DEVELOPING A NATURAL FOOD COLORANT FROM PECAN SHELLS

by

JILLIAN KAY ERICKSON

(Under the Direction of William L. Kerr)

ABSTRACT

Liquid extraction and spray drying conditions were optimized to produce a natural food colorant from pecan shells, an abundant agricultural byproduct in Georgia. The powdered colorant was evaluated for color stability across different pH values, storage temperatures, and exposure to light over six weeks. Additionally, the colorant was added to a soft drink and color changes were monitored for four weeks. The optimum liquid extraction conditions found for extracting color and phenolic compounds were 70-mesh size shells, a ratio of 3 g shells/100 mL deionized water, and heating at 121°C for 30 minutes. Spray drying the colorant was most efficient with a 40% pump rate and an inlet temperature between 140°C and 160°C. The colorant was most stable when stored at 4°C in the dark and at low pH values. Lastly, the colorant exhibited minor changes in color when applied to a soft drink.

INDEX WORDS: Pecan shells, Natural food colorant, Phenolic compounds, Spray drying

DEVELOPING A NATURAL FOOD COLORANT FROM PECAN SHELLS

by

JILLIAN KAY ERICKSON

B.S.A, The University of Georgia, 2016

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2018

DEVELOPING A NATURAL FOOD COLORANT FROM PECAN SHELLS

by

JILLIAN KAY ERICKSON

Major Professor:	William L. Kerr
Committee:	Ronald B. Pegg
	George A. Cavender

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2018

ACKNOWLEDGEMENTS

Saying thank you doesn't quite cut it, considering the level of support I've received from my family and friends during this journey of obtaining my Masters degree. Without the constant love, prayers, and encouragement from all of you, I would not have made it this far in one piece. You kept me laughing and never failed to crack jokes about pecan shells all along the way. In a nutshell – I love you all. I am also incredibly grateful for Dr. Kerr for serving as my mentor throughout graduate school, Carl Ruiz for constantly challenging me to produce the best work possible, and Po-An Chi for keeping work in the FPL fun and never failing to lend a helping hand. Thank you to Shaun Kerr for always taking over the "bad" rotations during Processing III Lab and endlessly giving me a hard time about it. I will miss working with all of you! I am grateful for Clint Wills and South Georgia Pecan Company for providing the pecan shells for my research. I would also like to thank Dr. Pegg and Dr. Cavender for graciously serving on my committee. Thank you for shaping my project and serving as resources to me throughout my time in graduate school.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

The appearance of a food product, particularly its color, strongly influences a consumer's initial perception of food quality (Loughrey, 2000). Vibrant color can entice consumers to purchase a product, while discolored or dull products may be less appealing. Colorants can serve multiple roles such as adding color to a colorless product, restoring color lost from processing, and standardizing color throughout batches of products (Martins, Roriz, Morales, Barros, & Ferreira, 2016). As consumer demand for more healthful, "clean label" products increases, the sources of food colorants are becoming increasingly important to consumers (Loughrey, 2000). Colorants derived from natural materials, rather than chemical synthesis are more marketable to consumers and can impart additional health benefits compared to synthetic colors (Cortez, Luna-Vital, Margulis, & de Mejia, 2017).

Nutshells are a source of natural reddish-brown pigments that have been explored in the textile industry, but not yet applied to food. Dyes produced by boiling water with hazelnuts, chestnuts, and almonds have successfully imparted colors ranging from tan to burgundy reddish-brown to fabrics (Ismal, Ozdogan, & Yildirim, 2013; Tutak & Benli, 2012; Zhao, Feng, & Wang, 2014). Additionally, these dyes are cost effective because they are produced from an agricultural byproduct (Ismal et al., 2013; Zhao et al., 2014).

Pecan shells are a major agricultural byproduct in Georgia and typically considered waste and discarded. Assuming 40-50% of the weight of the pecan comes from its shell, Georgia produced more than 43 million pounds of pecan shells in 2016 alone (do Prado et al., 2104;

NASS, 2017). Utilizing the pigments present in pecan shells for a food colorant would create a value-added product that fills an increasing need for naturally sourced colorants.

This study focused on developing a natural food colorant from pecan shells and testing its color stability. The first phase of the study consisted of optimizing liquid extraction conditions for extracting color and phenolic compounds from pecan shells. Next, spray drying conditions were optimized in the second phase of the study to produce a powdered colorant from the liquid extract. In the third phase of the study, the stability of the powdered colorant was assessed in response to pH, storage temperature, and exposure to light over six weeks. Additionally, the colorant was added to a soft drink and color change was monitored for four weeks.

CHAPTER 2

REVIEW OF LITERATURE

Pecan Shell Background

Size of the Pecan Industry

Pecans are a vital crop to the state of Georgia and are increasing in popularity worldwide. Global production of pecans has increased 59% over the past 10 years. Based on a six-year average, the United States is the leading producer of pecans with 59% of the world's pecan kernel production by weight, followed by Mexico with 35% (INDFC, 2015). In 2016, the United States produced 268,770,000 lbs of pecans valued at $696,806,000. Within the United States, Georgia is the leading state in pecan production, followed by New Mexico, Texas, and Arizona (NASS, 2017). In 2016, Georgia produced 109,000,000 lbs of pecans valued at $272,500,000. The majority of pecans are sold in shelled form, as traditionally the pecan kernel is the only portion of the nut that is consumed. Based on the production values for 2016, roughly 91% of the pecans were sold shelled (NASS, 2017). This high volume of shelling consequently generates a high volume of pecan shells. In fact, approximately 40-50% of the weight of a pecan is due to its shell (do Prado et al., 2014). Therefore, using the values previously stated for 2016 production, Georgia alone produced over 43 million pounds of pecan shells in one year (NASS, 2017). Pecan shells are an abundant byproduct of one of the leading crops in Georgia and it would be economically beneficial to investigate potential uses for pecan shells.

Current Uses for Pecan Shells

Although the pecan industry generates millions of pounds of pecan shells each year, current uses for pecan shells are limited. On a basic level, ground pecan shells can act as mulch. Pecan shell mulch provides a reddish-brown color for ornamental landscapes and a study from Stafne, Rohla, and Carroll (2009) found that pecan shell mulch is effective at controlling weeds and provided similar or better fruit yield and tree growth for Peach trees than a traditional herbicide treatment. Nutshells have also been widely studied as a cost effective alternative source of granular activated carbons (GACs) for water treatment to replace coal and expensive imported coconut shells (Bansode, Losso, Marshall, Rao, & Portier, 2004). Pecan shell-based GACs were shown to have higher adsorptive efficiency than a coal-based commercial carbon (Bansode et al., 2004). In the food science sector, research was conducted in the UGA Department of Food Science and Technology on substituting pecan shells for pecan and other woods in smoked chicken products (Fu, 2015). Overall, smoking with pecan shells yielded chicken with good consumer sensory acceptability and similar properties to chicken smoked with hickory, mesquite, and apple tree wood (Fu, 2015). Furthermore, pecan shell extract was found to be a viable natural antioxidant in margarine. Following extended storage, the margarines with pecan shell extract exhibited the same quality as margarines with the synthetic antioxidant butylated hydroxytoluene (BHT) (Ribeiro, Policarpi, Dal Bo, Barbetta, & Block, 2017). While some applications for pecan shells exist, none are widely practiced. Most pecan shells are considered waste and discarded by processors.

Composition of Pecan Shells

Before exploring applications for pecan shells, it is important to know their composition. Pecan shells are mostly composed of insoluble fiber (cellulose, lignin, hemicellulose) making up

45.5% of the shell by weight (do Prado, Aragao, Fett, & Block, 2009). Following fiber, other carbohydrates make up 29.6% of the shell. Lipids comprise 1.1% of shell weight and protein is present at 2.2%. The moisture content of pecan shells is 16.8% by weight (do Prado et al., 2009). Outside of the three macronutrients, pecan shells contain exceptionally high contents of polyphenols (4.5%) and proanthocyanidins (PACs) or condensed tannins (10%) (do Prado et al., 2009). While pecans are often touted as one of the most antioxidant rich nuts, pecan shells were shown to have about 4.5 times higher antioxidant capacity than pecan kernels (Villarreal-Lozoya, Lombardini, & Cisneros-Zevallos, 2007). Additionally, pecan shells contain 6-18 times higher total phenolics and condensed tannins than pecan kernels (Villarreal-Lozoya et al., 2007). Prior to 2018, six specific phenolic compounds were identified with high performance liquid chromatography (HPLC) in pecan shells. Compounds identified included four phenolic acids: gallic acid, chlorogenic acid, p-hydroxybenzoic acid, and ellagic acid, along with the flavanols epigallocatechin and epicatechin gallate (de la Rosa, Alvarez-Parrilla, & Shahidi, 2011; do Prado et al., 2014). These specific phenolic compounds, especially gallic acid and epicatechin gallate, contribute to the high antioxidant activity of pecan shells (do Prado et al., 2014). Recently, Hilbig et al. (2018) also used HPLC to identify 24 additional phenolic compounds in pecan shell extracts. Newly identified phenolic compounds present in the highest amounts included two flavanols: (+)-catechin and (-)-epicatechin, another phenolic acid: vanillic acid, and a flavonol: myricetin (Hilbig et al., 2018).

Health Benefits of Pecan Shells

Several recent studies have found potential health benefits from consuming antioxidant rich aqueous pecan shell extracts. In a study with rats by Müller et al. (2013), aqueous pecan shell extract was found to help prevent liver disease caused by ethanol consumption. Even

relatively small concentrations of pecan shell extract were able to reduce thiobarbituric acid reactive substances (TBARS), which are lipid peroxidation byproducts. In a study by Reckziegel et al. (2011), aqueous pecan shell extract helped prevent oxidative damage and reduce anxiety from smoking cessation in mice passively exposed to cigarettes. Furthermore, aqueous pecan shell extract exhibited antidiabetic and antihypercholesterolemic effects in Wistar rats (Porto et al., 2015). These studies both support the traditional medicinal use of pecan shell tea, which is produced by steeping pecan shells with water (Porto et al., 2015). Pecan shells represent an underutilized source of health promoting antioxidant compounds that could be valuable to the food industry.

Phenolic Compounds Background

Classes of Phenolic Compounds in Pecan Shells

Perhaps the most widely studied and useful compounds isolated from pecan shells are phenolic compounds. Phenolic compounds are secondary plant metabolites that contain an aromatic ring with at least one hydroxyl group attached. According to their structure and number of phenolic rings, phenolic compounds are further broken down into several groups (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). Recent studies have focused on three main groups of polyphenols in pecan shells: phenolic acids, flavanols, and proanthocyanidins (PACs) (de la Rosa et al., 2011; do Prado et al., 2014; Hilbig et al., 2018). Phenolic acids may be further divided into two classes: derivatives of benzoic acid or cinnamic acids (Manach et al., 2004). The phenolic acids that have been isolated in pecan shells are benzoic acid derivatives such as, gallic acid, chlorogenic acid, p-hydroxybenzoic acid, ellagic acid, and vanillic acid (de la Rosa et al., 2011; do Prado et al., 2014; Hilbig et al., 2018). The other major classes of polyphenols found in pecan shells are PACs, a type of tannin, and their monomers, flavan-3-ols. Tannins are

highly hydroxylated and polymerized compounds with molecular weights ranging between 500 and 30,000 Da (Bravo, 1998; Serrano, Puupponen-Pimia, Dauer, Aura, & Saura-Calixto, 2009). They are often concentrated in peels or outer portions of crops (Serrano et al., 2009). Depending on monomeric structure, tannins are divided into two main groups: hydrolyzable tannins and condensed tannins or proanthocyanidins (PACs) (Bravo, 1998). Flavan-3-ols, such as catechin and epicatechin, are the monomeric units of PACs (Bravo, 1998). Two additional examples of flavan-3-ols that have been identified in pecan shells are epigallocatechin and epicatechin gallate (do Prado et al., 2014). Another identifying characteristic of tannins is their ability to form complexes with carbohydrates and proteins (Bravo, 1998). Many foods rich in tannins have an astringent or bitter taste caused by the complex between salivary proteins and tannins (Manach et al., 2004).

Chemical Analysis of Phenolic Compounds

With the widespread nature of phenolic compounds and potential health benefits from their antioxidant capacity, chemical analyses are needed to quantify phenolics content and antioxidant activity within foods. Originally in order to quantify the total phenolics content of wine, Singleton and Rossi developed a colorimetric method using Folin and Ciocalteu's phenol reagent (FCR) to determine total phenolics content (TPC) (Singleton & Rossi, 1965). This method is now used for a variety of foods and beverages. Prior to reaction with phenolic compounds, the molybdenum component of the phosphotungstic-phosphomolybdic complexing reagent in the acidic FCR is yellow. After electron reduction by phenolic compounds, the color of the molybdenum component turns blue under alkali conditions from the addition of saturated sodium carbonate solution. The absorbance of the blue compound is measured with a

spectrophotometer and compared to a standard curve of a phenolic compound, most commonly gallic acid, to calculate TPC (Singleton, Orthofer, & Lamuela-Raventos, 1999).

Colorimetric methods have also been developed to specifically measure the amount of PACs in food products (Serrano et al., 2009). Perhaps the most specific and sensitive of these methods involves the reaction of PACs with the reagent 4-dimethylaminocinnamaldehyde (DMAC) (Payne et al., 2010). The yellow DMAC reagent reacts with PACs meeting the following three structural conditions to form a green color measurable at 640 nm: meta-substituted dihydroxybenzene rings, a single bond between C2 and C3, and the lack of a carbonyl at C4 (McMurrough & McDowell, 1978; Payne et al., 2010). A figure displaying the ring structure of flavanols, including the locations of C2, C3, and C4, is shown in Figure 2.1. A variety of standards can be used to calculated PAC content, such as (+) - catechin and procyanidin B2 (Payne et al., 2010).

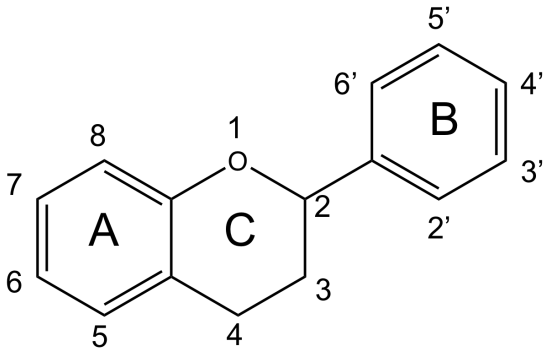

Figure 2.1: Ring structure of flavanols

One straightforward method for measuring antioxidant activity from phenolic compounds is the reaction with 2,2'-diphenyl-1-picrylhydrazl (DPPH). Antioxidants within the sample quench the free radical in DPPH by transferring a proton and an electron. As a result, DPPH loses its initial purple color and the absorbance loss is measured by a spectrophotomer at 517 nm (Plank et al., 2012). Samples are compared to a standard curve of dilutions of Trolox ®, a water-

soluble vitamin E analog, to calculate antioxidant activity (Plank et al., 2012). Overall, these three methods are useful for measuring total phenolics content, specifically quantifying PACs, and analyzing the activity of the antioxidants present to gain a better understanding of the composition and usefulness of phenolic compounds present in samples.

Food Colorants

Demand for Natural Colorants

Color is one of the first factors considered by a consumer when evaluating appearance and selecting a food product (Delgado-Vargas & Paredes-Lopez, 2003). In order to create the most desirable appearance, colorants may be added to foods. These colorants are also useful for providing color to a colorless product, adding back color lost during processing, or providing uniform color to products (Martins, Roriz, Morales, Barros, & Ferreira, 2016). Consumers are increasingly demanding natural alternatives to synthetic food colorants. In fact, from 2015 to 2019, the global market for natural food colorants is projected to increase at a compound annual growth rate of 6.22% (Cortez, Luna-Vital, Margulis, & de Mejia, 2017). As a whole, the food colors industry is anticipated to increase 10 to 15% annually (IFT, 2016). Ingredients with a natural origin falling under the "clean label" category appear more familiar to consumers and could provide additional health benefits compared to their synthetic counterparts (Cortez et al., 2017). Furthermore, artificial food colorants have been associated with hyperactivity in children among other harmful side effects that can negatively impact consumers' opinion of synthetic colorants (Martins et al., 2016). The European Food Safety Authority (EFSA) has taken steps within the past few years to limit the use of some artificial colorants by lowering the acceptable daily intake for three artificial colors and requiring a warning label stating that the product "may

have an adverse effect on activity and attention in children" (Ghidouche, Rey, Michel, & Galaffu, 2013). Moving forward, high demand exists for natural food colorants.

Natural Colorants and Nutshells

In addition to providing antioxidant activity and health benefits, current research highlights nutshells as a source of reddish brown pigments. While nothing was found in the recent literature about nutshells as a natural colorant for food, many studies have been conducted on nutshells as a natural dye source for textiles. As with the natural food colorant industry, textile manufacturers are seeking natural alternatives for environmentally harmful synthetic dyes (Tutak & Benli, 2012). Nutshells are also cost effective sources of pigments, because they are underutilized byproducts of nut processing (Ismal, Ozdogan, & Yildirim, 2013; Zhao, Feng, & Wang, 2014). Hazelnuts, chestnuts, and almonds can all be boiled with water to produce dyes suitable for coloring fabrics in shades ranging from tan to burgundy reddish-brown (Ismal et al., 2013; Tutak & Benli, 2012; Zhao et al., 2014). Applying this knowledge to a natural food colorant has not been explored and would be advantageous both economically and environmentally.

Color Measurement

Difficulties arise when attempting to accurately and precisely describe color, which is subjectively observed by the eye. In order to avoid such difficulties and produce repeatable and quantifiable color measurements, two main methods have been developed to objectively measure color (Loughrey, 2000). A colorimeter measures color by illuminating a sample and then passing the reflected light through red, green, and blue filters to mimic the types of color receptors in the human eye. Values are calculated from the light passed through filters and can be reported in various scales (Loughrey, 2000). Two of the most popular color scales in the food industry today

are the CIE L*a*b* scale and the CIE L*c*h color scale (Wrolstad & Smith, 2010). In both scales, L* represents lightness and 100 corresponds to white and 0 corresponds to black. The amount of red or green color in a sample is represented by the value of a*, with positive values being more red and negative values being more green. Similarly, the amount of yellow or blue color in a sample is represented by the value of b*, with positive values being more yellow and negative values being more blue (Wrolstad & Smith, 2010). In the L*c*h color scale, c* is a measure of the chroma of a sample. Chroma is the saturation of the color and lower values indicate dull colors, whereas colors with higher values are more vibrant. Lastly, h is a measure of the hue or basic color of the sample and is reported as an angle. A value of 0° corresponds to red, 90° corresponds to yellow, 180° corresponds to green, and 270° corresponds to blue (Loughrey, 2000). Color can also be measured using a spectrophotometer. This instrument measures the amount of light absorbed by a sample at individual wavelengths, which can also be converted to color scale values (Loughrey, 2000). The absorbance curve obtained from a spectrophotometer is unique for each color. When comparing the two methods, colorimeters are less expensive, easier to use, and faster than spectrophotometers. On the contrary, spectrophotometers can be more suitable for transparent samples and provide more detailed data than a colorimeter (Loughrey, 2000; Wrolstad & Smith, 2010). Both methods for measuring color are valuable depending on the type of sample to be analyzed.

Spray Dried Powder Colorants

Food colorants are often manufactured into powdered forms due to increased storage stability, improved ease of handling, and lower weight and cost of transport compared to liquid colorants (Obón, Castellar, Alacid, & Fernández-López, 2009). A popular method for producing such powdered forms of food colorants is spray drying. The spray drying process begins by

atomizing a feed of liquid into a spray of smaller droplets, and then moisture is evaporated from the resulting spray by contact with hot air to produce a final dried powder (Masters, 1976). As one of the leading drying methods in the food industry, spray drying has many advantages. The main advantages include the ability to continuously produce high volumes of product in one-step with equipment that is relatively simple to operate, the ability to dry heat-sensitive materials and maintain their nutrients, control over product properties and quality, and production of powders with uniform particle sizes and shapes (Passos & Ribeiro, 2010). Because of the advantages of powder colorants over liquid colorants and the aforementioned benefits of spray drying, producing a spray dried pecan shell colorant from the optimized liquid colorant was a practical way to generate a more useable product for food applications.

Properties of Powders

Properties of dry powders such as moisture content, bulk density, and solubility are all important factors in the functionality and final application of the powder. These properties can be manipulated by operating conditions during spray drying. Feed rate, feed solids content, inlet drying temperature, and spray-air contact velocity are some of the most influential operating conditions (Masters, 1976). Drying aids or carriers can also be added to feeds of spray-dried products to increase product yield and stability (Nadeem, Torun, & Özdemir, 2011). Partially hydrolyzed starch products, typically maltodextrins or dried glucose syrups, are common carriers for spray drying due to their lack of taste and smell, white color, and high digestibility (Obón et al., 2009). Bulk density, specifically tap bulk density, refers to the volume that a defined mass of powder occupies following tapping the measuring vessel to tightly pack the particles. This powder property is especially important in packaging powders, as the volume of a product with bulk density that is too low will not fit in the package and a product with bulk density that is too

high will not adequately fill the package (Barbosa-Cánovas, Ortega-Rivas, Juliano, & Yan, 2005). Solubility is another important property of powders, because most powders will be rehydrated in their final application and difficulty when dissolving powders is problematic and undesirable to consumers (Fitzpatrick et al., 2016). Manipulating spray drying operating conditions can alter the solubility of the product by changing the surface characteristics of the particles (Jafari, Ghalenoei, & Dehnad, 2017). Measuring the effects of various spray drying operating conditions on the properties of the powder produced is key to understanding the optimum processing method for the most functional end product.

Stability of Natural Colorants

Despite the consumer appeal and potential health benefits of natural colorants, their stability is often inferior to synthetic colorants. Color change or fading in a food product from unstable colorants may indicate quality loss to consumers (Sutherland, Varnum, & Evans, 1986). Therefore, formulating colorants that are stable at a minimum for the shelf life of the target food product is crucial (Ghidouche et al., 2013). Factors such as pH, temperature, light, humidity, and oxygen are all known to impact the stability of phenolic compounds (Hernández-Herrero & Frutos, 2014). The mechanisms of fading reactions vary based on the type of pigment. Examples include oxidation in carotenoids, hydration and oxidation in anthocyanins, and metal ion loss and oxidation in chlorophylls (Ghidouche et al., 2013). Little is known about the stability of the condensed tannins and other polyphenolic compounds found in pecan shells as natural food colorants. However, many studies have been conducted on the stability of natural food colorants sourced from anthocyanins (Cortez et al., 2017). A purple-red extract of the leaves of *Rhoeo spathacea* (Swartz) Stearn, an herbal plant rich in anthocyanins, was studied for color stability under 17 different pH values, two temperatures, and two lighting conditions for 60 days. The

results showed 80% color stability in samples at or below pH 6.0 stored at 25°C for 15 days. Also, colors were generally more stable when stored at 8°C compared to 25°C (Tan, Lim, & Lee, 2014). While natural food colorants can exhibit good stability, it is important fully assess their performance under a variety of stressors to ensure long lasting color quality.

Colorant Food Applications

Caramel Color and Concerns

One of the most widely used classes of food colorants is caramel color. In fact, greater than 80% (by weight) of colorants added to foods and beverages are caramel color (Sengar & Sharma, 2014). Some of the most common foods employing caramel color are baked goods, beer, soft drinks, and confections. Caramel color is divided into four classes based on the composition of the color. Among these classes, classes III and IV have been studied for toxicity due to the presence of 4(5) methylimidazole (4(5)-MI) (Lee & Lee, 2016). This compound produced by the Maillard reaction could have carcinogenic effects on humans according to the International Agency for Research on Cancer (IARC, 2012). In light of recent toxicity studies finding lung cancer in male and female mice after long-term exposure to 4(5)-MI, regulations have become stricter for using caramel color (NTP, 2007). 4-Methylimidazole is listed as a carcinogen on California's Proposition 65, mandating a warning label at certain concentrations of the compound (OEHHA, 2016). Research is ongoing for natural brown colorants from fruit and vegetable materials without the presence of potentially carcinogenic compounds. A recent study examined coffee melanoidins from instant coffee as an antioxidant-rich alternative to caramel color in baked goods (Passos et al., 2017). While some natural alternatives to caramel color are commercially available, nutshell colors are an unexplored source of brown color that could be applied to foods and beverages.

Pecan Shell Safety

Few studies have been published regarding the toxicity of pecan shells and their extracts; however, the current information does not indicate any harmful effects from consuming pecan shell extract in the doses to be created in this experiment. Porto et al. (2013) examined the toxicity and mutagenic activity of aqueous pecan shell extract in Wistar rats. Rats were given one dose of 300 or 2000 mg/kg of the extract or 10 or 100 mg/kg for 28 days. No toxic effects, point mutations, or chromosomal mutations were observed in any of the groups, suggesting that pecan shell extract appears to be safe to consume in small doses. A more recent study by Porto et al. (2016) found that the median lethal dose (LD_{50}) of aqueous pecan shell extract was 1166.3 mg/kg for mice, and the accumulation of inorganic elements such as copper and manganese from the shells could be harmful at high doses. Furthermore, a safety study with pecan shell fiber and rats supported the idea that pecan shells are safe to consume. The no observable adverse effect level (NOAEL) for pecan shell fiber (ground pecan shells) over 91 days of feeding Sprague-Dawley rats was 150,000 ppm, corresponding to 9947.5 mg/kg bw/day in males and 11,082.8 mg/kg bw/day in females. Additionally, results from a mouse peripheral blood micronucleus assay and a bacteria reverse mutation assay indicated that pecan shell fiber is non-genotoxic (Dolan, Matulka, Worn, & Nizio, 2016). The results from these three studies support that it would be safe to consume the pecan shell extract colors created in the proposed experiment.

While tree nuts are one of the eight major allergens of concern, the allergenicity of pecan shells is unclear. Three allergenic proteins have been identified in pecan kernels, but no work has been conducted to identify proteins in pecan shells (Sharma et al., 2011a; Sharma et al., 2011b; Zhang et al., 2016). The extremely low protein content of pecan shells suggests that even if any allergenic proteins were present, the content of the allergen would be incredibly low (do Prado et

al., 2009). Further studies would be needed to determine if pecan shell products are safe to consume for those with a tree nut allergy.

References

Bansode, R. R., Losso, J. N., Marshall, W. E., Rao, R. M., & Portier, R. J. (2004). Pecan shell-based granular activated carbon for treatment of chemical oxygen demand (COD) in municipal wastewater. *Bioresource Technology, 94*, 129-135. doi:10.1016/j.biortech.2003.12.009

Barbosa-Cánovas, G. V., Ortega-Rivas, E., Juliano, P., & Yan, H. (2005). *Food powders: physical properties, processing, and functionality*. New York, NY: Kluwer Academic/Plenum Publishers.

Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews, 56*, 317-333.

Cortez, R., Luna-Vital, D. A., Margulis, D., & de Mejia, E. G. (2017). Natural pigments: stabilization methods of anthocyanins for food applications. *Comprehensive Reviews in Food Science and Food Safety, 16*, 180-198. doi:10.1111/1541-4337.12244

de la Rosa, L. A., Alvarez-Parrilla, E., & Shahidi, F. (2011). Phenolic compounds and antioxidant activity of kernels and shells of Mexican pecan (*Carya illinoinensis*). *Journal of Agricultural and Food Chemistry, 59*, 152-162. doi:10.1021/jf1034306

Delgado-Vargas, F., & Paredes-Lopez, O. (2003). Natural colorants for food and nutraceutical uses. *Trends in Food Science & Technology*, *14*, 438. doi:10.1016/S0924-2244(03)00076-1.

do Prado, A. C. P., Aragao, A. M., Fett, R., & Block, J. M. (2009). Antioxidant properties of Pecan nut *Carya illinoinensis* (Wangenh.) C. Koch shell infusion. *Grasas y Aceites, 60*, 330-335. doi:10.3989/gya.107708

do Prado, A. C. P., da Silva, H. S., da Silveira, S. M., Barreto, P. L. M., Vieira, C. R. W., Maraschin, M., . . . Block, J. M. (2014). Effect of the extraction process on the phenolic compounds profile and the antioxidant and antimicrobial activity of extracts of pecan nut *Carya illinoinensis* (Wangenh) C. Koch shell. *Industrial Crops and Products, 52*, 552-561. doi:10.1016/j.indcrop.2013.11.031

Dolan, L., Matulka, R., Worn, J., & Nizio, J. (2016). Safety studies conducted on pecan shell fiber, a food ingredient produced from ground pecan shells. *Toxicology Reports, 3*, 87-97. doi:10.1016/j.toxrep.2015.11.011

Fitzpatrick, J. J., van Lauwe, A., Coursol, M., O'Brien, A., Fitzpatrick, K. L., Ji, J. J., & Miao, S. (2016). Investigation of the rehydration behaviour of food powders by comparing the behaviour of twelve powders with different properties. *Powder Technology, 297*, 340-348. doi:10.1016/j.powtec.2016.04.036

Fu, W. (2015). Utilization of pecan shells in smoked chicken products. (Master's thesis). Retrieved from http://dbs.galib.uga.edu/cgi-bin/getd.cgi?userid=galileo&serverno=16&instcode=publ&_cc=1

Ghidouche, S., Rey, B., Michel, M., & Galaffu, N. (2013). A Rapid tool for the stability assessment of natural food colours. *Food Chemistry, 139*, 978-985. doi:10.1016/j.foodchem.2012.12.064

Hernández-Herrero, J. A., & Frutos, M. J. (2014). Colour and antioxidant capacity stability in grape, strawberry and plum peel model juices at different pHs and temperatures. *Food Chemistry, 154*, 199-204. doi:10.1016/j.foodchem.2014.01.007

Hilbig, J., Alves, V. R., Muller, C. M. O., Micke, G. A., Vitali, L., Pedrosa, R. C., & Block, J. M. (2018). Ultrasonic-assisted extraction combined with sample preparation and analysis

using LC-ESI-MS/MS allowed the identification of 24 new phenolic compounds in pecan nut shell *Carya illinoinensis* (Wangenh) C. Koch extracts. *Food Research International, 106*, 549-557. doi:10.1016/j.foodres.2018.01.010

IARC Monographs. (2012). *4-Methylimidazole.* Retrieved from Monographs.iarc.fr/ENG/Monographs/ vol101/mono101-015.pdf.

Institute of Food Technologists. (2016). *Coloring foods & beverages*. Retrieved from http://s36.a2zinc.net/clients/IFT/IFT16/Public/ eventmap.aspx?shmode=E.

International Nut & Dried Fruit Council. (2015). *Global statistical review 2014-2015.* Retrieved from http://www.nutfruit.org/global-statistical-review-2014-2015_101779.pdf.

Ismal, O. E., Ozdogan, E., & Yildirim, L. (2013). An alternative natural dye, almond shell waste: effects of plasma and mordants on dyeing properties. *Coloration Technology, 129*, 431-437. doi:10.1111/cote.12047

Jafari, S. M., Ghalenoei, M. G., & Dehnad, D. (2017). Influence of spray drying on water solubility index, apparent density, and anthocyanin content of pomegranate juice powder. *Powder Technology, 311*, 59-65. doi:10.1016/j.powtec.2017.01.070

Lee, S., & Lee, K. G. (2016). Analysis and risk assessment of 4(5)-methylimidazole in brown colored foods and beverages. *Food Additives & Contaminants Part B-Surveillance, 9*, 59-65. doi:10.1080/19393210.2015.1127294

Loughrey, K. (2000). The measurement of color. In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants: science and technology* (pp. 273-287). New York, NY: Marcel Dekker, Inc.

Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition, 79*, 727-747.

Martins, N., Roriz, C. L., Morales, P., Barros, L., & Ferreira, I. (2016). Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. *Trends in Food Science & Technology, 52*, 1-15. doi:10.1016/j.tifs.2016.03.009

Masters, K. (1976). *Spray drying; an introduction to principles, operational practice, and applications*. Cleveland, OH: CRC Press.

McMurrough, I., & McDowell, J. (1978). Chromatographic separation and automated analysis of flavanols. *Analytical Biochemistry, 91*, 92-100. doi:10.1016/0003-2697(78)90819-9

Müller, L. G., Pase, C. S., Reckziegel, P., Barcelos, R. C. S., Boufleur, N., Prado, A. C. P., . . . Burger, M. E. (2013). Hepatoprotective effects of pecan nut shells on ethanol-induced liver damage. *Experimental and Toxicologic Pathology, 65*, 165-171. doi:10.1016/j.etp.2011.08.002

Nadeem, H. S., Torun, M., & Özdemir, F. (2011). Spray drying of the mountain tea (*Sideritis stricta*) water extract by using different hydrocolloid carriers. *LWT - Food Science and Technology, 44*, 1626-1635. doi:10.1016/j.lwt.2011.02.009

National Agricultural Statistics Service. (2017). *Noncitrus fruits and nuts 2016 summary.* Retrieved from http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-06-27-2017.pdf

National Toxicology Program. (2007). *Toxicology and carcinogenesis of 4-methylimidazole (CAS No. 822-36-6) in F344/N rats ad B6C3F1Mice (fee studies)*. NTP Technical Report Series, No.535 (NIH Publication No. 07-4471). Research Triangle Park, NC: U.S. Department of Health and Human Service, NTP.

Obón, J. M., Castellar, M. R., Alacid, M., & Fernández-López, J. A. (2009). Production of a red–purple food colorant from Opuntia stricta fruits by spray drying and its application in food model systems. *Journal of Food Engineering, 90*, 471-479. doi:10.1016/j.jfoodeng.2008.07.013

Office of Environmental Health Hazard Assessment. (2016). *4-Methylimidazole (4-MEI) A Fact Sheet.* Retrieved from https://oehha.ca.gov/proposition-65/4-methylimidazole-4-mei-fact-sheet.

Passos, C. P., Kukurova, K., Basil, E., Fernandes, P. A. R., Neto, A., Nunes, F. M., . . . Coimbra, M. A. (2017). Instant coffee as a source of antioxidant-rich and sugar-free coloured compounds for use in bakery: Application in biscuits. *Food Chemistry, 231*, 114-121. doi:10.1016/j.foodchem.2017.03.105

Passos, M. L., & Ribeiro, C. P. (2010). *Innovation in food engineering: new techniques and products*. Boca Raton, FL: CRC Press.

Payne, M. J., Hurst, W. J., Stuart, D. A., Ou, B. X., Fan, E., Ji, H. P., & Kou, Y. (2010). Determination of total procyanidins in selected chocolate and confectionery products using DMAC. *Journal of AOAC International, 93*, 89-96.

Plank, D. W., Szpylka, J., Sapirstein, H., Woollard, D., Zapf, C. M., Lee, V., . . . Baugh, S. (2012). Determination of antioxidant activity in foods and beverages by reaction with 2,2'-diphenyl-1-picrylhydrazyl (DPPH): collaborative study first action 2012.04. *Journal of AOAC International, 95*, 1562-1569. doi:10.5740/jaoacint.CS2012_04

Porto, L. C. S., da Silva, J., Ferraz, A. D. F., Correa, D. S., dos Santos, M. S., Porto, C. D., & Picada, J. N. (2013). Evaluation of acute and subacute toxicity and mutagenic activity of the aqueous extract of pecan shells *Carya illinoinensis* (Wangenh.) K. Koch. *Food and Chemical Toxicology, 59*, 579-585. doi:10.1016/j.fct.2013.06.048

Porto, L. C. S., da Silva, J., Ferraz, A. B. F., Ethur, E. M., Porto, C. D. L., Marroni, N. P., & Picada, J. N. (2015). The antidiabetic and antihypercholesterolemic effects of an aqueous extract from pecan shells in Wistar rats. *Plant Foods for Human Nutrition, 70*, 414-419. doi:10.1007/s11130-015-0510-9

Porto, L. C. S., da Silva, J., Sousa, K., Ambrozio, M. L., de Almeida, A., dos Santos, C. E. I., . . . Picada, J. N. (2016). Evaluation of toxicological effects of an aqueous extract of shells from the pecan nut *Carya illinoinensis* (Wangenh.) K. Koch and the sossible association with its inorganic constituents and major phenolic compounds. *Evidence-Based Complementary and Alternative Medicine*, *2016*, 1-8. doi:10.1155/2016/4647830

Reckziegel, P., Boufleur, N., Barcelos, R. C. S., Benvegnu, D. M., Pase, C. S., Muller, L. G., . . . Burger, M. E. (2011). Oxidative stress and anxiety-like symptoms related to withdrawal of passive cigarette smoke in mice: Beneficial effects of pecan nut shells extract, a by-product of the nut industry. *Ecotoxicology and Environmental Safety, 74*, 1770-1778. doi:10.1016/j.ecoenv.2011.04.022

Ribeiro, P. C. E., Policarpi, P. D., Dal Bo, A., Barbetta, P. A., & Block, J. M. (2017). Impact of pecan nut shell aqueous extract on the oxidative properties of margarines during storage. *Journal of the Science of Food and Agriculture, 97*, 3005-3012. doi:10.1002/jsfa.8141

Sengar, G., & Sharma, H. K. (2014). Food caramels: a review. *Journal of Food Science and Technology-Mysore, 51*, 1686-1696. doi:10.1007/s13197-012-0633-z

Serrano, J., Puupponen-Pimia, R., Dauer, A., Aura, A. M., & Saura-Calixto, F. (2009). Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Molecular Nutrition & Food Research, 53*, S310-S329. doi:10.1002/mnfr.200900039

Sharma, G. M., Irsigler, A., Dhanarajan, P., Ayuso, R., Bardina, L., Sampson, H. A., . . . Sathe, S. K. (2011). Cloning and characterization of an 11S legumin, Car i 4, a major allergen in pecan. *Journal of Agricultural and Food Chemistry, 59*, 9542-9552. doi:10.1021/jf2017447

Sharma, G. M., Irsigler, A., Dhanarajan, P., Ayuso, R., Bardina, L., Sampson, H. A., . . . Sathe, S. K. (2011). Cloning and characterization of 25 albumin, Car i 1, a major allergen in pecan. *Journal of Agricultural and Food Chemistry, 59*, 4130-4139. doi:10.1021/jf104319d

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In L. Packer (Ed.), *Oxidants and Antioxidants, Pt A* (Vol. 299, pp. 152-178). San Diego: Elsevier Academic Press Inc.

Singleton, V. L. & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144-158.

Stafne, E. T., Rohla, C. T., & Carroll, B. L. (2009). Pecan shell mulch impact on 'Loring' peach tree establishment and first harvest. *HortTechnology, 19*, 775-780.

Sutherland, J. P., Varnum, A. H., & Evans, M. G. (1986). *A color atlas of food quality control.* Weert: CRC Press.

Tan, J. B. L., Lim, Y. Y., & Lee, S. M. (2014). Rhoeo spathacea (Swartz) Stearn leaves, a potential natural food colorant. *Journal of Functional Foods, 7*, 443-451. doi:10.1016/j.jff.2014.01.012

Tutak, M., & Benli, H. (2012). Dyeing properties of textiles by Turkish hazelnut (Corylus colurna): leaves, coat, shell and dice. *Coloration Technology, 128*, 454-458. doi:10.1111/j.1478-4408.2012.00399.x

Villarreal-Lozoya, J. E., Lombardini, L., & Cisneros-Zevallos, L. (2007). Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. *Food Chemistry, 102*, 1241-1249. doi:10.1016/j.foodchem.2006.07.024

Wrolstad, R. E., & Smith, D. E. (2010). Color analysis In *Food Analysis* (pp. 573-586). Boston, MA: Springer US.

Zhang, Y. Z., Lee, B. R., Du, W. X., Lyu, S. C., Nadeau, K. C., Grauke, L. J., . . . McHugh, T. H. (2016). Identification and characterization of a new pecan Carya illinoinensis (Wangenh.) K. Koch allergen, Car i 2. *Journal of Agricultural and Food Chemistry, 64*, 4146-4151. doi:10.1021/acs.jafc.6b00884

Zhao, Q., Feng, H., & Wang, L. J. (2014). Dyeing properties and color fastness of cellulase-treated flax fabric with extractives from chestnut shell. *Journal of Cleaner Production, 80*, 197-203. doi:10.1016/j.jclepro.2014.05.069

CHAPTER 3

LIQUID EXTRACTION

Introduction

Color is a primary aspect of appearance, and consequently a key attribute of foods and beverages (Loughrey, 2000). In order to be selected by consumers, products must have an appealing appearance and colorants can significantly enhance the appearance of a food. For example, colorants added to a colorless beverage add visual appeal and can help indicate flavor to a consumer (Kamuf, Nixon, & Parker, 2000). The source of food colorants is becoming increasingly important, because consumers are demanding more natural, healthful, and "clean label" foods (Cortez, Luna-Vital, Margulis, & de Mejia, 2017). Natural colorants are defined most simply as colors derived from living cells, whereas synthetic colorants are derived from chemical synthesis (Hendry & Houghton, 1996).

Caramel color is one of the most commonly used synthetic food colorants (Hendry & Houghton, 1996). In North America, caramel color is primarily used in soft drinks (Kamuf et al., 2000). Despite its widespread use, recent concerns over the carcinogenicity of compounds, such as 4(5) methylimidazole, formed by the Maillard reaction in the production of certain types of caramel color have spurred demand for natural alternatives (Lee & Lee, 2016). Of the major natural food colorants, little to none exhibit the same shade range as caramel color. Therefore, a natural brown food colorant could be extensively used and highly marketable.

Recent studies indicate that nutshells are a natural source of reddish brown pigments (Ismal, Ozdogan, & Yildirim, 2013; Tutak & Benli, 2012; Zhao, Feng, & Wang, 2014). Similar

to colorants in the food industry, natural alternatives to synthetic dyes are increasingly popular among textile manufacturers (Tutak & Benli, 2012). Fabrics have been successfully colored by reddish brown dyes produced from boiling shells from hazelnuts, chestnuts, and almonds in water (Ismal et al., 2013; Tutak & Benli, 2012; Zhao et al., 2014). This knowledge could be applied to food colorants with pecan shells in order to fill the void for a natural substitute for caramel color, while utilizing an abundant agricultural byproduct.

Millions of pounds of pecan shells are generated and discarded in Georgia alone each year, despite containing a wealth of phenolic compounds (do Prado, Aragao, Fett, & Block, 2009a; NASS, 2017). In comparison to pecan kernels, pecan shells contain 6-18 times more total phenolic compounds and proanthocyanidins (PACs), along with exhibiting roughly 4.5 times more antioxidant capacity (Villarreal-Lozoya, Lombardini, & Cisneros-Zevallos, 2007). Recent studies have shown potential health benefits from consuming aqueous extracts of pecan shells with high antioxidant capacity (Müller et al., 2013; Porto et al., 2015; Reckziegel et al., 2011). Therefore, extracting the phenolic compounds from pecan shells could serve as an inexpensive, natural source of reddish brown pigments with possible health benefits.

In order to produce vibrantly colored, phenolic rich extracts from pecan shells, it is important to determine the optimum conditions for extraction. Supercritical extraction with CO_2, extraction with water, and extraction with ethanol were all conducted on pecan shells in a study by do Prado et al. (2014). While this study examined the effect of extraction conditions on the composition of phenolic extracts in pecan shells, no color measurements were recorded. Additionally, liquid extraction was only examined for one grind size of pecan shells, one temperature, and one ratio of pecan shells to solvent. Several aspects of solvent extraction including extraction temperature and time, solid particle size, and solvent-to-solid ratio are all

important factors in extraction efficiency (Dai & Mumper, 2010). Understanding the relationships between extraction conditions and the resulting color and phenolic makeup of the extracts would be advantageous to effectively produce a liquid pecan shell colorant with rich color.

The first objective of this study was to optimize conditions for extracting color and phenolic compounds from pecan shells. The experimental factors included grind size, ratio of pecan shells to solvent, extraction temperature, and solvent type. Color was assessed by measuring CIE L*c*h color values and visible absorbance spectra. The second objective was to determine the relationship between extraction conditions and the phenolic components of pecan shell extracts. The phenolic components of the pecan shell extracts were assessed through estimating total phenolics content (TPC), condensed tannin content (CT), and antioxidant activity (AA).

Materials and Methods

Pecan Shell Preparation

Eastern Schley variety pecan shells were received from South Georgia Pecan Company (Valdosta, GA, USA) and stored in a freezer at -20°C until needed. Larger pieces of shells were separated from stray pieces of pecan kernel and very fine pieces of shell by shaking with a ¼ inch sieve (6.3 mm) and 6-mesh sieve (3.35 mm), blowing away fine particles with compressed air, and collecting the resulting large pieces by hand. Shells were ground with a Homoloid Machine grinder (Model JT, The Fitzpatrick Co., Elmhurst, IL, USA) fitted with the 1521 0125 screen. Ground shells were passed through a series of sieves by a vibrating shaker to obtain two fractions with uniform particle sizes. Sieve sizes included 50-mesh (297 μm) and 70-mesh (210

μm). Next, the shells were dried in a vacuum oven (1430MS, VWR Scientific Products, USA) at 40°C overnight and then stored in airtight plastic bags in the freezer until needed.

Liquid Extract Preparation

A 2x3x3 design with grind size, concentration, and temperature was employed to produce a total of 18 different aqueous extracts. Experiments were conducted in triplicate. A summary of sample numbers and corresponding liquid extraction conditions is presented in Table 3.1.

The dried, ground pecan shells were added to deionized (DI) water in three different concentrations: 2 g/100 mL, 2.5 g/100 mL, 3 g/100 mL in 125 mL Erlenmeyer flasks covered with aluminum foil. The concentration range was based on the work of do Prado et al. (2009a) and preliminary experiments. Samples underwent heating for 30 minutes. Three different temperatures were assessed. One set of samples was placed in a shaking water bath (Model G-76, New Brunswick Scientific Co., New Brunswick, NJ, USA) set to 70°C and a shaking speed of 180-200 rpm. The second set of samples was heated to 100°C on hot plates with constant stirring. The third set of samples was heated to 121°C at 15 psi in an autoclave (Type LS-2036, American Sterilizer Co., Erie, PA, USA). After 30 minutes of heating, the extracts were left to cool to room temperature. Additionally, the volumes of the boiled extracts were standardized to 100 mL with DI water after heating. Vacuum filtration (Grade 415, VWR Scientific Products, USA) was used to remove the pecan shells from the aqueous extracts. Extracts were stored in plastic re-sealable bags in the dark at -20°C until further use.

Extraction was also conducted with ethanol following the procedures of do Prado et al. (2014) with minor modifications. Ground shells (70-mesh size) were added to ethanol at three concentrations: 2 g/100 mL, 2.5 g/100 mL, and 3 g/100 mL. Samples were stirred constantly for one hour at room temperature. Then, samples were filtered and stored in the same manner as the

aqueous extracts until further use. A total of 21 different extracts were produced in triplicate for a final total of 63 extracts.

Dry Weight of Liquid Extracts

The dry weights of the liquid extracts were determined by gravimetric analysis. Aliquots (5 mL) of each aqueous extract were dried in a vacuum oven (1430MS, VWR Scientific Products, USA) at 105°C until reaching a constant mass (do Prado et al., 2009a). Dry weights of ethanol extracts were determined in the same manner, except at 85°C.

Total Phenolics Content

Total phenolics content (TPC) was determined by the Folin-Ciocalteu colorimetric assay with gallic acid as a standard (Singleton & Rossi, 1965). Aliquots (2.0 mL) of diluted samples were added to test tubes with 6.5 mL of DI water and mixed 0.5 mL of the Folin-Ciocalteu reagent, followed by neutralization with 1.0 mL of saturated sodium carbonate. Test tubes were thoroughly vortexed after the addition of all reagents. After incubating for 40 minutes in the dark at room temperature, the absorbance of each sample was measured with a spectrophotometer (Genesys 2 Model 336009, Spectronic Instruments, USA) at 750 nm in a 1 cm cuvette. A standard curve of gallic acid was prepared to quantify total phenolics. Results were reported as mg gallic acid equivalents (GAE)/ml extract and mg GAE/g of dry weight.

Condensed Tannin Content

Condensed tannin content was determined by the DMAC method following the procedures of Payne et al. (2010). The DMAC reagent was prepared before each run by dissolving 0.0300 ± 0.001 g of DMAC into 30 mL of 1:9 (v/v) of HCl and ethanol. Dilutions of a 600 ppm solution of (+)-catechin dissolved in 1:1 methanol-water were used to prepare the standard curve. For analysis, 50 µL of standard solutions, 50 µL of samples diluted with ethanol,

and a blank of 50 µL of ethanol were added to a 96-well plate. Aliquots of 250 µL of the DMAC reagent were added to each well. Plates were read with a microplate reader (FLUOstar Omega, BMG LABTECH Inc., Cary, NC, USA) set to 25°C and 640 nm. After adding the DMAC reagent, plates were shaken for 3 s and absorbance readings were taken every 1 min for 12 min. The maximum absorbance for each sample during this time period and the standard curve of (+)-catechin were used to determine CT. Results were reported as mg (+)-catechin equivalents (CE)/ml of extract and mg CE/g of dry weight.

Antioxidant Activity

The DPPH assay was performed on all samples to assess antioxidant activity of the liquid extracts according to the methods of Brand-Williams, Cuvelier, and Berset (1995) and Zhang and Hamauzu (2004). First, a 0.1 mM solution of DPPH was prepared by dissolving DPPH in 80% methanol. Aliquots of 4 mL of the DPPH solution were added to 0.2 mL of the diluted samples and 0.2 mL of DI water as a control. Samples were allowed to stand at room temperature in the dark for one hour before the absorbance was measured in a 1 cm cuvette at 517 nm with a spectrophotometer (Genesys 2 Model 336009, Spectronic Instruments, USA). A standard solution of 0.05 mg/ml Trolox ® was also prepared with 80% methanol. A standard curve was produced with solutions of 0.01, 0.02, 0.03, and 0.04 mg Trolox ®/mL and the results of the DPPH assay were reported as mg Trolox ® equivalents (TE)/ml of extract and mg TE/g of dry weight.

Color Assessment

Color of the liquid extracts was examined by two methods. First, a colorimeter (Model CR-410, Minolta Co. Ltd., Tokyo, Japan) was used to assess color in terms of the CIE L*c*h values under the D65 illuminant system. A petri dish was placed on top of sheets of white paper

with the color values L* = 91.77, c* = 4.02, h = 281.78°, and filled with 20 mL of the liquid extract. The colorimeter was placed directly into the petri dish filled with the extract and measurements were recorded. The colorimeter was regularly calibrated with a white calibration tile.

Secondly, a spectrophotometer (Genesys Model: G10S UV-Vis, Thermo Fisher Scientific, Madison, WI, USA) was used to record the visible absorbance spectra of samples from 380-700 nm to determine the maximum wavelength for each sample. Ethanol extracts and aqueous 70°C and 100°C extracts were diluted 1:5, while 121°C extracts were diluted 1:10 prior to taking the spectra. The absorbance of each sample at the λ_{max} was recorded and compared across samples.

Statistical Analysis

One-way ANOVA and Tukey HSD were performed with JMP 13 (SAS Institute, Cary, NC, USA) to determine statistically significant differences between means with $p<0.05$.

Results and Discussion

Dry Weight

The dry weights of all liquid extracts are found in Table 3.1. The amount of dry weight was significantly different for corresponding samples across grind size, concentration, and temperature. Therefore, all three extraction conditions are important to the amount of dry matter extracted from the pecan shells. Finer grind size, higher temperature, and higher concentration all yielded higher dry weights in aqueous extracts. Sample 18 (121°C, 70-mesh, 3g) had the highest dry weight at 0.9491 g/100 g extract, while sample 1 (70°C, 50-mesh, 2 g) had the lowest dry weight at 0.3040 g/100 g extract. Ethanol extracts had relatively high dry weight values, despite lower extraction temperatures. Similar to the aqueous extracts, higher concentrations of shells

within the ethanol extracts yielded higher dry weights. Sample E3 (Ethanol, 25°C, 70-mesh, 3 g) had the overall highest dry weight of all liquid extracts at 1.214 g/100 g. This value is nearly four times higher than that of sample 1, which had the lowest dry weight of all liquid extracts.

Increased extraction temperatures can increase phenolic extraction efficiency by increasing the solubility of the phenolic compounds (Dai & Mumper, 2010). This increased temperature can also lower the viscosity and surface tension of the solvent, which facilitates more contact with the sample matrix and improves the rate of extraction (Dai & Mumper, 2010). Higher ratios of solid-to-solvent have been found to increase efficiency of phenol extraction, because more phenolics are available at the onset of extraction. This effect has been studied in phenolic compounds from black currants and grape pomace (Cacace & Mazza, 2003; Pinelo, Rubilar, Jerez, Sineiro, & Núñez, 2005). Finally, reducing the particle size of solids is another method of increasing phenol extraction efficiency due to the increased surface area of the solid material exposed during extraction, which has been shown in studies on extracting phenolic compounds from grape byproducts where optimum extraction conditions were those with the lowest particle size tested (Pinelo, Del Fabbro, Manzocco, Núñez, & Nicoli, 2005).

Total Phenolics Content

TPC of all liquid extracts are listed in Table 3.2 and Table 3.3. When analyzed by volume of extract, TPC increased with increasing temperature and concentration and finer particle size. TPC was significantly different for all corresponding samples across grind size, temperature, and concentration, with the exception of E1 and E2. Sample 18 (121°C, 70-mesh, 3g) had the highest TPC at 4.586 mg GAE/ml, while sample 1 (70°C, 50-mesh, 2 g) had the lowest TPC at 1.358 mg GAE/ml. The results of the TPC of ethanol extracts were dispersed throughout the range of TPC

in the aqueous extracts when analyzed by volume. The highest TPC for an ethanol extract was for E3 at 3.401 mg GAE/ml.

In terms of dry weight, TPC values were much more similar across samples than in terms of volume. The range of TPC values for aqueous extracts in terms of dry weight was from 431.0 mg GAE/g (Sample 14) to 483.2 mg GAE/g (Sample 18), corresponding to 11.4% difference between the values. For the TPC values per volume of extract, values ranged from 1.358 mg GAE/mL (Sample 1) to 4.586 mg GAE/ml, (Sample 18) which corresponds to 108.6% difference. No significant differences were detected between corresponding aqueous samples across concentration or temperature. Some significant differences were detected between corresponding samples across grind size groups (2 and 5, 13 and 16, 14 and 17, 15 and 18). Finer grind sizes yielded slightly higher TPC than coarser grind sizes in these cases. Ethanol extracts had significantly lower TPC, ranging from 280.1 mg GAE/g to 308.4 mg GAE/g, when compared to all aqueous extracts.

Previous experiments have been conducted to analyze the TPC of aqueous and alcoholic pecan shell extracts. In a study by do Prado et al. (2014) extracts were prepared with a concentration of 20 g/L (dry basis) of 60-mesh sieve pecan shells to water and heating at 98°C for 10 minutes. The results indicated 181.49 mg GAE/g for TPC, which is significantly lower than the aqueous extracts in this experiment. The TPC for ethanol extracts from do Prado et al. (2014) were still lower than the results of this study at 167.85 mg GAE/g. Another study by do Prado et al. (2009a) with aqueous extracts produced under the same conditions as the previous study had a similar TPC of 138 mg GAE/g. Hilbig et al. (2018) recently generated pecan shell extracts with ultrasonic-assisted extraction, which had much higher TPC values that more closely matched the results of this study. The TPC values for the aqueous extracts and hydroalcoholic

extracts respectively were, 426.15 mg GAE/g and 581.90 mg GAE/g. However, the extraction procedures followed in this study were less expensive and easier to conduct than ultrasonic-assisted extraction.

Overall, it appears that the increase in TPC across the samples on a volume basis is due to the increase in solids extracted. When compared on a dry basis, the results are much more similar. As the amount of phenolic compounds extracted increases, the TPC increases accordingly. The discrepancy of the TPC values in this study and those found in the literature can be due to a number of factors related to variable polyphenol content in plants. Factors such as cultivar, storage conditions, germination, degree of ripeness, and processing conditions can all greatly affect the amount of polyphenols available in pecan shells (Bravo, 1998). Villarreal-Lozoya et al. (2007) examined the phenolics content and antioxidant activity of shells and kernels from several pecan cultivars. Their results revealed differences in TPC ranging from 290 mg chlorogenic acid equivalents (CAE)/g in the Desirable cultivar to 633 mg CAE/g in the Kanza cultivar (Villarreal-Lozoya et al., 2007). Therefore, it is difficult to directly compare results between studies of different pecan cultivars not stored under uniform conditions.

Condensed Tannins

The CT contents of all liquid extracts are found in Table 3.2 and 3.3. For all corresponding aqueous extracts except 3 and 6, CT was significantly higher for finer grind sizes. Higher temperatures also generally yielded extracts with higher CT content. Overall, almost all 121°C extracts had higher CT than their 70°C and 100°C counterparts. Fewer significant differences were found between pairs of 70°C and 100°C extracts. Increased concentrations of pecan shells yielded extracts with higher CT, however only some differences were statistically significant. Sample 18 (121°C, 70-mesh, 3g) had the highest CT at 6.503 mg GAE/ml, while

sample 1 (70°C, 50-mesh, 2 g) had the lowest CT at 1.718 mg GAE/ml. Similar to the TPC results, the results of the CT of ethanol extracts were dispersed throughout the range of CT in the aqueous extracts when analyzed by volume. The highest CT for an ethanol extract was for E3 at 5.290 mg GAE/ml.

CT content was more similar across samples in terms of dry weight than in terms of volume, but this trend was not as strong as the TPC results. The range of CT values for aqueous extracts in terms of dry weight was from 429.3 mg CE/g (Sample 6) to 732.5 mg GAE/g (Sample 17), corresponding to 52.19% difference between the values. For the CT values per volume of extract, values ranged from 1.718 mg CE/mL (Sample 1) to 6.503 mg CE/ml, (Sample 18) which corresponds to 116.4% difference. No significant differences were detected between corresponding aqueous samples across concentration. Some significant differences were found between corresponding samples across grind sizes (3 and 6, 13 and 16, 14 and 17). Additionally, some significant differences were found in the 70-mesh samples across temperatures. The 121°C samples with 70-mesh shells (16, 17, 18) all had significantly higher CT content than 70°C samples with 70-mesh shells (4, 5, 6). This suggests that the combination of higher temperature and finer shells could lead to more extraction of condensed tannins. Ethanol extracts CT contents were comparable to several of the aqueous extracts, ranging from 461.4 mg CE/g to 435.7 mg CE/g.

CT in pecan shell extracts has been measured in previous studies, but all of the analyses were conducted by reaction with Vanillin rather than DMAC. The CT values for liquid pecan shell extracts in this study were much higher than those found in the literature. Do Prado et al. (2014) and Hilbig et al. (2018) produced aqueous extracts with 36.94 mg CE/g and 76.82 mg CE/g, respectively. For ethanol extracts, the CT value from do Prado et al. (2014), 412.1 mg

CE/g, was much closer to the values in Table 3.3. On the contrary, the CT value of the hydroalcoholic extract produced with ultrasonic-assisted extraction from Hilbig et al. (2018) was much lower at 71.08 mg CE/g.

As with TPC, CT values appear to increase accordingly with increased amount of phenolics extracted. The same principles of variations inherent to different cultivars and storage conditions apply to CT (Villarreal-Lozoya et al., 2007). Finer particle sizes, higher temperatures, and higher concentration of pecan shells tend to produce extracts with more phenolic compounds and therefore higher CT.

Antioxidant Activity

The antioxidant activities of all liquid extracts are listed in Table 3.2 and Table 3.3. The AA of samples generally increased with increased extraction temperatures and concentrations and finer grind size. All corresponding aqueous extracts were significantly different across temperature, grind size, and concentration, with the exception of samples 4 and 10. The lowest AA of the aqueous extracts came from sample 1 (70°C, 50-mesh, 2 g) at 3.679 mg TE/ml and the highest AA was sample 18 with 11.18 mg TE/ml (121°C, 70-mesh, 3g). Like TPC and CT, the AA values of the three ethanol extracts fell among the range of aqueous values. The highest AA for an ethanol extract was sample E3 with 9.818 mg TE/ml.

The results for AA are much more similar on a dry basis than by volume. The range of AA values for aqueous extracts in terms of dry weight was from 1108 mg TE/g (Sample 3) to 1348 mg TE/g (Sample 17), corresponding to 19.54% difference between the values. For the AA values per volume of extract, values ranged from 3.679 mg TE/mL (Sample 1) to 11.18 mg TE/ml (Sample 18), which corresponds to 101.0% difference. Although there are some significant differences between AA values of the aqueous extracts, no clear trend was observed

with respect to concentration, grind size, or temperature. The AA values of ethanol extracts were significantly lower than all aqueous extracts when reporting AA on a dry basis, ranging from 808.7 mg TE/g (E3) to 908.1 mg TE/g (E1). This could be due to the difference in polarity of the solvents. Ethanol is less polar than water and may be less efficient at extracting more polar antioxidant-rich compounds.

The AA of liquid extracts presented in Table 3.4 (1268.03 mg TE/g) were similar to those reported for aqueous and hydroalcoholic pecan shell extracts produced with ultrasound (1287.08 mg TE/g) (Hilbig et al., 2018). The AA of other pecan shell extracts produced by infusion had much lower antioxidant activity, such as 612.24 mg TE/g in aqueous extracts and 524.77 mg TE/g in ethanol extracts (do Prado et al., 2014).

Based on the increased amount of solids extracted with increased temperature, smaller particle size, and higher concentrations of pecan shells, increased AA was expected. Extracting more phenolic compounds that exhibit high antioxidant activity should lead to increased AA in extracts with high TPC and CT. All three measures of the phenolic components of the liquid pecan shell extracts (TPC, CT, AA), increased in accordance with more favorable extraction conditions. The three measures all examine aspects of the same class of chemical compounds and should follow the same trends (Bravo, 1998).

Color

Color values of the liquid extracts are listed in Table 3.4. Differences in extraction temperature had the most significant effect on color values. As temperature was increased, samples appeared darker with lower L* values. Some significant differences were shown between the lightness of 70°C and 100°C samples of equal concentration and grind size (3 and 9, 5 and 11), however all 121°C extracts were significantly darker than corresponding samples at

70°C and 100°C. Chroma values greatly increased with temperature, indicating more saturated colors. Significant differences were detected between corresponding samples across all three temperature groups. Furthermore, all 121°C extracts had significantly higher c* values than all other extracts. In terms of hue angle, all 121°C extracts had significantly lower values than all corresponding 70°C extracts and were slightly significantly lower for most corresponding 100°C extracts. Therefore, higher extraction temperatures appear to correspond to more red hues, while lower extraction temperatures yielded more yellow tinted extracts.

With very few exceptions, concentration and grind size did not make significant differences in L*, c*, or h values between corresponding samples. Ethanol samples were comparable in lightness to 70°C and 100°C samples, but significantly lighter than 121°C extracts. The color of the ethanol extracts was similar in saturation to 70°C extracts, but much less vivid than the highly saturated 121°C extracts. Finally, ethanol extracts had the most yellow hue compared to all other samples. Hue values for ethanol extracts were not significantly different from all 70°C aqueous extracts and most 100°C aqueous extracts.

Spectra of the liquid extracts are displayed in Figures 3.1, 3.2, 3.3, and 3.4 and absorbances at the λ_{max} of 456 nm for all extracts are displayed in Table 3.4. Overall, as particle size was reduced and concentration and temperature were increased, the absorbance at 456 nm increased. These results follow similar trends to the c* values discussed earlier and found in Table 3.4. As the absorbance increased, the colors appeared more vivid and darker. Ethanol extracts exhibited similar absorbance values to 70°C extracts. The overall shapes of the spectra followed the same pattern for all extracts, with a peak around 456 nm and decreasing absorbance as the wavelength increased to 700 nm.

Differences in color are likely linked to the changes in the amount and relative proportion of color-providing phenolic compounds extracted from each set of conditions. Of the CIE L*c*h values, hue was the most similar across the 21 samples. The saturation of the color extracted, c*, changed the most, particularly with increased temperature. This indicates that similar color was extracted from the shells, but in differing intensities. Furthermore, do Prado, Aragao, Fett, & Block (2009b) measured the color of pecan kernel cakes following extraction with multiple solvents and found that samples with higher condensed tannin contents also exhibited hue values closer to red than samples with lower condensed tannin contents. This observation supports the findings in this study, as 121°C extracts had significantly lower hue angles and significantly higher CT values than 70°C extracts. Despite relatively high concentrations of condensed tannins, ethanol extracts exhibited the most yellow hue angles. This may be due to the low extraction temperature of 25°C, since temperature appeared to affect color values the most.

Similar to the color value results, with more intense color and higher concentrations of phenolic compounds, the absorbance values of extracts with higher temperatures, higher concentrations of pecan shells, and smaller particle sizes were greater. Also, the similar shape of the spectra of all liquid extracts suggests that similar phenolic compounds are being extracted across extraction conditions, but the amount of these compounds extracted increases with decreased particle size and increased temperature and concentration. Based on this information, no new compounds appear to be forming as a result of high extraction temperatures. Lastly, the absorbance peak at 456 nm is consistent with the yellow-orange appearance of the diluted samples.

Photos of all liquid extracts are included in Figures 3.5, 3.6, and 3.7. Samples 1-6, produced at 70°C, have a light orange appearance. Samples 7-12, produced at 100°C, have a

darker, burnt orange appearance, resembling tea. Samples 13-18, produced at 121°C, have the darkest appearance with reddish-brown pigments, which resemble cola.

Conclusion

Liquid extracts of ground pecan shells were more rich in phenolic compounds, condensed tannins, and exhibited higher antioxidant activity when produced with higher temperatures, higher ratios of pecan shells to solvent, and finer particle sizes of ground shells. When comparing results for color, dry weight, TPC, CT, and AA, the most significant differences of the extraction conditions tested were found across temperature groups. Sample 18, which was produced by heating 70-mesh pecan shells at a concentration of 3 g/100 mL water at 121°C for 30 minutes, had the highest dry weight, TPC, CT, and AA. In addition, Sample 18 had the darkest, most saturated reddish-orange color of all the extracts. The sample with the lightest color, least dry weight, TPC, CT, and AA was sample 1, which was produced with 50-mesh pecan shells at a concentration of 2 g/100 mL at 70°C. In the end, pecan shells can be used to produce a dark, saturated, reddish-orange extract that is rich in phenolic compounds and could serve as an inexpensive, natural colorant.

Figures and Tables

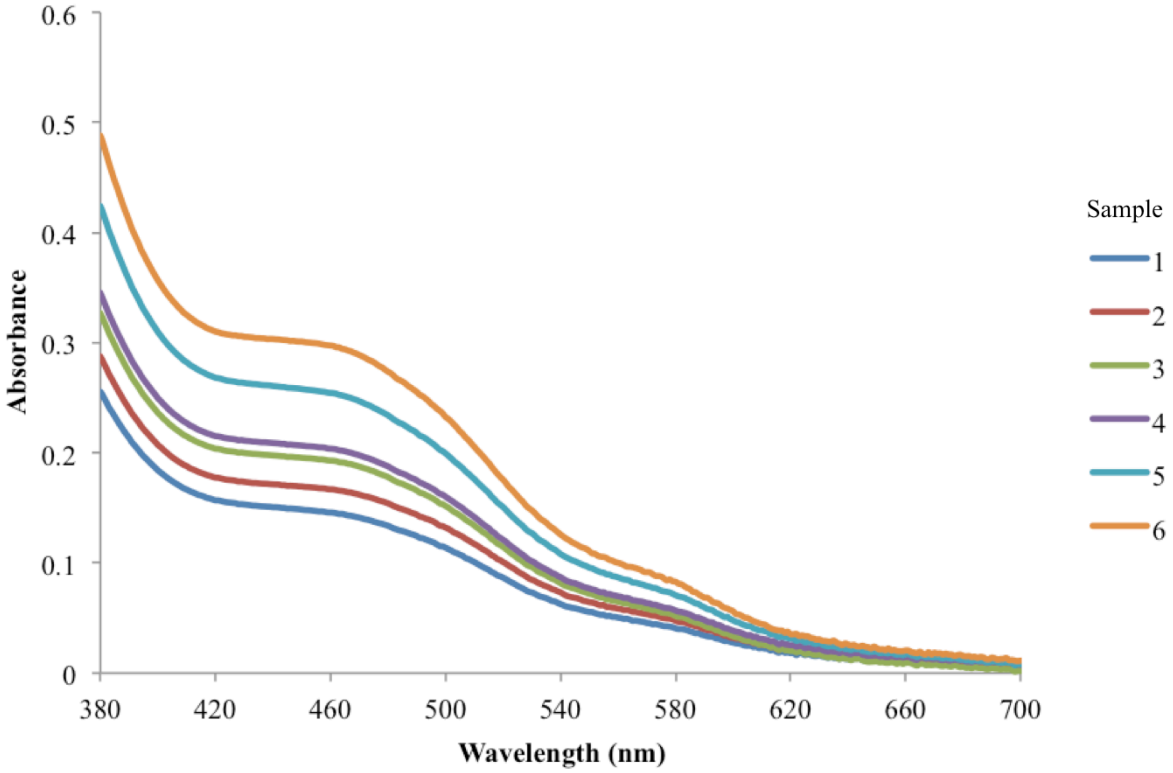

Figure 3.1: Absorbance spectra of 70°C extracts, samples 1-6

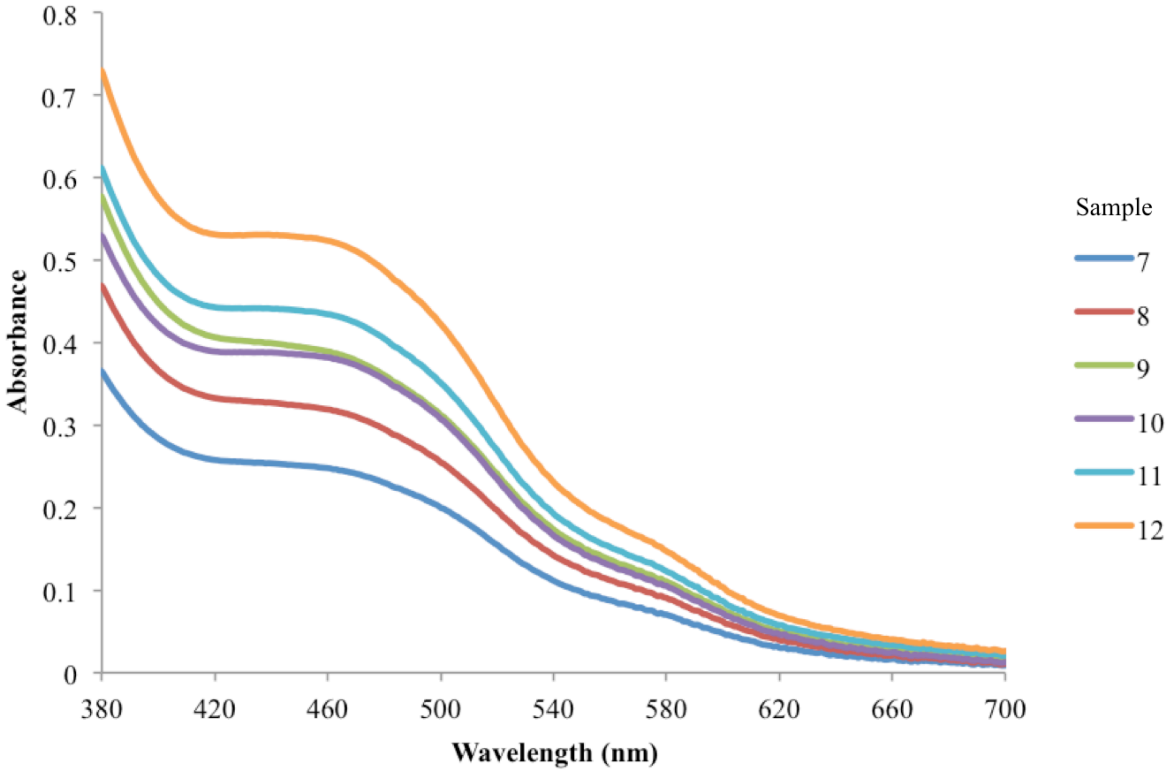

Figure 3.2: Absorbance spectra of 100°C extracts, samples 7-12

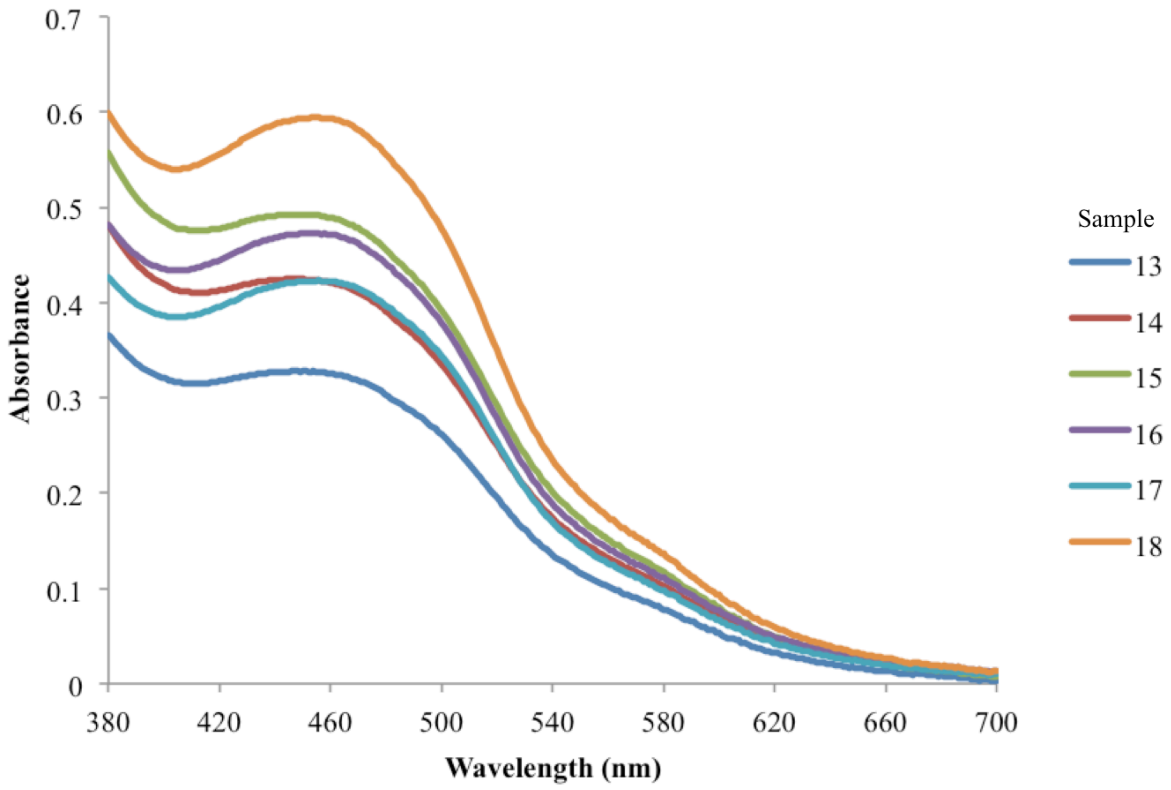

Figure 3.3: Absorbance spectra of 121°C extracts, samples 13-18

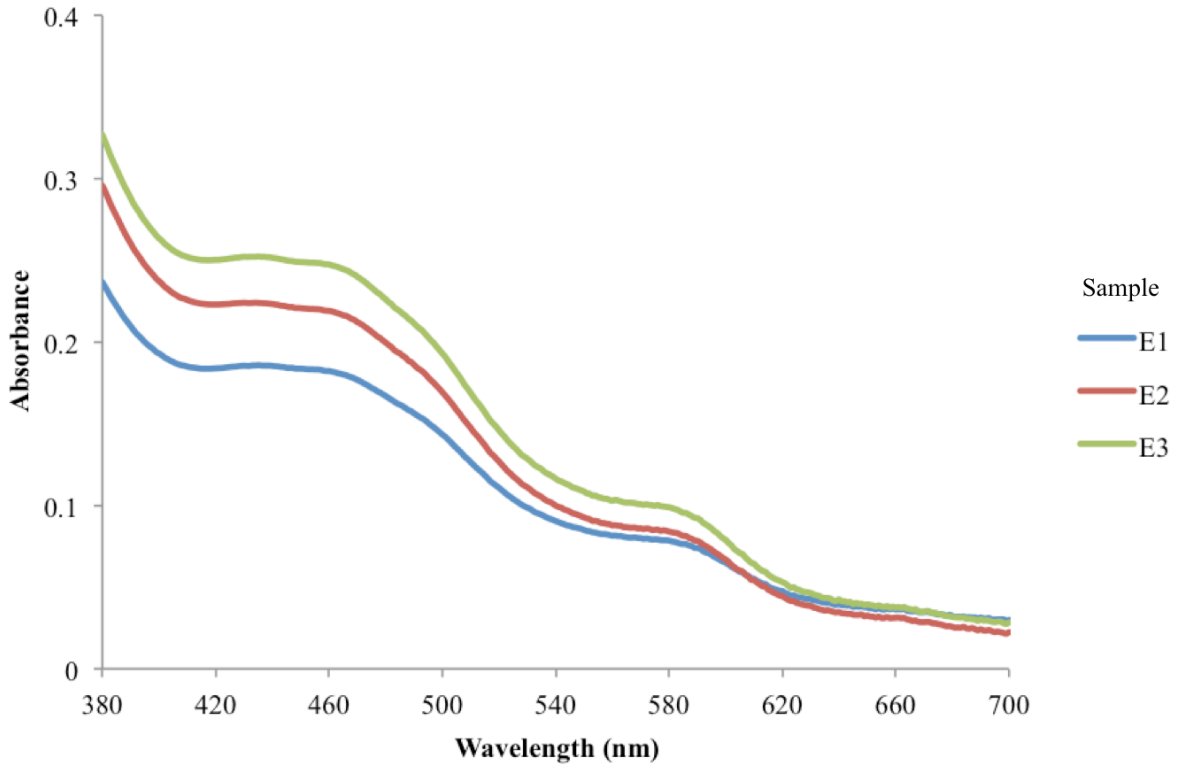

Figure 3.4: Absorbance spectra of ethanol extracts, samples E1-E3

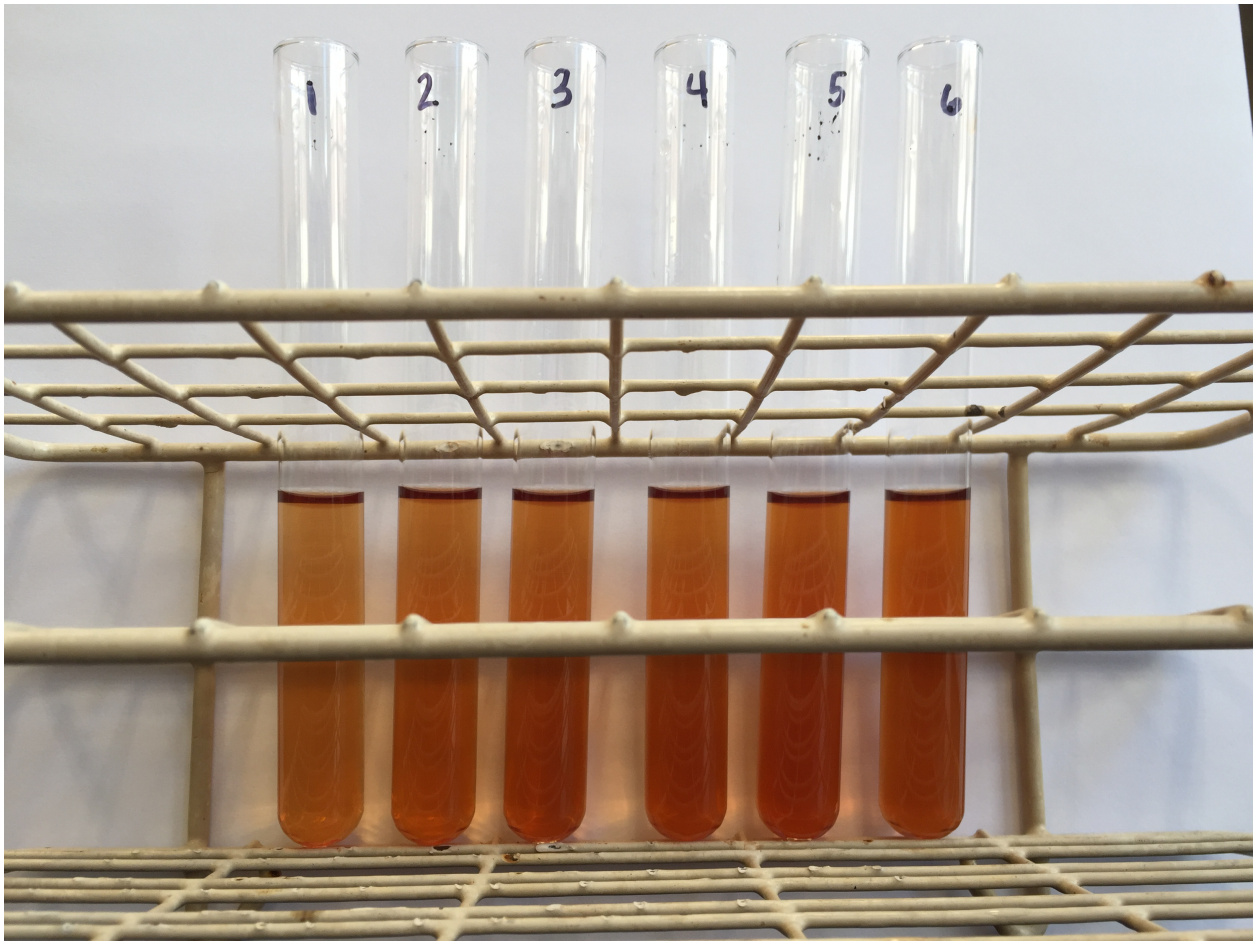

Figure 3.5: Color of 70°C extract, samples 1-6

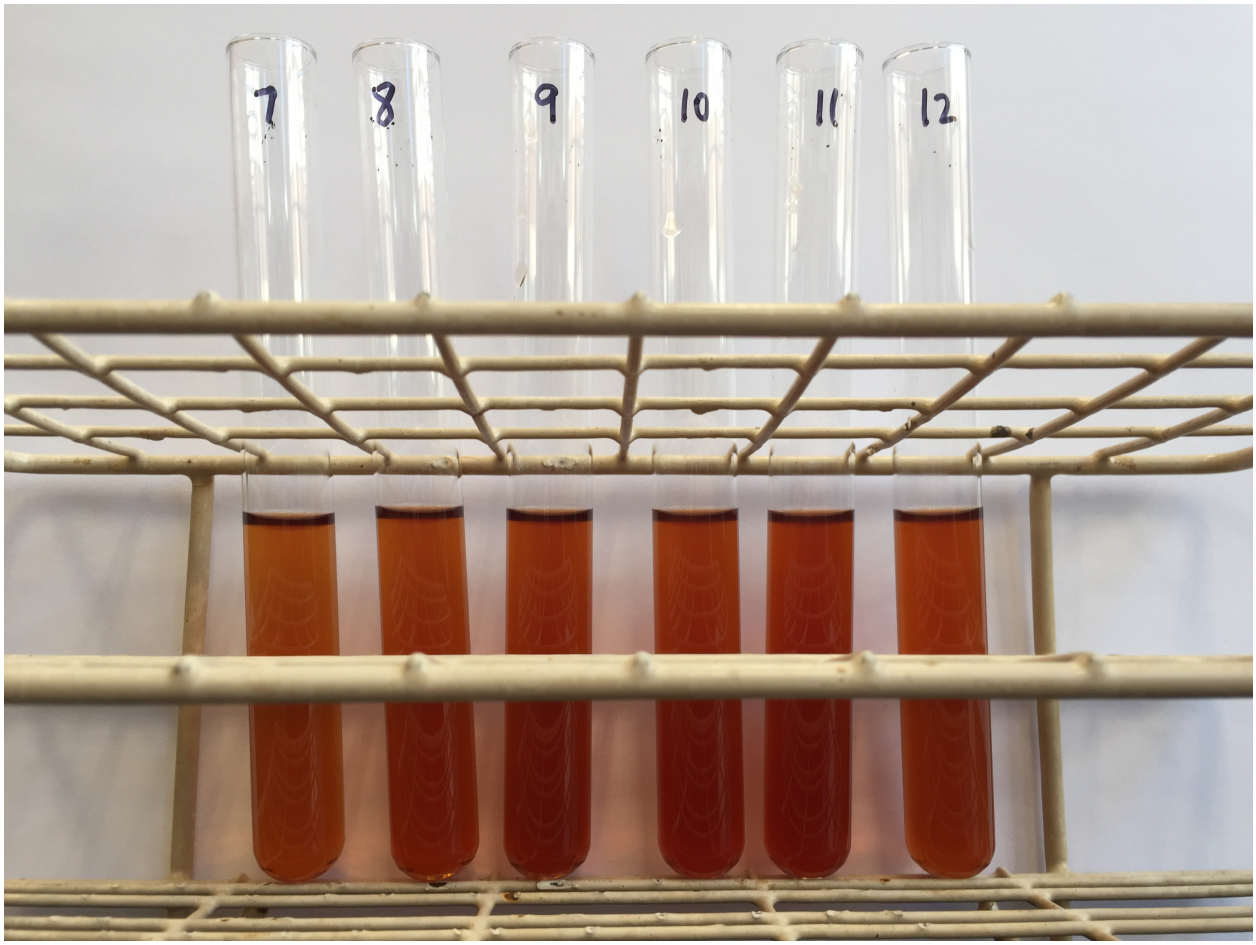

Figure 3.6: Color of 100°C extracts, samples 7-12

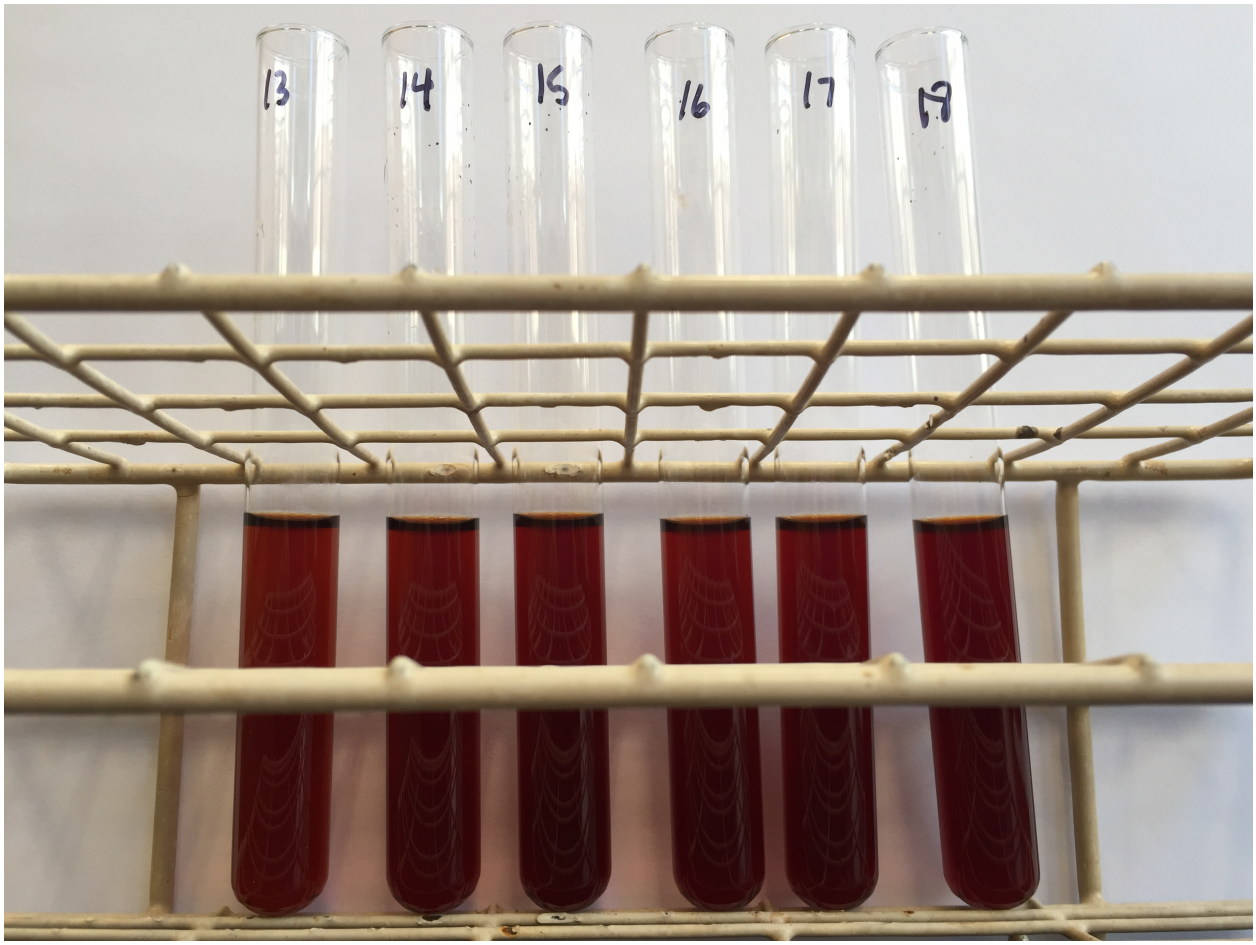

Figure 3.7: Color of 121°C extracts, samples 13-18

Table 3.1: Sample numbers, corresponding liquid extraction conditions, and dry weight

Sample	Solvent	Temperature (°C)	Grind Size (mesh size)	Concentration (g shells/100 mL)	Dry Weight (g dry extract/100 g extract)[1,2]
1	Water	70	50	2	0.3040 ± 0.0064^{n}
2	Water	70	50	2.5	0.3896 ± 0.0130^{m}
3	Water	70	50	3	0.4591 ± 0.0016^{l}
4	Water	70	70	2	0.5122 ± 0.0065^{jk}
5	Water	70	70	2.5	0.6243 ± 0.0107^{h}
6	Water	70	70	3	0.7539 ± 0.0105^{e}
7	Water	100	50	2	0.3826 ± 0.0120^{m}
8	Water	100	50	2.5	0.4643 ± 0.0018^{l}
9	Water	100	50	3	0.5507 ± 0.0034^{ij}
10	Water	100	70	2	0.5733 ± 0.0083^{i}
11	Water	100	70	2.5	0.7067 ± 0.0067^{f}
12	Water	100	70	3	0.8367 ± 0.0092^{d}
13	Water	121	50	2	0.4885 ± 0.0201^{kl}
14	Water	121	50	2.5	0.5817 ± 0.0087^{i}
15	Water	121	50	3	0.6715 ± 0.0142^{fg}
16	Water	121	70	2	0.6474 ± 0.0088^{gh}
17	Water	121	70	2.5	0.7766 ± 0.0101^{e}
18	Water	121	70	3	0.9491 ± 0.0074^{c}
E1	Ethanol	25	70	2	0.8128 ± 0.0172^{d}
E2	Ethanol	25	70	2.5	1.016 ± 0.009^{b}
E3	Ethanol	25	70	3	1.214 ± 0.025^{a}

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Measurements were performed on all three replicates of each sample (n=3)

Table 3.2: TPC, CT, and AA of liquid extracts[1]

Sample	TPC (mg GAE/ml extract)[2]	CT (mg CE/ml extract)[3]	AA (mg TE/ml extract)[3]
1	1.358 ± 0.041^{m}	1.718 ± 0.139^{m}	3.679 ± 0.088^{n}
2	1.702 ± 0.052^{l}	2.068 ± 0.324^{lm}	4.429 ± 0.074^{m}
3	2.018 ± 0.071^{k}	2.686 ± 0.133^{ijkl}	5.088 ± 0.087^{l}
4	2.325 ± 0.024^{ij}	2.271 ± 0.365^{ghi}	6.884 ± 0.131^{i}
5	2.923 ± 0.048^{fg}	3.220 ± 0.465^{ghij}	7.791 ± 0.179^{g}
6	3.489 ± 0.079^{cd}	3.237 ± 0.452^{ghij}	8.845 ± 0.087^{de}
7	1.723 ± 0.094^{l}	1.867 ± 0.317^{lm}	4.975 ± 0.288^{l}
8	2.043 ± 0.044^{k}	2.346 ± 0.405^{klm}	5.647 ± 0.150^{k}
9	2.412 ± 0.046^{i}	3.109 ± 0.162^{hijk}	6.333 ± 0.146^{j}
10	2.680 ± 0.064^{gh}	3.380 ± 0.470^{fghi}	6.779 ± 0.192^{i}
11	3.239 ± 0.114^{de}	4.044 ± 0.174^{defg}	8.961 ± 0.154^{de}
12	3.905 ± 0.070^{b}	4.195 ± 0.934^{def}	10.29 ± 0.17^{b}
13	2.125 ± 0.134^{jk}	2.499 ± 0.449^{jklm}	5.968 ± 0.169^{jk}
14	2.507 ± 0.038^{hi}	3.348 ± 0.509^{fghi}	6.815 ± 0.084^{i}
15	2.893 ± 0.074^{ef}	4.059 ± 0.271^{defg}	8.383 ± 0.299^{f}
16	3.111 ± 0.133^{ef}	4.566 ± 0.464^{cde}	9.055 ± 0.089^{d}
17	3.693 ± 0.033^{bc}	5.689 ± 0.329^{ab}	10.47 ± 0.18^{b}
18	4.586 ± 0.053^{a}	6.503 ± 0.539^{a}	11.18 ± 0.22^{a}
E1	2.507 ± 0.040^{hi}	3.750 ± 0.107^{efgh}	7.381 ± 0.092^{h}
E2	2.674 ± 0.356^{gh}	4.638 ± 0.239^{cd}	8.622 ± 0.169^{ef}
E3	3.401 ± 0.511^{d}	5.290 ± 0.245^{bc}	9.818 ± 0.494^{c}

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Triplicate measurements were performed on all three replicates of each sample (n=9)
[3]Duplicate measurements were performed on all three replicates of each sample (n=6)

Table 3.3: Dry weight, TPC, CT, and AA on dry basis of liquid extracts[1]

Sample	Dry Weight (g dry extract/100 g extract)[2]	TPC (mg GAE/g dry extract)[3]	CT (mg CE/g dry extract)[4]	AA (mg TE/g dry extract)[4]
1	0.3040 ± 0.0064^{n}	446.6 ± 13.5^{bcdefg}	565.0 ± 45.6^{cdefgh}	1210 ± 29^{cdef}
2	0.3896 ± 0.0130^{m}	436.9 ± 13.4^{efg}	530.8 ± 83.1^{defgh}	1137 ± 19^{gh}
3	0.4591 ± 0.0016^{l}	439.6 ± 15.4^{cdefg}	585.1 ± 29.0^{bcde}	1108 ± 19^{h}
4	0.5122 ± 0.0065^{jk}	453.9 ± 4.7^{abcdefg}	443.4 ± 71.2^{fgh}	1344 ± 26^{a}
5	0.6243 ± 0.0107^{h}	468.2 ± 7.8^{abc}	515.7 ± 74.4^{defgh}	1248 ± 29^{bcd}
6	0.7539 ± 0.0105^{e}	462.8 ± 10.4^{abcdef}	429.3 ± 59.9^{h}	1173 ± 12^{efg}
7	0.3826 ± 0.0120^{m}	450.2 ± 24.7^{bcdefg}	488.0 ± 82.9^{defgh}	1300 ± 75^{ab}
8	0.4643 ± 0.0018^{l}	440.1 ± 9.5^{cdefg}	505.4 ± 87.2^{defgh}	1216 ± 32^{cde}
9	0.5507 ± 0.0034^{ij}	438.0 ± 8.3^{defg}	564.6 ± 29.3^{cdefgh}	1150 ± 27^{fgh}
10	0.5733 ± 0.0083^{i}	467.5 ± 11.2^{abcd}	589.6 ± 82.0^{bcde}	1182 ± 34^{efg}
11	0.7067 ± 0.0067^{f}	458.4 ± 16.1^{abcdefg}	572.2 ± 24.7^{bcdefg}	1268 ± 22^{bc}
12	0.8367 ± 0.0092^{d}	466.7 ± 8.3^{abcde}	501.4 ± 111.6^{defgh}	1230 ± 20^{cde}
13	0.4885 ± 0.0201^{kl}	434.9 ± 27.5^{fg}	511.5 ± 92.0^{defgh}	1222 ± 35^{cde}
14	0.5817 ± 0.0087^{i}	431.0 ± 6.5^{g}	575.6 ± 87.5^{bcdef}	1172 ± 14^{efgh}
15	0.6715 ± 0.0142^{fg}	444.3 ± 11.1^{cdefg}	604.5 ± 40.4^{abcd}	1248 ± 44^{bcd}
16	0.6474 ± 0.0088^{gh}	480.6 ± 20.6^{a}	705.3 ± 71.7^{ab}	1201 ± 12^{def}
17	0.7766 ± 0.0101^{e}	475.6 ± 4.2^{ab}	732.5 ± 42.4^{a}	1348 ± 24^{a}
18	0.9491 ± 0.0074^{c}	483.2 ± 5.6^{a}	685.1 ± 56.7^{abc}	1178 ± 23^{efg}
E1	0.8128 ± 0.0172^{d}	308.4 ± 4.9^{h}	461.4 ± 13.2^{efgh}	908.1 ± 11.4^{i}
E2	1.016 ± 0.009^{b}	263.2 ± 35.0^{i}	456.5 ± 23.5^{efgh}	848.6 ± 16.6^{ij}
E3	1.214 ± 0.025^{a}	280.1 ± 42.1^{hi}	435.7 ± 20.2^{gh}	808.7 ± 40.7^{j}

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Measurements were performed on all three replicates of each sample (n=3)
[3]Triplicate measurements were performed on all three replicates of each sample (n=9)
[4]Duplicate measurements were performed on all three replicates of each sample (n=6)

Table 3.4: Color values of liquid extracts[1]

Sample	L*[2]	c*[2]	h (°)[2]	Absorbance at 456 nm
1	84.21 ± 1.48^{a}	14.17 ± 2.65^{g}	78.74 ± 0.89^{abc}	0.147
2	83.79 ± 1.32^{a}	14.93 ± 3.04^{fg}	78.49 ± 0.41^{abc}	0.168
3	82.25 ± 1.54^{a}	18.37 ± 3.63^{efg}	77.96 ± 0.37^{abc}	0.194
4	81.72 ± 1.13^{a}	20.57 ± 2.50^{defg}	78.87 ± 0.44^{abc}	0.205
5	80.65 ± 0.85^{a}	22.27 ± 1.31^{cdef}	78.13 ± 0.48^{abc}	0.256
6	78.02 ± 1.06^{ab}	28.16 ± 2.46^{cd}	77.31 ± 0.42^{abcd}	0.299
7	80.24 ± 2.41^{a}	22.65 ± 4.45^{cdef}	76.86 ± 1.09^{abcd}	0.249
8	78.63 ± 1.39^{ab}	25.95 ± 2.36^{cde}	76.59 ± 0.63^{abcd}	0.321
9	71.58 ± 6.60^{bc}	38.16 ± 10.29^{b}	72.83 ± 3.61^{cde}	0.392
10	77.00 ± 1.87^{ab}	29.41 ± 4.65^{c}	76.07 ± 0.78^{abcd}	0.384
11	72.00 ± 1.44^{bc}	38.96 ± 2.94^{b}	73.62 ± 0.75^{bcd}	0.437
12	71.58 ± 1.45^{bc}	39.15 ± 2.41^{b}	72.92 ± 0.97^{cde}	0.526
13	65.32 ± 2.31^{cd}	51.59 ± 2.98^{a}	70.61 ± 1.50^{def}	0.656
14	59.15 ± 7.36^{de}	53.78 ± 1.20^{a}	64.43 ± 8.14^{fg}	0.848
15	60.23 ± 7.27^{de}	53.49 ± 3.24^{a}	65.90 ± 6.89^{efg}	0.984
16	58.70 ± 7.87^{de}	55.67 ± 2.84^{a}	64.36 ± 8.19^{fg}	0.946
17	57.00 ± 5.93^{e}	57.74 ± 1.88^{a}	63.07 ± 5.75^{g}	0.846
18	56.48 ± 3.30^{e}	59.01 ± 0.81^{a}	63.16 ± 3.84^{g}	1.188
E1	83.30 ± 1.88^{a}	15.56 ± 3.88^{fg}	81.50 ± 0.55^{a}	0.183
E2	82.03 ± 1.82^{ab}	17.68 ± 3.83^{fg}	81.07 ± 0.51^{a}	0.220
E3	80.59 ± 1.64^{a}	19.98 ± 3.23^{efg}	80.53 ± 0.57^{ab}	0.248

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Duplicate measurements were performed on all three replicates of each sample (n=6)

References

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free-radical method to evaluate antioxidant activity. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie, 28*, 25-30.

Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews, 56*, 317-333.

Cacace, J. E., & Mazza, G. (2003). Optimization of extraction of anthocyanins from black currants with aqueous ethanol. *Journal of Food Science, 68*, 240-248. doi:10.1111/j.1365-2621.2003.tb14146.x

Cortez, R., Luna-Vital, D. A., Margulis, D., & de Mejia, E. G. (2017). Natural pigments: stabilization methods of anthocyanins for food applications. *Comprehensive Reviews in Food Science and Food Safety, 16*, 180-198. doi:10.1111/1541-4337.12244

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15*, 7313-7352. doi:10.3390/molecules15107313

do Prado, A. C. P., Aragao, A. M., Fett, R., & Block, J. M. (2009). Antioxidant properties of Pecan nut *Carya illinoinensis* (Wangenh.) C. Koch shell infusion. *Grasas y Aceites, 60*, 330-335. doi:10.3989/gya.107708

do Prado, A. C. P., Aragao, A. M., Fett, R., & Block, J. M. (2009). Phenolic compounds and antioxidant activity of Pecan *Carya illinoinensis* (Wangenh.) C. Koch kernel cake extracts obtained by sequential extraction. *Grasas y Aceites, 60*, 458-467. doi:10.3989/gya.129708

do Prado, A. C. P., da Silva, H. S., da Silveira, S. M., Barreto, P. L. M., Vieira, C. R. W., Maraschin, M., . . . Block, J. M. (2014). Effect of the extraction process on the phenolic

compounds profile and the antioxidant and antimicrobial activity of extracts of pecan nut *Carya illinoinensis* (Wangenh) C. Koch shell. *Industrial Crops and Products, 52*, 552-561. doi:10.1016/j.indcrop.2013.11.031

Hendry, G. F., & Houghton, J. D. (1996). *Natural food colorants*. Glasgow: Blackie Academic & Professional.

Hilbig, J., Alves, V. R., Muller, C. M. O., Micke, G. A., Vitali, L., Pedrosa, R. C., & Block, J. M. (2018). Ultrasonic-assisted extraction combined with sample preparation and analysis using LC-ESI-MS/MS allowed the identification of 24 new phenolic compounds in pecan nut shell *Carya illinoinensis* (Wangenh) C. Koch extracts. *Food Research International, 106*, 549-557. doi:10.1016/j.foodres.2018.01.010

Ismal, O. E., Ozdogan, E., & Yildirim, L. (2013). An alternative natural dye, almond shell waste: effects of plasma and mordants on dyeing properties. *Coloration Technology, 129*, 431-437. doi:10.1111/cote.12047

Kamuf, W., Nixon, A., & Parker, O. (2000). Caramel color. In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants: science and technology* (pp. 253-272). New York, NY: Marcel Dekker, Inc.

Lee, S., & Lee, K. G. (2016). Analysis and risk assessment of 4(5)-methylimidazole in brown colored foods and beverages. *Food Additives & Contaminants Part B-Surveillance, 9*, 59-65. doi:10.1080/19393210.2015.1127294

Loughrey, K. (2000). The measurement of color. In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants: science and technology* (pp. 273-287). New York, NY: Marcel Dekker, Inc.

Müller, L. G., Pase, C. S., Reckziegel, P., Barcelos, R. C. S., Boufleur, N., Prado, A. C. P., . . . Burger, M. E. (2013). Hepatoprotective effects of pecan nut shells on ethanol-induced liver damage. *Experimental and Toxicologic Pathology, 65*, 165-171. doi:10.1016/j.etp.2011.08.002

National Agricultural Statistics Service. (2017). *Noncitrus fruits and nuts 2016 summary.* Retrieved from http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-06-27-2017.pdf

Payne, M. J., Hurst, W. J., Stuart, D. A., Ou, B. X., Fan, E., Ji, H. P., & Kou, Y. (2010). Determination of total procyanidins in selected chocolate and confectionery products using DMAC. *Journal of AOAC International, 93*, 89-96.

Pinelo, M., Del Fabbro, P., Manzocco, L., Núñez, M. J., & Nicoli, M. C. (2005). Optimization of continuous phenol extraction from *Vitis vinifera* byproducts. *Food Chemistry, 92*, 109-117. doi:10.1016/j.foodchem.2004.07.015

Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., & Núñez, M. J. (2005). Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *Journal of Agricultural and Food Chemistry, 53*, 2111-2117. doi:10.1021/jf048810

Porto, L. C. S., da Silva, J., Ferraz, A. B. F., Ethur, E. M., Porto, C. D. L., Marroni, N. P., & Picada, J. N. (2015). The antidiabetic and antihypercholesterolemic effects of an aqueous extract from pecan shells in Wistar rats. *Plant Foods for Human Nutrition, 70*, 414-419. doi:10.1007/s11130-015-0510-9

Reckziegel, P., Boufleur, N., Barcelos, R. C. S., Benvegnu, D. M., Pase, C. S., Muller, L. G., . . . Burger, M. E. (2011). Oxidative stress and anxiety-like symptoms related to withdrawal

of passive cigarette smoke in mice: Beneficial effects of pecan nut shells extract, a by-product of the nut industry. *Ecotoxicology and Environmental Safety, 74*, 1770-1778. doi:10.1016/j.ecoenv.2011.04.022

Singleton, V. L., & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16,* 144-158.

Tutak, M., & Benli, H. (2012). Dyeing properties of textiles by Turkish hazelnut (Corylus colurna): leaves, coat, shell and dice. *Coloration Technology, 128*, 454-458. doi:10.1111/j.1478-4408.2012.00399.x

Villarreal-Lozoya, J. E., Lombardini, L., & Cisneros-Zevallos, L. (2007). Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. *Food Chemistry, 102*, 1241-1249. doi:10.1016/j.foodchem.2006.07.024

Zhang, D. L., & Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry, 88*, 503-509. doi:10.1016/j.foodchem.2004.01.065

Zhao, Q., Feng, H., & Wang, L. J. (2014). Dyeing properties and color fastness of cellulase-treated flax fabric with extractives from chestnut shell. *Journal of Cleaner Production, 80*, 197-203. doi:10.1016/j.jclepro.2014.05.069

CHAPTER 4

SPRAY DRYING

Introduction

A consumer's first impression of a food or beverage can be greatly influenced by color. This aspect of appearance has the power to entice or push consumers away from selecting a product. Furthermore, color can indicate flavor to a consumer, add visual appeal to an originally colorless product, and protect flavors that are susceptible to changes from light (Kamuf, Nixon, & Parker, 2000). With increased consumer interest in healthy products, natural food additives that fall under the "clean label" category are in high demand (Loughrey, 2000). In general, natural food colorants are defined as substances derived from plants, animals, or minerals that are used to add color to a food (Mazza, 2000).

Nutshells are a natural source of reddish brown pigments that have not been applied to the food industry. In the textile industry, shells from hazelnuts, chestnuts, and almonds have been used to produce dyes for fabrics (Ismal, Ozdogan, & Yildirim, 2013; Tutak & Benli, 2012; Zhao, Feng, & Wang, 2014). No current research has been found for dyes produced specifically from pecan shells. Nutshell dyes for textiles are more environmentally friendly and inexpensive compared to synthetic alternatives, because they utilize an abundant agricultural byproduct (Tutak & Benli, 2012). Assuming approximately 40-50% of the weight of a pecan comes from its shell, more than 43 million pounds of pecan shells were produced in 2016 in Georgia alone (do Prado et al., 2014; NASS, 2017). Processors discard the majority of these shells, despite their

pigment content. Like other nutshells, pecan shells show potential to produce vivid reddish brown dyes that could serve as a natural colorant for food.

Food colorants come in different forms ranging from powders to liquid dyes to suit a variety of food products. Powdered colorants typically exhibit greater storage stability, are easier to handle, and cost less to transport due to lower weight than liquid colorants (Obón, Castellar, Alacid, & Fernández-López, 2009). The transformation from liquid raw materials to dried powders can occur in several ways. Some of the most common methods to produce powders are spray drying, freeze drying, drum drying, belt drying, and crystallization (Bhandari, Bansal, Zhang, & Schuck, 2013). Out of these drying options, spray drying is especially popular due to its unique benefits. Spray drying is a rapid drying method that operates continuously, can produce high volumes of powders in one step, and the equipment is not difficult to operate. This method operates by drying a spray of atomized droplets with hot air to produce fine solid particles (Bhandari et al., 2013). Furthermore, spray drying is useful when working with heat-sensitive materials, as it can maintain nutrients well. Product properties and quality can be well controlled with spray drying and the powders produced have uniform particle sizes and shapes (Passos & Ribeiro, 2010). These benefits of spray drying make it a viable option to produce a powdered form of the liquid pecan shell colorant.

During spray drying, the selection of processing conditions influences the properties of the final powder. Additionally, the properties of the powder impact its ultimate functionality in a food product. Key properties of powders that are influenced by processing conditions are moisture content, bulk density, and solubility. A few of the operating parameters of spray driers that can be manipulated to alter powder properties are the flow rate of the feed material, concentration of the feed material, inlet air temperature, and outlet flow temperature (Bhandari et

al., 2013). In addition to changing operating parameters, high molecular weight drying aids or carriers without color or odor can be added to feed material to produce more stable products with higher yield (Bhandari et al., 2013; Nadeem, Torun & Özdemir, 2011). Overall, a good understanding of the influence of processing conditions during spray drying on powder characteristics is necessary to produce a natural food colorant with high functionality.

The objective of this study was to optimize spray drying conditions to produce powdered forms of liquid pecan shell extract. Evaluations of the products were based on physical properties of spray dried powders, as well as the chemical properties of the reconstituted extracts. Physical properties measured included color, moisture content, water activity, bulk density, and water solubility. Chemical properties of the reconstituted extracts measured included total phenolics content (TPC), condensed tannin content (CT), and antioxidant activity (AA).

Materials and Methods

Preparation of Feed Liquid

Pecan shells of the Eastern Schley variety were obtained from South Georgia Pecan Company (Valdosta, GA, USA). Shells were stored at -20°C prior to use. Before grinding, pecan shells were separated from fine pieces of shell and kernel through shaking with a ¼ inch sieve (6.3 mm) and 6-mesh sieve (3.35 mm) and blowing with compressed air. The remaining large pecan shells were collected by hand. A Homoloid Machine grinder (Model JT, The Fitzpatrick Co., Elmhurst, IL, USA) fitted with the 1521 0125 screen was used to grind the pecan shells. Then, the ground shells were sieved to a 70-mesh (210 μm) size. Finally, the shells were dried overnight in a vacuum oven (1430 MS, VWR Scientific Products, USA) at 40°C and then stored in airtight plastic bags at -20°C. Extraction protocols were based on previous work found in Chapter 3: Liquid Extraction. In short, 70-mesh pecan shells were added to DI water at a

concentration of 3 g/100 mL and autoclaved (Type LS-2036, American Sterilizer Co., Erie, PA, USA) at 121°C and 15 psi for 30 minutes. The cooled shell and water mixture was vacuum filtered (Grade 415, VWR Scientific Products, USA) to obtain the final liquid extract. The liquid extract was stored in plastic re-sealable bags at -20°C in the dark until spray drying.

Dry Weight of Feed Liquid

The dry weight of the feed liquid was determined by gravimetric analysis. Aliquots of 5 mL of the feed liquid were added to aluminum pans and dried in a vacuum oven (1430 MS, VWR Scientific Products, USA) at 105°C until a constant mass was reached (do Prado, Arago, Fett, & Block, 2009).

Spray Drying Liquid Extracts

The liquid pecan shell extract was spray dried using a Mini Spray Dryer B-290 (Büchi, Switzerland). Compressed nitrogen was used for the heated inlet air. Three different inlet temperatures (140°C, 150°C, and 160°C) and three different feed rates (8 g/min, 12.5 g/min, 15.3 g/min) were tested when spray drying. The three feed rates corresponded to pump settings of 25%, 40%, and 50%. All other conditions were kept constant and were as follows: aspirator = 100%, Q flow = 40, and vacuum = -60 mbar. The mass of the feed liquid prior to spray drying and the mass of the final powder were recorded in order to calculate the yield with the following equation. The outlet temperature for each trial was also recorded.

$$\frac{Mass\ of\ powder * \%\ solids}{Mass\ of\ liquid\ feed * \%\ solids} * 100 = \%\ solids\ yield \qquad \text{(Eq. 1)}$$

Finally, maltodextrin (STAR-DRI® 100 Maltodextrin, Tate & Lyle, Decatur, IL, USA) was added as a carrier to increase the solids content by 10% in the liquid extract. The extract with maltodextrin was spray dried with an inlet temperature of 150°C and 40% pump (12.5

g/min). Powders were collected from the cyclone and bottom collection vessel of the spray dryer and stored in amber vials at room temperature.

A 3x3 design with inlet temperature and feed rate was employed, along with the additional extract with maltodextrin, to produce 10 different spray dried powders. Spray drying parameters for all 10 samples are listed in Table 4.1. Spray drying runs were conducted in duplicate.

Analysis of Spray Dried Powders

The color values of the spray dried powders were measured using a colorimeter (Model CR-410, Minolta Co. Ltd., Tokyo, Japan) under the D65 illuminant system. For each color measurement, 1.5 g of powder were spread and packed evenly in the bottom of a petri dish placed on top of white paper. Then, the colorimeter was placed onto the powder and measurements were recorded in terms of the CIE L*c*h values. The colorimeter was regularly calibrated with a white calibration tile.

The solids content of each powder was measured with a halogen moisture analyzer (HR73, Mettler Toledo, Switzerland). The water activity of each powder was measured with a water activity meter (Aqua Lab Series 3, Decagon Devices, Inc., Pullman, WA, USA).

Bulk density was measured according to the methods of Obón et al. (2009). For each measurement, 2 g of powder were added to a 10 mL graduated cylinder. The powder was tamped down with a plastic rod to completely pack the powder. The lowest volume achieved after packing was recorded and the bulk density was reported as g/mL.

Water solubility of powders was determined according to the procedures by Cano-Chauca, Stringheta, Ramos, and Cal-Vidal (2005) modified by Nadeem et al. (2011). Powders were dissolved in DI water to match the solids content of the feed liquid found from gravimetric

analysis. Next, the reconstituted extracts were centrifuged at 3000x g for 5 min (Sorvall RC 6 Plus, Thermo Fisher Scientific, Asheville, NC, USA). Aliquots of 8 mL of the supernatant were dried in a vacuum oven (1403 MS, VWR Scientific Products, USA) at 105°C for 24 h until reaching a constant mass and the percent solubility was calculated by mass difference on a dry basis.

Analysis of Reconstituted Extracts

Color

Spray dried powders were reconstituted with DI water to match the initial solids content of the feed liquid found by gravimetric analysis. In order to measure color in terms of CIE L*c*h values, a colorimeter (Model CR-410, Minolta Co. Ltd., Tokyo, Japan) was used under the D65 illuminant system. A petri dish was placed on top of sheets of white paper with the color values L* = 91.77, c* = 4.02, h = 281.78°, and filled with 20 mL of the liquid extract. The colorimeter was placed directly into the petri dish filled with the extract and measurements were recorded. The colorimeter was regularly recalibrated with a white calibration tile. The color of the feed liquid was also recorded in this manner and ΔE color change values between the feed liquid and each sample were calculated with the following equation: $\Delta E = (\Delta L^{*2} + \Delta c^{*2} + \Delta h^2)^{1/2}$.

In addition, a spectrophotometer (Genesys Model: G10S UV-Vis, Thermo Fisher Scientific, Madison, WI, USA) was used to record the visible absorbance spectra of samples from 380-700 nm to determine the maximum wavelength for each sample. The reconstituted extracts were diluted 1:10 prior to taking the spectra. The absorbance of each sample at the λ_{max} was recorded and compared across samples. Additionally, a spectrum of the feed liquid was taken in the same manner for comparison.

Total Phenolics Content

The Folin-Ciocalteu colorimetric assay was used to determine TPC with gallic acid as a standard (Singleton & Rossi, 1965). Reconstituted extracts were diluted and 2.0 mL were added to test tubes containing 6.5 mL of water. Then, 0.5 mL of the Folin-Ciocalteu reagent was added along with 1.0 mL of saturated sodium carbonate to neutralize the reaction and the test tubes were vortexed. The test tubes were placed in a dark environment at room temperature to incubate for 40 minutes. Following the incubation period, the absorbance of each sample was measured with a spectrophotometer (Genesys 2 Model 336009, Spectronic Instruments, USA) at 750 nm in a 1 cm cuvette. A standard curve was prepared with gallic acid to quantify TPC. Results were reported as mg gallic acid equivalents (GAE)/ml extract and mg GAE/g of dry weight.

Condensed Tannin Content

The DMAC method, according to the procedures of Payne et al. (2010), was used to determine CT. To begin, the DMAC reagent was prepared by dissolving 0.0300 ± 0.001 g DMAC into 30 mL of 1:9 (v/v) of HCl and ethanol. This reagent was prepared before each run. Next, (+)-catechin was dissolved in 1:1 methanol-water at a concentration of 600 ppm and then diluted to make a standard curve. Using a 96-well plate, 50 µL of standard solutions, 50 µL of samples diluted with ethanol, and a blank of 50 µL of ethanol were pipetted for analysis. Each well received 250 µL of the DMAC reagent and a microplate reader (FLUOstar Omega, BMG LABTECH Inc., Cary, NC, USA) set to 25°C and 640 nm was used to analyze the plates. Once the wells all received the DMAC reagent and the plates were shaken for 3 s, absorbance readings were taken every 1 min for 12 min. For calculations, the maximum absorbance for each sample over the 12 min time period was used in conjunction with the standard curve of (+)-catechin to

determine CT. Results were reported as mg (+)-catechin equivalents (CE)/ml of extract and mg CE/g of dry weight.

Antioxidant Activity

The DPPH assay according to the procedures of Brand-Williams, Cuvelier, and Berset (1995) and Zhang and Hamauzu (2004) were used to measure the antioxidant activity of the reconstituted extracts. DPPH was dissolved in 80% methanol to produce a solution with a concentration of 0.1 mM. Reconstituted extracts were diluted and 0.2 mL of the diluted samples were added to test tubes with 4 mL of the DPPH solution. An aliquot of 0.2 mL of DI water was used as a control. Test tubes were placed in a dark environment to incubate for one hour at room temperature. Next, absorbance was measured at 517 nm in a 1 cm cuvette with a spectrophotometer (Genesys 2 Model 336009, Spectronic Instruments, USA). Trolox ® was added to 80% methanol to produce a standard solution with a concentration of 0.05 mg/mL. The Trolox ® solution was further diluted to solutions with concentrations of 0.01, 0.02, 0.03, and 0.04 mg Trolox ®/mL, which were used to produced a standard curve. Results of the DPPH assay were reported as mg Trolox ® equivalents (TE)/ml of extract and mg TE/g of dry weight.

Statistical Analysis

One-way ANOVA and Tukey HSD were performed with JMP 13 (SAS Institute, Cary, NC, USA) to determine statistically significant differences between means with $p<0.05$.

Results and Discussion

Spray Drying Parameters and Yields

As seen in Table 4.1, adjusting the inlet air temperature and feed rate altered the outlet flow temperature during spray drying. as seen in Table 4.1. When inlet temperature was increased, outlet temperatures increased. When the pump rate was increased, outlet temperatures

decreased. Table 4.1 also lists the average yield for each sample. Trials with conditions producing higher outlet temperatures also tended to have higher yields than trials with lower outlet temperatures. The lowest yield (60.6%) was for sample 14050, which also had the lowest outlet temperature (35°C). The highest yield (77.5%) was for sample 15025, which had the second highest outlet temperature (71°C). Additionally, solids yield was only slightly changed by the addition of maltodextrin. There was less than a 1% difference between the yields for samples 15040M and 15040.

Manipulating feed rate and inlet temperature are the two main ways to control outlet temperature, which explains the changes in outlet temperature for different treatments in this study (Masters, 1976). The trends of decreasing outlet temperature with decreasing inlet temperature and increasing feed rate follow the general trends for spray drying found in the literature (Masters, 1976; Obón et al., 2009). Additionally, outlet temperature is reportedly the most effective parameter to control in order to maintain product quality, including bulk density, moisture content, and color (Masters, 1976). Therefore, changes in physical properties throughout the samples are expected based on different outlet temperatures.

Nadeem et al. (2011) reported lower yields when spray drying a tea extract when using increased feed rate because of sticking on the walls from particles that were not fully dry. This may also explain the lower yields for samples produced with 50% pump (15.3 g/min) and relatively low outlet temperatures. Similar to the results of this study, an optimization study on spray drying pomegranate juice found that changing inlet temperature had no significant effect on product yield (Horuz, Altan, & Maskan, 2012). Other factors, such as the addition of a carrier at high concentration appear to have a much higher impact on product yield (Horuz et al., 2012; Nadeem et al., 2011). The insignificant effect of adding maltodextrin in this study could be

attributed to the relatively low concentration (10% increase) added. In the end, it appears that samples produced with a 40% pump (12.5 g/min) at an inlet temperature between 140°C and 160°C would be the most efficient. Although the 25% pump (8 g/min) samples had the highest yield, using a 40% pump (12.5 g/min) saved time while producing outlet temperatures high enough to maintain product quality. In order to achieve maximum thermal efficiency, spray drying should be conducted at an outlet temperature as low as possible (Masters, 1976). While the 50% pump samples exhibited the lowest outlet temperatures, their yields were also the lowest. The 40% pump samples exhibited lower outlet temperatures than 25% pump samples and higher yields than 50% pump samples. Therefore, spray drying with the 40% pump setting appeared to be the most efficient, because this setting produced the lowest outlet temperatures that still maintained high yields.

Powder Color

The CIE L*c*h color values of the spray dried powders were very similar and are listed in Table 4.2. There were no significant differences in chroma or hue angle for all 10 samples. Therefore, the saturation and color of all powders were essentially the same. The hue angles between 58° and 59° indicate a reddish-orange appearance. Slight differences were observed in lightness values, but the differences do not appear to follow any trends with changes in processing conditions. L* values ranged from 54.22 in sample 15050 to 56.67 in sample 15040M. Sample 15040M with maltodextrin was slightly darker than 15040 without maltodextrin, but overall the saturation and color were unaltered.

The color values of spray dried pecan shell extract have not been reported in the literature, but manipulating parameters during spray drying does not appear to greatly change product color. A study on spray drying mountain tea extract found that increasing inlet

temperature significantly darkened powder color, possibly because of non-enzymatic browning during spray drying (Nadeem et al., 2011). In this study, the feed liquid was previously subjected to high temperatures near those of spray drying for an extended period of time. Any changes in color due to exposure to high temperature may have already occurred during the production of the feed liquid and would not be expected to occur again while spray drying. Therefore, the colors of the powders were mostly unchanged.

Physical Properties of Powders

All results for physical properties of powders, including water activity, solids content, bulk density, and water solubility are listed in Table 4.3.

The most significant changes in water activity were observed in powders produced with 50% pump (15.3 g/min), the highest feed rate. All 50% pump extracts had significantly higher water activity than all powders produced with 25% and 40% pump. No significant differences in water activity were found between samples with different inlet temperatures within the feed rate groups. Furthermore, the addition of maltodextrin did not significantly alter water activity. Water activities ranged from 0.186 (Sample 16025) to 0.380 (Sample 15050).

Results for solids content of powders changed in accordance with the results for water activity. Samples with lower water activities exhibited higher solids content. Some significant differences were detected for higher solids content in samples with the same inlet temperature, but lower feed rates. Only one significant difference was found among samples with different inlet temperatures within the same feed rate group (14025 and 16025). Again, the addition of maltodextrin did not significantly affect solids content. Solids content ranged from 89.91 g dry matter/100 g in sample 15050 to 94.74 g dry matter/100 g in sample 16025.

Solids content of the pecan shell extract powder was in the lower range of results reported in studies on other spray dried phenolic powders, but the water activity was in a similar range to these powders. Nadeem et al. (2011) found spray dried tea extract exhibited solids contents greater than 95% and water activities ranging from 0.253-0.402. Phenolic-rich spray dried pomegranate juice had moisture contents between 3.44 and 9.13% (Horuz et al., 2012). Obón et al. (2009) reported that spray dried juice from the fruit of *Opuntia stricta* for use as a red-purple food colorant had solids contents around 96%.

Temperature has also been shown to affect the moisture content and water activity of powders by increasing the drying rate (Horuz et al., 2012). Higher temperatures often lead to more solids and lower moisture content (Horuz et al., 2012; Obón et al., 2009). The increase in solids content and decrease in water activity generally followed an increase in outlet temperature in this study. Therefore, higher inlet temperatures and lower feed rates are expected to produce lower moisture and lower water activity powders.

Bulk density significantly increased with increased feed rate, except between samples 16025 and 16040. In the 25% and 40% pump groups, inlet temperature did not appear to have a consistent effect on bulk density. In the 50% pump group, bulk density significantly decreased with increased inlet temperature. There was no significant difference in bulk density with the addition of maltodextrin. The lowest bulk density was in sample 15025 at 0.485 g/mL and the highest bulk density was in sample 14050 at 0.684 g/mL.

The increase in bulk density with the increase in feed rate can be explained by an increase in particle size with higher feed rate (Masters, 1976). Furthermore, when inlet temperature is increased, droplets expand to occupy more volume and bulk density is decreased (Masters, 1976). The range of bulk densities in this study is similar to the ranges for pomegranate juice

powder (0.3540 g/mL to 0.4720 g/mL) and the aforementioned *Opuntia stricta* powder (0.52 g/mL to 0.60 g/mL) (Horuz et al., 2012; Obón et al., 2009).

Overall, the spray dried powders were highly soluble in water and only a few significant differences were observed in solubility among the 10 samples. Of the conditions manipulated, the addition of maltodextrin was associated with the most significant difference in water solubility. Sample 15040M was significantly less soluble than all other samples. The most water-soluble sample was 16050 with 99.28% solubility, while the least water-soluble sample was 15040M with 94.02% solubility.

The high water solubility of the spray dried pecan shell extract was expected, because of the aqueous feed liquid. The compounds were soluble in water prior to spray drying and maintained that functionality after spray drying. For comparison, spray dried aqueous tea extracts were also found to be highly soluble in water and ranged from 97.35% to 98.60% soluble. Although maltodextrin is water-soluble, previous studies indicate a slight decrease in water solubility with increased maltodextrin concentration (Jafari, Ghalenoei, & Dehnad, 2017; Moreira et al., 2009). Despite the decreased solubility observed in both studies, samples with maltodextrin added still maintained high water solubility above 90% (Jafari et al., 2017; Moreira et al., 2009). The high water solubility of all the spray dried powders in this study are promising for the colorants' ease of use in aqueous systems.

Color of Reconstituted Extracts

The color of pecan shell extracts was well maintained through spray drying and the L*c*h values of the reconstituted extracts are listed in Table 4.4. Most of the reconstituted extracts did not have significantly different L*c*h values from the original liquid extract. No significant differences were detected in L* or h across all 10 samples and the feed liquid. Few

differences were shown in chroma (c*) and only three samples exhibited significantly lower c* than the feed liquid. Sample 15050 was the most saturated, while sample 16025 was the least saturated. Furthermore, the ΔE color change values show only small differences in color between the reconstituted extracts and the feed liquid. Values range from 4.33 in Sample 15040 to 10.76 in Sample 16025.

The absorbance spectra of the reconstituted extracts also had a similar shape to the absorbance spectra of the feed liquid. Spectra are pictured in Figures 4.1, 4.2, and 4.3 according to feed rate group. As with the feed liquid, the spectra have a strong absorbance peak around 460 nm and steadily decrease in absorbance as the wavelength was increased to 700 nm. For comparison, the absorbances of the reconstituted extracts at 456 nm (λ_{max} of feed liquid) are listed in Table 4.4. Absorbances range from 0.848 in 16050 to 0.522 in 14040.

Overall, the color measurements of the reconstituted extracts showed few significant differences from each other, similar to the effect observed between the color measurements of the powders. Liquid extracts were subjected to high temperatures during extraction, and the short exposure to high temperatures in spray drying may not cause any further changes to pigments than what already occurred during liquid extraction. Many studies reporting significant color changes from spray drying cite high concentrations of carriers or exposure of mildly heat-treated feed materials to high temperatures as the main sources of change (Horuz et al., 2012; Jafari et al., 2017; Nadeem et al., 2011). Both of these factors were not present in this study.

Phenolic Components of Reconstituted Extracts

The TPC of the liquid pecan shell extract per volume was not significantly changed by spray drying in nine out of 10 samples (Table 4.5) and for eight out of 10 samples when calculated per dry weight (Table 4.6). Within the reconstituted extracts, TPC did not significantly

differ across samples produced with different inlet temperatures within the same pump group. Only one slight significant difference was found in TPC across samples produced with the same inlet temperature, but different feed rate. Regardless of processing conditions, phenolic compounds appear to have been maintained during spray drying. Also, the addition of maltodextrin did not significantly alter TPC by volume. However, in terms of dry weight, adding maltodextrin resulted in lower TPC when compared to all other reconstituted extracts and the feed liquid. This is likely due to the decreased proportion of phenolic compounds to total solids present because of the addition of another solid.

Condensed tannin content was also preserved during spray drying. Two out of 10 samples had significantly lower CT than the feed liquid (Tables 4.5 and 4.6). Processing conditions did not have a clear effect on CT. With one exception, samples produced with the same feed rate, but different inlet temperatures did not differ in CT. Some differences were found in samples produced with the same inlet temperature, but different feed rates. However, these differences do not follow a clear trend with the change in feed rate. Adding maltodextrin to sample 15040M did not significantly change CT either. Similar to TPC, CT values were mostly unaffected by spray drying under various conditions.

Unlike TPC and CT, AA was significantly higher in all but one reconstituted extract when compared with the original liquid pecan shell extracts. The AAs of the reconstituted extracts are listed in Tables 4.5 and 4.6. Values range from 13.01 mg TE/mL extract in 14025 to 11.75 mg TE/mL extract in 15040M. Processing conditions did not greatly affect the AA of the reconstituted extracts. No significant differences were found with the change in inlet temperature among samples with the same feed rate. There was a very slight decrease in AA with increased feed rate, but only one significant difference was detected (14025 and 16050). The addition of

maltodextrin did not significantly affect AA when analyzed by volume. Conversely, when analyzed by dry weight, the addition of maltodextrin significantly decreased AA. While TPC and CT of pecan shell extracts were mostly unchanged by spray drying, AA was slightly increased by spray drying irrespective of the spray drying conditions.

One other study involving spray drying aqueous pecan shell extract was found (do Prado et al., 2014). In this study, 60-mesh size ground pecan shells were heated with 98°C water at a concentration of 20 g/L for 10 minutes to produce the liquid extract. Then, the extract was spray dried at an inlet temperature of 150°C, outlet temperature of 50°C, and 25% pump rate. The resulting spray dried pecan shell extract exhibited slightly higher TPC at 590.78 mg GAE/g than the TPC of the feed liquid and reconstituted extracts in this study as seen in Table 4.6. However, these differences can be attributed to differences in pecan cultivars, storage conditions, and processing conditions (Bravo, 1998). CT content was also measured by do Prado et al. (2014), but the analysis was conducted with the Vanillin reagent and not the DMAC reagent used in this study. This could explain the much lower result of 48.70 mg CE/g compared to the results ranging from 507.7 mg CE/g to 763.7 mg CE/g in this study (do Prado et al., 2014). The AA from do Prado et al. (2014) of 1210.97 mg TE/g was comparable to the results of this study, which ranged from 1081 mg TE/g to 1281 mg TE/g.

The decreases in TPC and AA with the addition of maltodextrin in 15040M can be explained by the dilution of the phenolic compounds extracted by adding another solid. A similar effect was seen in spray dried tea extract (Nadeem et al., 2011). Furthermore, the effect of increased antioxidant activity following spray drying has been observed in other studies (do Prado et al., 2014; Horuz et al., 2012). Generally, this effect is linked to increases in overall phenolics content after spray drying from concentration of phenolic compounds. The dry storage

conditions of a spray dried powder could also be more favorable to preserve antioxidant activity compared to the liquid feed material.

Conclusion

Liquid pecan shell extract was successfully spray dried with high yield, while maintaining saturated reddish-orange color and high TPC, CT, and AA. The combination of 40% pump (12.5 g/min feed rate) and inlet temperatures ranging from 140°C-160°C produced powders most efficiently out of the conditions tested in this study. Differences in physical properties were detected across the 10 spray dried samples, however the ranges of values for color, water activity, solids content, bulk density, and solubility were narrow. The color, TPC, and CT of the reconstituted extracts were not significantly different from the feed liquid for most samples. After spray drying, AA was slightly increased in reconstituted extracts compared to the feed liquid. Overall, spray drying efficiently generated a powder form of pecan shell extract that has potential to serve as a natural food colorant rich in reddish-orange color, phenolic compounds, and antioxidant activity.

Figures and Tables

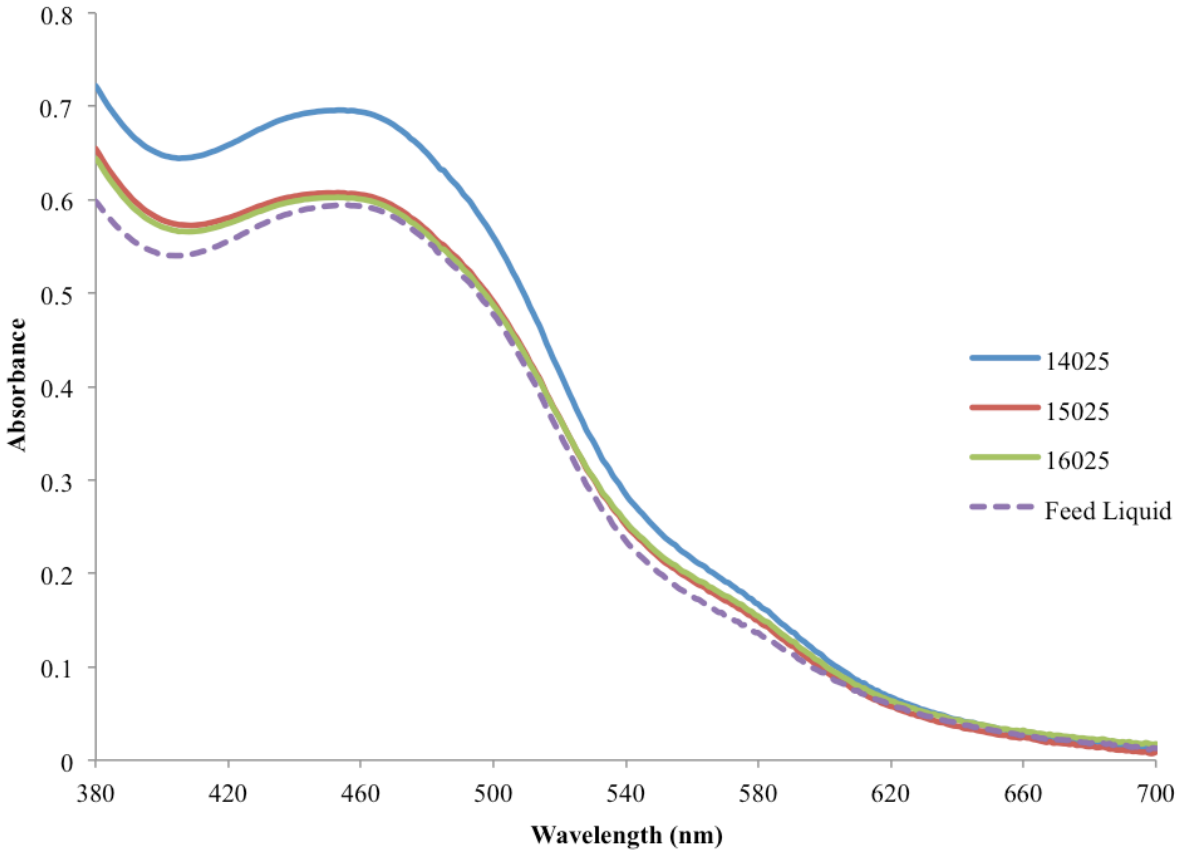

Figure 4.1: Absorbance spectra of 25% pump reconstituted extracts and feed liquid

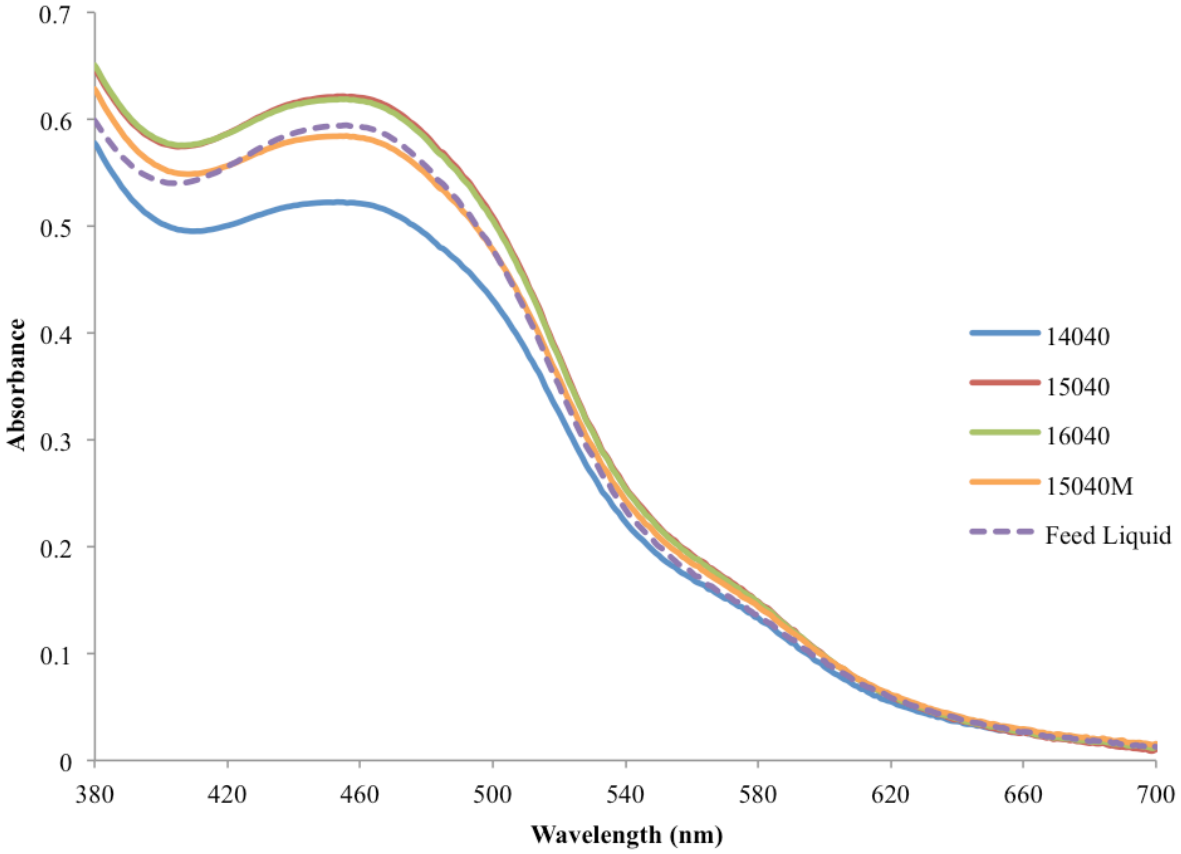

Figure 4.2: Absorbance spectra of 40% pump reconstituted extracts and feed liquid

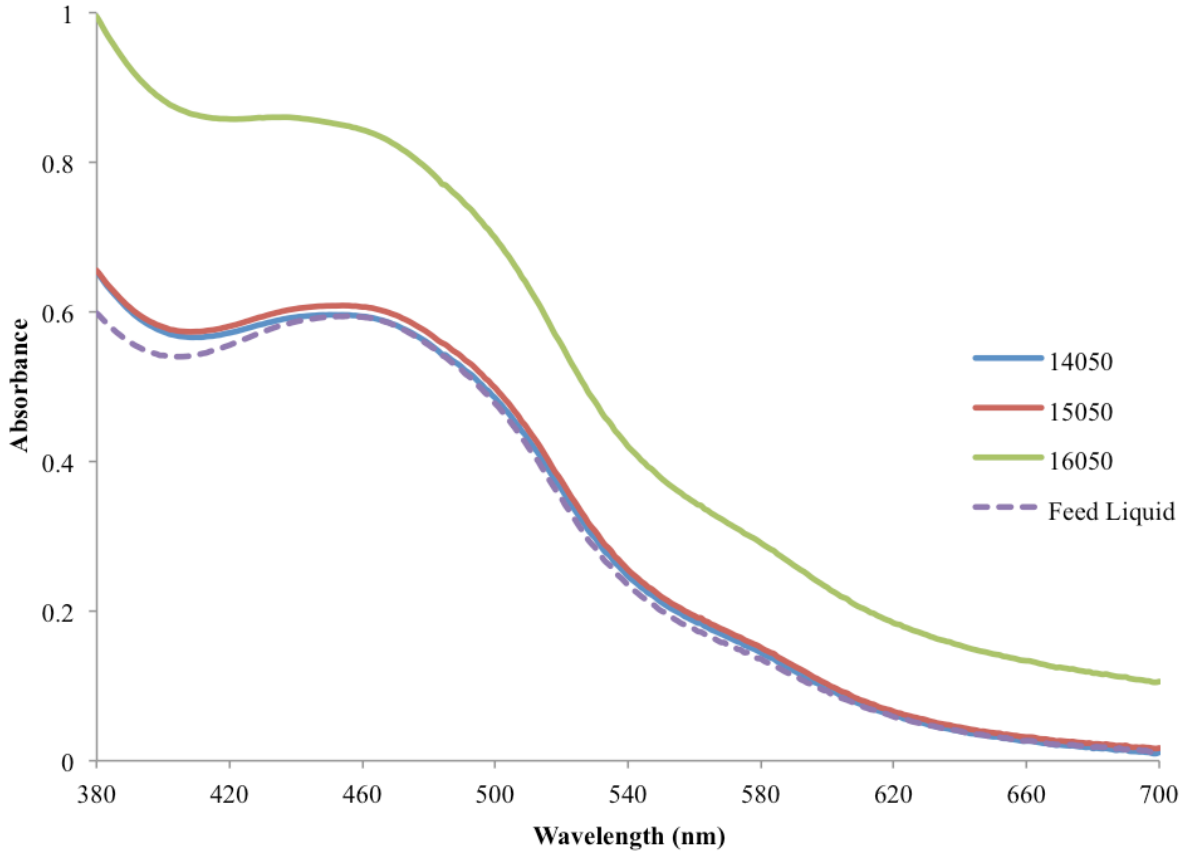

Figure 4.3: Absorbance spectra of 50% pump reconstituted extracts and feed liquid

Table 4.1: Spray drying parameters and yields

Sample	Inlet Temperature (°C)	Outlet Temperature (°C)[1]	Pump (%)	Feed rate (g/min)	Maltodextrin Level (%)	Yield (%)[1]
14025	140	68	25	8	0	75.1
15025	150	71	25	8	0	77.5
16025	160	78	25	8	0	77.2
14040	140	52	40	12.5	0	73.2
15040	150	59	40	12.5	0	77.4
16040	160	64	40	12.5	0	74.6
14050	140	35	50	15.3	0	60.6
15050	150	41	50	15.3	0	69.8
16050	160	47	50	15.3	0	72.8
15040M	150	54	40	12.5	10	76.6

[1]Mean of duplicate runs for each sample (n=2)

Table 4.2: Color values of spray dried powders[1,2]

Sample	L*	c*	h (°)
14025	55.34 ± 0.26^{cd}	27.69 ± 1.41^{a}	58.79 ± 1.34^{a}
15025	56.51 ± 0.50^{ab}	27.82 ± 1.48^{a}	59.26 ± 1.04^{a}
16025	54.92 ± 0.17^{de}	28.24 ± 0.43^{a}	58.68 ± 0.38^{a}
14040	56.27 ± 0.66^{abc}	28.15 ± 0.31^{a}	59.60 ± 0.23^{a}
15040	54.93 ± 0.57^{de}	29.07 ± 0.34^{a}	59.78 ± 0.41^{a}
16040	55.55 ± 0.54^{bcd}	29.09 ± 0.21^{a}	59.76 ± 0.60^{a}
14050	55.16 ± 0.30^{de}	28.33 ± 1.17^{a}	59.92 ± 0.85^{a}
15050	54.22 ± 0.32^{e}	29.19 ± 0.56^{a}	59.52 ± 0.50^{a}
16050	55.23 ± 0.53^{cde}	29.45 ± 0.20^{a}	59.82 ± 0.31^{a}
15040M	56.67 ± 0.21^{a}	29.33 ± 0.51^{a}	59.99 ± 0.22^{a}

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Duplicate measurements were performed on two replicates of each sample (n=4)

Table 4.3: Physical properties of spray dried powders[1,2]

Sample	a_w	Solids Content (g dry matter/100 g)	Bulk Density (g/mL)	Water Solubility (%)
14025	0.220 ± 0.023^{cd}	92.46 ± 1.15^{bcd}	0.491 ± 0.006^{ef}	99.05 ± 0.57^{ab}
15025	0.213 ± 0.046^{cd}	94.48 ± 0.92^{ab}	0.485 ± 0.011^{f}	96.88 ± 1.74^{abc}
16025	0.186 ± 0.052^{d}	94.74 ± 0.45^{a}	0.513 ± 0.011^{e}	98.34 ± 1.59^{ab}
14040	0.274 ± 0.014^{bc}	91.28 ± 1.91^{cde}	0.544 ± 0.014^{d}	97.78 ± 1.33^{ab}
15040	0.238 ± 0.016^{bcd}	92.59 ± 0.85^{abcd}	0.516 ± 0.007^{e}	97.34 ± 1.64^{ab}
16040	0.226 ± 0.021^{cd}	93.38 ± 0.95^{abc}	0.497 ± 0.006^{ef}	98.30 ± 1.12^{ab}
14050	0.367 ± 0.015^{a}	90.53 ± 0.60^{de}	0.684 ± 0.011^{a}	96.37 ± 1.09^{bc}
15050	0.380 ± 0.030^{a}	89.91 ± 0.80^{e}	0.625 ± 0.016^{b}	97.75 ± 0.31^{ab}
16050	0.362 ± 0.020^{a}	90.10 ± 0.38^{e}	0.580 ± 0.010^{c}	99.28 ± 0.35^{a}
15040M	0.294 ± 0.004^{b}	91.49 ± 0.35^{cde}	0.505 ± 0.016^{ef}	94.02 ± 1.12^{c}

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Duplicate measurements were performed on two replicates of each sample (n=4)

Table 4.4: Color values of reconstituted extracts[1,2]

Sample	L*	c*	h (°)	ΔE	Absorbance at 456 nm
14025	56.08 ± 4.58[a]	57.86 ± 0.95[ab]	61.81 ± 5.25[a]	4.78 ± 4.88[a]	0.695
15025	59.17 ± 3.94[a]	56.90 ± 2.68[abc]	64.75 ± 3.41[a]	5.05 ± 4.36[a]	0.607
16025	63.47 ± 2.80[a]	52.29 ± 2.91[c]	68.20 ± 2.03[a]	10.76 ± 4.44[a]	0.602
14040	61.55 ± 2.29[a]	54.34 ± 2.22[bc]	66.59 ± 1.77[a]	7.61 ± 3.52[a]	0.522
15040	59.59 ± 1.24[a]	57.44 ± 0.87[ab]	64.78 ± 1.67[a]	4.33 ± 1.42[a]	0.621
16040	61.12 ± 3.26[a]	55.34 ± 2.69[abc]	66.46 ± 2.63[a]	6.86 ± 4.61[a]	0.619
14050	59.90 ± 5.22[a]	56.06 ± 3.10[abc]	65.06 ± 4.78[a]	7.06 ± 4.86[a]	0.596
15050	58.23 ± 4.64[a]	57.66 ± 2.36[ab]	63.76 ± 4.45[a]	5.34 ± 3.97[a]	0.608
16050	59.93 ± 2.77[a]	57.41 ± 1.12[ab]	65.59 ± 2.49[a]	5.19 ± 2.43[a]	0.848
15040M	57.53 ± 2.28[a]	53.28 ± 0.99[bc]	64.45 ± 1.99[a]	6.29 ± 1.70[a]	0.584
Feed liquid	56.48 ± 3.30[a]	59.01 ± 0.81[a]	63.16 ± 3.84[a]		0.594

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Duplicate measurements were performed on two replicates of each sample (n=4)

Table 4.5: TPC, CT, and AA of reconstituted extracts by volume[1]

Sample	TPC (mg GAE/ml extract)[2]	CT (mg CE/ml extract)[3]	AA (mg TE/ml extract)[3]
14025	4.515 ± 0.056^{abc}	7.248 ± 0.192^{a}	13.01 ± 0.12^{a}
15025	4.438 ± 0.024^{bc}	6.369 ± 0.467^{abc}	12.82 ± 0.38^{ab}
16025	4.599 ± 0.056^{abc}	6.865 ± 0.353^{ab}	12.74 ± 0.48^{ab}
14040	4.656 ± 0.027^{a}	4.818 ± 0.456^{d}	12.73 ± 0.20^{ab}
15040	4.515 ± 0.084^{abc}	5.258 ± 1.466^{cd}	12.38 ± 0.25^{abc}
16040	4.563 ± 0.036^{abc}	6.417 ± 0.229^{abc}	12.31 ± 0.23^{bc}
14050	4.404 ± 0.115^{c}	5.476 ± 0.395^{bcd}	12.25 ± 0.26^{bc}
15050	4.488 ± 0.056^{abc}	4.864 ± 0.210^{d}	12.31 ± 0.27^{bc}
16050	4.508 ± 0.286^{abc}	5.394 ± 0.579^{cd}	11.95 ± 0.31^{c}
15040M	4.588 ± 0.057^{abc}	5.598 ± 0.374^{bcd}	11.75 ± 0.22^{cd}
Feed liquid	4.586 ± 0.053^{ab}	6.503 ± 0.539^{abc}	11.18 ± 0.22^{d}

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Triplicate measurements were performed on two replicates of each sample (n=6)
[3]Duplicate measurements were performed on two replicates of each sample (n=4)

Table 4.6: TPC, CT, and AA of reconstituted extracts on dry basis[1]

Sample	TPC (mg GAE/g dry extract)[2]	CT (mg CE/g dry extract)[3]	AA (mg TE/g dry extract)[3]
14025	475.7 ± 5.9^{abc}	763.7 ± 20.2^{a}	1371 ± 13^{a}
15025	467.6 ± 2.6^{bc}	671.1 ± 49.2^{abc}	1351 ± 40^{ab}
16025	484.6 ± 5.9^{abc}	723.4 ± 37.2^{ab}	1343 ± 50^{ab}
14040	490.6 ± 2.9^{a}	507.7 ± 48.1^{d}	1342 ± 21^{ab}
15040	475.7 ± 8.8^{abc}	554.0 ± 154.5^{cd}	1305 ± 26^{abc}
16040	480.8 ± 3.8^{abc}	676.2 ± 24.1^{abc}	1297 ± 24^{bc}
14050	464.0 ± 12.1^{c}	577.0 ± 41.6^{bcd}	1291 ± 27^{bc}
15050	472.9 ± 5.9^{abc}	512.4 ± 22.2^{d}	1297 ± 29^{ab}
16050	475.0 ± 30.1^{abc}	568.3 ± 61.0^{cd}	1259 ± 32^{c}
15040M	439.5 ± 5.5^{d}	589.8 ± 39.4^{bcd}	1126 ± 21^{d}
Feed liquid	483.2 ± 5.6^{ab}	685.1 ± 56.7^{abc}	1178 ± 23^{d}

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Triplicate measurements were performed on two replicates of each sample (n=6)
[3]Duplicate measurements were performed on two replicates of each sample (n=4)

References

Bhandari, B., Bansal, N., Zhang, M., & Schuck, P. (2013). *Handbook of food powders: Processes and properties*. Cambridge: Woodhead Publishing Limited.

Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews, 56*, 317-333.

Cano-Chauca, M., Stringheta, P. C., Ramos, A. M., & Cal-Vidal, J. (2005). Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Innovative Food Science & Emerging Technologies, 6*, 420-428. doi:10.1016/j.ifset.2005.05.003

do Prado, A. C. P., Aragao, A. M., Fett, R., & Block, J. M. (2009). Antioxidant properties of Pecan nut *Carya illinoinensis* (Wangenh.) C. Koch shell infusion. *Grasas y Aceites, 60*, 330-335. doi:10.3989/gya.107708

do Prado, A. C. P., da Silva, H. S., da Silveira, S. M., Barreto, P. L. M., Vieira, C. R. W., Maraschin, M., . . . Block, J. M. (2014). Effect of the extraction process on the phenolic compounds profile and the antioxidant and antimicrobial activity of extracts of pecan nut *Carya illinoinensis* (Wangenh) C. Koch shell. *Industrial Crops and Products, 52*, 552-561. doi:10.1016/j.indcrop.2013.11.031

Horuz, E., Altan, A., & Maskan, M. (2012). Spray drying and process optimization of unclarified pomegranate (*Punica granatum*) juice. *Drying Technology, 30*, 787-798. doi:10.1080/07373937.2012.663434

Ismal, O. E., Ozdogan, E., & Yildirim, L. (2013). An alternative natural dye, almond shell waste: effects of plasma and mordants on dyeing properties. *Coloration Technology, 129*, 431-437. doi:10.1111/cote.12047

Jafari, S. M., Ghalenoei, M. G., & Dehnad, D. (2017). Influence of spray drying on water solubility index, apparent density, and anthocyanin content of pomegranate juice powder. *Powder Technology, 311*, 59-65. doi:10.1016/j.powtec.2017.01.070

Kamuf, W., Nixon, A., & Parker, O. (2000). Caramel color In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants: science and technology* (pp. 253-272). New York, NY: Marcel Dekker, Inc.

Loughrey, K. (2000). The measurement of color. In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants: science and technology* (pp. 273-287). New York, NY: Marcel Dekker, Inc.

Mazza, G. (2000). Health aspects of natural colors In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants: science and technology* (pp. 289-314). New York, NY: Marcel Dekker, Inc.

Moreira, G. E. G., Costa, M. G. M., de Souza, A. C. R., de Brito, E. S., de Medeiros, M. D. D., & de Azeredo, H. M. C. (2009). Physical properties of spray dried acerola pomace extract as affected by temperature and drying aids. *LWT-Food Science and Technology, 42*, 641-645. doi:10.1016/j.lwt.2008.07.008

National Agricultural Statistics Service. (2017). *Noncitrus fruits and nuts 2016 summary.* Retrieved from http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-06-27-2017.pdf

Nadeem, H. S., Torun, M., & Özdemir, F. (2011). Spray drying of the mountain tea (*Sideritis stricta*) water extract by using different hydrocolloid carriers. *LWT - Food Science and Technology, 44*, 1626-1635. doi:10.1016/j.lwt.2011.02.009

Obón, J. M., Castellar, M. R., Alacid, M., & Fernández-López, J. A. (2009). Production of a red–purple food colorant from Opuntia stricta fruits by spray drying and its application in food model systems. *Journal of Food Engineering, 90*, 471-479. doi:10.1016/j.jfoodeng.2008.07.013

Passos, M. L., & Ribeiro, C. P. (2010). *Innovation in food engineering : new techniques and products*. Boca Raton, FL: CRC Press.

Singleton, V. L., & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16,* 144-158.

Tutak, M., & Benli, H. (2012). Dyeing properties of textiles by Turkish hazelnut (Corylus colurna): leaves, coat, shell and dice. *Coloration Technology, 128*, 454-458. doi:10.1111/j.1478-4408.2012.00399.x

Zhao, Q., Feng, H., & Wang, L. J. (2014). Dyeing properties and color fastness of cellulase-treated flax fabric with extractives from chestnut shell. *Journal of Cleaner Production, 80*, 197-203. doi:10.1016/j.jclepro.2014.05.069

CHAPTER 5

COLOR STABILITY OF PECAN SHELL EXTRACTS

Introduction

Initial consumer perception of food quality is strongly linked to a food's appearance, particularly its color (Loughrey, 2000). For example, consumers are less likely to purchase discolored produce or browning meats than their more colorful counterparts (Hendry & Houghton, 1996). Even the perception of flavor and apparent level of sweetness can be judged by the color of a food product (Hendry & Houghton, 1996). Food colorants are useful for several purposes: increasing the vibrancy of color already present, adding color to a colorless product, adding back color diminished during processing, and providing uniform color throughout batches of products (Martins, Roriz, Morales, Barros, & Ferreira, 2016).

In recent years, consumers have expressed more desire for natural, healthful food additives as opposed to synthetic additives. Synthetic colors are derived from chemical synthesis, whereas natural colors are obtained from edible sources occurring in nature (Hendry & Houghton, 1996). Aside from being more approachable on a food label, natural colors are often rich in antioxidants and could provide added health benefits to foods, such as reduced risk of cardiovascular disease and cancer, antimicrobial properties, and anti-inflammatory activities (Mazza, 2000).

While natural food colorants are popular with consumers and can potentially add health benefits to food products, their stability can be problematic. As mentioned earlier, the color in a food product is crucial to its consumer appeal. Changes to color over time, including fading or

darkening, can negatively impact a consumer's perception of the product (Hendry & Houghton, 1996). Therefore, the influence of storage conditions and the composition of the food product must be considered when developing a natural food colorant (Hendry & Houghton, 1996). Temperature, pH, light, humidity, and oxygen are a few factors known to affect the stability of phenolic compounds, which can be used as natural food colorants (Hernández-Herrero & Frutos, 2014). For example, the color of a product stored in a clear container at room temperature will likely be less stable than the color of a product stored at a low temperature without exposure to light or oxygen.

In order to monitor the stability of a food colorant, its color must be measured over time. Two main methods exist to objectively assess the color of food colorants: use of a colorimeter or a spectrophotometer (Loughrey, 2000). With a colorimeter, a sample is illuminated and the reflected light is passed through red, green, and blue filters to mimic color receptors in the human eye (Loughrey, 2000). The resulting values are reported in various scales, most commonly the CIE L*a*b* scale and the CIE L*c*h scale (Wrolstad & Smith, 2010). When using a spectrophotometer, an absorbance curve detailing the amount of light absorbed by a sample at each wavelength is generated. The data from the absorbance curve can also be converted to color scale values (Loughrey, 2000). Depending on the nature of the sample and specificity of data needed, both methods are useful.

The mechanisms behind changes in natural colors differ with the chemical class of the pigment. Carotenoids may undergo oxidation, while metal ion loss and oxidation may occur in chlorophylls (Ghidouche, Rey, Michel, & Galaffu, 2013). The degradation of phenolic compounds has been extensively studied for anthocyanins as food colorants, but little is known about the stability of condensed tannins as a food colorant (Cortez, Luna-Vital, Margulis, & de

Mejia, 2017). Anthocyanins are typically susceptible to changes when exposed to UV or visible light, highly affected by pH, but most stable in acidic conditions, and less stable when stored at higher temperatures (Hendry & Houghton, 1996).

In this study, phenolic compounds from pecan shells were explored as a natural source of reddish brown pigments for foods. Previous studies have harnessed reddish brown pigments from nutshells to dye textiles, but this knowledge has not been applied to a food colorant (Ismal, Ozdogan, & Yildirim, 2013; Tutak & Benli, 2012; Zhao, Feng, & Wang, 2014). In Georgia alone, more than 43 million pounds of pecans were produced in 2016 when assuming that 40-50% of the pecan weight is due to its shell (do Prado et al., 2014; NASS, 2017). Most of these shells are deemed an agricultural byproduct and thrown away. Considering the abundance and potential coloring properties of pecan shells, creating a natural food colorant from pecan shells would be economically and environmentally advantageous.

To prove its usefulness, this pecan shell colorant should be tested for its stability in a variety of storage conditions and its performance in a model food. The model food selected was a soft drink, because the color extracted from nutshells is similar to caramel color. Additionally, caramel color is one of the most widely used classes of food colorants and it is most popularly used in soft drinks in North America (Kamuf, Nixon, & Parker, 2000; Sengar & Sharma, 2014). Natural alternatives to synthetic caramel color are in demand, because of the potential carcinogenic effects of 4(5) methylimidazole (IARC, 2012; Lee & Lee, 2016). This compound is only produced in classes III and IV of caramel color, but these are the classes used in soft drinks (Lee & Lee, 2016). Successful application of pecan shell colorant to a soft drink would fill a need for a natural substitute for caramel color.

The first objective of this study was to evaluate the stability of pecan shell extract color over time in response to a range of pH values, temperatures, and lighting conditions. The last objective was to evaluate the stability of pecan shell extract color in a model soft drink system over time.

Materials and Methods

Preparation of Spray Dried Pecan Shell Extract

A liquid pecan shell extract was produced based on extraction protocols from previous work found in Chapter Three: Liquid Extraction. This optimized extract was produced by autoclaving (Type LS-2036, American Sterilizer Co., Erie, PA, USA) 70-mesh pecan shells and DI water at a concentration of 3 g/100 mL at 121°C and 15 psi for 30 minutes. Then, the liquid extract was spray dried using a Mini Spray Dryer B-290 (Büchi, Switzerland) according to the methods in Chapter Four: Spray Drying. Compressed nitrogen was used for the heated inlet air. The powdered extract that was analyzed for color stability in buffer solutions was produced with the following spray drying conditions: inlet temperature = 150°C, feed rate = 12.5 g/min, aspirator = 100%, Q flow = 40, and vacuum = -60 mbar.

Color Stability in Buffer Solutions

Preparation of Buffer Solutions

Color stability of the powdered pecan shell colorant under different pH values, temperatures, and lighting conditions was assessed by methods modeled after Tan, Lim, and Lee (2014) and Hernández-Herrero & Frutos (2014). First, seven buffer solutions of pH values 2.4, 3.1, 4.0, 5.0, 6.0, 7.0, and 8.1 were made according to the ratios listed in Table 5.1 with 0.1 M solutions of KHP, KH_2PO_4, HCl, and NaOH. Spray dried pecan shell extracts were dissolved in each buffer solution at a concentration of 0.1 g/50 mL. Each buffer solution was divided into

four 10 mL portions, which were stored in capped test tubes under four sets of conditions to assess the effects of light and temperature on color stability over time.

Storage Conditions

The four storage conditions included: 30°C dark, 30°C light, 4°C dark, and 4°C light. Samples stored at 30°C were kept in an incubator (Isotemp Incubator, Fisher Scientific, Asheville, NC, USA) and 4°C samples were stored in a refrigerator. Dark samples were placed in sealed boxes, while 1000 lumen LED portable work lights (Model MPL1022-LED12K040, Utilitech Pro, USA) were used for light samples. The lights emitted neutral white light with a color temperature of 4000 K. Each light was placed 10 inches away from the sample racks, corresponding to an illuminance of 5164.8 lux, which was measured with a light meter (Model L-398 Studio Deluxe, Sekonic Co., Ltd., Tokyo, Japan).

Color Measurement

Absorbance spectra were measured from 380-700 nm with a spectrophotometer (Genesys Model: G10S UV-Vis, Thermo Fisher Scientific, Madison, WI, USA) one hour after mixing the colorant with the buffer solutions to determine the maximum wavelength and initial spectra for the solutions. Prior to scans, samples were diluted 1:5 with DI water. An absorbance spectra for each solution was measured at 1, 2, 4, 7 days and 2, 4, 6 weeks. The absorbance at λ_{max} for each solution was also recorded at each time point. Color stability was assessed as the change in absorbance at the λ_{max} following each storage period.

Color Stability in Model Beverage

Preparation and Storage of Soft Drink

Color stability of the powdered pecan shell extract was assessed in a model soft drink system over time. First, 3.0 g each of six different pecan shell extracts spray dried with the

following conditions were mixed to create a blend of powdered pecan shell extracts: 140°C, 150°C, and 160°C inlet temperatures and feed rates of 8 g/min and 12.5 g/min. Then, 1.5 g of the powder blend were added to glass bottles. Aliquots of 222 mL of a clear, lemon-lime flavored soft drink (Sprite®, The Coca-Cola Company, Atlanta, GA, USA) were poured into each bottle in a cold environment and capped immediately. Bottles were thoroughly shaken to evenly disperse the colorant with the beverage. The soft drinks were stored in an incubator (Isotemp Incubator, Fisher Scientific, Asheville, NC, USA) at 30°C with indirect light from a 1000 lumen LED portable work light (Model MPL1022-LED12K040, Utilitech Pro, USA) placed on the rack below the soft drinks. The light emitted neutral white light with a color temperature of 4000 K. The illuminance of the light received by the soft drinks was 1721.6 lux, which was measured with a light meter (Model L-398 Studio Deluxe, Sekonic Co., Ltd., Tokyo, Japan).

Color Measurement

The color of each soft drink was measured with a colorimeter (Model CR-410, Minolta Co. Ltd., Tokyo, Japan). Color was assessed in terms of the CIE L*c*h values under the D65 illuminant system. Aliquots of 20 mL of the soft drink were added to a petri dish placed on top of sheets of white paper with the color values L* = 91.77, c* = 4.02, h = 281.78°. The colorimeter was placed directly into the petri dish filled with the drink and measurements were recorded. Periodically, the colorimeter was calibrated with a white calibration tile. Each soft drink was stirred under vacuum to remove carbonation prior to measuring color. Color measurements were taken weekly for four weeks and immediately after the soft drinks were produced. Total color difference was calculated between each time point with the following equation: $\Delta E^* = (\Delta L^{*2} + \Delta c^{*2} + \Delta h^2)^{1/2}$, similar to the procedures described by Duangmal, Saicheua, & Sueeprasan (2008) and Obón, Castellar, Alacid, & Fernández-López (2009).

Results and Discussion

Color Stability in Buffer Solutions

The initial absorbance spectra of the buffer solutions are found in Figure 5.1. All buffer solutions exhibited one peak between 455 and 501 nm and then decreased in absorbance as wavelength increased to 700 nm. Buffers with pH values of 2.4, 3.1, 4.0, and 5.0 all had very similar observed color and maximum wavelengths. For pH 2.4 and 4.0 the maximum wavelength was 456 nm, while the maximum wavelength for pH 3.1 and 5.0 was 455 nm. As pH was increased, the buffer solutions appeared to have more red color, due to absorbance peaks at higher maximum wavelengths. The maximum wavelengths for pH 6.0, 7.0, and 8.1 were 461 nm, 494 nm, and 501 nm, respectively. Additionally, the buffer solution at pH 8.1 exhibited the highest absorbance values and appeared darker than the other buffer solutions.

The changes in absorbance at the maximum wavelength for each buffer solution over time are listed in Figures 5.2, 5.3, 5.4, and 5.5 according to storage conditions. Overall, the buffer solutions stored at 4°C in the dark showed the least changes in absorbance, as seen in Figure 5.2. Conversely, buffers stored at 30°C with exposure to light showed the greatest changes in absorbance, as seen in Figure 5.5. Furthermore, buffers stored at 4°C with exposure to light showed less changes than buffers stored at 30°C in the dark. Some buffers showed darkening at higher storage temperatures, whereas only fading and decreasing absorbance were observed at 4°C. Temperature appears to more strongly affect color stability than exposure to light, although both variables appear to influence stability of the pecan shell colorant.

In the storage group 4°C dark (Figure 5.2), some initial fluctuations in absorbance occurred during the first week. Few changes were observed after week one. Therefore, after an initial period of fading, the buffer solutions of all pH values tested appear relatively stable. In the

4°C light storage group (Figure 5.3), stronger fading, as detected by decreasing absorbance values, was observed in the more acidic buffers (2.4, 3.1, 4.0, 5.0) compared to the 4°C dark storage group. The remaining buffers with pH values of 6.0, 7.0, and 8.1 exhibited similar trends in absorbance over time across both 4°C groups. As a result, light appears to mostly affect the color stability of acidic solutions at low storage temperatures.

Buffer solutions stored at 30°C in the dark (Figure 5.4) mostly increased in absorbance and darkened over time. The least amount of change in absorbance occurred at pH 2.4, 3.1, and 8.1. The buffer solution at pH 6.0 showed the greatest increase in absorbance and darkened considerably. In the extracts that darkened over time (pH 4.0, 5.0, 6.0, 7.0), increases in absorbance steadily occurred after week one. Exposure to light did not appear to greatly affect the stability of buffer solutions at pH 6.0, 7.0, and 8.1 stored at 30°C, as seen by similar curves in Figures 5.4 and 5.5. However, in buffers of pH 2.4 and 3.1, exposure to light at 30°C greatly decreased absorbances to the point of almost no color remaining after 42 days. Finally, pH 4.0 and 5.0 solutions both decreased in absorbance and faded with exposure to light at 30°C, rather than increasing in absorbance and darkening without exposure to light at 30°C. Overall, the buffer solutions at pH 6.0 that were stored at 30°C with and without exposure to light showed the most darkening over time, whereas the buffer solution at pH 2.4 stored at 30°C with exposure to light showed the most fading over time.

High storage temperatures and exposure to light are known stressors for many natural food colorants, such as annatto, anthocyanins, and beetroot (Hendry & Houghton, 1996; Hernández-Herrero & Frutos, 2014). As a result, the buffer solutions stored at 30°C with exposure to light were expected to exhibit the most changes in absorbance, while the solutions stored at 4°C in the dark were expected to show the least changes. In a study on the color and

antioxidant capacity stability in strawberry juice, color was severely deteriorated in juices stored at 23°C versus juice stored at 6°C (Hernández-Herrero & Frutos, 2014).

Another critical factor in colorant stability is pH (Hendry & Houghton, 1996). Anthocyanins, a class of phenolic compounds, have been widely studied for their color changes in response to changes in pH (Tan et al., 2014). Typically, anthocyanins are most stable under acidic conditions (Hernández-Herrero & Frutos, 2014). Less information is available on the color stability of tannins, which are phenolic compounds that have been isolated in pecan shells, in response to changes in pH. One study on the stability of tea catechins, a class of compounds also found in pecan shells, in response to different pH and storage temperatures showed that catechins were more stable at lower storage temperatures (4°C and 25°C) and acidic pH values (Zeng, Ma, Li, & Luo, 2017). Additionally, Zeng et al. (2017) noted the formation of a brown color in solutions with higher pH and higher storage temperatures. This supports the increases in maximum wavelength observed for the more basic solutions in this study, as well as the increases in absorbance observed in the 30°C storage groups. Increased absorbance and darkening were not observed in the most acidic buffer solutions. Overall, the buffer solutions at pH 6.0 and 7.0 showed the greatest changes in absorbance over the 42 days. The buffer solutions at pH 2.4, 3.1, and 4.0 were generally stable except at 30°C with exposure to light. The combination of stress from temperature and light appeared to be strong enough to degrade the colorant, despite the acidic environment.

Upon mixing the colorant with the buffer solutions, a precipitate was observed in all acidic buffer solutions. No precipitate was present in buffer solutions at pH 7.0 or 8.1. The precipitate was present immediately after mixing in the powdered colorant and all of the

precipitate settled at the bottom of the test tube after the first day. No additional precipitate formation was observed over time.

Several natural colorants can precipitate out of solution given the right set of conditions. For example, carmine forms a precipitate at pH values less than 3.5, extracts from grape skins can precipitate in the presence of proteins, and norbixin, a water soluble pigment from annatto, can precipitate at low pH or with hard water (Hendry & Houghton 1996). Thus, the precipitation encountered in this experiment is not uncommon for a natural food colorant. Filtration or centrifugation could be employed to remove the precipitate from the final food product.

A photo of the buffer solutions on day zero is presented in Figure 5.6 to further illustrate the initial colors observed. Photos of the buffer solutions on day 42 are presented in Figures 5.7, 5.8, 5.9, and 5.10 according to storage conditions to show the color changes of the solutions. Additionally, the precipitates can be seen in these figures, especially in Figure 5.10.

Color Stability in Model Beverage

After an initial change in color, the color of the model beverage appeared very stable according to the color results in Table 5.2. There was a significant decrease in L* from 64.28 on day zero to 58.32 on day seven. Therefore, some darkening occurred after mixing the colorant with the beverage. No significant changes were detected in L* after day seven. One slight statistically significant change occurred in chroma at day 28. The saturation of the color of the beverage increased from 54.31 at day 21 to 57.99 in day 28. After day zero, there was a significant decrease in hue from 69.98° to 61.67°. This indicates more red tint than yellow after storing the beverage. Results in hue for days 14, 21, and 28 were not significantly different from day 7. Lastly, the ΔE color change values indicate a small, but noticeable change in color between day zero and day seven (ΔE = 9.87). However, the ΔE values for days 14, 21, and 28

were not significantly different from day seven. Values ranged from 9.15 to 10.19. Therefore, following an initial color change in the first week, almost no difference was found in color of the soft drinks over the next three weeks. The color of the soft drinks after 28 days of storage can be seen in Figure 5.11.

A very thin layer of precipitate was observed in the bottom of the soft drink bottles. This precipitate was observed at the first time point for measuring color (seven days) after initial mixing and the amount of precipitate did not appear to change with time. Therefore, the significant change in color between days zero and seven could be due to colorant settling out of solution over time. Once the product was allowed to fully settle, color was stable for the remaining time period. The slower rate of settling in the soft drink versus the buffer solutions could be attributed to increased viscosity in the soft drink.

The color values of Coca-Cola® (The Coca-Cola Company, Atlanta, GA, USA) were measured for comparing the color of soft drinks using caramel color to soft drinks colored with pecan shells. When measured in quadruplicate with a colorimeter in the same manner as the pecan shell colored drink, Coca-Cola® had the following average L*c*h values: L*= 78.27, c*= 22.01, h= 87.15°. After 28 days, the L*c*h values of the pecan shell colored soft drink were L*= 58.53, c*= 57.99, h= 62.05°. The pecan shell colored soft drink had lower L*, much higher c*, and lower h than Coca-Cola®, indicating a lighter, more saturated red color. In Figure 5.11, bottles of the soft drinks show that visually, the color of the model beverage appears similar to commercial cola drinks. The pecan shell colored beverage has a more vivid reddish-orange color than the typical brown shade of other colas, but further studies would be needed to determine if this difference in color is acceptable to consumers.

Previous studies indicate high color stability for model soft drinks colored with natural phenolic pigments over at least one month of storage (Duangmal et al., 2008; Obón et al., 2009). The fruit from cacti, *Opuntia stricta*, contain betacyanin pigments that were spray dried to add red-purple color to a soft drink (Obón et al., 2009). This soft drink was stored at 4°C for one month and exhibited a ΔE value less than 5, which indicates very little color change and stable color under refrigerated conditions (Obón et al., 2009). Another colorant rich in anthocyanins was produced from freeze drying roselle, a species of hibiscus, and applied to a model soft drink (Duangmal et al., 2008). The soft drink was stored for 84 days at 30°C an exhibited a low ΔE value of approximately 12 (Duangmal et al., 2008). In the end, the phenolic compounds concentrated in the pecan shell colorant proved to be at least as stable in a soft drink as the anthocyanins in these studies.

Conclusion

Spray dried extracts from pecan shells show variations in color stability depending on pH, temperature, and lighting conditions. The colorant showed the most color stability over a six week period when stored at 4°C without exposure to light, regardless of pH. Buffer solutions stored at 30°C with exposure to light exhibited noticeable fading in the most acidic solutions and darkening at pH 6.0 and 7.0. Overall, colorants can be applied to solutions of pH 2.4 or 3.1 and stored at 4°C with or without light or at 30°C in the dark for six weeks with only minor color changes. Despite color stability over time, storage in acidic environments led to some precipitation that would have to be removed with an additional processing step. Furthermore, the colorant was darker and relatively stable at pH 8.1. However, very few food products exhibit such a high pH value and the colorant would not realistically be used in that application. When applied to a soft drink, the colorant successfully imparted reddish-brown color that resembled

soft drinks colored with caramel color for four weeks. Ultimately, pecan shell colorant represents a value-added, natural reddish-brown food colorant that can be incorporated into a soft drink with good color stability.

Figures and Tables

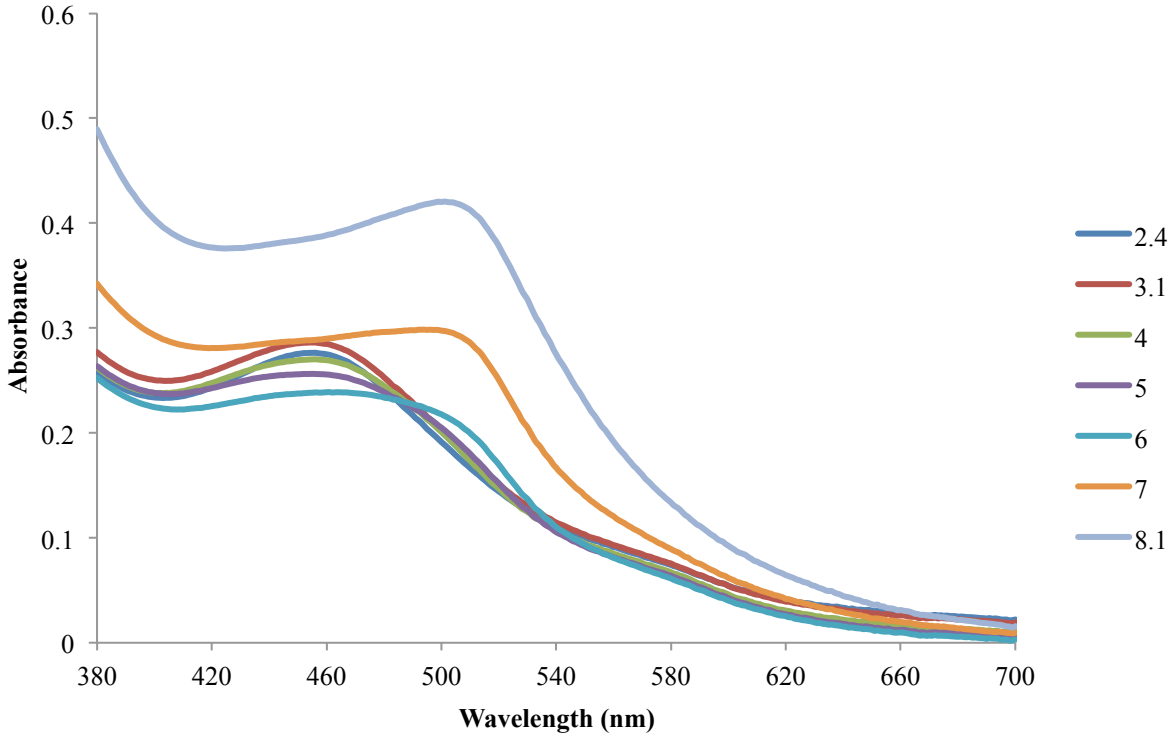

Figure 5.1: Initial absorbance spectra of buffer solutions ranging from pH 2.4-8.1

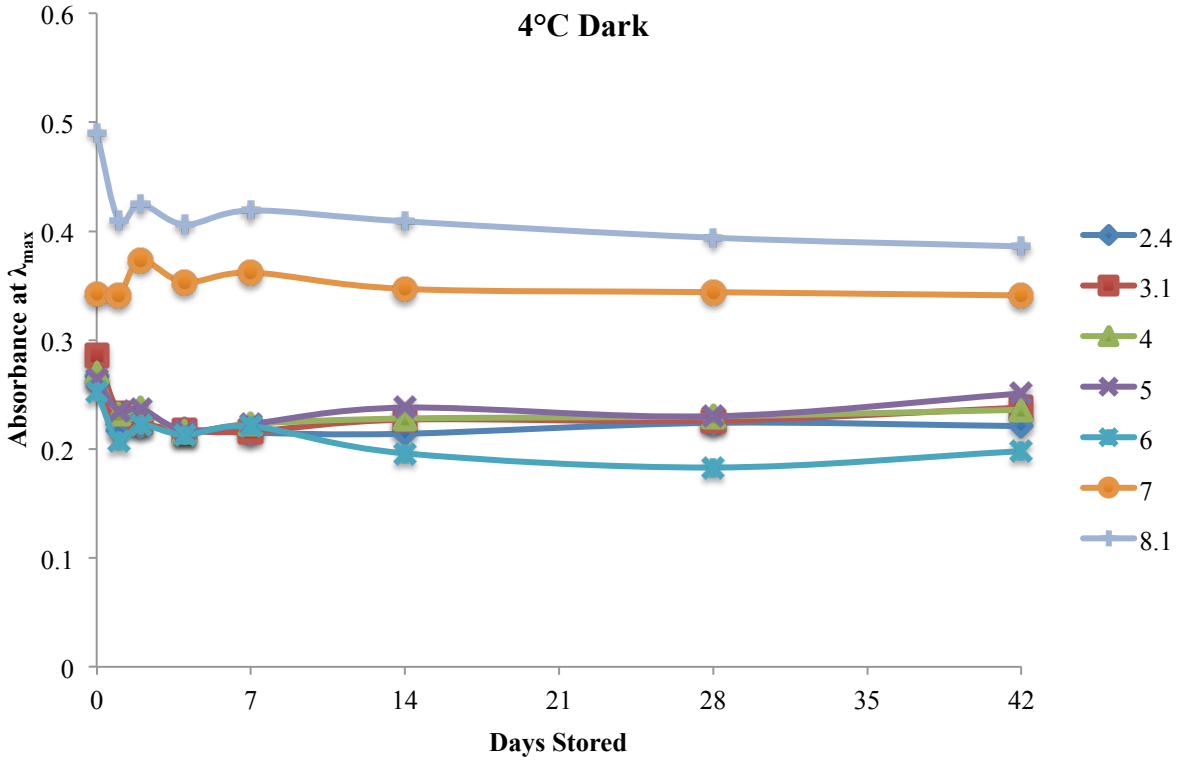

Figure 5.2: Absorbances at λ_{max} over time for buffer solutions stored at 4°C in the dark of pH 2.4-8.1

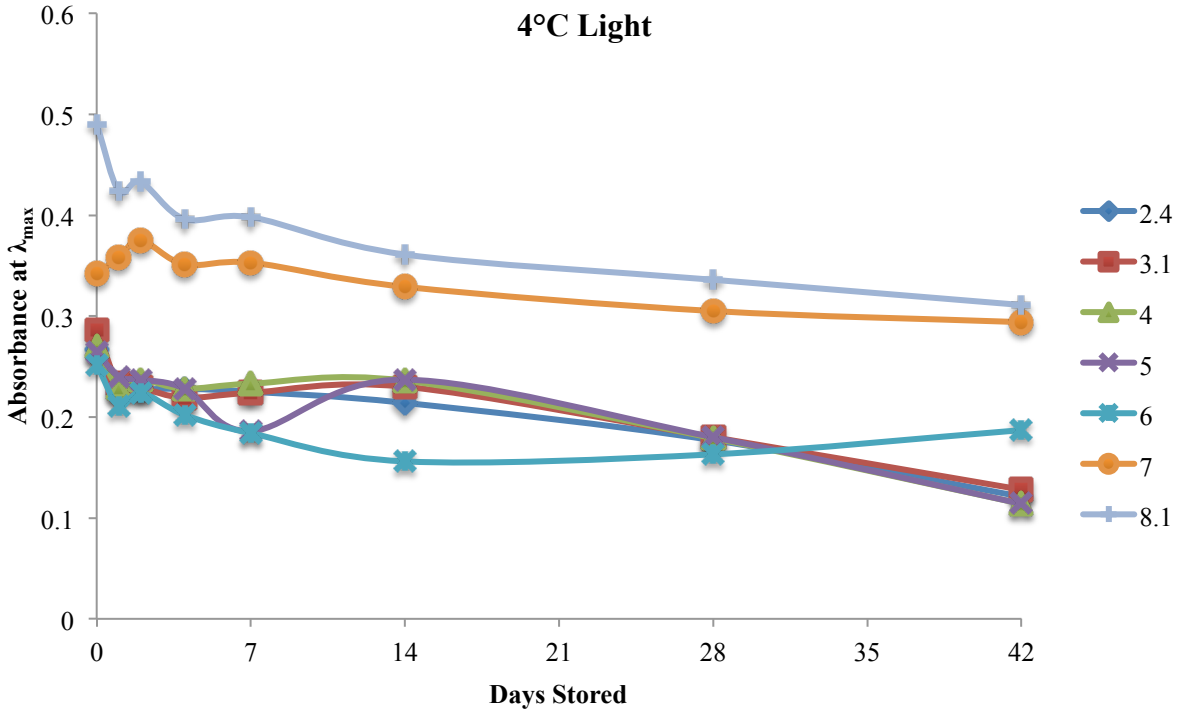

Figure 5.3: Absorbances at λ_{max} over time for buffer solutions stored at 4°C in the light of pH 2.4-8.1

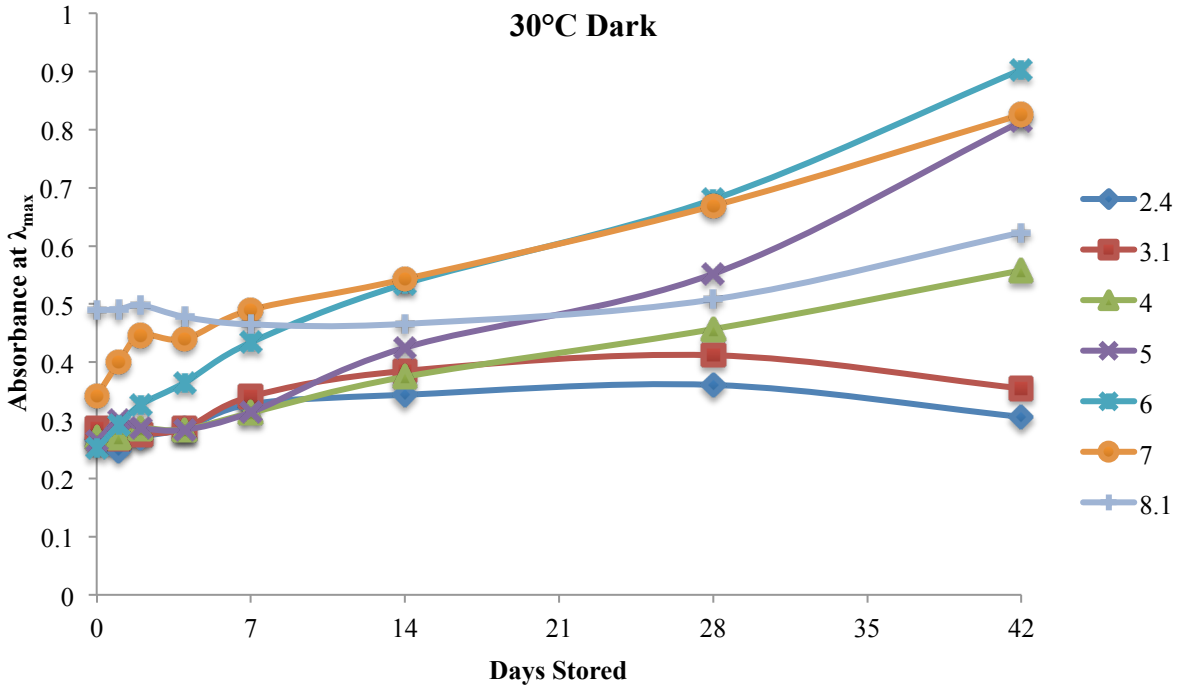

Figure 5.4: Absorbances at λ_{max} over time for buffer solutions stored at 30°C in the dark of pH 2.4-8.1

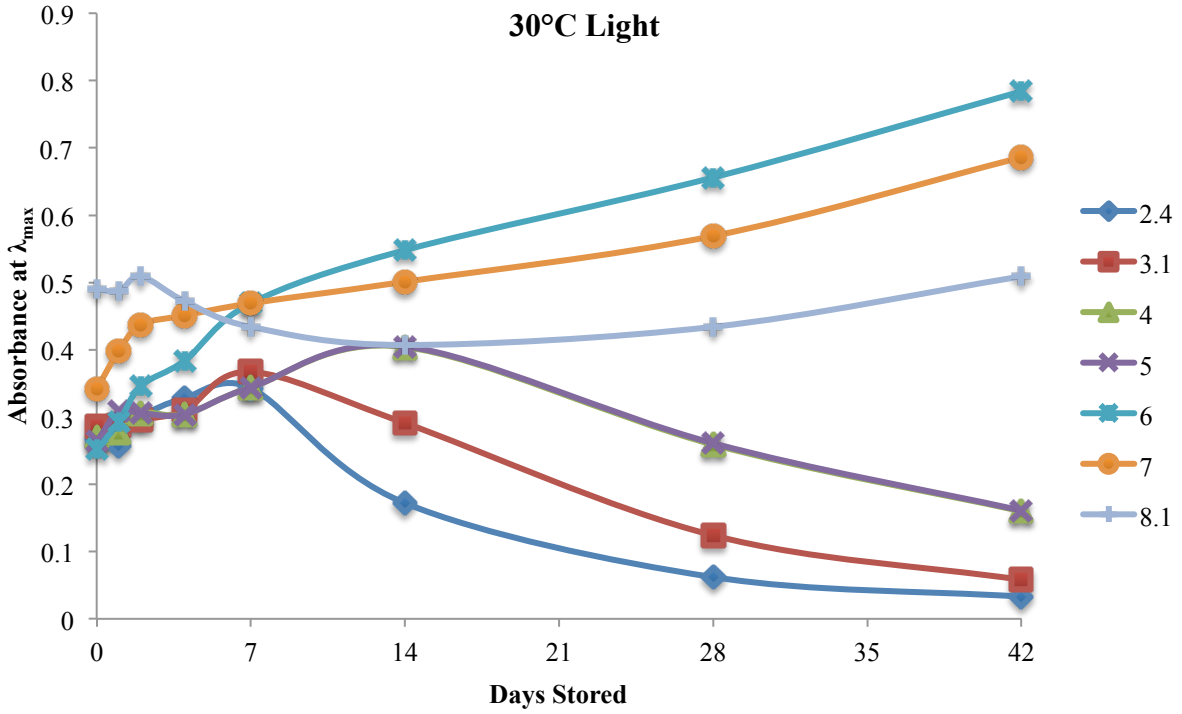

Figure 5.5: Absorbances at λ_{max} over time for buffer solutions stored at 30°C in the light of pH 2.4-8.1

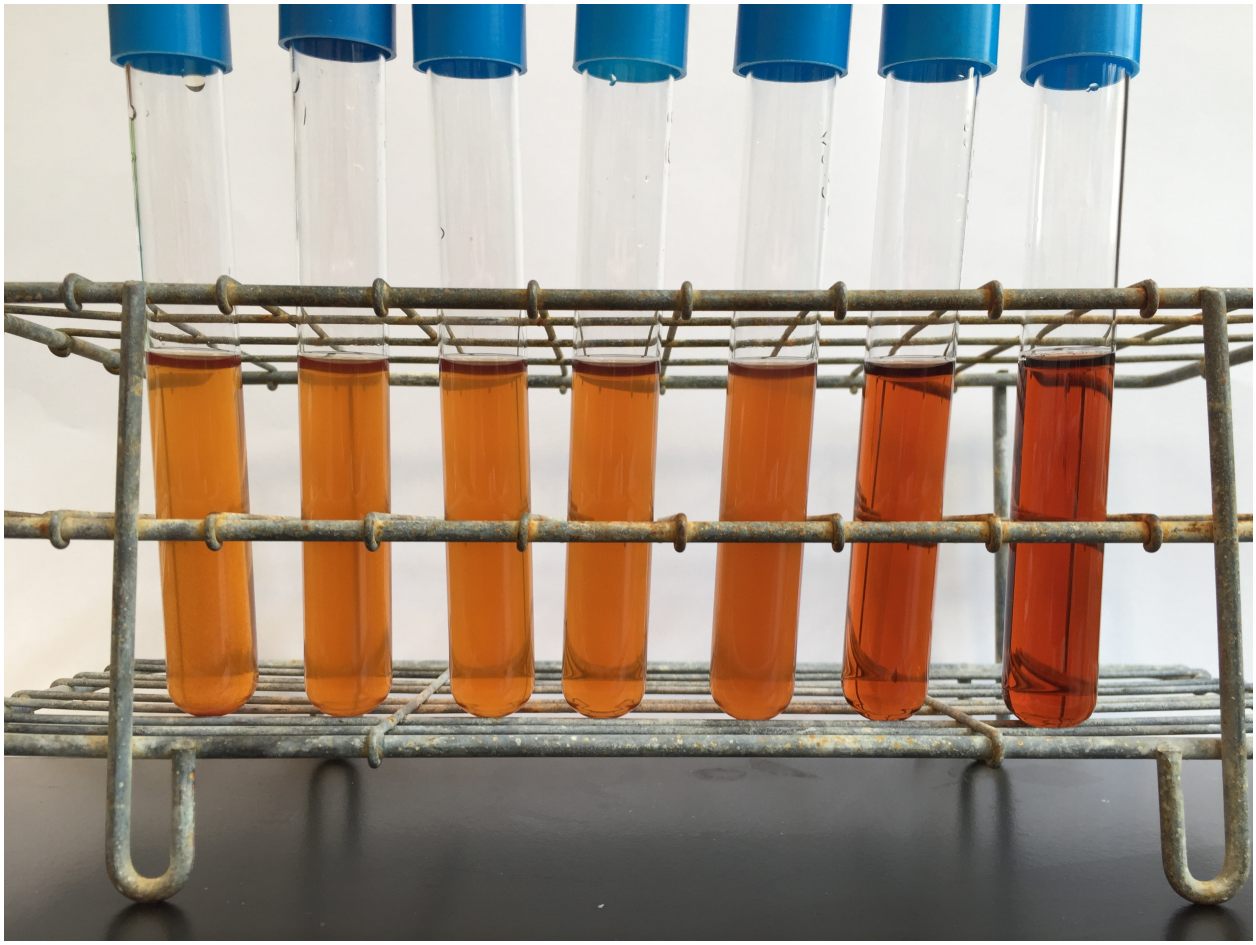

Figure 5.6: Color of buffer solutions of pH 2.4, 3.1, 4.0, 5.0, 6.0, 7.0, and 8.1 (left to right) on day zero

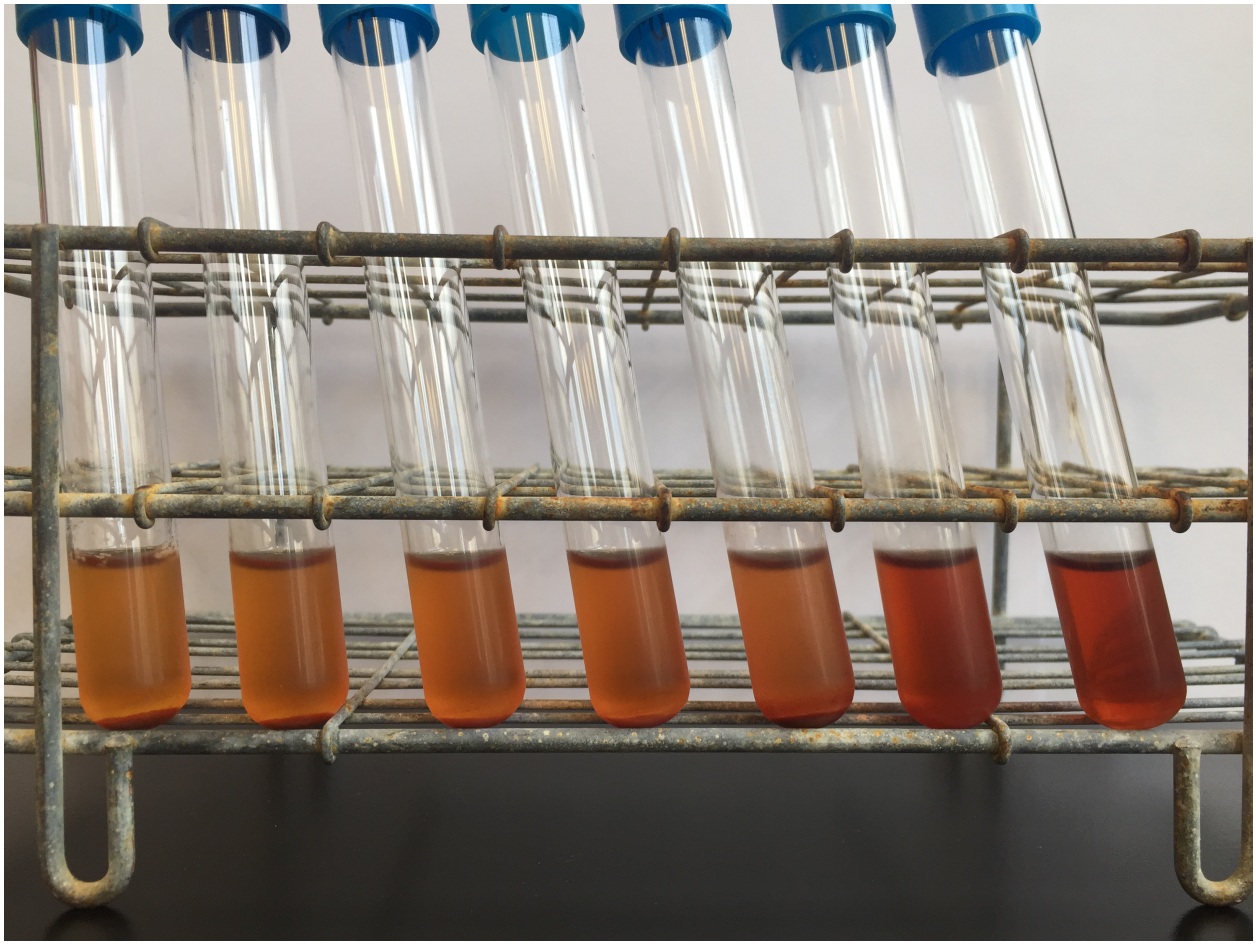

Figure 5.7: Color of buffer solutions of pH 2.4, 3.1, 4.0, 5.0, 6.0, 7.0, and 8.1 (left to right) stored at 4°C in the dark on day 42

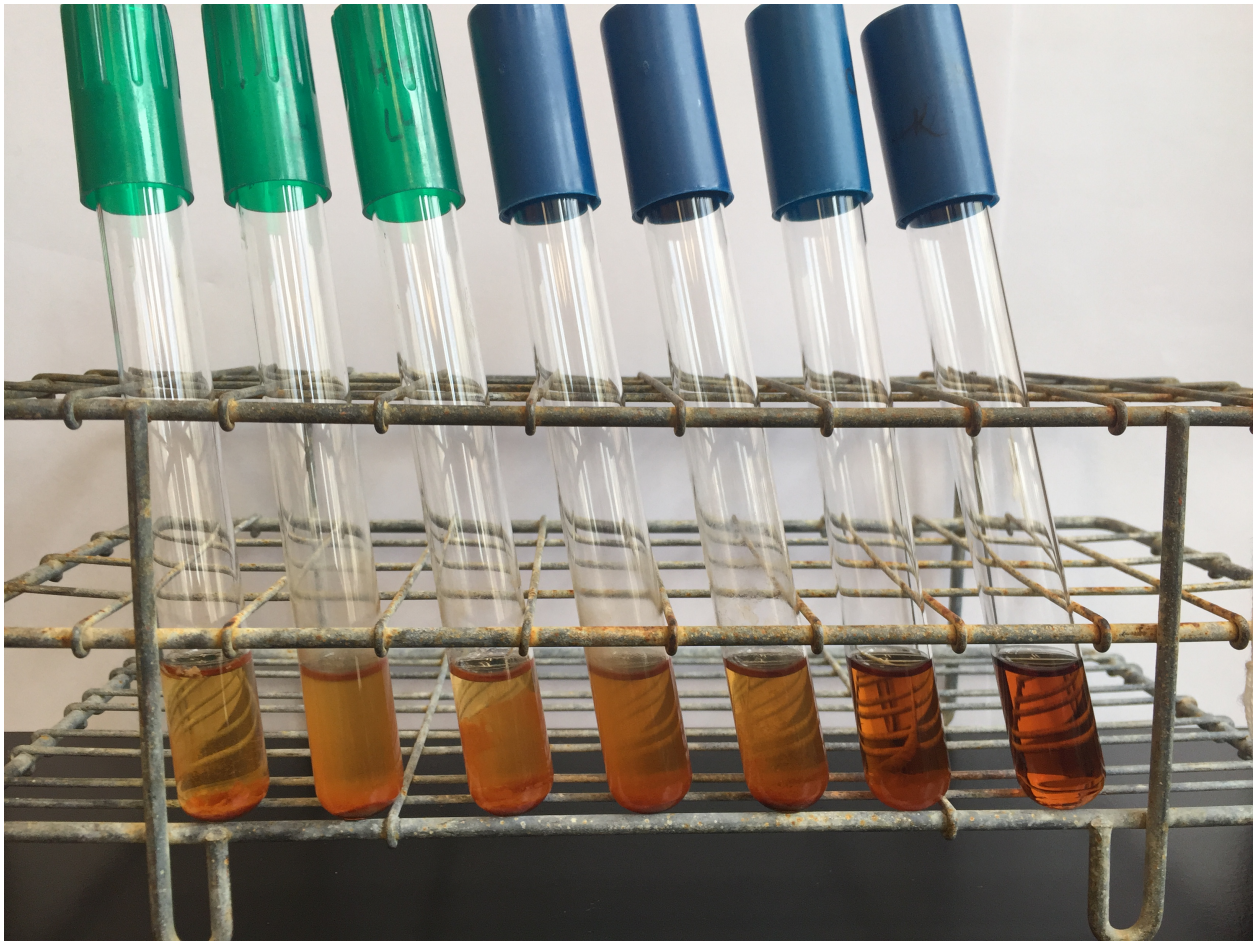

Figure 5.8: Color of buffer solutions of pH 2.4, 3.1, 4.0, 5.0, 6.0, 7.0, and 8.1 (left to right) stored at 4°C with exposure to light on day 42

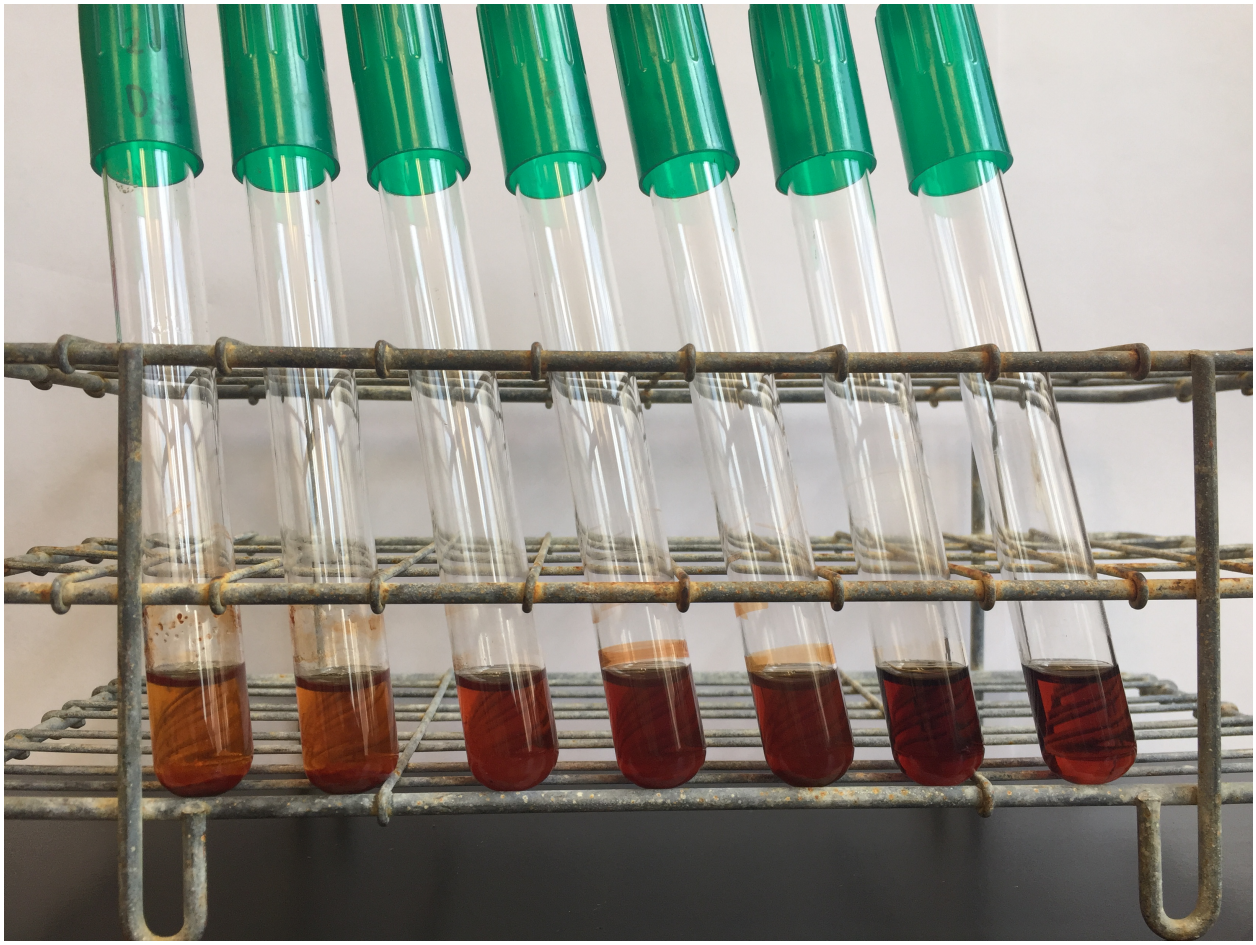

Figure 5.9: Color of buffer solutions of pH 2.4, 3.1, 4.0, 5.0, 6.0, 7.0, and 8.1 (left to right) stored at 30°C in the dark on day 42

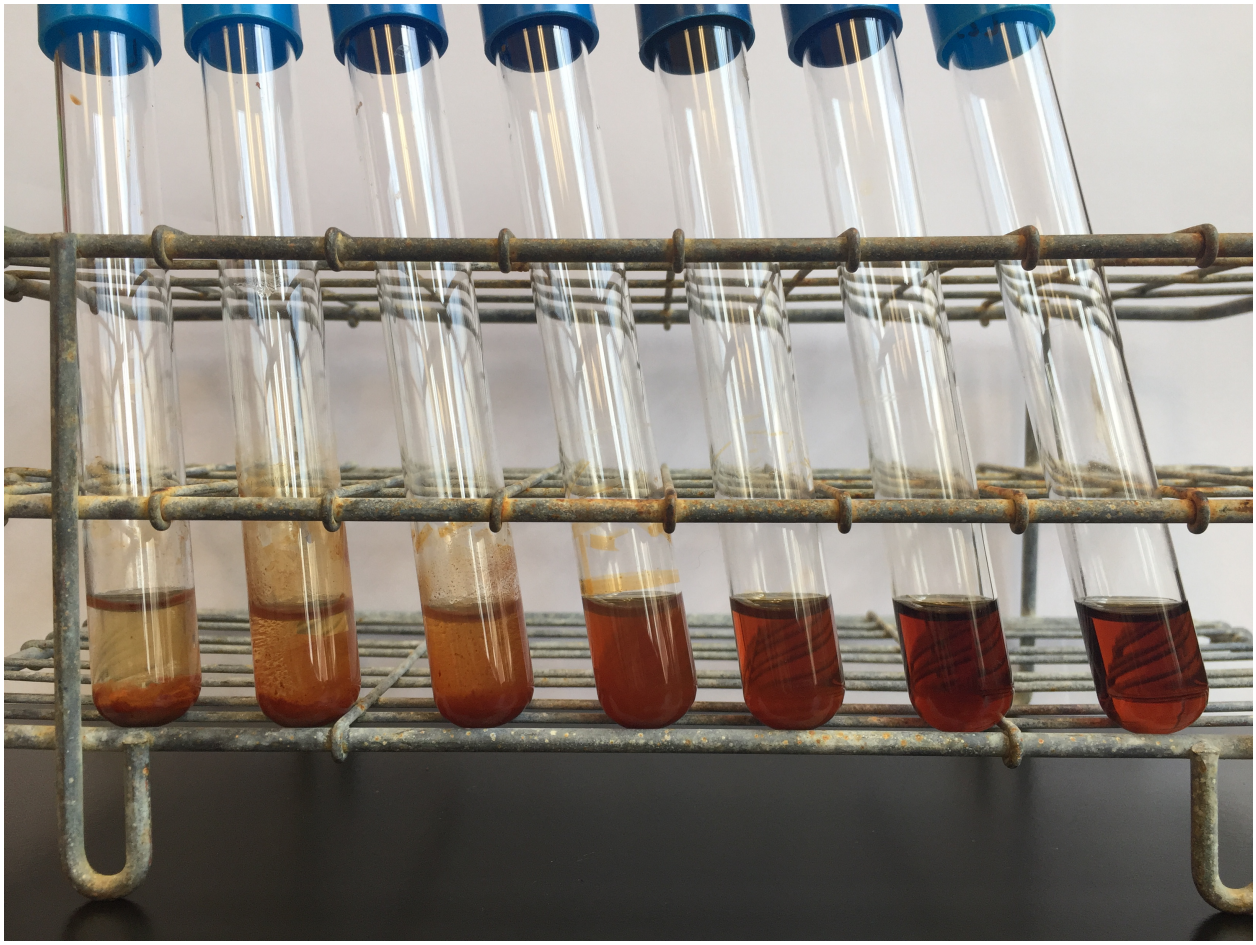

Figure 5.10: Color of buffer solutions of pH 2.4, 3.1, 4.0, 5.0, 6.0, 7.0, and 8.1 (left to right) stored at 30°C with exposure to light on day 42

Figure 5.11: Color of soft drinks after 28 days

Table 5.1: Solvent proportions (v/v) used to make buffer solutions for color stability experiments[1]

pH	KHP 0.1 M	KH_2PO_4 0.1 M	HCl 0.1 M	NaOH 0.1 M
2.4	100		103.7	
3.1	100		46.0	
4.0	100		0.3	
5.0	100			45.6
6.0		100		13.9
7.0		100		59.3
8.1		100		97.1

[1]Adapted from Hernández-Herrero and Frutos (2014)

Table 5.2: Soft drink color values and color change from Day 0[1]

Day	L*[2]	c*[2]	h (°)[2]	ΔE from Day 0
0	64.28 ± 1.37^{a}	56.72 ± 1.42^{a}	69.98 ± 1.17^{a}	
7	58.32 ± 1.24^{b}	56.93 ± 0.78^{a}	61.67 ± 1.09^{b}	9.87 ± 1.69^{a}
14	59.34 ± 1.85^{b}	55.64 ± 1.61^{a}	61.41 ± 1.81^{b}	10.19 ± 1.59^{a}
21	59.71 ± 1.55^{b}	54.31 ± 0.73^{ab}	62.25 ± 1.13^{b}	9.94 ± 2.20^{a}
28	58.53 ± 0.79^{b}	57.99 ± 0.45^{b}	62.05 ± 1.48^{b}	9.15 ± 1.49^{a}

[1]Means ± SD followed by the same letter in a column do not significantly differ ($p<0.05$)
[2]Duplicate measurements were performed on two replicates of each sample (n=4)

References

Cortez, R., Luna-Vital, D. A., Margulis, D., & de Mejia, E. G. (2017). Natural pigments: stabilization methods of anthocyanins for food applications. *Comprehensive Reviews in Food Science and Food Safety, 16*, 180-198. doi:10.1111/1541-4337.12244

do Prado, A. C. P., da Silva, H. S., da Silveira, S. M., Barreto, P. L. M., Vieira, C. R. W., Maraschin, M., . . . Block, J. M. (2014). Effect of the extraction process on the phenolic compounds profile and the antioxidant and antimicrobial activity of extracts of pecan nut *Carya illinoinensis* (Wangenh) C. Koch shell. *Industrial Crops and Products, 52*, 552-561. doi:10.1016/j.indcrop.2013.11.031

Duangmal, K., Saicheua, B., & Sueeprasan, S. (2008). Colour evaluation of freeze-dried roselle extract as a natural food colorant in a model system of a drink. *LWT-Food Science and Technology, 41*, 1437-1445. doi:10.1016/j.lwt.2007.08.014

Ghidouche, S., Rey, B., Michel, M., & Galaffu, N. (2013). A rapid tool for the stability assessment of natural food colours. *Food Chemistry, 139*, 978-985. doi:10.1016/j.foodchem.2012.12.064

Hendry, G. F., & Houghton, J. D. (1996). *Natural food colorants*. Glasgow: Blackie Academic & Professional.

Hernández-Herrero, J. A., & Frutos, M. J. (2014). Colour and antioxidant capacity stability in grape, strawberry and plum peel model juices at different pHs and temperatures. *Food Chemistry, 154*, 199-204. doi:10.1016/j.foodchem.2014.01.007

IARC Monographs. (2012). *4-Methylimidazole.* Retrieved from Monographs.iarc.fr/ENG/Monographs/ vol101/mono101-015.pdf.

Ismal, O. E., Ozdogan, E., & Yildirim, L. (2013). An alternative natural dye, almond shell waste: effects of plasma and mordants on dyeing properties. *Coloration Technology, 129*, 431-437. doi:10.1111/cote.12047

Kamuf, W., Nixon, A., & Parker, O. (2000). Caramel color. In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants: science and technology* (pp. 253-272). New York, NY: Marcel Dekker, Inc.

Lee, S., & Lee, K. G. (2016). Analysis and risk assessment of 4(5)-methylimidazole in brown colored foods and beverages. *Food Additives & Contaminants Part B-Surveillance, 9*, 59-65. doi:10.1080/19393210.2015.1127294

Loughrey, K. (2000). The measurement of color. In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants: science and technology* (pp. 273-287). New York, NY: Marcel Dekker, Inc.

Martins, N., Roriz, C. L., Morales, P., Barros, L., & Ferreira, I. (2016). Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. *Trends in Food Science & Technology, 52*, 1-15. doi:10.1016/j.tifs.2016.03.009

Mazza, G. (2000). Health aspects of natural colors. In G. J. Lauro & F. J. Francis (Eds.), *Natural food colorants: science and technology* (pp. 289-314). New York, NY: Marcel Dekker, Inc.

National Agricultural Statistics Service. (2017). *Noncitrus fruits and nuts 2016 summary.* Retrieved from http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-06-27-2017.pdf

Obón, J. M., Castellar, M. R., Alacid, M., & Fernández-López, J. A. (2009). Production of a red–purple food colorant from *Opuntia stricta* fruits by spray drying and its application in food model systems. *Journal of Food Engineering, 90*, 471-479. doi:10.1016/j.jfoodeng.2008.07.013

Sengar, G., & Sharma, H. K. (2014). Food caramels: a review. *Journal of Food Science and Technology-Mysore, 51*, 1686-1696. doi:10.1007/s13197-012-0633-z

Tan, J. B. L., Lim, Y. Y., & Lee, S. M. (2014). *Rhoeo spathacea* (Swartz) Stearn leaves, a potential natural food colorant. *Journal of Functional Foods, 7*, 443-451. doi:10.1016/j.jff.2014.01.012

Tutak, M., & Benli, H. (2012). Dyeing properties of textiles by Turkish hazelnut (*Corylus colurna*): leaves, coat, shell and dice. *Coloration Technology, 128*, 454-458. doi:10.1111/j.1478-4408.2012.00399.x

Wrolstad, R. E., & Smith, D. E. (2010). Color analysis In *Food Analysis* (pp. 573-586). Boston, MA: Springer US.

Zeng, L., Ma, M. J., Li, C., & Luo, L. Y. (2017). Stability of tea polyphenols solution with different pH at different temperatures. *International Journal of Food Properties, 20*, 1-18. doi:10.1080/10942912.2014.983605

Zhao, Q., Feng, H., & Wang, L. J. (2014). Dyeing properties and color fastness of cellulase-treated flax fabric with extractives from chestnut shell. *Journal of Cleaner Production, 80*, 197-203. doi:10.1016/j.jclepro.2014.05.069

CHAPTER 6

CONCLUSION

The purpose of this study was to develop a natural food colorant from pecan shells and assess the colorant's stability over time. When producing a liquid extract from pecan shells, higher extraction temperatures, finer particle sizes of ground shells, and higher ratios of pecan shells to solvent yielded extracts more rich in color, phenolic compounds, condensed tannins, and antioxidant activity than extracts produced under other conditions. Of the 21 samples produced, optimum extraction conditions for color and phenolic compounds included water as the solvent, 70-mesh shells at a concentration of 3 g/100 mL, and heating at 121°C for 30 minutes.

The optimized liquid extract was spray dried to produce a powdered pecan shell colorant with high yield. Spray drying with a feed rate of 12.5 g/min (40% pump) and inlet temperatures between 140°C and 160°C produced powders faster than the 8 g/min (25% pump) feed rate without sacrificing product quality or yield. Minor differences in physical properties were found across the 10 samples. Also, the color, total phenolics content, and condensed tannin content of the liquid colorant did not significantly change after spray drying for most samples, while antioxidant slightly increased after spray drying.

When analyzing color stability over time of the powdered pecan shell colorant, pH, temperature, and exposure to light all induced changes in color. The colorant was most stable over six weeks when stored at 4°C in the dark. However, colorants in buffer solutions of pH 2.4 and 3.1 showed minor changes in color over six weeks when stored at 4°C in the dark, 4°C with

exposure to light, and 30°C in the dark. In a soft drink system, the colorant showed a slight change in color over the first week of storage at 30°C with exposure to light. No significant changes in color were observed over the following three weeks.

In the end, pecan shells show potential as a natural food colorant. Saturated reddish-brown color was extracted from shells and successfully applied to a food system that remained relatively stable over one month. Considering the abundance of pecan shells and the demand for natural food colorants, this product could be highly marketable to consumers. Further studies assessing the sensory characteristics of the colorant and consumer acceptability would be beneficial.