirm the identification.

Resveratrol Contents in the Peanut Roots of Spring and Fall Crops. The resveratrol contents of the peanut roots of Tainan 9, Tainan 11, and Tainan 12 of 2000 fall and 2001 spring crops were determined with HPLC. For each extraction, 0.1 g of dehydrated peanut root was deposited into a 10 mL centrifuge tube. The powder was homogenized with 4 mL of 80% methanol (Kinematica Polytron, 15000 rpm, 1 min with an aggregate probe), and the probe was cleaned with an additional 1 mL of 80% methanol and pooled. The tubes were screw-capped, heated in a water bath at 70 °C, and shaken occasionally for 30 min. Then the tubes were centrifuged at 8000*g* at 20 °C for 15 min, and the supernatants were membrane-filtered (0.45 μM) for HPLC analysis.

For HPLC analysis, a reversed phase column (250 mm × 4 mm, Thermal Hypersil ODS) was run with the same gradient solvent system as described above. The injection volume was 20 μL, and the flow rate was 1.0 mL/min. Absorbance of the eluate at 254 nm was monitored. Standard resveratrol (Sigma Chemical Co.) of 0.02 mg/mL in ethanol was run under the identical condition for quantitation.

Antioxidative Characterization of Peanut Roots. The dehydrated peanut root powders of Tainan 9, Tainan 11, and Tainan 12 of 2000 fall and 2001 spring crops were respectively combined and subjected to antioxidative characterization following the procedure reported previously (*19*). Aliquots (60 g) of ground pork-fat patties were placed in a series of porcelain bowls and respectively mixed with 0.6 g of the pulverized peanut root powders and cooked for 2 h in a forced-air oven at 125 °C. After the patties had been cooked and cooled to ambient temperature, aliquots (1.5 mL) of the top layer of oils were withdrawn and deposited into 1.5 mL microfuge tubes for centrifuging (8000*g* for 1 min). One milliliter of oil was deposited in a series of 20 mL brown vials and placed in an oven at 60 ± 1 °C. During storage, 2.5 μL of oil was withdrawn periodically and dissolved in 2.5 mL of iso-octane, and the absorbance at 234 nm was measured. The conjugated diene hydroperoxide (CDHP) content was expressed as absorbance at 234 nm of 0.1% (v/v) oil solution in iso-octane. Oils prepared from the ground pork-fat patties cooked alone were stored and determined as control.

Statistics. At least duplicate experiments were conducted. Means of determinations with standard deviation and followed by pair comparisons through Student's *t* test are reported.

Table 1. Resveratrol Contents in Peanut Roots As Affected by Cultivar and Crop Season

peanut cultivar	resveratrol content, mg/g		
	average[a]	highest	lowest
	Fall Crops of 2000		
Tainan 9	0.554 ± 0.225[a]	0.898	0.328
Tainan 11	0.620 ± 0.408[a]	1.018	0.130
Tainan 12	0.905 ± 0.311[a]	1.330	0.583
	Spring Crops of 2001		
Tainan 9	0.027 ± 0.012[c]	0.039	0.015
Tainan 11	0.031 ± 0.020[bc]	0.063	0.017
Tainan 12	0.037 ± 0.002[b]	0.052	0.020

RESULTS AND DISCUSSION

On the basis of the obtained EIMS spectrum, the pure powder has a molecular mass of 228 amu, corresponding to a molecular formula of $C_{14}H_{12}O_3$. The ^{1}H NMR signals at δ 6.14 (1 H, t, J = 2.2 Hz), 6.43 (2 H, d, J = 2.2 Hz), 6.75 (2 H, d, J = 8.6 Hz), 6.78 (1 H, d, J = 16.3 Hz), 6.95 (1 H, d, J = 16.3 Hz), and 7.34 (2 H, d, J = 8.6 Hz) indicated the presence of a trans double bond, a 1,4-disubstituted benzene, and a symmetrical 1,3,5-trisubstituted benzene. By addition of three hydroxyls, these partial structures were assembled as 3,4′,5-trihydroxystilbene (resveratrol). It is further confirmed by the same retention time in HPLC analysis as a genuine resveratrol. This finding of resveratrol in peanut roots was in agreement with the report of Ingham (*8*), who had isolated two distinct compounds identified as *cis*- and *trans*-3,4′,5-trihydroxystilbene (resveratrol) from the fungus-infected hypocotyls of an African-grown cultivar of *A. hypogaea* L. However, other stilbene compounds, *cis*- and *trans*-3,4′,5-trihydroxy-4-isopentenylstilbene, have been isolated from the extracts of germinating seeds or from stems of American peanut plants after challenges with microorganisms (*5*−*7*).

The resveratrol contents of peanut roots of Tainan 9, Tainan 11, and Tainan 12 of 2000 fall and 2001 spring crops are shown in **Table 1**. As affected by growing season, resveratrol contents in the roots of fall crops were much higher than those of spring crops. Among the cultivars grown in each crop season, resveratrol contents varied in a limited range. In Taiwan, the weather pattern during the planting period of spring and fall crops varies in a reverse manner. Spring crops are harvested in summer and fall crops are harvested in winter (*17*). In addition to variation as affected by crop season as relevant to weather pattern, resveratrol content varied widely among peanut root samples from different plants. In comparison, the highest and lowest resveratrol contents in the roots of 2000 fall and 2001 spring crops were 1.330 and 0.130 mg/g and 0.063 and 0.015 mg/g, respectively. The observed resveratrol contents in the peanut roots were higher than the reported contents detected in the wounded cotyledons, edible peanuts, and commercial products (*13*−*15*). Thus, peanut roots provide a source of resveratrol.

Increases of CDHP content in the oils separated from ground pork-fat patties (60 g) cooked alone (control) or cooked with 0.6 g of peanut root powders of spring and fall crops and subjected to storage at 60 °C are shown in **Figure 1**. CDHP contents in the control oils increased rapidly after 3 days of storage. CDHP contents in the oils separated from ground pork-fat patties cooked with peanut roots of 2001 spring crop increased after 9 days of storage. In the oils separated from the ground pork-fat patties cooked with peanut roots of 2000 fall crop, CDHP content did not increase after 15 days of storage. The difference in oil stability was observed in close relation to

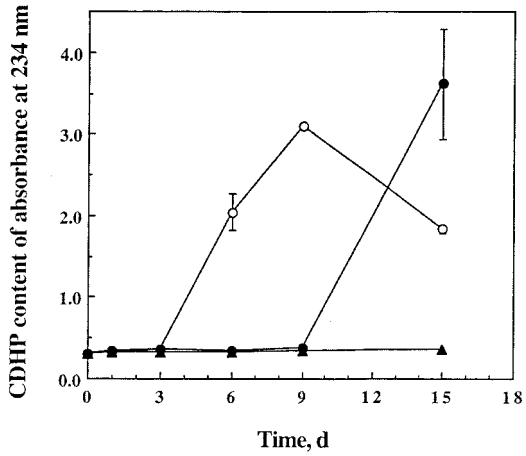

Figure 1. Changes of conjugated diene hydroperoxide (CDHP) content during storage at 60 °C for 15 days of the oils separated from 60-g ground pork-fat patties after cooking: (○) cooked alone (control); (▲) cooked with 0.6 g of peanut root powder of 2000 fall crop; (●) cooked with 0.6 g of peanut root powder of 2001 spring crop. Error bars represent ±1 SD.

the resveratrol contents detected (**Table 1**). Nevertheless, other antioxidants in addition to resveratrol should also be involved in the overall antioxidative performance. From the viewpoint of food processing and food safety, meats cooked with a small amount of peanut root to effectively enhance oxidative stability are worthy of further investigation.

In conclusion, resveratrol, a potent antioxidant, was extracted, purified, and identified from peanut roots. The resveratrol content of peanut roots is large enough to indicate they are a significant source and add value to this component of the plant, usually left as agricultural waste in the field. In further studies, the variables and mechanism affecting resveratrol synthesis in peanut tissues and related antioxidative or chemopreventive effects are worthy of intensive investigation.

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