

Chemical Composition and Antioxidant Activity of Portuguese Diospyros Kaki Fruit by Geographical Origins

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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 (106.2 mg/100g sample) and lower in ethanol (21.9 mg/100g sample) considering *Diospyros* fruits with peel. The antioxidant activity (AA) was evaluated by an in vitro model system through 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity (DPPH[•]) assay. Significant variations were found in the levels of these parameters according to different geographic regions. It was possible to describe correlations between the antioxidant compounds (polyphenolics, ascorbic acid and lycopene) and the antioxidant activity. Aqueous solvent was more effective on the extraction of antioxidant compounds from Portuguese *Diospyros* fruits with 52% radical scavenging activity. 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*, Antioxidant activity, (DPPH[•]) assay, Total polyphenolics, Lycopene, Geographic origin

1. Introduction

Fruits and vegetables have acquired the status of functional foods in Mediterranean diet (Vinha *et al.*, 2002; Alonso *et al.*, 2004). They seem to be capable of delivering health benefits besides fulfilling physiological needs. Epidemiological data as well as in vitro studies strongly suggest that foods containing bioactive phytochemicals

with antioxidation potential have strong protective effects against some major diseases, including cancer and cardiovascular diseases (Clinton, 1998; Jang, *et al.*, 2007; Céspedes, *et al.*, 2008).

Diospyros fruit is highly nutritious compared to other fruits. The protective action of fruits has been attributed to the presence of antioxidants, especially antioxidants vitamins, including ascorbic acid, β -carotene (Giraldi, *et al.*, 2003; Céspedes, *et al.*, 2008; Chen, *et al.*, 2008). Besides carotenoids, *Diospyros kaki* fruits contain a variety of natural antioxidants (Davies & Hobson, 1981; Clinton, 1998; Davey, *et al.*, 2000; Kim, *et al.*, 2006).

Antioxidants are compounds that can delay oxidation of lipids or other molecules by inhibiting the beginning or propagation of oxidative chain reactions (Velioglu, *et al.*, 1998). According to some authors, the antioxidant content of orange/red fruit mostly depends on genetic, environmental factors and ripening stage (Kim, *et al.*, 2006; Chen, *et al.*, 2008). Fruit ripening is a complex, genetically programmed process that culminates in dramatic changes in color, texture, flavor and chemical compositions (Vinha, *et al.*, 2005). It has been shown that ripening processes and storage temperature can severely affect the final nutrient composition of fruits (Davey, *et al.*, 2000; Javanmardi & Kubota, 2006; Kim, *et al.*, 2006; Patthamakanokporn, *et al.*, 2008). Lycopene, which is responsible for the orange/red color of *Diospyros kaki* (Clinton, 1998; Giraldi, *et al.*, 2003), changes significantly between cultivars, stage of maturity and growing conditions (Stahl & Sies, 1996; 2003) and also is regarded as an antioxidant with high biological activity among humans (Sahlin, *et al.*, 2004).

The genus *Diospyros* belongs to the family *Ebenaceae* and comprises about 500 species distributed in the tropical and temperate zone, including Portugal. This fruit is very appreciated by the Portuguese population and is highly nourishing, in comparison with other seasonal results. Besides his nourishing value, *Diospyros* is described for its applications for medicinal and therapeutic ends. Unfortunately, few researches on its bioactive activities have been reported, especially on bioactive compounds of the Portuguese fruit from different geographic areas. A single fruit weighs 250 g and can reach up to 610 g. Therefore, many attempts have been made to extend the storage and shelf life (Turk, 1993).

The aim of this study was to evaluate chemical profile 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 (Jung, *et al.*, 2005; Lee, *et al.*, 2008; Fukai, *et al.*, 2009), 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. Just about 5Kg of *Diospyros kaki* fruits were randomly harvest from 10 different trees in the same plantation area from different geographic plantation, as mentioned above and according to the seasonal growth. Freshly collected fruits were cleaned and prepared according to requirements of the intended analysis and stored at 4°C. 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, and after homogenized using a wet blender (National; model: MX-291N) for 1 minute. Homogenized samples were transferred into an air-tight container and kept at -20°C before extracts preparation.

2.2 Qualitative Parameters

Diospyros kaki samples were analyzed on moisture content, soluble solids, pH, water activity (a_w) and maturity index (color). Total soluble solids were quantified in a fruit puree using an Atago, NAR-3T refractometer, expressed as °Brix, a quick estimate of sugar content. pH value was measured by using a pH-meter (Hanna instruments 8417), for three times. Water content was determined by gravimetric assay. The 5g of each fresh sample in porcelain capsule were placed in stove (WTC binder Klasse 2.0, Tuttlingen, Germany) at $105^\circ\text{C} \pm 1^\circ\text{C}$, followed from regular weighting up to constant weight and results expressed in percentage of water (%). The water activity (a_w) was measured using a Rotronic Hygropalm 9 VCD model. Three color reading were taken from each sample, after homogenization of whole fruits, using a Minolta Chromameter II Reflectancia CR-2000 (Minolta Limited, Milton Keynes, UK). The L^* (red-green) and b^* (yellow-blue) values were used to calculate the hue angle value, $\tan^{-1}(b^*/a^*)$, which provides information about color index (color intensity) of fruit samples.

2.3 Antioxidant Compounds Quantification

2.3.1 Total Polyphenolic Content Assay

The amount of total polyphenolic was determined according to Jang (2007) using Folin-Ciocalteu reagent. Total polyphenolic compounds were extracted using three different solvents; water at ambient temperature (aqueous), methanol (MeOH 80%) and ethanol (EtOH 80%) for one hour, in order to assess the extraction effect in fruits antioxidant properties. A 1 g of fresh sample was added of 50 ml of each solvent, under constant agitation for 1 hour. Filtration took place under vacuum and checked for a final volume of 100 ml. A volume of 0.2 ml of extracts (1 mg/ml) was added of 0.5 ml of Folin-Ciocalteu reagent for 3 min at room temperature. After, 0.2 ml of sodium carbonate 6% was added and gently mixed. After standing at room temperature for 120 min, absorbance was read at 720 nm using a UV-Vis spectrophotometer. Total polyphenolic 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.

2.3.2 Ascorbic Acid Assay

Ascorbic acid content was determined according to the modified 2,6-dichlorophenolindophenol (DIP) method (Franck *et al.*, 2003). Each fruit sample (5 g) was extracted using 20 ml of oxalic acid (0.4%) for 1 hour. Extracts were filtered and 5 ml was added to 10 ml of oxalic acid and 1ml of HCL (1M). Quantification was obtained from a standard curve within the linear range of 0-0.8 mg ascorbic acid per mL.

2.3.3 Lycopene Assay

Lycopene content was determined according to Sadler *et al.* (1990). Briefly, 5g of homogenized sample was extracted adding 50 ml of a mixture of hexane/acetone/ethanol (2:1:1, v/v/v) for 30 minutes. The absorbance of supernatant (hexane layer) containing lycopene was read using Beckman DU-64 spectrophotometer at 472 nm (specific to lycopene). Absolute hexane was used as blank. The amount of lycopene in fruit was then estimated by the using the formula:

$$\text{Lycopene } (\mu\text{g/g}) = [(A \times v \times 10^6) / (3.450 \times W \times 100)]$$

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 Radical Scavenging Activity using DPPH Assay

DPPH was used for the estimation of antioxidant capacity based on the reaction of a specific reagent with electron donating or hydrogen radical producing anti-oxidant compounds. The radical scavenging activity of the Diospyros fruit extracts was determined as previously described (Jang *et al.*, 2007). Briefly, 5 ml of DPPH in MeOH (0.03 g/dm³) were added to 0.05 ml of polyphenolic extracts for 30 minutes in the dark. Absorbance (A) was measured every 10 minute over a 60 minute time period at 517 nm on a UV spectrophotometer UV-60. Radical scavenging activity was expressed as inhibition percentage and calculated using the equation:

$$\% \text{ Radical scavenging activity} = [(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100.$$

2.5 Statistical Analysis

Statistical analysis was performed using Prisma 5. Data of all analysis were expressed as mean \pm standard error mean (SEM) from three independent samples in triplicate. Results were statistically evaluated by variance analysis (ANOVA). Statistical differences with P-values under 0.05 were considered significant and means were compared by 95%.

3. Results and Discussion

Various parts of different plants could be used as fruits or vegetables for human consumption. They can be roots, tubers, bulbs, stems and shoots, flowers, leaves and fruits, or pods and seeds. It is crucial to study and learn about the plant cell and compounds responsible for flavor, texture and color to obtain reliable information about the effects of different treatments on these quality characteristics. However, geographic variation may also contribute for some specific nutritional and chemical variation in fruits pulp. 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. 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. Physicochemical results are presented in Table 1.

<Table 1>

Weight loss variation of *Diospyros kaki* fruit was not significantly different with peel and without peel ($p > 0.05$). It appears that fruit water content is in average around 80%. 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 significant 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 (h°) determination, in statistical analysis was observed a no significance results ($p > 0.05$) 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, with the exception of two samples from the South. The color angle value for peel sample in South showed a lower angle, which means more color intensity, than without peel fruit (74.93 and 79.20, respectively), reinforcing the idea that the climate and sun exposure has an influence on fruit color that might be related with fruit chemical composition, namely the lycopene content. These results are according to other published studies (Sadler *et al.*, 1990; Franck *et al.*, 2003; Furlong *et al.*, Jang *et al.*, 2007; Halilova & Yildiz 2009).

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 (Furlong *et al.*, 2003). 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, extraction procedure and environmental conditions (Howard *et al.*, 2003; Caldwell *et al.*, 2005; Hinneburg & Neubert 2005; Mukhopadhyay *et al.*, 2006).

<Table 2>

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/100g to 1.3079 mg/100g and 0.6834 mg/100g to 1.1290 mg/100g, 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/100g) ($p > 0.05$). The most significant lost in ascorbic acid was observed by peel remotion in *Diospyros* from North ($p < 0.001$). Usually, ascorbic acid content is directly related with pH value in a direct proportion. The weather in Center is smoother between seasons and in South is warmer. However these results are according with mean pH values from samples collected in different geographic regions.

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.

In Table 3 is represented lycopene content obtained in different *Diospyros kaki* fruit samples studied through different geographical areas from Portugal.

<Table 3>

Lycopene values ranged between 3.635 mg/100g and 8.064 mg/100g, 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/100g were reported by Homnava *et al.* (1990) and Kondo *et al.* (2004) respectively, probably owing to environmental effects and/or yearly fluctuations. Persimmons are described as a very good source of lycopene, superior to most of commonly consumed fruits, with a standard content of 0.159 mg/100 g fresh weight (USDA). 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. Lycopene content increases from north to south reinforcing results published by Kim *et al.* (2006) and Takahashi *et al.* (2006) showing increases in carotenoids and polyphenols concentrations in fruit skins compared to those found in pulps. The same authors had concluded that lycopene concentrations were related with different growing regions, particularly to those with higher sun exposure. A great interest has recently been focused on lycopene due to its preventive activity against several pathologies, such as cardiovascular disease (Rao 2002), hepatic fibrogenesis

(Kitade *et al.*, 2002), solar light induced erythema (Stahl & Sies 2002) and some cancer types, such as prostate, gastrointestinal and epithelial (Leong & Shui 2002; Livny *et al.*, 2002). Finally, it is also important to consider the synergic action of carotenoids with other bioactive compounds present in *Diospyros kaki* fruit, like total polyphenolic content.

Polyphenolic compounds represent a large and important group of abundant secondary plant metabolites in fruit. Total polyphenolic content of *Diospyros kaki* fruit extracts were obtained using methanol 80%, ethanol 80% and aqueous (water) solvents are shown in Table 4.

Table 4. Influence of geographic region on total polyphenolics content (mg Ec^a/100g sample) of *Diospyros kaki* fruit with peel and without peel using three different solvent extracts.

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 4 reveal, that peel samples showed higher values than those obtained without peel, in which concentrations ranged between 21.9 and 106.2 mg/100g for with peel *Diospyros* samples and 18.7 and 47.0 mg/100g 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/ 100g) and analyzed by the same procedure (Park *et al.*, 2006). 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.

The antioxidant activity of *Diospyros* fruits has been chemically assessed by determining the radical scavenging activity through the 1,1-diphenyl-2-picrylhydrazil (DPPH[•]) method (Leong & Shui 2002; Kondo *et al.*, 2004; Chen *et al.*, 2008; Del Bubba *et al.*, 2009). The relationship between total polyphenolics content and the radical scavenging activity of the Portuguese fruits along with controls (BHT) was investigated based on scavenging free radicals species recognized by antioxidants. According to Leong & Shui (2002) this method has been a useful tool to evaluate the antioxidant capacity of fruits. The DPPH[•] inhibition levels by discoloration of synthetic antioxidant BHT were used as positive control for comparison between samples and to validate analytical method. Statistical analysis had shown a positive and highly significant relationship between total polyphenolics content and radical scavenging activity ($p < 0.05$). Aqueous extracts from south revealed higher activity (52% and 44.8% from *Diospyros* fruits with peel and without peel, respectively, both in south samples). Less antioxidant activity was observed in methanolic extract corresponding to 34.8% sample with peel and 15% without peel. Ethanolic extract had revealed the lower antioxidant ability (33% and 18%, with peel and without peel, respectively). These values were higher than those found in *Diospyros* fruit varieties analyzed in Italy (Del Bubba *et al.*, 2009) but lower than Israeli persimmon fruits (Jung *et al.*, 2005). Again, it appears that the study of antioxidant activity is directly related to the content of polyphenolic compounds, giving more emphasis to the importance of the extraction process, mainly on the solvent used.

<Figure 1>

By statistical analysis it was found a relationship between the concentrations of polyphenolics and antioxidant activity of the same sample, as there is a relationship between the origin of persimmon fruits and antioxidant activity ($p < 0.001$). A good correlation was registered between catequin and DPPH[•] values (R^2 ranges between 0.86 and 0.89).

4. Conclusion

Some researchers have shown that persimmon is one of the most bioactive fruits. In this work the role of *Diospyros kaki* fruits as a source of primary and secondary metabolites (vitamin C, lycopene and polyphenols), was studied. The main function of antioxidants is the delaying of the oxidation process to other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals reducing oxidative damage to human body. The occurrence of such oxidative damage may be a significant causative factor in the development of many chronic diseases, such as cancer and cardiovascular diseases. Several epidemiological studies have shown a negative association between intake of fruits and certain diseases. *Diospyros kaki* have shown to possess a high free radical scavenging activity. This study indicates that the antioxidant activity of *Diospyros* fruits is related to polyphenolic compounds, ascorbic acid and lycopene content and the removal of fruits peel decreases their amounts. This study also highlighted the influence of different geographic regions on chemical composition

of fruits. The *Diospyros kaki* is a highly nutritious fruit in comparison to other fruits, and currently carries great importance in research for medicinal purposes, for its potential in promoting public health. Apart from all that was mentioned earlier, this study becomes a pioneer one, since there are no records of a similar study in Portuguese *Diospyros kaki*.

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Table 1. Influence of geographic region and presence of fruit peel on moisture (%), soluble solids contents (°Brix), pH values, water activity (%) and angle value (°h) of Portuguese *Diospyros kaki* fruit, cv. chocolate

Chemistry Characterization	Sample preparation	Geographic region from Portugal		
		North*	Center*	South*
Moisture (%)	Peel with	79.5±0.05	80.1±0.12	79.4±0.15
	Peel without	82.4±0.07	80.0±0.02	80.3±0.35
pH	Peel with	6.05±0.02	5.86±0.09	5.66±0.04
	Peel without	5.95±0.04	5.90±0.03	5.68±0.01
Soluble solids (°Brix)	Peel with	1.90±0.02	1.80±0.03	1.85±0.01
	Peel without	1.90±0.04	1.90±0.02	1.85±0.01
a _w (%)	Peel with	95.7±0.01	95.2±0.01	95.2±0.02
	Peel without	95.4±0.02	95.1±0.03	95.8±0.01
Hue angle value (°h)	Peel with	77.7±0.07	73.3±0.12	74.9±0.04
	Peel without	81.2±0.05	78.6±0.08	79.2±0.12

*All values are expressed as means ± 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 ($p < 0.05$).

Table 2. Influence of geographic region and presence of peel *Diospyros* fruit on ascorbic acid content.

	Sample preparation	Geographic areas from Portugal		
		North†	Center‡	South ^{ns}
Ascorbic acid (mg/100 g)	Peel with*	0.9479±0.02	1.3079±0.07	1.1268±0.01
	Peel without**	0.6834±0.05	1.1271±0.04	1.1290±0.07

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}.

Table 3. Influence of geographic region and peel on lycopene of *Diospyros kaki* fruit

	Sample preparation	Geographic areas from Portugal		
		North†	Center†	South†
Lycopene (mg/100 g)	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. *Compare results between same columns, peel with and without peel samples with different superscript were significantly different ($p < 0.001$); †Compare results between rows with different superscript were significantly different ($p < 0.05$), comparing different geographic regions from Portugal.

Table 4. Influence of geographic region on total polyphenolics content (mg Ec^a/100g sample) of *Diospyros kaki* fruit with peel and without peel using three different solvent extracts

Geographic areas from Portugal				
Sample fruit preparation	Solvent	North*	Center*	South*
Peel With†	Ethanol	21.9±2.43	35.9±1.62	79.3±1.34
	Methanol	39.0±1.23	52.5±10.6	106.2±2.80
	Water	30.9±5.19	54.1±0.92	99.3±1.83
Peel Without‡	Ethanol	18.7±0.84	24.3±5.75	26.8±4.40
	Methanol	20.2±1.20	41.4±1.31	47.0±2.39
	Water	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 ($p < 0.05$).

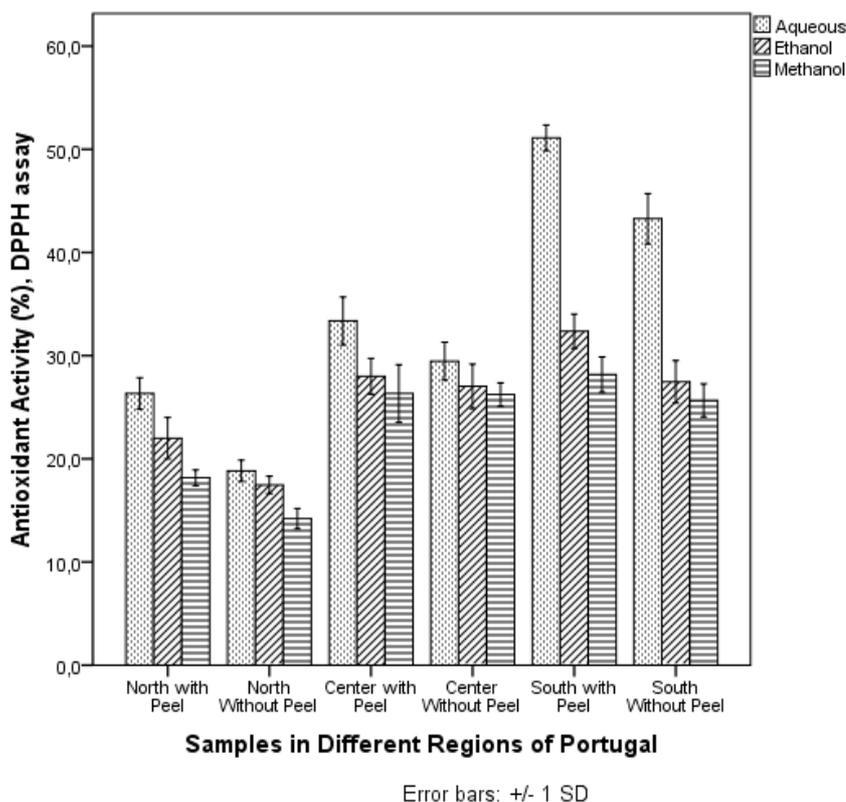


Figure 1. The antioxidant activity values of Portuguese *Diospyros kaki* fruits using the radical scavenging activity DPPH[•] method. N (north samples – peel and without peel); C (center samples – peel and without peel); S (south samples – peel and without peel)