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# Elicitation of Resveratrol in Peanut Kernels by Application of Abiotic Stresses

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Application of no postharvest stress or by size reduction, grinding, chopping, or slicing and exposure to UV light or ultrasound were investigated for the elicitation of *trans*-resveratrol, total phenolic compounds, and antioxidant activity (AOA) in peanut kernels. AOA and total phenolic compounds did not increase in stressed peanuts over incubation times 0−48 h at 25 °C; however, an increase in *trans*-resveratrol concentration over time occurred. Slicing (2 mm), ultrasound exposure for 4 min at 25 °C, and incubation for 36 h produced the highest level of *trans*-resveratrol synthesis in peanuts, increasing concentrations from 0.48 ± 0.08 μg/g in untreated peanuts to 3.96 ± 0.96 μg/g. AOA in stressed peanuts was negatively correlated with *trans*-resveratrol concentration, indicating that as AOA decreased *trans*-resveratrol concentration increased.

**KEYWORDS: Resveratrol; UV; ultrasound; wounding; total phenolics; antioxidant activity**

## INTRODUCTION

The antioxidative activity (AOA) provided by phenolic compounds has been shown to inhibit the oxidation of low-density proteins (*2*), thereby decreasing heart disease risks (*3*). Phenolic compounds have also been shown to have anti-inflammatory (*4*) and anticarcinogic activity (*5*).

Resveratrol (*trans*-3,5,4′-trihydroxystilbene), a stilbene phytoalexin, is a phenolic compound possessing antioxidant activity. Resveratrol has been shown to provide health-promoting activities such as lowering the incidence of coronary heat disease (*6*), to provide cancer chemopreventive activity (*7*), and to be a phytoestrogen exhibiting variable degrees of estrogen receptor agonism (*8*).

Red wine is the most common food source of resveratrol, having 0.99−5.01 mg/L (*9*). *trans*-Resveratrol has also been identified in peanut kernels and processed peanut products. Roasted peanuts contain the lowest content of resveratrol, 0.055 ± 0.023 μg/g, peanut butter contains a significantly higher amount, 0.324 ± 0.129 μg/g, and boiled peanuts have the highest concentration, 5.138 ± 2.849 μg/g (*10*). Because peanuts naturally contain lower concentrations of resveratrol, it is of interest to increase these levels to that of wine in order to increase health benefits.

Biotic and abiotic treatments have been used to increase AOA, total phenolic compounds, and resveratrol concentration in plant material. Biotic factors such as cultivar type (*11*−*13*), maturity level (*11*−*13*), and microbial exposure (*14*−*18*) have been tested for their elicitation effect on peanuts. In addition, abiotic factors such as wounding by slicing, in peanuts (*13*, *19*, *20*); ultraviolet light exposure, on grape and peanut leaves (*17*, *18*, *21*−*23*); ultrasound (*24*) exposure, applied to *Panax ginseng* cells; and processing methods, such as roasting of peanuts (*25*), have also been studied as elicitors. Among these methods only the effect of slicing on resveratrol synthesis was studied on peanut kernels.

The objective of this study was to determine the elicitation of AOA, total phenolic compounds, and *trans*-resveratrol in peanut kernels after exposure to postharvest stress. Specific objectives were to determine the effect of size reduction method by slicing, chopping, or grinding; to determine the effect of stress application by UV light or ultrasound exposure; and to determine the effect of incubation time on AOA, total phenolic compounds, and *trans*-resveratrol concentration in peanut kernels.

## MATERIALS AND METHODS

**Experimental Design.** Raw peanut kernels were subjected to postharvest treatments using a 4 × 3 × 4 factorial design. Factors studied were size reduction method (chopping, slicing, grinding, and no size reduction treatment), post size reduction stress applications (UV light, ultrasound, and no stress application), and incubation times (0, 24, 36, and 48 h). Three replications of the 48 treatments were conducted for at total of 144 samples. Peanut samples were analyzed for AOA and total phenolic compound concentration each in duplicate for a total of 288 samples per analyses. Duplicate analysis for AOA and total phenolic compounds was conducted to reduce time and cost of analysis; however, triplicate analysis was conducted when large variation occurred between results. *trans*-Resveratrol analyses were conducted in triplicate. Control samples were prepared from untreated raw peanuts.

**Sample Preparation.** Peanuts used in the analysis were Georgia green medium runners (McCleskey Mills Inc.) harvested in Smithville, GA, in 2002. The 22.68 kg bag (50 lb) had been stored under refrigerated storage in a seed storage room at 7 °C for approximately

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2 weeks prior to the study. Approximately 3.6 kg of raw peanuts were surface sterilized in 4 L of 20% hydrogen peroxide (Sigma, St. Louis, MO) for 15 min (*19*) and then rinsed in a sterile colander with 4 L of sterilized deionized water, sterilized by passing through a 0.2 $\mu$m nylon filter (Millipore Corp., Bedford, MA). The sterilized peanuts were soaked in 4 L of sterile deionized water for 16 h to reach the maximum water holding capacity of the peanut as determined by preliminary studies. The entire process was conducted under yellow light to avoid resveratrol isomerization (*28*).

**Size Reduction.** Approximately 1.2 kg of sterilized fully imbibed peanut kernels was reduced by one of the four size reduction methods as follows. Peanuts were ground into 1−2 mm pieces using a food processor (model 14181, Sunbeam Oskar, China) for 20 s in six batches of 200 g each. Kernels were chopped into 0.5 cm pieces in two batches of 600 g each using a commercial food cutter (model 84142, Hobart, Troy, OH) at 1725 rpm for 30 s. Peanuts were sliced into 2 mm thick pieces, by hand, with a razor blade; a ruler was used as a guide for size. Whole peanut kernels were also collected to represent samples with no size reduction treatment. Each of the size reduction batches was collected and mixed by hand with a spoon. All equipment used was sterilized prior to use with 20% hydrogen peroxide.

**Post Size Reduction Stress Application.** Peanuts from each of the size reduction treatments were divided into three batches of 400 g each and then stressed with UV light or ultrasound or had no further stress application. Peanuts arranged 1 cm in depth on a plastic tray were exposed to UV light for 10 min (*22*, *23*) using a germicidal lamp (254 nm, 30 W, model UVSL-58, Ultra Violet Products, Inc., San Gabriel, CA) placed 40 cm (*23*) above the surface of the peanuts. Peanuts were stirred after 5 min to ensure exposure of UV light to all sides of the kernels.

Ultrasound treatment was performed in a sonic cleaner (model FS60120V, 29.5 L × 15.5 W × 14.5 D cm, 260W, 2.2 A, 50/60 Hz; Fisher Scientific, Fair Lawn, NJ) with a power density of 39.2 mW/cm$^3$. Approximately 400 g of peanuts was placed into a 1 L glass beaker containing 800 mL of sterile deionized water. The beaker was placed into the sonic cleaner filled with enough water to completely surround the beaker and sonicated for 4 min at room temperature (*24*). After the sample was sonicated, the water was drained from the peanuts by placing them into a sterile colander for 5 min.

Approximately 100 g of sample was stored in 240 mL glass Mason jars (Ball Corp., Muncie, IN) with two-piece lids (Alltrista Corp., Muncie, IN). The Mason jars were wrapped with aluminum foil to protect samples from light exposure. Samples receiving none of the stress treatments were immediately placed in jars and incubated. Stress application was done in dim light to prevent *trans*-resveratrol isomerization (*28*).

**Sample Incubation.** Mason jars were incubated at 25 °C (Environmental Growth Chamber, Chagrin Falls, OH) for 24, 36, and 48 h. After incubation, the samples were stored for approximately 1 month at −23 °C until analyzed.

**Extraction of Antioxidants and Total Phenolic Compounds.** Each peanut sample was extracted for antioxidants and total phenolic compounds in duplicate. Ten grams of each peanut sample was ground, and 5 g was placed in a 250 mL centrifuge tube (Nalgene, Rochester, NY) with 50 mL of methanol (Fisher Scientific). Tubes were shaken for 12 h with a wrist action shaker (model 75, Burrell Corp., Pittsburgh, PA) at ambient temperature, 25 °C. The extracts were filtered (Whatman no. 42, Maidstone, U.K.) into a round-bottom flask, and the residue was extracted again under the same conditions. The combined filtrate in a round-bottom flask was placed in a water bath (Buchi 461 water bath, Switzerland) at 40 °C and evaporated under vacuum in a rotary evaporator (Brinkmann RE 111, Buchi), for ∼30 min, to a final volume of 5 mL.

**Analysis of AOA.** Determination of the AOA of the 144 peanut extracts was performed according to the thiocyanate method of Osawa and Namiki (*29*) for eucalyptus leaves as adapted by Duh and Yen (*12*).

AOA was calculated as percent inhibition of oxidation of linoleic acid relative to the control after a 60 min incubation according to the method of Emmons et al. (*31*) using the equation

$$\text{AOA (\%)} = 100[\ln(\text{control abs}_0/\text{abs}_{60}) - \ln(\text{sample abs}_0/\text{abs}_{60})][\ln(\text{control abs}_0/\text{abs}_{60})] \quad (1)$$

where AOA is the antioxidant activity and abs is the absorbance of the control or peanut sample tested at 0 time and after 60 min at 25 °C, ambient.

**Analysis of Total Phenolic Compounds.** The concentration of total phenolic compounds present in the peanut sample was determined using a spectrophotometric method with Folin−Denis reagent (*32*) established by Yen and Duh (*11*). The blue color was measured at 726 nm (*33*) using a spectrophotometer (model 8451A, diode array spectrophotometer, Hewlett-Packard, Palo Alto, CA). Absorbance was measured in triplicate for all peanut samples and standard solutions. Total phenolic compound concentration was determined in peanut kernels using the regression equation

$$y = [44.37(\text{AU})x + 0.019(\text{AU/mg/g})] \quad (2)$$

where $y$ is total phenolic compounds (mg/g) and $x$ is peak area.

**Analysis of *trans*-Resveratrol.** *trans*-Resveratrol was analyzed using an HPLC method. *trans*-Resveratrol was extracted following a procedure established by Sanders et al. (*34*) and modified by Rudolf et al. (*35*) using phenolphthalein as the internal standard. Approximately 35 g of peanut sample was prepared as described by Rudolf et al. (*35*). The entire procedure was conducted under yellow light to prevent resveratrol isomerization (*28*).

The dried peanut residue was prepared for HPLC analysis by adding 0.40 mL of 10% ethanol to a vial mixed and filtered into a 300 $\mu$L polypropylene plastic insert (National Scientific Co., Lawrenceville, GA), to minimize sample volume, which was placed in a 2 mL HPLC amber vial (National Scientific Co.). The vial was sealed with a screw cap that was fitted with a Teflon/silicone septum (National Scientific Co.).

HPLC analyses were performed using a Waters (Milford, MA) system with a W717 sample injector, a W2695 separations module, and a W996 photodiode array detector (PDA) set to monitor the UV spectrum from 240 to 400 nm. An Econosphere (Alltech Associates, Inc., Deerfield, IL) $C_{18}$ reversed-phase column (250 × 4.6 mm, 5 $\mu$m particle size) preceded by an Econosphere $C_{18}$ (Alltech Associates, Inc.) guard column (7.5 × 4.6 mm, 5 $\mu$m particle size) was used for analysis.

*trans*-Resveratrol was analyzed using a reversed-phase HPLC method developed by Rudolf (*35*). The mobile phase consisted of 0.1% acetic acid (J. T. Baker, Phillipsburg, NJ) in double-deionized water, which was filtered through a 0.2 $\mu$m nylon filter by vacuum as solvent A and 100% acetonitrile (HPLC grade, Aldrich, Milwaukee, WI) as solvent B, with a flow rate of 1.5 mL/min, and the column temperature was maintained at 25 °C, ambient. The gradient elution increased acetonitrile linearly from 5 to 41.8% over 23 min, then increased to 77% over 5 min, and finally returned to 5% over 1 min and was held for an additional 5 min. Waters Millenium[32] software, version 3.05, was used to control the HPLC autosampler, gradient conditions, PDA, and data acquisition. Peanut extracts were injected at a volume of 80 $\mu$L.

Peak areas of *trans*-resveratrol and phenolphthalein were quantified at 307 nm (*10*) and 254 nm (*35*), respectively. Ratios of *trans*-resveratrol and phenolphthalein peak areas from the analysis of peanut samples and standards were used to calculate the concentration of *trans*-resveratrol using the equation (*36*)

$$\mu\text{g of } i \text{ in sample} = \left[\frac{\left(\frac{\mu\text{g of } i \text{ in standard}}{\text{PA of } i \text{ in standard}}\right) \times \text{PA of } i \text{ in sample}}{\left(\frac{\mu\text{g of IS in standard}}{\text{PA of IS in standard}}\right) \times \text{PA of IS in sample}}\right] \times \mu\text{g of IS in sample} \quad (3)$$

where $i$ is *trans*-resveratrol, IS is phenolphthalein (internal standard), and PA is the peak area. The five levels of standards, 5, 3.125, 1.250, 0.625, and 0.375 ppm ($\mu$g/mL), for *trans*-resveratrol and phenolphthalein were analyzed at the beginning of each HPLC sample set. *trans*-Resveratrol concentrations were reported as $\mu$g/g of peanut on a dry weight basis.

**Table 1.** Mean Values for Percent Antioxidant Activity (AOA), Measured Spectrophotometrically at 500 nm, in Peanut Kernels Treated with Four Size Reduction Methods and Three Stresses and Then Incubated[a,b]

| post size reduction stress | incubation time (h) | | AOA[c] (%) | | | |
|---|---|---|---|---|---|---|
| | | | ground | chopped | sliced | whole |
| none | 0 | | 42.30 ± 1.91a | 39.93 ± 1.66 | 40.45 ± 1.41 | 40.81 ± 1.40 |
| | 24 | | 38.41 ± 0.46b | 38.35 ± 0.44 | 39.43 ± 1.29 | 38.93 ± 1.00 |
| | 36 | | 39.46 ± 1.35b | 38.59 ± 1.03 | 41.96 ± 1.01 | 39.58 ± 0.98 |
| | 48 | | 39.83 ± 1.25b | 40.08 ± 1.11 | 40.18 ± 1.06 | 40.59 ± 1.05 |
| | | *F value* | *8.93* | *2.83* | *1.25* | *1.83* |
| | | *p value* | *0.0007* | *0.0713* | *0.323* | *0.1797* |
| UV | 0 | | 40.59 ± 0.36a | 41.34 ± 1.13a | 43.09 ± 1.01a | 42.97 ± 1.02a |
| | 24 | | 38.42 ± 1.01b | 38.07 ± 0.21b | 38.36 ± 0.67b | 38.11 ± 0.56b |
| | 36 | | 38.53 ± 0.62b | 40.41 ± 1.51a | 38.99 ± 1.76b | 38.66 ± 1.03b |
| | 48 | | 38.38 ± 0.73b | 40.38 ± 1.25a | 42.22 ± 1.54a | 43.50 ± 1.29a |
| | | *F value*[d] | *68.84* | *10.27* | *19.79* | *7.78* |
| | | *p value*[d] | *0.0035* | *0.0005* | *<0.0001* | *0.0017* |
| ultrasound | 0 | | 43.05 ± 1.15a | 40.27 ± 0.65 | 42.21 ± 1.36a | 39.35 ± 1.33 |
| | 24 | | 37.99 ± 0.72b | 38.44 ± 0.24 | 38.59 ± 1.08bc | 38.30 ± 0.47 |
| | 36 | | 38.62 ± 0.94b | 40.46 ± 1.17 | 38.41 ± 1.46c | 39.84 ± 1.78 |
| | 48 | | 38.62 ± 0.70b | 40.75 ± 1.19 | 40.71 ± 1.17ab | 39.66 ± 1.66 |
| | | *F value* | *87.29* | *1.61* | *5.82* | *0.72* |
| | | *p value* | *<0.0001* | *0.2218* | *0.0085* | *0.5522* |

[a] Means were calculated from three replications with duplicate extractions measured for a total of six analyses at each incubation time. When large variation in AOA occurred between samples, triplicate analysis was conducted. Outliers were removed prior to statistical analysis. [b] For each size reduction method, means within the same stress treatment not followed by the same letter are significantly different at $\alpha < 0.05$ as determined by Fisher's least significant difference mean comparison test. [c] Untreated control peanuts had an AOA of 39.34 ± 0.87%. [d] The General Linear Model (PROC GLM) was used to determine the level of significance.

**Statistical Analysis.** Data were analyzed using SAS (*37*) statistical software, version 8. Regression analysis (PROC REG) was used to relate total phenolic compound concentrations as the dependent variable with peak area as the independent variable. The General Linear Model (PROC GLM) was used to detect significant differences in stress treatments for AOA, total phenolic compounds, and *trans*-resveratrol concentration. Fisher's least significant difference (LSD) test was used to compare means of stress treatments on AOA, total phenolic compounds, and *trans*-resveratrol concentration. Pearson's product correlation coefficients (PROC CORR) were calculated between mean AOA, total phenolic compounds, and *trans*-resveratrol concentration.

## RESULTS AND DISCUSSION

**AOA.** Incubation time was the only significant factor ($p < 0.01$) that affected AOA (data not shown). Size reduction and post size reduction stress did not significantly affect AOA. Means and their significant differences of AOA are shown in **Table 1**. AOA ranged from 37.99 to 43.50%, which is low compared to 96%, 87, and 99% in the methanolic extract of green tea and the antioxidants α-tocopherol and butylated hydroxylanisole(BHA), respectively (*38*).

AOA of untreated peanut kernels was 39.34 ± 0.87%, which is similar to results from stressed peanuts not exposed to post size reduction stress at 0 h of incubation, 39.93−42.30%, regardless of size reduction (**Table 1**). No literature comparison could be made due to the lack of documentation on peanut kernels. However, results are lower than those reported for peanut hulls, ranging from 94.8 to 93.9% in hulls at varied levels of maturity, 74−144 days after planting (*11*), and 99.7% in untreated hulls (*26*). Peanut hulls contain large concentrations of phenolic compounds (*11*, *12*), such as the three flavoniods 5,7-dihydroxychromone, eriodictyol, and luteolin (*39*), which function as antioxidants (*1*). Therefore, hulls should have higher levels of AOA than peanut kernels.

AOA of chopped, sliced, and whole peanuts not exposed to post size reduction stress did not change over incubation from 0 to 48 h. However, ground peanuts not exposed to post size reduction stress had a significant decrease ($\alpha < 0.05$) in AOA after 24 h of incubation and then remained stable until 48 h (**Table 1**).

All peanut samples exposed to UV light had a significant decrease ($\alpha < 0.05$) in AOA after 24 h of incubation (**Table 1**). At 36 h of incubation AOA remained stable except for chopped peanut samples, for which AOA increased to levels not significantly different from those at 0 h of incubation. After 48 h of incubation, AOA of sliced and whole peanuts increased to the initial level (0 h). AOA of ground and chopped peanuts after 36 h remained at the same level after 48 h of incubation. The decrease in AOA for ground peanuts after 24 h through 48 h in this study is contradictory to results found in the literature (*26*) for methanolic extracts of peanut hulls, where UV exposure did not significantly ($p < 0.05$) affect AOA.

Ultrasound exposure did not have any affect on AOA of chopped and whole peanuts. However, AOA in sliced peanuts exposed to ultrasound decreased after incubation for 24 and 36 h but increased to levels found at 0 h after 48 h of incubation (**Table 1**). AOA in ground peanuts did not significantly change after 24−48 h of incubation. No literature comparison can be made on the effect of ultrasound exposure to peanuts.

**Total Phenolic Compounds.** Time of incubation was the only significant factor ($p < 0.01$) affecting the total phenolic compound concentration in peanut samples (data not shown). Size reduction and postharvest size reduction stress did not significantly affect total phenolic compound concentration. Mean values and significant differences of total phenolic compounds are shown in **Table 2**.

Untreated peanut samples contained 1.35 ± 0.46 mg/g of total phenolic compounds. Imbibition of peanuts with water has no bearing on total phenolic compounds because in comparison to the means of the ground, chopped, and sliced peanuts at 0 h of incubation, levels were similar to that in raw kernels. Low concentration of total phenolic compounds in whole peanuts not exposed to post size reduction stress may be a result of method variability due to interfering compounds, described

**Table 2.** Mean Values for Total Phenolic Compound Concentration, Measured Spectrophotometrically at 726 nm, in Peanut Kernels Treated with Four Size Reduction Methods and Three Stresses and Then Incubated[a,b]

| post size reduction stress | incubation time (h) | | total phenolics[c] (mg/g) | | | |
|---|---|---|---|---|---|---|
| | | | ground | chopped | sliced | whole |
| none | 0 | | 1.07 ± 0.28 | 0.96 ± 0.43a | 1.19 ± 0.21 | 0.69 ± 0.51c |
| | 24 | | 1.24 ± 0.11 | 1.23 ± 0.36a | 0.92 ± 0.33 | 1.37 ± 0.19a |
| | 36 | | 0.95 ± 0.32 | 0.39 ± 0.21b | 1.13 ± 0.24 | 0.94 ± 0.35b |
| | 48 | | 1.34 ± 0.13 | 1.31 ± 0.28a | 0.87 ± 0.46 | 1.14 ± 0.23b |
| | | *F value*[d] | *0.97* | *8.48* | *0.59* | *19.22* |
| | | *p value*[d] | *0.4583* | *0.0099* | *0.6461* | *0.0018* |
| UV | 0 | | 1.08 ± 0.32 | 1.16 ± 0.22 | 2.06 ± 0.14 | 1.38 ± 0.16 |
| | 24 | | 1.01 ± 0.24 | 1.35 ± 0.13 | 1.32 ± 0.30 | 1.42 ± 0.15 |
| | 36 | | 1.18 ± 0.17 | 1.20 ± 0.27 | 1.41 ± 0.34 | 1.08 ± 0.61 |
| | 48 | | 1.51 ± 0.24 | 1.41 ± 0.20 | 1.41 ± 0.21 | 1.44 ± 0.18 |
| | | *F value* | *1.2* | *0.22* | *1.96* | *1.4* |
| | | *p value* | *0.4001* | *0.8758* | *0.2390* | *0.3191* |
| ultrasound | 0 | | 1.40 ± 0.16a | 0.72 ± 0.43 | 1.58 ± 0.11 | 1.45 ± 0.25 |
| | 24 | | 0.85 ± 0.34b | 1.38 ± 0.21 | 1.09 ± 0.19 | 1.31 ± 0.12 |
| | 36 | | 0.38 ± 0.26c | 1.09 ± 0.36 | 1.23 ± 0.32 | 1.36 ± 0.18 |
| | 48 | | 0.99 ± 0.16ab | 1.30 ± 0.19 | 1.19 ± 0.27 | 1.47 ± 0.21 |
| | | *F value* | *10.03* | *1.92* | *1.12* | *0.09* |
| | | *p value* | *0.0063* | *0.2150* | *0.4414* | *0.9627* |

[a] Means were calculated from three replications with duplicate extractions measured for a total of six analyses at each incubation time. When large variation in total phenolic compounds occurred between samples, triplicate analysis was conducted. Outliers were removed prior to statistical analysis. [b] For each size reduction method, means for a stress not followed by the same letter are significantly different at $\alpha < 0.05$ as determined by Fisher's least significant difference mean comparison test. [c] Untreated control peanuts had 1.35 ± 0.46 mg/g of total phenolic compounds. [d] The General Linear Model (PROC GLM) was used to determine the level of significance.

previously. Results show that the peanut kernels contain lower concentrations of total phenolic compounds than peanut hulls, 7.80 mg/g (*26*), which is expected because hulls are darker in color, which is associated with increased levels of phenolic compounds (*39*). Total phenolic compound concentration of ground and sliced peanuts not exposed to post size reduction stress was not affected over incubation for 0−48 h. Total phenolic compound concentration in chopped peanuts not receiving post size reduction stress significantly decreased ($\alpha < 0.05$) after 36 h of incubation and then increased to levels not significantly different from those at 0 and 24 h after 48 h (**Table 2**). Concentration of total phenolic compounds in whole peanuts not exposed to post size reduction stress significantly increased ($\alpha < 0.05$) to a maximum after 24 h of incubation, then decreased at 36 h, and remained constant after 48 h.

Exposure to UV light had no significant affect on total phenolic compounds regardless of size reduction or incubation time (**Table 2**). Results found in the study are contradictory to the literature (*26*), where a significant ($p < 0.05$) decrease in total phenolic compounds occurred in methanolic extracts of peanut hull powder after exposure to UV light. However, hull samples were exposed to UV light over a longer period of time, 6 days (*26*), compared to 10 min in the present study.

Ultrasound had no significant affect on total phenolic compound concentration in chopped, sliced, and whole peanuts (**Table 2**). Only ground peanuts exposed to ultrasound had a significant decrease ($\alpha < 0.05$) in total phenolic compounds after 24 and 36 h of incubation (**Table 2**). However, the concentration of total phenolic compounds in ground peanuts increased to levels not significantly different ($\alpha < 0.05$) from those at 0 and 24 h after 48 h (**Table 2**). A comparison with findings in the literature cannot be made due to the lack of reports on this effect.

A large variation occurred in total phenolic compound concentration in chopped and whole peanuts not exposed to post size reduction stress and ground peanuts exposed to ultrasound (**Table 2**). Because no trend in total phenolic compound concentration can be identified in these samples, variation could be attributed to method variability.

***trans*-Resveratrol.** Evaluation of the UV spectrum for the peak corresponding to *trans*-resveratrol in peanut extracts revealed an irregular shape compared to that in standards. However, previous analysis, in this laboratory, of peanut extracts by GC-MS confirmed that the compound identified was *trans*-resveratrol. The compound identified had an identical retention time and a similar fragmentation pattern compared with that of *trans*-resveratrol standard and published data (*34*, *40*).

Size reduction, post size reduction stress, incubation time, and the interaction between size reduction and incubation time were the factors that significantly ($p < 0.01$) affected *trans*-resveratrol concentration in peanut kernels (data not shown). Significant differences in mean values of *trans*-resveratrol concentrations are shown in **Table 3**. The highest amount of *trans*-resveratrol was 3.96 ± 0.96 $\mu$g/g, found in sliced peanuts exposed to ultrasound after 36 h of incubation.

Untreated peanut kernels contained 0.48 ± 0.08 $\mu$g/g of *trans*-resveratrol, where as approximately half of that amount (from 0.18 ± 0.21 to 0.25 ± 87 $\mu$g/g) was found in stressed peanuts not exposed to post size reduction stress at 0 h of incubation. Lower concentrations of *trans*-resveratrol in the stressed peanut kernels may be a result of soaking the peanuts in water for 16 h prior to size reduction treatment. However, results for *trans*-resveratrol concentration of untreated peanuts are similar to those reported in the literature (*34*), where peanuts in storage for up to 3 months contained 0.02−1.79 $\mu$g/g.

*trans*-Resveratrol concentration in all peanut samples not receiving post size reduction stress significantly increased ($\alpha < 0.05$) from 0 to 24 h of incubation (**Table 3**). Findings are consistent with those of Cooksey et al. (*19*), who observed resveratrol concentration to increase to a range of 4.3−23.8 $\mu$g/g in 11 different cultivars of peanuts sliced 2 mm thick and incubated for 24 h. In all size-reduced peanuts *trans*-resveratrol remained constant or decreased in concentration from 24 to 36 h. *trans*-Resveratrol concentration increased in whole kernels

**Table 3.** Mean Values for *trans*-Resveratrol Concentration, Measured by Reverse-Phase High-Performance Liquid Chromatography Analysis at 307 nm, in Peanut Kernels Treated with Four Size Reduction Methods and Three Stresses and Then Incubated[a,b]

| post size reduction stress | incubation time(h) | | *trans*-resveratrol concn[c] (μg/g) | | | |
|---|---|---|---|---|---|---|
| | | | ground | chopped | sliced | whole |
| none | 0 | | 0.18 ± 0.21b | 0.25 ± 0.87c | 0.22 ± 0.26c | 0.20 ± 0.08c |
| | 24 | | 0.65 ± 0.26a | 1.47 ± 0.67a | 1.43 ± 0.54ab | 0.96 ± 0.22b |
| | 36 | | 0.76 ± 0.49a | 0.89 ± 0.17b | 1.06 ± 0.90bc | 1.46 ± 0.51a |
| | 48 | | 0.49 ± 0.16a | 0.74 ± 0.91b | 2.15 ± 0.63a | 1.44 ± 0.43a |
| | | *F value*[d] | *4.93* | *31.33* | *8.31* | *47.97* |
| | | *p value*[d] | *0.0074* | *0.0001* | *0.0015* | *0.0001* |
| UV | 0 | | 0.17 ± 0.16c | 0.30 ± 0.12c | 0.33 ± 0.60c | 0.20 ± 0.15c |
| | 24 | | 0.49 ± 0.37b | 0.76 ± 0.35b | 1.31 ± 0.54b | 0.97 ± 0.27b |
| | 36 | | 0.86 ± 0.14a | 1.52 ± 0.46a | 1.70 ± 0.54b | 1.76 ± 0.72a |
| | 48 | | 0.67 ± 0.58ab | 1.64 ± 0.70a | 3.42 ± 0.95a | 0.99 ± 0.58b |
| | | *F value* | *18.98* | *31.27* | *63.13* | *12.74* |
| | | *p value* | *0.0001* | *0.0001* | *0.0001* | *0.0001* |
| ultrasound | 0 | | 0.26 ± 0.91c | 0.16 ± 0.13c | 0.20 ± 0.18b | 0.10 ± 0.17c |
| | 24 | | 0.75 ± 0.23a | 1.30 ± 0.36a | 2.54 ± 0.54a | 0.76 ± 0.15b |
| | 36 | | 0.69 ± 0.20ab | 0.93 ± 0.21b | 3.96 ± 0.96a | 1.47 ± 0.97a |
| | 48 | | 0.67 ± 0.28b | 0.80 ± 0.37b | 2.68 ± 0.98a | 1.60 ± 0.42a |
| | | *F value* | *78.52* | *38.75* | *8.75* | *67.53* |
| | | *p value* | *0.0001* | *0.0001* | *0.0007* | *0.0001* |

[a] Means were calculated from three replications with triplicate extractions measured for a total of nine analyses at each incubation time. Outliers were removed prior to statistical analysis. [b] For each size reduction method, means for a stress not followed by the same letter are significantly different at α < 0.05 as determined by Fisher's least significant difference mean comparison test. [c] Untreated control peanuts had 0.48 ± 0.08 mg/g of *trans*-resveratrol. [d] The General Linear Model (PROC GLM) was used to determine the level of significance.

from 0 to 48 h of incubation. After 48 h of incubation, *trans*-resveratrol concentration increased from levels at 0 h of incubation. On the other hand, from 36 to 48 h of incubation, the concentration remained equal or decreased except for sliced kernels, which increased in resveratrol content. Peanuts that were exposed to size reduction increased in *trans*-resveratrol concentration as particle size increased from ground to sliced (**Figure 1**). Slicing at 48 h of incubation produced the largest amount of *trans*-resveratrol (2.15 ± 0.63 μg/g) in peanuts not exposed to post size reduction stress.

Exposure of UV light to peanut kernels caused a significant increase (α < 0.05) in *trans*-resveratrol concentration as the incubation period increased from 0 to 36 h (**Table 3**). *trans*-Resveratrol concentration increased from 0 to 24 h of incubation, and then from 24 to 36 h the concentration increased even further. Results from this paper are consistent with other studies in which UV light exposure to grape leaves (*18*) and grapes (*21*) increased the synthesis of resveratrol after incubation for 24 and 23 h, respectively. *trans*-Resveratrol remained level or increased significantly (α < 0.05) from 36 to 48 h in size-reduced kernels, but not in whole kernels (**Table 3**). As particle size increased *trans*-resveratrol concentration increased, but not in whole kernels. The highest *trans*-resveratrol concentration (3.42 ± 0.95 μg/g) in peanuts exposed to UV light was found in sliced peanuts after 48 h of incubation. Creasy and Coffee (*21*) also found that UV light exposure to grapes and incubation for 48 h increased *trans*-resveratrol concentration. Fritzemeier and Kindl (*41*) found that leaves of Vitaceae exposed to UV light stimulated the production of stilbene synthase and catalyzed the reaction of 4-hydroxycinnamoyl-CoA and malonyl-CoA to produce resveratrol.

Ultrasound exposure caused a significant increase (α < 0.05) in *trans*-resveratrol concentration in all samples after 24 h of incubation (**Table 3**). From 24 to 36 h of incubation *trans*-resveratrol concentration decreased or was not significantly different (**Table 3**). *trans*-Resveratrol concentration in peanuts incubated for 36−48 h did not change (**Table 3**). In size-reduced peanuts *trans*-resveratrol concentration increased as particle size increased from ground to sliced. The highest *trans*-resveratrol concentration in peanuts exposed to ultrasound occurred in sliced kernels at 24, 36, or 48 h of incubation. Results from this study are similar to findings for saponin production in *Panax ginseng* cells, where a sharp increase occurred 2−3 days after ultrasound exposure and remained higher than the control through most of the remaining culture period (*24*). Lin et al. (*24*) attributed the increase in saponin to acoustic cavitation, which is the develop-

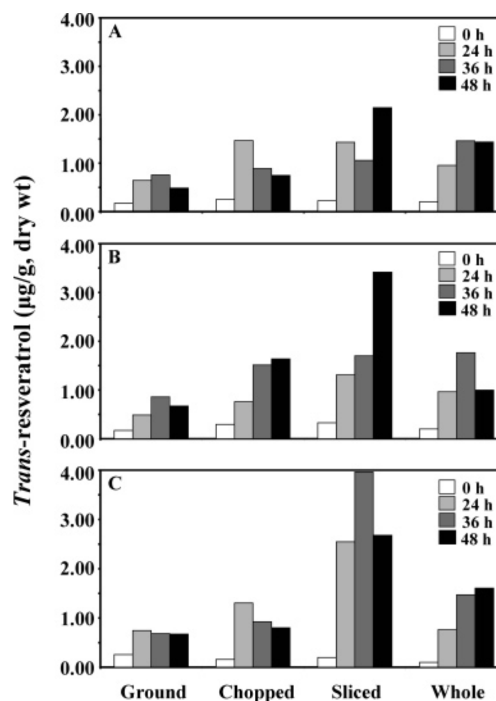


**Figure 1.** Mean values for *trans*-resveratrol concentration in peanut kernels whole or exposed or not to postharvest stress by size reduction, grinding, chopping, or slicing and (**A**) without post size reduction stress or with post size reduction stress by exposure to (**B**) ultrasound or (**C**) UV light over incubation times from 0 to 48 h.

**Table 4.** Pearson Product Correlations Coefficients (*r*) among *trans*-Resveratrol Concentration, Total Phenolic Compounds, and Antioxidant Activity in Chopped, Ground, Sliced, and Whole Peanut Kernels Exposed to UV Light and Ultrasound and Incubated for 0–48 h at 25 °C

| | *trans*-resveratrol (μg/g) | | total phenolics (mg/g) | | antioxidant activity (%) | |
|---|---|---|---|---|---|---|
| | *r* | *p* | *r* | *p* | *r* | *p* |
| *trans*-resveratrol (μg/g) | | | NS[a] | | 0.2086 | 0.0023 |
| total phenolics (mg/g) | NS | | | | NS | |
| antioxidant activity (%) | 0.2086 | 0.0023 | NS | | | |

[a] Not significant.

ment of gas or vapor bubbles in the tissue and the development of hydrogen peroxide, which is the early event of plant defense response to pathogenic attack and also acts as a secondary messenger to signal subsequent defense reactions in plants.

**Relationship between AOA, Total Phenolic Compounds, and *trans*-Resveratrol Concentration.** Significant Pearson's product correlation coefficients (*r*) obtained between AOA, total phenolic compounds, and *trans*-resveratrol concentration are shown in **Table 4**. Significant *r* was found between *trans*-resveratrol concentration and AOA; however, the magnitude of correlation was low. Because *trans*-resveratrol has been shown to provide antioxidant activity (*1*), correlation between the two factors is expected. None of the other combinations tested had significant correlations. These findings are similar to those of Kahkonen (*42*), who observed no significant correlations between the total phenolic content and AOA of plant extracts, berries, fruits, vegetables, cereals, herbs, plant sprouts, seeds, and tree leaves and bark. However, these results are contradictory to findings by Emmons (*31*), who observed total phenolic content to be significantly correlated with AOA in oat fractions. Emmons et al. (*31*) findings suggest that phenolic compounds in the oat might be responsible for a large proportion of the AOA (*31*).

In conclusion, application of postharvest stress by size reduction and post size reduction stress by UV light or ultrasound exposure did not increase AOA or total phenolic compounds over incubation time; however, these stresses were effective in increasing *trans*-resveratrol concentration. Slicing produced the highest increase of *trans*-resveratrol synthesis compared to other size reduction methods and post size reduction stress applied at each incubation time, except for whole peanuts exposed to UV or no additional stress and incubated for 36 h. *trans*-Resveratrol concentration in samples exposed to UV light, ultrasound, or no further stress showed similar increases at each incubation time for the individual size reduction methods, except for sliced peanuts exposed to ultrasound.

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