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Antioxidant activity and phenolic acid composition in different parts of selected cultivars of mangoes in Thailand

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Abstract

Total polyphenol contents, phenolic acid composition and antioxidant properties in peel, flesh and seed kernel of green mature (GM) and fully ripe (FR) mangoes of six cultivars (Khiew Sawoey, Nam Dokmai, Rad, Chok Anan, Fah Lan and Kaew Dum) were investigated. Results showed that all mango seed kernels contained highest content of total polyphenols, followed by those observed in peel and flesh. For GM and FR mango flesh, the highest amount of total polyphenols was found in Kaew Dum whereas Fah Lan showed the lowest amount. Mango seed kernel of all cultivars in FR stages exhibited the highest DPPH scavenging activity, ferric reducing antioxidant potential (FRAP) and H_2O_2 scavenging activity, followed by those observed in peel and flesh. Peel and flesh of all FR mango samples tended to possess higher antioxidant capacities compared to those of GM mangoes, while GM mango seed kernels showed higher antioxidant potentials over the ripe ones. For all mango samples, the correlation among DPPH, FRAP and H_2O_2 as well as their correlation with the total polyphenol content were highly correlated with positive correlation coefficients. Phenolic acid, as determined by high performance liquid chromatography, which found in all parts of mangoes was caffeic acid. Different types and contents of phenolic acids were found in all parts of each mango cultivar. The results indicated that mangoes, especially in peel and seed kernels, can be good sources of phenolic compounds with strong antioxidant.

Introduction

Mango (*Mangifera indica* Linn.) is one of the most important commercial tropical fruits worldwide in terms of production, marketing and consumption. There are over one thousand different cultivars of mangos growing around the world. As many as 172 cultivars have been recorded in Thailand and about ten have been grown commercially (Phakawatmongkol *et al.*, 2004). The leading mango cultivars in Thailand are 'Nam Dok Mai', 'Khiew Sawoey', 'Fah Lan', 'Chok Anan', 'Kaew', 'Okrong', 'Rad', 'Tongdum' and 'Nungklangwun'. Mango is known to be an excellent source of dietary fiber, vitamin C, ß-carotene and phenolic compounds (Ribeiro *et al*., 2008; Barreto *et al*., 2008), and many studies have been conducted on the potential nutritional and health-effects of mangoes (Hernández *et al.,* 2006). Several studies reported on phenolic compounds found in mango including quercetin, iso-querectin, kaempferol, mangiferin, gallic acid, m-digallic acid, m-trigallic acid, ellagic acid, and hydrolyzable tannin such as gallotannin. Gallic acid and hydrolyzable tannins are especially known as major phenolic compounds found in mango (Soong and Barlow, 2004, 2006; Masibo and He, 2008). It has been reported that mangoes are sources of valuable phytochemicals with antioxidant properties (Barreto *et al*., 2008; Masibo and He, 2008; Kim *et al.*, 2010). Mango pulp has been reported to have antilithiatic and free radical scavenging properties, which reduce lipid peroxidation and enhance antioxidant enzymes against isoproterenol (Bafna and Balaraman, 2005). Mango peel extracts have been reported to show strong antioxidant activity (Ajila *et al.*, 2010). There are also reports about the antioxidant activity of mango flesh and seed (Ribeiro *et al*., 2008; Masibo and He, 2008). Recently, an antioxidant study on mango flesh has been reported by Poovarodom *et al.* (2010) and Patthamakanokporn *et al.* (2008), antiproliferative activities in mango flesh and peel by Kim *et al.* (2010), tyrosinase inhibitory activity of mango seed kernel by Maisuthisakul and Gordon (2009) and anti-hemorrhagic and anti-dermonecrotic activities of mango seed kernel extract against snake venoms by Leanpolchareanchai *et al.* (2009) and Pithayanukul *et al.* (2009). Apart from the antioxidant activity, mango seed extract has also exhibited potent antibacterial activity (Abdalla *et al.,* 2007b; Khammuang and Sarnthima, 2011) and has

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been used as an immunomodulating agent in animals (Sahu *et al.*, 2007). Kittiphoom (2012) has reviewed the utilization of mango seed. He mentioned that mango seed kernel contained of several mineral elements, amino acids and others which can be used as functional food ingredients and having high potential applications. Hence, Thai varieties of mango should be investigated for feasibility of use. In this study, different fruit parts, namely peel, flesh, and seed kernel of green mature and fully ripe Thai mango of six commercial cultivars were chosen in an attempt to make systematic comparisons among their total polyphenol contents, antioxidant capacities and phenolic acids composition.

Materials and Methods

Chemicals

All chemicals and solvents used were of analytical grade. 1,1-Diphenyl-picrylhydrazyl (DPPH•), butylated hydroxyanisole (BHA), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®) and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Folin-Ciocalteu reagent was obtained from Merck (Darmstadt, Germany). Solvents used for HPLC analysis were of HPLC grade. Ultrapure water was produced using a Milli-Q system (Millipore, USA). Standards used for identification and quantification purposes by HPLC were as follows: Gallic acid (3,4,5-trihydroxybenzoic acid), Caffeic acid (3,4-dihydroxycinnamic acid), p-Coumaric acid (3-(4-hydroxyphenyl)-2-propenoic acid), Sinapic acid (4-Hydroxy-3,5-dimethoxycinnamic acid) and Ferulic acid (trans-4-Hydroxy-3-methoxycinnamic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Mango cultivars

The 6 cultivars of mango (*Mangifera indica* L.) studied were obtained from local fruit retail market in Pathumthani, Thailand during March to June. The mango samples were randomly selected off the shelves based on size, color and freshness. Then, mango samples were classified using criteria based on the ripening of mangoes following the method described by Department of Industrial Promotion, Thailand (2001). In this study, the ripening at stage 1 were the fully green mature stage (GM) and the ripening at stage 6 were obtained by wrapping individual GM mangoes with 2 layers of newspaper and placing in a paper box. The GM mangoes were allowed to ripen at at room temperature (30±1 °C) for 3 days or until 100% yellow color (fully ripe stage; FR). The cultivars studied were Khiew Sawoey (KS), Nam Dokmai (ND), Rad (Rd), Chok Anan (CA), Fah Lan (FL) and Kaew Dum (KD). The mango fruits were cleaned and separated into peel, flesh and seed kernel. The small cut pieces of the samples were kept in Polyethylene Terephthalate/Aluminium/Polyethylene (PET/AP; 15x20 cm, thickness: 57 micron) and stored in a freezer at -70°C until further analysis.

Determination of moisture content, color, ph, acidity and reducing sugar

The parts of mangoes samples from 6 cultivars were determined for the physicochemical properties including; moisture content was measured following the method of AOAC (2000), color (CIE L^*, a^*, b^*) was analyzed using colorimeter (Minolta CR-400, Japan), pH was measure with pH meter following method of Chen *et al.* (2007), total acidity was measured following the method of AOAC (2000), total soluble solid was measured using Milwaukee digital refractometer (MA871 Refractometer, 0-85% Brix, Romania) and reducing sugar was measured by the method of Neilson (1998).

Preparation of ethanolic extract from mango peel, flesh and seed kernel

The extracts of peel, flesh and seed kernel of GM and FR mangoes were prepared by using ethanol. Pieces of peel and kernel (0.5–1 g) and flesh (20 g) were homogenized (Moulinex, Mexico) with 100 ml of 95% ethanol at high speed for 30 sec, and the homogenates were placed in water bath at 80±1°C for 1 hr with an occasional shaking to increase the extraction capacity. The mixtures were cooled at room temperature and filter through Whatman filter paper No. 4, and the supernatant were adjusted to 100 ml with 95% ethanol. An aliquot of these extracts were used for the quantification of total phenolic contents and antioxidant activities.

Determination of total polyphenol contents

Total polyphenol contents in the extracts were determined according to the Folin-Ciocalteu colorimetric method based on the procedure described by Singleton and Lamuela-Raventos (1999) with some modifications. The extract solution (500 µl) was transferred to a test tube, and then distilled water was added to adjust the total volume to 10 ml. Folin-Ciocalteu reagent (500 µl) was added to the reaction. After ten minutes, 2 ml of 7% (w/v) sodium carbonate solution was added. The mixture was allowed to stand for 30 min at room temperature in the dark. The sample was shaken well before being measured at

730 nm using UV-Vis spectrophotometer (Shimadzu UV-1601, Japan). The experiment was carried out in triplicate and the total polyphenol content was expressed as milligrams of Gallic acid equivalents (GAE) per gram of fresh weight basis.

DPPH assay

The free radical scavenging of samples was evaluated using the DPPH radical discoloration method of Murakami *et al.* (2008) with some modifications. An appropriate amount of the extract sample (70 µl) was mixed with 95% ethanol to give a total volume 5.4 ml. Then, 600 µl of 0.8 mM DPPH in ethanol solution was added into each sample solution. The mixture was shaken vigorously and left to stand at room temperature (~30°C) for 30 min in the dark. Control was prepared without the sample solution. The absorbance at 517 nm was determined with a Shimadzu UV-1601 spectrophotometer (Japan). The calibration curve was performed with Trolox® (5-35 µg/ml), and the unit of DPPH radical scavenging activity is defined as the concentration of Trolox® having equivalent antioxidant activity expressed as milligram per gram sample on the fresh weight basis.

Ferric reducing antioxidant power (frap) assay

The FRAP assay was performed according to the method of Benzie and Strain (1999) with some modifications. The FRAP reagent was prepared as a mixture of 2.5 ml of 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM hydrochloric acid and 2.5 ml of 20 mM Iron (III) Chloride Hexahydrate ($FeCl_3 \bullet 6H_2O$) in 25 ml of 3 mM acetate buffer pH 3.6. One hundred microliters of a sample was mixed with 3 ml of the FRAP reagent. The reaction mixture was shaken and incubated at room temperature in the dark for 8 min. The absorbance at 593 nm was determined with a Shimadzu UV-1601 spectrophotometer (Japan). The calibration curve was performed with Trolox® (5-30 µg/ml), and the unit of total antioxidant activity is defined as the concentration of Trolox® having equivalent antioxidant activity expressed as milligram per gram sample on the fresh weight basis.

Scavenging of hydrogen peroxide

Hydrogen peroxide scavenging activity was measured by the method of Yen and Chen (1995). A hydrogen peroxide solution (H_2O_2, 90 mM) was prepared in phosphate-buffered saline (PBS, pH 7.4). The extract (100 µl) was added to a test tube containing 600 µl hydrogen peroxide solutions. The reaction mixture was shaken and incubated at room temperature in the dark. After 10 min, absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing the extract in PBS without hydrogen peroxide and then was plotted as a function of Trolox® concentration (50-400 µg/ml). The unit of scavenging of hydrogen peroxide is defined as the concentration of Trolox® having equivalent antioxidant activity expressed as milligram per gram sample on the fresh weight basis.

Analysis of phenolic acid composition by RP-HPLC

The extraction and analysis of phenolic acids in mango samples were carried out as described by Singh *et al.* (2004) with slight modification. Two grams of chopped mango samples was suspended in methanol (10 ml) and ground individual samples using mortar and pestle for 5 min, then transferred to beaker and cover with watch glass and allowed to stand for 30 min at room temperature in the dark. The mixture was filter through no. 4 Whatman filter paper and the supernatant were concentrated to 1 ml using 99.99% nitrogen gas flush. Of this extract, 1.0 ml was filtered quickly through a 0.22 µm membrane filter (Milles-HV). The filtrate (1.0 µl) was directly injected into the HPLC. The separation of phenolic compounds was performed using an Agilent HPLC series 1100 system (Agilent, USA) equipped with a 250 mm x 4.60 mm i.d., 4 µm, C18, Synergi 4µ Hydro-Rp 80A column (Phenomenex, USA). The phenolic compounds were detected in the eluant with an UV diode-array detector set at 300 nm. The mobile phase consisted of 2% (v/v) citric acid in water (eluent A) and of 0.5% acetic acid in water and acetonitrile (50:50 v/v; eluent B). The gradient programme was as follows: 0-25% B (15 min), 25-30% B (35 min), 30-80% B (10 min), 80-100% B (5 min), 100-0% B (0.5 min). The flow rate was 0.5 ml/min.

Statistical analysis

Results are presented as mean values±standard deviation (three replicate experiments). Analysis of variance and significant differences among means were determined by one-way ANOVA using computer software. Significant differences were declared at $p \leq 0.05$.

Results and Discussion

Determination of the physicochemical properties

The flesh of all mango cultivars harvested at the GM stage had a lower pH, total soluble solid (TSS) and reducing sugar (RS) compared to those harvested at the FR stage (Table 1). The pH values increased slightly but the TSS increase was much higher from GM to FR in all the cultivars. Also the flesh of mangoes harvested at GM stage had a higher total

Table 1. Physicochemical property of different Thai mango cultivars

	Parts	Mango cultivars					
	(Stages)	KS	ND	Rd	CA	FL	KD
pH	Flesh (GM)	4.12±0.02^{b}	3.33±0.00^{d}	3.41±0.00^{c}	2.97±0.00^{e}	4.30±0.00^{a}	2.68±0.00^{f}
	Flesh (FR)	5.05±0.00^{b}	5.24±0.01^{a}	4.89±0.00^{c}	4.48±0.00^{e}	5.20±0.10^{a}	4.57±0.00^{d}
TA	Flesh (GM)	2.13±0.00^{f}	6.40±0.21^{b}	4.69±0.00^{d}	5.55±0.00^{c}	3.04±0.05^{e}	8.68±0.25^{a}
	Flesh (FR)	0.85±0.00^{a}	0.64±0.00^{c}	0.85±0.00^{a}	0.78±0.02^{b}	0.77±0.00^{b}	0.78±0.02^{b}
TSS	Flesh (GM)	13.60±0.00^{a}	8.00±0.00^{c}	7.40±0.00^{d}	7.80±0.00^{c}	9.50±0.40^{b}	7.00±0.00^{e}
	Flesh (FR)	21.50±0.00^{a}	20.20±0.00^{b}	14.00±0.00^{e}	12.00±0.00^{f}	19.60±0.60^{c}	18.00±0.00^{d}
RS	Flesh (GM)	12.69±0.43^{c}	14.13±0.09^{b}	11.78±0.40^{d}	15.86±0.47^{a}	10.91±0.03^{e}	10.76±0.12^{e}
	Flesh (FR)	14.82±0.22^{c}	17.62±0.40^{b}	12.44±0.37^{d}	19.05±0.28^{a}	17.41±0.33^{b}	14.78±0.87^{c}
MC	Peel (GM)	67.62±0.02^{e}	70.93±0.39^{cd}	79.33±0.74^{a}	76.66±0.22^{b}	70.75±0.38^{d}	71.57±0.17^{c}
	Peel (FR)	69.80±0.45^{bc}	68.62±2.23^{c}	78.16±0.37^{a}	71.24±0.68^{b}	68.36±1.69^{c}	71.83±0.60^{b}
	Flesh (GM)	75.99±0.34^{d}	80.69±0.46^{bc}	84.82±0.34^{ab}	86.62±0.41^{a}	77.10±6.01^{cd}	84.62±0.43^{ab}
	Flesh (FR)	75.98±0.44^{cd}	78.46±0.34^{bc}	84.53±0.10^{a}	83.67±0.64^{a}	75.23±3.55^{d}	80.31±0.44^{b}
	Seed (GM)	56.24±0.48^{e}	60.84±0.49^{d}	68.42±0.03^{b}	71.81±0.96^{a}	65.32±3.82^{c}	49.65±0.33^{f}
	Seed (FR)	59.36±0.64^{b}	49.21±4.09^{c}	54.93±0.32^{bc}	55.83±0.44^{b}	76.03±7.18^{a}	39.25±0.33^{d}

KS = Khiew Sawoey, ND = Nam Dokmai, Rd = Rad, CA = Chok Anan, FL = Fah Lan and KD = Kaew Dum.

TA = total acidity (% as citric acid), TSS = total soluble solid (^{o}Brix), RS = reducing sugar (mg glucose/gram sample), MC = moisture content (% wet basis). GM = green mature stage 1 and FR = fully ripe stage 6, Values are means±SD, n = 3. Numbers on the same row with different superscripts are significant different at $p \leq 0.05$.

acidity (TA) than FR samples in all cultivars tested. Moisture content of the different parts of the mango fruit varied significantly between the cultivar and harvest fruit maturity stage. Seed kernel moisture content within each cultivar significantly decreased with the increasing maturity, excepted KS and FL where they increased during ripening. The moisture content was 67-79%, 75-86% and 49-76% for peel, flesh and seed kernel, respectively. However, the moisture content of the flesh for all the cultivars tested was within the range of the value previously reported (72-86%) (Po, 2007).

Table 2 showed the color changes *(L^*, a^*, b^*)* of mango peel and mango flesh of different cultivars between GM and FR fruits. After GM stage changes to FR stage, the L^* value of mango peel increased drastically in all cultivars, while a gradual reduction in the L^* value was observed in all cultivars for the mango flesh. On the other hand, the a^* and b^* values increased with the increasing degree of ripeness. These changes in a^* and b^* values agreed with the visual color in that at GM stage the mango was green and at FR stage it was orange or yellow color. This indicated that GM mango samples contain higher amounts of green color pigment such as chlorophyll. It is also well known that an increase in carotenoids content is responsible for the yellow color development during the ripening of mangoes (mature state and accelerate treatment).

Determination of total polyphenol content

Phenolic compounds are widely distributed in plants and have gained much attention, due to their antioxidant activities and free radical scavenging capacities, which potentially have beneficial implications for health (Petti and Scully, 2009). In this study (Table 3), a great variation in total phenolic contents was observed for GM and FR mango extract which ranging from 9.56±0.05 to 21.35±1.14, 0.29±0.01 to 0.83±0.04 and 27.82±0.51 to 66.95±2.33 mg gallic acid/g fresh wt in peel, flesh and seed kernel, respectively. It was also found that, seed kernel extract of all cultivars contained higher total polyphenol content than the flesh and peel, which indicated that seed kernel would be important reservoir of phenolic compounds. Yield of total polyphenolic content in flesh from different mango cultivar varied, with FR of CA presenting the highest yield. The total polyphenolic content of GM mango flesh are as follow: KD>ND>CA>KS>Rd>FL, and FR mango flesh are as follow: KD>CA>ND>Rd>KS>FL. The average of all six varieties for GM and FR equaled 0.59 and 0.57 mg gallic acid/g fresh wt, with a range of 0.34 to 0.79 and 0.29 to 0.83 mg gallic acid/g fresh weight for GM and FR mango flesh, respectively. These results indicated that both GM and FR of KD flesh exhibited the highest phenolic content. In addition, total polyphenol content found in ripe mango peels was higher than the green peels. On the other hand, flesh and seed kernel of ripe mangoes contained the lower content of total polyphenols than those of green mangoes. The total polyphenolic contents of Thai mango are similar to values previously reported for Maxico mangoes (var. Atailfo) which contain the total polyphenolic content 0.56 mg gallic acid/g fresh wt (Noratto *et al.,* 2010). Previous studies showed total polyphenolic content in mango flesh range from 0.48 to 2.09 mg gallic acid/g fresh wt (Ribeiro *et al*., 2007) and the variation of the total phenolic content in mango may also be due to the growing condition like temperature, light, plant species and even cultivars which have been reported by Klepacka *et al.* (2011)

Table 2. Color parameters of peel and flesh of green mature and fully ripe Thai mangoes

Mango cultivars	Parts (Stages)	L*-value	a*-value	b*-value
KS	Peel (GM)	46.18±1.73	-13.47±0.52	16.95±1.61
	Peel (FR)	62.79±1.74	-5.95±2.19	47.51±3.56
ND	Peel (GM)	55.71±2.56	-17.5±1.13	29.61±3.12
	Peel (FR)	67.24±2.49	4.01±1.65	37.91±3.00
Rd	Peel (GM)	59.17±1.04	-18.03±0.63	31.57±1.56
	Peel (FR)	71.02±1.68	-1.38±2.63	43.91±3.42
CA	Peel (GM)	54.46±3.65	-17.35±0.91	27.61±1.99
	Peel (FR)	71.60±1.48	-3.11±1.30	46.80±3.66
FL	Peel (GM)	46.59±1.13	-16.56±0.24	22.90±0.69
	Peel (FR)	60.67±2.53	-8.40±3.16	37.51±2.05
KD	Peel (GM)	49.56±2.15	-18.36±0.81	26.99±2.89
	Peel (FR)	71.28±1.89	5.48±2.29	54.31±3.91
KS	Flesh (GM)	83.54±0.68	-5.23±0.53	32.70±3.06
	Flesh (FR)	73.93±2.74	-1.46±0.89	59.57±1.36
ND	Flesh (GM)	84.93±1.68	-5.36±1.18	13.51±2.07
	Flesh (FR)	69.40±1.71	9.49±1.06	53.32±1.58
Rd	Flesh (GM)	86.57±3.59	-5.71±1.28	18.31±1.65
	Flesh (FR)	79.56±1.26	-3.04±0.37	40.55±1.76
CA	Flesh (GM)	84.89±0.95	-6.69±0.99	22.55±0.95
	Flesh (FR)	73.15±0.83	6.05±1.86	56.52±1.35
FL	Flesh (GM)	84.44±1.15	-6.68±0.23	23.50±0.64
	Flesh (FR)	72.59±0.54	-0.75±0.03	49.44±0.53
KD	Flesh (GM)	84.78±0.78	-6.54±1.35	16.87±2.25
	Flesh (FR)	66.49±4.19	16.93±2.90	64.87±2.89

KS = Khiew Sawoey, ND = Nam Dokmai, Rd = Rad, CA = Chok Anan, FL = Fah Lan and KD = Kaew Dum.GM = green mature stage 1 and FR = fully ripe stage 6, Values are means±SD, n = 3.

Table 3. Total polyphenol content of different Thai mango cultivars

Mango cultivars	Stages	Total polyphenol (meq gallic acid/g fresh wt)		
		Peel	Flesh	Seed kernel
KS	GM	12.64±0.08^{b}	0.50±0.01^{c}	38.88±0.13^{a}
	FR	14.93±1.35^{b}	0.43±0.01^{c}	35.96±0.81^{a}
ND	GM	15.51±0.27^{b}	0.77±0.05^{c}	48.15±3.13^{a}
	FR	13.85±0.87^{b}	0.60±0.05^{c}	27.82±0.51^{a}
Rd	GM	10.22±0.51^{b}	0.45±0.04^{c}	42.44±0.37^{a}
	FR	11.70±0.66^{b}	0.44±0.00^{c}	33.14±0.47^{a}
CA	GM	12.55±0.22^{b}	0.70±0.04^{c}	66.95±2.33^{a}
	FR	16.05±2.69^{b}	0.81±0.09^{c}	58.89±0.33^{a}
FL	GM	9.86±0.16^{b}	0.34±0.01^{c}	43.46±0.48^{a}
	FR	9.59±0.05^{b}	0.29±0.01^{c}	39.32±1.69^{a}
KD	GM	19.66±0.57^{b}	0.79±0.02^{c}	41.76±3.18^{a}
	FR	21.35±1.14^{b}	0.83±0.04^{c}	27.95±2.33^{a}

KS = Khiew Sawoey, ND = Nam Dokmai, Rd = Rad, CA = Chok Anan, FL = Fah Lan
and KD = Kaew Dum.GM = green mature stage 1 and FR = fully ripe stage 6,
Values are means±SD, n = 3. Numbers on the same row with different superscripts are significant different at p≤0.05.

and Papoulias *et al.* (2009)

Antioxidant capacity

In Figure 1, the antioxidant capacity methods, i.e. DPPH, FRAP and H_2O_2 were on x-axis and the total polyphenolic content was on y-axis. For fruits harvested at the GM stage of maturity: (a) is for peel, (c) is for flesh and (e) is for seed kernel, while for fruits harvested at the FR stage of maturity: (b) is for peel, (d) is for flesh and (f) is for seed kernel. The cultivar with high polyphenol content had consistently high antioxidant activity in all parts of fruit whatever detection method used. The correlation between the different cultivars and the method of antioxidant detection in peel (GM) and peel (FR) showed good correlation in DPPH method (R^2= 0.43, 0.56) and FRAP method (R^2=0.72, 0.85). The slope of line indicated the sensitivity of polyphenol acting as an antioxidant. For DPPH method, since its slope value was less the capacity of radical scavenging was better than the FRAP or H_2O_2 methods. However, the correlation was not found in the H_2O_2 method in every part of mango.

DPPH radical scavenging activity

DPPH is a stable free radical and can accept an electron or hydrogen radical to become a stable molecule which is widely used to investigate radical scavenging activity of plant extracts (Huang *et al*., 2005). The purpose of DPPH assay was to assess the potential antioxidant activity of the extracts from peel, flesh and seed kernel of GM and FR mango fruits of different cultivars. The extracts of seed kernel from all mango cultivars and maturity stage had the DPPH radical scavenging activity ranging from 70-170 mg Trolox®/g fresh wt, excepted for cultivar Fah Lan (FL) showed lower activity of 50 and 30 mg Trolox®/g fresh wt in GM and FR, respectively. Interestingly, the radical scavenging activity of the FR mango peel of all varieties had the higher values compared to the GM mango peel. The scavenging activity was observed in mango peel and mango flesh of all samples, which was in the range of 20-45 and 0.2-2 mg Trolox®/g fresh wt, respectively. The results

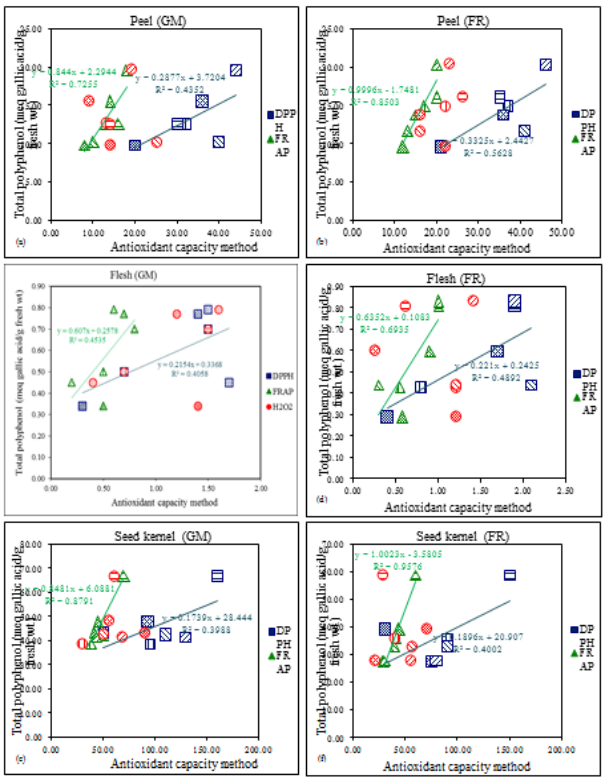

Figure 1. *In vitro* antioxidant capacity by DPPH radical scavenging assay (□), by ferric reducing antioxidant power assay (Δ) and by hydrogen peroxide assay (○); mgTrolox/gfresh sample
(▥) KS = KhiewSawoey, (▩) ND = Nam Dokmai, (▨) Rd = Rad, (▤)CA = Chok Anan, (▩) FL = FahLan and (▨) KD = Kaew Dum

also revealed that the mango seed kernel and mango peel are the major sources of antioxidant, which act as free radical scavenger and hence it is the primary antioxidant that react with free radical (Hamid *et al.*, 2010). The DPPH radical scavenging activity (average of all fruit parts) of these mango cultivars were in the order of CA>KD>Rd>KS>ND>FL and CA>Rd>KD≈KS>ND>FL for GM and FR, respectively.

Ferric reducing antioxidant power (FRAP)

To evaluate the reducing potential of mango fruit parts from GM and FR of different cultivars, the reduction of Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ in the present of the sample extracts were determined. The FRAP values of the extracts from mango fruit parts of different cultivars are shown in Figure 1. The results showed that the ferric reducing capacity were in the order of [mango seed kernel GM>FR]>[mango peel FR>GM]>[mango flesh FR>GM]. Among all mango seed kernels from different varieties, GM-Chok Anan (GM-CA) and FR-Chok Anan (FR-CA) had the highest FRAP activities of 61 and 72 mg Trolox®/g fresh wt, respectively, and other cultivars exhibited activity ranging from 28-48 mg Trolox®/g fresh wt. The mango seed kernel exhibited high FRAP activity values; these may be due to the higher total phenolic content. This finding was similar to the earlier study of Abu Baker *et al.* (2009) who reported that the total phenolic content was strongly correlated with the FRAP assay. In addition, the mango flesh extracts of GM and FR of different cultivars had the lowest FRAP values, whereas the reducing power increased from the GM stage to the FR stage. However, the variations of reducing power of mango fruits may still exist, because the antioxidant activity could be influenced by the geographical origin, cultivar and harvest or storage time (Halvorsen *et al.*, 2002).

Hydrogen peroxide (H_2O_2) scavenging activity

The hydrogen peroxide scavenging activity in this study was measured by the direct reaction of H_2O_2 with the tested samples. The activity of the GM and FR of different parts and different cultivars showed varying results in H_2O_2 scavenging capacity. Both GM and FR mango seed kernel of all cultivars had the highest activity as compared to the mango peel and mango flesh (Figure 1). Hydrogen peroxide itself is not very reactive, it can generate the highly reactive hydroxyl radical (OH•) through the Fenton reaction which may be the origin of many of its toxic effect (Haliwell, 1996). Our results showed that the H_2O_2 scavenging were in the order of mango seed kernel>mango peel>mango flesh. The H_2O_2 scavenging in this study showed similar results compared to DPPH radical scavenging and FRAP assay. The scavenging of H_2O_2 by phenolic compounds is attributable to their electron-donating ability (Wettasinghe and Shahidi, 2000). In comparison, the H_2O_2 scavenging of mango peel, mango flesh and mango seed kernel were 8-27, 0.3-1.6 and 19-90 mg Trolox®/g fresh wt, respectively.

Correlation

The correlation between total polyphenol content and antioxidant activity and among antioxidant assays is summarized as follows. Total polyphenol content showed significantly positive correlation with antioxidant activity and the correlation coefficient was 0.934*, 0.993* and 0.814* for DPPH radical scavenging, FRAP and H_2O_2 assay, respectively. It revealed that total polyphenol content had the strong correlation with the FRAP value, followed by DPPH radical scavenging and H_2O_2 assay. This indicates that the phenolic compounds could be a major component with antioxidant power of mango parts which is in accordance with the previous finding that many phenolic compounds in vegetables and fruits are good sources of natural antioxidant (Vinson *et al.*,

Table 4. Phenolic acids composition in peel, flesh and seed kernel of selected Thai mango cultivars

Variety	Parts (Stages)	Phenolic acid contents (mg/100g fresh wt)					
		GaA	CaA	*p*CA	CiA	FeA	Total
KS	Peel (GM)	nd	9.51±0.22	nd	nd	nd	9.51
	Peel (FR)	nd	18.41±1.49	nd	nd	nd	18.41
	Flesh (GM)	5.35±0.13	0.26±0.07	nd	nd	nd	5.61
	Flesh (FR)	20.89±0.12	16.17±1.09	nd	nd	nd	37.06
	Seed kernel (GM)	nd	105.22±3.29	334.94±0.36	826.36±23.38	283.98±0.23	1550.50
	Seed kernel (FR)	nd	101.30±10.15	241.76±16.98	454.65±15.27	211.36±0.36	1009.07
ND	Peel (GM)	nd	6.36±1.00	1.36±0.02	nd	nd	7.72
	Peel (FR)	nd	8.89±0.28	1.03±0.03	nd	nd	9.92
	Flesh (GM)	8.33±0.00	nd	nd	nd	nd	8.33
	Flesh (FR)	27.03±0.03	nd	nd	nd	nd	27.03
	Seed kernel (GM)	nd	122.15±10.28	nd	588.80±45.62	191.16±26.35	902.11
	Seed kernel (FR)	nd	326.90±12.73	nd	1137.09±79.80	299.66±5.03	1763.65
Rd	Peel (GM)	nd	7.65±0.44	nd	nd	nd	7.65
	Peel (FR)	nd	14.29±0.84	nd	nd	nd	14.29
	Flesh (GM)	5.77±0.32	14.29±0.47	nd	nd	nd	20.06
	Flesh (FR)	7.53±0.51	5.65±0.39	nd	nd	nd	13.18
	Seed kernel (GM)	nd	98.12±2.52	nd	786.58±22.02	254.69±14.17	1139.39
	Seed kernel (FR)	nd	90.77±1.86	nd	658.79±43.63	207.00±2.84	856.56
CA	Peel (GM)	nd	16.23±3.08	23.45±0.93	nd	nd	39.68
	Peel (FR)	nd	36.60±0.80	42.06±0.32	nd	nd	78.66
	Flesh (GM)	11.76±0.91	13.55±1.39	8.09±0.10	1.88±0.15	nd	35.28
	Flesh (FR)	9.72±0.57	17.20±3.09	10.36±0.23	2.85±1.51	nd	40.13
	Seed kernel (GM)	nd	7.04±2.14	444.64±34.83	997.75±98.52	344.18±7.87	1793.61
	Seed kernel (FR)	nd	8.73±0.50	591.49±10.55	858.45±55.85	185.21±13.37	1643.88
FL	Peel (GM)	nd	2.84±0.13	nd	12.06±0.44	4.93±0.26	19.83
	Peel (FR)	nd	9.96±0.83	nd	27.22±1.34	12.27±1.85	49.45
	Flesh (GM)	2.29±0.00	13.74±1.35	nd	nd	nd	16.03
	Flesh (FR)	23.33±4.28	2.54±1.17	nd	nd	nd	25.87
	Seed kernel (GM)	nd	174.37±14.75	nd	1028.96±31.43	407.02±0.00	1610.35
	Seed kernel (FR)	nd	89.43±9.89	nd	845.61±35.00	267.22±10.97	1202.26
KD	Peel (GM)	nd	30.09±0.02	nd	158.72±6.74	73.30±3.68	262.11
	Peel (FR)	nd	38.20±0.00	nd	97.87±0.00	55.34±0.00	191.41
	Flesh (GM)	10.47±0.28	1.72±0.05	nd	nd	nd	12.19
	Flesh (FR)	7.17±0.00	0.03±0.00	nd	nd	nd	7.20
	Seed kernel (GM)	nd	149.77±2.09	nd	1375.58±33.39	293.56±8.27	1818.91
	Seed kernel (FR)	nd	187.44±15.07	nd	490.85±32.39	168.63±2.53	846.92

KS = Khiew Sawoey, ND = Nam Dokmai, Rd = Rad, CA = Chok Anan, FL = Fah Lan and KD = Kaew Dum.
GM = green mature stage 1 and FR = fully ripe stage 6, Values are means±SD, n = 3.
GaA = gallic acid, CaA = caffeic acid, pCA = p-coumaric acid, CiA = cinnapic acid and FeA = ferulic acid.
nd = not detected

2001). Moreover, the correlation among antioxidant assays was positive correlation between DPPH radical scavenging, FRAP and H_2O_2. However, the correlation of DPPH and FRAP was strongly positive correlated (0.935*). High correlation between DPPH radical scavenging and FRAP has been reported in fruit juices (Gardner *et al.,* 2000). Thus, DPPH radical scavenging and FRAP assay could be a better method to study the antioxidant properties of fruit samples containing mainly phenolic compounds than H_2O_2 assay.

Identification of phenolic acid by HPLC

The extracts from peel, flesh and seed kernel of different GM and FR mangoes were identified and quantified for their phenolic acids. The RP-HPLC-DAD chromatogram of the standard phenolic acids and the phenolic acid composition of each part of mango fruit showed (data not shown) that the elution order and retention time (R_t) in minutes were; gallic acid, 2.941; caffeic acid, 5.297; p-coumaric acid, 8.777; cinapic acid, 9.469 and ferulic acid, 10.179. The distribution of phenolic acids in peel, flesh and seed kernel of mangoes is presented in Table 4. Gallic acid was found in all mango flesh extract, with concentration 2.29±0.00 to 11.76±0.91 and 7.17±0.00 to 27.03±0.03 mg/100g fresh wt in GM and FR samples, respectively. These results are in agreement with those reported by Kim *et al*., (2007) who reported that the gallic acid has been identified as the major polyphenol present in mangoes fruit (var. Tommy Atkins). However, our results showed the higher concentration of gallic acid compared to the commercial mango puree concentrate from Germany (6.9 mg/kg) (Schieber *et al*., 2000). Caffeic acid was the major phenolic acid in the peel, flesh and seed kernel of all cultivars, while it was not detected in the flesh of both GM and FR Nam Dokmai (ND). In addition, cinapic acid and ferulic acid were the predominant phenolic acid in the seed kernel extract of all mango samples. Similarly, seed kernel from Egyptian mango were reported to contain higher amounts of coumaric acid (12.6%), ferulic acid (10.4%) and caffeic acid (7.7%) (Abdalla *et al.,* 2007a). The total phenolic acid content of all GM and FR mangoes were found to be 902.11 to 1818.91 and 846.92 to 1763.65 mg/100g fresh wt, respectively. Besides the peel and flesh, mango seed kernel is rich

in phenolic acid. The changes of phenolic acid in mango fruits might be due to the fruit development, and the total phenolics have been found to be higher in order seed kernel>peel (ripe>raw)>flesh at all stage (Masibo and He, 2008). Thus, the by-products of the mango fruits have attracted considerable interest as a source of phenolic compound, with much attention focused on the mango seed kernel.

Conclusion

Results clearly demonstrated that different maturity stage of mango and different fruit parts separated from mangoes have a significant impact on the physicochemical, antioxidant activity and phenolic acid composition. In addition, the cultivars also show some influence in terms of antioxidant activity and phenolic acid content. The strongest antioxidant activity was found in the seed kernel of green mature mango fruits. The degree in antioxidant activity found in mango fruits during the ripening stage would be due to the considerably change of total polyphenol content. These results highlight that seed kernel from mango cultivar Chok Anan is a rich source of total phenolic content with good radical scavenging and ferric reducing properties. Our findings provide a valuable basis for future developing of by-products from mango as valuable food additive to enhance human nutrition via their phytochemicals and antioxidant activity.

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