Antioxidant Properties and Hypoglycemic Potential of Genomically Diverse Bananas Cultivated in Southeastern United States

by

Gabriela A. Hernandez

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 1, 2015

Keywords: *Musa*, ethephon, physicochemical, ripening, maturity, antioxidant

Approved by

Floyd Woods, Chair, Associate Professor of Horticulture
Elina Coneva, Extension Specialist and Associate Professor of Horticulture
J. Raymond Kessler, Jr., Professor of Horticulture
Esendugue Greg Fonsah, Professor and Extension Specialist of Agriculture and Applied Economics, University of Georgia, Tifton Campus, Tifton, GA 31793

Abstract

There has been increased interest in growing and selecting cold-hardy short-season cultivars to offer an alternative to the industry standard, the Cavendish (genome AAA). In addition to expansion of production, these specialty cultivars have advantages such as increased nutritional qualities, resistance to disease, and favorable postharvest attributes. The determination of suitable alternatives to the Cavendish subgroup is a relatively new concept; therefore very little research has been done regarding the postharvest and nutritional properties of these specialty cultivars.

The goal of the first experiment was to determine the effect of common postharvest practices and length of storage on the quality and nutrition of specialty bananas grown in the southeastern US. The objective of the second experiment was to determine the effects of fruit maturity stage on antioxidant properties of short-season cold-hardy cultivars. Results from both experiments indicate that genotype had role in determining fruit physicochemical and antioxidant properties. Results of this study will assist banana producers and commercial retailers in selecting adaptable cultivars, optimal ripening stage and best management practices to enhance quality and nutritional content of short season banana cultivars adaptable to southeastern United States.

Acknowledgements

First, I would like to thank Dr. Floyd Woods for giving me the opportunity to continue my education and pursue a career in post-harvest physiology. Without your training, advice, encouragement, and patience, none of this would have been possible. Thank you for all of your wisdom and positivity, and for always reminding me of the importance of faith and family. I would also like to thank the members of my committee, Dr. Elina Coneva, Dr. J. Raymond Kessler, and Dr. E. Greg Fonsah for all of your help, support, and insight. Special thanks to the Alabama Nut, Fruit, and Vegetable Industries for making this project possible. I'd like to give a huge "thank you" to Nicholas Larsen for providing our samples during the second year of our trial and to Dr. Roland Dute for allowing me to use your laboratory camera equipment. Thank you Michael Barnhill, Camille Crosby, Jessica Bryars, and Nylan Holmes for all of your help in the laboratory; I could not have done this without you.

I'd like to give a special thanks to my parents, Francisco and Idil Hernandez, for your unwavering support and encouragement and for setting such a great example. I'd also like to thank my brother Francis, who I was very lucky to have here with me these past two years. You have no idea how much of a difference you made.

And finally, I'd like to extend my sincerest gratitude to Dr. Elizabeth Varriano-Marston, Dr. James Finn, and "Maggie". Thank you, Dr. Marston, for inspiring my interest in post-harvest physiology and for giving me the confidence to further my

education. Without your guidance, encouragement, generosity, and support, I would not be the person I am today. Thank you for always believing in me.

Style manual or journal used:
HortScience: A Publication of the American Society for Horticultural Science

Computer Software used: Microsoft Word 2011, Microsoft Excel 2011, SAS v 9.3 and SigmaPlot 13.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AA	Ascorbic acid, reduced form
ABTS	2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid)
ACP	African, Caribbean and Pacific Group of States
C	Celsius
°	Degrees
DHA	Dehydroascorbic acid, oxidized form
DPPH	2,2-diphenyl-1-picrylhydrazyl
EU	European Union
FHIA	Fundación Hondureña de Investigación Agrícola
FRAP	Ferric reducing ability of plasma
FW	Fresh weight
g	Gram
GAE	Gallic acid equivalent
GAEAC	Gallic acid equivalent antioxidant capacity
gdw	Grams dry weight
gfw	Grams fresh weight
HAT	Hydrogen atom transfer
kg	Kilogram
L	Liters
lb	Pound
m	Meters
MDA	Malonydialdehyde
meq	milliequivalents
MG	Mature green maturity stage
1-MCP	1-methylcyclopropene
mg	Milligram
mL	Milliters
MPA	m-phosphoric acid
OCA	Overall cosmetic appearance
OR	Over ripe maturity stage
%	Percent
PAL	Phenylalanine ammonia lyase
PCR	Polymerase chain reaction
PPO	Polyphenol oxidase
R	Ripe maturity stage

RCI	Ripening Color Index
RH	Relative humidity
SET	Single electron transfer
SSC	Soluble solids content
SSH	Suppression subtraction hybridization
TA	Titratable acidity
TCA	Trichloroacetic acid
TE	Trolox equivalent
TP	Total phenolics
TPTZ	Tripyridyltriazine
TR	Transitional maturity stage
US	United States
VCEAC	Vitamin C equivalent antioxidant capacity

I. Literature Review

BACKGROUND

The banana (*Musa* spp.) is one of the most popular tropical fresh fruit produced for world market trade, comprising approximately 16% of total fruit production (Mohapatra *et al*., 2010). Bananas have low fat and sodium and are high in carbohydrates, antioxidants, minerals, and vitamins A, B6, and C. The health benefits of bananas are associated with reduction in cardiovascular dysfunction, muscular degeneration, intestinal disorders, and chronic disease. Bananas can be eaten fresh, consumed in an assortment of value-added products, minimally processed (i.e. pureed, fruit juices, or dried as chips) and used for medicinal and culinary purposes when used as unripe ingredients. Globally, bananas are recognized as the second largest fruit produced following citrus, due to health benefits, nutritional properties, culinary versatility, and consumer appeal (Fonsah and Chidebelu, 2012).

"Banana" is a general term referring to a number of species or hybrids in the genus *Musa* of the family *Musaceae*. Most cultivated bananas are hybrids between two wild species *Musa acuminata* of the A genome *Musa balbisiana* of the B genome and either diploid, triploid, or tetraploid (Lescot and Staver, 2010; Robinson, 1996). Bananas have been reported as one of the first cultivated plants and are native to southern Asia (Slabaugh and Grove, 1982). The tropical regions in this area are conducive to the growth and production of this crop. Optimum growth temperature is

26 to 28˚C for shoots 29 to 30˚C for fruit, and 20˚C for dry matter accumulation (Hailu *et al*., 2012). The minimum temperature that allows growth is 14˚C. The plant requires 30 to 40 weeks of irrigation, otherwise flowering can be delayed and the fingers, or individual fruit, can be underdeveloped. The fruit is usually harvested at the fully matured green stage. The age of the bunch, period between flowerings and harvesting, filling of the fingers, color of the peel and pulp, and texture of the flower are used to indicate maturity. These criteria vary among cultivars, as genetic makeup can influence various factors such as nutrition and reaction to postharvest treatment. However, very little is known regarding the exact processes that are responsible. The lack of information regarding fruit sensitivity to postharvest practices such as ethylene degreening makes it imperative to determine the impact of ethylene treatment and maturity stage on nutritional determinants and consumer quality on genomically diverse cultivars.

BANANAS IN THE MARKET

Global production of bananas and plantains are commercially cultivated in more than 130 countries primarily in tropical and subtropical regions. In 2012, global production of fresh unprocessed banana was estimated to be in excess of 101,992,743 tons, of which approximately 24.4% were from India, 10.63% from China, and less than 0.008% from the United States (Fig. 1, Appendix B). In 2012, banana exports were in excess of 16.5 million tons, representing a 7.1% increase from 2011. In 2012, exports from South America declined 6.4% while exports from Central America and Mexico increased. This was likely a response to the Association Agreement, a treaty

mandating that African, Caribbean, and Pacific (ACP) countries were given priority for banana export to the European Union (EU). A tariff-only regime that eliminates the quota of banana exports for Latin American and ACP countries has been implemented to mend the relationship between Latin America and the EU. In other words, while this tariff-only regime is anticipated to reduce the prices of bananas from Latin America, it hurts ACP countries, which heavily rely on banana exports. As a result, Costa Rica and Guatemala's exports exceeded those of Columbia, formerly the second largest exporter in the world (FAOSTAT 2014; Reynolds-Allie, 2013).

Increased interest in organic and fair trade bananas along with climactic disasters has caused a reduction in banana exports from Caribbean countries. The only country in which the banana remains a primary export commodity is the Dominican Republic. Even so, between 2011 and 2012, banana exports from the Dominican Republic declined by 2.2%. Omitting the Dominican Republic, the combined exports from Caribbean countries decreased significantly from 88,000 to 12,100 tons. Banana exports from Asia reached a record of 2.82 million tons, with over 93% coming from the Philippines. This makes the Philippines the second largest banana exporter in the world, Ecuador being the first (Fig. 2, Appendix B) (Banana Market Review and Banana Statistics 2012-2013, 2014).

Bananas are the most popular fresh fruit consumed in the United States. During the past decade, fresh unprocessed banana is considered the leading tropical agriculturally exported commodity to the United States with a value of approximately $5 billion per year (FAOSTAT, 2003; Huang and Huang, 2007). However, there is limited capability to commercially cultivate traditional cultivars of bananas

domestically due to environmental and climacteric constraints. Commercial production of bananas mainly occurs in Hawaii, which only allows 1,200 to 1,500 acres for planting (Su *et al*., 2011). Hawaii's annual commercial production allotment unfortunately is not sufficient to meet domestic market demand, stimulating international import estimated between 3,800 and 4,100 tons of fresh bananas per year. The United States is the largest banana importer in the world, with an annual market value estimate in excess of $1 billion annually (Fonsah *et al*., 2007; Muhammed *et al*., 2015; Su *et al*., 2011). During the period of 1990 to 2012, United States banana imports have increased by over 1.4 million tons (Fig. 3, Appendix). The United States accounted for 27% of all banana imports in 2012 (Banana Market Review and Banana Statistics 2012-2013, 2014). Over 95% of the United States' fresh banana imports come from Latin American countries including Columbia, Costa Rica, Ecuador, Guatemala, and Honduras. Dole Food Company, Chiquita Brands International, and Fresh Del Monte Produce are the three primary global corporations that provide market dominance for fresh and minimally processed bananas (Muhammed *et al*., 2015; Su *et al*., 2011). Since the early 1960's, bananas imported for domestic US market are primarily of the Cavendish (AAA) subgroup, which are traditional long-season bananas and are not commercially cultivated in non-tropical environments. Due to disease, climate, and nutritional concerns, there has been increased interest in searching for suitable alternatives to the industry standard.

PREHARVEST DISEASES

There are two major preharvest diseases that affect bananas. Panama disease (Fusarium wilt) is caused by *Fusarium oxysporum* f. sp. *Cubense* and Sigatoka is caused by *Mycosphaerella musicola*. The three races of Panama Disease that affect bananas are races 1, 2, and 4. Race 4 is the most devastating because it affects cultivars that are susceptible to races 1 and 2 as well as Cavendish cultivars. Symptoms of Panama disease start in the feeder roots and move on the rhizomes. They then accumulate where the stele meets the cortex and take over the pseudostem, resulting in slight brown streaks or spots in the older leaves. Next, the xylem changes color to red or brown. The older leaves lose chlorophyll and turn yellow, begin to split, and eventually all of the leaves become necrotic (Ploetz, 2000).

Panama disease was first reported in Australia in 1876 and now affects almost all banana-producing regions. It is responsible for the significant loss of common industry cultivars and field devastation. For example, 30,000 hectares were lost to Panama disease in Honduras between 1940 and 1960. Other areas significantly affected include Suriname and Costa Rica. While 'Gros Michel' (AAA) was the dominant cultivar in the market, damage due Panama disease resulted in direct losses of millions of dollars. A similar trend was observed in Cameroon in the 1930's where all 'Gros Michel' bananas were replaced as the industry standard by other Panama Disease-resistant cultivars (Fonsah and Chidebelu, 2012; Ploetz, 2000).

Yellow Sigatoka was the most destructive foliar disease until the spread of black Sigatoka. It was first reported in 1902 and became a global epidemic for the next 40 years. Sigatoka is considered the most detrimental disease because it accounts for over 38% yield loss on plantain and controlling it compromises more than one

quarter of the total production costs. If left uncontrolled, bananas infected with Black Sigatoka will show symptoms in their foliage. Their symptoms appear in six stages: white or yellow mark on the lower surface; red or brown spot on the lower surface; red or brown streaks on both surfaces; streak increases in diameter and color turns to dark brown; black, sunken lesion with yellow "chlorotic" halo; center of the spot changes color to white or gray (Fonsah and Chidebelu, 2012; Marín *et al*., 2003).

Though there are methods for control of these diseases, they have proven to be insufficient. Some of these methods include sanitation and eradication, while other methods are more extreme. Before it was phased out in 2005, fumigation with methyl bromide (100 $g \cdot m^{-2}$) combined with solarization resulted in a noticeable reduction in Panama disease, however the treatment was not a long-term solution (Herbert and Marx, 1990). Fungicides can be used for Sigatoka, however when Panama disease is discovered in a plot, often times growers will move their crops to a new land or flood fallowing. With the increased interest in organic foods, consumers have become increasingly wary of the use of pesticides and other conventional methods of disease control. Another challenge is that even with a disease-resistant cultivar, when there is a monoculture, chances of pathogen mutating and adapting increase and resistance decreases. Therefore, it is important to continue developing multiple disease-resistant cultivars to limit the need for chemical control.

BANANA CULTIVARS

Until 1960, 'Gros Michel' was the most popular variety due to qualities such as scarring and bruising resistance, which allowed them to be shipped while still

attached to their stems (Slabaugh and Grove, 1982). 'Gros Michel' was eradicated by Panama Disease and was therefore replaced by the Cavendish subgroup of the triploid (AAA) *M. acuminata* variety. Typical Cavendish varieties include 'Giant Cavendish', 'Grand Naine', 'Williams', 'Robusta', and 'Dwarf Cavendish'. All Cavendish cultivars, with the exception of the dwarf varieties, are nearly identical to each other and are grown in areas according to climate preference. For example, 'Williams' fruits are more tolerant of wind and can grow in cooler environments while 'Robusta' fruits are less sensitive to water stress. Most Cavendish banana growth is limited to the tropical region and is optimal at 27 to 30°C (Newley *et al*., 2008).

Because Cavendish cultivars are long cycle bananas, there is limited production in the United States due to climate restrictions. Currently, work is being conducted to develop cold hardy and short cycle bananas that can withstand the colder and harsher temperatures of Northern Florida, Georgia, and Alabama. Recent studies suggest that growers have the capability of growing bananas in subtropical regions in the southeastern parts of the United States (Fonsah *et al*. 2007; 2010; 2011).

Florida contains a climacterically favorable environment for banana growth, however most of the fruit grown are consumed locally. Though 'Cavendish' (AAA) cultivars have the ability to grow in Florida and are resistant to Panama disease, they are susceptible to Sigatoka disease. 'Dwarf Cavendish' (AAA) is a highly successful cultivar grown in Florida, however it is susceptible to Black Sigatoka disease. Because 'Williams' (AAA) is a Cavendish cultivar that is tolerant to cool subtropical areas, it is a favorable industry standard for the United States. In addition, 'Williams'

bananas are less susceptible to cigar end rot and have good tolerance to wind (Crane *et al.,* 2006).

'Lady's Finger', also known as 'Pome', 'Brazilian', and 'Prata' is of the Musa AAB group. However, there are also reports claiming that 'Brazilian' cultivars are from the Musa AAA Group (Crane *et al*., 2006; Liu *et al*., 2009). 'Ele Ele' (AAB) or "black-black" contains a black or burgundy trunk, leaf stalks, and midribs. Its fruit is large and often consumed after cooking. 'Hua Moa' (AAB) is a popular cultivar in Florida although it is susceptible to Panama disease. Its fruit are unusually large and can be consumed fresh or cooked (Crane *et al.,* 2006; Ploetz *et al*., 2007).

The Fundación Hondureña de Investigación Agrícola (FHIA) Breeding Program in Honduras was initiated in 1959 to develop a 'Gros Michel' AAA type banana that is resistant to Panama disease. Many bananas in the FHIA program have been bred to be resistant to disease, highly productive, seedless, and have favorable taste. Their hardiness allows them to be grown in a wider variety of climates and perform well in Africa, Latin America, and Australia. 'FHIA 01', also known as 'Goldfinger', is a tetraploid variety of the AAAB group. It is a dessert banana that is resistant to both Panama disease and Black Sigatoka ("Banana and Plantain Program,") (Crane *et al*., 2006). When stored in 21˚C, FHIA-01 fruit have been reported to last up to 15 days. Total soluble solids and acidity increased throughout storage at 21˚C while pH and firmness decreased. Total soluble solids remained fairly constant at ~4-7 ˚Brix until day 11, then showed a significant increase at days 13 and 15 (~19 and ~23 ˚Brix, respectively). Acidity increased steadily while remaining at below 10 meq $100g^{-1}$ fresh weight (FW) throughout storage until day 11, then peaked

on day 13 (~20 meq 100 gfw^{-1}) and slightly decreased down 10 ~18 meq 100 gfw^{-1} by day 15. Throughout the 15 day period, pH gradually decreased from 5.5 (initial) to ~4.3 (final). Firmness remained fairly constant at ~50 N, then began to decrease linearly on days 13 (~30 N) and 15 (~5 N) (Gutiérrez-Martínez *et al*., 2015).

Professor Esendugue Greg Fonsah *et al*., 2007, conducted a three-year study evaluating the production of several cold-tolerant and short-cycle cultivars in Savannah, Georgia (Latitude 32.133°N, 81.2°W, average temperature 24.95 – 13.18°C, daily average 18.9°C). Data was collected on planting-to-shooting time, shooting-to-harvest time, bunch emergence, and bunch quality. Marketability was measured by cosmetic appearance (OCA), size, finger length, and curvature. In the first year, plants were grown on pine-bark mulch using drip irrigation. Because fertilizer was applied manually, it remained on top of the mulch in drought periods and did not reach the plants. The combination was not effective, as only three plants produced bunches after 25 weeks. Two of the plants that produced bunched were of the 'Kandarian' cultivar. Trials were more successful in 2004 when they switched to a solid-set under-tree irrigation system with sprinklers. However, there was variability throughout the five replications. 'Musa 1780' produced the first bunch in May, 13 months after planting. Additional production occurred in 'Brazilian,' 'Orinoco,' 'Dwarf Namwah,' and 'Ice Cream.' In November 2004, 'Musa 1780,' 'Manzano,' 'Sweet Heart,' and 'Raja Puri' cultivars had growth. In 2005 and 2006, each plant was fertilized with 2.4 lbs of 10-10-10 and 0.78 lbs of muriate of potash. In August of 2005, fifteen bunches emerged from 'Manzano,' 'Raja Puri,' 'Dwarf Namwah,' 'Sweet Heart,' 'Belle,' and 'Musa 1780' plants. Overall, the best results were in 2006

when 83 plants produced bunches. Fonsah *et al*. (2007) concluded that 'Dwarf Namwah,' 'Ice Cream,' 'Kandarian,' 'Sweet Heart,' and 'Belle' had excellent potential for marketing. In the climates of the southeastern United States (USDA Zones 8a and 8b), bananas are physiologically mature 10 to 12 weeks after flower emergence and suitable bunches of these specialty cultivars have a market window from October 15 to November 15. It was suggested that to harvest bunches before mid-November, or the first frost of the season.

Because it has been proven that banana production can be expanded to areas of the southeastern part of the United States, the next step is to determine optimal techniques for fruit distribution throughout the country while maintaining quality by the time it reaches the consumer. At least six hands, or clusters of fingers, need to be present per bunch for them to be harvested, packaged, distributed, and marketed. This excludes the one or two false bunches, which are removed during harvest. A commercial plantation is considered well managed if it produces an average of nine hands per bunch. Growers will typically harvest bananas at the mature green stage to ensure that they do not over-ripen before reaching the market (Fonsah *et al*., 2003; 2007).

GENOME VARIABILITY

In addition to shorter growing seasons, these specialty cultivars have a variety of advantages. Recent studies comparing genomic diversity of banana cultivars emphasize the variations in pH and titratable acidity (as malic acid) among genomic cultivars and among postharvest ripening stages. However, there is no definitive

research explaining the nature of these differences (Bugaud *et al*., 2013; Chacon *et al*., 1987). It has also been found that bananas with the AAB genome contain higher vitamin C content than bananas with the AAA genome, which includes Cavendish cultivars (Wall, 2006). The reduced vitamin C content in Cavendish bananas (AAA) is due to their higher moisture content when compared to AAB and AA banana cultivars (Wenkam, 1990). In other words, higher moisture in the fruit results in diluted vitamin content. There are few studies reporting physicochemical and nutritional properties of genomically diverse cultivars.

PHYSICOCHEMICAL PROPERTIES

The banana is one of the most popular fruits in the world and the most widely consumed fruit in the United States. There are several sensory attributes that consumers select for when purchasing. These include yellow peel color, pulp and peel color uniformity, firmness, ripe taste, sweetness, and overall flavor. The most accurate way to determine and compare acceptability is usually with a consumer panel, in which a designated group of people performs a taste test and each individual rates the aforementioned criteria as objectively as possible. For example, the consumers may rate certain qualities such as firmness, taste, color, and overall likeability on a numbered scale. The individuals may either be trained for these tests or chosen at random. However, no matter how objective the panel tries to be, there is still a degree of subjectivity (Salvador *et al*., 2007). Though they are not as accurate in determining consumer preferences, there are certain methods and technologies to predict acceptable consumer qualities using quantitative data.

Peel Color

There are several color models used for measuring the color of food surfaces. The HSB model is based on the hue, saturation, and brightness of an object. Hue is the color that is reflected from an object and is measured based on its location on the color wheel (360º). Saturation, or Chroma, analyzes how pure the color is and is measured on a 0% (gray) to 100% (white) scale. Brightness determines the lightness or darkness of a color on a scale of o% (black) or 100% (white). The RGB (red, green, and blue) model uses transmitted light to show colors. Specific combinations of these three colors can be used to create cyan, magenta, and yellow. Also, red, green, and blue are additive colors meaning they can combine to create white. These colors are used for everyday technologies such as television and computer screens. The CMYK (cyan, magenta, yellow, black) model is used for the visual effect of white light hitting ink printed on paper. Cyan, magenta, and yellow can combine to form black, making them subtractive colors (Hunter Associate Laboratory, Inc., 2000; Yam and Papadakis, 2004).

The CIE L*a*b* color model is device-dependent model that creates a consistent color. L^* defines lightness from 0 (black) to 100 (white); C defines Chroma ($C^* = a^2 + b^2)^{½}$; a^* denotes the green/red value where $-a^*$ is green and $+a^*$ is red; and b^* represents the blue/yellow value where $-b^*$ is blue and $+b^*$ is yellow. The color wheel spans 360˚, and hue (h^*) values refer to the relation of the L^*, a^*, and b^* values to each other (Adobe Systems Inc., 2008).

Certain technology has been developed to quantify color using the aforementioned scales. A colorimeter is an apparatus that measures the color composition of a small area of an object. The colorimeter contains a variable wavelength light filter with a light-receiving surface. The colorimeter releases a flash of light and the light-receiving surface receives the reflected light beam and diffuses it into a range of wavelengths. The color content is measured and recorded into a colorimeter-based computer system (Vincent, 1993).

Bananas designated for commercial use are harvested at the mature green stage. Since bananas are climacteric fruit, they continue to ripen after harvest and have a respiratory peak (production of CO_2) during storage (Ramaswamy, 2014). Peel color is used to predict ripeness by a change in color from green to yellow. Using the CIE $L^*a^*b^*$ scale, this can be corresponded with increases in a^* and b^* values and decreases in h^* values. In other words, the chlorophyll breakdown during storage causes the peel to lose its green color and develop yellow and red tones. Reported peel color values for mature green *M. cavendish* (AAA) bananas were -14.87 (a^*), 37.03 (b*), 33.17 (C^*) and 114.19 (h^*). However, throughout storage at 20˚C, a* values increased to above 0 and b values increased to about 60 indicating the presence of and orange-yellow color (Salvador *et al*., 2007). Analyzing peel color is a non-destructive way to estimate pulp qualities such as texture and taste.

Texture, pH, Acidity, Soluble Solids, and Moisture Content

Texture and taste are the most important quality attributes of consumer preferences. The most common method of determining firmness is a puncture test using a texturometer. A sample of tissue, usually a slice of fruit around 1 cm thick, is laid out. The texturometer contains a blunt blade with a flat tip used to break through the tissue. The blade is applied at a known force and speed and a calculating device measures the amount of force needed to puncture the fruit. This is known as the Kramer shear test (Cano *et al.,* 1997; Meullenet, 2012).

As the fruit ripens in most cultivars, the pulp typically changes color from white to light yellow. Starch is converted to sugar causing an increase in osmotic pressure and decrease in turgor pressure. The pulp softens and firmness decreases due to cell wall degradation. Studies on the mechanism of banana fruit softening during ripening were inconclusive because it is likely a combination of many factors. It is known that one of the major influences on softening is due to the degradation of starch into simple sugars. The average starch content in fruit at the mature green stage for most cultivars is about 25%, and then decreases to less than 1% during the climacteric period. At the same time, sucrose increases by 12 times from the mature green to climacteric period (Arêas and Lajolo, 1981). Dessert bananas contain lower levels of starch than cooking bananas, which is why they can be eaten without being cooked. It was reported that firmness is highly correlated to starch in 'Nanicão' (AAA) and 'Mysore' (AAB) bananas. Initial starch of 'Nanicão' fruit (220 $g \cdot kg^{-1}$) was converted to soluble sugars (180 $g \cdot kg^{-1}$) after 17 days of ripening at 20°C and 90% RH. However, starch is not always an accurate indicator of pulp firmness. 'Terra' (AAB) fruit had reduced firmness at the yellow ripe stage yet maintained high

residual starch content. In reference to dessert bananas, it can be argued that increased softening during ripening may be due to starch degradation into soluble sugars, however this may not be the case for cooking bananas (Shiga, *et al*., 2011).

It was reported that for Spanish and Latin-American Cavendish cultivars (AAA) at the transitional stage, firmness was between 6.68±0.50, and 5.53±0.41 N gfw^{-1}. The peels had a yellow/green color, with *L** values between 60.11±1.18 and 59.25±0.72, *a** values between 1.16±0.09 and -3.09±0.61, *b** values between 26.10± and 20.64±0.45, and Hue values of 87.45±0.15 and81.49±0.56. Titratable acidity was between 0.50±0.03 and 0.03±0.04 g citric acid·100 gfw^{-1}, pH was between 4.91±0.05 and 4.74±0.03, and soluble solids were between 24.56±0.33 and 16.30±0.03˚Brix. Moisture content was between 76.05±0.19 and 73.24±0.11 (Cano *et al*., 1997).

In another study comparing the physicochemical characteristics of 18 different dessert bananas at the ripe stage, firmness values were reported to be between 1.47 and 2.85 N. Dry matter was between 22.2 and 31.2 g, soluble solids were between 21.4 and 23.2˚Brix, pH was between 4.12 and 5.31, and the average titratable acidity was 5.7 meq. The main contributors to sourness and sweetness of the fruit were malate and citrate. The parameters in this study were used to predict sensory attributes by consumers. It was determined that higher titratable acidity and dry matter content lead to a higher firmness score by the consumer. This may be due to the increased citric acid inhibiting pectin hydrolase activity and therefore inhibiting cell wall degradation. Also, fruit with higher citrate and malate contents tended to have higher 'sourness' scores by panelists (Bugaud *et al*., 2013).

Banana pulp organic acids were reported to increase during ripening in most cultivars. In a ripening study using 'Gros Michel' (AAA) bananas, malic acid contents increased from 1.36 to 5.37 to 6.20 meq·100 g^{-1} during mature green, transitional, and ripe stages, respectively. Total organic acidity was 4.43, 8.74, and 10.90 meq·100 gfw^{-1} for mature green, transitional, and ripe fruit, respectively. At the mature green stage, the majority of total organic acids were made up of oxalic acid (50%), followed by malic acid (35%) and citric acid with certain phosphates (10%). However, as the banana ripens, oxalic acid levels decrease while malic acid and citric acid increases by 3 or 4 times their initial levels (Wyman and Palmer, 1963). Though maturity is a significant determinant of physicochemical properties, other postharvest practices have been proven to alter these qualities as well.

ARTIFICIAL RIPENING TECHNIQUES

Ripening in bananas can be characterized by several physiological changes in color, texture, aroma, flavor, nutritional content, and susceptibility to pathogens. Many factors can influence the rate of ripening, for example, ethylene exposure, storage temperature, and atmosphere. Exposure to ethylene affects hundreds of defense and stress related genes in bananas. Studies have been implemented to determine the biochemistry behind ethylene-induced ripening. One particular study identified the genes involved in ethylene regulation and ripening using 'Robusta Harichhal' (AAA) bananas, which require exogenous ethylene to ripen. In the first treatment, bananas were exposed to 100 $\mu L \cdot L^{-1}$ ethylene and stored at 22°C. The second group of bananas were first treated with 10 $\mu L \cdot L^{-1}$ 1-methylcyclopropene (1-

MCP) and treated with 100 $\mu L \cdot L^{-1}$ ethylene. Suppression subtraction hybridization (SSH) and polymerase chain reaction (PCR) techniques were used to identify the genes related to ripening pathways. Specific genes that are expressed under cold, heat, salt, and drought stress, were up-regulated during ripening. The study showed that ripening induced by ethylene in bananas resulted in expression of stress, defense, and detoxification genes. Also, genes involved in ethylene biosynthesis, cell wall loosening, and gene expression regulation, were affected (Kesari *et al*., 2007).

Bananas are typically harvested when they are at the mature green stage. Once senescence has begun, the fruit is more susceptible to mechanical damage and decay. Therefore, it is preferable that the bananas remain in the mature green stage throughout transportation so they do not become too damaged and unmarketable. Wills *et al*., 2014 compared the effects of various concentrations of ethylene (1.0, 0.1, 0.01, or 0.001 $\mu L \cdot L^{-1}$) of 'Cavendish' bananas (AAA) stored in 15, 20, or 25°C. As ethylene concentration increased, green life of bananas decreased. Even in 15°C, treatment with 1.0 $\mu L \cdot L^{-1}$ ethylene reduced the green life by 30 days compared to 0.001 $\mu L \cdot L^{-1}$ treatment. 'Lady Finger' bananas (AAB) treated with 1.0 $\mu L \cdot L^{-1}$ ethylene all had a green life of 4 days regardless of storage temperature. Meanwhile, there was a reduction in green life with increased storage temperature in Lady Finger bananas treated with 0.001 $\mu L^{\cdot L^{-1}}$ ethylene (38.7±0.9 days in 15°C, 21.7±1.3 days in 20°C, and 13.3±0.3 days in 25°C). Results were applied to transportation (5 days, 3,000 km) and it was determined that at a mean summer temperature of 25°C, bananas did not have to be refrigerated if the ethylene remained below 0.58 $\mu L \cdot L^{-1}$. During winter with an average temperature of 14°C, bananas did not have to be refrigerated if ethylene

levels did not exceed 0.90 $\mu L \cdot L^{-1}$. Ethylene can be controlled by air ventilation and ethylene scavengers (Wills *et al*., 2014).

As soon as bananas reach the market, it is beneficial to accelerate ripening to increase visual appeal. The application of exogenous ethylene is commonly used for artificial ripening. To ensure uniform application, bananas must be treated in a gas-tight ripening room. However, not all facilities have the means to accommodate this technology, in which case alternative treatments are available. Commercial liquids that release ethylene, such as Ethrel® (2-chloro ethyl phosphonic acid), can be used as a dip. Kulkarni *et al*. (2011) tested the ripening effects of 250-1,000 ppm Ethrel dips for 5 minutes on 'Robusta' (AAA) bananas. After dipping, the fruit were air dried and stored at 20±1˚C. The bananas responded best to 500-ppm of Ethrel compared to 0 (control), 250, and 1,000 ppm. Untreated bananas did not ripen after 8 days in storage while ethrel (500-ppm) treated bananas not only ripened in 6 days, but also they had excellent external color, taste, flavor, and overall quality. Ethrel treatment caused an increase in Hunter colors *L* and *b*, total soluble solids, acidity, total sugars, and total carotenoids. There was a decrease in Hunter color *a*, pulp moisture, and pulp pH in the pulp (Table 1, Appendix A) (Kulkarni *et al*., 2011).

BANANA FRUIT BIOACTIVE COMPOUNDS AND HEALTH BENEFITS

Popularity of bananas has increased due to expanding discoveries of their beneficial nutritional and health qualities. Bioactive compounds, or non-nutritional components in foods that induce biological responses in plant tissues, have been extensively examined to determine their effects on human health. It was suggested

that consumption of bananas can help fight diseases related to oxidative stress caused by free radicals such as cancer and cardiovascular diseases (Pereira and Maraschin, 2015). Bananas are one of the best sources of antioxidants, containing powerful reducing agents such as vitamin C.

Vitamin C

Ascorbic acid is a labile molecule, meaning it easily changed or degraded. It can be lost during cooking or processing and is highly sensitive to light and temperature (Naidu, 2003). Ascorbic acid, otherwise known as vitamin C, is a reducing agent than can be oxidized to dehydroascorbic acid to quench free radicals that are toxic to the body. Since humans do not have the capability of synthesizing this vitamin due to lack of the enzyme $_{L}$-gulonolactone oxidase, it must be obtained from the diet and fruits are the best source. Vitamin C aids in collagen, carnitine, and neurotransmitter synthesis, bone formation, prevention of scurvy, and iron and hemoglobin metabolism. In addition, it enhances immunological function, and reduces risk of cataract formation, cancer, and coronary heart diseases. More commonly, ascorbic acid is used for prevention or relief of the common cold. Its role in collagen synthesis aids in wound healing. (Mathews, 2014; Naidu, 2003).

Ascorbic acid can be interconverted between its reduced form, ascorbic acid, and its oxidized form, dehydroascorbic acid. There has been controversy as to whether or not dehydroascorbic acid serves a physiological role; therefore the primary focus of many researchers is on ascorbic acid. However, research shows that dehydroascorbic acid is the primary form that is absorbed in plant membranes

(Deutsch, 2000). Vitamin C in most fruits is primarily found in its reduced form, ascorbic acid. Though there have been studies reporting vitamin C values in bananas, the majority of them only provide information on Cavendish varieties. In addition, the majority of the studies only look at the reduced form (AA) or the total vitamin C content (reduced + oxidized). The few studies that were found did not provide conclusive evidence. Hernandez *et al*. (2006) reported that the 'Gran Enana' (AAA) has reduced ascorbic acid levels of 15.1, 14.6, and 6.0 $mg \cdot 100\ gfw^{-1}$ for unripe, half-ripe, and ripe fruit, respectively; and total ascorbic acid levels of 15.5, 15.1, and 6.61 $mg \cdot 100gfw^{-1}$ for unripe, half-ripe, and ripe fruit, respectively. However, other literature contradicts these findings and suggests that the majority of vitamin C is in its oxidized form in bananas. Szeto *et al*. found that of the total vitamin C (100 $mg \cdot kg^{-1}$ fresh weight), less than 10 $mg \cdot kg^{-1}$ fresh weight was in the reduced form (Szeto *et al*., 2002). It is possible that the primary form of vitamin C in bananas is dictated by external factors, such as environment and genetics.

Phenolic Compounds

Phenolic compounds are water-soluble secondary metabolites found in plants. They are comprised of simple structures, such as vanillin, gallic acid, and caffeic acid, and polyphenols or compounds containing more than one phenolic ring. Examples of polyphenols include flavonoids and stilbenes. When plants are injured, phenolic compounds are oxidized by the polyphenoloxidase enzyme resulting in enzymatic browning. The structures of these compounds play a vital role in many plant properties such as color, aroma, flavor, defense, and metabolism. For example,

anthocyanins are flavonoids responsible for red and blue pigmentation in certain flowers and fruits, while flavonols can help the plant fight UV radiation damage. Compounds such as anthocyanins, proanthocyanins, and hydrolysable tannins can accumulate in certain plants to protect against herbivores, fungi, and viruses (Cheynier, 2012).

Preexisting phenols involved in plant defense include simple phenols, phenolic acids, flavonols, and dihydrochalcones. These phenols, also known as phytoanticipins, are synthesized when the plant is being attacked by a pathogen. For example, when a pathogen is present in the plant tissue, enzymes such as phenylalanine ammonia lyase (PAL), peroxidase, and polyphenol oxidase are stimulated. The enzymes consume oxygen and produce fungitoxic quinones, preventing additional pathogens from developing. Lignin, a phenolic polymer associated with the cell wall, protects against disease by acting as a physical barrier between the plant nutrients and harmful toxins released by the pathogen (Ewané *et al*., 2012).

Foods derived from plants contain a wide variety of phenolic compounds. The levels of these compounds are affected by genetics, environment, and maturity. Bananas have been shown to contain high levels of phenolics, specifically tannins, gallic acid, catechin, gallocatechin, and naringenin-7-*O*-neohesperoside (Aurore *et al*., 2009; Del Mar Verde Méndez *et al*., 2003). Tannin values in genomically diverse green dessert bananas are reported as: 0.5-2.1 $\%\cdot FW^{-1}$ (Group AA); 0.6 $\%\cdot FW^{-1}$ (Group AB); 0.35-0.7 $\%\cdot FW^{-1}$ (Group AAA); 0.35-2.1 $\%\cdot FW^{-1}$ (Group AAB); 2.0 $\%\cdot FW^{-1}$ (Group ABB). However, no tannins were found in green cooking bananas

‘Guayabo’ and ‘Poingo’ (Group ABB). The majority of the tannins consisted of epigallocatechin (96-98% in all cultivars) (Uclés Santos *et al*., 2010).

It has been proven that total phenols vary among cultivars. In a study comparing 29 cultivars with varying genomes at maturity stage 6, the cultivar with the lowest total phenolic content was ‘Cairpira’ of Musa Group AAA with 2.01±0.14 mg GAE·100 gdw^{-1}. The highest was ‘Highgate’, also of Musa Group AAA with 95.3±1.35 mg GAE·100 gdw^{-1}. ‘Williams’, an industry standard, was reported to have 22.3±1.17 mg GAE·100 gdw^{-1}. Overall, the most prevalent phenolic compound of the cultivars was gallocatechin. Gallocatechin had a collective mean of 360 μg·100 g^{-1} throughout ‘Saba’ (ABB), ‘Jaran’ (AA), ‘Bucaneiro’ (AAAA), ‘028003-01’ (AA), ‘Gros Michel’ (AAAA), ‘Wasolay’ (AAA), ‘Champa Madras’ (ABB), ‘Nam’ (AAA), and ‘Highgate’ (AAA) cultivars. The average gallocatechin levels were significantly higher than average epicatechin (39.3 μg·100g^{-1}), gallic acid (3.97 μg·100 g^{-1}), and protocatechin levels (1.97 μg·100 g^{-1}) (Borges *et al*., 2014).

Total Antioxidants

By harvesting banana fruit from the mother plant, a series of stress-induced reactions occurs. The plant’s metabolism is affected and antioxidant compounds are produced. By definition, an antioxidant is a molecule with low reduction potential that helps prevent oxidation of other molecules by donating electrons to free radicals (Ramaswamy, 2014). Antioxidants help maintain fruit quality by inhibiting oxidative stress caused by improper storage, senescence, and mechanical injury. In addition, there is substantial evidence that maintaining an antioxidant-rich diet is beneficial to

human health. Therefore, there have been increased efforts to identify foods rich in antioxidants and determine their nutritional benefits (Villa-Rodriguez *et al*., 2015).

Several methods have been developed to analyze the antioxidant content in foods. The ferric reducing ability of plasma, or FRAP assay was developed to quantify antioxidant power. The method relies on the reduction of ferric ions to the ferrous form at low pH causing the formation of a ferrous-tripyridyltriazine (Fe^{III}-TPTZ) complex. This reaction results in the development of a deep blue color with an absorption maximum of 593 nm. The FRAP assay can be used with several standards including ascorbic acid, bilirubin, uric acid, Trolox (vitamin E), α-tocopherol, and albumin (Benzie and Strain, 1996) This method is implemented to determine the scavenger properties of phenols, catechols, and anilines. It is a single electron transfer (SET) based assay, meaning the antioxidant transfers a single electron to help reduce target compounds. Unfortunately, the FRAP Assay is limited in the aspect that only the reducing capability based on ferric (Fe^{3+}) ions can be measured (Gülçin, 2012).

Another method for evaluating antioxidant content in foods is the use of the stable free radical 2,2-diphenyl-1-picrylhydrazyl, also known as DPPH. The $DPPH^{\bullet}$ radical scavenging assay is used to study the structural effects on phenolic antioxidant activity. This assay measures the activity of antioxidants to reduce the $DPPH^{\bullet}$ radical. The preparation of DPPH in ethanol produces a violet-colored solution absorbing around 517 nm. As the substrate scavenges the $DPPH^{\bullet}$, the violet color begins to fade. Therefore, a substrate containing high antioxidant levels will have a lighter coloration than a substrate with low antioxidant levels. The $DPPH^{\bullet}$ radical scavenging assay is based on the hydrogen atom transfer (HAT) mechanism, meaning the antioxidant

donates a hydrogen atom to $DPPH^{\bullet}$ and the non-radical DPPH-H is formed (Gaikwad *et al.,* 2010; Gülçin, 2012).

Like DPPH, the ferromyoglobin/2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) method, also known as the ABTS radical scavenging assay, can be used to measure total antioxidant activity. ABTS reacts with $_{L}$-ascorbic acid and produces an $ABTS^{\bullet+}$ radical cation, which can be used to determine the antioxidant activity of hydrogen-donating and chain-breaking antioxidants. The assay is based on an SET mechanism, in which ABTS is oxidized to the radical cation $ABTS^{\bullet+}$. During this reaction, the antioxidant acts as an electron donor and $ABTS^{\bullet+}$ returns to its stable form. The decrease in $ABTS^{\bullet+}$ causes the absorbance at 414 nm to decrease. Therefore, like the DPPH assay, substrates with higher a total antioxidant content with have an increased loss of color (Cano et al., 1998; Gülçin, 2012; Miller *et al.*, 1993; Re *et al*. 1999).

Often times, multiple assays are used to estimate total antioxidant activity. Experiments conducted by Thaiphanit and Anpring (2010) assessed the antioxidant levels of 'Hom Thong' bananas using DPPH and ABTS assays. They found that completely yellow (stage 6) of the 'Hom Thong' banana had the least antioxidant activity while overripe fruit (stage 8) had the highest. Fruit from stage 8 were classified as fruit with 50% of their peels being yellow and 50% of their peel covered in brown patches caused by sugar spots (Table 2, Appendix A). In stages 6, 7, and 8, free radical scavenging activity increased from 2.25 to 2.95 and 3.06, respectively. In other words, antioxidant activity increased with ripening. Results validated the categorization of bananas as a functional food, or a food that improves human health,

when compared with other fruits and vegetables. Results from the DPPH assay showed that antioxidant levels in 'Hom Thong' bananas exceeded values of other herbs and vegetables reported in previous literature. Results from the ABTS assay indicated that the antioxidant activity was comparable to blueberries, which are known for their extremely high antioxidant activity (Thaiphanit and Anpring, 2010).

Effects of Peel and Pulp Browning on Bioactive Compounds

There are several events that can affect the bioactive activity of bananas. Natural processes associated with ripening are known to influence the accumulation of these compounds, however external activities can have a severe impact as well. This includes chilling injury, mechanical injury, bruising, or even peeling the fruit prior to consumption. One can visually acknowledge these reactions by the presence of brown coloration on the peel or pulp. This is due to the oxidation of dopamine (3.4-dihydroxyphenylethylamine) by the enzyme polyphenoloxidase. Phenol oxidases are copper proteins that oxidize phenol substrates to quinones, which in turn oxidize to melanins, accounting for the brown pigmentation (Palmer, 1963).

Storage at cold temperatures can cause many problems for bananas. To be safe, bananas should not be stored below 12 to 14°C. Chilling injury occurs when the fruit is kept at below favorable temperatures, resulting in abnormal ripening, loss of flavor, decay, and peel discoloration (Toraskar and Modi, 1984). Chilling injury occurs as a result of membrane polar lipids turning from liquid to gel, therefore lowering membrane-bound enzyme activity and losing membrane semipermeability (Promyou et al., 2007). Peel discoloration occurs due to enzymatic and non-

enzymatic browning (Hashim, 2012). Phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) become more active and the banana turns from green to yellow and eventually from brown to black. Polyphenols increase during chilling injury and chlorophyll content decreases.

Previous research suggests that chilling injury in bananas is directly related to the decrease in peroxidase activity. When bananas ripen normally, peroxidase content increases significantly. Reports by Toraskar and Modi (1984) indicate that when bananas were stored in 10°C, respiration rate decreased about 42% at the climacteric peak compared to those stored at 28°C. Bananas stored in 10°C for 3 to 4 days had pale skin and did not ripen normally when they were transferred to 28°C. When stored in 10°C for 8 to 10 days, the pulp became brown and hard. However, bananas stored in 28°C ripened normally after 8 to 9 days. After 4 days of storage in 10°C, the bananas had 30% less peroxidase activity than those stored in 28°C.

The accumulation of PAL and PPO lead to the decrease in total free phenolics. It was reported that when chilling injury is induced, the increase PAL and PPO activity is correlated to the decrease in free phenolic activity (Nguyen, 2003). It was found that when 'Kluai Khai' (AA) and 'Kluai Hom Thong' (AAA, also known as 'Gros Michel') bananas were stored in 10˚C, there was a significant increase in PAL and PPO activities. Total phenolics in storage at 10˚C deceased from 3.0 to 2.8 $mg \cdot gfw^{-1}$ and 0.9 to 0.8 $mg \cdot gfw^{-1}$ in 'Kluai Hom Thong' and 'Kluai Khai' bananas, respectively. There was an even stronger reaction in bananas stored in 6˚C. Total phenolics in storage at 6˚C deceased from 3.0 to 2.3 $mg \cdot gfw^{-1}$ and 0.9 to 0.4 $mg \cdot gfw^{-1}$ in 'Kluai Hom Thong' and 'Kluai Khai' bananas, respectively.

Though chilling injury significantly enhances oxidation, it is also a natural occurrence during ripening resulting in fruit softening. Yang *et al*., (2008) found that after 'Williams' (AAA) bananas were stored in 22°C, firmness decreased from 11.228±0.188 N to 10.028±0.164 N on day 4 and 3.342±0.083 N on day 7. Reactive oxygen species such as malonydialdehyde (MDA), protein carbonyl, and lipofuscin increased significantly with the decrease in firmness. The production of hydroxyl radical, hydrogen peroxide content, and superoxide anion radical decreased until day 4 (which softening initiated), then significantly increased until day 7. Therefore, it is possible that the accumulation of MDA, protein carbonyl, and lipofuscin may stimulate the production of oxidation products (hydrogen peroxide and hydroxyl radical) and be involved in the initiation of fruit ripening.

SUMMARY OF LITERATURE REVIEW

There are limited published studies pertaining to postharvest behavior of short-season banana and plantain cultivars cultivated in southeastern regions of the United States (Crane *et al*., 2006; MacLean and Krewer, 2009; Nunes *et al*., 2013). Therefore, there is a need to establish best management practices in regard to basic postharvest recommendations for specialty bananas and plantains cultivated in the southeastern region of the United States (Alabama, Georgia, and Florida) due to increased consumer demand for locally cultivated genotypes familiar to this population. Although many challenges exist in regard to commercial production of bananas in southeastern United States (i.e., environmental and climactic conditions are less than favorable when compared to tropical environmental conditions) recent

studies indicate joint collaborative efforts in developmental technology transfer emphasize the potential to economically create niche market for short-season bananas adaptable to this region and thus thereby enhancing local rural agricultural economy (Fonsah *et al*., 2003; 2007). Sustainability and consumer appeal for short-season and cold hardy banana cultivars adaptable to the southeastern United States will require the identification of cultivars with enhanced flavor and nutritional quality that reflect changing consumer demographics. Therefore, the goals of this research were to determine optimum postharvest conditions for specialty bananas grown in Alabama, Florida, and Georgia, by evaluating the effect of 500-ppm ethephon treatment and maturity stages on physicochemical and nutritional parameters.

II. Effect of Artificial Ripening on Physicochemical and Antioxidant Properties of Banana Fruit

ABSTRACT

The physicochemical properties and ripening behavior of six banana cultivars differing in genotype were compared following application of ethephon. Commercial physiologically mature green cultivars 'Ele Ele' (*Musa* AAB Group), 'Pace' (*Musa* AAB Group), 'Brazilian' (*Musa* AAB Group), 'Ice Cream' (*Musa* ABB Group), 'Kandarian' (*Musa* ABB Group) and 'Sweet Heart' (*Musa* AABB Group) bananas at the full three quarter stage were separated and either treated with aqueous solution of ethephon (500-ppm immersion for 5 minutes) or controls (water immersion for 5 minutes) and stored for 9 days at 20˚C and 95% RH. Fruit quality was characterized by measuring peel color, dry matter, pH, titratable acidity, soluble solids, and vitamin C (reduced, oxidized, and total).

Results indicate that perceived sensitivity to ethephon treatment and modification in ripening behavior was variable among genotypes and measured fruit quality parameters during storage. 'Kandarian' fruit were most sensitive to postharvest ethephon treatment when compared to the other cultivars, followed by 'Ice Cream'. Interactions were observed between ethephon treated 'Kandarian' fruit and days in storage for external peel color (RCI values) and corresponding changes in internal fruit quality (dry matter, soluble solids, pH and titratable acidity). 'Pace' and 'Sweet Heart' had moderate to weak physicochemical response to ethephon

treatment. The two cultivars least responsive to ethephon treatment were 'Brazilian' and 'Ele Ele', respectively, due to variable postharvest ripening behavior. Throughout storage in 'Kandarian', the concentrations of total, reduced, and oxidized vitamin C progressively declined and were higher when compared to 'Ele Ele' in response to ethephon treatment. In contrast, 'Brazilian' fruit tissue concentrations of total, reduced and oxidized vitamin C remained relatively unchanged during storage. Overall, results indicate that physicochemical properties and vitamin C (reduced, oxidized and total) content are reliable indicators of fruit maturation and ripening in banana hybrids of *Musa* sp. adaptable to southeastern United States. Findings from this study indicate that sensitivity or responsiveness to postharvest ethylene treatment and hence degreening process of banana to obtain optimal consumer eating quality is highly dependent on selected cultivars.

Keywords: Ethephon, banana, ripening, physicochemical, vitamin C.

INTRODUCTION

Commercial production of banana in the contiguous United States has historically been limited due to climactic constraints. Due to changing consumer demographics, production and demand for short season banana cultivars adaptable to southeastern US has increased during the past 10 years. To a large extent, peel color, sweetness and acidity are important quality attributes that define consumer demand for bananas. Prior research indicates considerable differences in pH and titratable acidity (TA) among banana cultivars and postharvest ripening (Bugaud *et al*., 2013;

Chacon *et al*., 1987; Tsamo *et al*., 2014). Consistent with these findings, there is considerable variation in antioxidant content between genomic groups (Fernando *et al*., 2014; Wall, 2006). Genomic (Fernando *et al*., 2014; Anyasi *et al*., 2013; Sulaiman *et al*., 2011; Davey *et al*., 2009) and cultivar (Bugaud *et al*., 2011) diversity of bananas greatly influence nutrition, quality and consumer appeal as well as response to postharvest treatment. The determination of suitable alternatives to the Cavendish subgroup is a relatively new concept. A better understanding of these properties opens up the possibility for niche market development for short-season cold-hardy bananas. Bananas have low fat and sodium and are high in carbohydrates, antioxidants, minerals, and vitamins A, B6, and C (Anyasi *et al*., 2013). Although there have been prior reports of vitamin C content in bananas, the majority of them only provide information on Cavendish varieties. In addition, these studies primarily indicate the reduced form (AA) or the total vitamin C content (reduced + oxidized). In 'Gran Enana' (AAA) cultivars, the ascorbate is primarily in its reduced form and decreases throughout storage (Hernandez *et al*., 2006). However, there is a lack of consistency with regard to vitamin C content within the literature. Szeto *et al*. (2002) reported that of the total vitamin C content (100 $mg \cdot kg^{-1}$ fresh weight), less than 10 $mg \cdot kgfw^{-1}$ was in the reduced form. The objective of the study was to determine the effect(s) of artificial ripening (ethephon) on the physicochemical and antioxidant properties of short season banana cultivars grown in southeastern US.

MATERIALS AND METHODS

Chemicals

The following chemicals for vitamin C analysis assays were obtained from Sigma-Aldrich, St. Louis, MO: metaphosphoric acid (Catalog No. 239275); trichloroacetic acid solution, 6.1N (TCA) (T0699); 2,2'-Dipyridyl ReagentPlus (D216305); Iron (lll) chloride reagent grade, 97% (F-7134); $_{L}$-ascorbic acid (A5960); and n-ethylmaleimide (N-med) (E^{-}1271). Ethylenediamine tetraacetic acid (EDTA) (4005) was obtained from EMD Millipore Corporation, Billerica, MA. The following chemicals were obtained from Fisher Scientific (Waltham, MA): glacial acetic acid, Reagent A.C.S. (A38^{c}-212); o-phosphoric acid (M-12461); potassium phosphate dibasic (P288-500); dithiothreitol (DTT) (BP172); and potassium phosphate monobasic (P285-500).

Plant Materials, Experimental Design and Treatments.

The experiment was conducted during the 2013 cultivation season from two established banana experimental sites from the UGA Coastal Georgia Botanical Gardens, Savannah, GA (Latitude 32.133° N, 81.2° W) and fields of University of Georgia's campus site in Tifton, GA (31.4633° N, 83.5100° W) and soil type was Pelham fine loamy sand. Soil pH was adjusted to 6.5 prior to planting with dolomitic limestone with and field experimental design was a randomized complete block with five single-plant replications. Adaptive cultural practices of local cultivars are as indicated in (Fonsah *et al.,* 2003). Six genomically diverse short-season, cold hardy banana cultivars 'Ele Ele' (*Musa* AAB Group), 'Pace' (Musa AAB Group), 'Brazilian' (*Musa* AAB Group), 'Ice Cream' (*Musa* ABB Group), 'Kandarian' (*Musa* ABB Group), and 'Sweet Heart' (*Musa* AABB Group) were harvested over three

dates, on October 1, 2013, October 18, 2013, and October 19, 2013. Cultivars were harvested from representative physiologically mature green fruit were harvested when fully mature according to commercial practice of a noticeable absence fruit surface angularity. Samples were transported overnight to Auburn University's Postharvest Physiology laboratory in Auburn, AL. Fruit samples with noticeable defects (bruising, leakage of latex, observable disease) were discarded. Homogenous fruit samples of size and color were sorted at random and stored in ventilated cardboard boxes (20˚C, 95% RH, maintained with humidified mister) until initial color break (~7 days, simulated shipping conditions, Stages 2 – 3).

Fruit and Postharvest Treatments

Fruit used for experiment were selected for uniform size and for absence of physical injuries or postharvest infection. The experimental design was randomized with 10 fruit per treatment and replicated six times. Fruit were either immersed in 500-ppm ethephon solution for 5 minutes or water (control) also for 5 minutes, air dried at 25˚C for 24 hours and treated fruit were stored in ventilated plastic containers (20˚C, 95% RH, maintained with humidified mister) for 0, 3, 6, and 9 days and peel surface color was determined. Subsequently, Peels were removed and fruit pulp was cut into slices (1 cm thick). Peels and pulp were stored in -80˚C until further analysis.

Physicochemical Analysis

Peel Color and Processing

External peel colors were quantified using a Minolta CR-300 tristimulus colorimeter (Konica Minolta, Chiyoda, Tokyo). Peel color was measured as *CIE-L*C*h*a*b* and values were taken on opposite sides of each banana. *L** defines lightness from 0 (black) to 100 (white); C defines Chroma ($C^* = a^2 + b^2)^{½}$; *a** denotes the green/red value where –*a** is green and +*a** is red; and *b** represents the blue/yellow value where –*b** is blue and +*b** is yellow. The color wheel spans 360˚, and hue (*h**) values refer to the relation of the *L**, *a**, and *b** values to each other. The peels were removed and the pulp was cut into 1 cm thick slices. Samples were stored in -80˚C until further analysis. Ripening color index (RCI) was expressed as:

$$\text{RCI banana} = 200 \cdot (1 - a/b)^{-1}$$ (Gomes *et al*., 2013).

Dry Matter

Pulp dry matter was determined by placing 10 g of pulp in a petri dish and drying it at 70˚C for 48 hours using an Excalibur® Food Dehydrator (Excalibur Dehydrators, Sacramento, CA). The weight of the sample was recorded before (Wt_0) and after (Wt_f) drying and the following equation was used to obtain dry matter values:

$$\text{Dry Matter (\%)} = (Wt_o \cdot Wt_f^{-1}) * 100$$

Pulp pH, Titratable Acidity, and Soluble Solids Content

Pulp was treated using a method reported by Maqbool, *et al*. 2011 with some modifications. Four random sample replicates consisting of 10 g, sample of frozen

banana fruit pulp was homogenized with 40 mL ultrapure water (Millipore Direct-Q® 3, EMD Millipore, Billerica, MA) using a kitchen blender (Oster®, John Oster Manufacturing Company, Warwick, RI) for a 1:5 dilution. The mixture was placed in a water bath at 90˚C for 20 minutes and stored overnight in in a Fisher Scientific refrigerated chromatography chamber maintained at 10˚C, Fisher Scientific model Isotemp Laboratory Refrigerator Model 13-986-1276 (Fisher Scientific, Raleigh, NC.).

Pulp homogenate obtained from above procedure was used to determine pH, titratable acidity (% malic acid) and soluble solids. Titratable acidity (TA) was determined using a portable pH meter (HI 9811-5 Portable pH/EC/TDS/˚C Meter, Hanna® Instruments, Woonsocket, RI). A 3.75 g sample of pulp: ultrapure water (1:5) was measured and diluted to 40 mL with ultrapure water. The initial measurable value (pH) and titratable acidity were recorded. All analyses were repeated 6 times to ensure accuracy.

Soluble solids content (SSC) was determined using a digital tabletop refractometer (Reichert® ABBE MARK II Refractometer, Reichert Inc., Depew, NY) at room temperature (25˚C). A drop of 1:5 homogenized pulp: ultrapure water solution was placed on the prism glass. Readings were multiplied by the dilution factor to obtain original SSC. Results were expressed as ˚Brix.

Sample Preparation and Vitamin C Determination

Care was taken to exclude any direct sunlight during extraction and quantification procedures with all sample operations being performed under amber

fluorescent lighting conditions (GE F40/G0, 40W). In addition, all glassware was shielded with aluminum foil during laboratory bench operations.

Three cultivars were chosen for analysis of total and reduced vitamin C depending on their physicochemical responses to ethephon treatment. 'Kandarian' had the best response meaning there were differences in physicochemical parameters between control and ethephon-treated fruit. 'Ele Ele' and 'Brazilian' had the least responses or sensitivity to ethephon treatment.

Extraction and Determination of Reduced Vitamin C

Vitamin C content was determined by extracting six random sample replicates of 2.5 g of frozen pulp tissue in 10 mL of cold m-phosphoric acid-acetic acid solution (30g metaphosphoric acid, 0.5g ethylenediamine tetraacetic acid, EDTA; and 80 mL glacial acetic acid diluted to 1 L with ultrapure water). Samples were sonicated for 15 minutes, centrifuged (Beckman Centrifuge, model J2-21, San Antonio, TX) at 13,000 g_n for 15 minutes at 4°C, filtered, the diluted to 20 mL. For vitamin C analysis, 100 µL of crude extract was added to 250 µL of KH_2PO_4 (150mM, pH 7.4 and 5 mM EDTA), and a series of reagents (200 µL of TCA, 200µL of O-phosphoric acid, 200µL of 4% [w/v] 2, 2-bipyridyl dissolved in 70% HPLC grade ethanol and 100µL of 3% [w/v] $FeCl_3$; 50 µL DTT and 50 µL N-med were additionally added to determine total vitamin C). A standard curve from 0-200 µM was prepared. The absorbance was read at 525 nm using a microplate reader using a microplate reader (Synergy HT, BIO-TEK Instruments, Inc., Winooski, Vermont) and results were expressed as vitamin C mg·100 gfw^{-1}.

Statistical Analysis

Analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.3 (SAS Institute, Cary, NC). A completely randomized design was used, and each species was analyzed separately. The treatment design was a factorial of ethephon treatment by days in storage. Where residual plots and a significant covariance test for homogeneity (COVTEST statement) indicated heterogeneous variance, a RANDOM statement with the GROUP option was used in the analysis. Single degree of freedom orthogonal contrasts were used to test linear and quadratic trends over days in storage. Least squares means comparisons between ethephon treatments at each day were compared using the Shaffer Simulated method. All significances were at $\alpha = 0.05$

RESULTS AND DISCUSSION

The preliminary study revealed that of the six cultivars studied, 'Kandarian', 'Ice Cream', and 'Pace' had the most sensitivity to 500-ppm ethephon treatment followed by 'Sweet Heart', 'Brazilian', and 'Ele Ele'. Data obtained for peel color (RCI) and internal quality (dry matter content, pH, titratable acidity, soluble solids content) illustrates postharvest ethephon treatment was effective in advancing uniform ripening during storage with greatest sensitivity with 'Kandarian' fruit (Tables 5-8). Results from this study were similar to reported findings of Kulkarni *et al*. (2011) where 'Robusta' (AAA) banana total soluble solids, total acidity, total sugars, total carotenoids and weight loss (%) increased during storage after 500-ppm

ethephon treatment (Table 1). Also, Hunter colors L, a, and b increased, meaning the peel colors became less green and more yellow.

Peel Color

'Ele Ele' fruits had the least response to 500-ppm ethephon treatment (Table 5, Fig. 4A). There was a very slight visual difference in peel color between control and ethephon-treated fruit, which was only noticeable at the end of the experiment. RCI values for both control and ethephon treated fruit were not different between days in storage at day 6, but by day 9 the RCI of the ethephon-treated 'Ele Ele' fruit was higher than the control fruit. Overall, the peel color values indicate that 'Ele Ele' peel color did not respond to ethephon treatment and neither of the treatment groups showed a color change until the end of the experiment. The control fruit had a nonsignificant trend throughout 9 days of storage in 20˚C and 95% RH while the ethephon-treated fruit had a linear increase. The lack of response in peel color was possibly due to premature harvest. Improper ripening would have occurred if the fruit were harvested before the mature green stage. It is also possible that for 'Ele Ele' fruit, a more highly concentrated treatment of ethephon is required in order to induce a ripening reaction.

'Brazilian' peel colors were also relatively insensitive to ethephon treatment (Table 5, Fig. 4C). Both control and ethephon-treated fruit showed a rapid change in peel color from green to yellow beginning at day 3. Both ethephon-treated and control 'Brazilian' fruit had a linear increase in RCI values throughout storage. At day 0, there was a significant difference between control and ethephon-treated fruit RCI

values (133.06 and 139.38, respectively), indicating that ripening may have initiated before the study began. Control and ethephon-treated fruit peel colors were not significantly different at day 3, but on days 6 and 9 ethephon-treated fruit peels had significantly higher RCI values than control fruit.

Like 'Brazilian' samples, 'Sweet Heart' fruit were relatively insensitive to the ethephon treatment in the sense that both control and ethephon-treated fruit had relatively high RCI values at day 0, meaning color change had initiated as shown in Table 5 and Fig. 4F. There was no significant difference between control and ethephon-treated fruit at day 0, but on day 3 ethephon-treated fruit had higher RCI values than control fruit. On day 6, the peels of the ethephon-treated fruit were too decayed to analyze peel color. On day 9, both control and ethephon-treated fruit were discarded due to extreme decay. It is possible that the fruit was harvested at a more advanced stage than mature green, meaning fruit had begun to ripen before the study began. It is also possible that due to their small finger size and genome group, the application of ethephon at 500-ppm was too concentrated for 'Sweet Heart' fruit.

'Pace' fruit peel colors had a stronger reaction to the ethephon treatment than 'Ele Ele', 'Brazilian', and 'Sweet Heart' fruit (Table 5, Fig 4B). At days 0 and 3, control and ethephon-treated fruit RCI values were relatively low and not different between treatments, indicating that the peels had not yet begun to change color. On days 6 and 9, the ethephon-treated fruit peel RCI values were higher than the control fruit, indicating that ethephon treatment stimulated peel color change in 'Pace' bananas. Both control and ethephon-treated fruit had a linear increase in RCI values during storage.

‘Ice Cream’ peel color was enhanced by ethephon treatment (Table 5, Fig. 4D). Both control and ethephon-treated fruit showed a linear increase in RCI values. At day 0, control and ethephon-treated fruit were not different, however on days 3-9 the ethephon-treated fruit had higher RCI values than control fruit.

‘Kandarian’ fruit peels were the most affected by ethephon compared to the other cultivars (Table 5, Fig. 4E). At day 0, the control fruit had higher mean RCI value than the ethephon-treated fruit (132.99 and 129.27, respectively), meaning the samples from the control group had initiated peel color change before the ethephon-treated group. This is likely due to the 24 hour drying period between treatment and peel color analysis. However, on days 3-9 the ethephon-treated fruit mean RCI values were significantly higher than the control fruit. Also, the ethephon-treated fruit was had a linear increase at α=0.001 and the control fruit linear increase at α=0.01.

Increases in peel RCI mean values were predictive of visual color change from green to yellow, as shown in Table 5 and Fig. 4. Peel sensitivity to ethephon was determined by the trend throughout days in storage as well as higher RCI mean values in ethephon-treated fruit compared to control fruit. ‘Kandarian’ and ‘Ice Cream’, both from the ABB genome group, had the strongest peel color reaction to ethephon treatment at 500-ppm compared to the rest of the cultivars.

Pulp Dry Matter

Pulp dry matter percentages of samples at 0, 3, 6, and 9 days of storage in 20˚C and 95% RH were measured as a determinant of tissue degradation and water accumulation (Table 5). It was anticipated that dry matter percentages would decrease in fruit during ripening due to metabolic processes during senescence. ‘Ele Ele’ fruit

had the least response in dry matter reduction during ripening. There was no difference in loss of dry matter in neither control nor ethephon-treated fruit. The lack of response in dry matter percentage during storage may be indicative of premature harvest or the concentration of ethephon used for treatment for 'Ele Ele' fruit, specifically. It is possible that this cultivar may not respond to ethephon treatment 500-ppm or lower. It is also possible that because 'Ele Ele' fruit are typically cooked before consumption, the metabolic processes during senescence are not as accelerated as those in "dessert" cultivars.

'Brazilian' pulp dry matter content was not different in control and ethephon-treated fruit during days 0 and 3, however during days 6 and 9 dry matter content was lower in ethephon-treated fruit (Table 5). Control fruit did not have a trend in dry matter content throughout storage while ethephon-treated fruit had a negative linear trend. Therefore, ethephon treatment at 500-ppm initiated the degradation of starch and tissue into simple sugars and water.

Due to excessive decay, dry matter content was only determined for days 0, 3, and 6 in 'Sweet Heart' fruit (Table 5). In 'Sweet Heart' fruit, dry matter content was not affected by ethephon treatment on day 3. On day 0, dry matter content in ethephon-treated fruit was higher than the control but on days 3 and 6 there were no differences between treatments. It is likely that tissue degradation associated with ripening had already begun in 'Sweet Heart' fruit before the treatments began

'Pace' pulp dry matter content was minimally affected by ethephon treatment (Table 5). At days 0 and 3, control and ethephon-treated fruit mean dry matter contents were not different. On days 6 and 9, dry mater contents of ethephon-treated

fruit were lower than control fruit, indicating that ethephon treatment at 500-ppm induced tissue degradation towards the second half of the experiment. However, the control fruits had a linear decrease at α=0.001 and ethephon-treated fruits had a linear decrease at α=0.05.

'Ice Cream' fruit dry matter amounts were reduced in fruit treated with ethephon compared to control fruit (Table 5). In fact, there was a linear increase of dry matter content in control fruit while there was a linear decrease in dry matter content in ethephon-treated fruit. Because bananas are a climacteric commodity, it is likely that control fruit was still undergoing anabolic reactions and starch was accumulating. The ethephon may have triggered senescence, meaning catabolic reactions were dominant and tissue was being degraded.

'Kandarian' pulp dry matter content was affected by ethephon treatment (Table 5). On days 0 and 3, there was no significant difference between control and ethephon-treated fruit. On days 6 and 9, ethephon-treated fruit had lower dry matter content than control fruit, meaning tissue degradation was enhanced. There was no trend in control fruit during days of storage, but there was a linear decrease in dry matter content of ethephon-treated fruit.

Pulp pH, Titratable Acidity, and Soluble Solids Content

Unlike peel RCI and dry matter content, 'Ele Ele' pulp pH had a response to ethephon treatment (Table 6). At day 0, control and ethephon-treated pulp pH levels were not different, however on says 3-9, pulp pH was lower in ethephon-treated fruit than control fruit. Control fruit did not have a trend throughout days in storage,

however ethephon-treated fruit had a linear decrease. Although most physicochemical parameters did not indicate an enhanced ripening patterns in 'Ele Ele' fruit after ethephon treatment, there was a slight response pulp pH activity. It is possible that the reduction in pH is one of the first metabolic changes that occurred during ripening. However, the response in pulp pH to ethephon treatment was not indicative of pulp titratable acidity (TA) (Table 6) and soluble solids content (SSC) (Table 7). Pulp TA was expressed as the percentage of malic acid. In 'Ele Ele' fruit, there was no change in TA throughout the storage period in either the control fruit or the ethephon-treated fruit. However, ethephon-treated fruit had a higher mean TA level than control fruit (0.07 and 0.06% malic acid, respectively). 'Ele Ele' pulp SSC also showed a minimal response to ethephon treatment. With the exception of day 3, there were no differences at between control and ethephon-treated fruit during storage. On day 3, the pulp SSC levels in ethephon-treated fruit were higher than control fruit. Both control and ethephon-treated 'Ele Ele' fruit had a nonsignificant trend throughout the storage period.

'Brazilian' pulp pH was not affected by ethephon treatment (Table 6). With the exception of day 6, where the pulp pH in ethephon-treated fruit was lower than in the control, there was no difference in pulp pH between treatments. Pulp pH had a negative linear trend in control fruit and a negative quadratic trend in ethephon-treated fruit. Both pulp TA and SSC contents only showed a difference between the control and ethephon-treated fruits on days 3 and 6 in 'Brazilian' fruit, where the TA content in ethephon-treated fruit was higher (Tables 6 and 7). It is likely that the metabolic reactions accounting for malic acid production and soluble solids only

occurred while the fruit was ripening and concluded when the fruit became over ripe at day 9. Both control and ethephon-treated fruit showed a linear increase in pulp TA and SSC throughout storage.

Due to excessive decay, physicochemical assays for pH, TA, and SSC were only performed on days 0, 3, and 6 in 'Sweet Heart' fruit (Tables 6 and 7). 'Sweet Heart' fruit pulp pH, TA, and SSC was only affected by ethephon treatment on day 3. It is likely that the 'Sweet Heart' fruit has a relatively short ripening period compared to the other cultivars. On day 3, ethephon treatment resulted in lower pH levels and higher TA and SSC content.

'Pace' fruit pulp pH in both control and ethephon-treated fruit had a negative linear trend throughout storage (Table 6). At day 0, there was no significant difference between control and ethephon-treated fruit. However, on days 3-9 the ethephon-treated fruit had significantly lower pH levels. Pulp TA in both control and ethephon-treated had a linear increase throughout storage (Table 6). However, on days 6 and 9, the ethephon-treated fruit had higher TA values than control fruit, indicating the increased production of malic acid. Pulp SSC content was enhanced by ethephon-treatment on days 6 and 9, though both control and ethephon-treated fruit had a linear increase throughout storage (Table 7).

'Ice Cream' pulp pH was reduced in ethephon-treated fruit compared to control fruit on days 3-9 (Table 6). There was no trend in pulp pH content throughout storage in control fruit, however there was a linear decrease in ethephon-treated fruit. Pulp TA in control fruit remained constant throughout storage (0.06% malic acid) while pulp TA content in ethephon-treated fruit showed a quadratic increase (Table

6). Therefore, ethephon treatment enhanced malic acid production in 'Ice Cream' fruit after 9 days of storage in 20°C and 95% RH. Pulp SSC was enhanced with ethephon treatment on days 3-9 (Table 7). Control fruit SSC had a linear increase at α=0.05 and ethephon-treated fruit had a linear increase at α=0.001.

'Kandarian' pulp pH, TA, and SSC contents were all affected by ethephon treatment at the end of the storage period (Tables 6 and 7). On days 0-6, there was no significant difference in pH, TA, and SSC levels between control and ethephon-treated fruit. On day 9, ethephon-treated fruit had lower pH and higher TA and SSC content than control fruit. Control fruit did not have a trend in pH, TA, and SSC throughout storage. Ethephon-treated fruit had a quadratic decrease in pH, and quadratic increases in TA and SSC.

Vitamin C

Because of their performances during the physicochemical assays, only the two least sensitive cultivars 'Ele Ele' and 'Brazilian' and the most sensitive cultivar 'Kandarian' to ethephon treatment were selected for vitamin C analyses (Table 8). Vitamin C sensitivity to postharvest ethephon treatment and shelf life was highly dependent on cultivar and genome.

Ethephon treatment was effective in advancing vitamin C content ripening patterns with greatest sensitivity in 'Kandarian'. Vitamin C content in 'Kandarian' cultivars was optimal with ethephon treatment until day 6, and then declined progressively until day 9. In 'Ele Ele', ethephon-treated fruit had lower content of vitamin C until day 6, and then was optimal on day 9. In 'Kandarian' and 'Ele Ele',

reduced and oxidized forms of vitamin C fluctuated throughout storage. In contrast, ethephon did not affect vitamin C content in 'Brazilian' fruit.

CONCLUSIONS

Physicochemical and antioxidant sensitivity to ethephon treatment at 500-ppm was highly dependent on cultivars. Overall, 'Kandarian' fruit was most sensitive to ethephon treatment, followed by 'Ice Cream', 'Pace', 'Sweet Heart', 'Brazilian', and 'Ele Ele' fruit. It can be assumed that at 500-ppm, ethephon treatment was successful in enhancing ripening in *Musa* ABB cultivars due to variation between control and ethephon-treated fruit within each day of storage. However, 500-ppm ethephon may have been too high for 'Sweet Heart' and 'Brazilian' fruit due to rapid senescence early in the study. In contrast, it is possible that 500-ppm ethephon is not sufficient for 'Ele Ele' fruit, as because ripening was not observed until the end of the study. Further physicochemical and antioxidant studies using a range of ethephon concentrations for comparison should be performed.

III. Antioxidant Properties and Hypoglycemic Potential of Genomically Diverse Bananas Cultivated in Southeastern United States

ABSTRACT

There is limited information concerning the influence of maturity at harvest of *Musa* cultivars adaptable to southeastern United States. This study was conducted to determine variation of banana fruit ripening stage on the nutritional content of three selected banana cultivars differing in genotype: 'Hua Moa' (*Musa* AAB), 'Kandarian' (*Musa* ABB) and 'Williams' (*Musa* AAA) adaptable to southeastern United States. In this study, changes in water soluble AA (reduced vitamin C) content, ethanolic extracts of TP (total phenol) content, FRAP (ferric reducing antioxidant potential), DPPH (1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity) and ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) antioxidant capacities of fruit pulp tissue representative from four physiological maturity stages (mature green, MG; transitional, TR; ripe, R; and over ripe, OR) of banana fruit during postharvest fruit ripening (20°C, 95% RH) were determined. The vitamin C content (mg AA·100 gfw^{-1}) ranged from 1.3 ('Hua Moa', R) to 25.7 ('Williams', MG). The total phenolic content (mg GAE·100 gfw^{-1}) ranged from 15.3 ('Hua Moa', MG) to 41.6 ('Williams', OR). FRAP, VCEAC (mg AA·100 gfw) ranged from 8.9 ('Hua Moa', MG) to 58.7 ('Williams', R). FRAP, TEAC (mg TE·100 gfw^{-1}) ranged from 87.7 ('Hua Moa') to 308.5 ('Williams'). FRAP, GAEAC (mg GAE·100 gfw^{-1}) ranged from 3.1 ('Hua Moa') to 13.1 ('Williams'). DPPH, VCEAC (mg AA·100 gfw^{-1}) ranged from below

limits of detection ('Hua Moa') to 41.3 ('Williams'). DPPH, TEAC (mg TE·100 gfw^{-1}) ranged from below limits of detection ('Hua Moa') to 90.5 ('Williams'). ABTS, VCEAC (mg AA·100 gfw^{-1}) ranged from 24.7 ('Hua Moa') to 89.2 ('Williams'). ABTS, TEAC (mg TE·100 gfw^{-1}) ranged from 136.7 ('Hua Moa') to 338.7 ('Williams'). Physiological maturity and cultivar had an interactive effect on 'Hua Moa', 'Kandarian', and 'Williams' fruit in reduced vitamin C, total phenolics, and antioxidant capacity (FRAP, VCEAC) assays. However, only cultivar had an effect for indicated antioxidant capacity assays (FRAP, TEAC and GAEAC; DPPH, VCEAC; and TEAC; and ABTS, VCEAC and TEAC). Banana fruit ethanolic extracts representing TP fractions were observed to correlate strongly between FRAP, GAEAC thus indicating this fraction may contribute more to the antioxidant capacity of ripened fruit tissues. In conclusion, results of this study will provide critical nutritional information to potential banana growers in identifying superior cultivars with desirable antioxidant traits to increase potential market demand, sustainability, fruit storage life, and daily antioxidant intake of residents in southeastern United States.

Keywords: Banana, maturity, ripening, antioxidant, vitamin C, total phenolics.

INTRODUCTION

The banana (*Musa* spp.) is one of the most popular fresh produce commodities in world markets, making up over one-quarter of total fruit production (Mohapatra *et al*., 2010). Bananas have low fat and sodium and are high in carbohydrates,

antioxidants, minerals, and vitamins A and C. The health benefits of bananas are associated with reductions in cardiovascular dysfunction, muscular degeneration, intestinal disorders, and chronic disease. The banana's favorable nutrition properties, versatility, and inexpensive production help make it the second largest produced fruit, after citrus. Most cultivated bananas are hybrids between two wild species *Musa acuminata* of the A genome *Musa balbisiana* of the B genome and are diploid, triploid, or tetraploid (Lescot, 2010; Robinson, 1996). Genetic makeup can influence various factors such as nutrition and reaction to postharvest treatment, however very little is known about the exact mechanisms.

Bananas are one of the best sources of antioxidants, containing powerful reducing agents such as vitamin C. Ascorbic acid, otherwise known as vitamin C, is a reducing agent than can be oxidized to dehydroascorbic acid to quench free radicals that are toxic to the body. Because humans do not have the capability of synthesizing this vitamin due to lack of the enzyme $_{L}$-gulonolactone oxidase, it must be obtained from the diet and fruits are the best source. Vitamin C aids in collagen, carnitine, and neurotransmitter synthesis, bone formation, prevention of scurvy, and iron and hemoglobin metabolism. In addition, it enhances immunological function, and reduces risk of cataract formation, cancer, and coronary heart diseases. More commonly, ascorbic acid is used for prevention or relief of the common cold. Its role in collagen synthesis aids in wound healing (Mathews, 2014; Naidu, 2003).

Phenolic compounds are water-soluble secondary metabolites found in plants. They are comprised of simple structures, such as vanillin, gallic acid, and caffeic acid, and polyphenols or compounds containing more than one phenolic ring.

Examples of polyphenols include flavonoids and stilbenes. When plants are injured, the polyphenoloxidase enzyme oxidizes phenolic compounds, which results in enzymatic browning. The structures of these compounds play a vital role in many plant properties such as color, aroma, flavor, defense, and metabolism. For example, anthocyanins are flavonoids responsible for red and blue pigmentation in certain flowers and fruits, while flavonols can help the plant resist UV radiation damage. Compounds such as anthocyanins, proanthocyanins, and hydrolysable tannins can accumulate in some plants to protect against herbivores, fungi, and viruses (Cheynier, 2012).

Foods derived from plants contain a wide variety of phenolic compounds. The levels of these compounds are affected by genetics, environment, and maturity. Bananas were shown to contain high levels of phenolics, specifically tannins, gallic acid, catechin, gallocatechin, and naringenin-7-*O*-neohesperoside (Aurore *et al*., 2009; Del Mar Verde Méndez *et al*., 2003). It was also shown that total phenols vary between cultivars. In a study comparing 29 cultivars with varying genomes at fruit maturity stage 6, the cultivar with the lowest total phenolic content was 'Cairpira' of *Musa* Group AAA and the highest was 'Highgate', also of *Musa* Group AAA. 'Williams', an industry standard, was reported to have 22.3±1.17 mg GAE·100 gdw^{-1}. Overall, the most prevalent phenolic compound of the cultivars was gallocatechin. The average of gallocatechin levels was higher than average epicatechin, gallic acid, and protocatechin levels (Borges *et al*., 2014).

Antioxidants are molecules with low reduction potential that helps prevent oxidation of other molecules by donating electrons to free radicals (Ramaswamy,

2014). Antioxidants help maintain fruit quality by inhibiting oxidative stress caused by improper storage, senescence, and mechanical injury. In addition, there is substantial evidence that maintaining an antioxidant-rich diet is beneficial to human health. Therefore, there have been increased efforts to identify foods rich in antioxidants and determine their nutritional benefits (Villa-Rodriguez *et al*., 2015). Several methods have been developed to analyze the antioxidant content in foods. The ferric reducing ability of plasma (FRAP) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferromyoglobin/2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging assays were developed to quantify antioxidant activity. Often times, these assays are used together to determine antioxidant capacities by separate mechanisms. For example, DPPH and ABTS radical scavenging assays showed that 'Hom Thong' (AAA) bananas had the least antioxidant activity at the ripe stage while overripe fruit had the highest (Thaiphanit and Anpring, 2008). In other words, antioxidant activity increased with ripening. Results validated the categorization of bananas as a functional food when compared with other fruits and vegetables. Results from DPPH assays showed that antioxidant levels in 'Hom Thong' bananas exceeded values found in other herbs and vegetables reported in previous literature. Results from the ABTS assay indicated that the antioxidant activity was comparable to blueberries, which are known for their extremely high antioxidant activity.

There is a lack of information comparing the nutritional and antioxidant properties of cultivars grown in southeastern US to Cavendish cultivars. Proof that these specialty cultivars have enhanced nutritional qualities could benefit the market

for these cultivars and reduce the need to import bananas from other countries. The objective of this study was to evaluate the effect of maturity stage on various antioxidant properties of specialty cultivars grown in southeastern United States compared to the industry standard.

MATERIALS AND METHODS

Chemicals

Chemicals for vitamin C analysis, total phenolics, FRAP, DPPH and ABTS assays were obtained from Sigma-Aldrich, St. Louis, MO: metaphosphoric acid (Catalog No. 239275); trichloroacetic acid solution, 6.1N (TCA) (T0699); 2,2'-Dipyridyl ReagentPlus (D216305); Iron (lll) chloride reagent grade, 97% (F-7134); gallic acid (G7384) Folin-Ciocalteu's phenol reagent, 2N (F9252); sodium bicarbonate (S5761); 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (T1253); $_{L}$-ascorbic acid (A5960); 2,2-Diphenyl-1-picryhydrazyl (DPPH) (D9132). Ethylenediamine tetraacetic acid (EDTA) (4005) was obtained from EMD Millipore Corporation, Billerica, MA. The following chemicals were obtained from Fisher Scientific, Waltham, MA: Ethanol (Reagent Alcohol, A995-4); sodium acetate trihydrate (S209-3); glacial acetic acid, Reagent A.C.S. (A38^{c}-212); hydrochloric acid, Reagent A.C.S. (A144^{c}-212); o-phosphoric acid (M-12461); potassium phosphate dibasic (P288-500); and potassium phosphate monobasic (P285-500). Methanol (J.T. Baker ®) (9093-03) was obtained from Avantor Performance Materials, Center Valley, PA. 2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (018-10311)

and 2,2'-azobis (2-amidinopropane) (AAPH) (V-50) were obtained from Wako Chemicals USA, Inc. (Richmond, VA).

Fruit Samples and Storage Conditions

Genomically diverse, physiologically mature green banana cultivars 'Kandarian,' (*Musa* Group ABB), 'Hua Moa' (*Musa* Group AAB) and 'Williams' (*Musa* Group AAA) were purchased from a local commercial banana producer, NK Lago Farms, Pahokee, FL (26.8247° N, 80.6597° W) and harvested on two dates: October 6, 2014 and November 10, 2014. Fruit samples obtained were transported overnight to Auburn University's Postharvest Physiology laboratory in Auburn, AL. Fruit samples with noticeable defects (bruising, leakage of latex, observable disease) were discarded. Homogenous fruit samples of size and color were sorted and immersed in chlorinated water containing (approximately 7000 ppm active chlorine) for 5 minutes and air-dried at room temperature with a circulating fan. Fruit were stored in ventilated plastic containers (20°C, 95% RH, maintained with humidified mister) and stored until indicated laboratory analysis. Postharvest ripened banana fruit fingers were sampled based on peel color of four physiological maturity stages (mature green, MG; transitional, TR; ripe, R; and over ripe, OR) using a Minolta CR-300 tristimulus colorimeter (Konica Minolta, Chiyoda, Tokyo) and expressed in accordance with the CIELAB system. Although peel color measurements were determined in order to accurately categorize maturity stages the data is not presented. Subsequently, peels were separated, removed from fruit pulp tissues and were cut into

slices (1 cm thick). Peels and pulp were stored separately in Ziploc® storage bags (SC Johnson, Racine, WI) in -80˚C until further analysis.

Sample Preparation and Antioxidant Analysis

Care was taken to exclude any direct sunlight during extraction and quantification procedures with all sample operations being performed under amber fluorescent lighting conditions (GE F40/G0, 40W). In addition, all glassware was shielded with aluminum foil during laboratory bench operations.

Extraction and Determination of AA

Reduced vitamin C (AA) content was determined by extracting six random sample replicates of 2.5 g of frozen pulp tissue in 10 mL of cold m-phosphoric acid-acetic acid solution (30 g metaphosphoric acid, 0.5 g ethylenediamine tetraacetic acid, EDTA; and 80 mL glacial acetic acid diluted to 1 L with ultrapure water). Samples were sonicated for 15 minutes, centrifuged (Beckman Centrifuge, model J2-21, San Antonio, TX) at 13,000 g_n for 15 minutes at 4˚C, filtered, the diluted to 20 mL. For vitamin C Analysis, 100 L of crude extract was added to 250 µL of KH_2PO_4 (150mM, pH 7.4 and 5 mM EDTA), and a series of reagents (200 µL of trichloroacetic acid [TCA], 200µL of O-phosphoric acid, 200µL of 4% (w/v) 2, 2-bipyridyl dissolved in 70% HPLC grade ethanol and 100µL of 3% [w/v] $FeCl_3$). A standard curve from 0-200 µM was prepared. The absorbance was read at 525 nm using a microplate reader using a microplate reader (Synergy HT, BIO-TEK Instruments, Inc., Winooski, Vermont) and results were expressed as AA mg·100 gfw^{-1}.

Extraction and Determination of TP, FRAP, DPPH, and ABTS Assays

Samples were prepared according to the method of Chen *et al*. (2014) with modifications. Six random sample replicates consisting of 2.5 g, of frozen pulp tissue was extracted with 22.5 mL of ethanol: water solution (50:50; v/v) and homogenized. Pulp homogenates were placed in 50 mL centrifuge tubes (Nalgene™, Thermo Fisher Scientific Inc., Waltham, MA) flushed with nitrogen, and sealed. The tubes were placed in a shaking water bath at 25˚C for 30 min, and then centrifuged at 4000 g_n for 40 min (Beckman Centrifuge, model J2-21, San Antonio, TX). Samples were diluted to a final volume of 30 mL with ethanol: water solution (50:50; v/v) and stored in 80˚C until further analysis.

TP Determination

A standard stock solution of gallic acid was prepared by dissolving 50 mg gallic acid in 5 mL ethanol and brought to 50 mL with ultrapure water. A working stock solution was made by diluting 1 mL of standard stock solution to 10 mL with ultrapure water. A standard curve was prepared ranging from 0-100 μL of gallic acid. An aliquot of 40 μL of standard or sample was added to 200 μL of 1:10 diluted Folin-Ciocalteu's phenol reagent, 2N, and 1 mL of ultrapure water. Samples were vortexed twice over an interval of 2-5 minutes. Next, 160 μL of 7.5% sodium bicarbonate solution (7.5 g sodium bicarbonate in 100 mL ultrapure water) was added to the samples. The samples were vortexed and incubated in a water bath at 40˚C in the dark

for 1 hour. The absorbance was read at 750 nm using a microplate and results were expressed as gallic acid mg·100 gfw^{-1}.

FRAP Determination

Antioxidant power was measured according to Benzie and Strain (1996) with some modification. A sodium acetate buffer, pH 3.6, was prepared by adding 1.55 g sodium acetate trihydrate and 8 mL glacial acetic acid and brought to 500 mL with ultrapure water. A TPTZ solution was prepared with 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) in 40 mM HCl. An iron chloride solution was prepared by dissolving 0.324 g $FeCl_3$ in 100 mL ultrapure water. A final FRAP reagent was prepared by combining 200 mL sodium acetate buffer, 20 mL TPTZ solution, and 20 mL iron chloride solution in an amber Erlenmeyer flask and heating the solution in a water bath at 37˚C for 15 minutes.

Three standards were used in the FRAP assay: gallic acid, $_L$-ascorbic acid, and Trolox. A standard stock solution of gallic acid was prepared by dissolving 50 mg gallic acid in 5 mL ethanol and brought to 50 mL with ultrapure water. A working stock solution was made by diluting 1 mL of standard stock solution to 10 mL with ultrapure water. The linear standard curve was prepared ranging from 0-100 μL of gallic acid. A standard stock of $_L$-ascorbic acid solution was prepared by dissolving 50 mg $_L$-ascorbic acid in 50 mL ultrapure water. A working stock solution of $_L$-ascorbic acid was prepared by diluting 1 mL standard stock in 9 mL of ultrapure water. A working stock solution of $1M \cdot L^{-1}$ (25.3 mg Trolox in 100 mL 80% methanol) was used to create a linear standard curve from 0-600 μM. Aliquots of

sample or standards (10 μL) were added to 290 μL FRAP reagent. The absorbance was read using a microplate reader at 620 nm for all three standards. Results were expressed as vitamin C equivalent antioxidant capacity (VCEAC) (mg AA·100 gfw^{-1}), Trolox equivalent antioxidant capacity (TEAC) (mg TE·100 gfw^{-1}), and gallic acid equivalent antioxidant capacity (GAEAC) (mg GAE·100 gfw^{-1}).

Radical $DPPH^{\bullet}$ Scavenging Activity

A DPPH solution was prepared by dissolving 7.89 mg DPPH in 200 mL 80% methanol. The solution was stirred for 20 minutes, and then adjusted to an absorbance of 650. A 193.33 μL aliquot of adjusted DPPH solution was added 6.67 μL standard or sample. A linear standard curve of 0-100 μM was used for ascorbic acid and a linear standard curve of 0-600 μM was used for Trolox. A microplate blank of 80% methanol (pH 7.4) was used to eliminate external interference. The samples were read at 517 nm and results were expressed as VCEAC (mg AA·100 gfw^{-1}) and TEAC (mg TE·100 gfw^{-1}).

Radical Cation $ABTS^{\bullet+}$ Scavenging Activity

A phosphate buffer solution (PBS) was prepared by adjusting a dibasic phosphate solution (100 mM K_2HPO_4 + 150 mM NaCl) to pH 7.4 using a monobasic phosphate solution (100 mM KH_2PO_4 + 150 mM NaCl). Stock solutions of ascorbic acid (100mM) and Trolox (1M) were used to make an ascorbic acid linear standard curve of 0-100 μM and a Trolox linear standard curve of 0-600 μM.

An ABTS solution was prepared with 2.5 mM ABTS + 1 mM AAPH in 100 mL PBS. The solution was heated in a shaking water bath set at 68˚C and 90 RPM for 1 hour, cooled, and filtered using a ZAPCAP®-CR filter (Whatman Inc, Florham Park, NJ). The ABTS solution was adjusted to 0.650±0.20 at 734 mM using PBS solution and incubated at 37˚C. The samples were read at 734 nm for 30 minutes at 37˚C. Results were expressed as VCEAC (mg AA·100 gfw^{-1}) and TEAC (mg TE·100 gfw^{-1}).

Statistical Analysis

Analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.3 (SAS Institute, Cary, NC). A completely randomized design was used. The treatment design was a factorial of maturity stage by cultivar. Where residual plots and a significant covariance test for homogeneity (COVTEST statement) indicated heterogeneous variance, a RANDOM statement with the GROUP option was used in the analysis. Least squares means comparisons between maturity stages and cultivars were compared using the Shaffer Simulated method. Pearson Correlation test was performed to determine correlations among responses. Statistical difference was determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

AA Analysis

There was an interactive effect between maturity and cultivar (Table 9). AA had varying trends for each of the three cultivars. AA was undetectable in the mature green and transitional stages of 'Hua Moa', but was highest at the over ripe stage (4.3

mg AA·100 gfw^{-1}). 'Kandarian' pulp reduced vitamin C contents were higher than 'Hua Moa' fruit at all maturity stages. 'Kandarian' AA content peaked the transitional stage (17.0 mg AA·100 gfw^{-1}), declined at the ripe and over ripe stages (8.8 and 9.5 mg AA·100 gfw^{-1}, respectively). 'Williams' fruit reduced vitamin C content was higher at the mature green and transitional stages (25.7 and 24.0 mg AA·100gfw^{-1}, respectively) and then decreased at the ripe and over ripe stages (7.8 and 8.5 mg AA·100 gfw^{-1}, respectively). However, 'Williams' reduced vitamin C content was higher than 'Kandarian' and 'Hua Moa' fruit at all four maturity stages. It is likely that the variation in AA contents in both 'Kandarian' and 'Williams' fruit between maturity stages is due to the cycling between ascorbic acid and dehydroascorbic acid mechanisms during ripening.

TP Analysis

There was an interactive effect between maturity and cultivar (Table 9). 'Hua Moa' fruit had the lowest TP content compared to 'Kandarian' and 'Williams' in mature green, transitional, and over ripe stages. 'Williams' fruit had the highest TP content compared to 'Hua Moa' and 'Kandarian at mature green, transitional, and over ripe stages. At the ripe stage, there were no differences in TP content of 'Hua Moa', 'Kandarian', and 'Williams' fruit.

At the mature green stage, 'Kandarian' and 'Williams' fruit had higher TP content than 'Hua Moa' fruit (32.2, 25.6, and 15.3 mg GAE·100 gfw^{-1}, respectively). TP content in 'Kandarian' fruit at the transitional stage was not different from 'Hua Moa' fruit (22.6 and 17.5 mg GAE·100 gfw^{-1}, respectively), but 'Williams' fruit TP

content (35.5 mg GAE·100 gfw^{-1}) was higher than 'Hua Moa' and 'Kandarian'. At the over ripe stage, 'Kandarian' fruit TP content was similar to both 'Hua Moa' and 'Williams' fruit. However, 'Williams' TP content was higher than 'Hua Moa' TP content at the over ripe stage. Physiological maturity did not have an effect on 'Hua Moa' and 'Kandarian' fruit. However, in 'Williams' fruit, TP content was lowest at the mature green stage and increased progressively until the over ripe stage.

Ferric Reducing Antioxidant Power (FRAP) Assay

Vitamin C Equivalent Antioxidant Capacity (VCEAC)

FRAP assay results using the ascorbic acid standard were expressed as vitamin C equivalent antioxidant capacity (VCEAC) (Table 9). There was an interactive effect between maturity and cultivar. 'Williams' and 'Kandarian' fruit had the highest VCEAC in mature green, transitional, and over ripe stages when compared with 'Hua Moa' and fruit. 'Kandarian' fruit VCEAC was consistent with 'Williams' fruit with the exception of the ripe stage, where 'Kandarian' VCEAC was lower (22.3 and 58.7 mg AA·100 $gfw^{-1}$for 'Kandarian' and 'Williams', respectively). In all maturity stages, 'Hua Moa' fruit had lower VCEAC than 'Kandarian' and 'Williams' fruit.

'Hua Moa' and 'Williams' VCEAC were not affected by physiological maturity. 'Kandarian' fruit did not have any differences in VCEAC in mature green, transitional, and over ripe stages, however 'Kandarian' VCEAC declined significantly in the ripe stage.

Trolox Equivalent Antioxidant Capacity (TEAC)

FRAP assay results using the Trolox standard were expressed as Trolox equivalent antioxidant capacity (TEAC) (Table 10). Only the cultivar main effect was significant. 'Hua Moa' fruit had the overall lowest TEAC (87.7 mg TE·100 gfw^{-1}), followed by 'Kandarian' (200.0 mg TE·100 gfw^{-1}) and 'Williams' (308.5 mg TE·100 gfw^{-1}) fruit. The wide variation in TEAC levels between replications within each maturity stage is likely responsible for the fact that physiological maturity did not have an impact on any of the selected cultivars. A Pearson's correlation analysis revealed that there was a strong relationship between FRAP, VCEAC and FRAP, TEAC (Table 11).

Gallic Acid Equivalent Antioxidant Capacity (GAEAC)

FRAP assay results using a gallic acid standard were expressed as gallic acid equivalent antioxidant capacity (GAEAC) (Table 10). Only the cultivar main effect was significant. 'Hua Moa' fruit had the overall lowest FRAP, GAEAC (3.1 mg GAE·100 gfw^{-1}). 'Kandarian' and 'Williams' fruit were not different from each other (11.4 and 13.1 mg GAE·100 gfw^{-1}, respectively); however both were higher than 'Hua Moa' fruit. The wide variation in GAEAC levels between replications within each maturity stage is likely responsible for the fact that physiological maturity did not have an impact on any of the selected cultivars. There was a strong relationship between FRAP, GAEAC and TP meaning the ethanolic extracts may contribute more to the antioxidant capacity of ripened fruit tissues (Table 11).

Radical DPPH$^{\bullet}$ and Radical Cation ABTS$^{\bullet+}$ Scavenging Activity

For DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging activity assays, a vitamin C standard was used to determine the capacity of water-soluble antioxidants (VCEAC) and a Trolox standard was used to determine the capacity of lipid-soluble antioxidants (TEAC) (Table 10). In all DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging activity assays, only the cultivar main effect was significant. 'Hua Moa' fruit DPPH$^{\bullet}$ activity was not estimable (VCEAC or TEAC). This is likely related to the fact that 'Hua Moa' VCEAC and TEAC contents were not detected at the mature green and transitional stages in the DPPH$^{\bullet}$ assay. 'Williams' fruit had higher DPPH, VCEAC than 'Kandarian' fruit (41.3 and 29.2 mg AA·100 gfw^{-1}, respectively). A similar trend was observed in DPPH, TEAC (90.5 and 62.2 mg TE·100 gfw^{-1} in 'Williams' and 'Kandarian' fruit, respectively).

In the radical cation ABTS$^{\bullet+}$ scavenging assay, 'Hua Moa' fruit had the lowest VCEAC and TEAC contents (24.7 mg AA·100 gfw^{-1}and 136.7 mg TE·100 gfw^{-1}), followed by 'Kandarian' (62.3 mg AA·100 gfw^{-1}and 250.1 mg TE·100 gfw^{-1}) and 'Williams' (89.2 mg AA·100 gfw^{-1}and 338.7 mg TE·100 gfw^{-1}) fruit (Table 10). The lack of effect of physiological maturity in DPPH and ABTS assays is likely due to extreme variation in VCEAC and TEAC between replications in each maturity stage. There was a strong relationship between ABTS, VCEAC and TEAC (Table 11). In addition, there was a strong relationship between DPPH, TEAC and ABTS, VCEAC and TEAC.

CONCLUSIONS

There are several events that can affect the bioactive activity of bananas. Natural processes associated with ripening are known to influence the accumulation of these compounds including cultivar, ripening processes, chilling injury, mechanical injury, and bruising. In all antioxidant determination and capacity assays, the general trend was that 'Williams' fruit had the highest antioxidant activity overall, followed by 'Kandarian' and 'Hua Moa' fruit. Physiological maturity and cultivar had an interactive effect on 'Hua Moa', 'Kandarian', and 'Williams' fruit in AA, TP, and antioxidant capacity (FRAP, VCEAC) assays. However, only cultivar had a significant effect in the following antioxidant capacity assays: FRAP, TEAC and GAEAC; DPPH, VCEAC; and TEAC, and ABTS, VCEAC and TEAC. This is likely a result of the extreme variation in VCEAC, TEAC, or GAEAC contents between replications in each treatment. It is possible from the time the fruit were processed to the time they were placed in cold storage; the browning of the pulp may have affected the antioxidant activities. It was reported that when pulp browning is induced, dopamine is oxidized and phenylalanine-ammonia lyase (PAL) and polyphenoloxidase (PPO) accumulate. The increase in PAL and PPO activity is correlated to the decrease in free phenolic activity in 'Kluai Khai' (AA) and 'Kluai Hom Thong' (AAA) bananas (Nguyen, 2003). It can be assumed that the accumulations of PAL and PPO have additional effects on antioxidant activity. Further studies on the relationship of browning enzyme accumulation and antioxidant activities should be performed.

IV. Conclusions

Results from both experiments indicated that artificial ripening with 500-ppm ethephon and physiological maturity affected various physicochemical and antioxidant activities of short-season cold-hardy cultivars grown in the southeastern United States. The application of ethephon had an effect on physicochemical properties of *Musa* ABB Group cultivars 'Kandarian' and 'Ice Cream'. In contrast, physicochemical properties of *Musa* AAB Group cultivars 'Ele Ele' and 'Brazilian' were least affected by ethephon treatment. In addition, 'Kandarian' fruit pulp reduced, oxidized, and total vitamin C contents were enhanced by ethephon treatment until the 6th day in storage at 20˚C and 95% RH. 'Ele Ele' fruit pulp reduced, oxidized, and total vitamin C were optimal in control fruit until day 9, where ethephon-treated fruit vitamin C content surpassed control fruit. All forms of vitamin C content in 'Brazilian' fruit were unaffected by ethephon treatment during the first half of the storage period, and then ethephon-treated fruit had lower vitamin C content than control fruit at the second half of the storage period. Results indicated that additional experiments need to be performed in order to determine optimal artificial ripening practices for varying genome groups of short-season cultivars grown in the southeastern United States.

In all antioxidant determination and capacity assays, the general trend was that 'Williams' fruit had the highest antioxidant activity overall, followed by 'Kandarian'

and ‘Hua Moa’ fruit. Physiological maturity and cultivar had interactive effects on reduced vitamin C, total phenolics, and antioxidant capacity (FRAP VCEAC) properties of ‘Hua Moa’ (AAB), ‘Kandarian’ (ABB), and ‘Williams’ (AAA) cultivars. However, only cultivar had an effect in antioxidant capacity (FRAP TEAC and GAEAC), $DPPH^{\bullet}$ radical scavenging, and radical cation $ABTS^{\bullet+}$ scavenging activity assays. This is likely due to the effect of accumulation of browning enzymes after the fruit was processed. Further research on the metabolic effects of browning enzyme accumulation should be performed in order to fully understand the antioxidant activity of short-season banana cultivars at various physiological maturity stages.

Results of this study will assist banana producers and commercial retailers in selecting adaptable cultivars, optimal ripening stage, and best management practices (degreening and storage) which enhance quality and nutritional content of short season banana cultivars adaptable to southeastern United States. In addition, information provided from these experiments will assist banana producers in identifying superior cultivars with desirable antioxidant traits to increase potential market demand, sustainability, fruit storage life and daily antioxidant intake of residents in southeastern United States.

V. References

Adao, R. 2005. Bioactive amines and carbohydrate changes during ripening of 'Prata' banana (*Musa acuminata* × *M. balbisiana*). Food Chem. 90(4): 705–711.

Adobe® Photoshop® 6.0 User Guide for Windows® and Macintosh. 2000. Adobe Systems Inc. 13(2): 111–122.

Applications Note: Hunter L, a, b Color Scale. 2008. p. 4. *In* Insight on Color. Hunter Assoc. Lab., Inc, Reston, VA.

Alvindia, D.G. 2012. Revisiting hot water treatments in controlling crown rot of banana cv. Buñgulan. Crop Protection. 33:59-64.

Anyasi, T.A., A.I.O. Jideani, and G.R.A. Mchau. 2013. Functional properties and postharvest utilization of commercial and noncommercial banana cultivars. Comprehensive Rev. Food Sci. and Safety. 12:509-522.

Arêas, J.A.G. and F.M. Lajolo. 1981. Starch transformations during banana ripening: I. the phosphorylase and phosphatase behavior in *Musa acuminata*. J. Food Biochem. 5:19-37.

Aurore, G., B. Parfait, and L. Fahrasmane. 2009. Bananas, raw materials for making processed food products. Trends in Food Sci. & Tech. 20(2): 78–91.

Banana Market Review and Banana Statistics 2012-2013. 2014. Food and Agr. Organization of the United Nations, Rome.

Benzie, I.F., and J.J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a

measure of “antioxidant power”: the FRAP assay. Anal. Biochem. 239(1): 70–76.

Borges, C.V., V.B. de O. Amorim, F. Ramlov, C.A. da S. Ledo, M. Donato, M. Maraschin, and E.P. Amorim. 2014. Characterisation of metabolic profile of banana genotypes, aiming at biofortified Musa spp. cultivars. Food Chem. 145: 496–504.

Bugaud, C., P. Cazevielle, M. Daribo, N. Telle, P. Julianus, B. Fils-Lycaon, D. Mbéguié-A-Mbéguié. 2013. Rheological and chemical predictors of texture and taste in dessert banana (*Musa* spp.). Postharvest Biol. and Technol. 84: 1-8.

Bugaud, C. E. Deverge, M.O. Daribo, F. Ribeyre, B.F. Lycaon, and D.M. A. Mbéguié. 2011. Sensory characterization enabled the first classification of dessert bananas. J. Sci. Food Agric. 91:992-1000.

Bugaud, C., P. Alter, M.O. Daribo and J.M. Brillouet. 2009. Comparison of the physico-chemical characteristics of a new triploid banana hybrid, FLHORBAN 920, and the Cavendish variety. J. Sci. Food Agric 89:407-413.

Cano, A., J. Hernandez-Ruiz, F. Garcia-Canovas, M. Acosta, and M.B. Arnao. 1998. An end-point method for estimation of the total antioxidant activity in plant material. Phytochemical Analysis 9(4): 196–202.

Cano, M.P., B de Ancos, M.C. Matallana, M. Cámara, G. Reglero, and J. Tabera. 1997. Differences among Spanish and Latin-American banana cultivars: morphological, chemical, and sensory characteristics. Food Chem. 59(3): 414-419.

Chacon, S.I., F. Viquez, and G. Chacon. 1987. Physico-chemical profile of banana ripening. Fruits. 42:95-102.

Chen, G.L., S.G. Chen, Y.Y. Zhao, C.X. Luo, J. Li, and Y.Q. Gao. 2014. Total phenolic contents of 33 fruits and their antioxidant capacities before and after in vitro digestion. Ind. Crops and Prod. 57, 150–157.

Cheynier, V., 2012. Phenolic compounds: from plants to foods. Phytochemistry Rev. 11:153–177.

Crane, J.H., C.F. Balerdi, and I. Maguire. 2006. Banana Growing in the Florida Home Landscape. HS-10 Univ. of Florida-IFAS, Coop. Ext. Serv., Gainesville, FL: 1–13.

Dadzie, B. 1998. Post-harvest characteristics of black Sigatoka resistant banana, cooking banana and plantain hybrids. Technical Guidelines INIBAP 4. Intl. Plant Genet. Resources Inst. Rome, Italy.

Davey, M.W., I. V.D. Begrgh, R. Markham, R.Wennen, and J. Keulemans. 2009. Genetic variability in *Musa* fruit provitamin A carotenoids, lutein and mineral micronutrient contents. Food Chem. 115:806-813.

Del Mar Verde Méndez, C., M.P. Forster, M.Á. Rodríguez-Delgado, E.M. Rodríguez-Rodríguez, and C. Díaz Romero. 2003. Content of free phenolic compounds in bananas from Tenerife (Canary Islands) and Ecuador. European Food Res. and Tech. 217(4): 287–290.

Deutsch, J.C., 2000. Dehydroascorbic acid. J. of Chromatography A. 881(1-2):299–307.

Ewané, C.A., P. Lepoivre, L. de Lapeyre de Bellaire, and L. Lassois. 2012.

Involvement of phenolic compounds in the susceptibility of bananas to crown rot. A review. Biotechnol. Arong. Soc. Environ. 16(3):393–404.

Fernando, H.R.P., V. Srilaong, N. Pongprasert, P. Boonyaritthongchai, and P. Jitareerat. 2014. Changes in antioxidant properties and chemical composition during ripening in banana variety 'Hom Thong' (AAA group) and 'Khai' (AA group). Intl. Food Res. J. 21:749-754.

Food and Agriculture Organization (FAO). 2003. FAO Stat. 19 Jul 2015. <http://faostat3.fao.org/faostat-gateway/go/to/home/E>.

Food and Agriculture Organization (FAO). 2014. FAO Stat.. 6 Jun 2015. <http://faostat3.fao.org/faostat-gateway/go/to/home/E>.

Fonsah, E.G., G. Krewer, and M. Rieger. 2003. Banana cultivar trials for fruit production, ornamental-landscape use, and ornamental-nursery production in South Georgia. J. of Food Distrib. Res. 35(1): 86–92.

Fonsah, E.G, Krewer, G. and M. Rieger. 2005. Second year banana cultivars trial in South Georgia". J. of Food Distrib. Research, 36(1): 48-54.

Fonsah, E.G., G. Krewer, R. Wallace, and B. Mullinix. 2007. Banana trials: A potential niche and ethnic market in Georgia. J. of Food Distrib. Res. 38.

Fonsah, E.G., B. Bogortti, P. Ji, P. Sumner, and W. Hudson. 2010. New banana cultivars trials in the Coastal Plain of South Georgia. J. of Food Distrib. Res. 41(1): 46-50.

Fonsah, E.G., W. Hudson, P. Sumner, and J. Massonnat. 2011. Growth performance and economical potential of 'Veinte Cohol': A short-cycle banana cultivar produced in the Coastal Plain of Georgia. J. of Food Distrib. Res. 42(1): 48.

Fonsah, E.G. and A.S.N.D. Chidebelu. 2012. Economics of banana production and marketing in the tropics: A case study of Cameroon. Langaa RPCIG, Bamenda, North West Region, Cameroon.

Gaikwad, P., A. Barik, K.I. Priyadarsini, and B.S.M. Rao. 2010. Antioxidant activities of phenols in different solvents using DPPH assay. Res. on Chemical Intermediates 36(9): 1065–1072.

Gomes, J.F.S., R.R. Vieira, and F.R. Leta. 2013. Colorimetric indicator for classification of bananas during ripening. Scientia Hort. 150:201–205.

Gülçin I. 2012. Antioxidant activity of food constituents: an overview. Arc Toxicol. 86:345-391.

Gutiérrez-Martínez, P., R.C. Avila-Peña, D. Sivakumar, and S. Bautista-Baños. 2015. Postharvest evaluation of Goldfinger banana (FHIA-01) at different storage temperatures followed by an acclimation time. Fruits 70(3): 173–179.

Hailu, M., T.S. Workneh, and D. Belew. 2012. Review on postharvest technology of banana fruit. Afr. J. Biotechnol. 12(7):635-647.

Hashim, N., R.B. Janius, L. Baranyai, R.A. Rahman, A. Osman, M. Zude. 2012. Kinetic model for colour changes in bananas during the appearance of chilling injury. Food Bioprocess. Technol. 5:2952-2963.

Herbert, J.A., and D. Marx. 1990. Short-term control of Panama disease of bananas in South Africa. Phytophylactica 22(3): 339–340.

Hernandez, Y., M.G. Lobo, and M. González. 2006. Determination of vitamin C in tropical fruits: A comparative evaluation of methods. Food Chem. 96(4):654-664.

Huang, S. and K. Huang. 2007. Increased imports of U.S. fresh fruits and vegetables. FTS-328-01. United States Department of Agriculture. <www.ers.usda.gov>.

Kader, A., B. Mitcham, and B. Hess-Pierce. 2011. Optimum Procedures for Ripening Bananas. p. 49–57. In: Fruit Ripening & Retail Handling. UC Davis Postharvest Technol.

Kesari, R., P.K. Trivedi, and P. Nath. 2007. Ethylene-induced ripening in banana evokes expression of defense and stress related genes in fruit tissue. Postharvest Biol. and Tech. 46(2): 136–143.

Kulkarni, S.G., V.B. Kudachikar, and M.N. Keshava Prakash. 2011. Studies on physico-chemical changes during artificial ripening of banana (*Musa* sp) variety "Robusta." J. of Food Sci. and Tech. 48(6): 730–734.

Lescot, T., and Staver, C. 2010. Bananas, plantains and other species of Musaceae. Quality Declared Planting Material. Protocols and Standards for Vegetatively Propagated Crops. 15-32.

Lima, O.S., E.G. Souza, E.P. Amorim, and M.E.C. Pereira. 2014. Ripening and shelf life of 'BRS Caipira' banana fruit stored under room temperature or refrigeration. Ciência Rural 44(4): 734–739.

Liu, J., B. Xu, L. Hu, M. Li, W. Su, J. Wu, J. Yang, and Z. Jin. 2009. Involvement of a banana MADS-box transcription factor gene in ethylene-induced fruit ripening. Plant Cell Rpt. 28(1): 103–111.

Lobo, M.G., M. Gonzalez, A. Pena, and A. Marrero. 2005. Effects of ethylene

exposure temperature on shelf life, composition and quality of artificially ripened bananas (*Musa acuminata* AAA, cv. "Dwarf Cavendish"). Food Sci. Technol. Int. 11(2): 99–105.

MacLean, D. and G. Krewer. 2009. Postharvest quality and ripening of a novel Georgia grown banana. Proc. Southeast Regional Veg Conference. p. 49. Savannah International Trade & Convention Center, Savannah, Georgia (Poster).

Maqbool, M., A. Ali, P.G. Alderson, N. Zahid, and Y. Siddiqui. 2011. Effect of a novel edible composite coating based on gum Arabic and chitosan on biochemical and physiological responses of banana fruits during cold storage. J. Agric. Food Chem. 59(10): 5474–5482

Marín, D.H., R.A. Romero, M. Guzman, and T.B. Sutton. Black Sigatoka: An increasing threat to banana cultivation. 2003. Plant Dis. 87(3):208.

Mathews, S., 2014. Chapter 14: Ascorbic Acid - Vitamin C. Auburn Univ. Auburn, AL. 6 Feb. 2014. Lecture.

Meullenet, J.F., 2012. Apparatus and method for predicting meat tenderness. US Patent 8225645B2.

Miller, N. J., C. Rice-Evans, M.J. Davies, V. Gopinathan, and A. Milner. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Sci. 84: 407.

Miller , N.J. and C.A. Rice-Evans. 1997. Factors influencing the antioxidant activity determined by the ABTS•+ radical cation assay. Free Radical Res. 26(3): 195-199.

Mohapatra, D., S. Mishra, C.B. Singh, D.S. Jayas. 2010. Post-harvest processing of banana: opportunities and challenges. Food Bioprocess Technol. 4:327-339.

Muhammed, A, Zahniser, S. and E.G. Fonsah. 2015. A dynamic analysis of US banana demand by source: a focus on Latin American suppliers. Intl. J. Trade and Global Markets.

Naidu, K.A., 2003. Vitamin C in human health and disease is still a mystery? An overview. Nutr. J. 2:7.

Newley, P., A. Akehurst, and B. Campbell. 2008. Banana growing guide: Cavendish bananas. 18 Aug 2014. <www.dpi.nsw.gov.au>.

Nguyen, T. 2003. Relationship between browning and the activities of polyphenoloxidase and phenylalanine ammonia lyase in banana peel during low temperature storage. Postharvest Biol. and Tech. 30(2):187–193.

Nunes, C.N., Y.Yagiz, and J.P. Emond. 2013. Influence of environmental conditions on the quality attributes and shelf life of 'Goldfinger' bananas. Postharvest Biol. Technol. 86:309-320.

Palmer, J.K. 1963. Banana polyphenoloxidase. Preparation and properties. Plant Physiol. 38(5):508.

Pereira, A., and M. Maraschin. 2015. Banana (*Musa* spp) from peel to pulp: Ethnopharmacology, source of bioactive compounds and its relevance for human health. J. of Ethnopharmacology 160:149–163.

Peroni-Okita, F.H.G., M.B. Cardoso, R.G.D. Agopian, R.P. Louro, J.R.O. Nascimento, E. Purgatto, M.I.B. Tavares, F.M. Lajolo, and B.R. Cordenunsi. 2013. The cold storage of green bananas affects the starch degradation during

ripening at higher temperature. Carbohydrate Polymers. 96:137-147.

Ploetz, R.C. 2000. Panama Disease: A classic and destructive disease of banana. Plant Health Progress.

Ploetz, R.C., A.K. Kepler, J. Daniells, and S.C. Nelson. 2007. Banana and plantain – an overview with emphasis on Pacific Island cultivars. 15 May 2015. <www.traditionaltree.org>.

Promyou, S., S. Ketsa, and W.G. van Doorn. 2007. Hot water treatments delay cold induced banana peel blackening. Postharvest Biol. Technol. 48:132-138.

Ramaswamy, H. 2014. Post-harvest technologies for fruits and vegetables. DEStech Publ., Inc.

Re, R. N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med. 26:1231-1237.

Reynolds-Allie, K.M. 2013. Three Essays on Factors Influencing the Demand of Local and International Food Products. Auburn Univ., Auburn, PhD Diss.

Robinson, J.C.,1996. Distribution and importance; taxonomic classification, cultivars and breeding. In: Robinson, J.C. Banana and Plantains,1–33.

Salvador, A., T. Sanz, and S.M. Fiszman. 2007. Changes in colour and texture and their relationship with eating quality during storage of two different dessert bananas. Postharvest Biol. and Tech. 43(3): 319–325.

Shiga, T.M., C.A. Soares, J.R. Nascimento, E. Purgatto, F.M. Lajolo, B.R. Cordenunsi. 2011. Ripening-associated changes in the amounts of starch and non-starch polysaccharides and their contributions to fruit softening in three

banana cultivars. J. Sci. Food Agric. 91:1511-1516.

Slabaugh, W.R. and M.D. Grove. 1982. Postharvest Diseases of Bananas and Their Control. Plant Dis. 66:746-750.

Su, C.H., A. Ishdorj, and D.J. Leatham. 2011. An analysis of the banana import market in the US. Annu. Mtg., Corpus Christi, 5-8 Feb. 2011. Southern Agric. Econ. Assn.

Sulaiman, S.F., N.A. Md. Yusoff, I.M. Eldeen, E.M. Seow, A.A.B. Sajak, Suprianto, and K.L. Ooi. 2014. Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (*Musa* sp.). J. Food Compos. Anal. 24:1-10.

Stover, R.H., and N.W. Simmonds. 1987. Classification of banana cultivars. In R. H. Stover & N. W. Simmonds (Eds.), Bananas (3rd ed., pp. 97–103). New York: Wiley.

Szeto, Y.T., B. Tomlinson, and I.F.F. Benzie. 2002. Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. Brit. J. of Nutr. 87(01): 55.

Thaiphanit, S. and P. Anprung. 2010. Physicochemical and flavor changes of fragrant banana (*Musa acuminata* AAA group 'Gross Michel') during ripening. J. of Food Processing and Preservation. 34:366-382.

Toraskar, M.V. and V.V. Modi. 1984. Postharvest and Chilling Injury in Banana Fruit. J. Agric. Food Chem. 32:1352-1354.

Tsamo, C.V.P., C.M. Andre, C. Ritter, K. Tomekpe, G.N. Newilah, H. Rogez, and Y. Larondelle. 2014. Characterization of *Musa* sp. Fruits and plantain banana

ripening stages according to their physicochemical attributes. J. Agric. Food Chem. 62:8705-8715.

Uclés Santos, J.R., F. Bakry, and J.M. Brillouet. 2010. A preliminary chemotaxonomic study on the condensed tannins of green banana flesh in the *Musa* genus. Biochem. Systematics and Ecol. 38:1010-1017.

Ummarat, N., T.K. Matsumoto, M.M. Wall, and K. Seraypheap. 2011. Changes in antioxidants and fruit quality in hot water-treated 'Hom Thong' banana fruit during storage. Sciencia Hort. 130:801-807

Villa-Rodriguez, J.A., H. Palafox-Carlos, E.M. Yahia, J.F. Ayala-Zavala, and G.A. Gonzalez-Aguilar. 2015. Maintaining antioxidant potential of fresh fruits and vegetables after harvest. Critical Rev. in Food Sci. and Nutr. 55(6): 806–822.

Vincent, K.D., 1993. Colorimeter and calibration system. US Patent 5272518A.

Wall, M.M. 2006. Ascorbic acid, vitamin A, and mineral composition of banana (*Musa sp*.) and papaya (*Carica* papaya) cultivars grown in Hawaii. J. Food Comp. Analysis. 19:434-445.

Wenkam, N.S. 1990. Fruits and Fruit Products: Raw, Processed, and Prepared, p. 1-20. In: Foods of Hawaii and The Pacific Basin. Research Extension. College of Trop. Agr. and Human Resources, Univ. of Hawaii.

Wills, R.B.H., D.R. Harris, L.J. Spohr, and J.B. Golding. 2014. Reduction of energy usage during storage and transport of bananas by management of exogenous ethylene levels. Postharvest Biol. and Tech. 89: 7–10.

Wyman, H. and J.K. Palmer. 1963. Organic acids in the ripening banana fruit. Plant Phys. 39:630-633.

Yam, K.L., and S.E. Papadakis. 2004. A simple digital imaging method for measuring and analyzing color of food surfaces. J. of Food Eng. 61: 137–142.

Yang, S., X. Su, K.N. Prasad, B. Yang, G. Cheng, Y. Chen, E. Yang, and Y. Jiang. 2008. Oxidation and peroxidation of postharvest banana fruit during softening. Pak. J. Bot 40(5): 2023–2029.

VI. Appendices

APPENDIX A. TABLES

Table 1. Physicochemical comparison assay means for ethrel treatments.

	Ethrel (ppm)	Initial	Day 2	Day 4	Day 6
L	0	48.4±1.23	48.4±1.73[z]	47.2±1.20	45.5±1.59
	500		63.5±1.97	68.0±1.27	62.7±1.78
a	0	-12.8±1.88	-2.8±1.71	-12.8±1.93	-11.7±1.02
	500		-8.7±0.46	-3.7±0.36	-1.2±0.42
b	0	20.9±1.49	20.8±1.51	21.2±1.02	20.1±1.53
	500		30.8±1.73	31.5±0.99	33.4±1.42
Pulp Moisture (%)	0	72.1±1.52	72.41±1.78	72.1±1.49	78.46±1.76
	500		72.26±0.28	72.6±1.94	73.88±2.30
pH	0	5.4±0.14	5.4±0.14	5.4±0.22	5.3±0.22
	500		4.6±0.28	4.7±0.16	4.8±0.29
Total Soluble Solids (˚Brix)	0	3.2±0.08	4.3±0.46	5.9±0.57	7.0±0.78
	500		14.4±0.16	23.5±1.08	24.0±1.41
Total Acidity (%)	0	0.24±0.02	0.27±0.02	0.32±0.03	0.34±0.01
	500		0.47±0.01	0.50±0.04	0.54±0.02

[z]Means for ethrel treatments and for storage period differ significantly ($p \leq 0.05$), (n=3).

(Kulkarni *et al*., 2011)

Table 2. Ripening stages for Cavendish bananas as related to skin color and changes in soluble starch and sugars.

Stage	Description
1	Deep Green
2	Green with Traces of Yellow
3	More Green than Yellow
4	More Yellow than Green
5	Yellow, Green Tips
6	All Yellow
7	Yellow with Freckles
8	Yellow with Large Brown Spots (Overripe)

(Cabrera-Padilha *et al*., 2014)

Table 3. Post-harvest qualities of Grand Naine and Williams compared to FHIA-01 and FHIA-02 bananas.

Characteristics	Grand Naine (AAA)	Williams (AAA)	FHIA-01 (AAAB)	FHIA-02 (AAAA)
Peel L value	59.90	57.31	57.29	58.00
Peel a value	-20.11	-20.62	-18.82	-20.87
Peel b value	35.54	34.81	35.14	36.65
Pulp color	white/creamy	white/creamy	white/creamy	white/creamy
Pulp firmness (kgf)	1.65	1.67	0.86	1.30
pH	5.95	6.02	5.77	5.98
Total titratable acidity ($meq \cdot 100\ g^{-1}$)	2.84	2.23	3.39	2.47
Pulp dry matter content (%)	25.95	27.38	23.90	23.79
Pulp moisture content (%)	74.05	72.62	76.10	76.21

(Dadzie, 1998)

Table 4. Optimum Quality Parameters for Ripening Bananas.

	Ripeness stage						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Skin color ('a' value)	-29 to -17	-28 to -14	-18 to -12	-9 to -4	-6 to -2	-3 to -0.5	-2 to -0.4
Pulp firmness	9-11	8-11	2-3	1-2.5	1.5-1.8	1.4-2	0.8-1.2
Soluble solids %	2.0-3.1	2.2-3.3	13.0-14.3	18.9-20.5	19.6-22.4	19.4-20.2	19.4-20.9
Titratable acidity %	0.28-0.31	0.28-0.32	0.46-0.50	0.36-0.40	0.35-0.42	0.35-0.41	0.32-0.34
pH	4.8-5.1	4.8-5.1	4.4-4.9	4.5-4.7	4.5-4.7	4.3-4.5	4.7-4.8

(Kader *et al*., 2011)

Table 5. Effects of storage time on peel ripening color index (RCI) and pulp dry matter content of selected banana cultivars.

			Peel RCI					Pulp dry matter content (%)				
Group	Cultivar	Trt[z]	Days[y]					Days				
			0	3	6	9	Trend[x]	0	3	6	9	Trend
AAB	'Ele Ele'	Control	129.9ns[w]	132.8ns	129.6ns	135.4a	NS	32.8a[v]				
		Ethephon	130.5	151.6	137.6	170.3b	L**	31.5b				
	'Pace'	Control	133.5ns	138.8ns	152.2b	172.3b	L***	30.6ns	31.9ns	32.1a	32.8a	L***
		Ethephon	135.1	148.8	185.9a	233.9a	L***	30.8	31.3	30.7b	29.8b	L*
	'Brazilian'	Control	133.1b	189.7ns	191.2b	292.3b	L***	33.7ns	33.3ns	33.4a	32.9a	NS
		Ethephon	139.4a	221.3	253.3a	343.9a	L***	34.2	32.5	32.0b	30.4b	L***
ABB	'Ice Cream'	Control	127.8ns	141.0b	135.6b	148.9b	L**	36.2a	36.7a	37.0a	37.3a	L***
		Ethephon	132.4	177.3a	209.1a	240.3a	L***	35.3b	35.0b	33.3b	33.0b	L***
	'Kandarian'	Control	133.0a	134.1b	135.3b	157.6b	L**	32.0ns	30.8ns	30.8b	31.0a	NS
		Ethephon	129.3b	169.7a	174.7a	242.0a	L***	31.3	28.9	32.5a	28.2b	L*
AABB	'Sweet Heart'	Control	148.6ns	167.2b	257.4	-[u]		36.2a	35.6ns	35.4ns	-	L*
		Ethephon	143.3	232.3a	-	-		35.7b	35.2	36.2	-	Q*

[z]Treatment (Trt) by immersion of fruit in 500-ppm ethephon or water (control) for 5 minutes at 20˚C.
[y]Number of days in storage (20˚C and 95% RH). The treatment by days in storage interaction was significant at $\alpha = 0.05$.
[x]Trend not significant (NS), linear (L), or quadratic (Q) using orthogonal polynomials at $\alpha = 0.05$ (*), 0.01 (**), or 0.001(***).
[w]Least squares means comparisons in Trt for each day (in columns) using the Shaffer Simulated method at $\alpha = 0.05$. ns=not significant.
[v]Only the treatment main effect was significant $\alpha = 0.05$.
[u]Data not collected due to extreme decay.

Table 6. Effects of storage time on pulp pH and titratable acidity of selected cultivars.

Group	Cultivar	Trt[z]	Pulp pH					Pulp Titratable Acidity (%)				
			Days[y]					Days				
			0	3	6	9	Trend[x]	0	3	6	9	Trend
AAB	'Ele Ele'	Control	6.2ns[w]	6.1c	6.2a	6.2a	NS	0.06b[v]				
		Ethephon	6.2	5.5b	5.8b	5.5b	L***	0.07a				
	'Pace'	Control	6.2ns	6.0a	5.4a	5.1a	L***	0.06ns	0.06ns	0.07b	0.08b	L***
		Ethephon	6.2	5.7b	4.8b	4.8b	L***	0.06	0.07	0.10a	0.12a	L***
	'Brazilian'	Control	5.7ns	4.9ns	5.3a	4.8ns	L***	0.07ns	0.10b	0.07b	0.14ns	L***
		Ethephon	5.77	4.70	4.54b	4.7	Q***	0.06	0.12a	0.16a	0.14	L***
ABB	'Ice Cream'	Control	6.3ns	6.4a	6.4a	6.2a	NS	0.06ns	0.06ns	0.06b	0.06b	NS
		Ethephon	6.8	5.8b	5.1b	4.8b	L***	0.06	0.06	0.07a	0.11a	Q***
	'Kandarian'	Control	6.2ns	5.9ns	6.1ns	5.8a	NS	0.07ns	0.06ns	0.06ns	0.06b	NS
		Ethephon	5.9	5.9	6.0	4.7b	Q***	0.07	0.06	0.06	0.14a	Q***
AABB	'Sweet Heart'	Control	6.6ns	6.1a	4.9ns	-[u]	L***	0.05ns	0.06b	0.08ns	-	Q***
		Ethephon	6.8	4.9b	5.0	-	Q***	0.05	0.08a	0.08	-	Q***

[z]Treatment (Trt) by immersion of fruit in 500-ppm ethephon or water (control) for 5 minutes at 20˚C.
[y]Number of days in storage (20˚C and 95% RH). The treatment by days in storage interaction was significant at $\alpha = 0.05$.
[x]Trend not significant (NS), linear (L), or quadratic (Q) using orthogonal polynomials at $\alpha = 0.05$ (*), 0.01 (**), or 0.001(***).
[w]Least squares means differences in Trt for each day (in columns) using the Shaffer Simulated method at $\alpha = 0.05$. ns=not significant.
[v]Only the treatment main effect was significant at $\alpha = 0.05$.
[u]Data was not collected due to extreme decay.

Table 7. Effects of storage time on pulp soluble solid content (˚Brix) of selected cultivars.

Group	Cultivar	Trt[z]	Days[y]				Trend[x]
			0	3	6	9	
AAB	'Ele Ele'	Control	4.4ns[w]	5.6b	5.0ns	5.6ns	NS[w]
		Ethephon	4.8	10.9a	6.3	7.8	Q*
	'Pace'	Control	3.5ns	3.5ns	5.7b	11.2b	L***
		Ethephon	3.5	5.3	12.9a	16.5a	L***
	'Brazilian'	Control	3.8ns	12.6b	7.9b	25.2ns	L***
		Ethephon	4.6	18.9a	27.8a	26.7	L***
ABB	'Ice Cream'	Control	3.8ns	4.3b	5.9b	5.4b	L*
		Ethephon	4.5	7.1a	14.4a	20.2a	L***
	'Kandarian'	Control	3.7ns	3.2ns	2.6ns	4.0b	NS
		Ethephon	2.7	3.4	4.2	22.5a	Q***
AABB	'Sweet Heart'	Control	3.3ns	7.4b	23.9ns	- [u]	Q**
		Ethephon	4.9	22.7a	25.3	-	Q***

[z]Treatment (Trt) by immersion of fruit in 500-ppm ethephon or water (control) for 5 minutes at 20˚C.
[y]Number of days in storage (20˚C and 95% RH). The treatment by days in storage interaction was significant at $\alpha = 0.05$.
[x]Trend not significant (NS), linear (L), or quadratic (Q) using orthogonal polynomials at $\alpha = 0.05$ (*), 0.01 (**), or 0.001(***).
[w]Least squares means differences in Trt for each day (in columns) using the Shaffer Simulated method
at $\alpha = 0.05$. ns=not significant.
[v]Data was not collected due to extreme decay

Table 8. Effects of storage time on pulp vitamin C content of selected cultivars.

Cultivar	Trt[z]	Reduced (mg·100 gfw^{-1})				Oxidized (mg·100 gfw^{-1})				Total[x] (mg·100 gfw^{-1})			
		Days[y]				Days				Days			
		0	3	6	9	0	3	6	9	0	3	6	9
'Kandarian'	Control	4.6b[w]	0.2b	3.4b	4.0a	5.6a	0.2b	3.3ns	4.6a	11.1b	0.4b	7.6b	9.6a
(ABB)	Ethephon	7.9a	4.8a	7.5a	0.7b	4.1b	4.4a	3.2	0.6b	13.0a	10.5a	11.3a	1.4b
'Ele Ele'	Control	2.8a	1.3a	3.0a	2.7b	2.8a	1.0a	2.4a	2.1b	5.6a	2.5a	5.9a	5.4b
(AAB)	Ethephon	2.6b	0.6b	2.3b	5.8a	2.3b	0.5b	1.3b	4.4a	5.1b	1.2b	4.1b	11.3a
'Brazilian'	Control	0.2ns	0.4ns	0.9ns	0.7a	0.4ns	0.8ns	0.8a	0.3ns	0.6ns	1.1ns	1.6a	1.0ns
(AAB)	Ethephon	0.3	0.6	0.8	0.3b	0.4	0.4	0.6b	0.3	0.80	1.0	1.4	0.6

[z]Treatment (Trt) by immersion of fruit in 500-ppm ethephon or water (control) for 5 minutes at 20°C.

[y]Number of days in storage (20°C and 95% RH). The treatment by days in storage interaction was significant at $\alpha = 0.05$.

[x]Total vitamin C content was described as the sum of reduced and oxidized vitamin C content

[w]Least squares means differences in Trt for each day (in columns) using the Shaffer Simulated method at $\alpha = 0.05$. ns=not significant.

Table 9. Interactive effects of maturity stage and genome on vitamin C, total phenolics, and antioxidant capacity (FRAP VCEAC) in selected cultivars.

Maturity Stage[z]	Reduced Vitamin C (mg AA ·100 gfw^{-1})			Total Phenolics (mg GAE·100 gfw^{-1})			FRAP VCEAC (mg AA·100 gfw^{-1})		
	'Hua Moa' (AAB)	'Kandarian' (ABB)	'Williams' (AAA)	'Hua Moa' (AAB)	'Kandarian' (ABB)	'Williams' (AAA)	'Hua Moa' (AAB)	'Kandarian' (ABB)	'Williams' (AAA)
MG	- [y]	3.1bB[x]	25.7aA	15.3nsB	32.2nsA	25.6cA	8.9nsB	31.4aA	50.5nsA
TR	-	17.0NS	24.0a	17.5B	22.6B	35.5bA	12.0B	38.9aA	47.5A
R	1.3bB	8.8aA	7.8bA	18.8NS	19.9	28.9bc	13.8C	22.3bB	58.7A
OR	4.3aB	9.5aA	8.5bA	21.1B	34.4AB	41.6aA	15.0B	56.3aA	55.3A

[z]Data was collected at mature green (MG), transitional (TR), ripe (R) and over ripe (OR) maturity stages during storage at 20°C and 95% RH. Antioxidant activity is defined by the following assays: reduced vitamin C and total phenolics. Antioxidant capacity is defined by FRAP (VCEAC). The cultivar by maturity stage interaction was significant at α=0.05.

[y]Vitamin C content was below limits of detection.

[x]Least square means comparisons within maturity stage (lower case) and cultivar (upper case) using the Schaffer Simulated Method at α = 0.05. (ns) and (NS) = not significant.

Table 10. Main effect of cultivar on antioxidant capacity[z] in selected cultivars.

Cultivar	FRAP		DPPH		ABTS	
	TEAC (mg TE· 100 gfw^{-1})	GAEAC (mg GAE· 100 gfw^{-1})	VCEAC (mg AA· 100 gfw^{-1})	TEAC (mg TE· 100 gfw^{-1})	VCEAC (mg AA· 100 gfw^{-1})	TEAC (mg TE· 100 gfw^{-1})
'Hua Moa' (AAB)	87.7c[x]	3.1b	-[y]	-	24.7c	136.7c
'Kandarian' (ABB)	200.0b	11.4a	29.2b	62.2b	62.3b	250.1b
'Williams' (AAA)	308.5a	13.1a	41.3a	90.5a	89.2a	338.7a

[z]Data was collected at mature green (MG), transitional (TR), ripe (R) and over ripe (OR) maturity stages during storage at 20°C and 95% RH. Antioxidant capacity is defined by FRAP (TEAC and GAEAC), DPPH Radical Scavenging Assay (VCEAC and TEAC), and ABTS Radical Cation Scavenging Assay (VCEAC and TEAC). Only the cultivar main effect was significant at $\alpha = 0.05$.

[y]Antioxidant capacity was below limits of detection.

[x]Least square means comparisons within cultivar using the Schaffer Simulated Method at $\alpha = 0.05$.

Table 11. Pearson's correlation coefficients (r) of antioxidant properties in banana pulp at mature green, transitional, ripe, and over ripe stages.

	AA[z]	TP	FRAPC	FRAPT	FRAPG	DPPHC	DPPHT	ABTSC	ABTST
AA	1.0000	0.11809[nsy]	0.22916[ns]	0.31923*	0.33768*	0.3886*	0.46605*	0.43411*	0.39059*
TP			0.50098*	0.42459*	0.94462*	0.33274*	0.35539*	0.58422*	0.54401*
FRAPC				0.9038*	0.65695*	0.31797*	0.61358*	0.81565*	0.80351*
FRAPT					0.62279*	0.32902*	0.65386*	0.83957*	0.83826*
FRAPG						0.41082*	0.46611*	0.69386*	0.65844*
DPPHC							0.60306*	0.63786*	0.60956*
DPPHT								0.91005*	0.90031*
ABTSC									0.97227*
ABTST									

[z]AA, reduced vitamin C, TP, total phenolics, FRAPC, FRAP assay with vitamin C standard, FRAPT, FRAP assay with Trolox standard, FRAPG, FRAP Assay with gallic acid standard, DPPHC, DPPH assay with vitamin C standard, DPPHT, DPPH assay with Trolox standard, ABTSC, ABTS assay with vitamin C standard, ABTST, ABTS assay with Trolox standard.

[y]ns= non significant.

[w]*=significant at $p \leq 0.05$

APPENDIX B. FIGURES

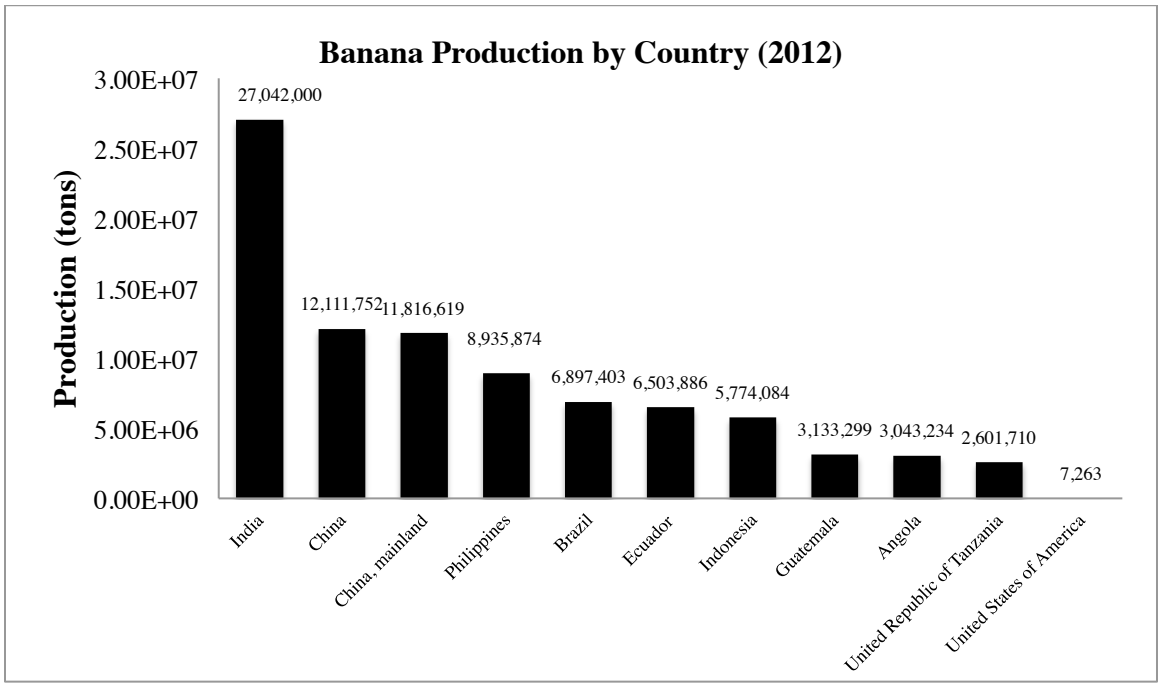

Fig. 1. Top 10 Banana Producing Countries Compared to the United States in 2012. (FAOSTAT, 2014).

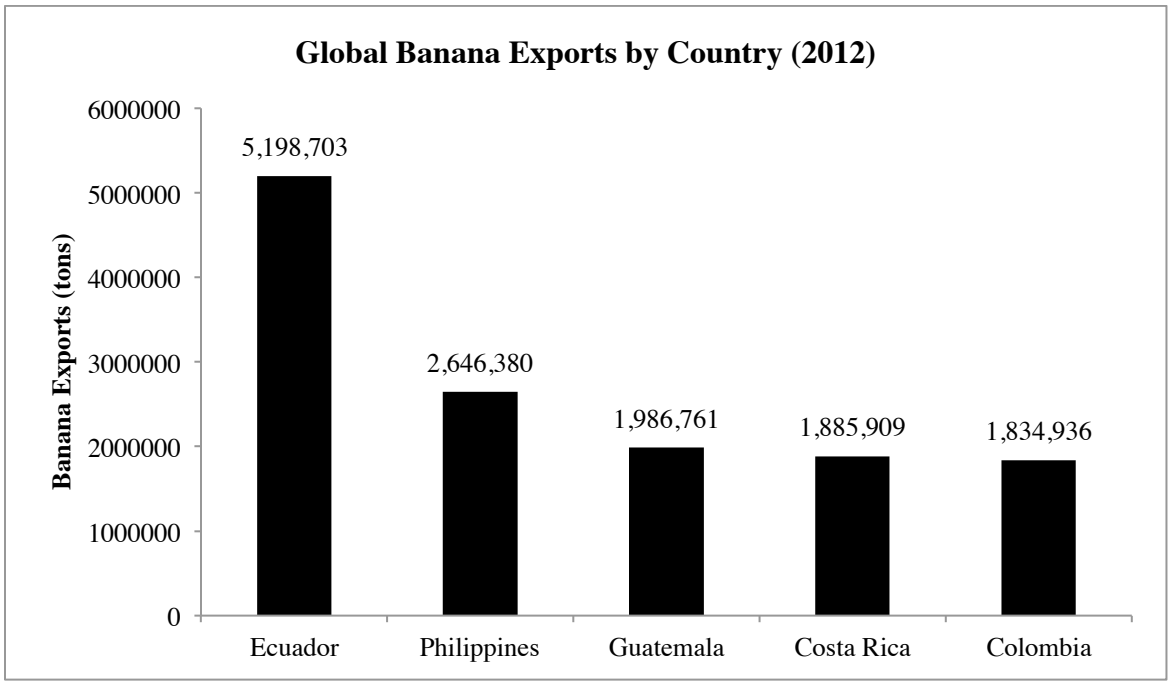

Fig. 2. Global leading banana exporters by country in 2012 (FAOSTAT, 2014).

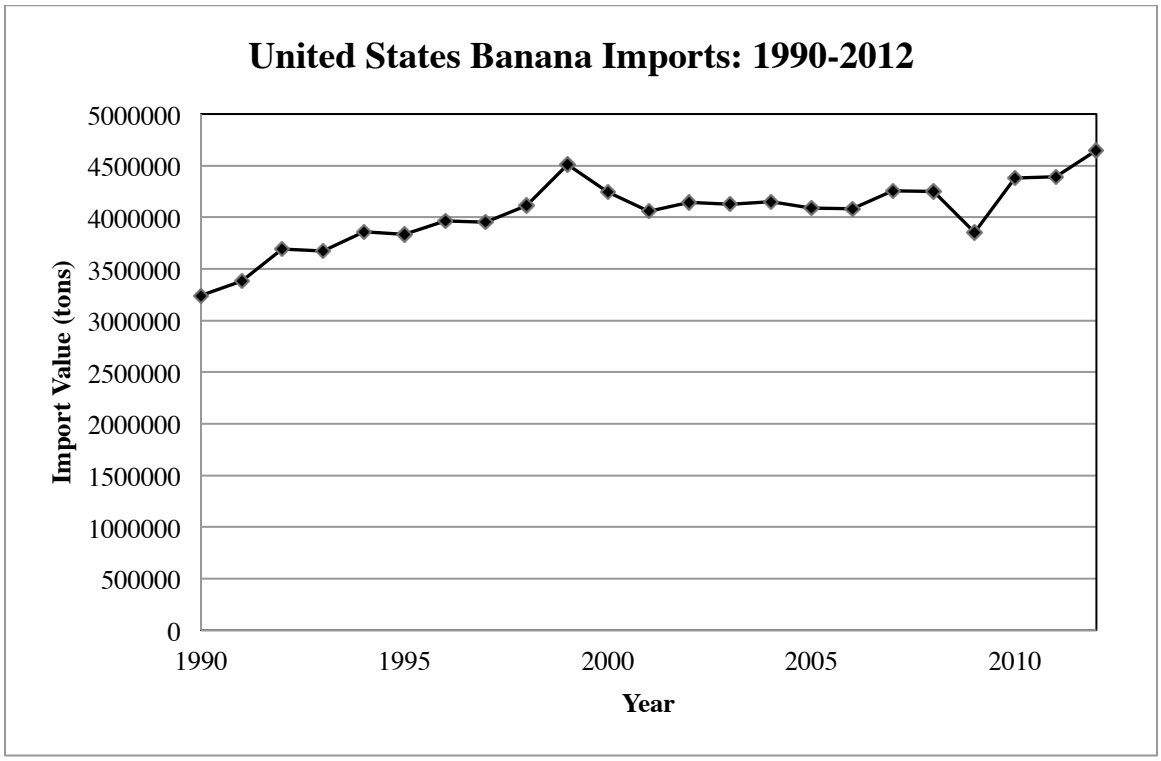

Fig. 3. United States Banana Imports from 1990-2012. (FAOSTAT, 2014).

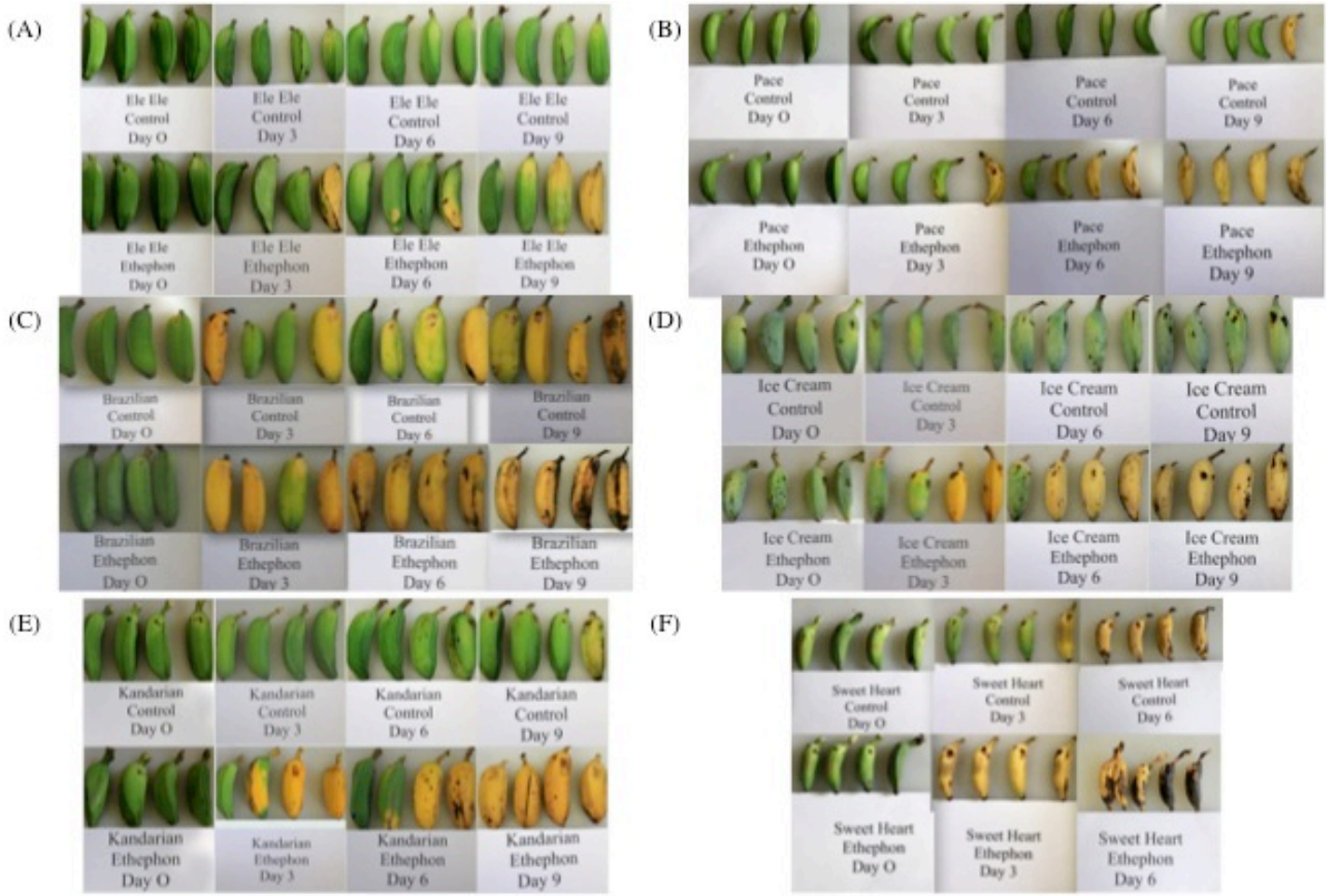

Fig. 4. Control and ethephon-treated selected cultivars after storage of 0, 3, 6, and 9 days in 20˚C and 90% RH.

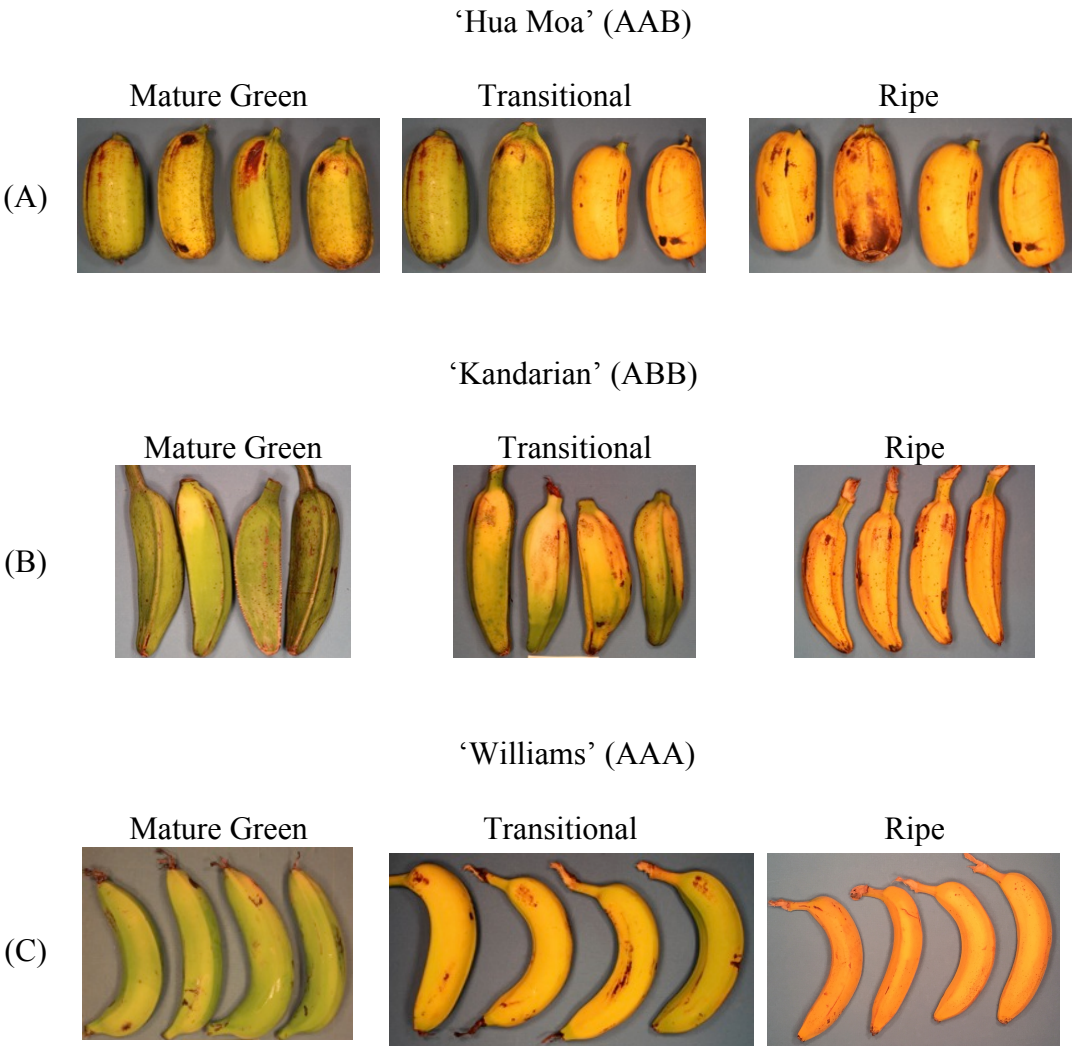

Fig. 5. Selected cultivars at the mature green, transitional, and ripe stages.