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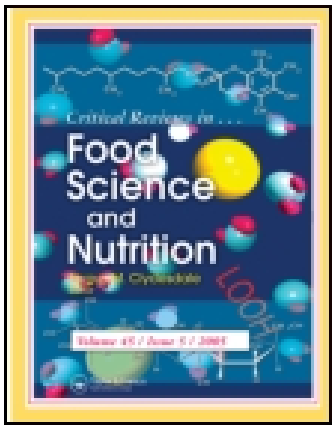

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Resveratrol in Peanuts

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Resveratrol in Peanuts

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Peanuts are important dietary food source of resveratrol with potent antioxidant properties implicated in reducing risk of cancer, cardiovascular and Alzheimer's disease, and delaying aging. Resveratrol is a naturally occurring stilbene phytoalexin phenolic compound produced in response to a variety of biotic and abiotic stresses. This paper is a review of trans-resveratrol and related stilbenes from peanuts—their chemical structures, mechanisms for their biosynthesis, and concentrations in comparison with other major food sources. It will also discuss trans-resveratrol's absorption, bioavailability, and major health benefits; processes to enhance their biosynthesis in peanuts by biotic and abiotic stresses; process optimization for enhanced levels in peanuts and their potential food applications; and methods used for its extraction and analysis.

Keywords Resveratrol, piceid, stilbenes, UV, ultrasound, biotic and abiotic stresses optimization

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INTRODUCTION

Peanut, *Arachis hypogaea,* is an important food crop in the United States. In 2008, total consumption was 6.5 pounds per capita (United States Department of Agriculture [USDA], 2008a) with over half consumed as peanut butter. Peanut butter had the highest per capita consumption of 3.3 pounds followed by 1.4 pounds in snack peanuts, 1.2 pounds in peanut containing candies, and 0.5 pounds in cleaned-in-shelled peanuts (USDA, 2008a). Stocks of shelled peanuts in commercial storage totaled 411 million pounds on August 31, 2008, of which 374 million pounds were edible grades and 37.3 million pounds were oil stocks (USDA, 2008b). In August 2008 alone, commercial processors utilized 96.9 million pounds as peanut butter, 30.1 million pounds as peanut candy, 33.7 million pounds as snack peanuts, and 26.3 million pounds as oil (USDA, 2008b).

Peanut contains bioactive compounds with health benefits, such as stilbenes, flavonoids, phenolic acids, and phytosterols (Table 1). These bioactive compounds may exert their effects by functioning as antioxidants, activating liver detoxification enzymes, blocking activity of bacterial or viral toxins, inhibiting cholesterol absorption, decreasing platelet aggregation, or destroying gastrointestinal bacteria (Pennington, 2002).

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The stilbene, resveratrol is a widely studied bioactive compound that has received much interest over the last 10 years due to its benefits to human health. It was first identified in 1940 in the roots of white hellebore, *Veratrum grandiflorum* O. Loes (Aggarwal et al., 2004) and later in 1963 as a component of *Polyganum cuspidatum* roots used in Japanese and Chinese folk medicine for treatment of disorders affecting liver, skin, heart and circulation, and lipid metabolism (Soleas et al., 1997). In 1976, resveratrol was synthesized in grapevine leaves after fungal infection and UV light exposure (Langcake and Pryce, 1976). However, increased interest by researchers in resveratrol, from the perspective of mammalian biochemistry or clinical science, began in 1992 (Soleas et al., 1997) after Siemman and Creasy (1992) found resveratrol in red wines.

The primary food and beverage sources of resveratrol in the human diet are peanuts, peanut butters, grapes, and red wines (King et al., 2006). The occurrence of resveratrol in edible peanuts was first reported by Sanders and McMichael in 1997 at the American Chemical Society meeting in Las Vegas, Nevada, which was followed by press releases and wide media coverage. Red wine, regularly present in the French diet, is associated with "French paradox," a phenomenon where the French had strikingly lower mortality from coronary diseases, only one-third of the average, despite large intake of high saturated fat diet, similar to that in Western countries such as in the United States (Stanley and Mazier, 1999). Resveratrol and other polyphenolic compounds in red wines were believed to be associated with this paradox.

There is evidence that resveratrol may decrease the risk of cardiovascular diseases (CVDs) (Frankel et al., 1993; Pace-Asciak et al., 1995; Boue et al., 2009). Resveratrol has also shown to inhibit initiation, promotion, and progression of

Table 1 Bioactive compounds in peanut plant materials and products

Compound	Peanut plant materials and products	Reference
Stilbenes:		
trans-Resveratrol	Peanut kernels, raw and roasted	Sobolev and Cole, 1999; Sanders et al., 2000; Rudolf and Resurreccion, 2005; Potrebko and Resurreccion, 2009; Sales and Resurreccion, 2009
	Peanut skins	Nepote et al., 2004; Francisco and Resurreccion, 2009
	Peanut leaves	Chung et al., 2003
	Peanut roots	Chen et al., 2002
	Peanut sprouts	Wang et al., 2005
	Peanut callus	Ku et al., 2005
	Peanut hairy roots	Medina-Bolivar et al., 2007
	Peanut butter	Ibern-Gomez et al., 2000
trans-Piceid	Peanut kernels, roasted and raw	Potrebko and Resurreccion, 2009; Sales and Resurreccion, 2009
	Peanut butter	Ibern-Gomez et al., 2000
Piceatannol	Peanut callus	Ku et al., 2005
Pterostilbene	Peanut hairy roots	Medina-Bolivar et al., 2007
Phenolic acids:		
ρ-Coumaric acid	Peanut kernels	Talcott et al., 2005
	Peanut skins	Yu et al., 2005; Francisco and Resurreccion, 2009
Hydroxybenzoic acid ester	Peanut kernels	Talcott et al., 2005
Ethyl protocatechuate	Peanut skins	Huang et al., 2003
Protocatechuic acid	Peanut skins	Francisco and Resurreccion, 2009
Chlorogenic acid	Peanut skins	Yu et al., 2005
Caffeic acid	Peanut skins	Yu et al., 2005; Francisco and Resurreccion, 2009
Ferulic acid	Peanut skins	Yu et al., 2005; Francisco and Resurreccion, 2009
Flavonoids:		
Dihydroquercetin	Peanut kernels	Pratt and Miller, 1984
Biochanin	Peanut kernels	Chukwumah et al., 2005
Genistein	Peanut kernels	Chukwumah et al., 2005
Quercetin	Peanut skins	Francisco and Resurreccion, 2009
Procyanidins (monomers, dimmers, trimers, tetramers, pentamers, hexamers, heptamers, octamers)	Peanut skins	Lazarus et al., 1999
Epicatechin-(2β O 7,4β 6)-[epicatechin-(4β 8)-catechin epicatechin-(2β O 7,4β 8)-[epicatechin-(4α 8)-catechin procyanidin B2 procyanidin B3 procyanidin B4	Peanut skins	Lou et al., 2004
Epigallocatechin epicatechin catechin gallate epicatechin gallate	Peanut skins	Yu et al., 2005;
Procyanidin dimer A1 [epicatechin-4β-8, 2β-O7)-catechin] procyanidin trimer A	Peanut skins	Verstatraeten et al., 2005
Procyanidin monomers A-type procyanidin dimers B-type procyanidin dimers A-type procyanidin trimers B-type procyanidin dimers A-type procyanidin tetramers B-type procyanidin tetramers	Peanut skins	Yu et al., 2006
Luteolin	Peanut hulls, mature	Daigle et al., 1988; Duh and Yen, 1995
Eriodictyol	Peanut hulls, immature	Daigle et al., 1988
Formononetin daidzein medicarpin	Peanut leaves	Subba Rao et al., 1996
Phytosterols:		
Beta-sitosterol campesterol stigmasterol	Peanut kernels peanut oil	Awad et al., 2000

cancer (Jang et al., 1997). More recently, resveratrol was implicated in reducing risk of Alzheimer's disease (AD) (Marambaud et al., 2005; Reviere et al., 2007) and in delaying aging (De la Lastra and Villegas, 2005; Baur et al., 2006).

Resveratrol is a phytoalexin, a group of low molecular weight secondary metabolites produced by a wide variety of plants (Aggarwal et al., 2004) in response to biotic and abiotic stresses, which enhance their synthesis (Boue et al., 2009). Biotic stresses result from natural infection or inoculation of the plant material with microorganisms such as molds (Keen, 1975) and yeast (Chung et al., 2003), whereas abiotic stresses include physical methods like wounding through slicing (Aguamah et al., 1981), chopping or grinding (Rudolf and Resurreccion, 2005), exposure to UV (Langcake and Pryce, 1977; Rudolf and

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Resurreccion, 2005), ultrasound (Lin et al., 2001; Rudolf and Resurreccion, 2005), ozone (Grimmig et al., 1997), heat (Yu et al., 2004) or far-infrared radiation (Lee et al., 2006), and treatment with chemicals such as cupric acid (Hanawa et al., 1992), aluminum chloride (Adrian et al., 1996), aluminum sulfate (Cantos et al., 2000), salicylic acid (Subba Rao et al., 1996), jasmonic acid (Chung et al., 2003), and ethylene (Chung et al., 2003).

This paper is a review of *trans*-resveratrol and related stilbenes from peanuts—their chemical structures, mechanisms for their biosynthesis, and concentrations in comparison with other major food sources. This review also discussed *trans*-resveratrol's major health benefits, absorption, and metabolism, processes to enhance their biosynthesis in peanuts and potential food applications, and methods used for its extraction and analysis.

TRANS-*RESVERATROL AND RELATED STILBENES*

Chemical Structure

The *trans*-resveratrol molecule consists of two phenolic rings linked by a styrene double bond to generate 3,5,4′-trihydroxystilbene (Fig. 1). The double bond facilitates *trans* and *cis* isomeric forms of resveratrol, with the *trans* isomer as stearically the more stable form (Trela and Waterhouse, 1996) and therefore occurs predominantly in nature and more biologically active (King et al., 2006). *trans*-Resveratrol is commercially available as an off-white powder, when extracted using methanol, with a molecular weight of 228 and a melting point of 253–255°C (Aggarwal et al., 2004).

Trela and Waterhouse (1996) investigated the isomeric molar absorptivities and stability of *trans*-resveratrol. They found that standard solutions of *trans*-resveratrol in 100% ethanol, in sealed, light-proof containers were stable for three months when protected from light, except in high-pH buffers. The *trans* form was converted to a maximum of 90.6% *cis* isomer after exposure to UV irradiation at 366 nm for 100 minutes, and only up to ≤63% *cis*-resveratrol at lower wavelength of 254 nm even after 10 hours. When exposed to fluorescent light, *trans*-resveratrol standard solutions were isomerized to about 80% *cis* form over 30 days. *cis*-Resveratrol was extremely light sensitive, which made it difficult to purify, remains stable in the dark only near neutral pH, and isomerized to *trans* form at low pH.

trans-Resveratrol is a better free radical scavenger compared with vitamins E or C but has similar activity as the flavonoids, epicatechin, and quercetin (Regev-Shoshani et al., 2003). Their antioxidant activities are believed to be due to their amphiphatic character with both hydrophilic and hydrophobic sites, which allow more effective oxidative protection for cellular and subcellular membrane components from oxidation compared with vitamin E (Sun et al., 1997).

The 4′-hydroxyl group of resveratrol was more reactive than the 3- and 5-hydroxyl groups (Fig. 1) because of resonance effects (Aggarwal et al., 2004). In terms of its antioxidant activity, the 4′-hydroxyl group is the most important functional group

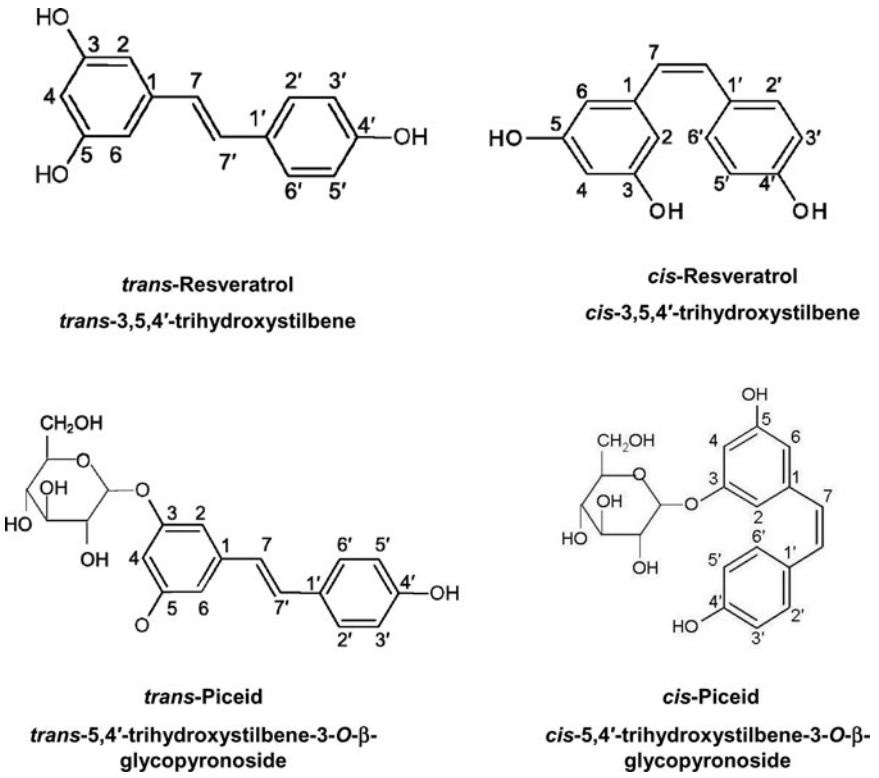

Figure 1 Structures of *trans*- and *cis*-resveratrol and piceid.

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in resveratrol as it is most reactive in scavenging free radicals compared with the 3′-hydroxyl group (Regev-Shoshani et al., 2003).

Synthetic Preparations of Resveratrol

Six analogues of resveratrol were synthetically prepared by Matsuoka et al. (2002). Those containing 4′-hydroxyl group were found genotoxic, based on their positive reactions to three genotoxicity tests including chromosomal aberration, micronucleus, and sister chromatid exchange tests in a Chinese hamster cell line and are therefore considered "unbeneficial" to humans (Matsuoka et al., 2002). Among the six analogues of synthetic resveratrol, both 3,4′-dihydroxy-*trans*-stilbene and 4′-hydroxy-*trans*-stilbene showed clear positive genotoxicity responses in a concentration-dependent manner in all three tests, but the 4′-hydroxyl analogue was the most genotoxic. The other four analogues without 4′-hydroxy group were not genotoxic. These findings may suggest that naturally produced resveratrol is safer and beneficial to humans than some synthetic forms that may be toxic.

Derivatives of Resveratrol

Resveratrol is the parent compound of a family of molecules, including glucosides and polymers existing in *cis* and *trans* configurations in a narrow range of spermatophytes or seed bearing plants (Soleas et al., 1997). A few naturally occurring derivatives of *trans*-resveratrol, such as piceid and piceatannol, have been identified, in which one or more of the hydroxyl groups are substituted with sugars, methyl, methoxy, or other residues (Soleas et al., 1997) with antioxidant and biological activities as well as water solubility and bioavailability different from the parent aglycon (Regev-Shoshani et al., 2003).

Piceid or 5,4′-dihydroxystilbene-3-O-β-D-glucopyranoside (Rogev-Shoshani et al., 2003) is the bound glucoside of resveratrol in *cis* and *trans* configurations (Fig. 1) found in peanuts (Ibern-Gomez et al., 2000), grapes, and wines (Lamuela-Raventos et al., 1995; Abert-Vian et al., 2005). In piceid, a glucose moiety replaces the hydrogen of the OH-group at the *meta*-position of resveratrol. Piceid has received as much attention as resveratrol because its concentration is usually significantly higher than resveratrol in grape products (Waterhouse and Lamuella-Raventos, 1994). The relative distribution between piceid and resveratrol in wines is dependent on a number of factors such as fermentation and ecological conditions such as region of growth (Moreno-Labanda et al., 2004).

Piceatannol, 3,4,3′,5′-tetrahydroxy-*trans*-stilbene, is a resveratrol derivative obtained from peanut roots, stems, and leaves (Lin et al., 2007) and found in high amounts in UV-irradiated peanut callus or stems (Ku et al., 2005; Lin et al., 2007). Piceatannol differs from resveratrol by having an additional hydroxyl group in one of the aromatic rings (Fig. 2).

Peanuts that were imbibed, sliced, and then allowed for natural microflora or inoculated microorganisms to grow produced several resveratrol derivatives. Three resveratrol derivatives, namely, arachidin I or *trans*-4-(3-methyl-but-1-enyl)-3,5,3′,4′-tetrahydroxystilbene; 4-(3-methyl-but-1-enyl)-3,5,4′-trihydroxy stilbene (4-isopentenylresveratrol); and 4-(3-methyl-but-1-enyl)-3,5,4′-trihydroxy stilbene were synthesized

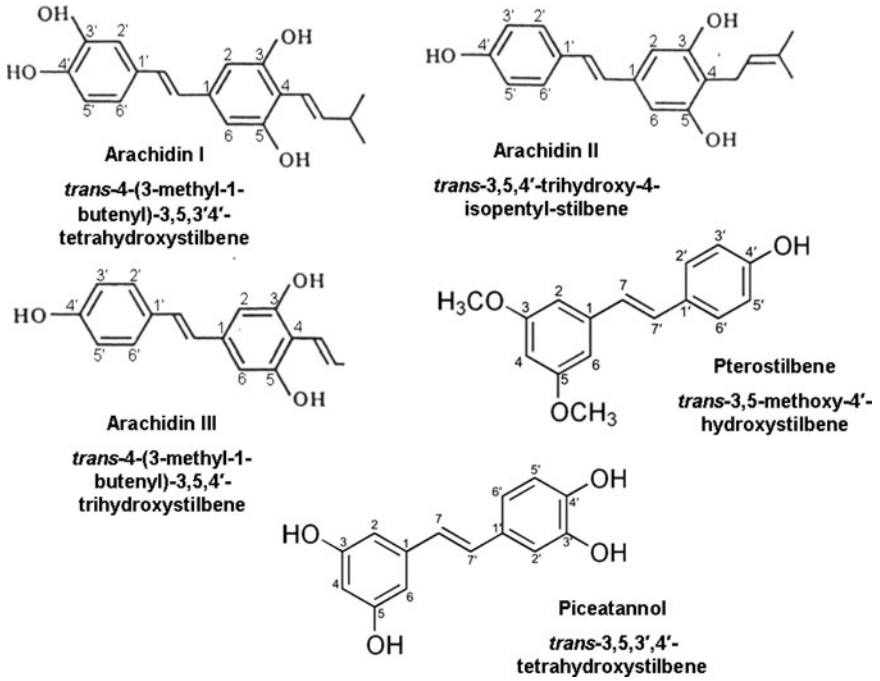

Figure 2 Structures of resveratrol derivatives found in peanuts.

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from sliced fully imbibed peanut kernels when natural microfloras were allowed to grow on them (Aguamah et al., 1981). Three stilbenes, arachidin I, arachidin II, and arachidin III or *trans*-4-(3-methyl-1-butenyl)-3,5,4′-trihydroxystilbene were also isolated from fully imbibed and sliced peanut kernels incubated for 0 to 144 hours at 25 and 37°C (Wotton and Strange, 1985). Arachidin II (Arora and Strange, 1991), the *cis* and *trans* isomers of 3,5,4′-trihydroxy-4-isopentenylstilbene were identified by Keen and Ingham (1976) from germinating American peanut seeds challenged with native microflora. Arachidin IV or 3-isopentadienyl-4,3′,5′-trihydroxystilbene was quantified from fully imbibed and sliced peanut kernels incubated for 24 and 48 hours at 25°C (Cooksey et al., 1988). Resveratrol derivatives, *trans*-3-isopentadienyl-4,3′,5′-trihydroxystilbene, and *trans*-4-(3-methyl-butyl-enyl)-3,5′,4′-trihydroxystilbene or *trans*-arachidin-III were isolated from fully imbibed and sliced peanut kernels inoculated with *Aspergillus flavus* (*A. flavus*) and *Aspergillus parasiticus* (Sobolev et al., 1995).

Biosynthesis of Resveratrol

Resveratrol is produced as a defense response to biotic and abiotic stresses. It is synthesized by plants from the condensation of one molecule of ρ-coumaroyl CoA and three molecules of malonyl CoA by the action of the enzyme, stilbene synthase (Fig. 3; Soleas et al., 1997). The ρ-coumaroyl CoA is derived from phenylalanine, an amino acid synthesized in plants from sugars via the shikimate pathway while malonyl CoA is derived from the elongation of acetyl CoA. Phenylalanine is converted to cinnamic acid by losing its amino group through oxidative deamination, catalyzed by enzyme phenylalanine ammonia lyase. Cinnamic acid is then enzymatically hydroxylated to ρ-coumaric acid by cinnamate-4-hydroxylase generating ρ-coumaroyl CoA from the free coenzyme by CoA ligase. For each molecule of *trans*-resveratrol synthesized, four molecules of CO_2 are released.

Biosynthesis of resveratrol specifically requires stilbene or resveratrol synthase (Aggarwal et al., 2004). Resveratrol synthase is normally unexpressed and inducible only by a range of biotic, such as infection, and abiotic stresses, which include UV irradiation (Soleas et al., 1997). Soleas et al. (1997) reported that after exposure of cultured peanut cells to sterilized insoluble fungal cell walls, increase in stilbene synthase was detected after 40 minutes and was 30-fold above the baseline after two hours. The first increase in translatable mRNA for stilbene synthase occurred within 20 minutes after application of the fungal cells. A stilbene synthase purified from peanut cell cultures was at least 10 times more active in producing resveratrol than other stilbenes (Soleas et al., 1997).

When the gene from peanuts that codes for stilbene synthase was transferred to tobacco plant together with a chimeric kanamycin-resistant gene, rapid expression of stilbene synthase with the accumulation of *trans*-resveratrol in tobacco cells occurred on exposure to UV (Hain et al., 1990). Stilbene synthase

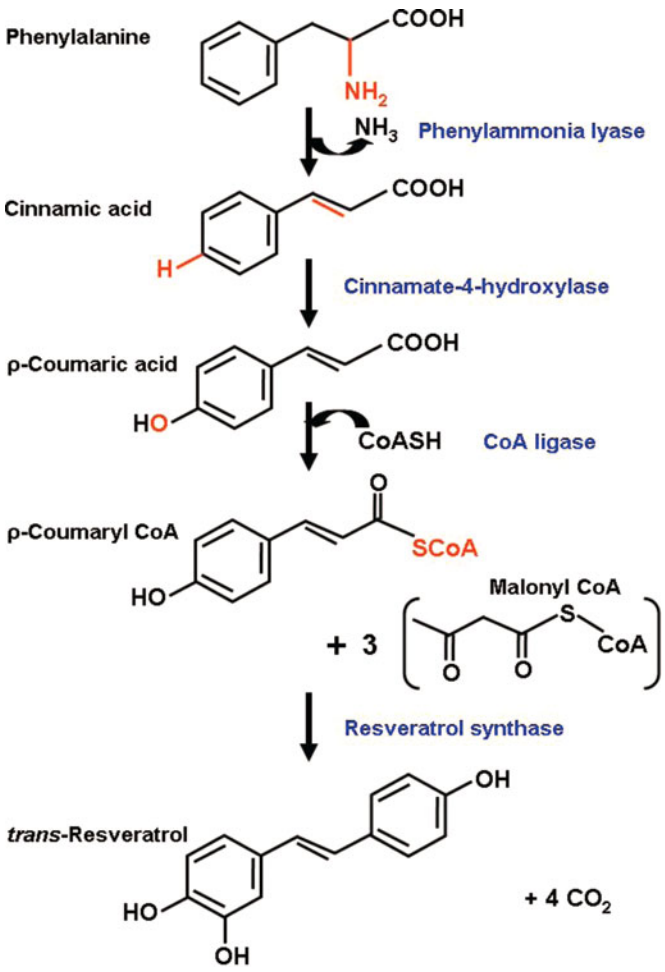

Figure 3 Biosynthesis of *trans*-resveratrol from one molecule of 4-coumaryl CoA and three molecules of malonyl CoA. (Color figure available online).

specific mRNA was detected 10 minutes after UV irradiation reaching a maximum between two and eight hours and ceasing after 24 hours (Hain et al., 1990).

Chung et al. (2003) investigated the regulation of resveratrol synthesis in peanut plants grown in the glasshouse and in the field. They found that resveratrol and RS mRNA were relatively abundant in roots and shells of peanut plants grown up to mid-maturity (40 days after flowering) compared with seed coats and seeds, indicating tissue-specific regulation of resveratrol synthesis. The levels of resveratrol in leaves, pods, and roots were 2.05-, 1.34-, and 1.19-μg/g fresh weight, respectively. In the pod, the amounts of resveratrol were 2.60-, 0.06-, and 0.05-μg/g fresh weight in the shell, developing seed, and seed coat, respectively. Mature peanut seeds had lower *trans*-resveratrol concentrations of 0.03–0.14 μg/g seed. A correlation existed between resveratrol and resveratrol synthase mRNA accumulation, indicating that resveratrol synthesis was regulated through the transcriptional control of resveratrol synthase genes. Chung et al. (2003) concluded that resveratrol was accumulated by elicitors and abiotic stresses such as wounding and UV light through the expression of resveratrol synthase genes in peanut leaves and roots. Peanut shells contain conjugated resveratrol at about half the amount of the free form mostly present in

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other peanut tissues. Resveratrol synthesis by the expression of resveratrol synthase in peanut tissues provides resistance to pathogen infection through direct antifungal effect of resveratrol and the reinforcement of shells by the synthesis of cell wall materials, with resveratrol as an intermediate.

The biosynthesis of *trans*-resveratrol was observed in sliced peanuts after UV exposure, increasing by sixfold to 3.42 μg/g from 0.48 μg/g in untreated controls (Rudolf and Resurreccion, 2005). Similarly, *trans*-resveratrol synthesis occurred in table grapes irradiated with 510-W UV lamp for 30 seconds at a distance of 40 cm followed by three days of incubation, which resulted in 11-fold increase (Cantos et al., 2001). In peanut leaves, a 200-fold *trans*-resveratrol increase was observed after exposure to UV light at 1.35 μE (m^2/second) for two hours, which was much higher than 20-fold increase in response to paraquat and 2–9-fold increase due to wounding (Chung et al., 2003).

Arora and Strange (1991) investigated the phytoalexins synthesis in peanuts during pod development from stage 1 (youngest developing pods) to stage 8 (mature) and reported that the capacity of peanuts to synthesize was reduced as they develop from stage 1 to 4 but increased thereafter from stage 5 (cotyledons are clearly seen) to stage 8. When peanuts at stages 6 and 8 were divided into pod, testa, and cotyledons, and mature pods and testas lost their abilities to synthesize phytoalexins, whereas mature cotyledons increased synthesis.

Concentrations in Peanuts

Resveratrol, piceid, and other stilbenes are naturally present in edible and inedible parts of peanut plant (Table 2). Being a source of these bioactive compounds, attention had been focused on the role of peanuts as phytochemicals with human health benefits and led to investigations on methods that will efficiently extract and quantify them in peanuts and peanut plant materials. This also led researchers to develop processes utilizing various parts of the peanut plant to elevate concentrations of resveratrol and its derivatives.

Edible Peanuts

Raw Peanut Kernels

Phytoalexin concentrations in peanuts were influenced by cultivar, length of storage, and viability of peanuts (Arora and Strange, 1991); therefore, variability in the amounts of *trans*-resveratrol in peanuts are expected as shown in Table 2. Earlier reports by Sanders et al. (2000) indicated that *trans*-resveratrol concentrations in raw peanuts ranged from 0.02 to 0.31 μg/g in 14 of 15 cultivars of three market types of raw peanuts. The *trans*-resveratrol content of 1.79 μg/g was way above range and reported for only one, a small white Spanish cultivar sample (Sanders et al., 2000) and therefore excluded from the range above. Later as equipment and procedures for extraction and analysis of these stilbenes became more sophisticated, a higher range of 0.09- to 0.30-μg/g *trans*-resveratrol was obtained in raw peanuts (Lee et al., 2004). Even higher concentrations were later reported from the analyses of six varieties and four market samples of raw peanuts ranging from 0.03–1.92 μg/g, with an average of 0.84 μg/g (Tokusoglu et al., 2005). Peanut cultivars PI 337394F and J11 reported to have resistance to seed colonization by *A. flavus* and aflatoxin contamination, accumulated more than three times as much arachidin IV as the susceptible cultivars Gangapuri and TMV2 (Arora and Strange, 1991). The observed differences in *trans*-resveratrol and phytoalexins concentrations may also be attributed to the maturity and quality of the peanuts. Peanut seed size is related to maturity where immature small seeds were found to have greater capacity for phytoalexin production than mature larger seeds (Sobolev and Cole, 1999). Discolored yellow split peanuts contain higher amounts of *trans*-resveratrol of as much as 7.09 μg/g compared with 0.23 μg/g in nondiscolored splits (Sobolev and Cole, 1999).

Conflicting effects of storage on the concentrations of *trans*-resveratrol in peanuts were reported in the literature. Peanuts from 15 cultivars and three market types, which had been cold stored for up to three years had higher *trans*-resveratrol of 0.02 – 1.79 μg/g compared with raw unstored peanuts containing 0.03 to 0.15 μg/g (Sanders et al., 2000). This finding contradicts the earlier report that peanuts stored at 15°C for nine months drastically reduced their ability to synthesize phytoalexins (Arora and Strange, 1991). Similarly, Potrebko and Resurreccion (2009) found that peanuts stored for 13 months and then roasted, had lower *trans*-resveratrol concentration of 0.016 μg/g compared with 0.03 μg/g in peanuts stored for only six months. Nonviable sliced peanut seeds accumulated lower total phytoalexins of 0.716 μM/g compared with 5.29 μM/g in viable sliced seeds (Arora and Strange, 1991).

Roasted Peanuts

Trace amounts to 0.13-μg/g *trans*-resveratrol were found in roasted peanuts (Lee et al., 2004), which were within the values of 0.10 to 0.80 μg/g earlier reported by Sobolev and Cole (1999). The concentrations of *trans*-resveratrol in roasted compared with raw Virginia and Spanish peanuts decreased by about half indicating that roasting decreased the concentrations of *trans*-resveratrol in peanut (Sanders et al., 2000). This contradicts the findings of Rudolf (2003) who reported that roasting significantly increased *trans*-resveratrol in ultrasound stressed peanuts from 2.73 to 6.8 μg/g *trans*-resveratrol before and after drying and roasting, respectively. Roasting considerably increased the concentrations of coumaric acid, a phenolic acid, from 28.3 and 23.2 μg/g in raw normal and high oleic peanuts to 78.5 and 62.7 μg/g in roasted samples, respectively (Talcott et al., 2005).

Peanut Butter

trans-Resveratrol contents in commercial peanut butters ranged from 0.265 to 0.671 μg/g in blended type (stabilizer

Table 2 Concentrations of *trans*-resveratrol and other stilbenes found in edible and inedible peanut and peanut plant materials

Source	Compound		Reference
	Name	Concentration (μg/g)	
A. Edible peanuts			
1. Raw peanut kernels			
Runners, six cultivars, cold stored for ~three years	*trans*-Resveratrol	0.022–0.069	Sanders et al., 2000
Spanish, five cultivars, cold stored for ~three years	*trans*-Resveratrol	0.023–1.792	Sanders et al., 2000
Virginia, four cultivars, cold stored for ~three years	*trans*-Resveratrol	0.048–0.306	Sanders et al., 2000
Spanish, seven cultivars	*trans*-Resveratrol	0.09–0.30	Lee et al., 2004
Virginia, eight cultivars	*trans*-Resveratrol	0.1–0.25	Lee et al., 2004
Six varieties and four market samples	*trans*-Resveratrol	0.03–1.92	Tokusoglu et al., 2005
Runners, Georgia Green	*trans*-Piceid	0.03	Sales and Resurreccion, 2009
Runners, Georgia Green	*trans*-Piceid	0.07	Sales and Resurreccion, 2009
2. Peanut products			
2.1 Roasted peanut kernels			
Commercial brands ($n = 8$)	*trans*-Resveratrol	0.18 –0.80	Sobolev and Cole, 1999
Commercial brands in Korea	*trans*-Resveratrol	trace amounts to 0.13	Lee et al., 2004
2.2 Peanut butter			
Commercial brands ($n = 15$)	*trans*-Resveratrol	0.148–0.504	Sobolev and Cole, 1999
Commercial brands ($n = 6$)	*trans*-Resveratrol	0.27–0.70	Lee et al., 2004
Commercial brands, blended ($n = 7$)	*trans*-Resveratrol	0.265–0.671	Ibern-Gomez et al., 2000
Commercial brands 100% natural ($n = 7$)	*trans*-Resveratrol	0.534–0.753	Ibern-Gomez et al., 2000
Commercial brands, blended ($n = 7$)	*trans*-Piceid	0.067–0.187	Ibern-Gomez et al., 2000
Commercial brands 100% natural ($n = 7$)	*trans*-Piceid	0.073–0.225	Ibern-Gomez et al., 2000
2.3 Boiled peanuts			
Boiled peanuts	*trans*-Resveratrol	0.02–1.79	Vaydorf, 2005
Boiled peanuts, canned, commercial brands	*trans*-Resveratrol		Sobolev and Cole, 1999
kernels		1.779–7.092	
hulls		2.415–7.873	
liquid		0.048–0.064	
B. Inedible peanut plant materials			
1. Peanut skins (seed coats)			
Runner variety	*trans*-Resveratrol	0.51	Sanders et al., 2000
Runner variety	*trans*-Resveratrol	4.30	Francisco and Resurreccion, 2009
Virginia variety	*trans*-Resveratrol	3.66	Francisco and Resurreccion, 2009
Virginia variety	*trans*-Resveratrol	0.78	Sanders et al., 2000
Spanish variety	*trans*-Resveratrol	15.04	Francisco and Resurreccion, 2009
Jinpoong variety (South Korea)	*trans*-Resveratrol	0.05 (fresh weight)	Chung et al., 2003
Florunner variety	*trans*-Resveratrol	9.07	Nepote et al., 2004
Florunner, in ethanolic extract	*trans*-Resveratrol	91.4	Nepote et al., 2004
Variety not specified	*trans*-Resveratrol	Not reported	Yu et al., 2005
2. Peanut leaves			
Jinpoong variety (South Korea)	*trans*-Resveratrol	2.05 (fresh weight)	Chung et al., 2003
3. Peanut roots			
Jinpoong variety (South Korea)	*trans*-Resveratrol	1.19 (fresh weight)	Chung et al., 2003
Tainan variety (Taiwan)	*trans*-Resveratrol	39 to 1330	Chen et al., 2002
4. Peanut hulls			
Peanut hulls (in boiled peanuts)	*trans*-Resveratrol	2.415–7.873	Sobolev and Cole, 1999
Peanut hulls (shells)	*trans*-Resveratrol	2.60 (fresh weight)	Chung et al., 2003

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added), 0.577 to 0.753 μg/g in 100% natural peanut butter (Ibern-Gomez et al., 2000), and in Korean commercial peanut butters, from 0.27 to 0.70 μg/g (Lee et al., 2004). Earlier, slightly lower range of *trans*-resveratrol concentrations of 0.148 to 0.504 μg/g from commercial peanut butter samples was reported (Sobolev and Cole, 1999). Differences in the values obtained by these investigators may be due to the efficiency of the methods of extraction, the analytical methods used for analysis, and the quality of raw materials used in the preparation of the peanut products.

trans-Piceid in peanuts was first reported by Ibern-Gomez and coworkers (2000). Natural peanut butters contained 0.073–0.225 μg/g *trans*-piceid, which were higher than the 0.067–0.187 μg/g in blended peanut butters (Ibern-Gomez et al., 2000). These levels of *trans*-piceid are about one-third lower than that of *trans*-resveratrol found in the same samples of peanut butter. Recently, slightly higher *trans*-piceid concentrations of 0.36 or 0.46 μg/g were reported in peanuts treated by UV and ultrasound, respectively (Potrebko and Resurreccion, 2009). Even higher amounts of *trans*-piceid were observed in

peanuts stressed by 27 treatments of UV and ultrasound with concentration ranges of 0.35–1.05 and 0.16–6.39 μg/g, respectively (Sales and Resurreccion, 2009).

Boiled Peanuts

Among commercial peanut surveyed, Sobolev and Cole (1999) found the highest *trans*-resveratrol concentrations of 1.779 to 7.092 μg/g in canned boiled. These authors attributed this to the presence of low-quality kernels, such as small, immature, and mechanically damaged pods, which are normally sorted out when manufacturing roasted peanuts and peanut butter. Resveratrol concentrations generally increased with decreasing seed size usually associated with more immature peanuts, and high concentrations were found in discolored seeds (Sobolev and Cole, 1999).

Peanut Sprouts

Peanut sprout is a novel product from peanuts being developed as a functional vegetable and found to have *trans*-resveratrol up to as much as 11.7 to 25.7 μg/g in experimental samples of three cultivars peanut seeds germinated for a maximum of nine days in the dark compared with initial concentrations of 2.3 to 4.5 μg/g (Wang et al., 2005). Among the sprout components, *trans*-resveratrol was found highest in the cotyledons with 12.0–47.1 μg/g, slightly lower in roots with 7.9 to 18.6 μg/g, and none in the stems (Wang et al., 2005).

Inedible Parts of Peanut Plant

Inedible peanut plant materials like leaves (Chung et al., 2003), roots (Chen et al., 2002; Chung et al., 2003), hulls (Sobolev and Cole, 1999; Chung et al., 2003), and skins (Nepote et al., 2004; Francisco and Resurreccion, 2009) contain beneficial phenolic compounds (Table 2). Recently, research studies on methods to enhance the concentrations of bioactive components in inedible plant materials are increasing due to their potential as natural inexpensive sources of dietary functional compounds for use as ingredient for dietary supplements and food product formulations. Peanut skins, the by-product in the manufacture of peanut butter and other peanut products, are used as animal feed of low economic value (Nepote et al., 2004) or discarded as waste.

Peanut Skins

Peanut skins are a good source of polyphenolic compounds containing 90–125 mg total phenolics/g (Yu et al., 2005). Polyphenols found by Yu et al. (2005) include the stilbene *trans*-resveratrol; phenolic acids-chlorogenic, caffeic, coumaric, and ferulic acids; and the flavonoids—epigallocatechin, epicatechin, catechin gallate, and epicatechin gallate. Francisco and Resurreccion (2009) identified and quantified *trans*-resveratrol, three phenolic acids, and five flavonoids in skins of three US peanut varieties using a reversed phase high-performance liquid chromatography (HPLC), which they developed for the simultaneous analysis of 16 phenolic compounds. They found that *trans*-resveratrol was higher in Spanish skins (15.04 μg/g) followed by Runners (4.3 μg/g) and Virginia (3.66 μg/g). The highest protocatechuic acid was obtained from Virginia (34.04 μg/g), followed by Spanish (15.45 μg/g) and lowest in Runner skins (7.62 μg/g). Caffeic acid (3.49 μg/g) was detected only in Spanish peanut skins. *p*-Coumaric acid was highest in Runners (32.34 μg/g), followed by Spanish (12.31 μg/g) then Virginia (4.98 μg/g). Epigallocatechin and catechin contents were higher in Virginia (1276 and 535 μg/g, respectively) and Spanish (1275 and 448 μg/g, respectively) compared with Runner skins (440 and 74 μg/g, respectively). Procyanidin B2, epicatechin, and quercetin were highest in Spanish skins with 107, 239, and 28 μg/g, respectively compared with those in Virginia and Runners.

Skins from Argentinian peanuts had 9.07 μg/g *trans*-resveratrol (Nepote et al., 2004). Lower concentrations of *trans*-resveratrol of 0.65 μg/g peanut skin equivalent to $<$0.04 μg/seed was found by Sanders et al. (2000). Much smaller amounts of 0.05 μg/g *trans*-resveratrol were reported from skins of developing seeds grown in the field (Chung et al., 2003).

Peanut skins also contain the flavonoid ethyl protocatechuate or 3,4-dihydroxybenzoic acid ethyl ester (Huang et al., 2003). Among the 17 fractions of compounds separated from the crude ethanol extracts of peanut skins, ethyl protocatechuate had the highest yield and marked antioxidant activity (Huang et al., 2003).

Peanut Hulls

Peanut hulls contain substantial amounts of *trans*-resveratrol in the range of 2.4 to 7.9 μg/g (Sobolev and Cole, 1999). Hulls from the developing seeds had relatively higher *trans*-resveratrol of 2.6-μg/g fresh weight compared with those in developing seeds and seed coats of field grown peanuts (Chung et al., 2003). Hulls from mature peanuts were found to contain the flavonoids—luteolin at 6.0 mg/g, eriodictyol at 3.8 mg/g, and 5,7-dihydroxychromone at 1.49 (Daigle et al., 1988). Lower concentration of 1.74-mg/g luteolin in peanut hulls was reported by Duh and Yen (1995). Peanut hulls had total phenolics content of 72.9-μM/L tannic acid equivalents, which increased to 90.3 μM after roasting at 150°C for 60 minutes (Lee et al., 2006).

Peanut Roots, Leaves, and Stems

Peanut roots contained 1.19-μg/g *trans*-resveratrol, whereas leaves had higher amounts of 2.05-μg/g fresh weight (Chung et al., 2003). Higher resveratrol concentrations of 6.34 μg/g in peanut roots but lower in leaves with 0.02-μg/g fresh weight were reported by Lin et al. (2007). Peanut roots and leaves also contain piceatannol of 2.955- and 0.06-μg/g fresh weight, respectively (Lin et al., 2007).

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In UV-irradiated peanut callus, Ku et al. (2005) did not detect *trans*-resveratrol and piceatannol right after irradiation using static cultivation, but concentrations increased up to 11.97 and 5.31 μg/g, respectively, after 18 hours of incubation. Using suspension cultures, *trans*-resveratrol but not piceatannol increased up to 6.93 μg/g after four hours from UV treatment and did not increase thereafter from 8 to 80 hours as calluses may have received shorter UV irradiation while constantly moving in the suspension (Ku et al., 2005).

Other Major Sources of Resveratrol

Grapes and Wines

Resveratrol is synthesized particularly in the skins of grape berries and none to only trace amounts are present in the fruit flesh (Creasy and Coffee, 1988; Becker et al., 2003). Compared with raw, roasted, and boiled peanuts with *trans*-resveratrol in the range of 0.02 to 7.09 (Table 2), grape skins had higher concentration up to 24.06 μg/g and contain *trans*- and *cis*-piceid of 42.1992.33 μg/g, respectively, but no detectable amounts of *cis*-resveratrol based on 13 samples of 7 varieties of grapes analyzed (Romero-Perez et al., 2001). Grape skins are also a major food source of other stilbenes including viniferins, astringin, and piceatannol or astringinin (Bavaresco, 2003).

Stilbene synthesis in grapes depends on grape variety, environment, and viticultural practices (Bavaresco, 2003). Red grapes have higher stilbene levels than white grapes. Red varieties of Merlot and Cabernet Sauvignon grapes contain mainly *trans*-piceid in the range of 1.5–7.3-μg/g fresh weight, whereas *trans*-resveratrol ranged from nondetectable to 0.5-μg/g fresh weight (Burns et al., 2002). Resveratrol in Concord grape products ranged from 0.002–1.042 and 0.002 μmol/g in grape juice (Wang et al., 2002). A positive correlation existed between vineyard elevation and grape stilbene concentrations and quality-oriented cultural practices produce grapes with higher levels of stilbenes (Bavaresco, 2003).

Because to its presence in grape skins, resveratrol is expected in wines due to skin contact during fermentation (Becker et al., 2003). When wine is made from grapes, resveratrol is released from the skins and maceration increased the extraction of resveratrol by 10-fold compared with nonmacerated wines (Jeandet et al., 1995). Paradoxically, lower concentrations of resveratrol were observed in wines made from highly *Botrytis* infected grapes than in those vinted from healthy and moderately infected grapes (Jeandet et al., 1995). Wines from various red and white grapes ranged 0.2–7.7 μg/mL (Aggarwal et al., 2004), which were higher than in raw and roasted peanuts but comparable with that in boiled peanuts (Table 2). Red wines have higher *trans*-resveratrol concentrations of 0.352–1.99 μg/mL compared with white varieties with 0.005–0.57 μg /mL (Gerogiannaki-Christopoulou et al., 2006). Italian red wines contained 8.63 to 24.84 μmol/L (Wang et al., 2002).

Lamuela-Raventos et al. (1995) found that *cis* and *trans* forms of resveratrol and piceid were present in 18 varieties of Spanish red wines with resveratrol concentrations higher than piceid in all samples. These authors postulated that the presence of *cis* isomers of resveratrol and piceid in wines arised from light exposure of must or wine during wine-making process and possibly from light exposure of wine bottles during storage. Pinot noir wines had the highest mean *trans*-resveratrol of 5.13 μg/mL compared with 3.99, 2.43, 1.42 and 1.33 μg/mL in Merlot, Grenache, Cabernet Sauvignon, and Tempralino, respectively. The *trans*- piceid concentrations of 2.98 mg/L in Merlot wines was highest compared with 2.63, 2.46, 1.13, and 1.07 mg/L in Grenache, Pinot noir, Tempranillo, and Cabarnet Souvignon wines, respectively. The ratios of *trans* to *cis* of both resveratrol and piceid in Spanish wines were always greater than 1 and highest at 20 (Lamuela-Raventos et al., 1995).

Similarly, Burns et al. (2002) found higher amounts of *trans*-resveratrol compared with *trans*-piceid in four red wine samples. *trans*-Piceid was only detected Cabernet Sauvignon (Bulgaria) with 1.89-μg/mL *trans*-piceid, while the other three samples had none. Pinot noir, 1994 (California), had the highest *trans*-resveratrol of 1.057 μg/mL and *cis*-resveratrol of 0.746 μg/mL, but no *trans*-piceid contributing to total resveratrol of 1.803 μg/mL compared with only 1.380 μg/mL in Cabernet Sauvignon, 1996 (Bulgaria), 0.20 μg/mL in Merlot, 1994 (Chile), and the least of 0.098 μg/mL in Cabernet Sauvignon, 1995 (California).

Vaccinum spp. Including Blueberries, Bilberries, and Cranberries

trans-Resveratrol but not *cis-resveratrol* was found in both fresh blueberry and bilberry samples (Lyons et al., 2003). These researchers found considerable regional variation in the concentrations of *trans*-resveratrol, with highbush blueberries from Michigan at 140.0 $\pm$ 29.9 pmol/g (or 0.00014 $\pm$ 0.00003 μmol/g), fresh bilberries from Poland at 71.0 $\pm$ 15.0 pmol/g (or 0.000071 $\pm$ 0.000015 μmol/g), whereas none was detected in highbush blueberries from British Columbia. The *trans*-resveratrol concentrations of blueberry and bilberries are much lower compared with raw, roasted, and boiled peanuts (Table 2). Heating by baking decreased *resveratrol* concentrations in blueberries between 17 and 46% after 18 minutes at 190°C and were expected to be lower compared with fresh fruits (Lyons et al., 2003). The level of resveratrol in fresh blueberries and bilberries was less than 10% that reported for grapes (Lyons et al., 2003).

Japanese Knotweed

Resveratrol was found in the dried roots of the Japanese knotweed, *Polyganum cuspidatum*, also known as Ko-jo-kon or the Itadori plant, a traditional Chinese and Japanese medicine to treat suppurative dermatitis, gonorrhea favus, athlete's foot, and hyperlipedemia (Aggarwal et al., 2004). The powder of *P. cuspidatum* has been used in China and Japan as a treatment for atherosclerosis, cough, asthma, hypertension, cancer, and for other therapeutic purposes (Vastano et al., 2000). However,

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the major stilbene found in *P. cuspidatum* is piceid (Lamuela-Raventos et al., 1995; Burns et al., 2002). Commercial Itadori root contained 1653-μg/g *trans*-piceid and 523-μg/g *trans*-resveratrol (Burns et al., 2002), which are much higher than those found in raw and roasted peanuts (Table 2). Itadori tea prepared by infusing 1 g of the commercial root preparation with 100 mL of boiling water for five minutes contained 9.05-μg/mL *trans*-piceid and 0.68-μg/mL *trans*-resveratrol (Burns et al., 2002). Piceid standard extracted from *P. cuspidatum* was used to establish the evidence for the presence of piceid in red wines butter (Lamuela-Raventos et al., 1995; Brandolini et al., 2002) and peanut butter (Ibern-Gomez et al., 2000).

Pistachio Nuts

Pistachio nuts are also a food source of *trans*-resveratrol with concentrations ranging from 0.09–1.67 μg/g (mean = 1.15 μg/g) in five varieties from Turkey (Tokusoglu et al., 2005), which were higher compared with raw and roasted peanuts but lower than boiled peanuts (Table 2). Turkish pistachios contain higher *trans*-resveratrol compared with 0.08–0.18 μg/g (mean = 0.12 μg/g) in 12 Sicilian varieties (Grippi et al., 2008). The difference in resveratrol concentrations could be attributed to differences in variety, quality of nuts, and analytical methods for extraction and analysis. The Sicilian pistachios also contained *trans*-piceid of 6.2–8.15 μg/g (mean = 6.97 μg/g), which were markedly higher than *trans*-resveratrol in all 12 samples examined, a result similar to that obtained generally in red grapes where the concentration of *trans*-piceid is more than its aglycon, *trans*-resveratrol (Grippi et al., 2008).

BIOAVAILABILITY AND HEALTH EFFECTS OF RESVERATROL AND OTHER STILBENES

Absorption and Bioavailability

The potential health benefits of resveratrol depend, in part, upon its absorption, bioavailability, and metabolism, which were characterized in several in vitro and in vivo models (King et al., 2006). Resveratrol is absorbed and metabolized and around 75% are excreted via feces and urine (Wenzel and Somoza, 2005). Absorption and metabolism studies using an isolated rat small intestine showed that the majority of *trans*-resveratrol was most likely to be absorbed in the form of resveratrol glucuronide after crossing the small intestine (Andluer et al., 2000; Kuhnle et al., 2000). Resveratrol bioavailability was 38% and its exposure was approximately 7- and 46-fold lower than resveratrol glucuronide after intravenous and oral administration, respectively (Marier et al., 2002). Even lower oral bioavailability of almost zero was reported by Wenzel and Somoza (2005) due to rapid and extensive metabolism and the consequent formation of various metabolites as resveratrol glucuronide and resveratrol sulfates.

The human digestive tract is known to have glucosidase activity, so it was possible that piceid could release the aglycon, resveratrol on ingestion (Lamuela-Raventos et al., 1995; Chen et al., 2001). Certain studies show that absorption of some phenols is enhanced by conjugation with glucose, so that it could be possible that *trans*-piceid would be more efficiently absorbed by the intestinal gut than its aglycon, *trans*-resveratrol (Ibern-Gomez et al., 2000). Henry-Vitrac et al. (2006) found that the transepithelial transport of *trans*-piceid in the small intestine and liver occurred at a high rate, and the compound was deglycosylated to its aglycon in two possible ways. The first is cleavage by the cytosolic-β-glucosidase, after passing the brush-border membrane by SGLT1 (sodium/glucose cotransporter 1). The second is deglycosylation on the luminal side of the epithelium by the membrane bound enzyme, lactase phlorizin hydrolase, followed by passive diffusion of the released resveratrol, which was further metabolized into two glucuronoconjugates—*trans*-resveratrol-3-β-glucuronide, the major glucuronide and *trans*-resveratrol-4′-*O*-β-glucuronide, the minor one.

The study on the cellular uptake and efflux of *trans*-resveratrol and *trans*-piceid on the apical membrane of the human intestinal caco-2 cells showed that *trans*-resveratrol had a higher rate of cellular accumulation than *trans*-piceid (Henry et al., 2005). *trans*-Resveratrol used passive transport to cross the apical membrane of the cells, whereas transport of *trans*-piceid was likely active. The involvement of the active SGLT1 in the absorption of *trans*-piceid was deduced using various inhibitors directly or indirectly exploiting the activity of this transporter.

Piceatannol, a known anticancer (Lin et al., 2007) and antileukemic agent, was confirmed to be the main metabolite in in vitro metabolism of resveratrol in rat liver microsomal incubation (Zhu et al., 2003). This result was consistent with the previous findings that in vitro metabolism of resveratrol by recombinant human cytochrome P450 enzyme CYP1B1 (Cytochrome P450, family 1, subfamily B, polypeptide 1) formed piceatannol (Potter et al., 2002). The enzyme CYP1B1 is overexpressed in a wide variety of human tumors and catalyzes aromatic hydroxylation reactions (Potter et al., 2002).

Health Effects

Antioxidative Effect

Much evidence suggests that resveratrol exerts antioxidant activity. It is a potent inhibitor of the production of reactive oxygen species (ROS), and this inhibitory action might be the major biochemical mechanism related to its anti-inflammatory and anticarcinogenic activities (Aggarwal et al., 2004). Antioxidants are substances, which when present at low concentrations compared with those oxidizable substrates, such as lipid containing polyunsaturated fatty acids (PUFAs), proteins, carbohydrates or DNA, significantly delay or prevent oxidation of the substrates (Aruoma, 2003) by inhibiting the initiation or propagation of oxidizing chain reactions (Zheng and Wang, 2001). An imbalance between antioxidants and ROS results in oxidative stress, leading to cellular damage (Buhler and Miranda, 2003). Oxidative

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stress has been linked to cancer, atherosclerosis, ischemic injury, inflammation, neurodegenerative diseases, such as Parkinson's and Alzheimer's, and aging (Buhler and Miranda, 2003). Emerging literature, pointing to the low intestinal absorbance of polyphenols, suggests that the ability of polyphenols and their metabolites in vivo to interact with cell-signaling cascades, such as apoptosis and redox-sensitive cell-signaling pathways, may be a major mechanism of action (Jang et al., 1997).

Cardiovascular Protective Effect

Cardiovascular disease is the leading cause of death in the United States with more than 1.4 million deaths each year accounting for more than 40% of all deaths (Centers for Disease Control and Prevention [CDC], 2007b). The economic burden of CVD has a profound impact on the US health care system and projected to be $421.8 billion in 2007, including health expenditures and loss of productivity resulting from death and disability (CDC, 2007b).

Atherosclerosis is the major cause of the coronary damages, particularly ischemic vascular disease, resulting from the disruption of normal reactions between blood (plasmatic proteins, lipoproteins, growth factors, lymphocytes, platelets) and normal cellular elements of the arterial wall (Delmas et al., 2005). Resveratrol protects against atherosclerosis by reducing peroxidative degradation of low-density lipoproteins (LDLs) mainly due to its capacity to chelate copper and scavenging of free radicals in vitro (Belguendouz et al., 1998; Delmas et al., 2005). The parahydroxyl group appeared to show a greater radical scavenging activity than the metahydroxyl groups, and the spatial position of hydroxyl groups was likely more favorable to the chelation of the *trans* than in the *cis* isomer (Delmas et al., 2005). Due to its hydroxylated structure, resveratrol can form a radical derivative stabilized by the delocalization of two electrons between the two aromatic cycles and the methylene bridge joining these two cycles (Delmas et al., 2005).

Resveratrol associates with lipoproteins in a linear dose–response curve increasing its concentrations as lipoprotein density decreases: high-density lipoproteins (HDLs) < LDL < very LDL (VLDL) (Belguendouz et al., 1998). When resveratrol was added to plasma prior to fractionation, it was more associated with lipoproteins than with lipoprotein-free proteins revealing its lipophilic character. Resveratrol also inhibited the formation of thiobarbituric acid reactive substances in preparations containing phospholipid unilamellar liposomes oxidized by 2,2′-azobis (2-amidinopropane) dihydrochloride, a water-soluble radical generator. A linear dose–response curve was obtained up to 30 μM when resveratrol was added in the final preparation and up to 200 μM when added before preparing liposomes, to facilitate its incorporation, suggesting that the soluble fraction of resveratrol scavenged free radicals in the aqueous phase before attacking PUFAs within membranes. All these results support the hypothesis that resveratrol may be efficient at different sites: in the protein and lipid moieties of LDL and in their aqueous environment.

Resveratrol stimulates the expression and activity of endothelial nitric oxide synthase (eNOS) as evidenced by increased eNOS mRNA expression up to 2.8 fold in a time- and concentration-dependent manner after incubation of human umbilical vein endothelial cells with resveratrol, thereby contributing to CVD protection from LDL oxidation (Wallerath et al., 2002). eNOS protein expression and eNOS-derived nitric oxide production were also increased after long-term incubation with resveratrol. Resveratrol also stabilized mRNA.

Resveratrol protects the heart by preconditioning it through the activation of adenosine receptors (Das et al., 2004). The results were consistent with many previous reports indicating the role of adenosine A1 and A3 receptors, but not A2a receptor, in preconditioning of the heart. Adenosine A1 transmits a survival signal through phosphatidylinositol (PI)3-kinase-Akt-Bcl-2 signaling pathway, and adenosine A3 protects the heart through a cAMP response element binding protein (CREB)-dependent Bcl-2 pathway in addition to an Akt-Bcl-2 pathway.

Resveratrol prevents the action of human squalene monooxygenase, a flavin adenine dinucleotide (FAD) containing microsomal enzyme that catalyzes the second step in the committed pathway for cholesterol biosynthesis (Laden and Porter, 2001). Resveratrol specifically inhibits purified human squalene monooxygenase in a noncompetitive manner with respect to both squalene, Ki = 35 micromolar and FAD, Ki = 69 micromolar.

Cancer Prevention and Therapy

Cancer is the second leading cause of death in the United States (CDC, 2007a). Cancer claims the lives of more than 556,000 Americans every year and costs the United States an estimated $206 billion, including $128 billion for lost productivity and $78 billion in direct medical costs (CDC, 2007a).

In 1997, Jang et al. published a paper describing the ability of resveratrol to inhibit diverse cellular events associated with the three major stages of carcinogenesis—initiation, promotion, and progression, which undoubtedly fired the imagination of the cancer chemoprevention research community (Gescher and Steward, 2003). Aggarwal et al. (2004) conducted a comprehensive review of the role of resveratrol in prevention and therapy of a wide variety of cancer, including lymphoid, myeloid, multiple myeloma, breast, prostate, stomach, colon, pancreas, thyroid, melanoma, head and squamous cell carcinoma, and ovarian and cervical carcinoma. Resveratrol induces apoptosis or initiation of cell death from inside of the cells, primarily from mitochondria, through a variety of pathways such as fas–a death domain containing member of the tumor necrosis factor receptor (TNFR) (Clement et al., 1998; Delmas et al., 2003), mitochondrial (Zheng and Ramirez, 1999; Dörrie et al., 2001), retinoblastoma (Rb-E2F/DP, Adhami et al., 2001), p53 activation (Huang et al., 1999; She et al., 2001), ceremide activation (Scarletti et al., 2003), and adenylyl-cyclase (El-Mowafy and Alkhalaf, 2003).

Resveratrol suppresses activation of the following (1) nuclear factor kappa B (NF-κB), a nuclear transcription factor that

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regulates the expression of various genes involved in inflammation, cytoprotection, and carcinogenesis (Holmes-McNary and Baldwin, 2000; Pellegata et al., 2003); (2) activator protein-1 (AP-1), a transcription factor transactivated by many tumor-promoting agents, such as phorbol ester, UV radiation, asbestos. and crystalline silica (Yu et al., 2001; Shen et al., 2003); (3) early growth response-1 (Egr-1) gene, a transcription factor belonging to a family of immediate early response genes that regulates a number of pathophysiologically relevant genes that are involved in growth, differentiation, immune response, wound healing, and blood clotting (Regione et al., 2003); (4) three mitogen-activated protein kinases (MAPK), i.e., extracellular receptor kinase (ERK 1/2) implicated in the proliferation of cells; c-Jun N-terminal kinase (JNK) and p38 MAPK, which are responsible for the activation of AP-1 (Nicolini et al., 2001); (5) protein kinases, which play a major role in tumorigenesis (Garcia-Garcia et al., 1999; Stewart et al., 1999); (6) growth factor and associated tyrosine kinases that mediate various growth factors for a variety of tumor cells (Kaneuchi et al., 1998; Palmieri et al., 1999); (7) cyclooxygenase-2 (COX-2). COX-2 and lipoxygenase (LOX), which play important roles in inflammation of cells (Subbaramiah et al., 1998; Chung et al., 2003); (8) cell cycle proteins that inhibit cell proliferation (Wolter and Stein, 2002; Yu et al., 2003); (9) various cell surface adhesion molecules, including intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and endothelial-leukocyte adhesion molecule (ELAM)-1, which play essential role in adhesion of tumor cells to endothelial cells (Ferrero et al., 1998; Pendurhi et al., 2001); (10) androgen receptors that play a role in prostate cancer etiology (Mitchell et al., 1999); (11) prostate specific gene (Hsieh and Wu, 2000) ; (12) expression of inflammatory cytokine (Wang et al., 2001); (13) angiogenesis, invasion, and metastasis. Angiogenesis plays an important role in tumor growth, other diseases, and wound healing. It is a process of formation of blood vessel that is mediated through modulation of proliferation and gene expression by endothelial cells. (Szende et al., 2000; Igera et al., 2001); (14) inflammation, by activating the transcription factor, NF-κB, suppressing proinflammatory cytokines such as tumor necrosis factor (TNF), interleukins (IL)-1, IL-6, and IL-8 (Wang et al., 2001) and suppressing the expression of proteins such as iNOS, COX-2, and 5-LOX that mediate inflammation (Kimura et al., 1983, 1995).

Resveratrol modulates the expression of nitric oxide (NO) and nitric oxide synthase (NOS). NO mediates antiproliferative effects in various cell types and has proinflammatory effects. Resveratrol both enhances and suppresses production of NO (Hsieh et al., 1999; Kageura et al., 2001).

Piceatannol, a resveratrol derivative extracted from UV-induced peanut calluses, was found to be more efficient inducer of apoptosis in an ex vivo assay of primary lymphoblasts than resveratrol (Ku et al., 2005). It has been demonstrated that resveratrol can be converted into piceatannol by cytochrome P450 enzyme CYP1B1 (Ku et al., 2005). Piceatannol is a potent inhibitor of the activity of protein tyrosine kinases, which are positive regulators of cell proliferation (Geahlen and McLaughlin, 1989). Both resveratrol and piceatannol induce apoptosis in many cancer cell lines, although there are also reports that piceatannol is a more efficient inducer of apoptosis (Lin et al., 2007).

Phytoestrogen Activity

Resveratrol is categorized as a phytoestrogen with structure similar to a synthetic estrogen, diethylstilberol, and exhibited variable degrees of estrogen receptor agonism in different test systems (Gehm et al., 1997). Resveratrol inhibited the binding of labeled estradiol to the estrogen receptor, and it activated transcription of estrogen-responsive reporter genes transfected in human breast cancer cells. It functioned as a superagonist producing a greater maximal transcriptional response than estradiol in some cell types (e.g., MCF-7 cells), whereas, in others, it produced activation equal to or less than that of estradiol. Resveratrol also increased the expression of native estrogen-regulated genes and stimulated proliferation of estrogen-dependent T47D breast cancer cells.

Resveratrol inhibits the growth of estrogen-receptor (ER) positive MCF-7 cells in a dose-dependent fashion (Lu and Serrero, 1999). Resveratrol antagonized the growth promoting effect of 17-β-estradiol (E_2) in a dose-dependent manner at both cellular (cell growth) and the molecular (gene activation) levels. At the cellular level, the antiestrogenic effect of resveratrol that abolished the growth-stimulatory effect mediated by E_2, was observed at a concentration of 10^{-6} M and above. At the molecular level, resveratrol antagonized the stimulation by E_2 of progesterone receptor gene expression in MCF-7 cells. Furthermore, resveratrol inhibited the expression of transforming growth factor-α and insulin-forming growth factor I receptor mRNA while significantly elevating the expression of transforming growth factor β2 mRNA. These results showed that resveratrol is a partial estrogen receptor itself, acting as an estrogen receptor antagonist in the presence of estrogen leading to inhibition of human breast cells (Lu and Serrero, 1999).

Treatment of Alzheimer's Disease

Alzheimer's disease is a progressive neurodegenerative disorder leading to the most common form of dementia. Beta-amyloid peptide is considered to be responsible for the formation of senile plaques that accumulate in the brains of patients with AD (Marambaud et al., 2005). There has been compelling evidence supporting the idea that beta-amyloid-induced cytotoxicity is mediated through the generation of reactive oxygen intermediates, ROIs (Jang and Surh, 2003). Considerable attention was focused on identifying phytochemicals that are able to scavenge excess ROIs, thereby protecting against oxidative stress and cell death.

The PC12 cells treated with beta-amyloid exhibited increased accumulation of intracellular ROI and underwent apoptotic death as determined by characteristic morphological alterations and positive in situ terminal end labeling (Jang and Surh, 2003).

The beta-amyloid treatment also led to the decreased mitochondrial membrane potential, the cleavage of poly (ADP-ribose) polymerase, an increase in the Bax/Bcl-X(L) ratio, and activation of JNK. Resveratrol attenuated beta-amyloid-induced cytotoxicity, apoptotic features, and intracellular ROI accumulation. Beta-amyloid transiently induced activation of NF-kappa B in PC12 cells, which was suppressed by resveratrol pretreatment.

The study of Marambaud et al. (2005) showed that resveratrol has potent antiamyloidogenic activity by reducing levels of intracellular beta-amyloid peptide produced from different cell lines. Resveratrol promotes the intracellular degradation of beta-amyloid peptide by a mechanism that implicates the proteasome. Evidence is compelling that a decrease in proteasome activity occurs in brains of individuals with AD. It has been proposed that beta amyloid itself may lead to proteasome inhibition, suggesting that high levels of beta amyloid in AD brains may create a vicious cycle by inhibiting the proteasome and blocking the degradation of critical regulators of its own clearance.

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Antiaging Property

Caloric restriction, a drastic, 30–40% reduction in daily caloric intake to a level that provides all nutrients sufficient for a healthy life, has been implicated in extending the lifespan. Delmas et al. (2005) reported that under caloric restriction, the altered oxygen consumption modifies the NAD+/NADH ratio and leads to an NAD+-dependent activation of sirtuin, an evolutionary conserved enzyme family, which chemically modifies proteins, especially p53, the tumor suppressor involved in longevity. Resveratrol and related compounds recently shown to mimic caloric restriction by lowering the Michaelis constant of sirtuin for both the NAD+ and the acetylated substrates leading to a sirtuin-dependent deacetylation of p53 both in yeast and in human cell cultures. Resveratrol also increased the DNA stability as shown by a strong decrease in the rDNA frequency.

Resveratrol increased the lifespan of brewer's yeast, *Saccharomyces cerevisiae* by 70%; roundworm, *Caernorhabditis elegans* by 14%, and fruit fly, *Drosophila melanogaster* by 29% (Delmas et al., 2005) implicating its potential as an antiaging agent in treating age-related human diseases (De la Lastra and Villegas, 2005). The lifespan extension in these organisms was dependent on Sir2, a conserved deacetylase proposed to underlie the beneficial effects of caloric restriction (Baur et al., 2006).

In a more recent study, Baur et al. (2006) reported that resveratrol shifts the physiology of middle-aged mice on a high-calorie diet toward that of mice on a standard diet, which significantly increase their survival. Resveratrol produced changes associated with extended lifespan of mice including increased insulin sensitivity, reduced insulin-like growth factors-1 (IGF-1), increased AMP-activated protein kinase (AMPK) and peroxisome proliferators-activated receptor-gamma coactivator 1alpha (PGC-1alpha) activity, increase mitochondrial number, and improved motor function.

Other Health Effects

Analgesic Effect or Antipain Property

trans-Resveratrol's analgesic effect was reported to be mediated via an opioidergic mechanism and produces tolerance to its analgesic effect similar to morphine (Gupta et al., 2004). The effect of graded doses of *trans*-resveratrol using a hot plate analgesiometer in rats showed that *trans*-resveratrol at graded doses of 5, 10, 20, and 40 mg/kg i.p. produced dose-dependent analgesia. Pretreatment for 20 minutes with naloxone (1 mg/kg i.p.) blocked the analgesic effect. A potentiation effect was observed when the submaximal dose of *trans*-resveratrol (5 mg/kg i.p.) was combined with a submaximal dose of morphine (2 mg/kg i.p.).

The effect of *trans*-resveratrol (20 mg/kg i.p.) was also studied on morphine tolerance of rats divided into different groups: 1-morphine (10 mg/kg i.p.), 2-*trans*-resveratrol (5 mg/kg i.p.) administered 10 minutes before morphine (2 mg/kg i.p.), 3-*trans*-resveratrol (20 mg/kg i.p.) with the treatment continued for 7 days (Gupta et al., 2004). The occurrence of tolerance was estimated by comparing the antinociceptive effect of morphine with *trans*-resveratrol on day 1 and day 8. Both morphine and *trans*-resveratrol produced tolerance. However, in the group that received the combination of submaximal doses of *trans*-resveratrol and morphine, tolerance was not significant.

The group of Kim et al. (2005) investigated the effects of resveratrol on tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) Na+ currents in rat dorsal root ganglion (DRG) neurons. Previous studies showed that resveratrol exhibited analgesic effects suggesting that COX inhibition and K+ channel opening were the underlying mechanisms. Resveratrol caused a hyperpolarizing shift of the steady-state inactivation voltage and slowed the recovery from inactivation of both Na+ currents. However, no frequency-dependent inhibition of resveratrol on either type of Na+ current was observed. The suppression and the unfavorable effects on the kinetics of Na+ currents in terms of the excitability of DRG neurons may make a great contribution to the analgesia by resveratrol.

Protective Effect on Hyperglycemia

Resveratrol blocks oxidative stress in high glucose-induced apoptosis and alterations in attachment ability by virtue of its antioxidant property (Chan, 2005). Hyperglycemia, a symptom of diabetes mellitus, induced hyperosmotic responses, including apoptosis, in vascular endothelial cells and leukocytes in mammals. The apoptotic biochemical changes such as caspase-3 activation and DNA fragmentation were blocked by antioxidant pretreatment during hyperosmotic shock-induced cell death. Resveratrol attenuated high glucose-induced apoptotic changes, including JNK activation and caspase-3 activation in human leukemia K562 cells. Experiments with the cell permeable dye, 20,70-dichlorofluorescein diacetate (DCF-DA), an indicator of ROS generation, revealed that high-glucose

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treatment directly increased intracellular oxidative stress, which was attenuated by resveratrol. In addition, high glucose-treated K562 cells displayed a lower degree of attachment to collagen, the major component of vessel wall subendothelium. In contrast, cells pretreated with resveratrol followed by high glucose exhibited higher affinity for collagen. These results collectively imply the involvement of oxidative stress in high glucose-induced apoptosis and alterations in attachment ability, which is blocked by resveratrol by virtue of its antioxidant property.

PROCESSES TO ENHANCE BIOSYNTHESIS OF RESVERATROL AND OTHER BIOACTIVE COMPOUNDS IN PEANUTS AND PLANT MATERIALS

Resveratrol and other stilbenes accumulate in plants in response to two types of stresses, biotic and abiotic, that activate stilbene synthase, the enzyme critical for resveratrol synthesis. Biotic stresses result from microbiological invasion and/or inoculation of biological agents such as molds and yeasts. Whereas abiotic stresses involve use of physical, mechanical, or chemical agents such as wounding, exposure to UV light and ultrasound, and treatment with metallic salts, salicylic acid, or ozone. Biotic stresses not only increase phytoalexin production but also leads to degradation of the plant material and production of toxic fungal metabolites such as aflatoxins produced by *Aspergillus flavus* in groundnuts, which renders the food unsafe for consumption. Problems associated with microbial infections could be eliminated using abiotic elicitors by subjecting the plant and/or plant materials to physical, mechanical, or chemical stresses.

Biotic Stresses

Microbial invasion and/or inoculation of microorganisms in plant and plant materials had been shown (Ingram, 1976) to increase the concentration of phytoalexins. Peanuts are known to synthesize many phytoalexins in response to microbial stress. Table 3 shows the concentrations of resveratrol and other resveratrol-related phytoalexins produced in peanut kernels, leaves, and roots after microbial invasion and/or inoculation.

Microbial Invasion and/or Inoculation of Molds

Ingham (1976) found that peanut hypocotyls accumulated a mixture of *cis*- and *trans*-resveratrol with concentrations ranging from 38–55 μg/mL after inoculation with 10 μl of a conidial suspension (ca 5×10^4 spores/mL) of *Helminthosporium carborum,* a nonpathogenic fungus, and incubation at 22°C for 24 hours. These compounds were not detected in extracts from noninoculated plants, suggesting that fungal infection initiated resveratrol biosynthesis.

In peanut kernels, as high as 3690 μg/g of a resveratrol derivative, 4-(3-methyl-but-1-enyl)-3,5,4′-trihydroxystlbene *trans*-resveratrol, was accumulated when imbibed peanuts were sliced and incubated for 48 hours in the dark at 25°C allowing natural microflora to grow (Aguamah et al., 1981). Other resveratrol derivatives produced in the same sample were 950 μg/g of 4-(3-methyl-but-1-enyl)-3,5,3′4′-tetrahydroxystilbene and 1160 μg/g of 4-(3-methyl-but-2-enyl)-3,5,4′,-trihydroxystilbene (4-isopentenylresveratrol).

Recently, four new stilbenoids, araphin-1, araphin-3, araphin-4, and araphin-5, were isolated from 3–6 mm sliced imbibed viable peanut seeds challenged by an *Aspergillus caelatus* strain, along with two known stilbenoids that have not been reported in peanuts, chiricanine, and araphin-2 and six other known stilbenes in peanuts, *trans*-resveratrol, arachidin-1, arachidin-2, arachidin-5, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene, and SB-1 (Sobolev et al., 2009). All stilbenoids were identified by nuclear magnetic resonance (NMR), mass spectrophotometry (MS), and UV spectra. Nonviable peanuts prepared by placing imbibed and sliced 3–6 mm seeds in distilled boiling water for 90 seconds were completely colonized by the fungus, whereas only few colonies were counted in viable peanuts, suggesting that viable peanuts were able to suppress fungal growth compared with nonviable samples. Viable sliced peanuts produced an array of stilbenoids at significant concentrations, whereas no stilbenoids were detected in sliced nonviable or sliced viable uninoculated control samples, indicating that slicing did not serve as elicitor (Sobolev et al., 2009).

In peanut leaves, increased concentrations of phytoalexins from 162–1830 nmol/g (1 nmol $= 10^{-3}$ μmol) fresh weight, a 55–618 fold increase, were elicited after spraying spore suspensions on leaves compared with control containing only 2.96-nmol/g fresh weight (Subba-Rao et al., 1996). Spore suspensions of *Cercospora arachidicola* (10^4 and 10^3 spores/mL), *Phaeoisariopsis personata* and *Puccinia archidis* (both at 10^4 spores/mL) in 1% Tween-80 (polyoxyethylene-sorbitan monooleate) were sprayed onto leaves of 3-week-old peanut plants and then enclosed for 24 hours in plastic bags, which had been wetted by spraying their inner surfaces with distilled water. The infected plants were sprayed with water in the evenings and covered with plastic bags for five consecutive nights of 16 hours and allowed to dry during the day thus providing a total intermittent leaf wetness for 80 hours. Extracts from leaves severely infected by *C. arachidicola* (10^4 spores/mL) contained the greatest amount of phytoalexins (1830-nmol/g fresh weight), those infected with *P. archidis* had the least (162-nmol/g fresh weight), and extracts from leaves infected with *P. personata* or mildly infected with *C. arachidicola* (10^3 spores/mL) were intermediates (664 and 745 nmol/g, respectively).

Similarly in grape, *Vitis vinifera*, vines and leaves, resveratrol was produced at the greatest concentrations, when the leaves were attacked by the fungus *Botrytis cinerea*. As the grape plant's production of resveratrol increases, so does its resistance to *Botrytis cinerea*, however, once a plant produced enough resveratrol to defend itself, the resveratrol concentration declined (Thomzik et al., 1997; Creasy, 1998).

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Table 3 Concentrations of resveratrol and other related compounds in peanut and grape plant materials stressed by microbial invasion and/or inoculation

	Postharvest stress treatment condition				Compound			
			Incubation			Concentration		
Plant material	Microorganism	Concentration (spores/mL)	Time	Temp.	Name	Control	Stressed	Reference
Peanut leaf hypocotyls, 2–4 cm	*Helminthosporium carbonum* Ullstrup	5×10^4	24 hours	22°C, ca 400 lux	*cis*- and *trans*-Resveratrol	0 μg/g dry weight (wt.)	38–55 μg/g dry wt.	Ingham, 1976
Peanut kernels	Natural microflora, wounding by slicing 1–2 mm	NR[1]	48 hours	25°C in the dark	4-(3-Methyl-but-1-enyl)-3,5,3′4′-tetrahydroxy stilbene	0 μg/g fresh wt.	950 μg/g fresh wt.	Aguamah et al., 1981
					4- (3-Methyl-but-2-enyl)-3,5,4′-trihydroxystilbene (4-isopentenylresveratrol)	0 μg/g fresh wt.	1,160μg/g fresh wt.	
					4-(3-Methyl-but-l-enyl)-3,5,4′-trihydroxystilbene	0 μg/g fresh wt.	3,690μg/g fresh wt.	
Peanut kernels	*Aspergillus flavus* *Aspergilus parasiticus*	NR	Five days	23–26°C, 100% RH	*trans*-Resveratrol	—	30 μg/g dry wt.	Sobolev et al., 1995
					trans-3-Isopentadienyl-4,3′4,-trihydroxystilbene	—	10 μg/g dry wt.	
					trans-4-(3-Methyl-butyl-enyl)-3,5,4′-trihydroxystilbene (*trans*-arachidin-3)	—	95 μg/g dry wt.	
Peanut leaves	*Cercospora arachidola*	10^4 Severe infection	Four weeks	NR	Total phytoalexins	2.96 nmoles/g fresh wt.	1830.21 nmoles/g fresh wt.	Subba-Rao et al., 1996
Peanut leaves	*Cercospora arachidola*	10^3 Mild infection	Four weeks	NR	Total phytoalexins	2.96 nmoles/g fresh wt.	745.17 nmoles/g fresh wt.	Subba-Rao et al., 1996
Peanut leaves	*Puccinia arachidis*	10^4 Severe infection	Four weeks	NR	Total phytoalexins	2.96 nmoles/g fresh wt.	161.59 nmoles/g fresh wt.	Subba-Rao et al., 1996
Peanut leaves	*Phaeoisariopsis personata*	10^4 Severe infection	Four weeks	NR	Total phytoalexins	2.96 nmoles/g fresh wt.	663.57 nmoles/g fresh wt.	Subba-Rao et al., 1996
Peanut leaves and roots	Yeast	25 mg/mL yeast extract	Six hours in the dark	NR	*trans*-Resveratrol -in leaves -in roots	0.15 μg/g	1.2 μg/g 2.8 μg/g	Chung et al., 2003
Grape leaf discs	*Plasmopora viticola*	NR	NR	NR	*trans*-Resveratrol ε-viniferin α-viniferin	—	—	Langcake and Pryce, 1977

[1]No data reported.

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Likewise, in leaves of transgenic tomato plants, a rapid accumulation of resveratrol occurred during the first six hours after inoculation of *Phytoptera infestans* and reached a maximum of 258-μg/g fresh weight after about 44 hours (Thomzik et al., 1997). Inoculation of transgenic tomatoes with *Botrytis cinerea* and *Alternaria solani* also followed a similar trend and afterward, resveratrol accumulation slowly declined. After the elicitor treatment, resveratrol content of 51.6-μg/g fresh weight was detected in immature tomatoes and 99-μg/g fresh weight in slices of ripe fruit. In whole tomatoes, inoculated with *P. infestans*, resveratrol increased from 1.7-μg/g fresh weight after 24 hours to 6.9-μg/g fresh weight after 96 hours. These were low compared with the amount detected in leaves and elicitor-treated fruit slices, which Thomzik et al. (1997) attributed to probable dilution with the noninfected tissue of the fruit pulp.

In peanut leaves, seven phytoalexins were extracted from peanut leaves infected with early leaf spot fungus, *Cercoscopora arachicola*, then separated and quantified by Edwards and Strange (1991). The three major phytoalexins include demethylmedicarpin, formononetin, 7,4′-dimethoxy-2′-hydroxyisoflavonone, and medicarpin while two minor components are 7,2′-dihydroxy-4′-methoxyisoflavonone and daidzein in infected peanut leaves. Except for the low concentrations of medicarpin, all other phytoalexins were essentially absent from the uninfected leaves, indicating that fungal infection elicited biosynthesis of these compounds.

Phytoalexins were also produced in rice leaves three days after 1-mm press-injured spots on leaves were infected with 27 μL of suspension containing 4×10^5 spores/mL *Pyricularia oryzae*, a rice blast fungus (Kato et al., 1993). Based on their experiment, phytoalexins were not produced in uninfected leaves. Infected leaves produced 37.7-ng/spot momilactone A, 11.4-ng/spot oryzalexin D, and 12.3-ng/spot oryzalexin E.

Citrus lemon seedlings inoculated with conidia from *Alternaria alternaria* or treated with fungal elicitors increase phenylpropanoid metabolism and synthesize umbelliferone and scoparone as part of the developed hypersensitive response (Castañeda and Perez, 1996). Fungal elicitation resulting in the transduction of the initial signal produced by oligomers of galacturonic acid containing 19 sugar residues caused by fungal elicitation is otherwise unknown to this species. Calcium ions also participated in signal transduction has been reported in several plant systems, as well as its role as a second messenger.

Microbial Invasion and/or Inoculation of Yeasts

Yeast extract was used to increase the levels of resveratrol in peanut leaves and roots (Chung et al., 2003). Leaves and roots of sterile peanut plants that had been grown in vitro for four weeks were placed under dark conditions for 48 hours. The roots of intact plants were then incubated in half strength Murashige and Skoog (MS) medium containing 25-mg/mL yeast extract and placed in the dark for six hours. Treatment of peanut plant materials with yeast extract accumulated resveratrol in the peanut leaves up to 1.2-μg/g fresh weight and in the roots up to 2.8-μg/g fresh weight, which corresponds to 8- and 19-fold increases relative to untreated control tissues. Levels of resveratrol synthase and mRNA also increased in leaves and roots treated with yeast extract, and these increases were correlated with the increase in resveratrol contents in the tissues. This indicates that yeast extract induces resveratrol accumulation via transcription of RS mRNA in peanut plants.

Microbial Invasion and/or Inoculation of Bacteria

Increase in the levels of phenolic compounds was enhanced in peanut leaves 50 days after planting of peanut hypocotyls inoculated with 1-mL cell suspension containing 1×10^7 cells/mL of *Bradyrhizobium* spp. (*Arachis*) strain (Azpilicueta et al., 2004). The inoculated and uninoculated plants were grown in a growth chamber at 28/22°C under 16/8-hour light/dark regime and watered every other day either with N-free (inoculated plants) or standard Hoagland solution (uninoculated plants). Total phenolics in leaves of inoculated plants ranged from 14.6- to 20.03-μg ρ-coumaric/mL compared with 5.71- to 9.82-μg ρ-coumaric/mL in uninoculated plants, a 2.6–3.5 fold increase.

Abiotic Stresses

Soaking or Imbibing in Water

Soaking raw peanuts in water for about 20 hours and then drying for 66 hours increased the resveratrol content between 45 and 65 times after treatment (Seo et al., 2005). Similarly, increase in resveratrol concentration from 0.48 in controls to 0.96 and 1.46 μ/g was observed when raw peanuts were imbibed in water for 16 hours and then incubated at 25°C for 24 and 36 hours, respectively (Rudolf and Resurreccion, 2005).

Wounding

Wounding by slicing has been shown to elicit synthesis of resveratrol and other phytoalexins in peanuts (Arora and Strange, 1991; Rudolf and Resurreccion, 2005). Table 4 shows the concentrations of resveratrol and phytoalexins in response to wounding of peanut and peanut plant materials.

The phytoalexin concentrations of 10 peanut cultivars imbibed with water, sliced and incubated at 25°C for 24 hours ranged from 28- to 935-μg/g fresh weight and vary depending on cultivar conditions, duration of incubation after slicing, and crop history (Wotton and Strange, 1985). Reduced phytoalexin yields were obtained when sliced peanut kernels of one cultivar studied were incubated in water at 37°C, and no phytoalexin was obtained when the slices were incubated under nitrogen gas or frozen before aerobic incubation (Wotton and Strange, 1985).

Arora and Strange (1991) imbibed the cotyledons of peanuts, wounded by slicing 1–2 mm thick, and incubated for 48 hours at 25°C in the dark, which resulted in the accumulation of up to 0.59-μM/g resveratrol, 0.02–0.98-μM/g arachidin III, and

Table 4 Concentrations of *trans*-resveratrol and other stilbenes in peanut kernels stressed by postharvest abiotic treatment of wounding through slicing, chopping, and grinding

Postharvest stress treatment condition			Compound			
	Incubation			Concentration (μg/g, dry basis)		
Wounding Treatment	Time (hours)	Temperature (°C)	Name	Control	Stressed	Reference
Slicing 2 mm	24	25	*trans*-Resveratrol	NR[1]	4.3–23.8	Cooksey et al., 1988
			3-Isopentadienyl-4,3′,5′-trihydroxystilbene	NR	38.6–105.8	
Slicing 2 mm	48	25	*trans*-resveratrol	NR	21.2–42.2	Cooksey et al., 1988
			3-Isopentadienyl-4,3′,5′-trihydroxystilbene	NR	89.5–157.5	
Slicing 1–2 mm	48	25	*trans*-Resveratrol	<22.82 (wet basis)	134.64 (wet basis)	Arora and Strange, 1991
Slicing 2 mm	0–144	25 and 37	Arachidon I, II, III	0 (wet basis)	>4000 (wet basis)	Wotton and Strange, 1985
Slicing 2 mm	24	25	*trans*-Resveratrol	0.22	1.43	Rudolf and Resurreccion, 2005
	36				1.06	
	48				2.15	
Grinding 1–2 mm	24	25	*trans*-Resveratrol	0.18	0.65	Rudolf and Resurreccion, 2005
	36				0.76	
	48				0.49	
Chopping 5mm	24	25	*trans*-Resveratrol	0.25	1.47	Rudolf and Resurreccion, 2006
	36				0.89	
	48				0.74	
Whole peanut	24	25	*trans*-Resveratrol	0.20	0.96	Rudolf and Resurreccion, 2006
	36				1.46	
	48				1.44	

[1]No data reported.

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1.11–4.38-μM/g arachidin IV. The concentrations of phytoalexins produced in wounded peanuts depend on cultivar, maturity, and viability of seeds. Peanut cultivars, PI 337394F and J11, which were resistant to seed colonization by *Aspergillus flavus* and aflatoxin contamination, accumulated more than three times as much arachidin IV as the susceptible cultivars, Gangapuri and TMV2. All parts of the developing peanut pod synthesized phytoalexins with diminishing capacity from immature stages of 1 to 4 but increased thereafter up to mature stage of 8. However, in contrast with mature cotyledons, mature pods and testa lost their ability to synthesize phytoalexins (Arora and Strange, 1991). Peanut seeds had been stored at 15°C for 9 months, then sliced drastically reduced their ability for the cotyledon to synthesize phytoalexins as evidenced by lower phytoalexins concentrations compared with fresh ones. Arora and Strange (1991) similarly found that stored peanuts for 6 months then sliced produced lower *trans*-resveratrol compared with unstored controls. Nonviable seeds, as a result of long-term storage, when sliced had lower total phytoalexin concentration of 0.716 μM/g compared with 5.29 μM/g in nonstored viable peanuts, a 7.4-fold increase (Arora and Strange, 1991).

Slicing of germinated peanut seeds followed by incubation at 23–25°C with artificial aeration tremendously increased the concentrations of *trans*-resveratrol and its derivatives (Chang et al., 2006). The *trans*-resveratrol, *trans*-arachidin I, and *trans*-arachidin III increased from initially trace or not detectable up to 147.3, 495.7, and 2414.8 μg/g, respectively, and corresponding to 20-, 16-, and 24-hour incubation, respectively. The concentration of *trans*-isopentadienylresveratrol (arachidin IV) continued to increase up to 4474.4 μg/g after 28 hours of incubation.

Grinding of fully imbibed raw peanuts then incubating for 48 hours at 45°C increased resveratrol to 0.85 μg/g compared with controls with 0.14 μg/g (Rudolf et al., 2002). In a subsequent study, comparing concentrations of sliced, chopped, and ground peanuts, wounding the peanuts by slicing 2 mm produced the highest resveratrol concentration of 2.15 μg/g, followed by chopping then grinding with 1.47 and 0.76 μg/g, respectively, compared with untreated controls having 0.48 μg/g (Rudolf and Resurreccion, 2005). Raw, whole imbibed peanuts produced only 1.46 μg/g resveratrol, a level lower than in sliced peanuts but similar to chopped samples (Rudolf and Resurreccion, 2005). These results showed that wounding by slicing produced maximum concentrations of resveratrol in peanut kernels, whereas chopping and grinding resulted in moderate and lowest concentrations, respectively, suggesting that as the severity of mechanical stress increased with size reduction, resveratrol production decreased (Sales and Resurreccion, 2009) but without application of mechanical stress (such as in whole kernels) did not result in significant resveratrol synthesis.

In healthy peanut leaves, wounding the trifoliate leaves by punching with fine fins increased resveratrol content up to twofold (Chung et al., 2003). The wounded leaves were floated on the sterile water, kept for the first 12 hours in the light with intensity of about 100 μE (m^2/second), and then for 12 hours in the dark at 25°C.

Similarly in tomato leaves, Pearce et al. (1998) found out that the amounts of two phenolic compounds, E-feruloyltyramine and E-ρ-coumaroyltyramine, increased 10-fold after 24 hours in response to mechanical wounding. The time course for the accumulation of the compounds increased steadily for about

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10 hours and then began to plateau until 72 hours. These researchers (Pearce et al., 1998) also assayed several elicitors at levels known to maximally induce defense related proteins in various plant species including tomato to test their ability to induce accumulation of feruloyltyramine in the leaves of young excised tomato leaves. After 24 hours from induction, feruloyltyramine increased by 25-fold in wounded leaves in response to the oligosaccharide elicitor, chitosan. Using fluorescence detection, feruloyltyramine was shown to accumulate in response to wounding but not to jasmonic acid or systemin, two elicitors of systemic wound-inducible defense responses in tomato. Pearce and his group (1998) observed that feruloyltyramine also accumulated in leaves of mutant tomato plants deficient in the octadecanoid signaling pathway in response to wounding, indicating that its synthesis is not regulated through this pathway. They concluded that these data support a role for hydroxycinnamate-tyramine conjugates as part of the array of defense chemicals and protective biopolymers induced in leaves and other plant tissues by wounding to protect the plants against pathogen and herbivore attacks.

Wounding combined with abiotic stress by allowing natural microflora to proliferate during a 3–5-day incubation period increased synthesis of phytoalexins in treated peanuts (Keen, 1975). The extracts from germinating seeds and stems of American peanuts, Virgina Jumbo, and Spanish cultivars, synthesized two phytoalexin compounds, which were identified by (Keen and Ingham, 1976) as the *cis* and *trans* isomers of 3,5,4′-trihydroxy-4-isopentenylstilbene (4-isopentenylresveratrol).

Three phytoalexin compounds were synthesized in sliced peanuts 1–2 mm thick, previously imbibed with water overnight, and then incubated in the dark at 25°C for 48 hours to allow natural microflora to proliferate (Aguamah et al., 1981). The major phytoalexin compound isolated has a concentration of 3.69 mg/g dry weight of seed and was suggested to have a structure of 4-(3-methyl-but-1-enyl)-3,5,3′4′-tetrahydroxystilbene based on proton NMR (H-NMR) spectral analysis. The second compound with a concentration of 1.16 mg/g dry weight of seed was confirmed to be 4-(3-methyl-but-2-enyl)-3,5,4′-trihydroxystilbene (4-isopentenylresveratrol), the same compound previously identified by Keen and Ingham (1976) from peanuts. The third compound had a concentration of 0.95 mg/g dry weight of seed and was suggested to have a structure of 4-(3-methyl-but-1-enyl)-3,5,4′-trihydroxystibene, also called arachidin III (Arora and Strange, 1991).

Exposure to Ultraviolet (UV) Light

Exposure to UV-light radiation was shown to elicit the biosynthesis of phytoalexins such as resveratrol in plant and plant materials (Langcake and Pryce, 1997; Cantos et al., 2000). UV induces the increase in enzymes such as phenylammonialyase responsible for biosynthesis of secondary metabolites such as flavonoids and resveratrol, which act as screens to prevent UV-induced damage to genetic material of plant cells (Cantos et al., 2000). Table 5 lists UV irradiation stress treatments applied by various researchers in peanuts and grapes to enhance synthesis of resveratrol and other bioactive compounds.

Earlier research on the role of UV irradiation on the biosynthesis of resveratrol and other bioactive compounds was conducted on grapes and grape plant materials and are therefore discussed in this section. In grape leaves, Langcake and Pryce (1977) found that the maximum concentration of resveratrol was produced at 260–270 nm. At wavelengths above 300–310 nm or below 230 nm, little or no resveratrol production occurred. Natural sunlight contains insufficient radiations in wavelengths below 300 nm, and this probably explains why resveratrol biosynthesis is not induced in field grown plants (Langcake and Pryce 1977). Sunlight does not act as an inducer under natural conditions, and it is explained that DNA itself is the actual photoreceptor for this response (Soleas et al., 1997). Resveratrol was consistently produced very rapidly after UV irradiation, rising to a maximum at 18 hours and decreasing thereafter (Langcake and Pryce, 1977). Resveratrol biosynthesis was confirmed to follow the phenylalanine–polymalonate pathway after incorporating radiolabeled precursors, 2–^{14}C-acetate, 2–^{14}C-malonate, U-^{14}C- phenylalanine, and U-^{14}C-tyrosine into resveratrol prior to UV irradiation of both leaves and immature grape berries (Langcake and Pryce, 1977).

A number of studies on the UV irradiation of grapes were reported by Cantos and coworkers. In their first report, Cantos et al. (2000) found that refrigerated storage and UV irradiation of table grapes increased the concentrations of potentially health-promoting phenolics, which include the anthocyanins malvidin 3-glucoside and its acetyl and *p*-coumaroyl derivatives, cyanidin 3-glucoside, peonidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, and delphinidin 3-glucoside. In addition, quercetin 3-glucoside and 3-glucuronide, caffeoyl tartaric, piceid, and resveratrol were also detected. The concentrations of most phenolics remained quite constant during postharvest refrigerated storage of 10 days at 0°C. However, postharvest treatments of Napoleon grapes with UVC and UVB lights induced a large increase in *resveratrol* derivatives, three- and twofold, respectively. The result of this study suggests that a 200 g serving of mature UV-irradiated Napoleon grapes provides approximately 1 mg of *resveratrol*, which is in the range of the amount supplied by a 140-mL glass of red wine. This resveratrol concentration can be increased to 2 or 3 mg per serving in grapes that have been irradiated with UVB or UVC, respectively.

In another study, Cantos et al. (2001) optimized the UV irradiation of grapes and concluded that the optimum process included exposure of grapes for 60 seconds under a 510-W UV lamp at a distance of 40 cm. The distance of 40 cm from UV light was the optimum, achieving the highest resveratrol content in UV-treated grapes compared with 20 and 60 cm. The lower resveratrol contents at 20 cm distance may have been "too strong" causing damage to the "biosynthetic system" of the resveratrol, whereas at 60 cm, delayed the induction of resveratrol biosynthesis occurred (Cantos et al., 2001).

Later, this optimum UV process for grapes (Cantos et al., 2001) was applied to irradiate seven grape varieties—red table

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Table 5 UV light stress treatments used to enhance resveratrol and other bioactive compounds in peanuts and grapes

	UV treatment and conditions					
Substrate	UV light parameters	UV light exposure time	Incubation time and temperature	Compound/concentration	Remarks	Reference
Peanut kernels, Runner, ground, chopped, sliced, whole	254 nm; 30W; 40 cm distance from the lamp; peanuts arranged 1 cm depth on plastic tray	10 minutes	0 to 48 hours at 25°C in the dark	*trans-r*esveratrol content in ground peanuts, 0.17–0.86 μg/g; chopped peanuts 0.30–1.64 μg/g; sliced peanuts, 0.33–3.42 μg/g; whole peanuts, 0.20–1.76 μg/g	Highest resveratrol content of 3.42 μg/g was found in sliced peanut kernels exposed to UV light for 10 minutes and incubated for 48 hours at 25°C.	Rudolf and Resurreccion, 2005
Peanut hulls, Spanish	UV 110 V, 60 Hz, 110 mm from UV light	0 to 6 days	25°C	Flavonoid: luteolin at 0 day: 1.74 mg/g after three days: 1.73 mg/g after six days: 1.21 mg/g		Duh and Yeh, 1995
Peanut hulls, Spanish	UV 110 V, 60 Hz, 110 mm from UV light	0 to 6 days	25°C	Total phenolics at 0 day: 7.8 mg/g (catechin equivalents) after three days: 7.53 mg/g after six days: 7.05 mg/g		Duh and Yeh, 1995
Peanut leaf	254 nm, 180 μW/cm^2 energy, 20 cm distance from lamp	48 hours	25°C for 96 hours at high humidity	Phytoalexin - abaxial (lower) surface 393 nmols/g (fresh weight) - adaxial (upper) surface 115 nmols/g (fresh weight)	*trans*-Resveratrol in the adaxial surface was not significantly different from control samples with 115 nmols/g phytoalexin (fresh weight)	Subba Rao et al., 1996
Peanut leaves, sterile	1.35 μE (m^2/s) UV lamps	two hours	0, 3, 12 hours in the dark	Resveratrol increased up to 225 fold after UV treatment and incubation for 12 hours	Free resveratrol contents of control, leaves, pods, and roots of healthy plants grown in the fields up to 40 days from flowering were 2.05, 1.34, and 1.19 μg/g fresh weight, respectively	Chung et al., 2003
Peanut callus	UVC light, 254 nm, 55 cm distance from the lamp	20 minutes	Static condition: 0 to 24 hours at 25°C in the dark Suspension condition: 0 to 80 hours at 25°C in the dark	Static condition: resveratrol: 0.25–11.97 μg/g, piceatannol: 2.17–5.31 μg/g Suspension condition: resveratrol: 3.14–6.93 μg/g, piceatannol: 0.32–0.52 μg/g	Static condition: *trans*-resveratrol increased from 0.25 to 11.97 μg/g after 6 to 18 hours of incubation then decreased to 1.42 after 24 hours of incubation. Piceatannol increased from 2.17 to 5.31 μg/g after 12 to 18 hours of incubation and then decreased to μg/g after 24 hours of incubation. Suspension condition: highest *trans*-resveratrol content of 6.93 μg/g was obtained after four hours of incubation suspension. Initial piceatannol content of 0.52 μg/g was maintained from 0 to 8 hours of incubation then decreased when incubation time was further increased	Ku et al., 2005

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Grape berries, immature	254 nm, 17 cm distance from lamp	10 minutes	18 hours at 26°C in the dark	Resveratrol U-^{14}C-phenylalanine = 0.139 μmol U-^{14}C-tyrosine = 0.009 μmol 2–^{14}C-malonate = 0.014 μmol 2–^{14}C-acetate = 0.124 μmol	Grape berries were cut longitudinally and radioactive substance was applied to the cut surfaces. Amounts of *trans*-resveratrol formed in berries were generally lower than in leaves.	Langcake and Pryce, 1977
Grape berries, mature	254 nm	30 minutes	10 days at 0°C followed by five days at 5°C	Resveratrol 100 μg/g	*trans*-Resveratrol production increased by twofold after 30 minutes exposure to UVC light with peak output at 254 nm.	Cantos et al., 2000
Grape berries, mature	340 nm	30 min	10 d at 0°C followed by 5d at 5°C	Resveratrol 65 μg/g	*Trans*-resveratrol production increased by to three-fold after 30 min exposure to UVB light with peak output at 340 nm.	Cantos et al., 2000
Grape berries, immature	254 nm	30 minutes	10 days at 0°C followed by 5 days at 5°C	Resveratrol 65 μg/g		Cantos et al., 2000
Grape berries, immature	340 nm	30 minutes	10 days at 0°C followed by five days at 5°C	Resveratrol 45 μg/g		Cantos et al., 2000
Grapes, Napoleon red	510-W irradiation power, 40 cm distance from lamp	30 seconds	Three days storage	Resveratrol 115μg/g of skin	A serving of UV-irradiated grapes could supply 7.5 mg/200 g serving *trans*-resveratrol equivalent to three glasses of red wine. Untreated grape contained 10 μg/g of skin.	Cantos et al., 2001
Grape berries	254 nm	10 minutes	24 hours at 24°C, 48 hours at 24°C	Phytoalexin 50 to 233.38 μg/g, 150 to 400.08 μg/g		Creasy and Coffee, 1988
Grape berry skin	254 nm, 2.5 W/m^2 energy	10 minutes	Four hours at 24°C, 48 hours at 24°C	Phytoalexin 0.8–0.12 μg/cm^2, 21 μg/cm^2		Creasy and Coffee, 1988
Grapevine leaves (Vitaceae)	254 nm, 3.7 W/m^2 energy	10 minutes	23 hours at 25°C in the dark	Resveratrol 50–100 μg/g	Infection of leaves by *Botrytis cinerea* produced greater *trans*-resveratrol content of 50 to 400 μg/g	Langcake and Pryce, 1976
Grape leaves	260–270 nm	Equivalent to 10 minutes at 250 nm	20 hours at 26°C in the dark in moist filter paper	Resveratrol 1.4–1.5 μg/14-mm-diameter disc	UV spectrum above 300 nm and below 230 nm produced little or no *trans*-resveratrol	Langcake and Pryce, 1977
Grape leaves	254 nm, 12 cm distance from lamp	15 minutes	20 hours	Resveratrol U-^{14}C-phenylalanine = 0.14 μmol U-^{14}C-tyrosine = 0.11 μmol 2–^{14}C-malonate = 0.21 μmol 2–^{14}C-acetate = 0.23 μmol	*trans*-Resveratrol was produced very rapidly rising to a maximum at 18 hours and decreasing thereafter. By incorporating radiolabeled precursors, 2–^{14}C-acetate, 2–^{14}C-malonate, U-^{14}C phenylalanine, and U-^{14}C-tyrosine, into resveratrol prior to UV light exposure, it was confirmed that resveratrol is biosynthesized by the phenylalanine-polymalonate pathway	Langcake and Pryce, 1977

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grape varieties of Flame, Red Globe, Crimson, and Napoleon, and white varieties of Superior, Dominga, and Moscatel Italica to determine elicitation of three most UVC inducible stilbenes in grapes, *trans*-resveratrol, *trans*-piceatannol, and viniferins (Cantos et al., 2002). The total resveratrol content on a fresh weight basis ranged from 0.69 mg/100 g in Dominga variety to 2.3 mg/100 g in Red Globe. The net resveratrol induction ranged from 3.4-fold in Flame variety to 2315-fold in Red Globe. The Flame variety had the highest viniferins content of 0.73 mg/100 g, although the Red Globe variety presented the highest viniferins induction of 175-fold. The highest piceatannol content of 0.17 mg/100 g and induction of 173-fold were observed in the Flame variety. They (Cantos et al., 2002) stressed that, taking into account the health-beneficial effects claimed for stilbenes, UV-C irradiated table grapes can be considered as new "functional fruits" that can supply resveratrol content equivalent to more than seven glasses of red wine (approximately 1.5 L), from a serving of 200 g unpeeled table grapes, depending on the variety (Cantos et al., 2002).

Furthermore, Cantos et al. (2003) found that UVC irradiation of "Monastrell" grapes increased stilbene content, of mainly *resveratrol* and piceatannol. The evolution of both compounds was followed through the different steps of an "analytical" traditional maceration wine-making process. The final wine made from UVC-irradiated grapes was enriched by about 2- and 1.5-fold *resveratrol* and piceatannol, respectively, compared with the control wine. No difference was detected regarding the standard enological parameters (color, acidity, etc.). These researchers suggested that the use of more susceptible wine grapes to induce bioactive stilbenes upon UVC irradiation can produce a much higher stilbene-enriched wine (Cantos et al., 2003).

UV-induced resveratrol biosynthesis in skins of developing grapes, red Shiraz and Cabernet Sauvignon, and white Semillon and Chardonnay (Bais et al., 2000). The developing grape berries were placed on orbital shaker set at 50 rpm, the rolling berries were irradiated for 10 minutes at a distance of 10 cm below UVC light with a peak output of 254 nm, and then incubated in the dark at 25°C for 72 hours. All four cultivars displayed similar patterns of UV-inducible resveratrol accumulation throughout berry development, and there were no major differences between the responses of white- and red-skinned cultivars. Preliminary experiments of Bais et al., (2000) revealed that resveratrol was induced only in berries that had been irradiated with UVC (<280 nm) but not in control berries, findings similar to those of Langcake and Pryce (1977).

There was a marked increase in the ability of the UV-irradiated berry skins to synthesize resveratrol, 1–5 weeks postflowering ranging from 150–330-μg/g fresh weight at week 1 to 810–1075-μg/g fresh weight at week 5. The capacity of berry skin resveratrol synthesis after week 5 progressively declined such that in week 16, resveratrol levels were only 11–113-μg/g fresh weight. These values are equivalent to an average 10-fold decline in Chardonnay, Shiraz, and Cabernet Sauvignon, and a 100-fold decline in Semillon, with regard to the ability of berry skin tissue to synthesize resveratrol 5–16 weeks postflowering (Bais et al., 2000).

The earliest work on UV elicitation of phytoalexins in peanuts was done on peanut leaves (Subba-Rao et al., 1996). When the abaxial (lower) or adaxial (upper) surfaces of the leaves were exposed 20 cm below the shortwave UV lamp (254 nm; 180 μW/cm^2) for 48 hours and then incubated for 96 hours at 25°C in the dark, the UV irradiated abaxial leaf resulted in 393-nmol phytoalexin/g fresh weight, which was more than three times higher than that produced in adaxial leaf surface with only 115-nmol phytoalexin/g fresh weight (Subba-Rao et al., 1996). Phytoalexin concentration in adaxial leaves was not significantly different from untreated controls with 104-nmol/g fresh weight. Similarly, Chung et al. (2003) reported accumulation of resveratrol in peanut leaves in response to UV light treatment using UV lamps at 1.35 microEinstein, μE (m^2/second) for two hours, which increased up to 225-fold after 12-hour incubation in the dark.

To our knowledge, the earliest work on UV irradiation of peanut kernels for resveratrol elicitation was conducted by Resurreccion and coworkers. Rudolf and Resurreccion (2005) exposed fully imbibed peanuts that were sliced (2 mm), ground (1–2 mm), chopped (0.5 cm), and whole at 40 cm below a UV germicidal lamp (254 nm, 30 W) for 10 minutes followed by incubation in the dark at 25°C for 24, 36, and 48 hours. Exposure to UV light of all peanut samples, regardless of size, resulted in a significant increase in *trans*-resveratrol concentration from 0.17 to 1.76 μg/g as incubation period increased from 0 to 36 hours. From 36 to 48 hours of incubation, the level of resveratrol increased in sliced peanuts from 1.70 to 3.42 μg/g but decreased in whole peanuts from 1.76 to 0.99 μg/g and did not change in ground (from 0.86 to 0.67 μg/g) and chopped (from 1.52 to 1.64 μg/g) peanuts. The highest *trans*-resveratrol concentration of 3.42 μg/g was obtained in UV-treated sliced peanuts that were incubated for 48 hours at 25°C. A similar maximum resveratrol concentration of 3.30 μg/g was obtained after peanuts were UV irradiated for 30 minutes at 40 cm distance from UV light and incubated for 36 hours at 25°C (Sales and Resurreccion, 2009). A lower concentration of 2.36-μg/g resveratrol was achieved when peanuts were exposed to UV for 20 minutes at 40 cm distance from UV light and incubated for 44 hours at 25°C (Potrebko and Resurreccion, 2009).

Tokusoglu et al. (2005) found that after exposing the samples of edible peanut and pistachio varieties in UV light for one minute, *trans*-resveratrol concentrations ranged from 0.02 to 1.47 μg/g and *cis*-resveratrol from 0.008 to 0.32 μg/g. They found that *cis*-resveratrol in pistachios was higher than in peanuts. The occurrence of resveratrol in peanut and pistachio was confirmed using total ion chromatograms (TICs) of bis[trimethylsilyl]trifluoroacetamide derivatives of resveratrol isomers, and by comparison of the mass spectral fragmentation data with those of a resveratrol standard.

UV irradiation of peanut callus produced not only resveratrol but also piceatannol, which was not present in untreated samples (Ku et al., 2005). Peanut callus were exposed to UV at 254 nm for 20 minutes at a distance of 55 cm from UV light and then incubated at 25°C for 0–24 hours producing 2.17–5.31-μg/g piceatannol after 12–24-hour incubation and

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0.25–11.97-μg/g resveratrol after 6–24-hour incubation, under static cultivation condition of reaching their maximum concentrations after 18 hours. Piceatannol produced by calluses in this study was much higher than the values reported in the literature, whereas the resveratrol produced was comparable with reported values (Ku et al., 2005).

UV-light exposure treatment alone decreased the amount of resveratrol in raw peanuts (Seo et al., 2005). However, the synergistic effect of UV-light exposure and soaking treatment induced resveratrol production increasing between 45 and 65 times after a soaking treatment compared with untreated raw peanuts (Seo et al., 2005).

In rice leaves, UV light was used for the biosynthesis of phytoalexins, diterpenes. Kodama et al. (1988) observed the accumulation of phytoalexins A, B, C, and D, momilactones A and B, and an unknown antifungal substance accompanied by the appearance of brown spots on the leaf surface after UV irradiation (15 W) of detached rice leaves for 20 minutes at 20 cm distance from UV light and incubation at 30°C in a moist box at high humidity. Momilactone A was detected in abundance and among the oryzalexins, oryzalexin D was a major substance. The maximum accumulation of these phytoalexins, except for oryzalexins C and D, was found three days after UV irradiation. Maximum accumulations of Oryzalexin D were observed after two days while that of oryzalexin C was after four days. Kato et al. (1993) later found diterpene phytoalexin oryzalexin E in rice leaves using the same irradiation treatment except incubating the leaves at 27°C at high humidity under dark conditions for 12 hours and then under light conditions for two days.

Ultrasound Processing

Ultrasound technology using high-frequency sound waves, above the upper range of human hearing or about 18 kHz, is a powerful technique with application in material analysis research and product development (Hancock, 1999). Ultrasonication produces cavitation phenomena when acoustic power inputs are sufficiently high to allow multiple productions of microbubbles that collapse and create shock waves that cause cells to disintegrate into very fine cell debris particles (Hancock, 1999).

Ultrasound is an emerging technology in the food processing industry for the enzymatic synthesis of glucose esters, in combination with extrusion process to improve the nutritional and functional quality of sorghum flour (Xiao et al., 2005). Lin et al. (2001) proposed that ultrasound treatment at low intensities of ginseng cell cultures, a nonfood material, stimulates growth and biosynthesis of secondary metabolites through mechanical stress and microstreaming induced by acoustic cavitation, which disrupts the cell wall. Ultrasound caused rapid increase in levels of enzymes, phenylammonia lyase, polyphenol oxidase, and peroxidase in ginseng cell cultures (Wu and Lin, 2002). Among these enzymes, phenylammonia lyase, the key enzyme of the phenylpropanoid pathway responsibles for stilbenes biosynthesis, was enhanced most dramatically by ultrasound with fivefold higher at power level 4 after four-day incubation compared with controls.

There are a very limited number of researchers who utilized ultrasound for the synthesis of bioactive compounds in food (Table 6). To our knowledge, major research on the application of ultrasound treatment to peanuts was conducted by Resurreccion and coworkers. Rudolf and Resurreccion (2005) investigated the effects of ultrasound treatment on increasing resveratrol concentrations in peanuts by sonicating whole and size-reduced (sliced 2 mm, ground 1–2 mm, and chopped 0.5 cm) fully imbibed peanut kernels in an ultrasonic bath cleaner with a power density of 39.2 mW/cm^3 for four minutes at 25°C and incubating at 25°C in the dark for 24, 36, and 48 hours. Significant increases in *trans*-resveratrol concentrations after 24-hour incubation were observed in all samples, from 0.10–0.26 at 0 hour to 0.75–2.54 μg/g after 24 hours with the largest increase to 2.5 μg/g in sliced peanuts. From 24 to 36 hours of incubation, the concentrations of *trans*-resveratrol significantly decreased in chopped peanuts from 1.3 to 0.93 μg/g, increased in whole peanuts from 0.76 to 1.47 μg/g, and did not change significantly in ground kernels (from 0.75 to 0.69 μg/g) and increased in sliced (2.54 to 3.96 μg/g) peanuts. No significant change in *trans*-resveratrol concentrations of all samples was observed from 36 to 48 hours of incubation. The highest *trans*-resveratrol concentration of 3.96 μg/g was obtained in sliced peanuts incubated after 36 hours. In more recent studies, the highest maximum *trans*-resveratrol concentration of 6.39 μg/g was achieved when peanuts were treated with ultrasound power density of 75 mW/cm^3 for two minutes followed by incubation at 25°C for 48 hours, among 27 ultrasound treatments (Sales and Resurreccion, 2009). In a separate study, using different parameters, sliced peanuts exposed to ultrasound power density of 40 mW/cm^3 for four minutes then incubated for 44 hours at 25°C produced 4.29-μg/g *trans*-resveratrol (Potrebko and Resurreccion, 2009). Ultrasound caused rapid increase in the concentrations of phenylammonia lyase, polyphenol oxidase, and peroxidase in ginseng cultures with phenylammonia lyase enhanced most dramatically (Wu and Lin, 2002). Phenylammonia lyase is responsible for the deamination of phenylalanine, the initial step in the biosynthesis of coumaryl CoA, one of the precursors for resveratrol synthesis. We believe that the release of phenylammonia lyase after ultrasound treatment could be responsible for increased concentrations of resveratrol in ultrasound-treated peanuts.

Chukwumah et al. (2005) used ultrasound at combined frequency of 25, 60, and 80 kHz for 30, 60, 90, 120, 150, and 180 minutes to extract selected phytochemicals from raw peanuts with 80% ethanol. They attribute improvement in extraction efficiency of ultrasound, a nonthermal procedure, to the enhancement of cell disruption, solvent penetration, and mass transfer (Chukwumah et al., 2009). The concentrations of resveratrol ranged from 0.11 $\pm$ 0.05 to 0.27 $\pm$ 0.01 mg/100 g; biochanin, 0.22 $\pm$ 0.01 to 0.32 $\pm$ 0.01 mg/100 g; and genistein, 0.01 $\pm$ 0.00 to 0.6 $\pm$ 0.02 mg/100 g. The highest amounts of resveratrol and biochanin were obtained after 150-minute

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Table 6 Ultrasound stress treatments used to enhance resveratrol and other bioactive compounds in peanuts and various food plants

Substrate	Ultrasound treatment and conditions: Ultrasound power density	Exposure time	Incubation time and temperature	Compound/concentration	Remarks	Reference
Raw peanut, Runner, whole kernel	39.2 mW/cm^3	Four minutes	24–48 hours at 25°C	*trans*-Resveratrol 0.97 to 1.76 μg/g	Highest *trans*-resveratrol of 1.76 μg/g was obtained when peanuts were incubated for 36 hours. Control had 0.2-μg/g *trans*-resveratrol	Rudolf and Resurreccion, 2005
Raw peanut, Runner sliced, 2 mm	39.2 mW/cm^3	Four minutes	24–48 hours at 25°C	*trans*-Resveratrol 1.31 to 3.42 μg/g	Highest *trans*-resveratrol of 3.42 μg/g was obtained when peanuts were incubated for 48 hours. Control had 0.2-μg/g *trans*-resveratrol	Rudolf and Resurreccion, 2005
Raw peanut, Runner, ground, 2 mm	39.2 mW/cm^3	Four minutes	24–48 hours at 25°C	*trans*-Resveratrol 0.49 to 0.86 μg/g	Highest *trans*-resveratrol of 0.86 μg/g was obtained when peanuts were incubated for 36 hours. Control had 0.17-μg/g *trans*-resveratrol	Rudolf and Resurreccion, 2005
Raw peanut, Runner, chopped, 5 mm	39.2 mW/cm^3	Four minutes	24–48 hours at 25°C	*trans*-Resveratrol 0.67 to 1.47 μg/g	Highest *trans*-resveratrol of 1.47 μg/g was obtained when peanuts were incubated for 36 hours. Control had 0.3-μg/g *trans*-resveratrol	Rudolf and Resurreccion, 2005
Roasted peanut, Runner, sliced, 7 mm	39.2 mW/cm^3	Four minutes	44 hours at 25°C	*trans*-Resveratrol 7.15 μg/g		Rudolf and Resurreccion, 2005
Raw peanut, Runner, sliced, 10 mm	39.2 mW/cm^3	Four minutes	44 hours at 25°C	*trans*-Resveratrol 0.57 μg/g		Rudolf and Resurreccion, 2005
Roasted peanut, Runner sliced, 10 mm	39.2 mW/cm^3	Four minutes	44 hours at 25°C	*trans*-Resveratrol 2.57 μg/g		Rudolf and Resurreccion, 2005
Raw peanut	High-frequency ultrasonication (combined frequency of 25, 60, 80 kHz)	30, 60, 90, 120, 150, and 180 minutes	NR[1]	*trans*-Resveratrol 0.11 ± 0.05 to 0.27 ± 0.01 mg/100g, biochanin 0.22 ± 0.01 to 0.32 ± 0.01 g/100g, genistein 0.01 ± 0.00 to 0.6 ± 0.02 mg/100g		Chukwumah et al., 2005
Ginseng roots	82 mW/cm^3	One–four minutes	NR	Ginsenoside saponins	Increased total saponin content up to 75%	Lin et al., 2001
Ginseng roots	Indirect sonication in water bath 38.5 kHz, 810 W	One hour Two hours	25°C 25°C	Ginsenoside saponins: yield is 2.1–4.10% by weight Ginsenoside saponins: yield is 2.11–4.0% by weight	Control using conventional thermal extraction (Soxhlet method) yielded 1.15–2.10% by weight. Ultrasound-assisted extraction is three times faster than conventional thermal method and can be carried out at lower temperature. No significant difference in the recovery of saponins between indirect and direct sonication	Wu et al., 2001
	Direct sonication with probe horn connected to 600 W ultrasound microprocessor, 20 kHz, no pulse, 22% amplitude	One hour Two hours	25°C 25°C	Ginsenoside saponins: yield is 2.1–4.332% by weight Ginsenoside saponins: yield is 2.11–4.0% by weight		
Organic solvents	20 kHz at 40, 100, 120 W using continual and intermittent treatment	One hour Two hours	NR NR	Glucose esters: at 120 W, 70% yield 94% yield	60 and 75% yield, respectively, under interval ultrasound (10 minutes ultrasound then 20 minutes shaking) High- power and continual operation gave better acceleration on the yields	Xiao et al., 2005

[1]No data reported.

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ultrasound-assisted extraction, after 180 minutes for genistein while daidzein was not detected. Chukwumah et al. (2009) later reported that ultrasound-assisted extraction using sample to solvent ratio of 1:2 and a 30-minute sonication at 25 kHz was sufficient to obtain significantly higher resveratrol.

Ultrasound technology was also used in the extraction of bioactive compounds in other substrates (Wu et al., 2001; Xiao et al., 2005). Wu et al. (2001) reported that the sonication-assisted extraction was simple and more effective alternative, about three times faster than the conventional extraction methods for the isolation of ginsenosides (tripentene saponins) from various types of ginseng roots. Direct sonication by probe horn provided much higher ultrasound energy (8.2 W) to the samples but did not show a clear advantage over the indirect sonication using a cleaning bath (3.5 W) for the extraction of saponins. The extraction rate with the cleaning bath was slightly higher than that of the probe horn, which is partially attributed to the agitation and higher temperature (38–39°C) in the sample tubes in the sonic bath compared with that of the sonicator probe horn (25–27°C) (Wu et al., 2001). The researchers concluded that an ultrasound cleaning bath may be more convenient and efficient for the extraction of large number of small volume samples for the following reasons: (a) the bath can process many samples at one time while probe horn only allows for one at a time; (b) sonication with the cleaning bath is nonintrusive to the sample, which will eliminate the possible contamination and loss of the extract; and (c) cleaning bath is usually much quieter than the probe horn during operation.

Xiao et al. (2005) used ultrasound for the enzymatic synthesis of glucose esters, in combination with extrusion process to improve the nutritional and functional quality of sorghum flour. Using three different powers of 20 kHz, namely, 50, 100, and 120 W using continual and intermittent ultrasound treatments, these researchers concluded that higher ultrasound power and continual operation gave better acceleration on the yields of glucose esters without changing the character and selectivity of the enzyme in the transesterification. Ultrasound showed a remarkable acceleration of the transesterification, and the yields under continual or interval ultrasound treatment were higher than shaking over the same reaction time. The yields using continual ultrasound at 120 W were 70 and 94% after one and two hours, respectively, whereas the yields using interval ultrasound (ultrasound for 10 minutes followed by 20 minutes shaking) were only 50 and 75%, respectively. It was also observed that three continual or ultrasound powers (120, 100, and 50 W) had higher conversions of glucose compared with shaking. A 98% conversion of glucose could be obtained after two hours in 120 W in an ultrasound bath, whereas only 48% was observed in shaking under the same conditions.

Ultrasound at 35 kHz and 37°C effectively shortened the long enzymatic hydrolysis time from six hours to 30 minutes involved with conventional thermostatic devices of edible seaweeds for the heavy metals analysis (Peña-Farfal et al., 2005). The ionic strength, a variable inherent to the enzymatic activity, appears to the most important factor controlling the enzymatic hydrolysis for both conventional and ultrasound-assisted enzymatic hydrolysis. This means that the metal releases are attributed to the enzymatic action and not to leaching procedures (Peña-Farfal et al., 2005).

Treatment with Metallic Salts

Treatment of plants with metallic salts to induce stilbene production was earlier reported by Hanawa et al. (1992) when they isolated two antifungal stilbenoids and their glucosides from the leaves of *Veratrum grandiflorum* treated with cupric chloride. These compounds were identified as *resveratrol*, oxyresveratrol, piceid, and the last compound, oxyresveratrol-3-O-glucoside, isolated for the first time from a natural source. In addition, three glucosides of flavonoid apigenin-7-O-glucoside, luteolin-7-O-glucoside, and chrysoeriol-7-O-glucoside were also found in the leaves.

Adrian et al. (1996) found that the metallic salt, aluminum chloride ($AlCl_3$) can act as a potent elicitor of resveratrol synthesis in grapevine leaves. They found out that all concentrations of $AlCl_3$ from 7 to 90 millimoles were capable of inducing a high resveratrol production in the leaves of *Vitis rupestris* while greater concentrations of $AlCl_3$ from 22 to 90 millimoles were required to obtain a similar response in the leaves of *Vitis vinifera* cv. Pinot noir. Commercially, resveratrol is produced by treating grape plants' vines and shoots with aluminum chloride or aluminum sulfate and by irradiating with UVB and UVC light (Cantos et al., 2000).

Treatment with Other Chemicals

Signal molecules such as salicylic acid, jasmonic acid, and ethylene play critical roles in plant responses to various biotic and abiotic stresses (Chung et al., 2003). Salicylic acid is responsible for the induction of genes involved in the systemic acquired resistance response, whereas jasmonic acid and ethylene activate certain genes involved in the salicylic acid-independent response (Chung et al., 2003).

Subba Rao et al. (1996) found that 0.01-M salicylic acid when applied as a foliar spray to abraded leaves of peanuts is an effective elicitor of phytoalexin. A total of concentration of 1270-nmol/g fresh weight of phytoalexin is obtained when salicylic acid was applied as a spray, which was higher than when it was used as a root drench giving only 590-nmol phytoalexin/g fresh weight.

Chung et al. (2003) investigated the accumulation of resveratrol and RS gene expression in response to hormone stresses in peanut plant tissues. They immersed peanut leaves with petioles in the sterile salicylic acid, jasmonic acid, and ethephon for 0, 3, 12, and 24 hours. Results showed that resveratrol was accumulated up to three-, two-, and eightfold in response to salicylic acid, jasmonic acid, and ethylene, respectively, in a time-dependent manner up to 24 hours but not in response to abscissic acid. RS mRNA increased in response to salicylic acid, jasmonic acid, and ethylene at least 12 hours after treatment and

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also increased in a time-dependent manner in response to salicylic acid and ethylene, which reached its maximum 24 hours after treatment. In contrast, RS mRNA was not induced by abscissic acid. The levels of RS mRNA increased in response to the hormones correlated with the amounts of resveratrol accumulated, indicating transcriptional control of RS gene expression.

Medina-Bolivar et al. (2007) found that sodium acetate (10.2 mM) was the most appropriate elicitor for stilbene induction in the hairy root cultures of peanuts, compared with laminarin, copper sulfate, cellulose, and chitosan. Approximately 60-fold increase (50 to 98 μg/mg) above the levels of *trans*-resveratrol detected in nonelicited culture extracts (692 to 1813 ng/mg extract dry weight) was achieved in elicited samples. In nonelicited cultures, *trans*-resveratrol concentrations were, 80-fold higher compared with *cis* isomers (8–31 ng/mg). The levels of *cis*-resveratrol found in elicited cultures were relatively low compared with those of nonelicited cultures, ranging from 47 to 399 ng/mg in the media extract to an average of 10 ng/mg root tissue extract. *trans*-Pterostilbene in the elicited hairy root culture media varied from 61 to 267 ng/mg reflecting approximately a twofold increase over nonelicited counterparts. The *cis* isomers of pterostilbene were not detected in both elicited and nonelicited culture media. Only *trans*-pterostilbene ranging from 44 to 136 ng/mg was found in the elicited root tissues while none was detected in the nonelicited root cultures. *cis*-Pterostilbene was not detected in both elicited and nonelicited hairy root cultures.

Exposure to Ozone

Ozone is a ubiquitous component of the terrestrial atmosphere and in the stratosphere, it provides a crucial barrier to incoming UV radiation (Samuel et al., 2000). Among its numerous phytoxic effects on plants, ozone is known to induce events usually elicited by various pathogens (Biolley et al., 1998). Ozone is likewise found to stimulate elicitation of functional compounds such as resveratrol and pterostilbene in plant and plant materials. Sarig et al. (1996) found out that exposure of ripe grape berries to a stream of air (airflow of 500 mL/minute) containing ozone at a rate of 8 mg/minute, elicited resveratrol and pterostilbene, with the resveratrol accumulating larger amounts. Production of these phytoalexins generally reached a maximum level 24 hours after 5–10-minute exposure, depending on cultivar, to ozone and was enhanced by inoculation with *Rhizopus stolonifer* isolated from diseased berries, either before or after ozone treatments. Ethanol treatment, on the other hand, reduced elicitation potential of ozone for both phytoalexins.

Grimmig et al. (1997) incorporated grapevine resveratrol synthase (Vst1) promoter combined with the β-1, 3-glucanase (GUS) reporter gene into a tobacco plant to identify the regions that control ozone-regulated gene expression. They reported that sequences located at within -430 to -280 of the Vst1 promoter were required for ozone regulation. They found out that in transgenic tobacco, a chimeric gene construct, containing the Vst1 promoter combined with GUS reporter gene, is rapidly induced by ozone at a concentration of 0.1 μl/L for 12 hours. Ozone was generated by electrical discharge in dry oxygen, and desired ozone concentration was computer controlled. The same construct was also strongly induced by ethylene at 20 μl/L for 12 hours.

Far-Infrared (FIR) and Heat Treatments

Many antioxidant phenolic compounds in plants are most frequently present as covalently bound forms with insoluble polymers. Far-infrared radiation and heat treatment are used to liberate and activate low molecular natural antioxidants in plants (Lee et al., 2006). Finely ground peanut hulls treated with far-infrared radiation for 5 to 60 minutes increased the total phenolic contents of water extracts from 79.3 to 141.6-μM/L () tannic acid equivalents compared with untreated samples containing 72.9-μM tannic acid equivalents (Lee et al., 2006). Similarly when peanut hulls were heat treated by roasting at 150°C for 5 to 60 minutes, total phenolics contents increased from 79.8- to 90.3-μM tannic acid equivalents (Lee et al., 2006).

Functional Peanuts and Products

Potential Product Usage for Functional Peanuts

Slicing of peanuts is necessary to produce peanuts with enhanced levels with bioactive compounds such as *trans*-resveratrol (Arora and Strange, 1994; Rudolf and Resurreccion, 2005). Therefore, for food applications, the required peanut ingredient should either be chopped, sliced, or ground peanuts. Potential products for functional peanuts include peanut butters, peanut bars, and peanut confections such as candies and granola bars that utilize ground or chopped peanuts.

Peanut Butter and Spreads

Peanut butters and spreads, which comprised 50% of all peanut products consumed in the United States, are potential food product application of resveratrol-enhanced peanuts (REP), which could deliver peanut products with additional health benefits to consumers. Rudolf and Resurreccion (2007) prepared peanut butters from REPs processed by ultrasound at 49.2-mW/cm^3 power density for minutes at 25°C to optimize parameters for the size of peanut slices and incubation time. Their results showed that *trans*-resveratrol increased to 1.38 μg/g in peanuts sliced to 0.6 cm and incubated for 48 hours compared with 0.29 μg/g in untreated peanuts. All natural peanut butters prepared from REP received slightly lower roasted peanutty aroma and flavor, peanut butter aroma and flavor, and sweet aromatic aroma, but higher oxidized, painty, and fishy flavors, although the magnitudes were low compared with peanut butters prepared from untreated peanuts. Reformulations of REP butters are therefore needed to mask the off-flavors inherently produced in the bioactive-enhanced peanut ingredient. Inclusion of fruit jams and chocolate in the REP butters or mixing REP with regular roasted peanuts may help in masking these off-flavors.

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Peanut Confections

The high protein content of peanuts makes them ideal for high-energy snacks. Six of the top 10 candy bars sold in the United States contain peanuts and/or peanut butter (Putnam et al., 2000), suggesting that REPs have potential in confectionery industry. The presence of sugar in these products could mask any inherent off-flavors developed in the process of enhancing *trans*-resveratrol resulting in lower acceptance of REPs by consumers.

Roasted peanuts from REPs treated by UV, ultrasound, and combined ultrasound-UV with resveratrol concentrations of received lower overall acceptance ratings of 5.0–6.3 (mean = 5.7), 4.2–6.0 (mean = 5.1), and 4.4–5.6 (mean = 4.9) compared with controls of 7.4–7.7 or like moderately (Sales and Resurreccion, 2009, 2010). There are a seemingly infinite number of varieties of candy products wherein REPs can be made available to the consumers. A large variety of candy bars combine peanuts (whole, chopped, or as peanut butter) with ingredients such as chocolate, nougat, marshmallow, caramel, dried fruits, and other nuts. Peanut brittles and chocolate-covered peanuts are always popular.

Peanut Flour

REP may have application in production of peanut flour. The flour from REP can be used in varieties of peanut-based products such as baked goods, high-protein drinks and snacks, breakfast or diet bars, and imitation peanut milk.

Roasted Peanuts/Snack Peanuts

The application of REP as roasted peanuts may be limited due to inherent off-flavors produced during processing resulting to products that may not be acceptable to consumers. Roasted REP produced from subjecting peanuts to nine treatment combinations consisting of fixed ultrasound process of 40-mW/cm^3 power density for four minutes, followed by UV exposure for 15, 35, and 55 minutes at 20, 40, and 60 cm distances from UV light, and then fixed incubation at 25°C for 44 hours significantly increased *trans*-resveratrol from 0.03 μg/g to 2.1–4.3 μg/g in untreated controls but significantly decreased consumers overall acceptance rating of 4.7–5.4 (5 = neither like nor dislike) compared with 6.3 or like slightly in controls (Potrebko and Resurreccion, 2010). However, incorporating roasted sliced or chopped REP as ingredient in snack products or with other nuts, such as pecans, almonds, and walnuts, or as ingredient in breakfast cereals may mask its off-flavors while providing consumers with health benefits from resveratrol.

Peanut Sprouts as Functional Vegetable

Functional peanut sprouts is a novel product developed by Wang et al. (2005) and Chang et al. (2006) was found to contain high concentrations of *trans*-resveratrol. This product would avail the consumers of a healthful vegetables enhanced with stilbenes beneficial to health.

Wang et al. (2005) found that when the rehydrated peanut kernels were germinated at 25°C and 95% relative humidity in the dark for nine days, resveratrol contents increased significantly from initial 2.3–4.5 to 11.7–25.7 μg/g depending on peanut cultivar. Resveratrol was highest in the cotyledons, slightly lower in roots, and not detected in the stems of peanut sprouts

Chang et al. (2006) detected four major fractionations in germinated and sliced peanuts identified as *trans*-resveratrol (RES), *trans*-arachidin-1 (Ara-1), and *trans*-archidin-3 (Ara-3), trans-and isopentadienylresveratrol (IPD). During incubation of germinated and sliced peanuts, concentrations of RES, Ara-1, and Ara-3 increased tremendously from initially trace or nondetectable amounts up to 147.3, 495.7, and 2414 μg/g, respectively, corresponding to 20, 16, and 24 hours of incubation, whereas IPD continued to increase up to 28 hours at 4474.4 μg/g.

Hairy Root Cultures of Peanuts

Many stilbenes recovered as an extract from a selected number of plants were not suitable for many applications in the food/pharmaceutical sectors due to high levels of impurities and overall low concentration of resveratrol and its derivatives in the extract (Medina-Bolivar et al., 2007). Medina-Bolivar et al. (2007) established and tested hairy root cultures of peanut as a bioproduction system for resveratrol and associated derivatives. Their results showed that a single 24-hour sodium acetate elicitation resulted in a 60-fold induction and secretion of *trans*-resveratrol into the medium of peanut hairy root cultures. *trans*-Resveratrol accumulated to 98 μg/mg of the dried extract from the medium representing 99% of the total resveratrol produced. *trans*-Pterostilbene was also detected in the medium at 0.24 μg/mg, a twofold increase compared with nonelicited cultures. Their results demonstrated the capacity of hairy root cultures as an effective bioprocessing system for valued nutraceuticals like resveratrol and resveratrol derivatives. Hairy roots may offer a scalable and continuous product recovery platform for naturally derived, high quality, enriched nutraceuticals as these effectively induced and recovered high levels of resveratrol and associated derivatives from the media fraction.

OPTIMIZATION OF PROCESSES FOR ENHANCED LEVELS OF BIOACTIVE COMPOUNDS

Optimization studies using response surface methodology are conducted to optimize parameters that would lead to optimum product with the highest consumer acceptance. Sensory affective tests are performed to evaluate the acceptance of a product using consumers—the ultimate users of the product.

Rudolf and Resurreccion (2007) optimized slicing and incubation times using response surface methodology for maximum enhancement of *trans*-resveratrol in peanuts treated with

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ultrasound at power density of 39.2 mW/cm^3 for four minutes while producing an acceptable REP butters. The optimized areas, which included sliced peanut sizes of 0.89, 0.72, and 0.64 cm and incubation times of 48, 41.5, and 48 hours, respectively, were predicted to produce REP butters with a *trans*-resveratrol $\geq$1.0 μg/g and slightly lower roasted peanut aroma (>24) and flavor (>43); peanut butter aroma (>14), and flavor (>31) compared with controls with attribute ratings of 33, 78, 38, and 51, respectively, and slightly higher oxidized aroma (<6), and painty (<0.5), fishy (<6), and cardboard flavor (>4) compared with controls rated 0 for all attributes using a 150-mm unstructured line scale. Rudolf and Resurreccion (2005, 2007) only used one dose of either UV or ultrasound processing treatment in enhancing *trans*-resveratrol in peanuts and reported results of descriptive sensory test but not consumer test to determine acceptance of REP.

Sales and Resurreccion (2009, 2010) investigated optimization studies using varying doses of UV, ultrasound, and combined ultrasound and UV, resulting in maximum levels of *trans*-resveratrol in sliced ($\sim$7 mm) REP with the highest consumer acceptance ratings $\geq$ 5 or neither like nor dislike. The optimum UV processes included all process combinations within the area of a triangle bound by the points, distance from UV light of 47, 41, and 33 cm for 20, 26.5, and 30 minutes, followed by incubation at 25°C for 36 hours, which produced REP with maximum *trans*-resveratrol of 2.1 μg/g (Sales and Resurreccion, 2009). Ultrasound process optimization achieved a maximum 4.4 μg/g *trans*-resveratrol and the optimum processes included all process combinations within a pentagon bound by points, ultrasound power density of 75, 75, 72, 67, and 66 mW/cm^3 for 5.2, 8, 8, 7.1, and 5.5 minutes, respectively, followed by incubation at 25°C for 36 hours (Sales and Resurreccion, 2009). The optimum combined ultrasound-UV processes, which produced a maximum of 4.8-μg/g *trans*-resveratrol, included all process combinations of ultrasound power densities of 74, 70, 62, 42, 48, and 58 mW/cm^3 for 8.3, 10.9, 11.2, 10.4, 8.3, and 9.1 minutes, respectively, followed by 50-minute exposure at 40 cm distance from UV light and 36-hour incubation at 25°C (Sales and Resurreccion, 2010).

ANALYSIS OF RESVERATROL AND OTHER STILBENES IN PEANUTS

Extraction Methods

Extraction of resveratrol and other bioactive compounds is a critical step in its quantitative analysis. Analytical methods have been developed that recover as much of the compounds from the sample and prevent their degradation and/or alteration up to the time that these are quantitatively analyzed. The following are important considerations during extraction of *trans*-resveratrol and other bioactive compounds.

Protection from Light

During extraction of samples for resveratrol analysis, it is important that extraction procedure should be protected from light as the *trans* isomer converts to *cis* form upon exposure to UV or fluorescent light. Trela and Waterhouse (1996) studied the stability of resveratrol standards in ethanol at various conditions in the laboratory, and their findings are summarized as follows: (1) Standards of *trans*-resveratrol in 100% ethanol can be stored for three months at −5°C in the dark and protected from stray light in a sealed light-proof containers, thus limiting the need for recalibration after three months. (2) *trans*-Resveratrol standards in 50% ethanol and kept unrefrigerated in light-proof, paraffin sealed containers appeared stable, although solvent evaporation was noticeable. (3) *trans*-Resveratrol standard solutions left unprotected from light and exposed to laboratory fluorescent lighting over 30 days isomerized to about 80% *cis*-resveratrol. (4) *cis*-Resveratrol was extremely light sensitive. When stored in 50% ethanol in the dark at ambient temperatures, *cis*-resveratrol remained stable for at least 35 days over the range of 5.3 to 52.8 μmol/L.

Wang et al. (2001) investigated the stability of 0.5-μM r*esveratrol* in 100% methanol and found that the standard solution was stable for five days at −20°C and three days at 4°C in the dark but not stable at room temperature. Resveratrol concentration decreased to $\sim$85% after four days at 4°C based on the reference standard stored at −80°C, which was assumed to be stable. At room temperature in the dark, reduction in resveratrol concentration was observed even only after two hours, reduced slowly to $\sim$75% after eight hours, and maintained at this level for 96 hours. When exposed to light at room temperature, only $\sim$65% of resveratrol remained after four hours and the concentration decreased to 30% after 48 hours. Wang et al. (2001) recommended that resveratrol should be protected from light during extraction, storage, and analysis. Standard solutions and extracts in methanol should store at $\leq$20°C and analyzed within five days. Fresh standard solutions should be prepared at least weekly. When using automatic injectors, these should be thermostated to $\leq$4°C and samples should be analyzed within two days.

The *cis*-resveratrol is extremely light sensitive, which made it difficult to purify (Trela and Waterhouse, 1996) and therefore not commercially available. It can remain stable in the dark only near neutral pH and isomerized to *trans* form at low pH (Trela and Waterhouse, 1996).

Solvents Used

Ethanol of varying concentrations has been used by a majority researchers for the extraction of resveratrol from different samples of peanuts and peanut plant materials including 95% (Aguamah et al., 1981; Arora and Strange, 1991) and 80% (Sanders et al., 2000; Chukwumah et al., 2005; Rudolf et al., 2005) in the extraction of peanut kernels; 80% in peanut butter

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(Ibern-Gomez et al., 2000); and 50% peanut leaves and roots (Azpilicueta et al., 2004).

Other organic solvents used in the extraction of stilbenes were (1) methanol: 80% in peanut roots (Chen et al., 2002) and in peanut kernels, leaves, and roots (Chung et al., 2003); (2) acetone: 80% in peanut kernels (Arora and Strange, 1991); and (3) acetonitrile: 100% in peanut kernels (Arora and Strange, 1991); 90% in roasted peanuts, peanut butter, and boiled peanuts (Sobolev and Cole, 1999; Lee et al., 2004).

Preliminary Reduction of Sample Size

Peanuts and other solid samples are reduced to fine particles by grinding using a blender (Sobolev and Cole, 1999); coffee mill (Sanders et al., 2000; Rudolf et al., 2005; Tokusoglu et al., 2005); and SMP process homogenizer at 15,000 rpm for one minute (Lee et al., 2004) to facilitate the extraction procedure.

Stilbenes in berry skin tissues were extracted by grinding the skins to a fine powder in a liquid N_2 using a prechilled mortar and pestle (Bais et al., 2003). Resveratrol from grapevine leaves was extracted by grounding in a mortar with sand and 80% methanol (Adrian et al., 1996).

Homogenizing, Centrifugation, and Agitation

Samples reduced to smaller particle sizes are mixed with the extraction solvent and homogenized, centrifuged, or agitated. Agitation with the solvent usually takes longer extraction time compared with homogenization alone or in combination with centrifugation.

Resveratrol was commonly extracted from peanut kernels by homogenizing the ground sample with the organic solvents followed by centrifugation. Sobolev and Cole (1999); Sanders et al. (2000); and Rudolf et al. (2005) homogenized ground peanuts with 80% ethanol at approximately 27,000 rpm for two minutes and then centrifuged in ice for five minutes at 1380*g*. Lee et al (2004) recovered resveratrol from peanuts and peanut butters by mixing the samples with acetonitrile/water (9:1, v/v), followed by homogenizing the mixture for two minutes using a Polytron, rinsing the Polytron with 5-mL extraction solvent and then filtration through a Whatman filter paper. Ibern-Gomez et al. (2000) extracted resveratrol and piceid from peanut butters with 80% ethanol at room temperature for 30 minutes followed by centrifugation for five minutes at 3000 rpm.

In dried peanut roots, Chen and coworkers (2002) recovered resveratrol by mixing the powdered roots with 80% methanol and homogenizing the mixture at 15,000 rpm for one minute with an aggregate probe. The probe was washed with 80% methanol adding the washings to the homogenate, and the mixture was heated in a water bath at 70°C with occasional shaking for 30 minutes. The heated mixture was centrifuged at 8000*g* at 20°C for 15 minutes, the supernatant was collected, membrane-filtered, and diluted with water to adjust to 20% methanol, and then the aliquot was loaded onto solid phase extraction.

Resveratrol from frozen leaf peanut tissue powder was extracted by Chung et al. (2003) by agitating in methanol for 16 hours at room temperature. Nepote et al. (2004) prepared the ethanolic extracts from peanut skins previously defatted in hexane, by macerating the defatted skins with ethanol for 24 hours in the dark at room temperature. The crude ethanolic extracts were purified by partition with a solution of dichloromethane, ethyl acetate, and water (90:300:55 v/v/v). The ethyl acetate fraction was evaporated in a rotary evaporator and separated with methanol in a minicolumn packed with Sephadex.

Adrian et al. (1996) extracted resveratrol from grapevine leaves by grounding in a mortar with sand and 80% methanol, followed by centrifugation at 10,000*g* for 15 minutes. The supernatant was prepurified on a Sep-Pak C_{18} cartridge through elution with 80% methanol and the eluate was evaporated to dryness at <40°C.

In powdered berry skins, Bais et al. (2003) mixed methanol (100%) to the sample and the mixture was shaken in the dark at room temperature for one hour. Extracted tissue was then pelleted via centrifugation at 7700*g* for 30 minutes at 4°C, and supernatant was taken for HPLC analysis.

Use of Clean-Up Columns

Crude extracts from high-fat containing samples, such as peanuts and peanut products, were cleaned up to remove the lipid and protein components that may interfere in the analysis by passing the extracts through clean-up columns (Sanders et al., 2000). The clean-up column is made up of a 1:1 mixture of aluminum oxide (Al_2O_3) and silica gel 60 R_{18} packed in a borosilicate glass disposable Pasteur pipette with cotton plug at the bottom (Sobolev et al., 1995; Sanders et al., 2000), or packed in a Teflon tube (Rudolf et al., 2005) or 3 mL disposable plastic syringe (Potrebko and Resurreccion, 2009) fitted with AP25 Millipore prefilter at the bottom. Commercially prepared clean-up columns are also available such as the Econo-column (Poly-Prep chromatography column) packed with 1.0–1.2 mL of mixture of Al_2O_3 and AccuBOND[11] SPE ODS C18 (Lee et al., 2004).

Facilitated Diffusion Technique

Azpilicueta et al. (2004) extracted phytoalexins in peanut leaves and roots using facilitated diffusion technique. The plant tissues were vacuum infiltrated with 50% ethanol and agitated overnight in a rotary shaker at 100 rpm and 20°C. The plant tissues were removed by gravitational filtration, and the filtrates were vacuum concentrated to approximately one-half volume at 45°C. The concentrated solutions were extracted twice with ethyl acetate using separatory funnel, and organic fractions were pooled, dehydrated with anhydrous sodium sulfate, and taken to dryness. Residues were redissolved in methanol at 0.2 mL/g of fresh tissue processed and kept at −20°C until analyzed.

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Vacuum Infiltration and Solid Phase Extraction

Phytoalexins were also extracted from peanut kernels by vacuum infiltration with acetonitrile in the dark for 48 hours at 25°C and partially purified by solid phase extraction (SPE; Arora and Strange, 1991). Chen and coresearchers (2002) purified the aliquots of supernatant from dehydrated peanut root extracts by loading onto SPE columns and eluted for cleanup with 28% methanol through an extraction unit. The absorbed resveratrol was eluted with 47.5% methanol, and eluates were repeatedly injected into a semipreparative HPLC column at 2.4-mL injection volume, then separated with a gradient solvent system initiated with 20 to 80% methanol in 16 minutes and held for an additional two minutes using a flow rate of 3 mL/minute. The active fractions were collected, pooled, and subjected to further separation for purification according to the same semi-HPLC procedure, and the collected solutions were evaporated to dry white powder.

Drying of Sample Extracts

After extraction, peanut extracts are evaporated to dryness under a stream of nitrogen or under vacuum or reduced pressure at a specified temperature to stabilize the samples while awaiting quantitative analysis. Sanders and co-workers (2000) dried their extracts under nitrogen at 60°C on a heating block; Rudolf et al. (2005) followed similar procedure but using a water bath. Eluates containing resveratrol extracted from various peanut, and peanut products were evaporated to dryness under nitrogen stream at 40°C in an evaporating unit (Sobolev and Cole, 1999) and Lee et al. (2004) adopted similar procedure at 50°C. Ibern-Gomez and her group (2000) concentrated the supernatant extracts collected from peanut butter samples to dryness under vacuum at temperatures below 40°C. The dried extracts were stable and can be kept at −20°C until quantitatively analyzed (Rudolf et al., 2005).

Quantitative Analysis of Resveratrol and Other Stilbenes in Peanuts

Standards Used

Currently, *trans* but not *cis* forms resveratrol, piceid, and piceatannol standards were commercially available and used by various authors as reference standards. In earlier studies, when *trans*-piceid standard was not commercially available, the standard was commonly prepared by extracting the roots of *Polygonum cuspidatum*, the known major source (Waterhouse and Lamuela-Raventos, 1994; Ibern-Gomez et al., 2000). The *cis* forms of the resveratrol and other stilbenes were usually prepared by exposing the *trans* forms to UV or white light. *cis*-Resveratrol was extremely light sensitive, and the preparation of standards, while executed in near total darkness, allowed enough light to cause slight isomerization to *trans* (Trela and Waterhouse, 1996). Trela and Waterhouse (1996) investigated the UV-induced isomerization of *trans*-resveratrol and found that pure *trans*-resveratrol at 418 μmol/L when subjected to UV light at 366 nm at an intensity of 180 μW/cm^2 for three hours converted to a maximum of 90.6% *cis* form. In contrast, at lower UV wavelength of 254 nm at an intensity of 750 μW/cm^2, the same concentration of *trans*-resveratrol (4.18 μmol/L) converted to only about 20% *cis* form after three hours, and extending UV irradiation time to as long as 10 hours resulted in only $\leq$63% conversion. Using higher concentration of 8.94-mmol/L *trans*-resveratrol, UV irradiation at 366 nm for three hours resulted in $\geq$80% conversion to *cis*-resveratrol.

Dominguez et al. (2001) prepared *cis*-resveratrol standard by exposing 100-mg/L stock solution of *trans*-resveratrol for 15 minutes in a climatic chamber equipped with solar radiation panel by xenon (1500 W) at controlled temperature of 28°C and 92% humidity. The concentration of *cis*-resveratrol was determined by based on the reduction in UV absorption and mass spectra of *trans*-resveratrol after its irradiation.

The standards of *cis* isomers of resveratrol and piceid were prepared by Burns and coworkers (2002) by exposing the *trans* isomers in methanol solution for 12 hours in high white light. Lamuela-Raventos et al. (1995) isomerized *trans*-resveratrol and *trans*-piceid to *cis* forms by exposing to sunlight but did not report the exposure time used.

Use of Internal Standard

Internal standards are used in the analysis of resveratrol in peanuts to efficiently quantify this compound in the samples. For HPLC samples requiring significant pretreatment or preparation before HPLC analysis, the use of a stable internal standard, which is not present in the sample being analyzed, is recommended (Francisco and Resurreccion, 2009). Fixed and known amount of an internal standard is added at the beginning of sample extraction. Phenolphthalein was used as an internal standard in the analysis of stilbenes in peanut samples by normal phase HPLC (Sobolev et al., 1995) and reverse phase (Rudolf et al., 2005; Potrebko and Resurreccion, 2009) due to its stability and suitable retention time relative to the stilbenes analyzed and therefore helped to quantitative analysis (Sobolev et al., 1995). Other internal standards such as 3,4,5-trimethoxycinnamic acid (Dominguez et al., 2001) and 2,5-dihydroxybenzaldehyde (Malovana et al., 2001) were used in the reverse-phase HPLC analysis of *trans*-resveratrol in wines as these were not known to be present in wines (Dominguez et al., 2001). β-Resorcylic acid was used as an internal standard for the simultaneous determination of 15 phenolic compounds in peanut skins (Francisco and Resurreccion, 2009).

Preparation of Dried Extracts Prior to Quantitative Analysis

Prior to quantitative analysis of the stilbenes, the dried residue was redissolved in organic solvents such as ethanol at low concentrations of 10–15% (Sanders et al., 2000; Rudolf et al., 2005; Potrebko and Resurreccion, 2009), methanol, 15% (Francisco and Resurreccion, 2009), or using HPLC mobile phase (Waterhouse and Lamuela-Raventos, 1994; Adrian et al.,

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1996; Sobolev and Cole, 1999; Ibern-Gomez et al., 2000; Lee et al., 2004). The reconstituted extracts were subjected to high-frequency ultrasonification (Chukwumah et al., 2005; Rudolf et al., 2005) to facilitate dissolution. Finally, the reconstituted residues were filtered through inorganic membrane filter (0.45 μm) prior to injection for quantitative analysis (Rudolf and Resurreccion, 2005; Potrebko and Resurreccion, 2009).

Methods for Quantitative Analysis of Resveratrol and Other Stilbenes

A number of analytical methods have been developed to measure resveratrol, piceid, and other stilbenes in peanuts, wines, and grapes. These include HPLC, gas chromatography (GC; Luan et al., 2000); gas chromatography mass spectrophotometry (GC-MS; Soleas et al., 1995; Medina-Bolivar et al., 2007); liquid chromatography-tandem mass spectrometry (LC/MS/MS; Lyons et al., 2003); and capillary electrophoresis (Gu et al., 2000; Gao et al., 2002). Among these methods, HPLC is regarded as a prime separation method and most widely used method even though it has some shortcomings including long analysis time, low resolution, and short lifetime of columns (Gao et al., 2002).

High Performance Liquid Chromatography (HPLC)

The HPLC methods developed by various researches for the analysis of resveratrol and other stilbenes in peanuts and peanut plant materials are listed in Table 7. HPLC separation modes were conducted as normal phase or reverse phase. In normal-phase HPLC, the stationary phase (column) is made of polar packing medium, such as silica, whereas the mobile phase is of nonpolar or low-polarity solvents, such as hexane, dichloromethane, chloroform, ethyl ether, and isopropyl alcohol. In reverse-phase HPLC, a nonpolar stationary phase and a polar mobile phase are used. The commonly used stationary phase packing material in the reverse-phase systems are chemically bonded phases of silica surface silanols with an organochlorosilane.

The resveratrol and other stilbenes in peanuts analyzed using HPLC commonly using gradient elution rather than isocratic methods (Rudolf et al., 2005). Isocratic elutions were used for the determination of stilbene phytoalexins in peanuts using acetonitrile in a reverse phase column (Aguamah et al., 1981), and n-heptane/2-propanol/water/acetonitrile/acetic acid (1050/270/17/5/1, v/v) in normal phase column (Sobolev et al., 1995; Sobolev and Cole, 1999). Phytoalexins were detected and quantified by gradient elution in reverse-phase HPLC using multichannel detector at 310 nm (Cooksey et al., 1988) and diode array detector (DAD) at 338 nm (Arora and Strange, 1991). Resveratrol and its isomers were detected using DAD in the range of 306–308 nm for *trans* form and 285 nm for *cis* form; UV detectors over the wavelengths of 254–320 nm; and fluorescence detector at 330 nm excitation and 374 emission (Table 7). The use of photodiode array detector in combination with a UV-transparent mobile phase (from 215 nm) helped to increase reliability of the method in the cases of low concentrations analyzed in the samples (Sobolev and Cole, 1999).

Simultaneous determinations of resveratrol and piceid were detected and quantified in peanuts and peanut butters using DAD at 285 for *trans* isomers and 306 nm for *cis*-resveratrol and piceid (Ibern-Gomez et al., 2000) and at 307 nm for both trans isomers of resveratrol and piceid (Potrebko and Resurreccion, 2009). Resveratrol and piceatannol were simultaneously detected in peanut callus using fluorescence detector at 343 excitation and 395 emission (Ku et al., 2005). The 16 phenolic compounds including an internal standard were quantified in peanut skins using DAD detector set at 250, 280, 306, 320, and 370 nm (Francisco and Resurreccion, 2009).

In grapevine berries of three *Vitis vinifera* varieties, an HPLC analysis was used to compare the levels of *resveratrol* and its derivatives, piceid, pterostilbene, and epsilon–viniferin, (Adrian et al. 2000). The concentrations of these compounds were evaluated in healthy and *Botrytis cinerea* infected grape clusters, both in natural vineyard conditions and in response to UV elicitation.

Vastano et al. (2000) analyzed the roots of two varieties of *Polygonum cuspidatum*, Hu Zhang and Mexican Bamboo, for *resveratrol* and analogues. The powdered roots were extracted with methanol and ethyl acetate, and the ethyl acetate fraction was subjected to fractionation and purification using silica gel column chromatography and semipreparative HPLC. In addition to *resveratrol*, three stilbene glucosides were identified as piceatannol glucoside (3,5,3′,4′-tetrahydroxystilbene 4′-O-beta-D-glucopyranoside), resveratroloside (3,5,4′-trihydroxystilbene 4′-O-beta-D-glucopyranoside), and piceid. The levels of the piceatannol glucoside and piceid were twice as high in Mexican Bamboo as compared with Hu Zhang variety.

Liquid Chromatography-Mass Spectrometry (LC/MS)

Wang et al. (2002) developed a LC/MS method, which simultaneously detected and quantified concentrations of resveratrol and piceid in fruit juices and wines, in a very wide range as low as 1.07 nmol/g in cranberry juice to as high as 24.84 μmol/L in Italian red wines. Samples were extracted using methanol, enzymatically hydrolyzed, and analyzed using reversed phase HPLC with positive ion atmospheric pressure chemical ionization (APCI) mass spectrometric detection. After APCI, the abundance of protonated molecules was recorded using selected ion monitoring of *m/z* 229. The LC/MS calibration curve showed a linear range over more than three orders of magnitude, from 0.52 to 2260 pmol of *trans-resveratrol* with a correlation coefficient 0.9999. The coefficient of variance (COV) of the response factor over the same concentration range was 5.8%, and the intra-assay COV was 4.2% ($n = 7$). The limit of quantitation, defined as signal-to-noise ratio 10:1, was 0.31 pmol injected on-column. The extraction efficiency of the method was 92%. R*esveratrol* was stable for five days at -20°C and three days at 4°C in the dark but not at room temperature without protection from light, indicating that resveratrol should be protected from light during extraction, storage, and analysis (Wang et al., 2001).

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Table 7 Comparison of published methods of high performance liquid chromatography (HPLC) analysis for resveratrol and resveratrol derivatives in peanuts and peanut plant materials

Sample	Separation mode[1]	Column	Column temperature (°C)	Mobile phase solvents		Elution	Flow rate (mL/minute)	Detector
				A	B			
Peanut kernels[2,3]	Reverse-phase	Hypersil octadecylsilyl (ODS) (250 mm L[20] × 4.6 mm i.d.[20]; 5-μm particle size)	NR[22]	acetonitrile/ water (1:1, v/v)	NA[21]	Isocratic	4	Pye-Unicam LC-UV 335 nm for stilbene phytoalexins
Peanut kernel[4]	Reverse-phase	Spherisorb 10 ODS (250 mm L × 4.6 mm i.d.; 5-μm particle size)	NR	water/ acetic acid (99:1, v/v)	Acetonitrile	Gradient: time (minutes), %B; 1, 40; 7, 45; 12, 45; 20, 65	1.5	Multichannel detector 310 nm and 0.04 A for phytoalexins
Peanut kernels[5]	Reverse-phase	Spherisorb 10 ODS (250 mm L × 4.6 mm i.d.; 5-μm particle size)	NR	water/ acetic acid (99:1, v/v)	Acetonitrile	Gradient: time (minutes),%B; 1, 30; 3, 30; 6, 35; 9, 35; 12, 40; 15, 40; 18, 50; 20, 50; 21, 30; 31, 30	1.5	Diode array detector (DAD) UV mode, 338 nm for phytoalexins
Peanut kernels[6]	Normal-phase	Ultrasphere-SI (250 mm L × 4.6 mm i.d.; 5-μm particle size)	NR	n-Heptane/ 2-propanol/ water/ acetonitrile/ acetic acid (1050:270: 17:5:1, v/v/v/v/v)	NA	Isocratic	1.5	Programmable multiwavelength 300 nm or 290–345 nm range (12 fixed wavelengths; 5 nm increments) in 0.005–0.005 AUFS range, for *trans*-resveratrol
Roasted and boiled peanuts and peanut butter[7]	Normal-phase	Zorbax-RX-SIL (250 mm L × 4.6 mm i.d.; 5 μm particle size)	ambient	n-Hexane/ 2-propanol/ water/ acetonitrile/ acetic acid (1050:270: 17:5:1, v/v/v/v/v)	NA	Isocratic	1.5	DAD 307 nm for *trans*-resveratrol of fresh and roasted peanuts; 320 nm for peanut butter
Peanut kernels[8]	Reverse-phase	Vydac C18 (150 mm L × 4.5 mm i.d.; 5-μm particle size)	NR	Water/TFA (9.9:0.1, v/v)	Acetonitrile	Gradient time (minute),%B; 1, 0; 3, 15; 23, 27; 28, 100; 29, 0; 39, 0	–	UV 308 nm for resveratrol
Peanut butter[9]	Reverse-phase	Nucleosil 120 C18 (250 mm L × 4 mm i.d.; 5-μm particle size)	40	Acetic acid/ water (52.6:900, v/v)	Solvent A/ acetonitrile (2:8, v/v)	Gradient Time (min),%B; 0, 16.5; 13, 18; 15, 18; 17, 23; 21, 25; 27, 31.5; 30, 0	1.5	DAD 285 nm for *trans* forms of resveratrol and piceid; 306 nm for *cis* forms
Peanut roots[10]	Reverse-phase	Thermal hypersil ODS (250 mm L × 4 mm i.d.; 5-μm particle size)	NR	Water	Methanol	Gradient time (minute),%B; 1, 20; 16, 80; 18, 80	1.0	UV 254 nm for resveratrol
Peanut leaves, pods and roots[11]	Reverse-phase	μ-Bondapak C18 column (3.9 mm × 300 mm)	NR	Water	Acetonitrile	Gradient (elution time and composition were not reported)	1.0	Fluorescence detector 330 nm excitation and 374 mm emission, for resveratrol
Peanut kernels and peanut butter[12]	Reverse-phase	Nucleosil 100–5 C18 (250 mm L × 4.0 mm i.d.; 5-μm particle size) Precolumn packed with nucleosil 5 C18 (4 mm × 4 mm)	NR	Acetonitrile/ water (40:60, v/v)	NA	Isocratic	0.3	UV detector 306 nm for *trsns*-resveratrol
Peanut skins[13]	Normal-phase	Nucleosil 120–5-C-18 column	NR	Methanol/ water (1:1, v/v)	NA	Isocratic	1.5	UV detector 320 nm for *trans*-resveratrol
Peanut sprouts[14]	Reverse-phase	Thermal hypersil ODS (250 mm L × 4.6 mm i.d.; 5-μm particle size)	NR	water	Methanol	Gradient time (minute), %B; 0, 30; 16, 90;2, 90	1.0	UV detector 307 nm for resveratrol
Peanut kernels[15]	Reverse-phase	Hypersil-ODS (250 mm L × 4.6 mm i.d.; 5 μm particle size)	30	Acetonitrile/ bidistilled water (40:60, v/v) plus 0.1N trifluoro-acetic acid, v/v	NA	Isocratic	1.0	DAD UV detector 308 nm for *trans*- and *cis*- resveratrol

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Peanut callus[16]	Reverse-phase	Mightsil RP C-18 column (250 mm L × 4.6 mm i.d.	NR	Water adjusted to pH 2.1 with formic acid	Acetonitrile adjusted to pH 2.1 with formic acid	Gradient: time (minute), %B; 0, 20; 20, 32; 30, 90; 35, 90	–	Fluorescence detector 343 nm excitation and 395 nm emission for resveratrol and piceatannol
Peanut kernels[17]	Reverse-phase	C18 Column (250 mm L × 4.6 mm i.d.; 5-μm particle size) proceeded by a C18 guard column, 7.5 mm L × 4.6 mm i.d.; 5-μm particle size)	NR	Water/acetic acid (9.999:0.001, v/v)	Acetonitrile	Gradient: time (minute), %B; 0, 5; 7, 22; 13, 23; 26, 63; 28, 80; 29, 5; 34, 5	1.5	DAD 307 nm for *trans*-resveratrol; 280 nm for internal standard, phenolphthalein
Peanut kernels[18]	Reverse-phase	C18 Column (250 mm L × 4.6 mm i.d.; 5-μm particle size) proceeded by a C18 guard column, 7.5 mm L × 4.6 mm i.d.; 5-μm particle size)	NR	Water	Acetonitrile	Gradient: time (minute), %B;0, 5; 23, 41.8; 28, 77; 29, 5; 34, 5	1.5	DAD 307 nm for *trans*-resveratrol and *trans*-piceid; 280 nm for internal standard, phenolphthalein
Peanut skins[19]	Reverse-phase	C18 column (250 mm L x 4.6 mm i.d.; 5 μm particle size) proceeded by a C18 guard column, 7.5 mm L x 4.6 mm i.d.; 5 μm particle size)	NR	Water/formic acid (9.999:0.001,v/v)	Acetonitrile/ formic acid (9.999:0.001, v/v)	Gradient: time (minute), %B; 0, 5; 7, 7; 75, 17; 110, 45; 117, 100; 124, 100	1.5	DAD 250 nm for benzoic acid derivatives and internal standard, β-resorcylic acid; 280 nm for flavanols; 320 for cinnamic acid derivatives; 306 nm for stilbenes, *trans*-resveratrol and *trans*-piceid; 370 nm for flavonol, quercetin

[1]Separation modes were conducted as normal or reverse phase. Normal-phase HPLC utilizes a polar adsorbent as the stationary phase (column), such as silica or silica to which nonionic functional groups have been chemically attached and nonpolar mobile phase. Reverse-phase utilizes a nonpolar stationary phase and polar mobile phase. The stationary phase in reverse-phase systems are chemically bonded phases of silica surface silanols with an organochlorosilane. Usually, the R_3 group is an octadecyl (C_{18} chain) as in octadecylsilyl (ODS) bonded phases.
[2]Aguamah et al. (1981).
[3]Three phytoalexins, 4-(3-methyl-but-1-enyl)-3,5,3′,4′-tetrahydroxy-stilbene, 4-(3-methyl-but-2-enyl)-3,5,4′-trihydroxystilbene (4-isopentenylresveratrol), and 4-(3-methyl-but-1-enyl)-3,5,4′-trihydroxystilbene, closely related to resveratrol were isolated from peanuts.
[4]Cooksey et al. (1988).
[5]Arora and Strange (1991).
[6]Sobolev et al. (1995).
[7]Sobolev and Cole (1999).
[8]Sanders et al. (2000).
[9]Ibern-Gomez et al. (2000).
[10]Chen et al. (2002).
[11]Chung et al. (2003).
[12]Lee et al. (2004).
[13]Nepote et al. (2004).
[14]Wang et al. (2005).
[15]Tokusoglu et al. (2005).
[16]Ku et al. (2005); simultaneous determination of resveratrol and piceatannol.
[17]Rudolf et al. (2005).
[18]Potrebko and Resurreccion (2009); simultaneous determination of *trans*-resveratrol and *trans*-piceid.
[19]Francisco and Resurreccion (2009); simultaneous analysis of 16 phenolic compounds: benzoic acid derivatives—gallic acid, protocatechuic acid, and internal standard, β-resorcylic acid; flavanols—epigallocatechin, (+)-catechin, porcyanidin B_2, (−)-epicatechin, (−)-epigallocatechin gallate, (−)-epicatechin gallate, (−)-catechin gallate; stilbenes—*trans*-piceid and *trans*-resveratrol; phenolic acids—caffeic-, coumaric-, and ferulic acids; and flavonol, quercetin.
[20]L means length; i.d. means internal diameter.
[21]Not applicable.
[22]No data reported.

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HPLC-Tandem Mass Spectrometry (LC-MS/MS)

Lyons et al. (2003) developed a new assay based on HPLC-tandem mass spectrometry (LC-MS/MS) to measure r*esveratrol* in bilberry *Vaccinium myrtillus* L., lowbush "wild" blueberry, *Vaccinium angustifolium* Aiton, rabbiteye blueberry (*Vaccinium ashei* Reade), and the highbush blueberry, *Vaccinium corymbosum* L. The LC-MS/MS assay provided lower limits of detection than previous methods for *resveratrol* measurement, 90 fmol of *trans-resveratrol* injected on-column, and a linear standard curve spanning >3 orders of magnitude. The recoveries of *resveratrol* from blueberries spiked with 1.8, 3.6 or 36 ng/g were 91.5 ± 4.5, 95.6 ± 6.5, and 88.0 ± 3.6%, respectively.

Gas Chromatography-Mass Spectrometry (GC-MS)

A GC-MS system was used to selectively target the detection of resveratrol and its derivative, pterostilbene in the medium, and root tissue of 12-day nonelicited and sodium elicited peanut hairy root cultures (Medina-Bolivar et al., 2007). Discrimination between *cis* and *trans* isomers of the compounds being analyzed was achieved by comparison of the retention time and mass spectra of authentic standards. Although GC-MS analysis provides excellent sensitivity and specificity, derivatization of resveratrol is required prior to analysis to increase its thermal stability and volatility (Wang et al., 2002). Furthermore, the high temperature of 250–300°C used at the injector, column, and ion source might cause partial isomerization or degradation of the sample resulting in inaccurate quantitation (Wang et al., 2002).

HPLC-Diode Array Detector (DAD)/Gas Chromatography-Mass Spectrometry (GC-MS)

Tokusoglu et al. (2005) analyzed *trans* and *cis* resveratrol in six edible peanut varieties and five pistachio (*Pistacia vera* L) varieties grown in Turkey and four market samples by HPLC diode array and gas chromatography-mass spectrometric detection. Resveratrol was confirmed by TICs of bis[trimethylsilyl] trifluoroacetamide derivatives of resveratrol isomers and comparison with the mass spectral fragmentation data with those of a resveratrol standard.

Capillary Electrophoresis

Capillary electrophoresis (CE) is becoming increasingly recognized as an important analytical separation technique for its speed, efficiency, reproducibility, ultra-small sample volume, and little consumption of solvent (Brandolini et al., 2002; Gao et al., 2002) and inexpensive capillary instead of expensive HPLC or GC columns (Brandolini et al., 2002). In addition, with electrochemical detection (ED), CE-ED offers high sensitivity and good selectivity for electroactive analytes. The group of Gao et al. (2002) used CE-ED as an alternative method for *trans*-resveratrol determination in some real samples of wines, medicinal herb, and health food, which was proven to be simple, convenient, sensitive, and selective.

Brandolini and coworkers (2002) developed an analytical method using capillary electrophoresis (CE) with diode array detection for the separation, identification, and quantification of *trans* and *cis* isomers of resveratrol and their corresponding glucosides, piceid, from synthetic and natural sources. The group optimized the process and they were able to obtain good separations of mixtures of *trans*-resveratrol, *cis*-resveratrol, *trans*-piceid, and *cis*-piceid. The effect of UV-irradiation time on the isomerization of resveratrol and *trans*-piceid showed that 50% isomerization was obtained after five minutes, and the equilibrium was achieved after 20 minutes of exposure, with 90% final conversion. In view of the limited availability of piceid, Brandolini et al. (2002) also developed a synthetic route using a simple and direct one-step glycosylation to synthesize adequate amount of *trans*-piceid. The synthesis was performed on *trans*-resveratrol monosodium salt by the addition of α-bromotetra-O-acetyl-D-glucose. The reaction proceeded with the concomitant deprotection of the hydroxyl functions of the sugar moiety to provide the expected 3-O-glycosylated derivative in a satisfactory yield. Results showed that the electrograms of both synthetic and natural *trans*-piceid showed the same migration time, the same corresponding peaks, and the same absorption spectra. and the stability to UV radiation was also investigated.

HPLC, UV Spectrophotometry, Electrospray Mass Spectrometry (HPLC-ESI-MS)

An HPLC method with electrochemical detection was used to determine the occurrence of *trans*-resveratrol and *cis*-resveratrol in various vegetables and fruits (Kolouchova-Hanzlikova et al., 2004). HPLC, UV spectrophotometry, electrospray ionization mass spectrometry (HPLC-ESI-MS) and enzymatic hydrolysis were used to detect and identify two new transgenic plant compounds, *trans* and *cis* isomers of resveratrol-3-glucoside (piceid) in poplar (Giorcelli et al., 2004).

CONCLUSIONS

*trans-R*esveratrol, *trans*-piceid, and other stilbenes are potent antioxidants naturally present in peanuts and can be enhanced in peanuts and peanut plant materials using biotic and abiotic stresses. The knowledge of the presence of these health beneficial bioactive compounds in peanuts led the researchers to investigate various processes that will further increase their biosynthesis not only in the edible parts of the plant but also in its inedible portions with the objective of obtaining cheaper sources for dietary supplements. Researchers developed analytical methods for the effective extraction and quantitative analyses of these bioactive compounds from the samples.

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