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Fresh Israeli Jaffa Blond (Shamouti) Orange and Israeli Jaffa Red Star Ruby (Sunrise) Grapefruit Juices Affect Plasma Lipid Metabolism and Antioxidant Capacity in Rats Fed Added Cholesterol

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The bioactivity of Israeli Jaffa blond (Shamouti) fresh orange and Israeli Jaffa red Star Ruby (Sunrise) grapefruit juices was investigated in vitro and in vivo. The contents of bioactive compounds of these juices were determined. The influence of bioactive compounds on plasma lipids and plasma antioxidant activity in rats fed cholesterol-containing and cholesterol-free diets was assessed. Significant differences in the contents of dietary fibers were not found. The contents of total polyphenols, flavonoids, and anthocyanins in fresh orange and grapefruit juices were 962.1 ± 27.2 and 906.9 ± 27.1; 50.1 ± 3.3 and 44.8 ± 3.2; and 69.9 ± 5.6 and 68.7 ± 5.5 μg/mL, respectively. The antioxidant potential measured by the scavenging activity against nitric oxide, the β-carotene−linoleate model system (β-carotene), and the 1,1-diphenyl-2-picrylhydrazyl and 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt assays was higher in orange juice but not significantly. A high level of correlation between contents of total polyphenols and flavonoids and antioxidant potential values of both juices was found. Diets supplemented with orange and to a lesser degree with grapefruit juices improved plasma lipid metabolism only in rats fed added cholesterol. However, an increase in the plasma antioxidant activity was observed in both groups. In conclusion, fresh orange and grapefruit juices contain high quantities of bioactive compounds, which guarantee their high antioxidant potential, and the positive influence on plasma lipid metabolism and plasma antioxidant activity could make fresh orange and grapefruit juices a valuable supplement for disease-preventing diets.

KEYWORDS: Citrus juices; bioactive compounds; plasma lipids; plasma antioxidant activity; rats

INTRODUCTION

Fruits in general and citrus fruits in particular have many healthful properties (*1*, *2*). The positive influence of these natural products is attributed to their essential bioactive compounds: phenolic acid (PA) and ascorbic acid (ASC) and certain parts of dietary fibers (*3*). Citrus fruits have a high content of these substances and, as a consequence, a high antioxidant potential (*4*−*7*). Many consumers prefer fruit juices instead of whole fruits (*8*, *9*). It was shown that fruit juices positively affect plasma lipid levels in animals (*9*, *10*). However, the influence on plasma antioxidant activity (AA) was not studied enough. Therefore, we decided to determine the contents of the essential bioactive compounds in fresh orange juices (OJs) and grapefruit juices (GJs) and to compare their influence on plasma lipids and AA in rats fed cholesterol-containing and cholesterol-free diets.

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In the past, we have used TRAP for the determination of the antioxidant potential of citrus fruits (*11*, *12*). However, this test is a relatively unspecific marker of the free radical scavenging activity in fruits and vegetables (*11*). Therefore, in the present investigation, other assays were used as follows: (i) the scavenging activity against the nitric oxide (NO) test (*13*, *14*); (ii) an antioxidant test using the β-carotene−linoleate model system (β-carotene) (*15*); (iii) the radical scavenging activity test using the DPPH method (*15*); and (iv) the TEAC (*16*). As far as we know, there have not been any comprehensive investigations of fresh OJs and GJs (without preserving substances) that also include experiments on laboratory animals.

MATERIALS AND METHODS

Chemicals. Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid), β-carotene, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Griess reagent, sodium nitroprusside, DPPH, and Folin−Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO), and ABTS was purchased from Fluka Chemie (Buchs, Switzerland). All reagents were of analytical grade.

Samples. Israeli Jaffa blond (Shamouti) oranges (*Citrus sinensis*) and Israeli Jaffa red Star Ruby (Sunrise) grapefruits (*Citrus paradisi*) of the same maturity degree were purchased from the same farmer. The OJs and GJs were prepared manually and were prevented from oxidizing. From 300 g of fresh oranges and grapefruits were obtained 100.3 and 98.8 mL of juices, respectively.

Determination of the Bioactive Substances. *Dietary Fibers.* Dietary fibers in the selected samples were analyzed by the modified AOAC method. Samples were treated with heat stable α-amylase, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000*g*) to separate the soluble and insoluble fractions and dialysis against water (*17*).

Total Polyphenols, PAs, and ASC. Total polyphenols and PAs and ASCs were determined as previously described (*11*).

Extraction and Hydrolysis of Total Polyphenols. A 50 mg aliquot of lyophilyzate was accurately weighed in a screw-capped tube. The total phenols were extracted with 5 mL of 1.2 M HCl in 50% methanol/water. The samples were vortexed for 1 min and heated at 90 °C for 3 h with vortexing every 30 min. After the samples were cooled, they were diluted to 10 mL with methanol and centrifuged for 5 min at 4000*g* with a benchtop centrifuge to remove solids. The phenols were measured at 750 nm after reacting for 10 min, using the Folin−Cocialteu reagent, diluted 5-fold before use, with gallic acid as the standard (*18*, *19*).

Flavonoids. The absorbance of flavonoids (extracted with 5% $NaNO_2$, 10% $AlCl_3 \cdot 6H_2O$, and 1 M NaOH) was measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as μg of catechin equivalents per mL of fresh juice (*19*).

Anthocyanins. Fifty milliliters of each fruit juice was added to 50 mL of acetonitrile containing 4% acetic acid and mixed and then centrifuged at 13 000*g* for 15 min at 4 °C. The pellet following centrifugation was washed with 50 mL of acetonitrile containing 4% acetic acid and centrifuged. The resulting supernatants were combined with the initial extract. The amount of anthocyanins was estimated by a pH differential method (*20*). The absorbance was measured in a Beckman spectrophotometer at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient of 29 600 for cyanidin-3-glucoside. The results were expressed as μg of cyanidin-3-glucoside equivalent per mL of fresh juice.

Determination of the Antioxidant Potential. *Scavenging Activity against the NO Test.* A 0.5 mL portion of a mixture (0.4 mL of fresh juice and 0.1 mL of sodium nitroprusside solution) was diluted with 0.3 mL of Griess reagent. The absorbance of the chromophore formed during the diazotination of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 570 nm and referred to the absorbance of standard solutions of sodium nitrite salt treated in the same way with Griess reagent. The nitrite concentration was calculated by referring to the absorbance of standard solutions of potassium nitrite. The results were expressed as the percentage nitrite production with respect to control values (*13*, *14*).

Antioxidant Test Using the β-Carotene−Linoleate Model System (β-Carotene). To the emulsion [β-carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg)] was added 40 mL of oxygenated water. Four milliliter aliquots of this emulsion were added to test samples containing 0.2 mL of fresh juice.

The AA of the extracts was evaluated in terms of bleaching of the β-carotene and measuring the absorbance at 470 nm, during $t = 180$ min at an interval of 15 min: $AA = 100[1 - (A_0 - A_t)/(A_0^\circ - A_t^\circ)]$, where A_0 and A_0° are the absorbance values measured at zero time and A_t and A_t° are the absorbance values measured in the test sample and control, respectively, after incubation for 180 min and after the kinetics was measured. Trolox, BHT, and BHA were used as the standards in these methods (*15*).

Radical Scavenging Activity Test Using the DPPH Method. Five milliliters of a 0.1 mM methanolic solution of DPPH was added to 100 μL of fresh juice and BHA and BHT standards. Changes in the absorbance of the samples and standards were measured at 517 nm. The radical scavenging activity was expressed as the inhibition percentage and was calculated as % radical scavenging activity = (control OD − sample OD/control OD) × 100 (*15*).

TEAC. The TEAC value is based on the ability of the antioxidants to scavenge the blue-green $ABTS^{\bullet+}$ radical cation relative to the $ABTS^{\bullet+}$ scavenging ability of the water soluble vitamin E analogue Trolox. The $ABTS^{\bullet+}$ radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After the addition of 990 μL of $ABTS^{\bullet+}$ solution to 10 μL of different extracts (0.2 mg/mL) or Trolox standards (final concentration 0−20 μM) in methanol or phosphate-buffered saline, the absorbance was monitored exactly 1 and 6 min after the initial mixing. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the extracts and of Trolox for the standard reference data. To calculate the TEAC, the slope of the plot of the percentage inhibition of absorbance vs concentration for the antioxidant was divided by the slope of the plot of Trolox (*16*).

The juices were also lyophilized and extracted with methanol. To compare the antioxidant activities of investigated samples by different scavenging radical methods, the same concentrations of the extracts and standards were used (*15*).

Rats and Diets. The Animal Care Committee of the Warsaw Agricultural University approved this study. Wistar male rats ($n = 60$) with a mean weight of 120 g at the beginning of the study were provided by the Institute of Animal Physiology and Nutrition of Polish Academy of Sciences (Jablonna, Poland). They were housed in plastic metabolic cages and were divided into six groups of 10. These groups were named control, chol, orange, chol/orange, grapefruit, and chol/grapefruit. During 4 weeks of the experiment, the rats of all six groups were fed a basal diet (BD), which included wheat starch, casein, soybean oil, and vitamin and mineral mixtures (*11*). The rats of the control group were fed a BD only. The BD of the five other groups was supplemented with 10 g/kg of NOC of analytical grade (chol group), 1−2 mL of OJ per day (orange group), 10 g/kg of NOC and 1−2 mL of OJ per day (chol/orange group), 1−2 mL of GJ per day (grapefruit group), and 10 g/kg of NOC and 1−2 mL of GJ per day (chol/grapefruit group). These juices were induced by intubation into the stomach. To get the rats used to the maximal quantity of juice (2 mL), for the first 2 weeks, every animal received only 1 mL of juice per day; in the third week, the animals received 1.5 mL of juice per day; and in the last week of the trial, the animals received 2 mL of juice per day. The dietary cholesterol was checked by high-performance liquid chromatography 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. The diets contained as percentages of energy 67% carbohydrates, 24% protein, and 9% fat. The calculated energy of the used diets was from 394.9 to 400.1 kcal/100 g, and this difference was not statistically significant.

Table 1. Content of Dietary Fiber in OJs and GJs (in %)[a]

juices	total	insoluble	soluble
grapefruit	2.85 ± 0.19 a	2.1 ± 0.19 a	0.75 ± 0.7 a
orange	2.75 ± 0.18 a	2.05 ± 0.17 a	0.70 ± 0.7 a

[a] Values are means ± SD of five measurements. Means in columns without letters in common differ significantly.

Table 2. Main Antioxidant Compounds in OJs and GJs[a]

juices	polyphenols[b]	anthocyanins[c]	flavonoids[d]
orange	962.1 ± 27.2 a	69.9 ± 5.6 a	50.1 ± 3.3 a
grapefruit	906.9 ± 27.1 b	68.7 ± 5.5 a	44.8 ± 3.2 a

[a] Values are means ± SD of five measurements. Means in columns without letters in common differ significantly ($p < 0.05$). [b] μg of gallic acid equivalent per mL of fresh juice. [c] μg of cyanidin-3-glucoside equivalent per mL of fresh juice. [d] μg of catechin equivalents per mL of fresh juice.

All rats were fed once a day at 10:00 h ad libitum. They had unrestricted access to drinking water. The food intake and body gains were monitored daily. It is generally accepted that the most reliable data of the blood lipid metabolism can be obtained from fasting animals, 14−16 h after the last feeding. Therefore, the food was removed from the cages at 6 p.m. the day before and the samples were collected at 9 a.m. the next day. The plasma was prepared and used for laboratory tests. After anesthesia, the abdomen was opened to take samples of the liver for TC determination.

Two time points were used in this experiment: before and after 28 days of feeding. At these time points, a wide range of laboratory tests was performed. The plasma TC was determined with a Randox kit reagents catalog no. CH 280, Appl. No. 7; the HDL-C was determined according to Izawa et al.; the LDL-C was determined using the method of Friedewald et al.; the TG level was determined with a Randox kit reagents catalog no. 1697, Appl. No. 8; and the TPH were determined with an ANALCO kit reagents catalog no. A-161 as described previously (*21*). For the determination of liver cholesterol, 0.5 g of liver tissue was homogenized in 2 mL of 0.9% NaCl. The homogenized liver was centrifuged two times for 10 min at 3000*g*. Then, the TC was determined, with the Randox kit reagents catalog no. CH 280, Appl. No. 7 (International Headquarters Randox Laboratories, Distributor Hand-Prod, Leszczyńskiego 40A, Warsaw, Poland).

In the past, we used TRAP and MDA tests for the determination of the plasma antioxidant capacity (*11*). However, these tests were not specific for this kind of investigation. Therefore, in this study, a more specific ABTS decolorization assay was applied. The plasma total AA was measured using the TEAC adopted for plasma investigation (*16*). The results were expressed as μM Trolox equivalent per L.

Statistical Analysis. The results of this investigation in vitro are means ± SD of five measurements. When appropriate, differences between groups were tested by two way analysis of variance (ANOVA). In the assessment of the antioxidant capacity, the Spearman correlation coefficient (*R*) was used. Linear regressions were also calculated. The *p* values of <0.05 were considered significant.

RESULTS

In Vitro. *Fibers.* The contents of total, soluble, and insoluble dietary fiber are summarized in **Table 1**. As can be seen, the content of dietary fibers in GJs was higher than in OJs. However, these differences were not significant.

Total Polyphenols, Anthocyanins, and Flavonoids. The results of the main antioxidant compounds are summarized in **Table 2**. As can be seen, the contents of all of the studied compounds are higher in OJ, but only with total polyphenols is the difference statistically significant ($p < 0.05$). Ascorbate gives a Folin reaction (an oxidation−reduction reaction) and interferes with the method. Ascorbate was destroyed in the total phenols extract under the acidic conditions and heat. Therefore, the total phenol concentration could be determined directly from the Folin assay (*18*, *19*). The values of anthocyanins and flavonoids were comparable and in some cases lower than those reported by other investigators, depending on the method of determination and variety of the citrus fruits (*6*, *18*, *20*).

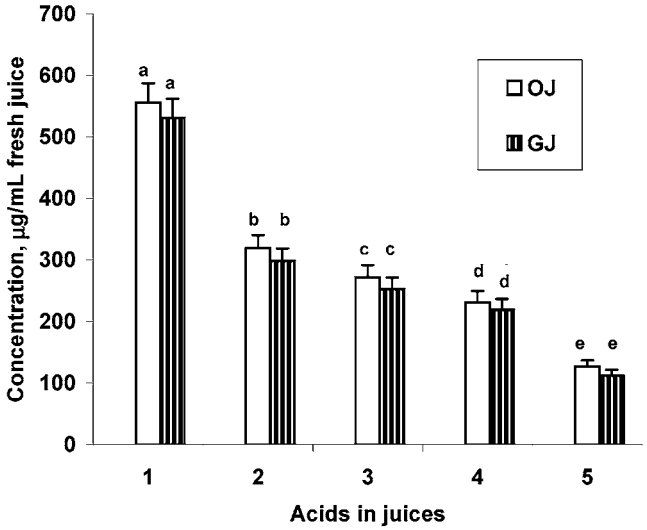

Figure 1. Comparative contents of PAs and ASCs in OJs and GJs. Means ± SD (vertical lines). Bars with different letters are significantly different ($p < 0.05$). ASC, FA, SA, *p*-CA, and CA, respectively.

PAs and ASCs. As can be seen, the contents of ferulic acid (FA), sinapic acid (SA), *p*-coumaric acid (*p*-CA), caffeic acid (CA), and ASC were higher in OJ than in GJ, but the differences were not significant (**Figure 1**). Among the PAs, the highest concentration was of FA and the lowest concentration was of CA. The content of ASC was significantly higher than the other PAs ($p < 0.05$).

Antioxidant Potential. To compare the antioxidant potential of the juices with the standards used in the methods, all compounds were of the same concentrations: 0.2, 0.1, and 0.05 mg/mL (*22*). OJs and GJs that were evaluated using the β-carotene−linoleate model system showed 31 and 29% AA, respectively. Similarly, the methanol extract of OJs and GJs using the DPPH radical scavenging activity method showed 37 and 34% AA, respectively. The scavenging activity against NO was about 20 and 19%, and TEAC showed 1.9 and 1.8 μM TE/mL. As can be seen, the free radical scavenging activity of OJ determined by all four assays was higher than of GJ but not significantly.

*Kinetics of the ABTS Scavenging Effect (**Figure 2A**,**B**).* As can be seen, juices (0.2 mg/mL) have shown a high percentage of inhibition, nearly close to BHA with the same concentration and higher than ASC. Naringin (NRG) has shown a very modest activity (**Figure 2A**). Juices of 0.05 mg/mL have shown inhibition values (**Figure 2B**) in the following order: the highest was the BHA curve and then GJ, OJ, and NRG at the same concentration. The two curves of BHA and GJ were close to each other in the end point of 6 min. The linearity of the method was comprised between 50 (**Figure 2A**) and 10% (**Figure 2B**). The obtained data were similar with others (*16*, *23*).

*Kinetics of the DPPH Scavenging Effects (**Figure 3A**,**B**).* It can be seen that OJ (0.2 mg/mL) was very close to BHA (0.2 mg/mL). OJ (0.05 mg/mL) and GJ (0.1 mg/mL) were closed to each other and to BHA (0.1 mg/mL). OJ and GJ (0.05 mg/mL) differ from each other only on 1.1% remaining DPPH (**Figure 3B**). OJs and GJs were nearly in the same remaining DPPH

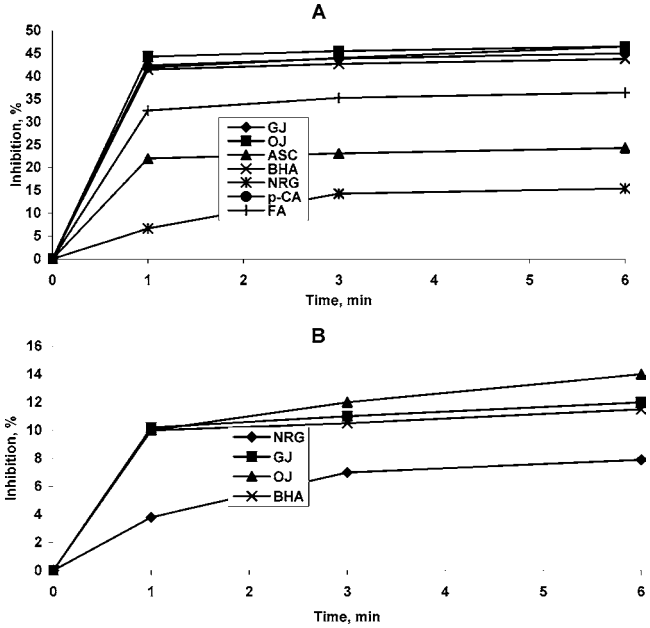

Figure 2. Kinetics of ABTS scavenging effect of (**A**) GJ, OJ, ASC, BHA, NRG, FA, and *p*-CA in a concentration of 0.2 mg/mL. (**B**) GJ, OJ, NRG, and BHA in a concentration of 0. 05 mg/mL.

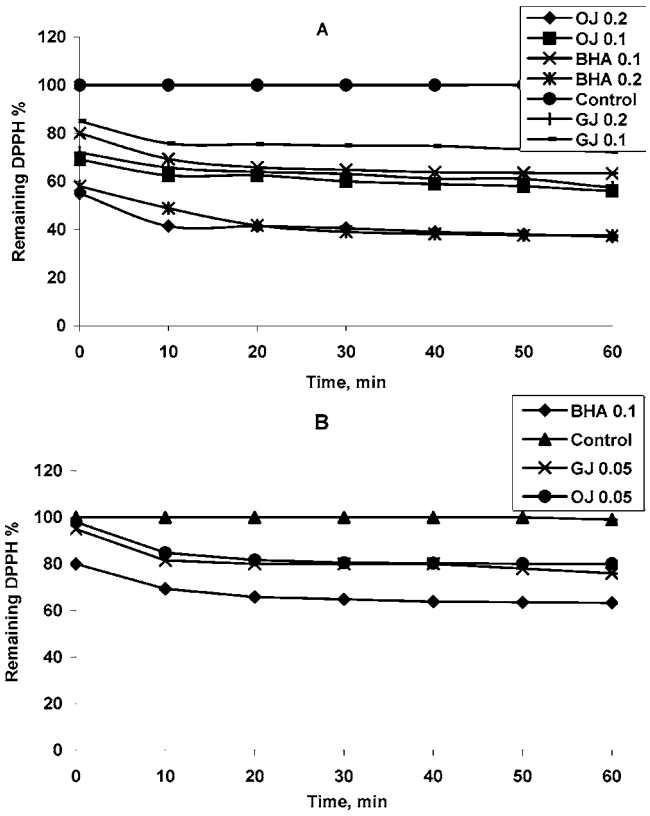

Figure 3. Kinetics of DPPH scavenging effects of (**A**) BHA at concentrations of 0.1 and 0.2 mg/mL; OJ/0.2 (0.2 mg/mL); OJ/0.1 (0.1 mg/mL); GJ (0.2 mg/mL); and GJ/0.1 (0.1 mg/mL). (**B**) OJ/0.05 (0.05 mg/mL); GJ/0.05 (0.05 mg/mL); and BHA at a concentration of 0.1 mg/mL.

percentage (difference of about 3%), and the closest standard to such a concentration was BHA at 0.1 mg/mL (**Figure 3A**,**B**). GJs and OJs of 0.2 and 0.1 mg/mL differ more (**Figure 3A**) than the samples of 0.05 mg/mL (**Figure 3B**). BHA at 0.2 mg/mL is significantly lower than others and showed the highest AA. The methanol extracts of juices have shown a strong AA as a function of their concentration and were comparable to the AA of BHA at the same concentration of 0.2 mg/mL. These results were comparable with others (*15*, *22*) that the AA depends on the concentration of the bioactive substances.

*Correlations of the AA and Some Antioxidant Compounds (**Figure 4A**−**D**).* As can be seen, a high degree of correlation was observed between the NO, the β-carotene, and the DPPH values and polyphenols and flavonoids (R^2 ranges between 0.9535 and 0.9934). The correlation between β-carotene, ABTS, and soluble dietary fiber (SDF) was relatively low (R^2 ranges between 0.4749 and 0.5007).

*Relationship between the Used Scavenging Methods (**Figure 5**).* It is shown that GJ and OJ scavenging effects in β-carotene and DPPH methods were higher than BHT and lower than CA. Oppositely, in the ABTS method, these samples have shown a much higher antioxidant capacity than BHT and CA. Our results are in accordance with others (*23*) showing similar results of juices as well as of standards (ASC, FA, and NRG).

In Vivo. The addition of OJs and GJs or/and cholesterol to the diets did not lead to significant differences in food consumption, body weight gain, and feed efficiencies between diet groups (**Table 3**). At baseline, the six diet groups did not differ from one another in plasma lipid concentrations (data not shown). The results of the changes in plasma lipid concentrations after the experiment are summarized in **Table 4**. As can be seen, the OJ- and GJ-supplemented diets in groups fed cholesterol significantly hindered the rise of plasma lipids. (a) TC: 2.97 vs 3.69 mmol/L, 20%, and 3.01 vs 3.69 mmol/L, respectively; (b) LDL-C: 1.36 vs 2.02 mmol/L, 32.6%, and 1.39 vs 2.02 mmol/L, 31.1%, respectively; and (c) TG: 0.73 vs 0.88 mmol/L, 17%, and 0.75 vs 0.88 mmol/L, 14.8%, respectively. These diets have also significantly decreased the level of TPH (1.34 vs 1.74 mmol/L, 23%, $p < 0.005$, and 1.37 vs 1.74 mmol/L, 21.3%, $p < 0.01$, respectively).

After 4 weeks of the different feedings, the TC liver concentrations in the rats of chol/orange, chol/grapefruit, and chol diet groups were 32.3, 33.1, and 48.7 μmol/g, 5.52, 5.66, and 8.32 times higher than in the control group, respectively. The TC concentrations in the livers of the chol group were 50.7 and 47.1% higher than in chol/orange and chol/grapefruit, respectively ($p < 0.001$ in both cases). Therefore, citrus fruit juice-supplemented diets significantly hindered the rise of TC in the liver. No significant changes in the lipid levels were registered in the groups of rats fed without cholesterol.

At the end of the trial, a significant increase in the plasma AA in the orange and grapefruit dietary groups was found (**Figure 6A**): a significant increase in the TEAC values. A decrease in the plasma AA in the chol/orange, chol/grapefruit, and chol diet groups was registered (**Figure 6B**). However, this decrease in the plasma AA in the chol/orange and chol/grapefruit diet groups was significantly less than in chol diet group (**Figure 6B**). No significant changes were observed in all studied parameters in the rats of the control group.

DISCUSSION

For the last 15 years, our team of biochemists, dieticians, and cardiologists has studied various kinds of nutritional products (*7*, *11*, *12*, *24*−*26*). It was shown in experiments on laboratory animals and in investigations of humans that citrus fruits possess high antioxidant activities (*10*, *27*, *28*). Previously, we investigated whole fruits or their parts (*7*, *24*−*26*). In the last years, most consumers prefer fruit juices (*8*−*10*, *29*). Therefore, we decided to study the contents of bioactive

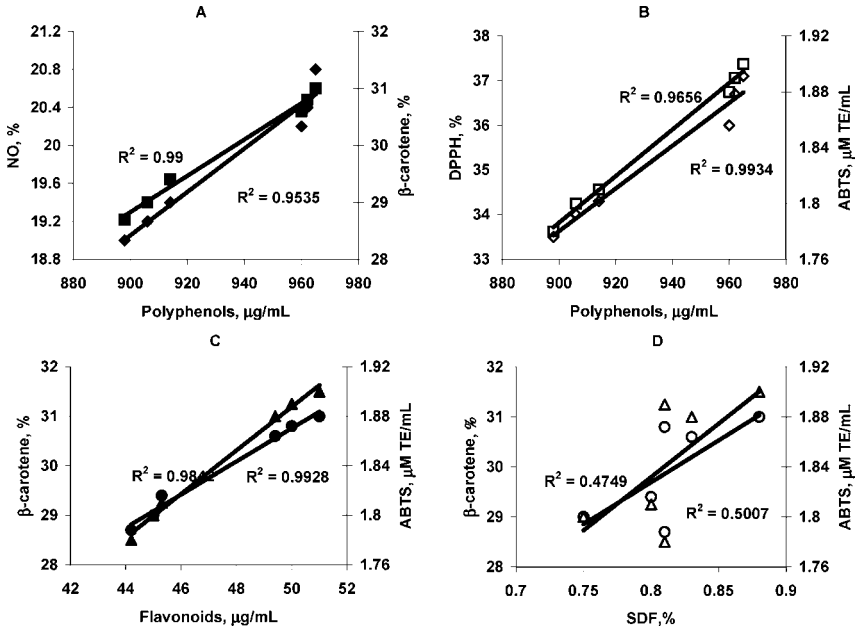

Figure 4. Relationship calculated by a linear regression analysis for OJs and GJs. (**A**) Polyphenols (μg/mL, X) to NO (%, Y_1), ◆; and polyphenols (μg/mL, X) to β-carotene bleaching effect (% inhibition, Y_2), ■. (**B**) Polyphenols (μg/mL, X) to DPPH scavenging effect (% inhibition, Y_1), ◇; and polyphenols (μg/mL; X) to ABTS (μM TE/mL; Y_2), □. (**C**) Flavonoids (μg/mL, X) to β-carotene (% inhibition, Y_1), ●; and flavonoids (μg/mL, X) to ABTS (μM TE/mL, Y_2), ▲. (**D**) SDF (%, X) to β-carotene (% inhibition, Y_1), ○; and SDF (%, X) to ABTS (μM TE/mL, Y_2), △.

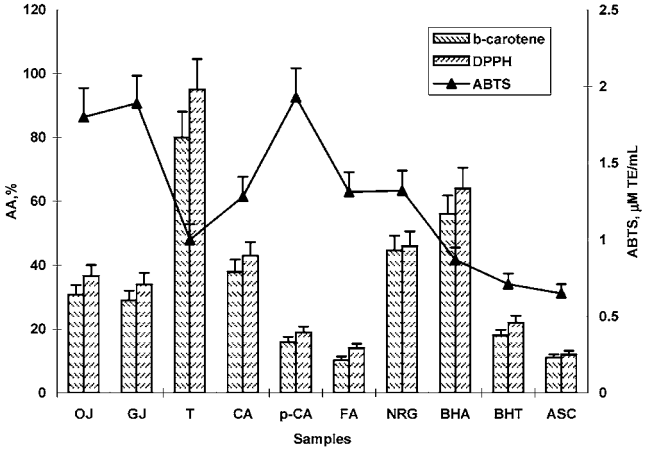

Figure 5. Relationship between three scavenging methods: β-carotene, DPPH, and ABTS radicals. Abbreviations: T, trolox; TE, trolox equivalent.

compounds in fresh OJs and GJs and to assess their influence on plasma lipids and plasma AA in rats fed cholesterol-containing and cholesterol-free diets.

High dietary fiber diets are positively associated with the prevention of some diseases (*1*). The results of this investigation have shown that the content of dietary fiber in both studied juices was relatively high and that the differences were not significant.

Some authors claim that dietary fiber possesses antioxidant properties (*30*, *31*). In our previous investigations in vitro, we found that the antioxidant potential of dietary fiber is not high (*12*). Also, in the present report, we have examined the correlation between dietary fiber and its antioxidant capacity. We have found that this correlation was relatively low (between ABTS and the dietary fiber content and between β-carotene and the dietary fiber content, the correlation coefficients were $R^2 = 0.5007$ and $R^2 = 0.4749$, respectively). Therefore, these results do not support those authors who claim that dietary fiber possesses high antioxidant properties (*30*, *31*).

Table 3. Weight Gains, Food Consumption, and Food Efficiency in All Groups of Rats[a]

groups	weight gain (g/day)	food consumption (g/day)	juice consumption (mL/day)[b]	feed efficiency ratio
control	5.85 ± 0.24 a	18.99 ± 0.62 a		0.31 ± 0.01 a
chol	5.54 ± 0.38 a	18.69 ± 2.00 a		0.30 ± 0.03 a
orange	5.19 ± 0.31 a	17.77 ± 0.77 a	1–2	0.29 ± 0.05 a
chol/orange	5.60 ± 0.38 a	19.37 ± 1.77 a	1–2	0.29 ± 0.02 a
grapefruit	5.58 ± 0.3 a	18.72 ± 1.03 a	1–2	0.30 ± 0.04 a
chol/grapefruit	5.68 ± 0.37 a	18.55 ± 1.80 a	1–2	0.29 ± 0.06 a

[a] Values are means ± SD of five measurements. Means in columns without letters in common differ significantly. [b] To get rats used to the maximal quantity of juice (2 mL), the first 2 weeks, every animal got 1 mL; the third week, every animal got 1.5 mL; and the last week of the trial, every animal got 2 mL of juice per day.

It was found that the contents of total polyphenols, FA, SA, *p*-CA, CA, and ASCs were relatively high in both juices. Also, these results are in accordance with others (*4*, *5*).

There are authors who claim that there is no correlation between the total phenolic content and the radical scavenging capacity (*32*). We have compared the total polyphenol content in the OJs and GJs with their antioxidant potential. The correlations between the polyphenols and the NO, ABTS, and DPPH assays were very high (R^2 ranges between 0.9535 and 0.9934). These results do not support the claims of Yu et al. (*32*) that there is no correlation between the total phenolic content and the radical scavenging capacity. Our data are in accordance with others who have shown that a high total polyphenol content increases AA and that there is a linear correlation between phenolic content and AA (*6*, *15*, *18*, *23*, *33*).

The PAs in the studied citrus fruits were in the following order: FA > SA > *p*-CA > CA. The AA of PAs is generally governed by their chemical structures. This activity increases with the number of hydroxyl groups. Therefore, our results are

Table 4. Plasma Lipids (mmol/L) and TC in Liver (μmol/g) of Rats Fed Diets with and without 1% Chol and with and without Juices[a–c]

diets	TC	LDL-C	HDL-C	TG	TPH	liver TC
control	2.85 ± 0.14 c	1.21 ± 0.09 c	1.64 ± 0.11 a	0.70 ± 0.06 b	1.77 ± 0.11 a	5.85 ± 0.21 c
chol	3.69 ± 0.19 a	2.02 ± 0.12 a	1.66 ± 0.11 a	0.88 ± 0.07 a	1.74 ± 0.11 a	48.7 ± 0.62 a
orange	2.81 ± 0.14 c	1.19 ± 0.09 c	1.62 ± 0.11 a	0.69 ± 0.06 b	1.74 ± 0.11 a	5.77 ± 0.21 c
chol/orange	2.97 ± 0.15 b	1.36 ± 0.10 b	1.61 ± 0.11 a	0.73 ± 0.06 b	1.34 ± 0.10 b	32.3 ± 0.59 b
grapefruit	2.83 ± 0.14 c	1.19 ± 0.09 c	1.62 ± 0.11 a	0.69 ± 0.06 b	1.75 ± 0.11 a	5.81 ± 0.21 c
chol/grapfruit	3.01 ± 0.15 b	1.39 ± 0.10 b	1.61 ± 0.11 a	0.75 ± 0.06 b	1.37 ± 0.10 b	33.1 ± 0.59 b
			two way ANOVA			
						p value
orange	NS	NS	NS	NS	NS	NS
grapefruit	NS	NS	NS	NS	NS	NS
chol	<0.001	<0.001	NS	<0.001	NS	<0.001
orange + chol	<0.001	<0.001	NS	<0.001	<0.005	<0.025
grapefruit + chol	<0.050	<0.050	NS	<0.050	<0.010	<0.050

[a] Values are means ± SD, $n = 10$. [b] Means in columns without letters in common differ significantly ($p < 0.05$). [c] Abbreviations used: NS, not significant ($p \geq 0.05$).

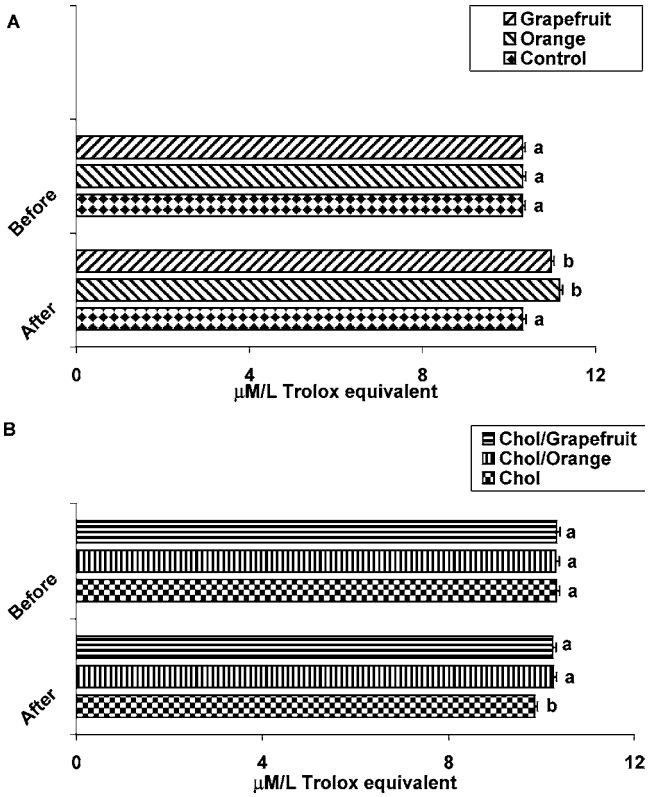

Figure 6. (**A**) Significant increase in the plasma AA in rats of the orange and grapefruit diet groups: an increase in the TEAC values. Means ± SD (horizontal lines). Bars with different letters are significantly different ($p < 0.05$). (**B**) Decrease in the plasma AA in rats fed added cholesterol (chol): decrease in the TEAC values. However, the decrease in the plasma AA is significantly less in the groups of rats fed added citrus juices. Means ± SD (horizontal lines). Bars with different letters are significantly different ($p < 0.05$).

of particular interest regarding the amount of FA found in OJs and GJs and are in agreement with others (*2*, *4*, *5*, *34*).

The contents of dietary fibers, total polyphenols, PAs and ASCs, and the total antioxidant potential in the OJs and GJs were comparable with these indices in a previously studied relatively new citrus fruit named sweetie (*7*, *11*).

We have found that OJs and GJs in rats fed a BD without cholesterol did not affect the lipid levels. Also, others have demonstrated that the hypolipidemic effect of fruits and vegetables is evident only when they are added to diets of rats fed cholesterol (*35*, *36*).

A significant increase in the plasma AA was found in the orange and grapefruit dietary groups. However, in groups fed added cholesterol, a decrease in the plasma AA was registered. It must be underlined that the decrease in groups whose diets were enriched with citrus juices (chol/orange and chol/grapefruit) was significantly less than in the chol group. Such results were expected; our previous investigations (*11*, *24*) and investigations of other authors (*37*, *38*) have shown that a cholesterol-supplemented diet decreases the blood AA, and this investigation demonstrates that the addition of citrus juices hinders this decrease.

In conclusion, we were able to show that (i) there are no significant differences in the content of dietary fiber, total polyphenols, PAs and ASC, anthocyanins, and flavonoids in the studied citrus juices. (ii) The antioxidant potential of OJs is higher than that of GJs. However, the differences are not significant. (iii) Diets supplemented with OJs and GJs exercise a hypocholesterolemic effect and increase the plasma AA. (iv) The above-mentioned properties of fresh OJs and GJs could make them a valuable supplement to disease-preventing diets.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; MDA, malondialdehyde assay; NOC, nonoxidized cholesterol; TC, total cholesterol; TEAC, trolox equivalent antioxidant coefficient; TG, triglycerides; DPPH, 1,1-diphenyl-2-picrylhydrazyl; TPH, total phospholipids; TRAP, total radical-trapping antioxidative potential.

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