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ARTICLE

Role of Specific Components from Commercial Inactive Dry Yeast Winemaking Preparations on the Growth of Wine Lactic Acid Bacteria

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The role of specific components from inactive dry yeast preparations widely used in winemaking on the growth of three representative wine lactic acid bacteria (*Oenococcus oeni*, *Lactobacillus hilgardii* and *Pediococcus pentosaceus*) has been studied. A pressure liquid extraction technique using solvents of different polarity was employed to obtain extracts with different chemical composition from the inactive dry yeast preparations. Each of the extracts was assayed against the three lactic acid bacteria. Important differences in the effect of the extracts on the growth of the bacteria were observed, which depended on the solvent employed during the extraction, on the type of commercial preparations and on the lactic acid bacteria species. The extracts that exhibited the most different activity were chemically characterized in amino acids, free monosaccharides, monosaccharides from polysaccharides, fatty acids and volatile compounds. In general, specific amino acids and monosaccharides were related to a stimulating effect whereas fatty acid composition and likely some volatile compounds seemed to show an inhibitory effect on the growth of the lactic acid bacteria. These results may provide novel and useful information in trying to obtain better and more specific formulations of winemaking inactive dry yeast preparations

KEYWORDS: Inactive dry yeast preparations; winemaking; lactic acid bacteria; pressure liquid extraction; wine

INTRODUCTION

In recent years, inactive dry yeast (IDY) preparations are gaining interest in the enological industry. These preparations are produced from enological yeasts (*Saccharomyces cerevisiae*) previously inactivated to eliminate their fermentative capacity. Depending on the treatment employed during their manufacturing, yeast extracts, yeast autolysates or cell walls can be obtained (*1*). Among all of them, yeast autolysates are the most commonly commercialized IDY preparations for winemaking applications. They are constituted by a soluble and an insoluble fraction from the cell wall and membranes, obtained after partial autolysis of the yeast (*2*). Depending on their composition IDY can be used for different applications in winemaking. Currently, one of their main applications is to be used for improving alcoholic fermentation and malolactic fermentation (MLF). However, many other IDY preparations are also claimed to enhance the organoleptic characteristics of wines or even to ensure wine safety (*1*, *3*, *4*).

The use of IDY preparations as fermentation enhancers is based on two different action mechanisms. The first one is related to the protective effect of IDY during the rehydration of active dry yeast (ADY) (*5*), and the second one is due to their ability to serve as fermentation nutrients. Regarding the first mechanism, IDY preparations can release insoluble fractions from the yeast cell wall into the rehydration medium, which may form groups of micelle-like sterols that can be incorporated into the ADY membrane, thereby repairing its possible damage (*6*). In addition, IDY preparations may help ADY to adapt their metabolism to the high sugar concentration in musts. Specifically, polyunsaturated fatty acids released from IDY might reduce the osmotic shock of ADY in the musts, thereby acting as protective agents (*7*).

The second mechanism is related to the use of IDY for promoting the growth of wine microorganisms. In this sense, IDY preparations could release yeast's cytoplasm soluble metabolites into the wine (*8*), which, it has been shown, may enhance the alcoholic fermentation rates in nitrogen deficient mediums (*9*). In addition, the insoluble fraction from IDY may also improve the fermentation efficiency in nondeficient nitrogen musts, due to the detoxifying effect of the yeast cell walls (*9*). This effect is based on the adsorption of some toxic metabolites, such as short and medium chain fatty acids, usually associated with stuck or sluggish wine fermentations (*10*, *11*).

Specific IDY preparations are currently being used for enhancing MLF (*1*). This process is important during winemaking for reducing wine's acidity and for improving wine aroma and flavor (*12*). MLF is mainly carried out by *Oenococcus oeni*, although other bacteria belonging to the genera *Lactobacillus* and *Pediococcus* can also be present during winemaking (*13*).

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Table 1. Inactive Dry Yeast (IDY) Preparations Employed in the Present Study

preparation	company	composition[a]
IDY1	1	inactive *S. cerevisiae* rich in polysaccharide + pectinase
IDY2	1	inactive *S. cerevisiae* rich in gluthatione + pectinase + β-glycosidase
IDY3	1	inactive *S. cerevisiae* rich in polysaccharides
IDY4	1	inactive *S. cerevisiae* with antioxidant properties
IDY5	2	inactive *S. cerevisiae* enriched in vitamins and minerals
IDY6	2	*S. cerevisiae* autolysate

[a] In agreement with the data sheet information supplied by the provider.

Although it has been shown that fractions with different molecular weights obtained from noncommercial yeast autolysates and yeast extracts can stimulate the growth of *O. oeni* (*14*−*16*), and besides the increasing number of different types of IDY preparations currently on the market, the literature concerning the effect of commercial winemaking IDY preparations on the MLF, and on their effect on specific wine lactic acid bacteria (LAB), is scarce.

The objective of this work is, therefore, to gain insight on the role of specific components from commercial IDY preparations on the growth of representative species of wine LAB trying to elucidate their action mode.

MATERIALS AND METHODS

Samples. Six commercial IDY preparations, widely used within the enological industry and provided by two different companies, were employed. **Table 1** shows their main characteristics and composition in agreement with the information provided by the manufacturers.

Lactic Acid Bacteria, Culture Media and Growth Conditions. Three bacterial strains corresponding to *Lactobacillus hilgardii* IFI-CA 49, *Pediococcus pentosaceus* IFI-CA 85 and *O. oeni* IFI-CA 96 were essayed. They belonged to the microbial culture collection of the Institute of Industrial Fermentations (CSIC). The bacteria strains were previously isolated from wines, and they were kept frozen at −70 °C in a sterilized mixture of culture medium and glycerol (50% v/v). A MRS culture media (Pronadisa, Madrid, Spain) based on the formula developed by Man et al. (*17*) was used for *L. hilgardii* and *P. pentosaceus*. They were cultivated for 48 h. In addition, a MLO culture media (Pronadisa) developed by Caspritz et al. (*18*) was used for *O. oeni*. This bacterium was cultivated for 3−4 days. In some experiments polyvinyl alcohol at a final concentration of 20 mL L^{-1} (Sigma-Aldrich, Steinheim, Germany) was added to the culture media to improve the solubility of the extracts. All the media were sterilized at 121 °C for 15 min, and in trying to be closer to wine conditions they were supplemented with ethanol to have a final concentration of 60 mL L^{-1}.

Pressure Liquid Extraction (PLE) To Obtain IDY Extracts. The extracts from IDY preparations were obtained by using an accelerated solvent extractor (ASE 200, Dionex Corporation, Sunyvale, CA) equipped with a solvent flow controller. Three solvents of different polarity, ethanol (Scharlau Chemie S.A., Barcelona, Spain), hexane (Panreac Quimica S.A., Barcelona, Spain) and water purified by using a Milli-Q system (Millipore, Inc., Bedford, MA), were employed for each IDY preparation. The extraction conditions were 150 °C, 10342 kPa and 20 min, and they were previously optimized in our laboratory (*19*). All the extractions were performed in 11 mL extraction cells containing 2 g of sample. In the case of water when used as solvent, the extraction cell was filled with three layers in order to prevent the clogging of the cell: first one of sea-sand (4 g) (Panreac Química S.A.), a second layer of the sample (2 g) and a final sand layer on the top of the cell (2 g). Between extractions, a rinse of the complete system was performed in order to overcome any extract carryover. The extracts obtained at all the assayed temperatures were quickly chilled in an ice−water bath to minimize the loss of volatiles and avoiding sample degradation. All the organic solvents were removed by using a Rotavapor R-200 (Büchi Labortechnik AG, Flawil, Switzerland) at 40 °C, while water extracts were dried in a lyophilizer (Labconco, KA, MS).

Determination of the Activity of the IDY Extracts on the Growth of Lactic Acid Bacteria. *Extract Dilution.* The IDY dry extracts that were previously obtained by using ethanol and water were dissolved in the culture media to have a final concentration of 20 mg of dry extract mL^{-1}. The solutions were centrifuged (13000*g*, 10 min) to obtain extracts as clean as possible. From the 20 mg mL^{-1} extract different serial dilutions ranging from 1.25 to 20 mg mL^{-1} were prepared. The IDY extracts obtained with hexane were dissolved in the culture medium supplemented with polyvinyl alcohol to have a final concentration of 5 mg of dry extract mL^{-1} using an Ultraturrax (IKA-Werke GMBH& Co. KG, Staufen, Germany). Serial dilutions ranging from 0.625 to 5 mg mL^{-1} were prepared from the most concentrated one.

Bacterial Inoculum. Briefly, 100 μL of the defrozen strain suspension was added to 10 mL of culture medium, incubated at 30 °C for 48 h for *L. hilgardii* and *P. pentosaceus*, and 72 h for *O. oeni*. Afterward, 100 μL of the suspension was added to 10 mL of medium, and incubated in the same conditions mentioned above. Adequate dilutions to have a final density in the wells of 5×10^5 colony forming units (CFU) mL^{-1} for *L. hilgardii* and *P. pentosaceus*, and 5×10^6 CFU mL^{-1} for *O. oeni* were prepared.

Activity of the IDY Extracts on the Growth of Lactic Acid Bacteria. The activity of the extracts was determined according to the method proposed by Rojo-Bezares et al. (*20*), previously modified in our laboratory (*13*). Prior to the assays, the growth curves of the strains *L. hilgardii* IFI-CA 49, *P. pentosaceus* IFI-CA 85, and *O. oeni* IFI-CA 96 were determined. The activity of the extracts was determined at 24 h for *L. hilgardii* and *P. pentosaceus*, and at 48 h for *O. oeni*, corresponding to a middle point of the exponential growth. For each assay, two 96-well multiplates (Greiner Bio-One, Frickenhausen, Germany) corresponding to the initial and final time were made. Control media wells (containing culture medium), control bacteria wells (containing the culture medium inoculated with bacteria) and sample wells (containing the extracts at different concentrations inoculated with the bacteria) were prepared in triplicate in each plate. The inoculum size was 10% of the total well volume, and the multiwell plates were incubated at 30 °C. Absorbance was measured using a Fluorimeter Fluostar Galaxy at 520 nm (BMG Labtech, Offenburg, Germany); previously the content of the wells was shaken. Finally, the activity of the extracts was determined by comparison of the bacterial growth in the sample wells and in the control bacteria wells, applying eq 1:

$$\%\ \text{activity} = (\Delta OD_{\text{sample}} - \Delta OD_{\text{controlbacteria}})/\Delta OD_{\text{controlbacteria}} \times 100 \quad (1)$$

where ΔOD was the increase in optical density in the final time compared to the initial time.

Chemical Characterization of the IDY Extracts. All the IDY dry extracts were reconstituted in their original solvent (the same employed during the PLE) to have a final concentration of 10 mg of extract mL^{-1}. All the analyses were made in duplicate, and the results were expressed in mg of each chemical component g^{-1} of dry extract.

Amino Acids. 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*-phthaldialdehyde (OPA) in the presence of 2-mercaptoethanol (Sigma-Aldrich) following the method described by Moreno-Arribas et al. (*21*). Separation was carried out on a Waters Nova Pack C18 (150 × 3.9 mm i.d., 60 A, 4 μm) column. Detection was performed by fluorescence ($\lambda_{\text{excitation}}$ n = 340 nm; $\lambda_{\text{emission}}$ n = 425 nm), and chromatographic data were collected and analyzed with a Empower-2-2006 system (Waters).

Free Monosaccharides. Monosaccharide analysis was performed according to Núñez et al. (*22*). Briefly, 1 mL of a reconstituted IDY extract in water at 10 mg mL^{-1} was dried in a rotavapor to obtain a dried residue. The dried residue was dissolved in 100 μL of anhydrous pyridine, 100 μL of (trimethylsilyl)imidazole, 100 μL of trimethylchlorosilane, 100 μL of *n*-hexane, and 200 μL of water, which were sequentially added and shaken during each step. Finally, 2 μL of organic phase was injected in split (1/40) into a Hewlett-Packard 6890 gas chromatograph with a flame ionization detector (GC-FID). The injector and detector temperatures were set at 270 °C. For separation, a fused silica Carbowax 20 M column (30 m × 0.25 mm i.d. × 0.5 μm; Quadrex Co., Woodbridge, CT) was used. The oven

temperature was programmed as follows: 175 °C as initial temperature, held for 15 min. In a first ramp, the temperature increased at 15 °C min^{-1} to 200 °C, then held for 13 min. In a second ramp, the temperature increased at 13 °C min^{-1} to 290 °C, held for 20 min. The system was controlled by HP ChemStation software. For quantification, a five point calibration curve of a standard solution including arabinose, xylose, galactose, fructose, glucose and mannose was prepared from 10 to 300 mg L^{-1} and injected in the same conditions as the sample.

Monosaccharides from Polysaccharides. The IDY extracts were hydrolyzed according to Núñez et al. (*22*). For this purpose, 1 mL of a reconstituted extract in water at 10 mg mL^{-1} was hydrolyzed at 110 °C in a stove during 24 h in a closed vial containing 1 mL of 2 M trifluoroacetic acid (Scharlau Quimica S.A.). Afterward, 1 mL of the hydrolyzed sample was dried in a rotavapor and derivatizated and analyzed by GC-FID in the same conditions explained above.

Fatty Acids. For fatty acid determination, the reconstituted extracts in hexane at 10 mg mL^{-1} were previously methylated. To do so, 0.5 mL of extract was dried in a rotavapor. The dried residue was dissolved in a mixture of chloroform:methanol (2:1) at 2 mg mL^{-1}, and then 1 mL of 0.5 N sodium methylate (Supelco, Bellefonte, PA) was added. The reaction took place at 65 °C for 20 min. Then, 0.5 mL of Milli-Q water and 2 mL of hexane were added. The upper layer was separated, and water was removed by anhydrous sodium sulfate. Three microliters of organic phase were injected in split mode (1/20) into an Agilent 6890 gas chromatograph coupled to an Agilent 5973 quadrupole mass spectrometer (GC−MS) (Agilent, Palo Alto, CA). The injector was set at 250 °C. For separation, a Carbowax 20 M (30 m × 0.25 mm i.d. × 0.5 μm; Quadrex Co.) was used. The oven temperature was programmed as follows: 100 °C as initial temperature; first ramp increased at 20 °C min^{-1} to 220 °C, held for 25 min; second ramp, increased at 15 °C min^{-1} to 270 °C and held for 10 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 compound was calculated using the data system Agilent MSD ChemStation software (D.01.02 16 version). The identification was carried out by comparison of the retention times and mass spectra of the samples in relation to a commercial standard solution of methyl ester of fatty acids (Supelco 37 Component FAME Mix). An estimation of the percentage of each compound in the sample was obtained by calculating the percentage of TIC area of each compound compared to the sum of TIC area of all the fatty acids identified in the sample.

Volatile Compounds. To determine the volatile compounds in the extracts, 3 μL of the extracts reconstituted at 10 mg mL^{-1} in hexane was directly injected in split mode (1/20) into the GC−MS. The injector was set at 250 °C. For separation, a HP-5 M fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent) was used. The oven temperature was programmed as follows: 40 °C as initial temperature held for 5 min. Then, a first ramp at 4 °C min^{-1} to 200 °C, and a second ramp at 2 °C min^{-1} to 250 °C, held for 5 min. The tentative identification of compounds was carried out by comparison of their mass spectra with those reported in the mass spectrum libraries, NIST98 and Wiley5; moreover, linear retention indexes were experimentally calculated with an *n*-alkane mixture (C5−C30) and compared with those available in the literature. To estimate the proportion of each compound present in the sample, the percentage of TIC area of each volatile compared to the sum of TIC area of all the volatile compounds detected in the sample was calculated.

RESULTS AND DISCUSSION

Pressurized Liquid Extracts from IDY Preparations. In the present work, PLE has been considered a useful technique to obtain extracts of different composition from IDY preparations. Other techniques such as ultrafiltration and dialysis have been also employed in previous works to obtain nitrogen fractions of different molecular weights from yeast autolysates (*14*−*16*, *23*). However, the possibility of using solvents of different polarities during the PLE allows one to obtain extracts with different composition, therefore making easier the study of the effect of compounds from IDY in the growth of lactic acid bacteria. Additional advantages of PLE are its rapidity and the lower amount of solvents required. In addition, the use of fluids at high pressure favors the extraction of analytes trapped into the matrix pores, which are difficult to extract by using other techniques that employ fluids under atmospheric conditions (*24*). In the present work, water, ethanol and hexane were employed as solvents due to the differences in their dielectric constants (78.5, 24.3, and 1.9 respectively), and therefore in their polarity (**Table 2**). As can be seen in **Table 2**, the extraction yields were very different depending on the solvent employed and, to a lesser extent, on the type of IDY preparation. The extraction yields when using water and ethanol (15.5% and 18.2% in average respectively) were much higher than the extraction yields obtained with hexane (2% in average). These results were already suggesting that most of the compounds present on these preparations were more polar than apolar in nature.

Table 2. Yields Obtained (% Dry Weight) in the PLE

	solvents		
type of IDY preparation	hexane (1.9)[a]	ethanol (24.3)	water (78.5)
IDY1	1.4	20.1	23.3
IDY2	0.8	20.1	26.5
IDY3	4.4	16.6	8
IDY4	2.6	15.5	12.2
IDY5	1.3	23.2	14.6
IDY6	1.5	13.7	8.2
average	2	18.2	15.5

[a] Dielectric constant of the solvents.

Effect of IDY Extracts on the Growth of Lactic Acid Bacteria. In general, most of the extracts obtained from the IDY preparations showed an effect on the growth of the three assayed LAB. However, depending on the extracts two opposite effects corresponding to a stimulation or an inhibition on the growth of LAB were found. This already showed that IDY preparations may include specific molecules in their composition that can promote or inhibit the growth of the assayed microorganisms. In addition, it was observed that, independently of the type of extract, the activity (stimulation or inhibition) was directly dependent on the concentration assayed (data not shown). **Table 3** summarizes these results and shows the effect (% activity) of the different extracts at the highest concentration essayed (20 mg mL^{-1} for the IDY extracts obtained with water and ethanol, and 5 mg mL^{-1} for those obtained with hexane) on the growth of the lactic acid bacteria. As can be seen, the differences in activity between different extracts were mainly dependent on the solvent employed during the PLE extraction. In general, the IDY water extracts either stimulated or did not show any effect. The stimulating effect may be due to the presence of some nitrogen compounds, that in the case of yeast autolysates, it has been shown that they may promote the growth of *O. oeni* (*14*−*16*, *25*). Surprisingly, the water extracts obtained from the IDY5 preparation inhibited the growth of all the assayed strains. In addition, the IDY6 water extract also inhibited the growth of *O. oeni*. This fact may be due to the inhibitory activity of some polar compounds, such as specific peptides with molecular weights between 5 and 10 kDa and released from the yeast, which in the presence of ethanol in the medium have been shown may inhibit the growth of *O. oeni* (*23*). On the contrary, the IDY extracts obtained with hexane, and therefore likely richer in nonpolar compounds, inhibited the growth of the three LAB strains. This effect may be related to a high concentration of short- and medium-chain fatty acids from the yeast, which have been shown can inhibit the growth of *O. oeni* (*10*, *26*). The IDY extracts obtained with ethanol showed

Table 3. Effect (% Inhibition or Stimulation) of the IDY Extracts Obtained by PLE Using Water (20 mg/mL), Hexane (5 mg/mL) and Ethanol (20 mg/mL) on the Growth of Lactic Acid Bacteria

		activity (%) of the IDY extract[a]		
type of IDY preparation	solvent[b]	*L. hilgardii*	*P. pentosaceus*	*O. oeni*
IDY1	W	+(186)	+(170)	+(124)
	H	−(59)	−(87)	−(58)
	E	+(149)	+ (24)	−(50)
IDY2	W	+ (12)	+ (29)	−(2)
	E	−(42)	−(36)	−(76)
IDY3	W	+(50)	+(67)	+(152)
	H	−(61)	−(54)	
	E	−(11)	n.a.	−(88)
IDY4	W	+(44)	+(28)	−(6)
	H	−(50)	−(57)	−(7)
	E	−(57)	−(57)	−(49)
IDY5	W	−(28)	−(68)	−(92)
	H	−(91)	−(101)	
	E	−(100)	−(96)	−(112)
IDY6	W	+(98)	n.a.	−(85)
	E	−(56)	−(83)	−(96)

[a] Activity (%) of the IDY extract compared to the control sample (without extract); + denotes a stimulatory effect, whereas − means an inhibitory effect; n.a., no activity was observed. [b] Type of solvent employed during the PLE: W, water; H, hexane; E, ethanol.

an intermediate effect on the growth of LAB between those obtained with water and hexane which could be explained by the intermediate polarity of this solvent and, therefore, by the presence of both types of compounds, those with stimulating and those with inhibitory activity of bacterial growth. Besides of the different effect of the IDY extracts depending on the type of solvent employed during the PLE, the activity of the extracts was also dependent on the type of IDY preparation. In this sense, **Figure 1** shows an example illustrating the effect of water extracts obtained from the six types of commercial IDY preparations on the growth of *O. oeni*. As can be seen, while IDY1 and IDY3 extracts showed a clear stimulation effect, IDY5 and IDY6 showed an inhibition on the growth of *O. oeni*. However, IDY2 and IDY4 did not show any effect. Interestingly, similar behaviors were found among IDY preparations supplied by the same provider and for the same type of application (**Table 1**). For instance, extracts obtained from IDY1 and IDY3 preparations, supplied by provider 1 and recommended for red wines, showed similar effect, while extracts from preparations IDY2 and IDY4 also supplied by provider 1 but for white wines did not show a clear effect on the bacteria growth (**Figure 1**). However, IDY5 and IDY6 extracts, which showed a clear inhibition effect (**Figure 1**), were supplied by a different provider.

Moreover, from **Table 3** it is worth underlining that the three lactic acid bacteria also showed a different susceptibility to the same extract. As an example, the water extract obtained from IDY3 greatly promoted the growth of *O. oeni* (152%), while it moderately stimulated the growth of *L. hilgardii* (50%) and *P. pentosaceus* (67%). These results show important metabolic differences between the three LAB species and/or strains.

To elucidate which compounds from the IDY preparations were the main ones responsible for the observed effects on the LAB growth, a chemical characterization of the extracts from the two IDY preparations which showed the most different activities was performed. Specifically, this study was performed with IDY1 and IDY5 extracts, which in general showed the highest stimulating and inhibition effect on bacterial growth respectively (**Table 3**).

Chemical Characterization of IDY Extracts. As it was explained above, IDY1 and IDY5 extracts were chosen to perform their chemical characterization. For the analysis of amino acids and monosaccharides the water extracts from both IDY preparations were used. In addition, the extracts obtained with hexane were employed to characterize the fatty acid and volatile composition.

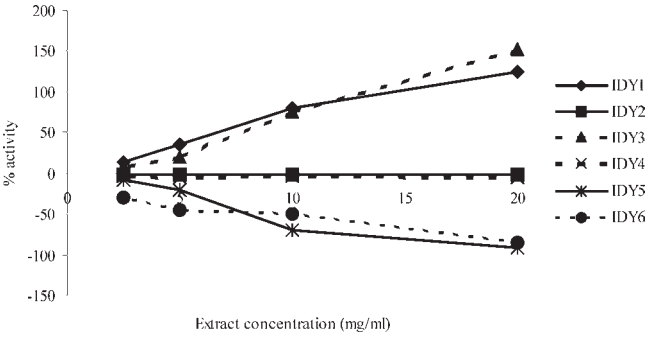

Figure 1. Effect (% activity) of IDY extracts obtained with water on the growth of *O. oeni* IFI-CA 96.

Amino Acids. The amino acid composition of IDY1 and IDY5 extracts is shown in **Figure 2**. As can be seen, the extracts from both preparations showed qualitative and quantitative differences. The total amino acid content was higher in the IDY1 extract (47 mg g^{-1} of dry extract) than in the IDY5 extract (27 mg g^{-1} of dry extract). Taking into consideration that wine LAB are able to use amino acids as a nitrogen source (*16*, *27*, *28*), the extract IDY1 should have provided a higher amount of these compounds for the development of LAB compared to the IDY5 extract. In addition, qualitative differences in the amino acid composition of both IDY extracts were also noticed (**Figure 2**). The major amino acids in the IDY1 extract were α-alanine, γ-aminobutyric, glutamic and aspartic acids, leucine and valine, which is in agreement with previous work performed with yeast autolysates (*14*). Nevertheless, the amino acid composition of the IDY5 extract was different, in which α-alanine was the major amino acid, while aspartic and glutamic acids, glycine, arginine, γ-aminobutyric acid and ornithine were found to a minor extent. The stimulation effect of alanine, valine, leucine, methionine and threonine on the growth of *O. oeni* has been shown in previous work (*28*). All of them were in a higher concentration in the IDY1 extract, which may explain the stimulating effect of this extract on the growth of the three LAB (**Table 3**). Despite the stimulating activity of some amino acids, Vasserot et al. (*29*) have shown that aspartic acid at high concentrations (above 19 mg L^{-1}) could inhibit the growth of *O. oeni*, although they also stated that the inhibition might be reduced in the presence of glutamic acid. In the present work, the aspartic acid concentration of both IDY1 and IDY5 extracts was very similar. However, the IDY1 extract presented higher concentration of glutamic acid compared to the IDY5 extract, and therefore, the former may have reduced the potential inhibitory effect of aspartic acid, which may explain why only the IDY1 extract promoted the growth of *O. oeni* (**Table 3**).

The lower inhibition of the IDY5 extracts in the growth of *L. hilgardii* compared to *P. pentosaceus* and *O. oeni* may be explained by its higher concentration in arginine and ornithine which may specifically promote the growth of *L. hilgardii* (*30*).

Free Monosaccharides and Monosaccharides from Polysaccharides. The results corresponding to the determination of monosaccharides in the IDY water extracts revealed that glucose was the only free monosaccharide detected, whereas mannose and glucose were identified in both extracts after their hydrolysis (**Figure 3**). The concentration corresponding to monosaccharides from polysaccharides was much higher (above 25 mg g^{-1} of dry extract) than that corresponding to free monosaccharides (above 0.5 mg g^{-1} of dry extract), which suggests that probably these preparations were rich in glucoproteins and mannoproteins from the yeast cell

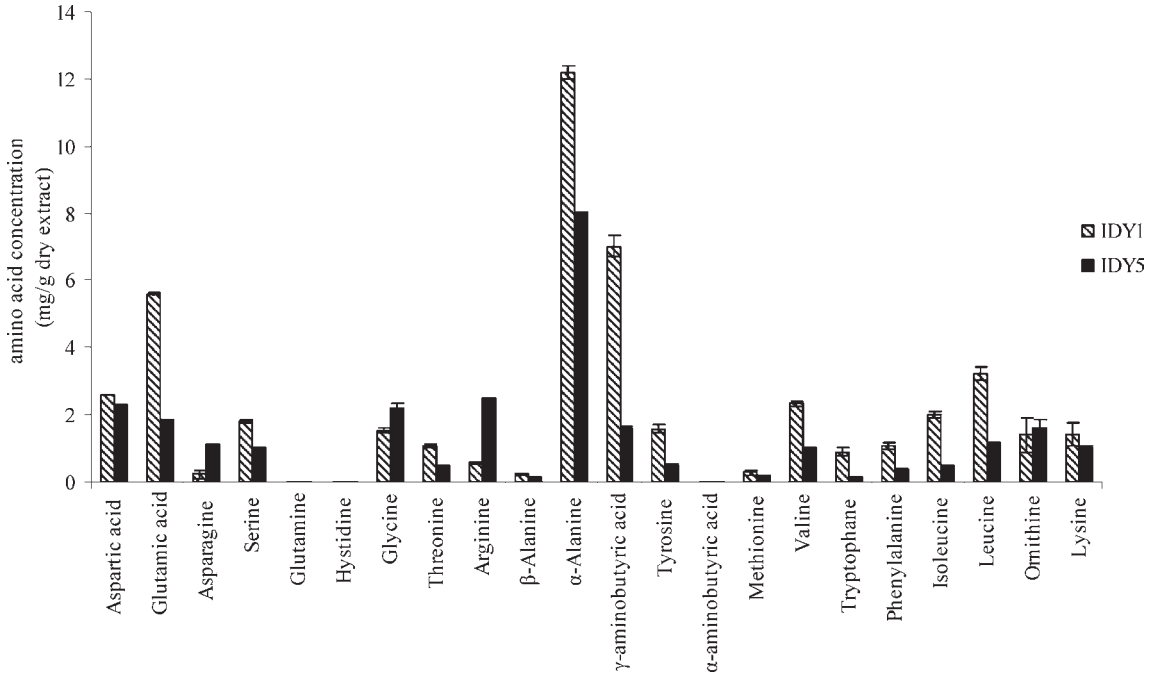

Figure 2. Free amino acid composition of the IDY1 and IDY5 extracts obtained with water.

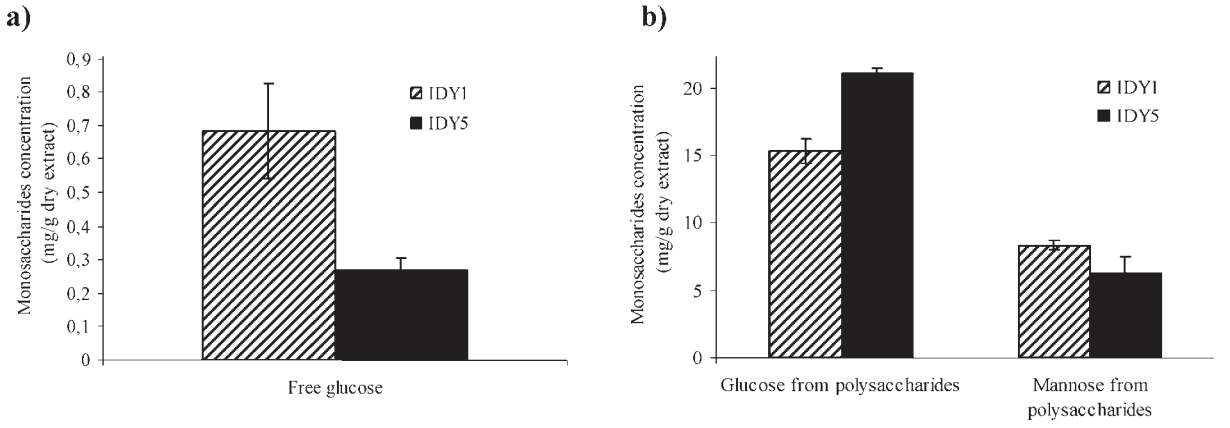

Figure 3. Concentration of free monosaccharides (**a**) and monosaccharides from polysaccharides (**b**) after the hydrolysis of IDY1 and IDY5 extracts obtained with water.

wall (*22*). Differences in monosaccharide concentration in both extracts were not as high as those we found for the amino acid composition. The IDY1 extract showed significantly higher concentration of free glucose, whereas the total content in monosaccharides from polysaccharides was very similar in both extracts, with values of 23.6 and 27.3 mg g^{-1} of dry extract for IDY1 and IDY5 extracts respectively. The ratio glucoproteins/mannoproteins (calculated from the glucose/mannose ratio after the hydrolysis) was 65/35 and 77/23 for IDY1 and IDY5 extracts respectively, showing in both cases a higher concentration of glucoproteins compared to mannoproteins, which is in agreement with the composition of the wall of *Saccharomyces cerevisiae* (*31*). The differences in the ratios between both extracts may be explained by differences during the manufacturing of both preparations, such as the nitrogen content and pH of the culture medium and the temperature and aeration conditions during the growth of the yeast, which, it has been shown, can influence the cell wall composition (*32*).

Free glucose is the most preferred monosaccharide to be consumed by wine LAB (*12*, *33*, *34*). However, the concentration of glucose in IDY1 and IDY5 extracts was very similar, which cannot explain the differences on the LAB growth exhibited by both extracts (**Figure 3**). On the other hand, the effect of polysaccharides from yeast on the growth of some LAB such as *O. oeni* has also been reported (*35*). This effect could be related to the capacity of mannoproteins to adsorb short- and medium-chain fatty acids that can inhibit the growth of some LAB such as *O. oeni* (*36*). In addition, the ability of some LAB with specific enzymatic activities to degrade yeast polysaccharides (e.g. $\beta(1-3)$ glucanase) may improve the nutritional content of the medium, thus promoting bacterial growth (*25*, *37*). Based on these explanations, both extracts IDY1 and IDY5 might have stimulated the growth of the three LAB under study, however, IDY5 not only did not show a promoting effect but rather showed an inhibition effect on the growth of the three LAB, and mainly, on the growth of *O. oeni* (**Table 3**). Therefore IDY5 extracts seemed to contain other components, that may be absent or in lower concentration in the IDY1 preparation.

Fatty Acids. The analysis of fatty acids in the extracts can be of great interest since they can affect the growth of LAB in wines (*36*, *38*). The composition in fatty acids in both extracts (IDY1 and IDY5) is shown in **Table 4**. The percentage of each compound in the sample was calculated as percentage of TIC

Table 4. Fatty Acids Composition of IDY1 and IDY5 Hexane Extracts

			IDY1		IDY5	
peak no.	RT	fatty acids	area ($\times 10^6$)	(%)[a]	area ($\times 10^6$)	(%)
1	3.15	octanoic acid	nd[b]	0	2.47 ± 0.13	0.34 ± 0.02
2	4.25	decanoic acid	nd	0	26.53 ± 0.23	3.65 ± 0.02
3	5.4	dodecanoic acid	nd	nd	2.27 ± 0.02	0.31 ± 0.01
4	6.52	myristic acid (C14:0)	nd	nd	4.97 ± 0.12	0.68 ± 0.01
5	7.92	palmitic acid (C16:0)	16.86 ± 2.61	8.82 ± 0.73	142.43 ± 1.19	19.58 ± 0.12
6	8.2	palmitoleic acid (C:16:1)	71.71 ± 4.81	37.65 ± 0.2	60.44 ± 1.57	8.31 ± 0.34
7	10.01	estearic acid (C18:0)	8.73 ± 1.15	4.57 ± 0.28	40.96 ± 2.72	5.63 ± 0.29
8	10.36	oleic acid (C18:1)	56.08 ± 6.02	29.40 ± 1.03	31.28 ± 4.53	4.30 ± 0.56
9	11.08	linoleic acid (C18:2)	3.63 ± 0.71	1.92 ± 0.51	43.62 ± 3.25	5.99 ± 0.36
10	12.27	α-linolenic acid (C18:3)	nd	nd	5.21 ± 0.59	0.72 ± 0.07
11	20.35	peak 11	33.48 ± 0.11	17.62 ± 1.34	147.43 ± 10.17	20.26 ± 1.10
12	30.8	peak 12	nd	nd	219.71 ± 10.43	30.22 ± 1.88
total			190.49 ± 13.77		727.33 ± 10.67	
∑MCFA[c]			nd	nd	31.28	4.30
∑SFA[d]			25.59	13.40	188.35	25.90
∑UFA[e]			131.42	68.98	140.56	19.32
UFA/SFA			5.14	5.15	0.75	0.75

[a] Normalized TIC signals = (TIC volatile compound/TIC from all volatile compounds) × 100. [b] Not detected. [c] Medium-chain fatty acids. [d] Long-chain saturated fatty acids. [e] Long-chain unsaturated fatty acids.

response compared to the sum of TIC responses from all the fatty acids in the sample. This allowed us to have a relative estimation of the percentage of each compound in the extracts. As can be seen in **Table 4**, the main fatty acids in the IDY extracts included medium-chain fatty acids, such as octanoic, decanoic and dodecanoic acids; long-chain saturated fatty acids such as myristic, palmitic and estearic acids and long-chain unsaturated fatty acids such as palmitoleic, oleic, linoleic and α-linolenic acids. All of them were identified in both extracts, and in general, this composition was in agreement with that corresponding to the plasmatic membrane of active dry yeast (*39*, *40*). Two other compounds that eluted at retention times of 20.35 and 30.80 min (peaks 11 and 12, respectively) were also found. Compared to the total fatty acids content, these compounds were found in larger amount in both extracts. The compound corresponding to peak 11 constituted 20% of the total fatty acid composition of both extracts, and it was tentatively identified as dioctyl adipate. This compound is widely used for the manufacturing of plastic and food packing material (*41*), and it may have migrated from the packaging into the IDY preparations. On the other hand, the compound corresponding to peak 12 was only detected in the IDY5 extract. It was tentatively identified as squalene, an intermediate in the synthesis of ergosterol in yeasts (*42*). Ergosterol can play an important role in the cell, reducing the damage of the plasmatic membrane during the rehydration of the ADY (*6*). Therefore, the ergosterol synthesis may have been promoted during the manufacturing of IDY5 preparation, which may explain the presence of intermediate metabolic products such as squalene. Comparing the fatty acid composition of both extracts, IDY5 showed a higher number of different fatty acids (twelve) compared to IDY1 (six) (**Table 4**). In contrast to what happened with the extract IDY1, the extract IDY5 showed some medium-chain fatty acids, such as α-linolenic acid and squalene. In addition, both extracts showed differences in the composition of saturated and unsaturated fatty acids. The percentage of unsaturated fatty acids (UFA) in IDY1 extract was almost five times higher than the concentration of saturated fatty acids (SFA) (**Table 4**). On the contrary, SFAs were more abundant in the IDY5 extract. These differences might be due to the effect of several factors related to the manufacturing conditions of both preparations, which can affect yeast plasmatic membrane composition such as differences in the nitrogen source (*40*), the aerobic and anaerobic conditions (*43*), the presence of lipids in the culture medium (*43*), the temperature and the species and strain of yeast (*39*) among others. It was previously shown that extracts obtained with hexane from IDY1 and IDY5 preparations inhibited the growth of LAB, although this effect was higher for the IDY5 extract (**Table 3**). This fact may be explained by the greater proportion of fatty acids in the IDY5 extract compared to the IDY1. This is in agreement with the results of Guilloux-Benatier et al. (*26*), who showed the inhibition on the growth of *O. oeni* by a mixture of fatty acids including short-, medium- and long-chain fatty acids. Besides, the proportion of short and medium chain fatty acids was also higher in the IDY5 extracts (**Table 4**). These compounds, and mainly decanoic acid, which represented the 3.6% of the total fatty acid content in IDY5 extract (**Table 4**), can inhibit the growth of some LAB as it has been widely described (*10*, *36*, *44*).

Volatile Compounds. Besides the fatty acid analysis the volatile composition of the hexane extracts from both preparations was also determined. **Table 5** shows the compounds tentatively identified in the samples. The percentage of TIC response of each compound compared to the sum of the TIC from the total volatiles identified in the samples was calculated to have an estimation of the proportion of each volatile compound in the extract. As can be seen, both extracts exhibited larger differences regarding the volatile composition. The IDY5 extract showed the highest number of different volatile compounds, and, in general, the TIC areas were also higher than in the IDY1 extract. In fact, the sum corresponding to the TIC areas of all the volatile compounds identified in the IDY5 extract was almost five times higher than those corresponding to the IDY1 extract. A total of 24 volatile compounds were identified in both samples, 17 of them were identified in the IDY5 extract and 12 in the IDY1. It is worth noticing that the volatile profile of IDY1 was mainly constituted by heterocyclic nitrogen compounds that are products from the reaction between sugars and amino acids and/or peptides present in the IDY preparations, which can take place during the thermal drying, in the last steps of their manufacturing (*19*, *45*). The major volatile compounds tentatively identified in the IDY1 extract were 2-pyrrolidone and 2-ethyl-3,5-dimethylpyrazine. However, IDY5 extract showed a different volatile profile, and besides the heterocyclic volatile nitrogen compounds from Maillard reaction, other compounds such as medium-chain

Table 5. Volatile Compounds Tentatively Identified in the IDY1 and IDY5 Hexane Extracts

			RI			IDY1		IDY5	
peak no.	RT	compounds	exptl[a]	lit.[b]	ID[c]	TIC ($\times 10^6$)	(%)[d]	TIC ($\times 10^6$)	(%)
1	11.25	2,5-dimethylpyrazine	908	913	RI, MS	2.01 ± 0.05	7.36 ± 0.17	nd[e]	nd
2	15.07	2-ethyl-6-methylpyrazine	997	997	RI, MS	0.35 ± 0.01	1.29 ± 0.04	nd	nd
3	15.16	2-ethyl-5-methylpyrazine	999	993	RI, MS	0.53 ± 0.09	1.95 ± 0.05	nd	nd
4	15.23	2,3,5-trimethylpyrazine	1000	1000	RI, MS	3.05 ± 0.1	11.18 ± 0.4	0.75 ± 0.04	0.49 ± 0.00
5	16.03	2-hydroxy-3-methyl-2-cyclopenten-1-one	1020		MS	nd	nd	0.91 ± 0.06	0.60 ± 0.01
6	17.44	2-acetylpyrrole	1055	1060	RI, MS	0.31 ± 0.01	1.15 ± 0.04	1.30 ± 0.26	0.85 ± 0.13
7	17.83	2-pyrrolidone	1064	1076	RI, MS	11.10 ± 0.24	40.69 ± 0.76	6.39 ± 0.61	4.22 ± 0.19
8	18.20	2-ethyl-3,5-dimethylpyrazine	1073	1083	RI, MS	6.37 ± 0.02	23.35 ± 0.01	2.05 ± 0.06	1.36 ± 0.03
9	19.13	isopropylmethoxypyrazine	1096	1097	RI, MS	0.89 ± 0.11	3.25 ± 0.40	nd	nd
10	19.50	3-hydroxy-2-methyl-4*H*-pyran-4-one	1106		MS	nd	nd	21.94 ± 2.62	14.49 ± 1.00
11	20.03	1*H*-pyrrole 5-methyl, 2-carboxaldehyde	1120	1105	RI, MS	nd	nd	1.50 ± 0.14	0.99 ± 0.04
12	21.30	2,3-diethyl-6-methylpyrazine	1153	1158	RI, MS	0.25 ± 0.00	0.91 ± 0.01	nd	nd
13	21.46	3,5-diethyl-2-methylpyrazine	1157	1160	RI, MS	0.82 ± 0.02	2.99 ± 0.1	nd	nd
14	22.11	octanoic acid	1175	1175	RI, MS	nd	nd	8.62 ± 0.12	5.71 ± 0.21
15	23.62	benzothiazole	1215	1221	RI, MS	0.36 ± 0.06	1.32 ± 0.03	1.36 ± 0.13	0.90 ± 0.04
16	24.72	benzeneacetic acid	1246	1254	RI, MS	nd	nd	1.46 ± 0.27	0.97 ± 0.23
17	27.10	2,5-dimethyl-3-isopentylpyrazine	1315	1315	RI, MS	1.24 ± 0.04	4.53 ± 0.16	nd	nd
18	27.65	benzenepropanoic acid	1331	1343	RI, MS	nd	nd	2.39 ± 0.27	1.59 ± 0.26
19	29.00	decanoic acid	1372	1380	RI, MS	nd	nd	81.97 ± 5.12	54.22 ± 0.64
20	29.61	ethyl decanoate	1391	1391	RI, MS	nd	nd	2.15 ± 0.31	1.42 ± 0.13
21	34.80	dodecanoic acid	1560	1567	RI, MS	nd	nd	10.26 ± 1.40	6.82 ± 1.27
22	35.66	dodecanoic acid ethyl ester	1589	1581	RI, MS	nd	nd	3.36 ± 0.47	2.22 ± 0.20
23	40.25	myristic acid	1755	1768	RI, MS	nd	nd	2.51 ± 0.71	1.67 ± 0.55
24	43.81	nonadecane	1893	1900	RI, MS	nd	nd	2.23 ± 0.36	1.47 ± 0.16
		total				27.27 ± 0.1		155.37 ± 0.1	

[a] RIs calculated with an alkane mixture (C5−C30). [b] RIs reported in the literature (NIST web database). [c] Identification method: RI identified by retention index, MS identified by mass spectra (Wiley libraries). [d] Normalized TIC signals = (TIC volatile compound/TIC from all volatile compounds) × 100. [e] Not detected.

fatty acids and their corresponding ethyl esters, such as ethyl decanoate and ethyl dodecanoate, were also identified. In this extract (IDY5), the major compounds corresponded to decanoic acid and the volatile compound tentatively identified such as 3-hydroxy-2-methyl-4*H*-pyran-4-one.

The volatile compounds identified in the two extracts may be responsible for the inhibition on the growth of LAB (**Table 3**). In fact, besides the higher amount of fatty acids detected in the IDY5 extract, the corresponding sterified forms present in greater amount in the IDY5 extract, may also have inhibited the LAB growth (*26*). In addition, the heterocyclic volatile nitrogen compounds present in both preparations could also contribute to the observed inhibitory effect. In fact, it has been previously shown that some of these compounds can have antimicrobial activities (*46*, *47*). However, the effect of these volatiles from IDY on wine LAB deserves further investigation.

In summary, the results from this work have shown that the PLE technique employing solvents of different polarity can be useful to obtain extracts from IDY preparations of different composition which have shown different effect on the growth of LAB. From the chemical characterization of the extracts, amino acids such as alanine, valine, leucine, methionine and threonine and mannose from polysaccharides promoted the growth of LAB while medium-chain fatty acids, such as octanoic, decanoic and dodecanoic acids, and their corresponding esters were more related to an inhibition of the bacterial growth. On the contrary, heterocyclic volatile nitrogen compounds also seemed to show an inhibition effect. Therefore, differences in the proportion of these compounds between the IDY preparations currently available in the market may have different consequences on wine LAB growth. As a whole, in spite of the limited number of LAB strains essayed, the results from this work should be considered as the starting point for deeper research with the objective of looking for more selective formulation of IDY preparations with specific enological applications and without provoking undesirable effects in wines.

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