DOI:10.1021/jf900667d

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effect of Ultraviolet Doses in Combined Ultraviolet−Ultrasound Treatments on *trans*-Resveratrol and *trans*-Piceid Contents in Sliced Peanut Kernels

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Peanuts are known to synthesize resveratrol, a stilbene phytoalexin, associated with cancer chemopreventive activity and cardioprotection. A modified HPLC method developed and validated to determine *trans*-resveratrol and *trans*-piceid concentrations in a single analysis run exhibited recoveries, precision, linearity, and limits of detection consistent with or better than those of methods previously reported in the literature. The effect of combined treatments, ultrasound (US) followed by varying UV light (UV) doses (three distances, three exposure times) on *trans*-resveratrol and *trans*-piceid concentrations in peanuts was determined. All nine US−UV treatments significantly increased *trans*-resveratrol contents from 0.03 μg/g, in untreated peanut control, to 2.10−4.73 μg/g and *trans*-piceid contents from 0.07 to 0.23−0.38 μg/g. Increases in *trans*-resveratrol were not influenced by UV distance and time. US and UV, individually, increased *trans*-resveratrol to 4.29 and 2.36 μg/g, respectively. The US and UV treatment combinations used did not exhibit synergistic effect compared to US treatments alone.

KEYWORDS: ***trans*-Resveratrol; *trans*-piceid; peanuts; stilbenes; UV; ultrasound; HPLC**

INTRODUCTION

trans-3,5,4′-Trihydroxystilbene or *trans*-resveratrol, a stilbene phytoalexin, is a widely studied compound due to its beneficial effect on the human body. It is associated with cancer chemopreventive activity (*1*) and reduced risk of cardiovascular diseases by inhibiting LDL oxidation (*2*) and also has a therapeutic potential in Alzheimer's disease (*3*). *trans*-Resveratrol-3-*O*-β-glucoside, or *trans*-piceid, the glucosylated form of *trans*-resveratrol, is a far less studied compound and has been reported to exhibit biological activity such as antiplatelet aggregation activity (*4*).

Substantial amounts of *trans*-resveratrol and *trans*-piceid were found in *Polygonum cuspidatum* roots, which are used in Chinese and Japanese folk medicine for promotion of good cardiovascular and overall health, with *trans*-resveratrol and *trans*-piceid concentrations of 2960−3770 and 2320−5310 μg/g, respectively (*5*). Food sources of *trans*-resveratrol and *trans*-piceid include dried grape berry skins with concentrations in white grapes of 11.04−47.6 μg/g (av = 22.03 μg/g) and from a nonquantifiable amount to 64.31 (av = 16.58 μg/g), and in red grapes, 18.32−38.26 (av = 25.79 μg/g) and 5.49−342.66 μg/g (av = 64.15 μg/g), respectively (*6*). In grape juices, *trans*-resveratrol and *trans*-piceid concentrations ranged, in white juices, from not detectable (nd) amounts to 0.19 μg/mL (av = 0.05 μg/mL) and from nd amounts to 0.83 μg/mL (av = 0.18 μg/mL), and in red juices, from nd amounts to 1.09 μg/mL (av = 0.5 μg/g) and from 0.77 to 7.34 μg/mL (av = 3.38 μg/g), respectively (*7*). According to Lamuela-Raventos et al. (*8*) the *trans*-resveratrol and *trans*-piceid concentrations in 18 Spanish red wines were found to range from 0.60 to 8.00 μg/mL (av = 2.48 μg/mL) and from 0.74 to 4.01 μg/mL (av = 1.85 μg/mL), respectively. Recently, *trans*-resveratrol and *trans*-piceid were found in dark chocolate at concentrations of 0.4 and 1 μg/g; in cocoa liquor, at 0.5 and 1.2 μg/g (*9*); in pistachios, at 0.07-0.18 μg/g (av = 0.12 μg/g) and 6.20−8.15 μg/g (av = 6.97 μg/g) (*10*); in hops, at 0.7−2.2 and 2.3−7.3 μg/g (*11*); and in hop pellets, at 0.5 and 2 μg/g (*12*), respectively; much lower concentrations were reported in four commercial beers, from nd to 0.005 μg/mL for each compound (*13*). In raw peanut kernels, concentrations of *trans*-resveratrol ranged from 0.02 to 1.79 μg/g (*14*) and 0.48 μg/g (*15*). In natural peanut butters *trans*-resveratrol was found at levels of 0.53−0.75 μg/g (av = 0.65 μg/g) and *trans*-piceid at levels of 0.07−0.23 μg/g (av = 0.14 μg/g) (*16*).

Preliminary runs to analyze *trans*-resveratrol and *trans*-piceid in peanut extracts using the HPLC method of Rudolf et al. (*17*) revealed that, although the method was effective in quantifying *trans*-resveratrol, it was not developed for *trans*-piceid analysis. Ibern-Gomez et al. (*16*) reported an analysis method for both compounds in peanut butters; however, their average percent recoveries for *trans*-resveratrol were 9% lower than that reported by Rudolf et al. (*17*).

Phytoalexins are produced by plants as a defense response to biotic stresses, such as fungal infection (*18*) and abiotic stresses, such as injury (*19*), and UV light (*18*). Arora and Strange (*20*) reported accumulation of resveratrol in response to wounding, such as slicing, of imbibed peanuts followed by incubation for 48 h

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 Published on Web 08/10/2009

in the dark. Cantos et al. (*21*) reported an increase of *trans*-resveratrol content in table grapes after UV irradiation. Chung et al. (*22*) found that resveratrol content increased in peanut leaves in response to various abiotic stresses and hormones including UV, wounding, H_2O_2, paraquat, salicylic acid, jasmonic acid, abscisic acid, and ethephon and found that UV light was the most effective among all of the stresses applied and increased resveratrol concentration 200-fold. Rudolf and Resurreccion (*15*) explored the effect of abiotic stresses, such as size reduction and subsequent UV or ultrasound (US) treatments, on *trans*-resveratrol content in peanut kernels followed by incubation for one of three time periods. Their results demonstrated effective increase in *trans*-resveratrol, from 0.48 to 3.42 μg/g, in sliced (to 2 mm) peanut kernels then exposed to UV light (distance of 40 cm, 10 min, and incubation of 48 h) and from 0.48 to 3.96 μg/g after US treatment (power density of 39.2 mW/cm^3, 4 min, and incubation of 36 h). In a later study, Rudolf and Resurreccion (*23*) optimized postharvest stress conditions such as varying slicing thickness, from 2 to 6 mm, and incubation time, from 24 to 48 h, to produce the highest amount of *trans*-resveratrol. Higher *trans*-resveratrol concentrations were found with increased incubation time. However, longer incubation time resulted in undesirable sensory changes, such as decreased peanutty flavor and increased off-flavors. The optimized area was bound by peanut sizes of 8.9, 7.2, and 6.4 mm and incubation times of 48, 41.5, and 48 h, respectively.

Rudolf et al. (*15*, *23*) studied the effect of either US or UV light at fixed operational parameters focusing mainly on the effect of size reduction methods and varying incubation times. In this study we investigated the possible additive effect of combining both US and UV treatments by varying UV doses, while holding US parameters and incubation time constant, on *trans*-resveratrol and *trans*-piceid in peanut kernels.

The specific objectives of this study were to (1) adapt and modify an existing HPLC method for quantifying *trans*-resveratrol and *trans*-piceid, (2) determine *trans*-resveratrol and *trans*-piceid concentrations in sliced peanuts treated by US followed by various UV doses and compare US−UV-treated peanuts with those treated with either UV or US only, and (3) study the effect of storage of peanuts on the *trans*-resveratrol and *trans*-piceid synthesis.

MATERIALS AND METHODS

Materials. Peanuts, Georgia green medium runners (McCleskey Mills Inc., Smithville, GA), harvested in fall 2006, were used in this study. The peanuts were stored in water-impermeable plastic bags (PrintPack Inc., Orange, TX) at 5 °C for either 6 or 13 months, as specified.

Standard stock solutions for *trans*-resveratrol (>99%, Sigma-Aldrich, St. Louis, MO), *trans*-piceid (>99%, Xian Guanyu, Bio-Tech Co., China), and phenolphthalein (>98%, Aldrich Chemical Co., Milwaukee, WI), each containing 200 μg/mL in 100% ethanol, were prepared and stored at −20 °C for up to 3 months in flasks flushed with nitrogen gas (Air Products & Chemicals, Inc., Allentown, PA) to remove oxygen, as recommended by Trela and Waterhouse (*24*). Working solutions (20 μg/mL) for each compound were prepared daily. To reduce light-induced resveratrol isomerization from trans to cis form, the flasks were wrapped with aluminum foil and the preparation of *trans*-resveratrol and *trans*-piceid solutions was conducted under yellow light (*24*). Adsorbents for the cleanup column were mixtures (1:1 w/w) of aluminum oxide neutral, activity 1, particle size = 0.063−0.200 mm (VWR Int., LLC, West Chester, PA) and silica gel 60 C18 (Fisher Scientific, Pittsburgh, PA) (*14*). Acetonitrile used was of HPLC grade (Fisher Scientific). Water used for HPLC and sanitizing peanuts was double deionized and then vacuum filtered through a 0.2 μm nylon filter (Millipore Corp., Bedford, MA). In the lieu of hydrogen peroxide (*12*), chlorinated water was used to sanitize peanuts at a concentration of 150 μg/mL, which was sufficient to suppress mold growth on peanuts without imparting chlorine flavor (*25*). A chlorine stock solution (10 g/L) was prepared using calcium hypochlorite granules (65% of available chlorine, Fisher Scientific).

Experimental Design. *Study 1. Effect of US−UV Treatments on trans-Resveratrol and trans-Piceid Concentrations.* Peanuts stored for 6 months were treated with combined US−UV treatments using a 3×3 factorial design; the treatment factors were UV exposure times of 15, 35, and 55 min and UV distances of 20, 40, and 60 cm, and US parameters were held constant at a power density of 40 mW/cm^3 for 4 min. Peanuts were also treated with either UV (distance of 40 cm for 20 min) or US (power density of 40 mW/cm^3 for 4 min). All of the treated peanuts were dried and roasted. The experiment was replicated after slicing with duplicate extractions of each replicate. Whole, untreated peanut kernels, dried and roasted under conditions identical to the treated samples, were used as control.

Study 2. Effect of Storage. In study 1 the peanuts stored for 6 months were treated with US, UV, and nine US−UV treatments. Of these nine US−UV treatments, one treatment that resulted in the highest *trans*-resveratrol concentration in peanuts stored for 6 months was applied for peanuts stored for 13 months. Peanuts stored for 13 months were also treated with either UV or US using similar parameters as described under Study 1, and the resulting *trans*-resveratrol concentrations were compared with those in peanuts stored for 6 months. The numbers of the replications and extractions and control sample used were as described above for study 1.

Sample Preparation. Raw peanuts were manually sorted to remove broken and defective kernels. All equipment, materials, instruments, and labware to be used to treat the peanuts were sanitized by rinsing with 200 μg/mL chlorinated water immediately followed by rinsing with sterilized water. The sorted peanuts were sanitized by soaking in 150 μg/mL chlorinated water for 15 min (1:1 v/v), rinsed with sterilized water, and then soaked in sterilized water (1:1 v/v) in a deep plastic tub for 16 h to reach maximum water-holding capacity (*15*). Fully imbibed peanut kernels were drained for 15 min and cut in half in the middle of the kernel widthwise to approximately 7 mm using a sterile kitchen knife with a 10 cm blade on a sterile plastic chopping board. The sliced peanuts were mixed in a bowl and then divided into two batches for two replications of the study.

Stress Application. Fully imbibed sliced peanut kernels were placed into a 1 L graduated plastic beaker, which was filled with sterile water to cover the peanuts up to 750 mL, then sonicated in batches of approximately 500 g using an ultrasonic processor, model CPX 500 (Cole Parmer Instruments, Vernon Hills, IL). Ultrasound at 20 kHz was applied with a 25 mm diameter probe operating continuously at an ambient temperature of 25 °C. The desired power delivered to the probe was achieved by adjusting the amplitude of vibration at the probe. The level of amplitude of 40% for 4 min was determined empirically from a series of preliminary sonications (data not shown) of peanut batches to obtain energy delivered to the volume of 500 g of imbibed peanuts in 750 mL of water, which corresponds to a power density of 40 mW/cm^3. The targeted parameter was selected to reproduce conditions used by Rudolf et al. (*15*), who used a power density of 39.2 mW/cm^3 to indirectly sonicate peanuts in an ultrasonic bath. We realize, however, that it is difficult to reproduce conditions and make direct comparisons between different studies performed with different ultrasonic systems, because the actual energy delivered to a sample was not measured.

The level of amplitude was empirically determined by calculating power density (PD) using the formula

$$\mathrm{PD\ (mW/cm^3)} = \frac{\text{power delivered to the probe (JorW·s)} \times 1000\ \text{(mW)}}{\text{volume of the container (cm}^3\text{)} \times 60\ \text{(s)}}$$

where the power delivered to the probe during sonication of the peanut batches of 500 g in 750 mL of water ranged from 1780 to 1849 J/min (readings of the instrument after sonication), with the volume of the container of 750 cm^3. The resulting calculated average PD was 40 mW/cm^3. The probe tip was held 4 cm from the bottom of the beaker. After sonication, the peanuts were transferred to a sterile colander to drain excess water for 15 min. Thirteen sonicated batches were collected in one bowl, mixed, and reseparated again into nine batches of approximately 700 g for the UV treatment. Each batch of sonicated peanuts was spread 1 cm in depth on a plastic tray (56 L×30.5 W×60 H cm), and three trays

were placed under a UV germicidal lamp (122 cm L, UPV XX 40S, 245 nm, 40 W, UltraViolet Products, Upland, CA) set up inside a fume hood. The peanuts were processed at three distances from the UV lamp with the trays being removed at a specific time according to the experimental design. The samples were mixed throughout each tray after half of the irradiation time to allow uniform exposure of peanut kernels to UV light. Thereafter, US−UV-treated peanuts were placed into 240 mL sanitized glass Mason jars (Ball Corp., Muncie, IN) without lids, wrapped completely with aluminum foil, and incubated for 44 h (*23*) at ambient temperature of 25 °C. After incubation, the samples were dried at 40 °C for 24 h in a mechanical convection oven (model 645, Precision Scientific, Winchester, VA) and roasted in fabricated perforated trays (38.1 × 40.6 cm; 0.4 cm i.d. perforations) in an impingement oven (Lincoln Impinger, Fort Wayne, IN) at 150 °C for 12 min. After cooling in the trays, the peanut pieces were manually deskinned, skins were separated, and the samples were stored at −20 °C for approximately 1 week until analyzed.

Analysis of *trans*-Resveratrol and *trans*-Piceid. *Extraction*. The peanut sample (approximately 25 g) was ground in a coffee mill (Braun coffee mill, Mexico) for 1 min; then 10 g was placed into a 250 mL centrifuge tube with 30 mL of ethanol (80%), and 2 mL of phenolphthalein (60 μg/g) was added as an internal standard. The contents of the tube were homogenized (PowerGEN 700, Fisher Scientific) for 2 min on ice on setting 5 (27000 rpm) and then centrifuged (Beckman, model J2-21M, Palo Alto, CA) at 1380*g* at 25 °C for 5 min using rotor 14. From each tube, 2 mL of clear supernatant was transferred to a cleanup column to separate interfering compounds coeluting with *trans*-resveratrol. The cleanup column was a 2 mL plastic disposable syringe fitted with a filter (AP25, Millipore, Bedford, MA), which was placed at the bottom of the column to prevent loss of packing. The column was filled with a 1 g mixture of aluminum oxide and silica gel 60 C18 (1:1, w/w) (*14*). The sample was drained by gravity through the column and collected in a 4 mL vial. The column was rinsed by adding 0.5 mL of 80% ethanol, and the filtrate was collected in the same vial. The vial with the filtrate was placed into a heating block (Dri-bath, Thermolyne, Dubuque, IA), and the contents were dried by blowing nitrogen directly over the sample in the vial through a hollow six-pin blowdown manifold, removing solvent from six samples at a time, in parallel, at 60 °C. When completely dried after approximately 1 h, the vials were capped with lids and wrapped with foil to prevent *trans*-resveratrol and *trans*-piceid degradation and then stored at −20 °C for approximately 1 week until analyzed by HPLC.

HPLC Analysis. Before HPLC injection, the dried peanut residue was reconstituted with 0.4 mL of 15% ethanol. The vials were shaken manually for 30 s, then immersed, six vials at a time, into water in a round plastic container (13 cm i.d. × 6 cm depth) for subsequent indirect sonication using the ultrasonic processor described in the US treatment of peanuts under Study 1. The probe was immersed into water and held 2 cm from the bottom of the container. The ultrasonic processor was set at an amplitude of 50% for 2 min. After sonication, the contents of each vial were poured into a 3 mL glass syringe with an attached inorganic membrane filter (Anotop 10, 0.2 μm, Fisher Scientific). The plunger was inserted into the syringe, and the contents were filtered into a 300 μL polypropylene plastic insert (Fisher Scientific), in a 2 mL HPLC amber vial (Fisher Scientific). The vial was sealed with a screw cap fitted with a Teflon/silicone septum (Fisher Scientific).

HPLC analysis was conducted using a Varian Pro Star (Varian Inc., Palo Alto, CA) HPLC system consisting of a Varian autosampler 410, Varian solvent delivery module 210, and diode array detector (DAD) 335. An Eclipse Plus (Agilent Technologies, Inc., Deerfield, IL) C18 reversed-phase column (250×4.6 mm, 5 μm particle size) preceded by an EclipsePlus C18 (Agilent Technologies, Inc.) guard column (7.5×4.6 mm, 5 μm particle size) was used for analysis.

A constant flow rate of 1.5 mL/min was used with two solvents: solvent A was 100% double-deionized filtered water; solvent B was 100% acetonitrile. The column was maintained at ambient temperature at 25 °C. The gradient elution profile was as follows: 0 min, 5% B; 7 min, 22% B; 13 min, 23% B; 26 min, 63% B; 28 min, 80% B; then finally returned to 5% B over 1 min and held at 5% B for an additional 5 min. Peanut extracts were injected at a volume of 40 μL using an autosampler. Varian Pro Star software, version 6.44, was used to control the HPLC autosampler, gradient conditions, DAD, and data acquisition.

Peak areas of *trans*-resveratrol and *trans*-piceid were quantified at 307 nm and of phenolphthalein at 280 nm (*17*). Ratios of *trans*-resveratrol/*trans*-piceid and phenolphthalein peak areas from the analysis of the standards were used to calculate the concentrations of *trans*-resveratrol and *trans*-piceid in peanut samples using the equation (*26*)

$$\mu\text{ g of } i \text{ in sample} = \left[\frac{\left(\frac{\mu\text{ g of } i \text{ in standard}}{\text{PA of } i \text{ in standard}}\right) \times \text{PA of } i \text{ in sample}}{\left(\frac{\mu\text{ g of IS in standard}}{\text{PA of IS in standard}}\right) \times \text{PA of IS in sample}}\right] \times \mu\text{ g of IS in sample}$$

where *i* is *trans*-resveratrol/*trans*-piceid concentration, IS (internal standard) is phenolphthalein concentration, and PA is the peak area. The five levels of standards, 0.5, 1.0, 1.5, 5.0, and 10.0 μg/mL, for resvertarol, *trans*-piceid, and phenolphthalein were analyzed at the beginning of each HPLC sample set. *trans*-Resveratrol and *trans*-piceid concentrations were reported as micrograms per gram on a dry weight basis.

HPLC Method Validation. *Accuracy*. Determination of accuracy was done by the recovery method (*27*), where extracts of raw peanuts were spiked by adding *trans*-piceid and *trans*-resveratrol at three different concentrations, 0.5, 5, and 10 μg/g each, and compared with peanut extracts without the added compounds. For each level of concentration 3 analysis replications were performed, resulting in 12 analyses and the control extract of raw peanuts.

Precision. To evaluate precision or reproducibility, a solution containing 5 μg/g of *trans*-resveratrol, *trans*-piceid, and phenolphthalein was injected 10 times at 10 μL. Precision was expressed as the standard deviation (SD) and relative standard deviation (RSD) or coefficient of variation (CV) of the data set as described by Snyder et al. (*27*).

Linearity. Linearity was determined by injecting seven levels, 0.025, 0.050, 0.125, 1.250, 2.500, 5, and 10 μg/g, of *trans*-piceid, *trans*-resveratrol, and phenolphthalein. At each level, standards were injected three times. A calibration curve was constructed by plotting concentrations of each analyte at seven concentrations against the peak areas obtained. A correlation coefficient (*r*) between peak areas and the concentrations of *trans*-resveratrol, *trans*-piceid, and phenolphthalein was analyzed for each compound.

Limit of Detection (LOD) and Limit of Quantification (LOQ). LOD is the concentration of analyte that results in a peak height of at least 3 times that of the noise (baseline deflection) when injected into HPLC. A solution containing *trans*-piceid, *trans*-resveratrol, and phenolphthalein was diluted in series with 15% of ethanol to obtain the lowest level of analyte that gave a measurable response. Analysis was conducted in triplicate. LOQ is the lowest concentration of the analyte in a sample that can be quantified with acceptable accuracy and precision measured by CV of the three replications (*28*).

Statistical Analysis. Data were analyzed using Statistical Analysis software (version 8, SAS Institute Inc., Cary, NC). Regression analysis (PROC REG) was used to obtain coefficients of determination (R^2) and to develop prediction models ($p \leq 0.05$ and $R^2 \geq 0.5$) for each dependent variable, either *trans*-resveratrol or *trans*-piceid concentration, and the following linear independent variables: distance and time. The general linear model (PROC GLM) was applied to detect significant differences ($p < 0.05$) in the *trans*-resveratrol and *trans*-piceid concentrations as a result of the stress treatments. Fisher's least significant difference (LSD) test was used to determine mean separation of *trans*-resveratrol and *trans*-piceid concentrations after the stress treatments.

RESULTS AND DISCUSSION

HPLC Method. The HPLC parameters of the modified and Rudolf et al. (*17*) methods are compared in **Table 1**. Before injection, the dry residue was reconstituted in 15% of ethanol, unlike 10% in the method of Rudolf et al., as better recoveries of the compounds were achieved with the increased ethanol concentration. Forty microliters or a half of the injection volume of 80 μL used by Rudolf et al. (*17*) was found to be sufficient for obtaining better chromatograms than if larger volumes were used. Solvents A and B used in the mobile phase system were

Table 1. Comparison of the Rudolf et al. (*17*) Method with the Modified Developed for This Study

	Rudolf et al.	modified method
reconstitution of dry residue	10% ethanol	15% ethanol
injection volume	80 μL	40 μL
reverse phase system		
solvent A	0.1% acetic acid in water	100% water
solvent B	100% acetonitrile	100% acetonitrile
gradient elution profile	time, solvent B	time, solvent B
	0 min, 5%	0 min, 5%
	23 min, 41.8%	7 min, 22%
	28 min, 77%	13 min, 23%
	29 min, 5%	26 min, 63%
	34 min, 5%	28 min, 80%
		29 min, 5%
		34 min, 5%
flow rate	1.5 mL	1.5 mL
retention time		
trans-piceid	13.1 min	11.7 min
trans-resveratrol	17 min	19 min
phenolphthalein	22 min	23.6 min

similar to those of Rudolf et al. (*17*) with the exception that acetic acid, which was used to suppress on-column ionic dissociation of *trans*-resveratrol, was omitted from solvent A (water) after no difference in the chromatographic peak shape was noted. The major changes in the method were in gradient elution profile, wherein segmented gradients were inserted with varying steepness to achieve good resolution for *trans*-piceid, *trans*-resveratrol, and phenolphthalein. Rudolf et al. (*17*) used in the first segment, from 0 to 23 min, a gradient steepness (GS) of 1.6, whereas in the current method it was necessary to insert three segments from 0 to 26 min. The first segment had a GS of 2.4, followed by the second from 7 to 13 min with the lowest GS of 0.17, allowing successful resolution of piceid with a retention time (t_R) for *trans*-piceid of 11.7 min. The GS of the third segment was increased to 3.7 to obtain good resolution between *trans*-resveratrol (t_R = 19 min) and phenolphthalein (t_R = 23.6 min) while eluting within 13−26 min. From 26 to 28 min the gradient of solvent B reached its maximum of 80% with GS of 8.5 and then finally returned over 1 min to the initial condition of 5% and held for an additional 5 min to recondition the column.

HPLC Method Validation. *Accuracy*. Recoveries of *trans*-resveratrol in peanut extracts were 99.23 ± 9.01, 108.02 ± 0.93, and 112.10 ± 9.17% and those of *trans*-piceid, 124.22 ± 2.05, 112.8 ± 15.6, and 113.3 ± 12.54% for the added *trans*-resveratrol and *trans*-piceid concentrations of 0.5, 5.0, and 10 μg/mL each. All calculations were adjusted to account for the natural concentrations of *trans*-resveratrol and *trans*-piceid, 0.017 ± 0.02 and 0.024 ± 0.03 μg/g of dry weight, respectively, in raw peanuts not containing added *trans*-resveratrol and *trans*-piceid standard solutions. The recoveries in this study for *trans*-resveratrol are similar to those of Sobolev and Cole (*29*) of 117.23 ± 8.87, 100.10 ± 2.49, and 100.45 ± 1.51% for the same *trans*-resveratrol concentrations. However, the recoveries for *trans*-resveratrol were higher than those found by Rudolf et al. (*17*) of 71.20 ± 3.42, 96.08 ± 1.00, and 93.02 ± 2.35% for the concentrations of 0.5, 1.0, and 1.5 μg/mL, respectively. Recoveries of *trans*-piceid in this study were higher than those of *trans*-resveratrol, meaning that *trans*-piceid had higher solubility in 80% ethanol in water (80:20, v/v), the solvent used during the extraction process.

Precision. The CVs for *trans*-piceid, *trans*-resveratrol, and phenolphthalein were 1.52, 1.89, and 2.17%, respectively. The results are within the acceptable limits of 1−2% recommended by Snyder et al. (*27*) for compounds of low-level concentrations.

Linearity. Linearity is expressed in terms of the correlation coefficient (r). The correlation coefficients of *trans*-piceid, *trans*-resveratrol, and phenolphthalein were 0.9994, 0.9997, and 0.9999, respectively, which are within the acceptable limits of $r > 0.999$ (*27*).

LOD and LOQ. The LODs were 0.0125, 0.0125, and 0.050 μg/g for *trans*-piceid, *trans*-resveratrol, and phenolphthalein, respectively. Results are similar to those reported by Rudolf et al. (*17*), who reported LODs for *trans*-resveratrol and phenolphthalein of 0.01 and 0.05 μg/g, respectively, and lower than those reported by Lamuela-Ravenotos et al. (*9*), who reported a LOD of 3 ppm in wine.

LOQs were 0.05, 0.05, and 0.125 μg/g for *trans*-piceid, *trans*-resveratrol, and phenolphthalein, respectively, with the corresponding CVs of 4.27, 6.82, and 13.07%. The precisions are well below acceptable limits of 20% (*28*). Rudolf et al. (*17*) found LOQs of 0.04 and 1 μg/g for *trans*-resveratrol and phenolphthalein, respectively, whereas Sanders et al. (*14*) and Sobolev and Cole (*29*) reported lower LOQs of a 0.02 and 0.01 μg/g for *trans*-resveratrol in peanuts, respectively.

***trans*-Resveratrol and *trans*-Piceid Analysis.** *Study 1. Effect of US−UV Treatments on trans-Resveratrol and trans-Piceid Concentrations*. All of the combined US−UV treatments increased the concentrations of *trans*-resveratrol from 0.03 μg/g, in untreated whole roasted peanut control, to 2.10−4.73 μg/g, a 70−158-fold increase, and of *trans*-piceid from 0.07 to 0.23−0.38 μg/g, a 3.3−5.4-fold increase (**Table 2**). The CVs ranged from 4 to 35% for both *trans*-resveratrol and *trans*-piceid concentrations. A typical HPLC chromatogram of *trans*-piceid, *trans*-resveratrol, and phenolphthalein in treated and untreated peanuts stored for 6 months is shown in **Figure 1**.

The highest amount of *trans*-resveratrol (4.73 μg/g) in US-UV-treated samples was found when peanuts were treated with US at a power density of 40 mW/cm^3 for 4 min followed by UV at a distance 40 cm for 35 min. However, results from analysis of variance showed that no significant difference was obtained between distance from UV source, time of UV exposure, or interactions and replication of the analysis. This suggests that any combination of UV exposure time (from 15 to 55 min) and UV source distance (from 10 to 60 cm) will result in similar increase in *trans*-resveratrol and *trans*-piceid concentrations. *trans*-Resveratrol content of 0.03 μg/g in untreated roasted peanuts in this study was lower than 0.48 μg/g in raw untreated peanuts, reported by Rudolf and Resurreccion (*15*). However levels found in this study were within the range of 0.02−0.08 μg/g in roasted peanuts reported by Sobolev et al. (*29*) and in the range of 0.02−0.3 μg/g in 14 of 15 cultivars representing 3 market peanut types found by Sanders et al. (*14*). *trans*-Piceid contents of 0.07 μg/g in this study were within the range of the 0.07−0.22 μg/g in natural and blended peanut butters, found by Ibern-Gomez et al. (*16*). Regression analysis showed (data not presented) that UV distance and UV time were not significantly ($p > 0.05$) related to *trans*-resveratrol or *trans*-piceid concentration. Comparison of combination US−UV treatments with US alone led to the conclusion that none of the combinations of treatments had a synergistic effect on increasing *trans*-resveratrol concentration. As can be observed, an equally high concentration of *trans*-resveratrol, 4.29 μg/g, was produced after US treatment, falling into the upper range (2.10−4.73 μg/g) of the US-UV-treated peanuts. Peanuts treated only with UV had a mean value for *trans*-resveratrol of 2.36 μg/g, falling into the lower range of US−UV-treated peanuts. One explanation for the lower concentrations after UV exposure could be a partial isomerization of *trans*-resveratrol into the cis form. López-Hernández et al. (*30*) reported a formation of *cis*-resveratrol from the trans form after a *trans*-resveratrol

Table 2. Mean Values[a] and Standard Deviations for *trans*-Resveratrol and *trans*-Piceid Concentrations in Peanut Kernels Stored for 6 Months and Treated with Ultrasound (40 mW/cm^3 for 4 min) followed by UV (Three Exposure Times and Three Distances)

	mean concentration (μg/g of dry weight)					
	trans-resveratrol at UV exposure distance of			*trans*-piceid at UV exposure distance of		
UV time (min)	20 cm	40 cm	60 cm	20 cm	40 cm	60 cm
15	3.47 ± 0.66 a	2.45 ± 0.17 a	2.67 ± 0.46 a	0.35 ± 0.10 a	0.25 ± 0.03 a	0.23 ± 0.04 a
35	3.14 ± 0.96 a	4.73 ± 1.20 a	2.10 ± 0.09 a	0.29 ± 0.13 a	0.35 ± 0.08 a	0.27 ± 0.15 a
55	2.44 ± 0.40 a	3.10 ± 1.10 a	3.15 ± 0.16 a	0.38 ± 0.26 a	0.30 ± 0.08 a	0.35 ± 0.11 a
control[b]	0.03 ± 0.01				0.07 ± 0.003	
US treated[c]	4.29 ± 1.53				0.46 ± 0.19	
UV treated[d]	2.36 ± 0.64				0.36 ± 0.16	

[a] Means were calculated from two replications with two extractions of each replication. The general linear model (PROC GLM) was used to determine significant difference. Means in a column for *trans*-resveratrol or *trans*-piceid not followed by the same letter are significantly different at $\alpha < 0.05$ as determined by Fisher's least significant difference mean separation test. [b] Control used was whole, dried, and roasted peanuts. [c] US-treated peanuts were sliced and exposed to US at power density of 40 mW/cm^3 for 4 min. [d] UV-treated peanuts were sliced and exposed to UV at a distance 40 cm for 20 min.

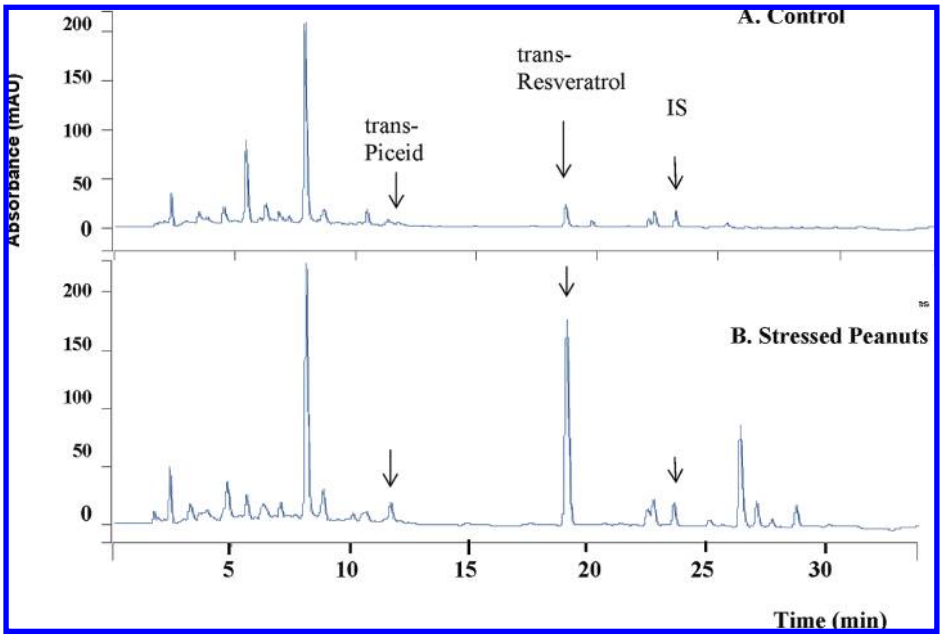

Figure 1. HPLC-DAD chromatogram (280 nm) of *trans*-resveratrol, *trans*-piceid, and phenolphthalein (IS, internal standard) in untreated peanut control (**A**) and in US−UV-stressed peanuts (**B**).

solution of 4 μg/mL in 15% of ethanol was exposed to UV (254 nm) at a 3.5 cm distance. After all of the applied treatments, US, UV, and US−UV, new unknown peaks were detected in the chromatograms, which were not present in controls, thus suggesting that the stress application to peanuts induced formation of new compounds not identified in this study. Likewise, we detected a new small peak, which could be *cis*-resveratrol as it was present only in chromatograms of UV- and US−UV-treated peanuts (280 nm) but not in ones of US-treated peanuts. This peak was very small, did not appear consistently in all of the UV or US−UV chromatograms, and, when present, was coeluted with another compound.

Combination US−UV treatments did not have any additive effect on *trans*-piceid concentrations, as we compare them with US or UV only. The concentration of the compound after US treatment of 0.46 μg/g is higher than the range of 0.23−0.38 μg/g after US−UV treatments. The concentration after UV of 0.36 μg/g falls into the upper range of US−UV-treated peanuts.

The *trans*-resveratrol concentration range of 2.10−4.73 μg/g in US−UV-treated peanuts, regardless of UV exposure time and distance, in this paper was similar to the levels of 3.42 and 3.96 μg/g in peanuts found by Rudolf and Resurreccion (*15*), after using only UV or US, respectively. However, the levels of *trans*-resveratrol in this paper are higher than those reported in a later study by Rudolf and Resurreccion (*23*) of 0.44−1.38 μg/g in US-treated peanuts. In this study we compared the effect of different stress treatments at the fixed incubation time. However, it might be difficult to assess these comparisons as the kinetics of *trans*-resveratrol synthesis might be different under combined or individual treatments and stilbenes may accumulate at different times after the stress. Therefore, even though 44 h was chosed as the optimum incubation time, according to the previous studies (*23*), future work may include different incubation times as an additional factor to assess maximum stilbene synthesis under these conditions.

UV exposure of sliced peanuts in this study resulted in an increase of *trans*-resveratrol concentration from 0.03 μg/g, in untreated whole roasted peanut control, to 2.36 μg/g, a 79-fold increase. Likewise, the induction of *trans*-resveratrol was reported by Cantos et al. (*21*), who observed an 11-fold increase of *trans*-resveratrol concentrations when table grapes were irradiated with UV of 510 W for 30 s at a distance of 40 cm followed by 3 days of incubation. Similarly, Chung et al. (*22*) reported a 200-fold *trans*-resveratrol increase in peanut leaves to UV light at

Table 3. Mean Values[a] and Standard Deviations for *trans*-Resveratrol and *trans*-Piceid Concentrations in Peanuts Treated with Three Different Stresses after 6 and 13 Months of Storage

	trans-resveratrol (μg/g) in peanuts stored for		*trans*-piceid (μg/g) in peanuts stored for	
treatment	6 months	13 months	6 months	13 months
US−UV[b]	4.73 ± 1.20 a	3.37 ± 0.38 a	0.35 ± 0.08 a	0.55 ± 0.09 a
US[c]	4.29 ± 1.53 ab	3.08 ± 0.28 ab	0.46 ± 0.19 a	0.49 ± 0.08 a
UV[d]	2.36 ± 0.64 b	2.68 ± 0.27 b	0.36 ± 0.16 a	0.43 ± 0.08 a
control[e]	0.03 ± 0.01	0.016 ± 0.002	0.07 ± 0.003	0.022 ± 0.003

[a] Means were calculated from two replications with two extractions. The general linear model (PROC GLM) was used to determine significant difference. Means in a column for *trans*-resveratrol or *trans*-piceid not followed by the same letter are significantly different at $\alpha < 0.05$ as determined by Fisher's least significant difference mean separation test. [b] US−UV-treated peanuts were sliced, sonicated (power density 40 mW/cm^3 for 4 min), exposed to UV (distance 40 cm for 35 min), incubated for 44 h, dried, and roasted. [c] US-treated peanuts were sliced, sonicated (power density 40 mW/cm^3 for 4 min), incubated for 44 h, dried, and roasted. [d] UV-treated peanuts were sliced, UV-irradiated (distance 40 cm for 20 min), incubated for 44 h, dried, and roasted. [e] The control used was whole, dried, and roasted peanuts.

1.35 μEinstein/(m^2/s) for 2 h. They found (*22*) that changes in *trans*-resveratrol content after UV irradiation were correlated with levels of RS mRNA, indicating a transcriptional control of *trans*-resveratrol synthase activity suggesting that abiotic stresses, such as UV, induced *trans*-resveratrol synthesis through the regulation of RS transcription.

US exposure of peanuts resulted in an increase of *trans*-resveratrol concentration from 0.03 μg/g, in untreated whole roasted peanut control, to 4.29 μg/g, a 143-fold increase, almost double that produced by UV exposure. To our knowledge, the possible mechanism of the ultrasound stress on *trans*-resveratrol synthesis has not been reported in the literature. However, ultrasonic irradiation was effectively employed over the past decade to accelerate chemical reactions to induce new reactivities leading to the formation of unexpected chemical species (*31*). Previously, Lin et al. (*32*) reported stimulation of biosynthesis of secondary metabolites, the ginsenoside saponins of ginseng cells, by US with increase of total saponins by 75%, stating that mechanical stress and microstreaming induced by acoustic cavitation were the most possible effects of US on ginseng cells. In particular, the authors considered that the enhancement of secondary metabolite synthesis may be a result of US-induced plant defense response. Xiao et al. (*33*) carried out studies on enzymatic synthesis of glucose esters under US and shaking in organic solvents. They observed remarkable acceleration of the transesterification of glycose and divinyl dicarboxylates by US, with yields higher than that of shaking over the same reaction time. They explained the enhanced effect of US to acceleration of the collision probability of the enzyme and substrate. Furthermore, they speculated that the effect of cavitations accelerated mass transport, resulting in faster product diffusion from the enzymatic site.

Study 2. Effect of Storage. *trans*-Resveratrol and *trans*-piceid concentrations in peanuts, which were stored for 6 and 13 months prior to application by a combination US−UV treatment and individually either with US or UV, are presented in **Table 3**. All of the treatments increased concentrations of *trans*-resveratrol and *trans*-piceid in peanuts stored for 6 or 13 months compared to untreated whole roasted peanut control. The effect of the treatments was more pronounced for *trans*-resveratrol (168−211-fold increase) than for *trans*-piceid (20−25-fold increase). The effect of the treatments and the pattern of the significant differences between the means of *trans*-resveratrol concentrations in peanuts stored for 13 months are similar to those of peanuts stored for 6 months. The maximum *trans*-resveratrol concentration was achieved when peanuts stored for 6 and 13 months were treated with combined US−UV treatments. However, the values of *trans*-resveratrol concentration were not significantly ($p > 0.05$) different from those obtained after US treatment, but it was significantly ($p < 0.05$) higher compared to that after UV exposure. No significant difference ($p > 0.05$) was obtained between the treatments on *trans*-piceid concentration.

The important observation from this storage experiment was that the peanuts stored for 13 months produced 28% less *trans*-resveratrol after combined US−UV treatments and 29% less resveratrol after the US treatment alone than those stored for 6 months, except for UV-treated peanuts, for which a slight increase of 13.5% was observed. Untreated roasted peanuts stored for 13 months contained 43% less *trans*-resveratrol than peanuts stored for 6 months, although both concentrations remained low. The lower *trans*-resveratrol concentrations could be due to two reasons. One is a degradation process occurring during storage of raw peanuts, resulting in reduced, almost in half, natural *trans*-resveratrol concentrations, probably due to oxidation or other mechanisms. The other reason is decreased *trans*-resveratrol synthesis. Arora and Strange (*20*) also observed reduced ability of peanuts to synthesize phytoalexins in response to slicing and incubation with increased storage time (9 months). They explained the phenomenon by decreased viability of cotyledons. Similar observations were made by Rudolf and Resurreccion (*23*), who attributed the reduced amount of *trans*-resveratrol in kernels in response to stress not only to longer storage conditions (1 year compared to peanuts stored for only 2 weeks) but also to a different geographic location of harvest. Hence, the greatest *trans*-resveratrol synthesis can be expected from processing of freshly harvested peanuts compared to stored ones. Interestingly, the amounts of *trans*-piceid after all treatments of peanuts stored for 13 months were 6−37% higher than those stored for 6 months. However, untreated roasted peanut control stored for 13 months contained 69% less *trans*-piceid than peanuts stored for 6 months.

The highest concentration of *trans*-resveratrol on a per gram basis (4.73 μg/g) obtained in this study is comparable to that of red wine (0.60−8.00 μg/g); however, when calculated on a per serving basis, wine delivers more *trans*-resveratrol. Yet for those individuals who drink wine occasionally or do not drink at all but consume peanut products on a daily basis, the functional peanuts with enhanced *trans*-resveratrol content, in the form of either peanut butter or peanut bars, might provide significant sources of *trans*-resveratrol and its beneficial effect.

In conclusion, accuracy, precision, linearity, and limit of detection were consistent with or better than those of methods previously reported in the literature for the quantification of *trans*-resveratrol and *trans*-piceid. UV or US individually, or combination of these two processing methods, proved to be effective in increasing *trans*-resveratrol concentration in peanut kernels. Because *trans*-resveratrol concentrations were equal to or higher than those obtained with US−UV treatments, the use of US alone, to increase *trans*-resveratrol concentration in peanuts, is recommended over the combined US−UV treatment. The results obtained from this and related studies conducted in our laboratory are the knowledge and the technology to be used by peanut processors or food industries for development of

resveratrol-rich peanuts (RRP), to use, for example, in resveratrol-rich peanut bars, confections, butters, and spreads for delivery of health benefits. The next steps for future studies would be to design, develop, process, and optimize these RRP products with high *trans*-resveratrol content and acceptable sensory qualities.

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Received February 26, 2009. Revised manuscript received July 11, 2009. Accepted July 24, 2009.