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Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits

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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 Folin–Ciocalteu reagent, phenolic acids (free, esters and glycosides) by HPLC analysis and anthocyanins spectrophotometrically. The antioxidant activities were estimated with two scavenging radicals: 2, 2′-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

1. Introduction

It is well known that eating fruits and vegetables lowers the risk of chronic diseases, such as heart disease and cancer. The question of what are the active ingredients is still unresolved. The initial hypothesis was that antioxidant vitamins were responsible. However, more recently the polyphenols have been investigated since they have been found to be beneficial as strong antioxidants (Vinson et al., 2002; Wang, Cao, & Prior, 1997). Recent studies are underline the importance of specific flavonoids as bioactive components of the diet in both in vivo and in vitro models. Thus, it is important to have a clear idea of the major phenolic families involved (Heller & Forkmann, 1988; Proteggente et al., 2002). It was shown that citrus fruits play a special role in decreasing the risk of ischemic stroke (Joshipura et al., 1999). This positive influence was attributed to some natural antioxidant phytonutrients (Paganga, Miller & Rice-Evans, 1999; Proteggente et al., 2002). Flavonols, flavanols, anthocyanins, and phenylpropanoids might act as antioxidants or as agents of other mechanisms contributing to cardioprotective action (Vinson et al., 2002; Wang et

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al., 1997). There are reports of the total antioxidant capacity of citrus fruits (Chen & Ho, 1997; Paganga et al., 1999; Wang et al., 1997). The antioxidant activities of sweet oranges, lemons and red grapefruits, in both fresh fruits and their extracts, were estimated (Sawamura, Kuriyma, & Li, 1988). Cinnamate conjugates and other polyphenols in citrus fruits (Bocco, Cuvelier, Richard, & Berset, 1998; Peleg, Naim, Rouseff, & Zehavi, 1991; Rapisarda et al., 1999) were widely studied. We have determined the antioxidant capacity of different fruits by total radical-trapping antioxidative potential (TRAP; Gorinstein et al., 2001, 2002). It was shown that the major source of antioxidant capacity of most citrus and other fruits is not vitamin C or dietary fibres, but their antioxidant compounds (Gorinstein et al., 2001, 2002).

In recent years Israel has produced and exported a new kind of citrus fruit, with the trade name Jaffa sweetie [(Oroblanco, pummelo-grapefruit hybrid (*Citrus grandis*×*C. paradisi*)]. On this new type of citrus fruit no investigations have been performed. Therefore, the aim of this study was to compare the contents of polyphenols, some phenolic acids, anthocyanins and antioxidative activities in sweeties with those of the better known white grapefruit using specific methods such as 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) and nitric oxide (NO). Free radical scavenging properties of sweetie and grapefruit were evaluated by a β-carotene bleaching (β-carotene) kinetic reaction. As far as we know, there are no such previous investigations of these fruits.

2. Materials and methods

2.1. Chemicals

2, 2′- azinobis (3-ethylbenzothiazoline-6- sulfonate)-(ABTS); 6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylic acid (Trolox); metmyoglobin; Greiss reagent; sodium nitroprusside; β-carotene; standard phenolic acids: gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, salicylic, ferulic, anisic and sinapic; butylated hydroxyanisole (BHA), naringin and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO).

2.1.1. Sample preparation

Jaffa sweeties (oroblanco) and Jaffa white grapefruits were harvested in the period December 2000–April 2001 in Israel. The fruits were cleaned with tap water and dried. The edible portion was weighed, chopped, and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. Then a weighed portion (50–100 g) was lyophilized for 48 h (Virtis model 10-324) and the dry weight was determined. The sample was ground to pass through a 0.5-mm sieve and stored at −20 °C until analyzed.

2.2. Phenolic acid analysis

Phenolic acids were extracted as described earlier (Cvikrová, Hrubcová, Meravỳ, & Pospišil, 1988). Free (F_1), ester-bound (F_2, released after alkaline hydrolysis) and glycoside-bound (F_3, released after acid hydrolysis) phenolic acids were obtained from a methanol extract of tissue ground in liquid nitrogen.

2.3. Extraction of the free phenolic compounds

Two grammes of sample were homogenized in liquid nitrogen in a blender IKA A10. The slurry was extracted three times with 30 ml of 80% methanol, with boiling for 30 min. The methanol extract was filtered through Whatman No.1 filter paper and washed three times with 40 ml of diethylether, then evaporated to dryness under vacuum at 40 °C. The residue was dissolved in 4 ml of dimethylformamide (DMF) and filtered on a 0.45 μm filter (Gelman GHP) for the identification and quantification of the free phenolic compounds. The aqueous phase, after extraction with diethylether, was divided into two parts.

2.4. Extraction of methanol-soluble phenolic esters

The aqueous phase was hydrolyzed with 200 ml of 2 N NaOH for 4 h under nitrogen at room temperature, acidified with 6 N HCl at pH 2 and then extracted three times with 200 ml of diethylether. The organic phase was evaporated to dryness under vacuum at 40 °C, and the residue was dissolved in 4 ml of DMF, filtered on a 0.45 ìm filter (Gelman GHP) and used for the identification and quantification of the soluble phenolic esters.

2.5. Extraction of methanol-soluble phenolic glycosides

The aqueous phase was hydrolyzed with 200 ml of 1 N HCl for 1 h at 100 °C at pH 2, extracted with diethylether, evaporated, dissolved, filtered, as with previous samples, and used for the analysis.

Phenolic acids were analyzed by HPLC using a Pye Unicam PU 4002-Video Liquid Chromatograph with a Spherisorb 5 ODS column (250×4.6 mm), using two solvents: A—5 m*M* citric acid + 5 m*M* sodium dihydrogen orthophosphate + 0.3 mM caprylic acid (adjusted to pH 2.0 by phosphoric acid) and B—80% (v/v) methanol. Elution conditions were as follows: flow rate 0.5 ml min^{-1}, linear gradient from 10 to 35% B for 70 min, then from 35 to 50% B for 15 min, from 50 to 100% B for 5 min and finally for 5 min 100% B and 5 min from 100 to 10% B. The column eluate was monitored at 260 and 300 nm using a Multichannel detector PU 4021.

2.6. Extraction of total anthocyanins

Fifty-gram samples of each fruit were added to 50 ml of acetonitrile containing 4% acetic acid and homogenized in a blender for 2 min. After the recovery of the homogenate, 25 ml of acetonitrile containing 4% acetic acid were used to wash the blender and pooled with the first homogenate. The pooled homogenate was left at room temperature with shaking every 3 min for at least 30 min and then centrifuged at 13000 *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.

Anthocyanins were estimated by a pH differential method (Cheng and Breen, 1991). Absorbance was measured in a Beckman spectrophotometer at 510 and at 700 nm in buffers at pH 1.0 and 4.5, using $A=[(A_{510}-A_{700})_{\text{pH1.0}}-(A_{510}-A_{700})_{\text{pH4.5}}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29,600. Results were expressed as microgrammes of cyanidin-3-glucoside equivalents per gramme of fresh weight (FW).

2.7. Extraction and hydrolysis of phenols

A 50 mg aliquot of lyophilized sample was accurately weighed in a screw-capped tube. For free phenols, 5 ml of 80% methanol/water and the sample were vortexed for 1 min and heated at 90 °C for 3 h with vortexing every 30 min. After cooling, the samples were diluted to 10 ml with methanol and centrifuged for 5 min at 5000 rpm with a bench top centrifuge to remove solids. Total phenols were extracted with 5 ml of 1.2 M HCl in 80%methanol/water and treated as above (Vinson, Su, Zubik, & Bose, 2001).

Phenols were measured in samples of each fruit at 750 nm, using the Folin–Ciocalteu reagent diluted 5-fold before use. Measurements at 750 nm, after reaction for 10 min by the method of Slinkard and Singleton (1997), were done and gallic acid was used as a standard. Total phenols were expressed as μmol gallic acid equivalents per gramme of fresh weight.

2.8. Total antioxidative potential determination

2.8.1. Total antioxidant status (TAA Test)

The TAA was estimated using the ferrylmyoglobin/ABTS method (Paganga, Miller, & Rice-Evans, 1999). This technique measures the relative ability of antioxidant substances to scavenge the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$), compared with standard amounts of the synthetic antioxidant Trolox, the water-soluble vitamin E analogue. The radical cation $ABTS^{\bullet}$, generated in the aqueous phase from ABTS through the peroxidation action of metmyoglobin, is a blue/green chromogen with characteristic absorption at 734 nm. Results are expressed as μmol Trolox equivalents (TE) per gramme of fresh weight.

2.8.2. Scavenging activity against nitric oxide (NO Test)

Nitric oxide interacts with oxygen to produce stable products, nitrite and nitrate. Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite. The concentration of nitrite in aqueous solution was assayed spectrophotometrically using the Greiss reagent, which reacts with nitrite to give a stable product absorbing at 542 nm (Marcocci, Packer, Droy-Lefaix, Sekaki, & Garde's-Albert, 1994; Saija et al., 1999).

Sodium nitroprusside solution was prepared immediately before the experiment, by dissolving 10 mM sodium nitroprusside in 20 mM phosphate buffer, pH 7.4, previously purged with argon. The samples were diluted in 20 mM phosphate buffer, pH 7.4, to obtain optimal concentrations. At the beginning of the experiment, 0.5 ml of the sample (at various concentrations) was diluted with 0.5 ml of sodium nitroprusside solution and incubated at 25 °C for 150 min. At the end of the incubation, 1 ml of Greiss reagent was added to each sample, and the absorbance was read at 542 nm. The nitrite concentration was calculated by referring to the absorbance of standard solutions of potassium nitrite. Results were expressed as percentage nitrite production with respect to control values (sample: 0 μl). The slope of the plot of percentage nitrite production vs. sample volume was calculated by first-order exponential regression analysis, and the antioxidant efficiency (AE) was arbitrarily assumed as (−slope)×100.

2.9. Kinetic reaction

2.9.1. β-carotene linoleate model system

Emulsion (4 ml) containing β-carotene, linoleic acid and Tween-40 (polyoxyethylene sorbitan monopalmitate), was added to 0.2 ml of citrus extracts (Jayaprakasha & Jaganmohan, 2000; Singh, Chidamdara, Jayaprakasha, 2002). The absorbance at 470 nm was taken at zero time ($t=0$) and continued until the colour of β-carotene disappeared ($t=$ 180 min) at intervals of 15 min. The antioxidant activity (AA) of the extracts was evaluated as a percentage of bleaching of β-carotene: $AA=100\,[1-(A_0-A_t)/(A^{\circ}_0-A^{\circ}_t)]$, where A_0 and A°_0 and At and $A^{\circ}t$ are the absorbance values measured at zero time and 180 min of the incubation for test sample and control, respectively. Trolox, BHA, naringin and phenolic acids were used as standards in these methods. The kinetics were done over 180 min.

2.10. Statistical analysis

To verify the statistical significance of all parameters, the values of means and ±SD were calculated. Where

appropriate, the data were tested by 2-way ANOVA. P values of less than 0.05 were adopted as statistically significant. All following data are means of five measurements.

3. Results

It was found that phenolic acids were present in both fruits in the following fractions: F_1, free phenolic acids, F_2, methanol-soluble ester-bound phenolic acids (soluble phenolic esters) and F_3, methanol-soluble glycoside-bound phenolic acids (soluble phenolic glycosides). The contents of gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, salicylic, ferulic, anisic and sinapic acids, in both grapefruits and sweeties, in pulp and peel, were comparable (Tables 1 and 2). Ferulic acid is the major component, followed by *p*-coumaric, sinapic, and caffeic acid. Total concentration (nmol/g) was higher in pulp (362) and peel (1513) of grapefruits than in sweeties (272 and 1277, respectively) for four of the hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic and sinapic). The sum of fraction F_1 for four hydroxycinnamic acids was lower in sweetie that in grapefruit and lower than in the F_2 fraction. Grapefruits and sweeties contained esters and glycosides of *trans*-hydroxycinnamic acids, which are prevalently derivatives of ferulic acid (Bocco et al., 1998; Peleg et al., 1991). The highest contents of ferulic and the lowest of caffeic acids were in peels of sweeties and grapefruits (Tables 1 and 2). The contents of sinapic acid are almost equal in the two fruits. The ratio between the concentrations of ferulic and sinapic acids and that of caffeic and *p*-coumaric acids, which was for pulp about 1.62 and 1.57 and for peel 5.8 and 5.0, for sweeties and grapefruits, respectively, may be a simple parameter for differentiating these two fruits.

Total polyphenols (μmol g FW) in peeled sweeties and grapefruits were 9.2 ± 0.9 and 7.0 ± 0.9 and, in peels of sweeties and grapefruits, 13.9 ± 1.1 and 8.4 ± 0.9, respectively. Free polyphenols (μmol/g FW) in peeled sweeties and grapefruits were 1.2 ± 0.1 and 0.8 ± 0.1 and, in peels of sweeties and grapefruits, 2.0 ± 0.2 and 1.5 ± 0.2, respectively. Total anthocyanins (μg/g FW) in peeled sweeties and grapefruits were 0.8 ± 0.1 and 0.5 ± 0.1 and in peels of sweeties and grapefruits 1.6 ± 0.3 and 1.1 ± 0.1, respectively. TAA (μM TE/g FW) in peeled sweeties and grapefruits were 6.95 ± 0.5 and 5.21 ± 0.9 and, in peels of sweeties and grapefruits, 8.52 ± 0.7 and 6.31 ± 0.5, respectively.

NO ($AE\times10^3$) in peeled sweeties and grapefruits were 15.4 and 11.6 and, in peels of sweeties and grapefruits, 19.3 and 14.5, respectively. The total and free phenol contents, anthocyanins and antioxidative activities, determined by

Table 1
Contents of individual phenolic acids (nmol/g FW) in fractions of free (F_1), ester-(F_2) and glycoside-(F_3) bound methanol-soluble phenolic acids in the pulps of sweeties and grapefruits

		GA	PA	pHBA	VA	CaA	pCA	SA	FA	AA	SiA	ΣPhA	Σ total
Sweetie	F_1	–	0.23	0.20	0.15	trs	0.11	–	0.31	0.18	–	1.18	376
	F_2	–	–	5.97	14.3	–	26.5	–	117	0.39	33.41	198	
	F_3	19.7	–	5.73	12.2	7.61	5.56	45.0	72.0	–	9.58	177	
Grapefruit	F_1	0.36	0.97	0.33	0.14	0.11	0.25	–	0.84	0.28	–	3.29	428
	F_2	0.70	2.25	8.04	14.8	–	38.2	–	185	1.05	26.47	276	
	F_3	–	–	6.81	11.7	12.7	8.88	19.3	89.4	–	–	149	

Data are means (M) of five measurements. Abbreviations: GA, gallic acid; PA, protocatechuic acid; *p*HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CaA, caffeic acid; *p*CA, *p*-coumaric acid; SA, salicylic acid; FA, ferulic acid; AA, anisic acid; SiA, sinapic acid; FW, fresh weight.

Table 2
Contents of individual phenolic acids (nmol/g FW) in fractions of free (F_1), ester-(F_2) and glycoside-(F_3) bound methanol-soluble phenolic acids in the peels of sweeties and grapefruits

		GA	PA	pHBA	VA	CaA	pCA	SA	FA	AA	SiA	ΣPhA	Σ total
Sweetie	F_1	0.08	0.26	0.21	0.14	0.08	0.77	–	2.66	–	2.65	6.85	1433
	F_2	1.66	10.9	13.6	25.8	–	447	–	432	–	174	1105	
	F_3	9.61	–	21.2	18.1	9.25	30.60	54.2	146	–	32.4	321	
Grapefruit	F_1	–	0.63	0.32	0.30	–	0.61	–	1.55	0.03	–	3.43	1705
	F_2	1.02	4.50	17.6	42.1	–	520	–	555	–	131	1272	
	F_3	17.29	–	24.3	31.9	9.08	59.2	47.4	198	4.59	38.14	429.52	

Data are means (M) of five measurements. Abbreviations: GA, gallic acid; PA, protocatechuic acid; *p*HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CaA, caffeic acid; *p*CA, *p*-coumaric acid; SA, salicylic acid; FA, ferulic acid; AA, anisic acid; SiA, sinapic acid; FW, fresh weight.

ABTS and nitric oxide in both pulps and peels of sweeties, were significantly higher than in grapefruits.

The antioxidant activities of sweetie and grapefruit extracts, standard antioxidants and some phenolic acids at 0.2 mg/ml concentration, as measured by the bleaching of β-carotene, are presented in Fig. 1, A and B. Sweetie extracts prepared from pulp and peel exhibited slightly varying degrees of antioxidative activities. The sweetie peel extract (Fig. 1B) showed the highest antioxidant activity (90%), followed by grapefruit peel (84%) and sweetie pulp (61%). BHA (94.4%) and trolox (80.0%) were found to give the maximum antioxidant activities. The antioxidant activity of sweetie pulp (61.0%) was between those of naringin (44.7%) and trolox in the extract of total phenols (Fig. 1B). Extract of free phenols from sweetie pulp showed 30.0% activity and the kinetic curve was between *p*-coumaric (16.0%) and caffeic (37.9%) acids (Fig. 1A), The determined activity depended on the method of extraction of free (Fig. 1A) and total phenols. All extracts of free phenols showed lower activities than the total ones (Fig. 1B). Sweetie extracts from pulp and peels showed stronger inhibition than the extracts from grapefruit.

The best correlation ($R^2 = 0.94$) was between total phenols and the total antioxidant activity (Fig. 2A). Good correlation levels were also observed for anthocyanins: $R^2 = 0.8068$ and $R^2 = 0.8612$, as determined by TAA and NO test, respectively (Table 3). However, hydroxycinnamic acids in free form showed a relatively lower correlation than for polyphenols and anthocyanins: $R^2 = 0.5498$ and $R^2 = 0.5849$, as determined by TAA and NO test, respectively (Fig. 2B). There was no significant difference between the quality of the free and total phenols in the fruits (Table 3), although the total polyphenol activity was higher ($R^2 = 0.9421$ and $R^2 = 0.9436$) than that of the free phenols ($R^2 = 0.8212$ and $R^2 = 0.8746$), as determined by TAA and NO test, respectively (Table 3).

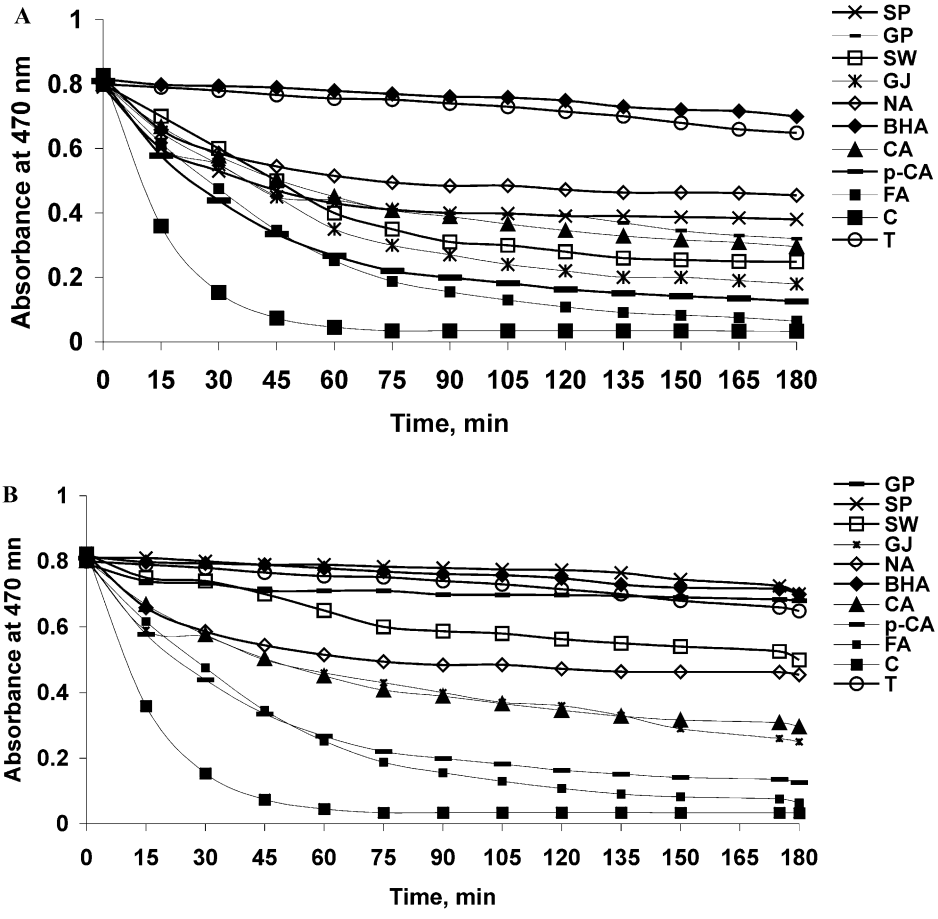

Fig. 1. Reaction kinetics: A, four citrus extracts with 50%Met from: sweetie peel (SP), sweetie pulp (SW), grapefruit peel (GP), grapefruit pulp (GJ); naringin (NA); butylated hydroxyanisole (BHA); *p*-coumaric (*p*-Ca), ferulic (FA) and caffeic (CA) acids; and trolox (T) with β-carotene bleaching. B, four citrus extracts with 50%Met/HCl from sweetie peel (SP), sweetie pulp (SW), grapefruit peel (GP), grapefruit pulp (GJ); naringin (NA); butylated hydroxyanisole (BHA); *p*-coumaric (*p*-Ca), ferulic (FA) and caffeic (CA) acids; and trolox (T) with β-carotene bleaching. The β-carotene concentration was 0.004 mg/ml and the samples were at 0.2 mg/ml in the reaction mixtures. Citrus extracts were obtained with 50% methanol/water/1.2 M HCl in Fig. 1B and with 50% methanol/water in Fig. 1A.

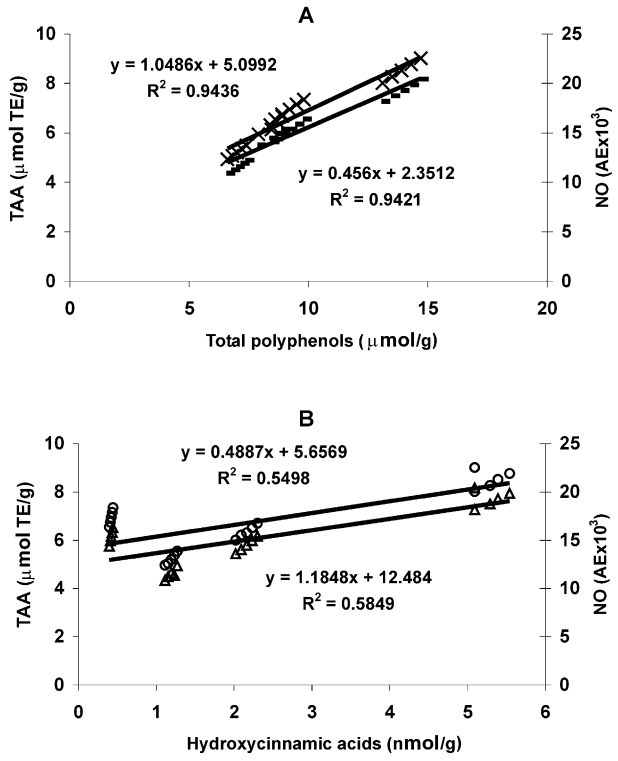

Fig. 2. A and B. Relationship, calculated by linear regression analysis for the studied sweeties and grapefruit, between: A, × total polyphenols (μmol/g; X) to TAA test (μM TE/g; Y_1) and total polyphenols (μmol/g; X) to NO test [(AE $\times 10^3$), Y_2]. B, ○ hydroxycinnamic acids (nmol/g, X) to TAA test (μmol TE/g; Y_1) and △ hydroxycinnamic acids (nmol/g, X) to NO test [(AE ($\times 10^3$), Y_2]. Abbreviations: TAA, total antioxidant activity; TE, Trolox equivalent, NO, scavenging activity against nitric oxide; AE, antioxidant efficiency.

Table 3
Correlation coefficients of linear regression between the concentrations of individual antioxidant constituents and the antioxidant activity of tested fruits

Antioxidant components	Correlation coefficient (R^2)	
	TAA, μM TE/g	NO test (AE$\times 10^3$)
Total phenols (μol/g)	0.94	0.94
Free phenols (μol/g)	0.82	0.87
Total anthocyanins (μg/g)	0.81	0.86
Free hydroxycinnamic acid (nmol/g)	0.55	0.58
Glucoside hydroxycinnamic acid (nmol/g)	0.46	0.52

4. Discussion

Phytochemicals, especially phenolics, in fruits and vegetables are suggested to be the major bioactive compounds for health benefits. However, the phenolic contents and their antioxidant activities in fruits and vegetables were underestimated in the literature, because bound phenolics were not included. This study was designed to investigate the profiles of total phenolics, including both soluble free and bound forms in sweetie and grapefruit, by applying solvent extraction and hydrolysis. Our results are in accordance with the data of Sun et al. (2002) on free and total phenolics and their antioxidative values.

The contents of phenolic acids in peels were significantly higher than in pulp of sweeties and grapefruit. These results are in accordance with others who indicate that peels are an important source of phenolic compounds (Bocco et al., 1998).

The role of hydroxycinnamic acid compounds as antioxidants and free radical scavengers has been pointed out (Chen & Ho, 1997). Our results are in accordance with others concerning the distribution of caffeic, *p*-coumaric, ferulic, and sinapic acids and the predominance of ferulic acid over the other hydroxycinnamic acids (Bocco et al., 1998; Fernandez de Simon et al., 1992; Peleg et al., 1991; Rapisarda et al., 1999). Hydroxycinnamic acids were highly correlated with each other and also with anthocyanins. Such correlations were expected because hydroxycinnamic acids are the precursors of the anthocyanins.

Recent studies represent the results of the measure of the total radical-trapping antioxidative potential (TRAP) of different fruits (Gorinstein et al., 2001; 2002) based on peroxidation, induced by the water-soluble radical initiator 2, 2′-azobis (2-amidinopropane) hydrochloride (ABAP). The peroxyl radicals, produced at a constant rate by thermal decomposition of ABAP, were monitored by luminol-enhanced chemiluminescence (CL). The results of these studies, using a peroxyl radical generator (ABAP), indicated that the antioxidant capacities of citrus and traditional fruits covered a considerable range and were slightly lower than the results of the present investigation. Apple pulps and peels have relatively high antioxidant capacities in comparison with pears and peaches and are preferable for disease-preventing diets (Gorinstein et al., 2002). It is not surprising that some variation of antioxidant activities was obtained in the present report; antioxidant activity of investigated samples depends upon which free radical or oxidant is used in the assay. It could be that such a method is not specific for methanol extracts of phenols (Gorinstein et al., 2002). It can also be explained by the use of a different variety of grapefruits. Each method for determination of antioxidants is based on the reactivity of different scavenging radicals (Gardner et al., 2000) and dependent, not only on this factor, but also on the pH of the testing system (Cano et al., 1998; Cervellati, Renzulli, Guerra, & Speroni, 2002). The most widely used chromogen compounds to measure the antioxidant activity of biological material are the ABTS(+) and the DPPH radicals. Even in these methods interferences were found at different wavelengths, caused by plant-derived materials of the studied samples (Arnao, 2000). Our results are in accordance with others that antioxidative potentials involve contributions from

polyphenols. The NO, TAA and β-carotene values for each extract were similar and well correlated with the total phenolic contents. These data are supported by others (Proteggente et al., 2002) on the basis of the comparison of different methods, as well as on the results of the citrus investigated. It was shown that the total phenolic content correlates highly with the antioxidant activity of sweeties and grapefruit and is in accordance with others (Henn and Stehle, 1998).

In the free phenol extract, the activity was a sum of the phenols and any vitamin C present (Vinson et al., 2001). Ascorbic acid is generally a minor component compared with the free phenols of fruits (Rapisarda et al., 1999). The contribution to quality is small: phenolics of fruits are more active antioxidants than ascorbate (Paganga et al., 1999; Vinson et al., 2001). Some authors claim that loss of total antioxidants in irradiated orange juice appeared to result from loss of ascorbic acid (Fan & Thayer, 2002). Vitamin C was found to account for 65–100% of the antioxidant potential of beverages derived from citrus. Although phenolics appear to be major contributors to the antioxidant potential of the non-citrus juices, their identity and bio-availability requires further investigation (Gardner et al., 2000). The correlation of the total phenolic content and the total antioxidant potential was very high ($R^2 = 0.94$). These data are in accordance with others, who have shown that high total polyphenol contents increase antioxidant activity (Henn & Stehle, 1998; Proteggente et al., 2002; Rapisarda et al., 1999) and there is a linear correlation between phenolic content and antioxidant activity.

In contrast to our previous investigations (Gorinstein et al., 2001,2002), in this report we used two different methods: the TAA and NO tests, as well as kinetic reaction with β-carotene. All tests have shown that sweeties have a higher level of antioxidant activity than grapefruits. As already reported, antioxidant activity of sweeties was determined for the first time in this study and therefore, no data are available to compare with our results.

In summary, the antioxidative activities of grapefruits and sweeties were measured using three assays with different reactive species. The antioxidant activity against peroxyl radicals of sweeties was higher than that of grapefruits. The studied fruits contain a group of natural antioxidants that have a high antioxidant activity.

5. Conclusion

The total phenol content and the antioxidative activity are higher in sweeties than in grapefruits. The higher antioxidative activity of sweeties could make this new type of citrus fruit a preferable choice for diets.

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