Aromatic characterization and total volatile composition of red wines from the region of Central Northern Bulgaria

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Abstract

Introduction. The aromatic potential of the wine is based on the presence and variety (quantitative and species) of compounds of the groups of esters, higher alcohols, aldehydes, monoterpenes, methoxypyrazines, aliphatics, phenylpropanoids and others.

Materials and methods. The object of the present study were red wines obtained from Rubin, Storgozia, Bouquet, Trapezitsa, Kaylashki rubin and Pinot Noir grapevine varieties cultivated in the Experimental Base of the IVE. The alcohol content of the obtained wines was defined by specialized equipment with high precision – automatic distillation unit – DEE Destillation Unit with Densimat and Alcomat, Gibertini, Milan, Italy. Gas chromatographic determination of the aromatic components in wine distillates was done.

Results and discussion. Twenty eight volatile compounds have been identified. The greatest variety of volatile compounds (22 identified) in red wine of the Rubin variety was found. The lowest content (11 identified compounds) in the wine from Storgozia was established.

Considering the total content of volatile compounds, their lowest amount was found in the Trapezitsa wine (368.41 mg.dm^{-3}). The highest total volatile content was found in the Kaylashki rubin wine (1202.55 mg.dm^{-3}).

The total amount of higher alcohols was lowest (101.48 mg.dm^{-3}) in the Rubin wine. This quantity was significantly lower compared to the results for this indicator in all red wines examined. The highest content of higher alcohols (504.84 mg.dm^{-3}) was found in the Kaylashki rubin wine. A single aldehyde – acetaldehyde was identified in the wines studied. Very high total ester content (501.79 mg.dm^{-3}) was found in the Kaylashki rubin wine. Five terpene alcohols were found in the wines studied.

Conclusions. The present study proved that red wines obtained from hybrid Bulgarian varieties (obtained by intra- and iter-species hybridization) were characterized by a complex and varied volatile composition similar to that of *Vitis vinifera* L.

Introduction

The content of a variety of volatile compounds with a pronounced and heterogeneous aromatic effect is an indicator of wine quality. More than 800 volatile compounds with a total quantity ranging up to 800 mg.dm^{-3} and higher – 1200.00 mg.dm^{-3} [1, 2] have been identified in the wine. Factors influencing the synthesis and quantitative accumulation of the volatile components in grapes and wine are highly variable. They are mainly concentrated in: the genetic potential of the varieties to synthesize and accumulate volatile components with pronounced aromatic influence [3]; climate, soils, geographical location of the vine growing [4, 5]; agro-technical measures [6]; phytosanitary status of the vine [7,8]; the technical and technological conditions of vinification [9]; metabolic potential and activity of yeasts microflora (alcoholic fermentation) and bacterial lactic acid microflora (malolactic fermentation) [10, 11]; the wine aging processes [12, 13].

The aromatic potential of the wine is based on the presence and variety (quantitative and species) of compounds of the groups of esters, higher alcohols, aldehydes, monoterpenes, methoxypyrazines, aliphatics, phenylpropanoids and others [14, 15].

The esters have the most significant contribution to the aroma profile of the wine. Their low threshold of perception distinguishes them as the main and strong factor for the quality of the aroma. Their formation begins in the grapevine. Their amounts in grapes are varietal characteristic and low (10.00 – 30.00 mg.dm^{-3}) [16]. The biological ester synthesis from the yeasts results in significant accumulations (up to 500.00 mg.dm^{-3}) in young wines after vinification [11]. The wine aging process leads to a chemical bonding between the available alcohols and acids (esterification), which significantly increases the total ester content (792.00 – 880.00 mg.dm^{-3}) in old wines [17].

The esters having a major influence on the aroma and bouquet of wines include ethyl acetate, ethyl hexanoate, ethyl butyrate, isobutyl acetate, isoamyl acetate, hexyl acetate, ethyl decanoate, phenyl acetate, ethyl lactate [18, 19].

The higher alcohols also represent an important group of compounds from the volatile composition of the wine. They have high thresholds of aromatic perception, in contrast to the esters. This determines their weaker aromatic influence. However, their important contribution is related to their role as precursors in the esterification process, forming different esters with the wine acids [13]. The higher alcohols are mainly synthesized during alcoholic fermentation as a product of yeasts amino acid metabolism. Their concentration in red wines can reach up to 600.00 mg.dm^{-3} [11]. The major representatives of this group of compounds are 3-methyl-1-butanol (isoamyl alcohol), isobutyl alcohol, phenylethyl alcohol, hexanol, heptanol, butanol [19, 20, 13].

The aldehydes are quantitated mainly by acetaldehyde. The permissible concentration of this substance in dry wines ranges from 10.00 to 200.00 mg.dm^{-3} [11].

The terpenic profile of the wine is represented by the terpene alcohols linalool, α-terpineol, β-citronellol, nerol and geraniol [21, 22]. They have a fundamental contribution to the aroma of wines obtained from muscat grapevine varieties [23, 24].

The aim of the present study is to perform the aromatic characterization and identification of the total volatile composition of red wines from the region of Pleven, Central Northern Bulgaria.

Materials and methods

Grapevine varieties and vinification.

The study was conducted at the Institute of Viticulture and Enology (IVE) – Pleven, Central Northern Bulgaria. The object of the present study were red wines obtained from Rubin, Storgozia, Bouquet, Trapezitsa, Kaylashki rubin and Pinot Noir grapevine varieties cultivated in the Experimental Base of the IVE.

Pinot Noir is a widespread variety of *Vitis vinifera* L. It was used for control variant in the present study. The remaining varieties were hybrids selected in the IVE-Pleven, via the intra- and interspecies hybridization, whose parental forms were as follows:

- Rubin – Nebiolo x Shiraz [25];
- Storgozia – Bouquet x Villar Blanc [26];
- Bouquet – Mavrud x Pinot noir [25];
- Trapezitsa – Danube Gamza x Marseilles early [27];
- Danube Gamza – Bouquet x Villar Blanc [25];
- Kaylashky rubin – (Pamid x Hybrid VI 2/15) x (Gamma noir x *Vitis amurensis*) [27];

The grapes from the different varieties were harvested (30 kg for each variety) and were vinified at the Experimental Wine Cellar of IVE. A classic scheme for the production of dry red wines [28] was applied – crushing and destemming, sulphitation (50 mg/kg SO_2), inoculating with pure culture dry yeasts *Saccharomyces cerevisiae* Siha Rubio Cru (EATON Begerow) – 20 g/hl, temperature of fermentation – 28 °C, separation from solids, further sulphitation, storage.

Determination of alcohol content of obtained wines

The alcohol content of the obtained wines was defined by specialized equipment with high precision – automatic distillation unit – DEE Destillation Unit with Densimat and Alcomat, Gibertini, Milan, Italy.

Aromatic content determination by GC-FID

Gas chromatographic determination of the aromatic components in wine distillates was done. The content of major volatile aromatic compounds was determined on the basis of stock standard solution prepared in accordance with the IS method 3752:2005 [29]. The method describes the preparation of standard solution with one congener, but the step of preparation was followed for the preparation of a solution with more compounds. The standard solution in this study include the compounds with purity > 99.0%. The 2 µl of prepared standard solution was injected in gas chromatograph Varian 3900 (Varian Analytical Instruments, Walnut Creek, California, USA) with a capillary column VF max MS (30 m, 0.25 mm ID, DF = 0.25 µm), equipped with a flame ionization detector (FID). The used carrier gas was He. Hydrogen to support combustion was supplied to the chromatograph via a hydrogen bottle. The injection was manually by microsyringe.

The parameters of the gas chromatographic determination were: injector temperature – 220 °C; detector temperature – 250 °C, initial oven temperature – 35 °C/retention 1 min, rise to 55 °C with step of 2 °C/min for 11 min, rise to 230 °C with step of 15 °C/min for 3 min. Total time of chromatography analysis – 25.67 min. The identified retention times of the compounds in standard solution were: acetaldehyde (3.141), ethyl acetate (3.758), methanol

(3.871), 2-propanol (5.170), isopropyl acetate (5.975), 1–propanol (6.568), 2–butanol (7.731), propyl acetate (9.403), 2–methyl-propanol (10.970), 1–butanol (11.509), isobutyl acetate (11.662), ethyl butyrate (12.710), butyl acetate (12.752), 2–methyl-1-butanol (13.054), 4-methyl-2-pentanol (13.629), 3–methyl-1–butanol (13.840), 1–pentanol (15.180), isopenthyl acetate (15.965), pentyl acetate (16.033), 1–hexanol (16.276), ethyl hexanoate (16.376), hexyl acetate (16.510), 1–heptanol (16.596), linalool oxide (16.684), phenyl acetate (18.055), ethyl caprylate (18.625), α-terpineol (19.066), 2-phenyl ethanol (19.369), nerol (19.694), β-citronellol (19.743), geraniol (19.831), ethyl decanoate (19.904). As an internal standard 1-octanol was used. The resulting chromatographic profile of the standard solution is shown in Figure 1.

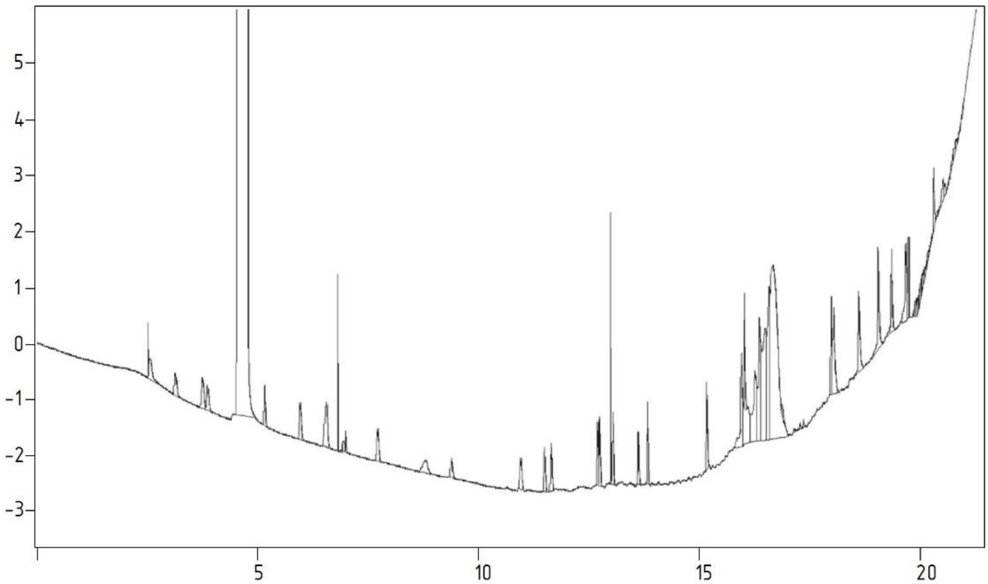

Figure 1. Chromatogram of standard solution

After determination of the retention times of aromatic compounds in the standard solution, we proceed to the identification and quantification of the volatile aromatic substances in the wines. The aromatic composition was determined based on injection of wine distillates. Prepared samples were injected in an amount of 2 μl in a gas chromatograph and was carried out an identification and quantification of the aromatic substances in each of them.

Results and discussion

The quantity of the identified compounds is presented in Table 1.

Table 1

Content of volatile aromatic compounds in red wines of Rubin, Storgozia, Bouquet, Trapezitsa, Kaylashki rubin and Pinot Noir

Identified compounds, $mg.dm^{-3}$	WINES					
	Rubin	**Storgozia**	**Bouquet**	**Trapezitsa**	**Kaylashki Rubin**	**Pinot Noir**
Ethyl alcohol, vol. %	12.72	11.39	13.53	14.75	12.65	14.91
Acetaldehyde	34.73	0.05	0.05	20.49	69.85	0.05
Methanol	22.02	75.75	36.63	40.91	126.07	50.59
1. Higher alcohols						
1-propanol	0.05	0.05	0.05	8.32	0.05	0.05
2-propanol	0.05	0.05	0.05	0.05	0.05	0.05
2-butanol	30.82	87.79	69.56	ND	62.00	44.71
2-methyl-1-buthanol	33.27	66.11	59.09	51.00	68.14	37.84
3- methyl -1-buthanol	37.19	273.63	220.74	181.39	241.42	199.12
4-methyl-2-pentanol	ND	ND	ND	ND	ND	0.05
1-pentanol	ND	ND	ND	ND	ND	31.45
1-hexanol	ND	ND	ND	ND	0.05	ND
1-heptanol	0.05	ND	ND	ND	ND	ND
2-phenyl ethanol	0.05	ND	ND	11.59	133.13	ND
Total higher alcohols	**101.48**	**427.63**	**349.49**	**252.35**	**504.84**	**313.27**
2. Esters						
Ethyl acetate	20.40	25.67	29.41	18.76	77.95	30.24
Propyl acetate	0.05	ND	ND	ND	ND	ND
Isobutyl acetate	ND	ND	ND	ND	273.23	ND
Ethyl butyrate	83.74	ND	ND	0.05	ND	ND
Ethyl hexanoate	ND	0.05	ND	ND	ND	ND
Pentyl acetate	ND	ND	ND	ND	0.05	0.05
Isopentyl acetate	156.85	ND	ND	ND	ND	ND
Hexyl acetate	ND	ND	ND	ND	0.05	ND
Phenyl acetate	0.05	ND	ND	ND	0.05	ND
Ethyl caprylate	0.05	0.05	ND	34.41	150.46	ND
Ethyl decanoate	0.05	ND	153.71	ND	ND	ND
Total esters	**261.19**	**25.72**	**183.12**	**53.22**	**501.79**	**30.29**
3. Terpen compounds						
α – terpineol	0.05	ND	0.80	0.97	ND	0.05
Linalool oxide	0.05	0.05	ND	ND	ND	0.05
Nerol	0.05	ND	0.0006	0.11	ND	ND
β – citronellol	0.05	ND	0.17	0.19	ND	ND
Geraniol	0.05	ND	ND	0.17	ND	0.05
Total terpenes	**0.25**	**0.05**	**0.97**	**1.44**	**ND**	**0.15**
Total volatile compounds	**419.67**	**529.20**	**570.26**	**368.41**	**1202.55**	**394.35**

Twenty eight volatile compounds have been identified. The greatest variety of volatile compounds (22 identified) in red wine of the Rubin variety was found. The lowest content number (11 identified compounds) in the wine from Storgozia was established.

Considering the total content of volatile compounds, their lowest amount was found in the Trapezitsa wine (368.41 $mg.dm^{-3}$). The highest total volatile content was found in the Kaylashki rubin wine (1202.55 $mg.dm^{-3}$). This wine was distinguished from all others by the higher and significant amounts of volatile and aromatic components. The results for the total quantitative volatile composition of the wines examined were in absolute correlation with the data of other researchers [2].

The total amount of higher alcohols was lowest (101.48 $mg.dm^{-3}$) in the Rubin wine. This quantity was significantly lower compared to the results for this indicator in all red wines examined. The highest content of higher alcohols (504.84 $mg.dm^{-3}$) was found in the Kaylashki rubin wine. Immediately after it, according to this indicator, the wine of the Storgozia variety (427.63 $mg.dm^{-3}$) was ranked. The established total alcohols in the wines examined correlated with the presented data (up to 600 $mg.dm^{-3}$) of Chobanova, 2012 [11].

2-methyl-1-butanol (active amyl alcohol) and 3-methyl-1-butanol (isoamyl alcohol), higher alcohols found in all wines tested, were dominated. 3-methyl-1-butanol was represented with higher content. Its concentration was lowest for Rubin wine (37.19 $mg.dm^{-3}$). The highest was for Storgozia wine (273.63 $mg.dm^{-3}$). The isoamyl alcohol is one of the major components of the higher alcohols group. It gave typical notes of malt and whiskey in the wine aroma [19].This compound was one of those with an important aromatic effect found in the study of red wines of Merlot and Cabernet Sauvignon varieties produced in California and Australia [30]. A study of aromatic compounds in Cabernet Sauvignon wines during aging in stainless steel tanks found that 3-methyl-1-butanol was the main representative of higher alcohols occupying the largest quantity [13]. The obtained data on the presence of 3-methyl-1-butanol in the present study were in full correlation with the studies of the scientific groups discussed above.

2-methyl-1-butanol (active amyl alcohol) was found in the highest amount (68.14 $mg.dm^{-3}$) in the Kaylashki rubin wine. Immediately after it was the wine of Storgozia (66.11 $mg.dm^{-3}$). The lowest was its amount in Rubin wine (33.27 $mg.dm^{-3}$). According to Chobanova (2012) [11] the average content of this alcohol is about 36.00 $mg.dm^{-3}$.

2-phenylethanol was found in the Kaylashki rubin wine (133.13 $mg.dm^{-3}$) and Trapezitsa (11.59 $mg.dm^{-3}$). This alcohol is important to the sensory of the wine. It was identified in white and red wines from Northwestern Spain [14]. It was responsible for the aroma of rose in the wine [31].

A high content of 2-butanol has been identified. The highest was its quantity (87.79 $mg.dm^{-3}$) in Storgozia wine followed by Bouquet wine (69.56 $mg.dm^{-3}$). The lowest concentration (30.82 $mg.dm^{-3}$) of this higher alcohol in the Rubin wine was established.

1-pentanol was found only in Pinot Noir wine (31.45 mg dm^{-3}).

1-hexanol was identified only in the Kaylashki rubin wine, and 1-heptanol was found only in the Rubin wine. The last two alcohols were available in minor amounts.

A single aldehyde – acetaldehyde was identified in the wines studied. The highest was its amount in the wine of Kaylashki rubin (69.85 $mg.dm^{-3}$). In the wines of Storgozia, Bouquet and Pinot Noir it was found in very low quantities. The established acetaldehyde levels were normal for red wines.

The ester fraction was represented by 11 identified compounds. The esters were of fundamental importance to the complexity of the wine aroma. Very high total ester content (501.79 $mg.dm^{-3}$) was found in the Kaylashki rubin wine.This wine was characterized by the highest aromatic complexity. Immediately after it was the Rubin wine, where a total ester content of 261.19 mg.dm^{-3} was established. The lowest overall concentration of esters (25.72 mg.dm^{-3}) in Storgozia wine was found.

The ethyl acetate was the main dominant ester. Its highest concentration (77.95 mg.dm^{-3}) was found in the wine of Kaylashki rubin variety. The lowest content of this ester (18.76 mg.dm^{-3}) in Trapezitsa wine was found. At concentrations up to 50.00 – 80.00 mg.dm^{-3} this ester provides a pleasant fruity aroma of the wine [11, 32]. At higher concentrations, its influence is negative [33]. The concentrations found in this study were in correlation with the above data, confirming the positive effect of ethyl acetate on the aroma of the wines studied.

With the highest amount of all identified esters isobutyl acetate was identified. This ester was found only in the Kaylashki rubin wine (273.23 mg.dm^{-3}).

The ethyl butyrate was found in the Rubin wine (83.74 mg.dm^{-3}) and in the Trapezitsa (0.05 mg.dm^{-3}). It was one of the esters with an important contribution to the fruit character of the wine [34]. It was also identified in Cabernet Sauvignon wines from China [32].

The isopentyl acetate was found only in the Rubin wine (156.85 mg.dm^{-3}). This ester was also established in Chinese Cabernet Sauvignon wines [32].

The ethyl caprylate was found in the wines of Rubin, Storgozia, Trapezitsa and Kaylashki rubin. Significant concentrations of this compound were found in the wines of the Kaylashki rubin (150.46 mg.dm^{-3}) and Trapezitsa (34.41 mg.dm^{-3}). Ivanova et al. (2013) [35] studied Macedonian and Hungarian wines and indicated higher amounts of ethyl caprylate in white wines than red wines.

The ethyl decanoate was identified in two of the wines – Bouquet (153.71 mg.dm^{-3}) and Rubin (0.05 mg.dm^{-3}). This ester was found to be a major in study on variations in the aromatic composition of Cabernet Sauvignon wines aging in stainless steel tanks [13].

Five terpene alcohols were found in the wines studied. The highest total amount of terpenes (1.44 mg.dm^{-3}) was determined in the Trapezitsa wine. The lowest total amount (0.05 mg.dm^{-3}) of these compounds was found in the Storgozia wine.

α-terpineol was identified in the Rubin, Bouquet and Trapezitsa wines. The highest was its content (0.97 mg.dm^{-3}) in the Trapezitsa sample, and the lowest (0.05 mg.dm^{-3}) in Rubin. α-terpineol was found to be one of the main terpenes in a Gewürztraminer wine [36].

Linalool oxide was identified in only three of the wines studied – Rubin, Storgozia and Pinot Noir. This compound was present in minor amounts.

Nerol was found in three of the wines studied – Rubin, Bouquet and Trapezitsa. The highest amount in Trapezitsa wine (0.11 mg.dm^{-3}) was established. The identified content of this terpen fully corresponded to its variance range (0.014 – 0.450 mg.dm^{-3}) presented by Chobanova (2012) [11].

β-citronellol has been identified in the Rubin, Bouquet and Trapezitsa wines. The highest was its concentration (0.17 mg.dm^{-3}) in the Trapezitsa wine. The content of this terpene in wines ranges from 0.014 – 0.450 mg.dm^{-3} [11], which fully corresponded to the concentrations obtained in this study.

The last identified terpene was geraniol. It was located in the wines of Rubin, Trapezitsa and Pinot Noir. The highest was its concentration in Trapezitsa wine (0.17 mg.dm^{-3}).

Another component of the volatile composition of the wine found in this study was methanol. This compound has no aromatic effect, but at high concentrations it is toxic. Its presence in wines is a normal phenomenon. Its presence is due to its precursor – fruit pectin, which is degraded to methanol from the pectolytic enzyme complex of the fruit [37]. The normal content of methyl alcohol in red wine ranges from 60.00 to 230.00 mg.dm^{-3} [16]. In this study this component of the volatile composition was found in all wines examined. The highest was its content in the Kaylashki rubin wine (126.07 mg.dm^{-3}), and the lowest in Rubin wine (22.02 mg.dm^{-3}). The concentrations of this compound found in this study correlated with the range of its normal presence.

Conclusions

The conducted study for aromatic characterization of the volatile and aromatic composition of red wines from the region of Central Northern Bulgaria established:

- Twenty eight volatile compounds from different aromatic groups were identified and quantified. The largest species diversity of volatile compounds (22 identified) were found in the Rubin wine, and the lowest (11 identified) in that of Storgozia;
- The highest total volatile content (1202.55 mg.dm^{-3}) was found in the red wine Kaylashki rubin. This wine was with significantly higher content of volatile compounds than all others in the study;
- The highest total content of higher alcohols (504.84 mg.dm^{-3}) was found in the Kaylashki rubin wine. The lowest (101.48 mg.dm^{-3}) in the Rubin wine was found;
- 3-methyl-1-butanol (isoamyl alcohol) and 2-methyl-1-butanol (active amyl alcohol) were presented in all tested wines. The established higher alcohols with significant effect on the wine aromatic profile were also 2-phenylethanol, 2-butanol, 1-pentanol, 1-hexanol and 1-heptanol;
- Only acetaldehyde was found from the group of aldehydes. The highest was its content in the Kaylashki rubin wine (69.85 mg.dm^{-3});
- The ester composition of the wines was represented by 11 identified compounds. The red wine Kaylashki rubin showed high quantitative (501.79 mg.dm^{-3}) ester complexity.
- The presence of the ester ethyl acetate was predominant. The ester group has also been found to be ethyl butyrate, isopentyl acetate, ethyl caprylate, ethyl decanoate. These esters have a serious impact on the aromatic quality and complexity of the wines analyzed;
- The highest total terpene content (1.44 mg.dm^{-3}) was found in Trapezitsa wine. From this group of aromatic volatile compounds in the wines were found linalool oxide, nerol, α-terpineol, β-citronellol and geraniol;
- Methyl alcohol was found in the wines. Its amount ranged from 22.02 mg.dm^{-3} (in Rubin wine) to 126.07 mg.dm^{-3} (in the Kaylaski rubin wine). Its concentrations were normal for red wines.

The present study proved that red wines obtained from hybrid Bulgarian varieties (obtained by intra- and iter-species hybridization) were characterized by a complex and varied volatile composition similar to that of *Vitis vinifera* L.

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