INTRODUCTION

Over 800 volatile compounds have been identified in the wine (Li, 2006). Different quantitative variations of individual volatile compounds - tens to hundreds of mg dm⁻³, as well as very low concentration ranges - µg dm⁻³ to ng dm⁻³ have been established (Rapp and Manderley, 1986; Li, 2006; Sanchez-Palomino, 2007). The total content of aromatic compounds in the wine can reach 800.00 mg dm⁻³ and more - 1200.00 mg dm⁻³ (Lakatolová et al., 2013). The volatiles composition of the wine is variable, because it is dependent on many different and diverse factors: Genetic contribution of the grapevine variety - its ability to accumulate compounds with a strong aromatic effect in grapes, passing through the wine (Abrasheva et al., 2008; Gonzalez-Barreiro et al., 2013); Climatic conditions and soils in the geographical location where the grapevine variety is grown (Mira de Orduna, 2010; Dal Santo et al., 2013); The degree of grapes maturity (Robinson et al., 2014); The impact of applied agro-technical measures (Bureau et al., 2000); The direct influence of irrigation events - induction and influence of water stress (Oliveira et al., 2003; Grimplet et al., 2007; Ou et al., 2010); The phytosanitary status of the vine - influence of fungal infections caused by Botrytis cinerea (Stech et al., 1991) and Aspergillus niger (Winterhalter and Skourroumouns, 1997; Selton and Williams, 1991); Technical and technological conditions of vinification (Oliveira et al., 2006); Metabolic activity of yeasts carrying the alcoholic fermentation (Rodriguez-Bencomo et al., 2002) and lactic acid bacteria carrying the malolactic fermentation (Chobanova, 2012); The wine aging (Cámara et al., 2006; Meng et al., 2011). The most important volatile compounds, which reflect the wine general aromatic profile, belong to the groups - esters, aldehydes, higher alcohols, terpene compounds (Lambrecht and Pretorius, 2000; Vilanova et al., 2013; Robinson et al., 2014). The main and essential role of the esters is due to their large quantitative presence (Mason and Dufour, 2000) as well as to the rich variety of aromatic nuances that they give: the aroma of red and black berries (Pineau et al., 2009), honey aroma (Escudero et al., 2007), general fruit character of the wine (Li et al., 2008) and others. The main quantitative and species esters diversity is a product of the wine-making process and related technological practices. The contribution of the grapevine to the final ester composition of the wine is very small, because only about 10.00 – 30.00 mg dm⁻³ of total esters are accumulated in the grapes (Abrasheva et al., 2008). The esters are formed in the wine by biological and chemical mechanisms. The biological is related to the yeasts vital activity (Chobanova, 2012). The other microflora may also have an impact (Swiegers et al., 2005). Chemical formation is due to the esterification process - interaction between wine acids and alcohols (Chobanova, 2012). This formation mechanism takes place throughout the wine aging period and it is important for the "bouquet" of old wines (Yankov et al., 2008). The ester content of young wines ranges from 50.00 to 250.00 mg dm⁻³ (Abrasheva et al., 2008) and may reach 500.00 mg dm⁻³ (Chobanova, 2012). In the aging process, the ester content may reach 780.00 - 880.00 mg dm⁻³ (Yankov et al., 2000). The main, predominantly quantitative ester is ethyl acetate. The higher alcohols are derived from the alcohol metabolism (Bell and Henschke, 2005) producing α-ketocacid processors. Also, by the ability of yeasts to decompose the amino acids directly by the Ethric path (Etievant, 1991; Swiegers et al., 2005). The higher alcohols are characterized with a high threshold of aromatic perception. This is an indicator of their less direct impact on the wine aromatic profile. However, they have an indirect influence on the wine aroma. It is due to their participation in the process of esterification. By their interaction with acids, a wide variety of esters are formed, which complicates and improves the aroma of wines during the aging process (Meng et al., 2011). Their quantitative variation in wine ranges from 150.00 - 550.00 mg dm⁻³ (Abrasheva et al., 2008). In red wines, their content may reach 600.00 mg dm⁻³ (Chobanova, 2012). When they exceed these concentrations, their contribution to the general flavor becomes negative (Simpson, 1979). The
exception to this thesis is only 2-phenylethanol (phenethyl alcohol) (Lopez et al., 2003). It gives the rose aroma (Simpson, 1979).

The group of aldehydes is represented mainly by acetaldehyde. Its optimal quantities range from 10.00 - 110.00 mg dm⁻³, but may reach 200.00 mg dm⁻³ in dry port (Chobanova, 2012). The acetaldehyde is an important component of the aromatic wine profile. It is produced by the yeasts Saccharomyces cerevisiae during fermentation. When it is available in its optimal quantities in dry wines, it gives a pleasant apple aroma, but when exceeding them it produces an oxidized tone in the wine (Chobanova, 2012).

An important group of aromatic composition are terpenic compounds. Of these, the terpenic alcohols linalool, α-terpineol, β-citronellol, nerol and geraniol (Arrhaenius et al., 1996; Luan et al., 2006; Oliveira et al., 2008) are found in wines. They are the product of the grapevine metabolism from where they accumulate in the grapes (Manito, 1980). They are primarily responsible for the aroma of wines obtained from muscat grapes (Villanová et al., 2013). The objective of this study is to determine the aromatic profile of red wines obtained from Rubin, Storgozia, Bouquet, Trapetiza, Kaylashky Rubin and Pinot Noir varieties from the region of Central Northern Bulgaria.

MATERIAL AND METHODS

Grape varieties and vinification

The study was conducted at the Institute of Viticulture and Enology (IVE) - Pleven in the period 2017-2018. The object of the present study were red wines, obtained from Rubin, Bouquet, Trapetiza, Kaylashky Rubin and Pinot Noir grape varieties, harvested in 2017, cultivated in the region of Pleven, Central Northern Bulgaria. The first five varieties were selected in IVE, inter-and intra-species hybrids. The latter being widespread introduced variety from Vitis vinifera. The parental forms of the selected hybrids were as follows:

- Rubin - Nebiolo x Shiraz (Petkov, 1977)
- Storgozia - Bouquet x Villar Blanc (Katerov et al., 1990)
- Bouquet - Mavrud x Pinot noir (Petkov, 1977)
- Trapetiza - Danube Gamsa x Marseilles early (Ivanov, 2016)
- Kaylashky Rubin - (Pamid x Hybrid VI 2/15) x (Gamma noir x Vitis amurenensis) (Ivanov, 2016)

The experimental vineyards on an area of 0.2 ha for each variety were grown in the Experimental Base of IVE. 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 red wine was used (Kolakovsky, 2002), crushing and destemming, sulphitation (50 mg/kg SO₂), inoculating with pure culture dry yeasts Saccharomyces cerevisiae Vitilevure CSM - 20 g/l dm³, temperature of fermentation - 28°C, separation from solids, further stabilization, 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 - Gibertiny BEE RV 10326 (Gibertiny Electronics Srl., Milano, Italy) and Gibertiny Densi Mat CE AM 148 (Gibertiny Electronics Srl., Milano, Italy).

Aromatic content determination by Gas Chromatography (Gas Chromatograph with Flame Ionization Detector)

Gas chromatography 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. 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 following compounds (purity > 99.0%): acetaldehyde, ethyl acetate, methanol, isopropyl acetate, 1-propanol, 2-butanol, propyl acetate, 2-methyl-propanol, isobutanol, 1-butanol, isobutyl acetate, ethyl butyrate, butyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl isovalerate, 1-pentanol, pentyl acetate, 1-hexanol, hexyl hexanoate, heptyl acetate, 1-heptanol, linalool oxide, phenyl acetate, ethyl caprylate, α-terpineol, β-citronellol, nerol, geranial. As an internal standard 1-octanol was used.

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 Helium. Hydrogen to support combustion was supplied to the chromatograph via a hydrogen bottle. The injection is manually by microsyringe.

The parameters of the gas chromatographic determination were: injector temperature - 220°C; detector temperature - 250°C, initial oven temperature - 35°C for 1 min, up to 55°C with step of 2°C/min for 11 min, up to 230°C with step of 15°C/min for 3 min. Total time of chromatography analysis - 25.67 min. After determination of the retention times: acetaldehyde (3.250), ethyl acetate (4.017), methanol (4.186), isopropyl acetate (5.897), 1-propanol (6.763), 2-
variety, it was found to be in normal concentration (6.64 mg dm⁻³), but in the wine from Kaylashky Rubin variety (15.48 mg dm⁻³) its quantity exceeds the threshold.

The terpenic wine profile was represented by 5 identified terpene alcohols. The highest total terpenic content was found in the wine from Trapezitsa (1.66 mg dm⁻³). The lowest was the content in the wine from Pinot Noir variety (0.27 mg dm⁻³). Geraniol was practically found in all wines.

The presence of a substance that is not a part of the aromatic matrix but found to be a normal component of the volatile composition in red wines – methanol was established. Its presence is due to its precursor - pectin present in the fruit, which is degraded by the pectolytic enzyme complex of the grapes (Marinov, 2005). The normal limited content of methyl alcohol in red wines should be in the range of 36.00 - 350.00 mg dm⁻³ (according to Chobanova, 2012) or 60.00 - 230.00 mg dm⁻³ (according to Abrasheva et al., 2008). The methyl alcohol must not exceed these amounts. In the present study, methanol was found at the lowest content in wine from Bouquet variety (87.27 mg dm⁻³), and the highest in the wine from Kaylashky Rubin (235.71 mg dm⁻³). The data are in agreement with the permitted thresholds of content for this alcohol in red wines.

Table 1 Content of volatile aromatic compounds in red wines from Rubin, Storgozia, Bouquet, Trapezitsa, Kaylashky Rubin and Pinot Noir grapevine varieties

<table>
<thead>
<tr>
<th>IDENTIFIED COMPOUNDS, mg.dm⁻³</th>
<th>WINES</th>
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</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>33.76</td>
<td>24.12</td>
<td>26.16</td>
<td>87.44</td>
<td>17.19</td>
<td>22.83</td>
</tr>
<tr>
<td>Methanol</td>
<td>110.03</td>
<td>141.95</td>
<td>87.27</td>
<td>228.88</td>
<td>235.71</td>
<td>116.27</td>
</tr>
</tbody>
</table>

1. Higher alcohols

| 1-propanol                   | ND    | ND    | 14.23 | ND    | ND    | 0.05  |
| 2-butanol                    | 0.05  | 0.05  | ND    | ND    | 10.31 | 13.04 |
| 3-methyl-1-butanol           | 373.02| 74.02 | 86.81 | 43.41 | 112.34| 48.15 |
| 2-methyl-1-propanol          | ND    | ND    | 39.67 | ND    | ND    | ND    |
| 1-pentanol                   | 0.05  | 0.05  | 0.05  | ND    | ND    | 0.05  |
| 1-hexanol                    | 6.64  | 0.05  | 0.05  | 0.05  | 15.48 | 0.05  |
| 1-heptanol                   | ND    | ND    | 0.05  | ND    | ND    | ND    |

Total higher alcohols 379.76 247.35 287.77 198.27 329.28 61.34

2. Esters

| Ethyl acetate                | 30.03 | 30.92 | 55.44 | 46.15 | 25.34 | 41.74 |
| Isopropyl acetate            | 0.05  | ND    | 0.05  | ND    | 0.05  | ND    |
| Isobutyl acetate             | ND    | 88.96 | 120.90| ND    | 82.82 | 162.29|
| Ethyl butyrate               | 18.61 | ND    | 12.93 | ND    | ND    | ND    |
| Ethyl hexanoate              | ND    | ND    | 0.05  | ND    | 0.05  | ND    |
| Ethyl isovalerate            | ND    | 0.05  | ND    | ND    | ND    | ND    |
| Pentyl acetate               | ND    | 25.27 | 13.63 | 0.05  | ND    | ND    |
| Phenyl acetate               | 0.05  | 0.05  | 5.68  | ND    | 2.40  | 0.05  |
| Ethyl caprylate              | 8.89  | 0.05  | 28.15 | ND    | ND    | ND    |

Total esters 57.63 145.30 195.75 87.28 110.66 204.08

3. Terpenes

| α - terpineol                | ND    | 0.05  | 0.09  | 0.57  | ND    | 0.05  |
| Linalool oxide               | ND    | ND    | 0.05  | 0.05  | ND    | ND    |
| Nerol                        | 0.09  | 0.05  | 0.05  | 0.87  | 0.11  | 0.05  |
| β – citronellol              | 0.14  | 0.09  | 0.05  | ND    | 0.074 | 0.015 |
| Geraniol                     | 0.97  | 0.31  | 0.54  | 0.17  | 0.95  | 0.16  |

Total terpenes 1.20 0.50 0.78 1.66 1.13 0.27

TOTAL VOLATILE CONTENT 582.38 559.22 597.73 603.53 693.97 404.79

*ND – Not Detected

Figure 1 Chromatographic profile of red wine from Rubin variety

Figure 2 Chromatographic profile of red wine from Storgozia variety
in wine from Rubin variety (379.36 mg/dm³), followed by Kaylashky Rubin (329.18 mg/dm³).

Five terpenes have been identified. Geraniol was present in all tested wines. Methyl alcohol was found in all wines. Its concentrations were typical for red wines.

An extensive aromatic characterization by gas chromatographic analysis of red wines obtained from grapevine varieties selected in the Republic of Bulgaria was carried out. The research proved that the red wines obtained in the conditions of Central Northern Bulgaria were characterized by a complex aromatic composition, due to the presence of different esters, higher alcohols and terpenes.

REFERENCES


MIRA DE ORDUÑA, R. 2010. Climate change associated effects on grape and wine quality and production. Food Research International, 43: 1844-1855. https://doi.org/10.1016/j.foodres.2010.05.001


