

ABSTRACT

MASSIE, BRIANNA JADE. Applications of Peanut Skin Extracts as a Functional Food Ingredient. (Under the direction of Lisa L. Dean.)

Each year 3.6 billion pounds of peanuts are harvested in the United States. Profits for peanut growers and shelling cooperatives, however, have shrunk to a mere 50 cents per shelled pound for the 2013 crop. As only the seed portion of the fruit is consumed, large amounts of low-value waste hulls and skins are produced. Peanut growers are further burdened by the associated costs of disposal of these waste products. The under-utilization of these waste biomasses is a lost economic opportunity to the industry. In particular, discarded peanut skins are a rich source of phenolic compounds which could be utilized to produce value-added products.

Despite being an ideal source of extractable antioxidants, peanut skin phenolic extracts present specific challenges to development as a functional food ingredient. The current study attempted to find solutions for two of these challenges; the possible high levels of arsenic and cadmium concentrated from peanut skins by the extraction process and the negative sensory attributes attributed to the phenolic profile present in peanut skins.

Adsorption technology using waste biomasses was chosen as an economically feasible method for the remediation of heavy metals from peanut skin extract. Two agricultural waste products were chosen; peanut hulls and chitosan cross-linked beads. Peanut skin extract solution was prepared from milled peanut skins extracted with aqueous ethanol (70%). After filtration, the supernatant was tested in batch adsorption systems with and without removal of the ethanol. Isothermal data evaluated using the Langmuir adsorption model determined that peanut hulls were the more efficient adsorbent of heavy metals. Hulls

were then evaluated at a range of pH values to optimize performance and determine adsorption kinetics. Peanut hulls removed 88.6 ± 1.9 % of cadmium from the 10ppm spiked solution within twenty minutes of contact. Adsorption isotherms, however, could not be completely explained using the Langmuir model indicating that sorption resulted from more than one mechanism. Successful removal of cadmium without reduction of the phenolic content of the extracts shows this strategy is feasible for heavy metal remediation of peanut skin extracts rendering them thus to be a viable source of antioxidants in food applications.

Peanut butter and chocolate candy, the leading uses of peanut products were chosen as the matrixes for peanut skin extract fortification. To decrease bitterness and astringency in the added peanut skin extracts, maltodextrin was investigated as an encapsulation agent for spray-drying applications. The effect of varying levels of maltodextrin for encapsulation and inclusion of the resulting spray-dried powder into peanut paste was investigated using response surface methodology. The resulting Trolox equivalence was measured as well as the bitterness and astringency intensities. The response surface returned an optimized formulation providing maximum trolox equivalence and minimal bitterness and astringency with 0.75g of 5.92% maltodextrin-encapsulated peanut skin extract per 100g peanut paste. Tests with the chocolate determined the threshold of inclusion for 10.5 (w/w)% maltodextrin-encapsulated peanut skin extract to be 2.50g/100g. These studies demonstrate peanut skin extracts are a viable functional food ingredient.

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Applications of Peanut Skin Extracts as a Functional Food Ingredient

by
Brianna Jade Massie

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DEDICATION

I would like to dedicate my thesis to my soon-to-be husband, Richard Channing Hess.
Nothing would be possible without your love and support. I love you a latte.

BIOGRAPHY

Brianna Jade Massie was born on August 29th, 1990 and grew up in the Appalachian mountains of Southwest Virginia. She received her Bachelor's of Science in Chemistry from Radford University in 2012. She then entered the Master's program in the department of Food, Bioprocessing, and Nutrition Sciences at North Carolina University under the direction of Dr. Lisa Dean in the USDA-ARS Market Quality and Handling Research Unit. After graduation, Brianna plans to continue her research of adsorption technology.

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CHAPTER 1: LITERATURE REVIEW

1.1 Peanuts

The peanut (*Arachis hypogaea*) originated in South America where it was originally cultivated by the indigenous peoples of the Caribbean islands, Mexico, Brazil, and Peru (Hammons 1982). Commandeered by Spanish colonizers, the peanut was spread to tropical, sub-tropical, and temperate climates throughout the world (Hammons 1982; Stalker 1997). Today peanuts are one of the major food and oil crops in the world with primary production in the United States, China, and India amounting to 35.98 million tons per annum as recorded in 2010-2011 (Francisco and Resurreccion 2008; USDA 2012).

Arachis hypogaea is known by many names: peanut, ground nut, earth nut, pygmy nut, pig nut, among others. Despite its many suggestive names, the peanut is a member of the legume family and is an annual herbaceous plant. The peanut is generally planted in the United States in April to May and the emerging peanut seedling is visible in the first few weeks after germination (Henning 1982; Stalker 1997). The peanut plant is anchored by a primary taproot which is capable of 135cm of growth beneath the soil surface and secondary lateral root growth that is typically 5-35cm in depth. The emerging stem ranges 12-65 cm in length growing in either a vertical or recumbent position (Stalker 1997). Lateral branches may also grow in either position with leaves growing opposite and pinnate with four leaves per branch (Stalker 1997; Ketring 1982). The peanut plant produces flowers 30-40 days after planting in the axils of its leaves, generally in groups of three. The flowers range in color from a light yellow to a deep orange (Stalker 1997). The peanut flower is unique among plant species in that after self-pollination, the flower wilts and the flower stalk elongates and bends

to push the remaining ovary into the soil where it develops into the mature peanut pod 90-140 days after planting (Stalker 1997; Sanders 1982). The peanut pod is elliptical in shape with varying degrees of constriction between the two to five peanut seeds within. The peanut seed is covered by a protective seed coat, or testa, which ranges in colour from off-white to deep purple (Stalker 1997). As the peanut pod matures the interior of the hull darkens. The peanut plant is harvested from August to October when the mesocarp of an estimated 75% of the pods is dark (Young 1982; Henning 1982).



Figure 1. Illustration of a Peanut Plant (Schoepke 2008)

In the United States, harvest is largely mechanized across 1.48 million acres resulting in 3.6 billion pounds of peanuts harvested every year (National Agricultural Statistics Service 2011). Machine-harvesting is carried out using a digger-inverter which extracts the plant from the soil by cutting the taproot and inverting the excised plant to dry in the sun (Stalker 1997). The pods are left in the field to dry to 20-25% moisture content. After field curing, the

peanut pods are then combined and dried in wagons with forced air to a moisture content of 10% (Young 1982).

Within the United States, four commercial market types are produced: Spanish (subsp. *fastigiata* var. *vulgaris*), Runner (subsp. *hypogaea* var. *hypogaea*), Valencia (subsp. *fastigiata* var. *fastigiata*), and Virginia (subsp. *hypogaea* var. *hypogaea*) (Stalker 1997). A particular market type may be preferred for specific applications due to differences in flavor, oil content, size, or shape (Henning 1982; American Peanut Council 2012). Spanish-type peanuts have a high oil content making the market type advantageous as an oilseed crop. Due to this and its bunch-type growth pattern, it is favored in less mechanized regions of the world (American Peanut Council 2012; Stalker 1997). Runners are the dominant market type in the United States, accounting for 80% of total production. Seeds from the Runner-type are typically used for peanut butter and candy. Virginia-type peanuts have the largest seeds and are usually eaten roasted or in-shell. The least commonly grown, the Valencia usually have three or more seeds per pod and are sweet lending themselves to roasting and fresh boiled-peanut preparations (American Peanut Council 2012).

Unless prepared for in-shell consumption, only the seed portion of the peanut pod is utilized and the rest is discarded in processing. Valued at only two dollars a ton, the commercially unimportant hulls are sold as mulch or go to waste (Francisco and Resurreccion 2008). The discarded skins are produced at a rate of 750,000 tons per year and are valued at less than twenty dollars a ton (Sobolev and Cole 2003). The skins have a minor use as cattle feedstock as they contain by weight approximately 12% protein, 16% fat, and 72% carbohydrates (Yu 2006). Inclusion into feedstock, however, is limited to 5-6% of total

composition due to the high content of phenolic compounds which can polymerize with dietary protein making it indigestible to livestock (Hill 2002).

1.2 Antioxidants

1.2.1 Extraction and Yield from Peanut Skins

Phenolic compounds are present in peanut skins in significant amounts; Nepote (2002) reported 140-150mg total phenolics per gram defatted dry skin. Yu (2005) investigated the effect of skin harvesting and solvent extraction on phenolic yield. It was found that roasting increased and blanching significantly lowered yield compared to direct peeling. Ethanol was determined to be the best solvent for extraction. Nepote (2005) went on to determine optimal extraction yield of 118mg g⁻¹ occurred with a 70% (v/v) concentration of ethanol/water solvent in a ratio of 20mL per gram uncrushed peanut skins with a three-stage shaking extraction procedure spanning a total of 30 minutes.

1.2.2 Function within the Plant

Phenolic compounds are particularly abundant in peanut skins as with all plant seed coats due to their protective effects in maintaining the seed's ability to germinate for a long periods of time(Dixon 2005). Phenolic compounds also serve a vast array of other functions within plants; deterrence of herbivores and insects, protection against UV radiation, coloration of plant parts and flowers, antifungal and antibacterial protection (Daniel 1999; Schijlen 2004; Dixon 2005). Phenolic compounds are components of a broad array of plant secondary metabolites synthesized from lipid precursors and aromatic amine, phenylalanine. Phenylalanine is deaminated by the enzyme phenylalanine ammonia-lyase (PAL) to produce cinnamate. By insertion a hydroxyl group into the phenyl ring of cinnamate by the enzyme cinnamate 4-hydroxylase, coumaric acid is formed. Coumaric acid serves as the precursor of

stilbenes and flavonoids (Daniel 1999). This metabolism pathway is referred to as the phenylpropanoid pathway and is illustrated in Figure 2.

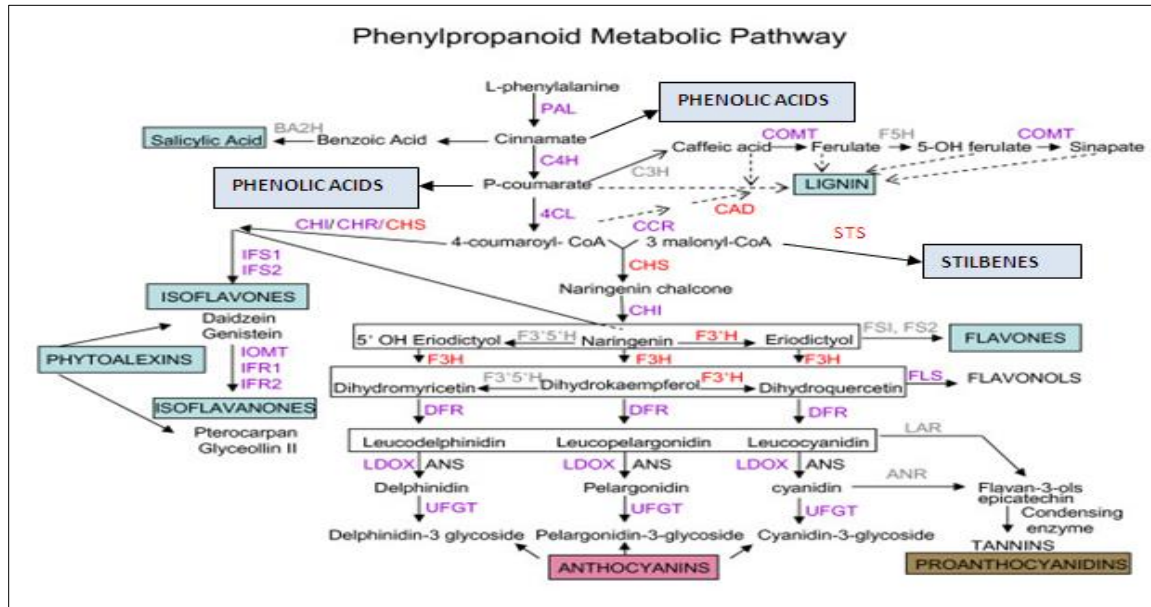


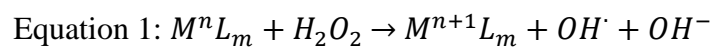
Figure 2. Phenyl propanoid pathway diagram (modified from Zabala 2006; Schijlen 2004).

PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate: CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; IFS, isoflavone synthase; F3'H, flavonoid 3'-hydroxylase; F3', 5'H, flavonoid 3',5'-hydroxylase; F3H, flavanone 3-hydroxylase; DFR, dihydroflavonol-4- reductase; ANS, anthocyanidin synthase also called LDOX, leucoanthocyanidin dioxygenase); UFGT, UDP-flavonoid glucosyltransferase; BA2H, benzoic acid 2-hydroxylase; C3H, p-coumarate 3 hydroxylase; COMT, caffeic O-methyltransferase; F5H, ferulic acid 5-hydroxylase; CCR, cinnamoyl CoA reductase; CAD, cinnamyl alcohol dehydrogenase; STS, stilbene synthase.

1.2.3 Oxidative Pathways

Many protective benefits of phenolic compounds are due to their antioxidant free-radical scavenging activity; donating electrons to unstable molecules which could cause cellular damage within the plant tissue (Rice-Evans 1997; Francisco and Resurreccion 2008). Unstable reactive oxygen species (ROS) produced in the plant tissue during plant metabolism are released as electrons from the mitochondrial electron transport chain or by the enzyme

superoxide-dismutase to oxygen molecules (Turrens 2003; Halliwell 1995). The released electron pairs with one of the two unpaired electrons in the O₂ outer shell creating a more reactive oxygen species, superoxide anion O₂^{-•}. Superoxide anion can then spontaneously or enzymatically be converted to hydrogen peroxide which in turn may be reduced to water or partially reduced to a strongly oxidant hydroxyl radical (Turrens 2003). Formation of the hydroxyl (OH[•]) radical is catalyzed by reduced transition metals, a process known as the Fenton reaction as shown in Equation 1 (Rachmilovich-Calis 2009).



Oxidative species can initiate free radical mediated chain reactions in long chain molecules such as proteins, polysaccharides, and lipids. Free radical chain reaction can be described in three steps; initiation, propagation, and termination. In a long chain molecule such as a lipid, initiation occurs when a hydrogen atom is removed from a carbon atom next to a double bond forming a free radical. Initiation requires an input of energy by heat or light and is catalyzed by the presence of metals. Propagation continues as the lipid radical reacts with a radical oxygen species such as ³O₂ or ¹O₂ forming a lipid peroxy radical. The lipid peroxy radical then abstracts a hydrogen atom from another lipid molecule thereby forming a new free radical. Free radicals will continue to propagate until oxygen is depleted.

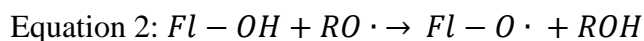
Termination occurs when two free radicals react with one another forming a stable final product such as a polyphenol compound scavenging a free radical (Choe and Min 2006).

1.2.4 Antioxidant Mechanism

The free-radical scavenging ability of a polyphenol compound is related to how easily the compound can transfer a hydrogen atom to the free radical. Hydrogen transfer may

proceed by one of three ways; single-step hydrogen transfer (HAT), single electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET).

As described by Amic (2013), a labile hydrogen atom is donated to a free radical thereby terminating the free radical in the HAT mechanism resulting in the formation a stable delocalized polyphenol radical. The newly formed phenolic radical is capable of quenching additional free radicals in the system. This process must occur more rapidly than the free radical can react to the substrate. In the SET-PT mechanism, a phenolic radical cation is formed first by the transfer of an electron. The formed cation is then deprotonated forming a phenolic radical and a free hydrogen proton which can terminate the long-chain free radical. The SPLET mechanism begins with the deprotonation of the polyphenol molecule forming a phenoxide anion. The hydrogen atom is then available to react with the free radical (Amic 2013). The three described mechanisms are thermodynamically identical and produce identical end products and therefore may be summarized by Equation 2.



The effectiveness of a polyphenol's antioxidant activity can be correlated to measures of how easily the phenolic O-H bond can be broken. Bond dissociation enthalpy of the O-H bond and the half-peak reduction potential of the compound are inversely correlated to the free radical scavenging ability of the polyphenol (Amic 2013; Rice-Evans 1997). By these measures, the phenolic structures with the greatest antioxidant activity can be identified. The ortho-3', 4'-catechol structure in the B ring yields a stable ortho-semiquinone radical through electron destabilization of the B-ring. Additional presence of a free hydroxyl in the 3 position and its corresponding planar structure allows ring conjugation for the formation of a more

stable radical. (Rice-Evans 1997; Hiem 2002). Steric hindrance of the 3', 4'-catechol structure significantly reduces antioxidant capacity of the compound as occurs with the 4'-O-methylation of quercetin to tamarixetin (Heim 2002). A meta-5, 7-dihydroxy in the A ring is shown to increase free-radical scavenging capacity in vitro. Finally, a 2, 3-double bond in conjugation with a 4-carbonyl group in the C ring has been indicated to increase antioxidant capacity (Hiem 2002; Rice-Evans 1997).

1.2.5 Phenolic Compound Classifications

Phenolic compounds found within the peanut skin can be separated into four broad categories; flavonoids, phenolic acids, stilbenes, and a structural phenolic compound known as lignin. Flavonoids are the pigments that color most fruits, flowers, and seeds and serve a myriad of biological functions including attracting pollinators, protection against UV radiation, and male fertility (Ferreira 2012). Flavonoids are polyphenols consisting of three phenolic rings as shown in Figure 3.

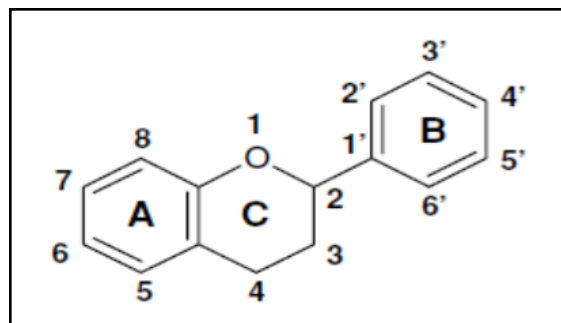


Figure 3. Generic structure of flavonoid (Francisco and Resurreccion 2008).

The large variety of over 6000 identified flavonoids are produced by a variety of enzymes which modify the three-ring chalcone base derived from the phenylpropanoid pathway. These enzymes include isomerases, reductases, hydroxylases, iron (II) and 2-

oxoglutarate dependent dioxygenases, and transferases which add sugars, methyl groups, and acetic acid moieties (Ferreyra 2012). Flavonoids are sorted into classes by the degree of saturation and pattern of substitution on six-member ring C; anthocyanidins, flavonols, flavones, flavanones, flavanols, flavanes, arones, and coumarins (Francisco and Resurreccion 2008). Individual flavonoids are identified by the varying degree of hydroxylation and methoxylation on the A and B rings (Yao 2004). Hydroxylation of the B ring is also primarily responsible for the differing colors of flavonoids (Winkel-Shirley 2001).

The most abundant class of flavonoids identified in peanut skins is the flavanols which includes catechins, epicatechins, galocatechin, epicatechin and their gallic esters (Francisco and Resurreccion 2008; Yu 2005). Oligomeric and polymeric combinations of catechins, proanthocyanidins (condensed tannins) are more prevalent in peanut skins than monomers (Lou 1999, 2001, 2004). Proanthocyanidins composed of only epicatechins, known as procyanidins are the most abundant. Procyanidins contribute to the red color of peanut skins due to their conversion to red-brown pigments when exposed to light and atmospheric conditions. Chukwumah (2009) found a positive correlation between the redness of the peanut skin and concentration of phenolic compounds. Karchesy and Hemingway (1986) determined that peanut skins are 17% by weight procyanidins; existing as both A and B-type procyanidin monomers, dimers, trimers, and tetramers (Lou et al 2004; Lazarus et al. 1999; Yu et al. 2006). Proanthocyanidins are distinguished as A or B type by their linkages between monomers. B-type procyanidin polymers are linked via a single C4→C8 or C4→C6 bond and A-type polymers have an additional linkage of units through a C2→C7 bond. The proanthocyanidins in peanuts are 51-65% A-type (Francisco and Resurreccion 2008).

Flavonoids have been associated with the prevention of age-related diseases such as cardiovascular disease. Their antioxidant effects prevent the oxidation of low-density lipoprotein (LDL), the precursor to the plaque formation of atherosclerosis. Consumption of flavonoids may strengthen blood vessel walls and inhibit platelet aggregation (Yao 2004). Flavonoids are also capable of chelating metal catalysts, activating antioxidant enzymes, reducing α -tocopherol radicals, and inhibiting oxidases to the benefit of human health (Heim 2002).

Phenolic acids exist as two classes; derivatives of benzoic acid and the more prevalent derivatives of cinnamic acid. In total, phenolic acids account for nearly one-third of dietary phenolic compounds (Francisco and Resurreccion 2008). Huang (2003) isolated ethyl protocatechuate, the most potent source of antioxidant activity in peanut skins. Yen (2005) demonstrated ethyl protocatechuate had a 84.6% scavenging rate on α,α -diphenyl- β -picrylhydrazyl (DPPH \bullet) radicals at a concentration of 100mg/L accounting for most of the activity in equivalently concentrated ethanolic peanut skin extract with a scavenging rate of 92.6%. Other phenolic acids identified in peanut skins are ellagic acid, chlorogenic acid, caffeic acid and coumaric acid (Yu 2005).

Stilbenes are formed by linkage of the pre-cursors malonyl CoA and 4-coumaroyl CoA by an ethylene bridge mediated by the enzyme stilbene synthase (Schijlen 2004; Francisco and Resurreccion 2008). Stilbenes are produced in the plant in response to injury and various stressors such as environmental conditions, UV radiation, and fungal infections (Sobolev 2003). The most well studied stilbene is resveratrol, which is present in peanut skins in its trans- conformation. Sanders (2000) reported resveratrol concentrations of 1.6-

3.7 $\mu\text{g/g}$ in a range of peanut cultivars. Peanut seed coats contained high concentrations, approximately 0.65 $\mu\text{g/g}$, but only accounted for a small percentage of overall resveratrol content by weight. Resveratrol has high bioavailability and has demonstrated protective effects against atherosclerosis and cancer cell proliferation (Francisco and Resurreccion 2008; Nepote 2004a, Yu 2005).

1.2.6 Health Benefits of Consumption

Research has suggested that phenolic compounds have beneficial and protective effects in the human body when consumed. Epidemiological studies have shown an inverse relationship to human disease and consumption of a diet rich in polyphenols. The Zutphen Elderly Study showed a correlation between increased consumption of catechins and lower heart disease mortality in a cohort study of eight hundred men in the Netherlands (Hertog 1993). A similar cohort of over thirty-three thousand Swedish women also found an inverse relationship between dietary antioxidant consumption and incident of heart failure (Rautiainen 2013). Polyphenols are consumed in the diet primarily from fruits and beverages such as tea, wine, or coffee. Polyphenols are also consumed from vegetables, dry legumes, cereals, and chocolates (Pandey and Rizvi 2009). Dietary polyphenols most often occur as O-glycosides of glucose or other sugars. Low molecular weight polyphenols are the most easily absorbed by the body while the absorption of larger polymers requires hydrolysis by enteric bacteria before absorption. Disease prevention may be due to the same ROS free-radical scavenging activity and prevention of lipid peroxidation exhibited in plant tissues. ROS have been shown to contribute to cellular aging, carcinogenesis, DNA damage, and oxidation of low-density lipoprotein cholesterol (Heim 2002). Polyphenols may therefore help prevent a

variety of human disease; such as atherosclerosis, cancer, diabetes, neurodegenerative diseases, and help mitigate negative effects of aging (Pandey and Rizvi 2009).

1.2.7 Value to the Food Industry

Antioxidant polyphenols may have economic value in several food industry applications. Antioxidants are added to food products to preserve shelf-life by preventing lipid oxidation, discoloration, and deterioration by autoxidation (Huang 2003; Nepote 2002). Additionally, antioxidant phenolic extracts are mildly antimicrobial (Yu 2010). Presently, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used most commonly to preserve food products. However, because of growing concern over the safety of synthetic antioxidants and consumer backlash against food additives, there is a growing interest in naturally derived antioxidants (Huang 2003; Nepote 2002). Peanut skin extracts have been investigated for this application in ground beef, honey roasted peanuts, potato chips, and cooking oils (Yu 2010; Nepote 2004b; Rehman 2003; Hoang 2008).

Antioxidants are also highly valued for their health benefits in the nutraceutical market. It is projected that the market for nutraceuticals such as added vitamins, minerals, and plant extracts will grow 6.3% in the 2009-2014 market period totaling an \$890 million dollar value in the U.S. and a worldwide value of \$21.8 billion. In a 2006 survey of food companies, 50% responded that antioxidants would play a more important part in their product lines. (O'Donnell 2011). Peanut skin antioxidants could play a part in this growing section of the food market.

1.3 Measuring Antioxidants

The efficiency of phenolic compounds to scavenge free radicals is described as its antioxidant capacity. Antioxidant capacity can be measured in a number of experimental methods such as the total phenolic content assay using Folin-Ciocalteu (FC) reagent, the oxygen radical absorbance capacity (ORAC) assay, the Trolox equivalent antioxidant capacity (TEAC) assay, and the DPPH• (2,2-diphenyl-1-picrylhydrazyl) method.

1.3.1 Folin-Ciocalteu Total Phenol Assay

The Folin-Ciocalteu method is a colorimetric assay based on the oxidation of the sample by the strongly oxidant reagent, a mixture of phosphotungstic and phosphomolybdic acids. The two metals exist in acidic solution as hydrated octahedral complexes coordinated around a central phosphate. The tungstate (VI) species is colorless while the fully oxidized molybdenum compounds give the reagent its yellow color. One to two electron reductions form blue species in both metal complexes (Singleton 1999). The natural range of antioxidants within the sample is compared to a standard of gallic acid or ferullic acid and is measured in equivalents of grams per liter. High sugar content and aromatic amines present in the sample can react with the reagents giving an artificially high value for total phenolic content (Francisco and Resurreccion 2008; Singleton 1999).

1.3.2 Oxygen Radical Absorbance Assay

The oxygen radical absorbance assay (ORAC) measures the oxidative degradation of a fluorescent probe molecule, fluorescein (3',6'-dihydrospiro[isobenzofuran-1[3H],9'[9H]xanthan-3-one) after mixing with a free radical generating azo compound, AAPH (2,2'-azobis(2-amidino-propane) dihydrochloride) (Ou 2001). The reagents are mixed in a 75mM potassium phosphate buffer (pH 7.4) solution and incubated to 37°C for 20

minutes initiating the generation of two free radical molecules from AAPH and the evolution of N_2 gas. The generated free radicals react with atmospheric oxygen gas forming peroxy radicals ($ROO\bullet$). Antioxidants in the sample matrix can quench the propagation of the peroxy radicals via a hydrogen atom transfer (HAT) mechanism to prevent the oxidation of the fluorescent molecule (Ou 2001). This mechanism is shown in Figure 4.

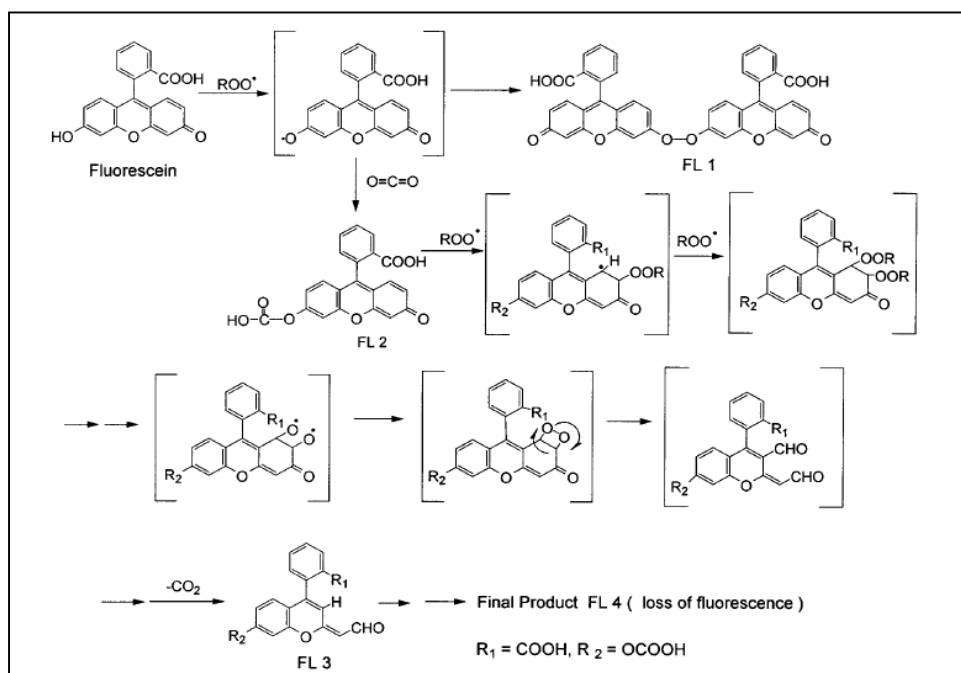


Figure 4. Degradation of Fluorescein via peroxy radicals generated by AAPH (Ou 2001).

Capacity to prevent fluorescence degradation may be measured by percentage and length of inhibition. Trolox, a synthetic water-soluble form of vitamin E, may also be used as a standard (Ou 2001). The ORAC method can be used with hydrophilic or with modification lipophilic antioxidant samples (Francisco and Resurreccion 2008). Ballard (2010) found a maximum ORAC value of 214,900 $\mu\text{Mol Trolox} / 100\text{g}$ for peanut skins using an optimized

micro-wave assisted extraction. Davis (2010) reported that ORAC of raw peanut skins to be 152,290 μ Mol TE / 100 g for peanut skins and increased 38% with roasting for seven minutes.

1.3.3 Trolox Equivalent Antioxidant Capacity Assay

The Trolox equivalent antioxidant capacity (TEAC) assay measures the ability of an antioxidants in a sample to scavenge the radical cation chromophore of 2, 2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS \cdot +) which is generated by oxidizing ABTS with potassium persulfate (Arts 2003). The decolorizing action of antioxidants on the chromophore can be observed spectrophotometrically at the wavelengths 660, 734, and 820nm in the near-infrared region. Results are expressed in comparison to the standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a synthetic antioxidant, in molar units (Francisco and Resurreccion 2008; Arts 2003).

1.3.4 DPPH Method

Similar to the ORAC assay, the DPPH \cdot method measures the ability of antioxidants to transfer a hydrogen atom or electrons to the synthetic paramagnetic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH \cdot) (Brand-Williams 1995). Efficiency of the antioxidant is measured spectrophotometrically as the absorbance of the deep purple DPPH solution at 515nm decreases as it changes color to a light yellow as the radical is quenched (Blois 1958). Brand-Williams (1995) investigated the reaction of DPPH \cdot with various phenolic compounds and found reaction kinetics to vary. Ascorbic acid, isoascorbic acid, and isoeugenol reached equilibrium state with DPPH \cdot in less than one minute. Slower kinetic reactions varied from one to six hours with varying stoichiometric efficiencies. It was hypothesized that different stoichiometric efficiencies of reaction resulted from three possible mechanisms in reducing

one DPPH molecule depending on the structure of the antioxidant. Phenolic compounds possessing two or more hydrogens on the carbon in the para-position of the aromatic ring may donate a second hydrogen following delocalization therefore reducing two DPPH radicals. Dimerization may occur between two phenoxy radicals with a free para- or ortho-position, regenerating two hydroxyl groups available to react with DPPH. Phenols with a free para- or ortho- position may also complex with one DPPH after its first hydrogen transfer and conversion to an aryl radical. Possible reactions are illustrated in Figure 5. Polyphenols were found to have a higher scavenging activity than monophenols.

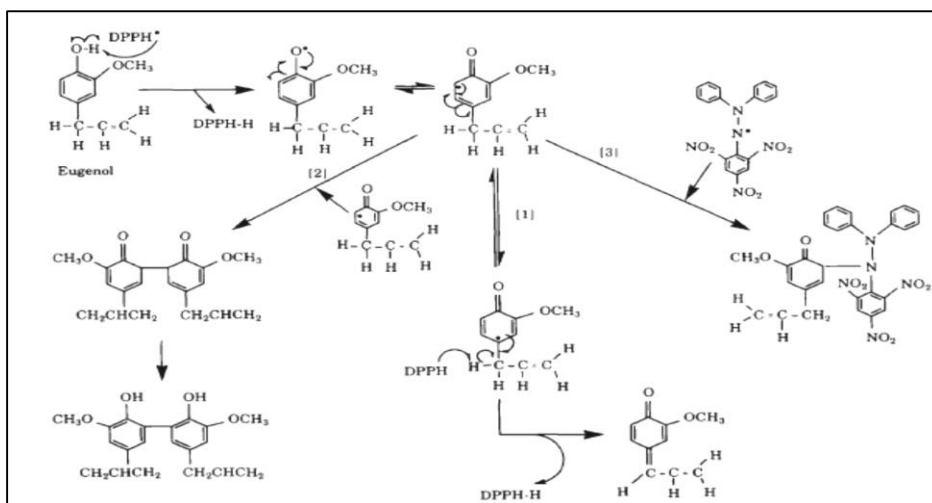


Figure 5. Potential reaction of DPPH with eugenol (Brand-Williams 1995).

Rxn 1: donation of a second hydrogen, Rxn 2: dimerization, Rxn 3: complexation.

1.4 Challenges of Peanut Skin Extract as a Food Ingredient

Despite the high content of antioxidants in peanut skins making it an ideal source of extractable antioxidants, peanut skin phenolic extracts present specific challenges. Given the allergenic nature of peanut protein, it is necessary to ascertain the allergenicity of peanut skin

extract. Constanza (2011) determined the content of allergens in spray-dried peanut skin extract powder and found it was below the limit of detection of the Veratox testing method. Constanza (2011) hypothesized that the majority of allergenic peanut protein remained in the insoluble phase during extraction while the heat of spray-drying denatured any traces of protein in the liquid phase. Despite a low risk of allergenic reaction, peanut skin extract powder should carry an allergen warning as according to Food Allergen Labeling and Consumer Protection Act of 2004 (U.S. FDA 2012). This restricts the practical inclusion of peanut skin extract to only peanut food products only at this time.

Another concern for the use of peanut skin extract is the content of metals concentrated by the extraction process. This is of special concern when using skins from peanuts grown in rotation with cotton crops. Pesticide use on previous cotton crops and use of fertilizer causes the uptake of the heavy metals, arsenic and cadmium in the peanut plant (Armbrust and Bridges 2002; McLaughlin 2000). McLaughlin (2000) determined peanut skins can accumulate cadmium concentrations of 2.92ppm, an order of magnitude higher than concentrations found in the peanut kernel. Constanza (2011) determined the arsenic and cadmium content of peanut skin extract to be 0.088ppm and 0.0056ppm respectively. Arsenic is an extremely toxic and carcinogenic element causing cancer of the lungs, kidneys, and liver (Chen 2006). Chronic exposure causes pigmentation changes and thickening of the skin, neurological disorders, and nausea. Acute arsenic poisoning results in vomiting, abdominal pain, and intestinal bleeding (Rana 2009). Similarly, cadmium also has deleterious effects on human health. Cadmium accumulates in the body from even low amounts of exposure causing irreversible damage to the kidneys, bones, and respiratory organs (Bernard 2008).

The FDA does not have regulations directly regarding allowed heavy metals in foodstuffs. However, both concentrations were above the allowable limits set by the United States Environmental Protection Agency for drinking water (United States Environmental Protection Agency 2011). The European Union allows 1.0ppm cadmium in food supplements as established by the Commission Regulation (EC) No1881/2006 (European Commission 2013).

Peanut skin extract also concentrates pro-oxidant transition metals such as iron and copper (Hathorne 2012). Transition metals catalyze the formation of free radicals by donating and accepting electrons; accelerating hydroperoxide decomposition, creating singlet oxygen and peroxy radicals, and directly reacting with lipids. Catalysis of oxidation can occur in food matrixes at metal concentration as low as 0.1ppm (Nawar 1985).

Another hurdle to using peanut skin extracts as a food ingredient are the negative sensory attributes, bitterness and astringency, attributed to the phenolic profile present in peanut skins. Peanut skins contain a mix of monomers, dimers, trimers, and tetramer procyanidins which account for 17% of the weight of peanut skins (Karchesy and Hemingway 1986; Yu et al. 2006). Monomeric procyanidins are the most intensely bitter while increasingly higher molecular weight procyanidins become less bitter and more predominantly astringent (Peleg 1999). Other low molecular weight phenolic acids such as caffeic acid, chlorogenic acid, and ellagic acid also contribute to bitterness (Yu 2005; Peleg 1999; Hathorn 2012). Concentrating these polyphenols into a final product will result in increased astringency and bitterness in the food matrix. Minimizing these negative sensory

attributes will be a major stumbling block to consumer acceptance of peanut skin fortified products.

1.5 Adsorption Treatment

Removal of toxic and pro-oxidant metals from peanut skin extract is a major obstacle to the utilization in food products. Adsorption is a promising method of removing metal from solution during the extraction process. Various adsorption agents include titanium dioxide, activated alumina, agricultural biomass-based systems, activated carbon, and chitosan (Demirbas 2008; Kratochvil and Volesky 1998). Choice of the most effective agent is dependent on a number of factors; the metal ion of interest, competing chelating agents in solution, and pH.

1.5.1 Polyphenol Metal Interactions

A complicating factor in removal of metal contaminants from peanut skin extracts is that polyphenols are capable of binding metal ions through complexation involving their o-diphenol groups located in the B ring and the ketol structures in the C ring of flavanols (Dixon 2004; Rice-Evans 1997). Quercetin is used as an example in Figure 6 where the three possible binding locations for metal ions are labeled.

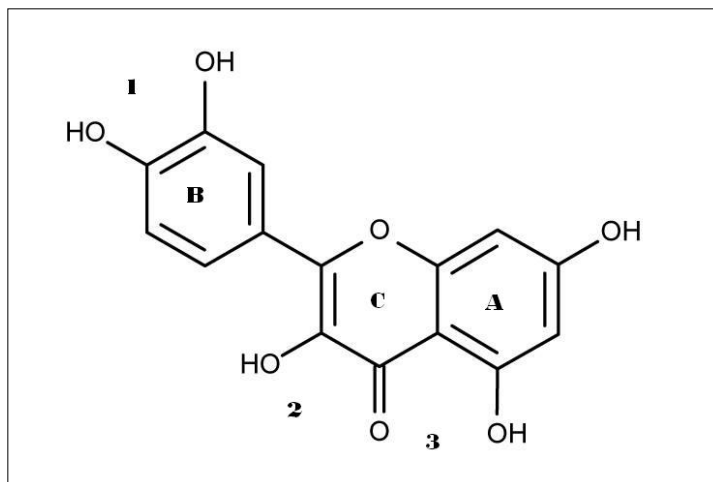


Figure 6. Quercetin metal ion binding sites.

Nearly all polyphenol metal binding involves the bidentate coordination of metal ions with two hydroxyl groups. The hydroxyl groups become deprotonated in basic pH conditions and function as hard Lewis bases. Hard bases favor binding with similarly hard Lewis acids such as arsenic (III), iron (III), or copper (II) and can occur at a pH as low as 5.0 (Hider 2001). At a higher pH range of 9.0-10.0, hydroxyl groups will readily bind to even soft acids such as cadmium (II) or lead (II). Therefore peanut skin extract should be kept at a lower pH to inhibit the binding of metals to the polyphenols and promote the availability of free ions in solution to the adsorbent material.

1.5.2 Speciation of Metals

The species distribution of the metal ions at a mildly acidic pH and reducing environment as encountered in peanut skin extract must also be considered. Figure 7 shows a possible distribution of arsenic species by pH and voltage potential.

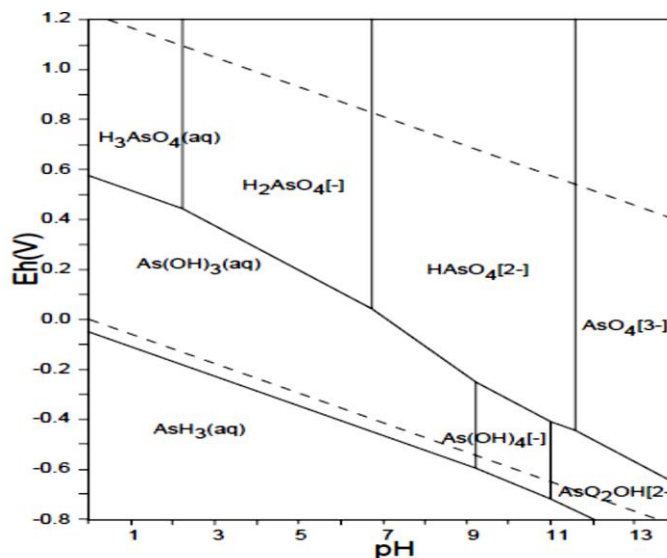
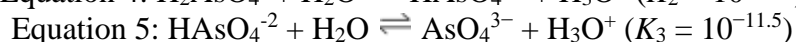
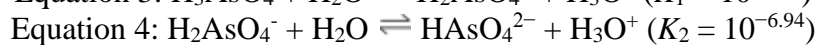
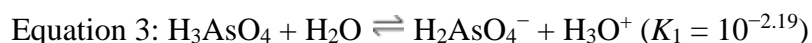


Figure 7. Arsenic Pourbaix diagram (Takeno 2005).

At a pH of 2.0-7.0, it may be possible for arsenic (III) to predominate in the extract as a neutral molecule as As(OH)_3 or HAsO_2 while smaller amounts of arsenic (V) may exist as arsenic acid (Haque 2007; Chen 2006). The equilibrium of arsenic acid is shown in Equations 3-5.



Hovanec (2004) and Armbrust's (2002) speciation of arsenic in peanut products also found organic arsenic present as two herbicides; dimethylarsonic acid ($\text{C}_2\text{H}_7\text{AsO}_2$) and methylarsonic acid (CH_5AsO_3). Other metals of concern such as cadmium, copper, iron, and lead exist primarily as divalent ions in acidic solution. Deviations from divalent charge can occur for iron and copper depending on the electrical potential of the solution as shown in their Pourbaix diagrams in Figure 8 & Figure 9.

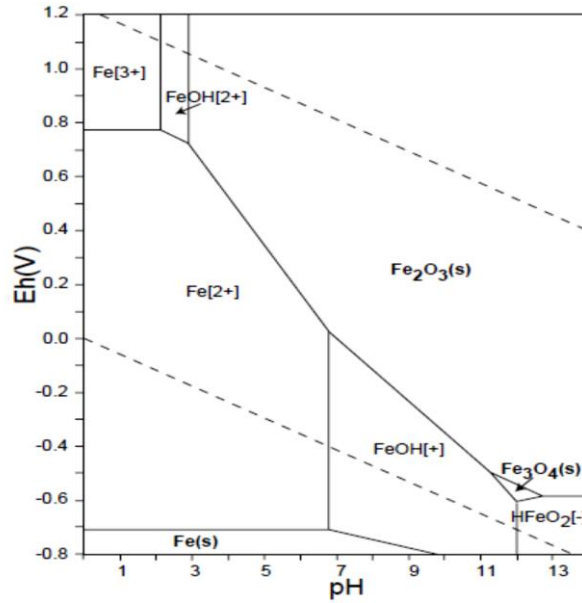


Figure 8. Iron Pourbaix diagram (Takeno 2005).

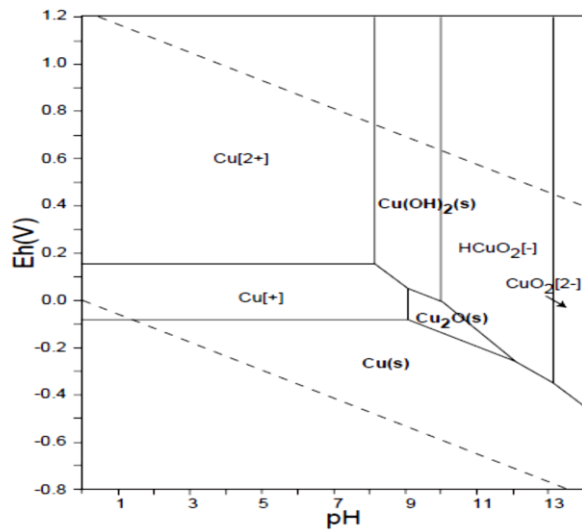


Figure 9. Copper Pourbaix diagram (Takeno 2005).

1.5.3 Chitosan

The most effective agent would be one that would bind cationic metal species in an acidic media. One such possibility is chitosan. Chitosan is a natural biodegradable

polyglucosamine derived from the deacylation of chitin, a structural element in crustacean shells, insect exoskeletons, and fungi (Nghah 2005, 2011). It is most often isolated from crustacean shells which contain 20-30% chitin (Young 1998). Chitosan has been reported to adsorb Cu(II), Hg(II), Zn(II), Cd(II), Cr(II), Fe(II), Fe(III), As(III) and As(V) (Gerente 2007; Nghah 2005; Chen 2006). Chitosan has a high affinity for metal ions due to the presence of amino (-NH₂) and hydroxyl (-OH) groups in its molecular structure. Chitosan amino acid groups become protonated at a pH lower than 6.5 and therefore can bind anionic molecules, such as H₂AsO₄⁻ through electrostatic attraction (Chaves 2009; Janegitz 2010). At very low pH values, however, metal ions must compete with protons for binding sites and increased protonation of amine groups causes repulsion of metal ions (Vold 2003). The ability of chitosan to adsorb metal cations is by the formation of complexes between the metal ion and the amino group whose nitrogen acts as an electron donor as shown in Figure 10 (Monteiro 1999). Chen (2006) proposed the formation of hydrogen bonds or Van der Waals forces were responsible for the adsorption of neutral As(III) molecules onto chitosan. Formation of metallic nodules onto the chitosan surface and absorption into the chitosan polymer structure has also been reported (Rana 2009).

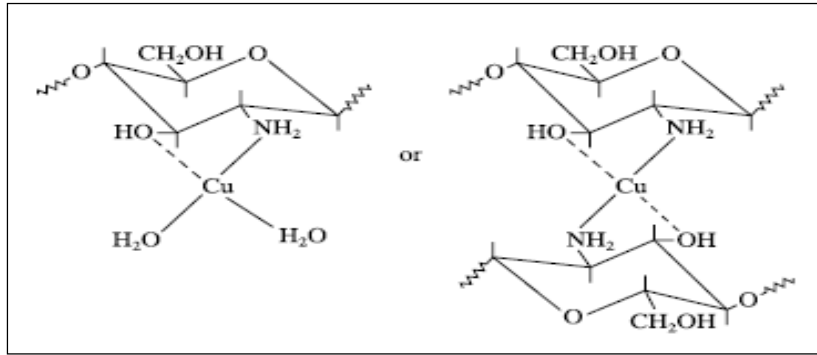


Figure 10. Complex of Copper and Chitosan (Monteiro 1999).

The chitosan adsorption system can be made more efficient by the crosslinking of chitosan to form beads. Chitosan beads exhibit increased porosity, more favorable mechanical properties, and increased stability in low pH solutions (Nghah 2010). Cross-linking of the chitosan can be achieved by a simple neutralization of an acetic acid chitosan solution into a molar equivalent base solution (Chen 2006). Chitosan beads may also be formed with the use of a cross-linking agent. Commonly used cross-linking agents are glutaraldehyde, epichlorohydrine and ethylene glycol diglycidyl ether. Unfortunately, these agents also exhibit toxicity and are not suitable for use in food applications (Nghah 2010). An alternative cross-linking agent is non-toxic sodium tripolyphosphate (STTP), a polyanion which creates ionic crosslinks with chitosan's amine groups. This creates additional hydroxyl groups available for cation adsorption but sacrifices a number of the available amino groups for anionic adsorption. Lee et al. (2001) showed a capacity of 248.5mg Cu (II) per gram of chitosan-STTP bead. Das et al. (2011) found adsorption followed pseudo-second order kinetics in a Langmuir monolayer model. The chitosan-STTP bead adsorbed equal amounts of cations Cr(III) and Fe(III) in a binary solution without lowering the adsorption capacity of

Cr(III) as determined in a single-cation system. This confirms the ability of chitosan to absorb several cations in a system as would be encountered in the peanut skin extract.

1.5.4 Peanut Hulls

Another possible adsorbent material for adsorbing heavy metals from peanut skin extract is peanut hull, an agricultural waste product. Over 1.5 billion pounds of hulls are produced annually in the United States, much of which is burned or buried in landfills (Chamarthy 2001). Peanut hulls composition is roughly 34-45% cellulose, 27-33% lignin, 8-10% moisture, 6-7% protein, 2-4% ash, and 1% fat (Brown 2000). Adsorption of metal ions occurs onto the cellulose, hemicelluloses, lignin, and also the polar functional groups of the hull's polyphenol content (Quiaser 2007; Zhu 2009). In aqueous solutions of pH above 3.5, hull cellulose becomes negatively charged and is able to absorb cationic species through electrostatic attraction (Zhu 2009; Oliveira 2009). As observed by Husaini (2011), the peanut hull acts as an acidic ion exchanger by releasing hydrogen atoms into solution as it binds metal ions causing a net decrease of solution pH. Peanut hulls have been demonstrated to be effective in the treatment of waste water to remove arsenic, cadmium, chromium, copper, lead, nickel, and zinc (Husaini 2011; Johnson 2002; Brown 2000; Chamarthy 2001). Isothermal adsorption data exhibits Langmuir behavior suggesting monolayer adsorption of metal ions (Zhu 2009). Peanut hulls are able to bind various metals with a max loading capacity of 23.7-45g metal ions per kilogram husk (Husaini 2011). Depending on the metal species, max efficiency of adsorption occurs at a pH of 4.5-6.0 (Zhu 2009; Husaini 2011). Adsorption occurs quickly with 90% of all uptake occurring within the first twenty minutes of contact and 98% occurring within two hours (Brown 2000; Zhu 2009). Adsorption follows

a pseudo-second order kinetic model which supports that adsorption occurs through a rate-limiting ion exchange step (Zhu 2009). Brown (2000) also found that adsorption capacity of the peanut hulls was somewhat dependent on particle size with the highest capacity for copper being 12mg/g for 80-mesh particles and the lowest capacity, 5mg/g occurring in below 20 mesh size particles. Heated treatment with acid can be used to increase the adsorption capacity of peanut hulls. Reaction with citric acid or phosphoric acid creates ester linkages to hydroxyls on the cellulose structure, creating additional carboxyl groups that can bind cationic metal ions (Wafwoyo 1999).

1.5.5 Langmuir Adsorption Model

Adsorption from solution can be modeled by the Langmuir isotherm model. The Langmuir model assumes monolayer coverage of the metal ions onto the surface area of the adsorbent and therefore a finite amount of material can be absorbed (Wong 2003). The Langmuir Isotherm model can be expressed by Equation 6:

$$\text{Equation 6: } q_e = \frac{K_L C_e}{1 + a_L C_e}$$

in which q_e is the solid-phase adsorbent concentration, C_e is the solution equilibrium concentration, K_L is the adsorbent material Langmuir isotherm constant ($L g^{-1}$), and a_L is the metal ion Langmuir isotherm constant ($L mol^{-1}$). The maximum solid-phase adsorbent concentration, Q_{max} , is defined by the K_L/a_L . The Langmuir model can also be written in a linear form as:

$$\text{Equation 7: } \frac{C_e}{q_e} = \frac{1}{K_L} + \frac{a_L}{K_L} C_e$$

The Langmuir parameters can be determined from the slope and intercept of the linear regression analysis. The Freundlich model is another commonly used adsorption model

in which adsorption occurs on an “amorphous” surface, and is derived by including an exponential decay energy function into the Langmuir equation to describe reversible adsorption (Wong 2003).

1.6 Quantitation of Metals

Analysis of heavy metal concentration in a matrix is achieved using atomic emission spectrometry using an inductively coupled plasma source (ICP) as described by Skoog 2007. Plasma is a conductive gaseous mixture of cations and electrons and can reach temperatures as high as 10,000K. An ICP source is referred to within the instrument as a torch and is composed of three concentric quartz tubes through which argon gas flows at a rate of 5-20L/min. Wrapped around the top of the outer tube is an induction coil powered by a radio-frequency generator. The induction coil radiates 0.5-2kW of power at 27.12MHz or 40.68MHz and must be cooled by a jacket of water. A tesla coil is used to ionize the flowing argon gas, separating the gas into cations and electrons. The magnetic frequency produced by the induction coil causes the ionized gas to flow within the tubes. The resistance of the ions and electrons to this flow produces ohmic heating of the plasma. Due to the high heat of the plasma, the outer quartz cylinder must be cooled by a separate flow of argon flowing in a spiral upward direction along the outer cylinder walls. This cooling path also centers the plasma within the quartz tubes. The resulting plasma’s intense, brilliant white core extends a few millimeters from the quartz tube in a flame-like tail.

Samples are introduced into the plasma by argon flowing through the central quartz tube. A concentric glass nebulizer is used to aspirate the sample into the plasma stream. The sample can be an aerosol, vapor, or a fine powder. Electrothermal vaporization, which uses

an open electrically heated graphite rod to vaporize samples prior to introduction, may be employed to achieve microsampling capabilities and lower detection limits. Samples are transported through the plasma stream through temperatures up to 8000K in mere milliseconds before they reach the spectral observation point 15-20mm about the induction coil where plasma temperatures are in the range of 6000-6500K. The samples by this point have been atomized into its constituent analytes. The chemically inert argon prevents the formation of oxides which cause chemical and spectral interference.

In the high heat of the plasma, the electrons in the atom's outer shells may be excited to higher orbitals. This excited state is brief and the absorbed energy is released as a photon emission as the atom returns to its ground state. Each element releases radiation in characteristic wavelengths as its electrons return to ground state and resulting radiation can be detected by the spectrometer (Taylor 2001). In a plasma-source AES a sequential spectrometer is most commonly used. Multichannel spectrometers are also available which increases the number of elements which can be quantified at a time but are more expensive and less versatile (Skoog 2007).

A sequential spectrometer measures the incoming radiation of one element's line intensity before moving to the next. This decreases the signal-to-noise ratio thereby increasing sensitivity but also increasing the sample consumption and time required. To separate incoming spectra into individual lines, a grating monochromator is employed. The radiation enters the monochromator through the entrance slit, bouncing off a concave mirror to the holographic reflection grating. Holographic gratings are produced using lasers to etch fine grooves into thin film of photosensitive material which is then coated with a reflective

material such as aluminum. Sequential instruments generally employ gratings with 2400-3600 groves per millimeter. The grating splits the beam of light into many different wavelengths branching out at different angles which is reflected through an exit slit. To reduce scanning time, a slew-scan method is used in which the grating is moved by a two-speed motor. This enables the monochromator to scan very quickly to a wavelength near a selected line of interest, minimizing time spent in regions containing no useful data. Once within the realm of interest, the scan rate is reduced to collect relevant data with a low signal-to-noise ratio. In this manner, a sequential spectrophotometer can measure up to fifteen individual elements in less than five minutes.

As the monochromator scans, wavelengths of radiation are directed into a fourth component, the radiation detector; a photomultiplier tube, which is used for measuring low radiant powers. As one photon of radiation enters the tube, it comes in contact with a cathode, called a dynode, coated in a photo-emissive material which when struck by radiation releases electrons. These electrons will then bounce off a second dynode which in turns emits more electrons. The electrons will bounce through a series of nine dynodes, each 90eV more positive than the last. By the end of the series of dynodes, the initial radiation will have propagated to about 10^6 - 10^7 electrons that will collect at the anode and the resulting current will be measured. In this manner, AES-ICP is able to detect arsenic at levels as low as 0.002ppm and cadmium as low as 7.0×10^{-5} ppm (Skoog 2007).

1.7 Peanut Products & Flavor

As previously discussed, the ingredient usage of peanut skin extract powder is limited to the addition to peanut products. Nearly all peanut products are roasted before

consumption. The roasting process is carried out in two ways; dry and oil roasting. Dry roasting heats raw peanuts in ovens heated to approximately 160°C for 40-60 minutes (Woodruff 1983). After roasting, the peanuts are cooled with circulating air to stop the roasting process and the skins and other foreign material is then removed by blanching. Blanching is the first step in oil roasting. The cleaned peanut kernels are submerged for 3-10 minutes in hot coconut, peanut, or cottonseed oil heated to 138-143°C. Similarly, the hot peanuts are then cooled to stop the cooking process (Woodruff 1983). During roasting, free amino acids and sugars in the peanut kernel undergo Maillard browning-type reactions to create pyrazine and carbonyl compounds, some of the primary compounds responsible for the unique flavor of roasted peanuts (Basha 1996). Specifically aspartic acid, asparagines, glutamine, histidine, and phenylalanine are responsible for pleasing peanut flavor while lysine, threonine, tyrosine, and an unidentified amino acid create less pleasing flavors (Newell et al. 1967).

Peanut flavors, desirable and undesirable, are described by a lexicon initially developed by Johnsen (1988) and expanded by Sanders (1989). The overall sense of flavor of a food is the combined sum of aromatic olfactory perceptions, perceptions of the basic tastes, and chemical feeling factors (Meilgaard, Civille, Carr, 1999) and therefore the peanut flavor lexicon is divided by those three categories. The aromatic category includes descriptors; roasted peanut, raw/beany, dark roast, sweet aromatic, and woody/hulls/skins. Included in the aromatic category are off-flavors; phenol, cardboard, burnt, musty, green, painty and fruity/fermented added by Sanders (1989). Basic tastes include sweet, sour, salt, and bitter and chemical feeling factors are astringent and metallic. Astringency is caused by

compounds such as polyphenols and aluminum salts binding and precipitating proteins in the tissues of the oral cavity resulting in a sensation of dryness or “puckering” (Bajec 2008). The metallic feeling factor is the result of the taste of metal ions in the oral cavity and olfactory perception of carbonyl compounds produced by lipid oxidation in the mouth by metal ions (Omar-Ozbek 2012).

Peanut flavors are often evaluated using the Spectrum® method in which trained taste panelists assign each flavor attribute an intensity using a universal fifteen-point intensity scale (Drake and Civille, 2003). The Spectrum® method uses reference points to establish a standardized intensity scale for example; the baking soda taste in a saltine cracker is 1, the cooked apple flavor in applesauce is 5, the orange flavor in orange juice is 7, and the cooked grape flavor of grape juice is classified as a 10. The roasted peanut flavor in commercial peanut butter is generally rated as a 7 (Meilgaard, Civille, Carr 1999).

National brands of peanut butter are rated fairly low (1-3) on the intensity scale for bitterness and astringency (Meilgaard, Civille, and Carr, 1999). Francisco and Resurreccion (2012) evaluated peanut skins for use as a tea-like beverage. Ten trained panelists developed a lexicon and described peanut skin tea infusions of three market varieties; Runner, Spanish, and Virginia peanut skins. Using a 150mm continuous scale, panelists described the infusions to have a woody/hulls/skins flavor ranging in intensity from 38.2 to 44.9 for all market types. Astringency was also comparable for all market varieties ranging from 79.6 to 89.5. Bitterness intensity ranked as follows for market varieties; Runner (108.6), Spanish (106.6), and Virginia (100.9). Hathorne (2012) fortified peanut paste with whole ground peanut skins in concentrations varying from 0-20% (w/w). Astringency and bitterness were found to be

2.9 and 2.8 respectively in unfortified peanut paste as rated on the Spectrum® scale. Scores were unaffected until more than 1% (w/w) peanut skins were added. At 10-15% bitterness and astringency ranked 4.3 & 4.4 and rose to 5.3 for both scores at a 20% peanut skin fortification level. Mitigating the bitterness and astringency of the polyphenols extract will be vital to consumer acceptance.

1.8 Minimizing Negative Sensory Attributes

Taste-masking is the act of downplaying the perception of an undesired taste in a food formulation. Taste-masking is described as occurring in the mouth or on a cognitive level in the brain as occurs with mixture suppression. An example of mixture suppression is adding sugar to a bitter tea. The sugar does not inhibit the interaction of bitter compounds to taste receptors but the taste of bitterness is suppressed as it is processed cognitively by the brain (Galindo-Cuspinera, 2011). The taste of bitterness can also be suppressed 5% up to 80% by the addition of sodium chloride depending on the bitter compound (Breslin 1996). Similar effects on bitterness by zinc salts, especially ZnSO₄, have been observed. The usefulness of zinc salts as a food additive for taste-masking purposes is tempered by the astringency of zinc salts and their ability to suppress the taste of sweet and thereby enhance other undesirable tastes (Keast 2006).

Taste inhibition occurring in the mouth is based on the physiological process of taste reception (Galindo-Cuspinera, 2011). Flavor molecules dissolved in the aqueous food matrix or saliva stimulate taste receptors “buds” in the oral cavity. Taste receptors respond to the basic tastes. Taste inhibition depends on the physical removal of taste molecules from the

taste receptors so that stimulation cannot occur. This is primarily achieved in the food product industry by microencapsulation.

1.8.1 Microencapsulation

Microencapsulation is the process of enclosing a core particle or liquid droplet within a thin outer coating of wall material (Gowthamarajan, 2004). Particles and droplets can be enclosed as singular capsules or aggregates of cores within a matrix of wall material (Fang and Bhandari, 2010). Suitable wall materials include gelatin, chitosan, waxes, alginates, sugars, gums, and other food-grade ingredients (Gowthamarajan 2004; Fang and Bhandari 2010). Benefits of encapsulation are protection and control over the release of core materials under different environmental conditions. For the purpose of taste-masking, it prevents the bitter core material from dissolving in the saliva to stimulate the taste receptors. Polyphenols especially benefit from microencapsulation technologies to protect from light, heat, oxygen encountered during processing and lessening the bitterness and astringency of concentrated polyphenols (Fang and Bhandari 2010).

There are many different methods to achieve microencapsulation applicable to polyphenols; spray-drying, coacervation, liposomes, inclusion, cocrystallization, nanoparticles, freeze-drying, yeast encapsulation, and emulsion encapsulation (Fang and Bhandari 2010). Of the many, spray-drying is the most promising for commercial scalability. Spray-drying is economical and its applications for microencapsulation have been used by the food industry since the 1950s. The wall material is hydrated and homogenized with the core material of polyphenols (Fang and Bhandari 2010). The fluid mixture is atomized through a small nozzle into a flow of air or inert nitrogen gas. The fine distribution of droplets in the

heated drying chamber quickly evaporates off the aqueous solvent. Due to the rapid evaporative process, the temperature of the particles remains low protecting the core material from damage (Murugesan 2012). The dried particles are then collected by suction into a cyclone where then the powder settles in a collection chamber (Moller and Fredsted 2009). The resulting particle morphology can be controlled by adjusting feed flow rate, spray dryer inlet and outlet temperatures, pressure and type of atomizer (Murugesan 2012). Problems may arise when the spray-drier temperatures exceed the wall material's glass transition temperature resulting in a soft and sticky product which may stick in the chamber causing low recovery.

1.8.2 Maltodextrin as an Encapsulation Agent

Maltodextrin is often used in spraydrying both as an encapsulating agent to improve stability and as a drying aid. It has been used with good success in improving the physical properties of spray-dried polyphenols (Fang and Bhadari 2010). Bakowska-Barczak and Kolodziejczyk (2011) spray-dried black currant ethanolic extract with maltodextrins of differing dextrose equivalences (DE). They found that the resulting powders were stable for at least nine months of storage with those encapsulated with lower DE values being the most stable. Saenz et al. (2009) used maltodextrin to encapsulate cactus pear ethanolic extract and achieved an above 100% recovery of polyphenols with a 3:1 core to wall ratio. Saenz (2009) theorized the increase in phenolic yield due to the hydrolysis of polyphenol conjugates during the spray-drying process. This is in agreement with the findings of Constanza (2011) who observed the depolymerization of peanut skin polyphenols during spray-drying. Zhang (2007) utilized a blend of maltodextrin and gum arabic to encapsulation procyanidins

extracted from grape seeds. With a 60% maltodextrin and gum arabic wall material and core to wall ratio of 30:70, Zhang (2007) was able to achieve an encapsulation efficiency of 99.2% and a phenolic recovery of 89.2%. Maltodextrin has not yet been extensively investigated for decreasing the bitterness and astringency of phenols but has been used with success to decrease the bitterness of milk protein hydrolysates. In the cited studies, the decrease in bitterness was attributed to the bitter hydrophobic moieties of the hydrolysates being less physically exposed within the core-wall matrix (Rocha 2009; Yang 2012). A similar process may occur in the encapsulation of polyphenols.

1.9 Ingredient Applications

The leading use of peanuts in the U.S. is as peanut butter (45%), followed by snack nuts (35%) and in candies and confections (25%). Half of the ten top selling candy bars in the U.S. contain peanuts (American Peanut Council 2012). For this reason, peanut butter and chocolate were chosen to be the matrices for peanut skin powder fortification. Antioxidant-enriched peanut butter is a novel concept, although, products such as Planters Nut-rition[®] (Kraft, Northfield, IL, USA) show consumer interest in the health benefits of peanut products. The market for polyphenol-rich chocolate, however, has already been bridged due to its natural high polyphenol content. Consumer demand is evident by the rising popularity of products such as CocoaVia[®] and branding of other chocolate products as a good source of antioxidants.

As an example Ares (2010) conducted a survey of 75 Uruguayan consumers and their pretrial expectations of chocolate milk desserts enriched with antioxidants. Ares (2010) then measured overall liking of milk desserts enriched with different concentrations of a

polyphenol extract of a native South American plant. Through word association exercises, consumers were divided into three clusters; 1) those concerned with hedonic sensory aspects, 2) those concerned with health but expected off-flavors, 3) and those who wished to find a healthy dessert they enjoyed and were concerned with health and sensory aspects. Overall liking of the desserts rose among the groups in that order as did willingness to purchase. Across all groups, overall liking dropped when the polyphenol concentration was greater than 0.4g/L though group 2 and 3 was significantly more tolerant than group 1. Assuming Ares (2010) results carry across nationalities, it shows a target pool of consumers who are interested and willing to purchase in polyphenol extract-enriched foods for perceived health benefits.

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CHAPTER TWO: REMOVAL OF HEAVY METAL CONTAMINATION FROM PEANUT SKIN EXTRACTS BY WASTE BIOMASS ADSORBENTS

2.1 Abstract

Each year 3.6 billion pounds of peanuts are harvested in the United States. Subsequent processing, however, generates large amounts of waste biomass as only the seed portion of the fruit is consumed. The under-utilization of waste biomass is a lost economic opportunity to the industry. In particular, discarded peanut skins are a rich source of phenolic antioxidant compounds which could be extracted to produce value-added products. However, certain obstacles prevent the utilization of some peanut skins such as the heavy metal content. This study attempted to develop an economically feasible method for the remediation of peanut skin extract using adsorption technology. Milled peanut skins from a commercial blanching operation were extracted with aqueous ethanol (70%). After filtration, the supernatant was tested with and without removal of the ethanol. Each matrix was spiked to a 10ppm concentration of arsenic and cadmium. Two agricultural waste products were chosen as adsorbent materials; peanut hulls and chitosan cross-linked beads. Batch adsorption experiments were used to determine the effect of adsorbent dosage by varying the amount of chitosan beads from 0.050 to 1.50g and the amount of peanut hull from 0.050 to 1.00 g. Isothermal data was evaluated using the Langmuir adsorption model to determine the best adsorbent material. The most efficient material was then evaluated under a range of pH values to optimize performance and determine adsorption kinetics. The Langmuir adsorption model determined peanut hulls were the more effective material. The optimized pH was found to be between pH 3-4. Peanut hulls removed 88.6 ± 1.9 % of added Cd within 20 min

of contact. Apparent removal of arsenic ($21.7 \pm 9.5\%$) showed no correlation to adsorbent dosage. Adsorption isotherms could not be completely explained using the Langmuir model indicating sorption resulted from more than one mechanism. Successful removal of Cd without reduction of the phenolic content of the extracts shows this strategy is feasible for heavy metal remediation of peanut skin extracts making them a viable source of antioxidants in food applications.

2.2 Introduction

Each year 3.6 billion pounds of peanuts are harvested in the United States (National Agricultural Statistics Board 2011). Only the seed portion of the peanut pod is consumed and consequent processing generates large amounts of waste biomass; approximately of 1.3 billion pounds hulls and 101 million pounds of skins (Brown 2000, Sobolev and Cole 2003). Valued at only two dollars a ton, the commercially unimportant hulls are sold as mulch or discarded (Francisco and Resurreccion 2008). The skins are valued at less than twenty dollars a ton (Sobolev and Cole 2003) as a minor component of cattle feedstock (Yu 2006). The under-utilization of waste biomass is a lost economic opportunity to the industry. In particular, discarded peanut skins are a rich source of phenolic antioxidant compounds which could be extracted to produce value-added products enriched in antioxidants (Hathorne and Sanders 2012).

Supported by a broad base of epidemiological studies such as the Zutphen Elderly Study, antioxidant compounds have been hailed as a silver bullet against human disease in popular media (Hertog 1993). This has resulted in an increased value in marketing for the food industry. Functional foods such as those with added antioxidants had a market value of

\$49 billion in 2011 and are projected to be worth \$67 billion by 2016. Nutraceuticals such as antioxidant supplements are estimated to be valued at \$207 billion in 2016 as well leaving a wide berth of opportunity for peanut skin antioxidant extracts to enter the market (Nutraceutical World 2013).

Unfortunately, obstacles exist that prevent the commercialization of waste peanut skin extracts. One of the most detrimental may be the high heavy metal content which can be concentrated in the extraction and spray-drying of peanut skin phenolic compounds (Constanza 2011). This is of special concern when using skins from peanuts grown in rotation with cotton crops. Pesticide use on previous cotton crops and use of fertilizer causes the uptake of heavy metals; arsenic and cadmium in the peanut plant (Armbrust and Bridges 2002; McLaughlin 2000). McLaughlin (2000) determined peanut skins can accumulate cadmium concentrations of 2.92ppm. Constanza (2011) determined the arsenic and cadmium content of peanut skin extract to be 0.088ppm and 0.0056ppm respectively.

Removal of toxic metals from peanut skin extract is a major hurdle to the utilization in food products. Adsorption is a promising method of removing metal from solution as it does not alter or damage the phenolic compounds and can be developed for column in-line application (Husaini 2011). Various commercial adsorbent materials such as activated carbon and resins are available but require a large capital investment and high operation costs. This study concentrated efforts on utilizing waste biomasses, peanut hulls and chitosan derived from shellfish waste as economically feasible adsorbent materials for the remediation of peanut skin extract.

Chitosan is a natural biodegradable polyglucosamine derived from the deacylation of chitin, a structural element in crustacean shells, insect exoskeletons, and fungi (Ngah 2005). The adsorption capacity and mechanical properties of chitosan can be improved by cross-linking to form beads. Chitosan beads exhibit increased porosity, more favorable mechanical properties, and increased stability in low pH solutions. Sodium tripolyphosphate was chosen for this study over more commonly used cross-linking agents due to its suitability for food-grade applications (Ngah 2010).

Peanut hulls were chosen as the second adsorbent material. Peanut hulls are a promising adsorbent material in a variety of applications due to its macro-porous nature and resistance to temperature, pH, mechanical stress, and prolonged submersion in water (Husaini 2011). In aqueous solution of pH above 3.5, the functional groups of the hull acquire a negative charge and are able to absorb cationic species through electrostatic attraction (Zhu 2009; Oliveira 2009). As observed by Husaini (2011), the peanut hull acts as an acidic ion exchanger by releasing hydrogen atoms into solution as it binds metal ions causing a net decrease of solution pH. Peanut hulls have been demonstrated effective in the treatment of waste water to remove arsenic, cadmium, chromium, copper, lead, nickel, and zinc (Husaini 2011; Johnson 2002; Brown 2000; Charmarthy 2001). Under optimal conditions, peanut hulls have been shown to have a metal loading capacity of 23.7-46mg/g hull (Husaini 2011).

This study presents the sorption of arsenic and cadmium onto peanut hull biomass and chitosan-tripolyphosphate cross-linked beads in peanut skin extract solutions using a batch system. The adsorbent materials were evaluated for effectiveness in two ethanol

concentrations of extract. The most efficient material was then characterized by initial pH and contact time.

2.3 Materials & Methods

2.3.1 Materials

Two adsorption materials were chosen for this study; chitosan-STTP cross-linked beads and peanut hulls. Peanut hulls obtained from Universal Blanching (Sylvester, GA, USA) were milled to 2.0mm particle size using a Wiley model 4 laboratory mill (Thomas Scientific, Swedesboro, NJ) to increase surface area for adsorption. All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

2.3.2 Chitosan-STTP Bead

A modified method of Ngah (2010) was used to produce chitosan beads; 2.0g of chitosan low-molecular weight flakes was dissolved overnight in 60mL of 5% (v/v) acetic acid. The chitosan solution was then added dropwise into a beaker of 0.05M of sodium tripolyphosphate solution to form translucent spheres. The beads were allowed to harden overnight with constant stirring. The resulting opaque white beads were filtered from the STTP solution, rinsed three times with deionized water and allowed to air dry. Chitosan beads were left intact due to their favorable mechanical qualities with expectation for future in-line column use.

2.3.3 Extraction & Preparation Procedure

Blanched peanut skins were obtained from Universal Blanching (Sylvester, GA, USA). Peanut skins were stored at 4°C in their original packaging to minimize exposure to light and oxygen prior to milling. Peanut skins were milled into a fine powder using a Blixer

food processor (Robot Coupe, Jackson, MS, USA). Milled skins were stored at 4°C until extraction in plastic bags in an opaque plastic container.

Extraction was performed under low actinic lighting in foil-wrapped beakers to minimize polyphenol degradation. The milled skins were extracted with 70% (v/v) ethanol (Fisher Scientific, Pittsburg, PA, USA) and water solution in a 1:5 skins to solvent ratio. Skins were stirred in solution using a Wheaton overhead stirrer (Wheaton Industries, Inc. Millville, NJ, USA) for 20 min at a speed setting of 3.0. The skin-solvent slurry was vacuum-filtered using Whatman #50 filter paper (Whatman International Ltd., UK) to separate the extract from the insoluble plant matter.

Two ethanol concentrations of extract were prepared for adsorption experiments; one liter 70% ethanol extract solution was reserved and additional extracts were evaporated to one-third the original volume using a Buchi Rotovap (BÜCHI Labortechnik AG, Switzerland) at 55-60°C. The reduced extract was then reconstituted to original volume with deionized water to produce <10% ethanol aqueous peanut skin extract.

2.3.4 Batch Adsorption Experiments

The method of standard additions was used in the testing to accurately measure the low concentration of arsenic and cadmium in peanut skin extracts. Stock peanut skin extract solutions of 10mg/L of As(III) and Cd(II) ions were prepared using AAS TraceCERT® standard 1000mg/L Cd and As solutions (Sigma-Aldrich, St. Louis, MO), . Spiked solutions were prepared immediately before each adsorption experiment was performed to minimize settling out of metal ions. Adsorption experiments were conducted in 50mL conical tubes

with 25mL of spiked extract solution. All experiments were conducted at ambient room temperature.

The effect of adsorbent dosage was conducted by varying the amount of chitosan beads from 0.050 to 1.50g and the amount of peanut hull from 0.050 to 1.00 g. Adsorbent materials were allowed to equilibrate in solution for 120 minutes with occasional shaking to facilitate mass transport of metal ions. After equilibration time, approximately 10mL of solution was decanted from each sample for further analysis. Sample treatments were performed in triplicate and the results averaged. The Langmuir isotherm equation was used to evaluate adsorption data.

The Langmuir model assumes monolayer coverage of metal ions onto the surface area of the adsorbent and therefore a finite amount of material can be absorbed (Zhu 2009). The Langmuir Isotherm model can be expressed by the equation:

$$\text{Equation 8: } q_e = \frac{K_L C_e}{1 + a_L C_e}$$

in which q_e is the solid-phase adsorbent concentration, C_e is the solution equilibrium concentration, K_L is the solute adsorptivity constant (L/g), and a_L is the energy of adsorption constant (L/mmol). The maximum solid-phase adsorbent concentration, Q_{\max} , is defined by the K_L/a_L . The Langmuir model can also be written in a linear form as:

$$\text{Equation 9: } \frac{1}{q_e} = \frac{1}{K_L} \left(\frac{1}{C_e} \right) + \frac{a_L}{K_L}$$

2.3.5 Kinetic Adsorption Assay

The effects of pH on the adsorption of As(III) and Cd(II) ions onto peanut hulls was studied in a range of pH 2-8. Additionally, the kinetics of adsorption at each pH was observed from 20 to 120 minutes. The appropriate volume of metal standard solutions was

added to 450mL of peanut skin extract before the pH was adjusted using 1M HCl and 4M NaOH solutions. The solutions were then brought to volume in a 500mL volumetric flask and the final pH was recorded. Spiked solutions were prepared immediately before each adsorption experiment was performed to minimize settling out of metal ions. A 15mL aliquot was reserved to serve as a zero-time point control. Adsorption experiments were conducted in 50mL conical tubes with 25mL of spiked extract solution. At the zero time point, 0.25g of accurately measured peanut hulls were added to each aliquot and shaken to mix. Ten milliliter samples were taken from three tubes at 20 minute intervals using a Restek syringe filter 0.45 μ m (Restek Corp., Bellefonte, PA). This procedure was repeated for a range of pH levels; 2, 4, 5, 6, and 8. All experiments were conducted at ambient room temperature.

2.3.6 Total Phenolic Assay

The Singleton (1999) method for Folin-Ciocalteu assay was scaled to one-tenth volume to determine the change in total phenolic content of the peanut skin extracts with increasing adsorbent material used in treatment versus an untreated sample. Gallic acid was used as a standard at concentrations ranging from 0-750mg/L. A 0.1mL of each standard concentration was added to approximately 4mL of deionized water in a 10mL volumetric flask. 0.1mL aliquot of each treatment sample was added to approximately 25mL of deionized water in a 50mL volumetric flask. After gently swirling to mix, 0.5mL of Folin-Ciocalteu reagent was added to standards and samples and swirled again to mix. After one minute but less than eight minutes, 1.5mL of 20% Na₂CO₃ was added to initiate color development. Flasks were filled to final volume with deionized water and inverted 20 times to thoroughly mix. Samples were incubated for 2 hours and measured for absorbance at

765nm and 27°C using a Tecan Safire microplate reader (Tecan Group Ltd., Switzerland).

The gallic acid equivalence (GAE) of samples was determined by comparison to the gallic acid standard curve.

2.3.7 Sample Preparation & Metal Analysis

Five milliliters of each ethanol matrix adsorption samples was pipeted into a round bottom flask and reduced in volume using a Buchi Rotovap at 60°C. Samples were then reconstituted to 5mL in a volumetric flask with deionized water. Aqueous matrix samples were analyzed as is. Samples were accurately weighed into 250mL Teflon bottles and 5mL of Optima™ grade nitric acid (Fisher Scientific, Pittsburg, PA, USA) was added to each bottled, loosely capped, and allowed to sit overnight. The samples were then heated at 95°C for two hours. After cooling to ambient temperature, 2mL of 30% hydrogen peroxide (Fisher Scientific, Pittsburg, PA, USA) was added to each bottle, loosely capped, and heated for one hour at 95°C. Solutions were allowed to cool and were brought to 25mL volume with deionized water. Samples were diluted to half the concentration with deionized water and analyzed on a Varian 820 ICP-mass spectrometer (Palo Alto, CA, USA) using RH and In as internal standards against a multi-calibration curve matching acid backgrounds.

2.3.8 Statistical Methods

Statistical analysis was performed using Windows Excel 2007. Student's t-test with a 95% confidence interval ($p < 0.05$) was used to differentiate metal removal efficiency by ethanol content.

2.4 Results & Discussion

2.4.1 Effect of Ethanol Concentration on Sampling Error

Two adsorbent materials were tested for effectiveness in two matrices; 70% ethanol and >10% ethanol aqueous solution. Two matrices were evaluated due to the expectation that the need for additional sample preparation for analysis of the ethanol matrix samples could potentially result in erroneous metal removal due to precipitation.

Analysis of results showed that peanut hulls removed more cadmium than chitosan while arsenic removal was not significantly different ($p = 0.3448$) between the two adsorbents. Overall significantly more metal ions were removed from the 70% ethanol matrix than the aqueous <10% ethanol matrix. Standard deviations were also on average larger in the 70% ethanol matrix for peanut hull adsorbent material samples. An accurate assessment could not be made for chitosan bead data due to metal ion concentrations falling below instrument detection limits; 0.5ppm arsenic and 0.2ppm cadmium. It is unknown if this was a result of sample treatment, precipitation or instrumental error.

Table 1. Effect of sample matrix on sampling error.

Cadmium						Arsenic				
g/L Hull	Aqueous		Ethanol		p-value	Aqueous		Ethanol		p-value
	% Removal	SD	% Removal	SD		% Removal	SD	% Removal	SD	
2	49.5a	12.3	42.6a	1.7	0.391	22.5a	16.3	32.8a	3.9	0.345
4	60.4a	5.7	58.4a	12.4	0.809	21.3a	8.2	42.2a	11.9	0.066
10	68.8a	2.4	71.1a	9.6	0.710	18.3a	9.4	49.0a	16.8	0.051
20	81.4a	2.8	79.2a	4.6	0.517	18.9b	5.6	42.4a	6.4	0.009
40	88.6b	1.9	94.4a	2.7	0.037	27.8b	9.4	66.8a	16.1	0.022
Average		5.0		6.2			9.8		11.0	
g/L Chitosan	Aqueous		Ethanol		p-value	Aqueous		Ethanol		p-value
	% Removal	SD	% Removal	SD		% Removal	SD	% Removal	SD	
2	46.2b	10.4	67.5a	5.6	0.035	24.5a	16.6	58.85a	17.6	0.070
4	53.9a	12.8	26.8b	3.7	0.024	37.8a	15.2	47.39a	2.4	0.341
20	55.2a	24.0	45.9b	5.4	0.546	37.2a	33.6	22.59a	27.7	0.594
40	43.4b	5.2	97.3a	n/a	0.00006	37.0b	19.9	94.52a	n/a	0.007
60	55.1b	8.9	97.3a	n/a	0.0012	38.2b	8.7	94.52a	n/a	0.0004
Average		10.4		n/a			18.8		n/a	

^a Different letters within the same row indicate a significant difference ($\alpha=0.05$)

These results suggest that precipitation is likely to have occurred during the sample preparation for analysis of 70% ethanol samples making the available data less suitable for the determination of adsorption efficiency. It does however have promising implications for the practical preparation of peanut skin extracts which requires evaporation of the ethanol prior to spray-drying. Therefore, both sets of data will be presented for efficiency of removal.

2.4.2 Chitosan-STTP Bead Adsorption Isotherms

Chitosan and sodium tripolyphosphate cross-linked beads were prepared according to a modified method outlined by Ngah (2010). Ngah (2010) investigated the adsorption of divalent lead and copper ions and found adsorption capacities of 57 and 26mg/g respectively. In the present work, sorption of As(III) and Cd(II) onto uncrushed beads was studied over a dosage range of 0-60g/L.

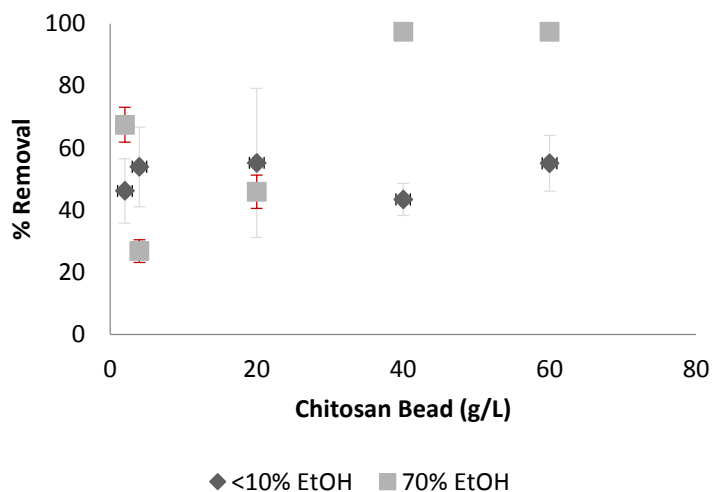


Figure 11. Adsorption isotherms of Cd(II) onto chitosan beads in two ethanol concentration of skin extract.

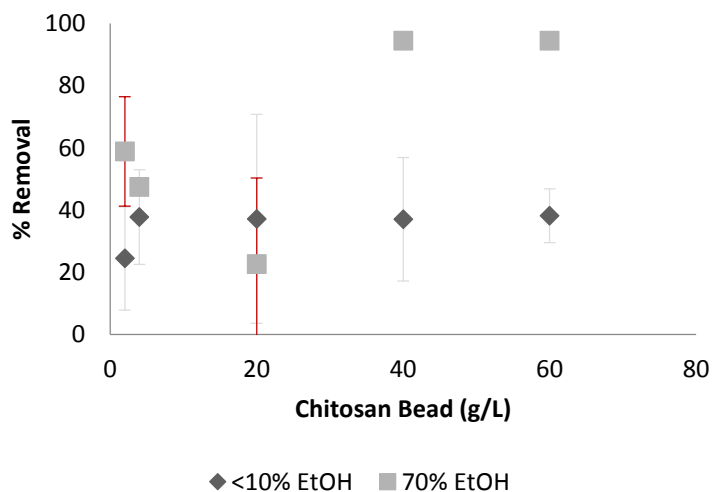


Figure 12. Adsorption isotherms of As(III) on chitosan bead in two ethanol concentrations of skin extract.

In the peanut skin extract, chitosan-STTP beads showed variable removal of metal ions ranging 25-97% but were not dependent on absorbent dose in the tested range as shown

in Figure 11 and Figure 12. Analysis using the Langmuir model depicted in Figure 13 shows no correlation between dose and metal uptake in the aqueous matrix.

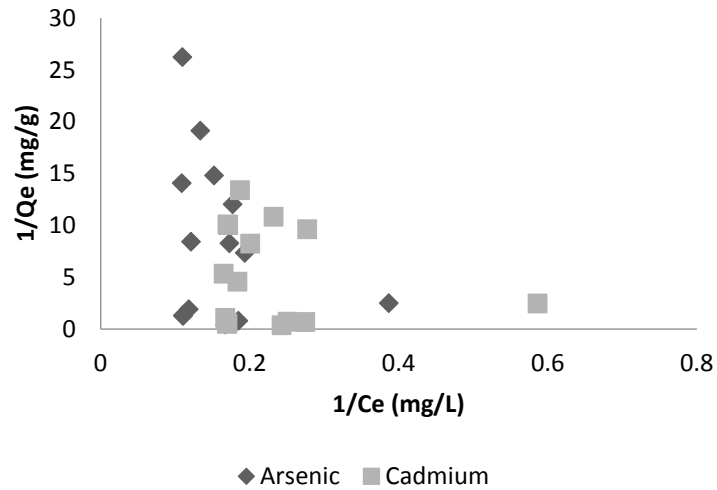


Figure 13. Langmuir isotherm of metal adsorption onto chitosan bead in <10% EtOH extract.

2.4.3 Peanut Hull Adsorption Isotherms

Peanut hulls are an ideal adsorbent material for the application at hand as they originate from the same waste stream. Additionally, raw unmodified hulls have been previously shown to be effective for the adsorption of the metals of concern; arsenic and cadmium (Husaini 2011; Brown 2000). The present investigation studied the sorption of As(III) and Cd(II) over a hull dosage range of 0 to 40g/L for a contact time of 120 minutes. In the peanut skin matrix, removal of cadmium occurred similar to previously defined characteristics for cadmium sorption onto hulls while removal of arsenic followed another proposed mechanism.

2.4.4 Peanut Hull Adsorption of Cadmium

Prior peanut hull adsorption studies have found modest adsorption capacities (Q_{\max}) for cadmium, Brown (2000) recording 6mg/g and Wafwoyo (1999) citing a maximum of 10mg/g. Adsorption of the positively charged cadmium ion occurs onto the hydroxyl and carboxyl functionalities of the peanut hull surface. The present study investigated cadmium adsorption from a 10ppm cadmium solution with a pH of 3.2 and achieved a max removal of $88.6\pm 1.9\%$ cadmium at the highest adsorbent dosage level as shown in Figure 14.

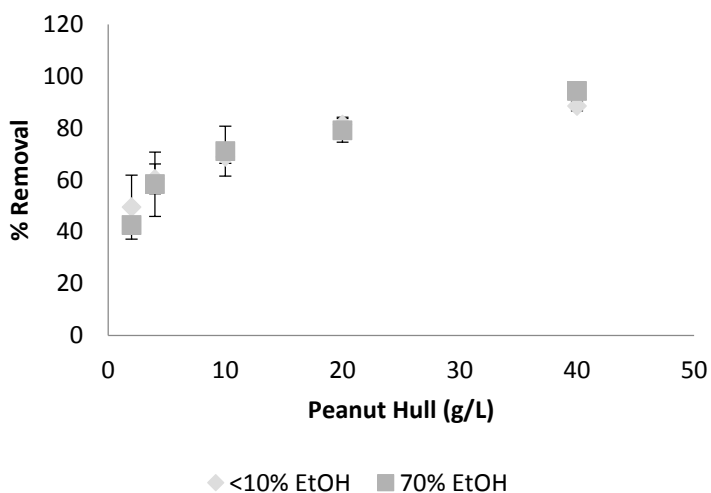


Figure 14. Adsorption isotherms of Cd(II) on peanut hull in two ethanol concentrations of skin extract.

In Figure 15, adsorption data was fit to the linear Langmuir model and showed a strong linear correlation ($R^2=0.991$) between equilibrium metal uptake (Q_e) and equilibrium metal concentration of the solution (C_e). This indicates that adsorption onto the peanut hull material was responsible for metal removal. However, the Langmuir model was violated by a negative y-intercept and therefore the theoretical Q_{\max} could not be calculated. This is not

discouraging as the Langmuir equation and its constants are merely a description of data in a convenient mathematical form. Although it is widely applied to numerous adsorption systems with great success, the Langmuir model assumes monolayer coverage of the ideal adsorbate onto the ideal adsorption surface and cannot take into account the many competing and complex interactions which occur in solution as this data suggests.

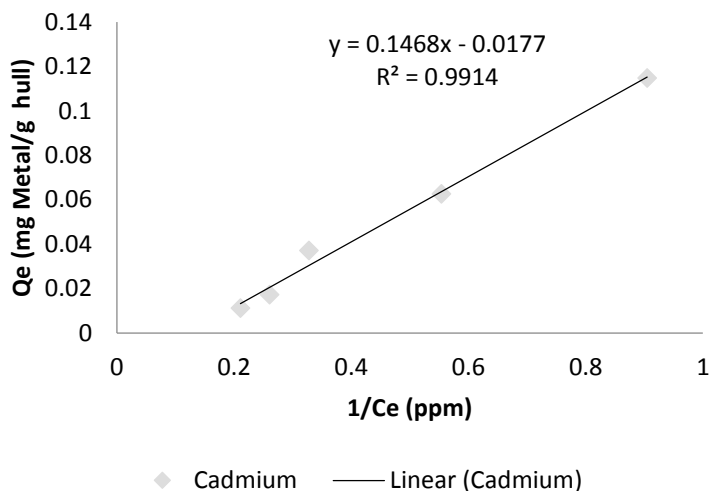


Figure 15. Linear Langmuir isotherm of cadmium adsorption onto hulls.

2.4.5 Peanut Hull Adsorption of Arsenic

Arsenic has been successfully adsorbed using various cellulose-based agricultural byproducts including; rice bran, sorghum biomass, and peanut hulls (Amin 2006; Haque 2007; Husaini 2011). Although application has been tested in industrial effluents and drinking water, heavy metal remediation of complex solutions such as a plant extracts has been largely excluded from current literature (Amin 2006; Husaini 2011).

Complex solutions such as peanut skin extract present additional challenges to arsenic removal due to its abundance of polyphenols. Not only are polyphenols competitive for metal

binding but they also create a reducing environment. In neutral pH water, arsenic exists in as a mix of valence states and species; As(V) as the oxyanions H_2AsO_4^- and HAsO_4^{2-} and to a lesser extent As(III). Within a reducing environment, the dominant arsenic species shifts towards its uncharged As(III) compound H_3AsO_4 . This can both help and hinder overall removal efficiency; uncharged As(III) is more resistant to co-precipitation with other metals as it cannot form a salt complex as its oxyanion forms do. However, there is no longer electrostatic repulsion between itself and the negatively charged peanut hull surface. The geometric configuration of the compound may theoretically then allow the positively charged central arsenic atom to bond to the adsorption site (Haque 2007).

The adsorption of arsenic was studied over a hull dosage range of 0 to 40g/L for a contact time of 120 minutes. Efficiency of removal was independent of adsorbent dosage and indicates that adsorption was not the primary mechanism. An average of $20.7 \pm 9.8\%$ of arsenic was removed from <10% ethanol PSE and while a higher percentage of $46.6 \pm 11\%$ was removed from the 70% ethanol matrix.

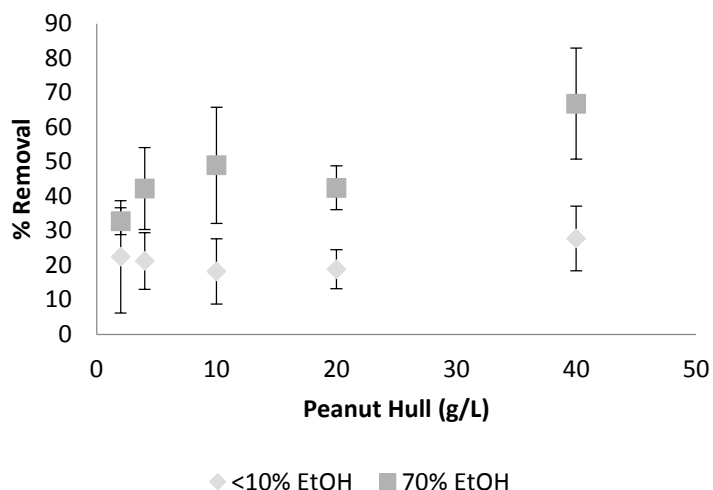


Figure 16. Adsorption isotherms of As(III) on peanut hull in two ethanol concentrations of skin extract.

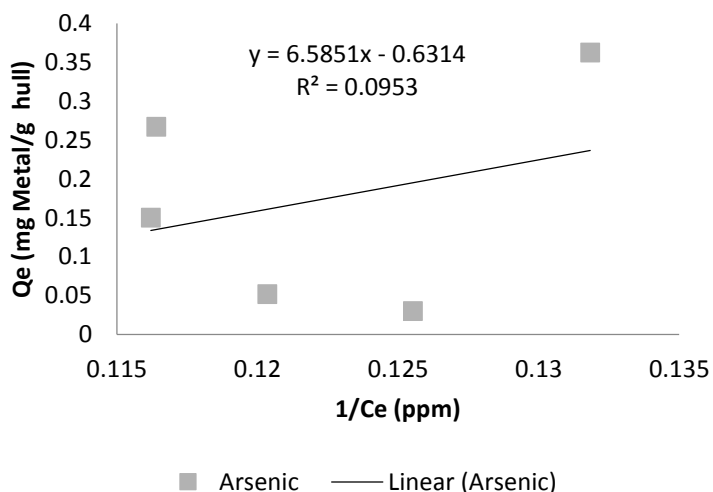


Figure 17. Linear Langmuir isotherm of arsenic adsorption onto hulls.

One possible mechanism explaining the increase in efficiency between the <10% and 70% ethanol matrices is the co-precipitation of As(V) oxyanions with other metal ions in solution as shown in Equation 10. The 70% ethanol matrix was stirred and heated to reduce the ethanol content for analysis. This promotes precipitation in two ways: 1) introducing

oxygen and heat into the system and thereby encouraging oxidation of the arsenic species and 2) concentrating the metal ions to facilitate precipitation. Being that commercial spray-drying of peanut skin extract products would require a similar process around 25% of the arsenic content could be reduced via concentration and filtration alone.



2.4.6 pH-Kinetic Study of Peanut Hull Adsorption for Optimization

Initial investigations of adsorption efficiency were carried out in cadmium and arsenic-spiked peanut skin extract with no attempt to adjust the pH of 3.2. This provided sufficient information to choose peanut hulls as the preferred adsorbent material. To further optimize the efficiency of metal removal, the effect of the pH on adsorption was then investigated. Initial pH determines several important variables; surface charge of the adsorbent and speciation and degree of ionization of the metal species to be adsorbed.

The adsorption of Cd(II) & As(III) were investigated over a pH range of 2-8 with a constant hull amount of 10g/L at 25°C. The <10% ethanol peanut skin extract was chosen as the matrix for investigation so that results would be a closer reflection of the efficiency of adsorption only rather than precipitation. Additionally, samples were withdrawn at 20 minute intervals across the full contact time of 120 minutes to study the kinetics of adsorption and better understand the mechanisms of removal. Figure 18 describes the overall removal efficiency of each pH after the solution came to equilibrium. A sharp peak for efficiency of removal of cadmium was observed at a pH of 5. Removal of arsenic, however, reached a maximum of 13% removal occurring at a pH of 6 with a second lower peak of 8.4% occurring at a pH of 4.

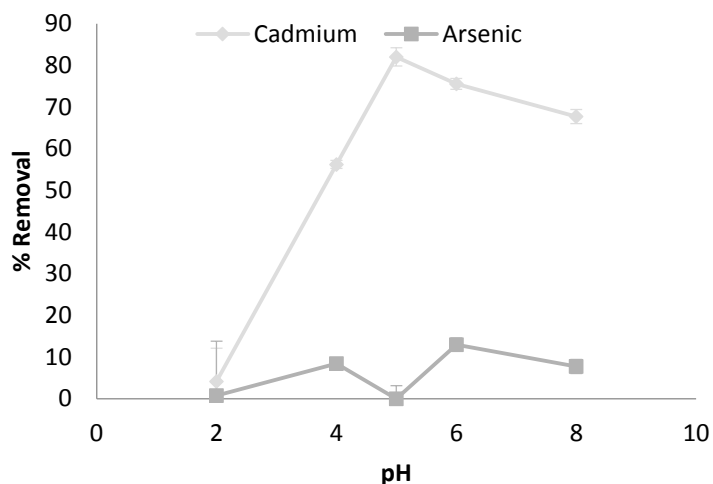


Figure 18. Effect of pH on removal efficiency at 120min contact time.

2.4.7 pH-Kinetic Study of Cadmium Adsorption onto Hulls

As shown previously shown in Figure 18, the removal efficiency rose sharply as the pH of the solution increased until a peak was reached at a pH of 5. This is concurrent with previously reported pH trends which placed maximum efficiency of adsorption occurring at a pH of 4.5-6.0 (Zhu 2009; Husaini 2011). This is because the peanut hull surface has a zero-point charge of 3.5 above which the net negative charge of the hull increases and therefore the adsorption until maximum capacity is reached (Zhu 2009).

Looking at the kinetics throughout the adsorption process in Figure 19 shows a wealth of new insight into the mechanisms occurring in solution. The primary mechanisms of removal are strongly dependent on the pH of the solution. At a pH of 2, no adsorption occurred as the adsorbent surface was uncharged and free hydrogen ions in solution competitively bound to available adsorption sites. As the pH rose to 4, there was a slight initial precipitation of metal ions out of solution but removal occurred primarily in the first 20 minutes by adsorption followed by a slower period of equilibrium. This quick primary

adsorption is in agreement with previous kinetic surveys which cite that 90% of all uptake occurs within the first twenty minutes of contact and 98% occurring within two hours (Brown 2000; Zhu 2009). Although this and other studies chose time intervals of 10-20 minutes, Husaini (2011) who surveyed the kinetics of adsorption over intervals of 20 to 240 seconds showed that equilibrium may be reached as quickly as 60 seconds for some metal ions which is promising for development of column applications.

Overall the available data indicates that at least in the pH range of 3.2 to 4 that adsorption of cadmium occurs as previously described in other studies on peanut hull adsorption of metal ions. Deviation from this established mechanism begins to occur as the peanut skin extract solution is adjusted to a pH above 5 when the primary mechanism of removal is that of immediate precipitation followed by a much more gradual removal by adsorption. Interestingly at the highest pH of 8, adsorption occurred similar to that occurring in the 3-4 range but was then followed by a re-release of ions into solution until equilibrium.

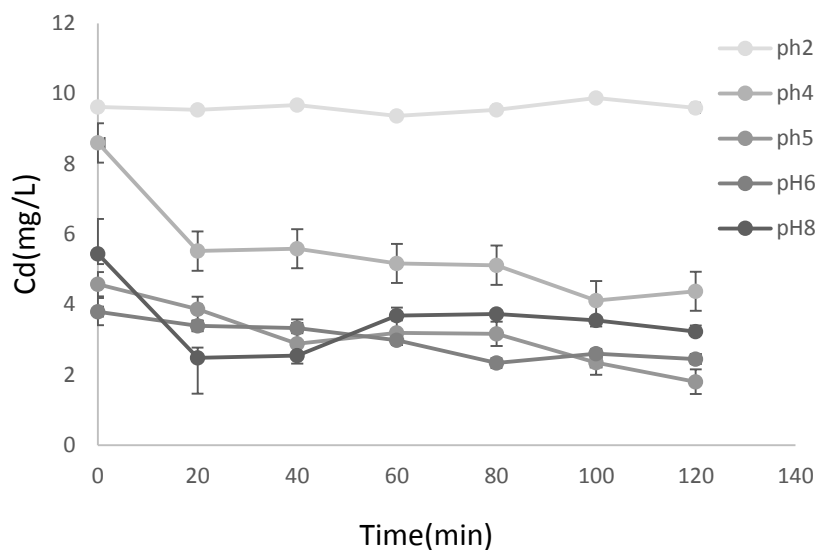


Figure 19. Kinetic survey of cadmium adsorption at varying pH levels.

Normally cadmium will not precipitate as a hydroxide species until a pH of above 8, although, some of its salt precipitates are less soluble. There are several possible explanations for the precipitation occurring at such a low pH such as co-precipitation with another metal ion such iron which can occur at a pH as low as 6.5. Another explanation is that the reductive environment is pushing the cadmium into a +1 oxidation state and therefore requiring less available hydroxide species to precipitate. Lastly, the cadmium metal may be binding with polyphenol compounds in solution strongly enough to form flocs. To determine the exact mechanism, the solid precipitate must be captured and analyzed in future studies.

2.4.8 pH-Kinetic Study of Arsenic Adsorption onto Hulls

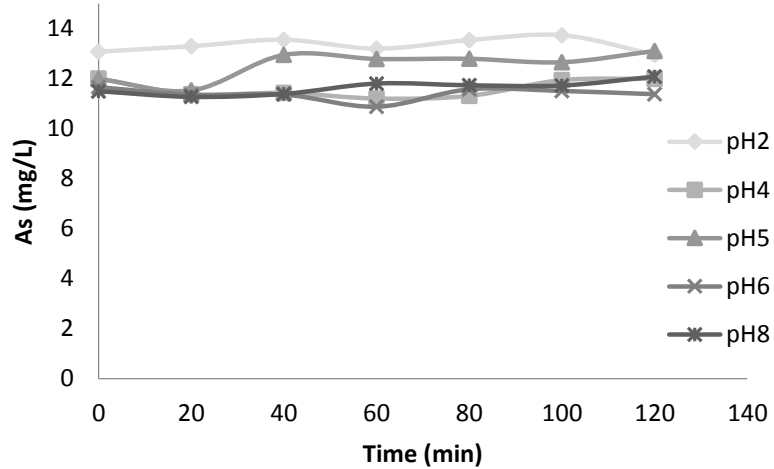


Figure 20. Kinetic survey of arsenic adsorption at varying pH levels.

In the <10% ethanol matrix, arsenic could not be coaxed out of solution at any appreciable level although two small peaks were observed at pH 4 and pH 6. Across the relevant pH levels, arsenic displays a special behavior unlike other metal ions and forms a mix of uncharged and negatively charged oxyanion compounds (Haque 2007). The formation of these compounds is described below:



One would assume these compounds would be either not attracted to or exhibit repulsion to the negatively charged peanut hull surface and therefore resist adsorption. However, adsorption of such arsenic species onto various biomasses with similar functional groups has been previously described. Specifically, Husaini (2011) demonstrated adsorption

of arsenic from industrial effluents onto peanut hull. Haque (2007) who achieved adsorption onto sorghum proposed that the geometric configuration of the arsenic complex allows for attraction between the positively charged central arsenic atom and the negatively charged functional groups on the adsorbent surface. These previous studies, however, researched arsenic adsorption in the confines of nearly pure aqueous solutions without the presence of competitive species. It seems likely in the complex matrix of the peanut skin extract that there would exist interactions competitive to the binding of the arsenic to the peanut hull surface.

Despite failure to achieve adsorption, initial data indicated that during the concentration of the 70% ethanol matrix that around 25% or more of the arsenic content could be removed via precipitation during concentration. Depending on the speciation of the arsenic present, this may be satisfactory. Arsenic not only exists as a mix of inorganic complexes but may also be present as various organic complexes. Inorganic complexes are considered the most toxic, followed by methylated arsenic forms, ribose-sugar species, and finally arsenobetaine is considered non-toxic (Hovane 2004).

2.4.9 pH-Kinetic Study of Total Phenolics in Solution

Lastly it is important to ensure that removal of heavy metals does not come at significant cost to the valuable polyphenol compounds of interest. For that reason, the total phenol content of the solutions was determined. Figure 21 shows that there was very little change across the wide pH range with a decrease occurring only at the highest pH of 8. This indicates that the procyanidin content of the peanut skin extract is more robust than

previously assumed and can withstand the large pH adjustments which may be necessary to facilitate the removal of heavy metal contamination.

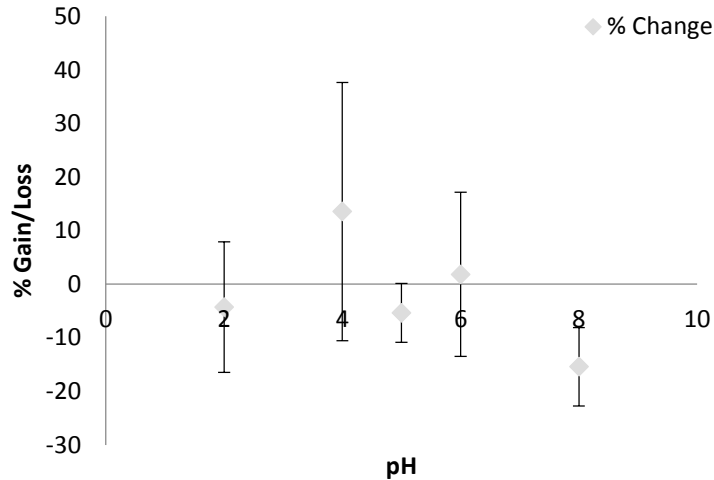


Figure 21. Effect of pH on total phenolic content after 120 minutes.

2.5 Conclusions

As the objective of using of peanut skin extracts as a function food ingredient is pursued, the issue of heavy metal contamination will remain as a challenge to be addressed. As more often than not with complex problems, finding a solution must take a multi-faceted approach. This means using a multi-step clean-up process for peanut skin extracts. Based on the results of this study, the author envisions a four-step process; 1.) concentrating the ethanol solution and thereby removing 25% or more of the arsenic content by precipitation 2.) pH adjustment to facilitate the precipitation of cadmium 3.) using peanut hulls as an adsorbent to remove the remaining cadmium and 4.) investment in a commercial adsorption resin to remove the remaining arsenic if needed. Taking advantage of the tendency of the

metal ions to precipitate and use of peanut hulls as a low-cost pre-treatment adsorbent will extend the lifespan and cost-effectiveness of investing in a commercial adsorption resin.

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CHAPTER THREE: MINIMIZING THE NEGATIVE FLAVOR ATTRIBUTES OF PEANUT SKIN EXTRACT USING RESPONSE SURFACE METHODOLOGY

3.1 Abstract

Each year one hundred million pounds of peanut skins are produced as a byproduct of the peanut crop. Most of this polyphenol-rich biomass is currently sold as low value animal feed or discarded. As consumer demand for antioxidant polyphenol products grows into a multi-billion dollar industry, peanut skins are a vastly under-utilized economic opportunity to the peanut industry. The current study attempted to further the development of spray-dried peanut skin polyphenol extracts for use as a functional food ingredient in peanut butter and chocolate, the leading uses of peanut products. To reduce the negative sensory aspects of concentrated polyphenols, maltodextrin was investigated as an encapsulation agent. The effect of varying levels of maltodextrin for encapsulation and inclusion of the resulting spray-dried powder into peanut paste was investigated using response surface methodology. The resulting Trolox equivalence was measured as well as the bitterness and astringency intensities. The response surface returned an optimized formula of 0.75g of 5.92% maltodextrin-encapsulated peanut skin extract per 100g peanut paste resulting in a Trolox equivalence of $8.41 \pm 2.87 \mu\text{mol/g}$, bitterness intensity of 2.43 ± 0.105 , and astringency of 1.92 ± 0.31 . Preliminary tests in chocolate determined the threshold inclusion of 10.5 (w/w)% maltodextrin-encapsulated peanut skin extract to be 2.50g/100g.

3.2 Introduction

Each year one hundred million pounds of peanut skins are produced as a byproduct of the peanut crop. Most of this waste product is discarded or sold for less than twenty dollars a

ton (Sobolev and Cole 2003) for use as a minor component in cattle feedstock (Yu 2006).

Peanut skins are a rich source of phenolic antioxidant compounds which could be extracted to produce value-added products enriched in antioxidants (Hathorne and Sanders 2012). As consumer demand for antioxidant polyphenol products grows into a multi-billion dollar industry, peanut skins are a vastly under-utilized economic opportunity to the peanut industry.

Antioxidant phenolic compounds have been extracted from peanut skins with significant yields; Nepote (2002) reported 140-150mg total polyphenols per gram defatted skin. Extracted polyphenols may then be spray-dried to further concentrate polyphenols and increase ease of use. Constanza (2012) reported 55.6 mg of phenolics could be extracted per gram of skins using 70% (v/v) ethanol in a 1:5 skins: solvent ratio with 20 minutes of stirring. The extract was then spray-dried to yield 2g powder per 100 grams skins with a phenolic content of 700mg/g gallic acid equivalents.

The high efficiency of yield gives peanut skin phenolic extracts significant potential in the growing health food market. Supported by a broad base of epidemiological studies such as the Zutphen Elderly Study, antioxidant compounds have been hailed as a silver bullet against human disease in popular media (Hertog 1993). This has resulted in an increased value in marketing for the food industry. Functional foods such as those with added antioxidants had a market value of \$49 billion in 2011 and are projected to be worth \$67 billion by 2016. Nutraceuticals such as antioxidant supplements are estimated to be valued at \$207 billion by 2016 as well leaving a wide berth of opportunity for peanut skin antioxidant extracts to enter the market (Nutraceutical World 2013).

The general motivation in current studies has been limited to the use of peanut skins as antioxidant-rich health beverages. Sobolev and Cole (2003) evaluated the use of peanut skins to enhance brandy and sweet ice tea. An informal panel (n=23) described the peanut skin brandy as “intense, bold, and balanced with a pleasant aftertaste”. The same panel described the peanut tea as having “a pleasant bittersweet chocolate background” and “subtle notes of peanut butter”. Francisco and Resurreccion (2012) also evaluated peanut skins for use as a tea-like beverage. Ten trained panelists developed a lexicon and described peanut skin tea infusions of three market varieties; Runner, Spanish, and Virginia peanut skins. Using a 150mm continuous scale, panelists described the infusions to have a woody/hulls/skins flavor ranging in intensity from 38.2 to 44.9 for all market types. Astringency was also comparable for all market varieties ranging from 79.6 to 89.5. Bitterness intensity ranked as follows for market varieties; Runner (108.6), Spanish (106.6), and Virginia (100.9).

Peanut skin extract usage would be restricted to peanut food products as it would carry an allergen warning as according to Food Allergen Labeling and Consumer Protection Act of 2004 (U.S. FDA 2012). The leading use of peanuts in the U.S. is as peanut butter (45%), followed by snack nuts (35%) and in candies and confections (25%). Half of the ten top selling candy bars in the U.S. contain peanuts (American Peanut Council 2012). For this reason, chocolate and peanut butter was chosen as the matrix for peanut skin powder fortification in the present study.

Antioxidant-enriched peanut butter is not yet a commercial product, although, products such as Nut-rition® by Planters shows consumer interest in the health benefits of

peanut products. Hathorne (2012) conducted a preliminary study of antioxidant-rich peanut butter. Hathorne fortified peanut paste with whole ground peanut skins in concentrations varying from 0-20% (w/w). Astringency and bitterness were found to be 2.9 and 2.8 respectively in unfortified peanut paste as rated on the Spectrum® scale. Scores were unaffected until more than 1% (w/w) peanut skins were added. At 10-15% bitterness and astringency ranked 4.3 & 4.4 and rose to 5.3 for both scores at a 20% peanut skin fortification level. Increase in bitterness and astringency was thus deemed less acceptable as national brands of peanut butter are rated fairly low (1-3) on the intensity scale (Meilgaard, Civille, and Carr, 1999). Ma (2013) also investigated inclusion of whole ground peanut skins into peanut butter at lower levels, 1.25-5%, and found decreased spreadability above 2.5% inclusion. Negative changes in textural aspects could be avoided with the use of spray-dried extract powder which would require less material addition to achieve the same antioxidant level.

The market for polyphenol-rich chocolate is already established due to its natural high-polyphenol content. Consumer demand is evident by the rising popularity of products such as CocoaVia® and branding of other chocolate products as a good source of antioxidants. In anticipation of increased consumer demand, Mars Incorporated proactively holds Patent #8,337,917 for products, including chocolate-containing confectionaries, containing nut skins or procyanidin-containing peanut skin extract (Chevauz 2012).

Ares (2010) conducted a survey of 75 Uruguayan consumers and their pretrial expectations of chocolate milk desserts enriched with antioxidants. Ares then measured overall liking of milk desserts enriched with different concentrations of a polyphenol extract

of a native South American plant. Overall liking dropped when the polyphenol concentration was greater than 0.4g/L. However, consumers who concerned with the health benefits of the antioxidant-enriched dessert were more tolerant of negative sensory aspects than those who were not.

It is important to establish to what point are consumers willing to sacrifice sensory hedonics for perceived health benefits. Harwood (2013) determined consumer rejection thresholds for chocolate made with underfermented cocoa, which is more bitter and astringent than traditional cocoa but higher in polyphenols. Through 2-alternative forced choice preference tasks, Harwood determined the rejection threshold to be 80.7% underfermented cocoa in a 73% cacao bar. Due to the limitations of the experimental design it could not be determined if rejection was a result of increased bitterness and astringency or reduced “chocolate-y” flavor. Prior work by Harwood (2012), however, established that those who self-identified as preferring milk-chocolate had a lower rejection threshold for milk chocolate dosed with the bitterant sucrose octa-acetate than those who preferred dark chocolate.

The current study attempted to reduce perceived bitterness and astringency utilizing encapsulation of the peanut skin polyphenol extract during spray-drying. Maltodextrin was chosen as the encapsulating agent as it is commonly used to increase yield efficiency and stability. It has been used with good success in improving the physical properties of spray-dried polyphenols. Benefits of encapsulation also include protection from light, heat, and oxygen. For the purpose of taste-masking, it prevents the bitter core material from dissolving in the saliva to stimulate the taste receptors (Fang and Bhandari 2010). The effect of varying

levels of maltodextrin for encapsulation and inclusion of the resulting spray-dried powder into peanut paste was investigated using response surface methodology. A central composite design was used to create a response surface for bitterness, astringency, and resulting antioxidant content. Desirability of the peanut paste formulation was maximized to increase antioxidant content and decrease bitterness and astringency. Consumer thresholds were also calculated for maltodextrin encapsulated peanut skin extract powder in milk chocolate.

Previous studies have focused on overall consumer perception and liking to polyphenol enriched chocolate products. Studies, however, suggest that increased bitterness and astringency plays a significant role in consumer acceptance of the products (Harwood 2012, 2013). Mitigating those negative sensory aspects will be critical in achieving consumer acceptance.

3.3 Materials and Methods

3.3.1 Materials

Jumbo Georgia-O6G peanuts grown at the USDA-ARS National Peanut Research Lab facility (Dawson, GA) were used for the current study. Blanched peanut skins were obtained from Jimbo's Jumbos (Edenton, NC, USA). Peanuts and peanut skins were stored at 4°C in their original packaging to minimize exposure to light and oxygen. Maltrin M150 maltodextrin was provided by Grain Processing Corp. (Muscatine, IA, USA). Confectioner's coating chocolate was purchased from Chocolate Smiles (Cary, NC, USA). Pure pharmaceutical grade ethanol (Decon Laboratories Inc., King of Prussia, PA, USA) was used for the preparation of food-grade peanut skin extracts. All other reagents were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.3.2 Peanut Skin Extraction Procedure

Peanut skins were milled into a fine powder using a Blixer-3 food processor (Robot Coupe, Jackson, MS, USA). Milled skins were stored at 4°C until extraction in plastic bags in an opaque plastic container. Extraction was performed under low actinic lighting in foil-wrapped beakers to minimize degradation of polyphenols. The milled skins were extracted with 70% (v/v) pure ethanol and deionized water solution in a 1:5 skins to solvent ratio. Skins were stirred in solution using a Wheaton overhead stirrer (Wheaton Industries, Inc. Millville, NJ, USA) for 20 min at a speed setting of 3.0. The skin-solvent slurry was vacuum-filtered using Whatman #50 filter paper (Whatman International Ltd., UK) to separate the extract from the insoluble plant matter. Extracts were evaporated to one-third the original volume using a Buchi Rotovap (Buchi Labortechnik, Switzerland) at 55-60°C. Reduced ethanol extracts were combined and stirred to homogenize before separating into aliquots for microencapsulation.

3.3.3 Preparation of the Microcapsules

Microcapsules were prepared as follows: 500g peanut skin extract was mixed with maltodextrin under constant stirring and brought to a final weight of 1000g to obtain maltodextrin concentrations of 1.3-19.7% (w/w). Prepared maltodextrin solutions were allowed to hydrate for a minimum of 24hours and stored at 4°C until use. The prepared soluble extracts were fed into a Buchi B-290 mini spray-dryer with an inlet temperature of 175 °C and an outlet temperature of 90 ± 5 °C. The solution feed pump rate was set at 30% (10 mL/min), the nitrogen flow rate was set at 35psi, and the aspirator was set at 100% for each of the runs. The powders obtained were stored to exclude light exposure and were kept at 4°C until use.

3.3.4 Preparation of Peanut Paste Formulations

Peanuts were roasted in a pilot plant scale roaster (Aeroglide, Cary, NC) in a bed depth of 8cm at 177 °C with an air flow rate of 1.0m/s for 20 minutes to the equivalent surface color of Medium 48.5±1 as determined by a HunterLab D25 colorimeter (Reston, VA). The air flow direction was changed from up-flow to down-flow at the ten minute time interval. A forced air blower was used to cool the peanuts to ambient temperature following roasting. Peanuts were manually blanched and ground into paste using a Blixer-3 food processor. Formulations were prepared following a central composite design with five replicates of the central point indicated in the scheme shown in Table 2. Peanut skin microencapsulated powder (0.19-0.90%) was incorporated into the paste with constant stirring to homogenize. Each unique formulation was assigned a random three-digit code and kept frozen at -20C in glass jars until evaluation.

Table 2. Peanut paste formulations.

Formula	(w/w) % Maltodextrin Encapsulation	g powder/100g paste
A	10.5	0.55
B	10.5	0.55
C	17	0.30
D	10.5	0.90
E	1.31	0.55
F	4	0.3
G	4	0.80
H	10.5	0.55
I	10.5	0.20
J	10.5	0.55
K	19.7	0.55
L	10.5	0.55
M	17	0.80

3.3.5 Preparation of Chocolate Formulations

Candy-coating milk chocolate pieces were weighed into a crockpot (Proctor Silex Co., Glen Allen, Virginia, USA) and allowed to melt on low heat. Peanut skin 10.5% maltodextrin microcapsule powder (0.1-6.4%) was incorporated into the melted chocolate with constant stirring. Molten chocolate was poured into molds, cooled, cut into square ¼” inch pieces and stored at 4°C.

3.3.6 Sensory Evaluation of Phenolic-Enhanced Peanut Paste

Evaluation of peanut paste was conducted by a trained descriptive panel (females, n=6 ; males, n=4). Samples were evaluated utilizing the 15-point Spectrum® universal

intensity scale and described using the peanut lexicon described by Johnsen (1988) and Sanders (1989) as shown in Table 3 with an additional descriptor being total off-flavor. An unaltered peanut paste reference was used as a warm-up sample prior to evaluating test samples. Panelists were instructed to take a drink of water and a bite of Muenster cheese or unsalted cracker to cleanse the palate between samples. The order of sample presentation was randomized for three replications and samples were presented labeled as random three-digit codes.

Table 3. Lexicon of peanut flavor descriptors. (Johnsen 1988)

Flavor Descriptors	Definition
Roast peanutty	The aromatic associated with medium-roast peanuts (about 3– 4 on USDA color chips) and having fragrant character such as methyl pyrazine
Sweet aromatic	The aromatics associated with sweet material such as caramel, vanilla, molasses
Dark roast	The aromatic associated with dark roasted peanuts (4+ on USDA color chips) and having very browned or toasted character
Raw/Beany	The aromatics associated with under-roasted peanuts or beans
Woody/Hulls/Skin	The aromatics associated with base peanut character (absence of fragrant top notes) and related to dry wood, peanut hulls, and skins
Sweet	The taste on the tongue associated with sugars
Bitter	The taste on the tongue associated with bitter agents such as caffeine or quinine
Astringent	A chemical feeling factor on the tongue and oral tissues, described as puckering/dry and associated with tannins or alum
Metallic	A chemical feeling factor on the tongue described as flat, metallic and associated with iron and copper
Total Off-flavor	A term summarizing the overall degree to which a sample exhibits off-flavors, as compared to the reference

3.3.7 Threshold Evaluation of Peanut Skin Phenolic Inclusion in Chocolate

The best estimate thresholds of peanut skin extract powder in chocolate was determined with 3-Ascending Forced Choice (AFC) methodology (Meilgaard, Civille, and Carr, 1999). Chocolate pieces were presented in plastic 2oz. soufflé cups and labeled with random three-digit codes. Panelists (n=24) were given dosed chocolate samples in a series with two blanks (unaltered pieces of chocolate). Five series were presented in ascending concentration and in randomized order. Series were presented on consecutive days to avoid signal saturation due to the fatiguing nature of the samples. Panelists were instructed to take a sip of water as needed between samples and to denote if their guess was “sure” or “not sure”.

3.3.8 Sample Extraction

Chocolate samples were pulverized to powder in a coffee mill (Proctor Silex Co., Glen Allen, Virginia, USA) for analysis. Peanut paste and chocolate samples were extracted using a Dionex Accelerated Solvent Extractor (Sunnyvale, CA, USA). One gram of sample was weighed analytically and mixed with approximately 25g of clean sand in a 22mL extraction cell. Filled cells were extracted with a 70:29.5:0.5 acetone: water: acetic acid solution (AWA). Extraction procedure was as follows; pressure, 1500psi; temperature, 80°C; 3x5minute flushing cycles; flushing volume, 60%; nitrogen purge, 60 seconds. Extracts were brought to a 50mL final volume with additional AWA.

3.3.9 Total Phenolic Assay

The Folin-Ciocalteu assay as adapted by Singleton (1999) was scaled to one-tenth volume to determine the change in total phenolic content of phenolic-enhanced peanut paste and chocolate. Gallic acid was used as a standard at concentrations ranging from 0-750mg/L. A 0.1mL aliquot of each standard concentration, treatment sample, or AWA blank was added

to 5mL deionized water in a culture tube. After gently vortexing, 0.5mL of Folin-Ciocalteu reagent (Sigma-Aldrich (St. Louis, MO) was added to standards and samples and swirled again to mix. After one minute but less than eight minutes, 1.5mL of 20% Na₂CO₃ was added to initiate color development. An additional 2.9mL of deionized water was added to each culture tube to bring to a final volume of 10mL. Samples were incubated for 2 hours at room temperature. After the incubation period, 200µL aliquots were pipetted in triplicate into 96-well plates and measured for absorbance at 765nm and 27°C using a Tecan Safire microplate reader (Tecan Group Ltd., Switzerland). The gallic acid equivalence (GAE) of samples was determined by comparison to the gallic acid standard curve.

3.3.10 DPPH Radical Quenching Assay

A 0.1mmol DPPH in methanol solution was prepared. A standard curve was prepared using 0-500µmol Trolox solutions. A 0.1mL aliquot of each sample was added to 2.9mL of DPPH solutions in a culture tube. Solutions were allowed to equilibriate in the dark for 24 hours at ambient temperature. AWA was used as a control and all solutions were measured against a methanol blank. The solution absorbance was measured at 517nm using a Pharmaspec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). Percentage of DPPH radical quenched was calculated using Equation 14.

$$\text{Equation 114: \% DPPH Quenched} = [1 - (A_{\text{sample}}/A_{\text{AWA control}})] \times 100\%$$

Percentage DPPH quenched for each standard solution was plotted against Trolox standards and the linear equation $y=0.1605x + 10.997$ ($R^2= 0.9959$) was generated. Antioxidant capacity of the samples in Trolox equivalence was thus calculated.

3.4 Statistic Design and Analysis

Statistical analysis was performed using JMP Pro 10 (SAS, Cary, NC, USA) and Windows Excel 2007.

3.4.1 Central Composite Design

Response surface methodology is a useful statistical method for designing experiments, building models and investigating the effect of several variables simultaneously. For this study, a central composite design was chosen over a full factorial design to reduce the number of needed experiments.

A total of 13 experiments was conducted for two factors at five levels with five replicates at the center point to account for random error. Independent variables included: weight percentage of maltodextrin used for encapsulation and grams encapsulated extract powder per 100g of food matrix.

Table 4 shows the independent variables and coded factor levels. Response variables included; bitterness, astringency, and Trolox Equivalence ($\mu\text{mol Trolox/g paste}$) as determined by DPPH.

Table 4. Experimental range and levels of independent variables.

Variable	Factor	Range and Level				
		$-\alpha$	-1	0	+1	$+\alpha$
Maltodextrin %	X ₁	1.3	4	10.5	17	19.7
Powder (g)	X ₂	0.2	0.3	0.55	0.8	0.9

The design of the model and the regression analysis was evaluated by analysis of variance (ANOVA). Post-hoc analysis included the F-test at a 95% confidence level to

evaluate the significance of individual variables and interactions. Optimized formulations were generated using the desirability function as a part of the prediction profiler.

3.4.2 Threshold Determination

The individual panelist best estimate thresholds (BET) was taken as the geometric mean between the last incorrect response concentration and the next concentration with a correct response. The correct response concentration was increased by a factor of 1.41 if the guess was indicated to be “not sure” to adjust for possibility of a chance correct response. Group thresholds were taken as a geometric mean of all 24 panelist BETs (Meilgard, Civille, and Carr, 1999).

3.5. Results & Discussion

3.5.1 DPPH versus Folin-Ciocalteu Assay

The Folin-Ciocalteu method is commonly used as a measure of total phenolic content. Reliance on this method for the current study was exercised with caution due to the chemical basis of the Folin-Ciocalteu reagent which reacts to all reducing compounds including maltodextrin (Singleton 1999). This suggested that the Folin-Ciocalteu method would not be sensitive to changes in polyphenol content in the peanut paste formulations as varying maltodextrin and product inclusion levels were arranged in a circular rather than linear design. Therefore, both the Folin-Ciocalteu method and the DPPH free radical quenching assay were used to measure the antioxidant contents of the peanut paste formulations. The two methods were compared to determine the most responsive measure of changes in antioxidant content as shown below in Figure 22.

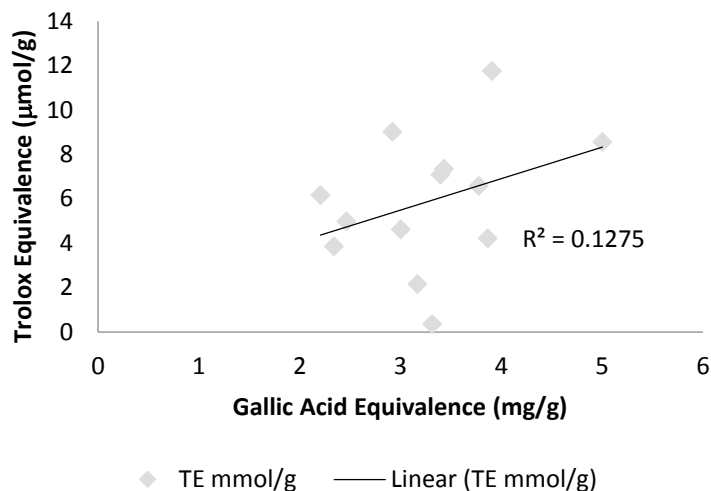


Figure 22. Correlation between Folin-Ciocalteu and DPPH assay results.

Each formulation's GAE was plotted against its Trolox equivalence as determined by the DPPH method. The correlation coefficient ($r = 0.4232$) was found to be weak and an averaging effect was observed for the GAE results which clustered from 2-4 mg/g although TE results ranged from 0-12µmol/g. Due to the greater sensitivity in formulation changes shown by the DPPH free radical quenching assay, it was chosen as the preferred response variable for building the response surface model.

3.5.2 Peanut Paste Central Composite Design

A total of 13 experiments was conducted for two factors at five levels with five replicates at the center point. Independent variables included: weight percentage of maltodextrin used for encapsulation and grams product per 100g of peanut paste. Three response variables were measured; astringency, bitterness, and Trolox equivalence (TE). Results of analysis of each formulation is shown in Table 5.

Table 5. Trolox equivalence, bitterness, and astringency results of central composite design.

Position	(w/w)% Maltodextrin Encapsulation	g powder /100g paste	TE ($\mu\text{mol/g}$)	Bitterness	Astringency
Axial	1.3	0.55	3.86 \pm 0.45	2.71 \pm 0.63	2.49 \pm 0.71
Factorial	4	0.3	0.355 \pm 0.39	2.49 \pm 0.31	2.66 \pm 0.68
Factorial	4	0.80	11.8 \pm 0.44	2.49 \pm 0.47	2.15 \pm 0.90
Axial	10.5	0.20	2.16 \pm 0.13	2.20 \pm 0.24	1.79 \pm 0.87
Center	10.5	0.55	4.99 \pm 0.21	2.30 \pm 0.31	1.93 \pm 0.52
Center	10.5	0.55	7.35 \pm 0.42	2.32 \pm 0.36	1.40 \pm 0.38
Center	10.5	0.55	4.62 \pm 0.08	2.41 \pm 0.32	1.88 \pm 0.42
Center	10.5	0.55	6.59 \pm 0.14	2.24 \pm 0.34	1.65 \pm 0.44
Center	10.5	0.55	9.02 \pm 0.36	2.25 \pm 0.26	1.54 \pm 0.29
Axial	10.5	0.90	7.09 \pm 0.27	2.30 \pm 0.46	1.59 \pm 0.36
Factorial	17	0.30	4.22 \pm 1.87	2.26 \pm 0.22	1.91 \pm 0.63
Factorial	17	0.80	6.17 \pm 0.15	2.44 \pm 0.43	1.80 \pm 0.65
Axial	19.7	0.55	8.56 \pm 0.13	2.24 \pm 0.39	1.51 \pm 0.42
LSD				0.216	0.336

Analysis of the raw data shows that bitterness and astringency responses fell in narrowly drawn ranges of less than a full point on the Spectrum[®] intensity scale for bitterness and 1.26 points for astringency. To assess panel performance, the panelists were asked to score roast peanut flavor of the samples which were prepared from the same batch of roasted peanuts; results across triplicate analysis of all 13 formulas averaged to 5.45 \pm 0.29. By comparison, the average standard deviation of the bitterness response was 0.38 points and astringency had a larger standard deviation of 0.56.

Means separation was conducted to find Fisher's least significant difference (LSD) for each attribute as shown in Table 5. Means separation of the raw sensory data revealed that means were broadly overlapping further suggesting that the panel experienced considerable difficulty differentiating the subtle changes in bitterness and astringency across the chosen variable ranges. In a central composite design, the five center replicates should be

statistically identical points as which occurred in the bitterness responses. However, for astringency the five central points were divided in three overlapping groups so that the highest and lowest means were not statistically identical to each other.

Although not well differentiated, both astringency and bitterness fell within 1 and 3 on the Spectrum® intensity scale as similarly reported for commercial peanut butters. This suggests that these two negative attributes would not be outside the range of consumer acceptance at the studied inclusion levels.

3.5.3 DPPH Trolox Equivalence Response Surface Model

Analysis of variance (ANOVA) results for the regression model obtained from the central composite design for the optimization of polyphenol rich peanut paste formulations is shown in Table 6. Statistical analysis was carried out using Fisher's test. Analysis of the model showed that it was not statistically significant within a 95% confidence level but was significant at a lesser confidence level of 90%. The lack of fit was not statistically significant ($p=0.294$). The coefficient of determination R^2 reports 68% of the total variation can be explained by the response model. Although not ideal, these results suggest that the regression model for Trolox equivalence is sufficient to roughly describe the response surface.

Diagnostic plots are shown in Figures 23 and 24. The actual versus the predicted Trolox equivalence values are given in Figure 23 which shows a linear regression model with tighter variance around the central points and larger deviations at the extreme axial points. Figure 24 shows the predicted TE versus the studentized residuals exhibits appropriate random scatter.

Table 6. ANOVA regression model for trolox equivalence in peanut paste formulations.

Source	Degrees Freedom	Sum of Squares	Mean Squares	F-value	p-value
<i>Model</i>	5	74.47	14.89	2.997	0.0924
X_1	1	60.76	60.76	12.23	0.010*
X_2	1	9.83	9.83	1.977	0.2024
X_1^2	1	0.105	0.105	0.0212	0.883
X_2^2	1	2.51	2.51	0.5051	0.5002
X_1X_2	1	1.084	1.084	0.2181	0.6547
<i>Lack of Fit</i>	3	19.76	6.59	1.755	0.294
<i>Pure Error</i>	4	15.02	3.75		
<i>Residual Error</i>	7	34.79	4.97		

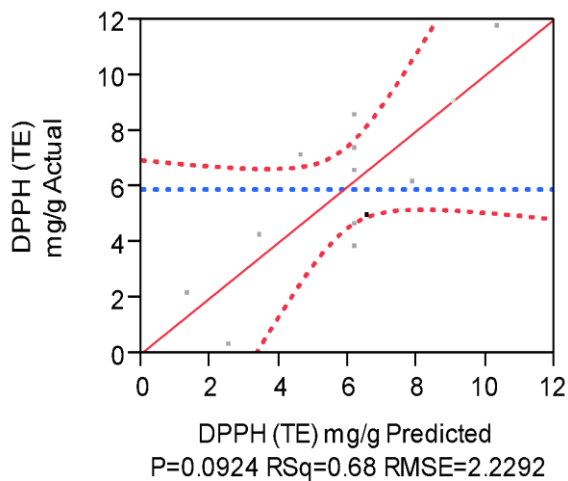


Figure 23. Actual vs. predicted DPPH trolox equivalence results.

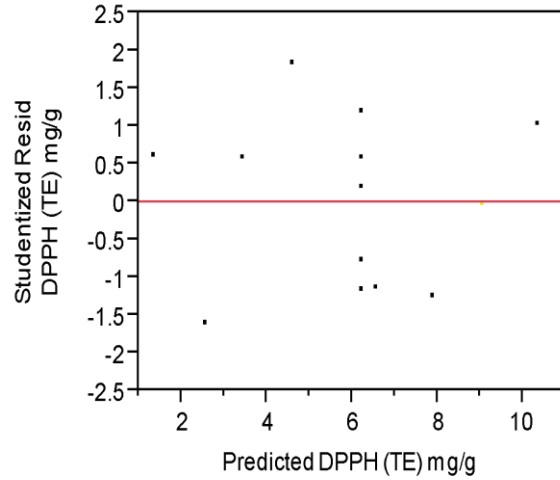


Figure 24. Predicted DPPH (TE) vs studentized residual plot.

The response surface is depicted as a contour plot in Figure 25. The contour plot shows that Trolox equivalence decreases with increasing maltodextrin weight percentage used for encapsulation. Maltodextrin encapsulation is indicated as the significant regression coefficient ($p=0.010$) in the regression model. Trolox equivalence did not vary significantly ($p=0.2024$) by grams of powder per 100g of peanut paste and there was not a significant interaction factor between the two independent variables.

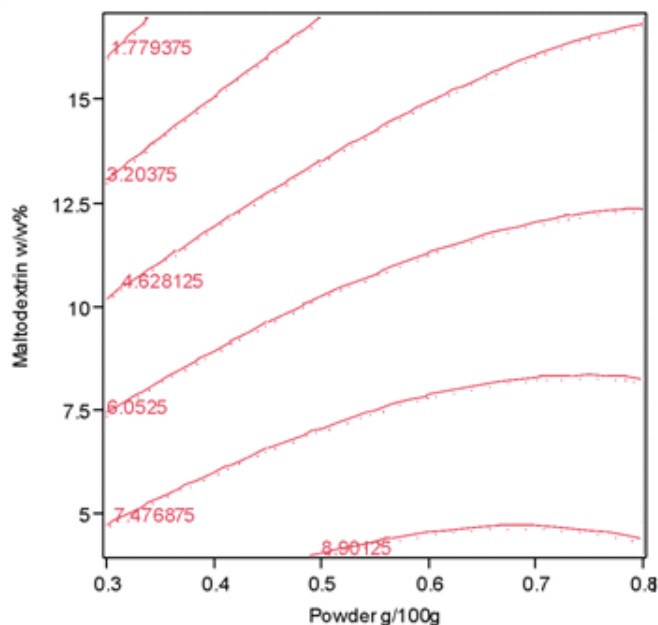


Figure 25. Contour plot of DPPH trolox equivalence response surface.

3.5.4 Bitterness Intensity Response Surface Model

Analysis of variance (ANOVA) results for the regression model for the optimization of bitterness in polyphenol-enriched peanut paste formulations is shown in Table 7. Analysis of the model showed that it was statistically significant ($p=0.0139$) within a 95% confidence level. The lack of fit was not statistically significant ($p=0.2858$). The coefficient of determination R^2 reported 83% of the total variation could be explained by the response model. These results suggest that the regression model for bitterness is adequate to describe the response surface. Diagnostic plots are shown in Figures 26 and 27. The actual versus the predicted bitterness values are given in Figure 26 which shows a linear regression model with increasing deviation in the higher response values. Figure 27 shows the predicted TE versus the studentized residuals with appropriate random scatter.

Table 7. ANOVA regression model for bitterness in peanut paste formulations.

Source	Degrees Freedom	Sum of Squares	Mean Squares	F-value	p-value
<i>Model</i>	5	0.21	0.042	6.61	0.0139*
X_1	1	0.11	0.11	17.8	0.0040*
X_2	1	0.013	0.013	2.06	0.1948
X_1^2	1	0.071	0.071	11.3	0.0121*
X_2^2	1	0.00094	0.00094	0.150	0.7104
X_1X_2	1	0.0081	0.0081	1.29	0.2935
<i>Lack of Fit</i>	3	0.025	.0084	1.81	0.2858
<i>Pure Error</i>	4	0.019	0.0047		
<i>Residual Error</i>	7	0.044	0.0063		*denotes significance

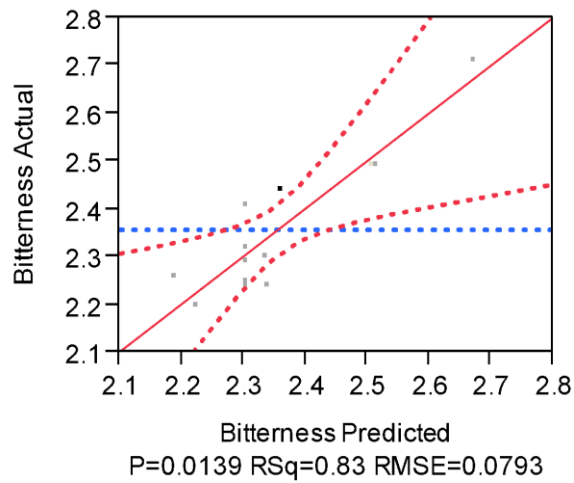


Figure 26. Actual vs predicted bitterness intensity response

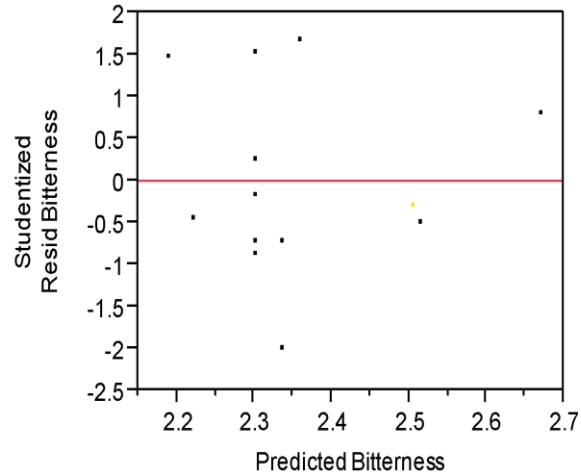


Figure 27. Predicted bitterness intensity vs studentized residual plot.

The bitterness response surface is depicted as a contour plot in Figure 28 which shows that bitterness decreases with increasing maltodextrin encapsulation. Maltodextrin encapsulation is again indicated as the significant regression coefficient ($p=0.0040$) in the regression model. Bitterness intensity did not vary significantly ($p=.1948$) by powder inclusion per 100g of peanut paste and there was not a significant interaction factor. This is likely a result of the sensory panel's inability to distinguish subtle changes in bitterness in the tested inclusion range.

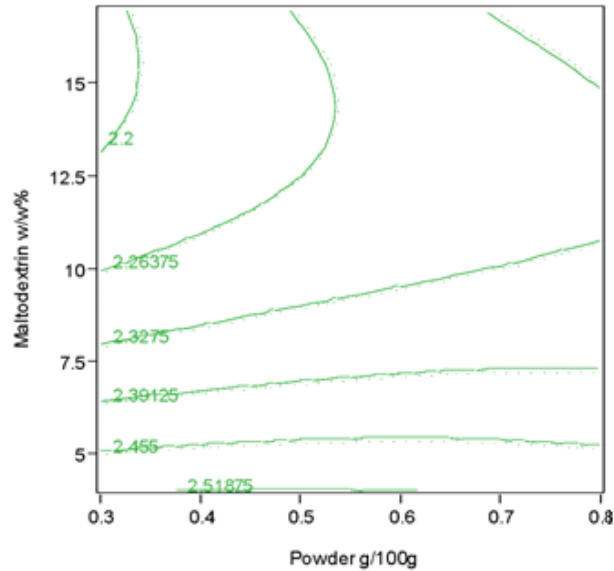


Figure 28. Contour plot of bitterness intensity response surface.

3.5.5 Astringency Intensity Response Surface Model

Analysis of variance (ANOVA) results for the regression model obtained for the optimization of astringency in polyphenol-enriched peanut paste formulations is shown in Table 8. Analysis of the model shows that it was statistically significant ($p=0.0338$) within a 95% confidence level. The lack of fit was not statistically significant ($p=0.4054$). The coefficient of determination R^2 reported 77% of the total variation could be explained by the response model. These results suggest that the regression model for astringency is adequate to describe the response surface. Diagnostic plots are shown in Figures 29 and 30. The actual versus the predicted astringency values are given in Figure 29 which shows an extremely tight cluster of astringency responses at 1.7 with three higher values falling near the prediction line. Figure 30 shows the predicted trolox equivalence versus the studentized residuals which more clearly indicates this pattern. Referring to the raw data, these three

higher values characterize the lower maltodextrin encapsulation values and are likely coherent with the model.

Table 8. ANOVA regression model for astringency in peanut paste formulations.

Source	Degrees Freedom	Sum of Squares	Mean Squares	F-value	p-value
<i>Model</i>	5	1.3	0.26	4.69	0.0336*
X_1	1	0.77	0.77	13.9	0.0073*
X_2	1	0.10	0.10	1.84	0.2171
X_1^2	1	0.37	0.37	6.71	0.0365*
X_2^2	1	0.040	0.040	0.729	0.4269
X_1X_2	1	0.040	0.040	0.721	0.4236
<i>Lack of Fit</i>	3	0.19	0.062	1.24	0.4054
<i>Pure Error</i>	4	0.20	0.050		
<i>Residual Error</i>	7	0.39	0.056		*denotes significance

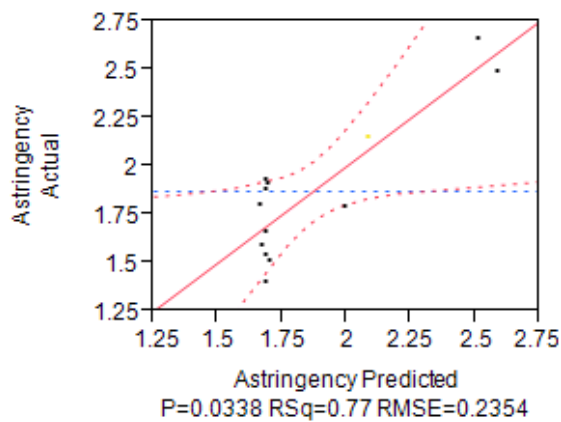


Figure 29. Actual vs predicted astringency intensity response.

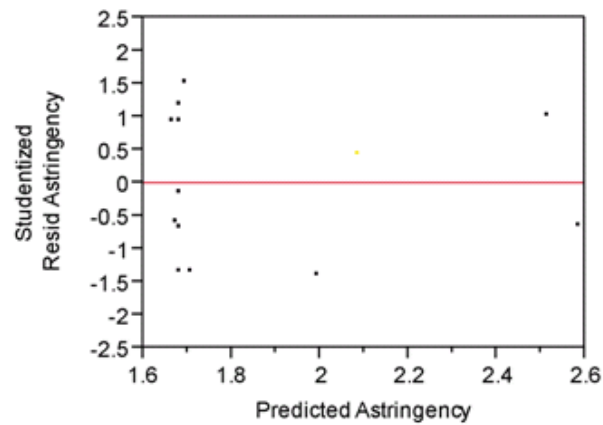


Figure 30. Predicted astringency intensity vs studentized residual plot.

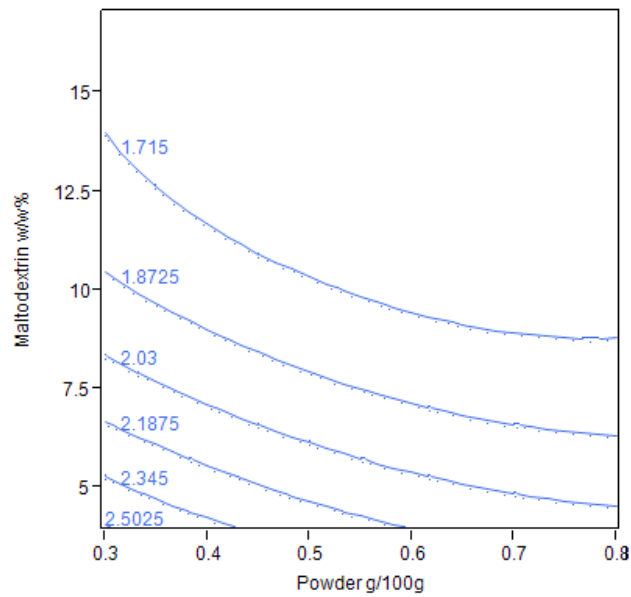


Figure 31. Contour plot of astringency intensity response surface.

The contour plot for astringency response is depicted in Figure 31 which shows that astringency decreases with increasing maltodextrin encapsulation, the significant regression coefficient ($p=0.0073$). Interestingly, product inclusion was slightly negatively correlated to

astringency ($r=-0.2459$) but the effect was not significant ($p=0.2174$). There was not a significant interaction factor.

3.5.6 Multivariate Analysis of Central Composite Design

Multivariate analysis was conducted on the two independent variables and the three response variables as shown in Figure 32. Overall, grams product per 100g peanut paste was a very poor predictor of the response variables as shown in Figure 32. Across all response variables, maltodextrin was consistently indicated as the significant regression coefficient with high correlation values. These results may be in part being due to the much larger dilution by the encapsulation process than occurred in product formulation. The low correlation ($r=0.2999$) between grams product in the formulation and the resulting Trolox equivalence versus its correlation with maltodextrin ($r=-0.7457$) supports this hypothesis.

A similar trend was observed in attribute intensity responses generated by the panel. This was further complicated due to the panel's inability to differentiate the products due to the very subtle changes in bitterness and astringency. Scores for bitterness and astringency ranged across less than 1.5 points on the Spectrum® scale for all 13 experiment formulations. Despite this maltodextrin was clearly negatively correlated to response intensities. As predicted, resulting Trolox equivalence of the formulations were positively correlated with bitterness ($r=0.6648$) and astringency ($r=0.5065$) intensity. The two attribute intensities were also highly correlated with each other ($r=0.7614$) as would seem logical as these attributes are directly related to Trolox equivalence.

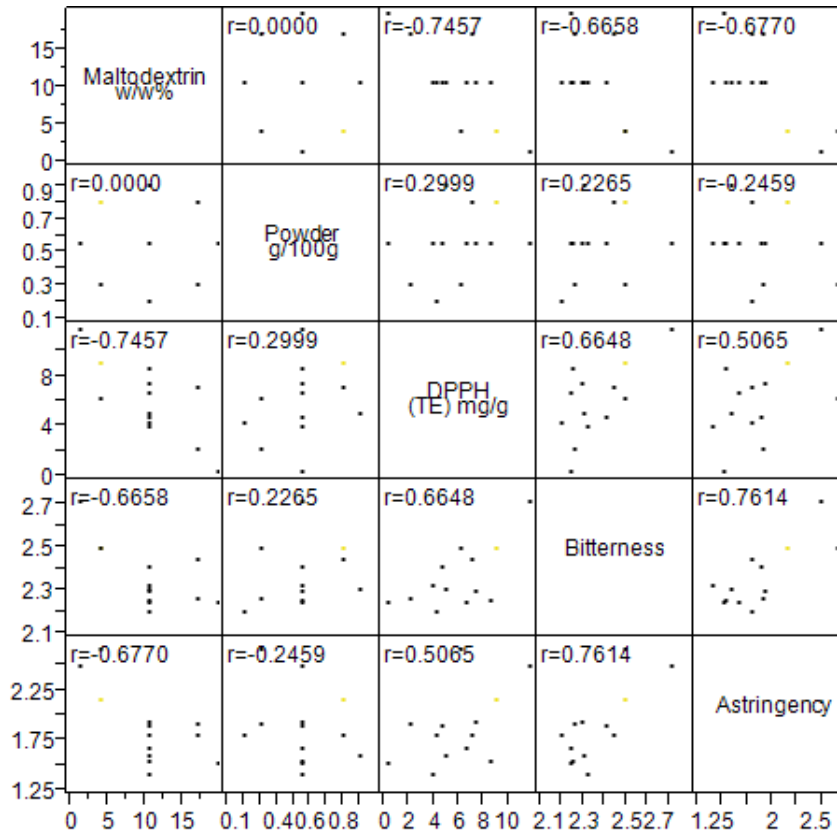


Figure 32. Multivariate analysis of independent and response variables.

3.5.7 Desirability Formula Optimization of Both Independent Variables

Desirability functions were generated to optimize the response variables; minimizing bitterness and astringency and maximizing Trolox equivalence. Desirability values of 1 and 0 were assigned to bitterness and astringency responses 1.0 and 3.0 respectively. A desirability value of 1 was assigned to a Trolox equivalence response of 12.0 and a desirability value of 0 to a 0 $\mu\text{mol/g}$ response. Each factor was assigned equal weight in the desirability function.

The desirability optimization was run with 20 trips, 250 iterations, and a convergence tolerance of 0.000001 for a max of 50 cycles.

For the model, the greatest desirability (0.61) was achieved with a maltodextrin (w/w) percentage of 5.92 and a product inclusion of 0.75 g per 100g paste. With these parameters, there is a 95% confidence that Trolox equivalence will lie in the range of $8.41 \pm 2.87 \mu\text{mol/g}$, bitterness intensity of 2.43 ± 0.105 , and astringency of 1.92 ± 0.31 .

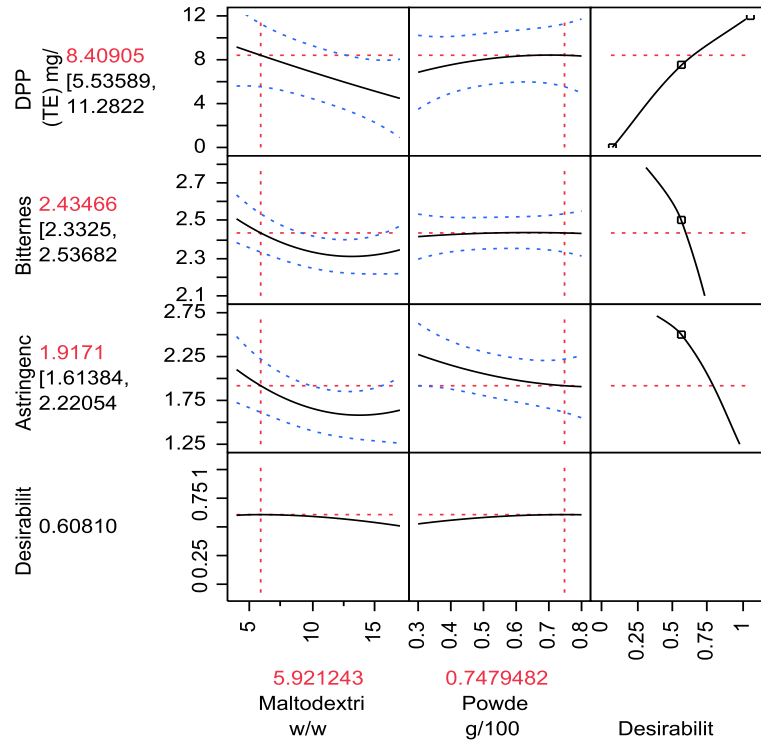


Figure 33. Desirability optimization

Analysis of the current response surface model suggests that the chosen range of 0-1.0g peanut skin extract powder per 100g peanut paste is too narrow to generate a reliable and meaningful response model. Product inclusion level was not a significant coefficient and was not highly correlated to the response variables. This can be largely contributed to two factors: 1) the greater dilutions created by the encapsulation process versus the different

dilutions created in product formulation and 2) the difficulty in differentiating changes in bitterness and astringency across the chosen variable ranges. This may have been an effect of panel habituation and/or a combination of panel timidity to use higher values on the scale as described by Meilgaard, Civille, and Carr (1999). Another possibility is that the product inclusion range tested lies below the recognition threshold of the panelists. Further analysis, however, would be required to validate either hypothesis.

The available data, however, does suggest that maltodextrin encapsulation lowers bitterness and astringency intensities. Yang (2012) also evaluated the effectiveness of maltodextrin encapsulation to decrease bitterness in whey protein hydrolysate using the taste dilution analysis method. To ensure decrease in bitterness was not an effect of dilution alone, mechanically blended whey protein and maltodextrin samples were included in the analysis. Yang found that encapsulation decreased bitterness intensity by a factor of 8 and was twice as effective as the mechanically blended samples. Inclusion of similar studies in future research could further validate the effect of maltodextrin encapsulation in mitigating the negative sensory attributes of polyphenol-enriched functional food products.

The results of this study are also promising for practical application in further developing peanut skin extract enhanced peanut butters. The thirteen formulations surveyed by the current study were consistently within the range of bitterness and astringency intensities reported in commercial peanut butter. Furthermore, the peanut paste was able to possess a Trolox equivalence as high as $841 \pm 287 \mu\text{mol}$ per 100g serving of peanut paste without significantly increasing bitterness or astringency. As a comparison, this is similar to strawberries ($1100 \pm 300 \mu\text{mol TE}/100\text{g}$) as reported by Vasco (2008). These results suggest

that the polyphenol content could possibly be increased to an even higher level without exceeding the acceptance threshold of consumers. In the future, a less conservative survey of maltodextrin-encapsulated peanut skin extracts may return a response surface model of higher quality and reliability.

3.5.8 Determining Absolute Recognition Threshold of Maltodextrin-encapsulated Peanut Skin Extract

After analysis of the central composite design, it became obviously necessary to determine the absolute recognition threshold level of product inclusion for future studies. Chocolate was chosen as the matrix as it is the third leading use of peanut products and is frequently associated with health due to its polyphenol content. The central concentration of maltodextrin (10.5% w/w) powder was chosen for use in determining the threshold in chocolate. A total of 24 panelists familiar with the triangle test were recruited from various USDA and university-based sensory panels. Individual BET values were calculated from results indicated on panelist ballots as shown in Table 9.

Table 9. Triangle 10.5% maltodextrin peanut skin extract powder in chocolate results.

Peanut Skin Extract Powder (w/w) % in Chocolate						
Panelist	0.2	0.4	0.8	1.6	3.2	IND BET
1	n	0	0	0	y	2.26
2	0	0	n	y	y	0.67
3	0	0	y	y	y	0.57
4	y	y	y	y	0	4.53
5	y	n	n	n	0	4.53
6	0	y	0	0	y	2.26
7	0	0	n	0	y	2.26
8	0	0	0	0	0	4.53
9	0	0	0	y	y	1.13
10	0	n	0	0	n	2.69
11	n	y	y	y	0	4.53
12	0	y	0	y	y	1.13
13	0	0	0	n	0	4.53
14	n	0	0	0	0	4.53
15	0	0	n	0	y	2.26
16	0	0	n	0	y	2.26
17	n	0	0	0	y	2.26
18	0	0	n	0	y	2.26
19	0	0	y	0	0	4.53
20	0	0	0	0	y	2.26
21	y	n	y	0	0	4.53
22	0	n	0	0	y	2.26
23	0	y	y	0	y	2.26
24	n	0	n	n	0	4.53
Group BET						2.50

*y denotes panelist indicated guess was “sure”

*n denotes panelist indicated guess was “unsure”

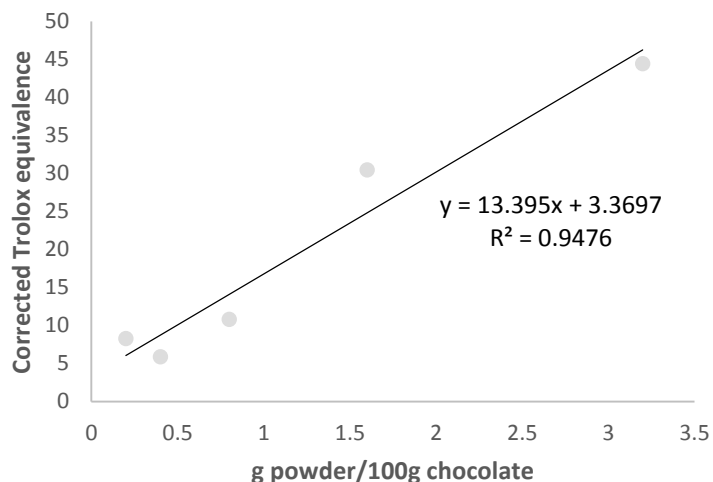


Figure 34. Corrected trolox equivalence of dosed chocolate.

This preliminary threshold study reported a best estimate group threshold of 2.50g per 100g food matrix for 10.5 (w/w) % maltodextrin-encapsulated peanut skin extract. The Trolox equivalence of the dosed milk chocolate samples was measured with the control chocolate having a value of $17\mu\text{mol/g}$. Shown in Figure 34, the Trolox equivalence of the dosed samples corrected for the natural content of the chocolate matrix is plotted against the product inclusion amount. The regression equation $y=13.395x+3.3697$ ($R^2=0.9476$) was generated and used to determine the group BET is equivalent to an increase of $36.9\mu\text{mol TE}$ per gram chocolate. This well surpassed the Trolox equivalence of dark chocolates which is approximately $21\text{-}24\mu\text{mol/g}$ (Brcanovic 2013). These results suggest peanut skin extract could be used to supplement the polyphenol content of milk chocolate to that of dark chocolate without surpassing the detection threshold. Further product inclusion, however, would be capped at the yet to be determined level which the maltodextrin content negatively effects the color and texture of the products.

3.6 Conclusions

In developing a response surface model, the chosen range of product inclusion levels proved to be too conservative to generate a reliable and meaningful model. This was further complicated by the difficulty in differentiating changes in the measured sensory attributes. However, the data tentatively suggests maltodextrin encapsulation lowered the intensity of bitterness and astringency in the final product. Further research will be needed to validate this hypothesis. Despite this, peanut pastes were fortified to a Trolox equivalence comparable to that of strawberries without exceeding the current range of bitterness and astringency intensities cited in commercially available peanut butters. A preliminary threshold test in chocolate determined a best estimate threshold of 2.50 (w/w)% of maltodextrin encapsulated peanut skin extract. The results of the current study strongly suggest that a broader range of product inclusion levels should be explored in future studies.

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SUMMARY AND SUGGESTIONS FOR FURTHER WORK

This research suggests that peanut skins are a viable source of polyphenols for use as a functional food ingredient. The current study found possible solutions to two challenges preventing the commercialization of peanut skin extracts: the possible high levels of arsenic and cadmium concentrated from peanut skins by the extraction process and the negative sensory attributes of the phenolic profile present in peanut skins.

Peanut hulls were shown to be effective for the removal of cadmium ions from peanut skin extract. Precipitation was also shown to contribute to overall removal of cadmium and arsenic in the peanut skin extract. Further work will be needed to characterize the degree and mechanism by which the two metal ions precipitate. Speciation of the forms of arsenic present in the peanut skin extract will also be critical to making final plans for the remediation process before scale-up experiments can be conducted.

Fortified peanut paste formulations with Trolox equivalences as high as 841 ± 287 μmol per 100g serving could be achieved without significantly increasing bitterness or astringency. Within a chocolate matrix, an antioxidant level of 3690 μmol per 100g was determined to be a best estimate detection threshold. Study results suggest that maltodextrin encapsulation decreased the negative sensory attributes of the polyphenol extract powder. Comparison of encapsulated peanut skin extract powder with a mechanically blended negative control will be needed to determine results were not an effect of dilution alone.

Finally, as peanut skin extract moves closer to use as a functional food ingredient, it will be important to determine consumer attitudes and perceptions of polyphenol-fortified

peanut products. Determining rejection thresholds and consumer acceptance tests will be critical to producing a healthy and enjoyable product for consumers.