



## Red Star Ruby (Sunrise) and blond qualities of Jaffa grapefruits and their influence on plasma lipid levels and plasma antioxidant activity in rats fed with cholesterol-containing and cholesterol-free diets

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### Abstract

Bioactive compounds of peels and peeled red Star Ruby (Sunrise) and blond qualities of Jaffa grapefruits were analyzed and their antioxidant potential was assessed. The dietary fibers were determined according to Prosky et al., the total polyphenol content by Folin–Ciocalteu method and measured at 765 nm, minerals and trace elements by atomic absorption spectrometer, phenolic and ascorbic acids by HPLC and the antioxidant potential by two different antioxidant assays (DPPH and  $\beta$ -carotene linoleate model system). It was found that the contents of most studied bioactive compounds in both qualities are comparable. Only the contents of total polyphenols and flavonoids were higher in red grapefruits, but not significant. The antioxidant potentials of red peeled grapefruits and their peels were significantly higher than of blond peeled grapefruits and their peels ( $P < 0.05$  in both cases). Diets supplemented with peeled red and blond qualities of Jaffa grapefruits and their peels have increased the plasma antioxidant capacity and

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improved plasma lipid levels, especially in rats fed with cholesterol added diet. In conclusion, both qualities of Jaffa grapefruits contain high quantities of bioactive compounds, but the antioxidant potential of red grapefruits is significantly higher. Diets supplemented with both qualities of Jaffa grapefruits improve the plasma lipid levels and increase the plasma antioxidant activity, especially in rats fed with cholesterol added diets. Jaffa grapefruits, especially their red Star Ruby quality, could be a valuable supplementation for diseases-preventing diets.

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*Keywords:* Red and blond grapefruits; Bioactive compounds; Rats; Plasma lipid levels and antioxidant activity

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## Introduction

Consumption of fruits and vegetables has been associated with reduced risk of some chronic diseases including the most dangerous—coronary atherosclerosis and cancer (Rimm et al., 1996a). The major bioactive compounds responsible for these natural product's health benefits are phytochemicals, especially phenolics (Bocco et al., 1998; Paganga et al., 1999). Phenolics possess antioxidant properties and prevent oxidation of low-density lipoprotein cholesterol (Paganga et al., 1999; Eberhardt et al., 2000). As a consequence, consumption of fruits and vegetables is inversely related to coronary atherosclerosis (Rimm et al., 1996a). Citrus fruits possess a high amount of these bioactive compounds, mostly phenolics (Kurowska et al., 2000; Vinson et al., 2001, 2002; Gorinstein et al., 2001, 2004). These fruits, especially different qualities of grapefruits, are very popular among European and North American consumers.

Recently were published the results of two important investigations of regularly consumed fruits and vegetables (Proteggente et al., 2002; Sun et al., 2002). The abovementioned authors have compared the antioxidant potential of these natural products. However, they did not investigate different qualities of the same fruits. Therefore, we decided to compare the main essential bioactive compounds and the antioxidant potential of blond and Star Ruby (red) qualities of the same Jaffa grapefruits and their influence on plasma lipid levels and plasma antioxidant activity in rats fed with cholesterol-containing and cholesterol-free diets.

Addition of citrus fruits to cholesterol-containing diets leads to hypocholesterolemic effect and to a decrease in the content of total cholesterol in liver in experiments on laboratory animals (Kurowska et al., 2000; Gorinstein et al., 2003; Kurowska and Manthey, 2004). Is it possible that the cholesterol-lowering effect of grapefruits is genuine or maybe it is a redistribution of cholesterol in the animal body? In order to answer this question, it was decided to determine the bile flow, the bile cholesterol and the bile acids concentrations before and at the end of the experiment.

As far as we know, there are no such comprehensive investigations of different qualities of the same Jaffa grapefruits including experiments *in vivo*.

## Materials and methods

### *Chemicals*

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid),  $\beta$ -carotene, butylated hydroxyanisole (BHA), Griess reagent, sodium nitroprusside, 1,1-diphenyl-2-picrylhydrazyl (DPPH) 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) diamonium salt (ABTS) was from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade.

### *Fruits and sample preparation*

Israeli blond and red Star Ruby qualities of Jaffa grapefruits (*Citrus paradisi*) were of the same ripeness. They were harvested in the period of 2003–2004 and were purchased from the same farmer. Samples (12 of each grapefruit's quality) were obtained from 24 randomly selected fruits for the determination of all studied variables. The grapefruits were cleaned with tap water and manually separated into peels and peeled fruits.

### *Determination of bioactive compounds*

Dietary fiber, trace elements, total phenols and individual antioxidants were determined as previously described (Gorinstein et al., 2001, 2004).

### *Determination of the antioxidant potential*

Previously, for determination of the antioxidant potential in citrus fruits, we used the total radical-trapping antioxidative test of Slavíková et al. (1998). However, this test is relatively unspecific marker of the free radical scavenging activity in fresh fruits (Gorinstein et al., 2001). Therefore, in the present investigation, two other assays already successfully tested by us on fresh fruits were used (Gorinstein et al., 2004):

1. Antioxidant assay using  $\beta$ -carotene linoleate model system [ $\beta$ -carotene] (Singh et al., 2002).  $\beta$ -Carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) were mixed. Chloroform was removed at 40 °C under vacuum, and the resulting mixture was diluted with 10 mL of water and mixed well. To this emulsion was added 40 mL of oxygenated water. Four-milliliter aliquots of the emulsion was pipetted into different test tubes containing 0.2 mL of fruit extracts or BHA in ethanol. BHA was used for comparative purposes. A control containing 0.2 mL of ethanol and 4 mL of the above emulsion was prepared. The tubes were placed at 50 °C in a water bath, and the absorbance at 470 nm was taken at zero time ( $t=0$ ). Measurement of absorbance was continued at an interval of 15 min until the color of  $\beta$ -carotene disappeared in the control tubes ( $t=180$  min). A mixture prepared as above without  $\beta$ -carotene served as blank. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the  $\beta$ -carotene using the following formula,  $AA=100 [1 - (A_0 - A_t)/(A_0^\circ - A_t^\circ)]$ , where  $A_0$  and  $A_0^\circ$  are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and  $A_t$  and  $A_t^\circ$  are the absorbance measured in the test sample and control, respectively, after incubation for 180 min.
2. Radical scavenging activity using DPPH method [DPPH] (Singh et al., 2002). Different concentrations of extracts of the peeled fruits and their peels were taken in different test tubes. The volume was adjusted to 100  $\mu$ L by adding MeOH. A 0.1 mM methanolic solution of 2, 2-diphenyl-L-picrylhydrazyl radical (DPPH) was added to these tubes and shaken vigorously and stand at 27 °C for 20 min. The control was prepared as above without any extract, and MeOH was used for

the baseline correction. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula: % radical scavenging activity =  $(\text{control OD} - \text{sample OD} / \text{control OD}) \times 100$ , where OD is optical density. Changes in the absorbance of the samples were measured at 517 nm. BHA was used for comparison.

### *Rats and diets*

The Animal Care Committee of the University had approved this study. The mean weight of the Wistar male rats ( $n=60$ ) at the beginning of the experiment was 100 g. They were housed in stainless steel metabolic cages and were divided into 10 groups of 6. These diet groups were named Control, RedGFpeel, RedGFpeeled, BlondGFpeel, BlondGFpeeled, Chol, Chol/RedGFpeel, Chol/RedGFpeeled, Chol/BlondGFpeel and Chol/BlondGFpeeled. During 4 weeks of the experiment, the 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 the BD only. The BD of the other 9 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 100 g/kg of red peeled grapefruit (Chol/RedGFpeeled), 10 g/kg of NOC and 100 g/kg of blond grapefruit peel (Chol/BlondGFpeel) and 10 g/kg of NOC and 100 g/kg of blond peeled grapefruit (Chol/BlondGFpeeled). Cholesterol of analytical grade (USP) was obtained from Sigma Chemical, St. Louis, MO. The dietary cholesterol was checked according to the HPLC method of [Ansary et al. \(1979\)](#) and was found not to contain cholesterol oxides. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. Several prior experiments on laboratory animals and human studies have shown that cellulose has not significant hypocholesterolemic effects ([Anderson et al., 1991](#)). Therefore, cellulose was used as a control fiber. The diets contained as percentage of energy 66% of carbohydrates, 25% of protein and 9% of fat. Their calculated energy was from 394.5 to 400.4 kcal/100 g and the differences were not significant.

All rats were fed once a day at 1000 h ad libitum. They had unrestricted access to drinking water. The food intake was monitored daily and body gains every week.

Before the experiment, the blood samples were taken from the tail vein. At the end of the experiment, the rats were anaesthetized using general urethan narcosis (1.8 g of urethan per 1 kg of the animal body weight) and the blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests. The abdomen was opened to take samples of the liver for the total cholesterol determination. Two time points were used in this experiment: before and after 28 days of different feeding. At these time points, a wide range of laboratory tests was performed. Total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), total phospholipids (TPH) and TC in liver, bile flow, bile cholesterol and bile acids concentrations were determined as previously described ([Krzeminski et al., 2003](#); [Gasowski et al., 2004](#)).

The total antioxidant status (TAA Test) was adopted for determination of the plasma antioxidant activity ([Rice-Evans and Miller, 1994](#)).

The TAA was estimated using the ferrylmyoglobin/ABTS method for total antioxidant activity. This technique measures the relative ability of antioxidant substances to scavenge the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS<sup>•+</sup>), compared with standard amounts of the

synthetic antioxidant Trolox, the water-soluble vitamin E analogue. The radical cation  $ABTS^{+\cdot}$ , generated in the aqueous phase from ABTS through the peroxidation action of metmyoglobin, is a blue/green chromogen with characteristic absorption at 734 nm. Results were expressed as  $\mu\text{mol/mL TE}$ .

### Statistical analyses

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

## Results

### *In vitro*

#### Dietary fibers

The contents of total, soluble and insoluble dietary fibers (Fig. 1) in peeled grapefruit's blond and red qualities and their peels were comparable ( $P>0.05$ ). The contents of fibers in peels were significantly higher than in peeled fruits ( $P<0.05$ ).

#### Minerals and trace elements

The results of the determination of the studied minerals show that the significantly highest content was of K following by  $\text{Mg}>\text{Ca}>\text{Na}$ . The results of the determination of the studied trace elements show

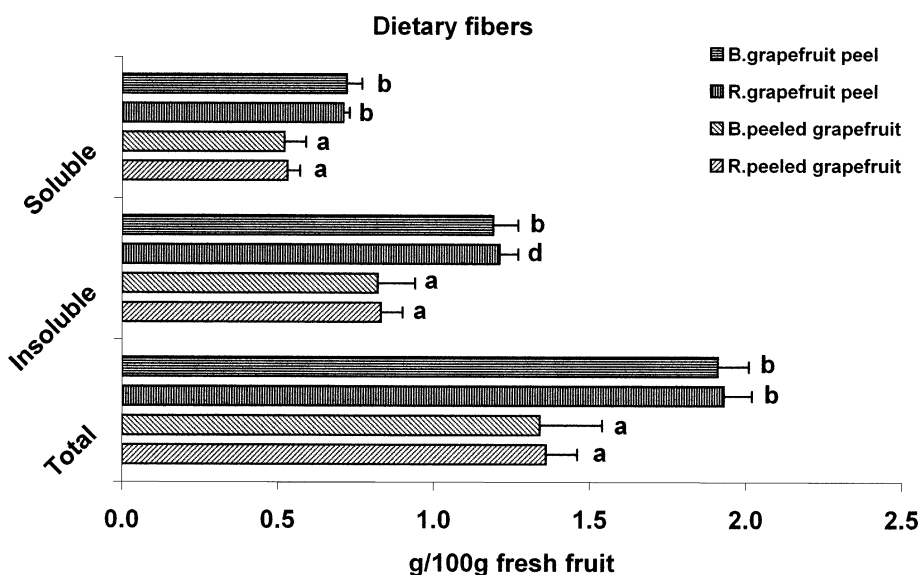


Fig. 1. Dietary fibers in peeled grapefruits and their peels.  $M \pm SD$  (horizontal lines). Bars with different letters are significant different ( $p<0.05$ ).

that the significantly highest content was of Fe following by Zn>Cu>Mn. The contents of all studied minerals and trace elements in peeled blond and red qualities of grapefruits and their peels were comparable ( $P>0.05$ ). The contents of all studied minerals and trace elements were significantly higher in peels than in peeled fruits ( $P<0.05$ ).

#### Total polyphenols

The contents of 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/100 g fresh weight (FW) of gallic acid equivalents in peeled blond and red grapefruit's qualities and their peels, respectively. The content of total polyphenols in peels and peeled red grapefruits was higher, but not significant ( $P>0.05$ ). The content of total polyphenols in peels of both qualities was significantly higher than in peeled grapefruits ( $P>0.05$ ).

#### Phenolic and ascorbic acids

The results of the determination of the contents of ferulic, sinapic, *p*-coumaric, caffeic and ascorbic acids in peeled grapefruits and peels of both qualities are summarized in Fig. 2. As can be seen, the contents of all studied compounds in red and blond grapefruit qualities were comparable. Among the phenolic acids, the highest concentration was of ferulic and the lowest of caffeic acid. The content of ascorbic acid was significantly higher than of every one of the phenolic acids ( $P<0.05$ ). The contents of ferulic, sinapic, *p*-coumaric, caffeic and ascorbic acids in peels of grapefruit's both qualities were significantly higher than in peeled grapefruits ( $P<0.05$ ).

#### Flavonoids

The contents of flavonoids in peeled red and blond grapefruits were  $21.2 \pm 1.3$  and  $19.1 \pm 1.2$  mg/100 g, respectively. The contents of flavonoids in peels of red and blond grapefruits were  $71.9 \pm 6.3$  and  $67.1 \pm 6.2$  mg/100 g, respectively. The contents of flavonoids in the red peeled grapefruits and their peels were higher than in blond quality, but the differences in both cases were not significant ( $P>0.05$ ). The content of flavonoids in peels was significantly higher than in peeled grapefruits ( $P<0.05$ ).

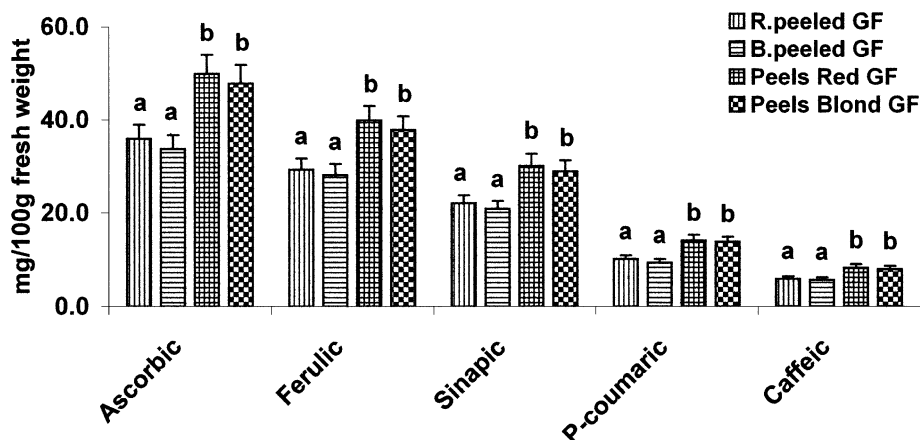


Fig. 2. Phenolic and ascorbic acids in peeled grapefruits and their peels.  $M \pm SD$  (vertical lines). Bars with different letters are significant different ( $p<0.05$ ).

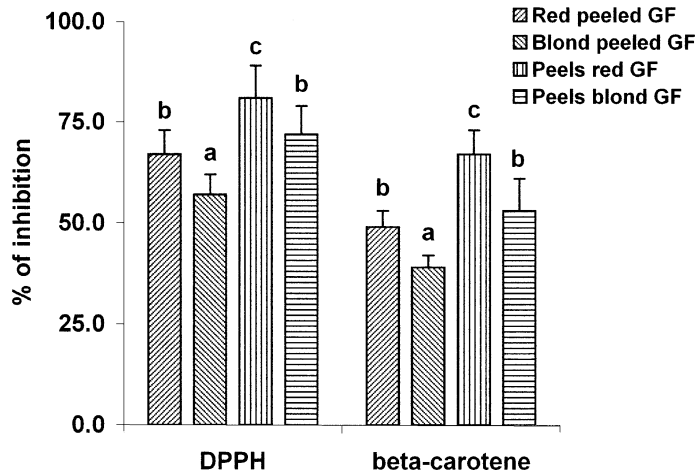


Fig. 3. The antioxidant activity in peeled grapefruits and their peels.  $M \pm SD$  (vertical lines). Bars with different letters are significant different ( $p < 0.05$ ).

#### The free radical scavenging activity

The results of the determination of total antioxidant potential in peeled red and blond grapefruits and their peels are summarized in Fig. 3. As can be seen, both antioxidant assays show that the antioxidant potential of grapefruit's red quality was significantly higher than of the grapefruit's blond quality ( $P < 0.05$ ). The antioxidant potential in peels of grapefruits' red and blond qualities was significantly higher than of the peeled fruits ( $P < 0.05$ ).

The calculated correlations showed that the dietary fibers contribution to the antioxidant potential of both grapefruit's red and blond qualities was minimal ( $R^2$  from 0.35 to 0.39), of ascorbic acid-moderate ( $R^2$  from 0.6203 to 0.6781). The contributions of the total phenols and flavonoids were high (Fig. 4A and B:  $R^2$  from 0.9424 to 0.9449 and  $R^2$  from 0.9831 to 0.9858 for flavonoids and total phenols, respectively).

#### In vivo

Addition of red or blond peeled grapefruits, their peels or/and cholesterol to the BD did not affect food intake, body weight gains and feed efficiencies (data not shown).

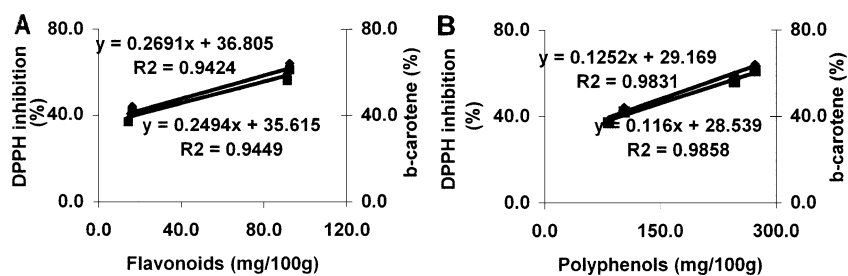


Fig. 4. Relationship, calculated by a linear regression analysis. (A)  $\blacklozenge$  Flavonoids (mg/100 g FW,  $X$ ) to DPPH scavenging effect (% inhibition,  $Y_1$ ) and  $\blacksquare$  flavonoids (mg/100 g FW,  $X$ ) to  $\beta$ -carotene (% inhibition,  $Y_2$ ); (B)  $\blacklozenge$  polyphenols (mg/100 g FW,  $X$ ) to DPPH scavenging effect (% inhibition,  $Y_1$ ) and  $\blacksquare$  polyphenols (mg/100 g FW,  $X$ ) to  $\beta$ -carotene bleaching effect (% inhibition,  $Y_2$ ). Abbreviations: DPPH—1,1-diphenyl-2-picrylhydrazyl radical scavenging test,  $\beta$ -carotene— $\beta$ -carotene bleaching.

At baseline, the 10 diet groups did not differ from one another in plasma lipid concentration (data not shown). The red and blond peeled grapefruits or their peel-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 (Table 1):

(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% and TC in liver—35.7%, 30.0%, 33.5% and 27.2%, respectively. Red and blond peeled grapefruits and their peel-supplemented diets significantly decreased the level of TPH (27.0%, 23.4%, 26.1% and 21.7% for the Chol/RedGFpeel, Chol/RedGFpeeled, Chol/BlondGFpeel and Chol/BlondGFpeeled diet group, respectively).

The red and blond peeled grapefruits or their peel-supplemented diets in rats fed with BD without cholesterol did not affect the variables measured.

Liver TC concentration in rats of the Chol group was 4.15 times higher than in the Control group. TC liver concentration in rats of the Chol/RedGFpeel, Chol/RedGFpeeled, Chol/BlondGFpeel and Chol/BlondGFpeeled was only 3.05, 3.19, 3.10 and 3.26 times higher than in the Control group, respectively ( $P < 0.005$  in all cases).

Table 1

Plasma lipids and total cholesterol concentration in liver of rats fed diets with and without 1% cholesterol (Chol) and with and without peeled grapefruits (GF) and their peels

Diets	Plasma lipids					Liver
	TC	LDL-C	HDL-C	TG	TPH	TC
	mmol/L					$\mu\text{mol/g}$
Control	2.86 ± 0.15 <sup>c</sup>	1.23 ± 0.05 <sup>c</sup>	1.62 ± 0.07 <sup>a</sup>	0.69 ± 0.04 <sup>b</sup>	1.76 ± 0.08 <sup>a</sup>	5.86 ± 0.44 <sup>c</sup>
RGFpeel	2.81 ± 0.15 <sup>c</sup>	1.19 ± 0.05 <sup>c</sup>	1.61 ± 0.07 <sup>a</sup>	0.07 ± 0.05 <sup>b</sup>	1.72 ± 0.08 <sup>a</sup>	5.74 ± 0.44 <sup>c</sup>
RGFpeeled	2.87 ± 0.15 <sup>c</sup>	1.22 ± 0.05 <sup>c</sup>	1.64 ± 0.07 <sup>a</sup>	0.71 ± 0.05 <sup>b</sup>	1.73 ± 0.08 <sup>a</sup>	5.81 ± 0.44 <sup>c</sup>
BGFpeel	2.81 ± 0.15 <sup>c</sup>	1.19 ± 0.05 <sup>c</sup>	1.61 ± 0.07 <sup>a</sup>	0.70 ± 0.05 <sup>b</sup>	1.72 ± 0.08 <sup>a</sup>	5.79 ± 0.44 <sup>c</sup>
BGFpeeled	2.87 ± 0.15 <sup>c</sup>	1.22 ± 0.05 <sup>c</sup>	1.64 ± 0.07 <sup>a</sup>	0.71 ± 0.05 <sup>b</sup>	1.73 ± 0.08 <sup>a</sup>	5.87 ± 0.44 <sup>c</sup>
Chol	3.69 ± 0.21 <sup>a</sup>	2.02 ± 0.12 <sup>a</sup>	1.61 ± 0.07 <sup>a</sup>	0.88 ± 0.05 <sup>a</sup>	1.74 ± 0.08 <sup>a</sup>	24.3 ± 1.15 <sup>a</sup>
Chol/RGFpeel	2.91 ± 0.18 <sup>b</sup>	1.31 ± 0.05 <sup>b</sup>	1.60 ± 0.07 <sup>a</sup>	0.75 ± 0.05 <sup>b</sup>	1.37 ± 0.06 <sup>b</sup>	17.9 ± 1.25 <sup>b</sup>
Chol/RGFpeeled	3.02 ± 0.18 <sup>b</sup>	1.41 ± 0.05 <sup>b</sup>	1.61 ± 0.07 <sup>a</sup>	0.79 ± 0.05 <sup>b</sup>	1.41 ± 0.06 <sup>b</sup>	18.7 ± 0.25 <sup>b</sup>
Chol/BGFpeel	2.95 ± 0.18 <sup>b</sup>	1.37 ± 0.05 <sup>b</sup>	1.68 ± 0.07 <sup>a</sup>	0.77 ± 0.05 <sup>b</sup>	1.38 ± 0.06 <sup>b</sup>	18.2 ± 1.26 <sup>b</sup>
Chol/BGFpeeled	3.06 ± 0.18 <sup>b</sup>	1.45 ± 0.05 <sup>b</sup>	1.61 ± 0.07 <sup>a</sup>	0.81 ± 0.05 <sup>b</sup>	1.43 ± 0.06 <sup>b</sup>	19.1 ± 1.26 <sup>b</sup>
<i>2-way ANOVA (P-value)</i>						
RGFpeel	NS	NS	NS	NS	NS	NS
RGFpeeled	NS	NS	NS	NS	NS	NS
BGFpeel	NS	NS	NS	NS	NS	NS
BGFpeeled	NS	NS	NS	NS	NS	NS
Chol	<0.001	<0.001	NS	<0.001	NS	<0.0005
Chol/RGFpeel	<0.050	<0.050	NS	<0.050	<0.010	<0.0005
Chol/RGFpeeled	<0.050	<0.050	NS	<0.050	<0.010	<0.0005
Chol/BGFpeel	<0.050	<0.050	NS	<0.050	<0.010	<0.0005
Chol/BGFpeeled	<0.050	<0.050	NS	<0.050	<0.010	<0.0005

Values are means ± SD ( $n=6$ ).

Means in columns without letters in common differ significantly ( $P < 0.05$ ).

Abbreviations: B—blond; GF—grapefruit; R—red.

### Bile flow, bile cholesterol and bile acids concentrations

The bile flow, bile acids and bile cholesterol concentrations in all groups of rats before the experiment were without significant differences.

The bile flow, bile cholesterol and bile acids concentrations in the groups of rats fed with cholesterol-free diets after completion of the experiment were without significant changes.

The changes in these indices in the groups of rats fed with cholesterol-containing diets after completion of the experiment are summarized in Figs. 5–7, respectively.

As can be seen (Fig. 5), a significant increase in the bile flow ( $P < 0.05$ ) was registered only in the Chol/RedGFpeel and Chol/BlondGFpeel groups.

A significant increase ( $P < 0.05$ ) in the bile cholesterol concentration (Fig. 6) was registered in Chol, Chol/RedGFpeel and Chol/BlondGFpeel groups. The increase in the bile cholesterol concentration in Chol/RedGFpeel and Chol/BlondGFpeel groups was significantly higher than in the Chol group ( $P < 0.05$ ).

Fig. 7 demonstrates that the bile acid concentration was increased significantly ( $P < 0.05$ ) only in Chol, Chol/RedGFpeel and Chol/BlondGFpeel groups. The increase in the bile acid concentration in Chol/RedGFpeel and Chol/BlondGFpeel groups was significantly higher than in the Chol group ( $P < 0.05$ ).

As calculated in percentages, the increase in the bile flow, bile cholesterol and bile acids concentrations was 33.2% and 22.9%, 31.9% and 16.5% and 59.0% and 36.4%, for Chol/RedGFpeel and Chol/BlondGFpeel groups vs. Chol group, respectively ( $P < 0.05$  in all cases).

After completion of the trial, a significant increase ( $P < 0.05$  in all cases) in the TAA values was registered in rats of the RedGFpeel, RedGFpeeled, BlondGFpeel and BlondGFpeeled diet groups vs. Control diet group (Fig. 8A). This significant increase in plasma antioxidant activity was expected: the diets of the abovementioned diet groups were supplemented with fruits containing high concentration of

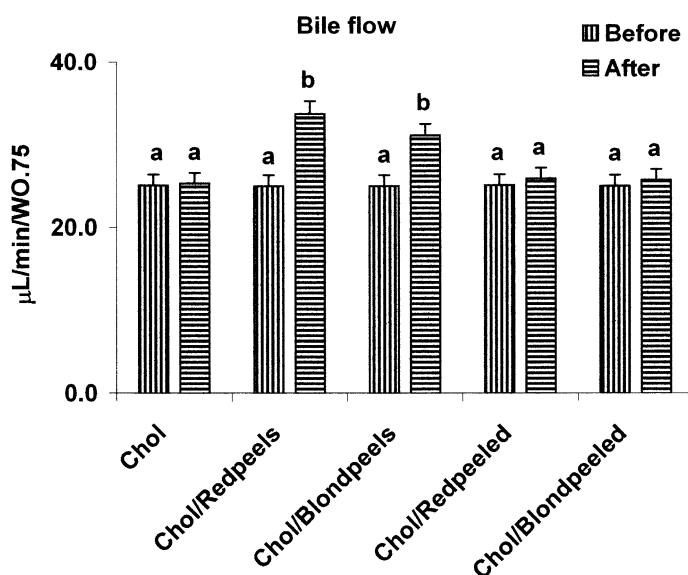


Fig. 5. Changes in the bile flow after the experiment. Means  $\pm$  SD (vertical lines). Bars with different letters are significantly different ( $p < 0.05$ ).

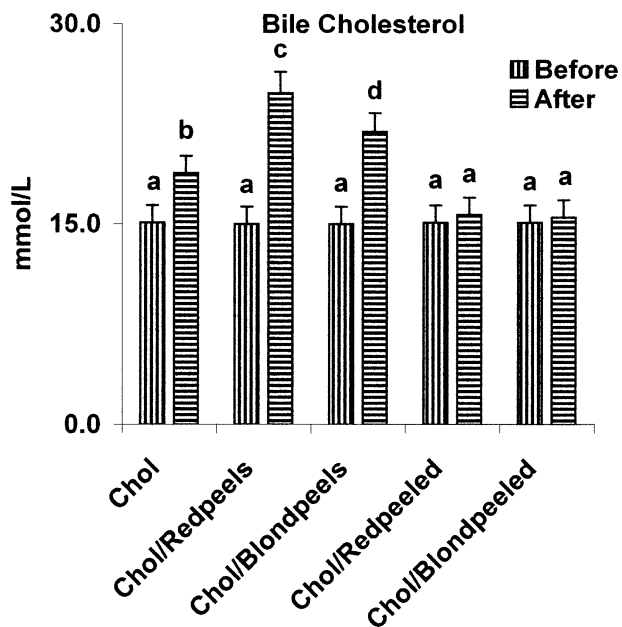


Fig. 6. Changes in the biliary cholesterol concentration after the experiment. Means  $\pm$  SD (vertical lines). Bars with different letters are significantly different ( $p < 0.05$ ).

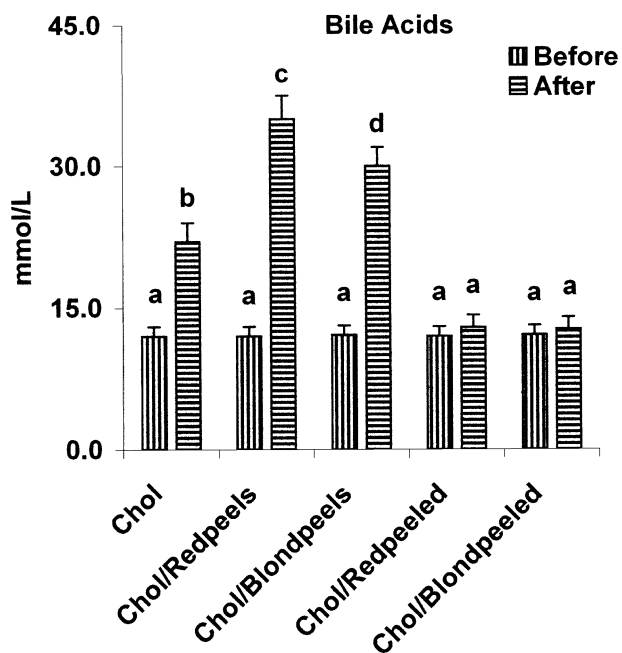


Fig. 7. Changes in the biliary bile acid concentration after the experiment. Means  $\pm$  SD (vertical lines). Bars with different letters are significantly different ( $p < 0.05$ ).

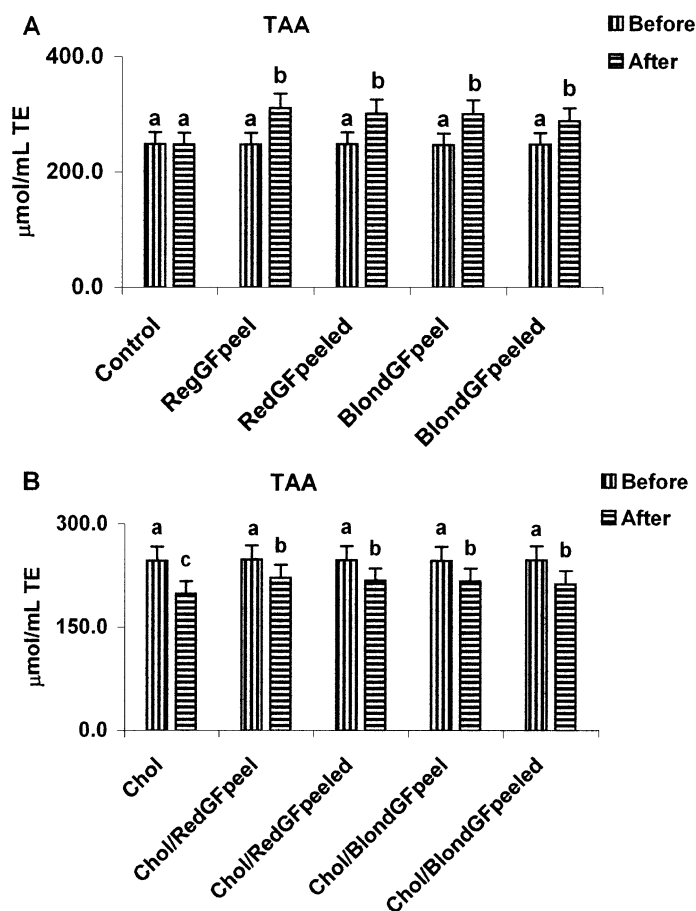


Fig. 8. (A) The plasma antioxidant activity before and after completion of the investigation in dietary groups fed with cholesterol-free diets.  $M \pm SD$  (vertical lines). Bars with different letters are significant different ( $p < 0.05$ ). (B) The plasma antioxidant activity before and after completion of the investigation in dietary groups fed with cholesterol-containing diets.  $M \pm SD$  (vertical lines). Bars with different letters are significant different ( $p < 0.05$ ).

natural antioxidants. However, a significant decrease in the TAA values in the rats fed with added cholesterol was found (Fig. 8B). This decrease in the plasma antioxidant activity was predictable: Tsai and Chen (1979), and Uysal (1986), observed that cholesterol-supplemented diet leads to a decrease in blood antioxidant activity. It must be underlined that the decrease in the antioxidant activity in the groups of rats fed with diets supplemented with fruits (Chol/RedGFpeel, Chol/RedGFpeeled, Chol/BlondGFpeel and Chol/BlondGFpeeled) was significantly less ( $P < 0.05$  in all cases) than in group of rats fed with diet without fruits (Chol).

## Discussion

Coronary atherosclerosis is still one of the most dangerous diseases in humans and is considered the principal cause of death in Western civilization (Hertog et al., 1995). Some authors have shown that diets

rich in fruits and vegetables are effective in the prevention of this disease (Rimm et al., 1996a,b). Therefore, many authors recommend inclusion of these natural products in disease preventive diets (Hertog et al., 1995; Vinson et al., 2001; Szeto et al., 2002).

Citrus fruits possess high contents of bioactive compounds and some authors claim that they has to be part of disease preventing diets (Kurowska et al., 2000; Gorinstein et al., 2001; Vinson et al., 2002).

As was shown, bioactive compounds are extranutritional constituents that typically occur in small quantities in foods (Kris-Etherton et al., 2002). They are being intensively studied to evaluate their effects on health. The impetus sparking this scientific inquiry was the result of many epidemiologic studies that have shown protective effects of plant-based diets on cardiovascular disease and cancer (Kris-Etherton et al., 2002).

As was indicated in the Introduction, recently were published results of two major investigations of regularly consumed fruits and vegetables (Proteggente et al., 2002; Sun et al., 2002). However, the authors did not investigate different qualities of the same fruits. Therefore, in this investigation, we decided to compare red and blond qualities of the same Jaffa grapefruits.

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 results of others and our previous data (Rapisarda et al., 1999; Gorinstein et al., 2001).

The contents of total polyphenols and flavonoids were higher in red grapefruits, but the differences were not significant. Also these results correspond with the results of others and our previous data (Peleg et al., 1991; Gorinstein et al., 2001).

It was found that antioxidant potentials of red peeled grapefruits and their peels were significantly higher than of blond peeled grapefruits and their peels. These data are in accordance with the data of Lotito and Frei (2004), who have shown that antioxidant activity of individual antioxidant compounds is not directly connected to total antioxidant potential of fruits.

We have found that diets supplemented with peeled red and blond grapefruits and their peels have increased the plasma antioxidant activity and improved plasma lipid levels, especially in rats fed with cholesterol-containing diets. Also others have demonstrated that hypolipidemic effect of fruits and vegetables is evident when they are added to diets of rats fed with cholesterol (Anderson et al., 1991, 1994).

The results of the present investigation have shown that the hypolipidemic effect of peeled grapefruits and their peels like of other natural products investigated by us is genuine (Krzeminski et al., 2003; Gasowski et al., 2004): diets supplemented with these fruits have increased the bile flow, bile cholesterol and bile acids concentrations.

Diets supplemented with both qualities of Jaffa grapefruits increase the plasma antioxidant activity in rats fed with cholesterol-free, and hindered the decrease of the plasma antioxidant activity in rats fed with cholesterol-containing diets. These results could be expected: peels and peeled grapefruits have high antioxidant potential.

In conclusion, (1) both qualities of Jaffa grapefruits contain high quantities of bioactive compounds, but the antioxidant potential of red grapefruits is significantly higher, (2) diets supplemented with both qualities of Jaffa grapefruits improve the plasma lipid levels and increase the plasma antioxidant activity, especially in rats fed with added cholesterol, (3) the cholesterol-lowering effect of diets supplemented with Jaffa grapefruits is genuine, and (4) Jaffa grapefruits, especially its red quality, could be a valuable supplement for diseases-preventing diets.

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