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Screening Underutilized Bananas for Carotenoid Content and Potential Health Benefits

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Abstract

Globally, banana (*Musa* spp.) is considered a daily dietary staple for millions of individuals that positively influences human health. Carotenoid enriched banana cultivars may serve as functional food sources to reduce the risk of non-communicable chronic diseases (NCD's). Although there is great diversity among commercial bananas, limited information is available concerning carotenoid content of non-traditional commercial cultivars adaptable to the southeastern United States and their market potential. Knowledge of diverse, underutilized, non-traditional, banana cultivars would readily assist in establishment and promotion of new niche markets, consumer demand and improvement in postharvest handling. Therefore, the objective of the current study was to compare and determine total carotenoid content of peel and pulp tissue of five underutilized, non-traditional, banana cultivars [‘Goldfinger’ (AAAB), ‘Hua Moa’ (AAB), ‘Kandarian’ (ABB), ‘Pisang Raja’ (AAB), ‘Saba’ (ABB)] ripening to the commercial industry standard ‘Williams’ (AAA), at four ripening stages. There was a significant ($p \leq 0.05$) cultivar by ripening stage interaction for total carotenoid content in peel and pulp tissues. In most cultivars and ripening stages, the results for peel were higher than pulp except for overripe ‘Hua Moa’ pulp, which had the highest total carotenoid content. Peel total carotenoid content ranged from 6.35 to 18.26 $\mu g \cdot g^{-1}$ FW in transitional ‘Goldfinger’ and overripe ‘Saba’, respectively. In pulp tissue, mean total carotenoid content was measured from 1.44 $\mu g \cdot g^{-1}$ FW in mature green ‘Kandarian’ to 19.08 $\mu g \cdot g^{-1}$ FW and in overripe ‘Hua Moa’. ‘Goldfinger’, ‘Pisang Raja’, and ‘Hua Moa’ had higher pulp total carotenoid content when compared to ‘Saba’, ‘Kandarian’, and ‘Williams’. These results indicate that the tetraploid cultivars had higher carotenoid content compared to the triploid ones. In addition, among triploid genomes, AAB hybrids showed higher carotenoid content compared to ABB and AAA genomes. The nutrient-rich cultivars should be promoted for production and consumption due to their health benefits.

A focal point of a consumer's decision to purchase and repurchase edible horticultural produce is color (Abbott, 1999; Barman et al., 2015; Vadiveloo et al., 2019). The dualistic aesthetic appeal of external and internal qualities of edible horticultural produce from the consumer's perspective is the motivating force for agricultural breeding, selection and future product enhancements (Buregyeya et al., 2018; Ghag and Ganapathi, 2017). Visual cues and consumer perception of food product attributes are the first impressions that influence consumer choice and perceived health benefits (Symmank et al., 2018). In fruits and vegetables, color serves as a reliable indicator for ripening process and decay. Color is the first sensory cue that generates overall quality expectations (Wei et al., 2012). Visual cues, fruit quality and nutritional status may not accurately reflect fruit phytonutrient content (Jaeger et al., 2018; Symmank et al., 2018). Maturation and ripening of peel and pulp tissues of many fruits may be biochemically coordinated (Bapat et al., 2010), or exist independently (Tadeo et al., 2008). In banana peel, coloration is used as a predictor of potential shelf life in both retailer and consumer environments and accurately reflects

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internal changes in pulp coloration (Dhandapani et al., 2017; Tsamo et al., 2014). There have been limited comparative studies that identify optimal carotenoid content of peel and pulp tissue of banana with the ripening process. This is very important because many commercial postharvest technologies rely on accurate color determination for handling, marketing, storage and consumer demand. Traditionally, positive correlation between banana pulp color and β-carotene concentration has been reported (Fungo and Pillay, 2011). Fu et al. (2018) noted that banana peels contain higher carotenoid content than pulp tissues. However, this occurrence may vary with genotype and cultivar.

Carotenoids are lipid-soluble pigments synthesized by plants and microorganisms. These compounds are responsible for yellow, orange and red coloration in fruits and vegetables (Saini et al., 2015). Diets rich in carotene are correlated with reduced risk of non-communicable chronic diseases (NCDs) such as cardiovascular diseases, cancer and diabetes due to associated antioxidant properties (Englberger et al. 2003).

Globally, banana (*Musa* spp.) is the fourth most important cultivated fruit crop with respect to human nutrition (Qamar and Shaikh, 2018; Adedayo et al., 2016; Singh et al., 2016; Manzo-Sánchez et al., 2015; Fonsah et at., 2004). Banana fruit provides a variety of phytochemicals and nutrients such as carotenoids, phenolic compounds, vitamin C, potassium and fiber (Arora et al. 2008a). Banana is a staple food in many countries because of its high nutritive value and positive health effects (Singh et al. 2016a). Edible *Musa* plants are classified according to their genomic groups and composition. Edible commercial bananas cultivars are primarily *M. acuminata* Colla (AA) and *M. balbisiana* Colla (BB), with a diversity of diploids (AA, AB, BB genomes), triploids (AAA, AAB, ABB, BBB genomes), and tetraploid (AAAA, AAAB, ABBB, AABB genomes) hybrids that reflect qualitative and quantitative compositional and chemical properties (Arvanitoyannis and Mavromatis, 2009; Englberger et al., 2006; Stover and Simmonds, 1987). Dessert bananas of the Cavendish subgroup (*Musa* sp., AAA group) comprise more than 95% of international and global banana trade (Daniells et al., 2013; Englberger et al., 2006). Most U.S. consumers are not familiar with non-Cavendish types of bananas (Englberger et al., 2006). Recently, there has been an increased interest in carotenoid enriched banana cultivars capable of reducing NCDs (Borges et al., 2014; Englberger et al., 2010, 2006, 2003; Sluijs et al., 2009). Although the Cavendish subgroup is considered the industry standard, it does not contain significant concentrations of carotenoids in comparison to certain subgroups and genotypes identified in international germplasm collections (Buah et al., 2016; Amorim et al., 2009). In particular, genotypes AAB contain higher carotenoid concentrations when compared to AAA genome (Davey et al., 2007; Englberger et al., 2003).

Critical to commercial sustainability and global trade of banana is the collection, conservation, characterization, and utilization of “desirable and novel consumer related properties” for commercial exploitation (Amah et al., 2019; De Clerck et al., 2017). Globally, identification and selection of enhanced carotenoid content of edible banana is an important and desirable trait for resolving food insecurity and nutritional concerns. (Ghag and Ganapathi, 2017; Buah et al., 2016; Borges et al., 2014).

Carotenoid content of banana is influenced by cultivar, genetics, geography and maturity at harvest and storage (Ding and Tee, 2012; Englberger et al., 2010; Davey et al., 2009; Newilah et al., 2009; Sangudom et al., 2014; Wall, 2006; Facundo et al., 2015). However, there is limited information concerning the effect of ripening on banana peel and pulp carotenoid content of non-traditional, diverse, banana cultivars (Aquino et al., 2018; Fu et al., 2018). In fact, most studies assume fully ripened banana fruit contain the highest carotenoid content (Englberger et al., 2010; Rodriguez-Amaya and Kimura, 2004). Limited studies have focused on the effects of genotype, ripening, tissue type, and their interactions on the carotenoid accumulation in non-traditional banana cultivars. There is growing interest in nutritive and carotenoid enriched functional food properties to improve human health and well-being. To ensure consumer demand and purchasing of these underutilized cultivars, it is essential to determine their carotenoid content to assist in banana breeding programs, as well as promotion and consumption of nutrient rich cultivars for residents of the southeastern United States. The purpose of this study was therefore; to determine the effects of cultivar and ripening on total carotenoid content of banana cultivars adaptable to the southeastern U.S. Information obtained from this study will assist in the promotion and the beneficial health effects of carotenoid-rich cultivars.

Materials and Methods

Material and Growth Condition. In 2015, six banana cultivars varying in genotype, 'Goldfinger' (AAAB), 'Hua Moa' (AAB), 'Kandarian' (ABB), 'Pisang Raja' (AAB), 'Saba' (ABB) and 'Williams' (AAA) were obtained from a commercial grower located in Pahokee, FL (26.8201° N, 80.6653° W). 'Goldfinger', 'Hua Moa', 'Pisang Raja', and 'Williams' fruits were obtained from a field in Canal Point, FL (26°53'48.62"N, 80°36'44.01"W) which was planted in 2012. 'Kandarian' and 'Saba' fruit were obtained from a field in Belle Glade, FL (26°43'11.63"N, 80°40'7.37"W), which was planted in 2009. The Canal Point field was irrigated by seepage from a canal connected to Lake Okeechobee, while the Belle Glade field was drip irrigated from a well daily during dry periods. Both locations were planted on a 4.6 m × 4.6 m spacing, and each plant was allowed to have five to seven suckers. Fertilization at each location was minimal due to soil fertility. Both fields were classified as Torry Muck soil type. The field in Canal Point was characterized as having sandier lower organic matter variant in the series. Weeds were controlled with a combination of herbicide treatment (glyphosate sprays) and mowing. Disease and insect outbreaks were rare at both fields due to planting of disease resistant cultivars and diversity of germplasm located at each site (≈ 20 cultivars across AAA, AAB, ABB, and Tetraploid genomes). The planting pattern and cultivars chosen were designed to create farms with low maintenance requirements.

Cultivar Selection. Cultivars utilized for initial carotenoid screening studies were selected based on consumer demand, familiarity, consumption and their availability in the local niche market (Crane and Balerdi, 2016; Krewer et al., 2008; Fonsah et al., 2007).

Sample collection. Four to six bunches from each cultivar were harvested at full three-quarter stage and absent of fruit surface angularity. Fruit samples were transported overnight to Auburn University Department of Horticulture Postharvest Physiology lab, Auburn, Alabama.

A minimum of four bunches per cultivar served as an individual replicate. Four replicates for each cultivar was designated. Sub-composite samples consisting of individual fingers were randomly selected following whorl detachment. Variability was minimized between bunches by exclusion of proximal and distal whorls within individual bunch and only median hands were considered comparable for postharvest ripening studies (Davey et al., 2007; Liu, 1976). Sub-composite sample replicates were maintained throughout postharvest ripening and sampling (approximately 50 fruit per replicate). Fruit bruising was minimized during whorl detachment by using a compact reciprocating saw with a stainless steel blade (Hyper Tough, Model No. 3308.2, Bentonville, AR) or a 6" stainless steel Hyper Tough jab saw (Walmart, Bentonville, AR). Prior to whorl detachment, stainless steel blades were sterilized with 1% bleach.

Uniform hands lacking visible damage were selected and individual fingers were immediately washed with water containing 100 mg/L sodium hypochlorite, air dried at ambient temperature and stored in ventilated, corrugated cardboard boxes at 20°C and 95% RH until they reached the desired ripening stage for carotenoid analysis. As individual fingers ripened, samples were selected for uniform size and weight. Four ripening stages (mature green, transition, fully ripe and overripe) were designated and analyzed for peel and pulp total carotenoid content. Ripeness was assessed visually according to the standard banana maturity, index color charts that describe the seven ripening stages (Von Loesecke, 1950; Li et al., 1997). In the mentioned chart, stages 1, 3, 5, and 7 were selected as mature green, transition, fully ripe and overripe, respectively.

Total Carotenoid Extraction and Quantification. Total carotenoid extraction and quantification was performed according to Talcott and Howard (1999). All sample extraction and measurement procedures were performed under amber fluorescent lighting conditions (GE F40/G0, 40W) to prevent photo oxidation. One gram composite sample of frozen fruit tissue was extracted using 5 mL of pre-chilled HPLC grade acetone/ethanol (1:1, v/v) with 100 mg/L butylated hydroxytoluene (BHT) as an internal antioxidant to prevent auto-oxidation of carotenoids. Following extraction, homogenates were vortexed for 1 minute, sonicated for 10 minutes, and centrifuged (Beckman, model J2-21, and JA-20 rotor, San Antonio, TX) at 12,000 × g for 15 min at 4°C. The supernatant was collected, and the remaining residue was re-extracted until colorless using the same method. The supernatant was combined and filtered through 4-fold Miracloth (Calbiochem, La Jolla, CA), and brought to 15 mL with the extraction solvent. Approximately 200 µL samples were loaded onto a microplate (Corning Polypropylene 96 well plates, Corning 3364, Kennebunk, ME). The absorbance of each aliquot was measured at 470 nm with a microplate reader (SNERGY HT, BIO-TEK Instruments, Inc., Winooski, VT). Total carotenoid content was calculated and expressed as $\mu g \cdot g^{-1}$ FW.

Statistical Analysis. An analysis of variance was performed using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The experimental design was completely randomized. The treatment design included cultivar, ripening stage and fruit tissue type (peel or pulp). Residual plots and a significant covariance test indicated heterogeneous variance among treatments, therefore RANDOM statement with the GROUP option was used to correct heterogeneity. Least squares mean comparisons were determined using the simulated method. All significances were at α=0.05.

Results

Interaction. A significant *cultivar × ripening stage × fruit tissue type* (peel *vs* pulp) interaction for total carotenoid content ($p \leq 0.05$) was observed (Table 1). In peel tissue, overripe fruit of 'Saba' and transitional fruit of 'Goldfinger' had the highest (18.26 $\mu g \cdot g^{1}$ FW) and the lowest (6.35 $\mu g \cdot g^{-1}$ FW) carotenoid content, respectively. The overripe stage of 'Goldfinger', 'Hua Moa', 'Kandarian', and 'Saba' had the highest total carotenoid content, whereas 'Pisang Raja' and 'Williams' had the highest carotenoid content at the mature green stage.

In pulp tissue, mean total carotenoid content ranged from 1.44 to 19.08 $\mu g \cdot g^{-1}$ FW in mature green 'Kandarian' and overripe 'Hua Moa', respectively. In all cultivars, carotenoid content increased from mature green to overripe stage. The results of transitional stage, ripe and overripe in 'Goldfinger', 'Pisang Raja' and 'Williams' were not different from ripe and overripe in 'Hua Moa' and 'Saba'.

In most cultivars and ripening stages, the results of peel tissue were higher than pulp with the exception of 'Hua Moa' overripe pulp, which indicated higher total carotenoid content. 'Goldfinger' mature green and transitional stage also had higher total carotenoid content in pulp tissue when compared to the

Table 1. Effect of ripening progression in peel and pulp tissues of genomically diverse banana cultivars on total carotenoid (µg.g^{-1} FW) content.[z]

Cultivar	Group	Peel				Pulp			
		Mature Green	Transitional Stage	Fully Ripe	Over Ripe	Mature Green	Transitional Stage	Fully Ripe	Over Ripe
‘Goldfinger’	AAAB	6.93 cBb[y]	6.35 dBb	11.53 bAns	12.69 bcAns	3.80 bBa	12.09 aAa	13.08 aA	9.65 bcdA
‘Hua Moa’	AAB	9.67 cABa	7.99 cB	8.30 cBns	12.22 bcAb	6.59 aBb	7.53 bcB	8.86 bcB	19.08 aAa
‘Kandarian’	ABB	12.05 abAa	7.74 cBa	11.45 bAa	13.25 bAa	1.44 cBb	2.75 dBb	7.71 bcAb	3.77 dABb
‘Pisang Raja’	AAB	13.94 aAa	13.44 aAa	10.30 bBns	13.14 bcAns	5.46 aBb	9.81 abAb	9.87 abA	14.24 abA
‘Saba’	ABB	12.88 abBa	10.12 bCa	13.60 aBa	18.26 aAa	3.93 bBb	4.29 cdBb	9.63 bAb	9.75 bcAb
‘Williams’	AAA	10.29 bcAa	7.31 cdBa	8.33 cABa	9.02 cABa	1.88 cBb	3.36 dABb	5.36 cAb	3.95 dABb

[z] The cultivar by ripening stage by tissue type interaction was significant at $P < 0.05$.

[y] Least squares means comparisons among cultivars (lower case in columns), among ripening stages (upper case in rows), and among tissue (underlined lower case in rows) using simulated method at $P < 0.5$. NS and ns = not significant.

peel. In addition, the data of ripe and overripe fruit in this cultivar were similar between peel and pulp tissue. ‘Pisang Raja’ ripe and overripe fruit and ‘Hua Moa’ transitional and ripe fruit were similar in terms of peel and pulp comparison.

Discussion

Visual cues are one of the first and foremost quality determinants that influence consumer appeal and purchase. Considering the above findings, the present study focused on screening of underutilized non-traditional banana cultivars as a potential functional food source for the development of local niche markets. As such, these cultivars may have additional relevance for modifying food security concerns and NCD’s associated with this geographical region. In the current study, total carotenoid content of non-traditional banana cultivars were higher than the Cavendish cultivar by 4-fold, confirming previous reports (Englberger et al., 2003; Wall, 2006). The darker color of these cultivars confirms the results and agrees with the Englberger et al., (2010) study. They reported yellow/orange-fleshed cultivars generally contained higher carotenoid content compared to cultivars with light-colored pulp. Carotenoid content may be influenced by several factors, including genotype, maturity, climate, geography, and storage conditions (Amorim et al., 2009; Setiawan et al., 2001). Globally, the commercial banana industry is comprised of mostly triploid cultivars, with the genome composition consisting of AAA, AAB and ABB. In the current study, the most important factors in carotenoid content were genotype and ripening.

Overall, the edible portion of banana fruit resulted in cultivars ‘Goldfinger’ (AAAB), ‘Pisang Raja’ (AAB), and ‘Hua Moa’ (AAB) having higher pulp total carotenoid content when compared to ‘Saba’ (ABB), ‘Kandarian’ (ABB), and ‘Williams’ (AAA). Results from this study indicate that cultivars with tetraploid genome may possess higher total carotenoid content than triploid cultivars among studied cultivars. In addition, in triploid genomes with the AAB, ABB, and AAA designations ranked the highest to lowest total carotenoid content, respectively. These results are in agreement with Amorim et al., (2009) and Englberger et al., (2003) studies. Amorim et al., (2009), spectrophotometrically determined total carotenoid content from forty-two cultivars including; 21 diploids, 19 triploid and two - tetraploid banana pulp samples. In this study, the typical pulp color included orange, orange-yellow, yellow, beige and white for diploids, and orange, orangish-yellow, cream for triploid and orangish-yellow and beige for tetraploid banana. Genotypes with pulp coloration ranging from yellow to orange had higher total carotenoid content when compared to white or beige pulp. Within the diploid group, the mean total carotenoid content was 5.24 $\mu g \cdot g^{-1}$ FW, with highest total carotenoid levels observed at 16.05 $\mu g \cdot g^{-1}$ FW. The mean content among triploids and ABB varied dependent on genomic group composition. Genomic AAA, AAB and ABB were, respectively, 2.93, 8.37 and 2.15 $\mu g \cdot g^{-1}$FW. In general, triploids with genomic composition AAB possessed higher total carotenoid content when compared to AAA and ABB triploids. This study emphasizes the importance of genotype selection and composition in carotenoid content.

Conclusions

In this study, the interactive effects of cultivar, ripening stage and tissue type on total carotenoid content of diverse underutilized banana fruit suitable for cultivation in the southeastern United States and development of local niche market were investigated. In most cultivars and ripening stages, peel total carotenoid content were higher than pulp total carotenoid content with the exception of overripe 'Hua Moa' pulp, which had the highest total carotenoid content. Increased knowledge of carotenoid enriched cultivars adaptable to southeastern United States will favorably assist interested banana fruit producers in selecting superior nutritional fruit quality for consumer demand. In addition, these cultivars will be utilized for promotional health enhancements of geographically diverse populations.

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