DETERMINATION OF NARINGIN AND HESPERIDIN IN CITRUS FRUIT BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY. THE ANTIOXIDANT POTENTIAL OF CITRUS FRUIT

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SUMMARY

Naringin and hesperidin in dimethyl sulfoxide extracts of citrus fruit (oranges, red and blond grapefruit, and sweeties – a hybrid of pummelo and blond grapefruit) have been analyzed by high-performance liquid chromatography with a mobile phase prepared from 2% aqueous acetic acid and acetonitrile. The detection wavelength was at 285 nm. Antioxidant assays based on hydrogen atom-transfer reactions (oxygen radical absorbance capacity, ORAC) and on electron transfer (total phenols by use of Folin–Ciocalteu reagent, FCR; trolox-equivalent antioxidant capacity, TEAC; ferric ion-reducing antioxidant power, FRAP; and reaction with 1,1′-diphenyl-2-picrylhydrazyl, DPPH) were used to compare the antioxidant potential of citrus fruit and their main flavonoids. The three antioxidant assays (FRAP, TEAC, and DPPH) were performed with prolonged duration of the assay time, because all fruit extracts require long reaction times to approach the end point in the scavenging reaction. Lipophilic and hydrophilic fractions from citrus fruit were investigated by the ORAC reaction. Relationships between the main flavonoids, total polyphenols, and antio-

xidant potential obtained by use of the FRAP, TEAC, and DPPH procedures showed that correlation coefficients are higher for polyphenols than for the main flavonoids (as markers of 100% citrus juices). HPLC is a precise method for obtaining reliable data on the bioactivity of citrus fruit grown under the same geographical and climatic conditions. The bioactivity of citrus fruit studied by use of five different antioxidant assays was in the order sweetie > red grapefruit > blond grapefruit = orange. Hesperidin and naringin are only partially responsible for the overall antioxidant activity of citrus fruit.

INTRODUCTION

The beneficial health effects of a diet supplemented with fruit and vegetables have enhanced interest in their bioactive compounds [1–4]. It has been shown that the positive effect of these natural products is usually connected with their antioxidant compounds [1–4,5]. Naringin and hesperidin are the main citrus flavonoids with physiological properties present in grapefruit and orange juice [1,2,6]. These flavonoids have been detected in human plasma after orange and grapefruit diets [6]. There are numerous reports of HPLC analysis of the composition of commercial juices, concentrates, fresh oranges, and grapefruit [5,7–10].

Commercial brands of grapefruit juice have been analyzed for their flavonoid content by HPLC. Naringin was identified in all grapefruit juices [7]. Other investigators obtained similar results [10–12]. Although hesperidin and naringin are the predominant flavonoids in oranges and grapefruit, few investigators have studied their antioxidant activity [1,2,13, 14]. The amounts of bioactive compounds in fruit, including citrus flavonoids, are a function of geographical region, climate, soil conditions, type of cultivar, growing season, harvest date, storage, low-dose irradiation, and other conditions [15,16]. It is natural that authors who study the bioactive compound content and antioxidant potential of citrus and other fruit from different geographical regions have obtained different results [9,12,17–19]. It must be emphasized that the work cited above did not include investigation of the relationship between the amount of flavonoids and the antioxidant potential of the fruit. It was therefore decided to use high-performance liquid chromatography to determine the naringin and hesperidin content of four types of citrus fruit from the same region (Sharon, Israel) and to determine the correlation between the amounts of these compounds and the antioxidant potential of the fruit. The novelty of this

investigation is assessment of the bioactivity of two cultivars of the same citrus fruit (Jaffa red Star Ruby (Sunrise) and blond grapefruit (*Citrus paradisi*)), Jaffa blond (Shamouti) oranges (*Citrus sinensis*), and pummelo–blond grapefruit hybrid (*Citrus paradisi* var Jaffa Sweetie) from the same harvest season grown under the same geographical and climatic conditions.

EXPERIMENTAL

Materials

Jaffa blond (Shamouti) oranges (*Citrus sinensis*), Jaffa red Star Ruby (Sunrise) and blond grapefruit (*Citrus paradisi*), and pummelo–blond grapefruit hybrid (*Citrus paradisi* var Jaffa Sweetie) of similar maturity were grown in Israel and harvested in the season 2004–2005.

Trolox (6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid); 1,1′-diphenyl-2-picrylhydrazyl (DPPH); butylated hydroxyanisole (BHA); 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); naringin, and hesperidin (95%) were from Sigma (St Louis, MO, USA). 2,2′-Azobis-2-methylpropanimidamide dihydrochloride (AAPH) and fluorescein were from Merck Eurolab (Darmstadt, Germany). All reagents were analytical grade.

Sample Preparation for HPLC

Fruit was peeled and the pulp was blended to a fine slurry. The juice was typically obtained by use of mechanical juice maker and was prevented from oxidizing. Juice was divided into portions and both juice and pulp were stored frozen at −80°C for future analysis. Slurry (7.1 g) was weighed and centrifuged at 3000 rpm in a refrigerated Eppendorf centrifuge. The supernatant was separated and stored in a 25 mL volumetric flask. The residue was washed with deionized water (3 × ~5 mL) and the aqueous solutions were combined in the volumetric flask. The concentration of the solution in terms of the total weight of edible fruit was 286 g L^{-1}. The residues were extracted with dimethyl sulfoxide (DMSO) three times; the final volume of DMSO was 25 mL. The aqueous and DMSO fractions were subjected to HPLC analysis for determination of naringin (sweeties and grapefruits) and hesperidin (oranges).

HPLC

HPLC was performed with a Shimadzu (Kyoto, Japan) DGU-14A system equipped with a model LC-10AT-VP liquid chromatography pump,

an autoinjector, and a diode-array detector. Shimadzu software was used to calculate peak areas. Compounds were separated on a Spherisorb ODS1 column from Waters Instruments (MA, USA). The mobile phase was a gradient prepared from 2% aqueous acetic acid, pH 2.58 (component A) and acetonitrile (component B). The composition of the gradient, given in Table I, enabled separation of most of the phenolic compounds of interest.

Table I

The solvent gradient used

Time (min)	A(%)	B (%)
0–15	100	0
15–45	100–70	0–30
45–50	70–0	30–100
50–55	0	100
55–60	0–100	100–0
60–90	100	0

The injection volume was 20 μL (Hamilton syringe; Reno, NV, USA), the mobile phase flow rate 1 mL min^{-1}, the oven temperature 40°C, and the detection wavelength 285 nm.

Commercial naringin and hesperidin samples were also measured under the same conditions.

Sample Preparation for Analysis of Polyphenols and Determination of Antioxidant Potential

Extraction and Hydrolysis of Total Polyphenols

Lyophilizate (50 mg) was accurately weighed in a screw-capped tube. Total phenols were extracted with hydrochloric acid (1.2 M, 5 mL) in 50% methanol–water. The samples were vortex mixed for 1 min and heated at 90°C for 3 h with vortex mixing every 30 min. The samples were cooled, diluted to 10 mL with methanol, and centrifuged for 5 min at 4000g with a bench-top centrifuge, to remove solids [20]. Extraction of polyphenols for determination of antioxidant potential was also performed with water, acetone, methanol, and DMSO.

Analytical Methods

Total polyphenols were determined by the Folin–Ciocalteu method and measured at 765 nm with gallic acid as standard [21].

Trolox-equivalent antioxidant capacity (TEAC) is based on the ability of antioxidants to scavenge the blue-green $ABTS^{\cdot+}$ radical cation (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) compared with the scavenging ability of the water-soluble vitamin E analogue trolox. The $ABTS^{\cdot+}$ radical cation was generated by reaction of ABTS (250 μM) with $K_2S_2O_8$ (40 μM). The absorbance was monitored exactly 1 and 6 min after addition of 990 μL $ABTS^{\cdot+}$ solution to 10 μL of fruit extracts or trolox standards (final concentration 0–20 μM) in methanol or phosphate-buffered saline (pH 7.4). The decrease in the absorbance at 734 nm was calculated and plotted as a function of extract concentration, or of trolox for standard reference data [12]. For the modified assay, ABTS was dissolved in 20 mM acetate buffer (pH 4.5) and prepared with potassium persulfate as described above.

The ferric-reducing antioxidant power (FRAP) assay measures the ability of the antioxidants in the fruit samples to reduce ferric tripyridyl-triazine (Fe^{3+}-TPTZ) to the ferrous form (Fe^{2+}) which absorbs light at 593 nm. The complexes of the ferrous and ferric forms of iron with TPTZ are the main products of this reaction. FRAP was calculated by plotting a standard curve of absorbance against concentration of Fe^{2+} standard solution or trolox [22,23].

In the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay the volume of fruit extracts in different test tubes was adjusted to 100 μL by addition of MeOH and a methanolic solution of DPPH (0.1 mM, 5 μL) was added to the tubes. The control was prepared in the same way but without extract, and MeOH was used for baseline correction. Changes in the absorbance of the samples were measured at 517 nm. BHA was used for comparison [24,25].

Three antioxidant assays (DPPH, ABTS and FRAP) were compared after the same periods of time (10, 30, 60, and 120 min) using methanolic extracts of the fruit of the same concentration. For each antioxidant assay trolox [25] was used to establish a standard curve. All data were then expressed as trolox equivalents (TE).

Pulp (50 g) was weighed and extracted with water, methanol, acetone, and DMSO for the oxygen radical absorbance capacity (ORAC) assay. The solutions were combined and subjected to ORAC assay, as described elsewhere [26], with minor modifications, on a fluorescent plate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT, USA). The results were expressed as μmol trolox equivalent (TE) per 100 g fresh weight (FW).

RESULTS AND DISCUSSION

It has been shown that fruit and vegetables are important parts of a disease-preventing diet [1–3,27]. Some investigators have recommended including fruits with high antioxidant capacity in such diets [1,2,6]. Recently published results for regularly consumed fruit and vegetables gave different estimates of their antioxidant potential [3,18]. One group of authors showed that antioxidant potential was in the order strawberry > red plum >>> grapefruit = orange > green grape > apple > pear > peach [3]. Others [12,18] claim that apples have the highest total phenolic content and the highest antioxidant potential, followed by red grapes, strawberries, peaches, lemons, oranges, pears, and grapefruits. In our opinion, such discrepancies are understandable – it is not valid to compare the bioactive compound content and antioxidant potential of fruit grown under different conditions. The unique conditions of the same harvest season and the same geographical and climatic conditions enabled assessment of the amounts of bioactive compounds and the antioxidant potential of the citrus fruit studied in this report.

HPLC chromatograms obtained from the DMSO fraction show that many peaks are detected at 285 nm (Fig. 1). The retention times (RT) of naringin (32.76 min) and hesperidin (33.2 min) in this work were similar to those in other work – RT = 36.2 and 35.4 min for hesperidin in orange and
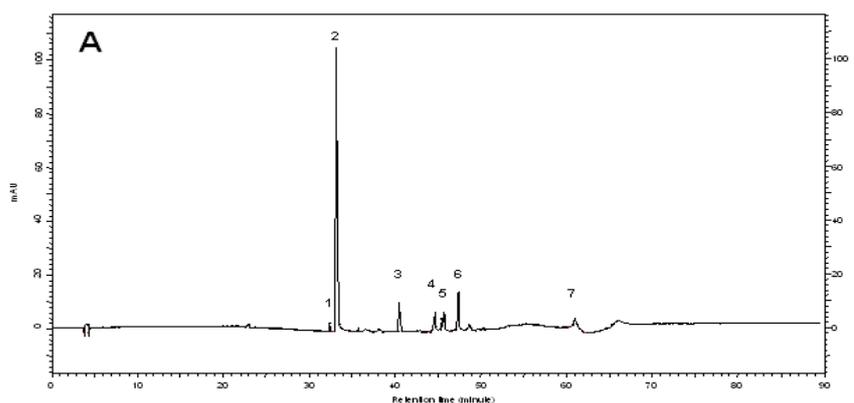

Fig. 1

HPLC chromatograms obtained from the dimethyl sulfoxide (DMSO) fraction of oranges and blond grapefruit. The main peak is that of hesperidin, the dominant phenolic compound in oranges. The dominant phenolic compound in grapefruit is naringin. A. Hesperidin (peak 2), RT = 33.23 min.
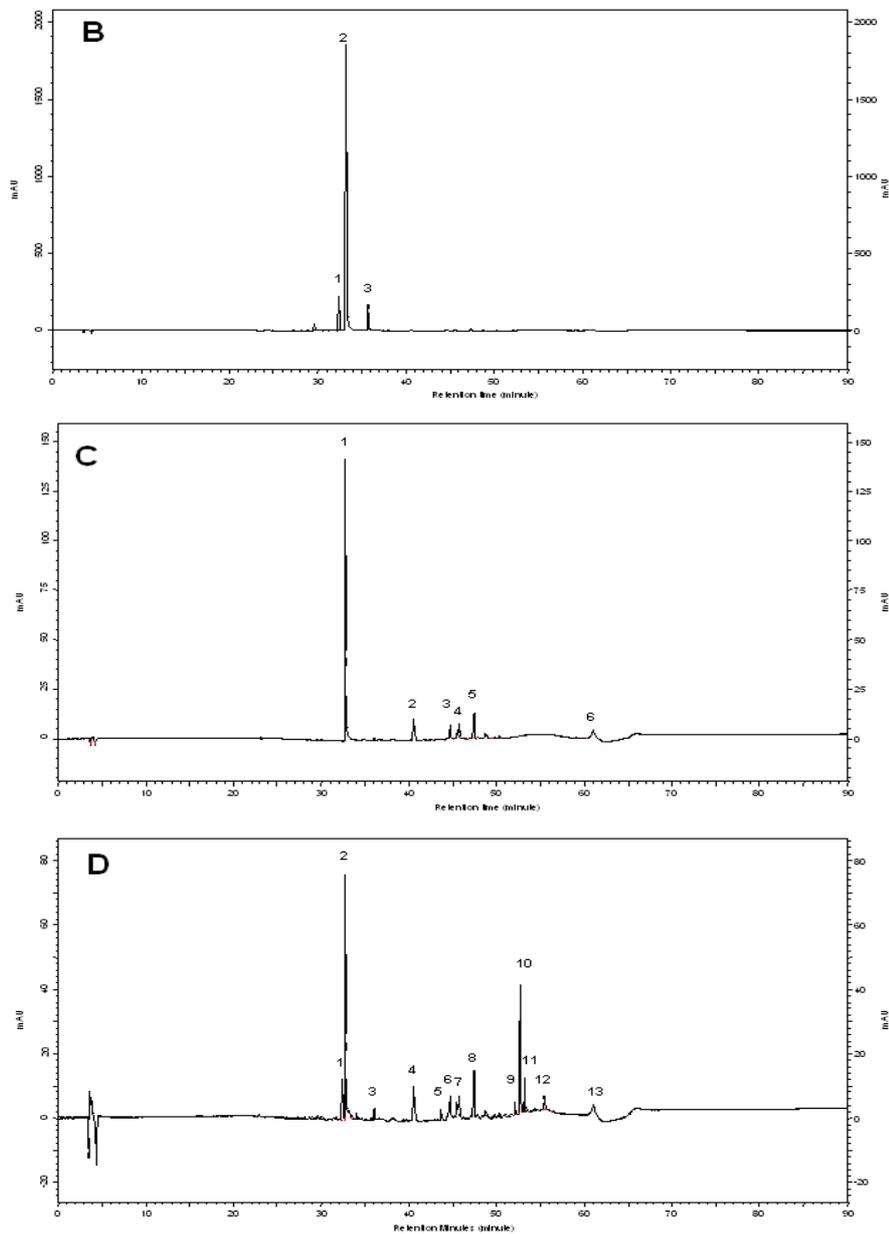
Fig. 1 (continued)

HPLC chromatograms obtained from the dimethyl sulfoxide (DMSO) fraction of oranges and blond grapefruit. The main peak is that of hesperidin, the dominant phenolic compound in oranges. The dominant phenolic compound in grapefruit is naringin. B. Orange (peak 2), RT = 33.203 min. C. Naringin (peak 1), RT = 33.754 min. D. Blond grapefruit (peak 2), RT = 32.759 min

naringin in grapefruit, respectively [4], and RT = 33.35 min for naringin and 34.20 min for hesperidin [8]. Slightly different HPLC conditions have been used for analysis of these compounds by other workers, for example a C_8 reversed-phase column with tetrahydrofuran–water–acetic acid, 21:77:2 (v/v), as mobile phase at 34°C [10], and use of a C_6 phenyl-phase column (and diode array detection) for analysis of naringin, hesperidin, and other flavonoid compounds [7]. These different conditions might explain why our results for naringin (52.5 ± 3.5, 49 ± 3, and 54.1 ± 2.9 mg per 100 g FW) and hesperidin (44.1 ± 3.7) (Tables II and III) were lower than in other reports [4,10].

Table II

The characteristics of the fruit and their flavonoids

Sample	Weight	Dilution	RT (min)	Peak area	Concentration $(mg\ mL^{-1})$ by HPLC
Hesperidin	5 mg	0.05 mL	33.227	892204	0.05
Naringin	5 mg	0.05 mL	32.754	1132011	0.05
Orange	7.15g	25 mL	33.207	15682745	0.879 (HE)
R. grapefruit	7.12g	25 mL	32.744	1772124	0.078 (NA)
B. grapefruit	7.12g	25 mL	32.759	607126	0.027 (NA)
Sweetie	7.14g	25 mL	32.748	1762022	0.076 (NA)

Abbreviations: RT, retention time; B, blond; R, red; NA, naringin; HE, hesperidin

Reported results for hesperidin in fresh, hand-squeezed juice from different cultivars of oranges were 12.2–25.4 mg per 100 g. Similarly, levels of naringin in grapefruit juice from different cultivars were 7.3–41.9 mg per 100 g [4,7,10]. Naringin and hesperidin have very similar structures but do not have the same antioxidant activity. Because most phenolic compounds absorb at the same wavelength, it is probable that other phenolic compounds contribute collectively to the radical-scavenging capacity (Fig. 1, Table I), as the major phenolic compounds in oranges and grapefruit are hesperidin and naringin, respectively. We also measured the antioxidant values of the two pure compounds obtained from commercial sources and quantified the concentrations of the compounds in each fruit (Table I). Naringin and hesperidin had different antioxidant activity in the TEAC (0.032 ± 0.001 and 0.99 ± 0.01 μmol TE μmol^{-1}, respectively) and FRAP (0.032 ± 0.001 and 1.69 ± 0.3 μmol Fe^{2+} μmol^{-1}, respectively) tests. Our results are in agreement with those of other authors, who showed that TEAC

Table III

Comparative data for the most important bioactivity indices for citrus fruit: total phenolic compounds (mg GAE per 100 g FW), naringin and hesperidin (mg per 100 g FW)

Indices[a]	Orange	R grapefruit	B grapefruit	Sweetie
Polyphenols[b]	138 ± 13	158 ± 7	147 ± 7.0	164 ± 17
Polyphenols [4]	126 ± 6.0	ND	150 ± 4.0	ND
Polyphenols [18]	81.2 ± 1.1	ND	49.6 ± 2.6	ND
Naringin[b]	ND	52.5 ± 3.5	49 ± 3.0	54.1 ± 2.9
Naringin [4]	ND	ND	197.1 ± 4.3	ND
Naringin [8]	ND	ND	44.7 ± 4.0	ND
Naringin [10]	ND	ND	58.5 ± 4.0	ND
Hesperidin[b]	44.1 ± 3.7	ND	ND	ND
Hesperidin [4]	98.5 ± 3.3	ND	ND	ND
Hesperidin [8]	39.2 ± 3.0	ND	ND	ND
Hesperidin [10]	24.7 ± 2.1	ND	ND	ND

Abbreviations: B, blond; R, red; GAE, gallic acid equivalents; FW, fresh weight; ND, not detected

[a]Numbers in square brackets are references to previous work

[b]Values are means ± SD from five measurements in this work

was 0.24 and 1.0 μmol TE μmol^{-1} for naringin and hesperidin, respectively [14]. Our results also confirm reports that the antioxidant properties of polyphenolic compounds were highest for quercetin, catechin, rutin, and epicatechin, at 4.7, 2.4, 2.4, and 2.2 μmol TE μmol^{-1}, respectively, and lowest for naringin, at 0.2 μmol TE μmol^{-1} [13].

We measured the methanol fraction of the fruit extracts, and the results were compared with literature data (Tables III and IV).

To obtain reliable data for possible changes in antioxidant potential the DPPH, FRAP, TEAC, and Folin assays were all used. These assays take into account the wide variety and range of action of antioxidant compounds present in citrus fruit [12], although all are based on electron transfer. The total polyphenol content and related total antioxidant (FRAP, DPPH, and TEAC) potentials (Tables III and IV) were significantly higher for sweeties and red grapefruit ($P < 0.05$). The polyphenol content in this work was similar to that measured by other investigators [4,18]. Our results for the antioxidant potential of the samples were within the wide range of reported literature data, and also in agreement with our recently published data. The results depended on the procedure used for extraction of the citrus fruits, i.e. the solvent used (acetone, methanol, or water) and the duration

Table IV

Comparative data for the most important bioactivity indices in citrus fruits: TEAC, ORAC (μmol TE per 100 g FW), and FRAP (μmol Fe^{2+} per 100 g FW)

Index[a]	Orange	R grapefruit	B grapefruit	Sweetie
TEAC[b]	722 ± 33	965 ± 64	783 ± 40	1007 ± 85
TEAC [4]	849 ± 25	ND	861 ± 53	ND
TEAC [9]	3740 ± 1.9	ND	ND	ND
TEAC [12]	874 ± 20	ND	ND	ND
FRAP[b]	801 ± 41	1024 ± 39	818 ± 39	1116 ± 36
FRAP [4]	1181 ± 6.0	ND	829 ± 6	ND
FRAP [9]	1880 ± 3.2	ND	ND	ND
FRAP [12]	2050 ± 4.1	ND	822 ± 7	ND
FRAP [17]	1500 ± 12	870 ± 8	820 ± 9	ND
FRAP [23]	942 ± 11	ND	808 ± 6	ND
ORAC[b]	1274 ± 16	1607 ± 14	1495 ± 47	1665 ± 19
ORAC [4]	1904 ± 259	ND	1447 ± 67	ND

Abbreviations: B, blond; R, red; FW, fresh weight; TEAC, trolox-equivalent antioxidant capacity; ORAC, oxygen radical absorbance capacity; FRAP, ferric-reducing/antioxidant power; ND, not detected
[a]Numbers in square brackets are references to previous work
[b]Values are means ± SD from five measurements in this work

and the temperature of extraction [1,2,9,17]. The antioxidant data also depend on the variety of fruit and on the geographical region in which it is grown. For oranges from Spain and Holland FRAP values were 1500 and 1080 μmol per 100 g, respectively; for grapefruit FRAP values were 810–870 μmol per 100 g for red from Honduras and 870 μmol per 100 g for yellow from Israel [17]. Comparative results for FRAP and TEAC covered a wide range and showed that the total antioxidant potential of citrus fruits and their juice is relatively high [3,4,6,12,23].

TEAC, DPPH, and FRAP values for each extract were similar and correlated well with total phenolic content. The TEAC and TRAP values for naringin and hesperidin were not similar, however (0.2 and 0.9 μmol TE μmol^{-1}, respectively). The relationship between values for total polyphenol concentrations and antioxidant activity (Fig. 2A) are rather interesting, because there is an excellent linear relationship (R^2 = 0.97–0.92). Correlations of naringin or hesperidin concentrations with antioxidant activity (Fig. 2B; R^2 = 0.84–0.70) were lower than for total polyphenols. It is worth remarking that TEAC and FRAP values for oranges are primarily a result of hesperidin whereas only a small fraction of the TEAC and
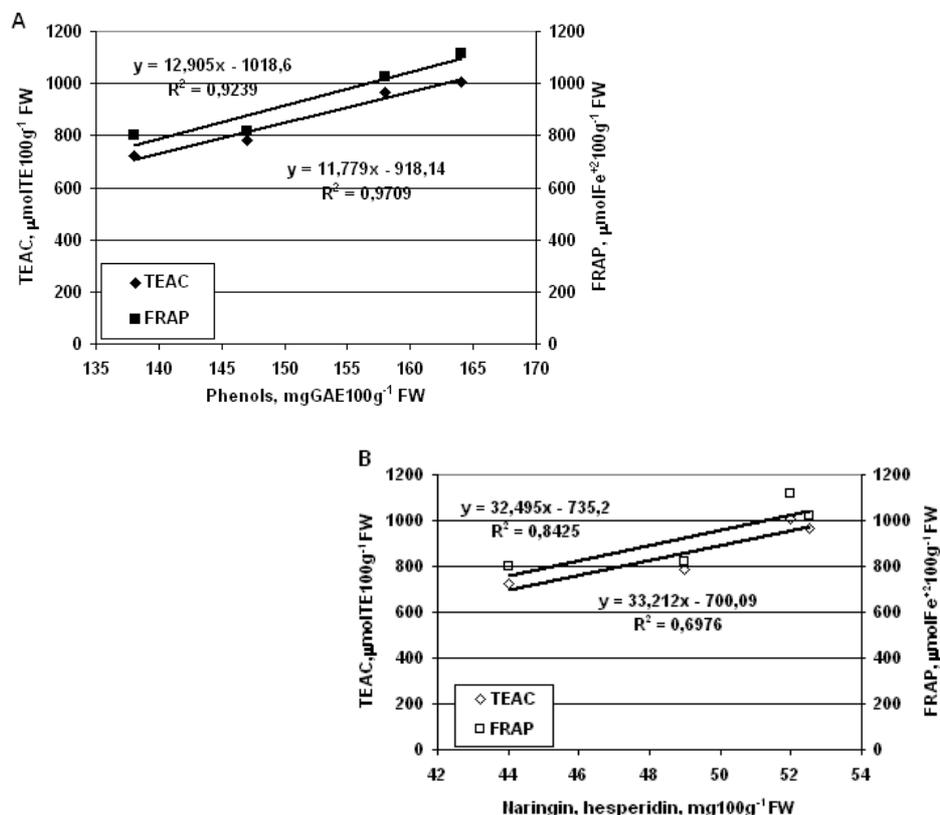
Fig. 2

Correlation, calculated by linear regression analysis, for citrus fruits between: A. ◆ polyphenol content (mg GAE per 100 g FW, X) and TEAC (μmol TE per 100 g FW, Y_1) and ■ polyphenol content (mg GAE per 100 g FW, X) and FRAP (μmol Fe^{2+} per 100 g^{-1} FW, Y_2); B. ◊ combined naringin and hesperidin content (mg per 100 g FW, X) and TEAC (μmol TE per 100 g FW, Y_1) and □ combined naringin and hesperidin constant (mg per 100 g FW, X) and FRAP (μmol Fe^{2+} per 100 g FW, Y_2). Abbreviations: GAE, gallic acid equivalents; TEAC, trolox-equivalent antioxidant capacity; FRAP, ferric-reducing/antioxidant power

FRAP values of grapefruit result from naringin. Most (>70%) of the TEAC and FRAP values arise from other compounds of unknown identity. The correlations between total polyphenols and the two scavenging antioxidant assays were similar to those obtained in other work [19] – there were strong correlations between antioxidant capacity and total phenols (TEAC, $R^2 =$ 0.91; FRAP, $R^2 = 0.83$) and total flavonoids (TEAC, $R^2 = 0.89$; FRAP, $R^2 = 0.82$). Our results for naringin and hesperidin were lower than those for

total flavonoids. Naringin is poorly soluble in water and no naringin was detected (HPLC, 285 nm) in the aqueous fraction from the juice. Other compounds that may not act as peroxy radical scavengers may be more important to its antioxidant properties.

The results from this investigation have shown that the total polyphenol, hesperidin, and naringin content and antioxidant potential differed from values reported in the literature, and depend on the extraction procedure used, geographical location, variety, and other variables.

The correlation obtained between the polyphenols and the DPPH, TEAC, and FRAP assays was expected, because all these assays are similar and work by the same mechanism (single-electron transfer).

Because the antioxidant potential of fruit extracts depends on the time of the assays, the samples were measured at the same concentration and after the same periods of time – 10, 30, 60, and 120 min. Antioxidant potentials varied among the four citrus fruits and among antioxidant assays. The highest was with FRAP, DPPH was intermediate, and the lowest was TEAC, irrespective of reaction time. The initial values (reaction time approximately 6–10 min) are comparable with those reported in the review articles cited [1–7]. The modified TEAC assay at lower pH (4.5) than in the previous used assay (pH 7.4) yields antioxidant values that are lower than those obtained by FRAP and DPPH [12,25]. The order of the antioxidant capacity of the samples was not affected by the antioxidant
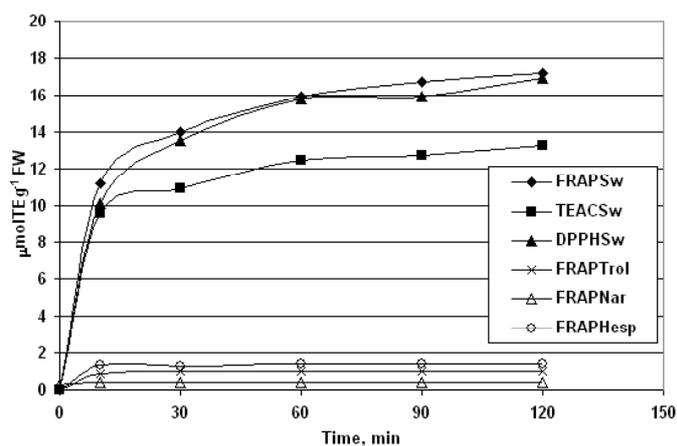

Fig. 3

Antioxidant potential (μmol TE per 100 g FW) of sweetie by the TEAC (at pH 4.5), FRAP, and DPPH assays, and of trolox, naringin, and hesperidin in the FRAP assay. Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl

method used. The sweetie had the highest antioxidant capacity, followed by red grapefruit, then blond grapefruit, and then orange. This pattern was the same for all the samples investigated, so only the kinetic changes for sweetie are presented in Fig. 3. Fruit extracts contain different classes of polyphenols, and true determination or comparison of the antioxidant potential of these fruits is complicated. The three assays gave different values, but the relative ranking was the same, and in agreement with other reports [25].

Results from the ORAC assay, based on hydrogen-atom transfer, were compared with the data reported above. Our ORAC results for total polyphenol antioxidant potential extracted with methanolic HCl differed slightly from those of other workers (Table IV, Fig. 4A). The results obtained for the water-soluble and DMSO fractions are plotted in Fig. 4B.
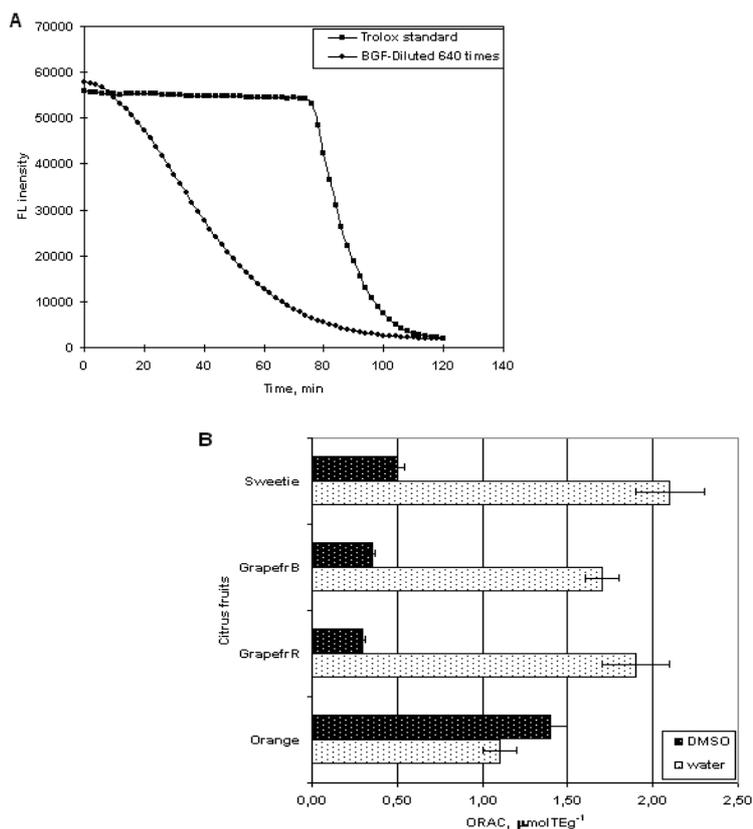

Fig. 4

ORAC antioxidant potential (μmol TE per g FW). A. Kinetic curves obtained for Trolox standard and blond grapefruit (BGF). B. Kinetic curves obtained for water and DMSO extracts of citrus fruits. Abbreviations: ORAC, oxygen radical absorbance capacity

For oranges the two fractions apparently have similar values whereas for grapefruit most of the ORAC value is in the water-soluble fraction. Total ORAC values for the four fruit are comparable.

The aqueous ORAC results for orange, sweetie, and blond grapefruit juice were 750 ± 9, 1558 ± 18, and 1384 ± 44 µmol TE per 100 g FW, respectively; the aqueous polyphenol results for the same fruit were 41.2 ± 4.1, 56.0 ± 5.4, and 66.3 ± 6.5 mg GAE per 100 g FW, respectively. For the acetone-extractable fraction the lipophilic ORAC results for orange, sweetie, and blond grapefruit juice were 524 ± 7.4, 107 ± 1.1, and 111 ± 1.5 µmol TE per 100 g FW and lipophilic polyphenol results for the same fruit juices were 9.0 ± 2.1, 2.4 ± 0.4, and 2.4 ± 0.5 mg GAE per 100 g FW, respectively. Results for fruit from different harvests also differed; in the previous year the hydrophilic ORAC results had been 1257 ± 18 for blond
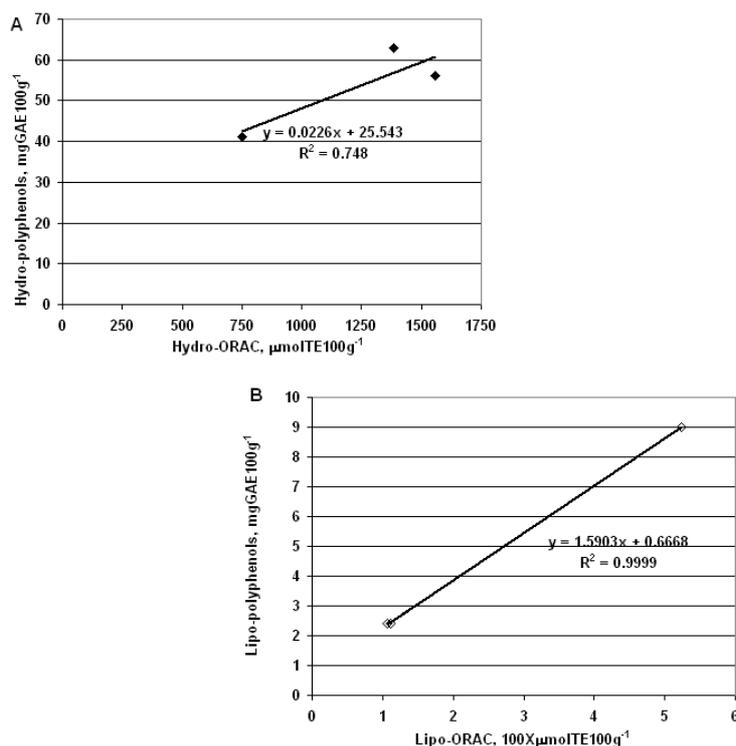

Fig. 5

Correlation, calculated by linear regression analysis, for citrus fruits between: A. ◆ polyphenol content (mg GAE per 100 g FW, X) and ORAC (µmol TE per 100 g FW, Y) in the hydrophilic fraction. B. ◊ polyphenol content (mg GAE per 100 g^{-1} FW, X) and ORAC (µmol TE 100 g FW, Y) in the lipophilic fraction

grapefruit juice and 1688 ± 24 for red grapefruit juice [27]. As shown in Fig. 5 there was high correlation ($R^2 = 0.99$) between lipophilic ORAC and polyphenols and low correlation ($R^2 = 0.75$) between hydrophilic ORAC values and total polyphenols, but the order for the investigated fruits was the same.

According to our data the bioactivity of these citrus fruits are in the order sweetie > red grapefruit > blond grapefruit = orange. These results are different from those of other workers who reported the order oranges > grapefruit [18]. Because these investigators purchased their fruit samples on three separate occasions from local supermarkets, we claim our data are more reliable.

CONCLUSIONS

Liquid chromatography is a precise method for obtaining reliable data about the bioactivity of citrus fruits grown under the same geographical and climatic conditions. According to five different antioxidant assays the bioactivity of the citrus fruit studied was in the order sweetie > red grapefruit > blond grapefruit = orange.

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