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OPTIMIZATION OF EXTRACTION PARAMETERS ON THE ANTIOXIDANT PROPERTIES OF BANANA WASTE

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ABSTRACT

Background. Banana is grown worldwide and consumed as ripe fruit or used for culinary purposes. Peels form about 18–33% of the whole fruit and are discarded as a waste product. With a view to exploiting banana peel as a source of valuable compounds, this study was undertaken to evaluate the effect of different extraction parameters on the antioxidant activities of the industrial by-product of banana waste (peel).

Materials and methods. Influence of different extraction parameters such as types of solvent, percentages of solvent, and extraction times on total phenolic content (TPC) and antioxidant activity of mature and green peels of *Pisang Abu* (PA), *Pisang Berangan* (PB), and *Pisang Mas* (PM) were investigated. The best extraction parameters were initially selected based on different percentages of ethanol (0–100% v/v), extraction time (1–5 hr), and extraction temperature (25–60°C) for extraction of antioxidants in the banana peels. Total phenolic content (TPC) was evaluated using Folin-Ciocalteu reagent assay while antioxidant activities (AA) of banana peel were accessed by DPPH, ABTS, and β-carotene bleaching (BCB) assays at optimum extraction conditions.

Results. Based on different extraction solvents and percentages of solvents used, 70% and 90% of acetone had yielded the highest TPC for the mature and green PA peels, respectively; 90% of ethanol and methanol has yielded the highest TPC for the mature and green PB peels, respectively; while 90% ethanol for the mature and green PM peels. Similar extraction conditions were found for the antioxidant activities for the banana peel assessed using DPPH assay except for green PB peel, which 70% methanol had contributed to the highest AA. Highest TPC and AA were obtained by applying 4, 1, and 2 hrs extraction for the peels of PA, PB and PM, respectively. The best extraction conditions were also used for determination of AAs using ABTS and β-carotene bleaching assays. Therefore, the best extraction conditions used have given the highest TPC and AAs.

Conclusions. By-products of banana (peel) can be considered as a potential source of antioxidants in food and pharmaceutical industry.

Key words: antioxidant activity, banana peel, extraction parameters, total phenolic content

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INTRODUCTION

Malaysia is well known to have rich biodiversity of many tropical nutritious fruits and vegetables found easily in the region of Peninsular Malaysia, Sabah and Sarawak. Banana (*Musa* species) is a nutritious fruit and it has large, fleshy and upright stalks topped with soft and smooth leaves. Banana originates from the family of Musaceae and can be classified into two main species, which are *Musa acuminata* and *Musa balbisiana* (Valmayor et al., 2002). Bananas are an important food crop in the subtropics and tropics where it is a good source of nutrients and energy. Bananas are often categorized as 'dessert' sweet bananas, which are ripened and eaten raw, but also edible when fully ripe (Perrier et al., 2011).

The peel of banana is a typical waste after consumption or disposed by banana crisp industries (Oliveira et al., 2008). The constituents of banana peel were 0.9, 1.7, 8.9–12.96, 59 and 31.7% of protein, lipid, ash, carbohydrates and fiber, respectively (Nagarajaiah and Prakash, 2011). Due to the high percentage of ash (8.9–12.95%), banana peel contains various minerals, such as, potassium, calcium, sodium, iron, manganese and bromine (Anhwange, 2008; Nagarajaiah and Prakash, 2011). Banana peel contains potent antioxidant compounds. Vitamin A, vitamin C, and carotenoids are the most abundant antioxidants in banana pulp as well as in banana peel (Arora et al., 2008; Pereira and Maraschin, 2015; Tsamo et al., 2015; US Department..., 2014). Banana peels are also known to have higher antioxidant activity (AA) than the pulp (Oliveira et al., 2008; Pereira and Maraschin, 2015; Someya et al., 2002). This shows that banana peel could be one of the natural sources of antioxidant. Someya et al. (2002) demonstrated that total phenolic content (TPC) was high in banana peels, accounting for 907 mg/100 g of dry weight of sample, where the phenolic compounds identified were mainly consisted of gallocatechin, catechin, and epicatechin. Kanazawa and Sakakibara (2000) found that banana peel, not its pulp, contained naringenin and rutin. In a recent study, Tsamo et al. (2015) demonstrated that hydroxycinnamic acids (sinapic and ferulic acids) are the predominant phenolic acids in banana pulp whereas flavonols (rutin and myricetin) dominated in banana peel. Dopamine was also identified in banana peel, where dopamine concentration in the banana peel was higher than the pulp (Teeranud et al., 2005). Besides, saponin (24 mg/g) has also been found in *Musa sapientum* peels (Anhwange, 2008).

Based on the significant findings obtained from previous studies, banana peel is a potential waste product that could be utilized for its nutraceutical properties. Extraction is an important stage in isolation and identification of phenolic compounds (Naczk and Shahidi, 2006). Extraction conditions such as solvent type, solvent concentration, extraction temperature, extraction time and solid-solvent ratio are the major concerns to enhance the efficiency in order to obtain highest yields of anti-oxidative compounds from natural resources (Pinelo et al., 2005; Spigno et al., 2007; Thoo et al., 2010). Each extraction parameter should be applied to single type of plant sample since different types of plant sample has its own range of phytochemical content (Wong et al., 2014). Studies determining the optimized extraction conditions using various plant extracts, such as *Inga edulis* (Silva et al., 2007), *Morinda citrifolia* (Thoo et al., 2010), grape marc (Spigno et al., 2007), flaxseed (Anwar and Przybylski, 2012) and passion fruit peel (Wong et al., 2014) had increased considerably in the recent years. To the best of our knowledge, the studies on optimum extraction conditions of antioxidants from natural sources are still scarce.

Therefore, this study aimed to establish a standard antioxidant extraction method by investigating the influence of extraction parameters on total phenolic content (TPC) and antioxidant activity (AA) of the peels of mature and green banana from three different local varieties. Then, the AA of banana peel will be determined at optimum extraction conditions by means of β-carotene bleaching activity (BCB), 2,2-diphenyl--1-picrylhydrazyl (DPPH) radical scavenging ability and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ABTS as well as TPC. Finally, the correlation between AC and TPC will be determined. Since most of the published studies have focused on Cavendish species, here we aimed to study the TPC and AA of the peels of mature and green banana from local Malaysian varieties (*Musa acuminate* Colla).

MATERIAL AND METHODS

Chemicals and reagents

All the chemicals and reagents were of analytical grades. Ethanol, hexane, gallic acid, linoleic acid, Trolox, Tween 40, sodium carbonate anhydrous, chloroform, iron (III) chloride anhydrous, and potassium persulfate were purchased from Fisher Scientific Co. (Fisher Scientific, Loughborough, UK). Acetone, methanol, Folin-Ciocalteu's reagent, and 2,2'-azino-di[3-ethyl-benzthiazoline sulfonate] (ABTS) were from Merck KGaA (Lichrosolv, Darmstadt, Germany), while 2,2-diphenyl-1-picryhydrazyl (DPPH), β-carotene, butylated hydroxy anisole (BHA), acetic acid, and sodium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Distilled water used was obtained from a Milli-Q water purification unit (Millipore, Milford, MA, USA).

Samples preparation and extraction

Three different varieties of banana (*Musa acuminata*), namely *Pisang Abu*, *Pisang Berangan*, and *Pisang Mas* were purchased from local market (Table 1). All bananas were washed under running tap water to remove the impurities. The banana peels were oven-dried (Memmert, Germany) at 40–45°C for 24 hr and then milled into powder through a 1.0 mm sieve using a miller (IKA-WERKE MF10B, Germany). The banana peel powder (10 g) was initially extracted with 100 mL of the selected solvents (methanol, ethanol, acetone, deionised water, and hexane) and subjected to agitation at 140 rpm in room temperature (25°C) on a shaker (Green Seriker, Busan, Korea) for 2 hr. The residues were removed by filtration using Whatman No. 1 filter papers. The residues were re-extracted using the same conditions and the combined extracts were concentrated at 40°C (Rotavapor R-200, Zubich, Switzerland).

Experimental design

Single factor experiment was used to determine the optimum extraction condition for banana peel. The influence of extraction parameters on the AA of banana peel, namely types of solvents, solvent concentration, and extraction duration were studied where one parameter was varied at a time while other parameters were kept constant (Thoo et al., 2010; Wong et al., 2014).

Extraction solvent and its concentration. Based on the selected solvents (acetone, ethanol, hexane, methanol and water), the samples were extracted using different percentages (10, 30, 50, 70 and 90%, v/v) of the best extraction solvent for 2 hr with other variables remained constant at room temperature (25ºC) for 60 min according to previous study (Wong et al., 2014).

Extraction duration. Different extraction times of 1, 2, 3, 4 and 5 hr at room temperature were applied using the best solvent concentration determined. The best solvent and its concentration, together with the best extraction duration were determined based on the highest total phenolic content (TPC) followed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Based on the screening tests, 500 µg/mL extract yielded the highest TPC and DPPH radical scavenging activity compared to other extract concentrations (100, 200, and 300 µg/mL). Therefore, 500 µg/mL of the extract was applied in this study. Finally, the best extraction conditions (solvent type, solvent concentration, and extraction time) were used to further determine the TPC and AAs (DPPH, ABTS, β-carotene bleaching assays) of the mature and green banana peels. To determine the EC_{50} values for DPPH and ABTS assays, 100–500 µg/mL of the extract and synthetic antioxidant (BHA) was used to plot the standard calibration curve for calculation of EC_{50} value that

Table 1. List of the three Malaysian cultivated banana cultivars

Cultivars	Classification	Local name
M. acuminata × *balbisiana* Colla cv. 'Bluggoe'	ABB	*Pisang Abu* (PA)
M. acuminata Colla cv. 'Masak Hijau'	AAA	*Pisang Berangan* (PB)
M. acuminata Colla cv. 'Lady's Finger'	AA	*Pisang Mas* (PM)

defined as the concentration of the extract to reduce the initial DPPH concentration by 50%, where EC_{50} was obtained from a linear regression equation.

Total phenolic content (TPC) analysis

The TPC was determined based on the method of Singleton and Rossi (1965) with slight modifications. Briefly, this colorimetric-based assay was performed by mixing 1.0 mL of sample with 1.0 mL of diluted Folin-Ciocalteu's reagent (1:10 ratio) and agitated for 5 to 10 s using a vortex mixer (VORTEX V-1, BPECO, Germany). The mixture was left to stand for 3 min in the dark. Then, the mixture was added with 1.0 mL of 7.5% of Na_2CO_3 solution and topped up to 10 mL with distilled water. The reacting mixture was kept in dark at room temperature for 90 min. The absorbance was read at 725 nm using a spectrophotometer (PRIM, Secomam, Alés Gard, France) against the blank. TPC was expressed as µg gallic acid equivalents (GAE)/mL extract. All analyses were performed in triplicate.

DPPH radical scavenging ability

Measurement of the ability of the sample extract to scavenge free radicals was determined based on a method described by Brand-Williams et al. (1995) with slight modifications. An aliquot of 4 mL of extract was mixed with 1.0 mL of methanolic solution containing DPPH radicals (final concentration of 0.2 mM). The mixture was vortex vigorously for 10 sec and left to stand at room temperature in dark for 30 min. The absorbance was measured at 517 nm against blank using a UV spectrophotometer (XTD 5, Secomam, Alés Gard, France) after 30 min. Each experiment was carried out in triplicate. BHA was used as the comparative standard for this assay. Antioxidant activity (AA) was expressed as the percentage of scavenging activity, calculated based on the following equation:

$$\text{Scavenging activity, \%} = [1 - \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}] \times 100 \quad (1)$$

ABTS radical cation inhibition activity

Determination of the scavenging ability of banana peel extracts was performed based on a method described by Re et al. (1999) with slight modifications. To begin, the stock solution of ABTS radicals was prepared by mixing 5 mL of 7 mM ABTS powder with 88 µL of 140 mM potassium persulfate in reagent bottle and kept in dark at room temperature for 18 hr to allow the complete generation of ABTS radical. Briefly, 1 mL of ABTS stock solution was diluted with 70 mL of ethanol to obtain absorbance of 0.70 ±0.05 measured at 734 nm. An aliquot of 100 µL of extract was added to 1 mL of ABTS reagent and vortexed vigorously for 10 s. The reacting mixture was allowed to stand in dark at room temperature for six min. The absorbance was measured at 734 nm against ethanol blank using a UV spectrophotometer (XTD 5, Secomam, Alés Gard, France). BHA was used as comparative standard. AA was calculated as percentage of inhibition activity relative to control, which was calculated based on the following equation:

$$\text{Inhibition activity, \%} = [1 - \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}] \times 100 \quad (2)$$

β-Carotene bleaching assay

β-Carotene bleaching (BCB) assay was performed based on a method described by Cheung et al. (2003) with slight modifications. Working reagents for BCB assay was prepared by mixing 1 mL of β-carotene solution (0.2 mg of β-carotene powder in 1 mL chloroform) with 0.02 mL of linoleic acid and 0.2 mL of Tween 40. Then, 1 mL of β-carotene solution was transferred into a round bottom flask and the chloroform was removed under vacuum at 40°C. The remaining solution was diluted with 50 mL of oxygenated water. The assay was started by adding 4.8 mL of the emulsion mixture to 0.5 mL of extract. The mixture was shaken vigorously for a while until liposome is formed. The absorbance of the mixture taken at 0 min measured at 470 nm using a UV spectrophotometer (XTD 5, Secomam, Alés Gard, France). The mixture was instantly placed into a water bath (WB/OB 7–45, Germany) of 50°C for 2 hr after the absorbance was taken. The absorbance of the reacting mixture was taken at 20 min interval until 120 min of incubation. The β-carotene bleaching rate (R) was calculated based on the following equation:

$$R = \ln (a/b) / t \quad (3)$$

where:

ln – the natural logarithm
a – the absorbance at 0 min
b – absorbance reading obtained at 20, 40, 60, 80, 100 and 120 min
t – the time, min.

AA was calculated as the percentage of inhibition activity relative to control based on the following equation:

$$\% \text{ inhibition activity} = [1 - \frac{R_{sample}}{R_{control}}] \times 100 \quad (4)$$

where:
$R_{control}$ and R_{sample} – the bleaching rates of β-carotene in the emulsion without antioxidant and with sample extract, respectively.

Statistical analysis

All independent analyses were performed in triplicate. The data obtained were statistically analysed using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA), where the results were expressed as mean ±standard deviation. Differences in the mean values were analyzed by one way ANOVA and followed by Bonferroni's test for comparison, as a post hoc test to determine the significant difference ($p < 0.05$) among the mean values of the samples. Pearson correlation was used to assess the relationships between TPC and AA (DPPH, BCB and ABTS).

RESULTS AND DISCUSSION

Effect of extraction solvents on TPC and AA

Applying a standardized 2 hr extraction time and 500 µg/mL of extract concentration, methanol has extracted the highest TPC from the peels of matured and immatured PA and PB as compared to other extraction solvents, while the peels of mature and immatured PM extracted by acetone and ethanol, respectively showed the highest TPC (Fig. 1). TPC determined for the types of solvent used for each matured or immatured peel of PA, PB, and PM is shown in Figure 1.

The AA of the banana peel extracts was initially determined using DPPH radical scavenging assay. Based on the different extraction solvents used, banana peel extracts extracted with methanol demonstrated the highest percentage of DPPH radical scavenging activity for the peels of matured PA and immatured PB, while ethanol for the peels of matured PB, matured PM, and immatured PM. The peel of PA demonstrated the highest percentage of AA extracted using acetone compared to other extraction solvents (Fig. 2). The percentages of AA for the peels of PA, PB, and PM that extracted using different extraction solvents are shown in Figure 2.

This study demonstrates that different extraction solvents have their own ability to extract different types of phytochemicals, such as phenolic compounds. Water extracts of the immatured peels of banana samples have shown to contain higher TPC than the matured counterparts. This is true since water, a polar solvent is able to extract more phenolic compounds. In order to extract phenolic compounds in banana peel, hexane is not suitable to be used since hexane extract has low TPC, which could be due to its low polarity (Chirinos et al., 2007). Non-polar antioxidants are known to have high solubility in hexane, therefore, hexane is more suitable for extraction of carotenoids in banana peels, instead of phenolic compounds (Naczk and Shahidi, 2006; Xu and Chang, 2007).

Generally, methanol as a universal organic solvent is recommended for the extraction of phenolic compounds, including the polar and non-polar forms (González-Montelongo et al., 2010; Tabart et al., 2007). High TPC was found for methanolic extracts of the banana sample besides ethanolic and acetone extracts. Liu et al. (2007) supported our findings that the TPC of *Xylaria* sp. was the highest in methanol extract as compared to hexane extract. Methanol is commonly known as the best extraction solvent for fruit peels (Naczk and Shahidi, 2006). In relation, highest TPC was also observed for apple residue, strawberry residue, and artichoke waste extracted with methanol compared to other extraction solvents (Peschel et al., 2006).

Solvents with intermediate polarity are often used for extraction compared to non-polar or highly polar solvent. Ethanol as one of the solvents with intermediate polarity has been used for extraction of phenolic compounds such as flavanoids, catecols, and tannins from plants materials (Spigno et al., 2007). Besides, ethanol has been used for the extraction of phenolic compounds in mango peels (Kim et al., 2010). Moreover, ethanol is also a more polar solvent which tend

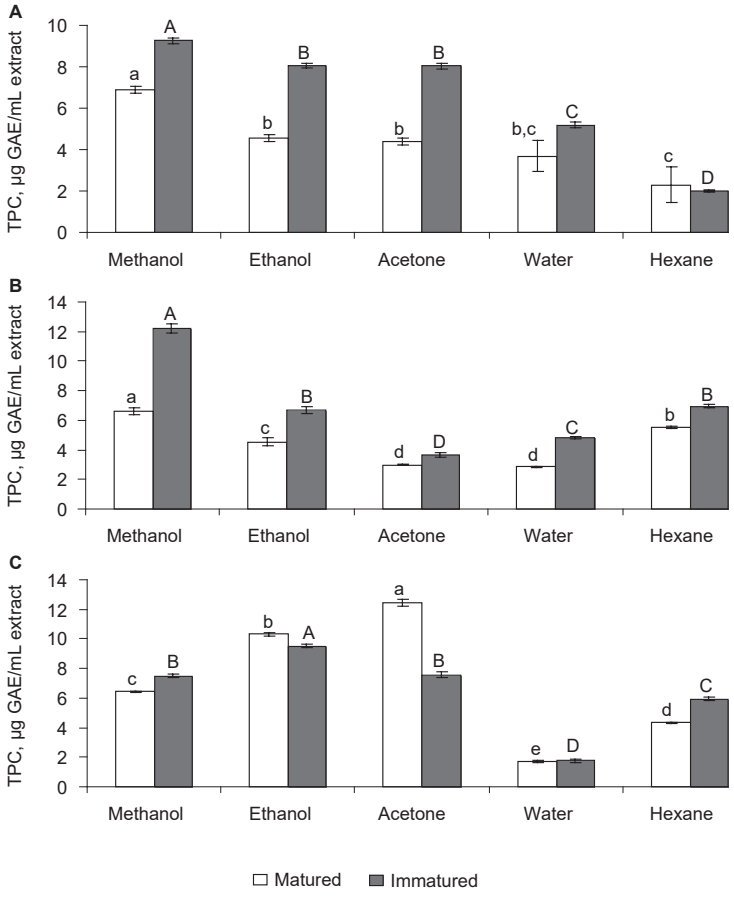

Fig. 1. Total phenolic content (TPC) of the mature and green peels of (**A**) PA, (**B**) PB, and (**C**) PM determined based on different extraction solvents (methanol, ethanol, acetone, water and hezane). Different capital letters (immatured) and small letters (matured) denote that they are significantly different ($p < 0.05$) amongst different extraction solvents

to highly solubilize hydroxylated aglycone forms of phenolic compounds (Arts and Hollman, 1998).

Methanolic and ethanolic extracts of banana samples have high percentages of scavenging activity. González-Montelongo et al. (2010) supported our findings that methanolic extract of the peel of banana from AAA group has the highest scavenging activity determined using DPPH assay compared to other extraction solvents used. Tabart et al. (2007) also demonstrated that methanol was the best solvent for extraction of catechin, where high concentration of catechin has been found in banana peels (Someya et al., 2002), which might contribute to its high AA. Besides, ethanol extract gave higher scavenging ability on sun dried mango seed kernel compared to water extract (Maisuthisakul and Gordon, 2009). Higher AA should be found in alcoholic extract compared to water extract because alcoholic solvent maximizes the interaction of DPPH radicals with antioxidants present in propolis as reported by Gregoris and Stevanato (2009).

Effect of solvent percentages on TPC and AA

As shown in Table 2, 70% methanol and 90% acetone have shown the highest TPC and AA in the peels of mature and green PA, while 90% ethanol and 70%

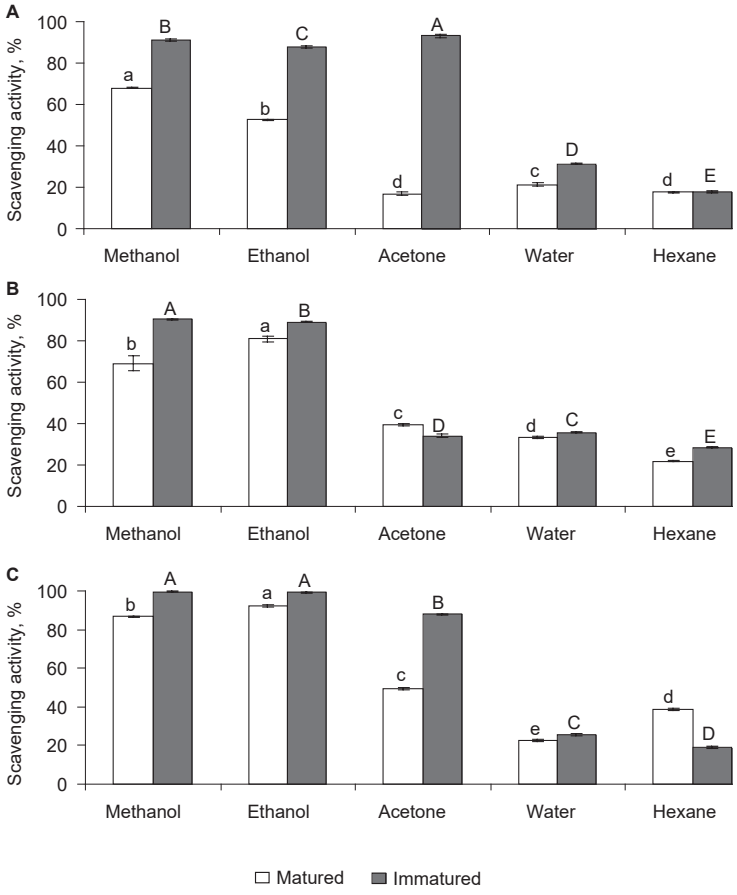

Fig. 2. AA of the mature and immatured peels of (**A**) PA, (**B**) PB, and (**C**) PM determined based on different extraction solvents obtained using DPPH radical scavenging assay. Different capital letters (immatured) and small letters (matured) indicate that the AAs are significantly different ($p < 0.05$) with each other

methanol demonstrated the highest TPC and AA in the peels of mature and green PB. Both mature and green peels of PM had the highest TPC and AA that were extracted by 90% ethanol. The highest values of TPC and AA obtained from the peels of banana extracted using the best solvents chosen are tabulated in Table 2. All extractions for this part of study were applied to a standardized 2 hr extraction time and 500 μg/mL extracts concentration.

The trend of aqueous methanol extraction was: 70% > 90% > 50% > 30% > 10% for TPC and AA determined in the peels of mature PA and green PB, except the peel of green PB had the highest TPC extracted with 90% methanol. For the ethanolic extracts, 10% ethanol demonstrated the lowest TPC and AA while 90% ethanol demonstrated the highest TPC and AA in the peels of matured PB, matured PM, and immatured PM. The TPC and AA of the samples extracted using 30–70% of ethanol were varied (Fig. 3). For acetone extracts, the descending order of TPC and AA in the peel of immatured PA extracted using different percentages of acetone was as followed: 90% > 70% > 50% > 30% > 10%.

Aqueous methanol has been commonly used as extraction medium for food samples. Chirinos et al. (2007) explained that 70% methanol was the best to

Table 2. Experimental protocol for choosing the best extraction conditions for determination of TPC and AA in the matured and immatured banana peels

Sample	Extraction solvent %	Extraction time hr	Total phenolic content μg/mL	Scavenging activity %
Optimized based on different percentages of extraction solvent				
Mature				
PA peel	70% methanol	2	5.91 ±0.04^{b}	44.43 ±0.73^{c}
PB peel	90% ethanol	2	7.14 ±0.12^{b}	56.51 ±1.95^{b}
PM peel	90% ethanol	2	12.58 ±0.04^{a}	72.55 ±0.58^{a}
Immatured				
PA peel	90% acetone	2	33.00 ±0.23^{a}	82.91 ±0.49^{a}
PB peel	70% methanol	2	9.24 ±0.08^{c}	62.94 ±0.22^{b}
PM peel	90% ethanol	2	19.09 ±0.21^{b}	81.13 ±1.37^{a}
Optimized based on different extraction times				
Mature				
PA peel	70% methanol	4	7.20 ±0.16^{b}	49.93 ±0.67^{b}
PB peel	90% ethanol	1	7.17 ±0.06^{b}	59.67 ±0.31^{a}
PM peel	90% ethanol	2	12.81 ±0.08^{a}	72.73 ±0.29^{a}
Immatured				
PA peel	90% acetone	4	44.05 ±0.13^{a}	95.9 ±0.57^{a}
PB peel	70% methanol	1	12.56 ±0.07^{b}	68.78 ±0.25^{b}
PM peel	90% ethanol	2	20.26 ±0.2^{b}	80.16 ±0.29^{b}

Different letters indicate that the TPCs and the scavenging activities are significantly different ($p < 0.05$) within each group of the fruit peel.

extract antioxidants in plants, where it was able to inactivate polyphenol oxidases for enhancing the extraction of flavonoids. However, 90% methanol yielded a maximum TPC in the immatured PB, but not the AA. The use of 70% methanol as extraction solvent was able to extract phenolic compounds and other polar antioxidants. Therefore, 70% methanol extract has the higher AA compared to 90% methanol extract. Besides, ~70% methanolic has also been used as an optimized extraction solvent for extraction of phenolic compounds in defatted *dabai* peel (Khoo et al., 2012).

Results demonstrated that matured PB and PM peels required less polar solvent to obtain maximum TPC and AA. As mentioned earlier, the ripen fruit tend to have more polar phenolic acid converted to semi-polar polyphenols. Less polar solvent such as ethanol with a reduced amount of water (10% water) was needed to extract these antioxidants from the matured fruit. On the other hand, TPC of the matured PM peel at lower extract concentrations (<500 μg/mL) were higher than the TPC at 500 μg/mL extract concentration that extracted using 70% ethanol. Aqueous ethanol (70%) has been reported to be the optimum solvent for the extraction of phenolic compounds in ground cocoa beans (Othman et al., 2007). Besides, Zhang et al. (2007) found that 70% ethanol had

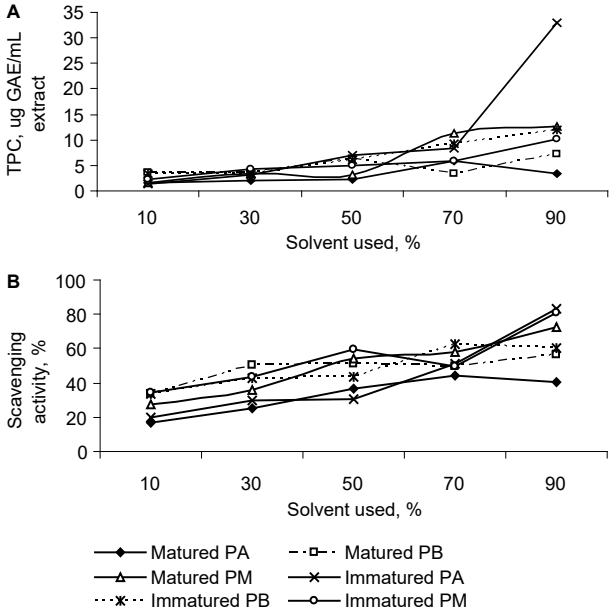

Fig. 3. (**A**) TPC and (**B**) DPPH radical scavenging activity (%) of the matured peels of PA and PB (methanol), and matured peels of PB and PM, immatured peel of PM (ethanol) and immatured PA (acetone) obtained based on different percentages of the specific extraction solvent optimized from the results shown in Table 2

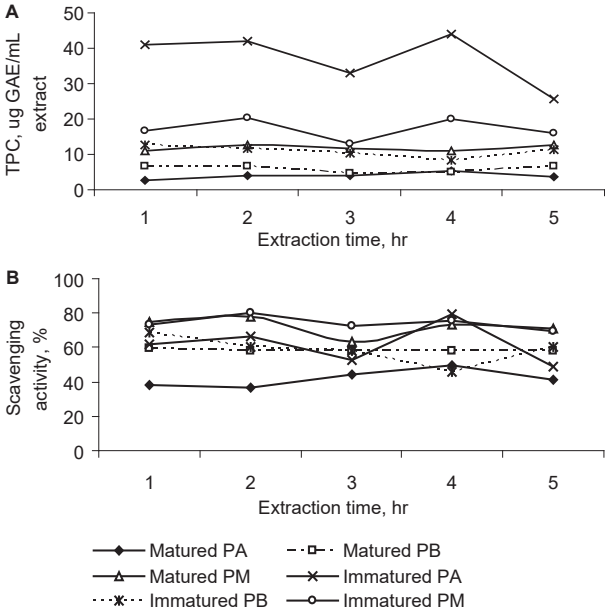

Fig. 4. (**A**) TPC and (**B**) DPPH radical scavenging activity (%) of the matured peels of PA and PB (methanol), and matured peels of PB and PM, immatured peel of PM (ethanol) and immatured PA (acetone) obtained based on different percentages of the specific extraction solvent optimized from the results shown in Table 2 determined based on different extraction times

effectively extracted flavonoid from *Houttuynia cordata* Thunb. Highest TPC (28.8 mg GAE/100 g fresh weight) was found in *Pisang Mas* extracted with 70% as compared to 30% and 90% ethanol (Alothman, 2009). Chirinos et al. (2007) reported that 50–80% methanol was needed for the extraction of antioxidants (phenolic acids and flavonoids). Similarly, subgroup of flavonoids such as catechin found in banana peels was postulated to contribute to the high AA. The solvent with medium polarity may yield the strongest radical-scavenging ability (Wang et al., 2009). The highest scavenging ability was in 90% ethanolic extract of some tropical fruits compared to 50% and 70% ethanolic extracts (Alothman, 2009). This happens because of different solubilities and polarities of different antioxidant compounds in the samples. Hence, it can be say that no single concentration of any solvent was able to recover all the phenolic compounds from a particular sample (Thoo et al., 2010; Wong et al., 2014).

Effect of extraction times on TPC and AA

Applying the best extraction conditions identified previously, the results reviewed that 4, 1, and 2 hr extraction yielded the highest TPC and AA for the matured and immatured peels of PA, PB, and PM, respectively (Table 2). The other extraction times had shown lower TPC and AA in all studied samples (Fig. 4). The use of different extraction times to extract phenolic compounds had been explained by Thoo et al. (2010) and Silva et al. (2007), where the key factors are the degrees of phenolic polymerization, solubility of phenolics and interaction between phenolic compounds and sample extract. In fact, the final equilibrium between solvent and solid diffusion was attained exactly at an optimum extraction point (Wong et al., 2014). Longer extraction time used is not advisable as it is not helpful to increase the yield of polyphenol. In fact, prolonged extraction time has been shown to increase the chances of exposure to environmental factor such as light, oxygen and temperature, increasing the oxidation of

phenolic compounds, thus contributing to lower AA (Lafka et al., 2007; Naczk and Shahidi 2006; Thoo et al., 2010; Wong et al., 2014).

Similar as for TPC, the AA of the samples extracted with 4, 1, and 2 hr extraction time were the highest for PA, PB, and PM, respectively (Table 2). Pinelo et al. (2005) described that DPPH radical scavenging activity was affected by the concentration of specific compounds, such as gallic acid, catechin, epicatechin, and quercetin, as well as non-polymeric compounds in banana peels. At longer extraction time, polymerization may occur as conformation of OH bonding and ring structure of the antioxidant compounds varies. This might result in the decrease of antioxidant activity. However, shorter extraction time is insufficient to completely extract the bound-phenolic compounds that lead to a decrease in AA indicated by the lowest TPC obtained at 1 hr extraction for PA samples (Vasco et al., 2008). Specifically, González-Montelongo et al. (2010) reported that scavenging activity of banana peels was the highest at 2 hr extraction at room temperature.

TPC and AA of the samples applying the best extraction conditions

The TPC and AA of the banana samples extracted based on the following extraction conditions were determined: matured peel of PA (70% methanol; 4 hr extraction), matured peel of PB (90% ethanol; 1 hr extraction), matured peel of PM (90% ethanol; 2 hr extraction), immatured peel of PA (90% acetone; 4 hr extraction), immatured peel of PB (70% methanol; 1 hr extraction), immatured peel of PM (90% ethanol; 2 hr extraction). BHA (200 µg/mL) was used as reference compound for AA determination. The TPC of matured banana peels were in the following order: PM > PB > PA, while the TPC for immatured banana peels were PA > PM > PB (Fig. 5). Both matured and immatured PB peels demonstrated low TPCs. For PA, the peel of immatured fruit had TPC about 11 times higher than the matured fruit. The TPC of all immatured banana peels were higher than the matured counterparts.

The result shows that all immatured banana peels had significantly higher AA than the matured banana peels ($p < 0.05$; Fig. 6). Determined using DPPH radical scavenging assay, the percentages of scavenging activity (%SA) for the matured banana peels were the highest in PM, followed by PB and PA, while for the

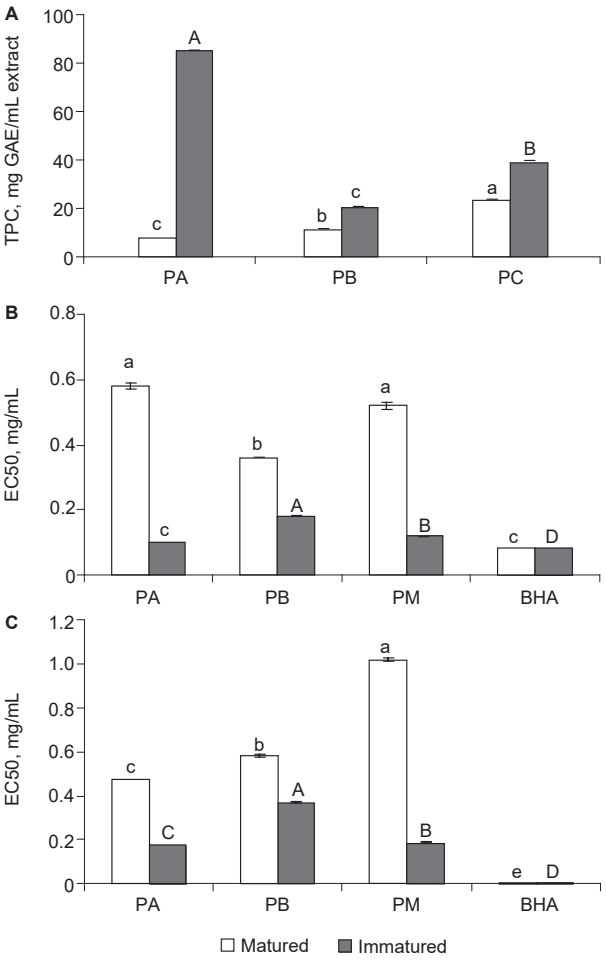

Fig. 5. TPC and EC_{50} of the matured and immatured peels of PA, PB, PM, and BHA (200 µg/mL as reference) determined based on the best extraction conditions using (**A**) Folin-Ciocalteu's reagent assay, (**B**) DPPH radical scavenging assay, and (**C**) ABTS radical scavenging assay. Different capital letters (immatured) and small letters (matured) indicate that the TPCs and EC_{50} values are significantly different ($p < 0.05$) with each other

immatured banana peel, PB had the lowest %SA. The %SA for the immatured PA and PM peels were not significantly different ($p > 0.05$). The percentages of inhibition activity (%IA) for the matured and immatured banana peels showed similar trend as found for the %SA. We observed that both matured and immatured PB peels had the lowest %IA assessed by both ABTS and β-carotene bleaching assays. However, no significant difference was observed for the %IA assessed by

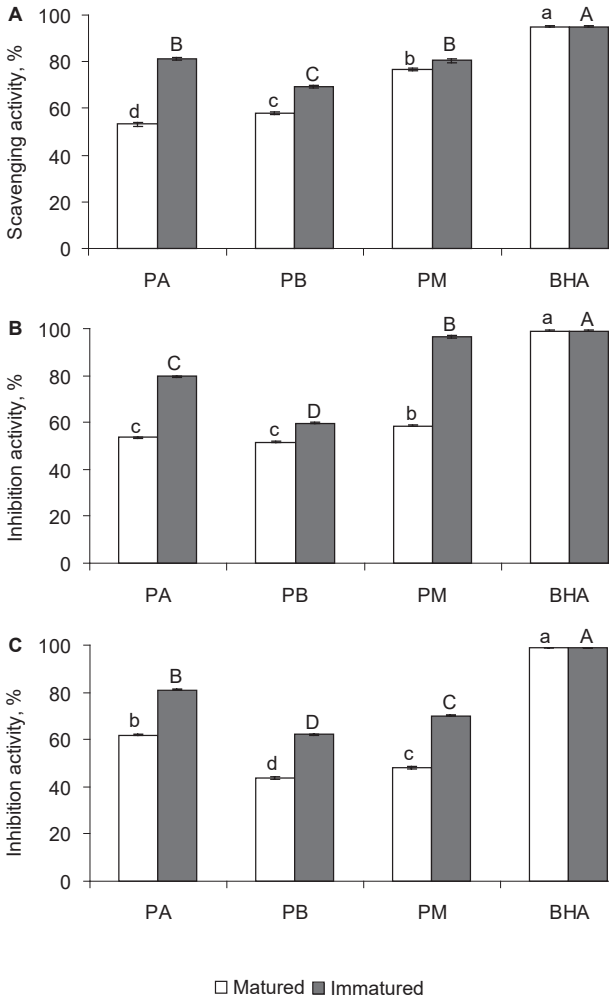

Fig. 6. AAs of the matured and immatured peels of PA, PB, PM, and BHA (200 μg/mL as reference) determined based on the best extraction conditions using (**A**) DPPH, (**B**) ABTS, and (**C**) β-carotene-linoleate bleaching assays. Different capital letters (immatured) and small letters (matured) indicate that the AAs are significantly different ($p < 0.05$) with each other

ABTS assay between the peels of matured PA and matured PB. Assessed using ABTS assay, matured PM peel had the highest %IA compared to other matured banana peels. But the result from β-carotene bleaching assay shows that the matured PM peel had the lower %IA than the matured PA peel.

The EC_{50} values for both DPPH and ABTS assays are shown in Figure 5. Assessed using DPPH and ABTS assays, the EC_{50} values for each studied sample were differed. The result from both DPPH and ABTS assays demonstrated that the peel of immatured PA had the lowest EC_{50} value, demonstrating that the peel of immatured PA extracted by 90% acetone for 4 hr at room temperature contains powerful antioxidants compared to other samples. The peels of immatured PM and PB had higher EC_{50} values than the peel of immatured PA, which ranked number second and third, respectively. The peel of matured PM had the highest EC_{50} value, followed by the peels of matured PA and PB. The reason is possibly due to loss of potential antioxidants during fruit maturity.

All immatured banana peels that have higher %IA than the matured banana peels show a similar trend for %IA assessed by both ABTS and β-carotene bleaching assays (Fig. 6). For the peels of PA and PB, not as found for the matured counterpart, the %IA for immatured PA peel was significantly higher than the immatured PB peel ($p < 0.05$). BHA that used for referencing had %SA and both %IA significantly higher than the all the samples studied. BHA demonstrated the lowest EC_{50} as compared to all matured and immatured banana peels. BHA has shown the lowest EC_{50} (<1 μg/mL) indicating that BHA has much higher ABTS radical cation inhibition activity compared to banana samples. Extraction conditions can greatly influence AA in plants. All the extraction parameters should be optimized as they are important for optimization of sample extraction procedures (Michiels et al., 2012; Wong et al., 2014).

Correlation analysis

Strong and positive correlations were found between TPC and AA determined in the peels of matured and immatured PA, PB, and PM. The r values (0.73 to 0.99) for the correlations of all samples are shown in Table 3. The correlation between TPC and AA assessed by β-carotene bleaching assay for the peel of green PA was moderately high ($r = 0.73$), while the correlations of other samples were very high (Table 3). The results showed that positive and very high correlations were found among the TPC and AAs. The strong correlations were in consistent with the results obtained by Alothman (2009) that TPC of banana was highly correlated to DPPH radical scavenging activity. Beside, high AAs of passion fruit peel

Table 3. Correlation coefficient (*r*) of TPC and AA (DPPH, FRAP and β-carotene bleaching assay) for the peels of mature (M) and immatured (IM) banana samples

Sample	TPC and DPPH			TPC and ABTS			TPC and β-carotene bleaching		
	PA	PB	PM	PA	PB	PM	PA	PB	PM
Matured	0.98	0.93	0.96	0.94	0.99	0.99	0.87	0.95	0.96
Immatured	0.97	0.93	0.95	0.93	0.98	0.99	0.93	0.99	0.99

have been reported to be high correlated to their phenolic content (Wong et al., 2014). A high correlation could be contributed by the low molecular weight phenolic compounds since DPPH is known to react preferentially with these phenolic compounds (Thoo et al., 2010). Thoo et al. (2010) reported strong correlation between TPC with DPPH for *Morinda citrifolia.* A high correlation between TPC and AA indicated that high amount of phenolic compounds in the banana peel will give rise to high AA. In fact, AA is not only contributed by phenolic compounds, other compounds such as ascorbic acid, tocopherols, carotenoids, reduced carbohydrates, and terpenes, as well as the synergistic effects among the antioxidant compounds could also contribute to the total AA of a particular food sample (Babbar et al., 2011, Naczk and Shahidi, 2006). The very high correlations between TPC and AAs (BCB, ABTS, or DPPH) were not surprising due to the similarity of the redox reactions between these assays. Hence, further work is also required for the isolation and identification of individual phenolic compounds present in these banana peels to identify the bioactive compounds responsible for the AA obtained. Perhaps, other unknown compounds in the banana peel could have contributed to the AA, thus more studies are needed to determine the relationship between these unknown and their AAs.

CONCLUSIONS

The optimized extraction conditions were used for extraction phenolic compounds in the peels of bananas. Methanol, ethanol, and acetone were chosen for extraction by applying 4, 1, and 2 hr extraction time for extraction of phenolic compounds in the peels of PA, PB, and PM, respectively. The AAs of the immatured banana peels were comparable with synthetic antioxidant, BHA. Since the banana peel extracts had high correlation between AA and polyphenol constituents, we concluded that the phenolic compounds in the banana peel could be the main component that contributed to the high AA.

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