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Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (*Diospyros kaki* L.)

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ABSTRACT

Primary metabolites (sugars, organic acids) and secondary metabolites (phenolics and carotenoids) were quantified by HPLC in fully ripe fruit of 11 kaki cultivars: 'Amankaki', 'Cal Fuyu', 'Fuji', 'Hana Fuyu', 'Jiro', 'O'Gosho', 'Tenjin O'Gosho', 'Thiene', 'Tipo', 'Tone Wase' and 'Triumph'. Amongst the analysed cultivars, 'Tone Wase' stands out as the richest in sugars, particularly glucose, and cultivars 'Tipo' and 'Triumph' contained the highest amounts of organic acids. Cultivars 'O'Gosho', 'Cal Fuyu' and 'Hana Fuyu' contained the least sugars and cultivar 'Jiro' the least organic acids. Amongst the individual phenolic compounds catechin and gallic acid were present in highest concentrations. The predominant carotenoid in both skin and pulp of ripe persimmon fruit was β-carotene, the highest content was measured in skin of cultivar 'Hana Fuyu', which also contained the highest level of total carotenoids. In persimmon pulp, much lower values for carotenoids were obtained, particularly in fruit of cultivar 'Cal Fuyu'.

1. Introduction

Japanese persimmon (*Diospyros kaki* L.) is a deciduous fruit, widespread in China, Japan and Korea, with more than 1000 local varieties estimated to be grown in Japan and numerous commercial cultivars popular worldwide (George & Redpath, 2008). Persimmon was traditionally used for medicinal purposes, e.g. treating coughs, hypertension, paralysis, frostbite, burns and bleeding; its fruit was consumed fresh or dried and trees were planted for ornamental purposes and wood (Ferrini & Pennati, 2008; Luo & Wang, 2008). As a good source of primary metabolites (particularly rich in sugars) and many nutritional antioxidants, carotenoids and polyphenols, it is now a popular and widespread fruit species in temperate to tropical regions (George & Redpath, 2008).

Persimmon fruit in Europe is mostly consumed fresh; therefore high contents of sugars and moderate concentrations of organic acids, which contribute greatly to the sensoric attributes, are preferred. The sugar/organic acid ratio and the content of individual sugars directly correlate with the sensation of sweetness and aroma in fruit, as was found in the research on peach (Colaric, Veberic, Stampar, & Hudina, 2005), where glucose, fructose and sucrose are responsible for the aromatic taste. However, consumers today are not only searching for a sweet tasting cultivar but also health-promoting compounds in fruit, such as polyphenols and carotenoids, which are equally important. In climacteric fruit, such as persimmon, the sugars (particularly sucrose) and total carotenoid contents increase in the final stages of ripeness and firmness, soluble tannins and titratable acidity levels decrease, resulting in an increase of flavour (Candir, Ozdemir, Kaplankiran, & Toplu, 2009).

Some researchers have shown that persimmon is one of the most bioactive fruits (Daood, Biacs, Czinkotai, & Hoschke, 1992; Gorinstein et al., 1998). Persimmon skin, in particular, has very high concentrations of polyphenols and carotenoids (George & Redpath, 2008) which contribute greatly to the vivid orange colour of the mature fruit. Higher concentrations of carotenoids in persimmon fruit thus, not only present an important biological property, but are also favoured from the marketing perspective. In recent studies, special focus was put on the antioxidant activity of persimmon fruit and the potent scavenging action against active oxygen free radicals was found to be mainly due to the group of flavonoids, namely flavan-3-ols with repeating units of catechin compounds (Lee, Cho, Tanaka, & Yokozawa, 2007). The content varies greatly amongst cultivars; comparatively non-astringent cultivars of persimmon appear to have far less polyphenols, catechins and tannins (lower antioxidant potential) than the astringent types (Chen, Fan, Yue, Wu, & Li, 2008; George & Redpath, 2008).

The beneficial effects of phenolic compounds in preventing cardiovascular diseases and the general favourable impacts of these substances on human health (Del Caro & Piga, 2008) could present important factors for selection. In the present study, the fully ripe fruits of 11 astringent and non-astringent persimmon cultivars grown in the northernmost Mediterranean region were analysed for their chemical composition. The differences in the concentrations of primary and secondary compounds determine

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the nutritional importance of the selected cultivars. Furthermore, they provide information on the marketing potential of the 11 cultivars and present an important chemical insight into the popular cultivars grown in this region.

2. Materials and methods

2.1. Plant material

Fruits of 11 cultivars of *D. kaki* L. ('Amankaki', 'Cal Fuyu', 'Fuji', 'Hana Fuyu', 'Jiro', 'O'Gosho', 'Tenjin O'Gosho', 'Thiene', 'Tipo', 'Tone Wase' and 'Triumph') were collected in October and November, 2007, in the Fruit Growing Centre Bilje, Slovenia, at their physiological maturity and allowed to ripen (full-ripeness, consumption maturity) at room temperature prior to analysis. Extractions were performed at consumption maturity, which was determined by the colour parameters obtained with a Minolta CR-10 Chroma portable colourimeter (Minolta Co., Osaka, Japan) with C illuminant. Fruit chromaticity was recorded in Comission International d'Eclairage (CIE) parameters, namely L^*, a^* and b^* colour space coordinates. Hue angle was calculated from parameters a^* and b^* and used as a reference, where 0° represents red colour, 90° yellow, 180° green and 360° blue (Jakopic, Veberic, & Stampar, 2007). Candir et al. (2009) report the parameter h° as an important maturity marker for persimmon fruit. Fruit was considered to be at consumption maturity when the parameter h° was between 45° and 55°, depending on cultivar (Table 1). For each cultivar, fruit from five trees were collected, i.e. three fruits per tree ($n = 15$).

2.2. Extraction and determination of sugars and organic acids

Fruit samples were analysed for the contents of individual sugars (glucose, fructose and sucrose) and organic acids (citric, malic and fumaric acid). Ten grams of mashed fruit were extracted with 50 ml of twice-distilled water, homogenised with the T-25 Ultra-Turrax macerator (Ika-Labortechnik, Staufen, Germany) and kept for 30 min at room temperature with occasional stirring. The extracted sample was centrifuged at 10 000 rpm for 7 min at 10 °C (Eppendorf Centrifuge 5810 R, Hamburg, Germany). The supernatant was filtered through a 0.45 μm cellulose ester filter (Macherey-Nagel, Düren, Germany), transferred into a vial and used for further analysis. Analysis of sugars was performed on a Thermo Separation Products HPLC with a refractive index (RI) detector (Thermo Scientific, San Jose, CA). Separation of sugars was carried out using a Rezex RCM-monosaccharide 300 × 7.8 mm column (Phenomenex, Torrance, CA), with column temperature maintained at 65 °C and flow rate of 0.6 ml min^{-1}; for each analysis, 20 μl of sample were used. The samples were eluted according to the isocratic method described by Sturm, Koron, and Stampar (2003). For the mobile phase, twice-distilled water was used, and RI detector for identification. Organic acids were analysed with HPLC, using an Aminex-HPX-87 H 300 × 7.8 mm column (Bio-Rad Laboratories, Hercules, CA) and a UV detector set at 210 nm, according to the method described by Sturm et al. (2003) with a flow rate of 0.6 ml min^{-1}, maintaining the column temperature at 65 °C. For the mobile phase, 4 mM sulphuric acid was used. The sugars and organic acids in persimmon extracts were identified by their retention time characteristics; the concentrations were calculated with the help of the corresponding external standard and expressed as g kg^{-1}FW for sugars and mg kg^{-1} FW for organic acids.

2.3. Extraction and HPLC analysis of phenolic compounds

Five grams of mashed whole fruit were extracted with 20 ml of MeOH and 1% 2,6-di-tert-butyl-4-methylphenol (BHT) in an ultrasonic bath (Escarpa & Gonzalez, 2000). After extraction, the treated samples were centrifuged for 7 min at 10 000 rpm and the supernatant was filtered through a Chromafil AO-45/25 polyamide filter, produced by Macherey-Nagel, to a vial, prior to injection into the HPLC system. The analysis was performed using a Surveyor HPLC system and a diode array detector (DAD), controlled by a Crom-Quest 4.0 chromatography workstation software system (Thermo Scientific). The phenolic compounds were analysed at 280 nm. The column used was a Phenomenex Gemini C18 (150 × 4.6 mm 3 μm), operated at 25 °C. The elution solvents were 1% formic acid in twice-distilled water (A) and 100% acetonitrile (B). The samples were eluted according to the linear gradient described by Marks, Mullen, and Crozier (2007), with the injection amount of 20 μl and a flow rate 1 ml min^{-1}. The phenolic compounds in persimmon extracts were identified by their spectral and retention time characteristics and the use of external standards. Concentrations were expressed as mg per kg FW.

2.4. Determination of total phenolic content

The extraction and determination of total phenolics was carried out according to the same protocol as for individual phenolics, with the difference that no BHT was added. The total phenolic content (TPC) of the extracts was assessed by using the Folin–Ciocalteu phenol reagent method (Singleton & Rossi, 1965). Six mililiters of twice-distilled water and 500 μl of Folin–Ciocalteu reagent were added to 100 μl of the sample extracts and, after waiting, between 8 s and 8 min at room temperature, 1.5 ml of sodium carbonate (20% w/v) were added. The extracts were mixed and allowed to stand for 30 min at 40 °C before measuring absorbance at 765 nm on a Lambda Bio 20 UV/VIS spectrophotometer (Perkin Elmer, Waltham, MA). A mixture of water and reagents was used as a blank. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg per kg of FW.

Table 1
Chromatographic parameters of fruit skin of 11 persimmon (*Diospyros kaki* L.) cultivars.

Diospyros kaki L. cultivars	a^*	b^*	L^*	C^*	h°
'Amankaki'	34.1 ± 2.14 ab	47.7 ± 2.16 bc	52.1 ± 1.69 abc	55.7 ± 3.78 a	51.6 ± 0.87 bcde
'Cal Fuyu'	38.4 ± 1.03 bc	46.2 ± 1.00 abc	54.0 ± 0.50 abc	30.2 ± 1.02 abc	50.3 ± 0.96 bcd
'Fuji'	38.1 ± 1.87 abc	43.3 ± 2.20 ab	51.1 ± 1.21 ab	56.7 ± 3.02 ab	50.0 ± 1.00 bc
'Hana Fuyu'	41.0 ± 1.19 c	50.0 ± 1.04 c	56.1 ± 0.68 c	64.9 ± 0.67 c	51.1 ± 1.27 bcde
'Jiro'	36.2 ± 1.05 abc	47.4 ± 1.54 bc	53.2 ± 3.46 abc	59.3 ± 1.56 abc	52.5 ± 0.87 cdef
'O'Gosho'	37.7 ± 0.72 abc	51.1 ± 1.29 c	55.9 ± 0.80 c	63.6 ± 1.39 bc	53.6 ± 0.45 ef
'Tenjin O'Gosho'	40.8 ± 0.69 c	47.5 ± 1.50 bc	53.9 ± 0.44 abc	62.6 ± 1.35 abc	49.3 ± 0.81 ab
'Thiene'	39.8 ± 1.61 c	51.2 ± 2.05 c	55.3 ± 1.08 bc	65.0 ± 2.49 c	52.1 ± 0.71 cdef
'Tipo'	33.6 ± 2.37 a	48.5 ± 2.00 bc	53.1 ± 1.59 abc	55.9 ± 3.88 a	52.9 ± 0.73 def
'Tone Wase'	39.4 ± 1.34 c	42.0 ± 1.78 a	49.9 ± 1.08 a	57.9 ± 22.1 abc	46.9 ± 0.56 a
'Triumph'	34.1 ± 1.35 ab	47.4 ± 1.16 bc	54.4 ± 0.50 abc	58.5 ± 1.57 abc	54.4 ± 0.80 f

Average values ± standard errors are presented. Different letters (a–f) in rows present statistically significant differences amongst cultivars at $p < 0.05$.

2.5. Extraction and HPLC analysis of carotenoids

Extract was prepared according to the method described by Pfeifhofer (1989), separately for skin and pulp: 0.5 g skin or 1 g pulp were extracted in the dark with 10 ml of ice-cold acetone and homogenised for 20 s on the ice with the T-25 Ultra-Turrax. The extracted sample was centrifuged at 4200 rpm for 5 min. The supernatant was filtered through a 0.45 μm cellulose filter, transferred into a vial and immediately analysed by the Thermo Finnigan Surveyor HPLC system with a diode array detector at 440 nm. The column used was a Phenomenex Gemini C18 (150 × 4.6 mm 3 μm), operated at 25 °C. Solvent A was acetonitrile/methanol/water (100/10/5 v/v/v), and solvent B was acetone/ethylacetate (2/1 v/v), linear gradient from 10% solvent B to 70% solvent B in 18 min, run time 30 min, flow 1 ml min^{-1}. The samples were eluted according to the linear gradient described by Pfeifhofer (1989) with the injection amount of 20 μl and a flow rate 1 ml min^{-1}. The carotenoid compounds in persimmon were identified by their spectral and retention time characteristics, as well as using the external standards. Concentrations were expressed as μg per kg FW.

2.6. Chemicals

The following standards were used for determination of sugars, organic acids, phenolic compounds and carotenoids: sucrose, fructose and glucose; citric acid, malic acid and fumaric acid; gallic acid and catechin; zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene from Fluka Chemie (Buchs, Switzerland). BHT, used in the extraction solution, was obtained from Sigma–Aldrich Chemie (Steinheim, Germany); methanol and acetonitrile, used as elutant, were from Mallinckrodt Baker (Deventer, Netherlands), and formic acid was from Fluka Chemie. Ethylacetate, used as elutant for carotenoids, was from Merck (Darmstadt, Germany) and acetone from Sigma–Aldrich Chemie. Water, used for sample preparation, solutions and analyses, was twice-distilled and purified with a Milli-Q water purification system by Millipore (Bedford, MA).

2.7. Statistical analysis

The results were statistically analysed with the Statgraphics Plus programme for Windows 4.0 (Herndon, VA), using one-way analysis of variance (ANOVA). The differences in the content levels of the analysed primary and secondary metabolites were estimated with Duncan's test. *p*-Values of less than 0.05 were considered statistically significant. Multivariate statistical analysis (hierarchical cluster analysis, discriminate analysis and classification) was conducted in order to interpret the differences in primary and secondary metabolites amongst persimmon astringency groups.

3. Results and discussion

3.1. Sugars

Glucose, fructose, sucrose and total sugar content levels (g kg^{-1} FW) in fully ripe fruit of 11 *D. kaki* L. cultivars are presented in Table 2. Generally, glucose was found in the highest concentrations (47.8 ± 3.56 to 87.9 ± 2.91 g kg^{-1}), followed by fructose (38.0 ± 5.00 to 77.8 ± 3.77 g kg^{-1}) and sucrose (9.16 ± 0.21 to 12.2 ± 0.46 g kg^{-1}), which is in accordance with the results of Candir et al. (2009). Taking into account the individual and total sugar concentrations cultivar, 'Tone Wase' had the highest content of glucose (87.9 ± 2.91 g kg^{-1}), fructose (77.8 ± 3.77 g kg^{-1}) and total sugars (178 ± 5.76 g kg^{-1}). This is in accordance with the results of Daood et al. (1992), who report somewhat higher values for glucose (110 g kg^{-1}) and fructose (101 g kg^{-1}) in an unknown cultivar of *D. kaki* L. The lowest content of total sugars was measured in fully ripe fruit of cultivars 'O'Gosho', 'Cal Fuyu' and 'Hana Fuyu', with an average value of 107 g kg^{-1}. Clearly, as sweetness is an important factor contributing to the internal quality and one of the most important objectives in persimmon breeding, cultivars with higher values of sugars should be promoted.

The content of total sugars in persimmon fruit is comparable to that in apples, where the concentration ranges from 115 to 183 g kg^{-1} (Hofer et al., 2005). However, in apple, the main sugar is fructose, followed by glucose and sucrose (Veberic, Zadravec, & Stampar, 2007). The persimmon cultivars with the highest concentrations of total sugars can also be compared with sweet cherry, which on average contains 150–230 g kg^{-1} and where, similarly to persimmon fruit, the fruit is richest in glucose, followed by fructose and sucrose (Usenik, Fabcic, & Stampar, 2008).

3.2. Organic acids

In persimmon fruit, three organic acids were determined, the predominant one being malic acid, followed by citric acid and fumaric acid (Table 3). Total organic acid content level ranged from 681 ± 26.4 mg kg^{-1} in cultivar 'Jiro' to more than twice as much in cultivars 'Triumph' and 'Tipo', which on average contained 1439 mg kg^{-1} of organic acids. Amongst the individual organic acids, the highest content of malic acid (1044 ± 43.2 mg kg^{-1} FW) was measured in cultivar 'Triumph', and the lowest (401 ± 16.7 mg kg^{-1} FW) in cultivar 'Jiro'. The content of malic acid in all analysed cultivars was much lower than that reported by Daood et al. (1992), who measured 3545 mg kg^{-1} FW in ripe fruit of an unknown cultivar. Compared to sweet cherry, which contains between 3530 and 8120 mg kg^{-1} FW of malic acid (Usenik et al., 2008), fully ripe fruit of the analysed persimmon cultivars contain much less malic acid. Cultivars contained between 196 ± 16.8 and

Table 2
Contents (g kg^{-1} FW) of individual and total sugars in fruit of 11 persimmon (*Diospyros kaki* L.) cultivars.

Diospyros kaki L. cultivars	Sucrose	Fructose	Glucose	Total sugars
'Amankaki'	10.4 ± 0.35 abc	55.9 ± 2.67 cd	60.5 ± 2.74 bc	127 ± 4.97 b
'Cal Fuyu'	9.90 ± 0.39 ab	42.0 ± 3.06 a	53.7 ± 2.74 ab	108 ± 5.08 a
'Fuji'	11.5 ± 0.61 cd	52.9 ± 2.79 bc	66.3 ± 1.89 cd	132 ± 3.85 b
'Hana Fuyu'	9.99 ± 0.36 ab	44.2 ± 2.49 ab	53.6 ± 1.78 ab	108 ± 2.26 a
'Jiro'	11.2 ± 0.57 bcd	44.2 ± 2.48 ab	60.5 ± 2.00 bc	120 ± 2.54 ab
'O'Gosho'	9.16 ± 0.21 a	38.0 ± 5.00 a	47.8 ± 3.56 a	106 ± 6.77 a
'Tenjin O'Gosho'	9.47 ± 0.18 a	46.0 ± 3.95 abc	61.8 ± 2.01 cd	117 ± 5.47 ab
'Thiene'	11.5 ± 0.46 cd	63.6 ± 2.71 de	79.9 ± 2.10 e	156 ± 3.54 c
'Tipo'	10.3 ± 0.48 abc	46.6 ± 2.67 abc	68.2 ± 1.69 d	126 ± 3.71 b
'Tone Wase'	10.2 ± 0.25 abc	77.8 ± 3.77 f	87.9 ± 2.91 f	178 ± 5.76 d
'Triumph'	12.2 ± 0.46 d	68.5 ± 2.62 e	76.7 ± 1.97 e	160 ± 2.88 c

Average values ± standard errors are presented. Different letters (a–f) in rows present statistically significant differences amongst cultivars at $p < 0.05$.

Table 3
Contents (mg kg^{-1} FW) of individual and total organic acids in fruit of 11 persimmon (*Diospyros kaki* L.) cultivars.

Diospyros kaki L. cultivars	Citric acid	Malic acid	Fumaric acid	Total organic acids
'Amankaki'	305 ± 36.0 abc	839 ± 42.6 c	125 ± 17.1 ef	1296 ± 71.8 ef
'Cal Fuyu'	251 ± 34.8 abc	576 ± 33.4 b	23.2 ± 4.99 ab	897 ± 61.2 bc
'Fuji'	285 ± 38.1 abc	556 ± 57.4 b	31.3 ± 7.76 ab	1007 ± 104 cd
'Hana Fuyu'	331 ± 64.0 bc	477 ± 79.3 ab	58.3 ± 7.22 bc	788 ± 59.8 ab
'Jiro'	230 ± 29.3 ab	401 ± 16.7 a	27.4 ± 3.06 ab	681 ± 26.4 a
'O'Gosho'	257 ± 47.0 abc	532 ± 63.5 ab	24.0 ± 3.73 ab	804 ± 81.8 ab
'Tenjin O'Gosho'	196 ± 16.8 a	499 ± 29.7 ab	14.7 ± 2.37 a	729 ± 31.5 ab
'Thiene'	351 ± 31.6 bc	853 ± 29.0 c	97.1 ± 18.2 de	1344 ± 44.0 ef
'Tipo'	352 ± 31.5 bc	934 ± 58.7 cd	146 ± 19.2 f	1436 ± 55.0 f
'Tone Wase'	701 ± 53.9 d	452 ± 16.7 ab	13.8 ± 3.38 a	1186 ± 58.2 de
'Triumph'	366 ± 28.2 c	1044 ± 43.2 d	68.8 ± 11.2 cd	1442 ± 54.8 f

Average values ± standard errors are presented. Different letters (a–f) in rows present statistically significant differences amongst cultivars at $p < 0.05$.

701 ± 53.9 mg kg^{-1} FW of citric acid, comparable to the results obtained by Daood et al. (1992), who measured 657 mg kg^{-1} citric acid in ripe persimmon fruit. Compared to apple, where citric acid levels are between 70 and 520 mg kg^{-1} FW (Hofer et al., 2005) and sweet cherry with citric acid content of 110–540 mg kg^{-1} FW (Usenik et al., 2008), ripe persimmon fruit is rich in this organic acid. But compared to elderberry, which averagely contains from 3080 to 4810 mg kg^{-1} (Veberic, Jakopic, Stampar, & Schmitzer 2008), persimmon has a significantly lower content of citric acid. Other tropical fruit, for example papaya and pineapple, have a much higher concentration of citric acid in ripe fruit (Hernández, Lobo, & González, 2008).

3.3. Sugar/organic acid ratio

The ratio between the analysed sugars and organic acids in persimmon at consumption maturity (Fig. 1) is a common quality index and a good indicator of internal fruit quality. Optimal ratio differs between cultivars and is crucial for a harmonious flavour. Although organic acids in persimmon fruit are present at lower concentrations than are sugars, their effect on the fruit flavour is considerable. The higher the ratio, the sweeter are the fruits and the lower the ratio, the more bitter (Colaric et al., 2005). The highest sugar/organic acid ratio was calculated for cultivar 'Amankaki', which had a high content of sugars and a very low content of organic acids. Cultivar 'O'Gosho', on the other hand, had a low content of sugars and a rather high content of organic acids and thus the lowest sugar/organic ratio.

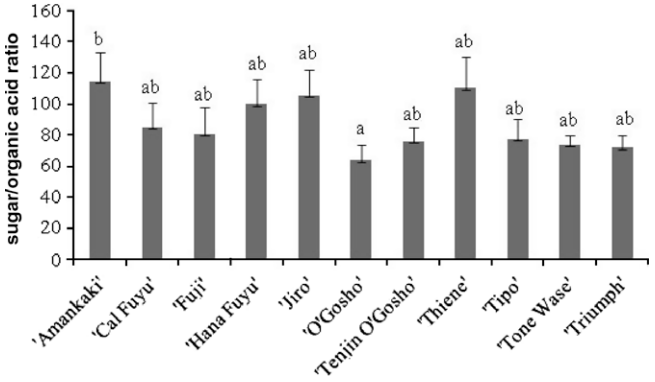

Fig. 1. Sugars/organic acids ratio in 11 cultivars of persimmon fruit (*Diospyros kaki* L.). Average values ± standard errors are presented. Different letters (a–b) denote statistically significant differences between sugars/organic acids ratio in 11 cultivars at $p < 0.05$.

3.4. Phenolic compounds

Amongst the phenolic compounds in fully ripe persimmon fruit, catechin and gallic acid were predominant (Table 4). Catechin was present at concentrations from 3.85 ± 1.92 mg kg^{-1} FW in cultivar 'Tenjin O'Gosho' to 19.0 ± 1.31 mg kg^{-1} FW in cultivar 'Jiro'. Chen et al. (2008) measured 58.1 mg kg^{-1} dry weight (DW) catechin in lyophilised samples of persimmon fruit which cannot directly be compared to our results as values are always lower in extraction of fresh fruit samples. Catechin concentrations in persimmon at consumption maturity are much like those in figs (Veberic, Colaric, & Stampar, 2008) but significantly lower than those in apple pulp and peel, which have from 52.8 to 205 mg kg^{-1} FW of catechin (Petkovsek-Mikulic, Stampar, & Veberic, 2007). The content of gallic acid ranged from 1.75 ± 0.60 ('Tenjin O'Gosho') to 24.3 ± 2.13 ('Triumph') mg kg^{-1} FW which is comparable to the results of Daood et al. (1992), who measured 6.5 mg kg^{-1} FW of gallic acid in ripe persimmon fruit. Chen et al. (2008) also report gallic acid as the main phenolic acid in persimmon fruit, with average values of 191 mg kg^{-1} dry weight. Similar values of gallic acid are reported by Gorinstein et al. (2001), who measured as much as 221 mg kg^{-1} FW in ripe persimmon. As gallic acid has been found to be a strong antioxidant, antimutagenic and anticarcinogenic agent (Gunckel et al., 1998), a high content in persimmon fruit is especially important. Gallic acid content in persimmon fruit is significantly higher than that in figs which contain between 1.4 and 3.8 mg kg^{-1} of gallic acid (Veberic, Colaric, et al., 2008). Epicatechin, caffeic acid, quercetin-3-rutinoside, quercetin-3-galactoside and quercetin-3-glucoside were detected in trace amounts; some of them were also reported by Gorinstein et al. (2001).

Table 4
Gallic acid and catechin contents (mg kg^{-1} FW) in fruit of 11 persimmon cultivars.

Diospyros kaki L. cultivars	Gallic acid	Catechin
'Amankaki'	7.18 ± 1.15 bc	8.13 ± 1.28 ab
'Cal Fuyu'	7.26 ± 1.25 bc	14.8 ± 1.70 cd
'Fuji'	7.50 ± 1.35 c	15.9 ± 1.76 cd
'Hana Fuyu'	3.61 ± 0.92 ab	6.85 ± 1.15 ab
'Jiro'	8.55 ± 1.47 c	19.0 ± 1.31 d
'O'Gosho'	3.29 ± 1.01 a	5.61 ± 0.77 ab
'Tenjin O'Gosho'	1.75 ± 0.60 a	3.85 ± 1.92 a
'Thiene'	9.23 ± 1.14 c	16.1 ± 1.90 cd
'Tipo'	9.68 ± 1.55 c	13.6 ± 1.47 c
'Tone Wase'	16.4 ± 0.98 d	9.19 ± 0.98 b
'Triumph'	24.3 ± 2.13 e	17.8 ± 1.78 cd

Average values ± standard errors are presented. Different letters (a–e) in rows present statistically significant differences amongst cultivars at $p < 0.05$.

3.5. Total phenolic content

Total phenolics were in the range 127 ± 17.9 mg GAE kg^{-1} FW in cultivar 'Triumph' to 295 ± 31.3 mg GAE kg^{-1} FW in cultivar 'Fuji' (Fig. 2). Much higher concentrations in persimmon fruit (1681 mg GAE kg^{-1} DW) have been reported in the research of Chen et al. (2008). In comparison with sweet cherry, which contains from 443 to 879 mg GAE kg^{-1} FW (Usenik et al., 2008) and apple, where the pulp contained 4225 mg GAE kg^{-1} FW and the peel 17546 mg GAE kg^{-1} FW (Petkovsek-Mikulic et al., 2007), our analysis of persimmon fruit showed significantly lower total phenolic content.

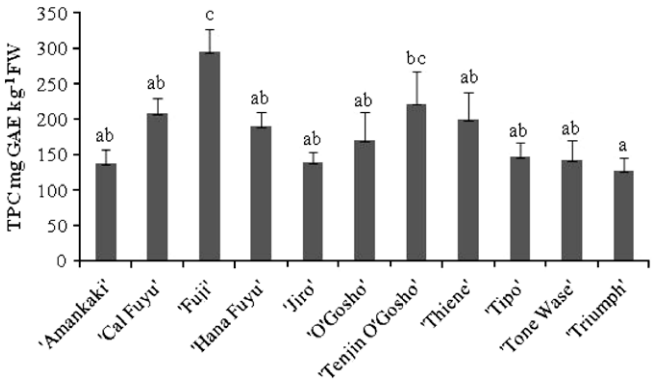

Fig. 2. Total phenolic content (TPC) in mg GAE kg^{-1} FW in 11 cultivars of persimmon fruit (*Diospyros kaki* L.). Average values ± standard errors are presented. Different letters (a–c) denote statistically significant differences amongst cultivars at $p < 0.05$.

3.6. Carotenoids

Zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene were detected in the skin and pulp of 11 persimmon cultivars (Tables 5 and 6). The predominant carotenoid in persimmon skin was β-carotene, as reported previously by Daood et al. (1992), considering only unbound carotenoids. When esterified forms are considered, the predominant carotenoids belong to the group of xanthophylls (Daood et al., 1992). Since the saponification step was emitted due to high probability of carotenoid degradation, in our study on 11 persimmon cultivars, xanthophylls were only present in minor amounts. Therefore, β-cryptoxanthin and zeaxanthin in non-esterified form, were detected in lowest amounts in persimmon skin (Table 5). Zeaxanthin was the minor carotenoid in the skin of all persimmon cultivars, ranging from 184 ± 25.8 μg kg^{-1} FW in cultivar 'Tipo' to 435 ± 42.0 μg kg^{-1} FW in cultivar 'O'Gosho'. The highest amount of β-carotene was measured in the skin of cultivar 'Hana Fuyu' (8747 ± 499 μg kg^{-1} FW) and the lowest in cultivars 'Thiene', 'Jiro' and 'Tipo', which on average contained 2592 μg kg^{-1} FW β-carotene. Total carotenoids in persimmon skin were highest in cultivars 'Hana Fuyu' (12235 ± 726 μg kg^{-1} FW); the lowest concentrations were detected in cultivar 'Thiene' (3517 ± 347 μg kg^{-1} FW). Similar concentrations of total carotenoids were measured in apple skin, where values ranged from 3 to 10 mg kg^{-1} and red and black grapes which contain 5 mg kg^{-1}, but in comparison with red pepper, which has 187 mg kg^{-1}, persimmon fruit has significantly lower concentrations of carotenoids (Lancaster, Lister, Reay & Triggs,1997).

The contents of zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and total carotenoids in persimmon pulp are much lower than those in skin (Table 6). Similar to the content in the skin, β-carotene was the predominant carotenoid in persimmon pulp,

Table 5
Individual and total carotenoid contents (μg kg^{-1} FW) in skin of 11 persimmon cultivars.

Diospyros kaki L. cultivars	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Total carotenoids
'Amankaki'	190 ± 17.3 a	327 ± 31.6 a	1088 ± 93.2 bc	4044 ± 340 b	5670 ± 431 bc
'Cal Fuyu'	300 ± 17.0 cd	767 ± 81.4 c	1368 ± 149 c	4179 ± 364 b	6975 ± 794 c
'Fuji'	300 ± 17.0 cd	784 ± 85.8 c	1385 ± 160 c	4286 ± 377 b	7127 ± 828 c
'Hana Fuyu'	415 ± 29.2 e	789 ± 84.8 c	2047 ± 140 d	8747 ± 499 e	12235 ± 726 e
'Jiro'	165 ± 29.0 a	477 ± 52.5 ab	784 ± 56.4 ab	2653 ± 169 a	4090 ± 272 ab
'O'Gosho'	435 ± 42.0 e	686 ± 72.1 c	1892 ± 158 d	5844 ± 438 cd	9181 ± 745 d
'Tenjin O'Gosho'	356 ± 32.9 de	1254 ± 71.1 d	1976 ± 132 d	6780 ± 373 d	10616 ± 500 de
'Thiene'	200 ± 17.2 ab	283 ± 19.6 a	652 ± 57.7 a	2390 ± 281 a	3517 ± 347 a
'Tipo'	184 ± 25.8 a	366 ± 40.3 ab	671 ± 76.2 a	2733 ± 374 a	3962 ± 488 ab
'Tone Wase'	310 ± 22.1 cd	581 ± 43.2 bc	1447 ± 140 c	4940 ± 508 bc	7000 ± 623 c
'Triumph'	267 ± 39.2 bc	1232 ± 156 d	1187 ± 140 c	4285 ± 529 b	6609 ± 759 c

Average values ± standard errors are presented. Different letters (a–e) denote statistically significant differences amongst cultivars at $p < 0.05$.

Table 6
Individual and total carotenoid contents (μg kg^{-1} FW) in pulp of different persimmon cultivars.

Diospyros kaki L. cultivars	Zeaxanthin	β-Cryptoxanthin	α-Caroten	β-Caroten	Total carotenoids
'Amankaki'	52.3 ± 8.30 abc	287 ± 80.6 b	72.6 ± 13.2 a	391 ± 77.7 ab	921 ± 172 bc
'Cal Fuyu'	35.1 ± 5.37 a	76.5 ± 16.8 a	75.6 ± 8.25 a	303 ± 33.8 ab	490 ± 44.8 a
'Fuji'	35.4 ± 5.37 a	79.7 ± 18.2 a	77.7 ± 8.83 a	314 ± 35.5 ab	507 ± 46.2 ab
'Hana Fuyu'	88.1 ± 15.5 cd	257 ± 59.0 ab	83.4 ± 11.2 ab	403 ± 71.8 ab	727 ± 117 abc
'Jiro'	48.7 ± 6.51 ab	178 ± 51.5 ab	121 ± 14.1 b	259 ± 15.2 a	576 ± 36.4 abc
'O'Gosho'	82.4 ± 21.0 bcd	148 ± 20.5 ab	83.1 ± 8.42 ab	459 ± 55.4 b	763 ± 83.2 abc
'Tenjin O'Gosho'	58.3 ± 7.71 abc	154 ± 22.9 ab	160 ± 14.8 c	360 ± 37.3 ab	758 ± 71.3 abc
'Thiene'	97.5 ± 16.7 d	206 ± 44.4 ab	92.0 ± 10.2 ab	384 ± 52.2 ab	795 ± 97.8 abc
'Tipo'	53.6 ± 8.53 abc	210 ± 57.0 ab	85.9 ± 10.8 ab	468 ± 85.2 b	936 ± 180 c
'Tone Wase'	37.0 ± 7.94 a	236 ± 80.5 ab	79.5 ± 9.26 a	305 ± 43.3 ab	814 ± 162 abc
'Triumph'	52.5 ± 9.70 abc	262 ± 89.1 ab	113 ± 17.7 ab	448 ± 63.8 ab	920 ± 167 bc

Average values ± standard errors are presented. Different letters (a–d) in rows denote statistically significant differences amongst cultivars at $p < 0.05$.

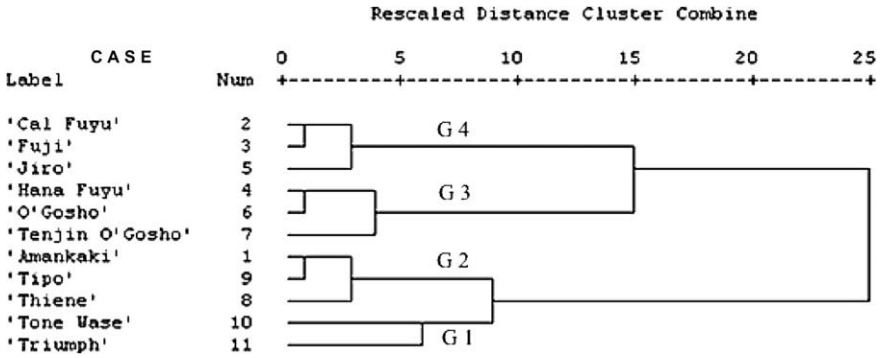

Fig. 3. Dendrogram of 11 persimmon cultivars using ward method based on square Euclidian distance from biochemical data. G1–G4 represent different groups.

followed by β-cryptoxanthin, α-carotene and zeaxanthin. The lowest value of β-carotene was measured in the pulp of cultivar 'Jiro' (259 ± 15.2 μg kg^{-1} FW) and the highest in cultivars 'O'Gosho' and 'Tipo' (464 μg kg^{-1} FW). Cultivar 'Tipo' also had the highest content of total carotenoids in pulp (936 ± 180 μg kg^{-1} FW) but the lowest content was measured in cultivar 'Cal Fuyu' (490 ± 44.8 μg kg^{-1} FW). Dias, Filomena, Camoes, and Oliveira (2009) measured much higher concentrations of β-cryptoxanthin in saponified samples of oranges and peaches and smaller concentrations of β-carotene were observed in apple pulp (0.29 mg kg^{-1} FW) and a somewhat higher α-carotene in cherries (0.29 mg kg^{-1} FW). The contents of zeaxanthin in the pulp of persimmon fruit are higher than that in apples (0.02 mg kg^{-1} FW) but significantly lower than that in oranges (1.07 mg kg^{-1} FW; Dias et al., 2009).

3.7. Contents of primary and secondary metabolites according to persimmon astringency classification

Eleven persimmon cultivars were classified into four astringency groups according to the research of Sugiura (1983):

- pollination constant non-astringent (PCNA) group (cultivars 'Cal Fuyu', 'Hana Fuyu', 'Jiro' 'O'Gosho', 'Tenjin O'Gosho') – cultivars with non-astringent fruit at harvest, regardless of the presence of the seeds;
- pollination variant non-astringent (PVNA) group (cultivars 'Amankaki', 'Tipo', 'Thiene') – cultivars with non-astringent fruit at harvest when seeds are present;
- pollination variant astringent (PVA) group (cultivars 'Tone Wase', 'Triumph') – cultivars with astringent fruit at harvest when fruit is seedless and mostly astringent when seeds are present;
- pollination constant astringent (PCA) group (cultivar 'Fuji') – cultivars with astringent fruit at harvest, regardless of the presence of the seeds.

Multivariate statistical analysis revealed a tight connection amongst cultivars of individual astringency groups. The dendrogram (Fig. 3), based on ward method using a square Euclidian distance, subdivided the cultivars analysed into four major groups: group 1 (G1) comprising cultivars classified as PVA and group 2 (G2) of cultivars belonging to the PVNA group. Groups 3 and 4 (G3, G4) were comprised of cultivars belonging to the PCNA group, with the exception of 'Fuji', which is classified in the PCA group. The detailed analysis of homogeneity of variances (data not shown) revealed that the classification was mostly influenced by the predominant sugars in persimmon fruit (fructose and glucose) and total carotenoids in skin. Organic and phenolic acids had no important influence on the analysis. As total sugar content directly influences the taste of persimmon cultivars and carotenoids are a clear visual attribute for the consumer preferences, it is interesting that this classification corresponds to persimmon astringency groups.

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