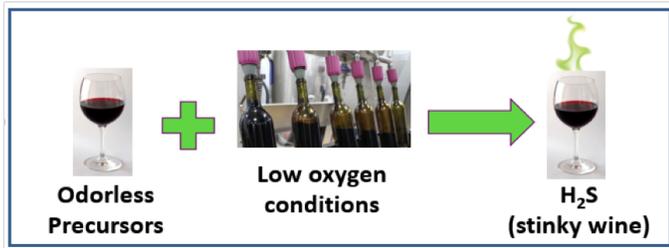# Research Focus

Sulfur Residues and Post-bottling Formation of Hydrogen Sulfide

Jillian Jastrzembski and Gavin Sacks
Department of Food Science, Cornell University, 411 Tower Road, Ithaca, NY

Hydrogen sulfide (H_2S, "rotten egg" aroma) can form in bottle under anaerobic conditions. The odorless precursors responsible for H_2S are still debated, and may include sulfur pesticide degradation products.

Photos by Jillian Jastrzembski

Winemakers are familiar with the stinky problems caused by sulfur pesticide residues during fermentation. But what if the problems continue after fermentation?

Recent work by the Sacks lab group shows that sulfur residues can be converted to odorless intermediates that continue to form a "rotten egg" (hydrogen sulfide) aroma after bottling and during storage. In other words, even if the hydrogen sulfide smell is removed prior to bottling, it will likely have returned by the time the wine is opened!

While this problem is not unique to wines fermented on sulfur residues, there are likely sulfur-derived precursors, i.e. sulfur residues from fungicide sprays, that serve as an additional reservoir for hydrogen sulfide in some wines. What, then, is the nature of these precursors, and how can we control their formation? To better understand this, we measured post-bottling formation of H_2S in wines with varying amounts of elemental sulfur, and found that S residues are a minimal problem in white and rose wines, if they are well clarified, and are unlikely to cause a problem for red wines in limited quantity (<1mg/kg).

Key Concepts

- Fungicidal sulfur (S) residues are well known to produce "rotten egg"-smelling hydrogen sulfide (H_2S) during fermentation.

- Recent work by our group shows that S-residues can also be converted to non-odorous, soluble intermediates that may continue to form H_2S post-fermentation.

- S-residue degradation products may contribute to phenomena of sulfur-like off-aromas (SLOs) increasing during bottle storage of some wines.

- S-residues should not exceed 1 mg/kg at harvest for skin-fermented wines to minimize risk of excess H_2S during fermentation and storage.

- In New York, this roughly corresponds to ceasing sprays four to six weeks prior to harvest for red wines.

- The risk to whites and rose wines is negligible if clarification is performed correctly.

- Convenient methods for S-residue quantification are available, and measurement methods of degradation products are being validated (See: Appellation Cornell ['Free' Doesn't Always Mean Free: Rethinking SO$_2$ Measurements in the Winery](#)).

Background (as in, "the stench lurking in the background"). Winemakers – have you ever had a wine go into the bottle smelling sublime, only to emerge months or years later smelling . . . a little stinky? Rotten egg, cabbage, burnt hair, or related descriptors? These off-aromas are typically the result of sulfur containing volatile compounds and are easily oxidized, so if you or your customer recognizes what's going on a little aeration can sometimes fix the smell. However, this is also a disappointing nuisance – How could a wine with so much promise have turned so funky in the bottle?

But, here's the good news: you've got esteemed company. At the International Wine Challenge in London, a competition that draws over 10,000 entries from prestigious wineries from around the world, reductive off-aromas accounted for 25-30% of all reported wine faults, comparable to the incidence of cork taint and oxidation (Goode et al. 2008).

So, what's responsible for the stink in a stinky wine? In a recent survey of off-aroma sulfur compounds in reduced wine, hydrogen sulfide (H_2S) was the one most often found in excess of its sensory threshold (Siebert et al., 2010). This may be due to a direct sensory effect, since H_2S has a nasty odor of "rotten eggs", or it may be an indirect precursor role, as H_2S can react with other compounds to make even more potent off-odorants.

H_2S formation during fermentation. The factors that increase H2S formation during fermentation are well studied (Waterhouse, Sacks, and Jeffrey 2016). First, yeast will produce H_2S as part of normal amino acid synthesis. This explains why accumulation of H_2S often occurs in cases of nutrient deficiency associated with amino acid production, such as low assimilable nitrogen, and also provides the justification for additions of nutrients before or early in fermentation. It also explains why some yeast strains are reported to be low or high H_2S producers – generally, it's related to genetic differences associated with amino acid production.

A second, independent pathway is the transformation of elemental sulfur (S) residues to H_2S. As discussed later, elemental sulfur is a popular control for powdery mildew. Formation of H_2S from S residues cannot be corrected by nutrient addition, and is much less dependent on yeast strains. H_2S is usually formed from S-residues in the latter half of the fermentation, which means that it does not coincide with carbon dioxide production. As a result, H_2S formed will not be lost immediately to carbon dioxide entrainment, and must be removed by other methods.

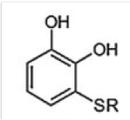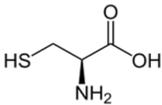There are several approaches to removing excess H_2S post-fermentation:

i) H_2S is highly volatile, and can be readily removed through sparging with inert gas, although this approach will be less effective against other (less-volatile) sulfurous off-aroma compounds.

ii) H_2S is also easily oxidized, so aeration (i.e. through racking) may also be used, particularly for reds. Although some texts claim that aeration should be avoided because of the danger of forming other noxious S-compounds, such as disulfides, there is little data to support this concern in recent scientific literature. However, oxidation can cause a loss of desirable S-containing compounds if done to excess, such as the fruity "grapefruit, passionfruit" compounds critical to Sauvignon Blanc and many other fruity white wines.

iii) Finally, copper additions are a common practice for the removal of H_2S, since the complexation of copper with thiols will cause them to precipitate. However, as discussed later, these complexes are challenging to remove from wine, and can potentially release H_2S (**Table 1**). In addition, copper might catalyze the release of H_2S from sulfur containing amino acids like cysteine, although this is still speculative (Ugliano et al. 2011).

Nature vs. nurture – the role of packaging in sulfurous off-aromas. Even after H_2S (or any related sulfurous off-aroma compound like methyl mercaptan) is removed, it can reappear during storage.

Table 1. Potential sources of H_2S during bottle storage.

Precursor	Structure	Evidence in Support	Reference
Quinone adduct	e.g.	Reverse reaction not yet shown in wine-like system	Kwasniewski 2013
Metal-sulfide complex	e.g. Cu_xS_y	Formation and reversibility demonstrated in wine	Franco-Luesma et al. 2014; Kreitman et al. 2016a
Cysteine		Shown to form H_2S under wine-like conditions	Ugliano et al. 2011; Pripis-Nicolau et al. 2000
Polysulfides	$R\text{-}(S)_n\text{-}R'$	Hypothesized precursor unique to S-fermented wines	Jastrzembski and Sacks 2015

Higher concentrations are reported to accumulate under low oxygen conditions (Ugliano 2013; Godden et al. 2001) – hence the term "reductive" off-aromas. That is why this type of wine fault is often found in bottles with a screw cap or synthetic cork, as these types of closures permit lower oxygen ingress compared to a traditional wine cork.

While one solution could be to use packaging materials with greater oxygen permeability, this also risks wine oxidation, e.g. the loss of desirable "fruity" smelling thiols.

Given our limited toolbox for dealing with sulfur-like off-aromas (SLOs) in bottle, the best option is to prevent the formation of these precursors in the first place.

So, what are the hidden precursors of H_2S?

The hidden menace – potential precursors of H_2S during bottle storage. Considering the importance of H_2S to SLOs, there have been surprisingly few studies on its potential precursors in finished wines. Recently, metal-thiol complexes – particularly copper sulfides – have been suggested as a key precursor of H_2S during bottle storage (**Table 1**). The formation and reversibility of these copper complexes has been demonstrated in model and real wines (Franco-Luesma et al., 2014). Additionally, contrary to conventional wisdom, the majority of added copper remains in wine and is not readily removed by racking or filtration (Clark et al. 2015). These results question the logic of using copper salts to treat reduced wines, as they will result in formation of solubilized copper – sulfur complexes that can serve as a ticking stink bomb during storage (Franco-Luesma et al. 2014)!

Yet, these complexes do not account for all of the latent H_2S present in wines, suggesting that additional precursor pools exist. H_2S has also been shown to bind to other compounds like "quinones" or oxidized polyphenols (Nikolantonaki et al. 2012), and potentially these complexes could be reversible (Kwasniewski 2013). H_2S can be generated through desulfurization of the amino acid cysteine (Ugliano et al. 2011). However, no firm evidence indicates that any of these H_2S reactions will occur to an appreciable extent in real wines. Formation of polysulfides through the reaction of thiols with H_2S under oxidizing conditions has also been observed in model wine systems (Nedjma et al., 1996; Kreitman et al. 2016b), but has not been characterized in real wines.

Elemental sulfur (S) pesticide residues as long-lived precursors of H_2S in wine. An alternate source of polysulfide precursors of H_2S are S-residues. As mentioned earlier, S-residues are widely used for powdery mildew control, and in the Northeast United States, may be applied 7-14 times in a growing season. Elemental S provides several advantages as compared to alternatives: it is effective, environmentally benign, and inexpensive (only $5/acre, as compared to $15-40/acre for alternatives), and commonly used as part of both organic and sustainable spray programs.

The ability of S-residues to generate H_2S during fermentation is well established – the earliest reports go back to 1869! However, the potential of S-residues (or their degra-

Figure 1. *Grape leaves infected with powdery mildew (top); Finger Lakes Riesling with sulfur spray residues, less than two weeks before harvest (bottom).*

Photos by Gavin Sacks

dation products) to serve as a latent source of H_2S has not been evaluated.

As part of Jill Jastrzembski's PhD thesis, we have been investigating if S-residues could form polysulfides, which could later release H_2S under reductive storage conditions. Elemental sulfur can react with disulfides to form polysulfides, which are shown to release H_2S upon reduction (Moutiez et al. 1994). Although these reactions have yet to be demonstrated in wine-like conditions, it is possible that polysulfides are a supplementary source of H_2S in S-fermented wines (**Table 2**).

Ongoing work in the Sacks lab group is investigating the presence of wine-relevant polysulfides – for example, glutathione trisulfide – that could be causing problems later on. A better understanding of this pathway will help us better understand how to prevent and solve H_2S problems in stored wine. In the meantime, the question is whether our 1 mg/kg limitation on S-residues is sufficient to prevent the reformation of H_2S in the bottle.

Table 2. Post-bottling Formation of H_2S in S-Fermented wines (Jastrzembski and Sacks 2015). nd = not detectable

S pre-fermentation (μg/L)	Free H_2S at bottling (μg/L)	Free H_2S at 3 months (μg/L)
0	nd	nd
20 × 103.	nd	2.4($\pm$0.2)
100 × 103	nd	10.0($\pm$0.9)
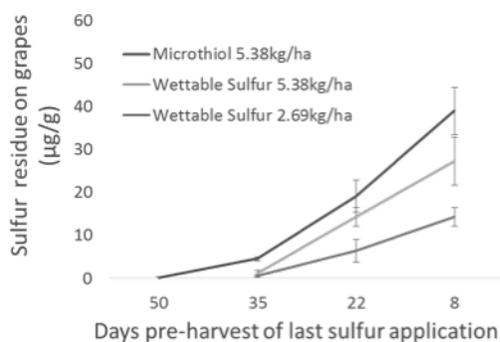
Figure 2. *S-residues on grape cluster samples taken throughout the 2010 season (Kwasniewski et al. 2014). The recommended limit for avoiding excess H_2S during fermentation or storage is 1-10 µg/kg.*

By correlating latent H_2S across a range of yeast strains to H_2S released three months after bottling, we estimated that S-residues should be limited to 7 mg/kg in must to prevent formation of excess H_2S precursors in stored wine. This is comparable to the 1-10 mg/kg limits recommended to avoid excess H_2S during fermentation (Kwasniewski et al. 2014).

Are my S-residues too high? When should I stop spraying? From the previous section, S-residues in must above 10 mg/kg will likely cause an increase in H_2S during and post-fermentation, 1-10 mg/kg will be borderline, and <1 mg/kg is unlikely to cause problems. Recent work by our lab has shown that ceasing sprays five weeks pre-harvest was sufficient to keep sulfur residues below 1 mg/kg for two out of three years.

However, a third vintage showed that ceasing spray applications 54 days before harvest still resulted in residues exceeding the 1 mg/kg cut-off (**Figure 2**). In general, ceasing spray four to six weeks before harvest should be sufficient, although differences in growing conditions, spray formulations and rates, and sprayer design make firm predictions challenging. Additionally, these are values for must prior to fermentation. S-residues stick to insoluble grape solids, and for well clarified white and rose juices, there will be negligible S-residues present.

What should be done if sulfur residues are too high? In the event that S-residues are too high come harvest, what can be done? Typically, about 50% of residues will be transferred from the grape skin to the must. If harvest can be delayed, then weathering can help to remove residues, and hand harvesting will minimize skin contact. For whites and roses, clarification can remove >90% of sulfur. For reds exceeding 1 mg/kg, a safe last-resort option is to minimize skin contact and make rosé wine. At the very least, performing a sulfur test can confirm whether or not elemental sulfur is the cause of H_2S production during fermentation.

What about adding copper salts during fermentation if things seem much stinkier than they should be? Anec-

SIMPLE, INEXPENSIVE METHOD TO TEST S-RESIDUES ON GRAPES

You don't have to rely on "typical" values for guessing if your grapes will have too much S-residue – you can measure it directly. The Sacks lab developed an inexpensive method for S-residue quantification (**Figure 3**). A reducing agent is added to buffered grape must or juice to convert elemental sulfur to H_2S. The sample is then de-aerated using an ant-acid tablet, and the H_2S is directed through the gas detection tube. The length of the color change in the tube is proportional to the amount of sulfur that was originally present in the sample. As this method costs less than $5 per run and takes less than 20 minutes, it can easily be performed by small operations.

Figure 3. *Simple, inexpensive method to quantify elemental sulfur residues on grape.*
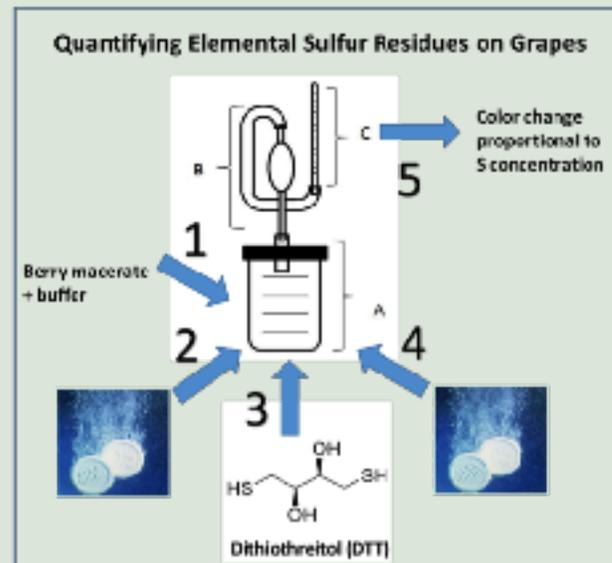
More information on this method is available in the literature (Kwasniewski et al. 2011), and at:

- Appellation Cornell Issue 23, 2015 'Free' Doesn't Always Mean Free: Rethinking SO$_2$ Measurements in the Winery

- Written protocol: Analyzing Elemental Sulfur Residues on Grapes

- Video Demo: http://www.youtube.com/watch?v=yH83vDX8ORQ

- New article by Chen, Jastrzembski, and Sacks (2016) in American Journal of Enology and Viticulture: Copper-Complexed Hydrogen Sulfide in Wine: Measurement by Gas Detection Tubes and Comparison of Release Approaches

dotally, we have heard of winemakers doing this. While it may decrease stink in the short term, it is advisable to check if residual copper remains after racking, and remediate if necessary. Otherwise, you could have a reservoir of latent SLOs in your wine, and potentially a wine with copper in excess of legal levels.

Testing for H₂S precursor and future work. Winemakers benefit from accelerated tests predicting if defects will occur - for example, chilling and heating tests are described for predicting formation of potassium bitartrate crystals or protein hazes, respectively. Similar routine tests for formation of SLOs are not available.

Our group and others are interested in developing approaches to quantify the risk of SLOs during storage. Recent work has shown that dilution with brine can release metal-sulfide complexes such as copper sulfides (Franco-Luesma et al., 2014), and that this release is correlated with release during accelerated or normal aging. Interestingly, this release can also be triggered by ascorbic acid (Chen et al. 2016) – in other words, the commonly used "ascorbic acid test" may not be detecting disulfides as is commonly assumed, but instead may be detecting copper sulfide (or copper mercaptide) complexes!

Because a gas detection tube (**Figure 3**) can detect release of free H₂S, this testing method is appropriate for implementation in small winery operations. However, the brine dilution method does not account for all H₂S released - as low as 11% in some wines (Franco-Luesma et al. 2016) – suggesting the need for complementary approaches to detect other H₂S precursors. We have recently shown that a reducing agent commonly used among biochemists (TCEP) can release a different pool of latent H₂S from wines than the pool released by brine dilution (Chen et al. 2016), and we are currently determining if this reagent will be useful for winemakers.

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Jillian Jastrzembski is a PhD student in the Sacks Lab in the Department of Food Science. Gavin Sacks joined the Cornell faculty in 2006. He is associate professor of food science and director of the undergraduate Viticulture and Enology program at Cornell.

Cornell University
College of Agriculture and Life Sciences
Cooperative Extension