Role of Carbonyl Compounds in SO_2 Binding Phenomena in Musts and Wines from Botrytized Grapes

Jean-Christophe Barbe, Gilles de Revel, Annick Joyeux, Aline Lonvaud-Funel, and Alain Bertrand*

Faculté d'Œnologie, Unité Associée INRA/Université Victor Segalen Bordeaux 2, 351 Cours de la Libération, F-33405 Talence, France

Carbonyl compounds play an important role in musts from botrytized grapes. Some of them, such as glyoxal and methylglyoxal, may explain a considerable part of bindable SO_2. Others, such as 2- and 5-oxogluconic acids, produced by gluconic acid oxidation in proportions respectively from 2.5 per 1 play an interesting role as SO_2 binding indicator. Finally, the levels of some compounds such as dihydroxyacetone, 5-oxofructose, and δ-gluconolactone in balance with gluconic acid are well correlated with SO_2 binding powers and also explain a large part of the bindable SO_2 in musts. During alcoholic fermentation, only dihydroxyacetone among these three compounds is metabolized by yeast. Thus, two compounds present in grapes, δ-gluconolactone and 5-oxofructose, with three yeast SO_2-binding byproducts, ethanal, pyruvic, and 2-oxoglutaric acids, explain much of the SO_2 binding power in wines from botrytized grapes.

Keywords: *Sulfur dioxide; wine; botrytized grapes; SO_2-binding compounds; carbonyl compounds*

INTRODUCTION

Sweet white table wines made from grapes infected by desirable *Botrytis cinerea* (called noble rot) generally contain many residual sugars. Alcoholic fermentation is stopped by sulfur dioxide addition, an operation called "mutage". These wines present an alcohol level ranging between 13 and 15 vol % and often contain more than 60 g/L residual sugars. Conservation of these wines which are not filtered is performed in barrels and lasts 12−24 months or even more.

Macris and Markakis (1974) showed that H_2SO_3, called "active" or molecular SO_2, is responsible for the anti-yeast activity of this additive. According to Sudraud and Chauvet (1985), about 1 mg/L active SO_2 is useful to avoid any fermentative phenomena.

When SO_2 is added to wine, an equilibrium between the various molecular forms of this compound is established. A part reacts with the carbonyl compounds to give carbonyl bisulfite and is called bound SO_2. The other part is called free SO_2. Most free SO_2 is present in hydrogenosulfite, HSO_3^-, form. The active form of SO_2 (H_2SO_3) is dependent on media pH and on the acidity constant which is itself dependent on media ethanol content and temperature. Thus, a pH decrease or an ethanol content or temperature increase causes an increase in H_2SO_3. Generally, in wines from botrytized grapes, at a temperature of about 20 °C, the active form of SO_2 is present at contents of about 1% free SO_2, whereas the sulfite form, SO_3^{2-}, is negligible. Thus, to have 1 mg/L active SO_2, about 50 mg/L free SO_2 and a few hundred mg/L total SO_2 are needed in these wines. In practice, the total SO_2 content is regulated, and hygienists always strive to reduce the authorized levels. The role of the winemaker is to obtain a wine with a satisfactory active SO_2 content for the lowest total SO_2 content possible.

Musts from botrytized grapes have levels of SO_2-binding compounds higher than those from healthy grapes (Asvany, 1985). In wine, according to Ribéreau-Gayon (1972), these compounds come either from alcoholic fermentation or from the oxidation of grape sugars. SO_2-binding compounds such as galacturonic, glucuronic, and 2- and 5-oxogluconic acids (Sponholz and Dittrich, 1984), xylosone and 5-oxofructose (Burroughs and Sparks, 1964) and α-dicarbonyl compounds such as glyoxal and methylglyoxal (de Revel et al., 2000) have already been described. High levels of SO_2-binding compounds produced by yeast during fermentation such as ethanal and pyruvic and 2-oxoglutaric acids are well-known. The arrest of the fermentation before the end of sugar consumption (mutage) implies the nonreduction of these three metabolic intermediates. However, according to their contents and their SO_2 affinities, all these compounds are of various interests in SO_2 binding phenomena.

The present work proposes a quantitative balance of SO_2-binding compounds in musts and wines from botrytized grapes. The evolution of the principal compounds responsible for SO_2 binding is also studied.

MATERIALS AND METHODS

Carbonyl Analysis. The method of detection of dihydroxyacetone and glyceraldehyde has been described by Guillou et al. (1997). *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride (PFBOA) reacts with the carbonyl function to form an oxime. A 0.1 mL sample of must or wine was mixed with 1.9 mL of microfiltered water (resistivity 18.2 MΩ cm), 5 mg of PFBOA (Aldrich 19,448−4), and 50 μL of lindane (Aldrich H-4500) at a concentration of 270 mg/L as internal standard. The reaction was performed in a stoppered flask for 1 h at room temperature. The oximes were extracted with 2 mL of ether/hexane (1/1, v/v, SDS, Pestipur) for 2 min. Two microliters of extract was injected into the GC with an MS detector.

* To whom correspondence should be addressed. Fax: 33 556846468. E-mail: alain.bertrand@oenologie.u-bordeaux2.fr.

10.1021/jf991359d CCC: $19.00
Published on Web 07/22/2000

Chromatographic conditions were the following: Hewlett-Packard HP 6890 gas chromatograph coupled with a mass spectrometer (HP 5972, electronic impact 70 eV, eMV = 2 kV, the detection mode was selected ion monitoring (SIM) with the ion of $m/z = 181$; column BP1 (SGE) (50 m × 0.32 mm, 0.4 μm); helium 5.6 Aga pressure, 70 kPa; injector temperature, 250 °C; detector temperature, 280 °C; oven temperature, 60 °C for 1 min programmed at a rate of 3 °C/min to 220 °C, the final step lasting 20 min; splitless time, 30 s; split flow, 30 mL/min.

5-Oxofructose was measured after the same derivatization reaction and analytic conditions, but the length of the capillary column was only 12 m, and an on-column injector was used. Standard 5-oxofructose was synthesized from D-fructose (Sigma, F2543) and 5-fructose dehydrogenase (Sigma, F5152) in the presence of 2,6-dichlorophenolindophenol (Sigma, D1878). Intralaboratory repeatability was determined by 10 successive analyses of the same sample containing 94 mg/L 5-oxofructose, and the variation coefficient was 7.2%. Method linearity was studied by adding 5-oxofructose (50, 100, 200, 400, 800 mg/L) to a wine which had no detectable level. The correlation coefficient between found and added levels was 0.995. α-Dicarbonyl compounds, such as glyoxal and methylglyoxal, were detected after reaction with 1,2-diaminobenzene according to the method adapted to wine by de Revel et al. (1999). Dicarbonyl compounds were purchased from Aldrich Chemicals and used without further purification: glyoxal (12,846-5), methylglyoxal (17,733-4), and hexane-2,3-dione (14,416-9). 1,2-Diaminobenzene (Sigma, *o*-phenylenediamine P-9029) was used as an aqueous solution. Fifty microliters of internal standard (1.2 g/L hexane-2,3-dione in hydroalcoholic solution, 50 vol %) was added to 50 mL of wine and 5 mL of an aqueous solution of 1,2-diaminobenzene at 6.5 g/L; the pH was adjusted to pH 8 (NaOH, 12 M). After 3 h at 60 °C, the mixture was acidified with 4 N sulfuric acid to pH 2 and extracted twice with 5 mL of dichloromethane. The organic phase was dried with sodium sulfate, and 2 μL was injected into the chromatograph. A Hewlett-Packard HP 5890 gas chromatograph was coupled with a nitrogen compound detector (NPD, Hewlett-Packard), the column was a HP5 (Hewlett-Packard, 50 m × 0.32 mm, 0.52 mm), and the Helium U (Air Liquide) pressure was 100 kPa. The injector and detector temperature was 220 °C, the oven temperature was kept at 60 °C for 1 min and programmed at a rate of 2 °C/min to 220 °C, the final step lasting 20 min, the splitless time was 30 s, and the split flow was 30 mL/min.

The determination of ethanal levels was done according to the method described by Bertrand (1990) and modified as follows. One hundred milliliters of a sample brought to pH 9 by 12 M NaOH was previously distilled. Fifty microliters of a hydroalcoholic (1/1, v/v) solution of 10 g/L 4-methylpentan-2-ol was mixed with 5 mL of distillate. One microliter was injected into a gas chromatograph with a flame ionization detector. Chromatographic conditions were as follows: HP 5890 gas chromatograph; column CPWax 57CB (Chrompack) (50 m × 0.25 mm, 0.2 μm); Hydrogen U (Air Liquide) pressure, 60 kPa; injector temperature, 200 °C; detector temperature, 240 °C; oven temperature, 40 °C for 5 min programmed at a rate of 4 °C/min to 100 °C; split injection with a split flow of 60 mL/min.

Pyruvic and 2-Oxoglutaric Acids. These were determined enzymatically using Boehringer-Mannheim (F-38242 MEYLAN) products.

Gluconic, 2-Oxogluconic, and 5-Oxogluconic Acids. The analysis was done with ionic HPLC. Their levels were calculated by comparison of peak areas with those obtained after injections of solutions containing reference products provided by Sigma (gluconic acid G1139, 2-oxogluconic acid K6250, and 5-oxogluconic acid K4125). The Dionex 4500 ion chromatograph was equipped with three columns, an Ion Pack anion trap column (Dionex ATC-1 10-32), an Ion Pack precolumn (Dionex AG11, 50 mm × 4 mm), and an Ion Pack column (Dionex AS11, 250 mm × 4 mm). The system functioned in gradient mode with a mobile phase of water/methanol (20%, v/v) and NaOH: 0/0.5, 2/0.5, 20/35 (time (min)/NaOH concentration (mM)). Samples were diluted 50 times and were introduced into the eluant flow by a 10 μL injection loop. Eluant hydroxide ions were neutralized before the conductometric detection cell with the ASRS Ultra suppressor (Dionex) working in chemical mode.

Measure of SO_2 Binding Power. The TL50 value was the total SO_2 level necessary to obtain 50 mg/L free SO_2. This determination was established on the basis of the work of Kielhöfer and Würdig (1960), who were the first to draw SO_2 binding curves representating free SO_2 versus combined SO_2. Then another parameter was determined: CL50, indicating the level of combined SO_2 necessary to have 50 mg/L free SO_2. Free and total SO_2 contents were determined by iodometry as described by Ribéreau-Gayon et al. (1972). Samples at pH 5 (NaOH, 12 M), to accelerate SO_2 binding phenomena, received four increasing and known SO_2 additions (thanks to a potassium bisulfite solution) to obtain a range of free SO_2 contents including the value of 50 mg/L. One hour after addition, free SO_2 was assayed. Then the straight line representing total SO_2 against free SO_2 was plotted to express the TL50.

SO_2 Combination Estimation. The dissociation constant of the bisulfitic combination represents the affinity of a compound for SO_2. K_d was calculated according to the mass action law:

$$K_d = \frac{C_{free}[HSO_3^-]}{C_{combined}}$$

where $C_{combined}$ is the concentration of the compound combined with SO_2 and C_{free} is the concentration of the compound not combined with SO_2.

The analytical methods used made it possible to establish only the total concentration of carbonyl compounds, so it was necessary to consider the following equation:

$$C_{total} = C_{combined} + C_{free}$$

Thus, by knowing the hydrogenosulfite level (which was nearly free SO_2), the estimation of the quantity of the compound combined with SO_2 was possible:

$$\frac{C_{combined}}{C_{total}} = 1 - \frac{K_d}{[HSO_3^-] + K_d}$$

Since the SO_2 binding power was TL50, these conditions ([free SO_2] = 50 mg/L) were used to estimate the fraction of the compound bound to SO_2 and to calculate its part in SO_2 binding. As it was not possible to determine the gluconolactone content, its level was considered to represent 5% of the gluconic acid, as is standard practice.

Appropriation of Grapes and Preparation of Musts. During the 1997 and 1998 harvests, grapes infected by desirable *B. cinerea* were harvested in the Sauternes area (close to Bordeaux) at different levels of maturation: full botrytis (the grape is completly covered by the botrytis, brown-colored berries with thin skins); "roasted" (the grapes have 50% more concentration and are shriveled and completely attacked by botrytis); advanced stage (grapes are further developed but not damaged); sour; mouldy. Each batch was composed of 1−2 kg of berries which were pressed with a small vertical wine press. The musts obtained were centrifugated at 9000*g* for 15 min and kept at −18 °C until analysis. Thirty-one different musts were prepared with this technique.

Alcoholic Fermentations. To study the evolution of SO_2-binding compounds in the presence of yeast, fermentations were carried out in the laboratory and wineries as follows. Musts were mixed with 200 mg/L dried yeasts previously rehydrated for 30 min at a temperature of 30 °C in 10 times their weight of half-diluted must. In the middle of the alcoholic fermentation, a control of the yeast strain implantation was done using PCR as described by Masneuf and Dubourdieu (1994).

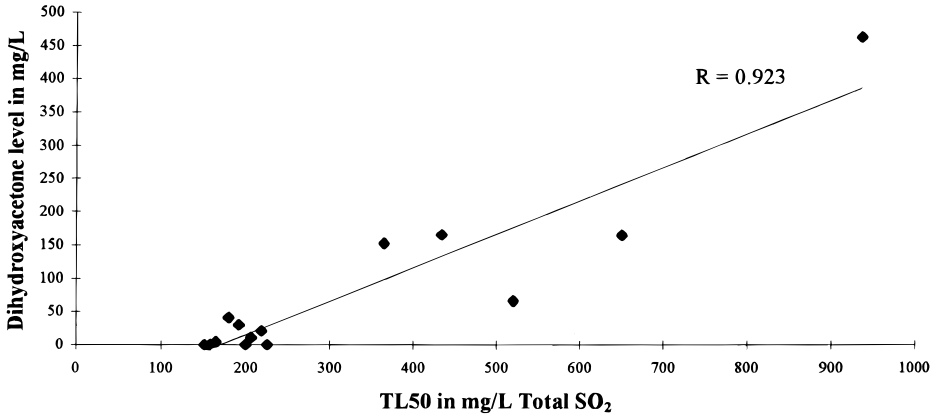

Figure 1. Dihydroxyacetone levels in musts from the 1998 harvest versus their SO_2 binding power (TL50).

To study the evolution of some carbonyl compounds, a must from healthy white grapes was mixed with dihydroxyacetone, 5-oxofructose, glycerol (10 g/L), and saccharose (to obtain a total sugar concentration of 330 g/L), a composition similar that of a must from noble rot. This must was inoculated with a commercial yeast strain. Fermentation lasted 10 days at 23 °C. When the must reached a density of 1.030, fermentation was stopped by addition of 250 mg/L SO_2 and centrifugation at 9000 rpm for 15 min.

In wineries, two experiments were carried out to study the influence of the yeast strain and the presence of SO_2 during alcoholic fermentation. Four commercial yeast strains were used in two different wineries: Maurivin PDM, Uvaferm BC, Zymaflore VL3, and Zymaflore ST. In each case different fractions of the same must were inoculated by the different yeast strains. In the middle of alcoholic fermentation, implantation controls showed good development of the tested strains. The mutage was done when the alcohol content reached 14.5 vol % by adding 320 mg/L SO_2 for winery A and at an alcohol content of 13 vol % by adding 250 mg/L SO_2 for winery B. To show the influence of the addition of SO_2 to the must, two barrels were filled with the same must. One received an addition of 50 mg/L SO_2 and the other none. Both were inoculated with Zymaflore ST. The mutage was done at the same time at a density of 1.035 by adding 320 mg/L SO_2 to the two barrels.

RESULTS AND DISCUSSION

Constitution of Musts. The initial musts presented a wide range of SO_2 binding power for TL50 between 119 and 936 mg/L total SO_2. Combination capacities were correlated to the health status of berries: only musts from advanced or mouldy stages had TL50 values higher than 400 (Table 1). The α-dicarbonyl compounds were generally present at relatively low concentrations and were not correlated with the SO_2 binding power of musts. The average (out of 31 samples) contents of glyoxal and methylglyoxal, respectively 1.22 ± 0.45 and 13.4 ± 14.6 mg/L, explained the combination at a value of approximately 10−15 mg/L SO_2 for 50 mg/L free SO_2. However, methylglyoxal had high levels (approximately 50 mg/L) in three musts paradoxically presenting low SO_2 binding power. Dihydroxyacetone and 5-oxofructose contents for 1998 musts correlated well with TL50 as shown in Figures 1 and 2, respectively. These figures show that the dihydroxyacetone contents increased with the SO_2 binding power, which is in agreement with the results of Guillou-Largeteau (1996). As for 5-oxofructose contents, they increased considerably from TL50 values of approximately 400 mg/L. Particularly high contents of this compound were previously described by Burroughs and Sparks (1973a) in a wine resulting from mouldy grapes.

Table 1. SO_2 Binding Power, Sugar Contents, pH, and Level of Maturity in Samples

picking date	harvested maturation level	TL50 (mg/L total SO_2)	sugar content (g/L)	pH
Sept 25, 1997	roasted	270	475	3.84
	roasted	210	494	3.78
	sour	270	325	3.32
Sept 30, 1997	full botrytis	120	155	3.53
	full botrytis	120	231	3.61
	roasted	340	380	3.73
Oct 13, 1997	roasted	250	303	3.59
	roasted	290	374	3.86
	roasted	240	376	3.79
	advanced stage	300	309	3.97
	sour	320	261	3.60
Oct 22, 1997	full botrytis	160	212	3.59
	sour	460	229	3.48
Oct 30, 1997	roasted	230	357	3.68
	roasted	210	421	3.75
	roasted	250	443	3.83
	advanced stage	460	465	4.02
Sept 17, 1998	full botrytis	190	207	3.50
	roasted	210	320	3.77
Oct 8, 1998	roasted	170	249	3.67
	advanced stage	180	215	3.52
October 16, 1998	roasted	230	344	3.96
	advanced stage	370	260	3.46
	mouldy	520	154	3.58
Oct 19, 1998	advanced stage	220	241	3.72
Nov 3, 1998	roasted	200	352	4.04
	roasted	430	335	3.64
	advanced stage	650	247	3.55
	advanced stage	940	315	3.11

Glyceraldehyde concentrations were weak because this compound was probably in a balance with dihydroxyacetone. Dihydroxyacetone (DHA) levels could reach 462 mg/L; Blouin et al. (1995) indicated the dissociation constant of this carbonyl bisulfite ($K_d = 2.8$ mM) and then that for 50 mg/L (0.781 25 mM) free SO_2, as demonstrated earlier:

$$\frac{[\mathrm{DHA_{combined}}]}{[\mathrm{DHA_{total}}]} = 1 - \frac{2.8}{0.78125 + 2.8} = 0.218$$

Since the dihydroxyacetone molecular weight is 90, then for a dihydroxyacetone level of 462 mg/L

$$[\mathrm{DHA_{combined}}] = 0.218 \times \frac{462}{90} = 1.12 \text{ mM}$$

Thus a dihydroxyacetone level of 462 mg/L corresponds to the combination of 1.12 mM or 72 mg/L SO_2 for 50 mg/L free SO_2. The 5-oxofructose contents reached 2.3 g/L, which corresponds to the combination of 575 mg/L

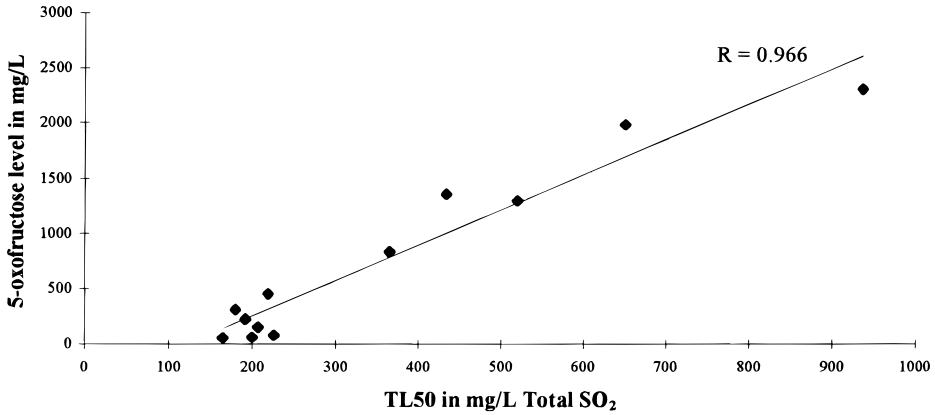

Figure 2. 5-Oxofructose levels in musts from the 1998 harvest versus their SO_2 binding power (TL50).

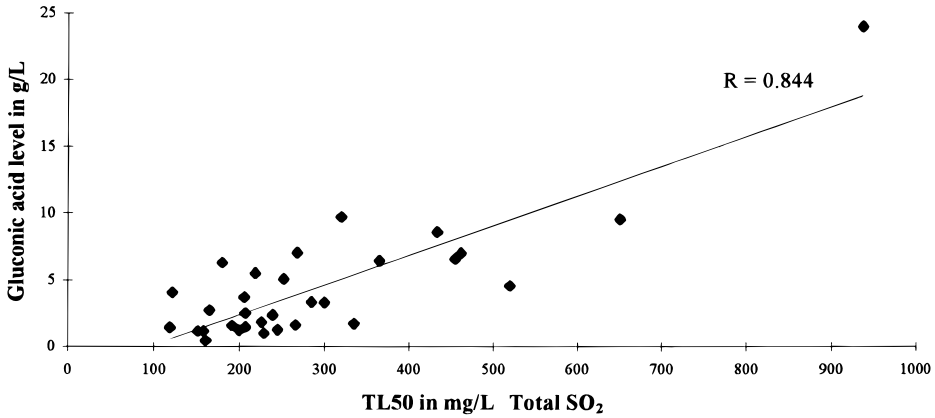

Figure 3. Gluconic acid levels in musts versus their SO_2 binding power (TL50).

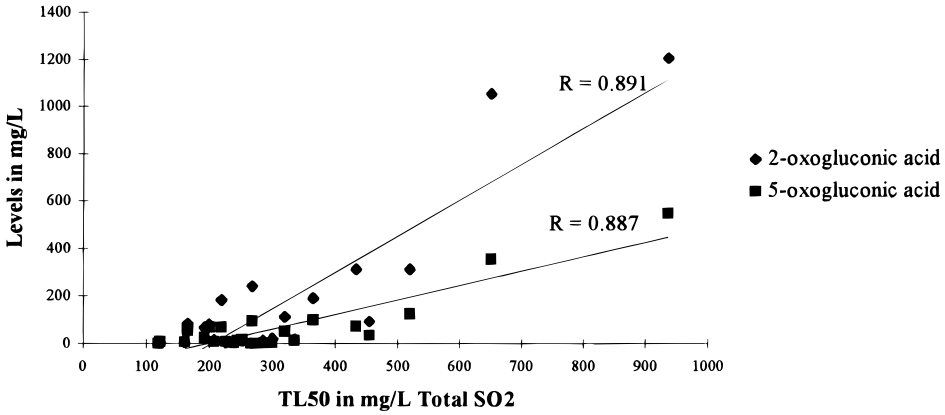

Figure 4. 2- and 5-oxogluconic acid levels in musts versus their SO_2 binding power (TL50).

SO_2 for 50 mg/L free SO_2 according to the dissociation constant of this carbonyl bisulfite ($K_d = 0.33$ mM) given by Burroughs and Sparks (1973b).

Gluconic and monooxogluconic acids presented a good correlation with SO_2 binding power (Figures 3 and 4). Between 0.5 and 24 g/L, gluconic acid presented a weak affinity for SO_2, but its content governed that of δ-gluconolactone, the latter playing a part in binding phenomena. Bertrand and Guillou (1999) indicated that 100 mg/L δ-gluconolactone explained 21.6 mg/L bound SO_2 for a free SO_2 level of 50 mg/L. 2-Oxogluconic acid, with contents ranging between traces and 1.2 g/L, was most of the time more abundant than 5-oxogluconic acid (between traces and 547 mg/L). Sudraud et al. (1986) already observed that these acids are present at their highest levels in wines presenting high SO_2 binding power. However, these authors noted that their contribution to the SO_2 binding balance is always weak. On the other hand, these compounds, which are directly produced by the oxidation of gluconic acid, play an interesting role as marker of SO_2 binding for samples with a TL50 higher than 400.

There was also a very good correlation between the contents of 2-oxogluconic and 5-oxogluconic acids, as shown in Figure 5. These two acids seemed systematically to be produced together from gluconic acid, 2-oxogluconic acid having higher concentrations than 5-oxogluconic acid (about 2.5 more).

Pyruvic acid, 2-oxoglutaric acid, and ethanal were generally present in low quantities in musts from botrytized grapes, but were higher than those described in healthy grapes (Blouin and Peynaud, 1963). Their role is weak in the binding power of musts because no correlation was noted.

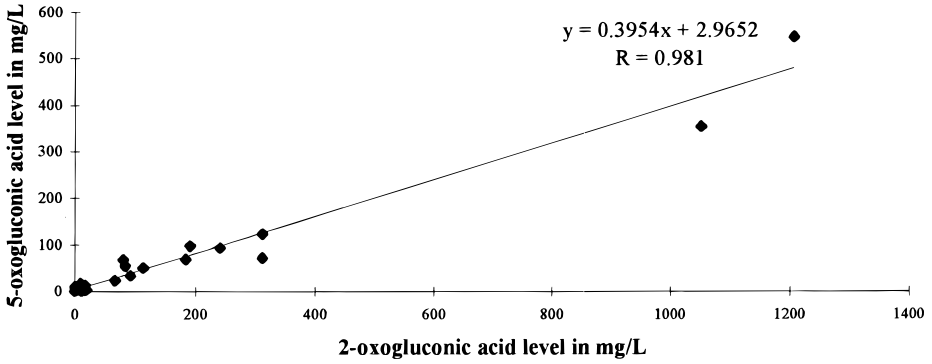

Figure 5. 2-Oxogluconic acid levels versus 5-oxogluconic acid levels in musts.

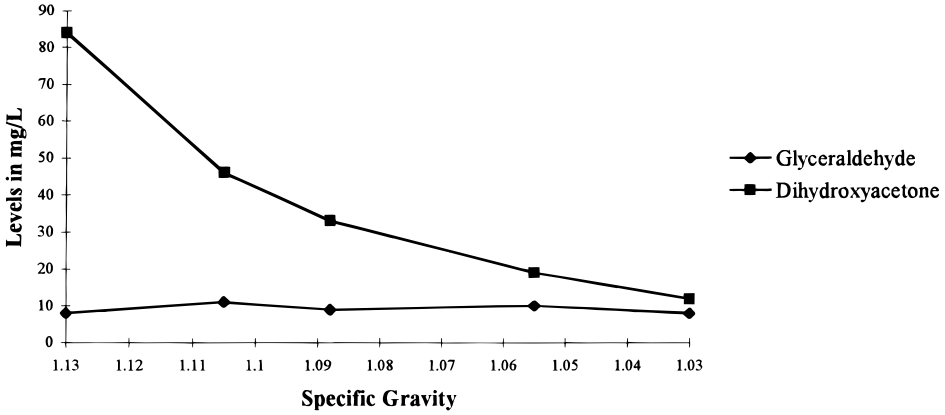

Figure 6. Glyceraldehyde and dihydroxyacetone level variations during alcoholic fermentation.

Table 2. Glyceraldehyde, Dihydroxyacetone, and 5-Oxofructose Levels before and after Alcoholic Fermentation

	glyceraldehyde level (mg/L)	dihydroxyacetone level (mg/L)	5-oxofructose level (mg/L)
before fermentation	8	84	265
after fermentation	8	12	280

Evolution during Alcoholic Fermentation. During alcoholic fermentation, we studied the evolution of the carbonyl compounds previously measured in musts. Under fermentation conditions of Sauternes wines, neither glyceraldehyde nor 5-oxofructose was metabolized by yeasts during alcoholic fermentation; on the other hand, dihydroxyacetone diminished, probably because it was metabolized through the triose phosphate pathway. The results are presented in Table 2 and Figure 6. They show that a must rich in 5-oxofructose will give a wine with the same content of this compound and thus a high SO_2 binding power. Thus, it is essential to select only grapes with noble rot.

Gluconic acid contents did not vary during alcoholic fermentation. On the other hand, those of pyruvic and 2-oxoglutaric acids increased considerably at the beginning of fermentation and decreased afterward as shown in Figure 7. These results are in agreement with those of Lafon-Lafourcade and Peynaud (1965), who observed that the ethanal content behaved in a similar way. The contents of these three compounds seem to depend on technological parameters.

Lafon-Lafourcade (1985) showed that the production of SO_2-binding compounds by yeast depends on the strains and species. This is why we wanted to test the influence of a few *Saccharomyces cerevisiae* commercial strains on this phenomenon. The yeast strain performing alcoholic fermentation plays an important role on ethanal and pyruvic and 2-oxoglutaric acids contents, and this has repercussions on the SO_2 binding power of the wines produced, as indicated in Table 3. Thus, Zymaflore ST gave the lowest TL50 and Uvaferm BC gave the highest SO_2 binding power (from 120 to 130 mg/L more than Zymaflore ST). In each case, the SO_2 only bound with ethanal and pyruvic and 2-oxoglutaric acids explained the difference of TL50 between the different wines. Therefore, yeasts produce these three compounds at different levels but reduce other SO_2-binding compounds present in musts in a similar way.

Sulfiting musts from botrytized grapes is an enological practice conducted by most wineries. This affects only the ethanal level (Table 5), the highest content, 22 mg/L, corresponding to an additional combination of 32 mg/L SO_2. Other SO_2-binding compounds present at high levels during alcoholic fermentation, such as pyruvic and 2-oxoglutaric acids, do not have their concentrations affected by must sulfiting. The rapid production of ethanal at the beginning of alcoholic fermentation and the particularly high affinity of this compound for SO_2 explain why it reacts as soon as it is present in the medium, as mentioned by Usseglio-Tomasset (1985).

Wine Constitution−SO_2 Binding Balance. Although well-known SO_2-binding substances such as ethanal and pyruvic and 2-oxoglutaric acids can explain the SO_2 binding phenomena in dry white and red wines (generally wines from healthy grapes), this is not the case for wines from botrytized grapes. Our results (Table 4) show that pyruvic and 2-oxoglutaric acids, gluconolactone, ethanal, dihydroxyacetone, glyceraldehyde, 5-oxofructose, glyoxal, and methylglyoxal may explain the totality or at least most of the SO_2 binding in wines from botrytized grapes. Among these compounds, the main ones are formed by yeast: ethanal, pyruvic and 2-oxo-

Table 3. Ethanal and Pyruvic and 2-Oxoglutaric Acid Levels and SO_2 Binding Power According to the Yeast Strain

site	yeast strain	ethanal level (mg/L)	2-oxoglutaric acid level (mg/L)	pyruvic acid level (mg/L)	TL50 (mg/L total SO_2)	bound SO_2 EOP[a] level (mg/L)
A	Maurivin PDM	55	78	162	300	181
	Uvaferm BC	55	147	308	390	271
	Zymaflore VL3	55	110	142	300	184
	Zymaflore ST	36	95	123	260	143
B	Maurivin PDM	45	133	211	380	209
	Uvaferm BC	53	167	326	460	284
	Zymaflore ST	32	137	156	340	167

[a] Bound SO_2 EOP = SO_2 bound only with ethanal and pyruvic and 2-oxoglutaric acids.

Table 4. SO_2 Binding Balance Sheet in Three Sauternes Wines from Vintage 1998

	wine 1		wine 2		wine 3	
	level (mg/L)	bound SO_2 level[a] (mg/L)	level (mg/L)	bound SO_2 level[a] (mg/L)	level (mg/L)	bound SO_2 level[a] (mg/L)
gluconolactone	270	58	155	33	260	56
2-oxoglutaric acid	110	42	248	96	78	30
pyruvic acid	95	41	254	111	43	19
ethanal	64	93	56	81	65	95
dihydroxyacetone	4	2	2	1	3	1
glyceraldehyde	13	6	6	3	14	6
5-oxofructose	150	38	80	20	130	33
glyoxal	1.1	2	1	2	2	4
methylglyoxal	1.3	2	1.7	2	5	7
bound SO_2 level for 50 mg/L free SO_2		284		351		255
CL 50 mg/L SO_2	290		330		290	
bound SO_2 explained (%)	98		106		88	

[a] By calculation from K_d.

Table 5. Ethanal and Pyruvic and 2-Oxoglutaric Acid Levels and SO_2 Levels and Binding Power According to Must SO_2 Addition

	ethanal level (mg/L)	2-oxoglutaric acid level (mg/L)	pyruvic acid (mg/L)	free SO_2 level (mg/L)	total SO_2 level (mg/L)	TL50 (mg/L SO_2)
wine from nonsulfited must	43	144	224	39	346	360
wine from sulfited must	65	139	227	41	379	400

glutaric acids, and others already present in must from botrytized grapes such as gluconolactone and 5-oxofructose, compounds absent in healthy grapes.

CONCLUSION

This work deals with the occurrence of several compounds related to the SO_2 binding power of musts from botrytized grapes. We demonstrate and confirm the importance of neutral carbonyl compounds such as dihydroxyacetone and 5-oxofructose as well as carboxylic acids (gluconic acid, 2- and 5-oxogluconic acids). Most of these compounds have already been described by Beech et al. (1979) as resulting from bacteria metabolism. Work is now underway in our laboratory on the importance of the microflora which, in addition to *B. cinerea*, are present on botrytized grapes. Their metabolism is being studied.

Among these compounds which correlate well with binding power, dihydroxyacetone and 5-oxofructose have an affinity for SO_2 which explains an important part of botrytized must combination. During alcoholic fermentation, dihydroxyacetone is metabolized by yeast but not 5-oxofructose. Moreover, during fermentation, the levels of SO_2-binding compounds such as ethanal and pyruvic and 2-oxoglutaric acids increase in wine. The levels of these compounds are strongly dependent on the yeast strain inoculated. Sulfiting the must increases only the ethanal concentration in wine and thus its SO_2 binding power.

In wine, the contents of pyruvic and 2-oxoglutaric acids, gluconolactone, ethanal, dihydroxyacetone, glyceraldehyde, 5-oxofructose, glyoxal, and methylglyoxal accounted for almost all the SO_2 combinations. Five compounds were of particular importance in this balance: the three compounds produced by yeast, ethanal and pyruvic and 2-oxoglutaric acids, and two compounds already present in botrytized must, gluconolactone and 5-oxofructose.

ACKNOWLEDGMENT

Isabelle Masneuf from the Faculté d'Œnologie, who did the implantation control of various yeast strains.

LITERATURE CITED

Asvany, A. Les techniques de vinification permettant de diminuer les doses de SO_2. *Bull. O.I.V.* **1985**, *652*−*653*, 621−623.

Beech, F. W.; Burroughs, L. F.; Timberlake, C. F.; Whiting, C. G. Progrès récents sur l'aspect chimique et l'action antimicrobienne de l'anhydride sulfureux. *Bull. O.I.V.* **1979**, *586*, 1001−1021.

Bertrand, A. Méthodes d'analyses des boissons spiritueuses d'origine viticole. *Feuill. O.I.V.* **1990**, 867.

Bertrand, A.; Guillou I. Sulphur dioxide combinations in sweet wines made from botrytised grapes. *Bull. O.I.V.* **1999**, *821*−*822*, 498−516.

Blouin, J.; Peynaud, E. Présence constante des acides pyruvique et α-cétoglutarique dans les moûts de raisins et les vins. *C. R. Hebd. Seances Acad. Sci.* **1963**, *256*, 4521.

Blouin, J.; Stonestreet, E.; Krivsky, A. Nouvelles connaissances sur les combinaisons carbonylées du SO_2 dans les vins. *C. R. Journ. Tech. CIVB* **1995**, 100−110.

Burroughs, L. F.; Sparks, A. H. The identification of sulfur dioxide-binding compounds in apple juices and ciders. *J. Sci. Food Agric.* **1964**, *15*, 176−185.

Burroughs, L. F.; Sparks, A. H. Sulphite binding power of wines and ciders. I Equilibrium constants for the dissociation of carbonyl-bisulfite compounds. *J. Sci. Food Agric.* **1973b**, *24*, 187−198.

Burroughs, L. F.; Sparks, A. H. Sulphite binding power of wines and ciders. III Determination of carbonyl compound in a wine and calculation of its sulphite binding power. *J. Sci. Food Agric.* **1973a**, *24*, 207−217.

de Revel, G.; Pripis Nicolau, L.; Barbe, J.-C.; Bertrand A. The detection of α-dicarbonyl compounds in wine by formation of quinoxaline derivatives. *J. Sci. Food Agric.* **1999**, *79*, 1−7.

Guillou-Largeteau, I. Etude de substances volatiles de faible poids moléculaire combinant le dioxyde de soufre dans les vins blancs issus de vendange botrytisée. Mise en évidence et importance du role de l'hydroxypropanedial. Ph.D. Thesis No. 425; University Victor Segalen Bordeaux 2, Bordeaux, France, 1996.

Guillou, I.; Bertrand, A.; de Revel, G.; Barbe, J.-C. Occurrence of Hydroxypropanedial in certain musts and wines. *J. Agric. Food Chem.* **1997**, 45, 3382−3386.

Kielhöffer, E.; Würdig, G. Die an unbekannte Weinbestandteile gebundene schwefflige Säure (Rest SO_2) und ihre Bedeutung für den Wein. *Weinberg Keller* **1960**, *7*, 313−361.

Lafon-Lafourcade, S. Role des microorganismes dans la formation des substances combinant le SO_2. *Bull. O.I.V.* **1985**, *652−653*, 590−604.

Lafon-Lafourcade, S.; Peynaud, E. Sur l'évolution des acides pyruvique et α-cétoglutarique au cours de la fermentation alcoolique. *C. R. Hebd. Seances Acad. Sci.* **1965**, 261, 1778−1780.

Macris, B. J.; Markakis, P. Transport and toxicity of sulphur dioxide in *Saccharomyces cerevisiae var. ellipsoideus*. *J. Sci. Food Agric.* **1974**, *25*, 21−29.

Masneuf, I.; Dubourdieu, D. Comparaison de deux techniques d'identification des souches de levures de vinification basées sur le polymorphisme de l'ADN génomique: réaction de polymérisation en chaîne (PCR) et analyse des caryotypes (électrophorèse en champ pulsé). *J. Int. Sci. Vigne Vin* **1994**, *28*, 153−160.

Ribereau-Gayon, J.; Peynaud, E.; Ribereau-Gayon, P.; Sudraud, P. In *Traité d'Œnologie;* Tome 1: Analyse et controle des vins; Dunod, Ed.; Paris, France, 1972; pp 458−460.

Ribereau-Gayon, P. Connaissance de la nature des combinaisons de l'anhydride sulfureux dans les vins. *Bull. O.I.V.* **1972**, *507*, 406−413.

Sponholz, W. R.; Dittrich, H. H. Über das Vorkommen von Galakturon- und Glucuronsaüre sowie von 2- und 5-Oxo-Gluconsaüre in Weinen, Sherries, Obst- und Dessertweinen. *Vitis* **1984**, *23*, 214−224.

Sudraud, P.; Chauvet, S. Action antilevure de l'anhydride sulfureux moléculaire. *Connaiss. Vigne Vin* **1985**, *19* (1), 31−40.

Sudraud, P.; Chauvet, S.; Joyeux, A. Acides monocétogluconiques et combinaison de l'anhydride sulfureux. *Rev. Fr. Oenol.* **1986**, *104*, 58−62.

Usseglio-Tomasset, L. Les technologies de vinification permettant de diminuer les doses de SO_2. *Bull. O.I.V.* **1985**, *652−653*, 606−616.

Received for review December 13, 1999. Revised manuscript received May 3, 2000. Accepted May 3, 2000. We thank the "Syndicat des Crus Classés de Sauternes et Barsac" and "Château d'Yquem" for their financial and technical support.

JF991359D