JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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# Health-Promoting Effects of Ethylene-Treated Kiwifruit 'Hayward' from Conventional and Organic Crops in Rats Fed an Atherogenic Diet

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**ABSTRACT:** Kiwifruit is a subtropical fruit that is very popular among consumers. Kiwifruit 'Hayward' (*Actinidia deliciosa* C.F. Liang et A.R. Ferguson) is an important source of bioactive compounds and possesses high antioxidant capacity, but its value can be changed during ripening. The aim of this study was to compare the levels of total polyphenols (TP), ascorbic acid (AA), and total antioxidant capacities (TACs) of ethylene-treated and nontreated kiwifruits 'Hayward' from conventional and organic farming. The influence of these fruits on lipid profile, TAC, and liver enzymes in plasma of rats fed diets with cholesterol was studied. Ethylene treatment shortened the ripening of kiwifruits. Ethylene-treated kiwifruits from organic farming (OHE) have the highest contents of TP and TAC. The experiment in vivo was performed during 33 days on male Wistar rats (111 ± 5 g), divided into six groups: one without cholesterol, control (C), and five groups with 1% of cholesterol (Chol). Four groups with cholesterol were supplemented with 5% of lyophilized kiwifruits: ethylene treated, organic (Chol/OHE) or conventional (Chol/CHE); and untreated, organic (Chol/OHC) or conventional (Chol/CHC). Cholesterol diets supplemented with kiwifruit influenced the palatability and feed intake, body gain, and FER. Diets containing kiwifruits significantly influenced the decrease of TG (61%), TC (29%), LDL-C (38%), atherogenic index TC/HDL-C (25%), and atherogenic index (AI, 32%), without differences between treatments. A significant increase of TAC in plasma of rats fed kiwifruit was obtained by DPPH (18%), FRAP (55%), and ABTS (55%). Aspartate aminotransferase (AST) activity in serum was significantly lower for all groups with kiwifruit supplementation. Alanine aminotransferase (ALT) was lower only in diet groups supplemented with conventional fruits in comparison with the cholesterol group. Glucose levels were higher in groups with kiwifruit supplementation than in C and Chol groups. Supplementation of Chol groups with organic kiwifruits influenced the prothrombin index and significantly decreased the amount of platelets (PLT) in blood. In conclusion, studied kiwifruit 'Hayward' can be a very good ingredient of the diet, especially for patients suffering from hypercholesterolemia and with other cardiovascular diseases, but not for diabetic patients.

**KEYWORDS:** *kiwifruit conventional and organic, ethylene treatment, bioactive compounds, lipids, coagulation indices, cholesterol, rats*

## INTRODUCTION

Consumption of fruits and vegetables plays a special role in the prevention of atherosclerosis and other cardiovascular diseases.[1−3] The health benefits of fruits, including kiwifruits, are attributed in part to bioactive compounds.[4,5] Kiwifruits are an exotic and climacteric crop from South Korea; however, even after maturation, they have hard flesh firmness and high acidity,[6−8] and can be eaten only after ripening. The natural ripening of kiwifruit is a long process and leads to a decrease in fruit quality.[9] Kiwifruit possesses a negligible content of ethylene at harvest, and its ripening can be induced by a very low concentration of exogenous ethylene.[8] To evaluate the effectiveness of the ethylene treatment, it is important to record the possible changes in bioactive compounds and in the total antioxidant capacities of kiwifruits.[8,10] The contents of polyphenols and ascorbic acid and the radical scavenging capacity of fruits treated and nontreated with ethylene were presented by Park et al.[11]

Nowadays, there is an increased interest by scientists in crops grown in organic versus conventional systems, utilization of exogenic ethylene, and evaluation of these conditions on the content of phenolic compounds and their bioactivities. According to Hassey et al.[12] for kiwifruit cultivation, an organic system is better than a conventional one. Benge et al.[13] reported that conventional kiwifruits, even with the same firmness, have a higher soluble solids content than organic, but softening and decay processes did not differ between the two

Received: January 15, 2013
Revised: March 25, 2013
Accepted: March 27, 2013
Published: March 27, 2013

systems. The bioactivity of kiwifruits grown in conventional and organic conditions has not been fully investigated.[10] The polyphenols may function individually to protect lipoproteins and vascular cells from oxidation or by other mechanisms, such as reducing plasma lipid levels (LDL-cholesterol and triglycerides), and also by increasing the ability of leukocytes to repair DNA breakage by free radicals.[14−16] Duttaroy and Jorgensen[3] reported that consumption of two or three kiwifruits per day by humans for 28 days lowered triglycerides by 15% and reduced platelet aggregation by 18%. It has been shown that kiwifruits possess antioxidant properties and diminish plasma lipids and have also anticoagulant activity.[3,17] This study was conducted for estimation of rats' reaction to the feeding of atherogenic diets supplemented with kiwifruit 'Hayward'. All kiwifruits were analyzed for their bioactivity. Lipid profiles, plasma antioxidant capacity, liver enzyme activity, and blood coagulation indices were determined in rats fed cholesterol with supplementation of conventional or organic kiwifruits, treated with ethylene or air. As far as we know, there are no published results of such investigations.

## MATERIALS AND METHODS

**Samples and Preparation.** Kiwifruits of 'Hayward' cultivar (organic and conventional) at their commercial maturity stage were harvested from an orchard (Heanam County, Jeonnam Province, South Korea, 2008). The kiwifruit samples [organic 'Hayward' ethylene treated (OHE) and conventional 'Hayward' ethylene treated (CHE)] were treated with 100 ppm ethylene for 24 h at 20 °C in a growth chamber (Percival Scientific Inc., Perry, IA, USA). The samples were put into an 18 L glass jar and ventilated with a humidified flow of air mixed with ethylene at 300 mL min $^{-1}$. The organic (OHC) and conventional (CHC) kiwifruit samples were put into an 18 L glass jar and ventilated with a humidified flow of air. Then the ethylene and air-treated kiwifruits were ripened separately using the same conditions, at 20 °C, in a growth chamber (Percival) for 10 days. All fruits were cleaned with tap water and dried, using five replicates of five fruits each. The fruits were peeled without using steel knives and then 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.

**Determination of Content of Bioactive Compounds.** Polyphenols were extracted from lyophilized fruits with 50% dimethyl sulfoxide (DMSO) (concentration = 25 mg/mL) at room temperature twice during 3 h. The polyphenols were determined by using the Folin−Ciocalteu method with measurements at 750 nm. The results were expressed as milligrams gallic acid equivalents (GAE) per gram dry weight (DW).[18] Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \cdot H_2O$, and 1 M NaOH, were measured at 510 nm. The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was 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). The measurements of optical densities were performed for polyphenols, flavonoids, flavanols, and tannins with a spectrophotometer (Hewlett-Packard, model 8452A, Rockville, MD, USA). Total ascorbic acid was determined by CUPRAC assay in a water extract (100 mg of lyophilized sample and 5 mL of water) according to the method of Ozyurek et al.[20] Dietary fiber was analyzed by using the modified Association of Official Analytical Chemists method of Prosky et al.,[21] whereby samples were treated with heat-stable amylase, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000*g*) to separate the soluble and insoluble fractions and dialysis against water.

**Determination of Total Antioxidant Capacity (TAC).** The TAC was determined by three complementary assays: (1) 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) was generated by the interaction of ABTS (7 mM/L) and $K_2S_2O_8$ (2.45 mM/L). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm.[22] (2) The 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.[23] (3) Scavenging free radical potentials were tested in a methanolic solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). In its radical form, DPPH has an absorption band at 515 nm, which disappears upon reduction by an antiradical compound. DPPH solution (3.9 mL, 25 mg/L) was mixed with the sample extracts in DMSO (0.1 mL), and then the reaction progress was monitored at 515 nm until the absorbance was stable.[24]

**Rats and Diets.** The Animal Care Committee of the Warsaw Agricultural University, Warsaw, Poland, approved this study. The mean weight of the male Wistar rats ($n$ = 36) at the beginning of the experiment was 111 ± 5 g. They were divided into six diet groups, each with six rats and named control (C), Chol, Chol/CHC, Chol/CHE, Chol/OHC, and Chol/OHE. During the first 5 days of adaptation, all groups were fed the basal diet (BD), which included wheat starch, casein, soybean oil, cellulose, vitamin (AIN-93-VX vitamin mix catalog no. 960402), and mineral mixtures (AIN-93-MX mineral mix catalog no. 960400) of the American Institute of Nutrition for laboratory animals. The rats were housed in metabolic cages (TECNIPLAST S.p.A., 21020, Italy). The rats of the control group during the 28 days of the experiment received the BD only, and the diets of the other groups were supplemented with 1% of cholesterol (Chol), 1% of cholesterol and 5% of lyophilized kiwifruits for Chol/CHC, Chol/CHE, Chol/OHC, and Chol/OHE, respectively. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. All rats were fed once a day at 10:00 a.m. ad libitum. They had unrestricted access to drinking water. The feed intake was monitored daily and body gain every week. At the end of the experiment after 24 h of starvation, the rats were anesthetized using Halothane (Narcotan, Zentiva), and blood samples were taken from the left atrium of the heart. Plasma and serum were prepared and used for a wide range of laboratory tests.

**Determination of Metabolic Indices.** As already stated above, the tests included determination of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) (analyzer Siemens Advia 1650), and plasma antioxidant capacity by DPPH, ABTS, and FRAP. As was mentioned above, the same assays were adopted for the determination of plasma antioxidant capacity of rats fed different diets. Instead of the kiwifruit extracts plasma of rats was used in all assays. TAC was evaluated as millimoles TE per liter. For the DPPH assay the results were expressed in percent radical scavenging capacity (RSC).

Liver enzyme activity for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in serum were determined, as previously described by Gorinstein et al.,[25] with utilization of a Siemens Advia 1650 analyzer, according to the principles of the Bayer Chemistry System.[26] Glucose in serum was determined with the utilization of a Siemens Advia 1650 analyzer, and platelets (PLT) in blood were estimated with a Siemens Advia 2120 analyzer. Coagulation indices (prothrombin index and prothrombin time) were carried out with a BCS Dade-Behring analyzer.

**Statistical Analysis.** The results of this study from in vitro evaluations are the mean ± 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 between groups' means. $P$ values of <0.05 were considered to be significant.

## RESULTS

**In Vitro Studies.** The results of the determination of all studied bioactive compounds and antioxidant capacities of

**Table 1. Content of Polyphenols, Ascorbic Acid, Total Dietary Fiber (TDF) and Its Fractions (IDF and SDF), and Total Antioxidant Capacity (by DPPH, ABTS, and FRAP Assays) in Kiwifruits from Different Crops and Treatments (per Gram DW)[a]**

| index | CHC | CHE | OHC | OHE |
|---|---|---|---|---|
| polyphenols, mg GAE | 7.1 ± 0.2 a | 8.7 ± 0.2 b | 7.9 ± 0.4 ab | 10.1 ± 0.4 c |
| flavonoids, mg CE | 2.0 ± 0.1 a | 2.3 ± 0.1 a | 2.0 ± 0.1 a | 3.0 ± 0.1 b |
| flavanols, μg CE | 165.0 ± 17.0 a | 210.0 ± 10.0 b | 181.0 ± 8.6 ab | 291.0 ± 10.2 c |
| tannins, mg CE | 1.8 ± 0.1 a | 2.3 ± 0.1 ab | 2.0 ± 0.1 a | 2.6 ± 0.1 b |
| ascorbic acid, mg AA | 0.17 ± 0.04 a | 0.20 ± 0.03 b | 0.19 ± 0.04 b | 0.19 ± 0.04 b |
| TDF, mg | 77.2 ± 0.3 a | 80.9 ± 0.3 ab | 82.5 ± 0.7 b | 84.5 ± 0.4 c |
| IDF, mg | 54.8 ± 0.6 a | 56.6 ± 0.2 b | 54.7 ± 0.4 a | 54.9 ± 0.3 a |
| SDF, mg | 22.4 ± 0.2 a | 24.3 ± 0.1 ab | 27.8 ± 0.4 b | 29.6 ± 0.2 c |
| DPPH, μmol TE | 10.2 ± 0.9 a | 12.7 ± 1.1 b | 11.3 ± 1.2 ab | 15.2 ± 1.3 c |
| ABTS, μmol TE | 20.0 ± 2.1 a | 27.0 ± 2.4 b | 22.3 ± 2.3 ab | 29.1 ± 2.2 c |
| FRAP, μmol TE | 14.4 ± 1.6 a | 18.4 ± 1.7 b | 16.7 ± 1.5 ab | 19.6 ± 1.9 c |

[a]Mean ± standard deviation. Averages in rows marked with different letters differ significantly ($P < 0.05$). Lack of letters indicates no difference between the means ($P > 0.05$). ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; CE, catechin equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl method; FRAP, ferric reducing/antioxidant power; GAE, gallic acid equivalent; trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, trolox equivalent; CHC, conventional kiwifruit air ripening; CHE, conventional kiwifruit ethylene treated; OHC, organic kiwifruit air ripening; OHE, organic kiwifruit ethylene treated.

**Table 2. Influence of Kiwifruits (Conventional and Organic) on Performance and Somatic Index of Organs in Rats Fed Atherogenic Diets (1% Cholesterol)[a]**

| index | Chol/CHC | Chol/CHE | Chol/OHC | Chol/OHE | C | Chol |
|---|---|---|---|---|---|---|
| food intake, g | 605.9 ± 17.3 bc | 619.3 ± 18.7 b | 606.8 ± 14.6 bc | 603.6 ± 11.9 bc | 571.1 ± 35.6 ac | 545.8 ± 53.4 a |
| body gain, g | 165.9 ± 3.9 b | 179.6 ± 30.1 b | 166.1 ± 18.2 b | 174.5 ± 18.2 b | 156.4 ± 14.6 b | 124.2 ± 31.9 a |
| FER, g/g | 3.9 ± 0.65 a | 3.53 ± 0.63 a | 3.68 ± 0.34 a | 3.49 ± 0.39 a | 3.66 ± 0.22 a | 4.6 ± 1.0 b |
| liver, % | 3.43 ± 0.27 ab | 3.67 ± 0.56 b | 3.44 ± 0.33 ab | 3.39 ± 0.26 ab | 3.39 ± 0.26 ab | 3.63 ± 0.33 b |
| kidneys, % | 0.62 ± 0.06 | 0.65 ± 0.05 | 0.65 ± 0.08 | 0.62 ± 0.06 | 0.67 ± 0.10 | 0.66 ± 0.08 |
| spleen, % | 0.16 ± 0.03 | 0.16 ± 0.02 | 0.16 ± 0.03 | 0.16 ± 0.02 | 0.19 ± 0.04 | 0.20 ± 0.03 |
| heart, % | 0.33 ± 0.03 | 0.32 ± 0.04 | 0.35 ± 0.05 | 0.34 ± 0.02 | 0.35 ± 0.02 | 0.34 ± 0.04 |

[a]Mean ± standard deviation. Averages in rows marked with different letters differ significantly ($P < 0.05$). Lack of letters indicates no difference between the means ($P > 0.05$). Chol/CHC, atherogenic diet with conventional kiwifruit air ripening; Chol/CHE, atherogenic diet with conventional kiwifruit ethylene stimulation; Chol/OHC, atherogenic diet with organic kiwifruit air ripening; Chol/OHE, atherogenic diet with organic kiwifruit ethylene stimulation; C, control; Chol, atherogenic diet (1% cholesterol).

**Table 3. Influence of Kiwifruit (Conventional and Organic) Treated with Ethylene on the Lipid Profile in the Serum of Rats Fed Atherogenic Diets (1% Cholesterol)[a]**

| index | Chol/CHC | Chol/CHE | Chol/OHC | Chol/OHE | C | Chol |
|---|---|---|---|---|---|---|
| TG, mmol/L | 0.9 ± 0.32 a | 0.87 ± 0.33 a | 0.76 ± 0.39 a | 0.94 ± 0.29 a | 1.07 ± 0.44 a | 2.23 ± 0.41 b |
| TC, mmol/L | 2.05 ± 0.48 a | 2.1 ± 0.28 a | 2.09 ± 0.26 a | 1.92 ± 0.19 a | 1.76 ± 0.16 a | 2.89 ± 0.55 b |
| HDL-C, mmol/L | 0.61 ± 0.12 | 0.63 ± 0.09 | 0.55 ± 0.07 | 0.59 ± 0.08 | 0.68 ± 0.13 | 0.63 ± 0.07 |
| LDL-C, mmol/L | 1.44 ± 0.4 b | 1.47 ± 0.25 b | 1.54 ± 0.2 b | 1.33 ± 0.21 ab | 1.08 ± 0.1 a | 2.26 ± 0.5 c |
| TC/HDL-C | 3.36 b | 3.33 b | 3.8 b | 3.25 b | 2.59 a | 4.59 c |
| AI | 2.36 b | 2.33 b | 2.8 b | 2.25 b | 1.59 a | 3.59 c |

[a]Mean ± standard deviation. Averages in rows marked with different letters differ significantly ($P < 0.05$). Lack of letters indicates no difference between the means ($P > 0.05$). AI, atherogenic index; TC/HDL-C, TC-HDL-C/HDL-C; Chol/CHC, atherogenic diet with conventional kiwifruit air ripening; Chol/CHE, atherogenic diet with conventional kiwifruit ethylene stimulation; Chol/OHC, atherogenic diet with organic kiwifruit air ripening; Chol/OHE, atherogenic diet with organic kiwifruit ethylene stimulation; C, control; Chol, atherogenic diet (1% cholesterol).

conventional and organic kiwifruits, treated or untreated with ethylene, are presented in Table 1. As can be seen, the contents of polyphenols, flavanols, tannins, and vitamin C in organic kiwifruits were higher than in conventional ones. It can be mentioned that the contents of polyphenols, flavonoids, flavanols, tannins, vitamin C, and dietary fiber and its fractions, IDF and SDF (Table 1), were significantly higher ($P < 0.05$) in both kiwifruits treated with ethylene. The antioxidant capacities of organic kiwifruit were significantly higher ($P < 0.05$) than those of conventional kiwifruit (Table 1). The capacity of the kiwifruits treated with ethylene was significantly higher ($P < 0.05$) than that of fruits untreated with ethylene. As calculated, the highest correlation was obtained between antioxidant capacities of fruits (DPPH assay) and polyphenols (TP) ($R^2 = 0.9638$), lower for flavonoids (TF) ($R^2 = 0.8703$), vitamin C ($R^2 = 0.8999$), and flavanols ($R^2 = 0.7288$).

**In Vivo Studies.** There was noted a significant decrease of feed intake, body weight gain, and increase of feed efficiency ratio (FER) in Chol group versus four groups supplemented with kiwifruits (Table 2). Supplementation of the Chol groups with kiwifruits increased feed intake (12%) and body gain (38%) and decreased FER values (21%).

The lipid profile in the serum of rats is presented in Table 3 and Figure 1. As shown, an increase of TC in the serum of the rats fed cholesterol versus control was 64% ($P < 0.05$). An increase of LDL-C was also registered in the Chol versus the control group by 109%. No significant changes in the level of HDL-C were registered. An increase of TG in the serum of rats in groups fed Chol versus the control group was 108% ($P < 0.05$). As presented, supplementation of the atherogenic diets with 5% kiwifruits prevented the increase of the lipids versus the Chol group by 29% (TC), 36% (LDL-C), and 61% (TG). As shown in Figure 1 and Table 3, no significant changes of TG, TC, and LDL-C between groups of rats fed diets supplemented with conventional and organic kiwifruits treated and nontreated with ethylene were determined. The atherogenic index TC/HDL increased from 2.59 (control) to 4.59 (Chol) ($P < 0.05$). Supplementation of the Chol diet with 5% lyophilized kiwifruits improved the atherogenic indices by about 25% and the atherogenic index (AI) by about 32%.

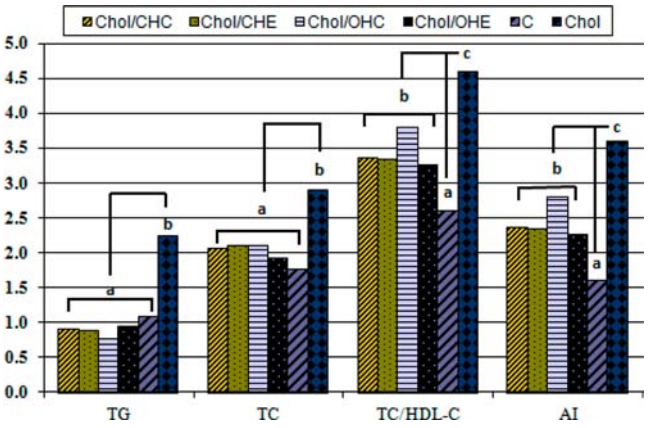


**Figure 1.** Lipid profile in serum (mmol/L) of rats fed atherogenic diets (1% cholesterol) and kiwifruits conventional and organic ethylene treated. Chol/CHC, atherogenic diet with conventional kiwifruit air ripening; Chol/CHE, atherogenic diet with conventional kiwifruit ethylene stimulation; Chol/OHC, atherogenic diet with organic kiwifruit air ripening; Chol/OHE, atherogenic diet with organic kiwifruit ethylene stimulation; C, control; Chol, atherogenic diet (1% cholesterol).

A significant decrease of plasma antioxidant capacity (DPPH, FRAP, and ABTS assays) was registered in the Chol group versus the control group (Table 4; Figure 2; $P < 0.05$). Supplementation of the Chol diet with 5% lyophilized kiwifruits increased the plasma antioxidant capacity evaluated by DPPH assay (18%), FRAP (55%), and ABTS (55%) (Table 4; Figure 2). Differences between conventional and organic fruits were not found. There was a highly negative correlation between the antioxidant capacity in plasma (DPPH test) and LDL-C in the blood serum of rats fed the atherogenic diet for 4 weeks. The resulting coefficient of determination ($R^2$) was high in rats of the Chol group and amounted to 0.9303 (Figure 3). A similar relationship was observed in rats loaded with cholesterol by 26 days,[6] where $R^2 = 0.9388$. AST activity in rat serum from the Chol group was higher than in the control (140.5 vs 102.3 IU/L) ($P < 0.05$). Activities of ALT and ALP did not differ significantly between these groups ($P > 0.05$) (Table 4). Kiwifruits lower the activities of AST and ALT ($P < 0.05$) (without Chol/OHE). Any significant changes were estimated between conventional and organic kiwifruits ethylene and untreated. The decrease of ALP activity was not significant ($P > 0.05$) in all rats fed diets with kiwifruits (Table 4).

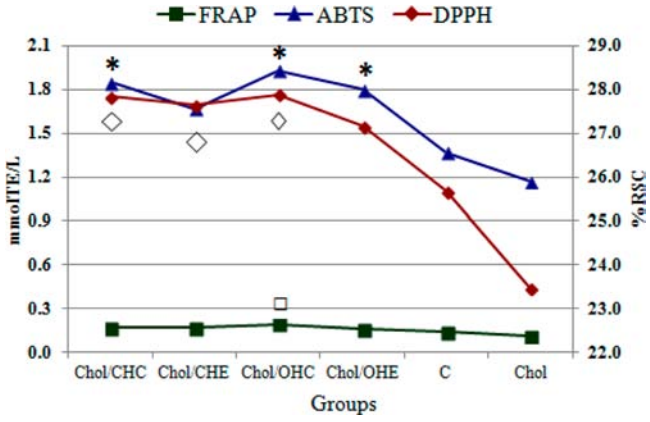


**Figure 2.** Antioxidant capacity in plasma of rats fed atherogenic diets (1% cholesterol) and kiwifruits conventional and ethylene organic treated. Chol/CHC, atherogenic diet with conventional kiwifruit air ripening; Chol/CHE, atherogenic diet with conventional kiwifruit ethylene stimulation; Chol/OHC, atherogenic diet with organic kiwifruit air ripening; Chol/OHE, atherogenic diet with organic kiwifruit ethylene stimulation; C, control; Chol, atherogenic diet (1% cholesterol). ∗, ◇, and □ indicate significant differences between the kiwifruit groups (CHC, CHE, OHC, OHE) and C and Chol groups.

**Table 4. Influence of Kiwifruit (Conventional and Organic) on Total Antioxidant Capacity in Plasma, Liver Enzyme Activity, and Glucose in Serum of Rats[a]**

| index | Chol/CHC | Chol/CHE | Chol/OHC | Chol/OHE | C | Chol |
|---|---|---|---|---|---|---|
| DPPH, %RSC | 27.83 ± 0.81 c | 27.64 ± 0.81 c | 27.89 ± 0.83 c | 27.16 ± 0.87 bc | 25.68 ± 0.85 b | 23.49 ± 1.01 a |
| FRAP, mmolTE/L | 0.17 ± 0.02 bc | 0.17 ± 0.03 bc | 0.19 ± 0.02 c | 0.16 ± 0.02 b | 0.14 ± 0.01 b | 0.11 ± 0.01 a |
| ABTS, mmolTE/L | 1.85 ± 0.31 cd | 1.67 ± 0.20 bc | 1.93 ± 0.30 d | 1.80 ± 0.32 c | 1.37 ± 0.23 b | 1.17 ± 0.20 a |
| AST, UI/L | 91.3 ± 17.1 a | 87.0 ± 7.6 a | 95.3 ± 18.6 a | 87.8 ± 7.6 a | 102.3 ± 12.5 a | 140.5 ± 28.8 b |
| ALT, UI/L | 25.2 ± 3.3 a | 25.8 ± 3.6 ac | 31.0 ± 6.0 bc | 29.2 ± 4.6 ab | 33.0 ± 1.6 b | 32.7 ± 4.5 b |
| ALP, UI/L | 299.8 ± 72 | 271.3 ± 24.9 | 294.5 ± 45.2 | 319.4 ± 48.6 | 319.4 ± 48.6 | 319.4 ± 48.6 |
| glucose, mmol/L | 5.2 ± 0.8 b | 5.3. ± 1.1 b | 4.9 ± 1.2 bc | 5.6 ± 0.8 b | 3.8 ± 0.9 ac | 3.6 ± 0.7 a |

[a]Mean ± standard deviation. Averages in rows marked with different letters differ significantly ($P < 0.05$). Lack of letters indicates no difference between the means ($P > 0.05$). ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; CE, catechin equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl method; FRAP, ferric-reducing/antioxidant power; GAE, gallic acid equivalent; trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, trolox equivalent; RSC, radical scavenging capacity; Chol/CHC, atherogenic diet with conventional kiwifruit air ripening; Chol/CHE, atherogenic diet with conventional kiwifruit ethylene stimulation; Chol/OHC, atherogenic diet with organic kiwifruit air ripening; Chol/OHE, atherogenic diet with organic kiwifruit ethylene stimulation; C, control; Chol, atherogenic diet (1% cholesterol).

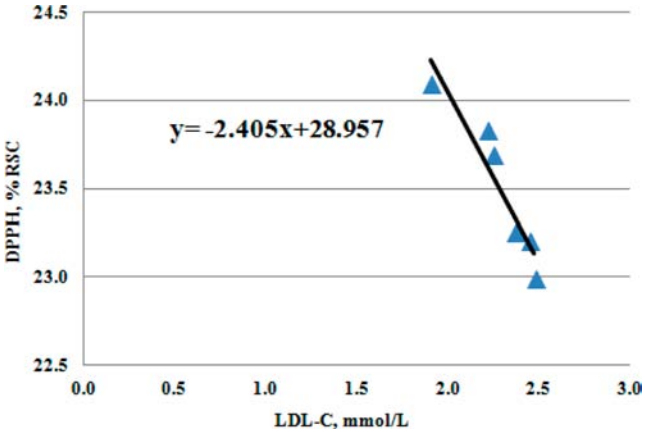


**Figure 3.** Correlation between RSC and LDL-C in rats fed diets with 1% cholesterol for 28 days. DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; RSC, radical scavenging capacity.

Some blood hematologic parameters are presented in Table 5. An increase of red blood cells (RBC) in blood and a decrease of platelets vs Chol group were registered in all rats fed kiwifruits diets ($P < 0.05$). It should be mentioned that there were no differences between the control group and the group with cholesterol for RBC and PLT amounts in blood. The prothrombin index was significantly lower, whereas the prothrombin time estimated was significantly higher in groups with kiwifruits than in the control ($P < 0.05$). All parameters of coagulation (prothrombin index, INR, and prothrombin time) in the groups with the addition of kiwifruits were comparable ($P > 0.05$).

## DISCUSSION

Multifunctional effects of bioactive compounds in fruits on human (and animal) bodies and their influence on disease prevention stimulate development of the study to elucidate the possible mechanisms of actions, especially in the case of new fruits. Fruits (also herbs and vegetables) are important sources of biologically active substances, such as polyphenols, dietary fiber, and vitamins. Burton-Freemann[27] showed that consumption of a phenolic-rich fruits led to an increase of the antioxidant capacity of the blood. According to Rankin et al.[28] a low level of plasma antioxidants can result in high mortality from coronary atherosclerosis. Proper diets play an important role in the prevention of atherosclerosis. As presented in experiments in vivo with rats, exotic fruits (kiwifruit, avocado, mango, persimmon, and others),[17,29] had pro-health properties. Fruits contain significant levels of biologically active substances with physiological and biochemical functions, which benefit human health. In recent years fruits have assumed the status of "functional food". This kind of food must meet nutritional requirements and bring several physiological benefits, such as the prevention of important pathologies, such as cancer,[30] cardiovascular diseases, and connected aging processes.

The content of bioactive compounds in the kiwifruit can be changed during maturation (for example, ethylene stimulation) and depends on the conditions of cultivation. These processes affect the antioxidant capacity of kiwifruit. In this paper, we compared different growth conditions of kiwifruits (conventional and organic) and ripening (ethylene treatment) and their influence on the content of bioactive compounds (polyphenols, flavonoids, flavanols, tannins, dietary fiber), vitamin C, and antioxidant capacity of fruits. The high correlation between vitamin C and antioxidant capacity ($R^2 = 0.8999$) corresponds with the data obtained by Tavarini et al.,[4] suggesting that vitamin C to a greater degree than other antioxidant compounds affects the value of this capacity. It has been shown that the content of vitamin C in the kiwifruit is higher than in oranges, strawberries, lemons, and grapes[4] and 10 times higher compared to apples and peaches.[31] Our results correspond with those obtained by Park et al.[10] Our results show that ethylene treatment significantly increases the contents of vitamin C, polyphenols, flavonoids, flavanols, tannins, and antioxidant capacity relative to kiwifruit without stimulation.[32,33]

In the in vivo study, Wistar rats fed during 28 days with diets containing 1% of cholesterol and kiwifruit from different cultivation conditions, ethylene or air treated after harvest, were chosen. In this paper we evaluated metabolic reactions of rats on the basis of lipid profile, antioxidant capacity, liver enzyme activity, and coagulation parameters in the blood. Phenolic compounds of kiwifruits, which determine their potential antioxidants, affect the antioxidant capacity in plasma of rats. Mahfouz and Kummerow[34] and Gorinstein et al.[2] showed that a diet supplemented with cholesterol leads to a reduction in plasma antioxidant capacity. This decrease was from 8.5% (DPPH assay) to 21.4% (FRAP assay) compared to group C (Table 4; $P < 0.05$). Introduction of kiwifruit in atherogenic diets shows a significant increase of the TAC in plasma of rats from 17.6% (DPPH) to 57.3% (FRAP) (an average for all rats).

**Table 5. Influence of Kiwifruit (Conventional and Organic) Ethylene Treated on Hematological and Coagulation Parameters in Blood of Rats Fed Atherogenic Diets (1% Cholesterol)[a]**

| index | Chol/CHC | Chol/CHE | Chol/OHC | Chol/OHE | C | Chol |
|---|---|---|---|---|---|---|
| RBC, m/$\mu$L | 7.86 ± 0.36 bc | 8.06 ± 0.36 b | 7.87 ± 0.26 bc | 8.07 ± 0.20 b | 7.61 ± 0.25 ac | 7.64 ± 0.28 a |
| HGB, g/dL | 13.40 ± 0.23 bc | 13.83 ± 0.53 b | 13.67 ± 0.64 b | 13.68 ± 0.45 b | 12.78 ± 0.70 ac | 12.90 ± 0.47 ac |
| HCT, % | 43.06 ± 1.26 bc | 44.45 ± 2.35 b | 43.07 ± 1.97 bc | 44.65 ± 1.24 b | 41.86 ± 1.78 ac | 41.37 ± 1.03 ac |
| PLT, K/$\mu$L | 779.5 ± 24.4 a | 793.2 ± 84.5 a | 857.8 ± 110.4 a | 825.3 ± 108.3 a | 1019.3 ± 66.9 b | 1111.8 ± 78.4 b |
| WBC, K/$\mu$L | 4.88 ± 1.62 | 5.53 ± 0.38 | 6.00 ± 1.83 | 4.55 ± 1.03 | 5.69 ± 1.20 | 5.45 ± 1.01 |
| prothrombin index, % | 62.4 ± 5.2 ac | 59.8 ± 5.7 a | 60.3 ± 6.9 a | 58.7 ± 5.5 a | 84.2 ± 4.1 b | 73.7 ± 4.3 bc |
| INR | 1.9 ± 0.2 b | 2.0 ± 0.3 b | 2.0 ± 0.3 b | 2.0 ± 0.3 b | 1.6 ± 0.2 a | 1.8 ± 0.3 ab |
| prothrombin time, s | 24.4 ± 1.9 b | 25.8 ± 2.8 b | 25.6 ± 2.9 b | 26.7 ± 2.5 b | 17.7 ± 1.5 a | 21.1 ± 1.1 ab |

[a]Mean ± standard deviation. Averages in rows marked with different letters differ significantly ($P < 0.05$). Lack of letters indicates no difference between the means ($P > 0.05$). Chol/CHC, atherogenic diet with conventional kiwifruit air ripening; Chol/CHE, atherogenic diet with conventional kiwifruit ethylene stimulation; Chol/OHC, atherogenic diet with organic kiwifruit air ripening; Chol/OHE, atherogenic diet with organic kiwifruit ethylene stimulation; C, control; Chol, atherogenic diet (1% cholesterol); INR, International Normalized Ratio.

An increase of TAC in the plasma of rats was presented also in our previous papers concerning supplementation of a cholesterol diet with 5% persimmon[35] or 5% durian.[36] It can be assumed that the addition of exotic fruits to the diet of 1% cholesterol improves endogenous antioxidant system of the body and increases the body's overall antioxidant capacity. The biological activity of polyphenols depends on their bioavailability. An indirect proof of the absorption of polyphenols in the gut increased plasma antioxidant activity, which was detected after consumption of the product.[37] The rate and extent of absorption in the intestine are determined by the chemical structure of polyphenols, affecting the amount of metabolites in the plasma. Polyphenols that are not absorbed from the intestine are metabolized by bacteria in the colon.[37] It should also be noted that the half-lives of each of the polyphenolic compounds vary; hence, a regular supply of them with foods, containing polyphenols, can increase their levels in the blood stream and tissues.

The high amounts of polyphenols, flavonoids, and vitamin C, as well as a high antioxidant capacity, influenced the values of TAC in the plasma of rats from groups containing conventional (Chol/CHE, Chol/CHC) and organic kiwifruit (Chol/OHC, Chol/OHE). A significant improvement in lipid parameters such as TC, LDL-C, and TG in rats fed diets containing kiwifruit was observed. Singh and Rastogi[38] showed that the increase in consumption of vitamin C was associated with an increase in HDL-C and a decrease in total cholesterol and triglycerides. In our study an increase in HDL-C was not shown in contrast to the results of Oliveira et al.[39] According to Oliveira et al.[39] the presence of cholesterol in the diet affects the growth of all its fractions in the serum, but for HDL-C to a lesser extent that for LDL-C. The contents of HDL-C in all experimental groups were comparable (no significant differences were noted), similar to the study of Valcheva-Kuzmanova et al.[40,41] Supplementation of kiwifruit resulted in a significant decrease (61%) in serum TG content in rats loaded with cholesterol (Table 3; Figure 1). These results are consistent with those of Duttaroy and Jorgensen,[3] who demonstrated a 15% reduction in TG content in humans. The present study found also a significant decrease in the value of the AI and atherogenic index TC/HDL-C in the groups with the addition of kiwifruit, without differences between them.

On the basis of these results, we conclude that a diet rich in kiwifruit has a positive effect on the metabolism of lipids and on plasma TAC and limits the negative impact of exogenous cholesterol.

Proanthocyanidins by the action of protecting and enhancing the function of vitamin C (involved in the transformation of cholesterol to bile acids in the liver) may accelerate the removal of cholesterol from the blood. Most of the bile acids secreted into the intestine are reabsorbed in the ileum and then returned with portal blood to the liver, where these acids are taken up by hepatocytes.[42,43] Bile acids, besides their role in lipid absorption, can also work as signaling molecules in metabolic homeostasis, including lipid metabolism and energy homeostasis.[42]

Improvement in lipid parameters and TAC due to the addition of exotic fruits in the diet is also confirmed by the studies of Haruenkit et al.[6] and Gorinstein et al.[44] As mentioned, 'Hayward' kiwifruit improved the lipid profile and plasma TAC in rats, but there was no significant difference between conventional and organic fruits and ethylene treated fruits after ripening. The protective mechanism of the antioxidant action is shown in removing from the blood already generated ROS and in preventing their formation. Inhibition of lipid peroxidation of cell membranes and the oxidation of LDL stimulates the formation of nitric oxide in the endothelium and inhibits inflammatory reactions and platelet aggregation. This prevents the development of plaques and blood clots. Therefore, regular consumption of plant polyphenols as found in kiwifruit can be recommended.

Important factors in the development of atherosclerosis are disorders of coagulation and fibrinolysis. No information in the literature on the impact of kiwifruit on the content of platelets in rats is shown. It is known that reducing the number of platelet influences blood coagulation. As shown in our study, the amount of platelets in all groups with kiwifruit was significantly lower than in C and Chol groups, without differences between groups with fruits (Table 5). It is believed that vitamin C and flavonoids reduce blood coagulation parameters by inhibiting platelet aggregation and reducing plasma fibrinogen content.[45,46] In our study, the groups fed with the addition of kiwifruit demonstrated a reduction of the prothrombin indices against the Chol group of about 20% (Table 5). INR rose by almost 10%, and the clotting time lengthened by an average of about 4 s. Pignatelli et al.[47] showed that the flavonoids (catechin, quercetin) in addition to inhibition of collagen-induced platelet aggregation also inhibit platelet adhesion to collagen, which is important in the development of atherosclerotic changes in vascular. Duttaroy and Jorgensen,[3] in the experience of volunteers eating two to three kiwifruits during the day, showed a significant decrease in platelet aggregation (test with collagen and ADP) by about 18%. This can be explained by the synergistic effect of vitamin C and flavonoids, which can play an important role in atherogenesis, affecting blood coagulation factors. Higher synergistic activity was shown when combinations of natural antioxidants were used.[48] The bioavailability of ascorbate is superior from some foods, such as kiwifruit.[49] These results have important implications for human nutrition.[48,49] After 2 weeks from the end of the experiment, platelet function returned to baseline (pre-experiment). The amounts of RBC, hemoglobin (HGB), and glucose in kiwifruit groups were significantly higher than in the group with cholesterol (Table 5). Kiwifruit significantly decreased serum AST activity in all rat groups and ALT activity only with kiwifruit of conventional crops. These results add credibility to the health benefits of consuming kiwifruits that have been reported in the scientific literature.[1,3,15,16] Dietary fiber, especially the soluble fraction (SDF), which is present in climacteric fruits, lowers cholesterol and triglycerides in the blood by binding exogenous cholesterol, reducing its absorption, increasing excretion of feces, lowering the reabsorption of fatty acids, and reducing the formation of LDL-C. In kiwifruit the proportion IDF/SDF is very profitable, because the amount of IDF is about twice higher than SDF (Table 1). According to Spiller[50] a proportion of 1−2.3:1 is good for physiological action of both fiber fractions. Dietary fiber anticholesterolemic action (mainly SDF) can be related to the decrease of cholesterol absorption, because its lowers synthesis in the liver, increasing degradation in the tissues and changes in lipoprotein synthesis. The content of cholesterol and a high content of fat in the diet are well-known as risk factors for atherosclerosis. These factors influenced the increase in serum lipids and development of histopathological lesions in the aorta and in the liver.[51,52] In this investigation of kiwifruits (and in the previous studies of Gorinstein et al.,[25] Valcheva-

Kuzmanova et al.,[41] and Leontowicz et al.[53,54]) no changes were seen in the atherosclerosis indices in rats fed cholesterol during 28 days. Longer cholesterol consumption can affect the occurrence of atherogenic changes in the vessels, after 42 days. These changes occurred in the aortic arch (lesions) and in the liver (steatosis), as examined by us in exotic fruits (persimmon, durian), and reduced their range.[35,36]

In conclusion, organic kiwifruits have higher amounts of bioactive compounds and antioxidant capacity than conventionally grown kiwifruits. Ethylene treatment increases the bioactivity of kiwifruit. Kiwifruits in atherogenic diets increase the plasma antioxidant capacity in rats, regardless of their growing and ripening after harvest. Hypolipidemic and hypocholesterolemic actions of kiwifruit are not dependent on their cultivation system and on ethylene treatment after harvest. Kiwifruits have anticoagulation properties, diminish liver enzyme activity, and influence the RBC system in blood. Kiwifruits 'Hayward', regardless of the crop and postharvest ripening, affect pro-health properties and can be recommended particularly in patients with hyperlipidemia and hypercholesterolemia.

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### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

We are thankful to Dr. Elena Katrich (School of Pharmacy, Hebrew University of Jerusalem) for her technical assistance in determination of antioxidant activity.

## DEDICATION

⊗(S.G.) This paper was written in memory of my dear brother Prof. Simon Trakhtenberg, who died in November 2011 and who encouraged me and our scientific group during all his life.

## REFERENCES


(1) Motohashi, N.; Shirataki, Y.; Kawase, M.; Tani, S.; Sakagami, H.; Satoh, K.; Kurihara, T.; Nakashima, H.; Musci, I.; Varga, A.; Molnar, J. Cancer prevention and therapy with kiwifruit in Chinese folklore medicine: study of kiwifruit extracts. *J. Ethnopharmacol.* **2002**, *81*, 357−364.

(2) Gorinstein, S.; Capsi, A.; Libman, I.; Katrich, E.; Lerner, Z. H.; Trakhtenberg, S. Preventive effects of diets supplemented with sweete fruits in hypercholesterolemic patients suffering from coronary artery disease. *Prev. Med.* **2004**, *38*, 841−847.

(3) Duttaroy, A. K.; Jorgensen, A. Effects of kiwifruit consumption on platelet aggregation and plasma lipids in healthy human volunteers. *Platelets* **2004**, *15*, 287−292.

(4) Tavarini, S.; Degl'Innocenti; Remorini, D.; Massai, R.; Guidi, L. Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit. *Food Chem.* **2008**, *107*, 282−288.

(5) Park, Y. S.; Leontowicz, H.; Leontowicz, M.; Namieśnik, J.; Suhaj, M.; Cvikrova, M.; Martincova, O.; Weisz, M.; Gorinstein, S. Comparison of the contents of bioactive compounds and the level of antioxidant activity in different kiwifruit cultivars. *J. Food Compos. Anal.* **2011**, *24*, 963−970.

(6) Haruenkit, R.; Poovarodom, S.; Leontowicz, H.; Leontowicz, M.; Sajewicz, M.; Kowalska, T.; Delgado-Licon, E.; Rocha-Guzman, N. E.; Gallegos-Infante, J.-A.; Trakhtenberg, S.; Gorinstein, S. Health properties and nutritional value of durian (*Durio zibethinus* cv Mon Thong): experiments in vitro and in vivo. *J. Agric. Food Chem.* **2007**, *55*, 5842−5849.

(7) Park, Y. S.; Jung, S. T.; Kang, S. G.; Drzewiecki, J.; Namieśnik, J.; Haruenkit, R.; Barasch, D.; Trakhtenberg, S.; Gorinstein, S. In vitro studies of polyphenols, antioxidants and other dietary indices in kiwifruit (*Actinidia deliciosa*). *Int. J. Food Sci. Nutr.* **2006**, *57*, 107−122.

(8) Park, Y. S.; Jung, S. T.; Kang, S. G.; Delgado-Licon, E.; Katrich, E.; Tashma, Z.; Trakhtenberg, S.; Gorinstein, S. Effect of ethylene treatment on kiwifruit bioactivity. *Plant Foods Hum. Nutr.* **2006**, *61*, 151−156.

(9) Park, Y. S. Storability of fresh-cut kiwifruit slices influenced by storage temperature. *J. Korean Soc. Hortic. Sci.* **2002**, *43*, 728−732.

(10) Park, Y. S.; Jung, S. T.; Kang, S. G.; Heo, B. G.; Arancibia-Avila, P.; Toledo, F.; Drzewiecki, J.; Namieśnik, J.; Gorinstein, S. Antioxidants and proteins in ethylene-treated kiwifruits. *Food Chem.* **2008**, *107*, 640−648.

(11) Park, Y. S.; Jung, S. T.; Kang, S. G.; Heo, B. G.; Lee, S. H.; Toledo, F.; Arancibia-Avila, P.; Drzewiecki, J.; Gorinstein, S. Radical scavenging capacity of ethylene treated kiwifruit. *J. Food Biochem.* **2009**, *33*, 674−692.

(12) Hassey, J. K.; Johnson, R. S.; Meyer, R. D.; Klonsky, K. An organic versus a conventional farming system in kiwifruit. *Acta Hortic.* **1997**, *444*, 223−228.

(13) Benge, J. R.; Silva, H. N.; Banks, N. H.; Jeffery, P. B. Empirical modelling of postharvest changes in the firmness of kiwifruit. *Postharvest Biol. Technol.* **2000**, *19*, 211−220.

(14) Jayaprakasam, B.; Vareed, S. K.; Olson, L. K.; Nair, M. G. Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits. *J. Agric. Food Chem.* **2005**, *53*, 28−31.

(15) Jung, K. A.; Song, T. C.; Han, D. S.; Kim, I. H.; Kim, Y. E.; Lee, Y. E. Cardiovascular protective properties of kiwifruit extracts *in vitro*. *Biol. Pharm. Bull.* **2005**, *28*, 1782−1785.

(16) Rush, E.; Ferguson, L. R.; Cumin, M.; Thakur, V.; Karunasinghe, N.; Plank, L. Kiwifruit consumption reduces DNA fragility: a randomized controlled pilot study in volunteers. *Nutr. Res. (N.Y.)* **2006**, *26*, 197−201.

(17) Dembitsky, V. M.; Poovarodom, S.; Leontowicz, H.; Leontowicz, M.; Vearasilp, S.; Trakhtenberg, S.; Gorinstein, S. The multiple nutrition properties of some exotic fruits: biological activity and active metabolites. A review. *Food Res. Int.* **2011**, *44*, 1671−1701.

(18) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152−178.

(19) Arnous, A.; Makris, D. P.; Kefalas, P. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red vines. *J. Agric. Food Chem.* **2001**, *49*, 5736−5742.

(20) Ozyurek, M.; Guclu, K.; Bektasoglu, B.; Apak, R. Spectrophotometric determination of ascorbic acid by the modified CUPRAC method with extractive separation of flavonoids−La (III) complexes. *Anal. Chim. Acta* **2007**, *588*, 88−95.

(21) Prosky, L.; Asp, N. G.; Schweizer, T.; De Vries, J. W.; Furda, I. Determination of insoluble and soluble dietary fiber in food and food products: collaborative study. *J. Assoc. Anal. Chem.* **1992**, *75*, 360−367.

(22) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical. Biol. Med.* **1999**, *26*, 1231−1237.

(23) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(24) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Food Sci. Technol. (London)* **1995**, *28*, 25−30.
(25) Gorinstein, S.; Leontowicz, H.; Leontowicz, M.; Jastrzębski, Z.; Najman, K.; Tashma, Z.; Katrich, E.; Heo, B. G.; Cho, J. Y.; Park, Y. J.; Trakhtenberg, S. The influence of raw and processed garlic and onions on plasma classical and non-classical atherosclerosis indices: Investigations in vitro and in vivo. *Phytother. Res.* **2010**, *24*, 706−714.
(26) Tietz, N. W. *Clinical Guide to Laboratory Tests*, 3rd ed.; W. B. Saunders: Philadelphia, PA, 1995.
(27) Burton-Frieman, B. Postprandial metabolic events and fruit-derived phenolics: a review of the science. *Br. J. Nutr.* **2010**, *104* (Suppl. 3), SI−S14.
(28) Rankin, S. M.; Whalley, C. V.; Hoult, J. R.; Wilkins, G.; Collard, J.; Leake, D. The modification of LDL by the flavonoids myricetin and glossypatin. *J. Biochem. Pharmacol.* **1993**, *45*, 67−75.
(29) Gorinstein, S.; Poovarodom, S.; Leontowicz, H.; Leontowicz, M.; Namieśnik, J.; Vearasilp, S.; Haruenkit, R.; Ruamsuke, P.; Katrich, E.; Tashma, Z. Antioxidant properties and bioactive constituents of some rare exotic Thai fruits and comparison with conventional fruits. *In vitro* and *in vivo* studies. *Food Res. Int.* **2011**, *44*, 2222−2232.
(30) Gescher, A.; Pastorino, U.; Plummer, S. M.; Manson, M. M. Suppression of tumor development by substances derived from the diet − mechanism and clinical implications. *Br. J. Clin. Pharmacol.* **1998**, *45*, 1−12.
(31) Beever, D. J.; Hopkirk, G. Fruit development and fruit physiology in kiwifruit. In *Kiwifruits: Science and Management*; Warrington, I. J., Weston, G. C., Eds.; New Zealand Society of Horticultural Science, Ray Richards Publisher: Auckland, New Zealand, 1990; Vol. *109*, pp 115−121.
(32) Leong, L. P.; Shui, G. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chem.* **2002**, *76*, 69−75.
(33) Goo, C. J.; Yang, J. J.; Wei, J. Y.; Li, Y. F.; CSU, J.; Jiang, Y. G. Antioxidant activity of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutr. Res. (N.Y.)* **2003**, *2*, 1719−1726.
(34) Mahfouz, M.; Kummerow, F. Cholesterol-rich diets have different effects on lipid peroxidation, cholesterol oxides, and antioxidant enzymes in rats and rabbits. *J. Nutr. Biochem.* **2000**, *11*, 293−302.
(35) Gorinstein, S.; Leontowicz, H.; Leontowicz, M.; Jesion, I.; Namieśnik, J.; Drzewiecki, J.; Park, Y. S.; Ham, K. S.; Giordani, E.; Trakhtenberg, S. Influence of two cultivars of persimmon on atherosclerosis indices in rats fed cholesterol-containing diets: investigation *in vitro* and *in vivo*. *Nutrition* **2011**, *27*, 838−846.
(36) Leontowicz, H.; Leontowicz, M.; Jesion, I.; Bielecki, W.; Poovarodom, S.; Vearasilp, S.; Gonzalez-Aguilar, G.; Roblem-Sanchez, M.; Trakhtenberg, S.; Gorinstein, S. Positive effects of durian fruit at different stages ripening on the hearts and livers of rats fed diet high in cholesterol. *Eur. J. Integr. Med.* **2011**, *3*, e169−e181.
(37) Scalbert, A.; Williamson, G. Dietary intake and bioavailablity of polyphenols. Chocolate: Modern Science Investigates an Ancient Medicine, Annual Meeting and Science Innovation Exposition of the American Association for the Advancement of Science in Washington. *J. Nutr.* **2000**, *130*, 2073S−2085S.
(38) Singh, R. B.; Rastogi, S. S. Effects of plasma ascorbic acid and coronary risk factors of adding guava fruit to the usual diet in hypertensive with mild to moderate hypercholesterolemia. *J. Nutr. Environ. Med.* **1997**, *7*, 5−10.
(39) Oliveira, T. V.; Maniero, F.; Santos, M. H. H.; Bydlowski, S. P.; Maranhao, R. C. Impact of high cholesterol intake on tissue cholesterol content and lipid transfers to high-density lipoprotein. *Nutrition* **2011**, *27*, 713−718.
(40) Valcheva-Kuzmanova, S.; Kuzmanow, K.; Mihova, V.; Krasnaliev, I.; Borisova, P.; Belcheva, A. Anthyperlipidemic effect of *Aronia melanocarpa* fruit juice in rats fed a high-cholesterol diet. *Plant Foods Hum. Nutr.* **2007**, *62*, 19−24.
(41) Valcheva-Kuzmanova, S.; Kuzmanov, K.; Tsanova-Savova, S.; Mihova, V.; Krasnaliev, I.; Borisova, P.; Belcheva, A. Lipid-lowering effects of *Aronia melanocarpa* fruit juice in rats fed cholesterol-containing diets. *J. Food Biochem.* **2007**, *31*, 589−602.
(42) Houten, S. M.; Watanebe, M.; Auwerx, J. Endocrine function of bile acids. *EMBO J.* **2006**, *25*, 1419−1425.
(43) Kramer, W.; Glombik, H. Bile acid reabsorption (BARI): novel hypolipidemic drugs. *Curr. Med. Chem.* **2006**, *13*, 997−1016.
(44) Gorinstein, S.; Bartnikowska, E.; Kulasek, G.; Zemser, M.; Trakhtenberg, S. Dietary persimmon improves lipid metabolism in rats fed diet containing cholesterol. *J. Nutr.* **1998**, *128*, 2023−2027.
(45) Furman, B.; Aviram, M. Flavonoids protect LDL from oxidation and attenuate atherosclerosis. *Curr. Opin. Lipidol.* **2001**, *12*, 41−48.
(46) Valenzula, A.; Sanhueza, J.; Nieto, S. Cholesterol oxidation: health hazard and role of antioxidants in prevention. *Biol. Res.* **2003**, *36*, 291−302.
(47) Pignatelli, P.; Pulcinelli, F. M.; Celestini, A.; Lenti, L.; Ghiselli, A.; Gazzaniga, P. P.; Violi, F. The flavonoids quercetin and catechin synergistically inhibit platelet function by antagonizing the intercellular production of hydrogen peroxide. *Am. J. Clin. Nutr.* **2000**, *72*, 1150−1155.
(48) Hait-Darshan, R.; Grossman, S.; Bergman, M.; Deutsch, M.; Zurgil, N. Synergistic activity between a spinach-derived natural antioxidant (NAO) and commercial antioxidants in a variety of oxidation systems. *Food Res. Int.* **2009**, *42*, 246−253.
(49) Vissers, M. C. M.; Bozonet, S. M.; Pearson, J. F.; Braithwaite, L. J. Dietary ascorbate intake affects steady state tissue concentrations in vitamin C-deficient mice: tissue deficiency after suboptimal intake and superior bioavailability from a food source (kiwifruit). *Am. J. Clin. Nutr.* **2011**, *93*, 292−301.
(50) Spiller, G. A. Suggestions of a basis on which to determine a desirable intake of dietary fiber. In *Handbook of Dietary Fibre of Human Nutrition*; Spiller, G. A., Ed.; CRC Press: Boca Raton, FL, 1986; pp 281−283.
(51) Balkan, J.; Kanbagh, O.; Hatipoglu, A.; Kucuk, M.; Cevikbas, U.; Aykac-Toker, G.; Uysal, M. Improving effect of dietary taurine supplementation on the oxidative stress and lipid levels in the plasma, liver and aorta of rabbits fed on a high-cholesterol diet. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 1755−1758.
(52) Balkan, J.; Dogru-Abbasoglu, S.; Aykac-Toker, G.; Uysal, M. The effects of high cholesterol diets on lipids and oxidative stress in plasma, liver, aorta of rabbits and rats. *Nutr. Res. (N.Y.)* **2004**, *24*, 229−234.
(53) Leontowicz, M.; Leontowicz, H.; Jastrzębski, Z.; Jesion, I.; Haruenkit, R.; Poovarodom, S.; Katrich, E.; Tashma, Z.; Drzewiecki, J.; Trakhtenberg, S.; Gorinstein, S. The nutritional and metabolic indices in rats fed cholesterol-containing diets supplemented with durian at different stages of ripening. *BioFactors* **2007**, *29*, 123−136.
(54) Leontowicz, H.; Leontowicz, M.; Haruenkit, R.; Poovarodom, S.; Jastrzębski, Z.; Drzewiecki, J.; Atala, A.; Jesion, I.; Trakhtenberg, S.; Gorinstein, S. Durian (*Durio zibethinus* Murr.) cultivars as nutritional supplementation to rats' diets. *Food Chem. Toxicol.* **2008**, *46*, 581−589.