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Original Article

Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (*Musa* sp.)

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ABSTRACT

Correlations between total phenolic and mineral contents with antioxidant activities of pulps and peels from eight banana (*Musa* sp.) cultivars were studied. The total phenolic contents were determined using Folin–Ciocalteu colorimetric method, and antioxidant activities were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant potential (FRAP) assay. The highest total phenolic content (76.37 ± 1.79 mg GAE/g d.w.) was obtained from the freeze-dried extract of fresh pulps of Raja cultivar. The maximum activity of DPPH (19.39 ± 0.15 mg TE/g d.w.) was recorded for the chloroform extract of dried peels of Mas cultivar. Meanwhile, the highest activity of FRAP was shown by most of the chloroform extracts of dried pulps, dominated by Awak cultivar (22.57 ± 0.13 mg TE/g d.w.). With few exceptions, peel extracts exhibited higher total phenolic content and stronger antioxidant activities than that of the pulps. Very weak correlation between total phenolic content and FRAP activity was observed, yet it is higher ($r^2 = 0.1614$, $p < 0.0001$) than that of total phenolic content and DPPH activity ($r^2 = 0.02339$, $p < 0.05$). A moderate correlation between DPPH and FRAP activities was obtained ($r^2 = 0.3533$, $p < 0.0001$). For mineral analysis, potassium (K) is the major element found in both fresh pulps and peels followed by P, Mg and Na. Fresh peels of Raja consisted the highest amount of K (1387.5 mg/100 g f.w.). With exception of Mn, no correlation was found between mineral content and antioxidant activity. A moderate correlation between Mn content and DPPH activity was observed ($r^2 = 0.2855$, $p < 0.0001$).

1. Introduction

Fruit antioxidants are scientifically proven for their synergistic effects and protective properties against various degenerative disorders including cancer, stroke, cardiovascular, Alzheimer's disease and Parkinson's disease (Giasson et al., 2002; Ndhlala et al., 2006; Kawasaki et al., 2008; Abdel-Hameed, 2009). The protection mechanism generally functions at several different levels within cells in human body by inhibiting the formation of free radical species, intercepting radical-chained reactions, converting existing free radicals into less harmful molecules and repairing oxidative damage (Du et al., 2009).

Banana is classed as one of the most popular fruits on the world market (Meechaona et al., 2007). It belongs to the genus *Musa* from the family Musaceae. This tropical fruit has a strong ability to protect itself from the oxidative stress caused by intense sunshine and high temperature by increasing its antioxidant levels (Kanazawa and Sakakibara, 2000; Mokbel and Hashinaga, 2005). Accumulating evidence has revealed that both banana pulp and peel contain various antioxidants, for instance vitamins (A, B, C and E), β-carotene and phenolic compounds such as catechin, epicatechin, lignin and tannins and anthocyanins (Someya et al., 2002; Wall, 2006; Lim et al., 2007). Banana is also notably enriched with minerals such as potassium and phosphorus (Hardisson et al., 2001; Leterme et al., 2006; Wall, 2006). Several reports indicated that banana peels possess higher phenolic compounds and antioxidant properties (Kondo et al., 2005; Someya et al., 2002) as well as mineral contents than that of banana pulps (Forster et al., 2002; Emaga et al., 2007). However, most of the previous studies have been focused mainly on only one cultivar that is the most well-known banana cultivar, i.e. *Musa acuminata* AAA (sub-group Cavendish) (Someya et al., 2002; Vijayakumar et al., 2008; González-Montelongo et al., 2010).

Various clones of edible bananas have evolved and been brought under cultivation in the rainy parts of southeast Asia, particularly in Malaysia. All of these cultivars are derived from two wild species, namely *Musa acuminata* and *Musa balbisiana*, which are native to southeast Asia. *M. balbisiana* is a hardy species and has a wider area of distribution than *M. acuminata* (Stover and Simmonds, 1987). Haploid contribution of those two species is

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designated as A and B. Intra- and inter-specific hybridization occurred resulting in eight genomic combinations in diploid (AA, AB and BB), triploid (AAA, AAB, ABB and BBB) and tetraploid (ABBB) forms. The hybrids should not carry a specific name but must use the letters A and B which indicate genome composition and ploidy with respect to parental origin (Valmayor et al., 1990). According to the banana germplasm collection at Malaysian Agricultural Research and Development Institute (MARDI), there are more than 50 edible banana cultivars in Malaysia (Nik Hassan, 2003). Among the numerous cultivars, only eight were selected in this study. They were selected due to their high consumption among local peoples and common availability in local markets. Various forms of banana products, such as juice (fresh juice form), flour (freeze-dried form), dessert (fresh form) and cracker/snack (dried form) are locally produced from the pulps of these selected cultivars (Mahmood, 2001). They can easily be differentiated from one another based on their distinct fruit characteristics, flavors and tastes. Most of the cultivars that belong to groups AA and AAA and some from group AAB are sweet and can be eaten raw as desert bananas, while others are starchy cooking bananas also known as plantains (Valmayor et al., 2000).

To date, no comparative study of antioxidant activity, total phenolic content and mineral composition of these banana cultivars has been reported. Therefore, this study aimed (i) to determine the influence of cultivars and extraction procedures to the quantitative analyses of the antioxidant activities, total phenolic and mineral contents of extracts obtained from peels and pulps of the cultivars; (ii) to correlate the total phenolic and mineral contents with the antioxidant activities. To date, no report on the correlation between antioxidant activity and mineral content of fruit is available. Investigating the role of minerals in antioxidant activity of banana is crucial due to its reputed enrichment with essential minerals; (iii) to determine the best extraction method for extracting antioxidants and phenolic compounds from banana tissues. Different extracts were obtained from the aqueous extraction of fresh pulps and peels including fresh juices/extracts and freeze-dried extracts; and sequential extraction of fresh and dry samples using different polarities of solvents. According to González-Montelongo et al. (2010), optimization of extraction condition and procedure is a key factor for accurate quantification of total phenolic content and antioxidant activities of banana.

2. Materials and methods

2.1. Plant materials

Fruits from eight cultivars of bananas were obtained from a farm at Batu Kurau, Perak, Malaysia. The identities were checked by morphological examination and comparison with authentic herbarium specimens, information from *Musa* Germplasm Information System (MGIS) and literature reviews (Daniells et al., 2001; Frison et al., 1999). Table 1 shows a list of cultivars used in this study, their groups, sub-groups, accession names and codes as recorded in MGIS (http://www.crop-diversity.org/banana/) and voucher numbers of voucher specimens that were deposited at the Herbarium of School of Biological Sciences, Universiti Sains Malaysia (USM).

2.2. Sample preparation and extraction

Commercial ripening stage of fruits from three hands (obtained from three different trees) of each cultivar of banana (approximately 800–900 g and 12–16 fruits per hand) were washed with clean sterile water and peeled. After that, 250 g of fresh pulps and peels from each hand were separately diced into small cubes, in order to optimize the extraction process. The size of each cube was approximately 2 mm (length) × 2 mm (width) × 2 mm (thickness). The interval between peeling and experimental work was specified to be less than 1 h. The cubes were then divided into three groups. The first and second groups were freshly extracted (50 g each) whereas the third group (150 g) was dried in an oven at 60 °C for two days prior to the extraction. All three extraction processes were carried out in triplicate, using fruits from different hands of bananas each time.

2.2.1. Water extraction of fresh samples

Water extraction of the samples was carried out by boiling 50 g of fresh banana pulps and peels separately in a 1000 mL conical flask containing 500 mL of distilled water and magnetic stirrer (at 1000 rpm) for 30 min on a stirring hot plate (Fisher Scientific, Pittsburgh, PA). The extracts were then filtered using a clean muslin cloth and centrifuged (Hittech EBA 20 Centrifuge, Japan) at 3000 rpm for 15 min. Half of the supernatant was stored at −21 °C over night and then freeze dried. The freeze-dried extracts were then kept at 4 °C for further test. Meanwhile, another half of the supernatant was freshly tested at concentration 100 milligrams of fresh sample per milliliter of water (100 mg/mL).

2.2.2. Sequential extraction of fresh and dried samples

Sequential extractions of fresh and dried samples were conducted using three different solvents with increasing polarity (i.e. hexane, chloroform and 80% methanol (v/v)). The solvents were purchased from Fisher Scientific (Springfield, NJ). For the fresh samples, 50 g fresh weight of each sample was soaked in the first solvent, i.e. hexane (500 mL) in a 1000 mL conical flask for 24 h on the stirring hotplate (Fisher Scientific, Pittsburgh, PA) with magnetic stirrer (at 1000 rpm). The extraction process was conducted in the fume cupboard at room temperature (25 ± 1 °C). The obtained extract was filtered using filter paper (Whatman No. 1, England) and the filtrate was concentrated using rotary evaporator (EYELA, Japan). The same extraction procedure was applied to the residue of sample. It was successively soaked in 500 mL chloroform (second solvent) followed by 80% (v/v) methanol (third solvent). Using the same type, volume and sequence of solvents as the fresh banana, 50 g dry weight of dried sample was successively extracted in a Soxhlet apparatus. All the dried extracts were stored at 4 °C for further study.

Table 1
Eight banana cultivars used in this study.

Banana cultivar	MGIS documentation				USM herbarium specimen
	Group	Sub-group	Accession name	Code	Voucher number
Mas	AA	Sucrier	Pisang Mas	ITC1403	USM11113
Kapas	AA	AA Ssp/sgr 732	Chuoi Tien	ITC1368	USM11115
Berangan	AAA	Lakatan	Lakatan	ITC0573	USM11118
Rastali	AAB	Silk	Rasthali	TRY0297	USM11117
Raja	AAB	Pisang Raja	Raja	RIF0037	USM11114
Nangka	AAB	Pisang Nangka	Pisang Nangka	ITC0004	USM11116
Awak	ABB	Pisang Awak	Pisang Awak	ITC0213	USM11111
Nipah	BBB	BBB Ssp/sgr 501	Saba	TRY0787	USM11112