Occurrence of Resveratrol in Edible Peanuts

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Resveratrol has been associated with reduced cardiovascular disease and reduced cancer risk. This phytoalexin has been reported in a number of plant species, including grapes, and may be one of the compounds responsible for the health benefits of red wine. Analytical methods for measuring resveratrol in wine and peanuts were adapted to isolate, identify, and quantify resveratrol in several cultivars of peanuts. Aqueous ethanol (80% v/v) extracts from peanuts without seed coats were purified over alumina/silica gel columns and analyzed by reversed phase HPLC using a C-18 column. Peanuts from each market type, Virginia, runner, and Spanish, produced in four different locations contained from 0.03 to 0.14 μg of resveratrol/g. Seed coats from runner and Virginia types contained ~0.65 μg/g of seed coat, which is equivalent to <0.04 μg/seed. Quantitative analysis of 15 cultivars representing 3 peanut market types, which had been cold stored for up to 3 years, indicated a range of 0.02−1.79 μg/g of peanut compared to 0.6−8.0 μg/mL in red wines.

Keywords: *Peanuts; resveratrol; 3,5,4′-trihydroxystilbene; Arachis hypogaea L.*

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene) has been associated with reduced cardiovascular disease (Goldberg, 1995) and reduced cancer risk (Jang et al., 1997). This phytoalexin has been reported in a number of plant species including grapes (Creasy and Coffee, 1988; Jeandel et al., 1991) and may be one of the compounds responsible for the health benefits of red wine consumption (Siemann and Creasy, 1992). Until our earlier report (Sanders and McMichael, 1997) that resveratrol was to be found in edible peanuts, resveratrol had been reported in peanut hypocotyls (Ingham, 1976), germinating seed (Keen, 1975), and seed (Arora and Strange, 1991; Sobolev et al., 1995) but only after inoculation with microorganisms and texts of these studies generally indicated that no resveratrol was detected in uninfected tissue. Arora and Strange (1991) reported the accumulation of resveratrol and other phytoalexins in imbibed peanuts after wounding (slicing and incubation). Although Arora and Strange (1991) did not mention preexisting phytoalexins in the text of their paper, data presented suggested the presence of resveratrol in control samples of the peanut cultivars examined. Recently, Sobolev and Cole (1999), who earlier reported no resveratrol in uninfected tissue (Sobolev et al., 1995), published a report on the concentrations of resveratrol in several commercial peanut butters, peanut products, and the Florunner cultivar.

A large number of analytical techniques have been developed to measure resveratrol in wine. They include HPLC-based methods (Lamuela-Raventos and Waterhouse, 1993; Pezet et al., 1994; McMurtrey et al., 1994; Lamuela-Raventos et al., 1995; Adrian et al., 1996) and GC-MS-based methods (Soleas et al., 1995; Lamikanra et al., 1996). Arora and Strange (1991) and Sobolev et al. (1995) presented methodology for the extraction and HPLC analysis of resveratrol and other phytoalexins from peanuts. Adaptation and application of red wine and peanut analytical methodology for resveratrol resulted in the first report of resveratrol in sound, edible peanuts (Sanders and McMichael, 1997), and that methodology has been further adapted in this study of resveratrol concentration in various peanut genotypes.

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MATERIALS AND METHODS

Materials. *trans*-Resveratrol used as a standard was purchased from Sigma Chemical Co. Conversion of *trans*-resveratrol to the *cis* form in samples and standards was accomplished by exposing samples to a UV lamp (366 nm, model UVL-S6, UVP, Inc., San Gabriel, CA) for 1 min (Siemann and Creasy, 1992). Peanut cultivars used for analysis were as follows: NC-9 (Virginia-type) from Lewiston, NC; Florunner (runner-type) from Dawson, GA; SunOleic 95R (runner -type) from Marianna, FL; and Starr (Spanish-type) from Caddo, OK. Samples were held in cold storage for ~6 months and hand blanched (seed coats removed) before analysis. Samples of runner and Spanish types were dry roasted at a maximum of 176 °C to a Hunter *L* color of 49−50. Samples of five peanut cultivars from each market type (runner, Virginia, and Spanish) obtained through the Uniform Peanut Performance Trials which had been held in cold storage for ~3 years were also hand blanched before analysis.

Extraction. All samples were ground in a Braun coffee mill. The meal was suspended in 80% v/v ethanol (3 mL/g of peanuts) and homogenized at high speed with a Polytron homogenizer on ice for 2 min. With protection from light, the extract was centrifuged to remove particulate matter and the supernatant recovered. Qualitative (GC-MS) and initial quantitative analysis was performed using this extract after concentration. A refined quantitative analysis was performed by passing 2 mL of the supernatant through a column containing 1 mL of a 1:1 w/w mixture of Al_2O_3 (Fischer) and silica gel 60 R_{18} (Sobolev et al., 1995). The column was washed with 2 mL of 80% ethanol, and the combined eluates were dried under N_2 at 60 °C on a heating block. Dried eluates were then suspended in 300 μL of 10% ethanol. Because *trans*-resveratrol is sensitive to UV light, the samples were protected from light after extraction. Data reported are the mean of six extractions with duplicate analyses of each extraction. Data

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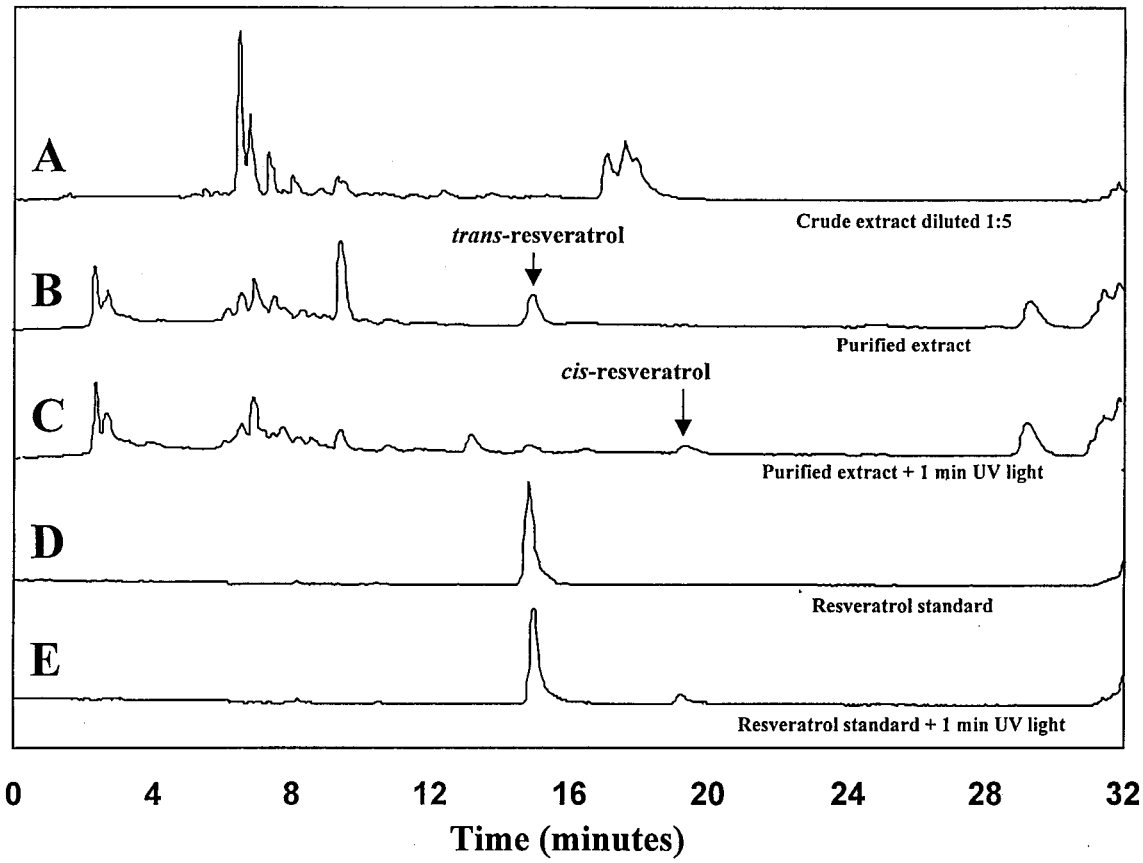

Figure 1. HPLC separation of peanut extracts and resveratrol standards.

were analyzed using a Statistical Analysis System (SAS, 1985) program package, and significant differences among means were determined by using the Waller Duncan test.

HPLC Analysis. The extracts were analyzed by reversed phase high-performance liquid chromatography (HPLC) using a C-18 column (4.5 mm × 150 mm, Vydac, with a 25 mL sample loop). The compounds were eluted with a linear gradient of acetonitrile in 0.1% v/v TFA as follows: 0% acetonitrile for 1 min, 0−15% acetonitrile in 2 min, 15− 27% acetonitrile in 20 min, 27−100% acetonitrile in 5 min. The column was returned to initial conditions for 10 min before the next injection. The column eluate was monitored for absorbance at 308 nm. A standard curve for *trans*-resveratrol in 10% ethanol was generated to allow quantitation of resveratrol in peanut extracts.

GC-MS Analysis. *trans*-Resveratrol was identified in peanut extracts by GC-MS following silylation with bis[trimethylsilyl]trifluoroacetamide (BSTFA) (Lamikanra et al., 1996). Aliquots (1.0 mL) of the extract were dried in vacuo, resolubilized in 0.2 mL of BSTFA, and incubated at 60 °C for 30 min and then at room temperature overnight. *trans*-Resveratrol standards were made by solubilizing 20 μg of the standard compound in 100 μL of BSTFA and heating at 60 °C for 30 min. Solutions of other concentrations were made by dilution of this stock mixture. Injections of 2 μL of the derivatized sample were made into an HP 5890 GC/5972 MS utilizing a 30 m, 0.25 mm i.d., DB-5MS column (J&W Scientific). Initial column temperature was 150 °C for 1 min followed by a 10 °C/min ramp to 300 °C. Ions (25−500 amu) were monitored using the SCAN mode of the instrument. *trans*-Resveratrol in the sample was identified by comparison to the standard and to published mass spectral data of the trimethylsilyl derivative of *trans*-resveratrol (Lamikanra et al., 1996).

RESULTS AND DISCUSSION

Detection of *trans*-Resveratrol in Peanuts. When 25 μL of filtered, centrifuged extract was diluted 1:5 with 80% ETOH and subjected to HPLC analysis (Figure 1A), resveratrol was not distinguishable, and no peak was consistently observed that coeluted with resveratrol. When the extract was concentrated by evaporation, a peak coeluting with the resveratrol standard was found and used for quantitation. The extract required further cleanup due to interfering substances that coeluted with resveratrol. Following the method of Sobolev et al. (1995), a 2 mL aliquot of peanut extract was partially purified on a cleanup column and then separated by reversed phase HPLC (Figure 1B). A peak (arrow, Figure 1B) with the same retention time as the *trans*-resveratrol standard (Figure 1D) was identified. A second aliquot of the purified extract was exposed to UV light and then analyzed on the same HPLC system (Figure 1C). The *trans*-resveratrol peak decreased and a peak (indicated by the arrow, Figure 1C) with the same retention time as the *cis*-resveratrol standard (Figure 1E, arrow) increased. The coelution of the peak from the extract with standard *trans*-resveratrol, along with its conversion by UV light to a form which coelutes with the *cis* form, strongly indicated that the compound in the extract was resveratrol.

Confirmation of the identification of resveratrol in the peanut extracts was provided by GC-MS analysis. As with HPLC, a compound from the extract with a retention time identical to that of the standard was recovered. This compound had a fragmentation pattern similar to that of the resveratrol standard (Table 1), which also concurred with published data (Lamikanra et al., 1996).

Survey of Peanut Cultivars. Initial analysis of the crude extract for various cultivars indicated concentrations of resveratrol in the range of 1.6−3.7 μg/g (Sanders

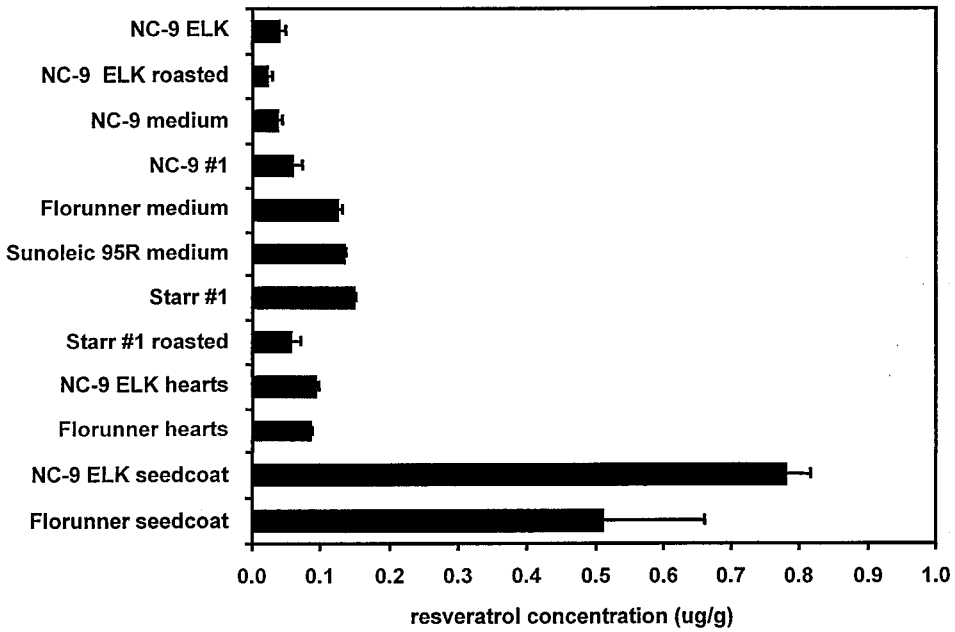

Figure 2. Resveratrol concentration in various peanut cultivars and seed parts. Bars represent standard deviation among sample means.

Table 1. Mass Spectral Data of Trimethylsilyl Derivatives

retention time (min)	sample	MS data *m/z*[a]
15.38	*trans*-resveratrol standard	73 (100), 207 (31), 75 (13), 208 (7), 444 (2)
15.34	compound from extract	73 (100), 207 (29), 75 (12), 208 (7), 444 (3)
	published data[b]	73 (100), 207 (35), 75 (16), 208 (9), 444 (7)

[a] Relative intensity [b] Lamikanra et al. (1996).

and McMichael, 1997). Refinement of the cleanup procedure as outlined above resulted in lower values and suggested the coelution of a compound or compounds with resveratrol in the crude extract.

Florunner, SunOleic 95R, NC-9, and Starr cultivars of peanuts from four different production locations were examined to provide a preliminary evaluation of the potential distribution of resveratrol in commercially available peanuts (Figure 2). Differences in resveratrol concentration among these four cultivars, 0.03−0.147 μg/g, were significant ($P < 0.0001$). Peanuts of three different commercial sizes, extra large kernels (ELK), medium, and no. 1 (determined by seed thickness), were examined in the NC-9 cultivar. The no. 1 size is composed of small seeds and represents a generally less mature lot of peanuts than the ELK or medium Virginia size. Peanut maturity has been related to changes in many compositional and quality characteristics (Sanders et al., 1995). Although the various sizes had different mean concentrations, the differences were less than those found among subsamples of the same type and size. Sobolev and Cole (1999) reported an inverse relationship between size and resveratrol concentration in a single lot of Florunner peanuts. They further indicated increased resveratrol concentrations in increasingly unacceptable peanuts such as color-sorted rejects from variously sized grades. Dorner et al. (1989) previously reported that immature (small) seeds had greater capacity for phytoalexin production than mature (larger) seeds.

Table 2. Concentration of Resveratrol in Peanut Cultivars

peanut type	genotype cultivar	resveratrol (μg/g)	SD[a]
Spanish	Small White Spanish	1.792	0.616
Spanish	Spanette	0.126	0.107
Spanish	Pearl	0.112	0.090
Virginia	NC-18016	0.306	0.146
Virginia	Early Bunch	0.129	0.194
runner	White's Runner	0.069	0.029
runner	Florunner C1	0.067	0.072
Spanish	GA 207-3-4	0.057	0.066
runner	GA 207-2	0.056	0.026
Virginia	NC-9	0.052	0.084
runner	Florispan C1	0.049	0.087
Virginia	NC-17291	0.048	0.070
runner	Dixie Runner	0.039	0.014
Spanish	PI-337396-FAV70	0.023	0.022
runner	Florispan C3	0.022	0.014

[a] Standard deviation.

Roasted NC-9 ELK and Starr peanuts appeared to contain less resveratrol than the same peanuts before roasting (Figure 2). Sobolev and Cole (1999) reported a similar finding in Florunner but found that commercial peanut butter generally contained similar concentrations of resveratrol as those found in raw peanuts. Further investigation of roasting on the concentration of resveratrol appears to be warranted.

Resveratrol concentration in seed coats on a gram basis appears to be high (Figure 2). However, an average seed coat weighs only ~0.04−0.07 g and thus contributes little resveratrol to the total on an individual seed basis. The presence of high concentrations of tannin-type antifungal compounds in peanut seed coats was demonstrated by Sanders (1977). Peanut hearts (embryos) from NC-9 and Florunner contained slightly <0.1 μg of resveratrol/g; however, as with seed coats, the total weight of the heart is small in comparison to the peanut (cotyledon) weight. Hearts weigh ~0.09 g, and the contribution of resveratrol on a per seed basis would be <0.009 μg.

Five cultivars from each of the three major market types (Spanish, runner, and Virginia) that had been stored for ~3 years were surveyed for resveratrol

content (Table 2). Standard deviations were high compared to the means, particularly between separate extractions of the same cultivar. Because such variation was not observed for standard samples run through the procedure, the variation in cultivar means must have been due to extraction differences or differences among subsamples taken for the extraction. Stilbene synthase in peanuts is an inducible enzyme (Ingham, 1976). Stimuli resulting in increased resveratrol concentrations include fungal invasion and mechanical damage. Studies have also demonstrated the production of resveratrol in response to various other abiotic stimuli (Adrian et al., 1996). The consistent presence of resveratrol in all samples examined suggests that either the expression of genes is "leaky" (i.e., some resveratrol production occurs even in the absence of the usual stimulus) or some stimulus for production of resveratrol is common in the production or early handling of peanuts. Concentrations of resveratrol due to fungal invasion may be hundreds or thousands of times higher than levels reported here (Arora and Strange, 1991; Sobolev et al., 1995). The levels we report may result from "leaky" gene expression; may be the result of the usual stimuli for resveratrol production, such as harvest or handling damage, in small areas on the peanut; or may be from some other stimuli not yet identified.

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