
APPELLATION CORNELL

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RESEARCH FOCUS

Sulphur Dioxide Content of Wines: the Role of Winemaking and Carbonyl Compounds

Nick Jackowetz[1], Erhu Li[1], and Ramón Mira de Orduña[2]

[1]Graduate Research Assistant and [2]Associate Professor
Dept. of Food Science, NYS Agricultural Experiment Station
College of Agriculture and Life Science, Cornell University

Winemaking practices influence production of SO_2 binders such as acetaldehyde and therefore the quantity of SO_2 a winemaker needs to add during the winemaking process.

Photo by Tim Martinson

Except for alcohol, sulphur dioxide (SO_2) is the only component that prompts a warning statement on wine labels, although some proteins potentially present in wine are also considered allergens and are under regulatory review in many countries. SO_2 serves many useful functions in winemaking. It can serve as enzyme inhibitor in musts to prevent juice browning and oxidation, as a microbiological control agent in musts and wines, and to prevent the oxidation of finished wines. It also has the ability to bind to undesirable volatile compounds thus reducing their sensory impact.

Unfortunately, SO_2 also is an irritant and may have serious effects on sensitive consumers. The U.S. Food and Drug Administration estimates that up to 1% of the U.S. population has an increased sensitivity to sulfites, and other studies suggest that 5% of asthmatics may risk adverse reactions upon ingestion of SO_2. However, it could be argued that considering its historic utilization in a number of foods the amount of health related data available is scarce.

KEY CONCEPTS

- For a given concentration of free SO_2, the total SO_2 content depends on bound SO_2 and hence, the concentration of SO_2 binders. Acetaldehyde and pyruvate are the most important SO_2 binders in most wines.
- Acetaldehyde may be formed biologically (by yeast at the start of alcoholic fermentation) and chemically (mainly after alcoholic fermentation when wines are not protected from oxidation by atmospheric oxygen).
- Yeast will degrade acetaldehyde during later stages of alcoholic fermentation if they are still viable and remain in contact with the wine. Malolactic bacteria will degrade acetaldehyde significantly during malolactic fermentation.
- All winemaking steps (racking, pumping, filtration, bottling) after the end of alcoholic or malolactic fermentation can lead to increased acetaldehyde levels.
- Addition of SO_2 in the presence of active yeast will lead to the formation of SO_2 binders. For every 10 mg/l of SO_2 added to the must, bound SO_2 levels in the final wine will increase by 3-7 mg/l.

Our laboratory has studied the microbial metabolism of carbonyl compounds since 2000 with the aim of controlling and reducing the use of SO_2. Because of the steadily increasing interest in low SO_2 wines, our results have been widely disseminated in scientific journals and at local and international conferences. In this article, we provide an overview of the results and information for winemakers interested in minimizing the production of these SO_2 binding compounds and final SO_2 levels.

Legal limits for SO_2 and consumer perceptions. Over the last few years, a number of New York winemakers have asked us for advice with regards to comments from customers asserting that European wines are lower in SO_2. The importance they place on SO_2 may lie in the overall negative connotations associated with its use, even though it has a long record of utilization as a preservative in a number of foods and is perfectly safe for the majority of consumers. The remarkable increase in the number of organic wineries in Europe and the steady increase in "sustainable"or "fresh" wines with lower SO_2 concentrations in the United States and Canada may also be indicative of the increasing consumer interest in minimally processed wines.

Are European wines lower in SO_2? It is true that the European Union (EU)−following previous reductions− has further reduced maximum limits for total SO_2 in red and white wines to 150 and 200 mg/l, respectively (EC 606/2009, Annex I B). However, these limits apply only to dry wines (<5 g/l of combined glucose and fructose). Numerous exemptions are listed in an additional four pages

Legal limits for total SO_2 in major winemaking nations (in mg/l)

Country	Wine type (RS)	Limit	Legal Reference/Description
USA	All	350	27 CFR 4.22(b)(1)
AUS	<35 g/l sugars	250	ANZFSC 4.5.1: Clause 5(5)(a)
	>35 g/l sugars	300	
NZ	<35 g/l sugars	250[1)]	
	>35 g/l sugars	400[1)]	
EU	white/rosé, <5 g/l	200	EC No 606/2009, Annex I B
	red, <5 g/l sugars	150	
	white/rosé, >5 g/l	250	
	red, >5 g/l sugars	200	
	specific wines	300	Eg.: Spätlese (can be dry), Bordeaux Sup., Côtes de Bordeaux, C. de Bergerac, Navarra, Penedès, several French VdP and Hungarian and some Greek sweets
	specific wines	350	E.g.: Auslese (can be dry), sweet wines from Romania, Czech Rep., Slovakia and Slovenia
	specific wines	400	E.g.: Beerenauslese, TBA, Eiswein, French sweet wines such Sauternes, Barsac, etc., sweet Greek with >45 g/l sugars, sweet Eastern European wines
CAN	All	350[2)]	Canadian Food & Drug Reg. B.02.100
India	All	450	Prevention of Food Adulteration Act & Rules, Appendix C, Table 3
Japan	All (>1% abv)	350[1)]	Japan's Specifications and Standards for Food Additives
RSA	white, <5 g/l sugars	160	Liquor Products Act 60 of 1989 Regulations Regulation 32 (Table 8)
	reds, <5 g/l sugars	150	
	All, >5 g/l sugars	200	
	specific wines	300	E.g.: noble late harvest and naturally dried

[1)] unit is mg/kg

[2)] Canada prescribes a maximum of 70 mg/l free or 350 mg/l combined SO_2

in this regulation, permitting up to 400 mg/l of total SO_2 in some wines—which is greater than the maximum of 350 mg/l allowed in any wine according to American regulations [27 CFR 4.22(b)(1)] (See box on page 2).

However, it is not particularly useful to compare the SO_2 content of dry Mediterranean wines to cool climate vintages that require some residual sugar for balance. If we compare apples with apples, we can see that in the EU whites exceeding 5 g/l of sugar may contain 250 mg/l of SO_2, Spätlese (even if dry) 300 mg/l, and Auslese even up to 350 mg/l of SO_2. The legal framework does not support the assertion that comparable European cool climate wines contain less SO_2 than New York State wines. In fact, there is some evidence to the contrary, since eastern French and German winemaking regions have not even been able to comply with the European SO_2 regulations in all years (See "German 2006 Vintage" box, page 5).

Admittedly, while they set the legal framework, regulations do not represent de facto concentrations. No thorough studies comparing the SO_2 content in wines have been published, which may be a blessing. However, typical winemaking approaches and regular sensory evaluations suggest that a good number of German wines tend to have higher SO_2 content than New York wines, while French wines may be equivalent.

SO_2 binders, their occurrence in wines, and their analysis. Compounds with carbonyl functions bind to SO_2. Relevant SO_2 binders in wine include glucose, acetoin, diacetyl, galacturonic, α-ketoglutaric and pyruvic acids, and acetaldehyde. A number of methods to quantify SO_2 binding compounds in wines are available. Individual analysis typically is tedious and cumbersome. In our laboratory, we can determine major SO_2 binders simultaneously using High Performance Liquid Chromatography (HPLC, **Figure 1**). However, the most important SO_2 binders—acetaldehyde, pyruvic and α-ketoglutaric acids—can also be determined with relatively cheap enzymatic tests using a standard spectrophotometer.

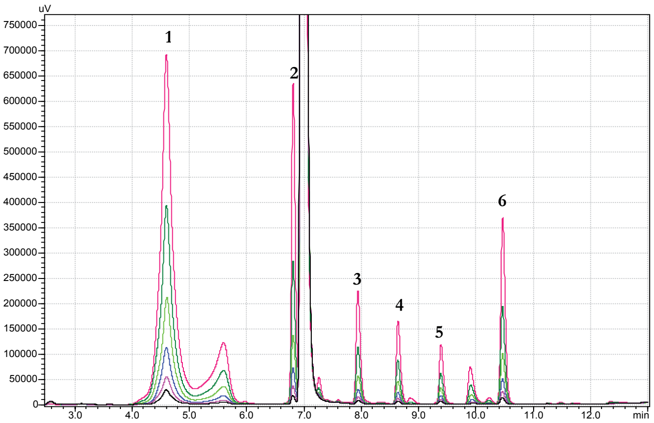

Figure 1. *Chromatogram of SO_2 binding compounds. 1. glucose, 2. galacturonic acid, 3. α-ketoglutaric acid, 4. pyruvic acid, 5. acetoin, 6. acetaldehyde.*

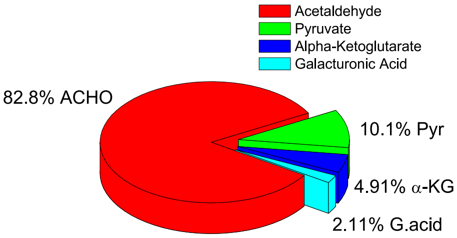

Figure 2. *Approximate share of major wine carbonyls for bound SO_2 of white wines.*

In recent years, we have analyzed over 200 New York State wines for SO_2 binders. In reds, galacturonic and α-ketoglutaric acids were found in higher concentrations, while white wines contained more pyruvic acid and acetaldehyde (**Table 1**).

Based on these results, we determined that the most relevant SO_2 binding compounds were acetaldehyde, pyruvic and α-ketoglutaric acids because of their binding properties and their usual concentrations in wines. Acetaldehyde typically accounts for over 80% of the bound SO_2 in wines **(Figure 2)** and its binding essentially is chemically irreversible. In sweet wines, glucose can bind some SO_2, although only weakly.

Because of its importance for bound SO_2, we have focused specifically on understanding the formation and control of acetaldehyde during the winemaking process.

Table 1. *Average concentrations (mg/l) of several SO_2-binding compounds in New York State white and red wines. A total of 237 wines were analyzed.*

Wine Type	Glucose	Galacturonic Acid	Alpha-Ketoglutarate	Pyruvate	Acetoin	Acetaldehyde
White	4750	267	31	25	10	40
Red	1400	810	74	14	11	25

Acetaldehyde in wines, its role, formation, and degradation. Acetaldehyde is the most important volatile wine carbonyl and can be formed both biologically (through yeast activity) and chemically (by wine oxidation). It is a small and highly reactive molecule with a green grass, apple-like or nutty aroma.

Formation. A common misconception is that the risk of acetaldehyde formation begins with the end of alcoholic fermentation, when wines may be exposed to atmospheric oxygen, leading to the oxidation of ethanol to acetaldehyde in the absence of SO_2. However, unless wines are treated carelessly after alcoholic fermentation, most of the acetaldehyde found in wine actually stems from yeast activity. Enological yeast, including commercial *Saccharomyces cerevisiae*, excrete acetaldehyde during the initial phases of alcoholic fermentation. After reaching a peak value, acetaldehyde is then re-utilized to a certain degree **(Figure 3)**. Typical residues found in Gewürztraminer and Riesling after alcoholic fermentation ranged from 22-49 mg/l of acetaldehyde. Chemical formation of acetaldehyde relies on exposure to oxygen, the presence of transition metals such as copper and iron, and phenolics.

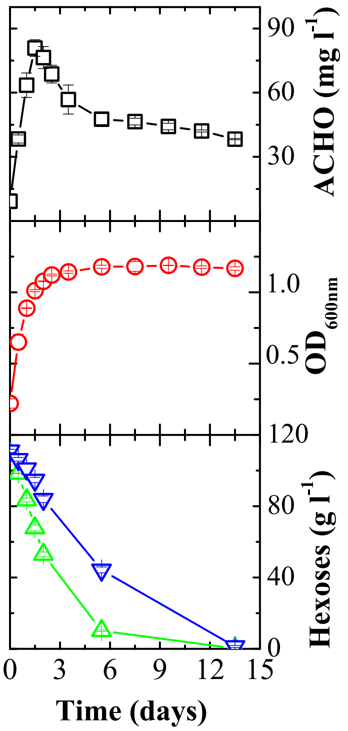

Figure 3. *Formation of acetaldehyde (ACHO in mg/l) during alcoholic fermentation by Saccharomyces cerevisiae.* □*, acetaldehyde;* ○*, growth,* △*, glucose,* ▽*, fructose*

Table 2. *Percent degradation of* SO_2 *binding compounds by 12 strains of wine lactic acid bacteria during malolactic fermentation in Riesling (average values represented)*

SO_2 Binding Compounds	% degradation
Galacturonic Acid	0
Alpha-Ketoglutarate	73
Pyruvate	87
Acetoin	20
Acetaldehyde	94

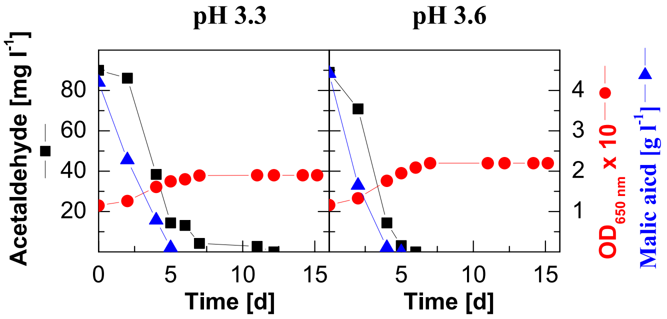

Figure 4. *Degradation of malic acid (g/l) and acetaldehyde (mg/l) during malolactic fermentation by Oenococcus oeni at pH 3.3 and 3.6 in Chardonnay.* □*, acetaldehyde;* ○*, growth,* △*, malic acid*

Degradation. In addition to the partial re-utilization of acetaldehyde by yeast in the second half of the alcoholic fermentation **(Figure 3)**, acetaldehyde is also degraded by lactic acid bacteria.

During malolactic fermentation, acetaldehyde is typically degraded simultaneously with malic acid or a little later (**Figure 4**). If a complete degradation of acetaldehyde is desired, wines should not be stabilized until five days after malic acid depletion. Malolactic fermentation also leads to the substantial reduction of pyruvic acid, and partial reduction of α-ketoglutaric acid. Hence, malolactic fermentation can make a significant contribution towards achieving lower bound and total SO_2 levels.

To some degree, acetaldehyde will also be used up by chemical reactions, e.g., the polymerization of phenolics in the stabilization of red wines.

Major factors influencing acetaldehyde residues in wines. Both biological and chemical factors are important in determining the level of acetaldehyde in wines.

Biological factors. Acetaldehyde residues will be higher, if (1) the initial formation is increased and/or (2) the re-utilization is reduced. A factor that affects initial formation

Residual Sugars and SO_2

Wines with residual sugar are popular in cool climates to achieve proper balance. A cheap, rapid method for wines with sufficient ethanol is to stop alcoholic fermentation with SO_2 before depletion of sugars. However, adding SO_2 to an active fermentation will invariably lead to yeast acetaldehyde formation and higher final bound and total SO_2 levels. To avoid or reduce this increase, rapid cooling with a heat exchanger is superior. However, if sufficient cooling energy is not available and back-sweetening with sucrose or sweet reserve is not desired, yeast flocculation and precipitation can be accelerated by addition of bentonite (20-50 g/hl), which can double as a partial or complete heat stability treatment.

The 2006 vintage in Germany and Eastern France: A Case Study

This vintage was characterized by a mild spring and hot July with early ripening. A cool August followed, and by mid-September grapes had high sugar but also unusually high acidity levels when strong rains commenced (would we know). This led to a surprisingly rapid onset of sour rot which resulted in frantic harvesting and hasty grape processing. In the final wines, bound SO_2 levels were sky-high, and consequently Germany and Alsace had to request a 40 mg/l increase in total SO_2 maxima for the 2006 vintage, which was granted (EC 423/2008 Article 23(4) and Annex XV) as it had been for the 2000 vintage. One reason for the elevated bound SO_2 levels were high pyruvate concentrations from grapes with a high percentage of rot. However, a good part of the problem was man-made. To control the microflora, winemakers made large SO_2 additions, which led to unusually high acetaldehyde levels from yeast production. The results were significant levels of bound SO_2 (due to the pyruvate and acetaldehyde) as well as a high incidence of musty-moldy aromas from rotten grapes.

In order to avoid going from the frying-pan into the fire in unfavorable harvest conditions, a careful winemaking approach should be delineated early on. A thorough selection in the vineyard followed by individualized grape sorting can prevent most problems for the premium wines. Instead of massive SO_2 additions, machine harvested and sorted out fruit can be subjected to stronger juice clarification (<30 NTU), possibly with the application of must bentonite and active carbon, for musts that are likely to develop musty-moldy aromas. The extent of clarification should be monitored and documented by turbidimetry. Over-clarified juices should never be enriched with the dregs, which will just re-create a problem. Instead, yeast hulls or microcrystalline cellulose may be used for yeast support. Flotation has proven to be an efficient, time and cooling-energy saving clarification method in such cases, too. Several New York State wineries have been using it successfully in recent years. Thermovinification is an interesting alternative in red wine production in difficult years (high microbial load, unripe aromas), as well.

is the yeast strain. *Saccharomyces cerevisiae* strains that predominate in alcoholic fermentation, regardless of whether inoculated or not, tend to produce more acetaldehyde than non-*Saccharomyces cerevisiae* yeast, with the exception of *Schizosaccharomyces pombe*, which we found to excrete large amounts. Among *Saccharomyces cerevisiae* strains, the acetaldehyde excretion and re-utilization was fairly uniform.

The most important factor for the biological formation of acetaldehyde is, however, the addition of SO_2 to musts **(Figure 5)**. Yeast produce more acetaldehyde in response to SO_2 additions. Across several studies with more than 20 yeast strains, we found that the addition of 1 mg/l of SO_2 typically increases the final acetaldehyde residue after alcoholic fermentation by 0.2-0.5 mg/l. Hence, a must SO_2 addition of 50 mg/l will increase final acetaldehyde levels by 10-25 mg/l and, thus, increase bound SO_2 levels by 15-37 mg/l.

With regards to re-utilization of acetaldehyde by yeast, we found that all factors that maintain a large number of viable yeast allow a better re-utilization of acetaldehyde.

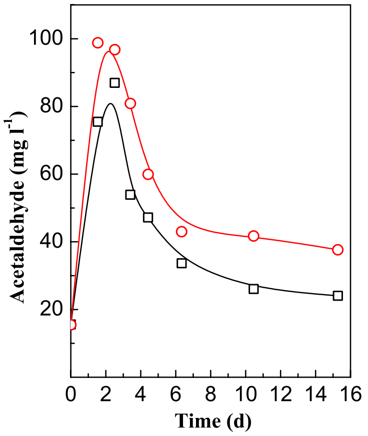

Figure 5. *Acetaldehyde formation and re-utilization during alcoholic fermentation by Saccharomyces cerevisiae.* ○ *shows the acetaldehyde values when 50 mg/l of SO_2 were added to the must before alcoholic fermentation.* □ *shows acetaldehyde values during alcoholic fermentation without SO_2 addition.*

Accordingly, adding yeast nutrients and maintaining moderate temperatures (20°C) led to reduced acetaldehyde residues, while maintaining cool temperatures (12°C) throughout fermentations and not adding any nutrients led to larger residues. Early racking of yeast lees or reducing the temperature reduces acetaldehyde re-utilization and leads to higher bound SO_2 levels.

With regards to the potential of malolactic fermentation to reduce acetaldehyde (and other SO_2 binders), recent results from our group show that malolactic bacteria are inhibited by bound SO_2 even in the absence of any free SO_2. In summary, large additions of SO_2 before alcoholic fermentation will result in large bound SO_2 levels, which in addition may prevent any further reduction of SO_2 binders, even by malolactic fermentation.

Chemical factors. Over the last two years, we have worked with ten New York State wineries spanning different grape growing and wine producing regions (Finger Lakes, Lake Erie, Niagara Escarpment, Hudson Valley, and Long Island) to document the formation and degradation of acetaldehyde. Winemakers sampled their

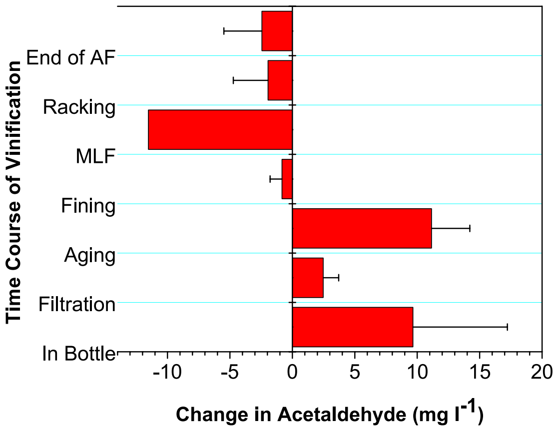

Figure 6. Data from ten New York wineries showing relative changes in acetaldehyde concentrations (mg/l) between different winemaking operations and steps.

wines after important winemaking steps (i.e., after racking, transferring, bottling, etc.), froze their samples, and sent them back to our laboratory for analysis. This has allowed us to identify major critical control points for acetaldehyde management. **Figure 6** shows average values obtained from this study. As long as yeast lees were present, acetaldehyde values were stable or decreased slightly. Malolactic fermentation significantly reduced the levels of acetaldehyde. All subsequent racking, filtration, ageing and bottling operations increased acetaldehyde values again, with the increase related to the degree of success in preventing unwanted aeration of wines.

On average, acetaldehyde concentrations in New York State wines were comparable with those determined in another large study we carried out with wines from Ontario (Canada) several years ago. White wines had average values around 40 mg/l and reds had approximately half as much (**Figure 7**). The reason for this difference is mainly malolactic fermentation!

Fermentation locks and tanks

After alcoholic fermentation and racking off yeast lees, wines are susceptible to oxidation and, hence, formation of acetaldehyde. Topping-up stainless steel tanks and using inert gases is optimal for post-alcoholic fermentation storage. Plastic (olypropylene and polyethylene) tanks are oxygen permeable to some degree as well as variable height tanks (along the inflatable rubber gasket). Some of the fermentation locks used by winemakers in New York may not be suitable for post-alcoholic fermentation storage either, e.g., the white polypropylene locks with marbles. Any lock which does not create a tight seal (e.g., spring loaded rubber seal with over-pressure protection) or barrier (e.g., traditional liquid filled fermentation lock) will allow air ingress.

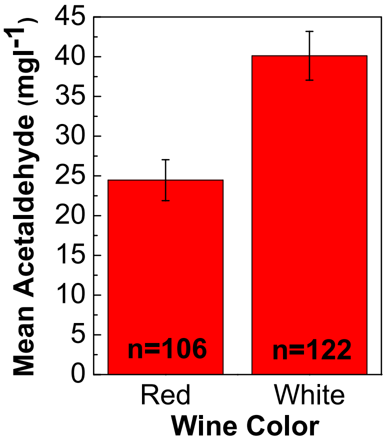

Figure 7. Average acetaldehyde concentrations (mg/l) in New York wines.

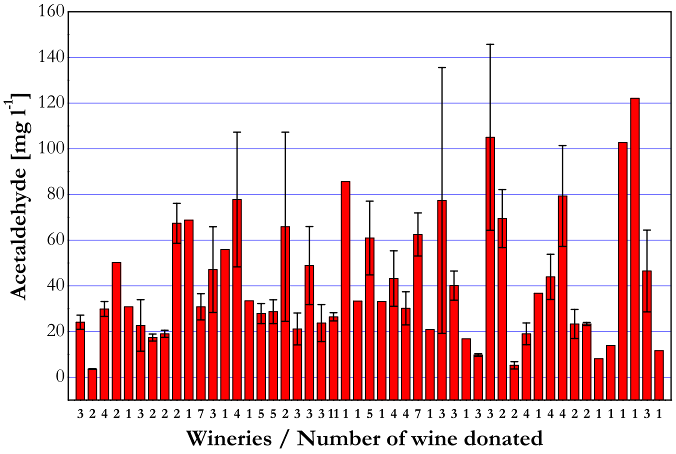

Figure 8. *Average acetaldehyde concentrations (mg/l) in wines provided by individual wineries. The number on the x-axis display the number of wines analyzed per winery.*

After wine color, the next important factor in the concentration of acetaldehyde and bound SO_2 is the winery (**Figure 8**). Some wineries produce wines with consistently low levels of acetaldehyde regardless of wine color. In some instances, we find wineries with occasional outliers. In cases where most wines present acetaldehyde levels that are significantly above the averages, there is room for optimizing vinification decisions, reducing post-alcoholic fermentation aeration, and hence reducing total SO_2 levels. A white wine with an acetaldehyde residue of 100 mg/l would require almost 90 mg/l more SO_2 than a comparable wine from an average winery.

In the future, new methods for the reduction of SO_2 binding compounds may be available. Bordeaux researchers have patented a method that would allow the reduction of SO_2 binding carbonyls in wines using an insoluble resin. The method was especially targeted at Sauternes, which tend to suffer from high SO_2 binder concentrations. So far, the method has not been scientifically scrutinized and is not permitted.

Distillates

The formation of acetaldehyde from yeast metabolism and chemical oxidation also applies to vinifications of other substrates, including the production of ciders or beers ("base wines") for distillations. Distillates from high acetaldehyde beers require larger cuts, hence reducing overall yields. Limitation of SO_2 utilization, longer yeast lees contact and—where applicable—malolactic fermentation, can reduce acetaldehyde levels.

Figure 9 shows an example of the changes in acetaldehyde values during vinifications. The initial biological formation by yeast is followed by a partial re-uptake. The second increase is due to involuntary chemical oxidation of ethanol. At 27 days, the wine was inoculated with bacteria to induce malolactic fermentation, which essentially removed all of the acetaldehyde.

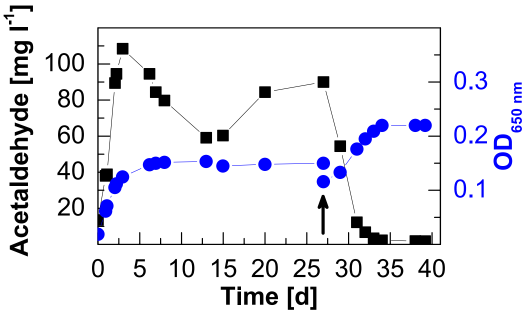

Figure 9. *Typical course of acetaldehyde levels (mg/l) during the vinification of a wine with malolactic fermentation. The blue line marks the turbidity and shows the growth of yeast and bacteria. Malolactic fermentation was induced after 24 days (indicated by arrow).*

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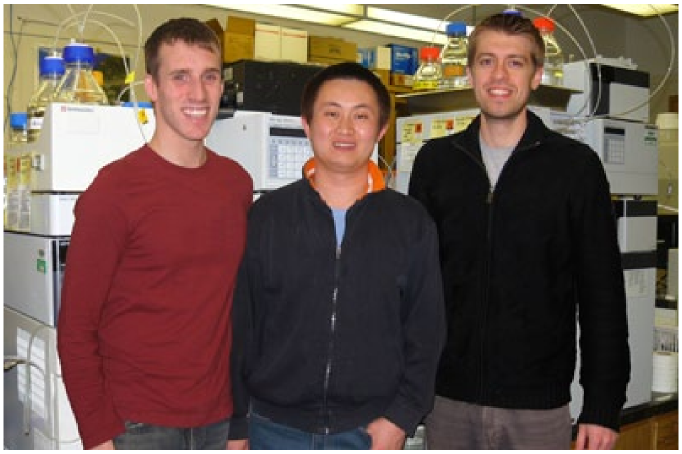

Students in Mira de Orduña laboratory, Left to right: Charles Frohman, Erhu Li, and Nick Jackowetz

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