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Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*)

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ABSTRACT

The composition of phenolic acids in various parts of mangosteen fruit (*Garcinia mangostana*) was determined by GC and MS. The total content of phenolic acids, identified by GC-FID ranged from 265.7 ± 12.7 (aril) to 5027.7 ± 188.0 (peel) mg per kg of dry matter of sample. Ten phenolic acids were identified in mangosteen fruit. Of these, protocatechuic acid was the major phenolic acid in the peel and rind, while *p*-hydroxybenzoic acid was the predominant phenolic acid in the aril. *m*-Hydroxybenzoic acid was detected only in the peel, while 3,4-dihydroxymandelic was present only in the rind. The phenolic acids liberated from esters and glycosidic bonds were the major fractions of phenolic acids in mangosteen fruit.

1. Introduction

Mangosteen (*Garcinia mangostana* L.), a tropical evergreen tree, may be found in Malaya, India, Thailand, Vietnam, Singapore, Philippines and Burma. It bears dark-purple to red-purple rounded fruits of 5–7 cm in diameter. The edible portion of fruit (aril) is white, soft with a slightly sour taste (Martin, 1980; Morton, 1987). The pericarp, 6–10 mm in thickness, has been used in folk medicine for the relief of diarrhea as well as for treatment of skin wounds and disorders (Mahabusarakam, Iriyachitra, & Taylor, 1987; Martin, 1980; Morton, 1987). Mangosteen fruit is a rich source of phenolic compounds such as xanthones, condensed tannins and anthocyanins (Fu, Loo, Chia, & Huang, 2007; Jung, Su, Keller, Mehta, & Kinghorn, 2006; Mahabusarakam et al., 1987). Of these, only xanthones have been extensively studied by various research groups (Ji, Avula, & Khan, 2007; Jung et al., 2006; Mahabusarakam et al., 1987).

The content of phenolics in fruits is affected by the degree of maturity at harvest, genetic differences (cultivar), preharvest environmental conditions, post-harvest storage conditions and processing (Shahidi & Naczk, 2004). Phenolic acids constitute about one-third of the dietary phenols and they are present in plants in the free and bound forms (Robbins, 2003). Bound-phenolics may be linked to various plant components through ester, ether, or acetal bonds (Chalas et al., 2001). Clifford (1999) estimated that daily consumption of phenolic acids ranged from 25 mg to 1 g. An increasing interest in determining the antioxidant activities exhibited by phenolic acids and their derivatives should also be noted (Lodovici, Guglielmi, Meoni, & Dolara, 2001; Rice-Evans, Miller, & Paganga, 1996, 1997).

To the best of our knowledge phenolic acid profiles of mangosteen fruits are still unknown. Therefore, the purpose of this study was to determine the composition of free and bound phenolic acids in locally available mangosteen fruits using analytical methodologies established in our laboratories (Zadernowski, 1987; Zadernowski, Naczk, & Nesterowicz, 2005; Zadernowski, Naczk, & Nowak-Polakowska, 2002).

2. Material and methods

2.1. Materials

Mangosteen fruits (*Garcinia mangostana*) imported from Mexico were obtained from a local food store in Antigonish, Nova Scotia, Canada. All fruits were cleaned and inspected to remove damaged, diseased or pest infested fruits. Following this the fruits were separated into three parts: the exocarp (referred here as peel), mesocarp (referred here as rind) and edible pulp (referred here as aril). Subsequently, the fruit parts were lyophilized and then stored in polyethylene bags at −20 °C until analysis. Before analysis, lyophilized fruit parts were crushed in a food processor.

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2.2. Chemicals

Caffeic, ferulic, *p*- and *m*-hydroxybenzoic, protocatechuic, sinapic, vanillic, and veratric acids, as well (+) catechin, sodium bicarbonate, sodium hydroxide, diethyl ether, methanol and *N,O*-bis(trimethylsilyl)acetamide were purchased from Sigma Chemical Co. (Sigma–Aldrich Sp. z.o.o.; Gliwice, Poland), while *p*-coumaric acid was obtained from Fluka (Sigma–Aldrich Sp. z.o.o.; Gliwice, Poland).

2.3. Preparation of crude phenolic extract

Soluble phenolics were extracted six times from crushed fruit parts into aqueous 80% (vol/vol) methanol (at a ratio of 1:1, wt/vol) at room temperature for 1 h using an orbital shaker at 250 rpm. The mixture was centrifuged at 1750*g* for 10 min and the supernatants were collected, combined, evaporated to near dryness under vacuum at $\leqslant$40 °C, and lyophilized (Zadernowski, 1987).

2.4. Fractionation of phenolic acids

Phenolic acids present in crude extract were fractionated into free and bound forms according to the procedure described by Kozlowska, Rotkiewicz, Zadernowski, and Sosulski (1983), Zadernowski (1987), Zadernowski, Naczk, and Nowak-Polakowska (2002) and Zadernowski, Naczk, and Nesterowicz (2005). A 0.5 g sample of dried crude phenolic extract was suspended in 50 mL of triply distilled water, acidified to pH 2 using 6 M HCl and extracted five times with diethyl ether (1:1, vol/vol) at room temperature. The ether extracts of phenolic acids (referred to as free phenolic acids) were combined and evaporated to dryness under vacuum at $\leqslant$40 °C. The water phase was adjusted to pH 7 with 2 M NaOH and then evaporated to almost dryness under vacuum at $\leqslant$40 °C. The residue was treated with 20 mL of 4 M NaOH under nitrogen for 4 h at room temperature. The reaction mixture was then acidified with 6 M HCl to pH 2 and extracted with diethyl ether as described above. The ether extracts of phenolic acids (referred to as phenolic acids liberated from ester bonds) were combined and evaporated to dryness under vacuum at $\leqslant$40 °C. The water phase was adjusted to pH 7 with 2 M NaOH and then evaporated to almost dryness under vacuum at $\leqslant$40 °C. The residue was heated with 50 mL of 2 M HCl for 30 min at 95 °C, cooled to room temperature and extracted with diethyl ether as described above. These ether extracts of phenolic acids (referred to as phenolic acids liberated from glycosidic bonds) were combined and evaporated to dryness under vacuum at $\leqslant$40 °C.

2.5. Purification of phenolic acids fractions

Each of the residues of phenolic acid fractions, obtained as described above, was dissolved in 50 mL of 5% (wt/vol) $NaHCO_3$ (pH 8) and extracted five times with diethyl ether to remove residual lipid material. The water phase was then acidified with 6 M HCl to pH 2 and extracted with diethyl ether as described above. The dry residues of phenolic acids were dissolved in 5 mL of 80% (vol/vol) methanol (Zadernowski, 1987).

2.6. Formation of trimethylsilyl derivatives

To 0.5 mL methanolic solution of purified phenolic acids in the reaction vial 20–50 μL of *N,O*-bis(trimethylsilyl)acetamide were added, depending on the phenolic acid concentrations. The vial was then tightly closed and left at room temperature for 24 h (Zadernowski, 1987).

2.7. GC–MS identification of phenolic acids

The trimethylsilyl derivatives of phenolic acids were identified using GC–MS methodology as described by Zadernowski (1987), Zadernowski, Naczk, and Nowak-Polakowska (2002), Horman and Viani (1971), Tian and White (1994), and Xing and White (1997). GC–MS analysis was carried out on a Hewlett–Packard 5890 Series II gas chromatograph interfaced with a MS Hewlett–Packard 5970 mass selective detector (Kennett Square, PA). Separations were performed using a 30 m × 0.25 mm (i.d.) SPB-1 silica-fused capillary column coated with 0.25 μm film of poly(dimethylsiloxane) as the stationary phase (Supelco Inc., Bellefonte, PA). Helium was used as the carrier gas at an average flow rate of 28 cm^3 per min. The injector and the transfer line temperatures were kept at 240 °C. The oven temperature program used was 120–260 °C at a rate of 20 °C per min. Initial and final temperatures were held for 2 min and 10 min, respectively. The injections were carried out in a split mode with a split ratio of 20:1. The mass spectrometer was operated with an ionization voltage of 235 eV and electron multiplier voltage of 1700 V and was scanned from 50 *m/z* to 500 *m/z* at 0.8 s per scan. The volume of injected samples ranged from 1 μL to 2 μL, depending on the sample. Caffeic, *p*-coumaric, ferulic, *p*-hydroxybenzoic, protocatechuic, vanillic and veratric (3,4-dimethoxybenzoic) acids were identified by using mass spectra of standard derivatives. The remaining phenolic acids (*m*-hydroxybenzoic, *p*-hydroxyphenylacetic and 3,4-dihydroxymandelic acids) as well as other acids (benzoic, cinnamic, mandelic (2-hydroxy-2-phenylacetic) and piperylonic (3,4-methylenedioxybenzoic) acids) were identified using the mass spectral library provided by the GC–MS supplier.

2.8. Quantitation of phenolic acids

The phenolic acids were quantified as described by Zadernowski (1987), Zadernowski, Naczk, and Nowak-Polakowska (2002) and Zadernowski, Naczk, and Nesterowicz (2005) using a Hewlett–Packard 5890 Series II (Kennett square, PA) gas chromatograph equipped with a flame–ionization detector. Separations of trimethylsilyl derivatives of phenolic acids were performed as described in the previous paragraph. Sinapic acid was used as an external standard. The contents of the phenolic acids are expressed as mg per kg of fruit part on a dry weight basis.

2.9. Chemical analysis

The total phenol content in crude extracts was estimated by the Folin-Ciocalteau assay (Shahidi & Naczk, 2004) and expressed in mg (+)-catechin equivalents per kg of fruit part on dry matter basis. The moisture content was measured by drying the ground berries at 105 °C until a constant weight was obtained (AOAC, 1980).

2.10. Data treatment

The results presented in the tables are mean values ($n = 6$ and $n = 3$ for Table 1 and $n = 3$ for Tables 2–5) ±SD (standard deviation). Statistical analysis of data (ANOVA and *t*-test) was performed using SigmaStat v.3.0 (SSPS, Chicago, IL). Differences at $P \leqslant 0.05$ were considered to be significant.

3. Results and discussion

3.1. Total phenols

About 80% (vol/vol) aqueous methanol–water is commonly used for the extraction of phenolic acids and their derivatives from plant materials (Naczk & Shahidi, 2004; Robbins, 2003; Zadernowski,

Table 1
Total phenols and contributions of phenolic acid fractions in various parts of mangosteen fruit

Sample	Moisture	Total phenols (TPH)	Free phenolic acids	Phenolic acids liberated from	
				Esters	Glycosides
	(%)	(g/kg d.m.[A])	(%)[B]	(%)[B]	(%)[B]
Peel	43.2 ± 0.4	70.2 ± 5.7	18.7 ± 1.5	41.4 ± 1.7[a]	39.9 ± 3.7[a]
Rind	60.0 ± 3.0	218.1 ± 18.0	5.5 ± 0.5	49.7 ± 4.2[a]	44.8 ± 4.0[a]
Aril	82.6 ± 0.2	6.4 ± 0.5	9.7 ± 1.3	76.5 ± 5.0	13.8 ± 3.3

Values for the same column marked by the same superscript letter are not significantly different (TPH: $n = 6$; ANOVA and t-test; $P > 0.05$; phenolic acids: $n = 3$; ANOVA and t-test; $P > 0.05$).
[A] g (+)-catechin equivalents per kg of dry matter of sample.
[B] % of the total phenolic acids as determined by GC-FID methodology.

Table 2
Total phenolic acid contents in various parts of mangosteen fruits [mg per kg of dry matter of sample]

Acid	Peel	Rind	Aril
Hydroxybenzoic acid derivatives (HBA)			
m-Hydroxybenzoic[B]	68.1 ± 4.6	–[A]	–
p-Hydroxybenzoic[B]	510.7 ± 37.2	941.4 ± 68.7	142.6 ± 10.8
Protocatechuic[B]	3812.2 ± 181.8	2301.3 ± 172.1	94.0 ± 6.3
Vanillic[B]	414.3 ± 27.3	163.9 ± 11.6	9.5 ± 1.3
Veratric[B]	84.3 ± 7.0	–	–
Hydroxycinnamic acid derivatives (HCA)			
Caffeic[B]	91.6 ± 6.0	–	–
p-Coumaric[B]	–	59.7 ± 4.9	18.7 ± 2.0
Ferulic[B]	12.0 ± 1.0	–	0.9 ± 0.0
Other phenolic acids (OtherPA)			
p-Hydroxyphenylacetic	34.5 ± 4.0	22.9 ± 3.0	–
3,4-Dihydroxymandelic	–	88.7 ± 9.5	–
Other acids (otherA)			
Benzoic[B]	24.5 ± 3.0[a]	–	26.1 ± 3.0[a]
Cinnamic[B]	27.9 ± 2.3	–	1.9 ± 0.2
Mandelic	7.4 ± 0.5	–	–
Piperonylic	203.3 ± 16.3	–	–
Total HBA	4889.6 ± 187.8	3406.6 ± 185.7	246.1 ± 12.5
Total HCA	103.6 ± 6.1	59.7 ± 4.9	19.6 ± 2.0
Total otherPA	34.5 ± 4.0	111.6 ± 10.0	–
Total PA	5027.7 ± 188.0	3577.9 ± 188.1	265.7 ± 12.7
Total otherA	263.1 ± 16.7	–	28.0 ± 3.0

Values in each row marked by the same superscript letter are not significantly different ($n = 3$; ANOVA and t-test; $P > 0.05$).
[A] Not detected.
[B] Identified using the mass spectrum of the standard derivative.

Table 3
Free phenolic acids content in various parts of mangosteen fruit (mg per kg of dry matter of sample)

Acid	Peel	Rind	Aril
Hydroxybenzoic acid derivatives (HBA)			
p-Hydroxybenzoic[B]	88.7 ± 10.2	–[A]	5.2 ± 0.8
Protocatechuic[B]	510.7 ± 60.0	153.5 ± 12.4	–
Vanillic[B]	286.0 ± 25.9	65.6 ± 5.6	0.8 ± 0.00
Veratric[B]	–	–	–
Hydroxycinnamic acid derivatives (HCA)			
Caffeic[B]	53.6 ± 4.5	–	–
p-Coumaric[B]	–	–	18.7 ± 2.0
Ferulic[B]	–	–	0.9 ± 0.00
Other acids (otherA)			
Cinnamic[B]	–	–	1.9 ± 0.2
Piperonylic	146.2 ± 15.9	–	–
Total HBA	885.6 ± 66.1	219.1 ± 13.6	6.0 ± 0.8
Total HCA	53.6 ± 4.5	–	19.6 ± 2.0
Total otherPA	–	–	–
Total PA	939.2 ± 66.3	219.1 ± 13.6	25.6 ± 2.2
Total otherA	146.2 ± 15.9	–	1.9 ± 0.2

[A] Not detected.
[B] Identified using the mass spectrum of the standard derivative.

Table 4
Content of phenolic acids liberated from esters in various parts of mangosteen fruit (mg per kg of dry matter of sample)

Acid	Peel	Rind	Aril
Hydroxybenzoic acid derivatives (HBA)			
m-Hydroxybenzoic[B]	30.3 ± 2.5	–[A]	–
p-Hydroxybenzoic[B]	220.0 ± 30.5	416.2 ± 40.0	122.7 ± 10.6
Protocatechuic[B]	1635.9 ± 15.9	1191.6 ± 110.8	72.0 ± 6.0
Vanillic[B]	54.5 ± 6.4	–	8.7 ± 0.9
Veratric[B]	56.4 ± 5.9	–	–
Hydroxycinnamic acid derivatives (HCA)			
Caffeic[B]	38.0 ± 4.0	–	–
p-Coumaric[B]	–	59.7 ± 4.9	–
Ferulic	12.0 ± 1.0	–	–
Other phenolic acids (otherPA)			
p-Hydroxyphenylacetic	34.5 ± 4.0	–	–
3,4-Dihydroxymandelic	–	88.7 ± 9.5	–
Other acids (OtherA)			
Benzoic[B]	24.5 ± 3.0[a]	–	26.1 ± 3.0[a]
Cinnamic[B]	27.9 ± 2.3	–	–
Mandelic	7.4 ± 0.5	–	–
Piperonylic	28.2 ± 3.0	–	–
Total HBA	1997.1 ± 35.6	1607.8 ± 117.8	203.4 ± 12.2
Total HCA	50.0 ± 4.1	59.7 ± 4.9	–
Total otherPA	34.5 ± 4.0	88.7 ± 9.5	–
Total PA	2081.6 ± 36.1	1756.2 ± 118.3	203.4 ± 12.2
Total otherA	88.0 ± 4.9	–	26.1 ± 3.0

Values in each row marked by the same superscript letter are not significantly different ($n = 3$; t-test; $P > 0.05$).
[A] Not detected.
[B] Identified using the mass spectrum of the standard derivative.

Table 5
Content of phenolic acids liberated from glycosides in various parts of mangosteen fruit (mg per kg of dry matter)

Acid	Peel	Rind	Aril
Hydroxybenzoic acid derivatives (HBA)			
m-Hydroxybenzoic[B]	37.8 ± 3.9	–[A]	–
p-Hydroxybenzoic[B]	202.0 ± 18.7	525.2 ± 55.9	14.7 ± 1.7
Protocatechuic[B]	1665.5 ± 170.9	956.2 ± 102.1	22.0 ± 2.0
Vanillic[B]	73.8 ± 5.9	98.3 ± 10.2	8.7 ± 1.0
Veratric[B]	27.9 ± 3.8	–	–
Other phenolic acids (otherPA)			
p-Hydroxyphenylacetic	–	22.9 ± 3.0	–
Other acids (otherA)			
Piperonylic	28.9 ± 2.0	–	–
Total HBA	2007.0 ± 172.1	1579.7 ± 116.8	45.4 ± 2.8
Total otherPA	–	22.9 ± 3.0	
Total PA	2007.0 ± 172.1	1579.7 ± 116.9	45.4 ± 2.8
Total otherA	28.9 ± 2.0	–	–

[A] Not detected.
[B] Identified using the mass spectrum of the standard derivative.

1987; Zadernowski, Naczk, and Nowak-Polakowska, 2002) and therefore, was selected for extraction of phenolics from mangosteen fruits. Table 1 shows the moisture and total phenol (TPH) contents in mangosteen fruit parts. In the literature, the reported TPH values are calculated per fresh weight of fruit or per dry matter of fruit. Moisture contents are provided here in order to aid the readers in comparing our results with those published. TPH values ranged from 6400 in the aril to over 218.1 g of (+)-catechin equivalents per kg of sample (dry matter; d.m.) in the rind (Table 1). The rind contains over threefold more TPH than the peel, while the edible aril is a very poor source of phenolics. The total phenols reported here are higher than those previously published for aril (Lim, Lim, & Tee, 2007) and peels (Maisuthisakul, Suttajit, & Pongsawatmanit, 2007). Factors such as regional differences, harvest time, storage conditions, and analytical procedures used for extraction and quantification of phenolics might contribute to these differences (Shahidi & Naczk, 2004).

3.2. Total phenolic acids

Many analytical procedures have been employed for the determination of phenolic compounds in plant materials (Antolovich, Prenzler, Robards, & Ryan, 2000; Robbins, 2003) but those currently used for the determination of plant phenolics only target major flavonoids and/or phenolic acids and their conjugates (Kähkönen, Hopla, & Heinonen, 2001; Thompson & Chaovanalikit, 2003). The total content of phenolic acids in mangosteen berries ranged from 265.7 ± 12.9 (aril) to 5027.7 ± 188.0 mg per kg, d.m., (peel) (Table 2). The phenolic acids are mainly located in the pericarp of mangosteen fruit. The peel, rind and aril of the mangosteen fruit contain eight, six and five phenolic acids, respectively. Hydroxybenzoic acid derivatives constituted from 92.6% (aril) to 97.3% (peel) of the total phenolic acids present. Of these, *m*-hydroxybenzoic, veratric and caffeic acids were only found in the peel and 3, 4-dihydroxymandelic acid in the rind. Protocatechuic acid was the major phenolic acid found in the peel and rind of the mangosteen fruit and this acid comprised 75.8% and 64.3% of the total phenolic acids present in these fruit parts, respectively. On the other hand, *p*-hydroxybenzoic and protocatechuic acids, the major phenolic acids found in the aril, constituted 53.7% and 35.4% of the total phenolic acids present in the aril, respectively. In addition, the peel and aril contained 263.1 ± 16.7 and 28.0 ± 3.0 mg per kg, d.m. of other acids (such as benzoic, cinnamic, mandelic and piperonylic), respectively (Table 2).

3.3. Free phenolic acids

Free phenolic acids comprised from 5.5% (rind) to 18.7% (peel) of the total phenolic acids present in mangosteen fruits (Table 1). Hydroxybenzoic acid derivatives were the major phenolic acids found in the peel and rind, while hydroxycinnamic acid derivatives dominated in the aril. Only six phenolic acids were identified in this fraction (Table 3). Of these, protocatechuic and vanillic were the major phenolic acids in the peel and rind, while *p*-coumaric acid was dominant in the aril. Furthermore, caffeic acid was detected only in the peel, while *p*-hydroxybenzoic acid was found in both the peel and aril. The levels of individual phenolic acids in the aril, however, did not exceed their taste thresholds reported in the literature (Maga & Lorenz, 1973). Thus, the fraction of free phenolic acids may not make a significant contribution to the aril flavour. Bunsiri, Ketsa, & Paull 2003) tentatively identified two free phenolic acids, namely sinapic and *p*-coumaric acids, in the pericarp of mangosteen fruit. These phenolics are involved with lignin synthesis in mangosteen pericarp leading to its hardening. Rapid decrease in the content of these phenolic acids was noticed in fruits subjected to mechanical impact. The rate of the disappearance these phenolic acids was, however, enhanced by fruit maturity and the presence of oxygen. In this study, we did not detect sinapic and *p*-coumaric acids in the free phenolic acids fraction isolated from either the peel or the rind.

3.4. Bound phenolic acids

Bound phenolic acids were the predominant phenolic acids in mangosteen fruits. Phenolic acids liberated from soluble esters comprised from 41.4% (peel) to 76.5% (aril) of the total phenolic acids present in the fruits (Table 1). Hydroxybenzoic acid derivatives comprised from 91.5% (rind) to 100% (aril) of phenolic acids identified in this fraction (Table 4). Of these, protocatechuic acid was the major phenolic acid in the peel and rind, while protocatechuic and *p*-coumaric dominated in the aril. *m*-Hydroxybenzoic, vanillic and veratric acids were unique, but minor phenolic acids in the peel. Furthermore, caffeic, ferulic and *p*-hydroxyphenylacetic acid were only detected in the peel, while *p*-coumaric and 3, 4-dihydroxymandelic acids were unique to the rind.

Phenolic acids linked to sugars by glycosidic bonds comprised from 13.8% (aril) to 44.8% (rind) of the total phenolic acids present in these fruits (Table 1). Hydroxybenzoic acid derivatives were the only class of phenolic acids found in this fraction. Table 5 shows the phenolic acid profiles for this fraction. Sugar moieties of glycosides were not identified in this study. Protocatechuic acid was the principal phenolic acid present in the peel, while *p*-hydroxybenzoic and protocatechuic acids were the major phenolic acids in the rind and aril. Moreover, *m*-hydroxybenzoic and veratric acids were only found in the peel, while *p*-hydroxyphenylacetic acid was only detected in the rind.

In conclusion, the results of this study indicate that phenolic acids are mainly located in the pericarp of mangosteen and that hydroxybenzoic acid derivatives are the major phenolic acids found in these fruits. The total content of phenolic acids in the mangosteen pericarp is similar to that reported for small berries. On the other hand, the edible aril contains up to 20 times less phenolic acids than small berries (Zadernowski, Naczk, & Nesterowicz, 2005). Free phenolic acids comprised only up to 20% of total phenolic acids. More research is still needed in order to establish the effect of the cultivar, preharvest environmental conditions, degree of maturity at harvest, post-harvest storage conditions and processing on phenolic acid profiles of mangosteen fruits.

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