trans-Resveratrol Content in Commercial Peanuts and Peanut Products

Victor S. Sobolev* and Richard J. Cole

National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 1011 Forrester Drive, Dawson, Georgia 31742

A modified high-performance liquid chromatographic (HPLC) method for determination of *trans*-resveratrol (resveratrol) in peanuts and peanut products has been developed. Resveratrol was extracted with acetonitrile−water (90/10, v/v) by blending with diatomaceous earth at high speed followed by purification of an aliquot of the extract on a minicolumn packed with Al_2O_3−ODS (C_{18}) mixture. The column was eluted with acetonitrile−water (90/10, v/v), eluate was evaporated under nitrogen, and residue was dissolved in HPLC mobile phase. Resveratrol in an aliquot of purified extract was quantitated by HPLC on silica gel with *n*-hexane−2−propanol−water−acetonitrile−acetic acid (1050/270/17/5/1, v/v) as a mobile phase. The recovery of resveratrol added to diatomaceous earth at 0.05 μg/g was 98.95 ± 17.79%; the recovery of the standard added to fresh peanuts (with 0.070 μg/g natural level of resveratrol) at 0.50, 5.00, and 10.00 μg/g was 117.23 ± 8.87, 100.10 ± 2.49, and 100.45 ± 1.51%, respectively. The quantitation limit of resveratrol in fresh peanuts was about 0.01 μg/g. Roasted peanuts had the lowest content of resveratrol of 0.055 ± 0.023 μg/g (n = 21), while in peanut butter its concentration was significantly higher, 0.324 ± 0.129 μg/g (n = 46), and boiled peanuts had the highest level of 5.138 ± 2.849 μg/g (n = 12). Resveratrol content in commercial peanut products was similar to the resveratrol content of the raw peanut fractions routinely used for making them.

Keywords: *trans-Resveratrol; resveratrol; Arachis hypogaea L.; peanuts; groundnuts; peanut maturity; roasted peanuts; peanut butter; boiled peanuts; commercial peanuts; peanut fractions; peanut brands; peanut shells; Florunner; HPLC analysis; phytoalexin*

INTRODUCTION

Resveratrol (*trans*-resveratrol, *trans*-3,5,4′-trihydroxystilbene) is one of the major stilbene phytoalexins produced by different parts of the peanut plant (*Arachis hypogaea* L.), including the peanut kernel. Stilbenes are produced by the peanut plant as a defense response to some exogenous stimuli, particularly, a fungal challenge (Ingham, 1976; Paxton, 1991; Sobolev et al., 1995). They have been shown to be naturally produced at high concentrations in immature peanut kernels with production capability dropping with maturity stage. Mature peanut kernels retain the capability to produce resveratrol and related metabolites but at a reduced level compared to growing kernels (Vidhyasekaran et al., 1972; Dorner et al., 1989).

Resveratrol has been shown to possess cancer chemopreventive activity in mice and to act as an antioxidant and antimutagen (Jang et al., 1997). It is also associated with reduced risk of cardiovascular disease by inhibiting or altering platelet aggregation and coagulation, or modulating lipoprotein metabolism (Arichi et al., 1982; Kimura et al., 1985, 1995; Chung et al., 1992; Bertelli et al., 1995; Pace-Asciak et al., 1995). It has been demonstrated (Klatsky et al., 1992) that wine preference, compared to beer or liquor preference, among moderately drinking people was associated with a significantly lower relative risk for cardiovascular death, which possibly could be explained by higher consumption of wines containing resveratrol.

Resveratrol has been found in grape skins and grape products, such as wines at 0.031−7.17 ppm levels (Mattivi, 1993; Celotti et al., 1996). However, a detailed analysis for the resveratrol content in commercially available peanuts and peanut products has not been reported except for a brief report on raw peanuts (Sanders and McMichael, 1997), although it is of great interest because of the importance of peanuts in the diet of people in many countries of the world (Sands, 1982). Because of its presence in some common foodstuffs and its beneficial properties, resveratrol became a subject of recent intense research (Kimura et al., 1995; Pace-Asciak et al., 1995; Jang et al., 1997).

The purpose of this work was to conduct an in-depth chemical analysis of the resveratrol content in peanuts and various peanut products commercially available in the United States.

MATERIALS AND METHODS

Apparatus. A high-performance liquid chromatograph equipped with a pump (model LC-10AT, Shimadzu), a diode array detector in the 220−450-nm range (model SPD-M10A with EZChrom software, version 3.2, Shimadzu), and an autosampler (model 712 WISP, Waters) was used. The separation was performed on a Zorbax Rx-SIL analytical column (250 × 4.6 mm i.d., packed with 5 μm of silica gel; MAC-MOD Analytical, Inc.) with *n*-hexane−2-propanol−water−acetonitrile−acetic acid (1050/270/17/5/1, v/v) as the mobile phase at a flow rate of 1.5 mL/min at room temperature. The column was equilibrated with the mobile phase within 2 h at 1.5 mL/

* To whom correspondence should be addressed (fax, 912-995-7416; e-mail, vsobolev@nprl.usda.gov).

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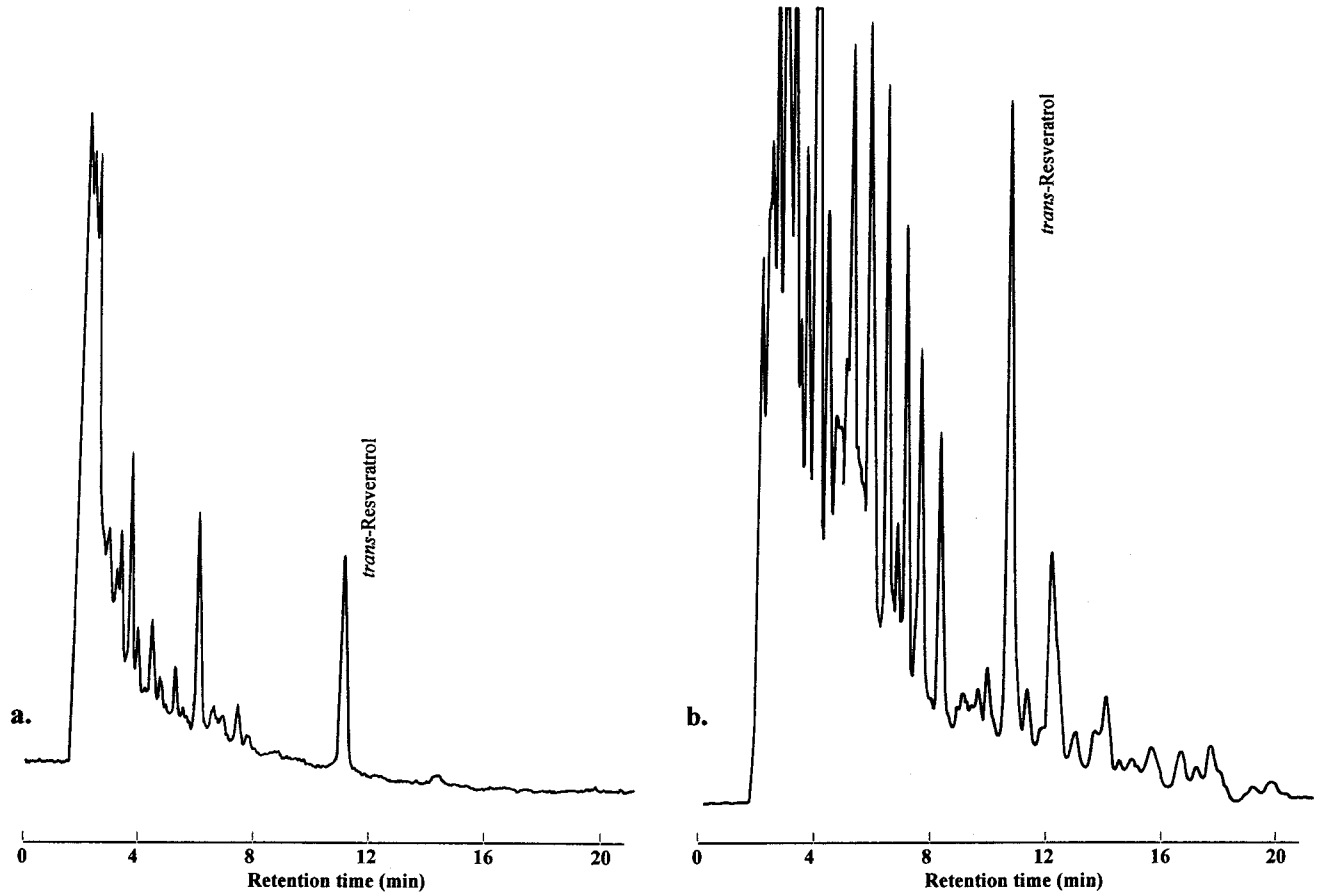

Figure 1. HPLC of roasted peanuts extract (a) and peanut butter extract (b) at 307 and 320 nm, respectively. Resveratrol concentrations are 0.079 and 0.290 μg/g, respectively.

min. Cleanup procedure was performed using an evaporating unit (Reacti-Vap evaporating unit, model 18780 with Reacti-Therm heating module, Pierce), an ultrasonic bath (Ultrasonic Cleaner, model T-9, L & R), a high-speed blender (13 000 rpm, with 100-mL stainless steel jar; General Electric), a cleanup column composed of a Pasteur pipet (borosilicate glass disposable Pasteur pipet, length 145 mm with a small cotton plug placed at its bottom) packed with 0.9−1.2 mL of a mixture of Al_2O_3 (neutral, Brockman activity 1, 80−200 mesh; Fisher) and Sorbsil C60 RP_{18} (40−60 μm; Fisher), a vial (4 mL, 45- × 15-mm diameter), borosilicate clear autosampler vial with screw cap and PTFE septa (National Scientific Co.) with a glass insert (500 μL polyspring; National Scientific Co., Lawrenceville, GA), and a microsyringe (100 μL; Hamilton).

Reagents and Products. Solvents for LC and extraction (*n*-hexane, 2-propanol, acetonitrile, water, and acetic acid) were LC grade (Fisher). An acid-washed diatomaceous earth was used (Sigma). Compressed nitrogen was used as a gas for extract evaporation (UN 1066, Air Products and Chemicals). Standard of *trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene, approximately 99%) was purchased from Sigma. Two stock solutions were prepared by dissolving 3.15 and 1.79 mg of the standard in 25 mL of the LC mobile phase. Working solutions were prepared daily by mixing 100 μL of one of the stock solutions with 2.9 mL of the LC mobile phase; 10−40 μL was injected into the LC system. All peanut products were purchased from April 5 to July 29, 1998, in Albany, GA (except for one sample that was available from Bisbee, AZ) at four different department and grocery stores. Fractions of shelled Florunner peanuts were supplied by Farmers Fertilizer & Milling Co., Colquitt, GA, and were analyzed for resveratrol content without delay.

Sampling Procedure. All peanut butter samples marked "natural" (no emulsifier in the formulation) were thoroughly mixed to a homogeneous condition with a stainless steel spatula. All other samples of roasted and boiled peanuts and peanut butter were taken with the help of disposable plastic teaspoons directly from cans and jars without any preliminary mixing or sorting. Peanut fractions (0.5−4 kg each) were thoroughly mixed on a stainless steel tray before random sampling (10 g each).

Cleanup Procedure. Ten grams of peanuts or peanut products, 2 g of diatomaceous earth, and 40 mL of a CH_3CN-H_2O mixture (9/1, v/v) were placed in a blender jar and blended for 1 min; 2 mL of particle-free extract was collected with a pipet from the jar close to the liquid surface. The aliquot was transferred to the cleanup column and allowed to drain by gravity into a vial. Then the column was eluted with 1 mL of a CH_3CN-H_2O mixture (9/1, v/v). Combined eluates were evaporated to dryness under a nitrogen steam at 40 °C in an evaporating unit. The residue was dissolved in 400 μL of LC mobile phase, sonicated in an ultrasonic bath for 10−15 s, and transferred into a glass insert.

Liquid from a can with boiled peanuts was prepared for the analyses by mixing 1 mL of the liquid with 9 mL of CH_3CN; 2 mL of the mixture was transferred to a minicolumn and further processed as above.

LC Quantitation. Purified extract (20−120 μL) was injected into the LC, and *trans*-resveratrol was quantitated at 307 or 320 nm by reference to the peak area of an external authentic standard of *trans*-resveratrol.

Data Analyses. Statistical analyses were conducted using the SigmaStat software program, version 1.00 (Jandel Corp., San Rafael, CA). An unpaired *t*-test and one-way ANOVA were used to compare two and three groups of data for a significant difference, respectively.

RESULTS AND DISCUSSION

A modified HPLC method (Sobolev et al., 1995) for the analyses of peanuts and peanut products was

Table 1. Resveratrol Content in Commercial Peanut Products

kind of product/brand	resveratrol content (mean ± SD, μg/g; $n = 3$ for the same sample)
roasted peanuts (in metal or foiled carton cans):	
Planters reduced fat honey roasted (45% less fat than regular peanuts)	0.018 ± 0.002
Staff unsalted fancy	0.034 ± 0.009
Staff dry roasted	0.046 ± 0.008
Planters salted	0.056 ± 0.003
fresh with skins, naturally air-dried (1997 local harvest, stored at room temperature)	0.060 ± 0.011
Planters cocktail	0.075 ± 0.002
Staff best Spanish (with skins)	0.075 ± 0.003
Planters grandstand	0.080 ± 0.002
peanut butter (in transparent plastic or glass jars):	
Jif, creamy (peanut butter spread, 60% peanuts)	0.148 ± 0.006
Skippy, super chunk	0.156 ± 0.044
Jif, extra crunchy	0.222 ± 0.029
Reese's, crunchy	0.224 ± 0.010
Reese's, creamy	0.255 ± 0.021
Jif, creamy	0.272 ± 0.031
Skippy, creamy	0.303 ± 0.008
Peter Pan, crunchy	0.307 ± 0.034
Smucker's natural style reduced fat, creamy	0.316 ± 0.008
Roddenbery's, creamy	0.332 ± 0.039
Peter Pan, creamy	0.400 ± 0.043
Shurfine, creamy	0.429 ± 0.025
Smucker's natural, chunky	0.472 ± 0.056
Safeway, chunky (100% natural)	0.472 ± 0.230[a]
American Fare, creamy	0.504 ± 0.095
boiled peanuts (in metal cans):	
Roddenbery's peanut patch (green) kernels	7.092 ± 3.401[a]
Roddenbery's peanut patch Cajun style kernels	3.422 ± 0.200
Roddenbery's peanut patch (green)	
kernels	6.468 ± 1.204
hulls	7.873 ± 1.638
liquid	0.064 ± 0.003
Roddenbery's peanut patch (green)	
kernels	1.838 ± 0.027[b]
hulls	2.661 ± 0.123[b]
liquid	0.051 ± 0.003[b]
Roddenbery's peanut patch Cajun style	
kernels	1.779 ± 0.021[b]
hulls	2.415 ± 0.075[b]
liquid	0.048 ± 0.002[b]

[a] Four replicates. [b] The content of the entire can was separated into kernels with skins, hulls, and liquid, which were separately homogenized. Three replicates of each homogenate are presented.

developed. Aqueous acetonitrile extraction of resveratrol from peanut butter with high oil content gave a low-mass fraction. An additional one-step purification procedure on a minicolumn resulted in an eluate with sufficient purity for HPLC quantitation of resveratrol. The use of diatomaceous earth provided excellent homogeneity of the extract after blending for 1 min. The extraction and cleanup procedures were fast, which completely prevented potential loss of light-sensitive *trans*-resveratrol. The recovery of resveratrol added to diatomaceous earth at 0.05 μg/g was 98.95 ± 17.79%; the recovery of the standard added to fresh peanuts (with 0.070 μg/g natural level of resveratrol) at 0.50, 5.00, and 10.00 μg/g was 117.23 ± 8.87, 100.10 ± 2.49, and 100.45 ± 1.51% ($n = 3$ for each level), respectively. The quantitation limit of resveratrol in fresh peanuts was estimated at 0.01 μg/g (signal-to-noise ratio 5:1). The use of photodiode array detector in combination with a UV-transparent mobile phase (from 215 nm) helped to increase reliability of the method in the cases of low concentrations of resveratrol in analyzed products. Quantitation of resveratrol in peanut butter was performed at 320 nm to increase selectivity. Roasted, fresh, and boiled peanuts gave cleaner extracts with no impurities coeluted with resveratrol (Figure 1). This permitted the use of the optimum wavelength (307 nm in the LC mobile phase used) for increased sensitivity that may be required for fresh and roasted peanuts with low concentrations of resveratrol. Typical chromatograms of purified extracts of roasted peanuts and peanut butter (Figure 1) show sufficient separation of *trans*-resveratrol from impurities, which, in combination with a photodiode array detector, allowed reliable quantitation of resveratrol.

The method was applied to various peanut products available on grocery store shelves, including the most popular brands. The concentrations of resveratrol detected in these samples are presented in Table 1. The data obtained showed that all analyzed peanuts and peanut products contained resveratrol. The concentration in roasted peanuts (Table 1) was low and on average varied from 0.018 to 0.080 μg/g with excellent repeatability of the results. Peanut butter showed significantly higher ($P < 0.001$) resveratrol content compared to roasted peanuts, varying from 0.148 to 0.504 μg/g (Table 1). The apparent explanation of this fact was that peanut products that could possibly contain more immature peanuts showed higher levels of resveratrol than peanut products composed of more mature kernels such as roasted peanuts. Edible "splits" along with "number 1's" fractions have usually been used for producing commercial peanut butter (Sands,

Table 2. Resveratrol Content in Fractions of Shelled Florunner Peanuts

fraction[a]	resveratrol content (mean ± SD, μg/g; $n = 3$ for the same sample)
edible:	
jumbos	0.070 ± 0.011
mediums	0.078 ± 0.007
splits	0.228 ± 0.084[b]
number 1's	0.716 ± 1.163[b]
inedible (oil stock):	
round + split pick-outs (P/O) + screening	1.281 ± 0.593
loose shelled kernels (LSK) oil stock (LSK that passed through 6.35-mm slotted screen)	2.687 ± 1.677
split P/O (color-sorted rejects from splits)	3.141 ± 2.308
oil stock (kernels that passed through 6.35-mm slotted screen)	4.335 ± 5.161
round P/O (color-sorted rejects)	5.355 ± 4.011
yellow split (collected manually from edible split)	7.092 ± 2.212

[a] Terms used in this table are described in Davidson et al. (1982). [b] Four replicates.

1982). These fractions represent peanuts that are supposed to differ from "jumbos" only by size of the kernels. This may be explained in part by the percentage of immature peanuts in the product. Unshelled boiled peanuts contained the highest amount of resveratrol (5.138 ± 2.849 μg/g; $n = 12$) among the analyzed products, exceeding the level of 11 μg/g in individual samples (Table 1). Such an unexpected high concentration of resveratrol, which was significantly higher than in peanut butter ($P < 0.001$), was also accompanied by high variability in the results. Close inspection of two brands showed that unsorted peanut pods were used for this kind of product—the samples represented a typical indeterminate peanut plant with pods of different size and maturity. Some pods were mechanically damaged. In other words, peanut pods used for making boiled peanut brands contained some of those peanut kernels, which would be removed from highest quality edible fractions if used for making roasted peanuts or peanut butter. It should be noted that peanut hulls (shells) showed a higher concentration of resveratrol compared to kernels with skins (testa) from the same samples, whereas no significant amount of resveratrol was found in the liquid.

The resveratrol content of various fractions of raw, shelled peanuts is presented in Table 2. Seed sizes in the edible fraction decrease from "jumbos" to "mediums" to "number 1's" with "splits" being those seeds that split during shelling. The inedible fraction includes seeds that fall through a 6.35-mm slotted screen, rejects from electronic color sorters, and certain manually removed, discolored seeds, particularly the "yellow split". Because of the lower quality of these seeds, they can only be used for oil stock. Data in Table 2 show that resveratrol content generally increases with decreasing seed size and that the highest resveratrol content was found in discolored seeds that were removed manually or by electronic color sorters. Larger seed size is generally associated with increased maturity, whereas smaller seed sizes are usually representative of more immature peanuts. Therefore, these data that generally show decreasing resveratrol content with increasing seed sizes support earlier findings that showed greater phytoalexin accumulation in more immature peanuts (Vidhyasekaran et al., 1972; Dorner et al., 1989).

Resveratrol concentration in "jumbos" and "mediums" was well-correlated with that in roasted brand peanuts (Table 1), while in "splits" and "number 1's" it was significantly ($P < 0.001$) higher. "Splits" showed a relatively consistent result at 0.228 μg/g. Individual samples of "number 1's" demonstrated high variability of resveratrol content from 0.101 to 2.460 μg/g. Among inedible (oil stock) peanut fractions "yellow split" is of particular interest since it was derived from edible "splits". "Yellow split" represents a fraction of yellowish and yellow-brownish peanuts that were not removed by electronic sorting machines but removed manually from a moving conveyer. This fraction showed the highest average resveratrol content of more than 7 μg/g. Obviously, visual inspection with manual collection of kernels that are slightly different in color is not perfect and does not guarantee their 100% removal from edible "splits". This fact, along with the use of edible "splits" and "number 1's" in making peanut butter, can explain the significantly higher resveratrol content in peanut butter compared to roasted peanuts. It is impossible, however, to quantitatively estimate the amount of "yellow split" fraction in peanut butter because of the great variability of peanut quality in commercial lots.

CONCLUSIONS

The proposed LC method was suitable for reliable resveratrol quantitation in peanuts and main peanut products at levels of 0.01−10 μg/g. This study demonstrated that *trans*-resveratrol content in commercial peanuts and main peanut products (roasted peanuts and peanut butter) was similar to the resveratrol content of the raw peanut fractions routinely used for making them.

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