Oregon Wine Symposium

Reduction Prevention Strategies

Gavin Sacks, Professor & Chair of Food Science, Cornell University
James Osborne, Associate Professor of Food Sci & Tech, Oregon State University

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Cornell CALS College of Agriculture and Life Sciences

Today's talk

1) "Reduced" aromas and the wine ecosystem

2) How are key "reduced" aroma compounds formed during fermentation?

3) How do these odorants disappear during treatment? How can they reappear?

First: what do we mean when we describe a wine as “reduced”?

Sensorially, we mean

- Rotten egg
- Sewer-like
- Cabbage
- Swampy
- Burnt hair
- Struck match

Chemically:

- Species responsible for “reduced” aromas contain reduced (*less oxidized*) forms of sulfur

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Major markers for "reduced" aromas in wine H_2S and MeSH

	Concentration range (μg/L) in "reduced wines"	
Variety (no. assessed)	**H_2S**	**Methyl mercaptan (MeSH)**
Chardonnay (4)	**1.5-5.0**	**3.0-8.0**
Pinot Gris (1)	**2.0**	**3.0**
Riesling (10)	**0.5-35.0**	**nd-3.0**
Sauvignon blanc (6)	**0.8-4.0**	**1.7-6.0**
Sauvignon blanc/Semillon (4)	**2.0-13.0**	**1.0-4.0**
Verdelho (1)	**1.0**	1.6
Viognier (1)	0.5	**3.0**
Sensory threshold	*1*	*2*

- Either H_2S or MeSH (or both) exceed sensory threshold in reduced wines
- Other S-compounds? Usually below threshold, e.g. dimethyl disulfide
- Dimethyl sulfide (DMS, "canned corn" aroma)?

From aging; DMS is not redox-active

Siebert, et al *JAFC* **2010**

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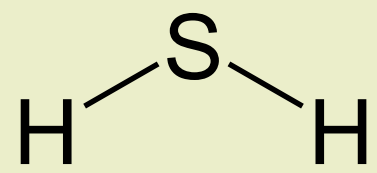
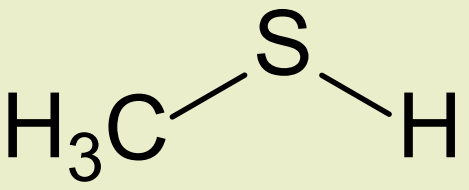
The key “reduced” aroma compounds are sulfhydryls (contain an –SH group)!

Key features of H_2S, MeSH, and other sulfhydryls

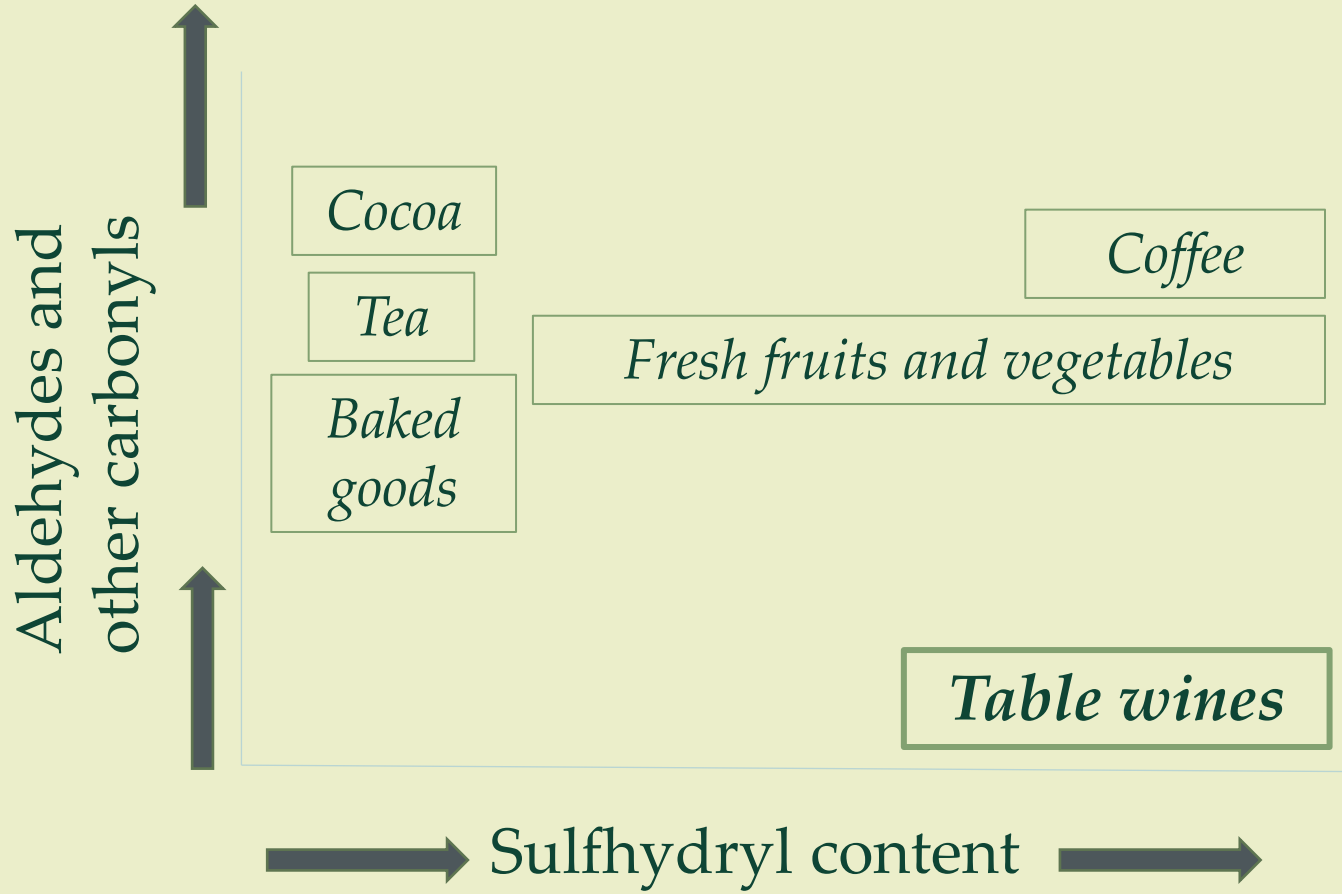


- Readily oxidized
- Form complexes with certain transition metals (e.g. Cu)

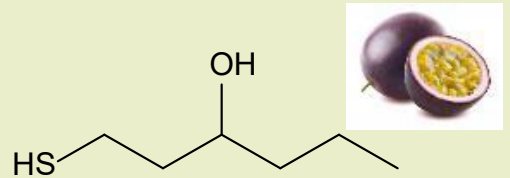


- Potent odorants (if volatile): ppb - ppt thresholds

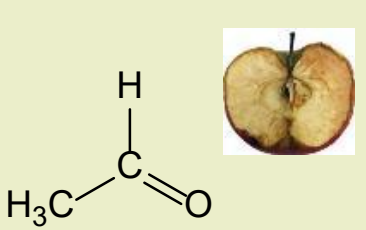
We should think of foods and beverages as ecosystems (instead of zoos)

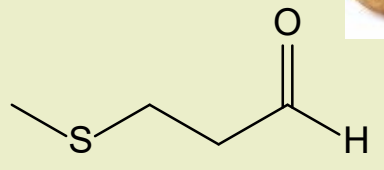


High sulfhydryls (-SH group) ("reduced", "tropical", fresh")



Low free aldehydes (-CHO) (the "oxidized" aromas)





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Changing the ecosystem. We change the concentrations of “reduced” and “oxidized” odorants

“Oxidized”

O_2

Original wine

Bisulfite (HSO_3^-)

“Reduced”

Aldehydes and other carbonyls

Sulfhydryl content

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To understand changes to odorants, we can focus on environment (redox potential) or seeds (precursors)

Redox potential perspective – *which way is the wine being pulled?*

Original wine

"More oxidizing"

O_2

"More reductive"

HSO_3^-

To understand changes to odorants, we can focus on environment (redox potential) or seeds (precursors)

Redox potential perspective – *which way is the wine being pulled?*

Original wine

"More oxidizing"

O_2

"More reductive"

HSO_3^-

Precursor and pathway perspective – *what's available?*

What are the seeds?

How much do we have?

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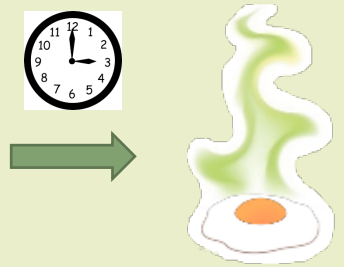
This talk will consider both, but focus more on the seeds of "reduced aromas"

H_2S and other sulfhydryls can reappear during storage . . . How?

Sauvignon blanc wine with undetectable H_2S before bottling, stored for 2 years

Adapted from Lopes, et.al. **2009**, *JAFC*

	Sealed ampoule	Saran-Tin Screwcap	Natural cork	Synthetic
H_2S after 2 years (µg/L)	29.6	21.1	15.5	3.5



Similar results for H_2S and MeSH seen in ~10 other papers, reviewed in:
Kreitman, Elias, Jeffery and Sacks **2019** *Crit Rev. Food Sci Nutr*

What is the source of this H_2S? What are the seeds?

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Where do H_2S and MeSH come from during fermentation?

How are they originally formed?

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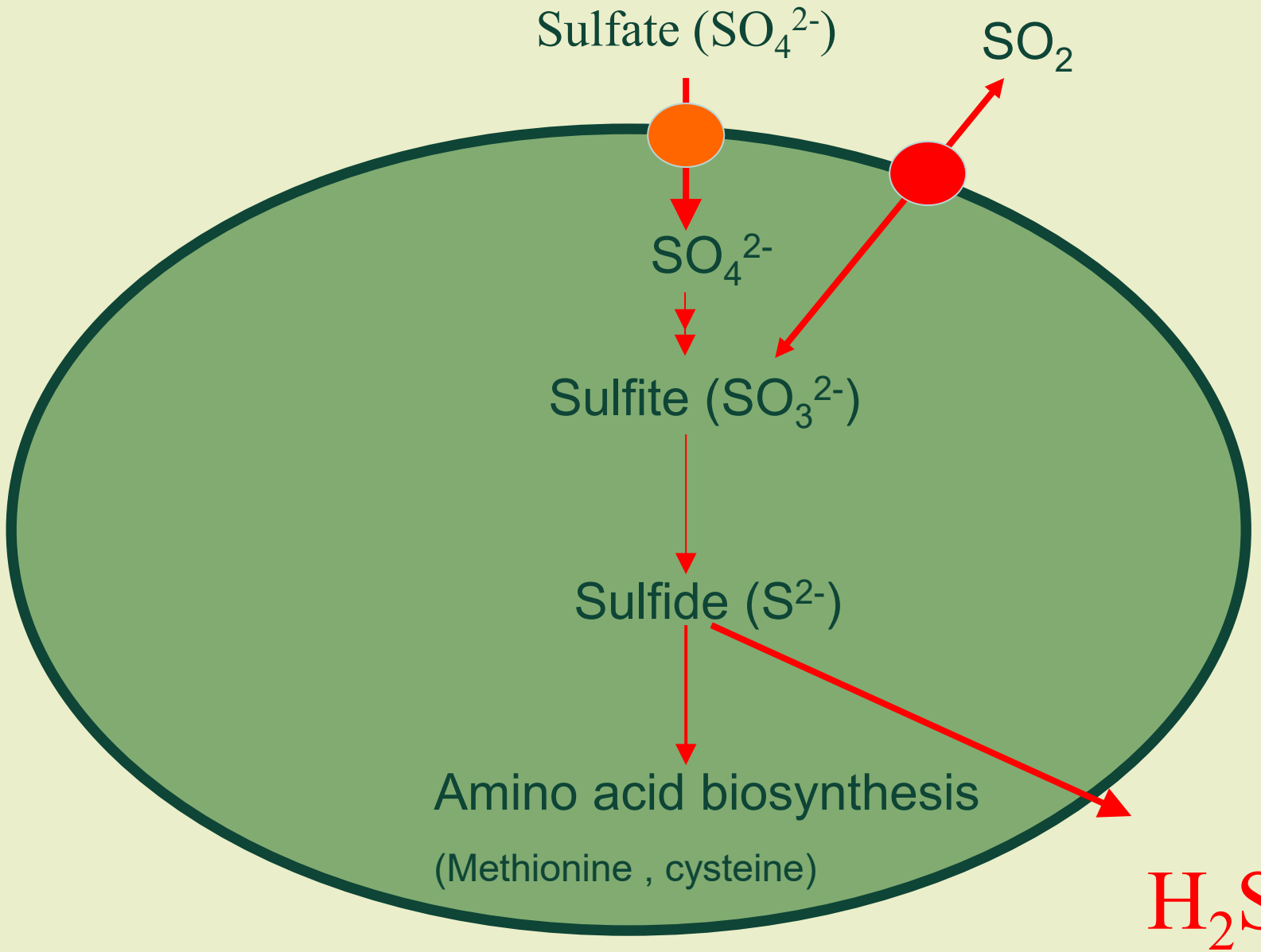
Sources of volatile sulfur compounds during fermentation

1. Yeast derived via metabolism
2. Yeast derived via degradation
3. Reduction of elemental sulfur

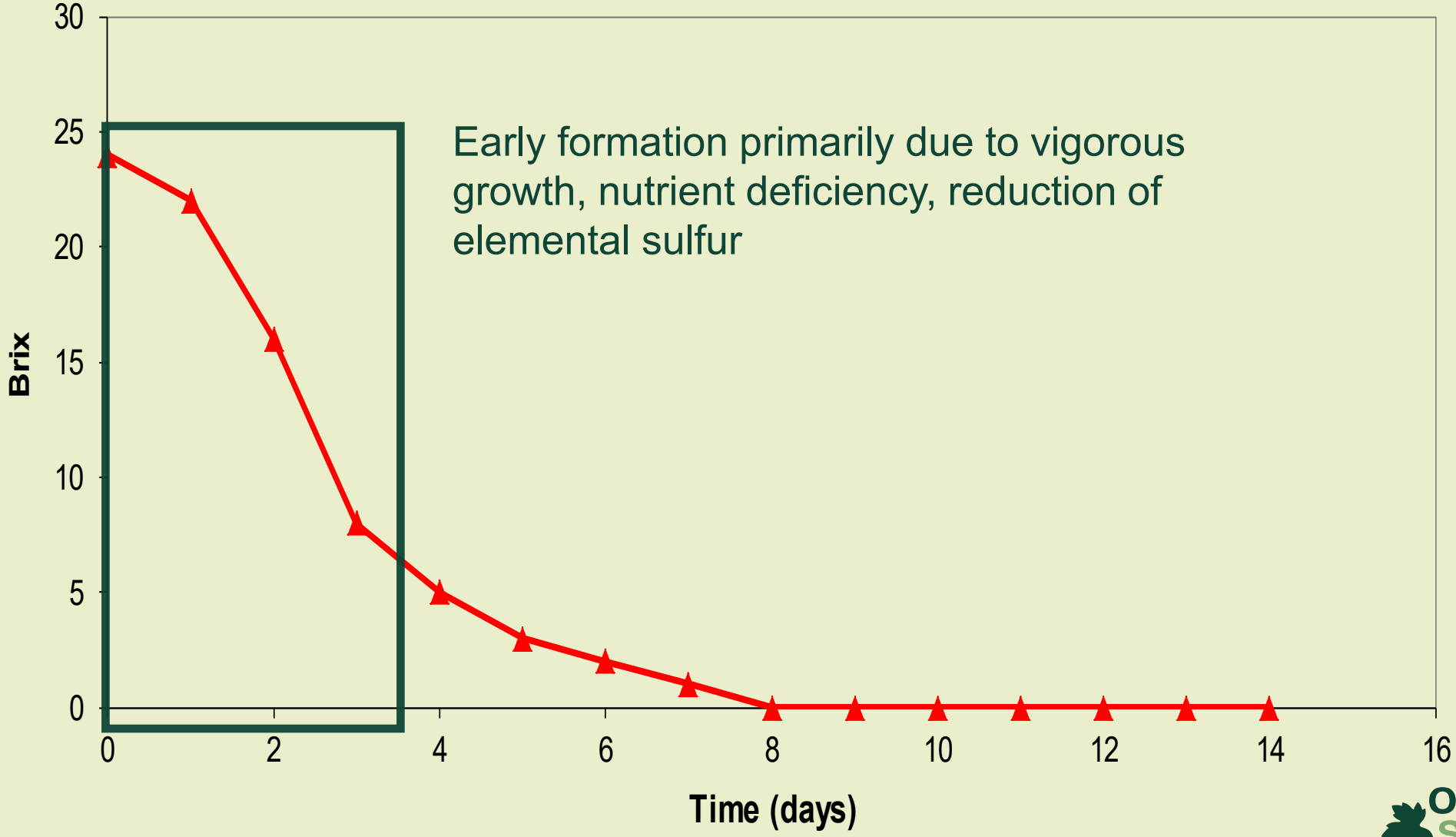
Origins – Yeast produced H_2S

- Yeast produce H_2S as an intermediate in the biosynthesis of sulfur-containing compounds
- An essential metabolic pathway is involved
  - Sulfite reduction sequence (SRS)
  - Incorporated into amino acids or expelled from the cell



Sulfur Reduction Pathway

When is H_2S produced?



Early formation primarily due to vigorous growth, nutrient deficiency, reduction of elemental sulfur

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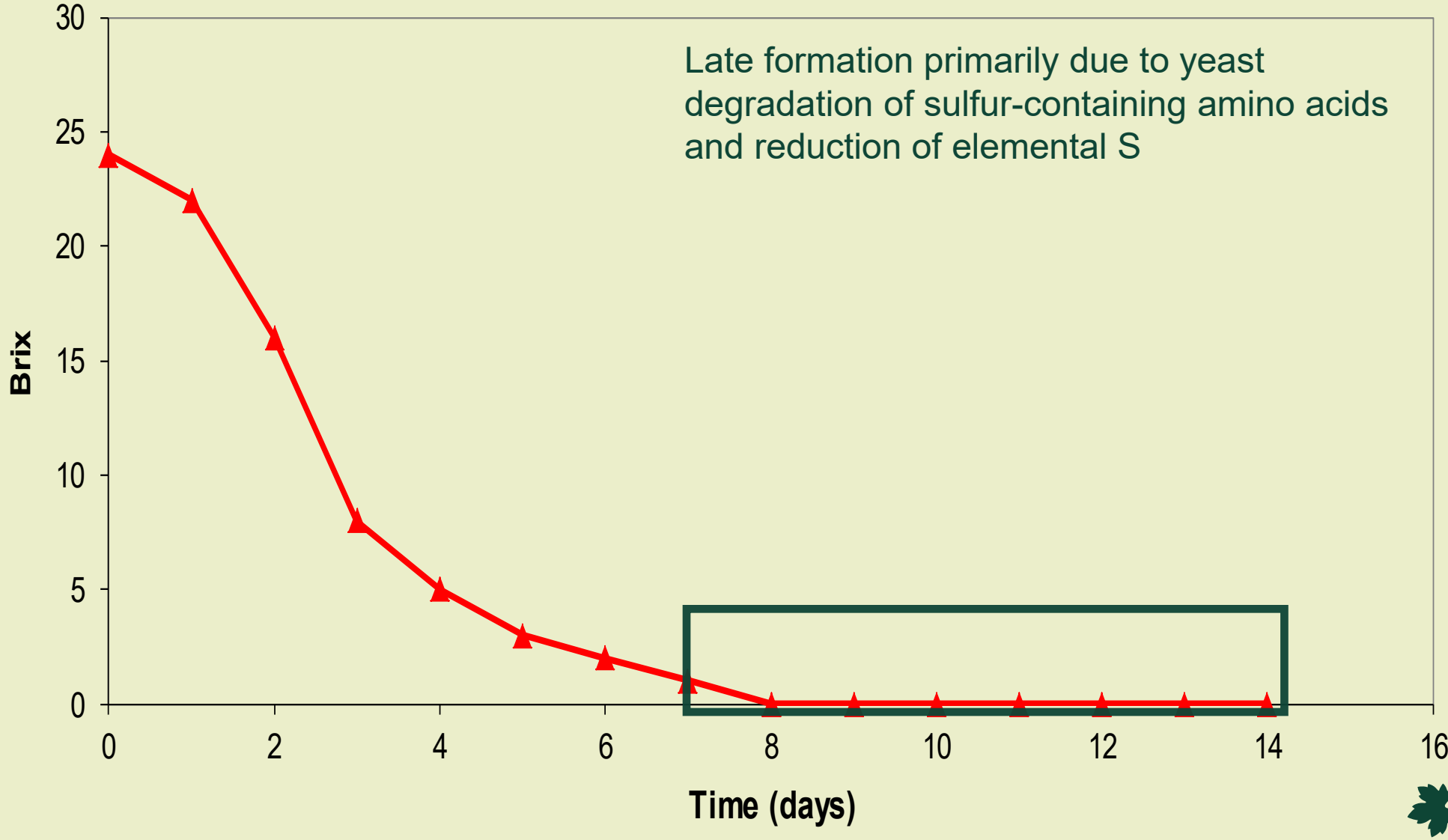
When is H_2S produced?



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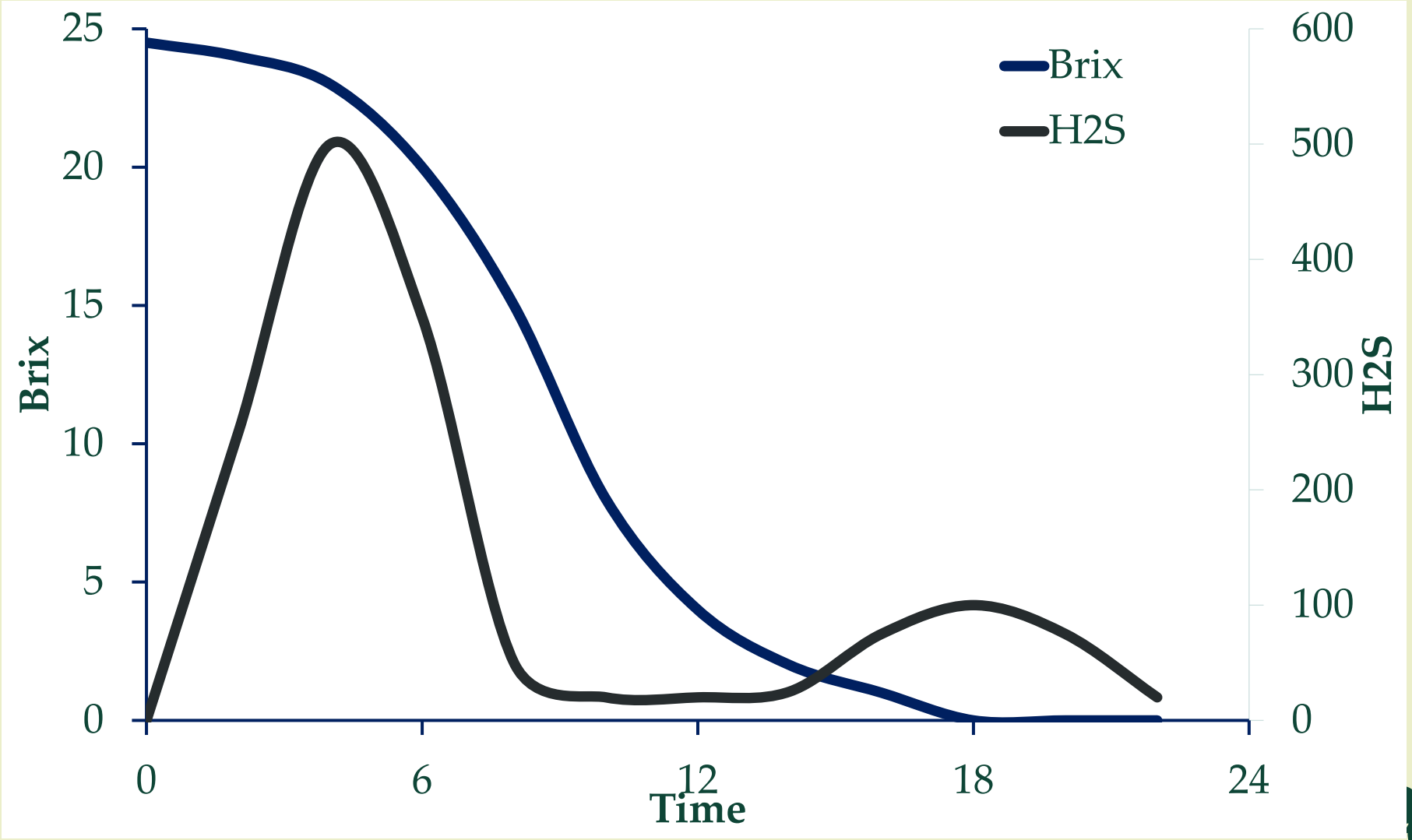
When is H_2S produced?



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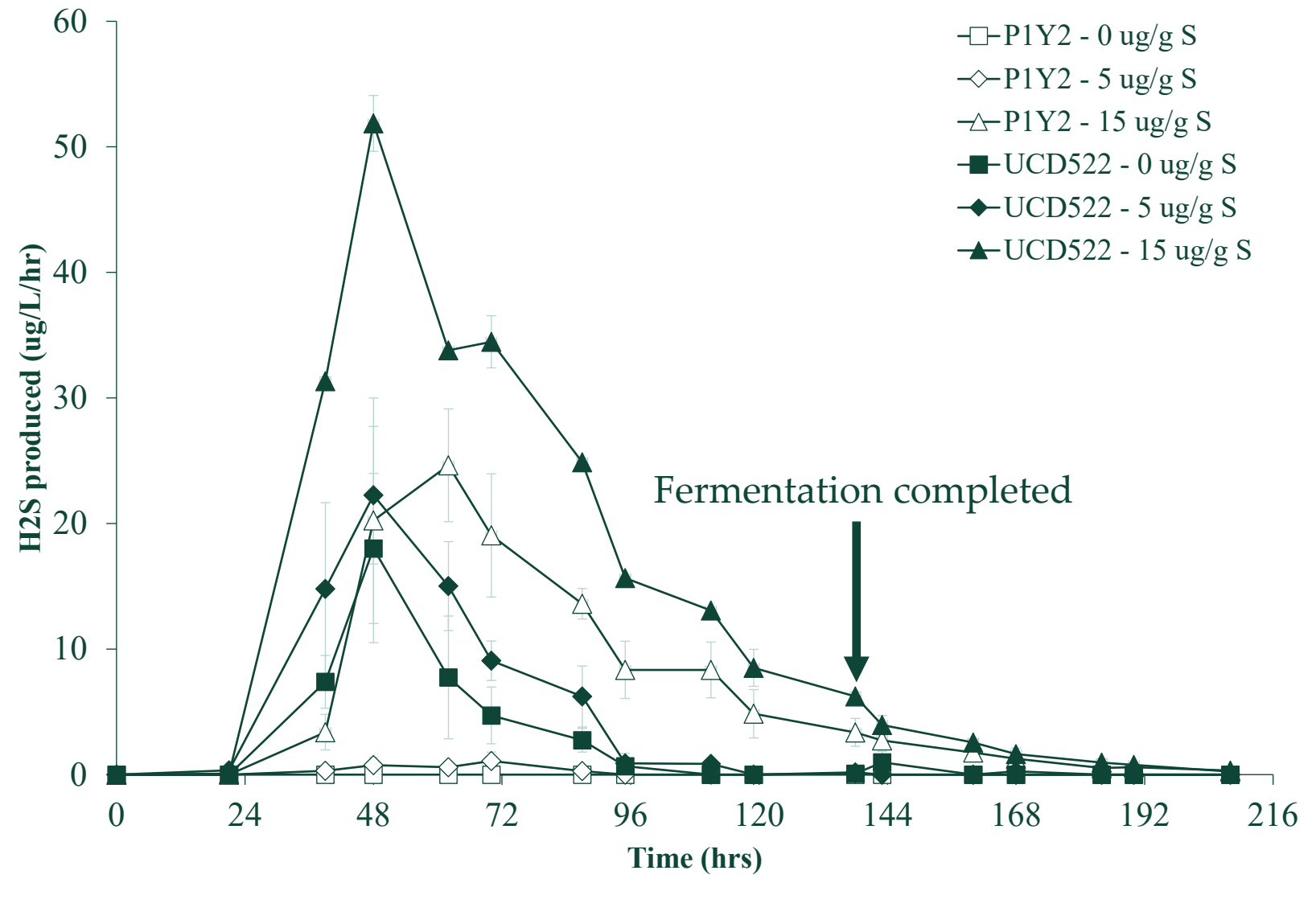

Kraft et al. 2023

Factors impacting yeast produced H_2S

- Yeast strain
- Nutrients
- Oxygen
- Temperature
- Juice turbidity
- Tank shape
- Others?

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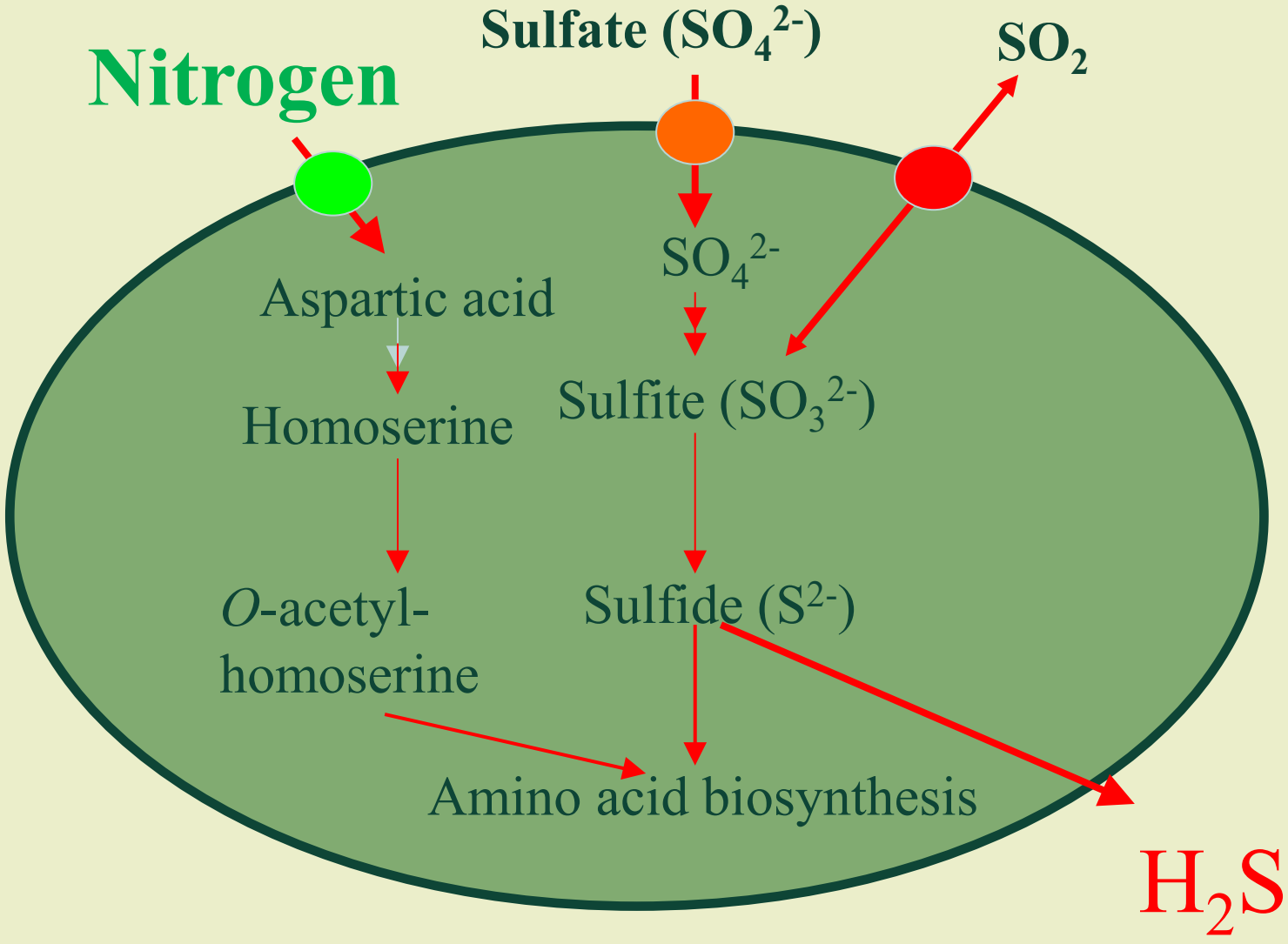
Yeast produced H_2S

- Yeast strain dependent
- High H_2S producing strains vs low
- "No/low H_2S" producing strains available from multiple companies
- Identification of genes responsible for sulfite reductase

Uglanio et al. 2010

Nutrients - Nitrogen

- Nitrogen required for amino acid production
- Lack of nitrogen may lead to build up of sulfide in cell which is toxic and diffuses from cell as H_2S
- Problem may be alleviated by addition of nitrogen



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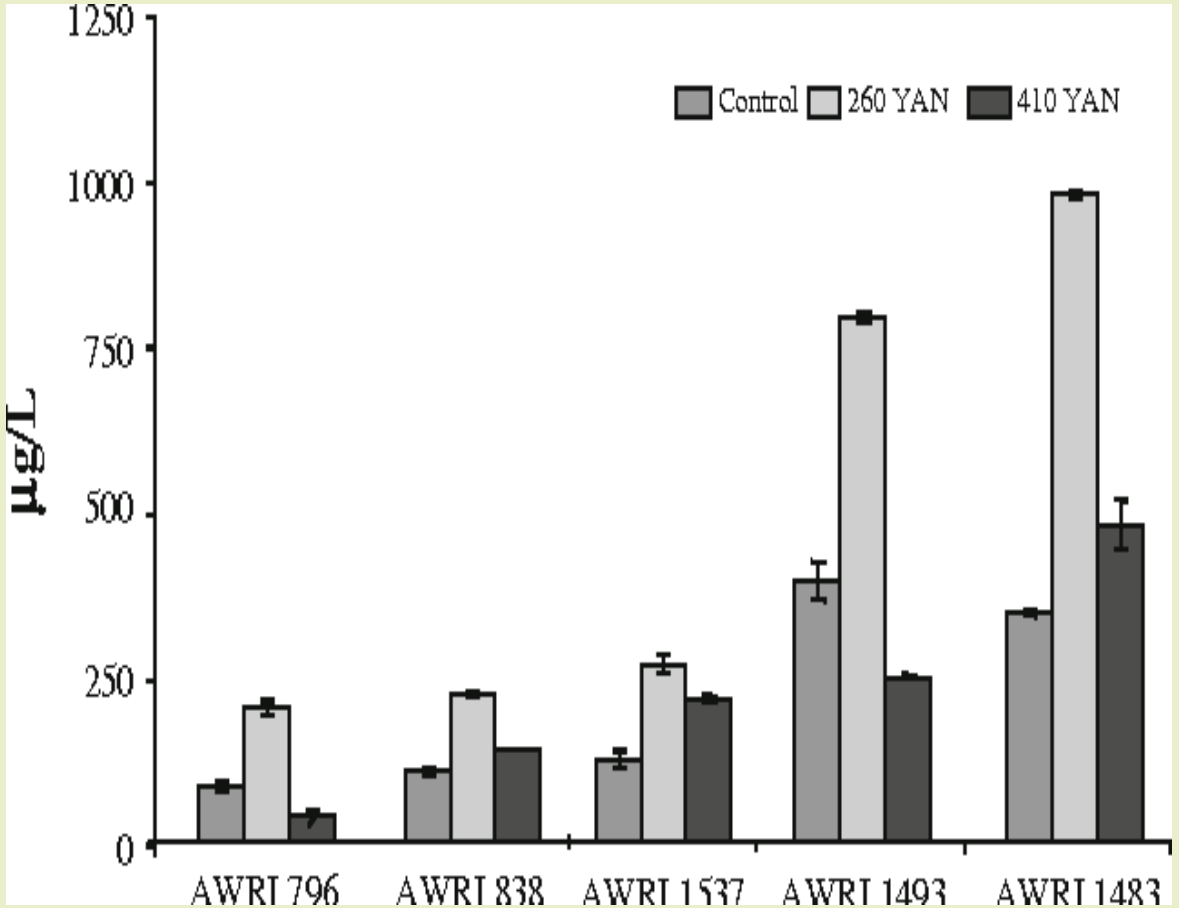
Nutrients - Nitrogen

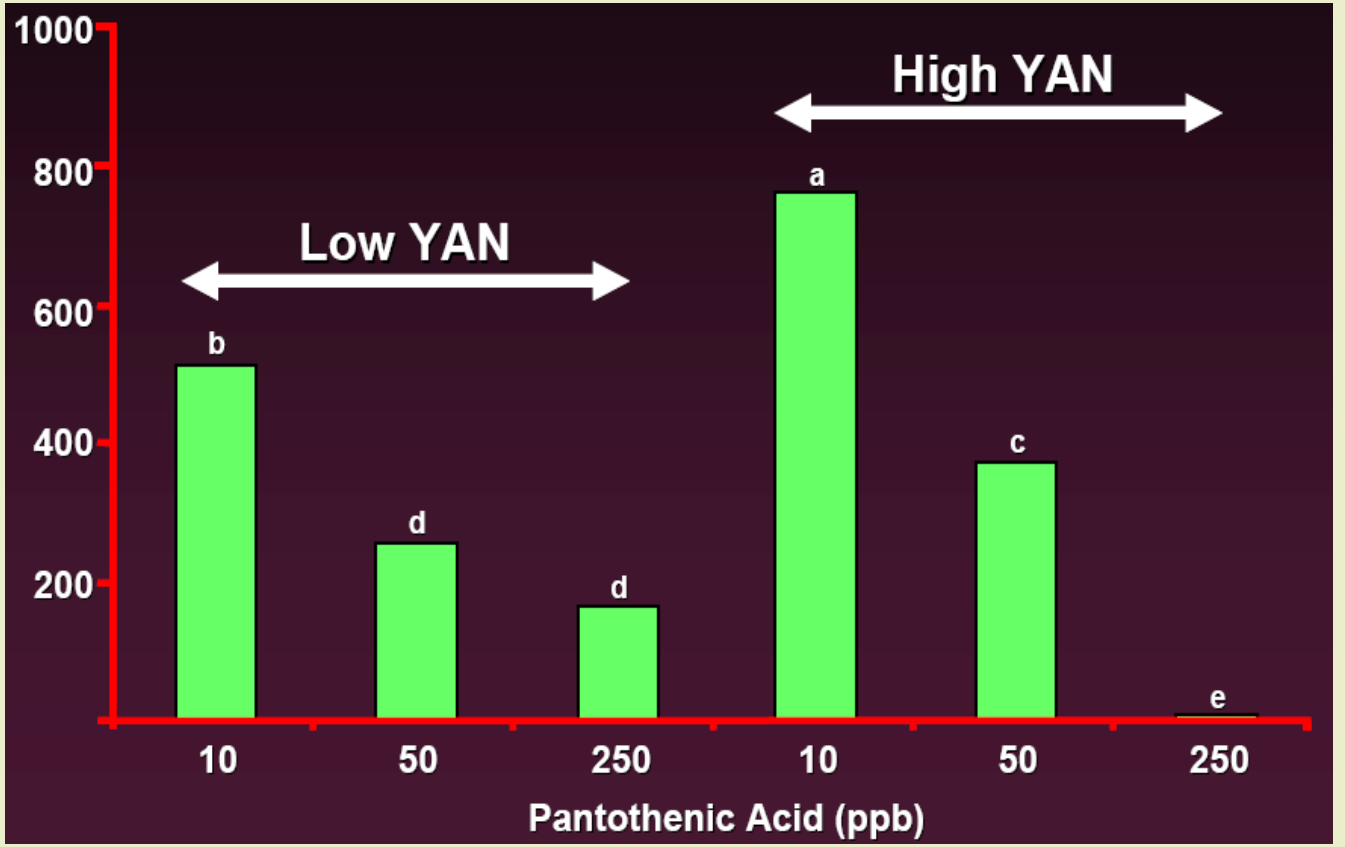
- Nitrogen content not well correlated with H_2S production
- Higher nitrogen can also result in elevated H_2S
- Rapid growth and increased biomass due to higher YAN?
  - Yeast strain variation



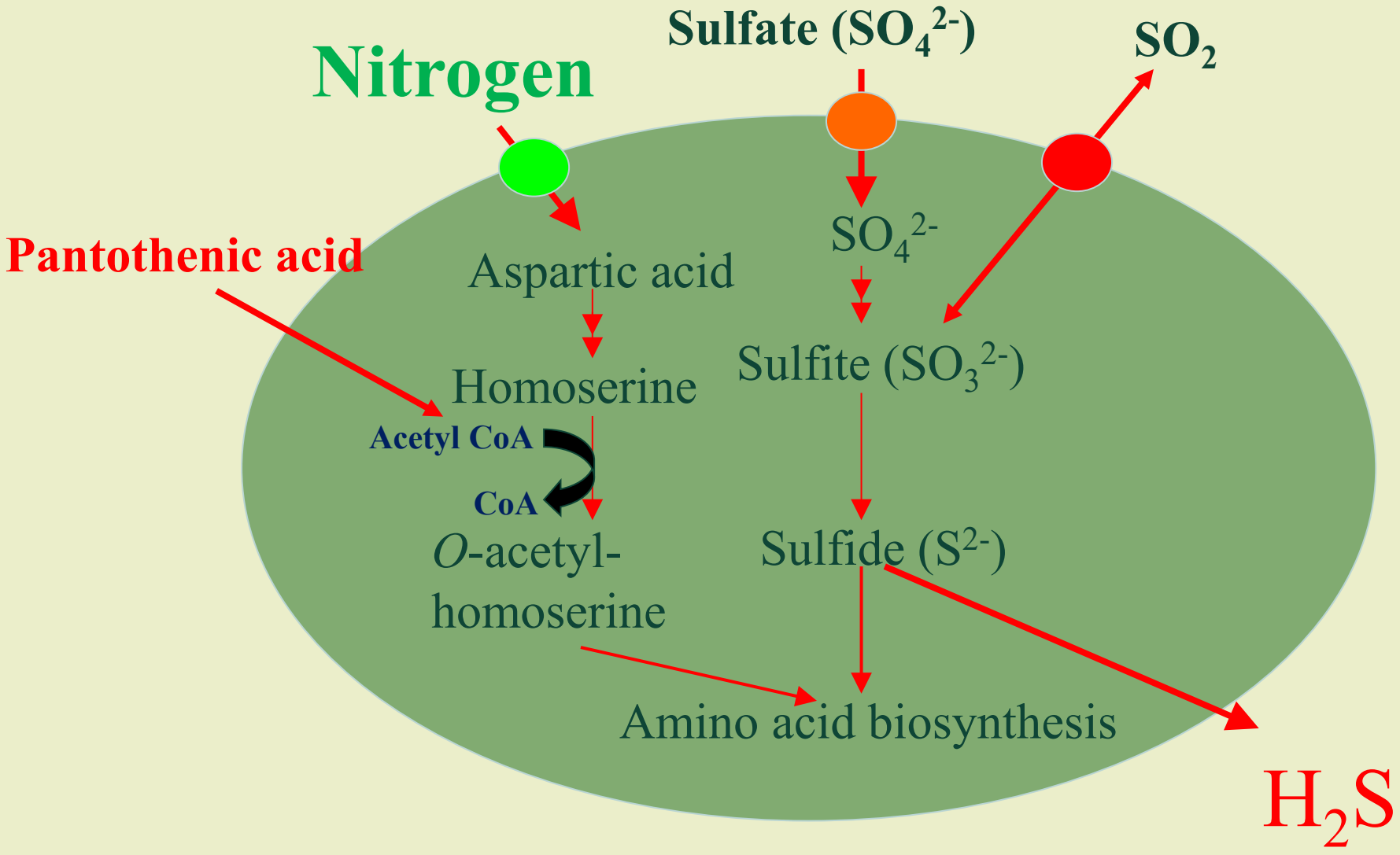
Uglianio et al. 2010

Nutrients - Vitamins

- Nitrogen content not well correlated with H_2S production
- Higher nitrogen can also result in elevated H_2S
- Rapid growth and increased biomass due to higher YAN?
  - Yeast strain variation
- **Other nutrient deficiencies**
  - **Pantothenic acid**



Wang et al. 2003



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Addition of air/oxygen

- Can stimulate yeast metabolism and reduce metabolic stress
- Prompts formation of cell membrane phospholipids
- May physically drive off some H_2S
- Recent studies suggest that impact of air/oxygen addition less to do with displacement of H_2S and more to do with reactions due to oxygen

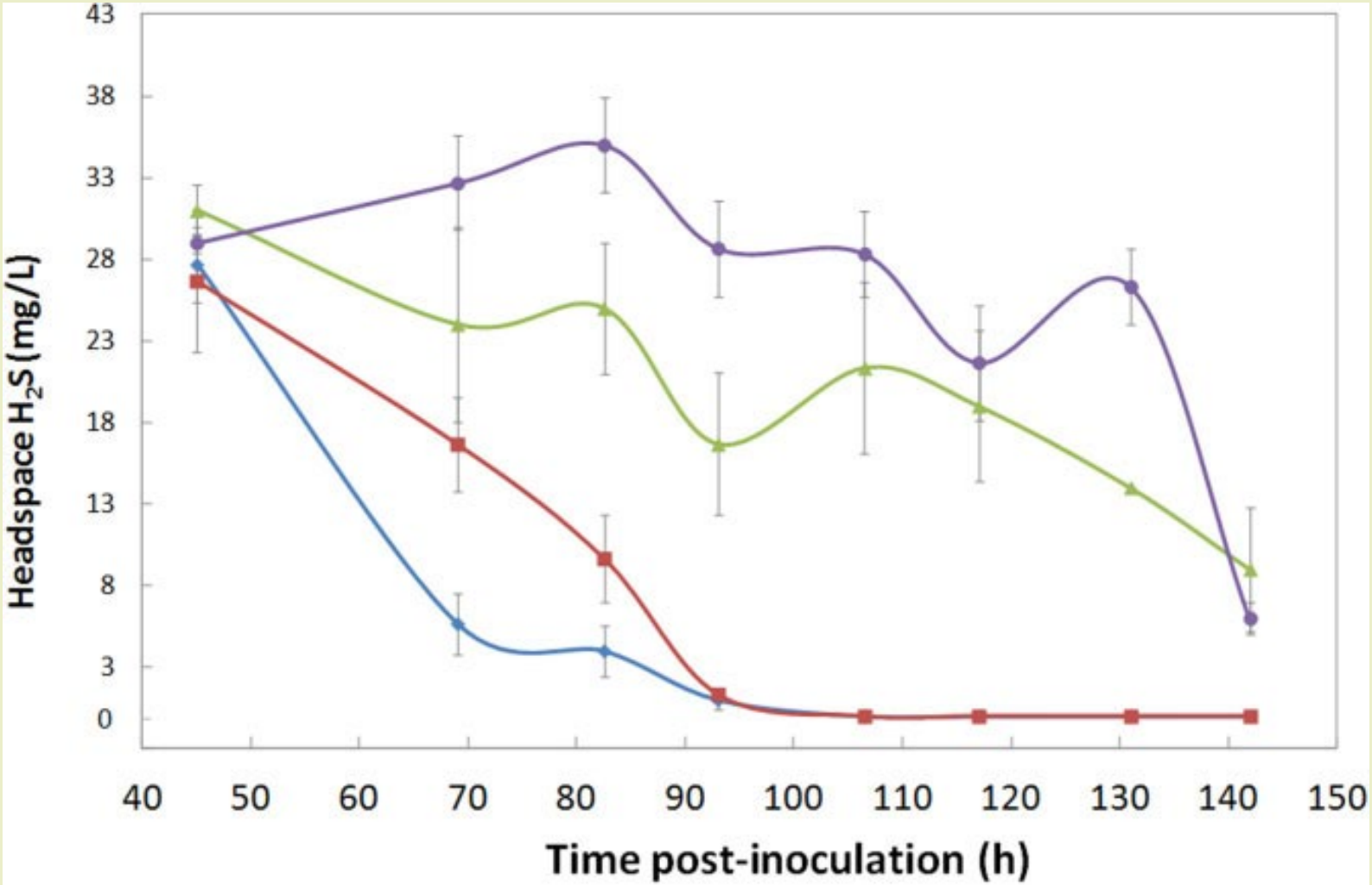

Figure 1. Effect of oxygenated [O240 (♦) and Air (■)] and non-oxygenated [Control (▲) and N2 (●)] treatments on the concentration of headspace hydrogen sulfide (H2S) in rotary fermenters measured with an environmental monitoring tube, loaded with lead acetate/copper sulfate (standard error bars).

Bekker et al 2016, AJGWR

- Application of oxygen, air, or nitrogen for 60 min every 12 hrs for 8 days using sparging stone
- Removal of H_2S was not primarily due to displacement

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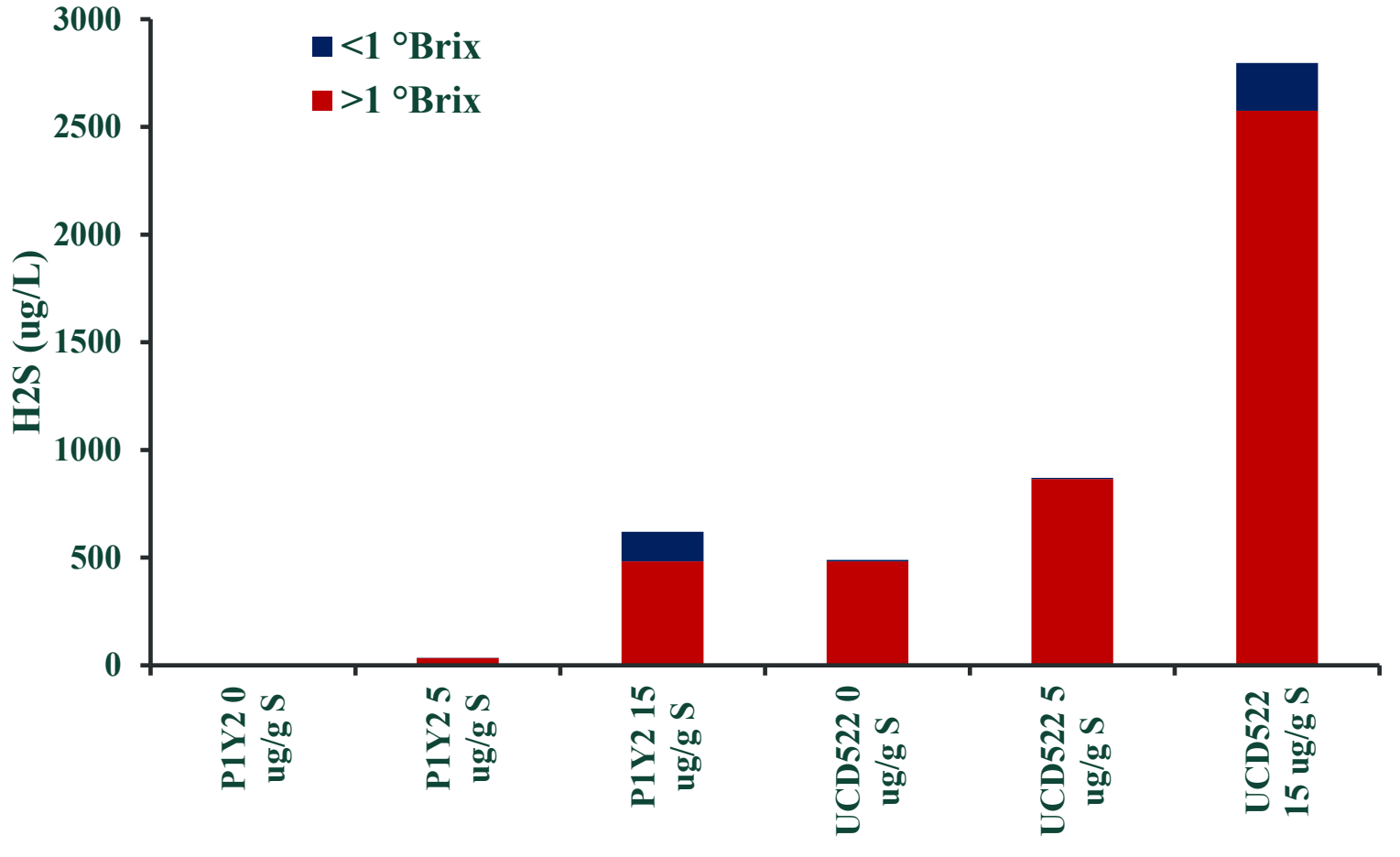
Use of oxygen

- Addition of oxygen
  - Early addition of air/oxygen during first 3-4 days of fermentation
  - Most efficient way is via a venturi during pump-overs or sparging stone
  - Rack and return less efficient and loss of H_2S mainly through ‘sparging’ rather than addition of oxygen

H_2S from elemental sulfur

- Elemental S common fungicide
- Residual S on grapes can cause H_2S issues during fermentation
  - Greater than1 ug/g can lead to an increase in H_2S
- Conversion of S to H_2S under reductive conditions of fermentation
- Formation of polysulfides



- S increased total H_2S
- High S resulted in production of H_2S late in fermentation
- Yeast strain interaction

Adapted from Kraft et al. 2023

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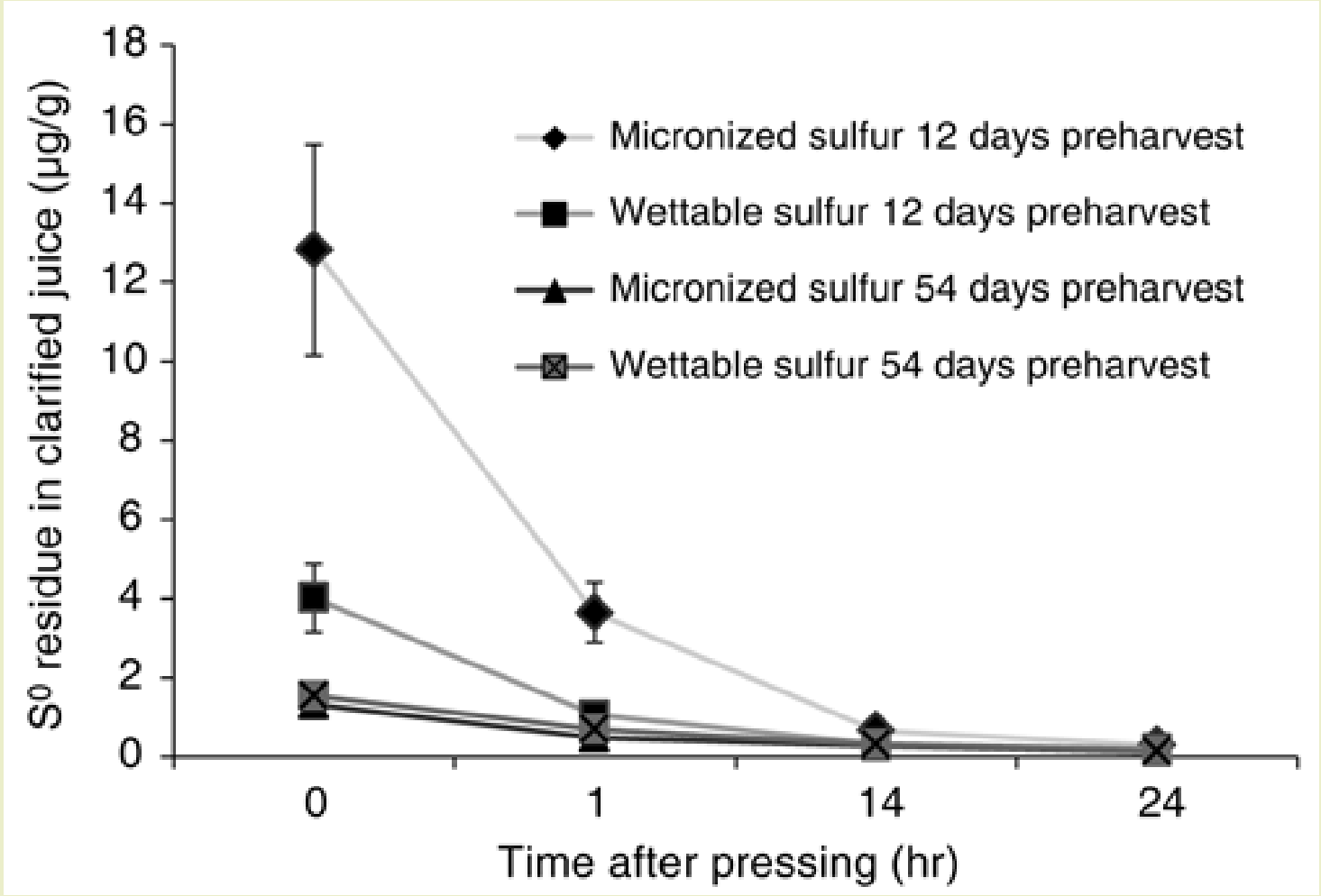
How do I prevent elemental S causing H_2S problems?

- No S applications post-veraison
  - Microthiol has longest residual
- Options once grapes are at the winery
  - Cold settling of whites juices is effective way to remove elemental sulfur residuals



In Cornell study 14 hrs settling was sufficient to remove 95% of residual S

Kwasniewski et al. 2014 AJEV

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Reducing H_2S formation

- Choice of yeast strain
  - A good starting point, particularly for historically reductive lots
  - Even low levels of H_2S may cause reductive issues post-fermentation
- Nutrition
  - Balance is important
  - Low or High YAN can be problematic
  - May need more than just N – complex nutrients
  - Split additions – Organic N first followed by DAP
- Avoid elemental S applications post-veraison

How do H_2S and MeSH disappear during storage?

And how can they re-form?

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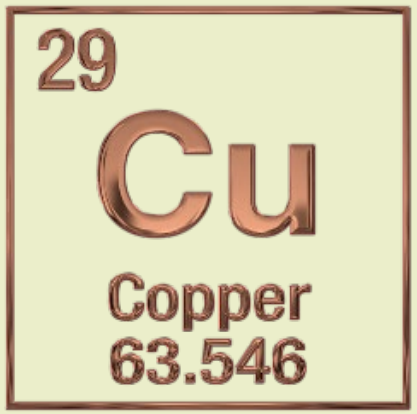
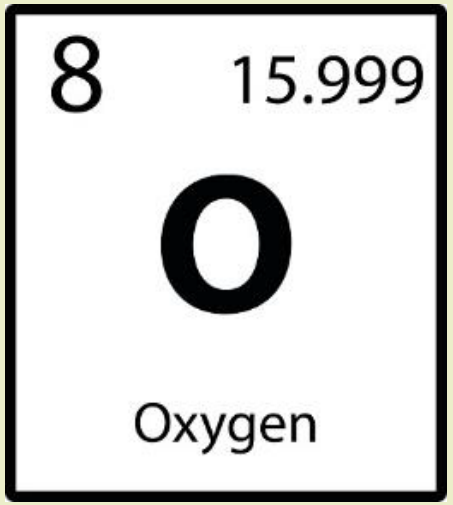
Fermentation ends, you've got a stinky wine - now what? Typical approaches

Copper salt addition



Aeration (splash-racking, bubbling in air, etc..)



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“I must leave, give me the take-home now!”
“What’s better, aeration or copper?”

- **Short answer:**

Both can likely lead to similar acceptable sensory outcomes, if done early

But, there’s not a lot of head-to-head comparisons in the literature, and what’s available is confusing!

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“I must leave, give me the take-home now!”
“What’s better, aeration or copper?”

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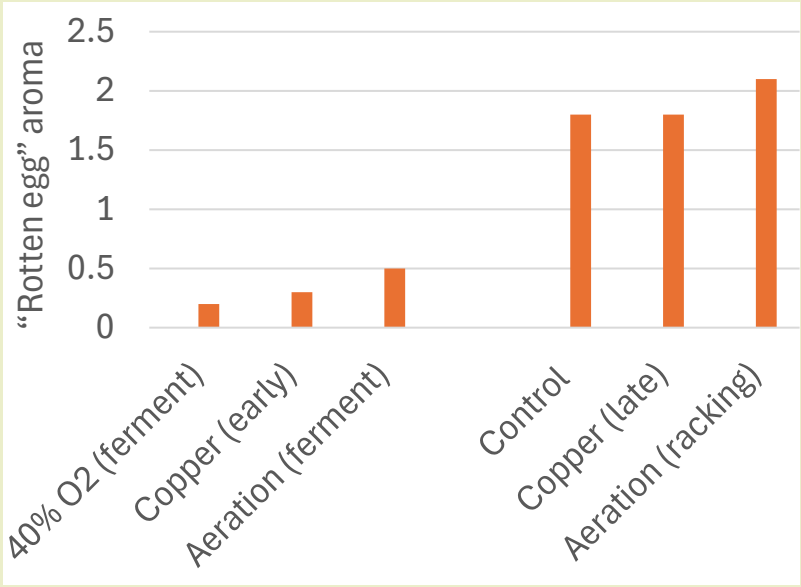
But, there’s not a lot of head-to-head comparisons in the literature, and what’s available is confusing!

At right: “rotten egg” aroma following 12 mo storage of different Shiraz treatments



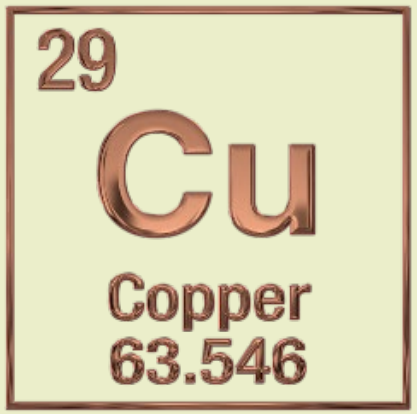
Early copper is best for limiting “reduced” aromas;
Late copper has no effect (makes some sense)

Aeration results in no decrease in “rotten egg”(??)
Aeration does not immediately decrease H_2S (??)

Bekker, et al *AJWGR* **2016**

What happens if we add copper to a wine with free sulfhydryls?

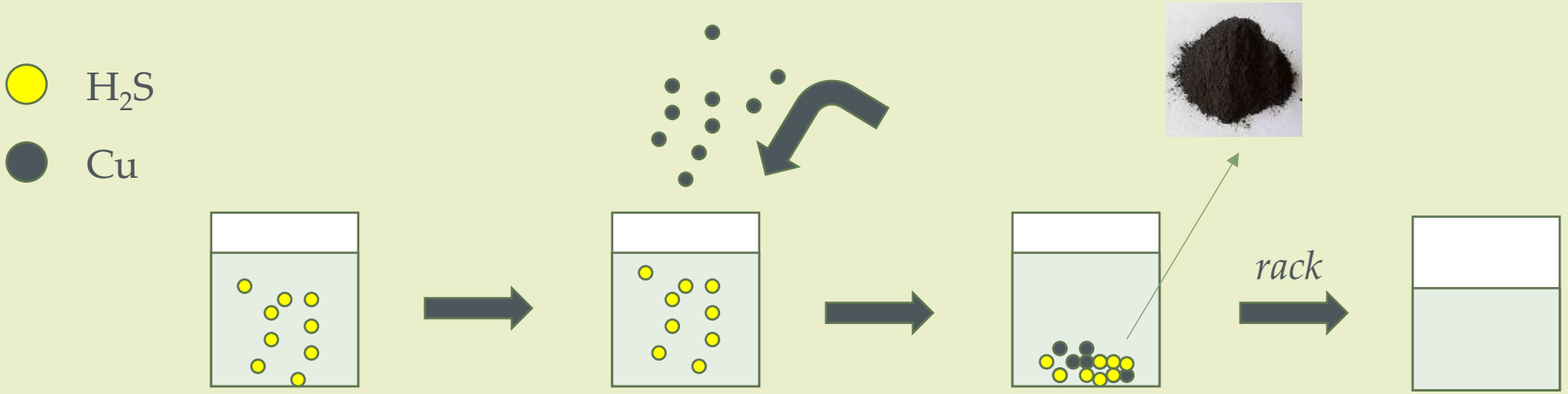
Copper salt addition



Aeration (splash-racking, bubbling in air, etc..)

Copper sulfhydryls precipitate in simple ethanol-water systems

In a simple ethanol-water system, insoluble copper-sulfhydryl precipitates form from H_2S and Cu(II)



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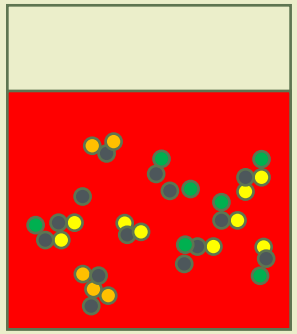
In real wine: stable, dispersed Cu-S nanoparticles appear to form

- H_2S
- Thiols
- Organic acids
- Cu



Real wine following Cu addition

No (or little) precipitation

Add Cu to a real wine with H_2S (Clark, et al, *Food Chem* **2016**)

Following storage, form stable nanoscale (10-100 nm) particles

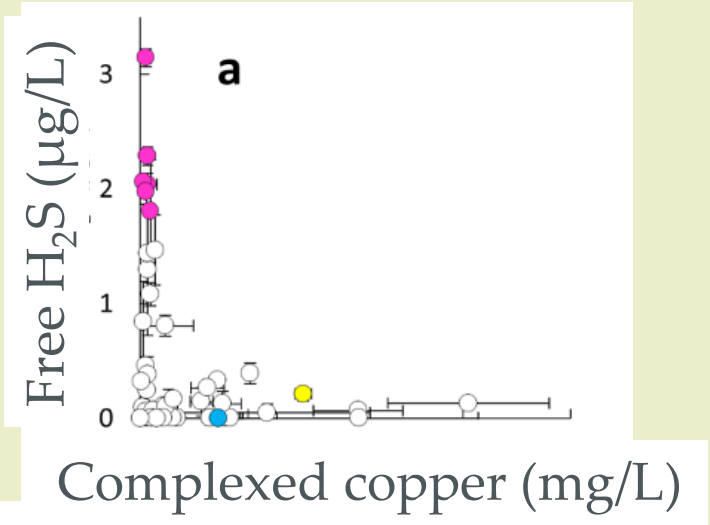
Complexes do not precipitate, <10% removed by filtration

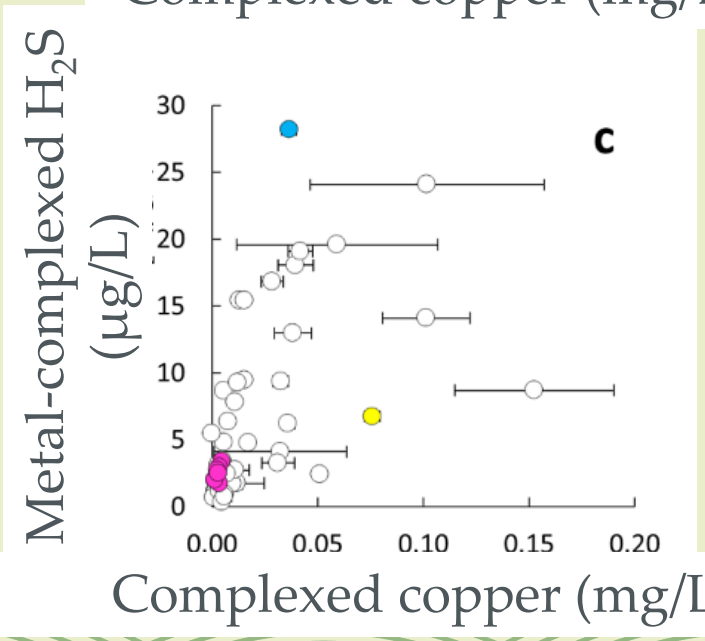
Proposed terminology (Andy Clark, Charles Sturt University)

Type I: Cu(II) - organic acids
Type II: Cu (I) - thiols
} Binds H_2S and MeSH; prevents free forms at >0.03 mg/L

Type III: Cu (I) - sulfides
} Majority of Cu; does not bind H_2S and MeSH

And, “free”, not “copper-bound” sulfhydryls contribute to aroma





Top left:

Wines with reductive character (wines in pink) have . .

- High free H_2S (> 2 µg/L)
- High free MeSH (> 4 µg/L)
- Low complexed Cu (< 0.03 mg/L)

Bottom left:

Wines with reductive character (wines in pink) have . .

- Low metal-complexed H_2S (< 5 µg/L)

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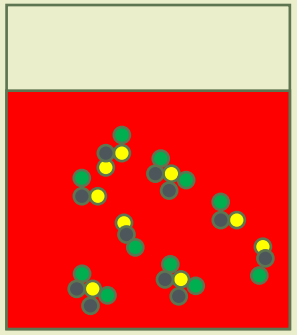
Zhang, et al *J Ag Food Chem* **2024**

Copper-sulfhydryls in wine: Could they be a source of sulfhydryls?

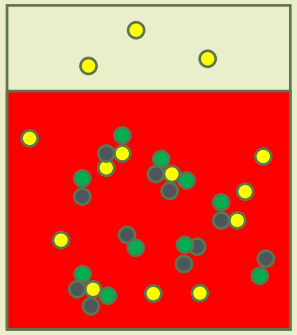
Wine w/ Cu-sulfhydryls



(little odor)

Months long anoxic bottle storage

Free sulfhydryls???



(stinky?)

YES, this happens! (Franco-Luesma, et al *J Ag Food Chem* **2016**)

NO, it doesn't! (Zhang, et, al *Food Chem* **2022**)

(I think the evidence is stronger for "No, it doesn't"

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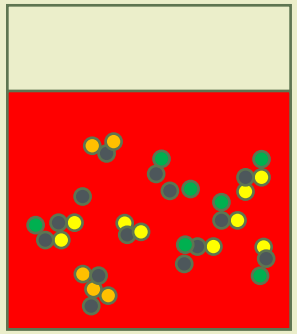
How about prophylactic addition of copper at bottling? Could that react with released sulfhydryls?

- H_2S
- Thiols
- Organic acids
- Cu



Real wine following Cu addition

No (or little) precipitation

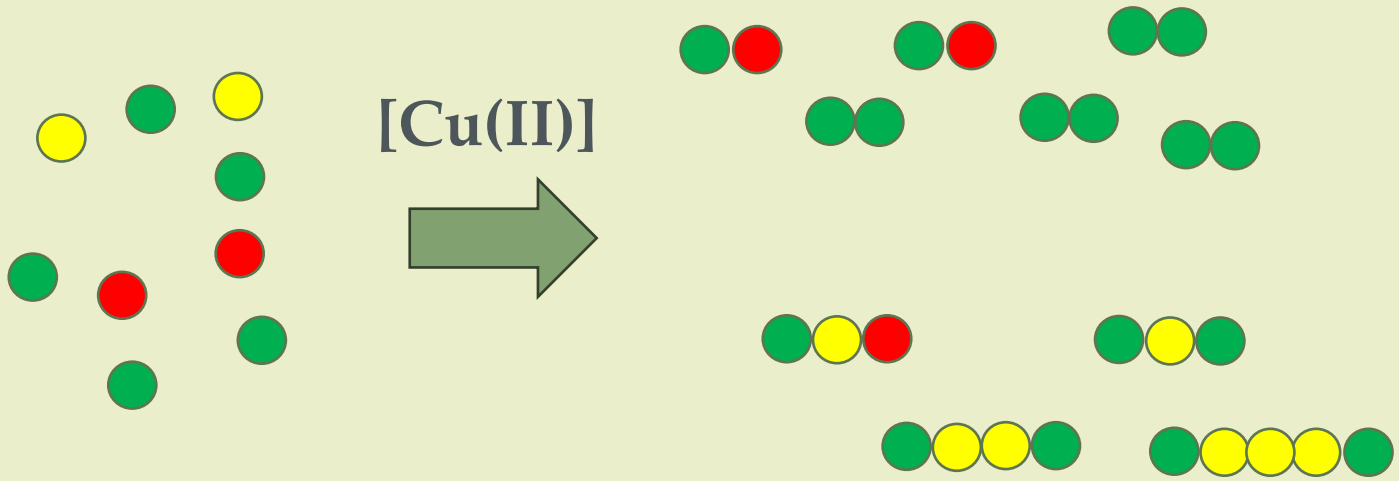
The problem: Type I/II Cu become Type III Cu during storage (doesn't bind) (Zhang, et al, *Food Chem* **2022**)

How long does this transformation take? Half-life is 1-2 months at room temperature

So, addition at bottling could provide protection for ~6 months

The other product of adding Cu salts: *Disulfides and polysulfides*

- H_2S or -S-
- Glutathione (GSH, GS-) or other non-volatile sulfhydryls
- MeSH or MeS- (methyl mercaptan)



Sulfhydryls

Disulfides
(one S-S bond)

Polysulfides
(more than one S-S bond)

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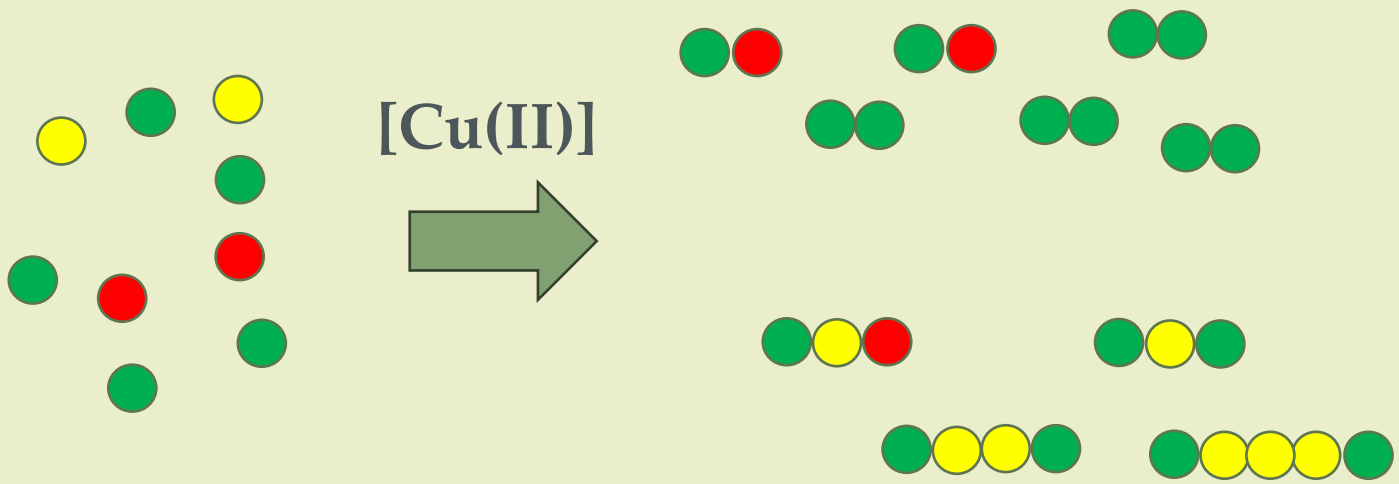
The other product of adding Cu salts: *Disulfides and polysulfides*

- These products are non-volatile, non-odorous
- Symmetric volatile disulfides are not favored!



Disulfides
(one S-S bond)

Polysulfides
(more than one S-S bond)

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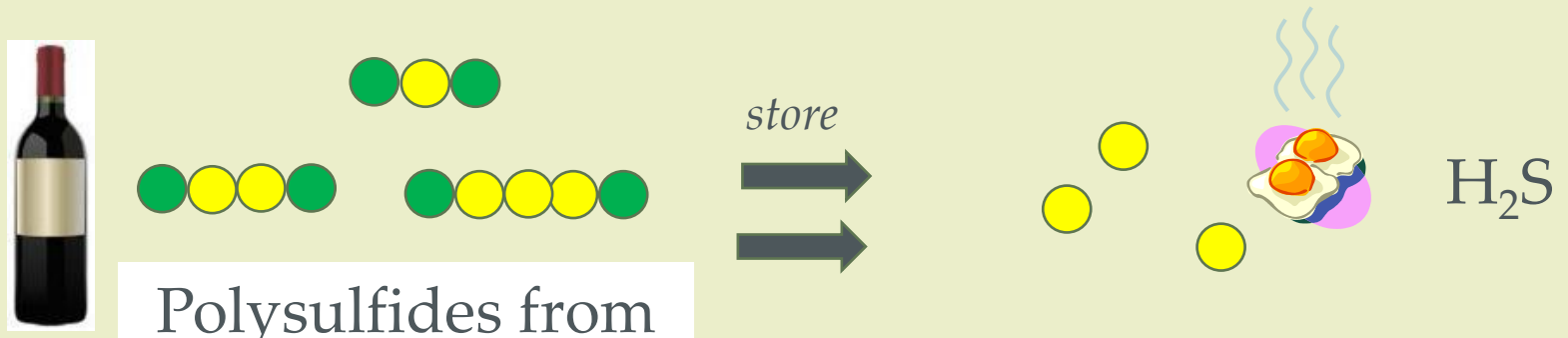
Another polysulfide source – degradation products from elemental S in late sprayed grapes

S^0 pre-fermentation (µg/L)	Free H_2S at bottling (µg/L)*	Free H_2S at 3 months (µg/L)
0	nd	nd
20×10^3	nd	2.4 (±0.2)
100×10^3	nd	10.0 (±0.9)

* Wines were sparged post-fermentation to remove H_2S



Polysulfides from elemental sulfur

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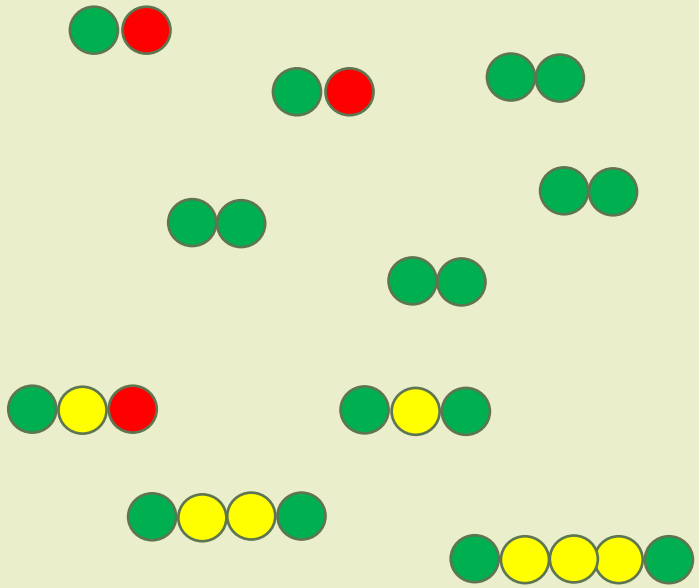
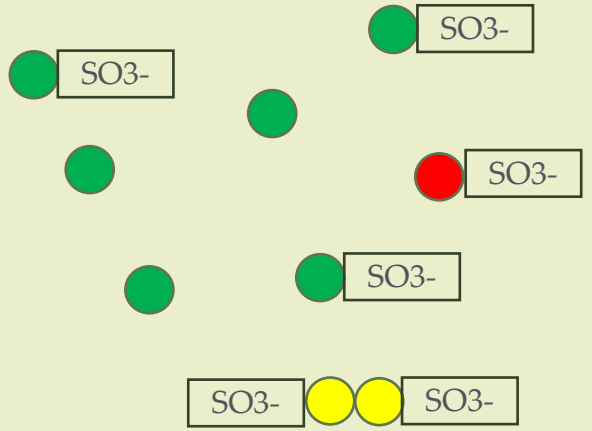
Jastrzembski, et al **2017** *J Ag Food Chem*

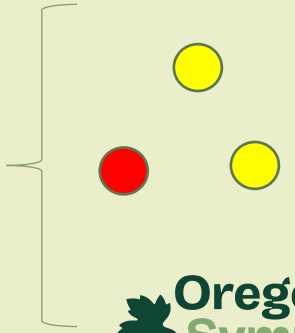
Established: H_2S and other volatile sulfhydryls reform in presence of sulfites



Reaction w/ bisulfite during bottle storage



Free H_2S and MeSH



Bekker, et al *Food Chem* **2018**
Kreitman, et al *Crit Rev. Food Sci Nutr* **2019**

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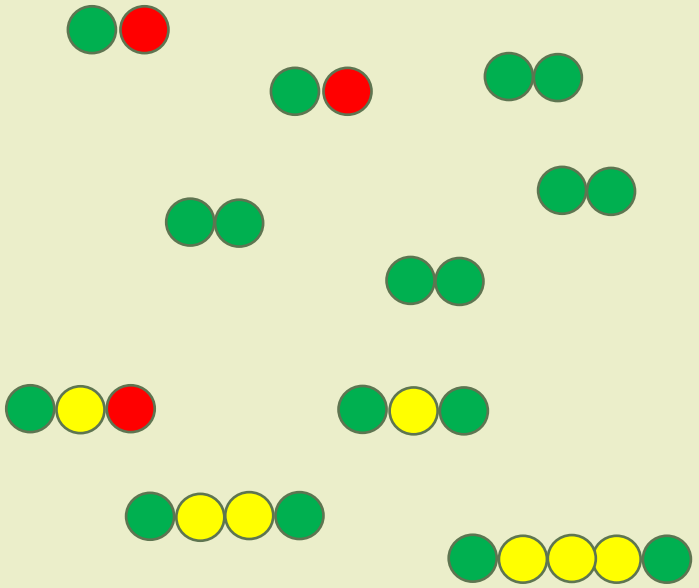
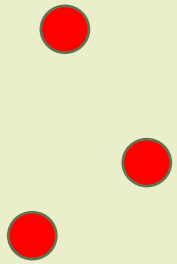
The same pathway can explain the appearance of other thiols



Reaction w/ bisulfite during bottle storage

Benzenemethanethiol

Furanmethanethiol (struck match)



Bekker, et al *J Ag Food Chem* **2025**

How long will these reactions take at room temperature and ~30 mg/L free SO_2?

- For Glutathione trisulfide? Half-life of **1-2 months**
- For Glutathione-BMT? Half-life of a **few years**

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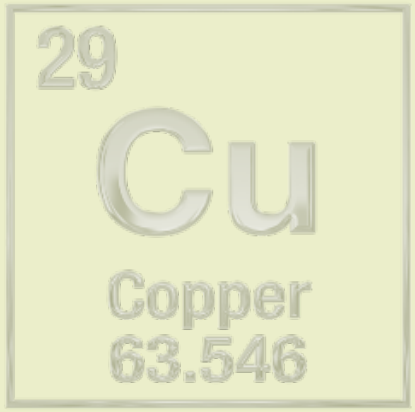
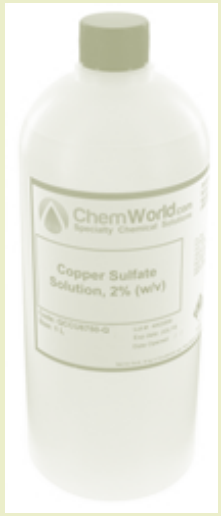
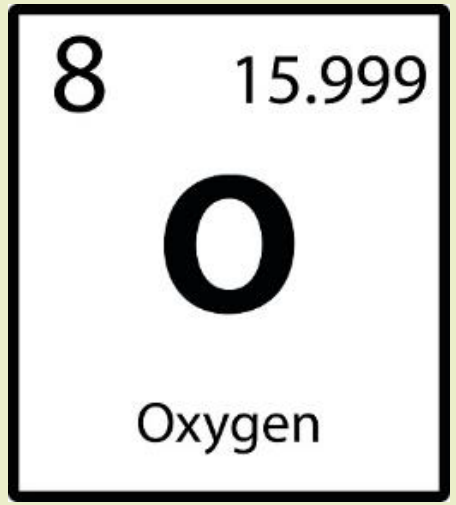
That’s what happens when you add copper. What about oxygen?

Copper salt addition





Aeration (splash-racking, bubbling in air, etc..)



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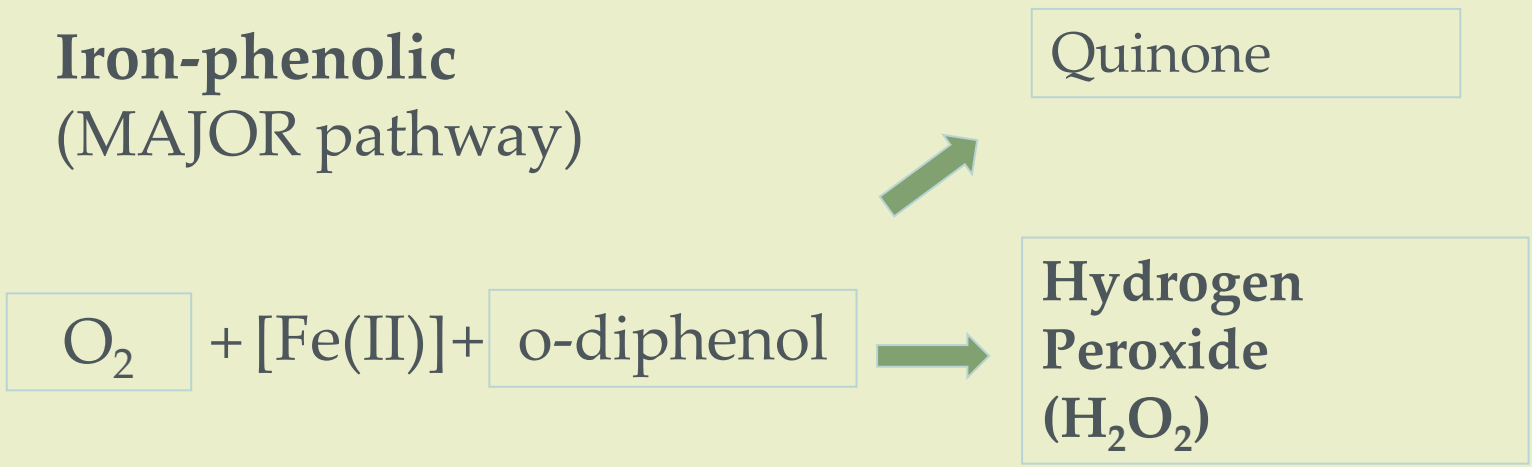
What happens to oxygen in a wine? The main pathway – "iron-phenolic"

Iron-phenolic
(MAJOR pathway)



If you've heard Dr. Andy Waterhouse (UC Davis, emeritus) speak about oxidation, you've heard about this pathway!

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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 → **Hydrogen Peroxide (H_2O_2)**

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

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

And, one of the intermediates (quinones) **irreversibly** reacts with sulfhydryls!

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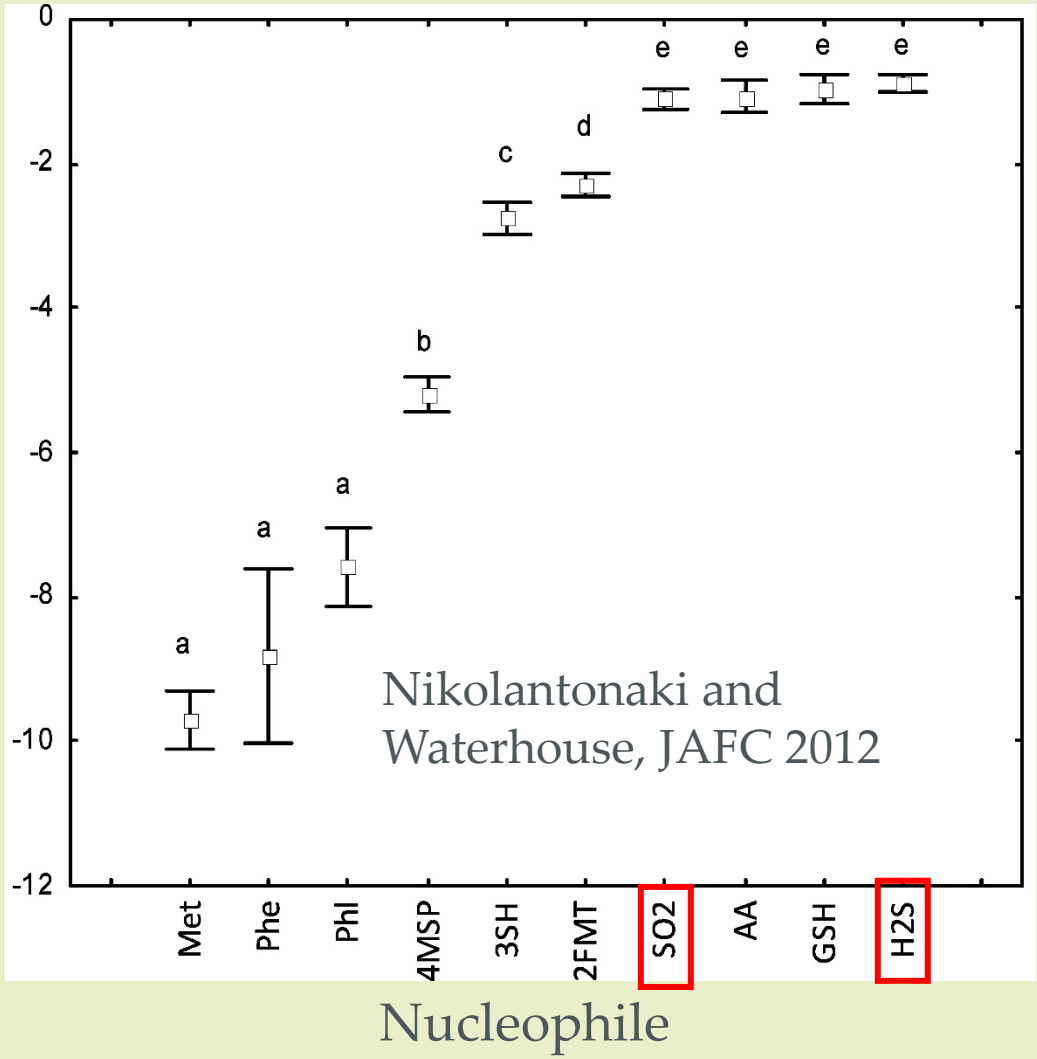
But, SO_2 will compete with H_2S for quinones



SO_2 and H_2S react w/ quinones at similar rates

But, there's 1000-fold higher SO_2 than H_2S in a typical packaged wine!

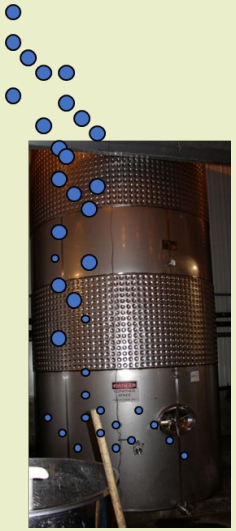
Outcome: this "quinone-scavenging" pathway only matters if . . .

i) The wine has very low free SO_2 (e.g. aeration immediately after fermentation)

ii) Or, a large fraction of free SO_2 is oxidized (e.g. 90% free SO_2 loss for 90% H_2S loss)

(Aha! Remember that earlier slide where aeration had no effect on H_2S and rotten egg?)

So, how else could aeration be decreasing H_2S and MeSH?



1) Entrainment (oxygen not required)

Two volumes of gas will remove ~50% of H_2S from one volume of wine at room temperature

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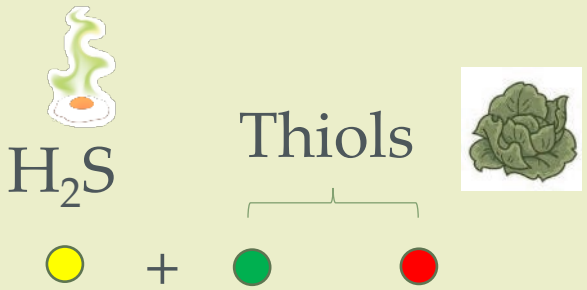
2) Metal-sulfhydryl pathway
(the MINOR oxidation pathway)



O_2

[Fe(II)]/[Cu(I)]

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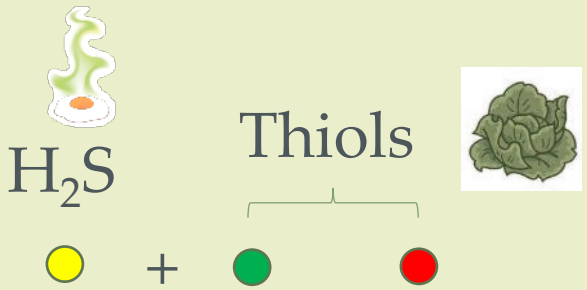
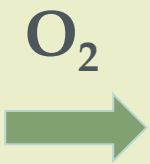
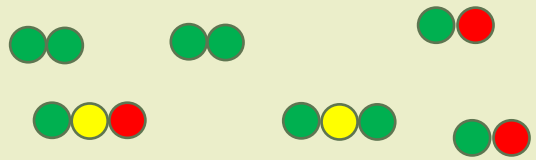




Mixed disulfides and polysulfides (negligible odor)

($RS\text{-}S_n\text{-}SR'$)



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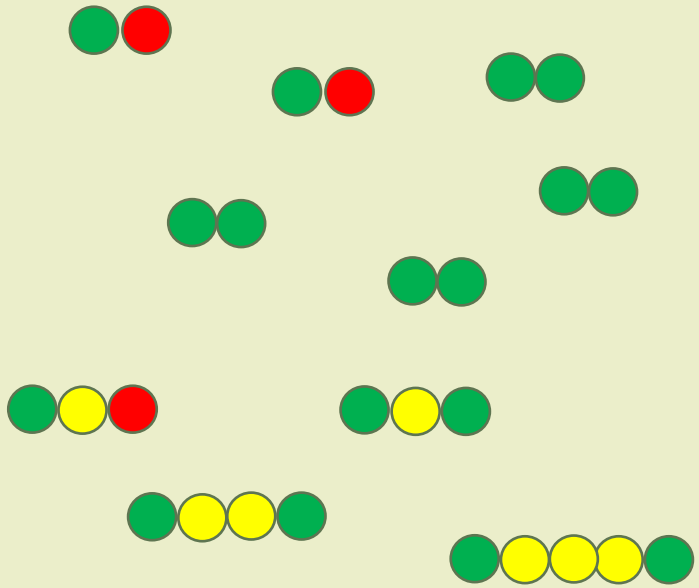
So, either Cu or O_2 addition can form di- and polysulfides …

Mixed disulfides and polysulfides (negligible odor)

($RS\text{-}S_n\text{-}SR'$)



Bekker, et al *Food Chem* **2018**
Kreitman, et al *Crit Rev. Food Sci Nutr* **2019**

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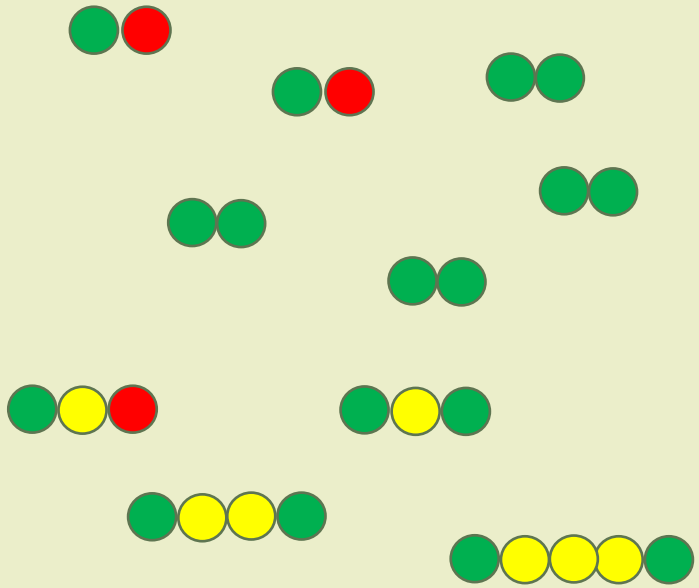
… and both can be effective in the short term, but risk reforming “reduced” aromas

Mixed disulfides and polysulfides (negligible odor)

($RS\text{-}S_n\text{-}SR'$)



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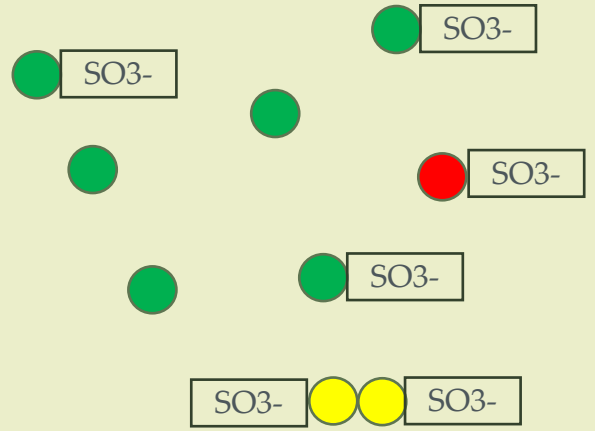
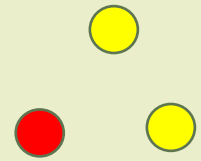


Free H_2S and MeSH



Bekker, et al *Food Chem* **2018**
Kreitman, et al *Crit Rev. Food Sci Nutr* **2019**

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“Wait . . . If treatments result in possible precursors, how do I treat a stinky wine?”

Test for residual copper, and remove with **PVI/PVP resin** if needed **(**Zhang, et al *Food Chem* **2021)**

- Still unclear if Cu-S complexes can reform volatile sulfhydryls, but they can catalyze formation of di-/polysulfides!

Early treatment reportedly more effective (Bekker, et al AJWGR **2016**)

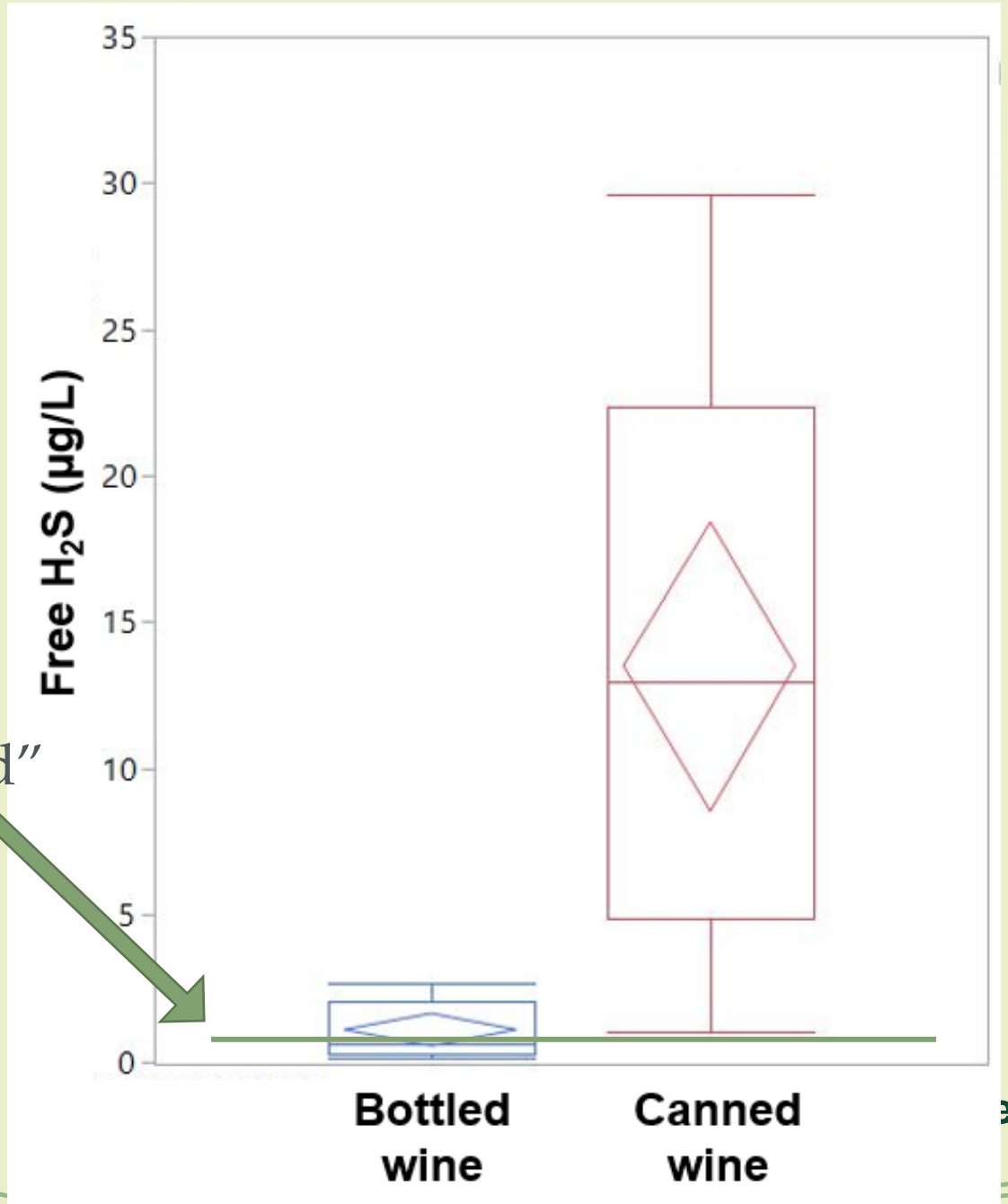
- Cu-S may co-precipitate
- Pre-sulfite aeration will favor irreversible reactions with quinones
- Di-/polysulfides will have time to react with bisulfite, if you form them

If it’s only **H_2S** . . .

Consider sparging with inert gas instead of adding Cu salts or O_2

One last source: why is H_2S higher in canned wines?

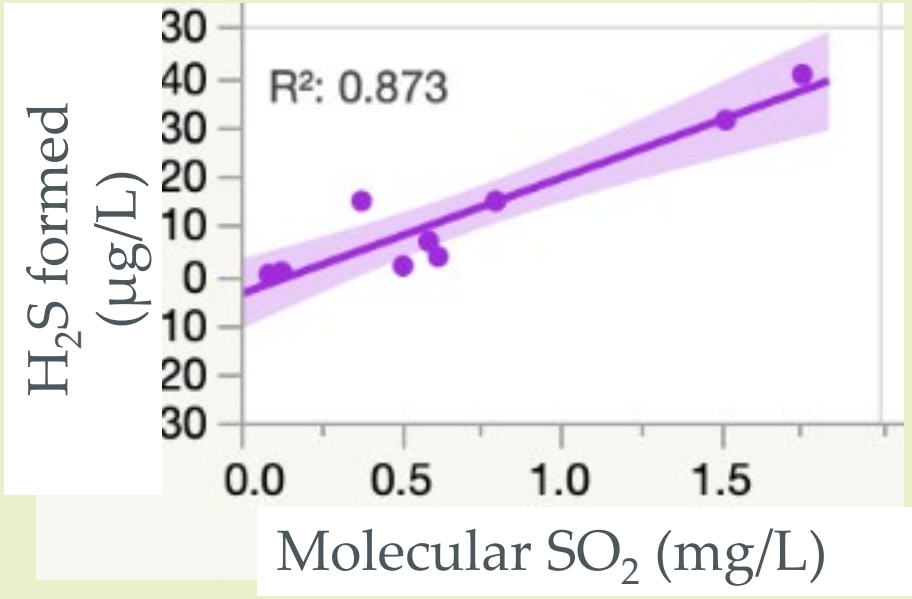
"Sensory threshold"



Allison, Montgomery, and Sacks;
AJEV-Catalyst, **2021**

Recent work by our group on cans→ H_2S is best predicted by molecular SO_2!

- High H_2S (> 10 µg/L) predicted by **molecular SO_2 > 0.5 mg/L**
- **Modest correlations with pH and Free SO_2**
- **Weak or no correlations** for Total SO_2, TA, ABV, Cu, Cl^-



$$3Al_{(s)} + SO_{2\,(aq)} + 6H^+ \rightarrow 3Al^{3+}_{(aq)} + H_2S_{(aq)} + 2H_2O$$

Montgomery, Allison, Goddard and Sacks, *Am. J. En Vit* **2023**

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And, there are so many unanswered questions

1) Can we direct sulfhydryls to react w/ quinones through the iron-phenolic pathway? Those products should be more stable.

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2) Are there valid approaches for predicting reduction issues? Note the **"ascorbic acid test"** should release both precursor classes; the validity of this test and others for predicting issues during storage is unclear

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1) Can we direct sulfhydryls to react w/ quinones through the iron-phenolic pathway? Those products should be more stable.

2) Are there valid approaches for predicting reduction issues? Note the **"ascorbic acid test"** should release both precursor classes; the validity of this test and others for predicting issues during storage is unclear

3) Anecdotally, H_2S and MeSH can react with other compounds to make even more potent sulfhydryls. How common is this in bottle? Can bound forms participate?

Today's talk – a review

- **Reduced sulfur compounds are critical to wine aroma**
  This can be undesirable compounds like H_2S, or desirable compounds like the tropical smelling "varietal thiols"

- **"Reduced aromas" are best correlated with suprathreshold free H_2S and MeSH**
  Causes: nutritional deficiencies, amino acid degradation, elemental S

- **Standard practices (aeration, Cu) to rid a wine of H_2S and MeSH can likely lead to similar outcomes, although there's more data in support of early Cu**
  In both cases, treatment could result in latent precursors (di/polysulfides) that stay in the wine. Recommendation . . . Bench trials! Treat early! Test for copper!