Role of Oxygen in Winemaking

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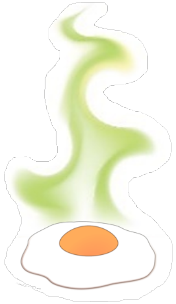


Oregon Wine Symposium 2022

Today's talk

The fate of oxygen, and its effects on wine, depend on timing

- Prefermentation
- During fermentation, or while in lees contact
- Post-fermentation, no lees contact

I'll try my best to hit the highlights of what happens at each of these stages . . .

But in a 30 min talk, there's bound to be some things left out

Quick terminology review – "Air Saturation"

The solubility of O_2 in wine and juice is limited

Solubility increases with decreasing temperature

- **10 mg/L at 15 °C**
- **8 mg/L at 25 °C**

Once this concentration is reached in the presence of air, the wine is "saturated" with oxygen

Outcome: unless you have an O_2 tank, aeration devices in the winery or the dining table are changing kinetics (the rate), not thermodynamics (the solubility)

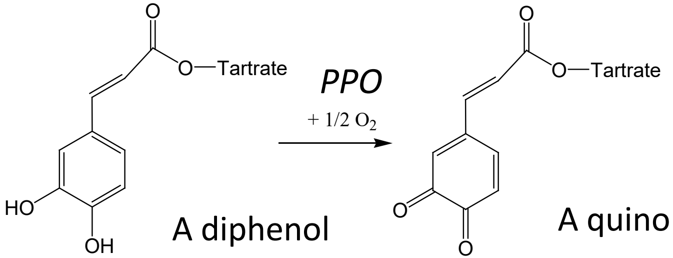
Fate of oxygen depends on "when" the exposure occurs

	Fresh must, no SO_2	Actively fermenting wine	Filtered wine w/ SO_2
Primary O_2 consuming reaction			
Typical O_2 consumption rate in air saturated system			
Potential danger of O_2 to wine quality			

Oxygen and wine: three different eras

	Fresh must, no SO_2	Actively fermenting wine	Filtered wine w/ SO_2
Primary O_2 consuming reaction	Polyphenol oxidase (PPO) from grape Laccase from botrytis		
Typical O_2 consumption rate in air saturated system	1-3 mg/L per min		
Potential danger of O_2 to wine quality	Low-medium		

Most obvious effect of oxygen → enzymatic browning (grapes, bananas, apples, potatoes, etc)



A diphenol
Caftaric acid

A quinone

Enzyme = Polyphenol oxidases (PPO)
Grapes and other fruits: *tyrosinases*
Molds: *laccase*

- Follows mechanical damage
- Rapidly oxidizes diphenols to "quinones"
- Unsulfited, freshly crushed grapes will consume 1-3 mg/L O_2 per min

- No peroxide formation

Enzymatic browning (PPO) results in, um, browning

Original juice

Oxidized juice

Catechin and related phenolics

↓ *PPO, O_2*

Quinones

↓

Brown reaction products

What will slow enzymatic browning?

"Antioxidants"

- Free SO2 (inactivates PPO; less effective on laccase)
- Ascorbic acid (reacts with quinone, reforms phenol)
- Glutathione (from grapes; forms "GRP")

Other treatments

- Heating (T > 55 °C) will denature enzymes
- Cooling slows enzymatic activity
- Fining agents (e.g. bentonite, charcoal) denature enzyme and remove brown products

Terminology time. Feel free to argue

"Hyperreductive"	"Reductive"	"Oxidative"	"Hyperoxidative"
SO_2 addition Minimize air contact Press under inert gas	SO_2 addition	No SO_2	No SO_2, add air Removal of oxidized products (e.g. by flotation) before fermentation

But, pre-fermentation reductive vs. oxidative practices often have small effects on finished wine

Hyperoxidized Chardonnay must

Partway through fermentation

Hyperoxidized must after fermentation

The well-established effects

Hyperoxidation decreases 'browning potential' in white wines

More oxygen (and low SO2) = more aerobic spoilage growth

Everything else . . . Minor effects, and often contradictory

Aroma, Taste, Mouthfeel? Small effects, when present.

Why? Some speculation

- Fermentation is a strongly 'reducing' environment (opposite of oxidizing). Many pre-fermentation reactions will be reversed
- Many wine flavor compounds, including oxygen-sensitive compounds, are released from precursors

Photos: R. Mira de Orduna

A case study – 3-mercaptohexanol (3-MH) “citrus” odorant in wine, not found in grapes

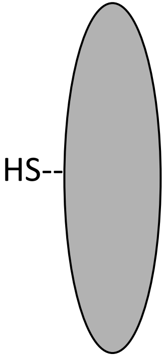
3-MH = ‘varietal thiol’, <u>easily oxidized</u>, key odorant in Sauv blanc and other whites/roses

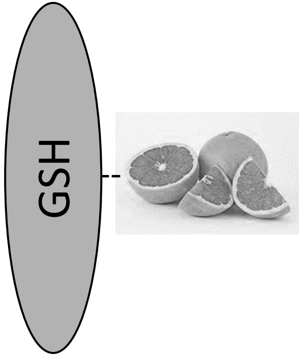
Several 3-MH precursors have been identified, including

“Grassy” **aldehydes** formed after crushing grapes

+

Glutathione (GSH) from grapes



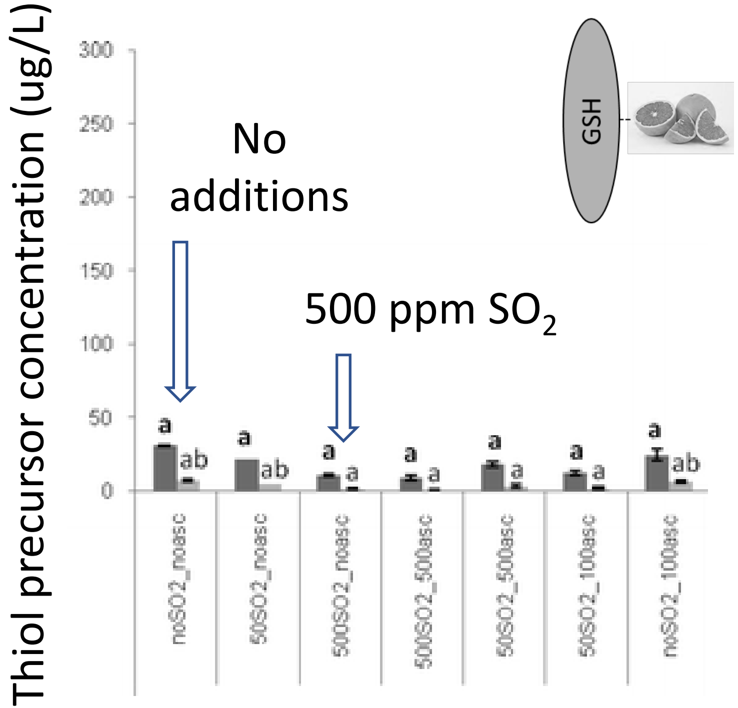


GSH – 3MH precursor (odorless)

fermentation

3-MH, “grapefruit”

3-MH, GSH and other thiols are easily oxidized. What's the effect of pre-fermentation anti-oxidants?



No transport

Hyperoxidation can decrease wine thiols slightly (Coetzee, et al 2015)

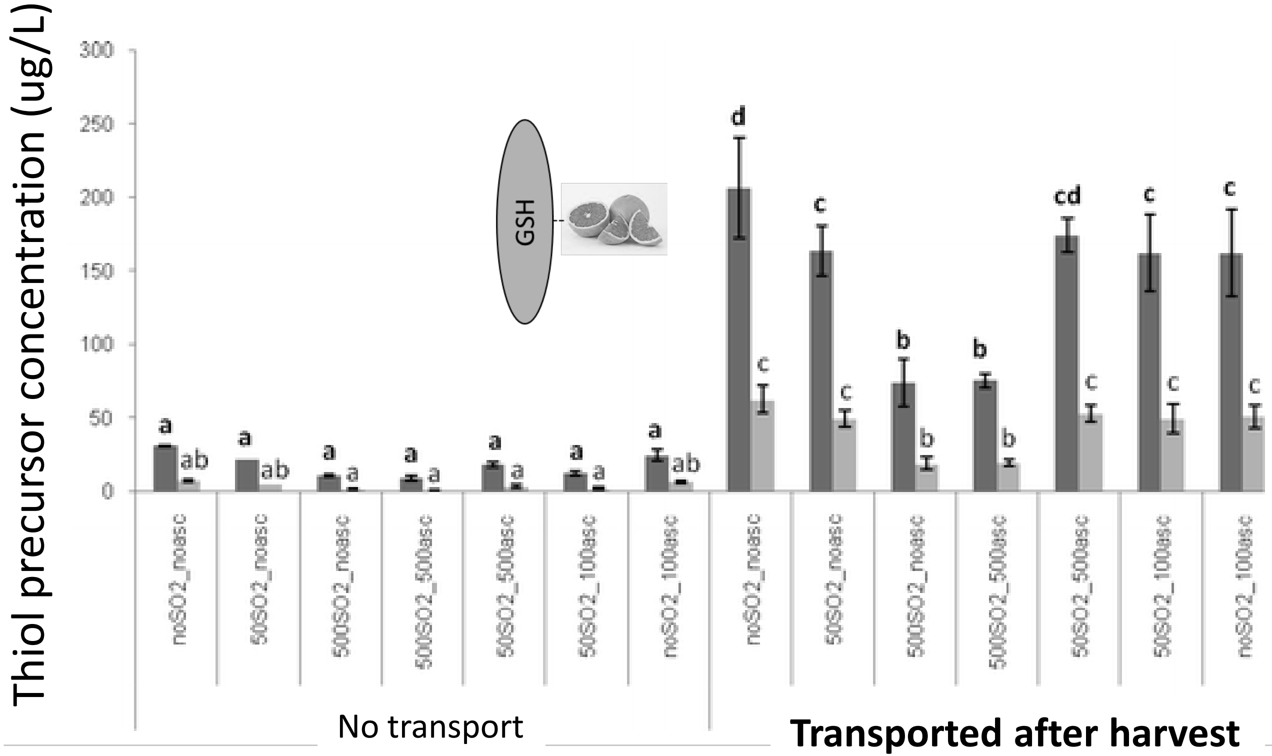
Why? Probably loss of glutathione, less precursors

But, adding SO_2 can have minimal or inhibitory effect on precursors!
(see data at left)

Why? SO2 may inhibit formation of grassy aldehydes (Capone and Jeffery, 2011)

Capone and Jeffery, JAFC 2011

But, oxidation matters much less than just letting the fruit sit around before fermenting



Longer time after harvesting =
More time for precursors to form!

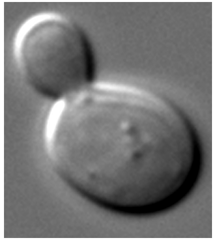
Oxidation (and antioxidants) =
Not so important.
High SO2 may inhibit precursor formation

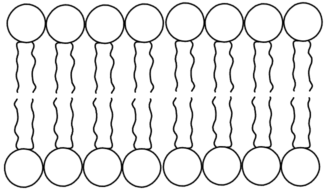
Capone and Jeffery, JAFC 2011

Oxygen and wine: fermentation

	Fresh must, no SO_2	Actively fermenting wine	Filtered wine w/ SO_2
Primary O_2 consuming reaction	Polyphenol oxidase (PPO) from grape Laccase from botrytis	Yeast enzymatic activity	
Typical O_2 consumption rate in air saturated system	1-3 mg/L per min	1-3 mg/L per min	
Potential danger of O_2 to wine quality	Low-medium	Medium	

Yeast have lots of uses for oxygen, but a big one is unsaturated fatty acid production





Yeast cell membranes contain fatty acids (as phospholipids)

Yeast adjust their fatty acid and sterol composition in response to environment

Colder temps? Less saturated, more unsaturated fatty acid, please

Saturated fatty acids
Higher melting point

O_2

Enzymatic transformation

Unsaturated fatty acids
Lower melting point

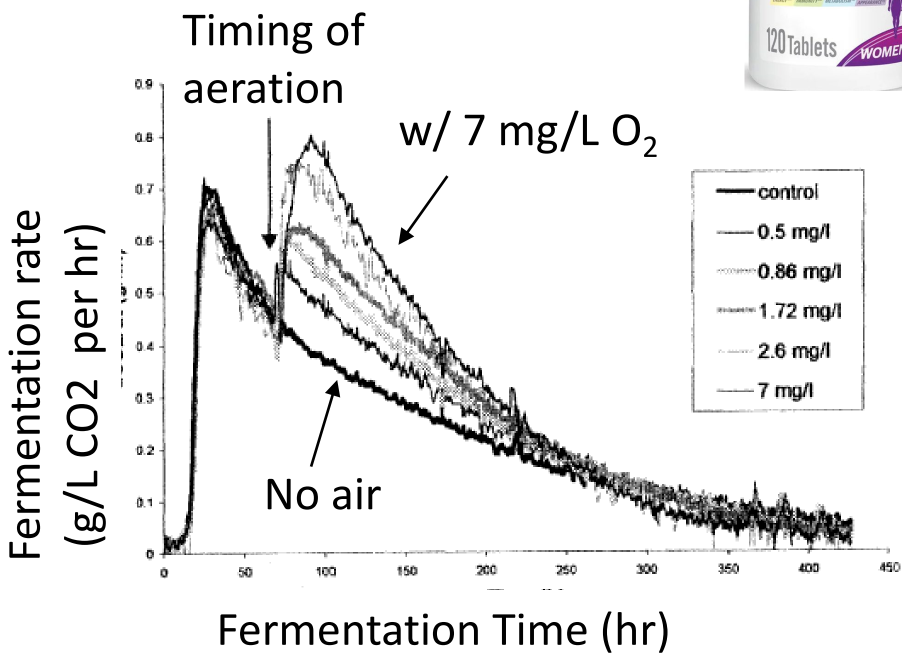
Oxygen as a **nutrient**

Low oxygen limits unsaturated fatty acid and sterol production

Limits yeast growth and fermentation rate

The effect is more acute with . . .
- Cool fermentations
- Closed fermenters
- Low insoluble solids (clarified musts)

In other words, greater issue with whites!



From Julien, et al AJEV 2000

But, there are other effects of oxygen addition during fermentation (of course)

Many microorganisms (non-Sacch. yeasts, Acetobacter, etc) grow faster with air
- Often will produce higher V.A. under aerated conditions (but, not *S. cerevisiae*)

O_2 affects amino and fatty acid production, and thus odorants
- Decreased acetate esters (banana-cherry-Beaujolais)
- Decreased straight chain ethyl esters ("fruit salad")
- Increased higher alcohols ("solvent, ethereal")
- Increased branched-chain (BC) fatty acids

→ Will form BC ethyl esters during storage ("dark-red fruit")

Often undesired in "fruit-forward" whites or roses

Biomass (lees), glycerol, succinic increase

See Understanding Wine Chemistry for references

Oxygen and wine: post-fermentation

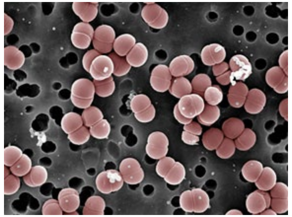
	Fresh must, no SO_2	Actively fermenting wine	Filtered wine w/ SO_2
Primary O_2 consuming reaction	Polyphenol oxidase (PPO) from grape Laccase from botrytis	Yeast enzymatic activity	Non-enzymatic Metals + Phenolics + SO_2
Typical O_2 consumption rate in air saturated system	1-3 mg/L per min	1-3 mg/L per min	0.1-1.0 mg/L per hour
Potential danger of O_2 to wine quality	Low-medium	Medium	High

Note: lees will continue to consume O_2 . Thus emphasis on "filtered"

Review: The major effects of O_2 on finished wine

1) Microbial growth, due to presence of O_2 and/or loss of SO_2

- Take your pick . . . Film yeasts, *Acetobacter*, LAB, Brettanomyces
- Off-flavor and haze formation
- Possible regulatory issues



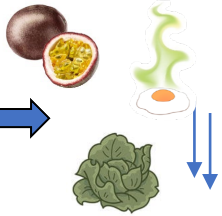
2) Chemical changes ("oxidation")

- Next slide!

What happens to oxygen in a wine with no SO_2? Two pathways

Iron-phenolic, pathway 1
(MAJOR)

O_2 + [Fe(II)] + o-diphenol → Quinone →



Loss of sulfhydryls (e.g. 3-MH, H_2S), tannin reactions

O_2 + [Fe(II)] + o-diphenol → **Hydrogen Peroxide (H_2O_2)** →

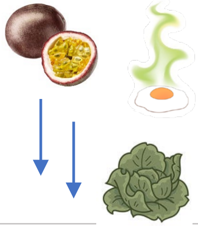
Aldehyde formation; browning, off-aromas

"wine pigment"

Metal-sulfhydryl, pathway 2
(MINOR)

O_2 + [Cu(I)] or [Fe(II)] + Sulfhydryl →

Loss of sulfhydryls (thiols and H_2S)



Kreitman, Elias, Jeffery, and Sacks; *Crit Rev. Food Sci Nutr.* **2019**

Chemical oxidation isn't always bad, an example "Micro-ox" and wine phenolics

Micro-ox reports from literature typically show

- Modest increases in **wine pigment** (also called **polymeric pigment**)
- Modest decreases in astringency

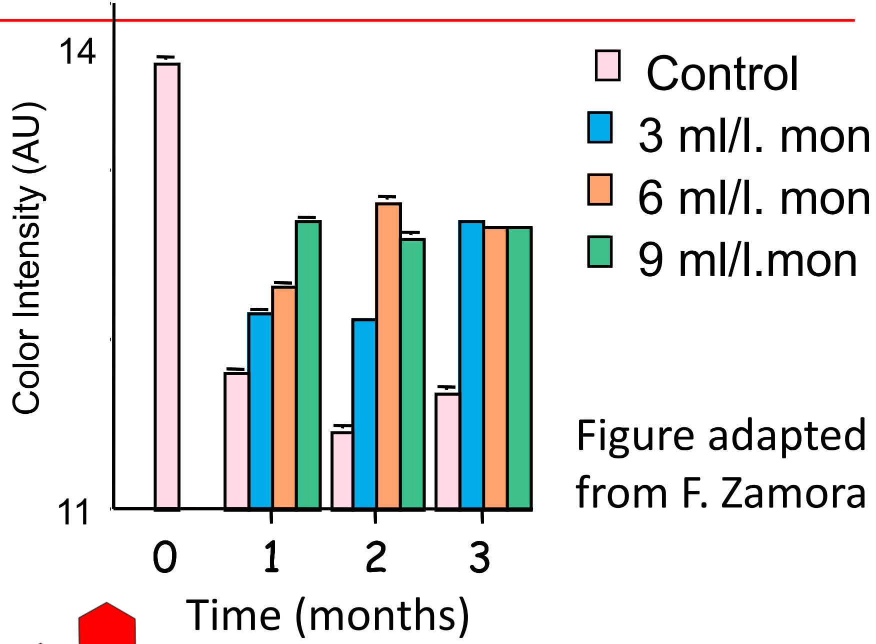
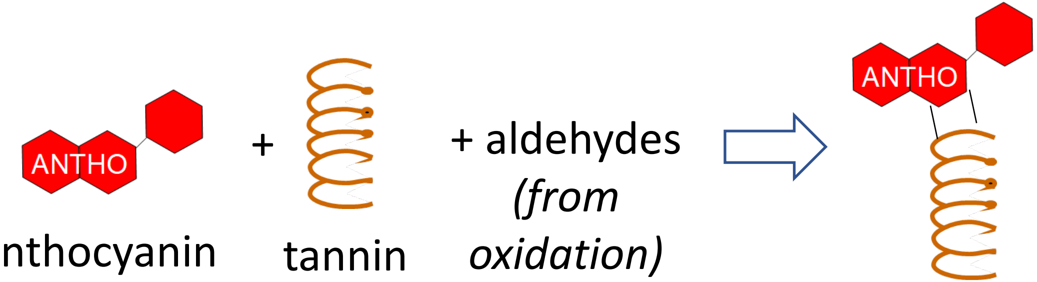


Figure adapted from F. Zamora



Wine pigment
Less affected by SO_2 and pH
Less prone to hydrolysis

Review: Sulfur dioxide (SO_2) is used to counteract the effects of O_2 on wine

"Free SO_2" = molecular + bisulfite

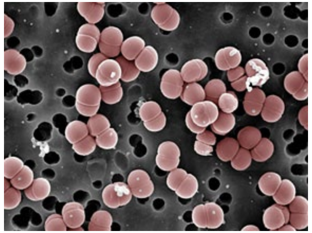
molecular SO_2		***Bisulfite***
$SO_2 + H_2O$	$\rightleftharpoons$	$H^+ + \mathbf{HSO_3^-}$

Bisulfite is the main contributor to free SO2 at wine pH, and the major antioxidant form

Review: Sulfur dioxide (SO_2) is used to counteract the effects of O_2 on wine

Anti-microbial

- Typically, 0.5-1.0 mg/L **molecular** SO_2 recommended to prevent spoilage



Anti-oxidant: reacts with products of oxidation

- At **<10 mg/L free SO_2** oxidized aromas are often evident
- Typically, 20-30 mg/L free SO_2 recommended at bottling
- Hardest function to replace

Review: Free SO_2 may form covalent bonds with binders to form "Bound SO_2"

"Free SO_2" in solution

molecular SO_2	**Bisulfite** *(major species)*

$$SO_2 + H_2O \rightleftharpoons H^+ + \mathbf{HSO_3^-}$$

$\updownarrows$ *+ binder*

$$\text{Binder-}HSO_3^-{}_{(aq)}$$

"Bound SO_2"

Bound SO_2 has less antimicrobial and antioxidant 'activity'

Bound SO_2 forms are in equilibrium with Free SO_2

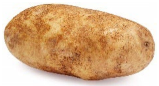
As free SO_2 is consumed, the "reservoir" of bound SO_2 will re-equilibrate, partially replenish free SO_2

Add free SO_2 to a wine, a portion is “bound”
A partial list of binders

Weaker binder

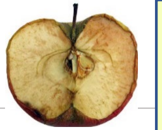
Glucose
(sweet wines)

Anthocyanins
(red-purple color)



Methional
(“potato” odor)

Stronger binder



Acetaldehyde
(“old apple” odor)

Free SO_2

Glucose — Bound SO_2

Bound anthocyanins (bleached) — Bound SO_2

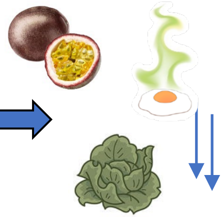
Bound methional (odorless) — Bound SO_2

Bound acetaldehyde (odorless) — Bound SO_2

Remember this? The two oxidation pathways?

Iron-phenolic, pathway 1
(MAJOR)

O_2 + [Fe(II)]+ o-diphenol → Quinone →



Loss of sulfhydryls, tannin reactions

→ **Hydrogen Peroxide (H_2O_2)** →

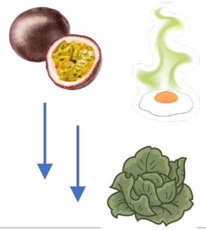
Aldehyde formation; browning, off-aromas

"polymeric pigment"

Metal-sulfhydryl, pathway 2
(MINOR)

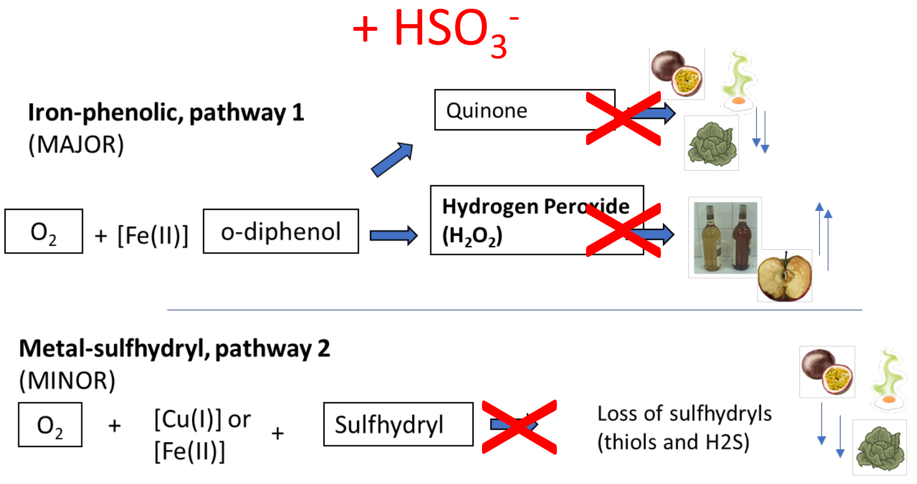
O_2 + [Cu(I)] or [Fe(II)] + Sulfhydryl →

Loss of sulfhydryls (thiols and H_2S)



Kreitman, Elias, Jeffery, and Sacks; *Crit Rev. Food Sci Nutr.* **2019**

Free SO_2 (as bisulfite, HSO_3^-) interrupts both pathways



The role of SO_2 is not to react with O_2 directly

It "reduces" (chemically speaking) oxidation products

Theoretically, up to **4 mg/L of Total SO_2 will be lost for every 1 mg/L of O_2**
2:1 Molar Ratio

So, there’s two reasons why a wine could accumulate free aldehydes following oxidation (and smell oxidized)

Option A – Oxidation results in **production of new aldehydes**

1) Iron-phenolic pathway forms hydrogen peroxide

H_2O_2

2) “Fenton Reaction” generates ·OH free radicals

3) Acetaldehyde (“bruised apple”) and other oxidation products are **formed** from alcohols

Option B – Oxidation **releases existing aldehydes**

1) Bound SO_2 pool exists in wine

Bound SO_2

2) Free SO_2 is consumed through oxidation reactions

3) Acetaldehyde (“bruised apple”) and other oxidation products are **released**

What's the evidence for forming aldehydes vs. releasing aldehydes?

Recent packaging evaluation trial

Three wines, multiple bag-in-box packages
Two storage temperatures (19 vs. 31 °C)
Free and total SO_2 measured at regular intervals, up to 400 d

Total SO_2 loss rate varied from 0.13-0.94 mg/L per day
(equivalent to 0.03-0.24 mg/L O_2 ingress per day)

Dissolved O_2 well below saturation over course of experiment

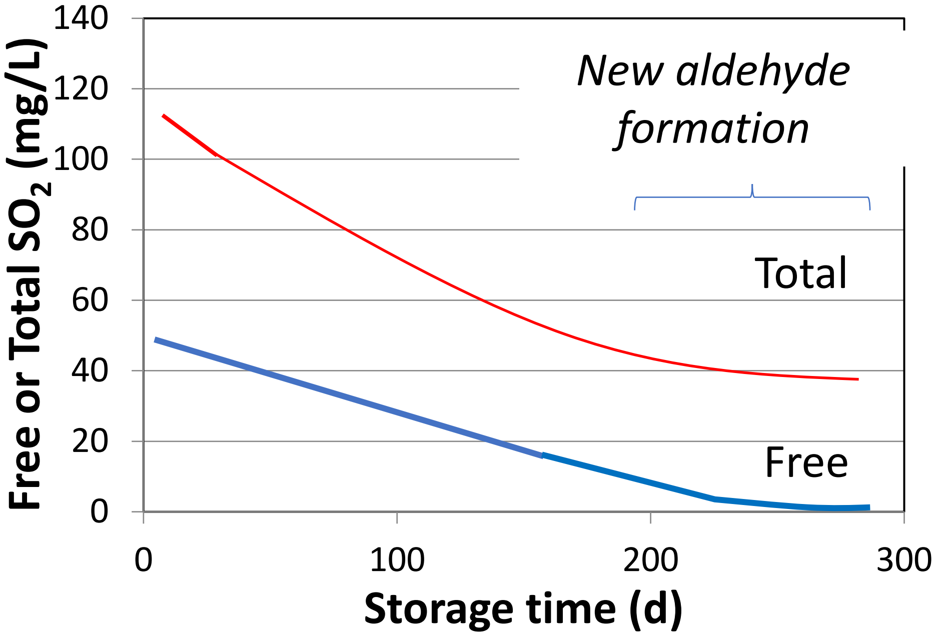
Free, Bound, and Total Sulfur Dioxide (SO_2) during Oxidation of Wines

Gavin L. Sacks,[1]* Patricia A. Howe,[2,5] Matthew Standing,[3,6] and John C. Danilewicz[4]

Sacks, Howe, Standing, and Danilewicz; *AJEV* **2020**

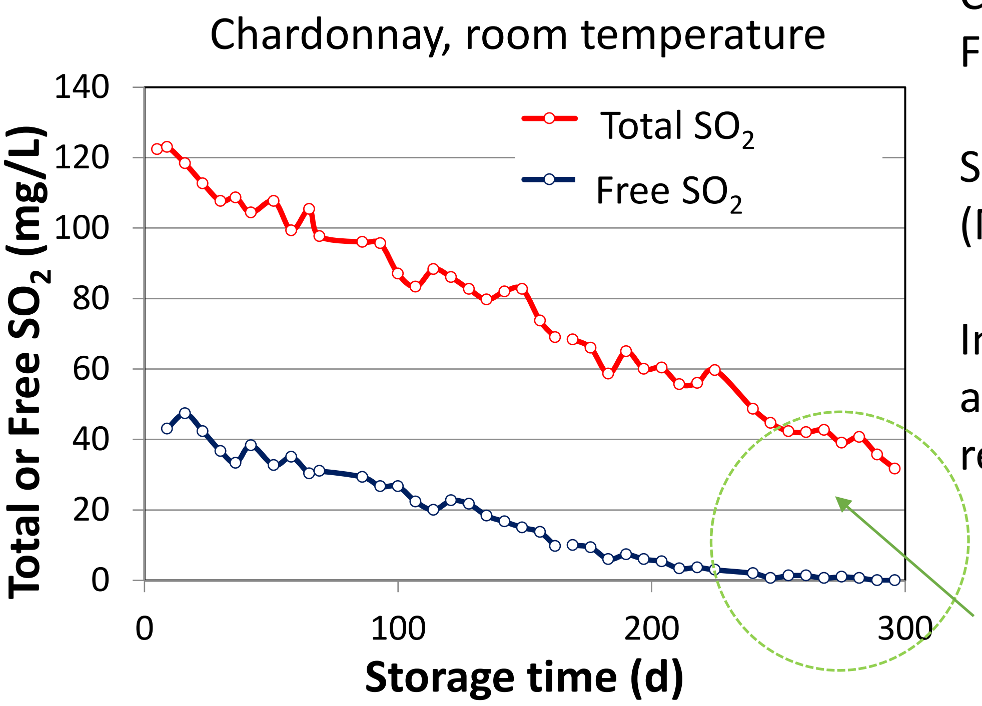
If “new” aldehyde formation is more important than aldehyde release, what should happens to SO_2

<u>Hypothetical</u> **Data for SO_2 in wine during oxidation if new aldehyde formation occurs**



- From previous slide, Fenton Reaction expected to become more important at low Free SO_2
- Rate of Total SO_2 loss should slow as Free SO_2 approaches zero.
- Why? Fenton generates acetaldehyde and other SO_2 binders, but would not change Total SO_2

Instead, we observe constant Total SO_2 loss . . . Even after Free SO_2 is no longer detectable



Constant rate of Total SO_2 loss even after Free SO_2 < 2 mg/L

Similar results observed for other wines (Merlot, Cab Sauv) in all treatments

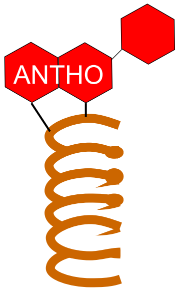
Interpretation: Bound SO_2 released from acetaldehyde is more important to H_2O_2 removal than the Fenton reaction!

Sacks, Howe, Standing, and Danilewicz; *AJEV* **2020**

Why does it matter if aldehydes are coming from release of “bound” forms? A couple thoughts.

1) If the key malodorous aldehydes (e.g. methional) are formed at the end of fermentation, can we get rid of them before sulfiting?
   - For example, through lees contact (akin to lagering in beer)?
   - Will this result in wines that smell fresh even at low levels of free SO2?

2) What’s the source of aldehydes during microoxygenation?
- Is it bound aldehydes, i.e. acetaldehyde-bisulfite?
- Is variability in micro-ox among wines a reflection of variability in this bound pool?



Today’s talk – a review

- **Prefermentation**
  Oxidized must looks bad; but unless there’s microbial spoilage, long-term effect are modest. A little O2 might increasethiol precursors!

- **During fermentation, or while in lees contact**
  Oxygen is a nutrient! But, its presence suppresses ester formation

- **Post-fermentation, no lees contact**
  Microbial growth and abiotic oxidation
  Did you know . . . Aldehydes are mostly coming from bound forms?

In a 30 min talk, I know I left things out. Time for Q&A?