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Different application dosages of a specific inactivated dry yeast (SIDY): effect on the polysaccharides, phenolic and volatile contents and color of Sauvignon blanc wines

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Abstract

Aim: The aims of this study were to (i) study the effect of different application dosages of a commercial specific inactivated dry yeast (SIDY) on several compounds (polysaccharides, phenolic and volatile compounds) and attributes (color parameters) related to the quality of white wines, and (ii) acquire better knowledge about the use of different dosages of SIDY in white wines with the objective to improve their quality.

Methods and results: Three different dosages were applied (10, 20 and 40 g hL^{-1}). Treated wines were followed after a contact time period of two months and after a bottle aging period of three months. Total phenolic content, color intensity, CIELab coordinates, polysaccharides, low molecular weight phenolic compounds and volatile compounds were evaluated.

Conclusions: Higher dosages of this SIDY resulted in a greater release of polysaccharides into the wine. In parallel, a positive effect on the reduction or prevention of wine oxidation was observed due to the interaction with certain phenolic compounds. The application of the highest dosage seems to lead to an adsorption or retention effect of the major identified volatile compounds. This effect seems to be more evident after the contact time period than after the bottle storage period.

Significance and impact of the study: This study can contribute to improve our knowledge on how applying different dosages of SIDY affects the physical and chemical quality of white wines.

Keywords: inactivated dry yeast, cell wall polysaccharides, phenolic compounds, volatile components, color

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Introduction

Recently, biotechnological companies have begun to offer winemakers the new winemaking tool of yeast-derived preparations. These preparations are obtained from *Saccharomyces cerevisiae* yeast strains and, as they can provide comparable positive effects without any potential drawbacks (Del Barrio-Galán *et al.*, 2011), they are supplied as an alternative to the on-lees wine aging technique. Due to the release of certain compounds from yeasts during autolysis, aging on lees is widely used to improve the sensory and technological quality of wines. However, it is a slow and complex process requiring several months – even years – to be completed and it may involve some enological issues such as microbiological and organoleptic alterations (Andújar-Ortiz *et al.*, 2013). Aging on lees also increases wine production costs, yet yeast-derived preparations can avoid or minimize the above problems because they can release major compounds of enological interest in a shorter period of time than traditional on-lees aging. Yeast-derived preparations used to shorten the aging time of wines are commonly classified as inactivated dry yeast, yeast autolysates, yeast cell walls, yeast protein extracts (Pozo-Bayón *et al.*, 2009), and purified mannoproteins, the most widely-used being specific inactivated dry yeasts (SIDY). In the SIDY production process, the complete yeast biomass is inactivated by a specific enzymatic or thermal process to stop its metabolism and enzymatic activities with the aim of obtaining a similar product to that obtained during natural autolysis, but more rapidly and in a more controlled process (Andújar-Ortiz *et al.*, 2013). Generally, these yeast-derived preparations are selected for their particular characteristics, such as their high polysaccharide content, mainly mannoproteins, which, due to their major influence on technological aspects and wine sensory characteristics (Doco *et al.*, 2003), appear to be those of main enological interest. These compounds can interact with some phenolic wine compounds acting as protective colloids, preventing or limiting the oxidation of white wines (Lopez-Toledano *et al.*, 2006; Del Barrio-Galán *et al.*, 2016), thereby decreasing astringency and bitterness while enhancing mouthfeel (Del Barrio-Galán *et al.*, 2012; González-Royo *et al.*, 2017). Polysaccharides and mannoproteins can modify the volatility of some volatile compounds in the wine (Bautista *et al.*, 2007; Chalier *et al.*, 2007; Juega *et al.*, 2015). In addition, some precursors present in the SIDY, such as amino acids and fatty acids, can be released into the wines, improving their flavor (Izzo and Ho, 1991; Mahadevan and Farmer, 2006). These compounds can also improve the protein and tartaric stability of white wines (Moine-Ledoux and Dubourdieu, 2002; Lomolino and Curioni, 2007) as well as the characteristics of sparkling wines (Núñez *et al.*, 2006). They can also remove some mycotoxins such as ochratoxin A (Caridi *et al.*, 2006).

Mannoproteins are synthesized and glycosylated in the yeast cytoplasm, particularly in the endoplasmic reticulum (Farkaš *et al.*, 1976). They are then transported through a secretory route to the extracellular region where they are assembled onto the yeast cell wall (Klis, 1994) and act as a selective and protective filter to prevent chemical and β-glucanase enzymatic attacks (Cid *et al.*, 1995). When yeast cells are inactivated and then added to the wine, they can release these polysaccharides and other intracellular components (Charpentier and Freyssinet, 1989).

In general, there is a relatively wide range of recommended dosages (5-40 g hL^{-1}) for applying yeast-derived preparations. It depends on the purity and solubility of the product, the type of wine, and the timing and purpose of the application. In addition, almost all of the research studies performed with these products have been undertaken using a medium-high dose (30-40 g hL^{-1}) (Del Barrio-Galán *et al.*, 2011, 2012, 2016). In these studies, it has been shown that the use of different yeast-derived preparations increased the content of polysaccharides and improved the technological and sensory characteristics of the wines with respect to a control wine. However, they have not studied the application of different dosages to improve the wine aging process. For these reasons, the objective of this work was to study the application of different SIDY dosages (low, medium and high) on a Chilean Sauvignon Blanc white wine in order to evaluate the impact on its polysaccharides, phenolic compounds, volatile compounds and color.

Material and methods

1. Winemaking and treatments

The study was performed with Sauvignon Blanc grapes supplied by the Popeta winery, located in the Maipo Valley (34° 27' 3.35» S and 70° 46' 42.17» W). The alcoholic fermentation was carried out in a 300-hL stainless steel tank. The grape juice was inoculated with 20 g hL^{-1} of Lalvin QA23® *Saccharomyces cerevisiae* yeast strain (Lallemand-Chile). The initial parameters of the fermented wine were pH 3.62; total acidity 2.94 (g L^{-1} of sulphuric acid); volatile acidity 0.26 (g L^{-1} of acetic acid); and alcoholic degree 13.1 (% vol.). Free SO_2 was

adjusted to 35 mg L^{-1} and then 15 L of the wine were transported in 2.5-L food-grade plastic tanks to the Department of Agro-industry and Enology (Faculty of Agronomical Sciences) of the University of Chile to apply the different treatments. The treatments lasted two months and were performed in duplicate: SIDY10 (wines with 10 g hL^{-1} of SIDY); SIDY20 (wines with 20 g hL^{-1} of SIDY); SIDY40 (wines with 40 g hL^{-1} of SIDY). Treatments were performed in a refrigerated room at a temperature of 4-6 °C. The addition of the different dosages of SIDY was as follows: the amount to be added to each treatment was weighed and resuspended in 10 times its weight in white wine. When the mixture was homogeneous, it was added to the final volume of white wine used in each treatment (2.5 L).

For a quick and optimized impact, all of the wines were stirred once a week during the first month and once every two weeks during the second. Two months after the treatment period (2MT), the wines were filtered (not clarified) through a cellulose plate filter, bottled and stored underground in a cellar for three months (3BS) at controlled temperature (14-15 °C).

SIDY, named PURE-LEES LONGEVITY™, was supplied by Lallemand-Chile. It is a specific inactivated dry yeast exhibiting a high dissolved oxygen consumption capacity, developed as a natural tool to help keep the wine in optimal conditions during storage in the cellar and aging (Sieczkowski *et al.*, 2016).

2. Reagents and materials

The standards of gallic, protocatechuic, caffeic, syringic, *p*-coumaric, ferulic, ellagic and caftaric acids, tyrosol, thyptophol, quercetin, myricetin, astilbin, (+)-catechin and (−)-epicatechin, dextrans and pectins were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Polyethylene membranes of 0.45 µm and 0.22 µm pore size were acquired from EMD Millipore (Billerica, MA, USA). Sodium sulphate (anhydrous), potassium metabisulfite, vanillin (99%), ethyl acetate, diethyl ether, sodium hydroxide, acetic acid, formic acid, sulphuric acid, ethanol, hydrochloric acid and high-performance liquid chromatography (HPLC)-grade acetonitrile, methanol and ammonium formate were purchased from Merck (Darmstadt, Germany). All the reagents were of analytical grade or higher.

The internal standard used for volatile compound determinations, 4-methyl-2-pentanol, was purchased from Merck (Darmstadt, Germany). Sodium chloride was obtained also from Merck.

The polydimethylsiloxane (PDMS) polymeric phase used for the extraction of volatile compounds was supplied from Gerstel (Mülheim and der Ruhr, Germany). The length of the PDMS Twisters was 10 mm and volume (thickness) was 24 mL (0.5 mm).

3. Analytical methods

Titratable acidity (TA), volatile acidity (VA), pH (Mettler-Toledo Seven Compact pH/ion S220, Columbus, OH), SO_2F (free sulphur dioxide), SO_2T (total sulphur dioxide) and alcoholic strength (% vol.) were evaluated following the OIV official methods (OIV, 2015). Total phenolic content (TP) was quantified by using gallic acid as external standard (mg L^{-1}) (Ribéreau-Gayon *et al.*, 2006). Color intensity (CI) was analyzed according to Glories (1984); L*, a* and b* CIELab coordinates were evaluated using the MSCV® method (simplified method to determine the color of wines) developed by the color group laboratory of the University of La Rioja (Spain) (Ayala *et al.*, 2014). The total color difference (ΔEab*) between all samples was obtained using the expression $\Delta Eab^* = [(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2]^{1/2}$. These measurements were performed using a UV/Vis 1700 Pharmaspec spectrophotometer (Shimadzu, Kyoto, Japan).

The polysaccharides were extracted using the methodology described by Ayestarán *et al.* (2004) and analyzed by HRSEC-RID. HRSEC-RID was performed using an Agilent 1260 Infinity Series liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a G1362A refractive index detector (RID), a G1311B quaternary pump, a G1316A column oven equipped with two Shodex columns, an OHpak SB-803 HQ and a SB-804 HQ (Showa Denko, Tokyo, Japan) connected in series (300 mm × 8 mm i.d.), and a G1329A autosampler. Nine analytical standards of dextrans from *Leuconostoc mesenteroides* were used for column calibration. One pectin, esterified potassium salt, from citrus fruit was used as external standard for quantification.

Low molecular weight phenolic compounds (LMWPC) were extracted, concentrated and analyzed using the methodology described in Peña-Neira *et al.* (2007). The samples were injected in an HPLC 1100 Series system (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1315B photodiode array detector (DAD), a G1311A quaternary pump, a G1379A degasser, and a G1329A autosampler. A reverse-phase Nova-Pak C18 column (4 µm, 3.9 mm i.d. × 300 mm; Waters, Milford, MA, USA) was used for HPLC–DAD analysis of

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LMWPC at 20 °C. Each major peak in the HPLC chromatograms of the extracts was characterized by retention time, the absorption and the spectrum form (from 210 to 360 nm). The calibration curves at 280 nm were produced by injecting the standard solutions before an extraction under the same conditions as the samples analyzed over the range of concentrations observed.

For volatile compound determination, the headspace sorptive extraction (HSSE) method described in Callejón *et al.* (2008) was used with slight modifications. In all cases, 7.5 mL of the sample were placed in a 20-mL vial and NaCl until saturation plus 10 µL of the internal standard were added. The headspace extraction was performed by placing a new twister in an open glass insert inside the vial and heating the sample in a water bath at 35 °C for one hour. The vial was tightly capped and, after extraction, the stir bar was removed with tweezers, rinsed with Milli-Q water, and dried with a lint-free tissue paper. Then, it was thermally desorbed in a gas chromatograph/mass spectrometer (GC/MS).

Gas chromatography analysis was carried out using a 7890B Agilent GC system coupled to a quadrupole mass spectrometer Agilent 5977 inert (Agilent Technologies, Palo Alto, CA, USA) equipped with a thermo desorption system and a cryo-focusing CIS-4 PTV injector (Gerstel). The thermal desorption was done in splitless mode with a flow rate of 70 mL min^{-1}. For the desorption, the temperature was held at 35 °C for 0.1 min, ramped at 60 °C min^{-1} to 210 °C, and then held for 5 min. The temperature of the CIS injector was held at -35 °C using liquid nitrogen for the entire desorption time and was then raised at 10 °C s^{-1} to 260 °C and held for 4 min. The solvent vent mode was used to transfer the sample to the analytical column.

A DB Wax capillary column with dimensions 60 m × 0.25 mm and 0.25 µm film thickness (J&W Scientific, Folsom, CA, USA) was used, and the carrier gas was helium at a flow rate of 1 mL min^{-1}. In the oven, the temperature was held at 35 °C for 1 min and then raised to 130 °C at 18 °C min^{-1} (held for 1 min). Then, the temperature was raised to 190 °C at 1 °C min^{-1} and subsequently to 220 °C. Electron ionization mass spectra in the full-scan mode were recorded at 70 eV with a scan range from m/z 35 to 300 amu.

Compound identification was based on mass spectra matching using the standard NIST library and the retention index (LRI) of authentic reference standards. The relative area was calculated by dividing the peak area of the target ion of each compound by the peak area of the target ion of the internal standard.

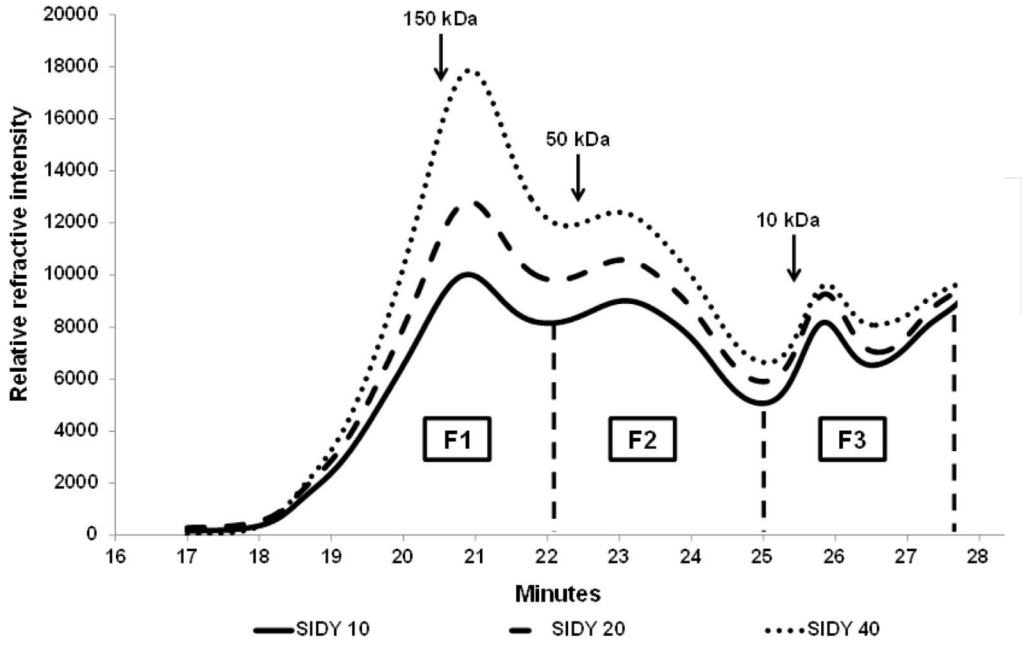

Figure 1. Chromatographic profile of polysaccharides of the different wines after the 2MT period. F1 : polysaccharides with an average molecular weight of 150 kDa ; F2 : polysaccharides with an average molecular weight of 48 kDa ; F3 : polysaccharides with an average molecular weight ≥ 10 kDa. SIDY10, SIDY20 and SIDY40 : wines treated with 10, 20 and 40 g hL^{-1} of SIDY, respectively.

4. Statistical analysis

The analysis of variance (ANOVA) and a Least Significant Difference (LSD-Fisher) post hoc test for multiple comparisons ($p < 0.05$) of the data was carried out with InfoStat v. 2012 software (FCA-Universidad Nacional de Córdoba, Argentina). Principal component analysis (PCA) was conducted using Statgraphics Centurion v. 15.2 (StatPoint Technologies Inc., Warrenton, VA) and Excel 2007 v. 12.0 (Microsoft Corp., Redmond, WA).

Results and discussion

Figure 1 shows the chromatographic profiles obtained for the different wines analyzed after the 2MT period. Three different polysaccharidic fractions (F1, F2 and F3) were obtained and classified according to their molecular weight. F1 had an average molecular weight (MW) of 150 kDa, F2 had an average MW of 48 kDa, and F3 had the lowest average molecular weight with MW ≥ 10 kDa.

Table 1 shows the concentrations of the different polysaccharidic fractions and the total content (sum of F1, F2 and F3 fractions) in the different treated wines. The polysaccharidic content of the wine after alcoholic fermentation was as follows: F1 73.4 mg L^{-1}; F2 153 mg L^{-1}; F3 43.3 mg L^{-1}; and total polysaccharide content 269.2 mg L^{-1}. As expected, after 2MT the polysaccharide content increased as the application dosage increased, and statistically significant differences were found in the different polysaccharidic fractions among the different dosages. Compared to the SIDY10-treated wine, the total polysaccharidic content increased in the SIDY20- and SIDY40-treated wines by 15.6 % and 42.5 %, respectively. Statistically significant differences were found in the F1 fraction between the three treatments applied, thus, the application of a higher dosage of SIDY resulted in a higher concentration of this fraction. Compared to SIDY10, the SIDY20- and SIDY40-treated wines showed an increased F1 fraction content of 26 mg L^{-1} and

Table 1. Total polysaccharide content in different fractions (mg L^{-1} ± SD of two biological replicates) and color parameters of the different treated wines.

Polysaccharides	SIDY10	SIDY20	SIDY40
2MT			
F1	110 ± 4.89a	136 ± 9.08b	193 ± 9.86c
F2	105 ± 3.93a	117 ± 9.31ab	133 ± 5.75b
F3	93.0 ± 3.76a	103 ± 5.39ab	113 ± 4.61b
Total	308 ± 16.6a	356 ± 23.79b	439 ± 20.22c
3BS			
F1	67.0 ± 2.90a	94.1 ± 0.074b	122 ± 1.88c
F2	110 ± 2.21a	123 ± 3.98b	126 ± 3.07b
F3	79.5 ± 0.485a	105 ± 1.54b	114 ± 2.77c
Total	257 ± 4.63a	322 ± 2.51b	362 ± 1.59c
Color parameters	**SIDY10**	**SIDY20**	**SIDY40**
2MT			
CI	0.064 ± 0.000	0.063 ± 0.001	0.062 ± 0.002
L*	98.3 ± 0.000a	98.5 ± 0.071b	98.5 ± 0.000b
a*	0.310 ± 0.085b	0.120 ± 0.099a	0.090 ± 0.057a
b*	3.22 ± 0.099	3.28 ± 0.255	3.27 ± 0.071
ΔE ab*	-	0.249	0.301
3BS			
CI	0.056 ± 0.001b	0.054 ± 0.003ab	0.048 ± 0.002a
L*	99.2 ± 0.020	99.3 ± 0.062	99.3 ± 0.018
a*	-0.212 ± 0.012c	-0.226 ± 0.025b	-0.288 ± 0.009a
b*	3.24 ± 0.018b	3.18 ± 0.090b	2.82 ± 0.167a
ΔE ab*	-	0.067	0.439

Different letters in a row indicate statistically significant differences ($p < 0.05$).
2MT: after two months of treatment; 3BS: after three months of bottle storage.
SIDY10, SIDY20 and SIDY40: wines treated with 10, 20 and 40 g hL^{-1} of SIDY, respectively.
CI: color intensity.

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83 mg L^{-1}, respectively. However, the only significant differences found in F2 and F3 fractions were between SIDY10- and SIDY40-treated wines. SIDY40-treated wines showed an increase in F2 and F3 fractions content of 28 mg L^{-1} and 20 mg L^{-1}, respectively, compared to the SIDY10-treated wines. As demonstrated in other studies, this increase in polysaccharide concentration is mainly due to the release of mannoproteins from the yeast cell walls during autolysis (Charpentier *et al.*, 2004; Del Barrio-Galán *et al.*, 2011) mainly caused by wine acids which can break the yeast cells. The total polysaccharide content decreased after the 3BS analysis. This phenomenon was probably due to the filtration treatment after 2MT that was undertaken to stabilize the wines. This filtration mainly affected the higher molecular weight polysaccharides (F1) because the F2 and F3 fraction contents remained stable during bottle aging. In general, after the 3BS period the differences among the treated wines remained unaltered. Indeed, the SIDY40-treated wines had higher F1 and F3 concentrations. Finally, the SIDY20- and SIDY40-treated wines presented similar F2 fraction contents, which were significantly higher than those of the SIDY10-treated wines.

Table 2 shows the wine TP and LMWPC contents. No significant differences were found in TP after the 2MT period. After the bottle aging period the SIDY20- and SIDY40-treated wines presented higher TP contents than SIDY10 wines. These results are contrary to those obtained by González-Royo *et al.* (2017) in synthetic and red wines where a reduction in TP was observed when three different SIDY were added. This difference in behavior may be explained by the fact that wine matrixes are different (red vs. white wine) and that different yeast-derived preparations were studied. However, these results are in agreement with those observed by Del Barrio-Galán *et al.* (2012) in white wines using several yeast derivative products. They explained that a higher dosage of these products could prevent the oxidation and/or precipitation of phenolic compounds. But this fact could be due to the higher oxygen consumption carried out by this specific inactivated yeast, as proved by Sieczkowski *et al.* (2016).

In terms of LMWPC, the compounds with the highest content after 2MT were, in descending order, tyrosol, catechin, protocatechic acid, epicatechin and gallic acid. The different LMWPC compounds identified and quantified were grouped in hydroxybenzoic acids, hydroxycinnamic acids, hydroxycinnamic acid derivatives, flavanols, stilbenes, phenolic alcohols and flavonols to gain a better understanding of the data obtained. After the 2MT period, only some statistically significant differences were found in hydroxycinnamic acids, hydroxycinnamic acid derivatives and stilbene contents. Therefore, the SIDY40-treated wines presented a lower content of these phenolic groups than the other treatments. As mentioned in several studies, yeast cell polysaccharides can interact with the LMWPC to form polymeric structures which can either remain soluble or precipitate, thus reducing phenolic compound concentration (Razmkhab *et al.*, 2002; Lopez-Toledano *et al.*, 2006; Del Barrio-Galán *et al.*, 2011, 2016). Some authors have recently shown evidence regarding the interaction (sorption) of polyphenols with yeast, inactivated yeast and yeast cell walls (Mekoue Nguela *et al.*, 2015a, 2015b). After the 3BS period, only SIDY40-treated wines presented a lower hydroxycinnamic acid derivative content. These compounds have been described as easily-oxidizable compounds (Razmkhab *et al.*, 2002; Del Barrio-Galán *et al.*, 2010). A reduction in these compounds can therefore imply a limitation of wine oxidation risk. On the contrary, the SIDY20- and SIDY40-treated wines showed higher contents of hydroxycinnamic acids and flavanols. In the case of flavanols, it was observed that the higher the dosage, the lower the decrease in these compounds. Some authors explain that the concentrations of certain phenolic compounds depend on the balance between the oxidation and polymerization reactions that will induce a decrease in their concentration, as well as in the hydrolysis of higher oligomers that will, in turn, increase the presence of these flavanols in wines (Dallas *et al.*, 1995). No significant differences were found in the other phenolic groups.

In this study, some statistically significant differences were found in the color of the white wines as a function of the application dosage (**Table 1**). After the 2MT period, no differences were found in color intensity (CI) between the different treated wines, but some differences were found in the CIELab parameters. Thus, SIDY20 and SIDY40 had lower a* values than SIDY10, but no differences were found between SIDY20 and SIDY40. As mentioned in the literature (Guzmán-Alfeo, 2010), this a* parameter is the coordinate that measures the color between red and green tones, whose usual values are lower than 1. Thus, the results obtained could indicate that SIDY20 and SIDY40 had more green notes than SIDY10, pointing out that these last wines could be getting oxidized more quickly. These results are well correlated with the lower L* values found in SIDY10 compared to SIDY20 and SIDY40. As was discussed in Guzmán-Alfeo (2010), the L* parameter is the chromatic coordinate that explains the lightness of the wines or their ability to reflect the white color,

Table 2. Low molecular weight phenolic compounds quantified and total phenolic content (mg L^{-1} ± SD of two biological replicates) of the different treated wines.

Phenolic compound	2MT			3BS		
	SIDY10	SIDY20	SIDY40	SIDY10	SIDY20	SIDY40
Hydroxybenzoic acids						
Gallic acid	1.37 ± 0.050	1.36 ± 0.105	1.35 ± 0.018	1.31 ± 0.057	1.35 ± 0.007	1.30 ± 0.045
Protocatechuic acid	2.48 ± 0.034	2.52 ± 0.028	2.42 ± 0.028	2.31 ± 0.051	2.43 ± 0.047	2.42 ± 0.051
Methyl gallate	0.052 ± 0.014	0.062 ± 0.000	0.087 ± 0.025	n.d.	n.d.	n.d.
Vanillic acid	0.337 ± 0.010	0.320 ± 0.022	0.337 ± 0.015	0.355 ± 0.050	0.420 ± 0.043	0.426 ± 0.043
Ethyl gallate	n.d.	n.d.	n.d.	0.197 ± 0.061	0.179 ± 0.021	0.176 ± 0.017
Total	**4.24 ± 0.016**	**4.26 ± 0.155**	**4.20 ± 0.020**	**4.18 ± 0.100**	**4.38 ± 0.110**	**4.32 ± 0.144**
Hydroxycinnamic acids						
t-caffeic acid	0.787 ± 0.018b	0.792 ± 0.011b	0.728 ± 0.012a	0.753 ± 0.004a	0.885 ± 0.017b	0.864 ± 0.031b
t-*p*-coumaric acid	1.06 ± 0.059	1.06 ± 0.080	1.05 ± 0.046	1.35 ± 0.032a	1.59 ± 0.019b	1.63 ± 0.059b
t-ferulic acid	0.568 ± 0.039	0.573 ± 0.051	0.551 ± 0.032	0.432 ± 0.003a	0.486 ± 0.011b	0.479 ± 0.013b
Total	**2.41 ± 0.002b**	**2.43 ± 0.018b**	**2.33 ± 0.025a**	**2.53 ± 0.033a**	**2.96 ± 0.047b**	**2.97 ± 0.103b**
Hydroxycinnamic acid derivatives						
t-caftaric acid	0.069 ± 0.022	0.055 ± 0.021	0.060 ± 0.009	0.138 ± 0.012b	0.123 ± 0.007ab	0.104 ± 0.002a
c-coutaric acid	1.26 ± 0.096b	1.22 ± 0.028b	1.08 ± 0.014a	1.03 ± 0.065	1.09 ± 0.056	0.97 ± 0.068
t-coutaric acid	0.602 ± 0.040	0.525 ± 0.065	0.599 ± 0.058	0.758 ± 0.040	0.646 ± 0.048	0.643 ± 0.042
t-fertaric acid	0.200 ± 0.008	0.201 ± 0.010	0.209 ± 0.012	0.256 ± 0.021b	0.214 ± 0.077ab	0.152 ± 0.008a
Total	**2.13 ± 0.037b**	**2.00 ± 0.061ab**	**1.95 ± 0.029a**	**2.18 ± 0.138b**	**2.07 ± 0.079ab**	**1.87 ± 0.120a**
Flavanols						
Catechin	2.98 ± 0.015	2.86 ± 0.058	2.84 ± 0.150	2.01 ± 0.042a	2.48 ± 0.086b	2.47 ± 0.149b
Epicatechin	2.34 ± 0.228	2.46 ± 0.012	2.54 ± 0.064	1.03 ± 0.041a	1.34 ± 0.062b	1.43 ± 0.096b
Procyanidin B2	1.08 ± 0.011	1.13 ± 0.016	1.11 ± 0.021	0.428 ± 0.005	0.436 ± 0.022	0.431 ± 0.004
Total	**6.40 ± 0.213**	**6.45 ± 0.046**	**6.48 ± 0.214**	**3.47 ± 0.083a**	**4.25 ± 0.023b**	**4.32 ± 0.246b**
Stilbenes						
c-resveratrol-3-glucosi	0.215 ± 0.004	0.207 ± 0.003	0.210 ± 0.001	0.186 ± 0.001	0.190 ± 0.001	0.184 ± 0.002
t-resveratrol	0.164 ± 0.005	0.159 ± 0.000	0.168 ± 0.002	n.d.	n.d.	n.d.
c-resveratrol	0.164 ± 0.003b	0.161 ± 0.004b	0.119 ± 0.000a	0.155 ± 0.000	0.159 ± 0.002	0.158 ± 0.000
Total	**0.543 ± 0.006c**	**0.527 ± 0.001b**	**0.497 ± 0.003a**	**0.341 ± 0.001**	**0.349 ± 0.001**	**0.342 ± 0.003**
Phenolic alcohols						
Tyrosol	5.53 ± 0.527	5.16 ± 0.065	5.11 ± 0.043	8.83 ± 0.657	8.82 ± 0.321	9.13 ± 0.491
Tryptophol	0.323 ± 0.050	0.352 ± 0.053	0.302 ± 0.015	0.348 ± 0.020a	0.416 ± 0.021b	0.418 ± 0.024b
Total	**5.86 ± 0.577**	**5.51 ± 0.124**	**5.42 ± 0.058**	**9.18 ± 0.677**	**9.24 ± 0.342**	**9.55 ± 0.466**
Flavonols						
Astilbin derivative 1	0.751 ± 0.023	0.743 ± 0.021	0.748 ± 0.072	n.d.	n.d.	n.d.
Astilbin derivative 2	0.387 ± 0.001	0.389 ± 0.001	0.436 ± 0.076	0.566 ± 0.023	0.615 ± 0.012	0.610 ± 0.009
Astilbin derivative 3	0.470 ± 0.003	0.462 ± 0.021	0.449 ± 0.001	0.510 ± 0.007	0.529 ± 0.032	0.499 ± 0.005
Total	**1.61 ± 0.021**	**1.59 ± 0.000**	**1.63 ± 0.149**	**1.08 ± 0.030**	**1.14 ± 0.044**	**1.11 ± 0.015**
Total phenolic conten	**238 ± 1.27**	**231 ± 1.70**	**236 ± 1.57**	**213 ± 2.41a**	**236 ± 3.54b**	**235 ± 4.15b**

Different letters in a row indicate statistically significant differences ($p < 0.05$).
2MT : after two months of treatment ; 3BS : after three months of bottle storage.
SIDY10, SIDY20 and SIDY40 : wines treated with 10, 20 and 40 g hL^{-1} of SIDY, respectively.
n.d. : not determined.

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pointing out that highest values of L* are well correlated with the clarity of the wines. Thus, higher values of this parameter could indicate that wines had lower oxidative or browning notes and these results indicate that applying SIDY at 20 and 40 g hL^{-1} doses had the same effect. However, as was mentioned by Guzmán-Alfeo (2010), most white wines have L* values close to 100 and for this reason, since all the wines analyzed in this study have values close to 100 it is difficult to draw clear conclusions regarding this L* parameter. No differences were found in the b* coordinate at this period.

On the other hand, after the 3BS period SIDY40-treated wines presented lower a* and b* value parameters than the other treatments, indicating that, in this case, the highest applied dosage of SIDY had a better effect than the other dosages for limiting or preventing wine oxidation during bottle storage. Thus, these treated wines presented better results in relation to color preservation, indicating that this specific inactivated yeast could be used as a natural tool to prevent or minimize wine oxidation during aging storage processes. The color results obtained for the SIDY40-treated wines were well-correlated with the lower values observed for some easily-oxidizable LMWPC, such as hydroxycinnamic acid derivatives. However, the content of other phenolic compounds that are also easily oxidizable, namely epicatechin, was higher in the SIDY40-treated wines. As supported by Cheynier and Ricardo da Silva (1991), flavanols are involved in chemical and enzymatic oxidative browning reactions. For these reasons, it is possible that dissolved oxygen comsumption prevented the oxidation of wine as described in the material and methods section.

Total color differences (ΔEab*) were also calculated (**Table 1**) in order to determine whether the differences obtained in wine color using different dosages of SIDY were great enough to be distinguished by the human eye; it is worthy of note that only $\Delta Eab^* \geq 3$ are perceptible to the human eye (Martínez *et al.*, 2001). In this study, no differences perceptible to the human eye were observed between the wines treated with the different SIDY dosages after the 2MT and 3BS periods, despite the differences in the spectrophotometric data.

Table 3 shows the volatile compounds identified in this study and the relative area of each compound. The compounds identified were grouped into esters (18), alcohols (7), volatile fatty acids (4), aldehydes (3) and ketones (2), their content depending on SIDY dosage and analysis timing. The ester group was further divided into subgroups, ethyl esters being the most abundant, followed by isoamyl, acetate and methyl esters. The ethyl octanoate was the most important ester compound (between 71.8% and 62.2% of total ester content), giving a sweet, fruity, green-apple aroma. Ethyl decanoate, isoamyl acetate, 2-phenylethyl acetate, and ethyl hexanoate also reached important levels in the wines. Other important compounds detected in the wines were 3-methyl-1-butanol, 1-hexanol, 2-phenylethanol, hexanoic acid and octanoic acid, all of which are common volatile compounds in wines. After the 2MT period the SIDY40-treated wines had a lower content of total esters, alcohols and fatty acids than the SIDY10- and SIDY20-treated wines. This was probably due the polysaccharides having an adsorption or binding effect on these volatile compounds (Comuzzo *et al.*, 2006; Bautista *et al.*, 2007). However, when the SIDY dosage is lower, this adsorption effect appears to be more intense in the case of several esters, alcohols and acids after the 3BS period. In the case of the ester group, this effect was mainly observed in ethyl, isoamyl and acetate ester subgroups and it was mainly due to the lower contents of ethyl octanoate, ethyl decanoate, ethyl hexanoate, isoamyl acetate and 2-phenylethyl acetate found. In the case of the alcohol group, we observed a similar trend to that of the esters. In general, lower SIDY dosages gave rise to wines with higher quantities of alcohols. Thus, SIDY20 and SIDY40 presented a lower content of most alcohols than SIDY10, mainly due to the lower 3-methyl-1-butanol and 1-hexanol content found. However, SIDY20 wines had a significantly higher 2-phenylethanol content than SIDY10 and SIDY40 wines. In the case of fatty acids, the SIDY20 wines presented the lowest content of isovaleric and isobutyric acids and the highest octanoic acid content. SIDY40 wines presented the lowest octanoic acid content. Some adsorption of short- and medium-chain fatty acids to SIDY was first described by Lafon-Lafourcade *et al.* (1984). This may have a positive effect on the sensory quality of wines because some of these compounds are responsible for certain unpleasant odors in wine (Comuzzo *et al.*, 2006). Finally, the same tendency was observed in the aldehyde and ketone group. Therefore, the SIDY20- and SIDY40-treated wines presented a lower content of total aldehydes and ketones due to the observed lower content in nonanal, furfural, acetol and acetophenone, respectively. In general, certain aldehydes and ketones are considered to be markers of wine aging because some of them are formed by the oxidation of other volatile compounds present in wine (Escudero *et al.*, 2002). A lower content and/or volatility of

Table 3. Volatile compound content (expressed in relative area) of the different treated wines.

Volatile compound	2MT			3BS			LRI	ID
	SIDY10	SIDY20	SIDY40	SIDY10	SIDY20	SIDY40		
Esters								
Ethyl esters								
Ethyl heptanoate	0.560 ± 0.093	0.501 ± 0.069	0.629 ± 0.074	0.214 ± 0.013a	0.216 ± 0.016a	0.350 ± 0.032b	C	1355
Ethyl-2-hexenoate	0.230 ± 0.006	0.214 ± 0.007	0.218 ± 0.004	0.142 ± 0.004	0.154 ± 0.018	0.150 ± 0.018	B	1375
Ethyl octanoate	358 ± 49.9b	350 ± 36.2ab	341 ± 23.3a	209 ± 0.067	208 ± 3.77	228 ± 14.4	A	1449
Ethyl decanoate	65.7 ± 7.22b	81.0 ± 6.12b	32.6 ± 4.53a	44.8 ± 4.76b	42.3 ± 0.709ab	35.3 ± 1.32a	B	1649
Ethyl dodecanoate	2.33 ± 0.399b	3.89 ± 0.174c	0.519 ± 0.060a	1.40 ± 0.022	1.67 ± 0.133	1.49 ± 0.178	B	1863
Ethyl hexanoate	12.5 ± 1.71b	11.9 ± 0.564ab	11.1 ± 0.241a	6.08 ± 0.663	7.12 ± 1.00	6.24 ± 0.749	A	1256
Ethyl *trans*-4-decenoate	0.082 ± 0.008b	0.091 ± 0.004b	0.058 ± 0.009a	0.066 ± 0.008	0.062 ± 0.004	0.051 ± 0.006	C	1693
Total	**440 ± 58.5b**	**447** ± 42.8**b**	**386 ± 27.7a**	**262 ± 4.04**	**259 ± 3.96**	**272 ± 16.7**		
Methyl esters								
Methyl octanoate	1.03 ± 0.158	1.01 ± 0.064	1.03 ± 0.070	0.587 ± 0.006	0.594 ± 0.045	0.637 ± 0.094	A	1392
Isoamyl esters								
Isoamyl acetate	54.5 ± 9.96b	40.4 ± 4.83ab	34.4 ± 1.95a	34.5 ± 10.8b	38.2 ± 3.05b	24.1 ± 1.75a	B	1134
Isopentyl hexanoate	1.24 ± 0.216a	1.83 ± 0.225ab	2.02 ± 0.187b	0.661 ± 0.020a	0.714 ± 0.043a	1.14 ± 0.149b	A	1517
Isoamyl octanoate	2.06 ± 0.07a	4.19 ± 0.009b	1.66 ± 0.235a	1.50 ± 0.076	1.42 ± 0.037	1.68 ± 0.207	B	1686
Isoamyl decanoate	0.373 ± 0.033b	0.731 ± 0.014c	0.068 ± 0.000a	0.277 ± 0.032b	0.235 ± 0.014ab	0.072 ± 0.928a	B	1894
Total	**58.2 ± 10.3b**	**47.2 ± 3.63ab**	**38.1 ± 2.38a**	**37.0 ± 11.0b**	**40.6 ± 3.05b**	**27.0 ± 2.20a**		
Acetate esters								
Nerol acetate	0.308 ± 0.008	0.364 ± 0.053	0.396 ± 0.058	0.204 ± 0.008b	0.160 ± 0.001a	0.154 ± 0.018a	A	1754
2-phenylethyl acetate	21.8 ± 3.76b	22.7 ± 3.69b	13.4 ± 0.838a	11.8 ± 1.80	12.1 ± 1.38	11.5 ± 0.831	A	1851
Hexyl acetate	13.3 ± 2.24	11.5 ± 1.75	11.4 ± 0.233	5.28 ± 0.504a	5.92 ± 0.649a	10.5 ± 0.522b	B	1304
Total	**35.4 ± 6.01b**	**34.6 ± 5.39b**	**25.2 ± 1.13a**	**17.3 ± 1.30a**	**18.2 ± 0.733a**	**22.2 ± 0.327b**		
Propyl octanoate	0.299 ± 0.051	0.329 ± 0.008	0.309 ± 0.037	0.137 ± 0.005	0.140 ± 0.003	0.167 ± 0.021	C	1539
Total esters	**540 ± 54.39b**	**528 ± 52.2b**	**461 ± 30.5a**	**323 ± 17.2**	**322 ± 0.204**	**330 ± 19.2**		
Alcohols								
2-Ethyl-1-hexanol	0.788 ± 0.001c	0.343 ± 0.040a	0.570 ± 0.052b	1.28 ± 0.208b	1.23 ± 0.190b	0.599 ± 0.016a	A	1569
Octanol	0.270 ± 0.032	0.385 ± 0.050	0.291 ± 0.031	0.289 ± 0.052	0.259 ± 0.031	0.192 ± 0.001	B	1606
2-furfuryl alcohol	1.62 ± 0.109b	0.990 ± 0.173a	1.06 ± 0.170a	1.03 ± 0.097a	0.871 ± 0.061a	1.33 ± 0.027b	C	1704
2-phenylethanol	9.88 ± 0.441a	17.1 ± 2.58b	8.19 ± 0.606a	9.56 ± 1.19a	11.6 ± 0.460b	11.2 ± 0.057b	A	1950
3-methyl-1-butanol	12.7 ± 0.046b	7.20 ± 0.828a	8.12 ± 0.533a	6.87 ± 0.472	7.19 ± 0.511	6.02 ± 0.128	A	1215
1-hexanol	12.7 ± 0.867b	7.48 ± 0.223a	9.33 ± 0.504a	8.37 ± 0.608b	8.86 ± 0.927b	5.43 ± 0.166a	A	1389
Alpha terpineol	0.159 ± 0.011b	0.079 ± 0.003a	0.097 ± 0.012a	0.246 ± 0.044b	0.154 ± 0.027a	0.164 ± 0.008a	A	1719
Total	**37.7 ± 0.000b**	**33.6 ± 4.31ab**	**27.7 ± 1.43a**	**27.6 ± 0.310ab**	**30.2 ± 0.11b**	**24.9 ± 0.234a**		
Fatty acids								
Isobutyric acid	0.355 ± 0.004c	0.185 ± 0.015a	0.297 ± 0.009b	0.229 ± 0.004b	0.232 ± 0.024b	0.151 ± 0.006a	B	1559
Isovaleric acid	0.618 ± 0.016c	0.394 ± 0.023a	0.489 ± 0.007b	0.480 ± 0.056b	0.446 ± 0.009b	0.335 ± 0.016a	B	1702
Hexanoic acid	4.84 ± 0.006	4.65 ± 0.584	5.62 ± 0.841	4.06 ± 0.570	4.28 ± 0.230	3.76 ± 0.029	A	1891
Octanoic acid	5.46 ± 0.256ab	7.58 ± 1.30b	3.24 ± 0.419a	4.97 ± 0.668	5.19 ± 0.078	4.73 ± 0.104	A	2100
Total	**11.3 ± 0.261b**	**12.8 ± 1.89b**	**9.6 ± 1.26a**	**9.7 ± 1.30**	**10.1 ± 0.292**	**9.0 ± 0.097**		
Aldehydes								
Nonanal	1.19 ± 0.184b	0.363 ± 0.052a	0.642 ± 0.059a	0.570 ± 0.031c	0.504 ± 0.007b	0.259 ± 0.004a	A	1416
Furfural	0.421 ± 0.054b	0.338 ± 0.011ab	0.304 ± 0.000a	0.292 ± 0.049	0.269 ± 0.041	0.334 ± 0.008	A	1492
Decanal	0.854 ± 0.148b	0.327 ± 0.045a	0.535 ± 0.087ab	0.426 ± 0.074b	0.412 ± 0.031b	0.193 ± 0.006a	B	1523
Total	**2.46 ± 0.386b**	**1.03 ± 0.108a**	**1.48 ± 0.146a**	**1.29 ± 0.092b**	**1.18 ± 0.078b**	**0.79 ± 0.019a**		
Ketones								
Acetol	1.87 ± 0.313b	1.17 ± 0.156ab	1.07 ± 0.158a	0.907 ± 0.041a	0.840 ± 0.002a	1.35 ± 0.032b	C	1380
Acetophenone	0.380 ± 0.027c	0.075 ± 0.000a	0.198 ± 0.014b	0.220 ± 0.033b	0.196 ± 0.013b	0.069 ± 0.004a	C	1775
Total	**2.25 ± 0.340b**	**1.25 ± 0.156a**	**1.27 ± 0.144a**	**1.13 ± 0.008a**	**1.04 ± 0.011a**	**1.42 ± 0.035b**		

Different letters in a row indicate statistically significant differences ($p < 0.05$).
2MT: after two months of treatment; 3BS: after three months of bottle storage.
SIDY10, SIDY20 and SIDY40: wines treated with 10, 20 and 40 g hL^{-1} of SIDY, respectively.
LRI: Linear Retention Index.

these compounds could be related to better-quality white wines.

ID: reliability of identification: A, mass spectrum and LRI agreed with standards; B, mass spectrum agreed with mass spectral data base and LRI agreed with the literature data (Tao *et al.*, 2008; Torrens *et al.*, 2010; Gómez García-Carpintero *et al.*, 2012); C, mass spectrum agreed with mass spectral data base.

According to the results obtained after the 2MT period, the wines treated with a higher SIDY dosage presented a lower content of most of the volatile compounds identified. This was probably due to a higher release of polysaccharides from the SIDY which could bind to the volatile compounds and reduce their content and/or volatility in the wines. This binding effect was previously described in white wines by other authors using different yeast-derived preparations and different dosages (Comuzzo *et al.*, 2006; Bautista *et al.*, 2007; Chalier *et al.*, 2007; Del Barrio-Galán *et al.*, 2010). Other authors explained that these products could have a "salting out effect", increasing the content of some volatile compounds related with other variables of wine, such as the type of macromolecules released from the yeast-derived preparations and their concentration, wine pH, alcoholic strength or other factors (Bautista *et al.*, 2007; Comuzzo *et al.*, 2011). This effect could have occurred to 2-phenylethanol in the case of SIDY20-treated wines, which presented a significantly higher content than the other treated wines.

In general, the differences between treatments were lower after the 3BS period. No significant differences were found in total esters, except in some subgroups. In the case of total isoamyl esters, the SIDY40-treated wines had a lower content compared to the other treated wines, mainly due to their effect on isoamyl acetate and isoamyl decanoate. An opposite effect was found in the total acetate ester content compared to 2MT because SIDY40-treated wines had a higher content than SIDY10- and SIDY20-treated wines, mainly due to the hexyl acetate content which remained stable in SIDY40 wines, while it decreased significantly in SIDY10 and SIDY20 wines. On the other hand, it was observed that SIDY20 and SIDY40 reduced the nerol acetate content, the latter having an important role in the floral, fruity, rose-like aroma perception (Cincotta *et al.*, 2015). In the case of alcohols, SIDY20 and SIDY40 wines had a higher 2-phenylethanol content and a lower 1-hexanol

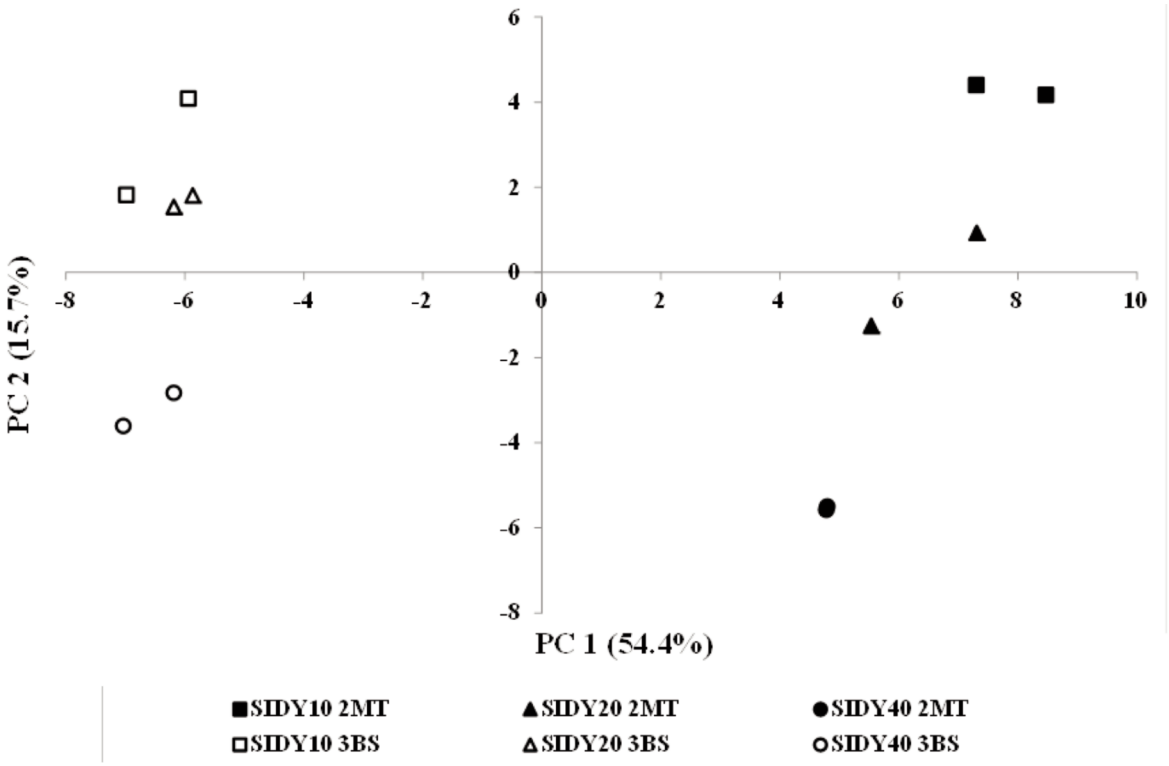

Figure 2. Distribution of the different wines defined by principal components 1 and 2.

content than SIDY10. No significant differences were found in the total acids content. However, SIDY40 wines had a significantly lower isobutyric and isovaleric acid content compared to the other treated wines. The SIDY40-treated wines had a lower nonanal and decanal content than the rest of the treated wines, reflecting a lower total aldehyde content. An opposite effect in total ketones was found after the 3BS period, observing that SIDY40 wines presented the highest content and SIDY10 the lowest, mainly due to the higher acetol content (t.i.) in SIDY40. This effect could be explained by the possible reversibility of the binding between the commercial products rich in polysaccharides and volatile compounds (Rodríguez-Bencomo *et al.*, 2010).

Principal component analysis (PCA) selected eight principal components (PC) with an eigenvalue greater than 1, explaining 98.4% of the total variance. **Figure 2** shows the distribution of the different wines on the plane defined by PC1 and PC2, which

Table 4. Principal component analysis and component weights of the most significant variables.

	Component 1	Component 2
Eigenvalue	46.2	13.4
Percentage of variance	54.4	15.7
Cumulative percentage	54.4	70.7
CI	**-0.994**	-0.016
Tryptophol	**0.991**	-0.115
Total hydroxycinnamic acid derivatives	**0.984**	-0.149
Vanillic acid	**0.984**	-0.149
Astilbin derivative 1	**-0.984**	0.111
Ethyl decanoate	**0.972**	-0.001
Total acetate esters	**0.970**	0.077
t-caffeic acid	**-0.969**	0.129
Total alcohols	**0.968**	-0.077
t-caftaric acid	**-0.966**	0.137
Ethyl heptanoate	**0.965**	-0.101
Total flavonols	**-0.965**	0.132
Ethyl hexanoate	**0.964**	0.025
Total flavanols	**0.963**	-0.171
Ethyl *trans*-4-decenoate	**0.957**	-0.157
Hexyl acetate	**0.949**	-0.219
Procyanidin B2	**0.945**	-0.297
Total hydroxycinnamic acids	**0.937**	-0.255
t-coutaric acid	**-0.933**	0.036
Epicatechin	**0.922**	-0.207
Ethyl gallate	**-0.905**	0.015
Total phenolic content	**0.900**	0.000
F2	0.365	**-0.877**
b*	-0.195	**-0.855**
Total hydroxybenzoic acids	0.049	**0.808**
a*	0.559	**-0.800**
F1	0.064	**-0.799**
Methyl octanoate	0.116	**-0.744**
Total ethyl esters	0.147	**-0.720**
c-resveratrol-3-glucoside	-0.194	**0.718**

Weight values higher than 0.900 were selected for component 1 and weight values higher than 0.700 were selected for component 2.
CI: color intensity. F1 and F2: polysaccharidic fraction 1 and 2, respectively.

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accounted for 70.1% of the cumulative variance and enabled significant separation of the samples.

Component 1 seems to explain the variance between the two different periods of analysis (2MT and 3BS), while component 2 seems to explain the variance between the different dosages of SIDY applied, mainly between SIDY10 and SIDY40. **Table 4** shows the component weights for the variables with an absolute value higher than 0.9 and 0.7 for PC1 and PC2, respectively, which contributes most significantly to the explanatory meaning of the components. As can be seen, most of these PC1-associated variables were CI and phenolic and volatile compounds, which allowed the differentiation of the wines between the two points of analysis (2MT and 3BS). In the case of PC2, the variables that contributed most significantly to the differentiation of wines by application dosages were the CIELab parameters and the F2 and F1 polysaccharide fractions.

Conclusions

In this study, an increased SIDY dosage led to an increased release of polysaccharides into the wine, principally those of high and medium molecular weight. This greater release of polysaccharides had a positive effect on the color of the wines because it prevented or reduced their degree of oxidation. This positive effect on color could have been due to the interaction of polysaccharides with certain phenolic compounds that are easily-oxidizable (hydroxycinnamic tartaric esters) and can cause browning of white wines' color. But other easily-oxidizable phenolic compounds (flavanols) did not seem to interact with polysaccharides. For this reason, the positive effect on color was mainly due to the oxygen consumption carried out by the highest dosage of SIDY.

A higher release of polysaccharides during the treatment period appears to be related to the adsorption of certain volatile compounds present in white wines, which could therefore modify their volatility, preventing their loss over time, as well as improving their persistence. However, this adsorption effect seems to be lower after bottle storage, with even the potential to be reversible, as suggested by the increase in the content of certain volatile compounds in wines with higher SIDY dosages. This effect could mean an increase and improvement of the intensity and aromatic persistence of wines over time.

Further studies should be carried out in this field to improve knowledge on how applying different dosages of SIDY affects the physical, chemical and sensory quality of Sauvignon Blanc wines and other white varieties.

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