ASSESSMENT OF VOLATILE COMPOUNDS OF SOME RED WINE SAMPLES FROM REPUBLIC OF MOLDOVA AND ROMANIA USING GC-MS ANALYSIS

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Abstract. Five monovarietal wines (Shiraz, Cabernet Sauvignon, Merlot, Rară Neagră from Republic of Moldova and Fetească Neagră from Romania) and two bivarietal wines (Codru and Vin Virgin also from Republic of Moldova) were submitted to a liquid-liquid extraction with dichloromethane and analysis by gas chromatography-mass spectrometry (GC–MS). A total of 23 volatile compounds were identified and quantified such as higher alcohols, esters, fatty acids and lactones. Considering all the volatile compounds detected, higher alcohols and esters are the main contributors to Romanian and Republic of Moldova red wines.

Keywords: red wine, liquid-liquid extraction, GC-MS analysis, Republic of Moldova wines, Romanian wines

INTRODUCTION

The aromatic profile of the wines is one of the main indicators determining its quality. The formation of aromatic components in wine is strongly dependent on the grapevine particularities.

There are many factors influencing the wine aroma: grape variety and its genetic potential to accumulate aromatic components (Jouanneau *et al.*, 2012); climatic conditions and geographical location of the vine growing area (Mendez-Costabel *et al.*, 2014a); the soils (Falcao *et al*., 2008; Bora et al., 2018); agro-technical management (Xie *et al.*, 2016; Mendez-Costabel *et al.*, 2014b); the degree of the grapes maturity (Talaverano *et al.*, 2017); the technological and technical conditions of winemaking (Moroşanu *et al.*, 2016); the applied practices of the grapes processing and wine-making (Antoce, 2012); the metabolism of the yeast cultures used for conduction of the alcoholic fermentation and the lactic acid strains used for the malolactic fermentation (de Revel *et al.*, 1999; Álvarez-Pérez *et al.*, 2014); the wine aging (Dumitriu *et al.*, 2014).

Republic of Moldova holds a rich tradition in winemaking dating back thousands of years. Cricova wineries are part of Chișinău winegrowing area. Low hills, slopes facing north-west, south, south-east and south-west provide a model conditions to grow different grapes varieties. Temperatures in summers are averaging between 22°C and 25°C, but can sometimes reach 35 to 40°C during heat waves. The winter months are moderately cold and dry, with daytime temperatures in January (the coldest month) between -3,2°C and -3,0°C, and minimum often far below -10°C. Annual rainfall ranges from around 500 to 540 mm (Nicolaescu, 2010).

The aim of our work was to characterize the main volatile component of the autochthonous red wine Fetească Neagră grown in Panciu vineyard in Romania and six red

wines from Republic of Moldova, Shiraz, Merlot (Vin Virgin), Codru (Cabernet Sauvignon/Merlot), Rară Neagră, Merlot, Cabernet Sauvignon. Vin Virgin from Cricova is the only Merlot wine in Moldova, which winemaking technique is close to that of an amarone wine, meaning that grapes are harvested a couple of weeks later after the full ripeness. Codru is a blended wine between Cabernet Sauvignon (75%) and Merlot (25%) and can be characterized like an almost perfect balance between body and structure, without losing its character.

In this study, we investigated the volatile compounds of five monovarietial and two bivarietal wines which were extracted by liquid-liquid extraction and detected by GC–MS.

MATERIAL AND METHOD

Grapes were harvested during the 2014 and 2015 season, at commercial harvest, in Cricova (47º08'4.51" N; 28º51'54.34" E) located in Codru wine region of Republic of Moldova and Panciu (45º54'29.19" N; 27º05'25.95" E) located in Vrancea County, Romania. Table 1 shows latitude, longitude and altitude of the areas determined with the global positioning system (GPS) apparatus and climatic conditions from the two studied sites.

Table 1

Climatic conditions and geographic coordinates of *Vitis vinifera* L. grapes from Republic of Moldova and Romania investigated in this study

Location	*Vitis vinifera* L. grape variety	Latitude	Longitude	Altitude (m)	Annual mean temperature (°C)	Mean relative humidity (%)	Annual mean rainfall (mm)
Cricova - Republic of Moldova	Shiraz, Merlot (Vin Virgin), Rară Neagră, Cabernet Sauvignon, Codru (Cabernet Sauvignon/ Merlot), Merlot	47º08'4.51" N	28º51'54.34" E	120-130	9.6	70-72	500-540
Panciu - Romania	Fetească Neagră	45º54'29.19" N	27º05'25.95" E	200-210	10.2	65-75	400-500

Wine samples. The wine samples consisted in seven red wines from Republic of Moldova and Romania: Shiraz, Merlot (Vin Virgin), Codru (Cabernet Sauvignon/Merlot), Rară Neagră, Merlot, Cabernet sauvignon (Republic of Moldova – Cricova Winery) and Fetească Neagră (Romania – Panciu, Vrancea County) were kindly donated by wineries. Rară Neagră, Merlot, Shiraz and Fetească Neagră were from 2015 vintage and Cabernet Sauvignon, Vin Virgin and Codru from 2014 vintage.

All wine samples were fermented in stainless steel fermenters at 24–26°C and the malolactic fermentation was performed ten days after the alcohol fermentation. Wines were aged in the same type of oak barrels provided by the same tonnellerie, for 6 months (Rară Neagră, Shiraz, Merlot and Fetească Neagră) and 12 months (Cabernet Sauvignon, Codru, Vin Virgin). Wines were elaborated and aged in the winery and cellars of Cricova Winery

(situated in Cricova – Republic of Moldova) and Panciu Wine (situated in Panciu – Vrancea County, Romania). All samples were kept at 4°C before the analysis.

The basic composition of the wine was analyzed according to the methods proposed by OIV, 2013 and the results are revealed in Table 2. The ethanol content of the red wine varied between 12,34 % vol. alcohol for Shiraz (Cricova) and 14,75 % vol alcohol for Vin Virgin (Cricova). Total and volatile acidity values of red wine samples were under the maximum allowable concentrations, according to European Union laws specified by OIV.

Table 2

Enological parameters of analyzed red wines

Wine location / Enological parameters	Cricova						Panciu
	Shiraz	Vin Virgin	Rară Neagră	Cabernet Sauvignon	Codru	Merlot	Fetească Neagră
% alcohol content ethanol	12.34	14.75	12.41	13.23	13.38	13.12	13.50
Total acidity (g/l acid tartric)	5.60	3.90	4.60	4.90	6.40	4.80	5.32
Volatile acidity (g/l acid acetic)	0.88	0.58	0.50	0.59	0.85	0.26	0.88
Dry extract (g/l)	29.00	31.90	28.00	28.90	28.40	46.10	24.00
Non-reducing dry extract (g/l)	27.00	29.00	26.00	26.10	26.00	33.00	22.06
Free SO_2 (mg/l)	25.00	22.00	20.00	29.00	39.00	26.00	45.00
Total SO_2 (mg/l)	120.00	93.00	87.00	141.00	193.00	131.00	85.00
Relative density at 20^o	0.9952	0.9960	0.9940	0.9960	0.9943	0.9940	0.9920
Total sugars (g/l)	3.30	3.90	1.50	2.80	2.80	3.00	3.90

In all red wine samples, the free SO_2 and total SO_2 values were less or equal with the specific limit existing for red wines of 35 mL/L, respectively 200 mg/L, proposed by EU (***, EC 2009).

Chemicals and Reagents

1-octanol was used as internal standard (IS), and as reference standards were used 2-phenyl ethanol, ethyl decanoate, butyrolactone and isoamyl alcohol. The standard stock solutions were prepared by dissolving 10 mg of each reference compound in 10 mL of dichloromethane.

The protocol used by Andujar-Ortiz *et al.*, 2009, was adapted and used for isolation of the volatilome from the samples. 50 mL of wine spiked with 1-octanol (IS) was added to 20 mL of dichloromethane in an Erlenmeyer flask equipped with a ground stopper. After separation, the organic layer was dried on Na_2SO_4, evaporated under a nitrogen stream of approximately 200 µL volume of the extract. From this solution, 1 µL was injected into the GC/MS system.

Analysis of wine volatilome was carried out using a Shimadzu QP 2010 PLUS Mass Spectrometer coupled with Gas Chromatograph (Shimadzu) equipped with a Carbowax type column from Agilent, with a dimension of 30 m x 0.32 mm ID and 0.50 µm film thicknesses. The carrier gas was He (6.0) with a flow rate of 1.7 mL/min. The working parameters were: injector temperature 250°C, the ion source temperature of 220°C, and the interface temperature of 250°C. The column temperature program was conducted as follows: 40°C was the initial temperature for 5 min, increasing at a rate of 4°C/min to 220°C, and holding

220°C for 15 min. The electron impact (EI) was set at 70 eV. A mass range of 35-500 m/z was recorded at one scan per second.

RESULTS AND DISCUSSIONS

A total of 23 volatile compounds were identified and quantified in red wine samples, grouped according to their chemical structure in 9 alcohols, 8 esters, 3 fatty acids, 1 lactone and 2 other compounds – furfural and ethyl vanillate (Table 3).

Higher alcohols are formed mainly during the first two stages of the alcoholic fermentation (Ivanova et al., 2013; Gil et al., 2006) and they are synthesized by yeast through the anabolic and catabolic pathway (Perestrelo et al., 2006). Volatile composition of wine depends on the variety of grape (Tao et al., 2008).

The composition of alcohols (9) differed both qualitatively and quantitatively among the wines. The total concentration of the alcohols ranged in large limits from 32677.33 µg/L (Codru wine from Cricova) to 5537.21 µg/L (Shiraz from Cricova), being 16.95 % and 76.23%, respectively, of the total volatile compounds detected (Table 3).

Volatile fraction of higher alcohols was mainly composed of isopentyl alcohol (cheese) and 2-phenyl-ethanol (roses, pollen, flowery). These two alcohols had concentrations >1,000 µg/l and existed in at least one of the studied wines. Isopentyl alcohol was the most abundant alcohol, accounting for > 80% of the total alcohols in all seven wines studied. Other alcohol components in the overall volatile content of the red wines were isobutanol (medicinal, wine-like), 1-hexanol (floral, green, cut grass; herbaceous, fatty; resinous) and 2,3-butanediol (chemical) as the major. In parentheses are presented the aromatic characteristics of volatile compounds. Slightly bitter taste of 2,3-butanediol may affect sensory qualities of wine, but it may contribute to the wine body because of its viscosity and its high content (Romano et al., 1996).

Higher alcohols in concentrations below 300 mg/L, contribute to the desirable complexity of wine, but when their concentrations exceed 400 mg/L, they have a negative quality factor (Rapp, Mandery, 1986).

Esters. An important family of between aroma compounds in wine are the esters. These compounds are formed by esterification of alcohols and acids followed by water molecule elimination (Perestrelo et al., 2006). There were also differences in the type and amount of esters present in the seven wines. In our study, 8 esters were detected in the wines analyzed. The total concentration of esters ranged from 1090.31 µg/L (Shiraz from Cricova) to 8533.47 µg/L (Codru from Cricova), being 15.99% and 19.91%, respectively, of the total volatile compounds detected (Table 3).

The ethyl esters (hexanoic acid ethyl ester, octanoic acid ethyl ester and ethyl lactate) have a positive contribution to quality of wine. The hexanoic acid ethyl ester (green apple; fruity; strawberries; anise note) is a volatile ester produced during fermentation process by yeasts and octanoic acid ethyl ester (pineapple, pear, floral) is an ester specific for the alcoholic beverages.

Ethyl lactate (lactic, medicinal, raspberry, strawberry) is formed mainly during the malolactic fermentation. In red wines the concentration is higher, the large variation between the wines can be explained by the achievement of this malolactic fermentation (Gil et al., 2006). This compound was not detected in Shiraz and Codru wines from Cricova, but had concentrations >1,500 µg/L in the rest of the red wines samples (Table 3).

The ethyl esters are among the key compounds in the fruity flavors and they make a general positive contribution to the general quality of wines. The presences of ethyl-3-hydroxybutanoate enhances the blackberry and fresh-fruit aromas in red wines.

During the malolactic fermentation, diethyl succinate (wine; fruity; caramel) is formed by lactic bacteria activity and the highest amounts of this compound being found in the red wines (Gil et al., 2006). Our results revealed that the diethyl succinate was found in all seven wine samples.

Table 3

Concentration of volatile compounds (µg/L) in the studied samples

Location / Wine / Volatile compounds	Cricova						Panciu
	Shiraz	Vin Virgin	Rară Neagră	Cabernet Sauvignon	Codru	Merlot	Fetească Neagră
Alcohols[a]							
Isobutanol	162.13	432.17	576.31	161.93	838.05	65.90	678.38
Butanol	12.93	27.11	30.61	-	52.15	-	-
Isopentyl alcool	4472.82	12454.17	21325.42	4656.66	22491.47	4556.68	18033.38
1-Hexanol	45.76	97.31	213.43	71.40	369.30	69.70	101.37
2,3-butandiol	76.49	127.67	187.39	-	77.34	-	-
3-(Methylthio)-1-propanol	31.27	51.10	89.67	-	109.43	-	44.85
Benzyl alcohol	-	-	42.14	24.48	141.88	84.66	-
2-Phenyl ethanol	735.81	4719.54	9615.95	1087.23	8568.03	2244.73	4530.10
Furfuryl alcool	-	-	-	-	29.68	-	70.67
Total alcohols (µg/L)	**5537.21**	**17909.07**	**32080.92**	**6001.7**	**32677.33**	**7021.67**	**23458.75**
%	81.24	77.25	69.76	70.07	76.23	52.50	74.93
Esters[b]							
N-amyl acetate	8.12	7.81	45.64	14.20	51.65	-	42.40
Hexanoic acid ethyl ester	-	11.44	32.26	10.72	55.09	-	33.88
Octanoic acid ethyl ester	-	10.88	41.85	6.87	43.77	35.41	52.20
Ethyl lactate	-	1716.43	3624.37	1355.55	-	1558.85	3127.84
Ethyl-3-hydroxy butanoate	419.91	279.33	496.60	57.87	26.4	-	251.90
Ethyl hydrogen succinate	518.59	2068.48	6957.90	584.76	5778.64	2620.97	2507.29
Diethyl malate	-	159.50	44.31	17.59	155.74	119.80	-
Diethyl succinate	143.51	531.39	1548.74	272.14	2422.18	1472.88	515.59
Total esters (µg/L)	**1090.13**	**4785.26**	**12791.67**	**2319.7**	**8533.47**	**5807.91**	**6531.1**
%	15.99	20.64	27.81	27.08	19.90	43.42	20.86
Fatty acids[a]							

Hexanoic acid	21.12	54.14	163.23	27.40	221.67	119.60	155.95
Octanoic acid	17.88	61.40	267.11	24.95	228.12	164.20	187.42
Decanoic acid	-	-	-	-	26.60	26.30	-
Total acids (µg/L)	**39.00**	**115.54**	**430.34**	**52.35**	**476.39**	**310.10**	**343.37**
%	0.57	0.49	0.93	0.61	1.11	2.31	1.09
Lactones							
Butyrolactone	**138.96**	**356.47**	**644.90**	**154.84**	**826.64**	**234.91**	**629.06**
%	2.03	1.53	1.40	1.80	1.92	1.75	2.00
Other compounds							
Ethyl vanilate	-	-	-	-	151.32	-	-
Furfural	9.78	15.94	35.33	11.23	198.49	-	0.31
Total other compounds (µg/L)	**9.78**	**15.94**	**35.33**	**11.23**	**349.81**	**-**	**0.31**
%	0.14	0.06	0.07	0.13	0.81	0	0.0009
Total volatiles (µg/L)	**6815.08**	**23182.28**	**45983.16**	**8564.77**	**42863.64**	**13374.59**	**31305.96**
%	100	100	100	100	100	100	100

a Expressed in equivalents of phenyl ethanol
b Expressed in equivalents of ethyl decanoate

Fatty acids. The composition of the must and fermentation conditions have a main contribution on the production of fatty acids (Schreir, Jennings, 1979). Three fatty acids were identified in all seven wine samples. The total concentration of those volatile compounds ranged from 39 µg/L (Shiraz from Cricova) to 476.39 µg/L (Codru wine from Cricova), being 0.58% and 1.12%, respectively, of the total volatile compounds detected (Table 3). Octanoic (rancidity; candy, cheese; animal, spicy; unpleasant) and hexanoic (cheese, greasy) acids, were detected and quantified in all seven wines samples. In our research, fatty acids might have a positive impact on the aroma of the wines due to the fact that their levels were far below 20 mg/L.

Lactones. The content and the amount of butyrolactone in wine sample depend on the grape variety and maceration (Bueno et al., 2003). Butyrolactone (fruity, coconut-like notes) was found in all seven red wine samples, due to the fact that all wines were aged for 6 or 12 months in oak barrels. The presence of butyrolactone in wines may be consider an indication of a wine ageing in oak barrels.

Other compounds. The composition of other compounds varied among the wines. Ethyl vanillate (vanilla, honey) was detected only in Codru wine from Cricova, but furfural (pungent, sweet, caramel, butterscotch) was detected in almost all wine samples, except Merlot from Cricova.

CONCLUSIONS

A total of 23 volatile compounds were determined by GC/MS in red wines produced in Republic of Moldova and Romania. Alcohols and esters were the main contributors to the overall volatile composition of the wines, which made up to 17–76% and 16–23% of the

total volatiles, respectively. Wines from Republic of Moldova were characterized by higher level of alcohols, esters and butyrolactones.

According to all volatiles detected, higher alcohols and esters are the main aromatic contributor for Romanian and Moldovian red wines.

Acknowledgements. Authors are grateful to Mr. Sergiu Bobutac and Mrs. Anamaria Vaduva (Cricova Wineries) and to Mr. Sorin Macoviciuc (Panciu Domain) for providing wine samples.

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