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ORIGINAL PAPER

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Effect of antioxidants and proteins on the quality of Israeli Jaffa red and blond grapefruits

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Abstract Some biochemical characteristics of Israeli Jaffa Star Ruby (red) and blond grapefruits were defined using atomic absorption spectrometry, antioxidant tests and the protein electrophoretic technique. It was found that the contents of dietary fibers, major and minor minerals, phenolic and ascorbic acids in both grapefruits were without significant differences. The contents of total polyphenols, anthocyanins and flavonoids were higher in red grapefruits, but also these differences were not significant. The antioxidant capacity was determined by two modified antioxidant methods, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\cdot+}$) and scavenging activity against nitric oxide, and was compared with the Folin–Ciocalteu assay. The two antioxidant assays used have shown that the antioxidant potential of the Star Ruby (red) grapefruit is higher than of the blond ($P<0.05$) grapefruit. Proteins were extracted and separated. Some small differences were found in the sodium dodecyl sulfate–protein bands in the region of 36 kDa. Antioxidants and proteins can be used for characterization of the quality of grapefruits as a fruit diet.

Keywords Differences of grapefruits · Antioxidant compounds · Proteins · Quality

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Introduction

Citrus fruits and their juices have been shown to produce a number of health benefits, including hypocholesterolemic and high-density lipoprotein cholesterol raising effects [1–3] and this was postulated to be largely due to the principal antioxidant compounds: flavanones and hesperidin from oranges and naringenin from grapefruit [4, 5]. In spite of the popularity of blond and Star Ruby (red) Jaffa grapefruits there are no comparative investigations of these citrus fruits grown in the same geographical region.

Therefore, we decided to compare the biochemical characteristics of the blond and red Jaffa grapefruits. Investigations of individual antioxidant compounds are important for understanding the health benefits of citrus fruits and their juices. However, the assessments of the bioactivity of individual compounds of fruits and vegetables do not reflect their true antioxidant values [6]. In this investigation not only individual bioactive compounds, but also the total antioxidant potential were assessed.

Antioxidant assay methods give different antioxidant activity trends [7, 8]. Therefore, it was decided to apply two other complementary assays, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\cdot+}$) and scavenging activity against nitric oxide (NO), and to compare them with the Folin–Ciocalteu method [9–11].

It is a common knowledge that proteins of foods supply the required building blocks for protein biosynthesis of humans. The role of all plant proteins is very important in the human metabolism [12, 13]; therefore,

the proteins of both grapefruits were studied. It was shown that determination of proteins is one of the major methods of quality control of citrus fruits [14, 15]. Major (Ca, K, Na, Mg) and minor (Fe, Cu, Zn, Mn) minerals play a very important role in biological processes. Iron, copper and manganese are very effective catalysts in prevention and treatment of atherosclerosis and its complications [16]. Mg and K are used in prevention and treatment of life-threatening arrhythmias, which are related to coronary artery disease [17, 18]. Ca is not only a basic part of the human skeleton but also insures the proper function of myocardium and heart vessels [18]. Fe is an integral part of hemoglobin and is used in the treatment of some forms of anemia. Recent investigations indicate that high stored Fe levels play a role in early atherogenesis, promote lipid peroxidation and can be an independent risk factor for coronary artery disease [19]. It was important to determine the contents of major and minor minerals. As far as we know there are no such investigations of both Jaffa grapefruits using two complementary antioxidant assays and electrophoretic techniques.

Materials and methods

Chemicals

6-Hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid (Trolox), sodium dodecyl sulfate (SDS), Griess reagent, sodium nitroprusside, Folin–Ciocalteu reagent and 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

Sampling procedure

Israeli Star Ruby (red) and blond Jaffa grapefruits (*Citrus paradisi*) of the same ripeness were harvested in the period of 2002–2003. The harvest time was 2 weeks around the following dates for each fruit: 10–20 October; orchard area Netanya. These harvest times are the best for obtaining the respective fruits at optimal maturity in Israel. At harvest time for Star Ruby (red) and blond Jaffa grapefruits the values of sugars (Brix) were 12–14 and 11–13%, of acidity were 1.6–1.9% and 1.7–1.9 and of juice content were 45–47 and 45–48%, respectively.

Samples (12 of each variety) were obtained from 24 randomly selected fruits for the determination of all the variables studied. These fruits were cleaned with tap water and manually separated into peels and peeled fruit.

Dietary fibers

Dietary fiber in the selected samples was analyzed by the modified AOAC method [20, 21]. Samples were treated with heat-stable α-amylase, protease and amyloglucosidase, followed by centrifugation (15 min, 3,000*g*) to separate the soluble and insoluble fractions and dialysis against water.

Major and minor minerals

Major (Na, K, Mg, Ca) and minor (Fe, Cu, Zn and Mn) minerals were determined as follows. The samples of peels and peeled fruit were lyophilized separately. Then 0.8 g of lyophilized samples was mineralized in a microwave oven with concentrated HNO_3. The concentrations of all the previously mentioned minerals were estimated using a PerkinElmer 5100 ZL atomic absorption spectrometer (PerkinElmer, Beaconsfield, Buckinghamshire, UK), using the flame method for Na, K, Mg, Ca, Fe, Cu and Zn and the flameless method for Mn [22].

Total polyphenols

Portions of 10 g of peeled grapefruits and their peels were separately homogenized with 125 ml 95% ethanol for 1 min and then gently boiled for 30 min. After this procedure, the fruit samples were cooled and filtered under vacuum using Whatman no. 1 filter paper. The filtrates were evaporated under vacuum at 60 °C until 10 ml and were then made up to 100 ml with distilled water. Total polyphenols were determined by the Folin–Ciocalteu method and were measured at 765 nm. The results were given in milligrams per 100 g fresh weight (FW) of gallic acid equivalent [23, 24].

Phenolic and ascorbic acids

Phenolic [25] and ascorbic [26] acids were determined by high-performance liquid chromatography. The results were expressed in milligrams per 100 g FW.

Anthocyanins and flavonoids

The total anthocyanins were measured by a pH differential method [27]. The absorbance was measured with a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = (A_{510} - A_{700})_{\mathrm{pH10}} - (A_{510} - A_{700})_{\mathrm{pH4.5}}$ with a molar extinction coefficient of cyanidin-3-glucoside of 29,600. The results were expressed as micrograms of cyanidin-3-glucoside equivalent per 100 g FW.

Flavonoids were extracted with 5% $NaNO_2$, 10% $AlCl_3{\cdot}6H_2O$ and 1 M NaOH and were measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed in milligrams per 100 g FW [23].

Total antioxidant capacity

As already mentioned, despite the high antioxidant capacity of individual polyphenols the antioxidant effect of the whole fruit could be low [6]. Therefore, in addition to the study of individual bioactive compounds we used two different complementary total antioxidant assays.

The antioxidant capacities were determined by two methods using ABTS with $K_2S_2O_8$ or with MnO_2 and scavenging activity against NO.

1. $ABTS^{\cdot+}$ radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After addition of 990 μl $ABTS^{\cdot+}$ solution to 10 μl of different extracts (0.2 mg/ml) or Trolox standards (final concentration 0–20 μM) in ethanol or phosphate-buffered saline, the absorbance was monitored exactly 1 and 6 min after the initial mixing [9].
2. $ABTS^{\cdot+}$ was prepared as well by passing a 5 mM aqueous stock solution of ABTS through manganese dioxide on a Whatman no. 5 filter paper. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2 μM Whatman poly(vinylidene difluoride) syringe filter. This solution was then diluted in 5 mM phosphate-buffered saline, pH 7.4, to an absorbance of 0.70. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the extracts and of Trolox for the standard reference data [9].

3. Scavenging activity against NO. Scavengers of NO compete with oxygen, leading to a reduced production of nitrite. The concentration of nitrite in the aqueous solution was assayed spectrophotometrically by using the Griess reagent and the absorbance was measured at 542 nm [10, 11]. The results were expressed as the percentage of inhibition.

Proteins

Approximately 30 ml of juice was filtered through filter paper. Twenty milliliters of filtrate was centrifuged at 15,000*g* for 20 min. The sediment was resuspended in 550 μl 0.5 M tris(hydroxymethyl)aminomethane–HCl (pH 8.3) buffer and mixed with an equal volume of Laemmli sample buffer. The solution was boiled for 3 min. After centrifugation at 15,000*g* for 15 min the supernatant was used for SDS-polyacrylamide gel electrophoresis (PAGE).

SDS-PAGE was carried out according to Laemmli [28], using a Hoeffer SE-600 apparatus. The Laemmli method was adapted for juice proteins: the resolving gel was 13.7% total acrylamide (T) and 1.7% cross linker (C); the stacking gel was 3.8% T and 1.8% C; the gel size was 140×160×1.5 mm. Five microliters of juice supernatant was loaded on the gel. The run was carried out at a constant current of 25 mA per gel. The gels were stained with 0.25% Coomassie Brilliant Blue R-250 in methanol/water/glacial acetic acid solution (5:5:1 v/v) and were destained in water.

Statistics

To verify the statistical significance of the parameters studied, the means and standard deviations of five measurements were determined. Where appropriate, differences between groups were tested by two-way analysis of variance. *P* values of less than 0.05 were considered significant.

Table 1 The contents of total, soluble and insoluble fibers in red and blond peeled grapefruits and their peels (g/100 g fresh fruit)

Fruits	Total	Soluble	Insoluble
Red peeled grapefruits	1.36±0.1[a]	0.53±0.05[a]	0.83±0.08[a]
Blond peeled grapefruits	1.34±0.1[a]	0.52±0.05[a]	0.82±0.08[a]
Peels of red grapefruits	1.93±0.2[b]	0.72±0.07[b]	1.21±0.1[b]
Peels of blond grapefruits	1.91±0.2[b]	0.72±0.07[b]	1.19±0.1[b]

Values are means ± standard deviations (*SD*) of five measurements. Means in columns without common superscript *letters* differ significantly ($P< 0.05$).

Results

The contents of total, soluble and insoluble dietary fibers (Table 1) in peeled red and blond grapefruits and their peels were comparable ($P>0.05$).

The contents of major (Na, K, Mg and Ca; Table 2) and minor (Fe, Mn, Zn and Cu; Table 3) minerals in peeled blond and Star Ruby (red) grapefruits and their peels were comparable ($P>0.05$).

The contents (Table 4) of total polyphenols, anthocyanins and flavonoids were higher in red grapefruit, but statistically were not significant ($P>0.05$).

The contents of fibers (Table 1), major (Table 2) and minor (Table 3) minerals, total polyphenols, anthocyanins and flavonoids (Table 4) in peels were significantly higher those than in peeled fruits ($P<0.05$).

The contents of ferulic, sinapic, *p*-coumaric, caffeic and ascorbic acids in peeled grapefruits (Fig. 1a) and peels (Fig. 1b) are comparable. Among the phenolic acids the highest concentration was of ferulic and the lowest was of caffeic acid. The content of ascorbic acid was significantly higher than for all the phenolic acids ($P<0.05$). The contents of ferulic, sinapic, *p*-coumaric, caffeic and ascorbic acids in peels were significantly higher than those in peeled grapefruits ($P<0.05$).

The results obtained with manganese dioxide (Fig. 2b) of red peeled grapefruit (RPGF1) and blond peeled grapefruit (BPGF1) and in peels of red grapefruit (PeelRGF1) and peels of blond grapefruit (Peel BGF1) were compared with the results of another variation of the ABTS decolorization assay where the $ABTS^{\cdot+}$ radical cation was produced by reacting ABTS (Fig. 2a) with potassium persulfate red peeled grapefruit (RPGF), blond peeled grapefruit (BPGF), peels of red grapefruit (PeelRGF) and peels of blond grapefruit (PeelBGF). ABTS with $K_2S_2O_8$ showed the following antioxidant capacities in millimoles of Trolox equivalents (TE) per g for RPGF, BPGF, PeelRGF and PeelBGF 9.65±0.6[b], 6.83±0.5[a], 13.82±0.7[c] and 10.34±0.5[b], respectively, and with MnO_2 the following antioxidant capacities were calculated for

Table 2 The contents of major minerals in red and blond peeled grapefruits and their peels (mg/100 g fresh fruit)

Fruits	Na	K	Mg	Ca
Red peeled grapefruits	2.6±0.2[a]	91.1±7.5[a]	4.5±0.4[a]	3.5±0.3[a]
Blond peeled grapefruits	2.5±0.2[a]	89.8±7.4[a]	4.3±0.4[a]	3.3±0.3[a]
Peels of red grapefruits	5.7±0.5[b]	151.3±11.7[b]	8.4±0.6[b]	8.1±0.6[b]
Peels of blond grapefruits	5.5±0.5[b]	149.7±11.6[b]	8.2±0.6[b]	7.9±0.6[b]

Values are means ± SD of five measurements. Means in columns without common superscript *letters* differ significantly ($P<0.05$).

Table 3 The contents of minor minerals in red and blond peeled grapefruits and their peels (μg/100 g fresh fruit)

Fruits	Fe	Mn	Zn	Cu
Red peeled grapefruits	110±10.1[a]	12.1±1.5[a]	49.5±4.2[a]	39.5±3.2[a]
Blond peeled grapefruits	108±10.0[a]	11.8±1.4[a]	47.3±4.1[a]	37.3±3.1[a]
Peels of red grapefruits	150±12.5[b]	19.3±1.7[b]	78.4±5.6[b]	63.1±6.1[b]
Peels of blond grapefruits	148±12.4[b]	18.7±1.6[b]	76.2±5.5[b]	61.9±6.1[b]

Values are means ± SD of five measurements. Means in columns without common superscript *letters* differ significantly ($P<0.05$).

Table 4 Contents of total polyphenols, anthocyanins and flavonoids in red and blond peeled grapefruits and their peels

Fruits	Total polyphenols (mg/100 g fresh weight)	Anthocyanins (μg/100 g fresh weight)	Flavonoids (mg/100 g fresh weight)
Red peeled grapefruits	158.3±7.1[a]	51.5±4.6[a]	21.61±1.3[a]
Blond peeled grapefruits	149.1±6.3[a]	49.3±4.5[a]	19.53±1.2[a]
Peels of red grapefruits	185.1±7.3[b]	87.6±7.3[b]	71.61±6.3[b]
Peels of blond grapefruits	168.2±7.0[b]	85.3±7.2[b]	69.53±6.2[b]

Values are means ± SD of five measurements. Means in columns without common superscript *letters* differ significantly (P<0.05).

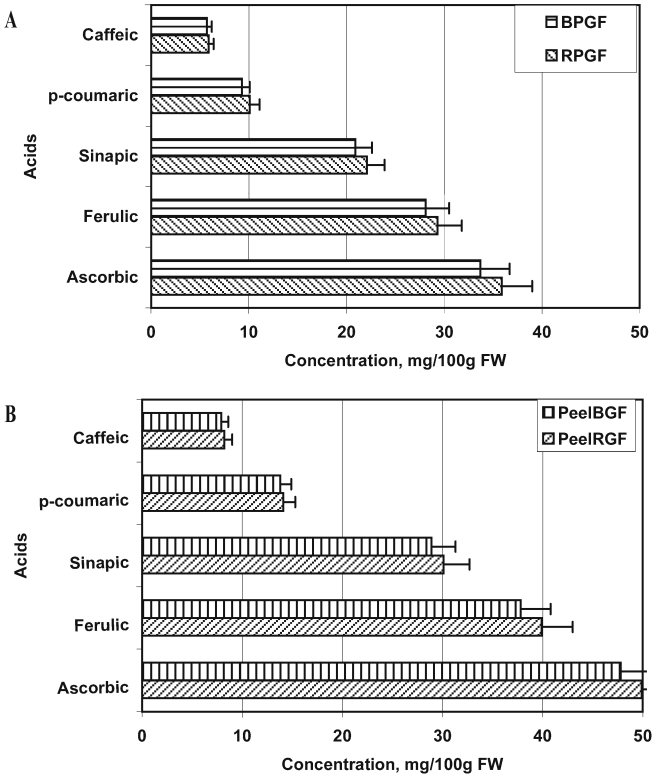

Fig. 1 Contents of phenolic and ascorbic acids in **a** peeled and **b** peels of red and blond grapefruits. Mean ± standard deviation (*vertical lines*). Red peeled grapefruit (*RPGF*), blond peeled grapefruit (*BPGF*), peels of red grapefruit (*PeelRGF*), peels of blond grapefruit (*PeelBGF*)

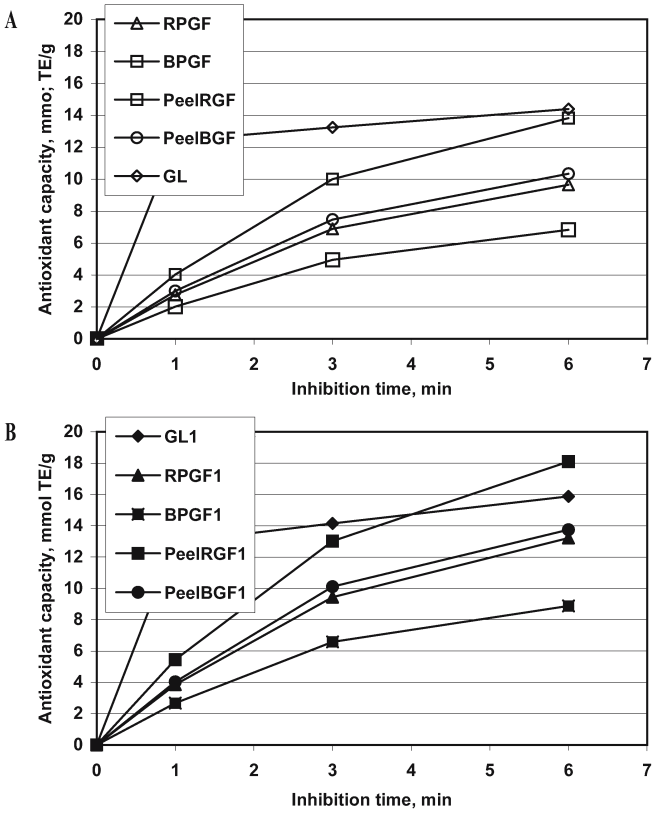

Fig. 2 Kinetics of 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (*ABTS*) scavenging effect of citrus extracts. The concentration of the samples was 0.25 mg/ml. **a** ABTS was produced by reacting potassium persulfate with RPGF, BPGF, PeelRGF and PeelBGF; *GL*, glutathione. **b** $ABTS^{\cdot+}$ radical cation was produced by reacting manganese dioxide with red peeled grapefruit (*RPGF1*), blond peeled grapefruit (*BPGF1*), peels of red grapefruit (*PeelRGF1*) and peels of blond grapefruit (*PeelBGF1*); *GL1*, glutathione.

RPGF1, BPGF1, PeelRGF1 and PeelBGF1 13.22±1.1[b], 8.88±0.8[a], 18.10±1.3[c] and 13.75±0.9[b].

The antioxidant capacity of these samples had comparative results against ABTS at the end point of 6 min as determined by spectrophotometric measurement (Fig. 2).

According to our results PeelRGF1 (Fig. 2b) had the highest percentage of inhibition, as well as the highest antioxidant capacity (18.10 mmol TE/g). BPGF showed the lowest antioxidant capacity (6.83 mmol TE/g). The samples were examined at the same concentration of 0.25 mg/ml and were comparable with glutathione. The manganese dioxide method gave slightly higher results than the potassium persulfate method (about 10% less). RPGF, BPGF, PeelRGF and PeelBGF samples showed the following percentage of inhibition with NO assay: 23.2±1.5[b]; 15.2±1.2[a]; 38.1±5.1[c]; 22.3±4.5[b], respectively. The values are means ± the standard deviations of five measurements. Means without common letters differ significantly (P<0.05).

The two antioxidant assays used show that the antioxidant capacity of the red grapefruit was significantly higher than that of the blond one (P<0.05). The antioxidant capacity in peels of both grapefruits was significantly higher than that of the peeled fruits.

The correlations showed that the dietary fiber contribution to the antioxidant potential of both grapefruits was minimal (R^2=0.4221–0.4864), the contribution of ascorbic

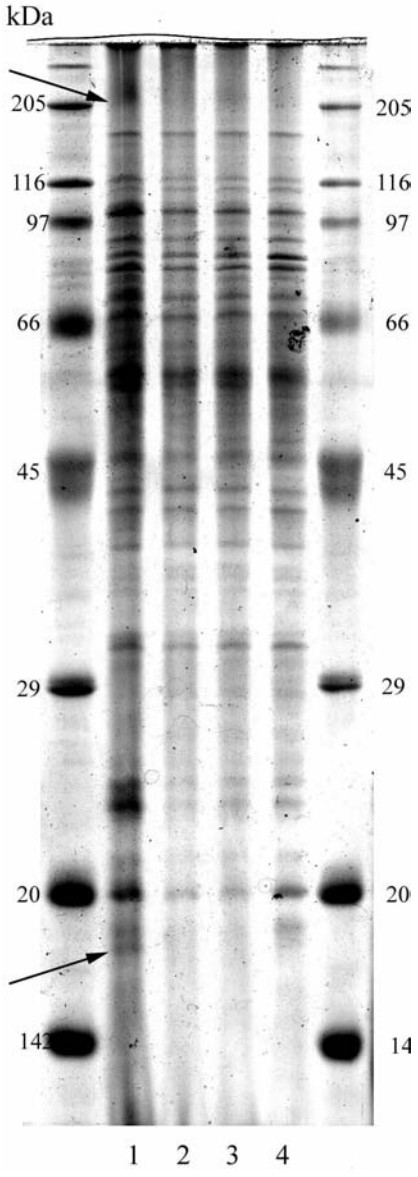

Fig. 3 Comparison of the band intensity of proteins extracted from citrus fruits and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis: blond (Shamouti) orange (*lane 1*), red grapefruit (*lane 2*), blond grapefruit (*lane 3*), Jaffa sweetie (*lane 4*). Molecular markers (kDa) from *left* to *right*: 205-myosin, 116-β-galactosidase, 97-phosphorylase b, 66-albumin, 45-ovalbumin, 29-carbonic anhydrase, 20-trypsin inhibitor, 14-α-lactalbumin

acid was moderate (R^2=0.6831–0.6918) and the contribution of total phenols was decisive (R^2=0.9487–0.9917).

In order to compare the SDS-PAGE electrophoretic protein patterns obtained samples from four citrus juices were applied to the gel. Thirty-four bands of Israeli Jaffa blond (Shamouti) orange (*C. sinensis*) (Fig. 3, lane 1), 32 bands of Jaffa Star Ruby (red) and blond grapefruits (*C. paradisi*) (Fig. 3, lanes 2 and 3) and a pummelo-grapefruit hybrid named Jaffa sweetie (Fig. 3, lane 4) were detected. In the range of 36 kDa the separation of proteins was sharp. Some aggregation appeared in the range of 20 kDa. The number of bands obtained was higher than in the very few reported works, especially in the range of 28 kDa [14, 15].

Discussion

Fruits and vegetables are an important part of disease prevention diets [6, 29–31]. It was shown that these natural products contain bioactive compounds, which are directly connected with their positive health effects [1, 2, 12, 13, 32]. In order to achieve the best health effects consumers have to choose natural products with high antioxidant potential [22, 25, 33]. It was demonstrated that the antioxidant potential of fruits and vegetables differs significantly [16, 32, 33]. However, the differences in the antioxidant potential of qualities of the same fruit had been studied less; therefore, we decided to investigate in vitro red and blond Jaffa grapefruits. In order to obtain reliable results the fruits were purchased from the same farmer and with the same ripeness.

Our results correspond with others [34, 35] that the contents of dietary fibers, major and minor minerals, total polyphenols, anthocyanins, flavonoids, phenolic and ascorbic acids are comparable in red and blond grapefruits and that the differences are statistically not significant (P>0.05). However, despite the data presented the two antioxidant assays used have shown that the antioxidant potential of red grapefruit is significantly higher than of the blond one (P<0.05), which is in accordance with previously published data. The individual bioactive compound values did not correspond with the total antioxidant potential [6].

Significantly higher amounts of bioactive compounds were found in peels in comparison with peeled fruits. Also these results are in accordance with the data of others, who found that citrus fruit peels are rich in dietary fibers and phenolics and are suitable for industrial processing [34].

According to our data the contribution of dietary fibers to the antioxidant potential was minimal in comparison with ascorbic acid and total phenols. These data are different from those of others [35]. The relative radical scavenging capacity of individual extracts against different testing radicals obtained may be explained by the different mechanisms involved in the radical-antioxidant reactions. In this study, $ABTS^{\cdot+}$ was generated by incubating ABTS with potassium persulfate or with manganese dioxide [9]. Chemical compounds that inhibit the potassium persulfate or manganese dioxide activity may reduce the production of $ABTS^{\cdot+}$. This reduction results in a decrease of the total $ABTS^{\cdot+}$ in the system and contributes to the total $ABTS^{\cdot+}$ scavenging capacity [9, 36]. The results obtained by the two methods demonstrated good agreement with published data [10, 11, 36] on citrus fruit antioxidant capacities.

The separation of proteins may have an application in the determination of the purity of the fruits used as an additive to the diet. Use of a longer gel (14 cm instead of 7 cm) gave us more distinct and sharper bands, especially

in the zone of 36 kDa. We suppose that the larger number of bands detected may be a favorable factor in searching for species-specific proteins in citrus fruit juices. Proteins have been separated into numerous components, but showed low polymorphism among different genotypes.

The differences between species were very small. Orange differs from the other fruits in the occurrence of two additional bands of 206 and 18 kDa (Fig. 3, see two arrows on lane 1). Both forms of grapefruit are indistinguishable (Fig. 3, lanes 2 and 3). The difference between sweeties and grapefruits is very weak. Sweeties differ from grapefruits only in the position of gel band 36 kDa (Fig. 3, see arrow on lane 4). It seems that this band corresponded to band 35 kDa of grapefruits, and sweeties are genetically the same as grapefruits.

Our results confirm the previous data [14, 15] that orange juice could be characterized by the occurrence of two additional bands in comparison with the other citrus fruits examined. Nevertheless, all the species examined are closely related and their protein pattern is very similar. One juice protein (82 kDa) occurred in all the citrus fruits investigated, and in two of the other fruits as well (65 and 46 kDa), but a species-specific protein appearing only in grapefruit could not be isolated [14, 15]. Unfortunately, we could not isolate any proteins of grapefruit showing species specificity during the present study, although this would be important as well. At present there is very little information about the isolation of specific proteins for these fruits [37].

The total antioxidant capacity of red grapefruit is higher than that of blond grapefruit and the total phenols are the main contributors to the antioxidant status this fruit. Electrophoretic analysis did not reveal big differences in the two citrus fruits studied, identified the main characteristic bands, which can be used to determine the purity of the fruits. On the basis of the results given in the present report the consumption grapefruits as a fruit diet can be recommended.

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