This Talk Contains Sulfites

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Cornell CALS College of Agriculture and Life Sciences

Today's talk

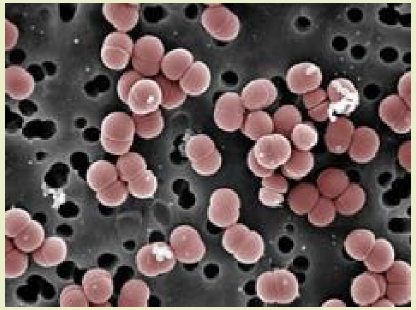
1) SO_2 – so many forms for such a short word
   - Review: Free, Bound, Total, Molecular
   - Apparent vs. Truly Free SO_2

2) SO_2 and wine oxidation
   - What happens to oxygen in wines?
   - Why is SO_2 hard to fully replace?
   - Why do wines start smell/appear oxidized?

Review: Why we measure and use sulfur dioxide (SO_2) in finished wines

Anti-microbial (Molecular SO_2)

- Typically, 0.4-0.8 mg/L **molecular** SO_2 recommended to prevent spoilage; Higher end to prevent yeast spoilage, lower end to prevent lactic or acetic spoilage
- Considerable variation among challenge studies;
  → don’t sweat 0.7 mg/L vs 0.8 mg/L!



Anti-oxidant: (Free SO_2)

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

Regulatory and QC

- **Total SO_2** best tracks oxygen exposure
- TTB limit: 350 mg/L **total** SO_2
- Sensory threshold: ~2 mg/L **molecular** SO_2

ALCOHOL AND TOBACCO TAX AND TRADE BUREAU

Review of SO_2 in wine: free vs. molecular

"Free SO_2" = molecular + bisulfite

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

Review of SO_2 in wine: free vs. molecular

"Free SO_2" = molecular + bisulfite

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

From the previous slide, we need to consider targets for molecular (antimicrobial) and free (antioxidant) separately!

Review: Proportion of molecular SO_2 increases as pH decreases

"Free SO_2" in solution

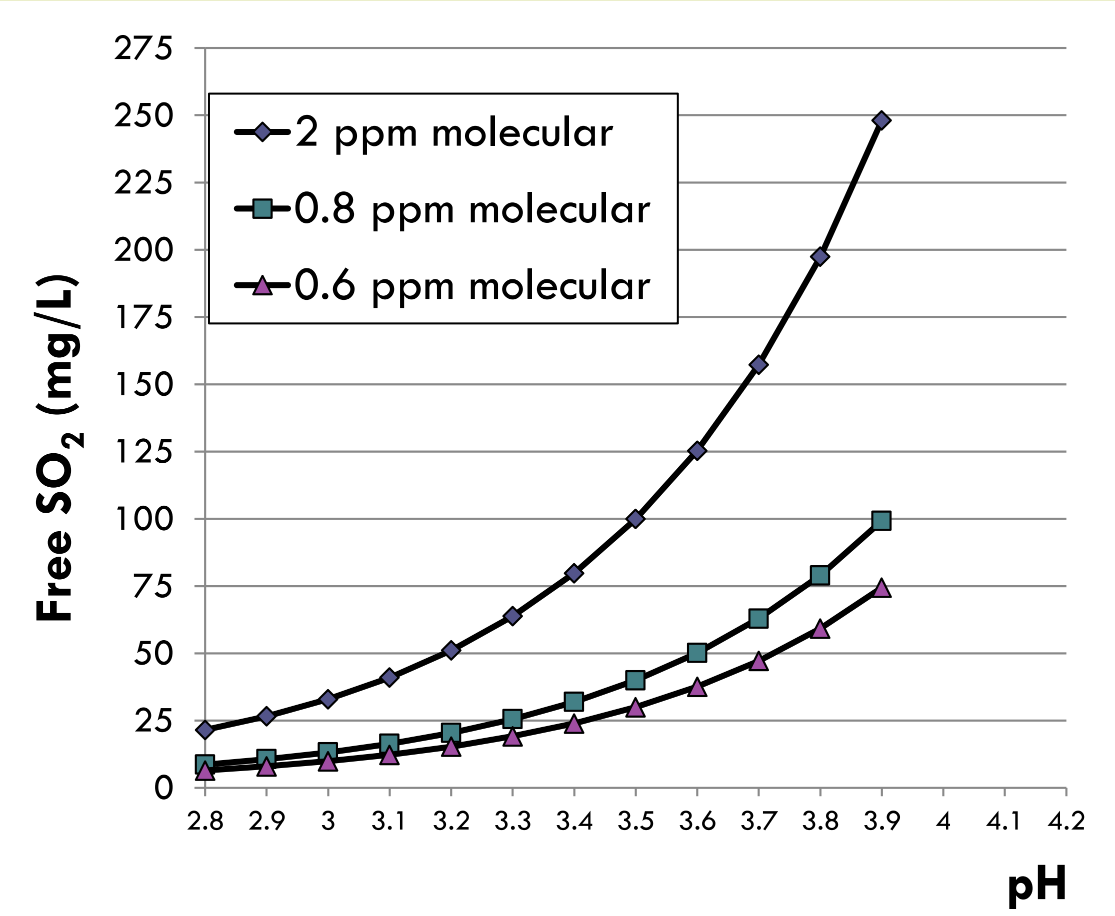
molecular SO_2 | ***Bisulfite*** *(major species)*

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

At higher pH, need more free SO_2 to achieve same molecular SO_2

At high pH (>4), achieving > 0.6 ppm molecular may require impractically high free and total SO_2

At low pH (<3.2) , hard to have sufficient free SO_2 without exceeding molecular sensory threshold (2 ppm)!



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Review: Molecular SO_2 is the volatile form of SO_2

"Free SO_2" in solution

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

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

$\updownarrow$

$SO_{2\,(gaseous)}$

Molecular SO_2 in solution will be in equilibrium with gaseous SO_2

(This is what your nose detects)
(It's also what gets distilled during A-O analyses)

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)*

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

$SO_2 \rightleftharpoons SO_{2\,(gaseous)}$

HSO_3^- + *binder* $\rightleftharpoons$ Binder-$HSO_3^-{}_{(aq)}$

"Bound SO_2"

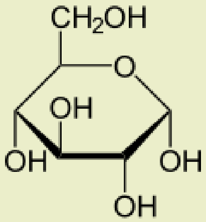
Bound SO_2 forms are in equilibrium with Free SO_2

Bound SO_2 has less antimicrobial and antioxidant 'activity'

Alkaline conditions (Ripper) and heating (A-O) favor dissolution of bound forms

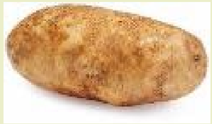
As free SO_2 is added to a wine, a portion is "bound" (partial list below)

Weaker binder



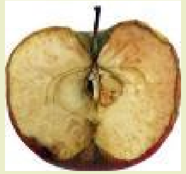
Glucose
(sweet wines)

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

Review: Total SO_2 is the sum of all previously discussed SO_2 forms

Total SO_2

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

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

SO_2 ⇅ $SO_{2\,(gaseous)}$

HSO_3^- ⇅ *+ binder* → Binder-$HSO_3^-{}_{(aq)}$

The sum of all SO_2 species (free and bound) is the Total SO_2

What's regulated in most countries

And, what best correlates with the extent of O_2 exposure (not free SO_2, more on this later)

“Enough review!”

“Tell me something I don’t know!”

(why did they fly this guy in from New York, anyway?)

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Today's talk

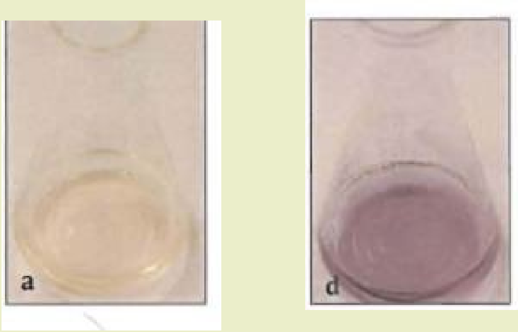
1) SO_2 – so many forms for such a short word
   - Review: Free, Bound, Total, Molecular
   - **Apparent vs. Truly Free SO_2**

2) SO_2 and wine oxidation
   - What happens to oxygen in wines?
   - Why is SO_2 hard to fully replace?
   - Why do wines start smell/appear oxidized?

Standard methods for "Free SO_2"
First steps are acidification and/or dilution

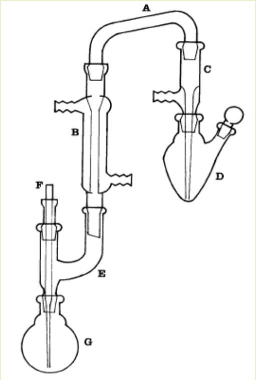
1) Acidification, followed by direct colorimetric

- Iodometric (Ripper)
  - Manual, Titrets, Autotitrator
- *p*-rosaniline (fuschin)

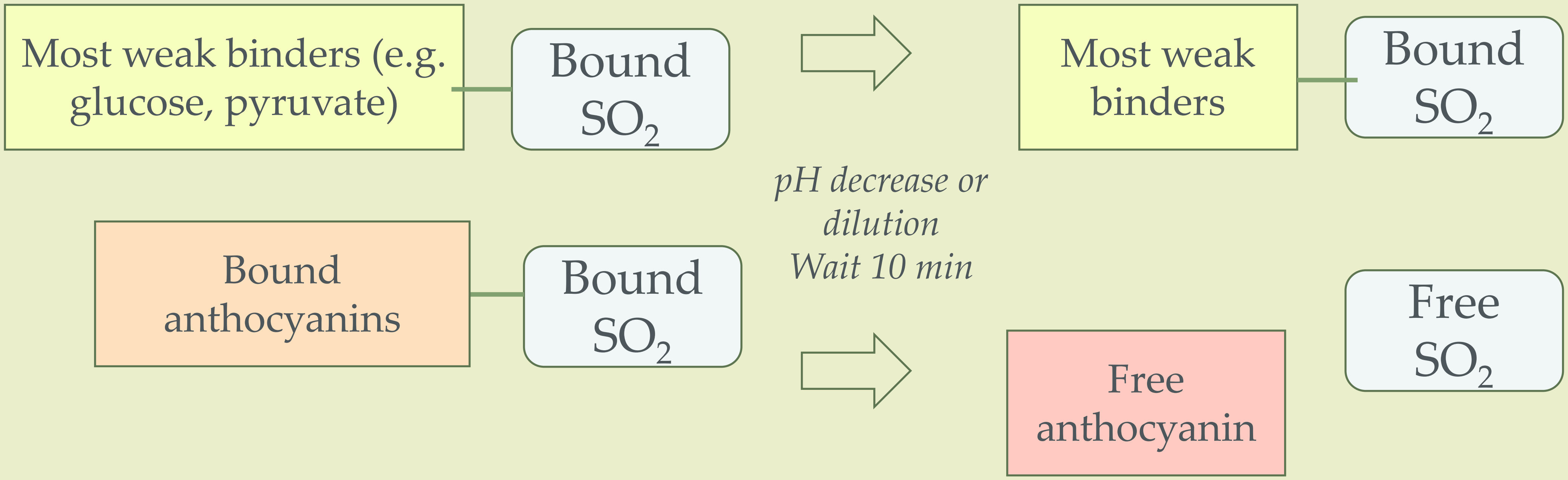


2) Acidification, SO_2 separation, measurement

- A-O
- Flow injection w/ membrane separation
- Flow injection w/ UV detection (Foss)



Acidification and dilution results in (unintentional) release of bound SO_2 from anthocyanins



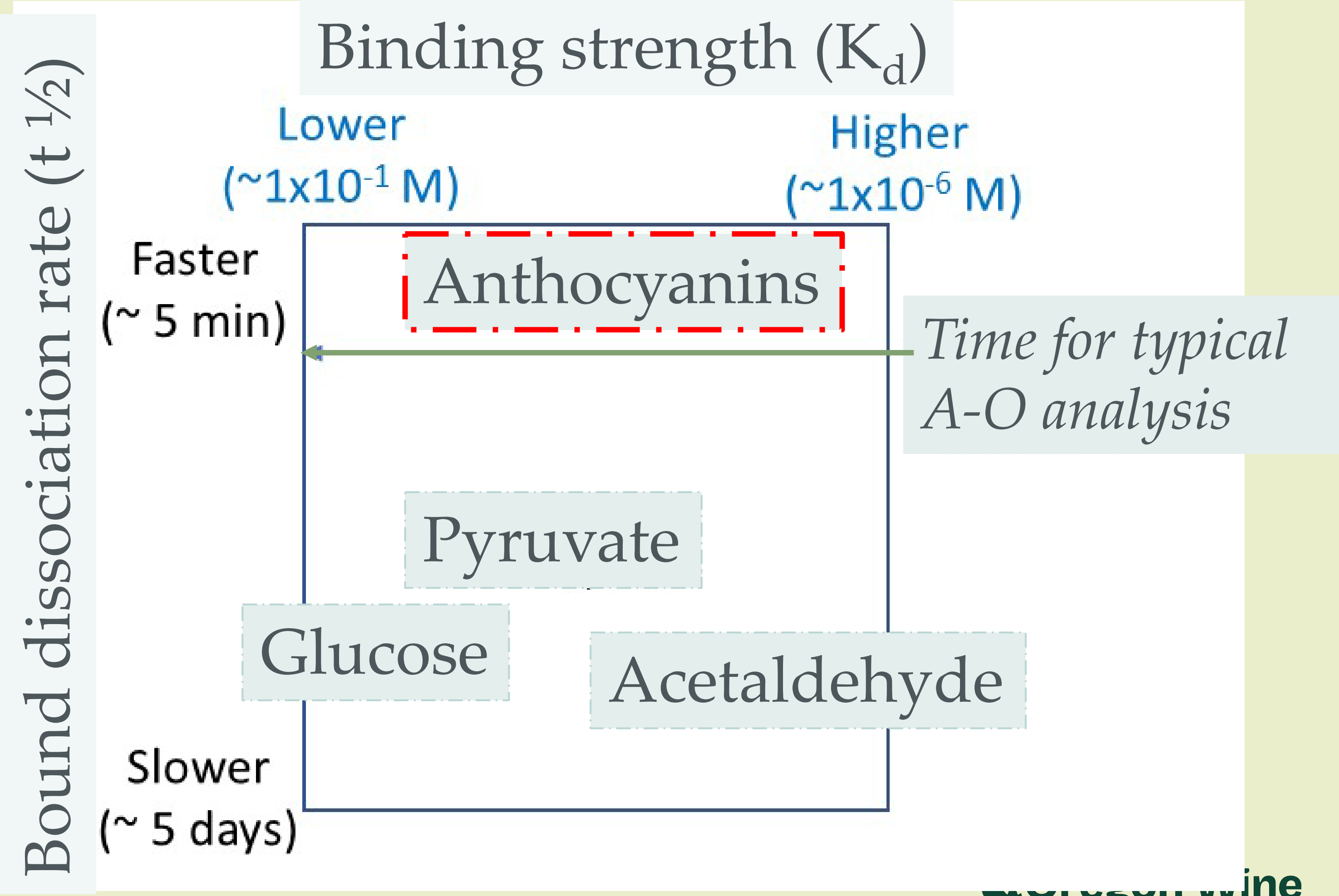
Result: fast dissociating SO_2 adducts (e.g. anthocyanins) etc… will dissociate during analysis, and will be counted as “Free SO_2”

Note that binding strength does not predict dissociation speed!

Contribution to "apparent" Free SO_2 is related to kinetics, not binding strength

Thermodynamics
- Anthocyanins are almost as strong a binder as acetaldehyde

Kinetics
- Anthocyanin-bisulfite complexes dissociate rapidly (seconds)

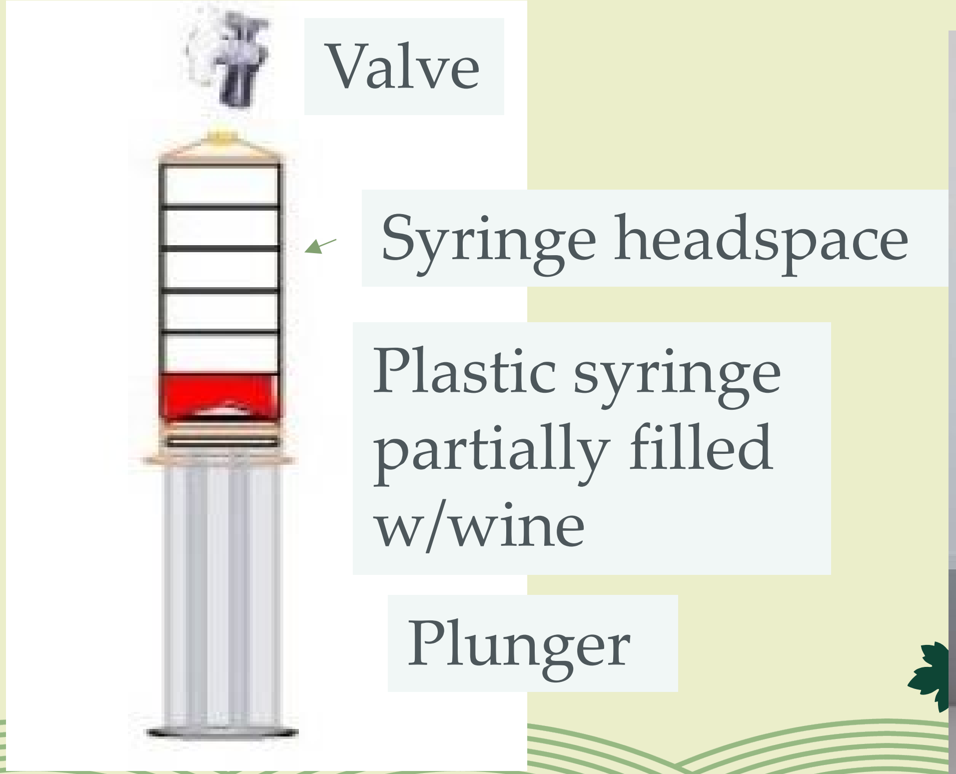


Waterhouse, Sacks & Jeffery; *Understanding Wine Chemistry* **2024**

Headspace gas detection tube (HS-GDT) approach to SO_2
"Non-perturbing" - no acidification, no dilution

- Draw liquid sample from tank, barrel, etc into plastic syringe

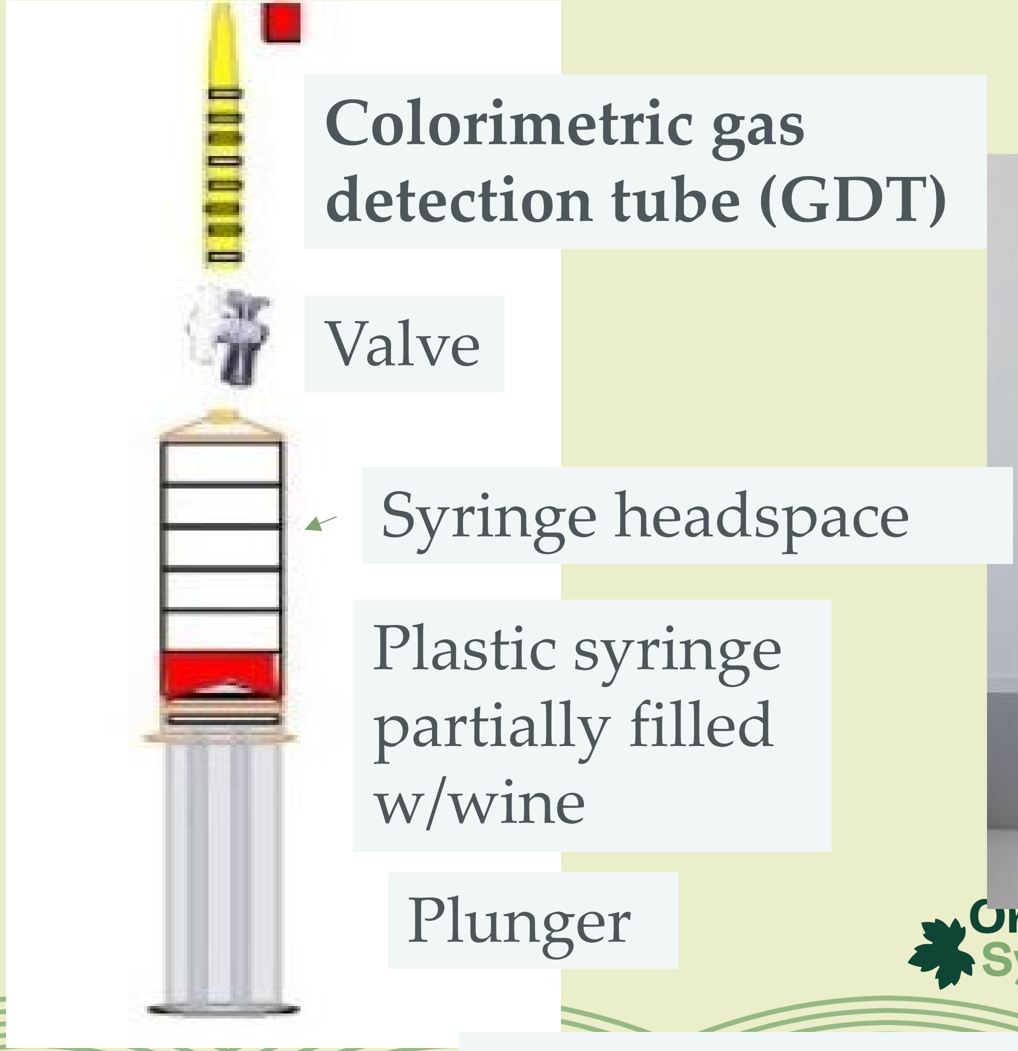
- Equilibrate ~5 min. Molecular SO_2 will enter syringe headspace.



Coelho, Howe, and Sacks, *AJEV* **2015**

Headspace gas detection tube (HS-GDT) approach to SO_2
"Non-perturbing" - no acidification, no dilution

- Draw liquid sample from tank, barrel, etc into plastic syringe
- Equilibrate ~5 min. Molecular SO_2 will enter syringe headspace.
- Expel headspace SO_2 into GDT
- Calculate Free from Molecular
- Color change on tube proportional to "True" SO_2



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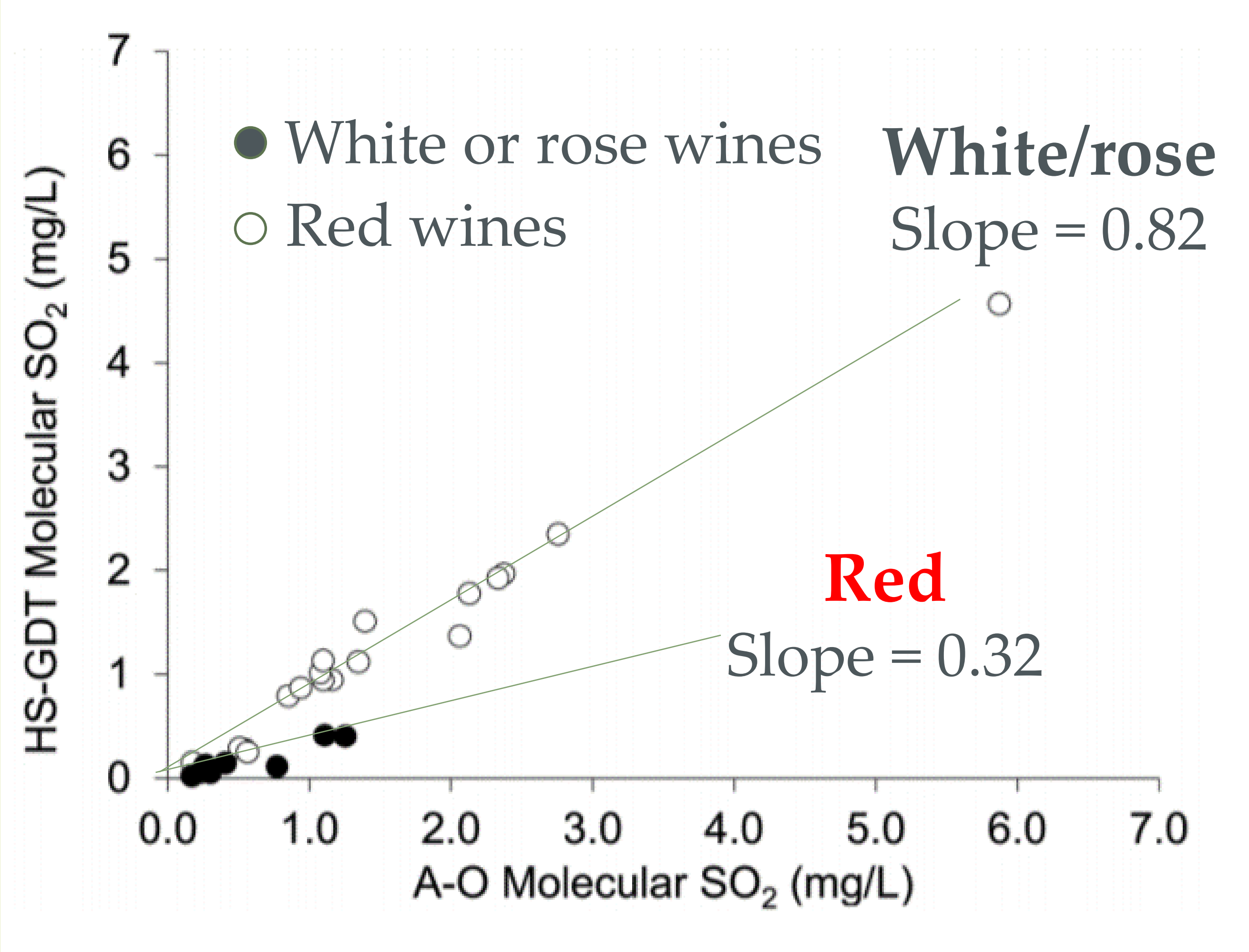
Coelho, Howe, and Sacks, *AJEV* **2015**

"Apparent" (A-O) and "True" (HS-GDT) molecular SO_2 correlate poorly in red wines



For whites and roses: agreement, slope is close to 1.0

For red wines: A-O averages ~3 fold higher values than HS-GDT

Put another way:
Most commercial reds have "true" molecular SO_2 < 0.2 mg/L

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Coelho, Howe, and Sacks, *AJEV* **2015**

When is "apparent" SO_2 by standard methods better? When are "true" methods better?

- These **standard methods** are likely better for predicting long-term oxidative stability

- These measure anthocyanin-bound SO_2, which can replenish free SO_2 as it is lost to oxidation

- But **non-disturbing "true" methods** are likely better for predicting outcomes dependent on SO_2 activity

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When should "true" SO_2 by HS-GDT or other methods be a better metric for SO_2?

- **Microbial stability**
- **Binding of carbonyls**
- **Sensory effects** (SO_2 "burn")

- **Rates of reactions** involving free or molecular SO_2
  - Formation of H_2S in canned wine (keep molecular <0.5 ppm)
  - Competition btw HSO_3^- and other components (e.g. polymeric pigment formation)
  - Release of H_2S and other volatile sulfur compounds from di- and polysulfides

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Example: Is True or Apparent Molecular SO_2 better at predicting microbial stability?

Challenge experiments

- Two wines, a white and a "red" (4 L, 0.22 µm filtered)
  - Low SO_2 commercial white wine vs. "red" wine with added anthocyanins
  - SO_2 added at varying concentrations
- Wines challenged with *S. cerevisiae*
  - EC 1118, ~10^6 cells/mL
- Samples taken over 10 days for SO_2 analysis (FIA and HS-GDT)
- Cell culturability determined on YM agar, viability by flow cytometry

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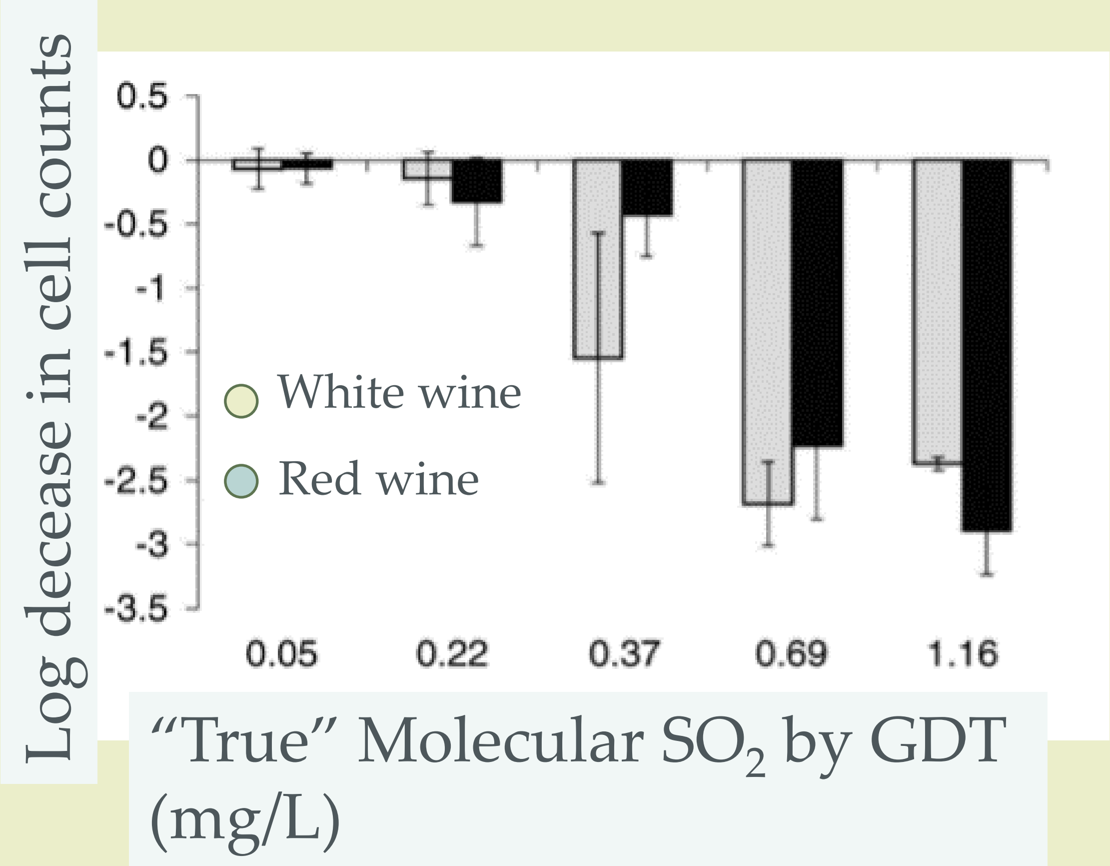
Howe, Worobo and Sacks; *AJEV* **2018**

True molecular SO_2 by GDT predicts antimicrobial SO_2 activity in both reds and whites

More live yeast

More dead yeast



Note that 0.69 mg/L molecular SO_2 is necessary for a significant reduction in yeast counts!

In line with the textbook recommended values!

Howe, Worobo and Sacks; *AJEV* **2018**

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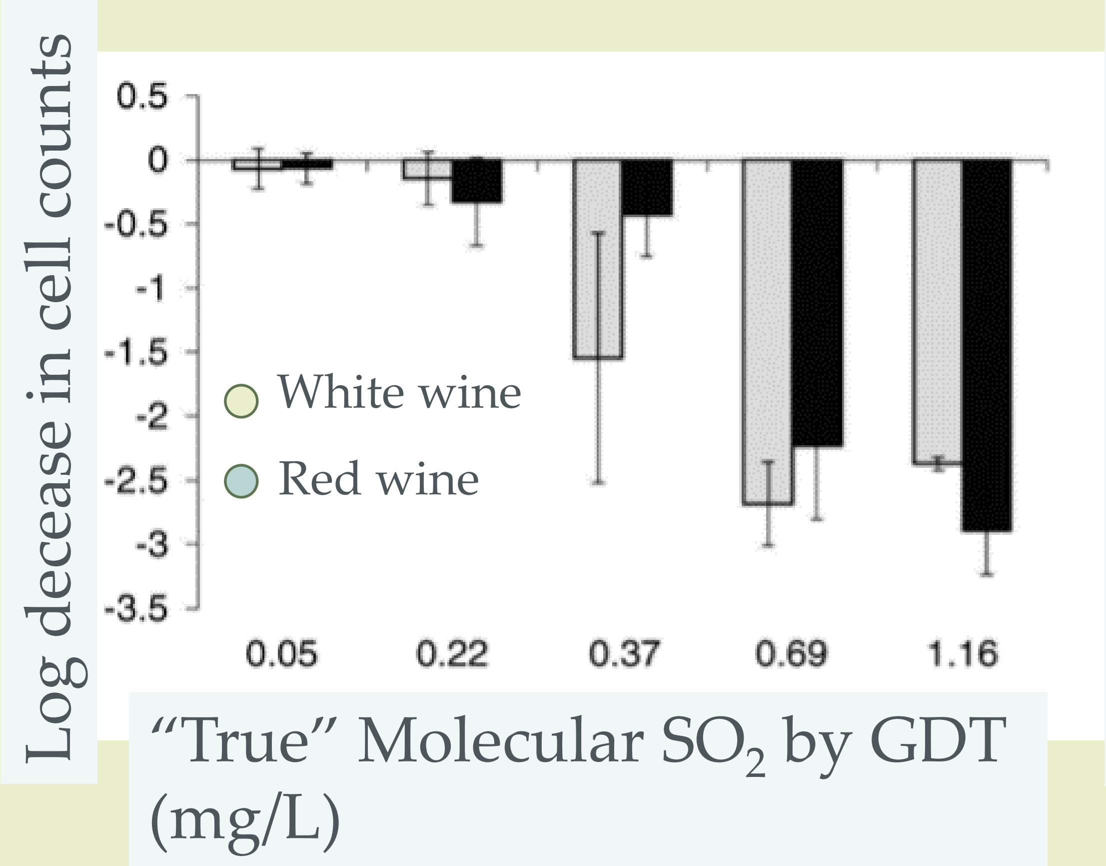
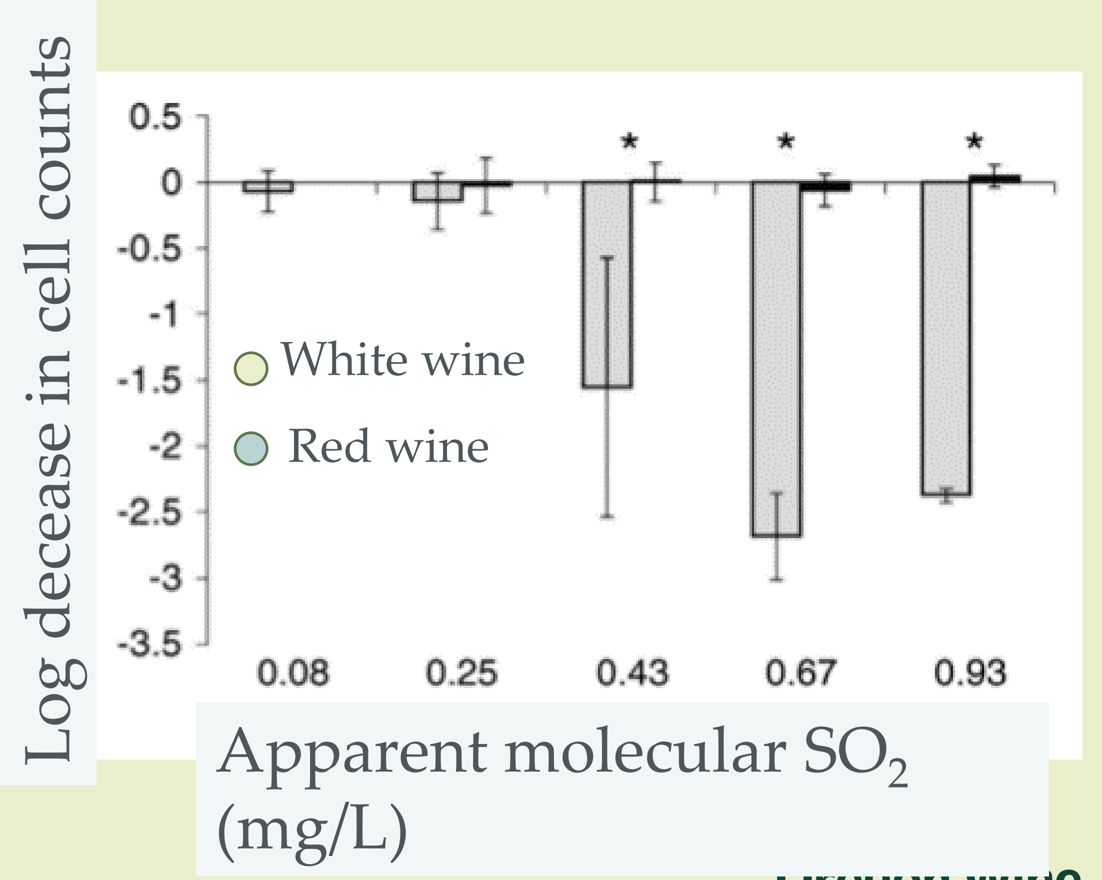
Apparent molecular SO_2 by FIA or AO is predictive of microbial stability in whites and . . . . useless in reds

More live yeast

More dead yeast





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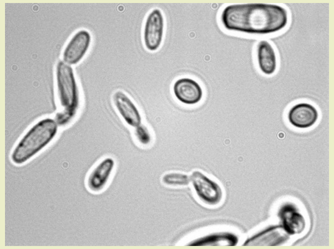
Howe, Worobo and Sacks; *AJEV* **2018**

“Wait, should I be tripling my free SO_2 in red wines?”

Answer: No, you’d bleach your color, and you may exceed regulatory limits. And, apparent SO_2 will still contribute to the reservoir of SO_2 during long-term aging

But, your darkly colored young red wines (high monomeric anthocyanins) are going to be at high spoilage risk!

Hmm . . . Maybe this is why Brett is a problem in reds, less so in whites?



And, if you are trying to interpret results where True SO_2 matters (e.g. why did this wine benefit from micro-ox and not this one?)

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- **What happens to oxygen in wines?**
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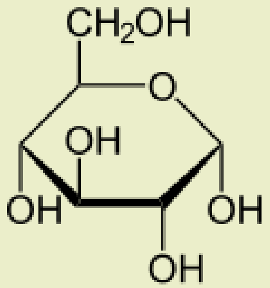
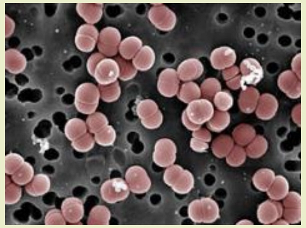
Review: Why the focus on finished wines? We use SO_2 pre-fermentation, too!

Typical pre-fermentation SO_2 additions: 30-50 mg/L
About ~50% of added SO_2 is bound by glucose in typical juice



Anti-microbial (Molecular SO_2)
- *S. cerevisiae* is more SO_2 tolerant than bacteria and most other yeast



Review: Why the focus on finished wines? We use SO_2 pre-fermentation, too!

Anti-oxidant and anti-oxidasic: (Free SO_2)

- Free SO_2 inhibits browning enzymes, reacts with oxidation products
- But, the impact on this pre-fermentation oxidation on finished wine is small (see later slide)

Downstream effects – more total SO_2

- Yeast generate aldehydes (especially acetaldehyde)
- In finished wine, approximately 50% of pre-fermentation SO_2 ends up as acetaldehyde bound SO_2

Why the emphasis on post-fermentation SO_2?

	Fresh must, no SO_2	Actively fermenting wine	Finished 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

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Note: lees may continue to consume O_2 for several months

Pre-fermentation reductive vs. oxidative practices often have small effects on finished wine

Hyperoxidized Chardonnay must

Partway through fermentation

Hyperoxidized must after fermentation

Photos: R. Mira de Orduna

The well-established effects

Hyperoxidation decreases 'browning potential' in white wines

More oxygen (and low SO_2) = 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

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What happens to oxygen in a wine? The main pathway – "iron-phenolic"

Iron-phenolic
(MAJOR pathway)

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

Quinone →

Loss of sulfhydryls (e.g. **H_2S, MeSH**), tannin reactions

Hydrogen Peroxide (H_2O_2) →

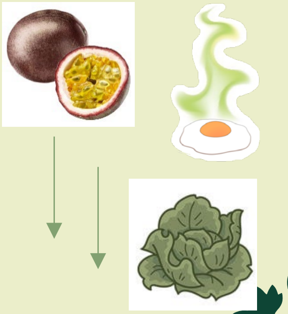
Aldehyde formation; browning, off-aromas
"Wine pigment"

Metal-sulfhydryl, pathway 2
(MINOR)

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

Loss of sulfhydryls (thiols and H_2S)

Formation of di- and polysulfides



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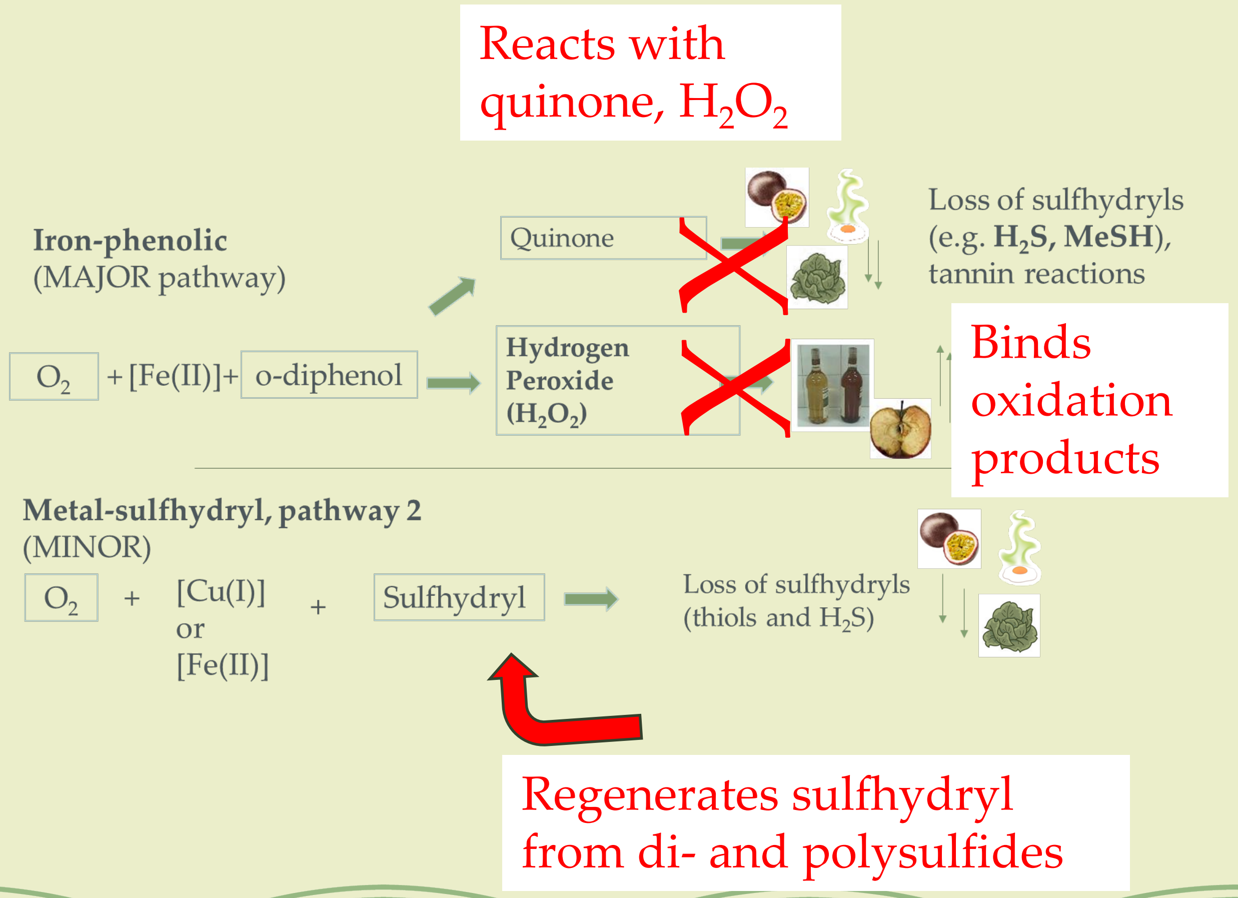
Kreitman, Elias, Jeffery, and Sacks; *Crit Rev. Food Sci Nutr.* **2019**

Free SO_2 (bisulfite) consumes wine oxidation products (quinones, H_2O_2, aldehydes, disulfides, etc)

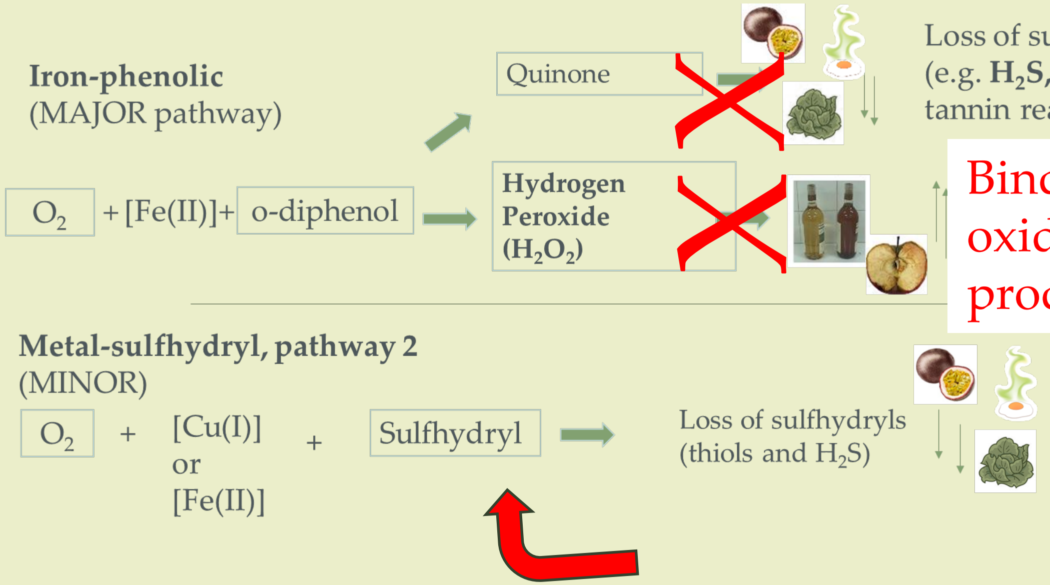
Roles of Free SO_2 in red



Free SO_2 (bisulfite) consumes wine oxidation products (quinones, H_2O_2, aldehydes, disulfides, etc)

Roles of Free SO_2 in red

Reacts with quinone, H_2O_2



Binds oxidation products

Regenerates sulfhydryl from di- and polysulfides

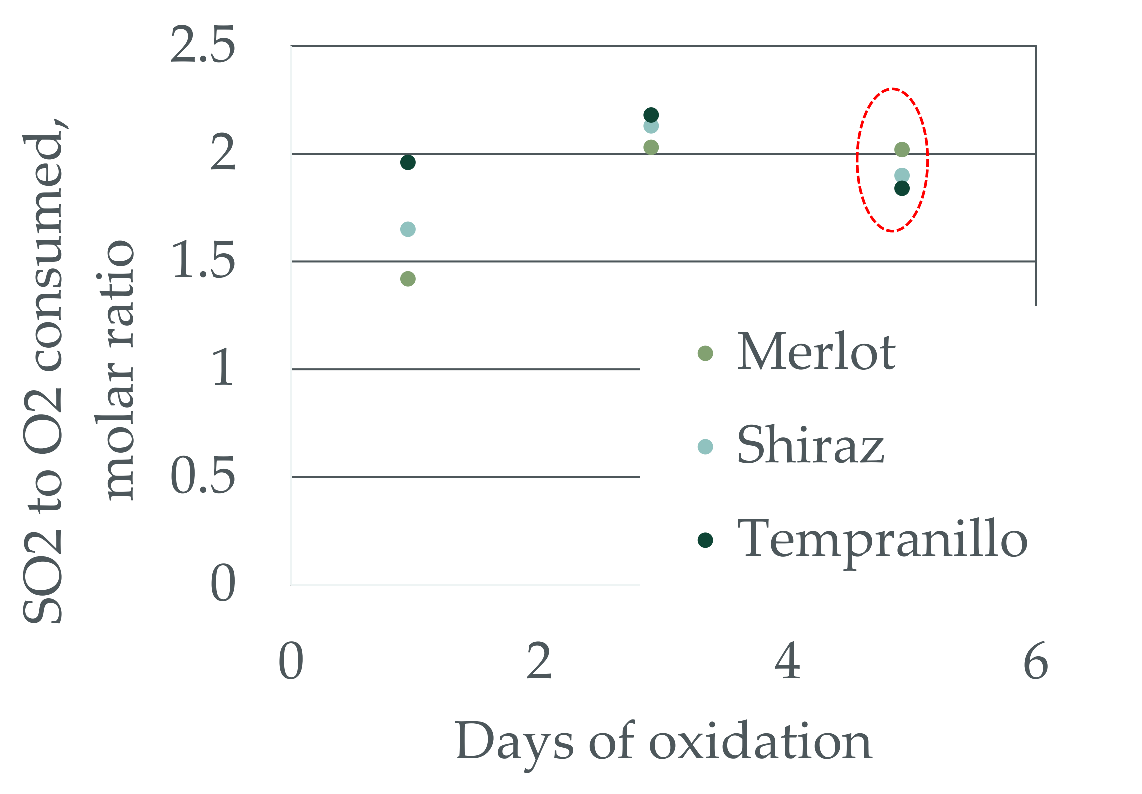
Put another way, the role of SO_2 is not to react with O_2 directly

It "reduces" (chemically speaking) oxidized 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)

In real wines, SO_2 consumed at a nearly 2:1 molar ratio (Total SO_2 to O_2)



That is, a ratio of 4 mg total SO_2 for every 1 mg O_2

Exception: ascorbic acid can react with the quinone; ratio can approach 2 mg SO_2 per 1 mg O_2

But, we have nothing other than free SO_2 that can rapidly consume H_2O_2!

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Data replotted from Danilewicz and Standing, *AJEV* **2018**

To repeat: the unique role of SO_2 is its ability to rapidly consume H_2O_2

Iron-phenolic
(MAJOR pathway)

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

→ Quinone

Quinone → 

Loss of sulfhydryls (e.g. **H_2S, MeSH**), tannin reactions

Hydrogen Peroxide (H_2O_2) → 

Aldehyde formation; browning, off-aromas "Wine pigment"

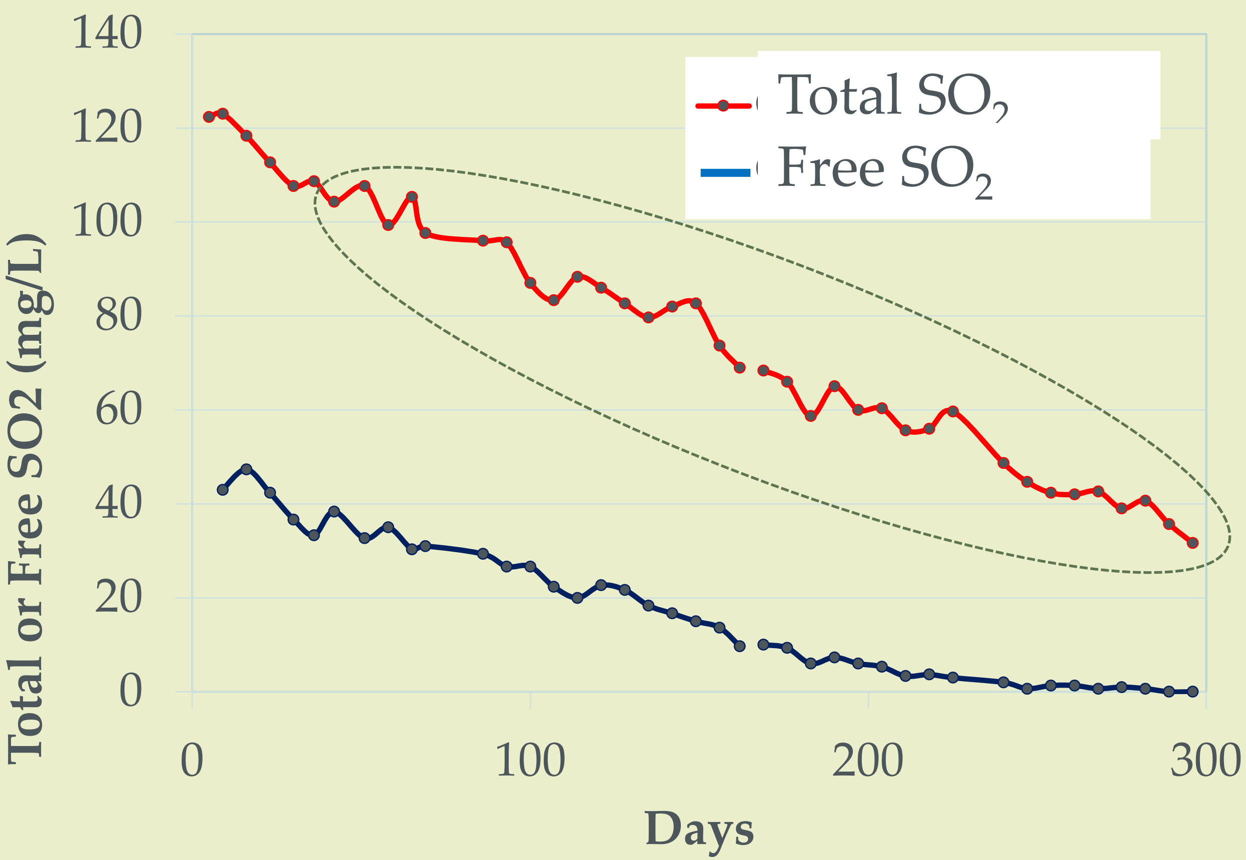
- Ascorbic, glutathione, and other wine components can consume quinones

- But only SO_2 can react with H_2O_2 before aldehydes and other oxidized aroma compounds and brown pigments are formed
- This is a challenge for no-/low SO_2 winemaking!

For some perspective: how much O_2 is introduced through packaging?

Packaging	Total package oxygen (mg O_2)	Ingress during first year (mg O_2)	Total SO_2 loss after 1 year (mg SO_2)
750 mL screwcap, Saranex liner	*1-4*	*0.2-0.5*	*5-18*
750 mL natural cork, high quality	*0.5-3*	*1-2*	*6-20*
750 mL synthetic, Nomacorc	*0.5-3*	*0.7-3*	*5-24*
1.5L Bag-in-box	*2-3*	*10-25*	*50-100*
Aluminum can	*0.5-4*	*<0.1*	*2-16*

Free SO_2 decreases slower than Total SO_2 because Bound SO_2 pool partially replenish SO_2



After 1 month, Total SO_2 decreases linearly during storage (~0.4 mg SO_2 /day) due to constant oxygen ingress (~0.1 mg O_2 /day)

Free SO_2 loss varies from ~0.25 mg/day to ~0 mg/day during storage as weakly bound SO_2 is depleted

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

During oxidation, binding reverses Adducts dissociate to release SO_2

Weaker binder

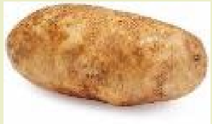
Stronger binder

Glucose	Bound SO_2	
Bound anthocyanins (bleached)	Bound SO_2	
Bound methional (odorless)	Bound SO_2	
Bound acetaldehyde (odorless)	Bound SO_2	

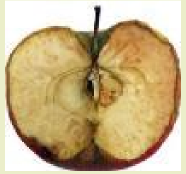
Free SO_2

Glucose (sweet wines)

Anthocyanins (red-purple color)



Methional (“potato” odor)



Acetaldehyde (“old apple” odor)

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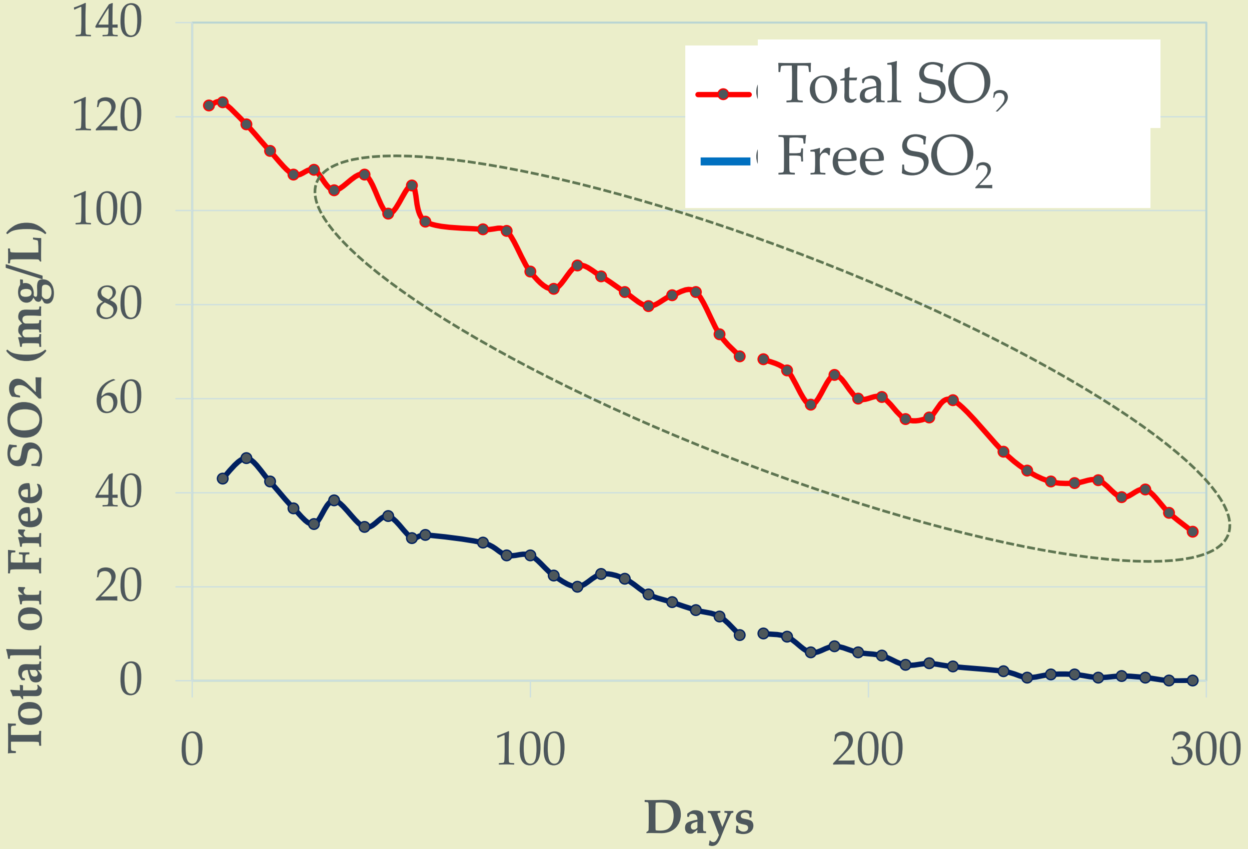
The decrease in Total SO_2 is a better parameter for determining the extent of O_2 exposure



With constant O_2 exposure, the decrease in total SO_2 is constant (4:1 ratio on mass basis, 2:1 on molar basis)

Free SO_2 and O_2 consumption

This is why tracking total SO_2 is useful for answering questions like . . .

- Microbial spoilage vs. oxidation?
- How much O_2 was introduced?

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Two ways 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

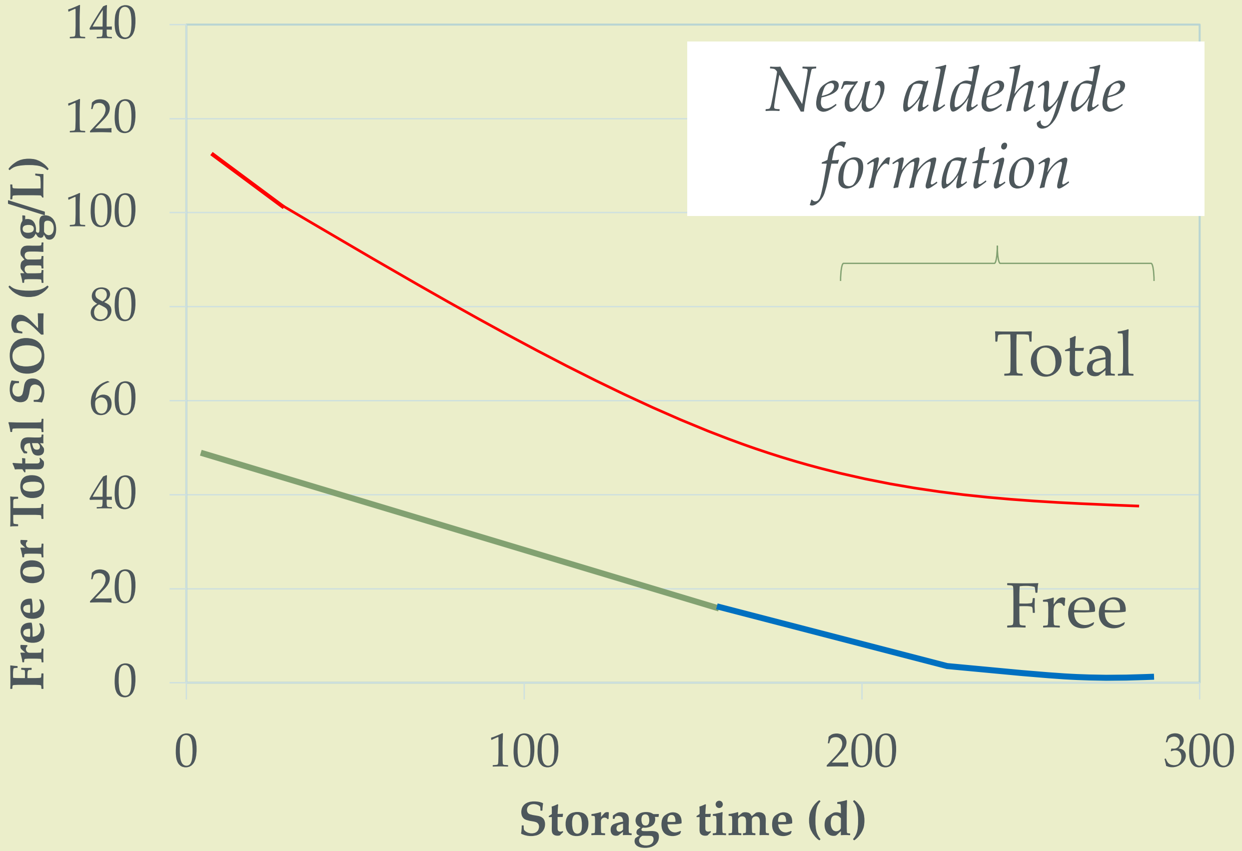
Bound SO_2

2) Free SO_2 is consumed through oxidation reactions

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

If "new" aldehyde formation is more important than aldehyde release, what should happen to SO_2?

Hypothetical Data for SO_2 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

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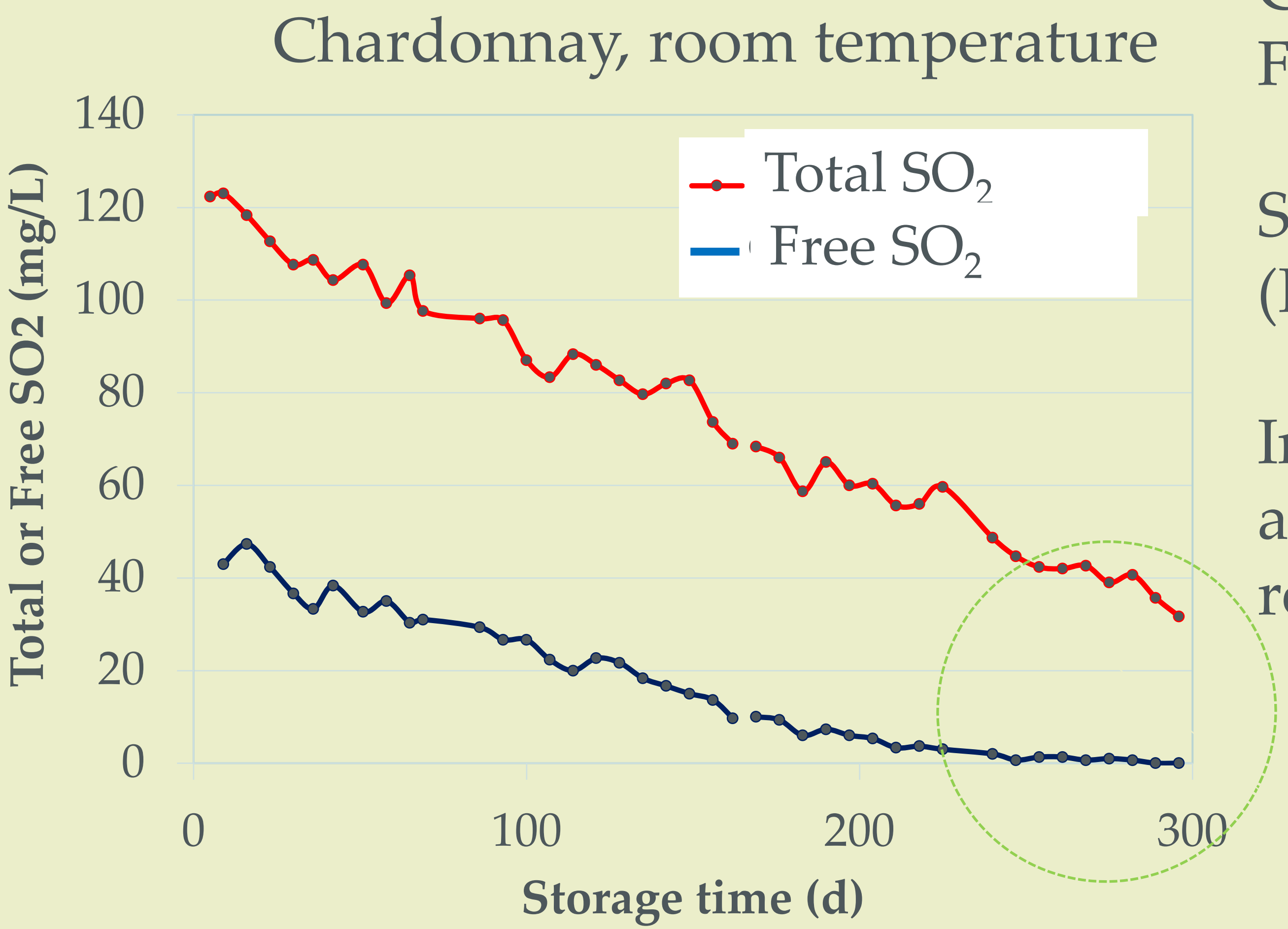
Instead, we observe constant Total SO_2 loss . . . Even after Free SO_2 is no longer detectable



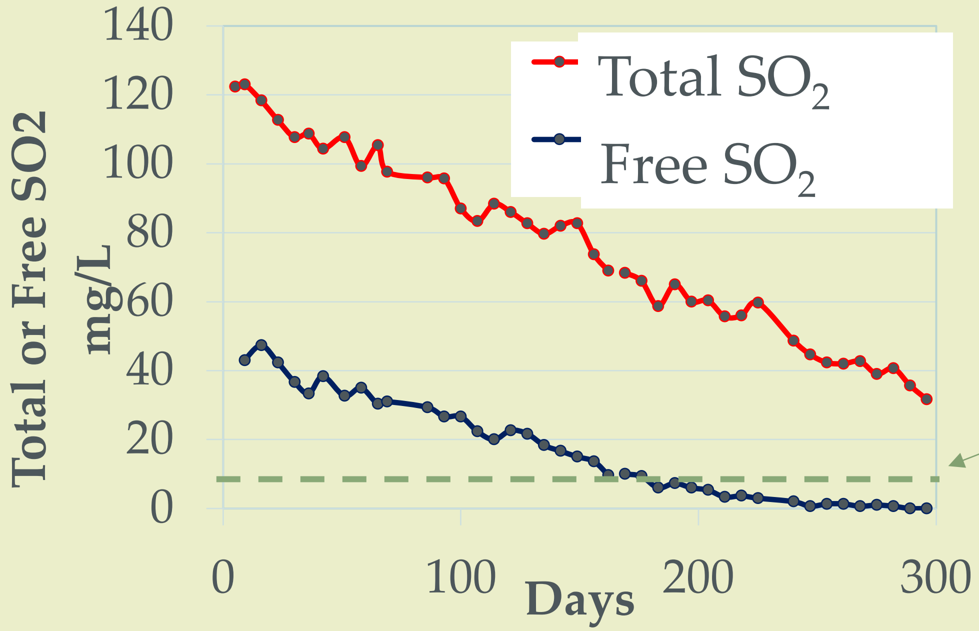
Constant rate of Total SO_2 loss even after Free SO_2 < 2 mg/L

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

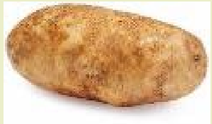
Malodorous compounds released from Bound forms if Free SO_2 gets too low!



Below ~10 mg/L free SO_2, oxidized aromas appear

Likely because of release of potent "bound" aldehydes, not new

Godden, et al *AJGWR* **2001**
Sacks, Howe, Standing, and Danilewicz; *AJEV* **2020**



Bound methional (odorless) — Bound SO_2

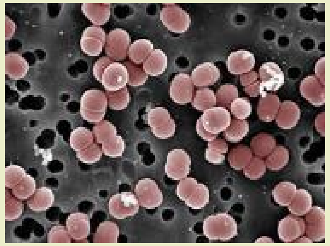
Loss of free SO_2 →

Methional ("baked potato")

Oregon Wine Symposium

The unique role of SO_2 as an antioxidant / oxidation product binder is what makes its elimination challenging

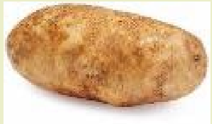
1) Molecular SO_2 as an antimicrobial – there are other options



There are other options! You don't need SO_2 to prevent spoilage!
DMDC, sterile filter, pasteurization (yikes?), and more

2) Free SO_2 (as bisulfite) as an antioxidant – lack of options

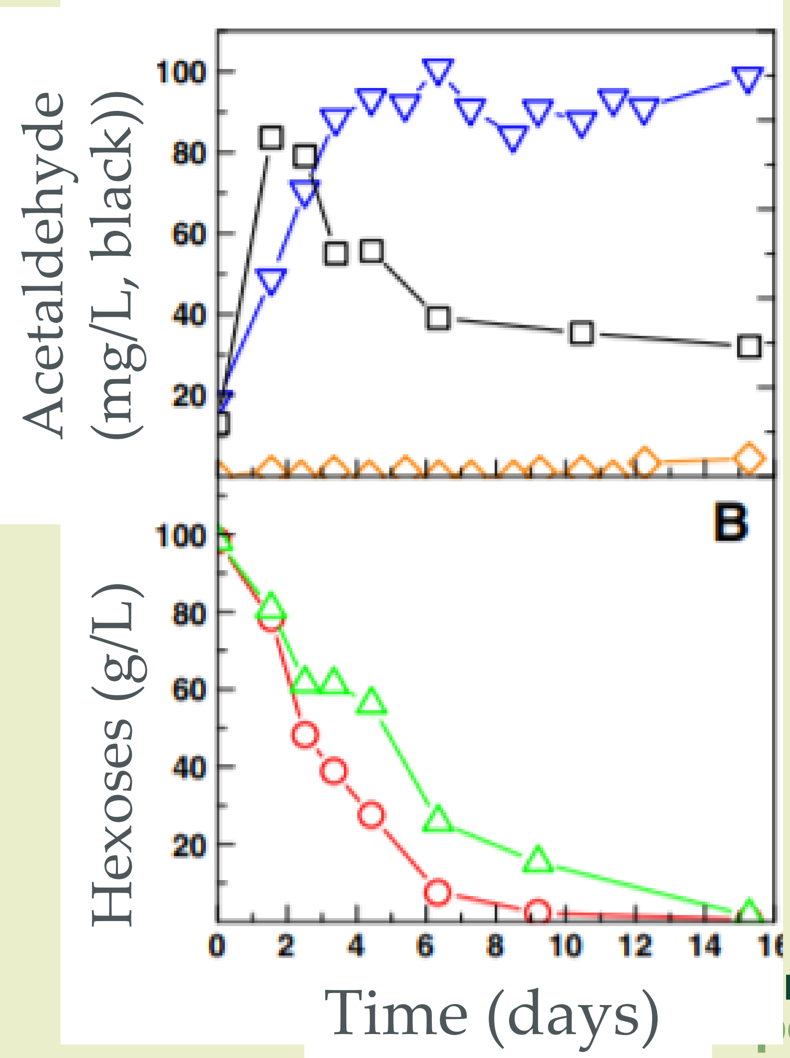
We currently use free SO_2 to bind malodorous aldehydes
We currently use free SO_2 to scavenge oxidation products (especially hydrogen peroxide)



How could one minimize volatile carbonyl concentration at the end of fermentation?

- What decreases acetaldehyde at the end of fermentation?
  - Prolonged lees contact (right)
  - Malolactic fermentation
  - Lower initial SO_2

- Not much data on weaker binding carbonyls (e.g. methional), but should be similar to acetaldehyde



Jackowetz and Mira de Orduna, *Food Res Int*, **2011**

Takeaways

- Free SO_2 (antioxidant) and Molecular SO_2 (antimicrobial): two separate targets
- Total SO_2: useful for tracking extent of oxygen exposure

- Commonly used methods measure “Apparent” Free or Molecular SO_2 due to dissociation of anthocyanin-bisulfite complexes
  - “Apparent” or “Free” SO_2 has dubious value for predicting microbial stability in red wines
  - What about for oxidation or carbonyl binding? TBD.

- We have alternatives for preventing microbial spoilage without SO_2 and consuming quinones, but not for consuming H_2O_2 from wine oxidation

- Wines initially smell oxidized because SO_2-bound carbonyls dissociate
  - Can we limit formation of bound carbonyls at the end of fermentation? Will that help?