JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

# INFLUENCE OF MACERATION AND ADDED FLAVOR – RELEASING ENZYME ON THE AROMATIC COMPOSITION OF WHITE WINES

*Dimitar Dimitrov*, Tatyana Yoncheva, Vanyo Haygarov*

***Address(es):***
Institute of Viticulture and Enology, Department of Enology and Chemistry, 1 Kala Tepe str., 5800, Pleven, Bulgaria, phone number: +359 885 54 02 45.

*Corresponding author: dimitar_robertov@abv.bg

**doi: 10.15414/jmbfs.2017/18.7.3.248-253**

ARTICLE INFO

Received 24. 5. 2017
Revised 24. 6. 2017
Accepted 3. 10. 2017
Published 1. 12. 2017

Regular article

OPEN ACCESS

**ABSTRACT**

Study on the impact of maceration and maceration with the addition of flavor-releasing enzyme on the aromatic composition of white wines from grapes grown in Central Northern Bulgaria was conducted. White wines from varieties Dimyat, Vrachanski Muscat, Aligote, Muscat Ottonel and Plevenska Rosa were examined. The research was performed at the Institute of Viticulture and Enology, Pleven, Bulgaria in the period 2015 – 2016 year. In the tested wines were identified twenty four volatile compounds related to the basic aromatic groups – esters, higher alcohols, terpenes. Presence of acetaldehyde and methyl alcohol was found. The study demonstrated the following trend: the use of oenological practices - maceration and maceration with the addition of flavor-releasing enzyme have a positive influence on the aromatic composition of wines, increasing the total content of esters, higher alcohols, and terpenes. The amount of methyl alcohol established in part of the wines was within the range 0.007 - 0.030% of the total alcohol content, typical characteristics for white wines composition. The established content of acetaldehyde in the wines (53.80 - 191.00 mg/dm$^3$) was three times lower than the maximum limit. The comprehensive analysis of the results leads to the conclusion that the application of oenological practices - maceration (12h) and maceration (12h) with the addition of flavor-releasing enzyme in the production of white wines from studied varieties results in improved overall aromatic composition and increase their chemical complexity.

**Keywords:** Aromatic composition, alcohols, white wines, esters, maceration, terpenes

## INTRODUCTION

The content of aromatic components in various ratios is one of the main indicators defining the quality and authenticity of the wines. World wine science at this stage has identified more than 800 volatile compounds that directly affect the aromatic matrix of different wines (**Rapp and Manderey, 1986; Guth, 1997; Ferreira *et al*., 1998; Ebeler, 2001; Aznar *et al*., 2001; Marti *et al*., 2003; Perestrelo *et al*., 2006; Li, 2006; Kobayashi, 2008; Sumby, 2010; Ivanova *et al*., 2013**). These compounds belong to a number of groups, but the greatest impact provides esters, higher alcohols, aldehydes, and last but not least - terpene alcohols. Their concentrations, distribution, and ratio are directly dependent of many factors, including: a genetically determined ability of the vine to accumulate aromatic components in the grapes; the degree of technological maturity of the grapes; the impact of soil and climatic conditions; geographical location of the area where the variety is cultivated; alcoholic fermentation; the technological winemaking practices; the wine aging process (**Gomez *et al*., 1994; Bureau *et al*., 2000; Camara *et al*., 2006a; Oliveira *et al*., 2006; Sanchez-Palomo *et al*., 2007; Skinkis *et al*., 2010**).
Higher alcohols are the products of the yeast metabolism (**Etievant, 1991**). The selected yeasts (*Saccharomyces cerevisiae*), and some wild yeasts, have the ability to convert the amino acids of the grapes to high levels of alcohols during fermentation (**Lambrechts and Pretorius, 2000**). According to **Velkov (1996)** the content of higher alcohols in white wines is in the range of 150.00 – 400.00 mg/dm$^3$. An important feature of higher alcohols is the fact that in excess of their concentration they begin to exert a negative effect on the organoleptic characteristics of the wine.
Esters are the products of the esterification process, related to the interaction between the acids and alcohols in the wine. The formation of esters due to the reaction between acetyl-CoA and alcohols of the wine (**Etievant, 1991; Lee *et al*., 2004**). It is known that certain esters can also be formed as by-products of yeast carbohydrate metabolism (**Peddie, 1990; Fujii *et al*., 1994**). Their presence and diversity is appreciated as a very important parameter of the high quality wines. According to **Yankov et al. (2000)** the total ester content in young wines is in the range of 2.00-260.00 mg/dm$^3$, while in the older wines their quantity varies within the concentration range 792.00 - 800.00 mg/dm$^3$. The amount of ethyl acetate is dominant.
Terpenes and their derivatives are other group aromatic components of the wines. They, in contrast to the higher alcohols and esters, are not products of the yeast metabolism or chemical transformations in aging process and are a distinctive characteristic of the variety. The main representatives in the wines are linalool, geraniol, nerol, citronellol and α - terpineol (**Luan *et al*., 2006; Oliveira *et al*., 2008**). Their biosynthesis begins with acetyl - CoA (**Manitto, 1980**). They have a significant influence on the formation of so-called varietal aroma of the wines. Numerous studies have shown that terpenes contained in various food composites showed high antioxidant activity against reactive oxygen species having expressed negative role in the pathological development of cardiovascular, neurodegenerative and other diseases affecting human organism (**Gonzalez-Burgoz and Gomes-Serramillos, 2012**). The content of terpenes in wines and grape juices ranged from 0.10 - 0.80 mg/dm$^3$ in low and middle-aromatic varieties to 1.00 – 3.00 mg/dm$^3$ in highly aromatic Muscat varieties (**Velkov, 1996**).
These three groups of compounds (esters, higher alcohols, and terpenes) determine the formation of the basic flavor of each wine. It is claimed that the total amount of volatile aromatic compounds varies in the range of 0.80 - 1.20 g/dm$^3$ (800-1200 mg/dm$^3$) (**Lakatošova *et al*., 2013; Ebeler, 2001**).
Different oenological practices are applied to increase the wine aromatic capacity. They include maceration and the addition of various enzyme systems able to improve and enhance the aromatic characteristics of wines.
Maceration is a technological method, which basically represents standing of the raw material for the production of alcoholic beverages with solid parts, intensifying the extraction and accompanied by complex biochemical and

physicochemical processes, increasing the content of phenolic and nitrogenous substances, vitamins, microelements in the final product (**Velkov, 1996**).

The application of added enzymes is mainly associated with their participation in the hydrolysis of glycosidic aromatic precursors (**Wang *et al*., 2012**). Therefore, the addition of glycoside enzymes, in particular β-glucosidase, results in a process of enzymatic hydrolysis of the precursors present in grape musts and wines (**Dignum *et al*., 2001**).

The aim of the study is to investigate the influence of maceration and the addition of flavor-releasing enzyme on the aromatic composition of white wines produced from varieties grown in the area of Pleven town, Northern Bulgaria.

## MATERIAL AND METHODS

### Varieties and vinification

The study was conducted in the Institute of Viticulture and Enology (IVE), Pleven, Bulgaria in the period 2015 – 2016 year. Objects of this research are white wines from varieties Dimyat, Vrachanski Muscat, Aligote, Muscat Ottonel and Plevenska Rosa. The last variety was selected in IVE - Pleven via interspecies hybridization. It is characterized by resistance to low winter temperatures and diseases (**Ivanov, 2016**).

The vineyards are situated at the Experimental base of IVE, an area of 0.3 hectares for each variety. The climatic conditions are continental type and are characterized by large temperature variations.

The grapes are harvested when reached technological maturity and vinified at the Experimental Winery of IVE - Pleven. Classic scheme for the production of dry white wines was applied (**Yankov *et al.*, 1992**) comprising the technological operations crushing, draining, pressing, sulphitation (50 mg/dm$^3$ $SO_2$), clarification of the musts, inoculation with pure culture yeasts *Saccharomyces cerevisiae Vitilevure B + C* in an amount of 20 g/100dm$^3$, temperature of fermentation 20°C, decantation, further sulphitation and storage.

The total quantity of grapes from five varieties is 150 kg, 30 kg for each type. Three technological schemes were applied, using 10 kg grapes for each of the different variants: V1 – control variant; V2 - maceration with the solids for 12 hours before alcoholic fermentation; V3 - maceration with the solids for 12 hours and addition of flavor-releasing enzyme Zymovarietal Aroma G (Sodinal, Bulgaria) in an amount of 3 g/100 kg, before alcoholic fermentation.

### Determination of alcohol content of wines obtained

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 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 presented in the same sequence as the peaks in Fig. 1 are shown.

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.

The resulting chromatogram of the standard solution is shown in Fig. 1.

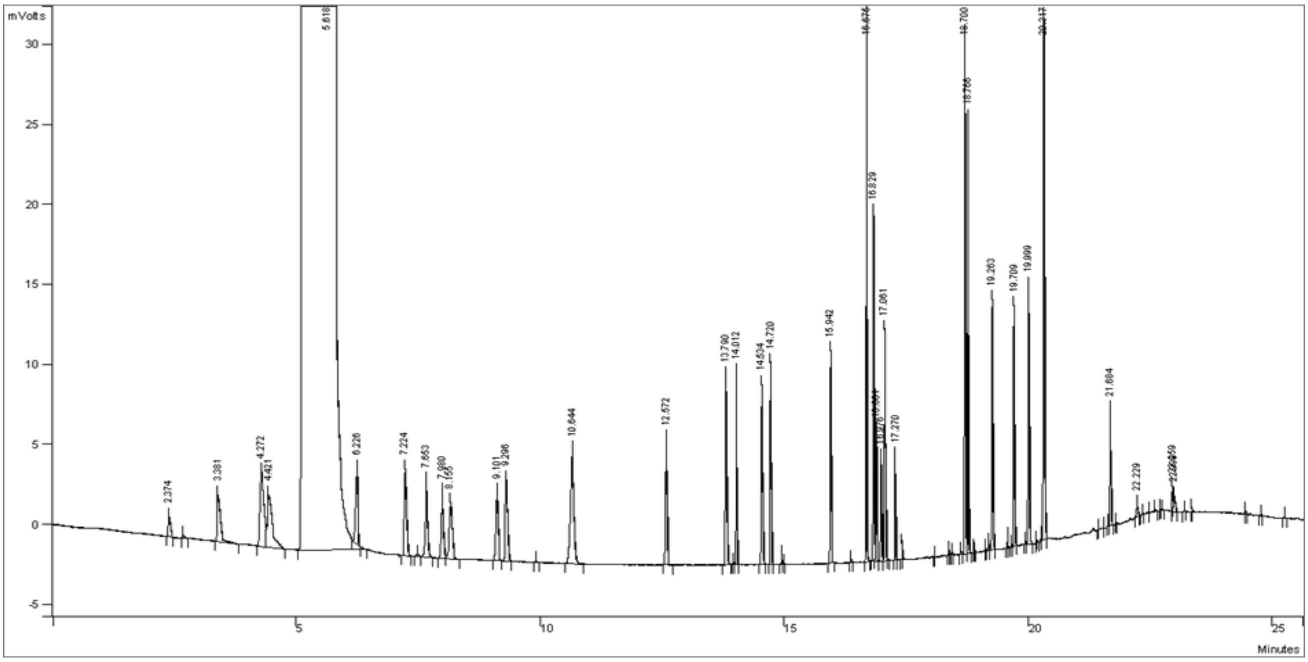


**Figure 1** Chromatographic profile of a standard solution of aromatic compounds

After determination of the retention times of aromatic compounds of 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. 5 cm$^3$ of each wine distillate and 1 cm$^3$ of internal standard solution (octanol) were placed in 10 cm$^3$ test tubes with a stopper. Identification and quantification of the aromatic substances in each of prepared samples were carried out (the samples were injected in an amount of 2 μl in the Chromatograph).

## RESULTS AND DISCUSSION

The results for the identification and quantification of the aromatic compounds and the alcohol content in the researched white wines are presented in Table 1.

Twenty four compounds were identified in wine samples examined using GC-FID.

The highest alcohol content was found in variant V3 of white wine from the Aligote (13.40 vol. %) variety, and the lowest in variant V1 from Dimyat variety (11.06 vol.%).

The total amount of volatile aromatic compounds (higher alcohols, esters, terpenes + acetaldehyde and methyl alcohol) in wines from Dimyat for the variants V1, V2 and V3, was distributed as follows: 105.27 mg/dm$^3$; 218.90 mg/dm$^3$; 256.77 mg/dm$^3$.

In the wine form Plevenska Rosa variety the quantitative trend of total aromatic composition was as follows for V1, V2 and V3: 279.25 mg/dm$^3$; 722.17 mg/dm$^3$; 260.12 mg/dm$^3$.

In the wine from Vrachanski Muscat the quantity of total volatile compounds for variants V1, V2 and V3, was as follows: 349.41 mg/dm$^3$; 509.95 mg/dm$^3$; 216.44 mg/dm$^3$.

The white wine from Aligote variety shows the total content of volatile aromatic components for variants V1, V2 and V3 respectively: 117.06 mg/dm$^3$; 301.47 mg/dm$^3$; 227.75 mg/dm$^3$.

The wine from Muscat Ottonel variety contain total amount of volatile aromatic compounds for the three variants V1, V2 and V3, as follows: 287.60 mg/dm$^3$; 290.19 mg/dm$^3$; 393.00 mg/dm3.

The obtained results for the total amount of volatile aromatic compounds in all studied white wines were typical for young wines. The total volatile composition concentration ranges up to 0.8 g/dm$^3$ (800.00 mg/dm$^3$), indicated in other studies (**Lakatošova *et al*., 2013; Ebeler, 2001**).

Almost in all tested wines (except for wine from Plevenska Rosa variety) was observed positive and similar trend - increasing total volatile composition of the wines after the application of maceration for 12h (V2) and maceration for 12h with the addition of flavor-releasing enzyme (V3). Compared to their controls, in samples V2 and V3 was observed proportional growth of aroma-defining compounds, which increases the complexity of the wine flavor when applying both oenological practices.

The results for total content of higher alcohols in white wines studied clearly indicate (in almost all experimental variants) an increase in the total content when the technological operations in V2 and V3 were applied. An exception of this trend was observed only in white wines from Dimyat, with final levels of higher alcohols in the experimental variants (V2 and V3) close to that of the control (V1).

Wine from Plevenska Rosa showed trend of significant growth in the total content of higher alcohols in the experimental variants (V2 and V3). Particularly active increase in quantity of higher alcohols was established in variant V2 - 357.05 mg/dm$^3$, in comparison with the control variant V1 - 75.05 mg/dm$^3$. This growth in the concentration was five times resulting in a significant improvement of the aromatic matrix, increasing the complexity of the aromatic composition of the white wine from Plevenska Rosa variety.

Similar trend was observed in the wine from Vrachanski Muscat. The highest amount of alcohols in wine was established in variant V2 - 233.56 mg/dm$^3$, compared with the control variant V1 - 31.75 mg/dm$^3$ - seven fold increase in the concentration of higher alcohols using application of the oenological practice maceration.

Wine from Aligote variety showed gradual augmentation of the total content of higher alcohols comparing the control variant V1 and experimental variants V2 and V3. This proves that both operations (maceration and maceration with the addition of flavor-releasing enzyme) have a positive influence on the processes of higher alcohols formation and reflected their total content.

The wine from Muscat Ottonel variety also showed a trend of enhanced flavor-releasing activity when applying the studied oenological practices. In variant V2 was reached five fold increase in the concentration of higher alcohols – 157.15 mg/dm$^3$ in comparison with the control – 29.82 mg/dm$^3$. For the variant V3 the increase was three times higher than the data obtained for the control – 96.40 mg/dm$^3$. The results for the influence of the maceration and maceration with the addition of flavor-releasing enzyme on the total concentration of higher alcohols are a clear indication for the capacitive capabilities of the applied technological practices. They lead to the quantitatively improvement of the higher alcohols fraction. Of all studied samples the highest obtained concentration of higher alcohols was found in variant V2 of wine Plevenska Rosa – 357.05 mg/dm$^3$. The lowest content of higher alcohols was obtained in the control variant V1 of wine from Muscat Ottonel – 29.82 mg/dm$^3$. The obtained results were in correlation with the statements of **Velkov (1996)**, who postulated that the total content of higher alcohols in white wines is in the range of 150.00 – 400.00 mg/dm$^3$.

The oenological practices applied in this study have a positive influence on changes in total ester content of white wines from Dimyat variety. Gradual growth in their total amount in the direction of the control V1 (50.65 mg/dm$^3$) to variant V2 - 12h maceration (63.06 mg/dm$^3$) and V3 - 12h maceration + add flavor-releasing enzyme (69.75 mg/dm$^3$) was established.

A similar trend was also observed in white wine from Aligote variety, increase in total ester content from the control variant V1 (73.14 mg/dm$^3$) to the experimental variant V2 (80.22 mg/dm$^3$), while the variant V3 showed lower concentration of esters in comparison with the control. Similar variations were also observed in the wine from Muscat Ottonel - a slight increase in the content of esters in the experimental variant V2 (122.40 mg/dm$^3$), compared to the control V1 (116.75 mg/dm$^3$).

The wine from the Plevenska Rosa variety also showed a trend of ester composition improvement when both oenological practices were applied. At 12 hour maceration total final content of esters - 310.80 mg/dm$^3$ was established, which demonstrated a significant increase, compared with the control variant V1 (23.70 mg/dm$^3$). At the experimental variant V3 the established content of esters was three times higher as the quantity (73.92 mg/dm$^3$), compared with the control variant V1.

Only wine from Vrachanski Muscat variety showed different characteristics - ester content was higher in the control variant V1, compared with the experimental variants.

With the highest ester content of all wines are characterized wine from Plevenska Rosa variant V2 - 310.80 mg/dm$^3$, while the lowest obtained was in the control variant of the wine from the same variety -V1 - 23.70 mg/dm$^3$. The obtained results for the total ester content of the wines are in correlation with the data of **Yankov *et al*. (2000)**.

During the research conducted was found that the dominat terpene alcohol in almost all samples tested, with the exception of the wine from variant V3 of the Vrachanski Muscat variety was β-citronellol. Five terpenes - α-terpineol, linalool oxide, nerol, β-citronellol and geraniol were identified.

The obtained results showed that the impact of oenological practices - maceration and maceration with the addition of flavor-releasing enzyme on the total terpene content was significant in white wines from Plevenska Rosa, Vrachanski Muscat, Aligote and Muscat Ottonel varieties.

In the wine from Plevenska Rosa variety was observed proportional growth in the total concentration of terpene alcohols in the order - control V1 (0.40 mg/dm$^3$), experimental variant V2 (0.47 mg/dm$^3$), variant V3 (0.60 mg/dm$^3$).

The wines from Vrachanski Muscat and Aligote varieties showed actively raising of the total terpene content in the experimental variants V2 (1.89 mg/dm$^3$ in Vrachanski Muscat and 0.91 mg/dm$^3$ in Aligote) against their respective control variants V1 (0.91 mg/dm$^3$ in Vrachanski Muscat and 0.21 mg/dm$^3$ in Aligote).

The two experimental variants of the Muscat Ottonel variety showed significant growth in total terpene content - 1.33 mg/dm$^3$ - variant V2, and 0.90 mg/dm$^3$ in variant V3, compared to the result for the control variant V1 (0.28 mg/dm$^3$).

The highest concentration of terpene alcohols of all tested wines was observed in variant V2 of wine from Vrachanski Muscat variety (1.89 mg/dm$^3$) and V2 of a wine from Muscat Ottonel (1.33 mg/dm$^3$). This is due to the fact that the Muscat varieties have genetic ability to form a strong flavor and their total final concentration of terpenes are in the range of 1 - 3 mg/dm$^3$ (**Velkov, 1996**).

In the comprehensive evaluation of the overall volatile composition of wines were established two compounds - methyl alcohol and acetaldehyde. According to **Yankov *et al*. (2000)** the amount of methyl alcohol in white wines is in lower percentage – 0.001-0.030% of the total alcohol content.

The data for the established methyl alcohol (in % of the total amount ethyl alcohol) in the tested wines are presented in Table 2.

**Table 1** Amount of ethyl alcohol and volatile aromatic compounds in white wines from varieties Dimyat, Plevenska Rosa, Vrachanski Muscat, Aligote, Muscat Ottonel; Variants: V1 - control variant; V2 - maceration for 12 h; V3 - maceration for 12 h + flavor-releasing enzyme

| IDENTIFIED COMPOUNDS, mg/dm$^3$ | WINES | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DIMYAT | | | PLEVENSKA ROSA | | | VRACHANSKI MUSCAT | | | ALIGOTE | | | MUSCAT OTTONEL | | |
| | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 |
| Ethyl alcohol, vol. % | 11.06 | 11.72 | 11.76 | 12.60 | 12.65 | 12.32 | 12.75 | 12.67 | 11.97 | 13.19 | 12.73 | 13.40 | 12.60 | 12.97 | 13.08 |
| Acetaldehyde | ≈0.05 | 116.00 | 125.00 | 180.00 | 53.80 | 78.60 | 164.40 | 191.00 | 94.30 | ≈0.05 | 167.00 | 108.40 | 140.80 | ND | 172.80 |
| Methanol | ≈0.05 | 8.24 | 8.81 | ≈0.05 | ≈0.05 | 21.50 | ND | 20.90 | 24.20 | ND | ND | ≈0.05 | ≈0.05 | 9.31 | 48.00 |
| 2-methyl-1-butanol | 43.50 | 31.40 | 53.00 | 62.60 | 204.00 | 70.20 | ND | 202.00 | ND | 20.20 | 24.32 | 27.32 | 19.25 | ND | 87.50 |
| 2- methyl -1-propanol | 10.60 | ND | ND | 12.40 | ND | ND | ND | 21.60 | ND | 18.21 | 22.30 | 25.25 | 10.52 | 21.10 | 11.90 |
| 3- methyl -1-butanol | ND | ND | ND | ND | 153.00 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 1-pentanol | ND | ≈0.05 | ≈0.05 | ≈0.05 | ≈0.05 | 15.30 | ≈0.05 | 9.96 | 21.00 | 5.20 | 6.72 | 7.20 | ≈0.05 | ≈0.05 | ND |
| 1-hexanol | ND | ND | ND | ND | ND | ND | 31.70 | ND | ND | ND | ND | ≈0.05 | ND | ND | ND |
| 1-heptanol | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 136.00 | ND |
| ***Total higher alcohols*** | ***54.10*** | ***31.45*** | ***53.05*** | ***75.05*** | ***357.05*** | ***85.50*** | ***31.75*** | ***233.56*** | ***21.00*** | ***43.61*** | ***53.34*** | ***59.82*** | ***29.82*** | ***157.15*** | ***99.40*** |
| Ethyl acetate | 23.80 | 33.80 | 30.80 | ≈0.05 | 13.00 | 10.00 | 16.10 | 27.60 | 20.60 | ≈0.05 | ND | 16.90 | 18.00 | 19.00 | 26.70 |
| Propyl acetate | ND | ≈0.05 | ≈0.05 | ND | ≈0.05 | 16.20 | ≈0.05 | ND | 23.39 | 22.12 | 21.25 | ≈0.05 | 34.90 | ND | ND |
| Isobutyl acetate | 26.70 | 22.40 | 26.40 | 23.40 | ≈0.05 | 30.60 | 39.80 | 34.90 | 32.80 | 38.92 | 35.41 | 17.10 | 63.70 | 27.80 | 45.10 |
| Ethyl butyrate | | 6.66 | 12.50 | ≈0.05 | ≈0.05 | 11.90 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Ethyl isovalerate | ≈0.05 | ND | ND | ND | 291.00 | ND | ≈0.05 | ≈0.05 | ≈0.05 | ND | ND | ND | ND | ND | ≈0.05 |
| Ethyl caprylate | ND | ≈0.05 | ND | ≈0.05 | ND | ND | 96.30 | ND | ND | 12.10 | 23.56 | 25.23 | ND | ND | ND |
| Ethyl hexanoate | ND | ND | ND | ≈0.05 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Pentyl acetate | ≈0.05 | ≈0.05 | ND | ≈0.05 | 6.65 | 5.12 | ≈0.05 | ND | ND | ND | ND | ND | ND | 24.45 | ND |
| Hexyl acetate | ≈0.05 | ≈0.05 | ND | ≈0.05 | ND | ≈0.05 | ND | ≈0.05 | ≈0.05 | ND | ND | ≈0.05 | ≈0.05 | ≈0.05 | ≈0.05 |
| Phenyl acetate | ND | ND | ND | ≈0.05 | ND | ≈0.05 | ND | ND | ND | ND | ND | ND | ND | 51.10 | ND |
| ***Total esters*** | ***50.65*** | ***63.06*** | ***69.75*** | ***23.75*** | ***310.80*** | ***73.92*** | ***152.35*** | ***62.60*** | ***76.89*** | ***73.19*** | ***80.22*** | ***59.33*** | ***116.65*** | ***122.40*** | ***71.90*** |
| α – terpineol | 0.20 | ND | ND | ND | ND | ND | ≈0.05 | ND | ND | ND | ND | ND | ND | ≈0.05 | ND |
| Linalool oxide | ≈0.05 | ND | ND | ND | ≈0.05 | ND | 0.19 | 0.90 | ND | ND | ND | ≈0.05 | ND | ≈0.05 | ND |
| Nerol | ≈0.05 | ≈0.05 | ND | ND | ND | ND | ND | ≈0.05 | ND | ND | ND | ND | ND | ND | 0.80 |
| β – citronellol | 0.12 | ≈0.05 | 0.11 | 0.40 | 0.21 | 0.15 | 0.18 | 0.20 | ND | 0.21 | 0.91 | ≈0.05 | 0.28 | 0.22 | ≈0.05 |
| Geraniol | ND | ≈0.05 | ≈0.05 | ND | 0.21 | 0.45 | 0.49 | 0.74 | ≈0.05 | ND | ND | ≈0.05 | ND | 1.01 | ≈0.05 |
| ***Total terpenes*** | ***0.42*** | ***0.15*** | ***0.16*** | ***0.40*** | ***0.47*** | ***0.60*** | ***0.91*** | ***1.89*** | ***0.05*** | ***0.21*** | ***0.91*** | ***0.15*** | ***0.28*** | ***1.33*** | ***0.90*** |

*ND – Not Detected

**Table 2** Percentage of methyl alcohol in white wines from studied varieties: Dimyat, Plevenska Rosa, Vrachanski Muscat, Aligote, Muscat Ottonel; Variants: V1 - control variant; V2 - maceration for 12 h; V3 - maceration for 12 h + flavor-releasing enzyme Zimovarietal Aroma G

| CONTENT OF METHYL ALCOHOL, % | WINES | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DIMYAT | | | PLEVENSKA ROSA | | | VRATCHANSKI MUSCAT | | | ALIGOTE | | | MUSCAT OTTONEL | | |
| | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 |
| Methyl alcohol | traces | 0.007 | 0.007 | traces | traces | 0.017 | ND | 0.016 | 0.020 | ND | ND | traces | traces | 0.007 | 0.03 |

*ND – Not Detected

In wines from varieties Vrachanski Muscat variant V1, Aligote variants V1 and V2, was not identified presence of methyl alcohol. In wines from varieties Dimyat variant V1, Plevenska Rosa variants V1 and V2, Aligote V3 and Muscat Ottonel variant V1, methanol has been identified, but in small amounts (traces). The highest amount was identified in wine from Muscat Ottonel in variant V3 (0.03%), and the lowest in V2 and V3 wines from Dimyat variety (0.007%).

All established amounts of methyl alcohol are correlated to the stated by **Yankov *et al*. (2000)** range of variation and correspond to the accepted doses in Bulgarian white wines.

The content of acetaldehyde in the wines from researched varieties was in the range of 53.80 - 191.00 mg/$dm^3$, which is correlated to the data of **Liu and Pilone (2000).** The highest content (191.00 mg/$dm^3$) was found in wine from variant V2 of the Vrachanski Muscat variety, and the lowest in the wine from Plevenska Rosa variety (53.80 mg/$dm^3$). In wine from Dimyat variant V1, and in the Aligote variety variant V1, acetaldehyde has been identified in traces.

## CONCLUSION

The study of the aromatic composition of white wines from varieties examined - Dimyat, Plevenska Rosa, Vrachanski Muscat, Aligote and Muscat Ottonel identified 24 volatile compounds. Identification of these compounds varies in relation to the different types of samples.

The applied oenological practices - maceration for 12 hours and maceration for 12 hours with the addition of flavor-releasing enzyme have a positive impact on the aromatic composition of wines towards increasing of the total content of higher alcohols, esters and terpene alcohols.

Presence of methyl alcohol in part of the wines was found. Its content as a percentage of total alcoholic content vary in the range 0.007 - 0.03%, which is typical for white wines.

The content of acetaldehyde in the tested wines is in the range 53.80 - 191.00 mg/$dm^3$. It makes its presence positive for the aromatic composition of the studied white wines.

## REFERENCES


AZNAR, M., LOPEZ, R., CACHO, J.F., FERREIRA, V. 2001. Identification and quantification of impact odorants of aged red wines from Rioja. GC-Olfactometry, quantitative GC-MS, and odor evaluation of HPLC fractions. Journal of Agricultural and Food Chemistry, 49:2924-2929. https://doi.org/10.1021/jf001372u

BUREAU, S., RAZUNGLES, A., BAUMES, R. 2000. The aroma of Muscat of Frogtignan grapes: Effect of the light environment of vine or bunch on volatiles and glycoconjugates. Journal of the Science of Food and Agriculture, 80:2012–2020. https://doi.org/10.1002/1097-0010(200011)80:14<2012::aid-jsfa738>3.0.co;2-x

CÂMARA, J.S., ALVES, M.A., MARQUES, J.C. 2006a. Changes in volatile composition of Madeira wines during their oxidative ageing. Analytica Chimica Acta, 563:188-197. https://doi.org/10.1016/j.aca.2005.10.031

COLDEA, T., SOCACIN, C., DAN VADNAR, M. 2011. Gas-chromatographic analysis of major volatile compounds found in traditional fruit brandies from Transylvania, Romania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 39(2):109-116.

DIGNUM, M.J.W., KERLER, J., VERPOORTE, R. 2001. b-Glucosidase and peroxidase stability in crude enzyme extracts from green beans of Vanilla planifolia Andrews. Phytochemical Analysis, 12(3):174–179. https://doi.org/10.1002/pca.578

EBELER, S. 2001. Analytical chemistry: Unlocking the secrets of wine flavor. Food Reviews International, 17:45–64. https://doi.org/10.1081/fri-100000517

ETIEVANT, P.X. 1991. Volatile compounds of food and beverages. In: Maarse, H. (ed). Publisher Dekker, New York. 1991.

FERREIRA, V., LOPEZ, R., ESCUDERO, A., CACHO, J. 1998. The aroma of Grenache red wine: Hierarchy and nature of its main odorants. Journal of the Science of Food and Agriculture, 50:538-543. https://doi.org/10.1002/(sici)1097-0010(199806)77:2<259::aid-jsfa36>3.0.co;2-q

FUJII, T., NAGASAWA, N., IWAMATSU, A., BOGAKI, T., TMAMAI, I., HAMACHI, M. 1994. Molecular cloning, sequence analysis, and expression of the yeast alcohol acetyltransferase gene. Applied and Environmental Microbiology, 6:2786-2792.

GÓMEZ, E., LAENCINA, J., MARTÍNEZ, A. 1994. Vinification effect on changes in volatile compounds of wine. Journal of Food Science, 59:406-409. https://doi.org/10.1111/j.1365-2621.1994.tb06978.x

GONZALEZ-BURGOS, E., GOMEZ-SERRAMILLOS, M.P. 2012. Terpene compounds in nature: a review of their potential antioxidant activity. Current Medicinal Chemistry, 19(31):5319-5341. https://doi.org/10.2174/092986712803833335

GUTH, H. 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. Journal of Agricultural and Food Chemistry, 45:3027-3032. https://doi.org/10.1021/jf970280a

IVANOV, M. 2016. Hybridization in the vine selection. Academic Press of Agricultural University. 2016; Plovdiv, Bulgaria.

IVANOVA, V., STEFOVA, M., VOJNOSKI, B., STAFILOV, T., BÍRÓ, I., BUFA, A., FELINGER, A., KILÁR, F. 2013. Volatile Composition of Macedonian and Hungarian Wines Assessed by GC/MS. Food and Bioprocess Technology, 6:1609–1617. https://doi.org/10.1007/s11947-011-0760-y

KOBAYASHI, M., SHIMIZU, H., SHIOYA, S. 2008. Beer volatile compounds and their application to low malt beer fermentation. Journal of Bioscience and Bioengineering, 106(4):317-323. https://doi.org/10.1263/jbb.106.317

LAKATOŠOVÁ, J., PRIESOLOVÁ, L., DOKUPILOVÁ, I., ŠMOGROVIČOVÁ, D. 2013. Characterisation of Slovak varietal wine aroma compounds by gas chromatography mass spectrometry. Potravinarstvo, 7:180 – 182.

LAMBRECHTS, M.G., PRETORIUS, I.S. 2000. Yeast and its importance to wine aroma - A review. South African Journal of Enology and Viticulture, 21, Special Issue.

LEE, S.J., RATHBONE, D., ASIMONT, S., ADDEN, R., EBELER, S.E. 2004. Dynamic changes in ester formation during chardonnay juice fermentations with different yeast inoculation and initial Brix conditions. American Journal of Enology and Viticulture, 55:346–353.

LI, H. 2006. Wine tasting. China Science Press. Beijing, China.

LIU, S.Q., PILONE, G.J. 2000. An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implifications. International Journal of Food Science and Technology, 35:49 – 61. https://doi.org/10.1046/j.1365-2621.2000.00341.x

LUAN, F., MOSANDL, A., GUBESCH, M., MATTHIAS, M., WÜST, M. 2006. Enantioselective analysis of monoterpenes in different grape varieties during berry ripening using stir bar sorptive extraction- and solid phase extraction-enantioselective-multidimensional gas chromatography-mass spectrometry. Journal of Chromatography Analysis, 1112:369-374. https://doi.org/10.1016/j.chroma.2005.12.056

LUKIC, I., MILICEVIC, B., BANOVIC, M., TOMAS, S., RADEKA, S., PERSURIC, D. 2011. Secondary aroma compounds in fresh grape marc brandies as a result of variety and corresponding production technology. Food Technology and Biotechnology, 49(2):277-293.

MANITTO, P. 1980. Biosynthesis of natural products. Ellis Horwood, Chichester. 1980.

MARINOV, M. 2005. Technology of alcoholic beverages and spirits. Plovdiv, Bulgaria. Academic Publishing of University of Food Technologies. 2005; ISSN 0477-0250.

MARTI, M.P., MESTRES, M., SALA, C., BUSTO, O., GUASCH, J. 2003. Solidphase microextraction and gas-chromatography olfactometry analysis of successively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. Journal of Agricultural and Food Chemistry, 51:7861–7865. https://doi.org/10.1021/jf0345604

OLIVEIRA, J.M., FARIA, M., SÁ, F., BARROS, F., ARAÚJO, I.M. 2006. C6-alcohols as varietal markers for assessment of wine origin. Analytica Chimica Acta, 563:300–309. https://doi.org/10.1016/j.aca.2005.12.029

OLIVEIRA, J.M., OLIVEIRA, P., BAUMES, R.L., MAIA, M.O. 2008. Volatile and glycosidically bound composition of Loureiro and Alvarinho wines. Food Science and Technology International, 14:341-353. https://doi.org/10.1177/1082013208097442

PEDDIE, H.A.B. 1990. Ester formation in brewery fermentations. Journal of the Institute of Brewing, 96:327-331. https://doi.org/10.1002/j.2050-0416.1990.tb01039.x

PERESTRELO, R., FERNANDES, A., ALBUQUUERQUE, F.F., MARQUES, J.C., CAMARA, J.S. 2006. Analytical characterization of the aroma of Tinta Negra Mole red wine: Identifcation of the main odorants compounds. Analytica Chimica Acta, 563:154-164. https://doi.org/10.1016/j.aca.2005.10.023

RAPP, A., MANDEREY, H. 1986. Wine aroma. Experientia, 42:873-884. https://doi.org/10.1007/bf01941764

SANCHEZ PALOMO, E., DIAZ-MAROTO, M.C., GONZALEZ VIÑAS, M.A., SORIANO-PÉREZ, A., PÉREZ-COELLO, M.S. 2007. Aroma profile of wines from Albillo and Muscat grape varieties at different stages of ripening. Food Chemistry, 18:398-403. https://doi.org/10.1016/j.foodcont.2005.11.006

SCHNEIDER, V. 2011. The Enological significance of acetaldehyde. Journal of Terroir Biodiversity. Accessed December 2011 from www.ithaka-journal (2009).

SKINKIS, P.A., BORDELON, B.P., BUTZ, E.M. 2010. Effects of sunlight exposure on berry and wine monoterpenes and sensory characteristics of Traminette. American Journal of Enology and Viticulture, 61:147–156.

STANDARD 3752:2005. Alcohol Drinks – Methods of Test (Second Revision).

SUMBY, K.M., GRBIN, P.R., JIRANEK, V. 2010. Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. Food Chemistry, 121:1–16. https://doi.org/10.1016/j.foodchem.2009.12.004

VELKOV, E. 1996. Encyclopedia of alcoholic beverages. Plovdiv, Bulgaria. Poligrafia Ltd. 1996. ISBN 954-698-002-1.

WANG, Y., XU, Y., LI, J. 2012. A novel extracellular b-glucosidase from Trichosporon asahii: Yield prediction, evaluation and application for aroma enhancement of Cabernet Sauvignon. Journal of Food Science, 77(8):M505–M515. https://doi.org/10.1111/j.1750-3841.2012.02705.x

YANKOV, A., KUKUNOV, S, YANKOVA, T. 2000. Technology of wine and higher alcohol drinks. Publisher: Teodoros, Sofia, Bulgaria. 2000.

YANKOV, A. 1992. Winemaking Technology. Sofia, Zemizdat. 1992.