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From grape to wine - Muscat Ottonel from Blaj-Târnave vineyard chemical and sensory analysis

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RESEARCH ARTICLE

Abstract
Following the global trend of selling high-quality wines, those produced in Blaj vineyard must be superior due to a highly competitive market and consumer tastes and demands. Because the quality of wine is given by both sensory and chemical properties this work presents the phenolic fingerprint, the general chemical characteristics, and the sensory properties of the 2021 Blaj Muscat Ottonel wine as well as the phenolic composition of the grapes from which this wine was produced. Grape phenolics were represented by flavanols (73%), flavonols (14%), hydroxybenzoic acids (9.08 %) and hydroxycinnamic acids (4%), while wine phenolics by flavanols (42%), hydroxycinnamic acids (33%) and hydroxybenzoic acids (15%). Catechin and procyanidin dimer B1 were identified both in grapes and in wine. More than half of the grapes' procyanidin dimer B1 (3.638 mg/g out of 6.379 mg/g) and more than one-tenth of grapes' catechin (1.570 mg/g out of 9.298 mg/g) were found in wine. As the general and sensory qualities of the Blaj Muscat Ottonel wine were kept within the limits of a Protected Designation of Origin (PDO) demi-sweet wine, the presence of resveratrol glucoside, catechin, and procyanidin in its content supports the idea of classifying this wine as a potential nutraceutical 'functional wine'.

Keywords: Muscat Ottonel wine; phenolics; general chemical characteristics, sensory properties

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INTRODUCTION

The impression of the vineyards' landscape extends beyond the terraces and grapevine rows generating a whole lifestyle of those working with vine and wine. "Wherever in the world, one enters a vine-growing region, the atmosphere is unmistakably unique, a combination of serenity and civility" (De Blij, 1983; Tiefenbacher and Townsend, 2019; Vosloban and Chedea, 2022). The economic importance of grapevine (V. vinifera and Vitis spp.) is given by the cultivated area of approximately 6.73 million ha which produced 73.5 million tons of fruits harvested in 2021 ("Food and Agriculture Organization of the United Nations," n.d.), as fresh table grapes, dried grapes, and grapes for wine production. Wine is a commonly used beverage with a millennial tradition as winemaking's history echoes that of civilization (Mitić et al., 2010; Vosloban and Chedea, 2022). Globally, in 2020 there were produced 26.7 million tons of wine, out of which Romania made 0.38 million tons, ranking 13th in the world ("Food and Agriculture Organization of the United Nations," n.d.). The grapevine was cultivated in Romania since antiquity because of the adequate climate and fertile soils (Chedea et al., 2021; Chiurciu et al., 2020; Muntean et al., 2022). Romania, located between 43°37′–48°15′ N lat and 20°15′–29°44′ E long, in Eastern Europe, having a temperate continental climate, Dfb and Dfa in a Koppen–Geiger climate updated classification, has eight important viticultural regions (Chedea et al., 2021; Irimia et al., 2018; Kottek et al., 2006; Muntean et al., 2022). The landscape of these areas has different environmental conditions influenced by the Carpathian Mountains (2500 m altitude), the Danube River, and the Black Sea, so different types of wines (white, red, rose, dry, demi-dry, demi-sweet, sweet, and sparkling) are produced from south to north (Chedea et al., 2021; Muntean et al., 2022).

The Blaj vineyards, situated on the Transylvanian Plateau, are a component of Romania's viticultural zone 1 and are situated at the junction of 46°–47° North latitude and 23°–24° East longitude (Călugăr et al., 2020; Chedea et al., 2021; Cudur et al., 2014; Donici et al., 2019; Iliescu, M.; Tomoiaga, L.; Farago, M.; Comsa, 2010). The most notable viticultural region in Transylvania, the prestigious Blaj vineyard, so named because the majority of the vineyards are situated on the slopes that separate the valleys of the rivers Târnava Mare and Târnava Mică (Călugăr et al., 2020; Donici et al., 2019; Iliescu, M.; Tomoiaga, L.; Farago, M.; Comsa, 2010), is renowned and praised for its high-quality wines with a distinct flavor and a good sugar/acidity balance (Chedea et al., 2021; Cudur et al., 2014; Donici et al., 2019; Iliescu, M.; Tomoiaga, L.; Farago, M.; Comsa, 2010). The area, grapevine cultivars, and clones planted here give the terroir and the importance of the Blaj vineyard. Original and noble wines obtained from the established cultivars Fetească albă, Fetească regală, Riesling Italian, Sauvignon blanc, Muscat Ottonel, Traminer Roz, Neuburger, as well as from the autochthonous cultivars Radames, Selena, Blasius, Brumăriu, Amurg, Astra are appreciated as dry, semi-dry, semi-sweet, sweet, semi-aromatic, aromatic, and sparkling POD (protected designation of origin) and PGI (protected geographic indication) wines (Călugăr et al., 2020; Chedea et al., 2021; Cudur et al., 2014; Iliescu, M.; Tomoiaga, L.; Farago, M.; Comsa, 2010; Marinela, 2012).

Following the global trend of selling high-quality wines, those produced in Blaj vineyard must be superior wines due to a highly competitive market and consumer tastes and demands. Generally, each wine producer (and merchant) claims that their wine is unique rather than ordinary, distinguishing their product not simply via standardized, external features but also because of the intrinsic features of a single wine, as well as the contrast with other comparable wines, and thus determining its value (Charters and Pettigrew, 2007; Chedea et al., 2021; Feinberg, 2020; Rahman and Reynolds, 2015; Rossi and Cortassa, 2020). Terroir is used by winemakers to adjust grapevine varietal characteristics to the specific vineyard environment to produce a distinctive wine (Chedea et al., 2021; Chironi et al., 2020).

The quality of wine is given by both sensorial properties and chemical fingerprints, with the presence/absence and quantity of a specific compound playing a significant influence (Kropek et al., 2023; Mitić et al., 2010). The chemical composition, the basis of the sensorial characteristics of a wine, is given by the concentrations of sugar, organic acids, ethanol and polyphenols. Even though the phenols are in much lower concentrations than the other compounds, they are highly relevant for the quality of grapes and wine (Drappier et al., 2019; Kropek et al., 2023). They have a significant role in the color and structure of wines on the palate, these being the first features that the consumers judge and are also taken into account when evaluating their authenticity and quality (Drappier et al., 2019; Kropek et al., 2023; Mitić et al., 2010). Phenolic compounds' contribution to the sensory and chemical wine’s value is due to their direct role and to their reactions with other molecules like polysaccharides, proteins or other polyphenols (Mitić et al., 2010; Silva et al., 2005). Polyphenols constitute a class of highly bioactive chemicals in grapes and wine that can be generated and altered during vinification (Kropek et al., 2023). In grapes and wine, the following subclasses of polyphenols were identified: phenolic acids, flavonoids, tannins, and stilbenes (Kropek et al., 2023; Merkyte et al., 2020).

When selling the wine, the trader recommends or even incites the customer to opt for a wine produced by a particular winery from a particular year and vineyard because of its special quality (Chedea et al., 2021; Hall and Mitchell, 2007; Tiefenbacher and Townsend, 2019), and in this framework, we aimed to focus on the Muscat Ottonel demi-sweet wine from Blaj vineyard. As, wine quality and implicitly, its phenolic pattern is strongly determined by three sets of factors: grapes’ characteristics (cultivar, their maturation stage, the terroir) (Andrade et al., 2001), the winemaking technology (Kropek et al., 2023; Ramos, R.; Andrade, P.B.; Seabra, R.M.; Pereira, C.; Ferreira, M.A.; Faia, 1999; Zafrilla et al., 2003) and the phenolics assessment method throughout wine aging (Bravo et al., 2006; Kropek et al., 2023; Mitić et al., 2010; Zafrilla et al., 2003), we present in this work the phenolic fingerprint, the chemical general characteristics and the sensory properties of the 2021 Blaj Muscat Ottonel wine as well as the phenolic composition of the grapes from which this wine originated.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were of analytical grade. Acetonitrile, HPLC grade was from Merck (Germany) and the ultrapure water was obtained using the Direct-Q UV system from de la Millipore (USA). Standards of chlorogenic acid (>98% HPLC), gallic acid (>99% HPLC), rutin and catechin (>99% HPLC) were from Sigma Chemical Co. (St.

Louis, MO, USA). Glucose, fructose, and maltose standards (99 % purity), lactic acid, acetic acid, and ethanol (purity >99%) were from Sigma-Aldrich (Germany). Iodine, sulfuric acid, starch solution, potassium hydroxide, sodium hydroxide, phenol red, phenolphthalein, tartaric acid, ethanol 96% vol., neutral lead acetate, nicotinamide adenine dinucleotide phosphate, adenosine-5'-triphosphate, hexokinase/glucose-6-phosphate-dehydrogenase, phosphoglucose-isomerase) were purchased from Nordic Chemicals (Cluj-Napoca, Romania).

Plant materials

The grapes (*Vitis vinifera* L., cultivar Muscat Ottonel) grown on the experimental plots of SCDVV Blaj, Blaj vineyard (Romania), were harvested at the optimal fruit maturity on 24th of September 2021, having the appropriate health status for further winemaking processing and analysis.

Phenolic compounds extraction from grape seeds and skin

The grape seeds and skin were dried at 40 °C using a laboratory oven (Memmert GmbH, Germany) and finely ground to obtain seed flour and skin flour, which were stored in dark at room temperature. Further 1 g of each flour was extracted using 10 ml methanol containing 1% of 37% HCl solution. The mixtures were vortexed for 1 min using a Heidolph Reax top vortex and then sonicated for 60 min using a sonication bath (Elmasonic E 15 H). At the end of the sonication process, the samples were centrifugated using an Eppendorf AG 5804 centrifuge for 10 min at a speed of 10000 rpm and a temperature of 24˚C. The clear extract was collected and further used in the chromatographic analysis. Until the analysis the extracts were stored at -20 ˚C.

Grape seeds and skin phenolic compounds determination by HPLC-DAD-ESI+

High-performance liquid chromatography (HPLC) Agilent 1200 was used for phenolic compound identification and quantification from the grape skin and seed extracts. The HPLC system was equipped with a quaternary pump, degasser and photodiode array DAD UV-Vis detector. The HPLC system was coupled with a 6110 Agilent single quadrupole mass spectrometer (MS) detector (Agilent Technologies, CA, USA). Compound separation was achieved using a Kinetex XB C18 column (4.6x 150 mm, 5 µm particle size, Phenomenex, USA) and mixture gradient obtained from mobile phase (A) water + 0.1% acetic acid and (B) acetonitrile + 0.1% acetic acid. The separation was performed at 25˚C using a 0.5 ml/min flow. The gradient elution (% B) was as follows: 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 compounds' absorption wavelengths were registered between 200-600 nm, while the chromatograms were registered at three different wavelengths (280 and 340 nm). The mass spectrometer was operated in ESI positive mode with the following operating parameters: capillary tension of 3000 V, temperature of 350 ˚C, nitrogen flow of 7 l/min, collision energy of 100 eV and the mass scan range between 120-1200 m/z. Data acquisition and results interpretation were performed using Agilent ChemStation software, B.02.01 SR2 version. The five-point calibration curves of standards used for quantification were performed in triplicate. Accordingly, the compounds belonging to the flavanols class were quantified as catechin equivalents, the compounds belonging to the hydroxybenzoic acids class were quantified as gallic acid equivalents, the compounds belonging to the hydroxycinnamic acids class were quantified as chlorogenic acid equivalents while compounds belonging to the flavonols class were quantified as rutin equivalents.

Prior injection to HPLC-MS system the extract samples were filtered using an 0.45 µm Chromafil Xtra nylon filter (Muso, Cluj-Napoca, Romania).

Wine production

The Muscat Ottonel wine was obtained at the winery of SCDVV Blaj (Blaj, Romania) in 2021 following the classical technology of the aromatic white winemaking process (Coldea and Mudura, 2015), except for the yeast inoculation stage. Wine production was performed without added yeast. The yeasts used were only the ones naturally existing on the berries at harvest. The maceration time was 65 hours. The treatments applied during winemaking productions were as follows: (1) addition of a 5-6% aqueous solution of sulfur dioxide (SO_2) at a rate of 1 L/t, to prevent the must oxidation following the destemming and crushing of the grapes, (2) sulfitation with 1 mL SO_2/L wine to end the alcoholic fermentation, (3) bentonization with 100 g/hL bentonite after being racked in another tank, (4) filtering through porous cellulose filters after being allowed to rest for 15 days (Sîrbu et al., 2022).

Wine phenolic compounds determination by HPLC-DAD-ESI+

Phenolic compounds in wine were performed as described in the previous section (skin and seeds). Prior injection to HPLC-MS system the wine samples were filtered using an 0.45 µm Chromafil Xtra nylon filter (Muso, Cluj-Napoca, Romania).

General characteristics of the 2021 Blaj Muscat Ottonel wine

Free and total sulfur dioxide content

The iodometric method from ASRO-SR 6182-13:2009 was used with slight modifications to quantify the free and total sulfur dioxide. 25 mL wine was mixed with 2.5 mL H_2SO_4 (1:3, v/v) and 1 mL starch solution of 1% and then titrated with 0.02N iodine until the color changed to determine free SO_2 (mg/L). The total SO_2 (mg/L) measurement was performed by mixing 25 mL of wine with 12.5 mL of 1N KOH. After 15 minutes, 5mL of H_2SO_4 (1:3, v/v) and 1 mL of 1% starch solution were added, followed by 0.02N iodine titration (ASRO - SR 6182-13, 2009.).

Alcohol content

The Dujardin-Salleron electric ebulliometer (AllaFrance-EB, MultiLab, Romania) was used to determine the alcohol content (% vol). The procedure was performed according to manufacturer instructions using a 10% alcohol standard solution prepared with 96% alcohol and distilled water (v/v) (https://www.dujardin-salleron.com/documents/fiches/589b20d4a2332--160350---ft-ebulliometer-en.pdf).

Total acidity measurement

10 mL of wine mixed with 10 mL of distilled water were titrated with 0.1N sodium hydroxide in the presence of phenol red indicator by stirring until the color changed to orange (ASRO - SR 6182-1, 2008). The total acidity was expressed as g/L tartaric acid ($C_4H_6O_6$).

Volatile acidity measurement

The steam distillation method was used to separate the volatile acids from wine. Before distillation, the wine was acidified using tartric acid to release the salts of volatile acids (ASRO - SR 6182-2:2008. Standard Roman (Romanian Standard)-ASRO. Wine, Part 2: Determination of volatile acidity., n.d.; Sîrbu et al., 2022). Afterward, volatile acids were titrated with 0.1 N sodium hydroxide solution using phenolphthalein as an indicator.

Density measurement

The method OIV-MA-AS2-01A (2012) was used to measure wine density at 20˚C with a 1 mg precision hydrostatic balance (Multi-Lab, Romania).

The total dry extract content

The total dry extract (g/L) was determined considering the de-alcoholized wine`s relative density. Accordingly, the density of the de-alcoholized wine is taken as the reference measurement unit, and the quantity of sucrose used to be dissolved in 1 L of water to obtain the same density as of the dealcoholized wine is measured (OIV-MA-AS2-03B, 2012).

The non-reducing dry extract

The difference between the total dry extract and the residual sugars (total sugars content), is calculated as the non-reducing dry extract (g/L) (Sîrbu et al., 2022).

Total sugar content

Total sugars were determined based on the ability of sugars to reduce the copper salt alkaline solution (OIV-MA-AS311-01A, 2009). Prior reaction the wine is clarified with neutral lead acetate. Afterward, 10mL of the clarified solution was mixed with 25mL of alkaline copper salt solution, 15mL of water and several tiny pieces of pumice stone. The combination was further boiled for 10 minutes using a reflux condenser, followed by titration with 0.1 M sodium thiosulfate solution. The amount of sugar was expressed as (g/L) inverted sugar (Sîrbu et al., 2022).

Glucose and fructose content

The quantification of glucose and fructose was determined using the enzymatic method according to OIV-MA-AS311-02 (2009). The measurements were performed using the automatic (Y15) oenological analyzer (BioSystems Romania). The results were expressed as g monosaccharides/L.

Sensory analysis of the 2021 Blaj Muscat Ottonel wine

A sensory (aromatic and gustatory) analysis of the Blaj Muscat Ottonel wine was performed. The panel for sensory analysis had six trained judges all with experience in sensory evaluation of wine. The tasting procedure took place at the evaluation laboratory of SCDVV Blaj, at a room temperature of 19 ± 2 ˚C. Tasting glasses were used for presenting the wine (Jesus et al., 2017).

RESULTS AND DISCUSSIONS

Phenolic composition of grapes

Nineteen phenolic compounds (59.700 mg/g) were found in the skin (17.024 mg/g) and seeds (42.676 mg/g) of the Blaj Muscat Ottonel grapes (Table 1). The seeds had 2.5 times more phenolics than the skin. As the phenolic fingerprint was determined separately for the skin and the seeds, to have a general view of polyphenols from the grape berry we summed the values of each identified molecule in the two components of the grape berry (Table 1).

Table 1. Identification and quantification of phenolic compounds (mg/g) extracted from the skin and seeds of grapes.

Peak No.	R_t (min)	UV λ_{max} (nm)	$[M+H]^+$ (m/z)	Phenolic compounds	Phenolic compounds subclass	Skin (mg/g)	Seeds (mg/g)	Grapes (skin+seeds) (mg/g)
1	3.24	270	139	2-Hydroxybenzoic acid	Hydroxybenzoic acid	0.15±0.01	0.59±0.03	0.74±0.05
2	6.06	270	322	Gallic acid-gallate	Hydroxybenzoic acid	0.15±0.02	0.17±0.02	0.32±0.04
3	9.68	280	579	Procyanidin dimer B3	Flavanol	1.78±0.01	1.98±0.08	3.77±0.10
4	11.36	332	343	Caffeic acid-glucoside	Hydroxycinnamic acid	0.96±0.07	n.d.	0,96±0.07
5	11.65	280	579	Procyanidin dimer B1	Flavanol	1.32±0.06	5.05±0.12	6.37±0.2
6	12.71	280	291	Catechin	Flavanol	1.23±0.06	8.06±0.15	9.29±0.22
7	12.93	332	355	Chlorogenic acid	Hydroxycinnamic acid	1.25±0.05	n.d.	1.25±0.05
8	13.35	280	579	Procyanidin dimer B4	Flavanol	n.d.	6.24±0.12	6.241
9	14.07	280	291	Epicatechin	Flavanol	0.77±0.05	8.48±0.16	9.25±0.21
10	14.85	280	579	Procyanidin dimer B2	Flavanol	0.44±0.06	4.42±0.11	4.87±0.17
11	15.78	360, 250	611,303	Quercetin-rutinoside (Rutin)	Flavonol	0.45±0.02	n.d.	0.45±0.02
12	15.98	280	443	Epicatechin-gallate	Flavanol	n.d.	2.29±0.11	2.29±0.11
13	16.42	360, 250	465,303	Quercetin-glucoside	Flavonol	4.04±0.07	n.d.	4.04±0.07
14	16.55	360, 260	303	Ellagic acid	Hydroxybenzoic acid	n.d.	4.35±0.12	4.35±0.12
15	16.95	360, 260	479,317	Isorhamnetin-glucoside	Flavonol	3.36±0.18	n.d.	3.36±0.18
16	17.51	360, 255	449,287	Kaempferol-glucoside	Flavonol	0.98±0.02	n.d.	0.98±0.02
17	17.64	280	458	Epigallocatechin-gallate	Flavanol	n.d.	0.99±0.02	0.99±0.02
18	21.75	360, 250	303	Quercetin	Flavonol	0.04±0.00	n.d.	0.04±0.00
19	23.35	360, 260	317	Isorhamnetin	Flavonol	0.04±0.00	n.d.	0.04±0.00
				Total phenolics		**17.02±0.7**	**42.67±1.0**	**59.70±1.83**

The main class of phenolic compounds in Muscat Ottonel grapes was represented by the flavanols which accounted for approximately 73% of total phenolics as identified by LC-MS, followed by the flavonols with approximately 14 % and hydroxybenzoic acids with 9.08 %. Hydroxycinnamic acids were also present, accounting for approximately 4% of total phenolics. When compared with literature data (Narduzzi et al., n.d.; Romero-Pérez et al., 2001; Savalekar et al., 2019), a great variation between concentrations and profile composition is observed. These differences are due to different climate conditions, growth locations, soil properties, grape cultivars, ripening time, as well as storage, processing steps, extraction, and detection procedures. For example, catechin concentration in the 2021 Blaj Muscat Ottonel grape is 9.28 mg/g as compared with 21.12 mg/g in the grape Moscatel cultivar from Spain, harvested at the maturation stage. The epicatechin gallate was 2.29 mg/g as compared to 1.05 mg/g of the same cultivar (Alonso Borbalán et al., 2003). Also, the non-flavonoid compounds, in the Spanish Moscatel grapes were represented only by the hydroxycinnamic acid like caftaric and coutaric while the Romanian Muscat Ottonel grapes had a different hydroxycinnamic acid profile, namely caffeic and chlorogenic acid derivatives. Additionally, hydroxybenzoic acids were also present.

In 2021 Blaj Muscat Ottonel grape skin the glucosides of quercetin (4.04 mg/g) and isorhamnetin (3.37 mg/g) were identified in the highest concentrations. These compounds were not found in the seeds. In grape berries, flavonols accumulate in the skin and offer protection from solar radiation, in particular UV-B by filtering the harmful wavelengths for DNA (Kropek et al., 2023).

Also, in the skin, the amounts of procyanidin dimer B_3 (1.78 mg/g), procyanidin dimer B_1 (1.32 mg/g), chlorogenic acid (1.25 mg/g) and catechin (1.23 mg/g) followed those of the flavonol glucosides (Table 1). In seeds catechin (8.06 mg/g), epicatechin (8.48 mg/g), procyanidin dimer B_4 (6.24 mg/g), procyanidin dimer B_1 (5.05 mg/g), procyanidin dimer B_2 (4.42 mg/g) and ellagic acid (4.35 mg/g) (Table 1) were found in the highest concentrations, demonstrating once more that the grape seeds are rich sources of the powerful antioxidants, catechins and procyanidins (Bunea, 2016; Chedea et al., 2010).

Phenolic composition of wine

The phenolics from the wine originating from the 2021 Blaj Muscat Ottonel grapes were analyzed and they are presented in Table 2. Quantitatively the wine phenolics as determined by LC-MS (Table 2) and related to the dry extract (Table 3) were 4.76 lower than the grape ones (skin+seeds) (Table 1). This result confirms the literature data showing that during the winemaking process, an important amount of phenolic compounds remain in grape pomace and thus not being found in wine (Vosloban and Chedea, 2022).

The polyphenols extracted in wine were mainly represented by flavanols found in 42%, followed by the hydroxycinnamic acids and hydroxybenzoic acids which accounted for approximately 33% and 15% of total phenolic, respectively. The hydroxycinnamates and flavanols are the most important phenolics in white wines, in terms of quantity and potential to participate in redox reactions (Mitić et al., 2010). These compounds are also linked to a wide spectrum of health benefits (Panche et al., 2016). Due to their known pharmacological properties, they are often used as key components in different nutraceutical products. Also, their demonstrated antioxidant, anti-inflammatory, anti-carcinogenic and anti-mutagenic effects make them excellent candidates for cosmetic, pharmaceutical and medicinal applications (Panche et al., 2016).

Stilbenes and flavonols were also identified accounting for approximately 9% and 2%, respectively of the total phenolics as quantified by the HPLC method. The results obtained indicate that the Blaj Muscat Ottonel wine is very rich in flavonols (227 mg/L). Similar results were reported for wines from Assyrtiko that ranged between 83 and 298 mg/L and the ones produced on the island of Kefalonia, Greece -between 14 and 194.75 mg/L (Karagiannis et al., 2000; Makris et al., 2003). The hydroxycinnamic acids (179 mg/L) were in the range of the ones identified in the Hellenic vineyard with concentrations reported between 64 and 197 mg/L, the richest being the ones produced in Asyrtiko and Monemvasia, Santorini and Paros islands (Makris et al., 2003; Psarra et al., 2002). When compared to the Croatian wines, the reported content of phenolic acids concentration (ranging between 113 to 140 mg/L) (Kropek et al., 2023) was lower than the one identified in the Romanian Blaj wine (259 mg/L). The profile of phenolic acids of the Romanian Blaj wine, which had coutaric acid (154 mg/L) as a major compound, was also different than the Croatian ones, with gallic acid as the most representative (ranging between 73 to 90 mg/L) (Kropek et al., 2023). In white wines, the oxidation products of caftaric and coutaric acids contribute to their yellowish-gold color (Mitić et al., 2010).

The stilbenes, that naturally occur in white wine are also represented in the Romanian Blaj wine by resveratrol glucoside, which is considered a good indicator of wine quality as a nutraceutical because of its positive physiological properties (Jeandet et al., 1991; Lamuela-Raventós and Waterhouse, 1993; Mattivi, 1993; McMurtrey et al., 1994; Pezet et al., 1994; Roggero and Archie, 1994; Romero-Pérez et al., 1996b, 1996a; Vrhovšek et al., 1995). According to Barriero-Hurlé et al. (2008) wines with high content in phenols, especially resveratrol have the future potential to be marketed as ”functional wines’’ (Ilak Peršurić et al., 2023).

The results obtained within this study also confirm the results reported in the literature highlighting the diverse phenolic compounds content and composition which is considerably influenced by the grape variety, the technological procedures, the yeast used for the alcoholic fermentation, as well as type and time of maceration process (Clarke et al., 2023; Kropek et al., 2023; Merkyte et al., 2020; Visioli et al., 2020). Only one flavonol was found in the Blaj Muscat Ottonel wine, quercetin-glucoside (10.462 mg/L).

Table 2. Identification and quantification of phenolic compounds (expressed as mg/L and mg/g total dry extract (TDE)) extracted from the 2021 Blaj Muscat Ottonel wine

Peak No.	R_t (min)	UV λ_{max} (nm)	$[M+H]^+$ (m/z)	Phenolic compounds' tentative identification	Subclass	Muscat Ottonel Wine (mg/L)	Muscat Ottonel Wine (mg /g TDE)
1	2.96	270	139	2-Hydroxybenzoic acid	Hydroxybenzoic acid	10.71±0.14	0.24±0.00
2	3.39	265	155	2,3-Dihydroxybenzoic acid	Hydroxybenzoic acid	30.31±0.19	0.69±0.00
3	5.63	280	199	Ethyl gallate	Hydroxybenzoic acid	15.14±0.16	0.34±0.00
4	6.54	290	332	Gallic acid-glucoside	Hydroxybenzoic acid	17.20±0.22	0.39±0.00
5	9.01	330	617	2-S-Glutathionyl caftaric acid	Hydroxycinnamic acid	13.05±0.18	0.29±0.00
6	9.44	322	297	Coutaric acid	Hydroxycinnamic acid	153.58±0.54	3.52±0.01
7	11.27	280	579	Procyanidin dimer B1	Flavanol	158.64±0.77	3.63±0.01
8	11.47	290	199	Syringic acid	Hydroxybenzoic acid	6.11±0.168	0.14±0.00
9	12.08	275	391	*cis*-Resveratrol-glucoside	Stilbene	49.61±0.32	1.13±0.00
10	12.53	280	291	Catechin	Flavanol	68.72±0.64	1.57±0.01
11	13.45	332	181	Caffeic acid	Hydroxycinnamic acid	12.43±0.18	0.28±0.00
12	15.97	360,250	465	Quercetin-glucoside	Flavonol	10.46±0.24	0.239
				Total phenolics		**546.01±4.46**	**12.52±0.10**

In our study, catechin and procyanidin dimer B1 were identified both in grapes and in wine. More than half of the grapes' procyanidin dimer B1 (3.638 mg/g out of 6.379 mg/g) and more than one-tenth (1.7) of grapes' catechin (1.570 mg/g out of 9.298 mg/g) were found in wine. Flavan-3-ols, hydrolysable tannins, proanthocyanidins (Monagas et al., 2003; Rosario Bronze et al., 1997), resveratrol, piceid (Bravo et al., 2006; Vitrac et al., 2002; Wang et al., 2002) are also reported in wine. Moreover, during the winemaking process, hydroxycinnamic and benzoic acids and their esters can be extracted from grapes in wine (Bravo et al., 2006).

General characteristics of Blaj Muscat Ottonel wine

In the climate conditions of Blaj region, with foggy days of late summer and early autumn during ripening, the grapes are harvested at complete maturity to obtain POD white wines with the best sugar-acidity ratio, and thus, having the appropriate characteristic acidity for the Blaj white wines. Moreover, in case of the aromatic wines like Muscat Ottonel, the flavors are preserved also (Călugăr et al., 2020). Muscat Ottonel grapevine cultivar is grown on 5547 ha (5.95% of total vineyard surface) in Romania, primarily in Moldavia (Eastern Romania), Transylvania (Central Romania), and Dobrogea (South-Eastern Romania) (Antoce and Calugaru, 2017; Călugăr et al., 2020).

As the quality is proven by the general characteristics, the following parameters were measured at the maturation stage, for the Blaj Muscat Ottonel wine: alcohol content, total sugars, glucose and fructose, total acidity, volatile acidity, dry extract, non-reducing dry extract, the relative density at 20°C, free and total SO2 (Table 3).

Table 3. General characteristics of the bottled Muscat Ottonel wine produced in 2021

General characteristics	Muscat Ottonel Cultivar
Harvest year	2021
Alcohol (min 8.50-max 15.00 % v / v)***	11.88±0.82
Total acidity (min 3.50- max 14.20 g/L C4H6O6)*	5.54±0.12
Volatile acidity (min 0.08-max 1.08 g/L CH3COOH)*	0.32±0.03
Total sugars (min 12.01-max 45.0 g/L)***	24.61±0.70
Total dry extract (min 21.00 g/L)**	43.60±0.20
Non-reducing dry extract (min 16.00 -max 25.00 g/L)**	19.08±0.57
Glucose + Fructose (min 12.01-max 45.0 g/L)***	24.33±1.00
Total SO2 (max. 200.0 mg/L)***	162.50±6.25
Free SO2 (max. 50.0 mg/L)***	35.00±0.78
Density (min 0.98-max 1.04 g/cm3)*	1.01±0.07

Note: *The maximal and minimal values were reported as published by Er and Atasoy (2016); ** the maximal and minimal values were reported as published by Țârdea (2007); *** the maximal and minimal values were reported as published in Romanian HG512/2016, ("HOTĂRÂRE nr. 512 din 20 iulie 2016 pentru aprobarea Normelor metodologice de aplicare a Legii viei şi vinului în sistemul organizării comune a pieţei vitivinicole nr. 164/2015," n.d.).

The alcohol content of the studied Muscat Ottonel was 11.88 % v / v, which is in line with the values of this parameter determined in other Muscat Ottonel wines from Romania, as follows: 12.49% v / v for Teaca wine (2017) which was produced from Lechința vineyard grapes (Călugăr et al., 2020), 12.40% v / v for the Drăgășani wine (2014) from Drăgășani vineyard grapes (Stoica, 2015) and 11.00% v / v for the Dealu Bujorului wine (2015) from Dealu Bujorului vineyard grapes (Bora et al., 2016). On the other hand, for the Chambave Muscat dry wine, at the end of alcoholic fermentation, the alcohol content was 13.48 v / v in 2006 and 14.95 v/v in 2007 (Lambri et al., 2012). Chambave Muscat is an aromatic white grape cultivar grown in Valle d'Aosta, northwest Italy, a region that typically produces dry wines with strong fruity and floral flavors (Lambri et al., 2012).

Other important parameters of wine quality are the total and volatile acidity. Total acidity is very important for wine stability, it`s lack gives a flat taste to the wine and a weak storage capacity (Bora et al., 2016). The wine must have a minimum total acidity of 3.5 g/L expressed as tartaric acid (46.6 milliequivalent/L) (GD no. 512/20.07.2016 - "HOTĂRÂRE nr. 512 din 20 iulie 2016 pentru aprobarea Normelor metodologice de aplicare a Legii viei şi vinului în sistemul organizării comune a pieţei vitivinicole nr. 164/2015," n.d.).

The total acidity of the studied Blaj Muscat Ottonel was 5.55 g/L tartaric acid (Table 3), which is higher than the values of this parameter determined in other Muscat Ottonel wines from Romania. Total acidity was 4.67 g/L tartaric acid, for Teaca wine (2017) which was produced from Lechinta vineyard grapes (Călugăr et al., 2020), 3.95 g/L tartaric acid for the Drăgășani wine (2014) from Drăgășani vineyard grapes (Stoica, 2015) and 4.40 g/L C4H6O6 for the Dealu Bujorului wine (2015) from Dealu Bujorului vineyard grapes (Bora et al., 2016). By contrary, the Italian Chambave Muscat dry wine, at the end of alcoholic fermentation, had a higher total acidity of 6.5±0.1g/L tartaric acid in 2006 and 7.2±0.4g/L in 2007 tartaric acid (Lambri et al., 2012) when compared with all the Romanian wines presented above.

The volatile acidity is the sum of all the volatile fatty acids mainly acetic acid but also formic, propionic, lactic and butyric acids and it is about one-tenth of the acid in wine (Bhattacharjee, 2016; Ţârdea, 2007). The volatile acidity of the studied Blaj Muscat Ottonel was 0.32 g/L acetic acid (Table 3), which is higher than the value of this parameter determined in other Muscat Ottonel wines from Teaca (2017) (0.21 g/L acetic acid) (Călugăr et al., 2020), and lower than the one of Dealu Bujorului wine (2015) from Dealu Bujorului vineyard grapes (0.54 g/L acetic acid) (Bora et al., 2016). However, none of the wines had volatile acidity over the admitted limit of 1.08 g/L acetic acid (GD no. 512/20.07.2016).

For the total sugars and glucose and fructose, the determined values of 24.60 g/L respectively 24.30 g/L are within the limits of the demi-sweet wines (Table 3) (GD no. 512/20.07.2016).

Blaj Muscat Ottonel wine had a total dry extract of 43.60 g/L (Table 3), a value higher than the one of Teaca (21.47 g/L) (Călugăr et al., 2020) and also than the ones of the Italian Chambave Muscat (16.7 ± 0.5 g/L in 2006 and 17.3 ± 0.3 g /L in 2007) (Lambri et al., 2012). Concerning the concentration of the non-reducing dry extract, this was for the studied wine of 19.00 g/L (Table 3) which was within the limits of the Blaj vineyard's high-quality wines (Sîrbu et al., 2022). This value was lower than the one of the Teaca Muscat Ottonel (20.07 g/L) (Călugăr et al., 2020), of Drăgășani Muscat Ottonel (22.18 g/L) (Stoica, 2015), or Dealu Bujorului Muscat Ottonel (29.00 g/L) (Bora et al., 2016).

To protect the wine from oxidation during the wine-making process sulphur is added. The free SO_2 of wine indicates the active molecular form of SO_2 that contributes to wine protection against oxidation and spoilage. It is difficult to predict the quantity and the rate of SO_2 loss due to aeration or binding (Sîrbu et al., 2022). The free and bound SO_2 (to aldehydes, sugars or pigments) represents the total sulfur dioxide (Sîrbu et al., 2022). Thus, in our case, the wine contained 35 mg/L free SO_2 and 162.50 mg/L total SO_2, values that are in the admitted limits (Table 3). In the literature, Teaca wine is reported with 20.33 mg/L free SO_2 and 129 mg/L total SO_2 (Călugăr et al., 2020) and Dealu Bujorului wine had 60.33 mg/L free SO_2 and 240 total SO_2 (Bora et al., 2016).

Sensorial characteristics of the Blaj Muscat Ottonel wine

As is presented in Table 4 the Blaj Muscat Ottonel wine had an excellent limpidity, a yellow-gold color, and a pleasant, typical aroma of basil. The wine is very pleasant, fruity and balanced having a demi-sweet taste and a medium acidity (Table 4).

Table 4. Sensorial analysis of 2021 Blaj Muscat Ottonel wine

Cultivar	Harvest year	Appearance	Color	Aroma/ Bouquet	Taste	Acidity
Muscat Ottonel	2021	excellent limpidity	Yellow-gold with medium-intensity	Very pleasant, floral, specific basil	Very pleasant, demi-sweet, fruity, balanced	medium

Muscat wines are distinguished by distinctive floral bouquets, which are mainly originating from the grapes (Jesus et al., 2017). The presence of powerful delicate aromas and protein stability for limpidity are two critical characteristics of aromatic white wines (Lambri et al., 2012).

CONCLUSIONS

As the general and sensory qualities of the Blaj Muscat Ottonel wine were kept within the limits of a PDO demi-sweet wine, the presence of resveratrol glucoside, catechin and pro-cyanidin in its content supports the idea of classifying this wine as a potential 'functional wine'. To our knowledge, this is the first study documenting the phenolic composition together with the chemical and sensory characteristics of the Muscat Ottonel wine from the Blaj-Târnave vineyard. There is a growing interest in this subject as winemakers become more aware of the significance of phenolic compounds in white wine. More knowledge of the phenolic levels in wines is anticipated to lead to the production of better-quality wines.

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Conflicts of Interest

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

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