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The Identification and Quantification of Terpenic Compounds from Aromatic and Semi-Aromatic Musts from Moldavia Region, Romania

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Abstract: The aim of this study is to identify and quantify terpenic compounds in musts from the Eastern region of Romania, Moldavia. Musts obtained for three consecutive years from four grape varieties, namely: Busuioaca de Bohotin, Feteasca neagra, Muscat Ottonel and Pinot gris were analyzed. The grapes were harvested from four wine centers from Bohotin, Cotesti, Cotnari and Husi. The monitoring of the terpenic compounds was assessed by using a spectrophotometric method. Results show that the free and bound terpenes had significant quantities in the aromatic varieties and lower quantities in the semi-aromatic ones. The accumulation of terpenes in musts are most likely to depend on the variety of the grapes than their region of provenience. The quantity of bound terpenes determined in musts is greater than that of free terpenes.

Keywords: must; free terpenes; bound terpenes; aroma

Introduction

Aroma is a fundamental element of wine. The compounds that provide this feature are multiple and they belong to different chemical classes (Sandru & Panaitescu, 2019). Literature provides studies regarding the identification and quantification of volatile compounds found in wines or in several stages of the vinification process, with the direct contribution of yeasts or technological parameters (Francis & Newton, 2005, Ferreira, 2010, Vilanova & Sieiro, 2006, Loscos *et al.,* 2009, Genisheva *et al.* 2014). The first aroma compounds are found in grapes, then they pass into the must and then, due to chemical, biological or technological factors, they vanish, increase in concentration or they are transformed and led to the formation of other new compounds (Vaschez-Pateiro *et al.,* 2020, Sefton *et al.,* 1993, Ugliano & Moio, 2008, Sanchez-Palomo *et al*., 2006).

The accumulation of these compounds depends on both ecological and climatic factors and the variety from which must or wine are obtained (Sandru, 2015, Rebenciuc & Tita, 2019). Terpenic compounds,

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that are considered to be flavor precursors, were studied using various methods, like dichloromethane extraction and GC-MS analysis for grape varieties from different geographical areas (Rusjan *et al.*, 2009, Green *et al.*, 2011, Genisheva & Oliveira, 2009, Vilanova *et al.*, 2015, Tominaga *et al.*, 1998, Baumes, 2009).

Rodriguez-Bencomo et al. in 2011 studied white grape varieties (Malvasia and Marmajuelo) from Spain (Canary Islands) that show significant differences between the content of terpenes, precursors of glycosidic flavors and volatile compounds identified in the must compared to those identified and quantified in wine. The main difference in the volatile composition of the wine was that the compounds cis-3-hexen-1-ol, ethyl acetate and γ-nonalactonic compounds had a significantly higher average content in Malvasía wines than in Marmajuelo wines. Of the flavor precursors analyzed, only α-terpineol, linalool oxides, 4-allyl-2,6-dimethoxyphenol, 2-methoxy-4-vinylphenol, 4-hydroxybenzaldehyde, vanillin and benzyl alcohol showed comparable quantities in grapes and wine (Rodriguez-Bencomo *et al.*, 2011).

Studies provided from grapes cultivated in Spain show higher concentrations of free terpenes than bound ones. They provide several flavors to the wine, like sweet, balsamic, floral and fruity. The aroma of the wine is influenced by factors like the chemical composition of the grape skins, the maturation methods and the types of utilized yeasts (Selli *et al.*, 2003, Oliveira *et al.*, 2008, Ugliano & Henschke, 2009, Ferreira & Lopez, 2019, Liu *et al.*, 2017, Vilanova *et al.*, 2017).

The formation of flavor is a complex process that largely depends on the concentration of chemical compounds, which can suppress the effects of odorant substances or potentiate certain chemicals that ultimately give a totally different taste and aroma to the wine (Ilc *et al.*, 2016, Vilanova *et al.*, 2012, Canosa *et al.*, 2012).

Wines have a common basic aromatic structure consisting of ethanol and 27 different aromatic compounds, most of them being by-products of fermentation. The mixture of these products provides the typical aroma of the wine and exerts a buffering effect, with the ability to suppress the effect of many added odorant substances, especially those with fruity characteristics. Different odorant chemicals have the ability to break down such a buffer and therefore transmit a different flavor to the wine. The relationship between the transmitted aroma hue and the aroma of the chemicals is used to define the different roles of aromatic compounds regarding the aroma of the wine. These compounds can be impact compounds, major contributors, net contributors, subtle flavor compounds, flavor enhancers and flavor depressants. They can be individual aromatic chemicals or well-defined mixtures of molecules that share chemical and odor properties (flavor families) (Ferreira, 2010).

Materials and Methods

The must samples used were from different sorts, like Busuioaca de Bohotin (BB), Feteasca neagra (FN), Muscat Ottonel (MO) and Pinot gris (PG), and the grapes were harvested from different cultivars, like Bohotin, Cotesti, Cotnari and Husi. Samples collected for three consecutive years were analyzed.

The used reagents were sodium hydroxide, phosphoric acid, linalool, α terpineol, citronellol, nerol, hotrienol, geraniol, β-D-glucopyranoside, 6-ortho-α-L-ramnopyranosyl-β-D-glucopyranoside, 6-ortho-α-L-apiofuranosyl-β-D-glucopyranoside, 6-ortho-α-L-arabinofurananosyl-β -D- glucopyranosides, and they were purchased from Sigma Aldrich – USA.

The identification and quantification of the terpenic aroma of musts is based on the separation of these chemical compounds by water vapor entrainment and a spectrophotometric determination by using a color reaction in the presence of vanillin. The spectrophotometer used was UV-VIS Cecil 1021, and the wavelength was 608 nm.

The aroma extraction was performed by the distillation of 100 mL of must, that was brought to pH 7,0 with a solution of sodium hydroxide 20%. The collection was performed up to 30 mL of distillate. The distillation was interrupted and 5 mL of phosphoric acid 20% were added and then it was continued until 40 mL of distillate was collected. The first distillate collected contains the free volatile terpenic compounds and the second one, terpenic compounds that are bound as precursors. For each compound, separately, a calibration curve was performed, and the results were calculated by using these curves (Țârdea, 2007).

Statistical Analysis

The results were evaluated with XLSTAT program Addinsoft 2014.5.03 version (Addinsoft Inc., USA), through Pearson correlation evaluation, principal component analysis (PCA) and discriminant analysis (DA). PCA was used for data reduction by identifying components that correlate with each other, thereby helping with the interpretation of DA that was used to discriminate between wines varietal origin.

Results and Discussion

Terpenes are extremely important chemical compounds found in grapes when they are subjected to the vinification process. Terpenes give several aromas to the wine, like sweet, floral, vegetal or pine ones (Lengyel, 2014, Popescu, 2019). In wines, terpenes often emit odors of oregano, resinous plants and rosemary, although the aromas of black peppercorns, roses and lavender can also be attributed to these chemical compounds.

Table 1 presents the Busuioaca de Bohotin terpenic compound variation. The linalool, a free terpene that provides floral aroma to the must was quantified in the Cotnari vineyard between 0.27 mg/L in 2017 and 0.35 mg/L in 2018. The quantity of α terpineol, a compound that provides a floral, lilac aroma to the must was on average 8 to 30% higher than linalool, the most substantial results were obtained in 2016 for the Cotnari vineyard. Nerol and citronellol are compounds that provide floral, rose aromas to the must and were quantified between 0.08 mg/L for the Cotnari vineyard in 2017 and 0.17 mg/L for the Husi vineyard in 2017 and Cotnari vineyard in 2018. Hotrienol was quantified between 0.18 mg/L for the Husi vineyard in 2016 and 0.24 mg/L for the Bohotin vineyard in 2018. Geraniol was determined at low quantities that were between 0.01 mg/L and 0.05 mg/L Low values of free terpenes in this case geraniol were detected in all year’s subject to the study, the values being between 0.01 mg/L determined for the Cotesti vineyard in 2016 and 0.05 mg/L determined for Cotnari vineyard in 2017.

Table 1. The Variation of Free and Bound Terpenic Compounds in Musts from the Busuioaca de Bohotin Variety

Compound of interest (mg/L)	Bohotin			Cotesti			Cotnari			Husi		
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018
Free terpenes												
Linalool	0.35 ±0.02	0.31 ±0.02	0.32 ±0.02	0.28 ±0.02	0.30 ±0.02	0.31 ±0.02	0.29 ±0.02	0.27 ±0.02	0.35 ±0.02	0.37 ±0.02	0.31 ±0.02	0.30 ±0.02
α terpineol	0.43 ±0.02	0.45 ±0.02	0.41 ±0.02	0.33 ±0.02	0.40 ±0.02	0.42 ±0.02	0.37 ±0.02	0.53 ±0.02	0.35 ±0.02	0.49 ±0.02	0.43 ±0.02	0.42 ±0.02
Citronellol	0.12 ±0.01	0.13 ±0.01	0.14 ±0.01	0.10 ±0.01	0.11 ±0.01	0.12 ±0.01	0.09 ±0.01	0.08 ±0.01	0.12 ±0.01	0.09 ±0.01	0.11 ±0.01	0.14 ±0.01
Nerol	0.15 ±0.01	0.12 ±0.01	0.17 ±0.01	0.12 ±0.01	0.15 ±0.01	0.16 ±0.01	0.12 ±0.01	0.14 ±0.01	0.17 ±0.01	0.14 ±0.01	0.17 ±0.01	0.12 ±0.01
Hotrienol	0.21 ±0.01	0.23 ±0.01	0.24 ±0.01	0.19 ±0.01	0.22 ±0.01	0.23 ±0.01	0.19 ±0.01	0.20 ±0.01	0.24 ±0.01	0.18 ±0.01	0.21 ±0.01	0.22 ±0.01
Geraniol	0.04 ±0.01	0.02 ±0.01	0.03 ±0.01	0.01 ±0.01	0.04 ±0.01	0.02 ±0.01	0.03 ±0.01	0.05 ±0.01	0.02 ±0.01	0.04 ±0.01	0.04 ±0.01	0.04 ±0.01
Total	**1.30**	**1.26**	**1.31**	**1.03**	**1.22**	**1.26**	**1.09**	**1.27**	**1.25**	**1.31**	**1.27**	**1.24**
Bound terpenes												
β-D-glucopyranoside	0.43 ±0.02	0.42 ±0.02	0.39 ±0.02	0.45 ±0.02	0.44 ±0.02	0.43 ±0.02	0.47 ±0.02	0.37 ±0.02	0.32 ±0.02	0.41 ±0.02	0.44 ±0.02	0.38 ±0.02
6-orto-α-L-ramnopyranosyl-β-D-glucopyranoside	0.56 ±0.03	0.58 ±0.03	0.59 ±0.03	0.54 ±0.03	0.56 ±0.03	0.59 ±0.03	0.61 ±0.03	0.44 ±0.03	0.62 ±0.03	0.58 ±0.03	0.56 ±0.03	0.59 ±0.03
6-ortho-α-L-apiofuranosyl-β-D-glucopyranoside	2.05 ±0.05	2.17 ±0.05	2.22 ±0.05	2.19 ±0.05	2.01 ±0.05	2.16 ±0.05	2.03 ±0.05	2.11 ±0.05	2.13 ±0.05	2.12 ±0.05	2.18 ±0.05	2.23 ±0.05
6-ortho-α-L-arabinofurananosyl-β -D-glucopyranosides	0.94 ±0.04	1.02 ±0.04	0.56 ±0.04	1.06 ±0.04	0.93 ±0.04	1.01 ±0.04	0.92 ±0.04	1.11 ±0.04	1.12 ±0.04	0.93 ±0.04	1.02 ±0.04	1.00 ±0.04
Total	**4.05**	**4.19**	**3.76**	**4.24**	**3.94**	**4.19**	**4.03**	**4.03**	**4.19**	**4.04**	**4.2**	**4.2**
Total free and bound terpenes	**5.35**	**5.45**	**5.07**	**5.27**	**5.16**	**5.45**	**5.12**	**5.30**	**5.44**	**5.35**	**5.47**	**5.44**

The bound terpenes that were identified in the Busuioaca de Bohotin musts had the highest quantities for 6-ortho-α-L-apiofuranosyl-β-D-glucopyranosides of more than 2 mg/L. β-D-glucopyranosides were quantified between 0.32 mg/L and 0.44 mg/L, depending on the year and origin of the harvest. 6-ortho-α-L-ramnopyranosyl-β-D-glucopyranosides, valuable aroma precursors, showed quantities that ranged for Cotnari vineyard between a minimum of 0.44 mg / L in 2017 and a maximum of 0.61 mg / L in 2016. 6-ortho-α-L-arabinofuranosyl-β-D-glucopyranosides were quantified for Cotnari vineyard between 1.11mg/L in 2017 and 0.9mg/L in 2016. The values of free and bound terpenic compounds vary from one year to another and from one vineyard to another, but for the same variety remains a constant average (Table 1).

Table 2. The Variation of Free and Bound Terpenic Compounds in Musts from the Muscat Ottonel Variety

Compound of interest (mg/L)	Bohotin			Cotești			Cotnari			Huși		
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018
Free terpenes												
Linalool	0.22 ±0.02	0.21 ±0.02	0.20 ±0.02	0.28 ±0.02	0.20 ±0.02	0.23 ±0.02	0.29 ±0.02	0.27 ±0.02	0.22 ±0.02	0.27 ±0.02	0.21 ±0.02	0.24 ±0.02
α terpineol	0.35 ±0.02	0.32 ±0.02	0.30 ±0.02	0.22 ±0.02	0.30 ±0.02	0.34 ±0.02	0.27 ±0.02	0.28 ±0.02	0.23 ±0.02	0.39 ±0.02	0.35 ±0.02	0.32 ±0.02
Citronellol	0.10 ±0.01	0.12 ±0.01	0.13 ±0.01	0.10 ±0.01	0.11 ±0.01	0.17 ±0.01	0.09 ±0.01	0.08 ±0.01	0.12 ±0.01	0.09 ±0.01	0.11 ±0.01	0.13 ±0.01
Nerol	0.15 ±0.01	0.11 ±0.01	0.17 ±0.01	0.12 ±0.01	0.12 ±0.01	0.16 ±0.01	0.17 ±0.01	0.13 ±0.01	0.15 ±0.01	0.13 ±0.01	0.17 ±0.01	0.14 ±0.01
Hotrienol	0.11 ±0.01	0.25 ±0.01	0.23 ±0.01	0.19 ±0.01	0.21 ±0.01	0.22 ±0.01	0.19 ±0.01	0.20 ±0.01	0.13 ±0.01	0.18 ±0.01	0.21 ±0.01	0.24 ±0.01
Garaniol	0.029 ±0.01	0.021 ±0.01	0.025 ±0.01	0.019 ±0.01	0.031 ±0.01	0.025 ±0.01	0.019 ±0.01	0.028 ±0.01	0.032 ±0.01	0.031 ±0.01	0.032 ±0.01	0.027 ±0.01
Total	**0.957**	**1.011**	**1.155**	**0.919**	**0.971**	**1.085**	**1.029**	**0.988**	**0.882**	**1.091**	**1.082**	**1.097**
Bound terpenes												
β-D-glucopyranoside	0.32 ±0.02	0.17 ±0.02	0.29 ±0.02	0.32 ±0.02	0.33 ±0.02	0.21 ±0.02	0.37 ±0.02	0.27 ±0.02	0.22 ±0.02	0.31 ±0.02	0.33 ±0.02	0.28 ±0.02
6-orto-α-L-ramnopyranosyl-β-D- glucopyranoside	0.26 ±0.02	0.28 ±0.02	0.29 ±0.02	0.23 ±0.02	0.26 ±0.02	0.29 ±0.02	0.61 ±0.02	0.33 ±0.02	0.62 ±0.02	0.28 ±0.02	0.26 ±0.02	0.29 ±0.02
6-ortho-α-L-apiofuranosyl-β-D-glucopyranoside	2.22 ±0.02	2.17 ±0.02	2.22 ±0.02	2.19 ±0.02	2.01 ±0.02	2.16 ±0.02	2.02 ±0.02	2.11 ±0.02	2.12 ±0.02	2.12 ±0.02	2.18 ±0.02	2.24 ±0.02
6-ortho-α-L-arabinofuranosyl -β -D-glucopyranosides	0.93 ±0.03	1.02 ±0.03	0.96 ±0.03	1.06 ±0.03	0.92 ±0.03	1.01 ±0.03	0.92 ±0.03	1.11 ±0.03	1.12 ±0.03	0.92 ±0.03	1.02 ±0.03	1.00 ±0.03
Total	**3.73**	**3.64**	**3.76**	**3.8**	**3.52**	**3.67**	**3.92**	**3.82**	**4.08**	**3.63**	**3.79**	**3.79**
Total free and bound terpenes	**4.68**	**4.65**	**4.915**	**4.719**	**4.491**	**4.755**	**4.949**	**4.808**	**4.962**	**4.721**	**4.872**	**4.887**

Musts from the Muscat Ottonel variety have relatively large amounts of both free and bound terpenes. Thus, linalool was quantified between 0.20 mg/L for Bohotin and Cotesti vineyards and 0.29 mg/L for Cotnari vineyard. α terpineol showed increased values, between 1.5% and 45% compared to linalool. The most significant values were detected for the Husi vineyard in 2016 where the values reached 0.39

mg/L. Citronellol and nerol had amounts around 0.1 mg/L, and hotrienol on average 24% -50% higher, or showed close values. Geraniol was found at values that did not exceed 0.031-0.032 mg/L, and the minimums was at 0.019 mg/L for Cotesti and Cotnari vineyards in 2016 (Table 2).

The bound terpenes had an ascending trend, maximum in the case of 6-ortho-α-L-apiofuranosyl-β-D-glucopyranosides, where the values reached 2.24 mg/L for Husi vineyard in 2018. Minimum values were obtained for Cotesti vineyard in 2017 of 2.01 mg/L. β-D-glucopyranosides and 6-ortho-α-L-ramnopyranosyl-β-D-glucopyranosides were quantified between 0.21 mg/L and 0.62 mg/L, the maximum values being specific to the years 2016 and 2018. 6-ortho-α-L-arabinofurananosyl-β-D-glucopyranosides were quantified between 0.92 mg/L for Cotesti and Husi vineyards and 1.12 mg/L for Cotnari vineyard in 2018 (Table 2).

The Feteasca neagra variety showed moderate values of free and bound terpenes, as shown in table 3. Linalool was quantified between 0.05 mg/L for the Bohotin vineyard in 2018 and 0.19 mg/L for the Cotnari vineyard in 2016. The quantities of α terpineol start from 0.08 mg/L for musts from Husi vineyard in 2017 and reach 0.33 mg/L for Bohotin vineyard in 2018. The quantities of free terpenes evaluated were situated in a very wide range, the mechanism of accumulation of these compounds is not yet fully understood. As far as citronellol, hotrienol, nerol and geraniol are concerned, these elements were quantified between 0.01 mg/L and 0.19 mg/L. Geraniol remained at minimum values of 0.01 mg/L (Table 3)

Table 3. The Variation of Free and Bound Terpenic Compounds in Musts from the Feteasca Neagra Variety

Compound of interest (mg/L)	Bohotin			Cotești			Cotnari			Huși		
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018
Free terpenes												
Linalool	0.07 ±0.01	0.08 ±0.01	0.05 ±0.01	0.18 ±0.01	0.10 ±0.01	0.08 ±0.01	0.19 ±0.01	0.17 ±0.01	0.08 ±0.01	0.17 ±0.01	0.08 ±0.01	0.10 ±0.01
α terpineol	0.24 ±0.01	0.31 ±0.01	0.33 ±0.01	0.18 ±0.01	0.30 ±0.01	0.31 ±0.01	0.17 ±0.01	0.19 ±0.01	0.08 ±0.01	0.39 ±0.01	0.31 ±0.01	0.31 ±0.01
Citronellol	0.02 ±0.01	0.03 ±0.01	0.1 ±0.01	0.10 ±0.01	0.08 ±0.01	0.05 ±0.01	0.09 ±0.01	0.08 ±0.01	0.07 ±0.01	0.06 ±0.01	0.08 ±0.01	0.13 ±0.01
Nerol	0.03 ±0.01	0.01 ±0.01	0.17 ±0.01	0.08 ±0.01	0.09 ±0.01	0.16 ±0.01	0.08 ±0.01	0.13 ±0.01	0.17 ±0.01	0.13 ±0.01	0.17 ±0.01	0.1 ±0.01
Hotrienol	0.03 ±0.01	0.04 ±0.01	0.03 ±0.01	0.09 ±0.01	0.07 ±0.01	0.08 ±0.01	0.11 ±0.01	0.10 ±0.01	0.13 ±0.01	0.12 ±0.01	0.08 ±0.01	0.05 ±0.01
Geraniol	0.03 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.03 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.03 ±0.01	0.03 ±0.01	0.03 ±0.01
Total	**0.42**	**0.48**	**0.69**	**0.64**	**0.67**	**0.69**	**0.65**	**0.68**	**0.54**	**0.9**	**0.75**	**0.72**

Bound terpenes												
β-D-glucopyranoside	0.33 ±0.01	0.31 ±0.01	0.19 ±0.01	0.32 ±0.01	0.33 ±0.01	0.34 ±0.01	0.37 ±0.01	0.17 ±0.01	0.28 ±0.01	0.31 ±0.01	0.33 ±0.01	0.18 ±0.01
6-orto-α-L-ramnopyranosyl-β-D-glucopyranoside	0.16 ±0.01	0.18 ±0.01	0.19 ±0.01	0.13 ±0.01	0.16 ±0.01	0.19 ±0.01	0.61 ±0.01	0.13 ±0.01	0.11 ±0.01	0.18 ±0.01	0.16 ±0.01	0.19 ±0.01
6-ortho-α-L-apiofuranosyl-β-D-glucopyranoside	1.08 ±0.01	1.17 ±0.01	1.08 ±0.01	1.19 ±0.01	1.01 ±0.01	1.16 ±0.01	1.01 ±0.01	1.24 ±0.01	1.08 ±0.01	1.19 ±0.01	1.18 ±0.01	1.05 ±0.01
6-ortho-α-L-arabinofurananosyl-β -D-glucopyranosides	0.93 ±0.03	1.01 ±0.03	0.96 ±0.03	1.06 ±0.03	0.91 ±0.03	1.01 ±0.03	0.91 ±0.03	1.08 ±0.03	1.08 ±0.03	0.91 ±0.03	1.01 ±0.03	1.00 ±0.03
Total	**2.5**	**2.67**	**2.42**	**2.7**	**2.41**	**2.7**	**2.9**	**2.62**	**2.55**	**2.59**	**2.68**	**2.42**
Total free and bound terpenes	**2.92**	**3.15**	**3.11**	**3.34**	**3.08**	**3.39**	**3.55**	**3.30**	**3.09**	**3.49**	**3.43**	**3.14**

The quantities of bound terpenes decrease compared to the previous analyzed varieties because the Feteasca neagra variety wines are classified as being semi-aromatic ones. 6-ortho-α-L-apiofuranosyl-β-D-glucopyranosides decreased by half compared to the aromatic varieties analyzed, and the quantity of 6-ortho-α-L-arabinofurananosyl-β-D-glucopyranosides was close to the quantity analyzed for the aromatic varieties. The difference between the two compounds was up to 10%. In contrast, β-D-glucopyranosides reached quantities up to 0.37 mg/L and 6-ortho-α-L-ramnopyranosyl-β-D-glucopyranosides up to 0.61 mg/L exceptionally for Husi vineyard in 2016 (Table 3).

Musts from white varieties such as Pinot gris stand out for their modest quantities of free and bound terpenes. Table 4 shows that these terpenes do not exceed quantities of 0.31 mg/L of α terpineol, and the minimum quantity determined for these compounds was 0.031 mg/L. Substantial differences were observed in all areas studied, and the average was approximately 0.3 mg/L. The quantity of linalool was between 0.03 mg/L for the Husi vineyard in 2018 and 0.08 mg/L for the Bohotin vineyard in 2017, Cotesti and Cotnari in 2018 and Husi in 2017. Citronellol, nerol and hotrienol are distinguished by quantities that were between 0.021 mg/L and 0.08 mg/L. Geraniol hardly exceeds 0.03 mg/L, the maximum quantity determined was determined for musts from the Husi vineyard in 2018.

The quantities of glycoside precursors, in particular 6-ortho-α-L-apiofuranosyl-β-D-glucopyranosides and 6-ortho-α-L-arabinofurananosyl-β-D-glucopyranosides were between 0.91 mg/L and 1.24 mg/L. The lowest quantities were reported in the case of 6-ortho-α-L-rhamnopyranosyl-β-D-glucopyranosides which were between 0.031 mg/L and 0.61 mg/L in the musts from Cotnari in 2016. β-D-glucopyranosides showed quantities close to those obtained for the Feteasca neagra variety, these being approximately 0.3 mg/L (Table 4).

Table 4. The Variation of Free and Bound Terpenic Compounds in Musts from the Pinot Gris Variety

Compound of interest (mg/L)	Bohotin			Cotești			Cotnari			Huși		
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018
Free terpenes												
Linalool	0.07 ±0.01	0.08 ±0.01	0.05 ±0.01	0.038 ±0.01	0.030 ±0.01	0.08 ±0.01	0.039 ±0.01	0.037 ±0.01	0.08 ±0.01	0.037 ±0.01	0.08 ±0.01	0.030 ±0.01
α terpineol	0.24 ±0.01	0.31 ±0.01	0.33 ±0.01	0.032 ±0.01	0.30 ±0.01	0.31 ±0.01	0.037 ±0.01	0.039 ±0.01	0.08 ±0.01	0.39 ±0.01	0.31 ±0.01	0.31 ±0.01
Citronellol	0.021 ±0.01	0.035 ±0.01	0.031 ±0.01	0.034 ±0.01	0.08 ±0.01	0.05 ±0.01	0.09 ±0.01	0.08 ±0.01	0.07 ±0.01	0.06 ±0.01	0.08 ±0.01	0.033 ±0.01
Nerol	0.03 ±0.01	0.01 ±0.01	0.037 ±0.01	0.08 ±0.01	0.09 ±0.01	0.036 ±0.01	0.077 ±0.01	0.033 ±0.01	0.035 ±0.01	0.033 ±0.01	0.031 ±0.01	0.03 ±0.01
Hotrienol	0.03 ±0.01	0.04 ±0.01	0.03 ±0.01	0.09 ±0.01	0.07 ±0.01	0.08 ±0.01	0.031 ±0.01	0.030 ±0.01	0.037 ±0.01	0.032 ±0.01	0.08 ±0.01	0.05 ±0.01
Geraniol	0.034 ±0.01	0.018 ±0.01	0.009 ±0.01	0.007 ±0.01	0.03 ±0.01	0.008 ±0.01	0.015 ±0.01	0.005 ±0.01	0.014 ±0.01	0.032 ±0.01	0.036 ±0.01	0.039 ±0.01
Total	**0.425**	**0.493**	**0.487**	**0.281**	**0.6**	**0.564**	**0.289**	**0.224**	**0.316**	**0.584**	**0.617**	**0.492**
Bound terpenes												
β-D-glucopyranoside	0.37 ±0.01	0.35 ±0.01	0.31 ±0.01	0.39 ±0.01	0.43 ±0.01	0.41 ±0.01	0.37 ±0.01	0.45 ±0.01	0.28 ±0.01	0.31 ±0.01	0.33 ±0.01	0.28 ±0.01
6-orto-α-L-ramnopyranosyl-β-D- glucopyranoside	0.036 ±0.01	0.038 ±0.01	0.039 ±0.01	0.033 ±0.01	0.036 ±0.01	0.039 ±0.01	0.61 ±0.01	0.033 ±0.01	0.031 ±0.01	0.038 ±0.01	0.031 ±0.01	0.035 ±0.01
6-ortho-α-L-apiofuranosyl-β-D-glucopyranoside	1.08 ±0.01	1.17 ±0.01	1.08 ±0.01	1.29 ±0.01	1.01 ±0.01	1.22 ±0.01	1.01 ±0.01	1.24 ±0.01	1.08 ±0.01	1.11 ±0.01	1.18 ±0.01	1.05 ±0.01
6-ortho-α-L-arabinofurananosyl -β -D-glucopyranosides	0.93 ±0.03	1.01 ±0.03	0.736 ±0.03	1.06 ±0.03	0.91 ±0.03	1.13 ±0.03	0.91 ±0.03	1.11 ±0.03	1.17 ±0.03	0.91 ±0.03	1.18 ±0.03	1.00 ±0.03
Total	**2.416**	**2.568**	**2.165**	**2.773**	**2.386**	**2.799**	**2.9**	**2.833**	**2.561**	**2.368**	**2.721**	**2.365**
Total free and bound terpenes	**2.841**	**3.061**	**2.652**	**3.054**	**2.986**	**3.363**	**3.189**	**3.057**	**2.877**	**2.952**	**3.338**	**2.857**

Figures 1-4 show the variation of the total terpenic compounds quantified in musts from the Busuioaca de Bohotin, Muscat Ottonel, Pinot gris and Feteasca negara varieties analyzed.

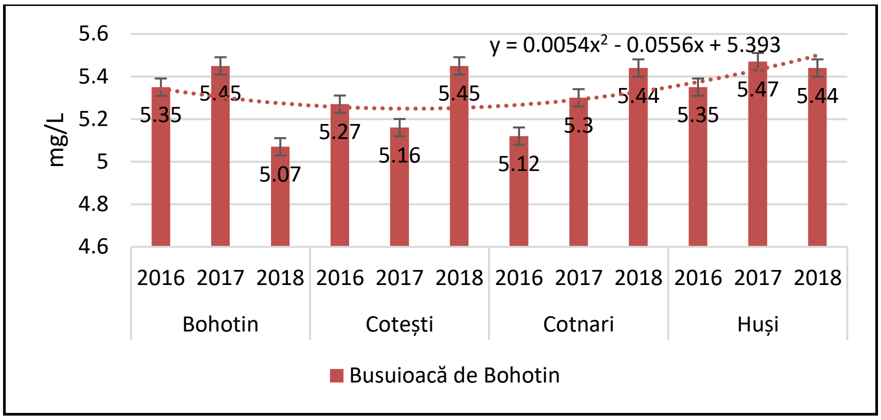


Figure 1. Total Free and Bound Terpenic Compounds in Musts from the Busuioaca de Bohotin Variety

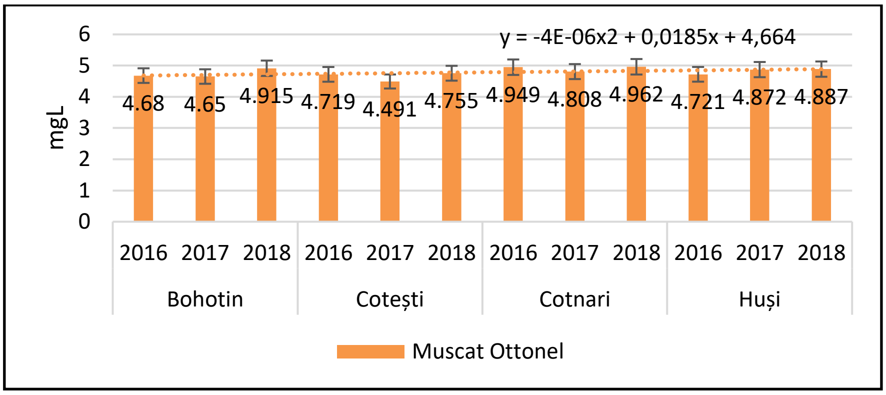


Figure 2. Total Free and Bound Terpenic Compounds in Musts from the Muscat Ottonel Variety

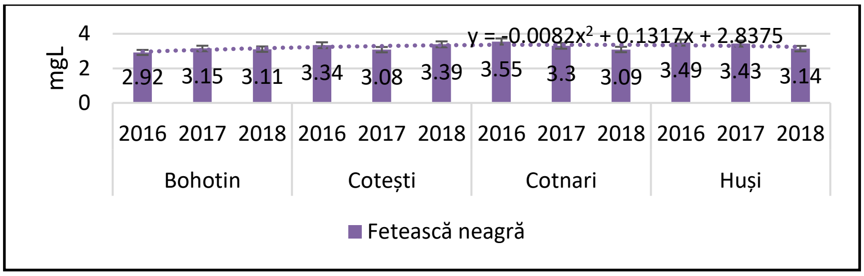


Figure 3. Total Free and Bound Terpenic Compounds in Musts from the Feteasca Neagra Variety

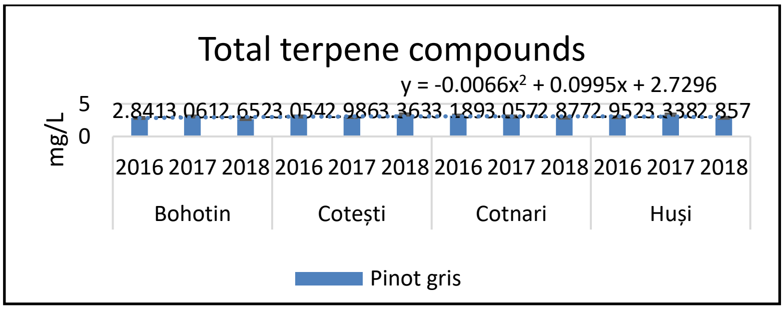


Figure 4. Total Free and Bound Terpenic Compounds in Musts from the Pinos Gris Variety

The highest quantities of terpenic compounds were found in the musts from the aromatic varieties, Busuioaca de Bohotin variety, followed by the Muscat Ottonel one. The semi-aromatic varieties, Feteasca neagra and Pinot gris had a large quantity of free and bound terpenic compounds, that are considered to be important for the formation of the aromatic pallet (Figures 1-4).

One preliminary way, approached in order to study the terpene data, was to explore the natural groupings among the samples. PCA was used to perform a preliminary data scan and to uncover the structure residing in the matrix formed by the total terpene compounds corresponding to all of the different wine samples. The PCA analysis allowed establishing a relationship between the different terpene compounds and the studied grapevines, as well as identifying the most important compounds in Busuioaca de Bohotin, Muscat Ottonel, Feteasca Neagra and Pinot gris varieties, while the correlations between different accessions of the same variety were evaluated using Pearson correlation analysis (figure 5). The maximum number of PCs was set at six, however, only the first three components presented an eigenvalue greater than 1 and explained 81.2% of the data variance.

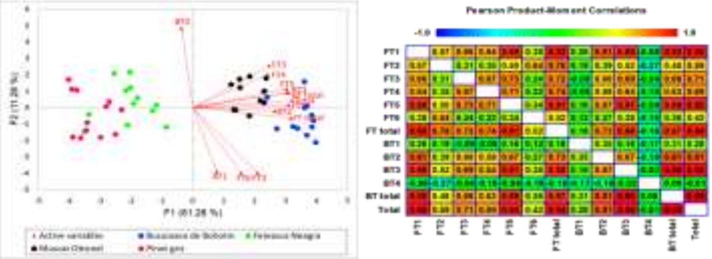
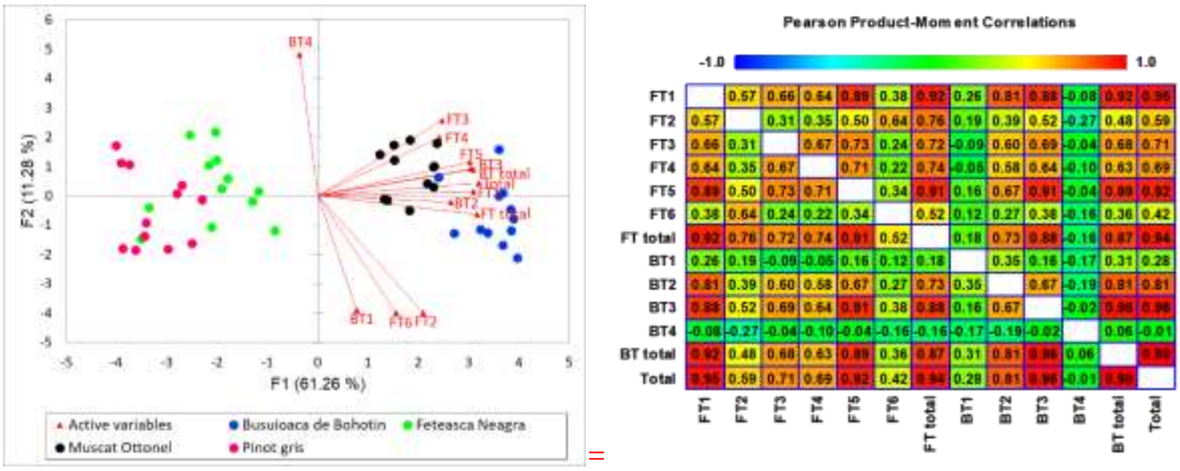


Figure 5. (A) Graphic representation of the two principal components for Busuioaca de Bohotin, Muscat Ottonel, Feteasca neagra and Pinot gris wines formed during 2016 and 2018, originating from Bohotin, Cotesti, Cotnari and Iasi vineyards; (B) Pearson correlation coefficients (r) between terpene compounds in the studied wines (Linalool – FT1; α terpineol – FT2; Citronellol – FT3; Nerol – FT4; Hotrienol – FT5; Geraniol – FT6; Total free terpenes – FT total; β-D-glucopyranoside – BT1; 6-orto-α-L-ramnopyranosyl-β-D- glucopyranoside – BT2; 6-ortho-α-L-apiofuranosyl-β-D-glucopyranoside – BT3; 6-ortho-α-L-arabinofurananosyl-β -D- glucopyranosides – BT4; Total Bound terpenes – BT5; Total free and bound terpenes – Total)



	FT1	FT2	FT3	FT4	FT5	FT6	FT total	BT1	BT2	BT3	BT4	BT total	Total
FT1		0.57	0.66	0.64	0.89	0.38	0.92	0.26	0.81	0.88	-0.08	0.92	0.95
FT2	0.57		0.31	0.35	0.50	0.64	0.76	0.19	0.39	0.52	-0.27	0.48	0.59
FT3	0.66	0.31		0.67	0.73	0.24	0.72	-0.09	0.60	0.69	-0.04	0.68	0.71
FT4	0.64	0.35	0.67		0.71	0.22	0.74	-0.05	0.58	0.64	-0.10	0.63	0.69
FT5	0.89	0.50	0.73	0.71		0.34	0.91	0.16	0.67	0.91	-0.04	0.89	0.92
FT6	0.38	0.64	0.24	0.22	0.34		0.52	0.12	0.27	0.38	-0.16	0.36	0.42
FT total	0.92	0.76	0.72	0.74	0.91	0.52		0.18	0.73	0.88	-0.16	0.87	0.94
BT1	0.26	0.19	-0.09	-0.05	0.16	0.12	0.18		0.35	0.16	-0.17	0.31	0.28
BT2	0.81	0.39	0.60	0.58	0.67	0.27	0.73	0.35		0.67	-0.19	0.81	0.81
BT3	0.88	0.52	0.69	0.64	0.91	0.38	0.88	0.16	0.67		-0.02	0.96	0.96
BT4	-0.08	-0.27	-0.04	-0.10	-0.04	-0.16	-0.16	-0.17	-0.19	-0.02		0.06	-0.01
BT total	0.92	0.48	0.68	0.63	0.89	0.36	0.87	0.31	0.81	0.96	0.06		0.98
Total	0.95	0.59	0.71	0.69	0.92	0.42	0.94	0.28	0.81	0.96	-0.01	0.98	

=

The score plot showed that almost all Pinot gris wine samples were clustered in the left lower quarter as well as Feteasca neagra wine samples situated in the left side. Considering the variables contribution, it is clear that the two varieties are characterized by a lower terpene concentration, but at the same time Feteasca Neagra has a more abundant aromatic profile than Pinot gris. Busuioaca de Bohotin and Muscat Ottonel wines varieties were clustered in the right quarter of the score plot, and distinguish from the other two by a higher total concentration of terpene compound. The PCA loadings showed that linalool – FT1, citronellol – FT3, nerol – FT4, hotrienol – FT5, 6-orto-α-L-ramnopyranosyl-β-D-glucopyranoside – BT2 and 6-ortho-α-L-apiofuranosyl-β-D-glucopyranoside – BT3 had the highest role in the formation of PC1. The Pearson correlation studied between the groups of aromas showed that FT3, FT4, FT5, BT2 and BT3 presented strong positive correlations. PC 2 was positively correlated with α terpineol – FT2, geraniol – FT6, β-D-glucopyranoside – BT1 and 6-ortho-α-L-arabinofurananosyl-β -D- glucopyranosides – BT4. PCA is known to be one of the most versatile of all chemometric methods that involves a mathematical procedure that minimize data dimensionality and enables hidden trends detection. Even though the results obtained from the PCA revealed obvious clustering of the samples according to their varietal origin, which indicated differences in the wine samples composition, for a more accurate differentiation between wine samples we applied discriminant analysis.

The forward step-wise method resulted in 3 components being most significant in botanical discrimination of wines assigning them to the 4 varieties (figure 6). Variables by order of entering in the stepwise DA are BT3, Total, FT1, FT total, FT5, BT2, FT4, FT3, BT1, FT2, FT6 and BT4. Each component and potential aromatic profile influence was discussed in detail above. For optimal separation of varieties, DA combined the 13 variables in 3 linear functions with standardized coefficients for each component listed. These are similar as factor loadings in PCA and can thus be used to interpret the correlations of variables with the function. For instance, Function 1 (F1) is predominant in FT1, FT3 and FT5, Function 2 (F2) in BT1 and BT2, and Function 3 (F3) in FT3 and FT4. Positive vs. negative loadings have no physical meaning unless inspected in combination with the coefficients given to each function.

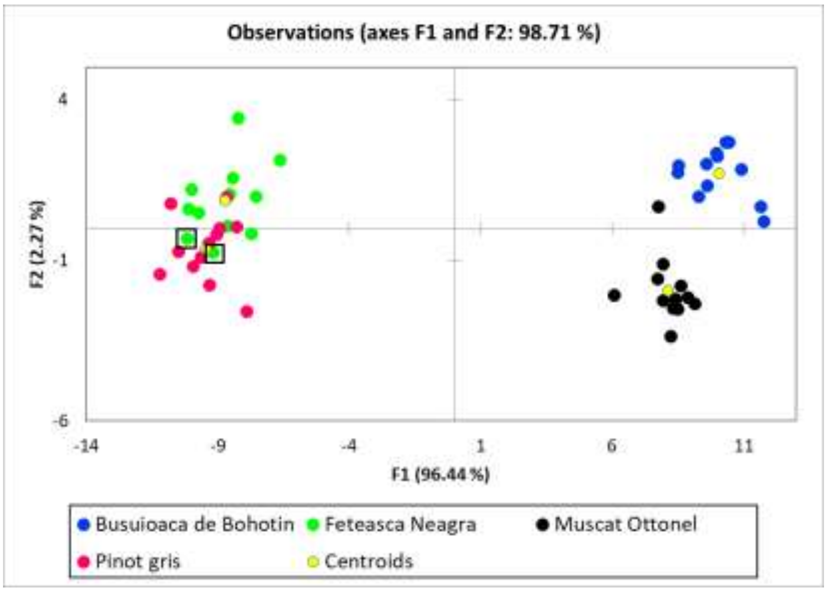


Figure 6. Discriminant Analysis Biplots Illustrating the Pattern of Terpenoids for Classifying Wines According to their Varietal Origin

Classification results of the model show that overall, 98.7% of samples were correctly assigned to their respective variety. Within each variety, classification was 100%, 83.33%, 100% and 100%, for Busuioaca de Bohotin, Feteasca Neagra, Muscat Ottonel and Pinot gris wines, respectively. Two misclassified cases consisted from 2 samples from Feteasca Neagra. In Fig. 6, all 48 wine samples are plotted using the first two functions from DA. Wine variety is color-coded and the 2 misclassified cases are encased. Examination of the plot together with the coordinates of the group centroids provides an indication of which functions are best at separating the various botanical origin. For instance, F1, with the high absolute loading of BT3, FT5 and FT1, has good discriminating power for wines relative to all origin. Analogously, F2 is best at discriminating between Busuioaca de Bohotin and Muscat Ottonel and all other varieties. F2 is associated with a positive coefficient for BT1 and BT2. The terpene compounds synthesis is generally determined by environmental and viticultural factors, but most important, the genetic profile of the cultivars has its unique fingerprint as observed in this study based on 4 wines varieties originating from 4 close but different regions and produced during 2016-2018. Since microclimate within a vineyard can affect the expression of certain genes and the final terpene profiles of grapes, by applying discriminant analysis, was obtained a separation between two white varieties. Therefore, through multivariate statistical analysis we were able to analyze the differences in terpene profiles of four varieties over three years. Although the development patterns of individual terpenoids varied significantly during the three harvest years, it was observed that the patterns of total monoterpene and individual terpenoids remained consistent to each grape cultivar.

Conclusions

The study shows that free and bound terpenes have significant values in the case of aromatic varieties and lower by about 50% in semi-aromatic ones. The values obtained do not show a strictly mathematical increase or decrease, for the same year were identified different accumulations of terpenes, even if the study areas are close. The amount of identified free and bound terpenes is related to the grape variety of the must rather than to the area of origin of the grapes. The quantity of bound terpenes analyzed was 3-5 times higher than the one of free terpenes.

Based on the multivariate analysis, it can be concluded that although free and bound terpene profiles of different grapes were not always well differentiated (as for Feteasca Neagra variety), significant changes could be observed and this approach enabled the identification of terpene evolution patterns for some grape varieties. The study revealed that although environmental variables may modify some individual terpene, the patterns of total profile is strongly linked with its genetic roots and remain unchanged for a grape cultivar.

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