International Journal of Food Sciences and Nutrition,
August 2008; 59(5): 415–427

informa
healthcare

Screening of the antioxidant and nutritional properties, phenolic contents and proteins of five durian cultivars

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Abstract

The antioxidant activities of different durian cultivars at the same stage of ripening (Mon Thong, Chani, Kan Yao, Pung Manee and Kradum) were compared in order to choose the best as a supplement in the human diet. Total polyphenols (mg gallic acid equivalent/100 g fresh weight (FW)) and flavonoids (mg catechin equivalent (CE)/100 g FW) in Mon Thong (361.4 ± 23.2 and 93.9 ± 7.4) were significantly higher ($P < 0.05$) than in Kradum (271.5 ± 11.2 and 69.2 ± 5.3) and Kan Yao (283.2 ± 16.5 and 72.1 ± 6.8). The free polyphenols and flavonoids showed lower results than the hydrolyzed ones. Anthocyanins (μg cyanidin-3-glucoside equivalent/100 g FW) and flavanols (μg CE/100 g FW) were significantly higher in Mon Thong (427.3 ± 23.8 and 171.4 ± 16.3) than in Kradum (320.2 ± 12.1 and 128.6 ± 9.7) and Kan Yao (335.3 ± 14.1 and 134.4 ± 11.7). Ultraviolet spectroscopy and high-performance liquid chromatography/diode array detection analyses showed that caffeic acid and quercetin were the dominant bioactive substances in Mon Thong cultivar.

The antioxidant activity (μM trolox equivalent/100 g FW) of Mon Thong cultivar (260.8 ± 20.2, 1,075.6 ± 81.4 and 2,352.7 ± 124.2) determined by ferric-reducing/antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) with Trolox equivalent antioxidant capacity (TEAC) assays was significantly higher ($P < 0.05$) than in Kradum (197.4 ± 8.9, 806.5 ± 31.2 and 1,773.2 ± 102.5) and in Kan Yao (204.7 ± 9.7, 845.5 ± 48.6 and 1,843.6 ± 107.5). The correlation coefficients between polyphenols, flavonoids, flavanols and FRAP, CUPRAC and TEAC capacities were between 0.89 and 0.98. In extracted and separated by electrophoresis durian proteins, some differences were found in the sodium dodecyl sulfate–protein bands in the region of 16 and 68 kDa for Kradum, 45 kDa for Mon Thong and three bands for Kan Yao. Antioxidants and proteins can be used for characterization of the quality of durian cultivars.

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ISSN 0963-7486 print/ISSN 1465-3478 online
DOI: 10.1080/09637480701603082

In conclusion, the bioactivity of durian cultivars Mon Thong, Chani and Pung Manee was high and the total polyphenols were the main contributors to the overall antioxidant capacity. The results of our investigation *in vitro* are comparable with other fruits that widely used in human diets. Therefore, Durian can be used as a supplement for nutritional and healthy purposes, especially Durian Mon Thong, Chani and Pung Manee.

Keywords: *Cultivars, durian, bioactive compounds, antioxidant activity, proteins*

Introduction

Total phenolics, flavonoids and flavanols of natural products and related to these compounds' antioxidant activity have a health protective effect (Andreasen et al. 2000; Shui & Leong 2005; Naczk et al. 2006; Leontowicz et al. 2006; Lim et al. 2007; Yang et al. 2007).

Durian cultivars are consumed all over the world, and the differences between them have not been studied until now**.** Some investigators reported about such cultivars as *Durio zibethinus Murr.* cv Chanee, but the research was focused on the correlation between the softening of the fruit and polygalacturonase activity. Imsabai et al. (2002) compared the softening between two cultivars of durian. The aril of Mon Thong durian was firmer and contained less water-soluble pectin and pectinesterase activity than that of Chanee, while their polygalacturonase activities were comparable during ripening (Ketsa and Daengkanit 1999). Very recent reports were focused on physicochemical, microbial and sensory changes of minimally processed durian (Voon et al. 2006). The physicochemical properties (pH, soluble solids, titratable acidity, sugars and organic acids), flavor and sensory properties of five Malaysian durian cultivars (D2, D24, MDUR78, D101 and Chuk) were studied (Voon et al. 2007). Another investigation showed that *D. zibethinus Murr.* cv. Mon Thong polysaccharide gel is able to entrap lipids and seems to have potential to be used as a medicinal dietary food for controlling lipid levels in patients (Tippayakul et al. 2005). All these reports introduced research on durian fruit from another point of view, related to the softening properties and carbohydrate composition. There is even one report found in the literature dealing with the antioxidant properties of durian and the characterization of its cultivars, such as, for example, about the bioactivities of different cultivars of the same fruit or different fruits, such as papaya, guava and dragon fruit (Mahattanatawee et al. 2006).

Therefore it was decided to study different cultivars of this fruit *in vitro* at the stage of their ripening. We used three other complemented assays for the determination of the total antioxidant potential: ferric-reducing/antioxidant power (FRAP), cupric-reducing antioxidant capacity (CUPRAC), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with Trolox equivalent antioxidant capacity (TEAC).

Methods

Sampling, chemicals, and polyphenol extraction

Samples. Five durian cultivars (Mon Thong, Chani, Kan Yao, Pung Manee and Kradum) at the same stage of ripening were investigated. The fruits, harvested in 2006, were purchased at a local market in Bangkok. Harvesting and determination of maturity was carried out by Thai skilled workers. They combined the following techniques: day count, character of fruit spines, tapping the fruit, color and shape of

fruit (Yaacob and Subhadrabandhu 1995). The mature durian cultivars were cut with the peduncle intact and brought down carefully. The samples were left for 1 day at room temperature and were cut open to obtain mature durian flesh with a firm texture and no smell. Some of the fruits were left for another 4 days at room temperature to ripen until their flesh became soft and normally smell. Overripe samples with a strong smell were obtained when fruits were left for another 3 days. The edible parts of the cultivars were prepared without using steel knives. The fruits were cleaned with tap water and dried. Then they were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model; Equipment Co Inc, MD, USA) for 1 min. A weighed portion (50–100 g) was lyophilized for 48 h (Virtis model 10-324; Bench Freeze Dryer, Gardiner NY, USA), and the dry weight was determined. The samples were ground to pass through a 0.5-mm sieve and stored at -20°C until analyzed.

Extraction of polyphenols. Defatted lyophilized fruit samples were extracted from a 50-mg aliquot with 5 ml of 60% methanol/water with heating at 90°C for 3 h for free polyphenols, and under the same conditions with 5 ml of 1.2 M HCl in 60% methanol/water for total polyphenols with some modifications. The samples were cooled, diluted to 10 ml with methanol and centrifuged for 5 min at 4,000 $\times g$ with a benchtop centrifuge to remove solids (Vinson et al. 2001).

Analytical methods

Polyphenol determination. The Folin–Ciocalteu method was used (Singleton et al. 1999) and the measurement was performed at 765 nm with gallic acid as the standard. The results are expressed as milligrams of gallic acid equivalents (GAE)/100 g fresh weight (FW).

Flavonoids. The absorbance of flavonoids (extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH) was measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results are expressed as milligrams of catechin equivalents (CE)/100 g FW.

Anthocyanin determination. The anthocyanins were measured by a pH differential method (Cheng and Breen 1991). Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29,600. The results are expressed as micrograms of cyanidin-3-glucoside equivalent (CGE)/100 g FW.

Flavanol determination. Flavanol content was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method. Methanolic extracts from durian samples (0.2 ml), diluted 1:100 with MeOH, were introduced into a 1.5-ml Eppendorf tube, and 1 ml DMACA solution (0.1% in 1 N HCl in MeOH) was added. The mixture was vortexed and allowed to react at room temperature for 10 min. The absorbance at 640 nm was then read against a blank prepared similarly without DMACA. Results are expressed as micrograms of CE/100 g FW (Arnous et al. 2001).

Identification of bioactive compounds. The contents of flavonoids and polyphenols were determined by high-performance liquid chromatography with diode array detection (HPLC/DAD) in the prepared extracts with the P580A LPG liquid chromatograph, equipped with the Gina 50 autosampler and the UVD340V DAD diode array detector (Gynkotek/Dionex, Germering, Germany); the column was the Tosoh Biosep cartridge filled with the TSK gel ODS-80 TM (5 μm, 250 mm, 4.6 mm i.d.; flow rate, 1 ml/min; catalogue number 08149; Tosoh Corporation, Tokyo, Japan). The chromatographic column was thermostated at 40°C. The 50-μl volumes of the extracts from the different durian samples were introduced to the HPLC/DAD system with autosampler in 50-min intervals. The standards quercetin, rutin, gallic and ferulic acids were analyzed as ethanol solutions, and their concentration was equal to 0.1 mg/ml (Andreasen et al.). The analyses were carried out with the gradient of the mobile phase composition (Table I).

Ultraviolet–visible spectophotometric analysis and fluorometry. The spectra of methanol extracts in concentrations of 1 mg/ml were measured on an Uvikon 930 (Bio-Teck-Kontron, Kontron instruments, Watford, UK) and were recorded from 180 to 300 nm (Sarni-Manchado et al. 2000). Fluorescence measurements were carried out using a model FP-770D/S Jasco Spectrofluorometer (serial N261332; Tokyo, Japan). Fluorescence emission spectra measurements for all samples at concentrations of 5 mg/ml were taken at excitation wavelengths of 274 and 295 nm, recorded over the frequency range from the excitation wavelength to a wavelength of 500 nm. The standards were used at concentrations of 1 mM (Gorinstein et al. 2001).

Antioxidant capacity. Three different assays were used:

1. The FRAP assay measures the ability of the antioxidants contained in the samples to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}) that absorbs light at 593 nm. Measurements of different durians using two variables such as different reaction times (1–4 min) and different concentrations (5–20 mg/ml) were performed (Szeto et al. 2002).
2. The CUPRAC assay is based on utilizing the copper (II)-neocuproine (Cu(II)-Nc) reagent as the chromogenic oxidizing agent. To the mixture of 1 ml Cu(II)-Nc and NH_4Ac buffer solution, antioxidant sample (or standard) solution (x ml) and H_2O [$(1.1 - x)$ ml] were added to make the final volume of 4.1 ml. The absorbance at 450 nm was recorded against a reagent blank (Apak et al. 2006).
3. The $ABTS^{\cdot+}$ radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). The absorbance was monitored exactly 1 and 6 min at 734 nm (Pellegrini et al. 2003) after the addition of 990 μl $ABTS^{\cdot+}$ solution to 10 μl

Table I. Applied composition gradient of the binary ACN+H_2O mobile phase.

Time (min)	Program	ACN (%)	H_2O (%)
0–2	Constant composition	5	95
0–22	Composition change	Rise from 5 to 25	Drop 95 to 75
22–32	Composition change	Rise from 25 to 55	Drop from 75 to 45
32–50	Constant composition	55	45

fruit extracts or Trolox standards (final concentration, 0–20 μM) in methanol or phosphate-buffered saline (pH 7.4).

Protein extraction and electrophoresis. Total proteins from defatted lyophilized durian samples of 20 mg each were extracted with 500 μl sample buffer (0.0625 M Tris–HCl, pH 6.25, containing 2% sodium dodecyl sulfate (SDS), 10% glycerol, 5% mercaptoethanol and 0.001% bromophenol blue. The extracts were allowed to stand overnight at room temperature. Samples were boiled for 5 min, and then centrifuged at 18,000 $\times g$ for 15 min at 15°C. A Hoeffer SE-600 apparatus (Hoeffer Pharmacia Biotech Inc., San Francisco, CA, USA) was used for SDS-polyacrylamide gel electrophoresis. The Laemmli (1970) method was used: the resolving gel was 12.7% total acrylamide and 1.3% cross-linker, and the stacking gel was 6% total acrylamide and 1.7% cross-linker. The gel size was $140 \times 160 \times 1.5$ mm^3. Supernatants (20 μl) were loaded on gel. The run was carried out at constant current 25 mA per gel. Gels were stained with 0.25% Coomassie Brilliant Blue G-250 in methanol/water/glacial acetic solution (5:5:1 v/v) and were destained in 1% solution of Brij 35.

Statistical analyses. The results of this investigation *in vitro* are the mean $\pm$ standard deviation of five measurements. Differences between groups were tested by two-way analysis of variance. In the assessment of the antioxidant potential, Spearman's correlation coefficient (R) was used. Linear regressions were also calculated. $P < 0.05$ was considered significant.

Results and discussion

Polyphenols

There were various contents of phenolic compounds in the extracts, depending on the extraction solvent. The methanolic total and free polyphenol extracts were very similar to catechin (standard) and show their ultraviolet (UV) spectra between 198.4 and 206.2 nm, which indicated that flavonoids predominated in the phenolic compounds.

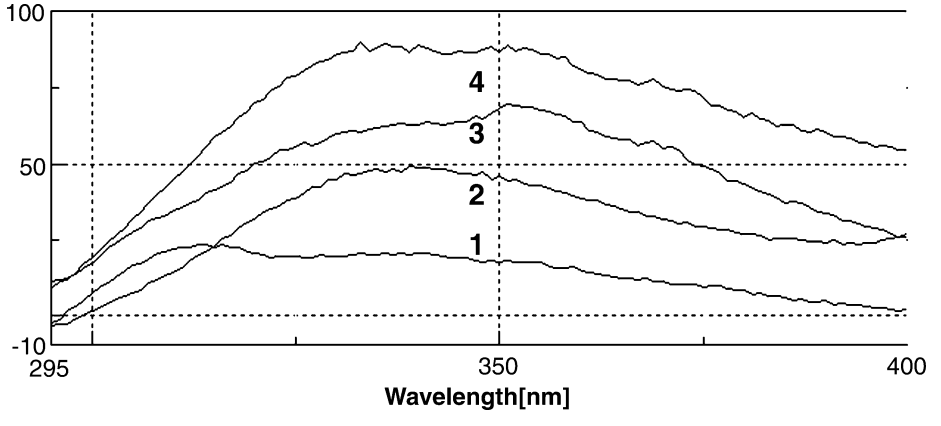

Figure 1. Absorption fluorimetric spectra at excitation wavelengths of 274 and 295 nm, recorded over the frequency range from the excitation wavelength to a wavelength of 500 nm, of different durian samples with a concentration of 5 mg/ml of free polyphenol extracts: 1, Kan Yao; 2, Chani; 3, Mon Thong; 4, Pung Manee.

The absorption units on the spectra with hydrolyzed polyphenols (methanol and acid) were higher than in the methanol one, showing higher yield during such extraction than in methanol solvent. The fluorimetric data (Figure 1) supported the UV results, therefore only these spectra are discussed as the peak maximum (nm) and the absorbancies (absorbance units (AU)) in the following order: for Chani (339 nm, 49.15 AU), for Mon Thong (351 nm, 70.03 AU), for Kan Yao (314 and 341 nm, 23.49 and 20.81 AU) and for Pung Manee (333 and 351 nm, 90.18 and 89.19 AU). The fluorimetric picture of the Kradum cultivar was without peak.

Identification of the compounds in the durian extracts was carried out through a comparison of their retention times (t_R) with those of the applied standards, and through a comparison of their UV spectra with those of the standards. In all studied cultivars the most important phenolic acids (gallic acid and ferulic acid) and flavonoids (quercetin and rutin) were determined, and registered their high antioxidant activity. Quercetin (μg/g dry weight) determined at 321 nm was in the following order: Mon Thong (68 μg/g), Chani (60 μg/g), Pung Manee (59 μg/g), and Kan Yao (5.66 μg/g). Gallic and ferulic acids at 253 nm were as follows: Mon Thong (56 and 11.2 μg/g), Chani (40 and 6.1 μg/g), Pung Manee (30 and 4.3 μg/g). Rutin at 321 nm was as follows: for Mon Thong (24.65 μg/g), Chani (13.91 μg/g), Pung Manee (19.87 μg/g) and Kradum (4.63 μg/g). The highest amount of the determined bioactive substances were found in Mon Thong and Chani, and the lowest in Kan Yao and Kradum (Table II). Mon Thong cultivar also showed the highest antioxidant activity. As it was shown by all the antioxidant methods including the FRAP measurements with variation of time (Figure 2, I and IV) in total and free polyphenol extracts and with variation of concentration (Figure 2, II and III), the absorption was higher in Mon Thong samples. As already mentioned, phenolic compounds are responsible for the antioxidant activity of fruits in generally and exotic fruits particularly (Leontowicz et al. 2006; Naczk et al. 2006). The obtained results in this investigation are in agreement with Naczk et al. (2006), who explained the distribution of phenolics between the skin, rind and aril (pulp) of mangosteen. The difference was in another kind of exotic fruit and in the employed extraction procedures for phenolics. In our case we used 50% methanol with and without acid, and these authors proposed 95% ethanol and 70% acetone. The free-radical scavenging activity of phenolic extracts was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ABTS assays, and in our case with FRAP, CUPRAC and ABTS. Such differences were minor. The most important was the conclusion that phenols displayed a strong antioxidant activity (Leontowicz et al. 2006; Naczk et al. 2006). The flavonoids and flavanols of the exotic fruits are responsible for their high antioxidant activity (Shui and Leong 2005). The HPLC analysis showed that the main flavonoids in durian cultivars are rutin and quercetin. There are no publications for comparison of their content in Durian but similar results were obtained with other exotic fruits (Shui and Leong 2005), where the antioxidants in salak (*Salacaa edulis Reinw*) were identified to be chlorogenic acid, (–)-epicatechin, and singly linked proanthocyanidins that mainly existed as dimers through hexamers of catechin or epicatechin. The phenolic content of the investigated different durian cultivars was between 361 and 272 mg GAE/100 g FW. These results corresponded with wild mulberry (373 ± 11 mg GAE/100 g FW) and were close to gala apple peel (309 ± 5 mg GAE/100 g FW) in Hassimotto et al. (2005) or with another tropical plant noni

Table II. Bioactive compounds and antioxidant activity in different cultivars of durian samples

Index	Durian cultivar				
	Mon Thong	Chani	Kan Yao	Pung Mance	Kradum
Total polyphenols (mg GAE/100 g FW)	361.4 ± 23.2^{a}	321.2 ± 22.7^{b}	283.2 ± 16.5^{c}	310.5 ± 21.4^{b}	271.5 ± 11.3^{c}
Free polyphenols (mg GAE/100 g FW)	42.1 ± 3.6^{a}	37.6 ± 3.2^{a}	31.3 ± 2.9^{b}	35.3 ± 3.4^{a}	29.9 ± 2.9^{b}
Total flavonoids (mg CE/100 g FW)	93.9 ± 7.4^{a}	81.6 ± 7.3^{b}	72.1 ± 6.8^{b}	78.8 ± 6.9^{b}	69.2 ± 5.3^{c}
Free flavonoids (mg CE/100 g FW)	22.9 ± 2.2^{a}	20.4 ± 1.8^{a}	18.1 ± 1.4^{b}	19.8 ± 1.6^{a}	17.3 ± 1.8^{b}
Anthocyanins (μg CGE /100 g FW)	427.3 ± 23.8^{a}	$379.1.1 \pm 18.9^{b}$	335.3 ± 11.1^{c}	367.3 ± 16.7^{b}	320.2 ± 12.1^{c}
Flavanols (μg CE/100 g FW)	177.4 ± 16.3^{a}	152.2 ± 14.1^{b}	134.4 ± 11.7^{c}	147.1 ± 13.1^{b}	128.6 ± 9.7^{c}
FRAP (μM TE/100 g FW)	260.8 ± 20.2^{a}	232.1 ± 16.3^{b}	204.7 ± 9.7^{c}	224.9 ± 14.4^{b}	197.4 ± 8.9^{c}
CUPRAC (μM TE/100 g FW)	$1{,}075.6 \pm 81.4^{a}$	955.4 ± 61.1^{b}	845.5 ± 48.6^{c}	924.9 ± 51.2^{b}	806.5 ± 31.2^{c}
ABTS (μM TE/100 g FW)	$2{,}352.7 \pm 124.2^{a}$	$2{,}091.4 \pm 118.4^{b}$	$1{,}843.6 \pm 107.5^{c}$	$2{,}020.4 \pm 118.5$	$1{,}773.2 \pm 102.5^{d}$

Data presented as the mean ± standard deviation of five measurements. Means in rows without superscript letters in common differ significantly ($P < 0.05$).
Abbreviations used: FW, fresh weight; TP, total polyphenols; FP, free polyphenols, TF, total flavonoids; FF, free flavonoids; GAE, gallic acid equivalent; CE, catechin equivalent; CGE, cyanidin-3-glucoside equivalent; AC, anthocyanins; FLAV, flavanols; FRAP, reducing/antioxidant power; CYPR, CUPRAC, cupric reducing antioxidant capacity; ABTS+, [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation; TE, trolox equivalent.

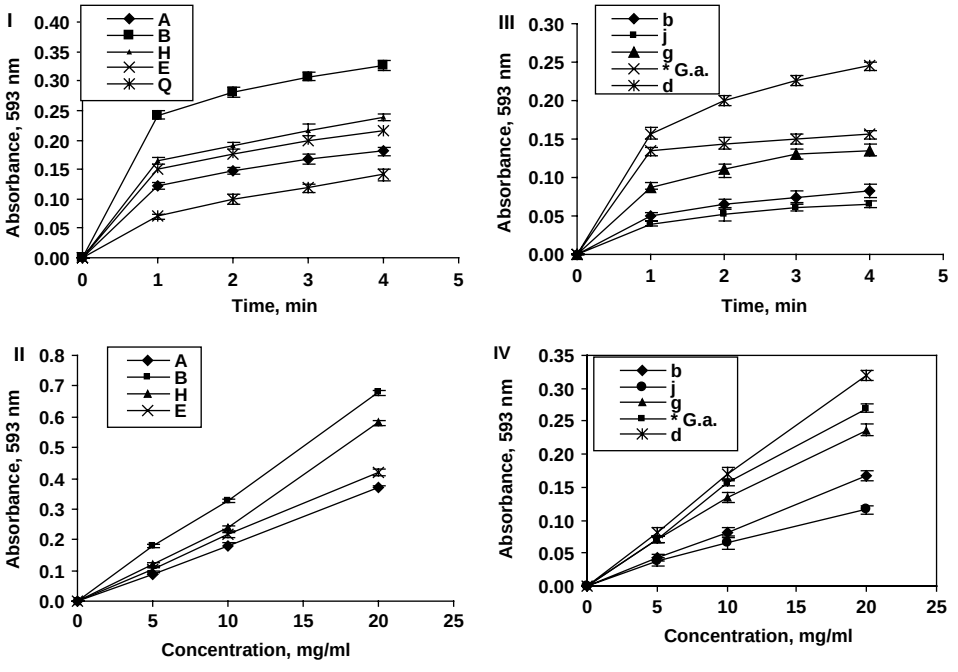

Figure 2. Kinetic FRAP measurements. (I) Total polyphenol extracts (B, Mon Thong; H, Chani; E, Pung Manee; A, Kradum at constant concentration (10 mg/ml) and with a change of time (1, 2, 3, and 4 min). Standard, quercetin (Q) (30 μg/ml). (II) Total polyphenol extracts (B, Mon Thong; H, Chani; E, Pung Manee; A, Kradum) at constant time (4 min) with a change of extract concentration (5, 10, 15 and 20 mg/ml). (III) Free polyphenol extracts (d, Mon Thong; g, Chani; b, Pung Manee; j, Kan Yao) at constant concentration (10 mg/ml) and with a change of time (1, 2, 3, and 4 min). Standard, gallic acid (G.a.) (10 μg/ml). (IV) Free polyphenol extracts (d, Mon Thong; g, Chani; b, Pung Manee; j, Kan Yao) at constant time (4 min) with a change of extract concentration (5, 10, 15 and 20 mg/ml).

(*Morinda citrifolia L.*), which possessed a high free-radical-scavenging activity with 1, 1-diphenyl-2-picrylhydrazyl at 140 mg equivalent ascorbic acid/100 ml and total phenols at 210 mg gallic acid/100 ml (Yang et al. 2007).

As already mentioned, the total polyphenols ranged from 3.61 ± 0.23 to 2.72 ± 0.11 mg GAE/g FW. These values of polyphenols were similar to those of Wu et al. (2004), where the amount of plums and Gala apples was 3.66 ± 1.09 mg GAE/g FW and 2.62 ± 0.29 mg GAE/g FW, respectively. Our results of total polyphenols ranged from 3,614 to 2,715 μg GAE/g FW, which corresponded to the result taken from the literature and cited by Mahattanatawee et al. (2006) of strawberry (3,680 μg GAE/g FW) and kiwi fruit (2,780 μg GAE/g FW). The results of Mahattanatawee et al. (2006) relate also to different cultivars (papaya, guava and dragon fruit). It was interesting to compare between different cultivars of these fruits because, as is seen from the comparison with the literature, our data correspond with guava, papaya and mango. The relationships between the amount of polyphenols were in white guava (1,589.3 μg GAE/g FW) and dragon fruit (1,075.8 μg GAE) a ratio of 1.48, in white guava and papaya (442.2 μg GAE/g FW) a ratio of 3.39 and in dragon fruit and papaya a ratio of 2.43. Our results between different cultivars of durian showed that

the relationship between Mon Thong (3,614 μg GAE/g FW) and Kan Yao (2,832 μg GAE/g FW) was a ratio of about 1.28, Mon Thong and Kradum (2,715 μg GAE/g FW) was 1.33, and Kan Yao and Kradum was 1.04. Our results were lower than in Mahattanatawee et al. (2006) because we compared different cultivars of the same fruit and did not compare between different cultivars of different fruits. The determined antioxidant activities by FRAP (1.27, 1.32 and 1.03), CUPRAC (1.27, 1.33 and 1.05) and ABTS (1.28, 1.32 and 1.04) assays showed the following relationships between Mon Thong and Kan Yao, Mon Thong and Kradum, and Kan Yao and Kradum, respectively. The antioxidant activity determined by the oxygen radical absorbance capacity (ORAC) showed for the compared different cultivars as white guava, dragon fruit and papaya values of 9.9, 3.0 and 5.3 μM TE/g puree, and by DPPH (radical scavenging activity) values of 298.6, 34.7 and 65.1 μg GAE/g puree. The relationship between white guava and dragon fruit, between white guava and papaya, and between dragon fruit and papaya in ORAC was 3.3, 1.86 and 0.57, respectively, and with DPPH the same fruits showed the following order: 8.6, 4.6 and 0.53. These relationships were different from the ones obtained in our study, because the comparison was with different cultivars of different fruits. But the comparisons of the amount of polyphenols (μg GAE/g puree), DPPH antioxidant activity (μg GAE/g puree) and ORAC (μM TE/g puree) between the same family of different cultivars such as guava:red guava (2,316.7, 609.3 and 16.7), guava:white guava (1,589.3, 298.6 and 9.9) and another family such as dragon:red dragon (1,075.8, 134.1 and 3.0) and dragon:white dragon (523.4; 34.7 and 3.0) showed completely different relationships. The polyphenol content ratio between red and write guava was about 1.48, and red and white dragon a ratio of 2.05, and the antioxidant activities by DPPH and ORAC for guava were 2.04 and 1.69 and for dragon were 3.86 and 1.0, respectively. These numbers were more similar with the ones obtained in this study.

The amount of the polyphenols and flavonoids obtained in this investigation can also be compared with other published recently reports (Chun et al. 2005). Our results of Mon Thong polyphenol content (361.4 ± 23.2 mg GAE/100 g FW) are similar to plums of 368.66 ± 12.66, and for Kradum (271.5 ± 11.25 mg GAE/100 g FW) are similar to strawberries (225.00 ± 2.60). The amount of flavonoids (mg CE/100 g FW) for Mon Thong was 93.9 ± 7.4 and showed lower data than in plums (194.42 ± 9.87), and for Kradum (69.2 ± 5.3) were higher than in strawberries (51.3 ± 1.40). The antioxidant activity (mg vitamin C equivalent/100 g FW) was for plums 481.43 ± 15.03 and for strawberries 347.20 ± 9.10. The FRAP values (mmol/100 g FW) of the samples were between 0.27 and 0.22, and corresponded with apple (0.26) and banana (0.20) according to Halvorsen et al. (2002). The ABTS values (μM TE/100 g FW) were between $2,353 \pm 28.6$ and $1,773 \pm 14.3$, and were lower than wild strawberry ($3,317 \pm 0.05$) and higher than cultivated strawberry ($1,317 \pm 0.08$) according to Scalzo et al. (2005). The FRAP values (μmol/g FW) of the samples were between 2.61 and 1.97, and were lower than banana (3.88) and similar to mango (2.28) according to Nilsson et al. (2005). The ABTS values (μmol/g FW) ranged from 23.53 to 17.73, and were slightly lower than lemon of 30.77 and nearly equal to guava of 19.72 according to Nilsson et al. (2005). The overall ratio of ABTS/FRAP was 9.2 and 9.0, in comparison with the water-soluble values of 1.7, 2.5 and 2.4 in Nilsson et al. (2005).

Only one cited report (Voon et al. 2007) was dealing with five Durian cultivars. There were significant differences ($P < 0.05$) among the five cultivars in terms of all

physicochemical characteristics tested, such as pH, soluble solids, titratable acidity, sugars and organic acids. It is impossible to compare the obtained results, because in the cited report other cultivars of durian were studied. The cultivars were also of Thai origin, but these fruits showed different bioactivity**.**

Nine tropical fruits were analyzed for total phenol contents, ascorbic acid contents and antioxidant activities. The antioxidant activities were evaluated based on the ability of the fruit extracts to scavenge DPPH, to reduce iron (III) to iron (II) and to bind to iron (II) ions (Lim et al. 2007). The results were compared with those of orange. It was found that guava, papaya and star fruit have higher primary antioxidant potential, as measured by scavenging DPPH and iron (III) reducing assays. Banana, star fruit, water apple, langsat and papaya have higher secondary antioxidant potential as measured by the iron (II) chelating experiment.

Protein profiles of durian samples

The results of electrophoretic run are shown in Figure 3. Two specific bands were detected (at 17 and 86 kDa, Figure 3a) for Kradum cultivar. Mon Thong showed one specific band at 46 kDa. Kan Yao differed from other varieties and showed three specific bands at 47, 70 and 99 kDa. Genotypes Pung Manee and Chani are non-distinguishable.There is only one work (Santoso et al. 2005) about suitable polymerase chain reaction–restriction fragment-length polymorphism markers of two chloroplast genes for distinguishing 27 species of Durio genus. Species were grouped into different clusters. Until now, no data concern distinguishing Durio (*D. zibethinus*) varieties on the basis of biochemical characters such as proteins. A new seed storage protein called zibethin was recognized as described by Charbonneau et al. (1991). Our purpose was to extract sample buffer 'total' proteins from fruit tissue and then to distinguish five durian varieties on the basis of the total protein pattern. Three of the five varieties possess a specific pattern, suitable for distinguishing from each other. Two varieties were non-distinguishable. Especially, variety Kan Yao differs from the others.

The differences in the samples with different stages of ripening are indicated in the range of 47 kDa (Figure 3b, arrow). The electrophoretic profiles of the samples are presented for the first time and can be an additional index for the determination of durian ripening, because the most intensive protein band at 47 kDa was detected in an overripe sample and the weakest one in a mature sample. There are no data for comparison of the obtained results. We found only one report dealing with reserve accumulation and protein storage vacuole formation during the development of recalcitrant seeds of *D. zibethinus* L. (Brown et al. 2001). Probably the storage proteins during the development (unglycosylated albumins, light and heavy zibethinin of 20.2 and 21.4 kDa) and after the ripening disappeared and the most intensive bands were detected in the range of 47 kDa. The obtained results also depend on the procedure of protein extraction. In our research, the proteins were extracted with a buffer containing SDS, therefore the albumins or prolamins are shown as subunits.

Conclusions

The bioactivity of durian Mon Thong, Chani and Pung Manee was high and the total polyphenols were the main contributors to the overall antioxidant capacity. The

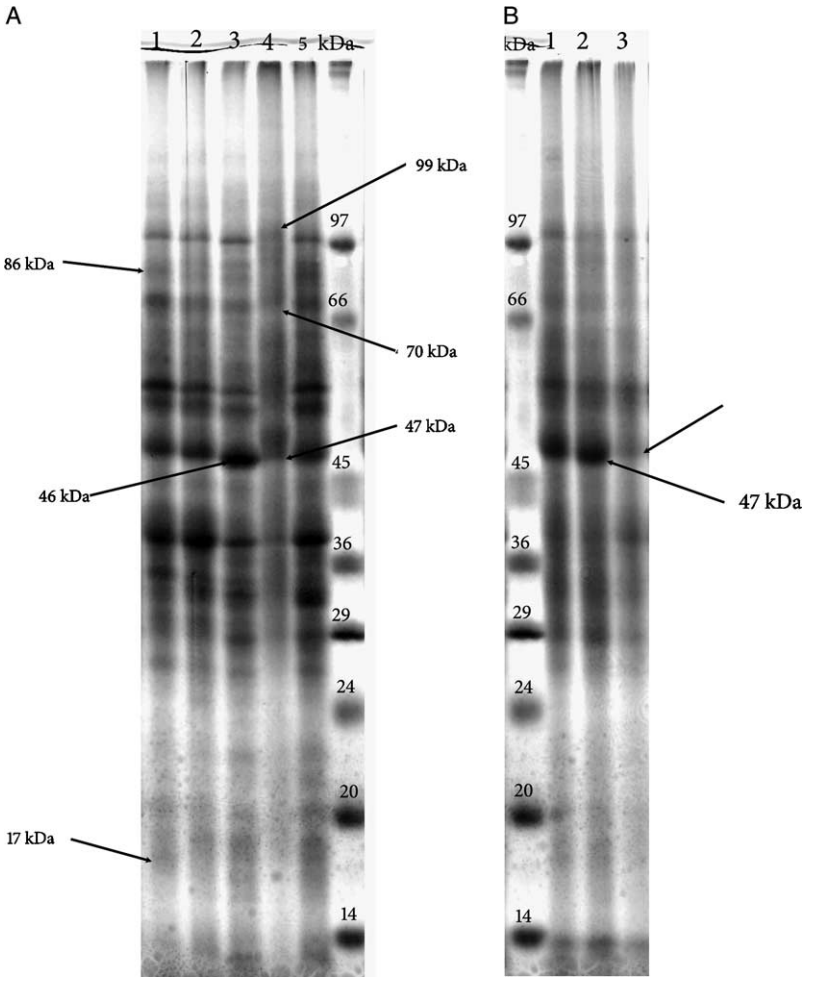

Figure 3. Comparison of the band intensity of proteins extracted from durian samples and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. (a) molecular weight marker: phosphorylase B, 97 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; glyceraldehyde-3 phosphate dehydrodrogenase, 36 kDa; carbonic anhydrase, 29 kDa; trypsinogen, 24 kDa; trypsin inhibitor, 20 kDa; α-lactaalbumin, 14 kDa. Lanes 1– 5, Kradum, Pung Manee, Mon Thong, Kan Yao, Chani, respectively. Arrows on the scan indicate specific bands: 17 and 86 kDa for Kradum; 46 kDa for Mon Thong; 47, 70 and 99 kDa for Kan Yao. (b) Lane 1, ripe; lane 2, overripe; lane 3, mature.

results of this investigation of durian cultivars are comparable with other fruits widely used in the human diet. Therefore, durian cultivars—especially durian Mon Thong, Chani and Pung Manee—can be used as a supplement for nutritional and healthy purposes.

Acknowledgements

The authors are grateful to Prof. Simon Trakhtenberg (Kaplan University Medical Center, Rehovot, Israel) for his intellectual assistance in discussion, to Dr Elena Katrich (Hebrew University of Jerusalem, School of Pharmacy) for technical

assistance in determination of the antioxidant activity, and to Dr Ratiporn Haruenkit and Dr Sumitra Poovarodom (King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok, Thailand) for supplying the durian samples. This work was partly supported by the Food Industrial Technology Innovation Center at Mokpo National University. Shela Gorinstein is affiliated with the David R. Bloom Center for Pharmacy.

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This paper was first published online on iFirst on 5 March 2008.