

Original article

Positive effects of durian fruit at different stages of ripening on the hearts and livers of rats fed diets high in cholesterol

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Abstract

Aim of the study: Diets containing high cholesterol levels led to atherosclerosis damage in the livers and hearts of rats. The aim of this study was to investigate the possible positive effects of durian fruit against high-cholesterol diets.

Materials and methods: Durians at various stages of ripening (young, mature, ripe and overripe) were chosen for in vitro and in vivo studies. In the in vivo study 36 male Wistar rats were divided into 6 groups and supplemented with cholesterol and durians. The bioactivity in vitro, plasma lipids, antioxidant activity, liver enzymes and histopathology of the aorta and liver were analyzed.

Results: Polyphenols and flavonoids were significantly higher in the overripe durian, while quercetin, ascorbic acid and anthocyanins were more abundant in the ripe fruit and tannins – in mature samples ($P < 0.05$). The highest antioxidant potential was in overripe fruit and only the value of FRAP – in ripe durian samples ($P < 0.05$). The interaction between polyphenol extracts of ripe durian and BSA had a strong ability comparable to that of quercetin – to quench the intrinsic fluorescence of BSA by forming complexes. The main histopathological changes were detected in the liver and aorta of rats fed a high-cholesterol diet without fruit supplementation. These changes were minor in rats of Chol/DRipe ($P < 0.05$).

Conclusion: Durian at different stages of ripening, especially ripe durian, constitute an excellent source of effective natural compounds with antioxidant and health-protective activity in general and liver and heart-protective effect in cholesterol fed rats in particular.

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Keywords: Durian; Composition; Rats; Cholesterol; Aorta and liver histology

Abbreviations: ABTS^{•+}, 2,2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt method; ALP, alkaline phosphatase; ALT, alanine amino-transferase; AST, aspartate aminotransferase; BSA, bovine serum albumin; CUPRAC, cupric reducing antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl method; FRAP, ferric-reducing/antioxidant power assay; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PAA, plasma antioxidant activity; TC, total cholesterol; TG, triglycerides; 2D-FL and 3D-FL, two and three-dimensional fluorescence spectra.

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Introduction

It was shown that cholesterol-containing diets lead to liver and heart damage in experimental animals [1–5]. The atherosclerotic lesions were detected in young male mice [3] and in rabbits [4] fed cholesterol-enriched diet [6,7].

Some authors claim that herbal medicine and vegetables exercise hepatoprotective activities in animals fed a high-fat diet [3,8]. So, Makni et al. [8] evaluated the hepatoprotective activities of flax and pumpkin seeds on 30 hypercholesterolemic male rats and found an improvement in histological sections of liver lipid hepatocyte storage only in rats fed the flax and pumpkin mixture. It was of great interest to investigate such influence of the most rare exotic fruits – durian.

Durian (*Durio zibethinus Murray*) is one of the important seasonal fruits in tropical Asia. Durian cultivars are derived from *D. zibethinus*, originating in the Malay Peninsula. This fruit is widely known and revered in Southeast Asia as the “king of fruits”. The durian is distinctive for its large size, unique odor, and formidable thorn-covered husk. The fruit can grow as large as 30 cm long and 15 cm in diameter and it typically weighs from 1 to 3 kg. Its shape ranges from oblong to round, the color of its husk is green to brown, and its flesh pale is yellow to red, depending on the species [9].

Durian is rich in dietary fibers, minerals and trace metals (P, K, Ca, Mg, Na, Fe, Mn, Cu, and Zn), sugar, vitamin C, potassium, and the serotonergic amino acid tryptophan, and is a good source of carbohydrates, proteins, and fats. The health properties of durian are based not only on the antioxidant properties, but also on its fatty acid composition. Durian is rich in *n* – 3 fatty acids, compared to some other fruits. The content of the major bioactive compounds (polyphenols, quercetin, flavonoids, flavanols, tannins, anthocyanins, ascorbic acid and carotenoids) is high and significantly higher in the Durian Mon Thong cultivar [10].

In Malaysia, a decoction of the leaves and roots used to be prescribed as an antipyretic. Another investigation showed that *D. zibethinus Murr. cv. Mon Thong* polysaccharide gel is able to entrap lipids and seems to have potential to be used as a medicinal dietary food for controlling lipid levels in patients [11]. 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 [10].

In our previous reports [12–14] the polyphenol and nutrient compounds, fatty acids, and antioxidant and antiproliferative activities of Mon Thong durian at different stages of ripening were studied. In vitro studies a wide range of antioxidant assays, FTIR, two-dimensional (2D-FL) and three-dimensional fluorimetry (3D-FL) was used [12–14].

In vivo studies [11–14] showed that diets supplemented with ripe and to a lesser degree with mature and overripe durian significantly hindered the rise in plasma lipids and 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. In our previous study it was no changes in

aorta heart and brain after 4 weeks loading rats with cholesterol [14].

Also other researchers found in different durian varieties antioxidant phytochemicals with relatively high amounts of quercetin [15]. It was found that Mon Thong durian was preferable in comparison with other varieties [13,14]. In the cited and as well as in our previous investigations the influence of durian supplemented diets on liver protection was not explored. Therefore it is of great interest to investigate if durian fruit of different stages of ripening also possesses liver and heart-protective properties for rats fed diets with cholesterol during 6 weeks. To support this hypothesis, durians of different stages of maturity were investigated in vitro. Then an investigation in vivo of Wistar male rats fed cholesterol-containing diets supplemented with durian of different stages of ripening was performed, and the histological changes in their aortas and livers were compared.

The interaction between flavonoids and BSA is important in the metabolism of drugs [16,17]. Such interaction between the extracted polyphenols and BSA can provide information about the better use of fruits in everyday consumption.

The objectives of this study were the following: in vitro to analyze the composition of durian samples at different stages of ripening; to study the interaction of quercetin and durian polyphenol extracts with BSA, and in vivo to determine the influence of durian supplemented diets on liver and aorta changes in rats. The results of the investigation of durian at the different stages of maturity in vitro and also in vivo on rats loaded with cholesterol would support our hypothesis and advances the use of this fruit in human nutrition.

As far as we know, no results of such investigation have been published before.

Methods and materials

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, $\text{FeCl}_3 \times 6\text{H}_2\text{O}$, $\text{CuCl}_2 \times 2\text{H}_2\text{O}$, 2,9-dimethyl-1,10-phenanthroline (neocuproine), quercetin and BSA 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.

Samples and preparation

All durian samples were harvested in May 2008 in the 25-year-old Mon Thong commercial durian orchard in Chantaburi province of Eastern Thailand.

The durian samples at various stages of ripening (young, mature, ripe and overripe) for in vitro and in vivo studies were chosen according to the criteria described in our previous investigations [13]: (1) young 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 were 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. All samples were cleaned with tap water and dried, and five replicates of five young, mature, ripe and overripe durian was used for each. The samples' edible parts were manually prepared for this investigation without using steel knives. The peeled samples were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then 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 the bioactive substances were analyzed.

Composition of durian samples

Polyphenols were extracted from lyophilized fruits with 50% dimethyl sulfoxide (DMSO) (concentration 25 mg/ml) at room temperature twice in 3 h. The polyphenols were determined by Folin-Ciocalteu method with measurement at 750 nm with spectrophotometer (Hewlett–Packard, model 8452A, Rockville, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW [18]. Flavonoids, extracted with 5% NaNO_2 , 10% $\text{AlCl}_3 \times 6\text{H}_2\text{O}$ 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 measured [19]. 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).

Total ascorbic acid was determined by CUPRAC assay in water extract (100 mg of lyophilized sample and 5 ml of water) [20]. The absorbance of the formed bis (Nc)-copper (I) chelate was measured at 450 nm.

The content of quercetin was determined by high-performance liquid chromatography with diode-array detection (HPLC/DAD) in the prepared extracts with the P580A LPG liquid chromatograph, equipped with the Gina 50 autosampler and the UVD340V DAD diode array detector (Gynkotek/Dionex, Germering, Germany) [21]. The column was the Tosoh Biosep cartridge filled with the TSK gel ODS-80 TM (5 mm, 250 mm, 4.6 mm i.d.; flow rate, 1 ml/min; catalogue number 08149; Tosoh Corporation, Tokyo, Japan). The chromatographic column was thermostated at 40°C . The 50- μl volumes of the extracts from the different durian samples were introduced to the HPLC/DAD system with autosampler in 50-min intervals. The standards of quercetin were analyzed as ethanol solutions, and their concentration was equal to 0.1 mg/ml. The analyses were carried out

with the gradient of the mobile phase composition: 0–2 min, constant composition: 5% ACN, 95% H_2O ; 0–22 min, composition changed: rise from 5% to 25% ACN and dropped from 95% to 75% H_2O ; 22–32 min, composition changed: rise from 25% to 55% ACN and dropped from 75% to 45% H_2O ; 32–50 min, constant composition: 55% ACN, 45% H_2O .

The anthocyanins were analyzed by a pH differential method [22]. Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700}) \text{pH}_{1.0} - (A_{510} - A_{700}) \text{pH}_{4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29,600. The results are expressed as micrograms of cyanidin-3-glucoside equivalent (CGE)/g DW.

Determination of antioxidant potentials (AP)

The AP was determined by four complementary assays:

- (1) 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS^{•+}) ABTS^{•+} radical cation was generated by the interaction of ABTS (7 mM/l) and $\text{K}_2\text{S}_2\text{O}_8$ (2.45 mM/l). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm [23].
- (2) Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine (Fe^{3+} -TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm [24].
- (3) Cupric reducing antioxidant capacity (CUPRAC): this assay is based on utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent. The absorbance at 450 nm was recorded against a reagent blank [25].
- (4) Scavenging free radical potentials were tested in a methanolic solution of 1,1-diphenyl-2-picrylhydrazyl method (DPPH). In its radical form, DPPH has an absorption band at 515 nm which disappears upon reduction by an antiradical compounds. DPPH solution (3.9 ml, 25 mg/l) in methanol was mixed with the samples extracts in DMSO (0.1 ml), then the reaction progress was monitored at 515 nm until the absorbance was stable [26].

Fluorimetry and Fourier Transform Infrared (FT-IR) spectra studies

Two-dimensional (2D-FL) and three-dimensional (3D-FL) fluorescence measurements were carried out using a model FP-6500, Jasco Spectrofluorometer, serial N261332, Japan. Fluorescence emission spectra for all durian samples at a concentration of 0.25 mg/ml were taken at emission wavelength (nm) of 330 and recorded from wavelength of 265 to a wavelength of 310 nm, at emission wavelengths of 685 nm from 300 to 750 nm; and at excitation of 350 nm from 370 to 650 nm. Quercetin was used as a standard. 3D-FL spectra of the investigated durian extracts were collected with subsequent scanning emission spectra from 250 to 750 nm at 1.0 nm increments by varying the excitation wavelength from 230 to 350 nm at 10 nm increments. The scanning speed was set at 1000 nm/min for all

Table 1
Composition of the diets.

	Control	Chol	DurianY	DurianM	DurianR	DurianO
Casein	150	150	150	150	150	150
Soybean oil	100	100	100	100	100	100
Wheat starch	691	681	631	631	631	631
DurianY	–	–	50	–	–	–
DurianM	–	–	–	50	–	–
DurianR	–	–	–	–	50	–
DurianO	–	–	–	–	–	50
Chol	–	10	10	10	10	10
Vitamin mixture ^a	10	10	10	10	10	10
Mineral mixture ^b	37	37	37	37	37	37
Cellulose	10	10	10	10	10	10
Choline	2	2	2	2	2	2

DurianY, durian young; DurianM, durian mature; DurianR, durian ripe; DurianO, durian overripe.

^a **AIN-93-VX** per kilogram of mix: 3 g nicotinic acid, 1.6 g Ca pantothenate, 0.7 g pyridoxine–HCL, 0.6 g thiamin–HCL, 0.6 g riboflavin, 0.2 g folic acid, 0.02 g biotin, 2.5 g vitamin B₁₂ (cyanocobalamin) (0.1% in mannitol), 15 g vitamin E (all-rac-alpha-tocopheryl acetate) (500 IU/g), 0.8 g vitamin A (all-trans-retinyl palmitate) (500,000 IU/g), 0.250 g vitamin D₃ (cholecalciferol) (400,000 IU/g), 0.075 g vitamin K₁ (phyloquinone), and 974.655 g powdered sucrose [27].

^b **AIN-93-MX** per kilogram of mix: 357 g of calcium carbonate anhydrous, 250 g potassium phosphate monobasic, 28 g potassium citrate, tripotassium monohydrate, 74 g sodium chloride, 46.6 g potassium sulfate, 24 g magnesium oxide, 6.06 g ferric citrate, 1.65 g zinc carbonate, 1.45 g sodium meta-silicate × 9H₂O, 0.63 g manganous carbonate, 0.30 g cupric carbonate, 0.275 g chromium potassium sulfate × 12H₂O, 81.5 mg boric acid, 63.5 mg sodium fluoride, 31.8 mg nickel carbonate, 17.4 mg lithium chloride, 10.25 mg sodium selenate anhydrous, 10 mg potassium iodate, 7.95 mg ammonium paramolybdate × 4H₂O, 6.6 mg ammonium vanadate, 209.806 g powdered sucrose [27].

measurements. All measurements were performed with emission mode and with intensity up to 1000 [16,17]. All solutions for protein interaction were prepared in 0.05 mol/l Tris–HCl buffer (pH 7.4), containing 0.1 mol/l NaCl. The final concentration of BSA was 2.0×10^{-4} mol/l. All solutions were kept in dark at 0–4 °C. The BSA was mixed with quercetin. The samples were mixed in the properties of BSA: extract = 1:1. The samples after the interaction with BSA were lyophilized and subjected to FTIR.

The presence of polyphenols in the investigated fruit samples and the interaction between polyphenols and bovine serum albumin (BSA) was studied by Fourier Transform Infrared (FT-IR) spectroscopy. A Nicolet iS 10 FT-IR Spectrometer (Thermo Scientific Instruments LLC, Madison, WI, USA), with the smart iTRTM ATR (Attenuated Total Reflectance) accessory was used to record IR spectra.

Animals and diets

The Animal Care Committee of the Warsaw University of Life Sciences (SGGW), Warsaw, Poland, approved this study. In this experiment, 36 male Wistar rats were used. The mean weight of the male Wistar rats at the beginning of the experiment was 95.4 ± 3.0 . They were divided into 6 groups each of 6 and were named Control, Control/Chol, Chol/DRipe, Chol/DMature, Chol/DOverripe and Chol/DYoung.

Before the beginning of the study, a five-day adaptation period was used. The rats were housed in plastic cages (TECNIPLAST S.p.A., 21020, Italy). During 42 days of the experiment, all 6 groups of rats were fed a basal diet (BD), which included wheat starch, casein, soybean oil, cellulose, vitamin and mineral mixtures: AIN-93-VX and AIN-93-MX, respectively [27]. The rats of the Control group were fed only the basal diet (BD). The BD of the other 5 groups was supplemented with 1% of nonoxidized cholesterol (NOC) (Chol group), 1% of NOC and

5% of lyophilized durian ripe (Chol/DRipe), 1% NOC and 5% of lyophilized durian mature (Chol/DMature), 1% of NOC and 5% of lyophilized durian overripe (Chol/DOverripe), and 1% of NOC and 5% of lyophilized durian young (Chol/DYoung). Table 1 shows the ingredient composition of the diets used in the study in comparison with the control diet [27]. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were fed to the rats. Cellulose was used as a control fiber. All rats were fed once a day at 10.00/h ad libitum. The rats had unrestricted access to drinking water. The feed intake was monitored daily, and weight gains were measured every week. Before the section the rats were not fed for 24 h. At the end of the experiment, the rats were anaesthetized using Narcotan[®] (Zentiva) for inhalation, and the blood samples were taken from the left atrium of the heart [28].

Plasma was prepared and used for laboratory tests, which included determination of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and plasma antioxidant activity (PAA). The liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were determined as previously described [28]. ABTS, DPPH and FRAP were adopted for determination of PAA.

Histological procedures

(a) Aortas were conserved in a formaldehyde buffered bath in distilled water, cleaned of loose adventitial tissue, and then cut lengthwise. Dye solutions of Sudan III and Sudan IV were used for 10 min. The surface area of aortic atheromatous lesions was measured by planimetry using a computer scanning system (Multi Scan Base 14.02) and expressed as a percentage of total intimal surface area. (b) From the liver of each experimental rat left lateral lobe and isolated specimens were dissected. The

Table 2

Polyphenols compounds in dimethyl sulfoxide (DMSO) extract^a of the studied durian samples at different stages of ripening.

Durian samples	Polyphenols mg GAE	Flavonoids μg CE	Quercetin mg	Flavanols μg CE	Tannins mg CE	Asc acid mg AA	Anthocyan mg
Young	2.20 \pm 0.1 a	385.2 \pm 18.6 c	58.7 \pm 2.8 a	28.11 \pm 1.4 b	1.76 \pm 0.08 a	2.11 \pm 0.1 b	1.18 \pm 0.05 b
Mature	2.14 \pm 0.1 a	182.1 \pm 9.1 a	62.1 \pm 3.1 b	16.35 \pm 0.8 a	2.68 \pm 0.1 b	1.87 \pm 0.09 b	1.03 \pm 0.04 b
Ripe	3.29 \pm 0.2 b	311.2 \pm 15.2 b	68.9 \pm 3.3 c	16.61 \pm 0.8 a	1.52 \pm 0.07 a	2.33 \pm 0.1 c	1.43 \pm 0.06 c
Overripe	3.63 \pm 0.2 b	490.5 \pm 23.1 d	61.9 \pm 2.9 b	26.33 \pm 1.3 b	2.25 \pm 0.1 b	1.47 \pm 0.07 a	0.28 \pm 0.01 a

CE, catechin equivalent; GAE, gallic acid equivalent; CGE, cyanidin-3-glucoside equivalent.

^a Values are means \pm SD of 5 measurements. Values in columns for every bioactive compound with the same solvent bearing different letters are significantly different ($P < 0.05$). Per g dry weight.

sections were fixed with buffered 10% formalin. After 10 days liver samples were cut in Criostat at a temperature of -20°C on slicks of 10 μm thickness and placed in distilled water (with few NH_3 drops). The slicks, which floated on the water surface, were stained in 10% Red Oil during 10 min and then were placed on fat free basal glass and closed in glycerol-gelatin. Morphology of liver lobules was evaluated by a light microscope. Also, the fat content in hepatocytes was evaluated. For this purpose, 100 cells were selected, and fat's integral density was measured using computer program, Lucia v.4.x. (Nikon), at 100 \times magnification, and the results were expressed in $\mu\text{g}/\mu\text{m}^3$. The results of morphometry were analyzed using statistics.

Statistical analysis

The results of this investigation from in vitro are means \pm SD of five measurements. One-way analysis of variance (ANOVA) for statistical evaluation of results in vivo was used, following by Duncan's new multiple range tests to assess differences group's means. The P values of <0.05 were considered significant.

Results

Polyphenols, flavonoids, quercetin, flavanols, tannins, anthocyanins and ascorbic acid

The contents of polyphenols and flavonoids were significantly higher in overripe samples, quercetin, ascorbic acid and anthocyanins – in ripe and tannins – in mature durian (Table 2, $P < 0.05$).

Antioxidant potential

According to ABTS, CUPRAC and DPPH the highest antioxidant activity was in overripe samples, and only according to

FRAP – in ripe samples, but not always significant (Table 3, $P > 0.05$).

Fluorimetry and Fourier Transform Infrared (FT-IR) spectra studies

One of the main peaks for BSA (Fig. 1A), BSA + durian ripe extract at 0 h time and 37°C (Fig. 1B), BSA + durian ripe extract at 1 h time and 37°C (Fig. 1C), and BSA + quercetin at 1 h time and 37°C (Fig. 1E) was found at $\lambda_{\text{ex/em}}$ of 225–230/335 nm. The second main peak appeared for these samples at $\lambda_{\text{ex/em}}$ of 280/345 nm with a shift of 10 nm (335 nm) for the durian sample in quenching BSA during 1 h reaction (Fig. 1C). The interaction between BSA and quercetin, and BSA and durian extracts showed the decrease in the fluorescence intensity of the second main peak. Peaks of BSA have been quenched in the presence of quercetin (Fig. 1F) and durian ripe extract (Fig. 1D). The intensity of BSA (Fig. 1D, curve 1) was 848 and λ_{em} 345 nm. The intensity of BSA in the presence of durian extract at 0 h (Fig. 1D, curve 2) was 748.61 and λ_{em} 345 nm. Only after 1 h the intensity of BSA (Fig. 1D, curve 3) decreased to 594 and λ_{em} 335 nm. The intensity of BSA with quercetin at 0 h (Fig. 1F, curve 1) was 876.79 and λ_{em} 335 nm. After one hour, it decreased to 727.43 and no change appeared in the location of the main peak (Fig. 1F, curve 2). The decrease of the intensity of the main peak with durian ripe was about 22.5% in comparison with quercetin of 23.6%. Other durian samples showed the following decrease (%): 21.8, 21.1 and 20.7 for durian mature, overripe and young.

FTIR spectra

The amide I and amide II peaks of BSA (Fig. 2c) were shifted from 1544 to 1540 cm^{-1} and from 1654 to 1626 cm^{-1} upon interaction with quercetin (Fig. 2a) and to 1548 and 1628 cm^{-1}

Table 3

The antioxidant activity of all studied durian samples ($\mu\text{MTE/g}$) in dimethyl sulfoxide (DMSO) extract.^a

Durian samples	ABTS	FRAP	CUPRAC	DPPH
Young	23.49 \pm 1.1 b	5.1 \pm 0.3 c	1.01 \pm 0.04 a	1.30 \pm 0.05 b
Mature	12.66 \pm 0.6 a	3.92 \pm 0.2 b	1.11 \pm 0.05 a	0.53 \pm 0.03 a
Ripe	11.06 \pm 0.5 a	5.87 \pm 0.4 c	2.21 \pm 0.1 b	1.12 \pm 0.04 b
Overripe	27.41 \pm 1.3 c	1.11 \pm 0.04 a	2.42 \pm 0.1 b	1.54 \pm 0.07 c

ABTS, 2,2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; CUPRAC, cupric reducing antioxidant capacity; FRAP, ferric-reducing/antioxidant power; DPPH, 1,1-diphenyl-2-picrylhydrazyl method.

^a Values are means \pm SD of 5 measurements. Values in columns for every value of antioxidant activity bearing different letters are significantly different ($P < 0.05$). Per g dry weight.

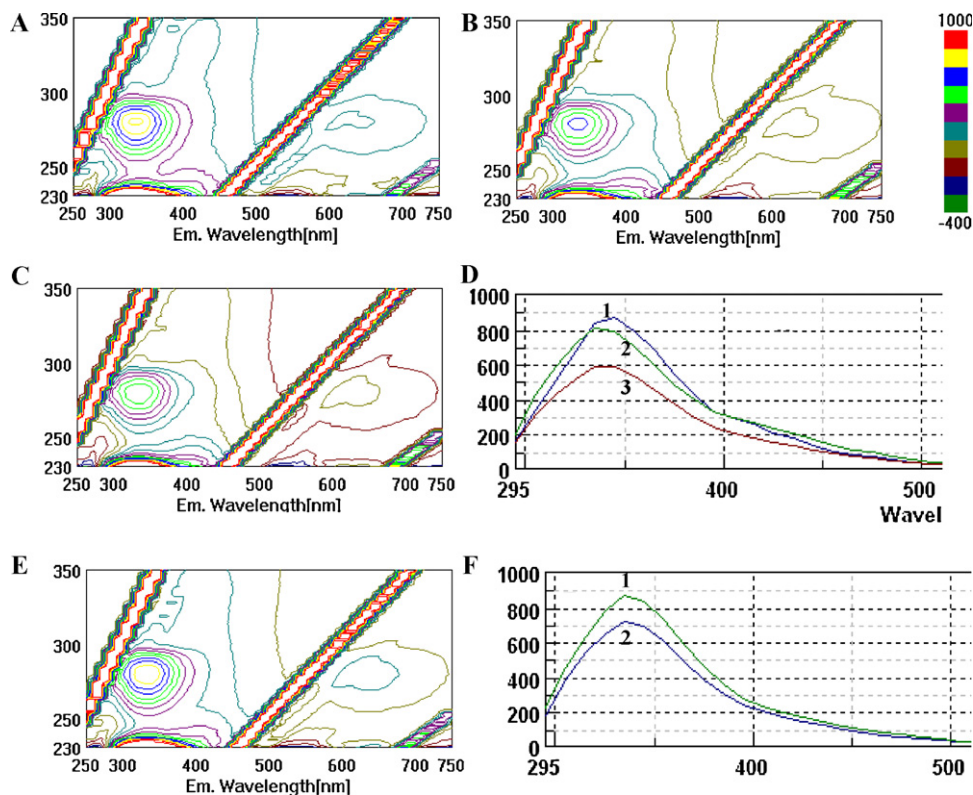


Fig. 1. Three-dimensional fluorescence (3D-FL) spectra illustrate the elliptical shape of the contours of 2.0×10^{-4} mol/l BSA (A), 2.0×10^{-4} mol/l BSA and $40 \mu\text{g/ml}$ extract of durian ripe at 0 h time (B), 2.0×10^{-4} mol/l BSA and $40 \mu\text{g/ml}$ extract of durian ripe at 1 h and 37°C time (C), change in fluorescence intensity (D) as a result of binding affinity of BSA and polyphenol extract of ripe durian: BSA (1), BSA + extract of ripe durian at 0 h time (2), BSA + extract of ripe durian at 1 h time (3); 2.0×10^{-4} mol/l quercetin and 2.0×10^{-4} mol/l BSA at 1 h and 37°C (E); change in fluorescence intensity (F) as a result of binding affinity of BSA and quercetin: BSA (1), 2.0×10^{-4} mol/l BSA + 2.0×10^{-4} mol/l quercetin at 1 h and 37°C (2). The 3D-FL were run emission mode and fluorescence intensity up to 1000, emission wavelengths from 250 to 750 nm and excitation wavelengths from 230 to 350 nm; scanning speed was 1000 nm/min, (D) and (F) excitation wavelength on x-axis and fluorescence intensity on y-axis; (A–C) and (E) emission wavelength on x-axis and excitation wavelength on y-axis.

upon interaction with durian ripe extract (Fig. 3b). FTIR of quercetin (Fig. 2b) shows broad phenolic OH band centred around 3400 cm^{-1} , characteristic $-\text{CO}$ stretching at 1663 cm^{-1} aromatic bending and stretching around 1091 and 1663 cm^{-1} , $-\text{OH}$ phenolic bending around 1197 and 1374 cm^{-1} . FTIR of quercetin could confirm the relative chemical stability of quercetin. FTIR spectra of durian ripe (Fig. 3c) showed a peak at 1744 cm^{-1} , which corresponds to the $-\text{CO}$ (stretching) and the peaks at 2917 and 2852 cm^{-1} are related to the $\text{C}-\text{H}$ bond of saturated carbons (Fig. 3c). As it was shown previously that the characteristic 1663 cm^{-1} of quercetin $-\text{CO}$ stretching (Fig. 2b) is seen as small shoulder due to the overlapping of the dominant $-\text{CO}$ stretching of durian (1744 cm^{-1} , Fig. 3c), but the phenolic OH corresponding to quercetin is seen around 3405 cm^{-1} for the quercetin–BSA at 3183 cm^{-1} (Fig. 2a).

Matching between the peaks in the range from 4000 to 400 cm^{-1} between (BSA + Quercetin):(Quercetin) = 12.4% ; (BSA + Quercetin):(BSA) = 49.1% (Fig. 2) and (BSA + DRipe):(DRipe) = 9.2% (Fig. 3). The comparison of the peaks in the interaction between BSA and quercetin and BSA and the durian ripe extract (BSA + Quercetin):(BSA + DRipe) was about 99.9% (Fig. 3). Other durian samples were similar (99.2% ; 98.5% and 98.1% for mature, overripe and young durian), but the best match was achieved with ripe durian extract in comparison with pure quercetin.

Plasma lipids and antioxidant activity

An increase in plasma lipids level was registered in all groups fed diets containing cholesterol (Table 4). However, diet supplemented with durian ripe decreased TC by 4.8% and LDL-C by 6.3% , both not significant, while in the case of TG this decrease was significant ($P < 0.05$) and amounted 26.3% .

Table 4

Plasma lipids profile in rats fed diets with cholesterol and durian fruits in different stages of maturity.^a

Diet groups	TC (mmol/l)	LDL (mmol/l)	TC/HDL	TG (mmol/l)
Chol/DY	2.3 ± 0.3 b	1.7 ± 0.1 b	3.8 ± 0.2 b	1.6 ± 0.3 b
Chol/DM	2.1 ± 0.3 b	1.6 ± 0.2 b	4.2 ± 0.2 b	1.5 ± 0.2 b
Chol/DR	2.0 ± 0.2 ab	1.5 ± 0.2 b	4.0 ± 0.2 b	1.4 ± 0.2 ab
Chol/DOR	1.9 ± 0.4 ab	1.4 ± 0.5 ab	3.8 ± 0.4 b	1.6 ± 0.4 b
Control	1.6 ± 0.3 a	1.1 ± 0.2 a	2.9 ± 0.2 a	0.9 ± 0.4 a
Control/Chol	2.1 ± 0.4 b	1.6 ± 0.4 b	4.3 ± 0.3 b	1.9 ± 0.3 c

Chol/DY, Chol/Durian Young; Chol/DM, Chol/DMature; Chol/DR, Chol/DRipe; Chol/DOR, Chol/DOverripe; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triacylglycerols.

^a Values are the means \pm SD of 6 measurements. Values in columns for every analysis bearing different letters are significantly different ($P < 0.05$).

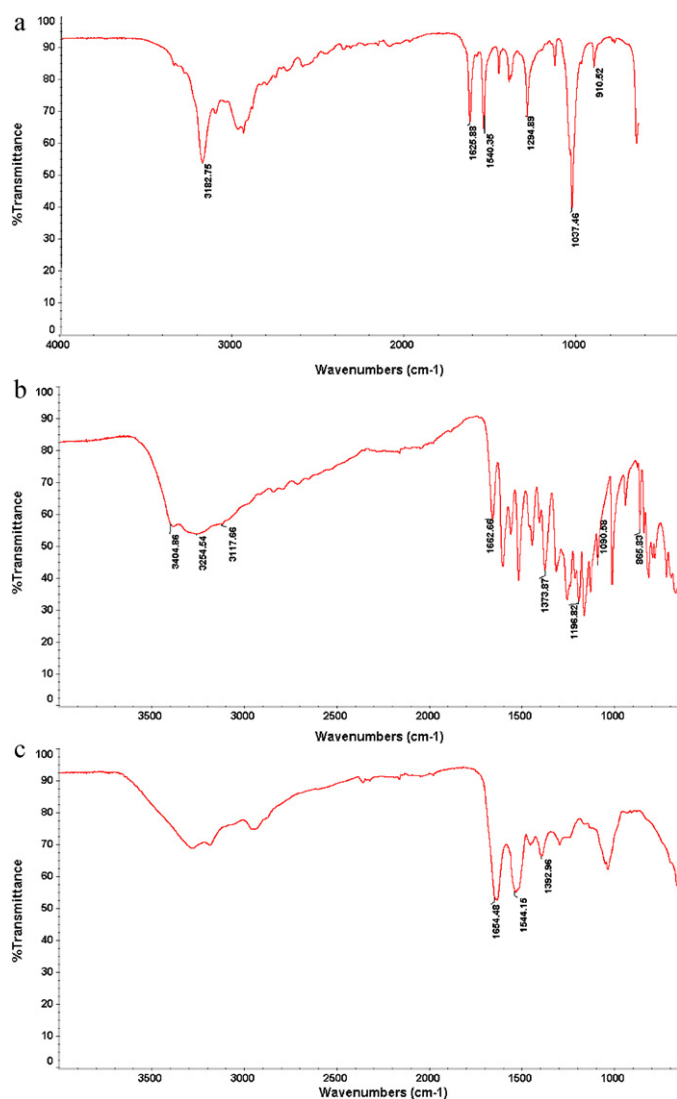


Fig. 2. Infrared study of FTIR spectra of (a) BSA and quercetin; (b) quercetin; (c) BSA. The interaction of 2.0×10^{-4} mol/l BSA and 2.0×10^{-4} mol/l quercetin was during 1 h, at 37°C , then after the fluorimetric measurements the sample was lyophilized.

A significant decrease of plasma antioxidant activity was registered in Chol group vs. Control group ($P < 0.05$), but only on the data obtained from the ABTS assay. Significant increase of plasma antioxidant activity was noted in the rat group supplemented with durian ripe vs. Chol Group: by 26.3% and 36.4%, according to ABTS and FRAP, respectively (Table 5).

It was registered a correlation between the increase of the plasma lipids and decrease in the plasma antioxidant activity: in group of rats with the highest increase of plasma lipids (Chol) the decrease of the antioxidant activity was significantly higher ($P < 0.05$) than in other diet groups.

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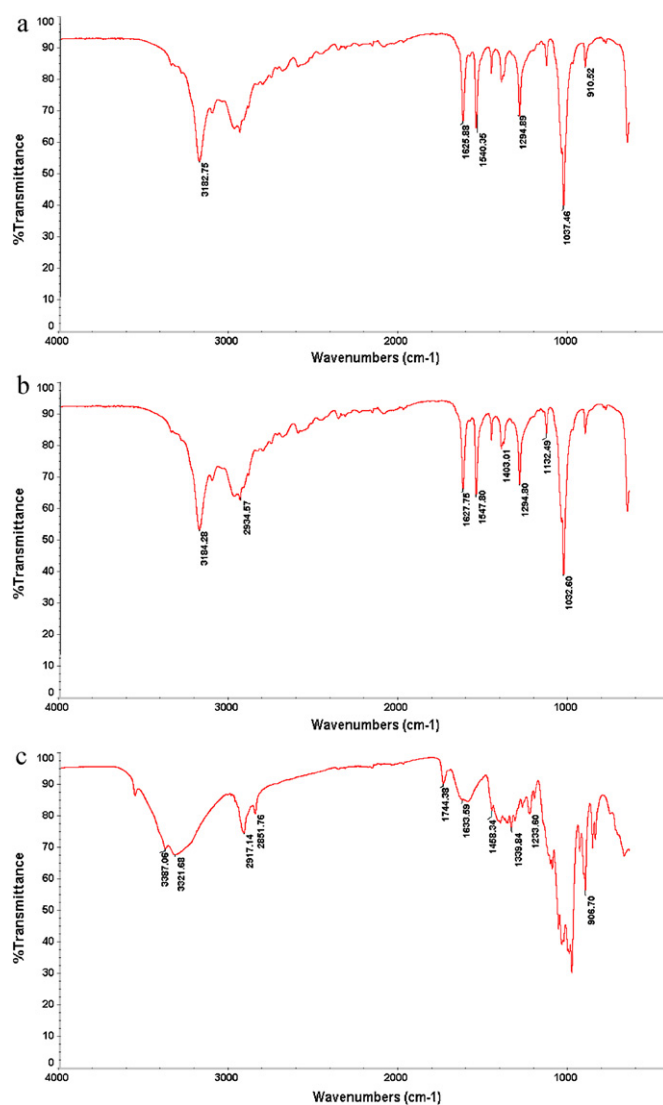


Fig. 3. Infrared study of FTIR spectra of (a) BSA and quercetin; (b) 2.0×10^{-4} mol/l BSA and $40 \mu\text{g/ml}$ durian ripe extract; (c) durian ripe extract. The interaction of 2.0×10^{-4} mol/l BSA and 2.0×10^{-4} mol/l quercetin (a) and 2.0×10^{-4} mol/l BSA and $40 \mu\text{g/ml}$ durian ripe extract (b) was during 1 h, at 37°C , then after the fluorimetric measurements the samples were lyophilized.

Table 5
The antioxidant activity in rats plasma.^a

Diet groups	ABTS (%RSA)	FRAP (mmolTE/l)	DPPH (mmolTE/l)
Chol/DY	1.22 ± 0.1 ab	0.13 ± 0.01 ab	24.7 ± 0.52 a
Chol/DM	1.23 ± 0.1 ab	0.13 ± 0.01 ab	25.0 ± 0.39 a
Chol/DR	1.49 ± 0.2 b	0.15 ± 0.01 b	25.8 ± 0.47 a
Chol/DOR	1.25 ± 0.1 ab	0.14 ± 0.01 ab	25.3 ± 0.62 a
Control	1.33 ± 0.1 b	0.13 ± 0.01 ab	25.2 ± 0.88 a
Control/Chol	1.18 ± 0.2 a	0.11 ± 0.01 a	23.6 ± 0.74 a

Chol/DY, Chol/Durian Young; Chol/DM, Chol/DMAture; Chol/DR, Chol/DRipe; Chol/DOR, Chol/DORripe; ABTS, 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diammoniu salt; FRAP, ferric-reducing/antioxidant power; DPPH, 1,1-diphenyl-2-picrylhydrazyl method; RSA, radical scavenging activity; TE, trolox equivalent.

^a Values are the means ± SD of 6 measurements. Values in columns for every analysis bearing different letters are significantly different ($P < 0.05$).

Table 6
The antioxidant activity in rats plasma.^a

Diet groups	AST (IU/l)	ALT (IU/l)	ALP (IU/l)
Chol/DY	98.8 ± 21.4	26.6 ± 5.2 a	224 ± 47.2 ab
Chol/DM	98.5 ± 10.2	31.2 ± 4.9 ab	235 ± 57.8 ab
Chol/DR	96.7 ± 10.1	30.3 ± 2.2 ab	208 ± 17.3 ab
Chol/DOR	93.0 ± 12.0	28.4 ± 3.6 a	234 ± 46.7 ab
Control	103.0 ± 14.7	34.2 ± 2.7 ab	192 ± 40.1 a
Control/Chol	105 ± 11.1	37.2 ± 8.1 b	282 ± 72.5 b

Chol/DY, Chol/Durian Young; Chol/DM, Chol/DMature; Chol/DR, Chol/DRipe; Chol/DOR, Chol/DOverripe; AST, asparagine aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

^a Values are the means ± SD of 6 measurements. Values in columns for every analysis bearing different letters are significantly different ($P < 0.05$).

Liver enzymes

The ALT activity was higher in Chol group than in the Control (37.2 vs. 34.2 IU/l) ($P > 0.05$). In the case of ALP activity the differences between these groups were significant (282.3 vs. 192.7 IU/l) (Table 6). Supplementation of cholesterol containing diets with durian samples decreases the ALT activity (significantly for Chol/DY and Chol/DOR). Diminishing of ALP activity in all durian groups was not significant ($P > 0.05$).

As can be seen, the supplementation of diet with ripe durian protects the rat's aorta and liver significantly better ($P \leq 0.05$) from damage from exogenous cholesterol loading than does durian at other stages of maturity.

The significant highest protection of the intimal surface area of aorta was in group, which diet was supplemented with ripe durian ($P < 0.05$).

Histopathology of the aorta and liver

(a) The histological changes in the aorta are shown in Fig. 4.

After the experiment in vivo the percentage of total intimal surface area of aortic atheromatous lesions was in the following order: Chol > Chol/DOverripe > Chol/DYoung > Chol/DMature > Chol/DRipe. The significant highest protection of the intimal surface area of aorta was in group, which diet was supplemented with ripe durian ($P < 0.05$).

(b) The results of the histology examination of liver are shown on Fig. 5. As can be seen, the histological architecture of rat's livers was correct. The hepatic cords radiantly surrounded the central venules. Histological changes (numerous fat droplets) concentrated inside hepatic cells of rats loaded with cholesterol. The fat integral density ($\mu\text{g}/\mu\text{m}^3$ of 100 hepatocytes) for Control group, Control/Chol, Chol/DRipe, Chol/DMature, Chol/DOverripe is 95.07 ± 6.37 ; 349.71 ± 29.13 ; 238.78 ± 15.29 ; 266.22 ± 14.05 ; 269.90 ± 8.02 ; 292.30 ± 23.73 . Cytoplasm of hepatic cells in Chol/DMature group is plumped of fat droplets which are saturated with different quality of red stain. In all hepatic cells fat droplets are of identical diameter. The fat droplets in Chol/DOverripe group filled cytoplasm of hepatic cells quite uniform. Hepatic cells cytoplasm fat droplets have a different

diameter. In Chol/DYoung group is full fat droplets, which have equal diameter in some hepatic cells. However, in different hepatic cells, the droplets are not identical. The fat integral density/100 hepatocytes are in the following order: Control/Chol > Chol/DYoung > Chol/DOverripe > Chol/DMature > Chol/DRipe > Control.

As can be seen, the supplementation of diet with ripe durian protects the rat's aorta and liver significantly better ($P \leq 0.05$) from damage from exogenous cholesterol loading than does durian at other stages of maturity.

Discussion

The present study showed in vitro results of determination of polyphenols, flavonoids, flavanols, tannins, anthocyanins and vitamin C in the investigated durian samples. Previously it was found that the content of polyphenols and their antioxidant potentials were the highest in overripe durian, while flavonoids – in ripe durian, and flavanols and antiproliferative activity – in mature durian ($P < 0.05$) [13].

The total phenolics reported by others [15] were in the range of 690.62–998.29 mg/l (about 2.32–3.34 mg/g DW), which are similar to our data. The total flavonoids and vitamin C contents were found in the range of 211.36–220.34 (approximately 0.71–0.74 mg CE/g DW) and 18.87–25.1 mg/l (0.063–0.084 mg/g DW), respectively. These values were twice as low for flavonoids and higher in the content of vitamin C [12]. Such variations in the content of bioactive compounds can be explained by different cultivars of durian, the year of growing, and the stages of ripening and mostly by the different extracts used in numerous studies

Durian samples at different stages of ripening showed relatively high amount of polyphenols and the overall antioxidant potentials. As it was mentioned above, the results were discussed in our previous publications and by other researchers [12–15].

The Mon Thong durian cultivar was used in the present study: it was found by ultraviolet spectroscopy and high-performance liquid chromatography/diode array detection analyses that caffeic acid and quercetin were the dominant bioactive substances in this cultivar [21]. In all studied samples the most important flavonoids quercetin was determined at 321 nm, and the highest amount was found in ripe durian (58.7 ± 2.8 , 62.1 ± 3.1 , 68.9 ± 3.3 and 61.9 ± 2.9 mg/g for young, mature, ripe and overripe samples, respectively). These results were different from the ones of our previous investigations [13]: the highest content of quercetin was detected in ripe samples (68.9 ± 3.3 mg/g). These results were predictable: the investigated durian was grown under different climatic conditions.

As it was discussed, quercetin is one of the most effective and abundant flavonoids in human diet [29,30]. Quercetin has been shown to exhibit a wide range of pharmacological properties. The bioavailability of quercetin and its conjugates are of significant importance due to the fact that little free quercetin is available in the blood after supplementation as a result of rapid glycosylation and vital role in its biological activity [29–31].

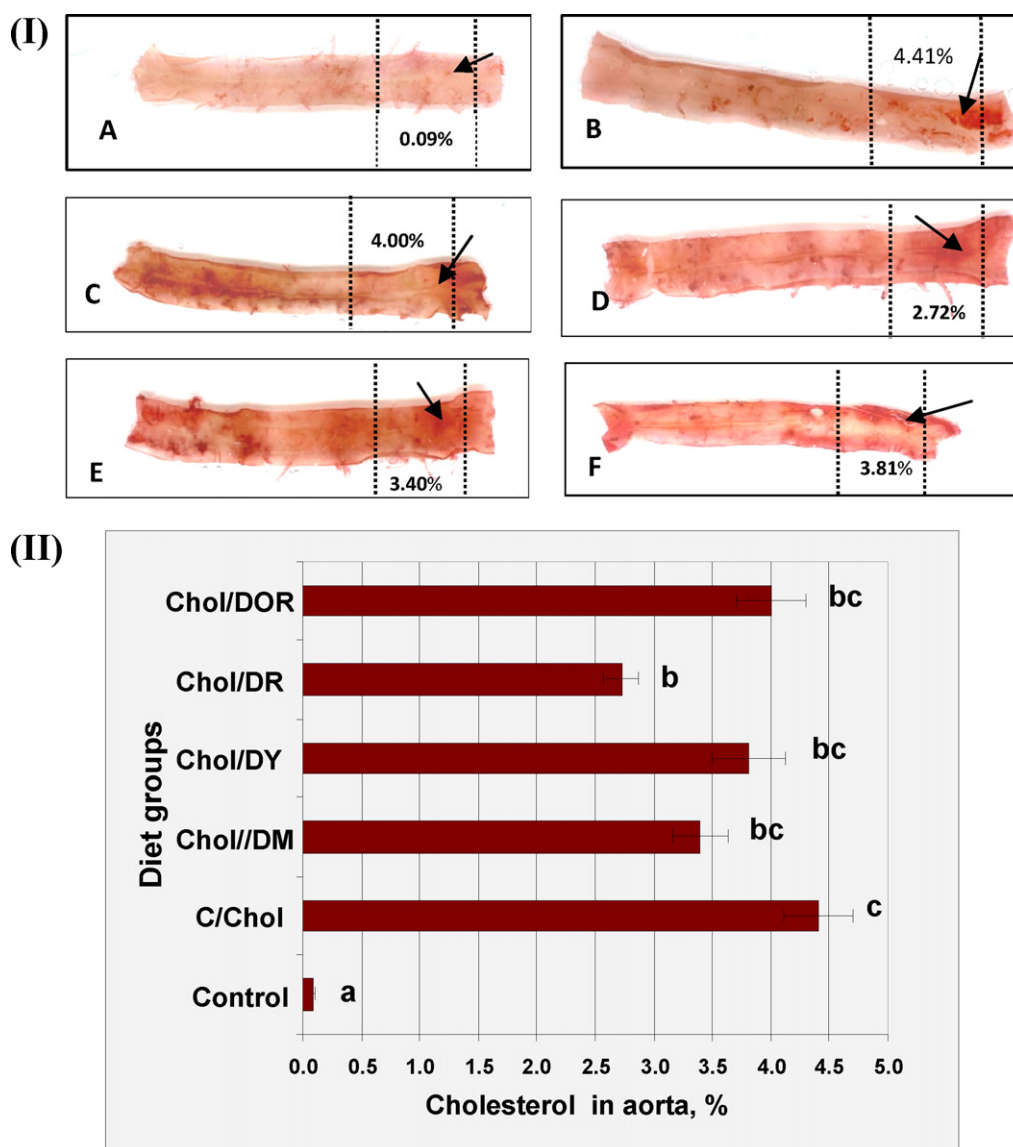


Fig. 4. (I) Aortic changes in the (A) Control, (B) Control/Chol, (C) Chol/Durian Overripe, (D) Chol/Durian Ripe, (E) Chol/Durian Mature, and (F) Chol/Values are the means \pm SD of 6 measurements. Values in bars for every analysis bearing different letters are significantly different ($P < 0.05$). Durian young diet groups. The marked positions show the most concentrated areas and the percentages of lesions in the arch of the aorta from the total amount. (II) Changes in lesions in the total aorta (percentage of Chol). C, Control; Chol, cholesterol; DM, durian mature; DY, durian young; DR, durian ripe; DOR, durian overripe.

The fruits are a rich source of dietary antioxidants and proanthocyanidins play one of the major roles, therefore in this report in vitro studies were carried out on interaction of quercetin with protein molecule such as BSA. The structure of the flavonoids appears to have some effect on the quenching process.

Based on such important properties of quercetin and the amount found in the ripe durian the interaction of BSA with quercetin was examined in an aqueous solution in physiological conditions similar to human metabolism.

FTIR, and 2D-FL, and 3D-FL methods were applied to determine the effects of flavonoid complexation on the secondary structure of the protein by the quenching of the albumin fluorescence and the enhancement of the flavonoid fluorescence.

In our previous study the FTIR-spectra data showed that the main bands in the durian samples were from 1700 to 800 cm^{-1} (1637, 1415, 1137, 1103, 1056, 995 and 923 cm^{-1}).

A shift in the difference between the standards and the investigated samples can be explained by the method of extraction of the main polyphenols. There are two regions (1700–1600 and 1550–1500 cm^{-1}) in the spectrum unique to the protein secondary structure (amides I and II). The shift in amide I and amide II peaks of BSA indicate the interaction between BSA and quercetin. The comparison of the peaks in the interaction between BSA and quercetin and BSA and the durian ripe extract (BSA + Quercetin):(BSA + DRipe) was about 99.9%, showing that the polyphenol extract of durian behaves similar to pure quercetin.

Our results show that the fluorescence at the wavelength of peak 2 is significantly quenched, because the conformation of the BSA changes in the presence of pure flavonoids and durian extracts. This interaction between quercetin and BSA was investigated using tryptophan fluorescence quenching. Our results are

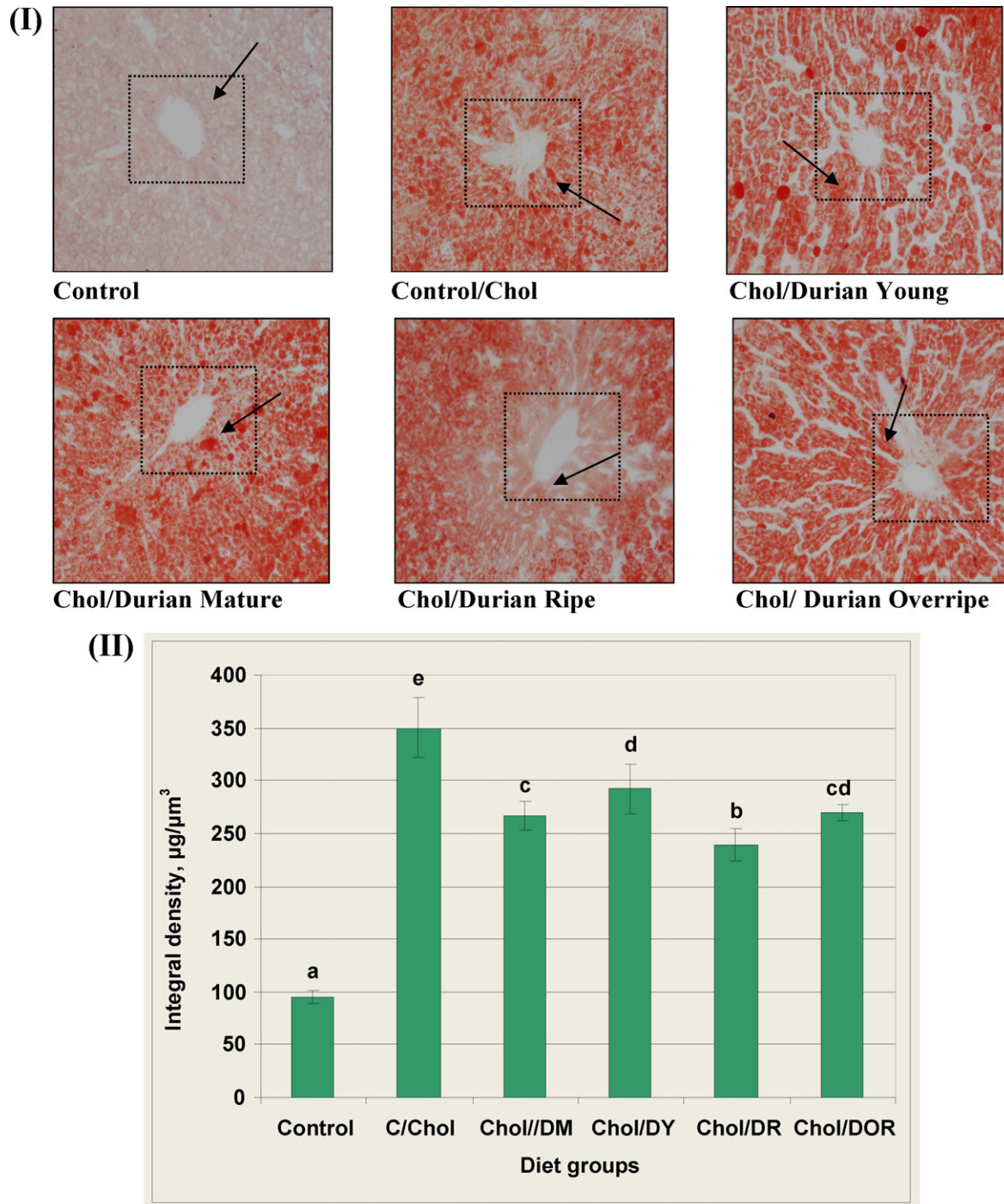


Fig. 5. Hepatic tissue morphology of Wistar rats of the following diet groups: (I). Control-view of normal rat hepatic tissue (without fat). Architecture shows radiated arranged hepatic cords around the central venule. Control/Chol – around the central venule are the hepatic cell cords. Intensive red coloring was the effect of high contents of fat inside hepatic cells. Chol/Durian Young – the liver presents full fat droplets. Chol/Durian Mature – droplets of fat show different saturation of red color. Chol/Durian Ripe – liver exposure on this factor shows small number of large fat droplets; Chol/Durian Overripe – the fat droplets filled cytoplasm of uniform hepatic cells. Red Oil stain was used, magnification $10\times$. The marked positions show the most concentrated areas of cholesterol in liver. (II). Changes in integral density ($\mu\text{g}/\mu\text{m}^3$) in liver: C, Control; Chol, cholesterol; DM, durian mature; DY, durian young; DR, durian ripe; DOR, durian overripe. Values are the means \pm SD of 6 measurements. Values in bars for every analysis bearing different letters are significantly different ($P < 0.05$).

in agreement with others that quercetin, as an aglycon, are more hydrophobic and demonstrates strong affinity toward BSA [29]. The obtained data about the binding of quercetin in our studies by 2D-FL, and 3D-FL methods correspond with Papadopoulou et al. [32]. Other results differ [17] from the reported by us,

probably because of the variety of antioxidant abilities of pure flavonoids and different ranges of fluorimetry scanning ranges used in a similar study.

Quercetin has a total quenching effect on BSA tryptophan fluorescence at a molar ratio of 1:1. The biological relevance

of such interaction in human organism is important from the point that this molecule of polyphenolic type extensively binds to human serum albumin (HSA), the most abundant carrier protein in the blood. The position of quercetin within the binding pocket allows simultaneous binding of other ligands such as warfarin, or sodium salicylate and to control the drug delivery in the human organism [16,17,32].

There are no publications on applications of 3D fluorescence spectra, therefore our present conclusions that 3-D fluorescence can be used as an additional tool for the characterization of the polyphenol extracts during different stages of ripening correspond with the previous data [14]. The highest content of quercetin in ripe durian leads to significantly highest protection from damages of aorta and liver in rats on cholesterol-containing diets supplemented with such samples.

Our *in vitro* results of interaction of BSA and quercetin can be compared with other reports *in vivo*, showing the protective effects of quercetin on hepatic injury induced by different chemical reactions. It was investigated that quercetin is effective against liver damage in rats with carbon tetrachloride-induced cirrhosis [33], inhibits dimethylnitrosamine-induced liver damage in rats [34] and protects against liver injury in chronically biliary obstructed rats [35]. The protective effects of chronic administration of the flavonoid quercetin (150 mol/kg body wt/day intraperitoneally) in rats with carbon tetrachloride-induced fibrosis during 3 weeks improved liver histology and reduced collagen content, iNOS expression, and lipid peroxidation [33]. Oral administration of quercetin (10 mg kg⁻¹ daily for 4 weeks) remarkably prevented dimethylnitrosamine-induced loss in body and liver weight and inhibited the elevation of serum alanine transaminase, aspartate transaminase and bilirubin levels [34]. The intermediate quercetin dose given for 2 or 4 weeks restored the reduction in glutathione concentration and partially prevented the increase in collagen concentration, TBARS and GSSG/GSH ratio. A significant preservation of the activities of antioxidant enzymes, a less pronounced fibrosis and a marked inhibition of bile ductular proliferation occurred during such treatment [35]. The authors [29–35] concluded that quercetin is effective in this model of liver damage. Our results are consistent with the cited *in vivo* results on application of pure quercetin, where was shown that the pure quercetin behaves as polyphenol extracts from durian fruit.

In our study, we investigated hepato- and heart-protective effects of durian at different stages of ripening in rats fed diets with cholesterol. Thus, in group of rats supplemented with ripe durian, the percentage of total intimal surface area of aortic atheromatous lesions was 2.63 vs. 4.47 and the fat integral density of 100 hepatocytes in liver was $238.78 \pm 15.29 \mu\text{g}/\mu\text{m}^3$ vs. $349.71 \pm 29.13 \mu\text{g}/\mu\text{m}^3$ of Chol group (P in both cases <0.05). These results were in agreement with Haidari et al. [7], who investigated the association between oxidative stress and regional susceptibility of the mouse aorta to atherosclerosis. In addition, the same tendency was shown in the results of the investigation of liver enzymes: ALT level in rats fed Chol diet (without durian fruits) than in the Control group ($P > 0.05$). Supplementation of cholesterol containing diets with durian samples decreased the ALT level, however significantly ($P < 0.05$) was

only for diet groups obtained durian overripe and young. In these stages of durian maturity amount of flavonoids, flavanols and polyphenols were the highest. It was also found that obese mice, compared to lean controls, had greater hepatic lipids and serum alanine aminotransferase [36]. Our results are in correspondence with Nakayama et al. [3], who found in young male mice fed a high-fat diet that the numbers of fatty droplets in the liver cytoplasm markedly increased as did as the liver weight. Our results were consistent with the cited report [4], where it was found that atherogenic diet (3% lard + 1.3% cholesterol) of rabbits after 50 days feeding enhanced lipid levels both in plasma and liver mitochondria, reduced plasma and mitochondrial concentrations of retinol and coenzyme Q-10. Vinaixa et al. [5] reported that dietary cholesterol increased the hepatic concentrations of cholesterol, triglycerides, and oleic acid, as well as the relative amount of long-chain polyunsaturated fatty acids in the liver. This was also accompanied by variations of the hepatic concentration of taurine, glutathione, methionine, and carnitine. Lesions in control mice increased dramatically by 6 months of CED. In contrast, lesion size did not increase during this time in CED-fed FHL2-null mice [6]. The cited references [3–7,36] were in correspondence with our results and the difference could be marked in the period of feeding of animals. So, Hernández-Espinosa et al. [2] found that a hyperlipidemic diet for six months led to a significant intrahepatic retention and aggregation of antithrombin, which correlated with hepatic steatosis, as revealed by immunohistological analysis.

The positive effect of durian supplemented diets depends not only on the biological properties of quercetin, but also on other polyphenols and phytochemicals [12–14,21,36,37]. Pre-treatment with date palm fruit extract restored the liver damage induced by dimethoate, as revealed by inhibition of hepatic lipid peroxidation and improvement of histopathology changes. The cited findings [37] indicate that *in vivo* date palm fruit may be useful for the prevention of oxidative stress induced hepatotoxicity. The authors [37] as well our conclusions decided that sufficient amount of antioxidant compounds such as polyphenols can give a good protection against liver damage: the amount of polyphenols in the palm fruit extract was similar to the durian fruit extract.

The contents of polyphenols, flavonoids, quercetin, flavanols, ascorbic acid and tannins in young, mature, ripe and overripe samples of Mon Thong durian cultivar and the antioxidant potentials as determined by four complementary assays (CUPRAC, DPPH, ABTS and FRAP) varied. The content of quercetin was the highest in ripe durian. Diets of rats fed cholesterol supplemented with ripe durian most significantly protect heart and liver from damages. The results of this investigation support our hypothesis and advance the use of this fruit in human nutrition.

Conclusion

Durian fruit at different stages of ripening, especially ripe, constitute an excellent source of effective natural compounds. This fruit possesses antioxidant and health-protective properties for liver and aorta in rats fed cholesterol-enriched diet.

Conflict of interest

No conflict of interest declared.

Disclosure

All research done by the authors.

Acknowledgments

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