INFLUENCE OF GREEN PRUNING OPERATIONS ON THE AROMATIC PROFILE OF RED WINES FROM STORGOZIA GRAPEVINE VARIETY

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Abstract
Gas chromatographic (GC-FID) study to determine the impact of green pruning operations on the aromatic profile of red wines from Storgozia grapevine variety was carried out. The wines examined were made from grapes subjected to the pruning operations: June topping shoot (V2), thinning cluster (V3), July topping shoot (V4), removal of leaves (defoliation) (V5). The control variant was without pruning operations (V1). Two harvests - 2016 and 2017 were investigated. At the harvest 2017, an additional operation - suckering was applied to all pruning operations. It has been found that suckering has a significant effect on improving and complicating the aromatic profile of wines, in the following aspects: Higher content of higher alcohols – tendency of higher content higher alcohols to be found in 2017 harvest wines (206.68 mg/dm3 - 294.68 mg/dm3) compared with harvest 2016 (25.43 mg/dm3 - 194.52 mg/dm3); Increased esters content - a higher final total ester content in wines from harvest 2017 compared with harvest 2016. The green pruning operation "suckering", as manipulation, is probably affect the subsequent synthesis of ethyl butyrate ester during fermentation by blocking it; Increased total terpene content – the terpenic profile was dominated by geraniol. Its content, as well as the total terpenes content were higher in the wines from harvest 2017 compared to 2016, except for the control variant V1 and V2 of the harvest 2016. Aldehydes were presented by acetadehyde. Methyl alcohol was found in concentrations normal for red wines.

Keywords: aromatic profile, red wines, esters, green pruning, suckering.

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1. INTRODUCTION

During the vegetation period in the vineyards, various operations are carried out with the green parts, in which some vegetative and generative organs of the vine are partially or completely removed. The main goal of green pruning operations is to regulate the growth of the remaining green parts and their ratio to provide a better phyto-climate of the vines. This results in higher and better yields. According to Gatti et al. (2015) summer pruning techniques can be used as flexible, powerful tools to directly achieve the desired crop composition.

Many authors (Nedelchev and Kondarev, 1962; Popov et al., 1972; Nikov, 1975; Kurtev et al., 1979; Radulov, 1988; Babrikov et al., 1989) adopt the suckering, which consists in the removal of the redundant shoots in the initial period of their growth, for one of the most important green operation. The authors have found that it regulates the ratio between fruitless and infertile shoots. Ensure the correct formation of the vines and maintain an appropriate ratio between the growth strength and the quantity of the yield. Avoid the thickening and shading of the shoots and leaves, thus creating the conditions for a more proper course of vine physiological

processes. Better ventilation and more efficient fight against diseases and pests, especially with downy mildew and gray rot of vines is achieve. With the tipping, the tops of the shoots are removed with 5-8 underdeveloped leaves. Some authors justified the need for tipping by the fact that for a specified time the growth of the shoots was stopped, as a result of which the plastic substances passed to the other parts, which improved the overall diet of the vine (Popov et al., 1972).

Thinning of the clusters is a viticultural operation that is use to correct the excessive load, for grape composition improvement, and for fruit growth and seed growth balance. In his study, Condurso

et al. (2016), pay particular attention to the volatile aromatic compounds that determine the sensory quality of the wine. They concluded that manual thinning of the clusters at an early stage of application reduced the yield, improved the maturation, improved the phenolic content of grapes and, therefore, the wine, and affected its volatile profile. The grapes from the thinning plants have a trend to accumulate more varietal and fermentative aromas. Irrespective of the economic impact, thinning of the clusters is a viable option because of improving the wine quality, especially for increasing of the amount of compounds that are responsible for the typical wine aroma and color.

The removal of leaves (defoliation) around the clusters is a common used practice especially in the northern regions, where the sunny days during the ripening period are few. By removing of the part of the leaves at the base of the shoots is achieve better ventilation and sunlight for the grapes, as a result of which the ripening is accelerate, to preserve the grapes from fungal diseases, to facilitate the fight with grapes parasites and to obtain higher quality grapes (Kurtev et al., 1969; English et al., 1998). Hunter and Visser (1990) reported that wines produced from partially defoliated vines were rated as being of higher quality than those obtained from non-defoliated plants. According to Šuklje et al. (2013) the suckering and thinning of the clusters have a direct or indirect effect on the concentration of primary and secondary metabolites of the grapes by influencing their biosynthesis, thus affecting the sensory properties and the style of the wines produced. In the study of various combinations of seasonal practices (suckering, topping, leaf thinning) performed during the growth period before veraison, Hunter et al. (2004) concluded that leaf thinning manipulations generally increased the content of monoterpenes (fruit flavor) and increased the content of 2-methoxy-3-isobutylpyrazine (grass/green pepper flavors) which increased the overall aromatic profile.

The volatile composition, which determines the aromatic character of the wine, is a major indicator of its quality. The wine composition includes variety of volatile components in different concentrations, over 800, some of which define its flavor (Li, 2006; Sanchez-Palomo et al., 2007).The compounds that have a major influence on the aromatic potential of the wines are from the groups of esters, aldehydes, higher alcohols, terpene compounds (Vilanova et al., 2013; Robinson et al., 2014).

The esters are the most active aromatic component of the wines. This is due to their species and quantity diversity, as well as to their low thresholds of aromatic perception. Their synthesis begins in grapes, where they are accumulate in small amounts - 10 - 30 mg/dm^3 (Abrasheva et al., 2008). The main amounts of esters are accumulate during the fermentation. This is due to the metabolic activity of yeasts, which produced esters with concentration up to 500.00 mg/dm^3 (Chobanova, 2012), accumulate in young wines. The esters continue to grow and complicate the aroma in the aging process. For older wines the higher ester content (792-800.00 mg/dm^3) (Yankov et al., 2000) is due to the esterification process - chemical bonding between acids and wine alcohols.

The higher alcohols are mainly product of the amino acid yeasts metabolism of *Saccharomyces cerevisiae* (Bell and Henschke, 2005, Swiegers et al., 2005a). Their amount ranges from 150.00 to 550.00 mg/dm^3 (Abrasheva et al., 2008). Due to their high thresholds of aromatic perception, their direct role for the general wine aroma is weak. Their indirect influence, however, is essential. They interact with the acids of the wine, forming esters that complicate the wine aroma (Meng et al., 2011).

The group of aldehydes is typically quantitated by acetaldehyde. For dry wines it can reach 100.00 mg/dm^3 (Velkov, 1996), and according to Chobanova (2012) from 10.00 to 200.00 mg/dm^3.

The group of terpenic compounds in the wine is mainly represented by the terpene alcohols a-terpineol, β-citronellol, linalool, linalool oxide, nerol and geraniol (Luan et al., 2006, Oliveira et al., 2008). They form the main aroma of wines from muscat grape varieties (Vilanova et al., 2013).

The aim of this study is to investigate the influence of green pruning operations on the aromatic profile of red wines from Storgozia grapevine variety.

2. MATERIALS AND METHODS

Grapevine varieties and vinification

The study was conducted at the Institute of Viticulture and Enology (IVE) - Pleven, in the period 2016 - 2018. The objects of the present study were red wines, obtained from the Storgozia variety, growned in the region of Pleven town, Central Northern Bulgaria. The following different green pruning operations have been applied to the vine, respectively:

V1 - control sample wine without pruning operations;

V2 - wine produced after the operation - June topping shoot (+ suckering for harvest 2017);

V3 - wine obtained after the operation - thinning cluster (+ suckering for harvest 2017);

V4 - wine obtained after the operation - July topping shoot (+ suckering for harvest 2017);

V5 - wine obtained after the operation - removal of leaves (defoliation) (+ suckering for harvest 2017).

The examined wines were of the harvests 2016 and 2017. The used grapevine variety was a hybrid selected in IVE. His parental forms were:

• Storgozia - Bouquet x Villar Blanc *(*Roychev, 2012*)*

Experimental vineyards were grown at the Experimental Base of IVE. The variety was located on an area of 0.2 ha. The grapes were harvested and vinified at the Experimental Wine Cellar of IVE. A classic scheme for the production of dry red wines (Yankov, 1992) was applied - crushing and destemming, sulphitation (50 mg/kg SO_2), inoculating with pure culture dry yeasts *Saccharomyces cerevisiae* Vitilevure CSM - 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 - 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 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 (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 included the following compounds (purity > 99.0%): acetaldehyde, ethyl acetate, methanol, isopropyl acetate, 1-propanol, 2-butanol, propyl acetate, 2-methyl-1-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, ethyl hexanoate, hexyl acetate, 1-heptanol, linalool oxide, phenyl acetate, ethyl caprylate, a-terpineol, nerol, β - citronellol, geraniol. As an internal standard 1 -octanol was used. The 2 ul 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 urn), equipped with a flame ionization detector (FID). The used carrier gas was He. Hydrogen for support the combustion was supplied to the chromatograph via a hydrogen bottle. The injection was manually, by microsyringe.

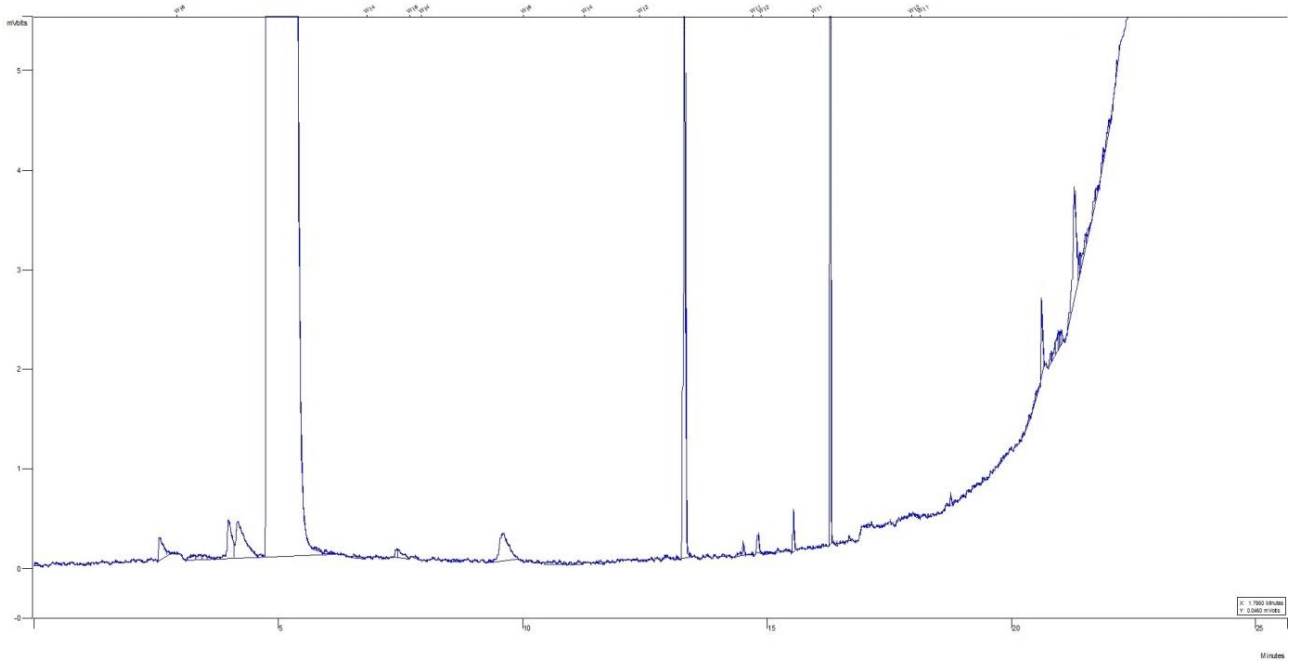
The parameters of the gas chromatographic determination were: injector temperature - 220 °C; detector temperature - 250 °C, initial oven temperature - 35 °C/retention 1 min, rise to 55 °C with step of 2 °C/min for 11 min, rise to 230 °C with step of 15 °C/min for 3 min. Total time of chromatography analysis - 25.67 min.

After determination of the retention times of the aromatic compounds in the standard solution the identification and quantification of the volatile aromatic substances in the wines was established. 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.

3. RESULTS AND DISCUSSION

The chromatographic profiles of the researched variants of red wines from the Storgozia variety are presented in Figures 1 - 5.



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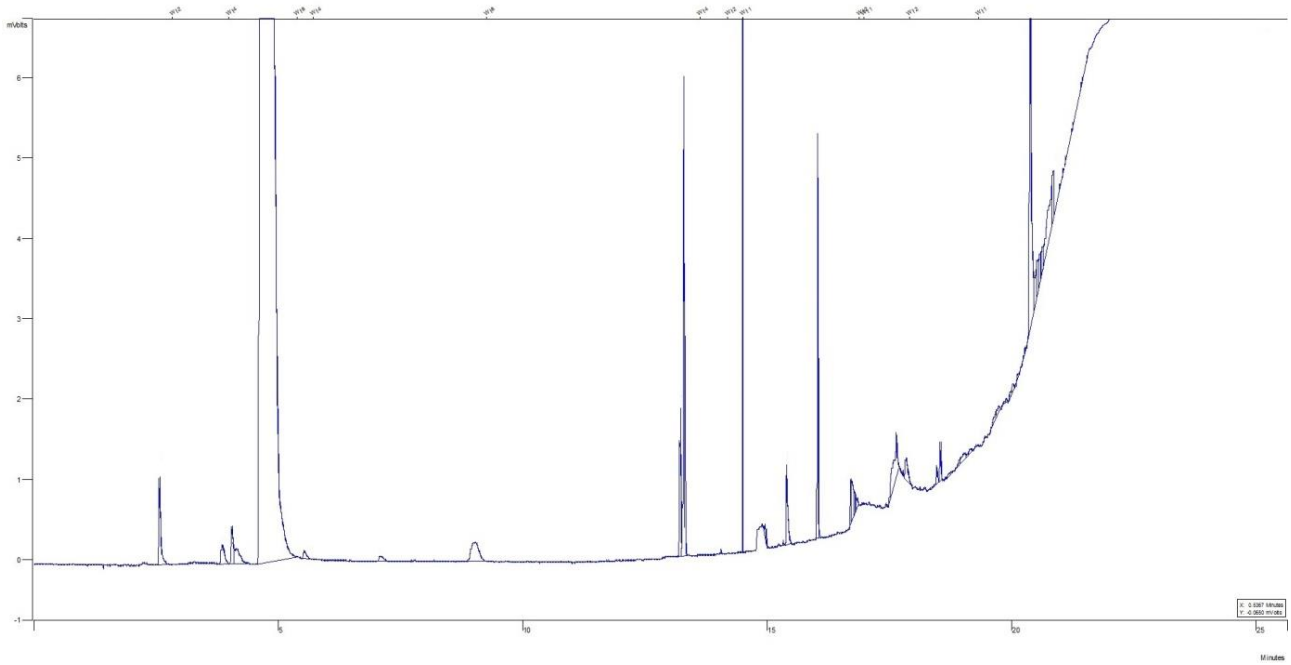
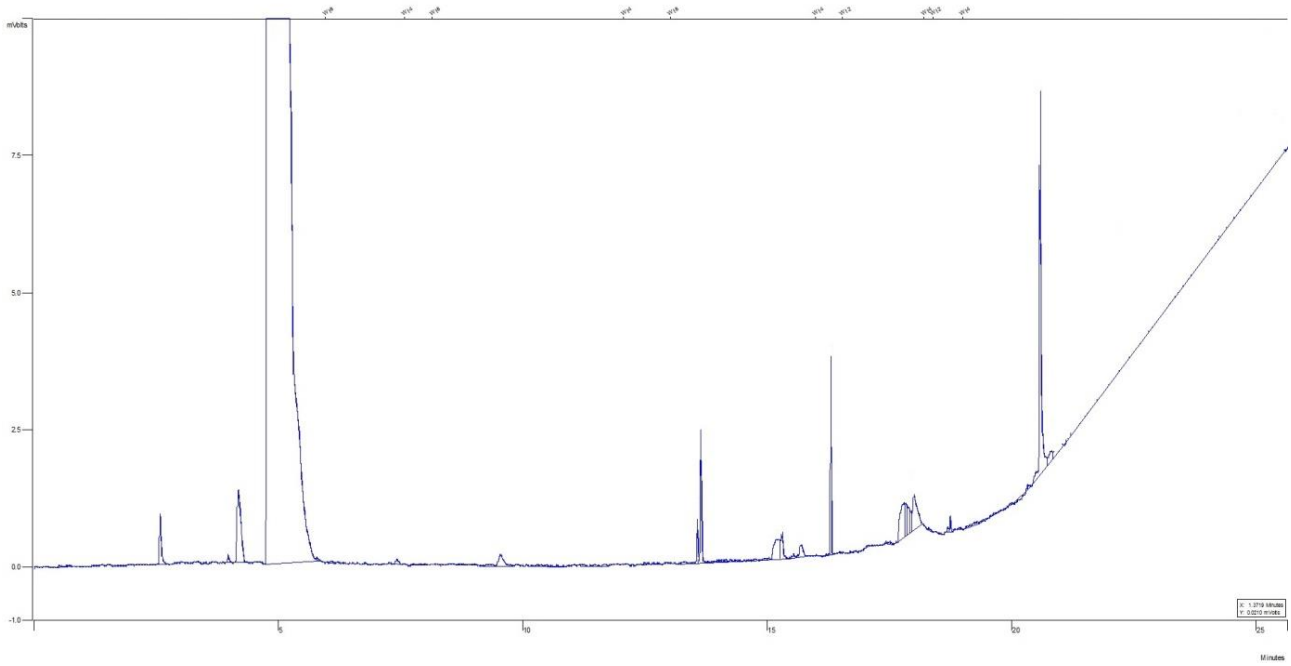


B)

Figure 1. Chromatographic profile of red wine from Storgozia variety, variant V1
A) Harvest 2016; B) Harvest 2017A



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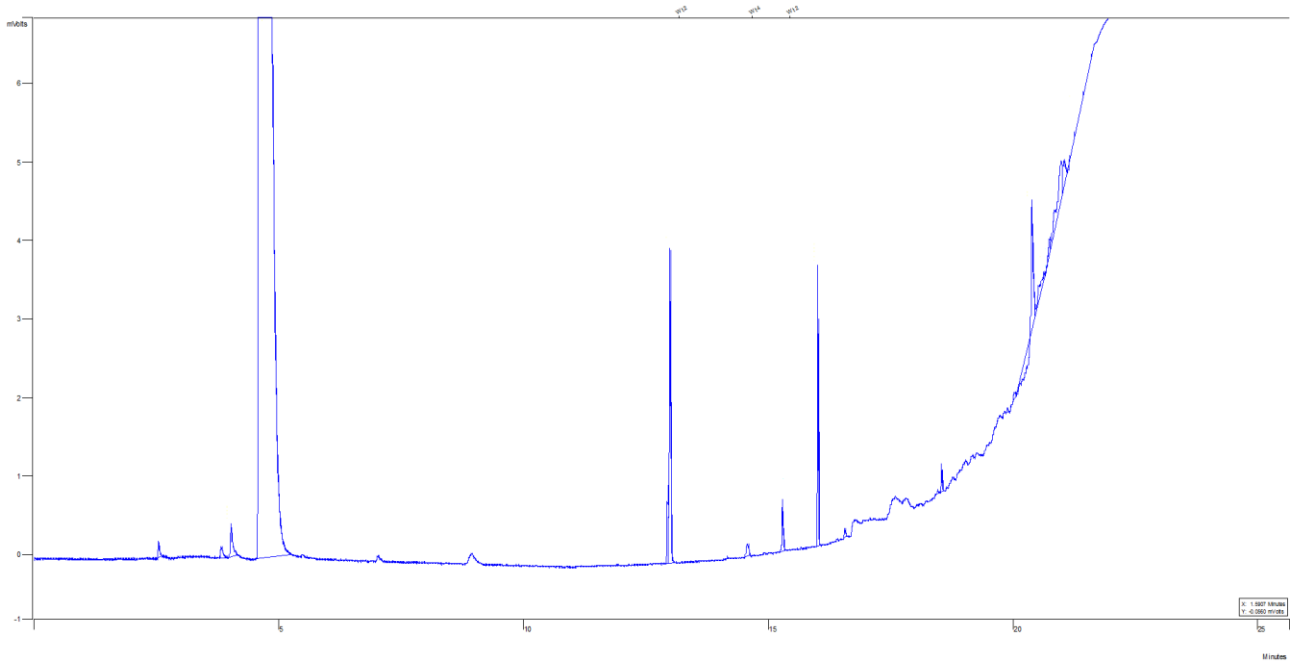

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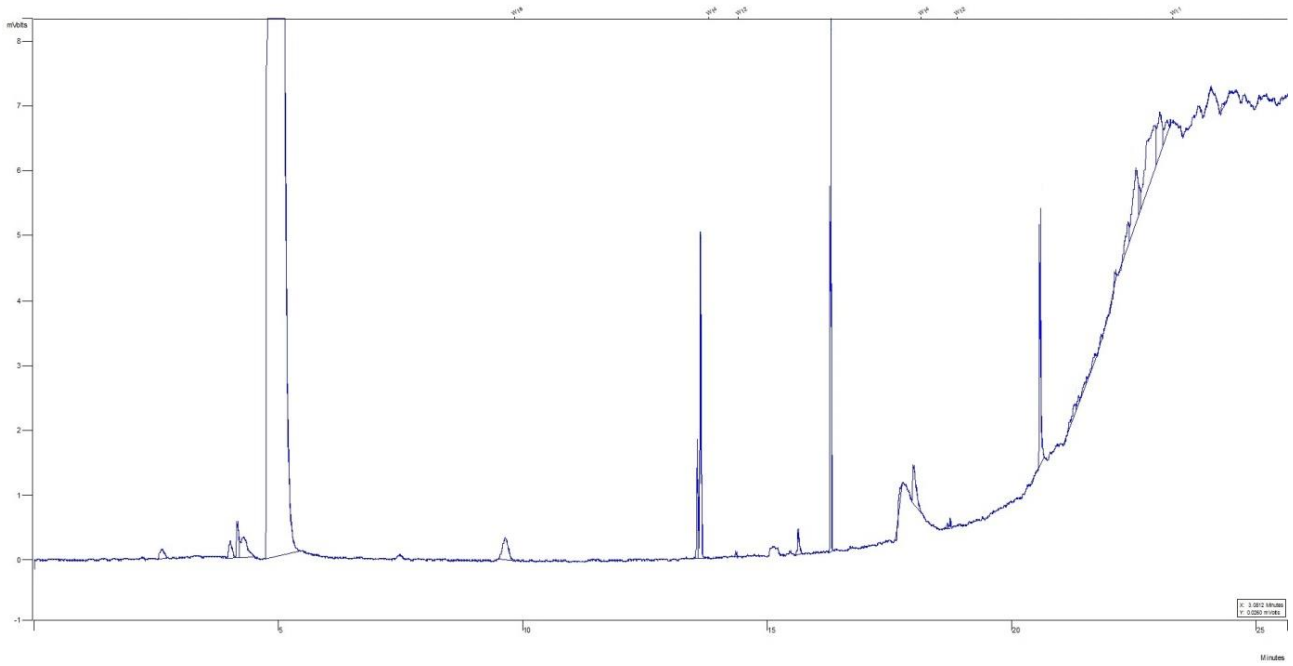
Figure 2. Chromatographic profile of red wine from Storgozia variety, variant V2
A) Harvest 2016; B) Harvest 2017



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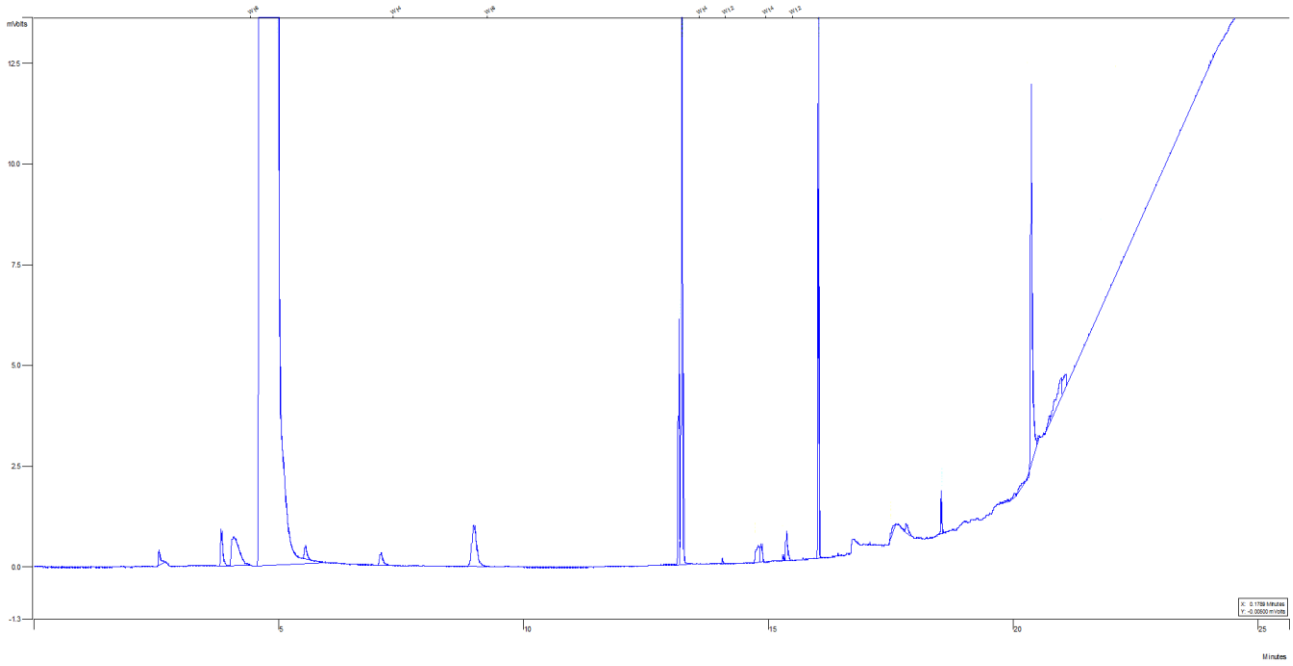
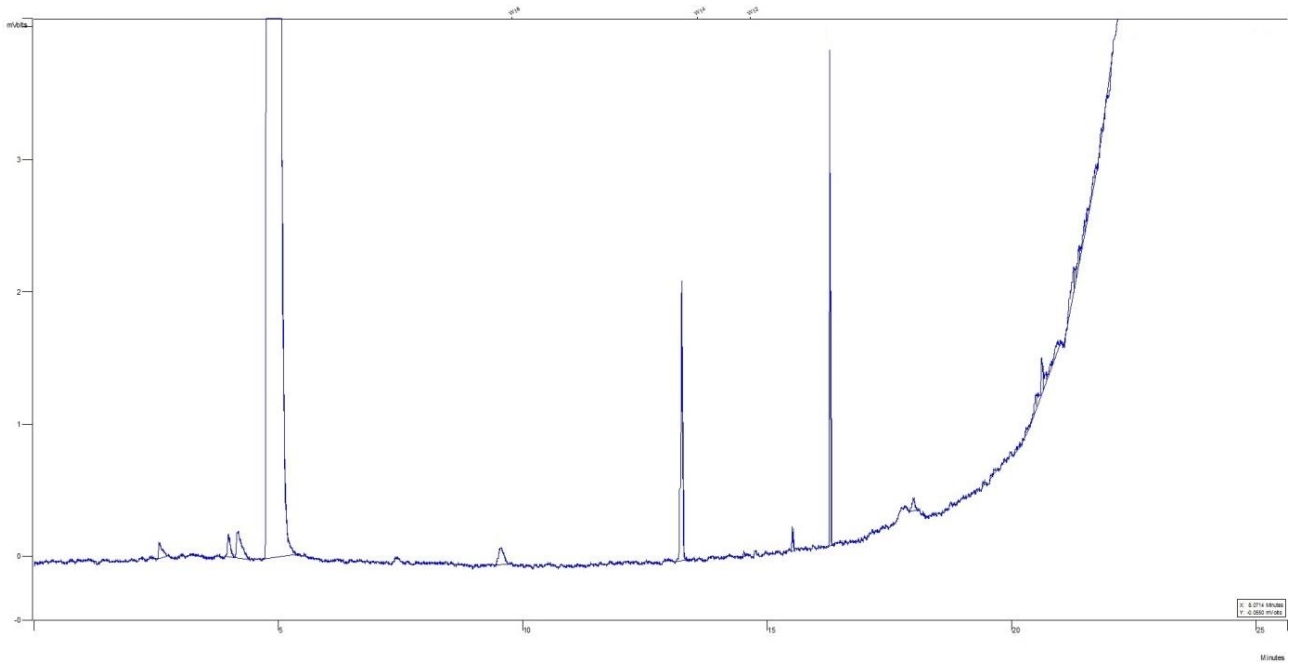


B)

Figure 3. Chromatographic profile of red wine from Storgozia variety, variant V3
A) Harvest 2016; B) Harvest 2017



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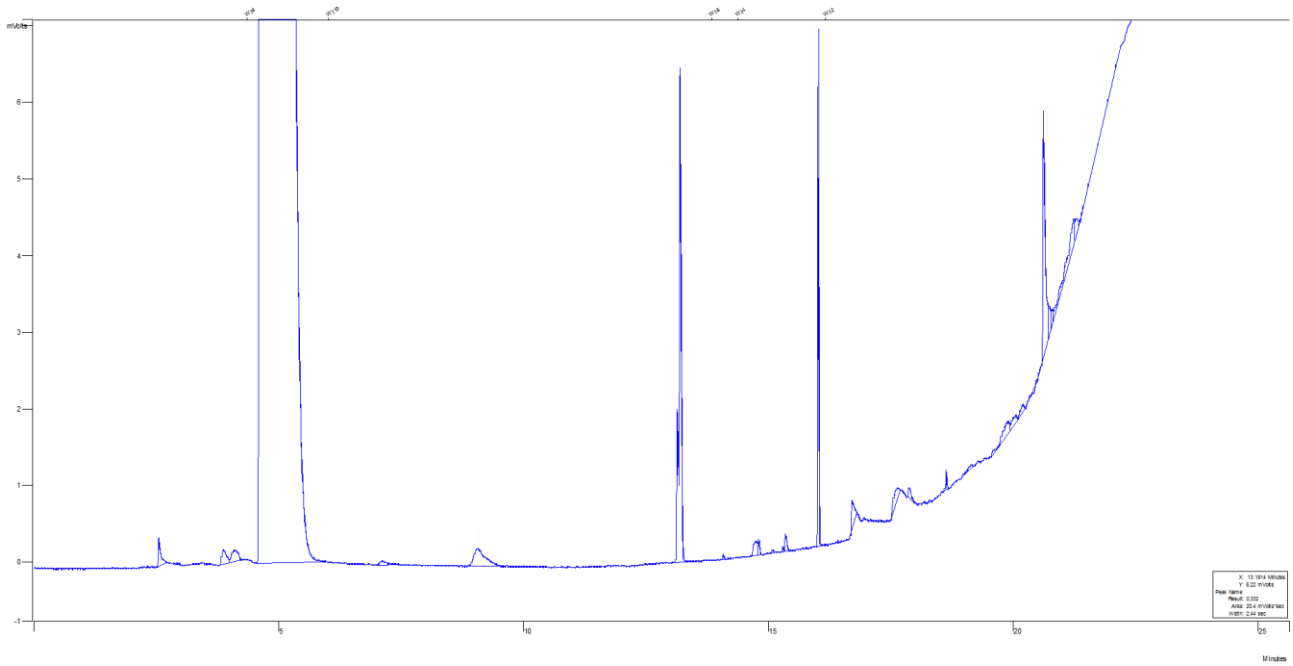
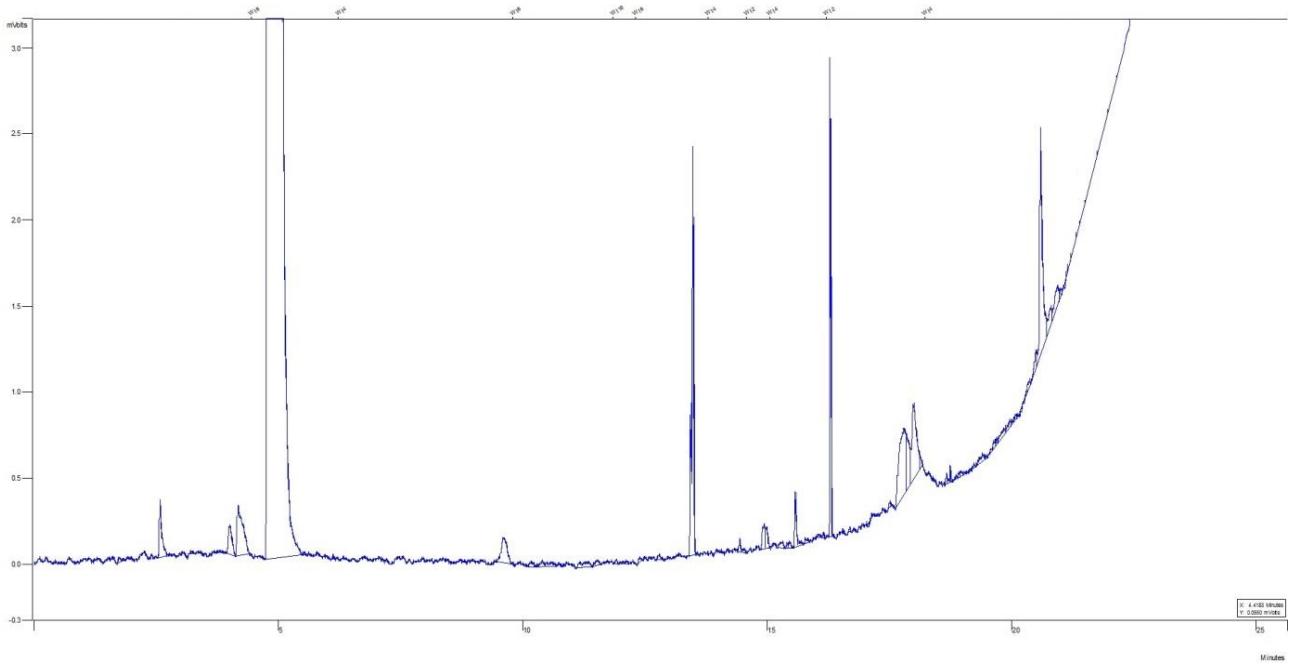


B)

Figure 4. Chromatographic profile of red wine from Storgozia variety, variant V4 B) Harvest 2016; B) Harvest 2017



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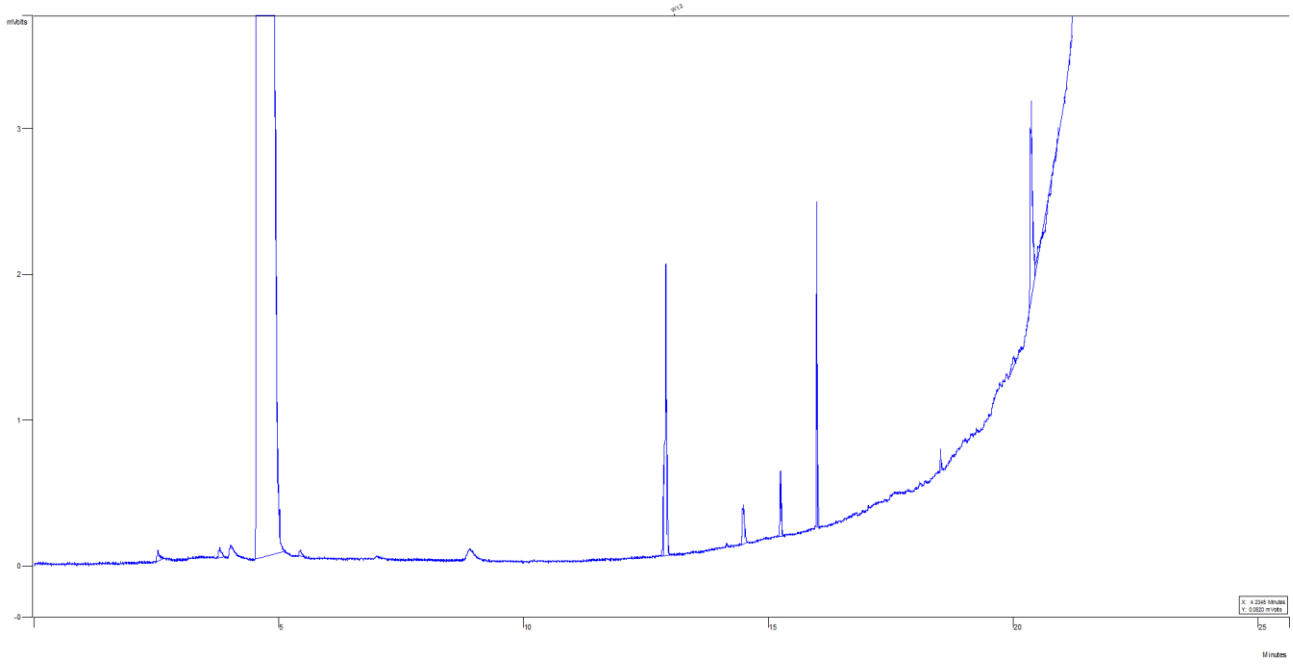

B)

Figure 5. Chromatographic profile of red wine from Storgozia variety, variant V5
A) Harvest 2016; B) Harvest 2017

Table 1. Content of volatile compounds in red wines from Storgozia variety, harvests 2016 and 2017, with various green pruning operations

IDENTIFIED COMPOUNDS, mg/dm^3	STORGOZIA /HARVEST 2016/					STORGOZIA /HARVEST 2017/				
	V1	V2	V3	V4	V5	V1	V2	V3	V4	V5
Ethyl alcohol, об.%	13.87	13.82	11.25	13.46	14.03	12.73	13.32	14.18	13.99	12.67
Acetaldehyde	26.04	130.89	35.36	40.32	59.27	56.81	36.23	17.85	38.75	35.40
Methanol	107.00	ND	110.00	≈0.05	ND	81.56	115.25	156.05	54.83	48.20
2-methyl-1-butanol	ND	49.13	ND	ND	ND	81.05	56.97	88.73	≈0.05	61.51
3-methyl-1-butanol	194.52	89.25	25.38	179.52	172.36	168.60	159.51	169.08	206.53	150.58
1-propanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-butanol	ND	ND	ND	ND	ND	≈0.05	ND	9.35	≈0.05	ND
1-pentanol	ND	≈0.05	ND	ND	ND	≈0.05	ND	≈0.05	≈0.05	ND
1-hexanol	ND	9.34	≈0.05	≈0.05	≈0.05	29.07	20.83	7.97	ND	≈0.05
1-heptanol	ND	ND	ND	ND	ND	15.86	ND	ND	ND	ND
Total higher alcohols	***194.52***	***147.77***	***25.43***	***179.57***	***172.41***	***294.68***	***237.31***	***275.18***	***206.68***	***212.14***
Ethyl acetate	56.45	291.81	37.19	63.98	89.31	46.33	38.45	29.46	28.11	24.30
Propyl acetate	≈0.05	≈0.05	ND	ND	ND	ND	ND	ND	ND	ND
Isopropyl acetate	≈0.05	≈0.05	ND	ND	ND	≈0.05	ND	17.28	ND	196.13
Butyl acetate	≈0.05	ND	ND	ND	≈0.05	ND	ND	ND	ND	ND
Isobutyl acetate	≈0.05	ND	ND	ND	ND	ND	ND	82.58	85.61	ND
Ethyl butyrate	11.57	14.29	13.72	10.09	11.45	20.85	ND	ND	ND	ND
Ethyl isovalerate	ND	ND	6.42	ND	ND	ND	ND	ND	ND	ND
Ethyl caprylate	≈0.05	≈0.05	ND	ND	≈0.05	≈0.05	ND	ND	ND	ND
Ethyl hexanoate	≈0.05	ND	ND	ND	ND	ND	≈0.05	ND	ND	ND
Pentyl acetate	ND	ND	≈0.05	ND	ND	33.74	≈0.05	ND	9.69	≈0.05
Hexyl acetate	≈0.05	≈0.05	ND	ND	≈0.05	34.24	ND	ND	31.15	ND
Phenyl acetate	≈0.05	34.72	ND	ND	ND	≈0.05	≈0.05	2.85	≈0.05	≈0.05
Total esters	***68.42***	***341.02***	***57.38***	***74.07***	***100.91***	***135.31***	***38.60***	***132.17***	***154.61***	***220.53***
α – terpineol	≈0.05	≈0.05	ND	≈0.05	ND	≈0.05	ND	ND	≈0.05	≈0.05
Linalool oxide	≈0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nerol	≈0.05	ND	ND	ND	≈0.05	≈0.05	≈0.05	≈0.05	≈0.05	≈0.05
β – citronellol	≈0.05	≈0.05	ND	≈0.05	≈0.05	≈0.05	ND	≈0.05	0.13	ND
Geraniol	0.48	0.13	0.29	≈0.05	0.40	0.34	0.12	0.10	0.52	0.65
Total terpenes	***0.68***	***0.23***	***0.29***	***0.15***	***0.50***	***0.49***	***0.17***	***0.20***	***0.75***	***0.75***
TOTAL CONTENT	***396.66***	***619.91***	***228.46***	***294.16***	***333.09***	***568.85***	***427.56***	***581.45***	***455.62***	***517.02***

V1 - control sample wine without pruning operations; V2 - wine produced after the operation - June topping shoot (+ suckering for harvest 2017); V3 - wine obtained after the operation - thinning cluster (+ suckering for harvest 2017); V4 - wine obtained after the operation - July topping shoot (+ suckering for harvest 2017); V5 - wine obtained after the operation - removal of leaves (defoliation) (+ suckering for harvest 2017); ND – Not Detected.

4. CONCLUSION

The content of ethyl alcohol of the wines from harvest 2016 ranged from 11.25 vol. % (Variant V3) to 14.03 vol. % (Variant V5). For the wines from harvest 2017 this range of variation was 12.73 vol. % (Control variant V1) - 14.18 vol. % (Variant V3). It was clear that slightly higher levels of ethyl alcohol were found in the wines from harvest 2017 compared to 2016. This is probably due to better sugar accumulation in grapes from harvest 2017.

Only 4 higher alcohols have been identified in the wines from harvest 2016. 3-methyl-1-butanol (isoamyl alcohol) was quantitative dominant. In the wines from harvest 2017 a much more complex species diversity of these components was found. In the red wines from this harvest 9 higher alcohols were found with predominant quantitative dominance of 2-methyl-1-butanol (active amyl alcohol) and 3-methyl-1-butanol (isoamyl alcohol). Comparing the established amounts of 2-methyl-1-butanol between the harvests, it was found that this compound in the wines from harvest 2016 was identified only in the wine of variant V2 at amount of 49.13 mg/dm^3. In harvest 2017, it was found in all analyzed red wines, in significantly higher content - 56.97 mg/dm^3 (variant V2) to 88.73 mg/dm^3 (variant V3).

An absolutely identical trend was observed with the other major higher alcohol - 3-methyl-1-butanol (isoamyl alcohol). For wines from harvest 2016 it ranged from 25.38 mg/dm^3 (variant V3) to 194.52 mg/dm^3 (variant V1). For the wines of the next harvest 2017, the 3-methyl-1-butanol levels were significantly higher - 150.58 mg/dm^3 (Variant V5) - 206.53 mg/dm^3 (Variant V4).

The established total content of higher alcohols in the wines showed a tendency for a higher quantities in the wines from harvest 2017 (206.68 mg/dm^3 - 294.68 mg/dm^3) compared with harvest 2016 (25.43 mg/dm^3 - 194.52 mg/dm^3).

It is clear from the obtained results for the concentration of higher alcohols (as individual representatives and total presence) that the application of the green pruning operation “suckering” in all variants of the harvest 2017 reflected to the higher final levels of higher alcohols in the obtained wines, compared to those from the harvest 2016. This operation probably has a positive effect on the vine metabolism, which is likely to form a high amount of amino acids in the grapes (one of the major precursors for the formation of higher alcohols in wines) and better sugar accumulation. This probably led to increased metabolic secretion of higher alcohols by the yeasts during fermentation.

It should also be noted that the established total content of higher alcohols in all wines examined correlated with the range of their normal presence, indicated by Abrasheva et al. (2008).

In the wines from harvest 2016, 12 esters were identified. In the wines from harvest 2017 the number of identified esters was the same. Dominant and practically established in all wines studied was ethyl acetate. In the wines from harvest 2016 the lowest content of this ester was observed in the wine from variant V3 (37.19 mg/dm^3). The highest content of ethyl acetate was found in the wine of variant V2 (291.81 mg/dm^3). In this variant, the amount of the ester exceeded the allowed concentrations for young wines - 50.00 - 80.00 mg/dm^3 (Chobanova, 2012). By overtaking the thresholds, it deteriorated the wine quality.

For the wines from harvest 2017, the amount of ethyl acetate ranged from 24.30 mg/dm^3 (variant V5) to 46.33 mg/dm^3 (variant V1). The trend of higher amounts of ethyl acetate in the wines from harvest 2016 was noticeable. Another important ester found in all variants of wines from harvest 2016 was ethyl butyrate. Its quantity in this wines ranged from 10.09 mg/dm^3 (variant V4) to 14.29 mg/dm^3 (variant V2). This ester was found in Cabernet Sauvignon wine from China (Tao and Li, 2009). In wines from harvest 2017, it was found only in the control variant V0 - 20.85 mg/dm^3.

From the obtained result, it can be concluded that the green pruning operation "suckering", as manipulation, was probably affected the subsequent synthesis of this ester during fermentation by blocking it.

The total ester content of the variants from harvest 2016 ranged from 57.38 mg/dm^3 (variant V3) to 341.02 mg/dm^3 (variant V2). The higher amount of ethyl acetate in variant V2 (291.81 mg/dm^3) was primarily responsible for the final high total ester content in this variant. In this case it affected wine negatively, giving an unpleasant acetic-acid taste.

For the wines from harvest 2017, the total ester content ranged from 38.60 mg/dm^3 (variant V2) to 220.53 mg/dm^3 (variant V5). By comparing the individual variants (except variant V2) between the different harvests, a tendency (as in the case of higher alcohols) to a higher final ester content in wines from harvest 2017 produced from grapes of grape vines with applied operation "suckering" was again observed. This confirms the impact of this practice on the

complication of the chemical volatile composition of the wines obtained.

The data for the ester content were in correlation with the range presented by Chobanova (2012).

The aldehyde fraction was represented by its main component, acetaldehyde. Its presence in variants of wines from harvest 2016 ranged from 26.04 mg/dm^3 (variant V1) to 130.89 mg/dm^3 (variant V2). For wines from harvest 2017 its content ranged from 17.85 mg/dm^3 (variant V3) to 56.81 mg/dm^3 (variant V1). Exceeding the allowable levels this compound may have a negative impact and give the oxidized tone to the wine. The data on the acetaldehyde content were correlated with the presented ranges of its presence by Chobanova (2012). Its influence on the aromatic profile of the analyzed wines in established concentration ranges was positive.

The group of terpenes was represented by identified 5 terpenic alcohols - α-terpinol, linalool oxide, β-citronellol, nerol and geraniol. They were identified in the wines of both harvests - 2016 and 2017. The main terpene, identified in all studied wines was geraniol. Its content in wines from harvest 2016 ranged from 0.05 mg/dm^3 (variant V4) to 0.48 mg/dm^3 (variant V1). In wines from harvest 2017 it was found in quantities of 0.10 mg/dm^3 (variant V3) to 0.65 mg/dm^3 (variant V5).

In the wines from the control variant V1 and variant V2 the geraniol content was higher in the wines from harvest 2016. In the other variants, the trend of higher geraniol content was observed for the wines from harvest 2017 compared to 2016. This confirmed the effect of the "suckering" operation on improving of the grapes quality.

The total terpenic content of the wines from harvest 2016 ranged from 0.15 mg/dm^3 (variant V4) to 0.68 mg/dm^3 (variant V1). For the wines from harvest 2017 it ranged from 0.17 mg/dm^3 (variant V2) to 0.75 mg/dm^3 (variants V4 and V5), confirming the effect of "suckering" on an increase in the total terpenic content of the grapes for the production of wine.

The presence of methyl alcohol was found in the examined wines. It is a normally present component of the volatile composition. Its presence is due to the presence of its precursor - pectin in the fruit, which is degraded to methanol from the pectolytic enzyme complex of the fruits (Marinov, 2005). The normal quantity of this component in the wines should be 60.00 - 230.00 mg/dm^3 (Abrasheva et al., 2008). For the wines from harvest 2016 it was found in concentrations of 0.05 mg/dm^3 (variant V5) - 156.05 mg/dm^3 (variant V4). For the wines from harvest 2017 its content was in the range of 48.20 mg/dm^3 (variant V5) - 156.05 mg/dm^3 (variant V3). Its presence was in normal concentrations, typical for red wines.

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