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Comparative Assessment of Phenolic Compounds from Authentic Wine Varieties from North-Western Romania from the 2021-2022 Harvest

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RESEARCH ARTICLE

Abstract
Wine is one of the most consumed beverages in the world. Romania, as a country, has a history in vineyards and wine making. This present study analyzed the phenolic compounds of 5 varieties of wine from Romania (Fetească Regală Muscat Ottonel, Riesling Italian, Pinot Gris and Merlot). The results suggested that some of the wines present a wide category of phenolic compounds where others present low values and some of the compounds are absent as detected with a developed HPLC-DAD-ESI+ method. Phenolic compounds were identified after the retention time. Merlot wine presented, in most cases, the highest values for phenolic compounds, with a few exceptions. As a conclusion, romanian wines are rich in phenolic compounds that possess a series of beneficial properties.

Keywords: phenols, variety, wine, HPLC

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INTRODUCTION

Wine is one of the most consumed beverages in the world (Hosu et al., 2014). Romanian traditional vineyards are becoming more and more scarce. One of the most important class of compounds that are found in wine are phenolic compounds, which present many beneficial properties for the human health. Varieties of wine such as Muscat Ottonel, Fetească Regală or Riesling Italian are well known and appreciated. The aroma, flavor and taste are different depending on the variety of grapes used, the soil and even weather. Among the components in red wine, we discuss antioxidant components like phenolic acids, stilbenes (e.g., resveratrol), flavonols (e.g., quercetin), flavan-3-ols (e.g., catechin and epicatechin), as well as procyanidins, and anthocyanins (Banc et al., 2014; Geana et al., 2014). Fetească albă grapes, for instance, make a wine that is characterized by finesse and balance with citrus flavors, lime blossom, field flowers, hay, ripe apricots. Also, the wine has a specific high acidity, giving freshness and a well–defined taste (Moroșanu, 2019). The specific taste of wines is influenced by many factors like their complex chemical composition. Wines, especially red wines are rich in polyphenols. Rodríguez-Vaquero et al. (2020) studied these polyphenols in red wine, their antioxidant activity and antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Listeria monocytogenes*. Polypohenols are also used as a wine authenticity tool (Palade and Popa, 2018; Proestos and Tzachristas, 2020).

The analyzes are part of the research objectives and topics proposed for the doctoral thesis with the title: "Research on the evaluation and characterization of some bioactive compounds in authentic wine assortments from the north-west of Romania", respectively the analysis of some wine compounds from the category phenols and polyphenols, compounds with a beneficial role for the health of consumers.

MATERIALS AND METHODS

Materials

Five varieties of wines (Muscat Ottonel, Pinot Gris, Fetească Regală, Merlot, Riesling Italian, Table 1) from Diosig vineyard (Diosig, Săcuieni, Siniob, Biharia and Tileagd wine producing centers) located in the north-west of Romania were subjected to investigations. The lithological substratum corresponds to sand and gravel and the relief is represented by the plains and the Piedmont hills of Oradea and Barcău. The climate is, in general, continental of central European type with influences of frosty air masses in winter and tropical in summer. The analyzed wine samples were taken from the varieties obtained from the harvest of 2021 and 2022.

Reagents

Acetonitrile, of HPLC purity, was purchased from Merck (Germany) and ultrapure water was purified with the Direct-Q UV system from Millipore (USA). Standards for chlorogenic acid (>98% HPLC), gallic acid (>99% HPLC), catechin and cyanidin (>99% HPLC) were from Sigma (USA).

Methods

Samples preparation

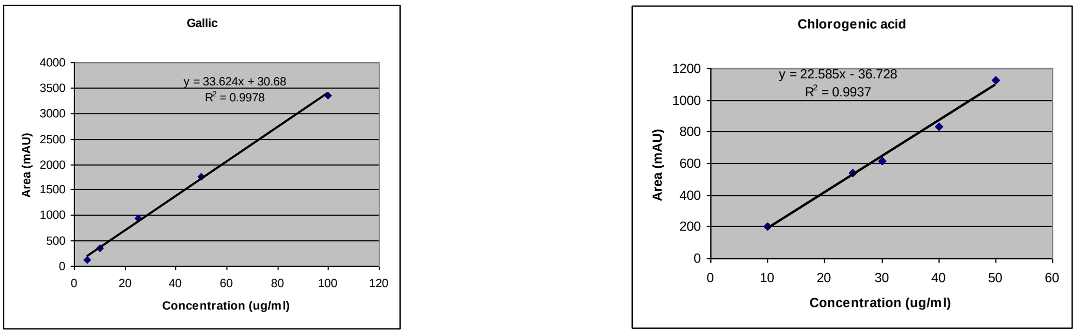
The determination of phenolic compounds was carried out by the HPLC-DAD-ESI+ Method in the Laboratories of the Institute of Life Sciences within USAMV Cluj-Napoca. The wine samples were kept at a temperature of 4-5^{o}C until their determination in the years of origin 2020 and 2021 respectively. The wine samples, used, were filtered through a 0.45 µm Chromafil Xtra nylon filter and 20 µl were injected into the HPLC system.

Chromatographic condition

Chromatographic measurements were carried out with an Agilent 1200 HPLC system (Agilent Technologies, CA, USA) equipped with a quaternary pump, solvent degasser, a diode array UV-Vis detector (DAD) and with a quadrupole Agilent 6110 mass spectrometer detector (MS). The separation of the compounds was carried out on a Kinetex XB C18 column, dimensions 4.6x 150 mm, with 5 µm particles (Phenomenex, USA), using the mobile phases (A) water + 0.1% acetic acid and (B) acetonitrile + 0.1% acetic acid in the gradient below, for 30 minutes, at a temperature of 25^{0} C, with a flow rate of 0.5 ml/min. Gradient (expressed in % B): 0 min, 5% B; 0-2 min, 5% B; 2-18 min, 5%-40% B; 18-20 min, 40%-90% B; 20-24 min, 90% B; 24-25 min, 90%-5% B; 25-30 min, 5% B. The spectral values were recorded in the 200-600 nm range for all peaks. The chromatograms were recorded at the wavelength λ = 280, 340 and 520 nm. For the MS, the full scan ESI positive ionization mode was used in the following working conditions: capillary voltage 3000 V, temperature 350^{0}C, nitrogen flow 7 l/min and m/z 120-1200. Data acquisition and interpretation of results was done using the Agilent ChemStation software.

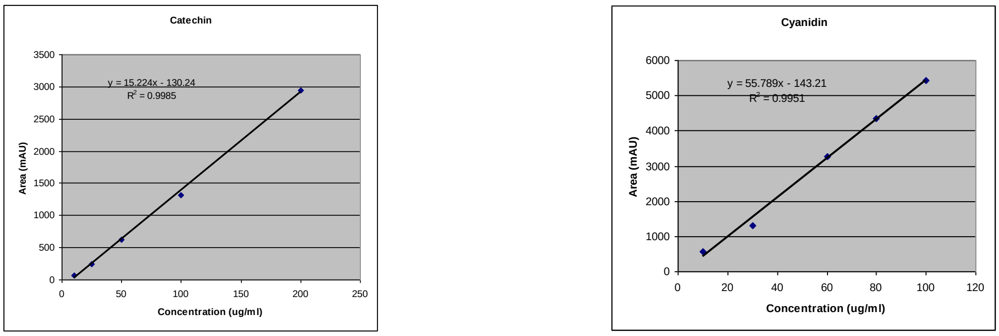
Calibration Curves

For the quantification of hydroxybenzoic acids and stilbenes, the calibration curve was made with gallic acid (R^2 = 0.9978), LOD = 0.35 µg/ml, LOQ = 1.05 µg/ml (Figure 1a). Hydroxycinnamic acids were quantified as chlorogenic acid equivalent (R^2 = 0.9937), LOD = 0.41 µg/ml, LOQ = 1.64 µg/ml (Figure 1b), flavanols as catechin equivalent (R^2 = 0.9985), LOD = 0.21 µg/ml, LOQ = 0.64 µg/ml (Figure 2a). For anthocyanins a calibration curve was made with cyanidin (R^2 = 0.9951), LOD = 0.21 µg/ml, LOQ = 0.84 µg/ml (Figure 2b).



(a) (b)

Figure 1. Calibration curves for gallic acid and chlorogenic acid

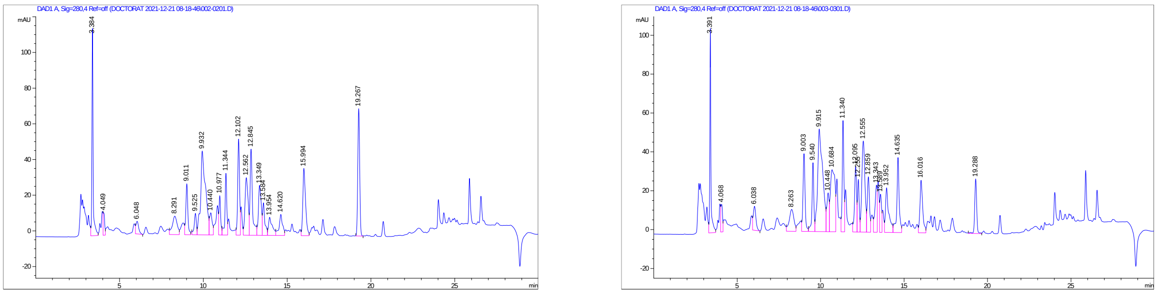


(a) (b)

Figure 2. Calibration curves for cyanidin (a) and catechin (b)

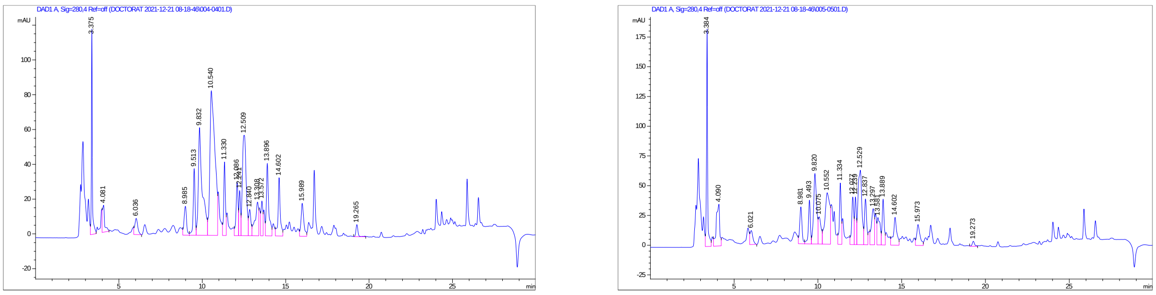
Cromatograme probe

Phenolic compounds were identified after the retention time (Table 1), UV-Vis absorbtion spectra (Figure 3-5) and mass spectra recorded for each peak. The wavelength λ=280 nm is specific for all subclasses of phenolic compounds (hydroxybenzoic acids, hydroxycinnamic acids, flavanols, stilbenes and anthocyanins), λ=340 nm is specific to hydroxycinnamic acids and the wavelength λ=520 nm is specific for anthocyanins.



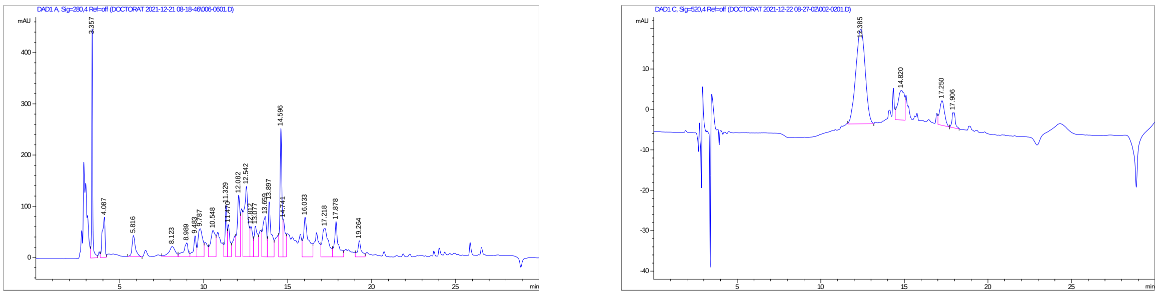
(a) (b)

Figure 3. Chromatogram probe for Muscat Ottonel wine-280 nm (a) and Fetească Regală wine- 280 nm (b)



(a) (b)

Figure 4. Chromatogram probe for Pinot Gris-280 nm (a) and Riesling Italian-280 nm (b)



(a) (b)

Figure 5. Chromatogram probe for Merlot- 280nm (a) and Merlot- 520nm (b)

In Table 1 all the phenolic compounds identified are listed, with the retention time, peak, class and subclass. All phenolic compounds identified are of interest both in wine flavor and authentication.

Table 1. The attempt to identify phenolic compounds in white and red wine samples

Peak	Rt (min)	UV λ max (nm)	M+H (m/z)	Compound	Sublcass
1	3.38	319	313	Trans-caftaric acid	Hydroxycinnamic acid
2	4.05	275	171	Gallic acid	Hydroxybenzoic acid
3	6.04	316	313	Caftaric acid	Hydroxycinnamic acid
4	8.29	275	332	Gallic acid-glucoside	Hydroxybenzoic acid
5	9.01	280	390	Resveratrol-glucoside	Stilbene
6	9.52	295	155	Protocatechuic acid	Hydroxybenzoic acid
7	9.93	321	327	Fertaric acid	Hydroxycinnamic acid
8	10.44	280	229	Resveratrol	Stilbene
9	10.75	322	297	Coutaric acid	Hydroxycinnamic acid
10	11.34	280	579	Procyanidin dimer I	Flavanol
11	12.10	280	139	p-Hydroxybenzoic acid	Hydroxybenzoic acid
12	12.38	530,280	493	Malvidin-glucoside	Antocyanin
13	12.56	280	291	Catechin	Flavanol
14	12.84	280	443	Epicatechin-gallate	Flavanol
15	13.34	322	181	Caffeic acid	Hydroxycinnamic acid
16	13.58	280	291	Epicatechin	Flavanol
17	13.95	322	209	Caffeic acid ethyl ester	Hydroxycinnamic acid
18	14.62	280	579	Procyanidin dimer II	Flavanol
19	14.82	530,280	535	Malvidin-acetyl-glucoside	Antocyanin
20	15.99	280	199	Syringic acid	Hydroxybenzoic acid
21	17.26	530,280	639	Malvidin-coumaroyl-glucoside	Antocyanin
22	17.90	531,280	609	Peonidin-coumaroyl-glucoside	Antocyanin
23	19.26	280	199	Gallic acid ethyl ester	Hydroxybenzoic acid

RESULTS AND DISCUSSIONS

In Table 2 and graphics from Figures 6-8 are represented the results obtained regarding phenol compounds identified from the authentic wine samples from the harvest in years 2021- 2022.

Table 2 presents in detail the phenol compounds from each wine variety, in both years. In general, Merlot variety presented the highest values for all compounds found with a couple of exceptions, namely protocatechuic acid for Pinot Gris in 2022, fertaric acid for Muscat Ottonel in 2021, coutaric acid for Pinot Gris in 2021 and gallic acid ethyl ester for Muscat Ottonel in 2021. This is similar to the literature where it is stated that red wines contain more phenolic compounds compared to the white wine.

Polyphenols have been shown to be correlated to wine quality (color, flavor, and taste) and health beneficial properties (antioxidant, neuroprotective, antimicrobial and cardio protective among others). We can divide them in two groups, Flavonoids (where we include anthocyanidins, flavonols, flavones and chalcones) and Non-flavonoids (where we include hydroxybenzoic acids, stilbenes, among others) (Gutiérrez-Escobar et al., 2021).

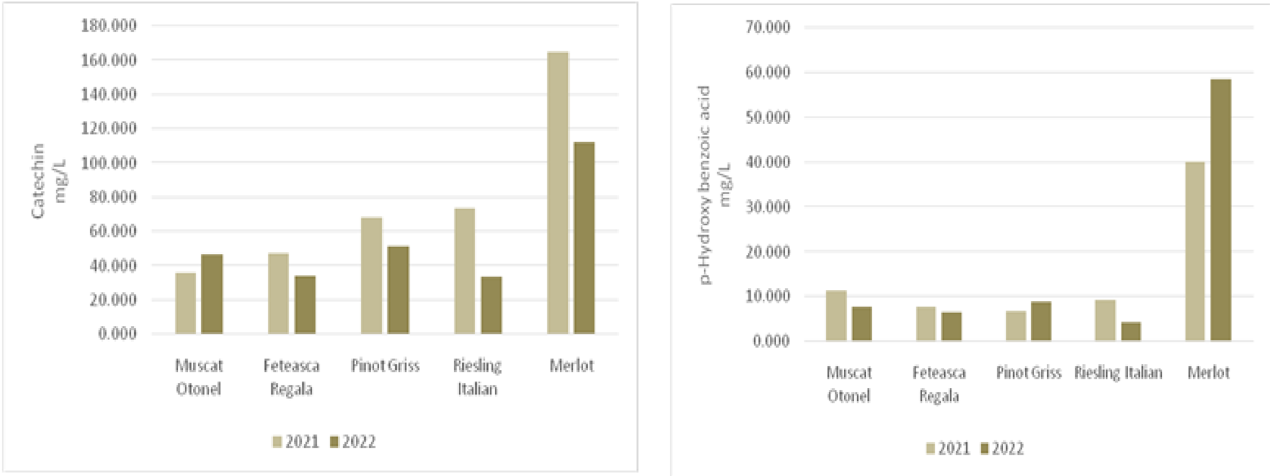
Protocatechuic acid (PCA) was proven to be strong in vitro and in vivo antioxidant activity as well as anti-inflammatory, anti-hyperglycemic and anti-apoptotic activities. More than that, PCA has been shown to put a stop to chemical carcinogenesis and start cell death as well as stops cell development in different cancerous tissues. Other studies showed PCA to have antimicrobial activities and can aide some antibiotics (Semaming et al., 2015).
As a naturally present compound in wines, many studies have showed that resveratrol possesses a very high antioxidant potential. Not only that, but it can also exhibit antitumor activity, neuroprotective and cardio protective effects (Bishayee, 2009; Salehi et al., 2018).

Catechins present different benefits including preventing or reducing skin damage. They are also known to have a powerful anti-oxidant, antimicrobial, antiallergenic, antiviral and anticancer activity (Bae et al., 2020).

Table 2. Comparative evaluation of phenolic compounds in white and red wine samples from years 2021 and 2022 [mg/L]

Peak no.	Rt (min)	Compound	Muscat Ottonel		Fetească Regală		Pinot Gris		Riesling Italian		Merlot	
			2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
1	3.38	Trans caftaric acid	27.4	36.0	22.5	20.1	22.3	22.0	39.5	25.9	80.7	55.9
2	4.05	Gallic acid	2.1	22.6	2.3	6.8	3.4	10.2	13.8	9.1	25.2	19.0
3	6.04	Caftaric acid	6.9	13.1	8.6	7.6	7.1	9.1	7.6	10.1	16.8	105.2
4	8.29	Gallic acid-glucoside	5.3	0.5	5.6	0.9	1.8	7.8	2.3	6.6	15.4	17.4
5	9.01	Resveratrol-glucoside	6.1	8.7	9.3	13.2	4.76	3.1	7.9	5.1	14.850	30.345
6	9.52	Protocatechuic acid	2.3	11.9	6.9	9.1	7.7	42.3	8.1	32.7	12.9	40.5
7	9.93	Fertaric acid	66.4	9.1	82.4	20.4	80.5	40.6	44.2	32.4	47.8	5.6
8	10.44	Resveratrol	3.2	22.1	4.7	36.4	6.4	46.5	6.9	17.0	10.6	59.5
9	10.75	Coutaric acid	8.4	61.0	37.7	87.2	121.3	107.8	57.6	41.6	66.2	48.0
10	11.34	Procyanidin dimer I	25.8	51.2	34.3	57.7	27.9	37.5	34.0	29.0	60.4	102.8
11	12.10	p-Hydroxybenzoic acid	11.4	7.6	7.7	6.5	6.7	8.9	9.4	4.2	40.0	58.5
12	12.38	Malvidin-glucoside	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.9	18.7
13	12.56	Catechin	35.7	35.7	47.0	47.4	34.2	68.3	51.8	73.9	33.7	164.8
14	12.84	Epicatechin-gallate	39.7	58.2	22.6	64.9	18.0	16.8	31.8	11.5	71.2	34.4
15	13.34	Caffeic acid	15.9	21.6	11.3	15.6	14.1	18.1	18.0	12.6	28.1	54.4
16	13.58	Epicatechin	19.9	42.1	18.1	46.5	18.8	34.6	27.6	22.0	90.1	218.2
17	13.95	Caffeic acid ethyl ester	8.8	28.7	15.5	13.0	19.8	5.4	15.9	6.9	63.0	15.6
18	14.62	Procyanidin dimer II	20.0	17.9	33.7	19.3	28.3	13.6	30.2	10.0	144.1	68.2
19	14.82	Malvidin-acetyl-glucoside	0.0	0.0.	0.0	0.0	0.0	0.0	0.0	0.0	7.4	5.3
20	15.99	Syringic acid	11.8	5.0	8.3	6.1	5.6	4.2	6.4	0.6	46.6	22.2
21	17.26	Malvidin-coumaroyl-glucoside	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.7	3.9
22	17.90	Peonidin-coumaroyl-glucoside	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	2.56
23	19.26	Gallic acid ethyl ester	15.9	0.7	6.0	0.1	0.9	0.9	0.3	0.3	13.4	1.5
		Total	333.9	465.9	385.8	466.4	464.5	482.2	436.2	312.3	1057.6	1101.2

Another compound with many beneficial properties is gallic acid ethyl ester. This phenolic compound presents properties like anticarcinogenic, antimicrobial, anti-mutagenic and anti-inflammatory (Choubey et al., 2015). Not only that but many studies proved that the compound has also neuroprotective and hepatoprotective potential (Pal et al., 2018).

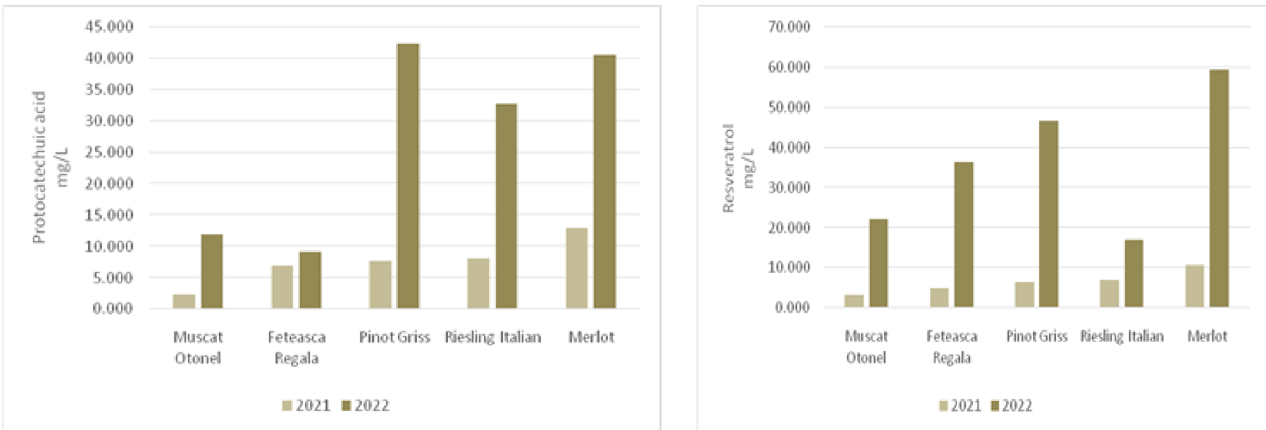


(a) (b)

Figure 6. Comparative evaluation of the catechin content for the varieties of wine investigated in the 2021-2022 years (a) and Comparative evaluation of the content of p-hydroxy benzoic acid for the varieties of wine investigated in the 2021-2022 years (b)

For catechin content all samples were tested, in both years (Fig 6). Merlot presented the highest values, in both years, followed by Riesling Italian, Pinot Gris, Fetească Regală and Muscat Ottonel. In 2012, Merlot showed the highest values recorded within the samples. In 2022, Merlot presented a high value but lower than in 2021. Only 3 varieties of wine presented higher values in 2021, namely Fetească Regală, Pinot Gris and Riesling Italian. Pinot Gris and Riesling Italian wines presented similar values for the year 2021. Wines made from Fetească Regală and Riesling Italian presented similar values for the year 2022.

Regarding p-hidroxi benzoic acid Merlot samples showed the highest content, in both years (similar to catechin). All other samples present much lower values, for both years taken for study. The values were similar within years and wine variety. For Fetească Regală, Pinot Gris, Muscat Ottonel and Riesling Italian values were similar both in 2021 and in 2022. There were small differences.

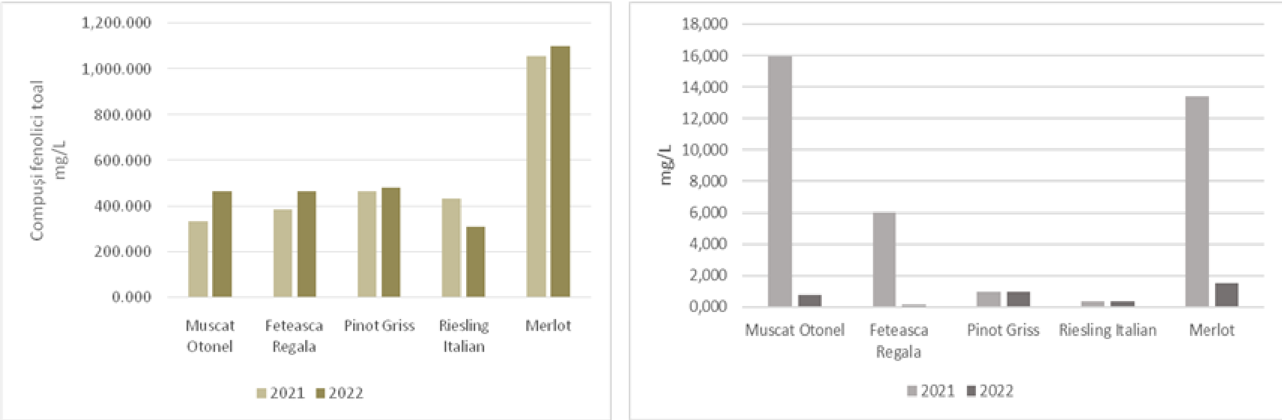


(a) (b)

Figure 7. Comparative evaluation of the content of protocatechuic Acid for the varieties of wine investigated in the 2021-2022 years (a) and Comparative evaluation of resveratrol content for the wine varieties investigated in the 2021-2022 years (b)

Protocatechiuc acid is an important compound in the hydroxybenzoic acid class. The results are presented in Figure 7. Muscat Ottonel and Fetească Regală varieties showed lower values in both years, with the lowest value registered in 2021. The rest of the samples showed higher values in the second year. For year 2021 Muscat Ottonel presented the lowest value registered from all samples and Merlot the highest. For year 2022 Pinot Gris presented the highest value from the ones registered and Fetească Regală the lowest.

Regarding resveratrol for all the samples of wine the greater values in mg/L were observed in 2022 (Figure 7). From these samples Merlot presented the highest value of resveratrol in 2022 and Riesling Italian the lowest. In 2021 all samples showed low values with Merlot presenting the highest value registered for the year and Muscat Ottonel the lowest.



(a) (b)

Figure 8. Comparative evaluation of the content of total phenolic compounds for the varieties of wine investigated in the years 2021-2022 (a) and Comparative evaluation of the content of gallic acid ethyl ester for the varieties of wine investigated in the years 2021-2022 (b)

For Merlot samples the content of phenolic compounds was the highest compared with the other samples of wine (Figure 8). Muscat Ottonel, Fetească Regală, Pinot Gris and Riesling Italian presented similar values with greater values in 2022, with the exception of Riesling Italian, which presented greater values in 2021 and lower values in 2022 and Merlot which presented the highest values for both years. For gallic acid ethyl ester the situation is a bit different (Figure 8). In this case Muscat Ottonel presents the highest values in year 2021, followed by Merlot in 2021 and Fetească Regală, in 2021. Muscat Ottonel also presented the highest value for fertaric acid (Table 2). Riesling Italian presented the smallest amount of the compound, in both years. In 2022, for all samples of wine the amount of the compound is extremely low, Merlot presenting the highest value. Fetească Regală presented the smallest amount for the year 2022, compared to the other wines.

CONCLUSIONS

The present study shows that wine produced from various grape varieties presents different phenols and polyphenols contents. Merlot samples usually presented the highest values for both years, with the exception of protocatechuic acid which presented the highest values for Pinot Gris in 2022, fertaric acid for Muscat Ottonel in 2021, coutaric acid for Pinot Gris in 2021 and gallic acid ethyl ester for Muscat Ottonel in 2021. For gallic acid ethyl ester Muscat presented the highest value followed by Merlot, both in 2021. Regarding phenolic content again, Merlot wine presented the highest values for both years. This is in agreement with the literature. Red wine contains more phenolic compounds compared to the white wine.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest

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