

# Characterization and Classification of Romanian Wines by Origin

## A chemometric approach based on some metals and phenolic composition

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*There is a growing trend in food industry in combining safety and quality attributes of products with their distinct regional identity. In this study we used data on several elements (Cd, Cu, Cr, Zn, Pb and Ni) and phenolic compounds (gallic acid, syringic acid, p-coumaric acid, (+)-catechin, (-)-epicatechin, trans-resveratrol, rutin, quercetin and vanillin) as complementary markers in the attempt to characterise and differentiate wines from three wine-growing areas (Murfatlar, Recas and Jidvei) of Romania, according to their geographical and varietal origin. Wines from south-eastern region (Murfatlar) were characterized by their lower content in Cu, Cr and Ni, compared with wines from western (Recas) and central (Jidvei) region, while the content of phenolic compounds in the wine samples showed a visible variation, depending both of the grape variety and geographical origin. The statistical processing tools applied to our results allowed robust differentiation between regions, wine varieties, and vintage year. The phenolic compounds (gallic acid, (+)-catechin, (-)-epicatechin, rutin, quercetin and resveratrol) showed the best correlations for the varietal discrimination of wines. Using discriminant analysis, a correct classification of the wines by variety was achieved in proportion of 88.89% (based only by phenolic compounds) and 92.59 % (based both on phenolic compounds and metals), revealing that the content of Cu, Cr and Ni is significant for discrimination. Moreover, a 100% successful classification of wines by region of origin was accomplished.*

**Keywords:** phenolic compounds, elemental composition, wine, FAAS, HPLC

The guaranteed quality (e.g. taste, texture, flavor, etc.) of many foodstuffs, including wine, is owed to the specific conditions encountered in the cultivation area. The local characteristics of climate and soil combine to produce crops with certain specific features. Sometimes, people are tempted to confer the consumption of products, mainly for certain foods such as wine, an aura of romance and tradition, reason for many consumers to demand for a guarantee of the product in terms of naturalness and authenticity.

Wine is a complex matrix which contains many classes of compounds such as sugars, alcohols, acids, flavonoids (tannins), non-flavonoids, minerals and proteins among others. Its composition and distinctive character are basically the result of interactions between several factors, including soil, climate, and environment, grape varieties, and local viticulture practices [1, 2].

Therefore, a thorough investigation of the wine regarding its chemical composition can provide essential information for both authentication and quality certification, but also data concerning the vintage year and area of origin [3].

Stable isotopes are primary origin markers used to authenticate wines [4], both in terms of geographic [5, 6] and variety [7] differentiation. Combined with other suitable indicators, as organic compounds [8], a better classification and assessment of origin may be provided. However, the possibility to distinguish between near small regions of appellation remains a challenging issue.

When considering the discrimination of the geographical origin of wine and wine authentication, another valuable tool for verifying the origin has been proved to be the quantification of trace elements in wine [9-12] and some metals isotope ratio, such as  $^{87}\text{Sr}/^{86}\text{Sr}$  [13-15].

The recent research studies revealed the existence of a good correlation between the content of cations (Zn, W, Mn,

Fe, Mg, Cu, Ca, Al, Sr, B, Na, K) in wines with the ones in soils from the area/vineyard of origin [16]. Basically, the multi-elemental composition of wine will reflect the geochemistry of provenience soil. However, there are several factors such as environmental contamination, agricultural practices, climate change or the winemaking processes that can significantly affect the multi-element composition of wines, endangering thus the direct correlation between the soil and wine composition. As a result, the applicability of multi-element analysis of the wine as fingerprinting technique could be narrowed only for characterization of those high-quality mono-varietal wines for which the influence of winemaking processes against wine pattern has been studied previously and is permanently controlled. In Romania, a multielemental analysis performed on benchmark wines originated from three major regions producing wine (Dobrogea, Moldova and Valea Calugareasca) highlighted that the elements Mn, Cr, Sr, Ag and Co can be useful indicators for differentiating region of origin [12].

Wines were also classified based on the phenolic compounds [17, 18] which are significant secondary metabolites of plants concentrated in the husks, seeds and pulp of beans grape and wine extracts [2, 19] contributing to the formation of specific characteristics of wine such as colour, aroma, mouth-feel (astringency) and flavour, the most abundant and functional polyphenols being flavan-3-ol monomers, -catechin and (-)-epicatechin. [20]. These compounds are responsible for different wine types [21]. The structure of these compounds differs depending on the fraction of the grape, through their mean degree of polymerization. [20].

In the attempt to find reliable methods of authentication, several other possibilities to differentiate wines were provided over time, based on the aromatic compounds [21, 22], amino acids, combinations between trace elements and stable

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isotopes or elemental composition and phenolic compounds [23].

The main goal of this work was to assess the usefulness of common investigated parameters for wine characterisation, like metals and phenolics, in classifying and differentiating wines in terms of their geographical and variety origin. Thus, a characterisation of three selected wine types from three different renowned wine-growing areas (Murfatlar, Recas and Jidvei) of Romania was performed based on data regarding several elements and phenolic compounds. For an accurate classification of the samples, the data were modelled by chemometrics.

## Experimental part

### Materials and methods

#### Samples

Twenty-seven samples of wine (crops 2010, 2011 and 2012) obtained from grapes of three different *Vitis vinifera* varieties, Chardonnay, Muscat Ottonel and Pinot Noir, grown in Murfatlar (Dobrogea region, south-eastern Romania), Recas (Banat region, western Romania) and Jidvei (Tarnave-Transylvania region, central Romania) vineyards were used in our work. In the experimental investigation, for each type of wine were selected samples from each vineyard and vintage year. Samples obtained directly from wineries were collected in 750 mL glass bottles and stored prior to analysis at 15°C and protected from light. All the wine samples were analyzed in two replications.

#### Characterization of the selected vineyards

Three vineyards situated in the central, western and south-eastern regions of Romania were selected for this work, due to their differentiation in climatic conditions and relief variability and diversity. Jidvei vineyard lies on the gentle hills of southern Transylvania, on the Tarnave's Valley, in central Romania. The land of the vineyard with its brown soil and the continental climate, not especially warm, with average temperatures of +9 °C, favours during the maturation of grapes special conditions for slow oxidation reactions which helps to continuously accumulates sugars and aromas and prevents the accumulation of acids. These natural conditions seem to be especially favourable to the cultivation of white grape varieties.

Located in western Romania, the Recas vineyard is situated on top of a hill, with gentle slopes. The climate is temperate continental with slight Mediterranean influences. Here the winters are generally mild, the summers are warm, the autumns are long and the transition from winter temperatures to summer ones is rather sudden. The oenoclimatic conditions in the area are favourably for the production of high quality red wines.

Murfatlar vineyard is situated in the south-eastern part of Romania, on hilly terrains situated between the Danube River and the Black Sea. The continental climate, the sheltering effect of the relief against the cold currents coming from the north-east, and the unique and extremely favourable influence of the Black Sea that results in less aggressive summer heat and less frosty winters, with early springs and late autumns, induce here longer vine vegetation offering thus the perfect microclimate to produce high-quality grapes and wines.

#### Reagents and standards

Unless specified, all the used reagents were of analytical grade.

Gallic acid, syringic acid, *p*-coumaric acid, (+) - catechin, (-)-epicatechin, trans-resveratrol, rutin, quercetin and vanillin were purchased from Sigma-Aldrich (Steinheim, Germany). Stock solutions of all these standards were prepared in

methanol. Working standards were made by diluting the stock solutions with mixture methanol: water (50:50, v/v). The linearity of the method was between 0 - 50 mg/L, for each compound. Both stock and working standards were stored at 4 °C until further use. Formic acid, acetonitrile and methanol were of LC grade, purchased from Merck. (Darmstadt, Germany). Twice distilled and demineralised water produced by a Milli-Q Millipore system (Bedford, MA, USA) was used for preparation of the aqueous solutions.

A multi-element standard solution XVI CertiPUR (mixture of 21 elements in diluted nitric acid), with a certified value of  $100 \pm 3$  mg/L, purchased from Merck (Darmstadt, Germany) was used for calibration curve in the quantitative analysis of metals (Cd, Cu, Cr, Zn, Pb and Ni). Solutions of varying concentrations were prepared for all the metals by diluting the standard solution. Nitric acid 65 % from Merck and ultrapure water, with a maximum resistivity of 18.2 MΩ/cm, obtained from a Milli-Q Millipore water purification system (Bedford, MA, USA), were used for sample treatment and dilution.

#### Phenolic compounds

Phenolic compounds were evaluated by reversed phase - high performance liquid chromatography (RP-HPLC) with direct injection. Chromatographic analysis was carried out with a Thermo Finnigan Surveyor Plus HPLC (Thermo Scientific, USA) equipped with a Surveyor Photodiode Array Detector (PDA), Surveyor autosampler, Surveyor LC Pump (Quaternary gradient) and Chrome Quest Chromatography Workstation.

Separation of phenolic compounds was carried out at 30 °C [19], on an Accuacore PFP (2.6 μm, 100 x 2.1 mm) column. The flow rate was 0.4 ml/min and injection volume 1 μL. Gradient elution of two solvents was used: solvent A consisted of water with 0.1% formic acid and solvent B: acetonitrile with

Time (minutes)	A%	B%
initial	98	2
30	70	30
35	25	75
40	98	2
50	98	2

**Table 1**  
SOLVENT GRADIENT  
CONDITIONS WITH LINEAR  
GRADIENT

0.1% formic acid. The used gradient programme is given in Table 1. Detection was made at 280 nm.

The wines were injected into HPLC system after filtering through a 0.45 μm pour size membrane filter. The amount of phenolic compounds in the extracts were calculated as mg/L wine using external calibration curves, which were obtained for each phenolic standard. Each determination was carried out in duplicate and the mean value was reported. Blank solution and control samples were analyzed in order to monitor performance related to variable factors or random error.

#### Metals

A series of six elements (Cu, Zn, Pb, Ni, Cd and Cr) were determined in wine samples according to the OIV official methods [24], using a novAA 300 (Analytic Jena, Germany) flame atomic absorption spectrophotometer, equipped with deuterium arc background corrector, in an air-acetylene flame (for Cu) and respectively a Zeenit 650 (Analytic Jena, Germany) atomic absorption spectrophotometer equipped with a tube-type pyrolytic graphite furnace. The light sources were hollow cathode lamps from Analytik Jena. Hollow cathode lamps (Analytik Jena, Germany) were used as radiation sources, and the analytical measurements were based on time average

absorbance. The analytical lines: 324.8, 213.9, 283.3, 232, 228.8 and 357.9 nm were used for registration of integral atomic absorbance values of Cu, Zn, Pb, Ni, Cd and Cr, respectively.

Previously, due to the high content of organic compounds in wine, the samples have undergone a pre-treatment procedure, a microwave assisted digestion, using a Mars 5 Microwave (CEM Microwave Technology Ltd, UK) system operated at max 1600W and equipped with fiber optic sensor for automatic temperature control and pressure sensor. Thus, the wine samples (2.5 mL) were introduced in Teflon digestion vessels, followed by addition of 2.5 mL of concentrated nitric acid. After sealing, the vessels were placed in the microwave oven set to run the following program: step 1- 20 min to reach 180°C and step 2 - 15 min cooling. After cooling to ambient temperature, the reactors were opened and sonicated to eliminate the nitrous vapors. The resultant solution (5 mL) was quantitatively transferred into a 50-mL volumetric flask and brought to the volume with ultrapure water.

Each sample was analyzed in duplicate, and each analysis was repeated five times. Calibration standards were prepared from multi-element standard solution XVI CertiPUR.

#### Data analysis

Considering the various factors (e.g. geographical origin, vine variety, vintage year and thus climatic conditions, and winemaking) that could influence the composition in metals and the phenolic profile of wine, a factorial analysis was applied to the obtained results for a better understanding of the studied variables considered the most appropriate to characterize and differentiate the wine samples. All the mathematical and statistical calculations (mean, standard deviation, median, range of variation) were performed using Microsoft Excel 2010 and XLSTAT software.

#### Results and discussions

The experimental results (minimum, maximum and average values) obtained for the analyzed wine samples are detailed further, for both metals and phenolic compounds, grouped by region of origin (vineyard) and wine variety.

#### Metals concentration

The results for metals measurement shown in Table 2 reveal that in some cases Cd and Pb concentration remained below the quantification limit and could not be detected.

A comparative assessment of the abundance of elements in wine according with the region of origin was performed; their tendency of breakdown is as follows:

for Murfatlar area: Cd < Pb < Ni < Cr < Cu < Zn

for Jidvei and Recas areas: Cd < Pb < Ni < Cr < Zn < Cu

If the variety of wines is the comparing term, then the trend is:

for Chardonnay: Cd < Pb < Ni < Cr < Zn < Cu

for Muscat Ottonel: Cd < Cr < Pb < Ni < Zn < Cu

for Pinot Noir: Cd < Pb < Ni < Zn < Cr < Cu

This classification is specific to this study not being generalized since the concentrations of elements in wine are influenced by several factors including among others the area of origin, type of soil, grape variety, climate (*terroir*) and wine and oenological practices. That is why the content of an element in wine could be detected in a wide range of values, even if it was made from the same grape variety, which can be observed in Figure 1.

Another overall observation is that the recorded values for Cu, Cr and Zn are lower in wines from Murfatlar (with average values for all wines of 363.24 µg/L for Cu, 101.82 µg/L for Cr and 722.81 µg/L for Zn) compared with that from Jidvei (657.33 µg/L for Cu, 250.23 µg/L for Cr and 892.21 µg/L for Zn) and Recas (1280.62 µg/L for Cu, 141.44 µg/L for Cr and 833.36 µg/L for Zn).

Copper (Cu), which is both an essential element and potentially toxic (when it is in excess), is normally associated with the use of pesticides and the production process. During the winemaking process, the copper content can vary. It decreases due to the formation of insoluble precipitates or increase as a result of corrosion processes that may affect the equipment used. Variation of Cu content in the analyzed wine samples is relatively high (Figure 1), ranging from 10.19 µg/L to 2733 µg/L. It may predominantly originate from the residual accumulation of Cu in soil due to the old farming practices of using copper sulphate or other copper-based fungicides to control the vine downy mildew. The results correspond to those reported in a previous study on Romanian wines from the

Parameters (µg/L)		Chardonnay			Muscat Ottonel			Pinot Noir		
		Murfatlar	Recas	Jidvei	Murfatlar	Recas	Jidvei	Murfatlar	Recas	Jidvei
Cd	Min	n.d.	0.78	0.64	0.53	0.77	0.02	n.d.	0.47	0.49
	Max	8.46	4.81	4.56	2.11	5.93	2.14	6.53	5.93	4.74
	Mean	3.25	2.33	2.83	1.27	3.40	0.96	2.55	2.41	2.63
	SD	4.51	2.17	1.99	0.79	2.58	1.08	3.49	3.05	2.12
Cu	Min	10.91	1639	627.90	438.00	307.30	10.19	30.12	377.40	395.60
	Max	506.6	2733	750.60	841.20	1048.00	715.60	314.60	1894.00	1803.00
	Mean	279.86	2111.33	672.43	599.40	685.23	338.83	210.47	1045.30	960.73
	SD	250.97	562.08	67.91	213.29	370.58	355.16	156.81	774.30	743.50
Cr	Min	9.02	3.03	4.06	7.01	3.41	n.d.	65.85	27.83	8.91
	Max	35.25	14.85	948.60	24.20	19.88	31.93	374.40	1062.00	1208.00
	Mean	21	8.9	323.35	16.88	12.53	18.14	267.58	402.88	409.20
	SD	13.26	5.91	541.52	8.88	8.38	14.51	174.81	572.63	691.78
Zn	Min	402.7	938.8	798.20	433.20	839.10	345.10	356.50	309.30	378.10
	Max	1676	1608	1733.00	993.10	994.88	1043.00	374.40	399.60	871.60
	Mean	1032.23	1209.93	1195.07	773.63	927.73	809.37	362.57	362.43	672.20
	SD	636.77	352.19	483.10	298.91	80.08	402.07	10.25	47.22	260.02
Pb	Min	56.58	n.d.	n.d.	8.75	n.d.	59.69	n.d.	7.93	65.07
	Max	66.1	3.06	66.81	56.58	21.01	66.83	1.24	76.64	66.23
	Mean	61.94	2.12	44.57	37.85	14.39	64.12	1.09	38.89	65.66
	SD	4.87	0.91	37.53	25.55	11.39	3.87	0.26	34.86	0.58
Ni	Min	50.1	75.26	33.91	43.21	20.40	41.10	77.53	90.12	65.56
	Max	83.11	92.63	63.63	64.82	54.94	126.90	97.46	103.00	105.90
	Mean	68.38	85.34	50.81	50.53	41.43	77.80	90.51	97.44	81.87
	SD	16.79	9.02	15.28	12.38	18.46	44.22	11.25	6.62	21.25

Results are expressed as µg/L; n.d. not detected

**Table 2**  
HEAVY METAL  
CONTENT OF WINE  
BY TYPE AND  
REGION



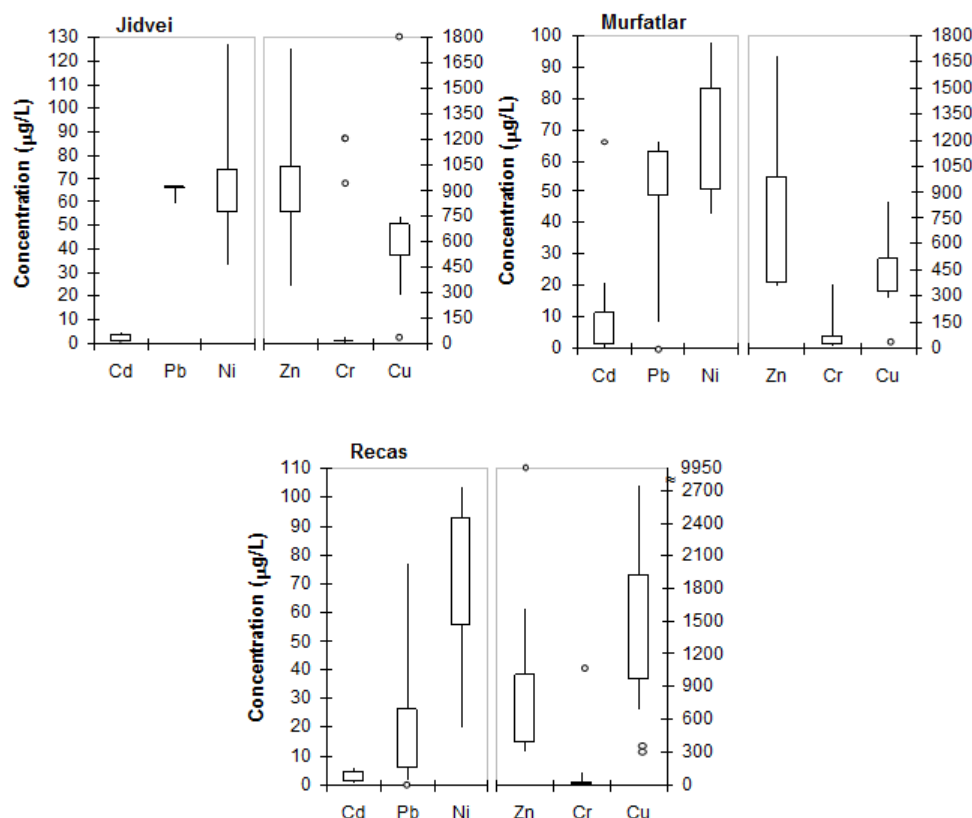


Fig. 1. The global representation of the metals concentrations ( $\mu\text{g/L}$ ) in wines produced in the three wine regions considered to this study

regions Dobrogea/Murfatlar, Muntenia/Valea Calugareasca and Moldova [12].

Regarding the content of zinc (Zn) in wine, it can be also related with the antifungal treatments of the vine. In all the analyzed samples, its variation was between  $309.30 \mu\text{g/L}$  and  $1733 \mu\text{g/L}$ , respectively with an average of  $816.13 \mu\text{g/L}$ . White wines had higher concentrations of Zn than red wine, the registered mean values ranging from  $1145.74 \mu\text{g/L}$  for Chardonnay and  $836.91 \mu\text{g/L}$  for Muscat Ottonel, to  $465.73 \mu\text{g/L}$  for Pinot Noir. All the samples considered in this study had lower values for Zn than the maximum acceptable imposed by the OIV, of  $5 \text{ mg/L}$ .

For chromium (Cr), the lowest values were recorded in Muscat Ottonel (mean value  $15.85 \mu\text{g/L}$ ), compared to Chardonnay ( $117.75 \mu\text{g/L}$ ) and Pinot Noir ( $359.89 \mu\text{g/L}$ ). Also, lowest levels of Cr were found in wines from Murfatlar and Recas vineyards, with mean value of  $101.82 \mu\text{g/L}$  and  $141.44 \mu\text{g/L}$  respectively, towards wines from Tarnave - Jidvei (mean value  $250.23 \mu\text{g/L}$ ). The wine storage conditions could influence the chromium content. Other possible causes of differences in wine chromium levels are related with the soil elemental composition [25], wine varieties and winemaking.

Regardless of the area or variety, the Pb content in the analyzed samples was lower than that imposed by the OIV (2011), below  $0.15 \text{ mg/L}$ . The wines from Recas region were characterised by low levels of Pb (mean value  $18.46 \mu\text{g/L}$ ), while in Murfatlar and Jidvei they registered mean values of  $33.63 \mu\text{g/L}$  and  $58.12 \mu\text{g/L}$ , respectively.

Nickel is one of the elements which can especially result from the stainless-steel containers. The mean value recorded for Ni in wines from Murfatlar was  $69.81 \mu\text{g/L}$ , less than that for wines from Jidvei and Recas, of  $70.16 \mu\text{g/L}$ , respectively  $74.74 \mu\text{g/L}$ . Reported to the variety, the Ni content registered slightly lower values in white wines, from  $33.91 \mu\text{g/L}$  to  $92.63 \mu\text{g/L}$  and a mean value of  $68.18 \mu\text{g/L}$  for Chardonnay and between  $20.4 \mu\text{g/L}$  and  $126.9 \mu\text{g/L}$ , with a mean of  $56.59 \mu\text{g/L}$  for Muscat Ottonel, compared to Pinot Noir that registered values from  $65.56 \mu\text{g/L}$  to  $105.9 \mu\text{g/L}$  (mean value  $89.94 \mu\text{g/L}$ ).

Cadmium is one of the natural components of the wine derived directly from the grapes, the maximum OIV admitted values being of  $10 \mu\text{g/L}$ . In large quantities, it is harmful for the human body, affecting the calcium in the bone structure and the kidneys. In our study, for Chardonnay the Cd content ranged from  $8.46 \mu\text{g/L}$  to n.d. (not detected), with a mean value of  $2.80 \mu\text{g/L}$  and for Muscat Ottonel from  $5.93 \mu\text{g/L}$  to  $0.02 \mu\text{g/L}$  (mean value  $1.88 \mu\text{g/L}$ ), while Pinot Noir had values from  $6.53 \mu\text{g/L}$  to n.d., with a mean value of  $2.53 \mu\text{g/L}$ .

All our results were in agreement with the literature, no significant difference being observed [1, 12, 25].

### Phenolic compounds

The content of phenolic compounds in the wine samples showed a visible variation, depending both of grape variety and geographical origin (Table 3). The main phenolic constituents identified in the wine samples were the syringic acid (average value of  $13.02 \text{ mg/L}$ ) as phenolic acid and quercetin (mean  $2.12 \text{ mg/L}$ ) and rutin or quercetin-3-O-rutinoside (mean  $2.09 \text{ mg/L}$ ) as flavonoids. However, quercetin and rutin were predominant in the red wine, Pinot Noir, registering values from  $2.43 \text{ mg/L}$  to  $11.15 \text{ mg/L}$  for rutin, with an average value of  $5.60 \text{ mg/L}$ , respectively between  $4.39 \text{ mg/L}$  and  $8.80 \text{ mg/L}$  for quercetin (average value of  $6.27 \text{ mg/L}$ ). In white wines the content of quercetin and rutin was very low, the quercetin recording values from n.d. to  $0.06 \text{ mg/L}$  in Chardonnay and  $0.01 \text{ mg/L}$  and  $0.25 \text{ mg/L}$  in Muscat Ottonel, while for rutin the values were from n.d. to  $0.78 \text{ mg/L}$  for Chardonnay and between  $0.13 \text{ mg/L}$  and  $1.07 \text{ mg/L}$  for Muscat Ottonel.

Another important flavonoid, the (+)-catechin, has recorded values between  $0.14 \text{ mg/L}$  and  $5.33 \text{ mg/L}$ , with an average of  $1.79 \text{ mg/L}$ . A significantly higher content of (+)-catechin was found in the red wine, Pinot Noir (from  $2.07 \text{ mg/L}$  to  $5.33 \text{ mg/L}$ , with an average of  $3.45 \text{ mg/L}$ ), compared to the white ones (from  $0.14 \text{ mg/L}$  to  $2.47 \text{ mg/L}$  and a mean of  $1.20 \text{ mg/L}$  for Chardonnay and between  $0.71 \text{ mg/L}$  and  $1.22 \text{ mg/L}$ , averaging  $0.72 \text{ mg/L}$  for Muscat Ottonel). In terms of geographical origin,

the Pinot Noir red wines produced to Jidvei averaged a higher content of (+)-catechin, of 4.03 mg/L, compared with those from Murfatlar (3.42 mg/L) and, respectively Recas (2.81 mg/L).

The content of (+)-epicatechin is found in significantly higher amounts in Pinot Noir, with values between 1.13 mg/L and 5.27 mg/L, with an average of 2.97 mg/L. The average values recorded for Pinot Noir produced in the areas Murfatlar (3.37 mg/L) and Jidvei (3.44 mg/L), are higher than those from Recas (2.81 mg/L). For white wines, (+)-epicatechin had values from n.d. to 3.43 mg/L for Chardonnay and between 0.10 mg/L and 2.02 mg/L for Muscat Ottonel.

From the category of hydroxybenzoic acids the syringic acid and gallic acid were analyzed. The registered values for syringic acid ranged between n.d. and 39.78 mg/L, with an average of 13.02 mg/L. The highest content of syringic acid was found in the varieties Pinot Noir (mean 16.08 mg/L) and Muscat Ottonel (average 16.20 mg/L) than in Chardonnay (6.78 mg/L). For Pinot Noir and Muscat Ottonel, the highest values were found in wines from Recas (23.56 mg/L, respectively 19.43 mg/L), followed by those from Murfatlar (14.51 mg/L, respectively 17.41 mg/L) and Jidvei (6.15 mg/L respectively 11.77 mg/L). For Chardonnay wines, the highest values were

found in wines of Jidvei (13.32 mg/L) and less in those of Murfatlar (6.91 mg/L) and Recas (0.09 mg/L).

Gallic acid was predominantly identified in all the samples of Pinot Noir, with values between 2.62 mg/L and 5.37 mg/L, with an average of 3.43 mg/L, and only in three samples of Muscat Ottonel (two from Jidvei - vintage years 2011 and 2012; one from Recas - vintage year 2012), with values between 0.04 mg/L and 1.68 mg/L and three samples of Chardonnay (two from Murfatlar - vintage years 2011 and 2012; one from Jidvei - vintage year 2011), with values from 0.002 mg/L to 0.34 mg/L. The results are consistent to other studies on wines from Romania [23] and Croatia [26].

From the hydroxy cinnamic acids, the *p*-coumaric acid was analyzed, which recorded values between n.d. and 2.34 mg/L, in agreement with other previously published data [23]. In the Pinot Noir samples, the mean content of *p*-coumaric acid was 0.22 mg/L, while in white wines samples was found only in two samples of Muscat Ottonel from Jidvei, years 2011 and 2013 (0.56 mg/L, respectively 2.34 mg/L) and two samples of Chardonnay, one from Recas, year 2013 (0.31 mg/L) and another one from Jidvei, year 2012 (0.34 mg/L).

Resveratrol, the main stilbene compound from grapes was found in samples of Pinot Noir, with values ranging from 0.52 mg/L and 1.26 mg/L (average of 0.87 mg/L) and only in one

**Table 3**  
THE EXPERIMENTAL RESULTS REGARDING THE CONTENT OF PHENOLIC COMPOUNDS IN WINE SAMPLES

Parameters (mg/L)		Chardonnay			Muscat Ottonel			Pinot Noir		
		Murfatlar	Recas	Jidvei	Murfatlar	Recas	Jidvei	Murfatlar	Recas	Jidvei
<i>Hydroxybenzoic acids (HBA)</i>										
gallic acid	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.80	2.62	3.38
	Max	0.32	n.d.	0.34	n.d.	0.04	1.68	4.11	3.16	5.37
	Mean	0.11	n.d.	0.11	n.d.	0.01	0.67	3.42	2.81	4.05
	SD	0.19	-	0.20	n.d.	0.02	0.89	0.66	0.30	1.14
syringic acid	Min	0.07	n.d.	n.d.	11.51	0.03	n.d.	2.15	8.89	2.40
	Max	20.15	0.20	39.78	24.83	34.30	21.73	39.13	38.23	12.99
	Mean	6.91	0.09	13.32	17.41	19.43	11.77	14.51	27.57	6.15
	SD	11.46	0.10	22.92	6.79	17.58	10.98	21.32	16.23	5.93
<i>Hydroxycinnamic acids (HCA)</i>										
<i>p</i> -coumaric acid	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.29	n.d.	0.09
	Max	n.d.	0.31	0.34	n.d.	n.d.	2.34	0.75	0.14	0.20
	Mean	n.d.	0.10	0.11	n.d.	n.d.	0.97	0.45	0.07	0.13
	SD	-	0.18	0.19	-	-	1.22	0.26	0.07	0.06
<i>Flavonoids</i>										
(+) -catechin	Min	0.14	1.23	0.93	0.64	0.71	0.43	2.07	2.47	2.88
	Max	1.07	1.59	2.47	0.69	1.22	0.76	4.51	3.15	5.33
	Mean	0.58	1.37	1.63	0.66	0.89	0.60	3.42	2.90	4.03
	SD	0.47	0.19	0.78	0.03	0.29	0.17	1.24	0.37	1.23
(+) -epicatechin	Min	0.24	n.d.	0.29	0.15	0.10	0.34	2.39	1.13	3.31
	Max	3.43	0.53	0.90	0.54	2.02	0.92	5.27	2.69	3.50
	Mean	1.41	0.34	0.53	0.37	0.82	0.60	3.37	2.10	3.44
	SD	1.76	0.30	0.33	0.20	1.04	0.30	1.64	0.85	0.11
quercetin	Min	0.02	n.d.	0.02	0.02	0.02	0.01	4.47	4.39	5.93
	Max	0.06	0.03	0.04	0.04	0.25	0.06	7.40	6.45	8.80
	Mean	0.03	0.02	0.03	0.03	0.10	0.03	5.72	5.32	7.78
	SD	0.02	0.02	0.01	0.01	0.13	0.03	1.51	1.04	1.60
rutin	Min	0.31	n.d.	0.25	0.19	0.16	0.13	4.13	2.43	4.92
	Max	0.47	0.32	0.78	0.26	1.07	0.38	11.15	4.42	6.90
	Mean	0.37	0.2	0.45	0.23	0.49	0.24	6.90	3.74	6.15
	SD	0.09	0.17	0.28	0.04	0.51	0.13	3.74	1.14	1.08
<i>Stilbenes</i>										
resveratrol	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.52	0.55	0.87
	Max	n.d.	0.4	n.d.	n.d.	n.d.	n.d.	1.01	0.91	1.26
	Mean	n.d.	0.13	n.d.	n.d.	n.d.	n.d.	0.77	0.78	1.05
	SD	-	0.23	-	-	-	-	0.24	0.20	0.20
<i>Others (coming from aging the wine in oak barrels)</i>										
vanillin	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.10	0.05	0.08
	Max	1.23	0.23	1.01	1.01	0.33	n.d.	0.50	0.58	0.34
	Mean	0.41	0.08	0.34	0.34	0.11	n.d.	0.25	0.24	0.20
	SD	0.71	0.13	0.58	0.58	0.19	-	0.22	0.29	0.13

Results are expressed as mg/L; n.d. not detected.

sample of Chardonnay originated from Recas, year 2013 (0.40 mg/L). Resveratrol content of the Pinot Noir samples recorded higher values in wines from Jidvei (average of 1.05 mg/L) than those from Murfatlar (average 0.77 mg/L) and Recas (average 0.71 mg/L).

Vanillin is part of those non-flavonoid phenolic compounds not originating directly from grapes. They derive from the process of wine aging in oak barrels. It was found in the Pinot Noir samples (mean 0.23 mg/L) and in three samples of Muscat Ottonel, two from Murfatlar, harvest 2011 and 2012 (0.005 mg/L, respectively 1.006 mg/L) and one from Reca°, harvest 2012 (0.33 mg/L) and three samples of Chardonnay, one from Jidvei, harvest 2013 (1.01 g/L), one from Murfatlar, harvest 2012 (1.23 mg/L) and one from Recas, harvest 2013 (0.23 mg/L).

#### Wines differentiation by variety

The main factor affecting the phenolic content in wine is the sun exposure of grapes during the growing/maturation season [26]. Moreover, the *terroir* effect leaves its mark on both the metals content and the phenolic compounds of wines.

The results achieved in this work highlighted the several variations in wine composition, both due to the grape variety, area of origin and year of harvest. Thus, the discrimination capability of the measurement results was verified by using a multivariate statistical analysis, based on discriminant analysis. In a first phase the definition of classes was made starting from the grape variety originating the analyzed wines (Figure 2). A very good differentiation was observed for red wine (Pinot Noir) versus white wines (Chardonnay and Muscat Ottonel). When both phenolic compounds and metals are used as discriminant parameters (Figure 2b) an improvement of the discrimination function can be observed.

Efficiency of classification is given by the grading matrix (Table 4). Therefore, the characterization data of a wine sample (considered unknown) are removed in the calculation model and then the origin of that wine is predicted. Chemometric

processing tools applied to our data were able to correctly classify the wines by variety in a proportion of 88.89% (when only the phenolic compounds are used for analysis) and 92.59% (when both phenolic compounds and metals are used for analysis). The influence of the metals is mainly observed to white wines, Chardonnay variety.

Elemental and phenolics data were also examined according to the variety using one-way ANOVA. The results indicate that the content of gallic acid, (+)-catechin, (-)-epicatechin, rutin, quercetin and resveratrol showed the best correlations with the grapes variety of wines origin (Table 5), being significant for differentiation.

#### Wines differentiation by geographical origin and harvest year

The ability of some elements like Ni, Ag, Cr, Sr, Zn, Cu, Rb, Mn, Pb, Ba, Li, Mg, and Na to discriminate the geographical origin of wines was already demonstrated in some studies on Romanian wines [6, 12, 23]. In our work, commonly used parameters for wine characterisation as heavy metals and phenolic compounds were assessed to see their usefulness in providing preliminary information on wines origin.

Based on the elemental contents, the cross-validation technique provided a 100 % percentage of predicted membership according to the wine geographic origin (71.79 % F1 and 28.21 % F2) (Figure 3a), but the efficiency of classification given by the confusion matrix for samples prediction was low (correct classification of wines by origin of provenance in proportion of 62.96%). A great improvement in the correct classification of wine geographical origin (predictive rate of 100%) was obtained by combining the elemental profile with the phenolic composition (82.69% F1; 17.31 % F2) (Figure 3). Among the investigated parameters, Cu and Pb were identified as the most significant for geographic differentiation of the wines (Figure 4).

Wines originating from Recas region can be well characterized based on parameters like Cd, Ni, Cu, and syringic acid, while wines from Murfatlar can be defined by the content in rutin, epicatechin and vanillin, and wines from Jidvei by

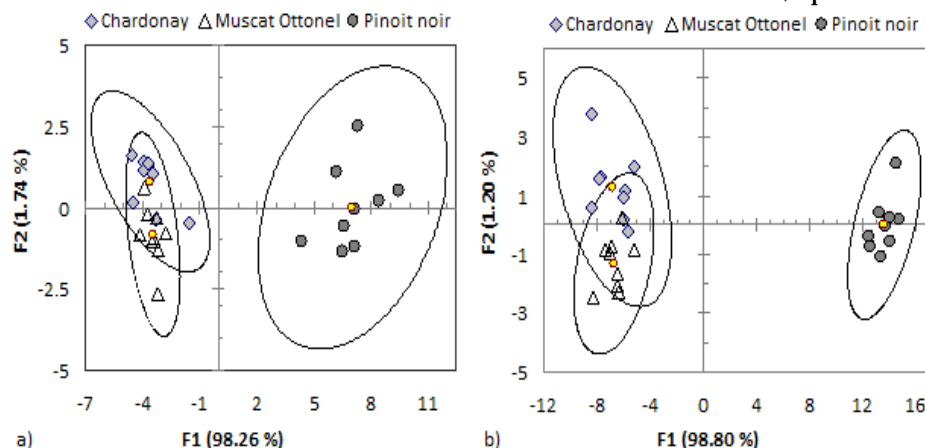


Fig. 2. Differentiation of wines depending on variety by discriminant analysis in terms of a) phenolic profile and b) phenolic composition and various heavy metals

	Chardonnay	Muscat Ottonel	Pinot noir	% correct
Discriminating elements: phenolic compounds in wines				
Chardonnay	7	2	0	77.78%
Muscat Ottonel	1	8	0	88.89%
Pinot noir	0	0	9	100.00%
<b>Total</b>	<b>8</b>	<b>10</b>	<b>9</b>	<b>88.89%</b>
Discriminating elements: phenolic compounds and metals in wines				
Chardonnay	8	1	0	88.89%
Muscat Ottonel	1	8	0	88.89%
Pinot noir	0	0	9	100.00%
<b>Total</b>	<b>8</b>	<b>10</b>	<b>9</b>	<b>92.59%</b>

**Table 4**  
PREDICTIVE RATE (n=27)

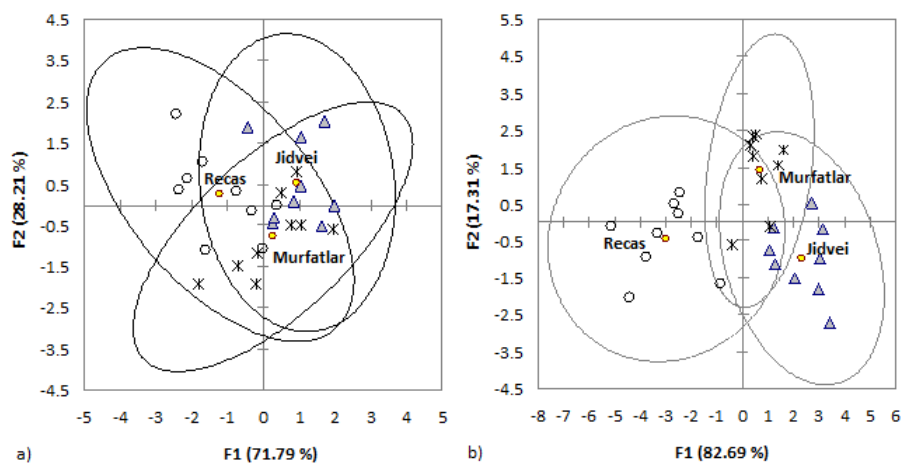


Fig. 3. Differentiation of wines according to geographic origin based on a) the elemental contents and b) the elemental contents and the phenolic composition

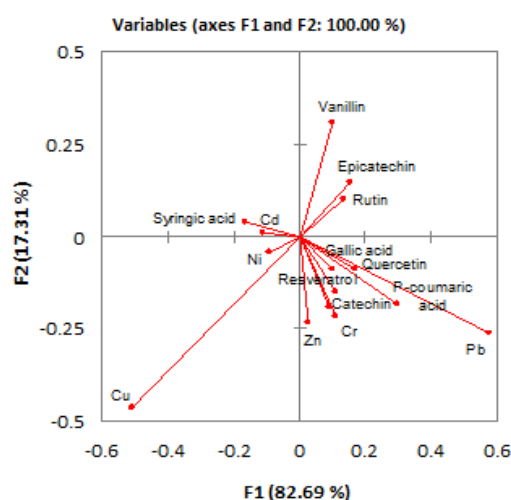


Fig. 4. Correlation between the analyzed parameters and the factors in discriminant analysis of wines geographic origin

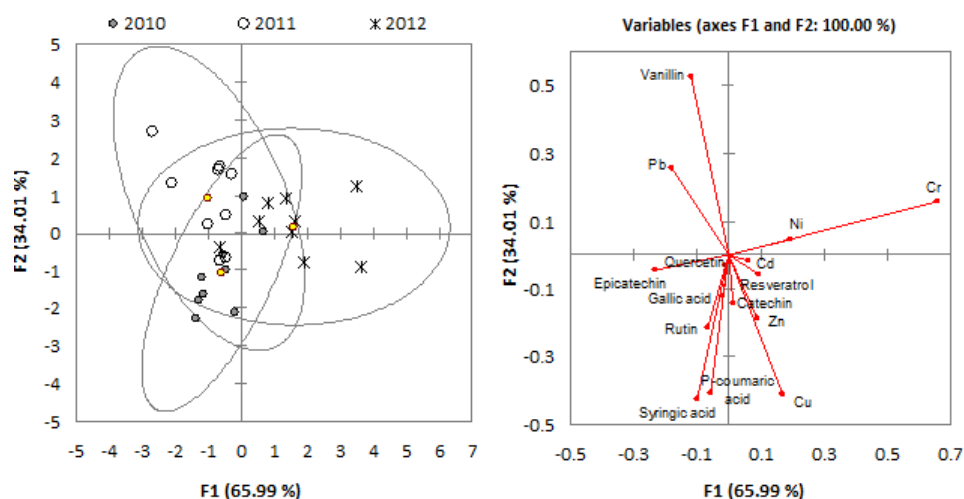


Fig. 5. Correlation chart between the selected variables and the discriminant functions for vintages 2010, 2011 and 2012

parameters like Zn, Cr, Pb, resveratrol, catechin, quercetin, gallic acid and *p*-coumaric acid.

The same set of elements that we used to classify the wine samples according to their geographical origin, namely Cd, Cu, Cr, Zn, Pb, Ni, gallic acid, (+)-catechin, syringic acid, vanillin, (-)-epicatechin, *p*-coumaric acid, rutin, resveratrol and quercetin, was applied to discriminate between 2010, 2011 and 2012 vintages. Figure 5 represents the correlation chart of loadings for the selected variables of the stepwise process, in the plane designed by the first two discriminant functions (F1 and F2). Function 1 express 65.99 % of the variance which provides the main separation between the vintage years and has a strong positive correlation with Cr, Ni, and Cd while function 2 (34.01 % of the variance) has a strong correlation with syringic acid, *p*-coumaric acid, vanillin and Pb concentrations. The efficiency of classification provided by

the confusion matrix has given a correct prediction proportion of 81.48%.

It was observed that the most useful variables in discriminating vintage 2012 from 2010 and 2011 were Cd, Cr, Ni and resveratrol, while significant for differentiating wines from 2010 to 2011 were the content of Cu, Zn, syringic acid, *p*-coumaric acid, rutin and quercetin (Table 6).

Concluding, the set of markers, six inorganic (Cd, Cu, Cr, Zn, Pb, and Ni) and nine organic ((+)-catechin, (-)-epicatechin, *p*-coumaric acid, gallic acid, syringic acid, rutin, quercetin, resveratrol and vanillin), that we used to characterize the wines, provide us a good classification of the samples in terms of grape variety and geographical origin, even vintage years. Our results confirm the powerful link between the wine composition and *terroir*, being in agreement with those reported by other authors [6, 23].



Parameters	Pinoit noir	Chardonnay	Muscat Ottonel	Pr > F	Significant
Cd	2.529 a a	2.803 a a	1.879 a a	0.708	No
Cu	738.836 a a	1021.210 a a	541.154 a a	0.329	No
Cr	359.888 a a	117.750 a a b	15.848 a b	0.087	No
Zn	465.733 b b	1145.744 a a	836.909 a b a	0.001	Yes
Pb	35.210 a a	36.210 a a	38.785 a a	0.968	No
Ni	89.941 a a	68.179 a b b	56.587 b b	0.012	Yes
Gallic acid	3.429 a a	0.076 b b	0.229 b b	0.000	Yes
Catechin	3.452 a a	1.196 b b	0.717 b b	0.000	Yes
Syringic acid	16.076 a a	6.781 a a	16.206 a a	0.289	No
Vanillin	0.230 a a	0.277 a a	0.152 a a	0.763	No
Epicatechin	2.969 a a	0.801 b b	0.596 b b	0.000	Yes
<i>p</i> -coumaric acid	0.229 a a	0.135 a a	0.384 a a	0.511	No
Rutin	5.597 a a	0.419 b b	0.320 b b	0.000	Yes
Resveratrol	0.866 a a	0.338 b b	0.330 b b	0.000	Yes
Quercetin	6.272 a a	0.029 b b	0.052 b b	0.000	Yes

Means followed by different lowercase letters in the line differ significantly by ANOVA complemented by Tukey's test of multiple comparisons; significance level of 5 %; Results expressed in mg/L for phenolic compounds and µg/L for metals

**Table 5**  
VARIATION OF THE  
METAL AND  
PHENOLICS  
CONTENT OF  
COLLECTED WINES  
OF DIFFERENT  
VARIETY

## Conclusions

The results of this study report that classical parameters, like phenolic compounds and metals, commonly used for characterization of wines may provide a picture of the oenoclimatic conditions variability across different regions and years, subsequently applicable to achieve reliable information for classifying wines by variety, origin of provenance and vintage.

By multivariate analysis based on the concentration of six inorganic (Cd, Cu Cr, Zn, Pb, and Ni) and nine organic ((+)-catechin, (-)-epicatechin, *p*-coumaric acid, gallic acid, syringic acid, rutin, quercetin, resveratrol and vanillin) compounds, it was possible to discriminate wines from three grape varieties (Chardonnay, Muscat Ottonel and Pinot Noir), obtained in three wine-producing regions of Romania (Recas, Murfatlar and Jidvei) during the production years, 2010, 2011 and 2012.

The content of gallic acid, (+)-catechin, (-)-epicatechin, rutin, quercetin and resveratrol revealed the best ability for discriminating the wine samples according to the grapes variety of origin. By using variance analysis by average comparative (ANOVA) and linear discriminant analysis (LDA) based on the concentration of the selected elements a correct classification of the wines by variety was achieved in proportion of 88.89% (based only by phenolic compounds) and 92.59 % (based both on phenolic compounds and metals), revealing that the content of Cu, Cr and Ni is significant for discrimination. Moreover, a 100% successful classification of wines by region of origin was accomplished, while a differentiation by year of production was achieved with a correct prediction rate of 81.48%.

The most important conclusion is the fact that based on wine characterization parameters, like phenolics compounds coupled with multi-element, a separation of wine samples in terms of variety and geographical origin can be achieved.

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