HONOR'S THESIS

The Effects of Degree of Ripeness on Phenolic Content and Radical Scavenging Activity of Banana Flesh and Peel

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INTRODUCTION

Fruits and vegetables are well known for their numerous health benefits and essentiality in the diet. Many of their beneficial properties are due to their high vitamin and mineral content. Additionally, fruits have been identified to contain various types of antioxidants. One of the most common fruits, the banana, is rich in minerals such as potassium and iron and contains different types of antioxidants (Kanazawa and Sakakibara, 2000). The banana is one of the most widely consumed fruit across the world and has become a topic of interest in aims to determine the value of its antioxidant properties.

One of the most abundant classes of antioxidants found in the diet is polyphenols. Fruits are one of the main sources of polyphenols. The antioxidant properties of polyphenols may give them the ability to protect against oxidative damage and reduce the risk of developing diseases linked to oxidative stress (Jiménez *et al.*, 2005). The protective effects of polyphenols are explained by their ability to neutralize free radicals and prevent or reduce the effects of reactive oxygen species and reactive nitrogen species. Free radicals are known to cause cell damage leading to the development of various health problems such as cancer, aging, heart diseases, and gastric problems (Saikat *et al.*, 2010). The known ability of polyphenols to reduce these types of cellular damage increases the importance of advocating a healthy diet in hopes to help prevent various health issues from arising.

In recent years, the antioxidant properties of various banana species native to different countries, such as India and Asia, have been studied. These studies looked at total phenolic content, flavonoid content, and diphenylpicrylhydrazyl (DPPH) radical scavenging abilities (Darsini *et al*., 2012; Sulaiman *et al*., 2011; Abbas *et al*., 2012, Someya *et al.*, 2002; Kanazawa and Sakakibara, 2000). Previous studies have reported the banana peel to have higher antioxidant

content than the fruit (Abbas *et al.*, 2012; Sulaiman *et al.*, 2011; Someya *et al.*, 2002; Kanazawa and Sakakibara, 2000). The study conducted by Abbas and colleagues (2012) in Malaysia investigated the effects of both variety and ripeness on the content of locally grown bananas, the Dream and Cavendish cultivars. This particular study focused on two stages of ripeness, green and ripe. They found the green bananas to contain greater phenolic content than the ripe bananas (Abbas *et al.*, 2012). Since previous studies have examined the antioxidant content and radical scavenging abilities of locally cultivated bananas, it was of interest to determine if the content would differ in the bananas imported to the US market (Stillwater, Oklahoma).

The purpose of the current study was to determine the phenolic content and radical scavenging abilities of the fruit and peel of imported Cavendish bananas available in the local grocery store (Stillwater, Oklahoma) and to compare to the results of previous studies conducted in the native areas of the Cavendish banana. Also, in order to further investigate the effects of ripeness on antioxidant content and radical scavenging activity, three stages of ripeness on the banana fruit and peel were examined: unripe (green peel), ripe (yellow peel), and over-ripe (brown peel). We hypothesized that the phenolic content and radical scavenging properties would increase with ripeness and be greater in the peels than the pulps, but overall would have lesser values compared to the bananas studied in their native countries as reported in the literature.

METHODS

Sample Preparation

A total of thirty Cavendish bananas were purchased from the local grocery store (Wal-Mart Stillwater, Oklahoma). All of the bananas were of green coloring and under-ripe. For ten of

the green bananas, the peels and flesh were immediately separated and placed into separate freeze-drier containers, frozen at -80ºC, and freeze-dried. These steps were repeated with the next group of ten bananas once they had ripened and became yellow in color and with the last group of ten bananas after they were allowed to ripen until they became brown in color. Ripeness was determined by both peel color and softness of the banana. Once all the peel and flesh were freeze-dried, it was ground into fine powder, poured into individual bags and returned to the -80ºC freezer.

Twenty five grams of each flesh and peel freeze-dried powder were extracted with 125 mL of methanol, placed into a shaking water bath set at 25ºC for 48 hours. After extraction, each solution was centrifuged three times at 9000 rpm, the supernatants were removed, and the methanolic extract was freeze-dried. Each dried methanolic extract was dissolved in 500 µl of methanol followed by 1500 µl of water, aliquoted, and stored in a freezer until used for analyses.

Determining Total Phenolic Content

A stock solution of gallic acid (1 mg/mL; Sigma Chemical, St. Louis, MO) in 25% methanol was prepared. A standard curve was prepared (0, 0.1, 0.2, 0.5, 0.75 mg/mL) from the stock gallic acid solution and 25% methanol as the diluent. Each flesh extract was diluted 1:10 while the peel extract was diluted 1:20 using 25% methanol. An aliqout (50 µl) of each standard, flesh, and peel extract was diluted with 1500 µl of distilled water. To this mixture, 250 µl of Folin-Ciolcalteu reagent was added. After 3 minutes, 1000 µl of 20% sodium carbonate was added and mixed thoroughly. The tubes were incubated in a boiling water bath for 1 minute then cooled to room temperature. The absorbance was measured at 650 nm against the reagent blank using the Synergy HT spectrophotometer (Biotek, Winooski, VT). Total phenolic content was

determined from the standard curve and expressed as milligrams gallic acid equivalent (GAE) per 100 g of fruit or peel.

Determining Flavonoid Content

A stock solution of catechin (80 mg in 15 ml 25% methanol) was prepared. A standard curve was prepared (0, 53, 106, 265, 530, 1060 µg/ml) from the stock catechin solution and 25% methanol as the diluent. The flesh and peel extracts were diluted 1:20 dilution using 25% methanol. To 100 µl of each standards and samples, 500 µl of distilled water and 50 µl of 5% $NaNO_2$ were added. After 6 minutes, 75µl of 10% $AlCl_3 \cdot 6H_2O$ was added. After 5 minutes, 250 µl of 1.0 M NaOH and 275 µl of distilled water were added. Immediately following this step, the absorbance was read against a reagent blank at 510 nm using the Synergy HT spectrophotometer (Biotek, Winooski, VT). Flavonoid content was determined from the standard curve and expressed as milligrams of catechin per 100 g of fruit or peel.

Determining DPPH Radical Scavenging Activity

A stock solution of butylated hydroxytoluene (BHT; 10 mg/ ml) was prepared using 25% methanol as solvent. A standard curve (0, 0.5, 1.0, 2.5, 5.0, 7.5, 10 mg/mL) was prepared from the stock BHT solution using 25% methanol as diluent. Each flesh extract was diluted to a 1:20 while each of the peel extract was diluted to a 1:10,000 using 25% methanol as diluent. Each standard concentration and sample dilution (10 µl) were pipetted into the 96-well plate followed by addition of 185 µl of 0.1mM DPPH. The plate was shaken at 300 rpm for 5 minutes at room temperature. Immediately following this step, the absorbance was read against a reagent black at 550 nm using the Synergy HT spectrophotometer (Biotek, Winooski, VT). DPPH radical scavenging activity was determined from the standard curve and expressed as milligrams of BHT equivalent per 100 g of fruit or peel.

Statistical Analysis

The results were expressed as mean ± standard deviation. Data were analyzed by one-way ANOVA (Analysis of Variance) using SAS version 9.1 (SAS Institute, Cary, NC). Difference in mean values were considered significant when $P < 0.05$.

RESULTS

Total Phenolic Content

Table 1 show the results for total phenolic content represented as mg gallic acid equivalent (GAE)/ 100 g dry weight for both the banana fruit and peel. The average total phenolic content ranged from 13.89 ± 4.3 to 120.5 ± 51.6 mg GAE/ 100 g dry weight. When comparing all the groups (flesh and peel as well as varying degree of ripeness), the yellow peel had the highest while the unripe flesh (with green peel) had the lowest total phenolic content. The yellow and brown peels have statistically similar total phenolic content and significantly higher than every other group. When comparing the degree of ripeness of the flesh, the over-ripe flesh (with brown peel) was significantly greater than the ripe (yellow peel) and un-ripe (green peel) flesh. The ripe flesh (with yellow peel) and un-ripe flesh (with green peel) have statistically similar total phenolic content. There is no effect of degree of ripeness on the phenolic content of the banana peels.

Flavonoid Content

Table 1 also show the results for flavonoid content and represented as mg catechin/ 100 g dry weight. The average flavonoid content ranged from 5.33 ± 0.3 to 178 ± 0.01 mg catechin/ 100 g dry weight. When comparing all the groups (flesh and peel as well as varying degree of

ripeness), the yellow peel had the highest while the ripe flesh (with yellow peel) had the lowest flavonoid content. All of the peels had significantly higher flavonoid content than the flesh ($p < 0.0001$) and the yellow peel was significantly higher than all other groups ($p < 0.0001$). When comparing the degree of ripeness of the flesh, the flavonoid content of the over-ripe flesh (with brown peel) was significantly greater than the ripe flesh (with yellow peel). The ripe flesh has flavonoid content that was statistically similar to the unripe flesh (with green peel). When comparing the degree of ripeness of the peels, the flavonoid content of the yellow peel was statistically higher than both the green and brown peel. The green peel had the lowest flavonoid content.

DPPH Radical Scavenging Activity

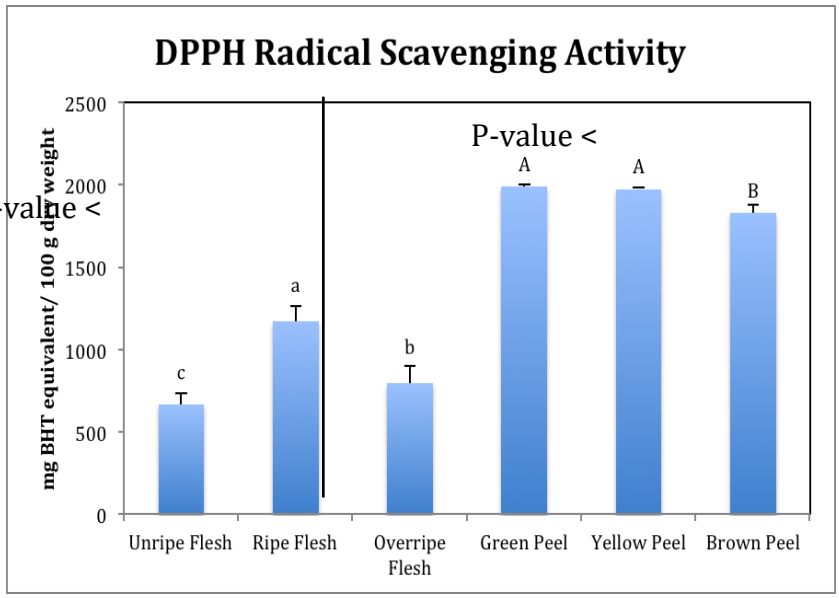
Figure 1 show the results for DPPH radical scavenging activity and represented as mg BHT equivalent/ 100 g dry weight. The average DPPH radical scavenging activity ranged from 667.1 ± 67.2 to 1989.7 ± 14.6 mg BHT equivalent/ 100 g dry weight. When comparing all the groups (flesh and peel as well as varying degree of ripeness), the green peel had the highest while the unripe flesh (with green peel) had the lowest DPPH radical scavenging activity. All the peels had statistically similar DPPH radical scavenging activity and were significantly greater than the flesh. When comparing the degree of ripeness of the flesh for its DPPH scavenging activity, all three were statistically different from each other with the ripe flesh having the highest and the unripe flesh having the lowest. When comparing the degree of ripeness of the peels, the DPPH radical scavenging activity of the green peel and yellow peel were statistically similar and significantly greater than the brown peel.

Table 1: Total Phenolic and Flavonoid Content of Flesh and Peels

Measures	**Flesh**				**Peel**				Overall P value*
	Unripe (Green)	Ripe (Yellow)	Over-ripe (Brown)	P value	Unripe (Green)	Ripe (Yellow)	Over-ripe (Brown)	P value	
Total Phenolic (mg GAE/100 g dry weight)	13.9 ± 4.1^{b}	22.7 ± 6.7^{b}	43.0 ± 18.8^{a}	0.0009	46.7±2.1	120.5±51.6	97.8±19.5	0.2049	<0.0001
Flavonoid (mg catechin/ 100 g dry weight)	5.3 ± 0.3^{b}	5.0 ± 0.5^{b}	5.7 ± 0.1^{a}	0.014	91.4 ± 0.07^{C}	178.0 ± 0.01^{A}	157.8 ± 0.4^{B}	<0.0001	<0.0001

Values represent mean ± SD. Values within a row with different letters (small and capital letters compare degree of ripeness of the flesh and peel, respectively) are statistically different ($P < 0.05$). * represents P-value comparing the flesh and the peel and the degree of ripeness (all six groups). GAE= gallic acid equivalent.

Figure 1: DPPH Radical Scavenging Activity of Flesh and Peels



Bars represent mean ± SD. Bars marked with different letters (small and capital letters compare degree of ripeness of the flesh and peel, respectively) are statistically different ($P < 0.05$). DPPH, diphenylpicrylhydrazyl and BHT, butylated hydroxytoluene.

DISCUSSION

The first objective of our study was to determine the phenolic content of the flesh and peel of Cavendish banana imported to our local grocery store and to compare our findings to those reported in the literature of locally cultivated bananas. Consistent with those reported in the literature, our findings showed that the peel had higher total phenolic content, flavonoid content, and DPPH radical scavenging activity than the flesh (Abbas *et al.*, 2012; Sulaiman *et al.*, 2011; Someya *et al.*, 2002; Kanazawa and Sakakibara, 2000). In support of our hypothesis, our reported values for total phenolic content and flavonoid content are quite low in comparison to those reported in the literature on the Cavendish cultivar of the banana. Abbas *et al.* reported the total phenolic content of the green and ripe peels to be 685.6 and 585.3 mg GAE/ 100 g dry weight while we found it to be 46.7 and 120.5 mg GAE/ 100 g dry weight. The literature reported the banana flesh to have total phenolic content ranging from 122.02 to 373.88 mg GAE/ 100 g dry weight (Darsini *et al.*, 2012; Abbas *et al.*, 2012) while we found it to be 13.9 to 43.0 mg GAE/ 100 g dry weight. Abbas *et al*. reported the flavonoid content of the peels as 225.9 (ripe) and 389.3 (green) mg catechin equivalents and the fruits as 196.5 (ripe) and 281.2 (green) mg catechin equivalents. We found the flavonoid content of the peels and flesh to range from 91.4 to 178.0 and 5.0 to 5.7 mg catechin/ 100 g dry weight. Our low values could be attributed to variations in extract preparation or, possibly, storage conditions necessary for importing the bananas. For example, Nguyen *et al* found the total phenolic content of banana peel to decrease over time while stored at low-temperatures. Additional research on the effect of methods taken during importation on phenolic content of bananas could further identify possible causes of decreased phenolic content.

The second objective of our study was to determine the effect of ripeness on phenolic content and radical scavenging activity of banana flesh and peel. In general, we found the antioxidant properties to be greater in the ripe and overripe flesh and peels than the unripe flesh and peel. Specifically, the yellow peel had higher total phenolic content and flavonoid content than both the green and brown peels. The yellow peel also had greater DPPH radical scavenging activity than the brown peel and only slightly less than the green peel. Our results are not in agreement with the findings reported by Abbas *et al*. They studied two degrees of ripeness, green and ripe, and reported the green flesh and peel to generally have higher antioxidant properties than the ripe flesh and peel (Abbas *et al.*, 2012). Our only similar finding was in the case of the DPPH radical scavenging activity of the peels. The green peel had significantly higher DPPH radical scavenging activity than the brown peel though it was statistically similar the yellow peel. Perhaps these differences could be attributed to dissimilarities in extract preparation.

Due to time restriction, we only conducted one method for assessing radical scavenging activity. Conducting additional assays such as ferric reducing power and hydroxyl radical scavenging activity could have strengthened our study and allowed us to more precisely determine the effect of ripeness on the antioxidant capacity of the banana fruit and peel. The ferric reducing power assay provides indication of the antioxidants' strength as reducing agents by measuring the ability of the antioxidants to stabilize free radicals through donation of electrons (Darsini et al., 2012). The hyrdoxy radical scavenging assay would help us determine the ability of the antioxidants to destroy hydroxyl radicals, which are extremely reactive and damaging to living cells (Darsini et al., 2012).

An important next step for this study would be to use animal models to investigate the *in vivo* effects of these banana fruit and peel extracts and study the mechanism by which they affect

animal health, specifically to examine the effect on a disease state. Eventually, a clinical study could look at the effect of banana antioxidants on disease state such as cancer or cardiovascular disease. Our findings suggest that the most benefit may be received from bananas that have ripened. Though the peel of bananas is not commonly consumed, perhaps it is necessary to find a way to consume the peel due to its greater antioxidant properties as compared to the fruit of the banana. For example, the benefits of the banana peel could be received through supplement form or by including the peel in a blended smoothie. Overall, consumption of bananas, along with other fruits and vegetables, should continue to be encouraged in order to promote a healthier society.

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