

# Characteristics of Blond and Red Star Ruby Jaffa Grapefruits (*Citrus paradisi*): Results of the Studies In Vitro, In Vivo and on Patients Suffering from Atherosclerosis

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## Abstract

The purpose of this study was to investigate blond and red Star Ruby Jaffa grapefruits (in vitro and in vivo) as a supplement to atherosclerosis preventing diets. It was found that the contents of the bioactive compounds, especially phenolics and the antioxidant capacity of the red grapefruits, are higher than in blond cultivar. Diets supplemented with red grapefruits significantly hindered the rise in the plasma lipid levels in cholesterol fed rats. Diets supplemented with red grapefruits significantly decreased the plasma lipid levels, especially triglycerides in patients suffering from coronary atherosclerosis and related hyperlipidemia: a) total cholesterol (TC) by 15.5% and 7.6% b) low density lipid cholesterol (LDL-C): by 20.3% and 10.7% c) total triglycerides (TG) by 27.2%, and 5.6%, for experimental group (EG1, red grapefruit) and EG2, blond grapefruit, respectively. Only the EG1 patients registered a significant decrease in hypertriglyceridemia. In conclusion, the combined results of the in vitro, in vivo and on humans investigations are a solid basis for recommendation to include red grapefruit in atherosclerosis preventive diet.

## INTRODUCTION

The health protective effect of natural products, such as fruits and vegetables, is mostly related to their antioxidants: phenolic compounds and to a lesser extend – to dietary fiber (Chun et al., 2005; Lairon et al., 2005). As a consequence, the consumption of fruits and vegetables is inversely related to coronary atherosclerosis (Dauchet et al., 2006). The interest in these natural products is growing. However, the differences between cultivars of the same fruits were investigated less.

It was shown that assessments of the bioactivity of individual compounds do not reflect the true antioxidant value of fruits (Lotito and Frei, 2004). Therefore, in addition to the investigation of individual bioactive compounds of both qualities of Jaffa grapefruits, their total antioxidant capacity was also determined. Each of the antioxidant methods has its limitations and some of them give different results, therefore, two other each

complemented assays for determination of the antioxidant capacity of the studied fruits were applied. The purpose of this study was to compare the main essential bioactive compounds and the antioxidant capacity of the popularity in US and Europe of blond and Star Ruby (red) cultivars of Jaffa grapefruits as a supplement to atherosclerosis preventing diets.

## **MATERIALS AND METHODS**

**Chemicals.** Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid),  $\beta$ -carotene, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co (St. Louis, MO, USA) and 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was from Fluka Chemie (Buchs, Switzerland). All reagents used were of analytical grade and obtained from Sigma Chemical Co (St. Louis, MO, USA).

Samples of Israeli blond and Star Ruby (red) Jaffa grapefruits *Citrus paradisi* (12 of each grapefruits) were obtained from 24 randomly selected fruits for the determination of all studied variables. The fruits were cleaned with tap water and peels separated manually. The juice was obtained by a fruit juice-maker from peeled fruits. Two antioxidant assays (radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and antioxidant activity (AA) using the  $\beta$ -carotene linoleate model system) for determination of total antioxidant capacity were used and compared with the Folin-Ciocalteu assay. As a complementation, all bioactive compounds (dietary fiber, minerals, trace elements, naringin, anthocyanins, flavonoids, phenolic and ascorbic acids) were determined to complete characterization of the studied citrus fruits as previously described (Gorinstein et al., 2005, 2006a).

## **In Vivo**

The Animal Care Committee of the University had approved this study. The mean weight of the Wistar rats (n = 60) at the beginning of the experiment was 100 g. They were divided into 10 groups of 6. These groups were named Control, RedGFpeel, RedGFpeeled, BlondGFpeel, BlondGFpeeled, Chol, Chol/RedGFpeel, Chol/RedGFpeeled, Chol/BlondGFpeel and Chol/BlondGFpeeled. During the experiment rats of all 10 groups were fed a basal diet (BD), which included wheat starch, casein, soybean oil, vitamin and mineral mixtures. The rats of the Control group were fed only the BD. The BD of the other nine groups was supplemented with 100 g/kg of red grapefruit peel (RedGFpeel), 100 g/kg of red peeled grapefruit (RedGFpeeled), 100 g/kg of blond grapefruit peel (BlondGFpeel), 100 g/kg of blond peeled grapefruit (BlondGFpeeled), 10 g/kg of nonoxidized cholesterol (NOC) of analytical grade (Chol group), 10 g/kg of NOC and 100 g/kg of red grapefruit peel (Chol/RedGFpeel), 10 g/kg of NOC and red peeled grapefruit (Chol/RedGFpeeled), 10 g/kg of NOC and 100 g/kg of blond grapefruit peel (Chol/BlondGFpeel), 10 g/kg of NOC and blond peeled grapefruit (Chol/BlondGFpeeled). The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. Before and after the experiment the blood samples were taken and plasma was prepared for determination of total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and triglycerides (TG).

## **Clinical Investigation**

The study population was recruited from patients-volunteers, who have previously undergone coronary bypass surgery due to coronary artery disease (CAD) at the Institute of Cardiology of the University Medical Center, Rehovot, Israel.

The subjects gave written, informed consent to a protocol approved by the responsible Institutional Committee on human experimentations, based on the Helsinki Declaration of 1975 as revised in 1983. Fifty-seven male patients between the ages of 47 and 68 years old were examined. No lipid-lowering medicine was used during the 30 days of the investigation.

These 57 hyperlipidemic patients were randomly divided into three groups: two experimental (EG1red and EG2blond) and one control (CG), each containing 19. All patients consumed the usual Israeli diet recommended for patients. For 30 consecutive days the diets of the EG1 and EG2 groups were supplemented once a day by one equal in weight with fresh red or blond grapefruits, respectively. The patients of the CG consumed only the usual diet. Before and after completion of the study, every patient was examined. Systolic and diastolic blood pressure, heart rate and weight were registered. A wide range of laboratory tests was performed. During the trial period, there were no treatment complications. Blood samples a day before and a day after investigation were collected after an overnight fast. Plasma total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG) were determined. Trolox equivalent antioxidant capacity test (TEAC) was performed to determine the plasma antioxidant activity (Gorinstein et al., 2006b).

### Statistical Analyses

The results of this investigation in vitro are means  $\pm$  SD of five measurements. Differences between groups were tested by two-way ANOVA using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA) followed by Duncan's new multiple-range test to assess differences between the groups means. In the assessment of the antioxidant potential, Spearman correlation coefficient (R) was used. The  $P < 0.05$  was considered significant.

## RESULTS

### In Vitro

The amount of total, soluble and insoluble dietary fibers in peeled blond and red grapefruits and their peels is presented on Figure 1. Major minerals (mg/100 g FW, fresh weight) were the following: Na for red (R) and blond (B) peeled grapefruits (G) as  $2.5 \pm 0.1$  and  $2.4 \pm 0.2$  and for peels of RG and BG  $-5.6 \pm 0.4$  and  $5.5 \pm 0.5$ ; K for red (R) and blond (B) peeled grapefruits (G) as  $90.5 \pm 7.5$  and  $88.9 \pm 7.3$  and for peels of RG and BG  $-150.8 \pm 10.9$  and  $149.9 \pm 11.2$ ; Mg for red (R) and blond (B) peeled grapefruits (G) as  $4.4 \pm 0.3$  and  $4.3 \pm 0.3$  and for peels of RG and BG  $-8.3 \pm 0.5$  and  $8.1 \pm 0.5$ ; Ca for red (R) and blond (B) peeled grapefruits (G) as  $3.4 \pm 0.2$  and  $3.2 \pm 0.3$  and for peels of RG and BG  $-7.9 \pm 0.5$  and  $7.9 \pm 0.5$ . The contents of minor minerals ( $\mu\text{g}/100\text{g}$  FW) were the following: Fe for red (R) and blond (B) peeled grapefruits (G) as  $112 \pm 11.4$  and  $109 \pm 10.2$  and for peels of RG and BG  $-153 \pm 12.9$  and  $149 \pm 11.7$ ; Mn for red (R) and blond (B) peeled grapefruits (G) as  $13.1 \pm 1.3$  and  $12.1 \pm 1.1$  and for peels of RG and BG  $-19.9 \pm 2.1$  and  $18.8 \pm 1.7$ ; Zn for red (R) and blond (B) peeled grapefruits (G) as  $50.3 \pm 4.4$  and  $48.8 \pm 4.1$  and for peels of RG and BG  $-79.5 \pm 5.9$  and  $77.1 \pm 5.8$ ; Cu for red (R) and blond (B) peeled grapefruits (G) as  $40.7 \pm 3.4$  and  $38.8 \pm 7$  and for peels of RG and BG  $-64.4 \pm 6.3$  and  $62.3 \pm 6.3$ . The results of fibers, major and minor minerals in peeled blond and red grapefruits and their peels, corresponded to our previous results (Gorinstein et al., 2005, 2006a) and did not show significant differences ( $P > 0.05$ ).

Total polyphenols were  $149.1 \pm 6.3$  and  $158.3 \pm 7.1$  and  $168.2 \pm 7.0$  and  $185.1 \pm 7.3$  mg GAE/100 g fresh weight (FW) in peeled blond and red grapefruit cultivars and their peels, respectively, and were higher in red cultivar. Ascorbic acid (mg/100g FW) for red and blond peeled grapefruits was  $62.2 \pm 6.8$  and  $61.6 \pm 6.1$ , respectively. The contents of all studied phenolic and ascorbic acids in red and blond grapefruits were comparable ( $P > 0.05$ ): with the highest concentration of ferulic and the lowest - of caffeic acid.

Anthocyanins in fresh peeled red and blond grapefruits were  $51.5 \pm 4.6$  and  $49.3 \pm 4.5$   $\mu\text{g}/100$  g, respectively, and in peels of these fruits showed  $87.6 \pm 7.3$  and  $85.3 \pm 7.2$   $\mu\text{g}/100\text{g}$ , respectively. Flavonoids (mg/100 g) in fresh peeled red and blond grapefruits and their peels were  $21.61 \pm 1.3$  and  $19.53 \pm 1.2$ ; and  $71.61 \pm 6.3$  and  $69.53 \pm 6.2$ , respectively. The contents of all studied bioactive compounds in peels of grapefruits of both cultivars were significantly higher than in peeled grapefruits ( $P < 0.05$ ). The

antioxidant capacity of red grapefruit was significantly higher than of the blond one as well as the naringin content ( $P < 0.05$ , Fig. 2).

### **In Vivo**

The red and blond grapefruits supplemented diets for the Chol/RedGFpeel, Chol/RedGFpeeled, Chol/BlondGFpeel and Chol/BlondGFpeeled diet groups significantly hindered the rise of plasma lipids vs. Chol diet group:

- a) TC - 26.8%, 22.2%, 25.1% and 20.1%
- b) LDL-C - 54.2%, 43.3%, 47.4% and 39.3%
- c) TG - 17.3%, 11.3%, 14.2% and 8.6%, respectively (Table 1).

### **Clinical Data**

The heart rate, the systolic and diastolic blood pressure and the weight of the patients after completion of the investigation were without significant changes (data not shown). It was found that plasma lipid levels in the EG1 (red grapefruit) and EG2 (blond grapefruit) vs. CG after treatment were decreased:

- a) TC: - 6.69 vs. 7.92, 15.5% and 7.32 vs. 7.92 mmol/L, 7.6% for EG1 and EG2, respectively
- b) LDL-C: 5.01 vs. 6.29, 20.3% and 5.62 vs. 6.29 mmol/L, 10.7.% for EG1 and EG2, respectively
- c) TG: 1.69 vs. 2.32, 27.2%, and 2.19 vs. 2.32 mmol/L, 5.6 %, for EG1 and EG2, respectively. Only in patients of the EG1 registered a significant decrease in hypertriglyceridemia.

After completion of the investigation, the plasma antioxidant activity was increased in patients of both EG1 and EG2 vs. CG: 1.91 vs. 1.40, + 36.4% and 1.65 vs. 1.40 mmol/L, + 17.8%), respectively. No significant changes in the TEAC values in patients of the CG group were registered. Similar results were reported by others (by us previously) (Gorinstein et al., 2006b). The calculated correlations, between the decrease of triglycerides and increase of antioxidant capacity of serum and citrus diet contribution to the antioxidant potential of both experimental groups (Fig. 3), was decisive for red grapefruit ( $R^2$  is about 0.98) and slightly lower for blond ( $R^2$  is 0.96). The obtained results in the present study were similar to the reported by Gorinstein et al., 2006b. The data in Figure 3 showed that the decrease of triglycerides in serum, which were obtained after the trial (27.2% for red grapefruits) and the polyphenols in fruits, were correlated.

### **DISCUSSION**

Coronary atherosclerosis is still one of the most common diseases in humans – the principal cause of death in the Western civilization. Some authors have shown that diets rich in fruits and vegetables are effective in prevention of this disease (Dauchet et al., 2006). Therefore, many authors insist on the inclusion in preventive diets these natural products and, among them, citrus fruits, which are especially important because of the high content of bioactive compounds (Chun et al., 2005; Gorinstein et al., 2005). In this investigation, two cultivars of Jaffa grapefruits, red and blond, were compared. It was found that the contents of dietary fibers, minerals and trace elements, phenolic and ascorbic acids in both grapefruit's qualities are high and comparable. These results correspond with the others' findings and our previous data (Peleg et al., 1991; Gorinstein et al., 2005, 2006a,b). Also, others have demonstrated that the hypolipidemic effect of fruits and vegetables is evident when they are added to the diets of rats fed cholesterol (Mahfouz and Kummerow, 2000).

The result of the investigation of patients suffering from atherosclerosis and related hyperlipidemia was a significant decrease in the level of triglycerides. Such results were registered only in patients whose diets were supplemented with red grapefruit. We did not find such significant decrease in the level of triglycerides in our previous investigations with other citrus fruits. It must be indicated that the use of medication (statins) in these patients was not effective.

## CONCLUSIONS

Both cultivars of Jaffa grapefruits contain high comparable quantities of bioactive compounds but the antioxidant capacity of red grapefruits is significantly higher. Diets supplemented with both cultivars of Jaffa grapefruits improve the plasma lipid levels in rats that are fed with added cholesterol. Only diets supplemented with red grapefruits significantly decreased the plasma lipid levels, especially triglycerides in patients suffering from coronary atherosclerosis and related hyperlipidemia. It is suggested that the Jaffa grapefruit red cultivar could be a valuable supplement for atherosclerosis preventive diet.

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## Tables

Table 1. Plasma lipid concentration (mmol/L) in rats fed cholesterol-containing and cholesterol-free diets supplemented with different grapefruit cultivars.

	TC	LDL-C	HDL-C	TG
Control	2.90 ± 0.17 <sup>a</sup>	1.24 ± 0.07 <sup>a</sup>	1.66 ± 0.07 <sup>a</sup>	0.71 ± 0.05 <sup>a</sup>
RGpeel	2.83 ± 0.16 <sup>a</sup>	1.18 ± 0.06 <sup>a</sup>	1.65 ± 0.07 <sup>a</sup>	0.70 ± 0.05 <sup>a</sup>
RGpeeled	2.85 ± 0.16 <sup>a</sup>	1.20 ± 0.06 <sup>a</sup>	1.65 ± 0.07 <sup>a</sup>	0.70 ± 0.05 <sup>a</sup>
WGpeel	2.86 ± 0.16 <sup>a</sup>	1.20 ± 0.07 <sup>a</sup>	1.66 ± 0.07 <sup>a</sup>	0.72 ± 0.05 <sup>a</sup>
WGpeeled	2.88 ± 0.16 <sup>a</sup>	1.22 ± 0.07 <sup>a</sup>	1.66 ± 0.07 <sup>a</sup>	0.72 ± 0.05 <sup>a</sup>
Chol	3.73 ± 0.19 <sup>c</sup>	2.06 ± 0.09 <sup>c</sup>	1.67 ± 0.09 <sup>a</sup>	0.90 ± 0.06 <sup>b</sup>
Chol/RGpeel	3.05 ± 0.19 <sup>b</sup>	1.41 ± 0.08 <sup>b</sup>	1.64 ± 0.07 <sup>a</sup>	0.77 ± 0.05 <sup>a</sup>
Chol/RGpeeled	3.15 ± 0.19 <sup>b</sup>	1.48 ± 0.08 <sup>b</sup>	1.67 ± 0.07 <sup>a</sup>	0.77 ± 0.05 <sup>a</sup>
Chol/WGpeel	3.07 ± 0.19 <sup>b</sup>	1.44 ± 0.19 <sup>b</sup>	1.63 ± 0.07 <sup>a</sup>	0.75 ± 0.05 <sup>a</sup>
Chol/WGpeeled	3.10 ± 0.19 <sup>b</sup>	1.47 ± 0.19 <sup>b</sup>	1.63 ± 0.07 <sup>a</sup>	0.75 ± 0.05 <sup>a</sup>

Values are means ± SD (n=8)

Abbreviations: HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; RG, red grapefruit; TC, total cholesterol, WG, white grapefruit; TG, triglycerides.

## Figures

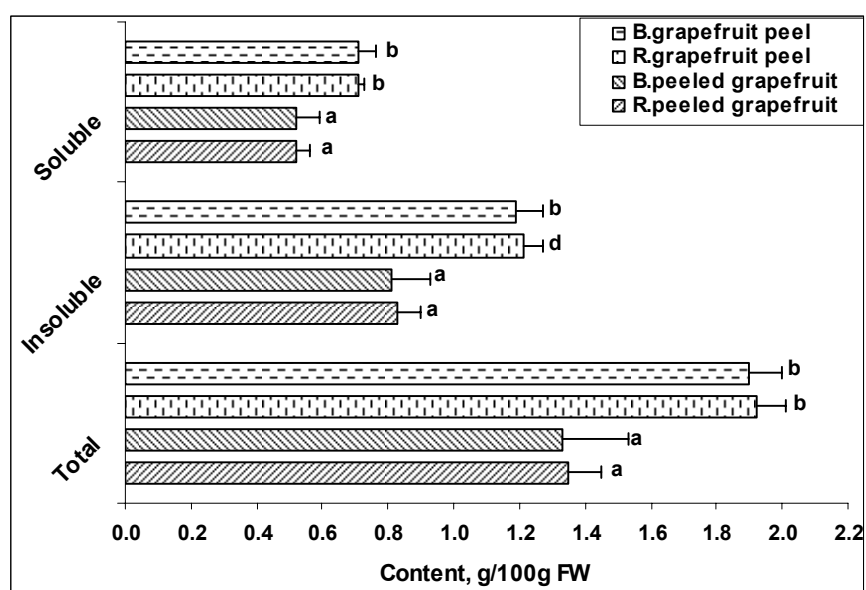


Fig. 1. Total, insoluble and soluble fibers in red (R) and blond (B) peels and peeled grapefruits. Mean ± standard deviation (vertical lines). Means with different letters are significantly different.

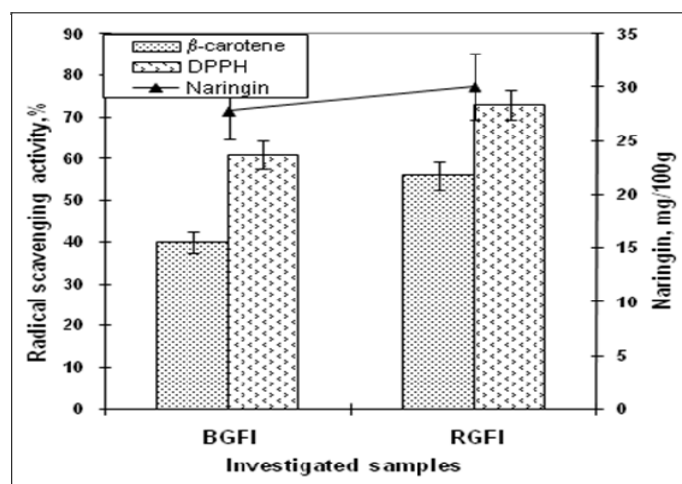


Fig. 2. Radical scavenging activity (%) by  $\beta$ -carotene and DPPH assays and naringin content of blond (BGFI) and red (RGFI) grapefruits.

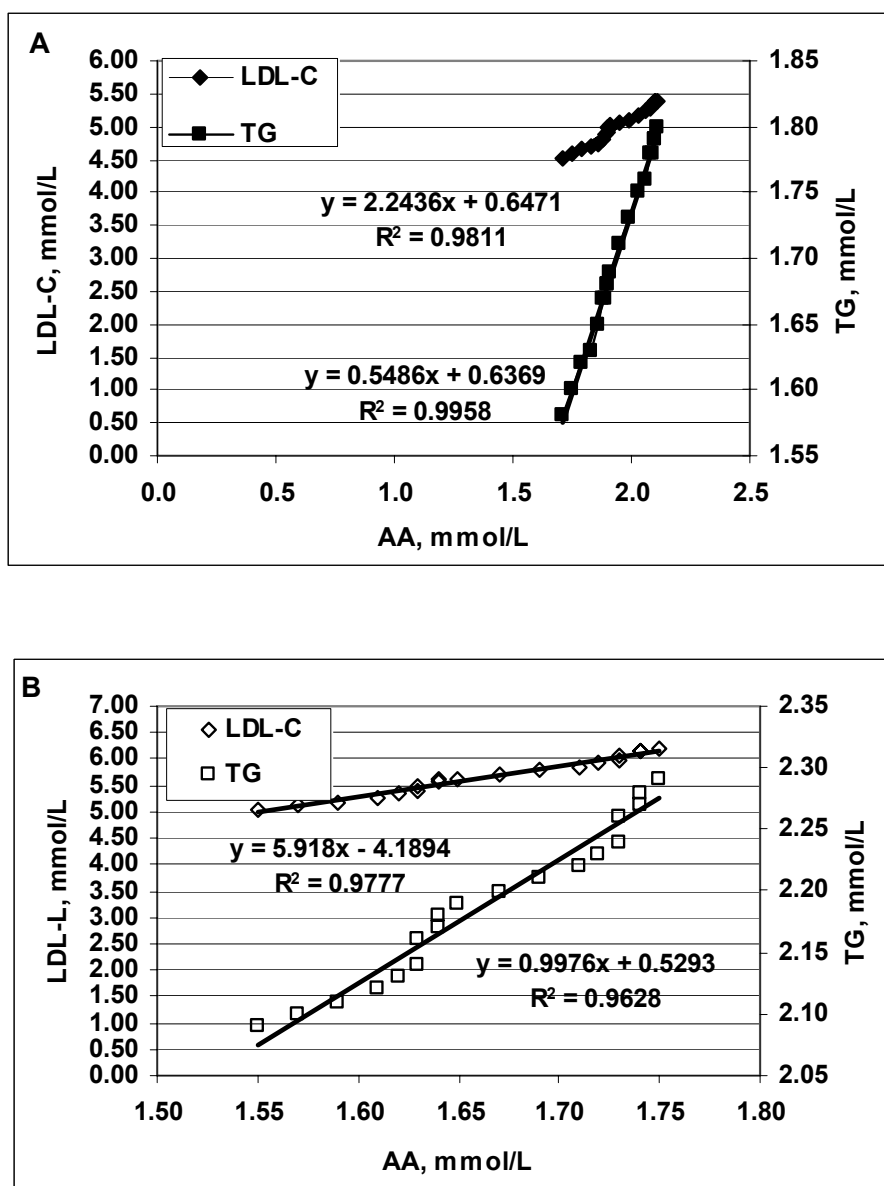


Fig. 3. Relationship, calculated by linear regression analysis, for Red (A) and Blond (B) grapefruits supplemented diets between: **A**, ◆ AA by ABTS scavenging radical (mmol/L, X) to reduction of cholesterol (mmol/L, Y<sub>1</sub>) and ■ Antioxidant activity by ABTS scavenging radical (mmol/L, X) to reduction of triglycerides (mmol/L, Y<sub>2</sub>). **B**, ◇ ABTS (mmol/L, X) to reduction of cholesterol (mmol/L, Y<sub>1</sub>) and □ ABTS (mmol/L, X) to reduction of triglycerides (mmol/L, Y<sub>2</sub>). Abbreviations: AA, Antioxidant activity; ABTS, 2,2'-azino-bis (3-ethyl-benzothiazoline- 6-sulfonic acid) diamonium salt.