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Polyphenolic and Vitamin C Contents and Antioxidant Activities of Aqueous Extracts from Mature-Green and Ripe Fruit Fleshes of *Mangifera* sp.

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ABSTRACT: Mature-green and ripe fleshes from 12 samples of *Mangifera* were selected for this study. The mature-green fleshes were found to have higher vitamin C contents than the ripe fleshes. However, not all higher total or individual phenolic contents were measured from the mature-green fleshes. The highest contents of vitamin C and total phenolics were respectively measured from the aqueous extracts of mature-green (255.86 ± 12.98 μg AAE/g sample) and ripe (142.57 ± 0.38 μg GAE/g sample) fleshes of *M. petandra* cv. Pauh. Gallic acid and mangiferin were detected in all aqueous extracts. The extracts of the mature-green flesh of *M. indica* cv. Chokanan and the ripe flesh of *M. indica* cv. Siku Raja, respectively, exhibited the greatest 1,1-diphenyl-2-picrylhydrazyl radical (DPPH)-scavenging activity (408.21 ± 5.37 μg TE/g sample) and metal chelating activity (93.68 ± 0.74%). The combined or potentiation effects of the moderate vitamin C, gallic acid, and mangiferin contents in both extracts may be responsible for the activities. The highest mangiferin content (31.72 ± 2.57 μg/g sample) in the mature-green *M. caesia* (Binjai) could be the major contributor to its highest FRAP activity (868.29 ± 2.71 μg TE/g sample). This paper reports apparently the first comparative study highlighting the antioxidant activities of these fruit fleshes.

KEYWORDS: *vitamin C, phenolics, antioxidant activity, Mangifera, fruit fleshes, mangiferin*

INTRODUCTION

Southeast Asia is believed to be the center of diversity for the genus *Mangifera*.[1] This genus comprises more than 60 species that are widely distributed throughout tropical and subtropical Asia. The fruits of 26 species are found to be edible[2] and are more preferable to be eaten either in ripe, mature-green, or in unripe form, depending on the species and cultivar. For instance, the fruits *Mangifera petandra* cv. Pauh and *Mangifera indica* cv. Sala and Siku Raja are often eaten in mature-green form due to their crispy and crunchy texture. The less sour and nonfibrous fruit fleshes are usually sliced and included in desserts, salads, ice cream, jellies, and beverages; processed into juice and puree; or dried, pickled, and canned in syrup.

The only *Mangifera* species that is well-known as a fruit crop is *M. indica* (mango). Several species such as *Mangifera caesia*, *Mangifera foetida*, *Mangifera odorata*, and *Mangifera petandra* are also cultivated on a small scale in various parts of Southeast Asia.[3] Others are categorized as wild species or underutilized fruits. To date, the antioxidant evaluations of *Mangifera* fleshes are limited to four of its species, that is, *M. foetida*,[4,5] *M. indica*,[6−14] *M. pajang*,[15,16] and *M. odorata*.[4]

Mango is a highly consumed fruit and is ranked fifth in global fruit production behind *Musa* sp. (bananas and plantains), *Citrus* sp., grapes, and apples.[17] Mango flesh is considered to be a rich source of dietary antioxidants such as ascorbic acid, carotenoids, and phenolic compounds. The main phenolics found in the fleshes of several cultivars of mango are mangiferin, isomangiferin, gallic acid, *m*-digallic acid, ellagic acid, quercetin 3-*O*-galactoside, and quercetin 3-*O*-glucoside.[7,8,18−20]

There are over 1000 cultivars recorded in more than 25 germplasm banks of mango cultivars worldwide.[3] Among the cultivars, only six popular cultivars from the northern part of Peninsular Malaysia were selected in this study. Among them, Harumanis is the most outstanding in the domestic markets due to its quality attributes demanded by consumers. It is cultivated in a large scale in Perlis, a state located in the extreme north of Peninsular Malaysia. Most of the selected *Mangifera* species (excluding *M. petandra* cv. Pauh and *M. indica* cv. Chokanan) are seasonal fruits that are mostly harvested between April and June, each year.

To our knowledge, only four samples that were selected for this study had been previously evaluated for their antioxidant activities, that is, 80% methanol extracts of lyophilized ripe flesh of *M. feotida* (Bacang) and *M. odorata* (Kuinin);[4] 80% acetone extract of ripe fresh flesh of *M. feotida*;[5] a combination of 80% ethanol and 80% acetone extracts of dried green and ripe fleshes of *M. indica* cv. Chokanan;[14] and methanol, water, acetone, and hexane extracts of lyophilized ripe flesh of *M. indica* cv. Nam Dok Mai.[10] Although various solvents are commonly used to extract antioxidant compounds from the fresh fruit fleshes, only water and ethanol are considered to be "green solvents" and are acceptable for food application. Thus, distilled water was used as an extraction solvent in the present study. The contents and types of phytochemicals may change during maturity and ripening processes, and these changes could affect the antioxidant activity of the extracts. The extract or sample that gives the highest antioxidant activity is more desired by consumers. However, to date, the comparative evaluation of the effect of ripening on the antioxidant activity of the selected *Mangifera* is limited to only a study on *M. indica* cv.

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Table 1. Harvesting Locations and Dates and Morphological Characteristics of the 12 Fruits of *Mangifera* Used in This Study

Mangifera species	local name	cultivar	harvest location	harvest date (2011)	length and width (cm)	shape	skin color		flesh color	
							mature-green	ripe	mature-green	ripe
caesia	Binjai		Sungai Petani, Kedah	June 26	10–15 and 6–8	obovate-oblong	pale greenish brown	yellowish brown	greenish white	milky white
foetida	Bacang		Bayan Lepas, Penang	June 5	9–14 and 6–8	ovoid-oblong	dirty dark green	yellowish green with brown spots	pale yellow	yellowish orange
indica	Mangga/mempelam	Harumanis	Batu Pahat, Perlis	May 19	8–22 and 6–8	ovoid-oblong	green	green	greenish yellow	yellowish orange
		Nam Dok Mai	Kangar, Perlis	May 8	10–15 and 6–8	oblong-oblique	green	yellowish green	greenish yellow	yellowish orange
		Siku Raja	Jejawi, Perlis	May 8	10–15 and 6–8	oblong-oblique	green	yellowish green	greenish yellow	yellow
		Chokanan	Kangar, Perlis	June 19	10–15 and 6–8	oblong	green	yellow	greenish yellow	yellow
		Apple	Kamunting, Perak	May 22	6–15 and 6–15	globular	green with crimson blush	green with crimson blush	greenish yellow	yellow
		Sala	Chuping, Perlis	May 15	12–20 and 6–10	oblong-oblique	green	yellowish green	greenish yellow	yellowish orange
odorata	Kuinin		Arau, Perlis	May 19	10–13 and 7–9	ellipsoid-oblong	green with brown lenticels	green with brown lenticels	pale yellow	yellowish orange
petandra		Pauh	Ayer Hitam, Penang	May 2	6–10 and 6–10	ovate-oblique	green	yellow	greenish yellow	yellow
		Bemban	Pauh, Perlis	May 15	7–10 and 7–10	ovate-oblique	dark green with dark spots	yellowish green with dark spots	greenish yellow	yellowish orange
quadrifida	Asam kumbang		Kuala Ketil, Kedah	June 19	7–10 and 7–10	globular	dark green with dark spots	dark green with purple spots	pale purplish green	pale purplish yellow

Chokanan.[14] Therefore, the aims of this study were (i) to measure the antioxidant activities (using three different colorimetric assays) and vitamin C and total phenolic contents in the aqueous extracts of the fresh fleshes from 12 Malaysian *Mangifera* prepared at two different ripening stages (mature-green and ripe) and (ii) to determine the phenolic profiles of the extracts.

MATERIALS AND METHODS

Plant Materials. Table 1 shows a list of 12 samples that were used in this study. They were harvested by hand in the morning. All of the ripe fruit samples were allowed to ripen on the trees, before being harvested, whereas all of the mature-green samples were picked during their early stage of maturity. We were assisted by skilled farmers for determination of the ripeness of the fruits to ensure identical stages (either mature-green or ripe) were used. The location of the plantations, harvesting dates, and morphological characteristics of the fruits are also indicated in Table 1. At least 30 fruits from 10 different trees for each sample were harvested. The interval between harvesting and experimental work was specified to be <4 h to retain the freshness of the sample.

Chemicals. All chemicals and reagents used in this study are of analytical grade. Folin–Ciocalteu phenol reagent, metaphosphoric acid, 2,6-dichloroindophenol (DCIP), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,4,6-tri-(2-pyridyl)-*s*-triozine (TPTZ), ferric chloride hexahydrate, ferrous chloride hexahydrate, ferrozine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), mangiferin, and ascorbic, gallic, protocatechuic, *p*-hydroxybenzoic, and vanillic acids were obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Sodium carbonate and sodium bicarbonate were purchased from Fluka (Buchs, Switzerland).

Extraction. The extraction process was carried out in triplicate, for each sample. The peel and seed kernel of each fruit were removed from the edible flesh. For each replicate, the fresh fleshes from 10 different fruits (200 g fresh weight) were washed with distilled water, and the remaining water at the external surface of the sample was dried using blotting paper (Whatman 3MM Chr). The sample was later ground for 2 min using a grinder to yield a fine puree (40 mesh or 400 μm of particle size). The puree (200 g) was then soaked in 200 mL of distilled water for 15 min at room temperature (27 ± 1 °C). The aqueous extract was then filtered using a clean muslin cloth and centrifuged using a Hittech EBA 20 centrifuge (Japan) at 704*g* for 15 min. The aqueous extracts were stored at 4 °C in the dark until further analysis. The interval between the extraction process and experimental work was specified to be <1 h. The concentration of the obtained aqueous extract was referred to fresh sample weight resulting in 1 g/mL (w/v).

Determination of Vitamin C Content. Vitamin C content was measured using a modified method of AOAC.[21] In the test, 2 mL of sample (aqueous extract (1 g/mL)) was mixed with 2 mL of 3% metaphosphoric acid for 30 min. The mixture was then centrifuged using a Hittech EBA 20 centrifuge (Japan) at 704*g* for 15 min. The supernatant (1 mL) was titrated with indophenol solution (3.5 mM DCIP in 2% sodium bicarbonate) until a light pink color appeared and persisted for 5 s. The amount of indophenol solution used for titration of each sample was compared to that of the ascorbic acid standard curve plotted at concentrations ranging from 0 to 2.5 mg/mL. Extraction and titration for each sample were performed in triplicate. The content of vitamin C of the aqueous extracts was expressed as micrograms of ascorbic acid equivalent per gram of fresh weight of sample (μg AAE/g sample).

Determination of Total Phenolic Content (TPC). TPCs from the samples were quantified using Folin–Ciocalteu's method[22] adapted to the 96-well plate assay, as described by Sulaiman et al.[23] Folin–Ciocalteu's reagent (25 μL) was added to 10 μL of aqueous extract (1 g/mL) in the well of a 96-well plate. After 5 min of incubation at room temperature (27 ± 1 °C), 25 μL of 20% (w/v) sodium carbonate was added to the mixture followed by distilled water to a final volume of 200 μL per well. After 30 min of incubation at

room temperature (27 ± 1 °C), the absorbance was read at 760 nm against a blank (distilled water) using a Multiskan EX microplate reader (Thermo Fisher Scientific, Finland). The experiments were carried out at least in triplicate. A standard curve was plotted using gallic acid (0−1 mg/mL). The results of the aqueous extracts were expressed as microgram gallic acid equivalents per gram of fresh weight of sample (μg GAE/g sample).

Quantification of Phenolic Constituents. The aqueous extract (1 g/mL) was filtered through a 0.2 μm of syringe filter. The phenolic compounds in the aqueous extracts were quantified in triplicate using an Acquity ultraperformance liquid chromatography (UPLC) system (Waters, Milford, MA, USA) equipped with a reverse-phase Acquity UPLC BEH C_{18} column, 1.7 μm (100 mm × 2.1 mm i.d.), and photodiode array detector. The mobile phase consists of solvent A (0.5% acetic acid) and solvent B (acetonitrile). The extract was separated using a gradient mode that was initially set at an A/B ratio of 90:10 and then linearly increased to 80:20 at 1 min, 30:70 at 2 min, and 10:90 at 3.5 min until 5.5 min. The detector was set at 270 nm with the flow rate of 0.20 mL/min, and the injection volume was 5.0 μL. A standard calibration curve of different concentrations (31.25−1000 μg/mL) of each phenolic compound (mangiferin and gallic, protocatechuic, *p*-hydroxybenzoic, and vanillic acids) was plotted. The concentration of phenolic compounds in each sample was calculated using the regression equation of its peak area to the peak area of known concentration of standard from the calibration curve. The results were expressed as micrograms of phenolic compound per gram of fresh weight of sample (μg/g sample).

DPPH Free Radical Scavenging Assay. The free radical scavenging activity of the samples was analyzed according to the method of Sulaiman et al.[23] Briefly, a 0.3 mM solution of DPPH in ethanol was prepared. An aliquot (50 μL) of aqueous extract (1 g/mL) was added to 150 μL of the DPPH solution in each well of a 96-well plate. For the blank, only 50 μL of distilled water was added to the DPPH solution. The decrease in absorbance was measured at 515 nm after 30 min of incubation at 37 °C using a Multiskan EX microplate reader (Thermo Fisher Scientific, Finland). All tests were performed in triplicate. Trolox was also used as a reference in this assay. A standard curve was obtained using different concentrations (0−1 mg/mL) of Trolox standard solution. The absorbance of the extract was compared to that of the Trolox standard, and the results of the aqueous extracts were expressed as microgram Trolox equivalents per per gram of fresh weight of sample (μg TE/g sample).

Ferric Reducing Antioxidant Potential (FRAP) Assay. The FRAP assay was conducted according to the method of Firuzi et al.[24] (adjusted to a pH of 3.6 by the addition of acetic acid to 1.0 mL of 20 mM ferric chloride hexahydrate (dissolved in distilled water) and 1.0 mL of 10 mM 2,4,6-tri-(2-pyridyl)-*s*-triozine (TPTZ) (dissolved in HCl 40 mM)). In a well of a 96-well plate, an aliquot (10 μL) of aqueous extract (at five different concentrations) was added to 190 μL of the FRAP solution. The aqueous extract (1 g/mL) was serially diluted into five different concentrations to obtain the optimized FRAP value. After 30 min of incubation at 37 °C, the absorbance of the reaction mixture was measured at 593 nm using a Multiskan EX microplate reader (Thermo Fisher Scientific, Finland). All tests were run in triplicate. Trolox (0−125 μg/mL) was used as a reference to produce a standard curve. The absorbance of the extract was compared to that of the Trolox standard, and the results of the aqueous extracts were expressed as microgram Trolox equivalents per gram of fresh weight of sample (μg TE/g sample). The experiments were carried out in triplicate.

Metal Chelating Assay. The metal chelating assay was performed in triplicate according to the method of Dinis et al.[25] adapted to a 96-well plate. Briefly, 50 μL of the aqueous extract (1 g/mL) was incubated with 5 μL of ferrous chloride hexahydrate (2 mmol/L) and 130 μL of deionized water for 5 min. The reaction was initiated by the addition of 15 μL of ferrozine (5 mmol/L). After the mixture had reached equilibrium (10 min), the absorbance was measured at 562 nm using a Multiskan EX microplate reader (Thermo Scientific, Finland). The negative control was prepared without the tested extract. The metal chelating percentage was calculated as follows: % metal chelating = [(absorbance of negative control − absorbance of sample)/absorbance of negative control] × 100%. All experiments were performed in triplicate.

Statistical Analysis. Data were analyzed using SPSS 11.5 for Windows software (SPSS Inc., Chicago, IL, USA). Values were expressed as the mean ± standard deviations. Analysis of variance was determined by one-way ANOVA using GraphPadPrism (San Diego, CA, USA). p values of <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Vitamin C Content. The vitamin C contents are shown in Table 2. All of the mature-green fleshes were found to have higher vitamin C contents than the ripe fleshes, and the results are in agreement with the literature for other cultivars of *M. indica* such as Chokanan[14] and Kiett.[26] Gomez and Lajolo[26] found about a 50% decrease in ascorbic acid content from initial development to full ripeness of mango fruit (*M. indica* cv. Kiett).

The mature-green flesh of *M. petandra* cv. Pauh gave the highest content (255.86 ± 12.98 μg AAE/g sample). The ripe flesh of Pauh also significantly showed the highest content of vitamin C in comparison to the other ripe fleshes (169.92 ± 5.95 μg AAE/g sample). Other vitamin C contents above 140 μg AAE/g sample were quantified from the aqueous extracts of mature-green fleshes of *M. caesia* (Binjai), *M. foetida* (Bacang), *M. indica* cv. Nam Dok Mai, Sala, and Siku Raja, and *M. petandra* cv. Bemban. The vitamin C contents in the aqueous extracts of ripe fleshes of six cultivars of *M. indica* (ranging from 90.85 to 133.82 μg AAE/g sample) were within the range of the reported values of other cultivars such as Palmer[6] and Tommy Atkins[8] but lower than those reported in Haden, Kent, Keitt, Ataulfo, and Uba cultivars,[6,8] all Chinese cultivars,[12] and *M. pajang*.[16]

Total Phenolic Content. As indicated in Table 2, not all of the mature-green fleshes were found to have significantly higher TPC than that of their ripe fleshes. This observation was found to be limited to only the aqueous extracts of mature-green fleshes of *M. caesia* (Binjai), *M. indica* cv. Sala, and *M. quadrifida* (Asam Kumbang). These results are comparable with those reported from the dried flesh of Chokanan[14] and fresh flesh of Tommy Atkins[13,27] that have determined the reduction in TPC during ripening.

No significant difference was observed between the TPC values of some mature-green and ripe fleshes of *M. indica* cv. Chokanan, Apple, Harumanis, and Nam Dok Mai. Kim et al.[9] also reported no variation of TPC in the unripe and ripe fleshes of Irwin cultivar. Furthermore, samples that demonstrated significant increase in TPC during ripening are *M. foetida* (Bacang), *M. indica* cv. Siku Raja, *M. odorata* (Kuinin), and *M. petandra* cv. Pauh and Bemban. The highest TPC was detected in the aqueous extract of ripe *M. petandra* cv. Pauh (142.57 ± 0.38 μg GAE/g sample). The obvious increase in TPC of these fleshes during ripening might also be associated with the increase of bitter taste or astringency. Thus, our findings may provide the basis of why these fruits are more preferable to be eaten in mature-green form. Other extracts that gave TPC values of >90 μg GAE/g sample are obtained from the fleshes of mature-green and ripe Binjai and Chokanan. The values of TPC obtained from this study were found to be lower than those reported in the ripe fleshes of *M. indica*, that is, in methanol/water (6:4) extracts of Haden, Palmer, Tommy Atkins, and Uba cultivars,[6] in methanol/acetone (1:1) extracts of Haden, Tommy Atkins, Kent, Keitt, and Ataulfo cultivars,[8] in

Table 2. Vitamin C and Total Phenolic Contents in *Mangifera* Samples[a]

		vitamin C (μg AAE/g sample)		total phenolic content (μg GAE/g sample)	
Mangifera species	local name/cultivar name	mature-green	ripe	mature-green	ripe
caesia	Binjai	142.41 ± 2.98de	126.94 ± 2.98fgh	122.82 ± 2.45b	116.24 ± 1.65c
foetida	Bacang	149.29 ± 2.98d	121.79 ± 2.98hi	60.51 ± 0.51h	72.91 ± 0.44g
indica	Chokanan	120.07 ± 5.16hi	102.88 ± 2.98j	93.17 ± 0.59d	91.63 ± 3.45d
	Apple	133.82 ± 5.95efg	125.22 ± 5.16fgh	36.42 ± 0.11l	36.74 ± 0.19l
	Harumanis	135.54 ± 5.16ef	121.79 ± 2.98hi	34.95 ± 2.75lm	33.86 ± 1.26m
	Nam Dok Mai	142.41 ± 2.98de	123.51 ± 2.98ghi	76.93 ± 0.29f	77.77 ± 2.45f
	Sala	163.04 ± 5.95c	133.82 ± 5.94efg	88.05 ± 0.40e	72.52 ± 0.11g
	Siku Raja	168.20 ± 7.88bc	90.85 ± 2.98k	57.20 ± 0.34i	70.52 ± 0.05g
odorata	Kuinin	113.19 ± 2.98ij	106.32 ± 2.98j	16.41 ± 0.19o	42.10 ± 3.27k
petandra	Pauh	255.86 ± 12.98a	169.92 ± 5.95bc	50.22 ± 0.40j	142.57 ± 0.38a
	Bemban	175.07 ± 14.89b	123.51 ± 2.98ghi	20.31 ± 0.62n	60.51 ± 0.88h
quadrifida	Asam Kumbang	123.51 ± 7.88ghi	113.19 ± 2.98ij	51.88 ± 1.00j	43.70 ± 0.73k

[a]Values are the mean ± standard deviation of triplicate analyses. Results for mature-green and ripe samples were analyzed together, whereas those from two different analyses were analyzed separately. Values per each analysis followed by different letters are significantly different ($p < 0.05$).

ethanol/water (1:1) extracts of Hainan and Shuixian cultivars,[11] and in ethanol/acetone (7:3) extracts of seven Chinese mangoes.[12] The low values of TPC might be due to the inefficiency of distilled water in extracting phenolic compounds from mango fleshes. Thus, further study to optimize the recovery of TPC in these *Mangifera* fleshes is highly required. On the basis of a previous study, acetone was found to be the most appropriate solvent for extraction of phenolic compounds from the fresh flesh of *M. indica* cv. Tommy Atkins.[13] Moreover, a study by Poovarodom et al.[10] discovered the efficiency of water and methanol in extracting higher amounts of polyphenolics from the lyophilized flesh sample of *M. indica* cv. Nam Dok Mai.

Quantification of Phenolic Constituents in the Aqueous Extracts. Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and mangiferin in the aqueous extracts of the fleshes were identified using UPLC with photodiode array detector based on the retention times and ultraviolet (UV) spectra of the peaks in comparison with the standard compounds, and these were confirmed by spiking the extracts with the corresponding standards. The contents of these compounds in the aqueous extracts are summarized in Table 3. All five identified compounds were detected only in the mature-green *M. indica* cv. Pauh and ripe *M. quadrifida* (Asam Kumbang). A UPLC profile of the phenolic compounds in the aqueous extract of mature-green Pauh is shown in Figure 1. Gallic acid and mangiferin were found in all tested extracts. The highest contents of gallic acid (291.47 ± 4.39 μg/g sample), protocatechuic acid (37.21 ± 11.84 μg/g sample), and *p*-hydroxybenzoic acid (28.71 ± 13.86 μg/g sample) were all significantly measured from the mature-green flesh of Nam Dok Mai. HPLC analyses by Poovarodom et al.[10] on Nam Dok Mai and by Kim et al.[27] on Tommy Atkins had also revealed gallic acid as the major constituent in the fleshes of these mangoes. Poovarodom et al.[10] had also estimated higher contents of *p*-hydroxybenzoic, vanillic, and ferulic acids in the methanol extract of the lyophilized ripe flesh of Nam Dok Mai. However, in this study, the two latter constituents were not detected in the aqueous extract of Nam Dok Mai.

In accordance with the results obtained by Kim et al.,[27] a significant decrease in gallic acid content during ripening was observed in *M. indica* cv. Chokanan, Nam Dok Mai, and Sala. The contents of protocatechuic and *p*-hydroxybenzoic acids in the ripe flesh of Nam Dok Mai were also significantly lower than in its mature-green flesh. In contrast, gallic acid content in the mature-green fleshes of *M. caesia* (Binjai) and *M. petandra* cv. Pauh, and protocatechuic acid content in the mature-green flesh of *M. feotida* (Bacang) were significantly lower than that of their ripe fleshes.

The ripe Binjai was found to contain the greatest vanillic acid content (72.89 ± 10.79 μg/g sample). The increase in vanillic acid content during ripening was observed only in the aromatic fleshes of Binjai, Bacang, *M. indica* cv. Harumanis, and *M. odorata* (Kuinin), suggesting its contribution as a flavoring constituent of the fleshes. A recent study on *M. indica* cv. Ataulfo had also reported an increase in protocatechuic acid and vanillic acid contents, with no change of gallic acid content during ripening.[28] In this study, no significant change of gallic acid content was determined from Bacang, *M. indica* cv. Apple, Harumanis, and Siku Raja, Kuinin, *M. petandra* (Bemban), and *M. quadrifida* (Asam Kumbang).

The highest content of mangiferin (31.72 ± 2.57 μg/g sample) was measured from the mature-green Binjai. Mangiferin was previously quantified from four Brazilian and five commercial varieties of mango fleshes, and the highest content was respectively measured from Uba[7] and from Ataulfo.[8] The mature-green Nam Dok Mai flesh (13.01 ± 4.87 μg/g sample) and ripe Kuinin (12.19 ± 0.58 μg/g sample) were ranked among the top three in mangiferin contents. Ellagic acid that was previously measured in Haden, Tommy Atkins, Kent, Ataulfo,[8] Hainan, and Shuixian cultivars,[11] was also not detected in this study.

Primary Antioxidant Activity. DPPH free radical scavenging and FRAP assays were used to measure the direct involvement of the aqueous extracts in enhancing the primary antioxidant activity. Although the extracts obtained from mature-green and ripe Pauh, respectively, contained the highest vitamin C and TPC (Table 2), they however do not exhibit the highest free radical scavenging activity. As shown in Table 4, the aqueous extract of the mature-green flesh of *M. indica* cv. Chokanan exhibited the greatest DPPH-scavenging activity (408.21 ± 5.37 μg TE/g sample). Abdul Aziz et al.[14] reported similar results showing the processed green Chokanan flesh gave higher scavenging activity than that of the ripe flesh. Moreover, the aqueous extracts of mature-green Binjai, Nam Dok Mai, and Sala and ripe Bacang were also ranked higher in the activity. Several earlier studies on the fleshes of *Mangifera* had also utilized the DPPH-scavenging assay.[7−10,12−16]

Table 3. Content of Phenolic Constituents in the Aqueous Extracts of *Mangifera* Samples[a]

Mangifera species	local name/ cultivar	gallic acid (μg/g sample)		protocatechuic acid (μg/g sample)		*p*-hydroxybenzoic acid (μg/g sample)		vanillic acid (μg/g sample)		mangiferin (μg/g sample)	
		mature-green	ripe	mature-green	ripe	mature-green	ripe	mature-green	ripe	mature-green	ripe
caesia	Binjai	25.13 ± 1.36d	113.00 ± 17.01b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0f	0.0 ± 0.0f	15.37 ± 1.60b	72.89 ± 10.79a	31.72 ± 2.57a	3.81 ± 0.96ghi
foetida	Bacang	1.44 ± 0.08g	9.43 ± 0.77efg	0.22 ± 0.08c	9.02 ± 3.64b	0.0 ± 0.0f	0.0 ± 0.0f	0.90 ± 0.17d	6.42 ± 0.63c	0.97 ± 0.03i	11.24 ± 4.36bcd
indica	Chokanan	41.46 ± 1.77c	13.00 ± 0.98ef	2.68 ± 1.16c	0.0 ± 0.0c	0.0 ± 0.0f	0.0 ± 0.0f	0.0 ± 0.0d	0.0 ± 0.0d	8.29 ± 1.82de	3.58 ± 0.58ghi
	Apple	7.58 ± 2.79fg	10.54 ± 2.15efg	2.29 ± 0.42c	1.82 ± 0.24c	7.75 ± 0.35de	10.33 ± 1.65cd	0.0 ± 0.0d	0.0 ± 0.0d	3.10 ± 1.71hi	9.26 ± 0.20cde
	Harumanis	2.46 ± 0.30g	1.24 ± 0.32g	1.07 ± 0.30c	0.43 ± 0.04c	1.30 ± 0.11f	0.0 ± 0.0f	0.0 ± 0.0d	3.34 ± 2.04cd	3.66 ± 0.62ghi	0.88 ± 0.22i
	Nam Dok Mai	291.47 ± 4.39a	17.85 ± 2.56de	37.21 ± 11.84a	1.81 ± 0.04c	28.71 ± 13.86a	1.54 ± 0.18f	0.0 ± 0.0d	0.0 ± 0.0d	13.01 ± 4.87b	7.76 ± 0.20ef
	Sala	42.46 ± 6.95c	2.34 ± 0.48g	4.96 ± 1.07bc	2.76 ± 1.12c	0.0 ± 0.0f	3.05 ± 1.36ef	6.64 ± 4.29c	0.0 ± 0.0d	8.63 ± 1.22de	6.57 ± 1.94efg
	Siku Raja	38.03 ± 5.50c	39.91 ± 2.41c	0.0 ± 0.0c	0.0 ± 0.0c	18.57 ± 2.34b	14.32 ± 2.87bc	0.0 ± 0.0d	0.0 ± 0.0d	6.69 ± 1.13efg	9.50 ± 2.13cde
odorata	Kuinin	9.83 ± 0.52efg	2.37 ± 1.39g	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0f	0.0 ± 0.0f	1.50 ± 0.65d	3.34 ± 1.82cd	4.56 ± 1.38fgh	12.19 ± 0.58bc
petandra	Pauh	19.12 ± 2.17de	37.47 ± 16.43c	2.91 ± 0.60c	0.0 ± 0.0c	7.73 ± 0.46de	3.98 ± 1.88ef	1.67 ± 1.03d	0.0 ± 0.0d	1.80 ± 0.78hi	3.84 ± 1.27ghi
	Bemban	3.07 ± 0.97fg	0.57 ± 0.39g	1.49 ± 0.64c	0.0 ± 0.0c	1.73 ± 0.49f	0.0 ± 0.0f	0.0 ± 0.0d	0.0 ± 0.0d	5.01 ± 0.78fgh	2.73 ± 0.73hi
quadrifida	Asam Kumbang	2.56 ± 0.37g	1.12 ± 0.04g	1.71 ± 0.33c	0.71 ± 0.17c	0.31 ± 0.01f	1.43 ± 0.12f	0.0 ± 0.0d	1.38 ± 0.14d	3.86 ± 0.87ghi	2.44 ± 0.71hi

[a]Values are the mean ± standard deviation of triplicate analyses. Results for mature-green and ripe samples were analyzed together, whereas the contents of different phenolic compounds were analyzed separately. Values per each phenolic compound followed by different letters are significantly different ($p < 0.05$).

However, due to the dissimilarity in sample matrices, solvent systems, extraction procedures, and quantification units, those literature data are not comparable with our results. Thus far, the results obtained from the previous comparative assessments of different cultivars of *M. indica* have revealed the promising scavenging activity of Uba,[7] Ataulfo,[8] and Tainong.[12] When the results in Tables 2 and 4 were compared, a similar trend of DPPH-scavenging activity and TPC in relation to ripening stages was observed, whereby only the ripe Bacang, Siku Raja, Kuinin, and Bemban possess higher activity than their mature-green samples.

With the exception of Siku Raja and Kuinin, the trend of FRAP values was also found to be related to TPC and DPPH values. Due to the decline in the levels of vitamin C during ripening, most of the FRAP values (excluding for Bacang, Pauh, and Bemban) were significantly higher in the mature-green samples than in the ripe samples. Therefore, TPC may be responsible for the higher activity of the ripe Bacang, Pauh, and Bemban, whereas vitamin C could possibly be the main attribute for the higher FRAP activity of mature-green Siku Raja and Kuinin. The aqueous extracts of the mature-green and ripe Binjai exhibited significantly higher FRAP activity. The highest activity shown by the mature-green Binjai (868.29 ± 2.71 μg TE/g sample) could be correlated with its higher contents of vitamin C and total phenolics (Table 2), as well as the highest mangiferin content (Table 3). These constituents were also abundantly quantified from the flesh extracts of Uba and Ataulfo.[6−8] The catechol moiety in the chemical structures of vitamin C and mangiferin is well-known as a key contributor to the FRAP activity.[24]

As indicated in Table 3, the higher content of gallic acid and the highest content of vanillic acid might be accountable in enhancing the FRAP activity of ripe Binjai (757.18 ± 4.41 μg TE/g sample) (Table 4). The pronounced activity of mature-green flesh of Nam Dok Mai (401.87 ± 7.86 μg TE/g sample) can be associated with the quantification of the highest contents of gallic, protocatechuic, and *p*-hydroxybenzoic acids in the extract (Table 3). The activity of the ripe flesh of Pauh (238.51 ± 6.96 μg TE/g sample) was in line with the highest TPC and higher vitamin C content (Table 2). Previous studies on the fleshes of *Mangifera* that have employed FRAP assay were mostly carried out on an individual cultivar or species,[5,10,11,14−16] and the comparative evaluation between the cultivars of *M. indica* by Ma et al.[12] had highlighted the highest FRAP value in Tainong cultivar. They also found the highest correlation between FRAP and TPC values of the cultivars.

Secondary Antioxidant Activity. The metal chelating assay measures the ability of extract to bind to ferrous (Fe(II)) ion (that catalyzes oxidation) and disrupt the formation of Fe(II)−ferrozine complex. Thus, an extract may act as a secondary antioxidant due to its indirect involvement in preventing the formation of free radicals. The aqueous extract of ripe Siku Raja with moderate contents of vitamin C and total phenolics (Table 2) possesses the greatest metal chelating activity (93.68 ± 0.74%; Table 4). To our knowledge, the only metal chelating study of mangoes was conducted by Ma et al.[12] on eight cultivars of Chinese mangoes. They reported that metal chelating activity was highly correlated with vitamin C ($r = 0.71$). Mallika cultivar with the highest content of vitamin C has the highest metal chelating activity. The metal chelating ability of polyphenols was found to be related with the presence an *o*-dihydroxyl moiety in their chemical structures or those bearing catechol or galloyl groups.[29] Several compounds that

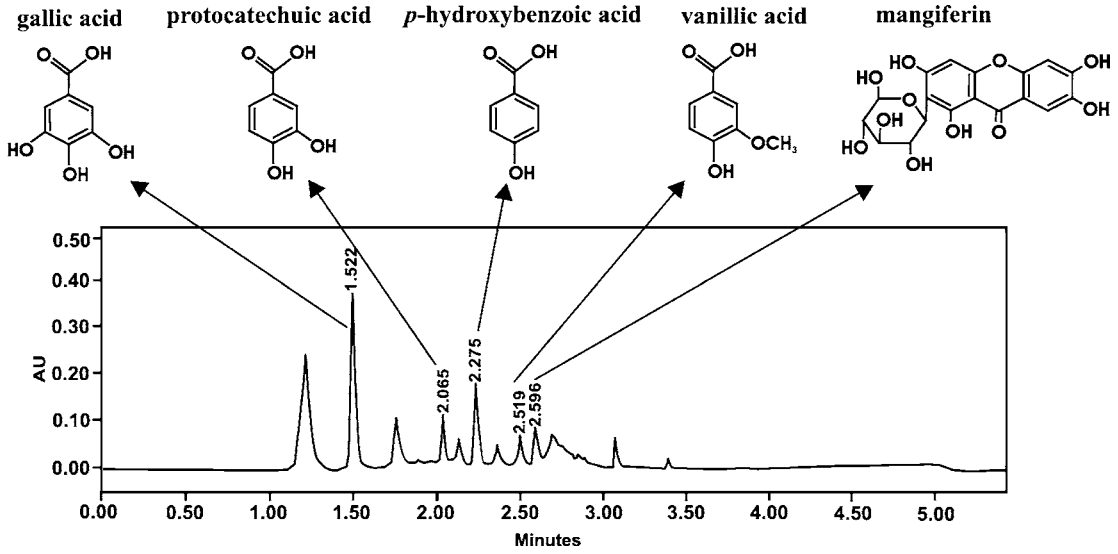

Figure 1. UPLC chromatograms of aqueous extract (1 mg/mL) of mature-green *Mangifera indica* cv. Pauh. Peak at retention time 1.522 min = gallic acid, 2.065 min = protocatechuic acid, 2.275 min = *p*-hydroxybenzoic acid, 2.519 min = vanillic acid, and 2.596 min = mangiferin.

Table 4. Antioxidant Activities of *Mangifera* Samples[a]

		DPPH (μg TE/g sample)		FRAP (μg TE/g sample)		metal chelating[b] (%)	
Mangifera species	local name/ cultivar	mature-green	ripe	mature-green	ripe	mature-green	ripe
caesia	Binjai	303.71 ± 21.11c	9.68 ± 3.61n	868.29 ± 2.71a	757.18 ± 4.41b	6.09 ± 2.21k	0.0 ± 0.0l
foetida	Bacang	107.27 ± 13.94ij	291.48 ± 25.21c	68.19 ± 3.30k	101.79 ± 3.84h	41.25 ± 6.46e	28.64 ± 2.53g
indica	Chokanan	408.21 ± 5.37a	197.14 ± 1.29gh	133.91 ± 1.43f	89.27 ± 2.34i	0.0 ± 0.0l	0.0 ± 0.0l
	Apple	209.09 ± 3.47efg	180.35 ± 5.23h	68.59 ± 0.36k	29.96 ± 0.46°	6.16 ± 2.25k	18.84 ± 0.32i
	Harumanis	53.71 ± 0.64lm	48.02 ± 1.59m	56.38 ± 2.64l	39.75 ± 1.35n	35.08 ± 4.47f	0.0 ± 0.0l
	Nam Dok Mai	296.97 ± 29.3c	67.89 ± 1.12klm	401.87 ± 7.86c	118.90 ± 5.81g	23.95 ± 0.42h	56.09 ± 0.76c
	Sala	370.60 ± 1.78b	87.85 ± 6.11jk	146.47 ± 0.84e	49.12 ± 0.39m	2.73 ± 0.56k	48.25 ± 1.40d
	Siku Raja	186.74 ± 17.17h	219.35 ± 21.56def	95.32 ± 0.64i	69.34 ± 2.46k	7.53 ± 0.03k	93.68 ± 0.74a
odorata	Kuinin	70.13 ± 0.46kl	114.74 ± 13.56i	94.35 ± 3.84i	71.67 ± 1.14k	41.95 ± 0.12e	48.88 ± 0.61d
petandra	Pauh	227.92 ± 5.26de	234.22 ± 4.59d	90.19 ± 7.02i	238.51 ± 6.96d	21.99 ± 0.87hi	12.54 ± 0.53j
	Bemban	47.61 ± 1.14m	100.46 ± 2.27ij	70.75 ± 0.32k	80.47 ± 2.08j	38.03 ± 0.36ef	88.94 ± 1.43b
quadrifida	Asam Kumbang	200.58 ± 13.75fgh	101.68 ± 4.80ij	78.82 ± 7.86j	28.36 ± 1.01o	0.0 ± 0.0l	3.92 ± 4.57k

[a]Values are the mean ± standard deviation of triplicate analyses. Results for mature-green and ripe samples were analyzed together, whereas those from three different antioxidant assays were analyzed separately. Values per each assay followed by different letters are significantly different ($p < 0.05$). [b]Resulted from assay using aqueous extract (1 g/mL fresh weight of sample).

were reported in the fleshes of *M. indica*,[7,8,18−20] such as vitamin C, mangiferin, isomangiferin, ellagic acid, protocatechuic acid, quercetin 3-*O*-galactoside, and quercetin 3-*O*-glucoside have an *o*-dihydroxyl or catechol group in their chemical structures, whereas gallic acid and *m*-digallic acid bear a galloyl moiety. Thus, the metal chelating activity of the aqueous extract of ripe Siku Raja flesh might be due to the synergistic effect of vitamin C (Table 2), mangiferin, gallic acid, and *p*-hydroxybenzoic acid (Table 3).

It is concluded that the samples used in this comparative study were varied in vitamin C and total phenolic contents and antioxidant activities. Higher contents of vitamin C were measured from the mature-green than from the ripe fleshes. Gallic acid and mangiferin were found in all aqueous extracts of *Mangifera* fleshes. On the basis of the results obtained from this study, further studies to optimize the recovery of polyphenolics in the mature-green fleshes of underutilized *M. caesia* (Binjai) and *M. indica* cv. Chokanan and the ripe flesh of *M. indica* cv. Siku Raja with outstanding FRAP, DPPH, and metal chelating activities, respectively, are definitely needed.

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Notes

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