

Some essential phytochemicals and the antioxidant potential in fresh and dried persimmon

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Abstract

Fresh persimmon contains high quantities of bioactive compounds, but is only available in the autumn and winter months. The aim of this investigation was to compare fresh and dried persimmon in order to determine whether the latter could be a substitute for fresh fruit. It was found that the contents of dietary fibers and trace elements in fresh and equivalent quantities of dried fruits were comparable. The content of total polyphenols in fresh persimmon was higher than in dried fruit, but not significantly ($P > 0.05$). Also the antioxidant potential in fresh persimmon as determined by all three used tests was higher than in dried fruit, but not significantly ($P > 0.05$). The methanol extracts of fresh and dried persimmon using the β -carotene–linoleate model system have shown 91% and 88% of antioxidant activity at 50 μ l, respectively. Radical scavenging activity with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method has shown 88% and 84% for the same extracts and the nitric oxide test showed similar results. The best correlation was found between polyphenols, β -carotene, DPPH and nitric oxide values (R^2 ranges between 0.9535 and 0.9934). In conclusion, both fresh and dried persimmon possess high contents of bioactive compounds and have a high antioxidant potential. When fresh fruits are not available, proper dried persimmon can be successfully used.

Introduction

At present tropical and subtropical fruits are available both in the USA and Europe. Among them is persimmon. Persimmon (*Diospyros kaki L.*) originated in Japan and has two widespread varieties: Fuio, which has seeds; and Triumph, which is seedless. The shape of this fruit is round, the texture is crispy, and the taste of ripe fruit is sweet. Some researchers have shown that persimmon is one of the most bioactive fruits (Uchida et al. 1989; Achiva et al. 1997). It was found that persimmon contains (in 100 g fresh fruit): water, 80.3 g; protein, 0.58 g; total lipids, 0.19 g; total

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carbohydrates, 18.6 g; and some minerals (magnesium, iron, zinc, copper, manganese and others), total dietary fiber up to 1.48 g, a high concentration of antioxidants like ascorbic acid (up to 7.5 mg), carotenoids (particularly β -cryptoxanthin, zeaxanthin and β -carotene), polyphenols and a specific group of polyphenols—tannins (Cerutti & Zappavigna 1977; Young & How 1986; Gross 1987; Uchida et al. 1989). The dry residue of persimmon includes among others: polyphenols, 0.16–0.25 g/100 g; carotenoids, 0.002 g/100 g; and soluble and non-soluble proteins, 0.64–1.3 g/100 g (Gross 1987; Pirretti 1991; Daood et al. 1992). Some authors indicate that particular components of persimmon are more active (Uchida et al. 1989). So Uchida et al. (1989, 1990) have reported that persimmon tannins prolong life and reduce the incidence of stroke in hypertensive rats. This effect was attributed to the fact that persimmon tannins are 20 times more potent than vitamin E—a classical antioxidant.

Persimmon is one of the most important fruit crops in Israel, which was widely investigated by our international team *in vitro* (Gorinstein et al. 1993, 1994, 1998a, 2001) and in experiments on laboratory animals *in vivo* (Gorinstein et al. 1998b, 1998c, 2000). We have found in the *in vitro* investigations that persimmon (cultivar Triumph) possesses high contents of dietary fibers, trace elements and total polyphenols, and has a high antioxidant potential. Diets supplemented with this fruit in the experiments on laboratory animals have decreased plasma lipid levels and increased plasma antioxidant activity mainly in rats fed cholesterol.

However, fresh persimmons are not available all year round: only in the period of September–December. Therefore, it was important to find a proper substitute for fresh fruits, which could be used when fresh persimmons are not available. Therefore, it was decided to prepare in our laboratory proper dried persimmons and to compare the contents of bioactive compounds and antioxidant potential in fresh and dried fruits in order to decide if the latter could be a substitute for fresh persimmons.

There are many methods for total antioxidant potential determination and every one has its limitations (Yu et al. 2002). Some antioxidant methods give different antioxidant activity trends (Ou et al. 2002). Therefore, the total antioxidant potential of fresh and dried persimmon was determined by the following tests, which were successfully tested in fruits:

1. Antioxidant assay using the β -carotene linoleate model system (β -carotene) (Singh et al. 2002).
2. Radical scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Singh et al. 2002).
3. Scavenging activity against nitric oxide (NO) (Marcocci et al. 1994; Saija et al. 1999).

As far as we know, there have previously been no such investigations.

Materials and methods

Chemicals

DPPH, β -carotene, butylated hydroxyanisole (BHA), Greiss reagent, sodium nitroprusside, phenolic acids (ferulic, gallic, protocatechuic, vanillic and *p*-coumaric), and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co. (St Louis, MO, USA). All reagents were of analytical grade.

Fruits and sample preparation

Ripened persimmons at the same maturity degree were purchased from an Israeli farmer. These persimmons were randomly divided into two groups: one for investigation as fresh fruits, and the second as dried fruits. After the drying process (lyophilizer Virtis model 10-324, 48 h, vacuum pressure of 1 mm Hg, temperature of -3°C), 19.9 g dried fruits were recovered from every 100 g fresh fruits. The dried persimmons were powdered in a grinder to obtain 40-mesh size powder.

Determination of the phytochemicals

Total, soluble and insoluble dietary fibers were determined according to Prosky et al. (1992).

The determination of trace elements (Fe, Cu, Zn and Mn) was carried out as follows. The samples of fresh and dried fruits were lyophilized separately. Then 0.8 g lyophilized samples was mineralized in a microwave oven with concentrated HNO_3 . The concentrations of all four aforementioned elements were estimated by a Perkin-Elmer 5100 ZL atomic absorption spectrometer (Perkin-Elmer Ltd, Beaconsfield, UK), using the flame method for Fe, Cu, Zn and the flameless method for Mn (Gorinstein et al. 2001).

Total polyphenols were measured at 765 nm using Folin–Ciocaltea reagent with gallic acid as standard (Gorinstein et al. 2003).

Phenolic acids were determined by high-performance liquid chromatography with a C_{18} column (250×4.6 mm, $10 \mu\text{m}$; Supelco, Inc., Bellefonte, PA, USA) using a solvent of 86% water/4% acetic acid/10% methanol with a flow rate of 2 ml/min. The column eluate was monitored at 320 nm using an ultraviolet detector (Bocco et al. 1998).

In the past for the determination of the antioxidant potential in fruits our team has used various antioxidant tests (Gorinstein et al. 2003). According to our experience the following three tests are the most suitable for investigation of fruits and therefore were used in this investigation.

1. Antioxidant assay using the β -carotene linoleate model system (Singh et al. 2002). β -carotene (0.2 mg) in 0.2 ml chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) were mixed. Chloroform was removed at 40°C under vacuum. The resulting mixture was diluted with 10 ml water. To this emulsion was added 40 ml oxygenated water. Four milliliter aliquots of the emulsion were added to 0.2 ml of the sample of ethanol extracts (50 and 100 μl). Synthetic antioxidant BHA in ethanol was used for comparative purposes. A control containing 0.2 ml ethanol and 4 ml of the aforementioned emulsion was prepared. The absorbance at 470 nm was taken at 50°C at zero time ($t = 0$). Measurement of absorbance was continued during 180 min at an interval of 15 min. A mixture prepared as already described, but without β -carotene, served as the blank. The antioxidant activity of the extracts was evaluated in terms of bleaching of the β -carotene: $\text{AA} = 100 [1 - (A_0 - A_t) / (A^{\circ}_0 - A^{\circ}_t)]$, where A_0 and A°_0 are the absorbance values measured at zero time of the incubation for the test sample and control, respectively, and A_t and A°_t are the absorbances measured in the test sample and control, respectively, after incubation for 180 min.

2. Radical scavenging activity using DPPH method (Singh et al. 2002). Different concentrations (50 and 100 μ l) of the sample of ethanol extracts and BHA (25 and 50 μ l) were taken. The volume was adjusted to 100 μ l by adding EtOH. Five milliliters of a 0.1 mM ethanolic solution of DPPH was added. The tubes were allowed to stand at 27°C for 20 min. The control was prepared as already described but without any extract, and EtOH was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage: % radical scavenging activity = (control OD – sample OD/control OD) \times 100.
3. Scavenging activity against NO (Marcocci et al. 1994; Saija et al. 1999). Scavengers of NO compete with oxygen, leading to a reduced production of nitrite. Samples (0.5 ml at various concentrations) were diluted with 0.5 ml sodium nitroprusside solution and incubated at 25°C for 150 min. At the end of the incubation, 1 ml Greiss reagent was added to each sample and the absorbance was read at 542 nm. The nitrite concentration was calculated by referring to the absorbance of standard solutions of potassium nitrite. Results were expressed as the percentage of nitrite production with respect to control values (sample, 0 μ l).

Statistical analyses

The results of this study are means \pm standard deviation of five measurements. When appropriate, differences between groups were tested by two-way analysis of variance. In the assessment of the antioxidant potential, the Spearman correlation coefficient (R) was used. Linear regressions were also calculated. $P < 0.05$ was considered significant.

Results

Total, soluble and insoluble dietary fiber

The dietary fiber contents in fresh and dried persimmons are summarized in Table I. If we take into account that after the drying process 19.9 g dried fruits were received from every 100 g fresh fruits, then the contents of dietary fiber in fresh and equivalent quantities of dried persimmon are comparable ($P > 0.05$).

Trace elements

The contents of the studied trace elements in fresh and dried persimmons are summarized in Table II. If we take into account that after the drying process 19.9 g

Table I. Dietary fiber contents in fresh and dry persimmon (g/100 g fresh or dried fruit).

	Fresh	Dried
Total	1.81 \pm 0.12 ^a	8.82 \pm 0.9 ^a
Soluble	0.68 \pm 0.06 ^b	3.21 \pm 0.3 ^b
Insoluble	1.13 \pm 0.11 ^c	5.61 \pm 0.5 ^c

Values presented as means \pm standard deviation of five measurements. Means in columns and rows without common superscript letters differ significantly ($P < 0.05$).

Table II. Some essential microelements in whole fresh and dried persimmon.

Samples	Na (mg/100 g)	K (mg/100 g)	Fe (µg/100 g)	Mn (µg/100 g)	Cu (µg/100 g)	Zn (µg/100 g)
Fresh persimmon	4.94±0.4 ^a	198.2±9.20 ^a	99.2±9.20 ^a	101.2±9.30 ^a	9.3±0.8 ^a	13.9±1.1 ^a
Dried persimmon	24.3.1±2.2 ^a	989.1±32.2 ^a	491.1±32.2 ^a	493.3±33.3 ^a	44.7±3.9 ^a	66.7±5.1 ^a

Values presented as means ± standard deviation of five measurements. Means in columns without common superscript letters differ significantly ($P < 0.05$).

dried fruits were received from every 100 g fresh fruits, then the contents of all studied trace elements in fresh and dried persimmon are comparable ($P > 0.05$).

In our previous investigation (Gorinstein et al. 2001) we found that the peel to pulp ratio for divalent elements is significantly higher ($P < 0.05$) than for monovalent elements. Also in the present investigation we found that the peel to pulp ratio for divalent elements is significantly higher ($P < 0.05$) than for monovalent elements (data not shown).

Total polyphenols

The content of total polyphenol was 1.51 ± 0.1 and 7.48 ± 0.6 g/100 g for fresh and dried persimmon, respectively. The content of the total polyphenols in fresh and equivalent quantities of dried persimmon was comparable ($P > 0.05$).

Phenolic acids

The mean contents of phenolic acids were as follows: 9.9 ± 0.9 and 48.9 ± 3.8 , 21.2 ± 2.1 and 104.8 ± 8.2 , 6.1 ± 0.5 and 29.1 ± 2.1 , 0.6 ± 0.1 and 2.3 ± 0.2 , and 60.1 ± 5.2 and 292.1 ± 19.7 mg/100 g fresh or dried persimmon for ferulic, gallic, protocatechuic, vanillic and *p*-coumaric acids, respectively. Among the phenolic acids the highest concentration was of *p*-coumaric and the lowest concentration was of vanillic acid. The contents of the studied phenolic acids in fresh and dried persimmon were comparable ($P > 0.05$).

The total antioxidant activity

The methanol extracts of fresh and dried persimmon using the β -carotene–linoleate model system have shown 91% and 88% antioxidant activity at 50 μ l, respectively (Figure 1). Similarly, the methanol extract of fresh and dried persimmon using the DPPH method have shown 88% and 84% antioxidant activity at 50 μ l, respectively (Figure 1) and the NO test showed 47% and 41% activity, respectively. As can be seen, the free radical scavenging activity of fresh and dried persimmon as determined by all three used tests was high in fresh fruit and comparable with equivalent quantities of the dry fruit ($P > 0.05$).

The kinetics of DPPH scavenging effects (Figure 2) has shown that fresh persimmon was close to BHA (0.2 mg/ml). Dry persimmon was close to BHA (0.1 mg/ml). Fresh persimmon and dry persimmon differ from each other only by 2.3% remaining DPPH. BHA of 0.2 mg/ml is significantly lower than the others and showed the highest antioxidant activity.

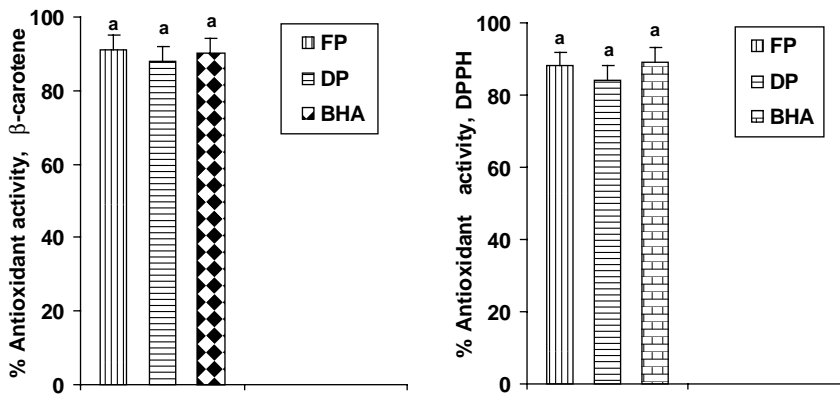


Figure 1. The antioxidant activity values of fresh and dried persimmon using the β -carotene–linoleate model system and the radical scavenging activity DPPH method. Mean \pm standard deviation (vertical lines). Bars with different letters are significantly different ($P < 0.05$). FP, fresh persimmon; DP, dry persimmon.

A low correlation was observed between β -carotene, DPPH and NO values and the dietary fiber content ($R^2 = 0.4741$, $R^2 = 0.4867$ and $R^2 = 0.4902$, respectively). As can be seen a high degree of correlation was observed between the NO, β -carotene and DPPH values and polyphenols (R^2 ranges between 0.9535 and 0.9934). A very good correlation was found between β -carotene, DPPH and NO values and the content of individual phenolic acids: the best between *p*-coumaric acid and β -carotene, and *p*-coumaric acid and DPPH values (R^2 ranges between 0.96 and 0.97) and between ferulic acid and β -carotene, and ferulic acid and DPPH values (R^2 ranges between 0.91 and 0.92). A good correlation was also registered between *p*-coumaric acid and β -carotene, and *p*-coumaric and DPPH values, and gallic acid, DPPH and NO values (R^2 ranges between 0.84 and 0.86).

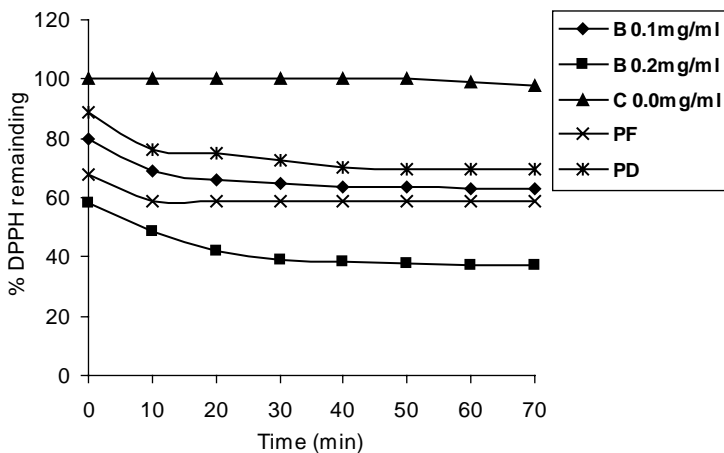


Figure 2. Kinetics of DPPH scavenging effects of BHA (B) at concentrations of 0.1 and 0.2 mg/ml, of fresh persimmon (PF) and dry persimmon (PD) extracts (60 mg/ml), and of control (C) (0.0 mg/ml).

Discussion

Despite all preventive measures, atherosclerosis still one of the most dangerous diseases in the Western industrial countries (Haskell et al. 1995). Atherosclerosis is a multifactorial process based on the action of various risk factors (Ross 1999). According to this author, the form and content of the advanced lesions of atherosclerosis demonstrate the results of three fundamental biological processes: (a) accumulation of intimal smooth muscle cells, together with variable numbers of accumulated macrophages and T lymphocytes; (b) formation by the proliferated smooth muscles cells of large amounts of connective tissue matrix, including collagen, elastic fibers, and proteoglycans; and (c) accumulation of lipids, principally in the form of cholesteryl esters and free cholesterol within the cells as well as in the surrounding connective tissues.

However, after the paper 'Beyond cholesterol: modifications of low density lipoprotein that increases its atherogenicity' was published (Steinberg et al. 1989), more and more authors support the theory that atherosclerosis is mainly an oxidative disease (Steinbrecher et al. 1990; Witztum & Steinberg 1991; Aviram 1993). The oxidative theory is widely used as a basis for prevention and treatment of this disease (Steinberg et al. 1989; Steinbrecher et al. 1990; Witztum & Steinberg 1991; Aviram 1993). According to this theory, only cholesterol-rich oxidized low-density lipoprotein-cholesterol particles are able to penetrate arterial walls, causing their occlusions (Steinberg et al. 1989). Such pathological damage can be found practically in every artery (Genest et al. 1992; Hodis et al. 1994). The most important damages are in the coronary arteries, which consequently lead to myocardial infarctions.

It was shown that proper diets can prevent the oxidation of low-density lipoprotein-cholesterol particles and therefore their penetration into arterial walls (Rimm et al. 1996a, 1996b).

In the past decades our international team has investigated some natural products in order to determine whether they could be used in antiatherosclerotic diets (Gorinstein et al. 1998a, 1998b, 1998c, 2000, 2001). In these investigations of fresh persimmon we have found that this fruit possesses a high amount of bioactive compounds and a high antioxidant potential (Gorinstein et al. 1993, 1994, 1998a, 2001). We have also found that diets supplemented with this fruit positively influence plasma lipid levels and plasma antioxidant activity in rats fed cholesterol (Gorinstein et al. 1998b, 1998c, 2000). However, fresh persimmons are not available all year round. It was important to find an alternative form, which could be used when fresh persimmons are not available. Therefore, a dried form of persimmon was prepared for the present investigation. The contents of dietary fiber, total polyphenols and phenolic acids were determined in fresh and dried persimmon, and their antioxidant potential was evaluated. It was found that both forms of this fruit possess high contents of bioactive compounds and that their antioxidant potential as determined by all three antioxidant tests is very high. The studied variables in fresh and equivalent quantities of dried persimmon and their antioxidant potential were comparable.

Therefore, according to our previous investigation *in vivo* (Gorinstein et al. 1998b, 1998c, 2000) it could be supposed that dried persimmon could also positively influence the plasma lipid levels and the plasma antioxidant activity. Of course, it has to be proved in investigations *in vivo*.

It must be mentioned that also in this investigation, like in our previous (Gorinstein et al. 2001), we have found that the peel to pulp ratio for divalent elements is

significantly higher ($P < 0.05$) than that for monovalent elements. Perhaps such a ratio is the result of higher antioxidant activity in the peel than in the pulp of persimmons.

Conclusions

1. The contents of dietary fiber, trace elements, total polyphenols and phenolic acids in fresh and equivalent quantities of dried persimmon are comparable.
2. Also the antioxidant potential of both fresh and equivalent quantities of dried persimmon as determined by all three used tests were very high and comparable.
3. Therefore, when fresh fruits are not available, the powdered dried persimmon could be successfully used as a substitute or a nutritional additive in functional foods.

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