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Antioxidant capacity and phenolic profile of table olives from the Greek market

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Abstract

Five different varieties of greek table olives (*Kalamon*, *Tsakistes*, *Crete*, *Amfissas* and *Thrubes Crete*) were investigated for the total polyphenol content and phenolic compounds, humidity, fat and total antioxidant capacity. Analysis was performed on the flesh and kernel of the table olives. Total polyphenol content was estimated with the Folin–Ciocalteau assay, humidity with freeze drying and total fat with Soxhlet extraction. Qualitative analysis of phenols and phenolic acids was performed with gas chromatography/mass spectrometry and thirteen compounds were identified in flesh as well in kernel except of oleanolic acid. Finally the antioxidant activity of olives was assessed by scavenging of the 1,1-diphenyl-2-picrylhydrazyl (DPPH·) radical. Total antioxidant capacity is in descending order: *Tsakistes* > *Amfissas* > *Kalamon* > *Crete* > *Thrubes Crete*. By the present work, the consumption of table olives, is considered to offer a high intake of antioxidants, mainly polyphenols, and so a health benefit for the prevention of many decadent diseases.

Keywords: Table olives; Polyphenols; DPPH; Antioxidant capacity; Radical scavenger

1. Introduction

Table olives (from olive tree *Olea Europaea* L.) are a traditional Greek product and one of the most important components of the Mediterranean diet. They are well-known sources of phenolic compounds with important biological properties (Boskou & Visioli, 2003). The benefits of table olives in nutrition are associated, beside the monounsaturated fat, with minor constituents such as phenolic compounds (Simopoulos, 2001). The phenolic fraction of table olives is very complex and can vary both in the quality and quantity of phenolic compounds (Uccella, 2001), as it is depended upon processing method (Romero et al., 2004), upon the cultivar (Romani, Mulinacci, Pinelli, Vincieri, & Cimato, 1999), upon irrigation regimes (Patumi et al., 2002) and upon the degree of drupe maturation (Ryan, Robards, & Lavee, 1999). The most important changes in the phenolic fraction are due to the depletion of oleuropein during the olive fruit development and the concentration increase of tyrosol and hydroxytysol (Ferreira et al., 2002; Piga, Gambella, Vacca, & Agabbio, 2001; Ryan et al., 1999). The major phenolic compounds present in table olives are tyrosol, hydroxytyrosol and oleanolic acid and the concentration of these compounds is depended upon the degree of maturation and the method of treatment of olive drupe till they become edible (Blekas, Vassilakis, Harizanis, Tsimidou, & Boskou, 2002; Owen

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et al., 2003; Romero et al., 2004; Romero, Garcia, Brenes, Garcia, & Garrido, 2002).

Table drupe treatment to edible grade, is mainly conduced, by three processing methods: the Spanish, the Californian and the Greek style. The Spanish processing method includes treatment with sodium hydroxide solution, for the total removal of the bitter compound oleuropein, washing, brining and fermentation, sorting and size grading and packaging (Romero et al., 2004). The Californian method of treatment includes lye treatment, washing, iron-salt treatment and air-oxidation, washing, sizing, canning and sterilization (Marsilio, Campestre, & Lanza, 2001). The Greek style method of treatment is milder and includes washing, natural fermentation in brine, air-oxidation for color improvement, sizing and packing (Piga et al., 2001). All these procedures result to a decrease of the total amount of phenols.

Polyphenols belong to the category of natural antioxidants and are the most abundant antioxidants in our diet (Boskou & Visioli, 2003). They play an important role in human nutrition as preventative agents against several diseases, protecting the body tissues against oxidative stress. Many studies indicate an antioxidant capacity of these polyphenols with respect to the oxidation of low-density lipoproteins (Andrikopoulos, Kaliora, Assimopoulou, & Papageorgiou, 2002) and oxidative alterations due to free radical and other reactive species (Soler-Rivas, Espín, & Wichers, 2000). Polyphenol intake is beneficial for human health because their antioxidant activity has been associated with lower risk of coronary heart disease (Keys, 1995; Simopoulos, 2001; Tapiero, Tew, Nguyen Ba, & Mathe, 2002; Trichopoulou & Lagiou, 1997), some type of cancer (Kris-Etherton et al., 2002; Simopoulos, 2001; Simopoulos, 2001; Trichopoulou & Lagiou, 1997), inflammation (Tapiero et al., 2002; Trichopoulou & Lagiou, 1997) and inhibition of platelet-activating factor activity (Andrikopoulos, Antonopoulou, & Kaliora, 2002).

The phenolic content of olive oil has been under investigation for many years. However the table olives have not been studied to equal extent. There are several studies about the quantity and quality composition of phenolic compounds in table olives (Blekas et al., 2002; Romero et al., 2002). However there are only a few studies that present the total antioxidant capacity of the phenolic fraction of table olives (Owen et al., 2003). Recently Owen et al. (2003), investigated the antioxidant capacity of two Italian brined olive drupe varieties, (one black and one green) by hypoxanthine/xanthine oxidase assay. The antioxidant activity of olive drupe extracts and the antioxidant activity of each purified phenolic compound were determinated. Black olives, which contain higher concentrations of phenolics compounds, present higher antioxidant activity compared to green olive extract. The IC_{50} value of the phenolic compounds investigated follows the order: hydroxytyrosol < tyrosol < phloretic acid < dihydrocaffeic acid < acteoside-1 (Owen et al., 2003).

Since 1995 (Brand-Williams, Cuvelier, & Berset, 1995) another effective method for the estimation of total antioxidant capacity has been applied by introduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$) as radical scavenger. By the DPPH method several foods have been tested, e.g., fruit (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998), grape seeds and wine (Saint-Cricq de Gaulejac, Provost, & Vivas, 1999), vegetable oils and oil fractions (Espin, Soler-Rivas, & Wichers, 2000) and olive oil (Goristein et al., 2003).

The aim of this study was to determine the total phenol content of table olives, to perform the qualitative and quantatitive analysis of polyphenols and to investigate the antioxidant activity of the phenol content of five different types of greek commercial table olives.

2. Materials and methods

2.1. Reagents

Methanol, hexane, petroleum ether of analytical grade, Folin–Ciocalteau reagent 50%, solution of saturated sodium carbonate (45 g Na_2SO_3/100 mL H_2O) and trimethylchlorosilane (TCMS) were obtained from Merck (Darmstadt, Germany). DPPH$^{\cdot}$, Bis(trimethylsilyl)trifluoroacetamide (BSTFA), *trans*-cinnamic acid and *p*-hydroxy-phenyl-propanoic acid were obtained from Aldrich, (Steinheim, Germany). Tyrosol, protocatechuic acid, caffeic acid, 3,4-dihydroxyphenylacetic acid, were obtained from Fluka, (Steinheim, Germany). 4-Hydroxy-benzoic acid, *p*-Hydroxy-phenyl-acetic acid, *p*-coumaric acid and ferulic acid, were obtained from Sigma, (Steinheim, Germany). Vanillic acid was obtained from Serva, (Heidelberg, Germany). Hydroxytyrosol by organic synthesis was kindly donated by the Laboratory of Food Chemistry and Technology, Aristotle University of Thessaloniki.

2.2. Materials

Five, commercial available, types of Greek table olives were purchased from the local market in Athens. *Kalamon:* Big, black color, elongated form olive fruit, in brine, *Tsakistes:* Small olive fruit, green color in brine, *Amfissas:* Big, black color olive fruit in brine, *Crete:* Small olive fruit, black color in brine and *Thrubes Crete:* Big, wrinkled, varicolored olive fruit.

2.3. Sample preparation

Certain quantity of table olives from each type was weighted, the flesh was separated from the kernel and then weighted apart to estimate the flesh and kernel

ratio. Then, both were subjected to freeze drying for further extraction and determination of humidity. The dry mass was crushed and stored in clean bottles in refrigeration. The determination of fat in flesh and kernel was performed by repeated extractions (Soxhlet method) with petroleum ether for 4 h. Finally the petroleum ether was evaporated in rotary evaporator.

2.4. Extraction of polyphenols/total polyphenol content

The procedure for the extraction of polyphenols it was the same in kernel and flesh. A quantity (0.5 g) of dry sample was extracted five times with 5 mL methanol, the extracts were combined, methanol was evaporated under nitrogen and the residue was dissolved in 5 mL methanol. The methanolic extraction has been reported to be superior to the mixtures of methanol/water (8/2) (Owen et al., 2000). Aliquots of these solutions were used for the determination of total phenol content with the Folin–Ciocaulteu assay according to the method of Gutfinger (Gutfinger, 1980). The olive extracts, from flesh and kernel, in methanol solution were stored in refrigeration for the determination of total antioxidant capacity. All the spectrophotometric data were acquired using an Uvikon 931 (Contron, Milano, Italy) UV–Vis spectrophotometer and all the experiments were performed in duplicate.

2.5. Total antioxidant capacity

The effect of the five methanol extracts from dry flesh and kernel, on DPPH· was estimated according to the procedure described by Brand-Williams et al. (1995). DPPH· is a stable radical in methanol solution. Extracts of antioxidants scavenge the DPPH· and the reduction of DPPH· is monitored by the decrease of the absorbance at 515 nm. The color from purple, in the initial solution, turns into yellow when all the amount of the free radical is blocked by the antioxidants. An aliquot of methanol solution (0.1 mL) containing different concentrations (1:2 serial dilutions from initial sample) of flesh and kernel extracts, was added to 3.9 mL of DPPH solution (6×10^{-5} M in methanol), mixed well and the absorbance was measured every 15 min until the reaction reached the plateau. The absorbance of the DPPH· without any antioxidant in methanol (control) was measured daily and kept in dark. The obtained data were used to determine the μg of polyphenols required to scavenge 50% of the DPPH· (EC_{50}). The percentage of remaining DPPH· was calculated as follows:

$$\%\ \mathrm{DPPH^{\bullet}}\ \text{remaining} = [\mathrm{DPPH^{\bullet}}]_t / [\mathrm{DPPH^{\bullet}}]_{t=0},$$

where t is the time necessary to reach the plateau. These values were plotted against μg of extract to obtain the amount of antioxidant necessary to decrease the initial DPPH· concentration by 50% (EC_{50}) using an exponential curve. All spectrophotometric data were acquired with the UV–Vis Spectrophotometer mentioned in 2.4 and the experiments were performed in duplicate.

2.6. Gas chromatography/mass spectrometry analysis

An Agilent (Wallborn, Germany) HP series GC 6890 coupled with a HP 5972 MS detector (EI, 70 eV), split–splitess injector and an HP 7673 autosampler were used for analysis. Prior to GC analysis 0.5 mL of methanol solution of extracts were evaporated to dryness under nitrogen and were derivatized by addition of 400 μL BSTFA and 10 μL TMCS at 70 °C for 30 min (Soleas, Diamandis, Karumanchiri, & Goldberg, 1997). An aliquot (3 μL) of each sample was injected into the gas chromatograph. Separation of sample was achieved using an HP-5 MS capillary column (5% phenyl–95% methyl siloxane, 30 m × 0.25 mm × 250 μm). Helium was used as a carrier gas at a flow rate 0.6 mL min^{-1}. The injector and transfer line temperature were set at 280 and 300 °C respectively. The oven temperature program was: initial temperature 70, 70–130 °C at 5 °C/min, 130–170 °C at 4 °C/min, held for 15 min and finally 170–300 °C at 10 °C/min where held for 30 min. A Single Ion Monitoring (SIM) GC/MS method was applied for detection of 13 target polyphenolic compounds. Detection of polyphenols was based on the +0.05 RT presence of target and qualifier ions of the standard polyphenols at the predetermined ratios. Target and qualifier ions (*T*, Q1, Q2) for the 13 polyphenolic compounds were set as following: cinnamic acid: *205*, 131, 103, tyrosol: *179*, 193, 282, *p*-Hydroxy-benzoic-acid: *267*, 223, 193, *p*-Hydroxy-phenyl-acetic acid: *179*, 296, 164, *p*-Hydroxy-phenyl-propanoic acid: *179*, 192, 310, vannilic acid: *297*, 267,312, Hydroxytyrosol: *267*, 193, 370, protocatechuic acid: *370*, 355,193, 3,4-dihydroxy-phenyl-acetic acid: *179*, 267, 384, *p*-coumaric acid: *308*, 293, 219, gallic acid: *458*, 281, 179, ferulic acid: *338*, 323, 308, caffeic acid: *219*, 396, 381 and oleanolic acid: *203*, 320, 482 (Kaliora, Mylona, Chiou, Petsios, & Andrikopoulos, 2004). Quantitation was performed based on a series of five external standards of the polyphenolic mixture. Correlation coefficients (R^2) varied from 0.9892 for ferulic acid to 0.999 for tyrosol and *p*-coumaric acid. Two control standards (low and high) were analyzed every 10 samples. Linearity was obtained in the range of quantitation limit in the samples analyzed and up to twentyfold concentration for each compound.

3. Results and discussion

The table olives and the characteristics of each type are presented in Table 1. The percentage of flesh in table olives varies between 76% and 82% and of kernel between 14% and 24%. The highest flesh/kernel ratio ap-

Table 1
Basic characteristics of table olives

Type	Characteristics	Mass (%) of one piece		Humidity (%)		Fat (%)	
		Flesh	Kernel	Flesh	Kernel	Flesh	Kernel
Tsakistes	Small, green colour in brine	76	24	48	26	35	17
Amfissa	Big, black in brine	82	14	71	30	19	8
Kalamon	Big, long, black in brine	81	16	63	35	28	8
Crete	Small, black in brine	82	18	54	26	39	22
Throubes Crete	Big, wrinkled, varicoloured	80	16	21	17	64	19

peared in *Amfissa* type, while the lower ratio in *Tsakistes* type. *Kalamon*, *Crete* and *Thrubes Crete* types appear similar flesh/kernel ratios. The total fat percentage in flesh is higher than that in kernel. The highest percentage of fat in flesh determined in *Thrubes Crete* variety and the lower in *Amfissas*. *Crete* type contains the greatest percentage of fat in kernel as compared to the other kernel types. The percentage of humidity in flesh varies between 21% and 71% and in kernel between 17% and 35%. *Amfissas* cultivar contains the highest percentage of humidity in flesh while *Kalamon* in kernel. *Thrubes Crete* variety contains the lower percentage of humidity in flesh and kernel, and especially in flesh presents considerable difference from the other cultivars.

3.1. Estimation of total polyphenol content

The mean values of total polyphenol content in all cultivars for flesh and kernel are presented in Table 2. The total polyphenol content, was expressed as mg caffeic acid per 100 g of sample (flesh or kernel). *Thrubes Crete* olives contain the higher polyhenol content, while *Amfissas* the lower. In relation to all other types *Thrubes Crete* contain the highest concentration of polyphenols in flesh and the smallest in kernel.

3.2. Evaluation of radical scavenging activity by DPPH· method

The methanol extracts of polyphenols of flesh samples were screened for their antioxidant capacity by DPPH· radical scavenging assay. Considering the study of total antioxidant capacity of samples with the DPPH· scavenging method, the lower the quantity of polyphenols required for the scavenging of half quantity of DPPH·, the higher the antioxidant capacity of sample, meaning that smaller quantity of polyphenols bounds the same quantity of DPPH· Table 3 presents the quantity of polyphenols (μg) and the respective quantity of table olive flesh (g), needed to decrease the initial DPPH· concentration to 50% (EC_{50}). The extracts of all cultivars, except *Thrubes Crete*, displayed high antioxidant capacity. The consumption of table olive needed to achieve the EC_{50} is similar for all cultivars, with the exception of *Thrubes Crete* type, where larger quantity is needed to display the same antioxidant capacity as that revealed by the other cultivars. According to these results, the total antioxidant capacity follows the order: *Tsakistes* > *Amfissas* > *Kalamon* > *Crete* > *Thrubes Crete*. Fig. 1 shows the reduction curve of the % remaining DPPH· as a function of polyphenols quantity (μg). *Thrubes Crete* type contains the highest polyphenol content according to Folin–Ciocaulteu assay while shows the lower antioxidant activity than the other cultivars tested. These contradictory results are explained by the fact that *Thrubes Crete* type contains the lower percentage of humidity as well as the lowest content in hydroxytyrosol and tyrosol.

Table 2
Total polyphenol (PP) content by Folin–Ciocalteau assay, expressed in mg caffeic acid per 100 g of flesh or kernel

Type	Flesh	Kernel
Tsakistes	145	256
Amfissa	82	122
Kalamon	155	234
Crete	130	127
Thrubes Crete	171	51

Table 3
Quantity of polyphenols (PP) and the respective quantity of olive flesh needed to decrease the initial DPPH· concentration by 50% (EC_{50})

Type	EC_{50} (μg PP)	Quantity of flesh (g)
Tsakistes	30	0.02
Amfissa	32	0.04
Kalamon	33	0.02
Crete	52	0.04
Thrubes Crete	587	0.3

3.3. Identification and quantification of phenolic compounds

The qualitative and quantatitive determination of polyphenols was performed both in flesh and kernel. Identification of chromatographic peaks was made by comparing the retention times and ratios of three fragment ions of each polyphenolic compound with those of reference compounds (Kaliora et al., 2004). Table 4 presents the results of GC/MS analysis in flesh and kernel for all examined types of greek table olives. The corresponding analytical chromatograms from flesh and

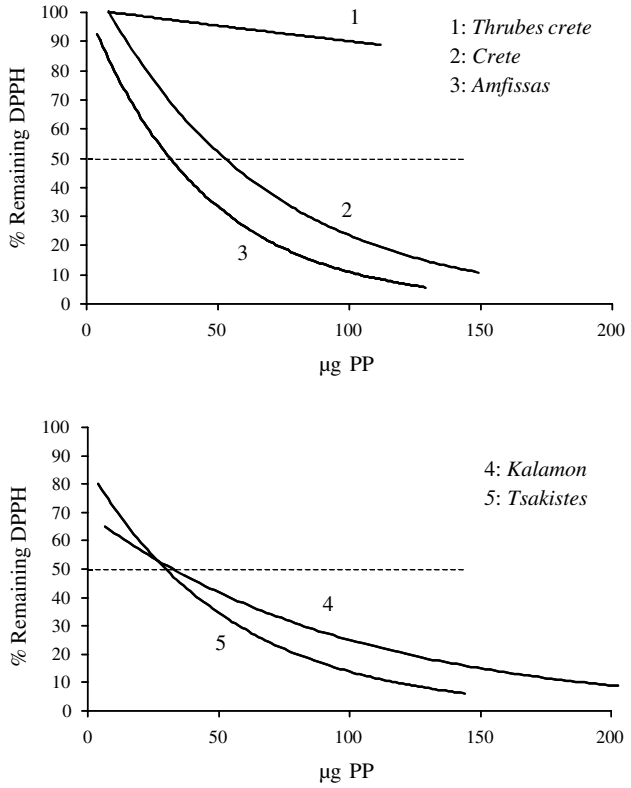

Fig. 1. The reduction curve of the % remaining DPPH· as a function of the containing amount of polyphenols (µg PP) in flesh for the five different table olive type.

kernel of *Kalamon* type are presented in Fig. 2, while the other types resulted similar TIC (Total Ion Chromatograms). GC/MS analysis resulted in determination of a mix of phenols and phenolic acids in both flesh and kernel of all olive types examined.

In the flesh samples, hydroxytyrosol presents the highest levels followed by oleanolic acid and tyrosol. *Tsakistes* cultivar have the highest concentration of hydroxytyrosol (114 mg/100 g), were *Thrubes Crete* have the lower hydroxytyrosol content (2 mg/100 g). The levels of oleanolic acid range between 12 and 38 mg/100 g and tyrosol between 1 and 21 mg/100 g. On the other hand in kernel samples, hydroxytyrosol presents the highest level from all the others polyphenols (0.7–81 mg/100 g) followed by tyrosol (0.7–22 mg/100 g). In *Thrubes Crete* the less phenolic compounds were determined, and in very low concentrations both in flesh and kernel. The richest qualitative and quantatitive phenolic content was found in *Tsakistes* cultivar followed by *Amfissas*, *Kalamon*, *Crete*, and *Thrubes* Crete type in decreasing order. Both in flesh and kernel have been identified the phenols tyrosol, hydroxytyrosol, and the phenolic acids, caffeic acid, ferulic acid, *p*-hydroxy-benzoic acid, *p*-hydroxy-phenyl-acetic acid, *p*-hydroxy-phenyl-propanoic acid, protocatechuic acid, *p*-coumaric acid, cinnamic acid, 3,4-dihydroxy-phenyl-acetic acid and vanilic acid, while oleanolic acid was detected only in flesh samples.

Thrubes Crete is a particular variety of olives that become edible by maturing on the olive tree, meaning that they undergo less post harvest treatment. While still on the tree with the influence of sunlight and natural catecholases and other enzymes the original polyphenol content may yield polyphenol derivatives with less antioxidant activity (Ryan et al., 1999; Tomás – Ferreira et al., 2002; Bárberán & Espın, 2001). For that reason the target polyphenols were not detected by the GC/MS method (in SIM mode) and the DPPH· inhibition was not as high as in the other samples. However Folin–Ciocalteau method gave high concentrations of catecholic compounds.

3.4. Nutritional evaluation of polyphenol content

Taking account of the results, table olives, which are available at the common Greek retail markets, are

Table 4
The results of GC/MS analysis in SIM mode in flesh and kernel for the five examined types of greek table olives expressed in mg of each polyphenol per 100 g of flesh or kernel (mg/100 g)

No	Phenolic compound	Retention time (RT)	*Crete*		*Kalamon*		*Amfissa*		*Tsakistes*		*Throubes Crete*	
			Flesh	Kernel	Flesh	Kernel	Flesh	Kernel	Flesh	Kernel	Flesh	Kernel
1	Cinnamic acid	16.70	3	1	2	5	0.9	0.6	4	2	ND	ND
2	Tyrosol	17.40	6	8	14	22	12	7	21	14	0.9	0.7
3	*p*-Hydroxy-benzoic acid	18.75	1	0.6	0.4	0.6	0.5	0.3	0.9	0.4	1	ND
4	*p*-Hydroxy-phenyl-acetic acid	19.07	0.6	1	0.9	0.9	0.5	ND	6	3	ND	ND
5	*p*-Hydroxy-phenyl-propanoic acid	22.54	2	2	7	8	2	6	6	3	0.2	0.4
6	Vanillic acid	22.77	0.2	0.2	0.2	0.2	0.1	0.03	0.3	0.2	0.3	0.1
7	Hydroxy-tyrosol	23.02	21	20	39	81	66	28	114	61	2	0.7
8	Protocatechuic acid	25.10	7	3	1	1	3	ND	2	0.5	4	ND
9	3,4-Dihydroxy–phenyl-acetic acid	25.60	ND	0.8	0.2	0.6	0.04	ND	10	4	ND	ND
10	*p*-Coumaric acid	31.26	1	0.5	0.2	ND	0.1	ND	0.7	0.2	0.7	ND
11	Ferulic acid	38.51	0.04	0.05	ND	ND	ND	0.01	0.04	ND	0.1	ND
12	Caffeic acid	39.54	4	3	0.6	1	0.8	0.6	4	ND	1	ND
13	Oleanolic acid	55.93	25	ND	12	ND	14	ND	25	ND	38	ND
Total			70.8	40.2	67.5	120.5	99.9	42.5	190.3	88.3	48.2	1.9

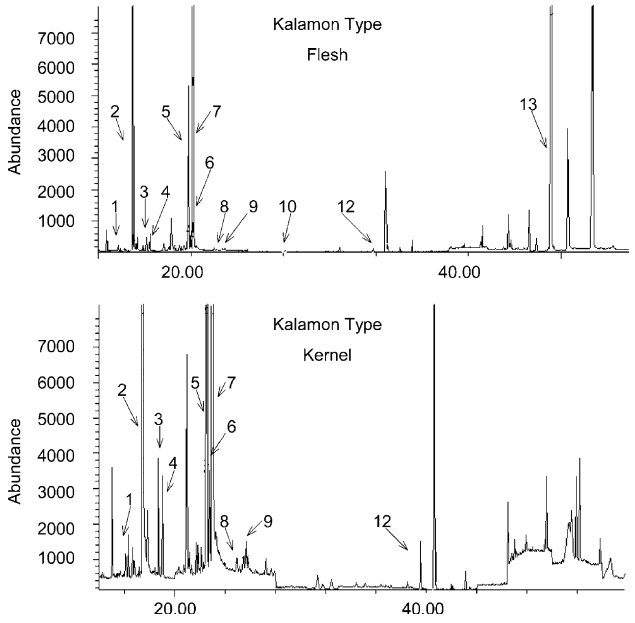

Fig. 2. GC–MS SIM chromatograms of phenolic extracts of the flesh and the kernel of *Kalamon* type with peaks according to Table 4. *Flesh*: 1, cinnamic acid; 2, tyrosol; 3, *p*-hydroxy-benzoic acid; 4, *p*-hydroxy-phenyl-acetic acid; 5, *p*-hydroxy-phenyl-propanoic acid; 6, vanillic acid; 7, hydroxy-tyrosol; 8, protocatechuic acid; 9, 3,4-dihydroxy-phenyl-acetic acid; 10, *p*-coumaric; 11, ferulic acid; 12, caffeic acid; 13, oleanolic acid. *Kernel*: 1, cinnamic acid; 2, tyrosol; 3, *p*-hydroxy-benzoic acid; 4, *p*-hydroxy-phenyl-acetic acid; 5, *p*-hydroxy-phenyl-propanoic acid; 6, vanillic acid; 7, hydroxy-tyrosol; 8, protocatechuic acid; 9, 3,4-dihydroxy-phenyl-acetic acid; 12, caffeic acid.

generally a very good source of polyphenols independently of cultivar. The consumption of 50 g (approximately 10 table olives) of table olive provides about 56 mg polyphenols from flesh. Thus, the consumption of table olives in combination with the consumption of olive oil, which are basic components of Mediterranean diet, provide a large amount of natural antioxidants as compared to the 23 and 28 mg of flavones and flavanones intake per day for Netherlands and Denmark, respectively, and of 115 mg per day for the United States, as reviewed by Ross and Kasum (2002). The beneficial health effects of olive oil have been reported and documented (Visioli & Galli, 2002), however little is known about the beneficial health effect of table olives. Therefore, given that the table olives are consumed a lot, mainly by Mediterranean population, and the consequent benefit from polyphenol intake is given, the phenolic fraction of table olives merits to be placed under further investigation.

4. Conclusions

- According to our results, all types of table olives have a similar phenolic profile with polyphenols in different quantities varying according to type.
- The total polyphenol content of phenolic compounds in all varieties is distinctly different in flesh and kernel except of Crete type.
- GC/MS qualitative analysis revealed in all varieties a sum of 13 different polyphenols ranged from 10 to 13 species for each table olive type.
- The qualitative and quantitative content of polyphenols in each type of table olives differentiates the total antioxidant capacity.
- About 5–10 table olives might cover the daily intake of polyphenols.

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