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ARTICLE

Application of Supercritical CO_2 Extraction for the Elimination of Odorant Volatile Compounds from Winemaking Inactive Dry Yeast Preparation

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A procedure based on the application of supercritical CO_2 extraction to reduce and/or to remove odorant volatile compounds from a winemaking inactive dry yeast (IDY) preparation has been set up. By applying a factorial design, a screening of different temperatures and pressure conditions was assayed in order to determine the optimal deodorization conditions, and afterward the effect of several sample pretreatments was investigated. The best extraction conditions were achieved at 200 atm and 60 °C, applying the cryogenic grinding of the sample and using 40% (w/w) ethanol as cosolvent. By using these conditions, it was possible to reduce to approximately 70% of the volatile compounds present in the samples that may be released into the wines and therefore affecting their sensory characteristics. Odorant volatile compounds such as 2-methylhydroxypyrrole, 2-ethyl-6-methylpyrazine, and 2,3,5-trimethylpyrazine completely disappeared from the deodorized sample as verified by GC-O analysis. Additional experiments in model wines confirmed the low release of volatile compounds from the deodorized samples, without provoking any change to their nonvolatile composition (nitrogen compounds and neutral polysaccharides) that is related to the technological properties of these preparations.

KEYWORDS: Supercritical CO_2 extraction; inactive dry yeast preparations; volatile compounds; deodorization; wine

INTRODUCTION

Inactive dry yeast preparations (IDY) have increased considerably within the winemaking industry. The main applications of these products are as fermentation nutrients or to enhance the wine's organoleptic characteristics (decreasing wine astringency, improving wine mouthful, avoiding wine oxidation, and enhancing wine color and aroma). A revision on the main applications during winemaking has been recently published (*1*).

Depending on their composition, the winemaking yeast preparations can be classified in yeast hulls or walls (mainly include insoluble yeast components), yeast mannoproteins, and yeast autolysates. The latter can include in their composition soluble (nitrogen, polysaccharides, nucleic acids) and insoluble components (yeast walls, and membranes) from *Saccharomyces cerevisiae* after thermal or enzymatic inactivation (*2*, *3*). During the last production steps, and with the objective of obtaining powdered yeast preparations easily to dose into the wines, different procedures such as roller drying or spray drying can be used, and therefore yeast components can be exposed to high temperatures (*4*). In this case, the thermal reaction between yeast's polysaccharides and nitrogen compounds may therefore be promoted, giving rise to different types of Maillard heterocyclic volatile compounds (*5*−*7*). It has recently been shown that some volatile compounds present in some types of IDY preparations, such as alkylmethylpyrazines, may be released into model wines (*7*). The release of these and other volatile compounds may be responsible for sensory changes that have been reported in wines supplemented with these preparations (*5*).

The application of supercritical CO_2 for deodorization of different types of food matrices has been successfully employed in extracts obtained from aromatic herbs (*8*), oils (*9*), and milk fat (*10*). In addition, the usefulness of supercritical CO_2 for the extraction of pyrazines, which is one of the most representative groups of volatile compounds in IDY preparations (*5*, *7*), has been previously shown by Shen and co-workers (*11*). Sanagi and co-workers (*12*) have also used supercritical CO_2 application for the selective extraction of pyrazines from cocoa seeds.

The main advantages of CO_2 for the extraction of odorant compounds from food matrices over more conventional extraction methods are due to its low viscosity and relatively high diffusivity. Supercritical CO_2 has therefore better transport properties than liquids and can easily diffuse through complex food matrices, resulting in faster extraction yields (*13*). In addition, the possibility of modifying the working pressure and/or temperature conditions can influence its solvent capacity and, consequently, the selective extraction of sample components (*14*). Other advantages associated with the use of supercritical CO_2 are related to its safety. It is generally recognized as a safe (GRAS) solvent and environmentally friendly, and it has a relatively low

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Table 1. Pressure and Temperature Conditions Assayed for Deodorization of IDY Preparations

pressure (atm)	temp (°C)	density (g L^{-1})
100	40	629.9
100	85	221.6
200	40	840.7
200	85	594.3
150	52	700.3
150	52	700.3

cost compared to other solvents (*13*). These advantages make the supercritical CO_2 extraction a suitable method for deodorization purposes.

Since the addition of IDY preparations into wines may affect wine aroma characteristics, the objective of the present work is therefore to develop and to optimize a procedure based on the application of supercritical CO_2 extraction to reduce or to remove odorant volatile compounds present in a widely used commercial oenological IDY preparation without provoking adverse effects on the nonvolatile composition, which can be of interest for the winemaking and biotechnology industries.

MATERIALS AND METHODS

Samples. A rich polysaccharide commercial powdered inactive *S. cerevisiae* yeast preparation (IDY) from a yeast autolysate (therefore including soluble and insoluble compounds in its composition) suitable as a red wine organoleptic enhancer was supplied by Lallemand (Madrid, Spain).

Model Wines. The model wines used to study the release of volatile compounds from the IDY preparations into the wine were constituted by ethanol (120 mL L^{-1}) and tartaric acid (4 g L^{-1}). The pH was adjusted to 3.5 with a 0.1 N NaOH solution. All the solvents and reactants were purchased from Panreac Química S.A. (Barcelona, Spain).

Supercritical CO_2 Extraction. A Suprex PrepMaster (Suprex, Pittsburgh, PA) supercritical fluid extractor was used for all of the experiments. A 2 g sample of powdered IDY preparation was placed into a 5 mL stainless steel extraction cell. In some experiments 2 g of sample was ground by using a cryogenic grinder (Retsch, Haan, Germany), 5 cycles for 3 min at 25 Hz. Moreover, a cosolvent (ethanol from Panreac) was tested in order to improve the extraction yield. These experiments were done by adding between 4% and 40% (w/w) of ethanol into the IDY preparation and mixing it thoroughly in the extraction cell. The supercritical CO_2 (industrial quality; Praxair, Madrid, Spain) flow rate was controlled using a needle valve as variable restrictor. Total extraction time was 120 min using dynamic extraction conditions.

Different extraction pressures and temperatures were selected as variables to study their effect on the ability of the supercritical CO_2 to reduce the volatile compounds in the samples. A two-level factorial design was carried out considering the pressure (atm) and temperature (°C) as design factors to select the best extraction conditions. The design describes in six experiments ($2^2 + 2$ replicates in the center point) the curvature of the response surface. **Table 1** shows the assayed extraction conditions. Pressure was selected between 100 and 200 atm and temperatures between 40 and 80 °C. This allowed covering of a wide range of CO_2 densities (0.2−0.8 g mL^{-1}).

Analysis of Volatile Compounds Using Solid-Phase Microextraction (SPME). The extraction was performed by exposing an 85 μm Carboxen-PDMS fiber (Supelco, Bellefonte, PA) into the headspace of the sample. Three extraction approaches were used for the analysis.

Analysis of Volatile Compounds in Water Slurry of the Sample. A water slurry of the sample containing 0.4 g of a deodorized or control sample was introduced in a 20 mL vial containing 8 mL of a NaCl solution (0.9% w/v). Fifty microliters of an ethanolic solution of methyl nonanoate (50 mg L^{-1}) was added to the sample to be used as internal standard. The vial was capped with a PTFE/silicone septum (Supelco). After 10 min of equilibrium time, extraction was performed for 20 min at 50 °C in constant stirring mode.

Analysis of Volatile Compounds Directly in the Powder Sample. One gram of deodorized or control sample was placed in a 20 mL vial and capped with a PTFE/silicone septum (Supelco). After 10 min of equilibrium time, extraction was performed for 40 min at 50 °C in static mode.

Analysis of Volatile Compounds in a Model Wine. To simulate the release of volatile compounds from the IDY preparations into the wine, 0.4 g of a deodorized or control sample was introduced in a 20 mL vial containing 8 mL of a model wine that was capped with a PTFE/silicone septum (Supelco). Previously, 50 μL of an ethanolic solution of methyl nonanoate (50 mg L^{-1}) was added to the sample to be used as internal standard. After 10 min of equilibrium time, extraction was performed for 20 min at 50 °C in constant stirring mode.

Gas Chromatography−Mass Spectrometry Analysis (GC-MS). Volatile compounds extracted by applying SPME were analyzed using an Agilent 6890N GC system with a split/splitless injector coupled to an Agilent 5973N quadrupole mass spectrometer (Agilent, Palo Alto, CA). The system was controlled by Agilent MSD ChemStation software (D.01.02 16 version). Separation was achieved using a coated capillary column with 30 m × 0.25 mm i.d. × 0.25 μm film thickness (HP-5M, Agilent). The SPME fiber was desorbed into the injector port at 280 °C for 5 min in splitless mode. Helium was used as the carrier gas (1 mL min^{-1}). The oven temperature was programmed as follows: 40 °C as the initial temperature, held for 5 min. In the first ramp, the temperature increased to 120 °C at 2 °C/min and in the second ramp, at 8 °C/min to 220 °C and held for 5 min. The temperatures of the quadrupole and transfer line were 150 and 230 °C, respectively; electron impact mass spectra were recorded at 70 eV, and the ionization current was 10 μA. The acquisitions were performed in full scan mode (from 35 to 450 amu).

The identification of compounds was carried out by comparing their mass spectra and/or retention times with those of standard compounds when possible or by comparison with those reported in the mass spectra libraries (NIST98 and Wiley 5). Moreover, linear retention indexes (LRIs) were experimentally calculated with an *n*-alkane mixture (C_5−C_{30}) and compared with those available in the literature. All of the reference compounds were provided by Sigma-Aldrich (St. Louis, MO).

Gas Chromatography−Olfactometry Analysis (GC-O). The most powerful odorants present in the IDY preparation were determined by high-resolution GC-O using a Hewlett-Packard (HP) 6890 gas chromatograph equipped with a split/splitless injector, a flame ionization detector (FID), and a sniffing port. The same column and chromatographic conditions described in Gas Chromatography−Mass Spectrometry Analysis (GC-MS) were used. Volatile compounds were extracted from a water slurry of the sample (IDY preparation), using SPME, following the extraction conditions described in Analysis of Volatile Compounds in Water Slurry of the Sample. A panel of six trained judges in GC-O analysis (same panel for all of the samples) evaluated the effluents enriched with purified, humidified air (100 mL min^{-1}). Each analysis was performed in duplicate in two different sessions. Sniffing of the chromatogram was divided into two parts of about 25 min. Each person participated in the sniffing of both parts but during two distinct sessions to avoid fatigue and lassitude. For each odor stimulus, panelists recorded the detection time and described the odor and intensity (low, medium, and high). Before the analysis, preliminary experiments were performed in order to choose the appropriate lexicon to be used for all the judges. Linear retention indices of the compounds were calculated using a series of *n*-alkanes (C_8−C_{30}).

Chemical Composition of Model Wines Supplemented with Control and Deodorized IDY Preparations. To establish the effect of the deodorization on the nonvolatile composition of model wines supplemented with control and deodorized IDY preparations (10 g L^{-1}), the concentration of neutral polysaccharides and nitrogen compounds was determined.

The concentration of neutral polysaccharides was determined by the phenol−sulfuric method, according to Segarra and co-workers (*15*). The absorbance was determined at 490 nm. The results were expressed in milligrams of mannose per liter.

The concentration of high molecular weight nitrogen compounds (HMWN) was determined following the Bradford method (*16*), based on the reaction of the HMWN compounds with the Coomassie blue G-250 reagent. The absorbance was determined at 595 nm, 15 min after the addition of the reactant in a spectrophotometer (DU 70; Beckman, Fullerton, CA). The results were expressed in milligrams of nitrogen per liter. The standard used was bovine serum albumin (BSA) from Sigma-Aldrich. In order to express the results, the molecular mass of BSA

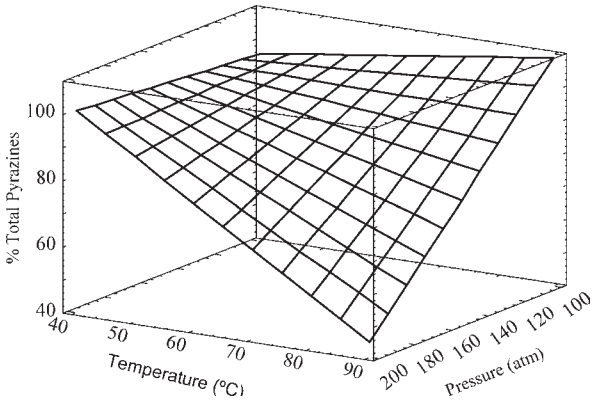

Figure 1. Response surface diagram corresponding to the percentage of reduction of TIC surface from total pyrazines in the IDY preparation depending on the CO_2 extraction conditions, pressure (P), and temperature (T): % residual pyrazine samples = $27.34 + 0.59P + 1.619T - 0.0013PT$ (R2 = 98.62%).

(66432 g mol^{-1}) and the number of nitrogen atoms present in the molecule (10276 g mol^{-1}) were taken into account.

Free amino acids were determined by the method of Doi and coworkers (*17*) 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 and peptides, following the conventional method of the ninhydrin (*17*), based on the reaction of the amino group with a mixture of ninhydrin/Sn. The absorbance was determined at 570 nm. A DU 70 spectrophotometer (Beckman) was used for both determinations. 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).

RESULTS AND DISCUSSION

Optimization of the Deodorization Conditions. *Supercritical CO_2 Temperature and Pressure Conditions*. As a first step in the optimization of IDY deodorization a factorial design 2^2 covering a wide range of temperatures and pressures was used (**Table 1**). This approach has been suggested by several authors for the extraction of volatile compounds (*9*, *18*−*20*). To evaluate the efficiency of the deodorization process, we focused on the extraction conditions that allowed the greatest reduction of the total ion current (TIC) response area of some target volatile compounds characteristic of these types of IDY, such as 2-ethyl-3,5-dimethylpyrazine, 2,5-dimethyl-3-isopentylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2,3,5-trimethylpyrazine, 2,3-diethyl-6-methylpyrazine, and 3,5-diethyl-2-methylpyrazine. (*1*, *5*). The reduction of the targeted volatile compounds was directly related to the increase in temperature and pressure (**Figure 1**). The increase in the pressure may have favored the solubility of volatile compounds in the CO_2, while the increase in the temperature may have improved the mass transfer coefficient of the volatile compounds into the extracting fluid (*21*). The most favorable extraction conditions were achieved by using 80 °C and 200 atm. However, by using these conditions, the formation of new volatile compounds was observed. This may have been promoted by the reaction between polysaccharides and nitrogen compounds present in the IDY preparation at high working temperatures. It was therefore decided for our deodorization purposes to set the extraction temperature at 60 °C but keep the pressure at 200 atm.

Sample Preparation Conditions. Once the CO_2 extraction conditions were set up, in order to increase the extraction yield of volatile compounds and to obtain the maximum deodorization of the IDY preparations, two improvements in the sample preparation

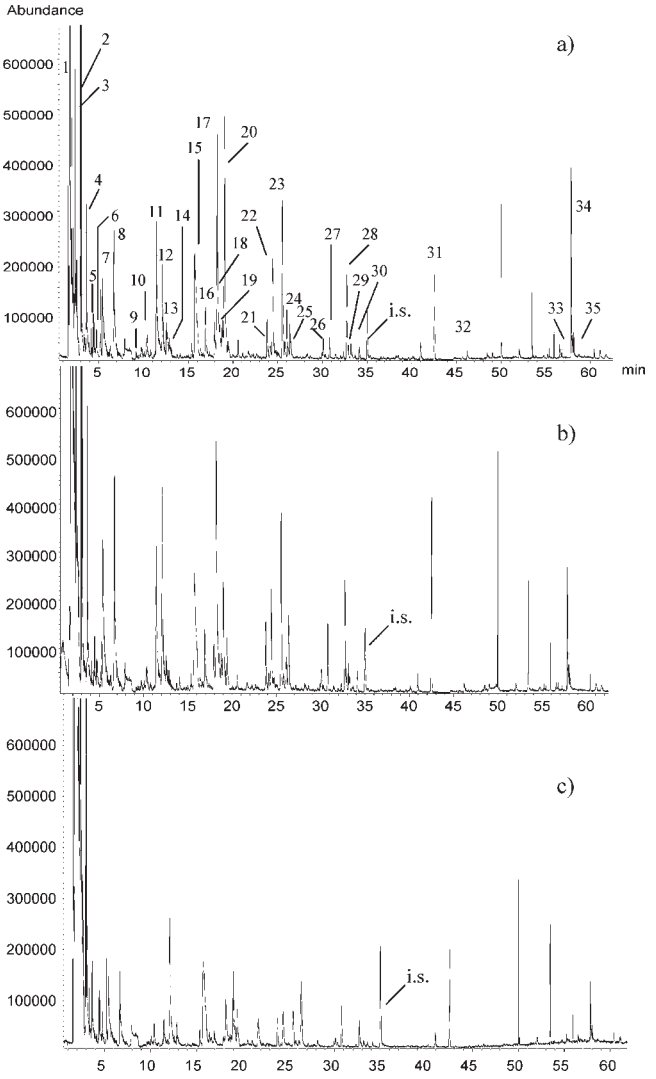

Figure 2. Chromatograms obtained by GC-MS using SPME corresponding to (**a**) control sample, (**b**) control sample after grinding, and (**c**) deodorized sample prepared with grinding and cosolvent addition (ethanol 40% w/w). Peak numbers correspond with those described in **Table 2**. i.s.: internal standard.

were made. The first one consisted of the cryogenic grinding of the sample, while the second one was the use of a modifier or cosolvent during the supercritical CO_2 extraction process. Panels **a** and **b** of **Figure 2** show an increase in the extraction of volatile compounds from IDY preparations, which may be due to the cell wall lysis (*22*) after the cryogenic grinding of the sample. Moreover, the reduction in sample size in the ground sample may have increased the contact surface between the sample and the solvent during the extraction promoting the mass transfer of volatile compounds into the solvent. To overcome the problem related to the low polarity of CO_2, the use of ethanol as cosolvent during the extraction process was also considered, since it has been shown that the use of modifiers, even at very low concentration, can produce substantial changes in the solvent properties of supercritical CO_2 (*23*). Although different concentrations of ethanol, ranging from 4% to 40% (w/w), were assayed, the highest extraction of volatile compounds without provoking the clogging of the sample in the extraction cell was achieved by adding 40% (w/w) ethanol into the IDY sample before the extraction. **Figure 2c** shows the chromatogram corresponding to the deodorized sample obtained with the optimized CO_2 extraction and

Table 2. GC-MS Identification and Percentage of TICr Reduction of Volatile Compounds from IDY Preparations after Deodorization

peak	Tr exp	LRI exp[a]	LRI ref[b]	compd	ID[c]	% TICr reduction[d]
1	2.37	600	600	hexane	RI, MS, R	57.4
2	2.95	644	641	3-methylbutanal	RI, MS	38.1
3	3.06	656	650	2-methylbutanal	RI, MS	49.8
4	3.62	700	700	heptane	RI, MS, R	74.9
5	4.68	736	785	dimethyl disulfide	RI, MS	87.0
6	4.79	740		ketone	MS	50.9
7	5.39	760		methylbenzene	MS	67.3
8	6.66	802	800	octane	MS, R	81.0
9	9.18	848	853	2-methylhydroxypyrrole	RI, MS	100
10	10.37	869		unknown		59.2
11	11.45	890	889	2-heptanone	RI, MS	91.4
12	12.10	902	902	heptanal	RI, MS, R	53.9
13	12.56	908	908	2,5-dimethylpyrazine	RI, MS	83.2
14	12.87	913		2,5-dihydrofuran	MS	22.5
15	15.71	954	961	benzaldehyde	RI, MS, R	54.4
16	16.95	972		acetyl-1*H*-imidazole	MS	89.6
17	18.23	991	980	2-octanone	RI, MS	86.6
18	18.50	994	997	2-ethyl-6-methylpyrazine	RI, MS	100
19	18.76	998	1000	2,3,5-trimethylpyrazine	RI, MS	100
20	19.04	1002	997	2-octanol	RI, MS, R	52.2
21	23.83	1068		3-ethyl-4,5-dihydro-1*H*-pyrazole	MS	74.7
22	24.43	1076	1082	2-ethyl-3,5-dimethylpyrazine	RI, MS	72.2
23	25.5	1090	1091	2-nonanone	RI, MS	87.1
24	26.14	1099	1089	ethyl heptanoate	RI, MS, R	100
25	26.39	1103	1108	nonanal	MS, R	18.1
26	29.99	1153	1153	2,3-diethyl-5-methylpyrazine	RI, MS	67.9
27	30.13	1155	1157	3,5-diethyl-2-methylpyrazine	RI, MS	80.1
28	32.75	1192	1184	2-decanone	RI, MS	86.8
29	33.20	1198	1189	ethyl octanoate	RI, MS, R	94.5
30	34.13	1211		methylpyrrole	MS	86.3
31	40.97	1313	1314	2,5-dimethyl-3-isopentylpyrazine	RI, MS	40.6
32	46.22	1397	1381	ethyl decanoate	RI, MS, R	81.9
33	55.39	1794	1780	ethyl tetradecanoate	RI, MS	100
34	57.83	1972	1955	ethyl 8-hexadecenoate	RI, MS	73.2
35	58.114	1993	1982	ethyl hexadecanoate	RI, MS	81.7

[a] LRIs calculated with an alkane mixture (C5−C30). [b] From Flavornet (http://www.flavornet.org, accessed Oct 2009) database and from NIST web chemistry book (2005) (http://www.webbook.nis.gov/chemistry). [c] Identification based on the Willey Mass Spectra Library (MS) by comparison of the experimental and literature retention index (RI) and by comparison with reference compounds (R). [d] % reduction of the relative TIC response = TICr control sample − (TICr deodorized sample)/(TICr control sample) × 100.

sample pretreatment conditions. When panel **a** and panel **c** of **Figure 2** are compared, the latter shows a higher reduction in the TIC surfaces for most of the volatile compounds. Therefore, the final selected conditions for the deodorization of IDY were cryogenic grinding, mixing with 40% ethanol (w/w), and extracting with CO_2 at 60 °C and 200 atm.

Effect of Deodorization on the Volatile Profile of the IDY Preparation. Table 2 shows the volatile compounds identified and tentatively identified in the control IDY sample and the percentage of TIC reduction produced by the application of the deodorization process. To do so, we have compared the relative TIC areas of each peak (TIC peak/TIC internal standard) between the control and the deodorized sample. As can be seen in **Table 2**, most of the volatile compounds corresponded to heterocyclic nitrogen volatile compounds. Most of them have been previously identified in yeast-derived preparations (*5*−*7*), and they were likely formed by thermal reactions during the last steps in their production, in which they can be exposed to high temperatures for drying and obtaining powdered preparations. Other volatile compounds in the sample included branched aldehydes (2- and 3-methylbutanal) produced by the strecker degradation of the corresponding amino acids or peptides (*24*). In addition, some straight chain aldehydes such as heptanal and nonanal were also identified. These compounds were most likely products of lipid oxidation during the thermal processing of IDY. Additional compounds identified in the samples were long chain fatty acids (LCFA) and their ethyl esters, such as ethyl decanoate, ethyl tetradecanoate, ethyl 9-hexadecenoate, and ethyl hexadecanoate (**Table 2**, peaks 32−35). These compounds are major fatty acids from *S. cerevisae* wine strains (*25*, *26*).

Most of the volatile compounds identified in the IDY preparation were reduced or eliminated after deodorization. In general, a reduction between 38% and 100% was observed in the TICr surfaces of all the volatile compounds identified in the samples (**Table 2**). The averaged percentage of TICr reduction was 72.7%. However, some volatile compounds, such as 2-methylhydroxypyrrole, 2-ethyl-6-methylpyrazine, 2,3,5-trimethylpyrazine, and the esters ethyl heptanone and ethyl tetradecanoate, practically disappeared from the deodorized sample.

A gas chromatography−olfactometry analysis was performed to determine the odorant impact of the volatile compounds identified in the control IDY sample (without deodorization). Only the odors described by four of the six judges whose results where coincident were considered. From the 18 odors identified in the chromatogram, only some of them were tentatively associated with some of the volatile compounds previously identified in the samples (**Table 3**). Most of them were associated with volatile compounds belonging to the alkylmethylpyrazine group. In the GC-O analysis, these compounds were associated with "popcorn", "roasted nuts", "burnt", and other thermal reaction-related odors. The volatile compounds associated to some of these descriptors were tentatively identified as 2-methyl-1*H*-pyrrole,

Table 3. Odor Compounds Identified in the IDY Preparation by SPME-GC-O Analysis

odor[a]	retention index	descriptors (intensity)[b]	tentative identification
A		burnt, yeast (++)	unknown
B		chips, burnt (++)	unknown
C	640	rancid, nuts (+)	2- or 3-methylbutanal
D	834	fruity, strawberry, candy (+)	ester (probable)
9	892	popcorn, nuts, bread (+++)	2-methyl-1-hydroxypyrrole
13	910	popcorn, corn snacks (+++)	2,5-dimethylpyrazine
15	958	candy, sweet (+)	benzaldehyde
E	994	toffee, vanilla, sweet (+)	unknown
18	1015	popcorn, corn snacks (+++)	2-ethyl-6-methylpyrazine
19	1036	popcorn, corn snacks, broth (+++)	2,3,5-trimethylpyrazine
21	1050	popcorn (+++)	3-ethyl-4,5-dihydro-1*H*-pyrazole
22	1064	burnt, smoke (++)	2-ethyl-3,5-dimethylpyrazine
F	1070	dry grass, mushroom, boiled potato (++)	ketone?
26	1120	toasted nuts, toasted corn (++)	2,3-diethyl-6-methylpyrazine
27	1130	roasted nuts, toasted corn (++)	3,5-diethyl-2-methylpyrazine
H	1146	rancid, dry flowers, moldy (++)	unknown
I	1172	yeast, burnt (++)	ketone?
30	1200	popcorn (+)	1-methylpyrrole

[a] Odor numbers correspond to those included in **Table 2**. Letters are used for unidentified compounds. [b] Odor intensity: (+) low; (++) medium; (+++) high.

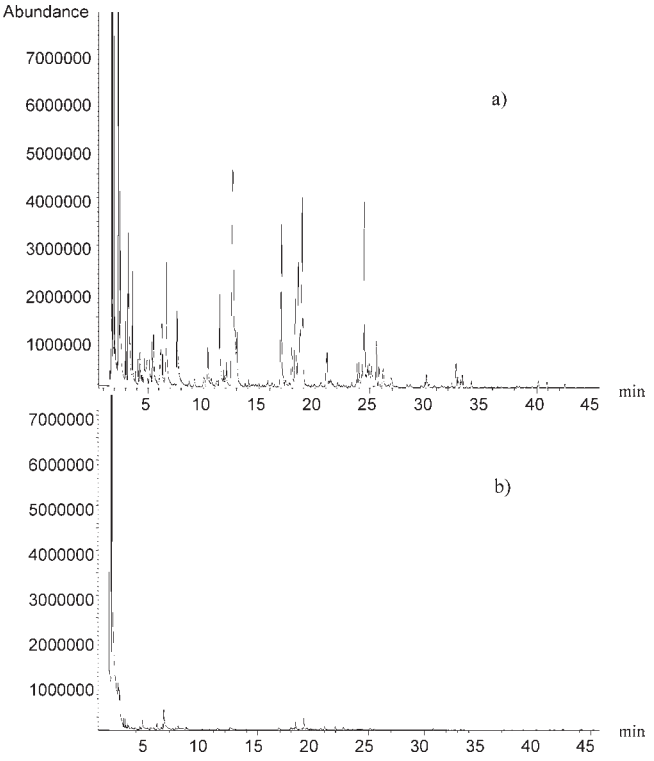

Figure 3. Chromatograms obtained by GC-MS and direct SPME of the sample powders in static extraction mode corresponding to (**a**) control sample and (**b**) deodorized sample.

2,5-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2,3,5-trimethylpyrazine, and 3-ethyl-4,5-dihydro-1*H*-pyrazole (peaks 9, 13, 18, 19, and 21, respectively). Peaks 9, 18, and 19 were completely absent in the deodorized samples (**Table 2**), while peak 13 corresponding to 2,5-dimethylpyrazine was reduced to more than 83% (**Table 2**). In addition, other impact compounds tentatively identified in the samples such as 2,3-diethyl-6-methylpyrazine, 3,5-diethyl-2-methylpyrazine, and 1-methylpyrrole (peaks 26, 27, and 30) showed a percentage of peak reduction between 67% and 86% in the deodorized sample compared to the control sample. According to sniffers' perception, peaks with retention times higher than 35 min did not provide any typical odor, meaning a little odor impact of the long chain fatty acids and their respective esters in the IDY preparations.

To confirm only the sensory relevance of the reduction of potent aroma compounds in the deodorized IDY sample, a simple sensory test was performed. A control and a deodorized sample were presented to 20 untrained judges who were asked to select the sample with least odor intensity. All the judges agreed on the lack of a typical odor in the deodorized preparation. However, the control sample was described as "baby food", "pet food", "broth", and "roast beef" like odor.

To have a closer knowledge on the volatile compounds the judges were perceiving during the sensory test, the same samples (control and deodorized IDY), which were presented to the judges, were directly extracted using SPME. Panels **a** and **b** of **Figure 3** show the corresponding volatile profiles. As can be seen, the chromatogram of the deodorized sample (**Figure 3b**) hardly showed volatile compounds compared to the nondeodorized sample, which is in agreement with the lack of odor noticed by the judges. It is also important to underline that the volatile profiles obtained by applying direct SPME on the IDY powders and those obtained from a water slurry of the sample (**Figure 2**) were different. The solubility properties of some of the volatile compounds present in the IDY preparations likely favor the transfer of volatile compounds to the headspace. In addition, the lower sensitivity of the static headspace SPME has been extensively documented (*27*).

Effect of Deodorization on the Volatile Profiles and Chemical Composition of Model Wines Supplemented with IDY Preparations. To better know the effect of the deodorization on the aroma release of volatile compounds from IDY preparations in more similar conditions to winemaking, deodorized and control IDY preparations were added to model wines. The volatile composition of the model wines was analyzed using SPME. **Figure 4** shows the volatile profiles of both types of model wines. The model wine supplemented with the deodorized sample (**Figure 4b**) showed in general fewer peaks and a reduction in the TIC of most of them compared to the wine supplemented with the control IDY preparation. It is also noticeable the great reduction observed in some powerful odorant volatiles previously identified by GC-O, such as 2-heptanone (peak 11), benzaldehyde (peak 15), 2-ethyl-6-methylpyrazine (peak 18), 2,3,5-trimethylpyrazine (peak 19), 3-ethyl-4,5-dihydro-1*H*-pyrazole (peak 21), and 3-ethyl-3,5-dimethylpyrazine (peak 22). The results clearly indicated the lower impact on the volatile profile of the wine of using deodorized IDY preparations compared to the original IDY sample.

However, since most of the applications for the use of IDY preparations are related to the nonvolatile composition and specifically to polysaccharides and nitrogen compounds (*2*, *3*, *7*), it was also important to establish whether these compounds were affected by the deodorization process. Therefore, the nitrogen and polysaccharide composition of the model wines supplemented with the deodorized and control IDY preparation was determined. The results showed that the concentrations of high molecular weight nitrogen (HMWN), free amino acids, peptides, and neutral polysaccharides were almost the same in both types of model wines (**Table 4**). In fact, the difference in composition between both types of wine samples was even lower than those

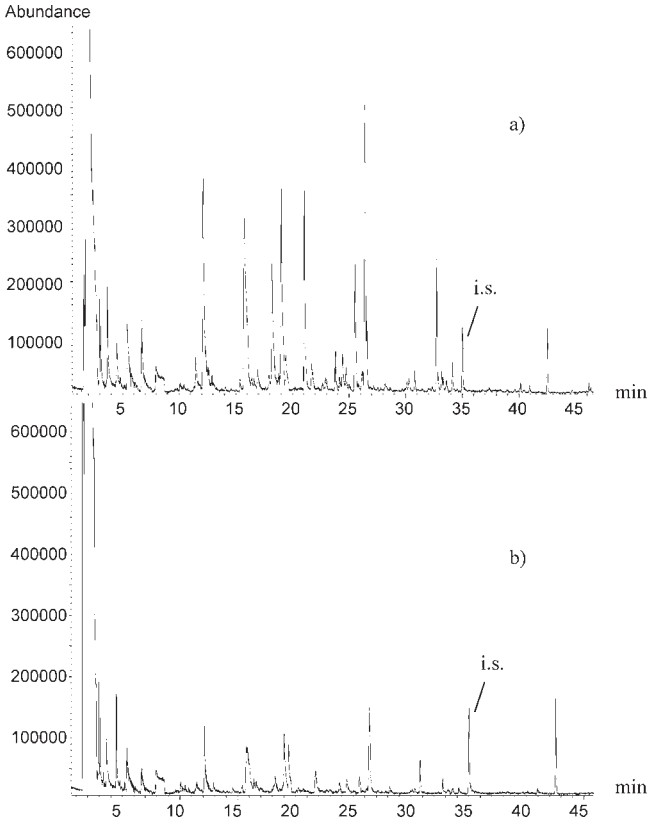

Figure 4. Chromatograms obtained by GC-MS using SPME corresponding to (**a**) model wine supplemented with a control IDY preparation and (**b**) model wine supplemented with a deodorized IDY preparation. i.s.: internal standard.

Table 4. Chemical Composition (Means ± SD) of Model Wines Supplemented with Control (C-IDY-W) and Deodorized (D-IDY-W) Preparations ($n = 4$)

	C-IDY-W	D-IDY-W
HMWN (mg of N L^{-1})	27.68 ± 0.4	26.5 ± 0.37
free amino acids (mg of N L^{-1})	48.53 ± 2.18	49.86 ± 0.3
peptides (mg of N L^{-1})	56.8 ± 10.86	55.73 ± 4.32
free polysaccharides (mg of mannose L^{-1})	2425 ± 63.12	2446 ± 76.21

observed when using enzymatic or thermal inactivation processes to obtain yeast autolysates (*28*). These results indicated that the CO_2 extraction of volatile compounds from the IDY preparations did not modify their nonvolatile composition, therefore keeping their technological aptitude to be used during winemaking.

CONCLUSION

The deodorization procedure for commercial winemaking IDY preparations from autolysated described in this work and based on the use of supercritical CO_2 allows the reduction of most of the volatile compounds (mainly produced by thermal generation) present in the IDY preparations, including powerful odorant compounds, thus greatly reducing their ability to be released into the wines and limiting possible changes in their sensory characteristics. In addition, it has been shown that the procedure did not alter the nonvolatile composition of the sample (nitrogen compounds and neutral polysaccharides), therefore keeping the technological aptitude of these preparations during winemaking. The procedure described herein may have many other applications in food technology such as the recovery of extracts enriched in potent odorant compounds from yeast or its application to recover volatile compounds from waste winemaking subproducts (such as lees).

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