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Impact of Commercial Inactive Yeast Derivatives on Antiradical Properties, Volatile and Sensorial Profiles of Grašac Wines

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Abstract: This study shows the impact of three different commercial inactive yeast derivatives (IYDs) (Opti Less™, Noblesse™, Optimum White™, Lallemand, Canada and Oenolees MP™ Lafort, USA) during the 6-month aging period on the volatile profile, sensory attributes and antiradical activity, including polyphenols and the total free sulfhydryl (-SH groups) content, of Grašac wines made in sequential fermentation with native *Hanseniaspora uvarum* S-2 and *Saccharomyces cerevisiae* QA23. The addition of IYDs helped in maintaining the constant values of antiradical activity during aging by increasing polyphenolic values and mitigating the decrease in -SH groups. HS-SPME-GC-MS analysis showed that esters were the major volatile compounds, with ethyl-acetate and 2-phenyl-ethyl-acetate being the most abundant among all the samples, followed by ethyl-dodecaonate, ethyl-decanoate and 3-methyl-butyl-octanoate, all of them contributing to fruity and floral aromas in wine. As the concentration of IYDs increased, a corresponding rise in the levels of certain volatiles, such as 2-methyl-1-propanol, phenyl-ethyl-alcohol and ethyl-octanoate, was observed. Sensory analysis showed that the addition of IYDs generally improved the taste and odor profile of the wine by reducing astringency and increasing fullness and complexity, regardless of the IYD type. The results demonstrated that different IYDs may have varying effects on wine, with each product having its specific purposes, providing the tools for winemakers to carefully regulate and obtain the desired sensory profile of the wine.

Keywords: non-*Saccharomyces yeasts*; sequential fermentation; wine aging; lees; inactive yeast derivatives

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1. Introduction

Current trends in scientific research, as well as in the modern food and wine industry suggest continuous improvement in product quality. One of the global trends in winemaking and viticulture is to strengthen the authenticity of wine and the balance and complexity of its flavor and aroma. Leading wine regions have already made progress in adapting to new customer segments by introducing new technological processes that will improve the complexity and quality of wine [1]. One of the techniques that has been becoming popular is the use of various inactive yeast derivatives (IYD), which are used as an alternative to the usual aging-on-lees procedure. The application of different IYDs (inactivated yeasts, yeast cell walls, extracts, and autolysates) offers similar benefits as traditional aging on the lees, such as the release of yeast cell components and valuable bioactive compounds in the wine over a much shorter period of time, significantly improving the overall quality and aroma profile of the wine, thus increasing cost efficiency [2]. Such alternative processes can fully meet the demands of consumers and winemakers for the most acceptable balance between wine quality and price. IYDs are a source of various compounds, such as mannoproteins, amino acids, peptides, proteins, polysaccharides, nucleotides, fatty acids, vitamins, and

minerals, that improve the stability, color, and mouthfeel of wine [3,4]. Some reports also indicate that these products absorb browning products in white wine and reduce wine oxidation and astringency [5,6]. IYDs affect the perception of wine aroma, with volatile compounds being gradually released during contact with wine.. The aroma compounds present in wine can interact with the polysaccharides and proteins of the IYDs, with the strength of the interaction depending on the type of aroma compound and the nature of the macromolecules [2,7]. In addition, glutathione (GSH), which is released from IYDs, is very important in the production of white wines due to its antioxidant properties and its ability to prevent the enzymatic darkening of white wines, but also because of its ability to protect the aromatic substances of the grape variety and reduce the occurrence of off-flavors. IYDs rich in GSH have an effect on the stabilization of varietal aromas, such as volatile thiols and terpenes [8–10].

Many types of YDs are sold commercially, claiming to achieve various wine improvements. However, their improper use may lead to the appearance of off-flavors and aromas, nutrient imbalances and reduction in polyphenols, thus increasing the risk of wine spoilage. Scientific knowledge on the chemistry underlying its use is rather insufficient, and the mechanisms responsible for the claimed effects in wine are not entirely clear. For that reason, the dosage and the selection of the particular type of YD must be optimized in accordance with the grape variety and the desired effect in the specific winemaking procedure. Additionally, recent studies have pointed out that the application of non-*Saccharomyces* yeast in pure or sequential fermentation has a great potential to improve the sensory attributes of wine [11–14]. Considering the central role that fermentation plays in the development of wine aroma, the yeasts that carry out the fermentation are very important for the development of specific aroma profiles. The non-*Saccharomyces* yeast genera, such as *Hanseniaspora* (*Kloeckera*), *Candida*, *Pichia*, *Kluyveromyces*, and *Metschnikowia*, are explored for their properties to reduce ethanol content and to develop the distinctive flavors of wine [15–20]. Due to the fact that non-*Saccharomyces* strains lack competitiveness during fermentation and have poor tolerance to stress, they are usually applied in sequential or mixed fermentation with traditionally used *Saccharomyces cerevisiae*. *Hanseniaspora uvarum* is one of the non-*Saccharomyces* yeast representatives that has been extensively investigated by our research group. We demonstrated in our previous work that *H. uvarum* modulates wine quality parameters along with the aroma and sensory profile of wine [14,21]. To our knowledge, a scarce number of studies have examined the combined effect of non-*Saccharomyces* yeasts and YDs on wine. The combined effect of yeast hulls and *Candida zemplinina/Saccharomyces cerevisiae* mixed starter cultures was evaluated before, indicating significant improvement on the volatiles, antioxidant activity, and sensory perception of aged wine [22].

Therefore, the aim of this research was to assess the volatile profile, sensory attributes and antioxidant activity of Grašac wines made in sequential fermentation with native *H. uvarum* strain, and to evaluate the impact of different commercial YDs during the 6-month aging period on the quality parameters of obtained wines.

2. Materials and Methods

2.1. Wine Production

The study was performed on Grašac grapes supplied by the "Molovin" winery (Šid, Serbia, 45°12′ N, 19°24′ E). The grapes were harvested at full technological maturity, destemmed and crushed. Must characteristics were: 21 °Brix, pH 3.19, total acids 6.37 g/L. The must was initially precipitated at 8 °C for 72 h. The fermentation was performed in a stainless-steel tank with a prepared culture inoculum of native strain *H. uvarum* S-2, containing 10^8 CFU/mL, followed by sequential co-inoculation with *Saccharomyces cerevisiae* QA23 (Lallemand, Montreal, QC, Canada, 25 mg/L). Co-inoculation was performed after the initial fermentation stages when °Brix decreased by 3 degrees. The selected yeast strain, *H. uvarum* S-2, had been previously isolated from locally grown blackberries and identified by PCR analysis according to their ITS sequence. Identification of yeast isolates was performed after DNA extraction and ITS sequence analysis using ITS1 (5′-

TCCGTAGGTGAACCTGCG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) primers for PCR analysis. Obtained sequences were compared with the Basic Local Alignment Search Tool (BLAST) database and identification was performed. The isolated strains are a part of the collection of the Faculty of Technology, University of Niš, Serbia. After the inoculation, polyvinylpyrrolidone (10 g/hL) and bentonite (100 g/hL) were added at 13 and 7 °Brix, respectively. The fermentation was performed until dryness. After the fermentation was complete, the obtained wine was divided into 8 tanks and treated with different commercial yeast derivatives originating from *S. cerevisiae* that are available on the market by the names: Opti Less™ (Lallemand, Montreal, QC, Canada), Noblesse™ (Lallemand, Montreal, QC, Canada), Optimum White™ (Lallemand, Montreal, QC, Canada), and Oenolees MP™ (Laffort, Petaluma, CA, USA). The specific characteristics of each IYD are given in Table 1, according to the data available in the manufacturer specifications. These products were applied in two concentrations, 20 g/hL and 40 g/hL, during a period of 6 months, with stirring every third day. After treatment, wine samples were filtered and bottled. Untreated wine was used as a control. Standard analyses of wine were conducted by Fourier transform infrared spectroscopy (FTIR), using an OenoFoss™ instrument (FOSS WineScan, Denmark) [23]. All experiments were performed in triplicate expressed as mean values with standard deviation.

Table 1. Specific characteristics of IYDs applied on Grašac wine during 6 months of aging.

IYD	Specification	Expected Results
Noblesse™ (Lallemand, Canada)	Specific inactivated yeast *S. cerevisiae*	Increase the perception of ripe fruit, contribute to a more intense structure, initial mouthfeel volume and rounded finish. It can decrease the perception of harsh, chemical and burning sensations. Can have a stimulatory effect on malolactic fermentation.
Optimum white™ (Lallemand, Canada)	Specific inactivated yeast *S. cerevisiae* with guaranteed glutathione levels	It helps protect white and rosé wines from oxidation, increases wine quality, fruitiness, freshness and longevity.
Oenolees™ (Lafort, USA)	Specific preparation of *S. cerevisiae* cell walls	The fining effect. Elevates midpalate sensations. Eliminating specific polyphenols responsible for bitterness and astringency.
Optilees™ (Lallemand, Canada)	Specific inactive *S. cerevisiae* yeast, rich in polysaccharides.	Faster maturation of the wine. The sensation of sweetness and fullness.

2.2. Total Free -SH Groups Determination

The total free sulfhydryl groups (-SH) in the wine samples were determined according to the method previously described by Kontogeoros and Roussis [24]. Briefly, a mixture of 2.4 mL of 200 mM K_2HPO_4/KH_2PO_4 and 0.6 mL of wine sample (diluted 1:10 with model wine) was prepared. The model wine was prepared as a mixture of 12% ethanol and 5 g/L tartaric acid in water, with the pH adjusted to 3.5 using 1N NaOH. Subsequently, 0.3 mL of a 1 mM 5,5′-dithio-bis (2-nitrobenzoic acid) DTNB solution in the same phosphate buffer was added to the mixture, which was then kept at 20 °C for an hour. The absorbance of each sample was measured at 412 nm against a blank containing only the buffer, and the absorbance of a mixture comprising 2.4 mL phosphate buffer, 0.6 mL model wine and 0.3 mL DTNB solution was subtracted from the sample absorbance. The spectrophotometer was set to zero using distilled water. The results were expressed as mg/L GSH equivalent (GE) using GSH solutions (0, 10, 20, 40, 80, 120 mg/L) in model wine instead of wine samples. The analyses were carried out in triplicate.

2.3. Total Polyphenols Determination

The total polyphenols of wine were determined using the adapted Folin–Ciocalteu method [25]. A total of 10 µL of diluted wine samples was mixed with 790 µL of distilled water and 50 µL of Folin–Ciocalteu reagent, and then vortexed in a 1.5-mL Eppendorf

tube. After 1 min, 150 µL of 20% aqueous sodium carbonate was added, and the mixture was vortexed and left in the dark at room temperature for 120 min. The absorbance was measured at 750 nm, and the total polyphenol concentration was determined by constructing a calibration curve with gallic acid as the standard. The results were reported as mg/L gallic acid equivalents (GAE).

2.4. Antioxidative Activity of Wine (DPPH Assay, Antiradical Activity)

The antioxidative activity of wine samples was determined using the method previously described by Psarra and coworkers [26]. All samples were diluted 1:10 with methanol prior to analysis. A total of 25 µL of diluted sample was mixed with 975 µL of DPPH solution (60 µM in methanol) and vortexed. The absorbance was measured at the 0 min and 30 min marks. The antioxidant activity was calculated using the following equation:

$$\%\Delta A_{515} = \frac{[A_{515}(0) - A_{515}(30)]}{A_{515}(0)} \cdot 100 \quad (1)$$

The calculation of antiradical activity was carried out by calibration curves obtained from the methanolic Trolox solution. Results are expressed as Trolox equivalents (mM TE).

2.5. Volatile Compound Analysis (HS-SPME-GC-MS)

Volatile aroma compounds were analyzed using solid-phase microextraction coupled with gas chromatography, using the experimental procedure previously described [21]. An SPME manual holder and fused silica fiber coated with Carboxen®/PolIYDimetilsiloxane (CAR/PDMS, 75 µm thickness) Supelco (Bellefonte, PA, USA) were used for the aroma compounds extraction by HS-SPME. GC/MS analysis was performed on Agilent Technologies 7890B gas chromatography, coupled with a 5977A mass detector. Components were separated on a polar column DB-WAX (30 m × 0.25 mm, 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA, USA). The concentration of volatile compounds (expressed in mg/L) in the wine samples was determined by an internal standard method and expressed as a mean of three injections of each replicate.

2.6. Sensory Analysis

A sensory analysis was conducted in accordance with ISO 6658, ISO 3591 [23]. The sensory panel consisted of 11 certified panelists aged between 30 to 55 years old, 4 of which were women and 7 were men. The panel evaluated various smell attributes, such as complexity, intensity, typicality duration, flowers, fresh fruit, dry fruit, tropical fruit, citrus, vegetable, tobacco, honey, spice, overall odor, as well as taste attributes, including harmony, intensity, typicality, complexity, fullness, acidity, astringency, and duration, using a nine-point intensity scale, ranging from 0 (not detected) to 9 (very intense). The evaluations were performed in duplicate, and the results were presented as the mean value.

2.7. Statistical Analysis

Statistical analysis of the obtained data was performed by one way ANOVA followed by Tukey's HSD post hoc test at a significance level of $p \leq 0.05$, using SPSS Statistics 21 (IBM, New York, NY, USA). Principal component analysis (PCA) was performed for the differentiation of samples based on the volatile profile of wines using STATISTICA 7 (StatSoft Inc., Tulsa, OK, USA, trial version).

3. Results and Discussion

3.1. Standard Oenological Parameters

The results of the standard oenological analysis of Grašac wines with the addition of different IYDs are presented in Table 2. All wine samples had similar content of ethanol (12.43–12.48%), which is typical for white wine varieties. There was a slight variation in the glucose and free and total SO_2 content. The acetic acid content in all wines was within the acceptable range (0.36–0.37 g/L). A tartaric acid content of 2.7 g/L in white wine is also

considered relatively low compared to the typical range of 4 to 10 g/L, while lactic and citric acids were within the typical ranges for white wines. In general, the analysis of the primary oenological parameters did not reveal any distinct trends that could be linked to the IYD applied, which is in accordance with previously published data [27].

Table 2. Standard oenological parameters of Grašac wine created with the addition of different IYDs.

Parameter	IYD Treatment *							
	NO20 **	**NO40**	**OL20**	**OL40**	**OW20**	**OW40**	**OE20**	**OE40**
Ethanol, % *v/v*	12.43 ± 0.10	12.43 ± 0.08	12.47 ± 0.18	12.46 ± 0.11	12.47 ± 0.02	12.45 ± 0.12	12.45 ± 0.09	12.48 ± 0.07
Total acids (as tartaric acid), g/L	5.1 ± 0.01	5.1 ± 0.01	5.2 ± 0.02	5.2 ± 0.10	5.2 ± 0.02	5.2 ± 0.01	5.1 ± 0.03	5.1 ± 0.00
Volatile acids (as acetic acid), g/L	0.37 ± 0.00	0.37 ± 0.01	0.36 ± 0.03	0.36 ± 0.02	0.37 ± 0.00	0.37 ± 0.01	0.36 ± 0.01	0.36 ± 0.00
Reducing sugar, g/L	2.3 ± 0.02	2.4 ± 0.00	2.3 ± 0.01	2.4 ± 0.02	2.5 ± 0.00	2.5 ± 0.00	2.5 ± 0.01	2.3 ± 0.00
Free SO_2, mg/L	6.2 ± 0.10	6.2 ± 0.04	6.6 ± 0.06	6.6 ± 0.02	6.4 ± 0.03	6.3 ± 0.00	6.0 ± 0.01	6.2 ± 0.00
Total SO_2, mg/L	88 ± 1.0	91 ± 2.0	92 ± 0.0	92 ± 0.0	92 ± 0.0	92 ± 0.0	89 ± 0.0	91 ± 0.0
pH	3.32 ± 0.00	3.32 ± 0.00	3.3 ± 0.00	3.30 ± 0.10	3.29 ± 0.00	3.30 ± 0.00	3.30 ± 0.00	3.29 ± 0.20
Glucose, g/L	0.9 ± 0.00	1.0 ± 0.00	1.2 ± 0.00	1.2 ± 0.00	1.1 ± 0.00	1.4 ± 0.00	1.1 ± 0.00	1.2 ± 0.01
Fructose, g/L	0.9 ± 0.00	1.0 ± 0.00	0.9 ± 0.00	0.9 ± 0.01	1.0 ± 0.00	1.0 ± 0.00	0.9 ± 0.00	0.9 ± 0.00
Tartaric acid, g/L	3.7 ± 0.10	3.8 ± 0.03	3.7 ± 0.00	3.8 ± 0.00	3.7 ± 0.10	3.8 ± 0.05	3.7 ± 0.00	3.7 ± 0.05
Citric acid, g/L	0.21 ± 0.00	0.20 ± 0.00	0.23 ± 0.00	0.22 ± 0.00	0.23 ± 0.00	0.24 ± 0.00	0.21 ± 0.00	0.22 ± 0.00
Malic acid, g/L	0.6 ± 0.00	0.6 ± 0.00	0.7 ± 0.00	0.7 ± 0.00	0.8 ± 0.00	0.7 ± 0.00	0.7 ± 0.00	0.7 ± 0.00
Lactic acid, g/L	0.8 ± 0.00	0.8 ± 0.00	0.7 ± 0.00	0.7 ± 0.00	0.7 ± 0.00	0.7 ± 0.00	0.8 ± 0.00	0.7 ± 0.00
Glycerol, g/L	6.3 ± 0.10	6.3 ± 0.00	6.2 ± 0.10	6.3 ± 0.00	6.2 ± 0.00	6.3 ± 0.00	6.2 ± 0.00	6.2 ± 0.10

* IYD treatments: NO20 Nobless™ 20 g/L, NO40: Nobless™ 40 g/L, OL20: Opty Less™ 20 g/L, OL40: Opty Less™ 40 g/L, OW20: Optimum White™ 20 g/L, OW40: Optimum White™ 40 g/L, OE20: Oennolees™ 20 g/L, OE40: Oenolees™ 40 g/L. ** There are no statistical differences between the samples ($p < 0.05$).

3.2. Polyphenols and Total Free Sulphydryl Groups (-SH) Content

Polyphenols are the major compounds that contribute to the antioxidant properties of wine and are responsible for the color, astringency and bitterness in the wine. Their concentration and composition can vary greatly, as it is influenced by factors such as grape variety, vinification, maturation and aging processes [28]. In the present study, the total polyphenolic content of all analyzed samples varied within the range 199.1–374.9 mg/L GAE (Table 3). These results are in accordance to those obtained for different white wine varieties [28–30]. The concentration of polyphenols in wine tends to decrease over time due to oxidation and their interaction with other chemicals that exist in wine [25]. In this research, we have demonstrated that the addition of IYDs can mitigate the degradation of polyphenols over time or even increase their content. After 6 months, all wine samples had a significantly higher content of total polyphenols than the control wine. A higher applied dose (40 g/L) had a more pronounced effect in preserving the polyphenols, which is explained by their higher potential to release different polyphenolic compounds, such as gallic acid, catechins and quercetin, by hydrolyzing larger oligomers [31]. Wines made with application of NO and OE had a lower concentration of polyphenols than the control, due to the ongoing oxidation and polymerization processes that occur simultaneously during aging [32]. Such a result is expected because of the manufacturer's claims that OE contains specific yeast cell walls formulation for eliminating the specific polyphenols responsible for bitterness and astringency. A recent study showed that among the six commercial IYDs applied, four of them had a positive effect on the polyphenolic content, when applied in the maximum allowed concentration (40 g/L), and that every IYD has a different capacity to absorb or release polyphenolic compounds [26]. Specifically, the IYDs that contain high molecular weight polysaccharides usually stimulate the decrease in polyphenolic compounds [33], which was the case for NO.

Table 3. Total polyphenols (mg/L gallic acid), antiradical activity (AR) (mM TE) and -SH groups (mg/L GE) content in Grašac wines treated with different IYDs after 3- and 6-month aging period.

Wine Treatment **	Aging Time, Months	Polyphenols	AR	-SH Groups
CONTROL	0	321.6 a * ± 7.63	0.75 ae ± 0.09	450.1 a ± 3.82
	3	222.4 b ± 5.20	0.64 bd ± 0.02	376.5 b ± 5.43
	6	199.1 c ± 3.87	0.52 c ± 0.00	330.1 c ± 3.32
NO20	3	309.1 d ± 6.29	0.67 bd ± 0.01	473.6 d ± 2.26
	6	320.8 a ± 2.55	0.62 d ± 0.05	416.7 eg ± 2.88
NO40	3	334.1 ae ± 6.17	0.77 e ± 0.04	488.1 d ± 3.14
	6	343.3 ef ± 6.61	0.73 a ± 0.00	476.0 d ± 2.34
OL20	3	337.4 afgh ± 10.41	0.71 ab ± 0.02	397.5 f ± 4.12
	6	347.4 eh ± 5.24	0.69 ab ± 0.01	345.7 c ± 3.82
OL40	3	334.1 afh ± 5.21	0.72 ab ± 0.01	395.7 f ± 6.55
	6	359.1 fhkl ± 9.46	0.71 ab ± 0.01	373.3 b ± 4.74
OW20	3	296.6 d ± 6.52	0.70 ab ± 0.01	402.2 ef ± 4.12
	6	326.6 afi ± 3.82	0.70 ab ± 0.01	408.5 ef ± 8.08
OW40	3	355.8 fhj ± 2.50	0.72 a ± 0.01	421.4 g ± 7.07
	6	364.9 jkl ± 7.63	0.71 ab ± 0.00	435.6 ag ± 1.66
OE20	3	321.6 a ± 5.21	0.77 ae ± 0.03	440.7 a ± 8.21
	6	342.4 egij ± 9.01	0.70 ab ± 0.02	438.7 a ± 4.02
OE40	3	335.8 afh ± 2.54	0.79 e ± 0.01	436.7 ag ± 8.85
	6	374.9 l ± 5.26	0.75 ae ± 0.01	443.7 a ± 4.23

* Different letters indicate statistically significant differences in the same column ($p < 0.05$). ** IYD treatments: NO20 Nobless™ 20 g/L, NO40: Nobless™ 40 g/L, OL20: Opty Less™ 20 g/L, OL40: Opty Less™ 40 g/L, OW20: Optimum White™ 20 g/L, OW40: Optimum White™ 40 g/L, OE20: Oennolees™ 20 g/L, OE40: Oenolees™ 40 g/L.

3.3. Volatile Compounds

The sensory profile of wine is characterized by a variety of volatile compounds that are formed during fermentation and wine aging. The non-volatile, active flavor precursor compounds are transformed through various chemical reactions into volatile aromatic compounds, including esters, higher alcohols, volatile fatty acids and many others [34]. Table 4 shows the content of volatile compounds in the examined wines. Esters were the major volatile compounds, with ethyl acetate and 2-phenyl ethyl acetate being the most abundant among all samples, followed by ethyl dodecaonate, ethyl decanoate and 3-methyl-butyl-octanoate, all of them contributing to different fruity and floral aromas. The major higher phenyl-ethyl alcohol was notably above its odor threshold level (OTL) in most samples at both time marks. Within the group of aldehydes, none of the compounds were found in the examined wines, but among fatty acids, octanoic, decanoic and dodecanoic acids were present in all wine samples, except for the wine treated with NO IYD at the 6-month mark, which did not contain dodecanoic acid.

As the concentration of IYDs applied increased, we observe a corresponding rise in the levels of certain volatile compounds, such as 2 methyl-1-propanol, phenyl ethyl alcohol and ethyl octanoate. Contrarily, the content of ethyl dodecanoate and 3-methyl-butyl-octanoate shows an inverse relationship with the dosage of IYD. In addition, most of the IYDs applied helped to preserve or even increase the amounts of specific volatiles during the aging period. This is the case for 2 methyl-1-propanol and 2-phenyl-ethyl acetate, while the concentration of methyl octanoate and 3-methyl-1-butanol tends to slightly decrease over time. On the other hand, even though the addition of IYDs resulted in an initial increase in the amount of ethyl octanoate, the aging period managed to diminish this effect, pointing out that the protective role of IYDs in the case of this compound is not long-term. None of the IYDs, regardless of application, had a significant impact on ethyl hexanoate content when compared to the control, although the concentration of this important wine aroma contributor tends to increase over time, especially in the wines treated with NO (40 g/L) and OL (both 20 and 40 g/L). The IYDs' dosage specifically comes to expression in the case

of ethyl acetate, where increasing the concentration of NO IYD from 20 to 40 g/L results in a 70% increase in the amount of this compound (from 16.73 mg/L to 28.55 mg/L).

Higher alcohols, also called fusel alcohols, are a quantitively predominant group of volatile compounds in wine usually produced by yeast during fermentation. The concentration of higher alcohols, up to 300 mg/L, is considered desirable, although the particular effect may vary depending on the style of wine [34]. In this study, the total amount of higher alcohols was in the range of 50.26–88.85 mg/L, with 3 methyl-1-butanol (isoamyl alcohol) being the most abundant. Among the samples, the highest concentration of higher alcohols was noted in the wines treated with OW. 3-methyl-1-butanol was beyond the OTL level in all samples except for the control and the wine treated with 20 g/L NO IYD. Although OL-, OW- and OE-treated wines initially had similar amounts of this volatile, after the aging period, only OE-treated wines managed to keep the same levels. This is explained by the fact that certain IYDs may release enzymes over time, which in turn break down those higher alcohols into simpler compounds [2]. Additionally, higher alcohols are involved in oxidation or esterification reactions with other compounds present in wine, which results in the synthesis of new compounds and an eventual decrease in the original higher alcohol [34]. On the other hand, IYDs may act stimulative to malolactic fermentation during the aging period [35], which leads to an increase in the level of some higher alcohols, such as phenyl ethyl alcohol, that contribute to flowery notes. This volatile was the most abundant in OW-treated wines after a 6-month aging period with the value being way above the OTL. Hexanol, mainly responsible for a grassy or herbaceous character, was present in the range of 4.52–6.98 mg/L, which is below the OTL. This is in accordance with the results obtained for white wines aging on commercial lees for two and seven months [36]. It should be noted here that the sensory effect of each individual compound on overall wine aroma and flavor is influenced by many factors besides concentrations, such as the complexity and balance of the wine.

Esters are an important contributor to the sensory perception of wine as they en-hance its fruity and floral aroma and flavor. A total of 14 esters were detected in the samples in a total concentration of 47.25–92.8 mg/L. All of them, except for the hexyl acetate, were identified as significantly above OTL. As for hexyl acetate, there is no clear consensus on the typical levels in wine due to variations between different types of wine. However, certain studies have reported concentrations of hexyl acetate ranging from undetectable levels to approximately 0.6 mg/L. It is noteworthy that hexyl acetate has a relatively low sensory threshold, with some tasters reportedly able to detect concentrations as low as 0.002 mg/L [37,38]. This implies that even small quantities of hexyl acetate, which are detected in all wine samples except for the control, can significantly influence the aroma and flavor of the wine. As a result, winemakers can carefully regulate the concentration of hexyl acetate during fermentation to achieve the desired sensory profile of the wine. The most abundant volatile ester was ethyl acetate, known for its fruity and floral aroma in wine with a sweet, ripe, fruit character [39]. The concentration range among the samples, 16.73–28.55 mg/L, is somewhat lower than the one obtained for Corvina red wines [39] and Chardonnay wine [39]. 2-phenyl-ethyl-acetate was the second most abundant ester in concentrations significantly over its OTL (0.25 mg/L), giving the wine a floral or honey-like character. The content of 3-methyl-1-butanol-acetate (0.1–0.91 mg/L), which is the carrier of a fruity, pear-like aroma, was slightly higher than those obtained for persimmon wine [40] and lower than the content of this compound in sparkling wines [7]. The highest amount of this compound (0.91 mg/L) was found in wines treated with 40 g/L NO IYD. This IYD also contributed to the highest content of ethyl acetate, while OE-treated wine had the highest content of methyl octanoate and ethyl-9-hexadecenoate, contributing to the orange and sweet fruity aroma, respectively.

Table 4. Concentration of volatile compounds (mg/L) found in Grašac wines produced with different IYDs after 3- and 6-month ageing period.

Compound	OTL, mg/L	Time	C	IYD Treatment *							
				NO20	NO40	OL20	OL40	OW20	OW40	OE20	OE40
3-Methyl-1-butanol	30.00 [41]	3	28.32 **	24.13	33.64	40.48	37.44	39.97	39.74	37.84	39.80
		6	23.1	22.30	12.59	13.70	18.59	13.85	29.28	37.82	39.82
2-Methyl-1-propanol	30.00 [42]	3	15.68	6.08	11.33	14.20	8.15	9.80	15.00	8.99	14.95
		6	19.21	5.22	18.55	19.87	14.31	10.28	18.69	8.69	16.39
n-Hexanol	8.000 [42]	3	4.52	5.51	5.88	6.49	6.98	5.16	6.02	5.97	4.96
		6	4.1	5.25	5.71	6.32	6.11	4.99	5.88	5.11	4.83
Phenyl ethyl alcohol	14.000 [42]	3	15.21	14.54	18.68	16.26	22.95	24.53	22.35	13.13	19.39
		6	15.55	19.30	26.26	22.73	23.72	38.94	35.00	20.81	25.10
Total alcohols		3	**63.73**	**50.26**	**69.53**	**77.43**	**75.52**	**79.46**	**84.11**	**65.93**	**79.1**
		6	**61.96**	**52.07**	**63.11**	**62.62**	**62.73**	**68.06**	**88.85**	**70.43**	**86.14**
Ethyl acetate	7.500 [42]	3	17.98	16.73	28.55	17.31	19.67	18.58	21.60	19.81	20.73
		6	12.02	14.25	18.30	21.79	16.59	19.39	14.11	20.63	16.25
Ethyl hexanoate	0.005 [42]	3	1.109	1.195	1.208	1.131	1.384	1.697	1.394	1.061	1.235
		6	1.85	1.518	3.249	4.408	3.451	1.96454	1.451	1.343	1.576
Ethyl octanoate	0.002 [42]	3	3.21	4.374	4.879	4.649	4.026	4.046	4.714	4.283	4.755
		6	tr	3.671	2.861	2.572	3.688	3.355	3.015	3.190	3.854
Methyl octanoate	0.017 [41]	3	tr	tr	1.67	3.06	2.86	2.02	3.30	3.80	3.26
		6	tr ***	tr	1.11	2.89	2.53	1.86	3.05	3.68	3.18
Ethyl decanoate	0.200 [42]	3	7.53	5.66	6.20	6.75	6.56	6.97	6.10	6.38	6.13
		6	6.23	6.72	6.02	6.15	6.18	7.08	6.64	6.96	6.56
Ethyl dodecanoate	1.500 [42]	3	10.977	6.028	5.406	6.041	4.080	6.164	6.640	5.115	4.010
		6	9.88	4.033	4.053	4.857	3.796	4.646	3.420	3.556	3.047
3-Methyl-1-butanol-acetate	0.030 [41]	3	0.40	-	0.91	0.10	0.10	0.91	0.36	0.40	0.43
		6	0.31	-	0.79	0.15	0.11	0.97	0.78	0.8	0.84
3-Methyl- butyl -octanoate	-	3	7.18	4.44	5.38	5.65	6.08	5.82	5.53	5.37	5.74
		6	6.98	5.44	5.51	4.81	5.01	6.20	5.34	5.57	5.49
3-methyl- butyl -pentanoate	-	3	0.29	0.14	0.19	0.24	0.22	0.24	0.20	0.26	0.20
		6	0.41	0.25	0.31	0.34	0.28	0.38	0.31	0.32	0.30
Ethyl-(9)-decenoate	0.100 [41]	3	3.55	3.59	3.75	4.18	4.03	4.36	4.24	3.95	4.20
		6	3.61	4.27	4.82	3.69	3.84	4.02	3.97	4.24	4.48
ethyl 9-hexadecenoate	2.000 [41]	3	7.4	tr	tr	10.11	16.87	16.49	16.74	12.34	16.23
		6	8.23	tr	tr	31.88	33.31	27.10	32.76	29.52	33.07
2-phenil-ethyl acetate	0.250 [42]	3	10.39	8.91	11.84	10.94	11.92	12.69	11.94	10.54	10.42
		6	8.22	7.1	14.53	13.81	14.1	15.21	14.98	12.78	13.11

Table 4. *Cont.*

Compound	OTL, mg/L	Time	C	IYD Treatment *							
				NO20	NO40	OL20	OL40	OW20	OW40	OE20	OE40
Hexyl acetate	0.670 [42]	3	-	0.56	0.164	0.147	0.178	0.177	tr	0.138	0.158
		6	-	tr	tr	tr	tr	tr	tr	tr	tr
Total esters		3	**70.02**	**51.07**	**69.98**	**70.16**	**77.81**	**79.99**	**82.76**	**73.31**	**77.34**
		6	**57.74**	**47.25**	**61.55**	**97.35**	**92.88**	**92.16**	**89.83**	**92.59**	**91.76**
Octanoic acid	0.500 [42]	3	2.132	0.98	2.23	2.812	2.78	3.55	3.08	2.09	2.31
		6	2.23	3.29	5.27	4.71	4.19	4.55	4.21	4.13	2.61
Decanoic acid	15.000 [42]	3	1.47	0.49	1.18	1.70	1.67	2.31	2.01	1.67	1.36
		6	1.98	2.29	3.84	3.823	3.02	3.02	3.32	3.12	1.78
Dodecanoic acid	10.000 [42]	3	10.13	-	-	9.07	10.40	13.44	12.06	9.52	7.72
		6	10.99	-	-	13.97	16.99	19.07	15.05	12.80	11.84
Total acids		3	**13.73**	**1.47**	**3.41**	**13.58**	**14.85**	**19.3**	**17.15**	**13.28**	**11.39**
		6	**15.2**	**5.59**	**9.12**	**22.50**	**24.20**	**26.64**	**22.58**	**20.05**	**16.23**

* IYD treatments: NO20 Nobless™ 20 g/L, NO40: Nobless™ 40 g/L, OL20: Opti Less™ 20 g/L, OL40: Opti Less™ 40 g/L, OW20: Optimum White™ 20 g/L, OW40: Optimum White™ 40 g/L, OE20: Oennolees™ 20 g/L, OE40: Oenolees™ 40 g/L; ** The values in the table represent the mean value of three replications. Standard deviations and statistical significances are given as a Supplementary File, Table S1; *** tr-trace.

The control wine generally contains a significant amount of variety of compounds, thus indicating the relevance of grape and yeast selection for the complexity of wine flavor. Specifically, the highest concentrations of ethyl decanoate and ethyl dodecanoate were found in the control, when compared to other wines treated with different IYDs. On the other hand, IYDs had a significant role during the aging process, where they helped to preserve the initial amount of those esters and prevent a further decrease in ethyl decanoate and ethyl dodecanoate concentration. Both of those compounds are important contributors to wine aroma: ethyl decanoate had a fruity, pineapple-like aroma, while, on the other hand, ethyl dodecanoate had a slightly more complex aroma, with fruity, waxy and floral notes. The ranges obtained in this study (6.02–7.53 mg/L and 3.04–10.97 mg/L) are in agreement with data published for red and white Greek wines [43]. IYD treatments did not show statistical significance on both compounds, although an increase in concentration was noted at the 6-month mark. This is related to lipid metabolic pathways when the content of the mentioned esters increases with an increase in the appropriate fatty acids [41].

3.4. PCA Analysis

In order to estimate the correlation between the IYD treatment and volatile composition of Grašac wines, a PCA analysis was performed for 18 selected volatile compounds with concentrations above the OTL (Table 3), which is shown in the PCA scores plot (Figure 1).

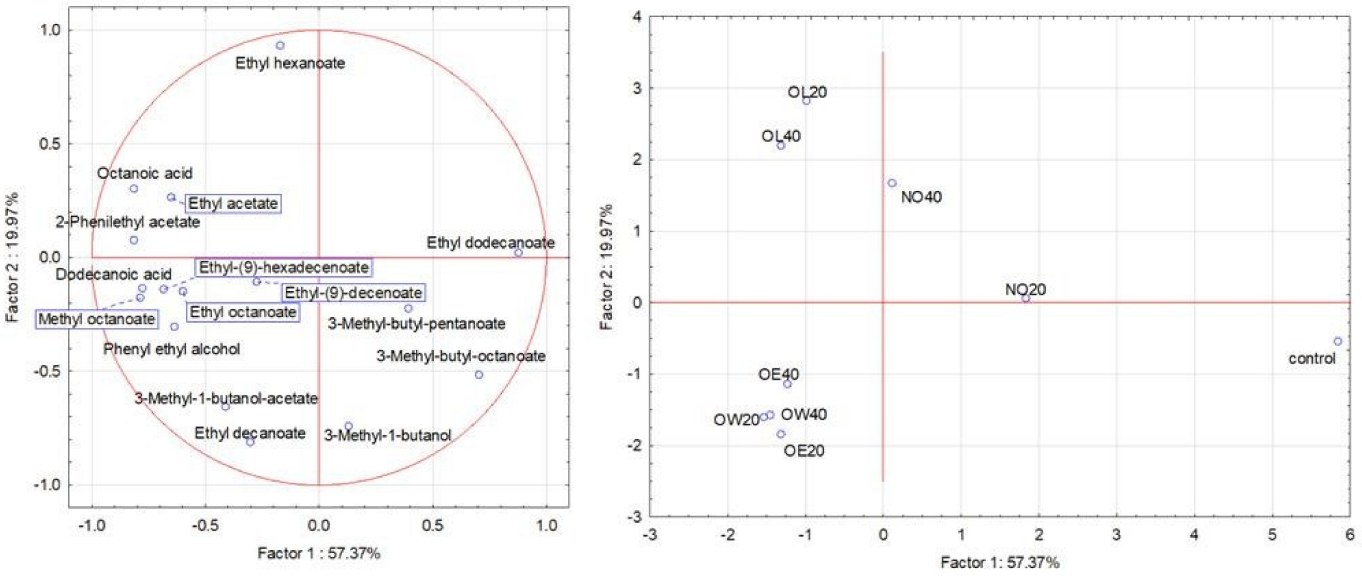

Figure 1. Principal component analysis (PCA) of volatile compounds with concentration above the odor threshold level (OTL).

According to the PCA, five factors had eigenvalues higher than 1, cumulatively explaining 96.65% of the total variance of the initial data set. PC1 and PC2 comprised 77.34% of the variability. Ethyl dodecanoate contributed to the positive aspect of PC1, while phenyl ethyl alcohol, methyl octanoate, ethyl-(9)-decenoate, ethyl 9-hexadecenoate and dodecanoic acid mostly contributed to the negative aspect of PC1. A clear separation among the Grašac wine samples can be observed, while it was found that samples treated by the same IYDs, regardless of concentration, were relatively similar and distributed closer to each other in the PCA plot. The samples treated by OE and OW in both concentrations were distributed in the lower negative quadrant. Compared to other samples, the number of volatile compounds that contributes significantly to the aroma of these wine samples was larger. Among them, ethyl decanoate, phenyl ethyl alcohol, 3-methyl-1-butanol-acetate and methyl octanoate were the most significant. Additionally, these samples were negatively correlated with ethyl hexanoate. The OL-treated samples were clearly different from the other wine samples, with ethyl hexanoate as the compound considered the most responsible

for aroma. These samples were characterized by the lower loadings of 3-methyl-1-butanol and 3-methyl- butyl -octanoate. On the other hand, the control wine sample separated well from the IYD-treated samples by PC1, and was located in the lower right quadrant (positive values for PC1 and negative for PC2), far apart from the treated wine samples. Ethyl dodecanoate showed a high positive loading score in PC1, which distinguished the control wine sample from the other samples.

3.5. Sensory Analysis

Grašac wines that aged for 6 months on different IYDs were subjected to descriptive sensory analysis. The results of the sensory analysis showed that the addition of IYDs generally improved the taste and odor profile of the Grašac wine by reducing astringency and increasing fullness and complexity in all wine samples, regardless of the type of added IYD. The addition of different IYDs into the wine resulted in a better olfactory perception of the samples (Figure 2). The least significant change was observed for the wine that aged with OE when it was added at a concentration of 20 g/L. On the other hand, the best taste score was recorded for the wines made with the addition of NO at 20 and 40 g/L. Those wines had improved taste harmony, decreased astringency, and increased richn-ess, complexity, duration, and intensity. All wine samples had similar levels of acidity and taste typicality. Increasing the concentration from 20 to 40 g/L of OE, OL and NP did not affect the gustatory attributes of the wine samples. Similar conclusions can be drawn for the smell profiles of the wines (Figure 3). NO had the best overall odor rating (7.2. range 6.5–7.2) when compared to the other samples, including the control. All wine samples, except NO (20 g/L), showed a significant increase in fresh fruit notes when compared to the control. A tropical fruit note was significantly pronounced for OW, OL, and NO, regardless of concentration, while OE (40 g/L) influenced the reduction in vegetable note perception. Higher concentrations of IYD (40 g/L) resulted in an enhancement of the dry fruit note in all samples, while the duration was prolonged in OPL (40 g/L) and NO (20 and 40 g/L). There was no significant difference between the samples and the control in terms of odor intensity, or typical floral, citrus or honey notes. Similarly, the addition of OW in sparkling Chardonnay wine increased in-the-mouth fruitiness and persistence, even at lower doses applied (10 g/L) [7]. The mannoproteins found in IYDs can explain the origin of the enhanced fruity aromas [5]. Another study related to sparkling wines showed that high scores for fruity aroma are achieved by the addition of autolyzed yeasts, while wines supplemented with yeast cell walls had high values of floral note, intensity and persistence [36]. The observed differences can be explained by the different duration of aging and the type of derivative added to the wine.

As for the dose of the derivative, increasing the concentration of IYDs had a significant effect only with OE for most attributes and with OW for odor overall. For other derivatives, the dose did not play a significant role in improving the olfactory perception of the wine, so it can be concluded that the lower applied concentration (20 g/L) was sufficient to obtain the desired effect. This is also confirmed by a previous study that examined the influence of different commercial IYDs on Pinot Gris, Traminer and Sauvignon. They concluded that dosage might be relevant in relation to grape variety and that each grape requires a preliminary test to optimize the IYDs concentration [4].

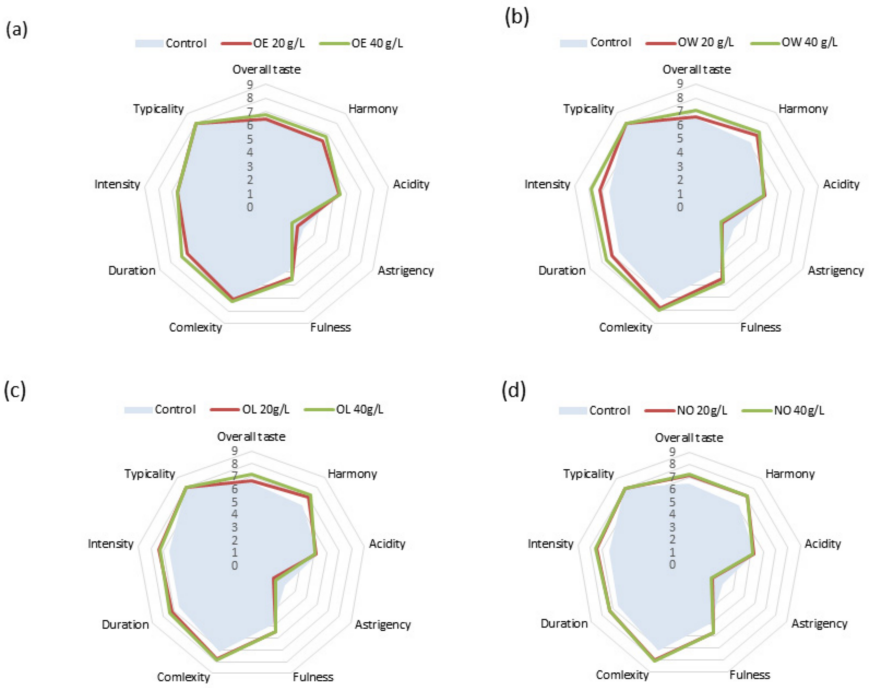

Figure 2. Sensory analysis of taste attributes of Grašac wines produced with the addition of different IYDs after 6 months of aging: (**a**) Oennolees™ (20 g/L and 40 g/L), (**b**) Optimum White™ (20 g/L and 40 g/L), (**c**) Opty Less™ (20 g/L and 40 g/L), (**d**) Noblesse™ (20 g/L and 40 g/L). The light-shaded area represents the control wine.

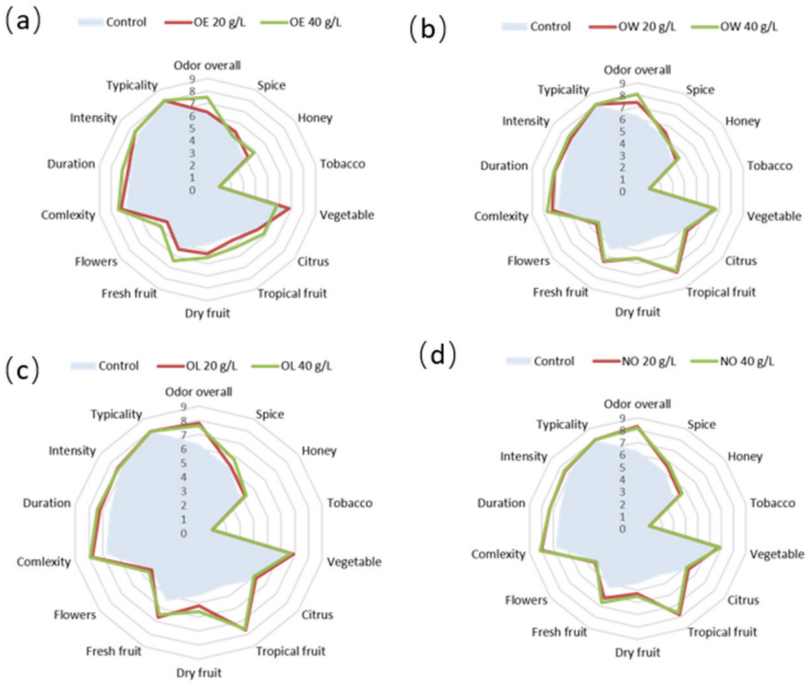

Figure 3. Sensory analysis of smell attributes of Grašac wines produced with the addition of IDYsafter 6 months of aging: (**a**) Oennolees™ (20 g/L and 40 g/L), (**b**) Optimum White™ (20 g/L and 40 g/L), (**c**) Opty Less™ (20 g/L and 40 g/L), (**d**) Noblesse™ (20 g/L and 40 g/L). The light-shaded area represents the control wine.

4. Conclusions

This study revealed that the application of *H. uvarum* S-2 in sequential fermentation with *S. cerevisiae* Q23 resulted in Grašac wine with a unique bouquet and a varietal character,

as it was noted for the control wine. Aging on IYDs helped to maintain the attributes of such wine. IYDs had a varying effect on wine, with each product having its specific purposes. Generally, the application of IYDs during the aging of Grašac white wine led to notable differences when compared to the control wines, in terms of antiradical activity, phenolic content, volatile composition and sensory attributes, which was noticeable after the 6-month aging period. The major volatile compounds were present within acceptable thresholds, but there were discernible variances among the wines produced, which were further corroborated by sensory assessments. Higher applied doses of IYDs had a more pronounced effect in increasing the amounts of certain volatiles. Aging on IYDs maintained the constant levels of antiradical activity and mitigated the decrease in -SH groups and polyphenol concentration. Additionally, IYD treatment improved the sensory profile of the samples described as fruity and floral with reduced astringency and increased their fullness and complexity. Hence, the production of unique and specific wines made with non-*Saccharomyces* yeast species with the addition of different IYDs that substitute the aging on the lees procedure should be considered as a good response to consumer and market requests and a step forward toward achieving excellence in winemaking.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9050494/s1, Table S1: Concentration of volatile compounds (mg/L) found in Grašac wines produced with different IYD treatments after 3- and 6-month ageing period—with standard deviations and statistical significances.

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