ABSTRACT

CHRISTMAN, LINDSEY MORGAN. Biological Activity of Peanut Skins as a Functional Food Ingredient. (Under the direction of Dr. Lisa Dean)

Peanut skins, which comprise about 3 grams per 100 grams of the peanut seed, are currently a waste product of the peanut industry. This byproduct is a rich source of phenolic compounds that may be beneficial to human health. The chemical properties of peanut skin phenolic compounds have been well recorded, however very few studies have investigated their biological activity. Also, few studies have been successful in fortifying a food with peanut skins without increasing the intensity of negative sensory attributes such as bitterness and astringency. The main objective of this study was to fill in these two gaps of research. The first objective was to incorporate peanut skins into a food, without the negative sensory attributes. The second objective was to investigate the anti-diabetic effect of peanut skins both in vivo and in vitro.

In order to investigate the use of peanut skins as a functional food ingredient, peanut skin extract was fortified into a coating for peanuts. To reduce the bitterness and astringency, the phenolic compounds were extracted from peanut skins using 70% (w/v) ethanol and this extract was then encapsulated in 10.5% maltodextrin (MD) with a spray drying process. The peanut skin extract (PSE) was incorporated into chili lime and honey roasted coatings for peanuts at varying levels. The highest level that could be incorporated without associated negative sensory attributes was determined by a threshold test and descriptive analysis. The total phenolic content and antioxidant activity of peanut skin fortified coated peanuts was determined using the Folin Ciocalteu, DPPH, and β-carotene bleaching assays. The sensory threshold was determined to be 12.8% and 16.6% for honey roasted and chili lime coated peanuts respectively. The antioxidant activity of the coated peanuts at the threshold was found to be significantly higher than the unfortified control peanuts for both assays done. The measured antioxidant activity of these
coated peanuts at the threshold was found to be comparable to or higher than common fruits, such as blueberries and strawberries.

The antidiabetic effect of peanut skins was investigating using HepG2 cells and an oral glucose tolerance study in humans. A state of toxic hyperglycemia was induced in HepG2 cells by exposing them to 160 mM glucose dissolved in media for 24 hours. The treatment of cells with the high glucose and 2.5% PSE was found to protect the cells from the decrease in cell viability caused by the hyperglycemic state. These results indicated that PSE may play a protective role in the liver during hyperglycemia. In the second part of this study, the effects of peanut skins on the glycemic response in humans to a glucose load was investigated. Fifteen participants (7 male and 8 female) aged 21-32 underwent an oral glucose tolerance test with five treatments: 1) 50 g glucose solution (reference); 2) 50-gram glucose solution, followed by 12 mg of vegi-capsulated maltodextrin (MD) (placebo); 3) 50-gram glucose solution, followed by 120 mg of vegi-capsulated MD-encapsulated PSE; 4) 50-gram glucose solution, followed by 28 grams (1 serving) of unfortified coated peanuts; 5) 50-gram glucose solution, followed by 28 grams of chili lime coated peanuts fortified with PSE. The glycemic response to each treatment was assessed by calculating the area under the 2.5-hour blood glucose curve. The glycemic profile was also calculated by dividing the incremental blood glucose response by the post-prandial glucose peak. The addition of peanut skin extract (PSE) and peanut skin extract fortified peanuts to the 50-gram glucose solution did not have a significant effect on the area under the curve. However, the addition of PSE did cause an increase in the glycemic profile, suggesting that they facilitate glucose regulation. This research further verifies the value of peanut skins as a functional food ingredient due to the benefits to human health.
Biological Activity of Peanut Skins as a Functional Food Ingredient

by
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DEDICATION

I dedicate this work to my parents and grandparents.
BIOGRAPHY

Lindsey Christman was born on September 22\textsuperscript{nd}, 1993 and grew up in Pittsburgh, PA. She received her Bachelors of Science in Biochemistry at Elon University in 2016. She then decided to work towards her Master’s degree in Food Science at North Carolina State University under the direction of Dr. Lisa Dean in the USDA-ARS Market Quality and Handling Research Unit. After graduation, Lindsey plans to pursue a career in food research and development.
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CHAPTER 1: LITERATURE REVIEW

Introduction

The peanut (*Arachis hypogea* L.) is one of the world's most popular and universal crops and is cultivated in nearly 100 countries on all six continents. The plant originated in South America and was carried to Africa by early explorers and missionaries. They were then brought from Africa to North America by slave traders in the early colonial days (Nwokolo 1996). The USA is now one of the world’s largest producers, producing 1.9 million tons of peanuts annually (American Peanut Council 2017).

The peanut is not a true tree nut but is a legume. It is the second most economically important legume worldwide behind soybean, and the fourth most important oilseed crop (Franscisco and Resurreccion 2008). Globally, peanuts are mainly used for oil production, however in the US 85% of production is processed for the production of peanut butter, confections, roasted peanuts, and snack products (American Peanut Council 2017). The use of peanuts in these foods is mainly due to the high protein, unsaturated fat, carbohydrate, vitamin, and mineral content, making it beneficial for nutrition (Arya and others 2015).

In the US, the most common peanut cultivars are virginia, spanish, valencia, and runner. Each has specific compositional and flavor characteristics, allowing them to be used for different food products. Runners are the dominant type in the US, accounting for 70% of the total US production, and are used mainly for peanut butter and candy production (American Peanut Council 2017). Virginia type pods and seeds are the largest size and account for most of the peanuts that are roasted and sold in shell. A total of about 22% of the US production is the virginia type and are produced mainly in Texas and the Virginia-Carolina area (Pattee and Stalker 1995). The spanish type peanuts account for only 4% of US production and are used
predominantly in peanut candy. They also have a higher oil content than other types of peanuts, making them useful when crushing for oil (American Peanut Council 2017). Valencias account for less than 1% of the total US production and are grown mainly in New Mexico. These are a very sweet peanut and are usually roasted and sold in shell (American Peanut Council 2017).

Consumption of peanuts has been associated with numerous health benefits, such as prevention of cardiovascular disease, type-2 diabetes, cancer, and other degenerative diseases (Arya and others 2015). There is substantial epidemiological evidence that tree nuts and tree nuts is related to a reduced incidence of coronary heart disease. In multiple studies examining the effects on cardiovascular health, diets with high consumption of peanuts lowered total cholesterol, LDL- cholesterol, the LDL: HDL ratio and serum triglycerides (Kris-Etherton and others 2008). These health benefits are mainly due to the low levels of saturated fatty acids, high levels of unsaturated fatty acids, micronutrients, and phytochemicals present in peanuts.

The edible portion of the peanut consists of both the kernel and the protective skin. The skin is a distinctive structure that is critical for development, as it functions to regulate nutrient uptake and defend against environmental stresses such as fungal infection (Pattee and Stalker 1995). They comprise about 2.6% (w/w) of the peanut seed and are removed before, or during, the blanching and roasting processes done in preparation for the production of most peanut products, such as peanut butter (Zhao and others 2011). Peanut skins are therefore a low-value byproduct of the peanut processing industry, with an estimated world production of 750,000 tons annually. They contain 12% protein, 72% carbohydrate, and can range in fat content from 8-35% depending on variety (Yu and others 2006). The skins have been found to contain the highest concentration of phenolic compounds in a peanut (Yu and others 2006). Regardless of
their low cost ($12-20/ ton) and high potential for health benefits, the use of this waste material is generally limited to use in animal feed or is incinerated (Zhao and others 2011).

**Plant Phenolics as Natural Antioxidants**

Phenolic compounds are ubiquitous to all plant organisms, and food products such as fruits, vegetables, whole grains, tea, chocolate, and wine are rich sources. They are secondary metabolites generally involved in defense against ultraviolet radiation or pathogens in plants (Pandey and Rizvi 2009). All phenolic compounds possess more than one phenolic hydroxyl group attached to one or more benzene rings, yet over 8000 different phenolic structures have been reported (Pandey and Rizvi 2009). Although all contain the same backbone, they are classified due to differences in the number of phenolic groups they contain and the structural elements attached to these rings (Balasundram and others 2006).

Recently there has been an increase in the interest in food phenolics due to their antioxidant and free radical scavenging abilities, and potential to enhance human health by alleviating or treating oxidative stress in the body (Pandey and Rizvi 2009). Oxidative stress has been linked to aging and multiple degenerative diseases, such as cancer, cardiovascular disease, and Alzheimer’s disease. Increased dietary intake of phenolic compounds with antioxidant activity has been recognized as a preventative measure against disease caused by oxidative stress and has also shown to have a beneficial effect in the treatment of disease (Pham-Huy and others 2008).

**Oxidative Stress**

Oxidation reactions, involving the transfer of electrons from one atom to another, are an essential part of aerobic respiration and metabolism. However, problems may arise when an excessive amount of free radicals are generated (Gülçin 2012). Free radicals contain an
unpaired electron, resulting in a highly unstable and reactive molecule that can either donate or accept an electron from other molecules. Free radical formation is a chain reaction, including initiation, propagation, and termination steps, as shown in Figure 1. In the initiation step, an electron is removed from a molecule. This removal of an electron can result from normal essential metabolic processes in the body, or from exposure to external sources such as X-rays, ozone, air pollutants, and industrial chemicals (Lobo and others 2010). After initiation, propagation of the free radical occurs, in which the free radical reacts with oxygen and other stable molecules to form reactive oxygen species (ROS), reactive nitrogen species (RNS), and new free radicals (Lobo and others 2010). As propagation continues, a state of oxidative stress can occur if the levels of free radicals become too high. Termination is the last step, in which the free radicals react to form a non-reactive product (Shahidi and Zhong 2007).

\[
\text{Initiation} : \ LH \rightarrow L \cdot + H \cdot \\
\text{Propagation} : L \cdot + O_2 \rightarrow LOO \cdot \\
LOO \cdot + LH \rightleftharpoons LOOH + L \cdot \\
\text{Termination} : 2LOO \cdot \rightarrow \text{non radical product} \\
LOO \cdot + L \rightarrow \text{non – radical product} \\
L \cdot + L \rightarrow \text{non – radical product}
\]

**Figure 1**: Schematic mechanism of free radical formation

Under normal conditions, there is a balance between the generation of these reactive species and innate antioxidant defenses. At these low concentrations, ROS and RNS are necessary for the maturation of cellular structures and can act as weapons for the host defense system (Pham-Huy and others 2008). However, when an imbalance occurs and free radicals are produced in excess, a state of oxidative stress occurs. At these high concentrations, free radicals
can react with and damage macromolecules, such as DNA, lipids, and proteins (Pandey and Rizvi 2009)

Mechanism of Antioxidant Activity

Antioxidants are classically defined as “any substance that delays, prevents, or removes oxidative damage to a target molecule” (Moon and Shibamoto 2009). Antioxidants are classified as primary or secondary antioxidants depending on their mechanism of action. Primary antioxidants neutralize free radicals resulting in the termination of the radical chain reaction. In the case of polyphenolic compounds, they are able to neutralize ROS by accepting or donating electrons (Shahidi and Zhong 2007). The structure of the resulting phenolic radical allows it to be stable due to resonance delocalization around the aromatic ring (Pham-Huy and others 2008). Secondary antioxidants can slow the oxidation rate through several mechanisms, including chelation of metal ions, regeneration of primary antioxidants, decomposition of hydroperoxides, and scavenging of oxygen (Shahidi and Zhong 2007)

The structure of phenolic compounds affects their radical scavenging ability. Their activity depends on the number and arrangement of the hydroxyl groups in the molecule of interest because this determines the level of delocalization that can occur when it becomes a radical itself (Heim and others 2002). Glycosylation or methylation of the hydroxyl groups has also been reported to affect the antioxidant activity of phenolic compounds. Cai and others (2006) found that almost all flavonoid glycosides had lower radical scavenging activity than their corresponding flavonoid aglycones. Glycosides of flavonoids have one or multiple sugar moieties linked to a hydroxyl group, resulting in a decrease in the number of free hydroxyl groups available for radical scavenging (Yokozawa and others 1998; Heim and others 2002)

Measurement of Antioxidant Activity
There are various methods for evaluating the antioxidant potential of a food (Shahidi and Zhong 2015). Depending on the type of reaction, these methods can be classified into two types; electron transfer reaction (ET) and hydrogen transfer reaction (HAT) based methods (Shahidi and Zhong 2015). HAT based methods measure the ability of an antioxidant to donate a hydrogen in order to neutralize free radicals, while ET based methods measure the ability of an antioxidant to donate an electron in order to reduce any compound (Shahidi and Zhong 2015). Common HAT methods include oxygen radical absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP) and \( \beta \)-carotene bleaching assay, while Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), and DPPH are common ET methods (Gülçin 2012).

The DPPH method measures the radical scavenging activity of antioxidants. This assay is based on the electron donation of antioxidants to neutralize a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Shahidi and Zhong 2015). The delocalization of the spare electron in the DPPH radical allows for a stable radical and results in the deep violet color that can be characterized by an absorbance band measured at 517 nm (Molyneux 2007). As a result, the reduction of DPPH\(^+\) by antioxidants causes a color change of the molecule to yellow and a decrease in absorbance measured at 517 nm (Dudonne and others 2009). The reaction of DPPH\(^+\) with an antioxidant is shown in Figure 2.
Figure 2: Reaction between DPPH$^+$ and an antioxidant to form DPPH (Moon and Shibamoto 2009)

The antioxidant activity measured by this method is often reported as EC$_{50}$, the effective concentration of antioxidant required to decrease the initial DPPH$^+$ concentration by 50%, or as Trolox Equivalents (TE) (Molyneux 2007). The lower the EC$_{50}$ and the higher the TE value, the greater the antioxidant activity. It has been argued that scavenging of DPPH$^+$ does not mimic real food or biological systems since it has no similarity to peroxyl radicals involved in lipid oxidation (Huang and others 2005). Nevertheless, antioxidant activity observed using DPPH for many grains, vegetables, and fruits had similar trends to those previously reported using ORAC, FRAP, and SOD, and is still one of the most widely reported method (Dudonne and others 2009).

An example of a HAT based antioxidant assay is the β-carotene bleaching assay. This method measures the ability of an antioxidant to inhibit the lipid peroxidation of lineolic acid. This is based on the principle that linoleic acid is oxidized by ROS produced in a heated system (Juntachote and Berghofer 2005). The linoleic acid free radicals will then oxidize β-carotene, resulting in a loss of the B-carotene color that is absorbed at 470 nm (Juntachote and Berghofer 2005). The rate of this color loss can be slowed in the presence of antioxidants. The antioxidant activity is then reported as the percent inhibition of β-carotene bleaching (Prior and others 2005).
The Folin Ciocalteu assay is a rapid and simple estimation of total phenolic content in food and plant extracts. (Shahidi and Zhong 2015). This assay is based on the reduction of the Folin Ciocalteu reagent by phenolic compounds transferring electrons to the molybdenum center of the reagent, yielding a blue colored chromophore with a maximum absorption at 765 nm (Ainsworth and Gillespie 2007). This assay was originally used for the analysis of proteins but now is often used for the characterization and standardization of botanical samples. However, high sugar content and aromatic amines that are present in the sample can react with the reagent and will give artificially high value for total phenolic content (Shahidi and Zhong 2015).

Although total phenolic content is not a direct measurement of antioxidant activity, various studies have found a correlation between antioxidant activity and total phenolic content (Velioglu and others 1998; Jayaprakasha and others 2008; Piluzza and Bullita 2011) Ghasemi and others (2009) studied this correlation in 18 citrus species and found that in general, the species with the highest radical scavenging activity showed the highest phenolic content as well. These results demonstrate that, although there may be other antioxidants present in plant materials, the phenolic compounds make a significant contribution to their antioxidant activity and therefore should be included in the analysis of antioxidant activity.

**Phenolic Compounds in Peanut Skins**

Peanut skins have been found to be rich in phenolic compounds that may be beneficial to human health. Phenolic compounds typically concentrate on the outer layers of plants to protect the inner core materials, leading to their high concentration in the skins (Ma and others 2014). Yu and others (2005) found that phenolic compounds in peanut skins belong to several classes including phenolic acids, stilbenes, and flavonoids. The phenolic content has been found to be sensitive to processing methods. Roasting has been found to increase the yield of phenolic
compounds, while water blanching resulted in a decrease in the yield (Yu and others 2006). During roasting, phenolics or phenolic like complexes may be formed as products of the Maillard browning reaction, contributing to this observed increase. Mild heat treatments may also lead to higher yields as a result of the number of free, reactive phenolic compounds due to the heat breaking covalent bonds between phenolic acids with components of the cell wall (Ma and others 2014). Extraction procedures have also been shown to have an effect on yield results. Nepote and others (2002) extracted phenolic compounds from peanut skins through solid-liquid extraction with hot water, ethanol, methanol, ethyl acetate, and aqueous mixes. The results demonstrated that methanol, ethanol and acetone extracts exhibited the highest phenolic content respectively, while water extracted the lowest amount of dry matter. The total antioxidant and free radical activity of water and ethanol extracts of peanut skins were found to be higher than that of green tea and vitamin C (Yu and others 2005). These results suggest that peanut skins could be used as a functional food ingredient because of their high antioxidant activity.

Classification of Phenolic Compounds

Peanut skins have been found to contain phenolic compounds within the classes flavonoids, phenolic acids, and stilbenes. Flavonoids are the most common and widely distributed group of plant phenolic compounds and are responsible for the color of most fruits, flowers, and seeds (Heim and others 2002). The general structure of a flavonoid is a 15 carbon backbone, consisting of two benzene rings (A and B) linked by a heterocyclic pyrane ring (C), as shown in Figure 3. They are divided into a variety of classes based on their level of oxidation and the pattern of substitution on the C ring (Balasundram and others 2006). The major different classes of flavonoids consist of flavonones, flavones, isoflavonoids, flavanols, anthocyanins and flavonols (Pandey and Rizvi 2009). Substitutions on the rings A and B then divide these
compounds into the different compounds in each class of flavonoid (Balasundram and others 2006).

![Flavonoid Backbone](image)

**Figure 3:** Basic structure of flavonoid backbone (Balasundram and others 2006)

Flavanols are the most abundant flavonoid found in peanut skins, including catechin, epicatechin, gallocatechin, and their gallic esters (Francisco and Ressurrecion 2008). Procyanidins, oligomeric and polymeric combinations of catechins and epicatechin units, are the most prevalent, making up 17% by weight of the peanut skin (Karchesy and Hemingway 1986). Peanut skins were found to primarily contain A-type procyanidins, in contrast to the B-type procyanidins that are found in apples and grape seed (Appeldoom and others 2009). Lou et al (2004) identified six A-type procyanidin dimers from the water-soluble fraction of peanut skins. Catechin, B-type procyanidin dimers, procyanidin trimers, tetramers, and oligomers were also reported to be present at a lower concentration (Lazarus and others 1999).

The next class of phenols present in peanut skins is phenolic acids. The basic skeleton consists of aromatic compounds that possess at least one carboxylic acid. Phenolic acids contain two distinctive carbon frameworks, hydroxycinnamic and hydroxybenzoic structures, as shown in Figure 4 (Pandey and Rizvi 2009). Yu and others (2005) identified several different phenolic acids, including chlorogenic acid, caffeic acid, coumaric acid, and ferulic acid.
The third class found in peanut skins is stilbenes, in the form of resveratrol. Stilbenes are characterized by two benzene rings linked via a 2-carbon methylene bridge (Pandey and Rizvi 2009). They are produced in response to injury and various stresses such as environmental conditions, UV radiation, and fungal infections. Peanut seed coats have been reported to contain significant concentrations of resveratrol, but only accounted for a small percentage of the overall resveratrol content of peanuts by weight (Sanders and others 2000)

**Benefits of phenolic compounds in peanut skins**

*Benefits to health*

Research has suggested that phenolic compounds are beneficial to human health because they are able to act as a natural source of antioxidants (Gülçin 2012). Normal biochemical reactions and increased exposure to the environment result in the generation of ROS and RNS. Oxidative stress is implicated as a cause of cancer, neurodegenerative diseases such as Alzheimer’s, cardiovascular diseases, and can cause cell death and tissue damage leading to heart attack and stroke (Pham-Huy and others 2008).

Epidemiological studies have shown the importance of phenolic compounds in the prevention of certain degenerative diseases such as cardiovascular disease. The effect of tea, apple, and onion consumption on the development of cardiovascular disease was followed in a
study involving 805 older men (Hertog and others 1993). The results of this study showed that an increased intake of flavonoids was inversely related to mortality from heart disease. Similarly, a cohort study on the consumption of soy isoflavones by 405 Japanese postmenopausal women, found that an increase in soy isoflavones reduced the risk of cerebral infactions (Kokubo and others 2007). Phenolics specific to red wine, such as procyanidins, anthocyanins, and resveratrol, are responsible for “the French Paradox”, or the observation that there is a lower mortality rate from cardiovascular disease among people who drink red wine (Yu and others 2005).

**Lipid Oxidation**

Antioxidants can also be helpful in extending the shelf-life of food by preventing lipid oxidation. Lipids are susceptible to oxidative processes in the presence of a catalyst such as heat, light, enzymes, metals, and microorganisms (Shahidi and Zhong 2015). Free radicals can cause this lipid oxidation, producing compounds that degrade the product quality and affect the texture, color, and nutrition of a food product. Susceptibility of fatty acids to lipid oxidation increases with the degree of unsaturation (Shahidi and Zhong 2015). Peanuts contain about 50-55% oil with 30% and 45% of the oil being linoleic and oleic acid, respectively (Nepote and others 2004). Linoleic acid is a polyunsaturated fatty acid and oleic acid is a monounsaturated fatty acid, causing peanuts to be susceptible to the development of rancid and off flavors through lipid oxidation during storage. The development of these undesirable flavors, such as oxidized, cardboard, and painty, is the result of secondary oxidation products such as aldehydes, ketones, and alcohols (Nepote and others 2004). Grosso and Resurreccion (2002) found that during storage, peanuts were reported to have an increased oxidized flavor and decreased roasted peanut flavor over time, which was related to a decrease in overall acceptance.
Antioxidants have the ability to reduce lipid oxidation in foods. Although synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used in many foods to prevent rancidity, their use is now limited and regulated by the Food and Drug Administration due to a growing concern on their health hazards (Gülçin 2012). As a result, natural antioxidants are gaining attention to replace these synthetic antioxidants and prevent lipid oxidation in food. Among others, peanut skins have been studied as food preservatives based on their antioxidant activity. Peanut skins have shown to not only preserve the color but also prevent lipid oxidation in raw ground beef (Yu and others 2010). This study also demonstrated that peanuts skin extract at a concentration of 0.6-1% was found to be as effective as 0.02% BHA, which is the most common synthetic antioxidant in commercial use. Similarly, the addition of 0.06-0.16% of peanut hull extract in sunflower oil was found to exhibit about the same antioxidant capacity as 0.02% BHA/BHT in potato chip during frying and storage (Nepote and others 2002). Munekata and others (2015) evaluated the use of peanut skin extracts on lipid oxidation of chicken patties. They found that when peanut skin extract was incorporated into the patties at 3%, it was able to reduce lipid oxidation, without causing noticeable color change. Peanut skin extracts, at inclusion levels as low as 0.02-0.1%, have also been shown to extend the shelf life of salami, sheep patties, and honey roasted peanuts (Nepote and others 2004; Larrauri and others 2012; Munekata and others 2016;).

**Bioactivity of Phenolics in Peanut Skins**

The antioxidant activity of polyphenols in peanut skins has been evaluated numerous times using *in vitro* chemical methods, however, these results simply imply the mechanisms of *in vivo* biological activity and do not directly show what is happening inside the human body. *In vivo* their biological activity is determined by many factors, including bioavailability. This
includes the absorption, transport, distribution, and retention in the biological fluids, cells, and tissues (Heim and others 2002). Bioavailability appears to differ among various phenolic compounds depending on size and structure of the molecule (Heim and others 2002). This is strongly affected by their metabolism, and the resulting metabolite may exhibit different antioxidant properties than the parent molecule.

The bioavailability of peanut skin phenolic compounds has not been studied extensively. Peanut skins are a rich source of procyanidins, which are not easily absorbed through the gut due to their polymorphic nature and high molecular weight (Manach and others 2004). Bansode and others (2014) used a targeted LC/MS analysis to evaluate the bioavailability of procyanidins present in peanut skins and found that A-type procyanidin was detectable, while B type procyanidin was undetectable. This is in agreement with studies that have found that procyanidin B2 is poorly absorbed, while procyanidin B3 is not absorbed at all in rats (Baba and others 2002; Donovan and others 2002).

Several studies have shown that even after metabolism, phenolic compounds from peanut skin exert a beneficial biological effect. Recent studies have found that these phenolic compounds are able to improve lipid homeostasis, lower cholesterol, and exert antioxidant and anti-inflammatory activity both in-vitro and in-vivo (Bansode and others 2013; Bansode and others 2014; Shimizu-Ibuka and others 2009; Lewis and others 2013). However, more research is needed to better understand the specific biological mechanisms responsible for their activity.

**Phenolics and Diabetes**

Several studies have reported that phenolic compounds may have an antidiabetic effect due to their ability to impact postprandial glucose concentrations and insulin secretion (Pandey and Rizvi 2009). Type 2 diabetes is a complex metabolic disorder that is characterized by
impaired glucose tolerance and insulin resistance leading to hyperglycemia (Bahadoran and others 2013). Hyperglycemia, or elevated blood glucose levels, is suggested to induce oxidative stress from the excessive production of ROS, which plays a role in the development of complications associated with diabetes (Bahadoran and others 2013). A meta-analysis by Liu and others (2014) found an association between a higher consumption of flavonoids and decreased risk of type 2 diabetes. A higher consumption of both tea and coffee, beverages abundant in phenolic compounds, has been linked with lower blood glucose levels and reduced prevalence of diabetes (Panagiotakos and others 2009; Wierzejska & Jorosz 2012). Therefore, phenolic compounds have demonstrated a beneficial effect on the management of blood glucose levels. Several different mechanisms have been suggested for this effect, including reduced intestinal absorption of dietary carbohydrates (Johnston and others 2005), inhibition of enzymes involved in carbohydrate metabolism (Tamura and others 2014), stimulation of insulin secretion, and their antioxidant activity (Bryans and others 2007). Regardless of the mechanism, phenolic compounds effect on postprandial glycemia has been investigated in in-vitro, utilizing animal models, and intervention studies.

**HepG2 cell studies**

The liver is the primary site of glucose metabolism and regulation; therefore, cultured liver cells are often used to study the effects of hyperglycemia on the liver (Chandreasekaran and others 2010). In these studies, hyperglycemic conditions are used to parallel in vivo conditions of hyperglycemic conditions and ketoacidosis of type II diabetes mellitus (Chandreasekaran and others 2010). The cultured liver cell line most commonly used for these studies is HepG2 cells, due to the ready availability and ease of cryopreservation and culture of this cell line (Iyer and others 2010). While HepG2 cells cannot be utilized as a substitute to in vivo experimentation,
immortalized HepG2 cells retain many of the morphological and biochemical characteristics of
_in vivo_ hepatocytes (Thabrew and others 1997) and therefore can serve as a tool to better identify
the cellular mechanisms involved in hyperglycemic conditions.

The 3-(4, 5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay is a
common method used to determine cell proliferation (Chandreasekaran and others 2010). This
colorimetric assay is based on the ability of viable cell’s mitochondrial enzymes to transform the
MTT tetrazolium salt into a dark blue formazan product. The amount of product formed can be
measured by change in absorbance at 570 nm and has been found to be directly proportional to
the number of viable cells (Gerlier & Thomasset 1986). However, the activity determined
through this MTT assay reflects cell metabolism, not specifically cell proliferation (Gerlier &
Thomasset 1986). Therefore, additional tests are needed to confirm this connection.

The trypan blue exclusion test is another commonly used method to determine cell
viability. This method is based on the principle that live cells possess intact cell membranes and
will not allow the negatively charged trypan blue dye to enter the cell. However, dead cells will
have a damaged cell membrane, allowing them to be stained by the blue dye (Tran and others
2011). The blue color can then be readily observed under a microscope and allows for viable
and nonviable cells to be easily distinguished.

To determine function, the activity of several enzymes primarily found in the liver are
commonly assayed. The activity of alanine aminotransferase (ALT) and aspartate
aminotransferase (AST), are commonly measured as a clinical measurement of liver function,
with increasing enzyme activity correlating to liver damage (Green and Flamm 2002). In 1955,
serum AST levels were reported to be elevated in hepatic diseases such as viral hepatitis, with
similar results being found for ALT levels (Haber and others 1995). AST and ALT are liver
enzymes that catalyze the transfer of amino groups to produce hepatic metabolites such as oxaloacetate and pyruvate (Green and Flamm 2002). Damage or injury to the liver causes both enzymes to be released into the plasma, leading to an increase in activity that can be used to evaluate the damage.

*In vitro* studies have shown that high glucose levels cause oxidative stress that leads to damage in the liver and initiates apoptosis in HepG2 cells. Chandrasekaran and others (2010) found that the cell viability of HepG2 cells was significantly decreased when incubated with concentrations equal to or higher than 50 mM glucose for 76 hours. This was accompanied by alterations in morphology and a significant increase in activity of liver enzymes, such as LDH, ALT, and AST. The cell death observed was suggested to be due to increased oxidative stress and increased ROS production, as a result of high glucose concentrations.

The protective effect of phenolic compounds in HepG2 cells exposed to high levels of glucose have been attributed to both their ability to reduce oxidative stress and their effects on regulatory proteins (Chandresekaran and others 2010). N-acetyl-cysteine (NAC), a precursor to an important antioxidant in the body, glutathione (GSH), was found to protect the cells against damage from high glucose levels by attenuating the decrease in cell viability and reversing the morphological changes (Chandresekaran and others 2010). This was accompanied by a reduction in lipid oxidation, suggesting that it reduced the levels of oxidative stress. Similarly, ceria nanoparticles, a free radical scavenger, were able to reverse the effects of high glucose levels on cell viability in HepG2 cells (Shokrzadeh and others 2016). This was most likely due to the ability of nanoceria to inhibit glucose-induced ROS formation. Procyanidins from cocoa have been found to improve liver functionality when exposed to high glucose levels by
impacting key proteins involved in glucose regulation and metabolism (Cordero-Herrera and others 2014).

**Human studies**

Although phenolic compounds have been shown to have positive effects *in-vitro* on hyperglycemia, these results must be confirmed using *in-vivo* studies. This is because there is controversy about the bioactivity of phenolics after metabolism. After metabolism, these compounds are often transformed into methylated, glucuronated and sulfated metabolites in key organs such as the intestine and the liver (Marament and others 2014). Further studies must be used to ensure that the phenolic compounds activity is not affected by their metabolism.

Oral glucose tolerance tests have been used experimentally to study the effect of phenolic compounds on the glycemic response in human and animal studies. The oral glucose tolerance test is a clinical measure of glucose tolerance and is used as a standard method for identifying people at risk of developing type 2 diabetes (Brand-Miller and others 2008). In this method, plasma glucose levels are measured after an overnight fast and after glucose loading. The glycemic response to the glucose loading can then be used as an index of how well a person is regulating the levels of glucose in the body. This glycemic response is often determined from the incremental area under the curve for the blood glucose response after the consumption of a food relative to a reference drink with 50 grams of glucose (Brand-Miller and others 2008). This method can be used to determine if phenolic compounds can lower the glycemic response to a 50-gram glucose reference. High postprandial glycemic responses are a known risk factor for type 2 diabetes and cardiovascular disease (Brand-Miller and others 2008).

A continuous glucose monitor (CGM), which measures the glucose level in the interstitial fluid, can be used to provide glucose profiles during an oral glucose tolerance test (Burge and
The device consists of a sensor that is inserted into the interstitial fluid and continuously generates an electric current that is proportional to the glucose concentration, and a recorder that digitally stores the average sensor current every 5 minutes. The concentration of glucose is then determined based on the electrochemical current generated from the oxidation of glucose to gluconolactone by glucose oxidase (Burge and others 2008). Reduction-oxidation reactions can generate a concentration-dependent current that can be measured by the electrode on the sensor. The glucose information is then calibrated using capillary blood glucose measurements taken 3-4 times a day. Frequent calibration is needed to correct for any variations in the reaction or any fluctuations in glucose and oxygen diffusion at the site of the sensor (Burge and others 2008). The goal of the CGM devise is to assess glycemic patterns and how they are influenced by diets.

Solomon and Blannin (2007) found that supplementation of cinnamon phenolics prior to the 50-gram glucose solution resulted in a significantly reduced plasma glucose response and an improvement in insulin sensitivity. Similar reductions in blood glucose were seen for red wine, coffee, berries, and apple juice (Gin and others 1999; Thom 2007; Torronen and others 2010; Johnston and others 2002). However, Torronen and others (2013) found when berries high in phenolic compounds were eaten with either wheat or rye bread, there was no significant reduction in the glucose area under the curve when compared to the control of bread alone. Instead they found that this treatment did increase the glycemic profile. Glycemic prolife was defined as the duration the postprandial blood glucose level above the baseline fasting level divided by the blood glucose peak and, an increase was suggested to indicate an increase in facilitated postprandial glycemic regulation.
The observation that phenolic compounds can improve glucose tolerance has been suggested to be the result of their ability to delay digestion and absorption of glucose (Tsujita and others 2014). Several studies have found that phenolic compounds have inhibitory activity against key enzymes involved in carbohydrate digestion. Tsujita and others (2014) found that phenolic compounds extracted from peanut skins were effective at inhibiting α-amylase, a key enzyme involved in carbohydrate absorption. Other studies have shown that phenolics such as proanthocyanidins are effective at inhibiting α-glucosidase, the enzyme responsible for breaking down sucrose into glucose (Schafer and Hogger 2007). Suggestion the inhibition of sucrose catabolism would reduce the intestinal absorption and consequently reduce blood glucose levels.

Phenolic compounds have also been found to interfere with intestinal absorption through interaction with glucose transporters. Phenolic acids, such as chlorogenic, ferulic and caffeic acids, have been found to competitively inhibit the Na+ dependent SGLT-1 glucose transporter (Welsh and others 1989). Flavonoids have also been shown to inhibit the GLUT2 transporter (Johnston and others 2005). These in vitro results agree with findings in human intervention studies. A study on the effects of apple juice on an oral glucose tolerance test showed that apple juice with high levels of phenolics affected plasma glucose, insulin, glucose dependent insulinotrophic peptide and glucagon-like peptide-1 concentrations that were consistent with a delay in absorption of glucose (Johnston and others 2002)

Previous attempts at use peanut skins as a functional ingredient

Due to the functional components in peanut skins, its use as a functional food ingredient has been investigated in multiple studies. Francisco and Resurreccion (2012) utilized the phenolic extract of peanuts skin for an antioxidant-rich infusion beverage. The peanut skin extract was spray dried with and without maltodextrin. The infusions were found to be rich in
phenolic content and have high antioxidant capacities, with those containing the spray dried extract having the highest phenolic content. However, the increase in phenolic compounds was accompanied by an increase in bitter and astringent taste reported by a sensory panel, especially those prepared from extracts from runner and Spanish peanuts. These negative attributes are a consequence of runner and Spanish peanuts containing the highest total phenolic content. This increase in bitterness and astringency was found to cause a decrease in overall acceptance of the product by the consumer, indicating this would be a challenge in developing a product with peanuts skins.

Multiple studies have evaluated the use of peanut skins in peanut butter. Similar to the result found by Francisco and Resurreccion (2010), Hathorn and Sanders (2012) found that when peanut skins were incorporated into peanut butter at a level higher than 1 g /100 g peanut butter (PB), it resulted in a decrease in roast peanut intensity, and an increased intensity of the attributes ‘woody’, ‘hulls’, ‘skins’, ‘bitter, and ‘astringent’. In contrast, Sanders and others (2014) found that overall acceptability of formulations with 2.5 g PS/ 100 g PB equaled that of the control. When only analyzing flavor, only formulations with 5.0 g PS/ 100 g PB subjected to roasted, but not blanching, were found to be significantly less acceptable than the control in flavor. An increase in bitterness and astringency was not indicated by taste panelists in that study.

**Negative Sensory attributes of phenolic compounds**

*Negative sensory attributes*

One of the major challenges of fortifying foods with peanut skins is the negative sensory attributes associated with it. Previous attempts to use peanut skins as a functional food ingredient have shown that it causes an increase in astringency and bitterness of the product (Francisco and Resurreccion 2012; Hathorn and others 2012). Taste and flavor are major driving
forces for the consumer when buying food products. In peanut products, consumer acceptability has been found to be positively associated with roasted peanut flavor, while woody/hull/skins flavor and bitter taste are not desirable to consumers (Young and others 2005).

Astringency is defined as a complex group of sensations involving dryness, roughness of oral surfaces and tightening, drawing or puckering of the muscles around the mouth (Lee and Lawless 1991). Polyphenols, specifically procyanidins, are known to be one of the four groups of compounds that contribute to astringency. Most consider astringency to be a tactile sensation because, in contrast to taste sensations, astringency increases upon repeated exposure, suggesting it involves mechanical rather than chemosensory processes (Green 1993).

The most accepted mechanism for astringency involves the interaction between tannins and proline-rich proteins (PRP) in saliva (Bate-Smith 1954). In this mechanism, astringency is the result of the ability of tannins to bind and precipitate proteins, resulting in a decrease in salivary lubrication properties that contributes to the astringency sensation. Charlton and others (2002) put forth a 3-step model for the binding and precipitation of PRPs by polyphenols. This model is shown in Figure 5. In step 1, hydrophobic associations occur between the tannin aromatic rings and hydrophobic sites of the proteins. Simultaneously, hydrogen bonding between the hydroxyl group of tannins and either the carbonyl or amino groups of proteins helps to stabilize the complexes. In step 2, the protein-tannin complexes self-associate through further hydrogen bonding to produce soluble larger protein-tannin complexes that will then aggregate. Finally, the aggregated complexes are large enough to form insoluble sediment and precipitate from solution.
Unlike astringency, bitterness is one of the five basic taste and the recognition is thought to protect the organism from the ingestion of poisonous food components (Chandrashekar and others 2006). Similar to the other five basic tastes, bitter taste reception is mediated by taste receptor proteins residing on the surfaces of taste receptor cells (TCR) within taste buds of the tongue. When bitter compounds bind to these taste receptors, a signal transduction cascade occurs through second messengers that cause changes in the cell, ultimately generating a nerve signal to the brain that is recognized as a bitter taste (Sun-Waterhouse and Wadhwa 2013).

Procyanidins have been identified as one of the key compounds in foods responsible for bitterness and astringency (Sun-Waterhouse and Wadhwa 2013). The molecular structure of phenolic compounds can affect whether they are perceived as bitter or astringent. Astringent polyphenols typically have a molecular weight between 500 and 3000 Da (Peleg and others 1999). This higher molecular weight results in a greater degree of polymerization and therefore an increased ability to bind and precipitate PRPs. Low molecular weight phenolic compounds tend to be more bitter (Hathorn and Sanders 2012). In peanut skins, the low molecular weight phenolic compounds that may cause bitterness are caffeic acid, chlorogenic acid, ellagic acid, and procyanidin monomers (Yu and others 2005). A-type procyanidin dimers, B-type procyanidin dimers, A-type procyanidin trimers, B-type procyanidin trimers, A-type
procyanidin tetramers, and B type procyanidin tetramers found in peanut skins tend to be more astringent (Yu and others 2005).

Reducing these Negative Sensory Attributes

As a result of the negative sensory attributes associated with phenolics, efforts have been taken to reduce the bitter taste and astringency of various food ingredients, both in the formulation and in the delivery technology. The use of certain food formulation has been suggested due to certain interactions between different flavors and tastes (Sun-Waterhouse and Wadhwa 2013). These approaches include the use of strong flavors and sweeteners, bitter blockers, matrix physical modifiers, or inhibitors for the bitter taste receptors. Complex formation with these bitter or astringent compounds has also been suggested to reduce these attributes since these complexes would prevent contact between the bitter molecules and the receptors (Sun-Waterhouse and Wadhwa 2013). For example, cyclodextrin has been shown to form complexes with organic molecules, causing either the solubility of bitter phenolics or the amount of bitter phenolics exposed to taste buds to decrease, resulting in a decrease in the perception of the bitter taste (Pandya 2008).

Taste masking has also been studied at the processing level with the use of processing and delivery technologies (Manlan and others 1990; Ceviker and Unal 2005; Lee and others 2009; Yang and others 2012; Rocha and others 2009). These technologies are based on structural modifications of the bitter compounds during mouth processing, the reduction of the solubility of bitter compounds in saliva, or the alteration of the pH, temperature, and physical properties (Sun-water and Wadhwa 2013). An example of this is encapsulation, as it allows polyphenolic compounds to be enclosed within a coating, reducing the solubility of these compounds by creating a physical barrier to the taste buds (Dziezak 1988).
Spray drying is one of the most used methods of microencapsulation (Yang and others 2012). The spray drying process generally consists of four steps; feed preparation, atomization, drying, and separation of the dried product (Figure 6). In the first step, the feed is prepared by hydrating and homogenizing the core material with the wall material that will encapsulate it. Next, atomization takes place and the particle size of the mixture is reduced. After atomization, the liquid is exposed to the drying gas, resulting in evaporation of the water contained in the droplets. The dried particles can then be collected after separation takes places in a cyclone (Murugesan and Orsat 2012).

Figure 6: Typical Set-Up of a Spray Dryer (Casanova and Santos 2015).

The choice of wall material is very important to ensure efficiency and stability of the product. The wall system is designed to protect the core material, limit volatile losses, and also allow controlled release of the material. Wall materials, such as gums, maltodextrin, soy protein isolate, pectin, and cyclodextrin, have been found to successfully attenuate the bitterness and hygroscopicity of food products (Favaro-Trindade and others 2010; Rocha and others 2009; Yang and others 2012).
Spray drying with maltodextrin with a dextrose equivalence (DE) of 10 or 20 was found to be successful in reducing the bitterness and hygroscopicity of casein hydrolysates (Rocha and others 2009). Similarly, spray dried encapsulated whey protein was found to be significantly less bitter than non-encapsulated whey protein (Yang and 2012). This was suggested to be due to the wall material diluting the concentration of the whey protein and to the wall material reducing the exposure of hydrophobic residues that result in the bitter taste.

The application of maltodextrin encapsulation on peanut skin extract was first reported by Constanza and others (2012). They found that spray dried powders had higher antioxidant activity, total phenolics, and increased solubility than peanut skins alone. Dean and others (2016) investigated the use of maltodextrin encapsulation to mask the negative sensory attributes of peanut skin extract when added to chocolate. It was found that encapsulation of peanut skin extract with maltodextrin at 10.5 % (w/w) allowed for the addition of peanut skin extract to be added at levels high enough to cause a substantial increase in antioxidant capacity, without the negative flavors.

**Conclusion and Summary**

Numerous studies have demonstrated the potential for peanut skins to be used as functional food ingredient. The high concentration and variety of phenolic compounds found in peanuts skins has caused them to demonstrate high antioxidant activity *in vitro*. However, limited studies have demonstrated that this extract can be incorporated in a food without negatively affecting the sensory attributes. More evidence is needed to verify that maltodextrin encapsulation of peanut skins can help to reduce the negative attributes, while increasing the antioxidant activity of a food. Additionally, very few studies have shown the biological activity of peanut skins both *in-vitro* and *in-vivo*. Evidence from both cell culture studies and human
studies is needed to further confirm the potential of this functional ingredient. Through reducing the negative sensory attributes of peanut skins and investigating their biological activity, there is increased opportunity for a potential market for this waste material.


CHAPTER 2: APPLICATION OF PEANUT SKINS AS A FUNCTIONAL FOOD INGREDIENT

Abstract

Peanut skins are a low-value byproduct of the peanut processing industry, with hundreds of thousands of tons being produced annually. Following their removal during the preparation of common peanut products, peanut skins are either discarded or used as a minor component of animal feed. Recent studies have found peanuts skins to be rich in health promoting phenolic compounds and thus have potential to create a market for this material. The aim of this study was to evaluate a new product that included encapsulated phenolic extract from peanut skins in a coating for peanuts. The phenolic compounds were extracted from peanut skins and then encapsulated in 10.5 % (w/w) maltodextrin in order to reduce the bitterness. The encapsulated phenolic extract was added at varying concentrations to both a honey roasted and chili lime coating that was then applied to roasted peanut through a panning process. The resulting total phenolic content and antioxidant potential of the coated peanuts was evaluated by the Folin-Ciocalteu, DPPH, and β-carotene bleaching assay. In order to produce a product that did not have the negative sensory attributes associated with peanut skins, a best estimate threshold for the encapsulated peanut skin extract in both the honey roasted and chili lime coating was found to be 12.8% (w/w) and 16.6% (w/w) respectively, based on the standard method (American Society of Testing Material; ASTM E-679). The total phenolic content and antioxidant capacity for both the honey roasted and chili lime coated peanuts at their threshold was found to be significantly higher that control coated peanuts coated peanuts that did not contain any peanut skins in the coating. The improved antioxidant activity and unchanged flavor profile at threshold levels of peanut skins demonstrate the potential success as a nutraceutical ingredient.
Introduction

Peanuts are an important crop worldwide and have become an important food staple and protein source to the world’s population. Peanut butter, snack nuts, and confections are the most common peanut products in the US, and require only the kernel (Yu and others 2006). The remaining byproducts, including the leaves, skins, hulls, and stems, generally have limited use in animal feed while the remainder is discarded as waste. These byproducts, specifically the skins, have been found to contain the majority of phenolic compounds in peanuts (Yu and others 2006).

Phenolic compounds typically concentrate on the outside of plants to protect the core material, resulting in high concentrations in peanut skins (Ma and others 2014). Peanut skins have been found to be abundant in phenolic acids, flavonoids, and stilbenes (Yu and others 2005). Specifically, procyanidins make up 17% by weight of the peanut skin, and are the predominate phenolic compound (Karchesy & Hemingway 1986). Procyanidins and other phenolic compounds have recently been given attention due to their ability to protect against oxidative stress, and therefore may be beneficial in the prevention of cardiovascular disease, diabetes mellitus, chronic inflammation, and certain types of cancer (Pandey and others 2009). As a result, there is a large potential market for this low-value byproduct as a functional food ingredient to benefit human health.

As consumer interest in healthy food has significantly increased in the last few years, there has been a steady trend in the production of functional foods. Functional foods remain undefined under current US regulation. The Academy of Nutrition and Dietetics position paper states, “Functional foods are whole foods along with fortified, enriched or enhanced foods that have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis at effective levels based on significant standards of evidence” (Sikand and others 2015).
The Global Functional Foods Market is poised to grow at an annual rate of around 7.2% over the next decade, and estimated to reach approximately $3.4 billion by 2025 (Businesswire 2017). The use of these functional food ingredients adds value to a product by addressing the consumer demand for foods with added health benefits (Sikand and others 2015)

Although consumer interest in the health benefits of foods has grown, taste is still a driving factor for consumer purchase (Verbeke 2006). Many nutraceutical products that claim to have high antioxidant activity have natural bitterness and astringency associated with them, due to their high concentrations of phenolic compounds. Bitterness and astringency are generally undesirable, and often inhibit consumer acceptability of food products (Sun-water and Wadhwa 2013). Tuorillo and Cardello (2002) reported that the occurrence of these off flavors decreased the probability of juice liking and consumption, despite the presence of health claims. Consumer acceptance is critical for long-term success of a nutraceutical product, and therefore the functional ingredient must not negatively influence taste.

Previous studies with the use of peanut skins as a functional ingredient have reported an increase in bitterness and astringency (Francisco and Resurreccion 2012; Hathorn and Sanders 2012). However, Dean and others (2016) found that the encapsulation of the peanut skin extract in maltodextrin allowed for it to be incorporated into milk chocolate at levels high enough to increase the antioxidant activity above that of dark chocolate, without impacting the flavor. The current study aimed to evaluate the use of peanut skins as a functional ingredient by incorporating it into a coating for peanuts. The main objectives of the present study are to (1) assess the effect of maltodextrin-encapsulated PSE incorporation into a coating on the flavor and textural attributes of coated peanuts and (2) to determine the effect of PSE incorporation on the potential antioxidant activity of the coated peanuts. The final goal is to determine if peanut skins
can be incorporated at levels high enough to increase the antioxidant activity without impacting the overall flavor and acceptance of the product.

**Materials and Methods:**

**Materials**

Peanut skins produced as a byproduct of the blanching process of Runner type peanuts were obtained from Jimbo’s Jumbos (Edenton, N.C., U.S.A). Maltrin M150 maltodextrin was provided by Grain Processing Corp (Mascatine, Iowa, U.S.A.). Pure pharmaceutical grade ethanol (Decan Laboratories Inc, King of Prussia, PA, USA) was used for preparation of peanut skin extract. TicaPAN 311 (Tic Gums, MD, USA) was used for precoating chili lime peanuts. All other reagents were obtained from Sigma Aldrich.

**Extraction of phenolic compounds**

Peanut skins extracts (PSE) were prepared according to the method of Constanza and others (2012). Briefly, peanut skins were ground to a fine powder using a Blixer-3 food processor (Robot Coupe, Jackson, MS, USA). Extraction was performed under low actinic lighting to minimize degradation of polyphenols under light. The milled skins were extracted with 70% (v/v) pure ethanol and deionized water solution in a 1:5 skins to solvent ratio. The solution was stirred using a Wheaton overhead stirrer (Wheaton Industries, Inc. Milville, NJ, USA) for 20 min at a speed setting of 2.8. The soluble extract was separated from the insoluble plant material through vacuum filtration using Whatman #40 filter paper (GE Healthcare, Marlborough, Mass, USA). Ethanol was then evaporated off of extract using a Buchi Rotovap (Buchi Labortechnik, Switzerland) at 60° C.

**Encapsulation of peanut skin extract**
The aqueous extract was mixed with 10.5% (w/w) maltodextrin. The prepared solution was allowed to hydrate for a minimum of 24 hours. The extract was fed into a Buchi Model B-290 bench scale spray dryer (Buchi Labortechnik, Switzerland) with an inlet temperature of 175°C and an outlet temperature of 90°C. The solution feed pump was set at 30%, the nitrogen flow rate was set at 35 psi, and the aspirator was set at 100%.

**Preparation of peanuts**

Peanuts were blanched by a whole nut blancher (Ashton Food Machinery Co. Inc., Newark, NJ) prior to processing. A P-H-T Fryer model 500 (Henry Penny Corporation, Eaton, Ohio) filled with 8 gallons of peanut oil (Stratas Food, Memphis TN) was used for oil roasting. Following roasting, the peanuts were spread on a wire mesh screen with a cooling fan installed above for cooling to ambient temperature prior to storage.

**Preparation of coatings:**

**Honey roasted peanuts**

This product was prepared as described by Nepote and others (2004). Briefly, roasted peanuts were coated with 33% syrup solution (w/w/w) and 67% dried solid mix. The syrup solution contained 50% sucrose, 35% honey (Burleson Honey, Waxahachi, TX), and 15% distilled water (w/w/w). The dried solid mix contained 70% impalpable sucrose (Domino Sugar, West Palm Beach, Fl), 20% sodium chloride (Morton Salt, Chicago Il), and 10% cornstarch (ACH Food Companies Inc, Oakbrook Terrace, Il) (w/w/w). PSE was incorporated into the syrup solution at seven varying levels (0.4-25.6% (w/w)). Peanuts were placed in a stainless steel confectionary coating pan attachment (Kitchen Aid, De buyer, France) rotating at 30 rpm. The syrup solution was applied first to the peanuts, followed by the dried solid mix to separate the
peanuts. This product was prepared with 85% roasted peanuts and 15% coating. Peanuts were rotated in the panner until evenly coated.

*Chili lime peanuts*

Roasted peanuts were coated in 33% Tic Precoat 311 solution and 67% chili lime seasoning blend (Trader Joe’s, Monrovia, CA). PSE was added to chili lime seasoning at varying inclusion levels (0.4-25.6%). Peanuts were placed in stainless steel panner rotating at 30 rpm. The Tic Precoat 311 solution was first applied to the peanuts, followed by the chili lime seasoning. The product was prepared with 85% roasted peanuts and 15% coating. Peanuts were rotated in panner until evenly coated.

*Human subjects*

Healthy adult subjects (minimum age 18 yr old) were recruited from the Department of Food, Bioprocessing and Nutrition Sciences at North Carolina State University (Raleigh, NC, USA) to participate in the study. The only demographic criteria used to recruit participants for the study was no allergy to peanuts or tree nuts. Testing procedures were approved by the North Carolina State University Institutional Review Board. Participants were compensated with a food treat after completion of the study.

*Threshold test*

A detection threshold of encapsulated PSE in both the chili lime and honey roasted coatings was determined using a 7-series-3-ascending forced choice threshold test with 80 participants. The standard method ASTM E-679 (American Society of Testing Material (ASTM) 2008) was used, where participants were forced to pick the odd sample in a set of 3. Seven series with a step factor of 2 were presented in ascending order and in randomized order. Crackers and water were provided for participants to cleanse the palate between samples.
Individual best estimate thresholds (BET) were calculated using Equation 1 (ASTM 2008). Using the geometric mean of these individual BETs, the group BET was computed.

Equation 1: \[
\text{BET} = \sqrt{\text{highest concentration missed} \times \text{lowest concentration without further misses}}
\]

Consumer test

A central location test (CLT) was performed to evaluate the consumer acceptance of the coated peanuts. The test was carried out in the Department of Food, Bioprocessing, and Nutritional Sciences at North Carolina State University with 100 consumers on one day. Participants were presented with commercially available coated peanuts and peanuts with PSE dosed coatings. The dosed honey roasted peanuts contained 12% (w/w) PSE and the dosed chili lime peanut contained 16% (w/w) PSE. The controls consisted of commercial honey roasted peanuts and commercial chili lime peanuts. Subjects were asked three liking questions based on the nine-point hedonic scale (Peryam and Giradordot 1952), along with three JAR questions, using the five point “just-about-right” scale (Rothman and Parker 2009). The liking questions investigated overall liking of the product, flavor liking, and flavor intensity liking. JAR questions evaluated the intensity of flavor, saltiness, and bitterness of the product by asking if the intensity was too little, just about right, or too intense.

Descriptive analysis

Coated peanuts were evaluated by a trained descriptive sensory panel (n=1, male, n=5 female) using the Spectrum™ universal 15-point intensity scale and the peanut lexicon described by Johnsen and others (1988) and modifications by Sanders and others (1989), as shown in Table 1, with additional textural attributes. Peanuts coated with an unfortified coating were used a reference. Panelists were instructed to take a drink of water and a bite of unsalted ...
crackers to cleanse the palate between samples. The order of presentation of samples was randomized for 3 replications and all samples were given random three digit codes.

Sample extraction

Coated peanut samples were ground to a powder using a coffee mill (Proctor Silex) for analysis. Ground peanut samples were extracted using a Dionex Accelerated Solvent Extractor (Sunnyvale, Calif, USA.). Five grams of sample was mixed with 20 g of sand in a 22-mL extraction cell. A 70:29.5:0.5 acetone:water:acetic acid solution (AWA) was used for extraction of the filled cells. The procedure for extraction was as follows: pressure, 1500 psi; temperature 80°C; 3x5 min flushing cycles; flushing volume, 60%; nitrogen pulse 60 s. Additional AWA was then added to the extracts to bring them to a final volume of 50 mL.

Total phenolic content

The total phenolic content of the peanut samples and the PSE was determined by the Folin Ciocalteu assay protocol adapted by Singleton (1999) but scaled to one-tenth volume. A standard curve was produced from gallic acid at concentrations ranging from 0-750 mg/L. Briefly, 0.1 mL of each sample was added to a test tube along with 6 mL distilled water and 0.5 mL Folin Ciocalteau reagent to a culture tube. After one minute, but less than 8 minutes, 1.5 mL of 20% Na₂CO₃ was added to initiate color development. Each culture tube was brought to 10 mL with the addition of 2.9 mL of deionized water. After the samples were incubated for two hours, the absorbance was measured at 765 nm at 27°C using a Tecan Safire microplate reader (Tecan Group Ltd. Switzerland). A gallic acid standard curve was generated to determine the gallic acid equivalents (GAE).

DPPH radical scavenging assay
The 2,2-diphenyl-1-picrylhydrazl (DPPH) radical quenching assay was performed on the samples according to the procedure by Brand-Williams and others (1995). Briefly, a 40 ppm solution of DPPH in methanol was used. A Trolox standard curve was produced at concentrations ranging from 0-2000 µM. Fifty microliters of each sample was mixed with 2.95 mL of DPPH solution in a labeled culture tube. AWA alone was used as a control. The absorbance values were read after 90 minutes at 515 nm using a Pharamspec UV-1700 spectrometer (Shimadzu, Kyoto, Japan). The percentage of DPPH radical quenched was calculated using Equation 2 (Brand-Williams and others 1995).

\[
\text{Equation 2: } \% \text{ DPPH Quenched} = [1 - (A_{\text{sample}} / A_{\text{control}})] \times 100\%
\]

A Trolox standard curve was generated and the samples antioxidant activity was then calculated as Trolox equivalents.

\textit{B-Carotene bleaching assay}

The β-carotene linoleate system was used to evaluate the antioxidant activity according to the protocol by Jayapraskasha and others (2001). Briefly, 0.2 mg β-carotene was dissolved in 0.5 ml chloroform and then mixed with 20 mL linoleic acid and 200 mL Tween 40. The chloroform was then evaporated off at 40°C using a Buchi Rotovap (Buchi Labortechink, Switzerland). This mixture was then diluted with 10 mL DI water. Oxygenated water was then added to this emulsion with vigorous shaking. Four mL of the emulsion were then added to different test tubes containing 0.2 mL of sample. A control emulsion was prepared as above without β-carotene. The absorbance was measured at 470 nm at time zero, then the tubes were placed in a water bath set to 50°C. After 180 minutes a final absorbance was taken using a Tecan Safire microplate reader (Tecan Group Ltd., Switzerland). The antioxidant activity was
evaluated as percent β-carotene bleaching inhibition using Equation 3 (Jayapraskasha and others 2001).

Equation 3: % inhibition = 100 \times \left(1 - \frac{A_0 - A_T}{A_0^0 - A_T^0}\right)

Where $A_0$ and $A_0^0$ are the absorbance measured at time zero for test sample and control respectively and $A_T$ and $A_T^0$ are the absorbance values measured after 180 minutes of incubation for the test sample and control.

Statistical analysis

Statistical analysis of data from antioxidant activity assays and descriptive analysis was performed using SAS version 9.4 (SAS Inst, Cary, NC). Analysis of variance (ANOVA) was generated for total phenolic content, DPPH, and Beta carotene using PROC GLM and comparison of means using Tukey studentized range test. Significance was established at $P >0.05$. Descriptive analysis was analyzed using ANOVA and means were differentiated using least square means at $P< 0.05$ significant level. Each test was done in triplicate.

Statistical analysis of CLT test was performed using a software package XLStat (Addinsoft, Paris, France). Data from the CLT was analyzed in two different ways. Means of hedonic scores obtained for liking levels of flavor, flavor intensity, and overall liking were calculated and $t$-test was used to determine significant different. JAR questions were analyzed using penalty analysis to determine how each attribute influenced overall mean liking of each sample.

Results and Discussion:

Threshold test

Phenolic compounds are often associated with negative sensory attributes such as bitterness and astringency. As a result, the addition of peanut skin extract to a peanut coating
may alter the flavor. Flavor and texture are the two main driving forces in consumer acceptance of a product, so enhancing the functional properties of the coated peanuts must not destroy the flavor (Sikand and others 2015). Therefore, the detection threshold inclusion level was evaluated to determine the highest concentration of PSE that could be incorporated into the peanut coating without the subjects perceiving it.

The group BET for the honey roasted coating and the chili lime coating was found to be 12.8% and 16.6% PSE inclusion, respectively. These results indicate that the group BET was within the range of concentrations tested (0.4%-25.6%). The results indicated a large amount of variation in individual BET for both coatings (Figure 1 and 2). This variation may be attributed to individual variation in sensitivity to bitter compounds (Drewnowski and others 1997). A small proportion (8% for honey roasted and 12% for chili lime coating) of people were unable to detect the PSE at even the highest concentration, indicating that they most likely have a decreased sensitivity to bitter compounds. Fortunately, this did not result in the group BET to fall outside of the range tested.

**Descriptive analysis**

In order to further verify that the encapsulated PSE did not affect the sensory attributes of the peanut product at threshold inclusion levels, a trained panel analyzed the samples through descriptive analysis. Descriptive sensory analysis can be used to describe and quantify flavor and texture in a food product (Stone and others 1974). The intensity of thirteen sensory attributes was analyzed using the Spectrum™ method (Table 2). The addition of peanut skins to the honey roasted coating at levels higher than 12.8% resulted in a significant increase in the attributes woody/hulls/skins and astringency. The panel did not indicate any significant difference in any other attributes evaluated by the lexicon. The addition of peanut skins to the
chili lime coating at a concentration higher than 12.8% resulted in a significant increase in woody/hulls/skins, astringency and bitterness and a significant decrease in sweet aromatic.

Phenolic compounds have been identified as key components responsible for bitterness and astringency in foods. Molecular weight and polymerization of phenolic compounds are important in the perception of these attributes. Astringency seems to increase, while bitterness decreases with the mean degree of polymerization (Leeschaeve and Noble 2005). Peanut skins contain both polymeric and monomeric phenolic compounds, therefore contributing to both astringency and bitterness (Yu and others 2006). As a result, the intensity of these attributes was increased when the level of peanut skin in the coating was above the threshold, due to a higher total phenolic concentration. In their study evaluating the use of peanut skins utilization in a functional beverage, Francisco and Resurreccion (2012) found that a higher total phenolic content was associated with a higher bitterness and astringency in the product. Bitter and astringency are often perceived as negative attributes in most products and are often cited as the reasons for consumer rejecting plant products (Leeschaeve and Noble 2005).

Encapsulation of peanut skin phenolic extract allowed for inclusion levels at or above 12.8% for the chili lime and honey roasted coating, before these undesirable attributes could be detected. For both products, there was a significant increase in the attributes woody/skin/hulls and astringency at inclusion levels above the threshold. In the chili lime coating there was a significant increase in bitterness above 12.8% inclusion of PSE, however, there was no significant increase in bitterness for the honey roasted coating. This observation can be attributed to the difference in the coating. Sugar has been identified as an important bitter-masking agent (Beck and others 2014). The extra sucrose in the honey roasted coating, in addition to the encapsulation of the phenolic extract in maltodextrin, allowed for the bitter taste.
to be masked at all levels tested. These data do suggest that the encapsulation of peanut skin extract in maltodextrin did help to reduce the bitterness intensity.

*Consumer test*

After determining the detection limit of PSE in a honey roasted and chili lime coating, consumer acceptance was evaluated. Due to the variability in individual BET, subthreshold inclusion levels of PSE in the honey roasted (12%) and chili lime (16%) coatings were used. Overall liking, flavor liking, and flavor intensity liking of the dosed honey roasted peanuts were similar to commercially available honey roasted peanuts (Table 3). Similar results were found for dosed chili lime peanut and commercial chili lime peanuts. However, the overall liking, flavor liking, and flavor intensity liking of the chili lime peanut were found to be significantly lower than that of the honey roasted peanuts.

The impact of the level of flavor, saltines, and bitterness on mean overall liking scores was investigated (Figures 3, 4, and 5). Just about right (JAR) scales are often used in consumer research to measure the intensity of a specific sensory attribute (Lawless and Heymann 2010). An increase in bitter taste is undesirable to consumers in peanut products, so the intensity of bitterness was evaluated to ensure acceptance of the product in the market (Young and others 2005). Penalty analysis combines JAR scores and overall liking tests to determine if the acceptability of a product is decreased if attributes are not optimal, or JAR (Lawless and Heymann 2010). The results of JAR analysis show that a majority (> 50%) of consumers felt that all attributes were just about right for all attributes tested. For the dosed honey roasted sample, penalty analysis showed that too much flavor had an impact on overall liking score. In comparison, too little flavor had an effect on overall liking of the commercial honey roasted peanuts. For the dosed chili lime sample, too much flavor, saltiness, and bitterness was found to
have an impact on overall liking. Unlike the dosed sample, too much saltiness did not affect the overall liking of the control commercial chili lime peanuts.

**Antioxidant activity**

**Total phenolic content**

Phenolic compounds tend to concentrate on the outer layers of plants, such as the peel, shell, and hull, in order to protect the inner core material. Peanut skins have been reported to be rich in phenolic compounds such as catechins, A and B-type procyanidin dimers, trimers, and tetramers (Lou and others 2004; Lazarus and others 1999; Yu and others 2006). The total phenolic content of the coated peanuts was determined by the Folin-Ciocalteu method, using gallic acid as the standard. The gallic acid equivalence (GAE) of the dosed coated peanuts was plotted against the inclusion level of PSE into the coating (Figure 6 and Figure 7). The resulting regression equation for the chili lime coating of \( y = 0.1021x + 0.1364 \) \((R^2 = 0.9912)\) demonstrated that at the threshold, the total phenolic content was 1.9314 ±0.01 mg GAE/g coated peanut. This was a significant increase in comparison to the total phenolic content of the control at 0.14 ± 0.11 mg GAE/g coated peanut. For the honey roasted peanuts, the resulting regression equation was \( y = 0.11939 + 0.07903x \) giving a total phenolic content of 1.13±0.04 mg GAE/gram coated peanut at the threshold. This was also a significant increase in comparison to the control with a phenolic content of 0.12±0.03 mg/g peanut. Both coated peanuts at the threshold had a phenolic content comparable to many common fruits such as apples (0.7396 mg/g), pineapple (0.9404 mg GAE/g), and kiwi (0.8754 mg GAE/g) as shown in Figure 8 (Fu and others 2011).

At the threshold, when the GAE was corrected to represent mg/gram of PSE, the value was found to be 80.8 and 82.7 for honey roasted and chili lime, respectively. There was no significant difference between this value and the GAE found for the PSE before being added to
the peanuts at 77.91±4.32 mg GAE /g PSE. These results were lower than those found by Francisco and Ressureccion (2009) who used a similar extraction conditions. In this study, they found that the total phenolic content ranged from 101.43-280.42 mg GAE/ g PSE depending on market type. Runner and Virgina type peanuts were reported to have a high total phenolic content. The peanut skins used in this study were a waste product of the blanching process and the exact market type is not known. The lower values found in this study may also be due to the additional step of spray drying and encapsulating the extract in maltodextrin. Constanza and others (2012) found spray drying of peanut skin resulted in a decrease in total phenolic content, most likely due to the destruction of phenolic compounds by the heat and the addition of maltodextrin.

**DPPH radical scavenging assay**

The DPPH assay was used to measure the ability of coated peanuts to scavenge free radicals (Yu and other 2006). DPPH is a stable nitrogen free radical that has a characteristic absorbance that can be measured at 517 nm. Reduction of this free radical by an antioxidant causes a color change and a loss of absorbance. The antioxidant activity can then be measured by the degree of discoloration of the solution (Brand-Williams and others 1995). The results of this assay were recorded as Trolox Equivalents (TE). The radical scavenging potential of the coated peanuts increased in a dose-dependent manner for both coatings (Figure 9 & 10). Based on linear regression of Figure 9, the radical scavenging activity of the chili lime coated peanuts without PSE and at the inclusion level threshold of 16.6% PSE was found to be 9.5 ± 0. 9 µmol /g peanut and 19.3 ± 0.9 µmol/ g peanut, respectively. This was a significant increase in radical scavenging activity. Based on the linear regression of Figure 10, the radical scavenging activity of the honey roasted peanuts without PSE and at the threshold was 8.776 ± 0.39 µmol/ g peanut.
and 16.068 ±0.4 µ/ g peanut, respectively. There was a significant increase in radical scavenging activity for both the chili lime and honey roasted peanut at the threshold when compared to the control. The TE of both coated peanuts at the threshold was found to surpass the natural levels in strawberry, which have a reported TE of 15.9 µmol/ gram (Ozgen and others 2006). These values are also higher or comparable to those found by Rodrigues and others (2011) for blueberries, a fruit well known for its antioxidant activity. In this study, the average TE found for blueberries was 16.39 µmol/g blueberry (Figure 11). These results indicate that the encapsulation of peanut skins allows for them to be added into the peanut coatings at a level high enough to make the antioxidant activity of the peanut comparable, or higher in the case of the chili lime coating, to that of a blueberry.

The mean DPPH value of the encapsulated PSE material was found to be 633.31 ± 26.66 µmol TE/ gram PSE (Table 3). This value was higher than that found by Costa de Camargo and others (2017), who reported a DPPH value to approximately 450 µM/ g. The TE of the chili lime and honey roasted coated peanuts corrected to represent per gram PSE was 676.4 and 570.51, respectively. There was a slight significant decrease in TE µmol/g PSE for the honey roasted coating compared to the raw material.

\[ \beta \text{-carotene bleaching assay} \]

The \( \beta \)-carotene bleaching assay was used to measure the coated peanuts’ ability to inhibit lipid peroxidation. This method is based on the principle that lipids, such as linoleic acid will form peroxide radicals in the presence of excess oxygen or heat (Jayaprakash and others 2001). This radical then reacts with \( \beta \)-carotene to form a \( \beta \)-carotene radical, resulting in a bleaching of its characteristic orange color. However, if an antioxidant is present in the solution, it will react and neutralize the linoleic acid radical. The antioxidant potential of the coated peanuts was
expressed as percent bleaching inhibition and plotted against the inclusion level of PSE (Figure 12 & 13). Based on the regression equation, the percent inhibition of the chili lime coated peanuts at the threshold was 46.65±1.09%. This was a significant increase from 2.75±0.105% inhibition measured for the control. The % inhibition for the honey roasted peanuts at the threshold was determined to be 41.09±1.16%. This was a significant increase from the control peanuts at 2.57%±0.69. Based on these results, the phenolic compounds in the extract can most likely reduce the β-carotene bleaching by neutralizing the linoleic free radical.

Conclusion:

Encapsulation of PSE in 10.6% (w/w) maltodextrin allowed for inclusion levels of 12.8% and 16.6% in a honey roasted and chili lime coating for peanuts, respectively. There were no reports of negative sensory attributes of bitterness or astringency at these levels. Furthermore, the overall liking of the dosed coated peanuts was not significantly different than commercially available coated peanuts, showing that they have a place in the market. Addition of the PSE at this level increased the total phenolic content and antioxidant activity as measured by DPPH and β-carotene assays. The total phenolics and DPPH values for the dosed product were comparable to common fruits. These results indicate that peanut skins have feasible potential as a functional ingredient. Additional studies should investigate the antioxidant activity of the PSE and dosed peanuts in vivo.
References


Ozgen M, Reese RN, Tulio AZ, Sceerens JC, Miller AR. 2006. Modified 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxident power (FRAP) and 2,2'-Diphenyl-1-pircylhydrazel (DPPH) method. J Agri Food Chem. 54(4):1151-1157.


Table 1: Lexicon of peanut flavor descriptors (Johnsen and 1988; Sanders and others 1989).

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatics</td>
<td></td>
</tr>
<tr>
<td>Roast Peanutty</td>
<td>The aromatic associated with medium roast peanuts (about 3-4 on USDA color chips) and having fragrant character such as methyl pyrazine.</td>
</tr>
<tr>
<td>Sweet aromatic</td>
<td>The aromatic associated with sweet material such as caramel or molasses.</td>
</tr>
<tr>
<td>Dark roast</td>
<td>The aromatic associated with dark roasted peanuts (4+ on USDA color chips) and having very browned or toasted character.</td>
</tr>
<tr>
<td>Raw/Beany</td>
<td>The aromatic associated with under-roasted peanuts or beans.</td>
</tr>
<tr>
<td>Woody/Hulls/Skin</td>
<td>The aromatic associated with base peanut character (absence of top notes) and related to dry wood, peanut hulls, and skins.</td>
</tr>
<tr>
<td>Basic Tastes</td>
<td></td>
</tr>
<tr>
<td>Sweet</td>
<td>The taste on the tongue associated with sugar.</td>
</tr>
<tr>
<td>Bitter</td>
<td>The taste on the tongue associated with bitter agents such as caffeine or quinine.</td>
</tr>
<tr>
<td>Salty</td>
<td>The taste on the tongue associated with sodium ions.</td>
</tr>
<tr>
<td>Sour</td>
<td>The taste on the tongue associated with acids.</td>
</tr>
<tr>
<td>Feeling Factors</td>
<td></td>
</tr>
<tr>
<td>Astringent</td>
<td>A chemical felling factor on the tongue and oral tissues, describing as puckering/dry and associated with tannins</td>
</tr>
<tr>
<td>Textures</td>
<td></td>
</tr>
<tr>
<td>Crispy</td>
<td>Amplitude of high pitched sound associated with mastication with incisors.</td>
</tr>
<tr>
<td>Crunchy</td>
<td>Amplitude of low pitched sound associated with mastication of sample with molar.</td>
</tr>
<tr>
<td>Hardness</td>
<td>Amount of force to break through the sample with molar.</td>
</tr>
<tr>
<td>Breakdown</td>
<td>Ease at which sample breaks as it chews with molars.</td>
</tr>
</tbody>
</table>
Table 2: Descriptive sensory analysis of peanut skin extract (PSE) fortified honey roasted and chili lime coated peanuts. PSE was fortified into a chili lime and honey roasted coating at varying concentrations. A trained descriptive sensory panel analyzed the coated peanuts using the Spectrum™ universal 15-point method and the peanut lexicon.

<table>
<thead>
<tr>
<th>Coating</th>
<th>% PSE in coating</th>
<th>Roast Peanuty</th>
<th>Sweet Aromatic</th>
<th>Dark Roast</th>
<th>Woody/Hulls/Skins</th>
<th>Sweet Taste</th>
<th>Bitter</th>
<th>Astringent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honey Roasted</td>
<td>0</td>
<td>3.13a</td>
<td>3.15a</td>
<td>2.58ab</td>
<td>2.81a</td>
<td>3.82bcd</td>
<td>2.03a</td>
<td>1.00a</td>
</tr>
<tr>
<td>Honey Roasted</td>
<td>.4</td>
<td>3.19a</td>
<td>2.77ab</td>
<td>2.68ab</td>
<td>2.78a</td>
<td>3.92cd</td>
<td>2.09ab</td>
<td>1.01a</td>
</tr>
<tr>
<td>Honey Roasted</td>
<td>.8</td>
<td>3.44ab</td>
<td>2.69b</td>
<td>2.75ab</td>
<td>2.94b</td>
<td>3.45abc</td>
<td>2.34ab</td>
<td>1.00a</td>
</tr>
<tr>
<td>Honey Roasted</td>
<td>1.6</td>
<td>3.53b</td>
<td>3.05ab</td>
<td>2.50a</td>
<td>2.61a</td>
<td>3.97d</td>
<td>2.14ab</td>
<td>1.00a</td>
</tr>
<tr>
<td>Honey Roasted</td>
<td>3.2</td>
<td>3.50ab</td>
<td>2.80ab</td>
<td>2.62ab</td>
<td>3.05b</td>
<td>3.77bcd</td>
<td>2.38ab</td>
<td>1.03a</td>
</tr>
<tr>
<td>Honey Roasted</td>
<td>6.4</td>
<td>3.79c</td>
<td>2.85ab</td>
<td>2.65ab</td>
<td>2.99b</td>
<td>3.52abcd</td>
<td>2.38ab</td>
<td>1.04a</td>
</tr>
<tr>
<td>Honey Roasted</td>
<td>12.8</td>
<td>3.63c</td>
<td>2.99ab</td>
<td>2.83b</td>
<td>3.03b</td>
<td>3.14a</td>
<td>2.48bc</td>
<td>1.17b</td>
</tr>
<tr>
<td>Honey Roasted</td>
<td>25.6</td>
<td>3.66c</td>
<td>3.09a</td>
<td>2.66ab</td>
<td>3.23b</td>
<td>3.38ab</td>
<td>2.41b</td>
<td>1.29b</td>
</tr>
<tr>
<td>Chili Lime</td>
<td>0</td>
<td>2.64ac</td>
<td>1.54a</td>
<td>2.38b</td>
<td>2.81a</td>
<td>2.29b</td>
<td>2.61a</td>
<td>1.00a</td>
</tr>
<tr>
<td>Chili Lime</td>
<td>.4</td>
<td>2.64ac</td>
<td>1.683a</td>
<td>2.38b</td>
<td>2.77a</td>
<td>2.17b</td>
<td>2.62a</td>
<td>1.0a</td>
</tr>
<tr>
<td>Chili Lime</td>
<td>.8</td>
<td>2.54abc</td>
<td>1.625b</td>
<td>2.3b</td>
<td>2.94ab</td>
<td>1.83a</td>
<td>2.62a</td>
<td>1.00a</td>
</tr>
<tr>
<td>Chili Lime</td>
<td>1.6</td>
<td>2.81a</td>
<td>1.641a</td>
<td>2.73a</td>
<td>1.6a</td>
<td>1.93a</td>
<td>2.53a</td>
<td>1.00a</td>
</tr>
<tr>
<td>Chili Lime</td>
<td>3.2</td>
<td>2.58ab</td>
<td>1.675a</td>
<td>2.63b</td>
<td>3.05ab</td>
<td>2.31b</td>
<td>2.49a</td>
<td>1.00a</td>
</tr>
<tr>
<td>Chili Lime</td>
<td>6.4</td>
<td>2.28b</td>
<td>1.691a</td>
<td>2.40b</td>
<td>2.99ab</td>
<td>1.93a</td>
<td>2.61a</td>
<td>1.03a</td>
</tr>
<tr>
<td>Chili Lime</td>
<td>12.8</td>
<td>2.83ac</td>
<td>1.958b</td>
<td>2.63ab</td>
<td>3.03ab</td>
<td>2.05a</td>
<td>2.74ab</td>
<td>1.13a</td>
</tr>
<tr>
<td>Chili Lime</td>
<td>25.6</td>
<td>2.44c</td>
<td>1.850b</td>
<td>2.40b</td>
<td>3.23b</td>
<td>2.33b</td>
<td>2.89b</td>
<td>1.25b</td>
</tr>
</tbody>
</table>

Mean sensory intensities in the same column with different letters are significantly different (P<0.05).
Table 3: Mean consumer liking response to control commercially available coated peanuts and peanuts with coating fortified with encapsulated peanut skin extract (PSE).
Subthreshold level inclusion levels of PSE were incorporated into the coatings at 16% for the chili lime coating and 12% for the honey roasted. 100 consumers were presented with all four samples.

<table>
<thead>
<tr>
<th></th>
<th>Overall Liking</th>
<th>Flavor Liking</th>
<th>Flavor Intensity Liking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial Honey Roasted</td>
<td>6.95a</td>
<td>6.85a</td>
<td>6.63a</td>
</tr>
<tr>
<td>Fortified Honey Roasted</td>
<td>6.73a</td>
<td>6.66a</td>
<td>6.56a</td>
</tr>
<tr>
<td>Commercial Chili Lime</td>
<td>5.58b</td>
<td>5.52b</td>
<td>5.36b</td>
</tr>
<tr>
<td>Fortified Chili Lime</td>
<td>4.99b</td>
<td>4.92b</td>
<td>4.99b</td>
</tr>
</tbody>
</table>

Means in the same column with different letters are significantly different (P<0.05).
Table 4: Total phenolic content (TPC), DPPH radical scavenging activity, and percent inhibition of β-carotene bleaching of peanut skin extract (PSE) raw material and PSE fortified coated peanuts. Total phenolic content was determined using the Folin Ciocalteu assay and is expressed as gallic acid equivalents (GAE) and DPPH radical scavenging activity is expressed as Trolox Equivalents (TE).

<table>
<thead>
<tr>
<th></th>
<th>Total Phenolic Content</th>
<th>DPPH</th>
<th>β-carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GAE (mg/g peanut)</td>
<td>GAE (mg/g PSE)</td>
<td>TE (µmol/g peanut)</td>
</tr>
<tr>
<td>PSE</td>
<td>77.91± 4.32</td>
<td>633.46±26.66a</td>
<td>68.65±3.42</td>
</tr>
<tr>
<td>16.6% fortified chili lime coating</td>
<td>1.83±0.14a</td>
<td>80.80±6.18a</td>
<td>19.36±0.9a</td>
</tr>
<tr>
<td>12.8% fortified honey roasted coating</td>
<td>1.13±0.04b</td>
<td>82.7±2.97a</td>
<td>15.44±0.4b</td>
</tr>
</tbody>
</table>

Values are mean ± standard error for three replicates. Mean values within each column with the same letters are not significantly different at P<0.05.
Figure 1: Frequency (# of people) at individual best estimate threshold (BET). Results from 80 subjects performed a 7 series ascending forced choice (AFC) threshold for peanut skin extract inclusion levels ranging from 0.4-25.6% (w/w) in a honey roasted coating for peanuts. For each concentration, subjects were given three samples and forced to choose the odd sample out of the three.
Figure 2: Frequency (# of people) at individual best estimate threshold (BET). Results from 80 subjects performed a 7 series ascending forced choice (AFC) threshold for peanut skin extract inclusion levels ranging from 0.4-25.6 (w/w) in a chili lime coating for peanuts. For each concentration, subjects were given three samples and forced to choose the odd sample out of the three.
Figure 3: Frequency of consumers for flavor just about right (JAR). 100 consumers were presented with four samples, roasted peanut coated with a chili lime coating fortified with 16.6% peanut skin extract, roasted peanuts coated with a honey roasted flavored coating fortified with 12.8% peanut skins extract, and commercially available chili lime and honey roasted coated peanuts. Consumers were instructed to rate the intensity of the flavor. Frequency shows the number of consumers who feel a certain way about the intensity of flavor of the peanut samples. * Indicates it caused a significant decrease in overall liking of the sample ($\alpha = 0.05$).
Figure 4: Frequency distribution of consumers for saltiness just about right (JAR). 100 consumers were presented with four samples, roasted peanut coated with a chili lime coating fortified with 16.6% peanut skin extract, roasted peanuts coated with a honey roasted flavored coating fortified with 12.8% peanut skins extract, and commercially available chili lime and honey roasted coated peanuts. Consumers were instructed to rate the intensity of the saltiness. Frequency shows the number of consumers who feel a certain way about the intensity of saltiness of the peanut samples.

* indicates it caused a significant decrease in overall liking of the sample ($\alpha = 0.05$).
Figure 5: Frequency distribution of consumers for bitterness just about right (JAR). 100 consumers were presented with four samples, roasted peanut coated with a chili lime coating fortified with 16.6% peanut skin extract, roasted peanuts coated with a honey roasted flavored coating fortified with 12.8% peanut skins extract, and commercially available chili lime and honey roasted coated peanuts. Consumers were instructed to rate the intensity of the bitterness. Frequency shows the number of consumers who feel a certain way about the intensity of bitterness of the peanut samples.

* indicates it caused a significant decrease in overall liking of the sample ($\alpha = 0.05$).
Figure 6: Total phenolic content of honey roasted coated peanuts fortified with varying levels of peanut skin extract (PSE) measured by the Folin Ciocalteu assay. The peanut skin extract (PSE) was fortified into the honey roasted flavored coating at levels ranging from 0-25.6%. The results of the assay are expressed as gallic acid equivalents (GAE)/g peanut. Values for each concentration were expressed as mean± SEM for three replicates.
Figure 7: Total phenolic content of chili lime coated peanuts fortified with varying levels of peanut skin extract (PSE) measured by the Folin Ciocalteu assay. The peanut skin extract (PSE) was fortified into the chili lime flavored coating at levels ranging from 0-25.6%. The results of the assay are expressed as gallic acid equivalents (GAE)/g peanut. Values for each concentration were expressed as mean± SEM for three replicates.
Figure 8: Total phenolic content of peanut skin extract (PSE) fortified chili lime and honey roasted coated peanuts at the threshold in comparison with common foods (Fu and others 2011). Threshold inclusion levels for peanut skins were 16.6% and 12.8% in the chili lime and honey roasted coatings, respectively. Total phenolic content was expressed as gallic acid equivalents (mg)/ dry weight. Values for each concentrations were expressed as mean± SEM for three replicates.
Figure 9: The radical scavenging activity of the honey roasted coated peanuts fortified with varying levels of peanut skin extract (PSE) measured by the DPPH radical scavenging assay. The peanut skin extract was fortified into the honey roasted flavored coating at levels ranging from 0-25.6%. The results of the assay are expressed as trolox equivalents (TE)/g peanut. Values for each concentration were expressed as mean± SEM for three replicates.
Figure 10: The radical scavenging activity of the chili lime coated peanuts fortified with varying levels of peanut skin extract (PSE) measured by the DPPH radical scavenging assay. The peanut skin extract (PSE) was fortified into the chili lime flavored coating at levels ranging from 0-25.6%. The results of the assay are expressed as trolox equivalents (TE)/g peanut. Values for each concentration were expressed as mean± SEM for three replicates.
Figure 11: Total phenolic content of peanut skin extract (PSE) fortified chili lime and honey roasted coated peanuts at the threshold in comparison with common foods ((Rodrigues and others 2011; Ozgen and others 2006). Threshold inclusion levels for peanut skins were 16.6% and 12.8% in the chili lime and honey roasted coatings, respectively. Total phenolic content was expressed as gallic acid equivalents (mg)/ dry weight. Values for each concentrations were expressed as mean± SEM for three replicates.
Figure 12: The percent inhibition of β-carotene bleaching of the honey roasted coated peanuts fortified with varying levels of peanut skin extract (PSE) measured by the β-carotene bleaching assay. The peanut skin extract was fortified into the honey roasted flavored coating at levels ranging from 0-25.6%. The % bleaching inhibition is based on the reduction in absorbance at 470 nm. Values for each concentrations were expressed as mean± SEM for three replicates.
Figure 13: The percent inhibition of β-carotene bleaching of the chili lime coated peanuts fortified with varying levels of peanut skin extract (PSE) measured by the β-carotene bleaching assay. The peanut skin extract was fortified into the chili lime flavored coating at levels ranging from 0-25.6%. The % bleaching inhibition is based on the reduction in absorbance at 470 nm. Values for each concentration were expressed as mean± SEM for three replicates.
Chapter 3: THE EFFECT OF PEANUT SKIN EXTRACT ON HYPERGLYCEMIA

Abstract:

Oxidative stress has been shown to play a role in the development and progression of type 2 diabetes. Natural phenolics have been suggested to have a protective effect on type 2 diabetes due to their ability to act as antioxidants. This study aimed to evaluate the effect of the phenolic extract from peanut skin on induced- hyperglycemia in an in-vitro and in-vivo study. In vitro, the phenolic extract was investigated for the effect on cell viability in a human hepatocellular liver carcinoma cell line (HepG2) exposed to high glucose concentrations. In vivo, the effect of peanut skin extract on the glycemic response to an oral glucose tolerance was investigated in human subjects. Peanut skin extract was found to attenuate the decrease in cell viability in high glucose treated HepG2 cells, showing a protective effect against hyperglycemia induced cell death. In vivo, the results of the glucose tolerance test showed no difference in the glucose area under the curve between any treatments. However, the treatment of the peanut skin extract with the glucose load resulted in a significantly lower peak blood glucose response, indicating that it was effective at reducing the glycemic response. The present study shows that the phenolic extract of peanut skins has an antidiabetic effect, further confirming their value as a functional food ingredient.

Abbreviations:
PSE, peanut skin extract; ROS, reactive oxygen species; DMEM, Dulbecco’s modified eagle’s medium; FBS, fetal bovine serum; EDTA, ethylenediaminetetraacetic acid; NAC, N-acetyl cysteine; MTT, (3-(4,5-Dimethylthiazol-2-yl)-2,5- Diphenyltetrazolium Bromide; ALT, alanine transaminase; MD, maltodextrin; DPPH, 2,2-diphenyl-1-picrylhydrazyl; CGM, continuous glucose monitor; AUC, area under the curve; GR, glutathione reductase; GPx, glutathione
peroxidase; GST, glutathione S-transferase; CAT, catalase; MAPKs, mitogen-activated kinases; ERK, extracellular signal-regulated kinase; JNK, c-Jun-N-terminal kinase; EEC, epicatechin-\((4\beta\rightarrow 6)\)-epicatechin-\((2\beta\rightarrow 0\rightarrow 7, 4\beta\rightarrow 8)\) catechin; SGLT, sodium glucose cotransporter; GLUT 2, glucose transporter 2; GLUT4, glucose transporter 4; ECg, epicatechin gallate; EGCg epigallocatechin gallate; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; AMPK, 5’adenosine monophosphate-activated protein kinase; PI3K, phosphoinositide 3-kinase.

**Introduction:**

Diabetes mellitus is a group of metabolic diseases characterized by deficient insulin secretion or action, resulting in chronic hyperglycemia (Bahadoran and others 2013). It is one of the most common occurring chronic diseases and its prevalence is continuing to increase worldwide. This disease affects approximately 346 million people worldwide and this number is likely to more than double by 2030 (Lavelli and others 2016). Type 2 diabetes is responsible for more than 90% of all diabetic cases, and its risk factors include poor nutrition, lack of physical activity, obesity, aging, and genetics (Tang and others 2013). Without effective management and prevention methods, the prevalence and corresponding complications will only continue to increase. The use of natural compounds, such as phenolic compounds, has been suggested as a potential therapeutic agent to help with the prevention or treatment of this disease (Cordero-Herrera and others 2014).

Oxidative stress has been suggested to play a role in the development and progression of type 2 diabetes (Bahadoran and others 2013). Oxidative stress occurs when there is an excess production of free radicals and reactive oxygen species (ROS) in the cell (Bahadoran and others 2013). Studies have shown that common risk factors for diabetes, such as obesity and unhealthy eating, cause an increase in oxidative stress (Styskal and others 2012). In addition,
hyperglycemia, or elevated blood glucose levels, causes injury to the innate antioxidant defense system resulting in an excessive production of reactive oxygen species (Bahadoran and others 2013). This is suggested to play a major role in the complications of diabetes, such as cellular dysfunction. As a result, compounds that are able to counteract this oxidative stress may be useful for diabetes prevention or management.

Natural antioxidants, such as phenolic compounds, have been suggested as an effective and economical way to help to prevent and manage the side effects of diabetes due to their ability to counteract oxidative stress (Shokrzadeh and others 2016). Epidemiological studies suggest an association between a higher consumption of flavonoid rich foods and a lower risk of type 2 diabetes (Wedick and others 2012). In addition to their antioxidant activity, phenolic compounds can also affect the digestion and absorption of carbohydrates (Bryans and others 2007). Phenolic compounds, such as procyanidins, have shown an ability to inhibit important enzymes involved in carbohydrate metabolism (Schafer and Hogger 2007). These same compounds have also been shown to interact with glucose transporters, thereby interfering with intestinal absorption (Welsch and others 1989; Johnston and others 2005).

Peanut skins have been reported to be rich in phenolic compounds, and their antioxidant activity has been reported in numerous studies (Nepote and others 2002; Lou and others 2004; Lewis and others 2013; Yu and others 2005; Yu and others 2006). However, there is very limited research on the antidiabetic effect. Tamura and others (2015) reported that peanut skin procyanidins inhibited the activity of alpha-amylase and decreased glucose transport in Caco-2 cells. However, the anti-diabetic effect of peanut skin phenolic compounds has not been reported in human studies.
The aim of this study was to investigate the effects of peanut skin extract on hyperglycemia using both in vitro and in vivo models. In vitro, our aim is to determine the effect of peanut skin phenolic extract on an induced hyperglycemic state was examined in HepG2 cells. In vivo, the objective was to determine the effect of peanut skin phenolics on postprandial hyperglycemia using an oral glucose tolerance test. Thus, this study gives more insight into the biological effects of peanut skin phenolics compounds.

Material and Methods

Materials

Peanut skins produced as a byproduct of the blanching process Runner type peanuts were obtained from Jimbo’s Jumbos (Edenton, N.C., USA). Maltrin M150 maltodextrin was provided by Grain Processing Corp (Mascatine, Iowa, USA). Pure pharmaceutical grade ethanol (Decan Laboratories Inc, King of Prussia, PA, USA) was used for preparation of peanut skin extract. TicaPAN 311 (Tic Gums, MD, USA) was used for precoating chili lime peanuts. Dulbecco’s Modified Eagle’s Medium (DMEM), Fetal Bovine Serum (FBS), trypsin-ethylenediaminetetraacetic Acid (EDTA), hepes (4-(2-hyrdoxyethyl)-1-piperazineethanesulfonic acid, penicillin-streptomycin, glutamine, non-essential amino acid solution, N-acetyl cysteine (NAC), glucose, and trypan blue were all purchased from Gibco (Invitrogen, Carlsbad, CA, USA). The MTT cell growth assay kit was purchased from Sigma- Aldrich (Milipore Corp. Bedford, MA, USA). The Alanine Transaminase assay kit (ALT) was purchased from Biovision (Minneapolis, MN, USA). Standard 50- gram glucose solutions for glucose load were obtained from Azer Scientific (Morgantown, PA, USA). Sterilized lancet, glucose meter equipment and strips for glucose meter were obtained from One Touch (Lifespan Inc., Wayne, PA, USA).

Preparation of peanut skin extract and coated peanuts
Peanuts skin extracts (PSE) were prepared by extracting the phenolic compounds using 70% ethanol. For the cell studies, the PSE was spray dried without encapsulation in maltodextrin. For the human study, PSE was encapsulated in maltodextrin (MD) as previously described in Chapter 1. The chili lime coated peanuts were also prepared as previously described in Chapter 1. The level of PSE added to the coating was the best estimate threshold (BET) as determined previously in Chapter 1 at 16.6% (w/w).

Cell culture

The human liver cancer cell line (HepG2 cells) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in complete media containing, Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) and 2% of penicillin streptomycin, glutamine, 4-(2-hyrdoxyethyl)-1-piperazineethanesulfonic acid (hepes), pyruvate, and non-essential amino acids under 5% CO$_2$ atmosphere at 37°C. When the cells reached 80-90% confluence, cells were sub-cultured using trypsin-ethylenediaminetetraacetic acid (EDTA) to detach from the flask. Cells were plated 48 hours prior to treatment.

Treatment of cells

The cytotoxicity of PSE on HepG2 cells was first screened to determine the efficacious dosage to use to evaluate the protective effect of PSE. HepG2 cells were treated with media containing doses of 0.5%, 1%, 2.5%, 5%, and 10% PSE and incubated for 24 hours at 37°C and 5% CO$_2$. Following the 24-hour incubation, cells were tested for cell proliferation, viability and ALT enzyme activity.

To induce a condition of oxidative stress in HepG2 cells, various glucose concentrations were screened. HepG2 cells were treated with media containing 0, 20, 40, 60, 80, 100, 130, 160
mM glucose for 24 hours. Following the treatment and 24-hour incubation, cells were analyzed for cell proliferation, viability and ALT enzyme activity.

Based on the screening results, cells were divided into 5 experimental groups: i. Control (Untreated); ii. Cells treated with glucose; iii. Cells treated with PSE; vi. Cells were treated with PSE and glucose; v. Cells treated with N-acetyl cysteine (NAC) (5 mM); iv. Cells treated with NAC and glucose (positive control). Following the treatment and 24-hour incubation, cells were analyzed for cell proliferation, viability and ALT enzyme activity.

*MTT cell growth assay*

To study the cell proliferation, cells were plated in 96-cell plates (4*10^5 cell/well) and measured using the MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Cell Growth Assay Kit according to manufacturer’s instruction. Briefly, 0.01 mL MTT reagent was added to each well. After 4 hour of incubation at 37 C, a solution of 0.1 ml isopropanol and 0.04 N HCl was added to each cell and the absorbance was read at 570 nm using an Epoch 25 Microplate Spectrophotometer (Biotech, Winooski VT, USA).

The values were corrected by subtracting out the background absorbance of media containing peanut skin extract with no cells. The viability of the control group was assumed to be 100%. The cell viability of the experimental group was compared to that of the control and expressed as percent viability relative to the control based on Equation 1.

Equation 1: 

\[
\text{Sample Absorbance/Control Absorbance) *100= \% relative cell viability}
\]

*Trypan blue exclusion method*

Cell viability was measured using the trypan blue exclusion method. Briefly, the cell suspension was mixed 1:1 with trypan blue stock solution. The mixture was placed in a TC20 Automated Cell Counter (BioRad, Hercules, CA, USA) to detect the cells that had absorbed the
dye to calculate the percentage of viable cells. Data was expressed as percent viability relative to
the control.

*ALT enzyme activity assay*

The Alanine Aminotransferase (ALT) activity colorimetric assay was done using the
manufacturer's instructions (Biovision, Minneapolis, MN, USA). Briefly, treated cells (1*10⁶)
were homogenized with ALT Assay Buffer and centrifuged to remove the insoluble material. A
pyruvate standard curve was produced by testing concentrations ranging from 0-1 nmol.

Samples and standards were added to 96-well plated (80,000 cells /well) along with a
reaction mixture containing ALT Assay Buffer, Oxired Probe, ALT Enzyme Mix, and ALT
Substrate. ALT catalyzes the transamination of α- ketoglutarate and alanine to form glutamate
and pyruvate. The amount of pyruvate was then determined by measuring the absorbance at 570
nm within ten minutes of plating (A₁) using the Epoch 25 Microplate Spectrophotometer
(Biotech, Winooski VT, USA). The plate was the incubated at 37 C for 60 minutes and then a
second absorbance was read (A₂). The amount of pyruvate generated during incubation is the
change in absorbance (A= A₂-A₁). A pyruvate standard curve was generated, and the nmol of
pyruvate (B) generated between T1 and T2 was calculated. The ALT activity was then
calculated using Equation 2

Equation 2: \[ \frac{B}{(T₂-T₁) \cdot v} \]

*Oral glucose tolerance test*

*Subjects*

Fifteen healthy subjects (7 males and 8 females) with an age range of 23-32 were
recruited from the staff and student population of North Carolina State University, Raleigh, NC
to participate. All participants were frequent consumers of peanut products and did not have an
allergy to peanuts. Exclusion criteria for participants in this study included: BMI > 35 kg/m², diagnosis of type 1 or 2 diabetes, anemia, a fasting glucose > 125 mg/dL, use of medication that affect glucose metabolism, renal, liver, pancreatic, or cardiovascular disease, uncontrolled hypertension, disorders of the esophageal or in gastrointestinal motility, hypo- or hyperthyroidism, allergic to peanuts, and pregnancy. The study was approved by the North Carolina State University institutional review board and all subjects gave written consent after a full explanation of the study procedures. Each subject completed five treatments in a randomized-crossover design.

Composition of experimental drinks:

The experimental treatments included: 1) 50 g glucose solution (reference); 2). 50-gram glucose solution, followed by 12 mg of vegi-capsulated MD (placebo); 3) 50-gram glucose solution, followed by 120 mg of vegi-capsulated MD-encapsulated PSE (Treatment); 4). 50-gram glucose solution, followed by 28 grams (1 serving) of unfortified coated peanuts; 5) 50-gram glucose solution, followed by 28 grams of chili lime coated peanuts fortified with PSE. The amount of peanut skin extract used was calculated to be equal to the antioxidant activity found by Rodrigues and others (2011) for blueberries measured by the DPPH reducing assay. The antioxidant activity of PSE was previously determined by the DPPH reducing assay in Chapter 2. The inclusion level of PSE for the coated peanuts was chosen based on the threshold found in Chapter 2 for chili lime coated peanuts. A randomized crossover design was used so that each subject received each of the five treatments on different days in randomized order. This was repeated three times for each subject with a week washout period between each replication.

Experimental design
Subjects were asked to maintain their usual dietary intake but to refrain from consuming alcohol during the time of the study. They were instructed to refrain from eating and any non-habitual exercise 10 hours before treatments. Subjects were also instructed to keep a dietary record and to track their steps for all days of the study.

Participants underwent continuous glucose monitoring for five days using the MiniMed continuous glucose monitoring system, iPro2 professional continuous glucose monitoring system (CGM; Medtronic). This CGM operates for 72 hours and takes a reading of interstitial glucose every five minutes using a subcutaneous microelectrode sensor. The CGM was inserted subcutaneously into the lower abdomen one day before the first day of treatments. On each of the five days, subjects were instructed to consume each treatment within ten minutes.

The blood glucose was determined for each subject before consuming the treatment (time zero) and 2.5 hours after treatment by finger prick using a blood glucose meter (OneTouch Verio IQ). The CGM system recorded glucose values continuously every five minutes for this 2.5-hour period. After the five days of treatments, CGMS were removed and the data that had been collected was downloaded and analyzed using CGMS Software version 3.0. The fasting blood glucose levels determined before the treatment was used as a baseline and the 2.5- hour postprandial glucose levels after each treatment were plotted and mean values under the curve were calculated.

The glycemic response for each treatment was estimated by the relationship between the total incremental area under the curve (AUC) for the treatment using the trapezoidal model (Brouns and others 2005). Peak heights were the incremental rises in blood glucose level above the baseline fasting glucose level. The glycemic profile was calculated by dividing the duration the glucose level was above the fasting glucose level by the incremental glucose peak (Rosen
and others 2009). When the glucose remained above fasting the entire 150 minutes after treatment, the duration value was set to 150 minutes.

**Statistical analysis**

Experiments were done in triplicate and data are expressed as mean +/- SEM. Statistical analysis was performed using SAS version 9.4 (SAS Inst., Cary, NC). Cell viability measured by both MTT assay and trypan blue exclusion method was expressed as percent viability relative to the control. Differences in cell viability and ALT enzymes activity between treatments was determined by one-way ANOVA followed by Tukey post hoc test. AUC, peak height, and glycemic profile were analyzed using a one-way ANOVA with repeated measures and were compared by Tukey post hoc test. P values<0.05 were considered significant.

**Results and discussion:**

**Toxicity of peanut skins**

The liver is the primary organ of glucose metabolism and regulation, therefore the human hepatoma cell line; HepG2 can be used as a model to investigate the effects of PSE on hyperglycemia in culture livered cells (Chandrasekaran and others 2010). To determine the nontoxic concentration of PSE on HepG2, cells were exposed to a range of concentrations (0-10%) for 24 hours. In the MTT assay (Figure 1), only viable cells are able to form the blue formazan crystals that result in absorbance at 570 nm (Gerlier and Thomasset 1986). Therefore, the absorbance is directly proportional to the number of viable cells. In the trypan blue exclusion method, the membrane of non-viable cells are permeable to the blue dye allowing for them to be differentiated from viable cells (Tran and others 2011). The concentrations of PSE tested did not result in significant changes in cell viability (Figure 1). This indicates that these concentrations of PSE did not compromise the cell integrity during the 24-hour incubation period.
Additionally, there was no significant increase in ALT activity when cells were treated with all tested levels of peanut skins (Figure 2). Instead, treatment with PSE was found to cause a significant decrease in ALT activity, suggesting the PSE may help to protect the cells against any natural stresses that were not controlled for. Cytotoxicity in HepG2 cells is often associated with increases in activity of liver marker enzymes, such as ALT (Eliza and others 2009). ALT is an enzyme that catalyzes the transfer of amino groups to form the hepatic metabolite, oxaloacetate. ALT is mainly found in the liver, and in healthy patients, the activity in the liver is about 3000 times that of the activity of the serum (Kim and others 2008). An increase in the measured activity in the cell medium demonstrates a release of ALT from the liver due to a condition of cellular stress and death (Kim and others 2008). The lack of increase in ALT activity in cells treated with all tested doses of PSE further demonstrates that these doses do not have a toxic effect on HepG2 cells.

Since there was no significant cytotoxicity associated with PSE observed in the HepG2 cells, a low yet realistic value of PSE was chosen for further analysis. Doses of 2.5% PSE were chosen since it was a midpoint of the range evaluated.

*Toxicity of glucose*

HepG2 cells were exposed to media with various concentrations of glucose (0-160mM) for 24 hours in order to determine the toxic concentration of glucose needed to induce hyperglycemia. This has been demonstrated to model the effect of the *in vivo* condition of diabetic ketoacidosis (Chandrasekaran and others 2010). Cell growth and viability was tested through MTT and the trypan blue exclusion method. As shown in Figure 3, the exposure of glucose did not have a significant effect on cell viability until it reached a concentration of 130 mM. This is higher than many other studies that have found that when exposed to a glucose
concentration of only 50 mM for 24 hours, the cell viability of HepG2 cells greatly decreases (Chandrasekaran and others 2010; Shokrzadeh and others 2016). The result of this screening was much higher, and as a result, a concentration of 160 mM was chosen for this experiment. The high glucose treatment also resulted in a significant increase in ALT activity (Figure 4), indicating injury to the liver cell.

Oxidative stress has been shown to play a role in the toxicity of high glucose levels in HepG2 cells. Chandrasekaran and others (2010) found the decrease in cell viability in HepG2 cells exposed to high glucose levels was accompanied by an increase in oxidative stress. Similarly, Cordero-Herra and others (2015) found that exposure to high levels of glucose resulted in overproduction of ROS. They also found that innate antioxidant enzymes, such as glutathione reductase (GR), glutathione peroxidase (GPx), glutathione S-transferase (GST), and catalase (CAT), had increased activity. These enzymes play an important role in the defense mechanism against free radicals, and their increased activity reflects the attempt of the cells to respond to elevated oxidative stress (Maritim and others 2003). This is similar to the effect of hyperglycemia in the body. Long-term hyperglycemia in diabetic patients has been identified as one of the major causes of increased ROS generation (Bahadoran and others 2013). As a result, diabetic patients have been reported to have an abnormally high level of free radicals, a decline in antioxidant defense systems, and lower vitamin levels. (Maritim and others 2003) This oxidative stress has been implicated as the cause of the major complications of diabetes, including nephropathy, retinopathy, neuropathy, and macro- and microvascular damage (Pourkhalili and others 2011).

Effect of PSE on high glucose treated cells.
Based on the results of the toxicity studies, HepG2 cells were exposed to media with both 2.5% PSE and 160 mM glucose for 24 hours in order to evaluate the potential protective effect of PSE on hyperglycemia. As shown in Figure 5, PSE showed protective effects against hyperglycemia induced cell death. When exposed to media with 160 mM of glucose, the relative cell viability was found to be decreased to 46.3% and 58.4%, when measured by MTT and trypan blue respectively. However, co-treatment of the cells with 2.5% PSE and 160 mM glucose brought the relative cell viability to above 100% measured by both methods. Therefore, treatment with PSE offered protection against glucose cytotoxicity. These results were similar to those found for cells treated with the positive control of 5 mM NAC and 160 mM glucose.

HepG2 cells exposed to high glucose levels had a significantly higher ALT activity (Figure 6). Several epidemiologic studies have reported that obesity and diabetes are risk factors for elevated ALT activity (Clark and others 2003). Treatment with PSE diminished this increase in ALT activity of high glucose treated cells. This effect suggests that PSE can help to prevent liver damage caused by hyperglycemia.

The protective effect of PSE against high glucose induced toxicity may be due to their ability to act as antioxidants. Antioxidants are able to scavenge free radicals and reduce oxidative stress. This is further demonstrated by the similar effects of PSE and the positive control, NAC, on high glucose treated cells. NAC is an antioxidant that leads to the generation of GSH, an innate antioxidant enzyme in the body. Chandrasekaran and others (2010) found that NAC was able to reverse the decrease in cell viability in HepG2 cells exposed to high glucose levels by lowering the ROS level. The similar results found between PSE and NAC suggest that they may have a similar mechanism of action. Similar results have been found in various other studies investigating the effects of natural phenolics on high glucose induced oxidative stress.
(Shokrzadeh and others 2016; Cordero-Herra and others 2015; Kapoor and Kakkar 2012). For example, Kapoor and Kakkar (2012) found that morin, a natural flavonoid found in many fruits, herbs, and wine, was able to inhibit the release of apoptotic proteins from the mitochondria as a result of their ability to reduce ROS levels in high glucose treated rat hepatocyte cells. Similar results were found for the phenolic extract of cocoa (Cordero-Herra and others 2015). In addition, it was found that this decrease in ROS production modulated key signaling pathways induced by stress. Oxidative stress causes the activation of mitogen-activated protein kinase (MAPKs) signaling pathways. MAPKs, such as extracellular signal-regulated kinase (ERK), c-Jun-N-terminal kinase (JNK), and p-38, are responsible for altering the expression of genes involved in high glucose induced apoptosis, inflammation, and oxidative stress (Evans and others 2002). Furthermore, these proteins have been shown to play a role in insulin resistance (Evans and others 2002). The co-treatment of high glucose exposed HepG2 cells with cocoa phenolic extract was found to prevent the activation of these kinases, suggesting this is the mechanism for how phenolics can alleviate the damages of oxidative stress in the hyperglycemic state.

**Oral glucose tolerance test**

In order to investigate the effects of PSE on glycemic response after consuming a glucose load made up of a 50-gram glucose solution, subjects participated in an oral glucose tolerance test. An oral glucose tolerance test is a common method used to assess the postprandial glycemic response (Ceriello 2004). Although ingesting a pure glucose solution does not incorporate many of the metabolic events that are associated with eating a mixed meal, it has been shown that the level of glycaemia in the oral glucose tolerance test is closely related to the level of glycaemia after a standardized meal (Wolever and others 1998). This suggests that this
method is a valid tool for assessing how different compounds, such as phenolics, can cause alterations in carbohydrate metabolism.

A total of 15 people (male=7, female=8) with a mean age of 25 +/- 4 years old completed the intervention study. Thirteen people had data for the complete three-week test period, while two people only had data for two weeks due to inaccuracies in the CGM. Inaccuracies in the CGM data are common due to calibration of the interstitial fluid glucose to the blood glucose levels (Vaddiraju and others 2010). The mean plasma glucose concentrations were plotted as a function of time for 150 minutes after each treatment (Figure 7). The consumption of the PSE capsule and PSE fortified coated peanuts with the glucose load resulted in a significantly lower peak plasma glucose response at 45 min (Figure 7). There was no significant difference when comparing the AUC for the different treatments after 150, 120 and 60 minutes (Table 1). However, several researchers have suggested that reducing the plasma spike in blood glucose 30 to 45 min after a meal is more important than decreasing the AUC (Coe and others 2013, Ceriello 2004). High postprandial plasma glucose spikes are associated with an increased risk of developing chronic diseases such as cardiovascular disease, metabolic syndrome, and type 2 diabetes (Ceriello 2004). This may be a result of the increase in oxidative stress that is induced by an acute rise in glycaemia. Ceriello and others (1998) found that the glycemic spike induced by an oral glucose tolerance test substantially decreased the plasma antioxidant capacity, suggesting that there is an increase in oxidative stress that leads to the consumption of antioxidant defense mechanisms. Furthermore, several epidemiological studies have shown that the glycemic spikes are associated with diabetic complications such as intima-media carotid thickening and atherosclerosis (Temelkova- Kurktscheiv and others 2000)
The glycemic profile was significantly improved by the treatment of the PSE capsule with the glucose load (Table 1). However, the glycemic profile was only slightly, but not significantly increased by the PSE fortified peanut treatment with the glucose load. The glycemic profile is a variable proposed by Rosen and others (2009) to quantify the change in the shape of the glucose curve. It is the duration of the postprandial blood glucose level above the baseline fasting level divided by the blood glucose peak. A higher glycemic profile is suggested to indicate facilitated glycemic regulation.

The decrease in peak plasma glucose levels by PSE treatments suggests that PSE delays the absorption of glucose. Consistent with these findings, peanut skin phenolic fractions, including (+)- catechin, procyanidin A1, and epicatechin-(4β→ 6)-epicatechin-(2β→ 0-->7, 4β→ 8)-catechin (EEC), have been shown to decrease the level of glucose uptake in Caco-2 cells (Tamura and others 2015). Although the mechanism of this effect by peanut skin phenolics has not been studied, various studies have found that phenolic compounds may interact with glucose transporters. Glucose absorption into the intestine is mostly mediated by active sodium-dependent transport via glucose co-transporter 1 (SGLT1) and facilitated sodium-independent transport via GLUT2. Tea polyphenols such as epicatechin gallate (ECg) and epigallocatechin gallate (EGCg) have been shown to inhibit SGLT1 through competitively binding to the transporter (Koboyashi and others 2000). Similar results were found by Manzano and Williamson (2010), who suggested that the inhibition of SGLT1 and GLUT2 by polyphenols extracted from strawberries and apples was due to their ability to bind and interact with the transporters. Welsh and others (1989) suggested a different mechanism for their observation that tannic acid and chlorogenic acid reduced glucose uptake in the brush border membrane vesicles of rats. They suggested that phenolic compounds could reduce the Na^+ electrochemical gradient.
that was required for SGLT-1 active transport. Regardless of the mechanism, inhibition of glucose transport would result in a lower postprandial glycemic response.

The ability of several phenolic compounds found in peanut skins to reduce glucose absorption has been further shown through their effect on glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide-1 (GLP-1) profiles. GIP and GLP-1 are the main incretin hormones and are secreted in response to a meal. Secretion of GIP occurs in the small intestine and is stimulated by the absorption of nutrients into the gut (Johnston and others 2003). A decreased rate of absorption would lead to a decreased secretion of GIP. In contrast, GIP-1 is secreted from the distal portion of the small intestine due to the presence of nutrients in the gut and its secretion can be increased when carbohydrate absorption is delayed (Johnston and others 2003). Several phenolic compounds found in PSE have been reported to promote GLP-1 secretion and decrease GIP secretion. Chlorogenic acid is a phenolic acid that has been found to be present in peanut skins (Yu and others 2005). The consumption of this compound has been shown to increase GLP-1 secretion and decrease GIP secretion in both mice and humans (Johnston and others 2003; Fujii and others 2015; Tunnicliffe and others 2011). Resveratrol is the major stilbene that is found in peanut skins (Sanders and others 2000). It has been found to increase GLP-1 secretion and consequently lower glycemia in high fat diet induced diabetic mice (Dao and others 2011).

Phenolic compounds have also been shown to have the potential to activate signaling pathways involved in glucose transport. The insulin sensitive glucose transporter, GLUT4, is mostly located within the intracellular compartment of adipose tissue, and skeletal and cardiac muscles (Yamashita and others 2012). Insulin causes the translocation of GLUT4 from the intracellular plasma membrane, allowing for a large amount of glucose to be brought into the
cell. This uptake of glucose is important for reducing blood glucose levels and maintaining glycemic homeostasis in the body. GLUT4 translocation is regulated by 5’adenosine monophosphate-activated protein kinase (AMPK) and phosphoinositide-3-kinsase (P13K) (Hardie 2004). Resveratrol has been shown to activate AMPK-dependent signaling pathways to results in the translocation of GLUT4 in cultured L6 myotubes (Minakawa and others 2011). Similarly, cacao liquor procyanidin extract was able to improve high-fat diet-induced hyperglycemia in mice by activating AMPK and causing translocation of GLUT4 in skeletal muscle, and white and brown adipose tissue (Yamashita and others 2012). This procyanidin extract was rich in monomeric epicatechin and catechin, and oligomeric procyanidins (Urpi-Sarda and others 2009). Peanut skin extract has been found to have a similar composition and contains catechin, A-type and B-type procyanidin dimers, trimers, and tetramers (Yu and others 2005). Since closely related phenolics to those found PSE have an effect on GLUT4 translocation, a similar mechanism may be involved.

The hypoglycemic effect of phenolic compounds has also been attributed to their ability to interfere with starch digestion. Phenolics have been found to inhibit digestive enzymes, thus reducing the amount of free glucose available to be released into the blood. Lin and others (2017) found that PSE exhibited high α-amylase inhibitory activity, similar to that of the anti-diabetes drug, Glycobay. In addition, Costa de Camargo and others (2017) found that PSE decreased the activity of α- glucosidase. α-glucosidase and α-amylase are key enzymes involved in carbohydrate digestion. Inhibition of these digestive enzymes interrupts the hydrolysis of carbohydrates in food and reduces their flow into the bloodstream.

The co-treatment of peanuts and the glucose solution caused a slight increase in the blood glucose peak (Figure 7). Previous studies have shown that peanut consumption can lead to a
reduced glycemic response as a result of the fat and protein content (Reis and others 2011). This was suggested to be due to an increase in gastric emptying and increase in insulin secretion. However, the contrasting results of this study may be due to the difference in form of the peanut. The previous study found that ground peanuts were able to significantly lower the glycemic response compared to whole peanuts. The amount of fat released and absorbed in the digestive system depends on the integrity of the cell wall (Ellis and others 2004). The ground nuts went through a grinding process that may cause more destruction to the cell wall than is possible with mastication alone. This explains why the un-fortified peanuts did not have any effect on the glycemic response AUC or the plasma glucose peak. Another reason may be that the treatment size of peanuts in this study was too small. The treatment size for this study was chosen as one serving because it was a realistic amount for a person to consume. However, Josse and others (2007) found that 60 grams of almonds were needed to decrease the glycemic response to white bread. The single serving size used for this study may not have been sufficient to cause a response.

**Conclusion:**

This work shows that the phenolic extract from peanut skins have a protective effect on hyperglycemia both in vitro and in vivo. PSE was found to protect HepG2 cells from the decrease in cell viability caused by exposure to high levels of glucose. PSE was also able to have an effect on the glycemic response to a 50-gram glucose solution by lowering the postprandial peak. These results suggest that PSE may help to maintain glucose homeostasis by having an effect on glucose absorption. A larger study on the effects of PSE on the glycemic response is needed to confirm these results. In addition, future studies should investigate the effect of PSE on glucose transporters in order to determine the mechanism behind the
hypoglycemic effect of PSE. This research shows that peanut skin phenolics exert biological activity, further confirming their potential as a functional food ingredient.
References


Lou H, Yuan H, Ma B, Ren D, Ji M, Oka S. 2004 Polyphenols from peanut skins and their free radical scavenging effects. Phytochemistry. 65(16): 2391-2399

Manzano S, Williamson G. 2010 Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells. Mol Nutr Food Res. 54(12): 1773-1780.


Table 1: Blood glucose responses in 15 healthy individuals after 5 treatments in an oral glucose tolerance test. The change in glucose level from fasting level was plotted against the time for 2.5 hours in glycemic response curve. Glucose levels were measured using a continuous glucose monitor. The area under the curve (AUC), glycemic profile, and glycemic incremental peak were determined from the glycemic response curve.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC (0-30)</th>
<th>AUC (0-60)</th>
<th>AUC (0-120)</th>
<th>AUC (0-150)</th>
<th>GP Min/mg/dL</th>
<th>Glycemic incremental peak (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 gram glucose solution</td>
<td>742.4±74.8b</td>
<td>1743.8±292.1a</td>
<td>3107.5±342.0a</td>
<td>3144.5±344.6a</td>
<td>3.06±1.47a</td>
<td>42.7±2.8b</td>
</tr>
<tr>
<td>Maltodextrin capsule</td>
<td>750.5±76.8b</td>
<td>2033.5±298.6a</td>
<td>2944.5±328.4a</td>
<td>3139.9±350.4a</td>
<td>3.07±1.51a</td>
<td>42.3±2.8</td>
</tr>
<tr>
<td>PSE capsule</td>
<td>466.2±75.8a</td>
<td>1880.4±299.1a</td>
<td>2971.9±330.9a</td>
<td>2984.14±347.4a</td>
<td>8.99±1.48b</td>
<td>27.8±2.8a</td>
</tr>
<tr>
<td>Unfortified chili lime coated peanuts</td>
<td>802.0±75.8b</td>
<td>2073.7±288.7a</td>
<td>3207.4±323.7a</td>
<td>3387.1±342.9a</td>
<td>3.15±1.46a</td>
<td>43.4±2.7b</td>
</tr>
<tr>
<td>PSE fortified chili lime coated peanuts</td>
<td>687.2±73.9b</td>
<td>1812.7±291.5a</td>
<td>3054.1±321.7a</td>
<td>3144.9±339.4a</td>
<td>5.94±1.47ab</td>
<td>33.5±2.7a</td>
</tr>
</tbody>
</table>

Each treatment was done in triplicate with a week washout period for each individual. Values are means ± SEM. Products in the same column not sharing the same letter were significantly different p< 0.05.
Figure 1: Effects of varying concentrations of PSE on HepG2 cells viability measured by MTT cells growth assay and trypan blue exclusion method. HepG2 cells were exposed to varying concentrations of peanut skin extract (0-10%) for 24 hours and their viability was determined. The viability of the control was assumed to be 100% and the data are expressed as viability relative to the control. For the MTT assay, the viability is corrected for by subtracting out the background absorbance of each treatment. Each treatment was done in triplicate for both methods and the data is expressed as mean +- SEM.

*Indicates statistically significant difference at P<0.05
Figure 2: Effect of varying concentrations of peanut skin extract on ALT leakage from HepG2 cells. HepG2 cells were exposed to varying concentration of peanut skin extract for 24 hours and the level of transaminase enzyme were measured. Each value was corrected for by subtracting out the background absorbance of the treatment without cells. Each treatment was done in triplicate and data are expressed as mean + SEM.

*Indicates statistically significant difference at P<0.05
Figure 3: Effects of varying concentration of glucose on HepG2 cells viability measured by MTT cells growth assay and trypan blue exclusion method. HepG2 cells were exposed to varying concentrations of glucose (0-160 mM) for 24 hours and their viability was determined. The viability of the control was assumed to be 100% and the data are expressed as viability relative to the control. For the MTT assay, the viability is corrected for by subtracting out the background absorbance of each treatment without cells. Each concentration was done in triplicate for both methods and the data is expressed as as mean ± SEM.

*Indicates statistically significant difference at P<0.05
Figure 4: Effect of varying concentrations of glucose on ALT leakage from HepG2 cells. HepG2 cells were exposed to varying concentrations of glucose for 24 hours and the level of transaminase enzyme were measured. Each value was corrected for by subtracting out the background absorbance of the treatment without cells. Each treatment was done in triplicate and data are expressed as mean ± SEM.

*Indicates statistically significant difference at P<0.05.
Figure 5: Effect of PSE on high glucose induced cytotoxicity in HepG2 cells viability measured by MTT cells growth assay and trypan blue exclusion method. HepG2 cells were exposed to either glucose, peanut skin extract, glucose and peanut skin extract, n-acetyl cysteine, or n-acetyl cysteine and glucose for 24 hours. N-acetyl cysteine is an antioxidant and used as a positive control. The viability of the control was assumed to be 100% and the data are expressed as viability relative to the control. For the MTT assay, the viability is corrected for by subtracting out the background absorbance of each treatment. Each treatment was done in triplicate and data are expressed as viability relative to the control and as mean ± SEM.

*Indicates statistically significant difference at P<0.05
Figure 6: Protective effect of peanut skin extract (PSE) on ALT leakage from HepG2 cells. HepG2 cells were exposed to either glucose, peanut skin extract, glucose and peanut skin extract, n-acetyl cysteine (NAC) or n-acetyl cysteine and glucose for 24 hours. N-acetyl cysteine is an antioxidant and used as a positive control. Each value was corrected for by subtracting out the background absorbance of the treatment without cells. Each treatment was done in triplicate and data are expressed as mean ± SEM.

*Indicates statistically significant difference at P<0.05
Figure 7: Postprandial change in glucose level from baseline (mean± SEM) during an oral glucose tolerance test. The fasting glucose level was used a baseline and the change in glucose level is plotted over time for 2.5 hours of the oral glucose tolerance test. Treatments for the oral glucose tolerance test include 50 gram glucose solution either alone (□), with capsule filled with maltodextrin (MD) (▲), with capsule filled with peanut skin extract (PSE) (♦), with PSE-fortified peanuts(●), or with non-fortified peanuts(*) in 15 healthy subjects. Each subject consumed each treatment in triplicate, with a week wash period between each replicate. Values are expressed as mean ±SEM.

*Indicates statistically significant difference between treatment (P<0.05)
Figure 8: Incremental area under the curve (Mean+_SEM) from 0 to 150 minutes for plasma glucose concentration following consumption of treatments in an oral glucose tolerance test. Treatments for the oral glucose tolerance test include 50 gram glucose solution either alone (□), with capsule filled with maltodextrin (MD) (▲) with capsule filled with peanut skin extract (PSE) (♦), with PSE-fortified peanuts (●), or with non-fortified peanuts (*) in 15 healthy subjects. The glycemic response was plotted and the area under the curve was calculated using the trapezoidal method. Each subject consumed each treatment in triplicate, with a week wash period between each replicate. Values are expressed as mean ±SEM.

*Indicates statistically significant difference between treatment (P<0.05)
Summary and Suggestions for Future Work

This research suggests that peanut skins have potential to be a valuable functional food ingredient. The study found that peanut skin extract can be incorporated into a food without any associated negative sensory attributes. In addition, peanut skin extract was shown to have biological activity and exert an antidiabetic effect both in-vivo and in vitro.

The encapsulation of peanut skin extract with maltodextrin was effective at reducing the bitterness and astringency. This allowed for the extract to be incorporated into the peanut coating at levels high enough to increase the antioxidant activity to that of blueberries, without changing the flavor or textural profile of the coated peanuts. Future studies should investigate the effect of peanut skin extract on lipid oxidation in coated peanuts. A decrease in lipid oxidation would demonstrate that the threshold level of peanut skin is sufficient to help extend the shelf life of the coated peanuts.

Peanut skin extract was found to have an effect on hyperglycemia both in vivo and in vitro. There was a protective effect by peanut skin extract against hyperglycemia in HepG2 cells. Future studies should investigate the mechanism behind this by testing oxidative stress markers in the cells. Peanut skin extract was also found to have effect on the glycemic response to a 50-gram glucose solution. Future work should confirm this finding in a larger study with a more diverse group of participants in order to show that the results are significant and are more representative of the whole population. It would also be interesting to study the effect of a pre-treatment of peanut skin extra if it was given prior to the oral glucose tolerance test. Additionally, future studies should investigate the effect of phenolic compounds from peanut skins on glucose transporters in order to determine the mechanism behind the change in glycemic response.