Advancing the Art of Winemaking through/with Science: Impact of Grape Ripening on Wine Phenolics and Sensory Attributes

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Introduction

- **Grape Ripening and its Influence on Wine Composition (DOA)**
- **Fruit and wine relationships are complex**
- **Not many relationships between grapes and wine are:**
- **WYSIWYG “wiz-ee-wig”**

Complex Relationships: Grape and Wine Flavor

- What you taste in fruit is not what you taste in wine?
  - Some flavor compounds are bound as precursors
  - Some flavor compounds are not
- Flavor compounds are changing during ripening
- Some influenced by vineyard practices
  - Sun exposure: IBMP, TDN
- How does wine composition influence this relationship?
  - Ethanol solvates hydrophobic compounds that we smell and taste

Complex Relationships: Color and Tannins

- **Relationship between fruit and wine tannin is awful (DOA)**
  - **Cell wall compounds: polysaccharide**
  - **Sponge of complex polysaccharides that must be satisfied (bound) before you can get free tannins into wine**
  - **Ethanol has some influence on extraction**
- **Relationship between fruit and wine anthocyanins isn’t great either (RBB)**
  - **Copigmentation: Influence of [A] & Co-factors**
  - **Extraction equilibrium (adsorption/desorption phenomenon)**
  - **Not quite like the sponge but similar**
  - **Ethanol has no influence on extraction**

How does fruit composition influence polymeric pigment formation?

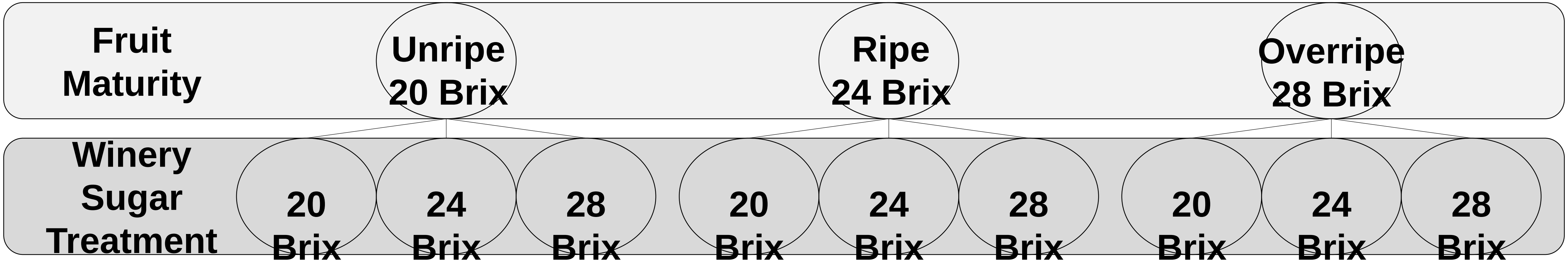
- Chemical Train Wreck: Polymeric Pigments (DOA)
  - Reaction between an anthocyanin and variety of wine components
  - Primarily tannins
  - Form stable color in wines
  - Coloration is less effected by pH changes and bisulfite bleaching
  - Modify mouth feel over time by decreasing astringency (theoretical still)
- Anthocyanin:Tannin thought to drive formation
  - Evidence in literature is negligible
  - Heat, O_2, Lack of O_2: all influence formation
  - Reactions take time so we will need a way around this

Experimental Design

- Pick fruit at different soluble solids: 20 Brix, 24 Brix, 28 Brix
- Represent different winemaking eras and extraction effect
  - ~ 12 %, 14%, & 16% (v/v) Ethanol
- Wines not skin or seed extracts
  - Phenolics extracted from wines Day 10 and subjected to heat treatments
    - Heat treatment based upon work done by Vidal et al. 2002
- Ethanol is controlled for at each harvest by dilution or sugar addition
- Cultivars that naturally have different A:T ratios selected for study
  - Syrah (High Pigment: Mid Tannin)
  - Cabernet Sauvignon (High Pigment: High Tannin)

Winemaking Procedure

- Syrah and Cabernet Sauvignon
- Wines replicated sugar content of other maturity treatments
  - Controlled for maturity vs. ethanol effects



- Experiment designed so wines would have a range of anthocyanin, tannin, and A:T

Winemaking Procedure

- Wines fermented in triplicate
  - 200 L scale, 54 total wines
  - TJ/Boulton Fermentors
- Inoculated with EC 1118 (10^6 cells/mL)
- Simultaneous ML fermentation (~48 hours post using VP41)
- Nutrient Addition
  - FermaidK (0.25 g/L), DAP (200ppm), GoFerm (0.3g/L)
- No acidity adjustments
  - Water for saignée/water back had 5 g/L tartaric acid
- Chaptalization with 80 Brix sugar solution
- 10 day maceration

Aging

- **Wines were collected at day 10 of fermentation**
  - **Remaining sugars and organic acids were removed (XAD7)**
  - **Dissolved in same volume model wine (14% alcohol, 5 g/L TA, pH=3.5)**
- **Aged at 30^{o}C for 4 months**
  - **Samples collected once a month**
  - **Cellar aged samples collected 6 months after fermentation**
- **Analysis of polymeric pigment, anthocyanin, tannin, and total phenolics performed by protein precipitation, HSO_3^- bleaching assays and $FeCl_3$**
- **HPLC methodologies also done but not shown today**

2015 Harvest Data

	Harvest (Pick Date)	Brix at Harvest	pH	TA (g/L)	Berry Weight (g)	Anthocyanin (mg/berry)
Cabernet Sauvignon	Unripe (DOY 233)	19.2 c	3.41 c	9.29 a	0.82 b	0.71 c
	Ripe (DOY 260)	25.1 b	3.72 b	7.23 b	1.02 a	0.91 a
	Overripe (DOY 289)	27.5 a	3.89 a	6.91 c	0.83 b	0.82 b
Syrah	Unripe (DOY 231)	20.0 c	3.47 c	7.95 a	1.37 a	0.90 c
	Ripe (DOY 252)	24.5 b	3.73 b	8.07 a	1.36 a	1.52 a
	Overripe (DOY 286)	27.9 a	4.01 a	4.72 b	1.12 b	1.14 b

- ~ 3-4 weeks between pick dates

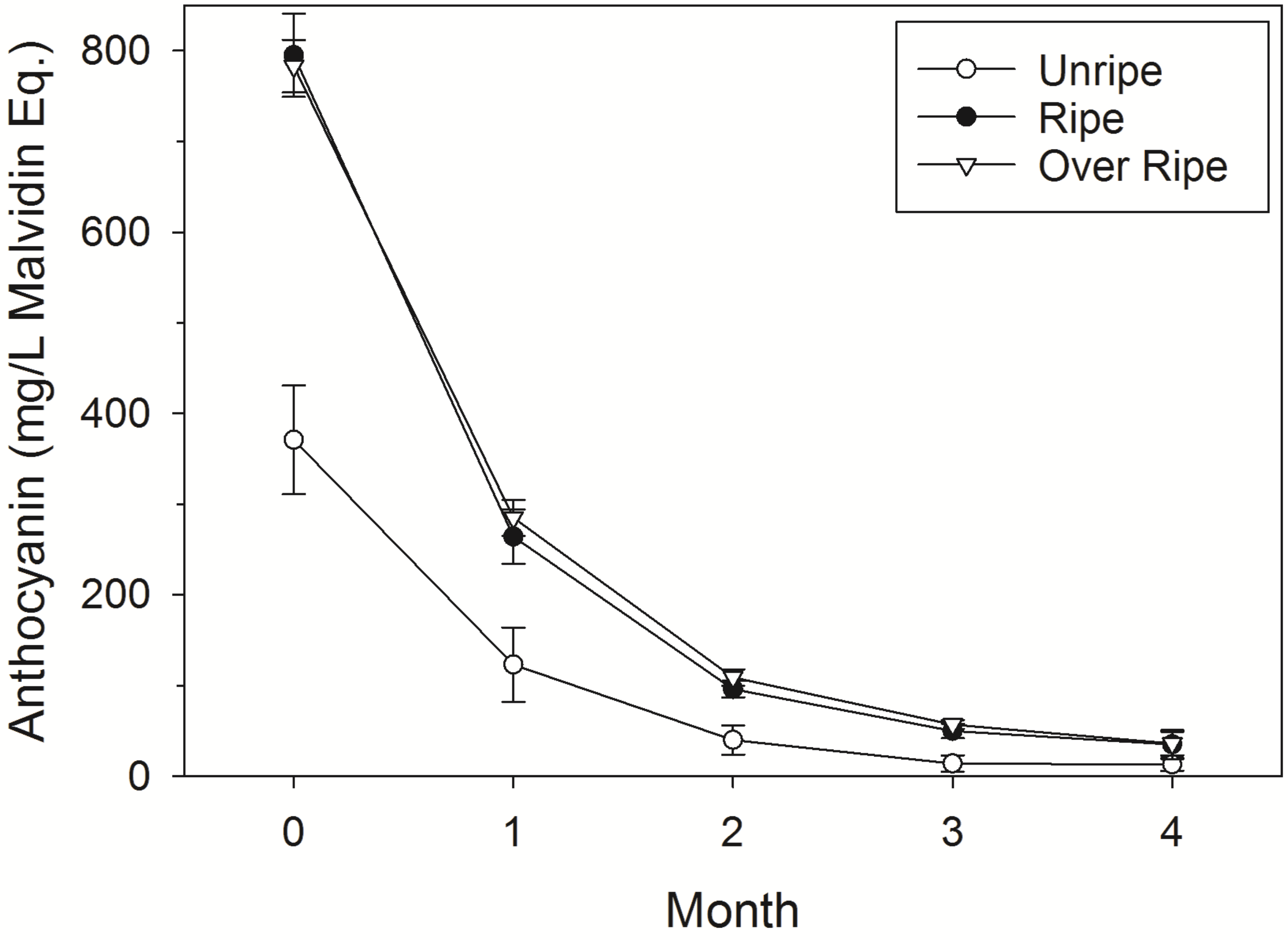
Cabernet Sauvignon Initial Wine Phenolic Data

Harvest Date	Anthocyanin (mg/L)	Tannin (mg/L CE)	Ratio A:T
Unripe DOY 233	371 a	1072 a	0.36 a
Ripe DOY 260	795 b	886 b	0.93 b
Overripe DOY 289	783 b	892 b	0.86 b
Alcohol Treatment			
Low	641	816 a	0.83
Medium	654	946 a	0.71
High	623	1189 b	0.61

Syrah Initial Wine Data

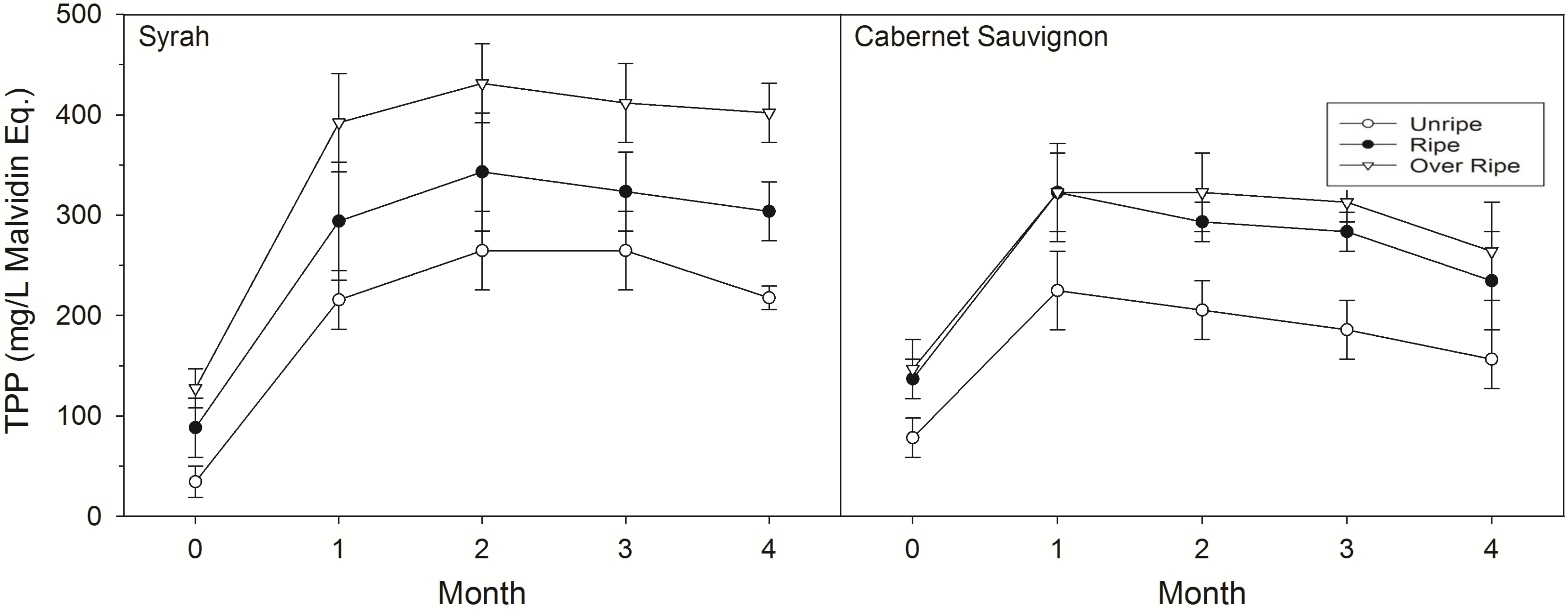
Harvest Date	Anthocyanin (mg/L)	Tannin (mg/L CE)	Ratio A:T
Unripe DOY 231	458 c	374 ab	1.3 b
Ripe DOY 252	726 b	351 a	2.1 a
Overripe DOY 286	832 a	429 b	2.0 a
Alcohol Treatment			
Low	640	302 b	2.1 a
Medium	700	416 a	1.7 b
High	680	437 a	1.6 b

Anthocyanin Changes Over Time



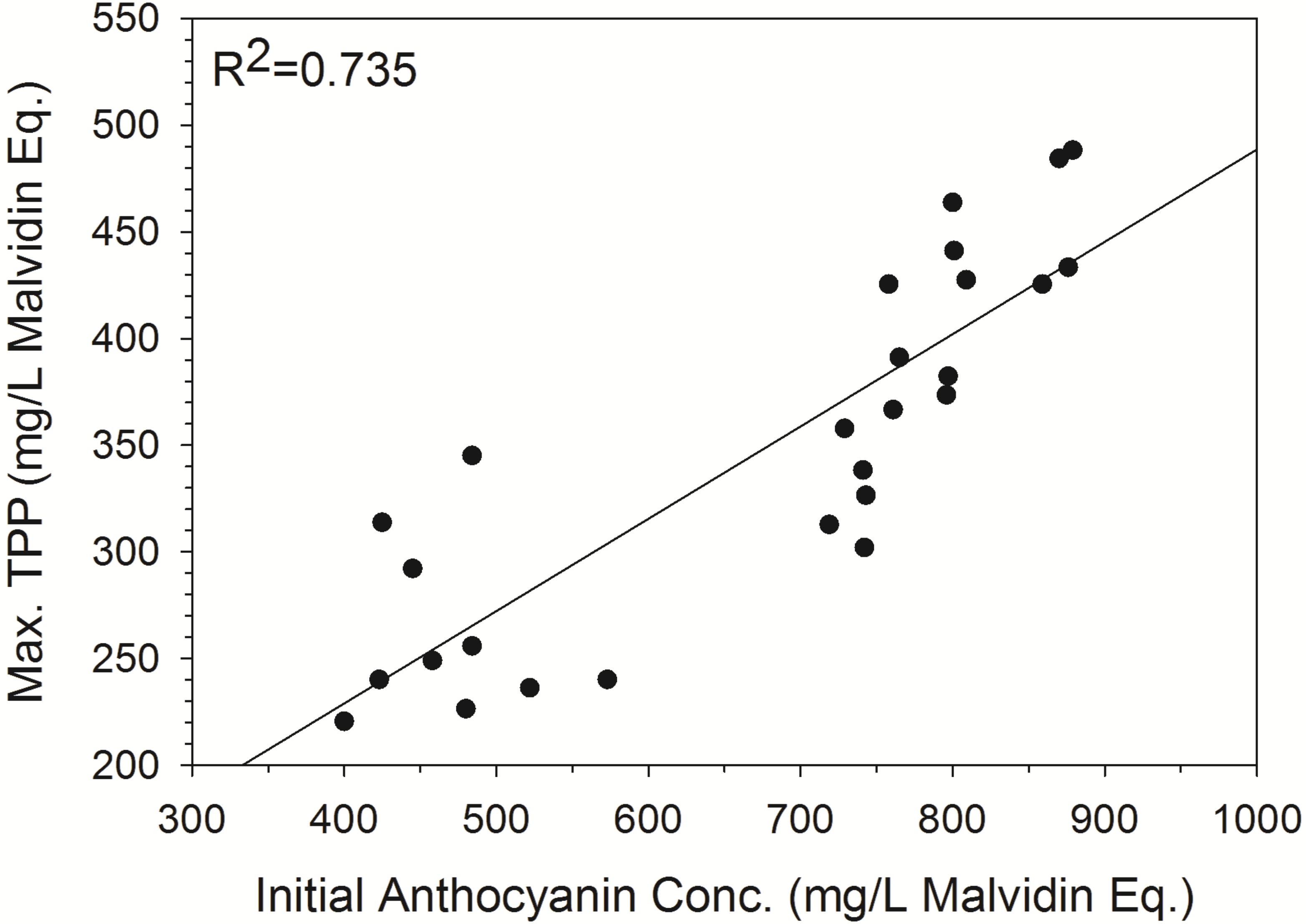
- **Independent of alcohol treatment Exponential decay R^2: 0.94-0.99**
- **1 month incubator=1 year cellar**

Polymeric Pigment Over Time



- **1 month incubator=1 year cellar**

Predicting Polymeric Pigment Content (SY)



R^2 Values for Other Predictors

- **Syrah**
  - **A:T=0.042**
  - **[Tannin]=0.392**
  - **[Anthocyanin]=0.735**
  - **[Tannin] + [Anthocyanin]=0.859**
- **Cabernet Sauvignon**
  - **A:T=0.405**
  - **[Tannin]=0.02**
  - **[Anthocyanin]=0.670**
  - **[Tannin] + [Anthocyanin]=0.767**

Conclusions

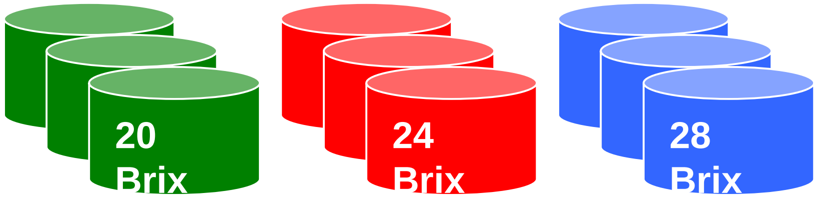
- Initial wine (not necessarily fruit) anthocyanin concentration best single predictor of long-term polymeric pigment
  - Higher initial anthocyanin (and tannin), higher polymeric pigment
    - More stable color and mouth feel modification over time
- Polymeric pigment formation occurs relatively rapidly
  - At equilibrium between formation and sedimentation after 1 month (1 year cellar)

Hang Time Experiment: Merlot Flavor

Harvest 1: Unripe
20.7 ± 0.5 Brix
Chaptalize to 24 Brix
5 September 2013
~21 Days
Brix

Low : Control (~20 Brix)
Medium:
High: Chaptalize to 28

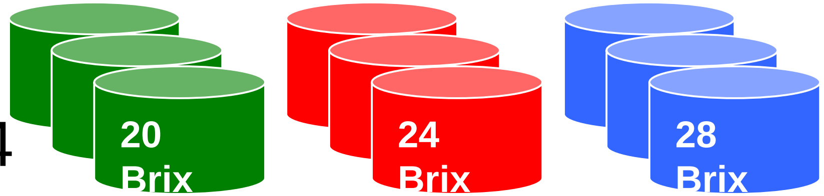
UNRIPE



Harvest 2: Ripe
Brix
24.0 ± 0.2 Brix
Brix)
~37 Days
26 September 2013

Low: Saignée – +H_2O to 20
Medium: Control (~24
High: Chaptalize to 28 Brix

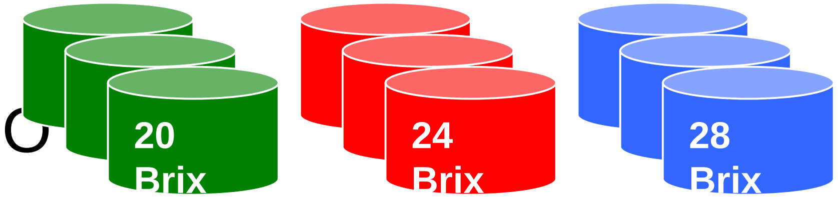
RIPE



Harvest 3: Overripe
20 Brix
27.4 ± 0.4 Brix
to 24 Brix
2 November 2013

Low: Saignée- +H_2O to
Medium: Saignée- +H_2O
High: Control (~28 Brix)

OVERRIPE



WINEMAKING

- 300 kg/Replicate
- 300 L Stainless Steel Tan
  - Treatment Replicates: n=3
- Yeast (EC-1118)
- 48 hrs. ML (VP41)
- 10 Days Contact Time
  - (26 ± 2°C)

Fruit Data

Harvest	Brix	pH	TA (g/L)	Berry Weight	Color (mg/g FW)	Skin Tannins (mg/g FW)	Seed Tannins (mg/g FW)
UNRIPE	20.7 a	3.57 a	7.83 c	0.98 a	0.65 a	0.60 a	3.68 b
RIPE	23.9 b	3.73 b	5.56 a	1.18 b	0.73 a	0.60 a	3.06 a
OVERRIPE	27.4 c	3.73 b	6.60 b	0.99 a	0.99 b	086 b	3.66 b

Overripe fruit characterized by concentration effects from dehydration

Intuitive Impacts: More color and skin tannins

Counter Intuitive Impacts: TA increase, Seed Tannin Increase

Drop in yield about 20-25% when ripening to 28 Brix

Harvest	EtOH % (v/v)	pH	TA (g/L)	RS (g/L)	Dynamic Viscosity (cP)	Density (g/cm^3)
UNRIPE	13.86	3.63 a	5.01 b	3.11 a	1.35 c	0.9857 a
RIPE	14.03	3.73 b	4.52 a	2.56 a	1.29 a	0.9860 a
OVERRIPE	13.95	3.73 b	5.15 b	4.11 a	1.32 b	0.9872 b
Ethanol						
Low	11.59 c	3.60 a	4.86	1.94 a	1.22 a	0.9884 c
Med	14.04 b	3.72 b	4.88	1.89 a	1.33 b	0.9860 b

OVERRIPE: Greater Viscosity and Lower Density

More EtOH: Yeast struggle (Higher RS); Greater Viscosity, Lower Density

Harvest	Anthos (mg/L)	SPP (A_{520nm})	LPP (A_{520nm})	Tannins (mg/L)	Total Iron Reactive Phenolics (mg/L)
UNRIPE	249 a	0.90 b	0.54 c	564 b	1571 a
RIPE	469 b	1.11 c	0.31 a	440 a	1521 a
OVERRIPE	524 c	0.82 a	0.40 b	792 c	2338 b
Ethanol					
Low	430	0.87 a	0.32 a	537 a	1655 a
Med	410	0.91 a	0.41 b	591 b	1766 b
High	403	1.06 b	0.52 c	669 c	2008 c

@ 6(… PE:
Tota

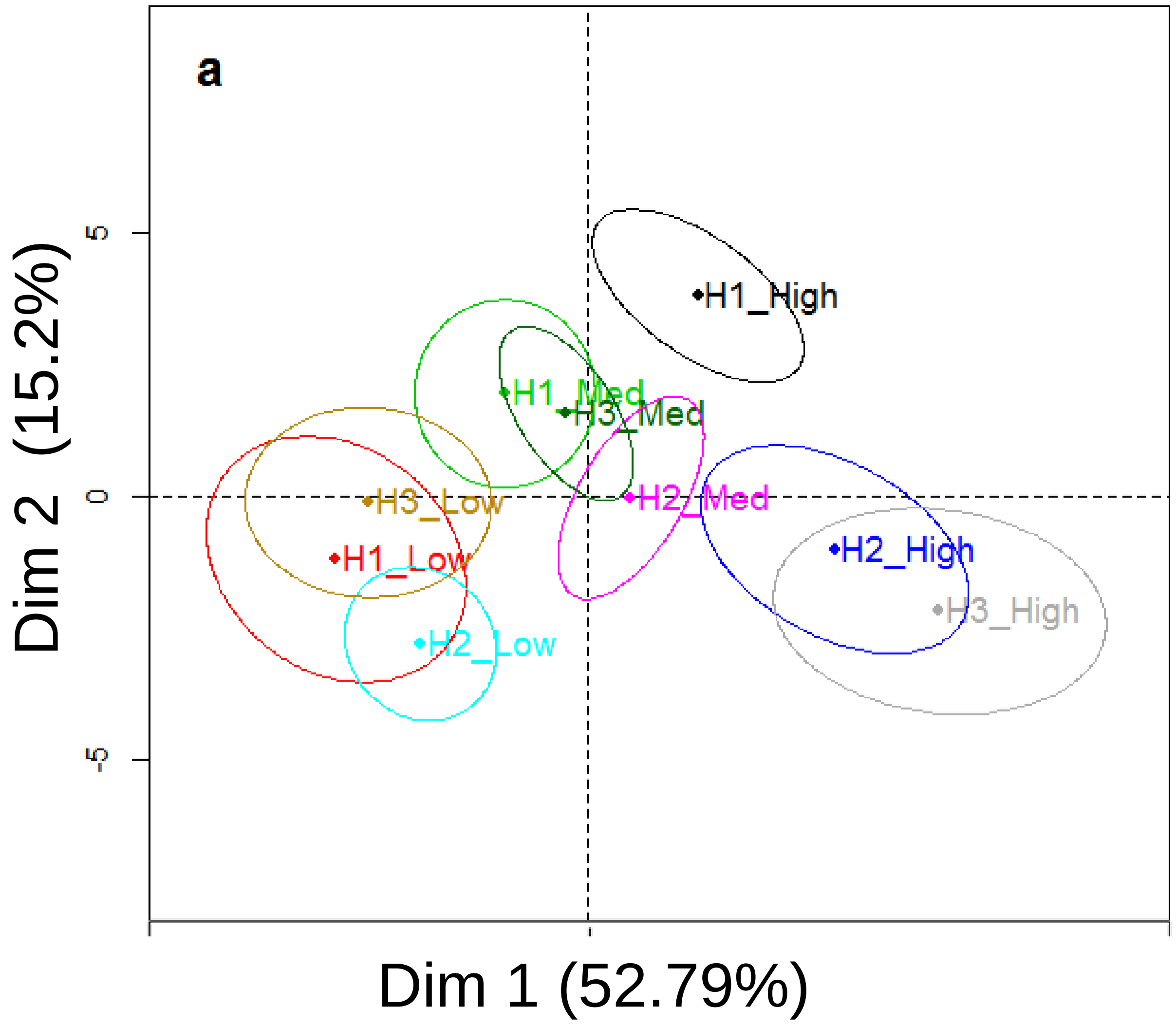
High Ethanol Impacted: Tannins, Total IRP

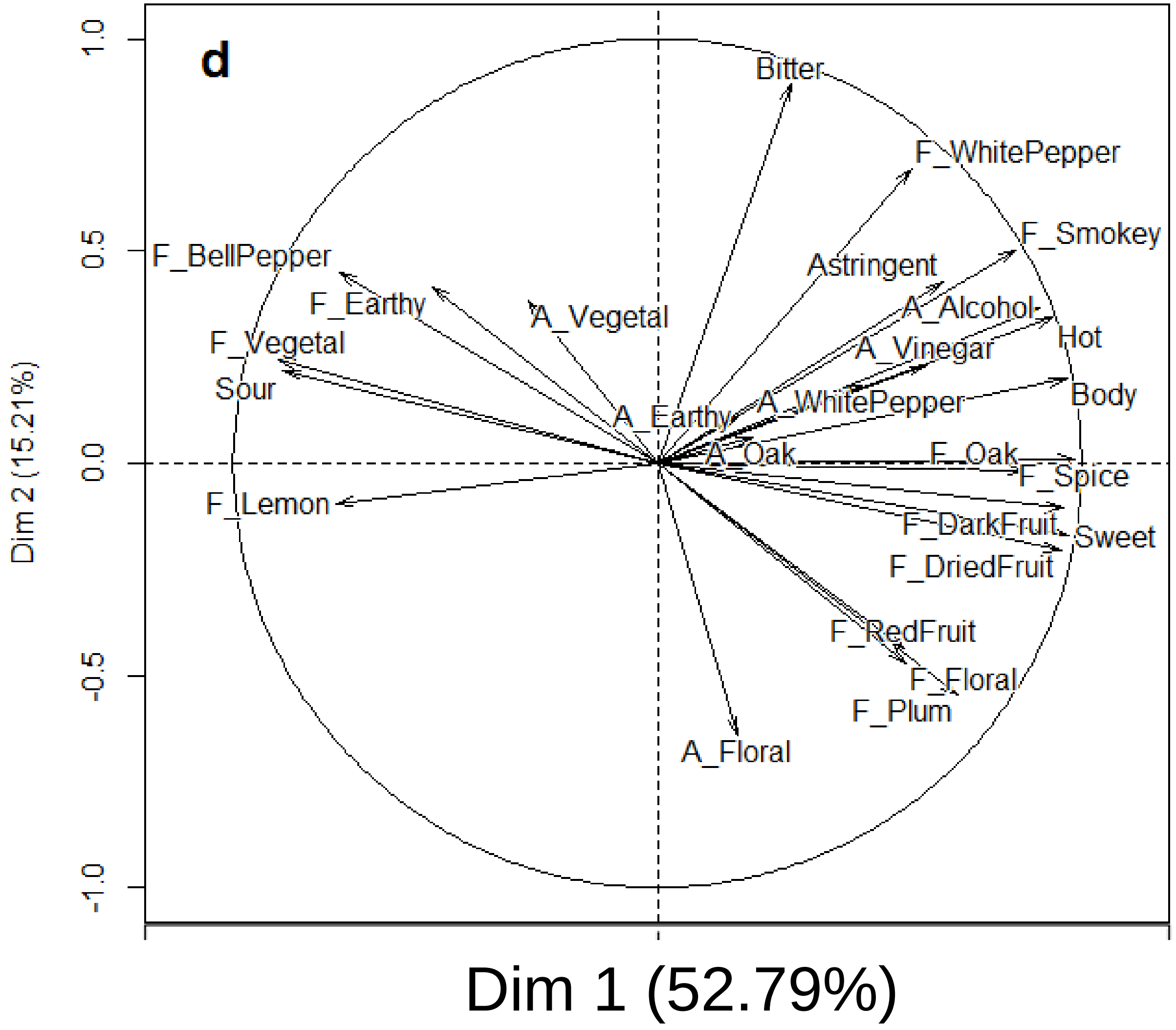
Sensory Panel Work

- **Descriptive Analysis: UC Davis Sherman & Heymann**
- **Sourness, Bitterness, Astringency, Sweet, Body**
- **Aromas by aroma and flavor**
    - **Vegetal, bell pepper, smokey, white pepper, floral, spice, red fruit, plum, dried fruit, oak**

Sensory Evaluation PCA
Dim 1 x Dim 2

SEPARATION BY ETHANOL
HIGH, MEDIUM, LOW





Ethanol Dominated Sensorial Evaluation

- Wines with similar ethanol were more similar to each other than the wines made from fruit picked at 20, 24 and ~28 Brix
- Low Ethanol: sourness, vegetal, bell pepper and earthy flavors.
- Medium Ethanol: vegetal, earthy and floral aromas.
- High Ethanol: Astringent, Bitter, Hot, Body, Sweet, Alcohol; Aromas & Flavors: Red Fruit, Plum, Oak, Smokey, White Pepper

Explain that one to me again

Henry's Law

- Tendency of molecule to partition between liquid and vapor phases
  - Henry's law is used in relatively dilute systems (Ethanol vs. aroma compounds)
    - 46- 49 M H_2O or 2-2.8 M Ethanol vs. mM, μM, nM Aroma Compounds
  - Vapor-liquid equilibrium data are represented in terms of K values
  - K value is vapor liquid distribution ratio
  - K = $\frac{y_i}{x_i}$
- Can be really complex of course:
  - Influenced by Chemical Equilibrium
  - Temperature (of course)
  - Ionic Strength (more salt tends decrease solubility of gases)
  - Solvent mixtures (EtOH + Water)!!!
  - Non-ideal solutions (sucrose)

Research Ongoing

- **GC-QTOF (untargeted & target) characterization of samples and standards**
- **Understand which compounds and how matrix of aroma compounds influence what we perceive**
- **Understand the relationship between the composition of our standards created for panelists and how the relate to wine compostion**

Take Away Messages

- Results suggest aroma/flavor partitioning into H_2O/EtOH is largely driving aroma profile in wine.
- Aroma profile in high ethanol wine is characterized by riper characteristics and greater viscosity (lower density).
- Riper fruit yields more color and tannins
  - More saturated color and more astringency.
- Disclaimer: These results may not apply to other cultivars and regions

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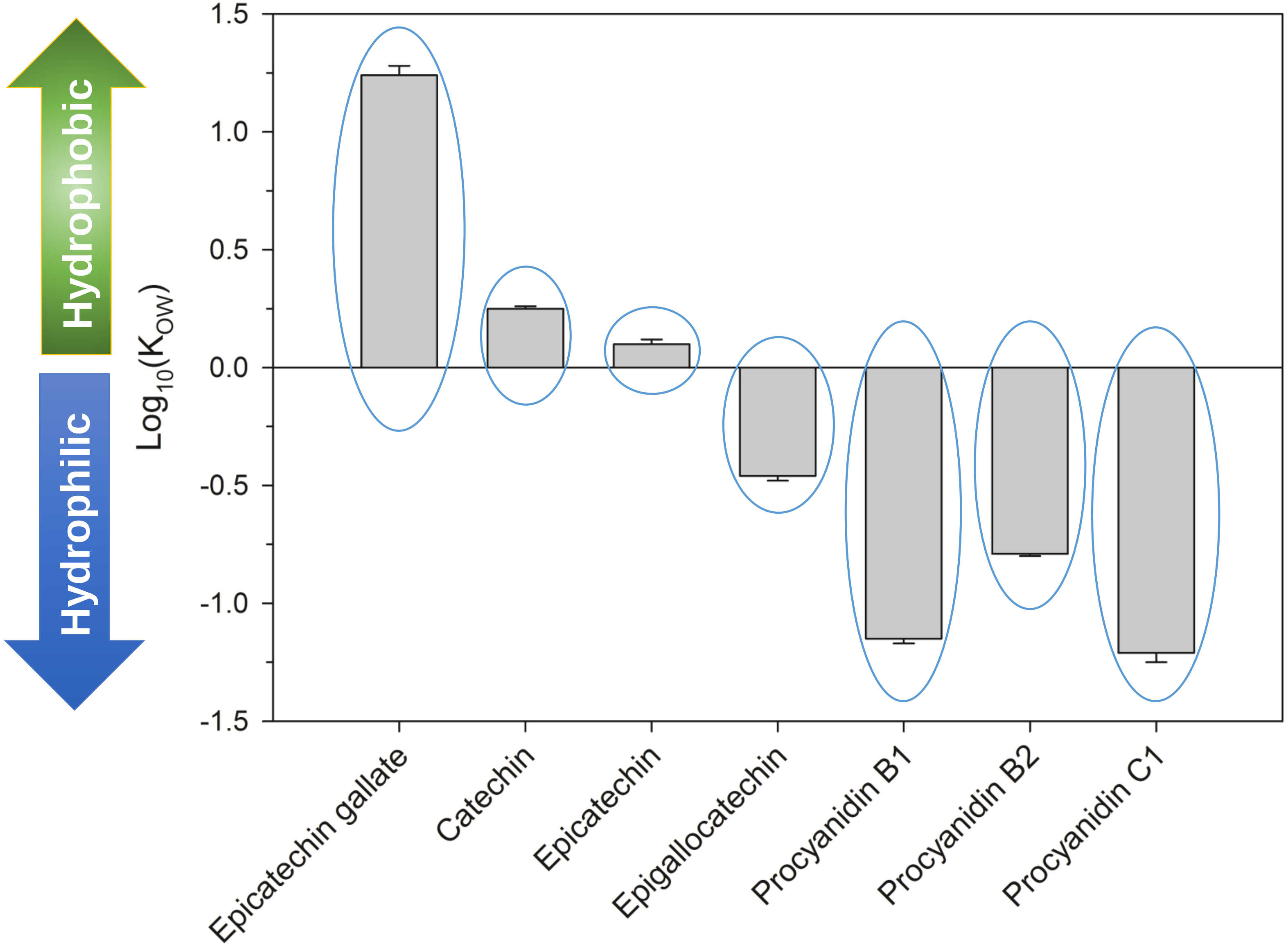
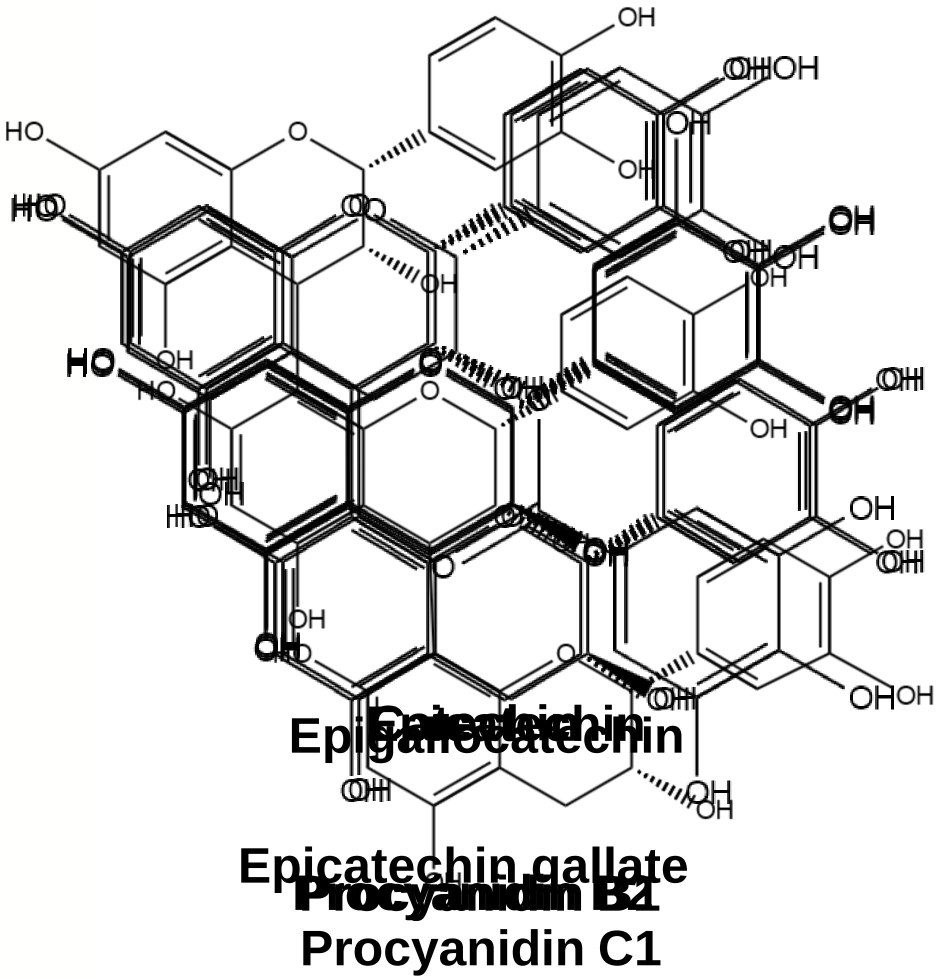
Change Gears!!!! Phenolic Hydrophobicity

- **Hydrophobicity is a measure of how much a compound will dissolve/react with water**
  - **Hydro-water**
  - **Phobia-fear**
- **Hydrophobicity of tannins relates to the strength of the protein-tannin complex**
  - **Number of hydrogen bonds vs hydrophobic interactions**
- **As a tannin polymer gets larger, it also gets:**
  - **More hydrophilic**
  - **More efficient at precipitating protein**
    - **More astringent**

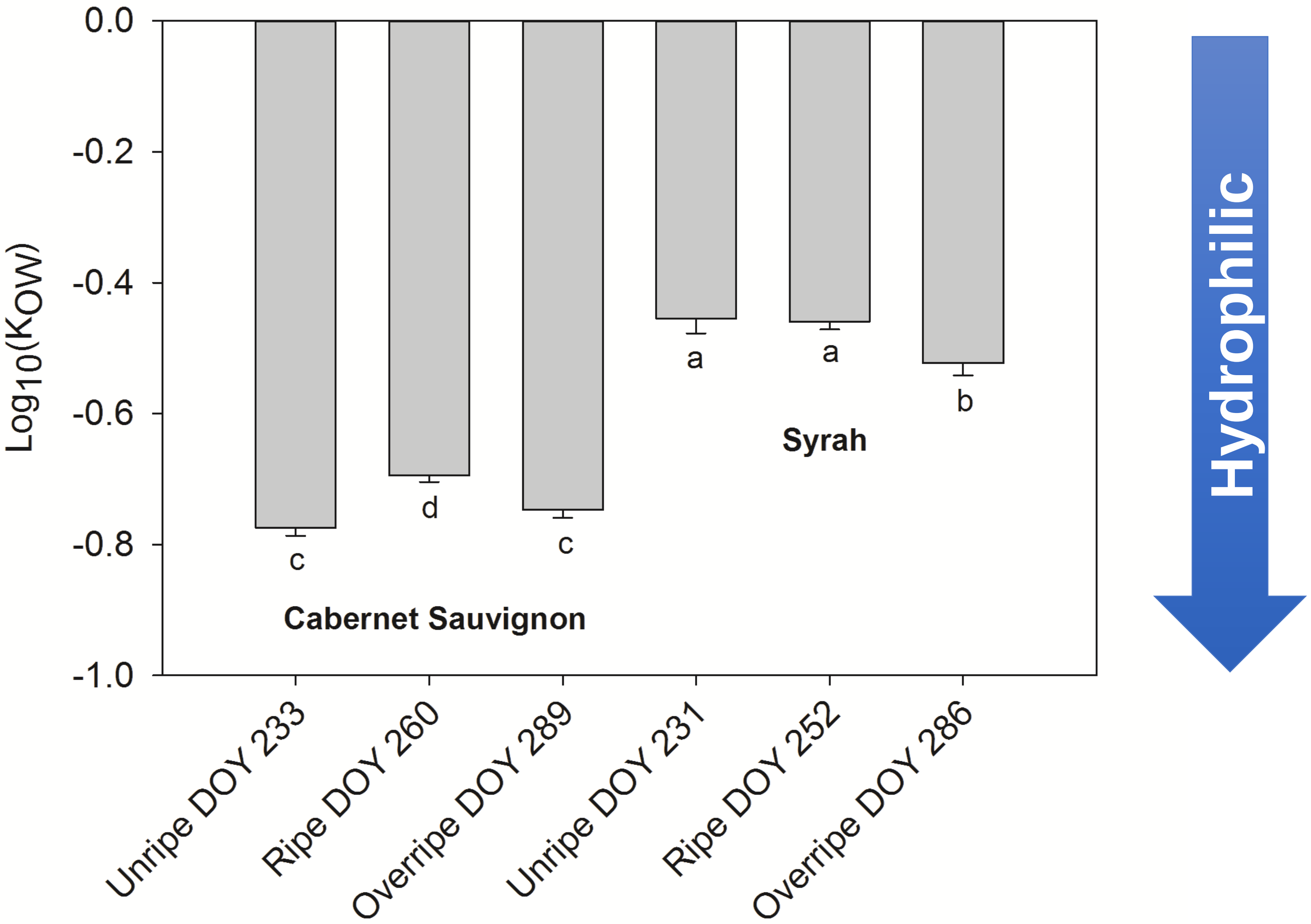
Phenolic Standards



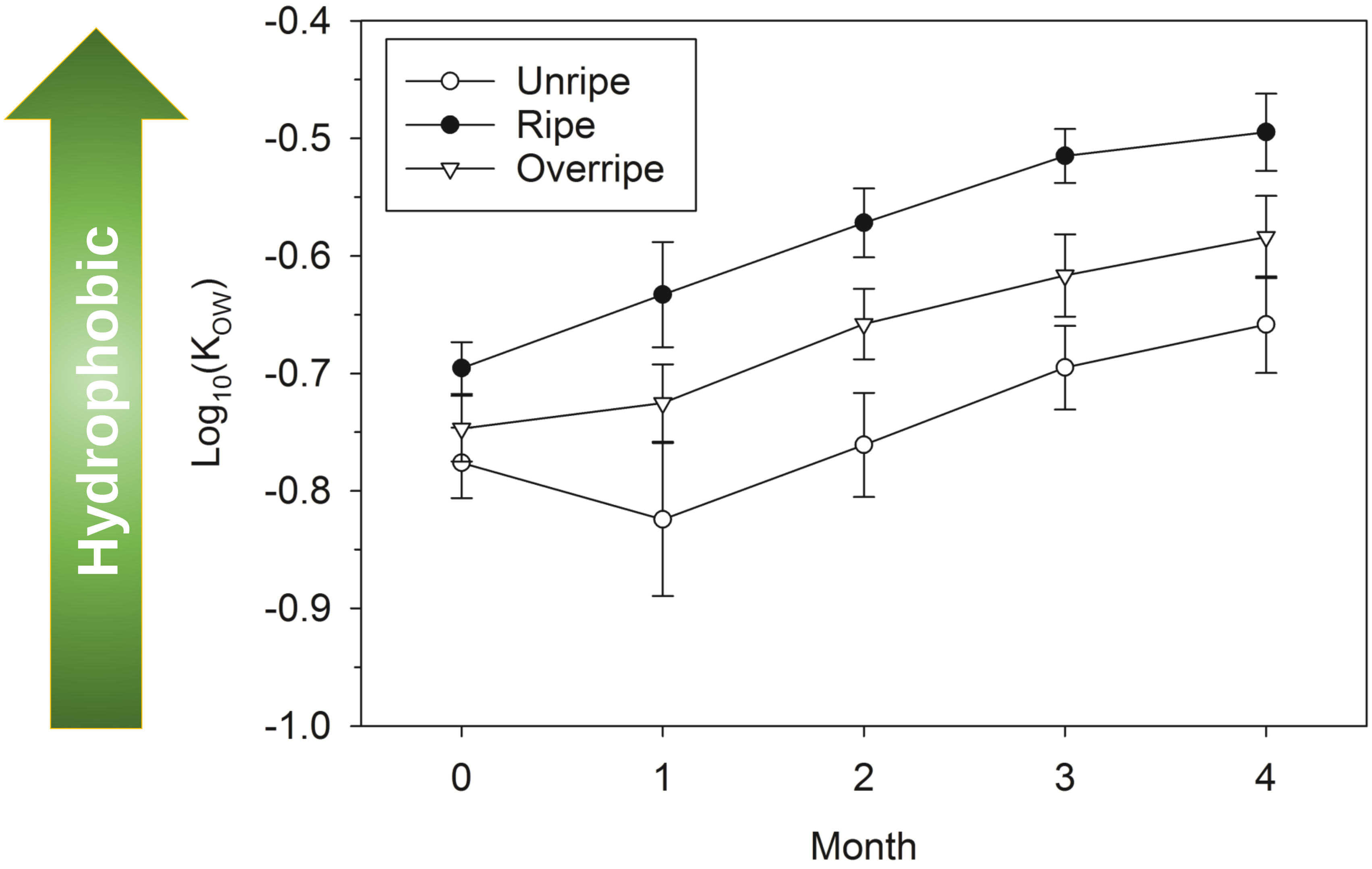


Wine Phenolic Hydrophobicity

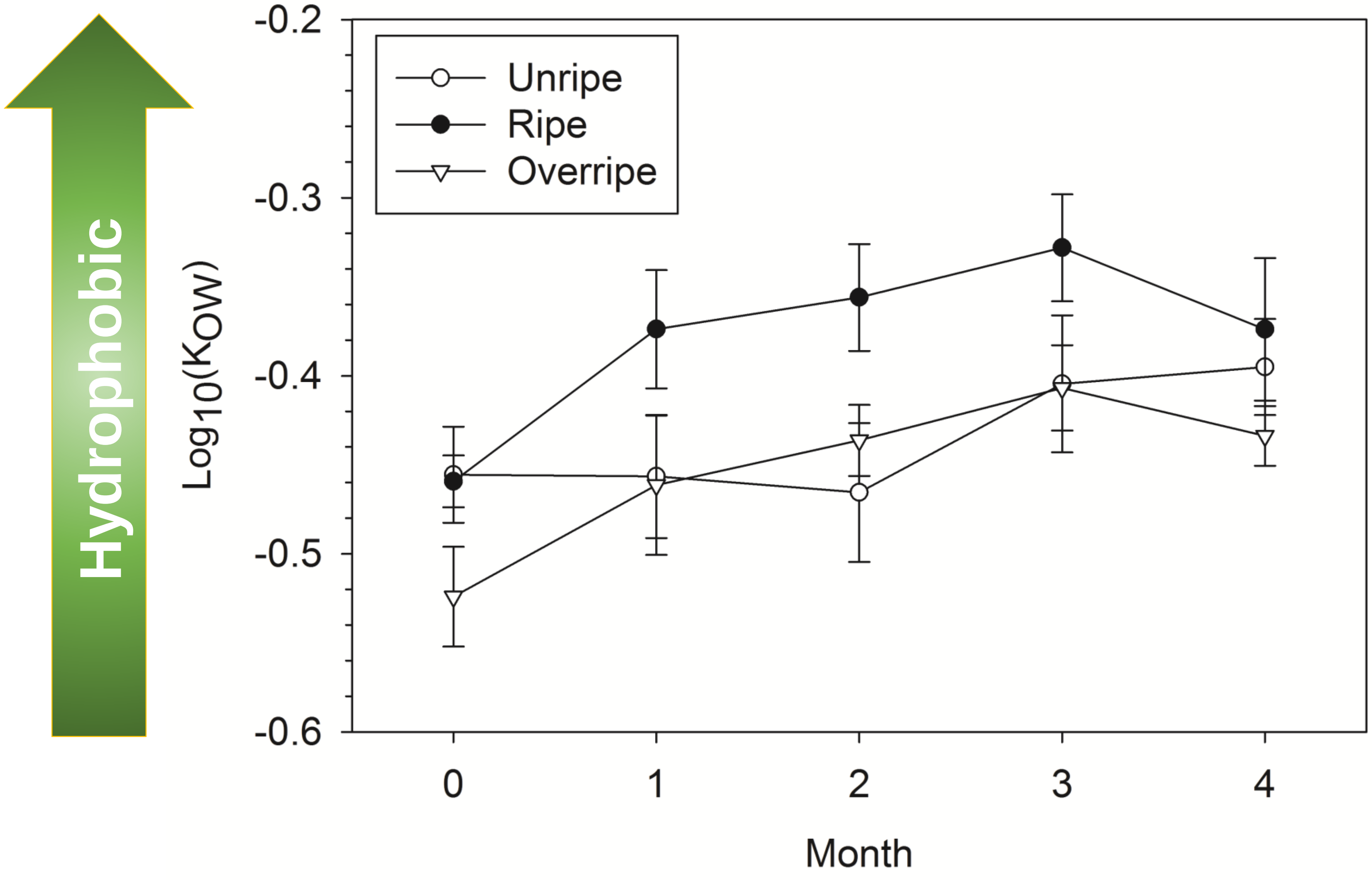
- **Dependent on varietal and berry maturity**
- **Independent of wine alcohol content**
  - **Alcohol content increased tannin concentration but did not change tannin composition**
  - **Anthocyanin content also independent of alcohol**
    - **But increasing anthocyanin would make hydrophobicity decrease**



Hydrophobicity Over Time-CS



Hydrophobicity Over Time-SY



What is happening over time?

- To increase K_{OW}, either:

$$\uparrow K_{OW} = \frac{\text{Area octanol phase} \uparrow}{\text{Area water phase} \downarrow}$$

- Area of octanol phase is not increasing, but area of water phase is decreasing
  - Losing hydrophilic compounds, not gaining hydrophobic ones
  - Tannin polymers NOT getting shorter

Conclusions

- Wine hydrophobicity suggests tannin polymers are relatively small
- Phenolic hydrophobicity is dependent on fruit maturity, not alcohol content
  - Tannin concentration is not changing with maturity but tannin composition/structure is changing
- Phenolic hydrophobicity increases over time
  - Due to structural transformations and losses of anthocyanin
    - Anthocyanin retention 40-60% over 4 months
  - Probably NOT due to shortening of tannin polymers over time