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Influence Evaluation of Enzyme Treatments on Aroma Profile of White Wines

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Abstract: Improving aroma profile represents one of the principal goals in winemaking. This paper focuses to evaluate the influence of enzymes applied before alcoholic fermentation of Fetească regală and Sauvignon blanc wines, even if most studies analyze their use in different winemaking stages. Fetească regală wines are described by higher proportions (1.07–4.28%) of ethyl octanoate (exotic fruits), 3-methylbutyl acetate (pear, banana), hexanoic acid (creamy, phenolic, exotic fruits), propan-2-yl acetate (ripe fruits, banana), and ethyl decanoate (floral, fruity, woody), while Sauvignon blanc wines are distinguished by significant proportions (2.77–42.15%) of 3-methylbutan-1-ol (exotic fruits), acetic acid (vegetal, sour), 1-phenylethanol (floral, honey), and diethyl butanedioate (fruity, floral). Variables as 3-methylbutyl acetate-ethyl decanoate, ethyl decanoate-hexanoic acid ($r > 0.8$) showed proportional levels in Fetească regală wines. In Sauvignon blanc samples, positive correlations were observed for 2-ethyl hydroxypropanoate-diethyl butanedioate or 2,3-butanediol-ethyl 4-hydroxybutanoate ($r > 0.7$). Data confirmed a significant influence of enzymes on wine's aroma profile ($p < 0.05$). The higher proportions of the most volatile compounds were obtained in samples treated with pectinases, for both varieties. In correlation with the sensory analysis, these variants showed the lowest intensity for negative descriptors such as phenolic sensation, the mineral or bitter taste, demonstrating that pectinases can give more acceptable results regarding the sensory perception compared to β-glycosidases.

Keywords: volatile fraction; sensory evaluation; winemaking optimization; pectinase; glycosidase

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1. Introduction

Enzymes preparations are efficient and versatile products, with a large application in food and beverages (especially wine, beer, or fruit juices) production [1]. These biocatalysts are used for decades during various winemaking phases ensuring wide chains of effects, such as to increase grape juice yield, to improve wine's stabilization and filtration characteristics [2]. Several authors proposed different perspectives for the application of enzymes in winemaking, with effective results in optimizing production process. Therefore, higher concentrations of phenolic compounds [3,4] and amino acids [5–8], enriched volatile [9–11] and sensory profiles [12,13] can be obtained when enzyme preparation are used. Moreover, previous publications have indicated a minor effect of enzymes on the physicochemical parameters of wines [14], but a major impact on chromatic parameters [3,15–17]. These hypotheses have also been confirmed in our previous works [18].

The interest in the consumption of high quality wine has increased in recent years. It is well-known that consumers require wines with a high commercial value (appearance, chromatic parameters, olfactory and gustatory particularities). Therefore, winemakers have always tried to apply modern technologies and treatments (including enzymes) that optimize their production process [19]. The sensorial perception of wines results from numerous interactions between chemical constituents and specific factors, including the applied technology, serving temperature [20], aging or storage conditions [21]. The sensory analysis of wines provides objective information on the consumer's understanding, the acceptance or rejection grade of stimuli, and the description of the evoked emotions [22].

Even if the management of the vineyard and grape characteristics (variety, chemical composition) is essential for the wine's final quality [23–25], many of the sensory characteristics of the wines cannot be detected in the grapes, being results of numerous biochemical reactions that take place during the winemaking period. During fermentation phase, yeast can release glucosidases [20,26]. These enzymes can convert odorless glycosidically bound precursors in aromatic compounds. Of these, glycosidases act by releasing aromas bound to sugars and sugar residues to form odorless glycosides, especially from monoterpene-rich varieties [27]. Some aromatic compounds such as monoterpenes, benzene derivatives, C13-norisoprenoids, and aliphatic alcohols are usually glycosylated in the grape berry cells, being released by enzyme hydrolysis [28]. Volatile thiols can also result from odorless precursors in fruits. The action of β-glycosidases that come from grapes or are produced by *Saccharomyces cerevisiae* yeasts is limited in winemaking, many of these enzymes being also present in the resulting young wines. Therefore, attention has been focused on using exogenous enzyme preparations, the filamentous fungi-originated pectinases being good alternatives [28].

It is well-known that wines contain numerous flavor- and aroma-active compounds that contribute to the final perception of wine. While the general composition of most grapes is similar, there are obvious aroma differences between them. This can be attributed to relatively minor variations in the proportions of the chemical constituents [20]. The principal chemical classes reported in wine aroma are esters, alcohols, terpenes, and acids. Of these, esters give a sweet-fruity aroma when are found in low proportions in wines. These components are generally alkyl acetates formed by condensation reaction of organic acids with alcohols. Terpenes are usually responsible for floral notes while free acids are correlated with aged wines [29]. Most fatty acids usually give off an unpleasant rancid, lactic, sour taste, but they contribute significantly to defining the aromatic balance and complexity of wines, preventing the hydrolysis of esters. Volatile acids are responsible for woody and almonds notes [30,31].

Even if many studies already postulated the importance of volatile compounds in winemaking, there has always been a challenge due to the complexity and diversity of these compounds in wines. Although the use of enzymes is often studied, most articles refer to terpenes and norisoprenoids enrichment of wines obtained from aromatic varieties. This paper focuses on numerous classes of volatile compounds present in musts and, respectively, in wines obtained from two semi-aromatic varieties. Limited data regarding the aroma profile of Feteacă regală and Sauvignon blanc wines from Romanian vineyards are available. Considering the mentioned, the purpose of this work is to determine the influence of some commercial enzymes on the aroma profile of white wines. The originality of this experiment consists in the application of enzymes before the fermentation stage, in must, as manufacturer's recommendations and most authors [2–18] analyze their action during other different phases of the winemaking. The results are a continuation of the team's previously published data, which focused on the effect of enzymes on the physicochemical characteristics, chromatic parameters, on the evolution of phenolic compounds and amino acids' profiles [18,32].

2. Materials and Methods

2.1. Vine Growing Cultural Conditions and Winemaking Processes

Fetească regală and Sauvignon blanc grapes were manually harvested from Iași-Copou vineyard (at 47°10′ north latitude and 27°35′ east longitude, Romania), with 220 g/L and 250 g/L sugars, respectively. The vines that were used in this study have semi-tall trunks (80 cm) and are trained with two canes containing an average of 12–14 buds per cane on a vertical shoot positioned trellis. These characteristics are meant to aid in combating frost damage and quality-driven production. Planting distances are 1.2/2.2 m. Regarding Fetească regală, yields range approximately around 4.5 kg per plant. Clusters vary as well, from 32 to 37 clusters per vine, while mass of one grape ranges from approximately 120 g to 140 g. Even if Fetească regală can genetically produce high quantities of grapes, cultural technologies were applied in order to maximize quality instead of quantity. Therefore, cane pruning and reduced bud load were decided. Concerning Sauvignon blanc, yields range around 5 kg per plant. Clusters vary as well, from 40 to 62 clusters per vine, while mass of one grape ranges from approximately 80 g to 125 g.

In the preparation of wine samples of the same variety, the only variable was the type of enzyme preparation used. So, after the quantitative (grapes weigh) and qualitative reception (sugar determination), the fruits were destemmed and pressed, and the must was separated in six parts into 50 L glass containers. In each aliquot, 20 g/hL of *Saccharomyces* yeast (*Levulia*® *esperide,* AEB, San Polo, Italy) was inoculated with 30 g/hL of yeast nutrient (FERMOPLUS® CH, AEB, San Polo, Italy). Five commercial enzymes based on pectinase and β-glycosidase activities were administrated before alcoholic fermentation and six variants were resulting for each variety: V1-Endozym Thiol®, AEB, San Polo, Italy; V2-Endozym® β-Split®, AEB, San Polo, Italy; V3-Zymovarietal® aroma G, SODINAL, Plovdiv, Bulgaria; V4-Endozym® Ice, AEB, San Polo, Italy; V5-Zimarom®, BSG WINE, Napa, CA, USA; V6-no enzyme), at a dose of 3 g/hL for Endozym® β-Split, Zymovarietal® aroma G, Zimarom® and 3 mL/hL for the Endozym Thiol® and Endozym® Ice. The dosages were in line with the producer's instructions and current OIV recommendations [30]. After enzymatic treatment, fermentation was carried out to dryness at 16–18 °C (for about 16 days). Samples were constantly collected in triplicate every five days (day 1-I, day 6-II, day 11-III, and day 16-IV) and kept at −20 °C until analysis. After that period, 1.5 mL/L of sulfur dioxide (6% concentration) was added. Filtration process was realized using 0.45 µm sterile membrane filters. The final samples were bottled and stored at constant temperature (8 °C), in the dark, and at 70–80% humidity. The samples were obtained in triplicate and analyzed after 6 months (indicated by the moment V).

The analyzed wines were dry, with approximately 1.3–1.8 g/L reducing substances in Fetească regală variants (alcoholic strength > 12.7% vol.) and 1.9–2.7 g/L reducing substances (alcoholic strength > 16.2% vol.) in Sauvignon blanc [29].

2.2. Volatile Compounds Determination

The evolution of over 65 volatile compounds was evaluated during alcoholic fermentation and final wines (after 6 months of storage). Volatile compounds were identified and separated using a gas chromatography system (Agilent 7890A) coupled with a mass spectrometer detector (5975 C inert XL EI/CI MSD) [31]. For sample extraction, a volume of 5 mL wine and 1 mL of dichloromethane was transferred into a 15 mL glass tube and the mixture was stirred for 45 min and then centrifuged at 7800× *g* for 10 min. The whole process was repeated and the extraction was carried out for 20 min. The two organic phases obtained were mixed and injected in the system. The Zebron ZB WAX fused silica capillary column had 60 m × 0.25 mm I.D., and 0.25 µm film thickness. Helium 6.0 was used as carrier gas under constant flow mode at 1 mL/min. Injection volume was 1 µL and the inlet temperature was set at 250 °C. The oven initial temperature was set at 40 °C held for 3 min, then programmed to increase with 3 °C/min to 230 °C, where it was kept for 4 min, then it was increased to a final temperature of 260 °C with 10 °C/min, that was maintained for 2 min. The mass spectrometer detector was used at 70 eV in the electron impact mode, using the mass range from 35 to 550 Da at 150 °C. Peak identification of the components was achieved by comparison of mass spectra with the mass spectral data collection from Wiley275 and NIST05a libraries. Both the samples and the laboratory determinations were performed in triplicate, under the same experimental conditions. Recorded data is expressed in proportion of the total area [18,33,34].

2.3. Sensory Analysis

The sensory characterization of the experimental wine samples was realized in accordance with the specifications indicated by the ISO8589:2010 [35] and ISO3591:1997 [36] standards and the OIV recommendations [37]. The tasting session was organized in the first part of the day to ensure a better perception of the studied descriptors. Samples were analyzed at 10–12 °C wine temperature. The sensory profile of the wines was evaluated by a panel of qualified tasters from the Iasi University of Life Sciences, represented by 11 men and 9 women. The evaluation of the sensory characteristics was made following some white wines key descriptors (vegetal, mineral, citric; ripe fruits; exotic fruits; dry fruits, hay, wildflowers, roses, sweet, honey, acid, bitter, phenolic, unctuous), and by giving grades from 0 to 5, depending on the perception intensity of the evaluated descriptors. The results were centralized and the arithmetic average of the resulting grades was made.

2.4. Statistics

Data processing including Anova and Fisher's Least Significant Difference was made with Statgraphics 19® centurion (Statgraphics Technologies, The Plains, VA, USA). Principal components analysis and Pearson correlation coefficient was realized using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). To perform the principal component analysis, ten predominant volatile compounds were selected for each variety. For the visual representation of sensory analysis results, the online version of RAWGraphs 2.0 beta (https://app.rawgraphs.io, accessed on 7 September 2022) was used.

3. Results

3.1. The Influence of Enzymes on Volatile Compound Fraction

The identified aroma compounds and their odor characteristics are presented in Tables 1–3.

Following the results obtained by gas chromatography, over 65 volatile compounds were identified, depending on the grape variety. Figures 1 and 2 demonstrate that V1 for Fetească regală and V2 for Sauvignon blanc wines have been highly differentiated from the others in volatile compounds proportions. Examples of chromatograms obtained in samples with and without enzymes are presented in Figure 3. From PCA (Figures 1 and 2) and Pearson correlation matrix, variable pairs as 3-methylbutyl acetate–ethyl decanoate (r = 0.87), ethyl octanoate–ethyl decanoate (r = 0.83), ethyl decanoate–hexanoic acid (r = 0.92) showed a high positive correlation in Fetească regală wines, their values being proportional.

Figures 1 and 2 demonstrate that four principal components for Fetească regală and five principal components for Sauvignon blanc samples were extracted (totaling 98.81% and respectively, 99.52%), with eigenvalues greater than 1.0.

Observing the PCA analysis, hexanoic acid have the greater influence on PC2, being positioned farther away from its origin, and ethyl decanoate have higher contribution in PC1. Regarding the Sauvignon blanc samples, positive correlations can be observed between the following pairs of compounds: 2-ethyl hydroxypropanoate–diethyl butanedioate (r = 0.88), acetic acid–1,6-anhydro-2,3,4-trimethylgalactose (r = 0.79), 2,3-butanediol–ethyl 4-hydroxybutanoate (r = 0.77), etc. From PCA analysis, the 1,6-anhydro-2,3,4-trimethylgalactose has the greater influence on PC2, while 1-phenylethanol has more impact on PC1.

The analyzed volatile compounds, their aroma descriptors, odor, and flavor thresholds are presented in Table 1. Tables 2 and 3 highlight the identified proportion of these compounds and homogenous groups (noted with letters). Thus, in Fetească regală variants, 25 esters, 12 alcohols, 12 hydrocarbons, 11 acids, and other compounds (carbonyl compounds, terpenes, nitrogen compounds, volatile phenols, etc.) were separated. Regarding the Sauvignon blanc samples, esters were predominant (20), followed by alcohols (15), hydrocarbons (16), and acids (7).

Table 1. Aroma descriptor and thresholds of the analyzed volatile compounds.

FR	SB	Volatile Compounds	Clasiff.	CAS	RT (Min)	OT (ppb)	FT (ppb)	AD	Ref.
1	1	propan-1-ol	alcohols	71-23-8	11.46	9000	-	ripe fruits	[38]
2	2	ethyl butanoate	esters	105-54-4	11.56	1	450	-	-
	3	2,6-dimethyldecane	hydrocarbons	13150-81-7	11.64	-	-	-	-
3	4	3-methylpropan-1-ol	alcohols	78-83-1	13.44	-	50,000	alcoholic, nail polish, pungent smell	[39]
4	5	3-methylbutyl acetate	esters	123-92-2	14.91	2	-	fruity, bananas, pears, acetone	[40]
5	6	butan-1-ol	alcohols	71-36-3	15.64	500	-	alcoholic, spirtuous	[38]
6	7	3-penten-2-ol	alcohols	1569-50-2	16.83	1.5	-	kiwi, fruity	[39]
34	8	undecane	hydrocarbons	1120-21-4	39.15	-	-	-	-
7		dodecane	hydrocarbons	112-40-3	18.16	-	-	petrol	[41]
8	9	3-methylbutan-1-ol	alcohols	123-51-3	18.54	250–300	170	alcoholic, nail polish, bananas	[38]
	10	decane	hydrocarbons	124-18-5	19.28	-	-	-	-
	11	5-ethyl-5-methyldecane	hydrocarbons	17312-74-2	19.60	-	-	-	-
9	12	ethyl hexanoate	esters	123-66-0	19.72	1	-	fruity, bananas, pineapple	[38]
	13	2,4,6-trimethylheptane	hydrocarbons	2613-61-8	20.07	-	-	-	-
	14	2-methyldecane	hydrocarbons	6975-98-0	20.20	-	-	-	-
10		dotriacontan	hydrocarbons	544-85-4	20.21	-	-	-	[38]
11		hexyl acetate	esters	142-92-7	21.46	2	-	fruity, pear	[18]
12	15	3-hydroxy-2-butanone	carbonyl compounds	51555-24-9	22.19	800	-	unctuous, milky	[39]
	16	3,9-dimethylundecane	hydrocarbons	17301-31-4	22.39	-	-	-	-
	17	3,3-dimethylhexane	hydrocarbons	563-16-6	22.53	-	-	-	-
	18	3-methylpentan-2-ol	alcohols	565-60-6	23.13	-	-	-	-
13		4-methyltetradecane	hydrocarbons	25117-24-2	22.39	-	-	pungent smell	[41]
14		2,6,10,14-tetramethylhexadecane	hydrocarbons	638-36-8	22.55	-	-	-	-
15	19	ethyl 2-hydroxypropanoate	esters	97-64-3	24.70	110,000	-	unctuous, ethereal	[39]
16	20	hexan-1-ol	alcohols	111-27-3	24.98	2500	-	vegetal, fruity, green apple peel	[40]
17	21	3-ethoxypropan-1-ol	hydroxyethers	1589-49-7	26.03	-	-	fruity	[41]
	22	nonadecane	hydrocarbons	629-92-5	28.27	-	-	-	-
18		isotetradecane	hydrocarbons	1560-96-9	26.97	-	-	-	-
19	23	ethyl octanoate	esters	106-32-1	28.59	-	15	fruity, banana, apple, pineapple, pear, floral, soapy	[42]
	24	2,6,10-trimethyldodecane	-	3891-98-3	-	-	-	-	-
20	25	eicosan	hydrocarbons	112-95-8	28.98	-	-	waxy, floral	[43]
	26	octadecane	hydrocarbons	593-45-3	29.46	-	-	-	-

Table 1. *Cont.*

FR	SB	Volatile Compounds	Clasiff.	CAS	RT (Min)	OT (ppb)	FT (ppb)	AD	Ref.
21		1-propylaziridine	nitrogen compounds	104549-74-8	30.43	-	-	-	-
22	27	acetic acid	acids	64-19-7	30.44	-	22,000	vegetable, rancid, sour	[39]
	28	pentacosan	hydrocarbons	629-99-2	-	-	-		
23	29	ethyl 3-hydroxybutanoate	esters	5405-41-4	32.24	14,000	-	fruity	[44]
	30	benzaldehyde	carbonyl compounds	100-52-7	32.53	350–3500	1500	almonds, nuts, fruity	[43]
24	31	2,3-butanediol	alcohols	107-88-0	33.14	150,000	-	fruity, fresh	[45]
25	32	1,3-butanediol	alcohols	107-88-0	34.63	-	-	-	-
26	33	2-methylpropanoic acid	acids	79-31-2	35.12	8100	-	fruity, tangy, ethereal, hint of rum	[40]
	34	ethyl acetamide	nitrogen compounds	625-50-3	36.68	-	-	-	-
27		hexadecanoic acid	acids	57-10-3	35.3	10,000	-	fatty, waxy	[44]
28	35	ethyl decanoate	esters	110-38-3	36.92	-	510	fruity, grape, pear, apple, waxy, oily	[18]
	36	1,2-hydrazinedicarboxaldehyde	carbonyl compounds	628-36-4	37.13	-	-	-	-
	37	10-methylnonadecane	hydrocarbons	56862-62-5	37.71	-	-	-	-
29		butanoic acid	acids	107-92-6	37.50	240	6200-6800	cheese, rancid, sweet, animal	[44]
30	38	diethyl butanedioate	esters	123-25-1	38.48	-	-	fruity, floral, waxy, dusty	[40]
31		docosan	hydrocarbons	629-97-0	38.78	-	-	wax	[44]
32		ethyl 9-decenoate	esters	67233-91-4	38.95	-	-	fruity, buttery	-
33	39	3-methylbutanoic acid	acids	503-74-2	39.02	120–170	-	rancid, cheese, fermented fruit	-
	40	3-methylsulfanylpropan-1-ol	sulfur compounds	505-10-2	40.09	-	-	sulphurous, onion, garlic, raw potato	[44]
35		1,3-dithiolane	hydrocarbons	4829-04-3	40.13	-	-	sweet, sulphurous, fried onions	[44]
36	41	2-propanyl acetate	esters	108-21-4	40.92	180–670	-	ethereal, bananas, sweet, ripe apples, fresh fruit	[18]
37	42	ethyl 4-hydroxybutanoate	esters	999-10-0	43.3	-	-	pineapple, roses, tropical fruits, honey, coconut, nectar	[44]
38	43	2-phenylethyl acetate	esters	103-45-7	43.69	250	-	fruity, rose, honey, vegetable, pollen, nectar	[39]
39		heneicosan	hydrocarbons	629-94-7	44.03	-	-	wax	[44]
40	44	ethyl dodecanoate	esters	106-33-2	44.52	-	200	floral, fruity, grassy, woody	[18]
	45	1-octadecene	hydrocarbons	112-88-9	44.63	-	-	-	-

Table 1. *Cont.*

FR	SB	Volatile Compounds	Clasiff.	CAS	RT (Min)	OT (ppb)	FT (ppb)	AD	Ref.
41		3-methylbutyl decanoate	esters	2306-91-4	45.17	-	-	bananas, waxy, fruity, cognac, vegetal, unctuous	[44]
42	47	n-(3-methylbutyl)acetamide	nitrogen compounds	13434-12-3	45.23	-	-	-	-
43	46	hexanoic acid	acids	142-62-1	45.40	3000	5400	cheese, phenolic, unctuous, ripe fruit, tropical fruit	[44]
	48	hydroxybutyric acid	acids	300-85-6	45.79	-	-	-	
44		butyl acetate	esters	123-86-4	45.81	66	-	fruity, pineapple	[18]
	49	1-phenyl methanol	alcohols	100-51-6	45.95	-	-	honey, bubble gum, fruity	[18]
45	51	1-phenylethanol	alcohols	60-12-8	47.19	10,000	-	rose, floral, honey	[39]
	50	ethyl 3-methylbutyl butanedioate	esters	28024-16-0	46.66	-	-	caramel, dried fruit, mineral, medicinal, burnt	[18]
	52	n-ethyl acetamide	nitrogen compounds	625-50-3	51.38	-	-	-	-
	53	diethyl hydroxybutanedioate	esters	141-05-9	51.46	-	-	caramel, fruity, vegetal	[44]
46		2,6-dimetil-3-7-octadiene-2,6-diol	terpene	13741-21-4	48.12	-	-	citrus	[18]
47		diethyl 2,3-dihydroxybutanedioate	esters	57968-71-5	51.44	-	-	fruity, caramel, red fruits	[44]
48	54	octanoic acid	acids	124-07-2	52.51	3000	5300	cheese, rancid, fatty, vegetable, sweet	[39]
	55	2-[ethyl(methyl)amino]ethanol	alcohols	2893-43-8	54.98	-	-	-	-
49		4-ethenyl-2-methoxyphenol	phenolic compounds	7786-61-0	56.46	-	-	dry wood, roasted hazelnuts, amber	[44]
50		5-propyl-2-oxolanone	lactones	105-21-5	57.80	-	-	fruity, grapey	[18]
	56	1-docosene	hydrocarbons	1599-67-3	57.70	-	-	-	-
	57	ethyl hexadecanoate	esters	628-97-7	57.83	2000	-	fruity	[44]
51	58	9-ethyl hexadecanoate	esters	54546-22-4	58.6	-	-	-	-
52	59	decanoic acid	acids	334-48-5	58.99	10,000	3500	rancid, sour, greasy, nasty, woody	[39]
53	60	2,4-di-tert-butylphenol	phenols	96-76-4	59.77	-	-	-	-
	61	hexaoxacyclooctadecane	hydrocarbons	17455-13-9	60.36	-	-	-	-
	62	11-octadecenal	carbonyl compounds	56554-95-1	60.56	-	-	-	-
54		3,7,11-trimethyl-2,6,10-dodecatrienol	terpenes	4602-84-0	60.82	-	-	lime blossoms, grapefruit, peach, anise, citrus, pear	[44]
	63	1,6-anhydro-2,3,4-trimethylgalactose	anhydrous sugars	-	62.50	-	-	-	-

Table 1. *Cont.*

FR	SB	Volatile Compounds	Clasiff.	CAS	RT (Min)	OT (ppb)	FT (ppb)	AD	Ref.
55		5-(1-hydroxyethyl)-furan-2-one	lactones	27610-27-1	61.98	-	-	-	-
56		4-ethenylphenol	phenolic compounds	2628-17-3	62.35	-	-	pungent smell, medicinal, gouache, medicinal, phenolic	[18]
57		2,3-dimethyl-1-pentene	hydrocarbons	3404-72-6	62.73	-	-	-	-
58		dimethyl butanedioate	esters	106-65-0	62.98	-	-	fruity, sweet, green fruit, floral, waxy, soapy	[44]
59	64	ethyl octadecanoate	esters	111-61-5	63.64	-	-	waxy	[44]
	65	4-hydroxy benzeneethanol	phenolic compounds	501-94-0	64.01	-	-	chemical, bitter, honey, wax, toast, smoke, cloves	[46]
60	66	ethyl 9-octadecenoate	esters	111-62-6	64.21	-	-	fresh, woody	[47]
61		methyl-10-octadecenoate	esters	13038-45-4	64.43	-	-	-	-
62		dodecanoic acid	acids	143-07-7	64.92	10,000	-	coconut, waxy, buttery	[44]
63	67	ethyl octadeca-9,12-dienoate	esters	544-35-4	65.55	-	-	sweet, freshness	[47]
64		2,3,5,8-tetramethyl-1,5,9-decatriene	hydrocarbons	230646-72-7	66.55	-	-	-	-
65		3,7,11,15-tetramethylhexadeca-1,6,10,14-tetraen-3-ol	esters	1113-21-9	66.55	-	-	fruity, floral, roses	[39]
66		9-octadecenamide	nitrogen compounds	3322-62-1	66.78	-	-	-	-
67		ethyl octadeca-9,12,15-trienoate	esters	1191-41-9	67.37	-	-	-	-
68		2-methyl-4-octanol	alcohols	40575-41-5	70.17	-	-	-	-
69		1-tetradecanoic acid	acids	544-63-8	71,01	10,000	-	waxy, buttery, pineapple, citrus peel	[44]
70		octadecanoic acid	acids	57-11-4	72.09	20,000	-	waxy, buttery	[44]
	68	n-(2-phenylethyl)acetamide	nitrogen compounds	877-95-2	67.30	-	-	-	-

FR—Fetească regală; SB—Sauvignon blanc; Clasif.—classification; CAS—Chemical Abstracts Service; RT—retention time; OT—odor thresholds; FT—flavor thresholds; AD—aroma descriptor; Ref. —references. Odor and flavor thresholds were presented according to www.leffingwell.com platform (accessed on 5 September 2022).

Table 2. Final proportion of identified volatile compounds in Fetească regală wines (% of total area).

C	V1	V2	V3	V4	V5	V6
1	0.51 ± 0.02 *	1.98 ± 0.01 c	1.63 ± 0.02 a	2.12 ± 0.03 *	2.24 ± 0.01 *	1.96 ± 0.01 c
2	0.19 ± 0.00 *	0.34 ± 0.01 a	0.42 ± 0.01 d	0.44 ± 0.01 *	0.35 ± 0.01 ab	0.42 ± 0.01 d
3	3.83 ± 0.02 *	4.35 ± 0.02 a	3.96 ± 0.00 *	4.46 ± 0.01 *	4.27 ± 0.00 *	4.36 ± 0.01 a
4	3.60 ± 0.02 d	1.25 ± 0.02 *	1.11 ± 0.00 b	1.40 ± 0.02 *	1.07 ± 0.00 a	1.33 ± 0.02 c
5	0.18 ± 0.00 bc	0.12 ± 0.02 a	0.15 ± 0.00 abc	0.15 ± 0.02 abc	0.12 ± 0.01 a	0.16 ± 0.02 abc
6	0.09 ± 0.01 a	0.12 ± 0.01 bc	0.09 ± 0.00 a	0.11 ± 0.00 abd	0.11 ± 0.01 ab	0.14 ± 0.01 c
7	0.13 ± 0.02 a	0.19 ± 0.02 c	0.18 ± 0.00 c	0.19 ± 0.01 c	0.14 ± 0.00 ab	0.14 ± 0.00 ab
8	37.19 ± 0.01 *	41.82 ± 0.03 *	42.39 ± 0.03 *	45.67 ± 0.02 *	45.51 ± 0.02 *	49.89 ± 0.00 *
9	1.15 ± 0.00 *	0.71 ± 0.03 d	0.70 ± 0.03 d	0.78 ± 0.00 *	0.64 ± 0.00 b	0.92 ± 0.02 *
10	0.25 ± 0.02 b	0.37 ±. 0.02*	0.32 ± 0.00 cd	0.33 ± 0.01 d	0.25 ± 0.01 b	0.26 ± 0.01 b
11	0.15 ± 0.07 *	nd	nd	nd	nd	nd
12	0.13 ± 0.00 *	nd	nd	nd	nd	nd
13	nd	nd	nd	nd	nd	nd
14	nd	nd	nd	nd	nd	nd
15	0.95 ± 0.00 *	1.86 ± 0.01 *	1.92 ± 0.01 *	2.07 ± 0.00 a	2.15 ± 0.00 *	1.57 ± 0.00 *
16	1.22 ± 0.04 *	0.85 ± 0.01 c	0.81 ± 0.04 ab	0.96 ± 0.00 de	0.78 ± 0.04 a	0.64 ± 0.00 *
17	0.69 ± 0.00 a	1.09 ± 0.00 bc	1.13 ± 0.00 d	1.22 ± 0.00 f	0.90 ± 0.00 *	1.21 ± 0.00 ef
18	0.19 ± 0.00 *	0.14 ± 0.01 c	0.15 ± 0.00 c	0.17 ± 0.00 b	0.15 ± 0.00 c	0.12 ± 0.00 b
19	2.33 ± 0.04 de	1.67 ± 0.3 b	1.80 ± 0.06 bc	1.90 ± 0.01 c	1.66 ± 0.00 bd	2.22 ± 0.00 *
20	0.50 ± 0.02 b	0.49 ± 0.10 b	0.05 ± 0.00 *	0.48 ± 0.00 b	0.38 ± 0.00 *	0.49 ± 0.00 b
21	nd	0.06 ± 0.00 ac	0.05 ± 0.00*	0.06 ± 0.00 ac	0.03 ± 0.00 b	0.07 ± 0.00 *
22	2.59 ± 0.02 *	0.57 ± 0.01 bc	0.56 ± 0.02 b	0.60 ± 0.00 *	0.59 ± 0.02 c	0.69 ± 0.03 *
23	0.15 ± 0.00 a	0.34 ± 0.00 c	0.34 ± 0.01 c	0.32 ± 0.02 bc	0.39 ± 0.02 d	0.39 ± 0.00 d
24	1.64 ± 0.02 *	1.04 ± 0.02 a	1.13 ± 0.02 b	1.00 ± 0.02 *	1.14 ± 0.01 b	1.05 ± 0.00 a
25	0.41 ± 0.08 bc	0.41 ± 0.08 abc	0.36 ± 0.08 ab	0.48 ± 0.04 cd	0.34 ± 0.06 a	0.42 ± 0.04 abc
26	0.15 ± 0.01 bcd	0.13 ± 0.01 ab	0.14 ± 0.01 ab	0.15 ± 0.03 bcd	0.11 ± 0.02 a	0.18 ± 0.04 d
27	0.14 ± 0.00 bcd	0.12 ± 0.00 abc	0.11 ± 0.04 ab	0.12 ± 0.04 abc	0.11 ± 0.03 ab	0.11 ± 0.02 ab
28	1.85 ± 0.04 *	1.15 ± 0.02 *	1.44 ± 0.03 *	1.26 ± 0.03 *	1.07 ± 0.03 *	1.38 ± 0.02 *
29	0.32 ± 0.01 d	0.29 ± 0.01 bc	0.27 ± 0.03 abc	0.29 ± 0.02 bc	nd	0.33 ± 0.03 d
30	0.31 ± 0.00 abc	0.43 ± 0.02 d	0.42 ± 0.04 d	0.44 ± 0.03 d	0.46 ± 0.03 d	0.51 ± 0.02 *
31	0.13 ± 0.02 bc	0.14 ± 0.01 b	0.16 ± 0.03 c	0.13 ± 0.04 bc	0.13 ± 0.00 b	0.16 ± 0.00 c
32	nd	nd	nd	nd	nd	nd
33	0.18 ± 0.01 *	0.31 ± 0.02 a	0.31 ± 0.03 a	0.31 ± 0.00 a	0.28 ± 0.04 a	0.36 ± 0.02 b
34	nd	0.10 ± 0.00 *	0.00 ± 0.00 a	nd	nd	nd
35	0.20 ± 0.04 d	0.15 ± 0.03 abc	0.15 ± 0.03 abc	0.12 ± 0.04 a	0.12 ± 0.00 a	0.18 ± 0.00 cd
36	1.08 ± 0.03 a	1.87 ± 0.03 bc	1.90 ± 0.02 c	1.53 ± 0.00 *	1.60 ± 0.00 *	2.16 ± 0.04 *
37	2.18 ± 0.02 *	0.92 ± 0.03 g	0.90 ± 0.02 fg	0.80 ± 0.02 bc	0.89 ± 0.00 ef	0.78 ± 0.00 ab
38	0.33 ± 0.03 *	nd	nd	nd	0.00 ± 0.00 a	nd
39	0.18 ± 0.01 bcd	0.18 ± 0.03 bcd	0.19 ± 0.03 cd	0.17 ± 0.00 bc	0.12 ± 0.00 a	0.12 ± 0.00 a
40	nd	nd	nd	nd	nd	nd
41	nd	nd	nd	nd	nd	nd
42	nd	0.18 ± 0.02 cd	0.21 ± 0.00 d	0.13 ± 0.00 ab	0.15 ± 0.02 abc	0.16 ± 0.00 bc
43	4.28 ± 0.05 *	2.42 ± 0.00 b	2.45 ± 0.00 b	2.09 ± 0.00 *	1.93 ± 0.00 *	2.31 ± 0.00 a
44	0.34 ± 0.04	0.41 ± 0.02	0.46 ± 0.00	0.42 ± 0.00	0.48 ± 0.04	0.38 ± 0.06
45	13.51 ± 0.02 *	12.85 ± 0.01 *	12.79 ± 0.00 *	12.65 ± 0.00 *	15.26 ± 0.00 *	10.80 ± 0.02 *
46	0.17 ± 0.07 b	0.09 ± 0.03 a	0.11 ± 0.00 a	0.11 ± 0.01 a	0.13 ± 0.00 *	0.09 ± 0.01 a
47	0.24 ± 0.03 a	0.46 ± 0.00 *	0.44 ± 0.02 de	0.53 ± 0.00 g	0.54 ± 0.02 g	0.27 ± 0.00 b
48	8.18 ± 0.00 *	4.44 ± 0.03 a	4.29 ± 0.02 *	5.19 ± 0.00 *	4.74 ± 0.00 b	3.63 ± 0.01 *
49	0.87 ± 0.05 g	0.74 ± 0.02 *	0.64 ± 0.02 ab	0.89 ± 0.00 g	0.73 ± 0.03 *	0.50 ± 0.03 *
50	0.19 ± 0.06 a	0.44 ± 0.02 ab	0.44 ± 0.50 a	0.49 ± 0.03 b	0.51 ± 0.02 b	0.37 ± 0.02 ab
51	nd	nd	nd	nd	nd	nd
52	2.19 ± 0.01 *	0.89 ± 0.01 *	0.93 ± 0.00 b	1.05 ± 0.01 d	1.06 ± 0.01 d	0.73 ± 0.00*
53	0.49 ± 0.05 fg	0.44 ± 0.03 de	0.42 ± 0.02 cd	0.53 ± 0.02 g	0.41 ± 0.01 cd	0.48 ± 0.04 ef
54	nd	nd	nd	nd	nd	nd
55	0.28 ± 0.00 *	0.14 ± 0.00 *	0.18 ± 0.00 *	0.11 ± 0.00 a	0.15 ± 0.00 *	0.16 ± 0.00 *
56	1.78 ± 0.04 *	0.61 ± 0.04 d	0.52 ± 0.03 c	0.53 ± 0.03 bc	0.62 ± 0.00 d	0.43 ± 0.03 a
57	nd	nd	nd	nd	nd	nd
58	2.41 ± 0.06 a	2.41 ± 0.04 a	2.47 ± 0.00 *	2.88 ± 0.00 *	3.04 ± 0.00 *	3.31 ± 0.00 *

Table 2. *Cont.*

C	V1	V2	V3	V4	V5	V6
59	nd	nd	nd	nd	nd	nd
60	nd	nd	nd	nd	nd	nd
61	nd	nd	nd	nd	nd	nd
62	nd	nd	nd	nd	nd	nd
63	nd	nd	nd	nd	nd	nd
64	nd	nd	nd	nd	nd	nd
65	nd	nd	nd	nd	nd	nd
66	nd	5.92 ± 0.01 *	6.04 ± 0.04 *	nd	nd	nd
67	nd	nd	nd	nd	nd	nd
68	nd	nd	nd	nd	nd	nd
69	nd	nd	nd	nd	nd	nd
70	nd	2.03 ± 0.03 *	1.97 ± 0.02 *	2.17 ± 0.01 c	2.21 ± 0.03 c	1.66 ± 0.02 b

C—analyzed compounds; V1—Endozym Thiol®, AEB; V2—Endozym β-Split®, AEB; V3—Zymovarietal aroma G®, SODINAL; V4—Endozym Ice®, AEB; V5—Zimarom®, BSG WINE; V6—control sample, no enzymes. 1—propan-1-ol; 2—ethyl butanoate; 3—3-methylpropan-1-ol; 4—3-methylbutyl acetate; 5—butan-1-ol; 6—3-penten-2-ol; 7—dodecane; 8—3-methylbutan-1-ol; 9—ethyl hexanoate; 10—dotriacontan; 11—hexyl acetate; 12—3-hydroxy-2-butanone; 13—4-methyltetradecane; 14—2,6,10,14-tetramethylhexadecane; 15—ethyl 2-hydroxypropanoate; 16—hexan-1-ol; 17—3-ethoxypropan-1-ol; 18—isotetradecane; 19—ethyl octanoate; 20—eicosan; 21—1-propylaziridine; 22—acetic acid; 23—ethyl 3-hydroxybutanoate; 24—2,3-butanediol; 25—1,3-butanediol; 26—2-methylpropanoic acid; 27—hexadecanoic acid; 28—ethyl decanoate; 29—butanoic acid; 30—diethyl butanedioate; 31—docosan; 32—ethyl 9-decenoate; 33—3-methylbutanoic acid; 34—undecane; 35—1,3-dithiolane; 36—2-propanyl acetate; 37—ethyl 4-hydroxybutanoate; 38—2-phenylethyl acetate; 39—heneicosan; 40—ethyl dodecanoate; 41—3-methylbutyl decanoate; 42—n-(3-methylbutyl)acetamide; 43—hexanoic acid; 44—butyl acetate; 45—1-phenylethanol; 46—2,6-dimethyl-3-7-octadiene-2,6-diol; 47—diethyl 2,3-dihydroxybutanedioate; 48—octanoic acid; 49—4-ethenyl-2-methoxyphenol; 50—5-propyl-2-oxolanone; 51—9-ethyl hexadecanoate; 52—decanoic acid; 53—2,4-di-tert-butylphenol; 54—3,7,11-trimethyl-2,6,10-dodecatrienol; 55—5-(1-hydroxyethyl)-furan-2-one; 56—4-ethenylphenol; 57—2,3-dimethyl-1-pentene; 58—dimethyl butanedioate; 59—ethyl octadecanoate; 60—ethyl 9-octadecenoate; 61—methyl-10-octadecenoate; 62—dodecanoic acid; 63—ethyl octadeca-9,12-dienoate; 64—2,3,5,8-tetramethyl-1,5,9-decatriene; 65—3,7,11,15-tetramethylhexadeca-1,6,10,14-tetraen-3-ol; 66—9-octadecenamide; 67—ethyl octadeca-9,12,15-trienoate; 68—2-methyl-4-octanol; 69—1-tetradecanoic acid; 70—octadecanoic acid. The results represent average values of laboratory determinations and the standard deviation, all samples being analyzed in triplicate. Different letters indicate homogeneous groups ($p > 0.05$) in correlation with the Fisher LSD test; *—presents a significant difference compared to all the variants, nd—not detected.

Table 3. Final proportion of identified volatile compounds in Sauvignon blanc wines (% of total area).

C	V1	V2	V3	V4	V5	V6
1	0.34 ± 0.01 bc	0.31 ± 0.02 b	0.65 ± 0.01 *	0.52 ± 0.02 *	0.47 ± 0.05 e	0.40 ± 0.00 d
2	0.26 ± 0.00 c	0.26 ± 0.00 c	0.07 ± 0.01 *	0.20 ± 0.01 b	0.15 ± 0.01 *	0.20 ± 0.01 b
3	0.22 ± 0.01 *	0.15 ± 0.01 ef	0.15 ± 0.01 ef	0.12 ± 0.01 de	0.17 ± 0.01 f	0.12 ± 0.01 de
4	4.34 ± 0.02 *	4.38 ± 0.01 *	4.51 ± 0.02 *	5.15 ± 0.01 *	4.10 ± 0.00 *	4.20 ± 0.01 *
5	0.16 ± 0.01 a	0.19 ± 0.02 *	0.16 ± 0.00 a	0.30 ± 0.00 c	0.10 ± 0.02 *	0.56 ± 0.00 *
6	0.11 ± 0.01 e	0.06 ± 0.01 bc	0.05 ± 0.02 b	0.06 ± 0.00 bc	0.09 ± 0.00 de	0.08 ± 0.01 cd
7	0.16 ± 0.00 *	0.12 ± 0.00 abc	0.13 ± 0.00 abc	0.21 ± 0.01 *	0.09 ± 0.05 a	0.14 ± 0.02 bc
8	nd	0.17 ± 0.01 b	0.18 ± 0.01 b	0.09 ± 0.00 *	nd	0.05 ± 0.00 *
9	39.21 ± 0.00 *	33.11 ± 0.00 *	38.60 ± 0.02 *	32.76 ± 0.00*	39.12 ± 0.02 *	42.15 ± 0.00*
10	0.26 ± 0.00	nd	nd	nd	nd	nd
11	0.05 ± 0.02 b	0.20 ± 0.02 *	0.25 ± 0.02 *	0.03 ± 0.05 ab	0.01 ± 0.05 a	0.28 ± 0.02 *
12	nd	nd	nd	nd	nd	nd
13	0.28 ± 0.00 *	0.05 ± 0.00 b	0.05 ± 0.00 b	0.02 ± 0.05 a	0.03 ± 0.00 ab	0.04 ± 0.00 ab
14	0.16 ± 0.01 c	0.25 ± 0.01 de	0.29 ± 0.01 e	0.15 ± 0.05 bc	0.14 ± 0.00 abc	0.23 ± 0.05 d
15	0.37 ± 0.01 *	0.18 ± 0.00 b	0.15 ± 0.01 b	0.10 ± 0.05 a	0.11 ± 0.00 a	0.10 ± 0.00 a
16	nd	0.39 ± 0.01 *	0.35 ± 0.02 *	0.02 ± 0.02 b	0.03 ± 0.00 bc	0.04 ± 0.01 c
17	0.13 ± 0.01	nd	nd	0.10 ± 0.01	0.11 ± 0.00	0.12 ± 0.00
18	4.98 ± 0.00 *	0.10 ± 0.02 a	0.13 ± 0.01 bc	0.15 ± 0.00 c	0.18 ± 0.02 *	0.15 ± 0.02 c
19	0.33 ± 0.01 *	6.41 ± 0.00 *	5.20 ± 0.00 *	5.40 ± 0.00 *	4.12 ± 0.00 *	4.00 ± 0.01 *
20	0.14 ± 0.05 ab	0.36 ± 0.00 *	0.31 ± 0.02 *	0.20 ± 0.00 *	0.15 ± 0.02 b	0.10 ± 0.05 a
21	nd	0.16 ± 0.02 cd	0.12 ± 0.00 ab	0.13 ± 0.05 abc	0.10 ± 0.00 a	0.15 ± 0.00 bc
22	0.52 ± 0.05 *	0.63 ± 0.05 *	nd	0.05 ± 0.00 *	nd	nd
23	nd	nd	0.51 ± 0.05 *	0.05 ± 0.00 *	0.10 ± 0.00 b	0.12 ± 0.05 b
24	nd	nd	nd	nd	nd	nd

Table 3. *Cont.*

C	V1	V2	V3	V4	V5	V6
25	nd	0.10 ± 0.00 c	0.06 ± 0.00 *	0.02 ± 0.00 *	nd	nd
26	nd	nd	nd	nd	nd	nd
27	4.05 ± 0.00 *	3.88 ± 0.00 *	3.20 ± 0.01 *	3.10 ± 0.01 *	3.33 ± 0.02 *	0.78 ± 0.00 *
28	0.11 ± 0.00 *	0.10 ± 0.00 *	0.08 ± 0.00 b	0.05 ± 0.00 *	0.06 ± 0.00 *	0.08 ± 0.00 b
29	0.08 ± 0.00 c	0.07 ± 0.00 bc	0.06 ± 0.02 ab	0.05 ± 0.00 a	0.07 ± 0.00 bc	0.05 ± 0.00 a
30	0.16 ± 0.00 *	0.13 ± 0.00 *	0.08 ± 0.00 a	0.08 ± 0.00 a	0.10 ± 0.00 *	0.09 ± 0.02 *
31	1.04 ± 0.00 *	1.02 ± 0.00 *	0.68 ± 0.00 b	0.56 ± 0.00 *	0.70 ± 0.00 *	0.68 ± 0.00 b
32	0.13 ± 0.00 c	0.48 ± 0.02 *	0.12 ± 0.00 *	0.11 ± 0.00 b	0.11 ± 0.00 b	0.13 ± 0.00 c
33	0.28 ± 0.00a	0.32 ± 0.00 *	0.19 ± 0.00 *	0.30 ± 0.00 *	0.35 ± 0.00 *	0.29 ± 0.00 *
34	0.83 ± 0.00 *	0.96 ± 0.00 *	0.80 ± 0.02 *	0.75 ± 0.02 *	0.89 ± 0.02 *	0.78 ± 0.00 *
35	0.04 ± 0.00 a	0.07 ± 0.04 b	0.07 ± 0.00 b	0.07 ± 0.00 b	0.05 ± 0.00 ab	0.05 ± 0.00 ab
36	nd	nd	nd	nd	nd	nd
37	nd	nd	nd	nd	nd	nd
38	2.77 ± 0.00 *	3.81 ± 0.00 *	3.36 ± 0.02 *	3.27 ± 0.05 *	3.00 ± 0.02 *	3.15 ± 0.00 *
39	0.23 ± 0.03 *	0.26 ± 0.00 *	0.18 ± 0.02 a	0.20 ± 0.00 a	0.29 ± 0.00 *	0.19 ± 0.02 a
40	0.26 ± 0.00 c	0.23 ± 0.02 b	0.19 ± 0.00 a	0.25 ± 0.01 c	0.20 ± 0.02 a	0.22 ± 0.01 b
41	0.40 ± 0.00 b	0.44 ± 0.00 *	0.35 ± 0.02 a	0.35 ± 0.00 a	0.38 ± 0.02 ab	0.30 ± 0.02 *
42	2.67 ± 0.00 *	2.43 ± 0.02 *	2.19 ± 0.00 *	2.32 ± 0.01 *	2.40 ± 0.05 *	2.22 ± 0.00 *
43	0.08 ± 0.00 cd	0.07 ± 0.00 bc	0.07 ± 0.02 bc	0.06 ± 0.00 ab	0.07 ± 0.01 bc	0.05 ± 0.00 a
44	nd	0.04 ± 0.00 *	0.03 ± 0.00 a	0.02 ± 0.00 *	0.01 ± 0.02 *	0.03 ± 0.00 a
45	0.40 ± 0.00 *	0.42 ± 0.02 *	0.04 ± 0.02 *	0.09 ± 0.00 a	0.20 ± 0.00 *	0.10 ± 0.00 a
46	0.52 ± 0.02 b	0.34 ± 0.05 *	nd	0.05 ± 0.00 a	0.17 ± 0.02 *	0.07 ± 0.00 a
47	0.55 ± 0.01 b	nd	0.55 ± 0.01 b	0.44 ± 0.02 a	0.09 ± 0.02 *	0.45 ± 0.00 a
48	0.07 ± 0.00 *	0.06 ± 0.00 *	0.10 ± 0.00 a	0.15 ± 0.01 *	0.18 ± 0.00*	0.14 ± 0.01 c
49	0.07 ± 0.01 a	0.07 ± 0.02 a	0.08 ± 0.02 ab	0.14 ± 0.00 d	0.12 ± 0.00*	0.09 ± 0.00 b
50	nd	0.08 ± 0.01 cd	0.10 ± 0.00 de	0.05 ± 0.01 ab	0.07 ± 0.02 bc	0.04 ± 0.01 a
51	16.00 ± 0.01 *	17.08 ± 0.05 *	17.17 ± 0.01 *	14.26 ± 0.05 *	13.67 ± 0.05 *	13.26 ± 0.00 *
52	nd	0.73 ±. 0.00 *	0.52 ± 0.02 *	0.15 ± 0.00 c	0.17 ± 0.00 c	0.12 ± 0.00 b
53	nd	nd	nd	nd	nd	nd
54	0.76 ± 0.03 *	nd	nd	nd	nd	nd
55	nd	nd	nd	0.05 ± 0.01 b	nd	nd
56	0.14 ± 0.02 c	0.10 ± 0.01 b	0.11 ± 0.00 b	0.08 ± 0.00 a	0.05 ± 0.00 *	0.08 ± 0.00 a
57	0.04 ± 0.00 c	0.03 ± 0.00 bc	0.02 ± 0.00 b	0.01 ± 0.01 a	0.02 ± 0.00 b	nd
58	0.19 ± 0.01 d	0.17 ± 0.05 cd	0.19 ± 0.00 d	0.07 ± 0.01 b	0.08 ± 0.00 b	0.15 ± 0.00 c
59	0.12 ± 0.01 *	0.06 ± 0.02 *	nd	0.28 ± 0.00 a	0.36 ± 0.00 *	0.30 ± 0.02 a
60	0.06 ± 0.00 cd	0.05 ± 0.01 bc	nd	0.04 ± 0.00 b	0.05 ± 0.02 bc	0.09 ± 0.00 f
61	nd	0.07 ± 0.02 *	nd	0.11 ± 0.02 c	0.15 ± 0.00 *	0.23 ± 0.00*
62	nd	nd	nd	0.10 ± 0.01 b	0.15 ± 0.05 *	0.07 ± 0.01 *
63	16.17 ± 0.05 *	18.73 ± 0.05 *	16.92 ± 0.02 *	14.00 ± 0.00 *	12.32 ± 0.05 *	10.14 ± 0.00 *
64	nd	nd	nd	nd	nd	nd
65	nd	nd	nd	nd	nd	nd
66	nd	nd	nd	nd	nd	nd
67	nd	nd	nd	nd	nd	nd
68	0.33 ± 0.00 *	0.31 ± 0.01 *	0.29 ± 0.01 *	0.15 ± 0.00 *	0.09 ± 0.00 *	0.05 ± 0.00 *

C—analyzed compounds; V1—Endozym Thiol®, AEB; V2—Endozym β—Split®, AEB; V3—Zymovarietal aroma G®, SODINAL; V4—Endozym Ice®, AEB; V5—Zimarom®, BSG WINE; V6—control sample, no enzymes; 1—1-propan-1-ol; 2—ethyl butanoate; 3—2,6-dimethyldecane; 4—3-methylpropan-1-ol; 5—3-methylbutyl acetate; 6—butan-1-ol; 7—3-penten-2-ol; 8—undecane; 9—3-methylbutan-1-ol; 10—decane; 11—5-ethyl-5-methyldecane; 12—ethyl hexanoate; 13—2,4,6-trimethylheptane; 14—2-methyldecane; 15—3-hydroxy-2-butane; 16—3,9-dimethylundecane; 17—3,3-dimethylhexane; 18—3-methylpentan-2-ol; 19—2-ethyl hydroxypropanoate; 20—hexan-1-ol; 21—3-ethoxypropan-1-ol; 22—nonadecane; 23—ethyl octanoate; 24—2,6,10-trimethyldodecane; 25—eicosan; 26—octadecane; 27—acetic acid; 28—pentacosane; 29—ethyl 3-hydroxybutanoate; 30—benzaldehyde; 31—2,3-butanediol; 32—1,3-butanediol; 33—2-methylpropanoic acid; 34—ethyl acetamide; 35—ethyl decanoate; 36—1,2-hydrazinedicarboxaldehyde; 37—10-methylnonadecane; 38—diethyl butanedioate; 39—3-methylbutanoic acid; 40—3-methylsulfanylpropan-1-ol; 41—2-propanyl acetate; 42—ethyl 4-hydroxybutanoate; 43—2-phenylethyl acetate; 44—ethyl dodecanoate; 45—1-octadecene; 46—hexanoic acid; 47—3-methylbutyl acetamide; 48—hydroxybutyric acid; 49—1-phenyl methanol; 50—ethyl 3-methylbutyl butanedioate; 51—1-phenylethanol; 52—n-ethyl acetamide; 53—diethyl hydroxybutanedioate; 54—octanoic acid; 55—2-[ethyl(methyl)amino]ethanol; 56—1-docosene; 57—ethyl hexadecanoate; 58—9-ethyl hexadecanoate; 59—decanoic acid; 60—2,4-di-tert-butylphenol; 61—hexaoxacyclooctadecane; 62—11-octadecenal; 63—1,6-anhydro-2,3,4-trimethylgalactose; 64—ethyl octadecanoate; 65—4-hydroxy benzeneethanol; 66—9-ethyl octadecenoate; 67—ethyl octadeca-9,12-dienoate; 68—n-(2-phenylethyl)acetamide. The results represent average values of laboratory determinations and the standard deviation, all samples being analyzed in triplicate. Different letters indicate homogeneous groups ($p > 0.05$); *—presents a significant difference compared to all the variants, nd—not detected.

The main acids identified in Fetească regală samples were octanoic, decanoic, and hexanoic acids. The proportion of octanoic acid was increasing during the fermentation process in most of the samples (except for the V4 variant), and decreased during storage for the V2, V3, and V6 samples. After six months of storage, V1 was remarking for the highest level of octanoic acid (8.18 ± 0.00%), while the blank presented the smallest value (3.63 ± 0.01%).

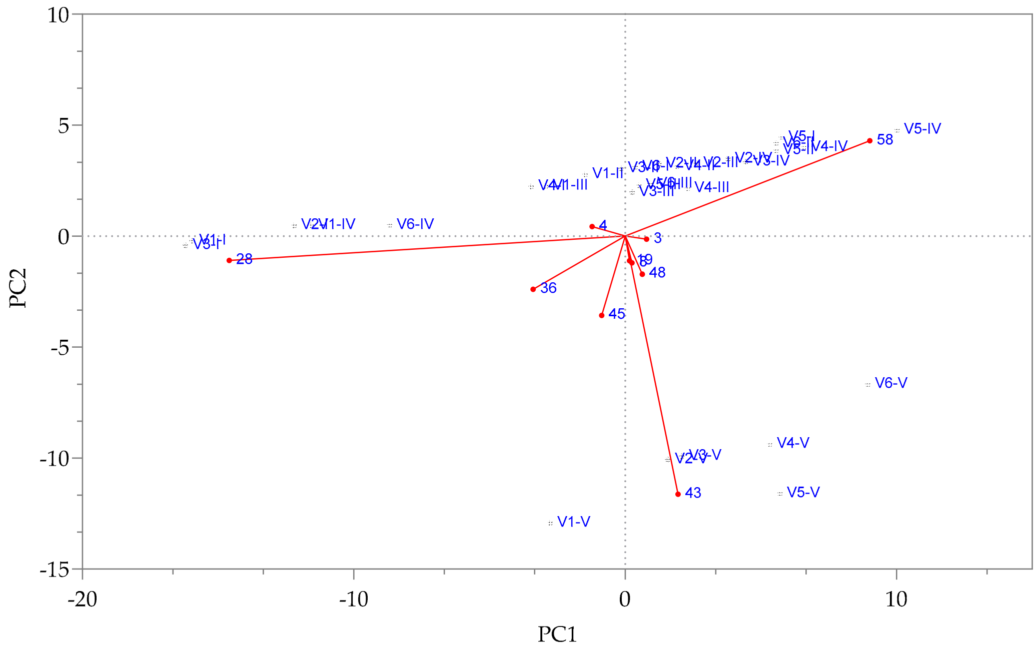


Figure 1. PCA analysis on Fetească regală samples. V1—Endozym Thiol®, AEB; V2—Endozym β-Split®, AEB; V3—Zymovarietal aroma G®, SODINAL; V4—Endozym Ice®, AEB; V5—Zimarom®, BSG WINE; V6—control sample, no enzymes. 3—3-methylpropan-1-ol; 4—3-methylbutyl acetate; 8—3-methylbutan-1-ol; 19—ethyl octanoate; 28—ethyl decanoate; 36—2-propanyl acetate; 43—hexanoic acid; 45—1-phenylethanol; 48—octanoic acid; 58—dimethyl butanedioate.

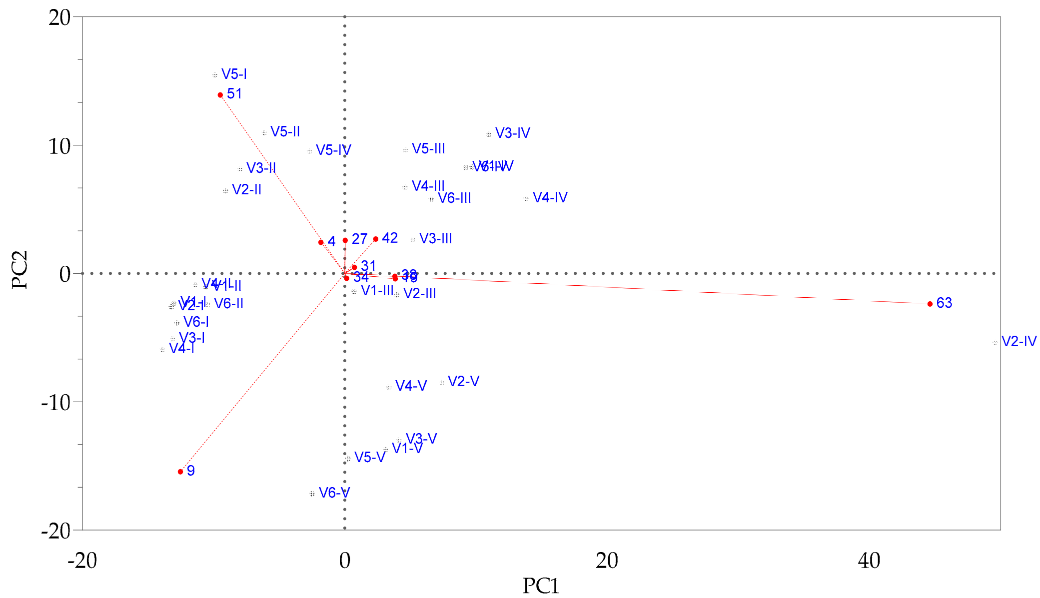
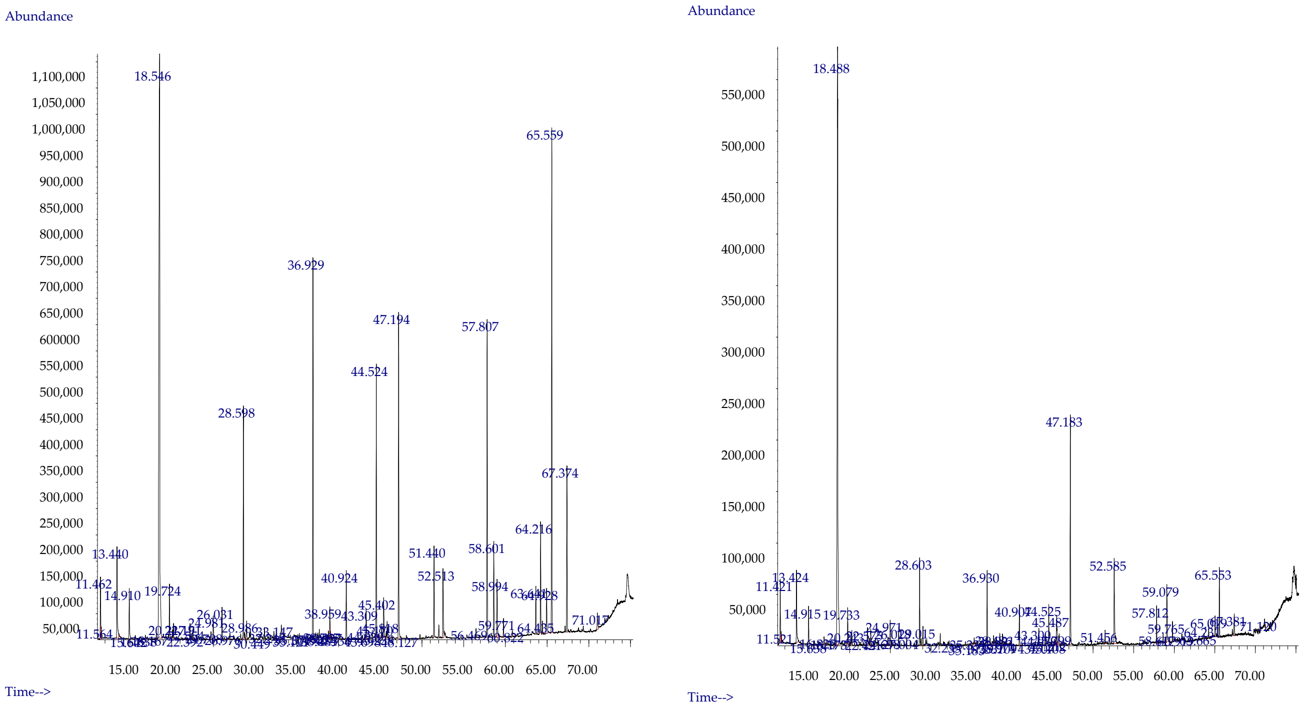


Figure 2. PCA analysis on Sauvignon blanc samples. V1—Endozym Thiol®, AEB; V2—Endozym β-Split®, AEB; V3—Zymovarietal aroma G®, SODINAL; V4—Endozym Ice®, AEB; V5—Zimarom®, BSG WINE; V6—control sample, no enzymes; 4—3-methylpropan-1-ol; 9—3-methylbutan-1-ol; 19—2-ethyl hydroxypropanoate; 27—acetic acid; 31—2,3-butanediol; 34—ethyl acetamide; 38—diethyl butanedioate; 42—ethyl 4-hydroxybutanoate; 51—1-phenylethanol; 63—1,6-anhydro-2,3,4-trimethylgalactose.



(**a**) (**b**)

Figure 3. Chromatogram example for V1-I (**a**) vs. V6-I (**b**). V1—Endozym Thiol®, AEB; V6—control sample, no enzymes; I—1st day of fermentation.

Decanoic acid was generally ascendant in the second phase of the fermentation, exception being made by V1 in which it is increasing during the biochemical process. It is well-known that during the alcoholic fermentation, significant amounts of the mentioned acids can be produced by yeasts [30]. During maturation and storage periods, the proportion of this acid was descending. The evolution of hexanoic acid's proportion was following the same trend in most samples; its value was increasing with the evolution of the fermentation process. Similar to decanoic acid, the amount of this compound showed significant decreases during storage. Its proportion was depending on the type of treatment. Thus, 3.63 ± 0.01% (V6) to 8.18 ± 0.00% (V1) octanoic acid was identified in Fetească regală wines. Hexanoic acid was fluctuating from 1.93 ± 0.00% (V5) to 4.28 ± 0.05% (V1), while the level of decanoic acid started from 0.73 ± 0.00% (V6) to 2.19 ± 0.01% (V1). High levels of these compounds in Fetească regală wines were also identified by Moroșanu et al. [46].

Regarding the Sauvignon blanc samples, acetic, octanoic, and hexanoic acids were most representative. The proportion of acetic acid was increasing in the first phase of the fermentation and decreased (being involved in esterification reactions) in most of the samples from the middle of the biochemical process (except V3 and V5) as well as in the storage phase. In the final samples (after 6 months of storage), the highest proportions of this compound was obtained in V1 (4.05 ± 0.00%), while the lowest value was registered in V6 variant (0.78 ± 0.00%). The octanoic acid's proportions presented different fluctuations, following which it decreases significantly toward the end of the alcoholic fermentation, as well as after bottling. After six month of storage, these compounds have been identified only in the V1 sample (0.76 ± 0.03%). Moreover, the level of hexanoic acid followed a downward evolution with the progress of the fermentation process and after it.

Higher alcohols usually give off a pungent smell, participating directly or indirectly (through the formation of esters) in the composition of the aging bouquet of wines [48]. Fetească regală samples was remarking by high content of 1-phenylethanol and 3-methylbutan-1-ol. The first mentioned compound showed higher values at the end of the fermentation, compared to the first day of sampling. Fetească regală wines presented between 10.8 ± 0.02% (V6) and 15.26 ± 0.01% (V5) 1-phenylethanol, which can give floral or honey flavors. The initial proportion of 3-methylbutan-1-ol increases in the first phase of fermentation (except V5) and registers various fluctuations, depending on the type of the administrated enzyme preparation. After the wine is stabilized and bottled, the amount of this compound decreases during storage and maturation in most samples, except V1 and V6. In the final samples (after 6 months of storage), a level between 37.19 ± 0.01% and 49.89 ± 0.00% of the total area was identified for 3-methylbutan-1-ol. Also called isoamyl alcohol, it usually comes from the enzymatic degradation of leucine [49]. These compounds are responsible for alcoholic and pungent odors in wines.

Sauvignon blanc variants are characterized by the presence of the 1-phenylethanol, 3-methylpropan-1-ol, and 3-methylpentan-2-ol. Their weight registers different fluctuations during fermentation, being lower on the last day of sampling compared to the beginning of the fermentation process. During storage in bottles, the level continues to decrease for the first two compounds but increases in the case of the last mentioned higher alcohol. The proportion of the main alcohols in Sauvignon blanc wines is between 13.26 ± 0.00% (V6) and 17.17 ± 0.05% (V3) in the case of 1-phenylethanol, between 4.10 ± 0.00% (V5) and 5.15 ± 0.01% (V4) for 3-methylpropan-1-ol, and between 0.10 ± 0.02% (V2) and 4.98 ± 0.00% (V1) for 3-methylpentan-2-ol, respectively. During alcoholic fermentation, numerous secondary reaction products can result, one of the most important being 2,3-butanediol. It participates in defining wine's bouquet, giving a bitter taste and viscosity. This compound is usually formed by yeasts metabolism. Thus, in the presence of acetoin reductase, yeasts reduce acetoin to 2,3-butanediol [50]. The proportion of this compound varies between 1.00 ± 0.02% (V4) and 1.64 ± 0.02% (V1) in Fetească regală samples. Regarding the Sauvignon blanc wines, levels from 0.56 ± 0.00% (V4) to 1.04 ± 0.00 (V1) were recorded.

Esters usually contribute to the definition of the fruity and floral aroma of young wines and the formation of the aging bouquet [47]. These compounds are responsible for the fruity aroma (lower aliphatic esters) and sweet aroma (higher esters) [39]. Fetească regală wine samples presented a large number of esters (23 such compounds), ethyl octanoate, 3-methylbutyl acetate (isoamyl acetate), and ethyl 4-hydroxybutanoate being representative. Ethyl alcohol esters are formed as a result of the reaction of ethanol with fatty acids, under the action of acetyl-coenzyme A [47]. The first specified compound comes from the raw material, with a proportion of approximately 3–4% on the first day of sampling in most samples, indicating different fluctuations in the fermentation process, depending on the administered treatment. Thus, its proportion increased to approximately 9% in the V1 variant on the last day of sampling. For the rest of the samples, this proportion was found to be lower at the end of the fermentation phase compared to the initial value. A high level of this compound was distinguished in V1 (2.33 ± 0.04%), followed by V6 (2.22 ± 0.00%), V4 (1.90 ± 0.01%), V3 (1.80 ± 0.06%), V2 (1.67 ± 0.03%), and V5 (1.66 ± 0.00%). Following the results, it can be assumed that 3-methylbutyl acetate came from the raw material, its proportion registering various fluctuations pending alcoholic fermentation. Thus, the level of this compound was two to four times higher in the last day of sampling compared to the first one. In the final samples (after 6 months of storage), the proportion of 3-methylbutyl acetate varies from 3.60 ± 0.02% in V1 variant, being about three times lower on the rest of the samples. Ethyl 4-hydroxybutanoate was found in proportions from 0.58 ± 0.00% to 0.77 ± 0.03% on the first day of sampling. Important levels were accumulated in the first phase of the fermentation process in all samples. This compound showed several decreases in the second stage of fermentation of V1, V3, and V5 variants and important increases in the other samples. Ethyl 4-hydroxybutanoate showed lower values in the resulting wines, except for the V1 variant. Thus, the final proportion varied from 2.18 ± 0.02% (V1) to 0.78 ± 0.00% (V6), after six months of storage.

Regarding the Sauvignon blanc variety, 20 esters were identified, ethyl 2-hydroxypropanoate (ethyl lactate), diethyl butanedioate (diethyl succinate), and ethyl 4-hydroxybutanoate being predominant. The first compound manifested an upward evolution throughout the wine's fermentation. Important proportions are found in the stabilized wines, the highest value being registered in V2 variant (6.41%), followed by V1 (6.33%), and the lowest value was identified in the control sample (4.00%). Diethyl butanedioate follows the same trend in all variants, being identified in more than ten times higher proportions on the last day of the fermentation process compared to the first. After stabilization and maturation period, V2 variant showed the highest proportion of diethyl butanedioate (3.81 ± 0.00%), followed by V3 (3.36 ± 0.02%), and the lowest level was recorded in V1 (2.77 ± 0.00%). Ethyl 4-hydroxybutanoate was identified in proportions of 1–2% on the first day of sample collection. Important amounts were formed during alcoholic fermentation, 4–6% being recorded on the last day. According to the results, a significant decrease of ethyl 4-hydroxybutanoate can be observed in the resulting Fetească regală wines, analyzed after 6 months. Thus, the highest amount was obtained in V1 variant (2.67 ± 0.00%), while the lowest was obtained in V3 sample (2.1 ± 0.00%).

Carbonyl compounds (aldehydes, ketones, and their derivatives) are of particular importance in defining wines' organoleptic characteristics. In high concentrations, aldehydes can usually give a pungent, irritating odor (acetaldehyde and formaldehyde), but also a pleasant aroma of bitter almonds (benzenaldehyde) [48]. The carbonyl compound 3-hydroxybutan-2-one was identified in Fetească regală samples. Also called acetoin, it is found in reduced proportions in most samples in the first day of sampling collection. This compound is present in V1 variant in all fermentation stages and was not identified by the second stage of the biochemical process for the rest of the samples. This constituent contributes to the formation of wines bouquet [50].

Sauvignon blanc samples were noted for the presence of some carbonyl compounds, including 3-hydroxy-2-butane, 1,2-hydrazinedicarboxaldehyde, 11-octadecenal, and benzaldehyde. The amount of the first two mentioned compounds showed important decreases from the first day of sampling to the last day of the fermentation process. The 11-octadecenal compound was identified in reduced proportions during alcoholic fermentation of V3, V4, V5, and V6 samples, being absent in V1 and V2 variants. After six months of storage, the highest proportion of this compound was obtained in V5 variant (0.15 ± 0.05%), followed by V4 (0.10 ± 0.01%), V6 (0.07 ± 0.01%) and it was not being identified in the rest of the Sauvignon blanc wines.

Benzaldehyde was generally formed during wine storage and maturation period. Reduced amounts were identified during the fermentation process in V5 and V6 samples. In the resulting wines, it was present in low proportions (0.08 ± 0.00%-V3 and V4; 0.16 ± 0.00%-V1). Among the volatile components of wine, lactones (δ-lactones, γ-lactones) play a major role in defining the aromatic profile. These compounds are responsible for the fruity (γ-hexalactone), floral (γ-dodecalactone), and coconut (γ-octalactone) odors. These substances are formed by the cyclization of γ-hydroxycarboxylic acids [51].

Belonging to γ-lactones, 5-propyloxolan-2-one (γ-heptalactone) and 5-(1-hydroxyethyl) oxolan-2-one (solerole) were identified in the Fetească regală resulting wines. These compounds have been found since the beginning of alcoholic fermentation and showed different variations depending on the type of administered enzyme. 5-propyloxolan-2-one proportion decreased during the fermentation process, and small amounts accumulated during the storage period. Regarding the 5-(1-hydroxyethyl)oxolan-2-one (solerole), it was formed after the wine stabilization and bottling stage, being associated with the racemization process during wine storage and maturation [52]. Thus, its amounts varied from 0.28 ± 0.00% in V1, 0.18 ± 0.00% in V3, and 0.11 ± 0.00% in V4.

Phenolic compounds such as 4-ethenylphenol (4-vinylphenol), 4-ethenyl-2-methoxyphenol (4-vinylguaiacol), and 2,4-ditert-butylphenol were identified in Fetească regală samples. Of these, vinylphenols are frequently found in white wines, positively contributing to the wine aroma profile under 440 μg/L amounts [48]. Higher proportions of 4-vinylphenol usually impart pungent, phenolic, medicinal odors [53]. 4-ethenylphenol was formed during alcoholic fermentation, reaching a maximum level at the middle of the biochemical process, after which decreases are recorded toward the end of fermentation. Its proportion was descendant during storage for most variants, but higher proportions were registered in enzyme-treated samples. Thus, proportions from 0.43 ± 0.03% (V6) to 1.78 ± 0.04% (V1) were registered for 4-ethenylphenol in Fetească regală wines. In this line, V1 also showed the highest level of 4-ethenyl-2-methoxyphenol (0.87 ± 0.05%), while the control sample presented the smallest one (0.50 ± 0.03%). For the third mentioned compound, V4 variant had the higher value (0.53 ± 0.02%).

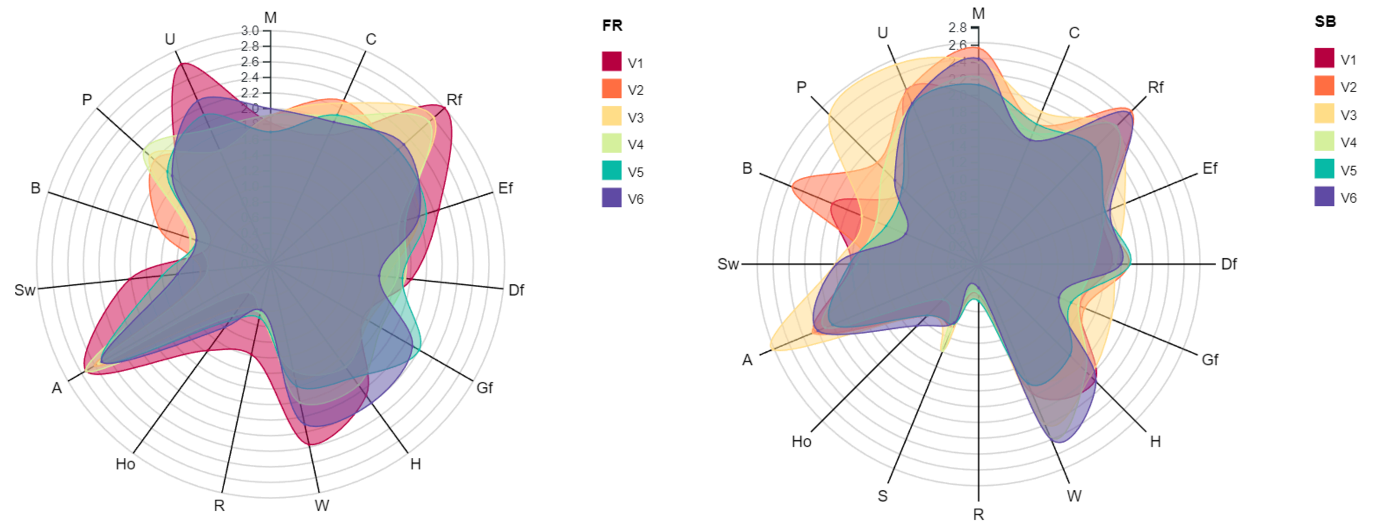
Terpenes are the main components responsible for the aroma of Muscat varieties (linalool, geraniol, nerol, hotrienol). Belonging to this class of substances, 2,6-dimethyl-3,7-octadiene-2,6-diol (citrus aroma) was identified in Fetească regală samples, resulting from the acid hydrolysis reaction [54]. It was found from the first day of sampling, in proportions of approximately 0.5–1%, registering different fluctuations, in dependence on the administered treatment. The highest amount of this constituent was determined in V1 variant (0.17 ± 0.07%), while the lowest proportion was identified in V2 and V6 (approximately 0.09%).

In Sauvignon blanc wines, 2,6,10-trimethyldodecane (farnesene) was predominant. This constituent was present in low proportions in V3, V4, V5, and V6 samples during alcoholic fermentation and it not identified in the final samples (after 6 months of storage).

To overview the major influence of enzymes on volatile compounds, Figure 3 illustrates chromatograms of a sample with and without any enzyme preparation.

3.2. The Influence of Enzymes on Sensory Perception

From a sensory point of view, the obtained wines differed primarily according to the variety (Figure 4). Within each variety analyzed, the samples showed significant differences depending on the type of the administered enzymes.



(**a**) (**b**)

Figure 4. The influence of enzymes on wine's sensory perception ((**a**)—Fetească regală; (**b**)—Sauvignon blanc). FR—Fetească regală; SB—Sauvignon blanc; V1—Endozym Thiol®, AEB; V2—Endozym β-Split®, AEB; V3—Zymovarietal aroma G®, SODINAL; V4—Endozym Ice®, AEB; V5—Zimarom®, BSG WINE; V6—control sample, no enzymes.; V—vegetal; M—mineral; C—citric; Rf—ripe fruits; Ef—exotic fruits; Df—dry fruits; H—hay; W—wildflowers; R—roses; S—sweet; Ho—honey; A—acid; B—bitter; P—phenolic; U—unctuous.

Therefore, Fetească regală wines were defined by an intense fruity aroma (exotic fruits, ripe fruits, dried fruits) and wildflower notes related to high proportions of ethyl octanoate, 3-methylbutyl acetate, hexanoic acid (OT = 3000 ppb; FT = 5400 ppb), ethyl decanoate

(FT = 510 ppb), and propan-2-yl acetate (OT = 180–670 ppb). Samples treated with enzyme preparations presented lower values for the vegetal and mineral character. The sensory descriptors usually less appreciated by consumers (e.g., phenolic and bitter sensation) were noted to have lower intensities in the V1 variant. In accordance to Pearson correlation test (Figure 5), 3-methylbutyl acetate is highly correlated to the sweet taste (r = 0.81) and roses flavor (r = 0.97). Ethyl octanoate is also highly correlated to floral (wildflowers) odor (r = 0.94), confirming its major contribution in defining the mentioned descriptor. Ethyl decanoate was positively correlated with ripe flowers odor. Sauvignon blanc wines have been described as more vegetal and spicy, with mown hay notes, minerals, and an intense citrus and exotic fruits aromas (1-phenylethanol, OT = 10,000 ppb; 3-methylbutan-1-ol, OT = 230–300 ppb). For these samples, the bitter sensation was the most pronounced in the V2 variant.

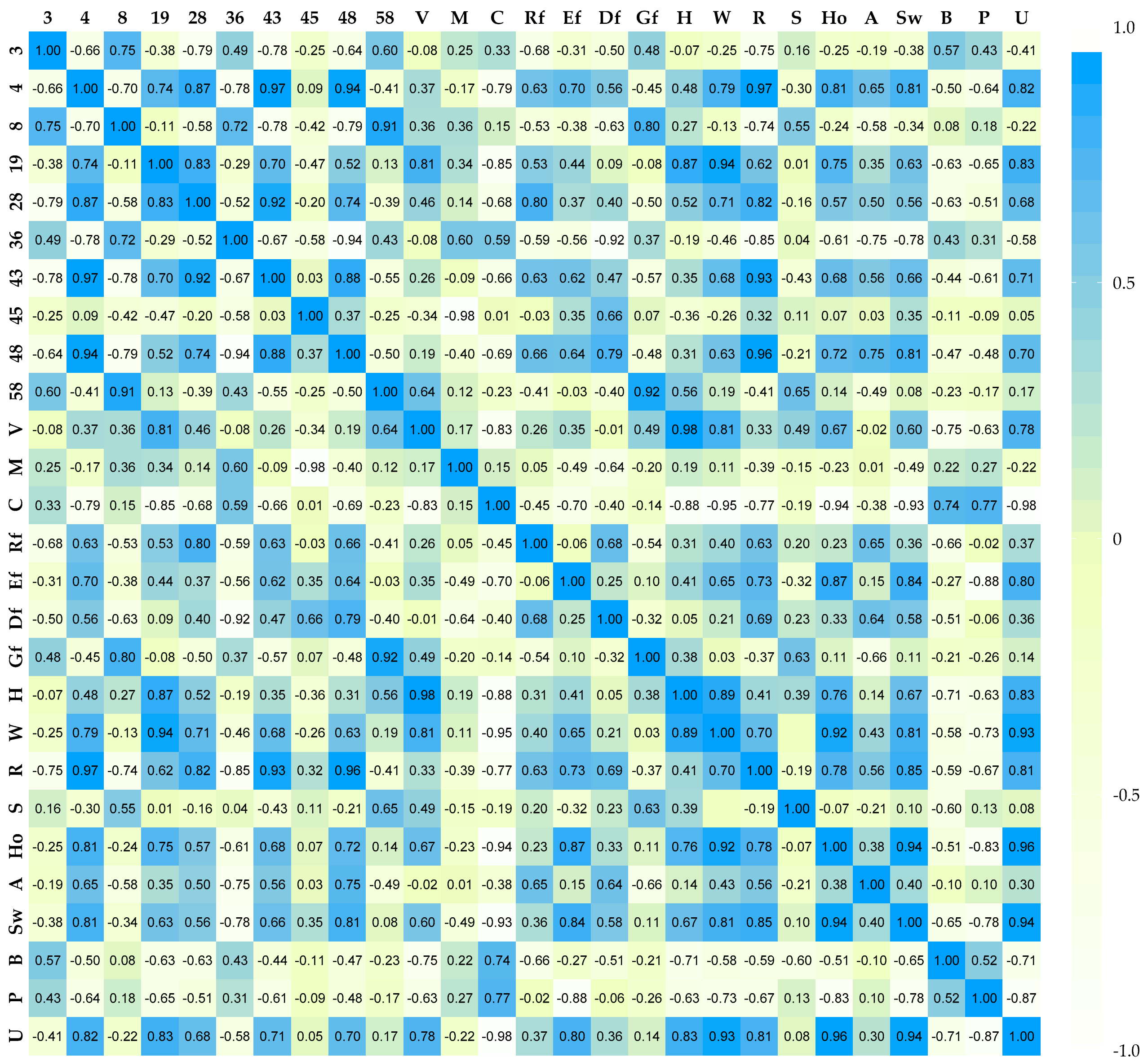


Figure 5. Pearson correlation on Fetească regală samples. 3—3-methylpropan-1-ol; 4—3-methylbutyl acetate; 8—3-methylbutan-1-ol; 19—ethyl octanoate; 28—ethyl decanoate; 36—2-propanyl acetate; 43—hexanoic acid; 45—1-phenylethanol; 48—octanoic acid; 58—dimethyl butanedioate; V—vegetal; M—mineral; C—citric; Rf—ripe fruits; Ef—exotic fruits; Df—dry fruits; H—hay; W—wildflowers; R—roses; S—sweet; Ho—honey; A—acid; B—bitter; P—phenolic; U—unctuous.

Regarding the identified volatile compounds, the sweet taste sensation can be associated to high levels of alcohols, such as 3-methylbutan-1-ol (r = 0.81) as shown in Figure 6.

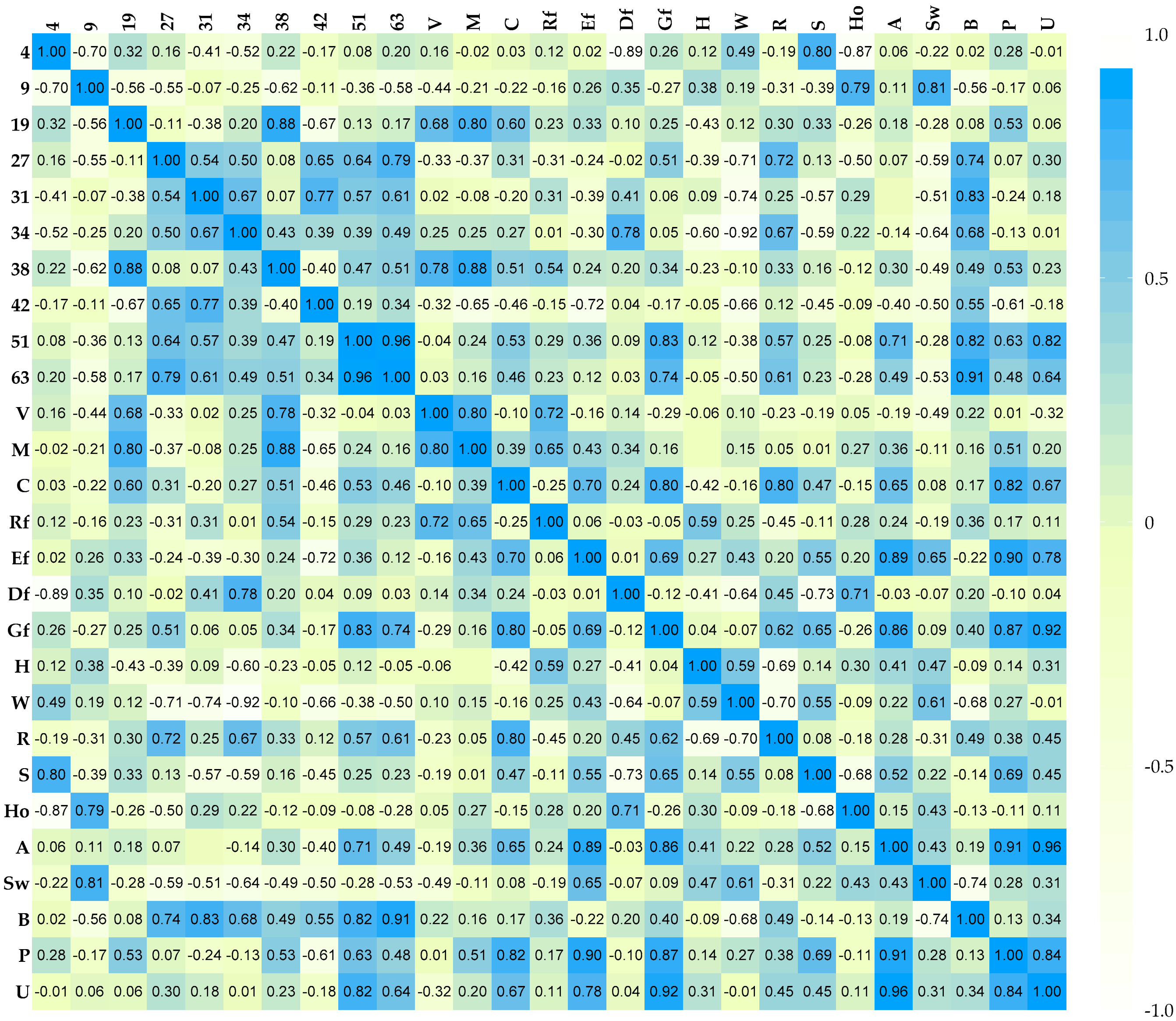


Figure 6. Pearson correlation on Sauvignon blanc samples. 4—3-methylpropan-1-ol; 9—3-methylbutan-1-ol; 19—2-ethyl hydroxypropanoate; 27—acetic acid; 31—2,3-butanediol; 34—ethyl acetamide; 38—diethyl butanedioate; 42—ethyl 4-hydroxybutanoate; 51—1-phenylethanol; 63—1,6-anhydro-2,3,4-trimethylgalactose; V—vegetal; M—mineral; C—citric; Rf—ripe fruits; Ef—exotic fruits; Df—dry fruits; H—hay; W—wildflowers; R—roses; S—sweet; Ho—honey; A—acid; B—bitter; P—phenolic; U—unctuous.

4. Discussion

This paper highlights the influence of enzymes on volatile compounds in musts and wines obtained from two semi-aromatic varieties. The samples were obtained from grape varieties cultivated in the same viticultural area and the wines were obtained by applying the same protocol. The pruning system, bud load, and yield took into account obtaining qualitative productions, similar in quantity. The proposed article contributes to the data consolidation already existing in the literature as regards the effect of enzymes on aroma profile of wine.

The identified compounds belong to different classes, such as acids, higher alcohols, esters, carbonyl compounds, compounds with a benzene ring, terpenes, etc. These volatile compounds can have different origins.

Acids can originate from the raw material (for example, malic, tartaric, citric acids), may result from chemical reactions during the fermentation process, or may appear from applied oenological treatments (such as hexanoic, decanoic, lactic, heptanoic, succinic acids, etc.,). Fatty acids are precursors to esters, terpenes, and alcohols [30].

Higher alcohols in wine can result from the degradation of some amino acids, from the catalysis of carbohydrates during fermentation or from aldehydes [55,56].

Esters are synthesized by yeasts during alcoholic fermentation, their amounts also being influenced by the presence of lactic acid bacteria in the wine. The formation of esters during fermentation can be influenced by the type of inoculated yeasts, pH, temperature, and fermentation conditions, the chemical composition of the must, the presence of oxygen during alcoholic fermentation, and the administered treatments [56,57]. Moreover, the content in amino acids influences the synthesis of esters in wine.

Carbonyl compounds formation is related to the presence of microorganism [58]. Acetoin is usually formed during alcoholic fermentation, being produced by *Saccharomyces* yeasts, from the condensation reaction of pyruvic acid with acetaldehyde (oxidative decarboxylation of diacetyl). Acetoin can also be formed by direct reduction of diacetyl, being a precursor in the synthesis action of diacetyl and 2,3-butanediol [50].

Compounds with a benzene ring (e.g., phenolic compounds) originate either from the raw material, or result during the alcoholic fermentation, under the action of *Saccharomyces cerevisae* yeasts (decarboxylation of hydroxycinnamic acids) from the chemical reactions (for example, degradation of phenolic acid) or by the contamination with *Brettanomyces* spp. [45,50]. Of these, vinylphenols are usually formed by enzymatic decarboxylation from cinnamic acids, under the action of yeasts [53].

Terpenes are usually synthesized from glucose, through acetyl-coenzyme A [36]. The amounts of terpene compounds in grapes and wines can be influenced by numerous factors such as cultivation technology, geographical region, and the applied winemaking technology [59].

Enzymes can generate significant increases in the concentrations of most volatile compounds and improve sensory characteristics in wines, with minimal equipment or energy consumption. Both pectinases and yeast activity can conduct to effective results regarding the volatile profile. Data showed that pectinases can generate more acceptable sensory features, such as intense fruity aroma. Thus, it can be said that the intensity of enzymatic hydrolysis depends on the type and activity of the enzyme preparation used. Moreover, the recommended application doses of the analyzed enzymes may not be sufficient to generate a measurable hydrolysis [60]. Numerous studies have indicated the enrichment of the aroma profile of wines following the application of different enzyme preparations. Although the use of enzymes is often studied, the majority of research are focused on terpenes and norisoprenoids enrichment of wines obtained from aromatic varieties. In this experiment, over 65 volatile compounds from different chemical classes were evaluated. Sun et al. [13] obtained a significant increase of the varietal compounds with β-D-glucosidase supplementation in Cabernet Gernischt wines. Pogorzelski and Wilkowska [54] studied the effect of different enzymatic preparations based on β-glucosidase, rhamnosidase, pectinase, and glycosyl hydrolase activities, to increase or release volatile substances in white wines. Masino et al. [4] obtained an increased level of vinylphenol in samples treated with pectinases. Cabaroglu et al. [28] also obtained increased concentrations of benzene derivatives in enzymes-treated Emir wine, these results being in accordance to those presented in this paper.

The action of pectolytic enzyme preparations and β-glycosidases in the production of white wines was also analyzed by Rusjan et al. [9], obtaining a significant increase in the concentrations of monoterpenes (such as geraniol, nerol, linalool, or α-terpineol), compared to the control wine. In accordance to the results of this study, Cabaroglu et al. [28]

obtained increased levels of terpenes in enzymes-treated Emir wines. Rusjan et al. [61] also studied the action of some enzyme preparations on terpenes in some white wines. In this experiment, the level of linalool did not register significant increases compared to the control sample. These results are supported by the use of enzyme preparations with a reduced activity of α-rhamnosidase, α-arabinosidase, and β-glycosidase. Thus, the choice of enzyme preparations suitable for the intended purpose is of particular importance.

Armada et al. [10] followed the effect of the application of some pectolytic enzymes to white wines obtained from the Albariño variety on the evolution of aroma compounds. All wines, regardless of the type of added enzyme, showed different aromatic characteristics compared to untreated wines, and the wines obtained after the application of maceration enzymes showed the highest level for ethyl esters or phenylethyl acetate. The use of maceration enzymes in combination with clarifying enzymes has been shown to be inappropriate due to the fact that glycosidic enzymes block the formation of some flavor compounds. The main analyzed components presented differences between the wines treated only with maceration enzymes (glycosidases), compared to the wines to which other types of enzymatic treatments were applied.

Rocha et al. [11] reported a significant increase in the concentrations of geraniol, terpenediols, phenols, alcohols, and esters in the Maria Gomez variety, but no major changes in these compounds were observed in the case of the analyzed Bical variety. The two varieties come from the same geographical area (Bairrada), which indicates that the extraction of flavor compounds under the influence of enzymes is closely related to the aromatic potential of the studied variety. According to other authors, the main volatile compounds of Sauvignon blanc wines are mercaptans (4-mercapto-4-methyl-2-pentanone) [62] but other studies consider methoxypyrazines (represented by 3-mercaptohexyl) as defining compounds for the mentioned variety [63]. The aromatic profile of the Sauvignon blanc variety is dependent on numerous factors, including the applied winemaking technology and the terroir [60]. These classes of chemical substances were not identified in the analyzed samples, probably due to the fact that pre-fermentative maceration was not made. So, winemaking technique is also an important variable in defining aroma profile. Dziadas et al. [63] demonstrated that glycosidases can increase monoterpenes' levels (linalool, α-terpineol, β-citronellol, nerol, geraniol) in white wines obtained from Perła Zali and Nachodka varieties. Even if the total proportion of these compounds decreased after 6 months of storage, enzyme-treated wines presented significantly higher concentrations than the blank. Thus, these samples were remarked by more intense notes of fruity and floral odors. The results are in accordance with those presented in this paper, the positive effect of glycosidases on wine aroma profile being confirmed.

The proportions of some volatile phenolic compounds largely increased in enzyme-treated wines, the results being in accordance with those obtained by Cabaroglu et al. [28]. Generally, the increase in volatile phenols concentrations in the enzyme-treated wines is related to the hydrolysis of their glycoconjugated forms, exception being made for vinylphenols and vanillin. Data highlighted that the proportions of vinylphenols were higher in the treated sample, suggesting the implication of cinnamate esterase activity. Thus, the must is enriched in cinnamic acids which are converted by yeasts to vinylphenols (4-vinylphenol and 4-vinylguaiacol). These compounds can manifest a positive sensory effect in white wines when they are found at levels below 725 g/L for the total of both 4-vinylphenol and 4-vinylguaiacol [28]. The enzyme-treated variants presented higher proportions, but a phenolic off-flavor was not perceived during sensory evaluation, the role of the wine matrix on the perception of volatile phenols being showed. Vinylphenol proportions were diminished over wine storage period. This phenomenon can be attributed to the reactivity of the unsaturated side chain [28].

The results initiate the possibility of using these enzymes before the installation of alcoholic fermentation to improve the aromatic properties of the wines, with regard to the liberation of esters and terpenes in wines [27]. Over 20 esters were identified in both Fetească regală and Sauvignon blanc wines, ethyl octanoate being representative for the first mentioned variety, while ethyl lactate was predominant in the second one. Therefore, in the present experiment, esters were predominant in both variants, but pectinases (from V1) were more effective in increasing their proportions. In accordance with the team's previous data, McKinnon [47] reported a positive correlation between ethyl octanoate formation and leucine levels. Fatty acid as octanoic, hexanoic, or decanoic acids were predominant in both the analyzed varieties. According to Csutoras, Bakos, and Burkus [64], the mentioned compounds and their ethyl esters can be potential markers of some defects in wines, high concentrations being undesirable (the sensory quality is descendant). In the analyzed samples, hexanoic acid was found in high proportions in Fetească samples, usually being responsible for buttery aroma, phenolic notes, and exotic fruits odors. Acetic acid was found in high levels in Sauvignon blanc variants, generally being responsible for vegetal and sour perceptions. This compound can be produced by *Saccharomyces cerevisiae* during the fermentation process from the hydrolysis of the compound acetyl-coenzyme A, following the oxidative decarboxylation of pyruvic acid and under the action of pyruvate dehydrogenase or by oxidation of acetaldehyde. McKinnon [47] also reported an increase in acetic acid concentration in samples with high proline content; this phenomenon being confirmed in our previous works [65].

In accordance with the data reported by Bakker et al. [66], pectinases supplementation can generate a significant increase in the intensity of positive sensory descriptors, compared to the control sample. McKinnon [47] highlighted a positive correlation between exotic fruits aroma and floral odor and leucine concentrations, this affirmation being confirmed in the analyzed samples in previous works [65]. The supplementation with pectinases was effective in increasing positive sensory descriptors in Fetească regală wines, although the β-glycosidases had more influence on Sauvignon blanc wines. In accordance to the team's previous work [65], the intense honey aroma was correlated with considerable leucine and phenylalanine, while samples with high fruity notes showed important amounts of valine, leucine, and isoleucine. The higher intensity of the sweet taste in V1-Fetească regală and V3-Sauvignon blanc was related to their glutamic acid and glycine proportions. Variants with intense sweet perception were characterized by a lower bitter taste, previously associated with considerable amounts of glutamic acid.

The release of volatile compounds from their glycoconjugates (by acid catalysis) during wine storage is generally low. The use of exogenous enzymes can accelerate the formation reaction of odor-active volatiles over the storage and maturation phase due to the reactivity of released aglycones in wine pH [28]. In this work, it has been observed that enzyme treatment of Fetească regală and Sauvignon blanc wines, followed by short-term bottle aging may contribute to the development of varietal aroma for commercial purposes. The inhibition of β-glycosidase preparations produced from fungal (*Aspergillus niger*) sources is an important disadvantage for aroma enhancement in winemaking [30].

The efficiency of the enzymes is also influenced by the stage of enzyme application. This experiment indicates the possibility of applying enzymes before the fermentation stage, in must, although according to the manufacturer's recommendations. The existing research [2–18] analyze their action during the other phases of winemaking. Thus, in the case of Fetească regală wines, the most effective were samples in which the enzymes complied with the manufacturer's application recommendations. However, it has been shown that β-glycosidases can produce effective results in increasing volatile compounds concentrations in Sauvignon blanc wines when administered at the beginning of the fermentation, in must, even if the producers recommend to be administered at the end of the biochemical process.

Enzymes are effective alternatives in improving the volatile compounds concentrations and sensory properties of wines. The results are relevant for the optimization of the winemaking process at a laboratory, but also at an industrial scale.

5. Conclusions

By testing the effectiveness of the application of enzymes on the aroma profile of wines, this article confirms their significant action on increasing the proportion of volatile compounds, but also on improving the sensory properties. The analyzed wines showed complex aroma profile but esters, alcohols, acids, and hydrocarbons were predominant. The applied treatments highlighted the specific varietal flavor of the analyzed samples. This suggests that although enzymes significantly impact the aroma profile, it is also necessary to consider the technology or grape variety as important variability factors. Therefore, Fetească regală wines were characterized by the fruity and floral odors, due to significant proportions of esters such as ethyl octanoate, 3-methylbutyl acetate, propan-2-yl acetate, and ethyl decanoate. Sauvignon blanc samples were described by more vegetal and spicy notes, but also fresh citrus and exotic fruits aromas due to high levels of higher alcohols (3-methylbutan-1-ol, 1-phenylethanol), esters (diethyl butanedioate), and acids (acetic acid). Pectinases treatment can determine an increase in the intensity of positive sensory descriptors, compared to the control sample. Being compared to β-glycosidases, pectinases can generate more acceptable sensory features, such an intense fruity aroma, when are administrated before alcoholic fermentation.

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