

Flavonoid profile and antioxidant activity of pink guava

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ABSTRACT: This article determines ascorbic acid, total phenolic content and the antioxidant capacity of whole fruit, flesh, and skin fractions of two varieties of pink guava widely produced in Malaysia (semenyih and sungkai). They were analysed and specific flavonoid compounds (apigenin, isorhamnetin, kaempferol, luteolin, myricetin, and quercetin) were determined. Ascorbic acid, total phenolic content and antioxidant capacity was found to be higher in semenyih than in sungkai, mainly in the skin fraction. The predominant flavonoid in all pink guava fractions was kaempferol, with sungkai flesh having the highest kaempferol content. The pink guava represents an important source of antioxidant flavonoid compounds that may have health benefits.

KEYWORDS: *Psidium guajava*, variety, DPPH, FRAP

INTRODUCTION

Several studies to determine the relationship between fruits and health have found that eating five or more servings of fruit and vegetables daily might reduce the chance of disease incidence^{1,2}. Since fruits and vegetables provide a mixture of phytochemicals, a hypothesis on the role of antioxidants in protective against chronic diseases has been proposed³.

Guava (*Psidium guajava*) is one of the most important commercial fruit crop in tropical and subtropical countries and claims superiority over different fruits by virtue of its commercial and nutritional values. Guava is considered a common man's fruit and is called the 'apple of the tropics'⁴. Guava, like many other fruits and vegetables, is rich in antioxidant compounds such as polyphenols⁵, ascorbic acid⁶, and carotenoids⁷. The chemical composition of the fruits depends on factors such as variety, maturity and the environmental conditions within which they are grown^{8,9}.

The objective of this study was to report the antioxidant activities of two varieties of Malaysian pink guava. Selected flavonoid compounds were quantified (apigenin, isorhamnetin, kaempferol, luteolin, myricetin, and quercetin). Various functions and actions such as antioxidants and anticarcinogens have been attributed to flavonoid compounds, making determination of their concentrations in food highly desirable.

MATERIALS AND METHODS

Samples

Two varieties of Malaysian pink guava namely sungkai and semenyih were collected from Perak State, Malaysia. Samples were transferred in ice on the same date to the Food Analysis Laboratory, University Kebangsaan Malaysia. The estimated time of transportation was about 4 h. For the purposes of this study approximately 20 fruits pooled sample portion (taken from a 60 sample lot) were used on the same day.

Physicochemical properties

Fruit weight and seed weight were measured using digital balance (SK-5001, A&D, Japan). The flesh weight was computed by subtracting seed weight from fruit weight. The fruit volume was determined by water displacement (ml) and specific gravity was calculated by dividing the weight of the fruit by the volume of the fruit. To determine the titratable acidity (TTA), samples were crushed and blended in a Waring blender (USA). Ten ml of blended samples were diluted with 50 ml of water before titrated with 0.1 N NaOH and calculated as percent citric acid. Blended undiluted sample was used to measure the total soluble solids, results were expressed as Brix using Abbe refractometer at 20 °C and the pH was determined using a pH meter from the undiluted samples. Lycopene was extracted using a mixture

of acetone, ethanol, and hexane¹⁰. For L. ascorbic acid determination, 1 g was extracted with 25 ml 1% cold metaphosphoric acid and determined using spectrophotometer¹¹.

Extraction of antioxidants

Fruits were deseeded and the skins were removed using fruit peeler to get skin and flesh fraction as well as whole fruit. Samples were extracted with 50% aqueous acetone¹².

Total phenolic contents

A 100 μ l aliquot of fivefold diluted pink guava extract was oxidized with diluted Folin-Ciocalteu reagent (500 μ l). After 5 min, the mixture was neutralized with 1 ml Na_2CO_3 (8%, w/v), and incubated for 120 min before reading absorbance at 765 nm¹³.

Ferric reducing antioxidant power (FRAP)

The FRAP assay method of Benzie and Strain¹⁴ was modified to determine antioxidant activity using trolox as the standard. The FRAP reagent was prepared using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, plus 16 ml glacial acetic acid made up to 1 l with distilled water). The acetate buffer was mixed with 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ at the ratio of 10:1:1 to produce the working reagent. For assays, 3950 μ l of freshly prepared FRAP reagent was mixed with 50 μ l sample, standard, or blank and incubated for 30 min before reading absorbance at 595 nm.

Radical scavenging activity

In the radical scavenging activity (RSA) assay, 3900 μ l methanolic DPPH solution (40 mg/l) and 100 μ l sample extract were mixed, incubated in the dark, and followed the absorbance change at 517 nm. The experiment was performed using different sample concentrations (10–50 mg fresh sample/ml extraction solvent). The experiment was also performed using sample extract of 50 mg fresh sample/ml extraction solvent at different time levels. The decrease in absorbance was monitored until the reaction reached a plateau, graphs were then constructed showing radical scavenging activity versus time. RSA was calculated as $(A_0 - A_c)/A_0$, where A_0 is the absorbance of DPPH solution without sample and A_c is the absorbance of DPPH and sample. The efficient concentration (EC_{50}) was also calculated as the amount of antioxidant necessary to decrease the initial DPPH by 50%.

Table 1 Physicochemical properties of pink guava fruits.

Characteristics	Sungkai variety	Semenyih variety
Fruit weight (g)	257 \pm 73 ^a	209 \pm 25 ^b
Flesh weight (g)	249 \pm 72 ^a	202 \pm 25 ^b
Seed weight (g)	8.1 \pm 2.5 ^a	7.0 \pm 3.2 ^b
Fruit volume (ml)	268 \pm 78 ^a	216 \pm 40 ^b
Specific gravity	0.96 \pm 0.05 ^a	0.98 \pm 0.10 ^b
Brix ^o	7.50 \pm 0.39 ^b	8.57 \pm 0.53 ^a
Brix ^o /TA	16.30 \pm 0.91 ^b	17.1 \pm 1.1 ^a
Acidity	0.46 \pm 0.00 ^a	0.50 \pm 0.00 ^a
Vitamin C (mg/100 g FW)		
- Fruit	135 \pm 28 ^b	202 \pm 13 ^a
- Flesh	130 \pm 27 ^b	190 \pm 12 ^a
- Skin	172 \pm 36 ^b	308 \pm 57 ^a
Lycopene (mg/kg)	40.5 ^b	52.8 ^a
L (lightness)	48.24 ^b	49.39 ^a
α^+ (redness)	11.25 ^b	16.06 ^a
b^+ (yellowness)	13.77 ^a	10.17 ^b
pH	4.27 ^a	4.21 ^a

Values are means of triplicate samples \pm SD. Different letters in the same row indicate significant differences ($p < 0.05$).

Extraction and determination of flavonoid compounds

Freeze-dried sample (0.5 g) was mixed with 10 ml of 2.0 M HCl in 80% aqueous methanol using an Ultra-Turrax disperser (IKA, Germany) and the mixture was heated up at 90 $^{\circ}$ C for 2 h. The extract was diluted with water and then filtered through a 0.22 μ m nylon filter (Whatman, Kent, UK) prior to injection into HPLC (Shimadzu, Japan). A sample of 10.0 μ l was injected to Symmetry-C18 column (Waters, USA) at 40 $^{\circ}$ C. The mobile phase used was 1% formic acid (55%) in methanol at 0.9 ml/min flow rate for 20 min.

Statistical analysis

Data collected were statistically analysed using MINITAB (14.20) software. A significance level of $p < 0.05$ was used.

RESULTS AND DISCUSSION

Physicochemical properties of pink guava

Table 1 shows the characteristics of the two Malaysian pink guava fruit varieties. Semenyih had smaller fruit, lower fruit and flesh weight, lower pH and higher TTA compared to sungkai. Semenyih had a higher sugar/acid ratio than sungkai, which might contribute towards better storability, since low sugar/acid ratio has been shown to be a good indicator for prolonging fruit quality during storage¹⁵. The

Table 2 Folin-Ciocalteu index and ferric reducing antioxidant power (FRAP) values for different pink guava fruit fractions from two different varieties (sungkai and semenyih).

Fruit fraction	Total phenolic content [†]	FRAP [*]
Sungkai skin	671 ± 32 ^b	66.9 ± 3.9 ^b
Sungkai fruit	227.9 ± 9.8 ^e	27.1 ± 1.1 ^e
Sungkai flesh	193.1 ± 4.9 ^f	25.8 ± 1.1 ^e
Semenyih skin	841 ± 22 ^a	82.7 ± 3.2 ^a
Semenyih fruit	383 ± 33 ^c	56.3 ± 1.9 ^c
Semenyih flesh	344.7 ± 7.6 ^d	39.2 ± 1.5 ^d

Values are mean ± SD. Different letters in the same column indicate significant differences ($p < 0.05$).

[†] mg gallic acid equivalent/100 g fresh weight.

^{*} μ Mtrolox equivalent/g fresh weight.

amounts of total ascorbic acid (TAA) in semenyih were significantly higher ($p < 0.05$) than sungkai. TAA contents in the skin of both varieties were significantly higher ($p < 0.05$) compared to their respective flesh or fruit fractions. Ascorbic acid content of fruits depended highly on the varieties and the cultivation conditions. Distinct varieties of the same fruit type showed significantly different concentrations¹⁶.

Compared to sungkai, semenyih fruit showed significantly ($p < 0.05$) higher lycopene content that gives the red colour for the pink guava fruit. Wilberg and Rodriguez-Amaya¹⁷ reported lycopene content in Brazilian pink flesh guava is in a range from 48.20–54.20 mg/kg fresh ripe fruits. The colour value of sungkai showed significantly ($p < 0.05$) lower redness value (a^+), lower lightness value (L), and higher yellowness value (b^+) when compared to semenyih.

Total phenolic content and ferric reducing antioxidant power (FRAP)

Individual fruit fractions showed a wide variation of their total phenolic content and FRAP value (Table 2). Maximum total phenolic content and FRAP values were obtained for semenyih fruit variety fractions when compared to sungkai variety. Among each variety fractions, skins showed the highest total phenolic content and FRAP values followed by fruit and flesh, respectively. The size of sungkai fruit is much bigger and this may affect the results, in small fruit the ratio of the skin will be higher compared to the total fruit. Total phenolic content and FRAP values for the pink guava fruit in this study were higher than the values reported by Thaipong et al⁵, Luximon-Ramma et al¹⁸, and

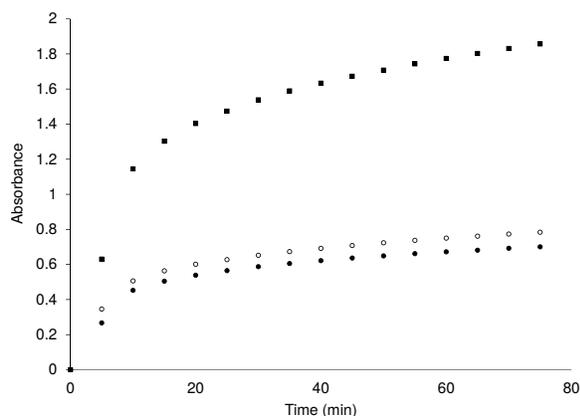


Fig. 1 FRAP reaction kinetics for skin (■), fruit (○), and flesh (●) fractions (sungkai variety), rate of increase in absorbance at 595 nm for 50 g/l solutions of sample.

Guo et al¹⁹. Differences in extraction methods and solvent¹², cultivation location^{20,21}, ripening stage²², harvested condition and seasons²³ could be the most likely factors.

In this study the reaction time of FRAP was prolonged and compared to the 4 min in the original procedure of Benzie and Strain¹⁴. The rate of the reactions of individual fraction differs substantially (Fig. 1). FRAP value was calculated at 30 min since the order of antioxidant efficiency of samples were maintained after 30 min. Trolox standards react quickly with FRAP reagent, reaching the maximum in less than 2 min and the absorbance was constant after that. Pulido et al²⁴ observed similar results based on different standards but not plant extracts. The results of our observation are in agreement with published data^{25,26}.

DPPH radical scavenging activity of pink guava

The radical scavenging activity values in Fig. 2 indicate a different activity of each pink guava fruit samples towards the stable free radical. Skin fraction extract reacted faster towards the DPPH radical and the reaction reached the plateau in 2 min for semenyih skin and 3 min for sungkai skin, whereas slow reaction took place for other samples. The flesh fractions in both variety reactions reached the plateau in 4 min for semenyih flesh and 12 min for sungkai flesh. Trolox standards also react quickly towards DPPH radical.

The RSA for pink guava extract at different concentrations are shown in Table 3. As anticipated, the higher the concentration of sample, the higher the scavenging activity. However, at certain concen-

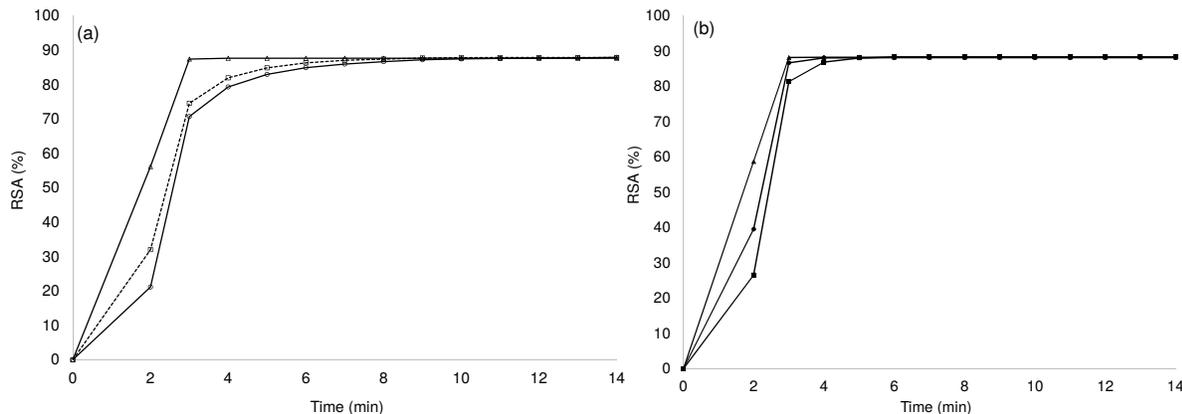


Fig. 2 DPPH radical scavenging activity of (a) sungkai variety and (b) semenyih variety (triangles: skin, squares: fruit, circles: flesh), with rate of increase in activity at 515 nm for 50 g/l solutions of sample.

Table 3 Radical scavenging activity as a function of sample concentration for pink flesh guava fruit.

Fruit sample	RSA (%)					EC ₅₀ [‡]
	C = 10 [†]	20	30	40	50	
Sungkai skin	67 ^b	86 ^a	86 ^a	86 ^a	86 ^a	7.57 ^e
Sungkai fruit	35 ^e	52 ^d	61 ^c	74 ^b	83 ^b	18.91 ^b
Sungkai flesh	35 ^e	50 ^d	60 ^c	69 ^c	77 ^c	20.15 ^a
Semenyih skin	74 ^a	85 ^a	86 ^a	86 ^a	86 ^a	6.73 ^f
Semenyih fruit	48 ^c	67 ^b	80 ^b	86 ^a	86 ^a	10.85 ^d
Semenyih flesh	43 ^d	62 ^c	77 ^b	86 ^a	87 ^a	13.57 ^c

[†] Sample concentration (mg fresh sample/ml solvent).
[‡] Radical scavenging activity expressed as the concentration that reduced the free radicals by 50% (mg weight/ml extracting solvent).
 Different letters in the same column indicate significant differences ($p < 0.05$).
 Different letters in the same row, except for the EC₅₀ column, showed significant differences ($p < 0.05$).

trations, there was no increase in the scavenging activity. At higher concentrations of any antioxidant, absorbance will not increase beyond the point at which all the available DPPH free radicals have been consumed. This is reflected in the results, which showed no change in DPPH radical scavenging activity at concentrations above 40 mg fresh sample/1 ml solvent. At high sample concentration, the RSA rank of the antioxidant activity value in the extracted samples was: semenyih skin = sungkai skin = semenyih fruit = semenyih flesh > sungkai fruit > sungkai flesh. Generally, any sample showed scavenging activity value higher than 80% should be diluted to get better and safer classification of samples. When samples classified by using sam-

ples at low concentration (10 mg/ml) of extract; the rank of the antioxidant activity value in the extracted samples was: semenyih skin > sungkai skin > semenyih fruit > semenyih flesh > sungkai fruit = sungkai flesh.

Results from single concentration may not be directly used in comparing between radical scavenging activities of different samples. The use of different sample concentrations may result in more information about antioxidant extracts. The radical scavenging activity was reported as percentage as effective concentration (EC₅₀) and compared to RSA. Sample extracts were easily and clearly classified when EC₅₀ was used. The rank of the antioxidant activity values in fruits was: semenyih skin > sungkai skin > semenyih fruit > semenyih flesh > sungkai fruit > sungkai flesh.

The EC₅₀ value is more convenient than the kinetic version but it does not give any information about the rapidity of the kinetics. However, using EC₅₀ requires more numbers of samples and work compared to RSA which is convenient, and save time and chemicals. RSA could be used for screening purposes while EC₅₀ could be used for sample classification and details.

Flavonoids in pink guava fruit

Kaempferol was the dominant flavonoid in fruits and quercetin was the minor flavonoid in both varieties (Fig. 3). The total flavonoid content was significantly higher ($p < 0.05$) in sungkai fruit and flesh compared to semenyih; while semenyih skin was higher ($p < 0.05$) compared to sungkai skin. As for luteolin, both sungkai and semenyih fruit showed no significant difference ($p < 0.05$) in their luteolin content while sungkai flesh and semenyih skin both

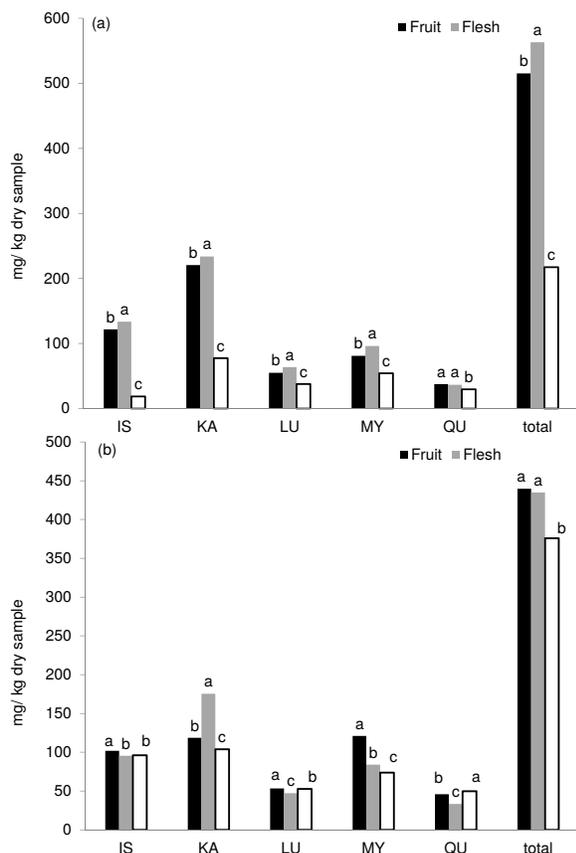


Fig. 3 Flavonoid contents in pink guava fruit fractions of sungkai variety (a) and semenyih variety (b). The flavonoids detected were myricetin (MY), quercetin (QU), luteolin (LU), kaempferol (KA), and isorhamnetin (IS). Means of each flavonoid marked by the same letter are not significantly different at $p < 0.05$.

showed higher values. The levels of myricetin in sungkai fruit, flesh, and skin were 80.38, 93.75, and 51.60 mg/kg, respectively, and for semenyih the levels were 83.05, 84.00, and 73.75 mg/kg for fruit, flesh, and skin, respectively. Apigenin was not detected in all fractions from both fruit varieties which is in agreement with published work²⁷.

Conclusion

Pink guava shows valuable nutraceutical properties in terms of high antioxidant activity as well as vitamin C and lycopene. The flavonoid content is reported with kaempferol as the main flavonoid compound. Moreover, since these fruits show the highest antioxidant content in the peel, they seem to be particularly suitable for unpeeled whole fresh fruit consumption and thus promote health related benefits.

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