Influence of Maceration and the Addition of Flavoring Enzyme on the Aromatic Profile of Red Wines from the Region of Central Northern Bulgaria

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Abstract: Gas chromatographic study (GC-FID) for determination of the influence of maceration and addition of flavoring enzyme on the aromatic profile of red wines from Central Northern Bulgaria was conducted. The wines were obtained from selected clones (Gamza 52-9-4, Gamza 52-9-5 and Pamid 5/76) and varieties (Kaylashky rubin, Trapezitsa). Nineteen volatile compounds have been identified. Of these, 5 higher alcohols, 9 esters and 4 terpene alcohols affected the aroma of the wines. Methyl alcohol has been found in wines. Its concentrations were normal for red wines. The highest total concentration of volatile compounds was found in the Gamza clone 52-9-5 control (363.10 mg/dm^3). Gamza 52-9-4 clone, Kaylashky rubin and Trapezitsa varieties have been observed to increase the content of higher alcohols after addition of flavoring enzyme, while in the wines from Gamza 52-9-5 clone and Pamid 5/76 clone, the trend was reversed. The ester composition of the experimental samples was diverse. Increased ester content, after the addition of flavoring enzyme was found in Gamza 52-9-4 clone, Kaylashky rubin and Trapezitsa variety. In Gamza 52-9-5 and Pamid 5/76, the trend was reversed. The dominant ester was ethyl acetate. The highest content of terpene alcohols was observed in the wine from the control variant of clone Pamid 52-9-4 (0.69 mg/dm^3).

Keywords: *Maceration, Red wines, Enzyme, Aromatic profile, Methanol, Esters, Aldehydes, Higher alcohols, Terpenes.*

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INTRODUCTION

The varietal and quantitative diversity of aromatic components in wines are the main indicators for their quality. These components are products of a variety of chemical, biochemical and microbiological changes occurring in the grape maturation process and consequently in the production and aging of the wines. A large variety of volatile components, more than 800, has been found in studies on different worldwide wines (Aznar et al., 2001; Marti et al., 2003; Li, 2006; Kobayashi, 2008; Sumby, 2010). A wide concentration range for individual compounds is reported. The compounds are found in different concentrations ranging from mg/dm^3, in $\mu g/dm^3$ to ng/dm^3 (Rapp and Manderey, 1986; Ebeler, 2001; Sanchez-Palomo, 2007).

The volatile compounds with the most significant influence on the aromatic matrix of wines are esters, aldehydes, higher alcohols, terpene alcohols (Lambrechts and Pretorius, 2000; Vilanova et al., 2013). The compounds of these groups are present in wines in wide concentration ranges and have different thresholds of aromatic perception. In this way, they reflect in the wide and varied aromatic perception of the wine composition.

The total quantity of volatile compounds, many of which actively influence the wine aromatic sensory activity moves within the range 800.00 - 1200.00 mg/dm^3 (Ebeler, 2001; Lakatosova et al., 2013).

By arranging the aromatic groups of compounds according to their importance for the wine aroma, the best reflection is provided by the esters (Mason and Dufour, 2000; Ivanova et al., 2013). In wines they can be products of the yeast vital activity and the metabolism of other microflora (Swiegers et al., 2005a; Chobanova, 2012). In the wine aging process their quantity increases on the basis of the chemical mechanism of their formation - the interaction between the wine acids and alcohols (Chobanova, 2012). Their total concentration in young wines ranges from 2.00 to 200.00 mg/dm^3 (Yankov et al., 2000), and in the process of wine aging their concentration can significantly increase and may reach 1000.00 mg/dm^3 (Velkov, 1996).

The higher alcohols are aromatic compounds with high thresholds of aromatic perception. They can be products of both carbohydrate and amino acid yeasts metabolism (Etievant, 1991; Bell and Henschke, 2005). At the study of the volatile composition of Macedonian and Hungarian red wines, a variation in the alcohols content of 32.30 - 45.50 mg/dm^3 was found (Ivanova et al., 2013). Velkov (1996) indicates a variation in the content of higher alcohols in wines - 150.00 - 400.00 mg/dm^3. The diversity of higher alcohols is an important parameter, since at the wine aging they take part in the esterification processes and form esters when interacting with the wine acids (Perestrelo et al., 2006).

Terpenes are also a group wine aromatic components. They have a particular influence of the flavor of wines obtained from muscat grape varieties (Mateo and Jimenez, 2000; Fenoll et al., 2009).

The main and most concentrated of this group are terpene alcohols - linalool, α-terpineol, β-citronelol, nerol and geraniol (Wilson et al., 1986; Lee and Noble, 2003; Chobanova, 2012). Terpenes are the products of the vine metabolism from where they pass into the wine. In the strong muscat varieties their quantity ranges from 1.00 to 3.00 mg/dm^3, followed by varieties with average aromaticity (about 0.50 mg/dm^3) and slightly aromatic (0.10 - 0.20 mg/dm^3) (Velkov, 1996).

Compounds which enter into the volatile composition but do not have an aromatic effect can also be found in the wine. Such a compound is the methyl alcohol. It is obtained in the process of maceration on the base of degradation of the pectin in the fruit under the influence of the pectolytic enzyme complex (Marinov, 2005). Its content in red wines ranges from 36.00 - 350.00 mg/dm^3 (Chobanova, 2012).

The maceration (continuous contact of liquid with solids) is a widely applied technological practice in winemaking, in order to increase the extraction, increase of phenolic substances, microelements, vitamins, nitrogen substances in the obtained wines (Velkov, 1996).

The addition of enzyme systems, in particular β-glucosidase, leads to the hydrolysis of the glycosidic aromatic precursors of grapes must and wine, which may reflect in complicating and improving of the wine aromatic profile (Dignum et al., 2001; Wang et al., 2012).

The aim of the present study is to investigate the influence of the addition of flavoring enzyme on the volatile aromatic composition of red wines obtained from newly selected clones and grape varieties grown in the region of Pleven town, Central Northern Bulgaria.

Materials and methods

Grape varieties and vinification

The study was conducted at the Institute of Viticulture and Enology (IVE) - Pleven. The object of the study are red wines from two consecutive vintages (2015 and 2016) obtained from selected clones and newly selected grapevine varieties:

- Gamza clone 52-9-4 (Nakov et al., 2017)
- Gamza clone 52-9-5 (Nakov et al., 2017)
- Pamid clone 5/76 (Nakov et al., 2011)
- Kaylashky rubin (interspecies hybrid, obtained in IVE - Pleven) - Pamid x Hybrid VI 2/15 x Gamay noir x *Vitis amurensis* (Ivanov, 2016)
- Trapezitsa (interspecies hybrid selected in IVE - Pleven) - Danube gamza x Marseilles early (Ivanov et al., 2012)

The grapes were harvested at a technological maturity and were vinified in the Experimental Wine Cellar of IVE. A classic scheme for the production of dry red wines (Yankov et al., 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.

The grapes from the five studied varieties and clones was divided into two technological variants with a quantity of 30 kg grapes for each variant, as follows:

V1 - control variant

V2 – experimental variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation.

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).

Volatile content determination by GC-FID

Gas chromatographic determination of the volatile components in wine distillates was done. The content of major volatile aromatic compounds was determined on the basis of standard solution prepared in accordance with the IS method 3752:2005. The method describes the preparation of standard solution followed by a preparation of a solution with more compounds. The standard solution in this study include the following compounds (purity > 99.0%): acetaldehyde, acetone, 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, ethyl hexanoate, hexyl acetate, 1–heptanol, linalool oxide, phenyl acetate, ethyl caprylate, α-terpineol, nerol, β-citronellol, geraniol.

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 generated and supplied to the chromatograph via a hydrogen generator Parker Chroma Gas: Gas Generator 9200 (Parker, United Kingdom).

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.

Results and discussion

The resulting chromatographic profiles of the wine variants tested are presented in Figures 1-5.

Table 1 presents the quantities of volatile compounds identified in the studied wines.

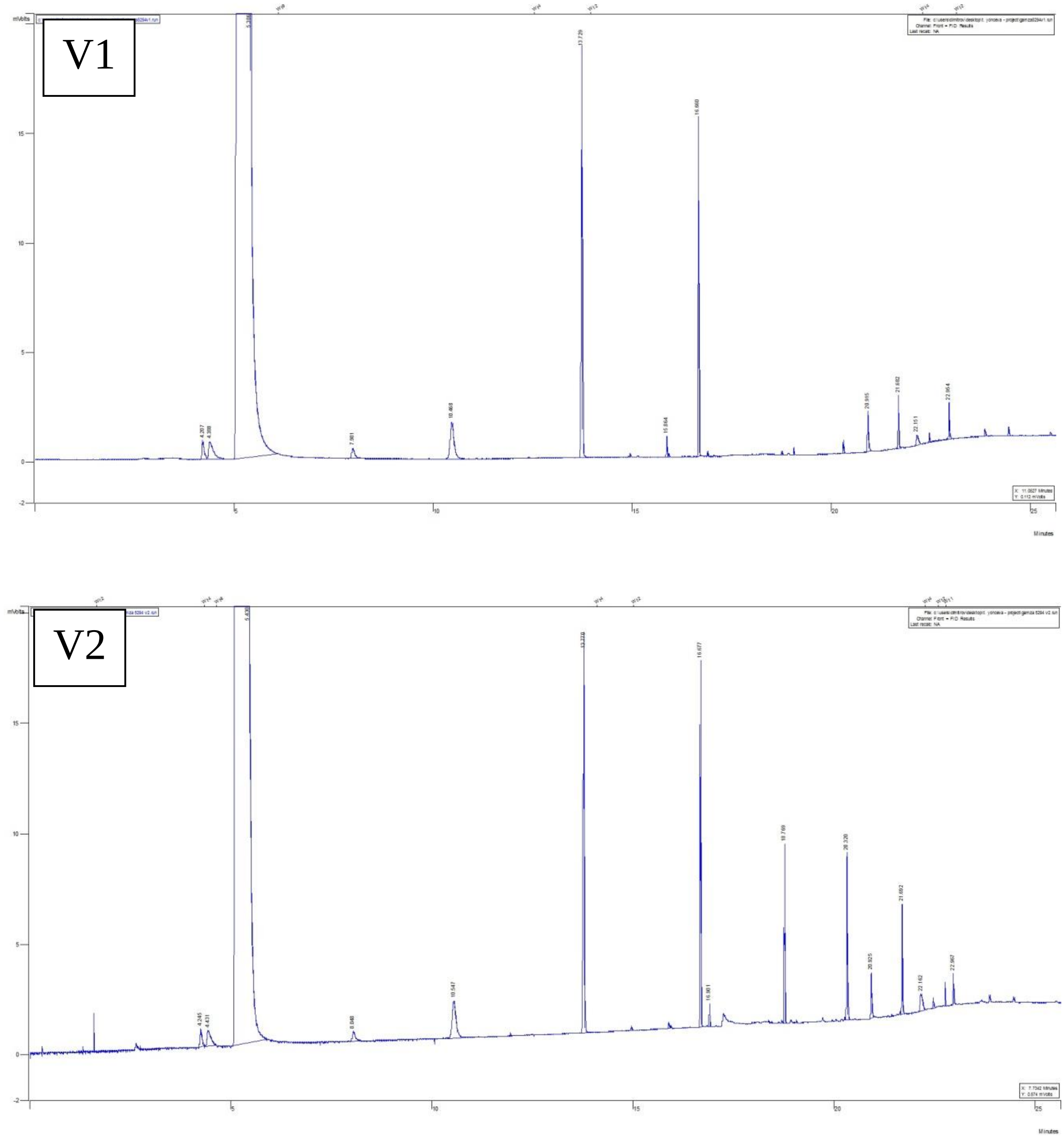

Figure 1. Chromatographic profile of red wine obtained from the selected clone GAMZA 52-9-4; V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation

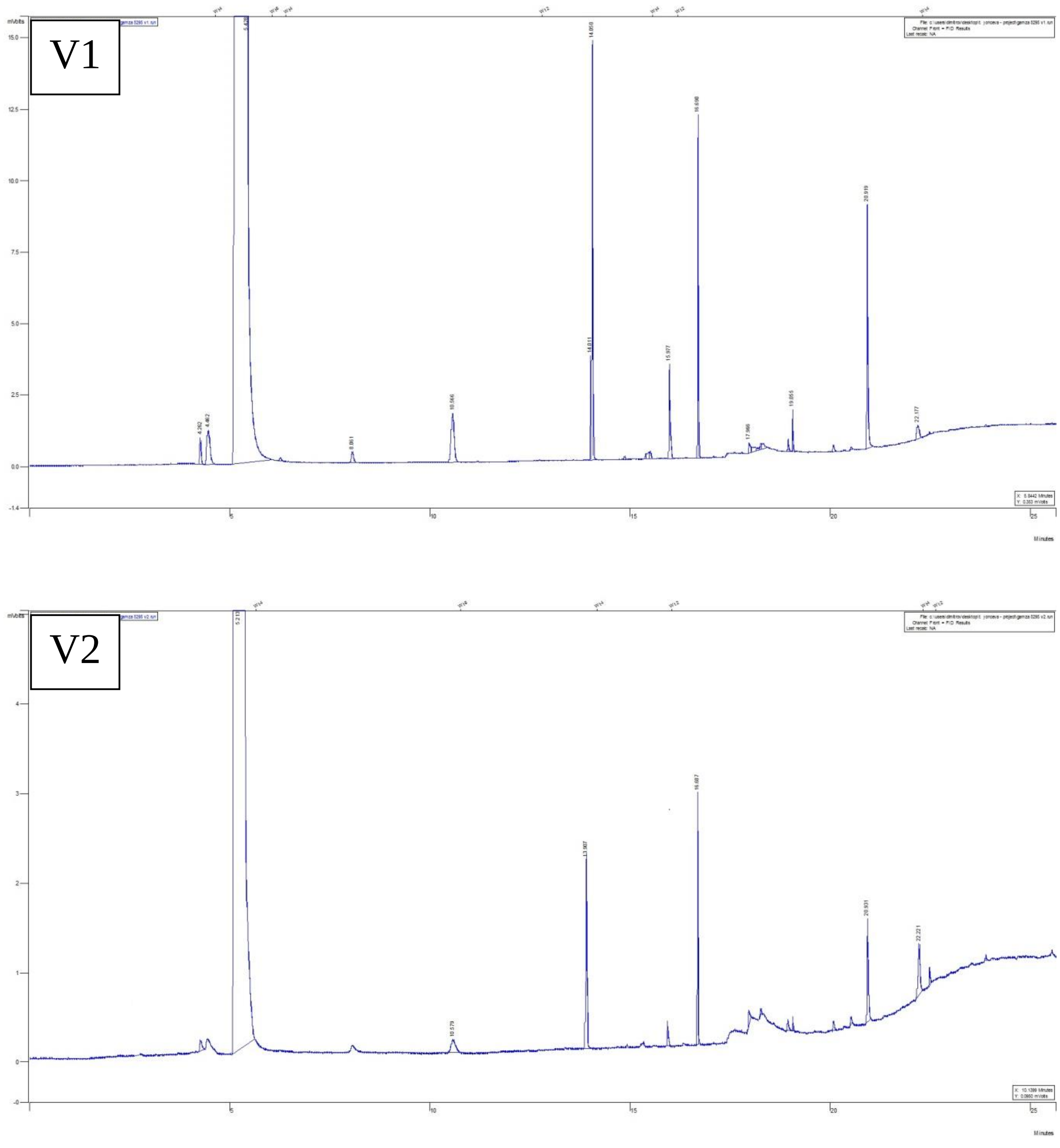

Figure 2. Chromatographic profile of red wine obtained from the selected clone GAMZA 52-9-5; V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation

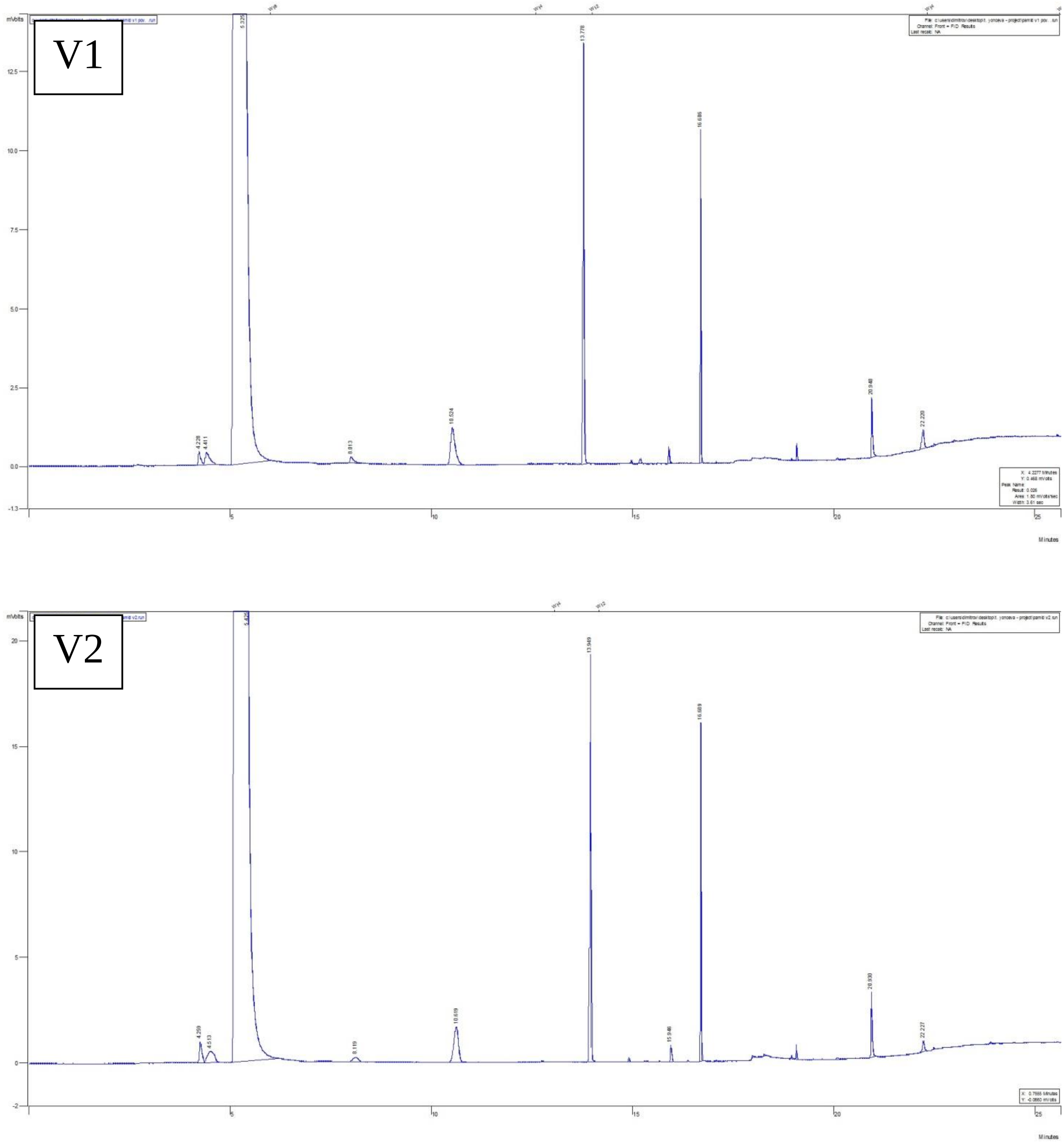

Figure 3. Chromatographic profile of red wine obtained from the selected clone PAMID 5/76, V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation

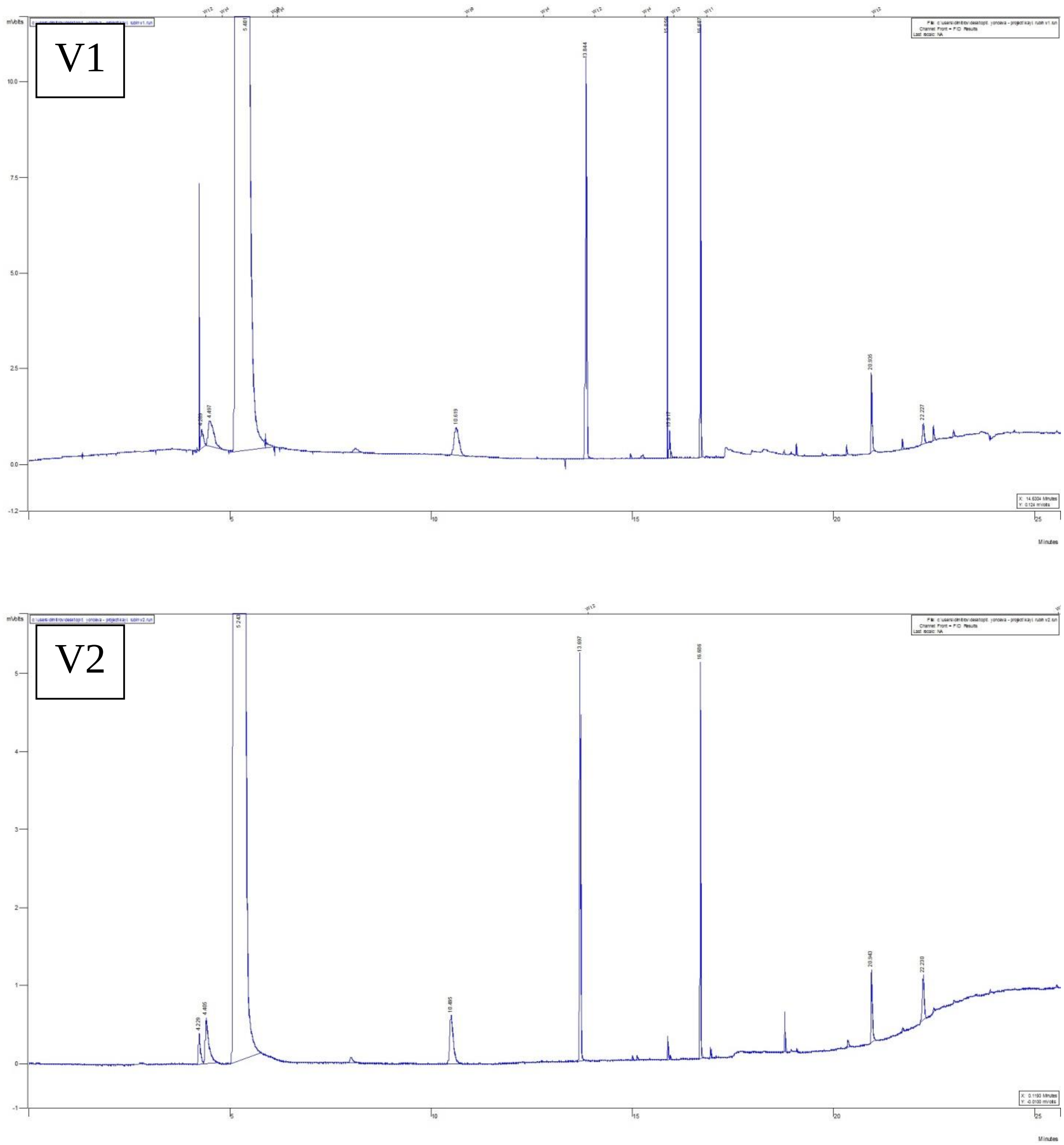

Figure 4. Chromatographic profile of red wine obtained from the selected variety KAYLASHKY RUBIN , V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation

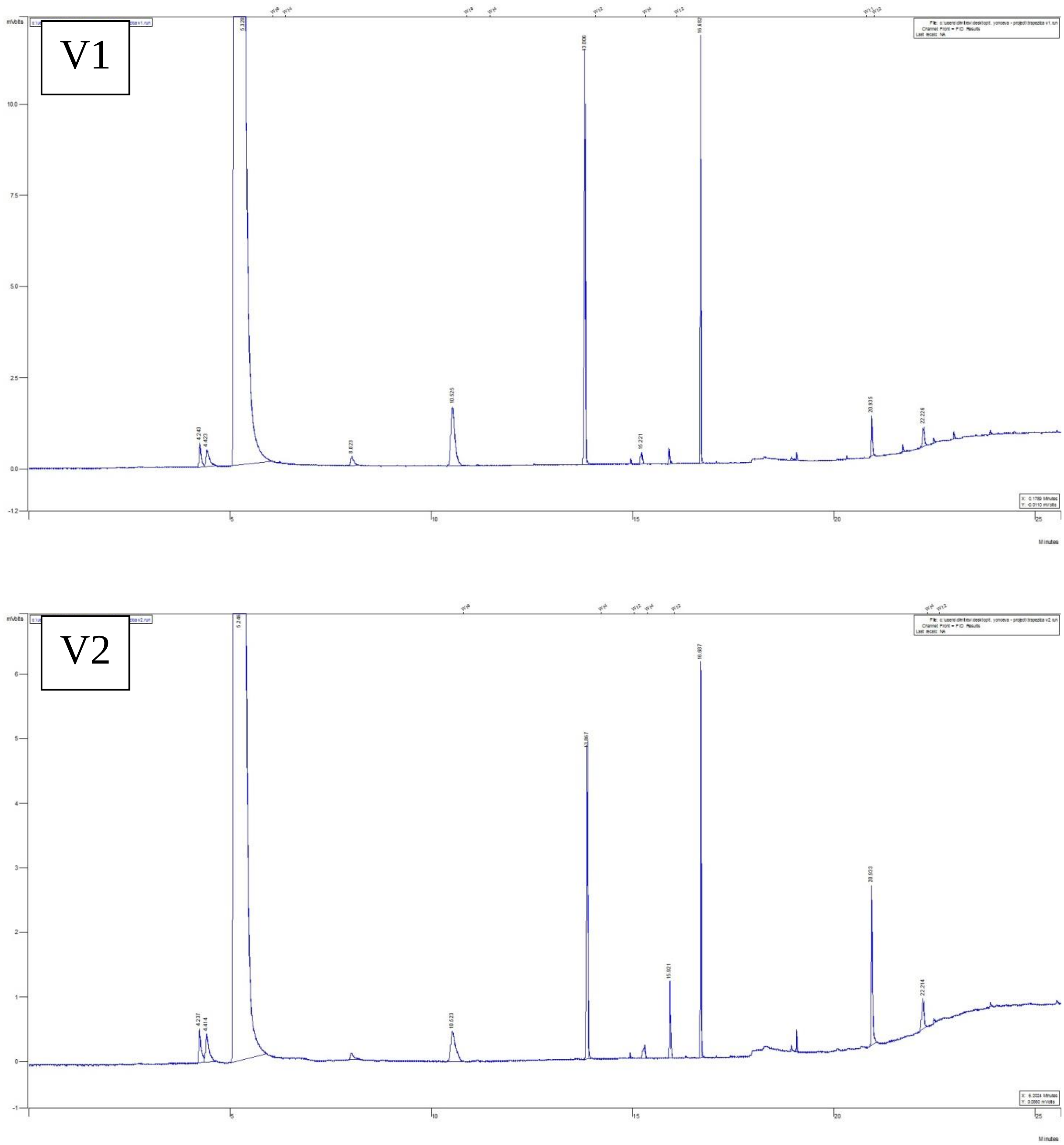

Figure 5. Chromatographic profile of red wine obtained from the selected variety TRAPEZITSA, V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation.

Table 1. Quantity of volatile compounds identified in the red wines examined

IDENTIFIED COMPOUNDS, mg/dm^3	**WINES**									
	GAMZA 52-9-4		**GAMZA 52-9-5**		**PAMID 5/76**		**KAYLASHKY RUBIN**		**TRAPEZITSA**	
	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
Ethyl alcohol, vol.%	15.51	15.56	14.60	14.64	13.39	12.97	13.08	13.29	12.55	12.43
Methanol	52.90	37.20	76.10	≈0.05	43.20	61.40	85.00	105.40	38.40	64.20
2-methyl-1-butanol	ND	ND	72.20	75.35	51.85	53.20	ND	48.25	ND	ND
2-methyl-1-propanol	ND	27.70	ND	ND	26.30	24.10	≈0.05	≈0.05	28.10	≈0.05
3-methyl-1-butanol	ND	ND	26.80	ND	ND	ND	ND	ND	ND	ND
1-pentanol	14.20	≈0.05	97.20	32.25	25.90	21.80	21.70	≈0.05	≈0.05	73.60
1-hexanol	≈0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total higher alcohols	***14.25***	***27.75***	***196.20***	***107.60***	***103.75***	***99.10***	***21.75***	***48.35***	***28.15***	***73.65***
Ethyl acetate	22.50	17.20	21.10	≈0.05	20.00	34.90	25.20	32.50	26.30	44.80
Propyl acetate	36.10	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isopropyl acetate	ND	ND	ND	ND	ND	ND	≈0.05	ND	ND	ND
Isobutyl acetate	63.40	57.90	69.60	36.90	86.90	87.90	53.80	75.30	153.90	97.20
Ethyl butyrate	ND	ND	ND	ND	≈0.05	≈0.05	≈0.05	ND	≈0.05	ND
Ethyl isovalerate	ND	≈0.05	ND	ND	ND	≈0.05	ND	ND	ND	ND
Ethyl hexanoat	ND	22.30	ND	ND	ND	ND	ND	≈0.05	ND	ND
Hexyl acetate	≈0.05	ND	≈0.05	ND	≈0.05	≈0.05	ND	≈0.05	≈0.05	ND
Phenyl acetate	≈0.05	50.30	ND	ND	ND	ND	≈0.05	≈0.05	ND	ND
Total esters	***122.10***	***147.75***	***90.75***	***36.95***	***107.00***	***122.95***	***79.15***	***107.95***	***180.30***	***142.00***
α – terpineol	ND	ND	ND	ND	ND	ND	≈0.05	ND	ND	ND
Nerol	ND	ND	≈0.05	≈0.05	≈0.05	≈0.05	ND	ND	ND	ND
β – citronelol	≈0.05	0.47	ND	ND	ND	ND	≈0.05	≈0.05	≈0.05	ND
Geraniol	0.64	0.12	ND	ND	ND	ND	≈0.05	≈0.05	≈0.05	ND
Total terpenes	**0.69**	**0.59**	**0.05**	**0.05**	**0.05**	**0.05**	**0.15**	**0.1**	**0.1**	**ND**
TOTAL CONTENT OF VOLATILE COMPOUNDS	**189.94**	**213.29**	**363.10**	**144.65**	**254.30**	**283.50**	**186.05**	**261.80**	**246.95**	**279.85**

The ethyl alcohol content of the studied red wines varied in the concentration range of 12.43 vol. % - 15.56 vol. %. This alcohol content meets the requirements for "dry wine" and is an indicator of sufficient and complete metabolic consumption of grapes sugars from yeasts.

Methyl alcohol has been identified practically in all wines. Only in variant V2 of the wine from Gamza clone 52-9-5 it was found in traces. The highest content of methyl alcohol was found in the wine variant V2 from the Kaylashky rubin variety - 105.40 mg/dm^3. Considering its concentrations in the different wine variants it can be observed that in the wines from the selected clones Gamza 52-9-4 and Gamza 52-9-5 there is a tendency of decrease in its levels between the two variants. In the case of Gamza clone 52-9-4 in variant V1 the methanol has been identified at content of 52.90 mg/dm^3 and a lower concentration of 37.20 mg/dm^3 was reported in the wine variant obtained with addition of enzyme - V2. The same trend was observed in the wine obtained from Gamza clone 52-9-5. In the other wines the trend was reverse. In the wine from Pamid clone 5/76, the methanol content in control variant V1 (43.20 mg/dm^3) was lower than the experimental (61.40 mg/dm^3). The same trend was observed in the wines obtained from Kaylashky rubin and Trapezitsa varieties.

The methyl alcohol is a normal product obtained as a result of maceration with solids. The concentrations of methyl alcohol in the wines found in this study were in full correlation with the range of its normal presence (up to 350.00 mg/dm^3) indicated by Chobanova (2012).

The gas chromatographic study identified 19 volatile compounds in the studied wines. There are 5 higher alcohols, 9 esters and 4 terpenes (terpene alcohols), which reflect the aroma of the wine. The highest total concentration of volatile compounds was established in the wine of variant V1 from Gamza clone 52-9-5 (363.10 mg/dm^3), followed by the wine variant V2 from Pamid clone 5/76 (283.50 mg/dm^3). The lowest content of volatile components was found in the wine variant V2 from Gamza clone 52-9-5 (144.65 mg/dm^3).

Regarding the presence of higher alcohols, higher levels of higher alcohols in the variant with added enzyme V2 (27.75 mg/dm^3) were observed in the wine from Gamza clone 52-9-4, compared to the control variant V1 (14.25 mg/dm^3). In the wine from Gamza clone 52-9-5, a reverse trend was observed - the content of higher alcohols was higher in the wine from control variant V1 (196.20 mg/dm^3) compared to the experimental variant V2 (107.60 mg/dm^3). It should be noted that in the wines from this clone was found to have higher quantities of higher alcohols than the other studied wines.

In the wine from clone Pamid 5/76, the total content of higher alcohols was similar, but with slightly higher concentration found in control variant V1 (103.75 mg/dm^3), compared to the experiment variant V2 (99.10 mg/dm^3).

In the case of red wine from the Kaylashky rubin variety, a reverse trend was found with the two-fold higher concentration of higher alcohols in the experimental wine V2 (48.35 mg/dm^3) compared to the control V1 (21.75 mg/dm^3). The same trend of distinct variations in higher alcohols concentrations of variant V2 (73.65 mg/dm^3) versus control (28.15 mg/dm^3) was observed in Trapezitsa wine.

As can be seen from the results obtained, a positive tendency to increase in the higher alcohols concentrations with the addition of flavoring enzyme in Gamza clone 52-9-4, Kaylashky rubin and Trapezitsa varieties was observed. While in the wines from Gamza clone 52-9-5 and Pamid clone 5/76 the trend was reversed.

The resulting concentrations of higher alcohols are in correlation with the studies and variability ranges represent from other researchers (Ivanova et al., 2013; Velkov, 1996).

The esters have the most significant influence on the aromatic characteristics of the wines. The diversity of higher alcohols and acids in wines leads to a diverse ester composition in the aging process, which reflects in different aromatic nuances.

Considering the total ester content of wine variants from clone Gamza 52-9-4, there was a tendency in increase of esters content after the addition of enzyme in variant V2 (147.75 mg/dm^3) compared to the control sample (122.10 mg/dm^3).

A trend of increased content of esters was also observed in the wine of Pamid 5/76. In this wine variant V2, obtained with the addition of flavoring enzyme (122.95 mg/dm^3) has a higher ester concentration compared with variant V1 (107.00 mg/dm^3). The same trend was observed in the wine of Kaylashky rubin - from 79.15 mg/dm^3 esters in control variant V1 to a significant increase in the experimental variant V2 - 107.95 mg/dm^3.

In the wines from clone Gamza 52-9-5 and Trapezitsa grape variety, a reverse trend was observed. Lower levels of total esters were found in the experimental variants compared to controls.

The highest total concentration of esters was found in the red wine variant V1 of Trapezitsa variety - 180.30 mg/dm^3, followed by the wine variant V2 obtained from clone Gamza 52-9-4 - 147.75 mg/dm^3.

The results for total ester content in wines correlate with the data presented by Yankov et al. (2000).

The main, dominant ester, found in all wines studied was ethyl acetate. This ester of ethanol and acetic acid are present in concentrations of 30 - 300 mg/dm^3, and in young wines in quantities of 50 - 80 mg/dm^3 (Chobanova, 2012). In the present study, the quantities found do not exceed the range indicated for young wines. This reflects on its positive aromatic influence. In the samples of Pamid clone 5/76, Kaylashky rubin and Trapezitsa varieties, its concentration was higher in the V2 variants compared to the control V1. In the wines of clone Gamza 52-9-4 and clone Gamza 52-9-5, this trend was reversed. The highest concentration of this ester is obtained in the experimental variant V2 of the Trapezitsa wine (44.80 mg/dm^3). The lowest was the level of ethyl acetate in the sample of variant V2 of Gamza 52-9-4 clone (17.20 mg/dm^3). At concentrations lower than 150 mg/dm^3, the ethyl acetate contributes to the wine fruit aroma (Tao and Li, 2009).

Ethyl hexanoate was identified in wine of variant V2 of clone Gamza 52-9-4. This ester was also available in variant V2 of the Kaylashky rubin variety, in traces. It is important and contribute to the fruit character of wines (Li et al., 2008).

Hexyl acetate was identified in wines from variants V1 of clone Gamza 52-9-4 and Gamza 52-9-5. Also in the two variants of the wine of the Pamid clone 5/76. It is also identified in the sample of variant V2 of the Kaylashki rubin variety and in variant V1 obtained from Trapezitsa grape variety. This ester gives characteristic pear aroma (Peinado et al., 2004).

Phenylacetate was identified in the wines obtained from Gamza clone 52-9-4 and Kaylashky rubin. This ester gives a floral aroma to the wine (Guth, 1997).

Terpene alcohols were also identified in the studied wines. The wine with the highest total concentration of terpene alcohols was variant V1 of the clone Gamza 52-9-4 (0.69 mg/dm^3), followed by variant V2 (0.59 mg/dm^3) from the same clone. The remaining wines have a very low content of terpene alcohols. With the highest concentration was found geraniol (0.64 mg/dm^3) in variant V1 of Gamza clone 52-9-4. This terpene was also found in the wine samples of the Kaylashky rubin and Trapezitsa varieties. The aroma of rose is the characteristic nuance it gives to (Hoffman et al., 1973).

β-citronelol was found in the highest amount in variant V2 of clone Gamza 52-9-4 (0.47 mg/dm^3). This terpene was also available in the wines of the Kailashky rubin and Trapezitsa varieties. The flavor that gives this terpene are lily and coniferous shrub (Lengyell, 2012).

Nerol was found in traces in wines from Gamza 52-9-5 and Pamid 5/76, and α-terpineol was found only in wine of V1 variant from the Kaylashky rubin variety.

Conclusions

1. The GC-FID study of red wines obtained from newly selected clones and varieties of vines identified a total of 19 volatile compounds. Of these, 5 higher alcohols, 9 esters and 4 terpene alcohols have been found to be aromatic.

2. Methyl alcohol was identified in all wines. It was in quantities typical for red wines.

3. The highest total concentration of volatile compounds was found in wine variant V1 of clone Gamza 52-9-5 (363.10 mg/dm^3) and the lowest in wine variant V2 obtained from the same clone (144.65 mg/dm^3).

4. There was a tendency for increasing of higher alcohols content after addition of flavoring enzyme at the wines from Gamza 52-9-4, Kaylashky rubin variety and Trapezitsa variety. In contrast,

in the samples of Gamza 52-9-5 and Pamid 5/76 a reverse trend was observed - a higher content of higher alcohols in the control variant.

5. The ester composition was diversed. A trend of increased ester content after addition of flavoring enzyme was observed in Gamza 52-9-4 clone, Kaylashky rubin and Trapezitsa varieties. At clones Gamza 52-9-5 and Pamid 5/76, the trend was reversed. The dominant ester was ethyl acetate. Its concentrations were normals for young wines. It exerts a positive influence on the aromatic profile.

6. The highest content of terpene alcohols distinguishes wine of variant V1 from clone Gamza 52-9-4 (0.69 mg/dm^3). With the highest concentration geraniol was identified. The wines of clone Gamza 52-9-4 have the highest total terpenes content compared to the others where they were low.

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