

The nutritional and metabolic indices in rats fed cholesterol-containing diets supplemented with durian at different stages of ripening

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Abstract. The aim of this investigation was to assess the nutritional and health properties of Mon Thong durian cultivar at different stages of ripening. The assessment was carried out *in vitro* and *in vivo*. The contents of dietary fibers, minerals and trace metals at different stages of ripening were comparable. Total polyphenols (mgGAE/100 g FW) and flavonoids (mg CE/100 gFW) in ripe durian (358.8 ± 31.4 and 95.4 ± 9.3) were significantly higher ($p < 0.05$) than in mature (216.1 ± 1 and 39.9 ± 3.8) and overripe (283.3 ± 26.2 and 53.5 ± 4.9).

Antioxidant capacity ($\mu\text{MTE}/100$ g FW) in total polyphenol extracts of ripe durian measured by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) assays (259.4 ± 23.6 and 2341.8 ± 93.2) were significantly higher ($p < 0.05$) than that of mature (151.6 ± 15.2 and 1394.6 ± 41.5) and overripe (201.7 ± 19.4 and 1812.2 ± 61.4) samples. The correlation coefficients between the bioactive compounds in different stages of ripening and their antioxidant capacities were high ($R^2 = 0.99$). Then 35 male Wistar rats were divided into 5 dietary groups each of 7 and named Control, Chol, Chol/Mature, Chol/Ripe and Chol/Overripe. During 30 days of the experiment the rats of all 5 groups were fed

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basal diet (BD), which included wheat starch, casein, soybean oil, vitamin and mineral mixtures. The rats of the Control group were fed a BD only. To the BD of the Chol group was added 1% of cholesterol. The BD of the Chol/Mature, Chol/Ripe and Chol/Overripe groups was supplemented with 1% of cholesterol and 5% of the mature, ripe and overripe durian as freeze-dried powder, respectively. Diets containing ripe and to a lesser degree mature and overripe durian significantly hindered the rise in plasma lipids and also hindered a decrease in plasma antioxidant activity. The nitrogen retention in rats of the Chol/Ripe group was significantly higher (63.6%, $P < 0.05$) than in other diet groups and the level of the plasma glucose remained normal. A decrease in fibrinogen fraction with ripe durian included in rat's diets was shown by electrophoretic separation. These changes were detected mostly in the low molecular weight proteins of rat's serum. Histological examination of aorta showed only slight differences in the tissue. In conclusion, ripe durian contains higher quantity of bioactive compounds, has higher antioxidant capacity and nutritional value. It positively affects the plasma lipid profile, the plasma glucose and the antioxidant activity in rats fed cholesterol enriched diets. Therefore, the ripe durian supplemented diet could be beneficial for patient suffering from hypercholesterolemia and diabetes mellitus.

Keywords: Ripe, overripe and mature durian, bioactive compounds, antioxidant capacity, nutritional value, rats, plasma lipid levels and antioxidant activity

1. Introduction

Fruits and vegetables possess health protective properties [25]. The protective effect of these natural products is mostly related to their antioxidants: phenolic compounds and to a less extent, dietary fiber [17, 32].

Nowadays the extensive trade relations make available many exotic tropical fruits to consumers practically in all countries [10]. Among these fruits durian (*Durio zibethinus* Murr. cv. Mong Tong) is less known [14]. Durian is consumed at different stages of ripening and the differences between them are practically unknown. Some authors have shown that there are significant differences in the content of bioactive compounds and antioxidant potential of traditional fruits at various stages of their ripening [41]. However, there are no such investigations of durian. Therefore it was decided to study this fruit *in vitro* at different stages of its ripening and then in an experiment on laboratory animals, to assess their nutritional values and their influence on plasma lipid, glucose levels and the plasma antioxidant activity.

It was shown that some antioxidant assays give different antioxidant activity trends [26]. Therefore, we used two each other complemented assays for the determination of the total antioxidant activity: [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and compared with the total polyphenols determined by Folin-Ciocalteu method.

There are no published articles describing investigations of durian at different stages of its ripening including experiments on laboratory animals.

2. Materials and methods

2.1. Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid); BHA (butylated hydroxyanisole); ABTS⁺, [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)]; Folin-Ciocalteu reagent; cholesterol of analytical grade (USP), DPPH (1,1-diphenyl-2-picrylhydrazyl); FeCl₃·6H₂O; CuCl₂·2H₂O and neocuproine (2,9-dimethyl-1,10-phenanthroline) were obtained 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. Deionized and distilled water were used throughout.

2.2. Samples

In this investigation samples of the Mon Thong cultivar at different stages of ripening were studied. Harvesting and determination of maturity was carried out by very skilled Thai workers. They combined the following techniques: day count, character of fruit spines, tapping the fruit, color and shape of fruit [39]. The mature durian fruits 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. Some of the fruits were left for another 4 days to ripe till their flesh became soft and normally smell. Overripe samples with strong smell were obtained when fruits were left for another 3 days.

The edible parts of the Mon Thong at different stages of ripening 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) for one minute. A weighed portion (50–100 g) was lyophilized 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 analyzed.

2.3. Determination of dietary fibers, polyphenols and flavonoids

Dietary fibers in the samples of durian at different stages of ripening were analyzed by the modified method of Prosky et al. [29]. Samples were treated with heat-stable α -amylase, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000 g) to separate the soluble and insoluble fractions and dialysis against water.

Extraction of total polyphenols. Defatted lyophilized fruit samples were extracted from a 50 mg aliquot with 5 mL of 1.2 M HCl in 50% methanol/water for total polyphenols (TP) with heating at 90°C for 3 h. The samples were cooled, diluted to 10 mL with methanol and centrifuged for 5 min at 4000 g with a benchtop centrifuge to remove solids [37].

Polyphenols determination. The Folin-Ciocalteu method was used and the measurement was performed at 765 nm with gallic acid as the standard [34]. The results were expressed as mg gallic acid equivalents (GAE)/100 g FW.

Flavonoids. The absorbance of flavonoids (extracted with 5% NaNO_2 , 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and 1 M NaOH) was measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as mg of catechin equivalents (CE)/100 g FW.

Minerals (Na, K, Mg, Ca) and trace elements (Fe, Cu, Zn and Mn). 0.8 g of lyophilized samples was mineralized in microwave oven with concentrated HNO_3 . The concentrations of above mentioned elements were estimated by a Perkin-Elmer 5100 ZL atomic absorption spectrometer (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, England), using the flame method for Na, K, Mg, Ca, Fe, Cu, Zn and the flameless method for Mn.

2.4. Determination of the antioxidant capacities in fruits

The following assays were used:

1. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay. The volume of durian extracts in different test tubes was adjusted to 100 μL by adding MeOH. A 0.1 mM methanolic solution of DPPH was added (5 μL) to these tubes. The control was prepared as above without any extract, and MeOH was used for the baseline correction. Changes in the sample's absorbance were measured at 517 nm on an Uvikon 930 spectrometer (Kontron Instruments, Watford, UK). Butylated hydroxyanisole (BHA) was used for comparison [33].

2. Trolox equivalent antioxidant capacity (TEAC). The ABTS^{•+} [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation was generated by the interaction of ABTS (250 μM) and $\text{K}_2\text{S}_2\text{O}_8$ (40 μM). The absorbance was monitored exactly 1 and 6 min after the addition of 990 μL of ABTS^{•+} solution to 10 μL of durian extracts or Trolox standards (final concentration 0–20 μM) in methanol or phosphate-buffered saline (pH 7.4). The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the extracts and of Trolox for the standard reference data [27].

2.5. Animals

The Animal Care Committee of the University had approved this study. The mean weight of the male Wistar rats ($n = 35$) at the beginning of the experiment was 111 g. Before the experiment all rats were put on 5 days of adaptation. These rats were housed in the first part of the experiment (1–25 days) in plastic cages and then in the last five days (26–30 days) in metabolic cages of the same Tecniplast, 21020, Italy. The rats were divided into 5 groups of 7. These groups were named Control, Chol, Chol/Mature, Chol/Ripe and Chol/Overripe.

2.6. Diets

During 30 days of the experiment the rats of all 5 groups were fed a basal diet (BD), which included wheat starch, casein, soybean oil, vitamin and mineral mixtures. The rats of the Control group were fed BD only. To the BD of the Chol group was added 1% of cholesterol. The BD of the three other groups (Chol/Mature, Chol/Ripe and Chol/Overripe) was supplemented with 1% of cholesterol and 5% of the mature, ripe and overripe durian as freeze-dried powder, respectively. Cholesterol of analytical grade (USP) was obtained from Sigma Chemical, St Louis, MO. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to rats. Several prior experiments on laboratory animals and human studies have shown that cellulose has not significant hypocholesterolemic effects [1]. Therefore, cellulose was used as a control fiber.

All rats were fed once a day at 10.00/h *ad libitum*. They had unrestricted access to drinking water. The feed intake was monitored daily and body gains every week.

2.7. Metabolic analyses

In order to assess the nutritional value of the used diets the following indices were determined: dry matter (DM) and crude protein (CP) in the diets, their digestibility and the influence of different diets on nitrogen retention. For the determination of the nitrogen retention from the diets, feces and urine were collected in the last 5 days of the investigation. The following procedure for the estimation of the dry matter of the diets and feces was used: samples were dried at the temperature of 80°C for 3 days and then at the temperature of 105°C for 3 additional days. The nitrogen was determined by Kjeldahl (Kjeltec-300 Tecator), according to AOAC, 1997. At the end of the experiment after 24 hours of starvation, all rats were anaesthetized using diethyl ether and the blood samples were taken from the left atrium of the heart. Plasma was prepared and used for some other laboratory tests: total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and TG were determined as previously described [9] and the plasma glucose level – according to the enzymatic method (Alpha Diagnostic: G6518-400).

For the determination of the plasma antioxidant activity four antioxidant tests were adopted: DPPH, ABTS, Ferric-reducing/antioxidant power (FRAP) and Cupric reducing antioxidant capacity (CUPRAC).

The determination of plasma antioxidant activity was done exactly as for the durian extract in ABTS method (10 μ L of plasma in the assay) and for the DPPH method 25 μ L of plasma were mixed with 75 μ L of MeOH and 800 μ L of 75 μ M DPPH. The reaction mixture was maintained in dark at room temperature for 90 min, and the absorbance at 517 nm was then recorded [31,38].

Ferric-reducing/antioxidant power (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+}) which absorbs light at 593 nm. The ferro- and ferric-iron form complexes with TPTZ reagent are the main products of this reaction. The antioxidant activity of 10 μ L of plasma was measured [5].

Cupric reducing antioxidant capacity (CUPRAC). This assay is based on utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent. To the mixture of 1 mL of 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. The antioxidant activity of 25 μ L of plasma was measured [3]. The results of antioxidant activity were expressed in mM of trolox equivalents (TE)/L.

Serum fibrinogen was precipitated with methanol, then purified by sequential DEAE anion-exchange chromatography, dialyzed against water for 72 hours, and lyophilized. Plasma samples were dissolved in sample buffer: 2% SDS; 10% glycerol, 2%-mercaptoethanol, 0.002% bromophenol blue and 0.62 M Tris HCl, pH 6.8. Electrophoresis was performed with the Hoeffer SE 600 vertical unit (Hoeffer Pharmacia Biotech Inc., San Francisco, CA 94107, USA) according to Laemmli method [19], using polyacrylamide gels (resolving gel $T = 13.7\%$, $C = 1.7\%$, stacking gel $T = 3.8\%$, $C = 1.8\%$) with gel size of $180 \times 160 \times 1.5$ mm. Sample size was 5 μ L. The run was carried out at 25 mA per gel until the end of electrophoresis. Gels were stained with 0.25% Coomassie Brilliant Blue R in methanol/water/glacial acetic acid (5:5:1 v/v), destained in water and scanned in transmission light with an Agfa SNAPSCAN 1236 (Agfa-Gevaert N.V Belgium, Agfa SnapScan 1236 s Color image scanner).

2.8. Histology

Samples of the aorta were analyzed. After a formalin fixation, segments of the aortas were processed by a common paraffin technique. Each sample was cut into 72 serial sections (thickness of 5 μ m) with a transversally oriented cutting plane, and stained with hematoxylin and eosin (HE) and green trichrome [6].

2.9. Statistical analyses

The results of this investigation *in vitro* are means \pm 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. The p values of < 0.05 were considered significant.

3. Results

3.1. *In vitro*

3.1.1. Dietary fiber

It was found that the contents of dietary fibers in all three studied durian samples were comparable (data not shown). The content of the soluble dietary fiber was in range of 0.35–0.40 g/100 g FW, and the differences were not significant ($P > 0.05$).

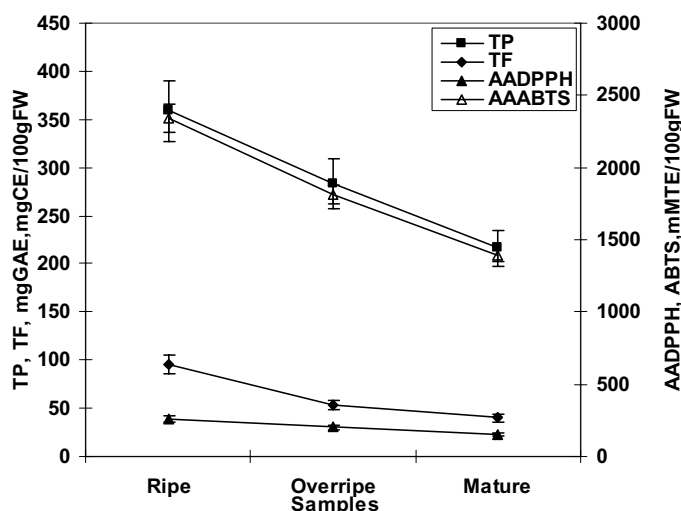


Fig. 1. Antioxidant capacities determined by different radical scavenging assays of Durian samples during ripening: (■) TP, polyphenols, mgGAE/ 100 g FW; (◊) TF, total flavonoids, mgCE/100 g FW; (Δ) AAABTS, μMTE/100 g FW; (▲) AADPPH, μMTE/100 g FW. Abbreviations: [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH).

No significant changes in the contents of minerals (Na, K, Mg and Ca) and trace elements (Fe, Mn, Zn and Cu) during ripening were registered ($P > 0.05$). The highest contents among the mineral and trace elements were of K and Fe, respectively.

3.1.2. Total polyphenols, flavonoids and antioxidant activities

The content of the total polyphenols and flavonoids was maximal in the ripe in comparison with the mature and overripe samples (Fig. 1). The DPPH and ABTS tests show a significant increase in the antioxidant potentials in the ripe and overripe samples together with similar increase in the content of total polyphenols ($P < 0.05$ in all cases).

A very good correlation was observed between the antioxidant potentials determined by DPPH and ABTS (Fig. 2A) and the total polyphenols ($R^2 = 0.9207; 0.9453$). Flavonoids showed lower correlation coefficients (Fig. 2B) than the total polyphenols ($R^2 = 0.6325; 0.6588$).

3.2. In vivo

3.2.1. Effects of the diets on feed intake, body weight gains, feed and protein efficiencies

The data of the feed intake, body gains, feed (FER) and protein efficiency ratios (PER) of all five diet groups show that only the feed intake and PER in the Chol/Ripe and Chol/Overripe are significantly higher than in the other three groups (Table 1).

3.2.2. Effects of the diets on digestibility

The dry matter (DM) in all diet groups, and the digestibility of DM (Table 2) and crude proteins (CP) were comparable ($P > 0.05$). However, the content of CP was increased significantly in the last stage of the ripening of durian ($P < 0.05$).

In order to assess the nutritional value of the used diets the nitrogen retention was calculated (Table 3). Because of relatively low nitrogen excretion in feces and urine in the Chol/Ripe group the nitrogen

Table 1
Diet groups feed intake, body gains, feed (FER) and protein (PER) efficiency ratios

Groups	Feed intake (g/day)	Body gains (g/day)	FER	PER
Control	13.77 ± 2.31 ^a	4.57 ± 0.79 ^a	0.353 ± 0.03 ^a	0.350 ± 0.08 ^a
Chol	14.58 ± 2.11 ^a	4.50 ± 0.71 ^a	0.308 ± 0.02 ^a	0.361 ± 0.022 ^a
Chol/Mature	13.478 ± 2.80 ^a	4.41 ± 0.64 ^a	0.338 ± 0.09 ^a	0.327 ± 0.08 ^a
Chol/Ripe	15.67 ± 1.36 ^b	4.86 ± 0.74 ^a	0.311 ± 0.03 ^a	0.398 ± 0.03 ^b
Chol/Overripe	16.50 ± 1.03 ^b	4.76 ± 0.74 ^a	0.287 ± 0.03 ^a	0.443 ± 0.04 ^b

Values are means and SD ($n = 7$). Values with different superscription letters in columns are significantly different ($P < 0.05$).

Abbreviations: Chol, cholesterol diet group; Chol/Mature, diet group supplemented with mature durian; Chol/Ripe, diet group supplemented with ripe durian; Chol/Overripe, diet group supplemented with overripe durian.

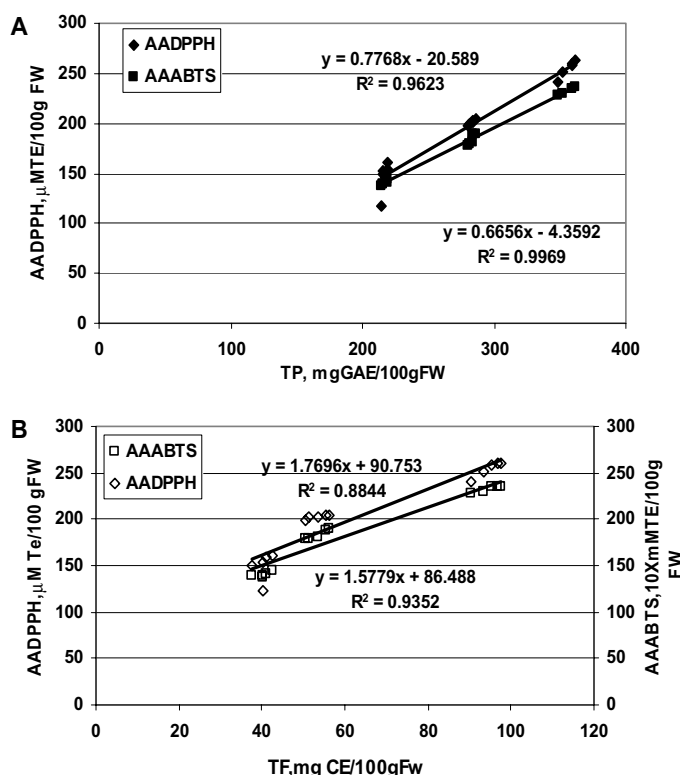


Fig. 2. Relationship, calculated by a linear regression analysis for durian samples and their antioxidant capacities. **A**, (\blacksquare) TP (mgGAE/100 g, X) and ABTS ($\mu\text{mol}/100\text{ g}$, Y_2) to (\blacktriangle) TP (mgGAE/100 g, X) and DPPH ($\mu\text{mol}/100\text{ g}$, Y_1). **B**, (\square) TF (mgCE/100 g FW, X) and ABTS ($\mu\text{mol}/100\text{ g}$, Y_2) to (\diamond) TF (mgCE/100 g FW, X) and DPPH ($\mu\text{mol}/100\text{ g}$, Y_1); Abbreviations: [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH).

retention remains significantly higher than in other four groups (0.224 ± 0.03 , 63.6%, $P < 0.05$). Opposite, in the rats of Chol/Overripe group the nitrogen excretion in feces and urine was relatively high. And in spite of highest intake of nitrogen from the diet the nitrogen retention remains lower than in Chol/Ripe group (0.198 ± 0.03 , 51.3%).

As can be seen, durian supplemented diets significant hindered the rise of plasma total cholesterol vs. Chol diet group (Fig. 3): a) TC – 8.4 %, 15.3% and 10.5% and b) LDL-C – 22.6%, 35.5 % and 24.7%

Table 2

Dry matter (DM) and crude protein (CP) in the diets and their digestibility as determined at the end of the experiment (%)

Groups	DM	CP	Digestibility of DM	Digestibility of CP
Control	98.29 ± 0.10 ^a	11.62 ± 0.42 ^a	95.9 ± 1.08 ^a	91.22 ± 2.54 ^a
Chol	98.45 ± 0.06 ^a	11.25 ± 0.20 ^a	95.45 ± 0.62 ^a	91.86 ± 1.17 ^a
Chol/Mature	98.19 ± 0.05 ^a	10.64 ± 0.64 ^a	94.96 ± 0.29 ^a	90.65 ± 0.70 ^a
Chol/Ripe	98.70 ± 0.22 ^a	12.46 ± 0.40 ^a	95.22 ± 0.19 ^a	92.51 ± 0.61 ^a
Chol/Overripe	98.24 ± 0.11 ^a	13.16 ± 0.06 ^b	94.94 ± 0.82 ^a	91.78 ± 1.80 ^a

Values are means and SD ($n = 7$). Values with different superscription letters in columns are significantly different ($P < 0.05$).

Abbreviations: Chol, cholesterol diet group; Chol/Mature, diet group supplemented with mature durian; Chol/Overripe, diet group supplemented with over ripe durian; Chol/Ripe, diet group supplemented with ripe durian.

Table 3

The influence of the different diets on nitrogen retention (one day average)

Groups	Intake of nitrogen from diets (g)	Nitrogen excretion in feces (g)	Nitrogen excretion in urine (g)	Nitrogen retention (in g and %)
Control	0.309 ± 0.008 ^a	0.038 ± 0.001 ^a	0.108 ± 0.02 ^a	0.163 ± 0.03 ^b (52.7%)
Chol	0.302 ± 0.03 ^a	0.034 ± 0.004 ^a	0.12 ± 0.01 ^a	0.148 ± 0.03 ^a (49.0%)
Chol/Mature	0.306 ± 0.02 ^a	0.040 ± 0.006 ^a	0.098 ± 0.02 ^a	0.168 ± 0.03 ^b (54.9%)
Chol/Ripe	0.352 ± 0.02 ^b	0.038 ± 0.004 ^a	0.090 ± 0.01 ^a	0.224 ± 0.03 ^d (63.6%)
Chol/Overripe	0.386 ± 0.01 ^b	0.050 ± 0.006 ^b	0.140 ± 0.01 ^b	0.198 ± 0.03 ^c (51.3%)

Values are means ± SD ($n = 7$). Values with different superscription letters in columns are significantly different ($P < 0.05$).

Abbreviations: Chol, cholesterol diet group; Chol/Mature, diet group supplemented with mature durian; Chol/Overripe, diet group supplemented with overripe durian; Chol/Ripe, diet group supplemented with ripe durian.

for the Chol/Mature, Chol/Ripe and Chol/Overripe, respectively.

At the end of the experiment, the plasma glucose levels were 6.51 ± 1.09 , 7.08 ± 1.23 , 7.24 ± 1.05 , 6.34 ± 1.09 and 7.25 ± 1.26 mmol/L for the Control, Chol, Chol/Mature, Chol/Ripe and Chol/Overripe groups, respectively. Therefore, only in the Chol/Ripe diet group the plasma glucose level remained unchanged.

A decrease in the plasma antioxidant activity values determined by four each other supplemented antioxidant assays in the rats fed added cholesterol was found (Table 4). However, this decrease was significant only in plasma of rats from the Chol and Chol/Mature diet groups. The decrease in the plasma antioxidant activity was predictable [23], however, it must be underlined that the decrease in the antioxidant activity in the groups of rats fed diets supplemented with ripe and overripe durian samples (Chol/Ripe, Chol/Overripe) was significantly less ($P < 0.05$ in both cases) than in Chol and Chol/Mature groups. Therefore, supplementation of diets with ripe and overripe durian hindered the decrease in the plasma antioxidant activity.

Electrophoretic bands of fibrinogen fraction show a difference between the durian diets: diet supplemented with mature durian (DMFch) in both for full and methanol precipitated plasma have more 14 kDa protein bands (Fig. 4A, B, C). It is especially noticeable for methanol precipitated variant (Fig. 4B).

Table 4

The changes in the plasma antioxidant activity values (mMTE/L) determined by four radical scavenging tests

Diet groups	DPPH	CUPRAC	ABTS	FRAP
Control	0.66 ± 0.06 ^b	0.25 ± 0.02 ^b	1.35 ± 0.1 ^b	0.246 ± 0.04 ^b
Chol	0.53 ± 0.05 ^a	0.17 ± 0.01 ^a	0.92 ± 0.08 ^a	0.197 ± 0.03 ^a
Chol/Mature	0.55 ± 0.05 ^a	0.19 ± 0.02 ^a	1.1 ± 0.09 ^a	0.204 ± 0.04 ^a
Chol/Ripe	0.65 ± 0.07 ^b	0.24 ± 0.02 ^b	1.27 ± 0.1 ^b	0.223 ± 0.04 ^b
Chol/Override	0.64 ± 0.05 ^b	0.23 ± 0.02 ^b	1.21 ± 0.1 ^b	0.211 ± 0.04 ^b

Values are means ± SD ($n = 7$). Values with different superscription letters in columns are significantly different ($P < 0.05$).

Abbreviations used: ABTS, [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)]; DPPH, 1,1-diphenyl-2-picrylhydrazyl; Ferric-reducing/antioxidant power (FRAP) and Cupric reducing antioxidant capacity (CUPRAC).

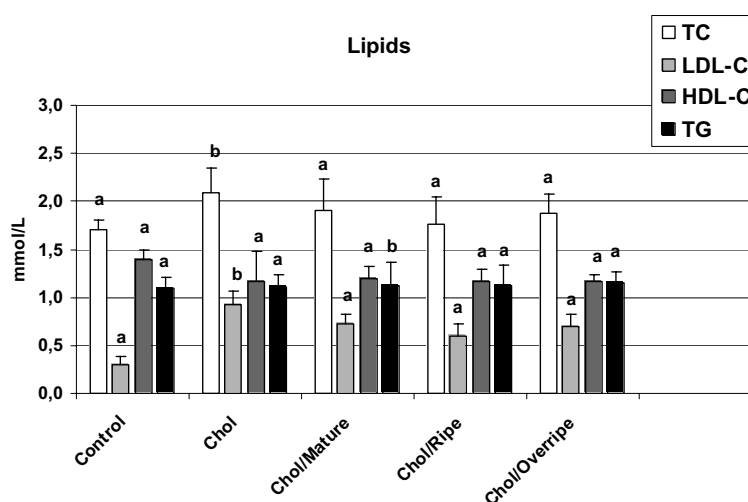


Fig. 3. Changes in the plasma lipid levels after completion of the experiment ($n = 5$). Values are means ± SD (vertical lines). The values with different superscript letters are significantly different ($P < 0.05$).

Three arrows which show thicker bands 14 kDa in variant of DMFch in comparison to other diets.

No lesions were found in the examined tissue of aorta (Fig. 5A, B, C).

4. Discussion

Consumption of fruits with high contents of bioactive compounds and high antioxidant capacity secure the best nutritional results [20,21]. Therefore, scientists recommend consuming fruits with high antioxidant capacity [30,35]. The contents of the bioactive compounds and the antioxidant capacity are changing during the ripening and these changes in traditional fruits were already reported [41]. The interest of the consumers in tropical and subtropical fruits is understandable, because they are more rear than others. Therefore, now some investigators are studying the changes, which occurred during ripening of tropical and subtropical fruits. The following authors have investigated these changes in mango [40], banana [7,12], persimmon [8] and guava [13]. However, the ripening of durian fruit was studied only by few investigators, who have described mainly the changes in ethylene production, respiration, solids, total

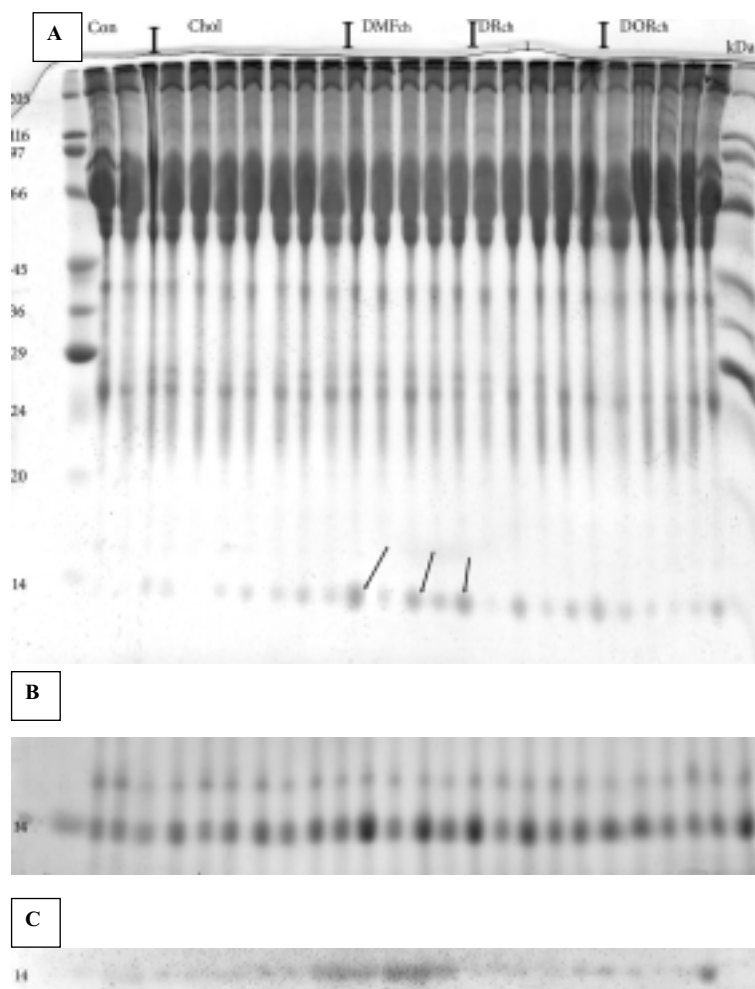


Fig. 4. Comparison of the band intensity of serum proteins after different durian diets extracted with sample buffer containing SDS and 2-ME and separated by SDS-PAGE. Molecular markers (kDa): 205- myosin; 116- β -galactosidase; 97- phosphorylase b; 66-albumin; 45- ovalbumin; 36- glyceralaldehyde-3-phosphate dehydrogenase; 29-carbonic anhydrase; 24- trypsinogen, PMSF treated; 20-trypsin inhibitor, 14- α -lactalbumin; loading 2 μ l: Con, control; Chol, cholesterol; DMFch, Durian Mon Thong, full mature+chol; DRch, Durian Mon Thong, ripe+chol; DORch, Durian Mon Thong overripe+chol. A, separation on the gel of full plasma; B, low molecular weight proteins of fibrinogen fraction; C, low molecular weight proteins of full plasma. Arrows indicate some differences visible on the gel: DMFch differ from others.

sugars, starch, firmness, pectic substances and activities of polygalacturonase and pectinesterase [14,15]. No results of comparative studies describing changes in contents of phenolic compounds and antioxidant activity during durian ripening and the influence of these changes in experiments *in vivo* were published to date [24]. Therefore, the present investigation was conducted.

It was found that the content of phenolic compounds is higher in the ripe durian samples than in the mature and overripe. It must be underlined that the content of phenolic compounds in the overripe samples were higher than in the mature. The same trends were observed in the changes of the antioxidant potential. So, antioxidant potential in the ripe durian samples was higher than in the mature and the overripe and in the overripe – higher than in mature samples.

These results correspond with data of the following authors, who evaluated some tropical fruits from

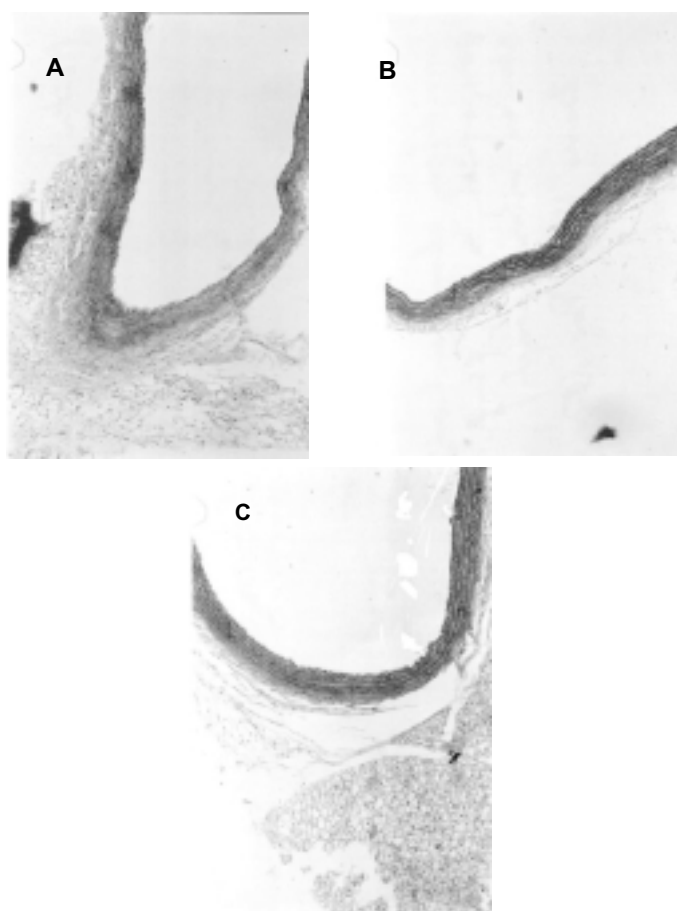


Fig. 5. (A, B, C). The histology of the aorta of rats. There were no lesions found in the examined tissue.

south Florida for antioxidant activity, total soluble phenolics, total ascorbic acid, total dietary fiber and pectin [22]. The most important is the comparison of the data obtained in this research with red and white guava, green and ripe mango, green and ripe papaya. These authors found that the content of the total soluble phenolics and the level of the antioxidant activity were influenced by the fruits ripening stage [22].

During the process of ripening major changes in the durian fruits are observed. The starch content decline rapidly between 0 and 6 day, however the concentration of soluble solids, total sugars and β -carotene rapidly increase [16]. The content of the water soluble pectin and activity of enzymes (polygalacturonase, pectinesterase) are higher in the mature aril of durian than in less mature [15]. The above-mentioned and other changes which are detected during the ripening stages of durian could be responsible for the different influence of diets supplemented with this fruit: increase in feed consumption and protein intake, feed utilization ratio, nitrogen retention and others. So, the nitrogen retention in Chol/Ripe group was the highest (0.224 g/day).

Nitrogen intake from the diets supplemented with ripe and overripe fruits (Chol/Ripe and Chol/Overripe) was comparable, but the amounts of nitrogen losses with feces (0.038 g/day) and urine (0.09 g/day) were significantly lower in Chol/Ripe than in the two other groups (0.04 and 0.098 and 0.05 and 0.14 g/day for Chol/Mature and Chol/Overripe, respectively).

The registered results of our investigation concerning the feed intake and body gains are different from the results of others [2]. These authors reported higher feed intake and body gains in rats fed diets supplemented with 10 or 20% of mango fruit bar during 12 weeks of their experiment. However, in our study the diets were supplemented only with 5% of durian.

The investigation *in vivo* have shown that after 30 days of different feeding, the diet supplemented with ripe and to a lesser degree with overripe and mature durian samples hindered a rise in the plasma lipids and hindered the decrease of plasma antioxidant activity in rats fed cholesterol-containing diet. No published data are available for comparison. Tippayakul et al. [36] investigated lipid entrapment property of polysaccharide gel extracted from fruit-hulls of durian by semi-permeable membrane dialysis technique using both cellulose membrane and gut sacs of dissected jejunum of rat. The above cited authors suggested that polysaccharide gel is able to entrap lipids and in their opinion it could be used as medicinal dietary food for lipid controlling patients [36].

The decrease in the plasma antioxidant activity was predictable: also other investigators observed that cholesterol supplemented diet leads to a decrease in plasma antioxidant activity [23].

The determination of the plasma glucose level demonstrated that only the diet supplemented by ripe durian did not increase the plasma glucose level. We did not find published data for comparison. However, it was shown that mango flour obtained from mango pulp containing 6.85% SDF, 11.96% IDF, 2.53% total protein supplemented to diets in dose of 10 or 20% significantly ($P < 0.05$) decreased glucose level in blood after 30 feeding days [28]. Also other authors claim that dietary fiber, mainly SDF, interfering in food digestion process, can alter the absorption of nutrients such as glucose [11].

According to the results of the present and our previous investigations the scavenging ability of studied tropical fruits was in following decreasing order: ripe Mon Thong durian > snake fruit > mangosteen > lichi > guava > ripe mango [10,21].

No significant changes in the plasma lipid levels and antioxidant activity were observed in the Control group.

Protein profile of plasma samples showed that in fibrinogen fraction of ripe and overripe durian were detected less protein bands and lower intensity than in other groups. The main patterns were located in the range of 50–116 kDa, showing that the amount of fibrinogen has decreased during such diet. Fibrinogen is one of the plasma proteins. Our findings indicate that one of the positive benefits of fruit consumption was to diminish the production of fibrinogen and its stability, which reduces the potential risk exerted by this protein. Therefore from the health point these results are positive. Our data corresponded with others who found improvement of rat blood fluidity and inhibitory effects on the thrombin-induced conversion of fibrinogen to fibrin using the Japanese apricot [18].

In spite of 1% cholesterol feeding, the histology of aorta in our experiment remained unaffected. Also other investigators have shown that only prolonged cholesterol feeding for three and six months induces changes in the histoarchitecture of aorta in the form of fatty streaks and atheromatous plaques followed by fibrous plaques [4]. Another experiment supports our findings [6]. These authors found that atherosclerotic lesions are significantly more developed in the experimental group fed a cholesterol diet for five months, than in the group fed the same diet for two months only. Therefore, the results of this investigation could justify the inclusion of ripe and even overripe durian samples in atherosclerosis preventing diets.

It is well known that the results of experiments on laboratory animals cannot be automatically applied to humans. Therefore, there is a need for further investigations of human volunteers, suffering from hyperlipidemia and diabetes mellitus.

5. Conclusion

In conclusion, the ripe Mon Thong durian, apart from a good taste, positively affects plasma lipid profile and plasma antioxidant activity in rats fed cholesterol-containing diets and did not raise the plasma glucose level. The durian fruit especially in the ripe stage possesses high nutritional value. According to the results of the present and our previous investigations the scavenging ability of tested tropical fruits was in decreasing order: ripe Mon Thong durian > snake fruit > mangosteen > lichi > guava > ripe mango. It is suggested that ripe Mon Thong durian supplemented diet could be useful for patients suffering from hypercholesterolemia and diabetic mellitus.

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