

The Use of Sulfur Dioxide in Must and Wine Treatment

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8.1 INTRODUCTION

The general use of sulfur dioxide (SO_2) appears to date back to the end of the 18th century. Its many properties make it an indispensable aid in winemaking. Perhaps some wines could be made in total or near-total absence of SO_2 but it would certainly be presumptuous to claim that all of the wines produced in the various wineries throughout the world could be made in this manner. It must also be taken into account that yeasts produce small quantities of SO_2 during fermentation. In

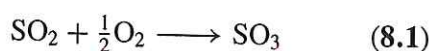
general, the amount formed is rarely more than 10 mg/l, but in certain cases it can exceed 30 mg/l. Consequently, the total absence of sulfur dioxide in wine is rare, even in the absence of sulfiting.

Its principal properties are as follows:

1. Antiseptic: it inhibits the development of microorganisms. It has a greater activity on bacteria than on yeasts. At low concentrations, the inhibition is transitory. High concentrations destroy a percentage of the microbial population. The effectiveness of a given

concentration is increased by lowering the initial population, by filtration for example. During storage, SO₂ hinders the development of all types of microorganisms (yeasts, lactic bacteria, and, to a lesser extent, acetic bacteria), preventing yeast haze formation, secondary fermentation of sweet white wines (Section 8.6.2), *Brettanomyces* contamination and the subsequent formation of ethyl-phenols (Volume 2, Section 8.4.4), the development of mycodermic yeast (flor) (Volume 2, Section 8.3.4), and various types of bacteria spoilage (Volume 2, Sections 8.3.1 and 8.3.3).

2. Antioxidant: in the presence of catalyzers, it binds with dissolved oxygen according to the following reaction:



This reaction is slow. It protects wines from chemical oxidations, but it has no effect on enzymatic oxidations, which are very quick. SO₂ protects wine from an excessively intense oxidation of its phenolic compounds and certain elements of its aroma. It prevents madeirization. It also contributes to the establishment of a sufficiently low oxidation-reduction potential, favoring wine aroma and taste development during storage and aging.

3. Antioxidasic: it instantaneously inhibits the functioning of oxidation enzymes (tyrosinase, laccase) and can ensure their destruction over time. Before fermentation, SO₂ protects musts from oxidation by this mechanism. It also helps to avoid oxidasic casse in white and red wines made from rotten grapes.
4. Binding ethanal and other similar products, it protects wine aromas and makes the flat character disappear.

Adding SO₂ to wine raises a number of issues. Excessive doses must be avoided, above all for health reasons, but also because of their impact on aroma. High doses neutralize aroma, while even larger amounts produce characteristic aroma defects, i.e. a smell of wet wool that rapidly becomes suffocating and irritating, together with

a burning sensation on the aftertaste. However, an insufficient concentration does not ensure the total stability of the wine. Excessive oxidation or microbial development can compromise its presentation and quality.

It is not easy to calculate the precise quantities required, because of the complex chemical equilibrium of this molecule in wine. It exists in different forms that possess different properties in media of different composition.

The concentration of sulfur dioxide in wine is habitually expressed in mg SO₂ per liter (or ppm) although this substance exists in multiple forms in wine (Section 8.3).

The words sulfur dioxide, sulfur anhydride or sulfurous gas can all be used equally, or even sulfurous acid, though the corresponding molecule cannot be isolated. The expression 'sulfur', however, is fundamentally incorrect. Additions made to wine are always expressed in the anhydrous form, in mg/l or in g/hl, regardless of the form effectively employed—sulfur dioxide gas or liquid solution, potassium bisulfite (KHSO₃) or potassium metabisulfite (K₂S₂O₅). The effect of the addition to wine is the same, regardless of the form used. The equilibrium established between the various forms is identical. It depends on the pH and the presence of molecules that bind with the sulfur dioxide.

Substantial progress in the understanding of the chemistry of sulfur dioxide and its properties have permitted the winemaker to reason its use in wine. As a result, the concentrations of SO₂ employed in wine have considerably decreased. Simultaneously, this technological progress has led to a decrease in authorized concentrations. In 1907, French legislation set the legal limit in all wines at 350 mg/l increased, in 1926, to 450 mg/l. Today, French wines are subject to EU legislation (Table 8.1), which has gradually reduced the permitted level to 160 mg/l for most red wines and 210 mg/l for the majority of white wines. Higher doses may only be used in wines with very high sugar content. They are generally premium wines produced in small volumes and consumed in moderate quantities.

In practice, the concentration used is even lower. For white French wines (excluding special wines)

Table 8.1. Maximum sulfur dioxide concentrations depending on wine type. EU regulations and OIV recommendations (values expressed in mg/l)

A. EU regulations no: 1493/1999 and 1622/2000, modified in 1655/2001

Types of wine	Sugar content <5 g/l	Sugar content = or >5 g/l
Red wines	160 (+40)*	210 (+40)*
White and rosé wines	210 (+40)*	260 (+40)*
Red <i>vins de pays</i>	125	150
White and rosé <i>vins de pays</i>	150	175
Dessert wines	150	200
<i>Vins de pays</i> (TAV >15% vol.; sugar >45 g/l)		
White AOC wines		
Bordeaux superieur, Graves de Vayres, Côtes de Bordeaux		
Saint-Macaire, Premières Côtes de Bordeaux, Sainte-Foy		
Bordeaux, Côtes de Bergerac suivie ou non de la denomination		
Côtes de Saussignac, Haut Montravel, Côtes de Montravel et		
Rosette, Gaillac		
White DO wines		
Allela, La Mancha, Navarra, Penedes, Rioja, Reuda, Tarragona		
et Valencia		
Alto Adige, Trentino "passito" "vendemmia tardiva"		
Vqprd Moscato di Pantelleria naturale and Moscato di Pantelleria		
United Kingdom Vqprd described as follows:		
botrytis, noble harvest, noble late harvested		
German wines		
Spätlese		300
Auslese and some Rumanian white wines		350
Beerenauslese, Ausbruch, Ausbruch-wein, Trockenbeerenauslese,		400
Elswein		
White AOC wines		
Sauternes, Barsac, Cadillac, Cérons, Louplac,		
Sainte-Croix-du-Mont, Graves supérieurs, Monbazillac,		
Jurançon, Pacherenc du Vic Bilh. Anjou-Coteaux de la Loire,		
Bonnezeaux, Quarts de Chaume, Coteaux de l'Aubance,		
Coteaux du Layon suivi du nom de la commune d'origine,		
Coteaux du Layon suivi du nom de Chauma, Coteaux de		
Saumur		
Alsace et Alsace grand cru suivi de la mention "vendanges		
tardives" ou "selection de grains nobles"		
Sweet wines from Greece (sugar = or >45 g/l)		
Samos, Rhodes, Patras, Rio Patron, Cephalonie, Limnos, Sitia,		
Santorin, Néméa, Daphines		
Certain Canadian white wines (Icewine)		400

*When required due to weather conditions in certain vineyard areas.

B. OIV—maximum acceptable limits: International Code of Winemaking Practices and Collection of International Wine Analysis Methods, 2001.

Types of wine	Sugar content = or <4 g/l	Sugar content >4 g/l
Red wines	150	300
White and rosé wines	200	300
Certain sweet white wines		400

the average concentration is 105 mg/l; for red wines it is 75 mg/l. The Office International de la Vigne et du Vin (OIV) recommends slightly higher values than those advocated by the EU in its member countries. In certain countries, the regulation of sulfur dioxide dictates a common limit for all wines. For example, this value is 350 mg/l in the USA, in Canada, in Japan and in Australia.

Due to the fluctuating equilibrium between free and bound forms of SO_2 , in general, the legislation of different countries exclusively refers to the total sulfur dioxide concentration. Certain countries, however, have regulations for the free fraction.

Today, especially for health reasons, the possibility of further reducing the authorized concentrations in different kinds of wines is sought after. Such an approach consists of optimizing the conditions and perfecting the methods of using this product. This supposes more in-depth knowledge of the chemical properties of the sulfur dioxide molecule and its enological role. Substitute products can also be considered. Due to the various effects of sulfur dioxide in wine, the existence of another substance performing the same roles without the disadvantages seems very unlikely, but, the existence of adjuvants, complementing the effect of SO_2 in some of its properties, is perfectly conceivable. Enological research has always been preoccupied by the quest for such a product or substitution process (Chapter 9).

In conclusion, sulfur dioxide permits the storage of many types of wine known, today that would not exist without its protection. In particular, it permits extended barrel maturation and bottle aging. In view of its involvement in a wide variety of chemical reactions, it is not easy to determine the optimum dose to obtain all the benefits of SO_2 without any of its unfortunate side-effects. The adjustment should be made within plus or minus 10 mg/l.

8.2 PHYSIOLOGICAL EFFECTS

The addition of sulfur dioxide to wine raises health-related objections. These should be taken into account, although this product boasts a long

history of use. Its use has always been regulated and enological techniques have always sought methods of lowering its concentrations. Since the beginning of the century, the possible toxicity of sulfur dioxide has been the subject of much research (Vaquer, 1988).

Acute toxicity has been studied in animals. The absorption of a single dose of sulfites is slightly toxic. Depending on the animal species, the LD_{50} (lethal dose for 50% of individuals) is between 0.7 and 2.5 g of SO_2 per kilogram of body weight. Sodium sulfite would therefore have an acute toxicity similar to inoffensive products such as sodium bicarbonate or potassium chloride.

Chronic toxicity has also been studied in animals (Til *et al.*, 1972). During several generations, a diet containing 1.5 g of SO_2 /kg was regularly absorbed. Three kinds of complications resulted: a thiamine deficiency linked to its destruction by sulfur dioxide; a histopathological modification of the stomach; and slowed growth. This study permitted the establishment of a maximum nontoxic concentration for rats at 72 mg/kg of body weight. This value led the World Health Organization to set the RDA (recommended daily allowance) at 0.7 mg of SO_2 /kg of body weight.

Concerning its toxicity in humans, studies carried out indicate the appearance of intoxication symptoms such as nausea, vomiting and gastric irritation at significantly high absorbed concentrations (4 g of sodium sulfite in a single concentration). No secondary effects were observed with a concentration of 400 mg of sulfur dioxide during 25 days. In humans, its possible toxicity has often been attributed to the well-known destruction of thiamine or vitamin B1 by sulfites, but the corresponding reaction has been observed to be very limited at a pH of around 2, which corresponds to stomach pH.

In 1973, allergic reactions to sulfites were proven to exist. They occur at very low ingested concentrations (around 1 mg) and primarily concern asthmatics (4–10% of the human population). Asthmatics are therefore urged to abstain from drinking wine. Although SO_2 sensitivity has not been clearly demonstrated for non-asthmatics, these allergic reactions led the US FDA (Food and Drug Administration) to require the mention of the

presence of sulfites on wine labels in the United States when the concentration exceeds 10 mg/l.

Considering an RDA of 0.7 mg/kg/day, the acceptable concentration for an individual is between 42 and 56 mg per day, depending on body weight (60 and 80 kg, respectively). The consumption of half a bottle of wine per day (375 ml) can supply a quantity of SO₂ higher than the RDA. If the total SO₂ concentration is at the maximum limit authorized by the EU (160 mg/l for red wines and 210 mg/l for white wines), the quantity of SO₂ furnished by half a bottle is 60 mg for reds and 79 mg for whites. The average SO₂ concentrations observed in France are much lower: 75 mg/l for red wines and 105 mg/l for white wines. Therefore, the daily consumption of half a bottle furnishes 28 and 39 mg of SO₂, respectively.

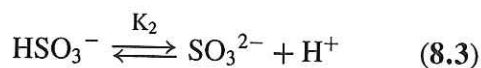
In any case, the figures clearly indicate that, with respect to World Health Organization norms, wines can supply a non-negligible quantity of SO₂. It is therefore understandable that national and international health authorities recommend additional decreases in the accepted legal limits.

Experts from the OIV estimate that the concentrations recommended by the EC can be decreased by 10 mg/l, at least for the most conventional wines. In this perfectly justified quest for lowering SO₂ concentrations, specialty wines such as botrytized wines must be taken into account. Due to their particular chemical composition, they possess a significant combining power with sulfur dioxide. Consequently, their stabilization supposes extensive sulfiting. The EU legislation authorizing 400 mg/l is perfectly reasonable, but this concentration is not always sufficient. In particular, it does not guarantee the stability of some batches of botrytized wines and will not prevent them from secondary fermentation.

8.3 CHEMISTRY OF SULFUR DIOXIDE

8.3.1 Free Sulfur Dioxide

During the solubilization of SO₂, equilibria are established:



The H₂SO₃ acid molecule would not exist in a solution. It nevertheless possesses two acid functions whose pKs are 1.81 and 6.91, respectively at 20°C. The neutralization of an acid begins at approximately pH = pK - 2. The absence of neutral sulfites (SO₃²⁻) at the pH of wine can therefore be deduced. But the first function is partially neutralized according to the pH. Knowing the proportion of free acid (active SO₂) and bisulfite (HSO₃⁻) is important, since the essential enological properties are attributed to the first. The calculation is made by applying the mass action law:

$$\frac{[\text{H}^+][\text{HSO}_3^-]}{[\text{SO}_2][\text{H}_2\text{O}]} = K_1 \quad (8.4)$$

The water concentration can be treated as a constant or very near to 1:

$$\frac{[\text{H}^+][\text{HSO}_3^-]}{[\text{SO}_2]} = K_1 \quad (8.5)$$

which results in:

$$\text{Log} \frac{[\text{HSO}_3^-]}{[\text{SO}_2]} = \text{pH} - \text{p}K_1 \quad (8.6)$$

Table 8.2 indicates the results for the pH range corresponding to various kinds of wine. The proportion of molecular SO₂, approximately corresponding to active SO₂, varies from 1 to 10. This explains the need for more substantial sulfiting when the must or wine pH is high.

Table 8.2. Molecular SO₂ and bisulfite percentages according to pH (at 20°C) in aqueous solution

pH	Molecular SO ₂	Bisulfite (HSO ₃ ⁻)
3.00	6.06	94.94
3.10	4.88	95.12
3.20	3.91	96.09
3.30	3.13	96.87
3.40	2.51	97.49
3.50	2.00	98.00
3.60	1.60	98.40
3.70	1.27	98.73
3.80	1.01	98.99
3.90	0.81	99.19
4.00	0.64	99.36

Table 8.3. Sulfur dioxide pK1 value according to alcoholic strength and temperature (Usseglio-Tomasset, 1995)

alcohol (% vol.)	Temperature (°C)							
	19	22	25	28	31	34	37	40
0	1.78	1.85	2.00	2.14	2.25	2.31	2.37	2.48
5	1.88	1.96	2.11	2.24	2.34	2.40	2.47	2.56
10	1.98	2.06	2.21	2.34	2.44	2.50	2.57	2.66
15	2.08	2.16	2.31	2.45	2.54	2.61	2.67	2.76
20	2.18	2.26	2.41	2.55	2.64	2.72	2.78	2.86

The pK value is also influenced by temperature and alcoholic strength (Table 8.3), and equally by ionic force—the concentration in salts. Usseglio-Tomasset (1995) calculated the effect of these factors on the proportion of sulfur dioxide in the form of active SO₂ (Table 8.4).

The bisulfite ion (HSO₃⁻) represents the corresponding fraction of the acid neutralized by bases, thus almost entirely in the form of ionized salts. Active SO₂ (or sulfurous acid in the free acid state) represents free sulfur dioxide as defined in enology. The difference between the chemical notion of a free acid and a salified acid should be taken into account.

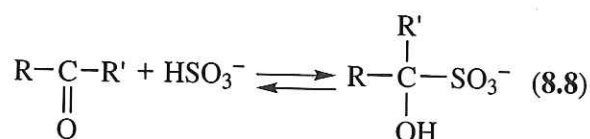
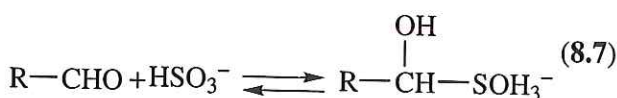
As a result, the antiseptic properties of a given concentration of free SO₂ towards yeasts or bacteria vary in function of pH, even if the HSO₃⁻ form is attributed with a certain activity. In the same manner, the disagreeable taste and odor of sulfur dioxide, for the same value of free SO₂, increase the more acidic the wine. The disagreeable odor of SO₂ is sometimes less the result of an exaggerated SO₂ addition than the nature of the wine—inferior quality, an absence of character and aroma, and very high acidity.

Table 8.4. Percentage of active molecular SO₂ at pH 3.0 according to alcoholic strength and temperature (Usseglio-Tomasset, 1995)

alcohol (% vol.)	Temperature (°C)		
	19	28	38
0	4.88		
10	7.36	15.40	27.55
20	10.95		

8.3.2 Bound Sulfur Dioxide

Bisulfites possess the property of binding molecules which contain carbonyl groups according to the following reversible reaction:



These additional forms represent bound sulfur dioxide, or bound SO₂ as it is defined in enology. The sum of free SO₂ plus bound SO₂ is equal to total SO₂. With respect to free SO₂, bound SO₂ has much less significant (even insignificant), antiseptic and antioxidant properties (Section 8.6).

In the reactions forming these combinations, the equilibrium point is given by the formula in Eqn (8.9), for the reaction in Eqn (8.7). This formula presents the molar concentration relationship between the different molecules:

$$\frac{[\text{R-CHO}][\text{HSO}_3^-]}{[\text{R-CHOH-SO}_3^-]} = K \quad (8.9)$$

K is a constant characteristic of each substance, with aldehydic or ketonic functions, able to bind SO₂.

This relationship can be written as follows:

$$\frac{[\text{R-CHOH-SO}_3^-]}{[\text{R-CHO}]} = \frac{[\text{HSO}_3^-]}{K} \quad (8.10)$$

For example, a concentration of 20 mg of free SO_2 per liter represents 25 mg of HSO_3^- per liter (molecular weights 64 and 81, respectively). The molar concentration is therefore:

$$[\text{HSO}_3^-] = \frac{25}{81} \times 10^{-3} = \frac{10^{-3}}{3.24} \quad (8.11)$$

The relationship in Eqn (8.10) becomes:

$$\frac{[\text{C}]}{[\text{A}]} = \frac{[\text{R-CHO-SO}_3^-]}{[\text{R-CHO}]} = \frac{10^{-3}}{3.24 \times K} \quad (8.12)$$

It expresses the proportion of carbonyl group molecules bound to SO_2 (C) and in their free form (A).

First case: K has a low value equal to or less than $0.003 \times 10^{-3} \text{ M}$, at equilibrium:

$$\frac{[\text{C}]}{[\text{A}]} = \frac{10^{-3}}{3.24 \times 0.003 \times 10^{-3}} = \frac{1}{0.01} = 100 \quad (8.13)$$

In this case, there exists 100 times more of the bound form than the free form. The binding molecule is considered to be almost entirely in the combined form. Free SO_2 can only exist when all of the molecules in question are completely bound. Furthermore, this combination is stable and definitive; the depletion of free SO_2 by oxidation does not cause an appreciable displacement of the equilibrium.

Second case: K has an elevated value equal to or greater than $30 \times 10^{-3} \text{ M}$:

$$\frac{[\text{C}]}{[\text{A}]} = \frac{10^{-3}}{3.24 \times 30 \times 10^{-3}} = \frac{1}{100} \quad (8.14)$$

In this case, there exists 100 times more of the free form than the combined form. The binding molecule is considered to be slightly combined and the corresponding combination is not very stable. When free SO_2 is depleted by oxidation, the dissociation of this combination, necessary for reestablishing the equilibria, regenerates free SO_2 .

Of course, $[\text{C}]$ plus $[\text{A}]$ represents the total molar concentration of the combining molecule as given by analysis, expressed in millimole per liter. It is therefore possible to establish overall reaction values of bound SO_2 for different free SO_2 values. In fact, by determining the quantity of each combining molecule, the amount of bound SO_2 can be calculated using the value of K and the concentration of free SO_2 (see Figure 8.3). The sum of the individual combinations must correspond with the total bound SO_2 determined by analysis (Section 8.4.3).

Figure 8.1 gives SO_2 combination curves for different values of K and for a combining molecular concentration of 10^{-3} M . The maximum bound SO_2 concentration is also 10^{-3} M , 64 mg/l.

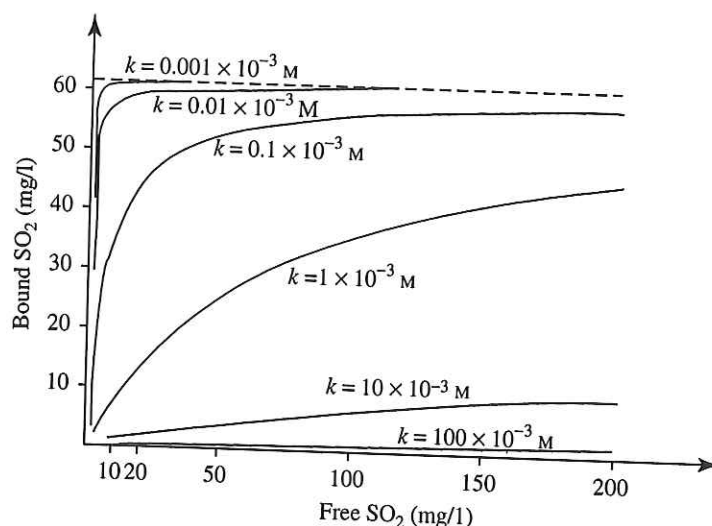


Fig. 8.1. Sulfur dioxide combination curves in accordance with the chemical dissociation constant K (concentration of carbonyled substance = 10^{-3} M (Blouin, 1965))

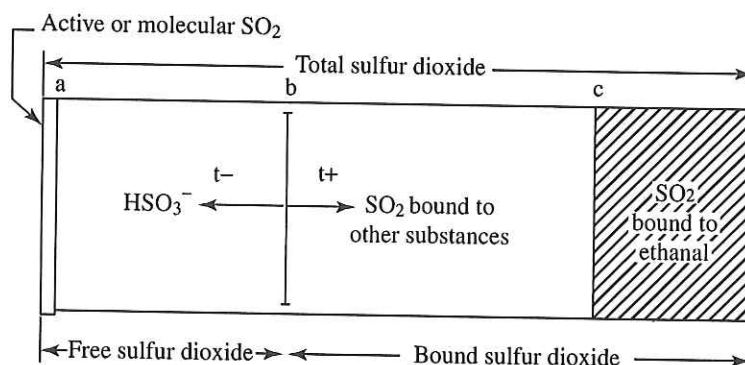


Fig. 8.2. The different states of sulfur dioxide in wine (Ribéreau-Gayon *et al.*, 1977)

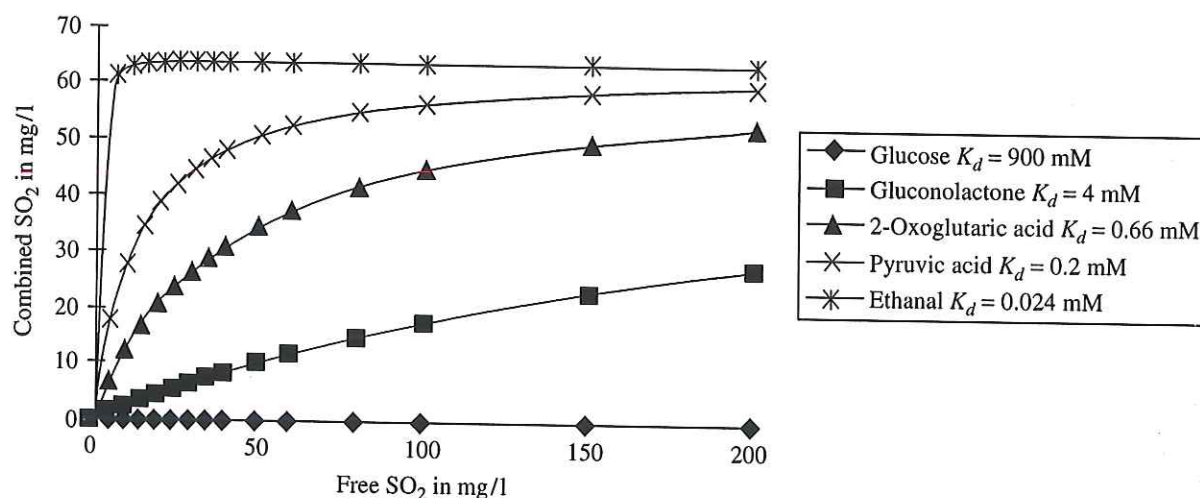


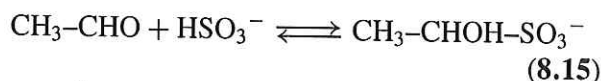
Fig. 8.3. Sulfur dioxide combination curves for various compounds at a concentration of 1 mM, in function of their K_d (Barbe, 2000)

In conclusion, the different forms of sulfur dioxide existing in wine are summarized in the Figure 8.2. Active SO_2 is located to the left; its separation (a) with HSO_3^- varies according to the pH. To the right, sulfurous aldehydic acid represents the SO_2 fraction combined with ethanal. Since K is low, this combination is very stable and depends on the ethanal concentration. The (c) separation line is definitive. On the other hand, the (b) separation between sulfur dioxide and sulfur dioxide combined with other substances varies, moving in one direction or the other according to temperature and the free SO_2 concentration.

8.4 MOLECULES BINDING SULFUR DIOXIDE

8.4.1 Ethanal

The reaction:



generally represents the most significant portion of bound SO_2 in wine. The value of K is extremely low (0.0024×10^{-3}) and corresponds to a combination rate of greater than 99%. The ethanal concentrations between 30 and 130 mg/l

correspond to possible bound SO_2 values between 44 and 190 mg/l.

In wine no longer containing free SO_2 , a weak dissociation of sulfurous aldehydic acid liberates a trace of ethanal. This ethanal is said to be responsible for the flat character in wine, but the presence of free ethanal is considered to be impossible in wine containing free SO_2 .

The combination is rapid. At pH 3.3, 98% of it is combined in 90 minutes and the combination is total in 5 hours. Within normal limits, the combination is independent of temperature. The amount of free SO_2 liberated by raising the temperature is very small. Concentrations in botrytized musts are of the order of 10 mg/l, up to a maximum of 20 mg/l. These concentrations may explain a mean combination of under 10 mg/l SO_2 .

Alcoholic fermentation is the principal source of ethanal in wine. It is an intermediary product in the formation of ethanol from sugars. Its accumulation is linked to the intensity of the glyceropyruvic fermentation. It principally depends on the level of aeration, but the highest values are obtained when yeast activity occurs in the presence of free SO_2 . The formation of sulfurous aldehydic acid is a means of protection for the yeasts against this antiseptic. Consequently, the level of grape sulfiting controls the ethanal and ethanal bound to SO_2 concentration.

Considering these phenomena, the addition of SO_2 to a fermenting must should be avoided. It would immediately be combined without being effective. When the grapes are botrytized, the variation in the ethanal content of different wines when 50 mg/l of SO_2 is added to the must accounts for a combining power approximately 40 mg/l higher than that of non-sulfited control wines. When stopping the fermentation of a sweet wine, a sufficient concentration should be added which

stops all yeast activity. This concentration can be decreased by initially reducing the yeast population, using centrifugation or cold stabilization (-4°C), for example. The highest ethanal concentrations are encountered when successive fermentations occur. The necessary multiple sulfittings progressively increase bound SO_2 concentrations.

The chemical oxidation of ethanol, by oxidation-reduction in the presence of a catalyzer, may also increase the ethanal concentration during storage—for example, during rackings. The combining power of the wine therefore also increases.

8.4.2 Ketonic Acids

Pyruvic acid and 2-oxoglutaric acid (formerly α -ketoglutaric acid) are generally present in wine (Table 8.5). They are secondary products of alcoholic fermentation. Considering their low K value, they can play an important role in the SO_2 combination rate. For example, a wine containing 200 mg of pyruvic acid and 100 mg of 2-oxoglutaric acid per liter has 93 mg of SO_2 per liter bound to these acids for 20 mg of free SO_2 .

Those two substances may combine with very different amounts of SO_2 . In wines made from botrytized grapes, for a free SO_2 content of 50 mg/l, 2-oxoglutaric acid is likely to combine with an average of 43 mg/l and pyruvic acid with 58 mg/l (Barbe, 2000). The average percentages of pyruvic and 2-oxoglutaric acids in the SO_2 combination balance are 20.7% and 16.7%, respectively.

It is therefore interesting to understand the formation and accumulation conditions of these acids during alcoholic fermentation. They are formed at the beginning of the fermentative process. After initially increasing, their concentration decreases towards the end of fermentation. This explains the higher concentration of these molecules in sweet

Table 8.5. The ketonic acids of wine (Usseglio-Tomasset, 1995)

Name	Formula	K	Average concentrations in wine
Pyruvic acid	$\text{CH}_3\text{-CO-COOH}$	$0.3 \times 10^{-3} \text{ M}$	10–500 mg/l
2-Oxoglutaric acid	$\text{COOH-CO-CH}_2\text{-CH}_2\text{-COOH}$	$0.5 \times 10^{-3} \text{ M}$	2–350 mg/l

Table 8.6. Action of thiamine on ketonic acids and free sulfur dioxide concentrations (mg/l), calculated for 250 mg of total SO₂ per liter (Ribereau-Gayon *et al.*, 1977)

Origin of wines	Control			+Thiamine		
	Pyruvic acid	2-Oxoglutaric acid	Free SO ₂ for total 250 mg/l	Pyruvic acid	2-Oxoglutaric acid	Free SO ₂ for total 250 mg/l
Monbazillac	10	traces	136	12	traces	134
Barsac	traces	128	104	traces	107	108
Cérons	traces	108	113	traces	82	111
Sauternes	264	121	44	40	73	108
Monbazillac	330	273	20	51	74	109
Sauternes	61	205	52	10	100	88
Cérons	108	72	48	41	70	81

wines with respect to dry wines. Elevated temperatures and pHs, along with aerations, favor the synthesis and accumulation of ketonic acids. In numerous fermentations, thiamine (at a concentration of 0.5 mg/l) has been shown to diminish the concentration of these acids and consequently sulfur dioxide combinations. The effect of thiamine is not surprising. It is an essential element of the carboxylase which assures decarboxylation of pyruvic acid into ethanal. This is an essential step of alcoholic fermentation. The accumulation of ketonic acids appears to result from a thiamine deficiency.

The figures in Table 8.6 show the effect of thiamine on the accumulation of ketonic acids and the corresponding combining power. In the first three wines made from slightly rotten grapes, the sulfur dioxide equilibrium is not modified after the addition of thiamine. In the other cases, the presence of thiamine decreases the ketonic acid concentration and often improves the sulfur dioxide equilibrium.

To be effective, thiamine needs to be added to clarified and sulfited must sufficiently early. It has no action on the accumulation of ethanal. In certain cases, useful secondary effects are observed: activation of the fermentation and diminution of volatile acidity. On average, in eight cases out of 10, thiamine increases the free SO₂ concentration in sweet wines by 20 mg, for the same bound SO₂ concentration.

8.4.3 Sugars and Sugar Derivatives

Considering the existence of aldehydic and ketonic functions in different sugar molecules, they can

be expected to have a combining power with sulfur dioxide. Fructose and saccharose, however, practically do not react.

Arabinose binds SO₂ at a rate of approximately 8 mg of SO₂ per gram of arabinose for 50 mg of free SO₂ per liter. Since the concentration of arabinose in wine is low (less than 1 g/l), this combination is not generally taken into account. Glucose has a much lower combining power. One gram combines 0.3 mg of SO₂ for 50 mg of free SO₂ per liter. Due to the high concentration of glucose in musts and sweet wines, this combination should be taken into account and it is included in the interpretation of the decrease in free SO₂ after sulfiting the grapes or the must.

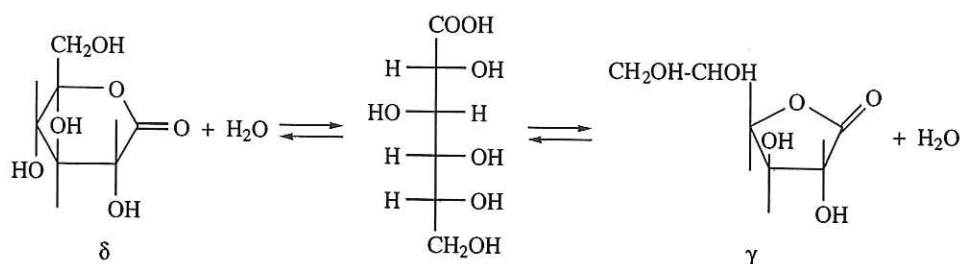
Burroughs and Sparks (1964 and 1973) identified the following substances: keto-5-fructose (5-oxofructose), xylosone, keto-2-gluconic (2-oxogluconic) and diketo-2,5-gluconic (2,5-dioxogluconic) acids (Table 8.7). Due to their concentrations in some wines (capable of attaining several dozen milligrams per liter), and their *K* values, some of them can play a significant role in binding with sulfur dioxide. These substances exist naturally in healthy, ripe grapes and they are also formed in large quantities by *Botrytis cinerea* and acetic acid bacteria (*Acetobacter* and *Pseudomonas*). Their development frequently accompanies various forms of rot.

According to more recent findings (Barbe *et al.*, 2002), among all the previously-mentioned compounds, 2-oxo and 2,5-dioxogluconic acids always present in a ratio of 2.5/1, do not have a significant affinity for SO₂. In contrast, at the pH of botrytized

Table 8.7. Sulfur-dioxide binding sugar derivatives (based on Burroughs and Sparks, 1964 and 1973)

	Uronic acid		Sugar oxidation products			
	Galacturonic acid	Glucuronic acid	Keto-2-gluconic acid	Diketo-2,5-gluconic acid	Keto-5-fructose	Xylosone
Formulae	$ \begin{array}{c} \text{CHO} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{HO}-\text{C}-\text{H} \\ \\ \text{HO}-\text{C}-\text{H} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{COOH} \end{array} $	$ \begin{array}{c} \text{CHO} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{HO}-\text{C}-\text{H} \\ \\ \text{H}-\text{C}-\text{H} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{COOH} \end{array} $	$ \begin{array}{c} \text{COOH} \\ \\ \text{C}=\text{O} \\ \\ \text{HO}-\text{C}-\text{H} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{CH}_2\text{OH} \end{array} $	$ \begin{array}{c} \text{COOH} \\ \\ \text{C}=\text{O} \\ \\ \text{HO}-\text{C}-\text{H} \\ \\ \text{H}-\text{C}-\text{H} \\ \\ \text{C}=\text{O} \\ \\ \text{CH}_2\text{OH} \end{array} $	$ \begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{C}=\text{O} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{HO}-\text{C}-\text{H} \\ \\ \text{C}=\text{O} \\ \\ \text{CH}_2\text{OH} \end{array} $	$ \begin{array}{c} \text{CHO} \\ \\ \text{C}=\text{O} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{HO}-\text{C}-\text{H} \\ \\ \text{CH}_2\text{OH} \end{array} $
<i>K</i>	20×10^{-3} M	20×10^{-3} M	0.4×10^{-3} M	0.4×10^{-3} M	0.3×10^{-3} M	0.15×10^{-3} M
Combination rate ^a	4.4	1.5	66	66	72	84

^aPercentage of combined substances per 50 mg of free SO₂.

**Fig. 8.4.** Formation of γ - and δ -gluconolactone from D-gluconic acid

musts and wines, gluconic acid (20 g/l) is in equilibrium with two lactones, γ - and δ -gluconolactone (Figure 8.4), representing about 10% of the concentration of the acid. The affinity corresponds to that of a monocarbonyl compound with a bisulfite combination dissociation constant $K = 4.22$ mM. Thus, the lactones of gluconic acid are likely to combine with up to 135 mg/l SO₂ for a free SO₂ content of 50 mg/l.

The 5-oxofructose content is also frequently of the order of 100 mg/l in wines made from botrytized grapes (Barbe, 2000). Concentrations increase with the combining power (Figure 8.5). According to Barbe (2000), 5-oxofructose may account for the combination of 4–78% of the sulfur dioxide. Concentrations of this compound are not altered by alcoholic fermentation or

any other aspect of yeast metabolism. Excessive concentrations can, therefore, only be avoided by monitoring grape quality. In the special case of must made from grapes affected by rot in the mature stage, it contributes, on average, over 60% to the combination balance. This compound is produced from fructose by acetic bacteria in the genus *Gluconobacter* (Section 7.5).

8.4.4 Dicarbonyl Group Molecules

In grapes affected by rot, Guillou-Largeteau (1996) identified molecules with two carbonyl groups (Table 8.8). They are probably formed during the development of *Botrytis cinerea* and other microorganisms involved in various types of rot. In view of the fact that concentrations do not

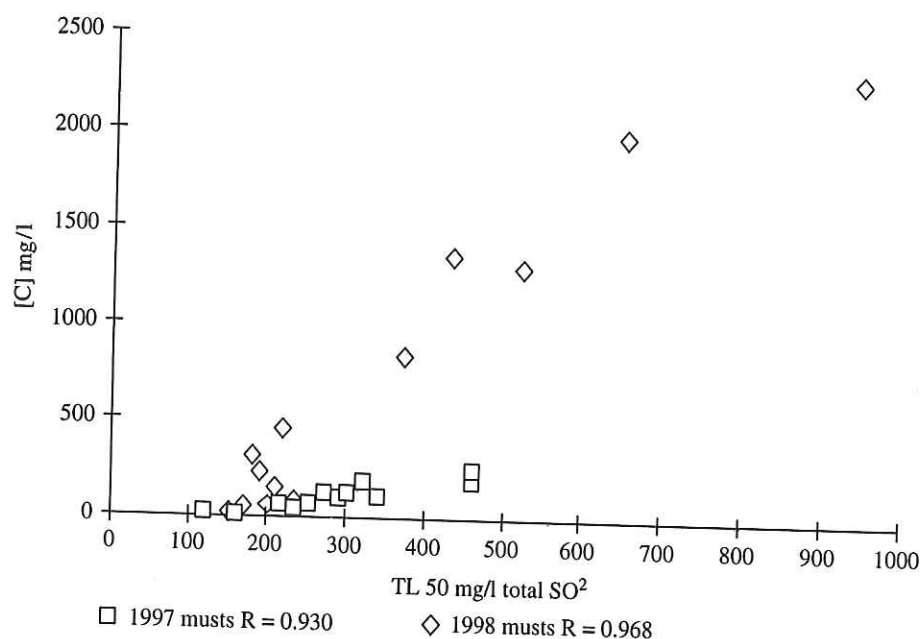


Fig. 8.5. Changes in the 5-oxofructose content according to the combining power of the must (Barbe *et al.*, 2000)

Table 8.8. Some dicarbonyl group molecules involved in sulfur dioxide combinations (hydroxypropanedial is a tautomer form of reductone) (Guillou-Largeteau, 1996)

Name	Chemical formula	Healthy grapes	Botrytized grapes
Glyoxal	$ \begin{array}{c} \text{C}=\text{O} \\ \\ \text{H} \\ \\ \text{C}=\text{O} \\ \\ \text{H} \end{array} $	Several mg/l	Several dozen mg/l
Methylglyoxal	$ \begin{array}{c} \text{CH}_3 \\ \\ \text{C}-\text{O} \\ \\ \text{C}=\text{O} \\ \\ \text{H} \end{array} $	Several mg/l	Several dozen mg/l

exceed 3 mg/l, the contribution of glyoxal to the SO₂ combination balance is practically negligible. Methylglyoxal makes a more significant contribution and may be responsible for combining over 50 mg/l SO₂ for a free SO₂ content of 50 mg/l (Barbe, 2000). Glyoxal and, especially, methylglyoxal, concentrations decrease during alcoholic fermentation, so these two α -dicarbonyl compounds

are only responsible for insignificant amounts of combined SO₂ in wine.

8.4.5 Other Combinations

Other substances likely to fix small amounts of sulfur dioxide have been identified: glucuronic, galacturonic acid and xylosone (Table 8.7), glyoxylic

acid, oxaloacetic acid, glycolic aldehyde, acetone, diacetyl, 5-(hydroxymethyl)furfural, etc. Their individual contribution is insignificant. In the case of dihydroxyacetone, 100 mg/l accounts for the combination of approximately 16 mg/l for 50 mg/l free SO₂, although this value may be as high as 72 mg/l in certain types of must (Barbe *et al.*, 2001c). While glyceraldehyde has a greater affinity for SO₂ ($K = 0.4$ mM, Blouin, 1995), it is only present in tiny amounts, so it makes a negligible contribution to the SO₂ combination balance.

SO₂ can also bind with phenolic compounds. In the case of proanthocyanic tannins, a solution of 1 g/l binds with 20 mg/l of SO₂ per liter. The combinations are significant with anthocyanins. These reactions are directly visible by the decoloration produced. The combination is reversible; the color reappears when the free sulfur dioxide disappears. This reaction is related to temperature (Section 8.5.2) and acidity (Section 8.5.1), which affect the quantity of free SO₂. The SO₂ involved in these combinations is probably titrated by iodine along with the free SO₂. In fact, due to their low stability, they are progressively dissociated to reestablish the equilibrium as the free SO₂ is oxidized by iodine.

8.4.6 The Sulfur Dioxide Combination Balance in Wines Made from Botrytized Grapes

Burroughs and Sparks (1973) calculated the SO₂ combination balance for two wines on the basis of the concentrations of the various constituents involved, determined by chemical assay and expressed in millimoles per liter (Section 8.3.2). The combined SO₂ calculated by this method was in good agreement with the combined SO₂ assay results, so it would appear that the SO₂ combinations were fully known in that case.

Blouin (1965) had previously demonstrated the particular importance of ketonic acids in this type of combination. In spite of all these findings, the sulfur dioxide combination balance cannot be considered complete and satisfactory. Progress has been made in establishing the combination

balance for wines made from botrytized grapes by finding out about other compounds, such as dihydroxyacetone, which is in balance with glyceraldehydes (Blouin, 1995; Guillou-Largeteau, 1996), and work on neutral carbonyl compounds in wines (Guillou-Largeteau, 1996). Finally, more recent research by Barbe and colleagues (2000; 2001a; b; and c; 2002) has improved control of sulfur dioxide concentrations by adding to knowledge of the origins of these compounds.

In wines made from botrytized grapes with high or low combination capacities, almost all of these combinations are accounted for by the concentrations of 5-oxofructose, dihydroxyacetone, γ - and δ -gluconolactone, ethanal, pyruvic and 2-oxoglutaric acid, glyoxal, methylglyoxal, and glucose (Table 8.9). In contrast, in must made from the same type of grapes, the high combining power is precisely accounted for by the quantities of SO₂ combined by 5-oxofructose, dihydroxyacetone, and gluconic acid lactones (Table 8.10).

Carefully-controlled fermentation of botrytized musts minimizes the accumulation of yeast metabolic products combining SO₂, although much higher concentrations of these compounds are implicated in stopping fermentation than those present in dry wines. Various technological parameters during fermentation make it possible to reduce the quantities of sulfur dioxide, by affecting only those combining compounds produced by fermentation yeasts. Wines with a lower sulfur dioxide combining power may be obtained by not sulfiting must, adding 0.5 mg/l of thiamine to must, choosing a yeast strain known to produce little ethanal or 2-oxoacids, and delaying mutage until the yeast metabolism has been completely shut down (e.g. by filtering or chilling the wine) (Barbe *et al.*, 2001c).

These compounds are produced due to the presence of microorganisms in botrytized grapes. Although yeasts represent a preponderant part of the microorganisms present, acetic bacteria, especially those in the *Gluconobacter* genus, are responsible for producing large amounts of these compounds, which act as intermediaries in their metabolism of the two main sugars in botrytized grapes (Barbe *et al.*, 2001a).

Table 8.9. Combining powers of compounds in a wine. SO₂ combinable by all the compounds assayed or only 6 of them (ethanal, pyruvic acid, 2-oxoglutaric acid, γ - and δ -gluconolactone, and 5-oxofructose) in 9 wines (Barbe, 2000)

Wines	CL50 mg/l Total SO ₂	Total combinable SO ₂		SO ₂ combinable by the 6 compounds accounting for the largest contributions	
		in mg/l	in % CL50	in mg/l	in % CL50
1	100	92	92	76	76
2	180	175	98	164	92
3	215	217	102	196	92
4	245	228	94	201	83
5	260	261	101	240	93
6	290	258	90	232	81
7	340	306	90	288	85
8	350	339	97	328	94
9	450	449	100	437	98

Table 8.10. Average quantities (mg/l) of sulfur dioxide combined by the compounds under study in different musts (Barbe *et al.*, 2001)

Compound	Musts (<i>n</i> = 24) with low combining power CL50 = 171 mg/l total SO ₂	Musts (<i>n</i> = 7) with high combining power CL50 = 498 mg/l total SO ₂
5-oxofructose	24	258
γ - and δ -gluconolactone	17	55
dihydroxyacetone	7	60
glucose	48	45
methylglyoxal	12	9
glyoxal	2	2
ethanal	10	14
2-oxoglutaric acid	14	15
pyruvic acid	5	9
other	32	31

The SO₂ combination balance varies considerably between different musts (metabolism of the acetic bacteria) and wines (fermentation parameters). Furthermore, the total content of these combinant compounds in wine may result from both sources, as shown in Table 8.11.

Finally, *Botrytis cinerea* indirectly plays two major roles in the accumulation of substances that combine with SO₂. Firstly, it causes in-depth modifications in the grape skins, which become permeable, thus facilitating access to the various substrates for acetic bacteria. Secondly, noble rot causes glycerol to accumulate in the grapes and is thus indirectly responsible for dihydroxyacetone production.

Table 8.12 recapitulates all the substances that combine SO₂ identified in musts and wines made from botrytized grapes.

8.5 PRACTICAL CONSEQUENCES: THE STATE OF SULFUR DIOXIDE IN WINES

8.5.1 Equilibrium Reactions

In a sulfited wine, an equilibrium exists between the free sulfur dioxide and the bound sulfur dioxide—more precisely, the bound sulfur dioxide with a high dissociation constant *K*. Sulfur dioxide

Table 8.11. Sulfur dioxide combination balance (in %) in two wines with similar TL50 (Barbe *et al.*, 2001b)Wine A: TL50 = 310 mg/l total SO₂ (i.e. CL50 = 260 mg/l total SO₂)Wine B: TL50 = 340 mg/l total SO₂ (i.e. CL50 = 290 mg/l total SO₂)

	Wine A	Wine B
5-oxo-fructose	2	32
γ - and δ -gluconolactone	11	16
trioses (glyceraldehyde + DHA)	1	2
ethanal	45	16
pyruvic acid	18	9
2-oxoglutaric acid	16	12
α -dicarbonyls (methylglyoxal + glyoxal)	2	1
glucose	5	6
other	—	6

Table 8.12. Concentrations found and K_d calculated for the main molecules identified in botrytized musts and wines (Burroughs and Sparks, 1964, 1973; Blouin, 1965, 1995; Guillou-Largeteau 1996; Barbe, 2000)

Molecules	Concentrations in wine min-max (mg/l)	K_d (Mm)	Combination ratio (mg/l)*
ethanal	20–100	0.0024	99.7
pyruvic acid	20–330	0.3	28
2-oxoglutaric acid	50–330	0.5	25
glyoxal	0.2–2.5	—	81
methylglyoxal	0.7–6	0.017	87
galacturonic acid	100–700	17	10
glucuronic acid	traces-60	50	1
5-oxo-fructose	traces-2500	0.48	22
dihydroxyacetone	traces-20	2.65	16
glyceraldehyde	traces-10	0.4	26
gluconic acid	1 000–25 000	20	—
2-oxogluconic acid	traces-1200	1.8	—
5-oxogluconic acid	traces-500	—	—
γ - and δ -gluconolactone	6% and 4% of the gluconic acid	4.22	5.6
glucose	± 100 g/l	800	0.03

*For a 100 mg/l concentration of the compound, the combination value in SO₂ for a free SO₂ content of 50 mg/l.

bound to ethanal does not participate in this equilibrium, since its combination has a very low K value and thus is very stable.

Any addition of sulfur dioxide to a wine results in the combination of a part of this sulfur dioxide. Conversely, the depletion of free sulfur dioxide by oxidation results in a decrease of the bound fraction to such a degree that the loss of free sulfur dioxide is less than the amount oxidized. This liberation mechanism is advantageous, since it

automatically prolongs the effectiveness of a given concentration of sulfur dioxide.

When the free sulfur dioxide concentration of a wine decreases to a very low level, it rarely falls completely to zero, unless yeasts are involved or other factors modify wine composition. The decombination of bound SO₂ progressively replaces the missing free sulfur dioxide.

As a result of these equilibria, the total sulfur dioxide concentrations of different wines cannot be

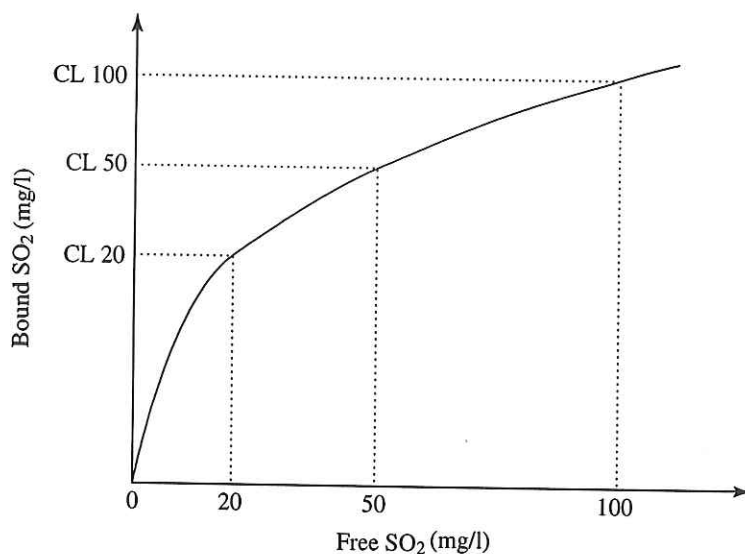


Fig. 8.6. Graphical representation of the binding of sulfur dioxide in wine at CL20, CL50 and CL100

compared if they do not have the same free sulfur dioxide concentration. For example, if a sweet wine has a total SO₂ concentration close to the legal limit, the consequence is not at all the same if it only contains 10 mg of free SO₂ per liter (insufficient for ensuring its stability) or 50 mg (largely sufficient).

To remedy this difficulty, Blouin (1965) recommended the use of the expressions 'CL20' and 'CL50' (Figure 8.6) which represent, respectively, the quantities of bound SO₂ necessary to have 20 or 50 mg of free SO₂ per liter. Known sulfur dioxide additions are used to obtain these numbers experimentally (Kielhöfer and Würdig, 1960). These considerations are most important in the case of sweet wines (Sauternes, Monbazillac, Coteaux de Layon, and Tokay), which require relatively high free SO₂ concentrations to ensure their stability. In practice, the combining power (TL50), or the amount of total SO₂ necessary in a must or wine to obtain 50 mg/l of free SO₂, is calculated by drawing a graph of total SO₂ against free SO₂ (Barbe, 2000).

8.5.2 Influence of Temperature

The determination of the free sulfur dioxide concentration in samples of a botrytized sweet

white wine with a strong binding power varies according to temperature, although the total SO₂ concentration remains constant (Table 8.13). The results for determining free SO₂ concentrations are therefore variable. Depending on the conditions, the results obtained can differ by as much as 20 mg/l.

The storage temperature of the wine must also be taken into account in the evaluation of the effectiveness of sulfiting, at least in the case of sweet wines. Finally, the influence of temperature becomes particularly important when heating wine. The SO₂ concentration can double, or even more. This liberation of sulfur dioxide singularly reinforces the effectiveness of heating.

Table 8.13. Influence of temperature on the state of sulfur dioxide (mg/l) in a botrytized sweet wine (sugar 74 g/l; ethanal 70 mg/l)

Sulphur dioxide	Temperature		
	0°C	15°C	30°C
Total sulfur dioxide	412	412	412
Free sulfur dioxide	68	85	100
Bound sulfur dioxide (SO ₂ C)	344	327	312
SO ₂ C (to ethanal)	104	104	104
SO ₂ C (to other substances)	240	223	208

At the time of bottling, wines can be sterilized at relatively low temperatures (between 45 and 50°C, for example), due in part to this phenomenon.

8.5.3 Empirical Laws of Combination

For a long time, enology has tried to determine applicable combination rules, both for sulfiting a new wine immediately following fermentation and for adjusting the free SO₂ during storage.

The most satisfactory solution consists of adding increasing concentrations of SO₂ to various samples of the same wine to produce a curve as in Figure 8.6. This operation is long and difficult; consequently, it is not always feasible. Laboratory tests are, however, recommended before the first sulfiting of unknown wines immediately following fermentation. Due to the diversity of harvests, a standard SO₂ concentration can lead to an insufficient free SO₂ concentration for ensuring stability, or, on the contrary, an excessively high concentration that would be difficult to lower.

The combination curve (Figure 8.6) clearly reveals that the bound SO₂ increases with the free SO₂. Yet the increase is slower and slower as the free sulfur dioxide concentration increases.

To increase the free SO₂ concentration of a wine already containing some, the combination of the added concentration must be taken into account. The lower the free SO₂ concentration, the more the added concentration combines. As a general rule in standard wines already containing

free SO₂, two-thirds of the supplementary concentration remains in a free state and one-third combines. As a result, 3 g/hl are necessary to increase the free SO₂ concentration by 20 mg/l. Eventual abnormal cases must also be anticipated, corresponding with a much higher combination rate.

In practice, a few days after the addition of SO₂ to wine, the free SO₂ concentration should be verified to ensure that it corresponds with the desired concentration and that the stabilization conditions are obtained.

8.6 ANTIMICROBIAL PROPERTIES OF SULFUR DIOXIDE

8.6.1 Properties of the Different Forms

The enological properties of sulfur dioxide were summarized at the beginning of this chapter (Section 8.1). It is essentially a multifaceted antiseptic and a powerful reducing agent that protects against oxidation. Its antifungal and antibacterial activities will be covered in Sections 8.6.2 and 8.6.3; the antioxidizing and antioxidasic properties will be covered in Section 8.7.2. The various forms of sulfur dioxide do not share these properties to the same extent (Table 8.14).

Its various properties can make sulfur dioxide seem indispensable in winemaking. The goal of enology is not to eliminate this substance completely but rather to establish responsible

Table 8.14. Wine conservation properties of the different forms of sulfur dioxide (Ribéreau-Gayon *et al.*, 1977)

Property	SO ₂	HSO ₃ ⁻	R-SO ₃ ⁻
Fungicidal	+	low	0
Bactericidal	+	low	low
Antioxidant	+	+	0
Antioxidasic	+	+	0
Gustatory amelioration:			
Reduction-oxidation potential	+	+	0
Neutralization of ethanal	+	+	+
Gustatory role of SO ₂	biting odor, SO ₂ taste	odorless, salty, bitter taste	odorless, tasteless at normal concentrations

concentration limits. This supposes a sufficient knowledge of its properties and conditions of use.

8.6.2 Antifungal Activities

The antiseptic action of SO_2 with respect to yeasts can appear in different ways. On one hand, it can be used to stop the fermentation of sweet wines (*mutage*) (Section 14.2.5b). It effectively destroys the existing population (fungicidal action). On the other hand, it protects these same sweet wines from possible refermentations—evaluated by the growth of a small residual population. It effectively inhibits cellular multiplication (fungistatic activity). Moderate sulfiting is also known to inhibit yeast growth temporarily without their total destruction. The subsequent disappearance of free SO_2 permits the revival of yeast activity. In practice, in the winery, new yeast activity may also come from new contaminations resulting from contact with non-sterile equipment and containers.

For these different reasons, the results concerning the action of SO_2 on wine yeasts cited in various research work and obtained in different conditions are not always easily compared. Moreover, the data on this subject seems incomplete.

Bound sulfur dioxide does not have an antiseptic action on yeasts. Yeasts make use of the formation of this combination to inactivate SO_2 . HSO_3^- also possesses a low but undetermined antiseptic activity. Table 8.15 indicates the concentrations of free SO_2 , titratable by iodine, that must be added to wines (according to their pHs) to have an antiseptic activity equal to 2 mg of active molecular SO_2 per liter. The antiseptic activity of the bisulfite form HSO_3^- is more or less significant, depending on the various hypotheses being considered. According to experience obtained on wine stability, HSO_3^- seems to be 20 times less active than SO_2 , notably in wines containing reducing sugars.

Sulfur dioxide is fungistatic at high pHs and at low concentrations, and it is a fungicide at low pHs and high concentrations. The HSO_3^- form is exclusively fungistatic. Each yeast strain

Table 8.15. Free sulfur dioxide concentrations necessary in wines to maintain an antiseptic activity equal to 2 mg of active molecular SO_2 per liter (Ribéreau-Gayon *et al.*, 1977)

Wine pH	Hypothesis: H-SO_3^- activity			
	None	100 times less than SO_2	20 times less than SO_2	10 times less than SO_2
2.8	22	20	14	11
3.0	34	29	19	14
3.2	54	43	24	16
3.4	87	61	28	18
3.6	134	81	31	19
3.8	200	100	33	20

probably has a specific sensitivity to the different forms of sulfur dioxide. Romano and Suzzi (1992) considered possible mechanisms that could explain these differences. According to these same authors (Suzzi and Romano, 1982), sulfiting must be before fermentation increases yeast resistance to SO_2 . Yeasts from a non-sulfited must, isolated after fermentation, are more sensitive to SO_2 than those coming from the same must which is sulfited before fermentation.

Concerning the *mutage* of sweet wines (fungicidal activity), the fermentation seems to stop abruptly after the addition of 100 mg of SO_2 per liter. The concentration of sugar remains constant, although carbon dioxide continues to be released for about an hour. During this time, the yeasts do not seem to be affected by the sulfiting—they are still capable of multiplying (Table 8.16), whatever the concentration used. It is necessary to wait at least 5 hours, and more often 24 hours, to observe a decrease in cell viability.

To ensure a complete cessation of fermentation, Sudraud and Chauvet (1985) estimated that 1.50 mg of molecular SO_2 per liter must be added to wine. According to the same authors, after the elimination of yeasts by different treatments, 1.20 mg of molecular SO_2 per liter seems sufficient for ensuring the proper storage of wines containing residual sugars (fungistatic activity). Lower concentrations could be recommended for wines

Table 8.16. Sulfiting to inhibit yeasts in a sweet wine at the end of fermentation (values are number of viable cells, capable of producing colonies in Petri dishes, per ml; initial population $58 \times 10^6/\text{ml}$) (Ribéreau-Gayon *et al.*, 1977)

SO ₂ concentration added (mg/l)	Time		
	1 hour	5 hours	24 hours
100	58×10^6	8×10^6	10^5
150	58×10^6	3×10^6	0
300	58×10^6	10^6	0

stored at low temperatures having a low yeast population.

Romano and Suzzi (1992) summarized the current understanding of the action of the sulfur dioxide molecule on yeasts. Molecular SO₂ penetrates the cell by either active transport or simple diffusion. Considering the intracellular pH, it must exist in the cell in the form of HSO₃⁻. Once inside the cell, it reacts with numerous constituents such as coenzymes (NAD, FAD, FMN), cofactors and vitamins (thiamine). It would also have an effect on numerous enzymatic systems and on nucleic acids. Finally, a significant decrease in ATP is also attributed to it.

8.6.3 Antibacterial Activities

The activity of free SO₂ on lactic acid bacteria is well known. It is even more influenced by pH than the activity with respect to yeasts. Yet the fraction combined with ethanal or pyruvic acid is also now known to possess an antibacterial activity. The combined SO₂ molecule has a direct action on bacteria. The mechanism is not explained by the decomposition of the combination by bacteria, resulting in the liberation of free SO₂.

The sulfur dioxide combined with ethanal (or pyruvic acid) seems to possess an antibacterial activity 5–10 times weaker than free SO₂, yet it can be 5–10 times more abundant.

A large number of bacteria are eliminated by 5 mg of free SO₂ per liter. The same concentration in the combined form lowers the population by

50%. *Oenococcus oeni* is less resistant to sulfur dioxide than *Lactobacillus* and *Pediococcus*.

Significant technical applications for controlling malolactic fermentation and storing wines have resulted from these observations. Sulfiting the grapes does not only act rapidly on bacteria in the pre-fermentation period; it acts by leaving a certain concentration of combined sulfur dioxide which effectively protects and retards bacterial growth until completion of alcoholic fermentation. In this manner, the medium that still contains sugar is protected from an untimely bacterial development which could lead to the production of volatile acidity (Section 3.8.1).

When malolactic fermentation is not sought (in dry white wines, for example), it should be noted that wine stability is not due solely to the bactericidal action of free SO₂ but rather to the concentration of combined SO₂ that the wine conserves after fermentation; its action is long-lasting during storage. In certain types of wine with too low a pH, combined SO₂ concentrations of 80–120 mg/l can make malolactic fermentation impossible.

Sulfur dioxide is also active on acetic acid bacteria but additional studies on this subject are needed. These bacteria resist relatively high concentrations. In the winery, acetic acid bacteria are most effectively prevented by avoiding contact with oxygen in the air and controlling temperature in the winery.

8.7 THE ROLE OF SULFUR DIOXIDE IN WINEMAKING

8.7.1 Advantages and Disadvantages

Although the use of sulfur dioxide in the storage of wine seems to be fairly ancient, its use in winemaking is more recent. It was recommended at the beginning of the 20th century—essentially for avoiding oxidasic casse. The very appreciable improvement in wine quality by sulfiting rotten grapes was an essential factor in the gain in

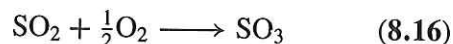
popularity of this process. Its antiseptic properties and its role in the prevention of bacterial spoilage were discovered later.

Nevertheless, the generalization of sulfiting in winemaking, or at least the establishment of a precise and homogeneous doctrine from one viticultural region to another, took a long time to come about. Besides its many advantages, sulfiting also presented some disadvantages; therefore, a sufficiently precise understanding of the properties of sulfur dioxide had to be obtained before defining the proper conditions of its use. These conditions permit the winemaker to profit fully from its advantages while avoiding its disadvantages.

When used in excessively high concentrations, this product has a disagreeable odor and a bad taste which it imparts to the wine; the taste of hydrogen sulfide and mercaptans in young wines can also appear when they are stored too long on their lees. The most serious danger of improper sulfiting is the slowing or definitive inhibition of the malolactic fermentation of red wines. Incidentally, for a long time sulfited grapes were observed to produce red wines with higher acidities. Before the understanding of malolactic fermentation, this observation was attributed to an acidifying effect of sulfur dioxide or an acidity fixation.

8.7.2 Protection Against Oxidation

The chemical consumption of oxygen by SO_2 is slow. It corresponds to the following reaction:



In a synthetic medium, SO_2 has been shown to take several days to consume 8.0–8.6 mg of oxygen per liter (this amount corresponds with the saturation of this medium). Such oxidation requires the presence of catalyzers, notably iron and copper ions. Yet musts are very oxidizable and should therefore be rapidly and effectively protected against oxidation. Sulfiting accomplishes this. Sulfur dioxide, however, cannot act by its anti-oxygen effect, that is to say by combining with oxygen which is no longer available for the oxidation of other must constituents.

Dubernet and Ribéreau-Gayon (1974) confirmed this hypothesis. The experiment consisted of saturating a white grape must with oxygen and measuring the oxygen depletion rate electrometrically (Figure 8.7). In the absence of sulfiting, the depletion of this oxygen is very rapid and is complete within a few minutes (4 to 20 on average). This phenomenon demonstrates the extremely high oxidability of grape must. If at a given moment

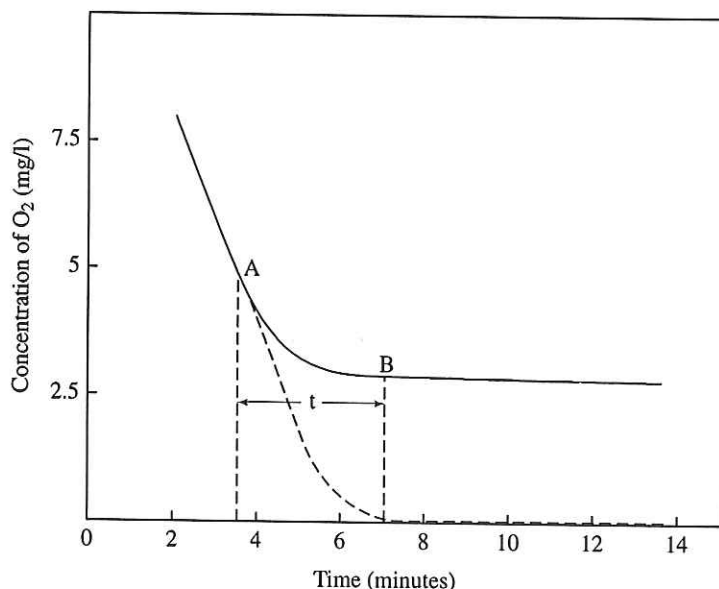


Fig. 8.7. Oxygen consumption in musts following sulfiting (Dubernet and Ribéreau-Gayon, 1974). (A) Addition SO_2 of (B) Stopping point of oxygen consumption (t = time necessary for oxygen consumption to stop)

Table 8.17. Protection of color of red wine made from botrytized grapes by sulfiting (Sudraud, 1963)

Level of harvest sulfiting	Composition of the wines obtained		
	Total phenolic compounds (index)	Color intensity ^a	Oxidasic casse potential
Without SO ₂	32	0.53	++++
+10g SO ₂ /hl	41	0.63	++
+20g SO ₂ /hl	45	0.83	0

^aColor intensity = OD 420 + OD 520 (under 1 mm thickness).

the must is sulfited, oxygen is no longer consumed and its concentration remains constant after a given time t , which varies depending on the conditions but is always fairly short. As an initial approximation, the value t varies between 1 and 6 minutes when sulfiting varies between 100 and 10 mg/l. The value t is much greater for must obtained from rotten grapes.

In summary, although the anti-oxygen effect of sulfur dioxide is involved in wine storage, its role is insignificant during winemaking. In this case, SO₂ protects against oxidations by destroying oxidases (laccase) or, at least, blocking their activity, if destruction is not total. The enzymatic oxidation phenomena are inhibited in this manner until the start of fermentation. From this point, the reductive character of the fermentation continues to ensure the protection. Yet oxidative phenomena can resume at the end of fermentation insofar as active oxidases remain after the depletion of free SO₂. The oxidasic casse test, or, even better, the determination of laccase activity, permits the evaluation of the risk and the necessary precautions to be taken.

In must, enzymatic oxidations are more significant than chemical oxidations because they are more rapid. In wine, however, chemical oxidations play an unquestionable role, since oxidative enzymes no longer exist. In this case, SO₂ reacts with oxygen to protect the wine.

Rot is responsible for the most serious oxidative phenomena. In fact, *Botrytis cinerea* secretes a laccase more active and stable than the tyrosinase of grapes. It is responsible for the oxidasic casse in red wines derived from rotten grapes. An appropriate sulfiting can protect against this phenomenon

to some extent. The figures in Table 8.17 show that intense sulfiting of rotten grapes (since they could be used in the past) increases the total phenolic compound concentration and the color intensity while decreasing the risk of oxidasic casse. Progress in phytosanitary vineyard protection has made such situations extremely rare.

From the start of fungal development, the oxidase secretion by *Botrytis cinerea* inside the berry can be considerable whereas the external signs are barely visible. This situation can be observed in the case of red grapes. The first brown blemishes are more difficult to observe on red grapes than on white grapes. During cold weather, the external vegetation of *Botrytis cinerea* is less developed. These factors must be taken into account when choosing the corresponding sulfiting concentration.

8.7.3 Inhibition, Activation and Selection of Yeasts

Sulfur dioxide is a general antiseptic with a multifaceted activity on different wine microorganisms. Its mode of action has been described in previous sections.

With respect to yeasts, sulfiting is used first and foremost to ensure a delay in the initiation of fermentation, allowing a limited cooling of the grapes. The fermentation is also spread out over a longer period in this manner, avoiding excessive temperatures. More and more often, natural tank cooling is complemented by controlled refrigeration systems.

In the case of white winemaking, the delay in the start of fermentation permits the settling and racking of suspended particles in must.

Sulfiting also makes use of the stimulating effect of sulfur dioxide when used in low concentrations. Consequently, the fermentation speed accelerates, as shown by the curves in Figure 8.8. After an initial slowing of the fermentation at the start, the last grams of sugar are depleted more rapidly. Finally, the fermentation is completed more rapidly in the lightly sulfited must.

During the running off of a tank of red wine that still contains sugar, a light sulfiting (2–3 g/hl) does not block the completion of the fermentation; on the contrary it is known to facilitate it more often than not.

This long-proven effect of sulfiting has been confirmed time and time again. It has been interpreted as the destruction of fungicidal substances by sulfur dioxide. These substances are toxic for the yeast and could come from the grape, *Botrytis cinerea* or even the fermentation itself. An increase in the must protease activity has also been considered. This activity would put assimilable amino acids at the disposal of the yeast (Section 9.6.1). Sulfiting probably acts by maintaining dissolved oxygen in the must. Not being

tied up in oxidation phenomena, it is available for yeast growth (Section 8.7.2).

Sulfiting has also been considered to affect yeast selection. Apiculated yeasts (*Kloeckera* and *Hanseniaspora*), developing before the others, produce lower quality wines with lower alcohol strength. These yeasts are more sensitive to sulfur dioxide. Therefore, a moderated sulfiting blocks their development. This result has been confirmed by numerous experiments (Romano and Suzzi, 1992), but the research of Heard and Fleet (1988) cast doubts on this generalization. In spite of sulfiting, these strains attained an initial population of 10^6 to 10^7 cells/ml in a few days before disappearing. Moreover, the advisability of eliminating apiculated yeasts and the interest of the successive participation of different yeast species for the production of quality wines are still being considered.

The problem of sterilizing musts by the total destruction of indigenous yeasts through massive sulfiting, or other processes such as heat treatments, followed by an inoculation using selected yeasts will be covered elsewhere.

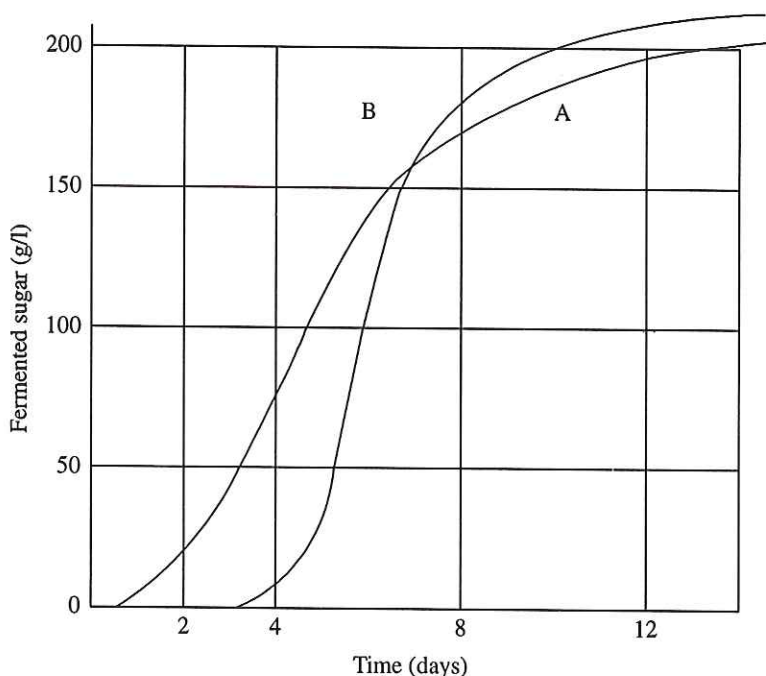


Fig. 8.8. Effect of moderate sulfiting (5–10 g/hl) on alcoholic fermentation kinetics of grape must. (A) Control must. (B) Sulfited must (5 g/hl)

8.7.4 Selection between Yeasts and Bacteria

Sulfur dioxide acts more on wine bacteria than on yeasts. Lower concentrations are consequently sufficient for hindering their growth or suppressing their activity. No systematic studies have been carried out on this subject but this fact is well known and is often demonstrated in practice. For example, in the case of a red wine still containing sugar (a site for simultaneous alcoholic and lactic fermentation), a moderated sulfiting (3–5 g/hl) can initially block the two fermentations. Afterwards, a pure alcoholic refermentation can take place without the absolute necessity of an yeast inoculation.

One of the principal roles of sulfiting in winemaking is to obtain musts much less susceptible to bacterial development, while undergoing a normal alcoholic fermentation. This protection is most necessary in the case of musts that are rich in sugar, low in acidity and high in temperature. The risks of stuck fermentations are highest in these cases.

In summary, sulfur dioxide delays, without blocking, yeast multiplication and alcoholic fermentation. The bacteria, supplied by grapes at the same time as the yeasts, are killed or at least sufficiently paralyzed to protect the medium from their development while the yeasts transform the totality of the sugar into alcohol. The serious danger of bacterial spoilage in the presence of sugar is an important factor in wine microbiology (Section 3.8.2).

In white winemaking and for wines in which malolactic fermentation is not sought, sulfiting can be adopted to inhibit bacteria completely. Incidentally, the light sulfiting of white musts undergoing malolactic fermentation can be insufficient to protect effectively against oxidation.

In red winemaking today, malolactic fermentation has become common practice. Generally speaking, the sulfiting of red grape musts favors wine quality. However, sulfiting must not compromise malolactic fermentation due to its conditions of use and the concentrations employed. To ensure the successful completion of alcoholic fermentation the amount of the sulfurous solution added to grape must should be regulated according to the pH, temperature, sanitary conditions and

Table 8.18. Influence of must sulfiting on the time necessary (expressed in days) for malolactic fermentation initiation in wine after running off (Ribéreau-Gayon *et al.*, 1977)

Sulfiting	Wine No. 1	Wine No. 2
Control 0	40	30
+2.5 g/hl	45	40
+5 g/hl	70	60
+10 g/l	100	100

other factors. Bacterial development must initiate rapidly after the depletion of sugar for exclusive malic acid degradation. The exact SO₂ concentration is difficult to determine, and it varies depending on the region. For red winemaking in the Bordeaux region, 7–10 g/hl seems to be an effective range: below this, the malolactic fermentation is not compromised; above this, it can be considerably delayed (Table 8.18).

8.7.5 Dissolving Power and General Effects on Taste

In red winemaking, sulfiting favors the dissolution of minerals, organic acids and especially phenolic compounds (anthocyanins and tannins) which constitute the colored substances of red wines. The dissolvent activity is due to the destruction of grape skin cells, which yield their soluble constituents more easily in this manner. In fact, the dissolvent effect of sulfur dioxide seems to have been exaggerated in the case of healthy grapes. The better color of wines derived from sulfited must is probably due to a better protection against the oxidasic casse in slightly rotten grapes.

The effectiveness of sulfitic maceration for extracting grape pigments is indisputable, and this process is used for the industrial preparation of commercial colorants. Yet when rigorous experiments are carried out on healthy red grapes, using classic winemaking techniques, no significant color improvement (anthocyanin and tannin concentration and color intensity value) is observed in the presence of a normal sulfiting. Since only the free SO₂ is active, and since this form rapidly disappears in crushed grapes, this effect of sulfiting appears to be exerted for only a brief moment. At

the end of fermentation, the effects of maceration time, temperature and pumping-over are more significant.

Nevertheless, the dissolvent effect of sulfiting, with respect to phenolic compounds, is obvious in the case of limited maceration. This operation is not recommended for crushed white grapes before must extraction by pressing. The sulfiting of grapes also has an impact on the color of rosé wines.

Sulfiting also has certain effects on wine quality which still remain poorly defined. The general properties of sulfur dioxide may possibly have indirect consequences (protection against oxidations and the binding of ethanal). In this way, sulfiting often improves the taste of wine—notably in the case of rotten grapes or mediocre varieties. It also protects certain aromas of 'new' wines. Moreover, grape sulfiting does not have an obvious impact on the subsequent development of the bouquet of mature wines.

Certain conditions, such as fermentations in strict anaerobiosis and especially prolonged aging on yeast lees, can lead to the formation of hydrogen sulfide and mercaptans from the added SO_2 . The odors of these compounds are disagreeable and can persist in wine.

8.8 THE USE OF SULFUR DIOXIDE IN THE WINERY

8.8.1 Winemaking Concentrations

Considering the rapidity of oxidative phenomena, grape and must sulfiting is only effective if the sulfur dioxide is intimately and rapidly incorporated into the total volume before the start of fermentation. If a fraction of the grape must ferments before being sulfited, it is definitively shielded from the action of the SO_2 , because it immediately combines with the ethanal produced by the fermenting yeasts.

In fact, a homogeneous distribution before the start of fermentation is not sufficient. Considering the rapidity of the oxygen consumption by grape must, each fraction of the grape harvest or the must should receive the necessary quantity of sulfur dioxide in the minutes that follow the crushing of the grape or the pressing of the harvest. This is the

only truly effective method of protecting against oxidations. It can be more effective to add 5 g of sulfur dioxide per hectoliter correctly to the harvest than to add 10 g/hl added in poor conditions. A poor sulfiting technique is certainly one of the reasons in the past that led to the use of excessive concentrations.

Based on these principles, the only rational sulfiting method for winemaking consists of regularly incorporating a sulfurous solution into the white grape must as it is being extracted, or for red grapes as soon as they are crushed. A few successive additions of SO_2 into the tank as it is being filled are not truly effective, even after a homogenization at the end of filling. During homogenization, part of the added sulfur dioxide is already in the combined form and thus inactive.

It is therefore also necessary to use a sufficiently diluted sulfur dioxide solution, capable of being correctly incorporated and blended into the must. The direct usage of metabisulfite powder or sulfurous gas in the tank should be avoided. When a tank of red grapes is sulfited by a few additions of a concentrated product during filling, the complete decoloration of certain fractions of the pomace is sometimes observed during the running off. In these cases, the sulfur dioxide was not properly blended, but was instead fixated on certain parts of the grapes, leaving the other parts unprotected.

When choosing the SO_2 concentration to add to the grapes or the must, grape maturity, sanitary state, acidity (pH), temperature and eventual contamination risks must all be taken into account. The choice can sometimes be difficult. Table 8.19 gives a few values for vineyards in temperate climates. The generalization of tank cooling systems and increased hygiene in the wineries, combined with a better understanding of the properties of sulfur dioxide, permit the lowering of the concentrations used in winemaking. Today, sanitary practices in the vineyard avoid grape rot, which once justified the intense sulfittings indicated in Table 8.19.

During the harvest, progressive increases in the sulfiting concentrations can compensate the increasingly significant inoculation (notably bacterial) resulting from the development of microorganisms on the equipment—the inner surface of

Table 8.19. Sulfur dioxide doses for winemaking in temperate climate zones

Status	Sulfur dioxide dose
Red winemaking:	
Healthy grapes, average maturity, high acidity	5 g/hl of wine
Healthy grapes, high maturity, low acidity	5–8 g/hl of wine
Rotten grapes	8–10 g/hl of wine
White winemaking:	
Healthy grapes, average maturity, high acidity	5 g/hl of must
Healthy grapes, high maturity, low acidity	6–8 g/hl of must
Rotten grapes	8–10 g/hl of must

the tanks and the walls of the winery. Problems of difficult final stages of fermentation and microbial deviations are frequently observed in the last tanks filled. Sufficient must sulfiting should avoid these contaminations.

In white winemaking, excessive concentrations (>15 g/hl) followed by a significant sulfiting at the end of fermentation (>4 g/hl) can be a source of reduction odors and should be avoided. Press wines, however, should be more intensely sulfited—especially in the case of continuous presses which cannot be disinfected regularly.

Concerning the sulfiting technique for red winemaking, the solution should be added after grape crushing to facilitate the blending and to avoid evaporation losses and attacks on metallic equipment. Taking into account the transfer of the crushed grapes by a pump with a constant delivery, the sulfurous solution should be injected into the tube immediately after the pump outlet. The sulfiting is suitably distributed and homogenized in this manner. Of course, the injection pump for the sulfurous solution must be properly adjusted and perfectly synchronized with the grape-pump.

The addition of the sulfurous solution after each grape load, by regularly spraying the surface, can only be practiced in small tanks and must also be sufficient in number. Even if it is not completely effective, a homogenization pumping-over is necessary after filling.

In the case of white winemaking, sulfiting must take place after must separation. Sulfiting of the crushed grapes is not recommended since it entails the risk of increasing the maceration phenomena

and a fraction of the SO_2 is fixated on the solid parts of the grape.

Considering the oxidation speed of white grape must, sulfiting (which ensures the appropriate protection) should be carried out as quickly as possible. Must extraction equipment (the press cage, mechanical drainer and continuous press) does not supply a constant delivery. Consequently, SO_2 cannot be injected with a pump adapted directly to these outlets. In order to sulfite in this manner, the must has to pass via a small tank through a constant delivery pump. The corresponding manipulation of the must, in particular the pumping, does not protect the must from a slight oxidation before sulfiting.

The sulfiting of white grape musts can also be calculated from the volume of the juice tray at the outlet of the press. During filling, a homogeneous distribution should be ensured.

The necessary volume of a sulfurous solution for sulfiting an entire tank during its filling at the chosen concentration should be prepared in advance. If the system is correctly adjusted, the entire volume of the sulfurous solution should have been injected in to the tank by the time the tank is full.

8.8.2 Storage and Bottling Concentrations

During storage, sulfiting is, first of all, thought to protect wine from oxidation. As an approximation, oxidative risks are present during prolonged storage below 5–10 mg/l for red wines, 20 mg/l for

Table 8.20. Recommended free sulfur dioxide concentrations (mg/l) in wines

Dose type	Red wines	Dry white wines	Sweet white wines
Conservation	20–30	30–40	40–80
Bottling	10–30	20–30	30–50
Expedition doses (cask or container)	25–35	35–45	80–100 ^a

^aThis type of wine should be bottled at the production site; bulk expedition should be avoided.

white wines made from healthy grapes and 30 mg/l for white wines made from more or less rotten grapes.

At the microbiological level, sulfiting dry wines must avoid yeast and bacterial development during storage. In dry white wines and red wines having undergone malolactic fermentation, the concentrations used for protection against oxidations are generally sufficient to avoid microbial developments. In red wines that have not undergone malolactic fermentation, the habitual free and total SO₂ concentrations can be insufficient to shield the wine completely from a malolactic fermentation—at least a partial one—during storage.

Of course, the sulfiting rules do not apply to certain kinds of wines (red or white, dry or sweet) with qualities derived from a certain oxidation state or containing ethanal.

Sulfiting also hinders the refermentation of sweet wines, generally provoked by SO₂-resistant yeast strains. The refermentation risks are independent of sugar concentrations, but are influenced by alcohol strength. In satisfactory storage conditions, 50 mg of free SO₂ per liter is required to ensure the storage of a sweet wine with a relatively low alcoholic strength (11%) and 30 mg/l for wines with a high alcohol content (13%).

In practice, carefully adjusted sufficient concentrations must be used to avoid accidental risks. The refermentation of a sweet wine can start in the lees of a tank containing a sufficiently high yeast population to ensure the combination of the SO₂. Simultaneously, at least for a certain amount of time, all of the liquid remains limpid without a refermentation, with 60 mg of free SO₂ per liter. If the fermentative process begins from the lees, the refermentation seems possible in spite of the high concentration of free sulfur dioxide.

The size of the yeast population should always be taken into account to evaluate the effectiveness of a sulfiting. All operations (fining and filtration) that eliminate a fraction of the yeasts permit the lowering of the free SO₂ concentration necessary for conserving sweet wines.

The possibility of lowering free SO₂ concentrations for stabilizing sweet wines results from steps taken in storing wine. Clean (if not sterile) conditions have diminished contaminating populations. These criteria for cleanliness should be applied not only to the product but also to the building, the containers and the material—all contamination sources. Microbiological controls that indicate the number of viable yeast cells are useful tools for adjusting sulfiting.

Table 8.20 indicates free sulfur dioxide concentrations that can be recommended in different situations.

8.8.3 Diminution of Sulfur Dioxide by Oxidation during Storage

The free sulfur dioxide concentration does not remain constant in wines stored in barrels or tanks. There is a continuous loss month after month. Over the years, its concentration decreases even in bottled wine.

The decrease in barrels or tanks results from an oxidation catalyzed by iron and copper ions. Although it is very volatile, a negligible quantity of free sulfur dioxide evaporates during storage in wooden barrels. Nor is it combined. A fairly common error is to consider that any decrease in free sulfur dioxide is the result of a combination with wine constituents. In reality, after the four or five days following the addition of SO₂, the wine constituents no longer bind. An equilibrium is attained and decreases occurring afterwards are

due to oxidation. For a new combination to occur, the chemical composition of the wine must be modified. For example, new binding molecules must be formed, such as ethanal, during a limited yeast development or by the oxidation of ethanol when a poorly clarified wine is racked.

The oxidation affecting sulfurous acid forms sulfuric acid. At the pH of wine, it is almost entirely in the form of sulfate. In botrytized and non-botrytized sweet wines with elevated free SO_2 concentrations, a considerable amount of sulfate can be formed (0.5 g/l). Less is formed in dry white and red wines, especially those stored in tanks. In the case of barrel-aged wines, the formation of sulfate by the oxidation of free SO_2 accumulates with the amount resulting from the combustion of sulfur in the empty barrels. This formation lowers the pH and harshens the wine. This phenomenon contributes to the decrease in quality of wines stored in barrels for an excessively long time.

When sulfiting is effected without a measurement beforehand, the wine can be excessively sulfited and its taste affected. In general, the characteristic odor appears at or above 2 mg of active molecular SO_2 per liter. Table 8.15 indicates the corresponding free SO_2 concentrations. To lower the concentration of free SO_2 of a wine, the most effective solution, when possible, is to use this wine to increase an insufficient concentration of free SO_2 of a similar wine.

If such an operation is not possible, the most generally recommended method is to aerate the wine. The effectiveness of this method is based on the slow oxidation of sulfur dioxide. During the days that follow, the higher the temperature, the more rapidly the concentration decreases. Aeration has a limited effectiveness, and 16 mg of oxygen per liter is required to oxidize 64 mg of total sulfur dioxide per liter. This approximately corresponds to a decrease of 42 mg of free SO_2 per liter, taking into account the dissociation of combinations.

The use of hydrogen peroxide is a radical means of eliminating an excess of free SO_2 . This method is too severe and is therefore prohibited; it compromises wine quality for a long time.

8.8.4 The Forms of Sulfur Dioxide Used

This antiseptic has the advantage of being available in various forms capable of responding to different situations: gaseous state (resulting from the combustion of sulfur), liquefied gas, liquid solution and crystallized solid.

Sulfurous gas SO_2 liquefies at a temperature of -15°C at normal atmospheric pressure or under a pressure of 3 bars at normal ambient temperature. It is a colorless liquid with a density of 1.396 at 15°C . Placed in 10–50 kg metallic bottles, this form is used for large-quantity additions that can be measured by weighing the bottle, which is placed directly on a scale. A '*sulfidoseur*' is used to treat smaller volumes of wine. The graduated container can be precisely filled from the metallic bottle by regulating a pair of small faucets—permitting the addition of precisely measured quantities of the gas.

Liquefied sulfur dioxide is still delivered in vials containing 25, 50 or 75 g of sulfur dioxide for example adapted for sulfiting wine in barrels with capacities of several hundred liters. A special tool perforates these small metal cap-stoppered bottles when they are inside the barrel to be treated.

For SO_2 additions to small volumes of wine, or to have a better incorporation, 5–8% solutions prepared in water or must (to avoid dilution) from liquefied sulfurous gas are used. The quantity needed is weighed. The concentration of the solution is regularly verified by measuring its density (Table 8.21) or by chemical analysis. It tends to decrease in contact with air.

Handling these solutions is disagreeable, since they give off a strong SO_2 odor. Prepared on the premises, they are well adapted to large winemaking facilities such as bulk wineries.

Concentrated 10% solutions, or 18–20% potassium bisulfite solutions, are also used. They are more easily handled than the preceding since they are less odorous. Being more concentrated, however, they are less easily incorporated into wine and must. Legislation limits their use to a single addition of 10 g of SO_2 per hectoliter. They acidify less than the preceding since the acidity in these solutions is partially neutralized. Potassium

Table 8.21. Density (at 15°C) of sulfur dioxide solutions prepared by the dissolution of sulfur dioxide gas in water

Sulfur dioxide (g/100 ml)	Density	Sulfur dioxide (g/100 ml)	Density
2.0	1.0103	6.5	1.0352
2.5	1.0135	7.0	1.0377
3.0	1.0168	7.5	1.0401
3.5	1.0194	8.0	1.0426
4.0	1.0221	8.5	1.0450
4.5	1.0248	9.0	1.0474
5.0	1.0275	9.5	1.0497
5.5	1.0301	10.0	1.0520
6.0	1.0328		

metabisulfite ($K_2S_2O_5$) solutions at 10% diluted in water can also be used. These solutions contain approximately 50 g of sulfur dioxide per liter (5%) and are suitable for limited-volume winemaking. The metabisulfite powder should be diluted in water before use. When added directly, it is difficult to blend into the must.

8.8.5 Sulfiting Wines by Sulfuring Barrels

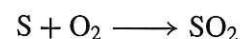
Sulfuring barrels, or small wooden tanks or containers, consists of burning a certain quantity of sulfur in these containers. It is probably the oldest form of using sulfur dioxide in enology. It is used for adjusting the free SO_2 concentration of wines at the moment of racking and also for avoiding microbial contamination when storing empty containers. It has a double sterilizing effect. It is exerted at once on the wine and the internal surface of the container. This practice is part of normal winery operations and could not be replaced by the simple addition of sulfurous solution to the wine. Due to the unpleasant odor imparted to the wine cellar by burning sulfur, its usage can be prohibited by the safety legislation of certain countries. Instead of coming from the combustion of sulfur, sulfurous gas can also be delivered as a bottle of compressed gas.

In any case, sulfur combustion is only applicable to wooden containers. In fact, sulfurous gas, coming from the combustion of sulfur, attacks the internal surface of cement tanks and the coating of

metallic tanks. It also accelerates the deterioration of stainless steel.

The sulfur is generally supplied in the form of a wick or ring. It may be coated on a cellulosic weave or mixed with a mineral base (aluminum or calcium silicate). The units most often used are 2.5, 5 and 10 g of sulfur. Chatonnet *et al.* (1993) demonstrated a certain heterogeneity in the quantity of SO_2 produced by the combustion of the same weight wick or ring according to their preparation conditions or storage (fixation of humidity).

From the equation:



$$32 + 32 = 64 \quad (8.17)$$

the burning sulfur combines with its weight in oxygen to give double the weight of SO_2 . In reality, 10 g of sulfur burned in a 225 l barrel produces only about 13–14 g of SO_2 —a 30% loss. One part of the difference is accounted for by the portion of the sulfur that falls to the bottom of the barrel without burning, and the other part by the production of sulfuric acid—a strong acid without antiseptic activity. The sulfiting loss and the acidification of wine (by repeated sulfurings) are explained in this manner.

The combustion of sulfur does not exert its effect by eliminating all of the oxygen from the barrel. The maximum quantity of sulfur that can burn in a 225 l barrel is 20 g, for the maximum production of 30 g of sulfurous gas. At this stage, the combustion stops because the sulfurous gas has the property of hindering its own combustion. It has been determined that approximately 32.5 liters of oxygen are present in the barrel at the moment when the combustion stops, compared with 45 liters beforehand.

These observations lead to the conclusion that the combustion of sulfur is limited. When a 40 g sulfur wick is burned, not all of the sulfur is consumed, even if the wick is burnt to a cinder. About half of it falls to the bottom of the barrel without burning.

The production of SO_2 by the combustion of sulfur in a barrel is therefore irregular. It is especially hindered in humid barrels; for instance 10 g

sulfur burned in dry barrel give 12 g SO₂ and only 5 g in humid barrel (Ribéreau-Gayon *et al.*, 1977). In addition, the dissolution of the SO₂ formed is generally irregular during the filling of the barrel. Depending on the filling speed and conditions (by the top or bottom, for example), a more or less significant part of the sulfurous gas is driven out of the barrel. Moreover, the distribution of the sulfiting in the wine mass is not homogeneous. The first wine that flows into the barrel receives more SO₂ than the last. In one example, the free SO₂ increased by 45 mg/l at the bottom of the barrel, by 16 mg/l in the middle and not at all in the upper portion. Consequently, the wine should be homogenized after racking—by rolling the barrel, for example. This sulfiting method should only be used for wines stored in small-capacity containers—say up to 6 hl.

As Chatonnet *et al.* (1993) stated, the combustion of 5 g of sulfur in a 225-liter wooden barrel increases the SO₂ in wine from 10 to 20 mg/l. Sulfur wicks are less efficient (10 mg/l) but more consistent than rings (10–20 mg/l). The latter are more sensitive to their external environment, i.e. moisture.

The combustion of sulfur for the storage of empty barrels will be covered in Volume 2, Section 13.6.2.

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