

# Influence of enzymatic treatments on white wine composition

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**Abstract.** Most biochemical reactions involved in the wine-making process are catalyzed by enzymes. The use of enzymes of exogenous origin in wine production is due to the numerous technological and economical advantages demonstrated over time in the winemaking process. Understanding the important role played by enzymes in wine making technology contributes to the development of optimization strategies for the production process to improve the final quality of the wine. In order to accomplish this study, the influence of five oenological preparations with pectolytic and  $\beta$ -glucosidases enzymes types on the volatile compounds of white wines obtained from Fetească regală variety was analyzed by monitoring their evolution during the alcoholic fermentation to the final product. Wine samples have been physically and chemically analysed (pH, acidity, alcoholic strength, density, malic acid, lactic acid, sugar content, SO<sub>2</sub>, total dry extract and non-reducing extract) according to OIV Standards. Separation and identification of flavor compounds was performed using an Agilent 7890 gas chromatograph coupled to a 5975 C inert XL EI/CI MSD spectrophotometer. The organoleptic evaluation of wines was made according to a wide range of sensory descriptors. An important evolution of volatile compounds during fermentation was observed, depending on the type of enzyme administered, compared to the control sample. Enzymatic treatments did not significantly affect the physico-chemical composition of the wines obtained. The chromatic parameters of the wine samples varied according to the type of enzyme applied. The results of the study showed a significant influence of the enzymes on the organoleptic characteristics of the wines. Therefore, the aromatic quality of a wine is directly proportional to the chemical composition of the grapes and to the technology.

## 1. Introduction

Enzymes are biological catalysers used in winemaking technology for their numerous advantages on the product quality. These compounds may come from indigenous microflora of grapes, yeasts or other bacteria correlated with wine cellar, vinification conditions or vineyard [1].

The wine-maker can enrich the activity of these endogenous enzymes by using commercial products, usually obtained by fermenting pure culture of selected microorganism [2]. Pectinases, proteases and glycosidases are some of the enzymes produced by yeast, that present significance in oenology for their technological actions and their participation to aroma constitution. Pectinolytic enzymes have the ability to cleave long pectin chains into shorter and more soluble chain segments, that facilitate pressing operation of the grapes and clarification of musts, enhance the extraction of color and aroma compounds and facilitate the filtration of resulted wine. These enzymes can also facilitate the stabilisation of the wines, due to the enzymatic hydrolysis of the protein into smaller molecules, containing more nitrogen. The aroma compounds concentration of wines can be increased by glycosidases, which hydrolyse nonvolatile glycosidic precursors of the grapes. Yeasts are also important producers of enzymes [3]. *Saccharomyces cerevisiae*, considered the fundamental wine yeast, is not appreciated as a considerable producer of extracellular proteases, lipases or proteolytic enzymes [4]. The

final quality of wines is influenced by a multitude of interactions between chemical compounds. During alcoholic fermentation, grapes derivatives are converted by yeast into a range of sensorial relevant metabolites that gives wine its vinous character [5]. Commercial enzymes are utilized in wine-making technology for diverse biotransformation reactions from pre-fermentation stage pending alcoholic fermentation, post-fermentation and wine aging period [6]. Understanding the important role of enzymes in winemaking technology contributes to the development of strategies to optimize the production process, improving the wine's composition and its sensory characteristics [7].

The purpose of this experiment is to investigate the influence of enzymes pre-treatments on the physico-chemical parameters, chromatic characteristics and aromatic profile.

## 2. Materials and methods

### 2.1. Grapes samples and winemaking

The grapes representing Fetească Regală variety were harvested in autumn of 2018, from Iasi vineyard, with approximately 250 g/L sugar content. After destemming and crushing operations, the must was transferred into 50 L glass vessels. A dose of 20 g/hL *Saccharomyces* yeast (*Levulia espede*, AEB) and 30 g/hL yeast nutrient (FERMO PLUS CH, AEB) (previously dissolved in warm

must) was inoculated in each variant. For this experiment, five commercial enzymes based on pectolytic and  $\beta$ -glucosidases activity were added to musts before alcoholic fermentation, thus contributing to the release of aroma compounds (Endozym Thiol®, AEB – V1; Endozym  $\beta$ -Split®, AEB – V2; Zymovarietal aroma G®, SODINAL – V3; Endozym Ice®, AEB – V4; Zimarome®, BSG WINE –V5 and no enzyme– V6), at doses of 3 g/hL (all enzyme preparations were diluted with must 1:10) and 3 mL/hL respectively. 6 variants were obtained. The fermentation was carried out at 16–18 °C for about one week. For this experiment, samples were collected at two days differences during the alcoholic fermentation and stored in the freezer until the analysis. When the alcoholic fermentation was finished, each variant was filtered using sterile membrane filter followed by sulphitation (to preserve wine from microbiological damage) and bottled. The wine samples were stored under controlled condition and analysed after three months.

## 2.2. Chemicals

Standard reagents and pure commercial substances were purchased from Merck KGaA (Germany).

## 2.3. Methods of analyses

**Chromatic parameters** were analysed using a Specord UV-VIS spectrophotometer, in accordance to the Commission Internationale d'Eclairage (CIE, 1976), using aspects of specific qualities of visual sensation: clarity, tonality, chromatic parameters, saturation, luminosity, hue [8]. CIELab space system is based on a sequential or continuous Cartesian representation of 3 orthogonal axes: L\*, a\*, and b\*. The vertical axis noted with L\* (clarity) measures from 0 – completely opaque, to 100 – completely transparent, and parameters “+a\*” red, “-a\*” green, “+b\*” yellow, “-b” blue were registered [9].

**Standard physico-chemical parameters** were reviewed according to International Organization of Vine and Wine Compendium methods of analysis [8]. Each variant was analysed for: total and volatile acidity, alcoholic strength, pH, density, total sugar, free and total sulphur dioxide, malic and lactic acid, total dry extract and non-reducing extract.

**Sensory characteristics** are important for the quality of wines. The wine samples were assessed for sensory characteristics by 10 tasters according to a wide range of sensory descriptors. The parameters were evaluated with ratings from 0 to 5, and the mean was registered in the tables. The sensory descriptors of samples were comparatively expressed in a chart.

**Separation and identification of flavour compounds** was performed using an Agilent 7890 gas chromatograph coupled to a 5975 C inert XL EI/CI MSD spectrophotometer. The column used for the analysis was HP-5MS fused silica capillary column (30 m · 0.25 mm I.D., 0.25  $\mu$ m with a split ratio of 50:1 and inlet temperature was set at 250 °C. The MSD was used at 70 eV in the electron impact mode (EI), using the mass range from 50 to 800 Da at 150 °C. Peak identification of the aroma components was achieved by comparison of mass spectra with the mass spectral data collection from Wiley275 and NIST05a libraries.

**Sample extraction:** In a 15 mL glass tube, 5 mL of wine sample and 1 mL of dichloromethane were added,

and the mixture was mechanically agitated for 45 minutes. The extract was separated from the aqueous phase by centrifugation at 7800 rpm for 10 minutes. This process was repeated twice, the extraction was performed after 20 minutes. The two organic phases were mixed and injected in the GC-MS.

## 3. Results and discussions

The present article highlights a comparative investigation on the action of enzymes on the quality of wines.

### 3.1. Effects of enzymatic pre-treatment on basic parameters of resulted wine

Table 1 shows the physical-chemical parameters of wines. The alcoholic strength of a wine is dependent of the total sugar content of the origin grapes. The obtained samples were dry wines with over 12.7% vol. Alcoholic strength of wines was not influenced by the type of administrated enzymes.

Acidity and pH are relevant to the sensory perception of wine, essentially defining its structure and equilibrium. Excessive acidity increases the perception of sourness since low acidity decreases the flavour balance. Acid content is relevant for preservation and is responsible for sensory features of wines. Its content in wine may be influenced by grape variety, ripeness, climatic conditions, wine-making process, wine storage and pH [9]. No significant differences were recorded in total and volatile acidity of wines, which means that enzymes have no influence on this indicator. The total dry extract shows values between 19 and 19.6 g/L. The content of non-reducing extract of most Romanian wines varies between 13 and 35 g/L, according to grape variety, health conditions, applied technology and wine treatments [10]. The analysed samples registered values of non-reducing dry extract between 17.6 and 17.9 g/L.

### 3.2. Chromatic characteristics

Colour is a primary attribute by which the consumer assesses wine and food. Chromatic parameters of analysed wines is presented in Table 2.

Analysed samples registered a high clarity, with more yellow and red shades, except V1 that presented more green and yellow shades of colour. Parameter “a\*” (green/red component) had the highest value on V6 (control sample) and the lowest on V1 sample (Endozym Thiol®). The highest values of “b\*” (blue/yellow component) were registered in the case of V1 sample (Endozym Thiol®), and the lowest in V3 (Zymovarietal aroma G®). Some differences were recorded for “L\*” that corresponded to brightness characteristics. It was expressed by a less green/red and more yellow colour of wine. The red pigment indicates the presence of low but still visible amounts of anthocyanins. Enzymatic treatment influenced chromatic parameters of analysed samples to varying degrees. The majority of wines treated with enzymatic preparations were characterized by the decrease in a\* compared to the control sample, a less intense green colour and more red colour respectively. Also, all samples treated with enzymes were evaluated by an increase in b\*, that means more yellow colour.

**Table 1.** Physical-chemical parameters of wines.

Sample	Total Acid g/L C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	pH	Alcoholic strength % vol.	Malic Acid	V.A. g/L C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	Density	Total Sugar g/L	Lactic Acid	Free SO <sub>2</sub> mg/L	Total SO <sub>2</sub> mg/L	T.D.E. g/L	N.E. g/L
V1	4.2	3.28	12.8	2.7	0.13	0.9908	1.8	0	23	56	19.6	17.8
V2	4.2	3.36	12.7	2.9	0	0.9909	1.7	0.1	23	56	19.6	17.9
V3	4.2	3.3	12.8	2.9	0.08	0.9907	1.6	0.2	25	58	19.3	17.7
V4	4.2	3.28	12.7	2.8	0.14	0.9907	1.4	0.2	25	58	19	17.6
V5	4.3	3.27	12.7	2.8	0.1	0.9907	1.3	0.2	23	58	19	17.7
V6	4.2	3.28	12.8	2.9	0.12	0.9907	1.4	0.3	25	56	19.3	17.9

**Table 2.** Chromatic parameters of wines.

Sample	L Clarity	Cromaticity		Crome C	Tonality H	Lightness	Hue	ΔE	ΔH
		a*	b*						
V1	98.3	-0.12	5.61	5.61	-88.82	0.12	3.32	2.72	1.92
V2	96.9	-1.44	4.92	5.13	-73.70	0.15	1.95	0.58	0.36
V3	97.1	-1.51	4.49	4.73	-71.43	0.14	1.88	0.55	0.29
V4	96.5	-1.63	5.56	5.79	-73.62	0.17	1.94	0.99	0.17
V5	97.1	-1.34	5.01	5.19	-75.02	0.15	2.02	0.77	0.46
V6	96.6	-1.80	4.59	4.92	-68.62	0.16	1.76	0.00	0.00

A perceptible colour difference between samples treated with enzyme preparations and control samples can be observed, suggesting that these products had a greater effect on colour. Delta values represent colour difference as compared to the control.

### 3.3. Volatile compounds

The aromatic profile of wines is influenced by numerous volatile compounds originating from the grapes or must, from yeast (fermentative aromas), or post-fermentative aromas (from aging processes) [11]. The strength and proportion of volatile substances set the category of wine odour. In Table 3, the most representative volatile compounds of analysed samples and their evolution during fermentation are represented.

Enzymatic treatments determine an increase in the total amount of volatile compounds compared to control samples. The results denote that the content of volatile compounds significantly fluctuate during fermentation and storage. Table 4 shows the concentration of volatile compounds identified in analysed wines and their odour descriptors.

Analysing V1 sample during fermentation, a significant increase of some compounds can be observed, such as: isoamylacetate, isoamylalcohol, ethyl caproate, 1-hexanol, ethyl octanoate, phenylethylacetate, hexanoic acid, benzeneethanol, octanoic acid. Also, other compounds registered a decrease, such as: 1-propanol, butanoic acid ethyl ester, ethyl decanoate, ethyl laurate, ethyl linoleolate, ethyl oleate. Variant V2 registered a relevant increase of isoamylalcohol, 3-ethoxy-1-propanol, isovaleric acid, ethyl 4-hydroxybutanoate, isopropyl acetate, hexanoic acid, benzeneethanol, 3,7-dimethyl-1,5-octadien-3,7-diol, and an important decrease of: ethyl caproate, ethyl decanoate, ethyl laurate,

decanoic acid, ethyl linoleate, phenol, lauric acid, ethyl linoleolate, ethyl oleate. A significant increase of isoamylacetate, 1-butanol, isoamylalcohol, ethyl lactate, 1-hexanol, isobutyric acid, ethyl decanoate, isovaleric acid, hexanoic acid, octanoic acid, 4-vinyl-2-methoxyphenol, was noted in V3, with a decrease of ethyl octanoate and eicosane. V4 sample can be characterized by an important increase of some compounds, such as: 1-propanol, isoamylacetate, isoamylalcohol, hexanoic acid, benzeneethanol, 4-vinyl-2-methoxyphenol, and a significant decrease of ethyl caproate, 1-hexanol, ethyl octanoate, ethyl decanoate, ethyl laurate, ethyl linoleolate. Isoamylalcohol, ethyl 4-hydroxybutanoate, hexanoic acid, benzeneethanol, octanoic acid and 4-vinyl-2-methoxyphenol showed an important increasing during alcoholic fermentation. Also, 1-hexanol, ethyl octanoate, 3-ethoxy-1-propanol, ethyl octanoate, eicosane, ethyl decanoate and isopropyl acetate were diminished during fermentation.

Control sample showed a considerable decrease of isoamylalcohol, ethyl decanoate, decanoic acid, ethyl linoleate, ethyl oleate, and an important increase of isoamylacetate, 3-ethoxy-1-propanol, hexanoic acid, benzeneethanol, octanoic acid.

The most abundant compound in all samples is isoamylalcohol, followed by benzeneethanol. By comparing the results of the V1, V2, V3, V4, V5 and V6 samples, of the 3 sampling days, the biggest differences are observed in case of fatty acids.

The concentration of the following esters derivatives decreases from the first day to the last day of sampling: ethyl caproate, ethyl octanoate, ethyl decanoate, ethyl laurate, ethyl palmitate, ethyl oleate, ethyl linoleate, ethyl linoleolate. Also, a decrease of fatty acids, such as decanoic acid and lauric acid, was registered during alcoholic fermentation.

**Table 3.** Evaluation of volatile compounds during alcoholic fermentation (percent of total area).

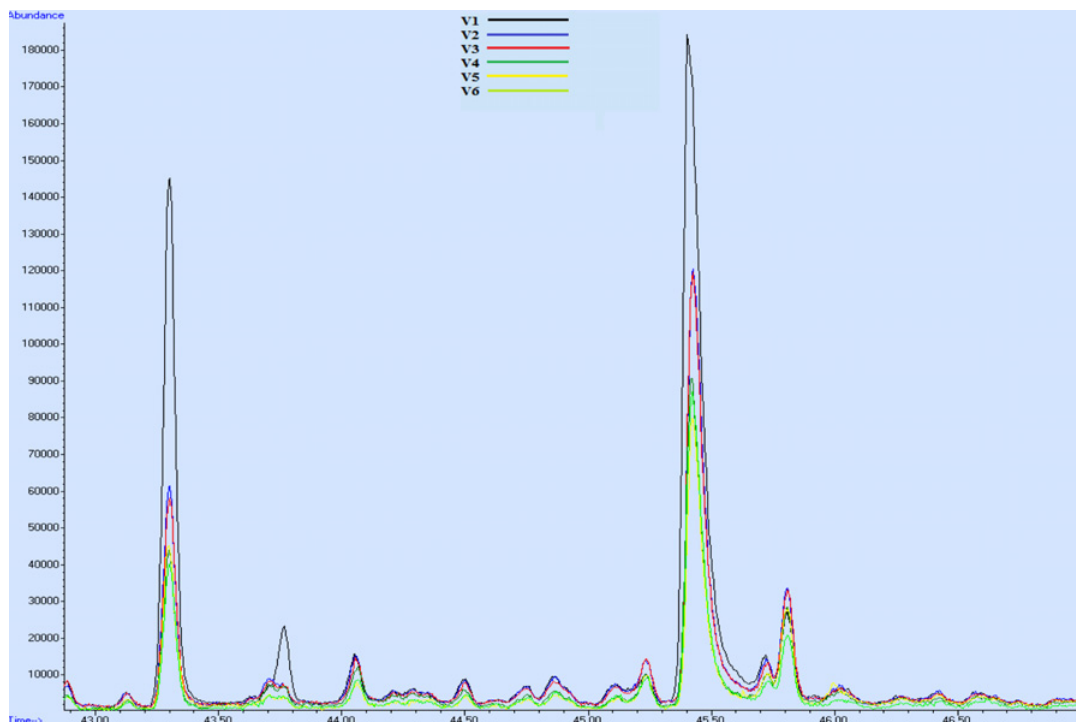
Volatile compounds	R.T.	V1			V2			V3			V4			V5			V6		
		D 1	D 3	D 7	D 1	D 3	D 7	D 1	D 3	D 7	D 1	D 3	D 7	D 1	D 3	D 7	D 1	D 3	D 7
1-propanol	11.46	1.26	1.86	0.98	1.34	1.54	1.68	1.23	1.91	1.73	1.61	2.36	2.47	2.71	2.12	2.03	2.34	2.28	2.41
butanoic acid ethyl ester	11.56	0.21	0.40	0.11	0.30	0.48	0.43	0.28	0.43	0.35	0.32	0.40	0.43	0.47	0.31	0.44	0.29	0.40	0.36
isobutyl alcohol	13.44	2.20	3.92	2.33	2.91	3.88	4.71	2.37	4.24	3.80	3.02	4.12	4.57	3.84	3.59	4.80	3.31	4.10	4.26
Isoamyl acetate	14.91	1.14	1.91	4.02	1.37	2.14	2.00	1.04	1.89	2.51	1.62	2.38	2.16	1.38	1.87	1.79	1.44	2.02	2.44
1-butanol	15.64	0.88	0.12	0.11	0.13	0.15	0.16	0.08	0.15	0.17	0.12	0.16	0.15	0.14	0.16	0.17	0.15	0.10	0.13
dodecane	18.16	0.13	0.21	0.14	0.07	0.18	0.13	0.09	0.20	0.06	0.16	0.19	0.22	0.20	0.23	0.23	0.37	0.21	0.13
isoamylalcohol	18.54	27.17	41.26	31.28	30.88	44.06	46.51	26.83	42.58	47.23	39.57	44.63	49.40	48.88	48.49	53.02	43.50	48.51	33.49
ethyl caproate	19.72	1.05	0.92	2.26	1.24	1.03	0.81	1.20	0.93	1.10	1.55	1.25	0.99	1.60	1.15	1.08	1.28	1.10	1.25
dotriacontane	20.21	0.26	0.48	0.30	0.16	0.33	0.28	0.20	0.38	0.12	0.53	1.27	0.38	0.36	0.38	0.47	0.50	0.33	0.38
phytane	22.55	0.19	0.31	0.22	0.15	0.27	0.28	0.17	0.31	0.12	0.25	0.46	0.31	0.21	0.32	0.39	0.62	0.23	0.34
ethyl lactate	24.70	0.23	0.39	0.20	0.27	0.40	0.52	0.25	0.41	0.49	0.30	0.39	0.66	0.49	0.45	0.44	0.44	0.38	0.62
1-hexanol	24.98	0.49	0.71	0.94	0.59	0.82	0.82	0.54	0.76	1.05	1.15	0.89	1.08	1.10	0.85	0.77	1.19	1.03	1.15
3-ethoxy-1-propanol	26.03	0.74	1.16	0.07	0.79	1.09	1.20	0.75	1.17	0.89	1.00	1.16	1.27	1.48	0.88	1.23	0.85	1.30	1.47
ethyl octanoate	28.59	4.37	3.42	8.50	3.82	3.14	2.33	4.62	2.98	2.71	3.40	3.05	2.49	3.24	2.36	1.78	3.23	2.08	2.99
eicosane	28.98	0.41	0.60	0.37	0.39	0.54	0.65	0.42	0.63	0.29	0.66	1.85	0.64	0.62	0.50	0.47	0.89	0.49	0.73
acetic acid	30.44	0.06	0.38	1.21	0.24	0.06	0.57	0.21	0.41	0.43	0.26	0.35	0.48	0.45	0.39	0.64	–	0.29	0.56
isobutyric acid	35.12	0.09	0.14	–	0.10	0.13	0.17	0.08	0.13	0.11	0.10	0.13	0.13	0.16	0.09	0.10	0.06	0.09	0.16
ethyl decanoate	36.92	7.41	2.73	5.51	5.81	1.97	1.43	7.78	2.28	0.97	3.60	2.78	1.05	2.53	1.18	1.29	3.19	2.06	1.77
butanoic acid	37.50	0.16	0.27	0.20	0.20	0.29	0.32	0.15	0.26	0.18	0.23	0.44	0.23	0.27	0.25	0.28	0.15	0.32	0.38
isovaleric acid	39.02	0.12	0.27	0.13	0.12	0.29	0.36	0.11	0.21	0.25	0.22	0.27	0.27	0.27	0.20	0.21	0.10	0.21	0.41
ethyl 4-hydroxybutanoate	43.30	0.64	1.17	0.26	0.73	1.23	1.35	0.58	1.19	1.08	0.86	1.29	1.13	0.75	0.94	1.02	0.77	0.99	1.63
ethyl laurate	44.52	5.24	0.67	1.89	4.37	0.46	0.20	4.80	0.54	0.07	2.17	0.42	–	0.79	0.26	0.16	–	–	–
1,3-dithiolane	40.13	0.11	0.17	0.06	0.09	0.16	0.18	0.07	0.14	0.12	0.10	–	–	0.14	0.12	0.13	–	0.11	0.21
isopropyl acetate	40.92	1.41	2.22	1.48	1.60	2.27	2.39	1.48	2.30	1.77	2.04	1.72	2.62	2.21	1.81	1.87	1.61	1.71	2.93
phenylethylacetate	43.69	0.12	0.25	0.41	0.11	0.18	0.18	0.13	0.25	0.13	0.17	0.17	0.18	0.13	0.19	0.25	0.30	0.15	0.27
hexanoic acid	45.40	1.05	1.81	2.73	1.21	2.13	2.63	1.17	2.18	2.95	1.77	1.95	2.28	1.54	1.74	2.02	1.68	2.71	3.47
butyl acetate	45.81	0.35	0.63	0.04	0.34	0.52	0.54	0.35	0.55	0.41	0.50	0.43	0.47	0.57	0.43	0.53	0.70	0.66	0.82
benzeneethanol	47.19	7.16	11.11	7.91	8.39	12.18	13.61	7.59	12.18	15.79	10.89	11.78	12.44	9.99	13.62	11.38	9.84	10.31	16.81
octanoic acid	52.51	1.76	3.42	5.45	2.41	4.01	4.84	2.18	4.05	6.06	3.35	3.78	4.31	2.63	5.13	3.96	4.79	3.86	6.48
3,7-dimethyl-1,5-octadien-3,7-diol	48.12	0.05	–	0.17	0.06	0.10	0.12	0.05	0.10	0.07	0.09	0.11	0.13	0.08	0.13	0.08	–	–	–
4-vinyl-2-methoxy-phenol	56.46	0.20	0.63	–	0.24	0.68	1.03	0.21	0.62	1.24	0.39	0.87	1.06	0.32	0.91	0.89	–	0.65	1.43
decanoic acid	59.01	1.50	1.21	4.51	1.77	1.32	1.12	1.99	1.32	1.67	1.93	1.18	1.18	1.21	1.61	1.39	2.94	1.24	1.80
4-vinylphenol	62.35	–	0.49	0.21	–	0.58	0.80	–	0.54	0.97	–	0.47	0.36	–	0.41	0.63	–	0.34	–
ethyl linoleate	65.55	10.44	1.54	0.30	8.60	0.77	–	9.95	1.07	–	4.03	0.76	–	–	–	–	2.58	0.59	–
phenol, 2,4-bis(1,1-dimethyl)	59.76	0.39	0.59	0.47	0.32	0.48	0.57	0.35	0.50	0.20	0.95	–	0.48	0.40	0.57	0.64	0.77	0.41	0.64
ethyl oleate	64.21	2.19	0.35	0.12	1.94	0.16	–	2.06	0.22	–	0.78	0.16	–	0.54	0.23	–	0.44	0.20	–
lauric acid	64.95	1.20	0.27	2.77	0.95	0.32	–	1.38	0.15	–	0.70	0.18	–	0.33	0.26	–	1.10	0.13	–
ethyl linoleolate	67.37	3.65	0.62	0.23	3.50	0.50	–	1.47	0.36	–	1.47	0.36	–	0.73	0.53	–	1.07	0.32	–
stearic acid	72.11	–	4.18	0.83	–	3.52	1.18	–	3.04	1.32	–	0.73	1.07	–	–	1.03	1.05	0.81	2.75

\*D – Day of sampling; RT – Retention time.

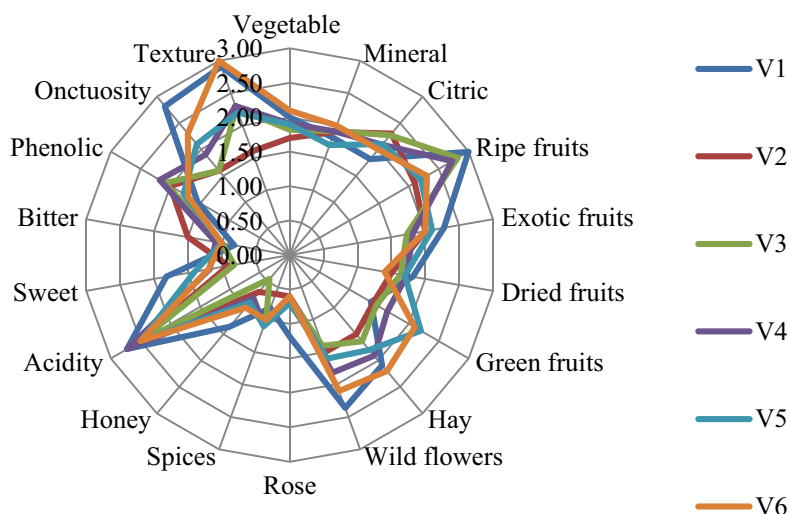
**Table 4.** Percent concentration of volatile compounds identified in analysed wines and their odour descriptors.

No.	Compound name	RT	FINAL WINES PERCENT OF TOTAL AREA						Odour descriptors	Ref.
			V1	V2	V3	V4	V5	V6		
<b>ALCOHOLS</b>										
1	1-propanol	11.46	0.50	1.97	1.63	2.11	2.23	1.95	Fruity	[5]
2	1-butanol	15.64	0.17	0.12	0.15	0.14	0.12	0.16	Fusel, spirits	[5]
3	1-hexanol	24.97	1.22	0.84	0.81	0.95	0.78	0.64	Vegetal, green, fruity, apple-skin, oily	[12] [13]
4	isoamylalcohol (isopentyl alcohol)	18.53	37.18	41.82	42.39	45.67	45.50	49.88	Harsh, nail polish, banana	[5]
5	benzeneethanol (Phenylethyl alcohol)	47.19	13.50	12.85	12.78	12.64	15.25	10.79	Floral, rose	[5]
6	3-penten-2-ol	16.83	0.09	0.12	0.09	0.11	0.11	0.14	Kiwi, fruity, acrid, pungent	[14]
7	3-ethoxy-1-propanol	26.02	0.68	1.09	1.13	1.21	0.89	1.21	Fruity	[15]
<b>ESTERS</b>										
8	ethyl octanoate	28.59	2.33	1.67	1.80	1.90	1.66	2.22	Fruity, banana, apple, pineapple, pear, floral, sweet, soap	[12] [5] [15]
9	ethyl caproate	19.73	1.15	0.71	0.70	0.78	0.64	0.92	Fruity, pineapple-banana	[12]
10	ethyl lactate	24.70	0.95	1.86	1.92	2.07	2.15	1.57	Ethereal, buttery odour	[16]
11	ethyl 3-hydroxy butyrate	32.23	0.15	0.34	0.34	0.32	0.39	0.39	Fresh fruits	[15]
12	butanoic acid ethyl ester	11.57	0.19	0.34	0.42	0.44	0.35	0.42	–	–
13	isoamylacetate (3-Methylbutyl acetate)	14.91	3.60	1.25	1.11	1.40	1.07	1.33	Fruity, banana, solvent, pear	[12] [5]
14	ethyl decanoate	36.92	1.85	1.15	1.44	1.26	1.07	1.38	Floral, soap, fatty	[5]
15	diethyl hydroxybutanedioate (Diethyl tartrate)	51.48	0.24	0.46	0.44	0.53	0.54	0.27	–	–
16	butanedioic acid diethyl ester (Diethyl succinate)	38.48	0.31	0.43	0.42	0.44	0.46	0.51	Faint, pleasant odour	[12]
17	butandienoic acid, diethyl ester	63.18	2.41	2.41	2.47	2.88	3.04	3.31	Fruity, waxy, floral, slightly musty	[16]
28	ethyl 4-hydroxybutanoate	43.30	2.18	0.92	0.90	0.80	0.89	0.78	–	
19	phenylethylacetate	43.76	0.33	–	–	–	–	–	Honey, floral, rose, green, hay, cocoa	[13]
20	1-hexyl acetate	21.46	0.15	–	–	–	–	–	Fruity, fresh, green, sweet, apple, pear, banana peel	[13]
21	isopropyl acetate	40.91	1.08	1.87	1.90	1.53	1.60	2.16	–	–
22	butyl acetate	45.80	0.34	0.41	0.46	0.42	0.48	0.38	Fruity, pineapple	[16]
<b>ACIDES</b>										
23	acetic acid	30.49	2.59	0.57	0.56	0.60	0.59	0.69	Green, sour, rancid	[9]
24	butanoic acid	37.54	0.32	0.29	0.27	0.29	–	0.33	Animal	[12]
25	isovaleric acid (3-methyl butanoic acid)	39.07	0.18	0.31	0.31	0.31	0.28	0.36	Rancid, cheese, rotten fruits	[17]
26	isobutyric acid	35.15	0.15	0.13	0.14	0.15	0.11	0.18	Pungent, ethereal, fruity, rum-like nuance	[16]
27	hexanoic acid	45.40	4.28	2.42	2.45	2.09	1.93	2.31	Rancid, fatty	[17]
38	octanoic acid	52.52	8.18	4.44	4.29	5.19	4.74	3.63	Fatty acid, dry, dairy, cheese, sweet	[17]
29	stearic acid	72.16	–	2.03	1.97	2.17	2.21	1.66	–	–
30	decanoic acid	59.04	2.19	0.89	0.93	1.05	1.06	0.73	Rancid, fat, dry, woody	[9]

\*Ref - References.



**Figure 1.** GC chromatogram for distribution of volatile components in analyzed wines.



**Figure 2.** Organoleptic parameters of obtained wines.

These differences can be generated by enzymes reactions and yeast. *Saccharomyces cerevisiae* yeast can have a major contribution to wine aroma profile.

The aroma profile of analysed samples is expressed especially by higher alcohols, esters and acids. The highest concentration in alcohols was detected in V5 variant, followed by V6, V3, V2, and V1. On the other hand, the richest variant in esters was V1, followed by V6, V4, V3, V2 and V5. V1 variant registered the highest amount of acids, followed by V4, V3, V5, V2 and V6. Isoamylalcohol and benzenethanol were the highest among the total of identified alcohols, imprinting complexity to the aroma. Esters constitute one of the most important classes of aroma compounds, with an important effect

on the fruity notes in wines (tropical fruits, banana, pineapple, pears). Butandienoic acid diethyl ester showed a high concentration in all variants. Organic acids enhance bitterness, freshness and astringency. Butanoic, hexanoic and decanoic acids presented the highest proportion. Phenylethylacetate and 1-hexyl acetate are specific for V1 sample (treated with Endozym Thiol®).

Significant differences can be observed referring to the quality of final wines, reported on the type of used enzyme. Enzymes released additional volatile compounds from the grapes, reducing the amount of alcohol, allowing a better perception and release of aroma compounds. The different distribution of volatile components in analysed wines is presented in Fig. 1.

### 3.4. Sensory characteristics

The final sensorial quality of wine is the result of a multitude of interaction between all the volatile components. The character of wine is influenced by the sweetness, acidity and astringency/bitterness, generated by non-volatile substances soluble in water or alcohol mixture. Together with volatile compounds, they are liable for the general aroma profile. Perceptible sweetness is significantly determined by numerous constituents, such as ethanol, acids or tannins. Detectable sweetness presents a diminishing effect on sourness and bitterness [18]. Sweet note was better remarked at V1 sample.

The principal organoleptic parameters of analysed wines are presented in Fig. 1. Following the sensory analysis, significant differences can be observed due to the type of enzyme preparation used as pre-treatment.

Enzymatic treatments generated an increase in flavor descriptors content compared to control sample. All wines were described as equilibrated, with a great acidity, that imprinted freshness and a good texture.

Control sample was characterized by hay notes, honey and vegetable flavour. V1 sample was remarked for its fruity taste (ripe fruits, exotic fruits, dry fruits), wild flowers and roses nose. Citric flavour was dominant in V2 sample and green fruits aroma is prevalent in V5.

Regarding the correlation between the chemical compounds and the results of sensorial analysis, the fruity notes (ripe fruits) of samples can be explained by the presence of 1-propanol, 1-hexyl acetate, 1-hexanol, ethyl octanoate, ethyl caproate. The presence of 1-hexanol explains the vegetal notes of samples. Ethyl caproate and isoamylacetate are the main responsible for the tropical aromas.

## 4. Conclusions

In this study, the pre-fermentative treatments did not appear to have a significant influence on the basic physical-chemical parameters. A perceptible colour difference between samples treated with enzyme preparations and control samples was observed, suggesting that enzymes preparation had a major effect on colour. Following the evolution of volatile compounds during fermentation, enzymes released additional volatile compounds from the grapes, reducing the amount of alcohol, thus allowing for a better perception and release of aroma compounds. Enzymatic treatments determine an increase in the total amount of volatile compounds compared to control sample. The most abundant compound in all samples is isoamylalcohol, followed by benzenethanol. Significant differences can be observed referring to the quality of final wines and on the type of used enzyme. The aroma profile

of analysed samples is expressed especially by higher alcohols, esters, and acids. Following the sensory analysis, significant differences can be observed due to the type of enzyme preparation used as pre-treatment.

Enzymes are effective, specific and convenient to use and the need for a more in depth search is clear.

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