Comparative Contents of Dietary Fiber, Total Phenolics, and Minerals in Persimmons and Apples

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Dietary fibers, major phenolics, main minerals, and trace elements in persimmons and apples were analyzed and compared in order to choose a preferable fruit for an antiatherosclerotic diet. Fluorometry and atomic absorption spectrometry following microwave digestion were optimized for the determination of major phenolics and minerals. Total, soluble, and insoluble dietary fibers, total phenols, epicatechin, gallic and *p*-coumaric acids, and concentrations of Na, K, Mg, Ca, Fe, and Mn in whole persimmons, their pulps, and peels were significantly higher than in whole apples, pulps, and peels ($P < 0.01−0.0025$). Conversely, the contents of Cu and Zn were higher in apples than in persimmons. In persimmons and apples all of the above components were higher in their peels than in whole fruits and pulps. The relatively high contents of dietary fibers, total and major phenolics, main minerals, and trace elements make persimmon preferable for an antiatherosclerotic diet.

INTRODUCTION

At the beginning of a new millenium atherosclerosis is the principal cause of death in Western civilization (*1*). Despite success in the prevention of atherosclerosis, coronary artery disease (CAD) is still responsible for one of every three deaths. Cholesterol is the "building material" for atherosclerotic plaque, which leads to the occlusion of arteries; therefore, it is very important to find proper means to influence its composition (*2*). It was established that only oxidized low-density lipoprotein (LDL-C) particles are able to penetrate the arterial walls in general and the coronary arterial walls in particular, causing their occlusion and leading inter alia to fatal myocardial infarctions (*3, 4*).

High dietary fiber diets are associated with the prevention and treatment of some diseases such as diverticular and coronary heart diseases (*5*). Thus, health organizations have recommended the ingestion of 30−45 g per day (*6*). High dietary fiber formulated food products are currently being developed (*7, 8*). Dietary fiber has different physiological effects (*9*). The viscosity of the soluble dietary fiber fraction is of more importance than the amount of soluble fiber in a food (*10*). Soluble dietary fiber became viscous when mixed with water. The viscosity is associated with delayed gastric emptying, altered mixing in the intestinal contents, and slower transit of digesta along the small intestine and, thus, with a slower rate of glucose, lipid, or sterol absorption along a greater length of the small intestine (*11*). The insoluble part is related to both water absorption and intestinal regulation, whereas the soluble fraction may influence the lipid metabolism in decreasing the levels of LDL-C and is associated with the reduction of cholesterol in blood (*12, 13*). Both fractions complement each other, and a 70−50% insoluble and 30−50% soluble dietary fiber is considered a well balanced proportion (*9, 12, 14*). Some investigators have demonstrated in vitro (*15, 16*) and in epidemiological studies (*17*) that nutritional antioxidants and particularly phenolics are able to prevent the oxidation of LDL-C and to delay the development of atherosclerosis.

Diets rich in vegetables and fruits lead to a significant decrease in the CAD mortality (*2, 18, 19*). Main minerals (Ca, K, Na, and Mg) and essential trace elements (Fe, Cu, Zn, and Mn) are very important in biological processes (*20*). The role of iron, copper, and manganese, as very effective catalysts in the prevention and treatment of atherosclerosis and its complications, was underscored by Wills (*21*). It was shown that Mg and K have been used in the prevention and treatment of life-threatening arrhythmias, which are related to coronary atherosclerosis (*22−24*). Ca not only is the basic part of the human skeleton but also ensures the proper function of myocardium and heart vessels. Fe is an integral part of hemoglobin and used in treatment of some forms of anemias. Recent investigations indicate that high stored Fe levels play a role in early atherogenesis, promote lipid peroxidation, and can be an independent risk factor

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Table 1. Total, Soluble, and Insoluble Fibers in Whole Persimmons and Apples, Their Pulps, and Peels (Grams per 100 g of Fresh Fruit)[a]

dietary fiber	whole fruit			pulp			peel		
	persimmon	apple	P <	persimmon	apple	P <	persimmon	apple	P <
total	1.5 ± 0.1	0.80 ± 0.08	0.002	1.31 ± 0.09	0.66 ± 0.07	0.0005	1.73 ± 0.11	0.91 ± 0.09	0.0005
soluble	0.71 ± 0.08	0.36 ± 0.06	0.002	0.61 ± 0.07	0.28 ± 0.05	0.0025	0.82 ± 0.08	0.43 ± 0.06	0.0005
insoluble	0.77 ± 0.08	0.43 ± 0.06	0.002	0.66 ± 0.07	0.37 ± 0.06	0.0025	0.87 ± 0.08	0.46 ± 0.06	0.0005

[a] Mean ± SD of eight measurements.

for CAD (*25*), but overloads of Fe in experimental animals decrease aortic arch lesion formation (*26*). It was shown that Cu catalyzes LDL-C oxidation. High serum copper and low zinc contents are associated with increased cardiovascular mortality (*27*). It is known that apples, which are part of an antiatherosclerotic diet, contain relatively high concentrations of polyphenols and fiber (dietary fiber = 2.3 g/100 g of fresh weight, *28*; total polyphenols = 0.1−0.4 g/100 g of fresh weight, *20*).

Recently we have studied the influence of dietary persimmons on lipid metabolism and antioxidant activity in rats (*29−31*). The positive influence of this fruit on lipid metabolism and antioxidant activity is connected to its dietary fiber and polyphenols. However, these investigations did not include the characterization of persimmon from the point of its major phenolics, mineral composition, and its comparison with other fruits. As far as we know there are no such comparative studies. Therefore, the aim of this investigation was to evaluate the compositions of persimmon and apple and to prove which is preferable for an antiatherosclerotic diet. Fluorometry and atomic absorption spectrophotometry are proposed and optimized for the determination of phenolics and minerals, respectively. Dietary fibers, total and major phenolics, main minerals, trace elements, and their relationships were determined in persimmons and apples.

MATERIALS AND METHODS

Materials. All reagents were of analytical grade and were purchased from Sigma Chemical Co. In this investigation were used Israeli seedless persimmons (*Diospyros kaki* L. var. Triumph) and apples (*Malus domestica* var. Lobo). These fruits were purchased from the same farmer, and each type of the fruit was of the same ripeness.

Samples. Samples (12 persimmons and 12 apples) were obtained from 24 randomly selected fruits for the determination of all studied components.

Determination of Dietary Fibers, Phenols, and Minerals. Determination of total, soluble, and insoluble dietary fibers was done according to the method of Prosky et al. (*32*).

Total phenols were extracted with ethanol as well as with methanol and ethyl acetate (*33, 34*). A portion of 10 g of whole fruit, pulp, and peel was separately homogenized with 125 mL of 95% ethanol for 1 min and then gently boiled. After this procedure, the fruit samples were cooled and filtered under vacuum using Whatman No. 1 paper. The filtrates were evaporated under vacuum at 60 °C to 10 mL and then made up to 100 mL by distilled water. Total phenols were determined according to the Folin−Ciocalteu method and measured at 675 nm (*35*). Epicatechin was measured on a Uvikon 930 UV spectrophotometer at 278 nm (Kontron AG Instruments, Zürich, Switzerland). Determination of major phenolics was done according to the method of García-Sánchez et al. (*36*), with our modifications and changes (*33, 37*).

Fluorescence emission was measured with a model FP-770, Jasco spectrofluorometer (Japan Spectroscopic Co., Ltd., Hachioji City, Japan) at excitation (λ_{ex}) and emission (λ_{em}) wavelengths suitable for each of the determined phenolic

acids: (a) gallic acid, λ_{ex} = 260 nm and λ_{em} = 357 nm, pH 4.63; (b) protocatechuic acid, λ_{ex} = 290 nm and λ_{em} = 363 nm, pH 10.7; (c) vanillic acid, λ_{ex} = 305 nm and λ_{em} = 378 nm, pH 9.3; (d) *p*-coumaric acid, λ_{ex} = 330 nm and λ_{em} = 443 nm, pH 10.7; (e) ferulic acid, λ_{ex} = 340 nm and λ_{em} = 460 nm, pH 11.2.

Determination of minerals (Na, K, Mg, and Ca) and trace elements (Fe, Cu, Zn, and Mn) was done as follows. The samples of whole fruits, their pulps, and peels were lyophilized separately. Then 0.8 g of lyophilized samples was mineralized in a microwave oven with concentrated HNO_3. The concentrations of all eight above-mentioned elements were estimated by a Perkin-Elmer 5100 ZL atomic absorption spectrometer (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, U.K.), using the flame method for Na, K, Mg, Ca, Fe, Cu, and Zn and the flameless method for Mn.

Statistics. To verify the statistical significance of the studied parameters, means and standard deviation (means ± SD) of eight measurements were determined. When appropriate, differences between groups were tested by two-way ANOVA. *P* values of <0.05 were considered significant.

RESULTS

Total, soluble, and insoluble dietary fibers in whole persimmons, their pulps, and peels were significantly higher than in whole apples, pulps, and peels (Table 1, *P* < 0.002−0.0005). As can be seen, the content of total, soluble, and insoluble dietary fibers in the studied apple cultivar was lower than found in the literature [1.8−2.3 g/100 g of fresh weight (*28*)]. Suni et al. (*38*) reported a content of 14−20 g/100 g of dry weight in seven apple cultivars (Summered, Aroma, Ingrid Marie, Cox Orange, Belle de Boskoop, Mutzu, and Jonagold) using a similar gravimetric procedure. Suni et al. (*38*) also reported a 26.8% difference in the content of total dietary fiber between Belie de Boskoop and other cultivars. Dietary fibers of both persimmons and apples were higher in their peels than in whole fruits and pulps (Table 1, *P* < 0.05). These results are in accordance with the data of Marlett (*39*), who found that removal of peel decreases total fiber content. Therefore, applesauce contains 33−39% less fiber than an unpeeled apple (*28*). Apple variety Lobo is poor in dietary fiber compared with other apple varieties. Apple cultivars different from Lobo have shown a lower proportion of soluble dietary fiber: Macintosh have shown only 17% soluble dietary fiber (*28*), and Summered, Aroma, Ingrid Marie, Cox Orange, Belle de Boskoop, Mutzu, and Jonagold presented an average of 31% (*38*). Thus, apple variety Lobo has a lower content of total dietary fiber but a better proportion of soluble fraction, which is more important from a physiological point of view. However, when dietary fiber was determined according to a modified Uppsala method, a lower content of the total dietary fiber in whole apples (1.8 g/100 g) was found due to the coprecipitation of simple sugars with the fiber polysaccharides when ethanol was added to recover the fiber (*38*).

Total phenols in whole persimmons, their peels, and pulps were significantly higher than in whole apples, peels, and pulps (Figure 1). In persimmon peel phenols
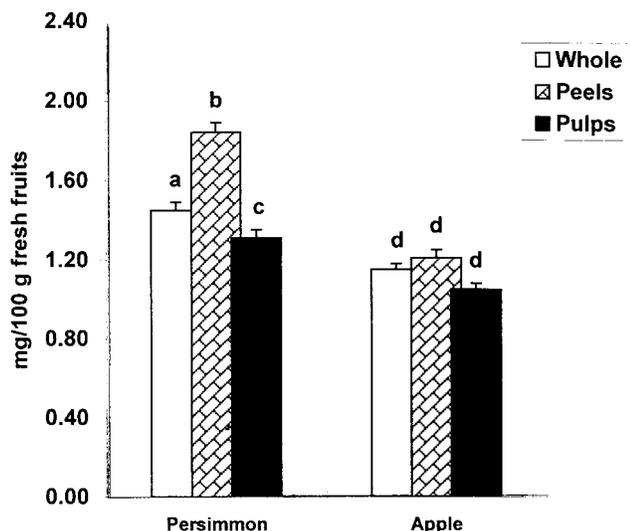
Figure 1. Contents of total phenolics in whole persimmons and apples, their pulps, and peels [mean ± SD (vertical lines)]. Bars with different letters are significantly different ($P < 0.05$).

were higher than in whole fruit and its pulp. These results are differed from those of Lachman et al. (*34*). The content of the studied components depends on the methods used.

ANOVA analysis indicates that the differences were statistically significant. Conversely, the differences in phenol content in whole apples, peels, and pulps were statistically not significant. These results are in the range described by others (*20*).

The amount of protocatechuic acid in persimmon, determined fluorometrically, shows the same maxima in the fruit extracts as for the standard phenolic presented on curve e (Figure 2). Some slight shoulders, which are shown on curves a–d (Figure 2), can be explained by the difference of phenolic extraction as well as by adjustment of the sample to a specific pH. Curve d shows a lower amount of protocatechuic acid in persimmon pulp extracted with ethyl acetate in comparison with pulp ethanolic extract presented on curve b. The recovery of phenolics was higher in ethanolic extracts than in ethyl acetate. The curves of other phenolics in persimmons and apples are not shown because they are similar and differ only with the excitation and emission wavelengths and concentrations of studied components. Corresponding curves for phenolic determination after different extraction procedures (methanol and ethyl acetate) are also omitted. Some major phenolics such as protocatechuic, ferulic, gallic, *p*-coumaric, and vanillic acids and epicatechin in whole persimmons and apples, their peels, and pulps are presented in Table 2.

Protocatechuic, ferulic, and vanillic acids have no significant differences between apples and persimmons (Table 2, $P < 0.1–0.15$). In contrast, gallic and *p*-coumaric acids and epicatechin in whole persimmons, their pulps, and peels were significantly higher than in whole apples, pulps, and peels (Table 2, $P < 0.01–0.0025$).

Major phenolics in persimmon and apple peels were higher than in whole fruits and their pulps, but not in all cases was the difference significant. The contents of ferulic, *p*-coumaric, gallic, and protocatechuic acids and epicatechin in apple peels were higher than in whole fruits and pulps, with significance only versus pulps
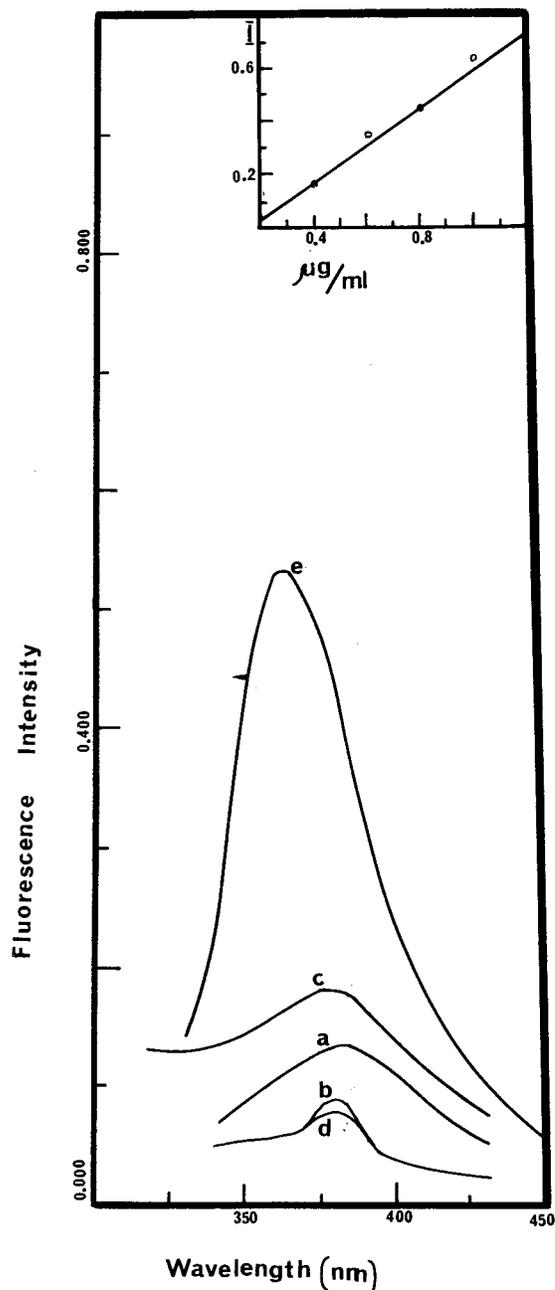
Figure 2. Fluorescence emission of ethanolic persimmon extracts from (a) whole fruit, (b) pulp, (c) peel, (d) pulp extracted with ethyl acetate, and (e) standard protocatechuic acid. (Inset) Calibration curve of protocatechuic acid (excitation at 290 nm).

($P < 0.01–0.0125$). For persimmons such a relationship was found only for ferulic and gallic acids ($P < 0.01–0.025$).

The concentrations of *p*-coumaric, protocatechuic, and vanillic acids and epicatechin in persimmon peels were significantly higher than in whole fruits and pulps. In apple peels such a correlation was found only for vanillic acid ($P < 0.05–0.0025$).

The results of our investigation of most major phenolics are in the range described by Scherz and Senser (*40*).

In whole persimmons the contents of Na, K, Mg, and Ca were significantly higher than in apples (Table 3, $P < 0.05–0.005$). This correlation was also significant for Mg versus pulps and for Ca versus peels (Table 3, $P < 0.05$).

Table 2. Major Phenolics in Whole Persimmons and Apples, Their Pulps, and Peels (Milligrams per 100 g of Fresh Fruit)[a]

	whole fruit			pulp			peel		
phenolic	persimmon	apple	$P <$	persimmon	apple	$P <$	persimmon	apple	$P <$
epicatechin	1.4 ± 0.1	0.9 ± 0.1	0.005	1.1 ± 0.1	0.7 ± 0.08	0.01	1.7 ± 0.1	1.1 ± 0.1	0.0025
ferulic acid	10.3 ± 1.0	12.2 ± 1.1	0.15	7.9 ± 0.6	9.8 ± 0.9	0.1	13.2 ± 1.3	14.9 ± 1.3	0.15
gallic acid	22.1 ± 1.8	16.2 ± 1.6	0.025	19.3 ± 1.4	15.1 ± 1.4	0.05	27.2 ± 2.1	19.4 ± 1.4	0.025
protocatechuic acid	6.3 ± 0.6	7.3 ± 0.6	0.1	5.5 ± 0.5	6.1 ± 0.6	0.2	8.3 ± 0.8	8.7 ± 0.8	0.4
vanillic acid	0.5 ± 0.06	0.6 ± 0.07	0.1	0.4 ± 0.05	0.5 ± 0.06	0.1	0.7 ± 0.08	0.8 ± 0.08	0.2
p-coumaric acid	61.4 ± 5.1	41.1 ± 4.9	0.01	54.2 ± 5.1	38.2 ± 4.0	0.025	82.6 ± 7.8	53.1 ± 5.0	0.01

[a] Mean ± SD of eight measurements.

Table 3. Main Minerals and Trace Elements in Whole Persimmons and Apples, Their Pulps, and Peels[a]

	whole fruit			pulp			peel		
element[b]	persimmon	apple	$P <$	persimmon	apple	$P <$	persimmon	apple	$P <$
Na	4.91 ± 1.34	0.6 ± 0.2	0.050	4.97 ± 1.36	0.63 ± 0.20	0.025	4.49 ± 1.2	0.48 ± 0.1	0.025
K	254 ± 24.0	81.9 ± 7.5	0.005	252.0 ± 25.0	78.0 ± 5.80	0.050	266 ± 15.0	107 ± 18.0	0.005
Mg	8.22 ± 1.10	5.02 ± 0.6	0.050	7.51 ± 1.10	3.82 ± 0.50	0.050	12.7 ± 1.40	12.6 ± 1.4	0.480
Ca	9.35 ± 1.80	4.26 ± 1.6	0.050	6.32 ± 0.90	3.48 ± 1.10	0.100	28.8 ± 7.70	9.15 ± 3.00	0.050
Fe	101.4 ± 31.0	94.3 ± 17.1	0.450	74.3 ± 24.1	63.7 ± 13.8	0.400	275 ± 37.2	287 ± 37.70	0.475
Mn	107.1 ± 30.0	30.7 ± 5.8	0.025	87.1 ± 16.1	24.3 ± 5.90	0.005	235.3 ± 35.0	71.5 ± 15.1	0.002
Zn	13.9 ± 3.10	18.1 ± 3.6	0.200	9.8 ± 3.10	15.1 ± 3.30	0.100	35.4 ± 10.1	40.2 ± 13.1	0.400
Cu	9.76 ± 3.10	24.1 ± 4.3	0.050	6.55 ± 2.10	20.5 ± 4.20	0.050	3 0.4 ± 5.50	47.1 ± 6.30	0.050

[a] Mean ± SD of eight measurements. [b] Na, K, Mg, and Ca in mg/100 g of fresh fruit; Fe, Mn, Zn, and Cu in μg/100 g of fresh fruit.

In whole persimmons the contents of Fe (Table 3, $P < 0.40-0.47$) and Mn (Table 3, $P < 0.025-0.005$) were higher than in apples. In contrast, Cu was significantly higher in whole apples (Table 3, $P < 0.05$). The differences between the Zn contents were found to be without significance (Table 3, $P < 0.1-0.4$). Peels contain higher amounts of phenolics as well as all of the above-mentioned minerals compared to whole fruits and pulps. The contents of Na, K, and Ca were significantly higher in persimmon peels than in whole fruits and pulps ($P < 0.0125-0.005$). The same relationship was found for Ca and Mg in apple peels, but the difference was not significant. The contents of Fe, Zn, and Cu in apple peels were significantly higher than in whole fruit ($P < 0.01$). Mn and Cu in persimmon and apple ($P < 0.005$) peels were higher than in fruit and pulp. The results of our investigation of most minerals and trace elements are in accordance with the data of Scherz and Senser (*40*).

DISCUSSION

It was shown in our recent studies that persimmon improves lipid metabolism in rats fed diets containing cholesterol (*29–31*). These experiments were conducted on male Wistar rats, which were randomly assigned to equal in number groups: experimental (EG) and control (CG). During 4 weeks semipurified diet with 1% cholesterol (BD) was used. The control group was supplemented with 7% cellulose and the EG with 7% whole dry persimmon or its peel. A significant increase in plasma total cholesterol (TC) and LDL-C was found only in CG ($P < 0.0005$). The increase of TC and LDL-C in the EG fed diet supplemented with persimmon was statistically not significant (P for both <0.1). The results of these experiments show that diets fortified with dry persimmon improve lipid levels and exert an antioxidant effect. A diet fortified with persimmon peel was more efficient than the diet with whole fruit (*30*). A diet fortified with whole persimmon was more efficient than a diet fortified with the phenol-free persimmon (*31*). Therefore, it was shown that persimmon possesses hypolipidemic and antioxidant properties that are evi-

dent when whole persimmon or its parts are added to the diet of rats fed cholesterol. These properties are attributed to its water-soluble dietary fiber and polyphenols. Our present results (Tables 1 and 2) demonstrate that hypolipidemic properties can be explained by the higher presence of major phenolics and fibers in peel than in whole fruits.

Because the contents of the studied compounds are influenced by some conditions, which include inter alia kind of fruits, region, climate conditions, and ripeness, the fruits were purchased from the same farmer in order to receive reliable results. In this way additional factors were eliminated, which could lead to doubtful results.

The beneficial influence of fibers on lipid metabolism are well-known (*5, 13, 31*). In this study it was found that the total, soluble, and insoluble dietary fibers in whole persimmons, their pulps, and peels were higher than in whole apples, their pulps, and peels. In both persimmons and apples these components were higher in their peels than in whole fruits and pulps, and the differences were significant. It can be pointed out that, within total dietary fibers, there is a 50% of each soluble and insoluble fraction in both persimmons and apples (Table 1), which is considered to be a well-balanced proportion between the fractions, according to Schneeman (*12*). Consequently, the ingestion of both persimmon and apples may have beneficial physiological effects due to both insoluble and soluble fractions. However, other products such as cereals may result in a very much lower effect, in some cases imperceptible, of the properties associated with the soluble dietary fiber fraction (*8, 9, 14*).

The role of phenolics and minerals in the prevention of atherosclerosis in general and coronary atherosclerosis in particular is widely described (*18*). In our previous study it was shown that the total phenols in whole persimmons, their peels, and pulps were significantly higher than in apples and their parts (*37*). However, not only the concentration of total phenolics but also the contents of major phenolics have characterized the intensity of the antioxidant activity (*15, 16, 31*). Fluorometric and atomic absorption methods were

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modified and applied with high efficiency to the determination of major phenolics and minerals in persimmons, apples, and their parts. It was found that in whole persimmons, epicatechin and gallic and *p*-coumaric acids were significantly higher than in whole apples. The contents of ferulic, protocatechuic, and vanillic acids were higher in whole apples, their pulps, and peels than in all parts of persimmon, but not significantly. Our results have good correlation with others (*40, 41*). The difference was only in the monitoring of the major phenolics at excitation and emission wavelengths corresponding to the reactivity of the individual compounds and measuring their intrinsic fluorescence.

The individual phenolics were in the following order in the investigated fruits: *p*-coumaric > gallic > ferulic > protocatehuic > epicatechin > vanillic acids (Table 2). It is known that the antioxidant activity of phenolic acids is generally governed by their chemical structures. Their activity improves with the number of increased hydroxyl groups. Therefore, our results on the major phenolics are of particular interest. Gallic, *p*-coumaric, and ferulic acids are present in persimmons in far higher concentrations than epicatechin and exhibit similar, if not greater, antioxidant and antiplatlet aggregation activities. Our results on major phenolics were in agreement with others, showing the dependence of their activity in the fruits (*16*).

The contents of Na, K, Mg, Ca, Mn, and Fe were higher in whole persimmons than in whole apples (Table 3). All main minerals and trace elements were higher in persimmon and apple peels than in whole fruits and their pulps (Table 3). These results were slightly different from those of others (*20*), but it was predictable because we studied different cultivars of apples, which were grown in completely different conditions of different geographic regions.

We have found that the quantities of studied minerals and essential trace elements both in persimmons and in apples are very small. It is known that the contents of most minerals and especially trace elements in plants are very low: it may be expressed in $10^{-4}-10^{-5}$ percent (*42*). However, in terms of biological activity they are strikingly strong. When they are incorporated into organomineral complexes, their ability is enhanced a thousandfold and sometimes a millionfold over the activity of simple ionic state (*42*). Therefore, the small quantities of these minerals and especially trace elements in persimmons and apples are acting in organomineral complexes, and therefore their ability is very enhanced. The activity of certain phenolics is due to the metal chelating property as was found in our studies. This corresponds with the data of Nardini et al. (*16*).

Relationships between the major phenolics, fibers, and minerals can be an explanation of high activity of persimmon as a free radical scavenger. These results correspond with others about the role of individual phenolics (*15, 18, 19*). The obtained results of this study also support the conclusions of others (*19, 43*) that supplementation with the persimmon with a balanced diet could be more effective and economical than consuming individual antioxidants and minerals in protecting the body against various oxidative stresses. The total antioxidant potential of persimmon is more important than any individual specific antioxidant constituents. In conclusion, dietary fibers, total phenolics, epicatechin, gallic and *p*-coumaric acids, Na, K, Mg, Ca, Fe, and Mn are higher in whole persimmons than in whole apples.

The above components are higher in persimmon and apple peels than in whole fruits and pulps. The higher content of the studied constituents in persimmons makes this fruit preferable for an antiatherosclerosis-preventing diet. Persimmon and apple peels are rich in all studied compounds and therefore can be used by individuals and in industrial processing.

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