EVALUATING THE STABILITY OF PURPLE CORNCOB EXTRACT IN

TORTILLA CHIPS

THESIS

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ABSTRACT

Color is the first attribute perceived by a consumer and often is used as an indicator of a number of different product traits such as flavor, quality, and texture. Recently there has been an increase in consumer demand for the introduction of cleaner product labeling and the replacement of synthetic colorants with those derived from natural sources. Anthocyanins are a class of compounds that have received a significant amount of attention due to not only their associated health benefits but also their potential to serve as artificial color replacements. These pigments are sensitive to pH, oxygen, increased temperature, metals, sulfites, ascorbic acids, and a number of other compounds that serve as co-pigments. This study was conducted to assess the stability of an anthocyanin-rich purple corncob extract in a tortilla chip matrix during processing and over a six-month storage period.

Purple corncob extract was produced by Sensus LLC (Hamilton, OH) by steam extracting the corncobs. At Wyandot Inc (Marion, OH) yellow corn was nixtamalized to prepare the masa to make tortillas. This extract was incorporated into masa at 1.84% just prior to tortilla chip production. The chips were bagged in foil bags and stored at ambient conditions. Chips were tested after production (time zero) and at one-month intervals for the duration of a six-month storage study. Samples were prepared by extracting the chips with a solution of 80% acidified methanol. The corncob and chip extracts were assayed for monomeric anthocyanin content, percent polymeric color, and
total phenolic content. The anthocyanin and phenolic profiles of the extracts were also monitored. Additionally, the color coordinates of the chips were tested throughout the storage study to monitor any color change.

In agreement with literature previously published, the anthocyanin profile of the corncob and tortilla chip extracts showed the presence of cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside (de Pascual-Teresa and others 2002, Jing and Giusti 2005, Yang and others 2009). The monomeric anthocyanin content of the corncob extract was 10.45 mg/g extract. In the tortilla chip extract, the monomeric anthocyanin content was found to be 0.038 mg/g tortilla chip. Percent polymeric color showed that in the chips the polymeric content increased up to three months, at which point polymers began to precipitate from solution. The total phenolic content of the corncob extract was 81.89 mg/g. Throughout the storage study, the monomeric anthocyanin content and total soluble phenolic content remained constant. The color coordinates, measured with CIELab, showed a slight decrease, corresponding to a decrease in the vibrancy of the chips.
DEDICATION

This is dedicated to my family and friends, the people who taught me more than I could ever hope to learn on my own. I dedicate this to the people who have helped me through the rough times and been there to celebrate the good times with. To my mom, for being a phenomenal role model and making sure that I know that I can do anything. To my dad, for always being there and showing me that it is never too late to try. To Evan, for never failing to have a surprisingly wise piece of advice or a funny story. To Tyler, for keeping me smiling and teaching me never to give up. To Mindy, for being a constant source of encouragement, support, and laughter since freshman year. I would like to thank specifically my aunt, Dr. Tatiana Oberyszyn, for taking me in as her adopted child for the duration of my time at Ohio State. Having her support, love, and care has helped me more than I can ever repay.
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Color is the first attribute of a product that is perceived by consumers. It provides an indication of flavor and is viewed as a marker of quality of a product. Due to the important role that color plays in product selection, it is of high priority to producers to ensure the color of their product meets the expectations of the consumer. While there are a wide range of synthetic lakes and dyes available to producers with which to color their products, recent research has linked artificial colorants with negative side effects in children (Bateman and others 2004, Lau and others 2006). These findings, along with the consumer desire for “natural” products and clean labeling, have lead food producers to seek out natural coloring alternatives (Jing and Giusti 2005).

Anthocyanins, a class of pigment compounds naturally occurring in various fruits, vegetables, and grains, provide a range of wide range colors from orange to blue. These phenolic compounds have recently received attention for their antioxidant activity and have been shown to possess numerous pharmacological properties (Tsuda and others 2003). In addition to their potential health benefits, anthocyanins have proven to be good natural alternatives to synthetic colorants (Fuhrman 2009).

Purple corn is a pigmented variety of Zea mays L. Originally, this crop was grown in the Andes but it has been introduced to various regions of the world including the US,
Canada, Mexico, and China. It has long been used in South American countries such as Peru and Bolivia to produce a fermented beverage, chicha morado (Jing and Giusti 2005). It has also been used in China to color jellies and candies (Yang and others 2009). Unlike other sources of anthocyanin extracts, such as purple sweet potatoes, radishes, grapes, and black carrots, the purple corncob is a waste product of the food industry.

The purpose of this study was to assess the feasibility of using purple corncob extract as a food colorant from natural sources, using a tortilla chip matrix. Tortilla chips were chosen because production of these chips is a relatively harsh processing method, especially for pH sensitive anthocyanins, as the nixtamalization process produces a basic environment.
2.1 Tortilla Chips

Nearly 75% of the corn grown in Mexico is used for food, the majority of which is made from nixtamalized products (McDonough and other 2001). These products include tamales, posole, tortillas, tostadas, and tortilla chips. The nixtamalization process, a process developed by the Mesoamericans, enhances palatability of the corn and increases the nutritional value. This alkaline cooking process consisted of cooking the corn kernels in lime or the leachate of wood ashes, steeping the corn mixture for 8-16 hours, washing the nixtamal to remove excess lime and the pericarp of the corn, and grinding the mix in a stone metate to produce a dough, masa. In the United States it is typical to use lye instead of lime to cook hominy, a practice that results in a different flavor. Other than the mechanization of the process, very little has changed since it was first developed.
2.2 Tortilla Chip Production

2.2.1. Preparation of Masa

The first step in producing masa from corn is the cooking step. A mixture of corn, typically, white and yellow dent corn, lime (calcium hydroxide), and water is placed in a cooking vessel and heated with agitation (Matz 1993). The corn begins to cook when the

Figure 1. Flow chart of masa production techniques.
mixture reaches above 65 °C (149 °F), the average gelatinization temperature for the corn starch (McDonough and other 2001). Cooking temperatures range from 65-100 °C with varying cooking time depending on what the end product is. Masa being used to make tortilla chips in a commercial setting is usually cooked at 90 °C between 15 and 25 minutes. The amount of time that the corn cooks depends on a variety of factors, such as the endosperm characteristics of the corn, lime concentration, size of the cooking vessel, and frequency of agitation. After cooking the nixtamal is cooled to below 60 °C and the steeping phase begins, where the mixture will rest, undisturbed, between 10 and 20 hours (Matz 1993). During the cooking step, the moisture content of the corn kernels increases from roughly 10-12% to 48-50%. Additional 4-7% water is absorbed during the steeping. After the steeping is complete, the steep liquor, nejayote, is washed away from the nixtamal removing excess lime and the pericarp.

Food-grade lime, quicklime or hydrated lime, consists mostly of calcium oxide, with less than 5% magnesium oxide (McDonough and other 2001). The amount of lime used can vary but typically is used in the cooking step at 1% by weight of the corn. Lime is used during cooking and steeping processes to cleave the bonds that hold together hemicelluloses in the corn kernel cell walls. Breaking these linkages aids in the removal of the pericarp from the kernels, a step that affects the texture, color, flavor, aroma, shelf life and nutritional value of the corn kernel. Although the pericarp is washed away from the masa portions of the pericarp that have been broken down remain in the mix and function as hydrocolloids, contributing to the desirable changes that occur during nixtamalization (McDonough and other 2001). Including lime in the cooking and
steeping mixture also helps to control microbial activity.

After the corn kernels have been cooked and the nixtamal steeped and washed the mixture is ground into masa. Traditionally, lava stones are used for this purpose, however synthetic stones have become popular alternatives (McDonough and other 2001). These synthetic stones last longer and require less maintenance but they cannot be resurfaced at the tortilla plant as lava stones can. Nixtamal that is ground for table tortillas is ground more finely than that for chips. As the masa is transported from the grinder to the sheeter it is kneaded, creating a plastic and cohesive dough. Two Teflon-coated rollers are used to press the kneaded masa into a thin sheet, out of which tortilla chips will be cut, baked, and ultimately fried.

2.2.2. Baking, Equilibrating, and Frying

Unlike corn chips, which are fried directly after sheeting, tortilla chips are baked before frying (Clark and others 1984). Baking not only reduced oil uptake during frying but it also produces a firmer texture and a more prominent alkaline flavor in the product (McDonough and other 2001). Prior to baking, the moisture content of the masa is roughly 50% and it is approximately 37% after baking. Tortillas intended for table use are baked only once but tortillas that will be fried for chips are passed through the oven three times (McDonough and other 2001). The temperature of the ovens ranges from 350 to 480 °C (660-890 °F) and the baking times can vary from 20 to 40 seconds. After baking, the tortillas are allowed to equilibrate for 12 hours at 4 °C. This equilibration time allows for moisture to come to the surface and evaporate, lowering the moisture content of the tortilla, therefore lowering the amount of oil that is absorbed into the
tortilla chip during frying. This equilibration period is essential to reduce the amount of pillowing and other defects that arise during frying. Additionally, the cold temperatures of the refrigerator enhance retrogradation, a process that improves the texture of fried products. After the equilibration process is complete, the chips are fried for roughly 60 seconds in 375 °C oil (Matz 1993). In both corn chips and tortilla chips the moisture content is between 1-3% after frying. The baking step prior to frying creates a drier product, which absorbs less oil during frying. Tortilla chips have an oil content between 21-25%, whereas the oil content of corn chips ranges from 30-40% (Clark and others 1984).

2.2.3. Tortilla Chip Quality Parameters

A number of different product quality attributes (PQA) are measured to ensure a product of optimal quality reaches the consumer. Some of these attributes include moisture content, dimensional changes, and texture. In a commercial setting, suggested quality control guidelines include monitoring the moisture content of the masa and chips, oil content of the chips, and the level of defects such as puffing, folding, or color deviations (McDonough and others 2001). The final oil content of the tortilla chip that is produced is dependent on a number of factors including the corn variety, cooking process, grinding conditions, baking time, and cooling after baking (Kawas and Moreira 2001a). A strong correlation has been established between the degree of cooking of the corn and the water uptake of the masa. The moisture content of the masa is one of the most important attributes to monitor during the production of tortilla chips due to the strong relationship observed between the masa moisture content and final oil content of
the chip (McDonough and others 2001, Kawas and Moreira 2001a, Kawas and Moriera 2001b, Gamble and others 1987).

A study by Meullenet and others (2002) evaluated consumer acceptance of tortilla chips and found that following flavor, texture was the most important attribute in overall acceptance. As with the oil content, the texture depends on a number of different factors including raw materials, processing and storage conditions (Kayacier and Singh 2003). Instrumental analyses utilizing texture analysis machines such as the Instron (Norwood, MA) and TA.XT2 Texture Analyzer (Texture Technology, NY) have been used to measure the hardness, crispness, and crunchiness of tortilla chips (Kawas and Moreira 2001a, 2001b, Katz and Labuza 1981, Bruwer and others 2007). While instrumental analyses and mathematical modeling can provide data about tortilla chip texture, it is always important to consider the relationship between instrumental and sensory analysis. Texture of tortilla chips is a complex concept that can be defined into a number of different descriptors (Meullenet and others 2002, Bruwer and others 2007). These include, but are not limited to, hardness, denseness, toughness/chewiness, fracturability, crunchiness, and crispness.

In addition to the masa and the chips, the oil that the chips are being fried in is also monitored for quality attributes. The parameters screened include the free fatty acid (FFA) content, level of oxidation, measured by peroxide values, and any changes in viscosity, heat transfer capacity, or the development of polymers (McDonough and others 2001). The Oil Stability Index (OSI) and Active Oxygen Method (AOM) are two methods that have been developed to determine the condition and stability of oil and fried products. Rapid methods have been developed that provide processors the ability to
monitored oil quality such as strips to test for FFAs and methods to determine peroxide values and polymer development.

2.2.4. Effects of Processing on Corn Components

Various studies have been conducted assessing the stability of polyphenolic compounds and anthocyanins in corn during nixtamalization and tortilla chip production (Del Pozo-Insfran and others 2006, 2007, de la Parra and others 2007). Ferulic acid, a compound shown to inhibit lipid oxidation and protect cells from oxidative damage, was identified as the major non-anthocyanin polyphenolic compound in white corn (de la Parra and others 2007, Del Pozo-Insfran and others 2006). Overall, white corn has been shows to have up to a 8.4-fold higher total polyphenolic content than pigmented corns. One of the main structural differences between white and pigmented corns is the harder endosperm of the white corn kernels, a trait most likely attributed to the higher free ferulic acid content. The harder endosperm of the white corn does mandate processing alterations between white and pigmented corns. White corns must be cooked at a higher temperature, up to 100 °C, versus the 90-95 °C that is sufficient for blue and purple corns. Additionally, the corns must be cooked for a longer period of time than pigmented corns to obtain the same texture in the final tortilla (Del Pozo-Insfran and others 2007).

In processing raw corn kernels into masa, tortillas, and tortilla chips, significant losses of polyphenolic content, antioxidant capacity, and anthocyanin content were observed in both white and pigmented varieties of corns. The highest losses were seen in white corn, most likely due to the longer cooking time that is necessary. Blue and purple corns, although having a lower total polyphenolic content, showed a higher antioxidant
activity (del Pozo-Insfran and others 2006). This is attributed to the presence of (+)-catechin and anthocyanins, compounds that have been suggested to be better radical scavengers than cinnamic acid derivatives (del Pozo-Insfran and others 2006, 2007). The nixtamalization step of processing had been shown to be the most detrimental to polyphenolic compounds in all types of corn (del Pozo-Insfran and others 2006, 2007, de la Parra and others 2007). The alkaline conditions, high temperatures, and leaching of polyphenolic compounds into the nejayote have been shown to cause the majority of polyphenolic compound degradation, with subsequent thermal techniques contributing only a minor amount to the overall polyphenolic content degradation.

Alternative processing methods have been explored, such as acidification of the masa after nixtamalization, nixtamalization after fractionation of the ground kernels, and extrusion of the masa (del Pozo-Insfran and others 2007, Cortes and others 2006, Mora-Rochin and others 2010). Acidification was conducted by adding fumaric acid to the masa after nixtamalization to return the pH to a range in which anthocyanins are more stable. This did increase anthocyanin retention in the tortilla chips by up to 17%, but results varied with the sample, indicating that cultivar and growth conditions affect the initial loss of anthocyanins during processing (del Pozo-Insfran and others 2007). Acidification of the masa does not address the step in processing that is more deleterious to anthocyanins, nixtamalization. A study done by Cortes and others (2006) assessed various methods of production while evaluating the losses of polyphenolic compounds and anthocyanins. This study found that increased concentration of calcium hydroxide during production lead to a decrease in anthocyanin retention. More recently, a study done by Mora-Rochin and others (2010) assessed the difference in polyphenolic and
anthocyanin retention in tortilla chips produced by traditional nixtamalization and extrusion techniques prior to baking and frying. Compared to the nixtamalized samples, masa that was extruded into tortillas had significantly higher retention of total polyphenolic content. The differences were attributed to the alkaline and thermal processing steps during nixtamalization. Producing tortilla chips by extrusion processing resulted in a retention of 96% of ferulic acid in blue maize, with retention of anthocyanins as high as 50%. The antioxidant capacity retained by this method was as high as 93% in blue maize tortilla chips.

2.3 Corn Kernel Structure

![Cross-section of corn kernel](image)

**Figure 2.** Cross-section of corn kernel (From Lucius 2009).
2.3.1. *Pericarp*

The corn kernel is a composite of protein, starch, and lipid (Lucius 2009). Figure 2 depicts a cross-section of a corn kernel, showing the pericarp, endosperm, and germ layers. The pericarp is the outermost layer of the corn kernel, made up of long cellulose chains, heteroxylans substituted with ferulic acids, and proteins. During the alkaline cooking process the pericarp is broken down and removed, step which improves the palatability and nutritional value of the corn kernel. It has been suggested that this pericarp degradation occurs when acidic groups are neutralized during alkaline treatment, releasing heteroxylan sugars and lignan gums into the nejayote (Sahai and others 2001).

2.3.2. *Endosperm*

The endosperm, composed of proteins, lipids, and starch granules, makes up the majority of the corn kernel (Lucius 2009). During nixtamalization the proteins in the endosperm are denatured and solubilized and the starch is partially gelatinized (Sahai and others 2001). The proteins of the endosperm are grouped into two groups based on their functionalities. Zeins, a class of storage proteins known as prolamins, are one of the types of proteins found in corn. In corn kernels there are four types of zein that exist, \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \)-zeins (Lucius 2009). Of these, \( \alpha \)-zein is most abundant, accounting for 75-85% of the prolamin in corn.

\( \beta \)-zein makes up 10-15%, \( \gamma \)-zein makes up roughly 20%, and \( \delta \)-zein comprises only 5% of the prolamin found in corn kernels. The other types of proteins found in corn kernels are collectively known as the glutelin fraction (Laztitzky 1996). These amorphous proteins are susceptible to alkaline conditions and reducing agents. When
exposed to these conditions the glutelin fraction protein alterations occurs such as the loss of amino acids such as cysteine and arginine and the deamination of glutamine.

The endosperm is further divided into the hard and soft endosperm, a designation made dependent upon the texture of the tissue found in these regions (Lucius 2009). Soft endosperm is composed of spherical starch granules that are surrounded by a thicker protein body. Under light, this portion of the kernel appears opaque because of the way that the voids between the starch granules and the protein matrix scatter incident light. Hard endosperm, on the other hand, is made up of more tightly bound protein-starch matrix (Hoseney 1998). Protein bodies are more abundant in this portion of the endosperm and because of the lack of space between starch granules and proteins; the cell walls appear thinner and translucent. The ratio of hard to soft endosperm determines the hardness of the kernel and subsequently its appropriate use (Hunter and others 2002). The snack food industry, for example, prefers harder kernels for nixtamalized corn-based snacks (Matz 1993). Softer kernels means more cracks in the kernel hulls, allowing water a more direct path to starches, resulting in over-hydration, a masa with elevated moisture content, and puffing or other defects in the final product.

The cooking and steeping processes of masa production also alter starch composition of the endosperm. Between 20-40% of the starch present in the endosperm is hydrated and loses crystallinity during cooking (Sahai and others 2001). Starch that melts during cooking recrystallizes to its native form during steeping however. During the cooking step, the intact aleurone layer slows water diffusion into the endosperm but the long steeping period provides enough time for water and lime to penetrate the kernel thoroughly (Rooney and others 2003).
2.3.3. Germ

The germ makes up roughly 10% of the dry weight of the corn (Lucius 2009). This portion of the kernel contains three quarters of the total lipids present in the kernel. During the nixtamalization of the corn the germ absorbs water and some of these lipids may become saponified. Although the germ is not broken into as many pieces as the endosperm, the germ does not contribute much to the flavor of the masa.

2.4 Pigmented Corn

Pigmented corns exist in a variety of colors such as green, red, orange, black, blue, and purple (Betran and others 2001). Traditionally, blue corn is a flour corn. It has a soft endosperm without dents or wrinkles. Although this corn is preferred for tortillas and atole, a hot beverage, it is not widely grown in Mexico and its presence on the central tablelands is limited. Blue corn has been associated with the Incas, Mayans, Aztecs, and the North American Native American Tribes, the Hopi, Zuni, and Navajo (Betran and others 2001). These cultures utilized corn throughout their lives but blue corn was reserved as a ceremonial corn. In the United States the commercial production of blue corn products began in New Mexico, due to the influence of the Native American tribes on southwestern US regional culture.

2.4.1. Plant and Kernel Characteristics

Compared to other types of hybrid dent corns, blue corn produces a significantly lower and less uniform crop yield (Betran and others 2001). This is due to the higher ratio of floury endosperm and lower bulk density of the kernels. Pigmentation can occur throughout the kernel but the blue pigments responsible for the color of blue corn are found in the outermost layer of the endosperm, the aleurone layer. Therefore, using small
kernels to produce a tortilla chip is favored as there is less endosperm to dilute the pigments of the corn kernel and the end product has a more intense color.

2.4.2. Blue and Purple Corn Products

Corn products fall into two categories, nixtamalized (alkaline cooked) and non-nixtamalized products. Foods made using the nixtamalization process include tortillas, tortilla chips, hominy, tamales, and atole (Betran and others 2001). None of these products are unique to blue corn but it has been suggested that the corn’s color influences acceptability and that these products are considered to have superior flavor and health benefits when compared to white or yellow corn products.

One of products that is made specifically with blue or purple corn is called chicha morado. This beverage made from the deeply pigmented corn is typical in South American countries (Betran and others 2001, Jing and Giusti 2005, de Pascal-Teresa 2002). This product can be alcoholic or non-alcoholic depending on the preparation. Chicha is not made specifically from the corn kernels; but rather using the whole corn or just the cobs, which are steeped in water. Other examples of products that can be made using blue corn include pika, “paper bread”, a thin bread made by Navajo peoples traditionally reserved for ceremonial occasions, pinole, a beverage made from toasted corn kernels, and chicos, steamed immature corn kernels that are used mainly as an ingredient in other dishes (Betran and others 2001).

Purple corn is a variety of pigmented corn that is indigenous to the Andes region of South America but has been adapted to grow in various locations worldwide, including China, the US, Mexico, and Canada. Unlike blue corn, the cob of purple corns is highly pigmented and has almost twice the anthocyanin pigment of that found in the kernels of
purple corn (Yang 2010). This is due to the fact that unlike the kernels, which have pigment only in the aleurone layer, the corncob is pigmented throughout. The anthocyanins that have been isolated from purple corn kernels include cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside. In the corncob of purple corn these anthocyanins along with their malonated counter parts have been isolated (Pascual-Teresa 2002, Yang 2010, Jing and Giusti 2005).

2.5 Anthocyanins

![Figure 3. General structure of anthocyanins](image)
Table 1. Common substitution patterns and wavelengths of maximum absorption in the visible region in methanol with 0.01% HCl (Mateus and Freitas 2009, Giusti and others 1999).

<table>
<thead>
<tr>
<th>Anthocyanin</th>
<th>R1</th>
<th>R2</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>R3 = H</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>H</td>
<td>H</td>
<td>520</td>
</tr>
<tr>
<td>Peonidin</td>
<td>OCH$_3$</td>
<td>H</td>
<td>532</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>OH</td>
<td>H</td>
<td>535</td>
</tr>
<tr>
<td>Malvidin</td>
<td>OCH$_3$</td>
<td>OCH$_3$</td>
<td>542</td>
</tr>
<tr>
<td>Petunidin</td>
<td>OH</td>
<td>OCH$_3$</td>
<td>543</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>OH</td>
<td>OH</td>
<td>546</td>
</tr>
</tbody>
</table>

2.5.1. Anthocyanin Structure

Anthocyanins are part of a large and widely spread group of plant compounds known as flavonoids. This family of compounds is a class of secondary plant metabolites that are based on a C6-C3-C6 backbone structure (Cavalcanti 2010). Anthocyanidins, the aglycone form of anthocyanins, are polyhydroxy and polymethoxy glycosylated derivatives of the flavylium cation (Frietas and Mateus 2009). Their three-ring structure, as seen in Figure 3, consists of an aromatic ring (ring A) bound to a heterocyclic ring (ring C) that contains an oxygen bonded to the third aromatic ring (ring B). The color of
these anthocyanin pigments comes from the resonant structure of the flavlylium cation and the double bonds, which absorb light around 500 nm (Castañedo-Ovando 2009, Cavalcanti 2010).

Although there are over 600 anthocyanins that have been identified 90% of anthocyanins are based on six common anthocyanidins, cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (Andersen and Jordheim 2006). The distribution of these anthocyanidins in nature corresponds to 50%, 12%, 12%, 12%, 7%, and 7%, respectively (Cooke 2005). As seen in the Table 1, the six common anthocyanidins differ in the substitution pattern of the B ring. The wide diversity of anthocyanins that have been identified arises from not only the various hydroxylation and methoxylation patterns of the aromatic rings, but also the presence of a variety of sugar moieties and acylation of these sugars with phenolic or aliphatic acids.

2.5.2. Anthocyanin Occurrence in Nature

Anthocyanins are the largest group of water-soluble plant pigments (Freitas and Mateus 2009). This class of pigments can be found in fruits, flowers, and leaves of plants. Most commonly known are the anthocyanins that occur in various types of fruit such as grapes, berries, and cherries, however these compounds can also be found in vegetables, roots, legumes, and cereal grains (Mazza and Miniati 1993). Table 2 highlights the wide variety of sources of anthocyanins. These pigments have also been isolated from eggplant, red onions, red cabbage, hibiscus flowers, the flowers of the morning glory plant (cultivar Heavenly blue), black rice, and pigmented corn varieties. The abundance, composition, and stability of the anthocyanins varies with cultivar differences as well as growth location and conditions (Francis 1989, Mazza and Miniati
1993, del Pozo-Insfran 2006). In fruits, the most common anthocyanins are 3-O-monoglycosides (Bridle and Timberlake 1997, Freitas and Mateus 2009). These are less stable than acylated anthocyanins, such as those found in pigmented corn (de Pascual-Teresa 2002, Del Pozo-Insfran 2006).

Table 2. Average amount of anthocyanin found in various foods. (Mazza and Miniati 1993, Mateus and Freitas 2009)

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberry</td>
<td>1150</td>
</tr>
<tr>
<td>Blueberry</td>
<td>825-4200</td>
</tr>
<tr>
<td>Cranberry</td>
<td>600-2000</td>
</tr>
<tr>
<td>Currant (black)</td>
<td>1300-4000</td>
</tr>
<tr>
<td>Red grapes</td>
<td>300-7500</td>
</tr>
<tr>
<td>Eggplant</td>
<td>7500</td>
</tr>
<tr>
<td>Red cabbage</td>
<td>250</td>
</tr>
<tr>
<td>Red wines</td>
<td>240-350</td>
</tr>
<tr>
<td>Port wines</td>
<td>140-1100</td>
</tr>
</tbody>
</table>

2.5.3. Anthocyanin Stability

Anthocyanins, when isolated, are highly unstable and susceptible to degradation. Several factors affect their stability including pH, temperature, light, oxygen, and the
anthocyanins’ chemical structure. Additionally, the presence of enzymes, sulfides, ascorbic acid, and sugars can alter the anthocyanin stability as well as the effects seen with concentration changes (Giusti and Wrolstad 2003, Rein 2005, Castañedo-Ovando 2009).

2.5.3.1. Structural Effects

Typically, anthocyanins are penta- or hexa-substituted at positions 3,5,7,3’, 4’ and 3,5,7,3’, 4’, 5’, respectively (Frietas and Mateus 2009). The position and presence of hydroxyl, methoxy, and glycoside groups on the anthocyanin molecule greatly affect its reactivity, stability, and appearance. Increasing the degree of hydroxylation on the B ring of the anthocyanin structure results in a decrease in anthocyanin stability as well as causing a bathochromic shift (Freitas and Mateus 2009, Rein 2005). This shift from a red to a more violet or blue color corresponds to a shift in the spectral band position of absorbance, transmittance, or reflectance toward a longer wavelength. This effect can be visualized when considering the transition from pelargonidin to cyanidin to delphinidin. As the degree of hydroxylation on the B ring increases, the color changes from the orange-red color of pelargonidin to the purple-blue color of delphinidin. Interchanging the hydroxyl substituents for methoxy groups subsequently decreases the stability of anthocyanins and reverses the color trend that is observed with increasing hydroxylation (Mazza and Brouillard 1987).

Anthocyanidins, the aglycone form of anthocyanins, are much less stable when not glycosylated and it is rare to find the aglycone form in nature (Andersen 2002). Like with the hydroxylation and methylation of anthocyanins, the presence and position of the sugar moiety alters the stability of the anthocyanin molecule. Typically,
glycosylation occurs at C-3, as the 3-glucosides are more stable than the 5-, 7-, and 4’-glucosides (Leon and others 1931). In the case of diglucosides, the second sugar moiety is usually attached at position 5, though structures have been elucidated in which the diglucoside nature arises from the glycosylation with a single disaccharide instead of two monosaccharides. The sugars that attach to anthocyanidin molecules can vary but the most common are glucose, galactose, rhamnose, and arabinose (Kähkönen and others 2003).

![Image: Sugars most commonly found associated with anthocyanins.](image)

Figure 4. Sugars most commonly found associated with anthocyanins.

Acylation of the anthocyanins is also possible. An acid, usually a phenolic or aliphatic acid, is attached to C6 of the glucoside through an ester bond, however structures have also been discovered in which the acids was attached to position 2,3, and 4 (Cabrita 1999). The most common phenolic acids that bond to anthocyanins are
hydroxycinnamic acid derivatives such as ferulic and caffeic acids (Francis 1989). The most common aliphatic acids that attach to anthocyanins are malonic, acetic, malic, succinic, and oxalic acids. The acylation of anthocyanins further enhances the stability, though the extent of stabilization is dependent upon the degree of acylation and the type of acyl that is attached (Asen 1976, Stintzing and Carle 2004). Polyacylated anthocyanins are more stable than those, which are monoacylated. Anthocyanins that are acylated with aromatic acids are more stable than those acylated with aliphatic acids. Work done by Francis (1992) highlighted that the position of acylation is also important in determining appearance and stability. Anthocyanins with acylation of the B ring were more stable and produced more intense coloration in the range of pH values from 4-5.5, a region in which anthocyanins are typically colorless.

![Figure 5. Acids most commonly found with anthocyanins.](image)

2.5.3.2. pH

The ionic nature of the flavylium cation allows for the change in anthocyanin molecular structure that occurs with a change in pH (Brouillard 1982). Figure 6 displays
the equilibrium that exists between the five structures of the anthocyanin molecules (Brouillard and Lang 1990). The flavylium cation, present on ring C, is the primary structure of anthocyanins (Freitas and Mateus 2009). This cation, the predominant structure at pH 1, is responsible for the intense red and purple colors observed at pH 3 and below. Between pH values of 2 and 4, the quinonoidal base predominates (Castañedo-Ovando 2009). This compound is formed by alkali oxidation of ring A. This proton transfer generates a quinonoidal base that appears blue or violet. Increasing pH values to 5 or 6 results in the transformation to two colorless compounds, the carbinol pseudobase and a chalcone (Cavalcanti 2010). The chalcone is formed by hydrating the flavylium cation and opening ring C. Closing the “C” ring of the anthocyanin, a reaction that occurs under mildly acidic conditions, forms the carbinol pseudobase. Above pH values of 7 anthocyanins are degraded, the end products dependent on their substitution patterns (Castañedo-Ovando 2009).

Even though varying the pH changes the color of anthocyanins in solution, it is interesting to note that the color intensity of anthocyanin solutions deviates from the Beer-Lambert law at high and low pH values (Bridle and Timberlake 1997). At highly acidic conditions, pH values <2, anthocyanins deviate negatively from Beer’s law and the color increases less than linearly with concentration increase. At higher pH values the color observed deviates positively from Beer’s law. The color of anthocyanin solutions increases more than linearly with increases in concentration, an effect attributed to self-association and co-pigmentation reactions (Bridle and Timberlake 1997).
2.5.3.3. **Temperature**

Anthocyanins are also susceptible to degradation when exposed to heat. As temperature increases the stability of anthocyanins decreases, eventually resulting in the formation of polymeric pigments, which appear brown. Interestingly, heating an anthocyanin solution can shift in the equilibrium toward the colorless chalcone and carbinol pseudobase forms (Freitas and Mateus 2009). Cooling, on the other hand, shifts
the equilibrium from the red flavylium cation to the blue quinonoidal base (Bridle and Timberlake 1997).

2.5.3.4. **Light**

Light plays a dual role in the life of anthocyanins. Early in the plant’s life light is essential for the biosynthesis of anthocyanins but its presence accelerates degradation (Markakis 1982). In a study done by Kearsley and Rodriguez (1981), a difference in color was observed in as little as 24 hours between two samples stored in the light and the dark. The extent and speed of degradation depends on the chemical structure of the anthocyanin (Inami and others 1996, Sapers and others 1981).

2.5.3.5. **Oxygen**

Along with other factors that cause degradation of anthocyanins, oxygen has both a positive and negative effect on the total phenolic and anthocyanin content of a sample based on when and at what levels it is present. Several studies have shown that oxygen enhances the effect of other anthocyanin degradation processes (Cavalcanti 2010). An example of this is the work done by Nebesky and others (1949) that shows that the removal of oxygen protects against thermal degradation of anthocyanins. Other studies however, show that 60-100% oxygen present in the early stages of cold storage increase the total phenolic and anthocyanin content but this effect decreases with prolonged storage (Perez and others 2001, Zheng 2003, Zheng 2007).

2.5.3.6. **Enzymes**

The main enzymes responsible for anthocyanin degradation are glycosidases (Huang 1955, Huang 1956). These enzymes cleave the covalent bond between the glycosyl residue and the aglycone, yielding a highly unstable anthocyanidin.
Other enzymes that act on anthocyanins include phenolases, such as polyphenol oxidase (PPO) and peroxidases (Kader and others 1997). These two classes of enzymes are commonly implicated in enzymatic browning of fruits and vegetables. Both PPO and peroxidases oxidize phenolic compounds into quinones, molecules that in turn polymerize and form brown pigments. Peroxidases unlike PPO, require the presence of hydrogen peroxide to be active. Kader noted that the levels of chlorogenic acid, a compound implicated in anthocyanin degradation pathways, in a fresh blueberry slurry decreased significantly during the first 10 minutes of reaction and that by 20 minutes of sitting at room temperature 90% of the chlorogenic acid had been degraded. The addition of catalase to this mixture did not alter the kinetics of degradation, indicating that peroxidases are not active in chlorogenic acid degradation and that only PPO should be considered in the anthocyanin degradation.

It has been suggested that PPO’s mechanism of anthocyanin degradation is not a direct oxidation reaction but that intermediate compounds are generated by PPO, which in turn act on the anthocyanins (Weshe-Ebeling and Montgomery 1990, Kader 1997, Kader 1998). PPO can oxidize anthocyanins but they are poor substrates, most likely because of the protective effect of steric hindrance caused by the presence of a sugar moiety (Mathew and Parpia 1971). It is the quinones produced by oxidation of other phenolic compounds, such as catechin, catechol, and chlorogenic acid, are responsible for anthocyanin degradation by a coupled oxidation mechanism (Kader 1997, Kader 1998). Anthocyanin structure also plays an important role in the rate of degradation. Delphinidin and petunidin-3-glucosides, along with other anthocyanins with o-diphenolic B rings have been shown to be degraded more rapidly than non-o-diphenolic

2.5.3.7. **Sulfites**

Sulfates and sulfites are utilized in the food industry due to their ability to be used in a variety of food products to produce a multitude of effects. These effects include inhibition of enzymatic and non-enzymatic browning in fruits and vegetables, dough conditioning, antimicrobial activity, and antioxidant effects (Timbo 2004). These compounds have also been implicated in anthocyanin discoloration, a desired effect when they are being used to bleach maraschino cherries but commonly an unwanted side reaction within a product. The color change that occurs is a result of the addition of the sulfate or sulfite to the C2 or C4 position of the anthocyanin structure (Cavalcanti 2010). This bleaching reaction can be reversed with acidification and heating, however concentrations over 10g/kg cause anthocyanin destruction.

2.5.3.8. **Ascorbic Acid**

Ascorbic acid is another food additive utilized for its antioxidant activity that causes discoloration of anthocyanins. The mechanism of anthocyanin degradation is due to direct condensation of ascorbic acid onto position C4 (Jurd 1972, Poei-Langston and Wrolstad 1981). This association causes both the anthocyanin and ascorbic acid to lose their color. Another mechanism, suggested by Iacobucci and Sweeney (1983), is that ascorbic acid acts as a molecular oxygen activator and produces free radicals that when quenched by anthocyanins results in a color change. Shrikhande and Francis (1974) showed that the addition of flavonol into a reaction mixture provides a protective effect against ascorbic acid, an effect most likely due to competition for the anthocyanins. As seen in the case of pH and oxygen, even though the presence of ascorbic acid has been
shown to enhance the rate of anthocyanin degradation, the conditions of ascorbic acid use can increase anthocyanin stability. Ascorbic acid has been suggested to indirectly protect anthocyanins from degradation as it reduces the amount of \( o \)-quinones that are formed prior to polymerization (Wilska-Jeszka 2007).

2.5.3.9. **Sugars**

Anthocyanins are naturally present in a multitude of food products that also contain sugar, such as berry juices and jams, and thus the interactions between sugars and anthocyanins must be explored. Work done by Rubinskiene (2005) and others showed that the effect of added sugar on anthocyanin stability depends not only on the structure, pH, and concentration of the anthocyanin but also on the type of sugar being added. Sucrose and aspartame exhibited similar behaviors when added to fruit preserves. From 0 to 20% sweetener the compounds had a destabilizing effect but as the concentration increased to 40% an increase in anthocyanin stability was observed. Fructose, on the other hand, showed an inversely linear relationship between sweetener concentration and anthocyanin stability. These findings go against other work that has been done with sugars which suggests that increased sugar concentration destabilizes anthocyanins, increasing the amount of polymerization and co-pigmentation that is seen (Tsai and others 2005). Yet another study showed that small concentrations of sugar, up to 20% had a protective effect on anthocyanin content and color in refrigerated berry extracts, though at higher concentrations this effect decreased (Nikkhah and others 2007).

2.5.3.10. **Concentration Effects**

Another variable that alters the stability of anthocyanins is the concentration of anthocyanins present in solution. An increase in anthocyanin concentration in solution
results in an increase in color stability (Giusti and Wrolstad 2003). The total anthocyanin content was shown to be more important to color stability than the types of anthocyanins present in solution (Skrede and others 1992). The effect of concentration on anthocyanin stability is a reaction that has been attributed to both copigmentation and self-association reactions.

2.5.4. **Anthocyanin Stabilization Mechanisms**

2.5.4.1. **Co-pigmentation and Self-association**

Co-pigmentation is a phenomenon in which anthocyanin pigments and other colorless compounds or metal ions can form molecular associations, which generate a change in the color of the anthocyanin (Boulton 2001). This occurs due to stacking of the hydrophobic acyl moieties and the flavylium nucleus of the anthocyanin molecule (Freitas and Mateus 2009). Co-pigments are generally colorless compounds and can be flavonoids, amino acids, alkaloids, organic acids, nucleotides, polysaccharides, metals, or even another anthocyanin (Castañedo-Ovando 2009). These compounds are rich in π-electrons, which can associate with the electron-poor flavylium nucleus. This association protects the flavylium cation, at C2, from nucleophilic attack by water (Matsufuji and others 2003). It also protects position 4 from being attacked by peroxides and sulfur dioxide, two compounds known to discolor anthocyanins (Mazza and Brouillard 1987).

There are four proposed mechanisms for co-pigmentation interactions, depending on the species involved in the reaction. The first is known as self-association (Castañedo-Ovando 2009). This reaction occurs when the co-pigment is another anthocyanin, also known as intramolecular co-pigmentation. The second type of reaction is when the co-pigment is a metal ion. The presence of o-dihydroxyl groups on the B
ring, as seen with cyanidin, delphinidin, and petunidin, allows for the complexation with metal ions (Boulton 2001). Although the complexation is weak, the binding of some di- and trivalent metal cations causes a bathochromic shift. An example of this can be seen in certain flowers where complexation with Fe (III) or Mg (II) at pH 5 is essential for the production of the blue color of the flower petals (Yoshida and others 2006). The third interaction is between anthocyanins and co-pigments with free electron pairs. This is known as intermolecular co-pigmentation. Lastly, it is possible that the co-pigmentation reaction is due simultaneously to a combination of an aglycone, sugar, co-pigment, and protons.

When the co-pigment is a phenolic compound the interaction is temporary due to the lack of chemical bonds (Castañedo-Ovando 2009). The reaction observed is due to the formation of a charge-transfer complex or π-π interactions (Foster 1969). Two oppositely charged compounds come into proximity and a weak bond, transferring electron density from the electron-rich to the electron-poor ring, links the rings. The π-π complex causes the spectral properties of the flavylium cation to change. The absorbance intensity and wavelengths increase, corresponding to a hyperchromic effect and a bathochromic shift, respectively (Dangles and Brouillard 1992). Additionally, the stability imparted by the π-π complex shifts the structural equilibrium toward the flavylium form, increasing the red color that is seen. The extent to which the co-pigmentation effect is observed is dependent on the pH. At a low pH anthocyanins are predominantly in the red flavylium cation form and there would be only a slight shift in the equilibrium. At a higher pH, between pH values 4 and 6 where anthocyanins exist in colorless forms, the shift in the equilibrium toward the red pigments would be much more
noticeable.

2.5.5. **Extraction, Isolation, and Identification of Anthocyanins**

2.5.5.1. **Extraction of Anthocyanins**

The first step in isolating anthocyanins is to physically extract the pigments from the food material. Using fresh plant material, it is often necessary to freeze, dry, or lyophilize the sample prior to grinding (Castañedo-Ovando 2009). It is also possible to soak the sample directly in the extraction solvent. Anthocyanins are most soluble in solvent less polar than water (Kim and Lee 2002). Accordingly, acidified methanol, acetone, ethyl acetate, ethanol, and their aqueous solutions are the most common extraction solvents (Kim and Lee 2002). While the ideal solvent of these depends on the matrix from which the anthocyanins are being extracted, ethanol is preferred by the food industry due to methanol toxicity (Castañedo-Ovando 2009). The extraction solvents are acidified to push the equilibrium toward the flavylium cation form of the anthocyanins, a step that aids in reducing the amount of degradation seen in non-acylated anthocyanins (Yang 2009). Strongly acidic media, however, can lead to the degradation of acylated anthocyanins as the glycosidic bond can be hydrolyzed in these conditions (Castañedo-Ovando 2009).

Various techniques can be utilized to optimize anthocyanin extraction. Among these are sonication and homogenization, two methods that have been shown to decrease the extraction time by disrupting the cellular structure of the anthocyanin-containing matrix (Kim and Lee 2002). The optimal time and temperature of extraction varies depending on the stability of the anthocyanins and the matrix from which they are being extracted. Work done by Yang (2009) showed the optimal extraction parameters for
anthocyanins from purple corn to be a 73 minute extraction, held at 70 °C with a solid to liquid ratio of 1:25, using acidified ethanol as the solvent. The enhanced extraction of anthocyanins with elevated temperature results from increased solubility and diffusion coefficients of the phenolic compounds (Cacae and Mazza 2003). An important fact to consider when conducting extractions at elevated temperatures is the heat labile nature of anthocyanins. When temperatures are increased too severely anthocyanins can polymerize or break down.

Other techniques and sample pretreatments that have been explored to optimize anthocyanin extraction include the use of pressurized extraction solvents (Gizir and others 2008, Seabra and others 2010). Microwave-assisted extraction is another method of extraction that has shown promise as a method to enhance anthocyanin yield while reducing degradation and time needed for extraction (Yang 2010). This technique showed improved efficiency with a reduced extraction time, solvent consumption and allows for a high degree of extraction automation. Additionally, a wider range of solvents could be utilized using this technique than traditional extraction procedures.

2.5.5.2. Purification of Anthocyanins

While extraction methods can be optimized to maximize the anthocyanin yield from a product, the extraction procedures are not selective to anthocyanins. Along with the anthocyanins, various phenolic compounds, sugars, and acids can be in the extraction solution (Kim and Lee 2002). Typically, the extraction mixture is filtered using vacuum filtration techniques to remove any particulate matter that may be present. Next, a chloroform or hexane partition step is employed to remove any excess non-aqueous
extraction solvent and lipid soluble compounds, such as cell wall components or
chlorophylls that may be in the extraction mixture. After removal of the solid and lipid-
soluble components of the extraction liquor, a filtration step using solid-phase extraction
is done to remove potential interfering materials, such as water-soluble phenolic
compounds.

Solid-phase extraction is done to minimize the effects of sample preparation or
purification on the integrity of the anthocyanin extract. Commonly, Sep-Pak C18 or
Sephadex cartridges are used for this step. Anthocyanins bind strongly to the hydroxyl
groups of the solid phase and are partitioned by changing the effluent polarity
(Castañedo-Ovando 2009). Prior to loading the aqueous sample, these pre-packed
cartridges must be activated with methanol (Kim and Lee 2002). Once the sample has
been loaded acidified water is used to rinse away organic acids and sugars. Acidified
methanol is then used to elute the anthocyanins still bound to the solid phase. Liquid-
liquid (LLE), countercurrent chromatography (CCC), and medium-pressure liquid
chromatography (MPLC) have also been suggested as purification techniques prior to
analysis with high-pressure liquid chromatography (HPLC) (Castañedo-Ovando 2009).

2.5.5.3. Identification of Anthocyanins

Anthocyanin identification is an important tool for adulteration and taxonomic
studies. The spectral characteristics of these compounds provide useful qualitative and
quantitative information and due to the low cost and simplicity, spectroscopy is the main
tool used for anthocyanin identification (Giusti and Wrolstad 2001, Wrolstad 2005).
High-pressure liquid chromatography with a photodiode array detector (HPLC-PDA), a
technique similar in principle to the solid-phase cartridges used for anthocyanin
purification, has been used for anthocyanin quantification and identification but there are
drawbacks to this methodology. Various anthocyanins have similar spectral
characteristics, making them difficult to separate. Additionally, obtaining reference
standards are difficult. For these reasons, mass spectrometry (MS) and nuclear magnetic
resonance (NMR), both $^1$H and $^{13}$C, have become the preferred techniques for
anthocyanin identification.

Mass spectrometry has been used to analyze anthocyanins as a method of structural
eucidation for a variety of studies. Anthocyanins have been analyzed with MS to
determine the anthocyanin profile in botanical extracts and as a tool to study the
transformation or polymerization of anthocyanins over time and as they react with other
flavonoids (Hillebrand and others 2004, Matsumoto and others 2001, Wu and others
2004, Wu and Prior 2005a, Wu and Prior 2005b). MS has recently been combined with a
variety of techniques, such as HPLC (HPLC-MS) and Electron Spray Ionization (ESI-MS),
to aid in identification of samples that are non-volatile or thermodynamically
unstable, such as anthocyanins. MS ionizes the samples either using electron impact or
chemical ionization to produce positive ions that, when passed through a magnetic field,
are separated based on their mass-to-charge ratio ($m/z$). The fragments of the sample that
are produced during ionization create a fragmentation pattern that is unique to the
specific molecule. Using the fragmentation pattern and the ($m/z$) ratio it is possible to
determine the structure of the sample in question.

Nuclear magnetic resonance (NMR) is another powerful technique that has been
used for structural elucidation of anthocyanins (Fossen and others 2001, Longo and
Vasapollo 2005, Košir and Kidrič 2002). NMR utilizes the interactions that occur
between spin resonances within isotopic nuclei and electromagnetic radiation fields to characterize molecules (Sorensen 1999). $^1$H NMR plays an important role in determining which chemical groups are present and the general structure of the molecule.

In order to determine fine structural characteristics such as the three-dimensional structure, two-dimensional NMR must be used.

2.5.6. **Health Benefits of Anthocyanins**

Phytochemical compounds that have been shown to exhibit antioxidant activity include flavonoids, tocopherols, carotenoids, and ascorbic acid (Castañedo-Ovando 2009). Anthocyanins are water-soluble pigments that are widely distributed in nature. These compounds occur in fruits, vegetable, legumes, and grains (Mazza and Miniati 1993). The average daily intake in the US diet has been estimated at 180-215 mg/day (Cooke 2005). Other dietary flavonoids, such as genistein, quercetin, and apigenin, however have been suggested to be only 20-25 mg/day.

Anthocyanins, in addition to being non-toxic and non-mutagenic have been suggested to have therapeutic properties (Bridle and Timberlake 1997). Anthocyanins have been reported to have antioxidant activity comparable to that of well-known antioxidants, such as $\alpha$-tocopherol, Trolox, catechin, and quercetin (Kähkönen and Heinonen 2003). It has also been suggested that certain anthocyanins have higher antioxidant capacity than vitamins C and E (Castañedo-Ovando 2009). This antioxidant activity has been implicated in the compounds’ anticarcinogen activity, anti-inflammatory effects and various other biological activities (Kong and others 2004). Anthocyanins have also been linked to the prevention of neuronal and cardiovascular

2.5.7. Food Colorants

Color is the first attribute of a product that is perceived by the consumer. Product color is not only an important part of product identity but also serves as a way for producers to draw consumers in. Color is used by the consumer as an indicator of flavor and quality and often acts as a food “fingerprint” (Bridle and Timberlake 1997). Consumers expect a strawberry flavored product to be red, while yellow indicates lemon or banana, and blue is often associated with a berry flavor. Color can be added to products to impart color that was not present originally or to replace color that was lost during processing.

Food colorants can be divided into three groups based on their origins, natural colors, nature-identical colors, and synthetic colors (Henry 1996). Natural colors are those that are derived from edible sources. Nature-identical pigments are those synthesized to be identical to colorants found in nature, an example of this is β-carotene. Synthetic colors are pigments that do not occur in nature but are manufactured by chemical synthesis, such as tartrazine (Freitas and Mateus 2009). The FDA however defines only two classes of colors, “color additives exempt from batch certification” and “color additives subject to batch certification” (FDA.gov). Both natural and nature-identical colors, as classified by Henry (1996) fall under the colors that are exempt from certification.

Recently, there has been much attention focused on the introduction of natural
alternatives to replace synthetic ingredients. “Synthetic” is a term that is psychologically linked with toxicological issues while “natural” products are linked with enhanced flavor and health promoting effects (Freitas and Mateus 2009, Fuhrman 2010). Legislative action, such as that passed in the European Union in July 2010, mandating the labeling of products that contain Red 40, Yellow 5, and Yellow 6 as “potentially causing attention and activity disorders in children” has not helped consumer perception of synthetic colorants (Kuntz 2010). Consumers have increased demand for natural colors and clean labeling (Fuhrman 2010, Cavalcanti 2010). Food producers are responding to these consumer concerns by increasing the ingredients that are considered more natural (Fuhrman 2010).

2.5.8. Anthocyanins as Food Colorants

Anthocyanins serve as an optimal source for natural replacements to synthetic colorants in food as they provide a wide range of colors, are water-soluble, and, when taken from fruit or vegetable concentrates, are exempt from certification. Carotenoids and anthocyanins are among the most widely used vegetable colorants in the food industry. Currently, anthocyanins are used to provide color to beverages in the United States and South America and to candies and jellies in Asian countries (Freitas and Mateus 2009, Yang 2009, Castañedo-Ovando 2009). These natural colorants have powerful coloring properties, sometimes exhibiting higher coloring capacities than some synthetic colorants, as determined by relative absorbances (Henry 1996). Examples of such cases are purple sweet potato extract, which has been shown to be more stable in beverages fortified with vitamin C than Red 40, and red cabbage extract, which exhibits superior light stability than Red 3 (Kuntz 2010).
Sources of anthocyanin extracts that have been explored include, among many, purple and red sweet potatoes, black carrots, and purple corn (Terahara 1999, Rodriguez-Saona 1998, Stintzing 2002, de Pascual-Teresa 2002, Yang 2009). In some cases, food waste products can be utilized to produce anthocyanin extracts (Jing and Giusti 2005, Jackman and Smith 1996, Pazmino-Duran 2001). The use of food waste products as a source of anthocyanin-rich extracts is an economical alternative to consuming edible foods to produce colorants.
CHAPTER 3

AIM OF THE STUDY

The objective of this study was to assess the stability of a naturally derived purple corncob extract as a colorant in tortilla chips. Prior work has shown that the nixtamalization step of production is the most detrimental to anthocyanin and phenolic content. There has been no published work studying the addition of an anthocyanin-rich extract into the corn tortilla masa after nixtamalization. The anthocyanin profile was identified and monitored in the purple corncob extract and in the chips to test for any changes occurring in the anthocyanin composition of the product. Various instrumental and sensory analyses were conducted over the course of a six-month storage study to monitor the physical properties, including color and texture of the chips. Additionally, monomeric anthocyanin, total soluble phenolic, and percent polymeric color contents were assayed throughout the study to monitor the stability of these compounds during processing and storage.
4.1 Tortilla Chip Production

Chips were produced in the method of commercial tortilla chip production. Nixtamal was produced by cooking and steeping a mixture of white corn kernels, water, and lye. The nixtamal was washed with water and ground to form masa. The purple corncob extract, produced by a steam extraction, was incorporated during the kneading of the masa at 1.84% w/w. The masa was then sheeted and cut into tortilla chip rounds. These rounds were baked in a triple-pass oven. The top oven was kept at 680-690 °F, the middle oven was 635 °F, the bottom oven was between 602 and 608 °F. In total the tortilla rounds were baked for 26 seconds. Prior to baking the moisture content of the chip was 47-50%, while after baking and equilibrating the moisture content was 30-35%. The chips were fried in 100% corn oil, held at 380-385 °F. Tortilla chip rounds were kept in the fryer approximately three minutes. The chips were cooled and bagged in PTE coated with aluminum. The bags had no window and were not gas flushed. Chips were stored at ambient conditions until sampling was conducted.

The white corn chips used for sensory analysis were Wyandot 100% White Corn Tortilla Chips, produced in the typical commercial methodology. The pigmented chips
used in sensory evaluation were Tostitos Organic Blue Corn Tortilla Chips, purchased at Kroger in Columbus, OH.

4.2 CIELab Analysis of Chip Color

Chips were assayed monthly using CIELab color coordinates to monitor any change in color that occurred during a six-month storage study. The CIELab color characteristics were measured using a Minolta CR-300 (Tokyo, Japan) handheld colorimeter. The chips were ground by hand to a uniform particle size and four replicates of each samples were taken, ensuring that each sampling area was different than the previous to account for natural variation in the chip color.

Utilizing CIELab color measurements, the chroma and color difference ($\Delta E$) of the samples can be calculated.

$$C = \sqrt{(a^2 - b^2)}$$
$$\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$$

4.3 Extraction of Anthocyanins

Prior to conducting the monomeric anthocyanin, total soluble phenolics, and percent polymeric color assays, the chips were extracted and an aqueous extract was produced. The chips were ground by hand and stirred in chloroform (Fisher Scientific, Fairlawn, NJ) for one hour at room temperature to remove oil from the chip. Eliminating
a majority of the oil in the chips aided with extraction of anthocyanins from the chip matrix as well as limited the amount of oil in the final aqueous extract. The chip-chloroform slurry was filtered using vacuum filtration and the chips were allowed to dry via this method, ensuring that all chloroform was removed from the chips. The dried chips were then ground using a mortar and pestle and macerated with 80% aqueous methanol (Fisher Scientific, Fairlawn, NJ) with 5% formic acid for 2.5 hours at room temperature. The extraction mixture was centrifuged at 2000 rpm for 10 minutes and the supernatant collected. The supernatant was partitioned with chloroform at a ratio of 1:2 and allowed to separate overnight. The next day another partition was conducted, using hexane (Fisher Scientific, Fairlawn, NJ) (1:2) to ensure that all residual oil was removed from the sample. The aqueous extract was collected and stored at -40 °C until further analysis. At each month’s time point a fresh extraction was conducted.

4.4 Monomeric Anthocyanin Content

Monomeric anthocyanin content of both the corncob and aqueous chip extracts was assayed at time zero, three months, and six months. The method used was that proposed by Giusti and Wrolstad (2001). Briefly, pH 1 and 4 buffers were produced using potassium chloride (Fisher Scientific, Fairlawn, NJ) and sodium acetate (Fisher Scientific, Fairlawn, NJ), respectively. 1 ml of sample extract was placed in a vial with 4 ml of the buffer solution, mixed thoroughly, and allowed to equilibrate at room temperature for 20 minutes. A HP 8452 UV-Vis spectrophotometer was used to read the samples at 520 and 700 nm. The machine was blanked with distilled water in a disposable cuvette. Using the extinction coefficient for cyanidin-3-glucoside in aqueous
pH 1 buffer, 26900, established by Jurd and Asen in 1966, and the molecular weight of cyanidin-3-glucoside, 449.2 g/mol, the absorbance of the sample was calculated.

\[
A = (A_{520} - A_{700})_{pH\,1} - (A_{520} - A_{700})_{pH\,4.5}
\]

Absorbance \( = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)} \)

A is absorbance. MW is molecular weight. DF is the dilution factor. 1000 is the conversion factor. \( \varepsilon \) is the extinction coefficient. 1 is the path length.

### 4.5 Percent Polymeric Color

Percent polymeric color assays were conducted on a monthly basis using the protocol from Current Protocols in Food Analytical Chemistry (Giusti and Wrolstad). A potassium metabisulfate bleaching solution was produced using reagents purchased from Fisher Scientific (Fairlawn, NJ) and was made fresh on the day of the assay. Samples and controls of the corn cob extract and the aqueous chip extract (2.8 ml) were mixed with the potassium metabisulfate solution or water (0.2 ml), respectively, and allowed to equilibrate for 20 minutes at room temperature. A HP 8453 UV-Vis spectrophotometer, blanked with distilled water in a disposable cuvette, was used to measure the absorbance at 420, 512, and 700 nm. Color density was calculated using the control sample, polymeric color was calculated using the bleached sample, and these values were used to obtain a value for percent polymeric color for the sample.
color density \( = \frac{(A_{412} - A_{700}) - (A_{520} - A_{700})}{DF_{\text{bleached}}} \)

polymeric color \( = \frac{(A_{412} - A_{700}) - (A_{520} - A_{700})}{DF_{\text{control}}} \)

percent polymeric color \( = \frac{\text{color density}}{\text{polymeric color}} \times 100 \)

4.6 Total Soluble Phenolic Content

Total soluble phenolic content was done according to the Waterhouse micro method, an adaptation on the Folin-Ciocalteau reagent method proposed by Singleton and Rossi in 1965. Folin-Ciocalteau reagent (2N) was purchased from Sigma (St. Louis, MO). Gallic acid (Sigma, St. Louis, MO) was used to make a gallic acid stock solution. Sodium carbonate (Sigma-Aldrich, St. Louis, MO) was used. 20 μL of sample was pipetted into disposable cuvettes and 1.58 ml distilled water was added. 100 μL of Folin-Ciocalteau reagent was added and mixed well. After waiting between 30 seconds and 8 minutes 300 μL of the sodium carbonate solution was added and the solutions were again mixed thoroughly and allowed to develop for 2 hours at room temperature. Once again, a HP 8453 UV-vis spectrophotometer was used to measure the samples. The wavelength monitored, 765 nm, corresponds to the presence of other phenolic compounds. Using a gallic acid standard curve, the total soluble phenolic content is determined and expressed as gallic acid equivalents (GAE mg/L). Samples were assayed at time zero, 3 months, and 6 months.
4.7 Identification of Anthocyanins- HPLC-MS

A Waters 2695 high pressure liquid chromatography system coupled with a Waters Micromass Q-Tof Premier mass spectrometer (HPLC-MS\textsuperscript{®}) was used to identify the anthocyanins present in the corncob and aqueous chip extracts. Capillary voltage used was 3.2kV/2.8kV, collision energy (CE) was 8 eV (low energy) and 25 eV (high energy). A 110\textdegree\ C source block, 450\textdegree\ C desolvation, with N\textsubscript{2} desolvation, running at 50/600 L/hr. A reverse-phase Waters Symmetry C18 column 3.5\textmu\text{m} (4.6 x 75 mm) was used with a gradient method. Solvent A: 5% formic acid in acetonitrile. Solvent B: 5% formic acid in water. Samples were monitored at 520 nm with a Waters 996 photodiode array (PDA) detector after initial structural identification.

4.8 Texture Analysis

Texture analysis was conducting using an Instron 5542 (Norwood, MA). Bluehill 2 software was used to collect and analyze data. The Instron was equipped with a 100 N load cell and the compression plate probe was used to measure average compression force at break (N). The Compression-Test Profiler Method was used with the compressive extension mode with an end point of 40%. The method assigned as “TPA Food Testing- no peak (failure)” . Twelve replicates were done of each sample and the average force required to break the sample was recorded.

4.9 Sensory Evaluation

Panelists (n=42) were recruited from the Food Science and Technology faculty, staff, students, and building visitors. Age of the panelists ranged from 18-56 with 24
females and 18 males. Those with allergies or intolerances to corn and those with known difficulty differentiating color were asked to refrain from participating.

Panelists were presented with and asked to sign an informed consent form prior to receiving any samples. Panelists were instructed not to eat the chips at the beginning and throughout the test but rather to hold the chip between the thumb and forefingers of each hand and bend the chip until it breaks. Subjects were seated in individual sensory booths under normal white light illumination. First, a 9-point hedonic scale was utilized to evaluate the overall visual liking of the chip sample. The panelists were then asked about the color intensity and crispness of the chips. They were asked to rate their overall liking of the color intensity on a 9-point hedonic scale. Then they were asked to use a 5 point JAR (Just-About-Right) scale to assess the intensity of color. The 9-point hedonic and 5-point JAR scales were then applied to the crispness of the chips. Samples were presented in a serial monadic fashion and removed after assessment of that sample was complete.

4.10 Statistical analysis

Microsoft Excel, versions 2007 and 2010, were used to analyze data from the monomeric anthocyanin, percent polymeric color, total soluble phenolic content, colorimetric, and textural assays. Statistical analysis of variance (ANOVA) was conducted using Minitab, version 16. Statistically significant results ($\alpha = 0.05$) were identified using Tukey’s post hoc analysis.
5.1 Analytical Testing

The purple corncob extract displayed a high levels of monomeric anthocyanin content (MAC) at 10.45 ± 1.32 mg/g. Previously reported data suggests that the anthocyanin content of purple corncobs ranges from 0.34 to 9.8 mg/g (de Pascual-Teresa and others 2002, Yang and Zhai 2010, Jing and Giusti 2007). Black carrots, another source rich in anthocyanins, have been reported to have total anthocyanin content ranging from 0.45 to 17.4 mg/g (Kammerer and others 2004). The anthocyanin levels reported in purple potatoes ranges from 0.35 to 1.75 mg/g (Wegener and others 2009). Growing conditions and cultivar differences have been shown to influence the anthocyanin content of the plant matter, accounting for the wide range of values reported for anthocyanin content of these plants (Mazza and Miniati 1993, Del Pozo-Insfran 2006, Castenedo-Ovando 2009). Compared to the range of values reported in the literature for a variety of plant matter, purple corncob extract proves to be a rich source of anthocyanins.

Tortilla chip production is a harsh process for anthocyanins to endure. These pH sensitive and heat labile compounds are subjected to alkaline conditions, high heat in both the triple-pass baking system and the frying step. Del Pozo-Insfran (2006, 2007) has
conducted studies assessing monomeric anthocyanin content of chips produced using blue corn kernels at each step of the production process. The results of this work shows that roughly 40-50% of monomeric anthocyanin content is lost during the nixtamalization process with an overall loss of up to 99% when processing is completed. Based on the inclusion of the corncob extract into the chip masa at 1.84% w/w, a theoretical value was calculated for the MAC contributed by the corncob extract. The theoretical MAC content of the colored masa was found to be 0.19 mg/g. This decreased in the tortilla chips to 0.04 mg/g, a 79% reduction as a result of processing. The high temperatures that the chips experienced during the baking and frying steps, as well as the high pH resulting from the alkaline cooking step in masa production, could contribute to MAC loss (Del Pozo-Insfran 2006, 2007, Cortes and others 2006, de la Parra and others 2007).

**Figure 7.** Monomeric anthocyanin content of the tortilla chip samples colored with purple corncob extract. Content was assayed using a pH differential method.
Measuring degradation of anthocyanins is not as straightforward as with some other pigmented compounds. Loss of color of anthocyanins does not necessarily indicate loss of pigment molecule, as the compounds can exist as colorless chalcone or carbinol base forms. The MAC assay indicates that there is indeed a loss in monomeric anthocyanin content but cannot specify if the losses are due to degradation or polymerization of the monomeric anthocyanins. To elucidate the mechanism of MAC losses, percent polymeric color was also monitored over the storage period.

Percent polymeric color in a sample is determined using an assay, which exploits the reactivity of anthocyanin molecules with sulfite compounds (Giusti and Wrolstad 2001). A sulfite solution will react only with monomeric anthocyanins in solution and the extent of reaction manifests as a bleaching effect on the sample. Percent polymeric color was assayed on a monthly basis to better visualize the variation in polymeric content over the course of the study. As seen in Figure 8, the percent polymeric color of the corncob extract is roughly 65%. It is expected that at such a low level of incorporation of the extract into the chips, the percent polymeric color would drop drastically in the time zero chips. However, the high heat of the baking and frying steps in tortilla chip production enhance the amount of polymerized anthocyanins. As the study progressed, the polymeric color increased steadily up until month three. At this point it is possible that the anthocyanin polymers reached a point at which they would precipitate out of solution. This behavior is typical of polymerized anthocyanins and would give the impression of a lower percent polymeric color, as seen at months 3, 4, and 5. Using the results collected from the polymeric color content assay, it is appropriate
to suggest that the monomeric anthocyanin losses seen as a result of processing are due mainly to polymerization rather than degradation.

**Figure 8.** Percent polymeric color of the purple corn cob extract and chip samples over duration of a six-month storage period.

Total soluble phenolic content of the chips was also monitored over a six-month storage period. Figure 9 shows the phenolic content, determined by a modified Folin-Ciocalteau method. The total soluble phenolic content of the purple corn cob extract was 81.89 mg/g. As with the MAC, a theoretical value or soluble phenolics in the masa was calculated using the 1.84% inclusion of the corn cob extract. Based on this theoretical value, 1.51 mg/g, 46% degradation in total soluble phenolics can be seen in the chips as a result of processing. Previously published reports show a wide range of the level of degradation of phenolics as a result of tortilla chip processing, from 44-80% (del Pozo-Insfran and others 2006, 2007, de la Parra and others 2007, Mora-Rochin and others 2007, Mora-Rochin and others 2008).
Throughout the duration of the storage study, the phenolic content of the chips remained constant at about 0.8 mg/g.

In addition to monitoring the anthocyanins over the six-month storage period, the anthocyanin profiles of both the corncob extract and the anthocyanins extracted from the test chips were determined. There are a variety of pigmented corns, ranging in color from green, red, orange, black, blue, and purple. While not each of these colors are derived from the presence of anthocyanidins, blue, black and purple corns have been shown to owe their color to three main anthocyanins and their glycosylated and acylated counterparts (de Pascual-Teresa 2002, Zhao and Corrales 2008, Yang and Zhai 2010a, 2010b). The predominant anthocyanins in purple corn kernels are cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside. It has been reported that in addition
to these anthocyanins, their malonated derivatives are present in the cobs of purple corn (de Pascual-Teresa 2002, Yang and Zhai 2010). Figure 10, showing the chromatogram of the purple corncob extract, confirms that these are the anthocyanins present in the extract used in this study. Identification of the compounds was tentatively based on retention time and was verified with mass spectrometry (MS). The broad peak centered at 10 minutes is characteristic of polymerized flavonoids, a spectral trait that is confirmed by the high percent polymeric color of the purple corncob extract (~65%).

![Figure 10. Chromatogram of purple corncob extract. Peak 1- cyanidin-3-glucoside, peak 2- pelargonidin-3-glucoside, peak 3-pelargonidin-3-glucoside, peak 4-cyanidin-3-malonylglucoside, peak 5-pelargonidin-3-malonylglucoside, and peak 6-peonidin-3-malonylglucoside.](image)

The anthocyanin profile of the chips produced using the purple corncob extract was also determined. As with the chromatogram of the purple corncob extract, cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside peaks are all present. It is also clear that cyanidin-3-malonylglucoside is observed in the chip extract. Figure 11 shows the unexpected results that new anthocyanin compounds are present in the chips.
Using MS to identify the peaks, peak 7 was labeled cyanidin-3-acetylglucoside, peak 8 was identified as pelargonidin-3-acetylglucoside, and peak 9 was labeled as peonidin-3-acetylglucoside. These compounds were likely formed during the production of chips and are breakdown products of the malonated anthocyanins in the purple corncob extract.

Figure 11. Chromatogram of chips produced using the purple corncob extract for color at 1.84%. Peak 1- cyanidin-3-glucoside, peak 2- pelargonidin-3-glucoside, peak 3- pelargonidin-3-glucoside, peak 4- cyanidin-3-malonylglucoside, peak 7- cyanidin-3-acetylglucoside, peak 8- pelargonidin-3-acetylglucoside, and peak 9- peonidin-3-acetylglucoside.

5.2 Colorimetric Evaluation

As highlighted previously, color is an important product attribute used to determine quality. Color characteristics of the chips were monitored using the CIELab color space over the six-month storage period to determine if any color changes were occurring in the chips. In addition to these coordinates, the chroma (C) was monitored to provide supplementary information about the chip color vibrancy. Color difference (ΔE), a term which measures the spatial difference of two colors using their Lab coordinates on the 3 dimensional color space of CIELab was also measured. During the storage period, the L, a, and b values of the chips each decreased slightly. A steady decrease was seen in the
chroma from the time zero chips (C = 4.30) to the six-month chips (C = 3.73), though not significant. The color difference between these two samples is 1.71. Previous work suggests that humans, although excellent at identifying differences between two side-by-side samples, cannot accurately determine a difference in color of two samples presented one at a time if the color difference is less than 2. Based on these finding, it is not expected that consumers would be able to consistently detect the difference in color between a freshly made chip and one that was produced six months prior.

**Figure 12.** Average L values of the test chip samples over a period of six months.
Figure 13. Average a and b values of the test chip samples over the duration of a six-month storage study.

To determine how the chips produced with the purple corncob extract compare to commercially available pigmented chips, color characteristics of chips already on the market were tested. The L value of Tostitos organic blue corn chips was 41.95, the a value was 4.97, and the b value was -1.15. Comparing these values to the test chips, it is clear that the Tostitos chips are slightly lighter, significantly redder and bluer. The \( \Delta E \) of the Tostitos and Wyandot chips was 3.01. Another type of chip, Garden of Eatin blue corn chips, had a L value of 42.67, a of 4.39, and b of 0.19. Though these chips were only significantly different in the b value, the color was much less uniform and the standard deviation was high.

5.3 Texture Analysis

Texture of tortilla chips is one of the most important factors in determining overall quality of the chips (Kayacier and Singh 2003). The vocabulary used to describe the
texture of a tortilla chip is extensive, including the surface characteristics, first bite, chewdown, and residual characteristics (Meullenet and others 2002). In this study, only the first bite characteristic of crispness was assessed. In the sensory portion of the test, panelists were asked to hold the chip between their thumb and forefinger and bend the chip until it broke. To mimic the motion of the panelists bending the chips between the thumb and forefinger of each hand, a compression plate probe was chosen. An Instron Texture Analyzer with a compression plate probe was used to measure compressive force at break of the chip.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average compressive load at break (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wyandot White Corn Chips</td>
<td>3.02 ± 0.89</td>
</tr>
<tr>
<td>Tostitos Organic Blue Corn Chips</td>
<td>3.16 ± 1.36</td>
</tr>
<tr>
<td>Purple Corncob Extract Chips</td>
<td>2.87 ± 0.83</td>
</tr>
</tbody>
</table>

As highlighted on Table 3, no significant differences were observed between the samples utilized in this study. This result could be due to the relatively large standard deviations that are seen. An indication that the setup used with these chips may not be the optimal method to measure tortilla chip texture.

5.4 Sensory Evaluation

Sensory testing was conducted to evaluate crispness and color intensity of the chips. Panelists were asked to hold the chip between the thumb and forefinger of each hand and break the chip, simulating the first bite into a tortilla chip, rating their overall liking of the
chip. They were then asked a series of questions about the crispness and the intensity of
the purple color of the chips. The first question was to rate the appropriateness of the
chips’ attribute. Panelists were then asked to rate the specified attribute of the sample
presented to them and what they would consider an ideal example of a tortilla chip with
relation to the specified attribute. Table 4 gives a summary of the samples used during
sensory evaluation.

Table 4. Tortilla chips presented to panelists during sensory evaluation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Chroma</th>
<th>Hue angle</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. White</td>
<td>74.71</td>
<td>1.48</td>
<td>22.11</td>
<td>------</td>
<td>86.17</td>
<td>39.88</td>
</tr>
<tr>
<td>W. Purple</td>
<td>41.1</td>
<td>4.11</td>
<td>0.80</td>
<td>4.03</td>
<td>11.01</td>
<td>------</td>
</tr>
<tr>
<td>Tostitos</td>
<td>41.95</td>
<td>4.97</td>
<td>-1.15</td>
<td>346.97</td>
<td>4.82</td>
<td>3.01</td>
</tr>
</tbody>
</table>

Super scripts denote significance

Overall, the white corn chips were rated higher in the overall liking, though the
difference was not significant. The only significant difference that was seen was between
the Tostitos blue corn and Wyandot White corn chips. Overall liking of the chip samples
showed no significant differences observed between the pigmented chips. The overall
liking of color intensity followed the same pattern; the only significant differences
observed were between the Tostitos and the Wyandot white corn chips. The color
intensity of the Wyandot purple chips was rated as slightly too little, while the Tostitos
chips were rated as having a color intensity that was slightly too much. JAR penalty
scores calculated for these two values were -0.02 and 0.36, respectively.
The human eye-brain combination has been likened to “an almost unsurpassed null detector”, being able to detect the minutest differences in color between side-by-side samples (Seghi and others 1989). However, it has been shown that when two samples, presented in a serial monadic fashion, have a color difference (ΔE) between one and two, panelists cannot accurately determine differences between samples. Comparing the average overall liking of color intensity of the pigmented chip samples presented, no significant differences are noted. Although the ΔE value of the purple corncob extract chip and the commercially available pigmented corn chip was higher than 2, at 3.01, it is possible that the CIELab color measurements of the tortilla chips are not wholly representative of the purple color of the chips. There is a natural color variation expected in tortilla chips, resulting from the rough grind of the corn kernels, which produces a flecked appearance. Even though the chips were ground to a uniform size prior to color analysis, the portions of the kernel that did not absorb the purple corncob colorant would influence the color measurements of the sample. This natural variation would lead to a skewed color measurement and a skewed ΔE of the chips.

The portion of sensory analysis focusing on crispness of the chips unexpectedly showed significant differences between each of the chip samples. Based on the results obtained from textural analysis, it was expected that no differences would be observed between the three types of chips used in the sensory panel. The overall liking of the crispness of the Tostitos was highest, followed by the Wyandot purple chips, and then the Wyandot white chips. The JAR penalty scores for the chips indicated that panelists desired a slightly crisper chip than the three varieties tested. The results from this section
of the sensory panel indicate that the incorporation of the corncob extract into the tortilla chips did not negatively affect consumer perception of the chip crispness. However, the disparity between instrumental and sensory results points out that there are other factors contributing to the consumer perception of crispness than were tested using the Instron Texture Analyzer.
CHAPTER 6

CONCLUSION

Monomeric anthocyanin content remained stable during storage of the tortilla chips. During processing, the monomeric anthocyanin content decreased roughly 79%. This was most likely due to the strong alkaline conditions that the anthocyanins in the purple corncob extract were exposed to when mixed with the masa. Additionally, the high temperatures used in the baking and frying steps of tortilla chip production enhance anthocyanin losses. The percent polymeric color assay was conducted in parallel with the monomeric anthocyanin content quantification to determine the mechanism of monomeric anthocyanin loss. Results from the percent polymeric color assay showed a slight, though not significant, increase in polymeric color during storage. Based on these findings it was concluded that a majority of any anthocyanin losses observed during storage were due to polymerization and not degradation. Total soluble phenolic content was also assayed to monitor losses during processing and storage. As with the monomeric anthocyanin content, significant degradation was seen as a result of processing through the storage period.

The anthocyanin profile of the purple corncob extract showed that the main anthocyanin was cyanidin-3-glucoside with pelargonidin and peonidin-3-glucosides present as well. In agreement with previously published literature (de Pascual-Teresa 2002, Jing and Giusti 2005, Yang 2010), the malonated counterparts of these three
anthocyanins were also observed in the purple corncob extract. In the chip extract, acetylglucoside derivatives of the three anthocyanins were identified in addition to the presence of the cyanidin, pelargonidin, and peonidin-3glucosides and cyanidin-3-malonylglucoside. This conversion from malonyl to acetyl derivatives is a reaction that has not been previously reported in anthocyanins but is seen with soy isoflavones and is thought to arise from the exposure to dry heat (Griffith and Collison 2001).

Colorimetric evaluation of the chips as compared to commercially available chips showed significant differences in the CIELab color space as well as in the chroma and hue angle. Though the color difference observed (ΔE) between commercially available pigmented chips and those produced in this study fell within or close to the range in which humans cannot accurately distinguish color differences between two samples. During the storage period, a general but not significant dulling of the chips was observed in the L*, a*, b* and chroma (C) values. The color difference between freshly produced chips and chips that had been stored for six months was 1.71, a difference that is not expected to be perceivable by consumers. The sensory testing that was completed showed that, as expected, there was no significant difference in preference of consumers between the pigmented chips but that the commercially available chips showed significant differences from the white corn chip that was tested.

In addition to the color, the texture of the chips was measured with an Instron Texture Analyzer, using the compression plate probe to assay the force required to break a chip. No significant differences were noted between the white corn chips, the test chips produced with purple corncob extract, and the commercially available colored chips. There was also no significance noted between the freshly produced and the chips that had
been stored for six months. However, in the sensory panel, significant differences existed between each of the chips in the overall liking of crispness. This is an indication that factors that were not included in the sensory testing play a role in tortilla chip crispness and further testing is required to correlate the sensory and instrumental data.

Overall, the results of this study suggest that purple corncob extract is an excellent source of anthocyanins and that when incorporated into a tortilla chip matrix, these pigments are a stable source of color. Additional research exploring the combination of some of the alternative production methods discussed in combination with the method of incorporating color after nixtamalization of the masa in order to further optimize anthocyanin retention in the tortilla chip matrix.
LIST OF REFERENCES


“Food, Exemptions from Labeling.” Code of Federal Regulations Title 21, Pt. 73, 2007


