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Original Article

Investigation on Phenolic and Aroma Compounds of Table Grapes from Romania

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Abstract

The chemical composition of Romanian table grape varieties was determined to evaluate their phenolic and aroma profiles because they are factors useful for the variety characterization and consumer acceptance. Two white table grapes ('Aromat de Iaşi' and 'Timpuriu de Cluj') and two red table grapes ('Napoca' and 'Someşan') were studied. The phenolic composition of berry skins and seeds was determined using spectrophotometric methods. The profile of anthocyanins and hydroxycinnamoyl tartaric acids (HCTs), and the content of *trans*-resveratrol were determined in the skins using high-performance liquid chromatographic (HPLC) methods. Free volatile compounds were quantified by gas chromatography/mass spectrometry (GC/MS). A different phenolic and aromatic composition was found in the varieties studied. Among white grapes, 'Aromat de Iaşi' stands out for the great number of aroma compounds, and 'Timpuriu de Cluj' showed high contents of polyphenols, particularly oligomeric flavanols in skins and seeds (1,171 and 1,189 mg kg^{-1}, respectively) and total skin HCTs (181.6 mg kg^{-1}). The red variety 'Napoca' had high contents of total anthocyanins (380 mg kg^{-1}) and total skin HCTs (183.2 mg kg^{-1}), and it is characterized by high percentages of acylated anthocyanin derivatives (28.5%) and *trans*-caffeoyltartaric acid forms (58.8%). 'Someşan' grapes showed high contents of free volatile compounds, particularly of 1-hexanol, (*Z*)-3-hexen-1-ol and (*E*)-2-hexenoic acid (167.0, 59.4 and 167.0 μg kg^{-1}). These results may contribute to the knowledge of Romanian table grapes perspective and to a better exploitation of these varieties.

Keywords: Romanian table grape varieties, polyphenolics, anthocyanins, hydroxycinnamoyl tartaric acids, flavor compounds

Introduction

Romania is a viticultural country that is member of the International Organization of Vine and Wine (OIV) from 1927, with areas planted with vines accounting for 176 thousands ha in 2014, of which 7183 ha for table grape varieties (INSSE, 2015). A large number of grape biotypes and cultivars are found mainly in the western part of Romania, representing an important source of biodiversity. This variability is also a valuable source for obtaining products with specific local features, which are desired by the consumers (Dobrei *et al.*, 2009).

Romanian table grape varieties were not enough exploited and studied, indeed they deserve more attention. The 'Aromat de Iaşi' cultivar was obtained by open pollination of 'Tămâioasă românească', and homologated in 1980. 'Timpuriu de Cluj' was obtained by controlled sexual hybridization between 'Crâmpoşie' and 'Frumoasă de Ghioroc' varieties. Homologated in 1979, this variety stands out for earliness, resistance to frost and high fertility. 'Napoca' was obtained in 1989 by hybridization of 'Alphonse Lavallee' × ('Regina viilor' × 'Muscat Hamburg') varieties. The 'Someşan' variety was obtained in 1987 by self-pollination of one hybrid elite from 'Muscat Hamburg' × 'Regina viilor'.

Grape phenolic substances or polyphenols include many classes of compounds such as phenolic acids, colored anthocyanins, flavanols, flavonols and stilbenes (Crupi *et al.*, 2012; Urcan *et al.*, 2016). Phenolic compounds content in grapes is variable depending on the variety, grape maturation, climatic conditions, geographical factors and cultural practices (Topalovic *et al.*, 2012). These compounds are responsible for nutraceutical properties of grapes, and health benefits of their consumption have been reported in many studies (Baiano and Terracone, 2011; Georgiev *et al.*, 2014; Topalovic *et al.*, 2012). Biologically active phytonutrients found in grapes have neuroprotective, antimicrobial, cardioprotective and antiaging properties (Heim *et al.*, 2002; Xia *et al.*, 2010).

In table grapes, aroma is a key attribute for consumers. In particular, the free forms of volatile compounds are directly involved in the sensory perception, and therefore it could be interesting to assess the aromatic potential of table grape varieties.

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Alcohols, aldehydes, acids, terpenes, norisoprenoids and benzene compounds have been identified and quantified in table grapes. The aromatic composition is mainly influenced by the variety, but other factors such as ripening, environmental conditions and cultural practices may have a role in the presence of these compounds in the grape berry (Fenoll *et al.*, 2009; Rolle *et al.*, 2015).

The present study represents an initial contribution to the chemical characterization of four table grape varieties from Romania ('Aromat de Iaşi', 'Timpuriu de Cluj', 'Napoca' and 'Someşan'). To our knowledge, there is no scientific literature regarding these varieties, and therefore it could be important to assess their peculiarities in the phenolic and aroma profiles. Total phenols, flavanols, anthocyanins, hydroxycinnamoyl tartaric acids and resveratrol were determined at harvest using spectrophotometric and high-performance liquid chromatographic (HPLC) methods. Free volatile compounds were quantified by gas chromatography/mass spectrometry (GC/MS).

Materials and Methods

Grape samples

The study was carried out in 2011 on 4 table grape cultivars (*Vitis vinifera* L.). Two Romanian white table grape varieties ('Aromat de Iaşi' and 'Timpuriu de Cluj'), and two Romanian red table grape varieties ('Napoca' and 'Someşan') were chosen. The grapes were collected in the collection vineyard of the University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca, Romania, when soluble solids concentration was higher than 14 °Brix. This collection vineyard was planted at 2.5 × 1 m, vertical shoot-positioned, and cane pruned.

For each cultivar, five hundred berries were randomly sampled directly from at least ten plants, from different parts of the cluster (shoulders, middle, and bottom) and with different solar exposure (shaded and sun-exposed). Sets of berries were randomly selected for the determination of the chemical composition of each cultivar. The remaining berries, subdivided into three replicates, were used for determining Brix degrees with a refractometer (Alla France, Chemillé, France) in the grape must obtained by manual crushing and centrifugation.

Chemical analysis

Solvents of HPLC-gradient grade and all other analytical-reagent grade chemicals were purchased from Sigma (Milan, Italy). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, UK). Standards for (+)-catechin, anthocyanins and *trans*-resveratrol were supplied from Extrasynthèse (Genay, France), volatiles from Sigma, and hydroxycinnamoyl tartaric acids (HCTs) from Fluka (Buchs, Switzerland).

Spectrophotometric methods. Three replicates of 10 berries each were randomly sampled and treated as described by Di Stefano and Cravero (1991). Once the 10 berries were weighed, the skins and seeds were obtained, weighed, and placed separately in two different flasks containing 25 mL and 10 mL, respectively, of a hydroalcoholic buffer (12% v/v ethanol, 5 g L^{-1} tartaric acid, 2 g L^{-1} sodium metabisulfite, adjusted to pH 3.2 with 1 mol L^{-1} sodium hydroxide) (Río Segade *et al.*, 2013). The skin extract was obtained by homogenization for 1 min at 8000 rpm using an Ultra-Turrax T25 (IKA Labortechnik, Staufen, Germany) and centrifugation at 3000 × g at 20 °C. The seeds were macerated at 25 °C for 7 days and the extract was then separated for the subsequent analysis (Río Segade *et al.*, 2013).

Phenolic compounds of berry skins (sk) and seeds (s) were determined by spectrophotometry (Di Stefano and Cravero, 1991) using a UV-1800 spectrophotometer (Shimazdu Scientific Instruments Inc., Columbia, MD, USA). Absorbance at 280 nm (A_{280}) was determined directly in the diluted sample. Total anthocyanins index (TAI) was determined using a hydroalcoholic solution (ethanol: water: 37% HCl, 70:30:1 v/v/v) and absorbance was measured at 540 nm. TAI was expressed as mg malvidin-3-glucoside chloride kg^{-1} berries (Di Stefano and Cravero, 1991). Proanthocyanidins (PRO) were determined after acid hydrolysis with heating (Bate-Smith reaction) using a ferrous salt ($FeSO_4$) as catalyst. They were expressed as mg cyanidin chloride kg^{-1} berries (Torchio *et al.*, 2010). Flavanols reactive to vanillin (FRV) were determined following the methodology proposed by Di Stefano and Cravero (1989), and they were expressed as mg (+)-catechin kg^{-1} berries.

Anthocyanin profile. The determination of individual anthocyanins in the skin extracts was performed after reverse-phase solid-phase extraction using a 1-g Sep-Pak C_{18} cartridge (Waters Corporation, Milford, MA) and elution with methanol (Rolle *et al.*, 2013a). The HPLC - Diode Array Detector (DAD) system and chromatographic conditions have been previously described (Rolle *et al.*, 2013a). A LiChroCART analytical column (25 cm × 0.4 cm i.d.) (Merck, Darmstadt, Germany) packed with LiChrospher 100 RP-18 5-μm particles (Alltech, Deerfield, IL) was used. The following mobile phases were used: solvent A, 10% v/v formic acid in water; solvent B, 10% v/v formic acid with 50% v/v methanol in water. The solvent flow rate was 1 mL/min, and the column temperature was 20 °C. A linear gradient was used starting at 72% A and decreasing to 55% A in 15 min, 30% A in 20 min, 10% A in 10 min, 1% A in 5 min and then back to 72% A in 3 min. An equilibrium time of 10 min was used (Rolle and Guidoni, 2007). Data treatment was carried out using the ChromQuest chromatography data system (ThermoQuest, San Jose, CA). Identification of the free forms of anthocyanins was performed by comparison with external standards. The acylated forms of anthocyanins were identified by matching the DAD spectrum at 520 nm and retention time of each chromatographic peak and by comparing these data with those available in the literature (Pomar *et al.*, 2005). The percentages of individual anthocyanins were determined by comparing the area of each individual peak with the total peak area. Each replicate was analyzed in duplicate.

Hydroxycinnamoyl tartaric acids. Three replicates of 10 berries each were processed as described by Ferrandino and Guidoni (2010). The skins were immersed into the hydroalcoholic solution buffered at pH 3.2, and incubated for 72 h at 30 °C. The berry skin extract was diluted 1.1-fold with 1 mol L^{-1} phosphoric acid and filtered through 0.2 μm hydrophilic polypropylene (GHP) membrane filters (Pall Corporation, New York, NY). The HPLC-DAD system and analytical column used for anthocyanins were again used to determine the HCTs. The chromatographic conditions have been previously reported in the literature (Ferrandino *et al.*, 2012). The mobile phases were as follows: A, 10^{-3} mol L^{-1} phosphoric acid; B, methanol. A linear gradient was established between 5 and 100% B over 49 min at a flow rate of 0.48 mL min^{-1}. After the identification on the basis of the DAD spectra at 320 nm and retention times, p-

coumaroyltartaric acid, caffeoyltartaric acid, and *trans*-feruloyltartaric acid were quantified as p-coumaric acid, caffeic acid, and ferulic acid equivalents, respectively. Then, the results were multiplied by the ratio between the molecular weight of each compound and the molecular weight of p-coumaric acid, caffeic acid, and ferulic acid for the p-coumaroyl, caffeoyl, and feruloyl derivatives, respectively. The total HCT concentration (mg kg^{-1} berries) was calculated as the sum of the concentrations of the individual compounds. HCT percentages were calculated. Each replicate was analyzed in duplicate.

trans-Resveratrol. Two replicates of 20 berries each were used. For each replicate, the skins were manually removed from frozen berries and then freeze-dried. 1 g of freeze-dried skins was treated as reported by Vincenzi *et al.* (2013). Briefly, the skins were immersed into a solution containing 40 mL methanol, 50 µL hydrochloric acid and 250 µL of an internal standard (*trans*-hydroxystilbene, 200 mg L^{-1} in ethanol). After Ultra-Turrax homogenization, the sample was stirred for 48 h at room temperature in the dark. The polyphenol-containing methanolic solution was recovered by centrifugation (5000×g, 5 min) and almost completely evaporated to dryness using a vacuum rotavapor (Buchi R-210, Switzerland) at 35 °C. The residue obtained was suspended in 20 mL water, and stilbene compounds were extracted twice with 10 mL ethyl acetate for 15 min. The upper organic phase containing stilbenes was carefully recovered, dried by adding anhydrous sodium sulphate, filtered through Whatman 589/3 paper and completely evaporated to dryness under a vacuum at 35 °C. The residue was then dissolved in 2 mL methanol and 50 mmol L^{-1} formic acid (1:1, v/v), and the extract was centrifuged at 14000×g for 10 min.

The HPLC-Dual Band UV system and chromatographic conditions have been previously reported in the literature (Vincenzi *et al.*, 2013). *trans*-Resveratrol was separated on the analytical column previously described for the anthocyanins and HCTs. The mobile phases were: solvent A, 50 mmol L^{-1} formic acid; solvent B, methanol. The solvent flow rate was 1 mL min^{-1}, and the column temperature was 40 °C. A linear gradient was used, starting at 0% B and increasing to 10% B in 3 min, 30% B in 5 min, 44% B in 35 min, 55% B in 2 min, 75% B in 15 min, and 100% B in 1 min, and then returning to 0% B in 3 min. The content of *trans*-resveratrol was expressed as µg g^{-1} skin. Each replicate was analyzed in duplicate.

Free volatile compounds. For each sample, two hundred berries were processed following the procedure proposed by Di Stefano (1991) and summarized by Rolle *et al.* (2012). The berries were deseeded and the pulp was separated from the skin with the addition of sodium metabisulfite (50 mg). The skins were treated with 20 mL methanol to avoid the enzymatic reactions. The pulps and skins were crushed separately under a nitrogen atmosphere with a laboratory blender (Waring Laboratory, Torrington, CT). The skin and pulp homogenates were then combined and centrifuged twice (7000 ×g, 15 min, 4 °C), washing the solid residue with tartaric acid buffer (pH 3.2). The extract (250 mL) was then clarified using a pectolytic enzyme (100 mg) without glycosidase activity (Rapidase X-Press, DSM, The Netherlands) at room temperature for 2 h. 1-Heptanol was added to the sample as an internal standard (200 µL, 44 mg L^{-1} in 10% v/v ethanol). Afterwards, an aliquot (100 mL for white grapes or 50 mL for red grapes, $n = 2$) was then loaded onto a 1-g Sep-Pak C_{18} RP-SPE cartridge (Waters Corporation). Free aroma compounds were eluted with 12 mL dichloromethane. The eluate was dried over anhydrous sodium sulphate, concentrated to approximately 200 µL under a stream of nitrogen and immediately analysed by GC-MS.

The GC-MS system and chromatographic conditions were previously reported by Rolle *et al.* (2012). A DB-WAXETR capillary column (30 m × 0.25 mm, 0.25 µm, J&W Scientific Inc., Folsom, CA) was used. The injection port temperature was 250 °C, the ion source temperature was 240 °C, and the interface temperature was 230 °C (solvent delay of 6.5 min). The temperature program started at 40 °C for 5 min, and increased at a rate of 2 °C/min to 200 °C for 10 min and at 5 °C/min to 220 °C. The oven was then held at 220 °C for 5 min before returning to the initial temperature. The detection was carried out by electron impact mass spectrometry in total ion current (TIC) mode, using an ionization energy of 70 eV. The mass acquisition range was *m/z* 30-330. Semi-quantitative data (µg kg^{-1} berries) were obtained by measuring the relative peak area of each identified compound, according to the NIST database (http://webbook.nist.gov/chemistry/), in relation to that of the added internal standard. Each replicate was analysed in duplicate.

Statistical analysis

Statistical analyses were performed using the SPSS Statistics software package (version 19.0, IBM Corporation, Armonk, NY). The Tukey-b test (at $p < 0.05$) was used to establish significant differences by one-way analysis of variance (ANOVA).

Results

Table 1 shows the sugar content and berry characteristics for the four table grape varieties analyzed at harvest. The ripeness reached was in agreement with historical data for these cultivars (data not shown). In general, the richest grapes in soluble solids, evaluated as juice °Brix degree, corresponded to the white 'Timpuriu de Cluj' variety, which was associated also with the lowest average berry weight. No significant differences were found in average berry skin and seed weight among the varieties studied.

Phenolic content

According to the values of A_{280}, the red 'Napoca' variety contains significantly higher amounts of total skin polyphenols when compared to the other varieties (Table 2). Among others, this fact could be due to the higher richness in total anthocyanins for 'Napoca' with respect to 'Someşan' (Table 3). 'Someşan' grapes presented high values of the PRO index in the skins, but the lowest values found in the seeds.

The white 'Timpuriu de Cluj' variety showed high amounts of total skins and seeds polyphenols, especially for oligomeric tannins that were evaluated by the FRV index (Rolle et *al.*, 2013b). On the opposite, 'Aromat de Iaşi' grapes showed significantly lower values of the PRO and FRV indices in the skin than the other varieties.

Anthocyanin content

Table 3 shows the total anthocyanin content and profile of the two red grape varieties. The 'Napoca' variety showed a significantly higher content of total anthocyanins (as mg kg^{-1} berries and mg g^{-1} skin) than the 'Someşan' variety. The

Table 1. Sugar content and berry characteristics of table grape varieties at harvest

Skin color	Variety	Harvest date (2011)	°Brix	Average berry weight (g)	Average berry skin weight (mg)	Average berry seed weight (mg)
White	'Aromat de Iaşi'	Sept 13	16.5 ± 0.4	2.95 ± 0.02 ab	270 ± 111	82 ± 49
	'Timpuriu de Cluj'	Sept 05	20.9 ± 1.6	2.42 ± 0.16 a	256 ± 16	66 ± 11
Red	'Napoca'	Aug 29	16.0 ± 0.5	3.38 ± 0.16 b	327 ± 12	98 ± 20
	'Someşan'	Sept 13	14.4 ± 0.8	2.66 ± 0.60 a	425 ± 40	84 ± 1
p value			-	0.014	0.077	0.629

Values are expressed as average ± standard deviation (n = 3 for °Brix, n = 30 for all other parameters). Different letters within the same column indicate significant differences (Tukey-b test; $p < 0.05$).

Table 2. Phenolic composition of berry skins and seeds of table grape varieties

Variety	A_{280} (kg^{-1} berries)		PRO (mg kg^{-1} berries)		FRV (mg kg^{-1} berries)	
	skins	seeds	skins	seeds	skins	seeds
'Aromat de Iaşi'	13.4 ± 1.6 a	15.3 ± 3.0	791 ± 106 a	854 ± 177	573 ± 37 a	1,109 ± 77 c
'Timpuriu de Cluj'	25.3 ± 1.4 b	22.5 ± 4.6	1,601 ± 149 b	898 ± 130	1,171 ± 216 b	1,189 ± 169 c
'Napoca'	33.2 ± 1.6 c	17.6 ± 1.9	1,866 ± 18 b	1,043 ± 115	1,134 ± 71 b	837 ± 10 b
'Someşan'	21.5 ± 3.5 b	13.9 ± 1.8	1,912 ± 356 b	714 ± 153	992 ± 235 b	580 ± 75 a
p value	< 0.001	0.067	< 0.001	0.179	0.007	0.001

Values are expressed as average ± standard deviation (n = 3). Different letters within the same column indicate significant differences (Tukey-b test; $p < 0.05$), A_{280} = absorbance measured at 280 nm, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin.

Table 3. Anthocyanin content and profile of berry skins of table grape varieties

Variety	Total anthocyanins index		Percentage of anthocyanin forms (%)							
	mg kg^{-1} berries	mg g^{-1} skins	Σ delphinidin-G	Σ cyanidin-G	Σ petunidin-G	Σ peonidin-G	Σ malvidin-G	Σ -G	Σ acetyl-G	Σ cinnamoyl-G
'Napoca'	380 ± 25	3.93 ± 0.20	3.8 ± 0.2	2.6 ± 0.2	5.5 ± 0.1	29.1 ± 1.7	59.0 ± 2.1	71.4 ± 1.7	3.9 ± 0.2	24.6 ± 1.5
'Someşan'	151 ± 24	0.93 ± 0.02	5.7 ± 1.9	4.1 ± 2.6	5.4 ± 0.8	30.4 ± 2.4	54.5 ± 2.9	96.4 ± 0.4	0.9 ± 0.1	2.7 ± 0.3
p value	0.002	< 0.001	0.150	0.177	0.102	0.015	0.006	< 0.001	< 0.001	< 0.001

Values are expressed as average ± standard deviation (n = 3). G = 3-glucoside.

Table 4. Skin hydroxycinnamoyl tartaric acids and *trans*-resveratrol contents of berry skins of table grape varieties

Variety	Total HCTs (mg kg^{-1} berries)	*trans*-caffeoylT (%)	*cis*-p-coumaroylT (%)	*trans*-p-coumaroylT (%)	*trans*-feruloylT (%)	p-coumaroylT / caffeoylT ratio	*trans*-resveratrol (μg g^{-1} skin)
'Aromat de Iaşi'	80.3 ± 16.5 a	41.3 ± 2.8 b	15.9 ± 1.6 b	41.4 ± 2.0 b	1.4 ± 0.4 b	1.39 ± 0.17 b	44.4 ± 5.9
'Timpuriu de Cluj'	181.6 ± 10.9 c	34.8 ± 1.2 a	14.8 ± 0.3 b	48.5 ± 1.1 d	1.8 ± 0.3 b	1.82 ± 0.09 c	71.0 ± 10.3
'Napoca'	183.2 ± 17.4 c	58.8 ± 0.4 c	8.3 ± 0.3 a	32.3 ± 0.2 a	0.7 ± 0.1 a	0.69 ± 0.01 a	46.2 ± 6.3
'Someşan'	131.4 ± 19.9 b	39.0 ± 0.8 b	15.2 ± 1.6 b	45.2 ± 0.9 c	0.6 ± 0.1 a	1.55 ± 0.05 b	60.5 ± 21.5
p value	< 0.001	< 0.001	< 0.001	< 0.001	0.002	< 0.001	0.260

Values are expressed as average ± standard deviation (HCTs n=3; *trans*-resveratrol n = 2). Different letters within the same column indicate significant differences (Tukey-b test; $p < 0.05$).

anthocyanin profile was similar for the two varieties considered, with significant differences in the free forms only for peonidin and malvidin. In particular, the percentage of cinnamoylglucoside forms was high in 'Napoca' grapes (about 25% of the total content), whereas the relative abundance of acylated forms was quite low in 'Someşan' grapes.

HCTs and trans-resveratrol content

Total content and profile of HCTs in the skins are shown in Table 4. 'Napoca' and 'Timpuriu de Cluj' varieties presented a significantly higher total skin HCTs content (more than 180 mg kg^{-1}) while this content in 'Aromat de Iaşi' grapes was only 80 mg kg^{-1}. The *trans*-caffeoyltartaric acid percentage ranged from 34.8% of 'Timpuriu de Cluj' to 58.8% of 'Napoca'. Instead, *trans*-p-coumaroyltartaric acid showed the lowest relative abundance in the latter variety (8.3%). The white varieties showed proportions of *trans*-feruloyltartaric acid above 1% of total HCTs, while the percentages achieved in the red varieties were not higher than 0.7%. The ratio between the sum of p-coumaroyltartaric acids and *trans*-caffeoyltartaric acids was higher than 1.3 in all the varieties except for 'Napoca' (0.69).

The highest skin contents of *trans*-resveratrol were found in 'Timpuriu de Cluj' grapes (71.0 μg g^{-1} skin), although the differences were not significant among varieties (Table 4).

Aroma compounds

Twenty-three free volatile compounds were identified and quantified (Table 5). As expected, most of the free aroma compounds identified were found in the 'Aromat de Iaşi' variety. The most abundant free volatile compound in this variety was geraniol, which represented about 38% of total content, followed by diol 1, linalool and geranic acid that represented about 11%, 10%, and 8%, respectively. These four aroma compounds were not found in the other white variety studied, whereas diol 1 and geranic acid were found only in 'Aromat de Iaşi'. This variety was also characterized by a large amount of geraniol (520 μg kg^{-1}

Table 5. Free volatile compounds of table grape varieties

Free compound (μg kg^{-1} berries)	'Aromat de Iași'	'Timpuriu de Cluj'	'Napoca'	'Someșan'	*p-value*
isoamyl alcohol	nd	nd	nd	2.5 ± 3.2	
(*E*)-2-hexenal	6.7 ± 2.5	nd	nd	10.0 ± 6.9	0.584
1-hexanol	38.0 ± 3.9 a	146.0 ± 6.0 bc	120.1 ± 4.1 b	167.0 ± 18.6 c	< 0.001
(*Z*)-3-hexen-1-ol	nd	27.0 ± 4.2 a	25.3 ± 5.5 a	59.4 ± 0.9 b	0.006
(*E*)-2-hexen-1-ol	46.0 ± 2.5 a	144.4 ± 8.5 b	61.1 ± 3.7 a	120.9 ± 11.3 b	< 0.001
cis-furanic linalool oxide	3.2 ± 1.2	nd	nd	nd	
trans-furanic linalool oxide	13.5 ± 0.2	nd	nd	nd	
linalool	131.7 ± 41.1	nd	nd	24.4 ± 1.1	0.066
geranial	7.6 ± 1.7	nd	nd	nd	
trans-pyranic linalool oxide	48.0 ± 4.6	nd	nd	42.3 ± 9.0	0.506
cis-pyranic linalool oxide	55.9 ± 6.8	nd	nd	18.8 ± 1.5	0.017
geraniol	520.0 ± 193.2 b	nd	8.7 ± 0.7 a	97.1 ± 24.1 a	0.038
hexanoic acid	54.1 ± 4.6 a	140.2 ± 6.5 b	116.7 ± 4.3 b	116.7 ± 10.4 b	< 0.001
benzyl alcohol	nd	nd	24.6 ± 27.1	20.3 ± 3.8	0.846
2-phenyl ethanol	21.8 ± 2.2	28.0 ± 3.8	21.0 ± 4.2	18.4 ± 3.2	0.175
(*E*)-2-hexenoic acid	nd	nd	56.3 ± 0.7	167.0 ± 13.2	0.007
4-vinylguaiacol	14.3 ± 3.5	40.9 ± 24.9	9.5 ± 1.5	30.0 ± 19.0	0.308
diol 1	154.5 ± 18.0	nd	nd	nd	
1-hydroxy linalool	12.2 ± 0.0	nd	nd	nd	
diol 2	61.4 ± 16.8	nd	nd	nd	
OH-geraniol	6.0 ± 0.5	nd	nd	nd	
3,7-dimethyl-6-octenal	46.6 ± 3.8	nd	nd	nd	
geranic acid	113.2 ± 5.3	nd	nd	nd	

Volatile compounds are ordered by their retention time. Values are expressed as average ± standard deviation ($n = 2$); nd =not detected. Different letters within the same column indicate significant differences between the varieties (Tukey-b test; $p < 0.05$).

berries), which was significantly different from all the varieties studied. The aroma profiles of 'Timpuriu de Cluj' and 'Someșan' grapes were characterized by high contents of 1-hexanol and *(E)*-2-hexen-1-ol, while (E)-2-hexenoic acid and isoamyl alcohol were also found in the 'Someșan' variety. In 'Napoca' grapes prevailed 1-hexanol, *(E)*-2-hexen-1-ol, hexanoic acid, and (E)-2-hexenoic acid with about 27%, 26%, and 13% of total content, respectively.

Discussion

According to the OIV Resolution VITI 1/2008 (OIV Standard on Minimum Maturity Requirements for Table Grapes), the table grape analysed in this study can be considered ripe at harvest. However, the ripeness level was different, particularly among white table grape varieties, and the soluble solids content was higher for 'Napoca' and 'Timpuriu de Cluj' table grapes even at earlier dates than 'Someșan' and 'Aromat de Iași' grapes, respectively. This seems to indicate longer maturity periods for the latter ones.

Berry weight and skin/pulp ratio are considered important factors for the table grape market. In this study, the skin weight/berry weight ratio varied between 9.1% ('Aromat de Iași') and 10.6% ('Timpuriu de Cluj'). These values are in agreement with those found in literature for other white table grapes (Rolle *et al.*, 2011). Only 'Someșan' grapes showed higher values of this parameter (16.0%), whereas 'Napoca' grapes were characterized by the heaviest berries. In red varieties, this ratio is particularly important because it is related to quality chemical parameters (Barbagallo *et al.*, 2011; Roby *et al.*, 2004; Rolle *et al.*, 2015).

In addition to physical properties, the interest of table grape consumers focused more on the nutraceutical values, and in particular the phenolic composition gained attention (Parpinello *et al.*, 2013). In this study, considering all the phenol compound classes (A_{280}), 'Timpuriu de Cluj' had the greatest amount of phenols among the white varieties while 'Napoca' among red varieties. With the exception of 'Aromat de Iași', the contents of phenolic compounds were always higher in skins than in seeds. The skin/seed ratio for total phenols ranged from 1.12 ('Timpuriu de Cluj') and 1.89 ('Napoca'). This fact could be positive from a sensory point of view because of a softening effect on the mouthfeel in relation to a lower astringency perception. The seeds of 'Aromat de Iași' were richer in total phenols than the skins in agreement with other table grape varieties (Baiano and Terracone, 2011). When compared with 'Muscat Hamburg' and 'Italia', which are ones of the most present varieties in the market for table grapes, the Romanian varieties studied here showed similar total contents of phenolic compounds (Liang *et al.*, 2011; Río Segade *et al.*, 2013; Rolle *et al.*, 2015), although climate and growing zone may influence these results. Particularly, 'Aromat de Iași' and 'Timpuriu de Cluj' white table grape varieties were richer than 'Italia' grapes in high molecular weight flavanols (PRO) and oligomeric flavanols (FRV) in skins and seeds. Instead, 'Napoca' and 'Someșan' red table grape varieties were richer in oligomeric flavanols, but the content of high molecular weight flavanols was similar to 'Muscat Hamburg' grapes.

From the red grapes compositional point of view, a possible indicator of the grape color is the skin anthocyanin content, which is directly related to the visual color (Rolle and Guidoni, 2007). Therefore, this factor has a huge contribution in the skin color for commercialization and fresh consumption of grapes. The total anthocyanin content of the red varieties studied was 151 and 380 mg kg^{-1} berries. These contents are in agreement with those published in previous studies on other varieties

(Crupi *et al*., 2012; Dami *et al*., 2006; Kamiloglu, 2011; Liang *et al*., 2011; Orak, 2006). The 'Napoca' variety had the highest amount of anthocyanins, with a prevalence of malvidin derivatives and high percentages of acylated anthocyanin forms, similar to the 'Regina nera' table grape cultivar (Rolle *et al*., 2013a). In the grape juice production, the phenolic content and the juice color are important factors, the latter also for the direct consumer perception (Kliewer, 1970). Therefore, special processing treatments were studied to achieve a higher phenolic content in the produced juices (Lima *et al*., 2015), and the use of phenolic-rich varieties such as 'Napoca' could give interesting products.

In winegrapes, smaller berries generally showed higher contents of anthocyanins (Barbagallo *et al*., 2011; Roby *et al*., 2004). For the 'Someşan' and 'Napoca' table grape varieties, this behavior was not detected. The results obtained in this study agreed with those observed in 'Muscat Hamburg' table grapes (Rolle *et al*., 2015).

The anthocyanin profile of grapes is primarily influenced by genetic factors, and the ratio among anthocyanin forms can be used for chemotaxonomical classifications (Carreno *et al*., 1997). In this study, many significant differences were found among the two varieties. Also the HCT profile could be a characteristic of the variety and could be used as a discriminating tool among cultivars (Ferrandino *et al*., 2012). The HCT compounds were yet less investigated in table grapes with respect to anthocyanins, flavanols, flavonols and stilbenes. 'Aromat de Iaşi' and 'Someşan' table grapes were characterized by a HCT content similar to those reported by Ferrandino *et al*. (2012) for winegrapes. Instead, the 'Timpuriu de Cluj' and 'Napoca' varieties were richer in these compounds. When compared with Muscat Hamburg table grapes (Ferrandino *et al*., 2012), all the Romanian varieties studied showed higher total HCT contents.

trans-Resveratrol content in the Romanian table grape varieties studied was in the range of winegrapes (Vincenzi *et al*., 2013). The beneficial effects of *trans*-Resveratrol on human health have been recognized, and the presence of relatively high amounts of this compound in table grapes is highly valued by consumers sensitized in the nutraceutical properties of foods.

Regarding aroma compounds, higher levels of linalool and geraniol in 'Aromat de Iaşi' variety may contribute to floral and fruity odour (Duan, *et al*. 2014). These terpenes are responsible for the aroma of Muscat grapes (Ruiz-García *et al*., 2014). Geraniol, linalool and *α*-terpineol are prevalent in 'Tămâioasă românească' with 17.6%, 38.5% and 43.7% respectively (Ţârdea, 2007). Although 'Aromat de Iaşi' was obtained from this last variety, *α*-terpineol was not detected.

Conclusions

Among white grapes, the 'Timpuriu de Cluj' variety has an important content of phenolic compounds, which can contribute greatly to the overall nutraceutical value. This characteristic could be particularly appreciated by consumers, especially in white grapes. However, this variety showed an aroma profile composed of few volatile compounds. 'Aromat de Iaşi', instead, had the more complex aroma composition among the Romanian table grape varieties studied with a relevant content of geraniol.

Red varieties are gaining interest by consumers in the table grape market. In this sense, 'Napoca' red grapes could be appreciated because of their greater content of skin anthocyanins and also total skin HCTs. Furthermore, these grapes are characterized by the highest berry weight among the studied varieties. Despite the lower anthocyanin content, 'Someşan' grapes showed an aroma profile composed of more compounds with generally higher contents in relation to the other Romanian red variety studied. This study permitted to improve the knowledge of minor Romanian table grape varieties in order to permit a better exploitation of these cultivars.

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