PROCESSES TO ENHANCE *TRANS*-RESVERATROL IN PEANUT KERNELS AND THEIR OPTIMIZATION

by

JOCELYN D. MASIGLAT-SALES

(Under the Direction of Anna V. A. Resurreccion and Louise Wicker)

ABSTRACT

Abiotic stresses including wounding through size reduction, UV, ultrasound (US), and combined US-UV were applied to peanut kernels to enhance *trans*-resveratrol biosynthesis. Slicing produced the highest *trans*-resveratrol followed by chopping, whereas whole peanuts had the least, suggesting that only mild damage to cells was needed for maximum biosynthesis. UV, US and US-UV treatments of sliced peanuts further increased *trans*-resveratrol from 0.37 to 3.29, 6.35 and 7.14 μg/g, respectively, corresponding to >100% that found in red wines, a known major food source. Resveratrol-enhanced peanuts (REP) had less roasted peanutty flavor and more bitter, astringent, cardboard, oxidized and fishy off-flavors resulting in lower acceptance rating (OA) ≥ 5.0 or neither like nor dislike compared to untreated controls of 7.4 or like moderately. *Trans*-resveratrol was positively correlated to astringent, bitter and painty flavors of REP.

Optimization using response surface methodology showed that optimum US-UV produced REP with highest *trans*-resveratrol of 4.8 μg/g followed by US and UV with 3.8 and 2.1μg/g, respectively, with maximum consumer acceptance ≤5 or neither like nor dislike. Furthermore,

US-UV had maximum 170 μg/g *p*-coumaric acid and 150 μM TE/g ORAC corresponding to >100% that found in red wines.

The shelf life of roasted REP was 52 days at 25°C, shorter than 90 days in regular roasted peanuts due to weaker peanutty flavor and more intense off-flavors, with a Q_{10} value of 2.2 for lipid oxidation. REP application in peanut bars increased shelf life at 25°C to 146 days probably due to sugar's protective effect which slowed down lipid oxidation or masked the effect on off-flavors. On a per serving basis, about 3 ½ REP bars containing 30 g peanuts/bar would provide equal resveratrol in 140 mL serving of red wine. REP will provide increased value and profitability for the food industry and health benefits to consumers.

INDEX WORDS: peanuts, UV, ultrasound, abiotic stress, trans-resveratrol, phenolic compounds, antioxidants, total phenolics, bioactive compounds, shelf life

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DEDICATION

My dissertation is dedicated to God Almightly who makes everything possible;

To my daughter, Adelou Jane who is my greatest source of strength and inspiration;

and

To my family and loved ones for their prayers, constant support and encouragements,

and unconditional love.

Thank you all for allowing me to achieve another milestone in my career.

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SECTION 1

INTRODUCTION

Interest in functional foods has been increasing in recent years due to their beneficial health effects in preventing the risk of cardiovascular disease, cancer and other diseases. Functional foods appear similar to a conventional food that is consumed as part of usual diet, but contains biologically active compounds that possess desirable physiological benefits and/or reduce the risk of chronic diseases beyond basic nutritional functions. *Trans*-resveratrol is a functional compound naturally occurring and synthesized in plants like grapes, peanuts and pines in response to biotic and abiotic stresses. It has powerful antioxidant properties which protect the tissue from oxidative damage. Many researchers devoted their research on *trans*-resveratrol as a result of several epidemiological studies showing inverse relationship between moderate consumption of red wines and incidence of coronary heart diseases, commonly known as the "French Paradox" (Stanley and Mazier, 1999) which was attributed to the *trans*-resveratrol contents of red wines.

Peanut has potential for developing into functional food as it contains not only *trans*-resveratrol but other health beneficial functional compounds such as piceid - the glucoside of *trans*-resveratrol, flavonoids, and other polyphenolic compounds. The USDA 2009 peanut crop production forecast was estimated at 3.52 billion lbs, with the Southeastern states, including Alabama, Florida, Georgia, Mississippi and South Carolina totaling 2.64 billion lbs (USDA, 2008). Increasing the value of peanuts provides economic benefits to farmers and the peanut processing industries and health benefits to consumers.

Biotic stresses used to elicit resveratrol in peanuts and grape plant and plant materials include invasion and/or inoculation of fungi, yeasts, and bacteria. Abiotic stresses include physical challenges such as wounding, exposure to ultraviolet light, ultrasound and ozone, and treatment with chemicals such as aluminum chloride, aluminum sulfate, cupric chloride, salicylic acid, jasmonic acid, and ethylene.

In peanuts, biotic elicitations of resveratrol and other functional compounds through microbial inoculation or invasion led to microbiological contamination that render peanuts and plant materials inedible and/or unsafe for human consumption due to production of fungal metabolites such as the carcinogenic aflatoxins produced by *Aspergillus sp.* (Wotton and Strange, 1985). The use of abiotic elicitors is a safe alternative for the generation of bioactive compounds to produce functional peanuts for food use and was effective in increasing concentrations of *trans*-resveratrol in peanuts. The study from the University of Georgia (UGA) showed that treatment of fully imbibed sliced (2 mm) peanuts with a single dose of UV (254 nm, 40 cm distance from UV light for 10 min) or ultrasound (39.2 mW/cm^3 power density for 4 min) followed by incubation at 25^oC increased *trans*-resveratrol to 3.42 and 3.96 μg/g or up to 7- and 8-fold increase, respectively, compared to untreated controls with 0.48 μg/g (Rudolf and Resurreccion, 2005). A Korean group of researchers found that UV exposure alone decreased *trans*-resveratrol in raw peanuts, but soaking in water before UV light exposure had synergistic effect increasing *trans*-resveratrol concentrations between 45 and 65 times (Seo et al., 2005). UGA's optimum ultrasound process which exposed 7 mm sliced peanuts to ultrasound power density of 39.2 mW/cm^3 for 4 min followed by incubation at 25^oC for 44 h increased *trans*-resveratrol to 6.80 to 7.15 μg/g in treated peanuts with and without skins, respectively, corresponding to 15- and 16-fold increases compared to 0.45 μg/g in controls (Rudolf, 2003) and

were higher than those found in red wines containing 0.99 to 5.51 μg/g (McMurtrey et al., 1994). However, peanut butter made from resveratrol-enhanced peanuts (REP) had low mean overall consumer acceptance of like dislikely due lower intensities of roasted peanutty flavor and higher off-flavors intensities such oxidized, painty, fishy and cardboard compared to untreated control (Rudolf, 2003).

Previous studies used single doses of either UV or ultrasound in enhancing resveratrol biosynthesis in peanuts (Rudolf and Resurreccion, 2005). The effects of varying doses of UV, ultrasound (US), and their combination, US-UV on the concentrations of *trans*-resveratrol, total phenolics, and antioxidant capacities, and sensory quality and acceptance in treated peanuts have not been investigated. Questions remaining are whether increasing exposure to UV or ultrasound or their combined treatments would result in increased biosynthesis of *trans*-resveratrol and other bioactive compounds, increased generation of off-flavors and decrease consumer acceptance of REP.

The goal of this research was to optimize the enhanced biosynthesis of *trans*-resveratrol and other bioactive compounds and antioxidants, through application of UV, ultrasound, and combined US-UV processing treatments, thereby producing resveratrol-enhanced peanuts with high antioxidant capacities and consumer acceptance, for use as ingredient in the manufacture of peanut and other food products, and deliver health benefits to consumers. The specific objectives of this research were to: (1) investigate the effects of varying doses of UV, US, and combined US-UV on the biosynthesis of *trans*-resveratrol and other phenolic compounds and antioxidant capacities, and consumer acceptance of peanut kernels; (2) optimize UV, US, and US-UV processes for enhanced biosynthesis of *trans*-resveratrol and other bioactive phenolics and antioxidants in peanuts while maintaining acceptable REP; (3) determine the stability of

selected REP products; and (4) identify the phenolic compounds in REP, assess and correlate the phenolic and sensory profiles of REP.

SECTION 2

REVIEW OF LITERATURE

I. INTRODUCTION

Peanuts, *Arachis hypogaea,* are an important food crop in the United States. In 2006, total consumption was 6.5 pounds per capita (USDA/ERS, 2008) with over half consumed as peanut butter. Peanut butter had a 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/ERS, 2008). 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/NAS, 2008). 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/NAS, 2008).

Peanuts contain bioactive compounds with health benefits, such as stilbenes, flavonoids, phenolic acids, and phytosterols (Table 2.1). 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).

The stilbene, resveratrol is a widely studied bioactive compound that has received much interest over the last ten 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

Table 2.1 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; Sales and Resurreccion, 2009; Potrebko and Resurreccion, 2009
	Peanut skins	Nepote et al., 2004; Francisco and Resurreccion, 2009b
	Peanut leaves	Subba Rao et al., 1996; 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	Sales and Resurreccion, 2009; Potrebko and Resurreccion, 2009
	Peanut butter	Ibern-Gomez et al., 2000
Piceatannol	Peanut callus	Ku et al., 2005
Pterostilbene	Peanut hairy roots culture	Medina-Bolivar et al., 2007
Phenolic acids:		
ρ-Coumaric acid	Peanut kernels	Talcott et al., 2005a; 2005b
	Peanut skins	Yu et al., 2005; Francisco and Resurreccion, 2009b
Hydroxybenzoic acid ester	Peanut kernels	Talcott et al., 2005a
Ethyl protecatechuate	Peanut skins	Huang et al., 2003
Protocatechuic acid	Peanut skins	Francisco and Resurreccion, 2009b
Chlorogenic acid	Peanut skins	Yu et al., 2005
Caffeic acid	Peanut skins	Yu et al., 2005; Francisco and Resurreccion, 2009b
Ferulic acid	Peanut skins	Yu et al., 2005 ; Francisco and Resurreccion, 2009b
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, 2009b

Table 2.1 continued…

Compound	Peanut Plant Materials and Products	Reference
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	Peanut skins	Lou et al., 2004
Epicatechin-(2β➢ *O*➢7,4β➢ 8)-[epicatechin-(4α➢8)-catechin		
Procyanidin B2		
Procyanidin B3		
Procyanidin B4		
Epigallocatechin	Peanut skins	Yu et al., 2005;
Epicatechin		
Catechin gallate		
Epicatechin gallate		
Procyanidin dimer A1 [epicatechin-4β-8,2β-O7)-catechin]	Peanut skins	Verstatraeten et al., 2005
Procyanidin trimer A		
Procyanidin monomers	Peanut skins	Yu et al., 2006
A-type procyanidin dimers		
B-type procyanidin dimers		
A-type procyanidin trimers		
B-type procyanidin dimers		
A-type procyanidin tetramers		
B-type procyanidin tetramers		
Luteolin	Peanut hulls, mature	Daigle et al., 1988; Duh and Yen, 1995
Eriodictyol	Peanut hulls, immature	Daigle et al., 1988
Formononetin	Peanut leaves	Subba Rao et al., 1996
Deidzen		
Medicarpin		
Phytosterols:		
Beta-sitosterol	Peanut kernels	Awad et al., 2000
Campesterol	Peanut oil	
Stigmasterol		

medicine (Soleas et al., 1997) for treatment of supporative dermatitis, gonorrhea favus, athlete's foot, and hyperlipemia (Aggarwal et al., 2004). In 1976, resveratrol was synthesized in grapevine leaves after fungal infection and UV light exposure (Langcake and Pryce, 1976). However, increased interest in resveratrol by researchers, 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). 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 developed countries such as in the US (Stanley and Mazier, 1999). Resveratrol and other polyphenolic compounds in red wines were believed to be associated with this paradox (Frankel et al., 1993).

There is evidence that resveratrol may protect against cardiovascular diseases (Pace-Asciak et al., 1995). Resveratrol has also shown to inhibit initiation, promotion, and progression of cancer (Jang et al., 1997). More recently, resveratrol was found to have therapeutic potential against Alzheimer's disease (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 fungi (Keen and Ingham, 1976) 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 Resurreccion, 2005), ultrasound (Lin et al., 2001; Rudolf and Resurreccion, 2005), ozone (Grimmig et al., 1997), heat 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), salicylic acid (Subba Rao et al., 1996), jasmonic acid (Chung et al., 2003) and ethylene (Chung et al., 2003).

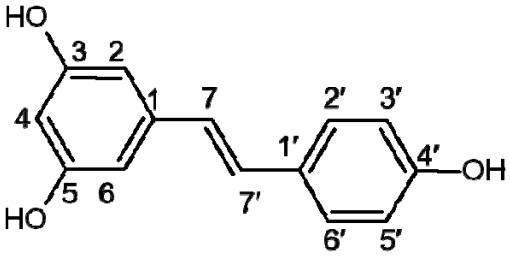
This section reviewed *trans*- resveratrol and related stilbenes from peanuts - their chemical structures, mechanisms for their biosynthesis, and concentrations in comparison with other major food sources. This review also discussed *trans*- resveratrol's major health benefits, absorption and metabolism,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

II. *TRANS*-RESVERATROL AND RELATED STILBENES

A. Chemical Structure

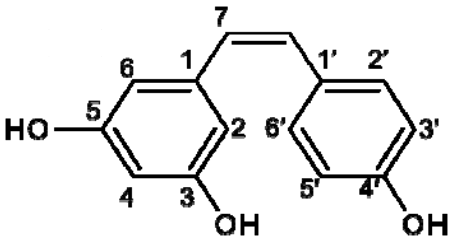
The *trans*-resveratrol molecule consists of two phenolic rings linked by a styrene double bond to generate 3, 5, 4′-trihydroxystilbene (Figure 2.1). The double bond facilitates *trans* and *cis* isomeric forms of resveratrol (Aggarwal et al., 2004) with the *trans* isomer as stearically the more stable 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^{o}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



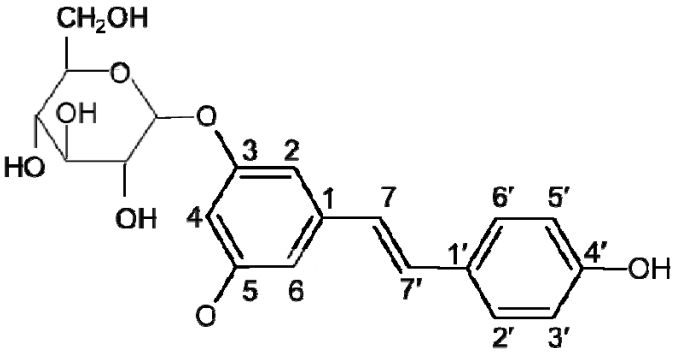
***trans*-resveratrol**

***trans*-3,5,4′-trihydroxystilbene**



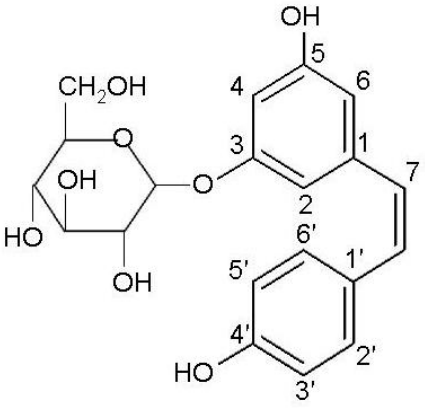
***cis*-resveratrol**

***cis*-3,5,4′-trihydroxystilbene**



***trans*-piceid**

***trans*-5,4′-trihydroxystilbene-3-*O*-β-glycopyronoside**



***cis*-piceid**

***cis*-5,4′-trihydroxystilbene-3-*O*-β-glycopyronoside**

Figure 2.1 Structures of *trans*- and *cis*- resveratrol and piceid.

sealed, light-proof containers were stable for three months when stored at -5^{o}C and protected from light, except in high-pH buffers. Trela and Waterhouse (1996) reported that the *trans* form was converted to a maximum of 90.6% *cis* isomer after exposure to UV irradiation at 366 nm for 100 min, and only up to ≤63% *cis*-resveratrol at lower wavelength of 254 nm even after 10 h. 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 (Trela and Waterhouse, 1996).

Trans-resveratrol is a better free radical scavenger compared to Vitamins E or C, but has similar activity as the flavonoids, epicatechin and quercetin (Stojanovic et al., 2001). 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 to vitamin E (Sun et al., 1997).

The 4′-hydroxyl group of resveratrol was more reactive than the 3- and 5-hydroxyl groups (Figure 2.1) because of resonance effects (Aggarwal et al., 2004). In terms of its antioxidant activity, the 4′-hydroxyl group is the most important functional group in resveratrol as it is most reactive in scavenging free radicals compared to the 3′-hydroxyl group (Regev-Shoshani et al., 2003).

B. 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 which may be toxic.

C. 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* configuration (Figure 2.1), 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 in peanut roots, stems and leaves (Lin et al., 2007) and in high amounts in UV-irradiated peanut callus (Ku et al., 2005; Lin et al., 2007). Piceatannol differs from resveratrol by having an additional hydroxy group in one of the aromatic rings (Figure 2.2).

Peanuts that were imbibed, sliced, and then allowed for natural microflora or inoculated microorganisms to grow produced several resveratrol derivatives. Keen and Ingham (1976) identified *cis*- and *trans*-isomers of 3, 5, 4′-trihydroxy-4-isopentenylstilbene, which was later named as Arachidin II (Arora and Strange, 1991), from the germinating American peanut seeds challenged with native microflora. In sliced fully imbibed peanut kernels where natural microflora were allowed to grow, Aguamah et al. (1981) isolated three resveratrol derivatives, namely: a) Arachidin I or *trans*- 4-(3-methyl-but-1-enyl)-3,5,3′,4′-tetrahydroxy-stilbene; b) 4-(3-methyl-but-1-enyl)-3,5,4′-trihydroxy stilbene (4-isopentenylresveratrol); and c) 4-(3-methyl-1-butenyl)-3,5,4′-trihydroxy stilbene. Arachidin I, Arachidin II, and Arachidin III were also isolated from fully imbibed and sliced peanut kernels incubated for 0 to 144 h at 25 and 37^{o}C (Wotton and Strange, 1985). The *cis* and *trans* isomers of 3, 5, 4′-trihydroxy-4-isopentylstilbene were identified by Keen and Ingham (1976) from germinating American peanut seeds challenged with native microflora which later named as Arachidin II by Arora and Strange (1991). Cooksey et al. (1988) quantified 3-isopentadienyl-4, 3′, 5′-trihydroxystilbene, which was later named as Arachidin IV by Arora and Strange (1991), from fully imbibed and sliced peanut kernels incubated for 24 and 48 h at 25^{o}C. Resveratrol derivatives, *trans*-3-isopentadienyl-4,3,5′-trihydroxystilbene and *trans*-4-(3-methyl-butyl-enyl)-3,5′,4′-trihydroxystilbene (*trans*-arachidin-III) were isolated from fully imbibed and sliced peanut kernels inoculated with *Aspergillus flavus* and *Aspergillus parasiticus* (Sobolev et al., 1995).

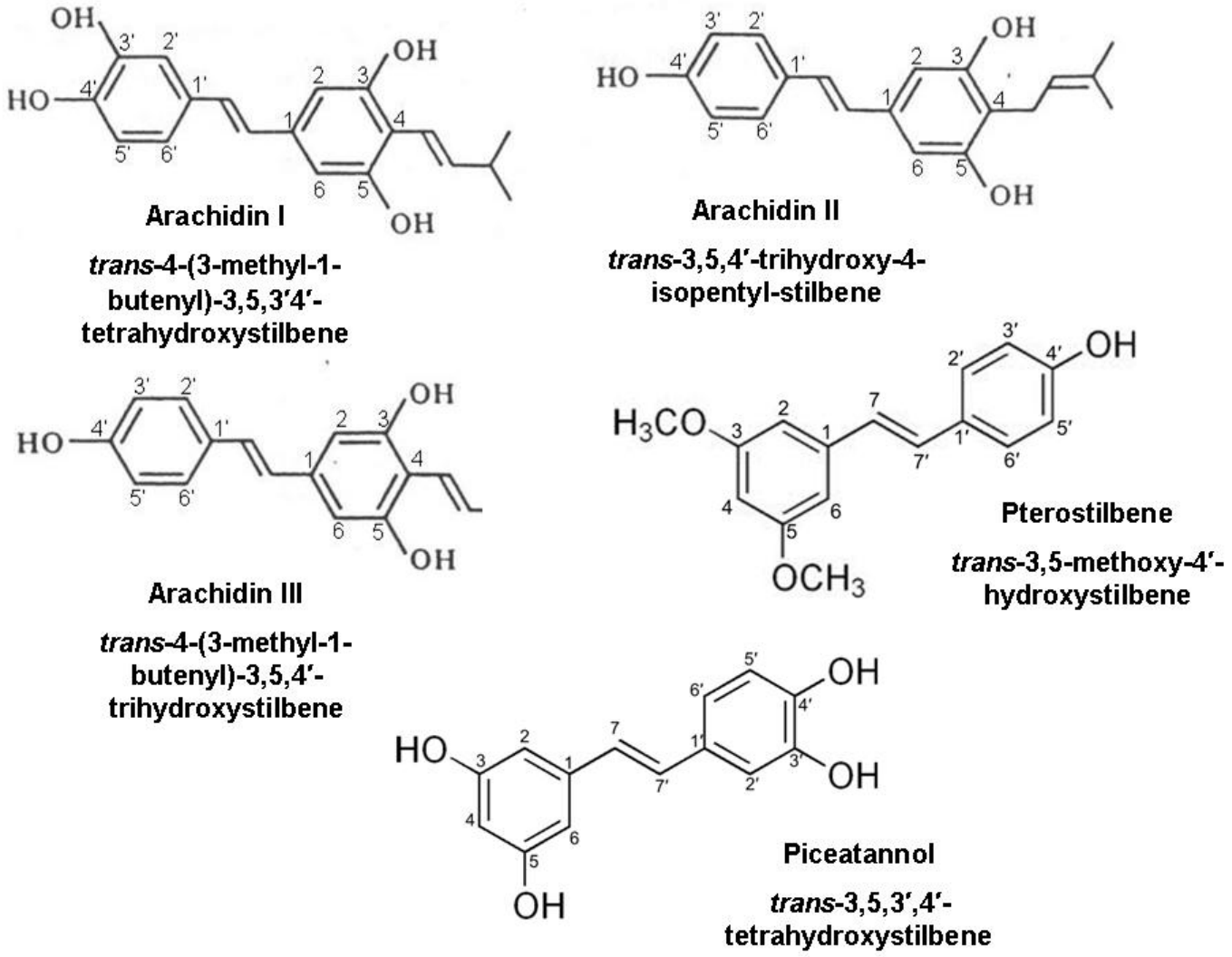


Figure 2.2 Structures of resveratrol derivatives found in peanuts.

D. 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 (Figure 2.3; 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 co-enzyme by CoA ligase. For each molecule of *trans*-resveratrol synthesized, four molecules of CO_2 are released.

Biosynthesis of resveratrol specifically requires stilbene 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 min, and was 30-fold above the baseline after 2h. The first increase in translatable mRNA for stilbene synthase occurred within 20 min 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).

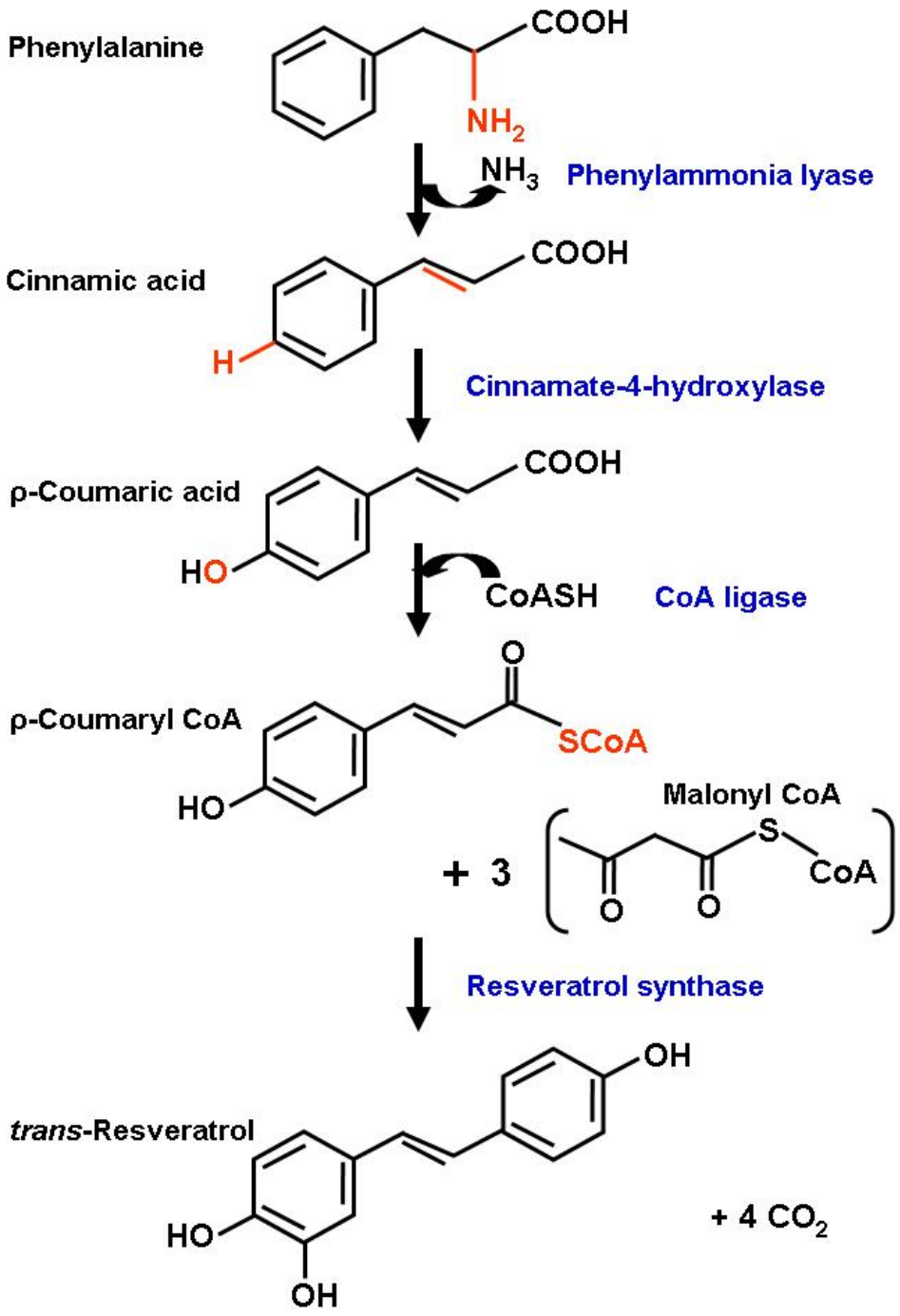


Figure 2.3 Biosynthesis of *trans*-resveratrol from one molecule of 4-coumaryl CoA and three molecules of malonyl (Soleas et al., 1997).

Stilbene synthase mRNA was detected 10 min after UV irradiation reaching a maximum between 2 and 8 h, and ceasing after 24 h (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 reported that resveratrol and resveratrol synthase (RS) mRNA were relatively abundant in roots and shells of peanut plants grown up to mid-maturity (40 days after flowering) compared to 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, resveratrol concentrations 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 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 6-fold 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 510W UV lamp for 30 s at a distance of 40 cm followed by 3 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/s) for 2 h 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 mature peanuts at stages 6 and 8 were divided into pod, testa, and cotyledons, Arora and Strange (1991) found that mature pods and testas lost their abilities to synthesize phytoalexins whereas mature cotyledons increased synthesis.

E. Concentrations in Peanuts

Resveratrol, piceid, and other stilbenes are naturally present in edible and inedible parts of peanut plant (Table 2.2). 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.

1. Edible peanuts

1.1 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 2.2. Earlier reports by Sanders et al. (2000) indicated that *trans*-resveratrol concentrations in raw peanuts ranged from 0.02 to 0.31μg/g in 14 of

Table 2.2 Concentrations of *trans*-resveratrol and other stilbenes found in peanuts and in edible and inedible peanut plant materials.

Source	Compound		Reference
	Name	**Concentration (µg/g)**	
A. Edible Peanuts			
1. Raw Peanut Kernels			
Runners, 6 cultivars, cold stored for ~3 years	*Trans*-resveratrol	0.022 - 0.069	Sanders *et al.*, 2000
Spanish, 5 cultivars, cold stored for ~3 years	*Trans*-resveratrol	0.023 - 1.792	Sanders *et al.*, 2000
Virginia, 4 cultivars, cold stored for ~3 years	*Trans*-resveratrol	0.048 - 0.306	Sanders *et al.*, 2000
Spanish, 7 cultivars	*Trans*-resveratrol	0.09 - 0.30	Lee *et al.*, 2004
Virginia, 8 cultivars	*Trans*-resveratrol	0.1 - 0.25	Lee *et al.*, 2004
6 varieties and 4 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

Table 2.2 continued…

Source	Compound		Reference
	Name	**Concentration (µg/g)**	
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	Vayndorf, 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 Materials			
1. Peanut skins (seed coats)			
Runner variety	*Trans*-resveratrol	0.51	Sanders *et al.*, 2000

Table 2.2 continued…

Source	Compound		Reference
	Name	**Concentration (µg/g)**	
Runner variety	*Trans*-resveratrol	4.30	Francisco and Resurreccion, 2009b
Virginia variety	*Trans*-resveratrol	3.66	Francisco and Resurreccion, 2009b
Virginia variety	*Trans*-resveratrol	0.78	Sanders *et al.*, 2000
Spanish variety	*Trans*-resveratrol	15.04	Francisco and Resurreccion, 2009b
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

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. Sobolev and Cole (1999) found that small seeds which were associated with immature peanuts have greater capacity for phytoalexin production than larger or mature peanuts. In terms of quality, discolored yellow inedible split peanuts contain higher amounts of trans-resveratrol up to 7.09 µg/g compared to 0.23 µg/g in non-discolored 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 3 years had higher *trans*-resveratrol of 0.02 – 1.79 µg/g compared to 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 9 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 to 0.03 μg/g in peanuts stored for only 6 months.

In terms of viability of peanut seeds, viable sliced peanuts accumulated higher total phytoalexins of as much as 5.29 μM/g compared to non-viable sliced seeds with 0.716 μM/g (Arora and Strange, 1991). Their results indicated that peanuts seeds should be viable for the maximum biosynthesis of phytoalexins in peanuts.

1.2 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 to 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., 2005b).

1.3 Peanut butter

Trans-resveratrol contents in commercial peanut butters ranged from 0.265 to 0.671 μg/g in blended type (stabilizer 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 were 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 co-workers (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).

1.4 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 peanuts. These authors attributed this to the presence of low quality kernels, such as small, immature, and mechanically damaged pods in boiled peanuts which were normally sorted out when manufacturing roasted peanuts and peanut butter. In the same study, resveratrol concentrations in raw shelled peanuts generally increased with decreasing seed size which usually associated with more immature peanuts; and highest concentrations were found in discolored seeds (Sobolev and Cole, 1999).

1.5 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 9 days in the dark compared to 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).

2. Inedible parts of peanut plant

Inedible peanut plant materials like leaves (Chung et al., 2003), roots (Chung et al., 2003; Chen et al., 2002), hulls (Sobolev and Cole, 1999; Chung et al., 2003) and skins (Francisco and Resurreccion, 2009a; Nepote et al. 2004) contain beneficial phenolic compounds (Table 2.2). 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.

2.1 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 (2009b) identified and quantified *trans*-resveratrol, three phenolic acids, and five flavonoids in skins of three U. S. peanut varieties using a reversed phase 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 to 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 to 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, fraction 17 identified as ethyl protocatechuate, had the highest yield and the fourth highest antioxidant activity (Huang et al., 2003).

2.2 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 and eriodictyol at 3.8 mg/g; and flavonoid decomposition product, 5, 7-dihydroxychromone, at 1.49 mg/g (Daigle et al, 1988). However, a 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^{o}C for 60 min (Lee et al., 2006).

2.3 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 was 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).

In UV-irradiated peanut callus, Ku et al. (2005) did not detect *trans*-resveratrol and piceatannol immediately after irradiation using static cultivation, but concentrations increased up to 11.97 μg/g and 5.31 μg/g, respectively, after 18 h of incubation. Using suspension cultures, *trans*-resveratrol but not piceatannol increased up to 6.93 μg/g after 4 h from UV treatment; and did not increase thereafter from 8 to 80 h as calluses may have received shorter UV irradiation while constantly moving in the suspension cultures (Ku et al., 2005).

3. Other major sources of resveratrol

3.1 Grapes and wines

Resveratrol is synthesized particularly in the skins of grape berries and none to trace amounts are present in the fruit flesh (Creasy and Coffee, 1988; Becker et al., 2003). Compared to raw, roasted and boiled peanuts with *trans*-resveratrol in the range of 0.02 to 7.09 (Table 2.2), 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 non-detectable to 0.5 μg/g fresh weight (Burns et al., 2002). Resveratrol in Concord grape products ranged from 0.002 – 1.042 μmol/g (1.56 – 1042 nmol/g) and 0.002 μmol/g (1.56 nmol/g) in grape juice (Wang et al. 2002). A positive correlation existed between vineyard elevation and grape stilbene concentrations (Bavaresco, 2003). Quality-oriented cultural practices produce grapes with higher levels of stilbenes (Bavaresco, 2003).

Due to its presence in grape skins, resveratrol is expected in wines due to skin contact during fermentation (Becker et al., 2003). 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). When wine is made from grapes, resveratrol is released from the skins and maceration increased the extraction of resveratrol by 9 and 13-fold in red and white wines, respectively, compared to nonmacerated wines (Jeandet et al., 1995). Paradoxically, lower concentrations of resveratrol were observed in wines made from grapes highly infected with *Botrytis* 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). In comparison, these values were higher than in raw and roasted peanuts but comparable with that in boiled peanuts (Table 2.2).

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 resulted 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 to 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 to 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 to *trans*-piceid in four red wine samples. *Trans*-piceid was only detected in Cabernet Sauvignon (Bulgaria) with 1.89 µg/mL *trans*-piceid while the other three samples had none. The total resveratrol or the sum of *trans*- and *cis*-resveratrol and *trans*-piceid in Cabernet Sauvignon, 1996 (Bulgaria) was 1.380 µg/mL. The three wines without *trans*-piceid were Pinot noir, 1994 (California), with the highest *trans*-resveratrol of 1.057 µg/mL and *cis*-resveratrol of 0.746 µg/mL contributing to total resveratrol of 1.803 µg/mL; Merlot, 1994 (Chile) and Cabernet Sauvignon, 1995 (California) with 0.20 and 0.098 µg/mL total resveratrol, respectively.

3.2 Vaccinum spp. including blueberries, bilberries, and cranberries

Trans- 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 ± 29.9 pmol/g (or 0.00014 ±

0.00003 μmol/g), fresh bilberries from Poland at 71.0 ± 15.0 pmol/g (or 0.000071 ± 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 to raw, roasted, and boiled peanuts (Table 2). Heating by baking decreased resveratrol concentrations in blueberries between 17 and 46% after 18 min at 190°C, and were expected to be lower compared to 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).

3.3 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, the major stilbene found in *P. cuspidatum* is piceid or *trans*-resveratrol glucoside (Lamuela-Raventos et al., 1995; Burns et al., 2002). Commercial Itadori root contained 1,653 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 5 min contained 0.905 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 (Lamuela-Raventos et al., 1995; Brandolini et al., 2002) and peanut butter (Ibern-Gomez et al*.,* 2000).

3.4 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 (Tokusoglo et al., 2005) which were higher compared to raw and roasted peanuts, but lower than boiled peanuts (Table 2.2). Turkish pistachios contain higher *trans*-resveratrol compared to 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).

III. BIOAVAILABILITY AND HEALTH EFFECTS OF RESVERATROL AND OTHER STILBENES

A. 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% is excreted via feces and urine (Wenzel and Somoza, 2005). Resveratrol had lower oral bioavailability of almost zero due to rapid and extensive metabolism and the consequent formation of various metabolites as resveratrol glucoronide and resveratrol sulfates (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 (Kuhnle et al., 2000; Andluer et al., 2000). Resveratrol biovailabity

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).

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). The human digestive tract is known to have glucosidase activity, so it could be possible that piceid could release resveratrol, on ingestion (Lamuela-Raventos et al., 1995; Chen et al., 2001). 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 compared to *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 transporter 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 at al., 2003). This result was consistent with the findings of Potter et al. (2002) that piceatannol was formed during *in vitro* metabolism of resveratrol by recombinant human cytochrome P450 enzyme CYP1B1 (cytochrome P450 family 1, subfamily B, polypeptide 1). In a wide variety of human tumors, the enzyme CYP1B1 is overexpressed and catalyzes aromatic hydroxylation reactions, so in the presence of resveratrol, this enzyme catalyzes the hydroxylation of resveratrol to form piceatannol (Potter et al., 2002).

B. Health Effects

1. 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 anti-carcinogenic 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, 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 stress has been linked to cancer, atherosclerosis, ischemic injury, inflammation, and 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).

2. Cardiovascular protective effect

Cardiovascular disease (CVD) including heart disease and stroke account for 34.3% of all deaths in the U.S., with heart disease as the first and stroke as the third leading causes of death (CDC, 2010b). The economic burden of CVD has a profound impact on the U. S. health care system and the cost including health expenditures and loss of productivity resulting from death and disability was estimated to be more than $503 billion in 2010 (CDC, 2010b).

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 (LDL) mainly due to its capacity to chelate copper and scavenge free radicals *in vitro* (Belguendouz et al., 1998; Frankel et al., 1993b). The para-hydroxyl group appeared to show a greater radical scavenging activity than the meta-hydroxyl 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).

Belguendouz et al. (1998) observed that when resveratrol was added to plasma prior to fractionation, it was more associated with lipoproteins than with lipoprotein-free proteins revealing its lipophilic character. For a given concentration of resveratrol added to the plasma, the amounts of resveratrol per mg lipoprotein protein increases in concentration as lipoprotein

density decreases; lowest with high density lipoproteins (HDL) intermediate with LDL, and highest with very low density lipoprotein (VLDL). To test the ability of resveratrol in protecting phospholipid polyunsaturated fatty acid (PUFA) from oxidation, phospholipid unilamellar liposomes supplemented with resveratrol were oxidized with a water-soluble radical generator, 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH) then thiobarbituric acid reactive substances (TBARS) were measured. Belguendouz et al. (1998) found that resveratrol inhibited the formation of TBARS in a linear dose-response curve when resveratrol was added up to 30 μM in the final preparation, and up to 200 μM when it was added before preparation of liposomes, suggesting that the soluble fraction of resveratrol scavenged free radicals in the aqueous phase before attacking PUFA within membranes. These researchers concluded that all these results support the hypothesis that resveratrol may be efficient at different sites in the cell, in the protein and lipid moieties of LDL and in their aqueous environment.

The stimulation of the expression and activity of endothelial nitric oxide synthase (eNOS) contributes to the cardiovascular disease protection from LDL oxidation (Wallerath et al., 2002). In their study, Wallerath et al. (2002) found that after incubation of human umbilical vein endothelial cells with resveratrol, increase in eNOS mRNA expression up to 2.8 fold in a time- and concentration-dependent manner was observed. eNOS protein expression and eNOS-derived nitric oxide production were also increased after long term incubation with resveratrol. Resveratrol also stabilized mRNA. These results indicate the cardiovascular protective effect of resveratrol.

Myocardial preconditioning is the exposure of myocardial tissue to brief, repeated periods of vascular occlusion in order to render the myocardium resistant to the deleterious effects of prolonged episodes of ischemia or reperfusion (Kalikiri and Sachan, 2004). The period of pre-

exposure and the number of times the tissue is exposed to ischemia and reperfusion vary, the average being 3 to 5 minutes (Kalikiri and Sachan, 2004). Resveratrol protects the heart by pre-conditioning it through the activation of adenosine A1 and A3 but not A2a receptors (Das et al., 2004). Adenosine A1 transmits a survival signal through phosphatidylinositol (PI)3-kinase-Akt-Bcl-2 signaling pathway whereas 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 (Das et al., 2004).

Squalene monooxygenase is 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 prevents the action of human squalene monooxygenase in a noncompetitive manner with respect to both squalene, Ki=35 micromolar and FAD, Ki=69 micromolar.

3. Cancer prevention and therapy

Cancer is the second leading cause of death in the United States (CDC, 2010a). According to the United States Cancer Statistics: 2006 Incidence and Mortality, which tracks cancer incidence for about 96% of U.S. population and mortality for the entire country, more than 559,000 Americans died of cancer and more than 1.37 million had a diagnosis of cancer in 2006 (CDC, 2010a). The financial cost of cancer was estimated at $228 billion in 2008 (CDC, 2010a).

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 (Escher and Steward, 2003). Agawam et al. (2004) conducted a comprehensive review of the role of resveratrol in prevention and therapy of a wide variety of

cancers including lymphoid, myeloid, multiple myeloma, breast, prostate, and stomach, colon, pancreas, thyroid, melanoma, head and squalors cell carcinoma, ovarian and cervical carcinoma. In their review, Agawam et al. (2004) reported that resveratrol induces apoptosis or initiation of cell death from inside of the cells, primarily from mitochondria, through a variety of pathways.

Resveratrol suppresses the activation of transcription factors, nuclear factor kappa B (NF-κB) that regulates the expression of various genes involved in inflammation, cytoprotection and carcinogenesis (Holmes-McNary and Baldwin, 2000); activator protein-1 (AP-1), a transcription factor transactivated by many tumor-promoting agents such as phorbol ester, UV radiation, asbestos and crystalline silica (Manna et al., 2000; Eferl and Wagner, 2003); and 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).

Resveratrol inhibits the activities of mitogen-activated protein kinases (MAPK) including extracellular receptor kinase (ERK 1/2) implicated in the proliferation of cells (Miloso et al., 1999), c-Jun N-terminal kinase (JNK), and p38 MAPK which are responsible for the activation of AP-1 (She et al., 2001); protein kinases which play a major role in tumorigenesis (Garcia-Garcia et al., 1999; Stewart et al., 1999); growth factor and associated tyrosine kinases that mediate various growth factors for a variety of tumor cells (Kaneuchi et al., 2003); cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) which play important roles in inflammation of cells (Subbaramiah et al., 1998); and cell cycle proteins that inhibit cell proliferation (Wolter et al., 2001; Yu et al., 2003).

Resveratrol suppresses the activities of 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., 2002); androgen receptors that play a role in prostate cancer etiology (Mitchell et al., 1999); and prostate specific gene (PSA) (Hsieh et al., 2000). It also prevents expression of inflammatory cytokine (Wang et al., 2001).

Resveratrol suppresses angiogenesis, 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) which plays an important role in tumor growth, other diseases and wound healing (Aggarwal et al., 2004). It also prevents inflammation, by activating NF-κB, suppressing proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL) -6 (Aggarwal et al., 2004; Wang et al., 2001), and suppressing the expression of proteins such as iNOS, COX-2, and 5-LOX that mediate inflammation (Kimura et al., 1995; Kimura et al., 1983).

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 (Kageura et al., 2001; Hsieh et al., 1999).

Piceatannol, a resveratrol derivative extracted from UV-induced peanut calluses was found to be a 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 reports that piceatannol is a more efficient inducer of apoptosis (Lin et al., 2007).

4. 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). In their study using human breast cancer cells, Gehm et al (1997) found that resveratrol inhibited the binding of labeled estradiol to the estrogen receptor and it activated transcription of estrogen-responsive reporter genes transfected into the cancer cells. Resveratrol 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. Gehm et al. (1997) found that resveratrol increased the expression of native estrogen-regulated genes and stimulated proliferation of estrogen-dependent T47D human breast cancer cells, which contradicted Jang et al (1997) who observed the anticarcinogenic effect of resveratrol in mouse mammary cultures. Ghem et al. (1997) explained this apparent contradiction that although many human breast cancer cells were mitogenically stimulated by estrogen, most mouse mammary cancers are estrogen insensitive.

Lu and Serrero (1999) observed that resveratrol inhibits the growth of estrogen-receptor (ER) positive MCF-7 cells in a dose dependent fashion. 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 concentrations $\geq 10^{-6}$ M. 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).

5. Treatment of Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to the most common form of dementia (Marambaud et al., 2005). Beta-amyloid peptide is considered to be responsible for the formation of senile plaques that accumulate in the brains of patients with AD (Jang and Surh, 2003). 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 Suhr, 2003).

Considerable attention was focused on identifying phytochemicals that are able to scavenge excess ROIs, thereby protecting against oxidative stress and cell death. Jang and Suhr (2003) found that cultured rat pheochromocytoma (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. The beta-amyloid treatment also led to the decreased mitochondrial membrane potential, 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-κB in PC12 cells, which was suppressed by resveratrol pretreatment.

Marambaud et al. (2005) showed that resveratrol has potent anti-amyloidogenic activity by reducing levels of intracellular beta amyloid peptide. Resveratrol promotes the intracellular degradation of beta amyloid peptide by a mechanism that implicates the proteosome. 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 proteosome inhibition suggesting that high levels of beta amyloid in AD brains may create a vicious cycle by inhibiting the proteosome and blocking the degradation of critical regulators of its own clearance.

6. Anti-aging 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 were 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 (Delmas et al., 2005). 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 anti-aging agent in treating age-related human diseases through the stimulation of Sirtuin (Sir) 2 activity (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 towards 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 activities of AMPK and PGC-1 alpha (peroxisome proliferators-activated receptor-gamma coactivator 1 aplha), increased mitochondrial number, and improved motor function.

7. Other health effects

7.1 Analgesic effect or anti-pain property

Trans-resveratrol's analgesic effect was reported to be mediated via an opioidergic mechanism (where opiods attenuate or weaken the affective component of pain) 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 intraperitoneal (i.p.) produced dose-dependent analgesia. Pretreatment for 20 min 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.). Potentiation effect indicates a synergistic action in which the effect of two drugs given simultaneously is greater than the sum of the effects of each drug given separately.

Gupta et al. (2004) studied the effect of *trans*-resveratrol (20 mg/kg i.p.) on morphine tolerance of rats in three different treatments administered continuously for 7 days including

treatment 1, morphine at 10 mg/kg i.p.; treatment 2, *trans*-resveratrol at 5 mg/kg i.p. administered 10 min before morphine at 2 mg/kg i.p.; and treatment 3, *trans*-resveratrol at 20 mg/kg i.p. The occurrence of tolerance was estimated by comparing the antinociceptive (reducing sensitivity to painful stimuli) 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 (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. According to these authors, resveratrol previously exhibited analgesic effects suggesting that cyclooxygenase inhibition and K+ channel opening were the underlying mechanisms. Kim et al (2005) reported that 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 dorsal root ganglion (DRG) neurons may make a great contribution to the analgesia by resveratrol.

7.2. Protective effect on hyperglycemia

Hyperglycemia, a symptom of diabetes mellitus, induced hyperosmotic responses including apoptosis in vascular endothelial cells and leukocytes in mammals (Chan, 2005). The apoptotic biochemical changes such as caspase-3 activation and DNA fragmentation, were blocked by antioxidant pretreatment during hyperosmotic shock-induced cell death. Chan (2005) found that resveratrol blocks oxidative stress in high glucose-induced apoptosis and alterations in

attachment ability by virtue of its antioxidant property. Resveratrol attenuated high glucose-induced apoptotic changes, including JNK and caspase-3 activation in human leukemia K562 cells. Experiments with the cell permeable dye, 20,70-dichlorofluorescein diacetate (DCF-DA), an indicator of reactive oxygen species (ROS) generation, revealed that high glucose 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 whereas cells pretreated with resveratrol followed by high glucose treatment 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 was blocked by resveratrol by virtue of its antioxidant property.

IV. 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 or ultrasound, and treatment with metallic salts, salicylic acid, or ozone.

Biotic stresses not only increase phytoalexin production but may also lead 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.

A. Biotic Stresses

Microbial invasion and/or inoculation of microorganisms in plant and plant materials had been shown (Ingham, 1976) to increase the concentration of phytoalexins. Peanuts are known to synthesize many phytoalexins in response to microbial stress as shown in Table 2.3 wherein synthesis of resveratrol and other resveratrol-related phytoalexins were enhanced after the proliferation or inoculation of various microorganisms in peanut kernels, leaves and roots.

1. Microbial invasion and/or inoculation of molds

Ingham (1976) found that peanut hypocotyls accumulated a mixture of 38-55 µg/mL *cis*- and *trans*-resveratrol after inoculation with 10µl of a conidial suspension containing approximately 5 $x10^4$ spores/mL of a non-pathogenic fungus, *Helminthosporium carborum,* and incubation at 22^oC for 24 h. These compounds were not detected in extracts from control non-inoculated peanut hypocotyls (Ingham, 1976) suggesting that fungal infection initiated resveratrol biosynthesis.

In peanut kernels, as high as 3,690 µg/g of a resveratrol derivative, 4-(3-methyl-but-1-enyl)-3,5,4′-trihydroxystilbene, was accumulated when imbibed peanuts were sliced and incubated in the dark at 25^oC for 48h 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 1,160 µg/g of 4-(3-methyl-but-2-enyl)-3,5,4′,-trihydroxystilbene (4-isopentenylresveratrol).

Recently, Sobolev et al. (2009) isolated four new stilbenoids, arahypin-1, arahypin-3, arahypin-4, and arahypin-5 from 3-6 mm sliced imbibed viable peanut seeds challenged by an unspecified concentration of *Aspergillus caelatus* spores, along with two known stilbenoids that have not been previously reported in peanuts, chiricanine A and arahypin-2, and six other

Table 2.3 Concentrations of resveratrol and other related compounds in peanut and grape plant materials stressed by microbial invasion and/or inoculation[1]

Plant material	Post-harvest Stress Treatment Condition				Compound			Reference
	Microorganism	Concen-tration (spores/mL)	Incubation		Name	Concentration		
			Time	Temp.		Control	Stressed	
Peanut leaf hypocotyls, 2-4 cm	*Helminthosporium carbonum* Ullstrup	5 x 10^4	24 h	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	48 h	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	5 d	23-26°C, 100% RH	*trans*-resveratrol	NR	30 µg/g dry wt.	Sobolev et al., 1995
					trans-3-isopentadienyl-4,3′5′-trihydroxystilbene	NR	10 µg/g dry wt.	
					trans-4-(3-methyl-but-1-enyl)-3,5,4′-trihydroxystilbene (*trans*-arachidin-3)	NR	95 µg/g dry wt.	

Table 2.3 continued…

Plant material	Post-harvest Stress Treatment Condition				Compound			Reference
	Microorganism	Concen-tration (spores/mL)	Incubation Time	Incubation Temp.	Name	Concentration Control	Concentration Stressed	
Peanut leaves	*Cercospora arachidocola*	10^4 Severe infection	4 weeks	NR	Total phytoalexins	2.96 nmoles/g fresh wt.	1,830.21 nmoles/g fresh wt.	Subba Rao et al., 1996
Peanut leaves	*Cercospora arachidocola*	10^3 Mild infection	4 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	4 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	4 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	25mg/mL yeast extract	6h in the dark	NR	*Trans*-resveratrol -in leaves -in roots	0.15µg/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	NR	NR	Langcake and Pryce, 1977

[1]NR means no data reported

known stilbenes in peanuts, *trans*-resveratrol, *trans*-arachidin-1, *trans*-arachidin-2, *trans*-arachidin-5, *trans* -3′-isopentadienyl-3,5,4′-trihydroxystilbene, and SB-1. All stilbenoids were identified by nuclear magnetic resonance (NMR), mass spectrophotometry (MS) and UV spectra. These authors found that non-viable peanuts prepared by boiling imbibed and sliced 3-6 mm seeds in distilled water for 90 s were completely colonized by the fungus, whereas, only few colonies were counted in viable peanuts suggesting that viable peanuts effectively suppress fungal growth compared to non-viable samples. Viable sliced peanuts that were inoculated produced an array of stilbenoids was produced at significant concentrations. In contrast, no stilbenoids were detected in uninoculated sliced non-viable and viable control samples indicating that slicing alone did not serve as elicitor (Sobolev et al., 2009), which is contrary to Aguamah et al. (1981) who found that uninoculated sliced peanuts after incubation for 48 h to allow natural microflora to proliferate induced accumulation of stilbene phytoalexins .

Subba Rao et al. (1996) found that extracts from peanut leaves that had been severely infected by *Cercospora arachidicola* at 10^4 spores/mL contained the greatest phytoalexins concentration of 1830 nmol/g fresh weight compared to uninoculated controls with 2.96 nmol/g fresh weight. Leaves severely infected with 10^4 spores/mL of *Puccina arachidis* had the least amount of phytoalexins with 162 nmol/g fresh weight, and those infected with 10^4 spores/mL of *Phaeoisariopsis personata* or mildly infected with *C. arachidicola* at10^3 spores/mL were intermediates at 664 and 745 nmol/g, respectively.

In leaves of transgenic tomato plants, a rapid accumulation of resveratrol occurred during the first 6 h after inoculation of *Phytoptera infestans* and reached a maximum of 258 µg/g fresh weight after about 44 h; afterwards resveratrol accumulation declined (Thomzik et al., 1997). Inoculation of transgenic tomatoes with *Botrytis cinerea* and *Alternaria solani* also followed a

similar trend and afterwards, resveratrol accumulation slowly declined. After fungi treatment of immature tomato fruits, resveratrol content of 51.6 µg/g fresh weight was lower than that detected in sliced ripe fruit at 99 µg/g fresh weight. In whole tomatoes, inoculated with *P. infestans,* resveratrol increased from 1.7 to 6.9µg/g fresh weight after 24 to 96 h, respectively, which were lower than those detected in inoculated leaves and fruit slices which Thomzik et al. (1997) attributed to probable dilution with the non-infected tissue of the fruit pulp.

In peanut leaves that were infected with early leaf spot fungus, *Cercoscopora arachicola*, Edwards and Strange (1991) extracted and quantified seven phytoalexins. The four major phytoalexins include dimethylmedicarpin, formononetin, 7, 4′-dimethoxy-2′-hydroxyisoflavonone, and medicarpin whereas two minor components are 7, 2′-dihydroxy-4′-methoxyisoflavonone and daidzen. 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 25 µL of suspension containing 4 x 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 whereas 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 fungal elicitation otherwise unknown to this species.

2. Microbial invasion and/or inoculation of yeasts

Chung et al. (2003) incubated the roots of sterile intact peanut plants grown *in vitro* for 4 weeks then placed in the dark conditions for 48h, in half strength Murashige and Skoog medium containing 25 mg/mL yeast extract to enhance the levels of resveratrol. After incubation in the dark for 6h, resveratrol accumulated in peanut leaves and roots up to 1.2 and 2.8 μg/g fresh weight, respectively, corresponding to 8- and 19-fold increases relative to untreated control tissues. The levels of resveratrol synthase and mRNA increased in the treated leaves and roots and increases were correlated with the increase in resveratrol contents in the tissues. Chung et al. (2003) indicated that yeast extract induces resveratrol accumulation via transcription of resveratrol synthase mRNA in peanut plants.

3. Microbial invasion and/or inoculation of bacteria

In peanut leaves, Azpilicueta et al. (2004) found that the levels of total phenolic was enhanced 0 d after planting peanut hypocotyls inoculated with 1 mL cell suspension containing 1 x 10^7 cells/mL of *Bradyrhizobium spp.* (*Arachis*) strain. The plants were placed in a growth chamber at 28/22^{o}C under 16/8 h light/dark regime and watered every other day with either N-free for inoculated plants or standard Hoagland solution for uninoculated plants. Total phenolics in leaves of inoculated plants ranged from 14.6 to 20.03 μg ρ-coumaric acid/mL of crude extract which were higher compared to 5.71 to 9.82 μg/mL in uninoculated plants, and correspond to 2.6 - 3.5 fold increase.

B. Abiotic Stresses

1. Soaking or imbibing in water

Soaking raw peanuts in water for about 20 h and then drying for 66 h increased the resveratrol content between 45 and 65 times after treatment (Seo et al., 2005). Similarly,

increases in resveratrol concentrations from 0.48 in controls to 0.96 and 1.46μ/g were observed when raw peanuts were imbibed in water for 16 h and then incubated at 25^{o}C for 24 and 36 h, respectively (Rudolf and Resurreccion, 2005). Kernels from drought stressed peanuts cultivars accumulated less phytoalexins of 17-65% than kernels from non-stressed (wet-treated) peanuts (Wotton and Strange, 1987).

2. 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 2.4 shows the concentrations of resveratrol and phytoalexins in response to wounding through slicing, chopping and grinding of peanut and peanut plant materials.

Wotton and Strange (1985) observed that phytoalexin concentrations of ten peanut cultivars previously imbibed in water, sliced and incubated at 25^{o}C for 24 h ranged from 28 to 935 μg/g fresh weight and vary depending on cultivar conditions, duration of incubation after slicing, and crop history. These authors found that phytoalexins accumulated within 24 h from wounding peanut kernels and reached its maximum concentrations in 96 to 120 h afterwhich they begun to decline (Wotton and Strange, 1985). Reduced phytoalexin yields were obtained when sliced peanut kernels of one cultivar studied, TMV2, were incubated in water at 37^{o}C; abolished phytoalexin accumulation when peanut slices were incubated under nitrogen gas; and prevented phytoalexin accumulation after alternate freezing and thawing before aerobic incubation (Wotton and Strange, 1985).

Arora and Strange (1991) observed that when imbibed cotyledons of peanuts were wounded by slicing 1-2 mm, and incubated for 48 h at 25^{o}C in the dark, phytoalexins accumulated of up to 0.59 μM/g resveratrol, 0.02 - 0.98 μM/g arachidin III, and 1.11 - 4.38 μM/g arachidin IV

Table 2.4 Concentrations of *trans*-resveratrol and other stilbenes in peanut kernels stressed by post-harvest abiotic treatment of wounding through slicing, chopping and grinding[1]

Post-harvest Stress Treatment Condition			Compound			Reference
Wounding Treatment	Incubation		Name	Concentration (µg/g, dry basis)		
	Time (h)	Temperature (^{o}C)		Control	Stressed	
Slicing 2 mm	24	25	*Trans*-resveratrol	NR	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	Arachidin 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 Resureccion, 2005
	36				1.06	
	48				2.15	
Grinding 1-2 mm	24	25	*Trans*-resveratrol	0.18	0.65	Rudolf and Resureccion, 2005
	36				0.76	
	48				0.49	

Table 2.4 continued…

Post-harvest Stress Treatment Condition			Compound			Reference
Wounding Treatment	**Incubation**		**Name**	**Concentration (ug/g, dry basis)**		
	Time (h)	**Temperature (^{o}C)**		**Control***	**Stressed**	
Chopping 5mm	24	25	*Trans*-resveratrol	0.25	1.47	Rudolf and Resureccion, 2006
	36				0.89	
	48				0.74	
Whole peanut	24	25	*Trans*-resveratrol	0.20	0.96	Rudolf and Resureccion, 2006
	36				1.46	
	48				1.44	

[1] NR means no data reported

depending on cultivar, maturity, and viability of seeds. Peanut cultivars resistant to seed colonization by *Aspergillus flavus* and aflatoxin contamination such as PI 337394F and J11, accumulated more than 3 times as much Arachidin IV compared to 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. When more mature pods at stages 6 and 8 where divided in to pod, testa, and cotyledon, mature pods and testa lost their ability to synthesize phytoalexins in contrast with mature cotyledon (Arora and Strange, 1991). Peanut seeds which had been stored at 15^{o}C for 9 months, then sliced drastically reduced their ability for the cotyledon to synthesize phytoalexins as evidenced by lower phytoalexins concentrations compared to fresh ones. Peanuts stored for 6 months then sliced produced lower *trans*-resveratrol compared to unstored controls. When non-viable peanut seeds, ascertained by viability test were sliced, lower total phytoalexin concentration of 0.716 μM/g was accumulated compared to 5.29 μM/g in sliced viable peanuts (Arora and Strange, 1991).

After slicing germinated peanut seeds followed by incubation at 23-25^{o}C with artificial aeration, Chang et al. (2006) found a tremendous increase in the concentrations of *trans*-resveratrol from trace to 147.3 μg/g after 20 h incubation. In the same samples, the biosynthesis of three resveratrol derivatives was elicited, resulting in 495.7 μg/g *trans*-arachidin I, 2414.8 μg/g *trans*-arachidin III and 4474.4 μg/g *trans*-isopentadienylresveratrol (IPD) corresponding to 16, 24, and 28 h incubation, respectively, from initially non-detectable concentrations .

Grinding of fully imbibed raw peanuts then incubating for 48 h at 45^{o}C increased resveratrol to 0.85 μg/g compared to controls with 0.14 μg/g (Rudolf et al., 2002). In a subsequent study Rudolf et al. (2005) compared the concentrations of peanuts wounded by

grinding 1-2 mm, chopping 5 mm, and slicing 2 mm and whole peanuts. Ground peanuts initially had 0.18 μg/g resveratrol which increased significantly to 0.65 μg/g after 24 h incubation but did not significantly change after incubating for 36 and 48 h with 0.76 and 0.49 μg/g, respectively. Chopped peanuts had an initial resveratrol concentration of 0.25 μg/g which increased significantly to 1.47 μg/g after 24 h incubation, and then decreased to 0.89 and 0.74 μg/g after 36 and 48 h. Sliced peanuts had 0.22 μg/g resveratrol at 0 h which significantly increased to 1.43 μg/g after 24 h incubation, did not change significantly after 36 h at 1.06 μg/g, and then increased significantly to 2.15 μg/g after 48 h, a concentration which was not significantly different from that incubated for 24 h. Whole peanuts had an initial resveratrol concentration of 0.20 μg/g which increased significantly to 0.96 μg/g after 24 h incubation, and further increased to 1.46 μg/g after 36h which did not differ from 1.44 μg/g after 48 h. Sliced peanuts incubated for 48 h produced the highest resveratrol of 2.15 μg/g followed by chopped peanuts incubated for 24 h and whole peanuts incubated for 36 h with 1.47 and 1.46 μg/g, respectively, and the least was ground peanuts incubated for 36 h with 0.76 μg/g compared to raw untreated control peanuts with 0.48 μg/g. These results showed that wounding by slicing produced maximum concentrations of resveratrol in incubated 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). Whole kernels without wounding will result in moderate resveratrol concentrations.

Wounding trifoliate peanut leaves by punching with fine pins followed by floating on the sterile water, then kept for 12 h in the light with intensity of about 100 μE (m^2/s) and then 12 h in the dark at 25^{o}C, increased resveratrol content up to 2-fold (Chung et al., 2003).

Wounding the germinating seeds of peanuts (previously soaked in water for about 24 h) by slicing 1-3 mm, combined with abiotic stress by allowing natural microflora to proliferate during a 3-5 day incubation period produced two antifungal compounds as determined by thin layer chromatography (Keen, 1975). Later, Keen and Ingham (1976) identified these compounds as the *cis* and *trans* isomers of 3,5,4′-trihydroxy-4-isopentenylstilbene which were not previously found in nature.

3. Exposure to ultraviolet (UV) light

Exposure to UV light radiation was shown to elicit biosynthesis of phytoalexins such as resveratrol in plant and plant materials (Langcake and Pryce, 1997; Cantos et al., 2000) and other phenolics (Cantos et al., 2000). UV induces the increase in enzymes such as stilbene synthase (Fritzemeier et al., 1983) responsible for biosynthesis of secondary metabolites such as resveratrol and flavonoids, which act as screens to prevent UV-induced damage to genetic material of plant cells (Cantos et al., 2000). The efficiency of the biosynthetic pathway and the form and maxima of enzyme profiles depended on the duration of UV exposure (Fritzemeier et al., 1983). Table 2.5 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 resveratrol biosynthesis 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 (1997) 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 is deficient in radiation wavelengths below 300 nm and this may explain why resveratrol biosynthesis is not induced in field grown plants (Langcake and Pryce, 1977). Sunlight does not act as an inducer under

Table 2.5 UV light stress treatments used to enhance resveratrol and other bioactive compounds in peanuts and grapes

Substrate	UV Treatment and Conditions			Compound/ Concentration	Remarks	Reference
	UV light parameters	UV light exposure time	Incubation time and temperature			
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 min	0 to 48 h at 25°C in the dark	*Trans-r*esvaratrol content in ground peanuts, 0.17-0.86 µg/g; chopped peanuts 0.30-1.64 µg/g ; sliced peanuts, 0.33-3.42µg/g; whole peanuts, 0.20-1.76µg/g	Highest resveratrol content of 3.42 µg/g was found in sliced peanut kernels exposed to UV light for 10 min and incubated for 48 h at 25°C.	Rudolf and Resureccion, 2005
Peanut hulls, Spanish	UV 110 V, 60 Hz, 110 mm from UV light	0 to 6 d	25°C	Flavonoid: luteolin At 0 d: 1.74 mg/g After 3 d: 1.73 mg/g After 6 d: 1.21 mg/g		Duh and Yen, 1995

Table 2.5 continued…

Substrate	UV Treatment and Conditions			Compound/ Concentration	Remarks	Reference
	UV light parameters	UV light exposure time	Incubation time and temperature			
Peanut hulls, Spanish	UV 110 V, 60 Hz, 110 mm from UV light	0 to 6 d	25^{o}C	Total Phenolics At 0 d: 7.8 mg/g (catechin equivalents) After 3 d: 7.53 mg/g After 6 d: 7.05 mg/g		Duh and Yen, 1995
Peanut leaf	254 nm, 180 µW/cm^2 energy, 20 cm distance from lamp	48 h	25^{o}C for 96h 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	2 hrs	0, 3, 12 h in the dark	Resveratrol increased up to 225 fold after UV treatment and incubation for 12 h	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

Table 2.5 continued…

Substrate	UV Treatment and Conditions			Compound/ Concentration	Remarks	Reference
	UV light parameters	UV light exposure time	Incubation time and temperature			
Peanut callus (stem)	UV-C light, 254 nm, 55 cm distance from the lamp	20 min	Static condition: 0 to 24 h at 25^{o}C in the dark	Static condition: Resveratrol: 0.25 to 11.97 µg/g Piceatannol: 2.17-5.31µg/g	Static condition: *Trans*-resveratrol increased from 0.25 to 11.97 µg/g after 6 to 18 h of incubation then decreased to 1.42 after 24 h of incubation. Piceatannol increased from 2.17 to 5.31 µg/g after 12 to 18 h of incubation and then decreased to µg/g after 24 h of incubation.	Ku et al., 2005
			Suspension condition: 0 to 80 h at 25^{o}C in the dark	Suspension condition: Resveratrol: 3.14 to 6.93 µg/g Piceatannol: 0.32-0.52µg/g	Suspension condition: Highest *trans*-resveratrol content of 6.93 µg/g was obtained after 4 hours of incubation of calluses in suspension. Initial piceatannol content of 0.52 µg/g was maintained from 0 to 8 h of incubation then decreased when incubation time was further increased	

Table 2.5 continued…

Substrate	UV Treatment and Conditions			Compound/ Concentration	Remarks	Reference
	UV light parameters	UV light exposure time	Incubation time and temperature			
Grape berries, immature	254 nm, 17 cm distance from lamp	10 min	18 h at 26^{o}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 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 min	10 d at 0^{o}C followed by 5d at 5^{o}C	Resveratrol 100 µg/g	*Trans*-resvaratrol production increased by two-fold after 30 min 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^{o}C followed by 5d at 5^{o}C	Resveratrol 65 µg/g	*Trans*-resvaratrol 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 min	10d at 0^{o}C followed by 5d at 5^{o}C	Resveratrol 65 µg/g		Cantos et al., 2000

Table 2.5 continued…

Substrate	UV Treatment and Conditions			Compound/ Concentration	Remarks	Reference
	UV light parameters	UV light exposure time	Incubation time and temperature			
Grape berries, immature	340 nm	30 min	10d at 0°C followed by 5d at 5°C	Resveratrol 45 µg/g		Cantos et al., 2000
Grapes, Napoleon red	510 W irradiation power, 40 cm distance from lamp	30 sec	3 days storage	Resveratrol maximum 115µg/g of skin	A serving of UV-irradiated grapes could supply 7.5mg/200g serving *trans*-resveratrol equivalent to 3 glasses of red wine. Untreated grape contained 10µg/g of skin.	Cantos et al., 2001
Grape berries	254 nm	10 min	24 h at 24°C 48 h 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 min	4 h at 24°C 48 h at 24°C	Phytoalexin 0.8-0.12µg/cm^2 21µg/cm^2		Creasy and Coffee, 1988
Grapevine leaves (Vitaceae)	254 nm, 3.7 W/m^2 energy	10 min	23 h 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

Table 2.5 continued..

Substrate	UV Treatment and Conditions			Compound/ Concentration	Remarks	Reference
	UV light parameters	UV light exposure time	Incubation time and temperature			
Grape leaves	260-270 nm	equivalent to 10 min at 250 nm	20h at 26^{o}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*-rresveratrol	Langcake and Pryce, 1977
Grape leaves	254 nm, 12 cm distance from lamp	15 min	20 h	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 radiolabelled 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

natural conditions (Soleas et al., 1997) suggesting that DNA itself is the actual photoreceptor for the UV irradiation induced response (Langcake and Pryce, 1997; Soleas et al., 1997). Resveratrol was produced very rapidly following UV irradiation, rising to a maximum at 18 h and decreasing thereafter (Langcake and Price, 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). Although all four precursors confirmed their roles in resveratrol biosynthesis, more resveratrol were isolated from leaves compared with those from immature fruits despite higher amounts of precursors fed (Langcake and Pryce, 1977)

A number of studies on the UV irradiation of grapes were reported by Cantos and co-workers. In their first report, Cantos et al. (2000) found that refrigerated storage and UV irradiation of table grapes increased 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, caffeoyltartaric, piceid, and resveratrol were also detected. While concentrations of most phenolics remained constant during postharvest refrigerated storage of 10 days at 0 degrees C, resveratrol derivatives increased 2-fold. Even larger increases in resveratrol derivatives of 3- and 2-fold were induced during postharvest treatments of Napoleon grapes with UVC and UVB light, respectively (Cantos, 2000). These authors suggested that a 200 g serving of mature non-irradiated Napoleon grapes provides approximately 1 mg of resveratrol, which is in the range of

the amount supplied by a 140 mL (5 oz) glass of red wine and can be increased to 2 or 3 mg per serving if grapes are UVB or UVC irradiated, respectively.

In another study, Cantos et al. (2001) optimized the UV irradiation of grapes and concluded that exposure of grapes for 60 s under a 510 W UV lamp at a distance of 40 cm was optimum for maximum resveratrol concentration. This optimum process resulted in 11-fold increase in resveratrol concentration compared to untreated grapes. The distance of 40 cm from UV light was the optimum, achieving the highest resveratrol content in UV-treated grapes compared to 20 and 60 cm distance. The lower resveratrol synthesized at 20 cm may have been due to too close a distance resulting in "too strong" irradiation causing damage to the "biosynthetic system" of resveratrol whereas at 60 cm, the distance was too far causing delayed induction of resveratrol biosynthesis (Cantos et al., 2001).

Later, Cantos et al. (2002) applied the optimum UV process for grapes (Cantos et al., 2001) was to irradiate seven grape varieties - red table grape varieties of Flame, Red Globe, Crimson, and Napoleon, and white varieties of Superior, Dominga, and Moscatel Italica to determine elicitation of stilbenes in grapes. These authors reported that the most inducible stilbenes included *trans*-resveratrol, *trans*-piceatannol, and viniferins. 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 (2.27 μg/g) in Flame variety to 2315-fold (2.31 μg/g) 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 (0.18 μg/g). 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 about 4.6 mg resveratrol to a 200 g serving of unpeeled table grapes depending on the variety, equivalent to more than seven glasses of red wine (approximately 1.5 L).

Furthermore, Cantos et al. (2003) found that UV-C irradiation using the optimum process (Cantos et al., 2001) of "Monastrell" grapes increased resveratrol and piceatannol in the resulting red wines while the stilbene glucosides such as piceid were not significantly induced. These authors tracked the concentration of both compounds was followed through the different steps of an "analytical" traditional maceration wine-making process which involved close contact between skins and must. The maximum resveratrol and piceatannol contents in the must (before pressing) were detected after 5 days at 1,170 and 655 μg/L, respectively, compared to 471 and 346 μg/L, respectively, in controls. After 10 days, resveratrol decreased to about 220 μg/L while piceatannol to about 420 μg/L. The final wine made from UV-C-irradiated grapes was enriched by about 2-fold (190.7 μg/L) resveratrol and 1.5-fold (311.0 μg/L) piceatannol compared to the control wine. No difference was detected regarding the standard enological parameters (color, acidity, density and alcoholic grade). These researchers suggested that the use of more susceptible wine grapes to induce bioactive stilbenes upon UV-C-irradiation can produce a much higher stilbene-enriched wine (Cantos et al., 2003).

Preliminary experiments of Bais et al., (2000) revealed that resveratrol was induced only in berries that had been irradiated with UV-C (<280 nm) but not in control berries. These authors studied the effects of UV-C-irradiation on the resveratrol biosynthesis in skins at various berry development of four grape cultivars, red Shiraz and Cabernet Sauvignon, and white Semillon and Chardonnay. The developing grape berries on an orbital 50 rpm shaker were irradiated for 10 min at 10 cm below UV-C light (254 nm), and then incubated in the dark at 25^{o}C for 72h. Bais

et al. (2000) found that 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. A marked increase in the ability of the UV-irradiated berry skins to synthesize resveratrol was observed 1-5 weeks post-flowering with concentrations ranging from 150-330 μg/g fresh weight at week 1, to 810 to 1075 μg/g fresh weight at week 5. The capacity of berry skin to synthesize resveratrol after week 5 progressively declined such that in week 16, resveratrol concentrations were only ~11 μg/g fresh weight or 100-fold decline in concentrations of Semillon and ~113 μg/g fresh weight or 10-fold decline in Chardonnay, Shiraz and Cabernet Sauvignon.

When Subba-Rao et al. (1996) exposed the abaxial (lower) or adaxial (upper) surfaces of peanut leaves, 20 cm below the shortwave UV lamp (254 nm; 180 $\mu W/cm^2$) for 48 h and then incubated for 96 h at 25°C in the dark, phytoalexins in abaxial leaves increased to 393 nmol/g fresh weight which was more than three times higher than that in adaxial leaf with only 115 nmol/g fresh weight and 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/s) for 2h which increased up to 225-fold (56.43 μg/g fresh weight) after 12 h incubation in the dark.

To our knowledge, the earliest work on UV irradiation of peanut kernels for resveratrol elicitation was conducted by Resurreccion and co-workers. 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 min followed by incubation in the dark at 25°C for 24, 36 and 48 h. Exposure to UV light of all peanut samples, regardless of size, resulted in significant increases in *trans*-resveratrol concentration from 0.17 to 1.76 μg/g as

incubation period increased from 0 to 36 h. From 36 to 48 h 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 incubated for 48 h at 25°C. A similar maximum resveratrol concentration of 3.30 μg/g was obtained after peanuts were UV irradiated for 30 min at 40 cm distance from UV light and incubated for 36 h 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 min at 40 cm distance from UV light and incubated for 44 h at 25°C (Potrebko and Resurreccion, 2009).

Tokusoglo et al. (2005) found that after exposing peanuts and pistachios in UV light for 1 min, *trans*-resveratrol concentrations ranged from 0.02 - 1.47 and 0.07 - 1.24μg/g, respectively; and *cis*-resveratrol from 0.008 - 0.32 and 0 - 0.44 μg/g, respectively. They found that *cis*-resveratrol in pistachios was higher than in peanuts. *Trans* and *cis* -resveratrol in the samples were confirmed using total ion chromatograms of the bis[trimethylsilyl]trifluoroacetamide derivatives of resveratrol isomers, and by comparison of the mass spectral fragmentation data with those of a resveratrol standard.

When peanut callus were UV irradiated by Ku et al. (2005), not only resveratrol but also piceatannol which was not present in untreated samples, were synthesized. Peanut callus exposed to UV at 254 nm for 20 min at 55 cm from UV light, and then incubated at 25°C for 0-18 h produced 2.17-5.31 μg/g piceatannol after 12-18 h incubation and 0.25-11.97 μg/g resveratrol after 6-18 h incubation under static cultivation, reaching their maximum concentrations after 18 h. In contrast, the concentrations of resveratrol and piceatannol in suspension cultures were lower at 3.93-6.93 and 0.30-0.52 μg/g, respectively, and did not

increase after 4 – 80 h incubation for resveratrol and throughout 0 – 80 h incubation for piceatannol. Kuh et al (2005) explained that because calluses were constantly moving in suspension, they may have received shorter UV irradiation than those in static cultures and produced less resveratrol and piceid. These researchers claimed that piceatannol produced by calluses in their study was much higher than the values reported in the literature, whereas the resveratrol produced was comparable to reported values.

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 to untreated raw peanuts (Seo et al., 2005).

In rice leaves, UV irradiation (15W) of detached leaves for 20 min at 20 cm from UV light and incubation at 30^{o}C in a moist box at high humidity, resulted in the accumulation of oryzalexins A, B, C, and D, and momilactones A and B, an unknown antifugal substance accompanied by the appearance of brown spots on the leaf surface (Kodama et al., 1988). 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 3 days after UV irradiation. Maximum accumulations of Oryzalexin D were observed after 2 days whereas oryzalexin C, was after 4 days. Kato et al. (1993) later isolated the novel diterpene phytoalexin, oryzalexin E in rice leaves using the same irradiation treatment of Kodama et al. (1988) except for incubating the leaves at 27^{o}C at high humidity under dark conditions for 12 h, followed by light conditions for 2 days.

4. Ultrasound processing

Ultrasound technology using high frequency sound waves, above the threshold of human hearing or >16 kHz (Soria and Villamiel, 2010) is a powerful technique with application in food quality and safety such as materials extraction and food technology (Mason, 2003). Ultrasonication produces cavitation phenomena when acoustic power inputs are sufficiently high to allow multiple production of microbubbles that collapse violently and create shock waves that cause cells to disintegrate into very fine cell debris particles (Chukwumah et al., 2005).

Ultrasound is an emerging novel technology in the food processing industry used in emulsification of mayonnaise, reduction of fat globule size in milk, milk homogenization before cheese making, increasing or improving viscosity of tomato puree and yoghurt, and improving extractions of food bioactives (Soria and Villamiel, 2010). Lin et al. (2001) proposed that ultrasound treatment at low intensities of ginseng cell cultures, a non-food 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 Panax ginseng cell cultures (Wu and Lin, 2002b). Among these enzymes, phenylammonia lyase, the key enzyme of the phenylpropanoid pathway responsible for stilbenes biosynthesis in Panax ginsenf cell cultures was enhanced most dramatically by ultrasound with 5-fold higher at power level 4 after 4 days incubation compared to controls (Wu and Lin, 2002b).

There are a very limited number of researchers who utilized ultrasound for the synthesis of bioactive compounds in food (Table 2.6). To our knowledge, major research on the application of ultrasound treatment in peanuts was conducted by Resurreccion and co-workers. Rudolf and Resurreccion (2005) investigated the effects of ultrasound treatment on increasing resveratrol

Table 2.6 Ultrasound stress treatments used to enhance resveratrol and other bioactive compounds in peanuts and various food plants[1].

Substrate	Ultrasound Treatment and Conditions			Compound/ Concentration	Remarks	Reference
	Ultrasound power density	Exposure time	Incubation time and temperature			
Raw peanut, Runner, whole kernel	39.2mW/cm^3	4 min	24 -48 h at 25^{o}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 h. Control had 0.2 µg/g *trans*-resveratrol.	Rudolf and Resureccion, 2005
Raw peanut, Runner sliced, 2mm	39.2mW/cm^3	4 min	24 -48 h at 25^{o}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 h. . Control had 0.2 µg/g *trans*-resveratrol.	Rudolf and Resureccion, 2005
Raw peanut, Runner, ground, 2mm	39.2mW/cm^3	4 min	24 -48 h at 25^{o}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 h. Control had 0.17 µg/g *trans*-resveratrol.	Rudolf and Resureccion, 2005
Raw peanut, Runner, chopped, 5mm	39.2mW/cm^3	4 min	24 -48 h at 25^{o}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 h. Control had 0.3 µg/g *trans*-resveratrol.	Rudolf and Resureccion, 2005
Roasted peanut, Runner, sliced, 7mm	39.2mW/cm^3	4 min	44 h at 25^{o}C	*Trans*-resveratrol : 7.15 µg/g		Rudolf, 2003

Table 2.6 continued…

Substrate	Ultrasound Treatment and Conditions			Compound/ Concentration	Remarks	Reference
	Ultrasound power density	Exposure time	Incubation time and temperature			
Raw peanut, Runner, sliced, 10mm	39.2mW/cm^3	4 min	44 h at 25^{o}C	*Trans*-resveratrol : 0.57 µg/g		Rudolf, 2003
Roasted peanut, Runner sliced, 10mm	39.2mW/cm^3	4 min	44 h at 25^{o}C	*Trans*-resveratrol : 2.57 µg/g		Rudolf, 2003
Raw peanut	High frequency ultrasonication (combined frequency of 25 kHz, 60 kHz, 80 kHz)	30, 60, 90, 120, 150, and 180 min	NR	*Trans*-resveratrol: 0.11±0.05 to 0.27±0.01mg/100g Biochanin: 0.22±0.01 to 0.32±0.01g/100g Geneistein: 0.01±0.00 to 0.6±0.02 mg/100g		Chukwumah et al., 2005
Ginseng roots	82 mW/cm^3	1-4 min	NR	Ginsenoside saponins	Increased total saponin content up to 75% over that of controls	Lin et al., 2001

Table 2.6 continued…

Substrate	Ultrasound Treatment and Conditions			Compound/ Concentration	Remarks	Reference
	Ultrasound power density	Exposure time	Incubation time and temperature			
Ginseng roots[2]	Indirect sonication in water bath 38.5 kHz, 810W	1 h	25°C	Ginsenoside saponins: Yield is 2.0-4.10% by weight	Control using conventional thermal extraction (soxhlet method) yielded 1.15-2.10 % by weight after 1 h and 1.45 – 2.95% after 2 h 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
		2 h	25°C	Ginsenoside saponins: Yield is 2.11-4.3% by weight		
	Direct sonication with probe horn connected to 600W ultrasound microprocessor, 20 kHz, no pulse, 22% amplitude	1 h	25°C	Ginsenoside saponins: Yield is 2.1-4.32% by weight		
		2 h	25°C	Ginsenoside saponins: Yield is 2.20- 4.75% by weight		
Alkaline protease from *Bacillus subtilis*	20 kHz at 40W, 100W, 120W using continual and interval treatment	1 h	NR	Glucose esters: At 120W, 70% yield	50% and 75% yield after 1 and 2h, respectively, under interval ultrasound (10 min ultrasound then 20 min shaking) High power and continual operation gave better acceleration on the yields.	Xiao et al., 2005
		2 h	NR	94% yield		

[1]NR means no data reported

[2]Indirect and direct ultrasound extraction used three solvents: pure methanol, water-saturated n-butanol, and water with 10% methanol.

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 a ultrasonic bath cleaner with a power density of 39.2 mW/cm^3 for 4 min at 25^{o}C, and incubating at 25^{o}C in the dark for 24, 36 and 48 h. Significant increases in *trans*- resveratrol concentrations after 24 h incubation were observed in all peanut sizes, from 0.10-0.26 at 0 h to 0.75-2.54 μg/g after 24 h with the largest increase to 2.54 μg/g in sliced peanuts. From 24 to 36 h incubation, the concentrations of *trans*-resveratrol significantly decreased in chopped peanuts from 1.3 to 0.93 μg/g; increased in sliced and whole peanuts from 2.54 to 3.96 and 0.76 to 1.47 μg/g, respectively; but did not change significantly in ground kernels from 0.75 to 0.69 μg/g. No significant change in *trans*-resveratrol concentrations of all samples was observed from 36 to 48 h of incubation. The highest *trans*-resveratrol concentration of 3.96 μg/g was obtained in sliced peanuts incubated after 36 h. 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 2 min followed by incubation at 25^{o}C for 48 h, 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 4 min then incubated for 44 h at 25^{o}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, 2002b). 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 (Soleas et al., 1997). 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. Improvement in extraction efficiency of ultrasound, a non-thermal procedure could be attributed 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/100g; biochanin, 0.22 $\pm$ 0.01 to 0.32 $\pm$ 0.01 mg/100g; and geneistein, 0.01 $\pm$ 0.00 to 0.6 $\pm$ 0.02 mg/100 (Chukwumah et al., 2005). The highest amounts of resveratrol and biochanin were obtained after 150 min ultrasound-assisted extraction, after 180 min for genistein while daidzein was not detected (Chukwumah et al., 2005). Later, (Chukwumah et al., 2009) reported that ultrasound-assisted extraction using sample to solvent ratio of 1:6 and a 30-min 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 ultrasound-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 using the 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 (3.5W) 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^{o}C) in the sample tubes in the sonic bath compared to that of the sonicator probe horn (25-27^{o}C) (Wu et al., 2001). These 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 non-intrusive 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, from the alkaline protease of *Bacillus subtilis* in eight different solvents, using three different powers of 50, 100, and 120 W at 20 kHz operating at continuous ultrasound or interval ultrasound (10 min ultrasound/20 min shaking without ultrasound). These researchers concluded that higher ultrasound power and continuous 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 treatments were higher than that of merely shaking over the same reaction time. For example, the yields of glucose esters using continuous ultrasound at 120W were 70% and 94% after 1 and 2 h, respectively, whereas the yields using interval ultrasound were only 50% and 75%, respectively. Xiao et al. (2005) found that 120, 100, and 50 W ultrasound powers had higher transesterification of glucose compared to shaking. A 98% transesterification of glucose could be obtained after 2 h in 120 W in an ultrasound bath, whereas only 48% was observed in shaking under the same conditions.

Ultrasound at 35 kHz and 37^{o}C, effectively shortened the long enzymatic hydrolysis time to 30 min from 6h involved using conventional thermostatic devices for the heavy metals analysis of edible seaweeds (Pena-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 which indicate that the metal

released are attributed to the enzymatic action and not to leaching procedures (Pena-Farfal et al., 2005).

5. 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 from the leaves of *Veratrum grandiflorum* treated with cupric chloride, two antifungal stilbenoids, resveratrol and oxyresveratrol, and their glucosides, piceid, and oxyresveratrol-3-O-glucoside. The last compound was 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 mM were capable of inducing a high resveratrol production in the leaves of *Vitis rupestris* while greater concentrations of $AlCl_3$ from 22 to 90 mM were required to obtain a similar response in the leaves of *Vitis vinifera* cv. Pinot noir suggesting that *Vitis rupestris* generally produces greater amounts resveratrol than Pinot noir. Aluminum sulfate used at the same concentrations as $AlCl_3$ had similar effects as those of $AlCl_3$ on phytoalexin induction, suggesting that aluminum was responsible for the enhanced elicitation of phytoalexins (Adrian et al., 1996).

6. 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

while 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 0.01M salicylic acid when applied as a foliar spray to abraded leaves of peanuts is an effective elicitor of phytoalexins. A total of concentration of 1,270 nmol/g fresh weight of phytoalexins was obtained when salicylic acid was applied as a foliar 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 resveratrol synthase (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 ethyphon for 0, 3, 12, and 24 h. Results showed that resveratrol was accumulated up to 3-, 2-, and 8- fold in response to salicylic acid, jasmonic acid and ethylene, respectively, in a time-dependent manner up to 24 h but none in response to abscissic acid. RS mRNA increased in response to salicylic acid, jasmonic acid and ethylene at least 12 h after treatment, and also increased in a time-dependent manner in response to salicylic acid and ethylene which reached its maximum 24 h 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 (Chung et al., 2003).

Medina-Bolivar and his co-workers (2007) found that sodium acetate (10.2 mM) was the most appropriate elicitor for stilbene induction in the hairy root cultures of peanuts, compared to laminarin, copper sulfate, cellulose and chitosan. The hairy root cultures treated with sodium acetate accumulated 50 to 98 μg/mg dry weight (50,339 – 97,992 ng/mg) *trans*-resveratrol, approximately 60-fold above the levels detected in untreated control culture media with 0.69-

0.81 μg/mg dry weight (692 to 1813 ng/mg). In treated cultures, *cis*-resveratrol concentrations of 0.047-0.399 μg/mg (47 to 399 ng/mg) were relatively low compared to *trans*-resveratrol, but higher than those in controls with 8-31 ng/mg. In control cultures, *trans*-resveratrol concentrations were 80-fold higher compared to *cis* isomers with 0.008-0.031 μg/mg (8-31 ng/mg). In treated hairy root tissues, *trans*-resveratrol ranged from 253-1134 ng/mg whereas untreated control root tissues had 0.210-0.587 μg/mg (210-587 ng/mg). *Cis*-resveratrol concentrations were not detected or very low at 10 ng/g in treated hairy root tissues and 3 ng/g in controls. *Trans*-pterostilbene in the treated hairy root cultures varied from 0.06 - 0.27 μg/mg (61-267 ng/mg) reflecting approximately a 2-fold increase over untreated controls with 0.02-0.14 μg/mg (22 – 140 ng/mg). The *cis* isomers of pterostilbene were not detected in both treated and control cultures. Only *trans*-pterostilbene ranging from 44 to 136 ng/mg was found in the treated root tissues while none was detected in the control root tissue. *Cis*-pterostilbene was not detected in both treated and control hairy root cultures and root tissues. Higher resveratrol and pterostilbene were observed in the cultures compared to root tissues of peanut hairy roots. Peanut hairy root cultures offer a novel and sustainable bioproduction system for resveratrol and associated derivatives (Medina-Bolivar et al., 2007).

7. 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 stimulated elicitation of phytoalexins in bean leaves (Biolley et al., 1988), and resveratrol and pterostilbene in grapes (Sarig et al., 1996). Sarig et al. (1996) found out that exposure of ripe grape berries to a stream of air (airflow of 500 mL/min) containing

ozone at a rate of 8 mg/min, elicited resveratrol and pterostilbene, with the resveratrol accumulating larger amounts. Production of these phytoalexins generally reached a maximum level 24 h after 5-10 min 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-glucoronidase (GUS) reporter gene into a tobacco plant to identify the regions that control ozone-regulated gene expression. They reported that sequences located within -430 to -280 bp of the Vst1 promoter were required for ozone-regulation. Grimmig et al. (1997) 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 0.1 µl/L for 12 h. The same construct was also strongly induced by ethylene at 20 µL/L for 12 h.

7. Far-infrared (FIR) and heat treatments

Generally, the outer layers of plants such as peel, shell, and hull contain large amount of polyphenolic compounds to protect inner materials and a number of phenolic acids are linked to various cell components (Lee et al., 2006). Far-infrared radiation and heat treatment may have capability to cleave covalent bonds and to liberate antioxidants such as flavonoids, carotene or polyphenols from repeating polymers (Lee et al., 2006). Far-infrared radiation for 5 to 60 min of finely ground peanut hulls increased the total phenolic contents of water extracts in a time-dependent manner from 79.3 to 141.6 micromole/L (µM) tannic acid equivalents compared to untreated samples containing 72.9 µM tannic acid equivalents (Lee et al., 2006). Similarly when peanut hulls were heat-treated by roasting at 150^{o}C, total phenolics contents increased

from 79.8 to 90.3 μM tannic acid equivalents as heating increased from 5 to 60 min. Results showed that FIR was more efficient than simple heating in increasing total phenolics contents of water extracts of peanut hulls (Lee et al., 2006).

V. FUNCTIONAL PEANUTS AND PRODUCTS

A. Potential Product Usage for Functional Peanuts

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

1. Peanut butter and spreads

Peanut butters and spreads which comprised more than 50% of all peanut products consumed in the U.S. (USDA/NASS, 2008), are potential food product application for resveratrol-enhanced peanuts (REP) that delivers additional health benefits to consumers. Rudolf and Resurreccion (2007) prepared peanut butters from REPs processed by ultrasound at 39.2 mW/cm^3 power density for min at 25^oC 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 h compared to 0.29 μg/g in untreated peanuts. All natural peanut butters prepared from REP had slightly lower intensity ratings for roasted peanutty aroma (22.5-30.2) and flavor (36.6-53.5), peanut butter aroma (10.5-16.7) and flavor (27.9-35.0), and sweet aromatic aroma (8.2-10.5) compared to controls with 33, 78, 38, 51, and 12. REP had higher intensity ratings, although the magnitudes were low, for oxidized (3.2-8.4),

and fishy (4.4-8.2) flavors; painty (0-8.4) off-flavors compared to peanut butters prepared from untreated peanuts with 0 ratings for these off-flavor attributes. Reformulations of REP butters are therefore needed to mask the off-flavors inherently produced in the resveratrol-enhanced peanuts. Inclusion of fruit jams and chocolate in the REP butters or mixing REP with regular roasted peanuts may help in masking these off-flavors.

2. Peanut confections

The high protein content of peanuts makes them ideal for high energy snacks. Four of the top ten candy bars sold in the U.S. contain peanuts and/or peanut butter (National Peanut Board, 2010) suggesting that resveratrol-enhanced peanuts have high potential in confectionery industry. REPs have inherent off-flavors developed in the process of enhancing *trans*-resveratrol resulting in its lower acceptance by consumers (Sales and Resurreccion, 2009; 2010). Roasted peanuts from REPs treated by UV, ultrasound, and combined ultrasound-UV with resveratrol concentrations received lower overall consumer 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), respectively, compared to untreated controls rated 7.4-7.7 or like moderately (Sales and Resurreccion, 2009; 2010). Addition of sugar and flavoring improved the perceived bitter flavor of peanut skins infusions and consumers significant gave higher acceptance ratings in sweetened compared to unsweetened infusions (Francisco, 2009) which suggest that adding sugar to REP may mask the off-flavors and improve the acceptance of REP products.

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 as chopped or as peanut butter, with other ingredients such as chocolate, nougat, marshmallow, caramel, and

dried fruit and peanut brittles and chocolate-covered peanuts are always popular (The Good Earth Peanut Company, 2010).

3. Peanut flour

Spadaro (1979) described the uses of defatted and partially defatted peanut flours in a variety of food products suggesting that peanut flour from resveratrol-enhanced peanuts may have the same potential applications. Peanut flour was acceptable as a protein supplement and functional ingredient in macaroni, bread dough and frankfurters at levels of 12, 11, and 10%, respectively; however, doughs prepared with the flour tended to be sticky which may be overcome by changes in processing (Spadaro, 1979). This author reported that the applications of peanut flour in cakes, cookies, soups, gruels, puddings spreads, gravies, meat loaf, ice cream, snack items and breakfast cereals have been evaluated. Peanut flour had been used satisfactorily to replace one-fourth to one half of the milk solids in ice cream (Spadaro, 1979). Peanut milks were prepared by adding one part of peanut flour to nine parts of water to produce skim milk. Incorporation of defatted peanut flour in gruel type blended food formulations, including general purpose and high calorie weaning blends containing either corn or wheat and peanut flour, whey protein concentrate and lysine hydrochloride was also evaluated (Spadaro, 1979).

4. Roasted peanuts/snack peanuts/other peanut products

The application of resveratrol-enhanced peanuts in snacks may be limited to products that will use them as ground, chopped or sliced, and butter. The National Peanut Board (2010) recently published in their website, new peanut products commercially available in the market for which REP may have applications which included peanut butter granola bar thins, peanut butter honey and peanut butter dark chocolate bars, peanut butter chunky cookies, peanut butter & caramel and peanut butter & raspberry duets, silky smooth milk chocolate peanut butter,

honey roasted peanut crunch, peanut butter & jelly and peanut butter cookie bar, gourmet peanut brittle, curry peanut and peanut soup, pure power and peanut bar with blueberry or tart cherries, chocolate peanut butter, and PB2, a powdered peanut butter with 85% less calories than regular peanuts.

5. 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^{o}C and 95% relative humidity in the dark for 9 days, resveratrol contents increased significantly from initial concentrations of 2.3-4.5 μg/g to a range of 11.7-25.7 μg/g depending on peanut cultivar. Resveratrol was highest in the cotyledons (12.0-47.1 μg/g), slightly lower in roots (7.9-18.3 μg/g) and not detected in the stems of peanut sprouts. When sprouts were heated in boiling water for 2 min, resveratrol contents were mostly unchanged indicating that resveratrol was fairly stable against heating or cooking (Wang et al., 2006).

Chang et al. (2006) detected four major fractions of stilbenoids in germinated and sliced peanuts as discussed previously which exhibited potent antioxidant and anti-inflammatory activities. Resveratrol showed equivalently potent 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity as butylated hydroxytoluene (BHT), and both had significantly higher activity than *trans*-arachidin-1, *trans*-archidin-3, and trans- isopentadienylresveratrol. In pork oil system, storage at 60^{o}C of all supplemented antioxidants at 100 μM showed inhibitory effects against formation of conjugated diene hydroperoxides.

6. 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 h 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, were also detected in the medium at 0.24 μg/mg, a 2-fold increase compared to non-elicited 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 (Medina-Bolivar et al., 2007).

B. Optimization of Processes for Enhanced Levels of Bioactive Compounds

Optimization studies are conducted to optimize parameters that would lead to optimal 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. Response surface methodology (RSM) is a statistical method used in optimization studies. RSM uses quantitative data from appropriate experimental design to determine and simultaneously solve multivariate equations which can be graphically represented as response surface, and can be used

in three ways: (1) to describe how test variables affect the response; (2) to determine the interrelationships among the test variables; and (3) to describe the combined effect of all test variables on the response (Giovanni, 1983).

Rudolf and Resurreccion (2007) optimized slicing and incubation times using response surface methodology for maximum enhancement of *trans*-resveratrol in peanuts treated with one level of ultrasound using a power density of 39.2 mW/cm^3 for 4 min in an ultrasonic bath while producing an acceptable resveratrol-enhanced peanut butters. The optimum processes included all treatments combinations within a triangle bound by three points, representing peanut size and incubation time combinations of 0.89 cm and 48 h; 0.72 cm and 41.5 h; and 0.64 cm, and 48 h, respectively. This optimum processes were predicted to produce REP butters with a *trans*-resveratrol ≥1.0 μg/g; and sensory intensity ratings for roasted peanutty aroma ≥24, and roasted peaunutty flavor ≥ 43, peanut butter aroma ≥ 14, peanut butter flavor ≥ 31, oxidized flavor ≤ 6, painty flavor ≤ 0.5, fishy≤ 6, and cardboard flavor ≤ 4 using a 150 mm unstructured line scale.

Optimization studies using varying doses of UV, ultrasound, and combined ultrasound and UV were conducted by Sales and Resurreccion (2009; 2010) resulting in maximum levels of *trans*-resveratrol in sliced (~7 mm) REP with the highest consumer acceptance ratings ≥ 5 or neither like nor dislike. The optimum UV processes which produced REP with maximum *trans*-resveratrol of 2.1 µg/g, included all process combinations within the area of a triangle bound by three points, representing a combination of distance from UV light and UV exposure time of 47 cm and 20 min, 41 cm and 26.5 min, and 33 cm and 30 min, followed by incubation at 25^{o}C for 36 h; (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 5 points, representing combination of ultrasound power density and exposure

times of 75 mW/cm^3 for 5.2 min, 75 mW/cm^3 for 8 min, 72 mW/cm^3 for 8 min, 67 mW/cm^3 for 7.1 min, and 66 mW/cm^3 for 5.5 min, followed by incubation at 25^{o}C for 36 h (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 within a hexagon bound by 6 points representing the combination of ultrasound power densities and exposure time of 74 mW/cm^3 for 8.3 min, 70 mW/cm^3 for 10.9 min, 62 mW/cm^3 for 11.2 min, 42 mW/cm^3 for 10.4 min, 48 mW/cm^3 for 8.3 min, and 58 mW/cm^3 for 9.1 min, followed by 50 min exposure at 40 cm distance from UV light and 36 h incubation at 25^{o}C (Sales and Resurreccion, 2010).

VI. ANALYSIS OF RESVERATROL AND OTHER STILBENES IN PEANUTS

A. Extraction Methods

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

1. 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). The stability of resveratrol standards in ethanol at various conditions in the laboratory was studied by Trela and Waterhouse (1996) and their findings are summarized as follows: (1) Standards of *trans*-resveratrol in 100% ethanol can be stored for 3 months at -5^{o}C in the dark and protected from stray light in a sealed light-proof

containers, thus limiting the need for re-calibration after 3 months. (2) *Trans*-resveratrol standards in 50% ethanol and kept unrefrigerated in light-proof, paraffin sealed containers appeared stable although solvent evaporation was noticeable over 3 months. (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 resveratrol in 100% methanol and found that the standard solution was stable for 5 days at -20^{o}C and 3 days at 4^{o}C in the dark but not stable at room temperature. Resveratrol concentration decreased to ~85% after 4 days at 4^{o}C based on the reference standard stored at -80^{o}C which was assumed to be stable. At room temperature in the dark, reduction in resveratrol concentration was observed even only after 2 h, reduced slowly to ~75% after 8 h, and maintained at this level for 96 h. When exposed to light at room temperature, only ~70% of resveratrol remained after 4h and the concentration decreased to 30% after 48 h. Wang et al. (2002) recommended that a) resveratrol should be protected from light during extraction, storage, and analysis; b) standard solutions and extracts in methanol should be stored at ≤20^{o}C and analyzed within 5 days; c) fresh standard solutions should be prepared at least weekly; d) automatic injectors maintained at ≤ 4^{o}C; and e) samples should be analyzed within 2 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).

2. Extraction solvents used

Ethanol/water, v/v 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 95:5 (Aguamah et al., 1981; Arora and Strange, 1991); 8:2 in the extraction of peanut kernels (Sanders et al., 2000; Rudolf et al., 2005; Chukwumah et al., 2005) and peanut butter (Ibern-Gomez et al., 2000); and 1:1 in peanut leaves and roots (Azpilicueta et al., 2004).

Other organic solvents used in the extraction of stilbenes were (1) methanol/water, 8:2, v/v in peanut roots (Chen et al., 2002) and in peanut kernels, leaves and roots (Chung et al., 2003); (2) acetone/water, 8:2, v/v in peanut kernels (Arora and Strange, 1991); (3) acetonitrile, 100% in peanut kernels (Arora and Strange, 1991); and acetonitrile/water, 9:1 v/v in roasted peanuts, peanut butter, and boiled peanuts (Sobolev and Cole, 1999; Lee et al., 2004) .

3. Preliminary size reduction of sample

Peanuts and other solid samples were reduced to fine particles by grinding to facilitate the extraction of bioactive compounds. Equipment for grinding used by researchers included blender (Sobolev and Cole, 1999); coffee mill (Sanders et al., 2000; Rudolf et al., 2005; Tokosoglu et al., 2005); SMP Process Homogenizer at 15,000 rpm for 1 min (Lee et al., 2004).

Skins of grapes were ground to a fine powder in a liquid nitrogen using a pre-chilled mortar and pestle (Bais et al., 2003) to extract resveratrol. Resveratrol from grapevine leaves was extracted by grinding in a mortar with sand and methanol/water 8:2, v/v (Adrian et al., 1996).

4. 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 to 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 ethanol/water (80:20, v/v) using PowerGen 700 homogenizer set at approximately 27,000 rpm for 2 min in ice bath, and then centrifuged for 5 min 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 2 min using a Polytron, rinsing the Polytron with 5 mL extraction solvent, and then filtration through a Whatman No.2. Ibern-Gomez et al. (2000) extracted resveratrol and piceid from peanut butters with ethanol/water 8:2, v/v at room temperature for 30 min followed by centrifugation for 5 min at 3000 rpm.

In dried peanut roots, Chen and co-workers (2002) recovered resveratrol by mixing the powdered roots with methanol/water, 8:2 v/v and homogenizing the mixture at 15,000 rpm for 1 min using a Polytron 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^{o}C with occasional shaking for 30 min. The heated mixture was centrifuged at 8000g at 20^{o}C for 15 min, the supernatant was collected, membrane-filtered, and diluted with water to adjust to 2:8, v/v methanol/water, 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 h 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 h 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 (100%) in a minicolumn packed with Sephadex.

Adrian et al. (1996) extracted resveratrol from grapevine leaves by grounding in a mortar with sand and methanol/water 8:2, v/v, followed by centrifugation at 10,000*g* for 15min. The supernatant was pre-purified on a Sep-Pak C_{18} cartridge through elution with methanol/water 8:2, v/v and the eluate was evaporated to dryness at <40^{o}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 1 hr. Extracted tissue was then pelleted via centrifugation at 7700 *g* for 30 min at 4^{o}C and supernatant was taken for HPLC analysis.

5. 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).

6. 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 ethanol/water 1:1, v/v 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 sulphate and taken to dryness. Residues were redissolved in methanol at 0.2 mL/g of fresh tissue and kept at -20°C until analyzed.

5. Vacuum infiltration and solid phase extraction

Phytoalexins were also extracted from peanut kernels by vacuum infiltration with acetonitrile in the dark for 48 h at 25°C and partially purified by solid phase extraction (SPE; Arora and Strange, 1991). Chen and co-researchers (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 min and held for an additional 2 min using a flow rate of 3 mL/min. 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.

6. 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^{o}C in an evaporating unit (Sobolev and Cole, 1999) and Lee et al. (2004) adopted similar procedure at 50^{o}C. Ibern-Gomez and her group (2000) concentrated the supernatant extracts collected from peanut butter samples to dryness under vacuum at temperatures below 40^{o}C. The dried extracts were stable and can be kept at -20^{o}C until quantitatively analyzed (Rudolf et al., 2005).

B. Quantitative Analysis of Resvertrol and Other Stilbenes in Peanuts

1. 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 prepared by exposing the *trans* forms to UV (Trela and Waterhouse, 1994) or white light (Bais et al., 2000). *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 3 h 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 3 h; and extending UV irradiation time to as long as 10 h resulted in only ≤63% conversion. Using higher concentration of 8.94 mmol/L *trans*-resveratrol, UV irradiation at 366 nm for 3 h resulted in ≥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 min in a climatic chamber equipped with solar radiation panel by xenon (1500 W) at controlled temperature of 28^{o}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 her co-workers (2002) by exposing the *trans* isomers in methanol solution for 12 h 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.

2. 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 pre-treatment 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, 2009b). Fixed and known amount of an internal standard is added at the beginning of sample extraction. Phenolphthalein was used 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, 2009b).

3. Preparation of dried extracts prior to quantitative analysis

Prior to quantitative analysis of the stilbenes, the dried residue was re-dissolved in organic solvents such as ethanol/water at low concentrations of 1:9 to 1.5: 8.5, v/v (Sanders et al., 2000; Rudolf et al., 2005; Potrebko and Resurreccion, 2009), methanol/water, 1.5:8.5, v/v (Francisco and Resurreccion, 2009b), or using HPLC mobile phase (Sobolev and Cole, 1999; Ibern-Gomez et al., 2000; Lee et al., 2004; Waterhouse and Lamuela-Raventos, 1994; Adrian et al., 1996). The reconstituted extracts were subjected to high frequency ultrasonification (Rudolf et al., 2005; Chukwumah et al., 2005) to facilitate dissolution. Finally, the re-constituted residues were filtered through inorganic membrane filter (0.45 μm) prior to injection for quantitative analysis (Rudolf and Resurreccion, 2005; Potrebko and Resurreccion, 2009).

4. 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 high performance liquid chromatography (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).

4.1 High Performance Liquid Chromatography (HPLC)

HPLC methods developed by various researches for the analysis of resveratrol and other stilbenes in peanuts and peanut plant materials are listed in Table 2.7. 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, while the mobile phase is of non-polar or low polarity solvents such as hexane, dichloromethane, chloroform, ethyl ether, and isopropyl alcohol. In reverse-phase HPLC, a non-polar stationary phase such 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 2.7). 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 and 306 nm for *trans*- and *cis*-resveratrol and piceid (Ibern-Gomez et al., 2000), respectively 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, 2009b).

In grapevine berries of three *Vitis vinifera* varieties, an HPLC analysis was used to quantify 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 to Hu Zhang variety.

4.2 Liquid Chromatography-Mass Spectrometry (LC/MS)

Wang et al. (2002) developed a liquid chromatography-mass spectrometry 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. Following APCI, the abundance of protonated molecules was recorded using selected ion monitoring (SIM) 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 10:1, was 0.31 pmol injected on-column. The extraction efficiency of the method was 92%. Resveratrol was stable for 5 days at -20^{o}C and 3 days at 4^{o}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).

4.3 High-performance Liquid Chromatography-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 resveratrol 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.

4.4 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 control (non-elicited) and sodium acetate-treated (elicited) peanut hairy root cultures (Medina-Bolivar et al., 2007). Discrimination between *cis* and *trans* isomers of the compounds 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 in order to increase its thermal stability and volatility (Wang et al., 2002). Furthermore, the high temperature of 250-300^{o}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).

4.5 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 total ion chromatograms of bis[trimethylsilyl] trifluoroacetamide derivatives of resveratrol isomers and comparison of the mass spectral fragmentation data with those of a resveratrol standard.

4.6 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 (Gao et al., 2002; Brandolini 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 (2002) used CE-ED as an alternative method for *trans*-resveratrol determination in samples of wines, medicinal herb, and health food, which was proven to be simple, convenient, sensitive and selective.

Brandolini and co-workers (2002) developed an analytical method using 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 *trans*-piceid and *trans*-piceid showed that 50% isomerization was obtained after 5 min, and the equilibrium was achieved after 20 min 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.

6. 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).

VII. 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**.**

Table 2.7 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/min)	Detector
				A	B			
Peanut kernels[2,3]	Reverse-phase	Hypersil Octadecylsilyl (ODS) (250 mm L[20] x 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 x 4.6 mm i.d.; 5 µm particle size)	NR	water/ acetic acid (99:1, v/v)	acetonitrile	Gradient: Time (min),%B; 1, 40; 7, 45; 12, 45; 20, 65	1.5	Mulitchannel detector 310 nm and 0.04 A for phytoalexins
Peanut kernels[5]	Reverse-phase	Spherisorb 10 ODS (250 mm L x 4.6 mm i.d.; 5 µm particle size)	NR	water/ acetic acid (99:1, v/v)	acetonitrile	Gradient: Time (min),%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 x 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

Table 2.7 continued…

Sample	Separation Mode[1]	Column	Column Temperature (°C)	Mobile phase solvents		Elution	Flow Rate (mL/min)	Detector
				A	B			
Roasted and boiled peanuts and peanut butter[7]	Normal-phase	Zorbax-RX-SIL (250 mm L x 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 x 4.5 mm i.d.; 5 µm particle size)	NR	water/TFA (9.9:0.1, v/v)	acetonitrile	Gradient Time (min),%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 x 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 x 4 mm i.d.; 5 µm particle size)	NR	water	methanol	Gradient Time (min),%B; 1, 20; 16, 80; 18, 80	1.0	UV 254 nm for resveratrol
Peanut leaves, pods and roots[11]	Reverse-phase	µBondapak C18 column (3.9 mm x 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

Table 2.7 continued…

Sample	Separation Mode[1]	Column	Column Temperature (°C)	Mobile phase solvents		Elution	Flow Rate (mL/min)	Detector
				A	B			
Peanut kernels and peanut butter[12]	Reverse Phase	Nucleosil 100-5 C18 (250 mm L x 4.0 mm i.d.; 5 µm particle size) Pre-column packed with Nucleosil 5 C18 (4 mm x 4 mm)	NR	acetonitrile/ water (40:60, v/v)	NA	Isocratic	0.3	UV detector 306 nm for *trans*-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 x 4.6 mm i.d.; 5 µm particle size)	NR	water	methanol	Gradient Time (min),%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 x 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
Peanut callus[16]	Reverse phase	Mightsil RP C-18 column (250 mm L x 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 (min),%B; 0, 20; 20, 32; 30, 90; 35, 90	-	Fluorescence detector 343 nm excitation and 395 nm emission for resveratrol and piceatannol

Table 2.7 continued…

Sample	Separation Mode[1]	Column	Column Temperature (°C)	Mobile phase solvents		Elution	Flow Rate (mL/min)	Detector
				A	B			
Peanut kernels[17]	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/acetic acid (9.999:0.001, v/v)	acetonitrile	Gradient: Time (min),%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, phenolpthalein
Peanut kernels[18]	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	acetonitrile	Gradient: Time (min),%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, phenolpthalein
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 (min),%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 non-ionic 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 a 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) simultaneous determination of *cis* and *trans* resveratrol and piceid
[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 (2009b); 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]NA means not applicable
[22]NR means no data reported

SECTION 3

MATERIALS AND METHODS

The goal of this research was to enhance biosynthesis of *trans*-resveratrol and other bioactive phenolics, through application of UV, ultrasound (US), and combined US-UV processing treatments, thereby producing resveratrol-enhanced peanuts (REP) with high antioxidant capacities and consumer acceptance. The intended use of REP for this study is that of an ingredient for preparing resveratrol-enhanced roasted peanuts and peanut bars to deliver health benefits to consumers.

I. STUDY 1 – ULTRAVIOLET LIGHT (UV) PROCESS OPTIMIZATION TO ENHANCE *TRANS*-RESVERATROL BIOSYNTHESIS IN PEANUTS

A. Experimental Design

The UV treatments for peanut kernels included a 3 x 3 x 3 full factorial design (Table 3.1). Parameters were distances from UV light of 20, 40 and 60 cm, UV exposure times of 10, 20 and 30 min, and incubation times at 25^{o}C for 24, 36 and 48h. The levels chosen for the three UV parameters were based on the work of Rudolf and Resurreccion (2005) who used one dose of UV at 40 cm distance from UV light for 10 min and incubation times of 24, 36 and 48 h to enhance elicitation of *trans*-resveratrol in peanuts; and Cantos et al. (2001) who used the range of 20 to 60 cm distances from UV light and exposure times of 5 s to 30 min to model postharvest induction of *trans*-resveratrol in grapes. Two replications of the 27 UV treatments and a control of untreated raw whole peanuts were prepared for a total of 56 samples (Table 3.2). Each sample was analyzed in duplicate for *trans*-resveratrol, *trans*-piceid, total phenolics, antioxidant capacity

Table 3.1 Treatment variables and levels for UV processes[1]

Factors (treatment variables)	Factor symbol code	Factor Levels (Coded Values)		
		-1	**0**	**+1**
Distance from UV light (cm)	X_1	20	40	60
UV exposure time (min)	X_2	10	20	30
Incubation time at 25°C (h)	X_3	24	36	48

[1] UV processing treatments to determine effects on the concentrations of *trans*-resveratrol, *trans*-piceid, total phenolics, and antioxidant capacities and consumer overall acceptance of sliced peanut kernels (7 mm).

Table 3.2 Experimental design for the three-level, three-factor response surface analysis for UV-treated peanuts.

Treatment	Coded values			Uncoded values		
	X1 UV irradiation distance (cm)	**X2 UV exposure time (min)**	**X3 Incubation time at 25°C (h)**	**X1 UV irradiation distance (cm)**	**X2 UV exposure time (min)**	**X3 Incubation time at 25°C(h)**
1	-1	-1	-1	20	10	24
2	-1	-1	0	20	10	36
3	-1	-1	+1	20	10	48
4	-1	0	-1	20	20	24
5	-1	0	0	20	20	36
6	-1	0	+1	20	20	48
7	-1	+1	-1	20	30	24
8	-1	+1	0	20	30	36
9	-1	+1	+1	20	30	48
10	0	-1	-1	40	10	24
11	0	-1	0	40	10	36
12	0	-1	+1	40	10	48
13	0	0	-1	40	20	24
14	0	0	0	40	20	36
15	0	0	+1	40	20	48
16	0	+1	-1	40	30	24
17	0	+1	0	40	30	36
18	0	+1	+1	40	30	48
19	+1	-1	-1	60	10	24
20	+1	-1	0	60	10	36
21	+1	-1	+1	60	10	48
22	+1	0	-1	60	20	24
23	+1	0	0	60	20	36
24	+1	0	+1	60	20	48
25	+1	+1	-1	60	30	24
26	+1	+1	0	60	30	36
27	+1	+1	+1	60	30	48

By two methods: trolox antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) using hydrophilic (H-ORAC) and lipophilic (L-ORAC) assays, and profile of phenolic compounds. When large coefficient of variation (COV) >35% occurred between two duplicates, a triplicate analysis was conducted to decrease COV to $\leq$35%. However when COV remained >35%, outlying results were dropped.

The 56 samples were analyzed using affective tests with 50 consumers, 25 panelists evaluated replication 1, while the other half evaluated replication 2 in two 1-hour tests, with one hour break in-between tests. During each test, panelists evaluated 4-5 samples in three sessions, each separated by 5 min compulsory breaks. Descriptive sensory analysis of the 56 samples was conducted using 10 trained panelists in two 2-hour tests, 28 samples/test, with 4-5 samples evaluated in six sessions, each separated by 5 min compulsory breaks.

B. Sample Preparation

Raw peanuts, *Arachis hypogaea* cv Georgia green medium runners, harvested in 2005 (Golden Peanut Co., Alpharetta, GA, USA) and stored for two months at 4^{o}C were used. All processing implements and surfaces were washed and sanitized with 200 ppm chlorine solution prior to use. About 1.8 kg peanuts were washed twice in 4 kg tap water, drained, surface sanitized in 2 kg of 150 ppm chlorine solution for 15 min, and rinsed with sterilized deionized water, prepared by passing through a 0.2 μm nylon filter (Millipore Corp., Bedford, MA, USA). Peanuts were fully imbibed in 2 kg sterilized water for 16 h, drained, and manually sliced to about 7 mm. Sliced peanuts, 3 batches of about 900 g each, were spread a single layer, about 1 cm deep on a plastic tray (56L x 30.5W x 60H cm, HDPE, Gage Industries Inc., Lake Oswego, OR, USA). Three trays were positioned side by side along their length, a total of 91.5 cm, to allow equal exposure to 122 cm UV light (UPV XX-40S, 40W, 254 nm, UltraViolet Products,

Upland, CA, USA) at the specified distances from UV light, and trays were removed after specified time according to the experimental design. Peanuts within a tray were mixed after half of exposure time for equal exposure of surfaces to UV.

UV-treated peanuts were packed in half gallon glass mason jars (Ball Corp., Muncie, IN, USA), covered and wrapped with foil and incubated at 25^{o}C (Environmental Growth Chamber, Chagrin Falls, OH, USA) for 24, 36, and 48 h. Incubated samples were accumulated in a walk-in freezer at -18^{o}C, and then thawed in a walk-in cooler, 5^{o}C for 24 h. Approximately 1 kg of peanuts was poured into aluminum trays (40.6 L x 38.1 W x 1.2 H, cm; 0.4 cm i.d. perforations) to one layer depth, and dried in a convection oven (645 Freas, Precision Scientific, Winchester, VA, USA) at 40^{o}C for 24 h to about 10% moisture by difference in weight before and after drying. Peanuts were roasted (Lincoln Impingement Oven Model 1452, Fort Wayne, IN, USA) at 158^{o}C for 4.5 min to an *L* value of 50$\pm$1 (Chroma meter, Model CR-200, Minolta, Japan), cooled using an industrial fan (Air Circulator, Model 9K961, Dayton Manufacturing Co., Niles, IL, USA). Skins were manually removed then blown away by the fan. Roasted peanuts were packaged (10.16 x 45.72 cm vacuum plastic bags, Koch Packaging, Kansas, MO), flushed with nitrogen gas (medical grade, South Air Gas, Griffin, GA, USA), and vacuum sealed (Ultravac, Koch Packaging, Kansas, MO, USA). The entire process was conducted under yellow light to avoid isomerization or degradation of stilbene compounds (Trela and Waterhouse, 1996). Samples were stored at -18^{o}C for 1 week prior to total phenolics and TEAC analyses, for 1 year prior to *trans*-resveratrol, *trans*-picied and ORAC analyses, and for 2 years prior to profiling of phenolic compounds.

C. Chemical Analysis

Chemical analyses were conducted on the samples to determine the concentrations of stilbenes - *trans*-resveratrol, *trans*-piceid, total phenolics, antioxidant capacities by trolox

equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) and profiled for 15 phenolic compounds**.**

1. *Trans*-resveratrol and *trans*-piceid

1.1 Extraction of trans-resveratrol and trans-piceid from samples

Only *trans* isomers of resveratrol and piceid were measured using the reverse HPLC method of Potrebko and Resurreccion (2009) as *trans* isomers are more abundant in nature and more stable than *cis* isomers (Trela and Waterhouse, 1996). About 30 g sample was ground using coffee mill (Model K9M2-4, BrAun, Mexico) for 1 min, then 10 g were weighed into a 250mL centrifuge bottle and 30 mL of 80% ethanol and 2 mL of 60 μg/mL freshly prepared phenolphthalein (Aldrich Chemical Company, Milwaukee, WI, USA) solution as internal standard were added. Contents were homogenized using PowerGen 700 (Fisher Scientific, Pittsburg, PA, USA) for 2 min on ice then centrifuged (Model J2-21M, Beckman, Palo Alto, CA, USA) for 5 min at 1380G at 25^{o}C. A 2 mL supernatant was drained by gravity, about 20 min, into a 5 mL tube through a clean-up column, to remove interfering compounds that co-eluted with resveratrol and piceid (Sanders et al., 2000). The clean-up column was a 3 mL disposable syringe fitted with about 5 mm diameter pre-filter (AP25, Millipore, Bedford, MA, USA) to prevent loss of packing, consisting of 1g mixture of aluminum oxide (neutral activity 1, particle size of 0.063-0.200 mm, EM Science, Gibbstown, NJ, USA) and silica gel 60 C_{18} (EM Science, Gibbstown, NJ, USA), 1/1, w/w. The column was washed with 0.5 mL ethanol and filtrate was collected in the same tube. Contents of 6 tubes placed parallel in a heating block (Thermolyne, Dubuque, IO, USA) set at #5 to maintain 60^{o}C, were dried for about 1h by blowing nitrogen directly over the samples through a hollow six-pin blowdown manifold, then tubes were capped, wrapped with foil and stored at -20^{o}C until analyzed. All extractions were conducted under yellow light.

1.2 Preparation of standard solutions

Stock solutions, 200 ppm each of standards of *trans*-resveratrol (Aldrich Chemical Co., Milwaukee, WI, USA), *trans*-piceid (Ningbo J & S International Trade Company Ltd., Ningbo, China) and phenolphthalein (Aldrich Chemical Company, Milwaukee, WI, USA) were prepared separately by weighing out 0.01g of powder of each standard and diluting to 50 mL with 100% ethanol in volumetric flasks. Prior to storage at -15^{o}C of the standard stock solutions, the flasks were flushed with nitrogen gas to remove oxygen and were wrapped with aluminum foil to reduce light-induced isomerization.

A working stock solution which was a mixture of the three standards prepared for every continuous run of approximately 1 week. The stock solution contain 10 ppm each of *trans*-resveratrol and *trans*-piceid, and 20 ppm of phenolphthalein by transferring of 1mL each of 200 ppm stock solutions of *trans*-resveratrol and *trans*-piceid, and 2 mL of 200 ppm of phenolphthalein stock solution in a 30 mL vial. To the mixture was added 16 mL of 15% ethanol for a total volume of 20 mL.

Calculations: $C_1V_1 = C_2V_2$

where C1 = Concentration of stock solution, 200 ppm

V_1= Volume (mL) stock solution

C_2 = Concentration (ppm) of working stock solution

V_2 = Volume (mL) of working stock solution

V_1 = (10 ppm) (20 mL)/ 200ppm = 1 mL each of 200 ppm *trans*-resveratrol and *trans*-piceid

V_1 = (20 ppm) (20 mL)/ 200ppm = 2 mL of 200 ppm phenolphthalein

Standard solutions for calibration curve with concentrations of 10, 5, 3, 1, and 0.5 ppm for *trans*-resveratrol and *trans*-piceid, and 20, 10, 6, 2, and 1 ppm for phenolphthalein for generating standard curves were prepared by diluting appropriate amounts of the working stock solution to 10 mL with 15% ethanol. An example of a calculation is as follows:

Calculation:

To prepare 5 ppm *trans*-resveratrol and *trans*-piceid, and 10 ppm phenolphthalein:

$$V_1 = \frac{C_2V_2}{C_1} = \frac{(5\text{ ppm})\ (10\text{ mL})}{10\text{ ppm}} = 5\text{ mL of 10 ppm working stock solution}$$

Where: V_1= volume (mL) of working stock solution

V_2= total volume of standard solution for calibration curve

C_1= concentration of working stock solution required

C_2= concentration of standard solution for calibration curve

All solutions were filtered by pouring into a glass syringe (Fisher Scientific, Fair Lawn, NJ, USA) and filtered through an inorganic membrane filter (Anotop 10, 0.2 μm, Whatman International Ltd., Maidstone, England) in 2 ml HPLC amber vial (National Scientific Co., Lawrenceville, GA, USA). The vial was sealed with a screw cap that was fitted with Teflon/silicone septum (National Scientific Co., Lawrenceville, GA, USA). All standard solutions were prepared under yellow light.

1.3 HPLC analysis of trans-resveratrol and trans-piceid

Before HPLC analysis, 0.40 mL ethanol (15%) was added to dried peanut extract, and mixed in a vortex mixer (Model VM 3000, VWR, Thorofare, NJ, USA) at setting #6.5 for 30 s. Six tubes at a time were immersed into water in a round plastic container (13 cm i.d. x 6 cm depth) for 2 min indirect sonication at 50% amplitude using ultrasonic processor (750W, 115 VAC,

50/60 Hz, Cole Parmer, Vernon Hills, IL, USA). Sample was poured into a 3 mL glass syringe with attached inorganic membrane filter (Anotop 10, 0.2µm, Whatman International Ltd., Maidstone, England), filtered into a 300 µL polypropylene insert placed in a 2 mL HPLC amber vial and sealed with a screw cap fitted with a teflon/silicon septum (National Scientific Co., Lawrenceville, GA, USA). HPLC analysis used a Varian ProStar HPLC system consisting of ProStar410 auto sampler, solvent delivery module 210, and ProStar 335 Diode Array Detector (DAD) to set UV spectrum from 240 to 400 nm, and controlled by LC Module Add-In Star version 6 and Star WS version 5.X, 6.41 software system (Varian Inc., Palo Alto, CA, USA). The Eclipse Plus C18 reverse column, 250 x 4.6 mm, 5µm particle size, preceded by an Eclipse Plus C18 guard column, 7.5 x 4.6 mm, 5µm particle size (Agilent Technologies, Deerfield, IL, USA) was maintained at ambient temperature, 25^{o}C. A 1.5 mL/min flow rate was used for solvent A, 100% double deionized water vacuum filtered through a 0.2µm nylon filter and solvent B, 100% acetonitrile (HPLC Grade, Aldrich, Milwaukee, WI, USA). Gradient elution included: 0 min, 5% B; 7 min, 22% B; 13 min, 23% B; 26 min, 63% B; 28 min, 80% B; then finally returned to 5% B in 1 min, and held at 5% B for an additional 5 min. Samples and standards were injected at 40 µL volumes.

Peak areas of *trans*-resveratrol and piceid, were quantified at 307 nm and of phenolopthalein, at 280 nm (Rudolf et al., 2005) and concentrations were calculated as follows:

$$\text{µg of } i \text{ in sample} = \frac{\left(\frac{\text{µg of } i \text{ in standard}}{\text{PA of } i \text{ in standard}}\right) \text{ x PA of } i \text{ in sample}}{\left(\frac{\text{µg of IS in standard}}{\text{PA of IS in standard}}\right) \text{ x PA of IS in sample}} \text{ x µg of IS in sample}$$

where *i*=*trans*-resveratrol or *trans*-piceid, IS (internal standard) =phenolphthalein, and PA=peak area. Five levels of standards, 10, 5, 3, 1, and 0.5 µg/mL for *trans*-resveratrol and piceid, and 20, 10, 6, 2 and 1 µg/mL for phenolphthalein were analyzed at the beginning of each HPLC sample

set. *Trans*-resveratrol and piceid were reported as µg/g peanut, dry weight basis, and their sum, as total stilbenes.

2. Total phenolics

Total phenolics contents of peanut extract samples were analyzed using Folin-Ciocalteu assay of Singleton et al. (1999). The Folin Ciocalteu assay is used as it is one of the three assays proposed for consideration as a standard antioxidant analytical procedure for measuring total phenolics to assess antioxidant capacity of foods and neutraceuticals, during the First International Congress on Antioxidants convened in Orlando, Florida in June 2004 (Prior et al., 2005). The modified Folin Ciocalteau assay of Singleton et al. (1999) is simple, speedy, economical, robust and does not require specialized equipment (Prior et al., 2005). This assay specifically reduces phenols and imposes mandatory steps and conditions to minimize variability and eliminate erratic results, such as addition of adequate reagent to react completely and rapidly with the oxidizable substances in the samples, use of sufficient mixing time of the samples, adding Folin Ciocalteau reagent solution before the alkali solution, and adequate time and temperature of holding conditions for color development. The modified Folin Ciocalteau assay of Singleton et al., (1999) was used in this study because it requires only 20 mL of reagents instead of the 100 mL used by Singleton and Rossi (1965).

2.1 Extraction of total phenolics from samples

Peanut extracts for total phenolics analysis were prepared (Talcott et al., 2005) by grinding about 20 g deskinned peanuts in a coffee mill (Model K9M2-4, Braun, Mexico) for 30 s. Ten grams were weighed into a 250 mL centrifuge bottle (Fisher Scientific, Fair Lawn, N, USA J) and 20 mL of 80% methanol (Fisher Scientific, Fair Lawn, NJ, USA) was added. The mixture was homogenized (PowerGen 700, Fisher Scientific, Pittsburg, PA, USA) for 1 min and the centrifuge

bottles were capped, wrapped with foil, and held for 24 h at ambient temperature, approximately 23^{o}C. After the holding period, the bottles were centrifuged (Model J2-21M, Beckman, Palo Alto, CA, USA) at 2500*g* for 5 min at 18^{o}C. Supernatant was filtered through Whatman No. 42 filter paper (Maidstone, U.K; Rudolf and Resurreccion, 2005) and collected in a 35 mL screw capped glass vial (Fisher Scientific, Fair Lawn, NJ, USA). The vials were capped, wrapped with foil, and stored in a freezer at -18^{o}C until analyzed.

2.2 Preparation of reagents and gallic acid standard solutions

The Folin-Ciocalteu's phenol reagent, 1:10, was prepared by mixing 100 mL of Folin Ciocalteau's phenol reagent, 2N (Sigma-Aldrich, St. Louis, MO, USA) and 1 L of deionized water. Sodium carbonate solution, 75g/1000 mL, was prepared by weighing 75 grams of sodium carbonate powder (Sigma-Aldrich, St. Louis, MO, USA), followed by dissolving and diluting to 1L volume with deionized water. Methanol, 80% solution, was prepared by mixing 800 mL of 99.99% methanol (Sigma-Aldrich, St. Louis, MO, USA) with 200 mL of deionized water.

A stock solution of gallic acid, 1 mg/mL, was prepared by accurately weighing 0.0100 g gallic acid powder (Sigma-Aldrich, St. Louis, MO, USA) in an empty 10 mL beaker. The powder was dissolved in small amount of 80% methanol and quantitatively transferred into a 10 mL volumetric flask. The volume was brought to 10 mL with 80% methanol. Calculations are as follows:

$$\frac{0.01 \text{ g Gallic Acid}}{10 \text{ ml of 80\% methanol}} = 0.001 \text{ g gallic acid/mL solution x } \frac{1000 \text{ mg}}{\text{g}} = 1 \text{ mg/mL}$$

Gallic acid calibaration standard solutions were prepared at concentrations of 5, 15, 30, 45 and 60 ppm for the generation of the standard curve. The formula, $C_1V_1 = C_2V_2$ was used to calculate the amounts of gallic acid stock solution to prepare the desired concentrations.

Where: C_1 = Concentration of gallic acid stock solution, 1 mg/mL

V_1= Volume (ml) gallic stock solution

C_2 = Concentration of working standards

V_2 = Volume of working solution

An example of a calculation used for the working standards is as follows:

For 30 µg/mL = 0.03 mg/mL

$$\text{mL gallic acid stock solution} = \frac{0.03 \text{ mg/mL} \times 20 \text{ mL}}{1 \text{ mg/mL}} = 0.6 \text{ mL}$$

2.3 Total phenolics analysis

In a 30 mL test tube, 2 mL of diluted sample or gallic acid calibration standard solution were mixed with 10 mL of 1:10 Folin Ciocalteau reagent. After 1 min but not exceeding 8 min, 8 mL of the 75g/1000 mL sodium carbonate was added for a total volume of 20 mL. The mixture was mixed in a vortex mixer, held for 2 h at ambient temperature at approximately 25^{o}C for the blue color complex generation. Absorbance was read at 760 nm (UV-Vis Diode Array Spectrophotometer, Model 8451A, Hewlett Packard, Palo Alto, CA, USA). Total phenolics concentration, expressed as mg gallic acid equivalents (GAE)/g, was calculated based on regression equation of gallic acid standard curve:

Total phenolic (TP) concentration (mg GAE/g) = [Abs/b] * Dilution Factor

Where: Abs = Absorbance at 760 nm

b= slope of the standard curve

Dilution Factor (DF) = amount of dilutions made on the analytical sample

$$\text{DF} = \frac{\text{20 mL of 80\% methanol}}{\text{g sample wet weight}} \times \frac{\text{2 mL diluted sample}}{\text{mL of sample extract}} \times \frac{\text{g sample wet weight}}{\text{g sample dry weight}}$$

3. Trolox equivalent antioxidant capacity (TEAC)

TEAC is one in a set of three assays recommended as an antioxidant standard test for antioxidant capacity (Prior et al., 2005); the two other tests in the set are ORAC and total phenolics. TEAC represents the electron transfer based method that measures antioxidant capacity and represents a reaction different from ORAC. TEAC of peanut kernels was determined using the 2,2′-azinobis (3-ethylbenzothiaziline-6-sulfonate) (ABTS) anion scavenging activity assay of Kim and Lee (2004). This procedure was modified by Kim and Lee (2004) from the TEAC procedure of van den Berg et al. (1999) by: (a) using 2,2′-azobis-2-amidinopropane dihydrochloride (AAPH), a thermolabile water-soluble radical initiator, rather than 2,2′-azobis-2-amidopropane hydrochloride (ABAP) as radical initiator of ABTS, and b) reducing the sample volume and reagents by half thus making the procedure more economical. The antioxidant capacity is expressed as Trolox Equivalent Antioxidant Capacity (TEAC). The higher the TEAC value of sample, the greater the antioxidant capacity

3.1 Extraction of antioxidants for TEAC analysis from samples

Samples for TEAC analysis were extracted along with total phenolics described previously in Section 3.C.2.1 above. Extracts were diluted appropriately as needed prior to analysis.

3.2 Preparation of reagents and trolox standard solutions

ABTS radical solution was prepared fresh on the day of analysis by mixing 2.5 mM ABTS (Sigma-Aldrich, St. Louis, MO, USA) and 1.0 mM AAPH (Sigma-Aldrich, St. Louis, MO, USA) in phosphate buffered saline (PBS) solution (pH 7.4; 100 mM potassium phosphate buffer containing 150 mM NaCl (Sigma-Aldrich, St. Louis, MO, USA). Details on preparing the reagents are discussed below. The mixture was heated in a water bath (Buchi 461 Water Bath, Switzerland) at 68^{o}C internal temperature for 13 min to produce a blue green $ABTS^{-}$ radical

solution. The concentration of the $ABTS^{\cdot}$ radical solution was adjusted with fresh PBS to an absorbance of 0.650 $\pm$ 0.020 at 734 nm (Spectrophotometer Model 8451A, Diode Array Spectrophotometer, Hewlett Packard, Palo Alto, CA, USA) at 37°C. The adjusted freshly prepared ABTS radical solution was stored at 37°C until needed for analysis. This ABTS radical solution was used to react with trolox standards and sample extracts using the ABTS anion scavenging activity assay.

PBS solution, 100 mL (pH 7.4, 100 mM with 150 mM NaCl) was prepared by mixing 1.3090 g KH_2PO_4 powder (MW=136.09; Sigma-Aldrich, St. Louis, MO, USA) with 0.8766 g of NaCl crystals (MW=58.44; Sigma-Aldrich, St. Louis, MO, USA), then dissolving and diluting to 100 mL with filtered deionized water. The pH of PBS was adjusted to 7.4 using 2M sodium hydroxide (Sigma-Aldrich, St. Louis, MO, USA) solution prepared by dissolving 8.000 g of NaOH pellets in 100 mL deionized water. In two separate 10 mL beakers, 0.1372 g of ABTS powder (Sigma-Aldrich, St. Louis, MO, USA) and 0.0271 g of AAPH powder (Sigma-Aldrich, St. Louis, MO, USA) were weighed, each was dissolved in about 5 mL of the prepared PBS, and both were added to the remaining PBS resulting in 2.5 mM ABTS and 1 mM AAPH in the final 100 mL mixture. Formula used in the calculation of weights of powdered or solid reagents to prepare ABTS radical solution is shown below.

$$W = \frac{mM \times MW}{1000}$$

Where: W = weight of reagent needed, g/L

mM = millimolar = millimoles / L = concentration of reagent needed

MW = molecular weight of reagent, g/mole

1000 = conversion factor = 1000 millimoles/mole

For example, to prepare 1 L of 100 mM KH_2PO_4,

$$\text{Weight of } KH_2PO_4\text{(g/L)} = \frac{(100 \text{ mmoles/L} \times 136.09 \text{ g/mole})}{1000 \text{ mmoles/mole}} = 13.609 \text{ g/L}$$

Twenty five mL of 5 mM trolox stock solution was prepared by dissolving 0.0313 g of Trolox powder (MW = 250.29 g/mole, Sigma-Aldrich, St. Louis, MO, USA) with 99.99% methanol to a final volume of 25 mL. The weight of trolox powder needed to prepare 25 mL of stock solution was calculated as follows:

$$\text{Weight of Trolox (g/L)} = \frac{(5 \text{ mmoles/L} \times 250.29 \text{ g/mole})}{1000 \text{ mmoles/mole}} = 1.25145 \text{ g/L}$$

$$= 0.0313\text{g}/25 \text{ mL}$$

The 5 mM or 5000 µM trolox stock solution was used to prepare 5 mL each of the working standard solutions with concentrations of 150, 300, 450, 600, and 750 µM to give the final calibration curve standard solution concentrations of 3, 6, 9, 12, and 15 µM, respectively, in the ABTS assay reaction tubes. For example 150 µM or 0.15 mM trolox standard was prepared by mixing 0.15 mL of 5 mM stock solution with 4.85 mL of 80% methanol for a total volume of 5 mL, then 0.02 mL of 150 µM was transferred to a 5 mL ABTS assay reaction test tube and added with 0.98 mL ABTS radical solution for a total volume of 1 mL and resulting to a final concentration of 3 µM trolox calibration curve standard solution. The volumes of 5 mM Trolox stock standard solution and 80% methanol used to prepare the different concentrations of trolox working standard solutions and the resulting calibration curve standard solutions after mixing 0.02 mL each of the working standards with 0.98 mL of ABTS radical solutions are shown below.

Table 3.3 Preparation of trolox working and calibration curve standard solutions for TEAC assay.

Preparation of trolox working standard solutions			Preparation of trolox calibration standard solutions in the reaction tube		
Volume of 5 mM trolox stock solution required (mL)	Volume of 80% methanol added (mL)	Resulting concentration of trolox working standard solution (μM)	Volume of trolox working standard solution (mL)	Volume of ABTS radical solution added (mL)	Resulting concentration of trolox calibration curve standard solution (μM)
0.15	4.85	150	0.02	0.98	3
0.30	4.70	300	0.02	0.98	6
0.45	4.55	450	0.02	0.98	9
0.60	4.40	600	0.02	0.98	12
0.75	4.25	750	0.02	0.98	15

3.3 TEAC analysis

Peanut extract, standard, or a control consisting of 50% methanol in deionized water (v/v; Kim et al., 2002), 20 µL each, was transferred to a 5 mL test tube. Then, 980 µL of ABTS radical solution was added to each tube and mixed manually for 5 s. A set of 12 test tubes containing the mixture, per run, was incubated in a water bath at 37^{o}C for 10 min under yellow light. Absorbance at 734 nm was measured after 10 min. Reduction in absorbance of the standards and the samples was calculated by subtracting the absorbance of control from those of standards or samples. ABTS radical scavenging activity of samples was expressed as TEAC in µM trolox equivalents (TE)/g using the formula below.

TEAC (µM TE/g) = [(ΔAbs – a)/b] x DF,

where: a = y intercept of the absorbance reduction curve of trolox standards

b = slope of the absorbance reduction curve of trolox standards

ΔAbs = the difference in absorbance at 734 nm of control and sample

DF = dilution factor.

$$DF = \frac{\text{20 mL of 80\% methanol}}{\text{g sample wet weight}} \times \frac{\text{1 mL diluted sample}}{\text{0.02 mL of sample extract}} \times \frac{\text{g sample wet weight}}{\text{g sample dry weight}}$$

4. Oxygen Radical Absorbance Capacity (ORAC)

Food antioxidants have hydrophilic or lipophilic fractions. Most methods available for analyzing antioxidant capacity in food measure only the hydrophilic fraction. In food containing appreciable amounts of fat, like peanuts, it is necessary to measure not only the hydrophilic but also the lipophilic antioxidants to measure their total antioxidant capacity. The ORAC assay of Prior et al. (2003) and Wu et al. (2004) measures both lipophilic (L-ORAC) and hydrophilic (H-ORAC) antioxidants and was used in this study. ORAC is the first of the set of three assays

recommended in the antioxidant standard tests for antioxidant capacity that include total phenolics and TEAC. It represents a hydrogen atom transfer reaction mechanism, which is most relevant to human biology (Prior et al., 2005). The ORAC analysis was carried out using a fluorescence microplate reader (FLUOstar Optima, BMG Labtechnologies, Duraham, NC, USA), fluorescence filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm, and 96 well Costar black microplates (Corning Incorporated, Corning, NY, USA).

4.1 Microplate assay

A stock fluorescein (FL) solution, Stock #1 was prepared by dissolving 0.0225g of fuorescein, Na salt (Fisher Scientific Co., Fairlawn, NJ, USA) in 50 mL of 0.075M phosphate buffer (pH 7.0). A second FL stock solution, Stock #2 was prepared by diluting 50 (L of Stock #1 in 10 mL 0.075M phosphate buffer. A 320 μL portion of Stock #2 was added to 20 mL of 0.075M phosphate buffer, of which 200 μL was added to each well. This provided 7.5 nmoles of fluorescein per well, or a final concentration of 14 μM.

In the standard L-ORAC or H-ORAC assays, 20 μL each of Trolox standard solutions (30, 60, 90, 120 μM) in 7% Randomly Methylated Cyclodextrin (RMCD), for the lipophilic assay or 0.075 M phosphate buffer (pH 7.0) for the hydrophilic assay, were pipetted into appropriate wells in triplicate based upon a set layout where samples were strategically positioned by placing the samples, blanks, and standards in a "forward-then-reverse" order to avoid possible positional errors (Wu, 2005) as shown in Figure 3.1. It is highly recommended to leave the edge well empty or with phosphate buffer to reduce the impact of "edge effect" on samples and standards (Wu, 2005).

The plate reader is equipped with an incubator and two injection pumps. The temperature of the incubator was set to 37^{o}C. The rate of peroxyl radical production from AAPH is temperature

sensitive, so timing and handling of the AAPH solution is critical (Wu et al., 2004). Therefore, a new AAPH solution was prepared for each run. Old fluorescein and AAPH solutions were flushed from the syringes and the syringes were primed with fresh fluorescein and AAPH before starting each run. In addition, to optimize the signal amplification to give maximum sensitivity, a gain adjustment was performed by manually pipetting 200 µL of fluorescein into a designated well, A1 (Figure 3.1) before starting the program.

The major parameters of ORAC assay for the FLUOstar Optima were: 35 cycle number; 693 s (11.55 min) cycle time for L-ORAC assay or 210 s (3.5 min) for H-ORAC assay; 8 s orbital shaking with 4 mm shake width before each cycle; 0.3 s position delay; 420 µL/s injection speed for both pumps 1 and 2. During each cycle, the instrument read the fluorescence in each well. In cycle 2, fluorescein at 200 µL volume was injected from pump #1 into the respective wells, to give a final fluorescein concentration of 14 µM in each well, followed by reading the fluorescence. During cycle 4, pump #2 was programmed to inject 150 µL of AAPH into the respective wells to give a final AAPH concentration of 4.8 mM per well, followed by reading the fluorescence.

The H-ORAC and L-ORAC values in µmolar Trolox Equivalents/L (µM TE), generated by Optima Software for FLOUstar (BMG LABTECH Inc., Durham, NC, U.S.A), were automatically calculated using a regression equation ($Y= a + bX$, linear; or $Y= a +bX + cX^2$, quadratic) between Trolox concentration ($\mu M = X$) and the net area under the fluorescein decay curve (net AUC=Y). Linear regression was used in the range of 30-120 µM Trolox Equivalents. The area under curve (AUC) was calculated by the software as:

$$AUC = (0.5 + f_5/f_4 + f_6/f_4 + f_7/f_4 + \ldots + f_i/f_4) \times CT$$

where: f_4 = initial fluorescence at reading at cycle 4,

	1	2	3	4	5	6	7	8	9	10	11	12
A	Gain	-	-	-	-	-	-	-	-	-	-	-
B	-	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	-
C	-	B	S1	S2	S3	S4	X15	X14	X13	X12	X11	-
D	-	X10	X9	X8	X7	X6	X5	X4	X3	X2	X1	-
E	-	X11	X12	X13	X14	X15	S4	S3	S2	S1	B	-
F	-	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	-
G	-	B	S1	S2	S3	S4	X15	X14	X13	X12	X11	-
H	-	-	-	-	-	-	-	-	-	-	-	-

Where: B = blank, dilution solvent, 20 µL
- = 200 µL of 0.075M phosphate buffer
Gain = gain adjustment well that contains 200 µL fluorescein
S1-S4 = standards (Trolox), in triplicate, 20 µL
X1-X15 = samples, in triplicate, 20 µL

Figure 3.1 Layout of the 96-well microplate used in hydrophilic and lipophilic oxygen radical absorbance capacity assay. The edges were not used for samples and filled-up with 200 µL of 0.075M phosphate buffer to reduce the impact of edge effect.

f_i = fluorescence reading at cycle *i*, and

CT = cycle time in minutes. The net AUC was obtained by subtracting the AUC of the blank from that of the sample.

Net AUC = AUC sample – AUC blankFrom the Net AUC, the software calculated the H-ORAC or L-ORAC in μM TE/g. The H-ORAC and L-ORAC (μM TE/g) in the sample or standard were calculated as follows:

$$\text{H-ORAC or L-ORAC } (\mu\text{mol TE/g}) = \frac{\mu\text{M TE}}{\text{L}} \times \text{DF} \times \frac{1}{\text{Weight of peanuts (g)}} \times \frac{1}{1000}$$

where: μM TE/L = H-ORAC or L-ORAC value generated by Optima Software for FLOUstar

The total antioxidant capacity (TAC) was calculated as the sum of H-ORAC and L-ORAC. Data for H-ORAC, L-ORAC and TAC were expressed as micromolar of Trolox equivalents (μMTE) per gram of sample (μM TE/g).

4.2 L-ORAC assay

The lipophilic (L-ORAC) and hydrophilic (H-ORAC) assays of Prior et al. (2003) and Wu et al. (2004) used the ASE 200 Accelerated Solvent Extractor to extract fat from the sample once with hexane. This procedure was modified in this study by manually extracting fat from peanuts for three times as follows: About 10 grams peanuts were ground using a coffee mill and 0.125 g ground peanuts were weighed and mixed with 2.5 mL hexane/dichloromethane (1:1, v/v, Fisher Scientific, Fair Lawn, NJ) in a 50 mL Teflon centrifuge tube (Fisher Scientific, Fair Lawn, NJ), mixed using a vortex mixer for 60 s, sonicated at 37^{o}C for 10 min and then centrifuged for 15 min at 24,000g (14000 rpm) at 10^{o}C. The hexane layer was transferred to a 30 mL vial using a Pasteur pipet and the residue was extracted again for two times with 2.5 mL hexane/dichloromethane each

time, for a total of three extractions sufficient to remove fat fraction from the sample. The residue after three extractions was set aside for H-ORAC determination. All hexane layers were combined in the same vial and dried under nitrogen to remove residual hexane from the extract. The dried hexane extract was dissolved in 2000 µL acetone, then a 50 µL aliquot was diluted with 500 µL of 7% randomly methylated β-cyclodextrin (RMCD, 7 g/100 mL; Trappsol, Pharmacy grade, Cyclodextrin Tech. Devt. Inc., High Springs, FL, USA) prepared by dissolving 7 g of RMCD in a 100 mL mixture of acetone and water (1:1, v/v), to completely dissolve the extract to obtain a clear sample solution. The 7% RMCD was used as a blank and to dissolve trolox standards and AAPH for L-ORAC assay. Twenty µL each of L-ORAC extracts, blank, and standards in triplicate were transferred to a 96-well microplate (Costar 3915, Corning Inc., Corning, NY, USA), and the plate was loaded to a microplate reader (FLUOstar Optima, BMG Labtech Inc., Durham, NC, USA) as described above.

4.3 H-ORAC assay

The residue after lipophilic extraction (Section 3.C.4.2 above) was dried under vacuum to remove residual hexane. Five mL acetone/water/acetic acid, AWA (70:29.5:0.5, v/v/v; VWR, St. Louis, MO, USA) was added to the dried residue, then the mixture was mixed in a vortex mixer for 30 s, and sonicated using an ultrasonic processor, Model CPX 750 (750 W, 50/60 Hz, Cole Parmer, Vernon Hills, IL, USA) as follows: Eight centrifuge tubes were immersed at one time and positioned along the periphery of a 11 cm x 11 cm x 9 cm cubical plastic container. The ultrasonic probe was positioned in the center of the container and sonicated at 50 % amplitude at 37^{o}C for 5 min shaking the vial to suspend the sample during half time of the sonication. The sonicated AWA extract was allowed to stand for 10 min at room temperature with occasional shaking, and then centrifuged at 14,000 rpm for 15 min at 10^{o}C. The AWA extract was transferred to a 30 mL

vial using a Pasteur pipet and the residue was discarded. A 20 µL portion of the pure H-ORAC extract was transferred to a 5 mL test tube and 500 µL of 0.075M phosphate buffer (pH 7.0) was added to result in H-ORAC levels within the range of the standard curve. Twenty µL each of the diluted H-ORAC extracts, blank, and standards in triplicate were transferred to a 96-well microplate (Costar 3915, Corning Inc., Corning, NY, USA), then loaded to a microplate reader (FLUOstar Optima, BMG Labtech Inc., Durham, NC, USA) as described above.

D. Physico-Chemical Analyses

The physico-chemical analyses including moisture and fat contents, and lightness color L* value were performed in samples of roasted UV-treated peanuts.

1. Moisture content

Moisture analysis (AACC 44-32) was performed as follows. Aluminum dishes, lids and liners (55 mm diameter, Fisher Scientific, Fair Lawn, NJ, USA) were dried overnight at 98-100 ºC in a mechanical oven (Model # M01440SC, Lindberg/Blue M, Asheville, NC, USA). The dried dishes and lids were cooled in a desiccator for 1 h and weighed to constant weight. Approximately 2 g of ground peanut kernels was weighed into the dried, pre-weighed aluminum dish with lid, weighing dish. This was the initial weight of the sample. The weighed sample was placed in a vacuum oven, (Vacuum Isotemp, Model 281A, Fisher Scientific, Fair Lawn, NJ, USA). The lid was loosened and the sample was dried at 98 to 100°C to constant weight (approximately 5 h) in partial vacuum at 25 mm Hg. After drying, dry air was admitted into oven to bring to atmospheric pressure and the oven door was opened. The lid was tightened on the dish immediately and the covered dish was placed in a desiccator to reach ambient temperature (23□C), for approximately 45 min under vacuum. The covered dish with sample was weighed to constant weight. The sample was returned to the vacuum oven to dry following

the procedure described above every h until a constant weight was reached. This was the recorded final weight of the sample. Percent moisture content of the roasted peanut was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\%$$

2. Fat content

Fat content analysis (AACC 30-25) was performed as follows: Two grams of moisture free dried ground peanut kernels (taken from moisture analysis) was weighed into a filter paper and placed inside a numbered oil extraction thimble. The Goldfisch for fat extraction apparatus (Model 35001, Laboratory construction Co., Kansas City, MO, USA) was used with petroleum ether (boiling point 35- 60 ºC, J.T. Baker, Phillipsburg, NJ, USA) as the extraction solvent. The thimble was placed into a glass sleeve and then into a pre-weighed extraction beaker previously dried (Model J466004, General Electric, Louisville, KY, USA) at 100^{o}C for approximately 2 h, cooled in the desiccator for approximately 1 h, to constant weight. The beaker was filled with 30 ml petroleum ether and then attached to the apparatus together with the glass sleeve. The peanut sample was extracted with petroleum ether for 16 h at a condensation rate of 5-6 drops/sec. Upon completion of the extraction, the thimble was removed and replaced with a reclaiming tube. The petroleum ether was collected from the extraction beaker, approximately 2h. The extraction beaker was placed on top of the heater, on the Goldfisch apparatus at an angle, and the remaining solvent was allowed to evaporate for approximately 30 min. The extraction beaker was transferred in a vacuum oven (Vacuum Isotemp, Model 282A, Fisher Scientific, Fair Lawn, NJ, USA) at 25 mm Hg at 70^{o}C for 2 h to dry off excess petroleum ether and then cooled to ambient temperature at 25^{o}C for 30 min in a desiccator for approximately 1 h. The extraction

beaker was weighed to constant weight. Redrying of samples in the vacuum oven as described above was done until a constant weight is achieved. Fat content of the sample was calculated using the following equation:

$$\% \text{ Fat} = \frac{\text{Weight of fat}}{\text{Weight of dried sample}} \times 100\%$$

3. Color

A handheld colorimeter (Chroma meter, Model CR-200/0(, Minolta, Japan) was used to measure the CIE L*(lightness) a* (redness) b*(yellowness) of the samples. The instrument was calibrated against a white (L*=97.84; a*=-0.85; b*=3.02) and yellow reference standard tiles (L*=85.46; a*=0.15; b*=54.57). The sample was filled completely into 60 x 15 mm diameter Petri dish placed on top of white paper. The colorimeter aperture was placed closely and perpendicular to the sample. Four readings was taken directly over each quadrant of the sample in the dish, by turning the dish clockwise after each reading and the average of four readings was reported as L*.

E. Sensory Evaluation

Descriptive analysis and consumer acceptance tests were conducted on all UV- treated roasted peanut samples. Before the screening and training sessions of the descriptive analysis test and the consumer acceptance test, the panelists completed and signed consent to participate forms approved by the University of Georgia Institutional Review Board. The detailed procedures for evaluating UV-treated roasted peanuts are as follows:

1. Sensory Evaluation Experimental Design

1.1. Descriptive analysis test

Each of the 10 panelists evaluated a total 56 samples over two days, 2 replications of 27 treatments and 1 control. Sensory evaluations were conducted each day with 28 samples evaluated in 6 sessions separated by mandatory 5 min breaks. Five samples were evaluated in the first 5 sessions, with the last session having only 3 samples. A time lag of 10 s was given between each sample evaluation. A complete block design was employed so that all panelists evaluated all treatment combinations. Samples were presented in a monadic sequential order. Evaluation sequence was based on balanced, randomized block design so that serving position and order of sample was balanced throughout the evaluation period. This was controlled by Compusense *five* (Version 5.0, Compusense Inc., Guelph, Ontario, Canada).

1.2 Consumer acceptance test

Each of the 25 panelists evaluated a total of 28 samples over 2 days. Each day, panelists evaluated 14 samples in 3 sessions separated by a mandatory 5 min break between sessions in a 1 h test. The second replicate of 28 samples was evaluated using another set of 25 panelists. Samples were presented using a balanced randomized design controlled by Compusense *five* (Version 5.0, Compusence Inc., Guelph, Ontario, Canada) in a monadic sequential order.

2. Descriptive Sensory Analysis Test

2.1 Panel recruitment

Prospective members of the descriptive analysis panel of at least 10 panelists were recruited from a database of trained panelists who had previously participated in descriptive analysis tests at the Department of Food Science and Technology, University of Georgia, Griffin campus or were recruited based on the following criteria: between 18 and 70 years of age, did not smoke,

not allergic to peanuts, consume peanuts at least twice per month, willing to evaluate peanuts, available for all training and testing sessions, and able to verbally describe characteristics of the sample. Panelists who passed the criteria were invited to screening tests.

2.2 Screening test

The potential panelists were screened to see if they would qualify by completing a taste and an aroma test. In the taste test, the panelists were asked to identify coded aqueous solutions of sucrose, sodium chloride, citric acid, caffeine and one unknown in 5 min. The panelists who correctly identified all 5 solutions also completed an aroma test; those who did not were dropped from further participation. In the aroma test, the panelists were asked to identify 7 commonly occurring aromatics in 10 min. The aromatics included banana, anise, pineapple, orange, vanilla, peppermint and lemon contained in 120 ml amber glass bottles with screw on lids. The panelists who identified at least 5 out of 7 aromatics correctly were qualified to undergo training.

2.3 Panel training

All training and test sessions were held in the sensory laboratory at the Griffin campus. Ten panelists, 1 male and 9 female, were selected. The panelists were trained and calibrated in 3 training sessions for 2 h each, for a total of 6 h over 2 days.

a) Orientation for new and untrained panelists

New and untrained panelists were given a 1-hour overview of sensory evaluation, one hour before the first training session. They were asked to complete a line scale exercise in 5 min and asked to estimate shaded regions of different circles by making a mark on 150-mm unstructured line scales with anchor points at 12.5 and 137.5 mm and were asked to measure their markings on the scale using a ruler and their responses were compared with the correct rating. Samples were evaluated using a “hybrid” descriptive analysis method (Einstein, 1991), a combination of

Spectrum Analysis Methods (Sensory Spectrum, Inc., Chatham, NJ, USA), which utilizes universal descriptive scales and extensive use of reference standards (Stone and Sidel, 2004); Quantitative Descriptive Analysis (Tragon Corp., Redwood City, CA), which employs the use of 150 mm linear graphic scale with anchors (Lawless and Heymann, 1998); and Texture Profile Techniques. Basic standard solutions of sucrose (2%, 5%, 10%, 15%), sodium chloride (0.2%, 0.35%, 0.5%), citric acid (0.05%, 0.08%, 0.15%) and caffeine (0.05%, 0.08%, 0.15%) were introduced corresponding to points on the universal line scales for sweet (20, 50, 100, 150), salty (25, 50, 85), sour (20, 50, 100), and bitter taste (20, 50, 100), respectively (Meilgaard et al., 2007). Additionally, new panelists were oriented in the use of the computer ballots.

b) First training session

The purpose of the first training session was to develop a sensory ballot and identify reference standards for each attribute. All panelists, trained and new, attended all succeeding sessions together. The new panelists were positioned between trained panelists around the discussion table. Each panelist was presented a total of 3 samples: roasted peanut treated with low UV dose at a 60 cm distance for 10 min, roasted peanut treated with high UV dose at a 20 cm distance for 30 min, and untreated roasted peanuts, representing the range of UV doses. Panelists were asked to describe all the perceptible sensory attributes in the samples by listing the descriptors under appearance, aroma, flavor, taste, and texture on a worksheet. To assist in attribute generation, lists of terms, definitions, references, and evaluation procedures compiled from lexicons previously published (Johnsen et al., 1988; Meilgaard et al. 2007, Walker, 2000; Gills and Resurreccion, 2000; Grosso and Resurreccion, 2002; Rudolf, 2003) and in-house terms generated during previous descriptive profiling tests on peanut products were provided to panelists. As intended, the lexicons were used as a starting point and panelists, as a group,

generated their own list according to their perception of the product. The panel leader wrote each descriptor on the whiteboard and then defined. Redundant terms were either combined and used to form a more precise definition or eliminated in favor of the most appropriate attribute. The panelists then decided on a comprehensive list of descriptors with definitions, references and evaluation procedures for the preliminary ballot.

Ratings of reference standards identified by the panelists for each descriptor were from published lexicons or from previous studies conducted in our sensory laboratory. When panelists did not come to a consensus, references were rated based on standard solutions intensities. To come to consensus, intensity ratings were determined by panelists by rating a reference with flash cards, simultaneously showing their ratings to the panel leader. Panelists with outlying responses were asked to re-evaluate the reference and adjust their rating accordingly. The final ratings used were based on the mean of the final intensity ratings. The additional reference standards identified by the panelists were purchased and evaluated on the next training session.

c) Second training session

The purpose of the second training session was to verify reference attribute intensities, determine ratings for additional references and warm-up sample, to calibrate the panelists against all the reface standards, and practice around the table. The panelists reviewed the preliminary ballot, generated additional terms, and revised definitions of the descriptive terms and suggested additional references, as needed. The reference standards that were not rated during the first training session were evaluated as described in the first training session section.

The panelists rated a warm-up sample made from roasted peanut treated with medium UV dose at 40 cm distance for 20 min using a paper ballot. This warm-up sample was used throughout the study. Intensity ratings for each attribute were called out by each panelist and

recorded on spreadsheet, projected on an active board (Promethean Ltd, Blackburn, Lancashire, UK). Panelists not within the 10% of the mean rating for each attribute were asked to re-evaluate the sample and adjust their rating. When a consensus ($\pm$10% of mean) was reached, the mean intensity ratings were used as attribute intensity ratings for the warm-up sample.

Panelists practiced by evaluating 3 samples around a table using paper ballots. The 3 samples are as follows: the warm-up sample, roasted peanut sample treated with high UV dose, and untreated roasted peanut control. Results were discussed as a group and panelists not within 10% of the mean rating for each attribute were reminded to focus when evaluating that attribute.

d) Third training session

The purpose of the third training session was to refine the ballot, calibrate, and practice descriptive sensory evaluation as a group and individually in partitioned sensory booths. The panelists calibrated as a group using basic solutions and reference standards. The panelists reviewed the preliminary ballot and discussed additional terms, definitions, references, and procedures, as necessary. The final descriptive terms (Table 3.4) and final ballot (Figure 3.2) were developed as agreed upon by all panelists.

Panelists practiced around a table on an unknown sample of roasted peanut using a paper ballot. This sample was the warm-up sample and was presented to determine panelist performance. The results were discussed as a group and panelists not within 10% of the mean rating for each attribute were reminded to focus when evaluating the attribute.

A practice session was conducted in the partitioned sensory booths using the electronic ballot using Compusense *five* (Version 5.0, Compusence Inc., Guelph, Ontario, Canada). The panelists were asked to evaluate three coded peanut samples. The results were discussed as a group and

panelists not within 10% of the mean rating for each attribute were reminded to focus when evaluating the attribute.

2.4 Monitoring of panelist performance and feedback to panelists

To provide feedback and improve panelist performance after each session, the mean ratings and standard deviations of the panel were calculated for each attribute and were presented on a table and discussed to the panelists before the next session. Each panelist attribute was highlighted when their individual ratings were not within $\pm$10% of the group mean. Ratings that were within $\pm$10% from the group mean were accepted as calibrated.

2.5 Test sample preparation

A total of 56 samples (10 g each) of roasted functional peanuts and control samples were placed into 28.57 g capacity plastic cups with lids (Solo Cups Co., Highland, IL, USA). Samples were coded with three digit random numbers and served at ambient temperature (23^{o}C) on a stainless steel tray lined with white paper.

2.6 Environmental Conditions

All tests were performed at the Department of Food Science and Technology, University of Georgia, Griffin campus. The panelists evaluated test samples in environmentally-controlled partitioned sensory booths, illuminated with two 50-watt white incandescent bulbs providing 739 lux of light.

Table 3.4 Terms, definitions, standard references, and respective intensity ratings used in the descriptive tests for roasted peanuts.

Sensory Attribute[1]	Definition	Reference sample	Brand/Type/Manufacturer	Intensity (mm)[2]
Appearance				
Brown color	The intensity of brown color from white to dark brown [3, 4, 5]	White bond paper	Georgia-Pacific (Georgia-Pacific Corp., Atlanta, GA)	0
		Dry cardboard (L=55)		42
		Chocolate syrup	Hershey (Hershey Foods Corp., Hershey, PA)	150
Aromatics				
Roasted peanutty	The aromatic associated with medium roasted peanuts [3, 4, 7]	Medium roasted peanuts (L=50±1) [3, 4, 5]	Georgia green, medium Runner (Golden Peanut Co., Alpharetta, GA)	73
		Roasted peanut 1 (L=55)	Georgia green, medium Runner (Golden Peanut Co., Alpharetta, GA)	41
		Roasted peanut 2 (L=48)	Georgia green, medium Runner (Golden Peanut Co., Alpharetta, GA)	77
Raw/beany	The aromatic associated with raw peanuts and raw green beans	Roasted peanut 1 (L=55)	Georgia green, medium Runner (Golden Peanut Co., Alpharetta, GA)	23
		Raw peanuts	Georgia green, medium Runner (Golden Peanut Co., Alpharetta, GA)	40
Woody/hulls/ skins	The aromatic associated with peanut skins and hulls [3,7,8]	Skins from medium roasted peanuts (L=50 ± 1)[4, 8]	Georgia green, medium Runner (Golden Peanut Co., Alpharetta, GA)	35

Table 3.4 continued…

Sensory Attribute[1]	Definition	Reference sample	Brand/Type/Manufacturer	Intensity (mm)[2]
Burnt	The aromatic associated with burnt peanuts/ espresso coffee [4, 7]	Burnt toast	Great Value white bread (Wal-Mart Stores, Inc., Bentonville, AR)	15
		Burnt peanuts (L=30)	Georgia green, medium Runner (Golden Peanut Co., Alpharetta, GA)	78
Cardboard	The aromatic associated with cardboard [7]	Wet cardboard, 1x1 cm piece with 1 tsp water [4, 8]		35
Oxidized	The aromatic associated with rancid fats and oils [4, 9]	Oxidized peanuts [4] using sweet basic standard solution for intensity	Georgia green, medium Runner (Golden Peanut Co., Alpharetta, GA)	48
Off-flavor	Any flavor that will be specified by the panelists which is not fishy, oxidized, stale, cardboard, burnt, woody/hull/skin, and raw/beany	Bitter, sweet, sour, and salty basic standard solutions		
Taste				
Salty	The taste on the tongue associated with sodium chloride solution [6, 7]	0.2% sodium chloride in deionized water [6]	Salt (Morton International, Inc., Chicago, IL)	25
		0.35% sodium chloride in deionized water [6]	Salt (Morton International, Inc., Chicago, IL)	50
		0.5% sodium chloride in deionized water [6]	Salt (Morton International, Inc., Chicago, IL)	85

Table 3.4 continued…

Sensory Attribute[1]	Definition	Reference sample	Brand/Type/Manufacturer	Intensity (mm)[2]
Sour	The taste on the tongue associated with acid solution [6, 7]	0.05% citric acid in deionized water [6]	Fisher Scientific, Fair Lawn, NJ	20
		0.08% citric acid solution in deionized water [6]	Fisher Scientific, Fair Lawn, NJ	50
		0.15% citric acid solution in deionized water [6]	Fisher Scientific, Fair Lawn, NJ	100
Bitter	The taste on the tongue associated with bitter agents such as caffeine solution [6, 7]	0.05% caffeine in deionized water [6]	Food grade caffeine (Fisher Scientific, Fair Lawn, NJ)	20
		0.08% caffeine in deionized water [6]	Food grade caffeine (Fisher Scientific, Fair Lawn, NJ)	50
		0.15% caffeine in deionized water [6]	Food grade caffeine (Fisher Scientific, Fair Lawn, NJ)	100
Feeling factors				
Toothpack	The amount of sample left in or on teeth after chewing [4]	Burnt toast	Great Value white bread (Wal-Mart Stores, Inc., Bentonville, AR)	15
				75
		Graham cracker [4]	Nabisco (East Hanover, NJ)	
Astringent	The puckering or drying sensation on the mouth or tongue surface	Milk of magnesia	Phillips (Bayer Healthcare LLC, Morristown, NJ)	40
		Grape juice	Welch's (Skaneateles Falls, NY)	65
Tongue sting/throat burn	The degree of sharp tingling sensation or feeling on the tongue or throat which leaves a burning sensation on the tongue surface	Big red gum	Wrigley (Chicago, IL)	57

Table 3.4 continued…

Sensory Attribute[1]	Definition	Reference sample	Brand/Type/Manufacturer	Intensity (mm)[2]
Texture				
Hardness	Force required to bite through the food	Raw peanuts	Georgia green, medium Runner (Georgia Peanut Co., Alpharetta, GA)	53
		Roasted peanut 2 (L=48)	Georgia green, medium Runner (Georgia Peanut Co., Alpharetta, GA	84
Crunchiness	Force needed and amount of sound (lower pitch) generated from chewing a sample with molar teeth [4]	Corn Chips [4]	Lay's (Frito-Lay, Plano, TX)	75
		Roasted peanut 2 (L=48)	Georgia green, medium Runner (Georgia Peanut Co., Alpharetta, GA	46

[1] Attribute listed in order as perceived by panelists
[2] Intensity rating based on 150 mm unstructured line scale
[3] Gills and Resurreccion (2000)
[4] Grosso and Resurreccion (2002)
[5] Rudolf and Resurreccion (2003)
[6] Meilgaard et al. (2007)
[7] Johnsen et al. (1988)
[8] Walker (2000)
[9] Muego-Gnanasekharan and Resurreccion (1992)

Descriptive Test Ballot for Roasted Peanuts

Panelist Code: _____ **Date: _____**

Appearance: Please look at sample as a whole and evaluate its **COLOR.**

Brown Color – the intensity of brown color from white to dark brown.
Reference: white paper = 0; dry cardboard (L=55) = 42; chocolate syrup=150; WUP=30

_____|_____|_____

Aromatics: Please take at least four pieces of sample and evaluate for the following **AROMATICS.**

Roasted peanutty- the aromatic associated with medium roasted peanuts.
Reference: medium roasted peanuts (L=50)= 73; roasted peanut 1 (L=55)= 41; roasted peanut 2 (L=48) =77; WUP= 21

_____|_____|_____

Raw/beany - the aromatic associated with raw peanuts and raw green beans.
Reference: roasted peanut 1 (L=55) =23; raw peanuts= 40; WUP=0

_____|_____|_____

Woody/hulls/skins – the aromatic associated with peanut skins and hulls.
Reference: peanut skins = 35; WUP = 18

_____|_____|_____

Burnt – the aromatic associated with burnt peanuts/espresso coffee.
Reference: Burnt toast=15; burnt peanuts (L=30) = 78; WUP = 0

_____|_____|_____

Figure 3.2 The ballot used in descriptive sensory analysis test for roasted peanuts.
A warm-up sample was used during test.

Cardboard– the aromatic associated with wet cardboard.
Reference: wet cardboard=35; WUP = 25

_____|_____|_____

Fishy – the aromatic associated with seaweed and fish oil .
Reference: Tuna= 50; Cod liver oil=78; WUP = 20

_____|_____|_____

Oxidized - the aromatic associated with rancid fats and oils.
Reference: oxidized peanuts =48 (using basic standard solutions sweet for intensity); WUP = 27

_____|_____|_____

Off-flavor- any flavor that will be specified by the panelists which is not fishy, oxidized, stale, cardboard, burnt, woody/hull/skin/ and raw/beany.
Reference: Basic standard solutions bitter, sweet, sour and salty; WUP=0
Please specify the off-flavor perceived on the paper provided on the tray.

_____|_____|_____

Tastes: Please take at least 4 pieces of sample and evaluate its **TASTES.**

Bitter - the taste on the tongue associated with bitter agents such as caffeine solution
Reference: bitter 20; bitter 50; bitter 100; WUP = 22

_____|_____|_____

Sweet – the taste on the tongue associated with sucrose solution
Reference: sweet 20; sweet 50; sweet 100; sweet 150; WUP = 15

_____|_____|_____
Sour- the taste on the tongue associated with acid solutions
Reference: sour 20; sour 50; sour 85; WUP = 0

_____|_____|_____

Figure 3.2 (continued)

Salty - the taste on the tongue associated with bitter agents such as caffeine
Reference: salty 20; salty 50; salty 85; WUP = 12

_____|_____|_____

Feeling factors: Please take at least four pieces of sample and evaluate its **FEELING FACTORS** and **TEXTURE**. After evaluating this sample, please click the 'next sample' button to go to the next sample.

Toothpack - the amount of sample left in or on teeth after chewing.
Reference: Burnt toast= 15; graham crackers= 75; WUP =59

_____|_____|_____

Astringent - the puckering or drying sensation on the mouth or tongue surface.
Reference: milk of magnesia = 40; grape juice=65; WUP=18

_____|_____|_____

Tongue sting/throat burn - the degree of sharp tingling sensation or feeling on the tongue or throat which leaves a burning sensation on the tongue surface.
Reference: Big red gum=57; WUP =0

_____|_____|_____

Texture:

Hardness – **force** required to bite through the food.
Reference: raw peanuts= 53; roasted peanut 2 (L=48) =84; WUP=91

_____|_____|_____

Crunchiness – the force needed and amount of sound (lower pitch) generated from chewing a sample with molars.
Reference: corn chips=75; roasted peanut2 (L=48) = 46; WUP=39
_____|_____|_____

Figure 3.2 (continued)

2.7 Ballot

The computer ballot used Compusense *five* (Version 5.0, Compusence Inc., Guelph, Ontario, Canada). The computerized ballot consisted of 18 questions regarding the attributes on several screen sequentially according to the order of their perception, each with a 150 mm unstructured line scale. Using a computer mouse, panelists clicked on each attribute marked the line scale indicating the intensity rating of the attribute. Panelists could return to each attribute before proceeding to the next sample but were not allowed to move backward or forward between samples. Instructions, definitions, references, control and reference intensities were provided as part of the ballot.

2.8 Calibration of panelists prior to testing

One hour prior to each test session, the panelists convened around the table in the sensory laboratory and calibrated against 4 standard basic solutions and the reference standards led by the panel leader. The warm-up sample was evaluated by each panelist using a paper ballot. Results from the warm-up sample were reviewed and panelists and those who were not within the 10% of the mean rating were asked to re-evaluate the sample and adjust to the mean rating.

2.9 Test procedure

The panelists individually evaluated the roasted peanut samples in partitioned sensory booths using the computer ballot. Test sessions were conducted between 10 am and 12 pm of each day. The samples composed of roasted peanuts made from UV-treated peanuts and controls according to the experimental design above. Water cup, expectoration cup, and napkin were supplied to each panelist. Samples were presented in a monadic sequential order. The panelists were instructed to expectorate and eat unsalted crackers and rinse with water between sample evaluations. Panelists were paid an honorarium of $12 per session.

3. Consumer Acceptance Test

Fifty consumers were recruited from a database of consumers who had previously participated in consumer tests at the Department of Food Science and Technology, University of Georgia, Griffin campus. The criteria for consumers were the following: between age of 18-70, non-smoker, not allergic to peanuts, consumes peanuts and peanut products at least once per month, willing to evaluate peanuts, and available for the session. The target sample for the recruitment was 50% males and 50% females in age groups corresponding to the population found in the US census (2006).

All tests were performed at the Consumer and Sensory Laboratories, Department of Food Science and Technology, University of Georgia, Griffin campus in 5 sessions hourly from 10:00 am to 5:30 pm. Panelists were greeted in a conference room and asked to sign in. Each consumer panelist was asked to fill-up a demographic questionnaire and sign two copies of consent to participate. Consumers were instructed using a PowerPoint presentation on how to evaluate their samples using a computer ballot and use of booth signal lights. Paper ballots were supplied to panelists not comfortable with using a computer. Once the consumers completely understood how to evaluate the samples, they were escorted to the partitioned sensory booths as described in the Descriptive Analysis Test section.

Panelists were asked to evaluate the overall acceptance and acceptance of aroma, flavor, appearance, color, and texture of the sample using a 9-point hedonic scale, with 1= dislike extremely, 5=neither like nor dislike and 9=like extremely (Peryam and Pilgrim, 1957) of roasted peanuts made from UV-treated peanuts and controls. An example of questions on a computer ballot (Compusense *five,* Version 5.0, Compusence Inc., Guelph, Ontario, Canada) and a paper ballot used to rate the roasted peanut samples is shown on Figures 3.3 and

3.4, respectively. Panelists were supplied with water cup, expectoration cup, and napkin. Panelists rinsed with water between sample evaluations and were instructed to expectorate into the supplied expectoration cup.

F. Statistical Analysis

Sensory data were collected from Compusense *five* software (Version 4.8, Compusense Inc., Guelph, Ontario, Canada). The data on the results of chemical, physico-chemical, and sensory analyses were analyzed using Statistical Analysis System (SAS), v 8 (SAS, Institute Inc., Cary, NC, USA, 1990). The General Linear Model (PROC GLM) was used to detect significant differences among treatments for *trans*-resveratrol, *trans*-piceid, total stilbenes (sum of *trans*-resveratrol and *trans*-picied), total phenolics, TEAC, ORAC and overall acceptance. The relative significance of the independent variables, distance from UV light (ID), UV exposure time (IT), and incubation time (IC) was established by Analysis of Variance (ANOVA) in terms of their percentage contribution to the response (Gaitonde et al., 2008) with the factor contributing the largest percentage to the total sum of squares as the most significant. Fisher's least significant difference (LSD) at $P \leq 0.05$ for mean separation test was used to compare means of response variable's *trans*-resveratrol, *trans*-piceid, total stilbenes, total phenolics, TEAC, ORAC and overall acceptance.

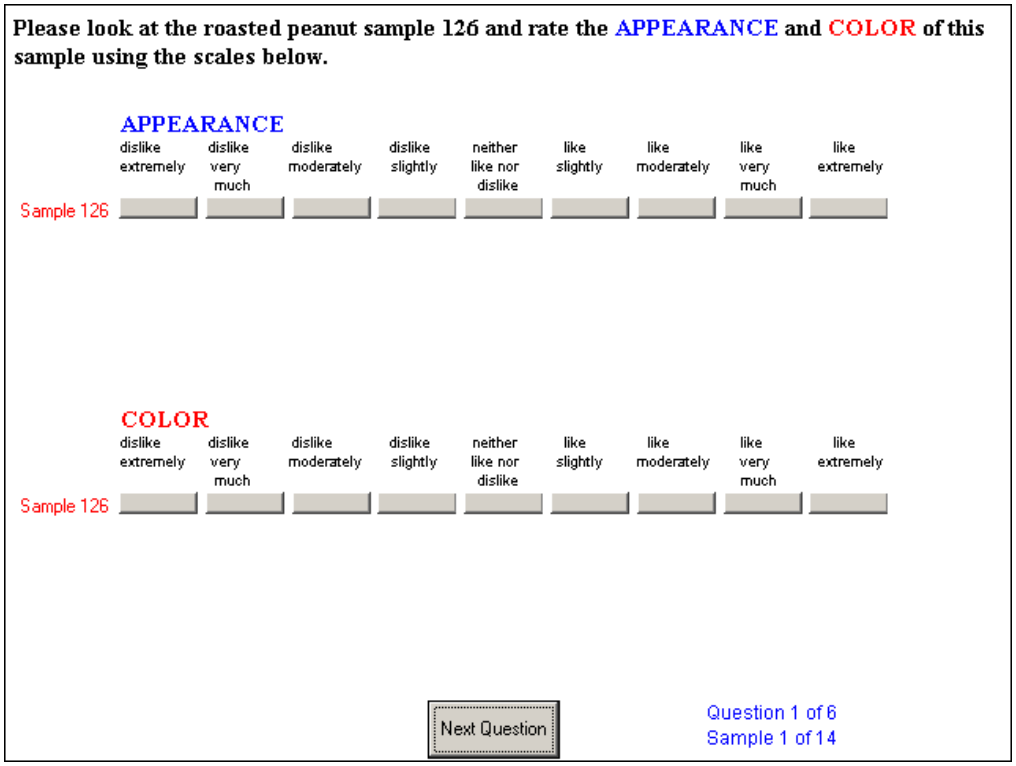


Figure 3.3 Computer ballot used in rating roasted peanuts during consumer test. Panelists used a 9-point hedonic scale to rate acceptance or liking.

Panelist Code: _____ **Sample Code: _____**
Date: _____

Ballot for Roasted Peanuts
Consumer Taste Test

Please check that the code number of the sample is exactly that shown in this paper ballot. You may spit out the sample using the inverted covered Styrofoam spit cup on your tray. Please keep you spit cup always tightly closed to prevent spills.

APPEARANCE AND COLOR

Please look at the roasted peanut sample and rate the APPEARANCE and COLOR of this sample using the scales below.

APPEARANCE

Dislike extremely	Dislike very much	Dislike moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

COLOR

Dislike extremely	Dislike very much	Dislike moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

AROMA

Now, please sniff the roasted peanut sample and rate the AROMA of this sample.

Dislike extremely	Dislike very much	Dislike moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

FLAVOR AND TEXTURE

Now, please put 4 pieces of sample into your mouth, chew it then rate the FLAVOR and TEXTURE of this sample. Do not swallow the samples. Spit sample out after you evaluate flavor and texture.

FLAVOR

Dislike extremely	Dislike very much	Dislike moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Figure 3.4 The ballot used in rating roasted peanuts during consumer test.
Panelists used a 9-point hedonic scale to rate acceptance.

TEXTURE

Dislike extremely	Dislike very much	Dislike moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

OVERALL ACCEPTANCE

Please put 4 pieces of sample into your mouth, chew it then rate the OVERALL ACCEPTANCE of this sample. Do not swallow the samples. Spit sample out after you evaluate the overall acceptance.

OVERALL ACCEPTANCE

Dislike extremely	Dislike very much	Dislike moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

You are now ready to evaluate the sample for the intensities of the following attributes:

1. **Roasted peanutty flavor**
2. **Off-flavors**

Please do not rate the sample for its acceptability.

Please put 4 pieces of sample into your mouth, chew it then answer the following questions. Please indicate the INTENSITY of the following attributes. Do not swallow the samples. Spit sample out after you evaluate intensities.

INTENSITY OF ROASTED PEANUT FLAVOR

How do you rate the INTENSITY of ROASTED PEANUT FLAVOR in the sample?

None	Very weak	Moderately weak	Slightly weak	Neither Weak nor Strong	Slightly strong	Moderately string	Very Strong	Extremely strong
☐	☐	☐	☐	☐	☐	☐	☐	☐

INTENSITY OF OFF-FLAVOR

How do you rate the INTENSITY of OFF-FLAVOR in the sample?

None	Very weak	Moderately weak	Slightly weak	Neither Weak nor Strong	Slightly strong	Moderately string	Very Strong	Extremely strong
☐	☐	☐	☐	☐	☐	☐	☐	☐

Figure 3.4 (continued)

G. Optimization of UV Process Parameters

Regression analysis (PROC REG) was conducted to develop prediction models for each dependent variable, resveratrol, piceid, total stilbenes, total phenolics, TEAC, ORAC and overall acceptance based on independent variables for UV treatment such as distance from UV light (ID), UV exposure time (IT), and incubation time (IC). A second order polynomial regression model with three linear terms was used as full model as follows:

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_1 x_1^2 + \beta_2 x_2^2 + \beta_3 x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 + \varepsilon$$

Where: Y = response variable, *trans*-resveratrol, *trans*-piceid, total stilbenes, total phenolics, TEAC, ORAC or overall acceptance;

β_o = intercept when x_1, x_2, *and* x_3 are equal to zero;

β_1, β_2, and β_3 = parameter estimates of ID (x_1), IT (x_2), and IC (x_3);

x_1^2, x_2^2, and x_3^2 = squared terms;

x_1x_2, x_1x_3, x_2x_3 and $x_1x_2x_3$ = cross product terms; and

ε = residual term.

Response surface methodology was used to determine the effects of the dependent variables on the concentrations of *trans*-resveratrol, *trans*-piceid, total stilbenes, total phenolics, TEAC, ORAC and overall acceptance of UV-treated peanut kernels. Significant regression models were identified ($P<0.05$) and were used to generate response surfaces and contour plots using PROC GCONTOUR. Plotting was done using two independent variables at a time, i.e., ID versus IT, while IC was fixed at one level.

Boundary regions of interest were shaded on the contour plots to at least 2.64 µg/g *trans*-resveratrol (McMurtrey et al., 1994); 1.85 µg/g *trans*-piceid (Lamuela-Raventos et al., 1995); 1.84

mg GAE total phenolics (USDA, 2007); 5.01 μM TE/g TEAC (Villaño et al., 2004); and 38 TE/g ORAC (USDA, 2007)), representing 100% of these compounds in red wines; and an overall acceptance rating ≥5.0. When response variables could not meet the set criteria for the region of interest, maximum values achievable were shaded in lieu of pre-determined regions of interest. When higher levels were achieved by the process, the contour plots were shaded starting at these higher values. Contour plots for all response variables were superimposed to identify the region of overlap that determined the optimum UV process parameters.

H. Verification of Prediction Models

The prediction models were verified for UV processes using parameters within and outside the region of overlap. The samples were analyzed for *trans*-resveratrol, *trans*-piceid, total stilbenes, total phenolics, TEAC, and ORAC as well as sensory consumer overall acceptance. The verified optimum UV process was used as basis for the combined US-UV processing treatments in Study 3.

II. STUDY 2 - ULTRASOUND PROCESS OPTIMIZATION TO ENHANCE *TRANS*-RESVERATROL BIOSYNTHESIS IN PEANUTS

A. Experimental Design

Raw peanuts kernels of varying sizes of chop (2-5 mm), slice (7 mm) and whole (12-14 mm) were treated with varying doses of US using a 3 x 3 x 3 full fractional design, composed of US power density (mW/cm^3), US exposure time (min), and incubation time at 25^oC. The three factors and levels for each factors were US power density (X_1) of 25, 50, 75 mW/cm^3; US exposure times (X_2) of 2, 5, and 8 min; and incubation times (X_3) at 25^oC of 24, 36 and 48 h. The factors, levels, symbols and codes are shown in Tables 3.5 and 3.6. Two replications of 27 treatments per size and 1 control of untreated raw peanuts were prepared for a total 170 samples.

B. Sample Preparation

Peanuts, *Arachis hypogaea* cv Georgia green medium runners (Golden Peanut Company, Alpharetta, GA) harvested in 2005 and stored for two months 4^{o}C were used. All processing implements and surfaces were washed and sanitized with 200 ppm chlorine solution. Peanuts were sorted, washed in water, sanitized with chlorine solution, soaked in water for full imbibition for 16 h as described previously in Study 1. Imbibed peanuts were chopped, sliced or kept whole prior to sonication. Chopping of peanuts to 2-5 mm by placing 600 g of imbibed peanuts in the sanitized bowl of a commercial food cutter (Model 84142, Hobart, Troy, OH) and chopped at 1725 rpm for 30 s. Slicing of peanuts 0.7 cm thick pieces was done as described previously in Study 1. Chopped, sliced and whole peanuts were subjected to different US treatments using power densities of 25, 50 and 75 mW/cm^3 for 4, 6 and 8 min in an ultrasonic processor with temperature controller (750W, 115 VAC, 50/60 Hz, Cole Parmer, Vernon Hills, IL) according to the experimental design in Table 3.8. Peanuts, 400 g, were weighed in a 1 L plastic beaker and filled with filtered deionized water up to 800 mL mark. A 25 mm diameter probe of the ultrasonic processor was placed at the center of peanuts and US was applied continuously at 25^{o}C at amplitudes that will supply the required power densities which were calculated using the formula,

$$\text{Power (Joule or Watt-s)} = \text{Power density (mW/cm}^3\text{)} \times \text{Volume level of water in beaker (cm}^3\text{)} \times \text{Time (s)} \times \frac{1\ \text{W}}{1000\ \text{mW}}$$

Table 3.5 Treatment variables and their levels for ultrasound processes[1].

Factors (treatment variables)	Factor symbol code	Levels (Coded Values)		
		-1	0	+1
Ultrasound power density (mW/cm^3)	X_1	25	50	75
Ultrasound exposure time (min)	X_2	2	5	8
Incubation time at 25°C (h)	X_3	24	36	48

[1]Ultrasound processing treatments to determine effects on the concentrations of *trans*-resveratrol, *trans*-piceid, total phenolics, antioxidant capacities, and consumer overall acceptance of sliced, chopped, and whole peanut kernels (7 mm).

Table 3.6 Experimental design for the three-level, three-factor response surface analysis[1] for ultrasound-treated chopped, sliced and whole peanut kernels.

Treatment	Coded values			Uncoded values		
	X1 Ultrasound power density (mW/cm^3)	X2 Ultrasound exposure time (min)	X3 Incubation time (h)	X1 Ultrasound power density (mW/cm^3)	X2 Ultrasound exposure time (min)	X3 Incubation time (h)
1	-1	-1	-1	25	2	24
2	-1	-1	0	25	2	36
3	-1	-1	+1	25	2	48
4	-1	0	-1	25	5	24
5	-1	0	0	25	5	36
6	-1	0	+1	25	5	48
7	-1	+1	-1	25	8	24
8	-1	+1	0	25	8	36
9	-1	+1	+1	25	8	48
10	0	-1	-1	50	2	24
11	0	-1	0	50	2	36
12	0	-1	+1	50	2	48
13	0	0	-1	50	5	24
14	0	0	0	50	5	36
15	0	0	+1	50	5	48
16	0	+1	-1	50	8	24
17	0	+1	0	50	8	36
18	0	+1	+1	50	8	48
19	+1	-1	-1	75	2	24
20	+1	-1	0	75	2	36
21	+1	-1	+1	75	2	48
22	+1	0	-1	75	5	24
23	+1	0	0	75	5	36
24	+1	0	+1	75	5	48
25	+1	+1	-1	75	8	24
26	+1	+1	0	75	8	36
27	+1	+1	+1	75	8	48

[1] Experimental design for three factors - ultrasound power density, ultrasound exposure time, and incubation time at 25°C that will determine the levels of *trans*-resveratrol, *trans*-piceid, phenolic compounds, total phenolics, antioxidant capacities, and consumer overall acceptance of sliced (7 mm) peanut kernels (Design-Expert v. 6.0.10, Stat-Ease, Inc., Minneapolis, MN).

Amplitudes that provided the required power in joules were empirically determined from preliminary sonication of peanuts immersed in water resulting in 21, 48, and 68% amplitudes to achieve power densities of 25, 50 and 75 mW/cm^3.

US-treated peanuts were drained in a sanitized colander for 15 min to remove excess water, packed in a sanitized half gallon glass mason jars, covered and wrapped with to prevent exposure to light, and incubated at 25^{o}C for 24, 36, and 48 h. The entire process was conducted under yellow light to avoid isomerization or degradation of light sensitive phenolic compounds. After incubation, the samples were stored in a walk-in freezer until these were dried and roasted as described previously in Study 1.

C. Chemical and Physico-Chemical Analyses

US-treated peanuts and controls were analyzed for *trans*-resveratrol, *trans*-piceid, total phenolics, TEAC, ORAC, moisture and fat contents following the procedure described previously in Study 1.

D. Sensory Evaluation

Descriptive analysis and consumer acceptance tests were conducted as described in Study 1 except for the samples used.

E. Statistical Analysis

The data collected from the chemical and sensory tests were analyzed statistically as described previously in Study 1.

F. Optimization of Ultrasound Process Parameters

Regression analysis to determine significant prediction models and response surface methodology to determine optimum US process parameters for were conducted following the procedure described previously in Study 1.

G. Verification of Prediction Models

The verification of the significant prediction models for US processes were conducted following the procedure described in Study 1, except for samples and parameters inside and outside the optimum region used.

III. STUDY 3- COMBINED ULTRASOUND-UV PROCESS OPTIMIZATION TO ENHANCE *TRANS*-RESVERATROL BIOSYNTHESIS IN PEANUTS

A. Experimental Design

A 3 x 3 x 3 full factorial design was used to treat peanuts with varying doses of combined US-UV based on a two-stage experimental design depicted in Figure 3.5. A first stage, represented by the smaller cube, shows the parameters from two separate experiments, reported previously to optimize either US or UV process (Sales and Resurreccion, 2009). It is embedded within the parameters of the larger cube representing the second stage for a combined ultarasound-UV process for this optimization study. The second stage was necessary to allow for modeling the synergistic potential of combined US-UV treatments.

Factors for the combined ultarasound-UV processing treatments included US power densities of 40, 80 and 120 mW/cm^3, US exposure times of 4, 8, and 12 min, and UV exposure times of 10, 30, and 50 min (Tables 3.7 and 3.8) at a fixed distance of 40 cm from UV light found optimal in the previous study (Sales and Resurreccion, 2009). All samples were incubated at the optimal time of 36 h at 25^{o}C (Sales and Resurreccion, 2009). Three controls were used: 1) untreated raw whole peanuts; 2) roasted peanuts treated with optimum UV process of 30 min at 40 cm distance from UV light and incubated for 36 h (Sales and Resurreccion, 2009); and 3) roasted peanuts treated with optimized US process of 6 min at 75 mW/cm^3 power density and incubated for 36 h (Sales and Resurreccion, 2009). Two replications of 27

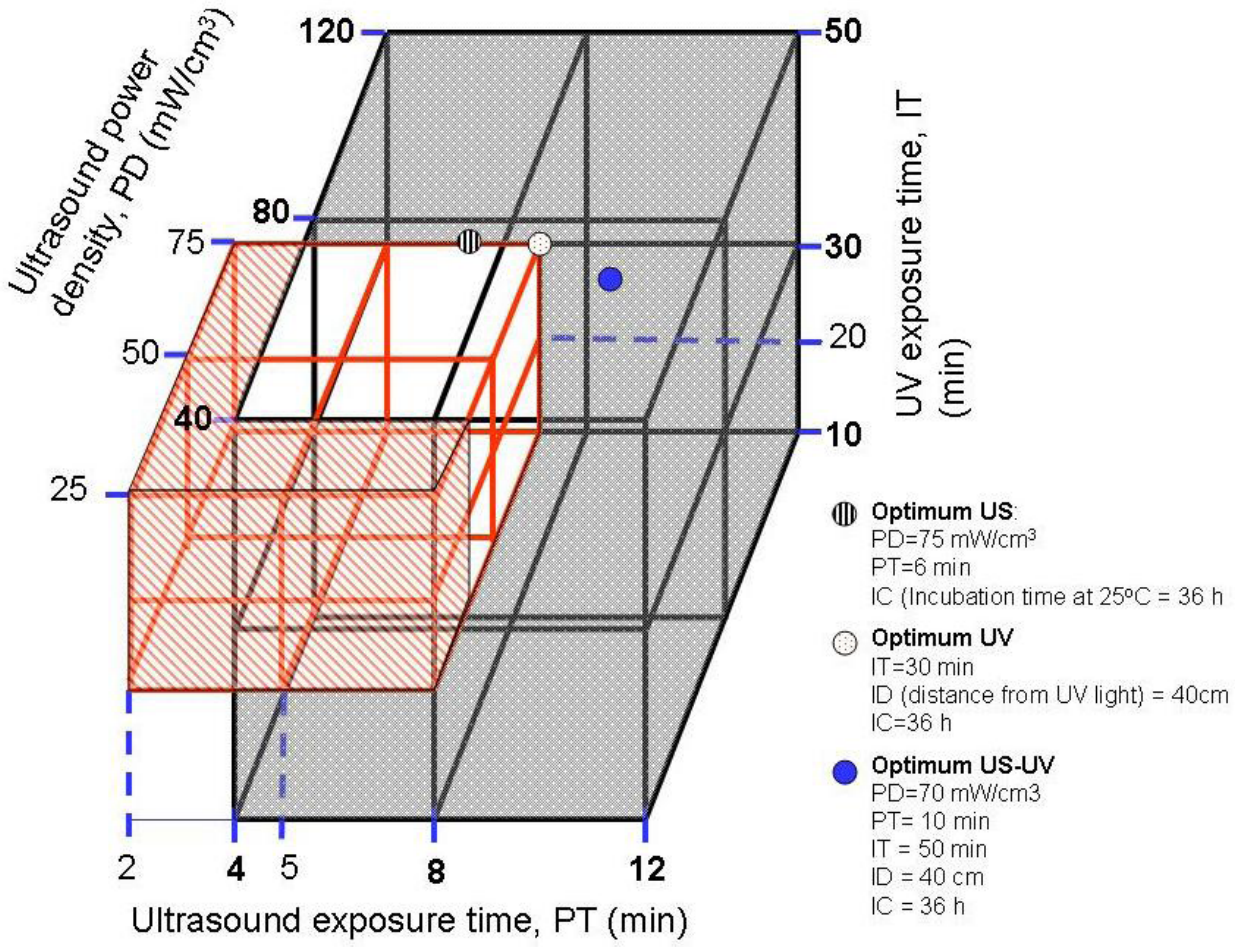


Figure 3.5 Two-stage experimental design for combined ultrasound and UV treatments of peanut kernels. The first stage, represented by the smaller cube, shows the parameters from two separate experiments conducted previously to optimize ultrasound or UV processes. The second stage, the larger cube where parameters of the first stage are imbedded, represents the design for optimizing a combined ultrasound-UV to allow for modeling the synergistic potential of ultrasound and UV. Ultrasound exposure time is plotted on the x-axis, UV exposure time on the y-axis, and ultrasound power density on the z-axis. (Reprinted from Food Chemistry, Vol. 122, Sales, J.M. and Resurreccion, A.V.A. Phenolic profile, antioxidants, and sensory acceptance of bioactive-enhanced peanuts using ultrasound and UV. Page No. 796, Copyright 2010, with permission from Elsevier.)

Table 3.7 Treatment variables and their levels for combined ultrasound-UV processes[1]

Factors (treatment variables)	Symbol code	Levels (Coded Values)		
		-1	**0**	**+1**
Ultrasound power density (mW/cm^3)	X_1	40	80	120
Ultrasound exposure time (min)	X_2	4	8	12
UV exposure time (min) at fixed distance from UV light of 40 cm	X_2	10	30	50

[1] Experimental design that will determine the ultrasound power density, ultrasound and UV exposure times that will be used to determine the levels of phenolic compounds, total phenolics, antioxidant capacities, and consumer overall acceptance of sliced peanut kernels (7 mm). Fixed factors are 40 cm distance from UV light and incubation time at 25^{o}C for 36 h found optimal in previous studies.

Table 3.8 Experimental design for the three-level, two factor response surface analysis[1] for combined ultrasound-UV treated peanuts.

Treatment	Coded values			Uncoded values		
	X1 Ultrasound power density (mw/cm^3)	X2 Ultrasound exposure time (min)	X3 UV exposure time (min)	X1 Ultrasound power density (mw/cm^3)	X2 Ultrasound exposure time (min)	X3 UV exposure time (min)
1	-1	-1	-1	40	4	10
2	-1	-1	0	40	4	30
3	-1	-1	+1	40	4	50
4	-1	0	-1	40	8	10
5	-1	0	0	40	8	30
6	-1	0	+1	40	8	50
7	-1	+1	-1	40	12	10
8	-1	+1	0	40	12	30
9	-1	+1	+1	40	12	50
10	0	-1	-1	80	4	10
11	0	-1	0	80	4	30
12	0	-1	+1	80	4	50
13	0	0	-1	80	8	10
14	0	0	0	80	8	30
15	0	0	+1	80	8	50
16	0	+1	-1	80	12	10
17	0	+1	0	80	12	30
18	0	+1	+1	80	12	50
19	+1	-1	-1	120	4	10
20	+1	-1	0	120	4	30
21	+1	-1	+1	120	4	50
22	+1	0	-1	120	8	10
23	+1	0	0	120	8	30
24	+1	0	+1	120	8	50
25	+1	+1	-1	120	12	10
26	+1	+1	0	120	12	30
27	+1	+1	+1	120	12	50

[1] Experimental design to determine the ultrasound power density, ultrasound exposure time, and UV exposure time that will determine the maximum levels of *trans*-resveratrol, *trans*-piceid, *p*-coumaric acid, cafeic acid, ferulic acid, total phenolics, and antioxidant capacities of sliced (7 mm) peanut kernels with high sensory consumer acceptance (Design-Expert v. 6.0.10, Stat-Ease, Inc., Minneapolis, MN).

combined US-UV treatments, and 3 controls were prepared for a total of 60 samples. Each sample was analyzed for 16 phenolic compounds simultaneously (Francisco and Resurreccion, 2009b) for benzoic acid derivatives: gallic-, protocatechuic-, and β-resorcylic (internal standard) acids; flavanols: epigallocatechin, catechin, procyanidin B_2, epicatechin, epigallocatechin gallate, epicatechin gallate, and catechin gallate; stilbenes: *trans*-resveratrol and *trans*-piceid; cinnamic acid derivatives: caffeic, *p*-coumaric- and ferulic acids; and flavonol: quercetin; total phenolics (Singleton et al., 1999), and antioxidant capacities by Trolox Equivalent Antioxidant Capacity (TEAC; Kim et al., 2002) and Oxygen Radical Absorbance Capacity (ORAC; Prior et al., 2003).

B. Sample Preparation

Peanuts (*Arachis hypogaea* cv Georgia green medium runners, 2008 crop year; McCleskey Mills Inc., Smithville, GA) were treated with US -UV following the procedure described in Study 1, except that the experimental design of this study was followed. US-treated peanuts were drained to remove excess water, then layered in plastic trays and subjected to UV treatments following the procedure described in Study 2 except that the experimental design of this study, was followed.

C. Chemical Analyses

The combined US-UV treated sliced peanut samples and controls were analyzed for 15 phenolic compounds simultaneously using reverse phase HPLC method (Francisco and Resurreccion, 2009b) as described below; and for total phenolics (Singleton et al., 1999) and for antioxidant capacities using ABTS anion scavenging assay (Kim et al., 2002) and ORAC (Prior et al., 2003) assays as described previously in Study 1.

1. Profiling of phenolic compounds

1.1 Preparation of dried crude phenolic extracts

The method of Talcott et al. (2005b) for extraction of total phenolics was adapted to prepare the liquid crude phenolic extracts of samples. Peanut skins were removed manually and approximately 20 g were ground in a coffee mill (Model K9M2-4, BrAun, Mexico) to the smallest obtainable particle size and the unground peanuts were discarded. Approximately 10 g of ground sample were weighed into a 250 mL centrifuge bottle (Fisher Scientific, Fair Lawn, NJ, USA) then 20 mL of 80% methanol was added. The method of Talcott et al. (2005b) was modified by adding 1 mL of 20 ppm internal standard, β-resorcylic acid, a compound not reported to be present in peanuts (Francisco and Resurreccion, 2009b) and possesses characteristics of an appropriate internal standard as described by Snyder et al. (1997). The mixture was homogenized using PowerGen 125 homogenizer (Fisher Scientific, Pittsburg, PA, USA) for 2 min. The bottle was shaken for 10 min at ambient temperature using a wrist action shaker (Model 75, Burrell Corp., PA, USA; Francisco and Resurreccion, 2009b) then centrifuged (Model J2-21M, Beckman, Palo Alto, CA, USA) at 2500G (3000 rpm using rotor # 14) for 5 min at 18^{o}C. The supernatant extract was passed through a filter paper, Whatman No. 42 (UGA Central Stores, GA, USA; Rudolf and Resurreccion, 2005) and collected in a 30 mL screw capped glass vial. The residue was extracted with 20 mL of 80% methanol under the same conditions as the first extraction, for a total of 3 times. After the last filtration, the filter paper was washed with 2 mL of 80% methanol and collected in the same vial. The combined filtrate was transferred into an evaporating flask, the vial was rinsed with 0.5 mL of 80% methanol, and rinsing was added to the flask to quantitatively transfer all the components. The filtrate was evaporated to dryness under vacuum in a rotary evaporator (RV05 Basic 1B, IKA, Fisher

Scientific Co., Fair Lawn, NJ, USA; Francisco and Resurreccion, 2009b) at 45°C in a water bath resulting in a dried crude methanolic extract of phenolics.

1.2 Preparation of purified phenolic extracts for HPLC analysis

The method of Francisco and Resurreccion (2009b) was adapted to purify the extract for HPLC analysis by removing protein and lipid before autosampler injection to obtain a good base line (Yu et al., 2005). To purity the extract, 3 mL of dichloromethane (DCM, 100%, VWR, West Chester, PA, USA) and 2 mL of filtered deionized water were added to the dried extract in the evaporating flask and the mixture was transferred to a test tube. The flask was rinsed with 1 mL of filtered deionized water and rinsing was added to the same tube. The tube was mixed for 1 min in a vortex mixer (Model VM 3000, VWR, Thorofare, NJ, USA) at setting #6.5 then centrifuged at 980G (2500 rpm, rotor radius: 14 cm) for 3 min 15 s using a table-top centrifuge (Vari Hi Speed Centricone, Chicago, IL, USA) to allow partitioning and phase separation. The water phase (upper layer) was collected in a second clean 20 mL test tube using a Pasteur pipette, and the lower layer in the test tube was discarded. The water layer was mixed with 5 mL ethyl acetate (100%, VWR, West Chester, PA, USA) for 1 min using the vortex mixer (setting #6.5) to cause a phase separation into water and ethyl acetate layers. The ethyl acetate layer containing phenolics (upper layer) was transferred into an evaporating flask using a Pasteur pipette, and dried in a rotary evaporator at 35^{o}C under vacuum. The dried phenolics were re-dissolved in 1.5 mL methanol and transferred to a 5 mL vial. The flask was rinsed with 0.5 mL methanol and rinsing was added to the same vial. Methanol from the vial was evaporated to dryness under nitrogen gas (Airgas South, Griffin, GA, USA), covered with parafilm and aluminum foil and stored in the freezer until analyzes. The entire process was completed under yellow light to prevent degradation of light sensitive phenolics.

1.3 Preparation of standard solutions and solvents for HPLC analysis.

a) Preparation of 16 standard stock solutions

A total of 16 standards including gallic acid, quercetin, catechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-epigallocatechin, procyanidin B_2, and β-resorcylic acid (internal standard, IS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). *Trans*-resveratrol, (-)-epigallocatechin gallate, caffeic acid, *p*-coumaric acid, protocatechuic acid, ferulic acid, and (-)-epicatechin were purchased from Fisher Scientific (Atlanta, GA, USA).

Stock solutions of gallic acid (1000 ppm), quercetin (1000 ppm), (+)-catechin (1000 ppm), (-)-catechin gallate (2000 ppm), (-)-epicatechin gallate (1000 ppm), (-)-epigallocatechin (2000 ppm), procyanidin B_2 (2000 ppm), β-resorcylic acid (1000 ppm), *trans*-resveratrol (1000 ppm), (-)-epigallocatechin gallate (1000 ppm), caffeic acid (1000 ppm), *p*-coumaric acid (1000 ppm), protocatechuic acid (1000 ppm), ferulic acid (1000 ppm), (-)-epicatechin (1000 ppm) and *trans*-piceid (1000 ppm) were prepared. The 1000 ppm stock solutions were prepared by dissolving 50 mg of each compound separately and diluting to 50 mL with 99.99% methanol (Fisher Scientific, Fair Lawn, NJ, USA). All stock solutions were stored in amber bottles (to protect against light) at -15(C until needed. To prepare a 2000 ppm stock solution, 100 mg powder of the compound was dissolved and diluted to 50 mL with 99.99% methanol. A sample calculation is as follows:

To obtain 1000 ppm gallic acid: $\frac{\text{1000 mg gallic acid x 50 mL 99.99\% methanol}}{\text{1000 mL 99.99\% methanol}}$ = 50 mg gallic acid in 50 mL 99.99% methanol

To obtain 2000 ppm catechin gallate: $\frac{\text{2000 mg catechin gallate x 50 mL 99.99\% methanol}}{\text{1000 mL 99.99\% methanol}}$ = 10 mg catechin gallate in 50 mL 99.99% methanol

b) Preparation of five calibration standard curve solutions from the standard stock solutions for the construction of standard calibration curves

Stock standard solutions were used to prepare standard mixture solution for calibration by mixing the required volumes, calculated by multiplying the final concentration desired in ppm with the total volume of standard mixture to be prepared and then dividing with the concentration of the stock standard solution as shown in Table 3.9.

A sample calculation showing the volume of β-resorcylic acid stock solution required in the preparation of the standard mixture is shown below.

$$\frac{200 \text{ ppm x } 25 \text{ mL}}{1000 \text{ ppm}} = \quad 0.5 \text{ mL or } 500 \text{ µL}$$

Calibration standard curve solutions consisting of 20, 40, 60, and 80% of the standard mixture solution were prepared by diluting the required volume of the standard mixture solution as shown in Table 3.10, with 15% methanol in a 5 mL vial. The 100% calibration standard curve solution used the standard mixture solution as is.

c) Preparation of Solvents for High Performance Liquid Chromatography (HPLC) Analysis

The solvents were prepared under the hood, using gloves and mask. Solvent A, deionized water in 0.1% formic acid was prepared by mixing 4 L deionized water previously filtered through 0.20µm type GN filter (Millipore Corp. Bradford, MA, USA) with 4.0 mL formic acid (VWR, West Chester, PA, USA). Solvent B, 100% acetonitrile with 0.1% formic acid was prepared by mixing 4.0 mL formic acid (VWR, West Chester, PA, USA) in 4 L of 100% acetonitrile.

Table 3.9 Volumes of individual stock solutions required for preparation of the standard mixture solution for phenolic profiling.

Standard	**Concentration (ppm) of stock solution**	**Required volume (µL) of stock solution**	**Final concentration (ppm) in standard mixture solution**
β-Resorcylic acid	1000	20	500
Gallic acid	1000	4	100
Protocatechuic acid	1000	4	100
(-)-Epigallocatechin	2000	20	250
(+)-Catechin	1000	20	500
Procyanidin B_2	2000	10	125
(-)-Epicatechin	1000	10	125
(-)-Epigallocatechin gallate	1000	10	125
(-)-Epicatechin gallate	2000	10	125
(-)-Catechin gallate	2000	10	250
Quercetin	1000	4	100
trans-Piceid	1000	4	100
trans-Resveratrol	1000	4	100
Caffeic acid	1000	4	100
p-Coumaric acid	1000	4	100
Ferulic acid	1000	4	100
Total volume (µL)		2,800*	

* The total volume of 2,800 µL was mixed in a 25 mL volumetric flask and diluted to 25 mL with 15% methanol.

Table 3.10 Volumes of standard mixture solution required for calibration standard curve for phenolic profiling.

Percent of Standard mixture solution	Volume (mL) of standard mixture solution required	Volume (mL) of 15% methanol added to standard mixture solution	Final volume (mL) of solution for HPLC analysis
20	1	4	5
40	2	3	5
60	3	2	5
80	4	1	5
100	2	0	2

1.4 HPLC analysis

The dried purified phenolic extract of UV-treated peanuts was redissolved in 0.75 mL of 15% methanol then transferred to a 2 mL HPLC amber vial. The 5 mL vial was washed with 0.25 mL of 15% methanol and washing was poured in the same HPLC vial for analysis. HPLC analysis was performed using a ProStar HPLC system (Varian Inc., Walnut Creek, CA, USA) with Eclipse Plus C18 reverse column, 250 x 4.6 mm, 5µm particle size, preceded by an Eclipse Plus C18 guard column, 7.5 x 4.6 mm, 5µm particle size (Agilent Technologies, Deerfield, IL, USA) using the conditions described by Francisco and Resurreccion (2009b) shown in Table 3.11. The Star Work Station software (Varian Inc., Walnut Creek, CA, U.S.A) version 6.41 was used to control the autosampler, gradient settings, diode array detector (DAD) and data acquisition. The mobile phase consisted of 0.1% (v/v) formic acid (VWR, West Chester, PA, USA) in filtered deionized water as solvent A and 0.1% (v/v) formic acid in 100% acetonitrile (VWR, West Chester, PA, USA) as solvent B, with a flow rate of 1.5 mL/min, and the column temperature was maintained at approximately 25°C, ambient temperature. The gradient elution increased solvent B linearly from 5 to 7% over 7 min, then to 17% from 7 to 75 min, then increased to 45% from 75 to 110 min, then to 100% from 110 to 117 min, and finally returned to its initial concentration of 5% from 117 to 124 min to allow stabilisation and return to initial concentration. The HPLC analysis of the samples and calibration standard curve solutions were evaluated by injecting 20 mL in duplicate.

Peak areas of standards of benzoic acid derivatives – gallic acid, protocatechuic acid, and internal standard, β-resorcylic acid were quantified at 250 nm; flavanols - (+)-catechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-epigallocatechin, procyanidin B_2, (-)-epigallocatechin gallate at 280 nm; stilbenes - *trans*-resveratrol and *trans*- piceid at 306 nm; cinnamic acid

Table 3.11 Chromatographic conditions for the determination of phenolic compounds in extracts of peanut kernels.

Chromatographic conditions	
Injection volume	20 μL
Guard column	C_{18} guard column, 4.6x12.5 mm, 5μm particle size (Eclipse Plus, Agilent Technologies, Wilmington, DE)
Analytical column	C_{18} reverse-phase column, 4.6x250mm, 5μm particle size (Eclipse Plus, Agilent Technologies, Wilmington, DE)
Mobile phase	Solvent A (formic acid in water, 0.1% v/v) Solvent B (formic acid in acetonitrile, 0.1% v/v) 1st segment: 0-7 min: 5 – 7% B 2nd segment: 7-75 min: 7 – 17% B 3rd segment: 75-110 min: 17 – 45% B Flushing step: 110-117 min: 45 – 100% B Conditioning step: 117-124 min: 100 – 5% B
Flow rate	1.5 mL/min
Temperature	Ambient temperature
Detection conditions	
Scanning	200 – 400 nm
Scan rate	10 Hz
Detection wavelength	Benzoic acid derivatives: 250 nm Cinnamic acid derivatives: 320 nm Flavanols: 280 nm Stilbenes: 306 nm Flavonols: 370 nm

derivatives – caffeic acid, *p*-coumaric acid and ferulic acid at 320 nm; and flavonol – quercetin at 370 nm using diode array detector (DAD). The chromatogram of each compound was verified against the known spectra of the compounds in the calibration standard curve solutions and at the specified wavelengths and retention time as established by Francisco and Resurreccion (2009b). The calibration curve for each compound was obtained by plotting peak areas (y-axis) versus concentrations (x-axis).

The concentration of each of the phenolic compound in µg/g, dry basis was calculated as follows:

$$\mu g \text{ of } i \text{ in dry sample} = \frac{\left(\left[\frac{\mu g \text{ of } i \text{ in standard}}{PA \text{ of } i \text{ in standard}} \right] \times PA \text{ of } i \text{ in sample} \Big/ \left[\frac{\mu g \text{ of IS in standard}}{PA \text{ of IS in standard}} \right] \times PA \text{ of IS in sample} \right) \times \mu g \text{ of IS in volume injected in HPLC} \times \text{volume of extract}}{\text{Volume injected in HPLC}}$$

where: *i* = phenolic compound

IS = internal standard, ß-resorcylic acid

PA = peak area.

D. Sensory Evaluation

Descriptive Analysis and Consumer Acceptance Tests were conducted as described in Study 1 except for the samples, ballot (Figure 3.6), and warm-up samples used.

E. Statistical Analysis

The data collected from the chemical and sensory tests were analyzed statistically as described previously in Study 1.

Descriptive Test Ballot for Roasted Peanuts

Panelist Code: _____ Date: _____

Appearance: Please look at sample as a whole and evaluate its **COLOR.**

Brown Color – the intensity of brown color from white to dark brown
Reference: white paper = 0; dry cardboard (L=55) = 42; chocolate syrup=150; WUP=34

_____|_____|_____

Aromatics: Please take at least four pieces of sample and evaluate for the following **AROMATICS.**

Roasted peanutty- the aromatic associated with medium roasted peanuts.
Reference: light roasted peanut (L=55) =55; medium roasted peanuts (L=50) = 65; dark roasted peanut (L=45) =76; WUP= 34

_____|_____|_____

Raw/beany - the aromatic associated with uncooked or raw peanuts.
Reference: light roasted peanut (L=55) =7; raw peanuts= 37; WUP=0

_____|_____|_____

Woody/hulls/skins – the aromatic associated with peanut skins and hulls.
Reference: peanut skins = 32; WUP = 10

_____|_____|_____

Burnt – the aromatic associated with very dark roast or burnt peanuts.
Reference: Burnt peanuts (L=30) = 73; Basic solutions; WUP = 27

_____|_____|_____

Cardboard– the aromatic associated with wet cardboard.
Reference: wet cardboard=36; WUP = 21

_____|_____|_____

Figure 3.6 The ballot used for the descriptive test of roasted combined ultrasound-UV treated peanuts.

Fishy – the aromatic associated with cod liver oil or old fish .
Reference: Cod liver oil=78; WUP = 0

_____|_____|_____

Oxidized - the aromatic associated with rancid fats and oils.
Reference: oxidized peanuts =48; WUP = 26

_____|_____|_____

Painty- the aromatic associated with linseed oil.
Reference: Linseed oil (diluted) =58; Linseed oil (pure) =115; WUP=0

_____|_____|_____

Tastes: Please take at least 4 pieces of sample and evaluate its **TASTES.**

Bitter - the taste on the tongue associated with bitter agents such as caffeine solution
Reference: bitter 20; bitter 50; bitter 100; WUP = 30

_____|_____|_____

Sweet – the taste on the tongue associated with sucrose solution
Reference: sweet 20; sweet 50; sweet 100; sweet 150; WUP = 12

_____|_____|_____

Sour- the taste on the tongue associated with acid solutions
Reference: sour 20; sour 50; sour 85; WUP = 8

_____|_____|_____

Salty - the taste on the tongue associated with sodium chloride solutions
Reference: salty 20; salty 50; salty 85; WUP = 10

_____|_____|_____

Figure 3.6 (continued)

Please take at least four pieces of sample and evaluate its **TEXTURE and FEELING FACTORS**.

Texture:

Crispness – force needed and amount of sound (high pitch) generated from chewing a sample with front teeth.
Reference: potato chips=75; WUP=29

_____|_____|_____

Crunchiness – the force needed and amount of sound (lower pitch) generated from chewing a sample with molars.
Reference: corn chips=75; medium roasted peanut (L=50) = 40; WUP=40

_____|_____|_____

Hardness – force needed to compress food between molar teeth.
Reference: raw peanuts= 45; medium roasted peanut (L=50) =37; WUP=37

_____|_____|_____

Feeling factors:

Toothpack - the amount of sample left in or on teeth after chewing.
Reference: graham crackers= 75; raw peanuts=86; WUP =59

_____|_____|_____

Astringent - the puckering or drying sensation on the mouth or tongue surface.
Reference: milk of magnesia = 40; grape juice=65; WUP=22

_____|_____|_____

Tongue sting - the degree of sharp tingling sensation or feeling on the tongue or throat which leaves a burning sensation on the tongue surface.
Reference: Basic solutions; WUP =0

_____|_____|_____

Figure 3.6 (continued)

F. Optimization of Combined Ultrasound-UV Process Parameters

Regression analysis to determine significant prediction models and response surface methodology to determine optimum process parameters for combined were conducted following the procedure described previously in Study 1, except that boundary regions of interest included 22 µg/g *p*-coumaric acid, 19 µg/g ferulic acid and 17 µg/g caffeic acid (Ghiselli et al., 1998) in addition to 2.64 µg/g *trans*-resveratrol (McMurtrey et al., 1994); 1.85 µg/g *trans*-piceid (Lamuela-Raventos et al., 1995); 1.84 mg GAE total phenolics (USDA, 2007); 5.01 µM TE/g TEAC (Villaño et al., 2004); and 38 TE/g ORAC/g (USDA, 2007)), representing 100% of these compounds in red wines; and an overall acceptance rating $\geq$5.0.

G. Verification of Prediction Models

The verification of the significant prediction models for the combined US-UV processes were conducted following the procedure described in Study 1, except for samples and parameters inside and outside the optimum region used.

IV. STUDY 4 - APPLICATIONS OF RESVERATROL-ENHANCED PEANUTS SELECTED PRODUCTS AND THEIR SHELF LIFE

A. Goal of the Study

The goal of this study was to apply the resveratrol-enhanced peanuts, prepared using optimal process of combined US-UV treatment determined in Study 3, as ingredient in peanut products. With increasing consciousness of consumers with regard to healthy foods, there is a need to find alternative natural and probably safer sources of food with enhanced levels of bioactive compounds and antioxidants to improve the quality of food. Products selected for application are roasted REP and resveratrol-enhanced peanut bars.

B. Experimental Design

1. Accelerated Shelf Life Test of Roasted Resveratrol-Enhanced Peanuts

An accelerated shelf life test of roasted REP packaged in polyethylene bags (Ziplock, S.C. Johnson & Son, Inc., Racine, WI) was performed using three accelerated storage temperatures of 30 (Model 3107, Hotpack Company, Philadelphia), 35 (Model 4-148-CY, American Instrument, Silver Spring, MD), and 40°C (Model 645 Treas, Precision Scientific, Winchester, VA). Temperatures higher than 40°C were not selected because it is not recommended that samples be stored at temperatures greater than 40°C (Taoukis and Labuza, 1996) as phase changes may occur which can accelerate the reaction such as fat changing from solid to liquid (Labuza, 2000). Samples were also stored at ambient temperature, about 25°C, for verification of shelf life at accelerated temperatures (Labuza and Schmidl, 1985). Control untreated samples were stored at two temperatures, 25°C and 4°C.

The sampling times for the shelf life study of roasted REP were estimated based on Q_{10} of 1.75 for the lipid oxidation (range is 1.5 -2.0; Labuza and Schmidl, 1985) and a shelf life of 90 days at 25°C for roasted peanuts using the equation (Labuza and Schmidl, 1985),

$$\theta_2 = \frac{\theta_1}{Q_{10}^{\Delta T/10}}$$

where: θ_1 = shelf life (day) at lower temperature (°C), T_1

θ_2 = shelf life (day) at higher temperature (°C), T_2

$\Delta T = T2 - T1$

and Q_{10} = the accelerating factor.

Based on these parameters, REP were predicted to have a shelf life of 68 days at 30°C, 51 days at 35°C and 39 days at 40°C. The sampling scheme (Table 3.12) for the study was a design

suggested by Labuza and Schmidl (1985), whereby the samples at each temperature were sampled at similar times for a minimum of six sampling time per temperature. To achieve similar days of sampling times, samples for prediction of shelf life at 30, 35, and 40^{o}C (day 0) were stored on day 29, day 44 and day 55 respectively, of the 25^{o}C storage temperature. In addition, three sampling times for the prediction of shelf life at 40^{o}C were conducted to provide a quicker estimation of shelf life at 40^{o}C and therefore, adjust the sampling scheme at other temperatures as needed. Verification of the shelf life at 25^{o}C was performed by storing samples at actual ambient temperature, approximately 25^{o}C.

Samples at every sampling time at each storage temperature were analyzed for analyzed for hexanal, *trans*-resveratrol, acceptance test by consumer panel, and descriptive sensory test by a trained panel using the procedures described previously in Study 1, except for the samples and the ballot for the consumer and descriptive sensory tests used in this study. Total phenolics and TEAC were analyzed at initial (0 day storage) and at the end of shelf life at each storage temperature using the procedure described previously in Study 1 except for the samples used in this study.

2. Storage Study of Resveratrol-Enhanced Peanut Bars

A storage study at 25 and 40^{o}C was conducted on resveratrol-enhanced peanut bars packaged in polyethylene bags (Ziplock, S.C. Johnson & Son, Inc., Racine, WI) using the scheme presented in Table 3.13. Samples were analyzed for trans-resveratrol, hexanal, total phenolics, TEAC, consumer acceptance and sensory descriptive analysis as described above in Item B.1 of this study.

Table 3.12 Sampling scheme for roasted resveratrol-enhanced peanuts stored at accelerated temperatures of 30, 35 and 40°C based on target shelf life of 90 days at 25°C using Q_{10} of 1.75 for lipid oxidation and verifed at 25°C

Storage Temperature (°C)	Sampling time, day number [1] (Estimated Shelf Life, ESL, %)[2]								No. of samples
Prediction:									
30		22 (0)	35 (19)	52 (44)	61 (57)	68 (67)	90 (100)	99 (113)	7
35			39 (0)	52 (15)	61 (43)	68 (56)	90 (100)	99 (118)	6
40				51 (0)	61 (25)	68 (43)	90 (100)	99 (123)	5
40		13 (0)	35 (56)	52 (100)					3
Verification:									
25	0 (0)	16 (18)	35 (39)	52 (58)	61 (68)	68 (76)	90 (100)	101(112)	8
Controls[3]:									
25	0 (0)			52 (58)					2
4				52 (58)					1
								Total	32

[1]The sampling time, day number, is the withdrawal time of sample from storage based on Day 0 at 25°C.
Samples at 30°C (Day 0 storage) was stored on Day 22 of 25°C. Estimated (predicted) end of shelf life at 30°C was 68 days.
Samples at 35°C (Day 0 storage) was stored on Day 39 of 25°C. Estimated shelf life at 35°C was 51 days.
Samples at 40°C (Day 0 storage) was stored on Day 51 at 25°C. Estimated shelf life at 45°C was 39 days.
Additional samples at 40°C (Day 0 storage) was stored on Day 13 of 25°C for quick estimation of shelf life for use a guide for adjusting the schedule of sample withdrawal at lower temperature, as needed.
[2]Numbers in parentheses indicate estimated shelf life in percent based on the estimated end of shelf life at the specified temperature.
[3]Controls are samples of roasted peanuts prepared from raw untreated peanuts.

Table 3.13 Sampling scheme for resveratrol-enhanced peanut bars stored at 25 and 40°C

Storage Temperature (°C)	**Sampling time, day [1] (Estimated Shelf Life, ESL, %)[2]**							**No. of samples**
Prediction:								
25	0 (0)	35 (39)	52 (58)	61 (68)	70 (77)	90 (100)	110 (122)	7
40			51 (0)	61 (26)	70 (48)	90 (100)	110 (120)	6
Control[3]:								
25	0 (0)		52 (58)					2
4			52 (58)					1
							Total	16

[1]The sampling time, day number, is the withdrawal time of sample from storage based on Day 0 at 25°C.
Samples at 40°C (Day 0 storage) was stored on Day 51 at 25°C. Estimated shelf life at 40°C was 39 days.
[2]Numbers in parentheses indicate estimated shelf life in percent based on the estimated end of shelf life at the specified temperature.
[3] Controls are peanut bars prepared from untreated roasted peanuts.

C. Sample Preparation

1. Roasted Resveratrol-Enhanced Peanuts

Raw REP were prepared using the optimal process parameters for the combined US-UV process determined previously: US processing for 10 min exposure time to US power density 70 mW/cm^3 followed by UV processing for 50 min at 40 cm distance from UV light and then incubation for 36 h at 25^oC. Raw REP were prepared in batches such that the samples were stored as per schedule in the sampling plan in Tables 3.12 and 3.13.

Raw peanuts (40 kg) were prepared for combined US-UV processing treatment as described previously in Study 3 except for the process parameters used. Sliced peanuts were prepared using optimal parameters for combined US-UV process of 70 mW/cm^3 US power density for 10 min followed by 50 min exposure at 40 cm distance from UV light and then incubated at 25^oC for 36 h. The incubated samples were dried and roasted as described previously in Study 1. The roasted REP were weighed, 320 g each, and packed in 1 quart (0.97 L) capacity Ziplock bags (17.78 cm x 19.69 cm, S.C. Johnson & Son, Inc., Racine, WI) according to the experimental design. Controls using untreated raw peanuts were also prepared. The roasted REP and controls were prepared in duplicate.

2. Resveratrol-Enhanced Peanut Bars

The ingredients for REP bar were weighed according to the formulation indicated in Table 3.14. Syrup was prepared by mixing refined sugar (Great Value, Walmart Stores, Inc., Bentonville, AR), light corn syrup (Karo, Best Food, Engelwood Cliffs, NJ), water, vegetable oil (Great Value, Walmart Stores, Inc., Bentonville, AR), and salt (Morton International Inc., Chicago IL) in a pan placed on top of an electric range (Kitchen Aid Model Superba, KitchenAid, Benton Harbor, MI) set to medium-high. The mixture was stirred continuously until

all solids were dissolved. Mixing was continued intermittently every 5 minutes until it started to caramelize at about 140^{o}C. Stove setting was set down to medium and stirring continued to a syrup temperature of 165^{o}C. The pan was removed from the stove top, while stirring of syrup was continued and temperature increased to 170^{o}C. The roasted REP were mixed with syrup thoroughly and transferred on the flattening board. The pin was rolled on top of the mixture to flatten the peanut-syrup mixture to about 0.5 cm thickness. The flattened mixture was cut with a knife to 10 cm long x 4 cm wide. A template was used to ensure uniform cut of bars. This resulted in approximately 50 grams per bar. The cut mixture was allowed to cool at room temperature. After cooling, the cut edges were bent to break into individual bars. The bars easily broke by bending with hands when adequately cooled. Three bars each were packed in Ziplock bags. Controls using untreated raw peanuts were also prepared. The roasted resveratrol-enhance peanut bars and controls were prepared in duplicate.

C. Sample Withdrawal and Analyses

Samples were withdrawn from storage incubators at pre-determined intervals according to the sampling schemes in Tables 3.12 and 3.13 and kept in walk-in freezers at -15^{o}C until analyzed. Samples withdrawn every sampling day were analyzed for hexanal analyses (Chu, 2003) on the day of sampling according to the procedure described below. Sampling scheme was modified based on the results of hexanal analyses and additional sampling times were performed for hexanal analysis toward the end of shelf life. Samples withdrawn every sampling day were also analyzed for *trans*-resveratrol (Potrebko and Resurreccion, 2009) after accumulation of at least 12 samples; and for consumer acceptance and descriptive tests at the end of sample withdrawals. Total phenolics (Singleton et al., 2009) and TEAC (Kim et al., 2002) were analyzed at initial (zero day storage) and at the end of shelf life. Control untreated samples

Table 3.14 Formulation for resveratrol-enhanced peanut bars

Ingredient	% in Formulation	Weight (g) for 1 k g formulation
Roasted resveratrol-enhanced peanuts	43.79	430.8
Water	25.86	258.6
Refined sugar	24.28	242.8
Light corn syrup	4.06	40.6
Oil	1.73	17.3
Industrial salt	0.26	2.6

were analyzed at initial at the end of shelf life of REP for all chemical and sensory analyses conducted for treated samples.

1. Chemical Analyses

Chemical analyses for *trans*-resveratrol, total phenolics, and TEAC were conducted following the procedures described previously in Study 1.

2. Hexanal Analysis Using Gas Chromatography

Hexanal, the secondary lipid oxidation product was analyzed at initial zero day and every sampling during storage of roasted REP and resveratrol-enhanced peanut bars bars at accelerated and ambient temperature conditions according to the conditions for hexanal analysis of Chu (2003), using 4-heptanone as internal standard.

2.1 Preparation of hexanal and heptanone (internal) standards

Stock solutions, 1000 ppm, each of hexanal and internal standard, 4-heptanone (Sigma, St Louis, MO, USA) were prepared separately by mixing 5 µL in 5 mL of canola oil (Great Value, Bentonville, AR, USA) and stored at 4^{o}C until used.

Working heptanone internal standard with concentration of 30 ppm was freshly prepared on the day of analysis by mixing 150 µL of 1000 ppm heptanone stock solution with 4.85 mL of canola oil.

Five calibration curve standard solutions, 5 mL each containing 20, 40, 60, 80 and 100 ppm of hexanal and fixed 30 ppm of 4-heptanone were prepared according to Table 3.15. The calibration curve standard solutions were placed in 10 mL Kimax cylindrical screw capped vials and sealed with a Teflon lined rubber septum (Supelco, 2-7451, 20 mm septa, ppfe/silicon, 1.5 mm thick, Supelco Bellefonte, PA, USA).

2.2 Sample preparation

About 30 g of peanut sample were ground in a coffee grinder to the smallest possible particles. Using analytical balance, 3.000 g of ground peanut was weighed into a 10 mL Kimax cylindrical screw capped vial, and spiked with 50 µL of 30 ppm 4-heptanone internal standard. The vial was sealed with a Teflon lined rubber septum and tapped to level off the surface of the sample.

2.3 Hexanal extraction

The sealed vial containing a calibration curve standard solution or a sample was placed into a Multi-Bloc heater (Lab-line Instruments, Inc., Melrose Park, IL, USA) set at 37^{o}C for 15 min to allow the volatiles to evaporate in the headspace. After 30 min, the rubber septum lining of the lid of the vial was initially punctured at the middle using a syringe needle used only for this purpose. The Solid Phase Micro Extractor (SPME) fiber, 100 µm Polydimethylsiloxane, coated fiber RED (Supelco, Bellfonte, Pa., USA) secured in a stainless steel needle (as purchased) was placed inside a SPME holder and dialed to 0.8 cm mark. The SPME needle was pushed through the pre-punctured septum of the vial and once it had gone through, it was dialed up to 1.6 mark then the SPME fiber was pushed down and locked into place. The standard or sample was heated for another 30 min to allow absorption of the volatiles in the vial headspace by the SPME fiber. After 30 min, the SPME fiber was unlocked, the needle was retracted to 0 cm mark and removed from the vial.

2.4 Hexanal analysis by gas chromatography

The needle with SPME fiber was dialed to 5 cm mark, pushed into the injection port of the HPLC, and immediately pushed down the SPME fiber and locked into place. The fiber was left

Table 3.15 Volumes of hexanal and 4-heptanone standard stock solutions used to prepare the five calibration curve standard solutions

Hexanal concentration, ppm	4-heptanone concentration, ppm	Volume of 1000 ppm hexanal, µL	Volume of 1000 ppm 4 - heptanone, µL	Volume of canola oil, µL
20	30	100	150	4750
40	30	150	150	4700
60	30	300	150	4550
80	30	450	150	4400
100	30	500	150	4350

in the HPLC for at least 5 min before removing from the machine. The fiber was held for 10 min at room before use in the next sample.

Hexanal analysis was performed using a gas chromatograph (Varian Chromopack CP-3800, Walnut, CA, USA) equipped with capillary column (Supelco Wax 10, 30 m x 0.25 m, 25 µm film, Supelco, Bellefonte, PA) using helium as the carrier gas. The initial oven temperature was set at 100°C and was programmed to increase at 10°C per min until it reached 220°C. The temperature of the injector was set at 220°C. The flow rate for helium was 1.2 mL/min. A flame ionization detector, set at temperature of 300°C was used to detect the peaks.

2.5 Calculations of hexanal concentrations

A total run time of 17 min was used for each standard or sample. Peak areas from the chromatographs were obtained for hexanal and heptanone with retention times of 3.59 ± 0.20 and 3.96 ± 0.03 min, respectively. Hexanal concentration in µg/g was calculated using the following equation (Chu, 2003).

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

Where: i = hexanal

IS = internal standard, heptanone

PA = area under the peak

3. Sensory Evaluation

The sensory properties and consumer acceptance of roasted REP were analyzed using descriptive analysis and consumer acceptance tests, respectively, as described in Study 1 except for the samples analyzed. Resveratrol-enhanced peanut bars were anayzed for their sensory

properties and consumer acceptance following the procedures for descriptive analysis and consumer acceptance tests, respectively, as described in Study 1 except for the samples and descriptive sensory ballot used as shown in Figure 3.7.

D. Prediction of Shelf Life

The results of hexanal of samples stored at 40°C were plotted at each sampling time immediately to predict the shelf life of the samples, and make necessary adjustments for sampling at lower temperatures of 25, 30 and 35°C based on kinetics using Arrhenius model described below. The predicted shelf life of roasted REP at 25°C was determined using shelf life model (Fu and Labuza, 2005) based on the shelf life at accelerated storage temperatures of 30, 35 and 40°C as described below.

1. Accelerated Shelf Life Test Using Arrhenius Model

1.1 Determination of order of reaction (n)

Loss of quality of most food can be represented by the following mathematical equation (Labuza 1984):

$$\frac{dA}{dt} = k[A]^n \qquad \text{Equation 1}$$

where:

A= quality factor measured, e.g., sensory rating, concentration of a compound

t = storage time

n = order of reaction

k = reaction rate constant, the slope of the plot of A or ln A against t.

Descriptive Test Ballot for Peanut Bars

Sample Code: _____

Panelist Code: _____ **Date: _____**

Appearance: Please look at sample as a whole and evaluate its **COLOR.**

Brown Color in Peanuts – the intensity of brown color from white to dark brown (**look at the cut side**)
References: white paper = 0; dry cardboard (L=55) = 42; chocolate syrup=150; WUP=27

_____|_____|_____

Brown Color in Caramel – the intensity of brown color from beige to brown (**look at the underside**)
References: white paper = 0; beige paper = 19; caramel = 75; chocolate syrup=150; WUP=68

_____|_____|_____

Aromatics: Please take at least ¼ portion of sample and evaluate for the following **AROMATICS.**
Caramel- the aromatic associated with caramel.
References: Kraft caramel candy= 65; WUP= 18

_____|_____|_____

Roasted peanutty- the aromatic associated with medium roasted peanuts.
References: light roasted peanut (L=55) =55; medium roasted peanuts (L=50)= 65; dark roasted peanut (L=45) =76; WUP=34

_____|_____|_____

Woody/hulls/skins – the aromatic associated with peanut skins and hulls.
References: peanut skins = 32; WUP = 5

_____|_____|_____

Burnt – the aromatic associated with very dark roast or burnt peanuts.
References: Burnt peanuts (L=30) = 73; Basic solutions; WUP = 19

_____|_____|_____

Figure 3.7 The ballot used for the descriptive test of resveratrol-enhanced peanut bars.

Oily– the aromatic associated with vegetable oil.
References: Vegetable oil = 24; WUP=2

_____|_____|_____

Fishy – the aromatic associated with cod liver oil or old fish.
References: Cod liver oil=78; WUP = 0

_____|_____|_____

Oxidized - the aromatic associated with rancid fats and oils.
References: oxidized peanuts =48; WUP = 18

_____|_____|_____

Painty- the aromatic associated with linseed oil.
References: Linseed oil (diluted) =58; Linseed oil (pure) =115; WUP=0

_____|_____|_____

Tastes: Please take at least ¼ portion of the sample and evaluate its **TASTES.**
Bitter - the taste on the tongue associated with bitter agents such as caffeine solution
References: bitter 20; bitter 50; bitter 100; WUP = 21

_____|_____|_____

Sweet – the taste on the tongue associated with sucrose solution
References: sweet 20; sweet 50; sweet 100; sweet 150; WUP = 106

_____|_____|_____

Sour- the taste on the tongue associated with acid solutions
References: sour 20; sour 50; sour 100; WUP = 6

_____|_____|_____

Salty - the taste on the tongue associated with sodium chloride solutions
References: salty 20; salty 50; salty 85; WUP = 17

_____|_____|_____

Figure 3.7 (continued)

Please take at least ¼ portion of the sample and evaluate its **TEXTURE and FEELING FACTORS**.

Texture:

First bite : Bite through a predetermined size of samples with incisors

Hardness – force required to bite through the sample.
References: almond = 110; WUP=93

_____|_____|_____

Fracturability– force with which the sample crumbles, cracks or shatters
References: graham cracker = 42; almond = 89; WUP=71

_____|_____|_____

Crunchiness – the force needed and amount of sound (lower pitch) generated from chewing a sample with molars.
References: corn chips = 75; medium roasted peanuts (L=50) = 40; WUP=93

_____|_____|_____

Chew down: Chew the sample with molars for a predetermined number of chews enough to mix sample with saliva to form a mass.

Cohesiveness of mass– degree to which samples hold together in a mass.
References: Baby Ruth = 89; Welch's fruit snack = 125; WUP= 68

_____|_____|_____

Chewiness– the force required to masticate the sample, at a constant rate of force application, to reduce it to a consistency suitable for swallowing.
References: Welch's fruit snack = 71; WUP=57

_____|_____|_____

Feeling factors:

Toothpack - the amount of sample left in or on teeth after chewing.
References: graham crackers= 75; WUP =87

_____|_____|_____

Astringent - the puckering or drying sensation on the mouth or tongue surface.
References: milk of magnesia = 40; grape juice=65; WUP=21

_____|_____|_____

Figure 3.7 (continued)

Most orders of reactions follow a zero order (n=0) or first order (n=1) (Labuza, 1984). The order of reaction, n at each accelerated storage temperature was determined by plotting A or ln A (dependent variable) on the y-axis versus t (independent variable) on the x-axis (Figure 3.7). Regression analyses for plots of A versus t and ln A versus t were conducted to determine which line was fitted best for the results. When R-square value is higher in plot of A versus t compared to plot of ln A versus t, the reaction order was determined to be zero. Otherwise, it was a first order reaction.

1.2 Calculation of reaction rate constant (k)

For zero order reaction, reaction rate constant, k at each accelerated storage temperature was determined from the slope of the plot of A versus t (Figure 3.8) or calculated by regression analysis using Equation 2. For first order reaction, k was determined from the plot of ln A versus time (Figure 3.8) or calculated by regression analysis using Equation 3.

$$A = c + kt \qquad \text{Equation 2}$$

$$\ln A = c + kt \qquad \text{Equation 3}$$

where:

A = quality factor measured

k = reaction rate constant or the slope of the line at each accelerated temperature

c = y-intercept

t = time

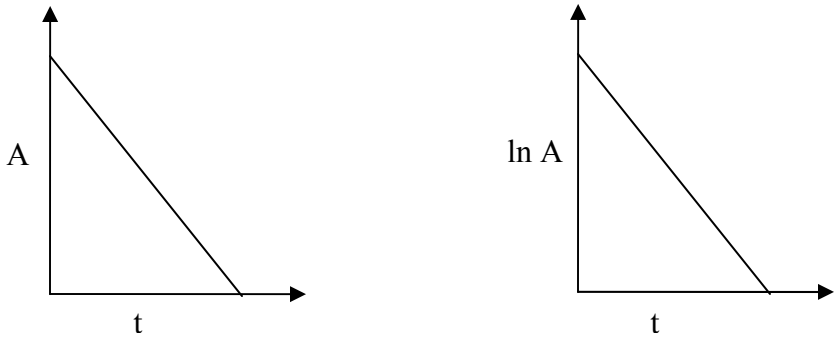


Figure 3.8 Plots of quality (A) against time (t) for zero order reaction and ln A against time for the first order reaction.

1.3 Calculation of Q_{10}, activation energy, E_A

Reaction rate constants, k were used to calculate the reaction rate constant (Labuza, 1984; Labuza and Schmidl, 1985).

a) Zero order reaction

For zero order reaction, Q_{10} was calculated using Equation 4:

$$Q_{10} = \frac{k \text{ at } T+10\ (^oC)}{k \text{ at } T(^oC)} \qquad \text{Equation 4}$$

where:

Q_{10} = the accelerating factor

k = slope of the line of the plot A against t, at temperature, T

T = storage temperature in ^oC

The Q_{10} obtained was used to calculate the activation energy, E_A, of the quality factor A using Equation 5, if E_A is expressed in kJ/mol K; and Equation 6 if expressed in kcal/mol K (Labuza, 1984):

$$\log Q_{10} = \frac{0.523\ E_A}{T\ (T+10)} \qquad \text{Equation 5}$$

$$\log Q_{10} = \frac{2.19\ E_A}{T\ (T+10)} \qquad \text{Equation 6}$$

where:

E_A = activation energy expressed in kJ/mol K or kcal/mol

T = storage temperature in K

b) First order reaction

For the first order reaction, an Arrhenius plot of ln k against the reciprocal of the absolute temperature (1/K) was constructed as shown in Figure 3.8, and the equation of the line was in the form:

$$\ln k = \ln k_o - \left(\frac{E_A}{R}\right)\left(\frac{1}{T}\right) \qquad \text{Equation 7}$$

where:

ln k = ln reaction rate constant or the slope of the line for each temperature from the plot ln A versus t

$\ln k_o$ = y-intercept

E_A = activation energy (J/mol)

R = universal gas constant, 8.314 J/mol K or 1.9872 cal/mol K

T = temperature in Kelvin (K)

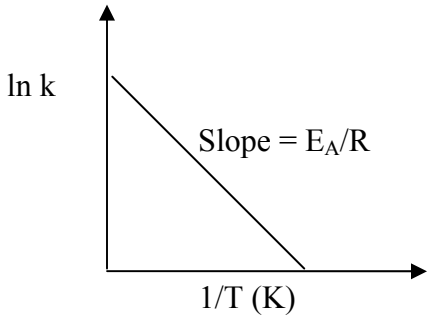


Figure 3.9 Arrhenius plot of reaction rate constants (ln k) against inverse absolute temperature (1/K).

The slope of the line was determined to calculate activation energy constant, E_A using the equation:

$$\text{Slope} = \frac{E_A}{R} \qquad \text{Equation 8}$$

2. Accelerated Shelf Life Test Using Shelf Life Model

The shelf life, θ, at 25°C was predicted using shelf life model based on the overall shelf life (end-point analysis) as a function of storage temperature (Figure 3.9; Fu and Labuza, 2005). The shelf life (days) at accelerated temperatures of 30, 35 and 40°C determined when the product's consumer overall acceptance rating was ≤ 5 or neither like nor dislike using a 9-point hedonic rating scale. Since the temperature range used is usually quite narrow, the following exponential relation exists between shelf life and storage temperature (Fu and Labuza, 2005):

$$\ln \theta = -bT + c \qquad \text{Equation 9}$$

where:

θ = shelf life (days) at temperature, T

b = slope of the plot of ln θ against T

T = storage temperature (^oC)

c = intercept

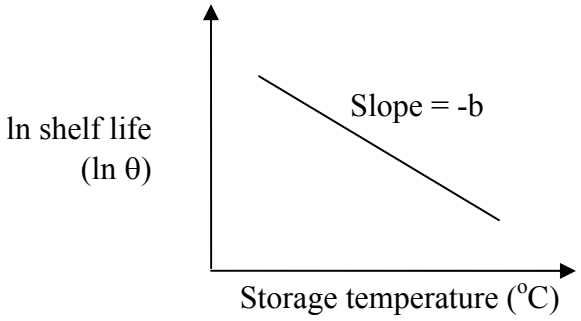


Figure 3.10 Shelf life plot showing the relation of ln shelf life and storage temperature (^oC).

The Q_{10} or the accelerating factor was calculated using the following equation (Labuza and Schmidl, 1985):

$$Q_{10}^{\Delta T/10} = \frac{\theta_1}{\theta_2} \qquad \text{Equation 10}$$

where:

θ_1 = shelf life (days) at lower temperature (^oC), T_1

θ_2 = shelf life (days) at higher temperature (^oC), T_2

$\Delta T = T_2 - T_1$ = difference in temperature

3. Verification of predicted shelf life

The predicted shelf life at 25^{o}C was verified by evaluating the samples stored at actual ambient conditions of about 25^{o}C. Verification samples were analyzed for *trans*-resveratrol, total phenolics, TEAC, consumer acceptance and descriptive sensory properties as described in Study 1, and for hexanal as described in this study.

4. Statistical analysis

Sensory data were collected from Compusense *five* software (Version 4.8, Compusense Inc., Guelph, Ontario, Canada). The data on chemical and sensory analyses were analyzed using SAS System v 8 (SAS, Institute, Cary, NC, 2001). The effects of storage time, and temperature on the levels of hexanal, *trans*-resveratrol, *trans*-piceid, ORAC, TEAC, and phenolic profile of roasted REP and peanut bars was evaluated by Analysis of Variance (ANOVA) at 5% level of significance using General Linear Models (PROC GLM). Fisher's least signicant difference mean separation test was performed to determine significant differences between samples at $P<0.05$.

V. STUDY 5 - PROFILING OF PHENOLIC COMPOUNDS AND SENSORY ATTRIBUTES OF PEANUTS TREATED WITH UV, ULTRASOUND AND COMBINED ULTRASOUND-UV PROCESSES

A. Goal of the Study

The goal of this study was to determine the correlation of phenolic compounds with the sensory attributes of resveratrol-enhanced peanuts (REP). The specific objectives were to (1) verify the peaks identified and quantified from representative UV and US-treated samples using liquid chromatography-mass spectrometry (LC-MS); (2) determine correlation of phenolic compounds and sensory attributes of UV, US, and combined US-UV treated peanuts.

B. LC-MS analysis

Identified peaks from representative peanut extracts of UV- and US-treated peanut samples were verified by analyzing their mass spectra. The HPLC unit was attached to a Perkin-Elmer Sciex API I Plus (Ontario, Canada) with an electrospray ion source. The eluant stream was split so that 23 (L/min went to the mass spectrometer. The mass spectrometry was operated in

negative mode at -3500 V and OR at -60V. The scan range was 145-685 m/z with a 0.2 m/z step size, and 2.0 msec dwell.

C. Profile of Phenolic Compounds in Resveratrol-Enhanced Peanuts

The profiles of phenolic compounds including *trans*-resveratrol, *trans*-piceid, *p*-coumaric acid, caffeic acid, and ferulic acid in peanuts treated with UV, US, and combined US-UV were obtained using the reverse –HPLC method of Francisco and Resurreccion (2009b) for profiling of phenolic compounds as described previously in the methods for Study 3.

D. Profile of Sensory Attributes of Resveratrol-Enhanced Peanuts

The profiles of the sensory attributes of peanuts treated with UV, US, and combined US-UV were determined using descriptive analysis tests as described previously in the methods for Study 1.

E. Statistical Evaluation of the Results of Sensory and Chemical Analyses of UV and Ultrasound Treated Peanuts

All data on the profiles of phenolic compounds and descriptive sensory attributes were statistically evaluated using PROC COR program in SAS. Correlations between phenolic compounds and sensory attributes were determined.

SECTION 4

RESULTS AND DISCUSSION

I. STUDY 1. ULTRAVIOLET LIGHT (UV) PROCESS OPTIMIZATION TO ENHANCE *TRANS*-RESVERATROL BIOSYNTHESIS IN PEANUTS

A. Effect of UV Treatment on the Concentrations of *Trans*-Resveratrol, *Trans*-Picied and Total Stilbenes in Peanut Kernels

1. *Trans*-resveratrol, *trans*-picied and total stilbenes concentrations in sliced UV-treated peanuts

Trans- rather than the *cis*- forms of the stilbenes were analyzed in this study due to their higher stability and abundance in nature (Trela and Waterhouse, 1997). The mean concentrations of *trans*-resveratrol, *trans*-piceid and total stilbenes of UV treated peanuts are shown in Table 4.1. UV increased ($P<0.05$) concentrations of resveratrol, range=0.54 $\pm$ 0.05 to 3.30 $\pm$ 0.58 µg/g, and total stilbenes, 0.66 $\pm$ 0.04 to 4.00 $\pm$ 0.07 µg/g, but not *trans*-piceid, of 27 treated peanuts compared to controls with 0.02 $\pm$ 0.002 and 0.05 $\pm$ 0.004 µg/g, respectively which correspond to 27-165 and 13-80 fold increase. *Trans*-piceid concentrations were increased ($P<0.05$) by UV exposure in only 4 of 27 treatments, with levels ranging from 0.35 $\pm$ 0.02 to 1.05 $\pm$ 0.78 µg/g compared to 0.03 $\pm$ 0.001 µg/g in raw whole untreated peanuts resulting in a 12-35 fold increase. The 4 treatments included exposure to UV at 40 cm distance from UV light for 10 min and incubated for 24 and 48 h; and for 30 min at the same distance from UV light then incubated for 24 and 36 h for which no trend was identified, while the other 23 treatments did not significantly differ from controls suggesting that UV was not effective in increasing *trans*-piceid in sliced peanuts. Thus, the contribution to total stilbenes is mostly from *trans*-resveratrol with only small

Table 4.1 Concentrations (mean ± standard deviation, dry basis) of *trans*-resveratrol, *trans*-piceid, and total stilbenes of roasted sliced UV-treated peanuts and controls[1]

Treat-ment #	ID	IT	IC	Resveratrol μg/g	Piceid μg/g	Total Stilbenes μg/g
1	20	10	24	2.2443 ± 0.1894cd	0.2584 ± 0.0208cd	0.3858 ± 0.0518cd
2	20	10	36	1.9579 ± 0.4861d	0.1904 ± 0.0417cd	2.1483 ± 0.5249d
3	20	10	48	0.7836 ± 0.3075ef	0.1388 ± 0.0213cd	0.9224 ± 0.3206efgh
4	20	20	24	0.6478 ± 0.0506f	0.1427 ± 0.0162cd	0.7904 ± 0.0483gh
5	20	20	36	0.6665 ± 0.1753f	0.1131 ± 0.0267cd	0.7797 ± 0.1955gh
6	20	20	48	0.5378 ± 0.0680f	0.1223 ± 0.0274cd	0.6601 ± 0.0416h
7	20	30	24	0.5554 ± 0.1160f	0.1632 ± 0.0315cd	0.7185 ± 0.1444gh
8	20	30	36	0.6402 ± 0.1176f	0.1521 ± 0.0403cd	0.7923 ± 0.1387gh
9	20	30	48	0.6686 ± 0.0100f	0.1345 ± 0.0412cd	0.8031 ± 0.0365gh
10	40	10	24	0.8883 ± 0.2216ef	0.3462 ± 0.1037cd	1.2346 ± 0.3103ef
11	40	10	36	0.7746 ± 0.0883ef	0.2405 ± 0.0557cd	1.0150 ± 0.0556efgh
12	40	10	48	0.8835 ± 0.3318ef	0.3498 ± 0.1521cd	1.2333 ± 0.4804ef
13	40	20	24	0.8077 ± 0.1478ef	0.3015 ± 0.0472cd	1.1091 ± 0.1895efg
14	40	20	36	2.4662 ± 0.4687bc	0.2455 ± 0.0420cd	2.7116 ± 0.4636c
15	40	20	48	2.6491 ± 0.3638b	0.2079 ± 0.0225cd	2.8570 ± 0.3859c
16	40	30	24	2.4254 ± 0.6579bc	1.0515 ± 0.7751a	3.4769 ± 0.4788b
17	40	30	36	3.2985 ± 0.5785a	0.6974 ± 0.6270b	3.9958 ± 0.7424a
18	40	30	48	1.9954 ± 0.4858d	0.1977 ± 0.0677cd	2.1931 ± 0.4997d
19	60	10	24	0.8553 ± 0.1139ef	0.1330 ± 0.0075cd	0.9883 ± 0.1107efgh
20	60	10	36	0.6555 ± 0.0375ef	0.1617 ± 0.0322cd	0.8172 ± 0.0595fgh
21	60	10	48	0.7990 ± 0.1475e	0.1655 ± 0.0438cd	0.9645 ± 0.1476efgh
22	60	20	24	1.1265 ± 0.2008f	0.1842 ± 0.0931cd	1.3107 ± 0.2934e
23	60	20	36	0.8281 ± 0.1724ef	0.1473 ± 0.0426cd	0.9754 ± 0.2135efgh
24	60	20	48	0.7022 ± 0.1517f	0.1791 ± 0.0383cd	0.8813 ± 0.1647efgh
25	60	30	24	0.7713 ± 0.0722ef	0.1862 ± 0.0450cd	0.9574 ± 0.0300efgh
26	60	30	36	0.7185 ± 0.0271f	0.1282 ± 0.0165cd	0.8467 ± 0.0367fgh
27	60	30	48	0.6561 ± 0.0466f	0.1677 ± 0.0437cd	0.8238 ± 0.0511fgh
Control[2]	120	0	0	0.0212 ± 0.0020g	0.0271 ± 0.0010d	0.0482 ± 0.0046i

[1] ID = distance from UV light (cm), IT = UV exposure time (min), and IC = incubation time (h) at 25°C.

Means ± standard deviation were calculated from two replications for a total of 4 analyses per sample each for *trans*-resveratrol, *trans*-piceid and total stilbenes. Means in the same column not followed by the same letter are significant ($P<0.05$) from each other as determined by Fisher's least significant difference mean separation test.

[2] Control is untreated raw whole peanuts.

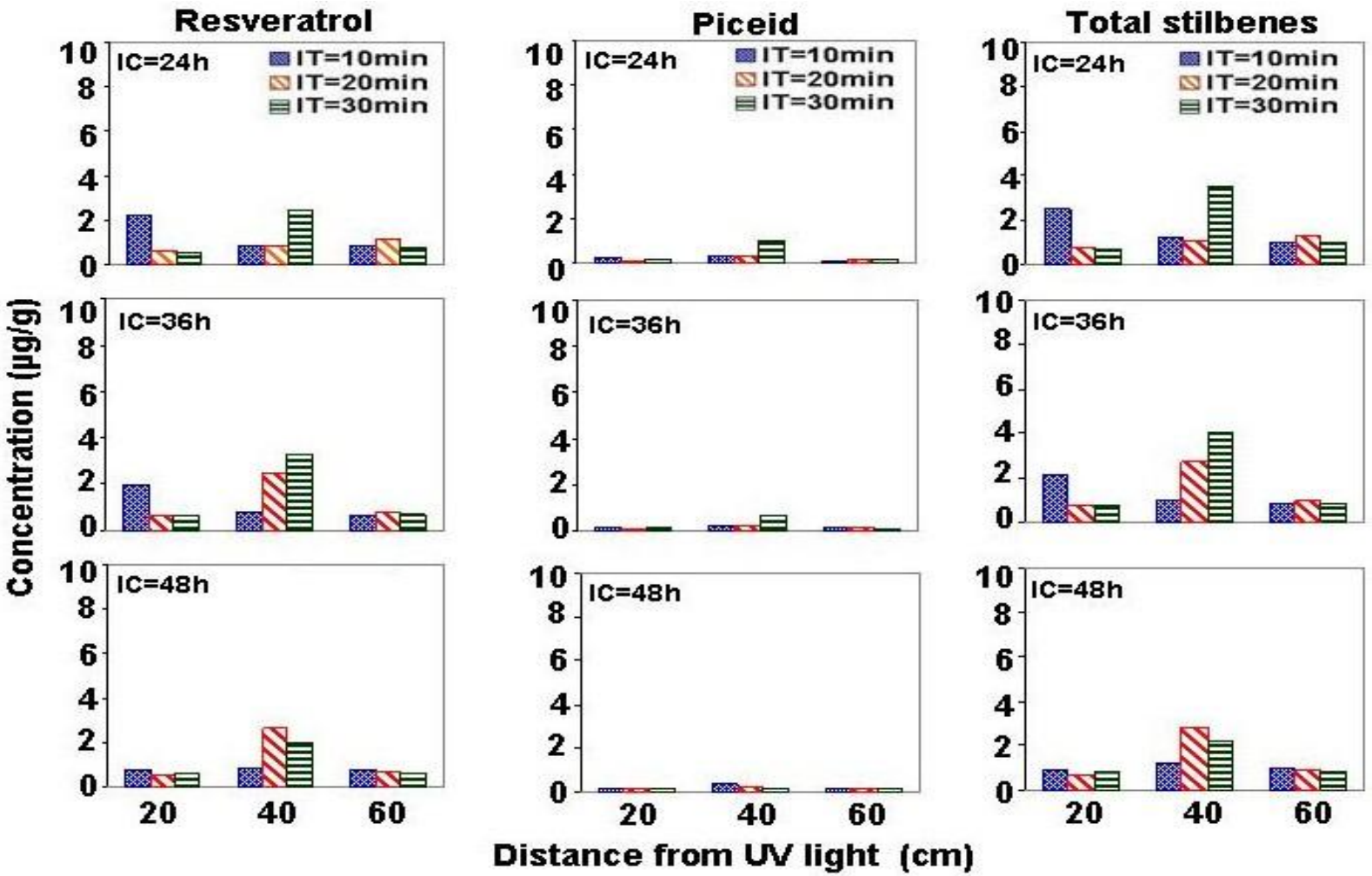


Figure 4.1 Mean *trans*-resveratrol, *trans*-piceid, and total stilbenes of sliced peanuts exposed to varying doses of UV.
IC is the incubation time at 25°C and IT is the UV exposure time. Control untreated raw whole peanuts had 0.02 ± 0.002 µg/g *trans*-resveratrol, 0.05 ± 0.001 *trans*-piceid µg/g and 0.05 ± 0.001 µg/g total stilbenes.

amounts from *trans*-piceid (Figure 4.1). UV induces increase in enzymes responsible for the 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) causing increased *trans*-resveratrol in UV-treated peanuts. UV light stimulated the coordinate induction of phenylammonia lyase, the enzyme that catalyzes the first step in ρ-coumaroyl CoA production from phenylalanine, and enzymes of stilbene synthesis in the leaves of the vine (Fritzemeier and Kindl, 1981 in Soleas et al., 1997).

Trans-resveratrol in controls were less than 0.29 to 0.48 μg/g found by Rudolf and Resurreccion (2005; 2007) but within 0.02-0.31μg/g analyzed 14 of 15 cultivars of three market types of raw peanuts (Sanders et al., 2000). Sanders et al. (2000) reported 1.79 μg/g *trans*-resveratrol only for a small white Spanish cultivar and therefore, we did not include it as basis for comparison. The highest ($P<0.05$) *trans*-resveratrol of 3.30 μg/g and 4.00 μg/g total stilbenes were achieved after 30 min UV treatment at 40 cm distance from light and incubated for 36h. Rudolf and Resurreccion (2005) obtained similar maximum *trans*-resveratrol of 3.42 μg/g in UV-treated peanuts for 10 min at 40 cm distance from UV light and incubated for 48 h.

Analysis of variance, ANOVA (Table 4.2) showed that all main effects of distance from UV light, UV exposure time and incubation time and their interactions significantly contributed to the concentrations of *trans*-resveratrol and total stilbenes in UV-treated peanuts. Except for the interactions of UV exposure time with either distance from UV light and incubation time, all main and interaction effects significantly affect the concentrations of *trans*-piceid in UV treated peanuts. Due to interaction effects, no factor used in the experiment could be singled out as the most significant contributor to the concentrations of *trans*-resveratrol, *trans*-piceid, and total stilbenes.

Table 4.2 Significant treatment effects on the concentrations of stilbenes, total phenolics, antioxidant capacities, and overall acceptance of sliced UV-treated peanuts.

Factors[1]	Trans-resveratrol	Trans-piceid	Total Stilbenes	Total Phenolics	TEAC	H-ORAC	L-ORAC	TAC	Overall Acceptance
Sliced peanuts									
Main effects									
ID	<0.0001	<0.0001	<0.0001	NS	NS	<0.0001	<0.0001	<0.0001	NS
IT	0.0069	0.0132	<0.0001	NS	NS	NS	NS	NS	NS
IC	0.0006	0.0351	0.0002	NS	NS	0.0044	0.0008	0.0006	<0.0001
Interactions									
ID x IT	<0.0001	NS	<0.0001	NS	NS	0.0222	<0.0128	NS	NS
ID x IC	<0.0001	0.0014	<0.0001	NS	NS	<0.0001	NS	<0.0001	0.0204
IT x IC	<0.0001	NS	<0.0001	NS	NS	0.0033	0.0106	<0.0001	0.0266
ID x IT x IC	<0.0001	0.0488	<0.0001	NS	NS	0.0003	0.0033	<0.0001	NS

[1]ID= distance from UV light ; IT=UV exposure time; IC=incubation time at 25^{o}C.
NS = not significantly different (P>0.05)

UV-treated peanuts had higher *trans*-resveratrol, *trans*-piceid and total stilbenes compared to blended peanut butters with 0.41±0.02, 0.13±0.01, and 0.54±0.03 µg/g, respectively, and 100% natural peanut butters with 0.65±0.02, 0.14±0.01, and 0.814±0.03 µg/g, respectively (Ibern-Gomez et al., 2000).

The amounts of *trans*-resveratrol of 0.54 - 3.30 µg/g produced in UV-treated peanuts were within the concentrations of 0.60 to 8.00 (mean=2.48) µg/mL in 18 Spanish red wines (Lamuella-Raventos et al. (1995) and higher those found in white and red grape juices with not detectable to 0.9 (mean=0.05) and not detectable to 1.09 (mean=0.5) µg/mL, respectively (Romero-Perez et al., 1999); dark chocolate and cocoa liquor with 0.40 and 0.50 µg/g, respectively (Counet et al., 2006); pistachios with 0.07-0.18 (mean=0.12) µg/g (Grippi et al., 2008); hops with 0.7-2.2 µg/g (Jerkovic and Collin, 2007); and hop pellets with 0.5 µg/g (Callemien et al., 2005). The levels of *trans*-resveratrol in UV-treated peanuts were lower compared to those in dried white and red grape berry skins with 11.04-47.60 (mean= 22.03) µg/g and 18.32-38.26 (mean=25.79) µg/g, respectively, (Romero-Perez et al., 2001).

The *trans*-piceid concentrations of 0.35-1.05 µg/g obtained by UV treatment of sliced peanuts were higher compared to white grape juices with non-detectable to 0.83 (mean=0.18) µg/mL (Romero-Perez et al., 1999); similar to 1.00 and 1.20 µg/g in dark chocolate and cocoa liquor, respectively (Counet et al., 2006); and within the lower limit of 0.74 – 4.01 (mean=1.85) µg/mL in 18 Spanish red wines (Lamuella-Raventos et al., 1995). The concentrations of *trans*-piceid in UV-treated peanuts were lower than in pistachios with 6.2-8.15 (mean=6.97) µg/g (Grippi et al., 2008); hops with 2.3-7.3 µg/g (Jerkovic and Collin, 2007); hop pellets with 2.0 µg/g (Callemien et al., 2005); dried white and red grape skins with non-quantifiable to 64.41 (mean=16.58) and 5.49-342.66 µg/g, respectively; and red grape juices with 0.77-7.34 µg/mL (Romero-Perez et al., 1999).

B. Effect of UV Treatment on the Total Phenolics Concentrations and Antioxidant Capacities of Sliced Peanut Kernels

The First International Congress on Antioxidant Methods was convened in June 2004 in Orlando, FL for the purpose of dealing with analytical issues relative to assessing antioxidant capacities in foods, botanicals, nutraceuticals, and other dietary supplements and proposing one or more analytical methods that could be standardized for routine assessment of antioxidant capacity. Based on the evaluation of data presented at the congress and in the literature, as well as consideration of potential end uses of antioxidants, it was proposed that procedures and applications for three assays be considered for standardization: the oxygen radical absorbance capacity (ORAC) assay, the Folin-Ciocalteu method, and the Trolox equivalent antioxidant capacity (TEAC) assay (Prior et al., 2005). ORAC represents a hydrogen atom transfer reaction mechanism, which is most relevant to human biology (Prior et al., 2005). The Folin-Ciocalteu method is an electron transfer based assay and gives reducing capacity, which has normally been expressed as phenolic contents. The TEAC assay represents a second electron-transfer based method. These three methods were used in this study to evaluate the antioxidant capacities of treated peanuts.

1. Total phenolics concentrations in sliced UV-treated peanuts

Figure 4.2 and Table 4.3 show the mean concentrations of total phenolics in UV-treated sliced peanuts. UV increased ($P<0.05$) the total phenolics of treated sliced peanuts to 1.38-1.82 mg GAE/g whereas controls of raw whole peanuts had only 0.84 mg GAE/g. At 24 and 48 h incubation times, the total phenolics of UV-treated peanuts were not different ($P<0.05$) from each other. At 48 h incubation time, peanuts treated at 40 cm distance from UV light for 10 min

Table 4.3 Concentrations (mean ± standard deviation, dry basis) of total phenolics, trolox equivalent antioxidant capacity (TEAC), and oxygen radical absorbance capacity (ORAC) total antioxidant capacity (TAC) of roasted sliced UV-treated peanuts and controls[1]

Treat-ment #	ID	IT	IC	Total Phenolics mg GAE/g	TEAC μM TE/g	H-ORAC μM TE/g	L-ORAC μM TE/g	TAC-ORAC μM TE/g
1	20	10	24	1.58 ± 0.08abc	3.05 ± 0.38a	29.09 ± 6.98efg	20.51 ± 3.21ab	49.60 ± 9.98defg
2	20	10	36	1.43 ± 0.42bc	2.84 ± 0.58a	30.58 ± 6.94defg	17.18 ± 2.41abcd	47.76 ± 8.61efg
3	20	10	48	1.63 ± 0.11abc	3.14 ± 0.4a	33.56 ± 9.83cdefg	19.88 ± 3.92abc	53.44 ± 6.66def
4	20	20	24	1.66 ± 0.09abc	3.33 ± 0.41a	35.19 ± 7.29cdef	15.48 ± 2.47cdefg	50.67 ± 9.19defg
5	20	20	36	1.62 ± 0.02abc	3.28 ± 0.38a	36.64 ± 7.74cdef	18.33 ± 5.41abcd	54.97 ± 8.60de
6	20	20	48	1.51 ± 0.11bc	3.27 ± 0.38a	27.30 ± 8.31efg	15.22 ± 2.98cdefgh	42.52 ± 8.17efgh
7	20	30	24	1.56 ± 0.12abc	3.28 ± 0.44a	33.66 ± 4.82cdefg	14.78 ± 3.24defghi	48.44 ± 7.90efg
8	20	30	36	1.59 ± 0.29abc	3.34 ± 0.45a	31.09 ± 3.70defg	13.74 ± 3.50defghij	44.83 ± 6.28efgh
9	20	30	48	1.53 ± 0.02abc	3.26 ± 0.44a	28.54 ± 8.80efg	17.48 ± 6.02abcd	46.02 ± 2.86efgh
10	40	10	24	1.54 ± 0.19abc	3.37 ± 0.37a	65.69 ± 19.01a	16.12 ± 2.24bcde	81.81 ± 17.00ab
11	40	10	36	1.38 ± 0.37c	2.92 ± 0.63a	42.89 ± 11.71bcd	9.37 ± 2.78jklm	52.26 ± 12.34defg
12	40	10	48	1.38 ± 0.17c	3.24 ± 0.49a	45.01 ± 5.20bc	17.20 ± 4.66abcd	62.21 ± 6.70cd
13	40	20	24	1.62 ± 0.06abc	3.19 ± 0.47a	36.98 ± 5.79cdef	14.53 ± 3.08defghi	51.52 ± 7.88defg
14	40	20	36	1.69 ± 0.02ab	2.89 ± 0.81a	29.44 ± 5.94efg	13.60 ± 3.62defghij	43.04 ± 6.20efgh
15	40	20	48	1.57 ± 0.11abc	2.89 ± 0.60a	50.99 ± 10.51b	21.31 ± 7.03a	72.30 ± 5.81bc
16	40	30	24	1.82 ± 0.50a	3.37 ± 0.48a	78.59 ± 24.07a	21.43 ± 3.54a	100.02 ± 24.44a
17	40	30	36	1.57 ± 0.08abc	3.33 ± 0.49a	38.72 ± 4.00bcde	13.67 ± 1.76defghij	52.38 ± 4.95defg
18	40	30	48	1.62 ± 0.11abc	2.97 ± 0.55a	35.87 ± 9.81cdef	10.31 ± 2.76ijklm	46.18 ± 10.49efg

Table 4.3 continued…

Treat-ment #	ID	IT	IC	Total Phenolics mg GAE/g	TEAC μM TE/g	H-ORAC μM TE/g	L-ORAC μM TE/g	TAC-ORAC μM TE/g
19	60	10	24	1.58 ± 0.10abc	2.91 ± 0.53a	39.00 ± 11.27bcde	11.38 ± 3.39efghij	50.38 ± 12.22defg
20	60	10	36	1.63 ± 0.03abc	3.05 ± 0.61a	30.18 ± 6.95defg	10.60 ± 3.46hijkl	40.77 ± 4.05fgh
21	60	10	48	1.58 ± 0.11abc	3.03 ± 0.57a	29.44 ± 5.97efg	15.66 ± 2.57cdef	45.09 ± 7.60efgh
22	60	20	24	1.54 ± 0.09abc	3.19 ± 0.44a	24.95 ± 5.76fg	7.69 ± 2.81klm	32.65 ± 3.57hi
23	60	20	36	1.47 ± 0.20bc	3.33 ± 0.53a	42.84 ± 9.33bcd	6.51 ± 1.59m	49.35 ± 10.56defg
24	60	20	48	1.50 ± 0.06bc	3.26 ± 0.51a	32.06 ± 9.44cdefg	11.17 ± 3.09fghijk	43.22 ± 12.37efgh
25	60	30	24	1.62 ± 0.52abc	2.95 ± 0.81a	28.68 ± 8.03efg	11.36 ± 2.887efghijk	40.04 ± 9.25abfghi
26	60	30	36	1.50 ± 0.12bc	3.28 ± 0.50a	30.84 ± 6.37defg	8.38 ± 1.67klm	39.21 ± 5.93ghi
27	60	30	48	1.54 ± 0.09abc	3.16 ± 0.59a	31.73 ± 3.24defg	10.68 ± 1.10ghijkl	42.41 ± 3.14efgh
Control[2]	120	0	0	0.84 ± 0.02d	0.61 ± 0.01b	21.20 ± 5.30g	5.61 ± 0.46m	26.81 ± 5.72i

[1] ID = distance from UV light (cm), IT = UV exposure time (min) and IC = incubation time (h) at 25°C.
Means were calculated from two replications with two extractions of each replication. Means in a column not followed by the same letter are significantly different from each other at $P < 0.05$ as determined by Fisher's significant difference mean separation test.
H-ORAC= hydrophilic; L-ORAC = lipophilic ORAC; TAC-ORAC = Total Antioxidant Capacity = H-ORAC + L-ORAC.
[2] Control is untreated raw whole peanuts.

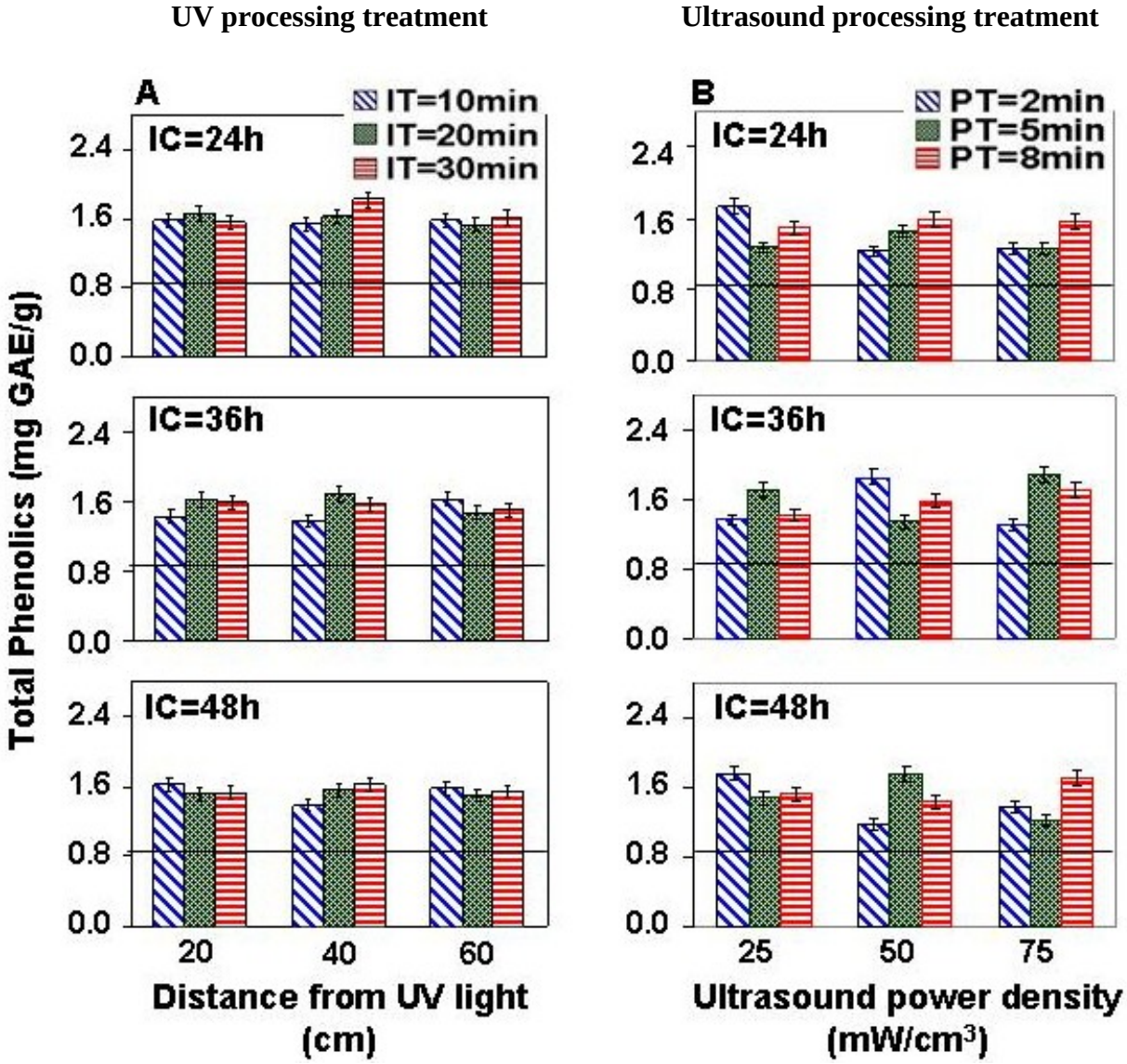


Figure 4.2 Mean concentrations for total phenolics (mg GAE/g) of sliced peanuts exposed to varying doses of UV (A) and ultrasound (B). IC is the incubation time at 25^{o}C, IT is the UV exposure time, and PT is the ultrasound exposure time. Control raw peanuts had total phenolics of 0.84 $\pm$ 0.02 mg GAE/g and represented on each graph by a horizontal line. Means were calculated from 2 replications for a total of four analyses per sample.

had higher (P<0.05) total phenolics than those treated for 10 min, but not from the remaining seven treatments which were not significantly different from each other.

ANOVA (Table 4.2) indicated that neither main effects nor interaction effects of the factors used in this experiment contributed significantly on the total phenolics concentrations of UV-treated samples. This finding agrees with Rudolf and Resurreccion (2005) who did not find significant differences in total phenolics of peanuts incubated for 24 to 48 h after exposure to UV for 10 min at 40 cm distance from UV light. In contrast, Duh and Yen (1995) reported a decrease in total phenolics of the methanol extract of peanut hulls as UV exposure time increased from 0 to 6 days due to the different biological system used and also their longer exposure times of days compared to only minutes in our study. Studies on grapes showed that the phenolic compound, resveratrol, was induced by UV particularly in the skins of berries and only trace amounts in the fruit flesh (Becker et al., 2003) suggesting the limited UV penetration in the flesh. In our study, UV light may not have penetrated the interiors of sliced peanut kernels and whatever concentrations of phenolics produced on its surfaces after treatment remained unchanged during incubation. The enzymes responsible for biosynthesis of phenolic compounds should be activated by UV to cause their formation in plants (Soleas et al., 1997). UV treatment was also reported to cause increase in total phenolics contents of fresh cut mangoes (Gonzales-Aguilar et al., 2007), strawberries (Erkan et al., 2008), and blueberries (Wang et al., 2008).

The total phenolics of UV-treated peanuts of 1.38-1.82 mg GAE/g were slightly higher than 0.91-1.14 mg/g in raw peanuts (Talcott et al., 2005). Our observed values were within the total phenolics concentrations of blueberry wines with 0.60-1.86 mg GAE/g (Sanchez-Moreno, et al., 2003), and within the lower limits of 1.81-4.58 mg GAE/g in fresh blueberry purees (Prior et al., 1998) and 1.31-2.39 mg GAE/mL in red wines (Fernandez-Pachon et al., 2004).

2. Trolox equivalent antioxidant capacity (TEAC) in sliced UV-treated peanuts

The mean concentrations of TEAC in UV-treated peanuts are presented in Figure 4.3 and Table 4.3. UV increased ($P< 0.05$) TEAC, a measure of antioxidant capacity, in all 27 treated peanuts to 2.84-3.37 μmol TE/g compared to 0.61 μmol TE/g in untreated controls. However, all UV-treated peanuts were not significantly different from each other suggesting that all UV treatments used will produce similar antioxidant activity. ANOVA (Table 4.2) indicated the main effects of distance from UV light, UV exposure time, and incubation time and their interactions did not contribute ($P< 0.05$) to the antioxidant capacities by TEAC of UV-treated peanuts which disagreed with Rudolf and Resurreccion (2005*)* who found that antioxidant capacities of UV treated sliced peanuts decreased ($P< 0.05$) after 24 and 36 h incubation, then increased after 48h to levels not different ($P< 0.05$) from those at 0h. This difference in results is mainly due to the different reaction mechanisms used in the analytical methods used to measure antioxidant capacity. The thiocyanate method of Osawa and Namiki used by Rudolf and Resurreccion (2005) measures the percent inhibition of oxidation of linoleic acid relative to control whereas the TEAC assay of Kim et al. (2002) that we used in the analysis measures the ABTS radical scavenging activity.

3. Oxygen radical antioxidant capacity (ORAC) in sliced UV-treated peanuts

3.1 Total Antioxidant Capacity in sliced UV-treated peanuts

The total antioxidant capacity is the sum of the hydrophilic and lipophilic antioxidants (Wu et al., 2004). The antioxidants in food are mostly hydrophilic in nature. However, it is important to measure the lipophilic antioxidants in peanuts due to its high fat content of about 50%. The oxygen radical absorbance capacity (ORAC) assay, analyzes both hydrophilic and lipophilic antioxidants.

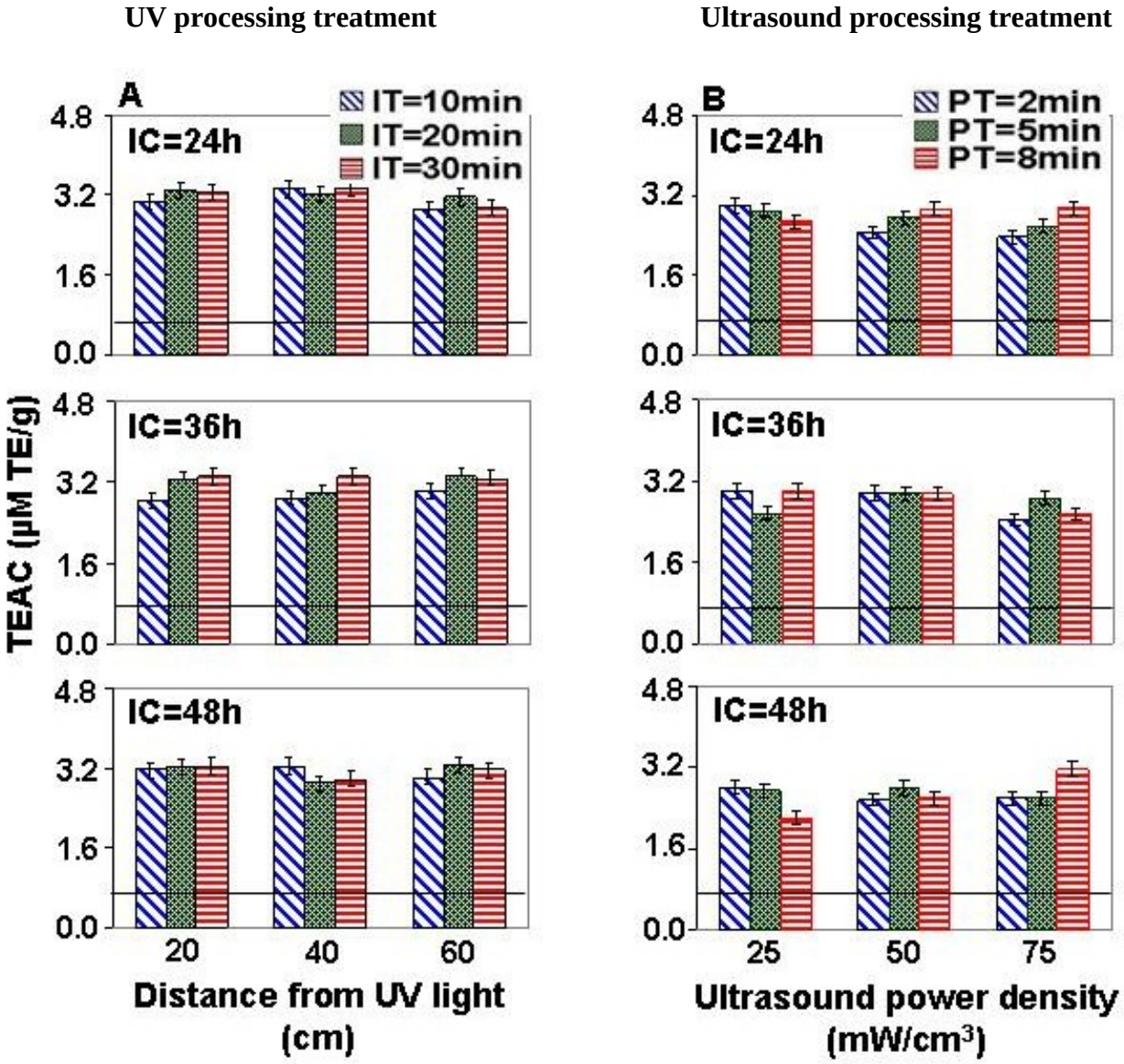


Figure 4.3 Mean trolox equivalent antioxidant capacities (TEAC, µM Trolox Equivalents/g) of sliced peanuts exposed to varying doses of UV (A) and ultrasound (B). IC is the incubation time at 25°C, IT is the UV exposure time, and PT is the ultrasound exposure time. Control raw peanuts had TEAC value of 0.66±0.003 µM TE/g and represented on each graph by a horizontal line. Means were calculated from 2 replications for a total of four analyses per sample.

The mean concentrations of ORAC total antioxidant capacity are shown in Table 4.3 and Figure 4.4. UV increased (P<0.05) the ORAC total antioxidant capacity (TAC) in 24 of 27 treated peanuts which ranged from 32.65-100.20 μM TE/g compared to controls with 26.81 μM TE/g. The three treatments (#22, 25 and 26) that did not differ (P<0.05) from controls received the lowest doses of UV and shorter incubation time. These were treated at 60 cm from UV light for 20 and 30 min then incubated for 24 h, and for 30 min at 36 h incubation time. The highest (P<0.05) total antioxidant capacity of 100.20 μM TE/g was produced a distance from UV light of 40 cm after exposure for 30 min and incubation for 24h.

The total antioxidant capacity increased as distance from UV light increased from 20 to 40 cm and then decreased at 60 cm, a trend similar to that observed in total phenolics. Likewise, UV-C treatment increased the antioxidant capacity of fresh-cut mangoes, with increasing ORAC and DPPH radical values as UV irradiation time increases (Ayala-Zabala et al., 2007). UV treatment of strawberries for 5 and 10 min produced the highest ORAC values (Erkan et al., 2008). Optimum UV-C doses for enhancing antioxidant capacities and phytochemical contents in blueberries was found to be 2.15 and 4.30 kJ m^{-2}, respectively (Wang et al., 2009).

ANOVA (Table 4.2) showed except for main effect of UV irradiation time and its interaction with distance from UV light, all other main and interaction effects significantly contributed to the total antioxidant capacity of UV-treated peanuts. Due to interaction effects, the main factors of distance from UV light and incubation time cannot singled out as the major contributors to the total antioxidant capacities of UV-treated peanuts.

3.2 H-ORAC in sliced UV-treated peanuts

The H-ORAC of UV-treated peanuts ranged from 24.95-78.59 μM TE/g whereas controls had 21.20 μM TE/g (Table 4.4 and Figure 4.5). Twelve of 27 treatments had significantly

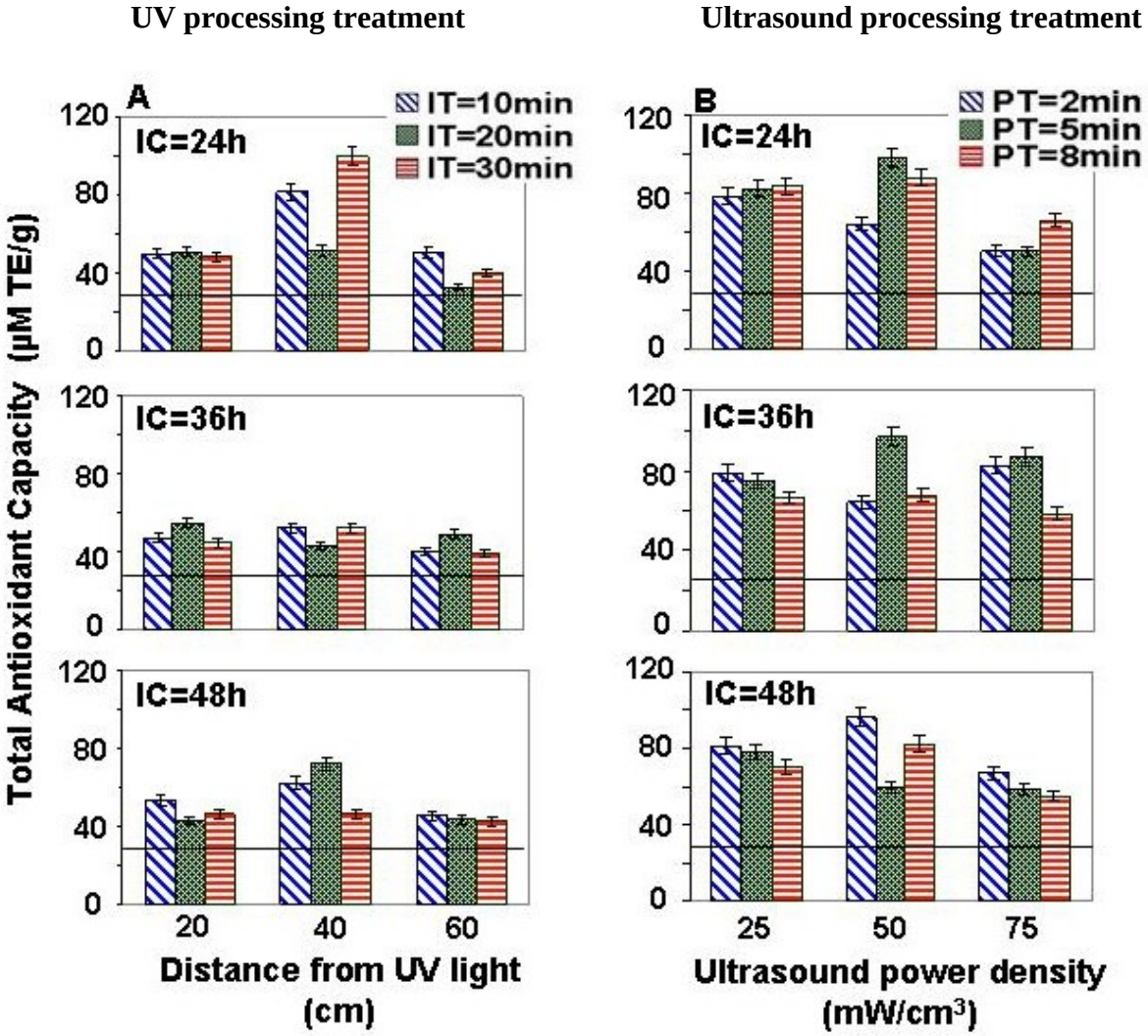


Figure 4.4 Mean total antioxidant capacities (TAC-ORAC, µM Trolox Equivalents/g) of sliced peanuts exposed to varying doses of UV (A) and ultrasound (B). IC is the incubation time at 25°C, IT is the UV exposure time, and PT is the ultrasound exposure time. Control raw peanuts had TAC of 26.81±5.70 µM TE/g and represented on each graph by a horizontal line. Means were calculated from 2 replications for a total of four analyses per sample.

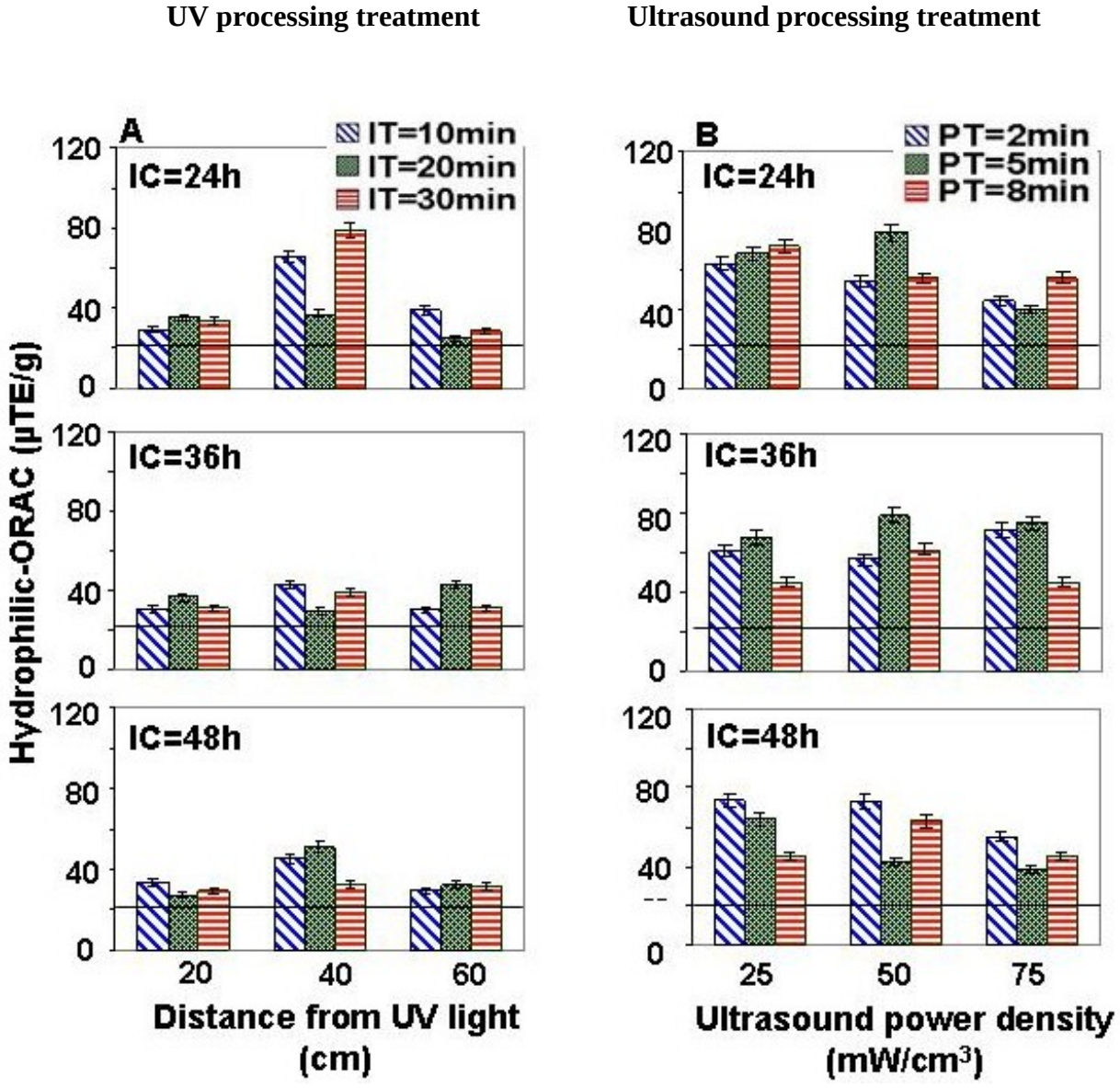


Figure 4.5 Mean hydrophilic ORAC (H-ORAC, µM Trolox Equivalents/g) of sliced peanuts exposed to varying doses of UV (A) and ultrasound (B). IC is the incubation time at 25°C, IT is the UV exposure time, and PT is the ultrasound exposure time. Control raw peanuts had H-ORAC of 21.20±5.30 µM TE/g and represented on each graph by a horizontal line. Means were calculated from 2 replications for a total of four analyses per sample.

(P<0.05) higher H-ORAC of 35.19- 78.59 μM TE/g compared to controls whereas the rest of the 15 treatments had the same (P<0.05) degree of antioxidant capacity as controls. Treatments with higher H-ORAC were exposed to medium distance of 40 cm from UV light suggesting that medium doses of UV are necessary to enhance the hydrophilic antioxidant capacity of UV-treated peanuts. The 15 processing treatments with same antioxidant capacities as controls were either treated at the shortest distance of 20 cm from UV light or at the longest distance of 60 cm distance from UV light indicating that either low or high doses of UV processing treatments will not enhance the hydrophilic antioxidant capacities of treated peanuts. These findings agreed with those of Cantos et al., (2001) who found that 40 cm distance from UV light enhanced the resveratrol synthesis in grapes.

ANOVA (Table 4.2) showed that except for the main effect of UV exposure time, all other main and interaction effects significantly contribute to the hydrophilic antioxidants of UV-treated peanuts. Due to interaction effects, the main factors of distance from UV light and incubation time cannot singled out as the major contributors to the total hydrophilic antioxidants of UV-treated peanuts.

3.3 L-ORAC in sliced UV-treated peanuts

The L-ORAC of UV treated peanuts ranged from 8.37-21.31 μM TE/g whereas controls had 5.61 μM TE/g (Figure 4.6). Twenty two of 27 treatments had significantly (P<0.05) higher lipophilic antioxidant capacities than controls whereas the rest of the five treatments (Trt# 11, 18, 22, 23, 26) had the same L-ORAC values (P<0.05) as untreated controls.

ANOVA showed that except for the main effect of UV exposure time and interaction effect of distance from UV light and incubation time, all other main and interaction effects significantly contribute to the lipoophilic antioxidants of UV-treated peanuts (Table 4.2). Due to interaction

effects, the main factors of distance from UV light and incubation time cannot singled out as the major contributors to the total hydrophilic antioxidants of UV-treated peanuts.

C. Effect of UV on the Overall Acceptance of Sliced UV-Treated Peanuts

Table 4.4 presents the consumers' mean overall acceptance hedonic ratings of UV-treated peanuts. Consumer test results showed that the overall acceptance ratings of UV-treated peanuts ranged from 5.0 $\pm$ 1.7 (neither like nor dislike) to 6.3 $\pm$ 1.5 (like slightly) which were lower ($P<$ 0.05) than that of controls with 7.4 $\pm$ 1.4 or like very much. UV exposure initiates lipid peroxidation resulting in the development of off-flavors to food (Duh and Yen, 1995) and this could have led to the lower slightly consumer acceptance of our UV-treated products compared to controls. The results, however, indicate that although UV treatment reduced the acceptance of peanut kernels, none of the samples were disliked with hedonic ratings $\geq$ 5.0.

Only incubation time and its interaction with either distance from UV light or UV exposure time significantly contributed to the overall acceptance of UV-treated peanuts (Table 4.2). The overall acceptance of UV-treated peanuts decreased with increasing incubation time indicating that the shortest incubation time of 24 h is conducive to high overall acceptance. Thus at 24 h, the highest mean overall acceptance of 5.9 or close to like slightly was achieved compared to the lowest rating of 5.4 or neither like nor dislike obtained when incubated for 48; while those incubated for 36 had an intermediate mean overall acceptance of had the lowest overall acceptance rating of 5.6 or neither like nor dislike.

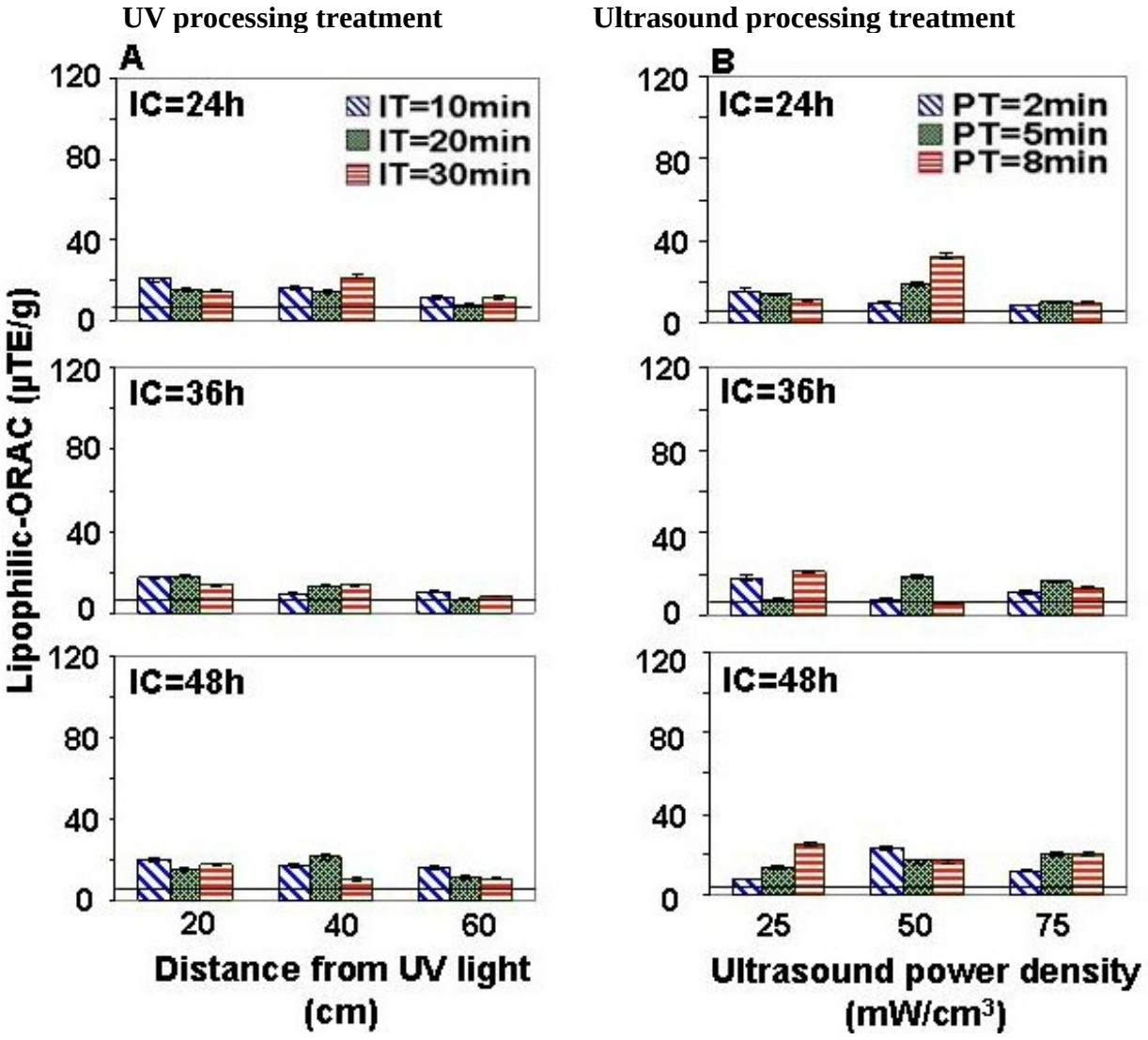


Figure 4.6 Mean lipophilic ORAC (L-ORAC, µM Trolox Equivalents/g) of sliced peanuts exposed to varying doses of UV (A) and ultrasound (B). IC is the incubation time at 25°C, IT is the UV exposure time, and PT is the ultrasound exposure time. Control raw peanuts had L-ORAC of 5.61±0.46 µM TE/g and represented on each graph by a horizontal line. Means were calculated from 2 replications for a total of four analyses per sample**.**

Table 4.4 Consumers' overall acceptance rating (mean ± standard deviation) of roasted sliced UV-treated peanuts and controls[1]

Treatment #	ID	IT	IC	Overall Acceptance[2]
1	20	10	24	6.3 ± 1.5b
2	20	10	36	5.8 ± 1.7bcdefg
3	20	10	48	5.0 ± 1.8hi
4	20	20	24	6.2 ± 1.5bcd
5	20	20	36	5.3 ± 1.8efghi
6	20	20	48	5.5 ± 1.6defghi
7	20	30	24	6.0 ± 1.8bcde
8	20	30	36	5.9 ± 1.8bcde
9	20	30	48	5.2 ± 1.7fghi
10	40	10	24	6.0 ± 1.9bcde
11	40	10	36	5.8 ± 1.6bcdefg
12	40	10	48	5.2 ± 1.5fghi
13	40	20	24	6.2 ± 1.3bcd
14	40	20	36	5.5 ± 1.4cdefghi
15	40	20	48	5.1 ± 2.4ghi
16	40	30	24	5.8 ± 2.0bcdef
17	40	30	36	5.4 ± 2.3efghi
18	40	30	48	5.6 ± 2.0cdefghi
19	60	10	24	5.3 ± 2.0efghi
20	60	10	36	5.8 ± 2.1bcdefg
21	60	10	48	5.8 ± 2.0bcdefg
22	60	20	24	6.0 ± 1.9bcde
23	60	20	36	5.0 ± 1.7hi
24	60	20	48	5.9 ± 1.9bcde
25	60	30	24	5.7 ± 2.3bcdefgh
26	60	30	36	6.2 ± 2.0bc
27	60	30	48	5.4 ± 1.7efghi
Control	120	0	0	7.4 ± 1.3a

[1] ID = distance from UV light, IT = UV exposure time, and IC = incubation time at 25°C. Means ± standard deviation were calculated from two replications for a total 50 analyses/sample for overall acceptance. Means in the column not followed by the same letter are significant ($P<0.05$) from each other as determined by Fisher's least significant difference mean separation test. Controls are roasted untreated peanuts that were sliced prior to evaluation by panelists.

[2]Rating based on 9-point hedonic rating scale where 1 = dislike extremely, 5= neither like nor dislike, and 9=like extremely.

D. Modeling, Mapping of Contour plots and Region of Overlap for UV Processing Treatments

Table 4.5 shows the regression coefficients, coefficients of determination (R^2) and *P*-values for the significant models of *trans*-resveratrol, total stilbenes, H-ORAC, L-ORAC total antioxidant capacity, and overall acceptance for UV-treated peanuts. The regression models for total phenolics and TEAC were not significant and are not shown or used in the optimization procedure. The *P*–values for the models of *trans*-resveratrol, *trans*-piceid, total stilbenes $\leq$0.01 indicating that the models were highly significant in predicting the concentrations of these compounds in UV-treated peanuts. However, their R^2 values of 0.41, 0.39, and 0.45 were low indicating that 42, 39, and 45% of the variability in *trans*-resveratrol, total stilbenes and overall acceptance, respectively, was explained by the predictors – distance from UV light, UV exposure time, and incubation time.

The *P*–values for the models of total antioxidant capacity, H-ORAC, and L-ORAC were all $\leq$0.0001 and for overall acceptance, 0.0003, indicating that the models were highly significant in predicting the concentrations of ORAC and overall acceptance of UV-treated peanuts. However, their R^2 values of 0.35, 0.32, 0.44 and 0.02, respectively, were low indicating that 35, 32, 44, and 2%, of the variabilities in the total antioxidant capacity, H-ORAC, L-ORAC and overall acceptance, respectively, were explained by the predictors – distance from UV light, UV exposure time and incubation time.

The contour plots were generated from full models because R^2 values were low and were not reduced further as removal of terms results in significantly different models compared to full models. The contour plots for the regression models for stilbenes are shown in Figures 4.7 and

Table 4.5 Regression coefficients, R^2, and *P*-values for the significant prediction models of response variables in UV-treated peanuts[1]

Coefficients	*Trans*-Resveratrol	*Trans*-Piceid	Total stilbenes	H-ORAC	L-ORAC	TAC-ORAC	Overall acceptance
Intercept	0.87601	-0.12828	0.74774	-10.5305	52.0253	41.4923	10.92222
Linear							
ID	0.08297	0.04020	0.12317	3.8211	-0.2774	3.5443	-0.10111
IT	-0.20424	-0.02291	-0.22715	1.0951	-0.7515	0.3449	-0.13133
IC	0.01339	-0.00985	0.00354	-0.6837	-1.5929	-2.2774	-0.14657
Quadratic							
ID*ID	-0.00230	-0.00061	-0.00291	0.0372	-0.0039	-0.0412	0.00014
IT*IT	0.00039	0.00088	0.00127	0.0308	0.0071	0.0378	0.00050
IC*IC	-0.00155	0.00011	-0.00144	0.0216	0.0191	0.0407	0.00035
Interaction							
ID*IT	0.00429	0.000322	0.00144	-0.0467	0.0180	-0.0288	0.02650
ID*IC	0.00020	0.000209	0.00221	-0.0211	0.0108	-0.0103	0.00239
IT*IC	-0.00042	-0.000272	0.00389	-0.0618	0.0084	-0.0535	0.00294
ID*IT*IC	-0.00008	-0.000007	-0.00009	0.0012	-0.00004	0.0008	-0.00007
Adjusted R^2	0.41	0.39	0.45	0.32	0.44	0.35	0.02
P-value	0.0069	0.0111	0.0017	<0.0001	<0.0001	<0.0001	0.0003

[1] ID=distance from UV light; IT=UV exposure time; IC=incubation time at 25^{o}C
H-ORAC=hydrophilic ORAC, L-ORAC=lipophilic ORAC, TAC= total antioxidant capacity by oxygen radical absorbance capacity (ORAC) assay, Overall Acceptance using 9-point hedonic rating scale: 1=dislike extremely, 5= neither like nor dislike, 9=like extremely.,

for total phenolics, antioxidant capacities and overall acceptance, in Figure 4.8. The superimposed regions of overlap showing the optimum processes are presented in Figure 4.9.

1. Predicted *trans*-resveratrol concentrations for UV-treated peanuts

The process combinations of distance from UV light, UV exposure time and incubation time did not achieve the targeted 2.64 µg/g (McMurtrey, 1994) corresponding to 100% resveratrol in red wines. Only 2.0 µg/g or 75% of the concentration in red wines was produced at incubation time of 36 h (Figure 4.7) shown shaded in the contour plots. The maximum resveratrol of 2.06 µg/g was obtained after UV treatment for 29-30 min at 40-44 cm distance from UV light and incubated for 36h. *Trans*-resveratrol was highest when incubated for 36 h and then decreased further when distance from UV was increased or decreased. With increasing exposure time at about 30-50 cm distance from UV light, *trans*-resveratrol increased except when incubated for 24h wherein *trans*-resveratrol concentrations decreased with increasing exposure time from 10-20 min and at 28-40 cm distance from UV light, and then increased when exposed for 20-30 min with increasing distance from UV light of 38-48 cm. These results indicate that distance from UV light of 30 to about 45 cm and exposure time for more than 30 min followed by incubation for 36h was necessary to allow maximum production of *trans*-resveratrol in UV-treated peanuts.

2. Predicted *trans*-piceid concentrations for UV-treated peanuts

The process combinations of distance from UV light, UV exposure time and incubation time did not achieve the targeted 1.85 µg/g (Lamuella-Raventos et al., 1994) or 100% in red wines. Only 30% of that in red wines or 0.55 µg/g *trans*- piceid was produced at 24 h incubation time (Figure 4.7). *Trans*-piceid increased with decreasing incubation time from 48 to 24 h and as exposure time increased from 20-30 min, regardless of incubation time. As exposure time increased from 10-20 min, *trans*-piceid increased as 40 cm was approached from 20 or 60 cm

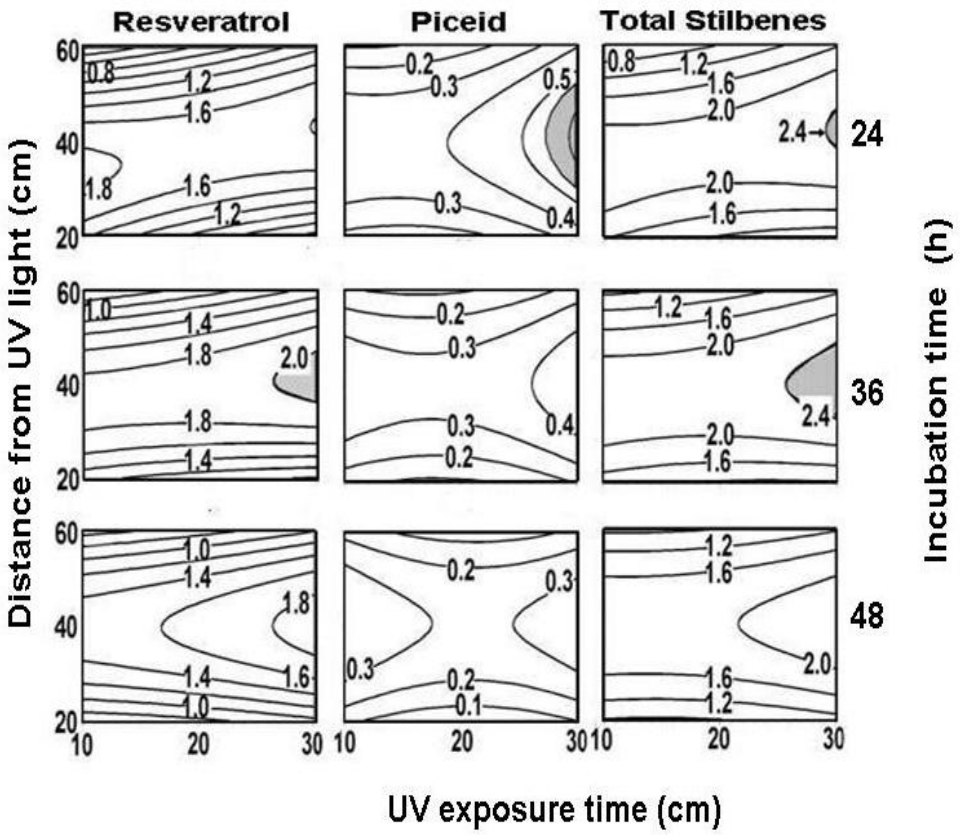


Figure 4.7 Contour plots for the significant prediction models for *trans*-resveratrol (µg/g), *trans*-piceid (µg/g), and total stilbenes (µg/g) of UV-treated peanuts as affected by distance from UV light and UV exposure times at specified incubation time (IC) at 25°C. Contours plots are shaded to cover the areas that meet the regions of interest, or the maximum concentrations achieved.

distance from UV light. As incubation time increased from 24-48 h, a longer exposure time to UV was needed to obtain equal increase in piceid content. The highest *trans*-piceid of 0.60 μg/g or 32% in red wines was achieved when peanuts were exposed to UV for 30 min at 35.5-46 cm distance from UV light and incubated for 24 h. Results indicated that a distance approaching 40 cm and exposure time greater than 30 min followed by incubation for 24 h would produce the highest amounts of *trans*-piceid in UV- treated samples.

3. Predicted total stilbenes concentrations for UV-treated peanuts

The process combinations of distance from UV light, UV exposure time and incubation time did not achieve the targeted 4.33 μg/g total stilbenes or 100% in red wines (Figure 4.7). Only 55% or 2.4 μg/g total stilbenes was achieved at 36 h incubation and shaded in the contour plots. The total stilbenes increased when distance of 40 cm was approached either from 20 or 60 cm, and increased further with increasing exposure time from 17-30 min, or decreasing exposure time from 17-10 min achieving a maximum 2.55 μg/g after 30 min UV exposure at a distance of 40-42 cm and incubation for 36h. Results show that a distance approaching 40 cm and exposure time greater than 30 in followed by incubation for 36h achieved the highest total stilbenes.

4. Predicted total antioxidant capacity by ORAC for UV-treated peanuts

The process combinations of distance from UV light, exposure time and incubation time met the targeted 38.73 μM TE/g corresponding to 100% of total antioxidant capacity in red wines (USDA, 2007). However, a maximum of 58.10 μM TE/g or 150% total antioxidant capacity in red wines was achieved and therefore shaded in the contour plots (Figure 4.8). The total antioxidant capacity increased as 40 cm distance from UV light was approached regardless of UV exposure time from 10-30 min. A maximum of 72 μM TE/g was produced when peanuts were incubated for 24 h and exposed to UV for 30 min at 35.25-37.25 cm distance from UV

light. The highest antioxidant capacity was obtained when peanuts were exposed to distance of 40 cm from UV light compared to 60 cm or 20 cm. Lower total antioxidant capacity was achieved when peanuts were exposed to either higher doses of UV (20 cm distance) or lower doses (60 cm distance). These results were similar to Cantos et al. (2001) where 40 cm distance from UV light produced highest *trans*-resveratrol in UV treated grapes compared to 20 and 60 cm.

5. Predicted H-ORAC for UV-treated peanuts

The process combinations of distance from UV light, exposure time and incubation time met the targeted 38.73 μM TE/g or 100% H-ORAC in red wines and shown shaded in Figure 4.8. Red wine had no lipid fraction, so its total antioxidant capacity is equal to H-ORAC of 38.73 uM TE/ g. The maximum H-ORAC was obtained as 40 cm distance from UV light was approached, regardless of UV exposure time from 10-30 min. The highest H-ORAC of 52 μM TE/g was obtained after 24 h incubation and exposure to UV for 28-30 min at 32-42 cm distance from UV.

6. Predicted L-ORAC for UV-treated peanuts

The process combinations of distance from UV light, exposure time and incubation time did not achieve the targeted 38.73 μM TE/g L-ORAC so the contour plot was not shaded (Figure 4.8). L-ORAC increased with decreasing distance from UV light from 60 to 20 cm and decreasing UV exposure time from 30 to 10 min. The highest L-ORAC of 19 μM TE/g was obtained when peanuts were exposed to UV for 10-12 min at 20-23 cm distance from UV light then incubated for 24h; and UV treated for 10-11 min at distance from UV light of 20-37 cm then incubated for 48h. These results indicate that shorter UV exposure time and distance from UV light will produce high L-ORAC values.

7. Predicted overall acceptance by consumers for UV-treated peanuts

The process combinations of distance from UV light, UV exposure time and incubation time met the targeted overall acceptance rating of 5 or neither like nor dislike (Figure 4.8). However, a higher maximum overall acceptance rating ≥5.5 could be achieved and shown shaded in the contour plots. A mean rating of 5.5 assumes that half of the panel rated the sample greater or equal to 6.0 or like slightly while the other half rated it lower or equal to 5.0 or neither like nor dislike. The overall acceptance increased with decreasing incubation from 48 to 24 h. The high overall acceptance ratings were met when incubation time, distance from UV light, and UV exposure time decreased indicating that all UV treatments will diminish acceptance. The highest rating of 6.3 or like slightly was achieved when peanuts were exposed to UV for 10-14 min at 20 cm distance from UV light and incubated for 24h.

8. The predicted optimum UV processing treatment parameters for sliced peanuts

The region of overlap (Figure 4.9) represents optimum UV processes that met at least 74% *trans*-resveratrol (2.0 μg/g), 55% (2.42 μg/g) total stilbenes, and 142% (55 μM TE/g) antioxidant capacity by ORAC in red wines and mean overall acceptance rating ≥5.5. Only UV-treated peanuts incubated for 36 h met these criteria. Thus, the optimum UV processes included all process combinations within the area of a triangle bound by the points distances from UV light of 47, 41, and 33 cm for 30 (point A), 26.5 (point B) and 30 (point C) min, respectively. At this optimum region of overlap, the predicted concentrations of phenolic compounds and antioxidant capacities were 2.00 to 2.06 μg/g *trans*-resveratrol , 2.44-2.55 μg/g total stilbenes (sum of *trans*-resveratrol and *trans*-piceid) , 55.00 μM TE/g total antioxidant capacity by ORAC assay, and overall acceptance ratings ≥5.6 or neither like nor dislike. The concentrations at the optimum UV processes correspond to 74-78% *trans*-resveratrol, 55-59% total stilbenes, and 142-

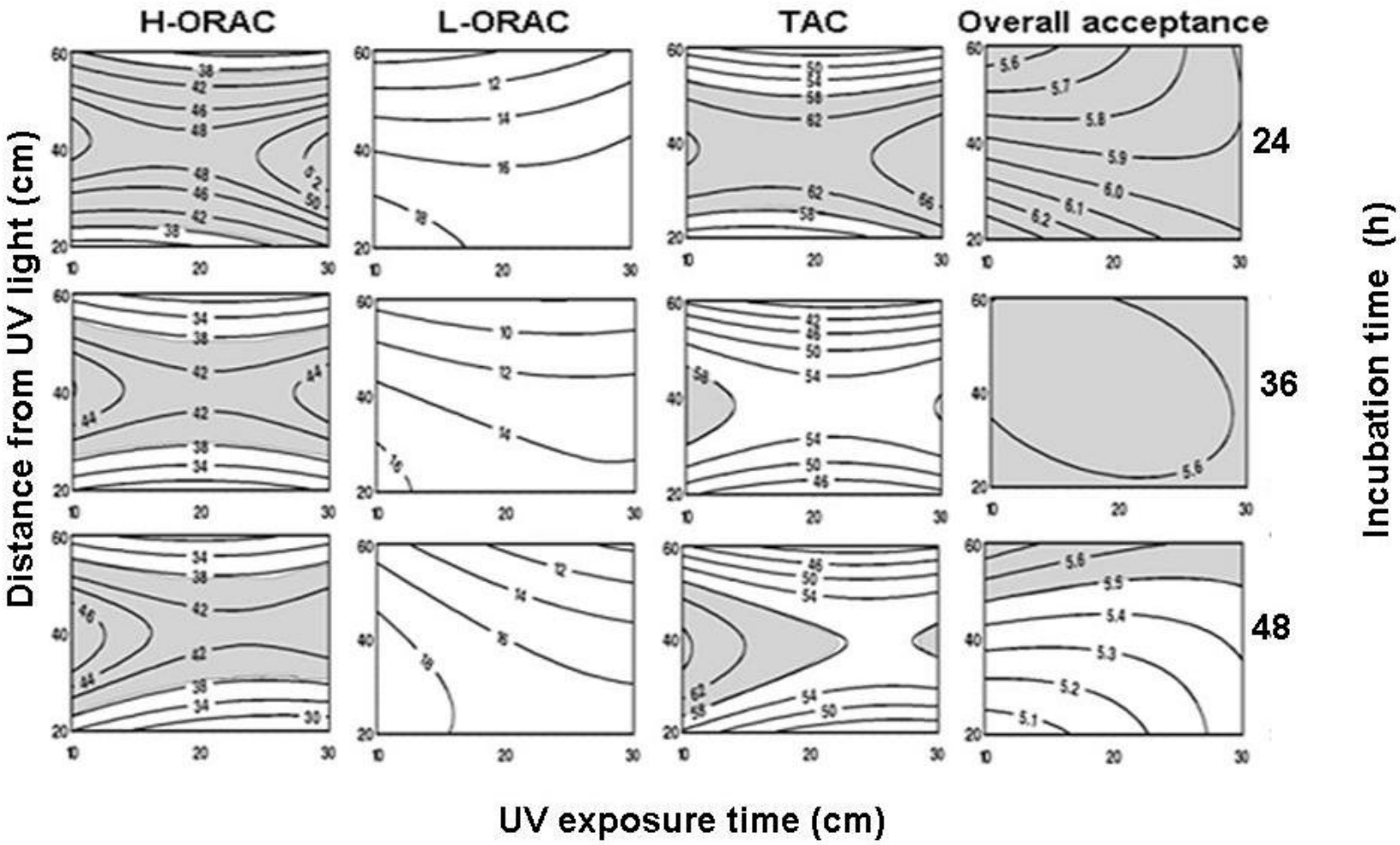


Figure 4.8 Contour plots for the significant prediction models for H-ORAC (hydrophilic ORAC, μM TE/g), L-ORAC (lipophilic ORAC, μM TE/g), TAC (total antioxidant capacity, μM TE/g), and overall acceptance (9-point hedonic rating) of UV-treated peanuts as affected by distance from UV light and UV exposure times at specified incubation time (IC) at 25°C. Contour plots are shaded to cover the areas that meet the regions of interest, or the maximum concentrations or OA ratings achieved.

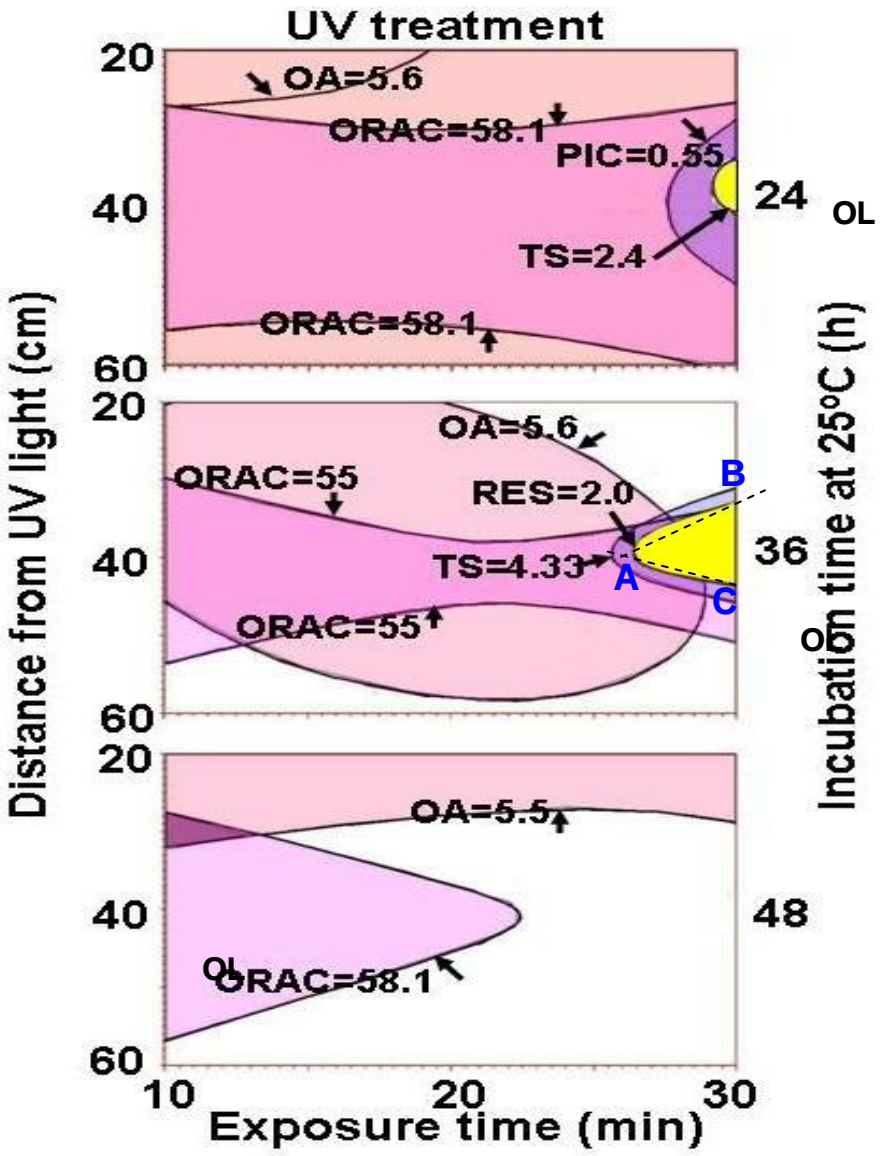


Figure 4.9 Superimposed contour plots for the significant (*P*<0.05) prediction models of *trans*-resveratrol (RES, µg/g), *trans*-piceid (PIC, µg/g), ORAC (total antioxidant capacity, µM TE/g) and overall acceptance (OA) based on 9-point hedonic rating where 1=dislike extremely, 5=neither like nor dislike and 9-like extremely. The regions of overlap (OL) represent the optimum UV processes at the specified incubation time. Arrows indicate directions of increasing concentrations or acceptance ratings.

150% total antioxidant capacities, respectively, of that in red wines. On a per serving basis, if a peanut bar containing 30 g of treated peanuts were prepared using these optimum processes, about 3 bars, each containing 30 g of peanuts will provide the same amount of total antioxidant capacity as a 120 mL serving of red wines or 4,950 μM TE.

D. Verification of Prediction Models for UV Processing Treatment Parameters

The prediction models for the UV processing treatment parameters of peanut kernels were verified by preparing samples of UV-treated peanuts using a process within and outside the optimum region and then analyzing the samples for *trans*-resveratrol, *trans*-piceid, and total antioxidant capacity. In this study, a sample was UV-treated at 40 cm distance from light for 30 min followed by 36 h incubation at 25^{o}C to represent a process within the optimum region and another sample was exposed at 20 cm distance from UV light for 10 min followed by followed by 36 h incubation at 25^{o}C representing a process outside the optimum process. The observed and predicted values of all response variables analyzed are shown in Table 4.6. Results showed that the observed and predicted values for *trans*-resveratrol, and *trans*-piceid had probabilities > 0.05 indicating that the paired values were not significantly different from each other and therefore verifying that the regression equations could predict the concentrations of these compounds. The observed values for ORAC within and outside the optimum regions were higher than the prediction values with *P* values of 0.0009 and 0.0019, respectively.

Table 4.6 Observed and predicted values of stilbenes concentrations and antioxidant capacity values of UV-treated peanuts for verification of prediction models.

Compound	Within Optimum Region			Outside Optimum Region		
	Observed	Predicted	PROB[1]	Observed	Predicted	PROB
Trans-resveratrol (μg/g)	2.06	2.06	0.5988 NS	1.28	1.27	0.9504 NS
Trans-piceid (μg/g)	0.49	0.47	0.1599 NS	0.15	0.14	0.8020 NS
Total Antioxidant Capacity (ORAC, μM TE/g)	227	127	0.0090**	134	58	0.0019**

[1] PROB = probability >0.05 means paired values are not significantly different from each other at 5% level of significance

NS = not significant, **significant at 0.01% level

II. STUDY 2. ULTRASOUND PROCESS OPTIMIZATION TO ENHANCE *TRANS*-RESVERATROL BIOSYNTHESIS IN PEANUTS

A. Effect of Ultrasound Treatment on the Concentrations of Stilbenes, Total Phenolics, and Antioxidant Capacities of Sliced Peanut Kernels

1. *Trans*-resveratrol, *trans*-picied and total stilbenes of sliced ultrasound-treated peanuts

The mean concentrations of *trans*-resveratrol, *trans*-piceid and total stilbenes in US- treated sliced peanuts are shown in Table 4.7 and Figure 4.10. US increased ($P<0.05$) the mean concentrations of *trans*-resveratrol, 0.32±0.16 to 6.39±2.15 µg/g, *trans*-piceid, 0.16±0.05 to 6.39±2.27 µg/g and total stilbenes, 0.48±0.16 to 9.86±0.82 µg/g, of all 27 sliced US-treated peanuts compared to controls with 0.02 ± 0.002, 0.03 ± 0.001, and 0.05 ± 0.004 µg/g, respectively, corresponding to 16-319, 5-213, and 10-197 fold increase, respectively. US treatment at low intensities of ginseng cell cultures, stimulated growth and biosynthesis of secondary metabolites through mechanical stress and microstreaming induced by acoustic cavitations which disrupted the cell wall (Lin et al., 2001). US caused rapid increase in levels of phenylammonia lyase, polyphenol oxidase, and peroxidase in ginseng cell cultures with phenylammonia lyase enhanced most dramatically with 5-fold higher at power level 4 after 4 days incubation compared to controls (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 *trans*-resveratrol synthesis (Soleas et al., 1997). We believe that the release of phenylammonia lyase after US treatment could be responsible for increased concentrations of *trans*-resveratrol and *trans*- piceid in US- treated sliced peanuts in this study.

Table 4.7 Concentrations (mean ± standard deviation, dry basis) of *trans*-resveratrol, *trans*-piceid, and total stilbenes of roasted ultrasound-treated sliced peanuts and controls[1].

Trt #	PD	PT	IC	*Trans*-Resveratrol µg/g	*Trans*-Piceid µg/g	Total Stilbenes µg/g
1	25	2	24	0.4100 ± 0.1068h	0.2950 ± 0.2679e	0.7050 ± 0.2358hij
2	25	2	36	4.1225 ± 0.5049bcd	0.5550 ± 0.4608e	4.6775 ± 0.8972de
3	25	2	48	1.9650 ± 1.5505efgh	2.1000 ± 0.8665cde	4.0650 ± 1.3328de
4	25	5	24	2.8650 ± 0.0742cdef	0.8325 ± 0.4322e	3.6925 ± 0.4711efg
5	25	5	36	0.7775 ± 0.2037gh	1.3575 ± 0.3508de	2.1375 ± 0.2257ghi
6	25	5	48	5.2075 ± 0.8898ab	1.4125 ± 0.5082de	6.6175 ± 1.2000bc
7	25	8	24	1.2250 ± 1.3261fgh	1.0575 ± 0.4840e	2.2825 ± .8736fgh
8	25	8	36	2.0725 ± 0.7905defgh	1.8675 ± 0.6070de	3.9375 ± 1.0808ef
9	25	8	48	2.7725 ± 1.0857cdefg	1.3200 ± 1.3638de	4.0950 ± 0.4585de
10	50	2	24	0.5825 ± 0.6286h	0.6400 ± 0.4807e	1.2200 ± 0.3391hij
11	50	2	36	0.7300 ± 0.6704gh	4.3200 ± 2.6532abc	5.0500 ± 2.0701cde
12	50	2	48	0.4450 ± 0.2322h	0.2025 ± 0.1511e	0.6475 ± 1.1127hij
13	50	5	24	0.4700 ± 0.2855h	6.3950 ± 2.2723a	6.8625 ± 2.2131b
14	50	5	36	0.8250 ± 1.2985fgh	4.4750 ± 3.0847ab	5.3000 ± 2.0842bcde
15	50	5	48	0.5175 ± 0.3111h	0.3325 ± 0.3732e	0.8500 ± 0.2205hij
16	50	8	24	0.3175 ± 0.1640h	0.1600 ± 0.0455e	0.4750 ± 0.1529ij
17	50	8	36	1.0925 ± 0.2765fgh	0.5225 ± 0.3709e	1.6125 ± 0.2139hij
18	50	8	48	0.5150 ± 0.5496h	0.7675 ± 0.7140e	1.2800 ± 0.2709hij
19	75	2	24	4.0000 ± 2.1527bcde	0.9375 ± 1.0621e	4.9375 ± 1.5378cde
20	75	2	36	1.3375 ± 0.6038fgh	0.2375 ± 0.1706e	1.5750 ± 0.4886hij
21	75	2	48	6.3892 ± 2.1501a	3.4725 ± 2.2721bcd	9.8625 ± 0.8220a
22	75	5	24	1.1675 ± 0.0634fgh	0.4825 ± 0.5619e	1.6525 ± 0.5126hij
23	75	5	36	6.00930 ± 0.3695ab	3.5525 ± 1.1474ab	9.5625 ± 2.1861a
24	75	5	48	4.7450 ± 2.7460abc	0.6650 ± 0.8077e	5.4075 ± 3.3050bcd
25	75	8	24	4.6150 ± 0.7677abc	0.2350 ± 0.2450e	4.8500 ± 0.9919cde
26	75	8	36	4.0025 ± 1.5115bcde	1.0550 ± 1.3090e	5.0575 ± 1.0514cde
27	75	8	48	3.7725 ± 1.4654bcde	2.0375 ± 1.0517cde	5.8100 ± 0.5854bcd
Control[2]	0	0	0	0.0212 ± 0.0020i	0.0271 ± 0.0010e	0.0482 ± 0.0046j

[1] PD= ultrasound power density (mW/cm^3), PT= ultrasound exposure time, and IC= incubation time at 25^{o}C.
Means ± standard deviation were calculated from two replications for a total of 4 analyses/sample.
Means in a column not followed by the same letter are significantly different from each other at $P < 0.05$ as determined by Fisher's significant difference mean separation test.
[2]Control is untreated raw whole peanuts.

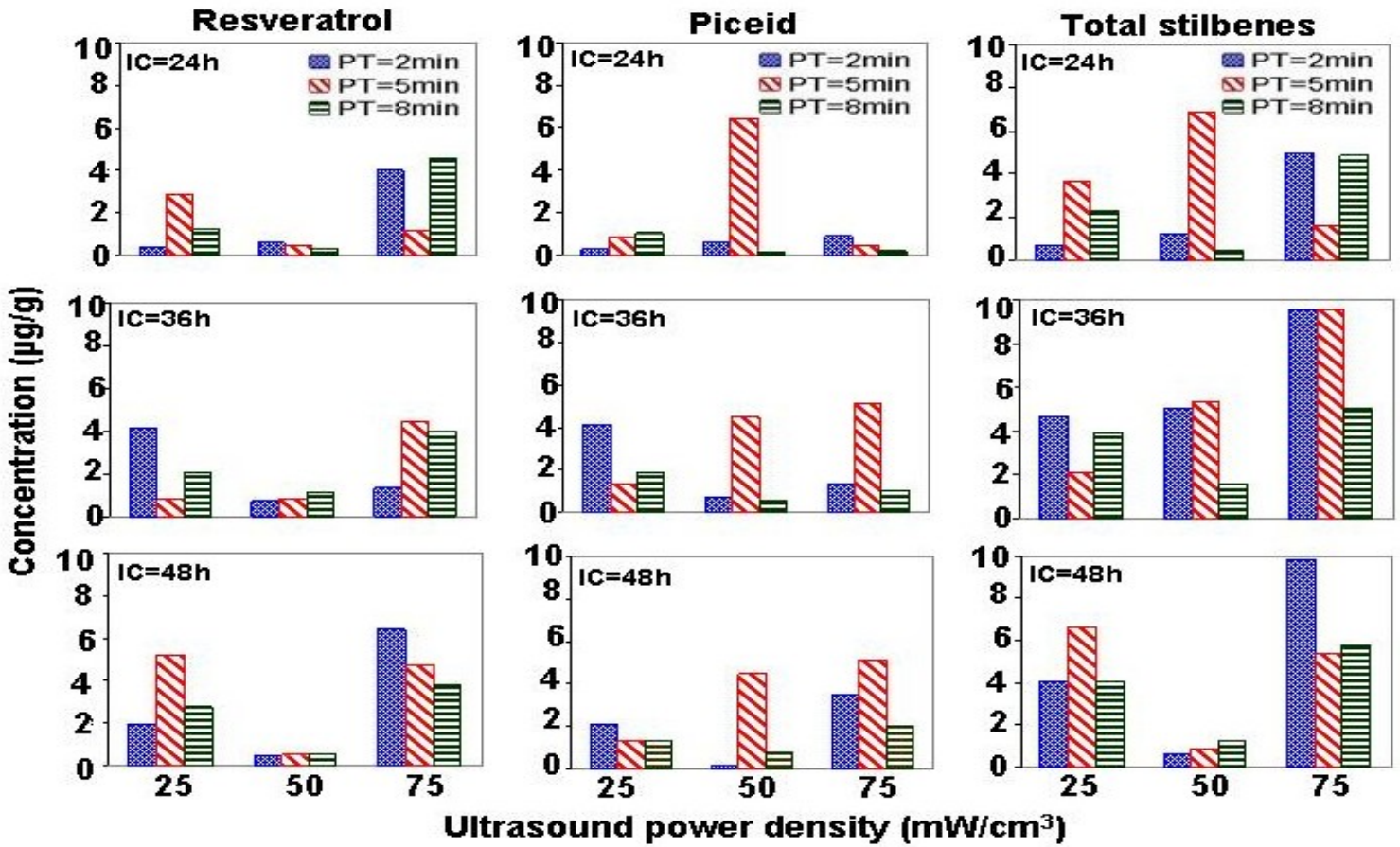


Figure 4.10 Mean *trans*-resveratrol (µg/g), *trans*-piceid (µg/g), and total stilbenes (µg/g) of sliced peanuts exposed to varying doses of ultrasound. IC is the incubation time at 25°C and PT is the ultrasound exposure time. Control untreated raw whole peanuts had 0.02 ± 0.002 µg/g *trans*-resveratrol, 0.05 ± 0.001 *trans*-piceid µg/g and 0.05 ± 0.001 µg/g total stilbenes.

The analysis of variance (Table 4.8) indicated that all factors of US power density, the main factors of US exposure time and incubation time, and their interactions significantly ($P<0.05$) contributed to the concentrations of *trans*-resveratrol in sliced peanuts. The concentrations of *trans*-piceid in sliced peanuts were significantly contributed by all factors and their interactions excluding the main effect of power density. All factors and their interactions, excluding the interaction of power density and exposure time significantly contribute to the concentrations of total stilbenes in sliced peanuts.

Compared to UV treatment, US resulted in higher ($P<0.05$) *trans*-resveratrol, *trans*-piceid and total stilbenes in sliced peanuts. US processed sliced peanuts had an overall mean concentrations of 2.27 $\pm$ 0.99 µg/g *trans*-resveratrol, 1.59 $\pm$ 1.09 µg/g *trans*-piceid and 3.86 $\pm$ 0.92 µg/g total stilbenes, while UV-treated sliced samples resulted in 1.19 $\pm$ 0.22, 0.24 $\pm$ 0.03, and 1.43 $\pm$ 0.23 µg/g, respectively. The average increase in concentrations in US compared to UV treated peanuts were 91% for resveratrol, 562% for *trans*-piceid, and 170% for total stilbenes.

Sliced US-treated peanuts had higher *trans*-resveratrol, *trans*-piceid and total stilbenes compared to blended peanut butters with 0.41$\pm$0.02, 0.13$\pm$0.01, and 0.54$\pm$0.03 µg/g, respectively, and 100% natural peanut butters with 0.65$\pm$0.02, 0.14$\pm$0.01, and 0.814$\pm$0.03 µg/g, respectively (Ibern-Gomez et al., 2000). Compared to red wines with mean concentrations of 1.85, 2.48 and 4.33 µg/mL *trans*- resveratrol, *trans*-piceid, and total stilbenes, respectively (Lamuela-Raventos et al., 1995), sliced US-treated peanuts had comparable *trans*-resveratrol but lower *trans*- piceid and total stilbenes. McMurtrey et al. (1994) reported even higher mean *trans*-resveratrol in red wines of 2.64 µg/mL, range: 0.99-5.01 µg/mL, but did not report values for *trans*-piceid.

Table 4.8 Significant treatment effects on the concentrations of stilbenes, total phenolics, antioxidant capacities, and overall acceptance of sliced, chopped, and whole ultrasound-treated peanuts.

Size/Factors[1]	Trans-resveratrol	Trans-piceid	Total Stilbenes	Total Phenolics	TEAC	H-ORAC	L-ORAC	TAC	Overall Acceptance
Sliced peanuts									
Main effects									
PD	<0.0001	NS	<0.0001	NS	NS	0.0007	<0.0001	<0.0001	0.4595
PT	0.0014	0.0034	0.0003	NS	NS	0.0253	<0.0001	NS	NS
IC	<0.0001	0.0399	0.0003	0.0051	NS	NS	<0.0001	NS	NS
Interactions									
PD x PT	<0.0001	0.0126	NS	0.0051	0.0061	NS	<0.0001	NS	NS
PD x IC	0.0004	0.0020	<0.0001	0.0269	NS	0.0059	<0.0001	0.0004	NS
PT x IC	0.0005	0.0032	0.0246	NS	NS	<0.0001	0.0425	<0.0001	0.0037
PD x PT x IC	<0.0001	0.0002	<0.0001	<0.0001	0.0256	0.0055	<0.0001	0.0016	<0.0001
Chopped peanuts									
Main effects									
PD	<0.0001	<0.0001	<0.0001	NS	NS	0.0449	0.0009	NS	NS
PT	<0.0001	<0.0001	NS	NS	NS	NS	<0.0001	NS	NS
IC	<0.0001	<0.0001	<0.0001	NS	0.0015	NS	0.0018	NS	<0.0001
Interactions									
PD x PT	<0.0001	NS	<0.0001	NS	0.0377	NS	<0.0001	NS	NS
PD x IC	<0.0001	<0.0001	<0.0001	0.0028	NS	0.0262	<0.0001	0.0060	0.0105
PT x IC	<0.0001	0.0005	<0.0001	NS	NS	NS	0.0231	NS	NS
PD x PT x IC	<0.0001	<0.0001	<0.0001	0.0094	NS	<0.0001	<0.0001	<0.0001	NS

Table 4.8 continued…

Size/Factors[1]	Trans-resveratrol	Trans-piceid	Total Stilbenes	Total Phenolics	TEAC	H-ORAC	L-ORAC	TAC	Overall Acceptance
Whole peanuts									
Interactions									
PD	0.0475	<0.0001	<0.0001	0.0023	NS	0.0034	<0.0001	<0.0001	0.0026
PT	NS	0.0379	<0.0001	0.0004	NS	0.0095	0.0209	0.0015	NS
IC	NS	<0.0001	<0.0001	0.0172	0.0177	0.0154	0.0006	0.0010	NS
Interactions									
PD x PT	0.0251	NS	0.0121	0.0074	NS	0.0002	NS	0.0005	0.0011
PD x IC	0.0197	<0.0001	<0.0001	NS	NS	NS	NS	NS	NS
PT x IC	0.0221	<0.0001	<0.0001	NS	0.0520	<0.0001	0.0191	0.0001	NS
PD x PT x IC	0.0072	<0.0001	<0.0001	NS	0.0321	<0.001	<0.0001	0.0009	<0.0001

[1]PD=ultrasound power density; PT=ultrasound exposure time; IC=incubation time at 25^{o}C.
NS = not significantly different (P>0.05)

The *trans*-resveratrol concentrations of 0.32-6.39 µg/g produced in US-treated peanuts were higher those found in white and red grape juices with non-detectable to 0.9 (mean=0.05) g/mL and non-detectable to 1.09 (mean=0.5) µg/mL, respectively (Romero-Perez et al., 1999), dark chocolate and cocoa liquor with 0.40 and 0.50 µg/g, respectively (Counet et al., 2006), pistachios with 0.07-0.18 (mean=0.12) µg/g (Grippi et al., 2008), hops with 0.7-2.2 µg/g (Jerkovic and Collin, 2007), and hop pellets with 0.5 µg/g (Callemien et al., 2005). The *trans*-resveratrol concentrations in US-treated peanuts, however, were lower compared to those in dried white and red grape berry skins with 11.04-47.60 (mean= 22.03) and 18.32-38.26 (mean=25.79), respectively, (Romero-Perez et al., 2001).

The amounts of *trans*-piceid of 0.16-6.39 µg/g obtained by US treatment of sliced peanuts were higher compared to white grape juices with non-detectable to 0.83 (mean=0.18) µg/mL (Romero-Perez et al., 1999); dark chocolate and cocoa liquor with 1.00 and 1.20 µg/g in respectively (Counet et al., 2006); Spanish red wines with 0.74 – 4.01 (mean=1.85) µg/mL (Lamuella-Raventos et al., 1995); and hop pellets with 2.0 µg/g (Callemien et al., 2005). The piceid concentrations in sliced US-treated peanuts were within that found in red grape juices with 0.77-7.34 µg/mL (Romero-Perez et al., 1999), hops with 2.3-7.3 µg/g (Jerkovic and Collin, 2007), and pistachios with mean of 6.97 (range= 6.2-8.15) µg/g (Grippi et al., 2008), but lower than those found in dried white and red grape skins with mean concentration of 16.58 (range= non-quantifiable to 64.41) and 64.15 (range =5.49-342.66 µg/g), respectively (Romero-Perez et al., 2001).

2. Total phenolics of sliced ultrasound-treated peanuts

Table 4.9 and Figure 4.3 show the mean total phenolics concentrations of US treated sliced peanuts. US increased ($P< 0.05$) the total phenolics of all 27 treated sliced peanuts

Table 4.9 Concentrations (mean ± standard deviation, dry basis) of total phenolics, trolox equivalent antioxidant capacity (TEAC), and oxygen radical absorbance capacity (ORAC) of roasted sliced ultrasound-treated peanuts and controls [1]

Trt #	PD	PT	IC	Total Phenolics mg GAE/g	TEAC μM TE/g	H-ORAC μM TE/g	L-ORAC μM TE/g	ORAC-TAC μM TE/g
1	25	2	24	1.74 ± 0.14abc	3.00 ± 0.02ab	62.80 ± 0.02abcde	15.69 ± 0.02fgh	78.37 ± 11.49cde
2	25	2	36	1.37 ± 0.19efghij	3.00 ± 0.16ab	60.81± 0.16bcdef	18.17 ± 0.16def	78.97 ± 2.93bcde
3	25	2	48	1.76 ± 0.02ab	2.81 ± 0.16abcde	73.29 ± 0.16abc	80.40 ± 0.16jklm	81.33 ± 12.31abcde
4	25	5	24	1.28 ± 0.29ghij	2.90 ± 0.17abc	68.14 ± 0.17abcd	13.78 ± 0.17ghi	81.86 ± 3.42abcd
5	25	5	36	1.72 ± 0.21abcd	2.56 ± 0.52bcdef	67.52 ± 0.52abcd	7.72 ± 0.52jklm	75.23 ± 8.39cdef
6	25	5	48	1.48 ± 0.08cdefghi	2.74 ± 0.58abcde	64.04 ± 0.58abcd	13.80 ± 0.58ghi	77.83 ± 7.54cde
7	25	8	24	1.50 ± 0.02cdefgh	2.68 ± 0.39bcde	71.99 ± 0.39abcd	11.32 ± 0.39hijk	83.37 ± 13.75abcd
8	25	8	36	1.42 ± 0.36efghij	3.00 ± 0.16ab	45.34 ± 0.16efgh	21.07 ± 0.16bcd	66.41 ± 12.23defgh
9	25	8	48	1.53 ± 0.12bcdefg	2.21 ± 0.08f	44.97 ± 0.08fgh	25.27 ± 0.08b	70.24 ± 12.98defg
10	50	2	24	1.23 ± 0.21ij	2.46 ± 0.43cdef	56.64 ± 0.43cdefg	9.58 ± 0.43cdefg	64.12 ± 17.94efgh
11	50	2	36	1.86 ± 0.35a	2.96 ± 0.04ab	54.54± 0.04efgh	7.41 ± 0.04lmn	64.09 ± 15.10efgh
12	50	2	48	1.18 ± 0.22j	2.56 ± 0.38bcdef	72.88 ± 0.38abc	23.40 ± 0.38bc	96.12 ± 23.13ab
13	50	5	24	1.46 ± 0.18defghi	2.76 ± 0.23abcde	78.75 ± 0.23a	19.17 ± 0.23cdef	97.92 ± 31.89a
14	50	5	36	1.34 ± 0.07efghij	2.94 ± 0.30ab	78.52 ± 0.30a	18.40 ± 0.30def	96.92 ± 14.38a
15	50	5	48	1.76 ± 0.08ab	2.99 ± 0.56abcde	12.51 ± 0.56gh	17.00 ± 0.56defga	59.41 ± 7.35fgh
16	50	8	24	1.60 ± 0.03bcde	2.99 ± 0.27ab	55.70 ± 0.27cdefgh	55.70 ± 0.27cdefgh	88.01 ± 7.58abc
17	50	8	36	1.58 ± 0.04bcde	2.95 ± 0.04ab	61.61 ± 0.04abcdef	7.00 ± 0.04m	67.59 ± 6.10defgh
18	50	8	48	1.44 ± 0.05efghij	2.60 ± 0.53bcdef	62.85 ± 0.53abcde	19.51 ± 0.53cdef	82.35 ± 10.10abcd

Table 4.9 continued…

Trt #	PD	PT	IC	Total Phenolics mg GAE/g	TEAC μM TE/g	H-ORAC μM TE/g	L-ORAC μM TE/g	ORAC-TAC μM TE/g
19	75	2	24	1.26 ± 0.23ghij	2.36 ± 0.24ef	44.67 ± 0.24fgh	8.26 ± 0.24klmn	50.22 ± 8.59gh
20	75	2	36	1.31 ± 0.16fghij	2.43 ± 0.22def	71.40 ± 0.22abcd	11.32 ± 0.22hijk	82.73 ± 9.61abcd
21	75	2	48	1.39 ± 0.04efghij	2.59 ± 0.45bcdef	55.11 ± 0.45defhg	18.11 ± 0.45defhg	66.95 ± 14.97defgh
22	75	5	24	1.27 ± 0.26ghij	2.59 ± 0.35bcdef	40.09 ± 0.35hi	11.09 ± 0.35hi	50.00 ± 9.75h
23	75	5	36	1.89 ± 0.15a	2.59 ± 0.05abcd	74.62 ± 0.05ab	16.45 ± 0.05efg	86.61 ± 18.27abc
24	75	5	48	1.22 ± 0.22ij	2.59 ± 0.42bcdef	38.29 ± 9.60hi	20.61 ± 0.42cde	58.82 ± 8.63fgh
25	75	8	24	1.56 ± 0.02abcdef	2.94 ± 0.27ab	56.02 ± 0.27bcdefg	9.72 ± 0.27ijklm	65.74 ± 10.91defgh
26	75	8	36	1.71 ± 0.36abcd	2.55 ± 0.49bcdef	45.07 ± 0.49fgh	13.47 ± 0.49ghi	58.54 ± 14.53fgh
27	75	8	48	1.71 ± 0.13abcd	3.18 ± 0.20a	44.99 ± 0.49fgh	10.03 ± 0.49ijkl	55.02 ± 2.83gh
Control[2]		0	0	0.84 ± 0.02k	0.61 ± 0.01g	21.20 ± 0.20i	5.61 ± 0.20m	26.81 ± 5.72i

[1] PD is ultrasound power density (mW/cm^3); PT is ultrasound exposure time; and IC is incubation time.
H-ORAC = hydrophilic ORAC; L-ORAC = lipophilic ORAC; TAC= total antioxidant capacity is the sum of H-ORAC and L-ORAC.
Means ± standard deviation were calculated from two replications for a total of 4 analyses/sample each for *trans*-resveratrol, *trans*-piceid and total stilbenes.
Means in a column not followed by the same letter are significantly different from each other at $P < 0.05$ as determined by Fisher's significant difference mean separation test.
[2] Control is untreated raw whole peanuts.

to 1.18-1.89 mg GAE/g compared to untreated controls with 0.84 mg GAE/g of this study and controls of 0.91-1.14 mg GAE/g reported by Talcott et al. (2005). These amounts were slightly higher than the total phenolics of 1.09 to 1.58 mg/g reported by Rudolf and Resurreccion (2005) in sliced peanuts treated with a single dose of US power density of 39.2 mW/cm^3 for 4 min at 24-48 h incubation.

ANOVA of our data (Table 4.8) indicated that incubation time and all interactions US power density, exposure time, and incubation time, except that of US exposure time x incubation time, significantly contributed to the concentrations of total phenolics of sliced US-treated peanuts. These results agreed with Rudolf and Resurreccion (2005) who found that incubation time did not cause significant differences in the total phenolics of sliced US-treated peanuts. Our results disagreed with Wu and Lin (2002) working with sonicated *Panax ginseng* cells who reported that increasing power density from 14 to 61 mW/cm^3 increased phenolic compounds and polyphenols produced due to increased enzyme activities needed for biosynthesis of these compounds.

The observed values of 1.18-1.89 mg GAE/g in sliced US-treated peanuts were within the total phenolics of blueberry wines with 0.60-1.86 mg GAE/g (Sanchez-Moreno et al., 2003), and within the lower limits of 1.81-4.58 mg GAE/g in fresh blueberry purees (Prior et al., 1998) and 1.31-2.39 mg GAE/mL in red wines (Fernandez-Pachon et al., 2004*)*.

3. Trolox equivalent antioxidant capacity (TEAC) of sliced ultrasound-treated peanuts

The mean TEAC values of sliced US-treated peanuts are shown in Table 4.9 and Figure 4.4. US increased ($P< 0.05$) TEAC of all 27 treated sliced peanuts with 2.21 to 3.18 μM TE/g compared to controls with 0.61 μM TE/g. The analysis of variance (Table 4.8) indicated that only the interaction of power density x exposure time and interaction of all three

factors but not any single factor, caused significant change ($P< 0.05$) in the TEAC values of sliced US-treated peanuts. Sliced US-treated peanuts had lower ($P< 0.05$) mean TEAC of 2.74 ± 0.37 μM TE/g compared to UV-treated sliced samples with mean TEAC of 3.15 ± 0.50 μM TE/g at all incubation times. The TEAC of 2.21-3.18 μM TE/g of sliced US-treated peanuts were in the lower limit of 16 types of red wines with 3.06 to 11.15 μM TE/mL (Fernandez-Pachon et al., 2004).

4. Oxygen radical antioxidant capacity (ORAC) of sliced ultrasound-treated peanuts

4.1 Total Antioxidant Capacity (TAC) sliced ultrasound-treated peanuts

The mean total antioxidant capacities of sliced US-treated peanuts are presented in Table 4.9 and Figure 4.5. US increased ($P<0.05$) the total antioxidant capacity of all 27 treated sliced peanuts with the range of 50.00-96.92 μmol TE/g compared to 26.81 μmol TE/g in controls. The analysis of variance (Table 4.8) showed that US power density and its interactions with incubation time, and with both exposure time and incubation time; and the interation of exposure time with incubation time significantly contributed to the total antioxidant capacity of sliced US-treated peanuts. The moderate power density of 50 mW/cm^3 produced higher ($P<0.05$) total antioxidant capacity than 75 mW/cm^3 whereas, the lowest power density of 25 mW/cm^3 produced concentrations which were not different from either power densities suggesting that moderate power densities will produce higher antioxidants compared to higher and lower power densities. Similarly, Lin et al. (2001) working on *Panax ginseng* cells found that US exposure at moderate power densities of 34-61 mW/cm^3 for 1-2 min produced higher saponins compared to those treated at 3 and 82 mW/cm^3 for the same exposure time. US processing treatment which produced an overall mean total antioxidant capacity of 73.80 ± 19.02 in sliced peanuts was more effective than UV processing treatment which achieved an overall mean of 51.21 ± 16.04.

4.2 H-ORAC sliced ultrasound-treated peanuts

Table 4.9 and Figure 4.5 show the mean H-ORAC values of sliced US-treated peanuts. The H-ORAC of sliced US-treated peanuts ranged from 38.21-78.75 μM TE/g, respectively, compared to controls with 21.20 μM TE/g. Similar to the total antioxidant capacity the analysis of variance (Table 4.8) US power density and its interactions with incubation time, and with both exposure time and incubation time; and the interation of exposure time with incubation time significantly changed the H-ORAC of sliced US-treated peanuts. Sliced US-treated peanuts achieved significantly higher H-ORAC values with an overall mean of 59.07 ± 17.70 μM TE/g compared to UV-treated sliced samples with an overall mean of 36.75± 14.26 μmol TE/g.

4.3 L-ORAC sliced ultrasound-treated peanuts

Table 4.9 and Figure 4.5 show the mean L-ORAC values of US-treated peanuts. The L-ORAC of sliced US-treated peanuts had 5.99-32.31 μM TE/g, compared to 5.61 μM TE/g in controls. US which produced an overall mean L-ORAC of 14.73 ± 6.84 μM TE/g was equally effective as UV (overall mean of 14.27 ± 5.12) in increasing L-ORAC of sliced peanuts.

The analysis of variance (Table 4.8) showed that all main and interactions effects significantly contributed to the lipophilic antioxidants of sliced US-treated peanuts. Due to interaction effects, no main factor can be singled out as the major contributor to the L-ORAC of UV-treated peanuts. Sliced US-treated peanuts achieved significantly higher H-ORAC values with an overall mean of 59.07 ± 17.70 μM TE/g compared to UV-treated sliced samples with an overall mean of 36.75± 14.26 μmol TE/g.

B. Effect of Ultrasound on the Concentrations of Stillbenes, Total Phenolics, and Antioxidant Capacities of Chopped Peanut Kernels

1. *Trans*-resveratrol, *trans*-picied and total stilbenes of chopped ultrasound-treated peanuts

The mean concentrations of *trans*-resveratrol, *trans*-piceid and total stilbenes in US- treated chopped peanuts are shown in Table 4.10. US increased ($P<0.05$) the mean concentrations of *trans*-resveratrol, 0.51±0.05 to 2.91±0.08 μg/g and total stilbenes, 0.77±0.04 to 5.06±0.18 μg/g in all 27 treated chopped peanuts compared to controls with 0.02 ± 0.002 μg/g. *Trans*-piceid increased in only 19 of 27 chopped US-treated peanuts with mean concentrations of 0.34±0.04 to 2.44±0.08 μg/g compared to controls with 0.03 μg/g; whereas eight of 27 treatments with piceid of 0.19±0.02 to 0.30±0.04 were not different from controls.

Chopped US-treated peanuts produced lower maximum concentrations of 2.91, 2.44, and 5.06 μg/g, *trans*-resveratrol, *trans*-piceid and total stilbenes, respectively, compared to sliced treated peanuts which produced maximum concentrations of 6.39, 6.39, and 9.86 μg/g, respectively. This finding suggests that greater severity of damage to peanut cells through size reduction such as chopping the less enhancement of the stilbenes synthesis in peanuts which agrees with Rudolf and Resurreccion (2005) who found that sliced peanuts produced the highest amounts compared to ground and chopped peanuts.

The analysis of variance (Table 4.8) indicated that all main factors of US power density, US exposure time and incubation time, and their interactions significantly ($P<0.05$) contributed to the concentrations of *trans*-resveratrol in chopped peanuts. The concentrations of *trans*-piceid in sliced peanuts were significantly contributed by main factors and their interactions excluding the interaction of power density and exposure time. Except for US exposure time, all other main

Table 4.10 Concentrations (mean ± standard deviation, dry basis) of stilbenes of roasted chopped ultrasound-treated peanuts and controls[1].

Trt #	PD	PT	IC	*Trans*-Resveratrol, μg/g	*Trans*-Piceid, μg/g	Total Stilbenes, μg/g
1	25	2	24	0.5921 ± 0.0207kl	0.2140± 0.0080ij	0.8061 ± 0.0287 l
2	25	2	36	0.5614 ± 0.0252l	0.2081± 0.0157ij	0.7695 ± 0.0366 l
3	25	2	48	0.7814 ± 0.0636jk	0.1891± 0.0207ij	0.9705 ± 0.0839jkl
4	25	5	24	1.0228 ± 0.0757hi	0.2749± 0.0383ij	1.2976 ± 0.1139ijk
5	25	5	36	0.8512 ± 0.0632ij	0.2582± 0.0396ij	1.1094 ± 0.0931jkl
6	25	5	48	1.1840 ± 0.0255h	0.4234± 0.0089hi	1.6074 ± 0.0337gh
7	25	8	24	0.6208 ± 0.0175kl	0.2590 ± 0.0050ij	0.8798 ± 0.0225 l
8	25	8	36	0.7002 ± 0.1373jkl	0.2556 ± 0.0442ij	0.9559 ± 0.1811kl
9	25	8	48	2.0755 ± 0.0648f	0.7144 ± 0.0255gh	2.7899 ± 0.0900f
10	50	2	24	1.2202 ± 0.0214h	0.3498 ± 0.0198i	1.5700 ± 0.0200ghi
11	50	2	36	1.4416 ± 0.0277g	0.4188 ± 0.0160hi	1.8600 ± 0.0424g
12	50	2	48	2.0411 ± 0.1531f	2.3322 ± 0.1514ab	4.3733 ± 0.3043b
13	50	5	24	1.4523 ± 0.0778g	0.3411 ± 0.0450i	1.7936 ± 0.0657gh
14	50	5	36	1.1788 ± 0.1164h	0.2996 ± 0.0340ij	1.4784 ± 0.1277hij
15	50	5	48	2.6174 ± 0.1567bcd	2.4442 ± 0.0672a	5.0617 ± 0.1784a
16	50	8	24	0.5631 ± 0.0040kl	0.7111 ± 0.0843gh	1.2742 ± 0.0881ijk
17	50	8	36	0.5098 ± 0.0490 l	1.2490 ± 0.1332cde	1.7588 ± 0.1821gh
18	50	8	48	1.9256 ± 0.0933f	2.1390 ± 0.1434b	4.0645 ± 0.2165bc
19	75	2	24	2.7949 ± 0.1936ab	1.4522 ± 0.8398c	4.2472 ± 0.6469b
20	75	2	36	2.6398 ± 0.0615bcd	0.688 ± 0.0216gh	3.3278 ± 0.0821e
21	75	2	48	2.9093 ± 0.0778a	0.9643 ± 0.1376efg	3.8737 ± 0.1484cd
22	75	5	24	2.7644 ± 0.3372abc	0.8366 ± 0.5613g	3.6001 ± 0.8473de
23	75	5	36	2.4528 ± 0.1713de	1.1424 ± 0.0714def	3.5952 ± 0.2368de
24	75	5	48	2.5655 ± 0.1871cd	0.7454 ± 0.0677g	3.3109 ± 0.2548e
25	75	8	24	2.6391 ± 0.2952bcd	0.8793 ± 0.0941fg	3.5184 ± 0.3684de
26	75	8	36	2.3406 ± 0.4277e	1.3421 ± 0.1738cd	3.6827 ± 0.2840de
27	75	8	48	2.7094 ± 0.1528abc	1.4185 ± 0.0636cd	4.1279 ± 0.2152bc
Control[2]	0	0	0	0.0212 ± 0.0020m	0.0271 ± 0.0010j	0.0482 ± 0.0046j

[1] PD= ultrasound power density (mW/cm^3), PT= ultrasound exposure time, and IC= incubation time at 25^{o}C.
Means ± standard deviation were calculated from two replications for a total of 4 analyses/sample.
Means in a column not followed by the same letter are significantly different from each other at $P < 0.05$ as determined by Fisher's significant difference mean separation test.
[2]Control is untreated raw whole peanuts.

factors and their interactions, significantly contributed to the concentrations of total stilbenes in chopped peanuts. Due to interaction effects, no main factor could be singled out as the major contributor to the concentrations of *trans*-resveratrol, *trans*-piceid, and total stilbenes in chopped US-treated peanuts.

Chopped US-treated peanuts had lower maximum *trans*-resveratrol but higher *trans*-piceid and total stilbenes compared to sliced UV-treated peanuts with 3.30, 1.05 and 4.00 µg/g, respectively. Chopped US-treated peanuts had higher *trans*-resveratrol, *trans*-piceid and total stilbenes compared to blended peanut butters with 0.41±0.02, 0.13±0.01, and 0.54±0.03 µg/g, respectively, and 100% natural peanut butters with 0.65±0.02, 0.14±0.01, and 0.814±0.03 µg/g, respectively (Ibern-Gomez et al., 2000).

The amounts of *trans*-resveratrol of 0.51- 2.91 µg/g produced in chopped US-treated peanuts were within the concentrations of 0.60 to 8.00 (mean=2.48) µg/mL in 18 Spanish red wines (Lamuella-Raventos et al. (1995) and higher those found in white and red grape juices with not detectable to 0.9 (mean=0.05) and not detectable to 1.09 (mean=0.5) µg/mL, respectively (Romero-Perez et al., 1999); dark chocolate and cocoa liquor with 0.40 and 0.50 µg/g, respectively (Counet et al., 2006); pistachios with 0.07-0.18 (mean=0.12) µg/g (Grippi et al., 2008); hops with 0.7-2.2 µg/g (Jerkovic and Collin, 2007); and hop pellets with 0.5 µg/g (Callemien et al., 2005). The levels of *trans*-resveratrol in chopped US-treated peanuts were lower compared to those in dried white and red grape berry skins with 11.04-47.60 (mean= 22.03) µg/g and 18.32-38.26 (mean=25.79) µg/g, respectively, (Romero-Perez et al., 2001).

The *trans*-piceid concentrations of 0.19-2.44 µg/g obtained by US treatment of chopped peanuts were higher compared to white grape juices with non-detectable to 0.83 (mean=0.18) µg/mL (Romero-Perez et al., 1999); dark chocolate and cocoa liquor with 1.00 and 1.20 µg/g,

respectively (Counet et al., 2006); within the concentrations of 18 Spanish red wines with mean of 1.85 (range=0.74 – 4.01) μg/mL in (Lamuella-Raventos et al., 1995) and hop pellets with 2.0 μg/g (Callemien et al., 2005); and within the lower limits of red grape juices with 0.77-7.34 μg/mL (Romero-Perez et al., 1999) and hops with 2.3-7.3 μg/g (Jerkovic and Collin, 2007). The concentrations of *trans*-piceid in chopped US-treated peanuts were lower compared to pistachios with mean of 6.97 (range=6.2-8.15) μg/g (Grippi et al., 2008) and dried white and red grape skins with 16.58 (range=non-quantifiable to 64.41) μg/g and 64.15 (range=5.49-342.66) μg/g, respectively.

2. Total phenolics of chopped ultrasound-treated peanuts

Table 4.11 shows the mean total phenolics concentrations of US treated chopped peanuts. US increased ($P< 0.05$) the total phenolics of 16 of 27 treated chopped peanuts to 1.37-1.80 mg GAE/g compared to untreated controls with 0.84 mg GAE/g whereas 11 of 27 treatments with 1.04 – 1.23 mg GAE/g were not different from controls of this study and controls of 0.91-1.14 mg GAE/g reported by Talcott et al. (2005). The amounts of total phenolics were slightly higher than the total phenolics of 1.09 to 1.38 mg/g reported by Rudolf and Resurreccion (2005) in chopped peanuts treated with a single dose of US power density of 39.2 mW/cm^3 for 4 min at 24-48 h incubation.

ANOVA of our data (Table 4.8) indicated that only interactions of US power density with incubation time, and with both exposure time and incubation time, significantly contributed to the concentrations of total phenolics of US-treated chopped. The main effects of US power density, US exposure time and incubation time did not significantly contribute to the total phenolics concentrations of chopped US-treated peanuts.

Table 4.11 Concentrations (mean ± standard deviation, dry basis) of of total phenolics, trolox equivalent antioxidant capacity (TEAC), and oxygen radical absorbance capacity (ORAC) total antioxidant capacity (TAC) of roasted chopped ultrasound-treated peanuts and controls[1].

Trt #	PD	PT	IC	Total Phenolics mgGAE/g	TEAC μM TE/g	H-ORAC μM TE/g	L-ORAC μM TE/g	TAC-ORAC μM TE/g
1	25	2	24	1.2124 ± 0.5466 cdefg	2.9511 ± 0.0128 ab	61.9800 ± 5.6897 abcde	8.9125 ± 2.6482 ijk	70.8950 ± 4.5109 bcdefgh
2	25	2	36	1.8022 ± 0.0150 a	2.7729 ± 0.2464 abcdefgh	57.9325 ± 4.7285 bcdef	15.3550 ± 0.4949 de	73.2825 ± 15.1567 abcd efgh
3	25	2	48	1.3712 ± 0.3752 bcdef	3.0065 ± 0.1503 a	36.7000 ± 9.3538 ghi	6.0275 ± 2.0586 klm	42.7275 ± 9.5065 kl
4	25	5	24	1.5569 ± 0.0673 abcd	2.81665 ± 0.1241 abcdef	50.1260 ± 5.7234 defgh	8.7160 ± 2.3031 jkl	58.8440 ± 7.9746 ghijk
5	25	5	36	1.2053 ± 0.3143 cdefg	2.9014 ± 0.0022 abcd	34.0800 ± 5.6013 hi	11.640 ± 1.3550 ghij	45.7175 ± 5.5737 jk
6	25	5	48	1.5563 ± 0.3728 abcd	2.9214 ± 0.4166 abc	58.4180 ± 9.2333 bcdef	22.2280 ± 2.8038 ab	80.6460 ± 20.0728 abcd
7	25	8	24	1.2090 ± 0.3404 cdefg	2.3897 ± 0.3771 defgh	50.4975 ± 10.7516 defgh	20.0775 ± 2.4692 bc	70.5750 ± 9.9056 bcdefgh
8	25	8	36	1.4901 ± 0.5439 abcde	2.9000 ± 0.1237 abcd	53.6650 ± 10.7756 cdef	11.4650 ± 0.4340 ghij	65.1350 ± 11.1192 cdefghi
9	25	8	48	1.1877 ± 0.3970 cdefg	2.4944 ± 0.6453 abcdefgh	59.7280 ± 13.8805 abcdef	21.1380 ± 3.7401 ab	80.8680 ± 12.0182 abc
10	50	2	24	1.5993 ± 0.1842 abcd	2.7367 ± 0.2663 abcdefgh	47.9025 ± 3.9253 efgh	12.4050 ± 1.5758 efgh	60.3050 ± 4.5662 fghij
11	50	2	36	1.1260 ± 0.2657 efg	3.0098 ± 0.0596 a	51.2125 ± 12.7687 defgh	13.4025 ± 3.0763 efg	64.6175 ± 15.1320 cdefghi
12	50	2	48	1.22955 ± 0.0971 cdefg	2.2966 ± 0.3563 gh	57.8550 ± 16.9097 bcdef	19.6500 ± 1.2700 bc	77.5050 ± 17.5028 abcdef

Table 4.11 continued…

Trt #	PD	PT	IC	Total Phenolics mgGAE/g	TEAC µM TE/g	H-ORAC µM TE/g	L-ORAC µM TE/g	TAC-ORAC µM TE/g
13	50	5	24	1.4592 ± 0.1471 abcdef	2.3514 ± 0.5853 efgh	52.0880 ± 10.3238 defg	10.1840 ± 1.5382 hij	62.2760 ± 10.879 efghij
14	50	5	36	1.5931 ± 0.1635 abcd	2.9404 ± 0.4839ab	65.0575 ± 9.3513 abcde	8.9450 ± 0.8868 ijk	74.0000 ± 8.4933 abcdefgh
15	50	5	48	1.6091 ± 0.2496 abc	2.3484 ± 0.4433 efgh	72.1500 ± 12.0976 ab	4.3475 ± 0.9181 m	76.4975 ± 12.9224 abcdefg
16	50	8	24	1.6719 ± 0.0190 ab	2.5414 ± 0.4381 abcdefgh	69.7600 ± 12.6688 abc	9.7550 ± 2.22082 hij	79.5175 ± 14.4563 abcde
17	50	8	36	1.1056 ± 0.2348 efg	2.8028 ± 0.5965 abcdefg	51.1350 ± 3.2806 defgh	17.1075 ± 5.9856 cd	68.2450 ± 5.3439 cdefgh
18	50	8	48	1.0455 ± 0.3506 fg	2.4161 ± 0.4753 cdefgh	57.5200 ± 11.6840 bcdef	12.5575 ± 3.9024 efgh	70.0750 ± 14.760 cdefgh
19	75	2	24	1.4393 ± 0.5878 abcdef	2.3427 ± 0.4353 fgh	62.0425 ± 17.9066 abcde	13.5600 ± 0.7442 efg	75.6025 ± 18.1250 abcdefg
20	75	2	36	1.1768 ± 0.2128 defg	2.5202 ± 0.4730 abcdefgh	49.3850 ± 4.51246 efgh	11.6075 ± 1.4875 ghij	60.9875 ± 5.1081 fghijk
21	75	2	48	1.6628 ± 0.1865 ab	2.4892 ± 0.6077 bcdefgh	58.3000 ± 17.9108 bcdef	15.1925 ± 3.06314 def	73.4925 ± 16.5316 abcdefgh
22	75	5	24	1.3951 ± 0.3616 abcdef	2.8616 ± 0.1078 abcde	70.7375 ± 16.4217 abc	12.0450 ± 0.3218 fghi	82.7800 ± 16.323 abc
23	75	5	36	1.1900 ± 0.2611 cdefg	2.9770 ± 0.1624 ab	76.6750 ± 20.4389 a	11.9050 ± 1.31186 ghij	88.5725 ± 19.4278 ab
24	75	5	48	1.6680 ± 0.2523 ab	2.2829 ± 0.4063 h	37.7225 ± 5.6900 fgh	11.3175 ± 0.7410 ghij	49.0400 ± 6.23136 ijk

Table 4.11 continued…

Trt #	PD	PT	IC	Total Phenolics mgGAE/g	TEAC µM TE/g	H-ORAC µM TE/g	L-ORAC µM TE/g	TAC-ORAC µM TE/g
25	75	8	24	1.2238 ± 0.4425 cdefg	2.7906 ± 0.1137 abcdefgh	43.7525 ± 3.9457 fgh	13.4100 ± 0.0200 efg	57.1600 ± 3.9295 hijk
26	75	8	36	1.6676 ± 0.1488 ab	2.9451 ± 0.1980 ab	67.0275 ± 17.6332 abcd	24.1100 ± 2.3978 a	91.1400 ± 19.8614 a
27	75	8	48	1.7360 ± 0.1378 ab	2.6021 ± 0.3832 abcdefgh	51.3120 ± 10.7960 defgh	11.0000 ± 2.1985 ghij	62.3140 ± 12.5966 defghij
Control [2]	0	0	0	0.8439 ± 0.0202 g	0.6062 ± 0.0029 i	21.1967 ± 5.3026 i	5.6100 ± 0.4574 lm	26.8100 ± 5.7197 l

[1] PD= ultrasound power density (mW/cm^3), PT= ultrasound exposure time, and IC= incubation time at 25°C.
Means ± standard deviation were calculated from two replications for a total of 4 analyses/sample.
Means in a column not followed by the same letter are significantly different from each other at $P < 0.05$ as determined by Fisher's significant difference mean separation test.
[2]Control is untreated raw whole peanuts.

The maximum total phenolics concentration of 1.80 mg GAE/g achieved in chopped US-treated peanuts were not significantly different from 1.89 mg GAE/g produced in sliced ultrasound-treated peanuts and 1.82 mg GAE produced in UV-treated peanuts. The total phenolics of 1.04-1.80 mg GAE/g in chopped US-treated peanuts obtained in this study were within the total phenolics of blueberry wines with 0.60-1.86 mg GAE/g (Sanchez-Moreno et al., 2003), below the lower limits of 1.81-4.58 mg GAE/g in fresh blueberry purees (Prior et al., 1998) and within the lower concentrations of 1.31-2.39 mg GAE/mL in red wines (Fernandez-Pachon et al., 2004*)*.

2. TEAC of chopped ultrasound-treated peanuts

Table 4.11 shows the mean TEAC values of chopped US-treated chopped peanuts. US increased ($P< 0.05$) TEAC of all 27 treated chopped peanuts with 2.28 to 3.01 μM TE/g compared to controls with 0.61 μM TE/g. The analysis of variance (Table 4.8) indicated that only incubation time and the interaction of US power density and exposure time contributed significantly ($P< 0.05$) to the TEAC values of chopped US-treated peanuts. Mean separation test showed that incubation time of 36 h produced the highest mean TEAC of 2.86 μM TE/g compared to those at 24 and 48 h were not significantly different from each other at levels of 2.64 and 2.54 μM TE/g, respectively.

The maximum TEAC of 3.01 μM TE/g produced in chopped US-treated peanuts was not different from 3.17 μM TE/g in sliced US-treated and 3.37μM TE/g in sliced UV-treated peanuts. The TEAC of 2.28-3.01 μM TE/g of chopped US-treated peanuts were below the lower limit of 16 types of red wines with 3.06 to 11.15 μM TE/mL (Fernandez-Pachon et al., 2004).

4. Oxygen radical antioxidant capacity (ORAC) of chopped ultrasound-treated peanuts

4.1 Total Antioxidant Capacity (TAC) of chopped ultrasound-treated peanuts

The mean total antioxidant capacities of chopped US-treated peanuts are presented in Table 4.11. US increased ($P<0.05$) the total antioxidant capacity of 26 of 27 chopped US-treated peanuts with the range of 45.72 ± 9.51 to 91.14 ± 19.86 μM TE/g compared to 26.81 ± 5.72 μM TE/g in controls whereas one treatment with 42.73 ± 9.50 μM TE/g was not different from controls. The analysis of variance (Table 4.8) showed that only the interaction of US power density and incubation time, and their interaction of exposure time significantly contributed to the total antioxidant capacity of chopped US-treated peanuts. No main factor significantly contributed to the total antioxidant capacities of chopped US-treated peanuts.

4.2 H-ORAC of chopped ultrasound-treated peanuts

Table 4.11 shows the mean H-ORAC values of chopped US-treated peanuts. US increased the H-ORAC in 24 of 27 chopped US-treated peanuts which ranged from 43.75±3.95 to 76.68 ± 20.44 μM TE/g, compared to controls with 21.20 ± 5.30 μM TE/g whereas all other 4 treatments with 34.08 ± 5.60 to 37.72 ± 5.69 μM TE/g were not different from controls. The analysis of variance (Table 4.8) showed that US power density and its interactions with incubation time, and with both exposure time and incubation time significantly contributed to H-ORAC of chopped US-treated peanuts. Due to interaction effect, the US power density could not be singled out as the major contributor to the hydrophilic antioxidants in chopped US-treated peanuts. The maximum H-ORAC of 76.68 μM TE/g in chopped US-treated peanuts were not significantly different from 78.75 μM TE/g produced in sliced US-treated peanuts suggesting that slicing and chopping will have the same degree of enhancements of hydrophilic antioxidants in peanuts.

4.3 L-ORAC of chopped ultrasound-treated peanuts

Table 4.11 shows the mean L-ORAC values of chopped US-treated peanuts. US increased the L-ORAC in 24 of 27 treated chopped peanuts with 8.91 ± 2.64 to 24.11± μM TE/g, compared to 5.61± 0.46 μM TE/g in controls whereas all other 3 treatments were not different from controls with 4.35 ± 0.92 to 8.72 ± 2.30 μM TE/g.

The analysis of variance (Table 4.8) showed that all main factors and their interactions significantly contributed to lipophilic antioxidants of chopped US-treated peanuts. The maximum L-ORAC of 24.11±2.40 μM TE/g in chopped US-treated peanuts was significantly lower than 32.31 μM TE/g in sliced ultraound-treated peanuts suggesting that slicing enhanced the lipophilic antioxidants greater than chopping of peanuts.

C. Effect of Ultrasound on the Concentrations of Stillbenes, Total Phenolics, and Antioxidant Capacities of Whole Peanut Kernels

1. *Trans*-resveratrol, *trans*-picied, and total stilbenes of whole ultrasound-treated peanuts

The mean concentrations of *trans*-resveratrol, *trans*-piceid and total stilbenes in whole US-treated peanuts are shown in Table 4.12. US increased (P<0.05) the mean concentrations of *trans*-resveratrol to 0.99±1.11 μg/g in only one of 27 treated whole peanuts compared to controls with 0.02 ±0.002 μg/g, whereas all other 26 treatments with concentrations ranging from 0.01±0.003 to 0.16±0.009 μg/g were not different from controls. *Trans*-piceid and total stilbenes increased in all 27 whole US-treated peanuts with mean concentrations ranging from 0.50±0.04 to 2.60±0.44 μg/g and 0.60±0.06 to 2.76±0.21 μg/g, respectively, compared to controls with corresponding concentrations of 0.03±0.001 and 0.05± 0.004μg/g. The concentrations of total stilbenes in whole US-treated peanuts were largely contributed by *trans*-piceid rather than *trans*-resveratrol.

Table 4.12 Concentrations (mean ± standard deviation, dry basis) of stilbenes of roasted whole ultrasound-treated peanuts and controls[1].

Trt #	PD	PT	IC	*Trans*-Resveratrol µg/g	*Trans*-Piceid µg/g	Total Stilbenes µg/g
1	25	2	24	0.0102 ± 0.0031b	1.0797 ±0.0054gh	1.0899 ±0.0055hi
2	25	2	36	0.0168 ± 0.0108b	0.9013 ±0.1887hi	0.9181 ±0.1953ij
3	25	2	48	0.0177 ± 0.0015b	1.0779 ±0.0173gh	1.0955 ±0.0188hi
4	25	5	24	0.0109 ± 0.0014b	1.5131 ±0.0392ef	1.5241 ±0.0386g
5	25	5	36	0.0111 ± 0.0030b	1.0682 ±0.0299gh	1.0793 ±0.0272
6	25	5	48	0.0109 ± 0.0030b	1.3062 ±0.1077fg	1.3171 ±0.1082gh
7	25	8	24	0.0141 ± 0.0012b	1.1041 ±0.0516gh	1.1182 ±0.0513hi
8	25	8	36	0.0343 ± 0.0315b	1.3650 ±0.0815fg	1.3992 ±0.0699g
9	25	8	48	0.0229 ± 0.0028b	1.3475 ±0.1110fg	1.3704 ±0.1114g
10	50	2	24	0.1006 ± 0.0075b	2.2509 ±0.0180abc	2.3515 ±0.0186de
11	50	2	36	0.1080 ± 0.0097b	0.5547 ±0.0194ij	0.6627 ±0.0100k
12	50	2	48	0.1405 ± 0.0101b	0.5523 ±0.0200ij	0.6928 ±0.0180jk
13	50	5	24	0.1132 ± 0.0129b	0.5280 ±0.0153ij	0.6412 ±0.0235k
14	50	5	36	0.1122 ± 0.0113b	0.5177 ±0.0132j	0.6300 ±0.0228k
15	50	5	48	0.1598 ± 0.0997b	2.2542 ±0.0585abc	2.4141 ±0.0590cd
16	50	8	24	0.1228 ± 0.0197b	2.5993 ±0.4408a	2.7221 ±0.4604ab
17	50	8	36	0.0959 ± 0.0590b	0.5620 ±0.0535ij	0.6578 ±0.0993k
18	50	8	48	0.1007 ± 0.0162b	0.4948 ±0.0433j	0.5955 ±0.0554k
19	75	2	24	0.0357 ± 0.0100b	2.0757 ±0.1549cd	2.1113 ±0.1620ef
20	75	2	36	0.0386 ± 0.0066b	2.0285 ±0.1704cd	2.0671 ±0.1768f
21	75	2	48	0.0256 ± 0.0030b	2.0850 ±0.0444cd	2.1106 ±0.0462ef
22	75	5	24	0.0292 ± 0.0055b	2.4818 ±0.2067ab	2.5109 ±0.2076bcd
23	75	5	36	0.0252 ± 0.0100b	1.9458 ±0.3356cd	1.9710 ±0.3448f
24	75	5	48	0.0242 ± 0.0069b	2.1028 ±0.1841cd	2.1270 ±0.1886ef
25	75	8	24	0.0321 ± 0.0208b	2.1303 ±0.4200bcd	2.1624 ±0.4289ef
26	75	8	36	0.9875 ± 0.1142a	1.7769 ±1.0638de	2.7643 ±0.1218a
27	75	8	48	0.0208 ± 0.0011	2.5965 ±0.1958a	2.6173 ±0.1969abc
Control[2]	0	0	0	0.0212 ± 0.0020h	0.0271 ±0.0010k	0.0482 ±0.0046 l

[1] PD= ultrasound power density (mW/cm^3), PT= ultrasound exposure time, and IC= incubation time at 25^{o}C.

Means ± standard deviation were calculated from two replications for a total of 4 analyses/sample.

Means in a column not followed by the same letter are significantly different from each other at $P < 0.05$ as determined by Fisher's significant difference mean separation test.

[2]Control is untreated raw whole peanuts.

The analysis of variance (Table 4.8) indicated that except for the main effects of US exposure and incubation times, their interactions with other factors and the main factor of power density significantly (P<0.05) contributed to the concentrations of *trans*-resveratrol in whole peanuts. The concentrations of *trans*-piceid in sliced peanuts were significantly contributed by all factors and their interactions excluding the interaction effect of power density and esposure time. All factors and their interactions significantly contributed to the concentrations of total stilbenes in sliced peanuts. Due to interaction effects, no main effect could be singled out as the major contributor to the concentrations of trans-resveratrol, trans-piceid, and total stilbenes in whole US-treated peanuts.

The maximum *trans*-resveratrol and total stilbenes concentrations of 0.99 and 2.76 μg/g in whole US-treated peanuts were significantly lower than those sliced peanuts with 6.39 and 9.81 μg/g, respectively (Table 4.10). The maximum *trans*-resveratrol 0.99 μg/g produced in whole US-treated peanuts, although lower than 2.91 μg/g in chopped peanuts, was not significant different. Whole US-treated peanuts had a maximum *trans*-piceid concentration of 2.60 μg/g which was not significantly different from chopped with 2.44 μg/g but significantly lower compared to sliced peanuts with 6.39 μg/g. These findings suggests that less severe size reduction through slicing will further enhance the synthesis of stilbenes in peanuts more than chopping. Whole US-treated peanuts had lower maximum *trans*-resveratrol and total stilbenes concentrations but higher *trans*-piceid compared to UV-treated peanuts with 3.30, 4.00 μg/g, and 1.05, respectively.

Whole US-treated sliced peanuts had lower *trans*-resveratrol but higher *trans*-piceid and total stilbenes compared to blended peanut butters with 0.41$\pm$0.02, 0.13$\pm$0.01, and 0.54$\pm$0.03 μg/g,

respectively, and 100% natural peanut butters with 0.65±0.02, 0.14±0.01, and 0.814±0.03 µg/g, respectively (Ibern-Gomez et al., 2000).

Whole US-treated peanuts produced 0.01-0.99 µg/g *trans*-resveratrol of producedwhich were higher than pistachios with 0.07-0.18 (mean=0.12) µg/g (Grippi et al., 2008); within the concentrations of 0.40 and 0.50 µg/g in dark chocolate and cocoa liquor, respectively (Counet et al., 2006) and 0.5 µg/g in hop pellets (Callemien et al., 2005); and within the lower limit of 0.60 to 8.00 (mean=2.48) µg/mL in 18 Spanish red wines (Lamuella-Raventos et al. (1995), in white and red grape juices with not detectable to 0.9 (mean=0.05) µg/mL and not detectable to 1.09 (mean=0.5) µg/mL, respectively (Romero-Perez et al., 1999), and in hops with 0.7-2.2 µg/g (Jerkovic and Collin, 2007). The levels of *trans*-resveratrol in whole US-treated peanuts were lower compared to 11.04-47.60 (mean= 22.03) µg/g and 18.32-38.26 (mean=25.79) µg/g in dried white and red grape berry skins, respectively (Romero-Perez et al., 2001).

The *trans*-piceid concentrations of 0.50-2.60 µg/g obtained by US treatment of whole peanuts were higher compared to white grape juices with non-detectable to 0.83 (mean=0.18) µg/mL (Romero-Perez et al., 1999); dark chocolate and cocoa liquor with 1.00 and 1.20 µg/g, respectively (Counet et al., 2006); within the concentrations of 18 Spanish red wines with mean of 1.85 (range=0.74 – 4.01) µg/mL (Lamuella-Raventos et al., 1995), and hop pellets with 2.0 µg/g (Callemien et al., 2005); and within the lower limits of red grape juices with 0.77-7.34 µg/mL (Romero-Perez et al., 1999) and hops with 2.3-7.3 µg/g (Jerkovic and Collin, 2007). The concentrations of *trans*-piceid in whole US-treated peanuts were lower compared to pistachios with mean of 6.97 (range=6.2-8.15) µg/g (Grippi et al., 2008) and dried white and red grape skins with 16.58 (range=non-quantifiable to 64.41) µg/g and 64.15 (range=5.49-342.66) µg/g, respectively.

2. Total phenolics of whole ultrasound-treated peanuts

Table 4.13 shows the mean total phenolics concentrations of US-treated whole peanuts. US increased ($P< 0.05$) the total phenolics of all 27 treated chopped peanuts to 1.14-1.84 mg GAE/g compared to untreated controls with 0.84 mg GAE/g. The amounts of total phenolics were slightly higher than the total phenolics of 1.31 to 1.47 mg/g reported by Rudolf and Resurreccion (2005) in whole peanuts treated with a single dose of US power density of 39.2 mW/cm^3 for 4 min at 24-48 h incubation. ANOVA of our data (Table 4.8) indicated that all main effects- US power density and exposure time and incubation time, and the interaction of power density and exposure time significantly contributed to the total phenolics concentrations of whole US-treated peanuts.

The maximum total phenolics concentration of 1.84 mg GAE/g achieved in whole US-treated peanuts were not significantly different from 1.89 and 1.80 mg GAE/g produced in sliced and chopped US-treated peanuts, respectively, and from 1.82 mg GAE/g obtained in UV-treated sliced peanuts. The total phenolics of 1.14-1.84 mg GAE/g in chopped US-treated peanuts obtained in this study were within the total phenolics of blueberry wines with 0.60-1.86 mg GAE/g (Sanchez-Moreno et al., 2003), and within the lower limits of 1.81-4.58 mg GAE/g in fresh blueberry purees (Prior et al., 1998) and 1.31-2.39 mg GAE/mL in red wines (Fernandez-Pachon et al., 2004*).*

3. TEAC of whole ultrasound-treated peanuts

Table 4.13 shows the mean TEAC values of US-treated whole peanuts. US increased ($P< 0.05$) TEAC of all 27 treated whole peanuts with 2.28 to 3.18 μM TE/g compared to controls with 0.61 μM TE/g. The analysis of variance (Table 4.8) indicated that incubation time, and its interactions with US exposure time and with US power density and exposure time, significantly

Table 4.13 Concentrations (mean ± standard deviation, dry basis) of total phenolics, trolox equivalent antioxidant capacity (TEAC), and oxygen radical absorbance capacity (ORAC) total antioxidant capacity (TAC) of roasted whole ultrasound-treated peanuts and controls[1].

Trt #	PD	PT	IC	Total Phenolics, mgGAE/g	TEAC, μM TE/g	H-ORAC, μM TE/g	L-ORAC, μM TE/g	TAC-ORAC, μM TE/g
1	25	2	24	1.3408 ± 0.0981 ef	2.1600 ± 0.0157 fgh	24.4350 ± 6.6097 ef	4.9700 ± 1.774 hi	29.4025 ± 7.6162 fgh
2	25	2	36	1.6148 ± 0.2855 abcd	2.6099 ± 0.5175 abcdefgh	24.2600 ± 5.3332 def	5.7600 ± 1.7138 ghi	30.0175 ± 6.7564 efgh
3	25	2	48	1.5906 ± 0.1340 abcde	2.8027 ± 0.4255 abcde	27.3050 ± 8.1658 cdef	6.3950 ±1.4325 ghi	33.7025 ± 7.7593 defgh
4	25	5	24	1.3764 ± 0.0968 def	1.9916 ± 0.0381 h	30.5975 ± 7.2396 bcdef	3.6050 ±1.3964 i	34.2025 ± 6.5433 defgh
5	25	5	36	1.4666 ± 0.0953 cde	2.9499 ± 0.1592 abcd	26.9300 ± 7.2457 cdef	10.4275 ± 2.2625 abcde	37.3550 ± 8.4741 bcdefgh
6	25	5	48	1.6210 ± 0.0430 abcd	2.5768 ± 0.5623 abcdefgh	29.9375 ± 7.0309 cdef	8.1125 ± 3.6847 defg	38.0500 ± 8.7599 bcdefgh
7	25	8	24	1.5824 ± 0.0148 abcde	3.1332 ± 0.2544 ab	20.3350 ± 3.8471 f	6.5675 ± 1.9980 fghi	26.9050± 2.5660
8	25	8	36	1.8159 ± 0.1981a	2.6040 ± 0.6969 abcdefgh	24.8375 ± 7.7150 def	4.9000 ± 2.8798 i	29.7400 ± 8.0889 efgh
9	25	8	48	1.7920 ± 0.1457ab	2.5996 ± 0.6192 abcdefgh	26.1375 ± 7.3142 cdef	10.7050 ± 2.9737 abcd	36.8425 ± 6.4548 cdefgh
10	50	2	24	1.5078 ± 0.1280 cde	2.4009 ± 0.7013 defgh	21.2450 ± 3.6711 ef	6.8950 ±1.3135 efgh	28.1375 ± 3.8209 gh
11	50	2	36	1.5400 ± 0.1281 bcde	2.8496 ± 0.2102 abcde	26.0650 ± 2.9290 cdef	8.2325 ± 3.9846 cdefg	34.2950 ± 6.3943 defgh
12	50	2	48	1.8355 ± 0.3939a	2.4704 ± 0.4190 cdefgh	24.5150 ± 5.4604 def	13.2575 ±3.5487 a	37.7725 ± 7.9370 bcdefgh

Table 4.13 continued…

Trt #	PD	PT	IC	Total Phenolics, mgGAE/g	TEAC, μM TE/g	H-ORAC, μM TE/g	L-ORAC, μM TE/g	TAC-ORAC, μM TE/g
13	50	5	24	1.4476 ± 0.1010 cde	2.0191 ± 0.1419 gh	29.9950 ± 1.3086 cdef	8.2175 ± 0.3743 defg	38.2150 ± 1.6168 bcdefg
14	50	5	36	1.1424 ± 0.3259f	2.3151 ± 0.4622 efgh	40.8000 ± 1.2706 b	7.5925 ± 1.3934 efgh	48.3950 ±11.0960 b
15	50	5	48	1.4250 ± 0.1224 de	3.1792 ± 0.2002 a	25.6875 ± 2.7687 cdef	8.1475± 1.3597 defg	33.8350 ± 4.0121 defgh
16	50	8	24	1.4167 ± 0.1523 de	2.5358 ± 0.2952 bcdefgh	28.8300 ± 1.7223 cdef	12.1625 ± 2.5016 ab	40.9900 ± 1.3305 bcde
17	50	8	36	1.4490 ± 0.0368 cde	2.7412 ± 0.5325 abcdef	24.1000 ± 2.0194 def	11.4975 ± 2.0545 ab	35.6000 ± 3.4908 defgh
18	50	8	48	1.5280 ± 0.1890 bcde	2.7173 ± 0.3862 abcdef	66.9425 ±18.0597 a	8.4350 ±1.0508 cdefg	75.3775 ±17.2889 a
19	75	2	24	1.6398 ± 1.6398 abcd	2.6780 ± 0.3009 abcdef	27.9925 ± 6.1507 cdef	6.3075± 2.6470 fghi	34.2975 ± 8.7363 defgh
20	75	2	36	1.8180 ± 0.2434a	2.4648 ± 0.6244 cdefgh	33.9900 ± 9.2933 bcd	10.8500 ±1.9915 abcd	44.8400 ± 7.5435 bcd
21	75	2	48	1.8437 ± 0.2770a	2.5890 ± 0.4122 abcdefgh	32.3375 ± 8.3851 bcd	10.9950 ±3.6861 abcd	43.3350 ± 8.4607 bcd
22	75	5	24	1.5297 ± 0.1750 bcde	2.4765 ± 0.6856 bcdefgh	29.5725 ± 2.8948 cdef	10.5225 ± 1.5572 abcde	40.0925 ± 2.9426 bcdef
23	75	5	36	1.5156 ± 0.1064 cde	3.0356 ± 0.2010 abc	26.8900 ± 8.2645 cdef	9.2475 ± 1.65999 bcdef	36.1425 ± 9.7593 cdefgh
24	75	5	48	1.5939 ± 0.1007 abcde	2.7358 ± 0.6568 abcdef	24.7875 ± 6.2986 def	11.2750 ± 0.5319 abc	36.0650 ± 6.4150 cdefgh

Table 4.13 continued…

Trt #	PD	PT	IC	Total Phenolics, mgGAE/g	TEAC, μM TE/g	H-ORAC, μM TE/g	L-ORAC, μM TE/g	TAC-ORAC, μM TE/g
25	75	8	24	1.7118 ± 0.0924 abc	2.6364 ± 0.6263 abcdef	34.1000 ± 9.6919 bcd	10.6550 ± 0.3627 abcd	44.7550 ±10.0036 bcd
26	75	8	36	1.6134 ± 0.0858 abcd	3.0280 ± 0.1389 abcd	31.7950 ±10.2943 bcde	10.3225 ±0.6939 abcde	42.1150 ±10.1592 bcd
27	75	8	48	1.5224 ± 0.5025 bcde	2.6327 ± 0.5859 abcdef	35.7825 ±10.6806 bc	11.1425 ±1.4593 abcd	46.9250 ±11.8918 bc
Control [2]	0	0	0	0.8439 ± 0.0202 g	0.6062 ± 0.0029 i	21.1967 ± 5.3026 ef	5.6100 ± 0.4574 ghi	26.8100 ± 5.7197 2 h

[1] PD= ultrasound power density (mW/cm^3), PT= ultrasound exposure time, and IC= incubation time at 25^{o}C.
Means ± standard deviation were calculated from two replications for a total of 4 analyses/sample.
Means in a column not followed by the same letter are significantly different from each other at $P < 0.05$ as determined by Fisher's significant difference mean separation test.
[2]Control is untreated raw whole peanuts.

($P< 0.05$) contributed to the TEAC values of whole US-treated peanuts peanuts. Due to interaction effects, incubation time could not be singled out as the major contributor to the TEAC of whole US-treated peanuts.

The maximum TEAC values achieved in whole US-treated peanuts were not significantly different from those produced in sliced and chopped US treated peanuts suggesting that size reduction had no effect in enhancing the TEAC values of US-treated peanuts. The TEAC values of 2.28 to 3.18 μM TE/g μM TE/g in whole US-treated peanuts were within the lower limit of 16 types of red wines with 3.06 to 11.15 μM TE/mL (Fernandez-Pachon et al., 2004).

4. Oxygen radical antioxidant capacity (ORAC) of whole ultrasound-treated peanuts

4.1 Total Antioxidant Capacity (TAC) of whole ultrasound-treated peanuts

The mean total antioxidant capacities of chopped US-treated peanuts are presented in Table 4.13. US increased ($P<0.05$) the total antioxidant capacity of only 10 of 27 US-treated whole peanuts with the range of 38.22 – 75.38 μM TE/g compared to 26.81 μmol TE/g in controls whereas all other 17 treatments with 26.91-38.05 μM TE/g were not different from controls. The analysis of variance (Table 4.8) showed that all factors and their interactions, excluding the interaction of US power density with incubation time significantly contributed to the total antioxidant capacity of whole US-treated peanuts. Due interaction effects, no main factor could be singled out as the major contributor to the total antioxidant capacities of whole US-treated peanuts.

4.2 H-ORAC of chopped ultrasound-treated peanuts

Table 4.13 shows the mean H-ORAC values of whole US-treated peanuts. US increased the H-ORAC in only seven of 27 whole US-treated peanuts with concentrations ranging from 31.80-66.94 μM TE/g, compared to controls with 21.20 μM TE/g whereas all other 20 treatments with

20.34-30.60 μM TE/g were not different from controls. The analysis of variance (Table 4.8) showed that except for the interaction of US power density and incubation time, all main and interaction effects significantly contributed to H-ORAC of whole US-treated peanuts. The maximum H-ORAC of 66.94 μM TE/g in US-treated wholed peanuts were not significantly different from 78.75 and 76.68 μM TE/g produced in sliced and chopped US-treated peanuts, respectively, suggesting that slicing and chopping will not further enhance of hydrophilic antioxidants in US-treated peanuts.

4.3 L-ORAC of whole ultrasound-treated peanuts

Table 4.13 shows the mean L-ORAC values of whole US-treated peanuts. US increased the L-ORAC in 18 of 27 treated chopped peanuts with 8.11-13.26 μM TE/g, compared to 5.61 μM TE/g in controls whereas all other 9 treatments were not different from controls with 3.61-7.59 μM TE/g. ANOVA (Table 4.8) showed that except for the interaction of US power density with either US exposure or incubation times, all main and interaction effects significantly contributed to lipophlic antioxidants whole US-treated peanuts. Due to interaction effects, no main factor could be singled out as the major contributor to the L-ORAC of whole US-treated peanuts.

The maximum L-ORAC of 13.26 μM TE/g in whole US-treated peanuts was significantly lower than 24.11 and 32.31 μM TE/g in chopped and sliced US-treated peanuts; and sliced had the greatest increase. This finding suggests that size reductions such as chopping and slicing further enhanced the lipophilic antioxidants in US-treated peanuts and less severe damage to the cells such as slicing produced the greatest enhancements of lipophilic antioxidants.

D. Comparison of the Maximum Levels of Stilbenes, Total Phenolics and Antioxidant Capacities of the Different Sizes of Ultrasound Treated Peanuts

Table 4.14 shows the comparison of the maximum concentrations of stilbenes and total phenolics, and antioxidant capacities produced in sliced, chopped and whole US-treated peanuts. Results showed that sliced peanuts obtained significantly highest (P>0.05) *trans*-resveratrol and *trans*-piceid concentrations, compared to chopped and whole peanuts. The *trans*-resveratrol concentration, although higher in chopped peanuts was not significantly different from whole peanuts. *Trans*-piceid concentrations were not significantly different between chopped and whole US-treated peanuts. This result confirms the previous findings of Rudolf and Resurreccion (2005) who observed increased *trans*-resveratrol concentrations of US-treated peanuts as the severity of damage to the cells through size reduction decreased, from 0.75 μg/g in ground (1-2 mm) peanuts to 1.3 μg/g in chopped (2-5 mm), and the highest concentration of 3.96 μg/g in sliced (~7 mm) peanuts. The whole US-treated peanuts had a maximum *trans*-resveratrol of 1.60 μg/g (Rudolf and Resurreccion, 2005) suggesting that wounding is necessary to achieve the maximum enhancement of trans-resveratrol biosynthesis in peanuts.

No significant differences in the total phenolics concentrations and TEAC values were observed between chopped, sliced and whole US-treated peanuts. This finding verified the results of Rudolf and Resurreccion (2005) who found no significant differences in the total phenolics and antioxidant capacities of ground, chopped, sliced and whole US-treated peanuts. Similarly, the hydrophilic ORAC and total antioxidant capacities did not differ significantly between chopped, sliced and whole US-treated peanuts. On the basis of lipophilic antioxidants, slicing produced the highest antioxidant capacity, followed by chopping and whole peanuts had the least,

Table 4.14 Maximum concentrations (mean ± standard deviation, dry basis) of stilbenes, total phenolics, and antioxidants in ultrasound-treated chopped, sliced, and whole ultrasound-treated peanuts[1]

Compound	Sliced	Chopped	Whole
Trans-resveratrol,µg/g	6.3892±2.1520a	2.9095± 0.0778b	0.9875±0.1142b
Trans-piceid, µg/g	6.3927± 2.2708a	2.4443±0.0672b	2.5993±0.4408b
Total stilbenes, µg/g	9.8620±0.8221a	5.0617± 0.1783b	2.7643±0.1217c
Total phenolics, mgGAE/g	1.8882±0.1454a	1.8022±0.0150a	1.8438±0.2770a
TEAC, µM TE/g	3.1741±0.1969a	3.0098±0.0596a	3.1793± 0.2002a
Hydrophilic ORAC, µM TE/g	78.75 ± 24.37a	76.68 ± 20.44a	66.94±18.06a
Lipophilic ORAC, µM TE/g	32.31± 7.24a	24.11± 2.40b	13.26± 3.55c
Total Antioxidant Capacity µM TE/g	97.92 ± 31.88a	91.14 ± 19.86a	75.38± 17.29a

[1]Means not followed by the same letter within each row are not significantly (P<0.05) different from each other as determined by Fisher's least significant difference mean separation

suggesting that mild damage to the cell through slicing is needed to achieve the greatest enhancement of lipophilic antioxidants in US-treated peanuts.

The above findings confirmed that slicing of peanuts prior to US treatment produced the highest maximum concentrations of *trans*-resveratrol in peanuts. Process optimization for US using sliced peanut was therefore performed to determine the process parameters that will produce peanuts with the highest concentrations of *trans*-resveratrol, total phenolics andantioxidant capacities.

E. Consumer Overall Acceptance of Ultrasound-Treated Peanuts

Table 4.15 presents the consumers' mean overall acceptance hedonic ratings of US-treated peanuts. Consumer test results showed that the overall acceptance ratings of all US-treated peanuts regardless of size ranged from 4.2 ± 2.5 to 6.0 ± 1.8 or neither like nor dislike, and were lower ($P< 0.05$) than that of controls with 7.4 ± 1.4. Sliced, chopped, and whole US-treated peanuts had overall acceptance rating of 5.0, 5.0 and 5.1 or neither like nor dislike which were not significantly different from each other, but significantly lower compared to UV treatment with 5.7 ± 1.7 or near like slightly. UV-treated peanuts were not expected to have high overall acceptance compared to US-treated peanuts as UV exposure results in the development of off-flavors in food due to the initiation of lipid peroxidation (Duh and Yen, 1995). The presence of higher levels of *trans*-resveratrol, *trans- piceid* and polyphenolic antioxidants in US- compared to UV-treated sliced peanuts may have resulted in lower acceptance of all sizes of US-treated samples compared to UV. In food, phenolics may contribute to the bitterness, astringency, color, flavor, and odor of food (Naczk and Shahidi, 2006) resulting in lower overall acceptance of US-treated peanuts. This finding poses challenge to the researchers in finding ways to mask the off-flavors/tastes in US-treated peanuts in food applications.

Table 4.15 Consumers' overall acceptance rating (mean ± standard deviation) for roasted ultrasound-treated sliced, chopped, and whole peanuts and controls[1]

Treatment #	PD	PT	IC	Sliced	Chopped	Whole
1	25	2	24	5.5 ± 1.8bcde	4.8 ± 2.2efghij	5.2 ± 1.4 bcdefghi
2	25	2	36	5.0 ± 1.8cdefghi	5.2 ± 2.0bcdef	5.2 ± 1.7bcdefghi
3	25	2	48	4.4 ± 2.0ij	5.2 ± 2.6bcdef	5.5 ± 2.0bcdefg
4	25	5	24	4.9 ± 1.8defghij	5.2 ± 1.8bcdefg	5.3 ± 1.8bcdefgh
5	25	5	36	5.3 ± 1.7bcdefg	5.7 ± 1.7bcd	5.0 ± 2.0defghi
6	25	5	48	5.2 ± 1.6cdefgh	4.9 ± 2.2defghi	4.5 ± 1.8ijk
7	25	8	24	4.6 ± 2.1ghij	5.3 ± 1.6bcdef	5.3 ± 1.4bcdefgh
8	25	8	36	4.9 ± 2.3defghij	4.7 ± 2.1fghij	5.5 ± 2.1bcdef
9	25	8	48	4.7 ± 2.2fghij	5.1 ± 2.5cdefgh	4.9 ± 2.1efghij
10	50	2	24	6.0 ± 1.8b	5.8 ± 2.0bc	5.1 ± 2.2cdefghi
11	50	2	36	4.5 ± 2.0hij	4.3 ± 2.1ghij	5.3 ± 2.1bcdefgh
12	50	2	48	4.2 ± 2.5j	4.2 ± 1.9ij	3.8 ± 1.9k
13	50	5	24	4.5 ± 2.3hij	5.4 ± 2.0bcdef	5.9 ± 1.6b
14	50	5	36	4.8 ± 2.0defghij	5.3 ± 2.1bcdef	4.8 ± 1.9fghij
15	50	5	48	5.4 ± 2.5bcdef	5.3 ± 2.0bcdef	5.7 ± 1.8bcd
16	50	8	24	5.4 ± 2.0bcdef	5.6 ± 2.2bcde	5.3 ± 1.5bcdefgh
17	50	8	36	5.8 ± 2.3bc	5.3 ± 1.8bcdef	5.6 ± 1.8bcde
18	50	8	48	4.7 ± 2.0efghij	5.0 ± 2.3defgh	5.8 ± 2.0bc
19	75	2	24	5.0 ± 1.9cdefghi	6.0 ± 2.0b	4.7 ± 2.2hij
20	75	2	36	5.3 ± 2.0bcdefg	5.0 ± 2.0defghi	5.1 ± 1.7cdefghi
21	75	2	48	5.2 ± 1.8cdefgh	4.1 ± 2.0j	4.6 ± 1.9hij
22	75	5	24	6.0 ± 1.6b	5.0 ± 2.1cdefgh	4.5 ± 2.3ijk
23	75	5	36	4.6 ± 2.5ghij	4.6 ± 2.1fghij	5.8 ± 2.0bc
24	75	5	48	4.5 ± 2.4hij	4.3 ± 1.9ghij	4.9 ± 2.4efghij
25	75	8	24	4.5 ± 2.4hij	5.4 ± 1.9bcdef	4.7 ± 2.6ghij
26	75	8	36	5.2 ± 2.2cdefgh	5.2 ± 1.9bcdefg	4.2 ± 2.2jk
27	75	8	48	5.6 ± 2.5bcd	4.3 ± 2.2hij	4.9 ± 1.9efghij
Control[3]	120	0	0	7.4 ± 1.3a	7.4 ± 1.3a	7.4 ± 1.3a
Overall mean				5.0 ± 2.1	5.0 ± 2.1	5.1± 2.0

[1] PD = distance ultrasound power density; PT = ultrasound exposure time; IC = incubation time at 25°C.

[2] Rating based on 9-point hedonic rating where 1 = dislike extremely, 5= neither like nor dislike, and 9=like extremely. Means ± standard deviation were calculated from two replications for a total 50 analyses/sample for overall acceptance. Means within the column not followed by the same letter are significant ($P<0.05$) different from each other as determined by Fisher's Least Significant Difference mean separation test.

[3] Control is untreated roasted whole peanuts

The analysis of variance (Table 4.8) showed that US power density, interactions of US exposure and incubation time, and their interaction with incubation time significantly contributed to the overall acceptance of sliced US-treated peanuts. In chopped US treated peanuts, the major significant contributors to overall acceptance were incubation time and its interaction with power density. The overall acceptance of whole US-treated peanuts were significantly contributed by US power density, its interaction with US exposure time, and their interaction with incubation time. Due to interaction effects, no main factor could be singled out as the major contributor to the overall acceptance of sliced, chopped, and whole US-treated peanuts.

F. Modeling, Mapping of Contour Plots, and Region of Overlap in Sliced Ultrasound-Treated Peanut Kernels

Only the parameters for sliced peanuts and notchopped or whole US-treated peanuts were subjected to process optimization study as findings showed that slicing produced the highest *trans*-resveratrol in all treated samples. Table 4.16 shows the significant regression models, coefficients of determination (R^2) and *P*-values for the response variables - *trans*-resveratrol, total stilbenes, H-ORAC, L-ORAC, total antioxidant capacity, TEAC, total phenolics, and overall acceptance for US treated peanuts. The *P*–values for the models of *trans*-resveratrol and total stilbenes were <0.0001 to 0.05 indicating that the models were highly significant in predicting these models. However, their R^2 values of 0.42, 0.29, 0.22, 0.27, 0.22, 0.29, 0.42 and 0.01 were low indicating that 42, 29, 22, 27, 22, 29, 42 and 1% of the variability in the resveratrol, *trans*-resveratrol, total stilbenes, H-ORAC, L-ORAC, total antioxidant capacity, TEAC, total phenolics, and overall acceptance, respectively, is explained by the predictors – US power density, US exposure time and incubation. Contour plots were generated from the full

Table 4.16 Regression coefficients for the significant prediction models for *trans*-resveratrol, total stilbenes, antioxidant capacities, total phenolics, and overall acceptance of sliced ultrasound-treated peanuts[1].

Coefficients	*Trans*-Resveratrol	Total Stilbenes	H-ORAC	L-ORAC	TAC-ORAC	TEAC	Total Phenolics	Overall Acceptance
Intercept	11.56110	-0.01809	2.3779	43.7372	14.6726	-0.0181	0.8203	6.89889
Linear								
PD	-0.45346	-0.33600	-0.0101	-0.1881	0.6453	-0.3360	-0.0098	-0.00603
PT	-0.51448	1.12147	-0.0478	-4.0247	-9.2275	-0.0478	0.0679	-0.36148
IC	-0.11287	0.34505	0.0471	-1.7566	1.8556	0.0471	0.0613	0.04417
Quadratic								
PD*PD	0.00399	0.00305	-0.00009	-0.0051	-0.0134	0.00009	0.000002	-0.00004
PT*PT	-0.01025	-0.13594	-0.0013	-0.0593	-0.5279	-0.0013	0.0019	-0.00370
IC*IC	0.00123	-0.00482	-0.0007	0.0123	-0.0233	- 0.0007	-0.0008	-0.00030
Interaction								
PD*PT	0.01733	-0.01041	0.0009	0.1154	0.0411	0.00092	0.00147	0.00186
PD*IC	0.00215	0.00211	0.00015	0.0205	0.0138	0.00015	-0.00003	0.00030
PT*IC	-0.15660	-0.00724	-0.0039	0.1711	-0.1162	-0.00388	-0.00039	0.09440
PD*PT*IC	-0.00045	-0.00034	-0.000007	0.0037	-0.0014	0.00004	0.000004	-0.00004
Adjusted R^2	0.42	0.29	0.22	0.27	0.22	0.29	0.42	0.01
P-value	<0.0001	<0.0001	0.0023	0.0001	0.0016	<0.0001	<0.0001	0.0532

[1] H-ORAC=hydrophilic ORAC, L-ORAC=lipophilic ORAC, TAC-ORAC (Total antioxidant capacity by oxygen radical absorbance capacity assay), TEAC=trolox equivalent antioxidant capacity by ABTS anion scavenging capacity assay, Total phenolics by Folin Ciocalteau assay, Overall acceptance using 9-point hedonic rating scale, PD=ultrasound power density, PT=ultrasound exposure time, and IC=incubation time at 25^{o}C.

models because their R^2 were low and could not be further reduced as determined by a significant difference when a variable was dropped from the model.

1. Predicted *trans*-resveratrol concentrations in sliced ultrasound-treated peanuts

The contour plot for the significant regression model for *trans*-resveratrol is shown in Figure 4.11. Some process combinations of US power density, exposure time and incubation time achieved the targeted 2.64 μg/g or 100% of t*rans*-resveratrol in red wines (McMurtrey, 1994). The *trans*-resveratrol increased as US power density approached 75 and 25 mW/cm^3, with higher maximum at 75 mW/cm^3 regardless of US exposure time from 2-8 min, and with minimum *trans*-resveratrol at 44-48 mW/cm^3 power density. The areas of minimum *trans*-resveratrol concentrations decreased further as incubation time increased from 24-48 h. The highest *trans*-resveratrol of 4.4 μg/g was obtained when peanuts were incubated for 48 h and treated with 75 mW/cm^3 power density regardless of exposure time from 2-8 min. The results indicated that at the longest incubation time of 48 h, high *trans*-resveratrol $\geq$ 3.96 μg/g or >150% *trans*-resveratrol that in red wines will be achieved by treatment with high power densities of 71-75 mW/cm^3 for 2-6 min. At medium power densities, e.g. 40-55 mW/cm^3, lower *trans*-resveratrol concentrations of 0.15 to 1.3 μg/g were predicted.

2. Predicted total stilbenes concentrations in sliced ultrasound-treated peanuts

Figure 4.11 shows the contour plot for the significant regression model of total stilbenes. The process combinations of US power density, exposure time and incubation time met the targeted total stilbenes of 4.33 μg/g (Lamuella-Raventos et al., 1995) or 100% in red wines. The total stilbenes is the sum of *trans*-resveratrol and *trans*-piceid concentrations. While *trans*-piceid concentrations vary throughout the experimental design, their models were not significant (P<0.05), but contributed t **o**

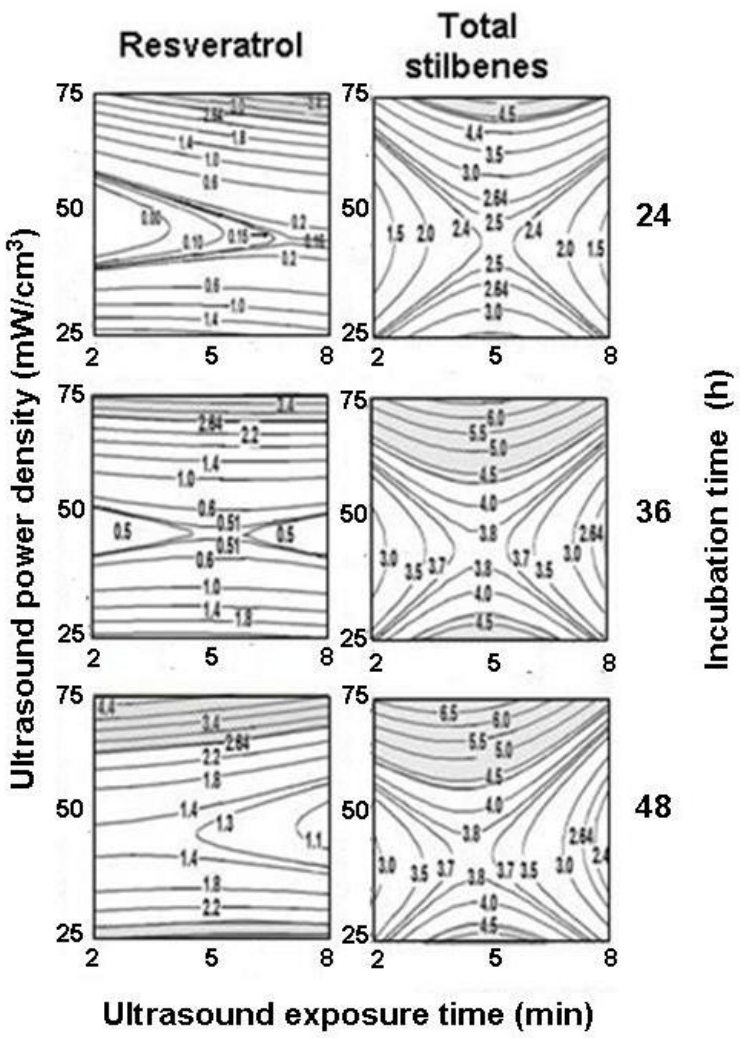


Figure 4.11 Contour plots for the significant prediction models for *trans*-resveratrol (µg/g) and total stilbenes (µg/g) of ultrasound treated peanuts, as affected by ultrasound power density and exposure times at specified incubation times at 25°C. Contour plots are shaded to cover the areas that meet regions of interest or the maximum concentrations achieved.

the total stilbenes. At 45 mW/cm^3, the amount of total stilbenes was minimum, and decreased as exposure time was either increased or decreased from 5 min. The highest predicted total stilbenes of 6.5 μg/g, was reached at 75 mW/cm^3 for 2-6 min when incubated for 48 and 36 h. Results indicated that both higher and lower power densities of 65-75 and 25-35 mW/cm^3 resulted in higher total stilbenes, reaching a maximum when using higher power densities, e.g., 75 mW/cm^3.

3. Predicted total antioxidant capacity in sliced ultrasound-treated peanuts

The contour plot for the significant regression model of total antioxidant capacity is shown in Figure 4.12. The process combinations of US power density, exposure time and incubation time produced the targeted 38.73 μM TE/g corresponding to 100% of total antioxidant capacity in red wines. However, a higher total antioxidant capacity of 67.78 μM TE/g or 175% total antioxidant capacity in red wines was achieved and reflected in the shaded area in the contour plot. A higher total antioxidant capacity was produced as 36 h incubation time was reached, and was minimum at incubation time of 24 and 48 h. A maximum total antioxidant capacity of 86 μM TE/g or 222% of TAC in red wines was obtained when peanuts were incubated for 36 h and US treated for 4.0-4.8 min at power densities of 38-45 mW/cm^3.

4. Predicted H-ORAC in sliced ultrasound-treated peanuts

Some process combinations of US power density, exposure time and incubation time met the targeted minimum of 38.73 μM TE/g or 100% H-ORAC in red wines as shown in Figure 4.12. However, 58.10 μM TE/g was achieved which correspond to 150% TAC in red wines and shaded areas of the contour plots reflect these values. The highest H-ORAC values was obtained as 36h incubation is reached, and decreased when incubation time was either decreased to 24h or increased to 48h. A maximum H-ORAC of 70 μM TE/g, equal to 181% H-ORAC in red wines

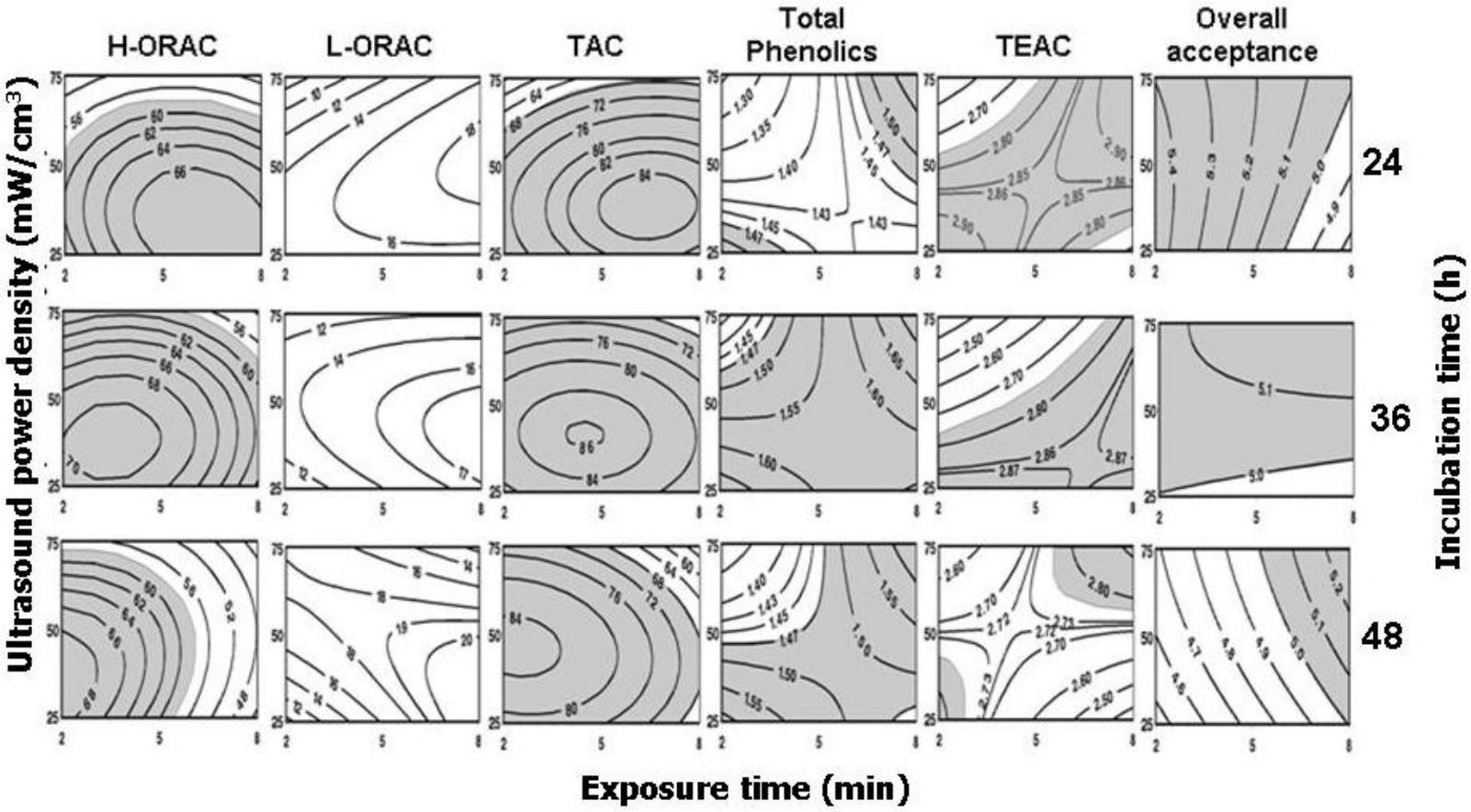


Figure 4.12 Contour plots for the significant prediction models for hydrophilic ORAC (H-ORAC), lipophilic ORAC (L-ORAC), total antioxidant capacity (TAC), total phenolics, TEAC, and sensory overall acceptance of ultrasound-treated peanuts as affected by ultrasound power density and exposure times at specified incubation times at 25^{o}C.

was produced when peanuts were incubated for 36h and exposed to US for 2.0-4.8 min at PD of 27-45 mW/cm^3.

5. Predicted L- ORAC in sliced ultrasound-treated peanuts

The process combinations of US power density, US exposure time, and incubation time did not achieve the targeted 38.73 μM TE/g L-ORAC so the contour plot was not shaded (Figure 4.12). The L-ORAC increased with increasing US exposure from 2-8 min. The highest L-ORAC of 20 μM TE/g was achieved when peanuts were incubated for 48 h after exposure to US for 8 min at power densities of 25-48 mW/cm^3.

6. Predicted total phenolics concentrations in sliced ultrasound-treated peanuts

No process combination of US power density, US exposure time, and incubation time produced the targeted total phenolics of 1.84 mg GAE/g (USDA, 2007) corresponding to 100% in red wines. Only a maximum of 1.47 mg GAE/g or 80% total phenolics in red wines was achieved and therefore shaded in the contour plots (Figure 4.12). Total phenolics increased with decreasing US power density from 75 to 25 mW/cm^3 and increasing US exposure time from 2-6 min, and then decreased when exposure time increase up to 8 min. The highest total phenolics of 1.72 mg GAE/g was achieved at 36 h incubation after US exposure for 7.8-8 min at 72-75 mW/cm^3 power densities.

7. Predicted TEAC in sliced ultrasound-treated peanuts

No process combination of US power density, US exposure time, and incubation time produced the targeted 5.01 μM TE/g TEAC (Villaño et al., 2004) corresponding to 100% in red wines. Only a maximum of 2.76 μM TE/g or 55% TEAC in red wines was achieved and reflected as shaded in the contour plots (Figure 4.12). TEAC increased as US power density decreased from 75 to 25 mW/cm^3 when US exposure time decreased from 6 to 3 min as

incubation time increased from 24 to 48h, otherwise it decreased. The highest TEAC of 2.95 μM TE/g was achieved at 36h incubation time when exposure to US for 7.8-8 min at 67-75 mW/cm^3 or 2 min at 25 mW/cm^3.

8. Predicted overall acceptance by consumers of sliced ultrasound-treated peanuts

Figure 4.12 shows the contour plot for the significant regression model of overall acceptance for sliced US-treated peanuts. A large proportion of process combinations of US power density, exposure time and incubation time will meet the targeted overall acceptance rating ≥ 5 or neither like nor dislike. The overall acceptance will increase with decreasing incubation time from 48-24 h. At 24 h incubation, the overall acceptance will decrease as exposure time to US is increased from 2-8 min, remains at the same ratings at 36h incubation time, and then increase at 48h incubation time, regardless of power densities used. The highest overall acceptance rating of 5.4 will be reached at the lowest incubation time of 24 h and lowest US exposure time of 2 min at any power densities. This indicates that the lower the doses of US, the higher will be the product acceptance.

9. Predicted optimum ultrasound processing treatment parameters for sliced peanuts

Figure 4.13 show the regions of overlap of the significant regression models for sliced US-treated peanuts. The regions of overlap that represent the predicted optimum US process parameters should produce at least 2.64 μg/g *trans*-resveratrol, 4.3 μg/g total stilbenes (Lamuella-Raventos et al., 1995), 67.8 μM TE/g (USDA, 2007), all representing 100% in red wines, and overall acceptance ratings ≥5 or neither like nor dislike. Some US processes at all incubation times will meet all these criteria (Figure 4.13). However, the largest number of US processes resulting in optimum products were observed at 36 h incubation time. Therefore, optimum US processes are all combinations within the area of a quadrilateral bound by US

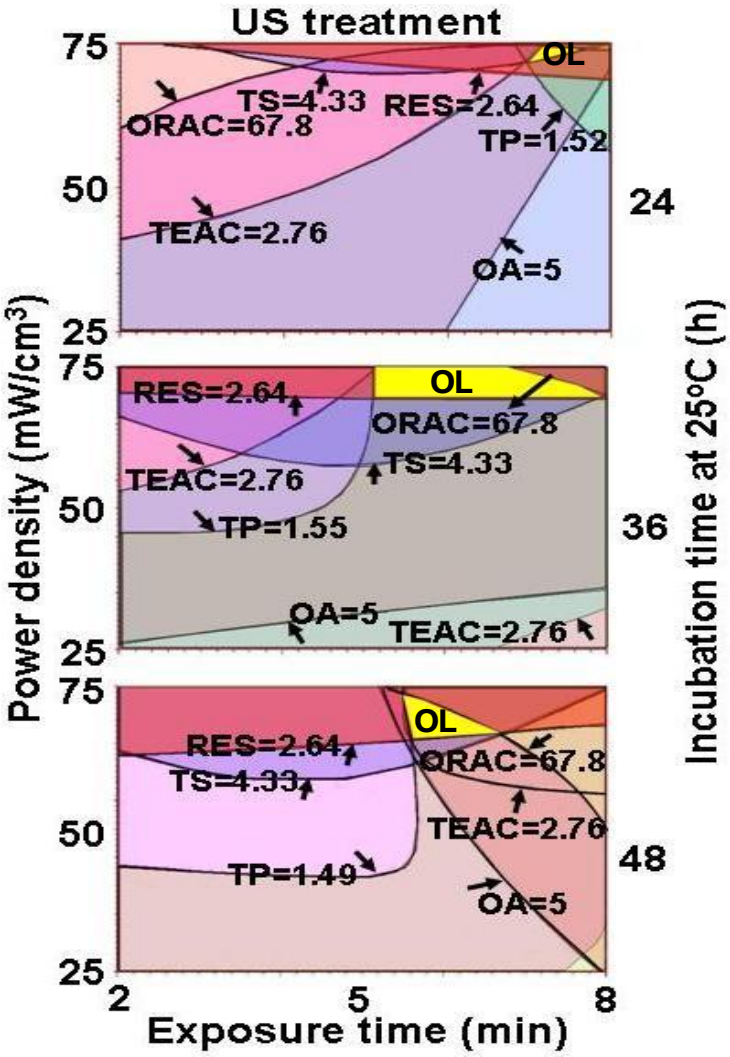


Figure 4.13 Superimposed contour plots for the significant (*P*<0.05) prediction models of *trans*-resveratrol (RES, µg/g), total stilbenes (TS, µg/g), ORAC total antioxidant capacity (µM trolox equivalents (TE)/g), TEAC (µM TE/g), total phenolics (TP, mg gallic acid equivalents/g), and overall acceptance (OA) based on 9-point hedonic rating where 1=dislike extremely, 5=neither like nor dislike and 9-like extremely. The regions of overlap (OL) represent the optimum ultrasound and UV processes at the specified incubation times. Arrows indicate directions of increasing concentrations or acceptance ratings.

power densities of 75, 75, 69 and 69 mW/cm^3 and corresponding exposure times of 5.1, 6.8, 7.9 and 5.1 min, respectively and incubated for 36 h.

G. Verification of Prediction Models Ultrasound Processing Treatment Parameters of Sliced Peanuts

The prediction models for the US processing treatments of peanut kernels were verified by preparing samples of US-treated peanuts using a process within and outside the optimum region and then analyzing the samples for *trans*-resveratrol, *trans*-piceid, and total antioxidant capacity. In this study, a sample was US-treated at 75 mW/cm^3 power density for 6 min followed by 36 h incubation at 25^{o}C to represent a process within the optimum region and another sample was exposed to 50 mW/cm^3 power density for 5 min followed by 36 h incubation at 25^{o}C representing a process outside the optimum process. The observed and predicted values for all response variables analyzed are shown in Table 4.18. Results showed that the observed and predicted values for *trans*-resveratrol, *trans*-piceid and total phenolics had probabilities > 0.05 indicating that the paired values were not significantly different from each other and therefore verifying that the models (regression equations) could predict the concentrations of these compounds. The observed values for ORAC within and outside the optimum regions were higher than the predicted values at probability levels of 0.0009 and 0.0019, respectively. The observed values for TEAC within and outside the optimum regions were slightly lower than predicted values.

Table 4.17 Observed and predicted values of stilbenes concentrations, total phenolics, and antioxidant capacities of ultrasound-treated peanuts for verification of prediction models.

Compound	Within Optimum Region			Outside Optimum Region		
	Observed	Predicted	PROB[1]	Observed	Predicted	PROB
Trans-resveratrol	3.79	3.81	0.2884 NS	0.57	0.55	0.0734 NS
Total phenolics	1.65	1.60	0.5387 NS	1.54	1.56	0.9338 NS
Total Antioxidant Capacity (ORAC)	430	69	0.0020**	203	84	0.0070**
Trolox Equivalent Antioxidant Capacity (TEAC)	2.63	2.82	0.0026**	2.68	2.85	0.0018**

[1]PROB = probability >0.05 means paired values are not significantly different from each other at 5% level of significance

NS = not significant, ** significant at 0.01% level

H. Verification of *Trans*-Resveratrol Biosynthesis in Peanuts

UV and US treatments increased the concentrations of *trans*-resveratrol in sliced imbibed raw peanuts. However, it was not clear whether increases of *trans*-resveratrol in stressed peanuts were due to efficient physical extraction of the compound in the peanut samples or to its biosynthesis; or whether non-viable peanut cells will produce *trans*-resveratrol after abiotic stress treatments. Thus, an experiment was conducted to verify if non-viable peanut cells prepared by roasting peanuts to make it non-viable, would generate increased levels of resveratrol in stressed peanuts.

Figure 4.14 showed the concentrations of *trans*-resveratrol in non-viable and viable peanuts stressed by wounding through slicing to ~7 mm, UV and US treatments. Results indicated that *trans*-resveratrol concentration of 0.02-0.23 μg/g all in non-treated and treated non-viable peanuts were not significantly different from each other, and from controls of raw whole untreated peanuts with 0.004 μg/g. In contrast, viable peanuts treated with UV and US had resveratrol concentrations of 1.46-2.71 μg/g which were significantly higher from all treated and un-treated non-viable peanuts, and controls. These findings confirmed that increases in concentrations in viable peanuts was due biosynthesis *trans*-resveratrol rather than physical extraction.

Results also confirmed the earlier finding that US was more effective than UV in increasing *trans*-resveratrol in sliced peanuts. US-treated peanuts had twice as much *trans*-resveratrol of 2.71 μg/g compared to UV-treated samples with 1.46 μg/g. US waves could penetrate deeper into the cells of peanut kernels where enzymes, phenylammonia lyase, responsible for resveratrol biosynthesis are released (Wu and Lin, 2002). UV stimulated the coordinate inductions of three enzymes responsible for resveratrol biosynthesis including stilbene synthase, phenylammonia

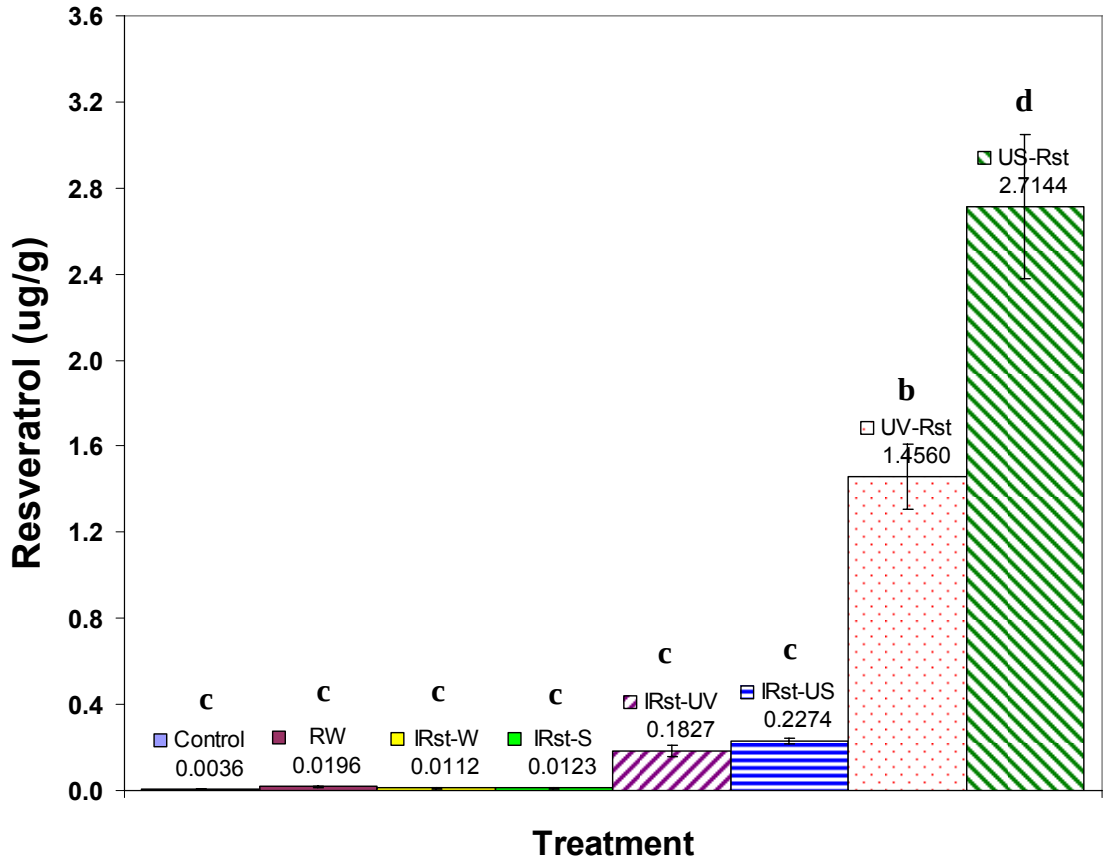


Figure 4.14 *Trans*-resveratrol concentrations (µg/g) in viable and non-viable peanuts to verify *trans*-resveratrol biosynthesis [1]

[1] Vertical lines on the bars represent standard deviations.
Means not followed by the same letter are significantly (P<0.05) different from each other.
Peanuts used had been stored for 1 year at 4°C. Non-viable peanuts were whole raw peanuts roasted at 153°C for 30 min, prior to treatments by any of the following: a) imbibition in filtered deionized water for 16 h; b) slicing to ~ 7mm; c) UV treatment for 30 min at 40 cm from UV light then incubated for 36 h at 25°C; or d) ultrasound treatment for 6 min at 75 mW/cc power density then incubated for 36 hr at 25°C. Viable peanuts were not roasted prior to treatment. Treatment descriptions are as follows:

Control	Viable: Control, untreated raw whole peanuts
RW	Non-viable: Whole roasted peanuts.
IRst-W	Non-viable: Whole roasted peanuts + imbibition.
IRst-S	Non-viable: Imbibed whole roasted peanuts, then sliced.
IRst-UV	Non-viable: Imbibed whole roasted peanuts, then sliced and UV-treated.
IRst-US	Non-viable: Imbibed whole roasted peanuts, then sliced and ultrasound treated.
UV-Rst	Viable: Whole raw imbibed peanuts, then sliced, UV-treated and roasted.
US-Rst	Viable: Whole raw imbibed peanuts, then sliced, ultrasound-treated, and roasted.

lyase, and cinnamic acid 4-hydroxylase, characterized by a maximum after 15 h of induction in grape leaves (Fritzemeier and Kindl, 1981). In grapes, resveratrol was only synthesized in UV-treated skins and not in the flesh (Coffee and Creasy, 1988) suggesting that in sliced peanuts, UV light could only pass through within outside surfaces, therefore only cells these areas were activated. These findings indicate that more enzymes needed for the resveratrol biosynthesis were released from cells using US compared to UV which could explain the less amounts of resveratrol in UV-treated compared to US-treated peanuts in this study.

III. STUDY 3. COMBINED ULTRASOUND-UV (US-UV) PROCESS OPTIMIZATION TO ENHANCE *TRANS*-RESVERATROL BIOSYNTHESIS IN PEANUTS

A. Effect of Combined Ultrasound –UV Processing Treatments on the Concentrations of Phenolic Compounds, Antioxidant Capacities, and Overall Acceptance of Roasted Peanut Kernels

Table 4.18 shows the mean concentrations of phenolic compounds, total phenolics, antioxidant capacities, and overall acceptance of combined US-UV treated peanuts and controls.

1. *Trans*-resveratrol concentrations in sliced US-UV treated peanuts

The concentration of *trans*-resveratrol in all 27 treatments of combined US-UV treated peanuts ranged from 1.63 ± 0.25 to 7.14 ± 2.03μg/g. Twenty four of 27 combined US-UV treated peanuts had mean *trans*-resveratrol concentrations of 2.53 ± 0.57 to7.14 ± 1.96 μg/g which were significantly (P<0.05) higher than untreated controls with 0.64 ± 0.10 μg/g. Three (#s 18, 19 and 20) of 27 combined US UV treatments had *trans*-resveratrol concentrations of 1.63 ± 0.25 to 1.81 ± 0.17 which were not significantly (P<0.05) higher than untreated controls. Seven (Trt# 4, 9, 10, 11, 21, 25 and 27) of 27 combined US-UV treatments resulted in *trans*-

Table 4.18 Concentrations (mean ± standard deviation, dry basis) of phenolic compounds, total phenolics, and antioxidant capacities, and overall acceptance ratings of roasted ultrasound (US)-UV treated peanuts[1] and controls.

Trt #	US PD	US PT	UV IT	Res[2] μg/g	Pic[2] μg/g	Caf[2] μg/g	Cou[2] μg/g	Fer[2] μg/g	TP[3] mgGAE/g	TEAC[4] μM TE/g	ORAC[5] μM TE/g	OA[6]
1	40	4	10	2.7661 ±0.6166	0.3278 ±0.0238	0.3858 ±0.0518	149.3852± 12.6713	2.3451 ±0.1857	1.3522 ±0.0775	3.9293 ±0.1569	127.1430 ±7.1011	4.7 ±1.9
2	40	4	30	2.5272 ±0.5739	1.2345 ±0.1855	0.4167 ±0.0406	156.9296± 34.2364	2.2018 ±0.5097	1.3953 ±0.0991	4.0004 ±0.0875	128.5160 ±39.0763	4.9 ±2.1
3	40	4	50	3.0044 ±0.2308	0.5454 ±0.0229	0.4804 ±0.0644	144.9869± 1.0940	2.1217 ±0.3838	1.3498 ±0.0515	3.8926 ±0.2577	143.2761 ±21.2873	5.2 ±2.0
4	40	8	10	6.0566 ±0.4734	1.1389 ±0.3902	2.1801 ±0.6355	266.4840± 78.6901	4.3077 ±1.4189	1.3740 ±0.1010	4.0475 ±0.0170	182.4258 ±22.9522	4.4 ±2.0
5	40	8	30	5.4159 ±0.6824	0.4450 ±0.1330	0.7482 ±0.0545	225.5779± 34.1292	3.8288 ±0.5375	1.3344 ±0.0364	4.0354 ±0.0462	132.4683 ±12.8255	5.0 ±1.9
6	40	8	50	3.6008 ±0.1182	0.5738 ±0.0012	1.1466 ±0.1076	173.5561± 10.4183	2.4803 ±0.4916	1.4206 ±0.0473	4.0119 ±0.0484	139.6811 ±21.0501	5.6 ±1.9
7	40	12	10	4.1839 ±1.0731	2.8468 ±0.0954	0.6037 ±0.0617	234.3938± 10.2305	4.6176 ±0.8491	1.3229 ±0.0972	4.1582 ±0.3007	116.0928 ±15.4584	5.0 ±1.9
8	40	12	30	3.1816 ±0.0585	0.8086 ±0.1420	0.4916 ±0.0721	160.8721± 21.9636	2.5359 ±0.5680	1.3191 ±0.0124	3.9103 ±0.1177	122.6631 ±20.3542	5.4 ±2.0
9	40	12	50	5.9495 ±1.3890	1.7151 ±0.2425	0.4018 ±0.0583	167.8978± 8.9095	3.2300 ±0.9039	1.4217 ±0.1901	3.8699 ±0.2288	145.7077 ±8.7401	4.9 ±1.9
10	80	4	10	6.3888 ±0.0618	1.7092 ±0.5110	0.2541 ±0.0429	151.4810± 3.5167	2.9795 ±0.0024	1.7360 ±0.5232	3.9436 ±0.0290	149.2960 ±10.9068	4.5 ±2.1
11	80	4	30	7.1198 ±0.7706	1.4465 ±0.1036	0.4567 ±0.0274	170.4419± 15.3316	3.0021 ±0.1862	1.4515 ±0.0455	3.9915 ±0.0325	116.6309 ±17.7256	5.0 ±1.9
12	80	4	50	3.6961 ±0.3738	0.5502 ±0.0029	0.4953 ±0.0083	145.8956± 19.3628	1.9136 ±0.1273	1.3994 ±0.0141	3.9688 ±0.0513	123.9076 ±5.2566	4.8 ±1.9
13	80	8	10	5.8608 ±1.9645	1.0430 ±0.3060	0.5652 ±0.0433	158.9971± 5.2433	1.5792 ±0.4215	1.4731 ±0.1640	3.9072 ±0.1721	127.6012 ±15.3089	5.1 ±2.2

Table 4.18 continued…

Trt #	US PD	US PT	UV IT	Res[2] µg/g	Pic[2] µg/g	Caf[2] µg/g	Cou[2] µg/g	Fer[2] µg/g	TP[3] mgGAE/g	TEAC[4] µM TE/g	ORAC[5] µM TE/g	OA[6]
14	80	8	30	4.0093 ±0.4343	1.3781 ±0.3520	0.3385 ±0.0962	143.0899± 30.0945	2.4858 ±0.3242	1.5159 ±0.0300	3.8983 ±0.2037	147.6709 ±8.6798	4.9 ±2.2
15	80	8	50	5.6341 ±1.0234	0.9889 ±0.2633	0.4406 ±0.0626	155.5378± 23.9280	2.7827 ±0.2640	1.5271 ±0.1132	3.9904 ±0.0237	127.4848 ±13.3346	4.7 ±2.0
16	80	12	10	3.9279 ±0.9950	1.5547 ±0.0620	0.6558 ±0.0340	161.4425± 37.0707	2.7921 ±0.8693	1.4051 ±0.0633	3.9817 ±0.0506	118.4970 ±29.0354	5.1 ±2.1
17	80	12	30	1.6304 ±0.2504	0.4665 ±0.0141	1.2266 ±0.0481	154.4166± 37.0374	2.6792 ±0.9122	1.4832 ±0.0436	3.8291 ±0.2182	167.7533 ±10.3434	5.1 ±2.1
18	80	12	50	1.6304 ±0.2504	0.3337 ±0.0491	0.3689 ±0.0013	139.6788± 4.8136	2.2921 ±0.1028	1.5216 ±0.0648	3.9095 ±0.1326	165.3135 ±43.5983	5.1 ±2.2
19	120	4	10	1.6681 ±0.3219	0.2383 ±0.0264	0.3185 ±0.0507	134.3292± 9.17555	1.8616 ±0.4295	1.4778 ±0.0690	3.9481 ±0.0824	175.6157 ±32.2755	4.6 ±1.9
20	120	4	30	1.8147 ±0.1663	0.2620 ±0.0109	0.2988 ±0.0438	148.4998± 36.3642	2.9784 ±0.9967	1.5162 ±0.0479	3.8404 ±0.0896	118.6089 ±10.6556	5.0 ±1.9
21	120	4	50	7.1425 ±1.9577	2.0034 ±0.6587	0.4914 ±0.0485	156.0460± 2.0153	2.4932 ±0.2692	1.5317 ±0.0612	3.9984 ±0.0164	219.9155 ±54.8525	4.7 ±2.1
22	120	8	10	2.8736 ±0.7004	0.2760 ±0.0080	0.4480 ±0.0414	145.1582± 21.1959	2.2726 ±0.1826	1.5535 ±0.1118	3.8139 ±0.1202	161.1010 ±40.2760	4.9 ±1.8
23	120	8	30	3.3838 ±0.6479	0.5249 ±0.0524	1.0482 ±0.2889	170.6680± 36.2477	2.5333 ±0.6051	1.4757 ±0.0697	4.0271 ±0.0636	145.8051 ±27.0411	5.0 ±1.9
24	120	8	50	3.6988 ±0.1050	0.9564 ±0.3055	1.5289 ±0.5093	158.8401± 6.1325	4.2065 ±0.1431	1.4598 ±0.0255	4.0270 ±0.0297	137.0786 ±32.3381	4.8 ±2.0
25	120	12	10	6.5963 ±1.8331	1.0424 ±0.3542	1.5485 ±0.2313	143.0337± 18.4045	3.8998 ±0.5430	1.6068 ±0.0389	4.0313 ±0.0196	172.2298 ±20.9343	4.6 ±2.2
26	120	12	30	3.5585 ±1.3245	0.4620 ±0.0113	1.8036 ±0.3564	149.8823± 27.8836	4.2574 ±0.9173	1.4919 ±0.0774	4.0120 ±0.0314	164.5487 ±4.4876	5.1 ±1.8

Table 4.18 continued…

Trt #	US PD	US PT	UV IT	Res[2] μg/g	Pic[2] μg/g	Caf[2] μg/g	Cou[2] μg/g	Fer[2] μg/g	TP[3] mgGAE/g	TEAC[4] μM TE/g	ORAC[5] μM TE/g	OA[6]
27	120	12	50	7.1049 ±2.0270	1.2621 ±0.0556	1.6082 ±0.5838	147.1393± 42.3031	4.1076 ±1.3305	1.4846 ±0.0498	3.9943 ±0.0254	158.2352 ±21.5829	4.6 ±2.0
Controls:												
Untreated whole peanuts, raw				0.6361 ±0.0970	1.1246 ±0.0436	0.5060 ±0.0229	35.1635 ±3.8031	0.1914 ±0.0661	1.2578 ±0.0426	3.4364 ±0.1277	44.6634 ±3.7497	7.7 ±1.0
UV-treated, roasted[7]				4.2502 ±0.4097	0.4003 ±0.1218	0.5787 ±0.0866	129.0268± 11.5084	2.1325 ±0.2538	1.4089 ±0.0263	3.9568 ±0.0086	164.5699 ±28.7394	5.8 ±1.1
Ultrasound-treated, roasted[8]				4.4767 ±0.6262	0.2320 ±0.0499	0.3983 ±0.1236	138.4985± 47.1958	2.6799 ±0.0282	1.4253 ±0.0927	3.9726 ±0.0371	169.5951 ±46.7394	5.4 ±1.2

[1] Process parameters were US PD=ultrasound power density (mW/cm^3); US PT=ultrasound exposure time (min); UV IT= exposure time (min); Fixed parameters were (1) distance from UV light of 40 cm and (2) incubation time at 25^{o}C for 36 h

[2] Francisco and Resurreccion (2009b) reversed-phase HPLC: Res= trans-resveratrol, Pic=*trans*-picied, Caf=caffeic acid, Cou=coumaric acid, Fer=ferulic acid, μg/g

[3] Singleton et al (1999) Folin Ciocalteau assay for total phenolics (TP), mg gallic acid equivalents (GAE)/g

[4] Kim et al. (2002) ABTS radical scavenging capacity assay, μM trolox equivalents (TE)/g

[5] Prior et al. (2003) ORAC (oxygen radical absorbance capacity) assay, Total antioxidant capacity = Hydrophilic ORAC + Lipophilic-ORAC, μM TE/g

[6] Resurreccion (1998) consumer test for overall acceptance (OA) using 9-point hedonic rating scale, 1=disliked extremely, 5=neither liked nor disliked, 9=liked extremely

[7] Optimal UV treatment of 30 min at 40 cm distance from UV light followed by incubation at 25^{o}C for 36 h

[8] Optimal ultrasound treatment of 6 min at 75 mW/cm^3 power density followed by incubation at 25^{o}C for 36 h

resveratrol of 5.95 ± 1.39 to 7.14 ± 2.03μg/g and were significantly (P<0.05) higher than either US- or UV-treated controls with 4.48 ± 0.63 and 4.25 ± 0.41 μg/g, respectively.

Exposing peanuts to 120 mW/cm^3 US power density for 4 min followed by UV exposure for 50 min at 40 cm was most effective in producing maximum *trans*-resveratrol of 7.14 μg/g. The maximum resveratrol of 7.14 μg/g in US-UV treated peanuts was higher than the maximum concentration of obtained previously in US or UV-treated peanuts of 3.96 and 3.42 μg/g, respectively (Rudolf and Resurreccion, 2005), 6.39 and 3.30 μg/g, respectively (Sales and Resurreccion, 2009), 4.29 and 2.36 μg/g, respectively (Potrebko and Resurreccion, 2010). Compared to combined US-UV treated peanuts, our maximum 7.14 μg/g was higher than 4.73 ± 1.20 μg/g *trans*-resveratrol produced when peanuts were treated using fixed US power density of 40 mW/cm^3 for 4 min followed by 35 min exposure at 40 cm distance from UV light (Potrebko and Resurreccion, 2010). We used higher doses of US up to 75 mW/cm^3 power density and exposure time up to 12 min with the same dose of UV.

Our maximum *trans* resveratrol of 7.14 μg/g was higher than that found in red grape juices with non-detectable to 1.09 μg/mL with a mean of 0.5 μg/mL (Romero-Perez, et al., 1999); and comparable to that in red wines with 0.60 to 8.00 μg/mL with a mean of 2.48 μg/mL (Lamuella-Raventos et al., 1995). A serving of roasted combined US-UV treated peanuts, approximately 30 g, would correspond to a maximum intake of 214 μg *trans*-resveratrol which is 2.3 times less than that in a serving of red wine (ca. 200 mL/serving) providing an average of 496 μg, and twice as much as that in a 200 mL serving of red grape juice with a mean of 100 μg. There are no other food sources than red wines and red grape juice that considerably contribute *trans*-resveratrol in the diet.

2. *Trans*-picied concentrations in sliced US-UV treated peanuts

The concentrations of *trans*-piceid ranged from 0.24 ± 0.03 to 2.85 ± 0.10 μg/g. Four (Trt# 7, 9, 10 and 21) of 27 combined US-UV treatments had *trans*-piceid concentrations of 1.71 ± 2.85 μg/g which were significantly higher (P<0.05) than untreated controls with 1.12 ±μg/g. These concentrations were also higher (P<0.05) than 0.40μg/g in UV-treated and 0.23 μg/g in US-treated controls.

Treating peanuts with US power density of 40 mW/cm^3 for 12 min followed by exposure at 40 cm distance from UV light for 10 min (Trt# 7) was most effective resulting in the maximum *trans*-piceid of 2.85 μg/g.

The highest *trans*-piceid of 2.85 ± 0.10 μg/g in combined US-UV treated peanuts were higher than those of white grape juices with non-detectable to 0.83 μg/g (mean=0.18 μg/g), but lower than in red grape juices with mean of 3.38 μg/g and a range of 0.77-7.34 μg/g (Romero-Perez et al., 1999) and in red wines with mean of 1.85 μg/g and a range of 0.74-4.01 μg/g (Lamuella-Raventos et al., 1995). One serving of roasted combined US-UV treated peanuts would provide a maximum intake of 85.5 μg piceid which is twice as much as a 200 mL serving of white grape juices with 36 μg/serving but much less than a serving of red grape juice with 676 μg/serving and red wine with 148-802 μg/serving.

3. *p*-Coumaric acid concentrations in sliced US-UV treated peanuts

All 27 combined US-UV processing treatments produced significantly (P<0.05) higher ***p***-coumaric acid concentrations of 134 ± 9.18 to 266 ± 78.7 μg/g compared to untreated controls with 35.2 ± 3.8 μg/g. The concentrations of *p*-coumaric acid in US- and UV-treated controls with 138.50 ± 47.2 and 129.03 ± 11.5 μg/g were also higher than those of untreated controls.

These results indicate that any UV, US, or combined US-UV processing treatments will increase ***p***-coumaric acid concentrations in peanuts. Three of 27 combined US-UV treatments had significantly higher ($P<0.05$) levels of *p*-coumaric acid with 225.58 ± 34.13 to 266.48 ± 78.69 μg/g compared to US- and UV-treated peanuts controls. Exposing peanuts to US power density of 40 mW/cm^3 for 8 min followed by 10 min exposure at 40 cm distance from UV light was most effective in increasing coumaric acid to a maximum level of 266.48 μg/g. A serving of roasted combined US-UV treated peanuts would provide up to 7994 μg coumaric acid which is about twice as much than in a serving of red wine with 4,400 μg/serving (Ghiselli et al., 1998).

4. Caffeic acid concentrations in sliced US-UV treated peanuts

The concentrations of 1.05 ± 0.29 to 2.18 ± 0.64 μg/g caffeic acid in eight of 27 combined US-UV treated peanuts were significantly higher ($P<0.05$) than untreated controls, UV-treated, and US-treated peanuts with 0.51±0.03, 0.58 ± 0.09, and 0.39 ± 0.12 μg/g, respectively. Similar to coumaric acid, treating peanuts with US power density of 40 mW/cm^3 for 8 min followed by UV exposure for 10 min at a distance of 40 cm from UV light was the most effective treatment producing the highest caffeic acid of 2.18 μg/g.

One serving of roasted combined US-UV treated peanuts will provide up to 65.4 μg caffeic acid which is 52 times much less than a serving of red wine with 3,400 μg caffeic acid (Ghiselli et al., 1998). Caffeic acid is a potent antioxidant comparable to butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) at concentrations of 10-20 μg/mL but higher than that of α-tocopherol and trolox on lipid peroxidation of linoleic acid emulsion at 20 μg/mL concentrations (Gulchin, 2006). It is also an effective $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and superoxide anion radical scavenger, has high, total reducing power and metal chelating properties with ferrous ions (Gulchin, 2006).

5. Ferulic acid concentrations in sliced US-UV treated peanuts

All of the combined US-UV treated peanuts had significantly higher ferulic acid contents of 1.86 ± 0.43 to 4.62 ± 0.85μg/g compared to untreated controls with 0.19 ± 0.07 μg/g. Similarly, the concentrations of ferulic acid in UV and US-treated peanuts of 2.13 ± 0.25 and 2.68 ± 0.03 μg/g, respectively, were also significantly higher than those in untreated controls. These findings suggest that any UV, US or combined US-UV effectively enhanced ferulic acid concentrations in untreated control peanuts.

Six of 27 combined US-UV treatments had significantly (P<0.05) higher ferulic acid concentrations ranging from 4.11±1.33 to 4.62±0.85 μg/g compared to US with 2.68±0.03μg/g. Seven of 27 treatments had significantly (P<0.05) higher ferulic acid of 3.83 ± 0.54 to 4.62 ± 0.85 μg/g than 2.13 ± 0.26 μg/g in UV-treated peanuts.

The most effective treatment producing the highest ferulic acid of 4.62 μg/g was US power density of 40 mW/cm^3 for 12 min followed by UV exposure for 10 min at a distance of 40 cm from UV light. On a per serving basis, roasted US-UV treated peanuts will provide up to 139 μg ferulic acid which is considerably less than a serving of red wine with 3,800 μg ferulic acid/serving (Ghiselli et al., 1998).

5. Total phenolics concentrations in sliced US-UV treated peanuts

The total phenolics assay of Singleton et al. (1999) used in this study measures all phenolic compounds including those compounds without antioxidant properties. Sixteen of 27 combined US-UV treatments had greater (P<0.05) *total* phenolics of 1.52 to 1.74 mg GAE/g compared to 1.26 mg GAE in untreated controls. Only 2 of 27 combined US-UV treatments with total phenolics concentrations of 1.61 ± 0.04 and 1.74 ± 0.52 mg GAE/g were significantly (P<0.05) higher than that of US-treated, and UV-treated with 1.42 ± 0.09, and 1.41± 0.03 mg GAE/g.

Treatment of peanuts with 80 mW/cm^3 US power density for 4 min followed by 10 min exposure at 40 cm distance from UV light produced the highest total phenolics of 1.74 mg GAE/g. The total phenolics of all 27 treated peanuts, however, were lower than found in red wines, raw blueberries, raw blackberries, and dark chocolate candies with 1.84, 5.31, 6.60, and 12.97 mg GAE/g, respectively (USDA, 2007). On a per serving basis, roasted US-UV treated peanuts will provide up to 52 mg GAE/serving total phenolics which is considerably lesser than a serving of dark chocolate candies (220 mg GAE/17g-bar), raw blueberries (293 mg/74g-half cup), red wine (368 mg/200 mL-glass), and raw blackberries (475 mg GAE/72g-half cup).

6. TEAC in sliced US-UV treated peanuts

All 27 treatments peanuts of combined US-UV and 1 each of UV and US treatment had significantly higher (P<0.05) TEAC values of 3.81 ± 0.12 to 4.16, 3.96 ± 0.01, and 3.97 ± 0.04 μM TE/g, respectively, compared to 3.44 ± 0.13 μM TE/g in untreated controls. These findings suggest that treatment of peanuts with UV, US, or combined US -UV will result in increased antioxidant capacities by TEAC.

Only 1 of 27 combined US-UV treatment had significantly (P<0.05) higher TEAC value of 4.16 ± 0.30 μM TE/g compared to US and UV-treated peanuts indicating that a combined US-UV process will not enhance TEAC over that of US or UV. US and UV- treated peanuts had similar (P<0.05) TEAC values.

The most effective treatment resulting in highest TEAC of 4.16 μM TE/g was exposure to 40 mW/cm^3 US power density for 12 min followed by 10 min exposure at 40 cm distance from UV light. The scavenging activity of 4.16 μM TE/g in roasted US-UV treated peanuts, however, were lower than red wines with mean of 5.01 μM TE/mL (n=16, Villaño et al., 2004), and those of blackberry, raspberry, black olives, redcurrant and wild strawberries with 20.24, 16.79, 14.73,

14.05, and 11.34 μM TE/g, respectively, (Pelligrini et al., 2003), and peanut skins extracts with 620-2560 μM TE/g (Francisco and Resurreccion, 2009a). A serving of roasted combined US-UV treated peanuts will provide up to 125 μM TEAC which is much less a serving of red wine (1002 μM TE), and those of blackberry (1457 μM TE/72g-half cup), raspberry (1209 μM TE/72g-half cup), wild strawberries (1383/122g-half cup), redcurrant (878 μM TE/56 g-half cup (ca. 56g), μM TE/half cup, black olives (3977 μM TE/10-pieces), and coffee espresso (1462 μM TE/240 mL-cup).

7. ORAC in sliced US-UV treated peanuts

7.1 Total Antioxidant Capacity in sliced US-UV treated peanuts

The ORAC values shown in Table 4.18 represent the total antioxidant capacity or the sum of hydrophilic and lipophilic ORAC values. All 27 combined US-UV-treated peanuts had significantly ($P<0.05$) higher total antioxidants of 114.92 ± 5.14 to 219.92 ± 54.85 μM TE/g compared to 44.67 ± 3.75 μM TE/g in untreated controls. Only 1 of 27 combined US -UV treatments produced significantly higher ORAC of 219.92 ± 54.85 μM TE/g compared to 169.60 ± 46.74 μM TE/g in US-treated and 164.57 ± 28.74 μM TE/g in UV- treated controls suggesting that a combined US -UV process will not enhance total antioxidant capacity over that of US or UV. US- and UV-treated peanuts had similar total antioxidant capacity values.

The treatment that resulted in highest total antioxidant capacity of 219.92 μM TE/g was exposure to 120 mw/cm^3 US power density for 4 min followed by exposure to 40 cm distance from UV light for 50 min. However, all treated peanuts had significantly higher total antioxidant capacity values compared to untreated control (44.67 ± 3.74 μM TE/g) indicating that either US or UV or combined US -UV will result in increased antioxidant capacities.

The total antioxidant capacity values of treated samples were higher than those of red wines, raw blackberries, raw blueberries, raw cranberries, and pecans with 38.00, 53.47, 62.52 and 179.40 μM TE/g, respectively (USDA, 2007). A serving of roasted US-UV treated peanuts would provide 6324 μM TE which is about 2/3 of a serving of red wine (9120 μM TE /200mL), about twice a serving of raw blackberries (3850 μM TE /74g half cup), raw blueberries (4626 μM TE /74g half cup), and raw cranberries (3126 μM TE /50g half cup), and a little more than a serving of pecans (5382 μM TE/30g).

7.2 H-ORAC in sliced US-UV treated peanuts

All 27 combined US-UV-treated peanuts had significantly ($P<0.05$) higher H-ORAC of 88.65 ± 20.46 to 173.53 ± 46.53 μM TE/g compared to 38.49 ± 3.34 μM TE/g in untreated controls. None of the 27 combined US -UV treatments produced significantly higher H-ORAC than 169.60 ± 46.74 μM TE/g in US-treated whereas one in 27 had higher ($P<0.05$) H-ORAC of 171.53 ± 46.53 μM TE/g compared to 164.57 ± 28.74 μM TE/g in UV-treated controls suggesting that a combined US -UV process will not enhance H-ORAC values over that of US or UV. US- and UV-treated peanuts had similar H-ORAC values.

7.3 L-ORAC in sliced US-UV treated peanuts

Twenty five of 27 combined US-UV treated samples had significantly ($P<0.05$) higher of L-ORAC values of 17.43 ± 2.90 to 55.01 ± 12.80 compared to 6.17 ± 0.81 in untreated controls. Three of the 27 combined US -UV treatments produced significantly higher L-ORAC values of 42.82 ± 2.74 to 55.01 ± 12.80 μM TE/g compared to 31.14 ± 11.07 μM TE/g in US-treated and 30.50 ± 3.19 in UV-treated peanuts suggesting that a combined US -UV process will not enhance H-ORAC values over that of US or UV. US- and UV-treated peanuts had similar H-ORAC values.

8. Overall acceptance of sliced US-UV treated peanuts

The combined US-UV treated peanuts obtained a highest overall acceptance rating of 5.6 ± 2.2 or neither like nor dislike, which was not significantly different from overall acceptance of UV- and US-treated peanuts with 5.8 ± 1.1 and 5.4 ± 1.2 ratings, respectively. All treated peanuts, however, had significantly lower overall acceptance compared to untreated controls with 7.7 ± 1.0 rating or liked moderately. The lower acceptance ratings of treated peanuts could be due to off-flavors such bitter and astringent, which resulted from increased concentrations of phenolic compounds (Naczk & Shahidi, 2006). Lower molecular weight phenolic compounds tend to be bitter whereas higher molecular weight polymers tend to be astringent (Drewnowski and Gomez-Carneros, 2000). Astringency is a puckering and drying sensation throughout the oral cavity (Drewnowski and Gomez-Carneros, 2000), and is related to the ability of dietary polyphenols to precipitate salivary proteins (Lesschaeve and Noble, 2005). Water soluble phenols with molecular weights between 500 and 3000 were reported to precipitate proteins, thus eliciting astringency (Lesschaeve & Noble, 2005).

B. Modeling and Mapping of Contour Plots for US-UV Processing Treatment Parameters

The results of regression analysis are shown in Table 4.19. All models of *trans*-resveratrol, *trans*-piceid, caffeic acid, coumaric acid, ferulic acid, total phenolics, total antioxidant capacity by ORAC, TEAC and overall acceptance were all significant ($P<0.05$) with *trans*-piceid, caffeic acid, and coumaric acid as the most significant having $P<0.0001$. The highest R^2 value obtained was 0.43 for caffeic acid indicating that 43% of the variance was explained by the model. The model for ORAC total antioxidant capacity had the lowest R^2 of 0.07 suggesting that only 7% of the variance was explained by the model. Due to low R^2 values, the full models were used in the generation of contour plots Contour plots for the significant models of *trans*-resveratrol,

trans-piceid, caffeic acid, coumaric acid, ferulic acid, total phenolics, total antioxidant capacity by ORAC, TEAC and overall acceptance and the superimposed regions of overlap are presented in Figure. 4.15.

1. Predicted trans-resveratrol concentrations in sliced US-UV treated peanuts

The process combinations of US power density, US and UV exposure times produced the targeted 2.64 μg/g corresponding to 100% *trans*-resveratrol in red wines. However, 4.3 μg/g corresponding to 163% in red wines was achieved and shaded as reflected in the contour plots (Figure 4.15). At lower UV exposure time of 10 min, *trans*-resveratrol decreased with increasing US power density from 52 to 120 mW/cm^3 regardless of US exposure time from 4 to 12 min, then reached a maximum as UV exposure time increased to 30 min and as US power density approached 80 mW/cm^3 at US exposure time of 7.5 to 11.5 min. As the UV exposure increased to 50 min, *trans*-resveratrol increased with increasing US power density from 40 to 120 mW/cm^3 regardless of US exposure time from 4 to 12 min. The maximum *trans*-resveratrol content of 5.7 μg/g was reached when peanuts were exposed to US power density of 120 mW/cm^3 for 4 min followed by 50 min exposure at 40 cm distance from UV light.

2. Predicted trans-picied concentrations in sliced US-UV treated peanuts

The process combinations of US power density, US and UV exposure times met the targeted *trans*-piceid of 1.85 μg/g or 100% in red wines only when exposed for 10 min at 40 cm from UV light (Figure 4.15). At lower UV exposure time 10 min, *trans*-piceid increased as US power density decreased from 120 to 40 mW/cm^3 and as US exposure time increased from 6 to 12 min, then further decreased as UV exposure time increased to 30 min As US exposure time reached 50 min, *trans*-piceid decreased as US power density decreased from 120 to 40 mW/cm^3. The

Table 4.19 Regression coefficients for the significant prediction models of the combined US-UV treated peanuts.

Parameters	Resveratrol	Piceid	Caffeic acid	Coumaric acid	Ferulic acid	Total Phenolics	ORAC-TAC	Overall Acceptance
Intercept	8.780200	1.656870	1.755580	126.441580	4.812280	1.431740	229.99536	3.102040
Linear								
Ultrasound power density, PD	-0.041800	-0.00131	-0.053250	-1.573280	-0.075850	0.006110	-1.377560	0.009700
Ultrasound exposure time, PT	-0.269070	0.015440	0.220490	35.706260	0.234520	-0.043660	-7.815110	0.161670
UV exposure time, IT	-0.297490	-0.06800	-0.010250	0.202370	-0.017600	-0.011820	-3.424880	0.071560
Quadratic								
PD*PD	-0.000293	-0.00003	0.000220	0.009080	0.000384	-0.000040	0.006600	0.000001
PT*PT	-0.016450	0.012350	-0.015910	-1.351170	-0.002950	-0.000005	0.064310	-0.002150
IT*IT	0.000173	0.000768	0.000037	-0.005080	-0.000129	0.000063	0.027710	-0.000494
Interaction								
PD*PT	0.008450	-0.00133	0.001680	-0.126830	-0.000579	0.000372	0.077670	-0.000872
PD*IT	0.002790	0.000512	0.001984	0.005180	0.000373	0.000055	0.018970	-0.000352
PT*IT	0.017120	-0.00209	-0.001550	-0.259880	-0.003800	0.001260	0.233830	-0.002830
PD*PT*IT	-0.000254	-0.000008	0.000025	0.002130	0.000037	-0.000011	-0.002450	0.000021
Adjusted R^2	0.0790	0.2701	0.4304	0.2443	0.1915	0.2034	0.0680	0.2892
P-value	0.0516	<0.0001	<0.0001	<0.0001	0.0005	0.0003	0.0543	0.0556

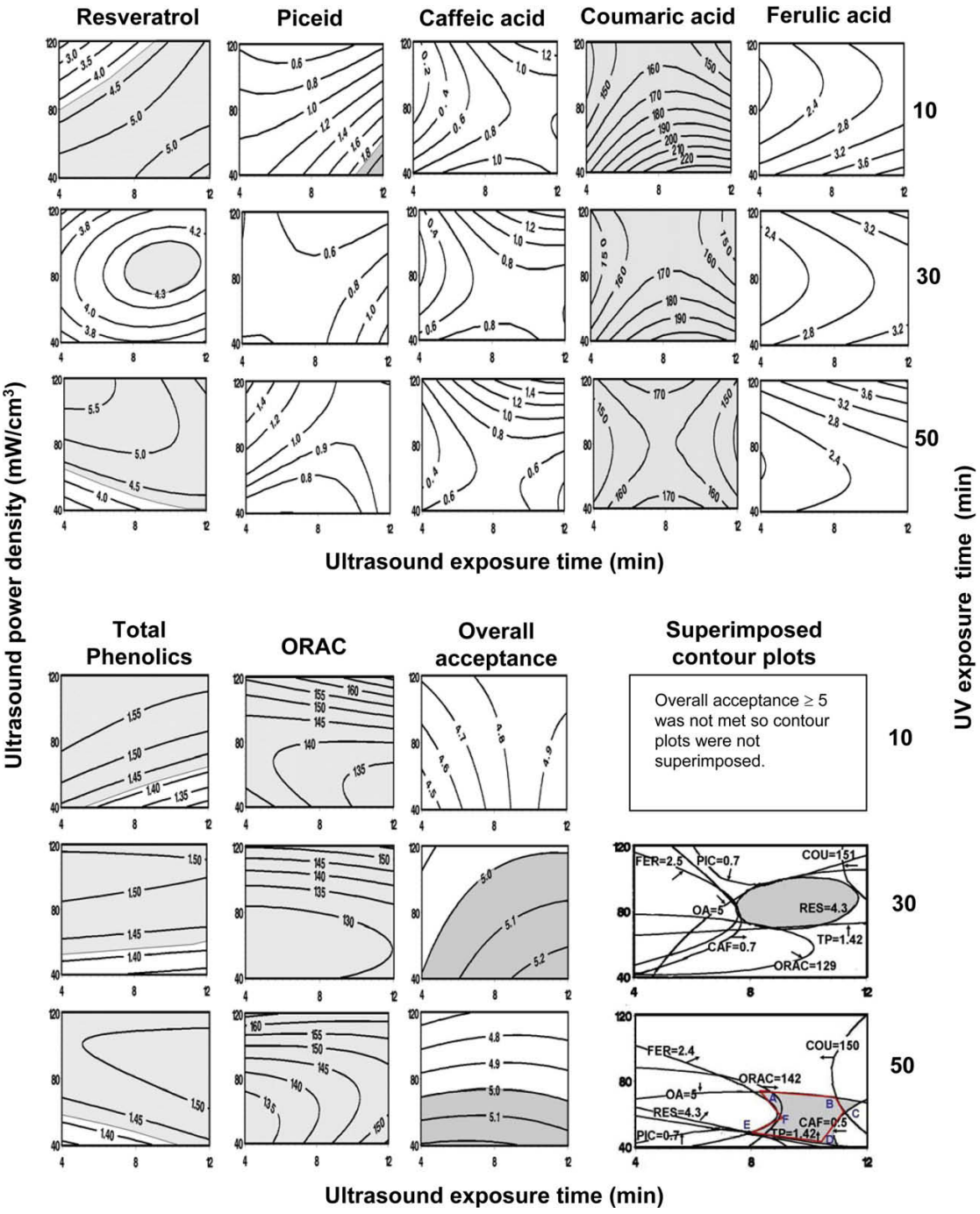


Figure 4.15 Contour plots for the significant prediction models of *trans*-resveratrol, *trans*-piceid, *p*-coumaric-, caffeic-, and ferulic-acids, total phenolics, ORAC, and overall acceptance. The superimposed contour plots show the regions of overlap (shaded areas) representing the optimum processes for the combined ultrasound-UV processes of peanut kernels. Arrows indicate directions of increasing concentrations or acceptance ratings. (Reprinted from Food Chemistry, Vol. 122, Sales, J.M. and Resurreccion, A.V.A. Phenolic profile, antioxidants, and sensory acceptance of bioactive-enhanced peanuts using ultrasound and UV. Page No. 802, Copyright 2010, with permission from Elsevier).

maximum *trans*-piceid of 2.2 μg/g was achieved when peanuts were exposed to US power density of 40 mW/cm^3 for 12 min followed by 10 min exposure at 40 cm distance from UV light.

3. Predicted p-coumaric acid concentrations in sliced US-UV treated peanuts

The process combinations of US power density, US and UV exposure times achieved the minimum concentration of 140 μg/g *p*-coumaric acid (Figure 4.15) which exceeded the targeted 22 μg/g or 100% in red wines. *p*-Coumaric acid increased with increasing UV exposure time 10 to 50 min, as US power density decreases from 110 to 40 mW/cm^3 and regardless of US exposure time from 8 to 10 min. The highest *p*-coumaric acid concentration of 230 μg/g was achieved when peanuts were exposed to US power density of 40 mW/cm^3 for 8.5 to12 min followed by UV exposure to 10 min at 40 cm distance from UV light.

4. Predicted caffeic acid concentrations in sliced US-UV treated peanuts

The process combinations of US power density, US and UV exposure times did not meet the targeted 17 μg/g or 100% caffeic acid in red wines and therefore contour plots were not shaded (Figure 4.15). Caffeic acid concentration increased as UV exposure time increased from 10 to 50 min and as US power density increased from 80 to 120 mW/cm^3 with increasing US exposure time from 8 to 12 min. The highest caffeic acid concentration of 1.6 μg/g was achieved when peanuts were exposed to US power density of 120 mW/cm^3 for 10-12 min followed by 10 min UV exposure at 40 cm distance from UV light.

5. Predicted ferulic acid concentrations in sliced US-UV treated peanuts

The process combinations of US power density, US and UV exposure times did not produce the targeted 19 μg/g ferulic acid or 100% in red wines and therefore contour plots were not shaded (Figure 4.15). Ferulic acid increased as UV exposure decreased from 10 to 30 min, and then increased as exposure time increased to 50 min. The maximum ferulic concentration of 3.8

μg/g was obtained when peanuts were US-treated for 10-12 min at 120 mW/cm^3 followed by 10 min UV treatment at 40 cm distance from UV light.

6. Predicted total phenolics concentrations in sliced US-UV treated peanuts

The process combinations of US power density, US and UV exposure times did not meet the targeted 1.84 mg GAE/g or 100% total phenolics in red wines. Only 1.42 mg GAE/g or 77% in red wines was achieved and shaded in the contour plots (Figure 4.15). The total phenolics content decreased as UV exposure time decreased from 10 to 30 min and remained at the same levels as UV exposure time further increased to 50 min. The highest total phenolics of 1.57 mg GAE/g was achieved when peanuts were US-treated at a power density of 120 mW/cm^3 for 4-8 min followed by 10 min exposure at 40 cm distance from UV light.

7. Predicted total antioxidant capacity by ORAC in sliced US-UV treated peanuts

The process combinations of US power density, US and UV exposure times achieved the minimum total antioxidant capacity by ORAC of 130 μM TE/g which exceeded 38 μM TE/g or 100% in red wines. The ORAC value decreased with increasing UV exposure time from 10 to 30 min and then increased when UV exposure increased to 50 min, with increasing US power density from 80 to 120 mW/cm^3, regardless of US exposure time from 4 to 10 min. The maximum ORAC value of 170 μM TE/g was reached when peanuts were US-treated for 11.5 -12 min at 120 mW/cm^3 then UV treated for 10 min at 40 cm distance from UV light.

8. Predicted overall acceptance of sliced US-UV treated peanuts

The process combinations of US power density, US and UV exposure times achieved the targeted overall acceptance rating of ≥5 was met when US treated peanuts were exposed to UV for 30 and 50 min, but not for 10 min. The overall acceptance of treated peanuts increased as UV exposure increased from 10 to 30 min and slightly decreased when UV exposure time was

further increased to 50 min. At lower UV exposure time of 10 min, no samples had an overall acceptance rating of $\geq$ 5 whereas about 80% of the treatments exposed to UV for 30 min received overall acceptance $\geq$ 5, and about half of those exposed to UV for 50 min were rated with overall acceptance $\geq$ 5. The overall acceptance ratings of treated samples did not reach higher than 5 due to off-flavors developed with increased levels of phenolic compounds and antioxidants.

D. Predicted Optimum Region for US-UV Processing Treatment Parameters of Sliced Peanuts

Figure 4.15 shows the superimposed contour plots indicating the optimum parameters for the combined US-UV processes. At 10 min UV exposure time, no overlap was observed because no treatment received an overall acceptance rating $\geq$ 5, which is the cut-off point for determining the optimum process for combined US-UV, as it is the limiting factor for product development applications. Some treatments that were exposed to UV for 30 and 50 min obtained had overall acceptance $\geq$ 5, and thus optimum processes were obtained. However, optimum processes using 50 min UV exposure achieved higher *trans*-resveratrol up to 4.8 μg TE/g compared to only 4.3 μM TE/g using 30 min UV exposure, and therefore, US-treated peanuts exposed to UV for 50 min were chosen as the optimum processes. The optimum US-UV processes included all process combinations within a hexagon bounded by six points (A to E in Figure 4.15), US 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 min US exposure time, respectively; followed by 50 min exposure at 40 cm distance from UV light. The optimum parameters for a combined US-UV process provided up to 4.8 μg/g *trans*-resveratrol, 170 μg/g *p*-coumaric acid, and 150 μM TE/g ORAC or >100% that found in red wines. Additionally, this process provided 1.0 μg/g *trans*-piceid, 2.6 μg/g ferulic acid, 1.48 mg GAE/g

total phenolics, and consumer acceptance ≥5. Compared to US or UV processes, the optimum combined US-UV process resulted in 1.3 times or 2.3 times the *trans*-resveratrol in US at 3.80 µg/g or UV at 2.06 µg/g (Sales and Resurreccion, 2009).

E. Verification of Prediction Models for US-UV Processesing Treatment Parameters for Sliced Peanuts

The prediction models for the combined US-UV processing treatments of sliced peanut kernels were verified by preparing samples of US-UV-treated peanuts using a process within and outside the optimum region and then analyzing the samples for *trans*-resveratrol, *trans*-piceid, and total antioxidant capacity. In this study, a process representing the parameters within the optimum region was conducted by exposing fully imbibed sliced peanuts to US at 70 mW/cm^3 power density for 10 min, followed by exposure to UV for 50 min at 40 cm distance from UV light, and then incubation at 25^oC for 36 h. Another sample of peanuts was exposed to 40 mW/cm^3 power density for 4 min, then exposed to UV for 50 min at 40 cm distance from UV light followed by 36 h incubation at 25^oC to represent a process outside the optimum region. The observed and predicted values for all response variables analyzed are shown in Table 4.21. Results showed that the observed and predicted values for *trans*-resveratrol, *trans*-piceid, ρ-coumaric acid, caffeic acid, ferulic acid, total phenolics, TEAC, and ORAC total antioxidant capacities had probabilities > 0.05 indicating that the paired values were not significantly different from each other and therefore verifying that the models (regression equations) could predict the concentrations of these compounds.

Table 4.20 Observed and predicted values of phenolic compounds concentrations, total phenolics, and antioxidant capacities of combined ultrasound-UV treated peanuts for verification of prediction models.

Compound	Within Optimum Region			Outside Optimum Region		
	Observed	Predicted	PROB[1]	Observed	Predicted	PROB
Trans-resveratrol	4.76	4.80	0.3214 NS	3.02	3.22	0.1374 NS
Trans-piceid	0.80	0.82	0.9367 NS	0.68	0.70	0.3201 NS
ρ-Coumaric acid	158.20	160	0.0931 NS	149.4	151	0.4521 NS
Caffeic acid	0.62	0.60	0.3651 NS	0.62	0.60	0.2557 NS
Ferulic acid	2.58	2.60	0.4319 NS	2.15	2.2	0.3419 NS
Total phenolics	1.48	1.50	0.5387 NS	1.54	1.56	0.9338 NS
Total Antioxidant Capacity (ORAC)	148	145	0.4297 NS	135	130	0.3245 NS

[1]PROB = probability >0.05 means paired values are not significantly different from each other at 5% level of significance
NS = not significant

D. Effect of Abiotic Stresses on the Concentrations of *Trans*-Resveratrol in Peanuts

The effects of abiotic stresses of wounding through size reduction by slicing and chopping, and treatments with UV, US, and combined US-UV on the concentrations of *trans*-resveratrol in peanuts are illustrated in Figure 4.16.

The untreated raw whole control peanuts had 0.02 μg/g *trans*-resveratrol and slicing increased the concentration to 0.37 μg/g or 17.5-fold. UV treatment of sliced peanuts further increase the concentration to 3.3 μg/g or an additional 8-fold increase from slicing or 164-fold cumulative increase from control. The combined US-UV treatments produced 7.14 μg/g corresponding an additional 1.2-fold increase from UV treatment, 18-fold increase from slicing and cumumulative increase of 356-fold from control.

US treatment of sliced peanuts increased *trans*-resveratrol to 6.35 μg/g which was higher than in chopped peanuts with 2.88 μg/g and whole peanuts with 0.99 μg/g, translating to 317-, 143-, and 49-fold increases from control, respectively. This finding suggests peanuts mild wounding of peanuts through slicing achieved the maximum enhancement of *trans*-resveratrol biosynthesis. The more severe wounding such as chopping resulted in less biosynthesis whereas no wounding such as in whole peanuts, provided the least.

US treatment which caused 317-fold increase in *trans*-resveratrol from 0.37 to 6.35 μg/g in sliced peanuts was twice as effective as UV which only increased by 164-fold from 0.37 to 3.30 μg/g. The effect of UV treatment in US sliced peanuts, however, only caused very minimal 0.12 fold increase in *trans*-resveratrol from 6.35 to 7.14 μg/g whereas the effect of US on UV-treated peanuts had an additive effect with a 2.16 fold increase in trans-resveratrol from 3.3 to 7.14 μg/g.

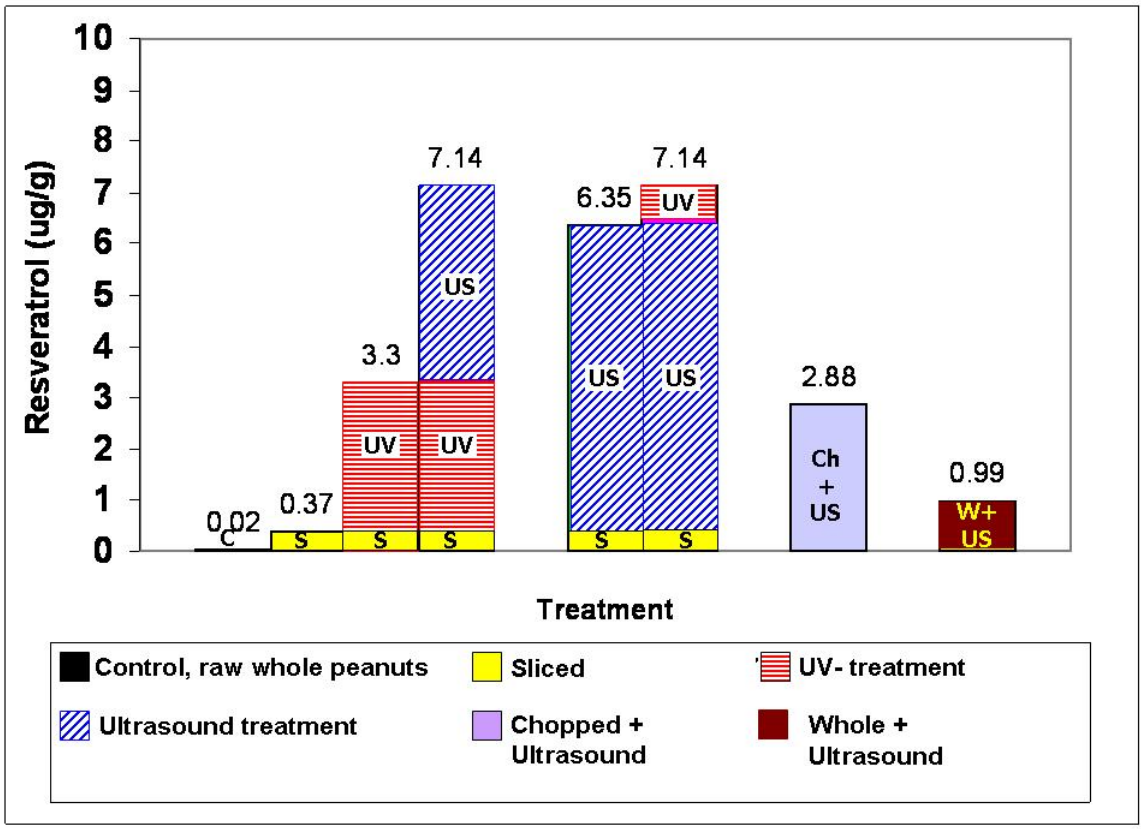


Figure 4.16 Increments of *trans*-resveratrol in peanuts due effects of abiotic stresses of wounding through size reduction and treatments with UV, ultrasound, and combined ultrasound-UV.

IV. STUDY 4. APPLICATIONS OF RESVERATROL-ENHANCED PEANUTS SELECTED PRODUCTS AND THEIR SHELF LIFE

A. Applications of Resveratrol-Enhanced Peanuts (REP)

REP were prepared using the optimal combined US-UV process determined in Study 3 with the parameters of 70 mW/cm^3 US power density, 10 min US exposure time and 50 min UV exposure time at 40 cm distance from UV light followed by incubation at 25^{o}C for 36 h. The REP were used to prepare roasted REP and resveratrol-enhanced peanut bars for which shelf life studies were conducted. An accelerated shelf life test (ASLT) was performed on roasted REP whereas, a storage study at ambient and at high temperature was done on resveratrol-enhanced peanut bars.

B. Accelerated Shelf Life Test Study of Roasted Resveratrol-Enhanced Peanuts

Peanuts contain about 50-55% fat, of which approximately is 30% linoleic acid, the fatty acid responsible for development of off-flavors as a result of lipid oxidation (Warner et al., 1996; Han et al., 2008). These oxidation reactions lead indirectly to the formation of numerous aliphatic aldehydes, ketones, and alcohols, of which hexanal is the most prevalent decomposition product (Warner et al., 1999). Hexanal has been used as indicator compound for the lipid oxidation in peanuts (Warner et al., 1996; Grosso and Resurreccion, 2002; Han et al., 2008; Jensen et al., 2005), chocolate peanut spread (Chu, 2003), pork scratchings, and oatmeal and muesli (Jensen, et al., 2005).

1. Changes in hexanal concentrations during storage

The changes in hexanal concentrations of roasted REP during storage indicated the degree of lipid oxidation of samples of 30, 35 and 40^{o}C as presented in Figure 4.17. Roasted REP had initial hexanal concentrations of 6.63, 10.19, and 11.55 µg/g at accelerated temperatures of 30,

35 and 40°C (Table 4.21) which were all significantly higher than untreated controls with 2.7 µg/g used in this study, suggesting that hexanal had been formed in REP immediately after processing treatments, which may be due to UV exposure known to initiate lipid peroxidation (Duh and Yen, 1995). Grosso and Resurreccion (2002) found an initial hexanal of 1.65 µg/g hexanal in untreated roasted peanuts which was slightly lower than that found in this study. Hexanal concentrations of roasted REP increased with increasing storage time at all accelerated storage temperatures of 30, 35, and 40°C used in the study, with faster rate of increase as temperature increased from 30 to 40°C (Figure 4.15). At about 40 days of storage, the hexanal concentrations of REP were 29.45 µg/g at 30°C, 36.84 µg/g at 35°C and 64.23 µg/g at 40°C (Table 4.23) which were much higher than 3.97 µg/g in normal roasted peanuts found by Grosso and Resurreccion (2002).

At verification temperature of 25°C, initial hexanal of 8.67 µg/g of REP increased to 106.07 µg/g after 101 days of storage. After 90 days, which is the industry's shelf life for normal untreated roasted peanuts, the hexanal of roasted REP increased 9.5-fold to 82.89 µg/g suggesting that roasted REP will have shorter shelf life than regular untreated roasted peanuts.

2. Changes in *trans*-resveratrol, total phenolics and TEAC levels during storage

The respective initial (0 day) *trans*-resveratrol concentrations of REP stored at 30, 35 and 40°C were 3.39, 3.39, and 3.34 µg/g; which decreased to 2.96, 3.12 and 2.80 µg/g or 13%, 8% and 16% reduction, as storage time increased to 61, 46 and 35 days, respectively (Figure 4.17). At 25°C, *trans*-resveratrol was reduced from 3.40 to 2.77 µg/g, an 18% decrease, after 101 days. Label claim requires that at least 80% of the concentration of the substance or ingredient should be present in the product during its shelf life (Fu and Labuza, 1993). Control samples had initial

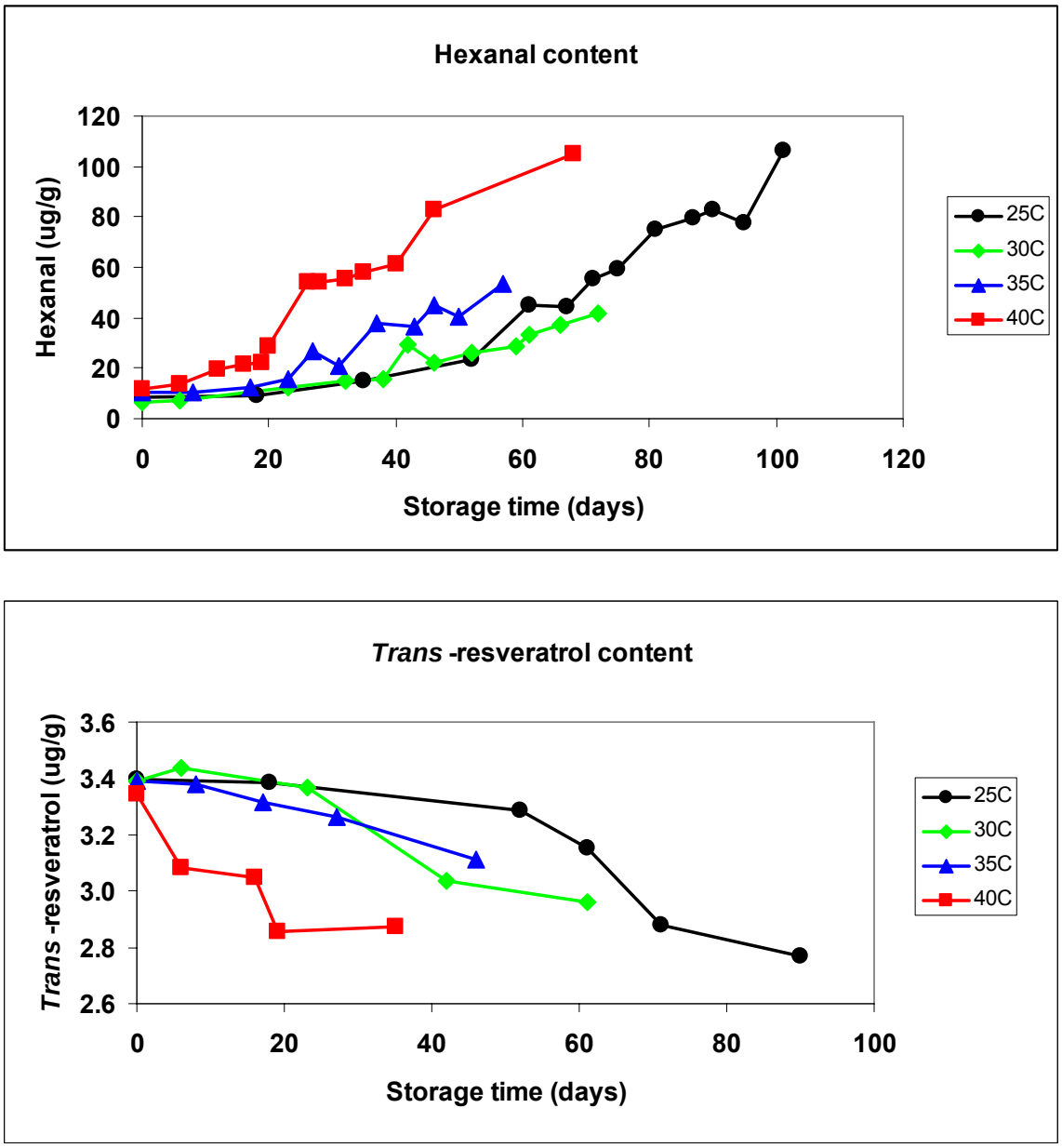


Figure 4.17. Changes in hexanal and *trans*-resveratrol concentrations of roasted resveratrol-enhanced peanuts during storage at accelerated temperatures of 30, 35 and 40°C and verified at 25°C.

Table 4.21 Mean hexanal concentrations (mean ± standard deviation) of roasted resveratrol-enhanced peanuts stored for varying times at accelerated temperatures of 30, 35, and 40°C, and verified at 25°C[1]

Treatment No.	Storage Temperature (°C)	Storage time (days)	Hexanal (µg/g)	*Trans*-resveratrol (µg/g)	Total Phenolics (mg GAE/g)	TEAC (µM TE/g)
1	25	0	8.67 ± 0.27f	3.40 ± 0.16	1.91 ± 0.13	8.26 ± 1.22
2	25	18	9.07 ± 1.80f	3.39 ± 0.36		
3	25	35	15.31 ± 1.46ef	-		
4	25	52	23.57 ± 7.47e	3.29 ± 0.25	1.76 ± 0.01	6.06 ± 1.63
5	25	61	45.07 ± 9.04d	3.15 ± 0.67		
6	25	67	44.26 ± 6.66d	-		
7	25	71	55.49 ± 3.56cd	2.87 ± 0.53		
8	25	75	59.62 ± 12.10c	-		
9	25	81	75.32 ± 26.52b	-		
10	25	87	79.69 ± 16.27b	-		
11	25	90	82.89 ± 8.21b	2.77 ± 0.94		
12	25	95	77.71 ± 10.69b			
13	25	101	106.07 ± 17.65a			
Controls						
54	25	0	2.77 ± 0.33i	0.29 ± 0.04	1.58 ± 0.00	6.47 ± 0.64
55	25	35	6.16 ± 2.29ij	-	-	
56	25	52	5.85 ± 0.87ij	0.28 ± 0.05	1.48 ± 0.04	6.36 ± 0.12
57	25	61	7.45 ± 1.65j	-	-	
58	4	35	3.21 ± 2.20i	-	-	
59	4	52	4.00 ± 0.89i	0.28 ± 0.03	1.57 ± 0.02	6.42 ± 0.21
60	4	61	3.44 ± 0.94i	-		

Table 4.21 continued…

Treatment No.	Storage Temperature (°C)	Storage time (days)	Hexanal (µg/g)	*Trans*-resveratrol (µg/g)	Total Phenolics (mg GAE/g)	TEAC (µM TE/g)
14	30	0	6.63 ± 0.70hg	3.39 ± 1.06	2.35 ± 0.07	9.29 ± 0.41
15	30	6	6.93 ± 0.55hg	3.44 ± 1.15	-	-
16	30	23	12.07 ± 0.34f	3.37 ± 0.88	-	-
17	30	32	14.77 ± 0.93f	-	-	-
18	30	38	15.62 ± 0.78f	-	-	-
19	30	42	29.45 ± 4.68cd	3.04 ± 0.60	2.02 ± 0.03	6.71 ± 1.93
20	30	46	22.33 ± 3.59e	-	-	-
21	30	52	25.78 ± 3.49de	-	-	-
22	30	59	28.47 ± 4.30d	-	-	-
23	30	61	33.19 ± 3.27bc	2.96 ± 0.27	-	-
24	30	66	36.91 ± 8.87b	-	-	-
25	30	72	41.87 ± 3.69a	-	-	-
Controls						
54	25	0	2.77 ± 0.33h	0.29 ± 0.04	1.58 ± 0.00	6.47 ± 0.64
55	25	35	6.16 ± 2.29gh	-	-	-
56	25	52	5.85 ± 0.87gh	0.28 ± 0.05	1.48 ± 0.04	6.36 ± 0.12
57	25	61	7.45 ± 1.65g	-	-	-
58	4	35	3.21 ± 2.20gh	-	-	-
59	4	52	4.00 ± 0.89gh	0.28 ± 0.03	1.57 ± 0.02	6.42 ± 0.21
60	4	61	3.44 ± 0.94gh	-	-	-

Table 4.21 continued…

Treatment No.	Storage Temperature (°C)	Storage time (days)	Hexanal (µg/g)	*Trans*-resveratrol (µg/g)	Total Phenolics (mg GAE/g)	TEAC (µM TE/g)
26	35	0	10.19 ± 0.76fgh	3.39 ± 0.33	2.05 ± 0.06	9.44 ± 0.75
27	35	8	10.24 ± 2.46fgh	3.38 ± 0.24	-	-
28	35	17	12.35 ± 0.26fg	3.31 ± 0.19	-	-
29	35	23	15.84 ± 2.53ef	-	-	-
30	35	27	26.45 ± 5.54d	3.21 ± 1.04	1.90 ± 0.12	6.87 ± 0.46
31	35	31	20.84 ± 3.18de	-	-	-
32	35	37	37.53 ± 5.77c	-	-	-
33	35	43	36.84 ± 2.72c	-	-	-
34	35	46	45.02 ± 6.89b	3.12 ± 0.74	-	-
35	35	50	40.14 ± 4.24bc	-	-	-
36	35	57	53.23 ± 12.64a	-	-	-
Controls						
54	25	0	2.77 ± 0.33i	0.29 ± 0.04	1.58 ± 0.00	6.47 ± 0.64
55	25	35	6.16 ± 2.29hi	-	-	-
56	25	52	5.85 ± 0.87hi	0.28 ± 0.05	1.48 ± 0.04	6.36 ± 0.12
57	25	61	7.45 ± 1.65ghi	-	-	-
58	4	35	3.21 ± 2.20i	-	-	-
59	4	52	4.00 ± 0.89i	0.28 ± 0.03	1.57 ± 0.02	6.42 ± 0.21
60	4	61	3.44 ± 0.94i	-	-	-

Table 4.21 continued…

Treatment No.	Storage Temperature (°C)	Storage time (days)	Hexanal (µg/g)	*Trans*-resveratrol (µg/g)	Total Phenolics (mg GAE/g)	TEAC (µM TE/g)
41	40	0	11.45 ± 2.77fghi	3.34 ± 0.34	2.01 ± 0.04	8.57 ± 0.46
42	40	6	13.89 ± 1.45effghi	3.08 ± 0.11	-	-
43	40	12	19.47 ± 1.83efefg	-	-	-
44	40	16	21.43 ± 2.05eefg	3.05 ± 0.70	-	-
45	40	19	22.27 ± 4.29ef	2.86 ± 0.98	1.82 ± 0.03	6.52 ± 0.11
46	40	20	28.95 ± 5.23e	-	-	-
47	40	26	53.98 ± 10.75cd	-	-	-
48	40	28	54.23 ± 3.90c	-	-	-
49	40	32	55.70 ± 10.75c	-	-	-
50	40	35	57.83 ± 3.90c	2.87 ± 0.58	-	-
51	40	40	61.36 ± 13.57c	-	-	-
52	40	46	82.81 ± 17.98b	-	-	-
53	40	68	105.12 ± 13.74a	-	-	-
Controls						
54	25	0	2.77 ± 0.33j	0.29 ± 0.04	1.58 ± 0.00	6.47 ± 0.64
55	25	35	6.16 ± 2.29hij	-	-	-
56	25	52	5.85 ± 0.87hij	0.28 ± 0.05	1.48 ± 0.04	6.36 ± 0.12
57	25	61	7.45 ± 1.65hij	-	-	-
58	4	35	3.21 ± 2.20j	-	-	-
59	4	52	4.00 ± 0.89ij	0.28 ± 0.03	1.57 ± 0.02	6.42 ± 0.21
60	4	61	3.44 ± 0.94j	-	-	-

[1] Means not followed by the same letter within the column at each specified storage temperature along with controls are significantly (P<0.05) different from each other as determined by Fisher's least significance difference mean separation test.

trans-resveratrol contents of 0.29 µg/g which did not change significantly during storage for 52 days at 4 and 25°C.

REP had mean initial total phenolics of 2.35, 2.05, and 2.01 mg GAE/g at 30, 35 and 40°C, respectively, which decreased to 2.02, 1.90, and 1.82 mg GAE/g or 14%, 17% and 19% reductions, after 42, 27, and 19 days of storage at respective temperatures. At 25°C, the total phenolics of REP decreased from 1.91 to 1.76 mg GAE/g or 8% after 52 days. Control samples had initial total phenolics of 1.58 mg GAEg/g which decreased to 1.48 mg GAE/g after storage for 52 days at 25°C and did not change during storage for 52 days at 4°C at 1.57 mg GAE/g.

The initial TEAC values of 9.29, 9.44 and 8.57 µM TE/g of REP stored at 30, 35 and 40°C, respectively, decreased to 5.21, 6.87, and 6.52 µM TE/g after 42, 27, and 19 days of storage at respective temperatures. At 25°C, TEAC decreased from initial value of 8.26 µM TE/g to 6.06 µM TE/g after 52 days of storage. Control samples had initial TEAC of 6.47 µM TE /g which decreased slightly to 6.36 and 6.42 µM TE /g after storage for 52 days at 25 and 4°C, respectively.

3. Changes in the consumer acceptance rating for the different sensory attributes of roasted REP by consumers

3.1 Overall acceptance of REP by consumers

The overall acceptance of roasted REP stored at varying times at accelerated storage temperature of 30, 35 and 40°C are shown in Table 4.22. At zero day storage, roasted resveratrol enhanced peanuts had significantly ($\alpha < 0.05$) lower overall acceptance ratings of 5.30, 5.14 and 4.90 or neither like nor dislike at accelerated storage temperatures of 30, 35 and 40°C, respectively, compared to controls with 7.10 or like moderately.

Table 4.22 Mean hedonic ratings ± standard deviation of consumer panel on roasted resveratrol-enhanced peanuts stored for varying times at 25, 30, 35, and 40°C.

Trt No.[1]	Temp. (°C)	Time (day)	Appearance[2]	Color[2]	Aroma[2]	Flavor[2]	Texture[2]	Overall Acceptance[2]
1	25	0	5.9 ±1.8a	5.9 ±1.7a	5.5 ±1.6bcd	5.1 ±1.7b	5.9 ±1.8d	5.1 ±1.7b
2	25	18	5.8 ±1.7a	5.9 ±1.7a	5.6 ±1.6bc	4.6 ±1.8bc	5.9 ±1.6d	5.0 ±1.7b
3	25	52	5.7 ±1.6a	5.9 ±1.6a	5.6 ±1.7b	4.7 ±1.9bc	6.0 ±1.6cd	5.0 ±1.6b
4	25	61	5.7 ±1.8a	5.8 ±1.8a	4.9 ±1.5de	4.6 ±1.7bc	6.0 ±1.8d	4.6 ±1.5bc
5	25	71	6.0 ±1.6a	5.9 ±1.7a	4.8 ±1.6de	4.5 ±2.0bc	6.0 ±1.7d	4.4 ±1.7c
6	25	90	5.8 ±1.7a	5.9 ±1.7a	4.9 ±1.5cde	4.3 ±1.6c	6.1 ±1.5cd	4.1 ±1.7c
7	25	101	6.0 ±1.4a	6.2 ±1.4a	4.9 ±1.9e	4.5 ±2.1bc	6.2 ±1.8bcd	4.1 ±1.9c
Control	25	0	6.3 ±1.5a	6.4 ±1.5a	6.8 ±1.9a	7.0 ±2.0a	6.6 ±1.5ab	7.1 ±1.9a
Control	25	52	6.6 ±1.2a	6.6 ±1.4a	6.8 ±1.6a	7.2 ±1.6a	6.9 ±1.4a	7.3 ±1.4a
Control	4	52	6.3 ±1.4a	6.2 ±1.5a	6.6 ±1.7a	7.1 ±1.4a	6.7 ±1.5ab	7.1 ±1.3a
	P-value		*0.1493*	*0.2810*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
8	30	0	6.2 ±1.6a	6.1 ±1.6a	5.7 ±1.8bc	5.6 ±1.6b	6.4 ±1.4abc	5.3 ±1.6b
9	30	6	5.9 ±1.5a	5.9 ±1.5a	5.6 ±1.2bc	5.3 ±1.2b	6.3 ±1.4bc	5.2 ±1.4b
10	30	23	6.0 ±1.4a	6.1 ±1.4a	5.8 ±1.3b	5.5 ±1.1b	6.3 ±1.2abc	5.3 ±1.5b
11	30	42	5.9 ±1.7a	5.9 ±1.7a	5.3 ±1.9bcd	5.1 ±1.5bc	6.1 ±1.6c	5.0 ±1.4bc
12	30	61	6.1 ±1.5a	6.1 ±1.7a	5.0 ±1.6cd	4.5 ±1.5c	6.2 ±1.6c	4.4 ±1.5cd
13	30	72	6.0 ±1.5a	6.1 ±1.6a	4.9 ±1.4d	4.6 ±1.7c	6.3 ±1.5c	4.1 ±1.7d
Control	25	0	6.3 ±1.5a	6.4 ±1.5a	6.8 ±1.9a	7.0 ±2.0a	6.6 ±1.5abc	7.1 ±1.9a
Control	25	52	6.6 ±1.2a	6.6 ±1.4a	6.8 ±1.6a	7.2 ±1.6a	6.9 ±1.4a	7.3 ±1.4a
Control	4	52	6.3 ±1.4a	6.2 ±1.5a	6.6 ±1.7a	7.1 ±1.4a	6.7 ±1.5ab	7.1 ±1.3a
	P-value		*0.3979*	*0.2136*	*<0.0001*	*<0.0001*	*0.0014*	*<0.0001*

Table 4.22 continued…

Trt No.[1]	Temp. (°C)	Time (day)	Appearance[2]	Color[2]	Aroma[2]	Flavor[2]	Texture[2]	Overall Acceptance[2]
14	35	0	6.1 ±1.4a	5.9 ±1.6bc	5.6 ±1.7bc	5.3 ±1.6bc	6.2 ±1.6cd	5.1 ±1.7b
15	35	8	6.0 ±1.2a	6.0 ±1.4bc	5.8 ±1.2b	5.6 ±1.3bc	6.2 ±1.3bcd	5.3 ±1.2b
16	35	17	5.8 ±1.7a	5.7 ±1.7c	5.1 ±1.6cd	5.0 ±1.7cd	6.0 ±1.3cd	4.8 ±1.6bc
17	35	27	5.9 ±1.3a	5.9 ±1.4bc	5.4 ±1.5cd	5.0 ±1.5bc	6.2 ±1.4cd	5.1 ±1.5b
18	35	46	6.1 ±1.3a	6.0 ±1.4abc	4.9 ±1.2e	4.4 ±1.4d	6.2 ±1.5cd	4.4 ±1.3c
19	35	57	6.0 ±1.7a	5.9 ±1.6bc	4.7 ±1.5e	4.4 ±1.6d	6.2 ±1.3bcd	4.4 ±1.6c
Control	25	0	6.3 ±1.5a	6.4 ±1.5ab	6.8 ±1.9a	7.0 ±2.0a	6.6 ±1.5abc	7.1 ±1.9a
Control	25	52	6.6 ±1.2a	6.6 ±1.4a	6.8 ±1.6a	7.2 ±1.6a	6.9 ±1.4a	7.3 ±1.4a
Control	4	52	6.3 ±1.4a	6.2 ±1.5abc	6.6 ±1.7a	7.1 ±1.4a	6.7 ±1.5ab	7.1 ±1.3a
	P-value		*0.1384*	*0.0096*	*<0.0001*	*<0.0001*	*0.0003*	*<0.0001*
20	40	0	5.9 ±1.5a	6.0 ±1.5a	5.4 ±1.8bcd	5.0 ±1.8b	6.0 ±1.6c	4.9 ±1.7b
21	40	6	6.0 ±1.5a	5.9 ±1.5a	5.9 ±1.4b	5.2 ±1.5b	6.2 ±1.4bc	5.1 ±1.4b
22	40	16	6.0 ±1.6a	6.0 ±1.6a	5.5 ±1.5bcd	5.0 ±1.8b	6.0 ±1.6c	5.0 ±1.4b
23	40	19	6.2 ±1.6a	5.9 ±1.6a	5.6 ±1.3bc	5.2 ±1.5b	5.9 ±1.5c	5.0 ±1.5b
24	40	35	6.0 ±1.5a	6.2 ±1.5a	5.1 ±1.4cd	4.3 ±1.5c	5.9 ±1.7c	4.2 ±1.3c
25	40	46	6.0 ±1.5a	6.0 ±1.6a	4.9 ±1.6d	4.6 ±1.7bc	5.9 ±1.8c	4.2 ±1.7c
Control	25	0	6.3 ±1.5a	6.4 ±1.5a	6.8 ±1.9a	7.0 ±2.0a	6.6 ±1.5abc	7.1 ±1.9a
Control	25	52	6.6 ±1.2a	6.6 ±1.4a	6.8 ±1.6a	7.2 ±1.6a	6.9 ±1.4a	7.3 ±1.4a
Control	4	52	6.3 ±1.4a	6.2 ±1.5a	6.6 ±1.7a	7.1 ±1.4a	6.7 ±1.5ab	7.1 ±1.3a
	P-value		*0.2775*	*0.1276*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

[1] Control samples were prepared from untreated peanuts.

[2] Mean ratings for acceptance using 9-point hedonic rating scale: 1="dislike extremely"; 5="neither like nor dislike"; 9="like extremely." Mean ratings within a column at each storage temperature along with controls not followed by the same letter are significantly different ($P<0.05$) from each other as determined by Fisher's least significance difference mean separation test.

The initial overall acceptance rating of ≥ 5 or neither like nor dislike did not change significantly (P<0.05) when REP were stored for up to 42 days at 30°C, up to 27 days at 35°C, and up to 19 days at 40°C. At these storage times, the levels of hexanal were 29.45, 26.45 and 22.27 μg/g for samples stored at 30, 35 and 40°C, respectively. After these storage times, all REP were disliked by the consumers with OA ratings <5 or dislike slightly, indicating that the shelf life of REP at accelerated temperature of 30, 35 and 40°C were 42, 27 and 19 days, respectively. At similar level of acceptance rating of ≥ 5, REP had higher hexanal contents compared to 6.01 μg/g in untreated roasted peanuts stored at 40°C for 66 days (Grosso and Resurrecion, 2002). The OA roasted control peanuts in this study did not change (P<0.05) after storage for 52 days at 25°C and 4°C.

At verification temperature of 25°C, the initial mean OA rating of REP was 5.10 or neither like nor dislike which did not change (P<0.05) after 52 days with equivalent hexanal of 23.57 μg/g. After 61-101 days, OA decreased to <5 and the samples remained disliked slightly with the corresponding increases in hexanal to 45.07-106.07 μg/g.

3.2 Acceptance ratings for the different sensory attributes of roasted REP by consumers

Table 4.22 shows the consumer ratings for their liking of the different sensory attributes of roasted REP.

Acceptance for Appearance and Color. Consumers had similar (P<0.05) acceptance ratings for the appearance of all REP and untreated controls, except for REP sample stored for 17 days at 35°C. This indicates that in terms of appearance, REP which needs to be sliced, have potential applications to peanut containing products such as peanut bars, granola, and other confections.

Similarly, consumers liked the color of treated stored REP (5.72 – 6.20 or like slightly) as much as the untreated controls (6.22-6.48 or like slightly). This implies that in terms of color, the REP can be used in any product applications where peanuts are visible.

Acceptance for Aroma and Flavor. In terms of aroma, consumers had lower (P<0.05) acceptance ratings for all REP (ratings = 4.8 – 5.8 or neither like nor dislike to dislike slightly) compared to untreated controls (ratings= 6.6-6.8 or like slightly). Except for REP stored at 35^{o}C, consumer's liking for aroma did not change (P<0.05) from its initial ratings at all storage temperatures, regardless of storage time and temperature suggesting that this was due to processing treatment of REP rather than storage effect. At 35^{o}C, consumers' liking for aroma decreased from 5.6 or neither like nor dislike to 4.9 or dislike slightly after 46 days of storage.

Although objective measurements of quality characteristics may strongly indicate quality in a product, in the final analysis, flavor largely determines product acceptance (Morris and Freeman, 1954). In terms of flavor, REP had lower (P<0.05) acceptance ratings of 4.30 to 5.78 or neither like nor dislike to dislike slightly, compared to untreated controls with 6.98-7.20 or like slightly to like moderately. The initial acceptance ratings for flavor did not change (P<0.05) when REP were stored for 42 days at 30^{o}C, 27 days at 35^{o}C, and 19 days at 40^{o}C, suggesting a processing treatment effect rather than storage effect. The acceptance for flavor compared to initial ratings decreased after 61 days at 30^{o}C, 46 days 30^{o}C, and 35 days 40^{o}C. At 25^{o}C, REP' initial flavor acceptance rating 5.10 did not change (P<0.05) after 71 days, then decreased to 4.3 after 90 days

Acceptance for Texture. Consumers liked (P<0.05) the texture of the stored roasted REP (rating= 6.04-6.42 or liked slightly) as much as untreated controls (rating=6.64-6.86 or liked slightly. At 25^{o}C, consumers had lower acceptance ratings for texture or 5.9 – 6.2 (6=like slightly) compared to untreated controls with ratings of 6.6-6.9 (7=like moderately).

4. Changes in the consumers' just-about-right (JAR) ratings for roasted peanutty flavor and bitterness

The just-about-right (JAR) rating scale had anchors of "high or too much" or "low or too little" of a product sensory attribute on either ends on the scale, with just-about-right at the middle. If not JAR, then consumers will indicate whether the product is higher or lower relative to their perceptual ideal level of that product attribute (Muñoz, 2004). The 7-point JAR rating scale with midpoint at 4=JAR was used in this study to determine the consumer perceptions of the roasted peanutty flavor and bitterness in REP (Table 4. 23), which were expected to be lower than JAR as a result of exposure to US and UV processing treatments.

4.1 Consumer JAR ratings for roasted peanutty flavor.

Roasted peanutty flavor is the most desirable sensory attribute in peanuts important to consumers. Roasting remove the tastes of raw peanuts to produce an appetizing product (Morris and Freeman, 1996). Roasted peanut flavor is composed of a complex blend of heterocyclic and other volatile compounds formed during roasting through thermal degradation reactions including Maillard reactions between carbohydrate, free amino acid, and protein (Beet and Boylston, 1992). Pyrazine derivatives are among the "character impact" compounds of typical roasted peanuts (Mason et al., 1966). REP used in this study were roasted to medium roast as this degree produced the most desirable flavor in peanut butters compared to light or heavy roasts (Morris and Freeman, 1996).

The JAR ratings for roasted peanutty flavor of all REP (ratings= 2.9-4.1) were lower (P<0.05) than unstored untreated controls (rating=4.5). REP stored for 52 days at 25^{o}C, 23 days at 30^{o}C, 27 days at 35^{o}C, and 19 days at 40^{o}C had JAR ratings which were not different (P<0.05) from controls stored for 52 days at 25^{o}C (rating = 4.2). This result was significant indicating that

Table 4.23 Consumer panel ratings (mean ± standard deviation) for just-about-right and intensity of sensory attributes of roasted resveratrol-enhanced peanuts stored for varying times at 25, 30, 35, and 40°C[1]

Trt. No.[2]	Storage Temperature (°C)	Storage Time (days)	JAR- Roasted peanutty flavor[3]	JAR- Bitterness[3]	Intensity of roasted peanutty flavor[4]	Intensity of off-flavor[4]
1	25	0	4.0 ±1.0b	4.6 ±1.0a	5.2 ±1.5b	5.4 ±1.5bc
2	25	18	3.9 ±1.0b	4.6 ±1.1a	5.0 ±1.2b	5.4 ±1.5bc
3	25	52	3.9 ±1.1b	4.4 ±1.2a	5.0 ±1.3b	5.3 ±1.6c
4	25	61	3.4 ±1.1c	4.2 ±1.3a	4.3 ±1.2c	5.7 ±1.2abc
5	25	71	3.4 ±1.4c	4.3 ±1.2a	4.3 ±1.6c	5.9 ±1.4abc
6	25	90	3.2 ±1.1cd	4.4 ±1.2a	4.2 ±1.8c	6.1 ±1.2a
7	25	101	2.9 ±1.1d	4.4 ±1.0a	3.5 ±1.2d	6.1 ±1.5ab
Control	25	0	4.5 ±1.1a	4.1 ±0.8a	6.1 ±1.5a	3.3 ±2.3d
Control	25	52	4.2 ±1.3ab	4.1 ±0.5a	5.8 ±1.5a	3.1 ±2.0d
Control	4	52	4.5 ±1.1a	4.1 ±0.7a	6.0 ±1.4a	3.1 ±1.9d
8	30	0	3.9 ±0.9bc	4.2 ±1.1a	5.1 ±1.0b	5.1 ±1.3b
9	30	6	3.9 ±0.9bc	4.2 ±0.9a	4.9 ±1.0b	5.0 ±1.2b
10	30	23	3.9 ±0.7bc	4.2 ±0.9a	4.8 ±1.0b	5.0 ±1.0ab
11	30	42	3.8 ±1.0c	4.3 ±1.0a	5.2 ±1.2b	5.3 ±1.3ab
12	30	61	3.2 ±0.9d	4.2 ±0.9a	4.1 ±1.1b	5.5 ±1.0ab
13	30	72	3.0 ±01.0d	4.2 ±0.8a	3.7 ±1.2c	5.6 ±1.2a
Control	25	0	4.5 ±1.1a	4.1 ±0.8a	6.1 ±1.5a	3.3 ±2.3c
Control	25	52	4.2 ±1.3ab	4.1 ±0.5a	5.8 ±1.5a	3.1 ±1.9c
Control	4	52	4.5 ±1.1a	4.1 ±0.7a	6.0 ±1.4a	3.1 ±1.9c

Table 4.23 continued…

Trt. No. [2]	Storage Tempera-ture (^{o}C)	Storage Time (days)	JAR- Roasted peanutty flavor[3]	JAR- Bitterness[3]	Intensity of roasted peanutty flavor[4]	Intensity of off-flavor[4]
14	35	0	4.0 ±1.0cd	4.4 ±1.0a	5.4 ±1.2bc	5.0 ±1.4b
15	35	8	3.9 ±1.0cd	4.1 ±1.0a	4.8 ±1.1d	4.9 ±1.1b
16	35	17	4.1 ±1.0bcd	4.5 ±1.1a	5.0 ±1.2cd	5.3 ±1.3ab
17	35	27	3.9 ±1.0cd	4.2 ±0.9a	4.8 ±1.0d	5.3 ±1.2a
18	35	46	3.2 ±0.8e	4.1 ±1.0a	4.0 ±1.2e	5.8 ±1.1a
19	35	57	3.2 ±1.1e	4.2 ±1.1a	3.8 ±1.4e	5.8 ±1.3a
Control	25	0	4.5 ±1.1ab	4.1 ±0.8a	6.1 ±1.5a	3.3 ±2.3c
Control	25	52	4.2 ±1.3abc	4.1 ±0.5a	5.8 ±1.5ab	3.1 ±1.9c
Control	4	52	4.5 ±1.1a	4.1 ±0.7a	6.0 ±1.4a	3.1 ±1.9c
20	40	0	4.1 ±1.2ab	4.2 ±1.0a	5.2 ±1.5b	5.0 ±1.5b
21	40	6	3.9 ±1.0ab	4.3 ±1.0a	5.1 ±1.2b	5.3 ±1.1b
22	40	16	3.9 ±1.0bc	4.3 ±1.0a	5.2 ±1.2b	5.4 ±1.3ab
23	40	19	3.8 ±1.0bc	4.3 ±1.0a	5.1 ±1.2b	5.4 ±1.4ab
24	40	35	3.5 ±1.2cd	4.2 ±1.0a	4.4 ±1.3c	5.9 ±1.4ab
25	40	46	3.2 ±1.2d	4.2 ±1.1a	4.3 ±1.4c	5.6 ±1.4ab
Control	25	0	4.5 ±1.1a	4.1 ±0.8a	6.1 ±1.5a	3.3 ±2.3c
Control	25	52	4.2 ±1.3ab	4.1 ±0.5a	5.8 ±1.5a	3.1 ±2.0c
Control	4	52	4.5 ±1.1a	4.1 ±0.7a	6.0 ±1.4a	3.1 ±1.9c

[1] Mean ratings within a column and storage temperature along with controls not followed by the same letter are significantly different (P<0.05) from each other as determined by Fisher's least significance difference mean separation test.
[2] Control samples were prepared from untreated peanuts.
[3] Consumer ratings using 7-point just-about-right rating scale: 1="much too weak"; 4="just-about-right"; 7="much too strong".
[4] Consumer ratings using 9-point intensity rating scale: 1="none"; 4="neither weak nor strong"; 9="extremely strong".

in terms of roasted peanutty flavor, product reformulations are not needed to meet the consumers' desired degree of roasted peanutty flavor in REP stored up to 52 days at 25^{o}C. The roasted peanutty flavors of REP were less than JAR after 61, 42, 46, and 35 days at 25, 30, 35, and 40^{o}C; and were lower than controls.

4.2 Consumer JAR ratings for bitterness.

The JAR ratings for bitterness of REP ranged from 4.16-4.37 or just-about-right, and did not change (P<0.05) during storage at 30, 35 and 40^{o}C. All REP samples had JAR bitterness ratings which were not different (P <0.05) from controls, except for REP stored at 25^{o}C for 18 days which had stronger bitterness than controls, although its rating of 4.6 is still within the JAR category. These results indicate that the bitterness of REP was just-about-right from consumers' perception of which is unexpected as REP were expected to be bitter due to production of phenolic compounds that impart bitterness in foods. This finding, however, was significant as REP will have food applications in products containing peanuts without imparting bitter taste.

5. Changes in the consumer intensity ratings for roasted peanutty flavor and off-flavor

5.1 Consumer intensity rating for roasted peanutty.

The most important sensory characteristic in roasted peanuts is the roasted peanutty flavor. The intensity of roasted peanutty flavor of all REP stored at 30, 35 and 40^{o}C (Table 4.23) ranged from 3.7-5.4 or moderately weak to neither weak nor strong, which were lower (P<0.05) compared untreated controls with ratings of 5.8-6.1 (6= slightly strong). Roasted peanutty flavor of all REP samples did not change (P<0.05) up to 61, 27 and 19 days of storage at 30, 35 and 40^{o}C, respectively, suggestive of a processing treatment effect rather than a storage effect; but decreased (P<0.05) after 72, 46, and 35 days at 30, 35, and 40^{o}C, respectively, indicating a storage effect.

At 25°C, all REP had weaker (rating=3.5-5.2) roasted peanutty flavor compared to controls; which decreased after 52 days from 5.2 (neither weak nor strong) to 4.3 (slightly weak), and further decreased to 3.5 (moderately weak) after 90 days. Warner et al (1996) also found that flavor scores for untreated roasted peanuts decreased slightly, leveled-off during storage whereas, hexanal increased during 38-day storage. The decreasing intensities in roasted peanutty flavor or 'flavor fade'of REP during storage could be attributed to masking of pyrazines and other 'roasted peanut' flavor compounds by large quantities of low-molecular weight aldehydes during lipid oxidation and not due to polymerization and/or degradation of the pyrazines that produce the characteristic roasted peanut flavor (Warner et al., 1996).

5.2 Consumer intensity ratings for off-flavors.

Lipid oxidation during storage has long been recognized as contributing to the development of undesirable flavors of peanuts (Bett and Boylston, 1992). All REP had stronger ($P<0.05$) off-flavors (ratings = 4.9-6.1 or neither weak nor strong to slightly strong) than controls (ratings = 3.1-3.3 or moderately weak) verifying the higher contents of REP compared to control found in this study. The initial off-flavors intensities in REP remained at the same levels until these increased after 90, 72, and 17 days at 25, 30, and 35; but remained at the same levels until 46 days at 40°C. Rancidity, the off-flavor developed due lipid oxidation reactions, makes a food unacceptable to consumers (Labuza, 1984; St. Angelo, 1996). Increased off-flavors intensities in REP resulted in decreased roasted peanutty flavor intensities and overall acceptance ratings of REP.

6. Intensity ratings for the different sensory attributes of roasted REP by a descriptive panel

6.1 Descriptive panel intensity ratings for brown color, roasted peanutty, and raw beany

Table 4.24 and Figure 4.18 show the intensity ratings by a descriptive sensory panel for brown color, roasted peanutty, and raw beany aromatics of roasted REP stored for varying times and temperatures.

Brown color. The initial intensities of brown color of all REP samples did not change during storage at all temperatures studied and were not different from 52-day stored controls at 25^{o}C. The brown color of controls did not change during storage at 25 and 4^{o}C for 52 days. These results suggest that storage time and temperature had no effect on the brown color of roasted REP and control peanuts.

Roasted peanutty flavor. The roasted peanutty flavor is the most important sensory attribute in peanuts. Generally, three types of reactions are responsible for the formation of roasted peanutty flavor and aroma which include Maillard reaction between sugar and amino acids, lipid thermal decomposition, and sugar degradation (El-Kayati et al., 1998). More than 300 volatile flavor components have been identified in roasted peanuts (El-Kayati et al., 1998) since Mason et al., 1966) first showed that low molecular weight pyrazines were important compounds. Soonafter, researchers identified additional new volatile compounds in roasted peanuts (Mason et al., 1967; Johnson et al., 1971; Walradt et al., 1971; Ho et al., 1981).

All REP had less (P<0.05) roasted peanutty flavor compared to controls. This finding agrees with the results of consumer test, where intensities for roasted peanutty flavor of all REP were lower (P<0.05) than controls, suggesting that reduction in roasted peanutty flavor was a processing

Table 4.24 Changes in the intensity ratings (mean ± standard deviation) for the brown color, roasted peanutty and raw beany aromatics of roasted resveratrol-enhanced peanuts stored for varying times at accelerated temperatures of 30, 35, and 40°C and verified at 25°C[1].

Trt. No.	Temp. (°C)	Time (day)	Brown color	Roasted peanutty	Raw beany
1	25	0	36.5 ± 5.2a	34.5 ± 5.2c	0.6 ± 2.0a
2	25	18	35.4 ± 2.1a	33.2 ± 1.0c	0.2 ± 0.4a
3	25	52	36.0 ± 5.1a	30.9 ± 2.5d	0.2 ± 0.7a
4	25	61	35.0 ± 1.8a	29.3 ± 2.2de	0.8 ± 2.5a
5	25	71	37.4 ± 2.2a	27.8 ± 2.0e	0.5 ± 1.8a
6	25	90	35.4 ± 2.4a	26.0 ± 1.9f	0.8 ± 2.4a
7	25	101	34.5 ± 2.1a	25.1 ± 1.8f	0.6 ± 2.3a
Control	25	0	36.6 ± 2.8a	58.9 ± 3.7a	0.2 ± 0.4a
Control	25	52	35.4 ± 4.0a	55.0 ± 4.2a	0.7 ± 2.2a
Control	4	52	37.0 ± 3.0a	58.4 ± 4.0a	0.5 ± 2.0a
	P-value		*0.0811*	*<0.0001*	*0.9989*
8	30	0	34.9 ± 1.7bc	34.8 ± 1.7c	0.8 ± 3.2a
9	30	6	34.3 ± 2.0c	33.4 ± 1.4c	0.1 ± 0.3a
10	30	23	35.9 ± 4.6abc	31.4 ± 0.2d	0.0 ± 0.2a
11	30	42	36.9 ± 5.1ab	29.7 ± 1.3e	1.7 ± 7.0a
12	30	61	35.4 ± 2.2abc	28.0 ± 1.6f	0.2 ± 0.4a
13	30	72	34.9 ± 3.5bc	26.5 ± 1.7f	0.2 ± 0.4a
Control	25	0	36.6 ± 2.8ab	58.9 ± 3.7a	0.2 ± 0.4a
Control	25	52	35.4 ± 4.0abc	55.0 ± 4.2b	0.7 ± 2.2a
Control	4	52	37.0 ± 3.0a	58.4 ± 4.0a	0.5 ± 2.0a
	P-value		*0.1574*	*<0.0001*	*0.7291*
14	35	0	35.0 ± 1.1c	34.8 ± 1.0c	0.1 ± 0.2a
15	35	8	34.6 ± 1.3c	32.9 ± 1.2d	0.8 ± 2.8a
16	35	17	34.8 ± 3.0abc	31.4 ± 1.2d	0.1 ± 0.3a
17	35	27	34.8 ± 2.8c	28.8 ± 2.4e	0.0 ± 0.2a
18	35	46	34.3 ± 1.8c	27.5 ± 2.1ef	0.6 ± 2.2a
19	35	57	34.8 ± 1.4c	26.1 ± 2.2f	0.1 ± 0.3a
Control	25	0	36.6 ± 2.8ab	58.9 ± 3.7a	0.2 ± 0.4a
Control	25	52	35.4 ± 4.0bc	55.0 ± 4.2b	0.7 ± 2.2a
Control	4	52	37.0 ± 3.0a	58.4 ± 4.0b	0.5 ± 2.0a
	P-value		*0.0199*	*<0.0001*	*0.6699*

Table 4.24 continued…

Trt. No.	Temp. (°C)	Time (day)	Brown color	Roasted peanutty	Raw beany
20	40	0	35.6 ± 5.3abc	34.9 ± 1.1c	0.5 ± 2.0a
21	40	6	34.9 ± 5.4abc	32.9 ± 1.2d	0.1 ± 0.3a
22	40	16	34.5 ± 2.7bc	30.7 ± 2.4e	0.2 ± 0.4a
23	40	19	34.1 ± 2.9c	28.7 ± 2.4f	0.2 ± 0.5a
24	40	35	34.3 ± 1.0c	27.9 ± 1.7fg	0.1 ± 0.2a
25	40	46	33.9 ± 1.6c	26.7 ± 1.5g	0.7 ± 2.5a
Control	25	0	36.6 ± 2.8ab	58.9 ± 3.7a	0.2 ± 0.4a
Control	25	52	35.4 ± 4.0abc	55.0 ± 4.2b	0.7 ± 2.2a
Control	4	52	37.0 ± 3.0a	58.4 ± 4.0a	0.5 ± 2.0a
P-value			*0.0125*	*<0.0001*	*0.8122*

[1]Intensity ratings using 150 mm unstructured line scale with anchors at 12.5 mm and 137.5 mm by 10 trained descriptive panel.

Means within a column at specified storage temperature along with controls, not followed by the same letter are significantly ($P<0.05$) different from each other as determined by Fisher's least significance difference mean separation test.

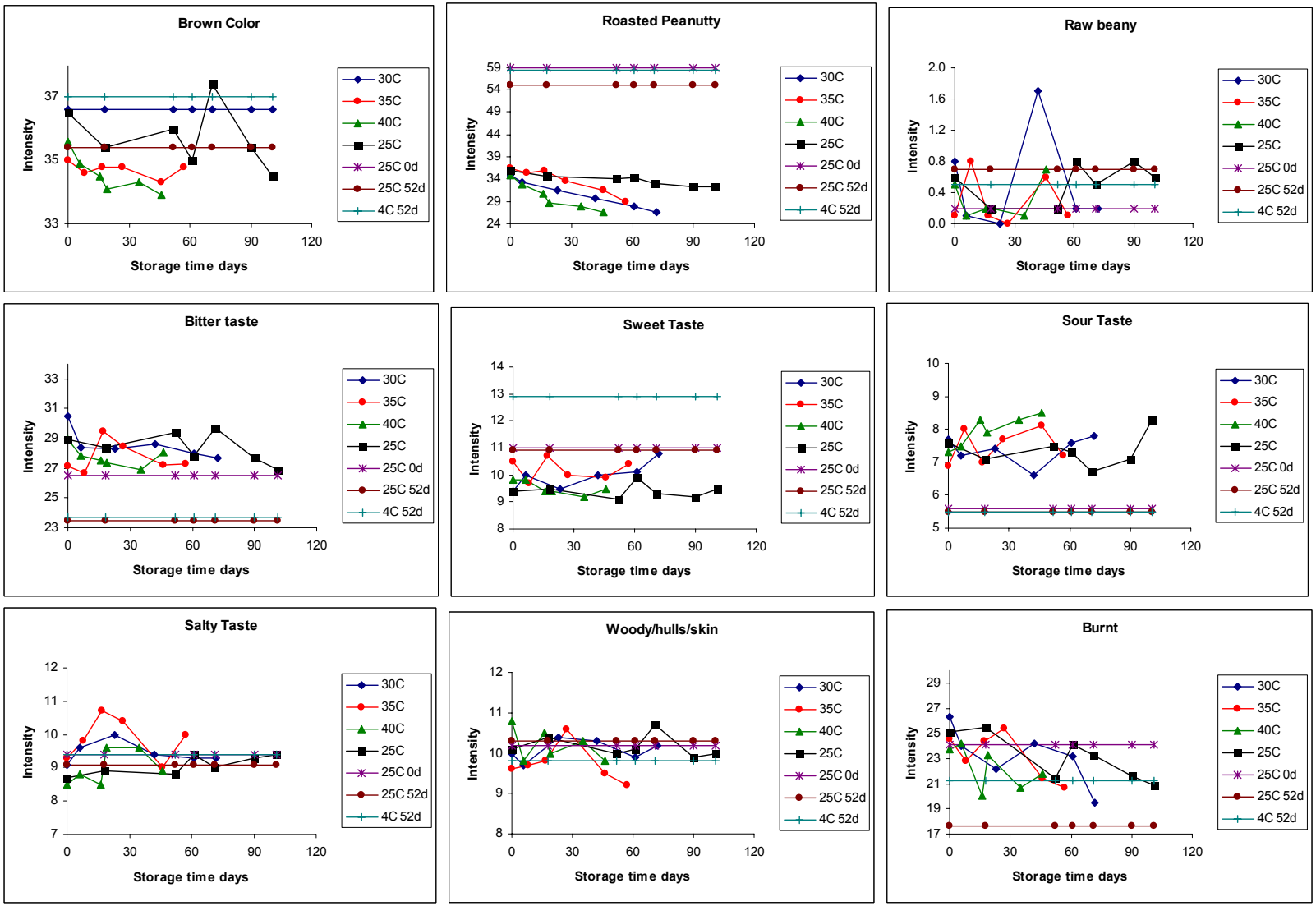


Figure 4.18 Changes in the intensity ratings of the different sensory attributes of roasted resveratrol-enhanced peanuts during storage at varying times and temperatures, by a descriptive panel. Horizontal lines indicate untreated controls.

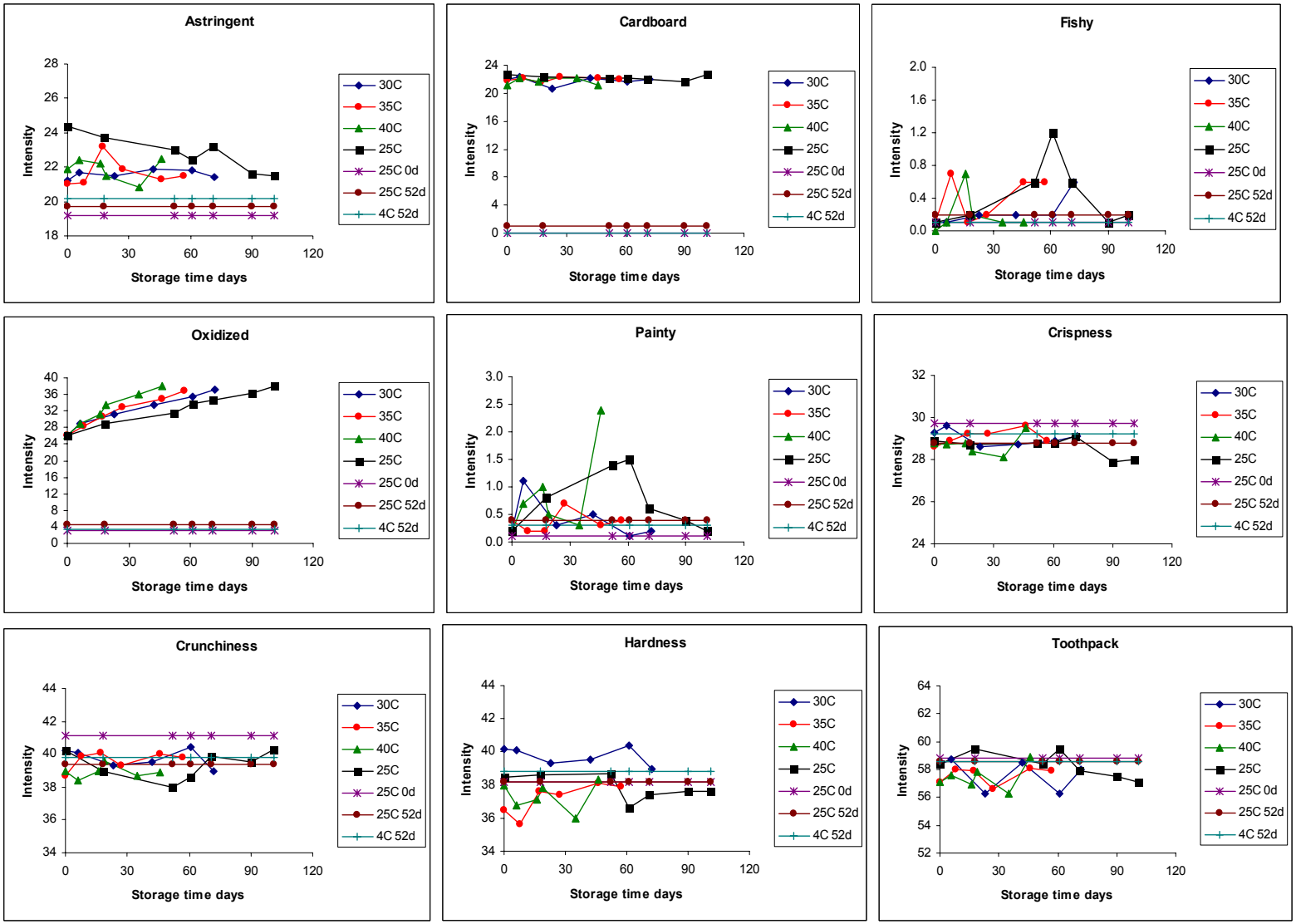


Figure 4.18 continued…

treatment effect. The initial intensities of roasted peanutty flavor of REP, decreased significantly during storage at 25°C indicating processing treatment rather than storage effect.

Raw beany flavor. Raw beany flavor is an unacceptable off-flavor in roasted peanuts which is removed by proper roasting of peanuts (Morris & Freeman, 1954). REP had low intensity ratings for raw beany of 0-1.7 which were not significantly different ($P<0.05$) from controls. These results indicate that roasting peanuts to medium roast with an L-value of 50 ± 1 was adequate to remove the raw beany flavor of REP and control peanuts and that processing treatments and storage had no effect on the raw beany flavor of treated and untreated control peanuts.

6.2 Descriptive panel intensity ratings for basic tastes

Table 4.25 and Figure 4.18 present the intensity ratings by a descriptive sensory panel for bitter, sweet, sour and salty tastes of roasted REP stored for varying times and temperatures. Significant differences between REP and controls were observed in bitter and sour tastes but not in sweet and salty tastes

Bitter taste. Significant differences in the intensity of bitterness between REP and controls were observed during storage at 25 and 30°C but not at 35 and 40°C. At 25 and 30°C, REP samples were significantly more bitter then stored controls, but not different from non-stored controls.

Sour taste. Significant differences in the intensity of sourness between REP and controls were observed only at the highest temperature of 40°C. At 40°C, no significant differences in the sourness of all REP samples were observed during storage, and were not significantly different from initial control and 52-day control stored at 4°C. However REP stored from 16 to 46 days (Trt#22, 23, 24 and 25) were significantly more sour than controls stored for 52 days at 25°C.

Table 4.25 Changes in the intensity ratings (mean ± standard deviation) for the basic tastes of roasted resveratrol-enhanced peanuts stored for varying times at accelerated temperatures of 30, 35, and 40°C and verified at 25°C[1].

Trt. No.	Temp. (°C)	Time (day)	Bitter	Sweet	Sour	Salty
1	25	0	28.9 ±7.2a	9.4 ±3.8b	7.6 ±3.3ab	8.7 ±3.5a
2	25	18	28.4 ±4.6a	9.5 ±4.0b	7.1 ±3.9ab	8.9 ±3.9a
3	25	52	29.4 ±4.6a	9.1 ±4.0b	7.5 ±3.7ab	8.8 ±3.7a
4	25	61	27.8 ±5.7a	9.9 ±3.8b	7.3 ±3.7ab	9.4 ±3.0a
5	25	71	29.7 ±4.6a	9.3 ±4.5b	6.7 ±3.0ab	9.0 ±3.1a
6	25	90	27.7 ±6.1a	9.2 ±3.9b	7.1 ±3.2ab	9.3 ±3.1a
7	25	101	26.9 ±5.8ab	9.5 ±3.3b	8.3 ±2.0a	9.4 ±4.0a
Control	25	0	26.5 ±7.8ab	11.0 ±5.9ab	5.6 ±2.0b	9.4 ±4.0a
Control	25	52	23.5 ±7.4b	10.9 ±5.5ab	5.5 ±4.2b	9.0 ±3.3a
Control	4	52	23.7 ±7.0b	12.9 ±4.7a	5.5 ±4.0b	9.4 ±4.1a
	P-value		*0.0057*	*0.2035*	*0.3954*	*0.9997*
8	30	0	30.5 ±4.6a	9.4 ±4.2b	7.7 ±2.7a	9.1 ±3.5a
9	30	6	28.4 ±3.7ab	10.0 ±4.7b	7.2 ±3.2a	9.6 ±3.9a
10	30	23	28.3 ±6.5ab	9.5 ±4.5b	7.4 ±3.5a	10.0 ±3.7a
11	30	42	28.6 ±3.9ab	10.0 ±3.7b	6.6 ±3.4a	9.4 ±3.0a
12	30	61	28.0 ±7.2ab	10.1 ±4.2b	7.6 ±2.9a	9.3 ±3.1a
13	30	72	27.7 ±6.7ab	10.8 ±3.9ab	7.8 ±2.2a	9.3 ±3.1a
Control	25	0	26.5 ±7.8bc	11.0 ±5.9ab	5.6 ±2.0b	9.4 ±4.0a
Control	25	52	23.5 ±7.4c	10.9 ±5.5ab	5.5 ±4.2b	9.0 ±3.3a
Control	4	52	23.7 ±7.0c	12.9 ±4.7a	5.5 ±4.0b	9.4 ±4.1a
	P-value		*0.0099*	*0.4669*	*0.3423*	*0.9911*
14	35	0	27.1 ±7.9ab	10.5 ±4.4ab	6.9 ±3.4ab	9.3 ±3.4a
15	35	8	26.7 ±7.3ab	9.7 ±3.9b	8.0 ±2.4a	9.8 ±3.9a
16	35	17	29.5 ±3.7a	10.7 ±4.4ab	7.0 ±2.7ab	10.7 ±2.3a
17	35	27	28.5 ±7.0a	10.0 ±3.3b	7.7 ±3.0ab	10.4 ±3.1a
18	35	46	27.2 ±5.4ab	9.9 ±4.1b	8.1 ±2.4a	9.0 ±3.4a
19	35	57	27.3 ±6.7ab	10.4 ±2.5ab	7.2 ±2.9ab	10.0 ±3.7a
Control	25	0	26.5 ±7.8ab	11.0 ±5.9ab	5.6 ±2.0b	9.4 ±4.0a
Control	25	52	23.5 ±7.4b	10.9 ±5.5ab	5.5 ±4.2b	9.0 ±3.3a
Control	4	52	23.7 ±7.0b	12.9 ±4.7a	5.5 ±4.0b	9.4 ±4.1a
	P-value		*0.0891*	*0.4943*	*0.1739*	*0.8480*

Table 4.25 continued…

Trt. No.	Temp. (°C)	Time (day)	Bitter	Sweet	Sour	Salty
20	40	0	28.9 ±5.6a	9.8 ±4.0b	7.3 ±2.4abc	8.5 ±3.0a
21	40	6	27.8 ±7.4ab	9.8 ±3.6b	7.5 ±3.0abc	8.8 ±3.4a
22	40	16	27.5 ±8.4ab	9.4 ±4.2b	8.3 ±3.2a	8.5 ±5.4a
23	40	19	27.4 ±6.8ab	9.4 ±3.8b	7.9 ±3.0ab	9.6 ±3.2a
24	40	35	26.9 ±6.2ab	9.2 ±4.1b	8.3 ±3.3a	9.6 ±3.4a
25	40	46	28.1 ±5.1a	9.5 ±4.1b	8.5 ±2.2a	8.9 ±2.0a
Control	25	0	26.5 ±7.8ab	11.0 ±5.9ab	5.6 ±2.0bc	9.4 ±4.0a
Control	25	52	23.5 ±7.4b	10.9 ±5.5ab	5.5 ±4.2c	9.0 ±3.3a
Control	4	52	23.7 ±7.0b	12.9 ±4.7a	5.5 ±4.0c	9.4 ±4.1a
	P-value		*0.0560*	*0.0583*	*0.0378*	*0.9782*

[1]Intensity ratings using 150 mm unstructured line scale with anchors at 12.5 mm and 137.5 mm by 10 trained descriptive panel.

Means within a column at specific storage temperature along with controls not followed by the same letter are significantly ($P<0.05$) different from each other as determined by Fisher's least significance difference mean separation test.

6.3 Descriptive panel intensity ratings for woody/hulls/skins, burnt and astringency aromatics

The intensity ratings by a descriptive sensory panel for woody/hulls/skins, burnt and astringency aromatics of roasted REP stored for varying times and temperatures are shown in Table 4.26 and Figure 4.19.

Woody/Hulls/Skins. All REP and control peanuts had the same (P<0.05) intensity of woody/hulls/skins flavor which did not change during storage. This results suggest that woody/hulls/skins flavor in all peanut samples was not affected by storage and processing treatments. The absence of peanut skins in all samples resulted in low intensity ratings of 9.7-10.8 for woody/hulls/skins.

Burnt Flavor. There is no significant difference (P<0.05) in the burnt flavor between REP and controls. This result suggests that roasting the samples to medium roast did not cause any burnt flavor in the samples.

Astringency. Polyphenols in the diet, of which *trans*-resveratrol, caffeic acid, coumaric acid, and ferulic acid found in REP are examples, precipitate oral proteins producing an astringent sensation (Baxter et al., 1997). The salivary proline-rich proteins, which are secreted into the oral cavity, form complexes with and precipitated dietary polyphenols and thus constitute the primary mammalian defense directed against ingestion of polyphenols (Baxter et al., 1997).

In this study, the intensity ratings for astringency of all REP stored at accelerated temperatures of 30, 35 and 40^{o}C were higher, but not significantly (P<0.05) different from controls. At 25^{o}C, the intensity of astringent aftertaste of REP significantly decreased after 71 days to levels that were significantly different from controls stored for 52 days at 25^{o}C but not from controls stored at 4^{o}C and REP samples stored from 90-101 days. The initial intensities of

Table 4.26 Changes in the intensity ratings (mean ± standard deviation) for woody/hulls/skins and burnt flavors and astringency of roasted resveratrol-enhanced peanuts stored for varying times at accelerated temperatures of 30, 35 and 40°C and verified at 25°C[1].

Trt. No.	Temp. (°C)	Time (day)	Woody/Hulls/ Skins	Burnt	Astringency
1	25	0	10.1 ±3.0a	25.1 ±6.6a	24.4 ±7.1a
2	25	18	10.4 ±2.9a	25.5 ±7.0a	23.7 ±5.7ab
3	25	52	10.0 ±2.5a	21.4 ±10.8a	23.0 ±3.2ab
4	25	61	10.1 ±2.3a	24.1 ±7.1a	22.4 ±2.4abc
5	25	71	10.7 ±3.4a	23.3 ±9.4a	23.2 ±3.4ab
6	25	90	9.9 ±2.6a	21.6 ±9.9a	21.6 ±5.6abcd
7	25	101	10.0 ±3.9a	20.9 ±8.6a	21.5 ±4.6abcd
Control	25	0	10.2 ±2.7a	24.1 ±10.1a	19.2 ±6.9d
Control	25	52	10.3 ±3.3a	17.6 ±10.8a	19.7 ±5.4cd
Control	4	52	9.8 ±3.8a	21.2 ±10.8a	20.2 ±5.3bcd
	P-value		*0.9989*	*0.1583*	*0.0250*
8	30	0	10.0 ±3.4a	26.3 ±7.2a	21.2 ±5.3a
9	30	6	9.7 ±2.6a	24.0 ±9.0a	21.7 ±5.0a
10	30	23	10.4 ±3.0a	22.2 ±8.8a	21.5 ±5.2a
11	30	42	10.3 ±2.6a	24.2 ±8.8a	21.9 ±5.8a
12	30	61	9.9 ±2.8a	23.2 ±10.1a	21.8 ±5.3a
13	30	72	10.2 ±3.5a	19.5 ±11.5a	21.4 ±5.3a
Control	25	0	10.2 ±2.7a	24.1 ±10.1a	19.2 ±6.9a
Control	25	52	10.3 ±3.3a	17.6 ±10.8a	19.7 ±5.4a
Control	4	52	9.8 ±3.8a	21.2 ±10.8a	20.2 ±5.3a
	P-value		*0.9939*	*0.1218*	*0.3725*
14	35	0	9.6 ±2.8a	24.6 ±9.3a	21.0 ±5.3a
15	35	8	9.7 ±2.7a	22.8 ±9.0a	21.1 ±5.1a
16	35	17	9.8 ±3.4a	24.4 ±7.9a	23.2 ±7.8a
17	35	27	10.6 ±3.0a	25.4 ±8.2a	21.9 ±5.3a
18	35	46	9.5 ±3.1a	21.4 ±9.8a	21.3 ±5.5a
19	35	57	9.2 ±3.5a	20.7 ±10.2a	21.5 ±5.5a
Control	25	0	10.2 ±2.7a	24.1 ±10.1a	19.2 ±6.9a
Control	25	52	10.3 ±3.3a	17.6 ±10.8a	19.7 ±5.4a
Control	4	52	9.8 ±3.8a	21.2 ±10.8a	20.2 ±5.3a
	P-value		*0.9645*	*0.2502*	*0.6541*

Table 4.26 continued…

Trt. No.	Temp. (°C)	Time (day)	Woody/Hulls/ Skins	Burnt	Astringency
20	40	0	10.8 ±2.7a	23.7 ±6.4a	21.9 ±2.2a
21	40	6	9.8 ±3.1a	24.2 ±6.2a	22.4 ±3.1a
22	40	16	10.5 ±2.0a	20.0 ±10.1a	22.2 ±5.4a
23	40	19	10.0 ±3.2a	23.3 ±7.5a	21.5 ±5.5a
24	40	35	10.3 ±1.8a	20.7 ±10.5a	20.8 ±5.2a
25	40	46	9.8 ±2.9a	21.8 ±10.9a	22.5 ±1.7a
Control	25	0	10.2 ±2.7a	24.1 ±10.1a	19.2 ±6.9a
Control	25	52	10.3 ±3.3a	17.6 ±10.8a	19.7 ±5.4a
Control	4	52	9.8 ±3.8a	21.2 ±10.8a	20.2 ±5.3a
	P-value		*0.9794*	*0.4271*	*0.2592*

[1]Intensity ratings using 150 mm unstructured line scale with anchors at 12.5 mm and 137.5 mm by 10 trained descriptive panel.

Means within a column at specific storage temperature along with controls not followed by the same letter are significantly ($P<0.05$) different from each other as determined by Fisher's least significance difference mean separation test.

astringency in REP did not change during storage at all temperatures indicating that astringency was a processing rather than storage effect.

6.4 Descriptive panel intensity ratings for off-flavors – cardboard, fishy, oxidized and painty flavors/aromatics

The oxidation of fatty acids in both raw and roasted peanuts leads to off-flavors in peanut products (Felland and Koehler, 1997). Lipid oxidation products such as low molecular weight pentanal, hexanal, heptanal, octanal and nonanal create cardboard or oxidized rancid flavor (Warner et al., 1996). The intensity ratings by a descriptive sensory panel for cardboard, fishy, oxidized and painty flavors/aromatics of roasted REP stored for varying times and temperatures are shown in Table 4.27 and Figure 4.19.

Cardboard Flavor. The initial intensities of cardboard flavors of all REP although higher were not different ($P<0.05$) from controls. The cardboard flavor of REP did not change during storage indicating that it was not affected by storage.

Fishy Flavor. The fishy flavor of REP and controls were not different ($P<0.05$) from each other; and did not change during storage. The magnitude of fishy intensity ratings of 0.1–1.2 were low in 150-mm scale and of no significant consequence.

Oxidized Flavor. All REP had more oxidized flavor (intensity ratings= 26.1-37.9) than untreated controls (ratings=3.1-4.6). As storage time increased, the intensities of oxidized flavor increased significantly until after 101, 72, 57 and 39 days of storage at 25, 30, 35 and 40^{o}C, respectively. These findings showed that the oxidized flavors in REP were both due to the effects of processing treatment and storage.

Painty Flavor. The intensities of painty flavors in REP stored at all temperatures were not significantly different from controls, except for samples stored at 40^{o}C for 46 days which had

Table 4.27 Changes in the intensity ratings (mean ± standard deviation) for the cardboard, fishy, oxidized, and painty aromatics of roasted resveratrol-enhanced peanuts stored for varying times at accelerated temperatures of 30, 35 and 40°C and verified at 25°C[1].

Trt. No.	Temp. (°C)	Time (day)	Cardboard	Fishy	Oxidized	Painty
1	25	0	22.7 ±4.3a	0.1 ±0.3a	26.1 ±4.3a	0.2 ±0.4a
2	25	18	22.4 ±3.7a	0.2 ±0.4a	28.9 ±4.5a	0.8 ±2.7a
3	25	52	22.1 ±3.8a	0.7 ±2.3a	31.5 ±4.5a	1.4 ±3.5a
4	25	61	22.2 ±2.8a	1.2 ±3.2a	33.7 ±4.9a	1.5 ±3.6a
5	25	71	22.0 ±3.5a	0.6 ±2.0a	34.7 ±3.2a	2.6 ±1.6a
6	25	90	21.7 ±2.9a	0.1 ±0.3a	36.3 ±2.7a	3.4 ±1.3a
7	25	101	22.7 ±4.1a	0.2 ±0.3a	37.9 ±2.6a	4.2 ±0.4a
Control	25	0	0.0 ±0b	0.1 ±0.3a	3.1 ±1.3b	0.1 ±0.3a
Control	25	52	1.0 ±0.5b	0.2 ±0.3a	4.6 ±1.9b	0.4 ±0.7a
Control	4	52	0.0 ±0b	0.1 ±0.3a	3.4 ±1.3b	0.3 ±1.0a
	P-value		*<0.0001*	*0.2322*	*<0.0001*	*0.3089*
8	30	0	22.0 ±3.2a	0.1 ±0.3a	26.2 ±4.2bc	0.2 ±0.4a
9	30	6	22.4 ±3.3a	0.1 ±0.4a	28.9 ±1.7bc	1.1 ±3.1a
10	30	23	20.6 ±5.5a	0.2 ±0.4a	31.1 ±3.1c	0.3 ±1.2a
11	30	42	22.1 ±3.8a	0.2 ±0.3a	33.5 ±3.4c	0.5 ±1.7a
12	30	61	21.7 ±1.9a	0.2 ±0.4a	35.5 ±3.1ab	0.1 ±0.3a
13	30	72	22.0 ±2.6a	0.6 ±2.2a	37.2 ±3.3a	0.2 ±0.3a
Control	25	0	0.0 ±0b	0.1 ±0.3a	3.1 ±1.3d	0.1 ±0.3a
Control	25	52	1.0 ±0.5b	0.2 ±0.3a	4.6 ±1.9d	0.4 ±0.7a
Control	4	52	0.0 ±0b	0.1 ±0.3a	3.4 ±1.3d	0.3 ±1.0a
	P-value		*<0.0001*	*0.6381*	*<0.0001*	*0.4848*
14	35	0	21.9 ±3.7a	0.1 ±0.3a	26.1 ±4.2b	0.4 ±1.0a
15	35	8	22.2 ±3.6a	0.7 ±2.3a	28.3 ±1.0b	0.2 ±0.4a
16	35	17	21.4 ±3.0a	0.1 ±0.3a	3.07 ±2.3b	0.2 ±0.3a
17	35	27	22.4 ±3.62a	0.2 ±0.3a	32.8 ±2.5b	0.7 ±1.7a
18	35	46	22.1 ±3.5a	0.6 ±1.5a	34.9 ±2.6a	0.2 ±0.3a
19	35	57	22.0 ±3.4a	0.6 ±2.0a	37.0 ±4.8a	0.4 ±1.2a
Control	25	0	0.0 ±0b	0.1 ±0.3a	3.1 ±1.3d	0.1 ±0.3a
Control	25	52	1.0 ±0.5b	0.2 ±0.3a	4.6 ±1.9d	0.4 ±0.7a
Control	4	52	0.0 ±0b	0.1 ±0.3a	3.4 ±1.3d	0.3 ±1.0a
	P-value		*<0.0001*	*0.5831*	*<0.0001*	*0.5234*

Table 4.27 continued…

Trt. No.	Temp. (°C)	Time (day)	Cardboard	Fishy	Oxidized	Painty
20	40	0	21.2 ±3.5a	0.0 ±0.2a	26.2 ±4.2b	0.2 ±0.4b
21	40	6	22.5 ±4.1a	0.1 ±0.3a	28.8 ±2.4ab	0.7 ±1.5b
22	40	16	21.6 ±6.1a	0.7 ±2.5a	31.3 ±3.6ab	1.0 ±2.8b
23	40	19	22.1 ±3.2a	0.2 ±0.6a	33.6 ±3.4ab	0.5 ±1.2b
24	40	35	22.2 ±3.6a	0.1 ±0.2a	35.9 ±3.4ab	0.3 ±1.1c
25	40	46	21.1 ±5.5a	0.1 ±0.4a	37.9 ±4.0c	2.4 ±4.2b
Control	25	0	0.0 ±0b	0.1 ±0.3a	3.1 ±1.3d	0.1 ±0.3b
Control	25	52	1.0 ±0.5b	0.2 ±0.3a	4.6 ±1.9d	0.4 ±0.7b
Control	4	52	0.0 ±0b	0.1 ±0.3a	3.4 ±1.3d	0.3 ±1.0b
	P-value		*<0.0001*	*0.3523*	*<0.0001*	*0.0111*

[1]Intensity ratings using 150 mm unstructured line scale with anchors at 12.5 mm and 137.5 mm by 10 trained descriptive panel.

Means within a column at specific storage temperature along with controls not followed by the same letter are significantly ($P<0.05$) different from each other as determined by Fisher's least significance difference mean separation test.

significantly higher painty flavor than controls. The magnitude of painty intensity ratings of 0–2.4, however, were low in 150-mm scale and of no significant consequence.

6.5 Intensity ratings for texture properties – crispness, crunchiness and hardness, and feeling factor, toothpack

The intensity ratings for the texture properties – crispness, crunchiness and hardness, and feeling factor – toothpack, of roasted REP stored for varying times and temperatures, by a descriptive sensory panel are shown in Table 4.28 and Figure 4.19. The ratings for crispness, crunchiness, hardness and toothpack of REP during storage at all temperatures were not different ($P<0.05$) each other and from controls. This result indicates that the texture properties and toothpack feeling factor were not affected by both processing treatment and storage.

7. Shelf life prediction of roasted resveratrol-enhanced peanuts using Accelerated Shelf Life Test (ASLT)

ASLT involves the use of higher testing temperatures in the food quality loss and shelf life experiments and extrapolation of the results to regular storage conditions through the use of the Arrhenius equation, which cuts down testing time substantially (Labuza, 2000). In this study the results of hexanal and overall acceptance of roasted resveratrol-enhanced peanut samples stored at accelerated temperatures of 30, 35, and 40^{o}C were used to predict the shelf life at any temperatures between ambient at 25^{o}C and 40^{o}C. As long as the temperature range is not greater than 30°C to 40°C, then extrapolation to lower temperature may be used to predict the expected product shelf life (Labuza, 2000). Good linearity and fit of the plot of measure quality (y-axis) against storage time (x-axis) indicates the order of reaction, whether zero-order (plot of Y versus time) or first-order (plot of ln Y versus time) (Taoukis and Labuza 1996).

Table 4.28 Changes in the intensity ratings (mean±standard deviation) for the texture attributes, and toothpack of roasted resveratrol-enhanced peanuts stored at varying times at accelerated temperatures of 30, 35 and 40°C and verified at 25°C[1].

Trt. No.	Temp. (°C)	Time (day)	Crispness	Crunchiness	Hardness	Toothpack
1	25	0	28.9 ±1.5a	40.2 ±3.1a	38.5 ±5.1a	58.4 ±6.7a
2	25	18	28.7 ±3.5a	40.0 ±4.3a	38.6 ±3.0a	59.5 ±5.2a
3	25	52	28.8 ±1.9a	38.0 ±8.2a	38.7 ±4.1a	58.4 ±6.7a
4	25	61	28.8 ±1.9a	38.6 ±7.0a	36.6 ±4.6a	59.5 ±7.6a
5	25	71	29.1 ±2.1a	39.9 ±1.3a	37.5 ±2.7a	57.9 ±6.0a
6	25	90	27.9 ±4.2a	39.5 ±2.6a	37.6 ±1.8a	57.5 ±5.7a
7	25	101	28.0 ±3.5a	40.3 ±1.7a	37.6 ±2.9a	57.1 ±5.3a
Control	0	0	29.7 ±3.1a	41.1 ±2.9a	38.2 ±2.7a	58.8 ±6.2a
Control	25	52	28.8 ±2.6a	39.4 ±4.8a	38.2 ±1.7a	58.6 ±6.1a
Control	4	52	29.2 ±2.9a	39.8 ±2.1a	38.8 ±2.2a	58.6 ±4.2a
	P-value		*0.7779*	*0.6615*	*0.6051*	*0.9818*
8	30	0	29.3 ±1.8a	40.2 ±2.9a	37.8 ±3.8a	58.3 ±5.4a
9	30	6	29.6 ±1.2a	40.1 ±1.6a	37.9 ±1.6a	58.7 ±6.1a
10	30	23	28.6 ±2.5a	39.3 ±2.3a	38.2 ±2.1a	56.3 ±7.8a
11	30	42	28.7 ±4.1a	39.5 ±1.9a	37.7 ±1.5a	58.5 ±5.1a
12	30	61	28.9 ±3.4a	40.4 ±1.5a	37.4 ±3.8a	56.3 ±6.9a
13	30	72	29.1 ±2.2a	39.0 ±3.4a	37.0 ±5.2a	58.0 ±6.2a
Control	0	0	29.7 ±3.1a	41.1 ±2.9a	38.2 ±2.7a	58.8 ±6.2a
Control	25	52	28.8 ±2.6a	39.4 ±4.8a	38.2 ±1.7a	58.6 ±6.1a
Control	4	52	29.2 ±2.9a	39.8 ±2.1a	38.8 ±2.2a	58.6 ±4.2a
	P-value		*0.7518*	*0.4677*	*0.7244*	*0.8798*
14	35	0	28.6 ±1.4a	38.7 ±4.3a	36.5 ±4.2a	57.1 ±7.0a
15	35	8	28.9 ±2.5a	39.9 ±3.9a	35.6 ±6.4a	58.0 ±5.8a
16	35	17	29.2 ±1.0a	40.1 ±1.1a	37.6 ±2.0a	57.9 ±5.7a
17	35	27	29.2 ±1.6a	39.3 ±3.4a	37.4 ±2.3a	56.6 ±6.6a
18	35	46	29.6 ±1.2a	40.0 ±1.2a	38.1 ±2.1a	58.1 ±5.6a
19	35	57	28.9 ±2.5a	39.8 ±2.2a	37.9 ±2.2a	57.9 ±6.4a
Control	0	0	29.7 ±3.1a	41.1 ±2.9a	38.2 ±2.7a	58.8 ±6.2a
Control	25	52	28.8 ±2.6a	39.4 ±4.8a	38.2 ±1.7a	58.6 ±6.1a
Control	4	52	29.2 ±2.9a	39.8 ±2.1a	38.8 ±2.2a	58.6 ±4.2a
	P-value		*0.9181*	*0.6501*	*0.1099*	*0.9835*

Table 4.28 continued…

Trt. No.	Temp. (°C)	Time (day)	Crispness	Crunchiness	Hardness	Toothpack
20	40	0	28.8 ±3.0a	39.0 ±5.3a	38.0 ±5.8a	57.1 ±7.5a
21	40	6	28.7 ±2.6a	38.4 ±5.1a	36.8 ±4.1a	57.6 ±5.6a
22	40	16	28.8 ±3.4a	39.0 ±4.9a	37.1 ±4.6a	56.9 ±5.9a
23	40	19	28.4 ±2.2a	39.6 ±3.1a	37.8 ±2.5a	57.8 ±7.0a
24	40	35	28.1 ±3.0a	38.7 ±5.3a	36.0 ±4.9a	56.3 ±7.9a
25	40	46	29.5 ±2.3a	38.9 ±4.8a	38.3 ±1.5a	58.9 ±6.9a
Control	0	0	29.7 ±3.1a	41.1 ±2.9a	38.2 ±2.7a	58.8 ±6.2a
Control	25	52	28.8 ±2.6a	39.4 ±4.8ab	38.2 ±1.7ab	58.6 ±6.1a
Control	4	52	29.2 ±2.9a	39.8 ±2.1ab	38.8 ±2.2ab	58.6 ±4.2a
	P-value		*0.6120*	*0.3992*	*0.1304*	*0.7615*

[1]Intensity ratings using 150 mm unstructured line scale with anchors at 12.5 mm and 137.5 mm by 10 trained descriptive panel.

Means within a column at specific storage temperature along with controls not followed by the same letter are significantly ($P<0.05$) different from each other as determined by Fisher's least significance difference mean separation test.

7.1 Reaction order (n) and rate constant (k) of lipid oxidation

The first step in the prediction of shelf life is to determine whether the reaction order of the mode of deterioration for the productis zero or first order based on the Arrhenius model. Figure 4.19 shows the Arrhenius plots of hexanal (A) and ln hexanal (B) against storage time. Based on the plots, the lipid oxidation which was measured by hexanal concentrations in REP, followed a first order reaction due to better fit of the plot of ln hexanal against storage time (Figure 4.19 B)**.** The plots with ln hexanal (B) had higher R^2 values of 0.90, 0.88 and 0.77 at storage temperatures of 30, 35 and 40^{o}C, respectively, compared to plots with hexanal (A) with 0.80, 0.82, and 0.64, respectively.

The reaction contants, k at different accelerated temperatures of 30, 35, and 40^{o}C correspond to the slopes of Arrhenius plots and were found to be 0.026, 0.033, and 0.042/day, respectively (Figure 4.20). The predicted k at 25^{o}C was 0.017/day.

7.2 Activation energy, E_A

The second step in the prediction of shelf life is the determination of activation energy constant, E_A which represents the measure of temperature sensitivity of the lipid oxidation in roasted REP and demonstrates how much faster the lipid oxidation goes as temperature is raised. The E_A for the lipid oxidation of REP was 300 cal/mol or 1,253 J/mol which was calculated by multiplying the slope (=150.7/K) of the Arrhenius plot in figure 4.20 by the universal gas constant, 8.314 kJ/mol K or 1.986 kcal/mol K.

7.3 Shelf life estimation based on shelf life plot method and calculation of Q_{10}

The shelf life of a food is defined as the time period within which the food is safe to consume and/or has an acceptable quality to consumers (Fu and Labuza, 2005). Based on Table 4.23, consumer tests showed that REP were acceptable with OA ratings of 5.00, 5.06 and 5.04 or

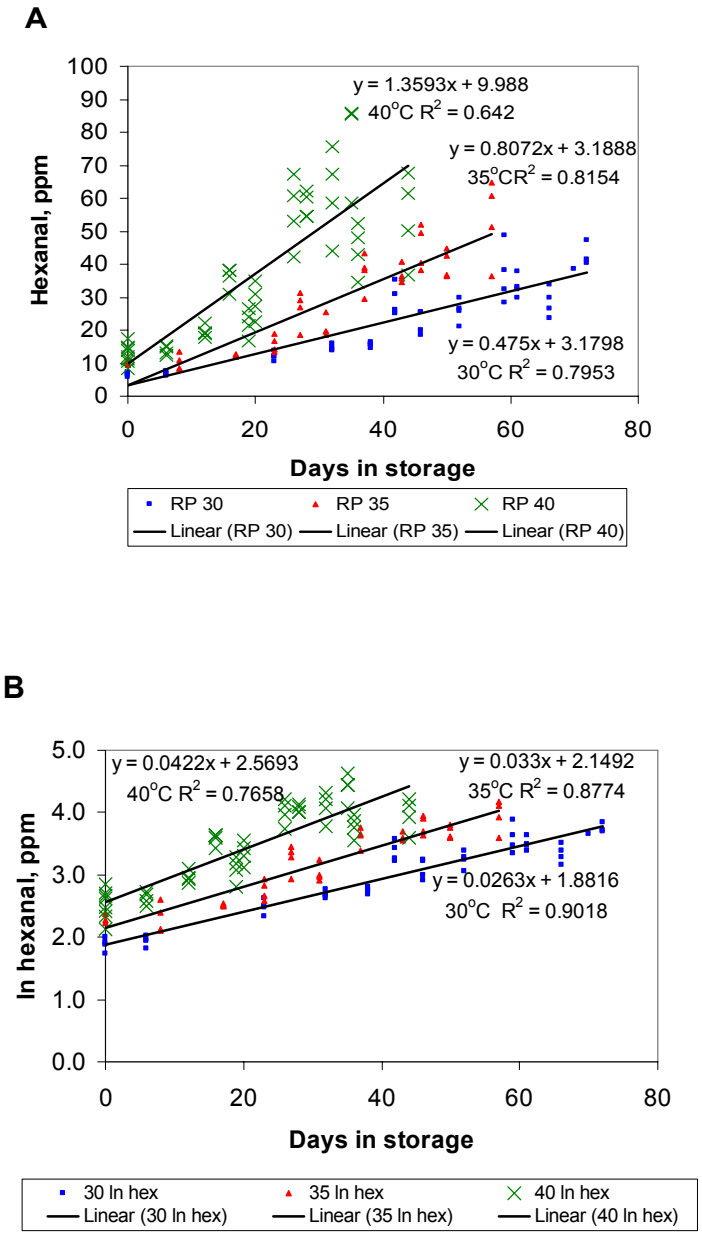


Figure 4.19 Plots of hexanal (A) and ln hexanal (B) concentrations against storage time used in the determination of reaction order for roasted resveratrol-enhanced peanuts stored at varying storage times and temperatures.

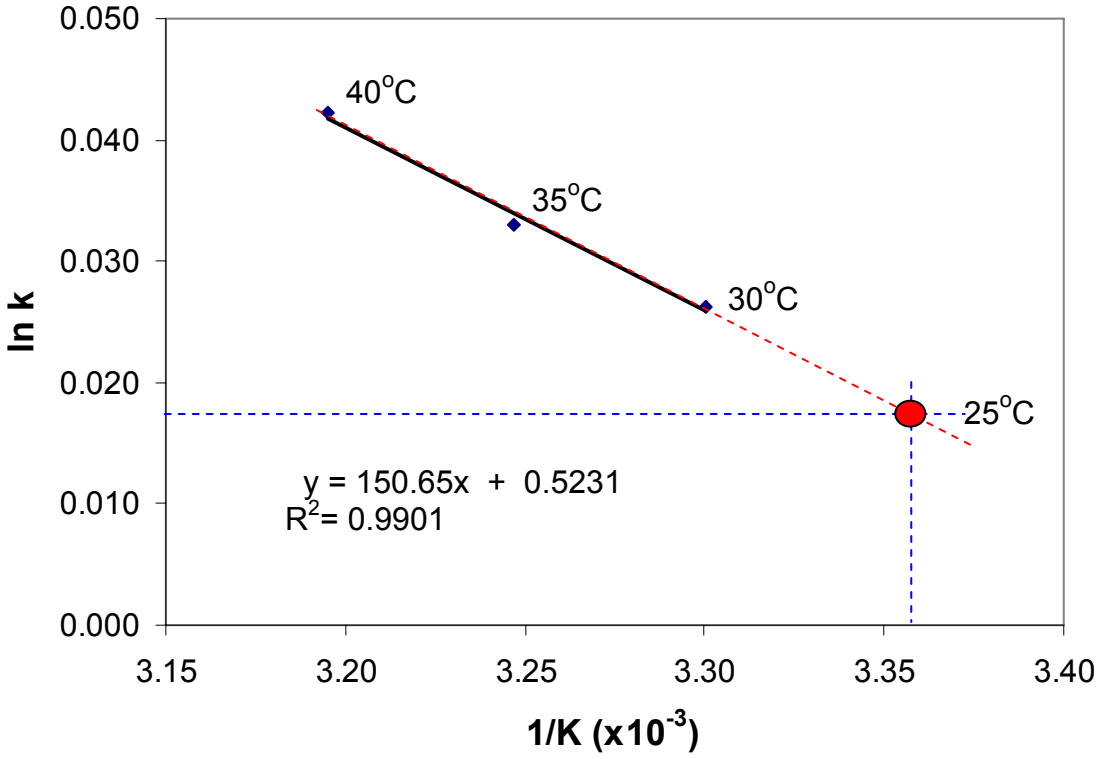


Figure 4.20 Arrhenius plot showing ln k (rate of increase in hexanal concentration) against reciprocal of absolute accelerated storage temperature in Kelvin at 30, 35, and 40°C used in the determination of activation energy for the lipid oxidation in resveratrol-enhanced peanuts. The dot shows the predicted ln k at 25°C.

neither like nor dislike when stored for 42 days at 30°C, 27 days at 35°C, and 19 days at 40°C, respectively. Using the shelf life model, which is a plot of ln shelf life in days on the y-axis against accelerated temperatures of 30, 35 and 40°C on the x-axis, the equation of y = -0.0793x + 6.1023, with a high coefficient, R^2 of 0.97 (Figure 4.21) was obtained. The predicted shelf life at 25°C of 61 days was calculated from the shelf life plot equation using regression analysis.

The predicted shelf life of 61 days at 25°C from ASLT, was not met as verification samples stored at actual ambient temperature conditions of about 25°C were not acceptable with overall acceptance rating of 4.6 or dislike slightly (Table 4.23) with high hexanal content of 45.07 μg/g (Table 4.22). Samples stored for 52 days, which was 9 days shorter than the predicted 61 days shelf life, had acceptable overall acceptance rating of 5.0 or neither like nor dislike, and hexanal content of 23.57 μg/g, and therefore 52 days was considered as the shelf life of REP at 25°C. This finding indicates that lipid oxidation proceeded faster at actual storage conditions ~25°C compared to those at higher temperatures. The water activity of dry foods, such as resveratrol-enhanced peanuts, can increase with temperature and causes an increase in reaction rates for products of low water activity in sealed packages resulting in over-prediction of true shelf life at the lower temperature (Labuza and Schmidl, 1985). Verification of the predicted shelf life based on ASLT must always be performed at actual storage conditions (Labuza and Schmidl, 1985). Compared to such as resveratrol- enhanced peanuts, controls after 52 days at 25°C had higher OA rating of 7.3 or like moderately, with low hexanal content of 5.8 μg/g. The shelf life of 52 days of REP at actual ambient conditions was 38 days shorter than the shelf life of regular roasted peanuts of 90 days. Applications of such as resveratrol- enhanced peanuts in products that will mask or minimize off-flavors such as in peanut bars and other peanut confections may increase its shelf life.

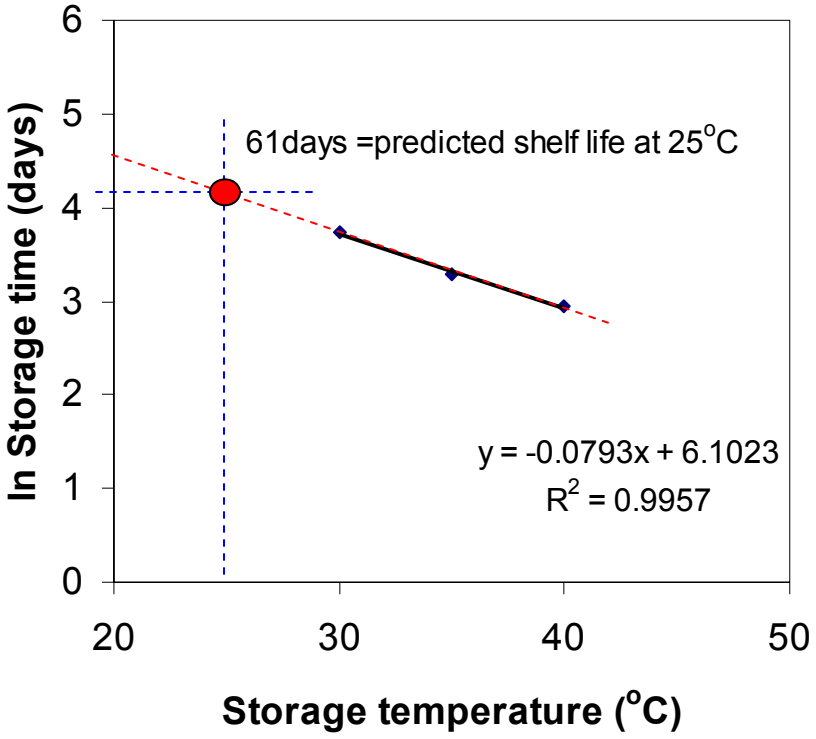


Figure 4.21 Shelf life plot of roasted resveratrol-enhanced peanuts showing the end of shelf life at different accelerated storage temperatures at 30, 35, and 40°C based on consumer overall acceptance ≤ 5 or neither like nor dislike and hexanal concentration. The predicted shelf life at 25°C of 61 days is represented by the dot.

At the end of shelf life of 52 days at 25°C, REP had *trans*-resveratrol content of 3.29 µg/g total phenolics of 1.76 mg GAE/g, and TEAC of 6.06 µM TE/g corresponding to reductions of 3, 8, and 27%, respectively, compared to initial concentrations of 3.40µg/g, 1.91 mg GAE/g, 8.26 µM TE/g, respectively. Product label requires that at least 80% of active ingredients or substance claimed should be the present in the product throughout its shelf life. Based on this, *trans*-resveratrol and total phenolics which were reduced by ≤20%, were stable in REP during shelf of 52 days at 25°C, whereas the antioxidant capacity, TEAC was not. When claimed in product label, the amount of TEAC should be adjusted such that at least 80% should remain in REP throughout the product shelf life.

Q_{10}, the accelerating factor used to describe relation between temperature and reaction rate constant, is defined as the ratio of the rate constants at temperature differing by 10°C (Labuza, 2000). The Q_{10} for roasted REP calculated based on the shelf life plot model was 2.2. This Q_{10} is higher than the reported Q_{10} of 1.6 in regular roasted peanuts of Evranuz (1993) indicating that lipid oxidation in REP proceeded faster compared to that in regular untreated peanuts. Using Q_{10} of 2.2, the shelf life of REP at temperatures other than 25°C can be calculated and the resulting estimated shelf lives were 35 days at 30°C, 24 days at 35°C, and 16 days at 40°C suggesting greater temperature abuse using higher temperatures during storage will shorten the shelf life of REP. This finding implies that REP and resveratrol-enhanced peanut containing products should be stored at low temperature as possible to maintain its shelf life.

C. Storage Study of Resveratrol-Enhanced Peanut Bars at 25 and 40°C

1. Changes in hexanal concentrations during storage of REP bars

The mean hexanal concentrations of REP bars during storage time at 25 and 40°C are presented in Figure 4.22 and Table 4.29. Two storage temperatures, 25 and 40°C were used in

this study. The high storage temperature at 40°C was used to predict the shelf life at 25°C using a critical hexanal value of 25 μg/g to mark the end of shelf life, which was then used as a guide to adjust the rate of sampling at 25°C, if needed. The data at ambient conditions at 25°C were used to determine the actual shelf life of resveratrol-enhanced peanut bars.

1.1 Storage at 40°C

REP bars stored at 40°C had an initial hexanal concentration of 14.27 μg/g of REP bars that were significantly higher than untreated controls with 2.11 μg/g indicating that hexanal was formed during UV and US processing treatments prior to storage study. UV exposure is known to initiate lipid oxidation food (Duh and Yen, 1995) and this could have caused the lipid oxidation in resveratrol-enhanced peanut bars. During storage at 40°C, the initial hexanal content of 16.91 μg/g was not significantly different from stored samples until 26 days indicating a processing rather than storage effect. From 26 to 57 days, there was a rapid significant increase in hexanal concentration to 25.77 μg/g (Figure 4.22) suggesting a storage effect. The rate of hexanal increase was 0.23 μg/g/day. Based on the equation, y = 0.2332x +14.034 in Figure 4.22 and an assumed critical hexanal value of 25 μg/g, the predicted shelf life at 40°C was 47 days. The shelf life at 25°C was then predicted to be 133 days based on 47 days predicted shelf life at 40°C and an assumed Q_{10} value of 2.0 for lipid oxidation (range=1.5-3.0; Labuza, 1984) using equation 10 in Section 3.1. This was longer than 90 days shelf life of regular roasted peanuts used in the experimental design and therefore, sampling time at 25°C was adjusted accordingly.

1.2 Storage at 25°C

REP bars stored at 25°C had initial hexanal concentration of 12.53 μg/g which were not significantly (P<0.05) different from those samples stored up to 87 days, suggesting that hexanal

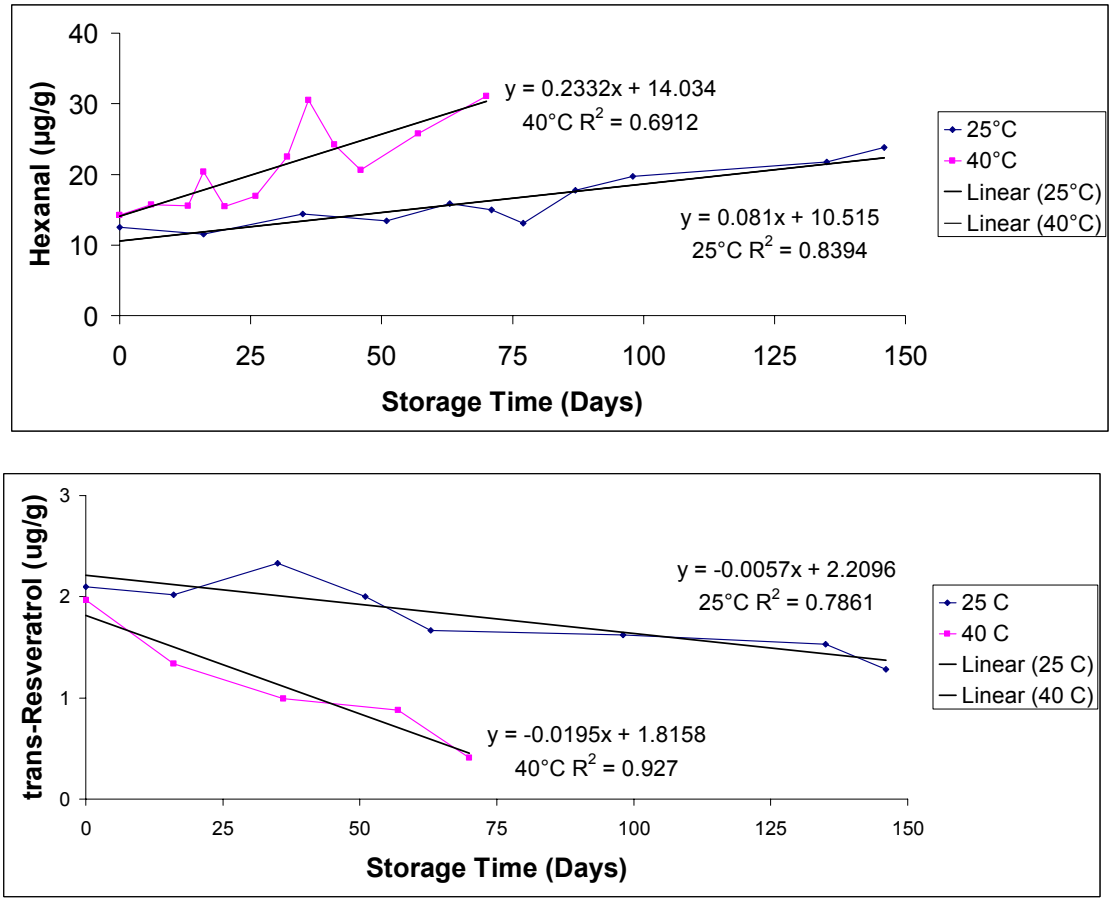


Figure 4.22 Changes in hexanal and *trans*-resveratrol concentrations of resveratrol-enhanced peanut bars during storage at 25 and 40°C.

Table 4.29 Changes in concentrations (mean ± standard deviation) of hexanal, *trans*-resveratrol, total phenolics, and TEAC values of resveratrol-enhanced peanut bars during storage at 25 and 40°C.

Tempe-rature (°C)	Time (days)	Hexanal (µg/g)	Resveratrol (µg/g)	Total Phenolics (mg GAE/g)	TEAC (µM TE/g)
25	0	12.53 ± 2.03ef	2.10±0.49a	1.80±0.06	6.12±0.31
25	16	11.54 ± 0.91f	2.02±0.05a	-	-
25	35	14.38 ± 3.97def	2.33±0.47a	-	-
25	51	13.39 ± 1.17def	2.00±0.55a	-	-
25	63	15.85 ± 3.06cdef	1.67±0.47b	-	-
25	71	14.98 ± 2.82cdef	-	-	-
25	77	13.07 ± 2.26def	-	-	-
25	87	17.79 ± 4.77bcd	-	-	-
25	98	19.71 ± 4.00abc	1.62±0.18b	-	-
25	135	21.74 ± 2.98ab	1.53±0.22b	-	-
25	146	23.78 ± 5.18a	1.28±0.13c	1.48±0.01	5.46±1.04
-18 Control[2]	0	2.11 ± 0.44g	0.20±0.001d	0.56±0.01	0.44±0.10
40	0	14.27 ± 4.16f	1.97±0.30a	1.98±0.11	8.02±1.79
40	6	15.68 ± 1.37ef	-	-	-
40	13	15.55 ± 1.38ef	-	-	-
40	16	20.40 ± 1.75cdef	1.34±0.40b	1.87±0.15	7.59±2.03
40	20	15.47 ± 3.05ef	-	-	-
40	26	16.91 ± 2.76def	-	-	-
40	32	22.47 ± 6.96cd	-	-	-
40	36	30.55 ± 4.75ab	0.99±0.15c	1.57±0.02	6.13±1.04
40	41	24.25 ± 4.28bc	-	-	-
40	46	20.65 ± 1.80cde	0.88±0.04c	-	-
40	57	25.77 ± 5.24abc	-	-	-
40	70	31.08 ± 9.59a	0.41±0.08d	-	-
-18 Control[2]	0	2.11 ± 0.44g	0.02±0.001e	0.56±0.01	0.44±0.10

[1]Means followed by the same letter within the same storage temperature are not significantly different at $P< 0.05$ as determined by Fisher's least significant difference mean separation test. (-) means not analyzed.

[2] Controls are peanut bars prepared from untreated raw peanuts.

formation is a processing rather than a storage effect. From 87 to 146 days, there was a rapid increase in hexanal concentration from 17.79 to 23.78 μg/g. The rate of hexanal formation was 0.08 μg/g/day which was slower than that at 40°C.

2. Changes in the concentrations of *trans*-resveratrol, total phenolics and TEAC during storage of REP bars

The concentrations of *trans*-resveratrol in REP bars decreased as storage time increased, with faster rate of loss of 0.020 μg/g/day at higher temperature, 40°C compared to 0.006 μg/g/day at 25°C. At 40°C, *trans*-resveratrol was reduced from 1.97 to 0.41 μg/g or 79% loss after 70 days whereas at 25°C, 39% loss was observed reducing the concentration from 2.10 to 1.28 μg/g after 146 days. Based on the regulations, at least 80% of the claimed value in the product label should be retained throughout the shelf life of the product (Fu and Labuza, 2005). Using the equation, y = -0.0057x + 2.2096, at 25°C (Figure 4.22), 80% of the initial *trans*-resveratrol concentration or 1.68 μg/g will be retained up to 93 days at 25°C.

The initial total phenolics of REP bar at 25 and 40°C were 1.80 and 1.98 mg GAE/g, respectively, and decreased to 1.48 and 1.87 mg GAE/g or 17.8 and 5.6% losses, after 146 and 36 days of storage at respective temperatures. The loss of >20% indicates that total phenolics in REP bars were stable during storage. Untreated controls had initial total phenolics of 0.56 mg GAE/g.

REP bars stored at 25 and 40°C had initial TEAC values of 6.12 and 8.02 μM TE/g respectively, and decreased to 5.46 and 7.59 μM TE/g or 10.8 and 5.4% losses after 146 and 36 days of storage at respective temperatures. The <20% losses in TEAC values suggest that the antioxidant capacities were stable in REP bar samples during storage. Untreated controls had initial TEAC of 0.44 μM TE/g. Untreated controls had initial TEAC of 0.44 μM TE/g.

3. Changes in the consumer acceptance of the sensory attributes of REP bars

Table 4.30 shows the mean hedonic ratings for the sensory attributes by consumer panel of the REP bars stored for varying times at 25 and 40^{o}C.

3.1 Overall acceptance of REP bars during storage

At 25^{o}C, all resveratrol-enhanced peanut bars except for Trt# 5 were acceptable to consumers with ratings of 5.8 or neither like nor dislike to 6.7 or close to like moderately. Trt#5 received the lowest overall acceptance rating of 4.5 or dislike slightly. Samples stored up to 135 days, except Trt# 5 had overall acceptance ratings which were not significantly different from controls with rating of 6.7 or like slightly. The overall acceptance of resveratrol-enhanced peanut bars stored for 146 days (Trt#7) with rating of 5.8, although significantly lower from control, were acceptable to consumers.

At 40^{o}C, all stored REP bars had significantly lower OA ratings of 4.4 to 5.2 (4= dislike slightly; 5=neither like nor dislike) compared to controls. The OA of all stored REP bars from 16 to 70 days except those stored for 36 days (Trt# 10) were not significantly different from non-stored samples. Trt#10 had the lowest OA rating which corresponds to a high hexanal of 30.55 μg/g.

Using OA ≥ 5 or neither like nor dislike as the critical value to mark the end of shelf-life at 25^{o}C, REP bars had shelf life of 146 days which was longer than 52 days in roasted resveratrol peanut kernels. The syrup in REP bars could have acted as a protective coating in each peanut kernel preventing them from exposure and/or as masking agent from the off-flavors/tastes produced during UV and US treatments of peanuts.

Table 4.30 Consumer hedonic ratings (mean ± standard deviation) for the sensory attributes of resveratrol-enhanced peanut bars[1] stored for varying times at 25 and 40°C[2]

Trt #[2]	Temp (°C)	Days	Appearance[3]	Color[3]	Aroma[3]	Flavor[3]	Texture[3]	Overall Acceptance[3]
1	25	0	6.4 ±1.6a	6.2 ±1.8a	5.7 ±2.0a	6.5 ±1.7ab	6.9 ±1.5a	6.6 ±1.8ab
2	25	35	6.3 ±1.9a	6.3 ±2.0a	6.2 ±1.7a	6.3 ±2.0ab	6.5 ±1.9ab	6.5 ±1.7ab
3	25	51	6.2 ±2.2a	6.3 ±2.1a	6.0 ±1.8a	6.5 ±2.2ab	6.6 ±2.1ab	6.3 ±2.5ab
4	25	63	6.4 ±1.7a	6.0 ±2.0a	6.3 ±1.8a	6.4 ±2.0ab	6.6 ±1.9b	6.4 ±1.9ab
5	25	87	6.8 ±1.9a	6.7 ±1.8a	6.3 ±1.9a	5.0 ±2.1c	5.7 ±2.0c	4.5 ±2.3c
6	25	135	6.4 ±1.8a	6.4 ±1.9a	6.2 ±1.7a	6.1 ±2.1ab	6.0 ±2.3bc	6.1 ±2.0ab
7	25	146	6.7 ±1.9a	6.7 ±1.9a	5.7 ±1.9a	5.9 ±2.1b	5.7 ±2.2c	5.8 ±2.3b
Control		0	6.5 ±2.0a	6.4 ±2.0a	6.7 ±2.0a	6.8 ±2.0a	6.7 ±2.1ab	6.7 ±2.1a
P-value			*0.2688*	*0.2758*	*0.3814*	*0.0005*	*0.0189*	*<0.0001*
8	40	0	6.5 ±2.1a	6.8 ±1.9a	5.9 ±1.9a	5.6 ±2.3b	6.4 ±2.2a	5.2 ±2.3b
9	40	16	6.3 ±2.1a	6.3 ±2.2a	5.8 ±1.9a	4.7 ±2.4cd	6.0 ±2.0a	4.4 ±2.3b
10	40	36	6.4 ±2.3a	6.3 ±2.4a	5.4 ±2.3a	4.1 ±2.2d	5.6 ±2.3a	3.3 ±2.1c
11	40	46	6.4 ±2.0a	6.6 ±1.9a	6.0 ±1.6a	5.3 ±2.3bc	6.0 ±2.0a	4.7 ±2.4b
12	40	70	6.9 ±1.7a	6.9 ±1.7a	5.8 ±1.9a	5.0 ±2.1bcd	6.1 ±2.0a	4.5 ±2.1b
Control		0	6.5 ±2.0a	6.4 ±2.0a	6.7 ±2.0a	6.8 ±2.0a	6.7 ±2.1a	6.7 ±2.1a
P-value			*0.1997*	*0.2981*	*0.1391*	*<0.0001*	*0.1801*	*<0.0001*

[1] Treated peanuts bars were prepared from peanuts processed using optimum ultrasound (US)-UV process of 70 mW/cm^3 US power density for 10 min, then exposed for 50 min at 40 cm distance from UV light followed by incubation at 25°C for 36h. Control peanut bars were prepared from untreated peanuts.

[2] Means followed by the same letter within the same storage temperature are not significantly different at $P< 0.05$ as determined by Fisher's least significant difference mean separation test.

[3] Consumer ratings for liking using 9-point hedonic rating scale: 1="dislike extremely"; 5="neither like nor dislike"; 9="like extremely".

3.2 Consumer acceptance for the appearance and color of resveratrol- enhanced peanut bars during storage

There were no significant (P<0.05) differences between the acceptance ratings ≥ 6.0 or liked slightly for the appearance and color of all REP bars and controls throughout all storage times at 25 and 40^{o}C. Results indicate that the consumer liking for appearance and color of REP bars were not affected by processing treatment and storage.

3.3 Consumer acceptance for aroma and flavor of REP bars during storage

No significant differences were observed in the acceptance ratings (5.4 - 6.7) for the aroma between REP bars and controls during all storage times at 25 and 40^{o}C. This finding suggests that the aroma of REP bars was neither affected by processing treatment and storage.

Significant differences in the consumers' liking for flavor were observed in REP bar stored at both temperatures. At 25^{o}C, all REP bars stored up to 135 days, excluding Trt#5, had acceptance ratings for flavor of 5.9 – 6.4 or liked slightly which were not significantly different from controls with 6.8 rating. After 146 days (Trt#7), REP bars had flavor acceptance rating of 5.9 which was significantly lower than controls but significantly higher that Trt#5 with rating of 5.0. Although Trt# 5 and 7 were significantly lower than controls, their ratings ≥ were acceptable.

At 40^{o}C, all REP bars had significantly lower acceptance for flavor than controls. Initially, REP had acceptable flavor (rating=5.6) which became unacceptable after 16 and 36 days (4.7 and 4.1, respectively) then increased to the same acceptable ratings as initial after 46 and 70 days of storage.

3.4 Consumer acceptance for texture of resveratrol-enhanced peanut bars during storage

Although all REP bars at all storage times at both temperatures had acceptable consumer acceptance ratings for texture >5 (range=5.6-6.9), significant differences were observed in

samples stored at 25°C but not at 40°C. At 25°C, consumer acceptance for the texture of all REP bars excluding samples stored for 87 (Trt#5) and 146 days (Trt#7) were not significantly different from controls. Trt# 5 and 7 had significantly lower acceptance for texture than controls and all other REP samples.

4. Changes in the consumers' just-about-right (JAR) ratings for roasted peanutty flavor and bitterness of REP bars during storage

4.1 Consumer JAR ratings for roasted peanutty flavor of REP bars during storage

No significant differences were observed in the JAR ratings for roasted peanutty flavor between REP bar and controls during storage at all times at 25 and 40°C which were all rated as just-about-right (Table 4.30). This result showed consumers found that REP bars had just-about-right roasted peanutty flavor as regular peanut bars and reformulations are not needed to meet the consumers' desired perceptual level for this attribute.

4.2 Consumer JAR ratings for bitterness

Significant differences were observed in the JAR ratings for the bitterness of REP bars where samples stored for 87 days at 25°C (Trt#5) and for 36 days at 40°C (Trt#10) had significantly stronger bitterness or greater than JAR whereas all other REP samples and controls were JAR. This result indicates that bitterness in REP bars was just-about-right in the perception of consumers and reformulation is not needed to adjust the bitterness of REP bars.

5. Changes in the consumers' intensity ratings for roasted peanutty flavor and off-flavors of REP bars during storage

5.1 Consumer ratings for the intensity of roasted peanutty flavor

Table 4.31 shows consumers' intensity ratings for roasted peanutty flavors and off-flavors in REP bars during storage. Although differences in intensity ratings for roasted peanutty flavor

Table 4.31 Just-about-right (JAR) and intensity ratings (mean ± standard deviation) of consumer panel on resveratrol-enhanced peanut bars[1] stored for varying times at 25 and 40°C[1]

Trt # [2]	Temp (°C)	Days	JAR- Roasted peanutty flavor[4]	JAR- Bitterness[4]	Intensity of roasted peanutty flavor[5]	Intensity of off-flavor[5]
1	25	0	4.0 ±0.8a	3.9 ±1.0b	5.3 ±1.2a	5.0 ±1.4b
2	25	35	4.0 ±1.0a	3.9 ±1.0b	5.0 ±1.2a	5.0 ±1.3b
3	25	51	3.8 ±1.1a	4.0 ±0.9b	5.0 ±1.6a	5.2 ±1.5b
4	25	63	3.9 ±1.0a	4.1 ±1.2b	5.3 ±1.2a	4.9 ±1.6b
5	25	87	4.3 ±1.4a	4.7 ±1.6a	5.4 ±1.8a	6.0 ±2.0a
6	25	135	4.0 ±1.0a	4.1 ±1.2b	5.5 ±1.6a	5.0 ±1.8b
7	25	146	3.7 ±1.0a	4.1 ±1.2b	4.9 ±1.5a	4.9 ±1.9b
Control[2]		0	3.9 ±1.0a	3.8 ±1.1b	5.2 ±1.3a	4.9 ±1.6b
	P-value		*0.2242*	*0.0034*	*0.4946*	*0.0255*
8	40	0	4.4 ±1.3a	4.8 ±1.3b	5.6 ±1.7a	5.9 ±1.9a
9	40	16	3.9 ±1.6a	4.9 ±1.6b	5.2 ±1.8a	6.1 ±1.9a
10	40	36	4.8 ±1.7a	5.6 ±1.4a	6.6 ±1.9a	6.6 ±2.2a
11	40	46	4.3 ±1.5a	4.9 ±1.5b	5.6 ±1.9a	6.1 ±1.9a
12	40	70	4.2 ±1.5a	4.9 ±1.6b	5.4 ±1.7a	6.3 ±1.7a
Control[2]		0	3.9 ±1.0a	3.8 ±1.1b	5.2 ±1.3a	4.9 ±1.6b
	P-value		*0.1683*	*0.0052*	*0.3340*	*0.0305*

[1] Treated peanuts bars were prepared from peanuts processed using optimum ultrasound (US)-UV process of 70 mW/cm^3 US power density for 10 min, then exposed for 50 min at 40 cm distance from UV light followed by incubation at 25°C for 36h.

[2] Control peanut bars were prepared from raw untreated peanuts.

Means followed by the same letter within the same storage temperature are not significantly different at $\alpha < 0.05$.

[3] Consumer ratings using 7-point just-about-right rating scale: 1="much too weak"; 4="just-about-right"; 7="much too strong"

[4] Consumer ratings using 9-point intensity rating scale: 1="none"; 4="neither weak nor strong"; 9="extremely strong".

were observed between REP bars and controls during storage at 25 and 40°C which ranged from 4.9-5.5 and 5.2-6.6 (5=neither weak nor strong; 6= slightly strong), respectively, these differences were not significant.

5.2 Consumer ratings for the intensity of off-flavor in peanut bars

Significant differences were observed for the intensity of off-flavors. At 25°C, REP bars stored for 87 days (Trt#5) had significantly more intense off-flavors (6.0=slightly strong) than all other REP bars and controls (4.9-5.2 or neither weak nor strong) which were not significantly different from each other. At 40°C, all REP bars had significantly higher off-flavors intensity ratings of 5.9-6.6 compared to controls with 4.9, but were not significantly different from each other. This result indicates that the off-flavor was a processing rather than a storage effect.

6. Changes in the intensity ratings of the different sensory attributes of resveratrol-enhanced peanut bars by a descriptive sensory panel

Figure 4.23 shows the mean intensity ratings by the descriptive sensory panel using a 150 mm line scale for the 21 sensory attributes of REP bar during storage at 25 and 40°C. Significant differences were observed in seven of 21 descriptive sensory attributes of REP bars including brown color of caramel, bitter taste, burnt and oxidized flavors, hardness and fructurability. No differences were observed all other 14 attributes of REP bars during storage were not significant from each other and from controls indicating that these attributes were not affected by processing treatments and storage.

6.1 Changes in the intensities of color, and roasted peanutty flavors in REP bars

Table 4.32 shows the changes in mean intensity ratings for color, caramel and roasted peanutty flavor of REP bars during storage at 25 and 40°C. Significant differences in intensities between REP bars and untreated controls were observed only in brown color of caramel whereas

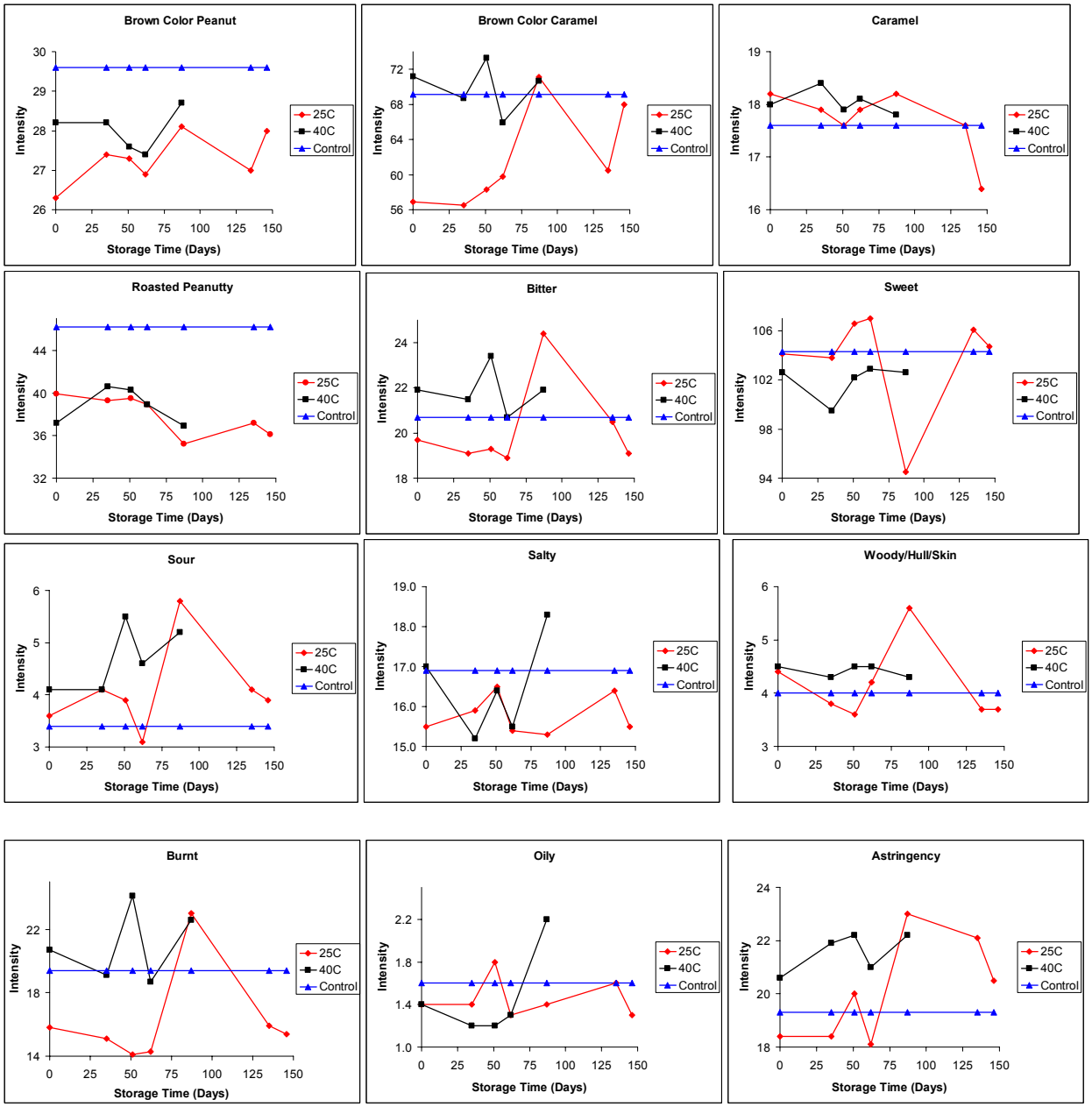


Figure 4.23. Changes in the mean intensity ratings for the different sensory attributes of roasted resveratrol-enhanced peanut bars during storage at 25 and 40°C by a descriptive panel using a 150 mm line scale. Horizontal lines indicate the mean intensity ratings of controls.

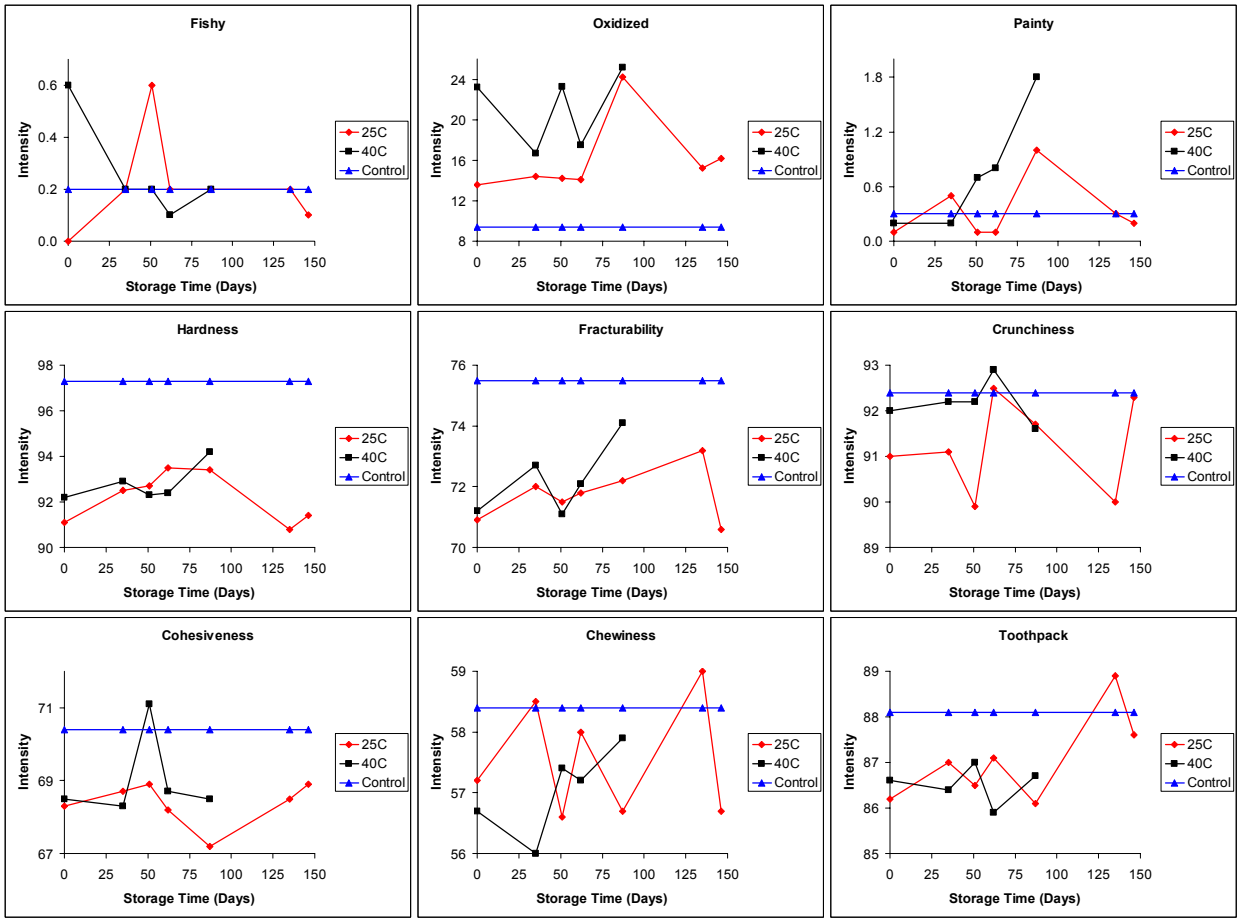


Figure 4.23 (continued)

no differences were found in brown color of peanuts, caramel and roasted peanutty flavor. This result indicate that REP bars were similar to regular peanut bars in terms of brown color of peanuts, caramel flavor and roasted peanutty flavor.

Brown color of caramel. Significant differences were observed during storage at 25°C but not at 40°C. At 25°C, the intensities of brown color in the caramel of REP bars stored up to 62 days with ratings of 57 to 60 using a 150 mm line scale were significantly lower than controls with 68 indicating a processing effect. After 87 days (Trt#5), the intensity of brown color increased significantly to levels not different from controls suggesting a storage effect.

6.2 Changes in the intensities of basic tastes in REP bars

Table 4.33 shows the changes in the mean intensity ratings for basic tastes of REP bars during storage at 25 and 40°C. Significant differences in the intensities of bitter and sweet tastes were observed between REP bars and controls but not in sour and salty tastes.

Bitter taste. Significant difference in the intensities of bitter taste was observed at 25°C but not at 40°C. At 25°C, all REP bars and controls, except Trt#5, had bitter intensities in the range of 18.9 – 20.7 which were not different from each other. Trt#5 with intensity rating of 24.4 was significantly more bitter than other REP bars and controls.

Sweet taste. Significant difference in the intensities of sweet taste was observed at 25°C but not at 40°C. The sweetness intensities of all REP bars, except Trt#5, and controls were not significantly different with ratings of 103.8 – 107. Trt#5 was less sweet than all other REP bars and controls.

6.3 Changes in the intensities of off-flavors in REP bars

Table 4.34 shows the changes in the mean intensity ratings for the off-flavors of REP bars during storage at 25 and 40°C. Significant differences in the intensities between REP bars and

controls were observed only in burnt and oxidized flavors but in woody/hulls/skins, oily, astringency, fishy and painty flavors.

Burnt flavor. At 25°C, all REP bars except Trt#5 had burnt flavor intensity ratings of 14.1-15.9 which did not significantly differ from each other. Trt#5 had significantly higher burnt flavor (rating=23.0) compared to all other REP bars and controls (rating=19.4). However, the burnt flavor of REP bars stored for 35, 51 and 62 days (Trt# 2, 3, & 4) were significantly lower than controls whereas initial and stored REP bars for 135 and 146 days were not different from controls.

At 40°C, the intensity ratings of 18.7-22.6 for burnt flavor of all REP bars, except samples stored for 36 days (Trt#10), were not different from controls at 19.4. Trt#10 (rating=24.1) had more burnt flavor than controls and REP bars stored for 16 and 46 days (Trt# 9, 11) but were not different from all other REP bars.

Oxidized flavor. Lipid oxidation products such as low molecular weight pentanal, hexanal, heptanal, octanal and nonanal create off-flavors such as cardboard or oxidized rancid flavors (Warner et al., 1996). The intensities of oxidized flavor of all REP bars stored at 25°C, except Trt#5, with ratings of 14.1-16.2 were not significantly different from each other and from controls at 9.4 intensity rating. Trt#5 had more oxidized flavor (rating= 24.2) compared to all other REP bars and controls. All REP bars stored at 40°C with 16.7-25.2 ratings had significantly more intense oxidized flavors compared to controls.

6.4 Changes in the intensities of texture attributes and feeling factors in REP bars

Table 4.35 shows the changes in the mean intensity ratings for the texture attributes and feeling factors of REP bars during storage at 25 and 40°C. Significant differences in the

intensities between REP bars and controls were observed in hardness and fracturability but not crunchiness, cohesiveness, chewiness anf toothpack.

Hardness. Hardness is the force required to bite through the sample using incisors. The hardness of all REP bars with intensities ranging from 90.8-93.5 did not significantly differ from each other during storage at either 25 or 40^{o}C but significantly less than controls (rating= 97.3), except for REP bars stored for 62 and 87 days (Trt#4 &5) which were significantly not different from controls. This result indicates a processing rather than storage effect.

Fracturability. Fracturability is the force with which the sample crumbles, cracks or shatters. At 25^{o}C, the fracturability of all REP bars with intensities ranging from 70.6-73.7 did not significantly change during storage but were significantly lower than controls with 75.5, except for sample stored for 135 days which did not differ from controls. This result indicates a processing rather than storage effect.

At 40^{o}C, except for samples stored for 70 days (Trt#12), all REP bars had significantly lower fracturability (ratings=71.1-72.1) compared to controls. Among the REP bars, initial and 36 day-stored samples (Trt#8 & 10) had significantly lower fracturability compared to Trt#12, but were not different from all other samples stored for 16 and 46 days (Trt#9 & 11). At higher temperature of 40^{o}C, the fracturability of REP bars increased with increasing storage time.

Table 4.32 Changes in the intensity ratings (mean±standard deviation) for color, caramel and roasted peanutty aromatics of resveratrol-enhanced peanut bars by a descriptive panel during storage at 25 and 40°C [1].

Trt #	Storage Temp. (°C)	Storage Time (day)	Brown color peanut	Brown color caramel	Caramel	Roasted peanutty
1	25	0	26.3 ± 2.3a	56.9 ± 12.5b	18.2 ± 1.6a	39.9 ± 10.3a
2	25	35	27.4 ± 6.9a	56.5 ± 13.3b	17.9 ± 2.9a	39.3 ± 10.3a
3	25	51	27.3 ± 1.9a	58.3 ± 14.2b	17.6 ± 1.5a	39.5 ± 11.5a
4	25	62	26.9 ± 2.5a	59.8 ± 10.3b	17.9 ± 1.8a	38.9 ± 12.3a
5	25	87	28.1 ± 2.5a	71.1 ± 10.1a	18.2 ± 2.7a	35.2 ± 9.4a
6	25	135	27.0 ± 2.8a	60.5 ± 12.5b	17.6 ± 2.5a	17.6 ± 2.5a
7	25	146	28.0 ± 6.2a	68.0 ± 6.3a	16.4 ± 4.7a	36.1 ± 8.3a
Control[2]	0	0	29.6 ± 3.0a	69.1 ± 3.6a	17.6 ± 2.9a	46.2 ± 13.4a
		P-value	*0.2338*	*<0.0001*	*0.5470*	*0.0740*
8	40	0	28.2 ± 2.8a	71.2 ± 9a	18.0 ± 2.6a	37.2 ± 8.3a
9	40	16	28.2 ± 2.6a	68.7 ± 12a	18.4 ± 1.7a	40.6 ± 12.2a
10	40	36	27.6 ± 2.1a	73.3 ± 8.9a	17.9 ± 4.1a	40.3 ± 10.0a
11	40	46	27.4 ± 1.4a	65.9 ± 6.7a	18.1 ± 1.3a	38.9 ± 11.9a
12	40	70	28.7 ± 3.3a	70.7 ± 6.2a	17.8 ± 2.7a	36.9 ± 10.1a
Control	0	0	29.6 ± 3.0a	69.1 ± 3.6a	17.6 ± 2.9a	46.2 ± 13.4a
		P-value	*0.0941*	*0.1014*	*0.9527*	*0.1021*

[1] Intensity ratings using a 150 mm unstructured scale with anchors at 12.5 and 137.50 mm
Means in within a column at the same storage temperature along with controls, not followed by the same letter is significantly different ($P<0.05$) from each other as determined by Fisher's least significant difference mean separation test.
[2] Controls were peanut bars prepared from untreated roasted whole peanuts

Table 4.33 Changes in the intensity ratings (mean standard deviation) for the basic tastes of resveratrol-enhanced peanut bars by a descriptive panel during storage at 25 and 40°C [1].

Trt #	Storage Temp. (°C)	Storage Time (day)	Bitter	Sweet	Sour	Salty
1	25	0	19.7 ± 3.7b	104.1 ± 12.8a	3.6 ± 2.6a	15.5 ± 4.0a
2	25	35	19.1 ± 3.9b	103.8 ± 12.9a	4.1 ± 1.3a	15.9 ± 2.6a
3	25	51	19.3 ± 4.5b	106.6 ± 3.1a	3.9 ± 3.2a	16.5 ± 3.1a
4	25	62	18.9 ± 5.6b	107.0 ± 3.1a	3.1 ± 2.6a	15.4 ± 3.3a
5	25	87	24.4 ± 6.0a	94.5 ± 23.1a	5.8 ± 3.7a	15.3 ± 5.2a
6	25	135	20.5 ± 8.6b	106.1 ± 4.5a	4.1 ± 2.7a	16.4 ± 2.6a
7	25	146	19.1 ± 5.8b	104.7 ± 7.1a	3.9 ± 3.3a	15.5 ± 3.9a
Control[2]	0	0	20.7 ± 2.9b	104.3 ± 12.4a	3.4 ± 2.7a	16.9 ± 1.1a
		P-value	*0.0323*	*0.0313*	*0.1666*	*0.7437*
8	40	0	21.9 ± 2.8a	102.6 ± 9.8a	4.1 ± 2.2a	17.0 ± 1.2a
9	40	16	21.5 ± 8a	99.5 ± 23.9a	4.1 ± 2.6a	15.2 ± 4.4a
10	40	36	23.4 ± 4.6a	102.2 ± 6.5a	5.5 ± 2.6a	16.4 ± 3.3a
11	40	46	20.7 ± 3.2a	102.9 ± 12.7a	4.6 ± 2.3a	15.5 ± 4.4a
12	40	70	21.9 ± 9.1a	102.6 ± 6.1a	5.2 ± 2.8a	18.3 ± 5.9a
Control	0	0	20.7 ± 2.9a	104.3 ± 12.4a	3.4 ± 2.7a	16.9 ± 1.1a
		P-value	*0.6809*	*0.9229*	*0.0943*	*0.1396*

[1] Intensity ratings using a 150 mm unstructured scale with anchors at 12.5 and 137.50 mm
Means in within a column at the same storage temperature along with controls, not followed by the same letter is significantly different ($P<0.05$) from each other as determined by Fisher's least significant difference mean separation test.

[2] Controls were peanut bars prepared from untreated roasted whole peanuts

Table 4.34 Changes in the intensity ratings (mean standard deviation) for the off-flavors of resveratrol-enhanced peanut bars by a descriptive panel during storage at 25 and 40°C [1].

Trt #	Storage Temp. (°C)	Storage Time (day)	Woody/ Hull/Skin	Burnt	Oily	Astringency	Fishy	Oxidized	Painty
1	25	0	4.4 ± 2.6a	15.8 ± 6bc	1.4 ± 1.6a	18.4 ± 4.7a	0.0 ± 0.2a	13.6 ± 10.3bc	0.1 ± 0.2a
2	25	35	3.8 ± 2.6a	15.1 ± 5.2c	1.4 ± 1.3a	18.4 ± 6.8a	0.2 ± 0.4a	14.4 ± 7.8bc	0.5 ± 1.2a
3	25	51	3.6 ± 2.3a	14.1 ± 6.3c	1.8 ± 1.5a	20.0 ± 4.9a	0.6 ± 2.3a	14.2 ± 8.5bc	0.1 ± 0.4a
4	25	62	4.2 ± 2.4a	14.3 ± 6.5c	1.3 ± 1.3a	18.1 ± 6.4a	0.2 ± 0.4a	14.1 ± 8.7bc	0.1 ± 0.4a
5	25	87	5.6 ± 6.8a	23.0 ± 9.3a	1.4 ± 1.6a	23.0 ± 12.0a	0.2 ± 0.4a	24.2 ± 11.3a	1.0 ± 2.7a
6	25	135	3.7 ± 2.1a	15.9 ± 7.0bc	1.6 ± 2.6a	22.1 ± 16.6a	0.2 ± 0.4a	15.2 ± 7.9b	0.3 ± 0.9a
7	25	146	3.7 ± 2.2a	15.4 ± 5.6bc	1.3 ± 1.4a	20.5 ± 2.4a	0.1 ± 0.3a	16.2 ± 9.5b	0.2 ± 0.3a
Control[2]	0	0	4.0 ± 3.3a	19.4 ± 4.9ab	1.6 ± 1.4a	19.3 ± 5.4a	0.2 ± 0.4a	9.4 ± 8.7c	0.3 ± 0.5a
		P-value	*0.6451*	*0.0002*	*0.9733*	*0.5216*	*0.6666*	*0.0004*	*0.1596*
8	40	0	4.5 ± 2.1a	20.7 ± 5.6a	1.4 ± 1.4a	20.6 ± 4.0a	0.6 ± 2.7a	23.2 ± 12.1a	0.2 ± 0.4a
9	40	16	4.3 ± 1.8a	19.1 ± 4.8a	1.2 ± 1.5a	21.9 ± 4.8a	0.2 ± 0.4a	16.7 ± 11.4a	0.2 ± 0.4a
10	40	36	4.5 ± 3.0a	24.1 ± 4.8a	1.2 ± 1.6a	22.2 ± 2.8a	0.2 ± 0.3a	23.3 ± 8.6a	0.7 ± 1.6a
11	40	46	4.5 ± 2.6a	18.7 ± 4.1a	1.3 ± 1.4a	21.0 ± 2.3a	0.1 ± 0.3a	17.5 ± 7.3a	0.8 ± 2.7a
12	40	70	4.3 ± 2.0a	22.6 ± 8.2a	2.2 ± 2.0a	22.2 ± 3.1a	0.2 ± 0.3a	25.2 ± 9.7a	1.8 ± 3.3a
Control	0	0	4.0 ± 3.3a	19.4 ± 4.9a	1.6 ± 1.4a	19.3 ± 5.4a	0.2 ± 0.4a	9.4 ± 8.7d	0.3 ± 0.5a
		P-value	*0.9870*	*0.0180*	*0.3579*	*0.1328*	*0.6980*	*<0.0001*	*0.0664*

[1] Intensity ratings using a 150 mm unstructured scale with anchors at 12.5 and 137.50 mm
Means in within a column at the same storage temperature along with controls, not followed by the same letter is significantly (P<0.05) different from each other as determined by Fisher's least significant difference mean separation test.

[2] Controls are peanut bars prepared from untreated roasted whole peanuts

Table 4.35 Changes in the intensity ratings (mean standard deviation) for the texture attributes and feeling factors of resveratrol-enhanced peanut bars by a descriptive panel during storage at 25 and 40°C[1].

Trt #	Storage Temp. (°C)	Storage Time (day)	Hardness	Fracturability	Crunchiness	Cohesiveness	Chewiness	Toothpack
1	25	0	91.1 ± 5.2b	70.9 ± 1.8c	91.0 ± 6.7a	68.3 ± 2.7a	57.2 ± 2.4a	86.2 ± 3.5a
2	25	35	92.5 ± 4.9b	72.0 ± 3.7bc	91.1 ± 4.9a	68.7 ± 2.7a	58.5 ± 3.8a	87.0 ± 4.1a
3	25	51	92.7 ± 3.8b	71.5 ± 4.1bc	89.9 ± 6.6a	68.9 ± 2.7a	56.6 ± 2.8a	86.5 ± 2.2a
4	25	62	93.5 ± 3.8ab	71.8 ± 5.1bc	92.5 ± 4.7a	68.2 ± 3.0a	58.0 ± 3.2a	87.1 ± 6.0a
5	25	87	93.4 ± 3.1ab	72.2 ± 4.9bc	91.7 ± 5.8a	67.2 ± 2.6a	56.7 ± 7.4a	86.1 ± 2.6a
6	25	135	90.8 ± 13.4b	73.2 ± 6.5abc	90.0 ± 5a	68.5 ± 3.1a	59.0 ± 5.4a	88.9 ± 1.7a
7	25	146	91.4 ± 5.4bc	70.6 ± 4.9c	92.3 ± 3.2a	68.9 ± 2.1a	56.7 ± 3.0a	87.6 ± 3.7a
Control[2]	0	0	97.3 ± 4.8a	75.5 ± 5.4a	92.4 ± 6.8a	70.4 ± 3.5a	58.4 ± 2.9a	88.1 ± 3.2a
		P-value	*0.0473*	*0.0383*	*0.6761*	*0.0593*	*0.4205*	*0.1857*
8	40	0	92.2 ± 3.6b	71.2 ± 1.8c	92.0 ± 2.6a	68.5 ± 2.6a	56.7 ± 3.5a	86.6 ± 3.3a
9	40	16	92.9 ± 4.0b	72.7 ± 5.6bc	92.2 ± 4.3a	68.3 ± 3.4a	56.0 ± 3.6a	86.4 ± 6.8a
10	40	36	92.3 ± 5.3b	71.1 ± 2.9c	92.2 ± 2.9a	71.1 ± 2.9a	57.4 ± 2.1a	87.0 ± 2.4a
11	40	46	92.4 ± 3.5b	72.1 ± 3.2bc	92.9 ± 5.2a	68.7 ± 2.5a	57.2 ± 2.4a	85.9 ± 4.6a
12	40	70	94.2 ± 3.1b	74.1 ± 5.5ab	91.6 ± 7.5a	68.5 ± 3.5a	57.9 ± 5.0a	86.7 ± 4.6a
Control	0	0	97.3 ± 4.8a	75.5 ± 5.4a	92.4 ± 6.8a	70.4 ± 3.5a	58.4 ± 2.9a	88.1 ± 3.2a
		P-value	*0.0007*	*0.0103*	*0.9878*	*0.1675*	*0.3144*	*0.6953*

[1] Intensity ratings using a 150 mm unstructured scale with anchors at 12.5 and 137.50 mm
Means in within a column at the same storage temperature along with controls, not followed by the same letter is significantly different ($P<.05$) from each other as determined by Fisher's least significant difference mean separation test.

[2] Controls are peanut bars prepared from untreated roasted whole peanuts

V. STUDY 5 – PROFILING OF PHENOLIC COMPOUNDS AND SENSORY ATTRIBUTES OF UV, ULTRASOUND AND COMBINED ULTRASOUND-UV TREATED PEANUTS

A. Results of LC-MS

LC-MS was conducted to verify presence of phenolic compounds in UV and US treated peanuts based on their mass, and retention time in the chromatograms, and spectra in comparison with those of known pure standards. Results confirmed presence of two stilbenes, *trans*-resveratrol and *trans*-piceid; and three phenolic acids, ρ-coumaric acid, caffeic acid, ferulic acid; and the internal standard, β-resorcylic acid, in the UV and US-treated samples as shown in Table 4.36. Quercetin, a flavonoid maybe present although its peak was not distinct. Due to its very low peak and difficulties of its identification in the chromatogram and spectra, quercetin was not quantified in this study. All other 7 flavanols, epigallocatechin, catechin, procyanidin B2, epicatechin, epigallocatechin gallate, epicatechin gallate, and catechin gallate; and 2 benzoic acid derivatives, gallic acid and protocatechuic acid, analyzed using LC-MS were not detected in US and UV-treated peanut kernels.

B. Phenolic Profiles of Peanuts Treated with UV, Ultrasound, and Combined Ultrasound-UV Processing Treatments

1. Phenolic profile of UV-treated peanuts

The phenolic profile of UV-treated peanuts consisting of *trans*-resveratrol, *trans*-piceid, *p*-coumaric acid, caffeic acid and ferulic acid obtained using the reverse HPLC method for the simultaneous analysis of 15 phenolic compounds and the internal standard, β-resorcyclic acid, by Francisco and Resurreccion (2009b) is shown in Table 4.37. The *trans*-resveratrol concentrations of treated peanuts ranged from 0.58 to 2.18 μg/g or overall mean of 1.05 μg/g

Table 4.36 Sixteen phenolic compounds assayed in peanut kernels using liquid chromatography-mass spectrometry (LC-MS).

Wavelength (nm)	Compound	Retention time in standards (min)	Retention time in sample (min)	Mass (g/mole)
	Hydroxybenzoic acids			
250	Gallic acid	4.6	ND	170
	Protocatechuic acid	9.1	ND	154
	β-resorcylic acid (internal standard)	21.1	21.88	153
	Flavan-3-ols			
280	Epigallocatechin	17.3	ND	290
	Catechin	20.1	ND	290
	Procyanidin B2	28.4	ND	578
	Epicatechin	32.8	ND	290
	Epigallocatechin gallate	35.1	ND	458
	Epicatechin gallate	60.2	ND	442
	Catechin gallate	63.8	ND	442
	Hydroxycinnamic acids			
320	Caffeic acid	23.0	23.97	179
	ρ-coumaric acid	36.4	36.75	163
	Ferulic acid	45.1	47.20	193
	Stilbenes			
306	*trans*-Piceid	55.13	55.49	389
	trans-Resveratrol	84.94	84.99	227
	Flavonol			
370	Quercetin	93.6	93.06*	301

ND means not detected

* Very low intensity, may be present in the sample

whereas control untreated raw peanuts had 0.31µg/g. These concentrations in the UV-treated samples were within the values of 0.54 to 3.30 µg/g *trans*-resveratrol previously found in UV-treated peanuts using the method of Potrebko and Resurreccion (2009). All 27 UV treatments had significantly higher *trans*-resveratrol compared to controls. The distance from UV light and UV exposure time were the significant factors affecting *trans*-resveratrol in UV-treated peanuts. The distance of 40 cm from UV light produced the highest mean overall *trans*-resveratrol of 1.20 µg/g which was significantly higher (P<0.0058) compared to 0.96 and 0.91 µg/g when exposed at 20 and 60 cm distance from UV light, respectively. At UV exposure times of 20 and 30 min, overall mean resveratrol of 1.14 and 1.15 µg/g were obtained which were significantly higher than 0.81 µg/g produced when exposed to UV for 10 min. These results suggest that a minimum distance of 40 cm from UV light and a moderate to high UV exposure time of 20 to 30 min are needed to produce the highest *trans*-resveratrol in UV-treated peanuts.

The amounts of *trans*-piceid produced in treated peanuts ranged from 0.47 to 1.74 µg/g or overall mean of 0.92 µg/g whereas untreated controls of 0.31 µg/g. The values obtained for treated samples were within the range of 0.35 to 1.05 µg/g previously reported using the method of Potrebko and Resurreccion (2009). Fourteen of 27 UV treatments had significantly higher *trans*-piceid compared to controls while the rest of 13 treatments had similar concentrations as in controls.

Caffeic acid concentrations of treated peanuts ranged from 0.13 to 0.90 µg/g or overall mean of 0.38 µg/g and untreated controls had 0.84 µg/g. None of the 27 UV-treated peanut had significantly higher caffeic acid than controls. Only 3 (Trt# 16, 18, 24 in Table 4.37) of 27 treatments had equal caffeic contents as controls while the rest of 24 treatments produced less caffeic acid compared to untreated controls indicating that UV process combinations of distance

from UV light and exposure time were not effective in increasing caffeic acid in UV-treated peanuts.

p-Coumaric acid is the major phenolic compound in UV-treated peanuts and their concentration ranged from 100.39 to 210.00 µg/g or overall mean of 147.81 µg/g whereas untreated controls had 63.01 µg/g. Twenty four of 27 UV-treated peanuts had significantly higher *p*-coumaric acid compared to untreated controls whereas the remaining three treatments (Trt#1, 19, 27) had concentrations similar to controls. The most significant factor affecting *p*-coumaric acid concentrations in treated peanuts was UV exposure time, with 20 and 30 min exposure times producing the highest overall mean concentrations of 157.65 and 154.62 µg/g, respectively, compared to 126.79 µg/g obtained when exposed for only 10 min. This result indicates that UV exposure time of at least 20 min is needed to produce high *p*-coumaric acid concentrations in treated peanuts.

Ferulic acid concentrations in treated peanuts ranged from 2.93 to 7.35 µg/g or overall mean of 4.73 whereas untreated controls had 1.4 µg/g. Twenty three of 27 UV treatments had significantly higher ferulic acid compared to controls while the rest of four treatments (Trt# 1, 17, 21, 27) produced the same concentrations as controls. UV exposure time was the most significant factor affecting ferulic concentrations in UV-treated peanuts with 20 and 30 min exposure times producing the highest overall mean concentrations of 5.22 and 4.93 µg/g, respectively compared to 3.82 µg/g obtained when exposed for 10 min.

The mean concentration of all phenolic compounds detected or the sum of *trans*-resveratrol, *trans*-piceid, caffeic-, coumaric, and ferulic acids in UV-treated peanuts ranged from 105.38 to 221.08 µg/g or overall mean of 154.90 µg/g while control had 66.93 µg/g. UV exposure time was the most significant factor affecting the mean concentration of all phenolic compounds with

20 and 30 min exposure times to UV producing the highest sum of all phenolic compounds of 165.34 and 162.16 μg/g, respectively, which were significantly higher than when exposed for 10 min obtaining only 132.54 μg/g.

2. Phenolic profile of ultrasound-treated peanuts

The phenolic profile of US-treated peanuts is shown in Table 4.38. The amounts of *trans*-resveratrol produced in treated peanuts ranged from 0.76 to 3.23 μg/g or overall mean of 1.57 μg/g whereas untreated controls had 0.31 μg/g. All 27 treatments had significantly higher *trans*-resveratrol compared to controls. US power density and incubation time were significant factors affecting the concentrations of *trans*-resveratrol in US-treated peanuts. Exposure of peanuts to 75 mW/cm^3 power density produced the highest overall mean *trans*-resveratrol of 1.87 μg/g compared to 1.41 μg/g obtained when exposed to 25 mW/cm^3 but not significantly different from 1.47 μg/g obtained when exposed to 50 mW/cm^3. Incubating US-treated peanuts for 24 h produced the highest overall mean *trans*-resveratrol of 2.21 μg/g which was significantly higher than those incubated for 36 and 48 h with mean concentrations of 1.31 and 1.38 μg/g, respectively,

Trans-piceid concentrations of US treated peanuts ranged from 0.33 to 3.64 μg/g or overall mean of 1.60 μg/g whereas untreated control had raw peanuts with 0.31 μg/g. Eight of 27 US-treated peanuts (Trt# 1, 3, 4, 10, 16, 20, 21, 22) had significantly higher, nine treatments (Trt# 5, 7, 9, 13, 15, 19, 23, 25, 27) had significantly lower *trans*-piceid compared to controls while the rest of the 10 treatments had concentrations equal to controls. Incubation time significantly affected the concentrations of *trans*-piceid of US-treated peanut with 24 h incubation time producing the highest overall mean concentration of 2.0 μg/g. This was significantly higher than

those incubated for 48 h with overall mean of 1.39 µg/g but not significantly different from 1.46 µg/g stored for 36 h.

Caffeic acid concentrations of treated peanuts ranged from 0.33 to 1.32 µg/g or overall mean of 0.79 µg/g while untreated controls had 0.85 µg/g. Three of 27 US-treated peanuts (Trt# 16, 20 and 24) had significantly higher caffeic acid than untreated controls, 21 treatments produced the same amounts of caffeic acid as controls and three treatments (Trt# 3, 13, 23) obtained lower significantly lower caffeic acid compared to controls.

p-Coumaric acid is the major phenolic compound in treated peanuts with 45.22 to 393.55 or overall mean of 176.79 µg/g whereas untreated controls had 45.22 µg/g. Twenty three of 27 US treatments produced significantly higher *p*-coumaric acid concentrations compared to controls whereas four treatments (Trt# 9, 13, 23, and 25) obtained similar concentrations as controls. US exposure time was the most significant factor affecting *p*-coumaric acid concentrations with 2 min exposure time producing the highest overall mean of 205.41 µg/g whereas 8 min exposure resulted in the lowest overall mean of 157.03 µg/g, and 5 min exposure to US with 170.65 was intermediate.

The concentrations of ferulic acid in treated peanuts ranged from 1.50 to 14.79 µg/g or overall mean of 6.82 µg/g whereas untreated controls had 1.4 µg/g. Twenty two of 27 US-treated peanuts obtained significantly higher ferulic acid compared to untreated controls and 5 treatments (Trt# 9, 13, 15, 23, and 25) had concentration similar to controls.

The overall mean concentrations of all phenolic compounds or the sum of *trans*-resveratrol, *trans*-piceid, caffeic-, *p*-coumaric- and ferulic acids in US-treated peanuts ranged from 50.70 to 413.08 µg/g or overall mean of 187.56 µg/g which were significantly higher ($P<0.05$) than control with 66.93 µg/g. Compared to overall mean concentration of all phenolic compounds

obtained previously in UV-treated peanuts (Study 5.A.1 above) of 152.66 µg/g, US processing treatment was more effective than UV in producing higher concentrations of all phenolic compounds evaluated in this study.

3. Phenolic profile of combined UV-US treated peanuts

The phenolic profile of peanuts processed with combined US-UV treatments was dicussed in Study 3 of this section and the mean concentrations of phenolic compounds are shown in Table 4.18. The combined US-UV processing treatments produced total (sum) concentrations of all phenolic compounds analyzed ranging from 136.39 to 280.17 µg/g compared to untreated controls of 39.8 µg/g. Compared to US-treated control peanuts with 146.29 µg/g or UV treated controls with 136.39 µg/g found in the same study (Table 4.20), the combined US-UV treated peanuts produced a higher maximum of 280.17 µg/g suggesting an additive effect of US and UV.

4. Comparison of concentrations of phenolic compound in peanuts treated with UV, ultrasound, and combined US-UV processing treatments

The comparison of the mean concentrations of phenolic compounds based on difference between concentrations of treated (UV, US or combined US-UV) peanuts and untreated controls is presented in Table 4.39. The differences in concentrations, rather than actual observed values, were used to compare the three treatments because treated samples were prepared at different times along with their controls. A positive value means that treated peanuts had higher concentration whereas a negative rating means lower compared to controls.

Results showed that the mean concentrations of *trans*- resveratrol, ρ-coumaric acid and ferulic acid in all treated peanuts were higher than controls whereas *trans*-piceid in combined US-UV and caffeic acid in UV and US treatments were lower than controls. *Trans*-resveratrol was highest in combined US-UV followed by US alone and lowest in UV treatment.

Table 4.37 Profile of phenolic compounds (mean ± standard deviation, µg/g, dry basis) of UV-treated peanuts[1].

Trt #	ID	IT	IC	*Trans*-resveratrol	*Trans*-Piceid	Caffeic acid	Coumaric acid	Ferulic acid
1	20	10	24	0.62±0.03c	0.47±0.10gh	0.31±0.00hijk	115.60±12.39ghi	2.93±0.45ij
2	20	10	36	0.58±0.08c	0.80±0.00cdefgh	0.11±0.04m	100.39±7.43abcdefg	3.50±0.19ghi
3	20	10	48	0.92±0.22ab	1.16±0.40c	0.34±0.01hij	128.07±30.35fgh	4.07±1.23defghi
4	20	20	24	0.87±0.14ab	1.15±0.17cd	0.13±0.04lm	148.21±8.37bcdefgh	5.44±0.40defghi
5	20	20	36	1.25±0.26b	0.74±0.04cdefgh	0.55±0.08cde	137.04±12.67cdefgh	4.38±1.12defghi
6	20	20	48	0.99±0.19ab	0.56±0.15fgh	0.16±0.00klm	132.26±27.61cdefgh	4.57±0.96defghi
7	20	30	24	1.12±0.40ab	1.15±0.36c	0.62±0.08cd	179.87±29.21abcde	5.56±0.46abcde
8	20	30	36	1.00±0.34ab	0.60±0.18efgh	0.42±0.04efgh	141.32±30.99bcdefgh	4.79±1.23cdefgh
9	20	30	48	1.37±0.02b	1.06±0.08cdef	0.53±0.02def	131.07±24.73cdefgh	3.96±0.77fghi
10	40	10	24	0.93±0.30ab	0.90±0.04cdefg	0.37±0.02fghi	133.96±21.37cdefgh	4.19±0.33defghi
11	40	10	36	0.90±0.1ab	0.49±0.08gh	0.38±0.10fghi	128.41±24.70defgh	3.34±0.86hi
12	40	10	48	0.86±0.12ab	0.90±0.14cdefg	0.21±0.02jklm	142.77±42.34bcdefgh	5.24±1.05bdefg
13	40	20	24	1.05±0.10ab	0.75±0.23cdefgh	0.52±0.03defg	142.63±14.68bcdefgh	4.01±0.91defghi
14	40	20	36	1.35±0.36b	1.08±0.31cde	0.23±0.08ijklm	161.52±30.20abcdefg	5.84±1.03abcd
15	40	20	48	0.86±0.06ab	0.81±0.27cdefgh	0.36±0.04fghi	142.60±30.30bcdefgh	3.66±0.18fghi

Table 4.37 continued…

Trt #	ID	IT	IC	*Trans*-resveratrol	*Trans*-Piceid	Caffeic acid	Coumaric acid	Ferulic acid
16	40	30	24	1.24±0.44b	1.19±0.34bc	0.83±0.15ab	195.90±31.43ab	7.35±0.59a
17	40	30	36	0.84±0.08ab	0.52±0.19gh	0.28±0.09hijkl	124.05±6.94hg	3.04±0.30hij
18	40	30	48	2.18±0.60a	1.74±0.53a	0.90±0.05a	183.27±29.58abcd	6.47±0.36abc
19	60	10	24	0.87±0.17ab	0.64±0.19defgh	0.42±0.06efgh	114.76±16.24ghi	3.24±0.53hi
20	60	10	36	1.00±0.18ab	0.97±0.16cdefg	0.15±0.01lmk	148.24±47.40bcdefgh	4.69±1.49cdefghi
21	60	10	48	0.66±0.10c	0.86±0.18cdefg	0.53±0.18def	128.95±14.43defgh	3.15±1.13hij
22	60	20	24	1.10±0.30ab	0.88±0.26cdefg	0.21±0.03jklm	172.65±25.29abcdef	5.31±0.61bcdef
23	60	20	36	0.85±0.21ab	0.94±0.27cdefg	0.56±0.20cde	155.81±50.34abcdefg	4.40±1.55defghi
24	60	20	48	1.13±0.33ab	1.70±0.41ab	0.69±0.04bc	210.60±14.87a	6.95±0.86ab
25	60	30	24	0.90±0.32ab	0.65±0.05cdefgh	0.38±0.03fghi	132.31±18.25cdefg	4.41±0.72defghi
26	60	30	36	0.89±0.03ab	0.70±0.14defgh	0.29±0.09hijkl	185.81±40.05abc	5.70±0.96abcde
27	60	30	48	0.81±0.22ab	0.75±0.26cdefgh	0.51±0.15defg	117.97±20.49fghi	3.12±0.99hij
Control[2]	120	0	0	0.31±0.01d	0.31±0.03h	0.84±0.07ab	63.01±10.30i	1.40±0.13j

[1]ID = distance from UV light (cm); IT = UV exposure time (min); IC = incubation time (h)
Means in a column not followed by the same letter is significantly different (P<0.05) from each other as determined by Fisher's least significant difference mean separation test.
[2] Controls are untreated raw whole peanuts.

Table 4.38 Profile of phenolic compounds (mean ± standard deviation, µg/g, dry basis) of ultrasound-treated peanuts[1].

Trt #	PD	PT	IC	*Trans*-resveratrol	*Trans*-Piceid	Caffeic acid	Coumaric acid	Ferulic acid
1	25	2	24	2.80±0.94a	2.78±0.84abc	1.09±0.18abc	261.98±36.45bc	8.89±1.89de
2	25	2	36	1.09±0.30efg	1.05±0.12ghijk	0.61±0.13efgh	164.75±47.38ghijk	7.11±1.87efg
3	25	2	48	0.99±0.16efg	1.04±0.32a	0.40±0.11gh	120.91±36.74 ijklmn	3.64±1.03 jkl
4	25	5	24	1.85±0.50c	3.64±1.22a	1.09±0.14abc	244.50±42.82bcd	10.64±2.46cd
5	25	5	36	1.00±0.18efg	0.81±0.25ijk	0.66±0.06defgh	168.01±24.66fghijk	7.09±1.10efg
6	25	5	48	0.83±0.02fg	1.32±0.42fghij	0.59±0.01defg	205.04±64.83cdefg	5.79±1.60ghij
7	25	8	24	1.99±0.52bc	0.52±0.15jk	0.71±0.16defg	157.99±46.84ghijkl	4.72±0.60hijk
8	25	8	36	0.98±0.16efg	0.89±0.17ghijk	0.59±0.08defg	123.10±14.41ijklmn	4.74±0.84hijk
9	25	8	48	1.14±0.11defg	0.49±0.08jk	0.49±0.03fgh	95.54±8.53mno	2.76±0.29klmn
10	50	2	24	2.00±0.25ab	3.37±0.54abcd	0.99±0.07abcd	257.06±33.45bc	10.57±0.24cd
11	50	2	36	0.89±0.03fg	0.92±0.30ghijk	0.60±0.04defg	113.22±6.08jklmn	3.80±0.17jkl
12	50	2	48	2.58±0.81ab	1.31±0.06fghij	1.17±0.41abc	185.61±1.20bcde	8.45±0.38def
13	50	5	24	1.50±0.14cdef	0.33±0.03k	0.45±0.04gh	103.37±13.31lmno	2.46±0.32lmn
14	50	5	36	1.33±0.23cdefg	1.83±0.49defg	0.87±0.26bcde	169.12±19.20efghijk	7.14±0.82efg
15	50	5	48	0.85±0.27fg	0.66±0.22jk	0.53±0.13defg	110.72±12.93klmn	3.09±0.67klmn
16	50	8	24	2.72±0.69a	2.70±0.68abcd	1.23±0.08ab	289.90±42.77b	14.79±2.97a
17	50	8	36	1.16±0.14defg	1.68±0.58efghi	0.70±0.07defg	175.08±26.67efghi	6.53±2.10fgh
18	50	8	48	0.76±0.04fg	1.77±0.03defgh	0.83±0.07cdef	158.43±8.34ghijkl	6.86±0.38efgh

Table 4.38 continued…

Trt #	PD	PT	IC	*Trans*-resveratrol	*Trans* - Piceid	Caffeic acid	Coumaric acid	Ferulic acid
19	75	2	24	1.63±0.29cde	0.85±0.30hijk	0.65±0.19defgh	124.73±20.38hijklm	4.14±0.26hijk
20	75	2	36	0.96±0.31efg	2.27±0.36bcde	1.28±0.05a	226.89±18.86cdef	10.02±0.02cd
21	75	2	48	2.65±0.01ab	2.86±0.68abc	1.00±0.19abcd	393.55±93.90a	13.19±0.81ab
22	75	5	24	1.80±0.20cd	3.14±0.93ab	1.09±0.21abc	228.04±12.47cdef	11.11±1.07ab
23	75	5	36	3.23±0.38a	0.54±0.02jk	0.33±0.06h	82.84±17.93nmo	1.52±0.21mn
24	75	5	48	1.82±0.56cd	2.18±0.64cdef	1.32±0.37a	230.27±6.38bcde	10.23±0.90cd
25	75	8	24	2.81±0.45a	0.69±0.23jk	0.48±0.00fgh	45.22±4.00o	1.50±0.08mn
26	75	8	36	1.09±0.15efg	1.68±0.10efghi	0.69±0.11defgh	172.82±8.77efghij	5.71±0.11efghi
27	75	8	48	0.85±0.06fg	0.87±0.15hijk	0.65±0.09defgh	195.18±45.31defg	6.27±1.40fghi
Control[2]	0	0	0	0.31±0.16h	0.31±0.1fg	0.85±0.10cdef	45.22±10.3o	1.40±0.02n

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IC = incubation time (h)
Means in a column not followed by the same letter is significantly different (P<0.05) from each other as determined by Fisher's least significant difference mean separation test.
[2] Controls are untreated raw whole peanuts.

Table 4.39 Comparison of phenolic compound mean concentrations, expressed as difference from control, in resveratrol-enhanced peanuts treated with UV, ultrasound, and combined ultrasound-UV.

Compound	Concentration (µg/g) expressed as difference from control of						Pr>F
	UV		Ultrasound		Combined US-UV		
	Difference[1]	Control[2]	Difference	Control	Difference	Control	
Trans-resveratrol	0.7986c	0.3110	1.4370b	0.3110	3.6375a	0.6362	<0.0001***
Trans-piceid	0.4238b	0.3149	1.711a	0.3149	-0.1655c	1.1247	<0.0001***
Caffeic acid	-0.4253c	0.8455	-0.0474b	0.8455	0.2649a	0.5060	<0.0001***
ρ-Coumaric acid	74.1240b	63.0068	117.569a	63.0068	126.896a	35.1635	0.0069**
Ferulic acid	5.2945a	1.4025	2.8133b	0.4025	2.698b	0.1914	<0.0001***

[1] Difference in mean concentrations between treated peanuts and controls were used because treated peanuts were prepared at different times. A total of 27 treatments and a control in 2 replications were analyzed for a total of 56 analyses. A positive rating means treated peanuts had higher concentration compared to controls. A negative rating means treated peanuts had lower concentration than controls. Means of the differences from control within a row followed the same letter are not significant different from each other as determined by Fisher's least significant difference mean separation test. NS= not significant; * = significant at $P<0.05$; **significant at $P<0.001$; ***significant at $P<0.0001$.

[2] Concentrations of control represent observed values of phenolic compounds.

ρ-Coumaric acid concentrations were higher in combined US-UV and US treatments compared to UV. Ferulic acid was highest in UV-treated peanuts compared to US and combined US-UV treatments which had similar concentrations. Caffeic acid concentration was only enhanced when peanuts were treated with combined US-UV whereas US and UV had no effect in increasing caffeic acid in treated peanuts. *Trans*-piceid concentrations were highest in US treated peanuts followed by UV but not enhanced using combined US-UV treatment. In summary, the concentrations of phenolic compounds except for piceid and ferulic acid were effectively enhanced when peanuts were treated with combined US-UV process, followed by US, and then UV process.

B. Sensory Profiles of Peanuts Treated with Treated with UV, Ultrasound, and Combined US-UV Processing Treatments

1. Sensory profile of UV-treated peanuts by a descriptive panel

1.1 Descriptive panel intensity ratings for brown color and roasted peanutty and raw beany flavors (Table 4.40)

Brown color. The brown color ratings of UV-treated peanuts ranged from 33.0 to 52.5 with a control rating of 32.5. Eighteen of 27 UV treated peanuts had significantly greater intensity of brown color of 38.0 to 52.5 compared to untreated controls with 32.5. The intensities of brown color of 33.0 to 36.4 of all other 9 treatments (Trt# 2, 4, 8, 10, 13, 14, 18, 24, 26) were not significantly different from untreated controls. Peanuts treated at a distance from UV light of 60 cm produced the most intense brown color with overall mean rating of 43.4 which was significantly higher than the brown color at 20 and 40 cm with overall mean ratings of 40.3 and 40.1. UV exposure time and incubation time had no effect on the intensity of brown color of UV-treated peanuts.

Table 4.40 Intensity ratings (mean±standard deviation) for the brown color, roasted peanutty and raw/beany aromatics of roasted sliced UV-treated peanuts by a descriptive panel.

Trt #	ID	IT	IC	Brown color	Roasted peanutty	Raw/beany
1	20	10	24	44.6 ± 6.7defgh	52.1 ± 16.5ab	0.0 ± 0.0c
2	20	10	36	33.9 ± 3.7m	30.0 ± 17.3ghijk	0.0 ± 0.0c
3	20	10	48	42.7 ± 6.7efghi	25.3 ± 10.5k	2.1 ± 4.3ab
4	20	20	24	36.4 ± 4.8klm	36.2 ± 16.3defghij	3.1 ± 5.1a
5	20	20	36	46.5 ± 6.2bcde	42.7 ± 15.8bcde	0.0 ± 0.0c
6	20	20	48	38.0 ± 2.7jkl	28.5 ± 6.7ijk	1.6 ± 4.1abc
7	20	30	24	45.2 ± 7cdefg	45.7 ± 17.1abcd	0.0 ± 0.0c
8	20	30	36	30.0 ± 3.1m	28.0 ± 9.9ijk	1.2 ± 4.1abc
9	20	30	48	42.8 ± 8.4efghi	26.2 ± 8.1k	2.7 ± 5.9ab
10	40	10	24	34.4 ± 4.2lm	33.6 ± 15efghijk	1.8 ± 4.2abc
11	40	10	36	49.2 ± 8.3abc	41.2 ± 15.1bcde	0.0 ± 0.0c
12	40	10	48	40.5 ± 4.6hij	29.9 ± 9ghijk	2.1 ± 4.3ab
13	40	20	24	36.4 ± 4.4lm	31.1 ± 9.2fghijk	2.2 ± 5.5ab
14	40	20	36	34.5 ± 4.2klm	26.8 ± 5.4jk	0.1 ± 0.3c
15	40	20	48	46.6 ± 5.5bcde	49.9 ± 19.1abc	0.0 ± 0.0c
16	40	30	24	38.2 ± 4.5jkl	38.8 ± 14.7efghijk	0.0 ± 0.0c
17	40	30	36	45.8 ± 9.8bcdefg	40.0 ± 17.8cdef	0.0 ± 0.0c
18	40	30	48	35.2 ± 4.3lm	30.9 ± 12.8fghijk	1.6 ± 4.7abc
19	60	10	24	46.3 ± 9.5bcdef	39.7 ± 14.3defg	1.2 ± 4.9abc
20	60	10	36	41.8 ± 7ghij	37.8 ± 16defghi	0.7 ± 2.8bc
21	60	10	48	42.5 ± 5.2fghi	39.1 ± 18.4defgh	0.8 ± 2.8bc
22	60	20	24	39.6 ± 5ijk	40.5 ± 14.7cdef	0.0 ± 0.0c
23	60	20	36	52.5 ± 6.5a	39.6 ± 18.4defg	0.0 ± 0.0c
24	60	20	48	36.4 ± 4.8klm	29.6 ± 13.6ijk	0.06 ± 0.2c
25	60	30	24	47.2 ± 4.6bcd	44.0 ± 16.3bcd	0.0 ± 0.0c
26	60	30	36	34.8 ± 3.5lm	30.0 ± 12.4ghijk	0.0 ± 0.0c
27	60	30	48	49.7 ± 7.1ab	43.9 ± 12.9bcd	0.0 ± 0.0c
Control[2]	120	0	0	32.5 ± 3.1m	54.9 ± 16a	0.0 ± 0.0c

[1]ID = distance from UV light (cm); IT = UV exposure time (min); IC = incubation time (h)
Means in a column not followed by the same letter is significantly different ($P<0.05$) from each other as determined by Fisher's least significant difference mean separation test.
[2]Controls are untreated raw whole peanuts.

Roasted peanutty flavor. The range of intensity ratings for roasted peanutty flavor of UV-treated samples was 25.3 to 52.1 with controls at 54.9. The intensities of roasted peanutty flavor of 45.7 to 52.1 three treatments (Trt# 1, 7, 15) were not significantly different from untreated controls. Twenty four of 27 treated peanuts had significantly lower intensities of roasted peanutty flavor of 25.3 to 44.0 compared to untreated controls. Distance from UV light, UV exposure time and incubation time had no effect on the roasted peanutty flavor on UV-treated peanuts.

Raw beany flavor. The range of raw beany intensity ratings of UV-treated peanuts was 0 to 3.1 with control at 0. Twenty two of 27 UV-treated peanuts had raw beany flavor intensities of 0 to 1.8 which were not significantly different from controls. Five treatments (Trt# 3, 4, 9, 12, 13) had raw beany flavor intensities of 2.1 to 3.1 which were significantly higher than that of control. Distance from UV light of 20 cm produced the most intense raw beany flavor with the overall mean rating of 1.2 which was significantly higher than the rating of 0.3 at 60 cm distance. Incubation for 48 h resulted in the highest overall mean raw beany flavor intensity of 1.2 which was significantly higher than 0.2 rating at 36 h incubation but not significantly different from 0.9 rating at 24 h incubation.

1.2 Descriptive panel intensity ratings for basic tastes (Table 4.41)

Bitter taste. The range of intensity ratings for bitter taste of UV-treated peanuts ranged was 18.9 to 23.6 and control was 18.4. Seventeen of 27 UV-treatments had bitter intensity ratings of 19.0 to 21.0 which were not significantly different from that of controls. The intensity ratings of 21.1 to 23.6 in ten treatments were significantly higher than controls. Distance from UV light, UV exposure time and incubation time did not significantly affect the intensities of bitter tastes in UV-treated peanuts.

Table 4.41 Intensity ratings (mean±standard deviation) for the basic tastes of roasted sliced UV-treated peanuts by a descriptive panel.

Trt #	ID	IT	IC	Bitter	Sweet	Sour	Salty
1	20	10	24	23.6 ± 2.6a	13.9 ± 1.8cde	0.9 ± 3.8ef	13.7 ± ab3.1
2	20	10	36	19.9 ± 3.9cdef	14.7 ± 2.4bcd	1.7 ± 4.7def	13.1 ± abc2.69
3	20	10	48	20.2 ± 7.4cdef	12.5 ± 4.0e	5.0 ± 8.4abc	12.6 ± 1.5bcd
4	20	20	24	20.2 ± 3.2cdef	14.7 ± 2.3bcd	1.5 ± 4.1def	12.2 ± 0.8cd
5	20	20	36	19.6 ± 3.8cdef	13.3 ± 1.9de	1.4 ± 3.7ef	12.7 ± 1.8abcd
6	20	20	48	21.0 ± 4.7abcdef	13.3 ± 3.8de	7.6 ± 6.2a	12.5 ± 1.1bcd
7	20	30	24	19.1 ± 3.8def	14.6 ± 2.5bcd	1.3 ± 3.6ef	12.8 ± 1abc
8	20	30	36	23.0 ± 4.1ab	14.1 ± 1.6cde	3.6 ± 5.6bcde	12.1 ± 1.3cd
9	20	30	48	20.2 ± 3.8cdef	14.0 ± 1.5cde	7.8 ± 6.3a	12.3 ± 1cd
10	40	10	24	20.3 ± 3.6cdef	14.1 ± 1.5cd	0.0 ± 0.0f	12.4 ± 1.9bcd
11	40	10	36	22.3 ± 4.5abc	14.2 ± 4.2cd	2.9 ± 5.2cdef	13.3 ± 2.1abc
12	40	10	48	20.3 ± 3cdef	14.6 ± 2.0bcd	3.7 ± 5.7bcde	12.1 ± 1.3cd
13	40	20	24	18.9 ± 3.9ef	16.0 ± 2.2ab	2.3 ± 4.8cdef	12.5 ± 1.5bcd
14	40	20	36	21.1 ± 2.8cdef	13.9 ± 1.4cde	4.7 ± 5.6abcd	12.2 ± 0.6cd
15	40	20	48	21.7 ± 3.9abcd	13.9 ± 1.7cde	3.6 ± 5.6bcde	13.3 ± 2.3abc
16	40	30	24	20.3 ± 3.0cdef	14.9 ± 1.4bcd	0.8 ± 3ef	12.6 ± 1.9bcd
17	40	30	36	21.5 ± 3.9abcde	14.2 ± 3.4cd	0.7 ± 2.8ef	12.6 ± 2.2bcd
18	40	30	48	20.2 ± 2.5cdef	15.0 ± 2.5bc	6.1 ± 7.2ab	11.4 ± 3.6d
19	60	10	24	21.3 ± 3.7abcde	13.9 ± 1.4cde	0.7 ± 2.8ef	12.6 ± 1.1abcd
20	60	10	36	21.3 ± 3.0abcde	14.9 ± 2.2bc	0.8 ± 3ef	12.4 ± 1.9bcd
21	60	10	48	19.0 ± 4.1ef	14.4 ± 2.8bcd	1.9 ± 4.1cdef	12.5 ± 1.1bcd
22	60	20	24	20.0 ± 3.2cdef	14.9 ± 1.9bcd	1.2 ± 3.3ef	12.8 ± 3.1abcd
23	60	20	36	20.7 ± 3.1bcdef	13.4 ± 1.5cde	1.7 ± 4.7def	13.3 ± 2.8abc
24	60	20	48	21.3 ± 2.9abcde	14.6 ± 1.6bcd	2.5 ± 4.5cdef	12.3 ± 0.9bcd
25	60	30	24	20.6 ± 3.1bcdef	14.5 ± 1.6bcd	0.8 ± 3ef	12.2 ± 0.9cd
26	60	30	36	20.7 ± 3.9bcdef	14.3 ± 1.7cd	0.7 ± 2.8ef	12.2 ± 0.9cd
27	60	30	48	22.1 ± 3.9abc	13.9 ± 1.4cde	3.1 ± 5.6bcdef	13 ± 2.4abc
Control	120	0	0	18.4 ± 5.0f	17.3 ± 3.2a	0.0 ± 0.0f	14 ± 3.6a

[1]ID = distance from UV light (cm); IT = UV exposure time (min); IC = incubation time (h)
Means in a column not followed by the same letter is significantly different ($P<0.05$) from each other as determined by Fisher's least significant difference mean separation test.
[2]Controls are untreated raw whole peanuts.

Sweet taste. The range of intensity ratings for sweet taste of UV-treated peanuts was 12.5 to 16.0 with control at 17.3. One of 27 UV treatments (Trt#1) with sweetness intensity rating of 16.0 was not significantly different from control with rating of 17.3. The rest of 26 treatments with intensity ratings of 12.5 to 15.0 were significantly lower than controls. Distance from UV light, UV exposure time and incubation time did not affect the intensity of sweetness of UV-treated peanuts.

Sour taste. The range of intensity ratings for sour taste of UV-treated samples was 0 to 7.8 with control at 0. The intensity ratings of sour taste of 0 to 3.05 in 19 of 27 treatments were not significantly different from control with intensity rating of 0. Eight treatments with intensity ratings of 3.61 to 7.8 were significantly higher than controls. ANOVA results indicated that distance from UV light and incubation time were the significant factors affecting the sour taste intensity of UV treated peanuts. The distance from UV light of 20 cm had the highest mean overall intensity rating of 3.4 which was significantly higher than 1.5 rating at 60 cm distance from UV light but not significantly higher than 2.7 rating at 40 cm. The incubation time of 48 h produced the highest intensity of sourness with overall mean rating of 4.6 compared to 2.0 and 1.0 ratings at 36 and 24 h incubation time, respectively.

Salty taste. Salty taste intensities of UV-treated peanuts ranged from 11.4 to 13.7 with control rating of 14.0. Ten of 27 UV treatments had salty taste intensity ratings of 12.6 to 13.7 which were not significantly different from controls with ratings of 14.0. The intensity ratings of 27 UV treatments were significantly less than the rating of salty taste in controls. The distance from UV light, UV exposure time and incubation time did not significantly affect the intensity of salty taste in UV-treated peanuts.

1.3 Descriptive panel intensity ratings for woody/hulls/skins and burnt flavors and feeling factors astringency and tongue sting(Table 4.42)

Woody/hulls/skins. The intensities of woody/hulls/skin flavor of UV-treated peanuts ranged from 15.1-18.0 with control rating as 0. In 26 of 27 UV-treated peanuts, ratings for woody/hulls/skin were not significantly different from those of controls with 18.0 intensity rating. Only one treatment (Trt #10) had significantly higher intensity rating of 18.0 compared to controls. ANOVA results indicated that distance from UV light, UV exposure time and incubation time were not significant factors affecting the woody/hulls/skins flavor of UV-treated peanuts.

Burnt flavor. The burnt flavor of intensities of UV-treated peanuts ranged from 0 to 20.3 with controls rated as 0.8. Sixteen of 27 UV-treated peanuts had burnt intensity ratings of 0 to 5.81 which are not significantly different from the intensity of burnt flavor in controls of 0.80. The burnt intensity ratings of 6.5 to 20.3 for 11 other treatments were significantly higher than controls.

Astringency. Astringent is the puckering or drying sensation on the mouth or tongue surface. The intensity rating for astringent aftertaste of UV-treated peanuts ranged for 13.3 to 20.2 with controls rated as 18.9. The intensity ratings of 17.9 to 20.2 for the astringent aftertaste in 26 of 27 UV treatments were not significantly different from untreated controls. Only one treatment (Trt# 6) was less astringent than control with a rating of 17.1. The factors distance from UV light, UV exposure time and incubation time were not significant in causing changes in the intensity ratings for astringency of treated peanuts.

Tongue sting. Tongue sting is the degree of sharp tingling sensation or feeling on the tongue or throat which leaves a burning sensation on the tongue surface. The intensity rating for tongue

Table 4.42 Intensity ratings (mean±standard deviation) for woody/hulls/skins, burnt, astringent, and tongue sting of roasted sliced UV-treated peanuts by a descriptive panel.

Trt #	ID	IT	IC	Woody/Hull/ Skin	Burnt	Astringent	Tongue sting
1	20	10	24	16.9 ± 2.5abcd	9.6 ± 10.6cdefg	18.6 ± 1.0b	2.6 ± 5.7abcd
2	20	10	36	17.8 ± 3.5ab	0.8 ± 2.6klm	18.6 ± 1.0b	0.8 ± 3cd
3	20	10	48	15.4 ± 6.6d	1.6 ± 5.3jklm	20.2 ± 4.9a	2.2 ± 4.4bcd
4	20	20	24	16.0 ± 2.4abcd	1.2 ± 4.9klm	18.9 ± 1.3b	0.8 ± 3.2cd
5	20	20	36	16.2 ± 2.9abcd	11.3 ± 12.3bcdef	19.1 ± 2.5ab	2.2 ± 4.2bcd
6	20	20	48	15.9 ± 2.2bcd	0.9 ± 3.8klm	17.1 ± 2.8c	2.8 ± 5.7abc
7	20	30	24	15.1 ± 1.9d	5.7 ± 7.1ghijk	18.4 ± 2.3bc	0.8 ± 3.2cd
8	20	30	36	15.1 ± 4.3d	0.4 ± 1klm	18.5 ± 0.8b	0.8 ± 3cd
9	20	30	48	16.7 ± 3abcd	7.6 ± 8.6efghi	17.9 ± 2.1bc	1.8 ± 4.4bcd
10	40	10	24	18.0 ± 4.7a	1.4 ± 5.5klm	18.8 ± 1.9b	4.2 ± 6.5ab
11	40	10	36	16.0 ± 2.1abcd	13.0 ± 9.2bcd	18.3 ± 3.1bc	2.8 ± 5.2abcd
12	40	10	48	16.6 ± 1.4abcd	3.7 ± 6.3hijklm	18.4 ± 1.7bc	0.7 ± 2.8cd
13	40	20	24	16.3 ± 2.3abcd	1.0 ± 3.1klm	18.5 ± 1.2bc	1.8 ± 4.7bcd
14	40	20	36	17.7 ± 2.0abc	2.6 ± 6.3ijklm	18.6 ± 1.0b	0.7 ± 2.3cd
15	40	20	48	16.8 ± 2.1abcd	20.3 ± 9.2a	18.6 ± 1.9b	2.7 ± 5.1abcd
16	40	30	24	15.6 ± 2.8cd	1.4 ± 3.9klm	18.3 ± 1.1bc	0.6 ± 1.9cd
17	40	30	36	17.1 ± 1.6abcd	12.3 ± 12bcde	17.9 ± 1.5bc	5.3 ± 6.6a
18	40	30	48	15.9 ± 1.8bcd	1.6 ± 5.3jklm	18 ± 1.8bc	2.4 ± 5.2bcd
19	60	10	24	17.0 ± 1.9abcd	9.9 ± 10cdefg	18.4 ± 1.3bc	1.6 ± 4bcd
20	60	10	36	17.5 ± 2.0abc	6.5 ± 9.4fghij	18.6 ± 1.6b	0.05 ± 0.2cd
21	60	10	48	17.1 ± 2.7abcd	5.4 ± 8.7ghijkl	18.5 ± 1.3b	1.1 ± 4.5cd
22	60	20	24	17.1 ± 2.5abcd	5.8 ± 8.7ghijk	17.9 ± 1.3bc	0.0 ± 0.0d
23	60	20	36	16.8 ± 2.4abcd	15.1 ± 5.4b	13.3 ± 1.6bc	0.0 ± 0.0d
24	60	20	48	16.9 ± 2.0abcd	0.7 ± 2.5klm	18.7 ± 1.2b	0.0 ± 0.0d
25	60	30	24	16.7 ± 2.0abcd	8.5 ± 9.2defgh	18.8 ± 1.5b	0.9 ± 3cd
26	60	30	36	16.1 ± 2.3abcd	0.0 ± 0.0m	18.8 ± 1.5b	1 ± 3.9cd
27	60	30	48	16.5 ± 5.2abcd	14.1 ± 11.5bc	18.4 ± 1.3bc	2.2 ± 5.5bcd
Control	120	0	0	15.8 ± 4.9bcd	0.8 ± 3.2klm	18.9 ± 1.8ab	0.0 ± 0.0d

[1]ID = distance from UV light (cm); IT = UV exposure time (min); IC = incubation time (h)
Means in a column not followed by the same letter is significantly different (P<0.05) from each other as determined by Fisher's least significant difference mean separation test.
[2]Controls are untreated raw whole peanuts.

sting of UV-treated peanuts ranged for 13.3 to 20.2 with controls rated as 18.9. The tongue sting intensity ratings of 2.8 to 5.2 in three of 27 UV treatments (Trt# 22, 23, 24) were significantly higher than that of controls. Twenty four of 27 UV treatments with intensity ratings for tongue sting of 0 to 2.75 were not significantly different from 0 rating in untreated controls which were exposed to medium doses of UV.

1.4 Descriptive panel intensity ratings for cardboard, fishy, and oxidized flavors (Table 4.43)

Cardboard flavor. The intensities of cardboard flavor in UV-treated peanuts ranged from 18.0 to 25.0 with control rated 16.9. Seven of 27 UV treatments (Trt# 1, 7, 11, 16, 21, 22, and 23) produced cardboard flavor with intensity ratings of 18.0 to 20.9 which were not significantly different from controls with a 16.9 intensity rating. The intensity ratings for cardboard flavor of all other 20 UV treatments of 21.0 to 25.0 were significantly higher than controls.

Fishy flavor. The intensities of fishy flavor in UV-treated peanuts ranged from 6.2 to 19.3 with control rated as 11.0. The intensity of fishy flavor in 15 of 27 UV treatments of 9.9 to 15.2 was not significantly different from controls. Eleven of 27 UV-treated peanuts had intensity ratings of 15.9 to 19.3 for fishy flavor which are significantly higher than controls. Incubation time was the significant factors affecting the intensity of fishy flavor in UV-treated peanuts. Incubation for 48 h produced the most intense fishy flavor with overall mean intensity rating of 15.8 which was significantly higher than at 24 h with intensity rating of 12.2 but not different from 36 h incubation time with fishy flavor rating of 14.3.

Oxidized flavor. The intensities of oxidized flavor in UV-treated peanuts ranged from 17.9 to 31.8 with control rated as 16.8. All treated samples had higher oxidized flavor than controls. Seven of 27 UV treatments had oxidized flavor intensities of 17.9 to 22.3 which were not significantly different from the intensity rating of 16.8 in untreated controls. The intensity

Table 4.43 Intensity ratings (mean±standard deviation) for the oxidation-related off-flavors of roasted sliced UV-treated peanuts by a descriptive panel.

Trt #	ID	IT	IC	Cardboard	Fishy	Oxidized	Off flavor
1	20	10	24	19.5 ± 4.7fgh	10.1 ± 8.4gh	19.9 ± 9.7ghij	0.0 ± 0.0b
2	20	10	36	22.6 ± 6.7abcdef	16.2 ± 5.8abcd	25.2 ± 8.1cdefgh	0.05 ± 0.2b
3	20	10	48	23.8 ± 3.7abcd	19.3 ± 5.4a	31.8 ± 7.6a	0.7 ± 2.6a
4	20	20	24	22.5 ± 3.9abcd	10.9 ± 8.2fgh	22.1 ± 7.6efghij	0.0 ± 0.0b
5	20	20	36	22.5 ± 6.1abcdef	12.6 ± 7.3defg	22.3 ± 8.2efghij	0.0 ± 0.0b
6	20	20	48	24.4 ± 4.7abc	14.4 ± 6.1abcdefg	26.2 ± 4.8bcde	0.0 ± 0.0b
7	20	30	24	18.0 ± 8.6gh	6.2 ± 8.0h	17.9 ± 10.6ij	0.0 ± 0.0b
8	20	30	36	21.4 ± 4.8abcdefg	16.4 ± 6.9abcd	26.2 ± 6.9bcde	0.0 ± 0.0b
9	20	30	48	24.2 ± 3.5abcd	18.3 ± 4.6ab	29.7 ± 5.3abc	0.0 ± 0.0b
10	40	10	24	21.6 ± 7.1abcdefg	13.0 ± 9.8defg	22.8 ± 10.6fghi	0.0 ± 0.0b
11	40	10	36	20.9 ± 5bcdefgh	10.1 ± 6.5gh	26.0 ± 7.2bcde	0.0 ± 0.0b
12	40	10	48	24.6 ± 3.6ab	16.8 ± 6.3abcd	31.4 ± 5.1ab	0.0 ± 0.0b
13	40	20	24	25.0 ± 6.1a	15.9 ± 8.4abcd	22.8 ± 10.2fghi	0.0 ± 0.0b
14	40	20	36	22.7 ± 5abcd	16.0 ± 8abcd	25.5 ± 3.6cdef	0.0 ± 0.0b
15	40	20	48	21.0 ± 4.3abcdefg	13.3 ± 7.6cdefg	27.1 ± 4.2abcde	0.0 ± 0.0b
16	40	30	24	20.5 ± 8.8cdefgh	14.6 ± 6.9abcdefg	23.4 ± 10.3defghi	0.0 ± 0.0b
17	40	30	36	21.2 ± 6.7abcdefg	9.9 ± 8.8gh	24.4 ± 5.5cdefgh	0.0 ± 0.0b
18	40	30	48	23.8 ± 4abcd	16.0 ± 5.7abcd	28.5 ± 4.8abcd	0.0 ± 0.0b
19	60	10	24	24.0 ± 4.7abcd	16.0 ± 4.0abcd	25.3 ± 5.8cdefgh	0.05 ± 0.2b
20	60	10	36	22.4 ± 6.7abcdef	15.3 ± 3.8abcdef	24.1 ± 7.2cdefgh	0.0 ± 0.0b
21	60	10	48	18.6 ± 6.5fgh	13.5 ± 6.1bcdefg	22.5 ± 7.7fghi	0.0 ± 0.0b
22	60	20	24	20.3 ± 6.4defgh	10.8 ± 7.1fgh	21.9 ± 8.1efghij	0.0 ± 0.0b
23	60	20	36	20.8 ± 9bcdefgh	14.4 ± 6.4bcdefg	20.0 ± 13.1fghij	0.0 ± 0.0b
24	60	20	48	24.0 ± 2.7abcd	16.9 ± 5.2abcd	25.5 ± 7.8cdefg	0.0 ± 0.0b
25	60	30	24	22.2 ± 6.7abcdef	12.5 ± 9.3defg	25.1 ± 8.1cdefgh	0.0 ± 0.0b
26	60	30	36	23.6 ± 3.1abcd	18.0 ± 4.3abc	25.0 ± 5.3cdefgh	0.0 ± 0.0b
27	60	30	48	21.3 ± 4.1abcdefg	13.2 ± 6.3cdefg	19.8 ± 11.3hij	0.0 ± 0.0b
Control	120	0	0	16.9 ± 8h	11.2 ± 8.2fg	16.8 ± 11.4j	0.0 ± 0.0b

[1]ID = distance from UV light (cm); IT = UV exposure time (min); IC = incubation time (h)
Means in a column not followed by the same letter is significantly different ($P<0.05$) from each other as determined by Fisher's least significant difference mean separation test.
[2]Controls are untreated raw whole peanuts.

ratings of 20 of 27 treatments at 22.5 to 31.8 were significantly higher than controls. Incubation time was the significant factor affecting the oxidized flavor of UV-treated peanuts with 48 h incubation time resulting in the highest overall mean intensity rating of 27.0 which was significantly higher than the overall mean rating of 22.4 when incubated for 24 h, but not significantly different at 36 h incubation.

Other off-flavors. The intensities of off-flavors ranged from 0 to 0.9 with control rated as 0. No other off-flavors were detected by the descriptive panel in all UV-treated and untreated peanuts except for only one treatment (Trt#3) had an off-flavor described as very low painty flavor with intensity rating of 0.66.

1.5 Descriptive panel intensity ratings for texture properties (Table 4.44)

Crunchiness. The intensities of crunchiness of UV-treated peanuts ranged from 38.4 to 44.3 with control rated as 43.2. Three treatments (Trt# 3, 7, 8) had crunchiness intensity ratings of 38.4 to 40.2 which were significantly lower than controls while the rest of 24 UV treatments had similar crunchiness as controls.

Hardness. The intensities of hardness ranged from 86.9 to 92.7 with control rated as 91.2. Two of 27 UV treatments had intensity ratings of 86.7 and 86.9 (Trt# 6 and 8, respectively) which were significantly less hard than controls. The remaining of 25 of 27 treatments with hardness intensity ratings of 88.0 to 92.7 were not significantly different from controls.

Toothpack. The intensities of toothpack ranged from 57.9 to 61.1 with control rated as 60.6. Except for one treatment (Trt# 3), the rest of 26 of 27 treatments were not significantly different from controls. Trt#3 had intensity rating of 57.9 which was significantly lower than controls and six treated peanuts (Trt# 15, 17, 18, 19, 20 and 21) but not significantly different from the rest of 20 treatments.

Table 4.44 Intensity ratings (mean±standard deviation) for the texture attributes and toothpack of roasted sliced UV-treated peanuts by a descriptive panel.

Trt #	ID	IT	IC	Crunchiness	Hardness	Toothpack
1	20	10	24	44.3 ± 4.8abcd	92.7 ± 3.0a	59.7 ± 2.1abcd
2	20	10	36	41.1 ± 3.3ab	91.0 ± 3.4abcd	58.9 ± 2.8abcd
3	20	10	48	38.4 ± 4.8d	88.7 ± 4.4cdef	57.8 ± 4.4d
4	20	20	24	38.6 ± 3.3abcd	88.0 ± 7.5def	59.3 ± 3.3abcd
5	20	20	36	41.3 ± 6.5abcd	89.7 ± 4.8abcdef	57.9 ± 3.3cd
6	20	20	48	40.2 ± 3.9bcd	88.7 ± 11.8f	57.9 ± 4.6cd
7	20	30	24	43.4 ± 5.1d	90.9 ± 3.3abcd	58.8 ± 1.9abcd
8	20	30	36	40.4 ± 2.9d	86.9 ± 5.7ef	59.3 ± 3.4abcd
9	20	30	48	39.0 ± 5.8abcd	88.0 ± 4.6def	59.3 ± 2.3abcd
10	40	10	24	41.5 ± 4.7a	90.5 ± 4.2abcd	60.3 ± 3.3abcd
11	40	10	36	42.0 ± 3.2abcd	90.9 ± 3.7abcd	59.7 ± 3.6abcd
12	40	10	48	39.9 ± 5.0abcd	88.8 ± 4.5cdef	58.9 ± 4.5abcd
13	40	20	24	41.0 ± 4.1abcd	88.8 ± 4.2cdef	60.1 ± 4.4abcd
14	40	20	36	38.8 ± 3.4abc	88.8 ± 3.4cdef	60.2 ± 3.0abcd
15	40	20	48	42.5 ± 3.6abcd	92.7 ± 5.1a	60.8 ± 5.6ab
16	40	30	24	39.1 ± 2.5cd	89.6 ± 3.2abcdef	58.8 ± 3.0abcd
17	40	30	36	42.3 ± 4.6abcd	90.8 ± 3.8abcd	61.1 ± 2.5ab
18	40	30	48	40.7 ± 3.9bcd	89.1 ± 6.1bcdef	61.0 ± 4.1ab
19	60	10	24	39.5 ± 4.1abcd	88.6 ± 4.0cdef	61.1 ± 3.1a
20	60	10	36	41.5 ± 4.9abc	90.0 ± 4.3abcde	60.8 ± 3.3ab
21	60	10	48	42.1 ± 4.5abcd	92.3 ± 2.9ab	61.0 ± 3.5ab
22	60	20	24	42.4 ± 3.4abcd	91.8 ± 3.1abc	59.8 ± 2.6abcd
23	60	20	36	42.5 ± 2.9abcd	91.7 ± 3.1abc	59.7 ± 2.6abcd
24	60	20	48	40.5 ± 1.9abcd	90.7 ± 3.8abcd	58.6 ± 3.0abcd
25	60	30	24	41.9 ± 2.9abcd	90.2 ± 4.7abcd	59.4 ± 2.8abcd
26	60	30	36	39.7 ± 4.2abcd	90.7 ± 4.3abcd	59.4 ± 4.2abcd
27	60	30	48	43.0 ± 5.1abcd	90.5 ± 4.6abcd	58.3 ± 11.4bcd
Control	120	0	0	43.2 ± 3.7bcd	91.2 ± 2.8abcd	60.6 ± 3.1abc

[1]ID = distance from UV light (cm); IT = UV exposure time (min); IC = incubation time (h)
Means in a column not followed by the same letter is significantly different ($P<0.05$) from each other as determined by Fisher's least significant difference mean separation test.
[2]Controls are untreated raw whole peanuts.

2. Sensory profile of ultrasound-treated sliced peanuts by a descriptive panel

2.1 Descriptive panel intensity ratings for the brown color and roasted peanutty and raw beany flavors (Table 4.45)

Brown color. The intensities of brown color of US-treated peanuts ranged from 26.0 to 43.2 with control rated as 29.8. Eighteen of 27 US-treated peanuts had brown color intensity ratings of 26.9 to 34.1 which were similar to untreated controls. The intensity ratings for brown color of 35.0 to 43.2 in nine treatments (Trt# 4, 5, 7, 19, 22, 23, 25, 26, 27) were significantly higher than that of controls. The brown color intensity of treated peanuts was affected by US power density, exposure time and incubation time. Peanuts treated with US power density of 25 mW/cm^3 had the most intense brown color with overall mean rating of 34.6 which was significantly higher that at 50 mW/cm^3 (mean rating of 29.0) but not significantly different from those at 75 mW/cm^3 (mean rating of 32.4). US exposure time of 8 min produced the highest overall mean brown intensity of 33.3 which was significantly higher than at 2 min with overall mean rating of 30.0 but not significantly different from the overall mean rating of 32.7 when exposed to 5 min. US-treated peanuts incubated for 24 h had the most intense brown color of 34.1 which was significantly higher than at 48 h with overall mean rating of 29.3 but not significantly different from 32.2 rating of 36 h incubation.

Roasted peanutty flavor. The intensities of roasted peanutty flavor of US-treated peanuts ranged from 20.6 to 32.7 with control rated as 3.9. The roasted peanutty flavor of 17 of 27 US treatments ranging from 25.1 to 32.7 were not significantly different from untreated controls with 30.9 rating. Ten of 27 treated peanuts had significantly lower intensity of roasted peanutty flavor of 20.6 to 24.7 compared to untreated controls. Exposing peanuts to US power density of 25 mW/cm^3 resulted in higher roasted peanutty flavor (rating of 27.9) compared to those exposed

Table 4.45 Intensity ratings (mean±standard deviation) for the brown color, roasted peanutty and raw/beany flavor of roasted sliced ultrasound-treated peanuts by a descriptive panel[1].

Trt #	PD	PT	IC	Brown color	Roasted peanutty	Raw/beany
1	25	2	24	26.0 ± 4.0i	24.4 ± 5.3efghi	4.5 ± 6.1ab
2	25	2	36	28.8 ± 3.7hi	24.3 ± 6.1efghi	0.9 ± 3.6cd
3	25	2	48	29.6 ± 3.2ghi	22.7 ± 7.9ghi	0.7 ± 2.8cd
4	25	5	24	43.2 ± 7.9a	25.1 ± 6.0cdefghi	0.0 ± 0.0d
5	25	5	36	36.6 ± 9.3bcd	30.8 ± 10.6abcd	0.3 ± 1.1d
6	25	5	48	27.8 ± 2.7hi	25.4 ± 6.4bcdefghi	2.9 ± 5.2abcd
7	25	8	24	36.4 ± 4.5bcd	31.4 ± 12.3ab	0.0 ± 0.0d
8	25	8	36	28.8 ± 6.6hi	25.1 ± 6.5cdefghi	3.7 ± 5.8abc
9	25	8	48	34.1 ± 5.2def	24.1 ± 8.1efghi	2.5 ± 5.5abcd
10	50	2	24	30.0 ± 5.8fghi	32.7 ± 11.2a	0.0 ± 0.0d
11	50	2	36	33.9 ± 11.7hi	27.1 ± 8.1abcdefgh	4.6 ± 6.5ab
12	50	2	48	28.4 ± 5.1hi	20.7 ± 3.3i	2.1 ± 4.4bcd
13	50	5	24	28.4 ± 4.3hi	26.2 ± 5.0bcdefghi	2.4 ± 5.3bcd
14	50	5	36	26.1 ± 4.4i	23.1 ± 4.4efghi	2.8 ± 6.3abcd
15	50	5	48	26.9 ± 4.3hi	24.7 ± 8.3defghi	2.5 ± 7bcd
16	50	8	24	30.7 ± 1.4efgh	21.8 ± 6.2hi	0.8 ± 3cd
17	50	8	36	28.7 ± 3.4hi	22.4 ± 5.4ghi	0.8 ± 3cd
18	50	8	48	27.5 ± 3.4hi	20.6 ± 2.6i	2.6 ± 5.6abcd
19	75	2	24	35.7 ± 5.6bcd	29.1 ± 15.3abcdef	0.0 ± 0.0d
20	75	2	36	28.4 ± 6.0hi	27.3 ± 9.3abcdefgh	5.8 ± 6.1a
21	75	2	48	29.5 ± 5.0ghi	25.2 ± 6.8bcdefghi	3.7 ± 6.4abc
22	75	5	24	36.3 ± 6.9bcd	25.8 ± 8.1bcdefghi	2.6 ± 5.6abcd
23	75	5	36	39.5 ± 8.4ab	28 ± 9.1abcdefg	0.8 ± 3.2cd
24	75	5	48	29.2 ± 4.9hi	29.4 ± 11.6abcd	2.4 ± 6.2bcd
25	75	8	24	39.9 ± 12.7ab	29.7 ± 14.2abcd	2.1 ± 6bcd
26	75	8	36	38.7 ± 9.1bc	29.7 ± 12.7abcd	0.9 ± 3.6cd
27	75	8	48	35.0 ± 7.9cde	26.5 ± 8.4bcdefghi	2.8 ± 4.9abcd
Control	0	0	0	29.8 ± 5.6fghi	30.9 ± 12.5abc	0.8 ± 3.0cd

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IC = incubation time (h). Means in a column not followed by the same letter is significantly different (P<0.05) from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts

to 50 mW/cm^3 with overall mean ratings of 24.4, but not significantly different from 75 mW/cm^3 with mean rating of 25.9

Raw beany flavor. The intensities of roasted peanutty flavor of US-treated peanuts ranged from 0 to 5.8 with control rated as 0.8. Twenty four of 27 US-treated peanuts had raw beany flavor intensities of 0.7 to 2.9 which were not significantly different from controls with 0.8 intensity rating for raw beany flavor. Three treatments (Trt# 1, 11 & 20) had raw beany flavor intensities of 4.5 to 5.8 which were significantly higher than controls.

2.2 Descriptive panel intensity ratings for basic tastes (Table 4.46)

Bitter taste. The intensities of bitter taste of US-treated peanuts ranged from 19.2 to 22.1 with control rated as 19.3. Twenty of 27 US treatments had bitter intensity ratings of 19.2 to 21.3 which were not significantly different from that of controls with 19.3 intensity rating. The intensity ratings of 21.4 to 22.1 in seven 7 of 27 treatments (Trt# 3, 4, 16, 20, 25, 26, 27) were significantly higher than controls.

Sweet taste. The intensities of sweet taste of US-treated peanuts ranged from 12.5 to 14.5 with control rated as 13.5. All 27 US treatments with sweetness intensity ratings of 12.5 to 14.5 were not significantly different from controls with rating of 13.5. Only one of 27 treatments (Trt#1) with intensity ratings of 14.5 was significantly higher than controls.

Sour taste. The intensities of sour taste of US-treated peanuts ranged from 1.4 to 9.5 with control rated as 3.0. The intensity ratings for sour taste of 1.4 to 6.7 in 25 of 27 treatments were not significantly different from controls. Only two of 27 treatments (Trt# 9, 12) with intensity ratings of 7.1 and 9.5 were significantly higher than controls.

Table 4.46 Intensity ratings (mean±standard deviation) for basic tastes of roasted sliced ultrasound-treated peanutsby a descriptive panel[1].

Trt #	PD	PT	IC	Bitter	Sweet	Sour	Salty
1	25	2	24	20.2 ± 2.6abcdef	14.5 ± 2.4a	4.4 ± 5.9bcde	12.4 ± 1.2ab
2	25	2	36	20.8 ± 3.5abcdef	13.0 ± 1.8abc	2.3 ± 4.8de	12.7 ± 1.4ab
3	25	2	48	21.7 ± 2.6abc	13.7 ± 2.2abc	5.4 ± 8.5bcde	12.6 ± 1.6ab
4	25	5	24	21.4 ± 2.4abcd	12.8 ± 2.5bc	5.3 ± 6.2bcde	12.1 ± 1.0ab
5	25	5	36	20.8 ± 2.7abcdef	13.5 ± 1.2abc	2.3 ± 4.8de	13.1 ± 4.0a
6	25	5	48	20.0 ± 5.6bcdef	13.6 ± 1.9abc	4.2 ± 5.6bcde	12.1 ± 0.6ab
7	25	8	24	20.7 ± 3.1abcdef	13.6 ± 1.9abc	1.4 ± 3.8e	12.3 ± 0.7ab
8	25	8	36	20.3 ± 2.5abcdef	13.7 ± 2.4abc	4.0 ± 5.3bcde	12 ± 1.0ab
9	25	8	48	20.9 ± 3.5abcdef	12.7 ± 1.1bc	7.1 ± 6.9ab	12.5 ± 2.4ab
10	50	2	24	19.5 ± 2.8def	13.2 ± 1.6abc	2.2 ± 4.8de	12.4 ± 2.1ab
11	50	2	36	19.2 ± 2.4f	13.0 ± 2.1abc	3.3 ± 6bcde	12.4 ± 2.2ab
12	50	2	48	20.3 ± 2.8abcdef	12.5 ± 4.1c	9.5 ± 6.8a	12.5 ± 1.8ab
13	50	5	24	20.4 ± 2.9abcdef	13.8 ± 1.9abc	4.5 ± 6.1bcde	12.1 ± 0.6ab
14	50	5	36	19.8 ± 1.8cdef	13.6 ± 2.6abc	6.7 ± 6.1abc	11.8 ± 0.8b
15	50	5	48	21.1 ± 3.0abcdef	13.5 ± 2.5abc	3.2 ± 5.9bcde	12.6 ± 1.0ab
16	50	8	24	22.0 ± 2.0ab	13.1 ± 1.9abc	2.9 ± 5.1cde	12.4 ± 1.8ab
17	50	8	36	21.3 ± 1.9abcde	13.5 ± 2.3abc	6.1 ± 6.4abcd	12.3 ± 1.3ab
18	50	8	48	20.4 ± 1.4abcdef	14.2 ± 2.3ab	4.1 ± 6.6bcde	12.3 ± 0.7ab
19	75	2	24	20.3 ± 2.3abcdef	12.8 ± 1.9bc	6.7 ± 7.2abc	12.4 ± 1.6ab
20	75	2	36	21.7 ± 2.0abc	14.1 ± 2.7ab	2.2 ± 4.6de	12.5 ± 1.7ab
21	75	2	48	21.2 ± 2.3abcdef	13.1 ± 1.6abc	2.2 ± 4.6de	12.5 ± 2.1ab
22	75	5	24	21.1 ± 2.7abcdef	13.2 ± 1.6abc	4.5 ± 6bcde	12.7 ± 1.3ab
23	75	5	36	20.6 ± 2.3abcdef	13.5 ± 1.9abc	2.6 ± 4.8cde	12.4 ± 1.6ab
24	75	5	48	20.7 ± 2.7abcdef	13.2 ± 1.7abc	3.6 ± 5.5bcde	12.5 ± 1.9ab
25	75	8	24	22.2 ± 3.3a	13.3 ± 2.0abc	6.3 ± 7.5abcd	12.5 ± 1.5ab
26	75	8	36	21.8 ± 3.3ab	13.3 ± 2.1abc	2.5 ± 5.6de	12.4 ± 1.4ab
27	75	8	48	21.5 ± 3.1abcd	13.3 ± 2.6abc	3.4 ± 6.3bcde	12.5 ± 1.1ab
Control	0	0	0	19.3 ± 4.5ef	13.5 ± 2.4abc	3.0 ± 5.4cde	12.7 ± 1.8ab

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IC = incubation time (h). Means in a column not followed by the same letter is significantly different (P<0.05) from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts

Salty taste. The intensities of salty taste of 27 US-treated peanuts ranged from 11.8 to 13.1 with control rated as 12.7. All of 27 US treated peanuts were not significantly different from controls suggesting that US treatment had no effect on the salty taste of treated peanuts.

2.3 Descriptive panel intensity ratings for woody/hulls/skins and burnt flavors and feeling factors astringency and tongue sting (Table 4.47)

Woody/hulls/skins. All 27 US treatments had intensity ratings of woody/hull/skins flavor of 16.2 to 18.0 which were not significantly different from those of controls with 16.6 rating. Result indicate that woody/hulls/skin flavor in peanuts was not affected by US treatment.

Burnt flavor. The intensities of burnt flavor of US-treated peanuts ranged from 0 to 8.0 with control rated as 1.0. The burnt flavor intensities of 0 to 3.8 in 21 of 27 US -treated peanuts were not significantly different from controls. The burnt intensity ratings of 4.3 to 8.0 in 6 of 27 other treatments (Trt# 4, 9, 19, 22, 23, 25) were significantly higher than controls. US power density and incubation time significantly affected the intensity ratings of burnt flavor in treated peanuts. The US power density of 25 mW/cm^3 produced the significantly highest burnt flavor with overall mean rating of 3.1 compared to 0.6 when exposed to 50 mW/cm^3 but not significantly different to the rating 2.2 when treated with 75 mW/cm^3. Incubation for 24 h resulted in significantly higher burnt flavor overall mean rating of 3.0 compared to 1.0 when incubated for 48 h but not significantly different to a rating of 1.8 when incubated for 36 h.

Astringency. The intensities of astringent flavor of US-treated peanuts ranged from 18.0 to 19.4 with control rated as 17.2. The intensity ratings of 18.4 to 19.4 for the astringent aftertaste in 26 of 27 US treatments were significantly different from 17.2 intensity rating of untreated controls. One treatment (Trt# 10) with a rating of 18.0 was as astringent as controls.

Table 4.47 Intensity ratings (mean±standard deviation) for woody/hulls/skins, burnt, astringent, and tonguesting of roasted sliced ultrasound-treated peanuts by a descriptive panel[1].

Trt #	PD	PT	IC	Woody/Hull/ Skin	Burnt	Astringent	Tongue sting
1	25	2	24	17.4 ± 2.3ab	0.0 ± 0.0e	19.2 ± 1.2ab	0.7 ± 2.8b
2	25	2	36	16.7 ± 1.9ab	0.0 ± 0.0e	18.5 ± 1abc	1.3 ± 3.5b
3	25	2	48	17.5 ± 2.6ab	0.8 ± 2.8de	19 ± 1.5abc	2.2 ± 4.8ab
4	25	5	24	17.3 ± 2.8ab	8 ± 8.4a	18.8 ± 1.9abc	4.2 ± 6.5ab
5	25	5	36	17.7 ± 2.4ab	3.7 ± 5.5bcd	18.8 ± 1.6abc	2.6 ± 5.7ab
6	25	5	48	16.8 ± 2.4ab	0.0 ± 0.0e	18.8 ± 1.3abc	2.3 ± 4.9ab
7	25	8	24	17.9 ± 3.3ab	1.8 ± 4.4cde	18.4 ± 1.0bc	2.3 ± 4.8ab
8	25	8	36	16.2 ± 2.4b	0.8 ± 3de	19.4 ± 2.0a	0.8 ± 3.2b
9	25	8	48	17.2 ± 2.5ab	4.5 ± 6.3bc	19.1 ± 1.5ab	2.3 ± 4.9ab
10	50	2	24	17.1 ± 2.3ab	0.8 ± 2.8de	18.0 ± 0.9cd	2.1 ± 4.6ab
11	50	2	36	16.8 ± 1.8ab	3.3 ± 5.6bcd	18.8 ± 1.4abc	2.4 ± 5.1ab
12	50	2	48	16.6 ± 1.7ab	0.0 ± 0.0e	19.3 ± 1.7ab	2.3 ± 4.9ab
13	50	5	24	17.5 ± 1.7ab	1.6 ± 4.1cde	18.5 ± 1.6abc	2.3 ± 4.9ab
14	50	5	36	17.5 ± 3.0ab	0.0 ± 0.0e	19.0 ± 1.0abc	2.3 ± 4.9ab
15	50	5	48	17.7 ± 3.1ab	0.0 ± 0.0e	18.6 ± 1.2abc	2.4 ± 5.2ab
16	50	8	24	17.7 ± 1.8ab	0.0 ± 0.0e	18.9 ± 0.9abc	2.6 ± 5.7ab
17	50	8	36	17.7 ± 2.0ab	0.0 ± 0.0e	19.4 ± 1.3ab	4.3 ± 6.2ab
18	50	8	48	17.5 ± 2.7ab	0.0 ± 0.0e	19.2 ± 1.1ab	2.6 ± 5.8ab
19	75	2	24	17.5 ± 1.8ab	4.6 ± 7.7bc	18.6 ± 1.3abc	3.8 ± 5.9ab
20	75	2	36	18.0 ± 1.8a	0.8 ± 3de	19.2 ± 1.6ab	2.8 ± 5ab
21	75	2	48	17.1 ± 2.1ab	0.0 ± 0.0e	18.6 ± 1.6abc	2.2 ± 4.7ab
22	75	5	24	17.2 ± 2.0ab	4.3 ± 7.2bc	18.6 ± 1.6abc	3.9 ± 6ab
23	75	5	36	17.3 ± 1.8ab	4.3 ± 7.0bc	18.6 ± 1.7abc	2.7 ± 6ab
24	75	5	48	16.4 ± 2.9ab	0.9 ± 3.6de	18.8 ± 1.47abc	3.9 ± 6ab
25	75	8	24	18.1 ± 2.0a	6.0 ± 6.4ab	19.3 ± 2ab	5.7 ± 6.9a
26	75	8	36	17.3 ± 4.9ab	3.8 ± 10.4bcd	19.4 ± 1.2ab	3.4 ± 6.5ab
27	75	8	48	17.0 ± 2.2ab	2.9 ± 6.4bcde	19.1 ± 1.7ab	4.3 ± 6.9ab
Control	0	0	0	16.6 ± 2.3ab	1.0 ± 4.0de	17.2 ± 2.3d	1.5 ± 4.0b

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IC = incubation time (h). Means in a column not followed by the same letter is significantly different (P<0.05) from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts

Tongue sting. The intensities of tongue sting of US-treated peanuts ranged from 0.7 to 5.7 with control rated as 1.5. Twenty six of 27 US treatments with intensity ratings for tongue sting ranging from 0.7 to 4.3 were not significantly different from 1.5 rating of untreated controls. The tongue sting intensity rating of 5.7 in one treatment (Trt# 25) was significantly higher than that of controls.

2.4 Descriptive panel intensity ratings for cardboard, fishy, and oxidized flavors (Table 4.48)

Cardboard flavor. The intensities of cardboard flavor of US-treated peanuts ranged from 19.9 to 25.1 with control rated as 18.0. Nine of 27 US treatments (Trt# 5, 7, 13, 19, 20, 23, 24, 27) with intensity ratings for cardboard flavor ranging from 19.8 to 21.5 were not significantly different from controls. The intensity ratings for cardboard flavor of 18 of 27 US-treated peanuts of 21.8 to 25.1 were significantly higher than controls.

Fishy flavor. The intensities of fishy flavor of US-treated peanuts ranged from 11.3 to 21.4 with control rated as 12.8. The intensity ratings of fishy flavor in 22 of 27 US treatments ranging from 11.8 to 17.4 were not significantly different from controls with 12.8 rating. Five of 27 US-treated peanuts (Trt# 3, 16, 17, 25, 27) had intensity ratings of 18.5 to 21.4 which were significantly higher than controls.

Oxidized flavor. The intensities of oxidized flavor of US-treated peanuts ranged from 21.8 to 31.6 whereas controls were rated as 22.4. Eighteen of 27 US treatments had oxidized flavor intensities of 21.8 to 27.2 which were not significantly different from the intensity rating of 12.4 in untreated controls. The intensity ratings of 27.8 to 31.6 in nine of 27 US treatments (Trt# 3, 4, 9, 12, 16, 17, 22, 25, and 27) were significantly higher than controls.

Table 4.48 Intensity ratings (mean±standard deviation) for lipid oxidation related off-flavor of roasted sliced ultrasound-treated peanuts by a descriptive panel[1].

Trt #	PD	PT	IC	Cardboard	Fishy	Oxidized	Off-flavor
1	25	2	24	24.0 ± 4.4abcd	16.5 ± 6.5bcdef	27.2 ± 5abcde	0.0 ± 0.0b
2	25	2	36	22.3 ± 3.4abcdef	14.9 ± 8.5def	26.9 ± 5.7abcde	0.2 ± 0.8b
3	25	2	48	23.4 ± 3.6abcdef	18.6 ± 5.2abcd	31.6 ± 5.4a	0.0 ± 0.0b
4	25	5	24	24.7 ± 2.5ab	12.7 ± 8.8ef	30.0 ± 5.3abc	0.0 ± 0.0b
5	25	5	36	20.7 ± 6.9defg	15.1 ± 6.8cdef	24.1 ± 7.7def	0.0 ± 0.0b
6	25	5	48	21.8 ± 3.7abcdef	11.8 ± 7.11f	24.2 ± 6.3def	0.0 ± 0.0b
7	25	8	24	21.5 ± 6.4abcdefg	13.5 ± 7.6ef	23.7 ± 7.4def	0.0 ± 0.0b
8	25	8	36	21.9 ± 3.8abcdef	13.3 ± 8.7ef	26.9 ± 5.7abcde	0.0 ± 0.0b
9	25	8	48	23.0 ± 3.3abcdef	16.1 ± 4.3cdef	31.1 ± 5.5a	1.6 ± 6.6a
10	50	2	24	19.8 ± 8.5fg	13.4 ± 6.7ef	21.8 ± 8.9ef	0.0 ± 0.0b
11	50	2	36	22.1 ± 7.0abcdef	14.3 ± 6.0def	23.9 ± 8.9def	0.0 ± 0.0b
12	50	2	48	24.8 ± 3.4ab	17.4 ± 5.6abcde	30.3 ± 6.1ab	0.0 ± 0.0b
13	50	5	24	21.5 ± 3.6abcdefg	14.6 ± 7.3def	25.4 ± 6.6cdef	0.0 ± 0.0b
14	50	5	36	23.1 ± 3.9abcdef	16.7 ± 6.4bcde	27.1 ± 3.8abc	0.0 ± 0.0b
15	50	5	48	22.2 ± 6.4abcdef	15.5 ± 8.0cdef	23.8 ± 8.5def	0.0 ± 0.0b
16	50	8	24	25.1 ± 2.8a	20.8 ± 3.8ab	29.6 ± 4.3abc	0.0 ± 0.0b
17	50	8	36	24.5 ± 2.2abc	19.7 ± 3.1abc	30.1 ± 3.0abc	0.0 ± 0.0b
18	50	8	48	23.9 ± 2.2abcde	16.7 ± 7.5abcde	25.8 ± 5.2bcdef	0.0 ± 0.0b
19	75	2	24	20.3 ± 6.9efg	14.8 ± 9.0def	26 ± 8.1bcdef	0.0 ± 0.0b
20	75	2	36	20.0 ± 7.9fg	16.3 ± 7.4bcdef	24.2 ± 7.9def	0.0 ± 0.0b
21	75	2	48	22.5 ± 4.9abcdef	14.8 ± 7.7def	23.6 ± 6.5def	0.0 ± 0.0b
22	75	5	24	23.2 ± 3.4abcdef	15.3 ± 6.0cdef	27.8 ± 5.7def	0.0 ± 0.0b
23	75	5	36	21.5 ± 6.4abcdefg	17 ± 5.6abcde	26.0 ± 8.7bcdef	0.0 ± 0.0b
24	75	5	48	21.2 ± 4.2bcdefg	14.1 ± 7def	24.8 ± 7.4def	0.0 ± 0.0b
25	75	8	24	24.1 ± 3.4abcd	21.4 ± 3.2a	30.9 ± 8.6a	0.0 ± 0.0b
26	75	8	36	22.6 ± 4.6abcdef	15.4 ± 7.7cdef	27.0 ± 8.2abcde	0.0 ± 0.0b
27	75	8	48	20.8 ± 8.1cdefg	18.5 ± 5.4abcd	28.3 ± 4.1abcd	0.0 ± 0.0b
Control	0	0	0	18.0 ± 6.7fg	12.8 ± 8.8ef	22.4 ± 11.6ef	0.0 ± 0.0b

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IC = incubation time (h). Means in a column not followed by the same letter is significantly different (P<0.05) from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts

Other off-flavors. No other off-flavors were detected by the descriptive panel in all US-treated and untreated peanuts except for two treatments, Trt# 2 and 9, where a low painty flavor intensity rating of 0.2 and 1.6, respectively were perceived.

2.5 Descriptive panel intensity ratings for texture properties (Table 4.49)

Crunchiness. The intensities of crunchiness of US-treated peanuts ranged from 33.9 to 42.9 with control rated as 41.6. All 27 US treatments had the same hardness intensity ratings as controls. This finding suggests that US treatment had no effect on crunchiness of treated peanuts.

Hardness. The intensity of hardness of 89.2 to 92.4 in all 27 US treatments was not significantly different from controls with 90.7 rating. This result indicates that US treatment had no effect on the hardness of treated peanuts.

3. Sensory profile of combined US-UV treated peanuts by a descriptive panel

3.1 Descriptive panel intensity ratings for brown color, roasted peanutty and raw beany flavor (Table 4.50)

Brown color. The intensities of brown color in combined US-UV treated peanuts ranged from 34.0 to 34.2 with controls rated as 33.5. Seventeen of 27 combined US-UV treated peanuts had the same intensity of brown color as controls whereas ten treatments had significantly lower brown color intensities than controls.

Roasted peanutty flavor. The intensities of roasted peanutty flavor in combined US-UV treated peanuts ranged from 32.6 to 36.7 with controls rated as 60.2. The intensities of roasted peanutty flavor of all 27 combined US-UV treatments of 32.1 to 36.7 were significantly lower than untreated controls. Results indicate that US and UV treatment reduced the roasted peanutty flavor of treated peanuts.

Table 4.49 Intensity ratings (mean±standard deviation) for texture attributes and toothpack off-flavor of roasted sliced ultrasound-treated peanuts[1].

Trt #	PD	PT	IC	Crunchiness	Hardness	Toothpack
1	25	2	24	41.1 ± 2.4ab	90.8 ± 3abc	59.3 ± 2.6abc
2	25	2	36	41.2 ± 2.6ab	91.7 ± 3.7ab	59.9 ± 1.8abc
3	25	2	48	41.4 ± 3ab	91.3 ± 2.9abc	59.1 ± 2.4abc
4	25	5	24	42.9 ± 3.3ab	92.3 ± 4.2ab	59.7 ± 2.8abc
5	25	5	36	42.8 ± 2.9ab	92.0 ± 3.6ab	59.6 ± 2.7abc
6	25	5	48	40.9 ± 3.7ab	90.3 ± 3.5abc	59.9 ± 1.9abc
7	25	8	24	40.4 ± 2.8ab	90.4 ± 2.5abc	59.2 ± 1.9abc
8	25	8	36	40.9 ± 3.9b	90.2 ± 4.0abc	59.9 ± 2.3abc
9	25	8	48	41.8 ± 4.3ab	90.2 ± 2.9abc	58.6 ± 1.8c
10	50	2	24	41.8 ± 3.1ab	90.7 ± 3.2abc	59.3 ± 3.4abc
11	50	2	36	42.1 ± 2.8ab	91.2 ± 3.3abc	59.8 ± 3.3abc
12	50	2	48	41.5 ± 3.6ab	91.1 ± 3.8abc	58.9 ± 1.7bc
13	50	5	24	42 ± 3.7ab	90.7 ± 3.2abc	59.8 ± 2.5abc
14	50	5	36	41.5 ± 3.4ab	90.3 ± 3.0abc	59.0 ± 2.2abc
15	50	5	48	41.5 ± 3.5ab	90.2 ± 3.0abc	59.6 ± 2.4abc
16	50	8	24	41.9 ± 3.1ab	91.7 ± 2.9ab	59.9 ± 2.7abc
17	50	8	36	41.8 ± 3.3ab	90.1 ± 3.1abc	60.2 ± 1.7ab
18	50	8	48	42.4 ± 3.2ab	90.8 ± 2.1abc	59.3 ± 1.6abc
19	75	2	24	42.8 ± 2.9ab	90.8 ± 1.7abc	59.6 ± 1.9abc
20	75	2	36	42.0 ± 2.5a	91.9 ± 3.2ab	60.2 ± 1.6ab
21	75	2	48	42.3 ± 3.1ab	91.5 ± 3.1abc	59.4 ± 2.0abc
22	75	5	24	42.7 ± 2.5ab	91.1 ± 2.6abc	60.6 ± 2.7a
23	75	5	36	42.5 ± 3.8ab	91.6 ± 3.9ab	59.4 ± 2.1abc
24	75	5	48	40.7 ± 2.5ab	89.2 ± 2.8c	59.5 ± 2.4abc
25	75	8	24	40.4 ± 2.9a	89.4 ± 4.1c	59.7 ± 2.2abc
26	75	8	36	41.3 ± 2.6ab	92.4 ± 3.2a	59.8 ± 1.7abc
27	75	8	48	39.9 ± 4.5ab	91.3 ± 3.2abc	60.0 ± 2.1abc
Control	0	0	0	41.6 ± 4.9ab	90.7 ± 3abc	59.2 ± 3.1abc

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IC = incubation time (h). Means in a column not followed by the same letter is significantly different (P<0.05) from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts

Table 4.50 Intensity ratings (mean±standard deviation) for the brown color, roasted peanutty and raw/beany aromatics of roasted sliced ultrasound-UV treated peanuts by a descriptive panel[1].

Trt #	PD	PT	IT	Brown color	Roasted peanutty	Raw/beany
1	40	4	10	32.7 ± 3.7abcd	33.6 ± 4.7bc	1.9 ± 3.7a
2	40	4	30	32.3 ± 3.2abcd	36.0 ± 9.0bc	0.1 ± 0.4cd
3	40	4	50	31.7 ± 3.3bdc	35.2 ± 7.6bc	1.0 ± 2.7abc
4	40	8	10	31.9 ± 3.5bdc	33.9 ± 7.0bc	0.2 ± 0.5cd
5	40	8	30	31.2 ± 4.3cd	36.3 ± 5.6bc	0.2 ± 0.6cd
6	40	8	50	31.1 ± 4.6cd	34.9 ± 8.0bc	0.9 ± 2.2bcd
7	40	12	10	31.5 ± 4.4bdc	33.2 ± 2.8bc	0.3 ± 0.5cd
8	40	12	30	32.0 ± 3.8abcd	34.5 ± 7.0bc	0.2 ± 0.5cd
9	40	12	50	33.1 ± 2.0abcd	36.3 ± 10.6bc	0.1 ± 0.4cd
10	80	4	10	32.6 ± 2.5abcd	35.3 ± 4.3bc	0.6 ± 2bcd
11	80	4	30	32.3 ± 4.0abcd	36.2 ± 5.9bc	0.6 ± 2.2bcd
12	80	4	50	32.9 ± 3.7abcd	36.3 ± 7.6bc	0.2 ± 0.7cd
13	80	8	10	32.3 ± 3.2abcd	36.4 ± 9.0bc	0.7 ± 2.1bcd
14	80	8	30	33.0 ± 2.4abcd	35.5 ± 6.8bc	0.6 ± 2bcd
15	80	8	50	32.3 ± 3.0abcd	34.9 ± 6.8bc	0.4 ± 0.8cd
16	80	12	10	32.0 ± 3.7bdc	35.6 ± 10bc	0.2 ± 0.5cd
17	80	12	30	33.0 ± 3.0abcd	34.7 ± 8.3bc	0.3 ± 0.6cd
18	80	12	50	31.9 ± 3.0bdc	32.1 ± 5.6c	0.4 ± 0.9bcd
19	120	4	10	32.6 ± 3.7abcd	33.6 ± 6.1bc	0.4 ± 1.1bcd
20	120	4	30	31.0 ± 5.9d	35.7 ± 3.4bc	0.5 ± 1.4bcd
21	120	4	50	32.2 ± 4.6abcd	36 ± 8.1bc	0.3 ± 0.6cd
22	120	8	10	33.2 ± 2.4abc	35.7 ± 4.9bc	0.3 ± 0.6cd
23	120	8	30	32.1 ± 3.7abcd	34.9 ± 8.1bc	0.3 ± 0.9cd
24	120	8	50	32.6 ± 2.5abcd	36.7 ± 7.2b	1.4 ± 3.8ab
25	120	12	10	32.6 ± 2.9abcd	33.4 ± 5.6bc	0.2 ± 0.8cd
26	120	12	30	31.7 ± 2.7bdc	34.4 ± 8.9bc	0.04 ± 0.8d
27	120	12	50	31.8 ± 3.7bdc	36.1 ± 6.3bc	0.1 ± 0.4cd
Control[2]	0	0	0	34.2 ± 3.4a	60.2 ± 4.2a	0.6 ± 2.0bcd

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IT = UV exposure time at fixed distance from UV light 40 cm and incubation time at 25^{o}C for 36 h. Intensity ratings using a 150 mm unstructured scale with anchors at 12.5 and 137.50 mm. Means in a column not followed by the same letter is significantly different from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts

Raw beany flavor. The mean intensities of raw beany flavor in combined US-UV treated peanuts ranged from 0.04 to 1.94 while controls had rating of 0.64. However, the magnitude of intensity ratings in 150-mm scale is too low and of no significant consequence.

3.2 Descriptive panel intensity ratings for basic tastes (Table 4.51)

Bitter taste. The mean intensities of bitter taste in combined US-UV treated peanuts ranged from 24.1 to 27.5 with controls rated as 11.1. All 27 treated peanuts had significantly higher intensities of bitter taste compared to controls. Among treated samples, two treatments (Trt# 1, 27) had significantly higher bitter intensity ratings of 27.4-27.5 compared to four other treatments (Trt# 4, 6, 8, and 22) with ratings of 24.1 to 24.4. This finding indicates that US and UV increase bitterness of treated peanuts which could be attributed to enhanced levels of phenolics in the treated peanuts.

Sweet taste. The intensities of sweet taste in combined US-UV treated peanuts ranged from 9.5 to 12.3 with controls rated as 11.9. The sweetness of 26 of 27 US treatments was not significantly different from controls. Only one treatment (Trt# 12) had significantly lower sweetness intensity compared to controls. Among the treated samples, Trt# 4 had significantly higher sweet intensity compared to three treatments, Trt# 5, 12 and 21, but not significantly different from all 23 other treated peanuts.

Sour taste. The intensities of sour taste in combined US-UV treated peanuts ranged from 4.4 to 7.8 with controls rated as 4.2. The intensity ratings for sour taste 26 of 27 treatments were not significantly different from controls. Only one treatment (Trt# 7) with mean intensity rating of 7.8 had significantly higher intensity of sourness compared to controls and to seven treated peanuts (Trt# 12, 13, 15, 16, 20, 22 and 26).

Table 4.51 Intensity ratings (mean±standard deviation) for the basic tastes of roasted sliced ultrasound-UV treated peanuts[1].

Trt #	PD	PT	IT	Bitter	Sweet	Sour	Salty
1	40	4	10	27.5 ± 3.9a	10.8 ± 3.0abc	6.0 ± 4.1ab	10.3 ± 1.9abcd
2	40	4	30	26.1 ± 5.0abcd	11.1 ± 2.7abc	4.9 ± 4.2b	10.8 ± 1.3abcd
3	40	4	50	26.0 ± 4.0abcd	10.4 ± 4.4abc	5.2 ± 4.7ab	10.2 ± 1.6abcd
4	40	8	10	24.4 ± 4.3cd	12.3 ± 3.4a	5.3 ± 4.3ab	10.7 ± 1.4abcd
5	40	8	30	26.3 ± 4.0abcd	9.8 ± 3.5bc	5.1 ± 4.4ab	11.5 ± 5.5abc
6	40	8	50	24.4 ± 6.0cd	11.4 ± 3.1abc	5.9 ± 4.7ab	10.0 ± 1.9abcd
7	40	12	10	24.6 ± 5.8abcd	10.1 ± 4.5abc	7.8 ± 5.2a	9.9 ± 2.5abcd
8	40	12	30	24.1 ± 6.8d	11.4 ± 3.6abc	5.4 ± 4.5ab	9.0 ± 3.6d
9	40	12	50	26.0 ± 6.1abcd	10.1 ± 3.1abc	6.4 ± 4.4ab	9.8 ± 2.4abcd
10	80	4	10	24.9 ± 6.0abcd	10.7 ± 3.5abc	5.5 ± 4.3ab	9.9 ± 2.9abcd
11	80	4	30	24.9 ± 5.6abcd	10.2 ± 3.3abc	5.2 ± 4.4ab	11.9 ± 9.3ab
12	80	4	50	27.2 ± 4.3abc	9.5 ± 4.8c	4.8 ± 4.4b	9.8 ± 2.0bcd
13	80	8	10	26.3 ± 3.9abcd	10.1 ± 4.4abc	5.0 ± 4.6b	9.4 ± 3.5cd
14	80	8	30	26.2 ± 4.0abcd	11.0 ± 4.7abc	5.5 ± 4.6ab	9.6 ± 3.0cd
15	80	8	50	25.4 ± 4.1abcd	10.4 ± 3.5abc	4.9 ± 4.2b	10.0 ± 1.9abcd
16	80	12	10	24.6 ± 4.7bcd	11.0 ± 3.9abc	4.4 ± 4.1b	9.3 ± 3.8cd
17	80	12	30	26.3 ± 5.3abcd	11.2 ± 4.3abc	5.4 ± 4.6ab	10.6 ± 1.8abcd
18	80	12	50	26.0 ± 4.4abcd	10.0 ± 3.6abc	5.7 ± 4.3ab	10.2 ± 1.9abcd
19	120	4	10	26.8 ± 3.3abcd	10.7 ± 3.3abc	6.2 ± 3.3ab	9.5 ± 3.2cd
20	120	4	30	26.0 ± 4.3abcd	10.6 ± 5.2abc	4.8 ± 4.5b	9.9 ± 3.1abcd
21	120	4	50	26.7 ± 3.8abcd	9.7 ± 3.7bc	6.3 ± 4.4ab	9.8 ± 2.0abcd
22	120	8	10	24.4 ± 4.2cd	10.3 ± 4.9abc	4.8 ± 4.6b	9.9 ± 2.8abcd
23	120	8	30	27.2 ± 3.7abc	11.4 ± 3.1abc	5.4 ± 4.1ab	9.8 ± 2.8abcd
24	120	8	50	25.8 ± 5.3abcd	10.4 ± 3.7abc	5.8 ± 4.4ab	9.5 ± 2.6cd
25	120	12	10	26.2 ± 3.8abcd	10.0 ± 3.4abc	5.8 ± 4.4ab	9.9 ± 2.7abcd
26	120	12	30	25.3 ± 4.1abcd	10.5 ± 3.3abc	4.8 ± 4.4b	9.9 ± 2.8abcd
27	120	12	50	27.4 ± 4.4ab	10.0 ± 4.1abc	6.2 ± 4.1ab	9.9 ± 3.3abcd
Control[2]	0	0	0	11.8 ± 3.7e	11.9 ± 4.6ab	4.2 ± 4.2b	12.1 ± 10.1a

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IT = UV exposure time at fixed distance from UV light 40 cm and incubation time at 25^{o}C for 36 h. Intensity ratings using a 150 mm unstructured scale with anchors at 12.5 and 137.50 mm. Means in a column not followed by the same letter is significantly different from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts

Salty taste. The intensities of salty taste in combined US-UV treated peanuts ranged from 9.1 to 11.9 with controls rated as 12.1. Twenty two of 27 combined US-UV salty taste had mean intensity ratings ranging from 9.8 to 11.9 which were not significantly different from controls with ratings of 12.1.

3.3 Descriptive panel intensity ratings for woody/hulls/skins and burnt flavors and feeling factors astringency and tooth pack (Table 4.52).

Woody/hulls/skins. Twenty six of 27 combined US-UV treatments had woody/hull/skins flavor mean intensity ratings ranging from 9.0 to 10.7 which were not significantly different from those of controls. One treatment (Trt#14) had significantly higher mean rating intensity of 11.4 for woody/hulls/skins flavor compared to controls and Trt# 15.

Burnt flavor. All 27 combined US–UV treated peanuts had burnt mean intensity ratings of 12.1 to 18.1 which were not significantly different from the intensity of burnt flavor in controls. Result indicated that US and UV had no effect on the burnt flavor of treated peanuts.

Astringency. The mean intensity ratings of 20.7 to 23.7 for the astringent aftertaste in 27 combined US-UV treatments were significantly higher than 3.0 intensity rating of untreated controls. The astringency of one treatment (Trt#14) was significantly (P<0.05) higher than 8 treatments (Trt# 4, 5, 16, 18, 20, 20, 24 and 26), but significantly lower than Trt# 12 and similar to all other 17 treated samples. Results suggest that US and UV treatments increased astringency of treated samples which could be attributed to enhanced concentrations of phenolic compounds, known to impart astringent aftertaste, in the treated samples.

Toothpack. All 27 combined US-UV treated peanuts had toothpack mean intensity ratings of 52.0 to 58.5 which were similar (P<0.05) to controls with mean rating of 56.6. This finding

Table 4.52 Intensity ratings (mean±standard deviation) for woody/hulls/skins, burnt, astringent and toothpack of roasted sliced ultrasound-UV treated peanuts by a descriptive panel[1].

Trt #	PD	PT	IT	Woody/Hull/Skin	Burnt	Astringent	Toothpack
1	40	4	10	10.7 ± 3.7ab	15.4 ± 10.6a	22.0 ± 2.1abcd	57.7 ± 6.2abc
2	40	4	30	10.2 ± 2.8ab	16.4 ± 2.8a	20.9 ± 1.8bcd	56.9 ± 6.4abc
3	40	4	50	9.9 ± 3.6ab	14.4 ± 11.5a	22.0 ± 3.1abcd	56.3 ± 6.0abc
4	40	8	10	11.0 ± 3.7ab	15.1 ± 11.1a	21.2 ± 1.8bcd	57.4 ± 6.7abc
5	40	8	30	10.3 ± 3.3ab	12.1 ± 11.4a	21.5 ± 3.9bcd	55.9 ± 6.5abc
6	40	8	50	10.2 ± 2.6ab	12.7 ± 11.5a	22.1 ± 2.9abcd	57.2 ± 4.8abc
7	40	12	10	9.2 ± 3.1ab	15.5 ± 10.5a	22.8 ± 4.0abc	57.8 ± 7.0abc
8	40	12	30	10.0 ± 3.3ab	14.3 ± 11.8a	21.8 ± 2.1abcd	56.4 ± 6.5abc
9	40	12	50	9.9 ± 4.0ab	14.8 ± 10.1a	22.9 ± 5.0ab	55.7 ± 8.2abc
10	80	4	10	10.0 ± 2.6ab	15.6 ± 10.9a	21.6 ± 2.5abcd	57.7 ± 6.8abc
11	80	4	30	10.2 ± 3.5ab	16.9 ± 11.5a	21.7 ± 2.5abcd	56.0 ± 6.9abc
12	80	4	50	9.8 ± 3.4ab	13.9 ± 12.0a	20.7 ± 3.4d	57.6 ± 6.3abc
13	80	8	10	10.7 ± 3.4ab	14.6 ± 12.2a	21.9 ± 2.2abcd	56.4 ± 9.4bc
14	80	8	30	11.4 ± 4.8a	18.1 ± 10.6a	23.7 ± 10.0a	55.2 ± 8.9abc
15	80	8	50	9.1 ± 3.5b	14.0 ± 12.1a	22.6 ± 2.3abcd	55.5 ± 8.2abc
16	80	12	10	9.3 ± 3.1ab	14.6 ± 9.9a	21.3 ± 3.1bcd	57.2 ± 6.9abc
17	80	12	30	10.0 ± 4.3ab	16.8 ± 12.2a	21.9 ± 2.3abcd	56.2 ± 7.6abc
18	80	12	50	9.9 ± 2.0ab	18.0 ± 10.3a	20.9 ± 3.4bcd	52.0 ± 14.2c
19	120	4	10	9.4 ± 4.2ab	14.7 ± 11.7a	20.8 ± 3.6abcd	55.1 ± 9.0abc
20	120	4	30	9.7 ± 4.0ab	15.2 ± 10.7a	21.0 ± 2.4bcd	55.3 ± 9.5abc
21	120	4	50	9.6 ± 4.5ab	15.7 ± 11.6a	22.5 ± 2abcd	56.0 ± 8.1abc
22	120	8	10	9.6 ± 4.5ab	14.3 ± 11.1a	22.2 ± 2.3abcd	57.7 ± 6.5abc
23	120	8	30	10.4 ± 3.0ab	16.7 ± 10.5a	22.3 ± 3.0abcd	55.8 ± 8.7abc
24	120	8	50	9.7 ± 2.5ab	14.5 ± 11.0a	21.6 ± 2.5bcd	57.2 ± 6abc
25	120	12	10	10.5 ± 2.8ab	15.1 ± 11.2a	22.4 ± 2.2abcd	57.5 ± 6.1abc
26	120	12	30	10.0 ± 4.2ab	14.2 ± 10.6a	21.3 ± 2.1bcd	55.6 ± 7.2abc
27	120	12	50	10.4 ± 3.1ab	15.2 ± 10.3a	21.6 ± 2.8abcd	58.5 ± 7.0a
Control[2]	0	0	0	9.0 ± 4.3b	12.0 ± 10.7a	3.0 ± 2.4e	56.6 ± 7.5abc

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IT = UV exposure time at fixed distance from UV light 40 cm and incubation time at 25^{o}C for 36 h. Intensity ratings using a 150 mm unstructured scale with anchors at 12.5 and 137.50 mm. Means in a column not followed by the same letter is significantly different from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts

indicates that toothpack feeling factor in treated peanuts was not affected by US and UV treatments.

3.4 Descriptive panel intensity ratings for cardboard, fishy, oxidized and painty flavors (Table 4.53)

Cardboard flavor. All 27 combined US-UV treatments with mean intensity ratings for cardboard flavor ranging from 14.4 to 19.1 were significantly higher than untreated controls with no detected cardboard flavor. This finding suggests that US and UV treatments caused cardboard flavor which is related to lipid oxidation, in treated peanuts.

Fishy flavor. All 27 combined US-UV treated peanuts had fishy intensity ratings of 0 to 1.27 which were not significantly different from controls where burnt flavor was not detected. However, the magnitude of intensity ratings in 150-mm scale was too low and of no significant consequence.

Oxidized flavor. Nine of 27 combined US-UV treated peanuts with mean intensity ratings ranging from 27.1 to 31.6 had significantly ($P<0.05$) higher oxidized flavor than controls with 22.4 mean rating. All other 18 treatments were not significantly different from controls.

Painty flavor. The painty flavor, the flavor related to advanced oxidation of food was detected in peanuts treated with combined US-UV. However, the magnitude of intensity ratings in 150 mm scale is low ranging from 0 to 1.2 and of no consequence of significance.

3.5 Descriptive panel intensity ratings for texture properties (Table 4.53).

Crispness. All 27 combined US-UV treated peanuts with intensity ratings of 28.4 to 32.1 were as crispy as controls with intensity ratings of 31.4. This finding suggests that any processing treatment used will not affect the intensity of crispnesss of treated peanuts.

Table 4.53 Intensity ratings (mean±standard deviation) for the oxidation related off-flavors of roasted sliced ultrasound-UV treated peanuts by a descriptive panel[1].

Trt #	PD	PT	IT	Carboard	Fishy	Oxidized	Painty
1	40	4	10	19.1 ± 5.7a	1.3 ± 3.1a	24.5 ± 6.1ef	0.3 ± 1.2a
2	40	4	30	16.4 ± 8.0ab	0.7 ± 2.3a	26.9 ± 26.9abcde	0.3 ± 1.2ab
3	40	4	50	16.8 ± 7.0ab	0.9 ± 3.8a	24.8 ± 3.9cdef	0.2 ± 0.7ab
4	40	8	10	17.6 ± 5.9ab	1.0 ± 2.7a	24.8 ± 3.1cdef	0.3 ± 1.2ab
5	40	8	30	17.4 ± 7.9ab	0.0 ± 0.2a	28.8 ± 6.1a	0.4 ± 1.5ab
6	40	8	50	17.7 ± 8.3ab	1.2 ± 3.1a	24.9 ± 8.1cdef	0.5 ± 1.7ab
7	40	12	10	17.4 ± 7.2ab	0.6 ± 2.2a	27.4 ± 6.8abcde	1.2 ± 3.2ab
8	40	12	30	18.8 ± 6.5ab	1.2 ± 3.2a	26.1 ± 4.9abcdef	1.1 ± 2.3ab
9	40	12	50	15.8 ± 9.4ab	1.0 ± 3.1a	28.5 ± 4.1ab	0.6 ± 2.0ab
10	80	4	10	18.3 ± 6.0ab	0.3 ± 1.3a	26.9 ± 8.1abcde	0.5 ± 1.4ab
11	80	4	30	16.9 ± 8.3ab	0.6 ± 2.3a	27.0 ± 5abcde	0.4 ± 1.8ab
12	80	4	50	17.1 ± 7.6ab	0.3 ± 1.0a	26.9 ± 3.7abcde	0.1 ± 0.5b
13	80	8	10	16.2 ± 8.6ab	0.6 ± 2.0a	26.6 ± 4.4abcde	0.8 ± 2.8ab
14	80	8	30	16.5 ± 8.0ab	0.0 ± 0.2a	24.5 ± 4.4ef	0.8 ± 2.7ab
15	80	8	50	15.4 ± 7.7ab	0.2 ± 0.5a	26.2 ± 3abcdef	0.3 ± 0.7ab
16	80	12	10	15.1 ± 8.8ab	1.2 ± 4.1a	24.8 ± 4.2def	0.1 ± 0.2b
17	80	12	30	16.2 ± 8.0ab	0.1 ± 0.2a	24.8 ± 4.6cdef	0.0 ± 0.2b
18	80	12	50	17.0 ± 5.9ab	0.6 ± 1.8a	24.9 ± 3.7cdef	0.4 ± 1.8ab
19	120	4	10	18.4 ± 5.3ab	1.2 ± 3.3a	23.1 ± 4.4f	0.8 ± 2.2ab
20	120	4	30	15.3 ± 8.2ab	0.2 ± 0.7a	25.2 ± 4.3bcdef	0.5 ± 1.2ab
21	120	4	50	14.4 ± 9.3b	0.6 ± 1.8a	27.5 ± 5.8abcde	0.5 ± 1.4ab
22	120	8	10	16.8 ± 7.0ab	0.3 ± 1.0a	26.4 ± 4.8abcdef	0.2 ± 0.3ab
23	120	8	30	16.4 ± 7.5ab	0.04 ± 0.2a	26.1 ± 6abcdef	0.1 ± 0.2b
24	120	8	50	17.3 ± 7.6ab	0.6 ± 1.8a	25.5 ± 5.3bcdef	0.2 ± 0.7ab
25	120	12	10	15.4 ± 8.5ab	0.8 ± 3.2a	28.0 ± 8.6bcd	0.4 ± 1.0ab
26	120	12	30	18.7 ± 4.4ab	1.0 ± 2.8a	24.6 ± 4.1def	1.0 ± 2.7ab
27	120	12	50	15.5 ± 7.7ab	0.6 ± 2.3a	28.4 ± 8.3ab	0.7 ± 2.4ab
Control[2]	0	0	0	0.0 ± 0.0c	0.0 ± 0.0a	0.0 ± 0.0g	0.0 ± 0.0b

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IT = UV exposure time at fixed distance from UV light 40 cm and incubation time at 25^{o}C for 36 h. Intensity ratings using a 150 mm unstructured scale with anchors at 12.5 and 137.50 mm. Means in a column not followed by the same letter is significantly different from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts.

Table 4.54 Intensity ratings (mean±standard deviation) for the texture attributes of roasted sliced ultrasound-UV treated peanuts by a descriptive panel[1].

Trt #	PD	PT	IT	Crispness	Crunchiness	Hardness
1	40	4	10	29.0 ± 2.8ab	38.8 ± 3.18a	37.8 ± 3.0ab
2	40	4	30	29.8 ± 3.3ab	37.9 ± 5.3a	37.0 ± 3.5ab
3	40	4	50	28.6 ± 4.2ab	37.7 ± 5.6a	36.5 ± 4.9ab
4	40	8	10	28.6 ± 2.6ab	39.5 ± 4a	36.6 ± 4.8ab
5	40	8	30	28.4 ± 4.0ab	39.2 ± 5.3a	36.3 ± 5.2ab
6	40	8	50	28.7 ± 2.3ab	39.3 ± 3.2a	38.2 ± 2.2ab
7	40	12	10	27.9 ± 4.1b	37.8 ± 4.1a	36.8 ± 3.4ab
8	40	12	30	28.7 ± 1.9ab	38.8 ± 3.8a	36.4 ± 5.7ab
9	40	12	50	30.1 ± 5.9ab	37.6 ± 7.3a	37.1 ± 5.5ab
10	80	4	10	30.0 ± 6.2ab	39.2 ± 3.0a	38.2 ± 3.2ab
11	80	4	30	29.2 ± 2.3ab	37.8 ± 6.5a	38.0 ± 6.7ab
12	80	4	50	30.0 ± 4.7ab	38.7 ± 3.9a	38.9 ± 8.8ab
13	80	8	10	31.2 ± 9.0ab	38.3 ± 6.9a	39.2 ± 12.3ab
14	80	8	30	29.9 ± 4.0ab	37.9 ± 7.3a	38.9 ± 5.6ab
15	80	8	50	29.7 ± 4.1ab	37.3 ± 7.7a	36.2 ± 7.3ab
16	80	12	10	30.6 ± 7.2ab	37.7 ± 6.8a	38.2 ± 7.3ab
17	80	12	30	31.0 ± 10.7ab	39.6 ± 4.9a	37.6 ± 3.4ab
18	80	12	50	29.1 ± 7.0ab	38.5 ± 5.2a	37.9 ± 9.1ab
19	120	4	10	30.0 ± 2.9ab	37.8 ± 5.6a	39.8 ± 9.0ab
20	120	4	30	30.2 ± 5.5ab	37.6 ± 7.3a	38.0 ± 3.8ab
21	120	4	50	29.8 ± 6.9ab	38.2 ± 5.2a	37.7 ± 7.3ab
22	120	8	10	30.6 ± 7.6ab	37.7 ± 8.1a	37.7 ± 4.4ab
23	120	8	30	32.1 ± 11.5a	38.8 ± 5.5a	39.4 ± 10.3ab
24	120	8	50	28.9 ± 4.1ab	39.0 ± 3.6a	35.8 ± 5.5b
25	120	12	10	30.9 ± 10.2ab	39.6 ± 7.6a	38.8 ± 3.9ab
26	120	12	30	29.5 ± 6.7ab	38.8 ± 2.8a	37.4 ± 5.9ab
27	120	12	50	29.9 ± 4.9ab	39.4 ± 2.4a	39.5 ± 9.6ab
Control[2]	0	0	0	31.4 ± 7.3ab	38.1 ± 5.2a	40.4 ± 9.6a

[1]PD = ultrasound power density (mW/cm^3); PT = ultrasound exposure time (min); IT = UV exposure time at fixed distance from UV light 40 cm and incubation time at 25^{o}C for 36 h. Intensity ratings using a 150 mm unstructured scale with anchors at 12.5 and 137.50 mm. Means in a column not followed by the same letter is significantly different from each other as determined by Fisher's least significant difference mean separation test.

[2]Controls are untreated raw whole peanuts.

Crunchiness. All 27 combined US-UV treated peanuts with intensity ratings of 332.3 to 39.6 were as crunchy as controls, UV-treated and US-treated peanuts with intensity ratings of 38.1, 37.7, and 38.2, respectively. This finding suggests that any processing treatment used will not affect the intensity of crunchiness of treated peanuts.

Hardness. The intensities of hardness in combined US-UV treated peanuts ranged from 35.8 to 39.8 with controls rated as 4.4. Only one combined US-UV treated peanuts (Trt # 24) with intensity rating of 35.8 was significantly less hard than controls and UV-treated peanuts (rating of 40.0) while all other 26 treatments were similar to controls and UV-treated peanuts.

4. Comparison of the sensory profiles of peanuts treated with UV, US, and combined US-UV processing treatments

The comparison of the intensity ratings for the different sensory attributes based on difference between ratings of treated (UV, US or US-UV) peanuts and untreated controls are presented in Table 4.55. The differences in intensity ratings between treated and control peanuts, rather than actual observed ratings, were used to compare the three treatments because treated samples were prepared at different times along with their controls. A positive rating indicates that treated peanuts had higher intensity ratings compared to controls whereas a negative rating means lower intensity. .

The roasted peanutty flavor is the most important sensory attribute of roasted peanuts (Mason et al., 1966). The roasted peanutty flavor of all treated peanuts was consistently lower (Table 4.54), compared to untreated controls. The least difference from control ($P<0.05$) in roasted peanutty flavor intensity of -4.4 was observed in US-treated peanuts, followed by UV treatment at -19.1, and by combined US-UV treatment at -25.1 which was the lowest.

Table 4.55 Comparison of the intensity ratings (difference from control) for the different sensory attributes of resveratrol-enhanced peanuts treated with UV, ultrasound, and combined ultrasound-UV by a descriptive panel[1].

Sensory Attribute	Difference from control ratings for						Pr>F
	UV		Ultrasound		Combined US-UV		
	Difference[2]	Control[3]	Difference	Control	Difference	Control	
Brown	8.3a	32.2	3.1b	29.8	-2.0c	34.7	<0.0001***
Roasted peanutty	-19.1b	49.7	-4.4a	26.5	-25.1c	60.3	<0.0001***
Raw beany	0.8a	0	1.2a	1.5	-0.2b	0.8	<0.0001***
Woody/ hulls/skins	0.7a	17.0	1.2a	17.1	1.1a	9.2	0.1837 NS
Burnt	5.0a	0	1.2c	0	3.1b	12.3	<0.0001***
Bitter	2.9b	18.6	2.8b	19.8	14.0a	12.1	<0.0001***
Sweet	-3.0c	16.4	0.4a	13.6	-1.3b	11.5	<0.0001***
Sour	2.5a	0	1.2b	3.0	1.3b	5.0	0.0004**
Salty	-1.5b	13.2	0.1a	12.6	-2.0b	12.3	<0.0001***
Cardboard	5.8b	20.4	5.5b	20.9	16.8a	0	<0.0001***
Fishy	2.8a	14.6	3.5a	20.9	0.6b	0	<0.0001***
Oxidized	8.4b	19.6	6.1c	24.0	26.1a	0	<0.0001***
Painty	0.1b	0	0.1b	0	0.5a	0	<0.0001***
Crispness	-0.1a	45.7	-0.1a	45.9	-1.6b	46.7	<0.0001***
Crunchiness	-1.8c	45.5	1.6a	41.0	0.4b	37.9	<0.0001***
Hardness	0.5b	90.5	3.3a	90.4	-2.6c	41.1	<0.0001***
Toothpack	0.4b	60.0	2.5a	59.1	-0.3b	56.9	<0.0001***
Astringency	1.2c	18.5	3.0b	17.4	18.8a	3.4	<0.0001***
Overall acceptance[4]	5.7a	7.4	5.1b	7.4	4.9b	7.7	0.0432*

[1] Intensity ratings using a 150 mm unstructured line scale with anchors at 12.5 and 137.50 mm.
[2] Differences in ratings between treated peanuts and untreated controls were used because treated peanuts were prepared at different times. A positive rating means treated peanuts had higher rating than controls. A negative rating means treated peanuts had lower rating than controls. Analyses are based on 54 samples/processing treatment rated by a 10-member descriptive panel. Means of the differences from controls within a row followed the same letter are not significant different from each other as determined by Fisher's least significant difference mean separation test. NS= not significant; * = significant at $P<0.05$; **significant at $P<0.001$; ***significant at $P<0.0001$.
[3] Intensity ratings of controls represent actual observed values for the specified sensory attribute.
[4] Mean overall acceptance by 50 consumers using 9-point hedonic rating scale where 1=dislike extremely; 5= neither like nor dislike; and 9=like extremely.

Astringent aftertaste and bitter taste are sensory attributes related to the phenolic compounds (Naczk and Shahidi, 2006). The astringent aftertaste of all treated samples was consistently higher than controls. The least difference from control of 1.2 in astringency was produced in UV treatment which was significantly lower than 3.0 in US and 17.4 in combined US-UV treated peanuts. The bitter taste of all treated peanuts were higher than controls. The least difference from control of 2.8 and 2.8 was observed in peanuts treated with US and UV, respectively, which were significantly lower than 14.0 in combined UV-US treatment.

Peanuts contain about 50% fat and are prone to lipid oxidation (Grosso and Resurreccion, 2002; Han et al., 2008). Off-flavors related to lipid oxidation such as oxidized, cardboard, fishy, raw beany and painty flavors were consistently higher in treated peanuts compared to controls. The highest ($P< 0.05$) difference from control rating in oxidized flavor of 26.1 was produced when peanuts were treated with combined US-UV, followed by 8.4 when UV treated, and then least at 6.1 when US treated. The highest difference from control in cardboard flavor at 16.8 rating was observed in combined US-UV which was significantly higher than UV and US, both with 5.5 ratings. Fishy flavor intensity rating was highest in both UV and US treated peanuts at 2.8 and 3.5 ratings which were significantly higher than 0.6 in combined US-UV treated peanuts. The difference from control ratings of -2 to 1.2 and 0.1 to 0.5 in raw beany and painty flavor , respectively, showed significant differences ($P<0.05$) among treated samples but were too low a magnitude in a 150 mm scale to establish differences between and among treated and control samples.

The highest difference from control ratings in brown color of 8.3 was observed in UV, followed by US at 3.1, while combined US-UV treated peanuts had less intense brown color compared to control at -2.0. The highest difference from control ratings in burnt flavor of 5.0

was also observed in UV-treated peanuts, followed by combined US-UV at 3.1 and US at 1.2 which was the least.

The difference from control ratings in sweet, sour and salty tastes ranging from -3.0 to 2.5 were too low a magnitude. Likewise, differences from control ratings of -1.8 to 3.3 in texture attributes including crispness, crunchiness, hardness were too low a magnitude to establish significant differences between and among treated and control samples.

In summary, all treated resveratrol-enhanced peanuts had less intense roasted peanutty flavor, more bitter and astringent, and more intense off-flavors such as oxidized and cardboard compared to controls. Among the treatments, the combined US-UV treated peanuts had the least intense roasted peanutty flavor and most intense bitterness, astringency, oxidized and carboard compared to US and UV treatments. As a result, US-UV treated samples had the least acceptance rating by consumers which was not significantly different from the rating of US- but significantly lower than UV-treated peanuts. US-treated peanuts had the highest roasted peanutty flavor, least oxidized flavor, and intermediate astringent flavor compared to UV and combined US-UV treated peanuts. UV-treated peanuts had intermediate intensity of roasted peanutty flavor and the least astringent flavor among the three treatments. However, it must be noted that the sensory ratings analyzed in this study were means of all 27 treatments in 2 replications. Optimization studies conducted as discussed in Study 1, 2 and 3 in of this section showed that US-UV treatment would produce acceptable products with the highest concentrations of resveratrol compared to US and UV.

D. Correlations of Profiles of Phenolic Compounds and Sensory Attributes of Resveratrol-Enhanced Peanuts

Regression models for the response variables, adjusted R^2 and Pearson correlation coefficient for the significant correlation of profiles of phenolic compounds and sensory properties of UV, US, and combined US-UV treated peanuts are shown n Table 4.56. *Trans*-resveratrol was the only phenolic compound found to have correlations ($|r| > 0.50$) with the sensory properties of peanuts treated with UV, US and combined US-UV. *Trans*-resveratrol was positively correlated with astringent, painty and burnt aromatics, and bitter taste suggesting that the higher the concentrations of *trans*-resveratrol, the higher will be the intensity ratings of these sensory properties. These results agreed with Naczk and Shahidi (2006) who reported that phenolics in food contribute to the off-flavors such as bitterness, astringency, color, flavor, and odor which resulted in the lower acceptance of treated peanuts compared to untreated controls.

Table 4.56 Regression models for the response variables, adjusted R^2 and Pearson correlation coefficient (r) for the significant correlation of profiles of phenolic compounds and sensory properties of UV, ultrasound, and combined ultrasound-UV treated peanuts.

Regression model	Adjusted R^2	Pearson correlation coefficient (r)
Resveratrol = -5.96686 + 0.42268 Astringent	0.3295	0.58057
Resveratrol = -6.74404 + 0.40519 Bitter	0.4545	0.63839
Resveratrol = 1.65133 + 3.84983 Painty	0.4008	0.63839
Resveratrol = -7.74613 + 0.37229 Burnt	0.4981	0.7843
Resveratrol = 7.67389 - 0.37229 Woody	0.4961	-0.70838
Resveratrol = 3.96027 -0.16597 Fishy	0.4665	-0.68739
Resveratrol = 12.82090 - 0.89674 Salty	0.4704	-0.69017
Resveratrol =16.22773 -0.05422 Hardness	0.5790	-0.76401

SECTION 5

SUMMARY AND CONCLUSIONS

Peanuts are a rich food source of *trans*-resveratrol next to red wines and grape skins. *Trans*-resveratrol among other phenolic compounds with antioxidant properties delay aging and reduce risk of cancer, cardiovascular, and Alzheimer's diseases. Previous studies reported that single dose of either UV or ultrasound (US) enhanced the levels of *trans*-resveratrol in peanuts.

The present study treated raw peanut kernels with abiotic stresses including wounding through size reduction, and varying doses of UV, US, and combined US-UV to enhance biosynthesis of *trans*-resveratrol and other bioactive phenolic compounds. The optimum parameters for each processing treatment that will produce acceptable resveratrol-enhanced peanuts (REP) with maximum concentrations of *trans*-resveratrol and other bioactive compounds were determined using response surface methodology. Food applications of REP in roasted peanuts and peanut bars were conducted and their shelf lives were determined. The correlations of the profiles of phenolic compounds and descriptive sensory attributes of REP were analyzed.

Raw peanuts had 0.02 μg/g *trans*-resveratrol which increased to 0.37 μg/g after slicing and increased further to 3.3 μg/g after UV treatment. US treatment of sliced, chopped and whole peanuts increased *trans*-resveratrol to 6.35, 2.88 and 0.99 μg/g, respectively, suggesting that only mild damage to the cells through slicing is needed to induce the maximum enhancement of *trans*-resveratrol biosynthesis in peanuts. UV treatment of sliced US-treated peanuts further increased *trans*-resveratrol to 7.14 μg/g which is 2.7 times more than the mean concentration of 2.64 μg/g in red wines, the major food source. These results indicated that US was more

effective than UV. UV treatment of US-treated peanuts provided minimal additional enhancement whereas US treatment of UV-treated peanuts had additive effect in *trans*-resveratrol biosynthesis of sliced peanuts. Viable but not non-viable peanuts significantly increased *trans*-resveratrol in sliced peanuts confirming that *trans*-resveratrol increase in stressed peanuts was due to elicitation of biosynthesis rather than the result of efficient physical extraction of the compound from the treated samples. All UV, US and US-UV treated REP had less roasted peanutty flavor and more bitter, astringent, cardboard, oxidized and fishy off-flavors which resulted in lower consumer mean overall acceptance rating of 5.0 or neither like nor dislike compared to untreated controls of 7.4 or like moderately.

Process optimization showed that the optimum US-UV processes produced the highest *trans*-resveratrol of 4.8 μg/g with the maximum consumer overall acceptance ratings ≤5 or neither like nor dislike, compared to optimum US and UV processes with 3.8 and 2.1μg/g, respectively. The optimum US-UV processes parameters included all process combinations within a hexagon bounded by six points with the combination of US power density and time 74 mW/cm^3 for 8.3 min, 70 mW/cm^3 for 10.9 min, 62 mW/cm^3 for 11.2 min, 42 mW/cm^3 10.4 min, 48 mW/cm^3 for 8.3 min, and 58 mW/cm^3 for 9.1 min, respectively, followed by 50 min exposure at 40 cm distance from UV light, and 36 h incubation at 25^{o}C. The optimum US-UV also produced the maximum concentrations of 170 μg/g *p*-coumaric acid and 150 μM TE/g ORAC total antioxidant capacity which correspond to >100% that found in red wines, as well as 1.0 μg/g *trans*-piceid, 2.6 μg ferulic acid, 1.48 mg GAE/g total phenolics and 1.52 μM TEAC/g.

Roasted REP had a shelf life 52 days at 25^{o}C which was shorter than 90 days in regular roasted peanuts due to weaker peanutty flavor and more intense lipid oxidation-related off-flavors. At least 80% of the initial *trans*-resveratrol and total phenolics but not TEAC were

retained in roasted REP during its shelf life. Application of REP in peanuts bars resulted in increased shelf life up to 146 days at 25^{o}C suggesting the sugar coating could have protected each kernels from exposure to oxygen which slowed down the lipid oxidation or sugar acted to mask the off-flavors in peanut bars. On a per serving basis, about 3.5 REP bars containing 30 g peanuts/bar would provide as much as a 140 mL serving of red wine. REP will provide increased value and profitability for the food industry while providing health benefits to consumers.

Correlation of the profiles of phenolic compounds and descriptive sensory attributes showed that *trans*-resveratrol was the only compound, among five phenolics studied, that was positively correlated to astringent, bitter and painty flavors/tastes.

In futher studies, the application of REP in many other food product preparations and the stability of *trans*-resveratrol and other bioactive compounds with antioxidant activities are worthy of investigation.

SECTION 6

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