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Comparison of bioactive compounds, antioxidant and antiproliferative activities of Mon Thong durian during ripening

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ABSTRACT

The aim of this investigation was to compare the bioactive and nutrient compounds, fatty acids, and antioxidant and antiproliferative activities of Mon Thong durian at different stages of ripening. It was found that the total polyphenols, flavonoids, flavanols, ascorbic acid, tannins and the antioxidant activity determined by four assays (CUPRAC, DPPH, ABTS and FRAP) differed in immature, mature, ripe and overripe samples. The content of polyphenols and antioxidant activity were the highest in overripe durian, flavonoids were the highest in ripe durian, and flavanols and antiproliferative activity were the highest in mature durian ($p < 0.05$). FTIR spectra of polyphenols, HPLC profiles of fatty acids, the antioxidant and antiproliferative activities can be used as indicators to characterise different stages of durian ripening.

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1. Introduction

Durian (*Durio zibethinus Murray*) is one of the most important seasonal fruits in tropical Asia. Durian cultivars are derived from *D. zibethinus Murray*, originating in the Malay Peninsula (Voon, Hamid, Rusul, Osman, & Quek, 2007). The importance of this fruit is mostly connected with its composition and antioxidant properties (Arancibia-Avila et al., 2008; Leontowicz et al., 2008; Toledo et al., 2008). It has been reported that durian has additional valuable health properties: polysaccharide gel, extracted from the fruit hulls, reacts on immune responses and is responsible for cholesterol reduction (Chansiripornchai, Chansiripornchai, & Pongsamart, 2008). The glycaemic index of durian was the lowest in comparison with papaya and pineapple (Daniel, Aziz, Than, & Thomas, 2008). The health properties of durian are based not only on the antioxidant properties, but also on

its fatty acid composition. Cholesterol hypothesis implied that reducing the intake of saturated fats and cholesterol while increasing that of polyunsaturated oils is effective in lowering serum cholesterol, and thereby in reducing coronary heart disease. The protective activity is linked with a high supply of $n - 3$ fatty acids coming from fish and seafood, and high consumption of wholegrain products, as well as fruits and vegetables (Siondalski & Lysiak-Szydłowska, 2007). Durian is rich in $n - 3$ fatty acids, compared to some other fruits (Phutdhawong, Kaewkong, & Buddhasukh, 2005).

Recently, it has been shown that individuals who eat daily five servings or more of fruits and vegetables have approximately half the risk of developing a wide variety of cancer types, particularly those of the gastrointestinal tract (Gescher, Pastorino, Plummer, & Manson, 1998). Therefore the antiproliferative activities of methanol extracts of Mon Thong durian at different stages of ripening on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma) were determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. As far as we know there are no published results of such investigations.

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2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent (FCR), lanthanum (III) chloride heptahydrate, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 2,9-dimethyl-1,10-phenanthroline (neocuproine), ascorbic acid and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co., St. Louis, MO. 2,4,6-Tripyridyl-s-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. Individual fatty acids were purchased from Sigma–Aldrich (Steinheim, Germany). All solvents used as reaction media were of analytical grade and were obtained from POCh (Gliwice, Poland). Acetonitrile (ACN) and tetrahydrofuran (THF) used as mobile phases were of HPLC grade and were purchased from Merck (Darmstadt, Germany).

2.2. Samples and preparation

All durian samples were harvested in May, 2008, from a 25-year old Mon Thong commercial durian orchard, in Chantaburi province, eastern Thailand.

The maturity of the durian fruits was determined by combined techniques: day count, character of fruit spines, tapping the fruit, colour and shape of the fruit (Yaacob & Subhadrabandhu, 1995). The Mon Thong durian samples at various stages of ripening (1, immature; 2, mature; 3, ripe; 4, overripe) were chosen according to the following criteria:

1. Immature durian (80% maturity) was taken 5 days before the harvest.
2. Mature durian (100% maturity): harvested around 120–125 days after fruit set, using several methods and was prepared 1 day after harvest. Harvesting and determination of durian maturity was carried out by skilled workers. The mature samples were cut with peduncle intact and brought down carefully. The samples were left for 1 day and cut open to get mature durian flesh with firm texture and no smell.
3. Ripe durian: mature samples were left to soften (the stage that can be consumed), which normally takes 3–5 days till the flesh is losing contact with the thick shell.
4. Overripe durian: ripe durian after 3–5 days of ripening.

2.3. Fourier-transform infrared (FTIR) spectra of polyphenols

The presence of polyphenols (flavonoids and phenolic acids) in the investigated durian samples was studied by Fourier-transform infrared (FTIR) spectroscopy. A Bruker Optic GmbH Vector FTIR spectrometer (Bruker Optic GmbH, Attingen, Germany) was used to record IR spectra. A potassium bromide microdisk was prepared from finely ground lyophilised powder of 2 mg of durian sample with 100 mg of KBr (Edelmann & Lendl, 2002).

2.4. Determination of nutrients, polyphenols, flavonoids, flavanols, tannins and ascorbic acid

Elemental analysis, minerals and trace elements were determined as previously described (Poovarodom & Phanchindawan, 2006). The following solvents were used for extraction of bioactive compounds: sample/methanol, sample/water and sample/acetone as 25, 25 and 40 mg/ml, respectively, at room temperature for 2 h twice as previously described (Leontowicz et al., 2008, 2007). To determine the total amount of polyphenols in the studied ex-

tracts, Folin–Ciocalteu reagent (FCR) was used, and the measurement was performed at 765 nm with gallic acid as the standard. Results were expressed as mg of gallic acid equivalents (GAE). Flavonoids, extracted with 5% NaNO_2 , 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 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 (procyranidins) 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).

Total ascorbic acid was determined by CUPRAC assay (Ozyurek, Guclu, Bektasoglu, & Apak, 2007). The water extract was prepared from 100 mg of lyophilised sample and 5 ml of freshly prepared nitrogen-bubbled water, and stirred for 30 min at 4 °C and centrifuged. This extract (1 ml) was mixed with 2 ml of 3.0 mM lanthanum (III) chloride heptahydrate, also prepared with nitrogen-bubbled water. Ethyl acetate (EtAc) was used for extraction of flavonoids, in order to avoid interference. The remaining tannin in aqueous solution of fruit extracts after ethyl acetate treatment was checked.

Ascorbic acid was quantified in the aqueous phase. One millilitre of Cu (II)–neocuproine (Nc), in ammonium acetate-containing medium at pH 7, was added to 1 ml of the obtained extract. The absorbance of the formed bis-(Nc)–copper (I) chelate was measured at 450 nm (Ozyurek et al., 2007).

2.5. Determination of the antioxidant activity

The following four tests were applied:

1. 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 durian sample (or standard) solution (x ml) and H_2O [(1.1 – x) ml] was added to make a final volume of 4.1 ml. The absorbance at 450 nm was recorded against a reagent blank (Apak, Guclu, Ozyurek, & Karademir, 2004).
2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) solution (3.9 ml, 25 mg/l) in methanol was mixed with the samples extracts (0.1 ml). The reaction progress was monitored at 515 nm until the absorbance was stable (Ozgen, Reese, Tulio, Scheerens, & Miller, 2006).
3. 2,2-Azino-bis(3-ethyl-benzothiazoline-6-sulphonic acid) diammonium salt (ABTS^+) was generated by the interaction of ABTS (7 mM) and $\text{K}_2\text{S}_2\text{O}_8$ (2.45 mM). This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm (Ozgen et al., 2006).
4. Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants contained in the samples to reduce ferric-tripyridyltriazine (Fe^{3+} -TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm (Ozgen et al., 2006; Szeto, Tomlinson, & Benzie, 2002).

2.6. Fatty acids

2.6.1. Chromatographic procedure

2.6.1.1. *Sample preparation.* Lyophilised durian samples (~150 mg) were hydrolysed with 1 ml of 2 M KOH in $\text{MeOH}:\text{H}_2\text{O}$ (1:1; v/v) at 80–85 °C for 1.5 h in PTFE linked screw-capped amber coloured tubes. After cooling, the hydrolysates were acidified with 4 M HCl (~0.5 ml) to pH ~2, and the free fatty acids were extracted twice with 1 ml of *n*-heptane. The upper organic layer was separated, dried with Na_2SO_4 , and then heptane was removed under

a gentle stream of nitrogen at 40 °C. The residue was used for derivatisation (Czauderna & Kowalczyk, 2001).

2.6.1.2. Derivatisation procedure. Fatty acid standards (0.5–100 µg/ml) and fatty acids released by saponification from durian lipid extract were converted to fatty acid *p*-bromophenacyl ester, according to the modified method of Wood and Lee (1983) (Fig. 1). To the residue in a PTFE linked screw-capped amber coloured tube 200 µl of α -bromoacetophenone solution (10 mg/ml in acetone) and 200 µl of triethylamine solution (10 mg/ml in acetone) were added. The contents were ultrasonicated and heated for 30 min at 50 °C in an ultrasonic bath. The resulting solution was evaporated to dryness under a gentle stream of nitrogen at 40 °C. A 250 µl volume of acetonitrile:acetone (1:1; v/v) was added to the tube. The resulting solution was filtered and injected into the column. Analytes were dissolved in a mixture of acetonitrile:acetone (1:1; v/v) and 5 µl of the solution were injected onto the chromatographic column. A mixture of **A**, water and **B**, ACN + THF (99:1; v/v), was used as a mobile phase in gradient mode at flow rate of 2.0 ml min⁻¹. Gradient at 5 min was 80% **B**; at 8 min – 85% **B**; at 20 min – 98% **B**. The UV detector was operated at 258 nm (diode-array detector in single wavelength mode). All analyses were thermostatted at 40 °C. The concentrations of fatty acids in biological samples were calculated using fatty acid standards and an internal standard (hexadecanoic acid) as a measure of extraction yield. The limit of detection (LOD) was calculated as a signal-to-noise ratio of three, while the limit of quantification (LOQ) was defined as 10 times the noise level (Czauderna & Kowalczyk, 2001; Meyer, 1999; Wood & Lee, 1983).

2.6.1.3. Apparatus. Chromatographic analyses were performed using liquid chromatograph Series 1200 (Agilent Technology Inc., Santa Clara, CA) equipped with quaternary pump, autosampler, thermostatted column compartment and diode-array detector. Supelcosil LC-18 HPLC column (150 × 4.6 mm; particle size 3 µm) was obtained from Supelco (Bellefonte, PA).

2.7. Determination of the antiproliferative activity

The antiproliferative activities of 100% methanol extracts of four stages of durian ripening on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma) were measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cell lines were purchased from Korean Cell Line Bank (KCLB; Seoul, Korea). Cells were grown in RPMI-1640 medium at 37 °C under 5% CO₂ in a humidified incubator. Cells were harvested, counted (3 × 10⁴ cells/ml), and transferred into a 96-well plate, and incubated for 24 h prior to the addition of methanol extracts of durian. Serial dilutions of the extracts were prepared by dissolving compounds in dimethyl

sulfoxide (DMSO), followed by dilution with RPMI-1640 medium to give final concentrations at 125, 250, 500, 1000 and 2000 µg/ml. Stock solutions of samples were prepared for cell lines at 90 µl and samples at 10 µl, and incubated for 72 h. MTT solution at 5 mg/ml was dissolved in 1 ml of phosphate buffer solution (PBS), and 10 µl of it were added to each of the 96 wells. The wells were wrapped with aluminium foil and incubated at 37 °C for 4 h. The solution in each well containing media, unbound MTT and dead cells were removed by suction and 150 µl of DMSO were added to each well. The plates were then shaken and optical density was recorded using a microplate reader at 540 nm. Distilled water was used as positive control and DMSO as solvent control (Chon, Heo, Park, Kim, & Gorinstein, 2009). The effect of the durian extract on the proliferation of cancer and normal cells was expressed as relative cell survival rate:

$$\text{percent survival rate} = 100 \times (\text{OD of durian extract treated sample} / \text{OD of non-treated sample}),$$

where OD is optical density (Kim et al., 2006).

2.8. Statistical analyses

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

3. Results

3.1. Bioactive compounds

The soil of the durian orchard was a sandy loam with pH 4.2, EC 224 µS/cm, organic matter 2.8%, available P (BrayII) 200 mg/kg, exchangeable (NH₄OAc) K, Ca and Mg, 156, 347 and 51 mg/kg, respectively; extractable (DTPA) Fe, Mn, and Zn, 17.7, 2.0 and 2.4 mg/kg, respectively. It was suggested that KCl could be used as an effective replacement for K₂SO₄ in this soil. These results were similar with our previous data (Poovarodom & Phanchindawan, 2006). According to the results summarised in Table 1, the contents of most nutrients were significantly higher (*p* < 0.05) in the immature samples (P, Ca, Mg, Fe, Cu, Zn and B). Durian samples at different stages of ripening are relatively rich in all nutrients.

The wavenumbers of FTIR spectra for catechin at 827, 1039, 1115, 1143, 1286, 1478, 1511 and 1610 cm⁻¹ were assigned to

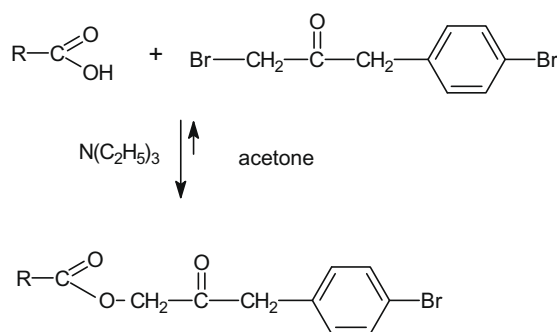


Fig. 1. Free fatty acids derivatisation reaction.

Table 1
Nutrient concentration in durian flesh at four different maturities (mg/kg DW).^{A,B}

Indices	Immature	Mature	Ripe	Overripe
N	9868 ± 412 ^b	8826 ± 4 ^a	11084 ± 419 ^c	11322 ± 421 ^c
P	1382 ± 64 ^b	946 ± 48 ^a	1094 ± 51 ^a	1103 ± 52 ^a
K	15907 ± 611 ^a	13811 ± 502 ^a	14493 ± 511 ^a	14167 ± 507 ^a
Ca	406 ± 19 ^b	172 ± 9 ^a	181 ± 9 ^a	181 ± 9 ^a
Mg	1100 ± 52 ^b	701 ± 32 ^a	628 ± 29 ^a	612 ± 28 ^a
Na	233 ± 12 ^b	212 ± 11 ^b	200 ± 10 ^a	187 ± 9 ^a
Fe	9.4 ± 0.5 ^b	5.7 ± 0.2 ^a	6.1 ± 0.3 ^a	6.2 ± 0.3 ^a
Mn	9.6 ± 0.5 ^b	8.3 ± 0.4 ^a	7.5 ± 0.4 ^a	9.2 ± 0.5 ^b
Cu	7.8 ± 0.4 ^b	4.8 ± 0.2 ^a	4.5 ± 0.2 ^a	4.3 ± 0.2 ^a
Zn	8.1 ± 0.4 ^b	6.5 ± 0.3 ^a	5.8 ± 0.3 ^a	6.3 ± 0.3 ^a
B	6.2 ± 0.3 ^b	7.0 ± 0.4 ^b	4.1 ± 0.2 ^a	3.9 ± 0.2 ^a

^A Values are means ± SD of five measurements.

^B Values in rows with different superscript letters are significantly different (*p* < 0.05).

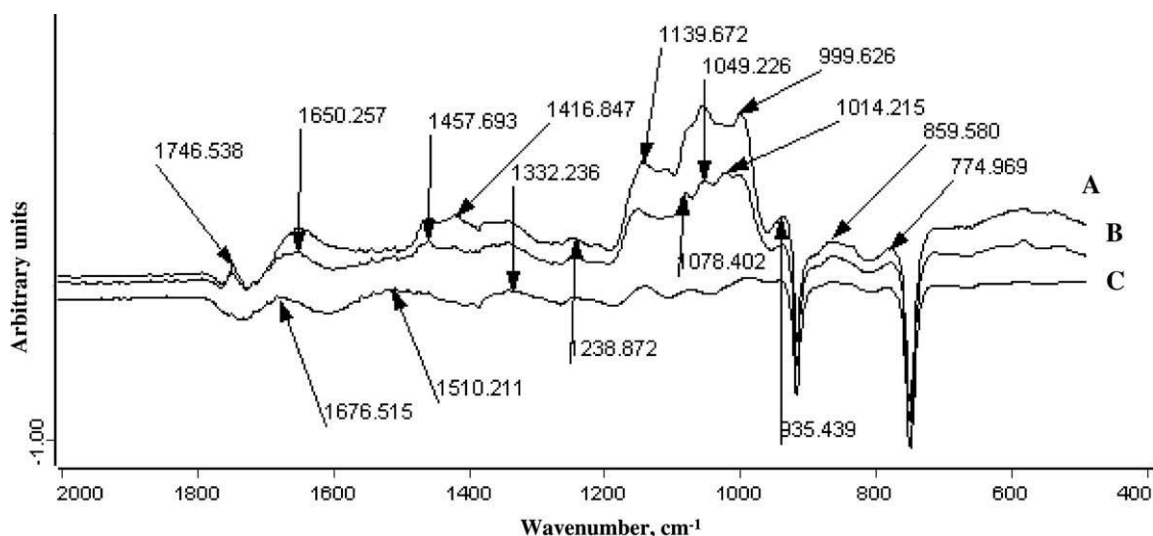


Fig. 2. FTIR spectra of ripe (C), mature (B) and overripe (A) durian samples.

C–H alkenes, –C–O alcohols, C–OH alcohols, –OH aromatic, C–O alcohols, C–H alkanes, C=C aromatic ring and C=C alkenes. Gallic acid showed the following wavenumbers (cm^{-1}) of 866, 1026, 1238, 1450, 1542 and 1618. Immature, mature, ripe and overripe durian samples in the region of polyphenols showed slightly different bands than the standards, but the wavenumbers of the bands were similar in this group (Fig. 2). The absorption bands at 1650, 1458, 1417 and 1014 cm^{-1} were absent in ripe durian, in comparison with the immature, mature and overripe samples. Oppositely, bands at 1677 and 1510 cm^{-1} were observed in ripe durian. Other bands in the durian samples were slightly shifted in comparison with the standards (Fig. 2).

3.2. Polyphenols, flavonoids, flavanols, tannins and ascorbic acid

Different solvents (water, acetone and methanol) showed variation in the amounts of the bioactive compounds. Methanol was the most effective solvent based on extraction yield. The amount of polyphenols (mg GAE/g) extracted from four durian samples with methanol, water and acetone was 12.3, 10.3 and 7.2, respectively (Tables 2 and 3). The contents (Table 2) of polyphenols in overripe durian, flavonoids in ripe durian, flavanols in mature durian, and tannins in mature and ripe durian were the highest in methanol extracts ($p < 0.05$). Polyphenols in acetone extracts were the highest in overripe samples (Table 2, $p < 0.05$). The contents of flavanols and ascorbic acid were the highest in overripe samples extracted with water (Table 3).

3.3. Antioxidant activity

The antioxidant activity of the methanol extract of overripe sample was the highest ($p < 0.05$), according to CUPRAC, DPPH, ABTS and FRAP tests. Acetone extract of immature sample by CUPRAC and ABTS (Table 4) showed the lowest value. The antioxidant activity of the water extracts (Table 5) was the highest in mature and ripe durian using ABTS test and in overripe durian using FRAP test samples ($p < 0.05$).

3.4. Fatty acids

The contents (Table 6) of capric acid was the highest in overripe durian, myristic and palmitic acids in overripe and ripe durian,

oleic acid in ripe and linoleic acid in immature and mature samples ($p < 0.05$). The HPLC profiles (Fig. 3) revealed that the major unsaturated fatty acids were oleic and linoleic, while the main saturated was palmitic. Capric, myristic, stearic and arachidic acids were determined in the samples. Palmitic (16:0), oleic (18:1) and linoleic (18:2) acids were among the major fatty acids throughout the maturation and ripening of the fruits. The levels of these fatty acids were found to be significantly different ($p < 0.05$) between the four maturity stages. These results show that durian has moderate levels of fatty acids that significantly changed during maturation and ripening.

Table 2

The contents of the studied biocompounds extracted with methanol and acetone at different stages of durian ripening^{A,B,C}

Samples	Methanol				Acetone
	Polyphenols (mg GAE/g)	Flavonoids (mg CE/g)	Flavanols ($\mu\text{g CE/g}$)	Tannins (mg CE/g)	Polyphenols (mg GAE/g)
Immature	1.3 \pm 0.2 ^a	1.0 \pm 0.1 ^a	116.3 \pm 8.6 ^a	0.7 \pm 0.07 ^a	1.2 \pm 0.1 ^a
Mature	3.4 \pm 0.3 ^b	1.2 \pm 0.1 ^a	135.5 \pm 8.9 ^c	0.8 \pm 0.07 ^b	2.0 \pm 0.2 ^b
Ripe	3.3 \pm 0.3 ^b	2.2 \pm 0.1 ^c	101.0 \pm 8.2 ^a	0.8 \pm 0.07 ^b	1.7 \pm 0.1 ^a
Overripe	4.3 \pm 0.4 ^c	1.7 \pm 0.1 ^b	121.6 \pm 8.7 ^b	0.7 \pm 0.07 ^a	2.3 \pm 0.2 ^b

^A Values are means \pm SD of five measurements.

^B Values in columns with different superscript letters are significantly different ($p < 0.05$).

^C Per g DW (dry weight).

Table 3

The contents of the studied biocompounds extracted with water at different stages of durian ripening^{A,B,C}

Samples	Polyphenols (mg GAE/g)	Flavonoids (mg CE/g)	Flavanols ($\mu\text{g CE/g}$)	Vit. C (mg Asc/g)	Tannins (mg CE/g)
Immature	2.0 \pm 0.2 ^a	0.5 \pm 0.03 ^a	34.9 \pm 2.6 ^a	4.3 \pm 0.3 ^a	0.2 \pm 0.02 ^a
Mature	2.8 \pm 0.2 ^b	1.5 \pm 0.1 ^b	62.0 \pm 5.6 ^b	9.4 \pm 0.7 ^c	0.6 \pm 0.05 ^c
Ripe	2.6 \pm 0.3 ^b	1.5 \pm 0.1 ^b	67.1 \pm 5.2 ^b	8.0 \pm 0.6 ^b	0.5 \pm 0.04 ^c
Overripe	2.9 \pm 0.3 ^b	1.6 \pm 0.1 ^b	73.9 \pm 6.4 ^c	11.3 \pm 1.1 ^d	0.4 \pm 0.03 ^b

^A Values are means \pm SD of five measurements.

^B Values in columns with different superscript letters are significantly different ($p < 0.05$).

^C Per g DW (dry weight).

Table 4The antioxidant activity ($\mu\text{mol Trolox equiv./g}$) of the studied biocompounds extracted with methanol and acetone at different stages of durian ripening.^{A,B,C}

Sample	CUPRAC ^D	CUPRAC ^E	DPPH ^D	ABTS ^D	ABTS ^E	FRAP ^D
Immature	17.3 \pm 1.1 ^a	5.0 \pm 0.4 ^a	4.6 \pm 0.3 ^a	12.4 \pm 1.6 ^a	3.8 \pm 0.3 ^a	10.3 \pm 1.1 ^a
Mature	22.5 \pm 1.4 ^b	7.1 \pm 0.6 ^b	8.0 \pm 0.6 ^c	20.6 \pm 1.8 ^b	4.9 \pm 0.4 ^b	12.0 \pm 1.2 ^a
Ripe	26.1 \pm 2.1 ^b	6.8 \pm 0.5 ^b	6.1 \pm 0.5 ^b	31.3 \pm 2.6 ^c	4.1 \pm 0.4 ^b	14.9 \pm 1.3 ^b
Overripe	29.3 \pm 2.5 ^c	7.1 \pm 0.6 ^b	10.0 \pm 0.8 ^d	44.1 \pm 3.3 ^d	4.4 \pm 0.4 ^b	17.0 \pm 1.4 ^c

^A Values are means \pm SD of five measurements.^B Values in columns with different superscript letters are significantly different ($p < 0.05$).^C Per g DW (dry weight).^D Extraction at room temperature in concentration of 25 mg lyophilised sample in 1 ml methanol.^E Extraction at room temperature in concentration of 40 mg lyophilised sample in 1 ml acetone.**Table 5**The antioxidant activity ($\mu\text{mol Trolox equiv./g}$) of the studied biocompounds extracted with water at different stages of durian ripening.^{A,B,C}

Sample	CUPRAC	DPPH	ABTS	FRAP
Immature	17.0 \pm 1.5 ^a	4.8 \pm 0.3 ^a	32.1 \pm 2.6 ^a	14.1 \pm 1.3 ^a
Mature	22.9 \pm 1.9 ^b	5.7 \pm 0.4 ^b	40.0 \pm 4.1 ^c	25.8 \pm 2.2 ^b
Ripe	22.1 \pm 1.9 ^b	5.2 \pm 0.4 ^b	39.4 \pm 3.9 ^c	17.1 \pm 1.3 ^a
Overripe	22.9 \pm 1.9 ^b	5.8 \pm 0.5 ^b	36.8 \pm 3.1 ^b	40.0 \pm 3.5 ^c

^A Values are means \pm SD of five measurements.^B Values in columns with different superscript letters are significantly different ($p < 0.05$).^C Per g DW (dry weight).**Table 6**Fatty acids composition ($\mu\text{g/g DW}$) of the durian samples.^A

Durian samples	Capric C 10:0	Myristic C 14:0	Palmitic C 16:0	Stearic C 18:0	Arachidic C 20:0	Oleic C 18:1	Linoleic C 18:2
Immature	2.9 \pm 0.1 ^a	26.2 \pm 5.4 ^a	2214.5 \pm 30.2 ^a	98.9 \pm 6.2 ^a	18.0 \pm 2.7 ^a	1789.7 \pm 37.8 ^a	711.3 \pm 12.5 ^b
Mature	3.5 \pm 0.1 ^a	48.9 \pm 10.9 ^a	2754.6 \pm 52.8 ^a	113.4 \pm 20.9 ^a	18.9 \pm 3.0 ^a	2113.8 \pm 41.6 ^a	513.0 \pm 10.6 ^b
Ripe	6.1 \pm 2.2 ^b	94.2 \pm 8.9 ^b	3421.3 \pm 26.6 ^b	125.5 \pm 10.1 ^a	18.8 \pm 1.8 ^a	2608.3 \pm 28.3 ^c	351.2 \pm 39.9 ^a
Overripe	8.8 \pm 0.5 ^c	102.9 \pm 11.5 ^b	3268.2 \pm 41.2 ^b	121.0 \pm 15.7 ^a	20.3 \pm 1.9 ^a	2314.6 \pm 30.1 ^b	354.6 \pm 48.5 ^a

^A Values are mean \pm SD of triplicate extractions.

3.5. Antiproliferative activity

It was observed that the antiproliferative activities of the methanol extracts of immature, mature, ripe and overripe durian samples on two cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma) were different (Fig. 4). The cell survival rate (%) for concentrations of 2000 $\mu\text{g/ml}$ for mature durian on Calu-6 was 86.8 ± 1.5 , and on SNU-601 was 88.5 ± 2.5 , showing the highest antiproliferative activity in comparison with other samples. Our investigation shows that antioxidant activity of the studied samples was not always correlated with their antiproliferative activity.

4. Discussion

Fruits and vegetables contain a variety of phytochemicals, including flavonoids, which have antioxidant and anticancer properties. The purpose of this study was to evaluate the bioactive and nutrient compounds, fatty acids, antioxidant activity and the antiproliferative effects of durian at different stages of ripening on human cell lines.

Our results in observation of FTIR polyphenol spectra were in accordance with others (Edelmann & Lendl, 2002; Sinelli, Spinardi, Di Egidio, Mignani, & Casiraghi, 2008). FTIR spectroscopy can be used as an additional tool to screen fruits for the content of phenolic compounds during their ripening.

Our results correspond with Harris and Brannan (2009), who reported that total phenolics were affected by ripeness, so the con-

centration of total phenolics in pawpaw pulp were in the order: underripe = ripe > overripe, while the concentration of flavonoids was in the order: ripe < underripe < overripe. Total phenolics were positive correlated with reducing and radical-scavenging potentials. These results indicate that pawpaw pulp of varying ripeness levels is a potential source of natural phenolic and flavonoid antioxidants that could lead to the development of value-added products from pawpaw. Durian, like pawpaw, can be used as a nutritional supplement to the everyday diet.

Our results were compared with Chaisuksant, Boonyuen, and Sunthornwat (2008), where immature green, mature green and orange ripe fruits of bullet wood (*Mimusops elengi*) were investigated. The methanol–acetone extracts were further separated into three different fractions designated as free phenolic acid (F1), soluble phenolic ester (F2) and insoluble phenolic acid ester (F3). The relative antioxidant capacities of extracts of immature and mature fruits, expressed as gallic acid equivalents, were $F2 > F3 > F1$ and of ripe fruit $F2 = F3 > F1$. The antioxidant capacity of crude methanol–acetone extract from immature fruit was higher than that of either the mature or the ripe fruit. Our results differ and showed that the antioxidant activity of overripe fruit was higher than the other samples.

The antioxidant activity of some fruits at different stages of ripening depends on the scavenging methods used for their determination (Lin, Wu, Tsai, Yang, & Chen, 2007). For example the oxygen-scavenging capacity of mature meifruit (*Prunus mume Seibu. et Zucc*) in water fraction was lower than that of Trolox. However, both oxygen- and hydroxyl-scavenging capacities of

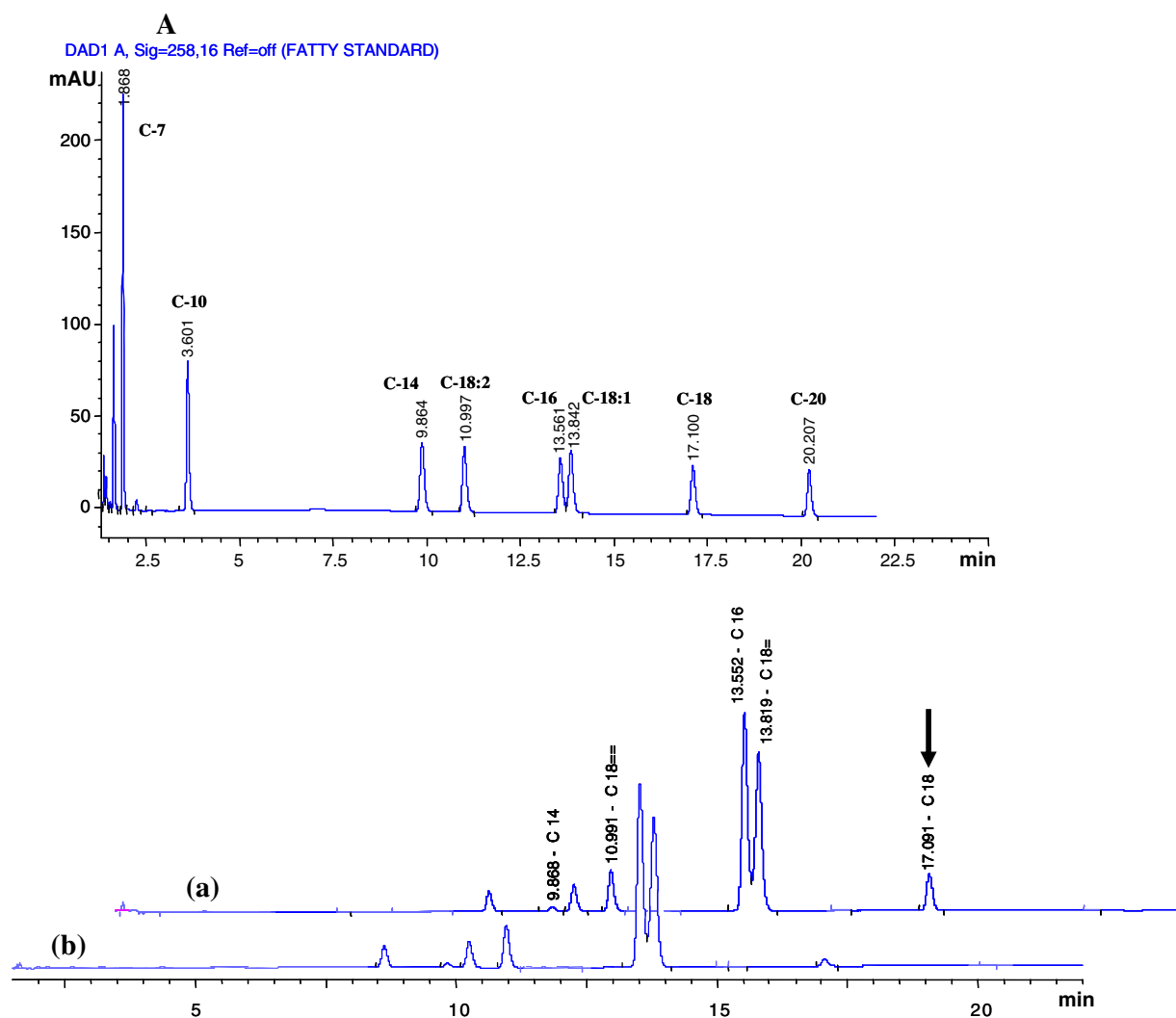


Fig. 3. (A) The typical chromatogram of fatty acids standard solution. Analytes: C-7, enanthic acid; C-10, capric acid; C-14, myristic acid; C-18:2, linoleic acid; C-16, palmitic acid; C-18:1, oleic acid; C-18, stearic acid; C-20, arachidic acid. (a) Chromatograms of derivatised mature durian hydrolysates spiked with C₁₈ fatty acid; (b) not spiked.

immature fruit and hydroxyl-scavenging capacities of mature fruit showed no significant difference from Trolox. Similar results were obtained with the ABTS-scavenging method in durian samples of water extracts. The amount of polyphenols in this fruit was about 5 times higher than in overripe durian water extract (Table 3).

The change in the amount of polyphenols during ripening was reported also in three apricot cultivars (Dragovic-Uzelac, Levaj, Mrkic, Bursac, & Boras, 2007). The content of individual polyphenols during ripening was quite similar, whereas their amounts differed significantly. Immature fruits showed the highest level of polyphenols, which decreased at semi-mature fruits and did not change remarkably in mature fruits. The quantity of polyphenols during fruits ripening depends on cultivars, therefore this comparison would not give results similar to those for durian fruit.

The fatty acid composition in durian samples similar to other reports with some slight differences (Phutdhawong et al., 2005): the most prominent component was stearic acid Me ester (35.9%), then palmitic acid Me ester (32.9%), oleic acid Me ester (4.67%), myristic acid Me ester (2.5%) and linoleic acid Me ester (2.2%). Other authors found that during ripening the content of fatty acids increases significantly. Wissem, Baya, and Brahim (2008), reported that total fatty acid contents of *Myrtus communis* var. *italica* fruit varied from 0.8% to 3.1% during fruit maturation

and the predominant fatty acids were linoleic (12.2–71.3%), palmitic (13.6–37.1%) and oleic (6.5–21.9%) acids. The linoleic acid proportions correlated inversely with palmitic and oleic acids during all the stages of ripening.

The content of fatty acids in durian was similar to the banana fruit. The most abundant fatty acids in the banana pulp (29–90% of the total amount of lipophilic extract), were linoleic, linolenic and oleic acids (Oliveira, Freire, Silvestre, & Cordeiro, 2008).

This information can be used by nutritionists and food technologists to improve the nutrition of local people and develop food products that would be beneficial to human health.

Epidemiological studies have consistently linked abundant consumption of fruits and vegetables to a reduction of the risk of developing several types of cancer. The methanolic fractions of different durian samples showed the highest antioxidant activity in comparison with the acetone and water extracts. The methanol fractions were selected for testing of their effect on cells. In most cases, however, the identification of specific fruits and vegetables that are responsible for these effects is still lacking, retarding the implementation of effective dietary-based chemopreventive approaches. Our previous investigations showed that the results of the antiproliferative effect of different plants of Korean salads were not consistent with the findings of DPPH radical-scavenging activ-

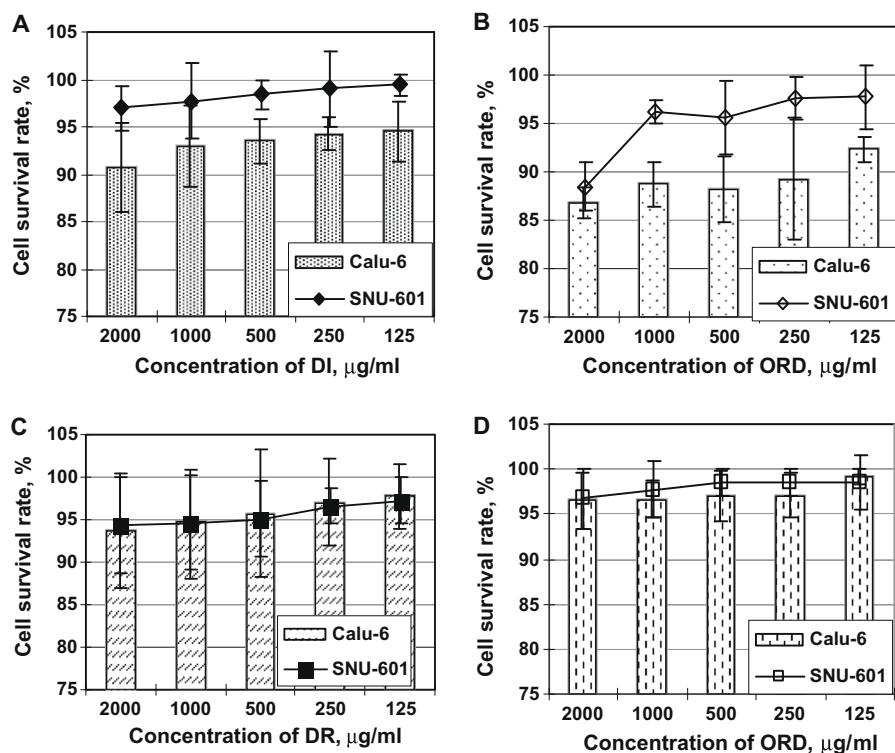


Fig. 4. Cytotoxic effect of methanol extracts from durian samples on human cancer cell lines Calu-6 (for human pulmonary carcinoma) and SNU-601 (for human gastric carcinoma): (A) DI, durian immature; (B) DM, durian mature; (C) DR, durian ripe; (D) ORD, overripe durian.

ity or total phenolic content (Chon et al., 2009). Mature durian sample showed the highest antiproliferative activity. This can be explained not only by relatively high antioxidant activity, but by the amount of flavonoids and other bioactive compounds. The results on cell proliferation can be explained as a synergistic effect of flavonoids, flavanols and ascorbic acid in mature durian. Our data correspond with others (Campbell, King, Harmston, Lila, & Erdman, 2006), that combinations of flavonoids, which are naturally present in whole fruits and vegetables, are more effective in cancer cell growth inhibition than the individual flavonoids. Durian can be used as a potential source of high-value phytochemicals with nutraceutical and functional food additive applications.

5. Conclusions

It was found that the contents of polyphenols, flavonoids, flavanols, ascorbic acid and tannins in Mon Thong durian cultivar in its different stages of ripening (immature, mature, ripe and overripe) and the antioxidant activity as determined by four complementary assays (CUPRAC, DPPH, ABTS and FRAP) was different. The content of flavonoids was the highest in ripe durian and flavanols in mature samples ($p < 0.05$). The antiproliferative activity of mature durian was the highest ($p < 0.05$).

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