
Recent Advances Regarding the Phytochemical and Therapeutic Benefits of *Diospyros kaki* Fruit

Ana F. Vinha^{1,2*}, Marta O. Soares³ and Marisa Machado³

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ABSTRACT

Lately, several studies have demonstrated the health benefits associated with natural compounds consumption. Fruit phenolics, lycopene and ascorbic acid, have strong antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic activities. In the present study parameters related to fruit quality and climacteric conditions, such as color, moisture, pH, water activity, total phenolic compounds, ascorbic acid and lycopene content were analyzed. *Diospyros kaki* cv. chocolate fruits were extracted with different solvents, such as methanol, ethanol and water. Total polyphenolics of each extract determined by Folin-Ciocalteu method were found to be higher in methanolic extract and lower in ethanol considering *Diospyros* fruits with peel. Significant variations were found in the levels of these parameters according to different geographic regions. Results support the importance of *Diospyros kaki* cv. chocolate fruits, Portuguese cultivar, as a functional food with high antioxidant potential that may have beneficial effects against oxidative human damage.

Keywords: Portuguese *Diospyros kaki*; chemical characterization; total polyphenolics; lycopene; geographic origin; fruit peel removal.

1. INTRODUCTION

Fruits are widely recommended for their health-promoting properties [1]. They seem to be capable of delivering health benefits besides fulfilling physiological needs [2]. In fact, fruits have historically held a place in dietary guidance because of their concentrations of vitamins, especially vitamins C, complex B, A and E; minerals, especially electrolytes; and phytochemicals, especially antioxidants [3,4]. Additionally, fruits are recommended as a source of dietary fiber, considering its effects on many aspects of the human body and metabolism. For instance, dietary fiber may reduce the risk of cardiovascular disease by improving serum lipids and reducing serum total and low-density lipoprotein (LDL) cholesterol concentrations. Increased fiber content decreases the glycaemic index of foods, which leads to a significant improvement in glycaemic response [5]. Thus, several studies have described a significant inverse association between fruit consumption and the incidence of several diseases, including cardiovascular diseases [3,6], different types of cancer [7,8], osteoporosis [9], and rheumatoid arthritis [10].

Currently, nutrition and health linkages focused on emerging strategy of diet based regimen to combat various physiological threats. In this context, consumption of fruits, including *diospyros kaki* fruit, is gaining considerable importance as safeguard to maintain human health. Likewise, their phytochemicals and bioactive molecules are also becoming popular as promising demulcent against several ailments. The current chapter is an effort to sum up information regarding persimmon (*D. kaki*) fruit with special reference to its phytochemistry and associated health claims. For instance, in recent

¹LAQV/Requimte, Department of Chemical Sciences, Faculty of Pharmacy of University of Porto, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

²FP-ENAS (UFP Energy, Environment and Health Research Unit), CEBIMED (Biomedical Research Centre), University Fernando Pessoa, R. Carlos da Maia, 296, 4200-150 Porto, Portugal.

³Drug Discovery, Delivery and Toxicology, IINFACTS /CESPU, Paredes, Portugal.

*Corresponding author: E-mail: acvinha@ufp.edu.pt;

years, it has been favored as tea for healthcare in Southeast Asia for its beneficial effects on homeostasis, hypertension, apoplexy, and atherosclerosis [11].

1.1 *Diospyros kaki*

Species of the genus *Diospyros* spp. belong to the family Ebenaceae. Nowadays, in Europe, the considerable interest of national economy to constitute such kinds of as: *Diospyros kaki* L. (Japanese persimmon) and *Diospyros virginiana* L. (American persimmon), fruit plants as food, *Diospyros lotus* L. (Date plum) as rootstock, *Diospyros virginiana* as a rootstock and also as a source of high winter hardiness at the hybridization [12]. It seems that native persimmons (*Diospyros kaki*) are originated in China, India and Japan, and were an important food source in those countries, from ancient history. Currently, it is one of the most important fruit crops in Asian countries, although from the 20th century it began to expand throughout Europe, however, China is the main producer, contributing about 77% of all global production [13]. Taking into account its botanical description, *Diospyros kaki* is a multi-stemmed or sometimes single-stemmed deciduous tree up to 6 m tall and typically round-topped, fairly open, erect or semi-erect, sometimes crooked or willowy; seldom with a spread of more than 4.5-6 m. Branches somewhat brittle and can be damaged in high winds [14]. The fruit is round, conical, oblate, or nearly square, capped by the persistent calyx, skin thin, smooth, glossy, yellow, orange, red or brownish-red; flesh yellow, orange, or dark-brown, juicy, gelatinous, seedless or containing 4-8 seeds. Generally, the flesh is bitter and astringent until fully ripe, when it becomes soft, sweet and pleasant, but dark-fleshed types may be no astringent, crisp, sweet and edible even before full ripening [14].

Among the nearly 350 species of the genus *Diospyros* that produce edible fruits is persimmon (*Diospyros kaki*), which has been considered as the species of major commercial interest in fruit growing due mainly to the nutritional and functional quality of its fruits [15]. Due to the utilization potential and nutraceutical and functional properties of persimmon fruits, several studies have been conducted to provide necessary and complete information on the quantification of these nutritional and phytochemical compounds. The fruit has a great use, especially when consumed in fresh form or when intended for agroindustrial processing. Recent studies indicate that persimmon fruit is rich in vitamins and substances (mostly polyphenols and carotenoids) which have nutraceutical, and functional properties and these compounds are directly responsible for the contribution of beneficial effects to human health and the reduction of risk and development of diseases [2].

1.2 Fruit Phytochemical Characterization

Functional foods and therapeutic agents are obtained either directly or indirectly from different natural sources. The therapeutic value of functional foods depends upon the presence of bioactive compounds. These bioactive compounds can offer several health benefits beyond the basic nutritional value of a food product. Thus, persimmon fruit has been shown a good quality for consumption *in natura*, mainly because they contain significant amounts of different bioactive compounds. Persimmon pulp (edible portion) is rich in vitamins, mostly vitamin C (~70 mg/100 g), and vitamin A (~65 mg/100 g), minerals like calcium (~9 mg/100 g), and iron (~0.2 mg/100 g) [16]. With regard to bioactive compounds, ferulic acid, *p*-coumaric acid, and gallic acid are de major phenolic acids described. These compounds possess antioxidant activity due to their chemical structures (number of hydroxyl groups attached). Carotenoids are the major pigment present in persimmon (pulp and peel). They contribute to both color and nutritional value [17,18]. Carotenoid contents rapidly increase as green mature fruit changes to soft mature persimmon, except for lutein and lycopene that decrease during fruit maturation. Among them β -cryptoxanthin content is the highest (50%), followed by lycopene (10%) ~ β -carotene (10%) > zeaxanthin (5%) > and lutein (5%) [19]. They are all excellent lipid-soluble antioxidants, especially lutein, astaxanthin, and zeaxanthin, having the ability to scavenge free radicals in a lipid-soluble environment and thus preventing the oxidation of lipids [20]. Different parts of the persimmon fruit also may contain different types and amounts of carotenoids. For example, the quantity of β -cryptoxanthin, β -carotene, lycopene or lutein is higher in the peel compared to the pulp. Nevertheless, the final composition and concentration of carotenoid contents are properly regulated to some extent by the different developmental stages of plant tissues [20].

Others carotenoids identified in persimmon fruits comprise *cis*-mutatoxanthin, antheraxanthin, zeaxanthin, neolutein, cryptoxanthins, α -carotene, and β -carotene and also fatty acid esters of β -cryptoxanthin and zeaxanthin [21]. Tannins are present in persimmon, composed mostly of epicatechin, epicatechin-3-O-gallate, epigallocatechin, and epigallocatechin-3-O-gallate, which are described as bioactive compounds capable of prolonged life and reduced the incidence of stroke in hypertensive rats [22]. This effect was attributed to the fact that persimmon tannins are 20 times more potent than antioxidant vitamin E [22].

Given de above, extensive research has related the consumption of persimmon with the reduced risk of several diseases and particularly highlighted the presence of bioactive compounds for their therapeutic properties. Unfortunately, few researches on its bioactive activities have been reported, especially on bioactive compounds of the Portuguese fruit from different geographic areas. Taking into account the increased production of this fruit in Portugal, which directly or indirectly promotes its consumption, this study focused on the chemical profile evaluation of Portuguese *Diospyros kaki* through different soil and climatic conditions in different geographic regions? At the same time, the content of total phenolics, ascorbic acid and lycopene were also determined. The radical scavenging activity of the *Diospyros* by employing the DPPH radical scavenging method was also analyzed. Although there are some reports regarding antioxidant activity of fruits and peels of persimmon, no work has been done on the comparative research about chemical profile, bioactive compounds content and antioxidant capacity of Portuguese *Diospyros kaki* and their relation with geographic regions.

2. MATERIAL AND METHODS

2.1 Samples

Diospyros kaki fruits cv. chocolate were collected from different locations, north (V. N. Famalicão), center (Guarda) and south (Portimão) of Portugal, at the same time, November 2009. Samples were prepared in two groups, with and without peel, in order to evaluate the antioxidant compounds lost with skin removal and also because persimmon fruits may be consumed with or without peel. Homogenized samples were transferred into an air-tight container and kept at -20°C before extracts preparation.

2.2 Qualitative Parameters

Fruit samples were analyzed on moisture content, soluble solids, pH, water activity (aW) and maturity index (color). Total soluble solids were quantified using an Atago, NAR-3T refractometer, expressed as °Brix. pH value was measured by using a pH-meter (Hanna instruments 8417). Water content was determined by gravimetric assay, expressed in percentage of water (%). The water activity (aW) was measured using a Rotronic Hygropalm 9 VCD model. Color index (color intensity) was observed, using a Minolta Chromameter II Reflectancia CR-2000. The a^* (red-green) and b^* (yellow-blue) values were used to calculate the hue angle value, $\tan^{-1}(b^*/a^*)$.

2.3 Bioactive Compounds

2.3.1 Total phenolics

The amount of total phenolic content was determined using Folin-Ciocalteu reagent, according to the methodology previously described [2]. Total phenolic contents were extracted using three different solvents; water at ambient temperature (H₂O 100%), methanol (MeOH 80%) and ethanol (EtOH 80%) for one hour, in order to assess the extraction effect in fruits. Total phenolic were quantified by calibration curve obtained from measuring the absorbance of a known catequin concentration standard. Concentrations were expressed as milligrams of catequin equivalents per 100 g of fresh weight (mg EC/100 g).

2.3.2 Ascorbic acid content

Ascorbic acid quantification was determined according to the modified 2,6-dichlorophenolindophenol (DIP) method, described in detail by Vinha et al. [2]. Quantification was obtained from a standard curve within the linear range of 0-0.8 mg ascorbic acid per mL.

2.3.3 Lycopene quantification

Lycopene content was determined according to Vinha et al. [2], by using a colorimetric assay. The amount of lycopene was estimated by the using the formula: Lycopene ($\mu\text{g/g}$) = $[(A \times v \times 10^6) / (3.450 \times W \times 100)]$, where v is the amount of hexanes (mL), W the weight of fruit sample (g), A the absorbance at 472 nm and 3.450 is the extinction coefficient.

2.4 Statistical Analysis

The results were submitted to descriptive statistical analysis (ANOVA), obtaining mean values and standard deviations of persimmon fruits. Analyzes were performed from three independent samples in triplicate. Statistical differences with P-values under 0.05 were considered significant and means were compared by 95%.

3. RESULTS AND DISCUSSION

It is crucial to study about the compounds responsible for organoleptic characteristics (color, texture, flavor and aroma) to obtain attested information about the effects of different treatments on these quality characteristics, mainly for food industry. However, we cannot discourage the fact that food by-products can also be integrated into the pharmaceutical and cosmetic industries, highlighting the importance of organoleptic attributes, also emphasizing the importance of organoleptic attributes in these areas. Furthermore, geographic variation may also contribute for same specific nutritional and chemical variation in fruits pulp, peel and seeds. All the compounds present in each fruit and its quantitative evaluation constitute an individual "fingerprint" in this case, a chemical profile that characterizes each fruit, where persimmons are no exception. This profile is not constant and there are several factors influencing their variation. Other factors that may influence the chemical profile are climate, sun exposure, soil nature and agricultural techniques. Physiochemical results are presented in Table 1.

Table 1. Influence of geographic region and presence of fruit peel on moisture (%), soluble solids contents ($^{\circ}\text{Brix}$), pH values, water activity (%) and angle value ($^{\circ}\text{h}$) of Portuguese *Diospyros kaki* cv. Chocolate

Chemical characterization	Sample preparation	Geographic region from Portugal		
		North	Center	South
Moisture (%)	Peel with	79.5 \pm 0.05	80.1 \pm 0.12	79.4 \pm 0.15
	Peel without	82.4 \pm 0.07	80.0 \pm 0.02	80.3 \pm 0.35
pH	Peel with	6.05 \pm 0.02	5.86 \pm 0.09	5.66 \pm 0.04
	Peel without	5.95 \pm 0.04	5.90 \pm 0.03	5.68 \pm 0.01
Soluble solids ($^{\circ}\text{Brix}$)	Peel with	1.90 \pm 0.02	1.80 \pm 0.03	1.85 \pm 0.01
	Peel without	1.90 \pm 0.04	1.90 \pm 0.02	1.85 \pm 0.01
a_w (%)	Peel with	95.7 \pm 0.01	95.2 \pm 0.01	95.2 \pm 0.02
	Peel without	95.4 \pm 0.02	95.1 \pm 0.03	95.8 \pm 0.01
Hue angle value ($^{\circ}\text{h}$)	Peel with	77.7 \pm 0.07	73.3 \pm 0.12	74.9 \pm 0.04
	Peel without	81.2 \pm 0.05	78.6 \pm 0.08	79.2 \pm 0.12

*†All values are expressed as means \pm S.D. in triplicate measures. Compare results between columns, peel with and without. Compare results between rows, geographic regions. Means in same column or rows with the same superscript were not significantly different ($p > 0.05$) with different superscript were significantly different ($p < 0.05$)

Taking into account the obtained results, it appears that fruit water content is around 80%. In fact, geographic region did not affect the moisture percentage. pH values ranged, in all samples collected North of Portugal, with statistical significance ($p < 0.05$) compared with samples collected in Center and South. Values from North *Diospyros* with peel and without peel were 6.05 and 5.95, respectively; samples from the South have lower values, 5.66 and 5.68 from samples with peel and without. Water activity values were similar in all samples with mean values corresponding to 95.5%, ($p > 0.05$). However, with regard to soluble solids, Center samples, collected in Guarda, showed significantly differences among *Diospyros* with and without peel ($p < 0.05$), did not observed the same relationship in samples collected in North and in South of Portugal. Regarding to color angle ($^{\circ}$ h) determination no significance results ($p > 0.05$) were observed between all samples when compared with geographical regions of cultivation. However, it is important to note that all samples with peel showed a greater color angle than samples without peel. This reinforces the idea of reusing food by-products, rich in valuable compounds, allowing their characterization and valorization into high value products with application in diverse biotechnological fields, such as Pharmaceutics, Food or Cosmetics, but also reduce the waste environmental impact and the related treatment costs [18,23].

The polyphenolic compounds and carotenoids are directly related to the organoleptic characteristics of fruit, particularly in color and flavor. The determination of phenolic compounds level in plant or fruit tissues is the initial step of any physiological functionality investigation for further stimulus to their consumption [24]. According with some authors the quantity and the composition of bioactive compounds, such ascorbic acid, lycopene and phenolic compounds present in fruits are influenced by genotype [25], extraction procedure [26,27], and environmental conditions [28-30].

Table 2. Influence of geographic region and presence of peel *Diospyros* fruit on bioactive compounds (mg/100 g)

Sample preparation		Geographic region from Portugal		
		North	Center	South
Ascorbic acid	Peel with*	0.9479±0.02	1.3079±0.07	1.1261±0.01
	Peel without**	0.6834±0.05	1.1271±0.04	1.1290±0.07
Lycopene	Peel with*	4.418±0.09	7.295±0.04	8.064±0.03
	Peel without**	3.635±0.04	4.986±0.01	5.349±1.2

Values expressed are means±S.D. of triplicate measurements. † $p < 0.001$; ‡ $p < 0.01$; ^{ns} $p > 0.05$. *North/Center[†]; North/South[‡]; †North/Center; **North/South[†]; Center/South^{ns}

Examining the results presented in Table 2, *Diospyros* fruit samples with peel in North and Center of Portugal have higher amount of ascorbic acid than samples without peel, collected in the same regions, ranging from 0.9479 mg/100 g to 1.3079 mg/100 g and 0.6834 mg/100 g to 1.1290 mg/100 g, respectively. South samples did not show any differences between them, with similar levels with peel (1.1268 mg/100g) and without peel (1.1290 mg/100 g) ($p > 0.05$). The most significant lost in ascorbic acid was observed by peel remotion in *Diospyros* from North ($p < 0.001$). Our results are in agreement with other published studies [31,32]. Usually, ascorbic acid content is directly related with pH value in a direct proportion. Thus, wide variations in the vitamin C content can be explained by environmental factors, intrinsic characteristics of the fruits, conditions of the crop and time of harvesting.

Fresh fruits contain antioxidant compounds like vitamin A, β -carotene, lycopene, lutein, zeaxanthin and cryptoxanthin. These compounds function as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that play a role in aging and various disease processes. Fruits are also a very good source of vitamin C, another powerful antioxidant. Regular consumption of foods rich in vitamin C helps body develop resistance against infectious agents and scavenge harmful, pro-inflammatory free radicals. Lycopene values ranged between 3.635 mg/100 g and 8.064 mg/100 g, showing higher concentration in whole with peel ($p < 0.05$). These results may be of greater interest considering others studies reported, mainly the difference on its concentration. For instance, in Saijo, concentrations of 0.7 and 3.90 mg/100 g were reported by Homnava et al. [33] and Kondo et al. [34] respectively, probably owing to environmental effects and/or yearly fluctuations. The present results showed that the persimmon fruits analyzed have low provitamin A activity, while other

non-provitamin A carotenoids are mainly present in these fruits. Special mention should be made for the lycopene, a non-provitamin A carotenoid widely studied due to its high antioxidant potential. Hence the wide variability of lycopene content could be attributed to genotype effect and could also depend on environmental factors. An interesting aspect is the increased level of lycopene in samples from different regions. On the other hand, opposite results were found in another study carried out with 46 different persimmon cultivars (32 astringent and 14 non-astringent) as the astringent varieties showed significant β -carotene amounts [35].

In this study we investigate the recovery of phenolic compounds from *Diospyros kaki* fruit using different solvents (Table 3). The main purpose of the study was to compare the extraction yields obtained with the three solvent chemistry nature and evaluate the content of total phenolics extracts.

Table 3. Influence of geographic region on total polyphenolics content (mg EC/100g sample) of *Diospyros kaki* fruit with peel and without peel using three different solvent extracts

Geographic areas from Portugal				
Sample preparation	Solvent	North	Center	South
Peel with [†]	EtOH	21.9±2.43	35.9±1.62	79.3±1.34
	MeOH	39.0±1.23	52.5±10.6	106.2±2.80
	H ₂ O	30.9±5.19	54.1±0.92	99.3±1.83
Peel without [‡]	EtOH	18.7±0.84	24.3±5.75	26.8±4.40
	MeOH	20.2±1.20	41.4±1.31	47.0±2.39
	H ₂ O	21.1±4.88	38.6±3.78	39.2±0.48

All values are expressed as means±S.D in triplicate measures. *Compare results between columns, peel with and without, observed with different solvent extractor. Different superscript were significantly different ($p < 0.05$); ^{†‡} Compare results between rows, with same solvent extractor by comparison different geographic areas, with different superscript were significantly different ($p < 0.05$)

The maximum extractable total polyphenolics content were recorded in methanol solvent, followed by aqueous and ethanol solvents, respectively. The extraction method is very important in experimental procedure, so we studied different extraction using the three different solvents showing a significant result between them ($p < 0.001$). Total polyphenolic content differences were significant ($p < 0.001$), however, total polyphenolic content in *Diospyros kaki* fruit seems to be higher in south samples, with and without peel. Results in Table 3 reveal, that peel samples showed higher values than those obtained without peel, in which concentrations ranged between 21.9 and 106.2 mg/100 g for with peel *Diospyros* samples and 18.7 and 47.0 mg/100 g to removed peel samples. These concentrations variation increase in a direct proportion with geographic regions ($p < 0.001$) and seems to be higher that polyphenol contents found in Triumph fruits harvested in different years (about 1.4 mg/ 100 g) and analyzed by the same procedure [36]. Fruits from South have higher total polyphenolic levels than North fruits contain lower levels of total polyphenolics, demonstrating that climatic conditions are important for their development in fruits metabolism. It should also be considered that the levels of these compounds are highly dependent on persimmon fruit variety and growth conditions and could be increased through appropriate cultivation practices.

4. CONCLUSIONS

Persimmon fruit contains bioactive compounds such as fiber, vitamin C, carotenoids, polyphenolics as well as micro- and macro-minerals with important health-promoting effects. The utilization of persimmon fruit and its bioactive components may be a good strategy to improve the health status of the population. Although the consumption of persimmon fruit has important health benefits, up to date no specific health claims are authorised for this fruit. Our results proved that peel removal interferes with the total content of bioactive compounds. In fact, *Diospyros* fruits are related to polyphenolic compounds, ascorbic acid and lycopene content and the removal of fruits peel decreases their amounts. In the south, where the temperature and sun exposure are higher, the content of ascorbic acid, lycopene and polyphenols is higher, verifying a direct relationship between edaphic and climatic conditions with the content of bioactive compounds. This study highlighted the influence of different geographic regions on chemical composition of fruits. Apart from all that was mentioned earlier, this

study becomes a pioneer one, since there are no records of an identical study in Portuguese *Diospyros kaki*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)



Ana F. Vinha

LAQV/Requimte, Department of Chemical Sciences, Faculty of Pharmacy of University of Porto, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal and FP-ENAS (UFP Energy, Environment and Health Research Unit), CEBIMED (Biomedical Research Centre), University Fernando Pessoa, R. Carlos da Maia, 296, 4200-150 Porto, Portugal.

Research and Academic Experience: PhD in Analytical and Food Chemistry.

Professional career: Assistant Professor at University Fernando Pessoa, Porto, Portugal.

Research Area: Food Science; Analytical Chemistry, Pharmacognosy and Phytotherapy.

Publications: 3 Books, 18 Book Chapters, 60 Scientific articles.

Research lines: Antioxidants; Plant chemical compounds; Food chemistry; Sustainability.

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