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SULFUR DIOXIDE (SO_2)

Learning Outcomes: *The reader will understand the three functions of sulfur dioxide in juice and wine: as an enzyme inhibitor, an antioxidant, and an antimicrobial agent. In the absence of sulfur dioxide, the plant enzyme tyrosinase oxidizes phenols while depleting must oxygen. Therefore, the addition of sulfur dioxide pre-fermentation may impact yeast growth and the ability to complete fermentation. There are limitations in the capabilities of sulfur dioxide as an antioxidant in wines, and SO_2 may be regarded as only a temporary preservative. In general, juice/wine bacteria are more sensitive to the effects of sulfur dioxide than are yeasts, while lactic acid bacteria vary in their sensitivity. The antimicrobial activity of sulfur dioxide is highly pH-dependent. Many yeast strains can reduce sulfate to produce sulfur dioxide, and new H_2S-free strains tend to produce higher concentrations of sulfur dioxide. Types of addition products and examples of rate calculations are provided.*

Chapter Outline

Section 1.

Introduction

Sulfur dioxide (SO_2) is widely used in the wine industry as a chemical preservative and antioxidant. Although, historically, sulfites were generally recognized as safe (GRAS), the U.S. Food and Drug Administration (FDA) has determined potential health problems to asthmatic individuals.

As a result, in 1987, the TTB (Alcohol and Tobacco Tariff and Trades Bureau) implemented labeling of sulfites present in alcoholic beverages at a level of greater than 10 mg/L (ppm). The maximum permissible level of total sulfur dioxide for both TTB and the OIV (*Organisation Internationale de la Vigne et du* Vin, Europe's wine governing body) is 350 mg/L.

A number of sensory characteristics in wines are associated with the presence of sulfur dioxide, even when it is present at levels well below the legal limit. These include the following:

- metallic (tinny) taste
- harsh character
- pungent aroma, a sharpness in the nose, and a "soapy" smell

Sulfur Dioxide as an Inhibitor of Browning

Juice and wines contain many readily-oxidizable compounds, including polyphenols. The role of SO_2 as an antioxidant in juice and wine lies in its competition with oxygen. As a reducing agent, SO_2 can, to a degree, inhibit oxidation caused by molecular oxygen. Aging may be considered a controlled oxidative process.

Ribéreau-Gayon (1933) recommended that oxygen absorption be limited to the rate at which it could be catalytically-reduced by oxidizable substrates, such as flavonoid phenols and SO_2. Thus, in the case of white wines, where oxidizable substrates are relatively low, SO_2 may play a more important role in consumption of oxygen.

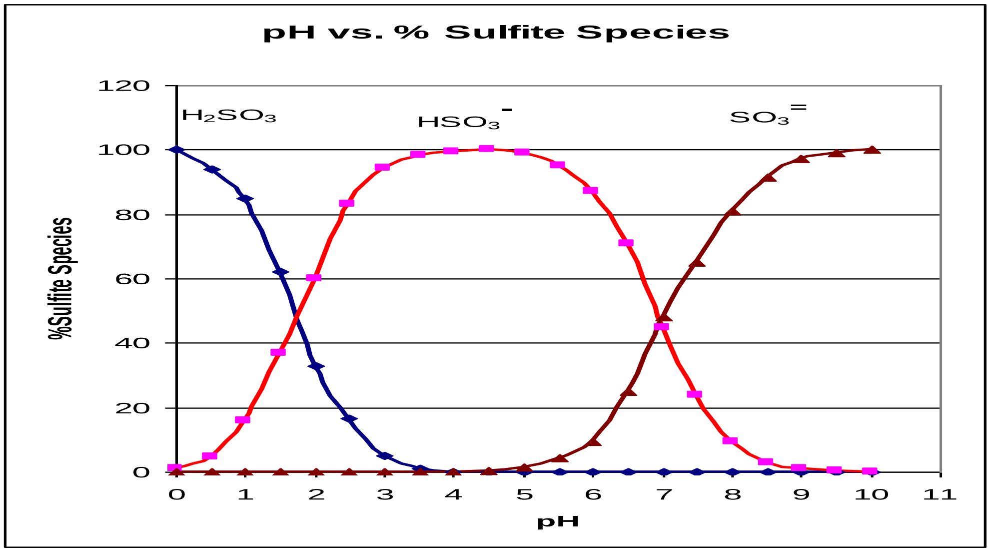
In contrast to the chemical oxidation described above, the commonly-observed browning phenomenon of freshly-cut fruit (and crushed grapes) is the result of the activity of a group of plant enzymes, the tyrosinases (formerly called polyphenoloxidases). These enzymes catalyze oxidation of non-flavonoid *ortho*-dihydroxyphenols (colorless) to their corresponding darker quinones or oxidized product. Sulfur dioxide additions, of 35 mg/L to must, are known to completely inhibit oxygen uptake by tyrosinase and prevent browning (White and Ough, 1973).

In addition to natural tyrosinases, grapes degraded by *Botrytis cinerea* contain the enzyme laccase. This enzyme rapidly oxidizes both *ortho*- and *para*-dihydroxyphenols (Peynaud, 1984) and, unlike tyrosinase, is significantly more resistant to SO_2.

Distribution of Sulfite Species in Solution

The presence of any form(s) of SO_2 in solution is pH-dependent (Figure 1). At the pH of wine, bisulfite and molecular SO_2 are the dominant species. With increasing pH, the percentage of sulfite also increases, whereas decreasing pH yields a relatively-higher percentage of molecular sulfur dioxide.

Figure 1. Distribution of Sulfite Species in Water as a Function of pH



Bound Sulfur Dioxide

Bound sulfur dioxide refers to the formation of addition compounds between the bisulfite ion and other juice/wine components, such as aldehydes, anthocyanins and sugars. The quantity of SO_2 fixed, and the rate of binding, is a pH-dependent reaction: the lower the pH, the slower the addition. Temperature affects the equilibrium in a similar manner. Bound SO_2 has little inhibitory effect against most yeasts and acetic acid bacteria. However, at levels greater than 50 mg/L, bound SO_2 (as its acetaldehyde-bisulfite complex) is believed to be inhibitory towards lactic acid bacteria.

Sulfur Dioxide Binders

Several compounds present in juice and wine are active in binding SO_2. In red wines, the major binding compounds are acetaldehyde and anthocyanins. At free SO_2 levels of 6.4 mg/L, 98% of the acetaldehyde present is bound. Grapes degraded by *Botrytis cinerea* and/or sour-rot complex are rich in compounds that can bind sulfur dioxide.

Compounds associated with sulfur dioxide binding are of interest in microbial management, including management of MLF (malolactic fermentation). There are currently over 300 commercial yeast strains on the market, some of which differ in their production of binding compounds.

Acetaldehyde

Produced as an intermediate during alcoholic fermentation, the majority of acetaldehyde is reduced to ethanol during this phase. Upon contact with oxygen, oxidation of ethanol, via coupled oxidation, may yield small amounts of acetaldehyde. The majority of acetaldehyde formed, however, results from microbial oxidation of ethanol under aerobic conditions. As an intermediate in bacterial formation of acetic acid, acetaldehyde may accumulate even under conditions of low oxygen concentration.

Bisulfite (HSO_3^-) helps protect juices and wines from oxidative browning reactions, as well as scavenging hydrogen peroxide (H_2O_2) formed from oxidative reactions. In cases where an aldehydic “nose” has developed in a wine, or if there is concern with excessive oxygen pick-up, an aroma screen can be conducted. With careful addition of SO_2, the aldehyde is bound to a heavier and more sensory-neutral addition product. Because this product is not volatile and, therefore, not odorous, this is a presumptive screen test for the presence of excessive oxidation.

Pigments

Anthocyanins also have a strong affinity for bisulfite, forming (among others) the colorless species anthocyanin-4-bisulfite. Such binding may significantly affect phenolic polymerization, color stability, and tannin suppleness in young red wines. Polymeric anthocyanins are resistant to SO_2 because of prior substitution at the same location on the molecule, which would be bound by other phenolic compounds. As such, this has been a method used to help quantify the pigmented versus non-pigmented anthocyanins.

Sugars

Several sugars that are present in must react with SO_2. These include glucose, xylose, and arabinose. From 50 to 70% of the total SO_2 addition to juice may be bound by sugars. The keto-sugar fructose does not form an addition compound with bisulfite ion (Braverman, 1963). Fructose comprises approximately one-half of the reducing sugar content of must, and this ratio increases as the fermentation reaches completion. Sugars combine with bisulfite (HSO_3^-) at a much slower rate than do aldehydes and ketones, and the products formed are less stable.

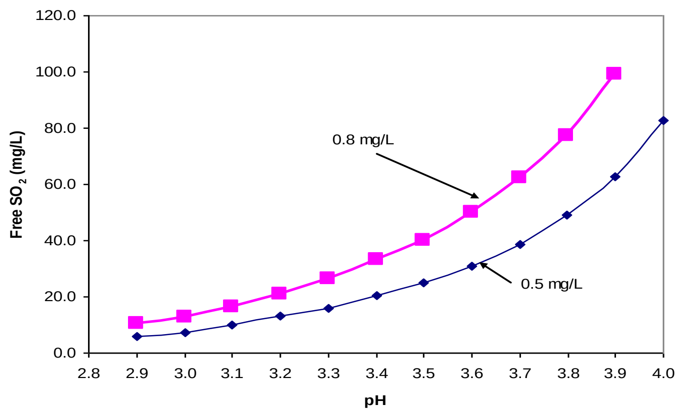
Free SO_2

Free molecular sulfur dioxide is the most important antimicrobial agent in wine. Within the pH range of juice and wine, the amount of free sulfur dioxide in the molecular form varies considerably (Figure 2). Attempting to control microbial growth with total or free SO_2, without reference to pH and molecular free SO_2, is of little value.

The level of molecular SO_2 (MSO_2) needed for the control of microbial growth will vary with viable cell number, temperature, ethanol content, etc. Beech et al.

(1979) determined that for white table wines, 0.8 mg/L molecular free sulfur dioxide achieved a 10,000-fold reduction in 24 hours in the number of viable *Brettanomyces* spp., certain lactic acid bacteria, and other wine spoilage organisms.

Figure 2. Free SO_2 Levels Needed to Obtain Molecular Free SO_2 Levels of 0.5 or 0.8 mg/L at Various pH Levels



Winemakers concerned about the negative sensory impact of excessive sulfur dioxide may elect to use a lower concentration, such as 0.5 mg/L molecular. The amount of free SO_2 needed to obtain 0.8 and 0.5 mg/L of the molecular form at various pH levels can be estimated from Figure 2 or by calculation:

$$[M\ SO_2] = \frac{[FSO_2]}{1 + 10^{pH-1.8}}$$

Depending upon wine pH, 30 mg/L free SO_2 may be excessive in one wine (lower pH) and deficient for control of microbial growth at another (higher pH).

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Section 2.

Sulfur Dioxide in Wine Production

There is a wine industry-wide trend toward reducing sulfur dioxide use whenever possible. Aside from health concerns, stylistic issues have sparked this interest, including utilization of MLF, enhanced delicacy resulting from lower levels of phenols in whites, and more rapid polymerization in reds.

Microbiological Control

SO_2 is probably taken up by yeasts as the molecular, rather than anionic, form. Because cytoplasmic pH is generally near 6.5, intracellular SO_2 rapidly ionizes, yielding HSO_3^- and SO_3^{2-}, resulting in yet more molecular SO_2 entering the cell to satisfy equilibrium demands. Eventually, inhibitory/lethal levels are accumulated by the cell.

Free molecular SO_2 is strongly inhibitory toward enzyme systems. Thiamine levels are also decreased by reaction with SO_2. During fermentation, yeasts are more resistant to SO_2 due to rapid fixation by aldehydes. Therefore, attempting to stop fermentation by the addition of sulfur dioxide within OIV or TTB limits (350 mg/L) is sometimes difficult, and may require the synergistic effects of lower temperature and increasing alcohol concentration to be effective.

Some non-*Saccharomyces* yeasts may exceed the resistance to SO_2 exhibited by wine strains of *Saccharomyces*. At SO_2 levels of 50 to 100 mg/L, high population densities (>10^6 colony forming units/mL) of *Hansenula,* *Kloeckera/Hanseniaspora*, and *Candida* spp. have been observed after the onset of fermentation. Some spoilage yeasts (i.e., *Zygosaccharomyces bailii*) exhibit extraordinary resistance to SO_2.

In general, juice and wine bacteria are more sensitive to the effects of sulfur dioxide than are yeasts. Acetic acid bacteria vary in their sensitivity to SO_2. *Acetobacter aceti* and *A. pasteurianus* have been reported as frequent isolates in wines with more than 50 mg/L total sulfites. *Gluconobacter oxydans*, on the other hand, was only found in wines with less than 50 mg/L SO_2 (Drysdale and Fleet, 1988).

Lactic acid bacteria (LAB) vary in their sensitivity to SO_2. In the case of *Leuconostoc oenos*, levels as low as 10 mg/L may be lethal (Widow et al., 1985). Bound levels of more than 30 mg/L delay the onset of growth (and MLF) and result in lower populations. Greater than 50 mg/L bound sulfite is strongly inhibitory toward LAB and MLF. *Lactobacillus* and *Pediococcus* spp. appear to be less sensitive to SO_2 than are strains of *Leuconostoc oenos* (Davis et al., 1988).

Grape Processing

Traditionally, sulfur dioxide additions were made at the destemmer-crusher. Levels used depended on the cultivar and maturity, as well as fruit integrity. In the United States, most white wine producers add sulfur dioxide at the press pan, settling tank, or only after primary fermentation.

Excessive sulfur dioxide added at crush increases extraction of flavonoid phenols, which can add a harsh character to the resultant wine. The trend for red wine production is to limit the free sulfur dioxide to less than 20 mg/L until after primary and malolactic fermentations, depending upon fruit conditions, maturity, and stylistic goals.

Cellar Considerations

Monitoring the concentration of SO_2 following addition shows a rapid increase in the levels of the free form. This is followed by a decrease over 2 to 8 hours, with corresponding increases in the percentage of bound SO_2. This is especially true in the case of sweet wines and those fortified with aldehydic spirits. Thus, SO_2 may be regarded as only a temporary preservative in wine, because of its combination with carbonyl groups or oxidation to sulfate.

There are limitations in the capabilities of sulfur dioxide as an antioxidant (see the module on Oxidation and Reduction). Because it reacts very slowly with molecular oxygen, SO_2 is impractical for scavenging oxygen from wines. On occasion, SO_2 additions accompany controlled aeration for removal of H_2S (see the module on Sulfur-Like Off Odor). As can be seen in the following reactions, elemental sulfur (S^o) and water are produced (Tanner, 1969):

$$2\ H_2S + O_2 \rightarrow 2\ H_2O + 2\ S^o$$

and

$$2\ H_2S + SO_2 \rightarrow 2\ H_2O + 3\ S^o$$

Barrel Maintenance

Several methods for sulfuring barrels are used, including sulfur wicks, pellets, gases and solutions. Pellets may provide more sulfur dioxide than wicks (Chatonnet, 1993a, b). When burned, oxygen is depleted and sulfur dioxide is released inside the barrel.

Pressurized liquid sulfur dioxide is also a common source for barrel sulfiting. Because SO_2 is now regarded as a pesticide, use of the pressurized gas requires the employee to obtain a pesticide applicator's license in certain states.

Alternatively, use of 1% SO_2 in acidified water also is effective in controlling microbial growth for longer-term storage. Unlike gaseous SO_2, use of the liquid form does not require special certification or licensing, but may represent a waste management problem.

Bottling Concerns

Most winemakers increase the concentration of free SO_2 immediately before bottling. Although amounts may vary, this adjustment may be calculated to yield free molecular SO_2 levels of 0.5 to 0.8 mg/L (see Figure 2). In pH range 3.2 to 3.5, this corresponds to additions of 20 to 40 mg/L.

Loss of free SO_2 in wine is proportional to the dissolved oxygen content in bottled wines. Such levels can be high in the absence of bottle purging gas, without vacuum filling and vacuum closing capabilities.

Rankine (1974) reported that the headspace in bottled wine may contain up to 5 mL of air, which amounts to 1 mL (1.4 mg) of oxygen that is available to initiate oxidation. In general, 4 mg of SO_2 is needed to neutralize the effects of 1 mg of oxygen, so an additional 5 to 6 mg of free SO_2 is needed to reduce molecular oxygen in the headspace.

This may represent a rather significant loss of free SO_2 that otherwise would be available as an antioxidant or antimicrobial agent. It should be noted that sulfur dioxide is not a good oxygen scavenger and that the reaction with the oxygen will take several days, during which oxidation of labile aroma/flavor components can occur. Purging bottles with inert gas before filling and/or vacuum fillers and corkers are helpful.

Sulfur Dioxide Addition Calculations

Originally, sulfur dioxide was obtained by burning sulfur but, today, alternative sources are available to the wine industry. Potassium metabisulfite and compressed sulfur dioxide gas, or solutions of the two, are the most common addition methods.

Typical additions of SO_2, using gas, potassium metabisulfite salt, and a concentrated solution of SO_2 are presented in the following examples, where a winemaker wishes to increase the amount of total SO_2 in 1,000 gallons (37.85 hL) of wine by 28 mg/L (ppm).

Addition of Sulfur Dioxide Gas (of 90% Activity)

Compressed gas has the advantages of being economical and avoiding the addition of potassium to the juice or wine. As already noted, use of compressed gas now requires certification/licensing.

(a) 28 ppm = 28 mg/L = 0.028 g/L

(b) 1,000 gal x 3.785 L/gal x 0.028 g/L = 106 g

(c) $$\frac{106 \text{ g}}{454 \text{ g/lb}} = 0.234 \text{ lb sulfur dioxide gas}$$

(d) Since the gas is 90% sulfur dioxide, the amount of SO_2 gas needed is:

$$\frac{0.234 \text{ lb/1,000 gal}}{0.90} = 0.260 \text{ lb/1,000 gal (37.85 hL)}$$

Addition of Potassium Metabisulfite (wt/vol)

Potassium metabisulfite (KMBS), is the water-soluble potassium salt of sulfur dioxide. Theoretically, available SO_2 makes up 57.6% of the total weight of potassium metabisulfite. Thus, the winemaker calculates the 28 mg/L SO_2 addition as follows:

$$\frac{0.028 \text{ g/L x 3.785 L/gal x 1,000 gal}}{0.576} = 184 \text{ g/1,000 gal}$$

During storage, dry forms of SO_2 may lose their potency. Elevated temperature and high humidity can also contribute to significant loss in strength. It is, therefore, desirable to monitor potency of these compounds, particularly if stored for extended periods.

Addition of Sulfur Dioxide (vol/vol)

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Some winemakers choose to use solutions of sulfur dioxide in water for additions. Such solutions are usually created by bubbling liquid sulfur dioxide, or dissolving KMBS, into a measured volume of water, thereby creating a saturated solution of $SO_2 \cdot H_2O$. At 20°C (68°F), the solubility of SO_2 in H_2O is 11.28%.

(a) In a well-ventilated, isolated area, create a saturated solution of $SO_2 \cdot H_2O$ solution. In cold water, solutions of 6-8% are readily achievable. Solutions of 15% SO_2 are commercially available.

(b) Using the data in Table 1 (Wilson et al., 1943), plot SO_2 concentration (% wt/vol) against its corresponding specific gravity at 20°C.

Table 1. Specific Gravity of SO_2 in Water

Concentration of SO_2 (% wt/vol)	**Specific Gravity at 20°C**
1.0	1.003
2.0	1.008
3.0	1.013
4.0	1.018
5.0	1.023
6.0	1.028
7.0	1.032
8.0	1.037

(c) Using a specific gravity hydrometer, determine the specific gravity of the solution. If the reading were 1.028, this corresponds to a 6.0% (60,000 mg/L) solution of SO_2.

(d) The volume of solution needed for a 28 mg/L addition of a 6% solution to 1,000 gallons is calculated as follows:

$$\frac{0.028 \text{ g/L} \times 3.785 \text{ L/gal} \times 1{,}000 \text{ gal}}{60 \text{g/L}} = 1.766 \text{ L}$$

Addition to 60-Gallon Barrels

A frequently-used formulation calls for dissolving 197 g KMBS in approximately 750 mL of slightly-warmed water and bringing to 1L final volume. From this stock solution, 20 mL = 10 mg/L in wine (Giannini, personal communication, 2008).

Practical Winemaking Summary

- There are limitations in the capabilities of sulfur dioxide as an antioxidant in wines.
- SO_2 may be regarded as only a temporary preservative in wine, because of its combination with various components and/or oxidation to sulfate.
- In the absence of sulfur dioxide, the plant enzyme tyrosinase will oxidize susceptible phenols while depleting must oxygen, which can impact yeast growth.
- The antimicrobial activity of sulfur dioxide is highly pH dependent.
- In general, juice and wine bacteria are more sensitive to the effects of sulfur dioxide than are yeasts.
- Lactic acid bacteria (LAB) vary in their sensitivity to SO_2.
- On occasion, SO_2 additions accompany controlled aeration for removal of H_2S.
- Many yeast strains can reduce sulfate to produce sulfur dioxide. Many new strains of H_2S-free yeasts tend to produce higher concentrations of sulfur dioxide.

- During storage, dry forms of SO_2 may lose their potency.