Banc R *et al.* (2020)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48(2):716-734
DOI:10.15835/nbha48211848
Research Article

Phenolic composition and antioxidant activity of red, rosé and white wines originating from Romanian grape cultivars

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Abstract

The objective of this work was to study the phenolic profile and composition in relation to antioxidant activity of fifteen samples of commercial red, rosé and white wines originating from six native grape varieties and produced in important wine regions from Romania. The profile and quantification of major phenolic compounds were performed by direct injection of wines in the LC-MS system, using DAD and ESI (+) MS techniques, in parallel with the total phenolic content (TPC) measured by spectrometry and the free radical scavenging activity, against 2,2-diphenyl-1-picrylhydrazyl (DPPH). There were identified 38 polyphenols in wines, including 3 flavan-3-ols, 17 flavonols, 12 anthocyanins and 6 stilbenes. The red wines had significant higher phenolic content and antioxidant capacity, followed by rosé and white wines. The richest phenolic content and antioxidant activity was obtained for 'Feteasca Neagra' (Tohani) among red wines and for 'Feteasca Regala' (Jidvei) among white wines. TPC values were positively correlated with the antioxidant capacity in all white wines and only for the red 'Feteasca Neagra' assortment, while for the 'Babeasca Neagra' assortment negative correlations were obtained. From the 38 variables, flavan-3-ols have exerted the greatest influence on wine differentiation, based on their colour (red, rosé and white). The study also revealed significant differences between cultivars, both qualitative and quantitative, in terms of their polyphenolic composition, that could be important in the cultivar authentication of wines from these varieties.

Keywords: antioxidants; HPLC-DAD-ESI(+)MS; multivariate data analysis; native Romanian wines; phenolics

Introduction

The consumption of wine is a common and ancient practice in Romania, our country being one of the main producers and consumers of wine. The European Union (EU) has a leading position on the world wine market, accounting for approximately 60% of global production. In 2013 Romania was the sixth wine producer

Received: 10 Mar 2020. Received in revised form: 15 Apr 2020. Accepted: 21 May 2020. Published online: 30 Jun 2020.

in EU, after Italy, Spain, France, Germany and Portugal, while in 2015 was ranked the same position in EU in terms of wine quantity, after Italy, France, Spain, Germany and Portugal (OIV, 2015).

Wines have been shown to be a significant source of dietary polyphenolic antioxidants, including benzoic and cinnamic acid derivatives, flavan-3-ols, flavonols, stilbenes and anthocyanins (Alén-Ruiz *et al*., 2009; Haseeb *et al*., 2019). The health benefits of regular, moderate wine consumption have been studied in depth and have been associated with the reduction of the incidence of many diseases such as cancer, cardiovascular diseases, atherosclerosis, hypertension, type 2 diabetes, neurological disorders, and metabolic syndrome (Artero *et al*., 2015; Haseeb *et al*., 2019) and these health-protective effects of wines are due to their antioxidant properties and their ability to scavenge free radicals. Also, the regular intake of wine, and especially red wine, has been associated with the so-called effect the "French Paradox", that means the reduction of mortality by cardiovascular diseases (Renaud and De Lorgeril, 1992).

The chemical composition of wines underlies their quality and authenticity. The polyphenolic profile of a given cultivar reflects to a great extent its genetic potential and, therefore, may be used as a tool to differentiate the various cultivars (Muccillo *et al*., 2014; Budić-Leto *et al*., 2017). Phenolic grape and wine compounds can be divided into two groups: non-flavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds (anthocyanins, flavan-3-ols and flavonols) (Gomez-Alonso *et al*., 2007).

Many different methods, including high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) in combination with different detectors, UV-Vis, photo diode array (PDA), mass spectrometry (MS), and electrochemical (EC) detectors, have been used to investigate the polyphenolic content and chemical composition of wine. Mass spectrometry (MS), especially, is responsible for great progress in the identification and characterization of polyphenols in wine (Šeruga *et al*., 2011). Characterization of wines, based on the phenolic profile, has been reported by numerous researchers from several countries and included the analysis of: flavan-3-ols, with catechin being the most important flavanol found in wines (Tinttunen and Lehtonen, 2001; Monagas *et al*., 2005; Gomez-Alonso *et al*., 2007; Budić-Leto *et al*., 2017); flavonols (Rastija *et al*., 2009; Vrček *et al*., 2011; Pereira *et al*., 2013; Budić-Leto *et al*., 2017), hydroxybenzoic and hydroxycinnamic acids (Alén-Ruiz *et al*., 2009; Budić-Leto *et al*., 2017); anthocyanins (Kelebek *et al*., 2010; Li *et al*., 2011; Bai *et al*., 2013; Figueiredo-González *et al*., 2014) and stilbenes, with resveratrol being the most important representative (Abril *et al*., 2005; Stervbo *et al*., 2007; Rodríguez-Cabo *et al*., 2014; Budić-Leto *et al*., 2017).

In our country, no detailed phenolic composition was reported for wines obtained from indigenous grape varieties. In this respect, until now, little attention has been paid to characterization of native Romanian wines, looking specifically to their phenolics' profile and composition. Thus, some wine samples, originating from autochthonous grape cultivars, were studied only for a few compounds, like catechin, epicatechin, resveratrol and phenolic acids (Geana *et al*., 2011; Geana *et al*., 2014a). Instead, for Romanian wines made from grape varieties of foreign origin, Ionete *et al*. (2019) reported the presence of hydroxybenzoic and hydroxycinnamic acids, flavonoids and stilbenes, in wines obtained from grapes of three different varieties, like Chardonnay, Muscat Ottonel and Pinot Noir.

The aim of this study is to characterize commercial samples of Romanian red, rosé and white wines from six local grape cultivars, 'Feteasca Alba' (FA), 'Feteasca Regala' (FR), 'Babeasca Rose' (BR), 'Busuioaca de Bohotin' (BB), 'Babeasca Neagra' (BN), 'Feteasca Neagra' (FN) and produced in six different regions of Romania (Patic, 2006). To estimate the phenolic profile and composition it was developed a fast HPLC method coupled with diode array (DAD) and electrospray ionization (ESI) - mass spectrometry detection. The total polyphenol content of all wines, performed by the standardized Folin-Ciocalteu (FC) method, aimed to be correlated with the antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging technique.

Materials and Methods

Wine samples

Fifteen Romanian wines, including red, rosé and white varieties of six different cultivars were used in this study. The samples used were produced during the 2010, 2011 and 2012 harvests from vineyards located in six important Romanian wine regions: Moldavia (wine no. 1, 5, 6, 7, 8, 11, 12), Transylvania plateau (wine no. 2, 3), Banat (wine no. 4), Dobrogea (wine no. 9, 15), South of the country (wine no. 10) and Muntenia and Oltenia (wine no. 13, 14) (Table 1). The wines were bottled after 1 or 2 years. The main criterion for the selection of wines was the one related to the native origin of the grape varieties. For this reason, it was necessary to purchase different wine types, with different sweetness index, to cover a sufficient number of indigenous varieties. The wines were commercialized in glass bottles, purchased from local supermarkets and stored at room temperature until analysed. The wines were examined shortly after bottling. Before analysis, wine samples were filtered through 0.45 μm polytetrafluoroethylene (PTFE) filters.

Table 1. List of wines investigated, based on cultivar type and wine producer, production year, wine colour and sweetness index. These Abbreviations are used in the text

Wine no.	Wine sample (abbreviation)	Name, producer	Year	Wine type, sweetness index
1	$FA_{Cot2011}$	Feteasca Alba, Coteşti	2011	White, Half-dry
2	$FA_{Jid2011}$	Feteasca Alba, Jidvei	2011	White, Dry
3	$FR_{Jid2011}$	Feteasca Regala, Jidvei	2011	White, Half-dry
4	$FR_{Rec2012}$	Feteasca Regala, Recas	2012	White, Half-dry
5	$BR_{Pan2012}$	Babeasca Rose, Panciu	2012	Rosé, Half-dry
6	$BB_{Hus2011}$	Busuioaca de Bohotin, Husi	2011	Rosé, Half-sweet
7	$BN_{Pan2011}$	Babeasca Neagra, Panciu	2011	Red, Half-dry
8	$BN_{Hus2010}$	Babeasca Neagra, Husi	2010	Red, Dry
9	BN_{SN2011}	Babeasca Neagra, Sarica-Niculitel	2011	Red, Dry
10	BN_{SC2010}	Babeasca Neagra, Sadova Corabia	2010	Red, Dry
11	$FN_{Cot2010}$	Feteasca Neagra, Cotesti	2010	Red, Half-sweet
12	$FN_{Pan2011}$	Feteasca Neagra, Panciu	2011	Red, Half-dry
13	$FN_{Cep2012}$	Feteasca Neagra, Ceptura	2012	Red, Dry
14	$FN_{Toh2010}$	Feteasca Neagra, Tohani	2010	Red, Half-dry
15	$FN_{Mur2011}$	Feteasca Neagra, Murfatlar	2011	Red, Half-sweet

Reagents and chemicals

All reagents used in the analysis were of analytical grade. For the determination of total phenolic content were used Folin Ciocalteu's phenol reagent, anhydrous sodium carbonate, Gallic acid and 40% ethanol analytical grade. Radical scavenging assay reagents used were: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). Solvents used for the determination of polyphenolic content by HPLC were of chromatographic grade: acetic acid, acetonitrile. HPLC grade purified water was obtained from a Milli-Q water purification system. Commercial standards used for flavonoids and stilbenes were quercetin dihydrate, (+)-catechin and *trans*-resveratrol and commercial standards used for phenolic compounds were gallic acid, cyanidin chloride.

HPLC-DAD-ESI/MS analysis

The HPLC separation, identification and quantification of wine phenolic compounds were performed on an Agilent 1200 HPLC Series system (Agilent, Santa Clara, CA, USA), equipped with a Diode Array Detector (G1315D) coupled with a MS system equipped with Electrospray Ionisation (ESI) source operated in the positive-ion mode and an Agilent Technologies 6110 Single Quadrupole mass spectrometer. The

chromatographic data were processed using ChemStation and DataAnalysis software from Agilent, USA. Compounds were separated on a reversed-phase column Zorbax Eclipse XDB-C18 (4.6 x 150 mm; 5 μm particle; Agilent), which was maintained at 25 °C. The mobile phase consisted of 10% acetonitrile and 0.1% acetic acid in aqueous solution (A) and 0.1% acetic acid in acetonitrile (B). The linear gradient for solvent B was as follows: 0 min, 5%; 2 min, 5%; 18 min, 40%; 20 min, 90%; 24 min, 90%; 25 min, 5%; 30 min, 5%. The flow rate was 0.5 mL min^{-1}, the injection volume was 5 μL and data were collected at 280, 340 and 520 nm. For identification, ESI-MS was used, setting the following parameters: positive ion mode; dry gas, N_2, flow rate, 8 L min^{-1}; drying temperature, 350 °C, nebulizer pressure 65 psi, capillary voltage, 3000 V, scan range, *m/z* 150-1000.

Identification and quantification of phenolics

Some of the phenolic compounds analysed were identified previously according to their order of elution, retention times and UV-Vis spectra of pure compounds ((+)-catechin, quercetin, *trans*-resveratrol and cyanidin) and the characteristics of the UV-Vis spectra published in different studies (Monagas *et al*., 2005). For the rest of individual polyphenols, the identification was performed by correlating the absorbance and mass spectra obtained with those previously reported in the literature and by study of their fragmentation patterns (Mazzuca *et al*., 2005; Monagas *et al*., 2005; Castillo-Muñoz *et al*., 2007). The quantification of phenolic compounds was carried out by using the DAD chromatograms obtained at wavelengths that show more sensitivity to each phenolic group: 280 nm for the flavanols and stilbenes; 340 nm for the flavonols; and 520 nm for the anthocyanins, by means of external standard calibration curves. The three replicated experiments were carried out for each variety of wine. Samples of each of the wine varieties were taken from three separate bottles. The concentration of the most representative compound for each group was measured after calibrations made with pure compounds analyzed in the same conditions and linear regression coefficients obtained were between 0.9935 and 0.9994. In general, more than one linear regression was performed for each compound, at different concentration levels. Calibration of a similar compound was used when the pure reference standard was not available: cyanidin was used for anthocyanidin 3-glucosides, quercetin for flavonol 3-glycosides and their free aglycones, (+)-catechin for flavan-3-ols and *trans*-resveratrol for stilbenes.

Determination of total phenolic compounds content

The total phenolic concentration was determined by spectrophotometry, according to the Folin-Ciocalteu (FC) method, using Gallic acid as reference standard (Singleton *et al*., 1999). A standard curve of gallic acid (ranging from 0 to 10 mg L^{-1}) was prepared and the results, determined from a regression equation:

$$\text{Phenolic Concentration} = 0.9443 \times \text{absorbance} + 0.0608,$$

$$R^2: 0.9945,$$

were expressed as mg gallic acid equivalents per liter of wine (mg GAE L^{-1}). Absorbance measurements were performed on a BIO-TEK Synergy HT multi-detection microplate reader (Biotek,Winooski, USA).

DPPH radical-scavenging assay

The antioxidant activity of wines was determined by DPPH radical-scavenging activity assay, a modified version of Brand-Williams *et al*. (1995). Free radical DPPH• (2,2-diphenyl-1-picrylhydrazyl) reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH•. The changes in colour (from purple to a residual pale-yellow colour) were measured at 515 nm on a spectrophotometer after 30 min of incubation. The decrease of radical absorbance is proportional to the concentration and activity of the sample analyzed. Absorbance measurements are transformed to antioxidant activity using as reference Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which is a commercial water-soluble analog of vitamin E that is used to standardize the results from different studies dealing with food antioxidant capacity. To this end, quantification of antioxidant capacity was made by calibration curve obtained from methanolic solutions of

Trolox, in a range of 0-500 μmol L^{-1}. The DPPH· stock solution (80 μM DPPH· in methanol) was prepared fresh daily, sonicated 15 min and kept in the dark at room temperature. The working protocol consisted of adding 250 μL of wine and 1750 μL of DPPH radical methanolic solution (8×10^{-5} mol L^{-1}) on the microplate, and, after 30 min, measuring the percentage of absorbance decrease at 515 nm. For the blank, 250 μL wine sample was replaced with 250 μL methanol. The capability of each wine sample to scavenge DPPH· was expressed as a percentage and calculated by using the formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{control} - A_{sample}) / (A_{control})] \times 100,$$

where $A_{control}$ is the absorbance of DPPH radical + methanol (containing all reagents except the sample) and A_{sample} is the absorbance of DPPH radical + wine sample / standard. Using Trolox as a standard, the total antioxidant activity (TAA) was expressed as milimoles of Trolox equivalents (TE) per L of wine (Brand-Williams *et al*., 1995; De la Cruz *et al*., 2013). Absorbance measurements were recorded on a BIO-TEK Synergy HT multi-detection spectrophotometer. Operating conditions were set at 25 °C.

Statistical analysis

All the samples were analyzed in triplicate; the average and the relative SD were calculated using the Excel software package. Data were subjected to one-way analysis of variance (ANOVA) and comparison between means was determined according to Duncan's test. Significant differences were accepted at $p \leq 0.05$. Pearson correlation coefficient between total phenolic content and scavenging activity was calculated using Microsoft Excel software package. Principal Component Analysis (PCA) was performed using Unscrambler Software, version 9.7 (CAMO Software AS, Norway).

Results and Discussion

Phenolics' profiling and quantification by HPLC-DAD-ESI (+) MS

A fast, direct injection HPLC-DAD-ESI(+)MS technique, optimized in the laboratory for phenolics analysis was applied and specific fingerprints were obtained for each wine sample. Figure 1 shows representative HPLC-DAD chromatograms of wines, recorded at 280 nm for the flavan-3-ols and stilbenes detection, e.g. 'Feteasca Regala', Jidvei ($FR_{Jid2011}$) white wine (Figure 1A), recorded at 340 nm for flavonols profile, as detected in 'Feteasca Neagra', Cotesti ($FN_{Cot2010}$) red wine (Figure 1B) and recorded at 520 nm for the anthocyanins detected in 'Feteasca Neagra', Ceptura ($FN_{Cep2012}$) red wine (Figure 1C).

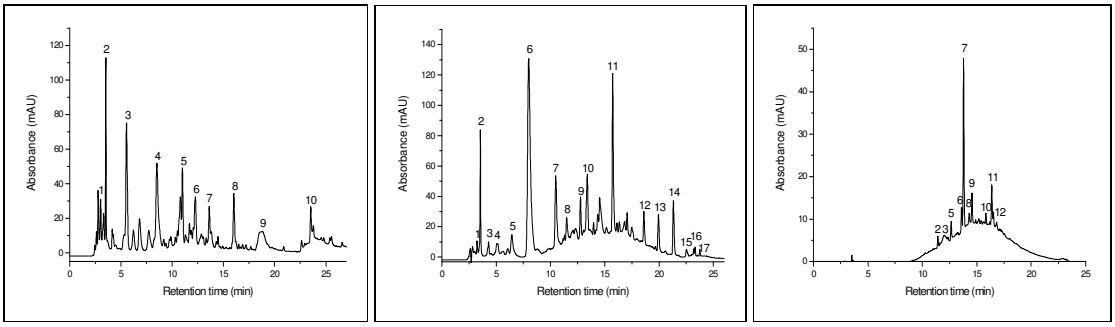


Figure 1. HPLC/DAD chromatogram recorded at: (A) 280 nm, showing the flavan-3-ols and stilbenes detected in $FR_{Jid2011}$ white wine; (B) 340 nm, showing the flavonols detected in $FN_{Cot2010}$ red wine; (C) 520 nm showing the anthocyanins detected in $FN_{Cep2012}$ red wine

A total number of 38 peaks corresponding to different phenolic classes were identified by comparison with pure standards and by mass spectrometry (molecular ion and fragmentation features) as presented in Tables 2, 3, 4.

Four classes of individual phenolic compounds were identified: flavan-3-ols (as monomers), flavonols (as glycosides and aglycones), stilbenes (monomeric aglycones, glycosides and dimers) and anthocyanins (monomeric anthocyanins). Based on the calibrated peak areas, the concentration of each individual phenol derivative was calculated and presented in Table 5. The total content of the four different groups of phenolic derivatives was then obtained from the sum of the individual concentration, expressed in mg L^{-1} of wine.

Table 2. HPLC-ESI(+)MS data used to identify flavan-3-ols and stilbenes in all wines after separation and DAD detection at 280 nm

Peak no	Identified compound	Retention time (min)	$[M-H]^+$ (*m/z*)	Fragment ion (*m/z*)
1	Resveratrol-5-O-glc	3.1	391	229
2	Resveratrol-3-O-glc	3.5	391	229
3	Non identified	5.6	213	
4	Resveratrol	8.7	229	
5	Gallocatechin	11.1	307	202
6	Catechin	12.3	291	202
7	Pallidol	13.7	455	213
8	Epicatechin gallate	16.2	443	202
9	Pterostilbene	19.1	257	229
10	Piceatannol	23.5	245	229

Notes: glc: glucoside

Table 3. HPLC-ESI(+)MS data used to identify flavonols in all wines after separation and DAD detection at 340 nm

Peak no	Identified compound	Retention time (min)	$[M-H]^+$ (*m/z*)	Fragment ion (*m/z*)
1	Myricetin-3-O-gal	3.1	481	319
2	Myricetin-3-O-glc	3.5	481	319
3	Quercetin-3-O-gal	4.2	465	303
4	Quercetin-3-O-glc	5.1	465	303
5	Isorhamnetin-3-O-gal	6.5	479	317
6	Isorhamnetin-3-O-glc	8.1	479	317
7	Laricitrin-3-O-glc	10.6	495	333
8	Quercetin-3-O-gluc	11.5	479	303
9	Laricitrin-3-O-(6-acetyl)-glc	12.8	537	333
10	Kaempferol-3-O-glc	13.5	449	287
11	Syringetin-3-O-glc	15.6	509	347
12	Syringetin-3-O-(6-acetyl)-glc	18.7	551	347
13	Myricetin	20.1	319	
14	Quercetin	21.3	303	
15	Isorhamnetin	22.5	317	
16	Laricitrin	23.7	333	
17	Kaempferol	24.5	287	

Notes: glc: glucoside; gal: galactoside; gluc: glucuronide

Table 4. HPLC-ESI(+)MS data used to identify anthocyanins in rosé and red wines, after separation and DAD detection at 520 nm

Peak no	Identified Compound	Retention time (min)	$[M-H]^+$ (*m/z*)	Fragment ion (*m/z*)
1	Cyanidin-3-O-glc	10.9	449	287
2	Petunidin-3-O-glc	11.4	479	317
3	Delfinidin-3-O-glc	12.0	465	303
4	Peonidin-3-O-glc	12.3	463	301
5	Cyanidin-3-O-(6-acetyl)-glc	12.6	491	287
6	Petunidin-3-O-(6-acetyl)-glc	13.5	521	317
7	Delfinidin-3-O-(6-acetyl)-glc	13.7	507	303
8	Peonidin-3-O-(6-acetyl)-glc	14.3	505	301
9	Delfinidin-3-O-(6-coumaryl)-glc	14.6	611	303
10	Malvidin-3-O-glc	15.8	493	331
11	Malvidin-3-O-(6-acetyl)-glc	16.5	535	331
12	Malvidin-3-O-(6-coumaryl)-glc	16.9	639	331

Notes: glc: glucoside

Table 5. Mean concentrations (mg L^{-1}) of phenolic compounds identified in all white, rosé and red Romanian wines (n = 3)

	White wines				Rosé wines	
Phenolic compounds	$FA_{Cot2011}$	$FA_{Jid2011}$	$FR_{Jid2011}$	$FR_{Rec2012}$	$BR_{Pan2012}$	$BB_{Hus2011}$
Flavan-3-ols						
(+)-Catechin	25.29 ± 0.50	42.05 ± 0.43	35.64 ± 0.18	33.30 ± 0.25	43.99 ± 0.34	42.30 ± 0.19
Epicatechin gallate[a]	24.03 ± 0.32	36.49 ± 0.15	32.74 ± 0.38	12.46 ± 0.08	40.09 ± 0.33	28.92 ± 0.22
Gallocatechin[a]	22.22 ± 0.21	33.10 ± 0.25	35.11 ± 0.41	38.81 ± 0.24	19.88 ± 0.10	28.12 ± 0.34
Total	71.54	111.64	103.49	84.57	103.96	99.34
Stilbenes						
Resveratrol-3-O-glc (Piceid)[b]	0.43 ± 0.02	0.50 ± 0.04	0.44 ± 0.06	0.36 ± 0.03	0.45 ± 0.06	0.37 ± 0.04
Resveratrol-5-O-glc[b]	0.27 ± 0.01	0.36 ± 0.02	0.23 ± 0.02	0.43 ± 0.05	0.17 ± 0.02	0.20 ± 0.03
trans-Resveratrol	0.67 ± 0.05	0.86 ± 0.06	0.73 ± 0.05	1.46 ± 0.09	0.61 ± 0.06	0.34 ± 0.02
Piceatannol[b]	0.35 ± 0.02	0.31 ± 0.01	0.29 ± 0.03	0.29 ± 0.01	0.29 ± 0.03	0.29 ± 0.05
Pterostilbene[b]	3.76 ± 0.11	0.48 ± 0.02	0.44 ± 0.03	0.46 ± 0.03	5.11 ± 0.19	5.63 ± 0.21
Pallidol[b]	0.38 ± 0.05	0.38 ± 0.05	0.24 ± 0.01	0.19 ± 0.02	0.24 ± 0.01	0.32 ± 0.03
Total	5.86	2.89	2.37	3.19	6.87	7.15
Flavonols						
Myricetin-3-O-gal[c]	-	-	-	-	2.39 ± 0.09	2.05 ± 0.08
Myricetin-3-O-glc[c]	1.17 ± 0.10	1.97 ± 0.09	2.35 ± 0.11	2.20 ± 0.06	2.95 ± 0.16	2.54 ± 0.12
Myricetin[c]	-	-	-	-	-	1.28 ± 0.05
Quercetin-3-O-gal (Hyperoside)[c]	3.89 ± 0.22	3.17 ± 0.17	2.91 ± 0.14	3.89 ± 0.12	2.64 ± 0.08	2.28 ± 0.12
Quercetin-3-O-glc (Isoquercetin)[c]	2.02 ± 0.14	8.50 ± 0.15	5.73 ± 0.20	-	10.43 ± 0.19	3.26 ± 0.16
Quercetin-3-O-gluc[c]	6.02 ± 0.19	6.29 ± 0.13	5.26 ± 0.11	6.37 ± 0.17	3.73 ± 0.15	4.32 ± 0.17
Quercetin	-	-	-	-	-	-
Isorhamnetin-3-O-gal[c]	14.04 ± 0.24	9.73 ± 0.14	12.31 ± 0.18	17.79 ± 0.20	11.17 ± 0.19	6.55 ± 0.15
Isorhamnetin-3-O-glc[c]	20.43 ± 0.31	30.23 ± 0.15	24.26 ± 0.32	53.66 ± 0.33	21.65 ± 0.23	11.63 ± 0.18
Isorhamnetin[c]	7.57 ± 0.16	3.30 ± 0.16	2.51 ± 0.09	0.96 ± 0.05	2.88 ± 0.09	2.96 ± 0.16
Laricitrin-3-O-glc[c]	5.22 ± 0.15	13.15 ± 0.14	9.62 ± 0.14	14.41 ± 0.11	5.80 ± 0.22	5.20 ± 0.19
Laricitrin-3-O-(6-acetyl)-glc[c]	-	-	1.34 ± 0.07	1.76 ± 0.09	1.72 ± 0.11	1.70 ± 0.07
Laricitrin[c]	1.61 ± 0.12	1.46 ± 0.08	1.24 ± 0.05	-	1.09 ± 0.08	1.14 ± 0.11
Kaempferol-3-O-glc (Astragalin)[c]	15.90 ± 0.18	8.58 ± 0.14	6.81 ± 0.11	4.13 ± 0.17	7.31 ± 0.13	9.49 ± 0.19
Kaempferol[c]	4.24 ± 0.11	-	1.63 ± 0.10	-	-	-
Syringetin-3-O-glc[c]	3.46 ± 0.09	2.69 ± 0.12	2.05 ± 0.11	0.86 ± 0.04	1.37 ± 0.06	2.78 ± 0.08
Syringetin-3-O-(6-acetyl)-glc[c]	-	-	-	-	-	-

Total	85.57	89.07	78.02		106.03	75.13	57.18
Anthocyanins							
Cyanidin-3-O-glc[d]	-	-	-		-	-	-
Cyanidin-3-O-(6-acetyl)-glc[d]	-	-	-		-	< 0.50	< 0.50
Petunidin-3-O-glc[d]	-	-	-		-	< 0.50	< 0.50
Petunidin-3-O-(6-acetyl)-glc[d]	-	-	-		-	< 0.50	< 0.50
Delfinidin-3-O-glc[d]	-	-	-		-	< 0.50	< 0.50
Delfinidin-3-O-(6-acetyl)-glc[d]	-	-	-		-	< 0.50	< 0.50
Delfinidin-3-O-(6-coumaryl)-glc[d]	-	-	-		-	< 0.50	< 0.50
Peonidin-3-O-glc[d]	-	-	-		-	-	-
Peonidin-3-O-(6-acetyl)-glc[d]	-	-	-		-	< 0.50	< 0.50
Malvidin-3-O-glc[d]	-	-	-		-	< 0.50	< 0.50
Malvidin-3-O-(6-acetyl)-glc[d]	-	-	-		-	< 0.50	< 0.50
Malvidin-3-O-(6-coumaryl)-glc[d]	-	-	-		-	< 0.50	< 0.50
Total	-	-	-		-	-	-

	Red wines								
Phenolic compounds	$BN_{Pan2011}$	$BN_{Hus2010}$	BN_{SN2011}	BN_{SC2010}	$FN_{Cot2010}$	$FN_{Pan2011}$	$FN_{Cep2012}$	$FN_{Toh2010}$	$FN_{Mur2011}$
Flavan-3-ols									
(+)-Catechin	112.39 ± 0.84	167.41 ± 0.49	203.38 ± 0.92	193.76 ± 0.55	192.38 ± 0.44	331.59 ± 1.10	202.88 ± 0.92	241.16 ± 0.78	212.11 ± 0.65
Epicatechin gallate[a]	63.75 ± 0.42	157.91 ± 0.60	146.08 ± 0.72	213.81 ± 0.74	192.11 ± 0.68	197.57± 0.88	141.30 ± 0.60	299.16 ± 1.20	206.68 ± 0.96
Gallocatechin[a]	48.98 ± 0.38	89.64 ± 0.36	77.39 ± 0.33	65.01 ± 0.35	101.29 ± 0.51	63.23 ± 0.32	53.79 ± 0.43	125.60 ± 0.80	60.59 ± 0.37
Total	225.12	414.96	426.85	472.58	485.78	592.39	397.97	665.92	479.38
Stilbenes									
Resveratrol-3-O-glc (Piceid)[b]	0.94 ± 0.08	1.21 ± 0.11	1.03 ± 0.08	1.20 ± 0.09	1.32 ± 0.08	0.98 ± 0.08	1.14 ± 0.06	1.02 ± 0.05	1.01 ± 0.07
Resveratrol-5-O-glc[b]	0.29 ± 0.04	0.36 ± 0.05	0.32 ± 0.04	0.29 ± 0.01	0.26 ± 0.04	0.27 ± 0.03	0.26 ± 0.01	0.25 ± 0.03	0.26 ± 0.03
trans-Resveratrol	2.70 ± 0.12	1.71 ± 0.09	1.33 ± 0.13	1.58 ± 0.14	1.68 ± 0.10	2.27 ± 0.15	0.72 ± 0.03	1.36 ± 0.09	1.33 ± 0.07
Piceatannol[b]	0.40 ± 0.04	0.26 ± 0.06	0.24 ± 0.03	0.20 ± 0.03	0.18 ± 0.03	0.21 ± 0.03	0.24 ± 0.02	0.37 ± 0.04	0.26 ± 0.05
Pterostilbene[b]	12.05 ± 0.16	1.04 ± 0.10	0.66 ± 0.06	0.27 ± 0.02	11.51 ± 0.21	9.10 ± 0.18	1.08 ± 0.08	13.42 ± 0.10	12.34 ± 0.14
Pallidol[b]	1.31 ± 0.07	1.24 ± 0.10	1.48 ± 0.11	2.04 ± 0.12	2.53 ± 0.19	1.91 ± 0.11	1.25 ± 0.08	3.78 ± 0.16	1.95 ± 0.06
Total	17.69	5.82	5.06	5.58	17.48	14.74	4.69	20.20	17.15
Flavonols									
Myricetin-3-O-gal[c]	-	3.51 ± 0.16	20.75 ± 0.35	3.29 ± 0.14	3.92 ± 0.16	1.95 ± 0.08	2.44 ± 0.07	-	-
Myricetin-3-O-glc[c]	7.08 ± 0.14	11.21 ± 0.12	6.25 ± 0.19	12.84 ± 0.19	15.79 ± 0.23	7.99 ± 0.16	17.15 ± 0.22	8.70 ± 0.15	9.71 ± 0.17
Myricetin[c]	6.59 ± 0.17	2.32 ± 0.09	6.34 ± 0.14	3.31 ± 0.17	7.80 ± 0.18	6.98 ± 0.14	1.48 ± 0.10	5.58 ± 0.12	2.66 ± 0.06
Quercetin-3-O-gal (Hyperoside)[c]	3.93 ± 0.13	4.49 ± 0.19	2.17 ± 0.11	3.38 ± 0.08	3.58 ± 0.17	3.20 ± 0.11	4.90 ± 0.16	2.79 ± 0.07	2.99 ± 0.13
Quercetin-3-O-glc (Isoquercetin)[c]	7.81 ± 0.11	2.38 ± 0.11	4.69 ± 0.17	4.70 ± 0.18	6.37 ± 0.13	5.42 ± 0.17	4.34 ± 0.12	2.99 ± 0.11	6.83 ± 0.16
Quercetin-3-O-gluc[c]	12.56 ± 0.14	9.88 ± 0.14	13.02 ± 0.24	10.52 ± 0.20	11.69 ± 0.19	9.86 ± 0.20	7.29 ± 0.13	8.65 ± 0.19	13.20 ± 0.20

Quercetin	-	5.95 ± 0.15	9.95 ± 0.15	5.07 ± 0.13	11.68 ± 0.11	2.88 ± 0.12	2.35 ± 0.05	6.52 ± 0.14	2.68 ± 0.08
Isorhamnetin-3-O-gal[c]	-	3.73 ± 0.07	10.09 ± 0.22	4.61 ± 0.12	8.94 ± 0.19	-	6.01 ± 0.12	10.35 ± 0.21	14.93 ± 0.19
Isorhamnetin-3-O-glc[c]	92.93 ± 0.33	42.72 ± 0.36	49.28 ± 0.38	46.30 ± 0.33	46.94 ± 0.37	82.33 ± 0.44	20.36 ± 0.25	26.22 ± 0.32	37.18 ± 0.29
Isorhamnetin[c]	6.57 ± 0.13	4.82 ± 0.12	1.94 ± 0.12	3.57 ± 0.07	2.35 ± 0.06	4.65 ± 0.18	0.98 ± 0.03	2.22 ± 0.06	2.23 ± 0.12
Laricitrin-3-O-glc[c]	30.68 ± 0.24	14.37 ± 0.21	35.96 ± 0.29	19.99 ± 0.19	30.92 ± 0.15	30.43 ± 0.32	11.13 ± 0.16	14.15 ± 0.17	14.66 ± 0.20
Laricitrin-3-O-(6-acetyl)-glc[c]	9.19 ± 0.18	9.59 ± 0.11	9.69 ± 0.18	10.87 ± 0.22	15.05 ± 0.14	9.54 ± 0.16	16.08 ± 0.18	9.39 ± 0.20	10.32 ± 0.14
Laricitrin[c]	1.37 ± 0.07	1.65 ± 0.05	-	1.31 ± 0.04	1.72 ± 0.09	1.49 ± 0.09	0.70 ± 0.02	1.09 ± 0.08	1.57 ± 0.07
Kaempferol-3-O-glc (Astragalin)[c]	19.15 ± 0.21	29.98 ± 0.26	16.32 ± 0.22	26.70 ± 0.18	26.93 ± 0.24	25.64 ± 0.24	10.42 ± 0.20	70.05 ± 0.35	24.56 ± 0.22
Kaempferol[c]	-	-	1.79 ± 0.07	-	1.32 ± 0.07	-	-	1.29 ± 0.06	-
Syringetin-3-O-glc[c]	34.35 ± 0.26	34.23 ± 0.43	45.98 ± 0.39	35.00 ± 0.25	46.15 ± 0.34	34.41 ± 0.25	34.37 ± 0.25	39.03 ± 0.45	18.81 ± 0.26
Syringetin-3-O-(6-acetyl)-glc[c]	4.68 ± 0.11	4.00 ± 0.13	6.70 ± 0.18	4.45 ± 0.15	7.93 ± 0.15	7.42 ± 0.18	2.98 ± 0.14	4.54 ± 0.15	4.19 ± 0.14
Total	236.89	184.83	240.92	195.91	249.08	234.19	142.98	213.56	166.52
Anthocyanins									
Cyanidin-3-O-glc[d]	-	-	4.55 ± 0.14	-	-	3.36 ± 0.14	-	-	1.62 ± 0.04
Cyanidin-3-O-(6-acetyl)-glc[d]	2.82 ± 0.11	1.27 ± 0.06	1.45 ± 0.05	1.30 ± 0.04	1.19 ± 0.05	1.52 ± 0.08	4.17 ± 0.15	1.22 ± 0.02	2.70 ± 0.10
Petunidin-3-O-glc[d]	2.06 ± 0.09	-	1.26 ± 0.07	1.22 ± 0.06	1.36 ± 0.09	1.69 ± 0.09	1.77 ± 0.09	-	-
Petunidin-3-O-(6-acetyl)-glc[d]	2.58 ± 0.12	1.28 ± 0.08	2.35 ± 0.11	1.26 ± 0.08	2.19 ± 0.13	1.60 ± 0.06	5.74 ± 0.14	2.38 ± 0.08	4.96 ± 0.14
Delfinidin-3-O-glc[d]	26.07 ± 0.22	-	5.71 ± 0.18	2.10 ± 0.09	13.06 ± 0.20	24.90 ± 0.18	6.50 ± 0.20	9.42 ± 0.16	32.62 ± 0.26
Delfinidin-3-O-(6-acetyl)-glc[d]	5.99 ± 0.14	3.09 ± 0.14	3.32 ± 0.17	2.79 ± 0.15	4.09 ± 0.14	4.41 ± 0.11	16.40 ± 0.19	4.57 ± 0.11	8.15 ± 0.16
Delfinidin-3-O-(6-coumaryl)-glc[d]	5.14 ± 0.17	1.58 ± 0.11	0.87 ± 0.03	1.78 ± 0.11	5.00 ± 0.17	4.67 ± 0.14	9.51 ± 0.17	4.52 ± 0.12	6.32 ± 0.18
Peonidin-3-O-glc[d]	-	-	2.22 ± 0.10	-	-	-	-	-	-
Peonidin-3-O-(6-acetyl)-glc[d]	6.47 ± 0.16	1.47 ± 0.08	1.86 ± 0.09	1.55 ± 0.07	4.30 ± 0.15	6.88 ± 0.18	8.53 ± 0.18	5.27 ± 0.14	12.10 ± 0.14
Malvidin-3-O-glc[d]	3.16 ± 0.11	1.22 ± 0.04	1.22 ± 0.08	1.17 ± 0.06	3.38 ± 0.11	3.34 ± 0.11	5.72 ± 0.21	4.31 ± 0.11	5.51 ± 0.11
Malvidin-3-O-(6-acetyl)-glc[d]	4.41 ± 0.15	1.96 ± 0.08	1.60 ± 0.12	2.02 ± 0.12	2.15 ± 0.14	4.11 ± 0.15	6.41 ± 0.19	5.31 ± 0.19	3.65 ± 0.14
Malvidin-3-O-(6-coumaryl)-glc[d]	6.10 ± 0.19	1.76 ± 0.05	1.44 ± 0.04	1.49 ± 0.09	5.49 ± 0.19	6.63 ± 0.13	4.11 ± 0.11	3.88 ± 0.12	7.48 ± 0.22
Total	64.80	13.63	27.85	16.68	42.21	63.11	68.86	40.88	85.11

Notes: - not found; < 0.50 - compounds detected but not quantifiable; [a] Expressed in equivalents of catechin; [b] Expressed in equivalents of *trans*-resveratrol; [c] Expressed in equivalents of quercetin; [d] Expressed in equivalents of cyanidin; glc: glucoside; gal: galactoside; gluc: glucuronide

The results obtained confirm a variation in the polyphenolic content amongst wines tested, due to their different grape cultivar, geographical origin, and wine colour, as expected. Comparisons with other data from

Romanian wines was difficult since only total phenolic content was available (Hosu *et al*., 2014), or concentration levels limited to a few compounds (resveratrol, catechin, quercetin and phenolic acids) and samples (Geana *et al*., 2014a; Luchian *et al*., 2018). However, the obtained concentration ranges are in agreement with the values reported in available literature (Tinttunen and Lehtonen, 2001; Castillo-Muñoz *et al*., 2007; García-Falcón *et al*., 2007; Tenore *et al*., 2011; Rodríguez-Cabo *et al*., 2014).

Flavan-3-ols

Three flavan-3-ol monomers were found in the studied wines: (+)-catechin, epicatechin gallate and gallocatechin. The concentration levels for (+)-catechin in red wines are in agreement with the values reported for French and German wines (Tinttunen and Lehtonen, 2001), except for the $FN_{Pan2011}$ wine with the highest amount of catechin (331.59 mg L^{-1}). In almost all samples catechin was the most abundant flavan-3-ol monomer, independently of the wine type or the aging time. Our results confirmed previous findings that reported catechin to be the most important flavanol found in wines made from different grape varieties (Monagas *et al*., 2005; Gomez-Alonso *et al*., 2007). The great variation obtained for the concentrations of individual flavan-3-ols, both between the three types of wines (white, rosé and red), as well as between the red wines, can be attributed to the vinification techniques, grape cultivar and climate characteristics. The high levels of flavan-3-ols in some samples (BN_{SC2010}, $FN_{Cot2010}$, $FN_{Pan2011}$, $FN_{Toh2010}$, $FN_{Mur2011}$) may be the consequence of a better extraction of grape tannins into the wine, during a longer maceration time. The total flavanol content in red wines was significantly higher than those in white and rosé wines. Our data are in accordance with the results of Spanish and China wines (Pérez-Magariño *et al*., 2006; Li *et al*., 2009), and are higher than those obtained for Greek, French and German wines (Arnous *et al*., 2001; Tinttunen and Lehtonen, 2001). The red wine sample with the highest content in total flavan-3-ols was $FN_{Toh2010}$; its content was nearly triple that in the $BN_{Pan2011}$ red wine. Larger variations on total flavanol content were registered in case of red wines, for which differences were obtained depending on the variety of grape from which they come. Thus, the FN wines had a higher content of total flavanols than the BN wines. Therefore, getting a high content of total flavanols for Romanian wines is a desirable result since flavanols have been shown to exhibit powerful antioxidant activities in different environments, and, even the concentrations of individual flavanols might be responsible for the antioxidant capacities of wines (Arnous *et al*., 2001). Additionally, the flavan-3-ols content is a key parameter for wine quality since these compounds were responsible for the astringency and bitterness. They also play an important role due to their interactions with other phenolic compounds during wine ageing (Boulton, 2001).

Stilbenes

Stilbenes are one minor group of phenolic compounds in wine, resveratrol being the principal stilbene. In all analyzed wines, there were found six stilbenes: five different forms of resveratrol-monomer stilbenes (*trans*-resveratrol, *trans*-piceid, resveratrol-5-O-glucoside, piceatannol, pterostilbene) and one form of resveratrol-dimer (pallidol).

Trans-resveratrol, a stilbene with multiple health benefits, was quantified, ranging from 0.67 to 1.46 mg L^{-1} in white wines, from 0.34 to 0.61 mg L^{-1} in rosé wines and from 0.72 to 2.70 mg L^{-1} in red wines. These amounts were comparable with the reported ranges found for white, rosé and red wines (Tinttunen and Lehtonen, 2001; Abril *et al*., 2005; Stervbo *et al*., 2007). Usually, *trans*-resveratrol contents in rosé wines are higher than those present in white wines and this difference in concentrations is linked to the winemaking process, especially to the contact of wine with the solid parts of the grape (Mattivi, 1993). However, we found that white wines have higher *trans*-resveratrol contents. This apparent contradiction can be explained by the environmental factors (different climatic conditions, soil quality, geographical origin) that can affect resveratrol contents, but also the concentration of resveratrol in wine varies considerably and appears to depend on the grape variety (Abril *et al*., 2005). In case of wines from regions of climatic similarity, these differences of concentrations can be due to the intrinsic resveratrol-synthesizing capacity of the different grape cultivars

employed (Goldberg *et al*., 1995). Resveratrol concentrations increase during fermentation of the skins in case of rosé wines, for example, but the amount extracted is dependent on the variety, wine production process, and stress exposure (Geana *et al*., 2014b).

Among the nine red wines tested, $BN_{Pan2011}$ showed the highest concentration of *trans*-resveratrol (2.70 mg L^{-1}), while $FN_{Cep2012}$ exhibited the lowest level of *trans*-resveratrol (0.72 mg L^{-1}). It has been observed that the red wines from Dealurile Moldovei wine region ($BN_{Pan2011,}$ $FN_{Pan2011,}$ $BN_{Hus2010}$ and $FN_{Cot2010}$) had higher amounts of *trans*-resveratrol than the red wines from the other Romanian wine regions, independently of the grape cultivar or the aging time. These differences regarding the *trans*-resveratrol levels in the analyzed wines may be related to vinification process (which influences the extraction and diffusion of phenolics from the grape to the wine) as well environmental factors (soil, geographical origin and climatic conditions).

Concerning the compound piceid, the natural main precursor of resveratrol found in grape, all the analyzed wines showed lower levels of *trans*-piceid than of *trans*-resveratrol, except the rosé wine $BB_{Hus2011}$ and the red wine $FN_{Cep2012}$ which had higher levels. Thus, lower concentrations of piceid, compared to those of resveratrol, can be explained by the hydrolysis of glycosylated forms (piceid) present in wine to aglycone form (resveratrol), with wine aging. The values of *trans*-piceid were similar to reported average data (Rodríguez-Cabo *et al*., 2014). Pallidol, alongside some other resveratrol dimers, are fungal metabolites of resveratrol and the occurrence of this compound in wine is due to the oxidation of resveratrol by fungus in infected berries used for vinification (Cichewicz *et al*., 2000). *Trans*-piceatannol and pterostilbene were also detected and quantified in all samples.

The levels of total stilbenes showed significant differences according to grape cultivar, winemaking region and vintage. An estimation of the total stilbene intake from $FN_{Toh2010}$ red wine, which is the richest in stilbenes from our samples, and considering a regular consumption of 250 mL day^{-1} of $FN_{Toh2010}$ wine, would mean a daily intake of stilbenes of approximately 5 mg day^{-1} $individual^{-1}$. For people drinking only FN Romanian wines, this value should be between 1.18 and 4.38 mg day^{-1} $individual^{-1}$, and between 1.27 and 4.43 mg day^{-1} $individual^{-1}$ for people drinking only BN Romanian wines. Therefore, one can conclude that Romanian wines, especially FN and BN varieties, can be an important source of daily intake of stilbenes. Our results indicate that Romanian red wines contained high levels of pterostilbene, and important levels of pallidol, *trans*-resveratrol and *trans*-piceid, which may constitute a significant proportion in stilbene dietary intake. Whereas resveratrol bioavailability by oral administration in rats is around 40% (Marier *et al*., 2002), the resveratrol plasma concentration obtained after ingestion of these red wines would probably be low, but might be increased after a long-term consumption. Since resveratrol glucoside may be deglycosylated and converted into bioavailable resveratrol during digestion, biological activities would be due both to the glycosylated and aglycone forms (Moreno-Labanda *et al*., 2004).

In order to explain the differences in stilbene levels between varieties, further studies are needed, regarding the soil composition, vinification and conservation process, and environmental conditions. It would also be necessary to investigate the bioavailability of the major stilbenes, such as pterostilbene and pallidol.

Flavonols

Flavonols are yellow pigments with significant role in the stabilization of the wine colour, since they participate in the co-pigmentation reactions with anthocyanins (Gomez-Alonso *et al*., 2007). As well as anthocyanins, flavonols occur naturally as glycosides and sugar substitution on flavonols usually takes place as the O-glycosides, mainly at the 3-position, the UV spectrum being the indicative of substitution position (Castillo-Muñoz *et al*., 2007). Seventeen flavonols were identified and quantified in Romanian wines, including twelve original grape flavonol O-glycosides and five free flavonol aglycones released from them by hydrolysis in wine. Taking into account the differences that occur in their biosynthesis pathway in grape berries, flavonols could be classified into six groups: myricetin derivatives, quercetin derivatives, isorhamnetin derivatives, laricitrin derivatives, kaempferol derivatives and syringetin derivatives. The concentrations of the

above-mentioned compounds varied according to variety, geographical origin and environmental conditions. Regarding the group of myricetin derivatives, none of the white wine varieties were found to contain myricetin aglycon and myricetin-3-O-galactoside and nor the $BR_{Pan2012}$ rosé wine to contain myricetin aglycon. These results are in agreement with previously reported data for other white wines (Rastija *et al*., 2009; Vrček *et al*., 2011; Pereira *et al*., 2013). Among the red wines, myricetin-3-O-galactoside was not detected in the following three samples: $BN_{Pan2011}$, $FN_{Toh2010}$ and $FN_{Mur2011}$. Only myricetin-3-O-glucoside was found in all red, rosé and white wines. Excepting the BN_{SN2011} red wine, in all other fourteen studied wines, myricetin-3-O-glucoside was the most abundant flavonol from the group of myricetin derivatives and this can be explained by the fact that this compound is not so easily hydrolyzed.

Among quercetin derivatives, only quercetin-3-O-galactoside and quercetin-3-O-glucuronide were detected and quantified in all fifteen wine samples. Quercetin-3-O-glucoside was not detected in the $FR_{Rec2012}$ white wine, while the quercetin aglycon was not found in any of the white and rosé wine varieties and neither in the $BN_{Pan2011}$ red wine, although previous studies reported the presence of quercetin aglycon in Spanish white wines (Pereira *et al*., 2013). These results confirmed that the rates of hydrolysis of the flavonol glycosides in wine were different, according to the type of flavonol aglycone and, also, with respect to the nature of the glycoside moiety. Since the quercetin-3-O-glucoside, one of the main flavonol glycosides found in grapes, seems to be easily hydrolysed, it is usual to observe an important level of free quercetin in wines due to its hydrolysis (Castillo-Muñoz *et al*., 2007). Therefore, this has been observed in five samples of red wine: $BN_{Hus2010}$, BN_{SN2011}, BN_{SC2010}, $FN_{Cot2010}$ and $FN_{Toh2010}$.

The isorhamnetin derivatives dominated the flavonol profiles in all white and rosé wines, and also in seven of the nine red wines investigated. For the other two red wine samples, the syringetin derivatives were the dominant in the $FN_{Cep2012}$ wine and the kaempferol derivatives in the $FN_{Toh2010}$ wine. At the same time, isorhamnetin-3-O-glucoside was the most abundant O-glucoside compound in all studied wines, except the $FN_{Cep2012}$ and $FN_{Toh2010}$ red wines that has the highest amount of syringetin-3-O-glucoside, respectively kaempferol-3-O-glucoside. Within each group of the isorhamnetin, laricitrin and kaempferol derivatives, the amounts of the 3-O-glucosides were much higher than those of their corresponding free aglycones. With regard to free aglycones, isorhamnetin was quantified in all samples, laricitrin was not found in two wine samples ($FR_{Rec2012}$ and BN_{SN2011}) and kaempferol was found only in two white wines ($FA_{Cot2011}$ and $FR_{Jid2011}$) and three red wines (BN_{SN2011}, $FN_{Cot2010}$ and $FN_{Toh2010}$).

To conclude, the concentrations of the flavonol O-glycosides found in all wines were higher than for flavonol aglycones, since the conjugates are more stable than the free forms, as confirmed by other studies (Castillo-Muñoz *et al*., 2007). All wine samples showed high levels of flavonols, decreasing from red wines (143.04-249.15 mg L^{-1}) to white wines (78.07-106.09 mg L^{-1}) and rosé wines (57.23-75.19 mg L^{-1}). This variability can be explained by several factors like the grape cultivar, the degree of grape ripening, the winemaking process and the aging time (Castillo-Muñoz *et al*., 2007). Also, it is known that the increased biosynthesis of polyphenols, especially flavonols, is greatly influenced by sunlight exposure and temperature, so the wines made from grapes which are grown in warmer, sunnier areas may have a higher level of flavonols (Tenore *et al*., 2011). On the basis of our results, it can be suggested that Romanian wines are richer in flavonols than wines from other countries, other authors reporting a lower number of flavonols in their wines, but similar or higher levels of individual flavonols (Tenore *et al*., 2011).

Anthocyanins

Anthocyanin pigments give the colour of grapes and young red wines. They occur naturally as glycosides and for *V. vinifera* red wines the glycosylation appears exclusively at the 3-position (Downey and Rochfort, 2008). The monomeric anthocyanins were eluted by HPLC and detected at 520 nm, by DAD and MS, as distinct individual peaks which overlapped over the polymeric anthocyanin hump (Figure 1C). The identification of individual peaks was made by comparing retention times, individual UV/Vis absorption

spectra and MS molecular ions by comparison with pure compounds and literature data (Mazzuca *et al*., 2005). A number of twelve anthocyanins were identified and quantified in the analyzed red wines, while in rosé wines, considering their small content, they could not be quantified.

The anthocyanins identified were monoglucoside derivatives of five anthocyanidins: cyanidin, petunidin, delphinidin, peonidin and malvidin, and derivatives were 6-*O*-acetyl and 6-*O*-coumaryl. It was observed that the anthocyanin profiles and composition of the wines were not similar; therefore, the vineyard location produced selective effects on individual anthocyanins, even in the case of the wines from the same grape variety. Differently from other studies (Kelebek *et al*., 2010; Li *et al*., 2011), delfinidin forms of the anthocyanins were the most abundant class of monomeric anthocyanins, followed by malvidin, peonidin, petunidin and cyanidin. Among the non-acylated anthocyanins, malvidin-3-O-glucoside was the only found in all wine samples. Peonidin-3-O-glucoside was found only in the BN_{SN2011} wine, and cyanidin-3-O-glucoside was quantified in three samples, $FN_{Mur2011}$, $FN_{Pan2011}$ and BN_{SN2011}. In addition, petunidin-3-O-glucoside was not found in the $BN_{Hus2010}$, $FN_{Toh2010}$ and $FN_{Mur2011}$ wines. The levels obtained for non-acylated anthocyanins were in accordance with those reported before for delfinidin-3-O-glucoside (Figueiredo-González *et al*., 2014), peonidin-3-O-glucoside (Bai *et al*., 2013), cyanidin-3-O-glucoside (Li *et al*., 2011) and petunidin-3-O-glucoside (Bai *et al*., 2013). The acylated anthocyanins consisted of five acetylated anthocyanins and two coumaryl derivatives of anthocyanins, quantified in all samples. They had a better stability and solubility than the non-acylated anthocyanins, but their contents could be influenced by several factors, such as the grape varieties, the vineyard location and climatic conditions (Garcia-Beneytez *et al*., 2003).

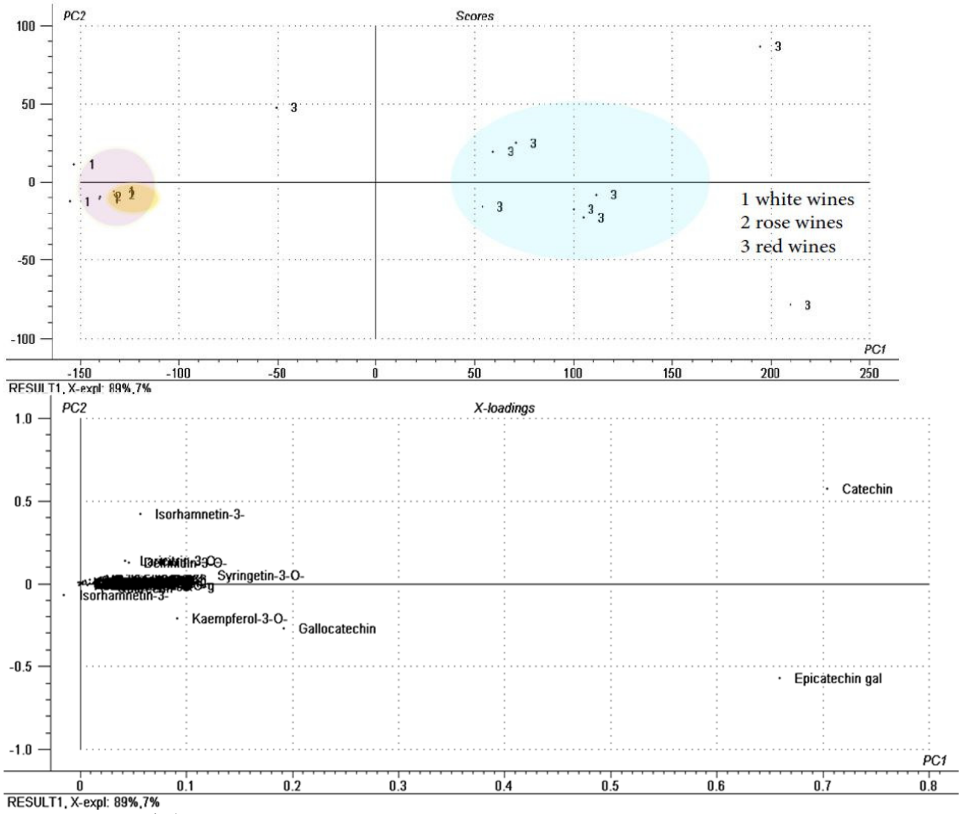
The total concentration of monomeric anthocyanins from the BN wines was lower than those from FN wines, excepting the $BN_{Pan2011}$ that had similar content with $FN_{Pan2011}$. Our results are similar with those reported for Spanish wines (García-Falcón *et al*., 2007) and Greek wines (Arnous *et al*., 2001), but much lower than those reported for Italian (De Nisco *et al*., 2013) and Turkish wines (Kelebek *et al*., 2010). These discrepant values can be due to the compositional differences between young and aged wines, degradation or condensation of monomeric anthocyanins with other compounds, to give more stable polymeric pigments. Furthermore, our findings showed for wines of 2011 and 2012 higher levels of anthocyanins (63.15-85.16 mg L^{-1}) than for wines of 2010 (13.66-42.25 mg L^{-1}), except for the BN_{SN2011} wine, whose total anthocyanin content was poor (27.92 mg L^{-1}).

Principal component analysis

The classification and discriminations between red, rosé and white wine samples, based on their phenolics profile and quantity, was performed by multivariate data analysis, using the Principal Component Analysis (PCA). Figure 2A shows two-dimensional scores plot of the fifteen analyzed wine samples, defined by the first two principal components, PC1 and PC2. The first principal component (PC1) accounted for 89% of the variability and the second principal component (PC2) accounted for 7% of the variability; together, PC1 and PC2 account for 96% of the total variance. Three main groups were identified: the first included white wines, the second rosé ones and the third included red wines. The first group partially overlapped the second one for two white wines, but overall, wines were obviously discriminated along the first axis (PC1) based on the wine colour. White and rosé wines are grouped mostly in the lower negative part of PC1, except for the $FR_{Rec2012}$ white wine which is placed in the left upper part of the plot. Within the third group, the red wines BN_{SN2011}, $FN_{Pan2011}$ and $FN_{Cep2012}$ are grouped in the right upper part of the plot, while $BN_{Hus2010}$, BN_{SC2010}, $FN_{Cot2010}$, $FN_{Toh2010}$ and $FN_{Mur2011}$ are grouped mostly in the lower positive part of PC1. Only the $BN_{Pan2011}$ red wine is located in the left upper side of PC1. These differences are due also to the sweetness index, as we reported also, using Fourier Transform Infrared spectroscopy coupled with chemometry (Banc *et al*., 2014).

Figure 2B includes the loading plots for PC1 and PC2, showing that catechin, epicatechin and gallocatechin are mainly responsible for the discriminations among clustered wine samples. The results obtained by PCA demonstrate that the differences between samples are due to varietal variability and to the

wine colour, but in terms of geographical region, it is more difficult to discriminate the samples considering only the phenolic composition.



(A)

(B)

Figure 2. (A) Scores of the fifteen wine samples in the plane defined by the first two principal components: PC1 and PC2; (B) Loading plots for PC1 and PC2

Total phenolic content

The total phenolic content of all analyzed wines is presented in Table 6. The TPC concentrations were expressed as mg Gallic acid equivalents per liter of wine (mg GAE/L), based on the linear equation obtained from Gallic acid standard calibration curve.

Table 6. Total phenolic content (TPC), DPPH radical scavenging activity (%) and total antioxidant activity (TAA) of 15 analysed wines (mean value (n = 3))

Wine no.	Wine sample	TPC (mg GAE/L)	DPPH radical scavenging activity (%)	TAA (mM TE/L)
1	$FA_{Cot2011}$	220.00^{a}	30^{a}	0.66^{a}
2	$FA_{Jid2011}$	230.00^{ab}	44^{c}	0.84^{b}
3	$FR_{Jid2011}$	245.00^{c}	51^{d}	0.93^{c}
4	$FR_{Rec2012}$	244.00^{c}	43^{bc}	0.82^{b}
5	$BR_{Pan2012}$	236.00^{bc}	45^{c}	0.86^{b}
6	$BB_{Hus2011}$	243.00^{c}	41^{b}	0.80^{b}
7	$BN_{Pan2011}$	801.00^{d}	86^{fg}	8.61^{f}
8	$BN_{Hus2010}$	2149.00^{h}	88^{gh}	8.95^{i}
9	BN_{SN2011}	2064.00^{g}	90^{h}	9.19^{j}
10	BN_{SC2010}	2196.00^{i}	85^{f}	8.54^{e}
11	$FN_{Cot2010}$	2311.00^{k}	87^{fg}	8.75^{gh}

12	$FN_{Pan2011}$	1877.00^{f}	87^{fg}	8.77^{h}
13	$FN_{Cep2012}$	2248.00^{j}	86^{fg}	8.69^{g}
14	$FN_{Toh2010}$	2359.00^{l}	95^{i}	9.84^{k}
15	$FN_{Mur2011}$	1660.00^{e}	63^{e}	5.54^{d}

Note: different letters (a, b, c...) on the same column indicate significant differences among wine samples at $p < 0.05$ (Duncan's test). GAE: Gallic acid equivalents; TE: Trolox equivalents

Therefore, the TPC values ranged from 220 to 245 mg GAE/L for the white wines, from 236 to 243 mg GAE/L for the rosé wines and from 801 to 2359 mg GAE/L for the red wines. This difference between red, rosé and white wines is in agreement with previously published results (Paixão *et al*., 2007; Li *et al*., 2009; Rastija *et al*., 2009). Among the red wines, the highest content of phenolics was found in $FN_{Toh2010}$ (2359 mg GAE/L) and the lowest in the $BN_{Pan2011}$ wine (801 mg GAE/L). $FN_{Cot2010}$ and $FN_{Cep2012}$ also contained a high content of phenolic compounds (2311 mg GAE/L, respectively 2248 mg GAE/L). Among the white wines, $FR_{Jid2011}$ and $FA_{Cot2011}$, respectively, represented the wines with the highest and lowest phenolic contents (245 mg GAE/L, respectively 220 mg GAE/L). The amounts of phenolic compounds vary in different wine samples according to grape variety, environmental factors in the vineyard, the wine processing techniques, soil and atmospheric conditions during ripening, aging process and berry maturation (Pérez-Magariño and González-San José, 2006). Our results confirm this variation in phenolic content among wine samples tested, the total phenols' content in red wine being up to 10 times higher than in rosé and white wine, explained by a higher content of condensed tannins and anthocyanins in red wines. These differences may be also the result of a better phenolics' extraction from grape skin and seed contact time, fermentation conditions and temperature for red wines, as opposed to white ones. The highest phenolic content of red wines contributed to their increased antioxidant activity in comparison to rosé and white wine.

Total antioxidant activity

The antioxidant activities of all wines were evaluated using DPPH free radical scavenging assay, and to express the Total Antioxidant Activity (TAA) Trolox was used as standard control for the samples' capacity to scavenge DPPH•, expressed as percentage values (%) or as mM Trolox equivalents (TE/L) (Table 6). The wines' total antioxidant activity ranged from 0.657 to 0.930 mM TE/L for the white ones, from 0.804 to 0.855 mM TE/L for the rosé ones and from 5.538 to 9.840 mM TE/L for the red wines. Our data is in accordance with the results of Croatian red and white wines (Vrček *et al*., 2011) and is higher than those obtained for Madeira red, rosé and white wines (Paixão *et al*., 2007). The free radical scavenging activities found by DPPH assay in the white, rosé and red wine varieties differed significantly, red wines' values were higher than those of white and rosé wines, in agreement with literature reports (Fernández-Pachón *et al*., 2004; Paixão *et al*., 2007; Vrček *et al*., 2011). The strongest antioxidant activity was found in the red wine $FN_{Toh2010}$, having the highest content of phenolics, while the lowest activity was obtained in white wine $FA_{Cot2011}$, with the lowest content of phenolics.

As regards the sulfur dioxide content of the examined wines, most labels, but not all, have the mention "contains sulfites". According to European Commission regulations (Ruling no 606/2009) (EC, 2009), the total sulfur dioxide content cannot exceed 150 mg L^{-1} in conventional red wines, and 200 mg L^{-1} in conventional white wines. In organic wines, the total sulfur dioxide content cannot exceed 100 mg L^{-1} in red wines and 150 mg L^{-1} in white wines. Studies conducted so far indicate that the presence of SO_2 in wines has no significant influence on the antioxidant activity of wines. Thus, the results obtained by Garaguso and Nardini (2015) indicate that organic red wines produced without sulfur dioxide/sulfites addition possess antioxidant activity, phenolics profile, total polyphenols and flavonoids content comparable to those of conventional red wines. In another study, Gabriele *et al*. (2018) followed the influence of SO_2 on the phytochemical profile and *in vitro* antioxidant activity of wines and they found comparable results for wines produced without SO_2 addition and those with 50 mg/L SO_2 added.

Correlations between total phenolic content and total antioxidant activity

The results of study show that, higher the concentration of antioxidants is, higher the free radical scavenging activity is. The antioxidant activity of wine polyphenols are related with their chemical structure since it has been reported that compounds with a high number of hydroxyl groups present higher activity. The contribution of each polyphenol to the antioxidant activity of wines is different, so the activity of wines depends on their phenolic profile.

The present study reveals for white wines a very strong correlation ($p < 0.001$) between TPC and TAA, representative for the 98.60% and respectively 81.70% of sample in case of $FA_{Cot2011}$ ($r = 0.993$) and respectively $FR_{Jid2011}$ ($r = 0.904$); a positive correlation ($p < 0.01$) representative for the 61.40% of sample for $FR_{Rec2012}$ ($r = 0.784$) and a medium positive correlation ($p < 0.05$) representative only for the 26.50% of sample for $FA_{Jid2011}$ ($r = 0.515$).

Regarding the rosé wines, it was obtained a weak to medium positive correlation ($p < 0.01$) for the $BR_{Pan2012}$ ($r = 0.440$), representative for the 19.40% of sample, and a medium negative correlation ($p < 0.01$) for the $BB_{Hus2011}$ ($r = - 0.565$), representative for the 31.90% of sample. Thus, it was observed that, although the TPC for $BB_{Hus2011}$ was slightly higher than for $BR_{Pan2012}$, the antioxidant activity was lower.

In case of red wines, for all BN samples, negative correlations were obtained, very strong ($p < 0.001$) for $BN_{Pan2011}$ ($r = - 0.976$) and BN_{SC2010} ($r = - 0.982$), and weak negative correlation statistically insignificant ($p > 0.05$) for $BN_{Hus2010}$ ($r = - 0.222$) and BN_{SN2011} ($r = - 0.327$). Positive correlations between TPC and TAA were obtained for all FN red wine samples. These were very strong ($p < 0.001$) for $FN_{Cot2010}$ ($r = 0.952$), medium ($p < 0.01$) for $FN_{Pan2011}$ ($r = 0.625$) and $FN_{Cep2012}$ ($r = 0.648$), and very weak statistically insignificant ($p > 0.05$) for $FN_{Toh2010}$ ($r = 0.169$) and $FN_{Mur2011}$ ($r = 0.213$).

Finally, our results showed that the antioxidant activity of wines has not been influenced by TPC, since wines having a highest TPC did not always show the highest values for antioxidant activity. These findings were in agreement with those reported by a few authors (Rivero-Pérez *et al*., 2007), but in contrast with others who have shown positive correlation between TPC of wines and their antioxidant activity evaluated by DPPH (Fernández-Pachón *et al*., 2004). Therefore, we can conclude that the antioxidant activity of wines is more related to the type of individual phenolic compounds found in the wines, than to the total phenolic content. Also, it has been suggested that, the antioxidant activity is mainly due to the flavan-3-ols fraction and not to anthocyanins. The polymeric phenolics and other pigments may not have similar antioxidant characteristics in comparison with monomeric anthocyanins, and, a possible synergy or antagonism among the different classes of polyphenols may influence the antioxidant capacity (Arnous *et al*., 2002; Di Majo *et al*., 2008).

Conclusions

This study represents the first phytochemical investigation of phenolic components from fifteen commercial Romanian wines, produced from six autochthonous grape cultivars. A total of 38 individual phenolic derivatives were identified and quantified in red, rosé and white wine samples by HPLC-DAD-ESI(+)MS analysis. The results obtained revealed qualitative and quantitative differences between the fifteen wine samples, depending on grape variety and wine type (white, rosé or red), red wine samples being characterized by a higher phytochemical concentration than the white and rosé wine samples. By Principal Component Analysis, significant discriminations between samples were noticed, due to varietal variability and to the wine colour, flavan-3-ols, e.g. catechin, epicatechin and gallocatechin being mainly responsible for the discriminations among clustered wine samples. Total and individual phenolic compounds content and antioxidant activity measured as radical scavenging activity were comparable to those of wines produced from international grape cultivars. The antioxidant activity of wines seems to be related more to the type of individual phenolic compounds, than to the total phenolic content and the antioxidant activity to be mainly

due to the flavan-3-ols. The results confirmed that the tested Romanian wines represent a good source of antioxidants and, therefore, a moderate consumption may have beneficial influence on human health. Further *in vivo* studies will be performed to confirm their potential beneficial effects on human health.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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