

Note

Antioxidative Properties of Jaffa Sweeties and Grapefruit and Their Influence on Lipid Metabolism and Plasma Antioxidative Potential in Rats

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The effective substances (polyphenols, phenolic and ascorbic acids, flavonoids and dietary fibers) and antioxidative activities, using different radical-scavenging tests, were determined for Jaffa sweeties and grapefruit. The antioxidative activities comprised the contributions from polyphenols, phenolic acids, flavonoids and ascorbate components, and were well-correlated with polyphenols and flavonoids. The correlation coefficient between the polyphenols and antioxidative activity varied from 0.73 to 0.99. All applied methods showed that sweeties had higher antioxidative activity than grapefruit. Experiments on laboratory animals show that diets supplemented with sweeties, and to a lesser extent with grapefruit, increased the plasma antioxidative potential and improved the lipid metabolism, especially in the rats fed with added cholesterol. These findings provide additional characterization of the nutritional value of citrus fruits and their influence on the lipid metabolism in rats.

Key words: antioxidant; Jaffa sweetie; grapefruit; rat

Israel has been producing and exporting a new kind of citrus fruit in recent years which is named Jaffa sweetie and originated by crossing grapefruit and pomelo (pummelo).¹⁾ This fruit is already known in Japan. In order to assess the biochemical potential of sweeties, the main antioxidative compounds, dietary fiber and antioxidative activities were investigated and compared with those of the best known white grapefruit. The influence of these two fruits on the plasma lipid metabolism and plasma antioxidative

activity in rats fed on cholesterol-free and cholesterol-containing diets was determined. As far as we know, this is the first such investigation of this new kind of citrus fruit.

The chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Israeli Jaffa sweeties and white grapefruits were purchased from the same farmer and at the same stage of maturity. Preparation of the fruit samples and determination of the total, soluble and insoluble dietary fiber were conducted as previously described.²⁾ Polyphenols were measured at 765 nm by using the Folin-Ciocalteu reagent with gallic acid as a standard. The absorbance of the flavonoids was measured at 510 nm with (+)-catechin as a standard.³⁾ Phenolic⁴⁾ and ascorbic⁵⁾ acids were determined by HPLC. The total antioxidative activity of each type of the fruit was determined by the following methods: Trolox equivalent antioxidant capacity (TEAC);⁶⁾ total radical-trapping antioxidative potential (TRAP);⁷⁾ 1,1-diphenyl-2-picrylhydrazyl radical scavenging test (DPPH);⁸⁾ β -carotene bleaching (β -carotene)⁸⁾ and nitric oxide inhibition (NO).⁹⁾ Trolox, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid, was used as the standard with these methods.^{6,7)}

In order to assess the comparative influence of the bioactive components of sweeties and grapefruit on the plasma lipid metabolism and plasma antioxidative activity in rats fed on cholesterol-free and cholesterol-containing diets, a suitable experiment on male Wistar rats (n = 60) with a mean weight of 120 g was conducted. The Institute of Animal Physiology

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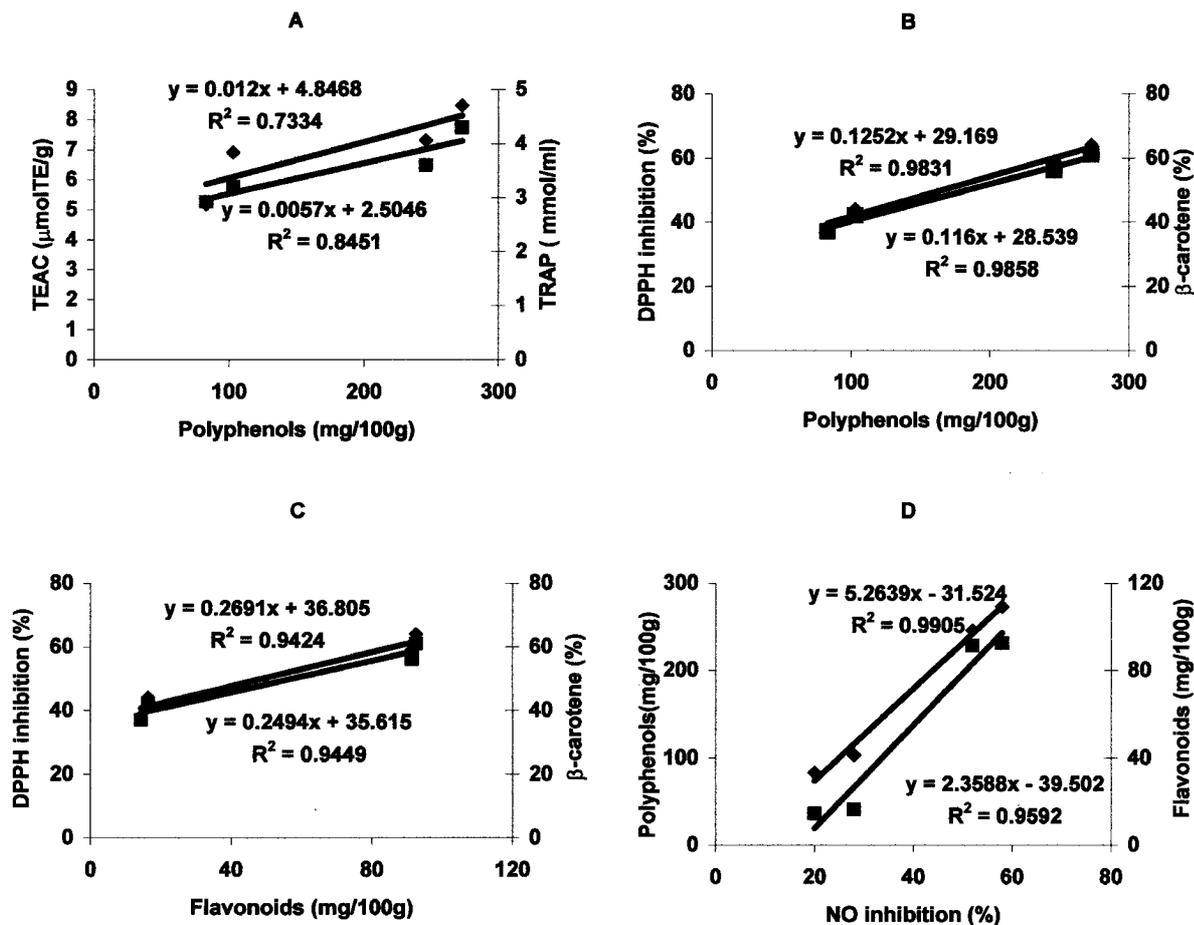


Fig. 1. Relationship, Calculated by a Linear Regression Analysis for Sweeties and Grapefruits.

A, \blacklozenge polyphenols (mg/100 g FW; X) to TEAC ($\mu\text{mol TE/g FW}$; Y_1) and \blacksquare polyphenols (mg/100 g FW; X) to TRAP (mmol/ml; Y_2). B, \blacklozenge polyphenols (mg/100 g FW; X) to DPPH scavenging effect (% inhibition, Y_1) and \blacksquare polyphenols (mg/100 g FW; X) to β -carotene bleaching effect (% inhibition, Y_2). C, \blacklozenge flavonoids (mg/100 g FW, X) to DPPH scavenging effect (% inhibition, Y_1) and \blacksquare flavonoids (mg/100 g FW, X) to β -carotene (% inhibition, Y_2); D, \blacklozenge NO scavenging effect (% inhibition, X) to polyphenols (mg/100 g FW, Y_1) and \blacksquare NO (% inhibition, X) to flavonoids (mg/100 g FW, Y_2).

Abbreviations: TEAC, Trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidative potential; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical scavenging test; β -carotene, β -carotene bleaching; NO, nitric oxide inhibition

and Nutrition of Polish Academy of Sciences (Jablonna, Poland) provided the male Wistar rats, and the Animal Care Committee of Warsaw Agricultural University approved this study. All groups of rats were fed on a basal diet (BD), which included wheat starch, casein, soybean oil, cellulose, and vitamin and mineral mixtures. The rats in the control group were fed with BD alone, while BD for the other five groups was supplemented with 10% of sweeties (sweetie group), 10% of grapefruit (grapefruit group), 1% of nonoxidized cholesterol, NOC (chol group), 1% of NOC and 10% of sweeties (chol/sweetie group), and 1% of NOC and 10% of grapefruit (chol/grapefruit group). Before and after the experiment, blood samples were taken from the tail vein, and a wide range of laboratory tests were performed. Malondialdehyde (MDA), as an index of lipid peroxidation, TRAP, total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-

C), total phospholipids (TPH), HDL-phospholipids (HDL-PH) and triglycerides (TG) were assayed as previously described.^{7,10} All results in this investigation are the mean of six measurements. $M \pm SD$ was calculated, and an analysis of variance (ANOVA) was applied, a p value of <0.05 being considered statistically significant.

There were no significant differences in the content range [g/100 g fresh weight (FW)] of total (1.34–2.51), soluble (0.43–0.88) and insoluble (0.87–1.62) dietary fiber in either the peel or peeled sweeties and grapefruits. Polyphenols (mg/100 g FW) in the peeled sweeties and grapefruits were 103.1 ± 4.2 and 83.2 ± 3.8 , and in peel of the sweeties and grapefruits were 273.1 ± 4.9 and 246.2 ± 4.2 , respectively. Flavonoids (mg/100 g FW) in the peeled sweeties and grapefruits were 16.40 ± 0.7 and 14.38 ± 0.8 , and in the peels of sweeties and grapefruits were 92.5 ± 1.9 and 91.4 ± 1.3 , respectively. The highest con-

Table 1. Plasma Lipids, Total Cholesterol Concentration in the Liver, TRAP and MDA in Rats Fed with Diets with Cholesterol and without 1% Cholesterol and with and without Sweetie and Grapefruit (Grapefr)

Diet	Plasma lipids			Liver			
	TC	LDL-C	TG	TPH	TC	TRAP	MDA
	mmol/L				$\mu\text{mol/g}$	nmol/ml	
Control	2.86 ± 0.15 ^c	1.23 ± 0.05 ^c	0.69 ± 0.04 ^b	1.76 ± 0.08 ^a	5.86 ± 0.24 ^c	242.9 ± 15.1 ^c	1.74 ± 0.08 ^c
Chol	3.69 ± 0.21 ^a	2.02 ± 0.12 ^a	0.88 ± 0.05 ^a	1.74 ± 0.08 ^a	24.3 ± 0.31 ^a	167.1 ± 10.1 ^a	2.91 ± 0.21 ^a
Grapefr	2.85 ± 0.15 ^c	1.21 ± 0.05 ^c	0.70 ± 0.05 ^b	1.72 ± 0.08 ^a	5.79 ± 0.24 ^c	293.4 ± 15.8 ^d	1.32 ± 0.07 ^d
Chol/Grapefr	3.01 ± 0.18 ^b	1.40 ± 0.05 ^b	0.73 ± 0.05 ^b	1.40 ± 0.06 ^b	18.3 ± 0.25 ^b	192.2 ± 11.9 ^b	2.36 ± 0.18 ^b
Sweetie	2.81 ± 0.15 ^c	1.19 ± 0.05 ^c	0.70 ± 0.05 ^b	1.72 ± 0.08 ^a	5.74 ± 0.24 ^c	301.7 ± 12.1 ^d	1.21 ± 0.11 ^d
Chol/Sweetie	2.91 ± 0.18 ^b	1.35 ± 0.05 ^b	0.75 ± 0.05 ^b	1.37 ± 0.06 ^b	17.9 ± 0.25 ^b	198.3 ± 12.1 ^b	2.24 ± 0.15 ^b

¹ Each value is the mean ± SD, n = 10.

² Means in columns without letters in common differ significantly ($p < 0.05$).

³ Abbreviations: Chol, nonoxidized cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; MDA, malondialdehyde test; NS, not significant; TC, total cholesterol; TG, triglycerides; TPH, total phospholipids; TRAP, total radical-trapping antioxidative potential

concentrations were of ferulic (29.9 ± 1.1), sinapic (26.1 ± 0.6) and *p*-coumaric (21.1 ± 0.2) acids and the lowest was of caffeic acid (12.2 ± 0.8) in both the peeled fruits and peel. The total concentration (mg/100 g FW) of the four hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic and sinapic) was higher in the pulp (89) and peel (117) of the sweeties than of the grapefruit (60 and 72, respectively) as was the amount of ascorbic acid ($p < 0.05$). Naringin was the most abundant flavonoid in both citrus fruits. TEAC values were significantly higher in the peeled sweeties and peel than in the grapefruits (6.91 ± 0.4 and 8.47 ± 0.6 vs. 5.18 ± 0.7 and 7.31 ± 0.4 $\mu\text{mol TE/g FW}$, respectively, $p < 0.05$). TRAP values were also significantly higher in the peeled sweeties and peel than in the grapefruits (3.2 ± 0.4 and 4.3 ± 0.6 vs. 2.92 ± 0.1 and 3.6 ± 0.2 mmol/ml, respectively, $p < 0.05$). A weak correlation existed between TRAP and dietary fiber ($R^2 = 0.3267$), and a strong correlation existed between TRAP and polyphenols ($R^2 = 0.8524$). A very strong correlation was found between TRAP and the individual phenolic acids: caffeic ($R^2 = 0.96$); ferulic ($R^2 = 0.92$); and *p*-coumaric and sinapic ($R^2 = 0.86$ in both cases), while the correlation between ascorbic acid and TRAP was relatively low ($R^2 = 0.64$). The DPPH scavenging effect of sweetie peel was 64%, and the least effect was with the methanol extract of grapefruit (38% vs. 44% for sweeties). The β -carotene inhibition was slightly lower than with the DPPH results, the highest value being for the sweetie peel (61%), this being followed by the grapefruit peel (56%), BHA (56%), sweetie pulp (42%) and grapefruit pulp (37%).

The NO test showed the highest correlation between polyphenols and flavonoids, and the highest percentage inhibition ($R^2 = 0.9905$) in comparison with the other four applied assays, as presented in Fig. 1. The respective correlation coefficients were as follow: 0.73, 0.85, 0.98, 0.99 and 0.99 with TEAC, TRAP, DPPH, β -carotene and NO. The antioxidative activity of sweetie was compared with that of

grapefruit, Trolox, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), some phenolic acids (caffeic, ferulic, sinapic and *p*-coumaric) and naringin by the NO test. The NO scavenging effect of the grapefruit peel extract (58%) was higher than that of each other extract, but was significantly lower than that of Trolox (70%) at the same concentration ($p < 0.05$). The NO scavenging effect of sweetie pulp was nearly equal to that of BHT (31%). Caffeic and ferulic acids, and naringin exhibited stronger effects on NO than *p*-coumaric acid. The variables and antioxidative activities studied in the peel were significantly higher than in the peeled fruit ($p < 0.05$). The highest scavenging effect of sweeties was with DPPH radicals 44% vs. 42% with the β -carotene and 28% with the NO test. All test results showed that the antioxidative value of peeled sweeties and peel were significantly higher ($p < 0.05$) than those of peeled grapefruits and peel. The plasma TC, LDL-C, TG, TPH and TC in the liver in all groups of rats before the investigation were similar to those of the control group (2.86 ± 0.15 ; 1.23 ± 0.05 ; 0.69 ± 0.04 ; 1.76 ± 0.08 mmol/l and 5.86 ± 0.24 $\mu\text{mol/g}$ for plasma TC, LDL-C, TG, TPH and TC in the liver, respectively). TRAP and MDA values before the investigation were 242.9 ± 15.1 and 1.74 ± 0.08 , 242.7 ± 15.0 and 1.73 ± 0.08 , 242.8 ± 15.1 and 1.74 ± 0.08 , 242.9 ± 15.1 and 1.72 ± 0.08 , 242.8 ± 15.1 and 1.75 ± 0.08 , and 242.7 ± 15.1 and 1.73 ± 0.08 nmol/ml for the control, sweetie, grapefruit, chol, chol/sweetie and chol/grapefruit diet groups, respectively.

Changes in the variables studied after completing the trial are summarized in Table 1.

The results reveal a decrease in the plasma antioxidative potential in all groups of rats fed with the cholesterol diet. However, this decrease in the antioxidative activity of the groups of rats fed on the diets supplemented with citrus fruit was significantly less than of the groups of rats fed on the diet without fruit (TRAP: 198.3 ± 12.1 and 192.2 ± 11.9 vs. 167.1 ± 10.1 for the chol/sweetie, chol/grapefruit and chol

groups, respectively; MDA: 2.24 ± 0.15 and 2.36 ± 0.18 vs. 2.91 ± 0.21 for the chol/sweetie, chol/grapefruit and chol groups, respectively).

A significant decrease in TRAP and an increase in MDA values in those rats fed with added cholesterol was predictable, other investigators have also observed a decrease in the blood antioxidative capacity in rats fed with added cholesterol.¹¹⁾

In conclusion, both fruits, and particularly sweeties, contained high quantities of antioxidative compounds and had high antioxidative potential. The diets supplemented with sweeties, and to a lesser extent with grapefruits, had increased plasma antioxidative potential and improved lipid metabolism, especially in those rats fed with added cholesterol.

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