UC DAVIS VITICULTURE AND ENOLOGY




OXIDATION MANAGEMENT

ANITA OBERHOLSTER, COOPERATIVE EXTENSION SPECIALIST IN ENOLOGY

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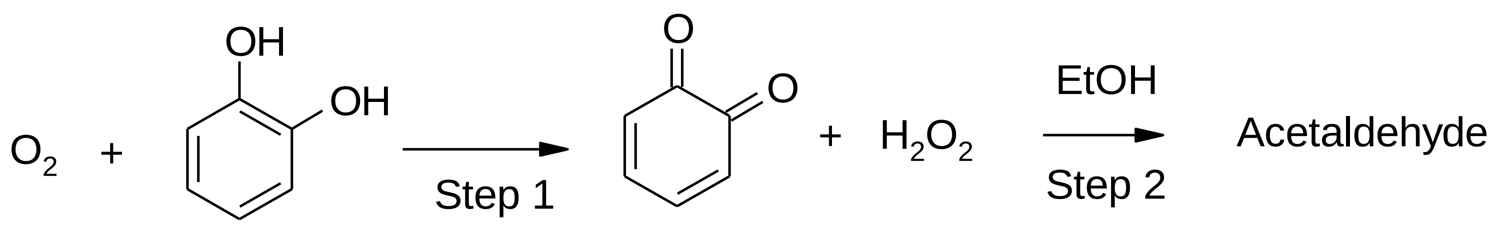
PRWCA WINERY SANITATION AND OXIDATION PREVENTION

Introduction

- Chemical oxidation
  - Metals
- Role of sulfur dioxide
  - Measurement
  - Antioxidant reactions
- Oxygen management
  - White wine
  - Red wine
- Micro-oxygenation
- Closure and bottle aging

Oxygen and Winemaking

- **Chemical oxidation**



- Oxygen reacts with phenolics to form quinones and hydrogen peroxide
- Quinones react with SO_2, other phenolics, thiols, others
- Hydrogen peroxide oxidizes ethanol to acetaldehyde (if no SO_2)

Role of metals

- **Metals can act as catalysts in oxidation reactions**

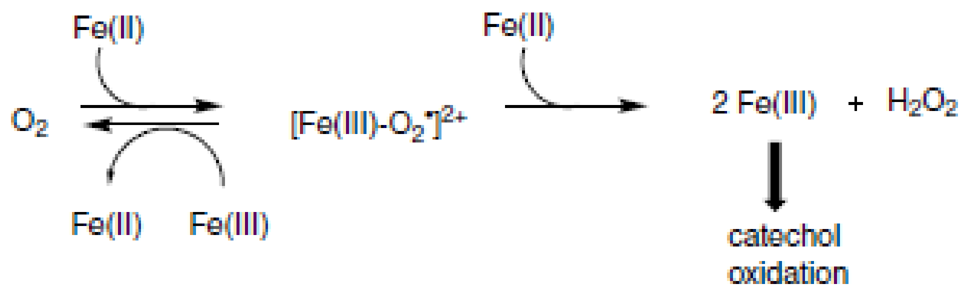


Figure 24.3 A two-step process for formation of o-quinone from an o-diphenol, showing potential roles of metal catalysts. Source: Danilewicz 2013 [19]. Reproduced with permission of AJEV

Understanding Wine Chemistry (2016) Waterhouse, Sacks, Jeffery

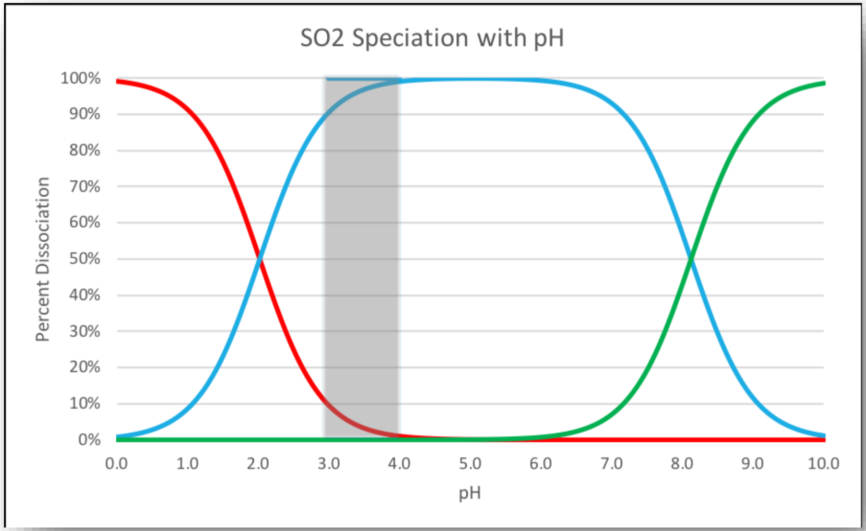
SO_2 Dissociation

$SO_2 + H_2O \rightleftharpoons HSO_3^- + H^+ \rightleftharpoons SO_3^{2-} + 2H^+$

Molecular — pKa = 2.0 — Bisulfite — pKa = 8.1 — Sulfite

(0.5-0.8 mg/L)

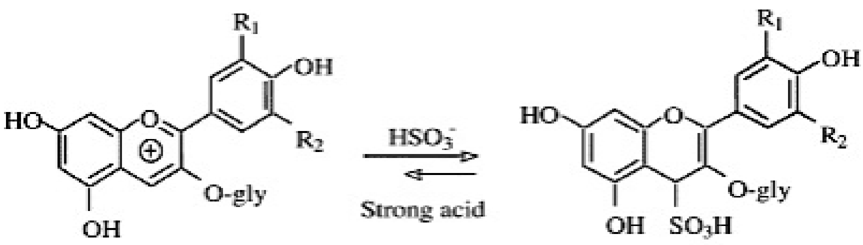
- Antimicrobial
- Antioxidant – Quenches H_2O_2
- Reacts with quinones
- Binding/addition reactions
- Inactivates polyphenoloxidase (PPO)
- Negligible amounts in wine
- Dissociation influenced by ethanol
  - Dielectric effect
  - ~13% ethanol
    - pKa_1 (~2.0)

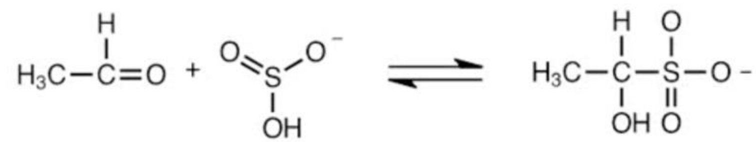


(Butler, 1964) Ionic Equilibrium: Solubility and pH Calculations
(Usseglio-Tomasset, 1984) *La prima costante di dissocianzione dell'acido solforoso*

Free and Bound SO_2

- **Bisulfite anion binds with carbonyls, particularly acetaldehyde**





“Truly Free”

Free

Weakly Bound (metastable)

Bound

Strongly Bound

SO_2 HSO_3^- SO_3^{2-}

SO_3^- bound to acetaldehyde

SO_3^- bound to phenolics, colors, sugars, proteins

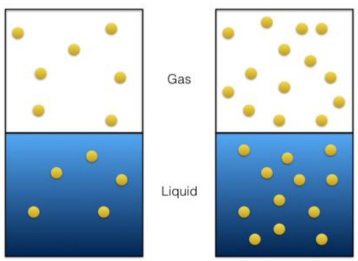
Total

Anthocyanins
Acetaldehyde
Pyruvate
α-ketoglutarate
Acetoin
Galacturonic acid
Sugars
Proteins

Diacetyl
B-Damascenone
B-Ionone
Hexanal
(E)-2-Pentenal
(E)-2-Nonenal

An alternative approach - Headspace

$$VP_{SO2} = K_H[SO_2]$$



$SO_{2\,(g)}$ $\rightleftharpoons$ $SO_{2\,(aq)}$

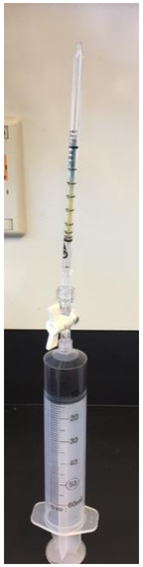
Binder-HSO_3^- $_{(aq)}$ $\rightleftharpoons$ HSO_3^- $_{(aq)}$

$SO_{2\,(aq)} \rightleftharpoons HSO_3^-{}_{(aq)} \rightleftharpoons SO_3^{2-}{}_{(aq)}$

Headspace SO2 methods

- **Headspace Gas Detection Tubes (HS-GDT) (Coelho et al. 2015)**

1) 10 mL sample) → **2)** 50 mL Headspace → **3)** Equilibrate → **4)** Expel headspace → **5)** Record color change



6) Back-calculations
- Alcohol
- pH
- Temp.

Headspace-Gas Detection Tubes

- **Strengths**
  - **Fast (5 minutes) – provided you already know alcohol and pH**
  - **Robust**
  - **Accessible to just about anyone (perfect for home-winemakers)**
  - **Can run multiple samples in batches**
  - **Does not disturb equilibrium (no pH shift)**
- **Weaknesses**
  - **Difficulty in endpoint determination**
  - **Potential cross sensitivities**
  - **No option for automation**
  - **Difficulty in controlling temperature (water bath)**

Gas Chromatography (HS-GC-SCD)

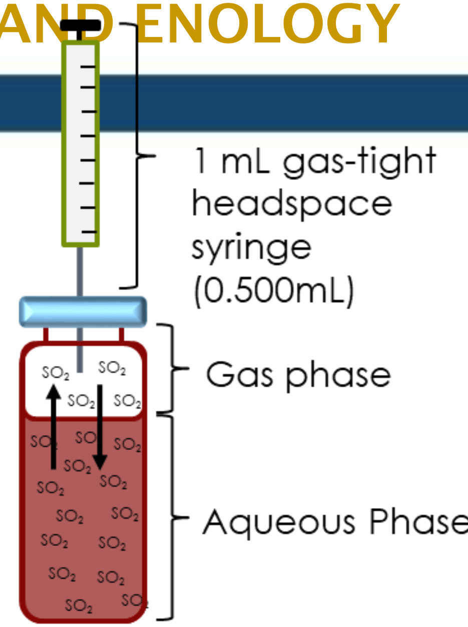
PAL RSI 85 Robotic Autosampler (With Static Headspace Syringe)

Agilent 7890B Gas Chromatograph (GC)

Agilent 8355 Sulfur Chemiluminescence Detector (SCD)

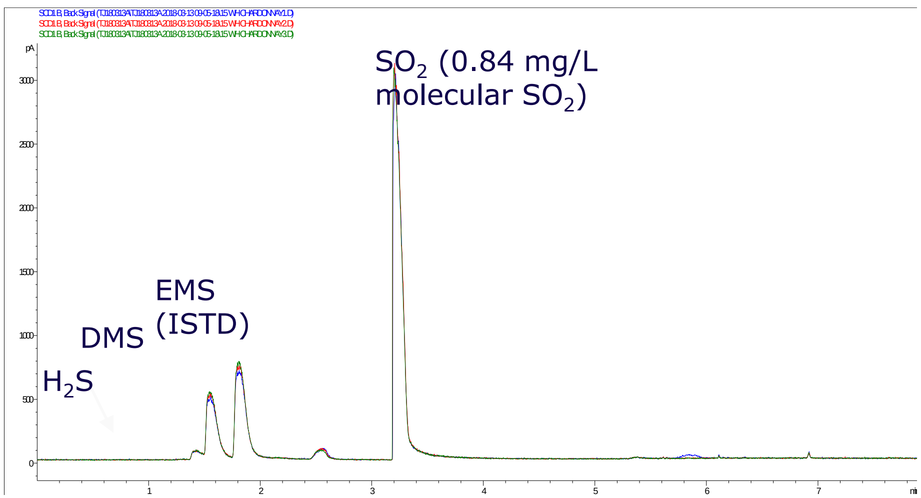
- Unpublished method developed by Waterhouse et al.

Example of data analysis (HS-GC-SCD)



- **14.3% ethanol (true pKa_1 = 2.20)**
- **3.45 pH**
- **25.0˚C**

- **Henderson-Hasselbalch**
  - **→ 15.9 ppm Truly Free SO_2**



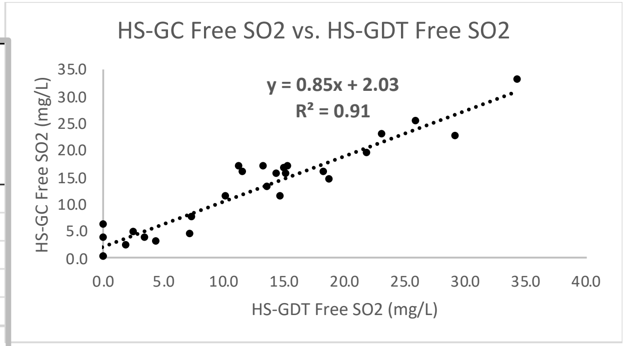
3 analytical replicates
% RSD = 2.15%

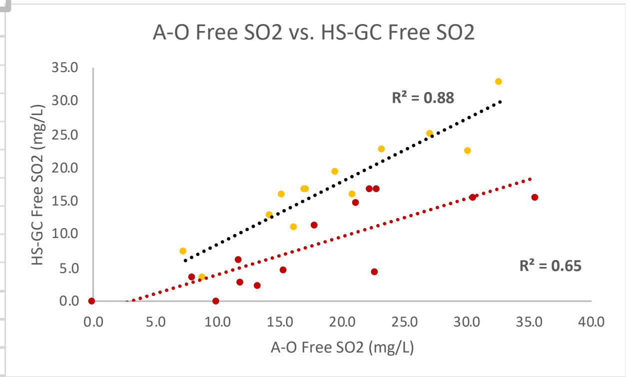
- LOD = 0.033 mg/L molec. SO_2
- LOQ = 0.067 mg/L molec. SO_2

9-20 mg/L difference

SO_2 method comparison

Sample ID	Wine Type	A/O (mg/L)	Ripper (mg/L)	HS-GC (mg/L)	HS-GDT (mg/L)	"Weakly Bound" (Metastable) SO2 (mg/L)	Percentage in Metastable complexes
2015 California Red Blend	Red	35.6	44.6	15.4	14.3	20.2	57%
2014 Sonoma County Zinfandel	Red	22.7	22.7	4.4	7.1	18.3	81%
2016 Monterey County Pinot Noir A	Red	30.6	36.7	15.5	15.1	15.1	49%
2015 Paso Robles Blaufrankisch	Red	13.3	16.3	2.2	1.9	11.2	84%
2015 California Cabernet Sauvignon	Red	15.4	19.8	4.5	2.4	10.9	71%
2014 Napa Valley Merlot	Red	10.0	15.5	0.0	0.0	10.0	100%
2013 Alexander Valley Zinfandel	Red	11.9	15.2	2.8	4.4	9.2	77%
2015 California Chardonnay	White	30.1	31.8	22.4	29.1	7.7	26%
2015 Central Coast Merlot	Red	17.8	23.0	11.2	10.1	6.5	37%
2016 Central Coast Rose	Rose	21.1	24.6	14.6	18.8	6.5	31%
2016 Monterey County Pinot Noir B	Red	22.8	30.4	16.9	11.3	5.9	26%
2013 Paso Robles Merlot	Red	11.7	15.9	6.1	0.0	5.6	48%
2015 Central Coast Pinot Noir	Red	22.2	28.4	16.7	15.0	5.5	25%
2014 California Moscato	White	8.8	9.5	3.5	3.4	5.4	61%
2015 Alexander Valley Fume Blanc	White	16.2	17.5	11.2	14.6	5.0	31%
2014 Napa Valley Chardonnay	White	20.9	25.7	15.9	18.2	4.9	24%
2013 California Zinfandel	Red	8.0	10.6	3.5	0.0	4.5	56%
N.V. Brut Sparkling	White	27.1	25.7	25.2	25.9	1.8	7%
2014 Central Coast White Blend	White	14.3	14.3	13.0	13.6	1.3	9%
2015 California Sauvignon Blanc	White	23.3	24.8	22.8	23.1	0.5	2%
2015 Napa Valley Chardonnay	White	17.2	18.1	16.8	15.3	0.4	2%
2015 Napa Valley Moscato	White	17.1	17.3	16.8	13.2	0.3	2%
2014 Central Coast Viogner	White	19.5	23.1	19.3	21.8	0.2	1%
2012 Napa Valley Port	Red	0.0	6.4	0.0	0.0	0.0	0%
2016 Sonoma County Moscato	White	7.4	10.5	7.5	7.3	0.0	0%
2014 Central Coast Chardonnay	White	32.7	34.5	32.9	34.3	0.0	0%
2015 Central Coast Viogner	White	15.2	17.3	15.9	11.5	0.0	0%
	Average Std. Dev. (mg/L)	0.8	0.7	0.4	1.4		
	Average % RSD	4.6%	3.8%	3.4%	11.8%		





%RSD 4.6% 3.8% 3.4% 11.8%

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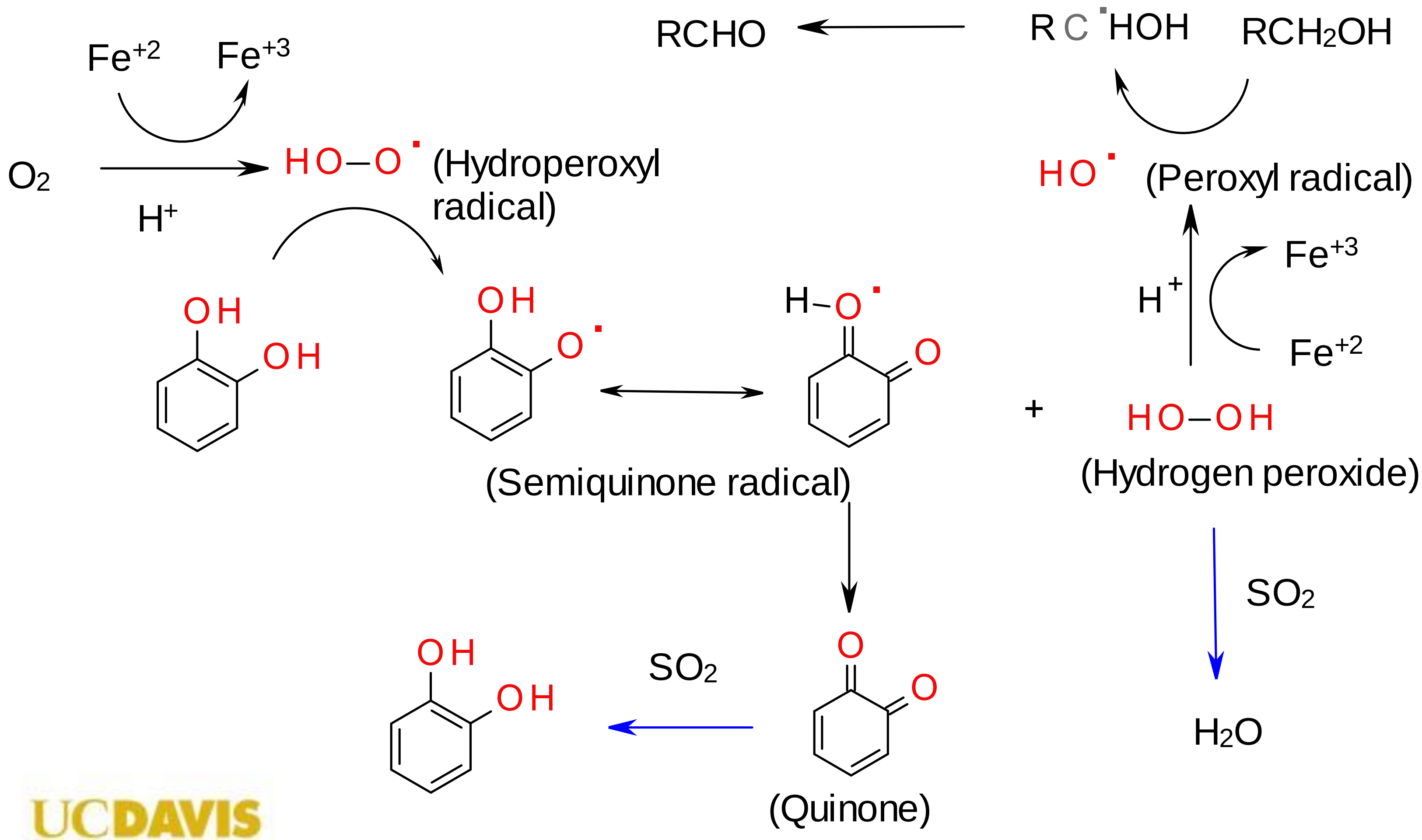
HS-GC Analysis

- **Strengths**
  - Fast (8-10 minutes) – provided you know alcohol and pH
  - AUTOMATED
  - Repeatable (%RSD < 3%)
  - No interferences
  - Does not disturb SO_2 equilibria-truly free SO_2
- **Weaknesses**
  - Skilled (GC) operator required
  - Needs periodic maintenance (SO_2 is acidic and shortens the column life)
  - Alcohol level, pH, temperature needed
  - High up-front cost ($70-120K)
    - S detector, headspace autosampler

SO_2 and O_2

- Theoretical Balanced Reaction
  - $2\ SO_2 + O_2 \rightarrow 2\ SO_3$
- Mass ratio, 4 SO_2 to O_2
- So, if 4/1 ratio of consumption (mg/L)
  - Balanced reaction
  - Complete protection from oxidation
- Ratio below 4: oxidation of other components!

Antioxidant Reactions of SO_2



O_2 management

- Wine is saturated with oxygen at ~6-8 mg/L
- How much oxygen can a wine take before it is compromised?
  - Matter of winemaking style and matrix

O_2 management

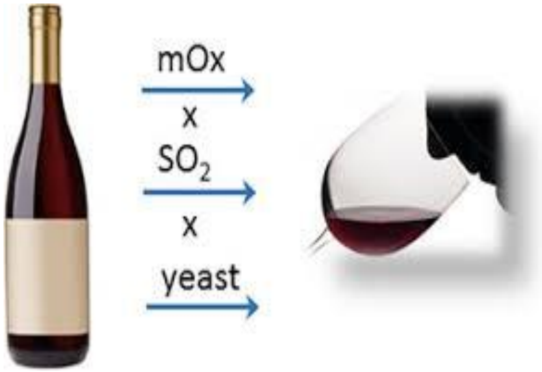
- Winemaking
  - O_2 good for yeast viability
    - Both yeast and bacteria grow under aerobic conditions
- Excess O_2 removed by CO_2 during fermentation
- Too little O_2 can result in reductiveness
- Too much O_2 can result in oxidation
  - Loss of varietal character for aromatic whites

O_2 management

- White Winemaking
  - Protective inert gas blankets and SO_2 have shown to preserve thiols and glutathione (GSH), a natural grape antioxidant
    - GSH 51% in juice and 40% in skins
  - Hyper-oxygenation – 50 mg/L just before cold settling
    - Protects against future browning
    - Lack of scientific study
    - Sensory of Chardonnay indicated ↑banana and ↓herbaceous and floral

O_2 management

- **Red Winemaking**
  - **Optimal use of O_2 can impact wine style greatly**
    - **Enhance fruit characters, limit reductive characters**
    - **Develop mouthfeel profiles**
    - **O_2 during fermentation ~ MOX following fermentation to remove reductive aromas**
    - **Optimal amount of oxygen still not determined**
      - **Matrix depended**



Micro-oxygenation

- **Goals:**
  - **soften tannin**
  - **stabilize color**
  - **reduce vegetal character**
- **Phase I: After primary fermentation, fast**
  - **30 mg/L/month**
- **Phase II: After malolactic fermentation, slower**
  - **2-5 mg/L/month**
- **Chemistry not well understood**
- **Application based on sensory and experience**
- **Optimal O_2 for matrix not known**

Micro-oxygenation

- Growth of residual viable yeasts is an important factor modulating MOx after MLF
- Yeasts consume oxygen and $SO_2 \rightarrow$ yield acetaldehyde during MOx
- Yeast growth during MOx results in aroma changes
- With active yeast, MOx changes tannin activity and astringency

Sáenz-Navajas et al., 2018 Food Res Int 108

Weak SO_2 Binding Agents

- Pyruvate
- Alpha-keto glutaric acid
- Galacturonic Acid
- Other aldehydes, ketones

Barrel Aging on Lees

- **Lower level of pyruvic acid!**
- **Yeast lees or bacteria seem to metabolize aldehydes and ketones, reducing them to the alcohol form**
- **Lees release yeast-derived antioxidants like glutathione**
- **Thus, the free SO_2 as currently measured, is more available for protective reactions**
- **Lees also consumes oxygen**
- **However, lees on its own is not an antioxidant but allow the use of lower SO_2 levels**

SO_2 – O_2 consumption ratios during bottle aging

- Wines aged on lees had higher SO_2/TCO ratios
  - More SO_2 available to react with oxygen
- O_2 available to wine during bottle aging depends on Total Packaged Oxygen (TPO):
  - Amount of dissolved oxygen (DO) at bottling
  - Headspace (HS) at bottling
  - Oxygen present in the closure
  - Amount of oxygen that diffuses into the bottle through closure (OTR)
- Most O_2 consumed in first 180 days
- Waterhouse et al., (2016) studied SO_2/TCO ratios when wines bottled with different DO and closures

SO_2 /TCO ratios during bottle aging

- When SO_2/TCO ratio less then 4 (based on stoichiometric only)
  - O_2 is reacting with substances other than SO_2
  - At very low SO_2–to-O_2 levels, most of oxygen entering the bottle was forming oxidation products rather than consuming SO_2
    - These wines showed oxidation characters and elevated levels of acetaldehyde
      - When free SO_2 < 10 mg/L according to Ripper or comparable methods
        - No protection
        - Oxygen reacts with thiols, glutathione, phenolics resulting in loss of aroma and browning

SO_2 vs TCO consumed

- TCO in bottle is a key new measurement
  - Based on consistent closures
- Comparison of SO_2 vs TCO appears to be useful
  - Ratio of 4 indicates no oxidation products
  - Ratio below 2 leads to significant oxidation
  - Value below 4 needed for development-phenolics
- However this study was based on Ripper SO_2 measurements

Waterhouse et al., (2016) AJEV 67

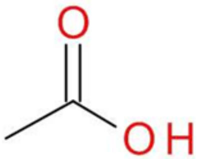
Impact of closure on wine post-bottling development

- For white wines: Sauvignon blanc
  - Very low OTR risk of reductive aroma development (<0.8 mg/L/y)
  - Low OTR, slow aging, maintain time at bottle character longer (<1.5 mg/L/y)
  - High OTR, oxidative character (nutty, bruised apple) and browning (< 6 mg/L/y)
  - Lopez et al., 2009 JAFC 57
- Synthetic and screw caps most consistent OTR's and natural corks most variable
- For red wines – optimal closure depends on:
  - Winemaking style
  - Phenolic content
  - Ageability
  - Expected bottle aging

O_2 and microbial spoilage

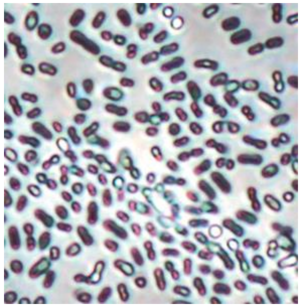
- **Acetaldehyde (nutty, bruised apple, sherry)**
  - **Ethanol + O_2 = CH_3CHO**
    - **Increase over time due to oxidation of EtOH due to aeration**
    - **Sensory threshold of 100 mg/L, typical conc. in wine 10-75 mg/L**
    - **Major intermediate in yeast fermentation**

O_2 and microbial spoilage



acetic acid

- **Aerobic acetic bacteria**
  - **Acetic acid (vinegar)**
    - **Acetic acid + ethanol = ethyl acetate**
    - **Small quantity of EA can be produced by yeast and by lactic acid bacteria**
      - **Aroma threshold 7.5 mg/L**
      - **Wine normal 22.5-63.5 mg/L, spoiled >150 mg/L**



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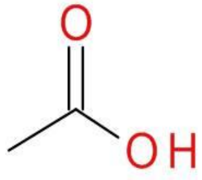
Acetic acid prevention/mitigation

- **Prevention**
  - **Protect against oxidation**
    - **No head space after AF**
    - **Adequate levels of SO_2**
  - **Mitigation**
    - **First hint of a problem**
      - **Sterile filtration**
  - **Reverse Osmosis**
    - **Will remove desirable aroma molecules as well**



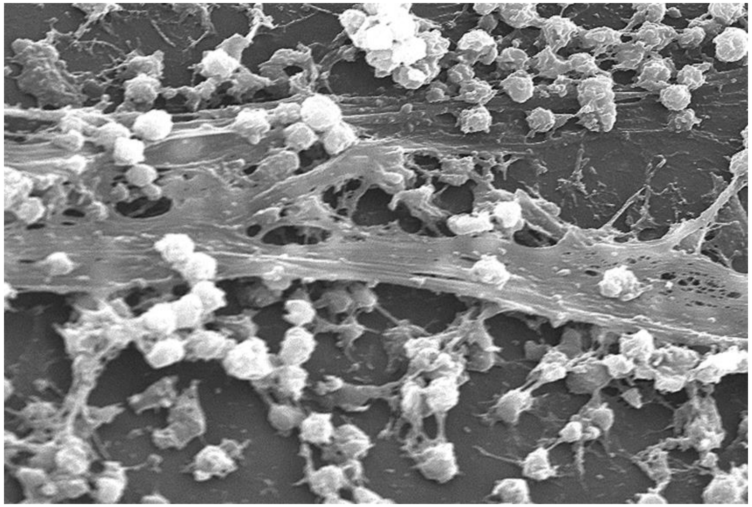
acetic acid

Preventing bacterial spoilage

- “Best practice”
  - Wine is hostile environment
    - pH =< 3.6
    - EtOH ±14%
    - High levels of phenolic compounds
    - SO_2 addition at crush
  - Cold storage at 15°C (60 ° F)
  - Avoid Stuck fermentations
  - Control nutrient additions
  - Control oxygen exposure
    - Headspace

Preventing bacterial spoilage

- **Cleaning and sanitation**
  - **Remove bacteria and biofilms**
  - **Kill bacteria**



Conclusions

- SO_2 is the main antioxidant in wine
- Current non-HS measurement of SO_2 can overestimate 10 – 20 mg/L
  - Using this measurement, below 10 mg/L indicate no protection from O_2
- Lees aging can reduce the amount of SO_2 needed for O_2 protection
  - Remove metabolites that can bind free SO_2
  - Consume O_2

Conclusions

- SO_2/TCO ratios may be an indicator of oxidation risk
  - Data needs to be updated using HS SO_2 measurements and perhaps TPO?
- Optimal OTR rates are difficult to predict due to complex wine matrix effects
  - General lower OTR for whites and light reds
- O_2 management during winemaking also has an impact on closure choice
  - Ageability