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ARTICLE

Characterization of Commercial Inactive Dry Yeast Preparations for Enological Use Based on Their Ability To Release Soluble Compounds and Their Behavior toward Aroma Compounds in Model Wines

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The characterization of commercial enological inactive dry yeast (IDY) with different applications in wine production has been carried out. This study was based on the yeast's ability to release soluble compounds (high molecular weight nitrogen, free amino nitrogen, peptidic nitrogen, free amino acids, and polysaccharides) into model wines and on its behavior toward the volatility of seven wine aroma compounds. Important differences in soluble compounds released into the model wines supplemented with commercial IDY were found, with the free amino acids being among the most released. The volatility of most of the aroma compounds was affected by the addition of IDY preparations at a concentration usually employed during winemaking. The extent of this effect was dependent on the physicochemical characteristics of the aroma compound and on the length of time the IDY preparations remained in contact with the model wines. Whereas shorter contact times (2, 4, and 6 days) mainly promoted a "salting-out" effect, longer exposure (9 and 13 days) provoked a retention effect, with the consequent reduction of aroma compounds in the headspace. The use of different commercial preparations also promoted different effects toward the aroma compounds that may be at least in part due to differences in their ability to release soluble compounds of yeast origin into the wines.

KEYWORDS: Inactive dry yeast preparations; wine; nitrogen compounds; polysaccharides; aroma compounds

INTRODUCTION

In recent years, winemaking additives based on inactive dry yeast (IDY) have been widely used within the enological industry to improve either technological processes or the sensory characteristics of wines. Inactive yeast, yeast autolysates, yeast extracts, and yeast hulls or walls can be included under the generic name of IDY preparations. Some of the compounds released during yeast autolysis, such as peptides, amino acids, and polysaccharides (mannoproteins), seem to be responsible for the great number of applications attributed to these preparations. It has been claimed they can be used as alcoholic and malolactic fermentation enhancers (*1*, *2*), as protective agents to improve active dry yeast rehydration (*3*, *4*), or as organoleptic enhancers stabilizing the color of red wines by using mannoprotein-rich IDY preparations (*5*−*7*). However, despite the fact that many of these preparations are currently in the market under different brands, which claim different wine improvements, scientific information about the chemistry behind their use and their action mode is still scarce. Therefore, scientific studies to better characterize the changes that these preparations induce in wines are required for the establishment of better criteria for their enological use.

On the other hand, compounds (soluble or insoluble) released by IDY can interact with wine aroma compounds and may alter wine sensory characteristics. For instance, early studies showed the ability of yeast cell walls and yeast mannoproteins to bind aroma compounds (*8*−*10*). More recently, it has been shown that mannoprotein fractions isolated from the autolysates of two different *Saccharomyces cerevisiae* strains added to wines at doses usually employed during winemaking have different binding abilities (*11*). However, these studies have not taken into consideration the effect of commercial IDY preparations, which are the additives used during winemaking and which also include in their composition soluble and insoluble compounds of yeast origin. As far as we know, in only one remarkable study, Comuzzo and collaborators (*12*) showed that the addition of yeast derivative preparations to wines modified their sensory characteristics, although whether the effect was produced by odorant compounds released by the IDY preparations themselves or because of modifications on the volatility of wine aroma compounds remains unclear.

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Table 1. Inactive Dry Yeast (IDY) Preparations Used for the Study and Their Main Applications during Winemaking

preparation	provider[a]	type of wine[b]	composition[c]	main applications in wines[d]
IDY-1	A	red	rich polysaccharide inactive *S. cerevisiae* + pectinase	increase color and aroma extraction
IDY-2	A	white	rich glutathione inactive *S. cerevisiae* + pectinase + β-glycosidase	increase aroma extraction
IDY-3	A	white	antioxidant inactive *S. cerevisiae*	protect wine from oxidation
IDY-4	A	red	rich polysaccharide inactive *S. cerevisiae*	improve color stability and mouthfeel
IDY-5	B	any type	vitamin and mineral enriched inactive *S. cerevisiae*	improve fermentations and wine organoleptic characteristics
IDY-6	B	any type	rich polysaccharide *S. cerevisiae* autolysate	improve fermentations and wine organoleptic characteristics

[a] IDY provider. [b] Type of wine in which the use of the IDY preparation is recommended. [c] Information exactly transcribed from the data sheet supplied by the manufacturers. [d] In agreement with the data sheet supplied by manufacturers.

Table 2. Aroma Compounds Employed in the Model Wine Systems and Some Physicochemical Characteristics

		physicochemical properties			
compound	CAS Registry No.	mol wt	log *P*[a]	boiling point (°C)	vapor pressure[b] (mmHg at 25 °C)
ethyl butyrate	105-54-4	116	1.85	125	14.6
isoamyl acetate	123-92-2	130	2.26	134	5.67
ethyl hexanoate	123-66-0	144	2.83	170	1.85
1-hexanol	111-27-3	102	1.82	157	0.28
linalool	78-70-6	154	3.38	204	0.083
ethyl phenylacetate	101-97-3	164	2.57	234	0.091
β-ionone	8013-90-9	192	4.42	262	0.0128

[a] Hydrophobicity (log *P*) estimated with EPISuit v3.20. [b] Values estimated with EPISuit v3.20.

With all of these antecedents in mind, the objective of the present study was to characterize commercial enological IDY preparations currently used during winemaking for different wine improvements, on the basis of their ability to release soluble compounds into model wines and their capacity to interact with relevant wine aroma compounds, altering their volatility and perception. This work constitutes a primary approach in understanding the action mode of these preparations and establishing better criteria for their use during winemaking.

MATERIALS AND METHODS

Model Wines. Model wines were prepared by mixing 12% ethanol (v/v) (VWR, Leuven, Belgium) and 4 g/L tartaric acid (Panreac, Barcelona, Spain). The pH was adjusted to 3.5 with NaOH (Panreac). Five hundred milliliters of model wine was transferred into 500 mL Erlenmeyer flasks. Depending on the experiment, the model wines were supplemented with different commercial IDY powders (**Table 1**) to have a final concentration of 0.4 or 0.8 g/L. The flasks were immediately sealed with a rubber septum (Sigma-Aldrich, Steinheim, Gemany) and stirred for 10 min. To study the effect of the IDY preparations on the volatility of aroma compounds, an aroma solution containing ethyl butyrate (50 μL), isoamyl acetate (50 μL), ethyl hexanoate (50 μL), 1-hexanol (100 μL), linalool (100 μL), ethyl phenylacetate (100 μL), and β-ionone (50 μL), from Sigma-Aldrich, was prepared in 100 mL of ethanol (VWR). The aroma compounds and some selected physicochemical properties are shown in **Table 2**. Five milliliters of the aroma solution was added into the model wines (500 mL) supplemented with the IDY preparations and into the control samples (without IDY added) with a syringe through the rubber septum of the flask to have a final concentration of 5 or 10 μL/L depending on the aroma compound. Samples were kept in an incubator chamber (Infors HT, Bottmingen, Switzerland) under controlled temperature (37 °C) and stirring conditions (150 rpm). Samples were analyzed after different contact times (2, 6, 9, and 13 days) depending on the experiment. All of the samples and controls from the same study were prepared at the same time and were left in the incubator under the conditions described until the moment of their analysis. Preliminary experiments confirmed good repeatability of the seven volatile compounds from flasks submitted to the same treatment (average RSD < 12%; calculated for the seven volatile compounds from three different flasks). In addition, prior experiments were carried out in model wines with the six IDY preparations to ensure that none of the aroma compounds used for the study were originally present in the preparations.

Analytical Characterization of Soluble Compounds Released into the Model Wines. *High Molecular Weight Nitrogen (HMWN) Compounds*. The concentration of HMWN compounds was determined following the Bradford method (1976), based on the reaction of the HMWN compounds with Coomassie blue G-250 reagent. The absorbance was determined at 595 nm, 15 min after the addition of the reactant to a DU 70 spectrophotometer (Beckman Instrument Inc., Fullerton, CA). The results were expressed in milligrams of nitrogen per liter. The standard used was bovine serum albumin from Sigma-Aldrich. To express the results, the molecular mass of BSA (66432 g/mol) and the number of nitrogen atoms present in the molecule (10276 g/mol) were taken into account.

Free Amino Acids and Peptides. Free amino acids were determined according to the method of Doi et al.(*13*) (method 5), based on the reaction of ninhydrin/Cd with the free amino group. The absorbance was determined at 507 nm. Similar conditions were used to determine the free amino acids plus peptides, but following the conventional method of the ninhydrin (*13*) (method 1), based on the reaction of an amino group with a mix of ninhydrin/Sn. The absorbance was determined at 570 nm. A DU 70 spectrophotometer (Beckman) was used to determine both free amino acids and peptides. The peptides were quantified by the difference between the results obtained with Doi's method 1 and method 5. The results were expressed in milligrams of peptide nitrogen per liter. The standard used was leucine (Leu) (14 g of N for each 131.17 g of Leu).

Amino Acid Analysis by HPLC. Amino acids were analyzed in duplicate by reversed-phase HPLC using a liquid chromatograph, consisting of a Waters 600 Controller programmable solvent module (Waters, Milford, MA), a WISP 710B autosampler (Waters), and a HP 104-A fluorescence detector (Hewlett-Packard, Palo Alto, CA). Samples were submitted to automatic precolumn derivatization with *o*-phthaldehyde (OPA) in the presence of 2-mercaptoethanol following the method described by Moreno-Arribas et al. (*14*). Separation was carried out on a Waters Nova Pack C18 (150 × 3.9 mm i.d., 60 A, 4 μm) column and the same type of precolumn. Detection was performed by fluorescence ($\lambda_{excitation}$ = 340 nm, $\lambda_{emission}$ = 425 nm), and chromatographic data were collected and analyzed with a Millenium 32 system (Waters).

Neutral Polysaccharides. The concentration of neutral polysaccharides was determined by using the phenol sulfuric method, according to Segarra et al. (*15*). The absorbance was determined at 490 nm. The results were expressed in milligrams of mannose per liter.

Headspace Solid Phase Microextraction (HS-SPME). HS-SPME was used to assess the effect of commercial IDY on the volatility of aroma compounds. Eight milliliters of model wine (with or without IDY added) was taken from the 500 mL flask containing the model wines with a syringe by piercing the rubber cap. The liquid was placed in a 20 mL headspace vial and sealed with a PTFE/silicon septum (Supelco, Bellefonte, PA). Samples were allowed to reach equilibrium for 80 min in a water bath at 40 °C. Extraction was performed by the exposure of an 85 μm Carboxen−PDMS fiber (Supelco) to the headspace of the sample for 5 min at 40 °C. After the extraction, the fiber was removed from the sample vial and desorbed in splitless mode in the GC injector port for 10 min. All of the analyses were performed in triplicate. Prior to use, the fiber was conditioned following the supplier's recommendation.

The ratio between the TIC signal of each aroma compound in the headspace of the sample supplemented with IDY compared to its respective control sample (without IDY) was used to determine the effect

Table 3. Mean and Standard Deviation (SD) ($n = 6$) Values of Soluble Compounds Released by Commercial IDY Preparations (0.4 g/L) into Model Wines (Data Corresponding to the Average Values Determined in Samples of 2 and 9 Days)[a]

	IDY-1		IDY-2		IDY-3		IDY-4		IDY-5		IDY-6	
compound	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
polysaccharides (mg of mannose/L)	101.30 d	6.19	45.34 b	1.19	24.77 a	1.05	61.29 c	1.71	60.47 c	4.93	57.55 c	1.10
HMWN (mg of N/L)	1.32 ab	0.02	1.24 a	0.07	1.61 b	0.29	1.22 a	0.04	1.28 ab	0.04	1.30 ab	0.03
free amino acids (mg of N/L)	2.54 b	0.05	2.99 c	0.02	3.25 c	0.22	2.53 b	0.14	1.82 a	0.06	1.76 a	0.10
peptides (mg of N/L)	1.17 ab	0.12	0.47 ab	0.26	0.17 bc	0.24	0.79 ab	0.21	1.15 b	0.60	0.45 b	0.17
aspartic acid (mg/L)	1.12 c	0.08	0.97 ab	0.02	1.06 bc	0.01	1.05 bc	0.03	0.87 a	0.02	0.95 a	0.03
glutamic acid (mg/L)	2.10 a	0.17	5.10 c	0.22	5.67 d	0.10	2.23 a	0.12	2.32 a	0.08	2.87 b	0.11
asparagine (mg/L)	0.78 a	0.04	0.87 b	0.02	0.95 c	0.03	0.79 ab	0.02	1.07 d	0.04	0.86 ab	0.03
serine (mg/L)	0.69 bc	0.05	0.53 a	0.03	0.61 ab	0.02	0.71 c	0.01	0.64 bc	0.01	0.55 bc	0.04
glutamine (mg/L)	nd a		0.98 b	0.04	0.98 b	0.03	nd a		1.09 c	0.03	0.94 b	0.07
histidine (mg/L)	nd a		1.12 c	0.29	0.97 bc	0.24	nd a		0.68 b	0.01	0.69 b	0.03
glycine (mg/L)	0.74 d	0.05	0.46 a	0.01	0.57 b	0.00	0.69 cd	0.03	0.95 e	0.06	0.63 bc	0.02
threonine (mg/L)	0.79 b	0.01	0.62 a	0.01	0.77 b	0.01	0.77 b	0.04	0.58 a	0.04	0.63 a	0.01
arginine (mg/L)	0.69 ab	0.05	1.05 c	0.06	0.83 b	0.02	0.58 a	0.05	1.21 c	0.08	1.06 c	0.12
β-alanine (mg/L)	0.54 bc	0.01	0.56 d	0.00	0.53 b	0.00	0.54 c	0.00	0.53 bc	0.00	0.00 a	0.00
α-alanine (mg/L)	4.10 c	0.37	4.24 c	0.23	4.07 c	0.04	4.20 c	0.19	2.74 b	0.14	1.89 a	0.03
γ-aminobutyric acid (mg/L)	3.12 c	0.28	1.03 a	0.05	1.44 b	0.02	3.26 c	0.16	0.87 a	0.04	1.05 a	0.03
tyrosine (mg/L)	0.99 c	0.04	0.72 a	0.01	0.79 b	0.01	0.98 c	0.01	0.85 b	0.02	0.70 a	0.02
α- aminobutyric acid (mg/L)	0.36 a	0.01	0.36 a	0.00	0.37 ab	0.00	0.36 a	0.00	0.49 c	0.02	0.39 b	0.00
methionine (mg/L)	0.62 b	0.01	0.50 a	0.01	0.53 a	0.00	0.64 b	0.04	0.52 a	0.00	0.52 a	0.02
valine (mg/L)	1.11 c	0.08	0.80 b	0.03	1.08 c	0.01	1.07 c	0.05	0.66 a	0.03	0.64 a	0.06
tryptophan (mg/L)	0.60 a	0.01	0.66 bc	0.02	0.66 bc	0.01	0.61 ab	0.01	0.59 a	0.01	0.68 c	0.04
phenylalanine (mg/L)	0.64 c	0.04	0.37 a	0.01	0.46 b	0.01	0.58 c	0.01	0.44 a	0.02	0.41 a	0.04
isoleucine (mg/L)	0.89 c	0.06	0.45 a	0.01	0.61 b	0.01	0.83 c	0.02	0.49 a	0.01	0.51 a	0.04
leucine (mg/L)	1.60 c	0.15	0.66 a	0.01	1.01 b	0.01	1.41 c	0.05	0.77 ab	0.03	0.86 ab	0.18
ornithine (mg/L)	2.09 b	0.01	3.55 c	0.14	3.67 c	0.13	2.02 ab	0.02	1.84 a	0.06	2.05 ab	0.02
lysine (mg/L)	1.92 b	0.13	1.51 a	0.06	1.47 a	0.01	1.58 a	0.02	1.35 a	0.03	1.59 a	0.25

[a] Letters denote statistical differences among values within the same line ($p < 0.05$). nd, not detected.

of the addition of IDY on aroma volatility. We will refer to this as TIC response ratio. Therefore, ratios <1 may indicate a retention effect, whereas values >1 may indicate a "salting-out" effect (*11*).

Gas Chromatography−Mass Spectrometry Analysis. An Agilent 6890N GC system (Agilent, Palo Alto, CA) with a split/splitless injector and coupled with an Agilent 5973N mass spectrometer was used for sample analysis. The injector was set at 250 °C. Agilent MSD ChemStation software (D.01.02 16 version) was used to control the system. For separation, a Carbowax 10 M fused silica capillary column (30 m × 0.25 mm i.d. × 0.5 μm film thickness; Quadrex Co., Woodbridge, CT) was used. Helium was the carrier gas (7 psi). The oven temperature was programmed as follows: 40 °C as initial temperature, held for 5 min; first ramp, increased to 60 °C at 1 °C/min; second ramp, increased at 5 °C/min to 160 °C and then held for 1 min; third ramp, increased to 180 °C at 20 °C/min and then held for 2 min.

For the MS system, the temperatures of the manifold and transfer line were 150 and 230 °C, respectively; electron impact mass spectra were recorded at 70 eV ionization volts and the ionization current was 10 μA. The acquisition was performed in scan mode (from 35 to 450 amu). The TIC signal for each aroma compound was calculated using the data system.

Liquid Extraction of Aroma Compounds Retained in the IDY Preparations. Model wines containing the IDY-1 commercial preparation (0.8 g/L) and with or without aroma were centrifuged (9000*g* and 10 min) at 5 °C. The precipitate was then extracted three times with 10 mL of dichloromethane (Merck, Darmstadt, Germany) stirred in a vortex for 2 min at maximum velocity and sonicated for another 10 min. The organic phase was collected and filtered through glass wool and dried over anhydrous sodium sulfate. The extract was concentrated to 1 mL using a Vigreaux column in a 70 °C water bath and then to a final volume of 200 μL under a helium stream. In the case of control model wines (without IDY added), 50 mL of model wine was extracted under the same conditions as described above. One microliter of the sample was injected in split mode (1:20) under the same chromatograph and chromatographic conditions described before.

Statistical Analysis. Data from the analysis of soluble compounds released into model wines were submitted to two-way ANOVA to test the effect of the two studied factors (type of IDY and time it remained in the wine) and principal component analysis (PCA) to examine the relationship among variables (soluble compounds released into the wines, contact time, and type of IDY preparation). Aroma retention results (TIC response ratios) were submitted to two-way ANOVA to test the effect of the two studied factors (concentration and the time the IDY remained in the wine) and one-way ANOVA to check the differences between commercial IDY preparations. The Scheffe test was used for means comparison. A one-sample t test was carried out to determine whether the means from the three TIC response ratios were statistically different from a fixed value ($=1$). STATISTICA for Windows (version 7.1) was used for data processing (StatSoft, Inc., 2005, www.statsoft.com).

RESULTS AND DISCUSSION

Characterization of Soluble Compounds Released by Commercial IDY Preparations into Model Wines. In the first experiment, soluble compounds (HMWN, free amino nitrogen, peptidic nitrogen, free amino acids, and polysaccharides) released into model wines supplemented with six different commercial IDY preparations (**Table 1**) at 0.4 g/L after 2 and 9 days of contact were determined. **Table 3** shows the values corresponding to the mean and standard deviation of the concentration of polysaccharides and nitrogen compounds determined in the model wines. In **Table 3**, the results obtained for both studied times (2 and 9 days) have been grouped together because the results from the two-way ANOVA (considering the effect of the type of IDY preparation and the time it remained in the wine) confirmed that there was only a small effect of the latter factor on the concentration of these compounds (data not shown).

As can be seen in **Table 3**, the concentration of neutral polysaccharides ranged between the lowest value of 24.77 mg/L in the case of the model wines supplemented with IDY-3 preparation and the highest (101.3 mg/L), corresponding to IDY-1. Although these values might seem low compared to those

corresponding to polysaccharides released during yeast autolysis (*16*), it is important to note that in the present study the amount of inactive yeast preparation employed was very low (0.4 g/L) compared to the amount of active yeast employed in the above-mentioned studies (above 80 g/L). In addition, **Table 3** shows that the amount of polysaccharides in the model wines was greatly dependent on the type of IDY preparation. In general, the model wines supplemented with IDY-2 and IDY-3 preparations clearly showed lower values (45.34 and 24.77 mg of mannose/L, respectively) compared to the rest. It is interesting to note that both of them are preparations specifically recommended for white wine production. On the contrary, the wines supplemented with IDY-1 and IDY-4, specifically recommended for red wine production, showed the highest values of polysaccharides released (101.3 and 61.29 mg of mannose/L, respectively). This could have a technological effect on wine, as it has been shown that polysaccharides can act as protective colloids to slow or prevent the self-aggregation of tannins in synthetic media, therefore stabilizing the color of red wine (*17*, *18*). Model wines supplemented with preparations without a specific application, but manufactured in the same facilities (wines supplemented with IDY-5 and IDY-6), released very similar amounts of polysaccharides (60.4 and 57.5 mg of mannose/L, respectively) and behaved more similarly to wines supplemented with IDY-4. In addition to the possible differences between the yeast strain employed in the manufacture of these preparations (*19*), the manufacturing method may be another explanation for the different amounts of polysaccharides released by the yeast preparations. For instance, Nunez and collaborators (*20*) have shown differences of up to 25% in the amount of neutral polysaccharides released during yeast autolysis by using enzymatic or thermal inactivation processes.

With regard to the nitrogen compounds, which can be seen in **Table 3**, free amino acids represented the greatest nitrogen fraction released into the model wines, followed by HMWN and peptide nitrogen. Different authors have shown that most of the nitrogen compounds released by yeast autolysates are peptides (*21*−*23*). Therefore, these results suggested that the conditions used for the manufacture of the IDY preparations which have been studied could have been more severe, allowing the hydrolysis of peptides and giving rise to a higher content of amino acids. Taking into consideration the sum corresponding to the nitrogen from HMWN, free amino acids, and peptides, the values for the different IDY preparations ranged between 3.51 mg of N/L for IDY-6 and 5.03 mg of N/L for IDY-1 and IDY-3. These values are in agreement with those found in model wines by Guilloux-Benatier and Chassagne (*24*) when considering the sum of nitrogen compounds in fractions of different molecular masses (<5 and >10 kDa) from an autolysate of active dry yeast. Significant differences between preparations were also observed in the free amino nitrogen content. Model wines supplemented with preparations IDY-2 and IDY-3 showed the highest free amino nitrogen release (2.9 and 3.2 mg of N/L, respectively). Surprisingly, both corresponded to preparations specifically formulated for white wines. Model wines supplemented with IDY-3 also showed the greatest release of HMWN compounds. Opposite from what was observed for the free amino acids, wines supplemented with IDY-1 and IDY-5 showed the highest values for peptide release, whereas the rest of the preparations all showed very similar values.

With regard to the amino acids released into the model wines, α-alanine, glutamic and γ-aminobutyric acids, and ornithine were found at higher concentrations in the model wines. The first three have also been found as major amino acids released during yeast autolysis in model wines (*22*). Ornithine has been described as one of the major amino acids of *Saccharomyces cerevisae* and in some fermented food products, such as bread, and is an important precursor to some nitrogen heterocyclic aroma compounds (*25*). The amount of free amino acids determined in the model wines (summing the amount of each free amino acid) ranged from the highest values (29.11 mg/L) found in the model wines supplemented with IDY-3 to the lowest (20.47 mg/L) for model the wines with IDY-6. These values are slightly higher than those found in model wines from a yeast autolysate (above 13 mg/L) (*22*). Furthermore, quantitative differences between model wines (**Table 3**) depending on the type of IDY preparation were found. This may have been due to differences in the manufacturing process or in the yeast strains employed, because of the use of different nitrogen sources, pH, and/or differences in the concentration of solutes during their growth (*23*, *26*). This could explain why the IDY preparations from the same provider (IDY-5 and IDY-6) behaved more similarly to each other.

PCA was applied to establish which variables revealed a relationship among the soluble compounds released by the IDY preparations into the model wines (considering the six types of IDY preparations essayed and the two contact times, 2 and 9 days). The two first components explained 74.1% of the total variance of the data. The first principal component (PC1), which explained 48.1% of the total variance, was strongly correlated with most of the amino acids: glutamine (0.94), α-aminobutyric acid (−0.98), tyrosine (−0.91), methionine (−0.93), phenylalanine (−0.96), isoleucine (−0.98), and leucine (−0.96). The second principal component, which explained 26% of the total variance, was mainly correlated with amino nitrogen (−0.92), glutamic acid (−0.90), and ornithine (−0.95). **Figure 1** shows the wines in the PCA plot defined by the first two principal components. In the plot, there is a group of samples with positive values in PC1 and negative values in PC2. These samples corresponded to model wines supplemented with IDY-2 and IDY-3 and in general exhibited higher values for glutamine but lower for the amino acids negatively correlated with PC1 (aminobutyric acid, methionine, phenylalanine, etc). In addition, these samples showed higher values for amino nitrogen, glutamic acid, and ornithine. It is interesting to note that both types of IDY preparations have been claimed to be used for white wines. The model wines supplemented with IDY-5 and IDY-6 preparations (which came from the same provider) are in the upper right corner of the plot. They showed positive values for both components and had the lowest values for amino nitrogen, glutamic acid, and ornithine. Finally, there was a third group of samples with negative values for PC1 but positive for PC2, corresponding to wines supplemented with IDY-1 and IDY-4. These wines exhibited a similar composition with regard to the amino nitrogen content and the amino acids; glutamic acid and ornithine but higher values for the other amino acids. Interestingly, both types of IDY preparations were specifically recommended for their use in red wines. In addition, and as it is shown, the time the IDY preparations remained in the wines seemed to be a minor factor for the distinction between model wines, as was previously noted using ANOVA.

Effect of Commercial IDY Preparations on the Volatility of Aroma Compounds. The differences found between commercial IDY preparations related to their ability to release chemical compounds into the model wines may also affect the aroma compounds already present in wine. In fact, previous studies have shown, for example, that mannoproteins isolated from different *Saccharomyces cerevisiae* strains may have different abilities to interact with aroma compounds in model wines (*11*). Therefore, to study the effect of the six commercial IDY preparations on aroma volatility, further experiments were performed using

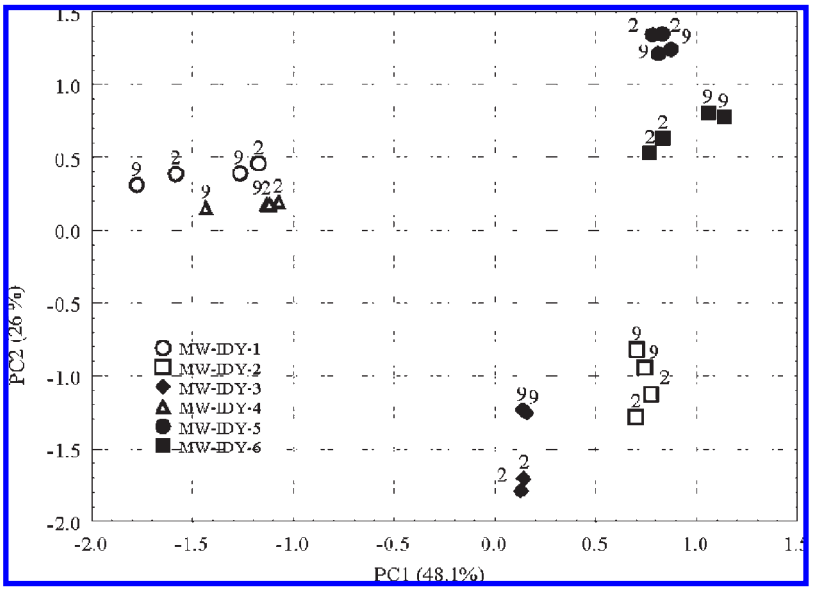

Figure 1. Plot of the model wines (MW) supplemented with commercial IDY preparations (0.4 g/L) in the plane defined by the first two principal components after 2 and 9 days of contact.

model wines supplemented with seven aroma compounds representing several chemical classes, all with different physicochemical properties (**Table 2**). These compounds were also chosen as they are important odor compounds often found in wines (*27*−*29*) and for having a grape or fermentative origin; thus, they could potentially be present in wines during the industrial application of these types of preparations.

In a first step, preliminary experiments were performed to set up the experimental conditions and to gain an insight into the effect of the relevant factors related to the handling of IDY preparations and on their effect on aroma volatility. For instance, it was important to determine the effect of the dose of the IDY employed, as previous studies have shown when these preparations have been added to wines (*12*). In addition, a systematic study showing the effect of the time the IDY preparations remained in the wine was performed, because this had not been previously studied, but it could be an outstanding factor for the adequate handling of these preparations during winemaking. To do so, model wines with the seven aroma compounds were supplemented with IDY-1 at two different concentrations, 0.4 and 0.8 g/L (both within the normal concentrations used during winemaking). Control wines with aroma compounds but without IDY were also analyzed. The headspace of the model wines with and without IDY preparations was analyzed after 2, 6, 9, and 13 days by HS-SPME. This technique has been shown to be appropriate in studying the effect of aroma retention by wine matrix macrocomponents (*30*−*32*). As was previously stated, the rate between the TIC signal of each aroma compound in the headspace of the sample supplemented with IDY compared to its respective control sample was used to determine the effect of the addition of IDY on aroma volatility (TIC response ratio). Ratios <1 may indicate a retention effect, whereas values >1 may indicate a salting-out effect (*11*). Moreover, a one-sample *t* test was carried out to determine whether the means from three TIC response ratios were statistically different from a fixed value ($=1$).

The means and standard deviations of the TIC response ratios of each aroma compound taking into account the two studied factors (concentration of IDY and the time the wine remained with the preparation) are shown in **Table 4**. Two-way ANOVA was applied to the data to test the significance of the studied factors (the interaction between factors and the error terms were pooled). The results (**Table 4**) revealed that the main factor affecting the concentration of the aroma compounds in the headspace was the time the IDY preparation remained in the wines ($p < 0.05$). The concentration of IDY only significantly affected the retention of ethyl hexanoate ($p < 0.01$) in model wines that were left for 6 days with the preparation and ethyl butyrate ($p < 0.05$) in the model wines after 6 and 13 days. In the three cases, the ratio was slightly, but significantly, higher when using a lower IDY dose (0.4 g/L). This showed an increase in the volatility of the aroma compounds or a salting-out effect. Similar results, which show an inhibition of the salting-out effect of some volatile compounds, have been previously described when the concentration of $CaCl_2$ in wines (*33*) was increased. However, the rest of the aroma compounds (1-hexanol, linalool, and β-ionone) were not significantly influenced by the dosage. On the contrary, Lubbers and collaborators (*10*) showed an increase in the retention of β-ionone (from 23 to 70%) when they increased the concentration of yeast cell walls in model wines from 1 to 10 g/L. These are very high concentrations compared to those used in the present study, and in addition, it is important to emphasize that they employed only yeast walls, therefore increasing the chance of interaction between the aroma compounds and the glycopeptides and lipids from the yeast walls (*8*, *9*).

Therefore, the time the IDY remained in the model wines was an outstanding factor affecting the volatility of the aroma compounds. There was a general trend showing that the TIC response ratios decreased with an increase in contact time (**Table 4**). **Figure 2** also shows an example for two aroma compounds (isoamyl acetate and 1-hexanol). After the application of a one-sample *t* test to determine the significance of these results, it was possible to establish which compounds were significantly affected by the addition of the IDY preparation. Some aroma compounds showed ratios significantly >1, which in general corresponded to wines that spent less time in contact with the IDY preparations. This was especially evident for the esters: ethyl butyrate, isoamyl acetate, and ethyl hexanoate in wines supplemented with IDY-1 after 2 and 4 days. Charlier and collaborators (*11*) have also reported a salting-out effect for isoamyl acetate in the presence of whole mannoprotein extracts in model wines. However, the TIC response ratios were

Table 4. Means and Standard Deviations (SD) of the TIC Response Ratios (TIC Compound$_{sample\ with\ IDY}$/TIC Compound$_{control\ sample}$) Calculated for Each Aroma Compound in Model Wines Supplemented with IDY-1 after Different Contact Times ($n = 3$) (Results of the Scheffe Test for Means Are Also Shown for Comparison)[a]

		2 days		6 days		9 days		13 days	
aroma compound	IDY concn (g/L)	mean	SD	mean	SD	mean	SD	mean	SD
ethyl butyrate	0.4	a **1.06** b	0.01	b **1.11** b	0.01	a 0.92 a	0.05	b 0.95 a	0.05
	0.8	a 1.05 b	0.05	a 1.04 b	0.04	a 0.90 ab	0.09	a 0.76 a	0.12
isoamyl acetate	0.4	a **1.09** b	0.03	a **1.18** b	0.02	a 1.00 ab	0.12	a 0.86 a	0.10
	0.8	a 1.01 ab	0.07	a 1.13 b	0.06	a 0.94 ab	0.10	a 0.73 a	0.12
ethyl hexanoate	0.4	a **1.21** b	0.08	b **1.52** c	0.13	a 0.96 ab	0.13	b **0.80** a	0.04
	0.8	a 1.27 b	0.22	a 1.14 b	0.08	a 0.89 ab	0.11	a **0.61** a	0.07
1-hexanol	0.4	a **1.06** b	0.02	a 0.95 ab	0.06	a **0.87** a	0.03	a 0.89 a	0.06
	0.8	a 1.03 a	0.21	a 0.92 a	0.04	a 0.84 a	0.09	a 0.86 a	0.10
linalool	0.4	a 1.04 b	0.06	a 0.92 ab	0.08	a 0.92 ab	0.06	a 0.77 a	0.14
	0.8	a 1.00 a	0.18	a 0.90 a	0.05	a 0.91 a	0.12	a 0.86 a	0.13
ethyl phenylacetate	0.4	a 1.02 a	0.07	a 1.03 a	0.12	a 0.99 a	0.16	a 0.89 a	0.10
	0.8	a 1.19 a	0.25	a 0.92 a	0.13	a 0.88 a	0.08	a **0.81** a	0.06
β-inone	0.4	a 0.93 a	0.06	a 0.88 a	0.10	a 0.79 a	0.13	a **0.73** a	0.09
	0.8	a 0.98 a	0.25	a 0.77 a	0.09	a 0.66 a	0.12	a **0.69** a	0.11

[a] Letters on the right denote statistical differences among values of different contact times ($p < 0.05$). Letters on the left denote statistical differences among values of different concentrations of IDY preparation ($p < 0.05$). Ratios in bold are significantly different from 1 ($p < 0.05$) (from one-sample t test).

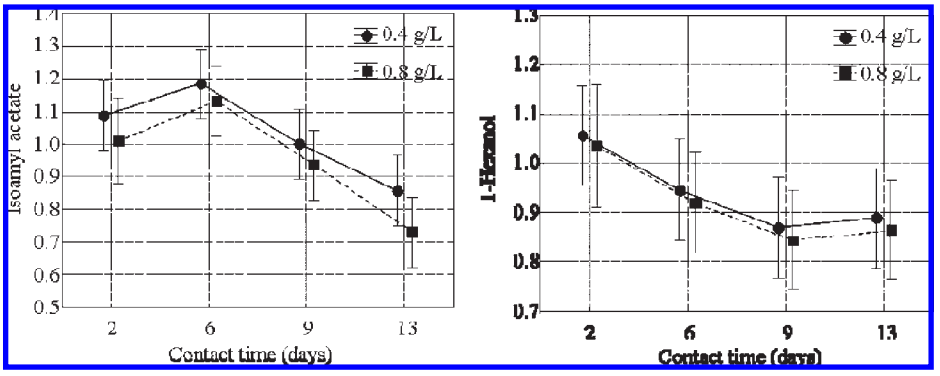

Figure 2. Evolution of the TIC response ratio of (**a**, left) isoamyl acetate and (**b**, right) 1-hexanol in model wines supplemented with two different concentrations of an IDY preparation at different contact times. Vertical bars denote 0.95 confidence interval.

significantly < 1, mainly in samples that had spent a longer time in contact with the IDY. This was the case of ethyl hexanoate in model wines supplemented with the yeast preparation for 9 days and 1-hexanol, ethyl phenylacetate, and β-ionone in wines at 13 days. In addition, β-ionone was the aroma compound that showed the highest reduction in headspace concentration (27−31%).

These results suggest a different effect of the addition of IDY toward the aroma compounds depending on the time these preparations remained in the wines. It seems that the salting-out effect observed by some aroma compounds in wines that remained in contact with IDY for less time could be mainly due to the effect of the soluble compounds immediately released into the wines from these preparations, such as amino acids and peptides. However, the retention effect was more evident in model wines supplemented with IDY for longer times. This may have been due to the progressive binding of the aromas on the insoluble matter (yeast cell wall residues), which remained in the Erlenmeyer flasks after each sampling. Therefore, the whole insoluble fraction seemed to be responsible for this interaction effect, which could be extended as long as the aroma molecules find available binding sites. This assumption is supported by the fact that the concentration of soluble compounds remained constant with an increase in the contact times (2 and 9 days). The potential contribution of the insoluble fraction from IDY preparations on the reduction of the volatility of aroma compounds, which was mainly observed in model wines that remained in contact with the preparation for a longer time, was also checked. To do so, the precipitates obtained after the centrifugation of the model wine with aroma and of the control wine (without aroma) supplemented with IDY-1 during 13 days were extracted with dichloromethane and further analyzed by GC-MS. **Figure 3** shows the corresponding chromatograms. **Figure 3b** clearly shows that all of the aroma compounds added to the model wines were also found in the precipitate of IDY. However, the most hydrophobic compounds (ethyl phenylacetate and β-ionone) were among the most retained compounds. This may explain the lower TIC response ratios signals (higher retention) found for these compounds in the SPME analysis of wines that remained in contact with the IDY for a longer time. The high hydrophobicity of these compounds seemed to favor the interaction not only with glycoproteins but also with lipids from cell walls (*9*).

Once it was confirmed that the main factor influencing the volatility of aroma compounds in model wines supplemented

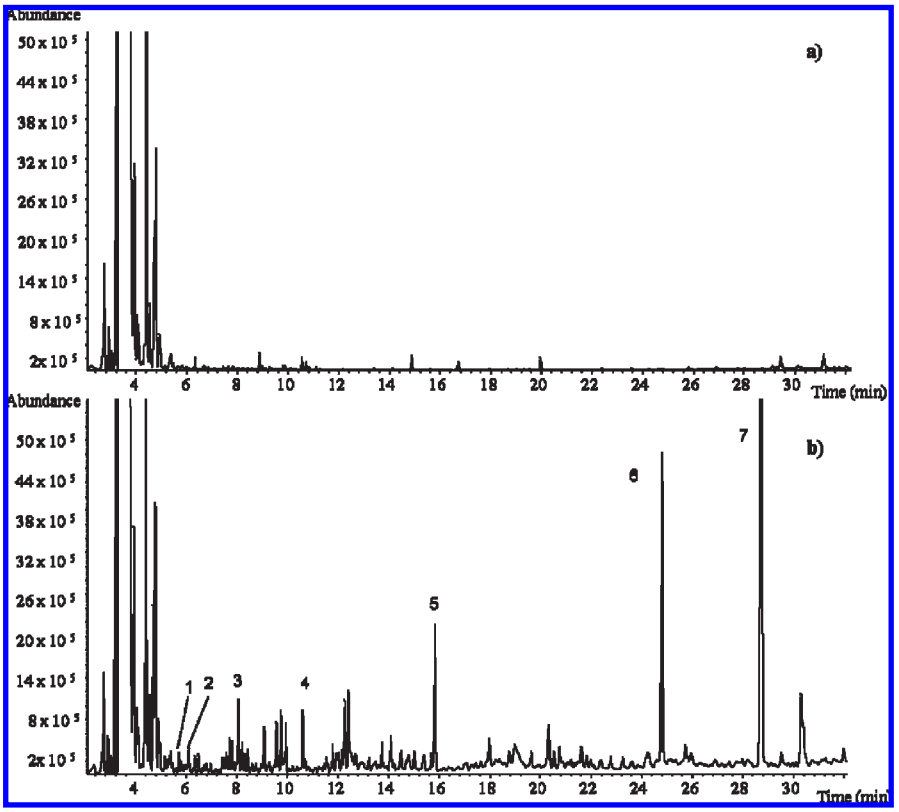

Figure 3. Chromatograms corresponding to dichloromethane extracts of the precipitate obtained from model wines without aroma (**a**) and with aroma (**b**) supplemented with an IDY preparation after 13 days of contact. Peaks: ethyl butyrate (1); isoamyl acetate (2); ethyl hexanoate (3); 1-hexanol (4); linalool (5); ethyl phenylacetate (6); β-ionone (7).

Table 5. Means and Standard Deviations (SD) of the TIC Response Ratios (TIC $\text{Compound}_{\text{sample with IDY}}$/TIC $\text{Compound}_{\text{control sample}}$) Calculated for Each Aroma Compound in Model Wines Supplemented with Different Commercial Preparations after 9 Days of Contact ($n = 3$) (Results of the Scheffe Test for Means Are Also Shown for Comparison)[a]

	IDY-1		IDY-2		IDY-3		IDY-4		IDY-5		IDY-6	
aroma compound	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ethyl butyrate	0.92 b	0.05	**0.67** a	0.07	0.90 b	0.02	1.18 c	0.14	1.03 b	0.06	**0.96** b	0.01
isoamyl acetate	1.00 b	0.12	**0.74** a	0.09	**0.92** b	0.00	1.20 c	0.15	1.01 bc	0.03	**0.99** b	0.00
ethyl hexanoate	0.96 b	0.13	0.87 ba	0.12	0.87 ba	0.05	1.34 c	0.23	0.85 ba	0.05	**0.72** a	0.04
1-hexanol	**0.87** a	0.03	0.97 ba	0.11	0.90 ba	0.04	1.17 c	0.12	1.06 b	0.10	1.02 b	0.02
linalool	0.92 a	0.06	0.98 a	0.09	0.85 a	0.08	1.16 a	0.08	1.01 a	0.22	**0.95** a	0.01
ethyl phenylacetate	0.99 a	0.16	1.05 a	0.06	0.87 a	0.06	1.00 a	0.12	0.95 a	0.12	0.86 a	0.07
β-ionone	0.79 a	0.13	1.06 a	0.07	0.82 a	0.07	1.10 a	0.17	1.00 a	0.23	0.84 a	0.08

[a] Letters denote statistical differences among values within the same line ($p < 0.05$). Ratios in bold are significantly different from 1 ($p < 0.05$) (from one-sample t test).

with IDY was the time these preparations remained in the wine, a new experiment was set up to determine if there were any differences in the behavior of the commercial IDY preparations toward the aroma compounds. To do so, the headspace of the model wines supplemented with the seven aroma compounds and the six different commercial preparations was analyzed after 9 days. They were all supplemented with the same concentration of IDY (0.4 g/L), because it was shown in the preliminary experiments that this factor did not significantly affect the volatility of the aroma compounds.

The corresponding TIC response ratios together with the results from the one-way ANOVA and the application of the Scheffe test to determine if there were statistically significant differences ($p < 0.05$) depending on the type of preparation are shown in **Table 5**. In addition, results from a one-sample t test to determine whether the values of the ratios were significantly different to one (= 1), are also shown in the table. The first conclusion extracted from this table was that the influence of the IDY on the aroma volatility depended on the type of IDY preparation and the type of aroma compound. In this sense, three aroma compounds, linalool, ethyl phenylacetate, and β-ionone, did not show any differences with the type of IDY preparation used. However, the esters ethyl butyrate, isoamyl acetate, and ethyl hexanoate and the alcohol 1-hexanol were more or less influenced depending on the type of commercial IDY preparation. Among the preparations, IDY-2 and IDY-4 were the most different. The addition of IDY-2 to the model wines produced a general reduction of the headspace concentration for most of the aroma compounds (lower TIC response ratios), which was statistically significant for isoamyl acetate and ethyl butyrate. However, the use of IDY-4 produced a higher aroma headspace concentration or a salting-out effect (although not statistically significant). It was previously shown (**Table 3**) that both types of preparations presented differences regarding the amount of some

soluble macromolecules released into the model wines. Whereas IDY-4 showed a higher release of peptides and mainly polysaccharides, IDY-2 released much lower amounts of these types of compounds. For instance, the polysaccharides released by IDY-4 were 1.4 times higher (62.4 mg of mannose/L) than the IDY-2 preparation (44.5 mg of mannose/L) (**Table 3**). These results seem to confirm that the greater the amount of soluble compounds released into the wines, the more significant the salting-out effect is. The rest of the commercial preparations showed different behaviors on the headspace volatility depending on the type of aroma compound. In general, most of them showed a reduction in the volatility of aroma compounds rather than a salting-out effect. For instance, IDY-3 and IDY-6 produced a significant decrease in the TIC response ratio of isoamyl acetate, which in the case of IDY-6 was also extensive to ethyl hexanoate, whereas IDY-1 significantly reduced the TIC response ratio of 1-hexanol. The reduction in volatility observed for most of the aroma compounds when using IDY preparations is in agreement with the results obtained from the first experiment, where there was an increase in the headspace concentration of some aroma compounds when the preparations remained in the wines for shorter times. However, a further aroma retention (or reduction in the headspace concentration) occurred when the wines remained in contact with the IDY preparations for longer periods of time (take into account that the model wines used for this experiment had been in contact with the IDY preparations for 9 days).

In conclusion, the results from this work have shown that the addition of IDY preparations to model wines using the same dosage usually used during winemaking can change the chemical composition of model wines and also have an effect on the headspace volatility of representative wine aroma compounds. However, the effect on volatility depends on the type of aroma compound and the length of time the IDY preparations remained in the wines. Whereas shorter periods of time (2, 4, and 6 days) mainly promoted a salting-out effect, longer exposure times (9 and 13 days) provoked a retention effect, with the consequent reduction in the volatility of the aroma compounds. The use of different commercial IDY preparations showed different effects toward the aroma compounds, not only because of the retention of aroma compounds in the insoluble matter present in these preparations but also due to differences in their ability to release soluble compounds into the wines. These results may have an effect on the sensory characteristics of wines, but this should be investigated in future works.

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