Phenolic and Antioxidant Properties of Male Bud Flowers and Fruit of *Musa* Genotypes with Different Ploidy

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Abstract:

Banana (*Musa X paradisiaca*) fruits are important foods and considered to be especially rich sources of polyphenolics. The bracts, abundant edible residues of banana production, were investigated as a potential source of natural colorants. The aim of this research was to investigate the total phenolic content and antioxidant activity of banana male bud flowers, unripe fruit peel and pulp at 80-90% mature of banana genotypes with different ploidy in Thailand. The total phenolics content and antioxidant activities were significantly different among banana cultivars. Total phenolic levels were higher in unripe peel than in male bud flowers and unripe pulp. The highest total phenolic contents in peel were 129.5 mg GAE/g DW in 'Thep Panom' (*Musa* ABB), followed by 128.9 mg GAE/g DW in 'Nam Thai' (*Musa* AA) and 72.9 mg GAE/g DW in 'Thep Parod' (*Musa* ABBB). This result is consistent with the antioxidant activity. The ABTS, DPPH and FRAP assays differed significantly ($p<0.05$) in different parts of banana with different ploidy (AA, ABB and ABBB). The highest DPPH antioxidant activity of unripe peel occurred in the 'Nam Thai' cultivar (970.2 mg TEAC/g DW), followed closely by 'Thep Panom' (963.9 mg TEAC/g DW) and 'Thep Parod' (887.9 mg TEAC/g DW). In conclusion, the amount of total phenolics and antioxidant activity were significantly different among banana cultivars with different ploidy. In addition, unripe peel extract showed stronger antioxidant activity than the male bud flowers and unripe pulp, respectively.

Keywords: Banana genotypes, Total phenolic content, Antioxidant activity, Fruit peel and pulp, Male bud flowers

Introduction

Early classification of banana (*Musa*) species was hindered by a poor understanding of the genetics of *Musa*, and a lack of knowledge of the complex hybridizations that readily occur within the genus. Yet, many diverse cultivars are now well studied and are known to occur as diploids (2n), triploids (3n), and tetraploids (4n). Simmonds and Shepherd [1] had previously suggested that edible bananas originated from two wild species, *Musa acuminate* Colla and *Musa balbisiana* Colla. The former species was designated as diploid genotype AA and *Musa balbisiana* Colla was designated diploid genotype BB. Because both species are native in common geographic areas, and because cross pollinations and hybridization readily occur among these two species and their hybrid progeny, numerous triploids (AAA, AAB, ABB, and BBB) as well as tetraploids (AAAA, AAAB, ABBB, and AABB) occur in nature and in commercial cultivation. Bananas contain bioactive compounds, such as phenolics, carotenoids, amines and phytosterols. Many of these compounds have antioxidant activities and potentially beneficial effects on human health [2]. Phenolic compounds are secondary metabolites produced in plants through the phenylpropanoid pathway and encompass a wide range of chemical classes, including phenolic acids, flavonoids, stilbenes and lignans [3]. Plant polyphenolics are generally considered essentials components of plant defense mechanisms, and exert health promoting effects in humans. They act as antioxidants and modulators of enzyme expression and thereby contribute to the allevation of wide range of chronic diseases [4]. The different parts of banana such as banana bracts [5], pseudostems [6], banana fruit peels and pulp have been found to be good source of antioxidants and food colorants [7]. Various phenolics present in banana have been identified as gallic acid,

catechin, epicatechin, tannins, and anthocyanins [8]. All six of the common anthocyanidins (delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin) have been detected in bracts of different species of *Musa* [9]. In a recent study, the antioxidant activity of the banana peel extract, against lipid autoxidation, was stronger than that of the banana pulp extract, and the gallocatechins in their tissues may account for their high antioxidant effects [10]. In addition, banana fruits are important foods and considered to be especially rich sources of polyphenolics in banana peel and pulp. The bracts, abundant edible residues of banana production, were investigated as a potential source of natural colorants.

The aim of this research was to investigate the total phenolic contents and antioxidant activities of banana male bud flowers of different banana genotypes in Thailand with different ploidy. The data will provide important information about the potentially beneficial effects of this plant tissue as a dietary component, and to suggest potential applications of new natural antioxidant-containing food ingredients in functional foods.

Materials and methods

Plant materials

The banana specimens were collected from various locations in Thailand, characterized and identified based on Simmonds [11-12]. Banana fruit (*Musa acuminate* Juss.) cultivars Nam Thai (*Musa* AA group), Thep Panom (*Musa* ABB group) and Thep Parod (*Musa* ABBB group) were harvested at 90% maturity stage in the same plantation located in the Burapha University Farm, Sakaeo campus, Thailand. Analyses were made using three replications for each cultivars and results were reported as averages with calculated standard deviations. Banana male bud flowers were harvested at the flowering stage, fruit peel and pulp were harvested at 80-90% fruit maturity stage. The banana specimens harvested for chemical analyses were freeze dried (Labconco Corporation, USA) and ground to a dry powder. The dry powder was stored at -20 °C for further analysis.

Sample extraction

The dried power of banana specimens were prepared using the method of Pothavorn *et al.* [13], with some modifications and made in triplicate. Ten grams of banana male bud dry powder were mixed with 50 mL of 80% ethanol containing 100 mM NaCl, 0.2 mM ascorbic acid, 40 mM citric acid, 0.1 mM $Na_2S_2O_5$, 0.25% Triton X-100 and 0.2 mM EDTA as antioxidants and inhibitors PPO. The sample was heated at 80°C for 30 min, then collected the supernatant and re-extract twice, each time using 50 ml of extraction solutions. These three extracts were combined and centrifuged at 5000 rpm for 30 min at 4°C using micro centrifuged (Beckman, J2-21, Beckman Instruments Inc., USA). The supernatants were collected and evaporated to dryness at 55°C (rotary evaporator, Buchi Rotavapor R-205, Switzerland). The remaining residue was dissolved in 80% ethanol to 6 mL and stored at 4°C under airtight and dark conditions for further analysis.

Total phenolic content (TPC) measurement

Total phenolics contents were determined by the Folin-Ciocalteu method, which was adapted from Singleton and Rossi [14]. The 20 µl of extract and 100 µl of 1:10 diluted commercial Folin-Ciocalteu reagent (Sigma-Aldrich) were combined and mixed well using a vortex. The solution was allowed to react for 1 min in the dark and then 80 µl of 7.5% (w/v) Na_2CO_3 was added and mixed well. The solution was incubated at room temperature (25°C) in the dark for 30 min. The absorbance was measured at 765 nm using a microplate reader (Epoch™ Microplate spectrometer, BioTek Instruments Inc., USA). The total amount of phenolics was expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (DW).

Antioxidant determinations

The 2,2□-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging (ABTS) assay followed the method of Thaipong *et al.* [15] with some modifications. The banana male bud extracts (10µl) were allowed to react with 190µl of ABTS cation radical reagent for 15 min in the dark. Then the absorbance

was taken at 734 nm using a microplate reader. The results were expressed in mg Trolox equivalents (TEA) per gram DW.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was run according to the method of Prior *et al.* [16] with some modifications. The banana male bud extracts (10µl) were allowed to react with 190µl of DPPH solution for 30 min in the dark. Then the absorbance was taken at 515 nm using the Epoch microplate reader. The results were expressed in mg Trolox equivalents (TEA) per gram DW.

The Ferric reducing antioxidant power (FRAP) assay was run according to Thaipong *et al.* [15] with some modifications. The banana male bud extracts (10µl) were allowed to react with 190µl of FRAP (Ferric reducing antioxidant power) reagent for 15 min in the dark. Readings of the colored product [Ferrous tripyridyltriazine complex] were then taken at 593 nm using the Epoch microplate reader. The results were expressed in mg Trolox equivalents (TEA) per gram DW.

Statistical analysis

The data of different parameters were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Least Significant Difference (LSD) test ($P<0.05$) using STATISTIX8 [17]. Values expressed were means of three replications ±standard deviation (SD).

Results and discussion

Characteristic of banana male bud flowers and fruit

Male bud shapes of banana show a diversity, including narrow ovate 'Nam Thai' (*Musa* AA), broadly ovate Thep Panom (*Musa* ABB), lanceolate Thep Parod (*Musa* ABBB) with acute and obtuse bract apex. Bract curling was bract reflex and roll back and wax on the bract. Colors were reddish-purple for the outside bract color, while reddish-orange to purple for the inside bract color. Bract color was reddish brown ('Thep Panom'), reddish purple ('Nam Thai'), and reddish brown with light purple ('Thep Parod'). The pigmentations of male flowers were creamy yellow in 'Nam Thai', light pink in 'Thep Panom' and pinkish-purple in 'Thep Parod' with yellow stigma in the three cultivars (Figure 1). The variation in bract color is correlated with their anthocyanin content. Pazmino-Duran *et al.* [5] reported that banana bracts (*Musa x Paradisiaca*) contained anthocyanins such as cyaniding-3-rutinoside (32 mg anthocyanin/100 g bracts), and 3-rutinoside derivatives of delphinidin, pelargonidin, peonidin, and malvidin. They have suggested the use of anthocyanins from banana bracts (male bud flowers) as natural colorants. Many factors other than anthocyanin contents influence bract color, including pigment accumulation patterns, vacuolar pH, and copigments in each individual cell or tissue area [18]. Anthocyanins are considered to be a good food colorants due to their attractive colors, water solubility, and stability in processed foods [19] as well as their established health benefits in humans [20]. The fruit bunches were harvested at 75 days from removal of the blossom in 'Nam Thai', 111 days in 'Thep Parod' and 124 days in 'Thep Panom' bananas with mature green peel color at 80-90% mature was shown in figure 1. Degree of fruit maturity at each age of fruit bunch was assessed based on the standard maturity index for banana according to fullness of fingers stages; full three-quarters – angularity not prominent,>80-90% mature.

Nam Thai (*Musa* AA) Thep Panom (*Musa* ABB) Thep Parod (*Musa* ABBB)

Nam Thai (*Musa* AA) Thep Panom (*Musa* ABB) Thep Parod (*Musa* ABBB)

Figure 1 Male bud flowers (upper) and fruit bunch (lower) of Nam Thai, Thep Panom and Thep Parod bananas.

Changes of TPC and antioxidant activities

The amount of total phenolics and antioxidants were significantly different among the three banana cultivars. Total phenolics were more abundant in peel than in male bud flowers and pulp, respectively (Table 1). The highest total phenolic contents in peel was 129.5 mg GAE/g DW in 'Thep Panom' (*Musa* ABB), followed by 128.9 mg GAE/g DW in 'Nam Thai' (*Musa* AA) and 72.9 mg GAE/g DW in 'Thep Parod' (*Musa* ABBB). The ABTS, DPPH and FRAP assay values differed significantly ($p<0.05$) for different parts of banana with different ploidy (AA, ABB and ABBB). The unripe peel extract showed stronger antioxidant activity than the male bud flowers and unripe pulp, respectively. The highest antioxidant activity based on the DPPH assay for unripe peel occurred for the 'Nam Thai' cultivar (970.2 mg TEAC/g DW), followed by 'Thep Panom' (963.9 mg TEAC/g DW) and 'Thep Parod' (887.9 mg TEAC/g DW). The DPPH and FRAP assays showed no difference among determinations, while the ABTS differed. Banana peel and pulp of *Musa Cavendish* are reported to contain higher levels of total phenolics in peel (907 mg GAE/100g DW) than in pulp (232 mg GAE/100g DW). The peel extract showed 2.2 times stronger antioxidant activity than the pulp extract and may be attributed to their phenolic contents [10]. In addition, banana peel extracts have been shown to be rich in antioxidant dopamine, L-dopa, and catecholamines [21]. The comparison of dopamine with other natural antioxidants, such as ascorbic acid and phenolic acids (*e.g.* gallocatechin gallate), the dopamine showed higher antioxidant activity *in vitro* (DPPH assay) [22]. Recently, Vu *et al.* [23] have also reviewed the phenolic compounds and their potential health benefits coming from banana peel. They have suggested the use of this valuable by-product from banana fruit processing industry in food and pharmaceutical industry. Banana peel and male bud flowers, which is usually discarded, should also be considered to be a good source of natural antioxidants and to be a functional food.

Table 1 Antioxidant activity of male bud flowers, unripe banana fruit peel and pulp by the ABTS, DPPH, and FRAP assays from three banana genotypes.

Banana cultivar	**Parts of banana**	**TPC[a] (mgGAE/gDW)**	**Antioxidant activity[b] (mgTEAC/gDW)**		
			ABTS	DPPH	FRAP
Nam Thai (*Musa* AA)	Male bud flowers	22.6±0.9c	600.1±9.6d	203.3±9.9d	33.6±1.3h
	Peel	128.9±5.2a	1246.2±49.1b	970.2±7.6a	681.6±48.4a
	Pulp	15.9±1.1d	196.9±2.4e	72.6±9.3g	68.3±5.6e
Thep Panom (*Musa* ABB)	Male bud flowers	17.01±1.3d	83.1±3.7gh	70.4±2.5h	49.7±2.5g
	Peel	129.5±2.8a	1737.5±17.5a	963.9±8.0b	670.2±28.2b
	Pulp	8.7±0.5e	117.7±6.8f	65.8±5.8i	34.6±2.7h
Thep Parod	Male bud flowers	21.9±2.2c	95.1±2.4g	106.6±7.1e	78.9±9.5d

(*Musa* ABBB)	Peel	72.9±1.9b	1096.4±28.4c	887.9±26.7c	458.3±23.2c
	Pulp	3.2±0.4f	74.0±4.6h	93.6±1.4f	52.5±5.3f

Value in each column marked by the same letter are not significantly different at $P<0.05$.
Results showed mean ±SD
[a] Total phenolic content milligrams of gallic acid equivalent (GAE) per gram of dry weight (DW)
[b] Milligrams of Trolox equivalent antioxidant capacity (TEAC) per gram of dry weight (DW)

Conclusions

The amount of total phenolics and antioxidant activity were significantly different among banana cultivars with different ploidy. Total phenolics were more abundant in peel than in male bud flowers and pulp. The highest antioxidant activity was shown in 'Nam Thai' (*Musa* AA), followed by 'Thep Panom' (*Musa* ABB) and 'Thep Parod' (*Musa* ABBB). The unripe peel extract showed stronger antioxidant activity than the male bud flowers and unripe pulp, respectively.

Acknowledgements

This work as financially supported by the Research Grant of Burapha University through National Research Council of Thailand (Grant no. 27/2562).

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