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Some Analytical Assays for the Determination of Bioactivity of Exotic Fruits

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ABSTRACT:

Introduction – The consumption of new exotic fruits, with their high nutritional and sensory value, has significantly increased in the past few years. Among the tropical fruits durian (*Durio zibethinus* Murr.) is less known than mango (*Mangifera indica* L.) and avocado (*Persea americana*). It has been shown that durian, mango and avocado possessed high nutritional and bioactive properties, but these data were determined using different methods. In order to obtain reliable results we investigated samples of durian, mango and avocado of the same stage of ripeness and unified methods were used for determination of the antioxidant potential. As far as we know, no results of such comparative investigation of three tropical fruits (durian, mango and avocado) and the use of such tests for phytochemical control have been published.

Objective – Lyophilised durian, mango and avocado samples harvested in 2008 in Thailand and Israel were investigated.

Methodology – The contents of crude protein, fat, carbohydrate, dietary fibre, total polyphenols, flavonoids, tannins and flavanols were determined by elemental analysis and UV spectroscopy. The presence of polyphenols (flavonoids and phenolic acids) in the investigated samples was studied by Fourier transform infrared (FT-IR) spectroscopy and three-dimensional fluorometry. Four complementary radical scavenging assays were used for antioxidant determination: ferric reducing antioxidant power (FRAP), 2, 2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\bullet+}$), 1-diphenyl-2-picrylhydrazyl method (DPPH) and cupric reducing antioxidant capacity (CUPRAC). Chemometrical processing was used for statistical comparison of the fruits.

Results – All spectrometric measurements were highly correlated. The contents of total fibre, proteins and fats were significantly higher ($p < 0.05$) in avocado, and carbohydrates were significantly lower in avocado ($p > 0.05$) than in the two other fruits. The wavelength numbers of FTIR spectra for three investigated fruits were in the same range (1700–600 cm^{-1}) as for catechin and gallic acid, used as standards. One main peak could be easily observed at the approximate location of ex/em 275/305 nm and the other one at ex/em 350/430 nm in the methanol polyphenol extracts of investigated fruits in three-dimensional fluorescence, in contour and cross fluorescence maps. Similarity was found between durian, mango and avocado in polyphenols (9.88 ± 1.0, 12.06 ± 1.3 and 10.69 ± 1.1, mg gallic acid equivalents/g dry weight, d.w.), and in antioxidant assays such as CUPRAC (27.46 ± 2.7, 40.45 ± 4.1 and 36.29 ± 3.7, μM Trolox equivalent (TE)/g d.w.) and FRAP (23.22 ± 2.0, 34.62 ± 3.4 and 18.47 ± 1.9, μM TE/g d.w.), respectively. The multisample median test between all possible pairs of groups is a Tukey–HSD type comparison and denotes the different groups in a case when a pair-wise test is significant and its q statistical value is greater than the table q parameter. The multisample median test of FRAP values were chosen from the compared fruits triplets as similar or homogenous subsets durian and avocado.

Conclusion – Nutritional and bioactive values of durian are comparable with these indices in mango and avocado. These fruits contain high, comparable quantities of basic nutritional and antioxidant compounds, and possess high antioxidant potentials. All fruits show a high level of correlation between the contents of phenolic compounds and the antioxidant potential. The methods used (three-dimensional fluorescence, FTIR spectroscopy, radical scavenging assays) are suitable for bioactivity determination of these fruits. In order to receive best results, a combination of these fruits has to be included in the diet. The methods used are applicable for bioactivity determination in phytochemical analysis in general.

Keywords: durian; mango; avocado; nutritional and bioactive properties; assays

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Introduction

The consumption of new exotic fruits, with their high nutritional and sensory values, has significantly increased in the past few years (Luximon-Ramma *et al*., 2003; Mahattanatawee *et al*., 2006; Haruenkit *et al*., 2007; Corral-Aguayo *et al*., 2008). Among the tropical fruits durian (*Durio zibethinus Murr*.) is less known than mango (*Mangifera indica* L.) and avocado (*Persea americana*) (Elez-Martinez *et al*., 2005; Terasawa *et al*., 2006; Khoo *et al*., 2008; Ribeiro *et al*., 2008; Takenaga *et al*., 2008; Robles-Sanchez *et al*., 2009).

It has been shown that durian (Khoo *et al*., 2008), mango (Ribeiro *et al*., 2008; Robles-Sanchez *et al*., 2009) and avocado (Elez-Martinez *et al*., 2005; Terasawa *et al*., 2006; Takenaga *et al*., 2008) possessed high nutritional and bioactive properties. Toledo *et al*. (2008) reported that five studied durian cultivars contained high quantities of bioactive compounds and high levels of antioxidant activity. Ribeiro *et al*. (2008) found that Brazilian mango has a high content of phenolic compounds and a high antioxidant capacity. Terasawa *et al*. (2006) recorded the high antioxidative activity of avocado epicarp hot water extract. Elez-Martínez *et al*. (2005) showed that avocado is nutritionally rich in many health-related components and is a valuable energy source due to its high-quality fat content.

The above data were determined using different methods. In order to obtain reliable data, we investigated samples of durian, mango and avocado of the same stage of ripeness (Haruenkit *et al*., 2010) and unified methods were used such as CUPRAC, DPPH, ABTS and FRAP (Szeto *et al*., 2002; Apak *et al*., 2004; Ozgen *et al*., 2006; Ozyurek *et al*., 2007) for determination of the antioxidant potential. As far as we know, no results of such comparative investigation of three tropical fruits (durian, mango and avocado) and the use of such tests for food control have been published.

Experimental

Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent (FCR), lanthanum (III) chloride heptahydrate, $FeCl_3 \times 6H_2O$, $CuCl_2 \times 2H_2O$, 2,9-dimethyl-1,10-phenanthroline (neocuproine), β-carotene and butylated hydroxytoluene (BHT), were purchased from Sigma Chemical Co., St Louis, MO, USA. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionised and distilled water was used throughout.

Samples and preparation

Durian (*Durio zibethinus Murr*. cv Mon Thong), mango (*Mangifera indica* L. cv Nam Dok Mai No. 4) and avocado (*Persea americana* cv Ettinger), harvested in 2008, were at the same stage of ripeness. Durian and mango samples were harvested from a 25-year-old Mon Thong commercial durian orchard, in Chantaburi Province, eastern Thailand. Avocado samples were donated by Mehadrin Tnuport Export (MTEX) L.P, Be'erot Yitzhak, Israel. The fruits were cleaned with tap water and dried, using five replicates of five fruits each. The relative standard deviation between five replicate sample preparations was <2%.

The edible parts of the above-mentioned fruits were prepared for this investigation manually without using steel knives. The peeled fruits were weighed, chopped and homogenised under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then lyophilised for 48 h (Virtis model 10-324), and the dry weight was determined. The samples were ground to pass through a 0.5 mm sieve and stored at −20°C until the bioactive substances were analysed (Haruenkit *et al*., 2010).

Determination of the basic nutritional, bioactive compounds and antioxidant potentials

The contents of crude protein, fat, carbohydrate, dietary fibre, total polyphenols, flavonoids and flavanols were determined as previously described (Gorinstein *et al*., 2004; Haruenkit *et al*., 2010). The presence of polyphenols (flavonoids and phenolic acids) in the investigated samples was studied by Fourier transform infrared (FT-IR) spectroscopy. A Bruker Optic GMBH Vector FT-IR spectrometer (Bruker Optic GMBH, Attingen, Germany) was used to record IR spectra. A potassium bromide microdisc was prepared from finely ground lyophilised powder of 2 mg of fruit samples with 100 mg of KBr (Edelmann and Lendl, 2002; Sinelli *et al*., 2008).

Fluorescence measurements

Fluorescence spectra for all fruit extracts in methanol at a concentration of 0.01 mg/mL were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan, equipped with 1.0 cm quartz cells and a thermostat bath. The widths of the excitation and emission slits were set to 10.0 and 5.0 nm, respectively. The three-dimensional spectra were collected with subsequent scanning emission spectra from 250 to 500 nm at 1.0 nm increments by varying the excitation wavelength from 250 to 450 nm at 10 nm increments. The scanning speed was set at 1000 nm/min for all measurements. All measurements were performed with emission mode and with intensity up to 500 (Wulf *et al*., 2005; Wang *et al*., 2009; Yin *et al*., 2009).

Extraction of total polyphenols

Lyophilised fruit samples were extracted from a 50 mg aliquot with 5 mL of 1.2 M HCl in 50% methanol–water for unconjugated plus conjugated ('total') polyphenols with heating at 90°C for 3 h (Durh, Manh and Avoh) and without HCl for non-hydrolysed polyphenols (Durnh, Mannh and Avonh). Then polyphenols were determined by the Folin–Ciocalteu method with measurement at 750 nm using a spectrophotometer (Hewlett-Packard, model 8452A, Rockville, MD, USA). The results were expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (d.w.). The extraction of total polyphenols was optimised to prove the completeness of extraction from selected fruits (Hertog *et al*., 1992; Perez-Jimenez and Saura-Calixto, 2005; Gorinstein *et al*., 2008).

Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH, were measured at 510 nm. The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm. (+)-Catechin served as a standard for flavonoids, flavanols and tannins, and the results were expressed as catechin equivalents (CE). Linearity range was determined for the spectrophotometric determinations and expressed as the determination coefficient (R^2).

Determination of the antioxidant potentials

Four complementary assays were used:

(1) The ability of the antioxidants contained in the samples to reduce ferric tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}) which absorbs light at 593 nm was measured. The antioxidant activity was determined at constant concentration and also with different concentrations of the fruits from 5 to 25 mg/mL.
(2) 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\cdot+}$) was generated by the interaction of ABTS (mmol/L) and $K_2S_2O_8$ (2.45 mmol/L). This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm.

(3) 1-Diphenyl-2-picrylhydrazyl method solution (3.9 mL, 25 mg/L) in methanol was mixed with the sample extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable.
(4) Cupric reducing antioxidant capacity (CUPRAC) is based on utilising the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidising agent. To the mixture of 1 mL of Cu (II), Nc and NH_4Ac buffer solution, extract of fruit 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.

Chemometrical processing

Samples with different proportions of fruit extracts (5, 10, 15, 20 and 25 mg/mL) were analysed by FRAP antioxidant activity assay (Ozgen *et al*., 2006). The FRAP data (μM Trolox equivalent TE)/g d.w.) set consisted of a 25×3 matrix in which rows represent the different extract concentrations and columns the three fruit species (durian, avocado and mango). Basic chemometric characterisation of the investigated fruit extract samples according to their ability to reduce the Fe^{3+} ions was carried out by summary, descriptive (normal probability, box/whisker and dot plots) statistics and multisample median testing using the statistical programme Unistat® (London, UK).

Data analysis

The results of this investigation are means ± SD of five measurements. Differences between groups were tested by two-way ANOVA. In the assessment of the antioxidant potential, Spearman's correlation coefficient (R) was used. Linear regressions were also calculated. p-Values of <0.05 were considered significant.

Results and Discussion

Major nutritional components

In durian, mango and avocado the following major nutritional components were determined, respectively: total fibre (g/100 g fresh weight), 3.2 ± 0.3^a, 2.9 ± 0.2^a and 6.2 ± 0.5^b; total proteins, 1.4 ± 0.1^b, 0.8 ± 0.06^a and 1.9 ± 0.2^c; total fats, 5.3 ± 0.4^b, 0.4 ± 0.03^a and 21.2 ± 1.3^c; carbohydrates, 27.1 ± 1.6^b, 28.2 ± 1.6^b and 8.3 ± 0.6^a. The different superscript letters show significant differences between the indices in three investigated fruits ($p < 0.05$). The contents of total fibre, proteins and fats were significantly higher ($p < 0.05$) in avocado, and carbohydrates were significantly lower in avocado ($p > 0.05$) than in the two other fruits.

FTIR spectra

Avocado, durian and mango samples (Fig. 1Aa–c) in the region of polyphenols showed slightly different bands than the standards (Fig. 1Ba, b), but the wavelengths of the bands were similar in this group of fruits. The absorption bands at 1408.1, 1101.7, 920.9, 868.3 and 777.9 cm^{-1} were absent in avocado (Fig. 1Ac) and durian (Fig. 1Ab) in comparison with mango (Fig. 1Aa); similar peaks were at 1656.1, 1647.3 and 1457.7 cm^{-1} for avocado and durian (Fig. 1Ac and b); at 1341, 1250.5, 1136.8, 1060.9 and 993.8 cm^{-1} similar peaks appeared for all fruits (Fig. 1Aa–c). In the region of 1638.6 and 1749.5 cm^{-1} the highest peak was measured for mango, and at 935.4 cm^{-1} one peak was shown for durian. Other bands in the fruit samples were slightly shifted in comparison with the standards (Fig. 1Ba, b). The wavelength numbers of FTIR spectra for catechin (Fig. 1Ba) at 831, 1040, 1112, 1144, 1285, 1478, 1512 and 1611 cm^{-1} were assigned to -C-H alkenes, -C-O alcohols, C-O-H alcohols, -OH aromatic, C-O alcohols, C-H alkanes, C=C aromatic ring and C=C alkenes, respectively. Gallic acid (Fig. 1Bb) showed the following wavelength numbers (cm^{-1}): 866, 1026, 1237, 1451, 1542 and 1619.

Fluorimetric measurements

The 2D fluorescence spectra showed the results described below. At emission 330 nm the recording of the main peak in the fluorescence spectra for mango and avocado was at 281.5 nm with fluorescence intensity (FI) 268.6 and 203.4, and for durian with a small shift at 282.5 nm with FI 202.4. The second peak was at 300 nm for mango, durian and avocado with FI 137.7, 122.7 and 90.1. Catechin 0.001 mM showed one peak at 282 nm with FI 700.4. For catechin with emission 685 nm, the spectra (290–400 nm) showed main peaks at 343.5 nm and a higher absorbance of 666.8; for mango the main peak was at 343.5 nm with FI 276.6; for avocado the main peak was at 343.5 nm with FI 258.5; and for durian the main peak was at 344 nm with FI 255.9. Emission of 740 nm showed the following data for the main peak (nm): for 0.001 mM of catechin and mango, 371.5 nm with FI 590.9 and 364.4; for avocado and durian exactly the same value, 371 nm, with FI 239.5. The second minor peak was for catechin and all fruits at the same wavelength, 739.5 nm, with different absorption intensities: catechin, 312; mango, –125.8; and avocado and durian, –55.3. At excitation wavelength 350 nm the following peaks appeared: the first peak (nm) for avocado, mango and durian was at 391.5, 391 and 390.5, respectively, with slightly different FIs of 77.3, 67.1 and 48.8. A shift appeared in the second peak: for avocado it was at 423.5 nm with FI 70.0; for mango it was at 429 nm with FI 45.2; and for durian it was at 448 nm with FI 11.1. Catechin (0.001 mM) showed only one main peak at 390.5 with FI 35.1. The wavelengths of catechin and durian in these conditions were exactly the same.

In three-dimensional fluorescence spectra the excitation and the emission wavelengths and the fluorescence intensity were used as the axes in order to investigate the information of the samples, and the contour spectra provided more information. Three-dimensional fluorescence spectra (Fig. 2) illustrated the elliptical shape of the contours. The x-axis represents the emission spectra from 250 to 500 nm, while the y-axis is the excitation spectra from 250 to 450 nm, for durian (A), avocado (B) and mango (C), catechin (D) and gallic acid (E). The contour maps (F–J) display the fluorescence spectra. The result shows that the three-dimensional fluorescence contour maps of durian, avocado and mango in comparison with standards catechin and gallic acid are obviously different. One main peak can easily be observed at the approximate location of ex/em 275/305 nm and the other one at ex/em 350/430 nm (Fig. 2F–J). There are some additional peaks for the samples and standards, depending on the fruit extract. Avocado extract showed the following peaks: ex/em 275/270 nm with FI 106, small; ex/em 275/310 nm with FI 2000, big; ex/em 275/330 nm with FI 643; ex/em 275/310 nm with FI 2000, big; ex/em 275/350 nm with FI 633. Only one peak is marked on Fig. 2G at ex/em 275/310 nm. The peak ex/em 275/305 nm is similar to durian and shifted to 310 nm. The second area of location was at ex/em 350/350 nm with FI 1000; ex/em 350/390 nm with FI 132; and ex/em 350/430 nm with FI 98. Mango (Fig. 2C, H) differed from the previous two fruits: the peak was at ex/em 275/270 nm with FI 126; ex/em 275/320 nm with FI 433; and ex/em 275/350 nm with FI 387. The peak ex/em 275/305 shifted to 275/320. At excitation of 350 nm the following peaks were indicated: ex/em 350/350 nm with FI 1000; ex/em 350/370 nm with FI 20; ex/em 350/390 nm with FI 95; and ex/em

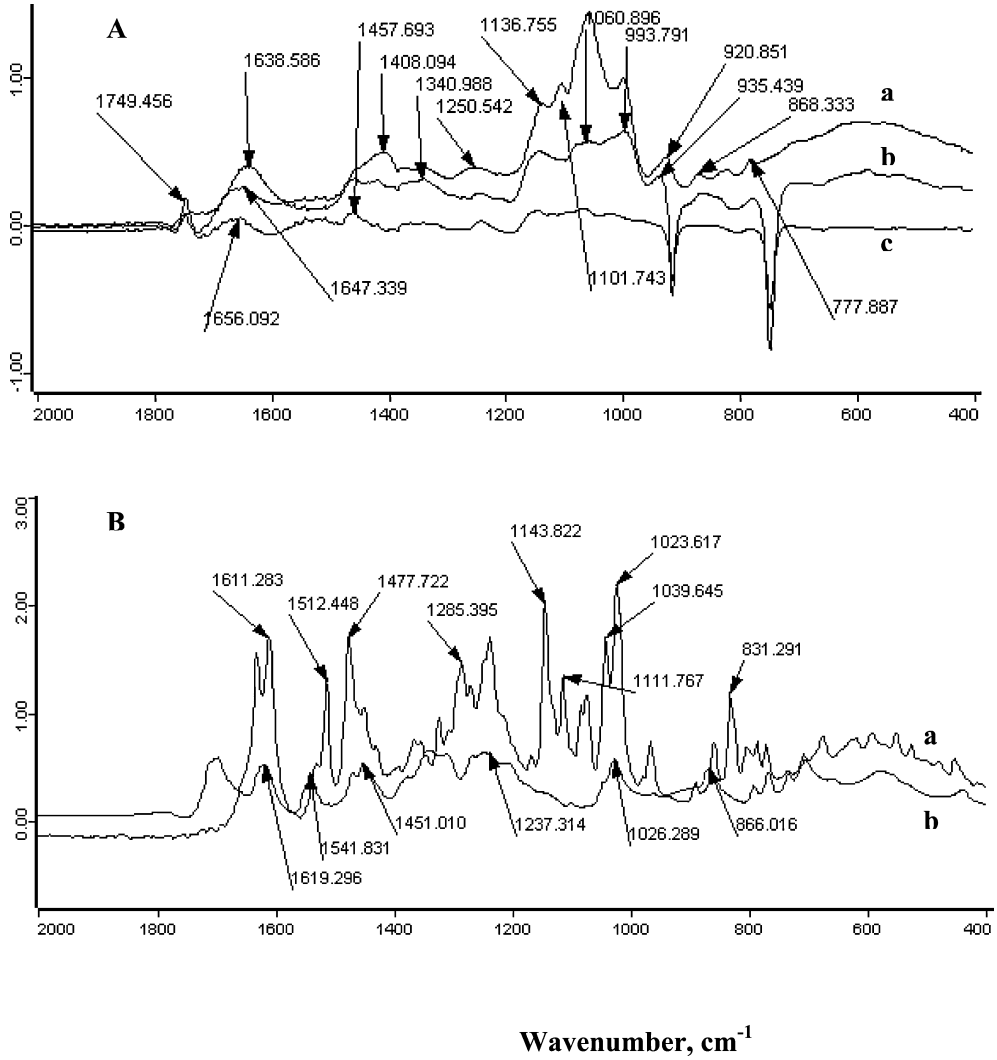


Figure 1. FTIR spectra of polyphenols from: (A) avocado (c), durian (b) and mango (a); (B) catechin (a) and gallic acid (b).

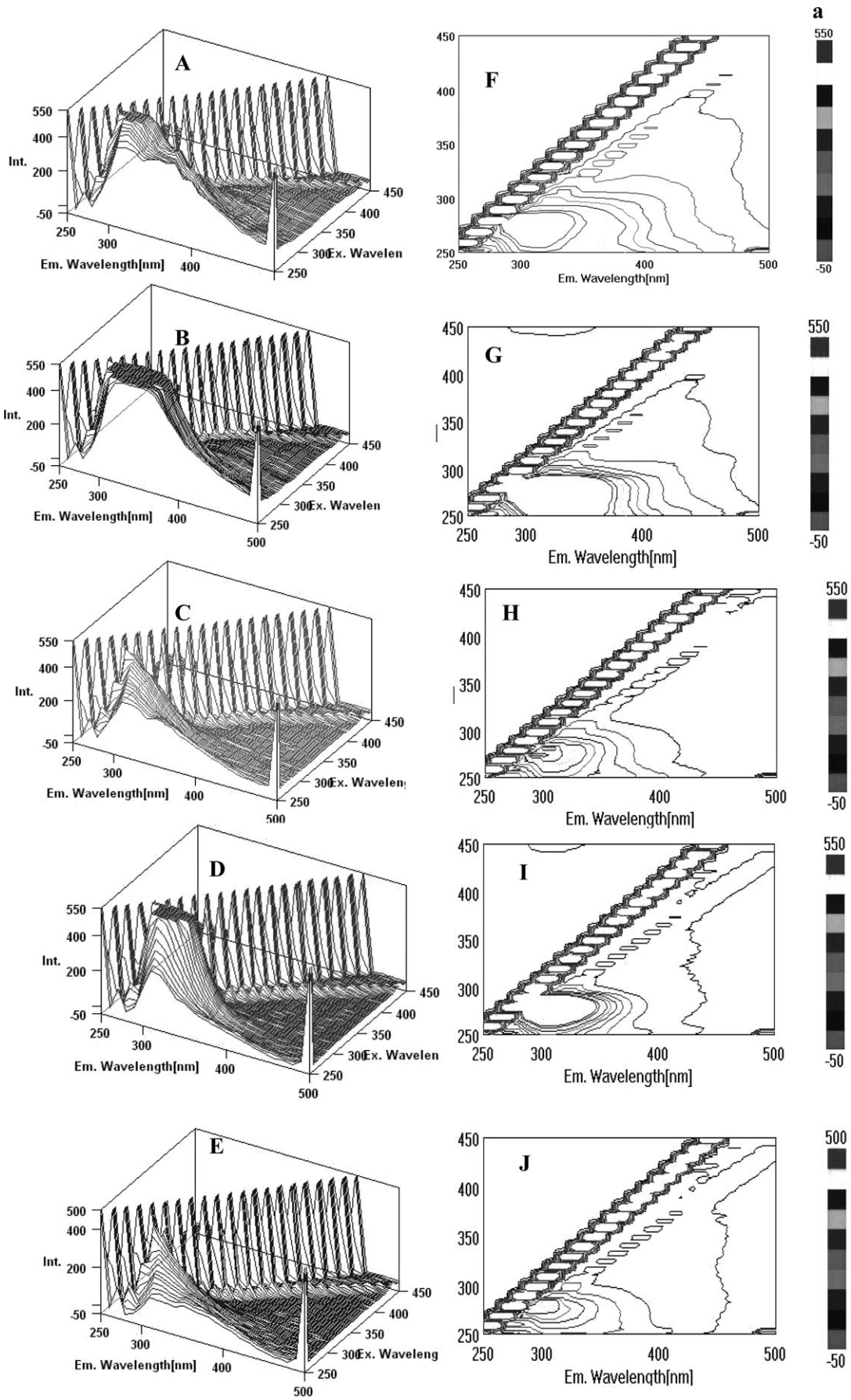
350/430 nm with FI 47. The standards Fig. 2(D, I, E, J) showed a peak at ex/em 275/300 and 350/440 nm for gallic acid and 350/390 nm for catechin.

Bioactive compounds and antioxidant potentials

The results of the determination of the studied bioactive compounds and their antioxidant potentials are summarised in Fig. 3. The results of this report support the conclusions of Perez-Jimenez and Saura-Calixto (2005) and our recent studies (Gorinstein *et al*., 2008), that the most efficient antioxidant extraction was achieved by using successively acidic methanol–water (50 : 50 v/v, pH 2). The extraction with methanol–HCl is based on the data of others (Hertog *et al*., 1992), showing that under acid hydrolysis with 2.0 M HC1 in boiling 50% aqueous methanol, flavonol 3-*O*-glucosides are hydrolysed completely within a few minutes, whereas complete hydrolysis of flavonol 3,7- and 4′-*O*-glucuronides takes 60–250 min. Optimisation of extraction and hydrolysis has shown that the highest yield was found using 1.2 M HC1 and a reaction period of 2 h. It appeared that extraction was most efficient with 50% aqueous methanol. The flavonoid glycosides were hydrolysed to their corresponding aglycons by refluxing in 1.2 M HCl containing 50% MeOH so as to exert maximal reducing power towards scavenging radicals used in the antioxidant assays. Increasing acid concentration and reaction time led to a significant degradation of quercetin; therefore, such extraction was suggested in this study for the extraction of total phenols (Hertog *et al*., 1992).

In hydrolysed methanol extracts (50% methanol and 1.2 M HCl with heating at 90°C), the total polyphenols (mg GAE/g d.w.,

Figure 2. Three-dimensional fluorescence map of 0.01 mg/mL of methanol extracts of durian (A), avocado (B), mango (C), 0.01 mM catechin (D) and 0.01 mM gallic acid (E), respectively. The contour maps (F–J) display the corresponding fluorescence spectra. The three-dimensional spectra used emission wavelengths from 250 to 500 nm and excitation wavelengths from 250 to 450 nm; scanning speed was 1000 nm/min, emission mode and fluorescence intensity up to 500. Abbreviations: A–E on *z*-axis—Int, fluorescence intensity; on *x*-axis—Em. Wavelength, emission wavelength; on *y*-axis—Ex. Wavelen, excitation wavelength; F–J, on *x*-axis—Em Wavelength, emission wavelength; *y*-axis—excitation wavelength. In position (a) all the fluorescence intensity values from –50 to 500 are presented. Arrows on the counter show the area of the location of the peaks.



Durh, Manh, Avoh, Fig. 3, R^2 = 0.9732) for durian, mango and avocado ranged from 9.88 to 12.06 and for non-hydrolysed (50% methanol with heating at 90°C, Durnh, Mannh, Avonh) from 0.75 to 3.11 (Fig. 3). In hydrolysed extracts flavonoids (mg CE/g d.w., R^2 = 0.9937) ranged from 0.63 to 3.57 and for non-hydrolysed from 0.178 to 0.023. Flavanols (mg CE/g d.w., R^2 = 0.9922) were found in hydrolysed extracts as follows: from 0.07 to 0.15, and in non-hydrolysed samples from 0.004 to 0.007. The antioxidant potentials for hydrolysed samples (μM TE/g d.w., Fig. 3, samples Durh, Manh, Avoh) by CUPRAC (R^2 = 0.9969), ABTS (R^2 = 0.9605), DPPH (R^2 = 0.9486) and FRAP (R^2 = 0.9788) assays were in the ranges 27.46–40.45, 39.98–66.72, 6.81–55.27 and 18.47–34.62, respectively. For non-hydrolysed samples (Fig. 3, samples Durnh, Mannh, Avonh) the antioxidant activities (μM TE/g d.w.) showed the following results for CUPRAC, ABTS, DPPH and FRAP, respectively: 3.58–11.03, 4.39–31.09, 3.27–27.14 and 2.48–6.18.

Descriptive statistics

Tables 1 and 2 show summary statistics (central tendency, such as the arithmetic mean, median or interquartile mean as well as statistical dispersion of the results like the standard deviation, variance and range) of the examination of the ferric reducing ability of durian, avocado and mango fruits in a quite wide-ranging concentration of fruit extracts from 5 to 25 mg/mL. To look at the relationship among fruit FRAP values side by side, box plots were generated. The box and dot plots in Fig. 4 show the frequency distribution of the durian, avocado and mango FRAP values expressed as Trolox equivalent in μM/g of fruit dry matter content, which were determined according to FRAP assay in the wide-ranking interval of fruit extract concentration from 5 to 25 mg/mL. For this reason FRAP data vary widely and all box plots have approximately the same variation. The data dots are plotted against their actual values on a vertical scale. Box plots of durian, avocado and mango display differences in distribution and median values of the fruits' experimental Trolox data and show that, of the tested fruits, mango has the highest ferric reducing ability, following by the durian and avocado. Notches on the boxes indicate dispersion of data about the median and whiskers' least and greatest experimental values excluding outliers. A multisample median test was used to determine whether the samples have been drawn from a population with the same median. The multisample median test made between all possible pairs of groups is a Tukey–HSD (honestly significant difference) type comparison and denotes the different groups in a case when a pairwise test is significant and its *q* statistical value is greater than the table *q* parameter. Multisample median test of FRAP values were chosen from the compared fruit juice triplets as similar or homogenous subsets durian and avocado (Table 2).

In our recent investigations (Toledo *et al.*, 2008), 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 to the human diet. It was proposed that durian Mon Thong is especially recommended for such purposes. Comparison of different

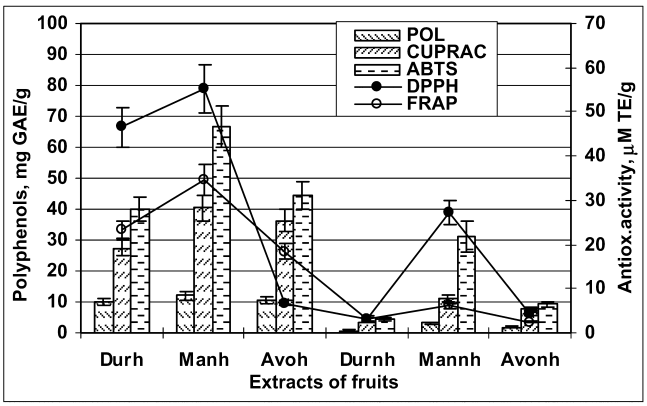


Figure 3. Polyphenols and antioxidant activities of different fruit extracts. Durh, Manh and Avoh, durian, mango and avocado, extracted with 50% methanol and hydrolysis; and Durnh, Mannh, Avonh, extracted with 50% methanol without hydrolysis. POL, polyphenols; CUPRAC, cupric reducing antioxidant capacity; ABTS, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt; DPPH, 1-diphenyl-2-picrylhydrazyl method; FRAP, ferric-reducing/antioxidant power; GAE, gallic acid equivalent; Antiox, antioxidant. All data calculated per gram d.w.

Table 1. Comparison of durian, avocado and mango fruits according to summary statistics of FRAP data (μM TE/g d.w.)

	Durian	Avocado	Mango
Valid cases	25.0000	25.0000	25.0000
Mean	23.2284	14.8352	34.6248
Median	22.1200	13.0800	34.3300
Variance	16.0319	15.8112	14.8398
Standard deviation	4.0040	3.9763	3.8522
Standard error	0.8008	0.7953	0.7704
Coefficient of variation	0.1724	0.2680	0.1113
Minimum	16.0800	11.0900	28.6200
Maximum	33.1100	23.7400	43.4900
Range	17.0300	12.6500	14.8700
Lower quartile	20.3200	12.3700	32.0300
Upper quartile	26.2400	15.9300	36.6800
Interquartile range	5.9200	3.5600	4.6500
Skewness	0.6617	1.2934	0.7075
Standard error of skewness	0.4637	0.4637	0.4637
Kurtosis	0.0214	0.2339	0.2128
Standard error of kurtosis	0.9017	0.9017	0.9017

Table 2. Comparison of mango, avocado and durian fruit extracts by multisample median test of FRAP values

Comparison	*q* Statistic	Table *q*	Significance	Lower 95%	Upper 95%	Result
Mango–avocado	9.1393	3.3145	0.0000	14.6587	31.3413	Different
Durian–avocado	3.1789	3.3145	0.0634	–0.3413	16.3413	Homogenous subsets
Mango–durian	5.9604	3.3145	0.0001	6.6587	23,3413	Different

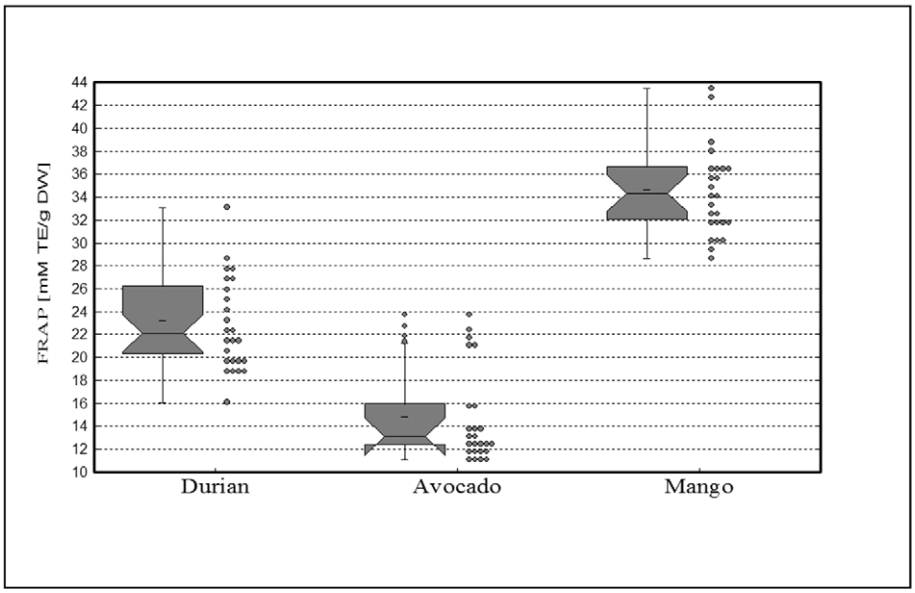


Figure 4. Box/whisker and dot plots showing FRAP data distribution of durian, avocado and mango fruits.

stages of ripening showed that ripe durian contains higher quantity of bioactive compounds, has higher antioxidant capacity and nutritional value (Haruenkit *et al*., 2010). It positively affects the plasma lipid profile, the plasma glucose and the antioxidant activity in rats fed cholesterol-enriched diets. In our previous investigations of tropical fruits we have found that snake fruit and mangosteen contain high quantities of total polyphenols and possess high antioxidant potentials (Haruenkit *et al*., 2007). A cholesterol-containing diet supplemented with these exotic fruits showed a positive affect on rat plasma lipid levels, especially on fibrinogen fraction, and on the antioxidant activity. In this study durian (*Durio zibethinus* Murr.), mango (*Mangifera indica* L.) and avocado (*Persea Americana*) were compared as dietary supplements. It was shown that durian (Khoo *et al*., 2008), mango (Robles-Sanchez *et al*., 2009) and avocado (Terasawa *et al*., 2006) possess high nutritional values and bioactive properties. In order to find out which of these three fruits is preferable for consumption, the above-mentioned properties of durian, mango and avocado were studied and compared as a supplement to food diets.

The contents of total fibre, total proteins and total fats are significantly higher and total carbohydrates significantly lower in avocado ($p > 0.05$). Similar results were reported by Mahattanatawee *et al*. (2006), who investigated 14 tropical fruits from south Florida.

The results show that total dietary fibre ranged from 0.9 to 7.2 g/100 g, in agreement with our data (Mahattanatawee *et al*., 2006). Others reported that avocado samples contained 5.23 ± 0.53 g of water-soluble dietary fibre and 11.3 ± 0.71 g of water-insoluble dietary fibre per 100 g edible portion on a dry-matter basis (Hirasawa *et al*., 2008).

It was found that the content of total phenols was high in all three studied fruits and comparable ($p > 0.05$). Conversely, the content of tannin ($R^2 = 0.9948$) was significantly higher in durian methanol extracts ($p < 0.05$).

It was shown in recent publications that the polyphenols in mango and avocado recalculated on dry weight were 2.57 and 0.70 mg GAE/g d.w. (Wolfe *et al*., 2008). The value of polyphenol content corresponds with our obtained results for mango in non-hydrolysed fraction (Mannh).

According to used antioxidant assays the antioxidant potential in all investigated fruits was high. The antioxidant potentials of fruit methanol extracts using hydrolysis for polyphenol extraction were significantly higher than without this procedure ($p < 0.05$). Also, other investigators show that the antioxidant potential of tropical fruits was high (Mahattanatawee *et al*., 2006). Therefore, these authors reported that the antioxidant potential according to Oxygen Radical Absorbance Capacity (ORAC) and DPPH was <0.1–16.7 μmol Trolox equiv/g puree, and 2.1–620.2 μg gallic acid equiv/g puree, respectively.

Others showed that the antioxidant activity determined by ORAC for avocado was 39.08 μM TE/g d.w. and for mango –46.56 μM TE/g d.w. (Wolfe *et al*., 2008). Our results by ABTS for avocado (Avoh) and for mango (Manh) were higher than those cited above (Wolfe *et al*., 2008).

Hydrolysed methanol extract of mango (Fig. 3, Manh) was slightly lower than the reported value of 41 μM TE/g d.w. The DPPH values for Avoh and Avonh were in the range 4.18–6.81 μM TE/g d.w. in comparison with 5.82 μM TE/g d.w. (Corral-Aguayo *et al*., 2008). Mango (Fig. 3, Manh, Mannh) showed 27.14–46.49 μM TE/g d.w. and these data corresponded to 82 μM TE/g d.w. (Corral-Aguayo *et al*., 2008). Mango in methanol contained total phenolics of 2.30 mg GAE/g d.w. in comparison with our results of 3.1 mg GAE/g d.w. for Mannh.

The experimental results of hydrophilic fractions extracted using different proportions of methanol with and without hydrolysis were close to literature values of total polyphenols and their antioxidant activities (Vinokur and Rodov, 2006).

Conclusions

Nutritional and bioactive values of durian are comparable with these indices in mango and avocado. These fruits contain high, comparable quantities of basic nutritional and antioxidant compounds, and possess high antioxidant potentials. All fruits show high level of correlation between the contents of phenolic

compounds and the antioxidant potential. The methods used (three-dimensional fluorescence, FTIR spectroscopy and radical scavenging assays) are suitable for bioactivity determination of these fruits. In order to obtain the best results, a combination of these fruits has to be included in diets.

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