Food Research International 44 (2011) 1752–1767

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Selected primary and secondary metabolites in fresh persimmon (*Diospyros kaki* Thunb.): A review of analytical methods and current knowledge of fruit composition and health benefits

Edgardo Giordani [a], Saer Doumett [b], Stefania Nin [a], Massimo Del Bubba [b,*]

[a] University of Florence, Department of Plant, Soil and Environmental Science, 50019 Sesto Fiorentino (Florence), Italy
[b] University of Florence, Department of Chemistry, 50019 Sesto Fiorentino (Florence), Italy

ARTICLE INFO

Article history:
Received 6 December 2010
Accepted 17 January 2011

Keywords:
Diospyros kaki
Sugar
Vitamin C
Carotenoid
Polyphenol
Health benefit

ABSTRACT

Diospyros kaki Thunb. (*Ebenaceae*) is a widely cultivated tree species in some countries of Asia, while in other continents persimmons are mostly considered "exotic" fruits. Peculiar characteristics of this species are a complex sex expression, parthenocarpy and fruit astringency at harvest time, associated with a different composition in polymerized flavan-3-ols. Analytical methods for determining sugars, vitamin C, carotenoids and polyphenols in persimmons were critically reviewed in order to evaluate the overall significance of literature results; the nutritional and nutraceutical properties, together with the health benefits of both astringent and non astringent cultivars, were also overviewed. To these aims, the available literature from the last twenty years and the most important formerly published papers were investigated using SciFinder®, Elsevier SciVerse, AGRIS, and PubMed search engines. Persimmons resulted rich in sugars (about 12.5 g/100 g FW), being fructose, glucose and sucrose the major components, and in total vitamin C, for which 100–150 g of fresh persimmon supplies the recommended daily amount. Astringent varieties supply higher amounts of sugars than nonastringent ones; conversely, higher concentrations of total vitamin C were found in nonastringent cultivars. The main carotenoid components are β-cryptoxantin (193 μg/100 g FW), β,β-carotene (113 μg/100 g FW) and β,ε-carotene (30 μg/100 g FW). Persimmons are also a good source of polyphenolic compounds such as p-coumaric acid, catechin, epicatechin, epigallo catechin, and condensed proanthocyanidins. This chemical composition, together with in vivo and in vitro studies, suggests a relevant role of persimmon in the protection against free radicals and in the prevention of some human diseases.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) is recognized as the most important species for fruit production in the *Diospyros* gender (Ebenaceae) (Yonemori, Yamada, & Sugiura, 2000). Persimmon is believed to have originated in China (Luo & Wang, 2008), before spreading to Korea and Japan (Sugiura, 1997), where it is a traditional crop, and then to other regions of the world, where it is considered an exotic fruit. World production of persimmons reached 3,627,575 t in 2008, with an upward trend since 1965 (FAOSTAT, 2010); the main producers are China (2,533,899 t), Korea (430,521 t), Japan (244,800 t), Brazil (169,000 t), Azerbaijan (132,179 t), Spain (70,000 t), Italy (50,000 t), Israel (30,089 t) and Uzbekistan (31,000 t).

From the botanical point of view, *D. kaki* is peculiar since sex expression, together with the variability of fruit astringency among cultivars, exert a direct effect on the nutritional and nutraceutical properties of fruits. Persimmon is generally recognized as an outstanding source of biologically active compounds related to both nutritional and nutraceutical values. In contrast to this fact, less efforts have been devoted to the investigation of primary and secondary

Abbreviations: VIG, Volatile Independent Group; PCNA, Pollination Constant Non Astringent; VDG, Volatile Dependent Group; PCA, Pollination Constant Astringent; PVA, Pollination Variant Astringent; PVNA, Pollination Variant Non Astringent; FW, Fresh Weight; GC, Gaschromatography; HPLC, High Performance Liquid Chromatography; SSC, Soluble Solid Content; NNDSR, National Nutrient Database for Standard Reference of the United State Department of Agriculture; DFCD, Danish Food Composition Databank of the Technical University of Denmark; AA, Ascorbic acid; DHAA, Dehydroascorbic acid; EDTA, ethylenediaminotetraacetic acid; AOAC, Association of Official Analytical Chemists; DCIP, 2,6-dichlorophenolindophenol; OPDA, 1,2-phenylendiamine; RP, reversed-phase; FCNT, Food Composition and Nutrition Tables; RDA, Recommended Daily Amount; BHT, Butylated hydroxytoluene; RAE, Retinol Activity Equivalent; CUDRINL, Committee on Use of Dietary Reference Intakes in Nutrition Labelling; PPO, Polyphenol oxidase; DP, Degree of Polymerization; GAE, Gallic Acid Equivalent; DW, Dry Weight; DPPH, 1,1-diphenyl-2-pycrylhydrazyl; ABTS, 2,2′-azino-bis (3-ethyl-benzoathiazoline-6-sulfonic acid) diammonium salt; FRAP, Ferric Reducing Antioxidant Power; LDL, Low Density Lipoprotein; MALDI, Matrix Assisted Laser Desorption Ionization; TOF, Time Of Flight; NMR, Nuclear Magnetic Resonance; IC_{50}, half maximal inhibitory concentration.

* Corresponding author. Tel.: +39 0554573282; fax: +39 0554573582.
E-mail address: delbubba@unifi.it (M. Del Bubba).

0963-9969/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2011.01.036

metabolites in persimmon fruits compared to other popular non exotic fruits and, to our knowledge, there is not a review published on this argument, while an overview of benefits of persimmon for human health has been presented by George and Redpath (2008) during the IV International Symposium on Persimmon. Hence, the aim of this paper is to report the state of the art related to the current knowledge of persimmon composition and to compare astringent versus non astringent fruits, in terms of selected primary and secondary metabolites.

A further relevant aspect to be considered when studying nutraceutical and nutritional characteristics of complex matrixes such as persimmon, is the possible occurrence of analytical artifacts. In fact, in order to evaluate the primary and secondary metabolite composition of such a complex matrix and to compare them in different genotypes, a compound by compound approach must often be followed and the uniformity of methods used for obtaining these data should be verified. In this regard, a further goal of this paper is to critically overview the main analytical methods applied to persimmon analysis.

Finally, aiming to elucidate the importance of persimmons for human health protection, antioxidant and antiradical activities of fruit extracts as measured by widely adopted chemical model systems, together with the results of some *in vitro and in vivo* studies, are discussed.

2. Botanical characteristics

Diospyros kaki Thunb., a deciduous, hardy, long-living tree has a complex sex expression, since it may bear only pistillate flowers, or both pistillate and staminate flowers, or hermaphroditic flowers together with pistillate and staminate flowers (Yonemori, Sugiura, Tanaka, & Kameda, 1993). The marketed fruits (yellow-reddish berries weighing about 300 g) derive from female flowers, since those originating from hermaphrodite flowers are undersized. Fruits can contain up to 8 seeds, but the most appreciated are seedless persimmons of parthenocarpic origin. The flesh colour varies from orange-yellow to reddish-brown depending on the genotype and the presence of seeds. All persimmons are edible when they are jelly soft (i.e. when they are over-ripe), but they can be astringent (not suitable for eating) or non-astringent (edible) at harvest time (when fruits are still firm) (Yonemori et al., 2000). The astringency at harvest time is associated with the amount of accumulated tannins in the fruit, tannin characteristics (Sugiura, Yonemori, Harada, & Tomama, 1979; Yonemori & Matsushima, 1987a,b; Yonemori, Matsushima, & Sugiura, 1983) and the ability of seeds to produce volatile compounds (Sugiura & Tomana, 1983; Sugiura et al., 1979). Sugiura (1984) divided persimmon cultivars into Volatile Independent Group — VIG (corresponding to the Pollination Constant Non Astringent — PCNA), and the Volatile Dependent Group — VDG, which includes Pollination Constant Astringent (PCA), Pollination Variant Astringent (PVA) and Pollination Variant Non Astringent (PVNA) types. In VIG type (nonastringent cultivars with edible fruits at harvesting time), tannins are usually water insoluble and present high molecular weights; furthermore, the accumulation of soluble tannins finishes at the early stage of fruit development and their final concentration is less than 1% on fresh weight basis (FW) in Jiro fruits (Taira, 1996). VDG cultivars show a higher concentration of water soluble low molecular weight tannins at harvesting (≈1.5% FW in Hiratanenashi fruits) (Taira, 1996), hence parthenocarpic fruits are not edible at ripening time. The presence of seeds in the fruits and their cultivar-dependent ability to exude different amounts of volatile compounds, namely ethanol, which insolubilises water soluble tannins, affects edibility of fruits at harvest time: in PCA and in PVA cultivars seeds do not remove fruit astringency since ethanol content in the flesh is almost nil (much less than 0.5 μl/g FW) or weak (from 0.4 to 0.9 μl/g FW) respectively; ethanol content is much higher in PVNA cultivars, ranging from 0.6 to 1.4 μl/g FW (Sugiura & Tomana, 1983). Therefore, only fruits of PCNA and pollinated PVNA cultivars are not astringent at harvest time; parthenocarpic fruits of PVNA cultivars, and both seeded and parthenocarpic fruits of PCA and PVA cultivars are edible only after artificial removal of astringency or when soft, overripe or processed (dried), i.e. when low molecular weight soluble tannins are coagulated, probably with pectins, into heavier water insoluble complexes (Taira, Ono, & Matsumoto, 1997). Most persimmons are consumed fresh (soft or hard) for dessert; dried fruits are used mainly in oriental countries. Hard fruits, naturally non-astringent or treated for astringency removal, are more marketable, because of handling, than soft fruits; on the other hand, consumer preferences are associated with geographical areas: in Europe soft fruits are consumed in areas were persimmon is traditionally grown, while hard fruits are mostly accepted by “new consumers” attracted by marketing.

3. Sugars

Free-sugars are the most important nutritional constituent of fruit. To our knowledge, free sugars have been determined in *D. kaki* fruits since the middle of the 20th century, showing that this fruit is an important source of readily available carbohydrates (Kitahara, Takeuchi, & Matsui, 1951). As previously mentioned, the critical evaluation of analytical methods adopted to assess sugar quali-quantitative composition is of paramount importance for assessing the overall reliability of the data obtained.

3.1. Methods of sugar extraction and analysis

The recovery of sugars from persimmon is usually performed using aqueous-alcoholic mixtures in different proportions (Del Bubba, Giordani, Cincinelli, Checchini, & Galvan, 2009; Ittah, 1993; Senter, Chapman, Forbus, Payne, & Payne, 1991), even if water-based extractants have occasionally been used (Table 1). Extraction methods have been reported to significantly affect the free-sugar composition determined in *D. kaki* fruits. In particular, according to Zheng and Sugiura (1990), the microwave irradiation of flesh tissue followed by methanol extraction effectively inhibited sucrose degradation during the sample treatment and afterwards. However, as reported by Hirai and Kondo (2002), heating treatments strongly change the original composition of the tannic fraction and are therefore incompatible with its investigation. Ittah (1993) evidenced the role of fructofuranosidase (e.g. invertase) in the modification of the original free-sugar composition of persimmon during extraction; in fact, very different glucose/fructose ratios and sucrose concentrations were found, depending on the extraction method adopted. In this regard, it should be noted that persimmon has a much higher invertase activity than other popular fruit (e.g. about two magnitude orders higher than apples) (Hirai & Kondo, 2002). According to Ittah (1993), the preventive immersion in methanol/water 80/20 (v/v) of the fruit flesh before its fine crushing is fundamental for inhibiting the invertase activity. For example, in fully ripened persimmons of Triumph cv., Ittah (1993) reported concentrations of about 10, 2.3 and 1.5 g/100 g fresh weight (FW), for sucrose, glucose and fructose respectively, while Veberic, Juhar, Mikulic-Petkovsek, Stampar and Schmitzer (2010), without any inhibition procedure for invertase, found a completely different sugar composition, with values of 1.2, 7.7 and 6.9 g/100 g FW for the same carbohydrates. These results suggest that great attention should be paid to the sample treatment, in order to avoid modifications of the original composition of free sugars. Therefore, a standard addition procedure should always be recommended to check both the recovery of analytes and the possible occurrence of degradation phenomena. In this regard, both the extraction procedure proposed by Zheng and Sugiura (1990) and that of Ittah (1993) can be considered reliable for sugar analysis, even

Table 1
Overview of methods of extraction and instrumental analysis of sugars in persimmons.

Extraction method	Instrumental technique	Reference
Microwave irradiation of the fruit followed by homogenisation in methanol.	GC analysis of TMS derivatives on a glass column packed with 3% SE-52 (5% Phenyl-dimethylpolysiloxane) on acid-washed and silanized Chromosorb W. Detection: FID.	Zheng and Sugiura (1990) Hirano et al. (1995)
Homogenisation of the mashed fruit in 2% aqueous metaphosphoric acid.	HPLC analysis on a Carbopack column at ambient temperature. Elution: isocratic with distilled water. Detection: refractive index.	Daood et al. (1992)
Immersion of the fruit in methanol/water 80/20 (*v*/*v*) followed by cutting and stirring.	HPLC on a cation-exchange polymer in the calcium form at T = 85 °C. Eluent: 0.02 mM $CaSO_4$ aqueous solution. Detection: refractive index.	Ittah (1993)
	HPLC on a cation-exchange polymer in the hydrogen form at ambient temperature after clean-up of the extract on a C18 SPE column. Eluent: water/formic acid at pH = 2.2. Detection: evaporative light scattering.	Del Bubba et al. (2009)
Homogenisation of the desiccated fruit in ethanol/water 70/30 (*v*/*v*).	GC analysis of TMS derivatives on a capillary 100% dimethylpolysiloxane column. Detection: FID.	Senter et al. (1991)
Mechanical pressure to obtain the fruit juice.	HPLC on a Zorbax (Agilent Technologies, Santa Clara, CA, USA) patented column at T = 30 °C. Eluent: water/acetonitrile 25/75 (*v*/*v*). Detection: refractive index.	Öz et al. (2005)
Treatment of the entire fruit in liquid nitrogen followed by blending in ethanol/water 95/5 (*v*/*v*).	HPLC on a silica-based amino modified column at T = 25 °C. Eluent: water/acetonitrile 20/80 (*v*/*v*). Detection: refractive index.	Candir et al. (2009)
Homogenisation of the mashed fruit in water.	HPLC on a cation-exchange polymer in the calcium form at T = 65 °C. Eluent: distilled water. Detection: refractive index.	Veberic et al. (2010)

though the latter is preferable owing to the absence of modifications of the original composition of other bioactive compounds.

Accurate instrumental analysis of free sugars in persimmons have been performed both by gaschromatographic (GC) and high performance liquid chromatographic (HPLC) techniques (Table 1), the latter increasingly more used owing to its advantages in terms of rapidity and reliability. In fact, due to the high polarity, hydrophilicity and low volatility of sugars, GC analysis requires their conversion into volatilizable and stable derivatives, i.e. trimethylsilyl or acetate derivatives, before their injection (Medeiros & Simoneit, 2007). Conversely, HPLC on cationic exchange resins in calcium, hydrogen or other forms and silica-based amino-alkyl modified phases can be successfully used for direct analysis of sugars. It should however be noted that the performance and life of cation exchange columns are strongly influenced by the eluent and matrix composition due to swelling of the resin and analyte retention. In this regard the entire analytical procedure of Ittah (1993) has recently been implemented by Del Bubba et al. (2009) by introducing the internal standard raffinose to check the recovery efficiency and possible change in sugar native composition, a replicated extraction approach to increase the recovery of analytes and, above all, a clean-up procedure of the extract to preserve the column performance and improve the chromatographic profile. In fact, HPLC detectors used for sugar analysis (refractive index and evaporative light scattering) are not selective and respond to a wide range of analytes.

The soluble solid content (SSC), estimated by the refractometric method, has been widely adopted, even recently, as a rapid measurement technique of total sugar levels in fruits (Besada, Arnal, & Salvador, 2009; Itoo, 1986; Sestari et al., 2009). However, the refractometric response is affected by numerous variable components other than sugar present in the fruit, and the need to correct it for certain fruits has been suggested for years (Scott, Morgan, & Veldhuis, 1961). Variable quantities and types of tannins influence refractometer response and the values observed for astringent fruit juices do not reflect the actual sugar content. A more reliable, but time-consuming, procedure to better determine actual sugar content via the refractometric method was developed and applied to astringent Japanese persimmon juice by using polyethyleneglycol as a tannin scavenger (Sugiura, Kataoka, & Tomana, 1983).

3.2. Sugar content and composition

The standard value of total sugar concentration in persimmon fruits is about 12.5 g/100 g FW, according to the National Nutrient Database for Standard Reference of the United State Department of Agriculture (NNDSR) (USDA, 2010) and the Danish Food Composition Databank of the Technical University of Denmark (DFCD) (Technical University of Denmark, 2010), being higher than those of other extensively consumed fruits such as apples, peaches, pears and oranges. The main sugars in the flesh of mature fruit are fructose, glucose and sucrose (Candir, Ozdemir, Kaplankiran and Toplu, 2009; Del Bubba et al., 2009; Ittah, 1993; Veberic et al., 2010); while galactose and arabinose are minor components (Senter et al., 1991; Testoni, 2002). Significant concentrations (1.6–2.2 g/100 g FW) of other monosaccharides, such as rhamnose and ribose, have been determined in ethanol extracts of four astringent persimmon cultivars (Golubev, Kostinskaya, & Khalilov, 1987).

During fruit development and ripening, a common finding observed in all studies performed for both astringent and nonastringent cultivars was the increment of total sugars (Candir et al., 2009; Del Bubba et al., 2009; Senter et al., 1991; Zheng & Sugiura, 1990). In some cases, this increase was the result of the rise of sucrose and reducing sugars, as for the astringent Nigoro-Konashiba, Kikuhira and Hiratanenashi (Zheng & Sugiura, 1990), Aizumishirazu, Gionbo, and the nonastringent variety Ichikikei Jiro (Senter et al., 1991). A different tendency was exhibited by the nonastringent types Fuyu and Jiro (Senter et al., 1991), and the astringent cultivars Kaki Tipo and Rojo Brillante (Del Bubba et al., 2009), for which an increasing–decreasing parabolic-like sucrose concentration trend was observed, together with a substantially constant increment of reducing sugars. An additional pattern, characterised by a relevant increase in sucrose and almost steady levels of glucose and fructose, was found in nonastringent Hana Gosho, as well as the astringent varieties Mikatani Gosho, Atago (Zheng & Sugiura, 1990) and Harbiye (Candir et al., 2009). Therefore, astringent and nonastringent varieties randomly followed one of the three described trends, complicating the interpretation of the results. Nevertheless, for the astringent cultivars Kaki Tipo and Rojo Brillante, Del Bubba et al. (2009) attributed the changes in sucrose, glucose and fructose during fruit growth to an increase of invertase activity coupled with a decrease in soluble tannins. The activity of this enzyme, in fact, has been reported to increase during on-tree fruit ripening (Hirai, Rokuhara, & Shimizu, 1986; Zheng & Sugiura, 1990) and a strong inhibition by gallic and tannic acids was demonstrated in an in vitro study (Chen, Ni, Sun, & Cheng, 2003). Hence, the establishment of a protocol for objectively determining the ripening stage of samples is of paramount importance for the comparative analysis of these compounds.

Total sugar content of fully ripened fruits has been reported by different authors and for various cultivars (Table 2) adopting the methods indicated in Table 1. The values ranged from 9.5 g/100 g FW

Table 2
Sugar composition (g/100 g FW) of astringent (normal character) and nonastringent (italic character) persimmon fruits at commercial harvest. b.d.l. = below detection limit.

Cultivars	Fructose	Glucose	Sucrose	Total sugars	Reference
Fude	4.1	6.1	0.8	11.0	Hirai and
Fuyu[a]	3.4	4.0	4.6	12.0	Yamazaki (1984)
Hachiya[a]	5.6	7.9	1.3	14.8	
Hime	5.6	6.9	0.5	13.0	
Hiratanenashi[a]	7.0	6.8	0.1	13.9	
Hyozaemon	1.7	2.5	5.9	10.1	
Ichida[a]	5.4	6.9	1.1	13.4	
Jiro[a]	4.6	5.8	3.1	13.5	
Kayabayashi[a]	4.9	5.9	2.6	13.4	
Koeda	6.3	8.0	1.0	15.3	
Kogaki	7.1	8.2	b.d.l.	15.3	
Myotan	3.0	4.2	4.8	12.0	
Nishikubo	4.7	5.3	5.0	15.0	
Super-hiratane	8.8	10.8	b.d.l.	19.6	
Tateishi[a]	5.2	6.6	1.2	13.0	
Wankari	6.8	9.2	b.d.l.	16.0	
Yatsubusa	3.4	5.6	4.5	13.5	
Atago	3.4[b]		9.3	12.7	Zheng and Sugiura
Hana-gosho	1.8[b]		9.9	11.7	(1990)
Hiratanenashi	9.0[b]		5.0	14.0	
Kikuhira	6.1[b]		3.8	9.9	
Mikatani-gosho	1.2[b]		12.9	14.1	
Nigoro-konashiba	7.3[b]		3.9	11.2	
Aizumishirazu	3.1	3.7	3.9	10.7	Senter et al. (1991)[c]
Fuyu	4.4	4.9	2.1	11.4	
Gionbo	3.9	4.2	2.4	10.5	
Ichikikei Jiro	3.3	3.8	4.4	11.5	
Jiro	4.5	4.7	3.3	12.5	
Triumph	1.5	2.2	10.1	13.8	Ittah (1993)
Hiratanenashi	4.2	4.7	4.0	12.9	Hirano et al. (1995)
Morali	2.0	2.5	7.0	11.5	Öz et al. (2005)[c]
Harbiye	2.5	6.0	12.5	21.0	Candir et al. (2009)
Kaki Tipo	5.1	5.1	4.8	15,0	Del Bubba et al. (2009)
Rojo Brillante	5.7	6.1	3.1	14,9	
Amankaki	5.6	6.1	1.0	12.7	Veberic et al. (2010)
Cal Fuyu	4.2	5.4	1.0	10.6	
Fuji	5.3	6.6	1.2	13.1	
Hana Fuyu	4.4	5.4	1.0	10.8	
Jiro	4.4	6.1	1.1	11.6	
O'Gosho	3.8	4.8	0.9	9.5	
Tenjin Gosho	4.6	6.2	0.9	11.7	
Thiene	6.4	8.0	1.1	15.5	
Kaki Tipo	4.7	6.8	1.0	12.5	
Tone Wase	7.8	8.8	1.0	17.6	
Triumph	6.9	7.7	1.2	15.8	

[a] Values are mean of cultivars grown in different habits.
[b] Sum of fructose and glucose.
[c] Data expressed on dry matter in the original paper have been herein converted considering an average water content of 80% (Piretti 1991).

of O'Gosho (Veberic et al., 2010) to 21.0 g/100 g FW of Harbiye (Candir et al., 2009), with an average of 13.2 g/100 g FW, in agreement with data from NNDSR and DFCD databases. Notwithstanding the obvious uncontrolled effects of environment, harvest year, fruit sampling and analytical methods, it is interesting to compare the gathered data. Taking into account fruit astringency, a relevant difference of total sugar content was observed, with a significantly higher value for astringent fruits (13.8 ± 2.5 g/100 g FW) than the one found for nonastringent cultivars (11.5 ± 1.0 g/100 g FW) according to the Student-T test (P = 0.005). The highest total sugar concentrations were observed in Harbiye (21 g/100 g FW), Super-hiratane (19.6 g/100 g FW) Tone Wase (17.6 g/100 g FW) for astringent varieties, and in Jiro (13.0 g/100 g FW), for nonastringent types.

When the mean sugar composition found in fully-ripened persimmons belonging to 33 different cultivars (Table 2) was considered, glucose was the most abundant (5.9 ± 1.9 g/100 g FW), followed by fructose (4.8 ± 1.7 g/100 g FW) and sucrose (3.3 ± 3.3 g/100 g FW), representing 44%, 36% and 20% of total sugar content, respectively. No statistically significant differences among glucose, fructose and sucrose were found in fruits of astringent cultivars, compared to those of nonastringent types.

In order to illustrate the differences in sugar composition of the cultivar samples, the relative percentage of each individual sugar with respect to total sugar concentration was calculated using data reported in Table 2, by excluding the samples with incomplete data. The obtained values were thereafter grouped by Cluster Analysis (Fig. 1). The first cluster, representing persimmons with high sucrose, low glucose, and, above all, low fructose percentages, was clearly identified by the astringent cultivars Morali, Hyozaemon, Harbiye and Triumph (upper part of the dendrogram). The nonastringent varieties Ichikikei Jiro, Jiro and Fuyu belonged to the central cluster together with several astringent cultivars, all of them showing comparable relative percentages of fructose, glucose and sucrose. The third cluster, grouping both astringent (such as Hiratanenashi, Wankari and Superhiratane), and nonastringent varieties (Jiro, Cal Fuyu, Hana Fuyu and O'Gosho), identified fruits with very low percentages of sucrose and almost identical amounts of fructose and glucose.

It should be noted that the dendrogram highlighted diverse sugar compositions for the same cultivars analysed by different authors. More specifically, the very low relative percentage of sucrose found by Veberic et al. (2010) in Jiro, Kaki Tipo and Triumph, compared to the values determined in Jiro by Hirai and Yamazaki (1984) and Senter et al. (1991), in Kaki Tipo by Del Bubba et al. (2009) and in Triumph by Ittah (1993), could be attributed to analytical artifacts rather than environmental effects. The low sucrose abundance found by Hirai and Yamazaki (1984) in contrast to that reported by Zheng and Sugiura (1990) and Hirano, Yonemori and Sugiura (1995), probably depended on the same factors.

4. Vitamin C

Vitamin C exists as two vitamers in fruits: L-ascorbic acid (AA) and its oxidation product, dehydro-L-ascorbic acid (DHAA). AA and DHAA have numerous important biological properties, some of which are related to their antioxidant and antiradical activities. AA and DHAA do not exert the same antioxidant and antiradical activities, as the former is more active than the latter; however, in the human body the oxidized form is readily and almost completely converted into the reduced one (Gregory, 1996) and the determination of the sum of the two vitamers (total vitamin C) is therefore important for assessing the overall biological activity of vitamin C.

4.1. Methods of extraction and analysis of C vitamers

Table 3 summarises the methods reported in literature for C vitamer extraction and analysis in persimmons. Extraction methods of C vitamers should be governed by the need to prevent their oxidation, especially for AA that has much higher reducing properties than DHAA. In order to avoid degradation and loss of C vitamers during the recovery procedures, some precautions must therefore be taken.

The extractants usually consist of aqueous or aqueous-alcoholic mixtures containing acids, such as metaphosphoric, acetic and citric acid. Acidity allows for keeping the carboxylic-like hydroxyls ($pK_{a1} = 4.04$) protonated, thus preventing their oxidation. A chelating agent like ethylenediaminotetraacetic acid (EDTA), can also be added to the extractant to sequestrate endogenous iron or copper cations that contribute to vitamin C oxidation; in fact, these metals have been found at significant concentrations in persimmons (Gorinstein et al., 2001). Further precautions consist of purging dissolved oxygen from the extraction mixture using nitrogen or helium. Moreover, the extraction should be conducted at low temperatures and avoid exposure of the sample to white light.

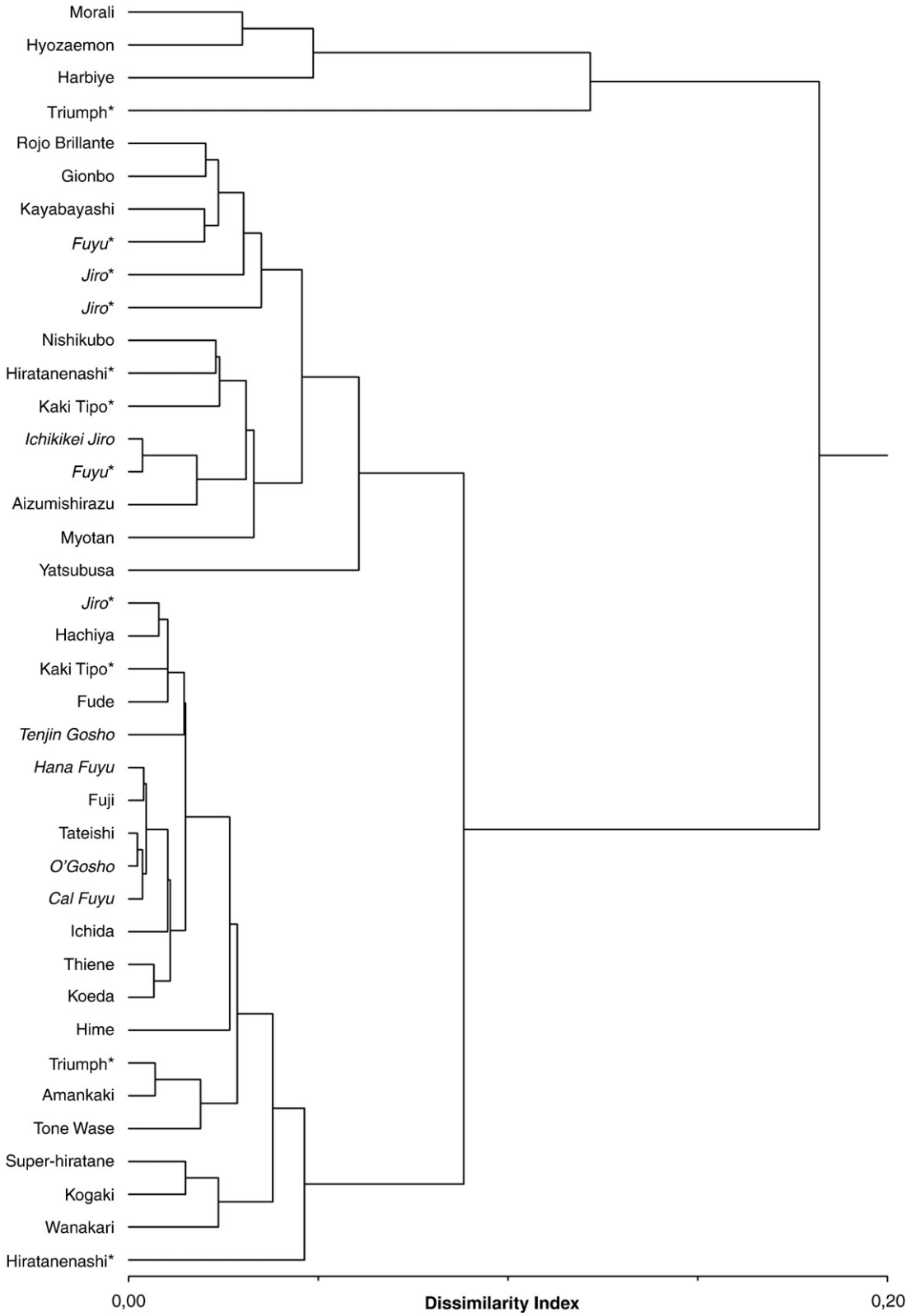

Fig. 1. Cluster analysis dendrogram based on fructose, glucose and sucrose relative percentages obtained from data reported in Table 2 for astringent (normal character) and nonastringent (italic character). (Cophenetic correlation R = 0.88; $P<0.01$). * cultivar analysed by various authors.

Diospyros kaki fruits are generally recognized as an important source of vitamin C, as reported by different nutrient databases (Souci, Fachmann, & Kraut, 1981; Technical University of Denmark, 2010; USDA, 2010), and both vitamers have been determined in this species (Del Bubba et al., 2009; Homnava, Payne, Koehler, & Eitenmiller, 1990; Wright & Kader, 1997). Nevertheless, Romero Rodriguez, Vazquez Oderiz, Lopez Hernandez and Simal Lozano (1992) did not find any AA in fresh persimmon pulp, showing that sample manipulation and/or extraction can affect vitamin C concentration and/or composition.

Table 3
Overview of methods of extraction and analysis of ascorbic (AA) and dehydroascorbic (DHAA) acids in persimmon.

Extraction method	Analysis technique	Reference
Homogenisation of the fresh fruit in glacial acetic acid containing metaphosphoric acid 4.69 M.	AA — 2,6-dichlorophenolindophenol titrimetric method. DHAA — Oxidation of AA to DHAA by aqueous bromine followed by reaction with 1,2-phenylenediamine (OPDA) to form a fluorescent derivatives determined with $\lambda_{excitation} = 350$ $\lambda_{emission} = 430$.	Homnava et al. (1990)
Homogenisation of the fresh fruit followed by immersion in metaphosphoric acid/glacial acetic acid.	HPLC on a silica-based C18 modified column at ambient temperature. Eluent: aqueous sulphuric acid, pH = 2.2. AA detection: UV — 254 nm.	Romero Rodriguez et al. (1992)
Homogenisation of the mashed fruit in 2% aqueous metaphosphoric acid.	HPLC on a silica-based C18 modified column at ambient temperature. Eluent: aqueous potassium phosphate 0.1 M/methanol/tetrabutylammonium hydroxide 97/3/0.5 (*v*/*v*/*v*), pH = 2.75. AA and DHAA detection: UV — 225 nm.	Daood et al. (1992)
Homogenisation of the fresh fruit in water/methanol 95/5 (*v*/*v*) mixture containing citric acid (0.1 M) and EDTA (5%) followed by clean-up on a C-18 SPE column.	HPLC on a silica-based C18 modified column at ambient temperature after derivatization of the extract using 1,2-phenylenediamine (OPDA). Eluent: water/methanol 95/5 (*v*/*v*) containing hexadecyltrimethylammonium bromide (5 mM) and potassium dihydrogen phosphate (50 mM), pH = 4.59. Detection: UV — 261 nm and 348 nm for OPDA derivatives of AA and DHAA, respectively.	Wright and Kader (1997) Del Bubba et al. (2009)
Homogenisation of the fresh fruit in 10% metaphosphoric acid.	Oxidation of AA to DHAA by aqueous bromine followed by reaction of DHAA with 2,4-dinitrophenylhydrazine and spectrophotometric determination.	Kondo et al. (2004)

The Association of Official Analytical Chemists (AOAC) has designed two official methods for the determination of vitamin C in food: the dye-titration method (AOAC, 1996a) and the fluorometric method (AOAC, 1996b). The former consists of determining AA via titration using 2,6-dichlorophenolindophenol (DCIP) as a titrimetric reagent and redox indicator; however, interference by other reducing agents and the lack of response to DHAA limits this approach. Conversely, the fluorometric method allows for determining the total vitamin C via oxidation of AA to DHAA and subsequent reaction with 1,2-phenylendiamine (OPDA) to form a highly fluorescent condensation product, without any distinction between the two forms. A similar method is based on the oxidation of AA to DHAA by aqueous bromine followed by reaction of DHAA with 2,4-dinitrophenylhydrazine; the product is then spectrophotometrically determined (Kondo, Yoshikawa & Katayama, 2004). Nevertheless, the latter method is less specific and sensitive than the one based on the formation of the OPDA derivative.

In order to overcome sensitivity and specificity problems, several HPLC methods have been proposed (Table 3). According to Romero Rodriguez et al. (1992) and Daood, Biacs, Czinkotai and Hoschke (1992) AA or both the C vitamers can be directly determined under reversed-phase (RP) or ion-pair RP conditions using silica based C18 columns and UV as a detection technique. However, DHAA has much lower UV-absorption than AA; in addition, in the chromatogram reported by Daood et al. (1992), only a partial resolution between AA and DHAA was achieved. For these reasons, the preventive OPDA derivatization of DHAA has been proposed for C vitamer determination by HPLC in fruits and vegetables (Zapata & Dufour, 1992) and also successfully applied to investigate vitamin C in persimmon, achieving a well-resolved chromatographic profile and quantification limits (AA: 1.0 mg l^{-1}; DHAA: 4.0 mg l^{-1}), low enough to meet the requirements of persimmon analysis (Del Bubba et al., 2009). It should also be noted that using the HPLC-OPDA method, the recovery of the whole analytical procedure can be evaluated by adding the internal standard isoascorbic acid to the matrix before extraction.

4.2. Vitamin C content

As previously described for sugars, significant changes in AA and DHAA have been observed during persimmon development and ripening. As a general finding of literature studies, total vitamin C concentration exhibited a decreasing trend as a function of fruit growth and maturation. For example, as reported by Kondo et al. (2004), total vitamin C decreased from 230 to 41 mg/100 g FW, during the last 130 days before full ripening in the astringent cultivar Saijo; a similar trend was observed by the same authors in nonastringent variety Fuyu, with a concentration decrease of about 150 mg/100 g FW in 120 days. However, as pinpointed by Del Bubba et al. (2009) for total vitamin C fruit contents in the astringent varieties Kaki Tipo and Rojo Brillante, this evolution mainly depended on fruit growth, rather than on the actual vitamin C degradation. This finding clearly evidenced that, in order to correctly evaluate the evolution of C vitamers and more generally of all primary and secondary metabolites during ripening, it is fundamental to express data on fruit content or dry weight concentration basis.

Total vitamin C, AA and DHAA concentrations of fully ripened persimmons are shown in Table 4 in different cultivars by various authors. For total vitamin C, the values ranged from 25 mg/100 g FW of Oku Gosho (Itoo, 1980) to 218 mg/100 g FW of Fuyu (Homnava et al., 1990), with an average concentration of 95 ± 63 mg/100 g FW that is six to ten times higher than the data reported by USDA-NNDSR and DFCD (7.5 mg/100 g FW) and by the FCNT (16 mg/100 g F.W.). In this regard it should be noted that no information concerning the genotype investigated is reported in the consulted databases, thus making difficult to explain these relevant discrepancies. The analytical methods adopted for obtaining the data cited in the above mentioned databases, which can contribute to obtain different results, are not reported as well.

As shown in Table 4, in some cases the same cultivar was analysed by different authors. Homnava et al. (1990) and Wright and Kader (1997) found a similar total vitamin C content in Fuyu (218 and 210 mg/100 g FW respectively), while the results of Itoo (1980) and Kondo et al. (2004) were much lower (52 and 40 mg/100 g FW respectively). Quite high differences were also observed when the results obtained by Itoo (1980) on Hana Gosho and Jiro were compared to those of Homnava et al. (1990). Some discrepancies also emerged from the comparison of AA data; the least matching results were those of Hiratanenashi, with 4.4 and 49 mg/100 g FW of AA found respectively by Mangarova (2005) and Homnava et al. (1990) and the ones obtained on Jiro by Itoo (1980) (24 mg/100 g FW) and Homnava et al. (1990) (143 mg/100 g FW). Very similar AA concentrations were found for nonastringent Fuyu and Jiro (116 and 118 mg/100 g dry weight, DW, respectively) by Gorinstein et al. (2011); these values were quite similar to the ones reported by Itoo (1980) when converted in FW, but much lower than the others. Irrespective of the lack of agreement among some literature results, it is interesting to compare total vitamin C concentrations and C vitamer composition found in astringent and nonastringent varieties. A relevant difference in total vitamin C was observed between the two groups with an average value for nonastringent fruits (121 ± 85 mg/100 g FW) significantly higher than that found for astringent cultivars (75 ± 27 mg/100 g FW), according to the Student-T test ($P = 0.075$). The nonastringent cultivars contributing to the highest values of vitamin C content (>210 mg/100 g FW) were Fuyu, Hana Fuyu and Jiro; Shogatsu (122 mg/100 g FW), Sheng (106 mg/100 g FW) and Kaki Tipo (104 mg/100 g FW) showed the highest concentrations among astringent types.

Table 4
Acorbic Acid (AA), dehydroascorbic acid (DHAA) and total Vitamin C content (mg/100 g FW) of astringent (normal character) and nonastringent (italic) persimmon fruits at commercial harvest. n.a. = not analysed.

Cultivars	AA	DHAA	Total vitamin C	Reference
Lycopersicon	44	n.a.	n.a.	Vidrich et al. (1978)[a]
Hana Gosho	33	12	45	Itoo (1980)
Saidoshi	31	15	46	
Fuyu	41	11	52	
Jiro	24	11	35	
OkuGosho	16	9	25	
Aizumishirazu	38	32	70	Homnava et al. (1990)
Saijo	19	25	44	
Gionbo	44	21	65	
Hiratanenashi	49	34	83	
Sheng	68	38	106	
Tanenashi	34	32	66	
Korean	54	41	95	
American type	63	23	86	
Hachiya	21	14	35	
Shogatsu	71	51	122	
Hana Gosho	56	34	90	
Hana Fuyu	146	68	214	
Jiro	143	69	212	
Ichikikei Jiro	109	75	184	
Fuyu	97	121	218	
Fuyu	110	100	210	Wright and Kader (1997)
Mikado	8.8	n.a.	n.a.	Oliveira et al. (1997)
Rama Forte	10.1	n.a.	n.a.	
Saijo	n.a.	n.a.	41	Kondo et al. (2004)
Fuyu	n.a.	n.a.	40	
Early	4.3	n.a.	n.a.	Mangarova (2005)
Hiratanenashi	4.4	n.a.	n.a.	
Costata	3.5	n.a.	n.a.	
Hyakume	3.9	n.a.	n.a.	
Unknown astringent cultivar	58	n.a.	n.a.	Bibi et al. (2007)
Hachiya	12	n.a.	n.a.	Celik and Ercisli (2008)
Kaki Tipo	49	55	104	Del Bubba et al. (2009)
Rojo Brillante	35	58	93	

[a] Data expressed on dry matter in the original paper have been herein converted considering an average water content of 80% (Piretti 1991).

The content of AA (mean values 47 ± 39 mg/100 g FW as average) varies greatly, from 3.5 mg/100 g FW in the astringent variety Costata to 146 mg/100 g FW in the nonastringent cultivar Hana Fuyu (Table 4). Astringent and nonastringent genotypes had actual different AA concentrations, the mean values of which were 34 ± 22 and 71 ± 51 mg/100 g FW respectively, and were significantly different according to the Student-T test (P = 0.07). Conversely, no statistic difference was found for DHAA, even though, on average, nonastringent cultivars were richer in DHAA (51 ± 41 mg/100 g FW) than astringent ones (34 ± 15 mg/100 g FW).

Based on the data reviewed and currently recommended daily amount (RDA) of vitamin C (40–90 mg/day), suggested by different organizations (e.g. United Kingdom Food Standards Agency and the United States Academy of Sciences), it is evident that in most cases, 100–150 g of persimmon (approximately half of a whole fruit) supply a daily quantity of AA plus DHAA included in the above-mentioned range. Moreover, some nonastringent cultivars are rich enough in AA and/or DHAA to give a dietary contribution almost equal to the upper RDA limit via the intake of only 50 g of fruit.

5. Carotenoids

Carotenoids consist of two main structural groups: hydrocarbon carotenes such as β,ε-carotene (i.e. α-carotene) and β,β-carotene (i.e. β-carotene) and xanthophylls (e.g. β-cryptoxanthin), composed of several derivatives containing hydroxyl, epoxy, aldehyde and keto groups; in addition, long-chain fatty acid esters of the hydroxylated carotenoids are often present in fruits.

Carotenoids, such as β,β-carotene and β-cryptoxanthin have nutraceutical relevance due to their role as precursors of vitamin A synthesis; more specifically, they are important dietary supplements for the reduction of degenerative human disease, since it has been reported that these molecules can prevent oxidative damage to important biological membranes owing to their antioxidant capacity (Rao & Rao, 2007).

5.1. Extraction and analysis of carotenoids

Table 5 contains an overview of extraction and analysis methods of carotenoids in persimmon. As previously reported for vitamin C, carotenoids are also strongly susceptible to oxidative degradation, due to the high number of conjugated double bonds; therefore, great attention should be paid to sample manipulation with care taken during extraction. Consequently, extraction must be performed in the dark or under yellow light, thus allowing to prevent photoisomerization phenomena, as well (Von Elbe & Schwartz, 1996). In some cases, an antioxidant compound, such as butylated hydroxytoluene (BHT) is added to the extractant to prevent oxidation phenomena, whereas a basic salt like magnesium carbonate is used to neutralize traces of organic acids that can cause structural transformations and actual degradation of carotenoids; other precautions consist in the extraction at low temperature or in the flushing of the solution with nitrogen to remove the dissolved oxygen (De Ancos, Gonzales & Cano, 2000; Homnava et al., 1990; Philip & Chen, 1988).

All classes of carotenoids are lipophilic compounds and therefore their extraction from fruits, which contain water, must utilize a suitable water-miscible organic solvent in which carotenoids are soluble. Alternatively, an extractant mixture consisting of a hydrophobic solvent and water miscible solvent, the latter acting as a "bridge" between hydrophilic and lipophilic phases, should be employed (Table 5).

Persimmons belong to the fruit class that contains carotenol fatty acid esters, together with carotenes and xanthophylls (Breithaupt and Bamedi, 2001); therefore, carotenoid analysis needs the use of chromatographic techniques, suitable to achieve a separation of the target compounds as complete as possible.

Carotenoid analysis is commonly performed by HPLC, under reversed-phase mode on octadecyl silica column, using spectrophotometry in the visible region (λ = 436–465 nm) as detection system (Table 5). The separation of carotenoids is carried out by applying an elution gradient from a aqueous polar to a non-polar phase or an isocratic flow with a non-aqueous mobile phase with low polarity; in both experimental conditions the elution order is xanthophylls, hydrocarbon carotenoids and long-chain fatty acid esters of xanthophylls (Daood et al., 1992; De Ancos et al., 2000). However, direct injection of the persimmon extract results in a complex chromatographic profile, the interpretation of which is quite difficult.

Saponification procedure is often adopted to hydrolyze esterified carotenols into the corresponding alcohols and the chromatographic investigation of both saponified and unsaponified extracts have been carried out to make easier the peak attribution and to get, at the same time, a complete picture of the carotenoid fraction (Daood et al., 1992; De Ancos et al., 2000). In this regard, it should be noted that, even if saponification has been reported to affect carotenoids composition (Ball, 2000), a method of carotenol fatty acid ester hydrolysis that does not seem to change native carotenoids has been proposed by Kimura, Rodriguez-Amaya, and Godoy (1990) for tomato, kale and papaya and used for characterizing carotenoids in persimmon (Homnava et al., 1990; Wright & Kader, 1997) (Table 5). According to Kimura et al. (1990) this procedure caused no loss or isomerisation of β,β-carotene and was able to completely hydrolize β-cryptoxanthin esters; moreover, losses of xanthophylls could be reduced to insignificant levels by extracting the matrix under an atmosphere of nitrogen or by adding an antioxidant to the extractant mixture.

Table 5
Overview of methods of extraction and instrumental analysis of carotenoids in persimmons.

Extraction method	Instrumental technique	Reference
Homogenisation of pureed fruits with methanol containing 0.2 M $MgCO_3$ followed by extraction with acetone/petroleum ether 80/20 (*v*/*v*) and purification on alumina column	HPLC analysis on a C18 column Elution: gradient with methanol and ethylacetate Detection: spectrophotometer set at 465 nm	Philip and Chen (1988)
Homogenization of fruits followed by saponification overnight with ethanol/water/saturated KOH 2/1/1 (*v*/*v*/*v*) containing 1 g of ascorbic acid, and extraction with hexane containing 0.01% BHT	HPLC analysis on a Zorbax C18 column Elution: isocratic with acetonitrile/dichloromethane (0.001% triethylammine)/methanol 350/150/1 (*v*/*v*/*v*) Detection: spectrophotometer set at 436 nm	Homnava et al. (1990, 1991)
Homogenisation of lyophilized samples with acetone containing 0.01 M $CaCO_3$ followed by extraction with acetone/diethyl ether 4/1 and 2/3 (*v*/*v*) and saponification with saturated methanolic KOH for 30 min	Spectrophotometric analysis Detection: spectrophotometer set at 453 nm	Forbus et al. (1991) Candir et al. (2009)
Extraction of frozen samples with carbon tetrachloride/ methanol 2/1 (*v*/*v*) followed by saponification with 10% methanolic KOH for 30 min	HPLC analysis on a Chromasil C18 column Elution: isocratic with acetonitrile/2-propanol/ethylacetate 53/40/7 (*v*/*v*/*v*) Detection: spectrophotometer set at 438 nm	Daood et al. (1992)
Homogenisation of frozen samples with cold ethanol followed by extraction with hexane and saponification with 10% methanolic KOH for 16 h	HPLC analysis on a Vydac C18 column Elution: isocratic with acetonitrile/methanol (0.05 M ammonium acetate)/dichloromethane 75/20/5 (*v*/*v*/*v*) containing 0.1% BHT and 0.05% triethylamine Detection: spectrophotometer set at 450 nm	Wright and Kader (1997)
Homogenisation of pureed fruits with tetrahydrofurane containing BHT 0.01% followed by extraction with diethyl ether and saponification with metanolic KOH (30 g/l) for 12 h	HPLC analysis on a Hypersil C18 column Elution: gradient with methanol/water 75/25 (*v*/*v*) and ethylacetate Detection: spectrophotometer set at 440 nm	De Ancos et al. (2000)
Homogenization of frozen fruits with 100% ethanol followed by extraction with diethyl ether and saponification with 10% methanolic KOH for 1 h	HPLC analysis on a YMC C30 column Elution: isocratic with methanol/t-butylmethyl ether 92/8 (*v*/*v*) containing acetic acid 0.1% Detection: spectrophotometer set at 450 nm	Kondo et al. (2004)
Homogenisation of samples with ice-cold acetone	HPLC analysis on a Gemini C18 column Elution: gradient with acetonitrile/methanol/water 100/10/5 (*v*/*v*/*v*) and acetone/ethylacetate 2/1 (*v*/*v*) Detection: spectrophotometer set at 440 nm	Veberic et al. (2010)

Spectrophotometric determination of total carotenoids on saponified persimmon extract has been reported by Forbus, Payne and Senter (1991) and Candir et al. (2009) (Table 5); this procedure allowed the determination of effective total carotenoid content, even if no information about the quali-quantitative composition of individual carotenoids could of course be achieved.

5.2. Carotenoid content and composition

The comparison and evaluation of data regarding carotenoids in fruits is very difficult, since many different analytes in this class, with various beneficial effects on human health, are known and authors have not always investigated the same compounds and/or used the same analytical approach. Individual carotenoids that have been commonly determined in persimmon by different authors using equivalent methods (based on extraction, extract saponification and HPLC analysis of saponified and non-saponified fractions) are β,β-carotene, β,ε-carotene and total β-cryptoxanthin (see Table 6). In two cases (Gorinstein et al., 2011; Veberic et al., 2010), tests were conducted on unsaponified esters, thus determining only the non-esterified β-cryptoxanthin (i.e. native free β-cryptoxanthin).

According to USDA-NNDSR and DFCD the standard values of β,β-carotene are 253 μg/100 g FW and 420 μg/100 g FW (range: 266–520 μg/100 g FW), respectively. These values were lower than those of apricots, mangoes and papayas, and higher than those of other extensively consumed fruits such as apples, peaches, oranges, and different small berries. By considering data reported in Table 6, an inferior average value (113 ± 123 μg/100 g FW) was obtained, with the lowest content (25.9 μg/100 g FW) in Jiro (Veberic et al., 2010) and the highest (550 μg/100 g FW) in Hana Gosho (Homnava et al., 1990), both nonastringent cultivars. High levels of β,β-carotene were also reported in one of the first papers devoted to carotenoid studies in persimmons (Brossard & Mackinney, 1963), with the concentrations of β,β-carotene in the nonastringent Fuyu, and the astringent varieties Tamopan and Honan Red equal to 303, 458 and 512 μg/100 g FW, respectively. Moreover, an outstanding value (1160 μg/100 g FW), which could be due to a specific fruit treatment during maturation, was determined by Daood et al. (1992) on persimmons of an unknown cultivar grown in Albania.

Among the 33 samples belonging to the 25 cultivars reviewed in Table 6, no statistically significant differences in β,β-carotene concentrations were observed between astringent and nonastringent groups, as the mean values and standard deviations were 110 ± 114 μg/100 g FW and 119 ± 143 μg/100 g FW, respectively.

USDA-NNDSR reported a nil content of β,ε-carotene in persimmons, whilst relevant concentrations of this compound were determined by different authors (Table 6), with a mean value of 30 ± 33 μg/100 g FW. The lowest concentration (7.3 μg/100 g FW) was found in the astringent variety Amankaki (Veberic et al., 2010) and the highest (167 μg/100 g FW) in the nonastringent cultivar Hana Gosho (Homnava et al., 1990). The mean concentration found in astringent genotypes (22 μg/100 g FW) was lower than the corresponding average value of nonastringent varieties (38 μg/100 g FW); however, the variation coefficients observed in both groups were about 100% and made the observed mean differences statistically insignificant.

The content of β,ε- and β,β-carotene of the cultivars Hana Fuyu, Jiro, Fuyu (nonastringent) and Triumph (astringent) were analysed by several authors, in some cases obtaining significantly different results; the concentrations of the two hydrocarbon carotenoids reported by Veberic et al. (2010) were approximately 30–80 % lower than those determined by Homnava et al. (1990) (for Hana Fuyu and Jiro), Wright and Kader (1997) (for Fuyu) and De Ancos et al. (2000) (for Triumph); in these cases the observed differences could be attributed to environmental factors, even if concurrent analytical artifacts cannot be excluded. Using the method of Veberic et al. (2010), Gorinstein et al. (2011) found concentrations of β,β-carotene equal to 156 and

Table 6
Concentrations (μg/100 g FW) of selected carotenoids in astringent (normal character) and nonastringent (italic character) persimmon fruits at commercial harvest. b.d.l. = below detection limit; n.a. = not analysed.

Cultivars	β, β-carotene	β,ε-carotene	β-cryptoxanthin	References
Aizumishirazu	65	23	54[a]	Homnava et al. (1990)
Saijo	70	33	70[a]	
Gionbo	56	19	78[a]	
Hiratanenashi	65	b.d.l.	49[a]	
Sheng	160	34	138[a]	
Tanenashi	114	27	260[a]	
Korean	95	18	75[a]	
American type	118	14	190[a]	
Hachiya	540	90	128[a]	
Shogatsu	121	29	48[a]	
Hana Gosho	550	167	162[a]	
Hana Fuyu	120	48	97[a]	
Jiro	93	37	80[a]	
Ichikikei Jiro	125	41	87[a]	
Fuyu	158	60	60[a]	
Hana Fuyu	100	29	59[a]	Homnava et al. (1991)
Sheng	163	39	127[a]	
Fuyu	105	20	205[a]	Wright and Kader (1997)
Triumph	163	n.a.	579[a]	De Ancos et al. (2000)
Rojo Brillante	124	n.a.	685[a]	
Fuyu	n.a.	n.a.	634[a]	Kondo et al. (2004)
Saijo	n.a.	n.a.	390[a]	
Amankaki	39	7.3	29[b]	Veberic et al. (2010)
Cal Fuyu	30	7.6	7.6[b]	
Fuji	31	7.8	8.0[b]	
Hana Fuyu	40	8.3	26[b]	
Jiro	26	12	18[b]	
O'Gosho	46	8.3	15[b]	
Tenjin Gosho	36	16	15[b]	
Thiene	38	9.2	21[b]	
Kaki Tipo	47	8.6	21[b]	
Tone Wase	31	8.0	24[b]	
Triumph	45	11	26[b]	

[a] Total β-cryptoxanthin (analysis carried out on saponified samples).
[b] Native free β-cryptoxanthin (analysis carried out on unsaponified samples).

153 μg/100 g DW in Fuyu and Jiro fruits, the latter being very similar to the value reported by Veberic et al. (2010) when converted on fresh weight. Lower variations were observed by comparing the content of hydrocarbon carotenoids in Sheng and Hana Fuyu persimmons harvested in two consecutive years (Homnava et al., 1990; Homnava, Payne, Koehler & Eitenmiller, 1991).

Xanthophylls are mainly present as mono- and di-esters of various fatty acids, such as lauric, myristic and palmitic acid; with concentrations about one magnitude order higher than the non-esterified forms (Breithaupt, Weller, Wolters, & Hahn, 2003; Daood et al., 1992; Philip & Chen, 1988). Among xanthophylls, β-cryptoxanthin has been reported as the most abundant in persimmon. The standard content of total β-cryptoxanthin reported by USDA-NNDSR for persimmons was 1447 μg/100 g FW, much higher than the average concentration obtained from data shown in Table 6 (193 ± 197 μg/100 g FW); the latter value was calculated after excluding the data reported by Veberic et al. (2010), which cannot be compared with the others due to the different methodological approaches adopted.

Within results obtained using the saponification procedure, significant differences were anyhow observed for total β-cryptoxanthin by comparing data determined on the same variety by different authors. For instance, in Saijo, concentrations of 70 and 390 μg/100 g FW were reported by Homnava et al. (1990) and Kondo et al. (2004), respectively, probably owing to environmental effects and/or yearly fluctuations. Higher values of total β-cryproxanthin were found by De Ancos et al. (2000) in Triumph (579 μg/100 g FW) and Rojo Brillante (685 μg/100 g FW) and by Kondo et al. (2004) in Fuyu (634 μg/100 g FW). For the latter cultivar, Homnava et al. (1990) determined a much lower content (60 μg/100 g FW). Wide variations were also observed by Brossard and Mackinney (1963) on a large set of cultivars; in this case total β-cryptoxanthin concentrations were higher than the USDA-NNDSR standard, as included in the range of 1708–3040 μg/100 g FW, for Tamopan and Honan Red, respectively.

Different concentrations for other xanthophylls, such as zeaxanthin (447, 880 and 732 μg/100 g FW), lutein (145, 160 and 275 μg/100 g FW), antheraxanthin (329, 736 and 1281 μg/100 g FW), mutatoxanthin (132, 40, 73.2 μg/100 g FW), violaxanthin (105, 144 and 872 μg/100 g FW), neoxanthin (66, 80 and 122 μg/100 g FW), were detected in fruits of Fuyu, Honan Red and Tamopan respectively, whereas luteoxanthin was only found in Tamopan (104 μg/100 g FW) by Brossard and Mackinney (1963). More recently, De Ancos et al. (2000) found similar concentrations for violaxanthin (111 and 218 μg/100 g FW), neoxanthin (135 and 114 μg/100 g FW), lutein (166 and 114 μg/100 g FW), antheraxanthin (36 and 55 μg/100 g FW) and zeaxanthin (332 and 367 μg/100 g FW), in Triumph and Rojo Brillante, respectively. For these xanthophylls, only the sum of lutein and zeaxanthin (834 μg/100 g FW) has been reported by USDA-NNDSR for persimmon, confirming the data, and assessing a much higher content of these carotenoids in persimmon than in more commonly marketed fruits.

Total carotenoids were spectrophometrically determined on various persimmon cultivars by Brossard and Mackinney (1963), Candir et al. (2009) and Forbus et al. (1991). Brossard and Mackinney (1963) reported a total carotenoid content in Tamopan, Fuyu and Honan Red equal to 6100, 6500 and 8000 μg/100 g, respectively, expressed as μg of β,β-carotene per 100 g FW. Forbus et al. (1991) found values ranging from 598 to 746 μg/g DW for the nonastringent cultivars Fuyu, Jiro and Ichikikei Jiro, while the astringent varieties Gionbo and Aizumishirazu showed concentrations of 565 and 581 μg/g DW, respectively; these values were higher than those observed by Brossard and Mackinney (1963) when converted to fresh weight concentrations on the basis of the water content commonly found in persimmons. Surprisingly, Candir et al. (2009), using the same method of Forbus et al. (1991), determined very low total carotenoid concentrations in the astringent cultivar Harbiye (81 and 92 μg of β,β-carotene per 100 g FW), grown at low and high altitudes.

Fig. 2 illustrates the estimated dietary intake of provitamin A carotenoids (β,β-carotene, β,ε-carotene and β-cryptoxanthin) expressed as Retinol Activity Equivalent (RAE), for the 15 persimmon cultivars reviewed in Table 6, for which all the three above-mentioned provitamin A-carotenoids have been investigated. The RAE values were calculated by the following equation:

$$RAE = \frac{C_{\beta,\beta-carotene}}{12} + \frac{C_{\beta,\varepsilon-carotene}}{24} + \frac{C_{\beta-Cryptoxanthin}}{24}$$

where carotenoid concentrations (C) were expressed in μg/100 g FW and 12 and 24 are the conversion factors from μg of carotenes to RAE, according to the Committee on Use of Dietary Reference Intakes in Nutrition Labelling (CUDRINL, 2003). Since fatty acid esters of β-cryptoxanthin are hydrolyzed in the human body, and the bioavailability of the esterified and non-esterified forms have been reported as comparable (Breithaupt et al., 2003), concentrations of native free β-cryptoxanthins, together with those of total β-cryptoxanthins were taken into account for the calculation of RAE. Astringent cultivar Hachiya and nonastringent Hana Gosho showed very similar RAE values (54 and 60 μg/100 g FW, respectively) and more than twofold higher than the others, indicating their greater contribution to vitamin A requirement; these values are lower than the USDA-NNDSR standard reference (81 μg/100 g FW), but still higher than the average values of other exotic and commonly consumed fruits. The differences observed between the RAE values calculated here, and the USDA-NNDSR values, are mainly due to the lower amounts of β-cryptoxanthin obtained from literature data, as referred above. Since the daily dietary reference intake of vitamin A is about 600 μg RAE/d

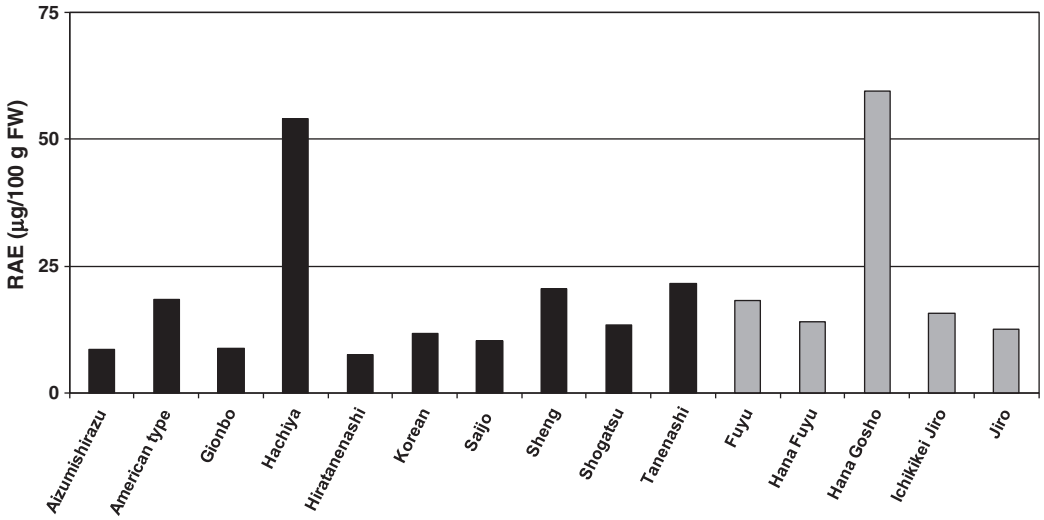

Fig. 2. Dietary intake of provitamin A carotenoids (β,β-carotene, β,ε-carotene and total β-cryptoxanthin, as reported in Table 6), expressed as µg Retinol Activity Equivalent (RAE) per 100 g of FW persimmon for different astringent (black bars) and nonastringent (grey bars) cultivars.

(CUDRINL, 2003), the contribution of 100 g of fresh persimmon represents an average of about 12% of the daily requirement according to the USDA-NNDSR standard reference; for most of the cultivars in Fig. 2 this contribution was in the range of 1–2% while for Hachiya and Hana Gosho it was about 9%.

Persimmons are also a very good source of lycopene, superior to most of commonly consumed fruits, with a standard content of 159 µg/100 g FW (USDA-NNDSR). Data reported in the few literature studies showed a sharp variation in the content of this compound among cultivars; for example, similar values to the USDA-NNDSR standard were found by Wright and Kader (1997) in Fuyu (242 µg/100 g FW), by Homnava et al. (1991) in Hana Fuyu (167 µg/100 g FW), two nonastringent cultivars, and by Philip and Chen (1988) in an unknown cultivar grown in California (110 µg/100 g FW). As regards astringent varieties, De Ancos et al. (2000) determined quite a high content of lycopene in Rojo Brillante (534 µg/100 g FW), whereas only traces were found in Triumph and a very low concentration (20 µg/100 g FW) was observed in Sheng by Homnava et al. (1991). Hence the wide variability of lycopene content could be attributed to genotype effect, rather than the type of cultivar in relation to astringency, and could also depend on both environmental factors (e.g. light exposure) and specific stage of ripening (Von Elbe & Schwartz, 1996). This hypothesis complies with the results already reported in 1963 by Brossard and Mackinney, who determined very different contents of lycopene concentrations in 40 persimmon varieties, irrespective of their astringency traits.

6. Polyphenols

Polyphenolic compounds represent a large and important group of abundant secondary plant metabolites in fruit and vegetables, often associated with sugar moieties (Sakakibara, Honda, Nakagawa, Assida, & Kanazawa, 2003). Polyphenols in plants contribute to several sensory properties and defence mechanisms (Taiz & Zeiger, 2002) and their role in human health protection, related to antioxidant and antiradical activities, has been repeatedly suggested (Dillard & German, 2000).

Many classes of polyphenols are known, classically distinguished in flavonoids and non-flavonoids; among flavonoids, monomeric and polymerized flavan-3-ols (i.e. proanthocyanidins or condensed tannins), with several structural variations are largely present in persimmon (Suzuki, Someya, Hu, & Tanokura, 2005; Taira, 1996). Non-flavonoids polyphenols such as benzoic and cinnamic acid derivatives have also been found in persimmon (Gorinstein et al., 1994; Jung et al., 2005).

Some literature studies also refer to insoluble and soluble tannins, the latter considered responsible for the typical astringency of unripe persimmons (Itoo, 1986; Taira, 1996). Both soluble and insoluble tannins belong to the proanthocyanidin class and differ in polymerization degree, as the latter is much more polymerized than the former and therefore water insoluble and unable to interact with salivary proteins giving rise to astringency perception (Piretti, 1991; Tarascou et al., 2010).

6.1. Extraction and analysis of polyphenols

Due to the large number of structurally different polyphenols present in vegetal matrixes, including persimmons, analytical procedures for the identification and quantification of individual compounds represent a difficult analytical problem.

The tests for phenolic compounds vary from simple spectrophotometric assays for quantifying total or selected classes of polyphenols, to the use of sophisticated instrumentation for the separation, quantification and structural characterization of individual components. In all cases sample manipulation and extraction of target compounds are important steps that can significantly affect the results.

As previously observed for sugars, an inactivation procedure of enzymes that can alter the native composition of polyphenolic fraction should be included in the recovery method of polyphenols from persimmon. For instance, the inactivation of polyphenol oxidases (PPO), as described by Suzuki et al. (2005), Chen, Fan, Yue, Wu, and Li (2008) and Gu et al. (2008), can be achieved by boiling the ungrounded persimmon pulp in the extraction solvent for 10–30 min. According to Del Bubba et al. (2009), PPO inactivation can also be obtained by adding sodium fluoride to the extraction mixture, without any thermal treatment. However, as reported by Piretti (1991), the PPO activity in the pulp of *D. kaki* is relatively low when compared to that of other popular fruits, such as apples and pears and therefore the lack of inactivation should have minor consequences on polyphenol native composition.

Extraction of polyphenols from persimmon has been carried out using methods that differ for many variables, such as the characteristics of the extractant, extraction time and temperature and number of extractions; consequently, the data reported in literature, obviously affected by the extraction procedure together with the instrumental quantification method, are not always easily comparable.

According to Taira (1996), extraction of the so-called soluble tannins can easily be achieved via homogenisation of the pulp at room temperature with CH_3OH/H_2O 8/2 (v/v); with this method three replicated extractions of astringent Kaki Tipo and Rojo Brillante flesh samples allowed for quantitatively recovering polyphenols that are

extractable under these experimental conditions (i.e. soluble polyphenols) (Del Bubba et al., 2009). Similar extraction methods consisted of single or replicated fruit extraction using aqueous-alcoholic mixtures in different proportions (Gorinstein et al., 1999; Katsube et al., 2004; Kondo et al., 2004; Park et al., 2006), alcohols alone (Chen et al., 2008; Ercisli, Akbulut, Ozdemir, Sengul, & Orhane, 2007; Nakatsubo et al., 2002) and ethyl acetate (Gorinstein et al., 2001) as extractants. Ultrasonic assisted extractions have also been tested for polyphenol recovery from persimmon (Chen et al., 2008; Nakatsubo et al., 2002).

A more complex procedure was proposed by Gorinstein et al. (1994) that extracted the persimmon pulp with methanol; the extract was then counter-extracted with light petroleum (boiling point 60–80 °C) and, after volume reduction by evaporation and dilution with water of the methanolic phase, polyphenols were finally recovered using ethyl acetate. A similar approach was adopted by Suzuki et al. (2005) that performed pulp extraction with hot water, followed by counter-extraction with chloroform and final recovery of polyphenols from the remaining water phase with ethyl acetate.

Extraction of soluble tannins has also been performed by using 80% aqueous acetone (Wu & Hwang, 2002), that is more effective in the recovery of higher molecular weight proanthocyanidins.

Using these kinds of recovery procedures, followed by spectrophotometrical determination with appropriate colorimetric reagents, polyphenols have been quantified and indicated by different authors as "total polyphenols"; nevertheless this locution can be questionable and misleading, since these extraction procedures obviously did not permit a complete recovery of the whole persimmon polyphenolic fraction.

A stronger procedure, consisting of hydrolysis of highly polymerized proanthocyanidins by homogenisation at high temperature of the pulp in aqueous HCl 0.56 N, was reported by Taira (1996) for determining both soluble and bounded polyphenols that are considered more representative of the total polyphenolic content of persimmon. An analogous hydrolysis treatment for the recovery of the whole polyphenolic fraction, based on the extraction with methanol acidified with HCl (1–4%) under reflux conditions at 90 °C, has been reported by Gu et al. (2008) and Gorinstein et al. (2011).

Extractable polyphenols have been spectrophotometrically determined by the Folin-Ciocalteu (Chen et al., 2008; Gorinstein et al., 1994, 2001; Katsube et al., 2004) or, less frequently, the Folin-Denis (Del Bubba et al., 2009; Suzuki et al., 2005) assays. In addition, in order to colorimetrically determine extracted flavanols the vanillin-HCl assay has been used (Gu et al., 2008; Nakatsubo et al., 2002).

Selected phenolic acids have been determined by Gorinstein et al. (1994) using a fluorometric method based on the measure of the height of fluorescence emission peaks of extracts obtained as described above versus reference standards. Moreover, based on fluorescence emission spectra of ethanol and ethyl acetate extracts, Gorinstein et al. (2001) concluded that phenolic acids were better extracted with ethanol than with ethyl acetate.

Also RP-HPLC with silica-based C18 columns has been employed for identification and quantitative determination of phenolic acids after alkaline hydrolysis of persimmon tissue, followed by pH adjustment to 3, extraction with petroleum ether, extract evaporation to dryness and final dissolution in dimethylformamide (Gorinstein et al., 1994). Under these experimental conditions, RP-HPLC gave rise to a retention time overlap of proanthocyanidin isomers extracted together with phenolic acids and having a different polymerization extent, causing the oligomers to coelute as a large unresolved hump, making the method unreliable. Under RP conditions (silica-based C22 column and isocratic elution with CH_3OH/0.1% H_3PO_4 22/78 v/v), Suzuki et al. (2005) reported the identification and quantification of some monomeric flavanols.

In order to make RP-HPLC also suitable for the analysis of persimmon proanthocyanidins, they are often depolymerised before chromatographic analysis in the presence of a suitable nucleophilic agent such as toluene-α-thiol (Gu et al., 2008; Li et al., 2010) or phloroglucinol (Akagi et al., 2010). This procedure leads to the progressive depolymerisation of the proanthocyanidin via the cleavage of the interflavanyl linkages, with the formation, as a final result of the reaction, of the thioether or phloroglucinol derivatives and the flavan-3-ol terminal unit. Therefore, the determination of the nature and proportion of constitutive units of proanthocyanidins, and the calculation of the average degree of polymerization (DP) are possible (Tarascou et al., 2010). Using this method, Li et al. (2010) and Akagi et al. (2010) showed that persimmon proanthocyanidins consist mainly of epigallocatechin-3-O-gallate, epicatechin gallate, epigallo catechin and epicatechin, with the former being the most abundant.

Another method for the purification of condensed tannins consisted of gel permeation chromatography, which allowed for obtaining fractions with different DP. This technique also enabled determination of the molecular weight range of tannins (Gu et al., 2008; Li et al., 2010) and consequently their DP.

In order to obtain more complete structural information of the proanthocyanidins, MALDI-TOF and NMR have been also employed; for example, Akagi et al. (2010) adopted these techniques for the identification of an unknown peak obtained by the HPLC analysis of a depolymerized extract, whereas Li et al. (2010) found a novel terminal unit (i.e. the flavonol myricetin) and confirmed for the first time the presence of A-type interflavan bonds besides the more common B-type interflavan linkages in persimmon tannins.

6.2. Soluble polyphenol content

The chemical constitution of the diverse kind of polyphenols occurring in persimmon may have important implications in the human diet. In spite of the incomplete knowledge of the chemical structure of persimmon polyphenols, it has been demonstrated that simple polyphenols together with highly polymerized flavanols are present in persimmons. As regards the former group (i.e. benzoic acid derivatives, monomeric flavanols, flavonols and flavanones) the availability for absorption during in vitro human digestion has been suggested (Mandalari et al., 2010). Likewise, in some *in vitro* experiments, proanthocyanidin dimers and trimers, but not polymers with an average DP = 7, were absorbed by human intestinal epithelium cells (Déprez, Mila, Huneau, Tome, & Scalbert, 2001); proanthocyanidin polymers were not absorbed even by chicken or sheep (Jimenez-Ramnsey, Rogler, Housley, Butler, & Elkin, 1994; Terrill, Waghorn, Woolley, McNabb, & Barry, 1994). Studies on human subjects showed that cocoa proanthocyanidins were stable during transit in the stomach (Rios et al., 2002), but some results suggest an extensive metabolism in the lower gastrointestinal tract (Rios et al., 2003). Intestinal bacteria with tannase activity have been isolated from human faeces (Osawa, Kuroiso, Goto, & Shimizu, 2000) and it has been demonstrated that polymeric proanthocyanidins were *in vitro* catabolised by human colonic microflora into low molecular weight phenolic acids (Déprez et al., 2000), which are similar to those resulting from the metabolism of monomeric flavonoids (De Eds, 1959).

In chemical terms, since insoluble persimmon tannins are hydrolysed by aqueous HCl 0.56 N (Taira, 1996), it can be hypothesized that a more or less partial hydrolysis of highly polymerized proanthocyanidins into simpler molecules will also occur during human gastric digestion. On the other hand, as pinpointed for the origin of astringency perception in the oral cavity, tannins bind readily with proteins to form quite indigestible complexes and can therefore be considered as antinutritional compounds in herbivores (Salunkhe, Chavan, & Kadam, 1989). Moreover, as suggested by Ben-Arie and Sonego (1993), tannins resolubilised during human digestion can be involved in the occurrence of persimmon bezoars, hard masses occasionally found in the human stomach or intestine (Seung et al., 2007), with a higher frequency in patients subjected to gastric surgery

(Moriel et al., 1983). In this regard, Ben-Arie and Sonego (1993) hypothesized that the formation of persimmon bezoars is caused by resolubilization of insoluble tannins by the high acidity occurring in the human stomach during digestion and successive re-coagulation of solubilised tannins with proteins and/or other macromolecules present in the stomach. The re-coagulating capacity of resolubilized tannins has been experimentally highlighted by Seung et al. (2007) on a patient with an intestinal persimmon bezoar resulting from the solubilisation of a previously diagnosed gastric bezoar.

It is therefore evident that proanthocyanidin digestion and absorption mechanisms are not fully understood and, as reported by Beecher (2004), two issues are crucially important for substantial advancement of the association of these dietary components with human health, that is, (i) to identify the biologically active compounds absorbed and their tissue distribution and, (ii) to assess the actual intake of proanthocyanidins in the dietary supplement.

Table 7 illustrates the concentrations of soluble polyphenols, which can presumably be considered digestible according to the previous consideration, as determined in ripened persimmons by different authors. Unfortunately the actual state of maturation, defined by means of comparable ripening indexes, is seldom clearly reported, thus compromising the comparison of results. This issue is of paramount importance since the composition of persimmon polyphenolic fractions, with particular reference to the so-called soluble and insoluble tannins, depends to a great extent on the fruit development stage. According to Del Bubba et al. (2009), in Rojo Brillante and Kaki Tipo astringent cultivars, a significant synthesis of polyphenols occurred in the early stages of fruit growth; thereafter a constant decrease in the water-methanol extractable polyphenols was observed during fruit development, followed by a sharp reduction of the water-methanol soluble fraction from commercial harvest stage to natural or artificial ripening, or after astringency removal; a similar trend was also observed by Taira (1996) in astringent Hiratanenashi. Conversely, in the nonastringent cultivar Jiro, soluble tannins spontaneously decreased to trace levels during on-tree fruit development (Taira, 1996).

Table 7
Total soluble phenolic concentration [TSP] of fresh (mg GAE/100 g FW) of astringent (normal character) and nonastringent (italic) persimmon fruits determinate with different extracting methods.

Cultivar	[TSP]	Extraction method	Reference
Hachiya	642	Replicated extractions with	Joslyn and Goldstein
Niu Nai	649	hot methanol and methanol/	(1964)
Fuyu	94	water 1/1 (*v*/*v*)	
Triumph	23	Extraction with methanol,	Gorinstein et al.
Fuyu	24	counter-extraction with light petroleum and final recovery with ethylacetate	(1994)[a]
Triumph	1.4	Extraction with hot ethanol/ water 95/5 (*v*/*v*)	Gorinstein et al. (1999)
Triumph	1.3	Extraction with hot ethanol/ water 95/5 (*v*/*v*)	Gorinstein et al. (2001) Park et al. (2006)
Triumph	1.5	Not reported	Jung et al. (2005)
Unknown	1480	Extraction with hot ethanol/	Katsube et al. (2004)
Unknown	34	water 7/3 (*v*/*v*)	
Hiratanenashi	9.9	Extraction with hot water,	Suzuki et al. (2005)[a]
Tonewase	8.0	counter-extraction with	
Ishibashi-wase	8.9	chloroform and final recovery	
Maekawa Jiro	2.2	with ethylacetate	
Matsumoto-wase-fuyu	1.7		
Various selections	314–846	Extraction with ethanol	Ercisli et al. (2007)[a]
Mopan	33.6	Ultrasonic assisted extraction with hot ethanol	Chen et al. (2008)[a]
Rojo Brillante	220[b] 31[c]	Replicated extractions with methanol/water 4/1 (*v*/*v*)	Del Bubba et al. (2009)
Kaki Tipo	262[b] 67[c]		
Fuyu	454	Methanol	Jang et al. (2010)[a]
Fuyu	427	Ethanol	
Fuyu	529	Acetone	
Fuyu	860	Water	

[a] Data expressed on dry matter in the original paper have been herein converted considering an average water content of 80% (Piretti 1991).
[b] Fruits treated with ethylene.
[c] Fruits treated with CO_2.

Among the 32 investigated varieties, soluble polyphenol concentrations ranged from 1.3 mg of gallic acid equivalent (GAE) per 100 g FW of the astringent cultivar Triumph (Gorinstein et al., 2001; Park et al., 2006) to 1480 mg GAE/100 g FW found in an unknown astringent genotype (Katsube et al., 2004). The wide range of variability observed can be explained on the basis of both cultivar and environment effects, even though the different applied extraction methods could also significantly influence the results. In fact, by considering the research of Jang et al. (2010), conducted on homogeneous samples of the nonastringent cultivar Fuyu, the lowest concentration of soluble polyphenols was 454 mg GAE/100 g FW when extracted with ethanol, about half the highest concentration determined with water extraction (860 mg GAE/100 g FW). Very similar values of soluble polyphenol contents were found in Triumph fruits harvested in different years (about 1.4 mg GAE/100 g FW) and analysed by the same procedure (Gorinstein et al., 2001, 1999; Park et al., 2006); conversely, when different extraction methods were applied, a much higher variation (1.4 to 24 mg GAE/100 g FW) was found in the same cultivar (Gorinstein et al., 2001, 1994; Park et al., 2006).

Besides these aspects, and taking into account fruit astringency, a common trend can be extrapolated from the results of studies on multiple cultivars; even if Gorinstein et al. (1994) found no differences between the nonastringent variety Fuyu (24 mg GAE/100 g FW) and the astringent Triumph (23 mg GAE/100 g FW), the content of soluble polyphenols is generally five to forty times higher in the astringent group (Joslyn & Goldstein, 1964; Katsube et al., 2004; Suzuki et al., 2005) than in nonastringent cultivars.

Among phenolic acids, gallic, chlorogenic, vallinic and protocatechuic acids (benzoic acids derivatives), caffeic, ferulic and p-coumaric acids (cynnamic acid derivatives) have been determined in fresh persimmon by different authors (Chen et al., 2008; Gorinstein et al., 2001, 1999, 1994; Jung et al., 2005; Suzuki et al., 2005). The most abundant was p-coumaric acid, the concentration of which was 8.5 mg/100 g FW in the nonastringent cultivar Fuyu, and ranged from 12.3 to 60.1 mg/100 g FW in the astringent variety Triumph (Gorinstein et al., 2001, 1994; Jung et al., 2005). For gallic acid a very wide variation was observed with concentrations in the range of 0.1–3.2 mg/100 g FW for Maekawa Jiro, Matsumoto-Wase-Fuyu and Fuyu, three nonastringent cultivars, and 0.2–21.2 mg/100 g FW in astringent cultivars. Surprisingly, the lowest and highest values were observed by the same author using the same fluorometric method in different samples of Triumph (Gorinstein et al., 1999; Jung et al., 2005). Minor variations were reported for protocatechuic acid, which was investigated in Triumph and Fuyu, determining values of 1.3–6.1 and 4.8 mg/100 g FW, respectively. Ferulic acid was only analysed in two Triumph samples, achieving similar results (7.9 and 9.9 mg/100 g FW) (Gorinstein et al., 2001; Jung et al., 2005).

Monomeric flavanols have been studied adopting HPLC methods by Suzuki et al. (2005) and Chen et al. (2008), showing the presence of catechin, epicatechin and epigallo catechin. The two nonastringent cultivars Maekawa Jiro (3.3 mg/100 g DW) and Matsumoto-Wase-Fuyu (2.1 mg/100 g DW) showed higher concentrations of catechin than those observed in the astringent varieties Hiratanenashi (1.7 mg/100 g DW), Tone-Wase (0.9 mg/100 g DW) and Ishibashi-Wase (1.1 mg/100 g DW). Conversely, higher concentrations of epigallo catechin (average 1.4 mg/100 g DW) and epicatechin (1.0 mg/100 g DW) were found in the same astringent cultivars (Chen et al. 2008; Suzuki et al., 2005).

7. Phytochemicals, anti-oxidant activity and human health benefits

In recent years, food and pharmaceutical studies have been focusing their attention on plant bio-active components considered beneficial for the treatment and prevention of human diseases, in the aim of scientifically demonstrating their real effectiveness (Beecher, 2004; Dillard & German, 2000). Epidemiological clinical studies should play a key role in helping to understand the phytochemical actions on human body and health; however, such studies are complex due to the multiplicity of nutraceutical compounds and the difficulty of the relative protocols and experimental designs. Alternative experiments consist of conventional chemical analyses of the matrices containing bio-active compounds (e.g. determination of the radical scavenging activity of fruits), studies on the effects of active principles on human cell cultures and the response of animals to artificial diets. Nevertheless, the correlation between the results obtained from these procedures and the findings of epidemiological studies has yet to be demonstrated, and this consideration is also applicable to the studies of phytochemicals in persimmons (George & Redpath, 2008).

The previous paragraphs address the quantitative and qualitative aspects of primary and secondary metabolites present in fresh persimmons. Irrespective of the variations deriving from analytical methods and genotype/environment effects, persimmons are particularly rich in vitamin C, carotenoids and polyphenols, all of which usually considered as powerful antioxidants that protect against free-radicals and prevent the risk of cardiovascular disease, diabetes and cancer (George & Redpath, 2008; Park et al., 2008; Piretti, 1991; Uchida et al., 1990).

The antioxidant activity of persimmons has been chemically assessed by determining the radical scavenging activity through the 1,1-diphenyl-2-pycrylhydrazyl (DPPH) method (Chen et al., 2008; Del Bubba et al., 2009; Gorinstein et al., 2011; Kondo et al., 2004), or with other methods, such as 2,2′-azino-bis (3-ethyl-benzoathiazoline-6-sulfonic acid) diammonium salt (ABTS), the measuring of ferric reducing antioxidant power (FRAP) and low-density-lipoprotein (LDL) oxidation (Gorinstein et al., 2011; Jang et al., 2010; Jung et al., 2005; Katsube et al., 2004; Park et al., 2006). Antioxidant activity against NO, β,β-carotene linoleate, β,β-carotene bleaching and various hydroxyl radical scavenging activity assays have also been adopted (Ercisli et al., 2007; Gu et al., 2008; Jung et al., 2005). Since sampling, extraction and determination methods exert a strong influence on the final analytical results, only data from samples analysed by the same author were compared. In this regard, Katsube et al. (2004) studied the antioxidant activity of one astringent and one nonastringent persimmon with LDL oxidation and DPPH assays. In both methods, the astringent persimmon showed values (111 ± 1 and 88 ± 4 μmol of epigallocatechningallate equivalent per g FW, respectively) about two order magnitudes higher than those of the nonastringent persimmon, of silvervine and fig samples, and about five or six times higher than the values observed in mulberries and blueberries. Garcia-Alonso, Pascual-Teresa, Santos-Buelga, and Rivas-Gonzalo (2004) using the ABTS radical method, determined a high aqueous-phase antioxidant activity in fruits of the astringent cv Rojo Brillante (400 μmol Trolox equivalent per g FW), which is twofold higher than that of blueberries and blackberries, both considered excellent sources of antioxidant compounds. Chen et al. (2008) showed that Mopan, a Chinese astringent persimmon, had similar DPPH and ABTS radical scavenging activity values (about 23 μmol Trolox equivalent per g FW), which were two, five and ten times higher than those found in grapes, apples and tomatoes respectively. Furthermore, Gorinstein et al. (2011), using FRAP, ABTS and DPPH methods evidenced a slightly higher antioxidant potential in Fuyu than in Jiro. Kondo et al. (2004) discovered that the IC_{50} value of DPPH radical scavenging activity in the astringent cultivar Saijo was about tenfold lower than that of the nonastringent cultivar Fuyu at commercial harvest; furthermore, the antioxidant activity of Saijo decreased drastically after astringency removal. Similarly, ethylene and CO_2 treatments of the astringent cultivars Kaki Tipo and Rojo Brillante caused a decrease of about 20–30% in the radical scavenging activity determined in fruits at harvesting time (Del Bubba et al., 2009). Conversely, drying of Triumph fruits seemed not to exert any influence on total radical scavenging activity (Jung et al., 2005; Park et al., 2006).

Irrespective of the different levels of assimilability of different molecular weight tannins, as indicated in paragraph 6.2, Gu et al. (2008) demonstrated that high molecular weight tannins in an astringent Chinese persimmon were more potent antioxidants than those with low molecular weight found in the same fruit and in grape seeds. Accordingly, Li et al. (2010) showed that high molecular weight tannin is the main antioxidant agent in persimmon. Santos-Buelga and Scalbert (2000) suggested that proanthocyanidins found in persimmon may reduce blood pressure and platelet aggregation and therefore exert a beneficial effect on coronary diseases. Moreover, diets supplemented with whole persimmon fruits have been found to improve lipid metabolism in rats by reducing LDL oxidation, which is a key step in the formation of an atherosclerotic lesion, regardless of the cultivar tested (Gorinstein, Bartnikowska, Kulasek, Zemser, & Trakhtenberg, 1998). Gorinstein et al. (2000) showed that Triumph, Jiro and Fuyu fruits lowered total plasma cholesterol by about 20%, LDL by 31% and triglycerides by 19% in rats fed cholesterol. This finding was mainly associated with the protective effects of the polyphenols of the fruit; similar conclusions were confirmed by Park et al. (2008). According to Gorinstein et al. (2011), when the diet of rats fed with cholesterol was supplemented with lyophilized Fuyu and Jiro fruits, the increase in plasma lipid levels and the decrease of plasma antioxidant activity caused by cholesterol intake were hindered; moreover, a significant decrease of atherosclerotic aorta lesions was observed. On the other hand, Lee, Cho, Tanaka, and Yokozawa (2007) observed in rabbits that polymers and oligomers from proanthocyanidins of persimmon peel could play a role as antidiabetic agents, by inhibiting either α-glucosidae or α-amylase activity, enzymes which increase glucose absorption in the gut. The protective role of proanthocyanidin from oxidative stress induced by diabetes was supported by the results found by Lee, Kim, Cho, and Yokozawa (2008) in their study on streptozotocin-induced diabetic rats. Persimmon peel was reported as a valuable source of antioxidants in the diabetic condition, since it reduced oxidative stress induced by hyperglycaemia (Yokozawa, Kim, Lee, & Nonaka, 2007).

Persimmons are suggested to exert a chemoprotective activity against different cancerous cells, such as oral carcinoma cells (Kawase et al., 2003), human lymphoid leukaemia cells (Achiwa, Hibasami, Katsuzaki, Imai, & Komiya, 1997, 1996, Achiwa, Hibasami, Katsuzaki, Kada, & Komiya 1996) and precancerous colon polyps in women (Takayuki 2005). Catechins and lycopene, both present in quite high amounts in persimmons, have been found to be active in both *in vitro* and *in vivo* tests against different kinds of cancers (Dorgan, Sowell, & Swanson 1998; Giovannucci 2002) and show a multi-drug resistant modulating inhibiting activity (Kawase et al. 2003). Recently, Jang et al. (2010) analysed the protective effect of nonastringent Fuyu persimmons on genotoxic oxidative DNA damage by H_2O_2 in human leukocytes; in this case the protective action was exerted by phenols, β-carotene and ascorbic acid.

Experiments have shown that persimmon tannins seem to reduce the incidence of brain haemorrhage and infarction in stroke-prone hypertensive rats, by inhibiting lipid peroxidation of their brain in a concentration-linked way (Uchida et al. 1990, 1995); furthermore, catechins in crude persimmon tannins have prevented episodes of epileptic symptoms in rat brains (Mori, Yokoi, Noda, & Willmore, 2004).

Other beneficial effects of persimmons are those associated with the reduction of various intoxications. Kim et al. (2001) reported that dried persimmon were effective in reducing alcohol concentration in human blood; similarly, the ingestion of dried persimmons before

drinking alcohol reduced the blood alcohol content by 40% and that of acetaldehyde by 30%, (George & Redpath 2008). Persimmon extracts also have detoxificant effects on snake venom, as well as other toxic substances produced by microorganisms (Okonogi & Hattori 1978; Okonogi, Hattori, Ogiso, & Mitsui, 1979).

8. Conclusions

In this review the role of persimmons as a source of primary and secondary metabolites (sugars, vitamin C, carotenoids and polyphenols), all of them important in human diet, was pinpointed. Astringent cultivars supply higher amounts of sugars than nonastringent ones, while vitamin C is present at higher concentrations in nonastringent varieties. However, literature data are affected by a number of variability sources, such as ripeness stage and analytical methods that should be much more controlled and uniformed by researchers in order to obtain more reliable and comparable results.

According to these considerations, new researches should be conducted using standardised strategies of sampling that can contribute to obtain more informative data from both pomological and nutritional point of views. For instance, ripening parameters of the analysed fruit, such as colour index and firmness, notwithstanding the influence exerted on them by cultivar and environmental factors should be always included in the experimental design. For nutritional purposes, fruits should be analysed at the "ready to eat" ripening stage, following the general consumer preference; additionally, information regarding environmental and postharvest factors, that can strongly affect ripening time and chemical composition of fruits, are often omitted in the papers and should be taken into major account in future studies.

From an analytical point of view, the determination of primary and secondary metabolites in complex matrixes such as persimmon needs the use of reliable methods and the recovery of analytes and the possible occurrence of degradation phenomena should always be verified by analysing certified quality control samples and/or by adding internal standards to real samples. Among the selected metabolites herein reviewed, polyphenols represent the more complex compound class, the composition of which is still far to be fully elucidated, especially for the condensed proanthocyanidins. More efforts should be done for accurately investigate the extent of polymerization and the structure of individual proanthocyanidins, since the structural characteristics of these compounds largely influence their health protective attributes (Yanagida et al., 2000). To achieve this goal the RP-HPLC capacities are probably not completely exploited, since, in contrast to the widely reported scarce separation efficiency of this technique towards high molecular weight proanthocyanidins, oligomers up to dodecamers have been recently identified in apple skin, quantifying them up to hexamers (Lamperi et al., 2008).

Finally, to better understand the effect of persimmon metabolites on human health, *in vivo* studies on animals and, when possible, on humans should be preferred to the ones *in vitro*, since the effect of a certain compound on human cells and tissues measured by in vitro tests could not represent a real evaluation of the in vivo effect.

References

Achiwa, Y., Hibasami, H., Katsuzaki, H., Imai, K., & Komiya, T. (1997). Inhibitory effects of persimmon (*Diospyros kaki*) extract and related polyphenol compounds on growth of human lymphoid leukemia cells. *Bioscience Biotechnology Biochemistry*, *61*, 1099−1101.

Achiwa, Y., Hibasami, H., Katsuzaki, H., Kada, H., & Komiya, T. (1996). Inhibitory effects of persimmon (*Diospyros kaki*) and constituents on the mutagenicity of C-nitro and C-nitroso compounds formed by reaction of sorbic acid with sodium nitrite. *Journal of the Japanese Society for Food Science and Technology*, *43*(5), 493−501.

Akagi, T., Suzuki, Y., Ikegami, A., Kamitakahara, H., Takano, T., Nakatsubo, F., et al. (2010). Condensed tannin composition analysis in persimmon (*Diospyros kaki* Thunb.) fruit by acid catalysis in the presence of excess phloroglucinol. *Journal of the Japanese Society for Holticultural Science*, *79*, 275−281.

AOAC (1996a). AOAC Official Method 967.21. Ascorbic acid in vitamin preparations and juices. 2,6-dichloroindophenol titrimetric method. *Official methods of Analysis of AOAC International* (pp. 16−17). Arlington, USA: Association of Analytical Communities.

AOAC (1996b). AOAC Official Method 967.22. Vitamin C (total) in vitamin preparations. Microfluorometric method. *Official methods of Analysis of AOAC International* (pp. 17−18). Arlington, USA: Association of Analytical Communities.

Ball, G. M. F. (2000). The fat-soluble vitamins. In L. M. L. Nollet (Ed.), *Food Analysis by HPLC* (pp. 321−402). New York: Marcel Dekker, Inc.

Beecher, G. R. (2004). Proanthocyanidins: Biological activities associated with human health.*Pharmaceutical Biology*, *42*, 2−20 Supplement.

Ben-Arie, R., & Sonego, L. (1993). Temperature affects astringency removal and recurrence in persimmon. *Journal of Food Science*, *58*, 1397−1400.

Besada, C., Arnal, L., & Salvador, A. (2009). Physiological changes of 'Rojo Brillante' persimmon during commercial maturity. *Acta Horticulturae*, *833*, 257−262.

Bibi, N., Khattak, A. B., & Mehmood, Z. (2007). Quality improvement and shelf life extension of persimmon fruit (*Diospyros kaki*). *Journal of Food Engineering*, *79*, 1359−1363.

Breithaupt, D. E., & Bamedi, A. (2001). Carotenoids esters in vegetables and fruits: A screening with emphasis on β-cryptoxanthin esters. *Journal of Agricultural Food Chemistry*, *49*, 2064−2070.

Breithaupt, D. E., Weller, P., Wolters, M., & Hahn, A. (2003). Plasma response to a single dose of dietary β-cryptoxanthin esters from papaya (*Carica papaya* L) or non-esterified β-cryptoxanthin in adult human subjects: A comparative study. *British Journal of Nutrition*, *90*, 795−801.

Brossard, J., & Mackinney, G. (1963). The carotenoid of *Diospyros kaki* (Japanese persimmon). *Journal of Agricultural and Food Chemistry*, *11*, 501−503.

Candir, E. E., Ozdemir, A. E., Kaplankiran, M., & Toplu, C. (2009). Physico-chemical changes during growth of persimmon fruits in the east Mediterranean climate region. *Scientia Horticulturae*, *121*, 42−48.

Celik, A., & Ercisli, S. (2008). Persimmon cv. Hachiya (*Diospyros kaki* Thunb.) fruit: Some physical, chemical and nutritional properties. *International of Food Science and Nutrition*, *59*, 599−606.

Chen, X. N., Fan, J. F., Yue, X., Wu, X. R., & Li, L. T. (2008). Radical scavenging activity and phenolic compounds in persimmon (*Dyospiros kaki* L. cv. Mopan). *Journal of Food Science*, *73*, 24−28.

Chen, J., Ni, H., Sun, J., & Cheng, D. (2003). Effects of major secondary chemicals of wheat plants on enzyme activity in *Sitobion avenae*.*Kunchong Xuebao*, *46*, 144−149 (In Chinese).

CUDRINL (2003). Committee on use of dietary reference intakes in nutrition labeling. www.nap.edu.

Daood, H. G., Biacs, P., Czinkotai, B., & Hoschke, A. (1992). Chromatographic investigation of carotenoids, sugars and organic acids from *Diospyros kaki* fruits. *Food Chemistry*, *45*, 151−155.

De Ancos, B., Gonzales, E., & Cano, P. (2000). Effect of high-pressure treatment on the carotenoid composition and the radical scavenging activity of persimmon fruit purees. *Journal of Agricultural and Food Chemistry*, *48*, 3542−3548.

De Eds, F. (1959). Physiological effects and metabolic fate of flavonoids. In J. W. Fairbairn (Ed.), *The pharmacology of plant phenolics* (pp. 91−102). Academic Press: London.

Del Bubba, M., Giordani, E., Cincinelli, A., Checchini, L., & Galvan, P. (2009). Nutraceutical properties and sugar contents in astringent persimmons durino on-tree growth and ripening and in response to different postharvest treatments. *Journal of Food Composition and Analysis*, *22*, 668−677.

Déprez, S., Brezillon, C., Rabot, S., Philippe, C., Mila, I., Lapierre, C., et al. (2000). Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *The Journal of Nutrition*, *130*, 2733−2738.

Déprez, S., Mila, C., Huneau, J. F., Tome, D., & Scalbert, A. (2001). Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxidants & redox signaling*, *3*(6), 957−967.

Dillard, C. J., & German, B. (2000). Review. Phytochemicals: Nutraceuticals and human health. *Journal of Science Food Agriculture*, *80*, 1744−1756.

Dorgan, J. F., Sowell, A., & Swanson, S. A. (1998). Relationship of serum carotenoids, retinol, alpha-tocopherol and selenium with breast cancer risk: Results from a prospective study in Columbia, Missouri (United States). *Cancer Causes Control*, *9*(1), 89-87.

Ercisli, S., Akbulut, M., Ozdemir, O., Sengul, M., & Orhane, E. (2007). Phenolic and antioxidant diversity among persimmon (*Diospyrus kaki* L.) genotypes in Turkey. *International Journal of Food Sciences and Nutrition*, *59*, 477−482.

FAOSTAT (2010). http://faostat.fao.org.

Forbus, W. R., Jr., Payne, J. A., & Senter, S. D. (1991). Nondestructive evaluation of Japanese persimmon maturity by delayed light emission. *Journal of Food Science*, *56*, 985−988.

Garcia-Alonso, M., Pascual-Teresa, S., Santos-Buelga, C., & Rivas-Gonzalo, J. (2004). Evaluation of the antioxidant properties of fruits. *Food chemistry*, *84*, 13−18.

George, A., & Redpath, S. (2008). Health and medicinal benefits of persimmon fruit: A review. *Advances in Horticultural Science*, *22*(4), 244−249.

Giovannucci, E. (2002). A review of the epidemiological studies of tomatoes, lycopene and prostate cancer. *Experimental Biolology and Medicine*, *227*(10), 825−859.

Golubev, V. N., Kostinskaya, L. I., & Khalilov, M. A. (1987). Carbohydrates of *Diospyros kaki*. In B. J. Hazzard (Ed.), *Chemistry of Natural Compounds*, *Vol. 23*. (pp. 115−116) New York: Plenum Publishing Corporation.

Gorinstein, S., Bartnikowska, E., Kulasek, G., Zemser, M., & Trakhtenberg, S. (1998). Dietary persimmon improves lipid metabolism in rats fed diets containing cholesterol. *The Journal of Nutrition*, *128*, 2023−2027.

Gorinstein, S., Kulasek, G., Bartnikowska, E., Leontowicz, M., Zemser, M., Morawiec, M., et al. (2000). The effects of diets, supplemented with either whole persimmon or phenol-free persimmon, on rats fed cholesterol. *Food Chemistry*, *70*, 303−308.

Gorinstein, S., Leontowicz, H., Leontowicz, M., Jesion, I., Namiesnik, J., Drzewiecki, J., et al. (2011). Influence of two cultivars of persimmon on artherosckerosis indices in fat rats fed cholesterol-containing diets: Investigation in vitro and in vivo. *The Journal of Nutrition*, doi:10.1016/j.nut2010.08.015.

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M., et al. (2001). Comparative content of dietary fiber, total phenolics and minerals in persimmon and apples. *Journal of Agricultural and Food Chemistry*, *49*, 952—957.

Gorinstein, S., Zemser, M., Haruenkit, R., Chuthakorn, R., Grauer, F., Martin-Belloso, O., et al. (1999). Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmons. *Journal of Nutritional Biochemistry*, *10*, 367—371.

Gorinstein, S., Zemser, M., Weisz, M., Halevy, S., Deutsch, J., Tilis, K., et al. (1994). Fluorometric analysis of phenolics in persimmons. *Bioscience Biotechnology and Biochemistry*, *58*, 1087—1092.

Gregory, J. F., III (1996). Vitamins. In O. R. Fennema (Ed.), *Food Chemistry* (pp. 531—616). New York: Marcel Dekker, Inc.

Gu, H. F., Li, C. M., Xu, Y., Hu, W., Chen, M., & Wan, Q. (2008). Structural features and antioxidant activity of tannin from persimmon pulp. *Food Research International*, *41*, 208—217.

Hirai, S., & Kondo, T. (2002). Problem in the saccharides analysis of the fruits and the countermeasure.*Iida Joshi Tanki Daigadu Kiyo*, *19*, 59—68 (In Japanese).

Hirai, S., Rokuhara, S., & Shimizu, S. (1986). Changes in invertase activity of Japanese persimmon fruit during maturation, storage and processing.*Nippon Shokuhin Kogyo Gakkaishi*, *33*, 369—374 (In Japanese).

Hirai, S., & Yamazaki, K. (1984). Studies on sugars components of sweet and astringent persimmons by gas chromatography.*Nippon Shokuhin Kogyo Gakkaishi*, *31*, 24 (In Japanese).

Hirano, K., Yonemori, K., & Sugiura, A. (1995). Involvement of sugar metabolism in persimmon growth inhibition by calyx lobe removal. *Journal of the American Society for Horticultural Science*, *120*(1), 75—77.

Homnava, A., Payne, J., Koehler, P., & Eitenmiller, R. (1990). Provitamin A (alpha-carotene, beta-carotene and beta-cryptoxanthin) and ascorbic acid content of Japanese and American persimmons. *Journal of Food Quality*, *13*, 85—95.

Homnava, A., Payne, J., Koehler, P., & Eitenmiller, R. (1991). Characterization of changes during ripening of oriental persimmon. *Journal of Food Quality*, *14*, 425—434.

Itoo, S. (1980). Persimmon. In S. Nagy, & P. E. Shaw (Eds.), *Tropical and Subtropical Fruits, Composition, Properties and Uses* (pp. 442—468). Westport, CT: Avi Publishing Inc.

Itoo, S. (1986). Persimmon. In S. P. Monselise (Ed.), *Handbook of fruit set development* (pp. 355—370). Boca Raton, FL, USA: CRC Press.

Ittah, Y. (1993). Sugar content changes in persimmon fruits (*Diospyros kaki* L.) during artificial ripening with CO_2: A possible connection to deastringency mechanism. *Food Chemistry*, *48*, 25—29.

Jang, I. C., Jo, E. K., Bae, M. S., Lee, H. J., Jeon, G. I., Park, E., et al. (2010). Antioxidant and antigenotoxic activities of different parts of persimmon (*Diospyros kaki* cv. Fuyu) fruit. *Journal of Medicinal Plants Research*, *4*(2), 155—160.

Jimenez-Ramnsey, L. M., Rogler, J. C., Housley, T. L., Butler, L. G., & Elkin, R. G. (1994). Absorption and distribution of 14 C-labeled condensed tannins and related sorghum phenolics in chickens. *Journal of Agriculture Food Chemistry*, *42*, 963—967.

Joslyn, M. A., & Goldstein, J. L. (1964). Change in phenolic content in persimmon during ripening and processing. *Journal of Agricultural and Food Chemistry*, *12*, 511—520.

Jung, S., Park, Y., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., et al. (2005). Some essential phytochemicals and the antioxidant potential in fresh and dried persimmon. *International Journal of Food Sciences and Nutrition*, *56*(2), 105—113.

Katsube, T., Tabata, H., Ohta, Y., Yamasaki, Y., Anuurad, E., Shiwaku, K., et al. (2004). Screening of antioxidant activity in edible plant productes: comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay, and Folin-Ciocalteu assay. *Journal of Agricultural Food Chemistry*, *52*, 2391—2396.

Kawase, M., Motohashi, N., Satoh, K., Sakagami, H., Nakashima, H., Tani, S., et al. (2003). Biological activity of persimmon (*Diospyros kaki*) peel extracts. *Phytotherapy Research*, *17*, 405—500.

Kim, S., Jeong, S., Kim, S., Jeon, K., Park, E., Park, H., et al. (2001). Effect of heat treatment on the antioxidative and antigenotoxic activity of extracts from persimmon (*Diospyros kaki* L.) peel. *Bioscience, Biotechnology and Biochemistry*, *70*(49), 999—1002.

Kimura, M., Rodriguez-Amaya, D. B., & Godoy, H. T. (1990). Assessment of the saponification step in quantitative determination of carotenoids and provitamins A. *Food Chemistry*, *35*, 187—195.

Kitahara, M., Takeuchi, Y., & Matsui, M. (1951). Biochemical studies on persimmon fruit. Part I. Comparison of some properties between bagged and unbagged fruit. *Journal of Japanese Society for Food Nutrition*, *4*, 138—141.

Kondo, S., Yoshikawa, H., & Katayama, R. (2004). Antioxidant activity in astringent and non-astringent persimmons. *Journal of Horticultural Science & Biotechnology*, *79*, 390—394.

Lamperi, L., Chiuminatto, U., Cincinelli, A., Galvan, P., Giordani, E., Lepri, L., et al. (2008). Polyphenol levels and free radical scavenging activities of four apple cultivars from integrated and organic farming in different italian areas. *Journal of Agricultural Food Chemistry*, *56*, 6536—6546.

Lee, Y., Cho, E., Tanaka, T., & Yokozawa, T. (2007). Inhibitory activities of proanthocyanidins from persimmon against oxidative stress and digestive enzymes related to diabetes. *Journal of Nutritional Science and Vitaminology*, *53*, 287—292.

Lee, Y., Kim, Y., Cho, E., & Yokozawa, T. (2008). Ameliorative effects of proanthocyanidin on oxidative stress and inflammation in streptozotocin-induced diabetic rats. *Journal of Agricultural and Food Chemistry*, *55*, 9395—9400.

Li, C., Leverence, R., Trombley, J. D., Xu, S., Yang, J., Tian, Y., et al. (2010). High molecular weight persimmon (*Diospyros kaki* L.) proanthocyanidin: A highly galloylated, A-linked tannin with an unusual flavonol terminal unit, myricetin. *Journal of Agricultural Chemistry*, *58*, 9033—9042.

Luo, Z., & Wang, R. (2008). Persimmon in China: Domestication and traditional utilizations of genetic resources. *Advances in Horticultural Science*, *22*(4), 239—243.

Mandalari, G., Tomaino, A., Rich, G. T., Lo Curto, R., Arcoraci, T., Martorana, M., et al. (2010). Polyphenol and nutrient release from skin almonds during simulated human digestion. *Food Chemistry*, *122*, 1083—1088.

Mangarova, M. (2005). Chemical contents of persimmon fruits (*Diospyros kaki*), grown in the conditions of the southern black sea region.*Plant Science*, *42*, 443—445 (In Bulgarian).

Medeiros, P. M., & Simoneit, B. R. T. (2007). Analysis of sugars in environmental samples by gas chromatography-mass spectrometry. *Journal of Chromatography A*, *1141*, 271—278.

Mori, A., Yokoi, I., Noda, Y., & Willmore, L. (2004). Natural antioxidants may prevent posttraumatic epilepsy: A proposal based on experimental animal studies. *Acta Medicine Okayama*, *58*(3), 11—118.

Moriel, E. Z., Ayalon, A., Eid, A., Rachimilewitz, D., Krausz, M. M., & Durst, A. L. (1983). An unusually high incidence of gastrointestinal obstruction by persimmon bezoars in Israeli patients after ulcer surgery. *Gastroenterology*, *84*, 752—755.

Nakatsubo, F., Enokita, K., Murakami, K., Yonemori, K., Sugiura, A., Utsunomiya, N., et al. (2002). Chemical structures of the condensed tannins in the fruits of *Diospyros* species. *Journal of Wood Science*, *48*, 414—418.

Okonogi, T., & Hattori, Z. (1978). Detoxifying effect of persimmon tannin against snake venom. *Snake*, *9*, 91—96.

Okonogi, T., Hattori, Z., Ogiso, A., & Mitsui, S. (1979). Detoxification by persimmon tannin of snake venoms and bacterial toxins. *Toxicon*, *17*(5), 524—527.

Oliveira, F. B., Vaitsman, D. S., Vidal, D. P., Meneguelli, N. A., & Matta, A. P. (1997). Preliminary studies on the chemical composition of *Dyospyros kaki*, L. from the Rio de Janeiro montains. *Anais da Associação Brasileira de Química*, *46*(1), 13—15.

Osawa, R., Kuroiso, K., Goto, S., & Shimizu, A. (2000). Isolation of tannin-degrading lactobacilli from humans and fermented foods. *Applied and Environmental Biology*, *66*, 3093—3097.

Öz, A. T., Özelkök, I. S., & Albayrak, B. (2005). Sugar and tannin content changes in persimmon fruit during artificial ripening with dry ice. *Acta Horticulturae*, *682*, 987—991.

Park, Y. S., Jung, S. T., Kang, S. G., Delgrado-Licon, E., Ayala, A. L. M., Tapia, M. S., et al. (2006). Drying of persimmons (*Dyospiros kaki L.*) and the following changes in the studied bioactive compounds and the total radical scavenging activities. *LWT-Food Science and Technology*, *39*, 748—755.

Park, Y. S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Jesion, I., & Gorinstein, S. (2008). Nutraceutical value of persimmon (Diospyros kaki Thunb.) and it sinfluence on some indices of atherosclerosis in an experiment on rats fed cholesterol-containing diet. *Advanced in Horticultural Science*, *22*(4), 250—254.

Philip, T., & Chen, T. S. (1988). Quantitative analyses of major carotenoid fatty acid esters in fruits by liquid chromatograph: Persimmon and papaya. *Journal of Food Science*, *53*, 1720—1722.

Piretti, M. V. (1991). Polyphenol constituents of the Dyospyros kaki fruit: A review. *Fitoterapia*, *62*, 3—13.

Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. *Pharmacological Research*, *55*, 207—216.

Rios, L. Y., Bennett, R. N., Lazarus, S. A., Remesy, C., Scalbert, A., & Williamson, G. (2002). Cocoa procyanidins are stable during gastric transit in humans. *American Journal Clinic Nutrition*, *76*, 1106—1110.

Rios, L. Y., Gonthier, M. P., Remesy, C., Mila, I., Lapierre, C., Lazarus, S. A., et al. (2003). Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *American Journal Clinic Nutrition*, *77*, 912—918.

Romero Rodriguez, M. A., Vazquez Oderiz, M. L., Lopez Hernandez, J., & Simal Lozano, J. (1992). Determination of vitamin C and organic acids in various fruits by HPLC. *Journal of Chromatographic Science*, *30*(11), 433—437.

Sakakibara, H., Honda, Y., Nakagawa, S., Assida, H., & Kanazawa, K. (2003). Simultaneous of all polyphenols in vegetable, fruits and teas. *Journal of Agricultural Food Chemistry*, *51*, 571—581.

Salunkhe, D. K., Chavan, J. K., & Kadam, S. S. (1989). *Dietary tannins: Consequences and remedies.* Boca Rato FL, USA: CRC Press, Inc.

Santos-Buelga, C., & Scalbert, A. (2000). Proanthocyanidins and tannin-like compounds-nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture*, *80*, 1094—1117.

Scott, W. C., Morgan, D. A., & Veldhuis, M. K. (1961). The determination of soluble solids in citrus juices. I. The effect of non-sucrose components on refractometer value. *Food Technology*, *14*, 423—428.

Senter, S. D., Chapman, G. W., Forbus, W. R., Payne, J. R., & Payne, J. A. (1991). Sugar and non volatile acid composition of persimmons during maturation. *Journal of Food Science*, *56*, 989—991.

Sestari, I., Edagi, F. K., Terra, F. A. M., Chiou, D. G., Kluger, R. A., & Antoniolli, L. R. (2009). Influence or ripening stage and astringency removal on quality of cold stored 'Rama Forte' persimmon. *Acta Horticulturae*, *833*, 263—268.

Seung, S. H., Hyun, S. L., Min, K. J., Seong, W. J., Chang, M. C., Sung, K. K., et al. (2007). Acute intestinal obstruction caused by a persimmon phytobezoar after dissolution therapy with Coca-Cola. *Korean Journal of Internal Medicine*, *22*(4), 300—303.

Souci, S. W., Fachmann, W., & Kraut, H. (1981). *Food Composition and Nutrition Tables 1981/82.* Stuttgart: Wissenschaftliche Verlagsgesellschaft MBH.

Sugiura, A. (1984). The origin of persimmon and its cultivar differentiation. In Y. Ohmura, T. Yomogihara, H. Ito, T. Kinoshita, & J. Kadota (Eds.), *Recent Advances in Plant Breeding, Vol. 25*. (pp. 30—37)Tokyo, Japan: Keigaku Publishers (In Japanese).

Sugiura, A. (1997). Proceedings of the First International Persimmon Symposium. Keynote address. *Acta Horticulturae*, *436*, 15—19.

Sugiura, A., Kataoka, I., & Tomana, T. (1983). Use of refractometer to determine soluble solids of astringent fruits of Japanese persimmon (*Diospyros kaki* L.). *Journal of Horticultural Science*, *58*(2), 241−246.

Sugiura, A., & Tomana, T. (1983). Relationships of ethanol production by seeds of different types of Japanese persimmons and their tannin content. *HortScience*, *18*, 319−321.

Sugiura, A., Yonemori, K., Harada, H., & Tomama, T. (1979). Changes of ethanol and acetaldehyde contents in Japanese persimmon fruits and their relation to natural deastringency.*Studies from Institute of Horticulture of Kyoto University*, *9*, 41−47 (In Japanese).

Suzuki, T., Someya, S., Hu, F., & Tanokura, M. (2005). Rapid communication. Comparative study of catechin compositions in five Japanese persimmons (*Diospyros kaki*). *Food Chemistry*, *93*, 149−152.

Taira, S. (1996). Astringency in persimmon. In H. -F. Linskens, & J. F. Jackson (Eds.), *Modern Method of Plant Analysis. Fruit Analysis*, *Vol. 18*. (pp. 97−109) Berlin: Springer-Verlag.

Taira, S., Ono, M., & Matsumoto, N. (1997). Reduction of persimmon astringency by complex formation between pectin and tannins. *Postharvest Biology and Technology*, *12*, 265−271.

Taiz, L., & Zeiger, E. (2002). Secondary metabolites and plant defense. In L. Taiz, & E. Zeiger (Eds.), *Plant Physiology* (pp. 283−308). Sunderland MA: Associates Inc. Publishers.

Takayuki, O. (2005). Persimmon: Your healthy autumn treats.*Asahikawa Information*, *108*, 1−2 (In Japanese).

Tarascou, I., Souquet, J. M., Mazauric, J. P., Carrillo, S., Coq, S., Canon, F., et al. (2010). Review. The hidden face of food phenolic composition. *Archives of Biochemistry and Biophysics*, *501*, 16−22.

Technical University of Denmark (). (The official Danish Food Composition Database). www.foodcomp.dk. Retrieved 1 July, 2010 from.

Terrill, T. H., Waghorn, G. C., Woolley, D. J., McNabb, W. C., & Barry, T. N. (1994). Assay and digestion of ^{14}C-labeled condensed tannins in the gastrointestinal tract of sheep. *British Journal of Nutrition*, *72*, 467−477.

Testoni, A. (2002). Post-harvest and processing of persimmon fruit. In E. Bellini, & E. Giordani (Eds.), *First Mediterranean symposium on persimmon. Options Mediterraneennes, Series A*, *51*. (pp. 53−70).

Uchida, S., Ohta, H., Niwa, M., Mori, A., Nonaka, G., Nishioka, I., et al. (1990). Prolongation of life span of stroke-prone spontaneously hypertensive rats (SHRSP) ingesting persimmon tannin. *Chemical & Pharmaceutical Bulletin*, *38*, 1049−1052.

Uchida, S., Ozaki, M., Akashi, T., Yamashita, K., Niwa, M., & Taniyama, K. (1995). Effects of (-)-epigallocatechin-3-O-gallate (green tea tannin) on the life span of stroke-prone spontaneously hypertensive rats. *Clinical and Experimental Pharmacology and Physiology Supplement*, *22*(10), S302−S303.

USDA (). (Nutritional Nutrient Database for Standard Reference). http://www.nal.usda.gov. Retrieved 1 July, 2010 from.

Veberic, R., Juhar, J., Mikulic-Petkovsek, M., Stampar, F., & Schmitzer, V. (2010). Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (*Diospyros kaki* L.). *Food Chemistry*, *119*, 477−483.

Vidrich, V., Cecconi, C. A., Fusi, P., Querci, E., & Fusi, P. (1978). Study of ascorbic acid content in kaki leaves and fruits. *Agricoltura Italiana*, *4–5*, 207−217.

Von Elbe, J. H., & Schwartz, S. J. (1996). Colorants. In O. R. Fennema (Ed.), *Food Chemistry* (pp. 651−722). New York: Marcel Dekker, Inc.

Wright, K., & Kader, A. A. (1997). Effect of slicing and controlled-atmosphere storage on the ascorbate content and quality of strawberries and persimmons. *Postharvest Biology and Technology*, *10*, 39−48.

Wu, P. W., & Hwang, L. S. (2002). Determination of soluble persimmon tannin by high performance gel permeation chromatography. *Food Research International*, *35*, 793−800.

Yanagida, A., Kanda, T., Takahashi, T., Kamimura, A., Hamazono, T., & Honda, S. (2000). Fractionation of apple procyanidins according to their degree of polymerization by normal-phase high-performance liquid chromatography. *Journal of Chromatography A*, *890*, 251−259.

Yokozawa, T., Kim, Y. A., Lee, Y. A., & Nonaka, G. (2007). Protective effect of persimmon peel polyphenol against high glucose-induced oxidative stress in LLC-PK$_1$ cells. *Food and Chemical Toxicology*, *45*(10), 1979−1987.

Yonemori, K., & Matsushima, J. (1987a). Morphological characteristics of tannin cells in Japanese persimmon fruit. *Journal of the American Society for Horticultural Science*, *112*, 812−817.

Yonemori, K., & Matsushima, J. (1987b). Changes in tannin cell morphology with growth and development of Japanese persimmon fruit. *Journal of the American Society for Horticultural Science*, *112*, 818−821.

Yonemori, K., Matsushima, J., & Sugiura, A. (1983). Differences in tannin of non-astringent and astringent type fruits of Japanese persimmon (*Diospyros kaki* Thunb.).*Journal of the Japanese Society for Horticultural Science*, *52*, 135−144 (In Japanese).

Yonemori, K., Sugiura, A., Tanaka, K., & Kameda, K. (1993). Floral ontogeny and sex determination in monoecious-type persimmons. *Journal of the American Society for Horticultural Science*, *118*, 293−297.

Yonemori, K., Yamada, M., & Sugiura, A. (2000). Persimmon genetics and breeding. *Plant Breeding Reviews*, *19*, 191−225.

Zapata, S., & Dufour, J. P. (1992). Ascorbic, dehydroascorbic and isoascorbic acid simultaneous determinations by reverse phase ion interaction HPLC. *Journal of Food Science*, *57*, 506−511.

Zheng, G. H., & Sugiura, A. (1990). Changes in sugar composition in relation to invertase activity in the growth and ripening of persimmon (*Diospyros kaki*) fruits.*Journal of the Japanese Society for Horticultural Science*, *59*, 281−287 (In Japanese).