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Drying of persimmons (*Diospyros kaki L.*) and the following changes in the studied bioactive compounds and the total radical scavenging activities

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Abstract

Fresh persimmons were subjected to two different processes: sun-drying during 1 month and dehydration at 60 °C during 12 h. To assess the effect of this process on nutritional and health-related properties of persimmons dietary fibers, minerals, trace elements, polyphenols and the total radical scavenging activities (TRSAs) were determined before and after processing. It was found that the contents of dietary fibers, minerals and trace elements in fresh and dried persimmons fruits were comparable. Total polyphenols in fresh persimmons was higher than in dried fruits (1.3 vs. 0.9 and 0.8 mg/100 g FW, respectively) and percentage of inhibition was higher than in dried fruits (70% vs. 59% and 55% and 58% vs. 53% and 46% for 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) [ABTS] radicals, respectively ($P > 0.05$ in all cases). In conclusion: (1) the differences in the contents of dietary fibers, minerals and trace elements in fresh and dried persimmons are not significant; (2) the contents of polyphenols and the level of the TRSA are higher in fresh persimmons than in dried fruits; however, both variables are also high in dried persimmons; (3) when fresh fruits are not available, proper dried persimmons could be used as a valuable substitute.

Keywords: Fresh; Dry; Persimmon; Bioactive compounds; Antioxidant activity

1. Introduction

The nutritional antioxidants and particularly phenolics are able to prevent oxidation of LDL-C and therefore to delay development of atherosclerosis in general and coronary atherosclerosis in particular (Gaziano, 1994; Kromhout, Menottim, Kesteloot, & Sans, 2002). Therefore, diets containing these nutritional antioxidants are in demand (Longeril et al., 1994; Partiff et al., 1994). The most known among them is the Mediterranean diet, which was proposed for prevention and treatment of hyperlipidemia (Longeril et al., 1994;

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Mattson, 1989; Spiller, 1991). Such diets are rich in dietary fibers, minerals, essential trace elements and phenolic compounds (Hertog et al., 1995; Kromhout et al., 2002).

The beneficial influence of fibers on the lipid metabolism is well known (Anderson, Smith, & Guftanson, 1994). It was shown that high dietary fiber diets are associated with prevention and treatment of coronary heart diseases (CAD). Thus, health organizations recommended the ingestion of 30–45 g of dietary fiber per day ((Bartnikowska, 1999; Spiller, 1986). Main minerals (Ca, K, Na, Mg) and essential trace elements (Fe, Cu, Zn, Mn) are very effective in prevention and treatment of atherosclerosis and its complications as life threatening arrhythmias, which are related to CAD (Baxter, Sumeray, & Walker, 1996; Wills, 1985). The role of minerals and trace elements in prevention of atherosclerosis in general and coronary atherosclerosis in particular was widely studied (Baxter et al., 1996; Wills, 1985).

Fe plays a certain role in early atherogenesis, promotes lipid peroxidation and can be an independent risk factor for CAD (Kiechl, Willeit, Egger, Poewe, & Oberhollenzer, 1997). Therefore, a subtropical fruit persimmon, which is rich in bioactive compounds, became a subject for investigation (Gorinstein et al., 1993, 1994; Uchida et al., 1989). This fruit possesses plasma lipid lowering and antioxidant properties and can be successfully used in antiatherosclerosis preventing diets (Gorinstein et al., 2000; Uchida et al., 1989). The bioactivity of persimmon is attributed to its water-soluble dietary fibers, minerals, trace elements and phenolics, which determine total antioxidant activity of the fruits (Hertog et al., 1995; Spiller, 1991). Diets supplemented with this fruit improve plasma lipid metabolism and increase total antioxidant activity in rats. The lipid lowering effect of persimmon was more evident when whole persimmon or its parts were added to the diet of rats with nutrition-induced hypercholesterolemia (Gorinstein et al., 2000).

However, fresh persimmon is not available all year around. Therefore, the contents of some important bioactive compounds and the total radical scavenging activities (TRSAs) in fresh and properly dried persimmons were determined and compared.

Fruits are generally dried under the sun or in a solar and an artificial dryer (Komiyama & Tsujji, 1987; Marder & Schomaker, 1995). A combination of solar and artificial drying has been used to provide high-quality products (Kitagawa & Glucina, 1984). There is very limited information about the drying process of persimmon fruits and the changes in their bioactive composition. Some authors have studied the changes in solar and sun-dried persimmons (Asgar, Yamauchi, & Kato, 2003; Chaudry, Bibi, Khan, & Sattar, 1998). However, in these reports were investigated only individual antioxidants: sinapine, catechin (CA), anthocyanidin, leucoanthocyanidin. It was shown by Lotito and Frei (2004) that the total antioxidant activity of fruits and vegetables is not determined only by individual antioxidants.

There are many methods for determination of the antioxidant activity, and each one has shortages and some of them give different antioxidant activity trends (Huang, Ou, & Prior, 2005). To avoid these shortages, the TRSA of fresh and dried persimmons was determined by two different radicals: 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) [ABTS] (Miller, Sampson, Candeias, Bramley, & Rice-Evans, 1996) and 1,1-diphenyl-2-picrylhydrazyl method [DPPH] (Singh, Chidamdara, & Jayaprakasha, 2002).

In this study, persimmon was dried by two different procedures: sun drying and dehydration at the temperature of 60 °C during 12 h. Dietary fibers, minerals, trace elements and polyphenols in fresh and proper dried persimmons and their TRSAs were evaluated in order to find a substitute for fresh fruits.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) [ABTS], potassium persulfate, butylated hydroxyanisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade. Deionized and distilled water were used throughout.

2.2. Samples

Seedless persimmons (*Diospyros kaki L.*, var. Triumph) of the same ripeness were harvested in the period of 2004 and were purchased from the same farmer. The fruits were randomly divided into three groups: one for use as fresh and second and third as dried fruits. The persimmons of the second and third groups were dried by two different methods: sun-drying during 30 days (Asgar et al., 2003) and dehydrated at 60 °C in a cabinet dryer during 12 h (Akyidiz, Aksay, Benli, Kiroğlu, & Fenercioğlu, 2004). These groups of fruits were named Fresh, Dried A and Dried B, respectively.

2.3. Determination of dietary fibers

Dietary fibers in all three groups were analysed with the method of Prosky, Asp, Schweizer, DeVries, and Furda (1992) with modification of Mañas, Bravo, and Saura-Calixto (1994). Samples were treated with

heat-stable α-amylase, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000 × *g*) to separate the soluble and insoluble fractions and dialysis against water. The results are given in g/100 g of fresh weight (FW).

2.4. Determination of minerals and trace elements

Minerals (Na, K, Mg, Ca) and trace elements (Fe, Cu, Zn and Mn) were determined as follows. The samples were lyophilized separately. Then 0.8 g of lyophilized samples was mineralized in microwave oven for 15 min with 5 ml of concentrated HNO_3 according to Jurkiewicz, Wiechula, Nowak, Gazdzik, and Loska (2004) with our modifications. The concentrations of all above-mentioned eight elements were estimated by a Perkin-Elmer 5100 ZL atomic absorption spectrometer (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, England), using the flame method for Na, K, Mg, Ca, Fe, Cu, Zn and the flameless method for Mn. The results are given in milligrams and micrograms in 100 g FW for minerals and trace elements, respectively.

2.5. Extract preparations and determination of total polyphenols

Portions of 10 g of the all three groups of persimmons were separately homogenized with 125 ml of 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. The filtrates were evaporated under vacuum at 60 °C until 10 ml. Total polyphenols were determined by Folin-Ciocalteu method with some modifications and changes (Gorinstein et al., 1994) and measured at 675 nm. The results were given in mg/100 g FW of gallic acid equivalent.

2.6. Determination of TRSA

The TRSA was determined in the same extracts which have been prepared for the determination of total polyphenols. For comparison the same volumes of 0.2 mg/ml of the ethanol extracts were used for two scavenging assays. These extracts were taken in different test tubes and evaporated to dryness.

The TRSA was determined by two complementary radical scavenging assays:

(1) 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\bullet+}$).
$ABTS^{\bullet+}$ radical cation was generated by the interaction of ABTS (250 μmol/l) and $K_2S_2O_8$ (40 μmol/l). After addition of 990 μl of $ABTS^{\bullet+}$ solution to 10 μl of Trolox standards (final concentration 0–20 μmol/l) in phosphate buffered saline (PBS), and the extracted persimmon samples with ethanol the absorbance was monitored exactly 1, 3 and 6 min after the initial mixing. The percentage decrease of the absorbance at 734 nm (Miller et al., 1996).

(2) 1,1-diphenyl-2-picrylhydrazyl method (DPPH).
The dry matter of 0.2 mg/ml of ethanolic extract was adjusted to 100 μl by adding EtOH. Five milliliters of a 0.1 mmol/l ethanolic solution of DPPH was added. The tubes were allowed to stand at 27 °C during 20 min. The control was prepared as above without any extract, and EtOH was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm (Singh et al., 2002). Radical scavenging activity was expressed as the inhibition percentage for comparison of both methods (Miller et al., 1996; Singh et al., 2002).

2.7. Statistical analysis

To verify the statistical significance of the studied parameters, means and standard deviation of five measurements were determined. Where it was appropriate, differences between groups were tested by 2-way ANOVA. Also Spearman correlation coefficient (*R*) was used. Linear regressions were also calculated. The *P*-values of less than 0.05 were considered significant. During the dehydration period in both processes (sun-drying and dehydration in cabinet drier) weight reductions of samples were about 70–75% and dry product yields were 25–30%. The final dry matter contents of dehydrated persimmons were 85%. In order to compare the data, the results of the investigation of the dried persimmon samples are given as an equivalent of fresh fruits.

3. Results

3.1. Dietary fibers

The contents of total, insoluble and soluble dietary fibers in fresh and two groups of dried persimmons are shown in Fig. 1. As can be seen, the differences were not significant ($P > 0.05$). Insoluble dietary fibers in both fresh and dried persimmons were significantly higher than of soluble ($P < 0.05$).

3.2. Minerals

The differences in the contents of all studied minerals (Fig. 2) were not significant ($P > 0.05$). The content of K in both fresh and dried persimmons was significantly

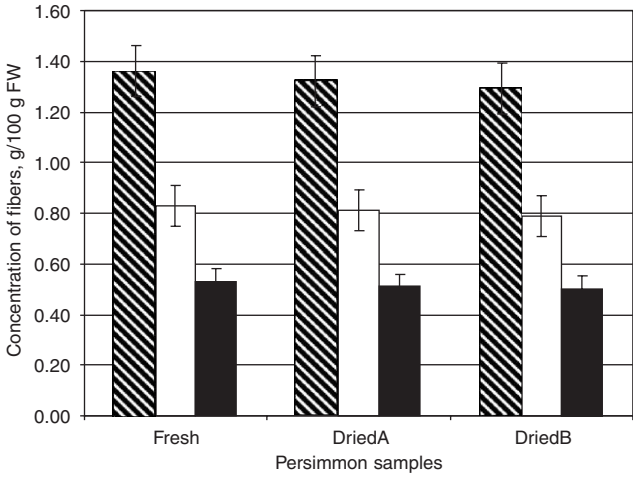

Fig. 1. Total, ▨ insoluble, □ and soluble ■ fibers in fresh (Fresh), and dried [sun-drying (Dried A) and dehydrated (Dried B)] persimmons. M ± SD of 5 measurements.

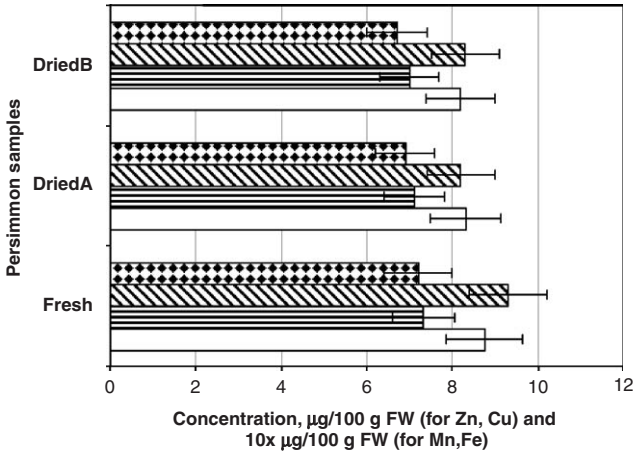

Fig. 3. Trace elements (□ Mn, ▬ Fe, ▨ Zn, ◆ Cu) in fresh (Fresh) and dried [sun-drying (Dried A) and dehydrated (Dried B)] persimmons. M ± SD of 5 measurements.

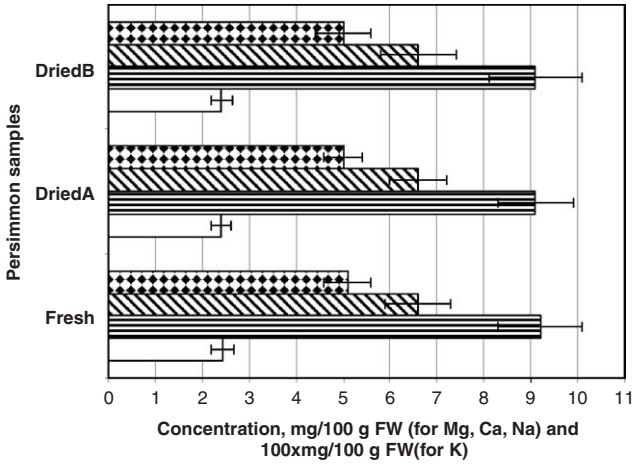

Fig. 2. Minerals (□ K, ▬ Mg, ▨ Ca, ◆ Na) in fresh (Fresh), and dried [sun-drying (Dried A) and dehydrated (Dried B)] persimmons. M ± SD of 5 measurements.

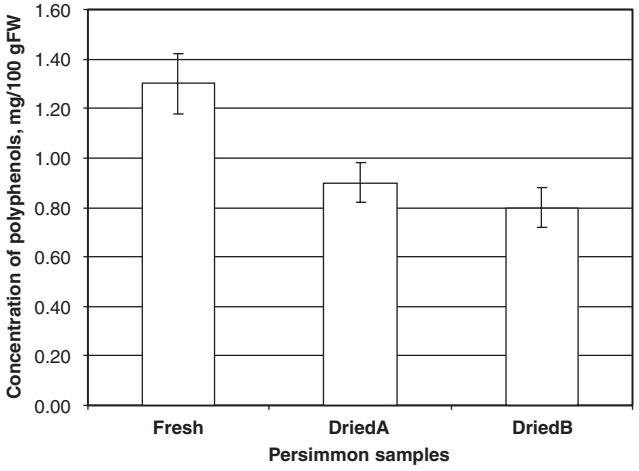

Fig. 4. Total polyphenols in fresh (Fresh), and dried [sun-drying (Dried A) and dehydrated (Dried B)] persimmons. M ± SD of 5 measurements.

higher than that of the three other studied minerals ($P < 0.05$).

3.3. Trace elements

The results of the determination of the contents of Fe, Mn, Zn and Cu in both fresh and dried persimmons are summarized in Fig. 3. As can be seen, the differences in contents of all studied trace elements were not significant ($P > 0.05$). The contents of Mn and Fe were significantly higher than that of Zn and Cu ($P < 0.05$).

3.4. Total polyphenols

The differences in the contents of total polyphenols (Fig. 4) were significant ($P < 0.05$): the highest content of the total polyphenols was in the fresh persimmons. The differences in the content of total polyphenols in dried groups were not significant ($P > 0.05$).

3.5. The total radical scavenging activity

The results of the determination of the TRSA of fresh and dried persimmons are summarized in Fig. 5. As can be seen, the total antioxidant activity of fresh persimmons as determined by DPPH and ABTS assays was significantly higher than that of the dried persimmons ($P < 0.05$).

4. Correlation

Based on the experimental data obtained from the total polyphenols and the antioxidant activities of the three investigated samples, a correlation of these data

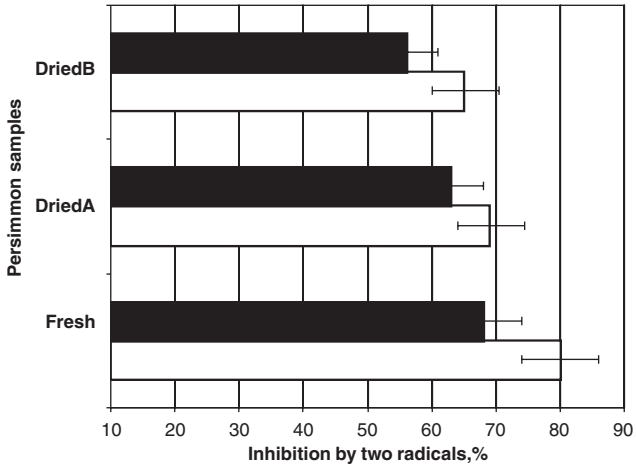

Fig. 5. The level of the total antioxidant activity (AA, %) in fresh (Fresh), and dried [sun-drying (Dried A) and dehydrated (Dried B)] persimmons as determined by DPPH □ and ABTS ■ assays. M ± SD of 5 measurements .

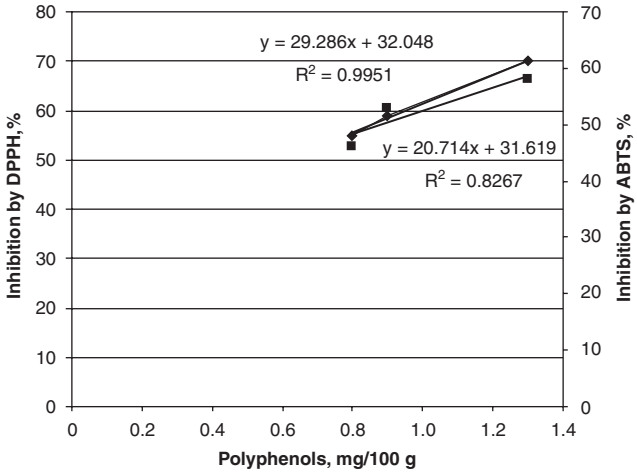

Fig. 6. The correlation between the content of total polyphenols and the percentage of inhibition determined by two radical assays: DPPH (◆) and ABTS (■) in Fresh (Fresh) and dried [sun-drying (Dried A) and dehydrated (Dried B)] persimmons.

were presented in Fig. 6. A very good correlation was observed between the percentage of inhibition determined by DPPH and ABTS scavenging assays (Fig. 6) and the content of total polyphenols ($R^2 = 0.9951$ and 0.8267, respectively).

4.1. Changes in content of total polyphenols and TRSA during drying process

The data of this investigation indicate that the sun-drying process was more effective than the dehydration. Based on the correlation coefficients the DPPH test is more sensitive than the ABTS assay. Therefore, we decided to measure the changes in the content of total polyphenols and total antioxidant activity measured by DPPH assay during 30 days of sun-drying. As can be seen, the major significant changes (Table 1) have appeared during the first 5 days of the sun drying ($P < 0.05$).

Table 1
The changes in the total polyphenol content in persimmon and in percentage of antioxidant activity (AA,%) as determined by DPPH assay during 30 days of the sun-drying process

Days	Total polyphenols content (mg/100 g FW)	AA,%
0	1.32 ± 0.12^a	71.2 ± 5.6^a
5	0.99 ± 0.11^b	60.1 ± 4.1^b
10	0.97 ± 0.10^b	59.8 ± 4.1^b
15	0.95 ± 0.10^b	59.6 ± 4.1^b
20	0.93 ± 0.09^b	$59.4 + 4.1^b$
25	0.91 ± 0.09^b	59.2 ± 4.1^b
30	0.91 ± 0.09^b	59.1 ± 4.1^b

Data are means ± standard deviations of five measurements.
Means in columns with different letters differ significantly ($P < 0.05$).

5. Discussion

It was found that the content of dietary fibers in both fresh and dried persimmon is high and the differences were not significant. These data are in accordance with Chaudry et al. (1998), Bibi, Chaudry, Khan, Ali, and Sattar (2001) and Asgar et al. (2003).

As was expected, the contents of minerals and trace elements after drying remained unchanged. The differences between fresh and dried fruits were not significant. Also these data were in accordance with Scherz and Senser (1994).

It was found that the contents of Fe, Cu, Zn and Mn in fresh and dried persimmons were very small. It is known that the contents of most minerals and especially trace elements in plants are very low: it can be 10^{-4}–10^{-5}% (Shkolnik, 1984). However, in terms of biological activity they are strikingly strong. When trace elements are incorporated into organo-mineral complexes, their ability is enhanced a thousand and sometimes a million fold over the activity of simple ionic state (Shkolnik, 1984).

The determination of the TRSA is more important than of any individual antioxidants (Lotito & Frei, 2004). Therefore, besides determination of the contents of total polyphenols two different supplementary radical scavenging assays were used in this investigation (Miller et al., 1996; Singh et al., 2002). TRSAs and polyphenols are the most popular methods; therefore, for the characterization of different persimmon samples such methods are discussed. For the determination of

phenolics Folin-Ciocalteu assay is preferable (Katsube et al., 2004). The radical scavenging activity determined by DPPH and ABTS assays using discoloration of these radicals has been applied due to their reproducibility (de Ancos, Gonzalez, & Cano, 2000; Katsube et al., 2004; Kondo, Yoshikawa, & Katayama, 2004; Pellegrini, Del Rio, Colombi, Bianchi, & Brighenti, 2003; Singh et al., 2002). In our previous investigations different extraction procedures and their yields were compared (Gorinstein et al., 1994, 2001). The optimum extraction was achieved with ethanol and methanol, and the same results were shown by Singh et al. (2002). The obtained contents of total polyphenols and the related TRSA have shown that both variables remained high in dried persimmons. However, the contents of total polyphenols and the TRSA in fresh persimmons were significantly higher than in dried persimmons. According to our data, the correlation coefficient between the Folin-Ciocalteu assay and the DPPH radical scavenging assay is high. These results correspond with the data of Katsube et al. (2004), who reported that the correlation between DPPH radical scavenging activity and total phenol content as estimated by the Folin-Ciocalteu method was significant and varied from 0.70 to 0.90. Such results indicate that DPPH, Trolox equivalent antioxidant capacity (TEAC) using ABTS, ferric ion reducing antioxidant power (FRAP) can be credibly predicted on the basis of the Folin-Ciocalteu assay and that these four methods depend on a similar mechanism: the property of electron transfer (Huang et al., 2005). The obtained results in this report are in the range of the data of others used in the same methods (Pellegrini et al., 2003). Huang et al. (2005) have used hydrogen atom transfer and reported the results by oxygen radical absorbance capacity (ORAC).

In the present research the polyphenols were extracted with 95% ethanol. Ethanol or methanol solvents of 40–70% show higher capacity to extract polyphenols than absolute alcoholic solvents (Katsube et al., 2004). From this point of extraction our results can be compared with Singh et al. (2002), where absolute methanol was used: obtained yield (%, w/w) was lower than with methanol (8.54 vs. 9.38).

The content of polyphenols and radical scavenging activity of persimmons was compared with other plant extracts. Katsube et al. (2004) studied 52 edible plants including as well persimmon. It was shown that the greatest activities in LDL oxidation assay were in akamegashiwa (*Mallotus japonicus*) leaf, Japanese privet (*Ligustrum japonicum*) leaf, green tea [*Camellia sinensis* (L.) O. Kuntze], and astringent persimmon (*D. kaki*). These results were obtained with 70% of ethanol extraction and astringent persimmon. The persimmon without astringent component had lower antioxidant activity, because the amount of soluble persimmon tannins was lower. Condensed tannins present in persimmon fruits are responsible for the astringent taste (Wu & Hwang, 2002).

According to our previous comparative investigations, the contents of polyphenols and other bioactive compounds and the radical scavenging activity of fresh persimmon were significantly higher than in traditional fruits (Gorinstein et al., 2001). In other reports it was found that (Guo et al., 2003) by FRAP the hawthorn was the strongest in antioxidant capacity among all fruit fleshes and followed by jujube, kiwifruit, mulberry, strawberry and pomegranate. The watermelon and persimmon were the weakest. In our previous reports (Gorinstein et al., 1994, 2001) we have studied the most popular varieties of persimmon (Triumph, seedless, and Fuyu with seeds) and comparable results were obtained. In other reports two cultivars Rojo Brillante and Sharon were compared for their antioxidant activity and carotenoid composition (De Ancos et al., 2000). Structural variety of the condensed tannins (proanthocyanidins) of 16 Diospyros species was investigated. Eleven species contained condensed tannins, mostly consisting of a mixture of CA and gallocatechin (GCA) repeating units; the other five species did not (Nakatsubo et al., 2002). In past were published only results of investigations of processed persimmons such as purees and liqueur (de Ancos et al., 2000; Gorinstein et al., 1993); therefore, the data discussed in the present report are shown for the first time. As it was mentioned the comparison can be done if the same extraction procedure, the same antioxidant method and the same variety was used.

In conclusion: (1) the differences in the contents of dietary fibers, minerals and trace elements in fresh and dried persimmons are not significant; (2) the content of total polyphenols and the related TRSA remained high in dried persimmons; (3) when fresh fruits are not available, proper dried persimmons could be used as a valuable substitute in diseases preventing diets.

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