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Impact Odorants Contributing to the Fungus Type Aroma from Grape Berries Contaminated by Powdery Mildew (*Uncinula necator*); Incidence of Enzymatic Activities of the Yeast *Saccharomyces cerevisiae*

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Powdery mildew due to the fungus *Uncinula necator* is an important disease for the vineyard. The development of the fungus at the surface of the berries leads to the occurrence of a very characteristic and sometimes intense mushroom-type odor cited as an important default for grapes quality. Gas chromatography/olfactometry, gas chromatography, and multidimensional gas chromatogaphy/mass spectrometry techniques were used to investigate the most important odorants of grapes diseased by powdery mildew. Among 22 odorants detected, strongly odorant compounds were identified or tentatively identified in purified extracts obtained from grapes diseased by powdery mildew. Aroma extraction dilution analysis (AEDA) analysis revealed that 1-octen-3-one (mushroom odor), (*Z*)-1,5-octadien-3-one (geranium-leaf odor), and an unidentified odorous zone (fishy-mushroom like odor) were the most potent volatiles of the diseased grapes. In the presence of nonproliferating *Saccharomyces cerevisiae* yeast cells, and consequently during alcoholic fermentation, the enzymatic reduction of 1-octen-3-one and (*Z*)-1,5-octadien-3-one to much less odorant compounds, namely 3-octanone and (*Z*)-5-octen-3-one, was shown. Those results explain to some extent the disappearance of the fungal aroma specific to powdery mildew grapes during alcoholic fermentation.

KEYWORDS: ***Vitis vinifera*; grape; powdery mildew; mushroom; 1-octen-3-one; (*Z*)-1,5-octadien-3-one; 1-octen-3-ol; (*Z*)-5-octen-3-one; *Saccharomyces cerevisiae*; enone reductase**

INTRODUCTION

Powdery mildew of the vine due to the fungus *Uncinula necator* represents one of the most serious diseases of the *Vitis vinifera* species and affects both the foliage and berries. This is very prejudicial for yields. The fungus develops on the surface of the leaves, then on the young berries in the form of a whitish mycelium gradually invading the whole bunch. As the attack continues, the epidermal cells become necrosed. If it continues, it dries out the skin. At the onset of veraison, the skin may split, thus favoring the development of gray rot (*1*, *2*). The disease, which made its first appearance in European vineyards in the middle of the 19th century, is usually well controlled by the use of sulfur-based fungicides or by organic products (*2*). However, the tendency in winegrowing practice at present is to limit the number of fungicide sprayings of the vine, thus raising the question of whether a certain degree of powdery mildew attack is tolerable without any repercussions on the wine. In a recent study (*3*), we set out to establish the incidence on some grape and wine compounds associated with enological quality, that is, grape sugar content, anthocyanin levels, and varietal aroma marker in wines, when various proportions of the harvest were affected by powdery mildew. Among other consequences usually related with the development of fungi on grapes, aroma defects in grapes and wines constitute one serious threat. In this respect, grapes contaminated by powdery mildew have a characteristic fungal aroma already noted by Viala in 1893 (*1*). This aroma is not perceived after alcoholic fermentation of Sauvignon Blanc and Cabernet Sauvignon wines obtained from partially diseased grapes, and at only a low level of intensity in wines obtained with most diseased grapes. This

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suggests that during the vinification process the main components responsible for the fungal aroma are at least transformed (*3*).

Some C8 alcohols and ketones (1-octen-3-ol and 1-octen-3-one) whose odors are reminiscent of mushroom, and (*Z*)-1,5-octadien-3-one with a more metallic and herbaceous odor, have been reported to be metabolites of various fungi (*4*−*8*). These ketones are also known to be the products of degradation from unsaturated fatty acids by chemical autoxidation reactions (*9*−*11*). In enology, knowledge is limited concerning the nature of the compounds in grape juices and wines which have a mushroom odor. 1-Octen-3-ol is a well-known compound associated with musts and wines obtained from grapes contaminated by gray rot (*12*, *13*). 1-Octen-3-ol, 1-octen-3-one, (*Z*)-1,5-octadien-3-one, and (*Z*)-1,5-octadien-3-ol have been tentatively identified by GC−olfactometry in wines contaminated by corks (*14*, *15*).

This study therefore aims to identify some of the major components responsible for the characteristic mushroom aroma related with powdery mildew development on grapes and to assess the incidence of the metabolism of *Saccharomyces cerevisiae* yeast on its evolution during alcoholic fermentation.

MATERIALS AND METHODS

Chemicals and Reference Compounds. Pentane (Pestipur quality) from SDS (Peypin, France) was distilled in order to improve its purity. 1-Octen-3-one, 1-octen-3-ol, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-nonadienal, (*E*,*E*)-2,4-decadienal, hexanal, octanal, and nonanal were provided by Interchim (Montluçon, France). 3-Octanone, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol were from Aldrich Chemical. 2-Methoxy-3-isobutylpyrazine was provided by Pyrazine Specialities (Atlanta, GA).

(*Z*)-1,5-Octadien-3-one and (*Z*)-5-Octen-3-one Synthesis and Purification. (*Z*)-1,5-Octadien-3-one was synthesized according to a protocol modified from that of Swoboda et al. (*8*). During the first step, (*Z*)-3-hexenol was oxidized to (*Z*)-3-hexenal according to the Fielder et al. (*16*) procedure. Then, (*Z*)-3-hexenal solution, cooled to 0 °C, was treated dropwise with 11 mL of vinylmagnesium bromide solution (1 M) in tetrahydrofuran. Stirring was maintained for 24 h at room temperature. The product of the Grignard reaction was then hydrolyzed with 50 mL of saturated ammonium chloride solution, and three successive extractions were performed with 50 mL of diethyl ether. The diethyl ether extract was dried with sodium sulfate, concentrated under nitrogen flow, and applied to a silica gel chromatography column. (*Z*)-1,5-Octadien-3-ol was eluted by pentane−diethyl ether solution (70:30, v/v)(0.6 g obtained). After resuspension in 10 mL of dichloromethane, this compound was added at 0 °C to a vigorously shaken Dess−Martin periodinane solution (2.42 g in 10 mL of dichloromethane; Lancaster, UK) (*17*). The mixture was then stirred at room temperature for 2 h and the disappearance of (*Z*)-1,5-octadien-3-ol was monitored by thin-layer chromatography [chromatography solvent: pentane−diethyl ether (80:20, v/v)]. The solution was treated with 0.5 M sodium thiosulfate in saturated sodium carbonate solution (2 × 20 mL). Aqueous phases were extracted with 20 mL of dichloromethane, and the pooled organic phases were rinsed with 5 mL of distilled water and dried. The solvent was finally eliminated under nitrogen flow and (*Z*)-1,5-octadien-3-one was purified by chromatography [pentane−diethyl ether (80:20, v/v) elution]. Purity of (*Z*)-1,5-octadien-3-one determined by GC−MS was 92%; MS/EI, *m*/*z* (relative intensity) 55 (100), 69 (6.6), 109 (4.8), 95 (4.4), 56 (4.4), 67 (4.7), 53 (4), 81 (3.3), 83 (3), 124 (M^+, 0.5). The protocol for (*Z*)-5-octen-3-one synthesis was very similar to that of (*Z*)-1,5-octadien-3-one except that (*Z*)-3-hexenal was treated with ethylmagnesium bromide solution (1 M) in tetrahydrofuran. Purity of (*Z*)-5-octen-3-one determined by GC−MS was 78%; MS/EI, *m*/*z* (relative intensity) 57 (100%), 41 (20.5), 69 (8.7), 126 (M^+, 6), 97 (4), 108 (3.5), 84 (2).

Grape Samples and Pentane Extraction. A total of 900 g of Cabernet Sauvignon and Sauvignon blanc grapes presenting powdery mildew aroma were collected near the harvesting period in experimental Bordeaux vineyards where no spraying had been done against powdery mildew. It should be noted that fungicide sprayings were applied during the vegetative period against downy mildew and gray rot. No gray rot was present on the grapes during sampling.

For the extraction of volatile compounds contributing to this aroma, each grape sample was put directly into pentane for 15 min. The pentane extract was then filtered through silica wool and vacuum-concentrated to 10 mL with a rotary evaporating apparatus at 4 °C to prevent the volatilization of aroma compounds, then concentrated to 500 μL under nitrogen flow (100 mL/min) before liquid chromatography on silica gel.

Partial Purification of Crude Powdery Mildew Extract by Adsorption Chromatography. The concentrated pentane extract was further purified by adsorption chromatography on silica gel (Aldrich, 70−230 mesh, 60 Å). Silica (15 g) was activated at 120 °C overnight, then cooled in a dehumifier and treated with pentane (100 mL) without previous deactivation with water, and put in a flash chromatography column (10 mm × 100 mm). The extract was then applied on the top of the column and the fractionation was obtained by an eluotropic series of solvents (40 mL), using 0.5 bar pressure of nitrogen [pentane (I) 40 mL, pentane−diethyl ether at the following volumes: (45 mL + 1 mL) (II), (40 mL + 2 mL) (III), (40 mL + 10 mL) (IV), and (25 mL + 25 mL) (V)]. Each fraction was concentrated under nitrogen flow to 100 μL, then analyzed by gas chromatography.

Capillary Gas Chromatography−Olfactometry (GC−O). The analysis was carried out alternatively by two operators on a Hewlett-Packard HP5890 gas chromatograph coupled with olfactory detection using the ODO1 installation [Scientific Glass Engineering (SGE), Ringwood, Australia]. A 1-μL portion of the extract was introduced by on-column injection at oven temperature (45 °C) onto a type HP5 fused silica capillary column [HP, 30 m, 0.32 mm internal diameter (i.d.), 0.25 μm film thickness] and onto a BP20 capillary column (SGE, 50 m, 0.25 mm i.d., 0.25 μm). For all analyses, the temperature program was as follows: 45 °C for 1 min, then 3 °C/min to 230 °C, then10 min isothermal. The carrier gas was hydrogen U (Air Liquide, France) with a column head pressure of 30 kPa (HP5 column) and 140 kPa (BP20 column).

GC−Mass Spectrometry (GC−MS). Analyses were carried out as previously described (*18*) on HP5970 and Saturn 2000 mass spectrometers. Extract was introduced in 1-μL aliquots by on-column injection on HP5 and BP20 columns.

Multidimensional GC Coupled with Mass Spectrometry (MDGC−MS). The analysis was performed on a HP 5890-I connected with a Varian 3400CX gas chromatograph coupled with the Saturn 2000 mass spectrometer. The connection between the two chromatographs was made with a thermoregulated transfer line at 150 °C. Multidimensional analysis was performed inside the HP oven using a Gerstel multi-column switching system (Gerstel, Germany). The precolumn (HP5) described previously was connected to the column switching device where the compounds eluting from the first column could be wasted or transferred directly into the analytical column (BP20, 50 m, 0.25 mm i.d., 0.25 μm). The column head pressure was programmed to allow the same helium flow during capillary analysis [i.e., 191 kPa at 45 °C for the (30 + 50) meter column, then 1.8 kPa/min increase]. Typically, samples (1 μL) were introduced at 45 °C by on-column injection. At 20 min, a cut was made for 3.5 min and the fraction was trapped on BP20 column at −50 °C (under liquid nitrogen) until the end of the temperature program (3 °C/min then 230 °C for 20 min). Then, the trap was warmed to 150 °C (temperature increase of 50 °C/min) and a new program was performed from 45 °C to 230 °C at 3 °C/min. The carrier gas was Helium N60 (Air Liquide, France).

Determination of Odorous Compounds on Crude Extract by Aroma Extract Dilution Analysis (AEDA). The analysis was performed using the method of Grosch (*19*). A total of 200 μL of crude powdery mildew extract, obtained from the 900 g of Cabernet Sauvignon grapes taken near harvesting period, was successively diluted in pentane [(1/5, 1/10, 1/20, 1/30); v/v] and 1 μL was injected for GC−olfactometry. Each GC−O analysis was performed twice. The flavor dilution (FD) factor was defined as the maximum dilution level at which the odor could be perceived by GC−O.

Table 1. Odorants from Cabernet Sauvignon Grapes Diseased by Powdery Mildew

	compound	fraction	Kovats indices HP5	Kovats indices Carbowax 20 M	odor quality	flavor dilution factor[a]	mode of identification	olfactory perception threshold[b] (μg/L)
1	hexanal	2	802	1073	green	1	MS, GC–O, KI	5[c]
2	(*Z*)-3-hexenal	crude	802	1126	powerful green	5	GC–O, KI	0.03[d]
3	(*Z*)-3-hexen-1-ol	3	877	1356	green	1	MS	200
4	(*E*)-2-hexenal	2.3	881	1207	green	1	MS, GC–O, KI,	40[c]
5	acetic acid	2.3	963	1452	acetic	<	MS	50000
6	1-octen-3-one	4	982	1291	mushroom	20	MDGC–MS, KI, GC–O	0.007
7	1-octen-3-ol	3	984	1445	mushroom	5	MS, KI, GC–O	7
8	(*Z*)-1,5-octadien-3-one	4	986	1362	geranium leaf	30	GC–O, KI	0.0007–0.0009
9	octanal	2.3	1008	1275	orange	<	MS, KI	0.7[c]
10	unknown 1	4	1042	-	green	1	GC–O	
11	unknown 2	4	1070	-	mushroom	1	GC–O	
12	nonanal	2.3	1087	1383	rancio	<	MS, KI	15[c]
13	(*E*,*Z*)-2,6-nonadienal	4	1148	1564	cucumber	1	MS, KI, GC–O	0.02[d]
14	2-methoxy-3-isobutylpyrazine	4	1173	1549	green strong	20	GC–O, KI	0.002
15	decanal	3	1209	1497	orange	<	MS, KI	5[c]
16	(*E*,*E*)-2,4-nonadienal	3.4	1211	1705	fatty	1	GC–O, KI	0.06[d]
17	benzaldehyde	3	1231	1465	almond	<	MS	300
18	(*E*,*E*)-2,4-decadienal	3.4	1313	1790	fatty	5	MS, GC–O, KI	0.05[d]
19	unknown 3	5	1327	-	fish and mushroom	5	GC–O	
20	unknown 4	5	1345	1690	fish and mushroom	30	GC–O	
21	unknown 5	5	1372	-	fish and mushroom	5	GC–O	
22	unknown 6	5	1591	-	green	1	GC–O	

[a] See Materials and Methods. [b] Thresholds were determined in water. [c] References from Vanderline (*29*). [d] References from Grosch (*19*).

Extraction of Volatiles from Must and Wines. Grape juice or wine (400 mL) was treated with 1 mL of 3-octanol (10 mg/L, internal standard) and with 100 μL of 2-octanone (100 mg/L, internal standard), then extracted in 1-liter flasks by three successive extractions with distilled pentane (20, 20, and 10 mL, respectively) with magnetic stirring for 5 min each time. The combined organic phases were then dried with anhydrous sodium sulfate and concentrated to 100 μL under nitrogen flow (approximately 100 mL/min).

Odor Threshold Determination. Odor perception thresholds (OP) of 1-octen-3-one, 1-octen-3-ol, 3-octanone, (*Z*)-1,5-octadien-3-one, (*Z*)-5-octen-3-one, (*Z*)-3-hexen-1-ol, acetic acid, and benzaldehyde were obtained by directional triangular tests of five increasing concentrations in ultrapure water (MilliQ, Millipore, Bedford, MA) produced from initially distilled water. The solutions were presented in glasses corresponding to AFNOR (Association Française des Normes) standards. The odor perception threshold corresponded to the minimum concentration below which 50% of 45 tasters statistically failed to detect the difference from the control. For (*Z*)-5-octen-3-one, a 10-person jury was constituted.

Incubation of Volatile Ketones and Alcohols in the Presence of Resting Cells. Active dry yeast (strain VL3c, *Saccharomyces cerevisiae*) was inoculated at 100 mg/L (equivalent to 5×10^6 cells/mL) in a model fermentation medium (*20*), (700 mL in 750-mL bottles equipped with bubblers) for 48 h at 20 °C. Then yeast cells obtained from 200 mL of fermentation medium were collected by centrifugation (5000*g*, 10 min). The resting yeast cells [approximately 5×10^6 cells/mL determined by optical density at 600 nm (1 unit OD corresponds to 2.4×10^7 yeast cells/mL)] were incubated with each volatile ketone and alcohol in tartaric acid solution [5 g/L, pH 3.5 (100 mL)] for 1 h at room temperature.

Fermentations in the Presence of Volatile Ketones and Alcohols. Fermentations were conducted using the same protocol as previously described. A model fermentation medium (1.3 L) was inoculated with active dry yeasts and fermented in 1.5-L bottles in the presence of each individual ketone and alcohol. Samples (100 mL) were taken at different fermentation times. A test sample was obtained by supplementing the model medium with an individual ketone and the alcohol with sodium fluoride to prevent the development of microorganisms.

Quantitative Analysis of Volatile Ketones and Alcohols in the Supplemented Fermentation Medium. A total of 100 mL of fermenting medium or tartaric acid solution incubated in the presence of resting yeast cells was supplemented with 100 μL of 2-octanone (100 mg/L, internal standard) and extracted in 200-mL flasks with pentane (8, 4, and 4 mL, successively) and magnetic stirring for 5 min each time. Organic phases were pooled and concentrated to 500 μL before injection by GC−MS (HP) in selected ion monitoring [*m*/*z* ions 55, 70, 97, 126 for 1-octen-3-one; *m*/*z* ions 55, 69, 109, 124 for (*Z*)-1,5-octadien-3-one; *m*/*z* ions 57, 72, 128 for 1-octen-3-ol; *m*/*z* ions 57, 71, 99, 128 for 3-octanone; *m*/*z* ions 57, 69, 126 for (*Z*)-5-octen-3-one; *m*/*z* ions 58, 71, 128 for 2-octanone] and total ion current.

RESULTS AND DISCUSSION

Identification of Volatile Odorous Compounds from Berries Diseased by Powdery Mildew. The crude concentrated extract obtained from diseased Cabernet Sauvignon bunches (900 g) was analyzed by gas chromatography with olfactometry (GC−O) and by gas chromatography with mass spectrometry (GC−MS) (**Table 1**). The analysis was then performed on fractions of the same extract obtained by chromatography on silica gel. GC−O analysis revealed a large number of odorous zones for which some compounds responsible could be identified by GC−MS and by comparing their retention time with that of pure products or only tentatively identified by analyzing retention times of odorous zones with those of pure products (**Table 1**). Hexanal (**1**), (*Z*)-3-hexenal (**2**), (*Z*)-3-hexen-1-ol (**3**), (*E*)-2-hexenal (**4**), acetic acid (**5**), 2-methoxy-3-isobutylpyrazine (**14**), and benzaldehyde (**17**) have already been identified in extracts of healthy berries (*21*−*24*). Moreover, GC−O analysis demonstrated other odorous zones reminiscent of the aroma of grapes infected with powdery mildew (**Table 1**). These are particularly zones having a fungal or heavy odor: KI HP5 982 (**6**), 984 (**7**), 986 (**8**), 1070 (**11**), 1313 (**18**), 1327 (**19**), 1345 (**20**), 1372 (**21**) (**Table 1**). The presence of these odorous zones was confirmed in other Cabernet Sauvignon and Sauvignon blanc samples obtained from grapes diseased by powdery mildew. They were not perceived by GC−O in healthy grape samples or they were perceived at a much lower intensity level.

The use of multidimensional GC coupled with mass spectrometry (MDGC−MS) revealed the mass spectrum of compound (**6**) which was responsible for the mushroom odor. The

Table 2. Change in Carbonyl Compounds Concentration in the Presence of Nonproliferating *Saccharomyces cerevisiae* Yeast Cells and during Alcoholic Fermentation

	1-octen-3-one (μg/L)	(*Z*)-1,5-octadien-3-one (μg/L)	1-octen-3-ol (μg/L)	3-octanol (μg/L)
before yeasts incorporation	10	20	20	20
day 2 after initiation of alcoholic fermentation (density 1.07)[a]	0.138 (7.8)[b]	< 0.01(8)[c]	19.5	18
day 4 middle alcoholic fermentation (density 1.03)	< 0.01	< 0.01	16	12
day 7 end of alcoholic fermentation (density 0.995)	< 0.01	< 0.01	16	10

[a] Yeast cells were collected by centrifugation and incubated in the presence of the 1-octen-3-one or in the presence of (*Z*)-1,5-octadien-3-one during 1 h at room temperature; see Materials and Methods. [b] Value in brackets indicates 3-octanone concentration after 1 h incubation of resting yeast cells with 1-octen-3-one. [c] Value in parentheses indicates (*Z*)-5-octen-3-one concentration determined after 1 h incubation of resting yeast cells with (*Z*)-1,5-octadien-3-one.

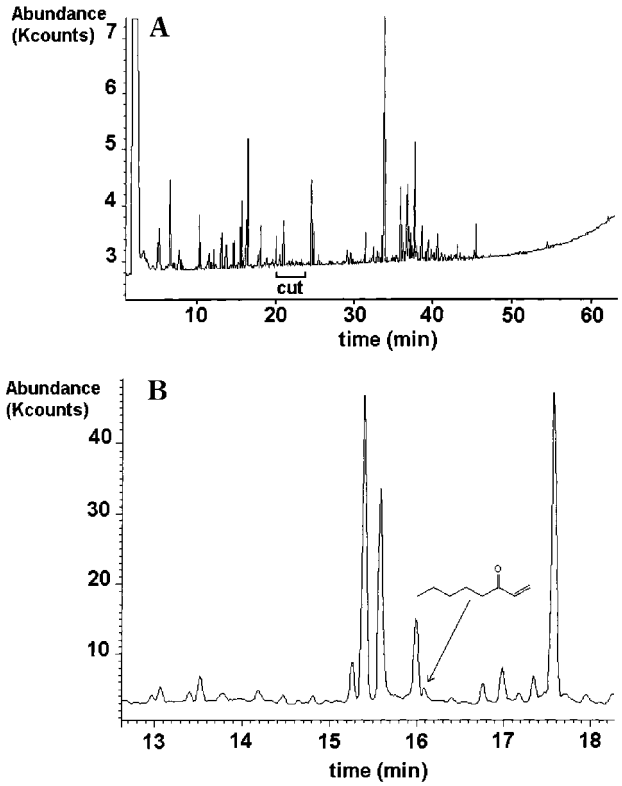

Figure 1. Multidimensional gas chromatography analysis of a purified fraction of Cabernet Sauvignon grapes diseased by powdery mildew (fraction 4): (A) precolumn chromatogram of purified fraction (10% of effluent detected by flame ionization); (B) main column chromatogram of purified fraction.

abundance of ions and the retention times coincided with those of 1-octen-3-one [(**Figure 1A**,**B**). This very odorous compound (OP = 7 ng/L in water) has a strong mushroom odor, which at high concentrations is sometimes described as being metallic (*8*). It is much more odorous than 1-octen-3-ol (**7**) (OP = 7 μg/L for the racemic form in water) identified in infected berries by direct analysis with GC−MS, which also possesses a fungal odor. In a Cabernet Sauvignon juice containing 50% of berries infected by powdery mildew, we detected 1.8 μg/L of 1-octen-3-ol, i.e., a concentration below its olfactory perception threshold in water (**Table 1**).

The synthesis of (*Z*)-1,5-octadien-3-one, a very odorous compound with a geranium-leaf odor (OP = 0.9 ng/L), demonstrated the coincidence between its odor and the retention time on two columns of different polarity, and that of the odorous zone (**8**) [KI HP5 986, KI BP20 1362]. GC−MS did not allow us to identify the other compounds with a fungal odor [KI HP5 1070 (**11**), 1327 (**19**), 1345 (**20**), and 1372 (**21**)]. Moreover, several polyunsaturated aldehydes known to be degradation products of unsaturated fatty acids (*9*) were identified by GC−MS in grape extracts infected by powdery mildew [(*E*,*Z*)-2,6-nonadienal (**13**); (*E*,*E*)-2,4-nonadienal (**16**); and (*E*,*E*)-2,4-decadienal (**18**)]. Saturated aldehydes [octanal (**9**), nonanal (**12**), and decanal (**15**)] which all can be formed by autoxidation of oleic acid (*9*) were detected by GC−MS in contaminated grapes (**Table 1**). The AEDA technique (*19*) allowed us to determine, in the same crude extract of powdery mildew-infected Cabernet Sauvignon berries, the most potent odorous zones (**Table 1**). The greatest impact was due to 1-octen-3-one (**6**), to (*Z*)-1,5-octadien-3-one (**8**), and to the odorous zone (**20**)(KI HP5 1345; KI BP20 1690) corresponding to an unidentified compound, then with a lesser extent to 1-octen-3-ol (**7**), (*E*,*E*)-2,4-decadienal (**18**), and to odorous zones (**19** and **21**) (**Table 1**).

It should be noted that (*Z*)-1,5-octadien-3-one possesses the same descriptor of geranium-leaf as has been cited for 2-ethoxy-hexa-3,5-diene, a metabolism product of sorbic acid by lactic bacteria in wines (*25*). However, the olfactory perception threshold of this compound is much more elevated [0.1 μg/L according to Wurdig (*26*)] than that of (*Z*)-1,5-octadien-3-one. (*E*,*E*)-2,4-nonadienal (**16**) and (*E*,*E*)-2,4-decadienal (**18**) are known as fungal metabolites (*27*) as 1-octen-3-one and (*Z*)-1,5-octadien-3-one (*4*−*6*, *8*−*11*).

Evidence of Role of *Saccharomyces cerevisiae* with Regard to Carbonyl Compounds Identified or Tentatively Identified in Diseased Grapes. The most marked odorous zones found in extracts of infected berries (**6**, **8**, **18**, **19**, **20**, and **21**) were either not perceived or perceived with a much lower intensity level at the end of alcoholic fermentation after GC−O analysis in wines made with infected berries. This shows that the compounds responsible are totally or partially degraded during fermentation. To assess the impact of the yeast *S. cerevisiae* on levels of 1-octen-3-one and (*Z*)-1,5-octadien-3-one during alcoholic fermentation, both of these compounds, as well as 1-octen-3-ol and 3-octanol, were placed in the presence of nonproliferating *S. cerevisiae* yeast previously cultivated on a model medium having a composition close to that of grape juice, or were directly added in a model medium, to monitor their evolution during alcoholic fermentation (**Table 2**). After 1 h incubation with resting yeast cells, 1-octen-3-one and (*Z*)-1,5-octadien-3-one had largely disappeared. On the other hand, in these conditions, the levels of 1-octen-3-ol and 3-octanol did not change. The disappearance of 1-octen-3-one was correlated with the appearance of a peak on the chromatogram identified as 3-octanone, a much less odorous compound than 1-octen-3-one (OP = 23 μg/L). The disappearance of (*Z*)-1,5-octadien-3-one was correlated with the appearance of a chromatographic

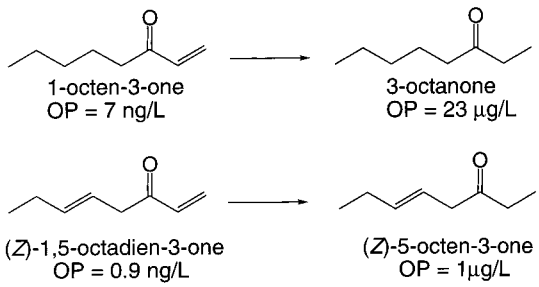

Figure 2. Bioconversion of 1-octen-3-one and (*Z*)-1,5-octadien-3-one, in the presence of nonproliferating *Saccharomyces cerevisiae* yeast cells.

peak whose mass spectrum was very close to that of 5-methyl-5-hepten-3-one. Synthesis of (*Z*)-5-octen-3-one confirmed that it was the compound formed when *S. cerevisiae* yeast was incubated with (*Z*)-1,5-octadien-3-one. Therefore, the yeast totally and selectively reduced the (*Z*)-1,5-octadien-3-one into (*Z*)-5-octen-3-one, which is a much less odorous compound (OP = 1 μg/L). In the added media analyzed both in the middle and at the end of alcoholic fermentation, neither 1-octen-3-one nor (*Z*)-1,5-octadien-3-one were detected, whereas at the end of alcoholic fermentation, 80 and 50% of the initial levels of 1-octen-3-ol and 3-octanol were found, respectively.

The specific reduction of 1-octen-3-one and (*Z*)-1,5-octadien-3-one by nonproliferating *S. cerevisiae* yeast sampled on day 2 of alcoholic fermentation, together with the appearance of 3-octanone and (*Z*)-5-octen-3-one, may be explained by the presence of a previously described (*28*) specific enzymatic activity (enone-reductase) from *S. cerevisiae* (**Figure 2**). Wanner et al. (*28*) demonstrated the selective enzymatic reduction of 1-octen-3-one to 3-octanone, but did not demonstrate the reduction of (*Z*)-1,5-octadien-3-one. Moreover, according to the same mechanism, i.e., α,β-unsaturated double bond reduction of carbonyl compounds as described by that group, (*E*,*E*)-2,4-decadienal, and probably (*E*,*E*)-2,4-nonadienal, and (*E*,*Z*)-2,6-nonadienal are enzymatically reduced. While other defects may be associated with the development of powdery mildew in grape berries (i.e., drop in anthocyanin levels; decreased levels of 3-mercaptohexanol, a varietal aroma marker in wine) beyond a certain amount of powdery mildew-infected berries (*3*), the present results explain to some extent the disappearance of the fungal aroma specific to powdery mildew during alcoholic fermentation.

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