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Currants (*Vitis vinifera* L.) content of simple phenolics and antioxidant activity

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Abstract

Currant (*Vitis vinifera* L.) is a dried vine product, produced almost exclusively in Southern Greece and used in the Mediterranean diet. The three different commercial sub-varieties of currants, namely *Vostizza*, *Gulf*, and *Provincial* were investigated for their total polyphenol content, individual phenolic compounds, and total antioxidant capacity. Total polyphenol content was estimated with the Folin-Ciocalteau assay while identification and quantification of individual target polyphenolic compounds was performed by gas chromatography/mass spectrometry. Total polyphenol content ranged between 151 mg/100 g and 246 mg/100 g currants. Among the polyphenols studied, 17 were identified in *Gulf* and *Provincial* currants and 16 in *Vostizza* currants. The predominant polyphenolic compound in all currants was vanillic acid at mean concentration 1.21 ± 0.23 mg/100 g currants. Caffeic acid, gallic acid, syringic acid, *p*-coumaric acid, protocatechuic acid, ferulic acid, and quercetin also predominated. The antioxidant activity of currants was assessed by the 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging assay. The amount of currants needed to scavenge 50% of DPPH radical (EC_{50}) was similar for all sub-varieties ranging from about 4 mg to 6.5 mg. Total antioxidant capacity is in descending order *Vostizza* > *Provincial* > *Gulf*. By the present work, the consumption of currants is considered to contribute to the intake of antioxidants.

Keywords: Currant; Raisins; Dried vine fruits; Polyphenols; Antioxidants; Antioxidant capacity

1. Introduction

Polyphenols are naturally occurring substances essentially in all plant material, and prominently ubiquitous in fruits, vegetables, seeds, and herbs but also in plant products, such as beverages, wine, cocoa (Bravo, 1998). Phenolic compounds are potent antioxidants that play an important role in human nutrition as preventative agents against several diseases, protecting the body tissues against oxidative stress. Epidemiological evidences indicate an inverse relationship between the intake of polyphenol-rich foods and the risk of coronary heart disease as well as some types of cancer (Bravo, 1998; Scalbert, Johnson, & Saltmarch, 2005).

Grapes are a rich source of phenolic compounds (Vinson, Su, Zubik, & Bose, 2001). Grape polyphenols range from simple compounds (monomers) to complex tannin-type substances (oligomers and polymers). Several classes of antioxidant polyphenols have been identified and quantified in grapes, such as phenolic acids (benzoic and hydroxycinnamic acids), stilbene derivatives (resveratrol), flavan-3-ols (catechin, epicatechin), flavonols (kaempferol, quercetin, myricetin), anthocyanins (Bonilla, Mayen, Merida, & Medina, 1999; Borbalán, Zorro, Guillén, & Barroso, 2003; Cantos, Espin, & Tomas-Barberan, 2002; Kallithraka, Mohdaly, Makris, & Kefalas, 2005). Several grape polyphenols are found in the form of esters with tartaric acid, while others are glycosylated. The polyphenolic composition of grape seeds has also been determined (Guendez,

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Kallithraka, Makris, & Kefalas, 2005; Yilmaz & Toledo, 2004). Among others, grape seeds contain gallic acid, flavanols, flavanol oligomers and polymers. Grape polyphenols are extracted into wines during the process of vinification. The polyphenolic content of red and white wines has been investigated in several studies (Del Alamo Sanza, Nevares Domínguez, Cárcel Cárcel, & Navas Gracia, 2004; Garcia-Beneytez, Cabello, & Revilla, 2003; Karagiannis, Economou, & Lanaridis, 2000; Proestos et al., 2005).

Currant (Corinthian raisin, *Vitis vinifera* L.) is a dried vine product that has been cultivated and processed for hundreds of years. Currant production corresponds to approximately 8–10% of dried vine fruits worldwide production, the other dried vine fruits being the raisins and the Sultanas. Raisins are produced mainly in California while Sultanas are produced mostly in Turkey, Iran, Australia, South Africa, Chile, and Greece. In the dried vine products market currant holds the highest price, possibly due to its shortage, elaborated cultivation, and drying procedures adopted but also due to its distinct colour and aroma. Currants are small sun-dried berries, coloured black to dark blue, produced almost exclusively in Southern Greece. They are classified in two main quality categories. The highest quality category is produced in north Peloponesse and comprises two subcategories, namely *Vostizza* currant that holds a PDO name and *Gulf* currant. The second category (*Provincial* currant) is produced in western Peloponesse and in two Ionian islands, Zante and Cephalonia. Quality categorization is related to product properties as well as to the applied agricultural practices and degree of cleanness and uniformity of the product.

Although grapes and wines have been widely studied, little research has been conducted for dried vine fruits with respect to their polyphenol content and antioxidant activity. In the study of Karadeniz, Durst, & Wrolstad (2000) the polyphenolic composition of sun-dried, dipped, and golden raisins obtained from Thompson seedless grapes (*V. vinifera* L. cv. sultanina) was reported. The raisins studied were found to contain oxidized cinnamics, caftaric acid, coutaric acid, protocatechuic acid, quercetin and kaempferol glycosides, and rutin. To our knowledge the antioxidant content of currants, another product of the Mediterranean diet, has not been studied yet.

The aim of this study was to determine the total phenol content of currants, to perform qualitative and quantitative analysis of currant polyphenols and to investigate the antioxidant capacity of the phenol content of three sub-varieties of currants.

2. Materials and methods

2.1. Reagents

Methanol and ethyl acetate of analytical grade, Folin-Ciocalteau reagent, and sodium carbonate were obtained from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$), bis-(trimethylsily)-trifluoroacetamide (BSTFA), *trans*-cinnamic acid, and *p*-hydroxyphenyl-propanoic acid (phloretic acid) were obtained from Aldrich (Steinheim, Germany). Caffeic acid, 3,4-dihydroxyphenylacetic acid, catechin, epicatechin, sinapic acid, tyrosol, and protocatechuic acid were obtained from Fluka (Steinheim, Germany). 4-Hydroxy-benzoic acid, *p*-hydroxy-phenylacetic acid, *p*-coumaric acid, vanillin, quercetin, syringic acid, gallic acid, resveratrol, kaempferol, and ferulic acid, were obtained from Sigma (Steinheim, Germany). Vanillic acid was obtained from Serva (Heidelberg, Germany). Solid phase extraction (SPE) columns, Isolute C8(EC) (500 mg, 3 mL) were purchased from International Solbent Technologies (Mid Glamorgan, UK).

2.2. Materials

Nine commercially available batches of currants (*V. vinifera* L.) originated from three regions of Southern Greece and corresponding to the three existing currant sub-varieties were provided by local processors: *Gulf*: three batches (from sub-areas of Nemea, Kiato, Xylokastro, namely G1, G2, G3, respectively), *Vostizza*: three batches (namely V1, V2, V3, respectively), and *Provincial*: two batches from western Peloponesse (one batch from the region of Olympia-Pyrgos, designated as O, and one from the region of Messinia designated as M) and one batch from an ionian island (island of Zante, designated as Z).

2.3. Sample preparation

A quantity of currants (approximately 20 g) from each batch was weighted, mechanically homogenized and subjected to freeze drying. The dry mass was stored in clean sealed bottles at 4 °C until analysis. The process was duplicated. All analyses were carried out in freeze dried samples. Results were subsequently expressed on fresh weight basis by means of water content. Water content of currants studied ranged from 5.4% (*Vostizza* batch V1) to 14.2% (*Vostizza* batch V2).

2.4. Polyphenol extraction

A weighed quantity of each dry homogenized sample (approximately 0.5 g) was mixed with methanol (5 mL), placed in a sonicator bath for 15 min and left for 24 h under stirring at room temperature. The mixture was then centrifuged at 3000 rpm for 5 min and methanol extract was collected. The remaining residue was extracted four additional times with methanol (5 mL). For each of the latter four extractions the mixture was placed in a sonicator bath for 15 min and then stirred for 10 min. All extracts were combined, methanol was evaporated under reduced pressure and the residue was dissolved in methanol (1 mL). Aliquots of these solutions (0.2 mL) were stored at 4 °C for the determination of total phenol content and

total antioxidant capacity. Subsequently, methanol was removed under a stream of nitrogen and the residue was reconstituted in bi-distilled water for further analysis. Except methanol a less polar solvent (ethyl acetate) and a more polar one (methanol/water 8:2) have also been used as extraction solvents, with methanol being the most appropriate for the extraction.

2.5. Total polyphenol content

Total polyphenols were determined in the methanol extracts of currants with the Folin-Ciocaulteu assay according to the method of Gutfinger (1980). Gallic acid was employed as calibration standard and results were expressed as gallic acid equivalents (GAE) (mg GAE/100 g of currants). All spectrophotometric data were acquired using an Uvikon 931 (Contron, Milan, Italy) UV–vis spectrophotometer. Each experiment was duplicated.

2.6. Total antioxidant capacity

The effect of currants methanol extracts on DPPH· was estimated according to the procedure described by Brand-Williams, Cuvelier, & Berset (1995). Briefly, each initial methanol extract was diluted 1:5 with methanol before analysis (working solution). Then an aliquot of working solution was subjected to six 1:2 serial dilutions with methanol. An aliquot (0.1 mL) of each of the seven solutions obtained (working solution plus six diluted solutions) was added to 3.9 mL of DPPH solution (6×10^{-5} M in methanol), thoroughly mixed, and the absorbance was measured after 30 min, that is when, according to literature, the reaction reached the plateau. The absorbance of DPPH· solution in methanol without any antioxidant (control) was also measured. The obtained data were used to determine the quantity (mg) of currants required to scavenge 50% of DPPH· (EC_{50}). The percentage of remaining DPPH· was calculated as follows:

$$\%\ \text{DPPH}^{\cdot}\ \text{remaining} = A_t \times 100/A_c$$

where A_t is the absorbance of sample after the time necessary to reach the plateau (30 min), A_c is the absorbance of control. These values were plotted against quantity of currants to obtain the currants amount 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 (515 nm). Experiments were performed in duplicate.

2.7. Polyphenol isolation

The isolation of currant polyphenols was performed by SPE of the extracts (obtained as described in Section 2.4) on an Isolute C8 column according to Soleas, Diamandis, Karumanchiri, & Goldberg (1997). Briefly, the SPE columns were washed with ethyl acetate (3 mL), methanol (3 mL), and bi-distilled water (5 mL), followed by the deposition of sample (0.5 mL). Solvent was allowed to dry under reduced pressure and polyphenols, retained in the column, were eluted with ethyl acetate (3 mL). Solvent was evaporated under a stream of nitrogen and the residue was redissolved in methanol (0.5 mL). A SPE vacuum device, which allowed handling 20 samples simultaneously, was used.

2.8. Acid hydrolysis of currant extracts

An aqueous solution of hydrochloric acid (3 M, 0.25 mL) was added to 0.5 mL of currant extract (Section 2.4). The mixture was maintained at 80 °C for 1 h. After cooling 0.5 mL of potassium hydrogen phosphate (1 M) were added, followed by SPE (as described in Section 2.7). This procedure was adopted in order to investigate the presence of conjugated forms of polyphenols, i.e. esters or glycosides, that would not be identified by GC/MS. The hydrolyzed residues were analyzed by GC/MS.

2.9. Gas chromatography/mass spectrometry analysis

An Agilent (Wallborn, Germany) series GC 6890N coupled with a HP 5973 MS detector (EI, 70 eV), split–splitess injector and a HP 7683 autosampler were used for analysis. Prior to GC analysis 0.1 mL of the obtained SPE extract was mixed with internal standard, 3-(4-hydroxy-phenyl)-1-propanol (19.2 μg/mL, 25 μL), evaporated to dryness under a stream of nitrogen, and derivatized by adding 250 μL BSTA followed by incubation at 75 °C for 20 min (Soleas et al., 1997). An aliquot (1 μL) of each sample was injected into the gas chromatograph at a split ratio 1:20. Separation of sample was achieved using an HP-5 MS capillary column (5% phenyl–95% methyl siloxane, 30 m × 0.25 mm × 250 μ). 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 °C and 300 °C, respectively. The oven temperature program was: initial temperature 70 °C for 5 min, 70–130 °C at 15 °C/min, 130–160 °C at 4 °C/min, held for 15 min, 160–300 °C at 10 °C/min, and finally held at 300 °C for 15 min. A selective ion monitoring (SIM) GC/MS method was applied for detection of 20 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 20 polyphenolic compounds were set as following: vanillin: 194, 209, cinnamic acid: 205, 220, tyrosol: 179, 267, 282, *p*-hydroxy-benzoic acid: 267, 223, 193, *p*-hydroxy-phenylacetic acid: 252, 296, 281, *p*-hydroxy-phenyl-propanoic acid: 179, 192, 310, vannilic acid: 297, 267, 312, protocatechuic acid: 193, 355, 370, 3,4-dihydroxy-phenylacetic acid: 384, 267, 179, *p*-coumaric acid: 308, 293, 219, gallic acid: 281, 458, 443, ferulic acid: 338, 323, 308, syringic acid: 327, 342, 312, caffeic acid: 396, 219, 381, sinapic acid: 368, 353, 338, resveratrol: 444, 445, 443, epicatechin: 368, 355, 474, catechin: 368, 355, 474, kaempherol: 559, 560, and

quercetin: 647, 559, 575. The terpenic acid, oleanolic acid, was also detected as following: oleanolic acid: 203, 320, 482 (Kaliora, Mylona, Chiou, Petsios, & Andrikopoulos, 2004).

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, while quantification was carried out by using 3-(4-hydroxy-phenyl)-1-propanol as internal standard at target ion m/z 206 and qualifiers 191 and 179.

Polyphenols were quantified by internal standard technique on currant matrix. For this purpose, standard curves were obtained after fortification of currant extracts with four different standard polyphenol concentrations followed by SPE. Polyphenols were added to the currants extract as a solution in methanol that was subsequently removed under a stream of nitrogen. Currant extract polyphenols quantity was calculated by extrapolation of the above standard curves. The construction of standard curves, used for the polyphenols quantification, was obtained after adding values derived from the extrapolation to the fortified ones. Linearity was obtained for all target phenolic compounds in the fortification range.

2.10. Statistical analysis

Statistical analysis was performed based on one way ANOVA Dunkan multiple range test, at confidence level 95%.

3. Results and discussion

3.1. Estimation of total polyphenol content

The total polyphenol content of the three currants sub-varieties is presented in Table 1. It was estimated according to the Folin-Ciocalteau assay and expressed as mg GAE/100 g of currants. Among the different batches studied, *Vostizza* currants revealed the greater variation, having both the highest and the lowest polyphenolic content. Considering the mean values of total polyphenol content for each sub-variety, *Provincial* currants contained the higher one. In the study of Pastrana-Bonilla, Akoh, Sellappan, & Krewer (2003) the phenolic content of muscadine grapes was reported. Total phenolics were measured separately for skins and pulp and the average values were 414.1 ± 119.1 mg GAE/100 g fresh weight and 23.3 ± 1.5 mg GAE/100 g fresh weight, respectively. Considering the fact that grape skins account for a small percentage of the whole grape, these values are analogous to the values found in this study for currants.

3.2. Evaluation of radical scavenging activity by the DPPH· method

The antioxidant capacity of currant methanol extracts was screened by the DPPH· radical scavenging assay. In this assay the lower the quantity of sample required for the scavenging of half quantity of DPPH· the higher the antioxidant capacity, since a smaller quantity of sample is required to bind the same quantity of DPPH·. The amount of currant methanol extract used for DPPH· scavenging was assigned to the initial amount of currants extracted, taking also into account the water loss during freeze drying. In Table 2 the quantity of currants (mg) needed to decrease the initial DPPH· concentration by 50% (EC_{50}) is presented. The amount of currants needed to achieve EC_{50} was similar for all cultivars ranging from 3.8 mg for *Vostizza* currants sample V2 to 6.5 mg for *Provincial* currants from Messinia (M).

Table 1
Total polyphenol (PP) content by Folin-Ciocalteau assay, expressed as gallic acid equivalents (GAE mg) per 100 g currants (*Vitis vinifera* L.)

Type	Sample	Region of origin	mg GAE/100 g
Vostizza	V1[a]	Vostizza, packed	246
	V2[a]	Vostizza, bulk	151
	V3[a]	Vostizza, bulk	155
	Mean value ($n = 6$)[b]		188 ± 50
Gulf	G1[a]	Xylokastro, bulk	188
	G2[a]	Kiato, bulk	190
	G3[a]	Nemea, bulk	167
	Mean value ($n = 6$)[b]		184 ± 17
Provincial	O[a]	Olympia, bulk	201
	M[a]	Messinia, bulk	205
	Z[a]	Zante, bulk	211
	Mean value ($n = 6$)[b]		206 ± 22
Average	Mean value ($n = 18$)[b]		191 ± 26

[a] Average value of two independent measurements.
[b] Mean value ± SD, n = number of samples.

3.3. Identification and quantification of phenolic compounds

Qualitative and quantitative determination of polyphenols was performed by GC/MS analysis after SPE of currants methanol extracts. The SPE procedure was adopted since in methanol extracts several compounds, mainly car-

Table 2
Quantity of currants (*Vitis vinifera* L.) needed to decrease the initial DPPH* concentration by 50% (EC_{50})

Type	Sample	Quantity of currants (mg)
Vostizza	V1[a]	4.3
	V2[a]	3.8
	V3[a]	6.2
	Mean value ($n = 6$)[b]	4.9 ± 1.5
Gulf	G1[a]	5.9
	G2[a]	5.3
	G3[a]	5.8
	Mean value ($n = 6$)[b]	5.6 ± 0.5
Provincial	O[a]	5.0
	M[a]	6.5
	Z[a]	4.9
	Mean value ($n = 6$)[b]	5.4 ± 1.5

[a] Average value of two independent measurements.
[b] Mean value ± SD, n = number of samples.

bohydrates, eluted as overlapping peaks to the ones of polyphenols. Moreover, this sample clean-up was followed to avoid sililation reagent consumption by carbohydrates. Internal standard quantification was performed based on standard curves obtained in currant matrix, in order to avoid recovery experiments in a range of polyphenol concentrations.

Results obtained for each batch of currants sub-varieties analyzed are presented in Table 3. A typical chromatogram obtained for *Vostizza* currants batch 1 is presented in Fig. 1. Among the polyphenols studied 17 were identified and quantified in *Gulf* and *Provincial* currants and 16 in *Vostizza* currants. In all studied samples no differentiation on the kind of polyphenols present was observed, with the exception of 3,4-dihydroxy-phenylacetic acid that was absent from all the samples of *Vostizza* currants studied. Vanillic acid was the predominant polyphenol among the polyphenols identified and quantified by GC/MS at mean concentration 1.21 ± 0.23 mg/100 g. Caffeic acid, gallic acid, syringic acid, *p*-coumaric acid, protocatechuic acid, ferulic acid, and quercetin were also predominating. Caffeic acid concentration was found higher in *Provincial* currants (0.83 ± 0.29 mg/100 g) than in the other two varieties. *p*-Hydroxy-benzoic acid (0.32 ± 0.06 mg/100 g), *p*-hydroxy-phenylacetic acid (0.17 ± 0.03 mg/100 g), protocatechuic acid (0.64 ± 0.05 mg/100 g), and gallic acid (0.95 ± 0.42 mg/100 g) were also found in higher concentration in *Provincial* currants. Resveratrol has gained considerable interest, since this compound has been found to have anticarcinogenic properties (Ider et al., 2000; Jang et al., 1997). Resveratrol was identified and quantified in all currants at mean concentration 0.19 ± 0.07 mg/100 g. Although not quantified, in all currants studied catechin and epicatechin were detected. The presence of the triterpenic acid oleanolic acid was also verified by GC/MS analysis (Fig. 1). In the study of Karadeniz et al. (2000) the concentration of protocatechuic acid in sun-dried raisins and dipped raisins was 0.68 ± 0.11 mg/100 g and 0.28 ± 1.7 mg/ 100 g, respectively, while it was not detected in golden raisins. This value is comparable to the one quantified in currants (average value 0.44 ± 0.16 mg/100 g). Quercetin glycosides and kaempferol glycosides were also quantified at overall concentrations that ranged between 0.42–0.79 mg/100 g and 0.14–0.46 mg/100 g, respectively. In currants the concentration of free quercetin (average value 0.33 ± 0.11 mg/100 g) and kaempferol (average value 0.06 ± 0.04 mg/100 g) is lower. Since the contribution of potential glycosidic forms of these phenolics was not evaluated in the present study their overall concentration in currants might be higher. Resveratrol, quantified in currants, was absent from the raisins in the study of Karadeniz et al. (2000).

The sum of the individual polyphenols identified and quantified by GC/MS ranged from 4.81 ± 0.99 mg/100 g to 6.71 ± 2.03 mg/100 g currants (mean value $5.60 \pm$

Table 3
Content of individual polyphenolic compounds (mg/100 g currants) identified and quantified in currants (*Vitis vinifera* L.) sub-varieties (*Vostizza*, *Gulf* and *Provincial*)

Polyphenols	Sub-variety												Average ($n = 18$[b])
	Vostizza				Gulf				Provincial				
	Samples			Mean ($n = 6$[b])	Samples			Mean ($n = 6$[b])	Samples			Mean ($n = 6$[b])	
	V1[a]	V2[a]	V3[a]		G1[a]	G2[a]	G3[a]		O[a]	M[a]	Z[a]		
1. Vanillin	0.14	0.16	0.15	0.15 ± 0.01^{A}	0.17	0.24	0.21	0.21 ± 0.05^{AB}	0.51	0.28	0.22	0.34 ± 0.19^{B}	0.23 ± 0.13
2. Cinnamic acid	0.17	0.16	0.16	0.16 ± 0.01^{A}	0.17	0.16	0.16	0.16 ± 0.01^{A}	0.17	0.16	0.17	0.17 ± 0.01^{A}	0.16 ± 0.01
3. Tyrosol	0.08	0.08	0.08	0.08 ± 0.00^{A}	0.16	0.10	0.11	0.12 ± 0.04^{A}	0.13	0.16	0.09	0.13 ± 0.05^{A}	0.11 ± 0.04
4. *p*-Hydroxy-benzoic acid	0.11	0.16	0.16	0.14 ± 0.03^{A}	0.22	0.20	0.23	0.22 ± 0.04^{B}	0.33	0.34	0.29	0.32 ± 0.06^{C}	0.23 ± 0.08
5. *p*-Hydroxy-phenylacetic acid	–	–	0.14	0.05 ± 0.07^{A}	0.16	0.15	0.08	0.13 ± 0.06^{B}	0.16	0.20	0.15	0.17 ± 0.03^{B}	0.12 ± 0.07
6. Phloretic acid	0.12	0.11	0.11	0.11 ± 0.00^{A}	0.12	0.12	0.12	0.12 ± 0.00^{A}	0.12	0.06	0.11	0.10 ± 0.05^{A}	0.11 ± 0.03
7. Vannilic acid	1.09	1.55	1.08	1.24 ± 0.25^{A}	1.05	1.19	1.28	1.18 ± 0.17^{A}	1.34	1.24	1.02	1.20 ± 0.31^{A}	1.21 ± 0.23
8. Protocatechuic acid	0.21	0.32	0.30	0.28 ± 0.06^{A}	0.47	0.40	0.36	0.41 ± 0.10^{B}	0.66	0.63	0.63	0.64 ± 0.05^{C}	0.44 ± 0.16
9. 3,4-Dihydroxy-phenylacetic acid	–	–	–	$-^{A}$	0.12	0.06	0.06	0.08 ± 0.06^{B}	0.12	0.14	0.12	0.13 ± 0.01^{C}	0.10 ± 0.06
10. Syringic acid	0.23	0.49	0.31	0.34 ± 0.12^{AB}	0.37	0.33	0.57	0.42 ± 0.17^{B}	0.30	0.26	0.21	0.26 ± 0.07^{A}	0.34 ± 0.13
11. *p*-Coumaric acid	0.35	0.30	0.30	0.32 ± 0.03^{A}	0.28	0.29	0.37	0.31 ± 0.07^{A}	0.53	0.29	0.50	0.44 ± 0.19^{A}	0.36 ± 0.12
12. Gallic acid	0.39	0.63	0.53	0.52 ± 0.15^{A}	0.58	0.86	0.39	0.61 ± 0.31^{AB}	1.10	0.51	1.26	0.95 ± 0.42^{B}	0.69 ± 0.34
13. Ferulic acid	0.30	0.30	0.29	0.29 ± 0.01^{A}	0.28	0.28	0.33	0.29 ± 0.03^{A}	0.40	0.33	0.40	0.38 ± 0.08^{B}	0.32 ± 0.06
14. Caffeic acid	0.63	0.51	0.50	0.55 ± 0.08^{A}	0.61	0.53	0.42	0.52 ± 0.12^{A}	0.73	0.58	1.19	0.83 ± 0.29^{B}	0.63 ± 0.22
15. Resveratrol	0.13	0.15	0.14	0.14 ± 0.01^{A}	0.29	0.21	0.10	0.20 ± 0.10^{AB}	0.28	0.21	0.21	0.23 ± 0.07^{B}	0.19 ± 0.07
16. Kaempherol	0.02	0.07	0.06	0.05 ± 0.02^{A}	0.04	0.04	0.04	0.04 ± 0.02^{A}	0.08	0.12	0.04	0.08 ± 0.05^{A}	0.06 ± 0.04
17. Quercetin	0.26	0.48	0.38	0.37 ± 0.11^{A}	0.31	0.28	0.17	0.26 ± 0.13^{A}	0.38	0.36	0.32	0.35 ± 0.10^{A}	0.33 ± 0.11
Sum	4.25	5.47	4.71	4.81 ± 0.99	5.40	5.45	5.00	5.28 ± 1.48	7.35	5.86	6.94	6.71 ± 2.03	5.60 ± 1.00

For sample names see Section 2.2.
[A–C] Mean values in the same row not sharing superscript capital letters are significantly different at confidence level 95%.
[a] Average value of two independent measurements.
[b] Mean value ± SD, n = number of samples.

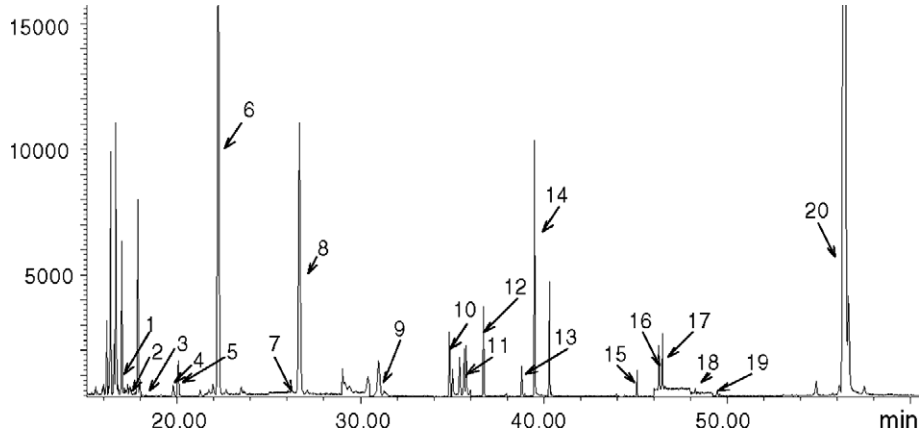

Fig. 1. GC–MS SIM chromatograms of *Vostizza* extract. Peaks: 1, vanillin; 2, cinnamic acid; 3, tyrosol; 4, *p*-hydroxy-benzoic acid; 5, *p*-hydroxy-phenyl-acetic acid; 6, internal standard; 7, phloretic acid; 8, vanillic acid; 9, protocatechuic acid; 10, syringic acid; 11, *p*-coumaric acid; 12, gallic acid; 13, ferulic acid; 14, caffeic acid; 15, resveratrol; 16, epicatechin; 17, catechin; 18, kaempherol; 19, quercetin; 20, oleanolic acid.

1.00 mg per 100 g currants). These values are lower than the ones estimated by the Folin-Ciocalteau assay (191 ± 26 mg GAE/100 g currants). These findings could be partially attributed to the presence of catechin and epicatechin that were not quantified in the present study as well as other polyphenols not identified. In an attempt to evaluate the presence of other conjugated polyphenols, such as esters derivatives, i.e. with tartaric acid or glycosilated, acid hydrolysis of currants extracts was performed. Preliminary results obtained by GC/MS analysis of the hydrolyzed extracts after SPE revealed an increase of the amount of 10 polyphenols, namely caffeic acid, *p*-coumaric acid, ferulic acid, protocatechuic acid, *p*-hydroxy-benzoic acid, syringic acid, vanillic acid, vanillin, *p*-hydroxy-phenyl-propanoic acid, cinnamic acid in decreasing order (data not shown). The presence of caffeoyltartaric and *p*-coumaroyltartaric acid in table grapes (Cantos et al., 2002; Flamini, 2003), raisins (Karadeniz et al., 2000), and wines (Arnous, Makris, & Kefalas, 2001; Borbalán et al., 2003) has already been reported. Thus, the increase in the amounts of these polyphenols might be at least partly attributed to such derivatives. On the other hand, the higher values in the Folin-Ciocalteau assay could be considered quite predictable, since this assay is rather unspecific because compounds such as aromatic amino acids could interfere in the measurement, leading to an overestimation of total phenolic content (Cantos et al., 2002). The presence of tyrosine in raisins has already been confirmed in the study of Karadeniz et al. (2000).

Based on the results of this study, the consumption of 18 g, one micro-portion as defined by the Nutrient database for the 2003 exchange lists for meal planning (Wheeler, 2003), of currants provides approximately 1 mg of polyphenols identified by GC/MS analysis, and 34 mg as estimated by the Folin-Ciocalteau assay. Thus, the consumption of currants in combination with the consumption of other products widely used in the Mediterranean diet, such as olive oil, will provide a large amount of natural antioxidants as compared to the 23 mg 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 & Kasum (2002). The beneficial health effects of grapes and wine have been previously reported and documented, however little is known about the beneficial health effect of currants. Despite their limited region of origin currants are consumed relatively widely, especially in Western Europe. Thus, the phenolic composition of these products merits to be placed under recognition and further investigation.

4. Conclusions

GC/MS qualitative analysis revealed a sum of 17 different polyphenols in currants, ranging from 16 to 17 species for each sub-variety. Among the polyphenols studied, all sub-varieties of currants have a similar phenolic profile. According to currants sub-varieties minor variations on the polyphenols quantity are observed. Based on the results obtained the contribution of currants consumption to the total daily polyphenol intake is important.

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