Bioactivities of Fruits and Vegetables for Human Health Improvement

(건강기능 개선을 위한 과채류의 생리활성)

Use of biomarkers (conventional and novel) in nutritional, agricultural, horticultural, ecological and clinical chemistry

Prof., Shela Gorinstein
Dept. of Medicinal Chemistry and Natural Products,
School of Pharmacy, The Hebrew University of Jerusalem,
P.O.B.12065, Jerusalem 91120, Israel,
Fax:972-2-6757076, E-mail: gorin@cc.huji.ac.il

Bioactivities of Fruits and Vegetables for Human Health Improvement

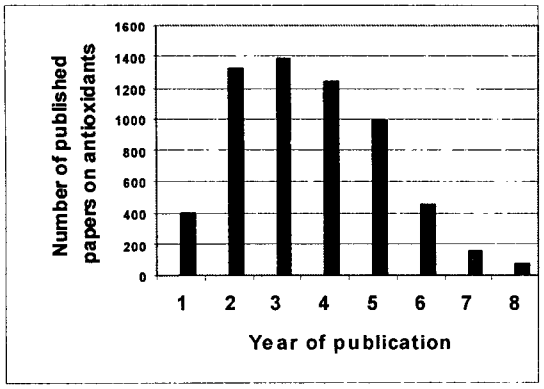
Prof., Shela Gorinstein
The Hebrew University of Jerusalem, Israel

On the example of our joint publication (Gorinstein et al., Journal of Agricultural and Food Chem, 2004, 52, 4853-4859) we will discuss the bioactivities of fruits and vegetables for human health improvement. The use of fruit and vegetable diets for prevention of diseases, screening of the fruits and vegetables for biomarkers such as total and individual polyphenols, antioxidants and antioxidant activity of a number of different edible plants will be screened.

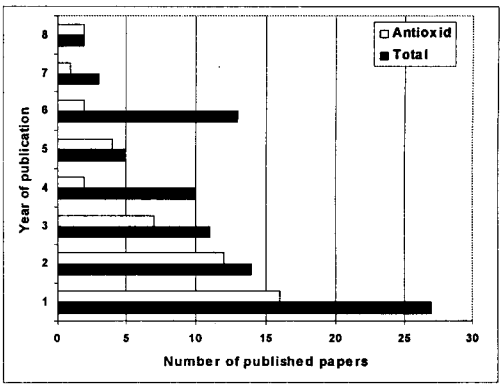
In this lecture will be discussed as well research on different fruits: traditional (apples, peaches, pears), citrus (lemon, sweeties, grapefruits, oranges), subtropical and tropical (kiwifruit, persimmon, etc), vegetables [pseudocereals (amaranth and quinoa) olives, garlic].

The studies were done *in vivo* and *in vitro.*

In order to see the importance of the subject on antioxidants we are presenting two histogramsof the science results analysis of generally published papers (A) in the world and our publications (B):



A
1=2005; 2=2004; 3= 2003; 4=2002;
5=2000; 6=1995; 7= 1985; 8=197



B
1=2003; 2=2004; 3=2002; 4= 2001;
5=2000; 6=1999; 7=1997; 8=1975

All the results of this lecture are based only on our very recent joint publications.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

2004, 52, 4853-4859

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

SHELA GORINSTEIN*, HANNA LEONTOWICZ, MARIA LEONTOWICZ, RYSZARD KRZEMINSKI, MIKOLAJ GRALAK, OLGA MARTIN-BELLOSO, EFREN DELGADO-LICON, 2 RATIPORN HARUENKIT, ELENA KATRICH, YONG-SEO PARK, SOON-TECK JUNG, AND SIMON TRAKHTENBERGr

ABSTRACT

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 g/mL, respectively. The antioxidant potential measured by the scavenging activity against nitric oxide, the ″-carotene-linoleate model system -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 metabolismonly 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.

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 "-carotene-linoleate model system (-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), -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 -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% NaNO2, 10% AlCl3x6H2O, and 1 M NaOH) was measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as g 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 using $A = [(A510 - A700)\ pH1.0 - (A510 - A700)pH4.5]$ with a molar extinction coefficient of 29 600 for cyanidin-3-glucoside. The results were expressed as g 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 where $A0$ and $A0$ are the absorbance values measured at zero time and At and At 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 L 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) X 100 (*15*).

ABTS Radical Cation Decolorization Assay. *The Trolox equivalent antioxidant coefficient (TEAC) value is based on the ability of the antioxidants to scavenge the blue-green 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt* (ABTS+) radical cation relative to the ABTS+ scavenging ability of the water-soluble vitamin E analogue Trolox. The ABTS+ radical cation was generated by the interaction of ABTS (250 M) and K2S2O8 (40 M) after the addition of 990 L of ABTS+ solution to 10 L of different extracts (0.2 mg/mL) or Trolox standards (final concentration) 0-20 M) in methanol or phosphate-buffered saline (PBS). 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 versus 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 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.

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, Leszczynskiego 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 M 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.

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.

Total Polyphenols, Anthocyanins, and Flavonoids. The results of the main antioxidant compounds are summarized in **Table 2**.

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* g of gallic acid equivalent per mL of fresh juice. *c* g of cyanidin-3-glucoside equivalent per mL of fresh juice. *d* g of catechin equivalents per mL of fresh juice.

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*).

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 "-carotene-linoleate model system showed 31 and 29% AA, respectively. Similarly, the methanol extract of OJs and GJsusing 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 MTE/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.

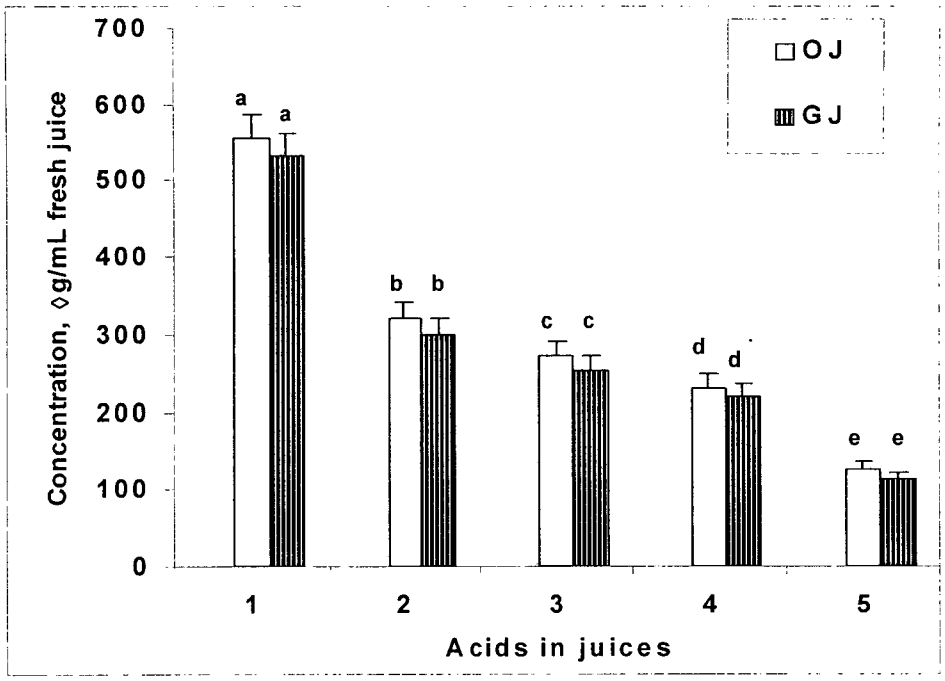


Figure 1. Comparative contents of PAs and ASCs in OJs and GJs. Means±SD (vertical lines). Bars with different letters are significantly different ($p < 0.05$). ASC, FA, SA, *p*-CA, and CA, respectively.

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 showninhibition 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*).

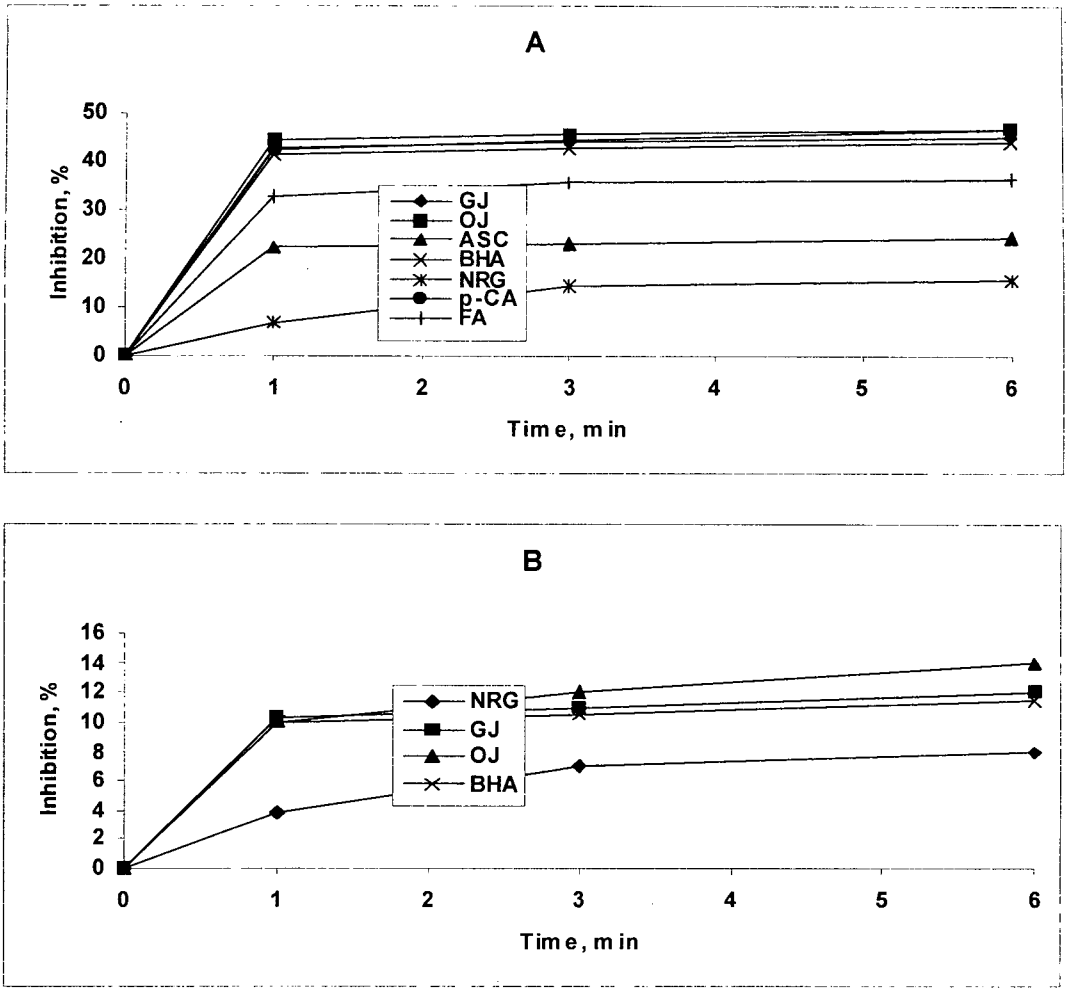


Figure 2. Kinetics of ABTS scavenging effect of (A) GJ, OJ, ASC, BHA, NRG, FA, and *p*-CA in a concentration of 0.2 mg/mL. (B) GJ, OJ, NRG, and BHA in a concentration of 0. 05 mg/mL.

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 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.

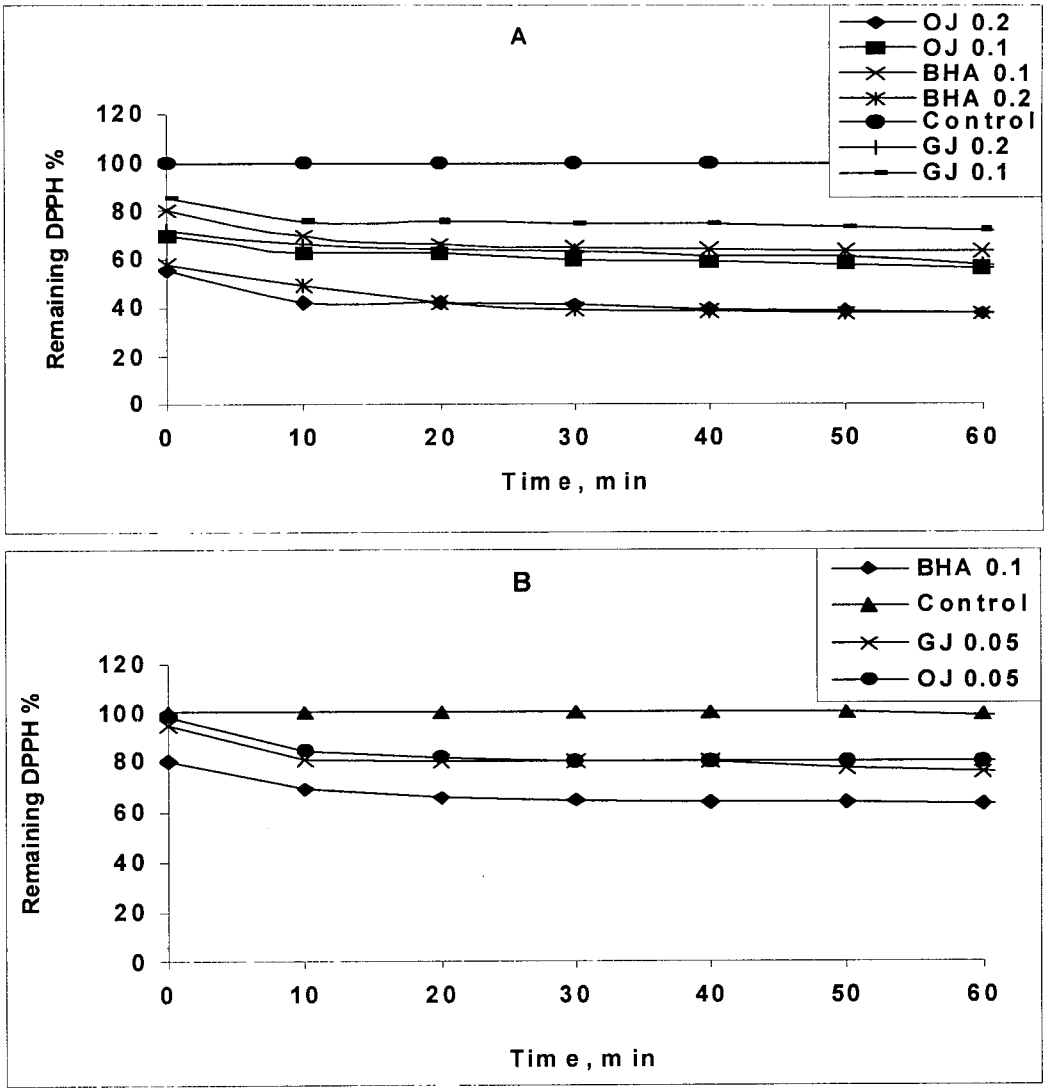


Figure 3. Kinetics of DPPH scavenging effects of (A) BHA at concentrations of 0.1 and 0.2 mg/mL; OJ/0.2 (0.2 mg/mL); OJ/0.1 (0.1 mg/mL); GJ (0.2 mg/mL); and GJ/0.1 (0.1 mg/mL). (B) OJ/0.05 (0.05 mg/mL); GJ/0.05 (0.05 mg/mL); and BHA at a concentration of 0.1 mg/mL.

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 -carotene, and the DPPH values and polyphenols and flavonoids (*R*2 ranges between0.9535 and 0.9934). The correlation between "carotene, ABTS, and soluble dietary fiber (SDF) was relatively low (*R*2ranges between 0.4749 and 0.5007).

Relationship between the Used Scavenging Methods (Figure5). It is shown that GJ and OJ scavenging effects in -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).

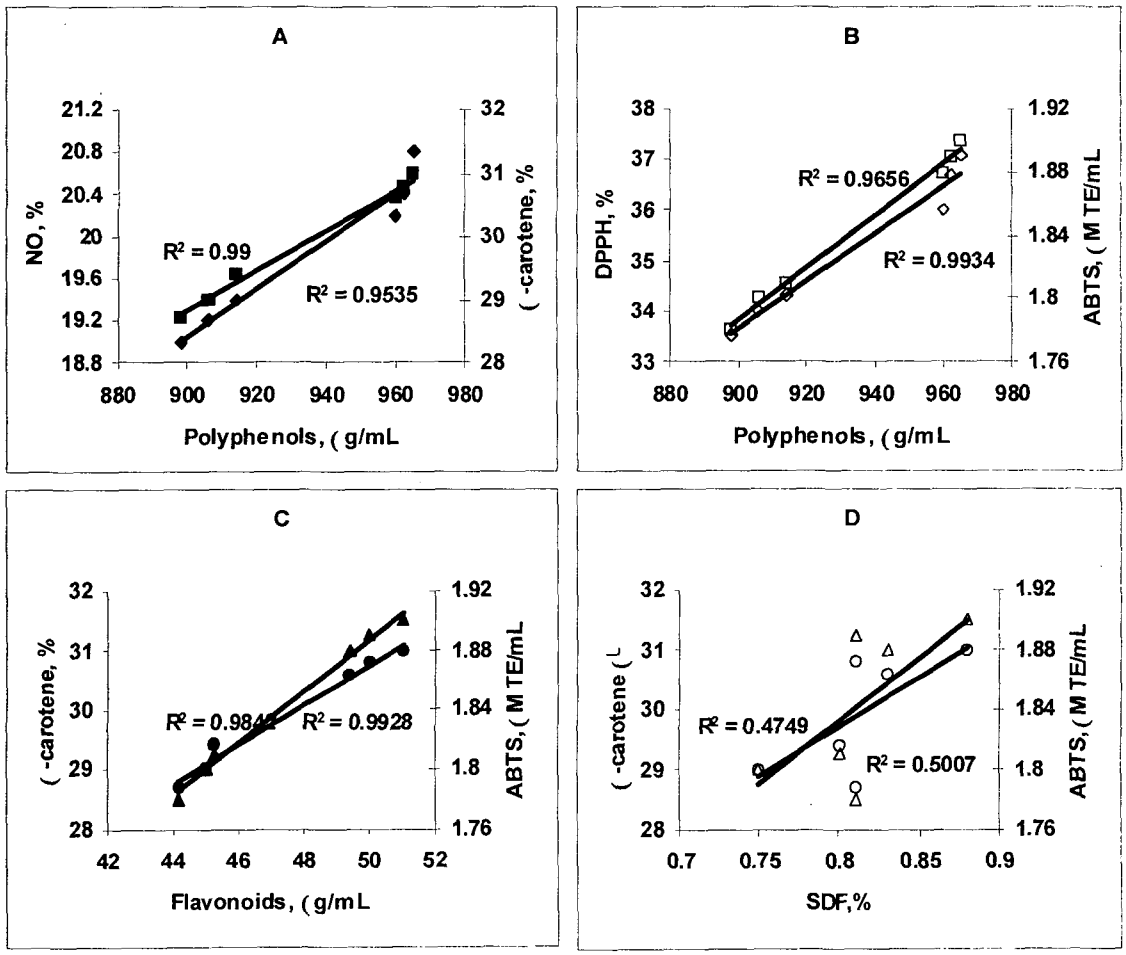


Figure 4. Relationship, calculated by a linear regression analysis for orange and grapefruit juices.

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 -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). Abbreviations: SDF, soluble dietary fiber.

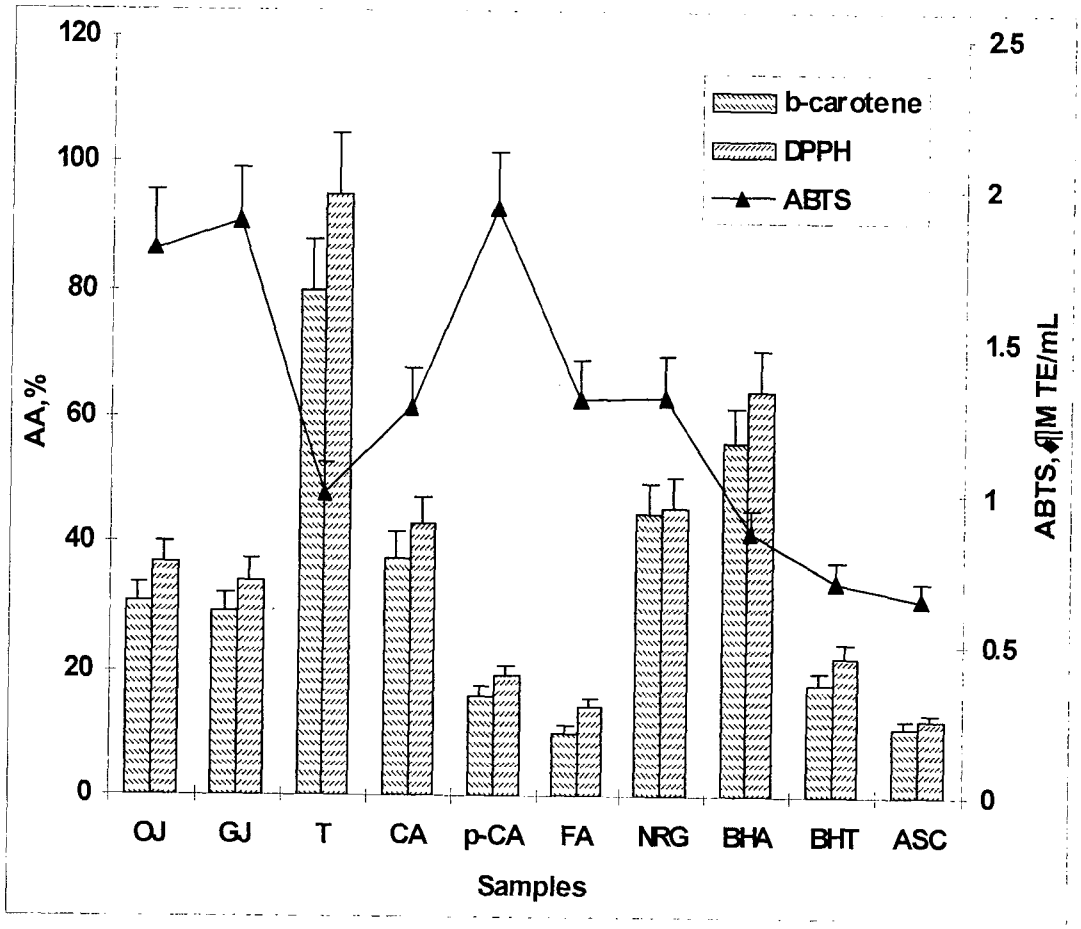


Figure 5. Relationship between three scavenging methods: -carotene, DPPH, and ABTS radicals. Abbreviations: T, trolox; TE, trolox equivalent.

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**).

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.

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**.

Table 4. Plasma Lipids (mmol/L) and TC in Liver (mol/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 (0.05).

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 mol/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.

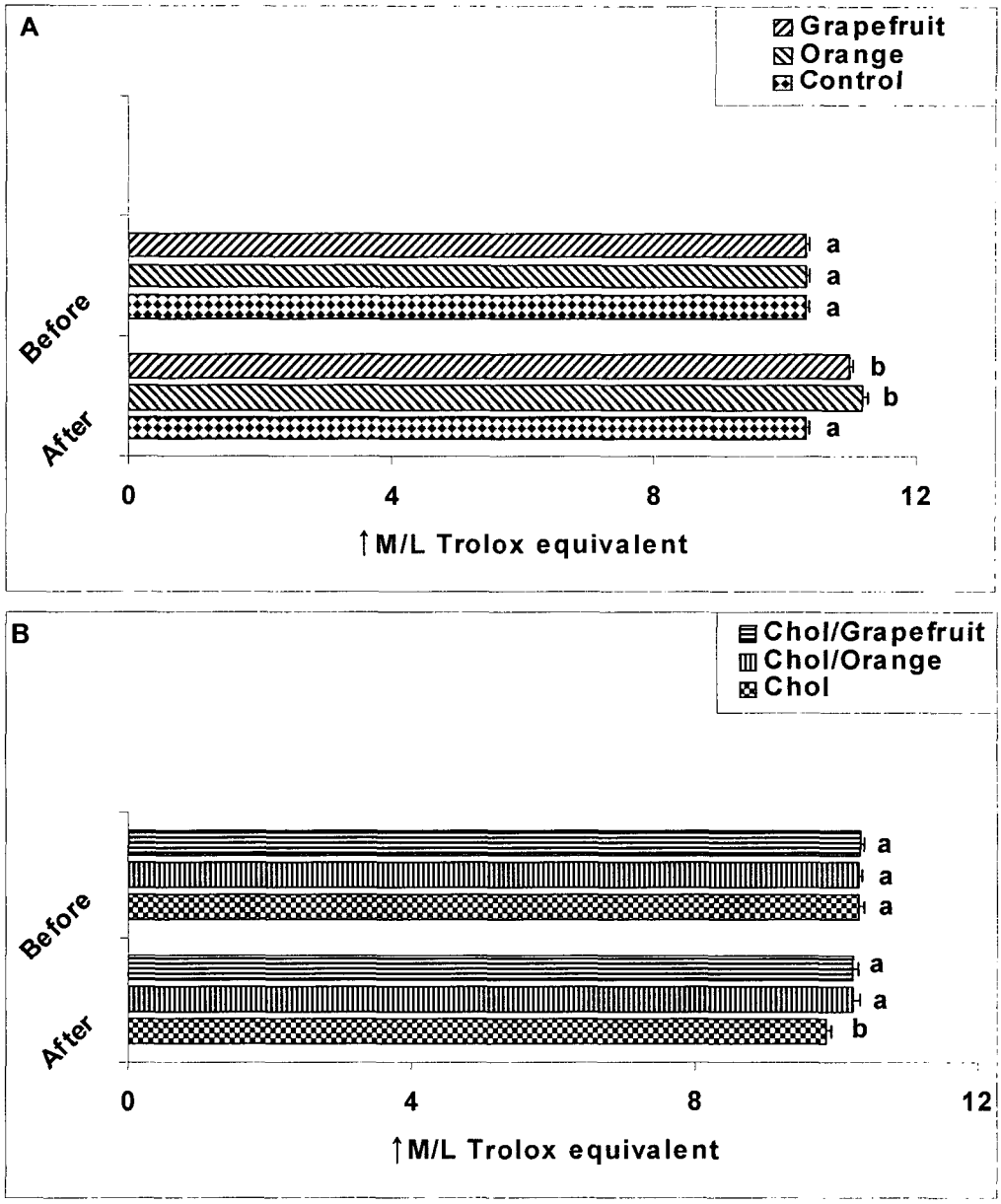


Figure 6. (A) Significant increase in the plasma AA in rats of the orange and grapefruit diet groups: an increase in the TEAC values. Means±SD (horizontal lines). Bars with different letters are significantly different ($p < 0.05$). (B) Decrease in the plasma AA in rats fed added cholesterol(chol): decrease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

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 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 -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*). 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 inthe 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 areof 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.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation; HDL-C, high-density lipoprotein-cholesterol; LDLC, 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.

Conclusion

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. Such conclusion is appropriate for many edible plants used as a supplement for diets.

LITERATURE CITED

(1) Rimm, E. B.; Ascherio, A.; Giovannucci, E.; Spiegalman, D.; Stampfer, M.; Willett, W. Vegetable, fruits, and cereal fiber intake and risk of coronary heart disease among men. *J. Am. Med. Assoc.* **1996**, *275*, 447-451.

(2) Bocco, A.; Cuvelier, M. E.; Richard, H.; Berset, C. Antioxidant activity and phenolic composition of citrus peel and seed extracts. *J. Agric. Food Chem.* **1998**, *46*, 2123-2129.

(3) Paganga, G.; Miller, N.; Rice-Evans, C. A. The polyphenolic content of fruits and vegetables and their antioxidant activities. What does a serving constitute? *Free Radical Res.* **1999**, *30*, 153-162.

(4) Stohr, H.; Hermann, K. U ber das Vorkommen von Verbindungen der Hydroxyzimtsauren, Hydroxybenzoesauren und Hydroxycumarine in Citrusfruchten. *Z. Lebensm. Unters. Forsch.* **1975**, *159*, 305-306.

(5) Peleg, H.; Naim, M.; Rouseff, R. L.; Zehavi, U. Distribution of bound and free phenolic acid in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). *J. Sci. Food Agric.* **1991**, *57*, 417-426.

(6) Rapisarda, P.; Tomaino, A.; Lo Cascio, R.; Bonina, F.; De Pasquale, A.; Saija, A. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J. Agric. Food Chem.* **1999**, *47*, 4718-4723.

(7) Gorinstein, S.; Cvikrova, M.; Machackova, I.; Haruenkit, R.; Park, Y.-S.; Soon-Teck Jung, S.-T.; Yamamoto, K.; Martinez Ayala, A. L.; Katrich, E.; Trakhtenberg, S. Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food Chem.* **2004**, *84*, 503-510.

(8) Vinson, J. A.; Liang, X. Q.; Proch, J.; Hontz, B. A.; Dancel, J.; Sandone, N. Polyphenol antioxidants in citrus juices: In vitro and in vivo studies relevant to heart disease. Flavonoids in cell function. *AdV. Exp. Med. Biol.* **2002**, *505*, 113-122.

(9) Pearson, D. A.; Tan, C. H.; German, J. B.; Davis, P. A.; Gershwin, M. E. Apple juice inhibits low-density lipoprotein oxidation. *Life Sci.* **1999**, *64*, 1913-1920.

(10) Kurowska, E. M.; Borradaile, N. M.; Spence, J. D.; Carroll, K. K. Hypocholesterolemic effect of citrus juices in rabbits. *Nutr. Res.* **2000**, *20*, 121-129.

(11) Gorinstein, S.; Yamamoto, K.; Katrich, E.; Leontowicz, H.; Lojek, A.; Leontowicz, M.; Ciz, M.; Goshev, I.; Shalev, U.; Trakhtenberg, S. Antioxidative properties of Jaffa Sweeties and Grapefruits and their influence on lipid metabolism and plasma

antioxidative potential in rats. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 907-910.

(12) Gorinstein, S.; Martin-Belloso, O.; Park, Y. S.; Haruenkit, R.; Lojek, A.; Ciz, M.; Caspi,A.; Libman, I.; Trakhtenberg, S. Comparison of some biochemical characteristics of different citrus fruits. *Food Chem.* **2001**, *74*, 309-315.

(13) Marcocci, L.; Packer, L.; Droy-Lefaix, M. T.; Sekaki, A.; Gardes-Albert, M. Antioxidant action of *Ginkgo biloba* extract EGb 761. *Methods Enzymol.* **1994**, *234*, 462-475.

(14) Saija, A.; Tomaino, A.; Lo Cascio, R.; Trombetta, D.; Proteggente, A.; De Pasquale, A.; Uccella, N.; Bonina, F. P. Ferulic and caffeic acids as potential protective agents against photooxidative skin damage. *J. Sci. Food Agric.* **1999**, *79*, 476-480.

(15) Singh, R. P.; Chidamdara, M.; Jayaprakasha, G. K. Studies on the antioxidant activity of pomegranate (*Punica granatu*m) peel and seed extracts using in vitro models. *J. Agric. Food Chem.* **2002**, *50*, 81-86.

(16) 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.

(17) Manas, E.; Bravo, L.; Saura-Calixto, F. Sources of error in dietary fibre analysis. *Food Chem.* **1994**, *50*, 331-342.

(18) Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: fruits. *J. Agric. Food Chem.* **2001**, *49*, 5315-5321.

(19) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152-158.

(20) Cheng, G. W.; Breen, P. J. Activity of phenylalanine ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *J. Am. Soc. Hortic. Sci.* **1991**, *116*, 865-869.

(21) Krzeminski, R.; Gorinstein, S.; Leontowicz, H.; Leontowicz, M.; Gralak, M.; Czerwinski, J.; Lojek, A.; C 埙z, M.; Martin-Belloso, O.; Trakhtenberg, S. Effect of different olive oils on bile excretion in rats fed cholesterol-containing and

cholesterol-free diets. *J. Agric. Food Chem.* **2003**, *51*, 5774-5779.

(22) Song, T.-Y.; Yen, G.-C. Antioxidant properties of *Antrodia camphorata* in submerged culture. *J. Agric. Food Chem.* **2002**, *50*, 3322-3327.

(23) Pellegrini, N.; Del Rio, D.; Colombi, B.; Bianchi, M.; Brighenti, F. Application of the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages. *J. Agric. Food Chem.* **2003**, *51*, 260-264.

(24) Gorinstein, S.; Bartnikowska, E.; Kulasek, G.; Zemser, M.; Trakhtenberg, S. Dietary persimmon improves lipid metabolism in rats fed diets containing cholesterol. *J. Nutr.* **1998**, *128*, 2023-2027.

(25) Gorinstein, S.; Zachwieja, Z.; Folta, M.; Barton, H.; Piotrowicz, J.; Zemser, M.; Weisz, M.; Trakhtenberg, S.; Martin-Belloso, O. Comparative content of dietary fiber, total phenolics and minerals in persimmon and apples. *J. Agric. Food Chem.* **2001**, *49*, 952-957.

(26) Gorinstein, S.; Martin-Belloso, O.; Lojek, A.; Ciz, M.; Soliva-Fortuny, R.; Park, Y. S.; Caspi, A.; Libman, I.; Trakhtenberg, S. Comparative content of some phytochemicals in Spanish apples, peaches and pears. *J. Sci. Food Agric.* **2002**, *86*, 1166-1170.

(27) Kurowska, E. M.; Spence, J. D.; Jordan, J.; Wetmore, S.; Freeman, D. J.; Piche, L. A.; Serratore, P. HDL-Cholesterolraising effect of orange juice in subjects with hypercholesterolemia. *Am. J. Clin. Nutr.* **2000**, *72*, 1095-1100.

(28) Bonina, F. P.; Leotta, C.; Scalia, G.; Puglia, C.; Trombetta, D.; Tringali, G.; Roccazzello, A. M.; Rapisarda, P.; Saija, A. Evaluation of oxidative stress in diabeticpatients after supplementation with a standardised red orange extract. *Diabetes, Nutr. Metab.* **2002**, *15*, 14-19..

(29) Aviram, M.; Dornfeld, L.; Rosenblat, M.; Volkova, N.; Kaplan, M.; Colemann, R.; Hayek, T.; Presser, D.; Fuhrman, B. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein

E-deficient mice. *Am. J. Clin. Nutr.* **2000**, *71*, 1062-1076.

(30) Lin, R. Nutritional antioxidants. In *Phytochemical and Antioxidant Functional Food* Goldberg, I., Ed.; Chapman and Hall: New York, London, 1994; pp 393-455.

(31) Larrauri, J. A.; Goni, I.; Martin-Carron, N.; Ruperez, P.; Saura-Calixto, F. Measurement of health-promoting properties in fruit dietary fibers: antioxidant capacity, fermentability and glucose retardation index. *J. Sci. Food Agric.* **1996**, *71*, 515-519.

(32) Yu, L.; Haley, S.; Perret, J.; Harris, M.; Wilson, J.; Qian, M. Free radical scavenging properties of wheat extracts. *J. Agric. Food Chem.* **2002**, *50*, 1619-1624.

(33) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113-4117.

(34) Nardini, M.; d'Aquina, M.; Tomassi, G.; Gentilli, V.; di Felice, M.; Scaccini, C. Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radical Biol. Med.* **1995**, *19*, 541-552.

(35) Kiryama, S.; Okazaki, J.; Joshida, A. Hypocholesterolemic effect of polysaccharides and polysaccharide-rich foodstuffs in cholesterol fed rats. *J. Nutr.* **1969**, *97*, 382-388.

(36) Anderson, J. W.; Jones, A. E.; Riddell-Mason, S. Ten different dietary fibers have significantly different effects on serum and liver lipids of cholesterol fed rats. *J. Nutr.* **1994**, *124*, 78-83.

(37) Tsai, A. C.; Chen, N. S. C. Effect of cholesterol feeding on tissue glucose uptake, insulin degradation, serum lipids and serum lipoperoxides levels in rabbits. *J. Nutr.* **1979**, *109*, 606-612.

(38) Uysal, M. Effect of cholesterol feeding on plasma lipids, lipid peroxides levels and lecithin-cholesterol acyl transferase activity in rabbits. *Biochem. Arch.* **1986**, *2*, 287-292.

The following publications have been used for discussion. The content of these data have been presented in attached slides.

TRADITIONAL, TROPICAL AND SUBTROPICAL FRUITS

Journal of the Science of Food and Agriculture *J Sci Food Agric* 82:1166–1170 (online: 2002)
DOI: 10.1002/jsfa.1178

Comparative content of some phytochemicals in Spanish apples, peaches and pears

Shela Gorinstein,[1]* Olga Martin-Belloso,[2] Antonin Lojek,[3] Milan Číž,[3] Robert Soliva-Fortuny,[2] Yong-Seo Park,[4] Abraham Caspi,[5] Imanual Libman[5] and Simon Trakhtenberg[5]

Abstract: Dietary fibre, total polyphenols and phenolic acids in Spanish apples, peaches and pears were analysed and compared with their total radical-trapping antioxidative potential (TRAP). There were no significant differences in the content of dietary fibre among the studied fruits. The content of total polyphenols was 2.40.4, 2.10.3 and 6.90.7g /kg in peeled fruits and 4.70.4, 4.50.4 and 11.11.2g/kg in their peels for peaches, pears and apples respectively. The contents of dietary fibre, total polyphenols, caffeic, p-coumaric and ferulic acids and the TRAP values were significantly ($P < 0.05$) higher in peels than in peeled fruits. The contents of all studied compounds and the TRAP values were significantly higher in peeled apples and their peel than in peaches and pears. We observed strong correlation between the contents of total polyphenols and phenolic acids and the total radical trapping antioxidative potential in all three fruits. The relatively high content of dietary fibre, the highest contents of total polyphenols, caffeic, p-coumaric and ferulic acids and the highest value of TRAP make apples preferable among the studied fruits for dietary prevention of atherosclerosis and other diseases.

Changes in Ethylene Treated Kiwifruit *(Actinidia deliciosa)* During First 10 Days of Ripening

Yong- Seo Park, Soon- Teck Jung, Seong- Gook Kang, Jerzy Drzewiecki, Ratiporn Haruenkit, Elena Katrich, Simon Trakhtenberg, and Shela orinstein
Submitted for publication in 2005

Evaluation of changes in protein and bioactive compounds of ethylene treated kiwifruit during the first ten days of ripening was the aim of the present report. Flesh firmness, sensory value, visual score, free sugars, soluble solids, ethylene biosynthesis, proteins, dietary fibers, total polyphenols and antioxidant potential were studied. Kiwifruit samples were randomly divided into two groups, which were named Treated and Untreated. Ethylene of ?g/ ml at 20 o C for 24 hours was used in Treated group. The ripening process at the same temperature was observed for 10 additional days.

Proteins were extracted from kiwifruit and separated by modified sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The separation was resolved into 14 protein bands. Some minor quality changes were found only in 32 kDa band, which was more pronounced in the treated samples. The flesh firmness and acidity were significantly decreased in the early stage of ripening. The contents of free sugars and soluble solids, the endogenous ethylene production, sensory value, polyphenols and related antioxidant potentials were increased in treated samples significantly higher than in untreated samples ($P<0.05$). The ethylene biosynthesis was increased simultaneously with the increase in 1-aminocyclopropane-1-carboxylic acid (ACC) content, ACC synthase and ACC oxidase activities and was significantly higher than in untreated samples ($P<0.05$). In conclusion, the ethylene treatment of the kiwifruit positively influences most of the studied compounds and increases fruit antioxidant potential. It shortens the ripening time and improves fruit quality by decreasing its flesh firmness and acidity and leads to some minor changes in the protein profile.

KEYWORDS: Ethylene treated and untreated kiwifruits; bioactive compounds; proteins; antioxidant potential

Drying of persimmons (*Diospyros kaki L.*) and the following changes in the studied bioactive compounds and the total radical scavenging activities. Lebensm.-Wiss. u.-Technol., 2005

Yong- Seo Park, Soon-Teck Jung, Seong-Gook Kang, Efren Delgado-Licon, Alma Leticia Martinez Ayala, Maria S. Tapia, Olga Mart?-Belloso, Simon Trakhtenberg, and Shela Gorinstein

Fresh persimmons were subjected to two different processes: sun- drying during one month and dehydration at 60℃ during 12 hours. To assess the effect of this process on nutritional and health-related properties of persimmons dietary fibers, mineral, trace elements, polyphenols and the total radical scavenging activities (TRSA) were determined before and after processing. It was found that the contents of dietary fibers, minerals and trace elements in fresh and dried persimmons fruits were comparable. Total polyphenols in fresh persimmons was higher than in dried fruits (1.3 vs. 0.9 and 0.8 mg/100g FW, respectively) and percentage of inhibition was higher than in dried fruits (70 vs. 59 and; 55 and 58 vs. 53 and 46% for 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) [ABTS] radicals, respectively ($P>0.05$ in all cases). In conclusion: 1) the differences in the contents of dietary fibers, minerals and trace elements in fresh and dried persimmons are not significant; 2) the contents of polyphenols and the level of the total radical scavenging activity are higher in fresh persimmons than in dried fruits; however both variables are also high in dried persimmons 3) when fresh fruits are not available, proper dried persimmons could be used as a valuable substitute.

Keywords: Fresh; dry; persimmon; bioactive compounds; antioxidant activity

Some essential phytochemicals and the antioxidant potential in fresh and dried persimmon

International Journal of Food Sciences and Nutrition, 2005

Soon-Teck Jung, Yong-Seo Park, Zofia Zachwieja, Maria Folta, Henryk Barton, Jadwiga Piotrowicz, Elena Katrich, Simon Trakhtenberg, and Shela Gorinstein

Fresh persimmon contains high quantities of bioactive compounds but is only available in the autumn and winter months. The aim of this investigation was to compare the fresh and dried persimmon in order to find out if the later could be a substitute for fresh fruit. It was found that the contents of dietary fibers and trace elements in fresh and equivalent quantities dried fruits were comparable. The content of total polyphenols in fresh persimmon was higher than in dried fruit, but not significantly ($P > 0.05$). Also the antioxidant potential in fresh persimmon as determined by all three used tests was higher than in dried fruit but not significantly ($P > 0.05$). The methanol extracts of fresh and dried persimmon using the -carotene-linoleate model system have shown 91 and 88% of antioxidant activity at 50 µL, respectively. Radical scavenging activity with 1,1-diphenyl-2-picrylhydrazyl method (DPPH) has shown 88 and 84% for the same extracts and NO test showed similar results. The best correlation was found between polyphenols, β-carotene, DPPH and NO values (R^2 ranges between 0.9535-0.9934). In conclusion, both fresh and dried persimmons possess high contents of bioactive compounds and have a high antioxidant potential and when fresh fruits are not available, proper dried persimmon can be successfully used.

Keywords: fresh and dried persimmon; antioxidant compounds; antioxidant potential

CITRUS FRUITS

Food Chemistry 74 (2001) 309315

Comparison of some biochemical characteristics of different citrus fruits

Shela Gorinstein, Olga Martin Belloso, **Yong-Seo Park, Ratiporn Haruenkit,** Milan Ciz, **Abraham Caspi, Imanuel Libman, Simon Trakhtenberg**

Abstract

The goal of this investigation was to evaluate the antioxidant properties of some citrus fruits. The contents of dietary fibre, total polyphenols, essential phenolics, ascorbic acid and some trace elements of lemons, oranges and grapefruits were determined and compared with their total radical-trapping antioxidative potential (TRAP). There were no significant differences in the contents of total, soluble and insoluble dietary fibre in the studied peeled fruits or their peels. The contents of total, soluble and insoluble dietary fibre in peels were significantly higher than in peeled fruits ($P < 0.05$ in all cases). The peeled lemons, oranges and grapefruits contain 164±10.3; 154±10.2 and 135±10.1 and their peels 190±10.6; 179±10.5 and 155±10.3 mg/100 g of total polyphenols, respectively. The content of total polyphenols in peeled lemons and their peels was significantly higher than in peeled oranges and grapefruits and their peels, respectively. The content of total polyphenols in the peels was significantly higher than in peeled fruits ($P<0.05$ in all cases). The same results were obtained in the investigation of essential phenolics and ascorbic acid. The content of Fe in peeled lemons and their peels was significantly higher than in peeled oranges and grapefruits and their peels, respectively. Also the TRAP was significantly higher in peeled lemons and their peels than in peeled oranges and grapefruits and their peels, respectively. In all three fruits, the TRAP was significantly higher in peels than in peeled fruits ($P<0.05$). In conclusion, lemons possess the highest antioxidant potential among the studied citrus fruits and are preferable for dietary prevention of cardiovascular and other diseases. The peels of all citrus fruits are rich in dietary fibres and phenolic compounds and suitable for industrial processing.

Keywords: Citrus fruits; Dietary fiber; Total polyphenols; Phenolic acids; Ascorbic acid; Trace elements; TRAP

Bioactive compounds and antioxidant potential in fresh and dried Jaffa® sweeties, a new kind of citrus fruit

Journal of the Science of Food and Agriculture 84:14591463 (2004)

Shela Gorinstein, Ratiporn Haruenkit, Yong-Seo Park, Soon-Teck Jung,

Zofia Zachwieja, Zenon Jastrzebski, Elena Katrich, Simon Trakhtenberg and Olga Martin Belloso

Abstract: Bioactive compounds in the edible parts of fresh and dried Jaffa sweeties, a new kind of citrus fruit, were analysed and their antioxidant capacities were assessed. Antioxidant-rich fractions were extracted from fresh and dried sweeties with 1.2M HCl in methanol/water (1:1 v/v), and the antioxidant activities of these extracts were evaluated. Using the β-carotene/ linoleate model system, the extracts from equivalent quantities of fresh and dried sweeties showed 89 and 87% antioxidant activity respectively. Similarly, using the DPPH radical-scavenging method, the extracts showed 87 and 85% antioxidant activity respectively. The best correlations were between caffeic acid content and -carotene and DPPH antioxidant activity values (r = 0.9849 and 0.9798 respectively, p = 0.005). Both fresh and dried sweeties are bioactive natural products; when fresh fruits are not available, properly dried sweeties could be used as a substitute.
Keywords: fresh and dried sweeties; antioxidant compounds; antioxidant potential

Characterization of antioxidant compounds in Jaffa sweeties nd white grapefruits. Food Chemistry 84 (2004) 503510

Shela Gorinsteina,Milena Cvikrova, Ivana Machackova, Ratiporn Haruenkit, Yong-Seo Park, Soon-Teck Jung, Kazutaka Yamamoto, Alma Leticia Martinez Ayala, Elena Katrich, Simon Trakhtenberg

Abstract Antioxidant compounds and the antioxidative activities of new Israeli citrus fruit sweetie [(Oroblanco, pummelo-grapefruit hybrid (Citrus grandis_C. paradisi)] were compared with the better-known white grapefruit. Total and free phenols were determined with the FolinCiocalteu reagent, phenolic acids (free, esters and glycosides) by HPLC analysis and anthocyanins spectrophotometrically.
The antioxidant activities were estimated with two scavenging radicals: 2,

20-azinobis (3-ethylbenzothiazoline-6-sulfonate)- (ABTS) and nitric oxide (NO). Free radical scavenging properties of sweetie and grapefruit were evaluated by β-carotene bleaching (β-carotene). The results of kinetic reactions showed that both fruits differed in their capacities to quench these radicals and sweetie showed more antioxidative activity than grapefruit. Trans-hydroxycinnamic acids (caffeic, p-coumaric, ferulic, and sinapic) were more abundant in grapefruits than in sweeties. High correlation was observed between antioxidative activities and phenols (R^2=0.94). Both fruits have high concentrations of natural antioxidants with high antioxidative activities. Phenol content and the antioxidative potential are significantly higher in sweetie than in grapefruit. The higher antioxidant capacity of sweetie could make these new kinds of citrus fruits preferable for diets. In summary, the studied citrus fruit has high total phenolics and high antioxidant activities in vitro. Consumption of this fruit may contribute to an adequate intake of antioxidant phytochemicals.
Keywords: Citrus fruits; Antioxidant compounds; Antioxidative activities

Characteristics of blond and Star Ruby (red) grapefruits by antioxidant and electrophoretic methods. **International Journal of Food Science and Technology, in print, 2005**

Shela Gorinstein, Jerzy Drzewiecki, YongSeo Park, Soon-Teck Jung, Seong-Gook Kang, Ratiporn Haruenkit, Fernando Toledo, Elena Katrich, & Simon Trakhtenberg

Summary Antioxidant and electrophoretic methods were applied in order to characterize the quality differences of blond and Star Ruby (red) grapefruits. The antioxidant potential was determined by two modified antioxidant assays: 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and -carotene linoleate model system and compared with the Folin-Ciocalteu method. Proteins were extracted from the fruits and separated by modified SDS-PAGE. Also the contents of dietary fiber, minerals and trace elements, total polyphenols, anthocyanins, flavonoids, phenolic and ascorbic acids were determined as an additional indicator for characterization of the studied citrus fruits. It was found that the antioxidant

potential of red grapefruit was significantly higher than of the blond fruit ($P < 0.05$) and well correlated with total polyphenols (R_2 from 0.9386 to 0.9840). The use of a longer gel (14 cm instead of 7 cm) gave more distinct and sharp bands, especially in the zone higher than 36 kDa. In both studied cultivars of grapefruits 32 electrophoretic bands were detected. The main electrophoretic bands occurred between 20 and 43 kDa in both grapefruits showing some minor electrophoretic differences between the varieties. In conclusion: a) the antioxidant potential of red grapefruit is higher than in blond grapefruits b) there are some minor differences in the SDS-electrophoretic patterns of the studied cultivars c) the applied antioxidant and electrophoretic methods are a preferable combination for characterization of differences of the same citrus fruits.

Keywords Blond and red cultivars of grapefruits; antioxidant potential; electrophoretic patterns; differences.

CEREALS AND PSEUDOCEREALS

J. Agric. Food Chem. 2003, *51,* 7798?7804

Identification and Differences of Total Proteins and Their Soluble Fractions in Some Pseudocereals Based onElectrophoretic Patterns

JERZY DRZEWIECKI, EFREN DELGADO-LICON, RATIPORN HARUENKIT, ELKE PAWELZIK, OLGA MARTIN-BELLOSO, YONG-SEO PARK, SOON-TECK JUNG, SIMON TRAKHTENBERG, AND SHELA GORINSTEIN

Genetic diversity and relationships of 11 species and cultivars belonging to different Angiosperms families were examined using sodium dodecyl sulfate seed protein markers. The protein was resolved into 36 bands (for soybean), 41 (for quinoa), 35 (for buckwheat), and 28 to 39 bands of Amaranth species, respectively. All species and cultivars can be distinguished from each other. Soybean, quinoa, and buckwheat species had a characteristic protein pattern showing a high degree of polymorphism. The protein patterns of soybean were

considerably different from other species. Amaranth species had similar seed protein electrophoretic profile. The similarity coefficients calculated on the basis of presence and absence of bands ranged from 0.08 to 0.97. Following the UPGMA algorithm of similarity coefficients, the examined species and varieties could be clustered into two similarity groups. Our results did not confirm the Tachtadzjan hypothesis that Polygonales (e.g., buckwheat) and Caryophyllales(e.g., quinoa and amaranth) are closely related. Our data rather indicate occurrence of significant genetic distance (similarity coefficients 0.05-0.10). Also, it is doubtful that amaranth and quinoa species are also closely related (similarity coefficients varied from 0.16 to 0.25). It seems that soybean, quinoa, buckwheat, and amaranth (as a genus) can be considered as phylogenetic distant taxa. Differences and similarities in the secondary structure were observed by circular dichroism spectra. Some similarity was found between these plants in their soluble protein fractions and amino acid composition.**These plants can be a substitution of each other as well as for cereals.**

KEYWORDS: Plants; proteins; properties; identification; differences; spectroscopy

International Journal of Food Science and Technology 2004, 39, 183189

Use of scanning electron microscopy to indicate the similarities and differences in pseudocereal and cereal proteins

Shela Gorinstein,Elke Pawelzik, Efren Delgado-Licon, Kazutaka Yamamoto, Shoichi Kobayashi, Hajime Taniguchi, Ratiporn Haruenkit, Yong-Seo Park, Soon-Teck Jung, JerzyDrzewiecki & Simon Trakhtenberg

Summary Isolated and separated protein fractions from cereal and pseudocereal grains were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis and scanning electron microscopy. Prolamin, the main storage protein in cereal such as maize, showed a difference in electrophoretic patterns and fine structure in comparison with those from amaranth and soybean. In contrast glutelins from amaranth, soybean and maize showed some similarity in the distribution of protein bands and in

microstructure. Amaranth and soybean were closely similar in distribution of protein fractions and their microscopic structure. As an addition to chemical analyses, microscopy helped to understand and visualize structural changes and textural differences in protein fractions. Pseudocereals can be used as a nutritive substitute of some cereals in functional foods.

Keywords Electrophoretic separation, isolated protein fractions, main storage proteins, nutritive substitution, structural changes.

OLIVES

Journal of Nutritional Biochemistry 14 (2003) 154159

Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests

Shela Gorinstein, Olga Martin-Belloso, Elena Katrich, Antonin Lojek, Milan Ciz, Nuria Gligelmo-Miguel, Ratiporn Haruenkit, Yong-Seo Park, Soon-Teck Jung, Simon Trakhtenberg

Abstract

The aim of this study was to compare the contents of the main biochemical compounds and the antioxidant capacity of five Spanish olive oils by four different antioxidant tests and to find out the most valuable oil for disease preventing diets. Fatty acids, sterols and individual antioxidant compounds in Arbequina, Hojiblanca, Extra Virgin, Picual and Lampante Spanish olive oils were determined. Antioxidant activities were done as well using different radical scavenging activities: total radical-trapping antioxidative potential by ABAP (TRAPABAP), radical scavenging activity by DPPH (RSA-DPPH), antioxidant assay by -carotene-linoleate model system (AA--carotene) and total antioxidant status by ABTS (TAA-ABTS). The highest content of all studied antioxidant compounds (353; 329; 4.6 and 2.7 mg/kg for tocopherols, tocotrienols, polyphenols and o-diphenols, respectively) was found in Extra Virgin oil. Also the highest antioxidant capacity was observed in Extra Virgin oil (668nmol/ml; 29.4%; 40.4% and 2.64 mmolTE/kg for TRAP-ABAP, RSA-DPPH, AA- -carotene and TAA-ABTS, respectively). The correlation between total phenols and antioxidant

capacities measured by four methods was very high, but the highest for the -carotene (R2 =0.9958). In conclusion, the best method for determination of the antioxidant capacity of olive oils is the -carotene test. Extra Virgin olive oil has high organoleptic properties and the highest antioxidant activity. The above-mentioned makes this oil a preferable choice for diseases preventing diets.
Keywords: Olive oils; Fatty acids; Sterols; Antioxidant compounds; Antioxidant activity