

Nutraceutical value of persimmon (*Diospyros kaki* Thunb.) and its influence on some indices of atherosclerosis in an experiment on rats fed cholesterol-containing diet

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Abstract: The nutraceutical value of persimmon (*Diospyros kaki* Thunb. cv. Triumph) and its influence on some indices of atherosclerosis were studied *in vitro* and in experiment on rats fed cholesterol-containing diet. It was found that persimmon possesses a high nutraceutical value: it contains soluble fibers, total polyphenols and phenolic acids. The content of dietary fiber was 1.83 ± 0.11 , 0.69 ± 0.07 and 1.14 ± 0.12 g/100 g fresh weight (FW) for total, soluble and insoluble fibers, respectively, 1.55 ± 0.11 g/100 g FW of total polyphenols and 10.29 ± 0.9 , 21.89 ± 2.2 , 6.5 ± 0.5 , 0.71 ± 0.05 and 58.2 ± 5.1 mg/100 g FW of ferulic, gallic, protocatechuic, vanillic and *p*-coumaric acids, respectively. Radical scavenging activity (RSA) with the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method, a nitric oxide and β -carotene tests has shown 88% for methanolic persimmon extracts. The properties of fresh and dry persimmon were compared. High correlation was found between polyphenols and RSA with different tests (R^2 ranges between 0.9535 and 0.9934). Addition of persimmon to the diets did not affect diet intake, its efficiency or body weight gains of rats. Other persimmon cultivars (Fuyu and Jiro) were compared with cv. Triumph *in vitro* and *in vivo* studies. 'Fuyu' was more effective than 'Jiro'. Diets supplemented with whole fruit positively influenced some indices of atherosclerosis in serum of rats fed a cholesterol-containing diet: it hindered the rise of lipid levels and the decrease in the antioxidant activity.

1. Introduction

Epidemiological studies, experiments on laboratory animals and investigations of humans show that consumption of fruits and vegetables is associated with a low risk of cardiovascular diseases and cancer (Chahoud *et al.*, 2004; Dauchet *et al.*, 2006). The health properties of these natural products depend on the contents of bioactive compounds, mainly phenolics, and partly on dietary fibers (Dauchet *et al.*, 2006). Persimmon (*Diospyros kaki* Thunb. cv. Triumph) is among widely consumed fruits. Some researchers have shown that persimmon is one of the most bioactive fruits, especially in polyphenols and tannins (Uchida *et al.*, 1990; Piretti, 1991; Daood *et al.*, 1992; Achiwa *et al.*, 1997). We also found in *in vitro* investigations that persimmon (cv. Triumph) possesses high contents of

dietary fibers, trace elements and total polyphenols, and has a high antioxidant potential (Jung *et al.*, 2005). The aim of the present investigation was to assess the nutraceutical value of persimmon (cv. Triumph) and its influence on some indices of atherosclerosis in an experiment on rats fed a cholesterol-containing diet. Two additional cultivars (Jiro and Fuyu) were used in the present study.

2. Materials and Methods

Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), β -carotene, butylated hydroxyanisole (BHA), Greiss reagent, sodium nitroprusside, phenolic acids (ferulic, gallic, protocatechuic, vanillic and *p*-coumaric) were purchased from Sigma (Germany).

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Samples

Seedless persimmon (*Diospyros kaki* Thunb.) 'Triumph' was purchased from an Israeli farmer. Fresh and dry persimmon samples were used. Persimmon 'Fuyu' and 'Jiro' were obtained from South Korea, Muan county.

Determination of the bioactive compounds and the antioxidant potential

The studied bioactive compounds were determined as previously described (Jung *et al.*, 2005). For the determination of the antioxidant potential the following tests were used: 1) the β -carotene linoleate model system; 2) 1,1-diphenyl-2-picrylhydrazyl (DPPH) method; 3) scavenging activity against nitric oxide (NO); 4) 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid diammonium salt (ABTS). These tests were performed as previously described (Jung *et al.*, 2005; Park *et al.*, 2006).

Animals and diets

Treatments and diets were formally approved by the Warsaw Agricultural University Animal Ethics Committee. The experiment was conducted on 36 male Wistar rats with a standard initial weight of 120 g. They were housed individually in stainless steel metabolic cages and were randomly assigned to four equal-in-number groups of nine individuals each: two experimental (EG1 and EG2), Chol and Control (CG). All were fed during the four weeks of the experiment a basal diet (BD) which included wheat starch, casein, soybean oil, mineral mixture and vitamin mixture and cholesterol (Chol). The BD of the CG animals was supplemented with 7% of cellulose; Chol with 7% of cellulose and 1% cholesterol; EG1 with 7% of whole dry persimmon fruit and 1% cholesterol; and EG2 with 7% of phenol-free dry persimmon and 1% cholesterol. Another experimental on animals was performed during 47 days on 4 groups of rats: CG, Chol and with 5% lyophilized persimmons CholJiro and CholFuyu.

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.

3. Results, Discussion and Conclusions

In vitro

The contents of total, insoluble and soluble dietary fibers in fresh and dried persimmons are shown in figure 1. The differences between the obtained results were not significant ($P > 0.05$). Insoluble dietary fibers in both fresh and dried persimmons were significantly higher than soluble fibers ($P < 0.05$).

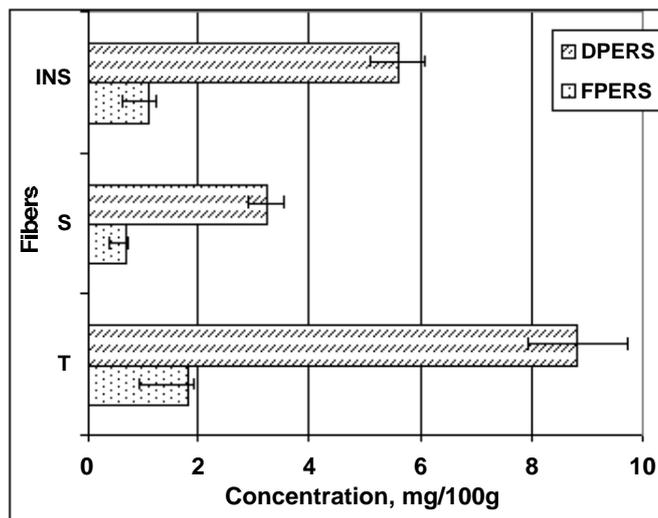


Fig. 1 - Total (T), soluble (S) and insoluble (INS) fibers in fresh (FPERS) and dry (DPERS) persimmons. $M \pm SD$ of 5 measurements.

Some essential microelements in fresh and dry persimmons were determined: Na- 4.94 ± 0.4 and 24.3 ± 2.2 ; K- 198.2 ± 9.20 and 989.1 ± 32.2 (mg/100g, respectively); Fe, Mn, Cu, Zn: 98.4 ± 8.4 and 487.4 ± 29.8 ; 99.5 ± 8.6 and 492.6 ± 31.3 ; 8.7 ± 0.7 and 40.6 ± 4.1 ; 13.1 ± 0.9 and 65.8 ± 4.9 ($\mu\text{g}/100\text{g}$, respectively).

The content of total polyphenols was 1.55 ± 0.11 g/100 g FW. The contents of phenolic acids were 10.29 ± 0.9 , 21.89 ± 2.2 , 6.5 ± 0.5 , 0.71 ± 0.05 and 58.2 ± 5.1 mg/100 g FW 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.

According to the β -carotene-linoleate test the antioxidant potential of the methanol extract of fresh and dried persimmons was 90% and 87%, respectively (Fig. 2). The antioxidant potentials of the methanol extracts of fresh and dry persimmons, using DPPH and other antioxidant scavenging assays, were slightly lower than the ones determined by β -carotene and ran-

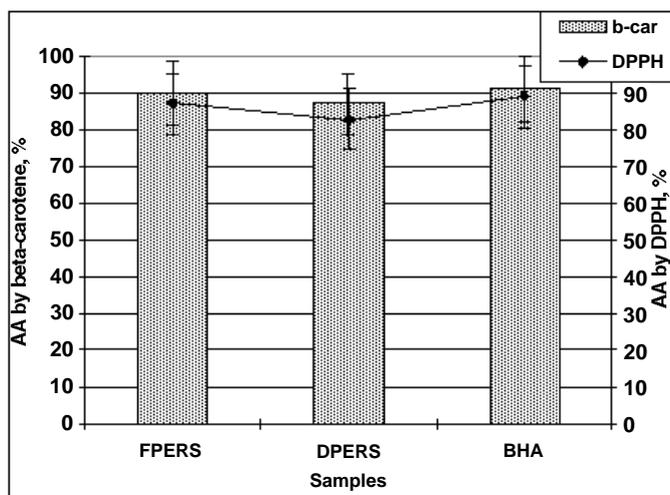


Fig. 2 - The antioxidant activity of fresh (FPERS) and dried (DPERS) persimmons, BHA (butylated hydroxyanisole), by β -car (β -carotene-linoleate model system) and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays.

ged from 87% to 83%. The free radical scavenging activity of fresh and dried persimmons as determined by 4 used tests was high in fresh fruit and comparable with equivalent quantities of the dry fruit. A low correlation was observed between β -carotene, DPPH, ABTS and NO values and the dietary fiber content ($R^2=0.4628$, $R^2=0.4749$, $R^2=0.5111$ and $R^2=0.4822$, respectively). A high degree of correlation was observed between the NO, β -carotene DPPH and ABTS values and polyphenols (R^2 ranges between 0.8267 and 0.9951) (Fig. 3). A

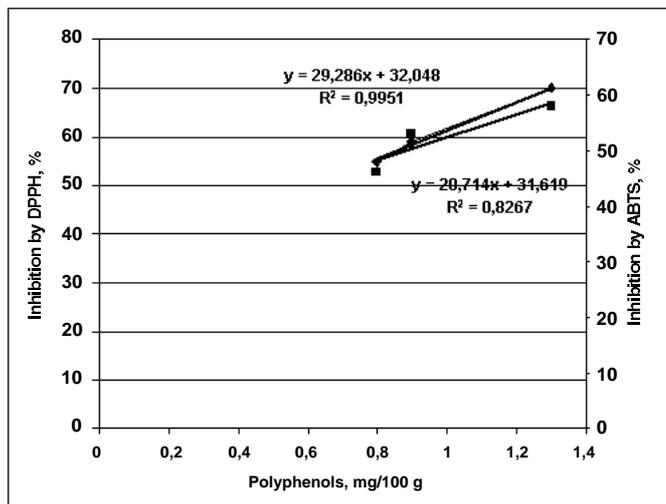


Fig. 3 - Correlation between the content of total polyphenols and the percentage of inhibition determined by two radical assays: DPPH and ABTS in fresh and dried persimmon.

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 gallic acid and DPPH and NO values (R^2 ranges between 0.84 and 0.86). Persimmons 'Jiro' and 'Fuyu' were compared by total polyphenols in the methanol extracts (mg GAE/g DW, 18.09 and 22.07, respectively) and by DPPH (mMTE/g DW, 47.44 and 51.34). As it can be seen the Fuyu cultivar showed higher antioxidant activity than 'Jiro'.

According to Piretti (1991), the mean composition of dry residue of persimmon fruit includes (in %): soluble and insoluble proteins (0.64), total sugars (14.7), reducing sugars (13.8), tannins (0.20), phenols (0.16). The phenolic components, after ripening of persimmon fruit, undergo prominent polymerization, resulting in formation of macromolecular substances, which are no longer water-soluble. Therefore, phenols were extracted by methanol or ethyl acetate.

Based on the present and previous results (Gorinstein *et al.*, 2001; Jung *et al.*, 2005; Park *et al.*, 2006) the relatively high contents of dietary fibers, total and major phenolics, main minerals, and trace elements make persimmon preferable for an antiatherosclerotic diet.

In vivo

Addition of persimmon to the diets did not affect diet intake, its efficiency or body weight gains of rats. The feed intake ranged: 17.58-20.4 g/d and body gain: 4.40-6.09 g/d (Fig. 4). Similar results were obtained using 5 or 7% of persimmon supplementation to cholesterol. At baseline, all groups did not differ from one another in plasma lipid levels. The changes in lipid levels after the trial are summarized in Table 1. The

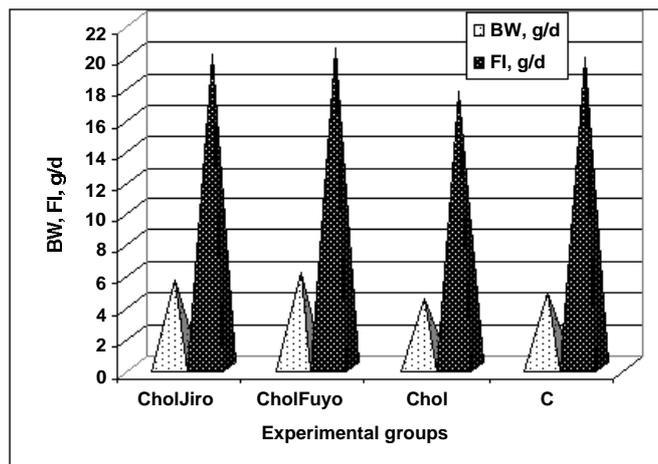


Fig. 4 - Body weight (BW, g/day) and feed intake (FI, g/day) during the experimental period of 47 days with two different persimmon cultivars in groups of cholesterol (Chol), Control (C), CholJiro and CholFuyu.

Table 1 - Plasma lipids (mM/L) and antioxidant activity (mMTE/L) after the experiment

Indices	Control	Chol	EG1	EG2
TC	2.21±0.09 a	2.69±0.11 b	2.29±0.10 a	2.45±0.11 b
LDL-Chol	0.87±0.05 a	1.25±0.07 b	0.98±0.07 a	1.10±0.08 b
HDL-Chol	1.34±0.08 a	1.44±0.08 a	1.31±0.08 a	1.35±0.08 a
TG	0.69±0.04 a	0.76±0.05 a	0.73±0.05 a	0.76±0.05 a
AA(ABTS)	1.49±0.08 a	0.95±0.07 b	1.33±0.07 a	1.11±0.06 b
AA(DPPH)	0.83±0.05 a	0.53±0.04 b	0.74±0.05 a	0.59±0.04 b

Values are means \pm SD, n = 9. Means in rows without letters in common differ significantly ($P < 0.05$).

AA= antioxidant activity;

ABTS= [2, 2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)];

Chol= cholesterol;

DPPH= 1, 1-diphenyl-2-picrylhydrazyl;

HDL-Chol= high density lipoprotein cholesterol;

LDL-Chol= low density lipoprotein cholesterol;

TC= total cholesterol;

TG= triglycerides.

increase in total cholesterol (TC) in all three groups fed cholesterol was registered. But only in rats of EG1, (diet supplemented with whole persimmon) was the increase statistically not significant ($P > 0.05$). Also an increase in the level of low density lipid cholesterol (LDL-C) in all three groups fed cholesterol was registered. The patterns of the changes in the LDL-C level were similar to those of TC. As in the case of TC, only in rats of EG1 was the increase statistically not significant ($P > 0.05$). No significant changes were registered in the level of high density lipid-cholesterol (HDL-C) and triglycerides (TG) in the three groups ($P > 0.05$). Liver TC concentration in rats of Chol, EG1 and EG2 were five and eight times higher than in CG. Whole persimmon supplemented diet (EG1) significantly hindered the rise of liver TC (34% vs. Chol group, $P < 0.05$). Only the whole persimmon supplemented diet for EG1 significantly hindered the decrease in the antioxidant activity vs. Chol group by 40% and 39.6% according to ABTS and DPPH assays, respectively (Fig. 5).

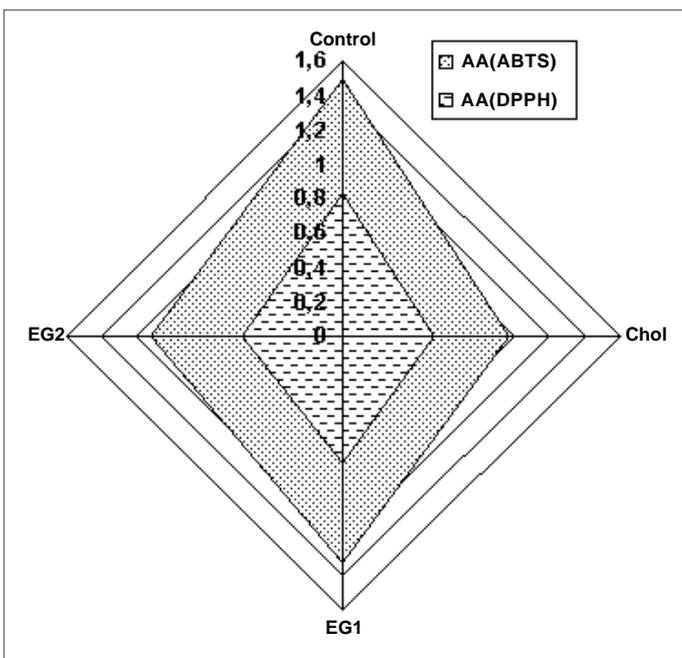


Fig. 5 - Total antioxidant capacity (mM TE/L) in plasma samples of rats after persimmon and cholesterol diets.

In rats fed persimmon diets supplemented with two additional cultivars Jiro and Fuyu in the second experiment a significant decrease in TC, TG, LDL-C, AI (atherogenic index), TC/HDL-C was found (Fig. 6). The data obtained with other cultivars were similar to 'Triumph'. A relative decrease in the lipid profile parameters between the Chol/Fuyu and Chol groups was as following (%): 19.4, 25.6, 29.7 and 23.4, respectively.

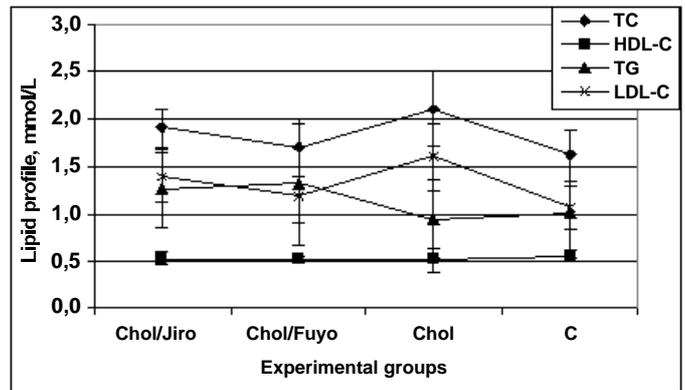


Fig. 6 - Lipid profile of serum in experiment with rats. Experimental groups with diets supplemented with persimmon (Chol/Jiro, Chol/Fuyo), Chol, cholesterol, C, control.

As was demonstrated by the previous (Gorinstein *et al.*, 2000) and this investigation, whole persimmon contains high quantities of nutraceuticals. Its antioxidant potential is high. It was found that supplementation of the cholesterol-containing diet with whole persimmon led to a hindering of the rise of plasma lipid levels and the decrease in plasma antioxidant activity. According to many authors this leads to prevention of the atherosclerosis process (Chahoud *et al.*, 2004). The four weeks of feeding different diets has shown that phenols in whole dry persimmon exercise a marked antioxidant effect, which was more statistically significant than the effects of phenol-free fruit. These results were expected; phenols of persimmon are 20 times more potent *in vitro* than the classical antioxidant vitamin E (Uchida *et al.*, 1990).

In conclusion, persimmon possesses a high nutraceutical value: it contains high quantities of dietary fibers, total polyphenols and phenolic acids. The main bioactive compound of persimmon is total phenolics: only diets supplemented with whole fruit positively influence some indices of atherosclerosis in rats fed a cholesterol-containing diet. When fresh fruits are not available, properly dried persimmons may be used as a valuable substitute. And finally, the studied three different cultivars were relatively similar and showed the decrease in the lipid profile of rats serum.

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