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DETERMINATION OF THE AROMATIC PROFILE IN VARIETAL WINES FROM GRAPE VARIETIES (*VITIS VINIFERA*) GROWN IN THE DRĂGĂȘANI VINEYARD

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Abstract

The aromatic profiles of eight white wines from Romanian and international grape varieties were analysed to characterize and also differentiate them. All wines come from the Drăgășani vineyard. Monoterpenes, higher alcohols, fatty acids ethyl esters, acetates were determined by gas chromatography-mass spectrometry (GC-MS). Alcohols were the most abundant class for Sauvignon, Muscat Ottonel varieties, esters for Italian Riesling, Fetească regală varieties. Terpenes (linalool, terpineol, nerol) were identified only in aromatic and semi-aromatic wines - Tâmâioasă românească, Muscat Ottonel and Sauvignon, Fetească regală. According to odor activity values (OAVs), not all of the determined compounds had concentrations higher than their odor thresholds.

Key words*: wine; aroma profile; volatile compound; Vitis vinifera, GC/MS.*

INTRODUCTION

In the expressiveness and quality of a wine, the aroma compounds play an extremely important role. Wine flavour can be varietal, fermentative or from ageing (Selli et al., 2006).

Wine, being the drink with the most complex aromatic profile, contains approximately 800 aroma compounds. The chemical compounds responsible for wine aroma are mainly alcohols, esters, volatile fatty acids, aldehydes and ketones, of which esters are particularly important (Rapp & Mandery, 1986). In addition to these aroma compounds, it must also be mentioned the terpenols, that contribute decisively to the character and typicality of aromatic wines. Many researchers have studied the volatile composition of different grapes and wines (Gunata et al., 1985; Etievant, 1991; Ferreira et al., 2000; Oliveira et al., 2004; Vilanova & Sieiro, 2007; Vilanova et al., 2013). The quantitative content of volatile compounds in wine can be influenced by several factors, such as grape variety, degree of ripening, climate conditions, fermentation conditions and winemaking practices, and last but not least, aging (Gunata et al., 1985; Rapp, 1998; Heroiu, 1998; Bueno et al., 2003; Oliveira et al., 2004; 2006; Stoica, 2003).

The quantitative analysis of wine aroma can be considered as an important asset for the wine industry, because of the need to have a reproducible analysis to differentiate wines of different varietal origins.

Using chromatographic methods, gas chromatography and especially gas chromatography coupled with mass spectrometry leads to the identification and quantification of aromatic compounds in wine. The predominant compounds identified in wine are monoterpenes, norisoprenoids, alcohols, acids, esters, carbonyls, phenylpropanoids, methoxypyrazines and volatile sulphur compounds (Ferreira et al., 2000; Francis & Newton, 2005).

The aromatic profile of Romanian wines has been studied over the years, but only on specific issues, such as determination of volatile compounds in grapes and wines (Țîrdea, 2007; Băducă-Cîmpeanu, 2016), the aroma profile of traditional Romanian and foreign aromatic varieties (Heroiu, 1998; Stoica, 2015), or the influence of wine processing on the flavour profile (Stoica et al., 2015).

The aim of this work was to define the aromatic profile by determining the major collate compounds of young wines produced from Romanian and foreign varieties (Tămâioasă românească, Fetească albă, Fetească regală,

Muscat Ottonel, Sauvignon, Italian Riesling, Chardonnay, Pinot gris) in Drăgășani (Vâlcea) and to identify the most active odour compounds.

MATERIALS AND METHODS

Grape samples

Eight Romanian and international *Vitis vinifera* white grape varieties (Tămâioasă românească, Fetească albă, Fetească regală, Muscat Ottonel, Sauvignon, Italian Riesling, Chardonnay, Pinot gris) from the Drăgășani -Vâlcea vineyard were considered in this study. The different varieties, from the 2021 harvest, were grown under the same conditions in the Drăgășani vineyard.

The Drăgășani vineyard extend on the right bank of the Olt, to the west and north of the town of Drăgăsani and to the south of Râmnicu Vâlcea, occupying the hills of the Piedmont area of the lower Olt and its main affluents, from the territory of Vâlcea and Olt counties.

The vineyard includes areas of major importance in the national viticulture. Located in the great geomorphological unit Getic Piedmont, the Drăgășani vineyard is located at this coordonate 44°39′40″N latitude and 24°15′38″E longitude, totaling 8,000 ha, at an altitude of 182 m. It is part of the wine-climatic zone A3, which includes regions and wine-producing centers mainly of high-class white, aromatic and red wines and secondary white and red wines for current consumption (Teodorescu et al., 2021).

The wines analysed in this study were obtained in the Oenology laboratory, Faculty of Horticulture, Craiova. The wines were produced in 25 L glass vessels. Before fermentation, sulphur dioxide (4 g/hL) and ascorbic acid (5 g/hL) were added to musts. The wines were elaborated using aromatic winemaking practices, which involves a period of maceration on the lees to favour the extraction of terpenes. For this purpose, a 36-hour maceration was applied, in the presence of the enzyme preparation Zymovarietal aroma G, in a dose of 3 g/100 L. After pressing, the fermentation was conducted using a strain of active selected yeasts (LSA) from the Institute Œnologique de Champagne - *Saccharomyces cerevisiae* var. *bayanus*, IOC-18-2007 (20 g/hL) at 18°C. After fermentation, the wines were filtered and transferred into 0.75 L bottles. The bottles were corked and stored at 16°C until analysis.

Reagents

The chemical standards for GC were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and Fluka (Buchs, Switzerland), having an average purity above 99.5%. Synthetic wine was made using calibration solutions. Synthetic wine consists of 12% v/v ethanol, 5 g/L tartaric acid, brought to 3.3 pH with 0.1N NaOH solution. As internal standard was used 2-Octanol. For each compound were prepared 5-points calibration curves for quantification. Regression coefficients of the calibration curves were above 98%

Volatile compound extraction and analysis

Volatile compounds of the wine samples were liquid-liquid extracted and analysed using gas-chromatography coupled with mass spectrometry (GC/ MS). 100 mL of wine sample was extracted 3 times with 10/5/5 mL of dichloromethane, at 600 rpm, for 20 min. The organic extract was dried and then, concentrated to a volume of 1.5 mL. Carbonyl compounds were determined with PFBOA (ortho-2,3,4,5,6-pentafluorobenzyl-ortho-hydroxil-amine) (Țîrdea, 2007).

The GC/MS used was a Varian 450GC/240MS. The column was a 60 m x 0.25 mm CPSil88, with 0.39 mm film thickness. The carrier gas was helium at a flow rate of 0.9 mL/min. 1 µL of sample was injected in the GC. The oven's starting temperature was 50°C, held for 2 min, then raised to 190°C, at a rate of 3°C/min and held at 190°C for 1.33 min. The mass spectrum in full mode electron impact at 70eV were recorded in the m/z range of 30 - 150. The volatile compounds were identified using standard compounds, mass spectra, mass spectra library and retention times (Milo, 2003; Popescu et al., 2014). Analyses were carried out in duplicate.

Odour activity value

The odour activity value (OAV) was determined to evaluate the contribution of a chemical compound to the aroma of a wine. OAV is a measure of the importance of a specific compound to the odour of a sample. If

OAV > 1 then indicates possible contribution to the wine aroma. This was calculated as the ratio between the concentration of the individual volatile compound and the perception threshold found in the speciality literature (Etievant, 1991; Francis & Newton, 2005; Vilanova et al., 2009; Popescu et al., 2014).

RESULTS AND DISCUSSIONS

The results regarding the concentration of volatile compounds from the 8 white wines, semi-aromatic and aromatic, are presented in Table 1. There were identified 20 different compounds: 3 terpenes 4 alcohols, 5 esters, 5 acids, and 3 aldehydes.

Monoterpenes

Terpenes are constituents of grapes that are in a free and bound state and whose biosynthesis begins with acetyl-coA (Yang et al., 2019). The bound form is not volatile and has a glycoside group, such as glucose, arabinose, rhamnose and apiose (Korenika et al., 2018). The grape varieties also contain β-glucosidase which, under normal winemaking conditions, can release the terpene compounds from the bound forms. This process is accentuated if maceration is used in the vinification of grapes. Therefore, besides the nature of the grape variety and the degree of ripening, the winemaking process plays an important role in the final terpene content of the wines. Terpene compounds such as α-terpineol, linalool, geraniol and nerol are volatile and responsible for the floral notes of the semi-aromatic grape varieties (Sauvignon and Fetească) and grape-aromatic varieties (Tămâioasă românească and Muscat Ottonel), but the distinctive note of the aroma is given both by the concentration in which are found as well as the proportions between them (Lukic et al., 2017).

Some monoterpenes are also found in other grape varieties, which are not usually considered aromatic, but in totally insignificant proportions compared to the aromatic varieties (Riesling Italian, Pinot gris and Chardonnay) (Luan et al., 2006; Oliveira et al., 2008).

In this study, the wines obtained from the aromatic varieties of Tămâioasă românească and Muscat Ottonel presented the highest concentrations of terpenes. It should be noted that the proportions of these compounds in the two wines are different. Thus, α-terpineol predominates in Tâmiaoasa românească wine, while in Muscat Ottonel the higher proportion is found in linalool. All three terpene compounds are found in the wines from the semi-aromatic Fetească and Sauvignon varieties, but in much lower concentrations. Also, in all three semi-aromatic wines, α-terpineol predominates quantitatively.

Differences in concentrations of volatile compounds between the samples in this study and the literature values can be justified by the difference between the preparation method, the place of origin for the wine and the harvest year. On the other hand, the qualitative differences regarding the number and type of detected compounds can be explained by the difference between the methods of preparation and analysis (extraction solvent, extraction time and steps, type of chromatographic capillary column, detector, analysis succession etc.) (Popescu et al., 2014).

Alcohols

Alcohols are also produced during the alcoholic fermentation of carbohydrates. With the exception of 2-phenylethanol, which has the aroma of roses (Etievant, 1991), the other alcohols do not have a pleasant contribution to the aroma of wines. 2-phenylethanol is the compound with the highest concentration in all analysed wines. However, from a quantitative point of view, semi-aromatic and aromatic wines stand out (Tămâioasă românească, Muscat Ottonel and Sauvignon). According to research by Quian et al. (2009), this alcohol can also be present in grapes, but mostly it is produced by yeasts during alcoholic fermentation. Isopropanol was identified only in Sauvignon. 1-hexanol was present in almost all the wines, with the exception of Muscat Ottonel in agreement with other researches (Popescu et al., 2014). The composition of alcohols differed both quantitatively and qualitatively between the varieties, Sauvignon having the largest number of alcohols (4), followed by Tămâioasă românească and Riesling Italian (3). All the other varieties have in composition only 2 alcohols. Tămâioasă românească and Muscat Ottonel wines have the highest alcohol concentration, 111 mg/L and 81 mg/l,

respectively. Chardonnay wine, considered neutral, also has high alcohol concentrations.

Esters

Esters are the compounds that have an important contribution to the aroma of young wine. Their origin in wine comes from the metabolism of yeast during alcoholic fermentation, but they are found in small quantities also in grapes (Perestrelo et al., 2006). Acetic esters (ethyl acetate and isoamyl acetate) result from the reaction between acetyl-CoA and higher alcohols. Ethyl esters of fatty acids are produced by enzymes during alcoholic fermentation and from acyl-CoA ethanolysis, being formed during the synthesis or degradation of fatty acids (Zhu et al., 2022). Most esters are formed at the beginning fermentation, their concentration varying to a small extent proportion during wine maturation (Popescu et al., 2014).

Ethyl butyrate, ethyl octanoate and isoamyl acetate were present in each variety. The most abundant ester was probably ethyl acetate formed in small amounts during yeast fermentation and in a larger quantity from the intervention of acetic bacteria, especially during the maceration process and maturation in the barrel, when the wine is still in contact with air.

Ethyl acetate is well perceived between 150-200 mg/L with specific aroma; if it exceeds 200 mg/L influences wine quality (Tarko et al., 2008).

Ethyl acetate from the studied wines, between 4.1 (Fetească wines) and 65 mg/L Riesling Italian wine) favourably influences the aroma, the aromatic variety had an ethyl acetate content within limits, of 18 mg/L in the Tămâioasă românească and 29 mg/L in Sauvignon (semi-aromatic).

In the Tămâioasă românească and Sauvignon varieties, all 5 esters were identified and quantified, analysing the largest number of esters, 6 compounds with total concentrations of 21.8 mg/L and 30 mg/L, respectively.

Table 1. Volatile composition (µg/L) of wines from Romanian and international varieties in Drăgășani vineyard

Compounds	Volatile composition (µg/L) of wines							
	Tămâioasă românească	Fetească albă	Fetească regală	Muscat Ottonel	Sauvignon	Riesling italian	Chardo-nnay	Pinot gris
Monoterpenic alcohols								
Linalool	1010.2	160.8	111.5	2287.8	290.2	18.5	ID/NC	ID/NC
α-Terpineol	3130.0	183.4	107.7	995.3	420.1	47.2	ID/NC	13.70
Nerol	420.1	93.0	90.5	380.1	119.0	1.20	-	ND
Alcohols								
1-Propanol	ID	-	-	-	ID/NC	ID/NC	ID/NC	ID/NC
1-Hexanol	1598.1	980.2	968.3	-	1450.1	972.0	1689.0	267.7
2-Phenyl ethanol	95280.0	99021.1	9804.0	81240.0	35710.0	33200.0	10900.0	9469
Isopropanol	-	-	-	-	50.3	-	-	-
Esters								
Ethyl butyrate	410.3	176.8	167.9	462.5	571.2	291.5	276.1	261.2
Ethyl octanate	720.4	871.0	810.4	2091.0	810.0	2390.0	1451.0	1125.9
Ethyl decanonate	332.3	-	-	291.1	210.0	-	560.2	218.8
Ethyl acetate	18528.1	41991.0	41880.2	-	29710.1	65720.0	-	-
Isoamyl acetate	1890.9	1170.2	1140.7	1941.6	1570.2	2716.8	1510.4	1763.6
Isovalerianic acid	-	-	-	-	1870.6	987.3	2070.1	1200.5
Diethyl acetic acid	1.9	-	-	1.8	-	-	1.9	-
Heptanoic acid	4.1	3.9	3.6	4.4	3.7	4.8	4.2	4.2
Dodecanoic acid	29.8	12.8	12.4	29.3	-	34.0	38.2	21.0
Aldehydes								
Acetaldehyde	39100.0	6820.1	6690.0	42600.0	24890.0	5480.0	-	-
Propion-aldehyde	-	63.4	61.9	-	49.1	48.8	-	-
Hexanal	20.3	20.7	19.8	22.9	21.5	23.5	23.8	-

ID/NC - identified but not quantified

Acids

The formation of acids depends on the composition of must and fermentation conditions (Heroiu, 1998). Isovaleric acid is the most abundant with concentrations between 1.2 mg/L and 2.0 mg/L, although it was not identified in all wines. Heptanoic acid is present in all analysed wine samples. Lactic acid was present only in Sauvignon but could not be quantified. The largest acids concentration is present in Chardonnay (2.1 mg/L), Sauvignon (1.8 mg/L), Pinot Gris (1.2 mg/L) and Riesling italian (1.0 mg/L). The wines of Feteasc ă and the aromatic ones of Tămâioasă and Muscat have the lowest concentration of acids. Acids give the wine freshness and a pleasant aroma at concentrations up to 10 mg/L. At concentrations above 20 mg/L, they have a negative effect on the sensory characteristics of the wine (Heroiu, 1998). The reported values for acids in the studied wines are in very good agreement with those reported in the literature.

Aldehydes

Aldehydes are formed in wine following the metabolism of amino acids and from the enzymatic oxidation of unsaturated fatty acids. The aldehyde content of the wines varied between 0.06 mg/L for Chardonnay and 42 mg/L for Muscat Ottonel.

Among the analysed aldehydes, acetic aldehyde registered the highest values 0.54 mg/L and 42 mg/L. Hexanal was not identified in 4 wines Tămâioasă românească, Muscat Ottonel, Chardonnay and Pinot gris. Aldehyde concentrations from the studied wines are similar to those reported in the specialized literature.

Odour activity value (OAV)

To evaluate the influence of the analysed volatile compounds on the general aromatic profile of wines, the odour activity value (OAV) was calculated by dividing the concentration of each compound by its perception threshold. Only compounds with an OAV greater than 1 individually contribute to wine aroma (Guth, 1997). However, studies by Francis and Newton (2005) showed that when the OAV of a certain compound is less than 1, it can still contribute to the aroma of a wine due to the additive effect of similar compounds (similar structure or smell). The odour descriptor, OAV and threshold for each analysed compound are listed in Table 2.

Table 2. Odour activity values (OAV) in varietal wines

Volatile compounds	Descriptor	Threshold µg/L	OAV							
			T.R.	F.A.	F.R.	M.O.	S.	R.I.	CH.	P.G.
Linalool	Floral, citrus	25.0	40.4	7.33	4.46	91.51	11.60	0.72	-	-
α-Terpineol	Pine, lilac	340	9.20	0.47	0.31	2.92	1.52	0.13	-	-
2-Phenyl ethanol	Rose, perfume	12000	7.94	0.82	0.81	6.77	2.97	2.76	0.90	-
Ethyl butyrate	Pineapple	20.0	20.5	8.79	8.39	23.12	28.56	14.57	13.80	13.06
Ethyl octanate	Pineapple, pear, floral	2.0	360.2	435.5	405.2	1045.5	405.0	1195.0	725.5	562.9
Ethyl decanonate	Grape, pleasant	200	1.66	-	-	1.45	1.05	-	2.8	1.09
Ethyl acetate	Fruity, sweet	75000	0.24	0.55	0.55	-	0.39	0.87	-	-
Isoamyl acetate	Banana	30.0	63.03	39.0	38.0	64.72	52.34	90.56	50.34	58.78
Isovalerianic acid	Fatty, rancid	33.0	-	-	-	-	54.77	29.91	62.73	36.37
Acetaldehyde	Ethereal, fruity	65000	0.60	0.10	0.10	0.65	0.38	0.08	-	-
Propion-aldehyde	Whiskey, nutty	24.0	0.84	0.86	0.82	0.95	0.89	0.97	0.99	-
Hexanal	Fresh, green	4.7	-	13.48	13.17	-	10.44	10.38	-	-
TOTAL										

Descriptors and flavour thresholds of volatile compounds found in the literature (Etievant, 1991; Ferreira et al., 2000; Francis & Newton, 2005; Vilanova et al., 2009)

Nine volatile compounds out of 20 analysed (45%), identified in the wines presented an OAV > 1, contributing to the aroma of the analysed wines. And other authors (López et al., 1999; Escudero et al., 2004; Jiang & Zhang, 2010) showed similar results in other young white and rosé wines. Ethyl-butyrate, isoamyl acetate, ethyl octanoate contributed to the wine aroma of all studied varieties. The floral character was represented by three compounds with OAV > 1. Linalool only contributed to the floral character of the wines Tămâioasă romanească, Fetească albă, Fetească regală, Muscat Ottonel and Sauvignon, α-terpineol in Tămâioasă românească, Muscat Ottonel and Sauvignon wines and 2-phenylethanol seemed to contribute only to the same character in Tămâioasă românească, Italian Riesling and Sauvignon wines, as the concentrations were above the odour threshold.

Ethyl decanoate, ethyl butyrate and isoamyl acetate also made an important contribution to the aroma of all monovarietal wines.

The most intense odorants in Tămâioasă românească and Muscat Ottonel wines produced in Drăgăşani vineyard are linalool, α-terpineol, 2phenyl-etanol, ethyl butyrate, ethyl octanate and isoamyl acetate.

CONCLUSIONS

The formation of volatile compounds in wines is complex process and depends on the chemical composition of the grapes and the maceration and fermentation process.

The main aim of this study was to characterize and differentiate wine varieties according to their volatility composition (flavour profile). It was found that some wines they are more aromatic than others. Tămâioasă românească, Muscat Ottonel and Sauvignon are the most aromatic, with the main floral aroma persisting. The compounds that greatly influence the aroma, in addition to the terpenes, are ethyl octanoate, isovaleric acid and isoamyl acetate. The overall aroma of the wine study is dominated by the maceration and fermentation stage, namely by ethyl esters of fatty acids (ethyl esters of butyric, octanoic and decanoic acids), which give the wine fresh, fruity notes. Also, the aroma profile of three Romanian autochthonous wine varieties (Tămâioasă românească, Fetească albă and Fetească regală) was quantified and classified, recording higher values than other international varieties. The results are preliminary, the interpretation provided being limited by the fact that all wines were produced for a single vintage, with no repetitions for variants.

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