ELSEVIER

Lebensm.-Wiss. u.-Technol. 37 (2004) 337–343

lwt

www.elsevier.com/locate/lwt

Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid

Shela Gorinstein[a,*,1], Zofia Zachwieja[b], Elena Katrich[a], Elke Pawelzik[c], Ratiporn Haruenkit[d], Simon Trakhtenberg[e], Olga Martin-Belloso[f]

[a] *Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University-Hadassah Medical School, P.O. Box 12065, Jerusalem 91120, Israel*

[b] *Department of Food Chemistry and Nutrition, School of Medicine, Jagiellonian University, Krakow 30084, Poland*

[c] *Institute of Agricultural Chemistry, Georg-August University, Göttingen D-37075, Germany*

[d] *Department of Agricultural Industry, Faculty of Agricultural Technology, King Mondkut Institute of Technology, Lankrabang, Bangkok, Thailand*

[e] *Kaplan Medical Center, Rehovot, Israel*

[f] *Department of Food Technology, University of Lleida, Lleida, Spain*

Received 24 May 2002; accepted 7 October 2003

Abstract

The aim of this study was to compare the main antioxidant compounds content and the antioxidant activity of white grapefruit and his new hybrid (Jaffa Sweeties). Total phenols were measured colorimetrically using the Folin-Ciocalteu reagent, phenolic acids—by HPLC, anthocyanins and flavonoids—spectrophotometrically. The antioxidant activity of these fruits was determined by total antioxidant activity (TAA) and nitric oxide (NO) methods. Trans-hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic, and sinapic) were more abundant in white grapefruit than in his hybrid. However, on a fresh weight basis, grapefruit's hybrid has a higher total phenol content as well as a higher antioxidant capacity in comparison with white grapefruit. A linear relationship existed between TAA and anthocyanins ($R^2 = 0.8068$), TAA and flavonoids ($R^2 = 0.9320$) and TAA and total phenols ($R^2 = 0.9446$). Our findings indicate the following: (1) Both studied fruits contain a high concentration of natural antioxidants that have not only a high antioxidant activity, but also a good antioxidant quality. (2) The total phenol content and the antioxidant potential are significantly higher in the grapefruit hybrids than in white grapefruits.

Keywords: Citrus fruits; Total phenols; Phenolic acids; Antioxidant potential

1. Introduction

It is widely accepted that fruits and vegetables have many healthful properties (Hertog et al., 1995). Consumption of fruits is beneficial to health and contributes to decrease of the mortality rate of cardiovascular and other diseases (Vinson, Dabbagh, Serry, & Jang, 1995; Wang, Cao, & Prior, 1996; Joshipura et al., 1999). A high intake of fruits also reduces blood pressure (Ascherio et al., 1992). In the Health Professionals Follow-up Study it was shown that citrus fruits play a special role in decreasing the risk of ischemic stroke (Joshipura et al., 1999). A prospective study of diet quality and mortality in women proved a significant inverse correlation of mortality with increasing consumption of healthy food (Miller, Appel, & Risby, 1998). This positive influence is attributed to some natural antioxidant phytonutrients (Rice-Evans & Diplock, 1993; Halliwell, 1994; Rice-Evans & Miller, 1994; Rice-Evans, Miller, & Paganga, 1996). It was shown that plant phenols such as flavonols, anthocyanins, and phenylpropanoids might act as antioxidants or as agents of other mechanisms contributing to cardioprotective action (Vinson et al., 1995; Wang, Cao, & Prior, 1997).

Abbreviations: TAA, total antioxidant activity; TE, Trolox equivalent; NO, scavenging activity against nitric oxide; AE, antioxidant efficiency

*Corresponding author. Tel.: +972-2-6758690; fax: +972-2-6757076.

E-mail address: gorin@cc.huji.ac.il (S. Gorinstein).

[1] Author is affiliated with the David R. Bloom Center of Pharmacy.

0023-6438/$30.00
doi:10.1016/j.lwt.2003.10.005

All the above-mentioned raises interest in further investigations of the bioactive compounds of fruits. Some attempts were done to quantify the total antioxidant capacity in fruits in general and in citrus fruits in particular (Cao, Sofic, & Prior, 1997; Chen & Ho, 1997; Wang et al., 1997; Prior et al., 1998; Paganga, Miller, & Rice-Evans, 1999). Several studies have already been realized on the antioxidant activity of sweet oranges, lemons and red grapefruits using both fresh fruits and their extracts (Sawamura, Kuriyama, & Li, 1988). In the last years Israel produces and exports a new kind of citrus fruit, a hybrid of white grapefruit. This fruit is originated from the same crossing as Melogold and is very tasty.

Some investigations were done on the nature of cinnamate conjugates and other phenols in citrus fruits (Peleg, Naim, Rouseff, & Zehavi, 1991; Larrauri, Ruperez, Bravo, & Saura-Calixto, 1996; Bocco, Cuvelier, Richard, & Berset, 1998; Rapisarda et al., 1999; Clifford, 2000; Tomás-Barberán & Clifford, 2000). However, this new kind of citrus fruits was not investigated yet.

Therefore, the aim of this investigation was to determine the content of the main antioxidant compounds and the antioxidant activity of the grapefruit hybrid and to compare with the better-studied white grapefruit.

It was shown that the major source of antioxidant capacity of most citrus and other fruits is not vitamin C and dietary fibers, but their antioxidant compounds (Rapisarda et al., 1999; Gorinstein et al., 2001a, b). In our recent investigations we have determined the antioxidant capacity of different fruits using the total radical-trapping antioxidative potential (TRAP) test (Gorinstein et al., 2001a, b, 2002; Leontowicz et al., 2002). However, TRAP is relatively unspecific marker of free radical scavenging activity. Therefore, in this investigation other more specific TAA and NO tests were used.

As far as we know, there are no such investigations of this grapefruit hybrid.

2. Materials and methods

2.1. Chemical materials

2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonate)-(ABTS); 6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylic acid (Trolox); metmyoglobin; Greiss reagent; sodium nitroprusside and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Sample preparation

As was mentioned in the Introduction, in the last years Israel produces and exports a new kind of citrus fruit, a hybrid of white grapefruit (*Citrus paradisi*). In this investigation this hybrid of white grapefruit (*Citrus paradisi* var Jaffa Sweetie) was compared with better-studied white grapefruit (*Citrus paradisi* var Jaffa White).

After 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 analysed.

2.3. Phenolic acid analysis

Phenolic acids were analysed by HPLC using a Pye Unicam PU 4002-Video Liquid Chromatograph with a Spherisorb 5 ODS column ($250 \times 4.6\,mm^2$) using two solvents: (A) 5 mM citric acid + 5 mM 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. Authentic compounds (SERVA, Germany) were used as references for quantitative analyses.

2.4. Extraction of total anthocyanins

These compounds were extracted from the studied fruits with acetonitrile/acetic acid. A 50 g sample of each fruit was added to 50 ml of acetonitrile containing 4 ml acetic acid/100 ml and homogenized in a blender for 2 min. After the recovery of the homogenate, 25 ml of acetonitrile containing 4 ml acetic acid/100 ml was 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 13,000*g* for 15 min at 4°C. The pellet following centrifugation was washed with 50 ml of acetonitrile containing 4 ml acetic acid/100 ml and centrifuged, and the resulting supernatants were combined with the initial extract.

The total anthocyanins were estimated by a pH differential method (Cheng & 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 $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29,600. Results were expressed as milligrams of cyanidin-3-glucoside equivalent per g of fresh and dry weights.

2.5. Phenol extraction and determination

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 five-fold before use (Singleton, Orthofer, & Lamuela-Raventos, 1999). 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/g gallic acid equivalent per g of fresh and dry weights.

2.6. Determination of flavonoids

The absorbance of flavonoids (extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH) was measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results are expressed as milligrams of catechin equivalents or mg per 100 g dry or fresh weights (Singleton et al., 1999).

2.7. Total antioxidant potential determination

The quality of the phenol antioxidants was measured by two following methods:

2.8. Total antioxidant status (TAA test)

The TAA was estimated using the ferrylmyoglobin/ABTS method (Rice-Evans & Miller, 1994). 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 gram of fresh or dry weights.

2.9. 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 by using the Greiss reagent, with which nitrite reacts to give a stable product absorbing at 542 nm (Marcocci et al., 1994; Saija et al., 1999).

Sodium nitroprusside solution was prepared immediately before the experiment, dissolving 10 mM sodium nitroprusside in 20 mM phosphate buffer, pH 7.4, previously bubbled with argon. The samples 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) $\times$ 100.

2.10. Statistical analysis

To verify the statistical significance of all parameters the values of means and $\pm$SD were calculated. Where it was appropriate, the data by two-way ANOVA were tested. The P values of less than 0.05 were adopted as statistically significant. All following data are means of five measurements.

3. Results and discussion

Phenolic acids were found in both fruits. The contents of gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, salicylic, ferulic, anisic and sinapic acids in peels of the white grapefruit and his hybrid were significantly higher than in their pulps. Ferulic acid was the major component in both pulp and peel, followed by *p*-coumaric, sinapic, and caffeic acid. Total concentration (nmol/g) of the four hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic and sinapic) was higher in pulp (362) and peel (1513) of white grapefruit than in its hybrid (272 and 1277, respectively).

The highest content of ferulic and the lowest of caffeic acids were in peels of both white grapefruit and its hybrid. The content of sinapic acid was almost equal in both fruits. The ratio between the concentrations of ferulic and sinapic acids and that of caffeic and *p*-coumaric acids may be a simple parameter for differentiating these two fruits, which was for pulp about 1.62

and 1.57 and for peel 5.8 and 5.0 for grapefruit hybrids and white grapefruits, respectively.

The role of hydroxycinnamic acid compounds as antioxidants and free radical scavengers has been pointed out (Chen & Ho, 1997). Our results are in correspondence with others concerning the distribution of caffeic, *p*-coumaric, ferulic, and sinapic acids and the predominance of ferulic acid over the other hydroxycinnamic acids (Peleg et al., 1991; Fernandez de Simon, Perez-Ilzarbe, Hernandez, Gomez-Cordovez, & Estrella, 1992; Bocco et al., 1998; Rapisarda et al., 1999).

Generally peels possess greater amount of bioactive components (Gorinstein et al., 2001a, b). Also the results of this investigation support this principle: the content of phenolic acids in peels were significantly higher than in pulp in both fruits. These results are in accordance with others who indicate that peels are an interesting source of phenolic compounds (Bocco et al., 1998).

Hydroxycinnamic acids were highly correlated with each other and also with anthocyanins. The latter correlations were expected because hydroxycinnamic acids are the precursors of the anthocyanins (Heller & Forkmann, 1988). The results of the determination of the content of phenols in the present study are in accordance with the results of others (Vinson et al., 2001).

The content of total flavonoids (mg/100 g FW) in peeled hybrids and white grapefruits were 47.12 ± 4.1 and 37.7 ± 3.2 and in their peels 92.5 ± 8.2 and 74.4 ± 6.9, respectively. The classification of the orange and grapefruit flavonoids has shown that the most abandon was naringin; therefore in our comparative studies instead of a common flavonoid, such as cathechin, we have used naringin (Bocco et al., 1998; Kawaii, Tomono, Katase, Ogawa, & Yano, 1999).

The antioxidant profile of white grapefruit and its hybrid is given in Table 1.

As can be seen, the total phenol content in both pulps and peels of grapefruit's hybrids is significantly higher than in white grapefruits.

In our recent investigations we have studied the total antioxidant potential of different fruits using total radical-trapping antioxidative potential test (Gorinstein et al., 2001a, b, 2002; Leontowicz et al., 2002). There are many methods for total antioxidant determination and every one has its limitations (Gorinstein et al., 2001a; Yu et al., 2002). It was shown that some antioxidant assay methods give different antioxidant activity trends (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). Therefore, in this investigation the free radical scavenging properties of the studied fruits were determined by two different methods (TAA and NO tests), which are more specific.

As far as we know, there are not such investigations of this grapefruit hybrid.

We have found that the hybrid has a higher level of antioxidant activity than white grapefruit (Table 1). As was mentioned, in the recent studies we have determined the antioxidant activity of different fruits by the TRAP test (Gorinstein et al., 2001a, b; Leontowicz et al., 2002), which is 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 had a considerable range and were slightly lower than the results of the present investigation. We have found that on the basis of wet weight that apples have relatively high antioxidant capacity in comparison with pears and peaches and are preferable for disease preventing diets (Gorinstein et al., 2002).

Our results of the TAA for white grapefruit were similar to those found by Wang et al. (1996) and Cao et al. (1997). Wang et al. (1996) have shown that the oxygen radical absorbance capacity (ORAC, μmol TE/g) for grapefruit, pink, is 4.83 of wet weight (WW) and 48.3 of dry weight (DW). The data of the present report of TAA (μmol TE/g) for WW and DW of white grapefruit pulp are slightly higher: 5.21 and 46.9 (Table 1) than in the literature (Wang et al., 1996). It is not surprising that some different results of the antioxidant

Table 1
Antioxidant profile of white grapefruit and its hybrid[a]

Fruits	Total phenols[b] (μmol/g)		Free phenols[b] (μmol/g)		Total anthocyanins[c] (μg/g)		TAA[d] (μmol TE/g)		NO test (AE) × 10^3
	WW	DW	WW	DW	WW	DW	WW	DW	
Hybrid (pulp)	9.2 ± 0.9	69.6 ± 6.1	1.2 ± 0.1	9.1 ± 0.8	0.8 ± 0.1	6.1 ± 0.5	6.95 ± 0.5	52.6 ± 4.9	15.4
Hybrid (peel)	13.9 ± 1.1	171.9 ± 9.1	2.0 ± 0.2	24.7 ± 2.1	1.6 ± 0.3	19.8 ± 1.7	8.52 ± 0.7	105.4 ± 7.1	19.3
White grapefruit (pulp)	7.0 ± 0.9	63.0 ± 5.9	0.8 ± 0.1	7.2 ± 0.7	0.5 ± 0.1	4.5 ± 0.3	5.21 ± 0.9	46.9 ± 4.1	11.6
White grapefruit (peel)	8.4 ± 0.9	134.0 ± 8.9	1.5 ± 0.2	23.9 ± 2.1	1.1 ± 0.1	17.5 ± 1.5	6.31 ± 0.5	100.6 ± 9.8	14.5

[a] Data are means (M) ± standard deviations (SD) of five measurements of white grapefruit and Jaffa sweeties (grapefruit hybrid).
[b] Data expressed as micrograms of gallic acid equivalents.
[c] Data expressed as micrograms of cyanidin-3 glucoside per g of wet weight (WW basis) or dry weight (DW basis).
[d] Data expressed as micromol of Trolox equivalents per g of wet weight (WW basis).

activities were obtained: antioxidant activity of investigated samples depends upon which free radical or oxidant is used in the assay.

Different assays with different free radical generators, end-points and quantification system were used in our previous investigations (Gorinstein et al., 2002; Leontowicz et al., 2002). It could be that this method is not specific for methanol extracts of phenols (Gorinstein et al., 2002). It also can be explained by the use of other variety of grapefruits.

The antioxidant activity of this grapefruit's hybrid was determined for the first time in this study and therefore, no data are available in the literature to compare with our results.

In the free phenol extract, the quality was a sum of the phenols and any vitamin C present. As was indicated, we have studied the total antioxidant potential of different fruits using total radical-trapping antioxidative potential test (Gorinstein et al., 2001a, b, 2002; Leontowicz et al., 2002). We have found in these investigations that ascorbic acid contributed less than phenols to the antioxidant capacity of fruits. Also others have found that ascorbic acid is generally a minor component, compared with the phenols of fruits (Prior et al., 1998; Rapisarda et al., 1999; Vinson et al., 2001).

There was no significant difference between the quality of the free and total phenols in the fruits, although the total polyphenols quality was higher ($R^2 = 0.9446$ and 0.9446) than that of the free phenols ($R^2 = 0.8212$ and 0.8746) as determined by TAA and NO test, respectively. These results are in agreement with Rapisarda et al. (1999), Paganga et al. (1999) and Vinson et al. (2001).

As can be seen, the correlation coefficients of linear regression between the concentration of individual antioxidants and total antioxidant activity have shown different correlation levels (Figs. 1 and 2). The best correlation was between total phenols and free phenols and the total antioxidant activity (Fig. 1A and B). Good correlation levels were observed also for anthocyanins: $R^2 = 0.8068$ and 0.8612 as determined by TAA and NO tests, respectively (Fig. 2A). Good correlation was observed between TAA and NO tests and flavonoids: $R^2 = 0.932$ and 0.889, respectively (Fig. 2B).

It was important to examine the correlation between the content of the total polyphenols and the total antioxidant potential because some authors have reported that there is no correlation between the content of these main antioxidant compounds and the radical scavenging capacity (Yu et al., 2002). The results obtained by us did not support these claims. The correlation of the total phenolic content and the total antioxidant potential was very high ($R^2 = 0.9466$ for both TAA and NO tests).

These data are in accordance with others, who have shown that high total phenols content increases

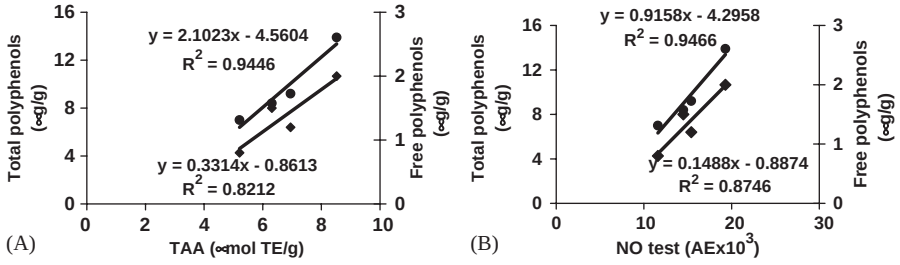

Fig. 1. Relationship, calculated by linear regression analysis for the studied Jaffa sweeties and grapefruits between antioxidant activities, total and free polyphenols: (A) •TAA test (μmol TE/g; X) to the concentration of total polyphenols (μmol/g; Y_1) and ♦TAA test (μmol TE/g; X) to the concentration of free polyphenols (μmol/g; Y_2). (B) •NO test [(AE ($\times 10^3$), X] to the concentration of total polyphenols (μmol/g; Y_1) and ♦TAA test (μmol TE/g; X) to the concentration of free polyphenols (μmol/g; Y_2).

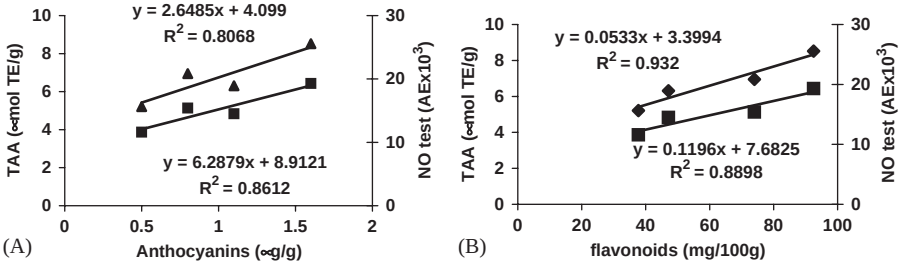

Fig. 2. Relationship, calculated by linear regression analysis for the studied Jaffa sweeties and grapefruits between antioxidant activities and anthocyanins and flavonoids: (A) ▲ concentration of anthocyanins (μg/g; X) to TAA test (μmol TE/g; Y_1) and ■ concentration of anthocyanins (μg/g; X) to NO test [(AE ($\times 10^3$), Y_2]. (B) ♦Concentration of flavonoids (mg/100 g, X) to TAA test (μmol TE/g; Y_1) and ■ concentration of flavonoids (mg/100 g, X) to NO test [(AE ($\times 10^3$), Y_2].

antioxidant activity (Velioglu, Mazza, Gao, & Oonah, 1998; Holasova et al., 2002) and there is a linear correlation between phenolic content and antioxidant activity (Gheldof & Engeseth, 2002).

In summary, the antioxidant activities of the studied grapefruit's hybrids and white grapefruits were measured using two assays with different reactive species. Based on the fresh weight of these fruits, the antioxidant activity against peroxyl radicals of grapefruit's hybrid is higher than of white grapefruit. The studied fruits contain a group of natural antioxidants that have not only a high antioxidant activity, but also a good antioxidant quality that could enrich lower density lipoproteins, thereby protecting them from oxidation and preventing development of atherosclerosis and other diseases. Therefore, the supplementation of natural antioxidants through a balanced diet containing enough fruits could be much more effective and economical than the use of individual antioxidants, such as ascorbic acid or α-tocopherol for protecting of the body against various oxidative stresses.

4. Conclusions

1. Both studied fruits contain a high concentration of natural antioxidants that have not only a high antioxidant activity, but also a good antioxidant quality.
2. The total phenol content and the antioxidant potential are significantly higher in grapefruit's hybrid than in white grapefruit.

References

Ascherio, A., Rimm, E. B., Giovannucci, E. L., Colditz, G. A., Rosner, B., Willett, W. C., Sacks, F., & Stampfer, M. J. (1992). A prospective study of nutritional factors and hypertension among US men. *Circulation*, *86*, 1475–1484.

Bocco, A., Cuvelier, M. E., Richard, H., & Berset, C. (1998). Antioxidant activity and phenolic composition of citrus peel and seed extracts. *Journal of Agricultural and Food Chemistry*, *4*, 2123–2129.

Cao, G., Sofic, E., & Prior, L. R. (1997). Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. *Free Radicals in Biology and Medicine*, *22*, 749–760.

Chen, J. H., & Ho, J. (1997). Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *Journal of Agricultural and Food Chemistry*, *45*, 2374–2378.

Cheng, G. W., & Breen, P. J. (1991). Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *Journal of American Society of the Horticultural Science*, *116*, 865–869.

Clifford, M. N. (2000). Chlorogenic acids and other cinnamates—nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture*, *80*, 1033–1042.

Fernandez de Simon, B., Perez-Ilzarbe, J., Hernandez, T., Gomez-Cordovez, C., & Estrella, I. (1992). Importance of phenolic compounds for the characterization of fruit juices. *Journal of Agricultural and Food Chemistry*, *40*, 1531–1535.

Gheldof, N., & Engeseth, N. J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry*, *50*, 3050–3055.

Gorinstein, S., Martin-Belloso, O., Park, Y. S., Haruenkit, R., Lojek, A., Číž, M., Caspi, A., Libman, I., & Trakhtenberg, S. (2001a). Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry*, *74*, 309–315.

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemzer, M., Weisz, M., Trakhtenberg, S., & Martin-Belloso, O. (2001b). Comparative content of dietary fiber, total phenolics, and minerals in persimmon and apples. *Journal of Agricultural and Food Chemistry*, *49*, 952–957.

Gorinstein, S., Martin-Belloso, O., Lojek, A., Číž, M., Soliva-Fortuny, R., Park, Y. S., Caspi, A., Libman, I., & Trakhtenberg, S. (2002). Comparative content of some phytochemicals in Spanish apples, peaches and pears. *Journal of the Science of Food and Agriculture*, *82*, 1166–1170.

Halliwell, B. (1994). Free radicals and antioxidants: A personal view. *Nutritional Review*, *52*, 253–265.

Heller, W., & Forkmann, G. (1988). Biosynthesis. In J. B. Harborne (Ed.), *The flavonoids. Advances in researches* (pp. 399–425). London: Chapman and Hall.

Hertog, M. G. L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., Nedeljkovic, S., Pekkarin, M., Simuc, B. S., Toshima, H., Feskens, E. J. M., Hollman, P. C. H., & Katan, M. B. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine*, *155*, 381–386.

Holasova, M., Fiedlerova, V., Smrcinova, H., Orsak, M., Lachman, J., & Vavreinova, S. (2002). Buckwheat—the source of antioxidant activity in functional foods. *Food Research International*, *35*, 207–211.

Joshipura, K. J., Ascherio, A., Manson, J. E., Stampfer, M. J., Rimm, E. B., Speizer, F. E., Hennekens, C. H., Spiegelman, D., & Willett, W. C. (1999). Fruit and vegetable intake in relation to risk of ischemic stroke. *Journal of American Medical Association*, *282*, 1233–1239.

Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., & Yano, M. (1999). Quantitation of flavonoid constituents in citrus fruits. *Journal of Agricultural and Food Chemistry*, *47*, 3565–3571.

Larrauri, J. A., Ruperez, P., Bravo, L., & Saura-Calixto, F. (1996). High dietary fibre powders from orange and lime peels: Associated polyphenols and antioxidant capacity. *Food Research International*, *29*, 757–762.

Leontowicz, H., Gorinstein, S., Lojek, A., Leontowicz, M., Číž, M., Soliva-Fortuny, R., Park, Y. S., Jung, S. T., Trakhtenberg, S., & Martin-Belloso, O. (2002). Comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. *Journal of Nutritional Biochemistry*, *13*, 603–610.

Marcocci, L., Packer, L., Droy-Lefaix, M. T., Sekaki, A., Garde, S., & Albert, M. (1994). Antioxidant action of *Ginkgo biloba* extract EGb 761. *Methods of Enzymology*, *234*, 462–475.

Miller 3rd, E. R., Appel, L. J., & Risby, T. H. (1998). Effect of dietary patterns on measures of lipid peroxidation: Results from a randomized clinical trial. *Circulation*, *98*, 2390–2395.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J., & Deemer, E. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry*, *50*, 3122–3128.

Paganga, G., Miller, N., & Rice-Evans, C. A. (1999). The polyphenol content of fruit and vegetables and their antioxidant activities. What does a serving constitute. *Free Radical Research*, *30*, 153–162.

Peleg, H., Naim, M., Rouseff, R. L., & Zehavi, U. (1991). Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). *Journal of the Science of Food and Agriculture*, *57*, 417–426.

Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., & Mainland, C. M. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry*, *46*, 2686–2693.

Rapisarda, P., Tomaino, A., Lo Cascio, R., Bonina, F., de Pasquale, A., & Saija, A. (1999). Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *Journal of Agricultural and Food Chemistry*, *47*, 4718–4723.

Rice-Evans, C., & Diplock, A. T. (1993). Current status of antioxidant therapy. *Free Radicals in Biology and Medicine*, *15*, 77–96.

Rice-Evans, C., & Miller, N. J. (1994). Total antioxidant status in plasma and body fluids. *Methods in Enzymology*, *234*, 279–293.

Rice-Evans, C., Miller, N. J., & Paganga, G. (1996). Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radicals in Biology and Medicine*, *20*, 933–956.

Saija, A., Tomaino, A., Lo Cascio, R., Trombetta, D., Proteggente, A., de Pasquale, A., Uccella, N., & Bonina, F. (1999). Ferulic and caffeic acids as potential protective agents against photooxidative skin damage. *Journal of the Science of Food and Agriculture*, *79*, 476–480.

Sawamura, M., Kuriyama, T., & Li, Z. (1988). Rind spot, antioxidative activity and tocopherols in the flavedo of citrus fruits. *Journal of Horticultural Science*, *63*, 717–721.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*, *299*, 152–178.

Slinkard, K., & Singleton, V. L. (1997). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture*, *28*, 49–55.

Tomás-Barberán, F. A., & Clifford, M. N. (2000). Flavonoids, chalcones and dihydrochalcones—nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, *80*, 1073–1080.

Velioglu, Y. S., Mazza, G., Gao, L., & Oonah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, *468*, 4113–4117.

Vinson, J. A., Dabbagh, Y. A., Serry, M. M., & Jang, J. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry*, *43*, 2800–2802.

Vinson, J. A., Su, X., Zubik, L., & Bose, P. (2001). Phenol antioxidant quantity and quality in foods: Fruits. *Journal of Agricultural and Food Chemistry*, *49*, 5315–5321.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry*, *44*, 701–705.

Wang, H., Cao, G., & Prior, R. L. (1997). Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural and Food Chemistry*, *45*, 304–309.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., & Qian, M. (2002). Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry*, *50*, 1619–1624.