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Total phenolics, flavonoids and antioxidant activity of banana pulp and peel flours: influence of variety and stage of ripeness

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Abstract: The influence of variety (Cavendish and Dream), stage of ripeness (green and ripe) and parts (pulp and peel) on antioxidative compounds and antioxidant activity of banana fruit was investigated. The TPC and TFC ranged widely from 75.01 to 685.57 mg GAE/100 g and 39.01 to 389.33 mg CEQ/100 g of dry matter respectively. Cavendish banana flour contained higher TPC and TFC compared to Dream variety. TPC and TFC values of banana peel were higher than those of banana pulp. Also, green banana showed higher TPC and TFC values than those of ripe fruit. Radical scavenging activities (inhibition of DPPH) of the extracts ranged from 26.55 to 52.66%. Although Dream banana peel extracts appeared to have low TPC and TFC, its antioxidant activities were ranked moderate to high. This implies that antioxidative compounds other than phenolics and flavonoids were probably responsible for inhibition of DPPH.

Keywords: Banana peel flour, banana pulp flour, total phenolic content, total flavonoid content, DPPH scavenging activity

Introduction

Lipid oxidation in food components is known as the main undesirable reaction which causes rancidity, polymerization and off-flavor compounds that eventually leads to reduction in shelf life and nutritive value of food. To minimize lipid deterioration, food industries have been relying on synthetic antioxidants such as 2,6-di-tert-butyl-p-hydroxytoluene (BHT), tertbutyl-4-hydroxyanisole (BHA) and propyl gallate (PG) to extend the shelf life of their products. The main drawback of using synthetic antioxidants is their potentials of causing health hazards. Thus, safer and natural alternatives of antioxidative compounds are desirable. In this respect, various types of fruit by-products with antioxidant properties have been demonstrated. For example, residues from star fruit (Shui and Leong 2006), grape pomace (Lafka *et al*., 2007), citric fruits peel (Xu *et al*., 2008), by-products from pomegranate (Singh *et al*., 2002) and banana peel (Gonzalez-Montelongo *et al*., 2010a) have been evaluated as inexpensive sources of antioxidants.

Banana peel represents about 40% of total weight of the fresh fruit (Anhwange *et al*., 2008). The total amount of phenolic compounds in banana peel has been reported from 0.90 to 3.0 g/100g dry weight (Someya *et al*., 2002; Nguyen *et al*., 2003). Gallocatechin is identified at a concentration of 160 mg/100 g dry weight (Someya *et al*., 2002). Other phytochemicals such as anthocyanin, delphinidin, cyanidin (Seymour, 1993) and catecholamines (Kanazawa and Sakakibara, 2000) have been identified in ripe banana pulp and peel. Recent studies demonstrated that banana peel generally include higher phenolic compounds than that of banana pulps (Someya *et al*., 2002; Kondo *et al*., 2005; Sulaiman *et al*., 2011). Subagio *et al.* (1996) reported carotenoids such as β-carotene, α-carotene and different xanthophylls in the range of 300–400 µg lutein equivalents/100 g. Someya *et al*. (2002) attributed antioxidant properties of banana peel to its gallocatechin content. Gonzalez-Montelongo *et al*. (2010a) identified the extraction conditions that produce maximum antioxidant activity (Acetone: water (1:1), 25ºC, 120 min). Moreover, the number of extraction steps, temperature and time, have been reported as the most effective factors associated with antioxidant properties of banana peel, respectively (Gonzalez-Montelongo *et al*., 2010b). According to Someya *et al*. (2002), total phenolics are more abundant in peel (907 mg/ 100 g dry wt.) than in pulp (232 mg/100 g dry wt.) in Musa cavendish.

Quite a number of work has demonstrated the occurrence of different types of antioxidants in both banana pulp and peel (Someya *et al*., 2002; Wall, 2006; Lim *et al*., 2007), however, the influence of variety of banana, stage of ripeness and parts of fruits

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on antioxidative compounds and antioxidant activity have not been studied. Therefore the objective of this study was to compare total phenolic content (TPC), total flavonoids content (TFC) and DPPH radical scavenging activity of banana flour taken from different varieties (i.e. Cavendish and Dream), parts (pulp and peel) and stage of ripeness (green and ripe).

Material and Methods

Preparation of banana flour and extraction for antioxidant attributes

Two of the most popular banana varieties in Asia, namely Cavendish (*Musa acuminata L*, *cv cavendshii*) and Dream (*Musa acuminata colla*. *AAA, cv 'Berangan'*) banana were obtained from the wet markets around Penang Malaysia. Green (stage 2 of ripening: all green) and ripe (stage 5 of ripening: yellow with green tip) (Aurore *et al*., 2009) bananas were washed, separated into pulps and peels and sliced into a thickness of 2 mm. Sliced bananas were dried overnight at 50°C using a hot-air dryer, grounded using a commercial blender, and passed through a 60 mesh sieve. Powdered material (20 g) for each sample was extracted with 200 ml of 80% methanol at room temperature by a magnetic stirrer. The extracts were separated from the residue by filtering through a clean muslin cloth and centrifuged at 3000 g for 15 min. Thereafter, the supernatant was concentrated under reduced pressure at 50°C using a rotary evaporator. The crude extracts were collected after 3 h, weighed to calculate the yield and then kept in dark glass bottles for three days in a freeze-dryer (Sanyo, Osaka, Japan) at -20°C for further use. Light exposure was avoided throughout the extraction process. Eight types of crude extract were prepared; ripe Cavendish pulp (RCPu), ripe Cavendish peel (RCPe), green Cavendish pulp (GCPu), green Cavendish peel (GCPe), ripe Dream pulp (RDPu), ripe Dream peel (RDPe), green Dream pulp (GDPu) and green Dream peel (GDPe). All samples were analyzed in triplicates and average values obtained.

Determination of total phenolic content (TPC)

Total phenolic content of banana flour extract was measured according to the method reported by Alothman *et al*. (2009) using Folin–Ciocalteu reagent with some modification. 50 mg of crude extract were mixed with 0.5 ml of Folin-Ciocalteu's phenol reagent and 7.5 ml deionized water. The mixture was kept at room temperature for 10 min, and then 1.5 mL of 20% (w/v) sodium carbonate added. The mixture was heated in a water bath at 40°C for 20 min and then cooled in an ice bath. Absorbance was measured at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1601PC, Tokyo, Japan). Amounts of total phenolic were calculated using the gallic acid calibration curve within the range of 10 – 100 ppm (R^2 = 0.9984). No information is available on the dominant phenolics compounds in Cavendish and Dream bananas in Malaysia; therefore the total phenolic contents were expressed as mg gallic acid equivalents (GAE)/100 g of dry matter. All samples were analyzed in triplicates and results averaged.

Determination of total flavonoids content (TFC)

TFC of the extracts were determined according to the colorimetric assay following the procedure of Sultana *et al*. (2008) with some modification. One milliliter of aqueous extract containing 0.01 g/ml of dry matter was placed in a 10 ml volumetric flask, and then 5 ml of distilled water was added. At zero time, 0.3 ml of (5% w/v) $NaNO_2$ was added. After 5 min, 0.6 ml of (10% w/v) $AlCl_3$ was added. After another 5 min, 2 ml of 1M solution of NaOH was added. After that, the volume was made up to 10 ml with distilled water. The mixture was shaken vigorously and the absorbance of the pink color of mixture was read at 510 nm using a UV-visible spectrophotometer (Shimadzu UV-1601PC, Tokyo, Japan). No information is available on the dominant flavonoids compounds in Cavendish or Dream bananas in Malaysia, therefore a calibration curve was prepared using a standard solution of catechin within the range 10 – 100 ppm (R^2 = 0.9962) and the results were expressed as mg catechin equivalents (CEQ)/100 g of dry matter. All samples were analyzed in triplicates and results averaged.

DPPH free radical-scavenging assay

The antioxidant capacity of the banana flour was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was assessed by using procedure reported by Sultana *et al*. (2008) with slight modifications. Briefly, 5.0 ml of a freshly prepared solution of 1, 1–diphenyl–2-picrylhydrazyl (DPPH) methanolic solution at concentration 0.025g/l was added to 1.0 ml of extract containing 25 µg/ml of dry matter in methanol. The mixture was shaken and kept in the dark and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured at 515 nm, against a blank of methanol without DPPH, using a UV-visible spectrophotometer (Shimadzu UV-1601PC, Tokyo, Japan). Results were expressed as percentage of inhibition of the DPPH radical which

was calculated according to the following equation:

$$\%\ DPPH = \left(\frac{Abs\ Control - Abs\ sample}{Abs\ control}\right) \times 100$$

where Abs control is the absorbance of DPPH solution without extracts.

Statistical analysis

Factorial experiment of type 2×4 to study the effect of type of banana (Cavendish and Dream) and four different samples (green pulp, green peel, ripe pulp and ripe peel) on four responses (yield, TPC, TFC and DPPH scavenging) measured from each sample. The experiment was replicated three times and the total number of runs was 24.

The results for yield, TPC, TFC, and DPPH scavenging obtained were analyzed using analysis of variance (ANOVA) and Tukey's test for multiple comparisons for different analyzed samples based on yield, TPC, TFC, and DPPH. SPSS version 18 was used for performing ANOVA and Tukey's test. Differences were considered significant at $P < 0.05$.

Result and Discussion

Yield of extract and polyphenol content

The effect of variety of banana (Cavendish and dream), banana parts (pulp and peel) and stage of ripeness (Green and ripe) on extract yield, TPC, TFC and DPPH scavenging activity of the banana flours was studied. The result of ANOVA (Table 1) exhibited that different varieties did not influence extract yield and DPPH inhibition, however varieties did exhibit significant differences ($P<0.0001$) on TPC and TFC. On the other, hand the results of ANOVA for different samples exhibited significant difference ($P<0.0001$) on all selected responses which means that different sample yielded different values of extract yield, TPC, TFC and DPPH scavenging activities ($P<0.0001$).

The actual average data of extract yield, antioxidative compounds analysis and antioxidant activity are given in Table 2. The extract yield of the banana flour varied over a wide range of 11.21 % to 28.35%. Sultana *et al*. (2008) showed the extract yield of banana peel with 80% ethanol was 24.6%, whereas Okonogi *et al*. (2007) obtained a yield of 7.66% with 95% of ethanol solvent and Gonzalez-Montelongo *et al.* (2010a) obtained a yield of extract ranged 3% to 54% with methanol : water and acetone : water extraction respectively. Thus the present data for banana peel and pulp flours fell in the typical range of banana extract yields. Tukey's test for yield showed that there was no significant difference ($P>0.05$) in yield extracted from RDPu and RCPu, RCPu and GDPu, and RCPe and GCPe, whilst the rest exhibited a significant difference among them and other samples ($P<0.05$). The yield was in the order; GCPe, RCPe > GDPe > RDPe > GCPu > GDPu, RCPu > RDPu. Variations in the yield of extracts might be attributed to the availability of different extractable components, defined by the chemical composition, nature of the soil and agro-climatic condition (Hsu *et al*., 2006). Other parameters and effectiveness of the extracting solvent to dissolve endogenous compounds might also be a contributing factor (Sultana *et al*., 2008).

Phenolic compounds are important fruit constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Maisuthisakul *et al*., 2007). The TPC determined in 80 % methanolic extracts of banana peel and pulp flours are shown in Table 2. The TPC ranged widely from 75.01 to 685.57 mg GAE/100 g of dry matter. Except between GDPu and RDPe, all samples showed significant differences ($P<0.05$) in the TPC. The TPC was in the order; GCPe > RCPe > GCPu > RCPu > GDPe > RDPe, GDPu > RDPu.

Table 1. The ANOVA for extract yield, TPC, TFC and inhibition of DPPH scavenging activitya

	S.O.V	D.F	S.S	M.S	F-value	P-value
			Type of Banana			
Yield	Between types of banana	30.827	1	30.83	0.621	<0.439
	Error	1092.015	22	49.64		
	Total	1122.842	23			
TPC	Between types of banana	789554.40	1	789554.40	44.215	<0.0001
	Error	392856.19	22	17857.10		
	Total	1182410.59	23			
TFC	Between types of banana	241101.26	1	241101.26	75.214	<0.0001
	Error	70521.79	22	3205.54		
	Total	311623.05	23			
DPPH	Between types of banana	66.43	1	66.43	0.742	<0.398
	Error	1970.88	22	89.59		
	Total	2037.31	23			
			Samples			
Yield	Between samples	1119.82	7	159.975	848.017	<0.0001
	Within samples	3.02	16	0.189		
	Total	1122.84	23			
TPC	Between samples	1182398.18	7	168914.025	217818.91	<0.0001
	Within samples	12.408	16	0.775		
	Total	1182410.59	23			
TFC	Between samples	311592.93	7	44513.28	23645.78	<0.0001
	Within samples	30.12	16	1.88		
	Total	311623.05	23			
DPPH	Between samples	2020.70	7	288.67	277.95	<0.0001
	Within samples	16.62	16	1.04		
	Total	2037.31	23			

[a] TPC (mg GAE/100g of dry matter), TFC (mg CE/100g of dry matter).

Table 2. Extract yield, TPC, TFC and inhibition of DPPH radicals of banana pulp and peel flours prepared from different variety and stage of ripenessm

Parameter	Cavendish banana				Dream banana			
	RCPu	RCPe	GCPu	GCPe	RDPu	RDPe	GDPu	GDPe
Yield of extract (%)	12.35 ± 0.40^{e,f}	27.28 ± 0.53^{a}	14.75 ± 0.47^{d}	28.35 ± 0.44^{a}	11.21 ± 0.28^{f}	23.84 ± 0.25^{c}	13.26 ± 0.35^{e}	25.34 ± 0.62^{b}
TPC	230.21 ± 1.19^{d}	585.29 ± 0.99^{b}	373.88 ± 0.92^{c}	685.57 ± 0.80^{a}	75.01 ± 0.82^{g}	94.9 ± 0.83^{f}	94.25 ± 0.53^{f}	160.77 ± 0.82^{e}
TFC	196.45 ± 0.89^{d}	225.91 ± 1.49^{c}	281.18 ± 1.88^{b}	389.33 ± 1.61^{a}	39.01 ± 1.17^{h}	72.46 ± 1.33^{g}	82.64 ± 1.07^{f}	96.92 ± 1.22^{e}
DPPH inhibition (%)	29.38 ± 0.70^{e,f}	45.08 ± 1.30^{b}	35.21 ± 0.98^{d}	52.66 ± 0.82^{a}	26.55 ± 0.86^{f}	40.01 ± 1.44^{c}	31.82 ± 0.93^{e}	50.64 ± 0.75^{a}

[m]Data are mean ± standard deviation (n = 3).
[a,b,c,d,e,f,g ,h] Values with different superscripts within a row are significantly ($P < 0.05$) different.
TPC (mg GAE/100g of dry matter), TFC (mg CE/100g of dry matter).

In general, this ranking shows that flours prepared from the pulp and peel of Cavendish had higher TPC than those prepared from Dream banana. For a particular banana component, the TPC was generally higher in the peel than in the pulp, and the green had higher TPC than the ripe components. The TPC of banana peel flour have been reported in the literatures as high as 1.1 g GAE/100g of dry matter (Sultana et al., 2008) when extracted with ethanol, and 907 mg CEQ/100 g when extracted with water-chloroform (Someya et al., 2002) and 1.4 g GAE/100 g when extracted with methanol (Gonzalez-Montelongo et al., 2010b). The TPC of banana pulp reported in the literatures were 56.1 mg GAE/100 g (Sun et al., 2002), 51-54 mg GAE/100 g for Pisang Mas banana (Lim et al., 2007; Alothman et al., 2009) and 90.4 mg GAE/100 g for Pisang Awak banana (Choo and Azis, 2010). The results of TPC of banana pulp flours in the present work were slightly higher than the literatures, however, the TPC of the banana peel flours were generally lower than the literatures.

Tukey's test for total flavonoid (TFC) showed significant difference ($P<0.05$) among samples and ranged from 39.01 to 389.33 mg CEQ/100 g of dry matter. The TFC order were as follows; GCPe > GCPu > RCPe > RCPu > GDPe > GDPu > RDPe > RDPu. In general, the TFC was higher in Cavendish as compared to Dream samples and for each type of banana the flours prepared from the green fruits had higher TFC than those obtained from the ripe fruits. In all types and stage of ripeness, it is evident that the peel always presented higher TFC than the pulp. The TFCs of the peel were quite low in comparison with previous report (0.62 g CEQ/100 g of dry matter) (Sultana *et al.*, 2008). The TFC of the pulp flours was higher than those reported by Alothman et al. (2010) (3.70 mg CEQ/100 g flesh weight of Pisang Mas banana). The variation in TPC and TFC among different plant materials might be attributed to factors such as natural chemical composition, maturity at harvest, soil state and conditions of post-harvest storage (Huang *et al*., 2005).

Antioxidant capacity

The ability of banana peel extracts to scavenge DPPH radicals has been demonstrated (Okonogi *et al*., 2007). Therefore DPPH scavenging activity of each sample was reported as the percentage of DPPH inhibition, with a higher value is associated to a stronger antioxidant activity. All extracts showed free radical scavenging properties in different levels (Table 2). The inhibition of DPPH radical of the banana flour ranged from 26.55 to 52.66%. This is consistent with those reported by Alothmant *et al*. (2009); Choo and Azis (2010). The tukey's test showed that DPPH scavenging activities differed significantly ($P<0.05$) among all samples except between GDPu, RCPu, and RDPu. Thus, the DPPH scavenging activity was in the order; GCPe, GDPe > RCPe > RDPe > GCPu > GDPu, RCPu, RDPu. The extracts prepared from Cavendish and Dream green banana peel (GCPe and DGPe) flours exhibited the highest scavenging activity, whilst the extracts prepared from green Dream, ripe Cavendish and ripe Dream banana pulp (GDPu, RCPu, RDPu) flours exhibited the lowest. It is evident that the extract obtained from the peel had higher antioxidant activity than that obtained from the pulp. Typically for plant materials, DPPH inhibition would follow a similar order of the TPC and TFC, i.e. as the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, the DPPH radical scavenging activity also increases (Alotman *et al*., 2009; Gonzalez-Montelongo *et al*., 2010b). This is however not the case in this study. Even though GDPe and RDPe were ranked quite low in terms of TPC and TFC, their antioxidant activities ranged from moderate (RDPe) to high (GDPe). These results imply that antioxidative compounds other than phenolics and flavonoids were also involved in inhibiting the

DPPH radicals. Compounds such as ascorbic acid, β-carotene, α-carotene and different xanthophylls (Subagio *et al*., 1996; Kondo *et al*., 2005) have been detected in banana and may have contributed to the antioxidant activity of the extracts.

Conclusion

Banana flours prepared from pulp and peel of Cavendish variety had higher antioxidative compounds than those prepared from the Dream variety. In most of the cases the antioxidative compounds were generally higher in the peel than in the pulp and in the green than in the ripe components. The free radical scavenging properties of the extracts were not directly related to the TPC and TFC, suggesting the presence of other antioxidative compounds that had also contributed to the inhibition of DPPH radicals.

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