Research in Plain English

Conventional Measurements of SO_2 in Red Wine Overestimate SO_2 Antimicrobial Activity

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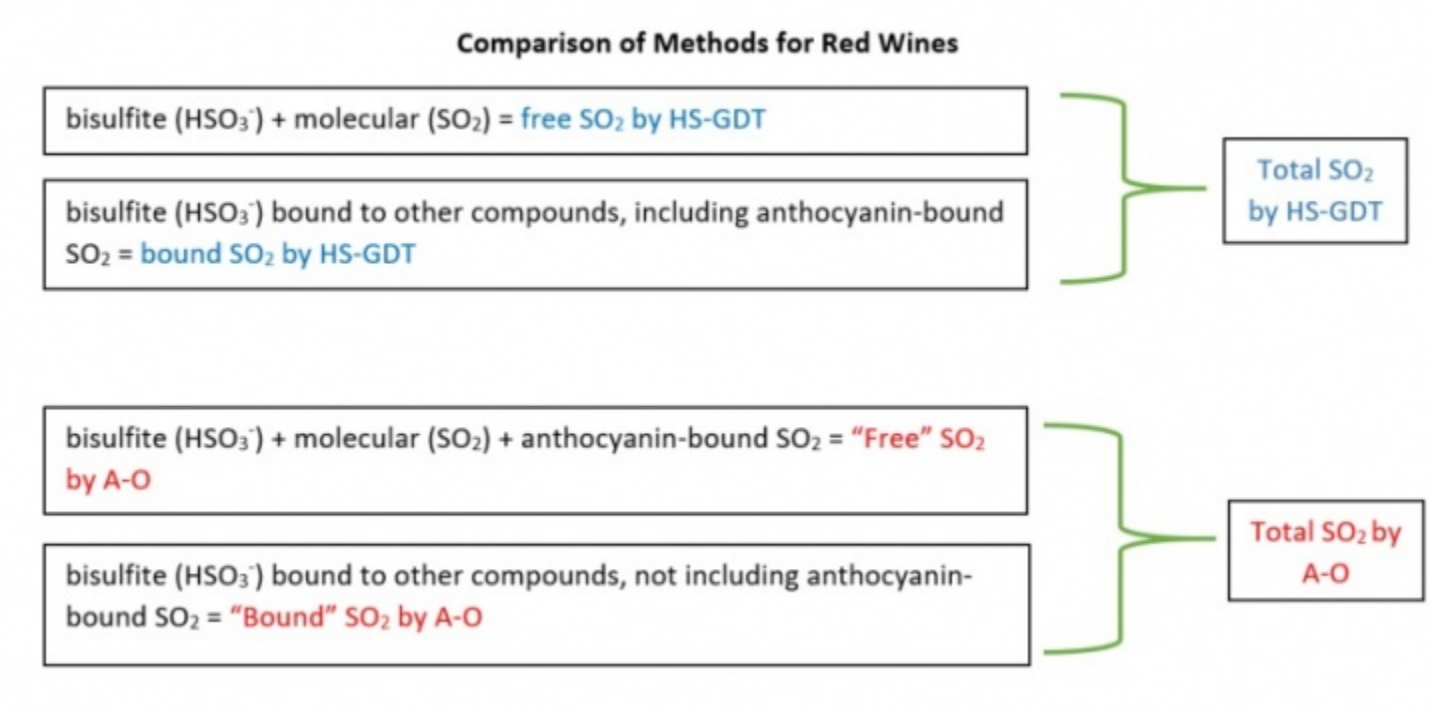
American Journal of Enology and Viticulture, February 2018. DOI: 10.5344/ajev.2018.17037

Summary by Raquel Kallas

Overview. Winemakers routinely add sulfur dioxide (SO_2) to wines to prevent microbial and oxidative spoilage. In red wines, a large portion of SO_2 is bound to anthocyanins (red pigments). Standard approaches to measuring SO_2 in wines involve an initial acidification step that releases this anthocyanin-bound SO_2, but it was unknown if this affected the accuracy of SO_2 measurements for the purposes of predicting microbial stability. Some authors have proposed that anthocyanin-bound SO_2 is also antimicrobial, but this work refutes that claim, and shows that standard approaches do not accurately predict microbial spoilage.

Background - SO_2 Terminology Reviewed. SO_2 can exist in several forms in wine: Molecular SO_2, bisulfite (HSO_3^-), and bound SO_2.The sum of molecular SO_2 and bisulfite is referred to as free SO_2. Protection against microbial spoilage in wines comes from molecular SO_2. The relative amount of molecular SO_2 to bisulfite in a wine varies with pH. Only a small portion of the free SO_2 in wine exists in the molecular form – usually, less than 5% at ordinary wine pH values. Typical recommended concentrations of molecular SO_2 range from 0.5-0.8 mg/L, depending on a wine's risk for spoilage.

Protection against chemical oxidation in wines comes from bisulfite. Bisulfite is the most abundant "free SO_2" form at wine pH, and free SO_2 recommendations are typically >30 mg/L. Finally, bisulfite can form bound SO_2 complexes by reacting with other wine components, such as aldehydes. In red wines, bisulfite may also form complexes with anthocyanins ("anthocyanin-bound SO_2"), particularly in young, highly pigmented red wines.



Background - SO_2 Measurements. Conventional industry methods for measuring free SO_2 in wine include aeration-oxidation (A-O), iodometric titration (the Ripper method), and flow injection analysis (FIA). The problem is that these methods disrupt anthocyanin-SO_2 complexes in red wine, breaking them apart which results in incorrectly high readings of free SO_2. Since molecular SO_2 is generally calculated using this free SO_2 value and the pH, the conventional measurement methods mentioned above can significantly overestimate molecular SO_2 levels.

The Sacks lab recently described a new approach to measuring molecular SO_2, called headspace measurements with gas detection tubes (HS-GDT), which does not disrupt anthocyanin-SO_2 complexes. Previous studies have shown that HS-GDT measurements of molecular SO_2 in red wine can result in only 32% of the molecular SO_2 levels obtained by A-O analysis, on average. In other words, A-O typically overestimates free and molecular SO_2 by a factor of 3. In contrast, HS-GDT measurements on white and rose wines averaged 86% of the molecular SO_2 measured by A-O.

Objectives. It has been well-established that molecular SO_2 is significantly overestimated using conventional measurement methods in comparison to HS-GDT. The objectives of this work were to:

1. Determine if anthocyanin-bound SO_2 has antimicrobial activity or not.
2. Determine if conventional measurements of molecular SO_2 are fit for practical purposes.

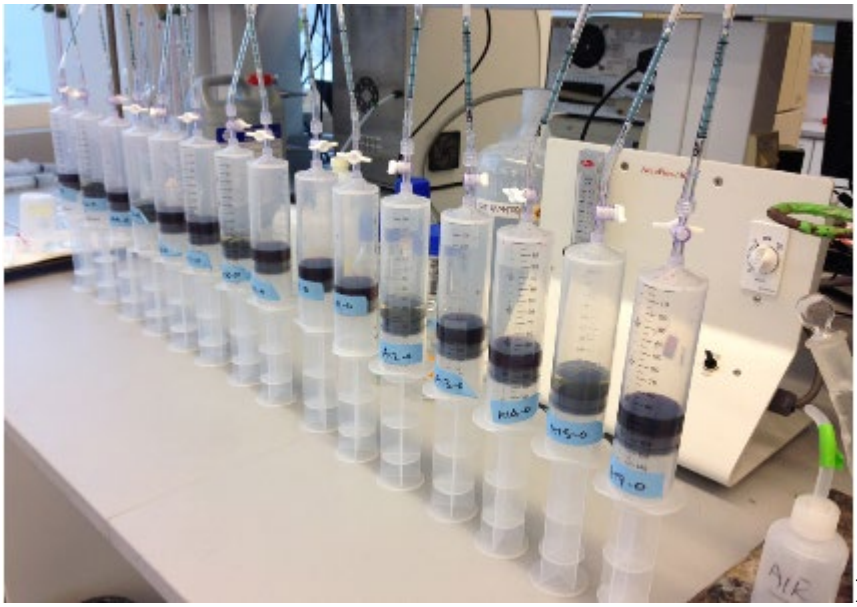
Experiment. "Red" wine was created by adding anthocyanin extract to a sterile-filtered white wine. This way, a comparison could be made between a red and a white wine with anthocyanin content being the only variable in the composition. A range of SO_2 concentrations were added to 1L bottles of each wine, from 0 mg/L (the control) up to 128 mg/L. To test the antimicrobial activity of the various SO_2 concentrations, the wines were inoculated with a commonly used S. *cerevisiae* strain, EC1118. Samples were then taken from the wines at time intervals over the course of 10 days to assess the survival and viability of the yeast, and to measure the free and molecular SO_2 concentrations using A-O and HS-GDT methods.

Results

Choice of Measurement Method is Critical. The results of this study were in agreement with the results from previous studies: molecular SO_2 concentrations in red wines are significantly exaggerated when the A-O method is used, compared to the value determined by the more accurate HS-GDT method. The white and red wines in the experiment showed the same level of molecular SO_2 when measured with A-O, but not with HS-GDT which showed lower molecular SO_2 in reds (in some cases < 0.2 mg/L molecular SO2, even when a recommended level of 30 mg/L free SO_2 was achieved). Molecular SO_2 was the same in white wines measured with both methods. All of this confirms that the anthocyanin-bound SO_2 complexes in red wines are responsible for the overestimated molecular SO_2.

Anthocyanin-bound SO_2 does not have Antimicrobial Activity. Pairs of red and white wine with the same level of molecular SO_2 as measured by each method were tested for yeast survivability. When the red and white wines had the same molecular SO_2 as measured by HS-GDT, there were no differences in yeast survivability. However, when a red and a white had the same molecular SO_2 as measured by A-O, there was significantly higher yeast survivability in the red wine, indicating that bisulfite-anthocyanin complexes don't have antimicrobial activity.

Conventional Measurements of Molecular SO_2 are not fit for Practical Purposes. You can't assume that molecular SO_2 measurements in red wine are accurate based on the results from conventional methods such as A-O. A method that does not disturb the anthocyanin-bound SO_2, such as HS-GTD, is necessary for an accurate measurement. You can't extrapolate what an HS-GTD measurement would be from an A-O measurement, since there is no linear relationship between them that would apply to any given red wine.


Headspace gas detection tubes. Photo by Patricia Howe

Conclusion and Practical Considerations. In red wine, molecular SO_2 will be overestimated by A-O, and should be measured by HS-GDT for best accuracy. *However, in some cases, the amount of molecular SO_2 actually needed is not practical to add because it would bleach the red wine. Instead, an HS-GDT measurement could indicate when more diligence is needed in monitoring wines.*

Another consideration is that the HS-GDT method is not automated, so it is best suited for small-scale wineries, or to check specific wines that may be at high risk for microbial spoilage.

Some examples of situations when there could be a high risk of microbial spoilage:

- Sweet or off-dry red wines, due to residual sugar content
- Unfiltered, or not sterile-filtered red wines
- Barrel aged red wines, especially in old barrels that may be infected with *Brettanomyces*
- Young red wines, due to the high quantity of monomeric anthocyanins present in young reds and their tendency to form anthocyanin-SO_2 complexes

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