The Impact of the timing and amount of SO_2 addition on Cab Franc Chemistry, Structure and Sensory Characteristics (2018)

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Summary

Sulfur dioxide is a traditional, inexpensive additive used widely at many different stages of modern wine making to combat oxidation and microbial spoilage. Despite all that is known about the chemistry and interactions of SO_2 in wine, many practical questions remain for winemakers. In this study, a single lot of Cabernet Franc wine was divided into six treatments with varying barrel age, dose and timing of SO_2 addition. The free and total SO_2, SO_2 addition rates, volatile acidity and microbiological evolution are reported. High initial dose led to higher total sulfur in each case. However, wines that received a high initial dose were also closer to the target SO_2 rate for aging for a higher proportion of the aging period. High initial dose may have helped preserve anthocyanins. Wines receiving high initial doses had lower overall accumulation of acetic acid during aging. New barrels had lower free SO_2 than their neutral counterparts on the same SO_2 schedule. They accumulated higher amounts of acetic acid and had high microbial populations of *Pediococcus* and acetic acid bacteria. If using new barrels, care should be taken to monitor SO_2 levels early in aging to accommodate for additional SO_2 binding by new barrels vs. older barrels. Delaying SO_2 addition by two weeks decreased total sulfur levels for both high and low dose wines. These wines began with higher acetic acid but experienced very little acetic acid accumulation during aging. The high, delayed dose was closest of all of the wines to the target SO_2 during aging. Though these wines were distinguishable from one another during sensory analysis, there was no significant preference for one wine over the other and no consistent trend in the differences (color, astringency, fruit or aromatic intensity).

Introduction

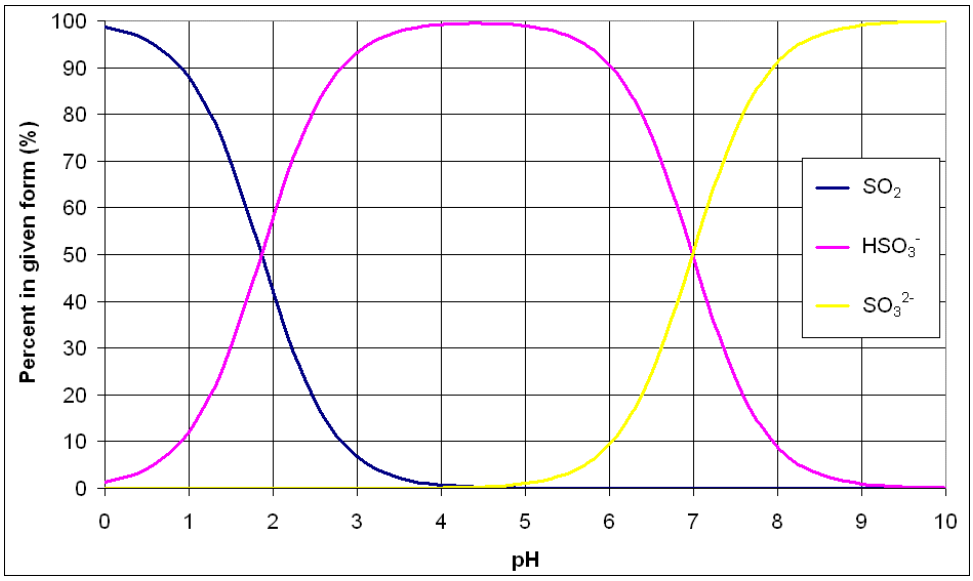
Sulfur dioxide is a traditional, inexpensive additive used widely at many different stages of modern wine making to combat oxidation and microbial spoilage. It can be used pre-fermentation to control microbial populations that come in on grapes and oxidation of juice, post-fermentation to protect wine from oxidation and spoilage during aging, for storage of barrels to prevent microbial spoilage, and even as a general cleaning agent in the winery (sprays on surfaces, etc…). Despite its widespread use, many questions of everyday practical use remain, including the best timing and magnitude of SO_2 additions. The following experiments focus on the use of SO_2 post-fermentation, during aging of Cabernet Franc wine.

When used in the winery, SO_2 is most often in a liquid form. When SO_2 dissolves in water, it doesn't remain only as SO_2. Instead, it interacts with the water in a way that takes three forms: molecular sulfur, bisulfite and sulfite (Figure 1). How much of any one form is

present depends on the pH of the solution, with the equation shifted to the left in low pH solutions and to the right in high pH solutions, meaning, the higher the pH, the less molecular sulfur dioxide is available[1–3]. Molecular sulfur is prized because of its antimicrobial activities. As the only form of sulfur dioxide that is not charged, molecular sulfur can penetrate the cell membranes of microbes and cause cellular damage and death. Once inside the cells of yeast and bacteria, which themselves have a pH of 6.5, molecular sulfur converts to the bisulfite form and denatures proteins, disrupts cell membranes, and ends cell functioning[1–3]. The bisulfite form of sulfur dioxide dominates in wine pH. Bisulfite is a potent inhibitor of enzymes such as tyrosinase (aka polyphenoloxidase) that causes enzymatic browning in juice and wine, though it is somewhat less effective against laccase, the oxidative enzyme produced by Botrytis[2]. However, the activity of bisulfite is constrained by the fact that it binds many constituents in the wine including acetaldehyde, anthocyanins, and sugars[1–3]. Once bound, it is no longer active as an antioxidant. At the pH of wine, the sulfite ion is nearly non-existent.

Figure 1: Forms of sulfur dioxide in solution are pH dependent. Image from Rotter n.d[4].

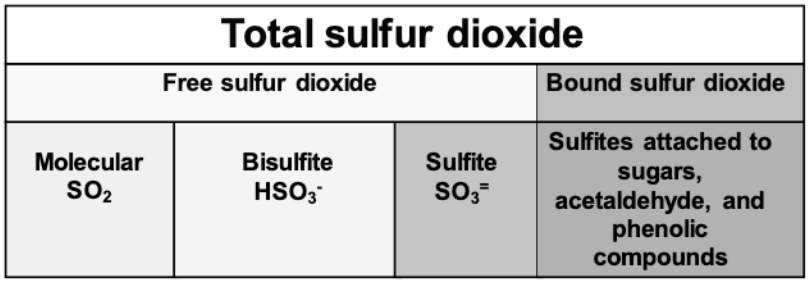
$$\underset{\text{water + molecular sulfur dioxide}}{H_2O + SO_2} \leftrightarrow \underset{\text{hydrogen ion + bisulfite}}{H^+ + HSO_3^-} \leftrightarrow \underset{\text{hydrogen ion + sulfite}}{2H^+ + SO_3^=}$$



When managing SO_2 in wine production, free and total sulfur dioxide are the most common measurements used. Free SO_2 refers to any form of SO_2 in wine that is not bound to another molecule. As mentioned above, the bisulfite form of SO_2 binds readily to many common constituents of wine, which also means it is no longer available for use as an antioxidant. Total sulfur dioxide, then, is the sum of the free sulfur dioxide (which includes all

unbound molecular, bisulfite and sulfite forms) and bound sulfur dioxide (mostly bisulfite bound to its many targets) (Figure 2)[1,3,5]. Since the proportion of SO_2 in any one form in wine is pH dependent, the concentration of molecular SO_2 can be calculated using the value for free SO_2 and the pH of the wine.

Figure 2: Forms of free and bound sulfur dioxide in wine. From Zoecklein[5]



Many guidelines used in winemaking regarding how much SO_2 to add to the wine, are based on known quantities needed to inhibit oxidation or microbial growth. Appendix 1 provides a compilation of these quantities from various sources. In many cases, ranges are given rather than firm numbers.

When determining how much SO_2 to add, it is important to keep in mind that some of the added SO_2 will be bound up by components of the wine. Estimates of SO_2 binding are that 1/3 to ½ of the SO_2 addition to finished wine will be bound up within the first few days of addition[1,2,6]. This figure will be higher in younger wines (for which more unbound components exist) as well as wines that have experiences Botrytis infection or infection with acetic acid bacteria, as both are known to produce more compounds that bind SO_2[1,7]

The decision of how much SO_2 to add to a wine is a good demonstration of the goldilocks principle: one wants to add just enough but not too much. SO_2 had many positive effects on sensory perception of wine. Judicious use can:

- preserve the freshness and fruit character of the wine by protecting it from oxidation[8]
- reverse the nutty, oxidative character of wines caused by acetaldehyde[8]
- increase extraction of color in red wines[9,10]
- prevent browning of white and red wines during aging[3,9,10]
- help prevent microbial spoilage that leads to ethyl acetate and acetic acid[8]
- prevent malolactic fermentation in crisp white wines[8]

However, SO2 also has negative sensory effects including

- loss of color due to anthocyanin bleaching[8,10]
- reduced rate of tannin polymerization and thus maturation in red wines[9]
- can neutralize other odors of the wine[1]

- can have negative odors itself such as a metallic, harsh pungent aroma[5], wet wool or burning characteristic[1]

Winemakers are often looking for ways to limit the use of SO_2. The best, least risky, way to limit SO_2 is to limit the bound fraction of SO_2 and maximize the unbound fraction, so that each SO_2 addition is more effective. When SO_2 is added to wine, it binds to several components including acetaldehyde, thiamine, enzymes, phenolics, and sugars[3,11]. In white wines, 80% of the bound SO2 is bound to acetaldehyde[11] whereas in red wines, most bound to either acetaldehyde or anthocyanins[5]. In sweet wines, glucose also binds SO_2. No matter the wine, reducing acetaldehyde will reduce the bound fraction of SO_2 and help limit total SO_2.

Acetaldehyde is formed by yeast as an intermediate in alcohol production during fermentation. Ethanol in wine can also be oxidized to acetaldehyde during aging [1,11]. It has sensory properties of its own, with a nutty, oxidized aroma sometimes compared to bruised apple[8,11]. Bisulfite binding converts acetaldehyde to a heavier , non-volatile compound with no sensory impact[5]. Bisulfite binding to acetaldehyde is relatively strong and rarely reverses, scavenging most SO_2 in solution. So ,if there is free SO_2, then the acetaldehyde is entirely bound up, packing the bound sulfur fraction[1,3,5].

There are three opportunities to control acetaldehyde production: at crush, at the end of fermentation, and during long cellar aging. *Saccharomyces cerevisciae* produces more acetaldehyde than non-*Saccharomyces* yeast, with a fairly uniform production rate among strains. The most important variable in acetaldehyde production is the addition of SO_2 at crush. SO_2 is toxic to *Saccharomyces* as well as other microbes, so when it is added, they take action to detoxify it. This includes cellular machinery to pump SO_2 out of the cell[12] as well as enhanced acetaldehyde production. Yeast produce more acetaldehyde as a means of detoxification; SO_2 will bind to acetaldehyde before it has a chance to enter their cells and shut down cellular function[1,11]. So, one way to limit acetaldehyde production is to limit SO_2 addition at crush.

Acetaldehyde production by yeast peaks during early fermentation after which time the balance shifts and yeast begin to consume it in the production of ethanol[11]. A strong fermentation with adequate nutrition produces a larger the number of viable cells at the end of fermentation, which are more likely to consume any remaining acetaldehyde. Other factors that affect acetaldehyde consumption include warmer temperatures (less acetaldehyde is produced) and contact with lees after completion of fermentation[11]. Malolactic bacteria also consume acetaldehyde simultaneously with and after completion of malolactic fermentation[11]. As a side note, malic acid bacteria also consume other compounds that bind SO_2 including pyruvic acid and alpha keto glutarate[11], adding another argument to allowing full malolactic fermentation to reduce SO_2 use. Waiting 1-2 weeks after the completion of malolactic fermentation allows time for the consumption of acetaldehyde (as well as diacetyl if it is present) by malic acid bacteria[8].

Zoecklein[5] states that most acetaldehyde results from microbial oxidation of ethanol under aerobic conditions. Acetaldehyde continues to form during aging as long as oxygen is present. Oxygen can be introduced through any number of means including un-topped tanks, racking and barrel storage. Switching out fermentation bungs for solid bungs, topping regularly, gassing with inert gas, and limiting racking can all diminish acetaldehyde formation during aging. Generally, SO_2 can't compensate for poor cellar practices[6].

SO_2 addition and effects on tannin evolution

In addition to its potential effects on preserving fruit flavors and aromas, preventing oxidation and spoilage, and potentially muting flavors, SO_2 has other impacts on the sensory perception of red wines through its interactions with phenolic groups. In red wines, the main binders of bisulfite are acetaldehyde and anthocyanins. When bisulfite binds to anthocyanins, it turns these pigments colorless, decreasing color intensity[2,7]. Bisulfite also binds in the same location on the anthocyanin molecule that tannins would bind, therefore SO_2 may delay or diminish formation more stable polymeric pigments[2]. Polymeric pigments that have already formed resist bleaching by SO_2 for this same reason[2]. Binding of SO_2 to anthocyanins is reversible, so over time, and with less SO_2 present, anthocyanins may be released and gain color[1,8], however at wine pH, the bound form is highly favored[7].

Another effect of SO_2 on polymerization of anthocyanins and tannins is its interaction with acetaldehyde. Acetaldehyde is an important component of tannin polymerization, as it forms bridges between tannin molecules as they form more stable, long-term bonds with each other and with anthocyanins. The presence of SO_2 slows the formation of these bonds because it binds with acetaldehyde, essentially taking it out of the equation. Without acetaldehyde, polymerization reactions are slower, including those among catechin molecules (formation of tannins) as well as between catechin and anthocyanins (formation of polymeric pigments)[10].

Polymerization of tannins is thought to diminish the astringency and bitterness of wine because larger tannins have less interaction with salivary proteins. Therefore, one would predict an increase in astringency and bitterness with sulfur dioxide use as a result of blocking tannin polymerization. Recent research by Panagiotis et al (2018)[13] has added a new wrinkle this prediction. This group has been documenting the presence of sulfonated versions of common sulfur dioxide targets including procyanidins (tannins) and epicatechin (a monomer of tannins). Sulfonation is simply the addition of a sulfonic acid group to an organic compound, in this case, to tannins, catechin and epicatechin, as one would expect to happen when wine is aged in the presence of SO_2. Previous studies by the same group had found sulfonated flavenols in red wines (like procyanidins and epicatechin) were positively correlated with age and storage conditions. Here, they surveyed wines of increasing age and found that as wines age in the bottle, these components become increasingly sulfonated.

In a talk at the 69th ASEV conference, Andrew Waterhouse also touched on this idea. He described discovering examples of sulfonated flavonoids (which include anthocyanins and the monomers that make up tannins) during an experiment examining quinone reactions during the phenolic cascade. In these experiments, the flavonoid was sulfonated at the position where tannins would normally bind. Normally, the interflavone bond that holds tannins together is broken by acid hydrolysis during aging. When this happens, the monomers that are released re-polymerize over time to form less astringent tannins. Waterhouse hypothesized that when this happens in the presence of SO_2, the SO_2 is binding to the subunits instead of another flavonoid monomer, essentially stopping the polymer from growing. He also described finding tannins modified with sulfonation, meaning the chain of monomers was capped by a sulfur group.

One would hypothesize that shorter tannin chains would be more astringent. However, they found that when tannin, acid and SO_2 were all present, protein precipitation products were abolished, indicating a softening of the astringency of wines with these conditions. These model wines also saw more SO_2 consumed than there was oxygen present, indicating SO_2 was binding something else, catechin and epigallocatechin (monomers) after acid hydrolysis.

Taken together, these studies reveal a new element of tannin evolution during aging that may significantly affect the sensory perception of astringency with aging. After discussing the current view of tannin softening by tannin polymerization during aging, Panagiotis et al (2018)[13] conclude their work by saying "alongside these reactions, we should now add sulfonated monomeric and dimeric flavenols, which are expected to direct interaction with proteins… sulfonated flavanols, a class of compounds so far neglected, could play a role in improving the sensorial quality of red wine with aging."

Despite all that is known about the chemistry and interactions of SO_2 in wine, many practical questions remain for winemakers. In the following experiment, a single lot of Cabernet Franc wine was divided into six treatments with varying barrel age, dose and timing of SO_2 addition. The free and total SO_2, SO_2 addition rates, volatile acidity and microbiological evolution are reported. Three questions were asked:

1. Is it better to add one large addition of SO_2 at the end of fermentation and allow the SO_2 to drift down during aging, or should many smaller additions be made? The magnitude of the initial addition may have consequences for the microbial population, sensory perception (both preserving fruit flavors as well as potentially producing off aromas), and tannin evolution.
2. Is there a difference in managing SO_2 in new barrels vs. neutral barrels? Here, do the additional potential binding sites for SO_2 on new wood make a noticeable difference in free SO_2, and do these have other consequences?
3. Can the total sulfur be altered if there is a 2 week lag period between the completion of malolactic fermentation and the addition of SO_2? Which is more important, the decrease in acetaldehyde or the risk of volatile acidity accumulation?

Methods

Cabernet Franc grapes were picked on September 26 from a single vineyard block and chilled overnight. On September 27, grapes were sorted, destemmed and loaded into Tbins for fermentation. SO_2 (50 ppm) was added to each bin. Bins were inoculated at a rate of 15g/hL of EC1118 yeast the next day (September 28). There was no acid addition. A total of 19 g/L of sugar was added on October 2. Each bin was monitored for Brix and temperature each day. All T-Bins were pressed together on October 10 after the completion of alcoholic fermentation to yield a single lot of wine. Wine was settled in tank for 7 days prior to racking with the addition of 1 g/225L barrel Scott Labs MBR process and 1.1 g/L tartaric acid. Malolactic fermentation was completed in tank. Malic acid depletion was checked every 4 days. Malic acid was determined to be 0.08 g/L on October 26. Wine was racked off lees on October 29, 1 g/L tartaric acid was added in tank, then wine was racked to individual barrels and SO_2 was added according to the appropriate treatment regime.

There were several comparisons made in this experiment. The difference in initial dose of SO_2 was determined by comparing a "high" dose of 75 ppm SO_2 with a "low" dose of 30ppm SO_2. For each high vs low comparison, there were three barrels: a new barrel (from Tonnellier Soud Oest with the same forest and toast levels), a neutral barrel (also from the same year, cooper and toast), and a neutral barrel with a delayed dose. The two "normally timed" barrels received SO_2 addition 3 days after the end of malolactic fermentation while the "delayed" barrel received SO_2 addition 19 days later. There were 6 barrels total (Table 1). Each barrel was periodically monitored for free and total SO_2. If needed, SO_2 addition was made to maintain a target of 0.5 molecular SO_2 (28 ppm).

Sensory Analysis

Sensory analysis was completed in two separate sensory sessions, the first at Bluestone Vineyards in the Shenandoah Valley and the second at Barboursville Vineyards in Central Virginia. At Bluestone, each flight was evaluated by a panel of 12 wine producers. In each flight, wines were presented blind in randomly numbered glasses. There were three tasting groups per flight with balanced tasting order among groups. In the first three flights, wine producers were presented with three wines, two of one type and one of another, and asked to identify which wine was different (a triangle test). Tasters were then asked to score each wine on a scale of 0 to 10 for color, aromatic intensity, fruit intensity and astringency. They were also given open ended questions to describe the wines. Results for the triangle test were analyzed using a one-tailed Z test. Descriptive scores were analyzed using repeated measures ANOVA. These flights included comparisons of (1) high dose vs. low dose wines from neutral barrels (2) high dose vs. low dose wines from new barrels and (3) high dose SO_2 addition at 3 days vs. 19 days. A fourth flight presented four wines in randomly numbered glasses: high and low dose

SO_2 at early and late timing. The wines from new barrels were not included in this flight. Producers were asked to rank the wines in order of preference, with open ended questions available to describe the reason behind the preference. At Barboursville, each flight was evaluated by a panel of 23 wine producers. Three of the four flights were re-tasted, with the sole exception the flight comparing sensory effects of SO_2 dose in new barrels.

Results

Initial juice chemistry was measured after destemming. Juice had 19.2°Brix with a pH of 3.85. After chaptalization and acidulation, the wine finished malolactic fermentation with pH of 3.54 and 11.9% alcohol.

Overall SO_2 addition and Total SO_2

A higher initial rate of SO_2 addition led to higher total SO_2 in the finished wine. In all cases, the high rate addition had higher total sulfur than its low dose counterpart (Table 2). The barrel receiving a delayed high dose required notably lower SO_2 addition during aging (Table 2) while maintaining a higher level of molecular SO_2 than the other treatments for the majority of the time of aging (Figure 3). This wine also had a lower total SO_2 at the end of aging than the other wines with an initial high dose (Table 2). Despite routine monitoring and addition, none of the barrels were able to maintain the target molecular sulfur throughout aging. The high dose barrels were closer to the target while the low dose barrels were considerably below this target at nearly every sampling event (Figure 1).

Differences in wine chemistry

Four of the six barrels maintained acetic acid accumulation rates within the expected range of 0.05 g/L per month (Table 3, Figure 4). The new barrels showed higher acetic acid accumulation rates than their counterparts, likely due to higher rates of SO_2 binding to oak components, leaving less free SO_2. The low dose new barrel had unacceptably high accumulation of acetic acid, likely due to infection with acetic acid bacteria. Though both delayed dose barrels had higher initial acetic acid than their counterparts, both maintained a lower overall level of acetic acid accumulation, leading to the lowest acetic acid levels at the end of the aging period (Table 3, Figure 4).

With the exception of the high dose barrel, all other barrels lost color through the course of aging (Table 4). Though the delayed dose barrels had a higher amount of color loss, they also started with higher color, leading to average color levels at the end of the aging period. There were not consistent trends in color loss based on initial SO_2 dose or timing. However, anthocyanins were always higher in the higher dose wines and lower in the lower dose wines (Table 5, Figure 5). Tannins tended to be slightly higher in low dose wines, with the exception of the new barrels. Tannins were generally higher in the new barrels (Table 5, Figure 3).

The new barrel receiving a low dose of SO_2 had much higher accumulation of acetic acid than the other three barrels. Sensory analysis indicated microbial spoilage, so microbiological testing using a PCR panel was done to describe the microbiological community of the wines in the new barrels (Table 6). The low dose barrel had much higher populations of acetic acid bacteria and *Pediococcus*. Both of these groups are relatively resistant to SO_2, so it is likely that the low dose was not sufficient to inhibit activities while the higher dose of SO_2 had a greater effect.

Results of sensory analysis

In a triangle test of high vs. low SO_2 dose wines, 8 out of 12 respondents were able to distinguish which wine was different in the Bluestone tasting and 12 out of 23 respondents were able to distinguish which wine was different at the Barboursville tasting. In both cases, the wines were significantly different ($Z=2.14$ and 1.7, $p=0.02$ and 0.04, respectively). This difference was also seen in the comparison of doses in wines aged in new barrels ($Z=1.81$, $p=0.04$). However, descriptions for what made the wines different in open ended questions, as well as scores for specific sensory descriptors (Table 7) did not show consistent results. For example, color was perceived to be higher in the high dose barrels at Bluestone but only in the neutral barrels. The new barrels showed the opposite trend.

In a triangle test comparing high dose SO_2 addition shortly after completion of malolactic fermentation with addition after two weeks, 7 out of 12 respondents were able to distinguish which wine was different in the Bluestone tasting and 10 out of 23 respondents were able to distinguish which wine was different at the Barboursville tasting. The difference was significant at the Shenandoah tasting ($Z=1.81$, $p=0.035$) but not at the Barboursville tasting ($Z=0.81$, $P=0.21$). Once again, trends were not consistent between the two tastings. For example, color was scored higher in the normally timed barrels at Bluestone but higher in the barrels with delayed timing at Barboursville. It is also important to note there are only small differences in means among any of the descriptors. When asked to rank the wines in order of preference, none of the wines was significantly preferred over any of the others (Table 9).

Conclusions

1. Is it better to add one large addition of SO_2 at the end of fermentation and allow the SO_2 to drift down during aging, or should many smaller additions be made? High initial dose led to higher total sulfur in each case. However, high initial dose was also closer to the target SO_2 rate for aging for a higher proportion of the aging period. High initial dose may have helped preserve anthocyanins and had lower overall accumulation of acetic acid during aging.
2. Is there a difference in managing SO_2 in new barrels vs. neutral barrels? New barrels had lower free SO_2 than their neutral counterparts on the same SO_2 schedule. They accumulated higher amounts of acetic acid and had high microbial populations of Pediococcus and acetic

acid bacteria. If using new barrels, care should be taken to monitor SO_2 levels early in aging to accommodate for additional SO_2 binding by new barrels vs. older barrels.

3. Can the total sulfur be altered if there is a 2 week lag period between the completion of malolactic fermentation and the addition of SO_2? Delaying SO_2 addition by two weeks decreased total sulfur levels at both dose levels. These wines began with higher acetic acid but experienced very little acetic acid accumulation during aging. The high, delayed dose was closest to the target SO_2 during aging.
4. Though these wines were distinguishable from one another during sensory analysis, there was no significant preference for one wine over the other and no consistent trends in the differences (color, astringency, fruit or aromatic intensity).

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Table 1: SO_2 treatment regimes based on dose, barrel age and timing

	SO_2 Dose	Barrel Type	Timing of SO_2 Addition
1	70 ppm	Neutral	3 days
2	70 ppm	New	3 days
3	70 ppm	Neutral	19 days
4	30 ppm	Neutral	3 days
5	30 ppm	New	3 days
6	30 ppm	Neutral	19 days

Table 2: SO_2 measurements and additions throughout aging (ppm)(in-house data)

	SO_2			Additions During Aging	
	Total	Free	Molecular	Total Number	Total Amount
Neutral High	103	18	0.47	2	30
Neutral Low	72	21	0.56	2	40
Neut High Delay	77	27	0.67	1	7
Neut Low Delay	62	20	0.53	2	33
New High	94	20	0.51	2	34
New Low	35	12	0.3	2	28

Table 3: Change in Acetic Acid (g/L) during aging for six treatments of Cabernet Franc (ICV labs)

	11/26/18	4/14/19	Change
Neutral High	0.39	0.64	0.25
Neutral Low	0.64	0.82	0.18
Neut High Delay	0.53	0.61	0.08
Neut Low Delay	0.51	0.62	0.11
New High	0.42	0.74	0.32
New Low	0.43	1.03	0.6

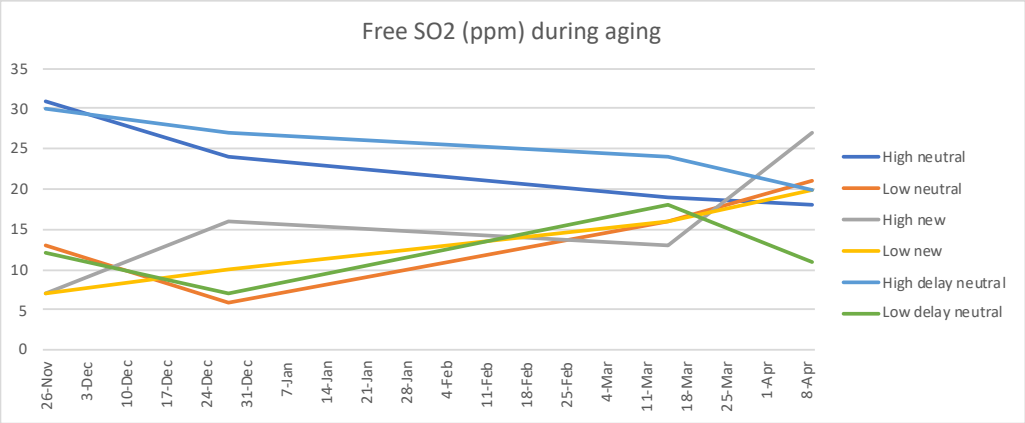
Table 4: Effect of SO_2 management on color (AU) over time for six treatments of Cabernet Franc (ICV labs)

	Color Intensity (420+520+620)			Hue		
	Initial	Final	Change	Initial	Final	Change
Neutral High	4.4	4.71	0.31	0.8	0.7	-0.1
Neutral Low	5.2	4.49	-0.71	0.7	0.7	0
Neut High Delay	5.7	4.71	-0.99	0.7	0.7	0
Neut Low Delay	5.8	4.6	-1.2	0.7	0.8	0.1
New High	4.6	4.55	-0.05	0.8	0.7	-0.1
New Low	5.4	5.04	-0.36	0.7	0.7	0

Table 5: Phenolic Measurements (mg/L) after aging for six treatments of Cabernet Franc (ETS labs)

	Polymeric Anthocyanins	Total Anthocyanins	Catechin	Tannin	PAC:Tannin
Neutral High	20	204	8	319	0.063
Neutral Low	22	178	7	346	0.064
Neut High Delay	19	205	8	314	0.061
Neut Low Delay	20	184	8	328	0.063
New High	21	202	8	331	0.063
New Low	20	192	8	323	0.062

Figure 3: Free and Molecular SO_2 measured during aging for each regime



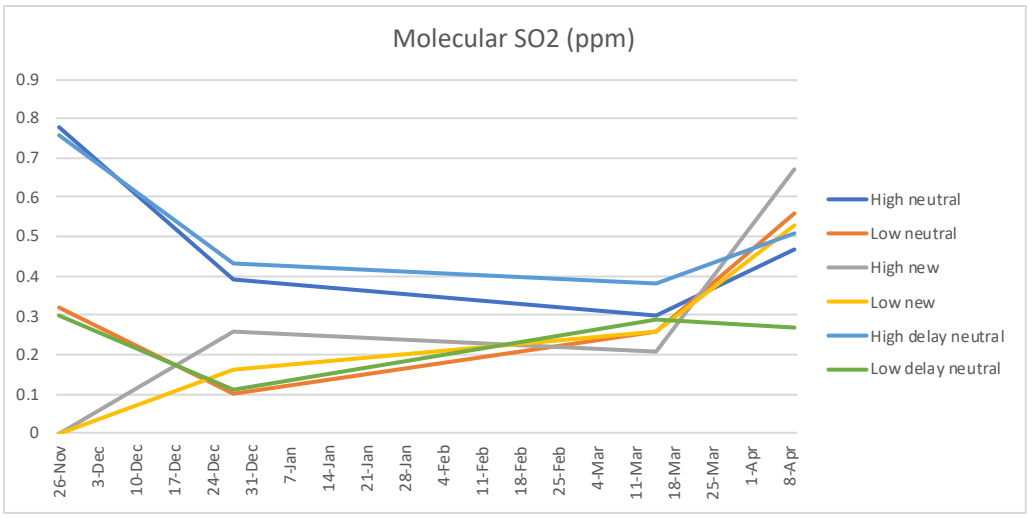


Figure 4: Change in Acetic Acid during aging for six treatments of Cabernet Franc (ICV labs)

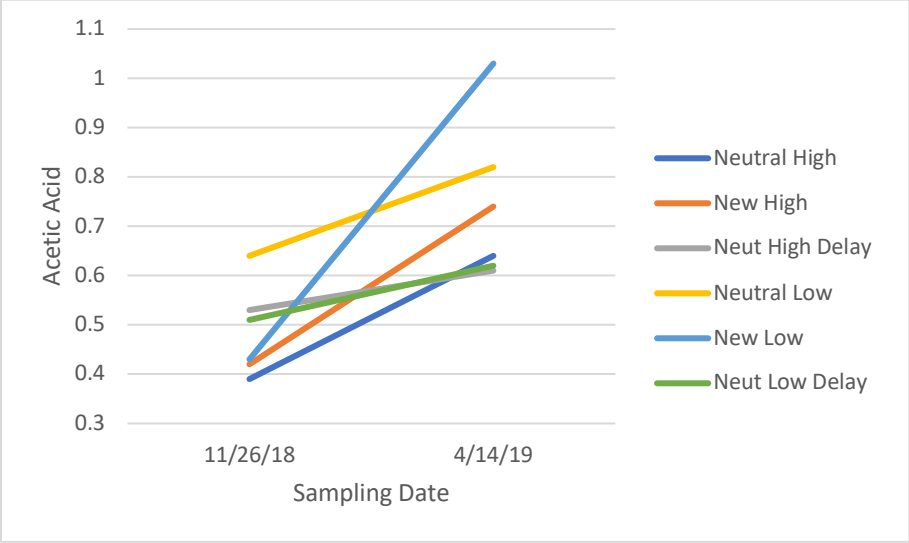


Figure 5: Total Anthocyanins and tannins for six treatments of Cabernet Franc (ETS labs)

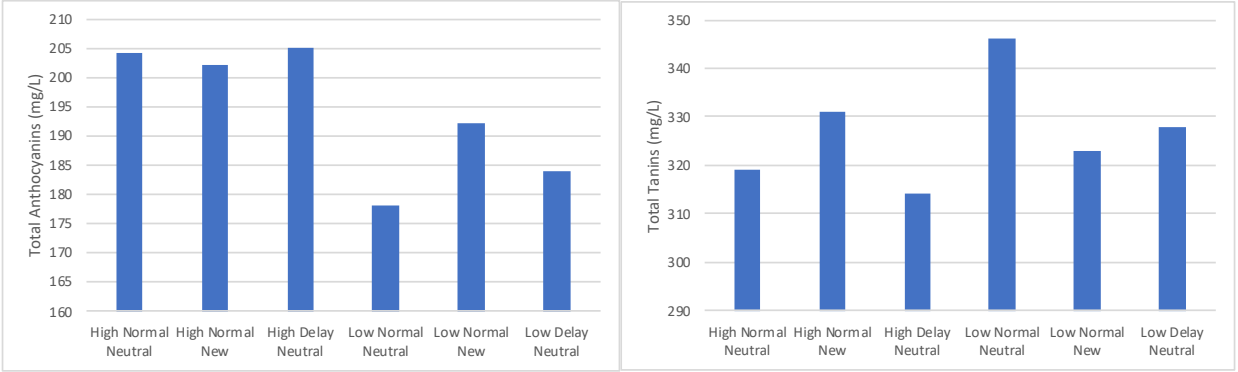


Table 6: Microbial community of wines in new barrels (ETS labs, scorpions)

	30 ppm	**70 ppm**
Acetic Acid Bacteria	*>1.0 x 10^7*	*2.2 x 10 ^6*
Brettanomyces	4490	4020
Lactobacillus	620	810
Oenococcus	7.2x10^6	7.2 x 10^6
Pediococcus	*3.3 x 10^5*	*470*
Saccharomyces	3850	3790
Zygossaccharomyces	40	10

Table 7: Sensory descriptor scores for high vs. low dose SO_2 from two sensory sessions

Color					
		Mean	SD	F	P
Bluestone	High	6.25	1.36	4.07	0.06
	Low	5.63	1.41		
Bluestone New Barrels	High	5.43	0.98	15	0.002
	Low	6.14	1.07		
Barboursville	High	5.50	1.17	0.31	0.58
	Low	5.67	1.37		
Aromatic Intensity					
		Mean	SD	F	P
Bluestone	High	5.88	1.55	0.62	0.45
	Low	5.31	1.39		
Bluestone New Barrels	High	5.57	1.27	0.46	0.51
	Low	5.86	1.35		
Barboursville	High	5.42	1.31	0.67	0.42
	Low	4.92	1.62		
Fruit Intensity					
		Mean	SD	F	P
Bluestone	High	5.75	1.39	1.84	0.20
	Low	5.13	1.55		
Bluestone New Barrels	High	5.43	1.27	1.88	0.20
	Low	4.71	1.80		
Barboursville	High	6.29	1.51	8.52	0.01
	Low	5.33	1.30		
Astringency					
		Mean	SD	F	P
Bluestone	High	5.71	1.38	0.26	0.62
	Low	5.29	1.25		
Bluestone New Barrels	High	5.14	1.35	0.07	0.80
	Low	5.29	1.50		
Barboursville	High	4.88	1.32	1.22	0.28
	Low	5.42	1.68		

Table 8: Sensory descriptor scores for normal timing vs. delayed dose of SO_2

	Color				
		Mean	SD	F	P
Bluestone	Normal	5.71	1.11	4.50	0.06
	Delay	5.29	0.76		
Barboursville	Normal	4.90	0.99	3.77	0.07
	Delay	6.00	1.83		
	Aromatic Intensity				
		Mean	SD	F	P
Bluestone	Normal	4.58	1.43	0.09	0.77
	Delay	4.92	2.38		
Barboursville	Normal	5.30	1.49	0.19	0.67
	Delay	5.10	0.77		
	Fruit Intensity				
		Mean	SD	F	P
Bluestone	Normal	4.83	1.17	0.04	0.85
	Delay	5.00	1.14		
Barboursville	Normal	4.70	2.06	0.33	0.57
	Delay	5.10	0.77		
	Astringency				
		Mean	SD	F	P
Bluestone	Delay	6.50	0.55	3.77	0.08
	Normal	5.33	1.21		
Barboursville	Normal	5.22	2.05	0.36	0.55
	Delay	5.61	0.70		

Table 9: Ranking in order of preference for four SO_2 regimes

	Bluestone		Barboursville	
	Mean	Std. deviation	Mean	Std. deviation
High Normal	2.27	1.27	2.29	1.10
Low Normal	2.50	1.07	2.57	1.12
High Delay	2.64	1.29	2.62	1.07
Low Delay	2.59	0.97	2.52	1.25
Q	0.52		0.83	
p	0.91		0.84	

Appendix 1: Compilation of SO_2 recommendations (with separate reference list)

Effect	**SO_2 level needed**	Ref(s)
Antimicrobial (fungicide, bacteriocide)	Generally: 0.6 ppm molecular for reds, 0.8 ppm molecular for whites	5
Againt yeast	Varies: up to 100 ppm free (Saccharomyces, Klockera, Candida); 0.8 - 1.5 ppm molecular	8
Against ML bacteria	10 ppm **total** slows, 50-80 total ppm prevents ML (0.6 ppm molecular); 50 gh/L inhibits ML, even if bound	5,8
Against Acetic Acid Bacteria	0.9 ppm molecular, >50 ppm free	8
Against Brett	0.3 ppm molecular to inhibit activity	3
	0.825 ppm molecular to eliminate viability (10,000 fold decreae in viable Brett)	8
Antioxidant	Target 20-40 ppm free during aging	3
Against enzymes at crush	20-80 ppm depending on fruit (50 ppm to healthy juice reduces PPO by 90%); 35 ppm will inhibit tyrosinases at crush	2,8
Red Wine oxidation	Risks below 10 ppm free	6
White Wine oxidation	Risks below 20 ppm free	6
Wine made from rotten grapes	Risks below 30 ppm free due to laccase	6
Guidelines		
SO_2 level	**Activity/Operation**	
150-200 ppm	General total sulfur limits for fine wine (sensory)	5,7
350 ppm	Legal limit of total sulfur	8
Aging (Free SO_2)		
20-30 ppm	Red wine aging; 0.6 molecular if done with ML, tannins allow antioxidant, 0.5 may be target if pH so high its hard to achieve	6
30-40 ppm	White wine aging, 0.8 molecular to prevent ML, oxidation	6
40-80 ppm	Sweet wine aging	6

Bottling (Free SO_2)	Generally 0.4-0.8 ppm molecular	3
Targets		
10-30 ppm (0.3-0.6 ppm molecular)	Red wine	*6,7,8*
20-30 ppm (0.4-0.8 ppm molecular)	White wine	*6,7,8*
30-50 ppm (0.8-1.2 ppm molecular)	Sweet wine	6
Additions		
50-70%	Proportion of SO_2 added to juice that binds to sugar, rest binds aldehydes and ketones	8
50-67%	Proportion of free SO_2 vs. bound; lower for initial additions, higher for subsequent additions	6,7
Operations		
8-10 ppm	Extra addition during bottling to offset oxygen intake due to filtration, racking	7
5-6 ppm free	Needed to offset O_2 in headspace of bottle (or, sparge bottles)	8
Other numbers		
>100 ppm	Amount of SO_2 addition needed to slow fermentation at crush, less if lower pH	1
10-30 ppm	Amount of SO_2 produced by yeast during fermentation	6
5ppm	Loss per month during normal barrel aging	7
8-10 ppm	Free SO_2 lost in bottle in the first year	7
Timing		
3-4 days	Time needed for bisulfite binding, lag time for SO_2 re-testing	7
5 days	Time needed for degradation of acetaldehyde post fermentation	5

Appendix 1 References

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