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Research Article

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# Characterization and Classification of Wines Based on Spectrophotometric Determination of Wine Bioactive Properties

Elisabeta-Irina Geana[1*], Corina Teodora Ciucure[1], Constantin Apetrei[2] & Victoria Artem[3]

[1]*ICSI Analytics Group, National R&D Institute for Cryogenics and Isotopic Technologies -ICSI Ramnicu Valcea, Romania*

[2]*Dunarea de Jos University of Galati, Faculty of Science and Environment, Galati, Romania*

[3]*Research Station for Viticulture and Oenology Murfatlar, Constanta, Romania*

[*]**Correspondence to:** Dr. Elisabeta-Irina Geana, ICSI Analytics Group, National R&D Institute for Cryogenics and Isotopic Technologies -ICSI Ramnicu Valcea, Romania.

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## Abstract

Wine is a product of high nutritional value, which, consumed in a moderate amount (especially red wines), contributes to the health of the human organism, increasing the quality and the life time, the main compounds responsible for this contribution being the phenolic compounds.

This work was aimed to investigate the bioactive properties (total phenolic - TP and total flavonoid - TF contents and antioxidant capacity) of different Romanian red and white wine varieties with different ageing periods, using quantitative UV-Vis spectrophotometric methods.

Our results indicated that the bioactive properties were higher in red wines compared to rose and white wine varieties and moderate positive correlations were observed between TP and TF with antioxidant capacity (DPPH method). Grape variety rather than vintage had stronger influence on

Thus, older wines, mainly Feteasca Neagra and Pinot Noire for red wines and Muscat Ottonel and Riesling Italian for white wines shows higher content of polyphenols and antioxidant capacity and are considered to be better quality.

The quantitative data were subjected to the unsupervised PCA pattern recognition method to investigate the possible classification of wines. No conclusive separation was achieved in the case of red wines, while some white wine varieties can be distinguished.

## Introduction

Modern society encourages consumption of foods that can treat and prevent different disease and increase longevity, like foods and beverages rich in antioxidant compounds [1,2]. Wine is one of the oldest beverages and has been used as a medicine from ancient times in numerous countries. In France, it was conclusioned that moderate wine consumption leads to a low mortality rate from ischemic heart disease and the prevalence of other risk factors, such as smoking (French Paradox) [3].

The quality of wines is dictated by its color, smell and taste, rather than on its content of bioactive compounds [4]. From chemical point of view, wine is a hydro-alcoholic solution (~78% water)) with a great chemical complexity, including numerous minority bioactive phytochemical constituents and their metabolites which act synergistically on human health [5].

A wide variety of compounds contributes to the health benefits of wine, among them: phenolic compounds, soluble proteins, sugars, vitamins, volatiles, ketones, lipids and organic acids, the most representative being phenolic compounds. Polyphenolic compounds are commonly known as plant secondary metabolites and are directly associated with health-promoting properties of wines [6,7]. Among them, phenolic acids, stilbenes (e.g., resveratrol), flavonols (e.g., quercetin and myricetin), flavan-3-ols (e.g., catechin and epicatechin), procyanidins and anthocyanins represent the most valuable phenolic phytochemicals [5,8].

In these latter days, phenolic compounds are the subject of increasing scientific interest due to their beneficial effects on human health [1], among: cardio-protective, anti-cancer, anti-diabetic, anti-aging and neuro-protective effects [6] as results to their antioxidant character, associated with the presence of numerous antioxidant wines. Antioxidants may be defined as inhibitors for the initiation and propagation of oxidative chain reaction, inhibiting the oxidation process of different molecules and protecting cells from oxidative stress. Also, they can also protect the human through the fight against free radicals in the body that cause disease and ageing.

The phenolic composition of wines is dependent on several factors; such as the grape variety, cultivation practices, winemaking techniques, ageing process and environmental factors [7,9]. Also, phenolic compounds play a major role in wine quality, contributing to the organoleptic properties such as color, flavor, astringency and to the oxidative stability [9].

Polyphenols are extracted during crushing and fermentation when the juice is in contact with the grape skins and seeds. Thus, the amount of phenolic compounds in red wine is higher compared with white wine

because red juice has longer contact time with the grape skins and seeds [10]. The phenolic content varies significantly in different types of wine depending on the presence of different classes of phenolic compounds [8], leading to difference in the measured bioactive properties, including total phenolic content, total flavonoids content and antioxidant capacity [9], thus allowing the classification of wines according to the geographical and varietal origins and vintage year .

The concentration of phenolic compounds in wines could be determined with low cost spectrophotometric methods, the most used being for the total phenolic content (Folin-Ciocalteu method), total flavonoid content assay, for the total anthocyanins quantification assay and for the antioxidant capacity estimation [11,12].

The evaluation of the antioxidant capacity of wine is an indirect index of phenolic compounds present in wine. Several well established analytical methods for the evaluation of the antioxidant capacity were proposed: spectroscopic (colorimetric: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), FRAP (ferric reducing antioxidant power), CUPRAC (cupric reducing antioxidant power); fluorescence - ORAC (oxygen radical absorption capacity) and chemiluminescence), electrochemical (cyclic voltammetry, amperometry), chromatographic - HPLC (determination of target antioxidants) [13]. Several studies on antioxidant capacity of wines have been published and among various analytical methods to evaluate total antioxidant capacity of wines, DPPH, ABTS and FRAP methods were commonly preferred [4,7].

Like any other food/feed matrix, wine requires authentication strategies based on suitable qualitative and quantitative analytical investigations of wine natural constituents which represents the specific fingerprint of each wine [14]. Various analytical approaches (chromatographic, spectroscopic, spectrometric, electrochemical) were applied in order to assess the profiles of wine bioactive constituents, including phenolic and volatile compounds, amino acids, thus, in combination with appropriate chemomteric approaches contributing to the development of different methodologies for the assessment of wine authenticity [15,16].

The present research aimed to evaluate the wine biochemical properties (total polyphenolic content, total flavonoids content and DPPH antioxidant capacity) of different red, rose and white wine varieties with different ageing times, produced at SCDVV Murfatlar, Romania during 9 year of production. All the data collected were analyzed by the multivariate statistical method of the Principal Component Analysis (PCA) in order to find the possible correlation between the total antioxidant activity measured and the concentration of each class of antioxidants analysed and for the classification of different red and white wine varieties and vintage years has been investigated.

## Materials and Methods

### Chemicals

All reagents for the determination of wine biochemical characteristics by UV-VIS measurements (anhydrous sodium carbonate, sodium acetate, methanol, 96% ethanol) were of analytical grade and were obtained from Merck (Darmstadt, Germany). Folin-Ciocalteu's phenol reagent, gallic acid, quercetin, 2-2-diphenyl-1-picrryl-hydrazyl (DPPH) and trolox (±)-6-Hydroxy-2, 5, 7, 8 - tetramethylchromane-2-carboxylic acid

acid were purchased from Sigma-Aldrich Corp. (USA). Ultra-pure water, produced by a Milli-Q Millipore system (Bedford, MA, USA), was used for preparation of the aqueous solutions.

**Wine Samples**

31 red wines (varieties: Cabernet Sauvignon, Merlot, Feteasca Neagra, Babeasca Neagra), 46 white wine (varieties: Chardonnay Riesling Italian, Columna, Sauvignon Blanc, Feteasca Regala, Pinot Gris and Muscat Ottonel) and 8 rose Mamaia wines produced at SCDVV Murfatlar during 9 year of production were analysed. The information about varieties, vintage years and specific notations were presented in Table 1.

***Table 1:*** *Investigated wine samples and the specific notation*

| Wine varieties | Harvest year | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 |
| **Red wines** | | | | | | | | | |
| **Merlot** | M09 | M10 | M11 | M12 | M13 | M14 | M15 | M16 | |
| **Cabernet Sauvignon** | CS09 | | CS11 | CS12 | CS13 | CS14 | CS15 | CS16 | CS17 |
| **Pinot Noire** | | PN10 | PN11 | PN12 | PN13 | | PN15 | | PN17 |
| **Feteasca Neagra** | FN09 | FN10 | FN11 | FN12 | FN13 | FN14 | FN15 | FN16 | FN17 |
| **Rose wines** | | | | | | | | | |
| **Mamaia** | MM09 | MM10 | MM11 | MM12 | | MM14 | MM15 | MM16 | MM17 |
| **White wines** | | | | | | | | | |
| **Chardonnay** | CH09 | CH10 | CH11 | CH12 | CH13 | CH14 | CH15 | CH16 | CH17 |
| **Columna** | C09 | C10 | C11 | C12 | C13 | C14 | C15 | C16 | C17 |
| **Riesling Italian** | RI09 | | RI11 | RI12 | RI13 | RI14 | RI15 | | |
| **Feteasca Regala** | | | FR11 | | FR13 | FR14 | FR15 | FR16 | FR17 |
| **Sauvignon Blanc** | | | | SB12 | SB13 | SB14 | SB15 | SB16 | SB17 |
| **Pinot Gris** | PG09 | PG10 | | PG12 | | | | PG16 | PG17 |
| **Muscat Ottonel** | MO09 | MO10 | MO11 | MO12 | MO13 | | | | |

**Spectrophotometric Investigations**

Spectrophotometric measurements of wine biochemical characteristics (total polyphenol -TP, total flavonoids (TF) and antioxidant capacity - AC were performed using an Specord 250 Plus UV-Vis spectrophotometer (Analytic Jena, Jena, Germany) equipped with 1 cm path length quartz cells. All the determinations were conducted in duplicate and results were averaged.

***Total polyphenols (mg GAE/L)*** was determined by the Folin-Ciocaltea method according to the method broposed by Syngleton *et al.* [17], with some modification. The ability of the wine phenolic compounds to get oxidized with the Folin-Ciocalteau reagent was measured. The resulting blue color has a maximum

absorbance at 675nm, the absorbance being proportional to the amount of wine phenolic compounds. In brief, 100µL of wine (10-fold dilution for red wines is necessary before) was added to test tubes and mixed with 5mL ultrapure water and 200µL Folin Ciocalteau reagent. After 5 min of reaction, 300µL of 20% sodium carbonate solution was added to stop the reaction and to develop characteristic blue color for 2 hours, at room temperature and protected from light. Total phenolic content (TP) of the wine sample was expressed as mg/L gallic acid equivalent (GAE) using a calibration curve in the concentration range100-1250mg/L.

***The Total Flavonoids Content (TF)*** of wines was determined by $AlCl_3$ method described by Hosu *et al.* [18], by treating 0.5mL of wine with 0.4mL of 25g/L $AlCl_3$ solution, 0.5mL of 100g/L CH3COONa solution and 4mL distilled water. The reaction was left for completion for 15 min, and then the absorbance of the mixture was measured at 430nm against water as blank. Total flavonoid content (TF) was quantified as mg quercetin equivalent/L of wine using calibration curve obtained in the concentration range of 0-125mg/L. Wine samples did not require dilution based on preliminary experiments.

***Antioxidant Capacity (AC)- DPPH Method.*** DPPH free radical scavenging assay was followed for the determination of antioxidant capacity (AC) of wine samples according to the method described by Hosu *et al.* [18], with some modifications. Synthetic radical, 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) was used to determine the antioxidant activity of wine. The decrease of absorbance of the radical is proportional to the concentration and activity of the sample analysed. Absorbance measurements are transformed to antioxidant capacity using trolox as reference. In this assay, 6mL of 0.09mg/mL DPPH methanolic solution was mixed with 0.5mL aliquots of appropriately diluted wine samples in distilled water (10-fold dilution for red wines and no dilution for white wines) and the absorbance was measured at 517nm, against methanol as blank, after 20 min at room temperature. Calibration was performed using trolox as standard, in the concentration range of 50-1000µmoli/L, and the antioxidant capacity was expressed as µmol/L Trolox equivalents.

### Statistical Analysis

Each chemical parameter was measured in duplicate. The obtained data were expressed as mean values and range of values and were processed according to the wine variety and vintage year. Data were examined separately by year and applied treatment in the vineyard using one-way analysis of variance (ANOVA). The Duncan test was used to discriminate the wine category ($p \leq 0.05$). The variability as influenced by red and white wine varieties and vintage years was investigated by correlation analysis and principal component analysis (PCA). Principal component analysis (PCA) was used to examine the possible grouping of samples according to the red and white wine varieties and vintage years and to visualize the presence of outliers. All the mathematical and statistical analyses were performed using Microsoft Excel 2010 and XLSTAT Addinsoft version 15.5.03.3707.

## Results and Discussions

### Biochemical Properties (TP, TF, AA) of Wines

The results obtained analysing each wine sample are presented in Table 2. As expected, since the wines chosen for the study are very heterogeneous, the experimental values obtained for the bioactive properties are

spread over a wide range. In particular, the total polyphenolic content (TP) ranged from 595.2 to 2333.0mg/L GAE for red wines and from 162.7 to 520.7mg/L GAE for white wines; the total flavonoids content (TF) ranged from 18.6 to 128.4mg/L GAE for red wines and from 4.3 to 10.9mg/L GAE for white wines; while the antioxidant activity (AC) ranged from 2225.6 to 9246.7μmoli/L Trolox for red wines and from 375.6 to 2029.8μmoli/L Trolox for white wines, respectively.

***Table 2:*** *Wine bioactive properties: total polyphenolic content (TP), total flavonoids content (TF) and Antioxidant capacity (AC)*

| Wine varieties | Total phenolic content (mg/L GAE) | Total flavonoid Content (mg/L QE) | Antioxidant activity (μmoli/L Trolox) |
|---|---|---|---|
| **Red wines** | | | |
| **Pinot Noire** | 1462.5 (1029.2-2333.0)[a] | 50.22 (35.8-70.1)[a] | 5130.7 (3572.2-7345.6)[a] |
| **Merlot** | 1208.8 (968.2-1347.9)[ab] | 47.04 (38.4-57.8)[a] | 5069.6 (3850.0-5941.1)[a] |
| **Cabernet Sauvignon** | 1056.9 (657.9-1502.0)[b] | 58.8 (34.5-128.4)[a] | 5531.9 (3112.2-8518.9)[a] |
| **Feteasca Neagra** | 1271.5 (833.1-1703.7)[ab] | 52.1 (25.8-74.6)[a] | 6052.2 (2456.7-9246.7)[a] |
| **Rose wine** | | | |
| **Mamaia** | 723.0 (595.2-873.6) | 25.8 (18.6-33.5) | 2775.0 (2225.6-3385.6) |
| **White wines** | | | |
| **Chardonnay** | 282.2 (207.0-366.6)[ab] | 7.7 (5.8-10.4)[a] | 800.8 (541.4-948.7)[b] |
| **Sauvignon Blanc** | 223.2 (166.2-296.3)[b] | 5.9 (5.2-7.3)[b] | 537.8 (384.3-724.7)[b] |
| **Feteasca Regala** | 262.4 (197.4-336.5)[b] | 6.1 (4.3-8.4)[ab] | 813.4 (462.3-1050.3)[b] |
| **Muscat Ottonel** | 348.9 (274.5-520.7)[a] | 8.0 (5.8-10.9)[a] | 1147.1 (747.6-2029.8)[a] |
| **Pinot Gris** | 282.4 (162.7-389.2)[ab] | 6.6 (4.4-8.3)[ab] | 600.9 (375.6-714.1)[b] |
| **Columna** | 229.4 (186.1-289.2)[b] | 5.5 (4.81-6.5)[b] | 569.3 (431.7-651.9)[b] |
| **Riesling Italian** | 253.2 (214.4-335.7)[b] | 6.8 (5.1-10.1)[ab] | 833.7 (559.1-1254.8)[b] |

The values represent the mean of the results obtained for the three replicates ± standard deviation. Means with different lowercase letters in the column differ significantly according to the Duncans multiple range test at p≤ 0.05

Our results are in agreement with the available literature for Romanian wines (with 2455.9mg/L GAE for red wines and 255.6mg/L GAE for white wines) [18,19], Spanish wines (1613.2mg/L GAE for red wines and 240.8mg/L GAE for white wines) [20] and wines from Czech Republic (1544.8mg/L GAE for red wines and 115.5mg/L GAE for white wines) [4].

The most phenolic compounds from wines come from the grape skin and, therefore, higher concentrations of phenolics can be expected in red wines [12]. According to Table 2, bioactive properties of red wines were higher compared with rose and white wines which is consistent to previous work reported [4,12]. The great differences in the contents of phenolic compounds in white and red wines indicate that anthocyanins (which are absent in white wines) represents the most important fraction of the phenolic compounds in red wines [4].

The TP is an important parameter widely used for evaluation of wines and other foods. Wines with higher TPC are considered to be better quality, in our case, Pinot Noire and Feteasca Neagra for red wines and Muscat Ottonel and Riesling Italian for white wines. The wines with higher TP tend to provide the higher antioxidant capacity indicating that the TP is responsible for the antioxidant capacity of the wine.

The antioxidant activity is a very relevant parameter to evaluate wine quality and its bioactive properties. For the analyzed wines, antioxidant capacity of red wines (expressed as μmoli/L Trolox) decrease in the order: Feteasca Neagra > Cabernet Sauvignon > Pinot Noire > Merlot, while for the white wines decrease in the order: Muscat Ottonel > Riesling Italian > Feteasca Regala > Chardonnay > Pinot Gris > Columna > Sauvignon Blank.

In the case of red wines, concentrations of total phenolics and antioxidant capacity were higher in wines from the 2010 and 2011 vintage years, with the exception of Pinot Noire wine from 2015. Young wines from 2017 presented the lowest values of antioxidant capacity (Figure 1).

For Muscal Ottonel and Feteasca Regala white wines, TP and antioxidant capacity were higher in old wines, while for Columna, Pinot Gris and Riesling Italian, TP and antioxidant capacity were higher in young wines. no semifinitive differences were observed between the Sauvignon Blank and Chardonnay wines wines obtained in different years (Figure 1).

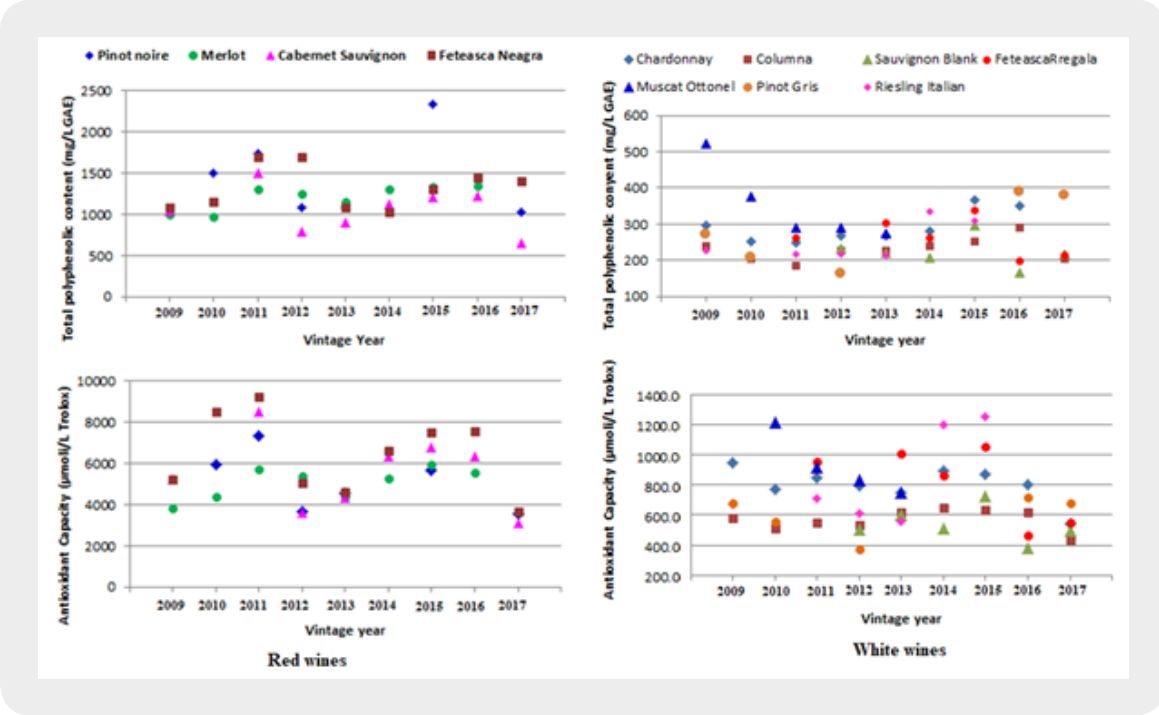


***Figure 1:*** *Total polyphenolic content (TP) and antioxidant capacity (AC) of red and white wines depending on vintage year*

**Relation Between Total Phenolic and Flavonoid Groups and Wine Antioxidant Activities**

Wine bioactive properties were examined according to the varietal origin of red and white wines using one-way ANOVA. The averages in the same row followed by the same lowercase letter were not significantly different according to the Duncan's multiple range test at $p \leq 0.05$ for varietal origin. The ANOVA results indicate that the TP was significantly different depending on the varietal origin of wines, while TF content was significantly different in the case of white wines. For the antioxidant capacity, no significant differences were observed.

The correlation analysis of the bioactive properties of red and white wines varieties (Table 3) shows moderate correlations between the parameters. The correlation coefficient refers to the relationship between two parameters. If the two parameters react in the same way, increasing or decreasing together, a positive correlation happens. The closer it is to 1, the higher the similarity between the two parameters.

The relationships between the red and white wines bioactive properties appear complex and are difficult to explain individually and generally; the interpretation of correlation analysis was done using correlation coefficients with values higher than 0.5. Higher correlations were obtained for TF and antioxidant capacity in the case of red wines, and for TP and antioxidant capacity in the case of white wines.

***Table 3:*** *Correlation matrix for the bioactive properties (total phenolic content – TP, total flavonoid cotent – TF and antioxidant capacity – AC) of red and white wines*

| **Red wines** | | | | **White wines** | | | |
|---|---|---|---|---|---|---|---|
| **Variables** | TP | TF | AC | Variables | TP | TF | AC |
| **TP** | **1** | | | TP | 1 | | |
| **TF** | 0.5108 | **1** | | TF | 0.6450 | 1 | |
| **AC** | 0.6852 | **0.7427** | **1** | AC | **0.8298** | 0.6853 | 1 |

The positive correlation indicates that when the concentrations of TP and TF increase, total antioxidant activity also increases. Moderate correlations suggest that besides the phenolic and flavonoids phytochemicals, in wines there are also other classes of compounds which contributed to the total antioxidant capacity, like anthocyanins in red wines and volatiles in white wines.

**Principal Component Analysis**

The experimental data indicate some differences among the different red and white wine varieties. Principal component analysis (PCA) was performed on red and white wine samples separately, using three variable components: Total phenolic content (TP), total flavonoid content (TF) and antioxidant capacity (AC).

The scree plot in Figure 2 shows that the first PC (PC1) explains about 63.61% and the second PC (PC2) about 25.18% of the total variance contained in the original dataset. No clear separation of wines into different classes was observed, due to the fact that the bioactive data of the investigated red wine varieties partially overlapped.

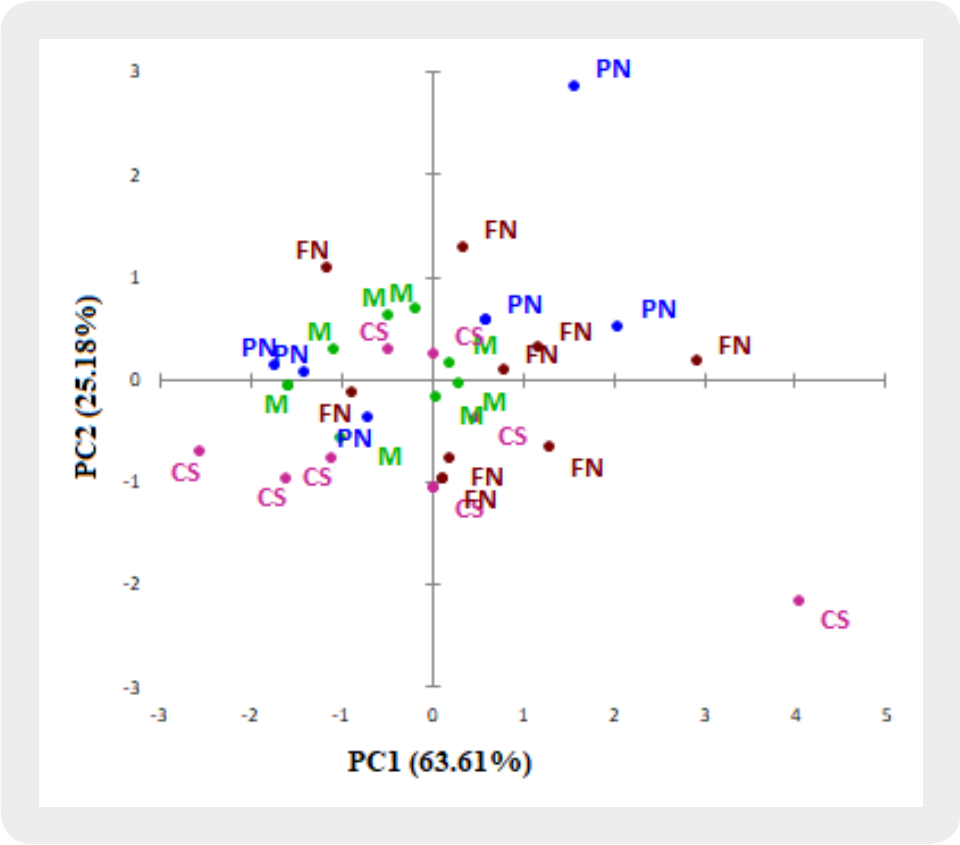


***Figure 2:*** *PCA scores plot for the red wines*

Apparently, PC1separate the Feteasca Neagra and Pinot Noire wines from Cabernet Sauvignon and Merlot wines, with the exception of Pinot Noire wines from 2010, 2011 and 2012 vintage year and Feteasca Neagra wines from 2011 and 2012 vintage year.

Figure 3 shows the PCA plot of white wine samples showing the separation between different varieties. It was found that PC1 accounts for 81.44% of total variance in dataset and PC2 accounts for 12.98% of total variance, the distribution of white wine samples along the two principal components showing a good discrimination of the wine samples.

PC1 separates the groups of Chardonnay and Muscat Ottonel wines (located on the right side) from the group of the Columna and Sauvignon Blank wines (located on the left), while PC2 separates the groups of Sauvignon Blank and Chardonnay wines (located at the top of the figure) from the group of Columna, Feteasca Regala and Muscat Ottonel wines (located at the bottom).

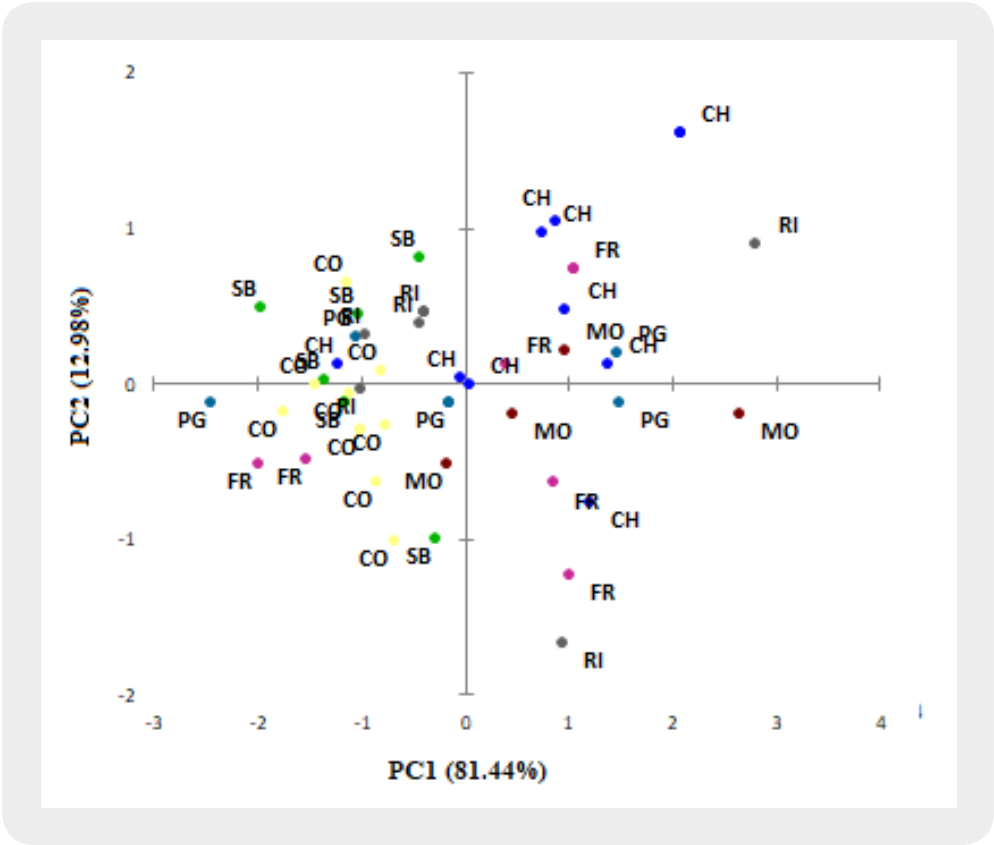


***Figure 3:*** *PCA scores plot for the white wines*

Feteasca Regala, Pinot Gris and Riesling Italian white wines were distributed in all the PCA plane, without the possibility of grouping, suggesting that, the blends made on the basis of these varieties will be very difficult to differentiate based on bioactive properties.

## Conclusions

In this work, 85 Romanian wine samples (31 red, 46 white and 8 rose) were characterized in term of some bioactive properties (total phenolic content, total flavonoid content and antioxidant capacity) using quantitative UV-Vis spectrophotometric methods.

The determination of wine bioactive properties using accessible and low cost spectrophotometric methods, could be a practical and simple measurement to evaluate the characteristics and potential beneficial effects on health of different wines. It was verified that the bioactive properties were significantly higher in red wines compared to white and rose varieties and total phenolics and flavonoids were moderated correlated with antioxidant capacity, for both, red and white varieties.

Based on quantitative spectrophotometric data in conjunction with PC analysis it was possible to address the wine classification problem. PCA analysis shows no clear separation for red wine varieties and the

possibility to distinguish between some white wine varieties including Chardonnay, Columna, Sauvignon Blank and Riesling Italian.

Future studies will be performed in order to investigate other bioactive properties such as total anthocyanin content, tannin content and phenolic and volatile compounds profiles, since they provide complementary results.

## Conflicts of Interests

The authors declare no conflict of interest.

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## Bibliography

1. Tarapatskyy, M., Kapusta, I., Gumienna, A. & Puchalski, C. (2019). Assessment of the Bioactive Compounds in White and Red Wines Enriched with a Primula veris L. *Molecules, 24*(22), E4074.

2. Wurz, D. A. (2019). Wine and health: A review of its benefits to human health. *BIO Web Conf., 12,* 04001.

3. Renaud, S., de Lorgeril, M. (1992). Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet, 339*(8808), 1523-1526.

4. Stratil, P., Kubáň, V. & Fojtová, J. (2008). Comparison of the phenolic content and total antioxidant activity in wines as determined by spectrophotometric methods. *Czech J Food Sci., 26,* 242-253.

5. Banc, R., Socaciu, C., Miere, D., Filip, L., Cozma, A., Stanciu, O. & Loghin, F. (2014). Benefits of Wine Polyphenols on Human Health: A Review. *Bull Univ Agric Sci Vet Med Cluj-Napoca Food Sci Technol., 71*(2), 79-87.

6. Fernandes, I., Pérez-Gregorio, R., Soares, S., Mateus, N. & de Freitas, V. (2017). Wine Flavonoids in Health and Disease Prevention. *Molecules, 22*(2), E292.

7. Sartor, S., Caliari, V., Malinovski, L. I., Toaldo, I. M. & Bordignon-Luiz, M. T. (2017). Bioactive profiling of polyphenolics and oenological properties of red wines from Italian grapes ( Vitis vinifera L.) cultivated in a selected subtropical region. *Int J Food Prop., 20*(Suppl 2), 1319-1328.

8. Snopek, L., Mlcek, J., Sochorova, L., Baron, M., Hlavacova, I., Jurikova, T., Kizek, R., Sedlackova, E. & Sochor, J. (2018). Contribution of Red Wine Consumption to Human Health Protection. *Molecules, 23*(7), 1684.

9. Fotakis, C., Christodouleas, D., Zervou, M., Papadopoulos, K. & Calokerinos, A. C. (2012). Classification of Wines Based on Different Antioxidant Responses to Spectrophotometric Analytical Methods. *Anal Lett., 45*(5-6), 581-591.

10. Yoo, Y. J., Saliba, A. J. & Prenzler, P. D. (2010). Should Red Wine Be Considered a Functional Food? *Compr Rev Food Sci Food Saf., 9*(5), 530-551.

11. Cassino, C., Gianotti, V., Bonello, F., Tsolakis, C., Cravero, M. C. & Osella, D. (2016). Antioxidant Composition of a Selection of Italian Red Wines and Their Corresponding Free-Radical Scavenging Ability. *J Chem., 2016,* 1-8.

12. Pandeya, A., Rayamajhi, S., Pokhrel, P. & Giri, B. (2018). Evaluation of secondary metabolites, antioxidant activity, and color parameters of Nepali wines. *Food Sci Nutr., 6*(8), 2252-2263.

13. Pisoschi, A. M. & Negulescu, G. P. (2012). Methods for Total Antioxidant Activity Determination: A Review. *Biochem Anal Biochem., 1*(1), 106.

14. Palade, L. & Popa, M. (2018). Polyphenol Fingerprinting Approaches in Wine Traceability and Authenticity: Assessment and Implications of Red Wines. *Beverages, 4*(4), 75.

15. Villano, C., Lisanti, M. T., Gambuti, A., Vecchio, R., Moio, L., Frusciante, L., Aversano, R. & Carputo, D. (2017). Wine varietal authentication based on phenolics, volatiles and DNA markers: State of the art, perspectives and drawbacks. *Food Control., 80,* 1-10.

16. Rocchetti, G., Gatti, M., Bavaresco, L. & Lucini, L. (2018). Untargeted metabolomics to investigate the phenolic composition of Chardonnay wines from different origins. *J Food Compos Anal., 71,* 87-93.

17. Singleton, V. L., Orthofer, R. & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol., 299,* 152-178.

18. Hosu, A., Cristea, V. M. & Cimpoiu, C. (2014). Analysis of total phenolic, flavonoids, anthocyanins and tannins content in Romanian red wines: prediction of antioxidant activities and classification of wines using artificial neural networks. *Food Chem., 150,* 113-118.

19. Hosu, A., Floare-Avram, V., Magdas, D. A., Feher, I., Inceu, M. & Cimpoiu, C. (2016). The Influence of the Variety, Vineyard, and Vintage on the Romanian White Wines Quality. *J Anal Methods Chem., 2016,* 1-10.

20. Gómez-Plaza, E., Gil-Muñoz, R., López-Roca, J. M. & Martínez, A. (2000). Color and Phenolic Compounds of a Young Red Wine. Influence of Wine-Making Techniques, Storage Temperature, and Length of Storage Time. *J Agric Food Chem., 48*(3), 736-741.