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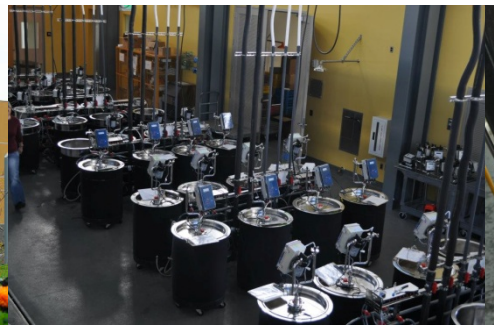
E&J. Gallo Winery






Understanding Cap Extraction in Red Wine Fermentations

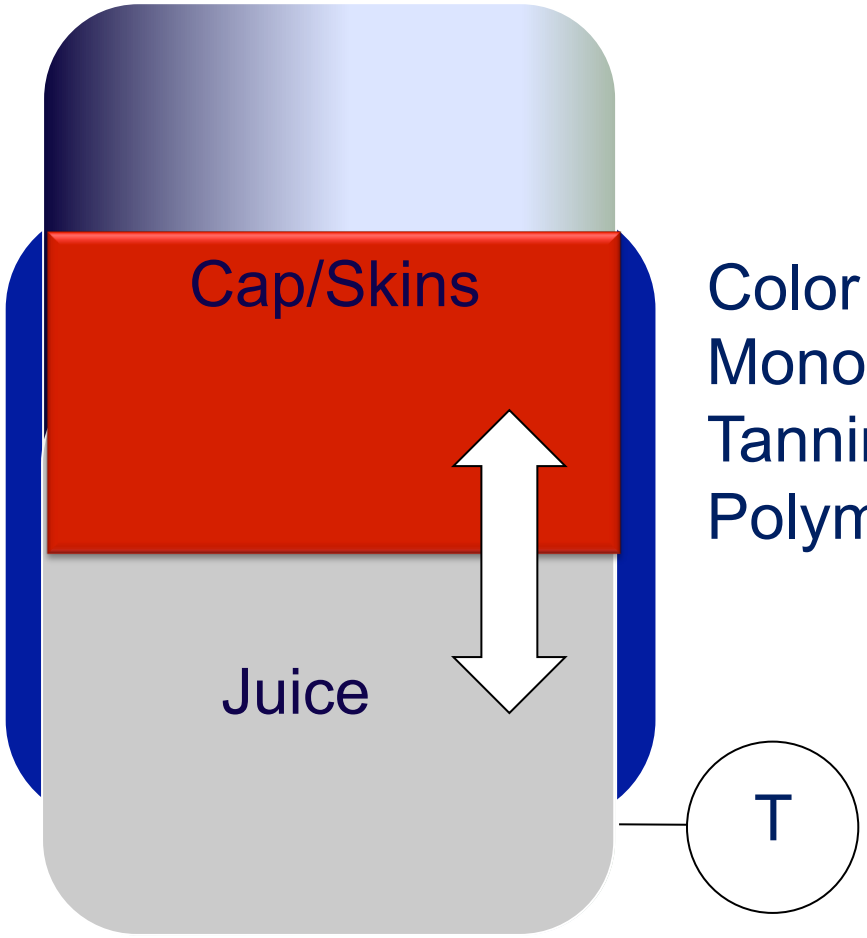
Max Reichwage, Larry Lerno, Doug Adams, Ravi Ponangi, Cyd Yonker, Leanne Hearne, Anita Oberholster, and David Block

Driving innovation in grape growing and winemaking

Understanding Cap Extraction in Red Wine Fermentors

- Motivation and key molecules
- Chemical gradients in red wine fermentors
- Effects of pumpover volume and frequency
- Effects of cap and must temperature on phenolic extraction

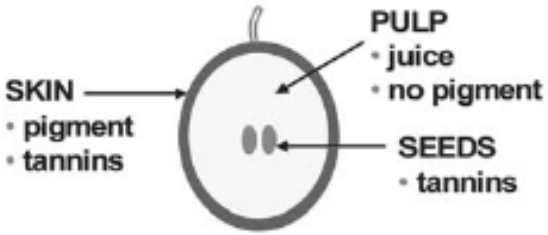
Cap Extraction in Red Wine Fermentors



Color
Monomeric Phenolics
Tannin
Polymeric Pigment

Closer look at the molecules extracted

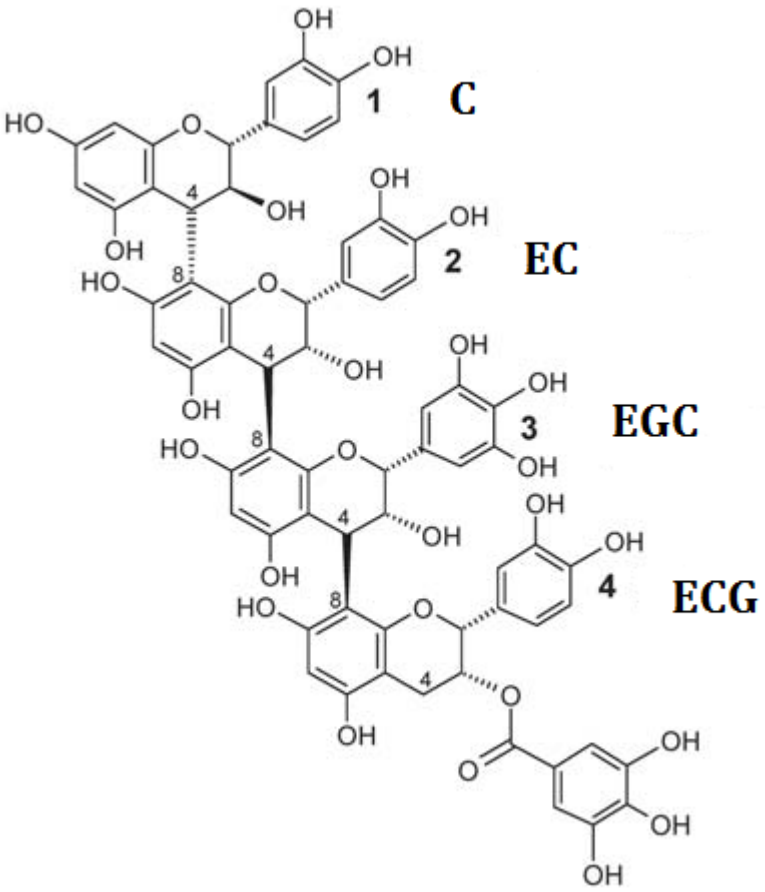
- **Polymeric flavan-3-ols (tannins)**
  - **The most abundant class of phenolics in grapes**
  - **Present in skins and seeds**
- **Anthocyanins**
  - **Malvidin-3-glucoside is the predominant anthocyanin**
  - **Found in the skin**
- **Hydroxycinnamates**
  - **Ex: caftaric acid, caffeic acid, coumaric acid**
  - **Found in the skin and pulp**



(Adams 2006)

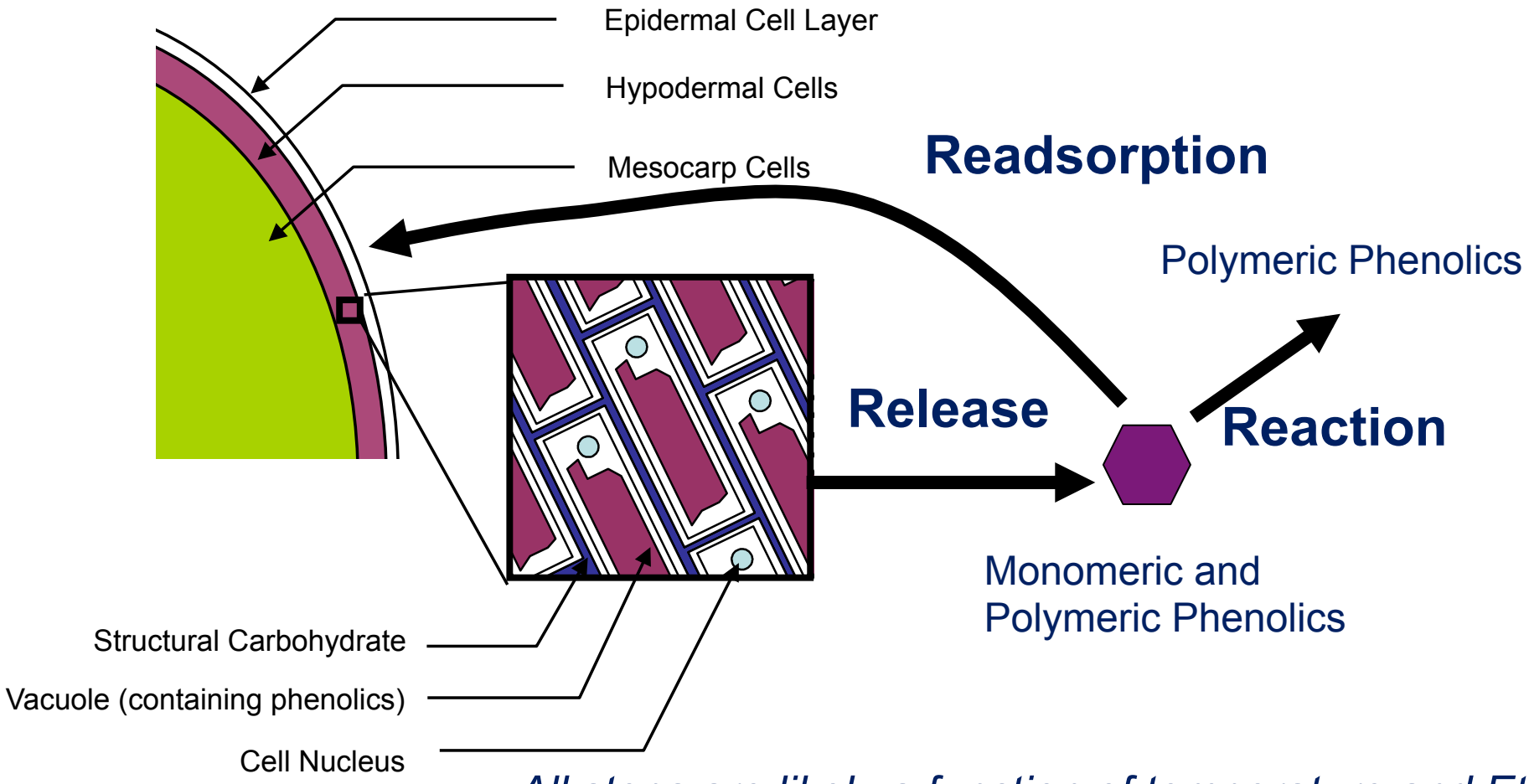
Closer look at the molecules extracted

- **Grape tannins-oligomers**
  - **catechin (C), epicatechin (EC), epigallocatechin (EGC), and epicatechin gallate (ECG)**
- **Differences between skin and seed tannins**
  - **Mean degree of polymerization (mDP) for skin tannin are ~30; seed tannins are ~10 (Souquet et al. 1996)**
  - **Proportion of ECG units is different in seeds (~30%) and skins (~5%) (Cheynier et al. 2006)**



Hypothetical tannin tetramer (Adams 2006)

A molecular mechanism for cap extraction



All steps are likely a function of temperature and EtOH

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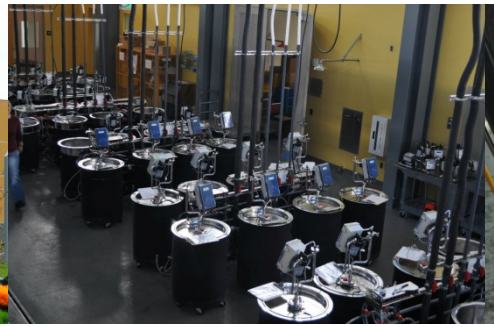



Chemical gradients in red wine fermentations

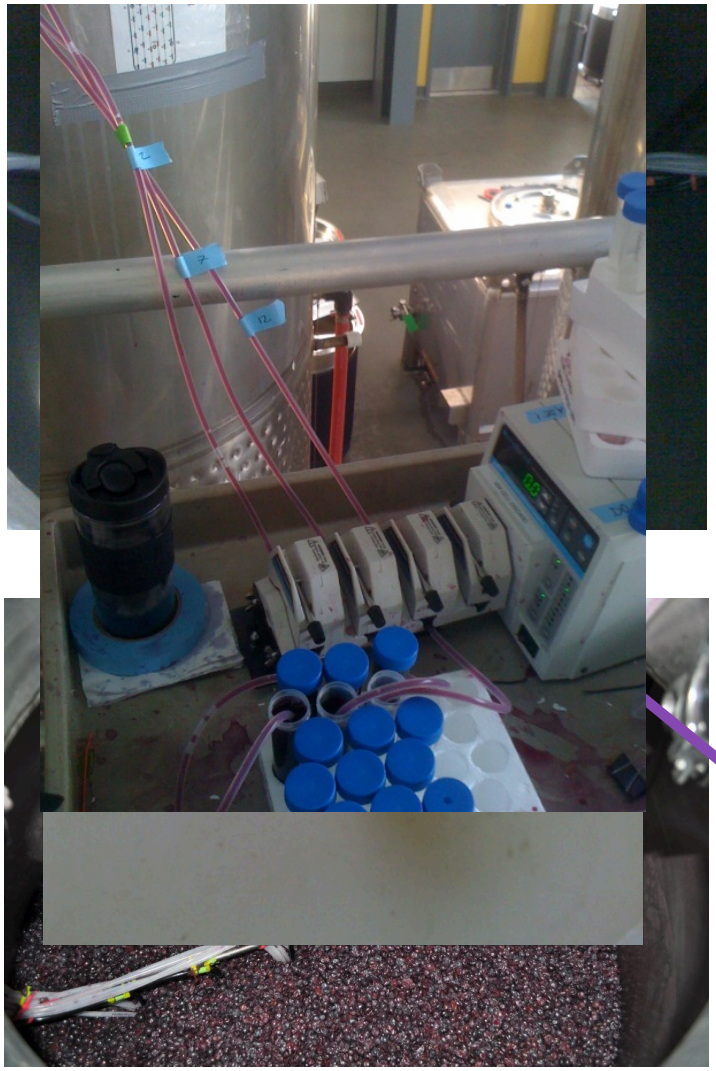
Driving innovation in grape growing and winemaking

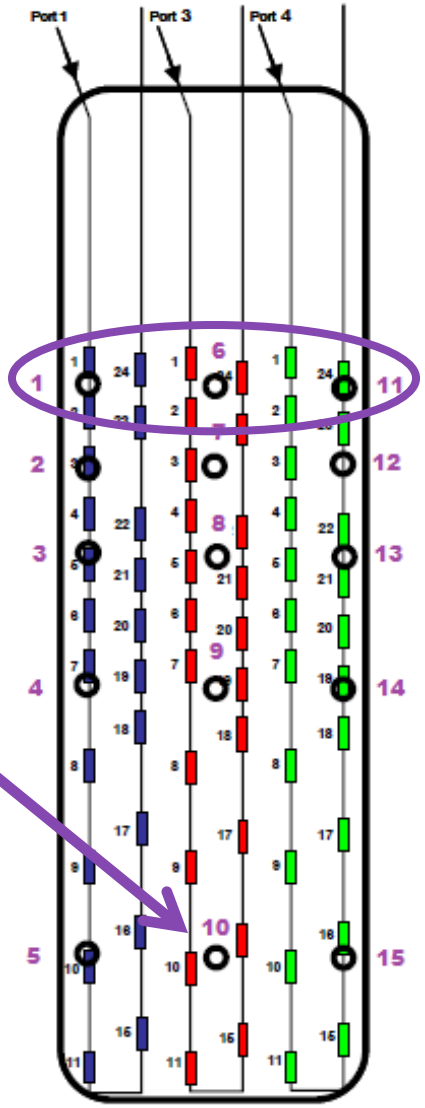
Experimental Design: UC Davis/E&J Gallo

- **Grapes: 2011 Cabernet Sauvignon from Lodi, CA**
  - **23.3°Brix, pH of 3.41, T.A. of 0.47 g/L**
  - **Hand-picked, destemmed and crushed**
  - **Inoculated with *S. cerevisiae* strain Lalvin D254®**
  - **YAN adjusted to 300 ppm, addition of 50ppm SO_2**
- **Pressed at dryness**

Are there chemical gradients in red wine fermentations?

- Installed a "curtain" of 66 temperature sensors throughout the cross-section of a 2000 L tank
- 15 sample extraction points
- Fermented 2 Tons of Cabernet Sauvignon
- Pumped-over 1 tank volume 2x per day
- Peristaltic pump on catwalk for sample extraction

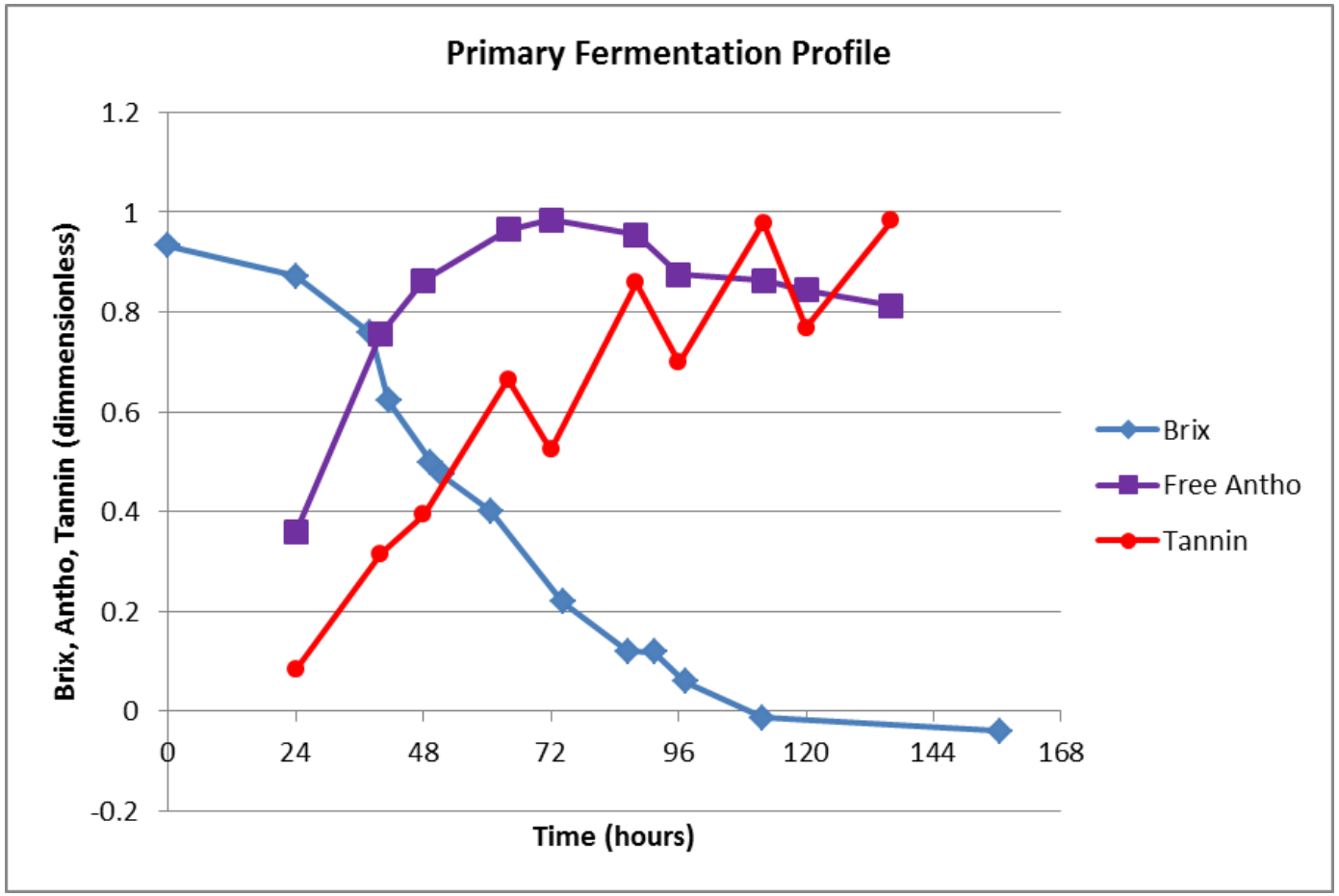




Analyses Performed

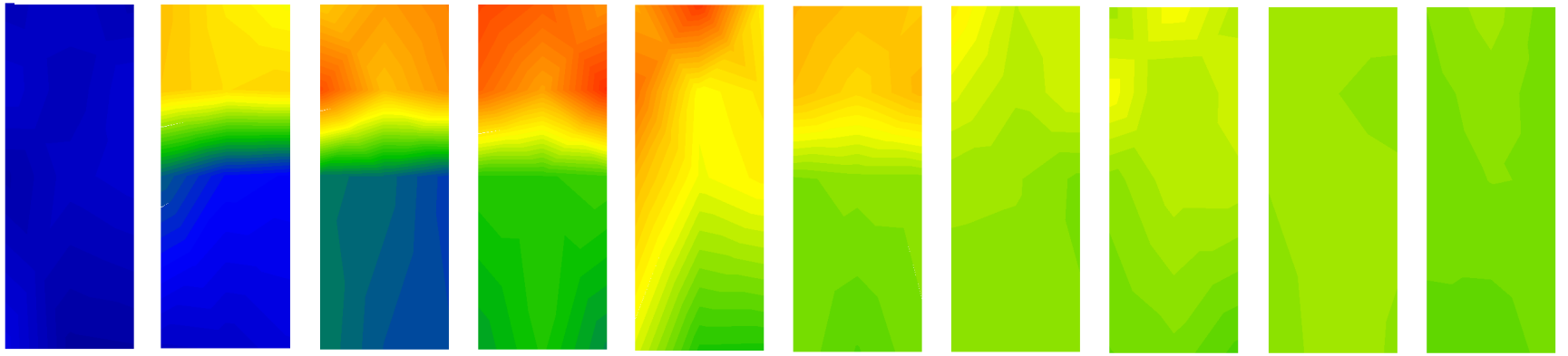
1. **Phloroglucinolysis of Isolated Tannins**
   - Tannin concentration, mDP, skin/seed contributions
2. **RP-HPLC Phenolic Assay**
   - Monomeric phenolic concentrations

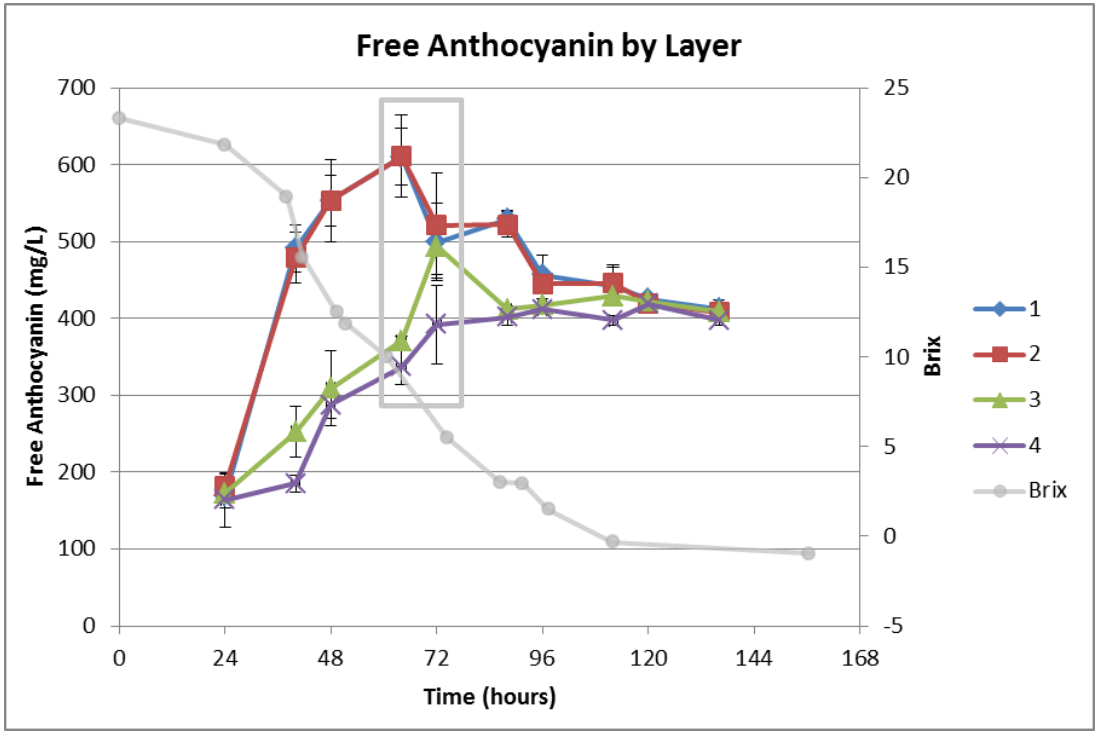
Fermentation Profile

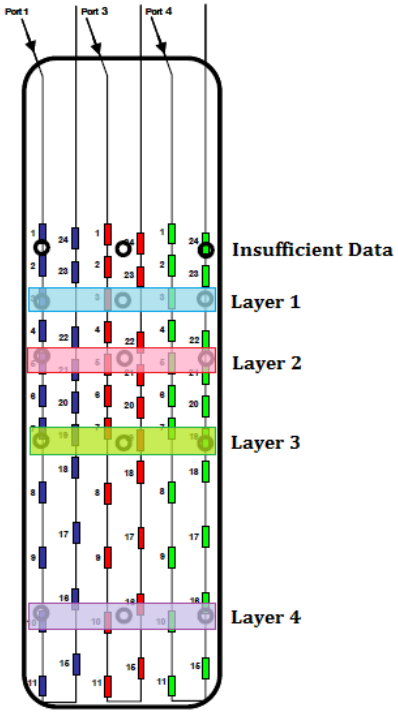


Normalized y-axis : Brix (1.0 = 25 Brix),
Free Anthocyanin (1.0 = 500 mg/L), Tannin (1.0 = 300 mg/L)

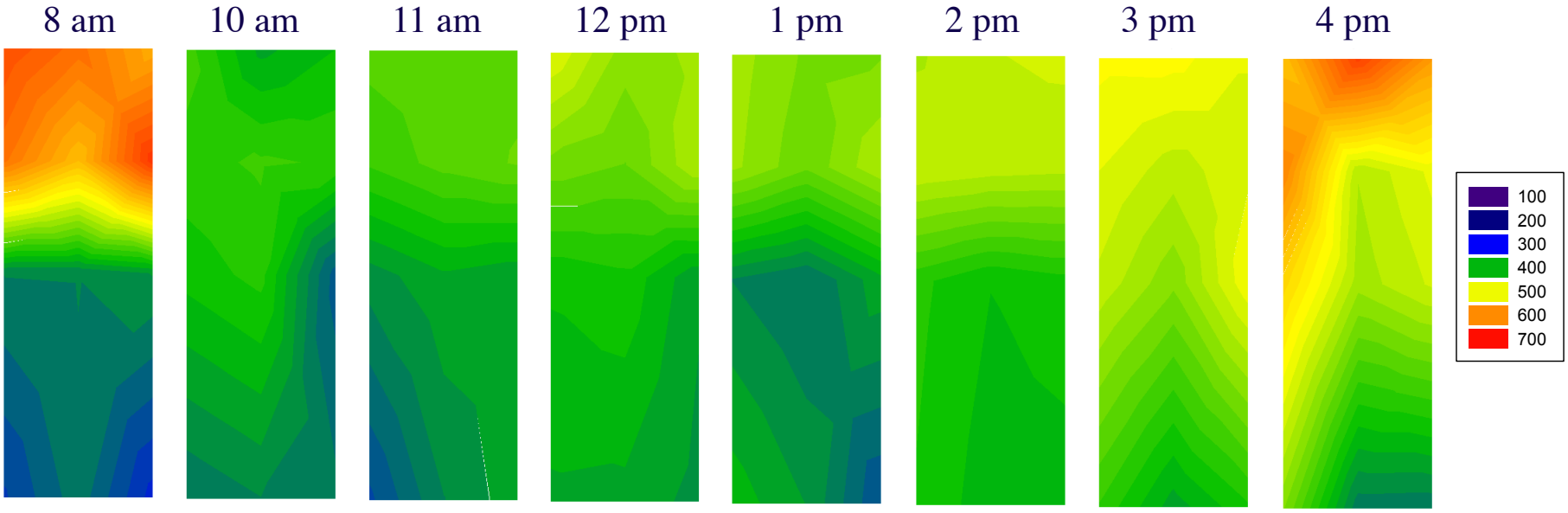
Results – Free Anthocyanin

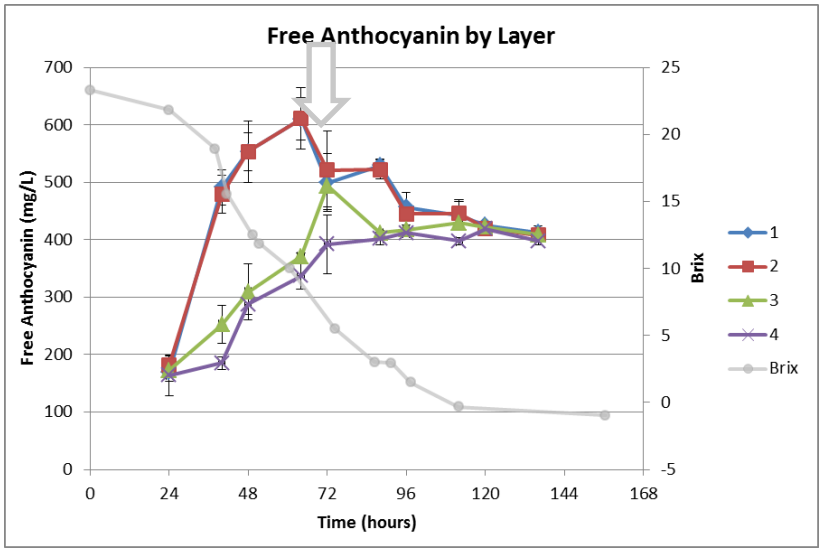




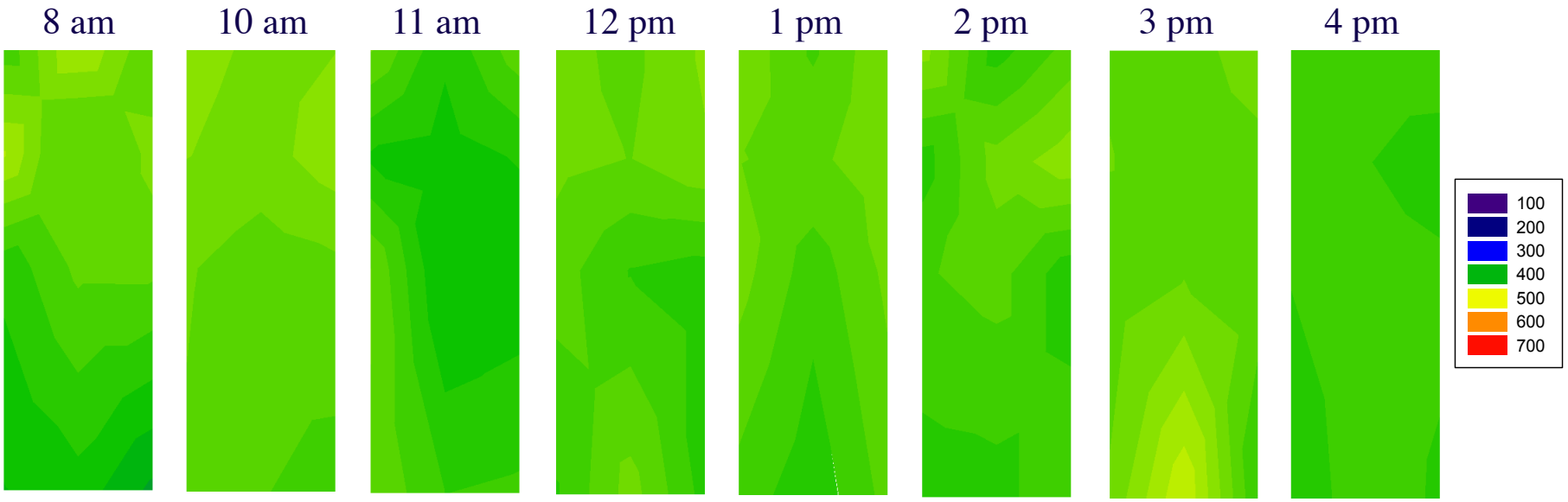


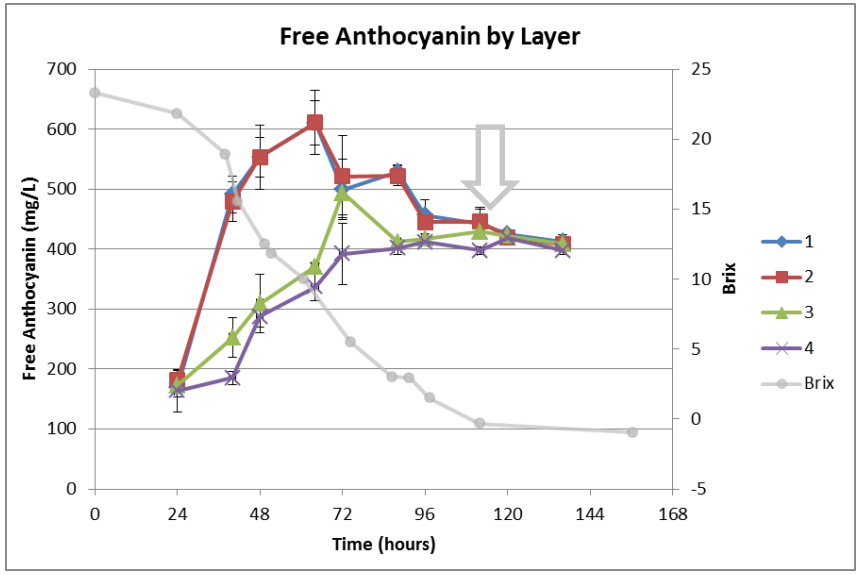
Free Anthocyanin Before and After A Pump-Over (Day 3)



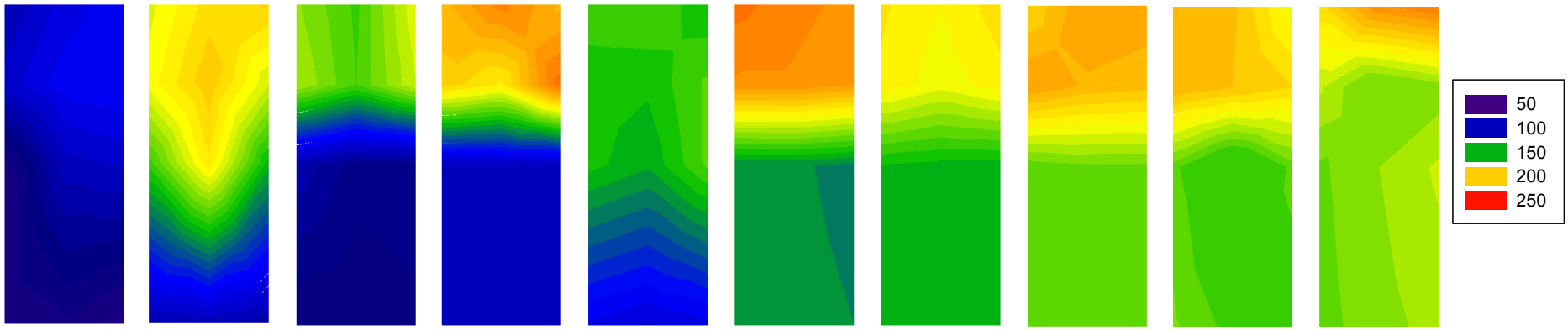


Free Anthocyanin Before and After A Pump-Over (Day 5)

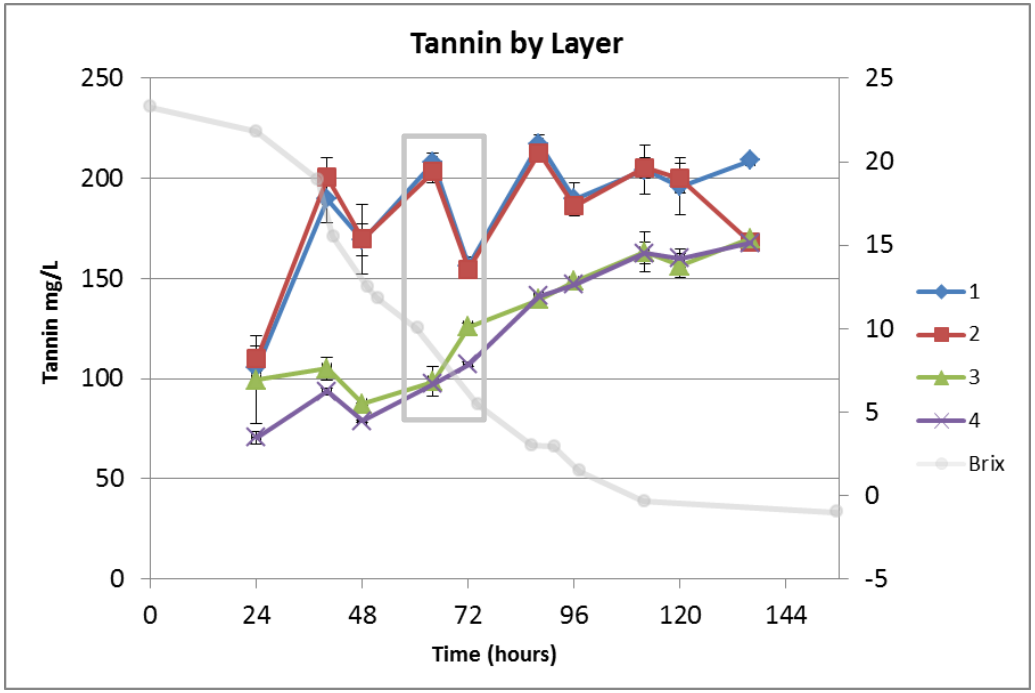


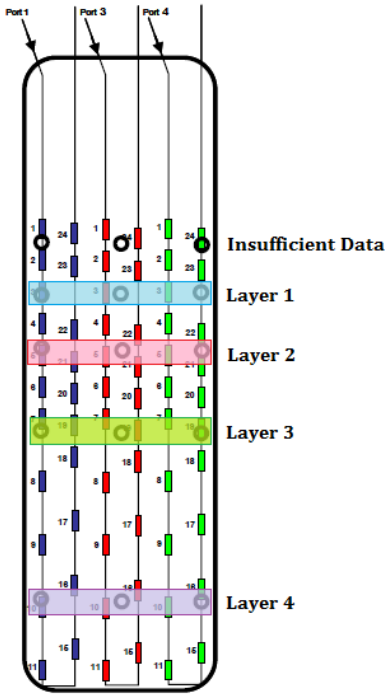


Tannin

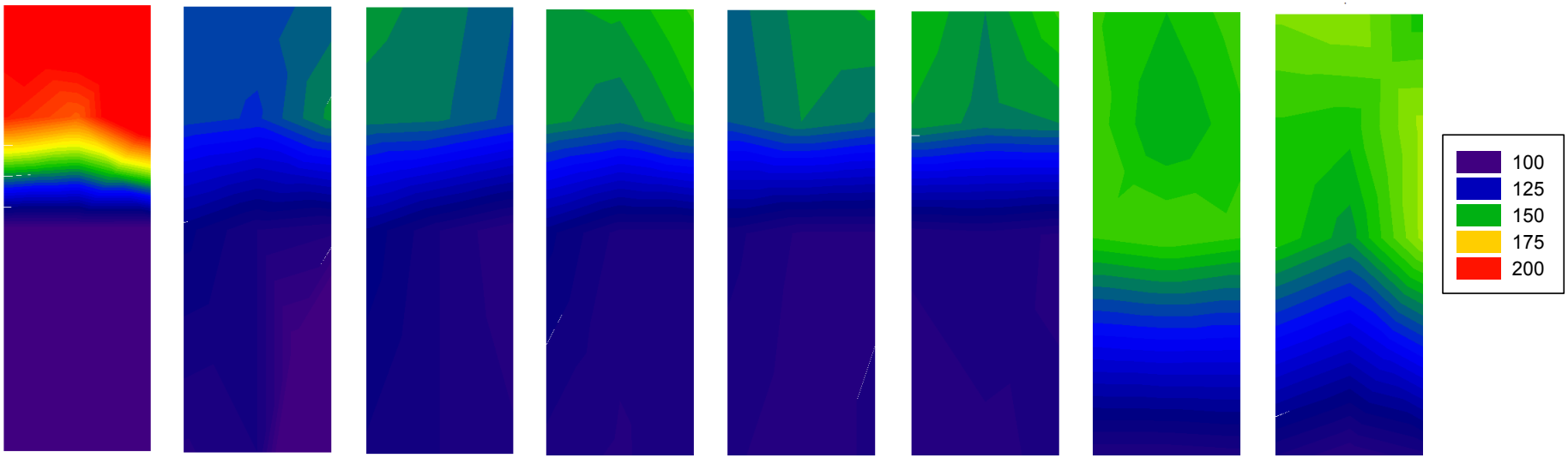


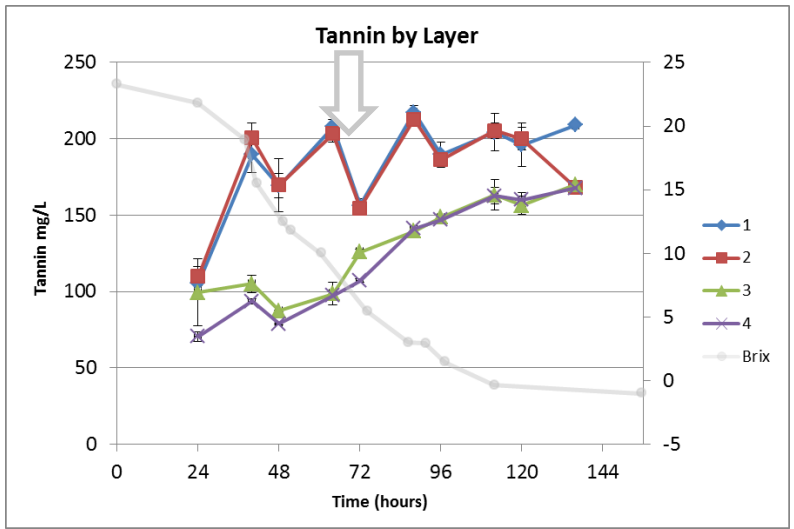
24 hr 40 hr 48 hr 64 hr 72 hr 88 hr 96 hr 112 hr 120 hr 136 hr



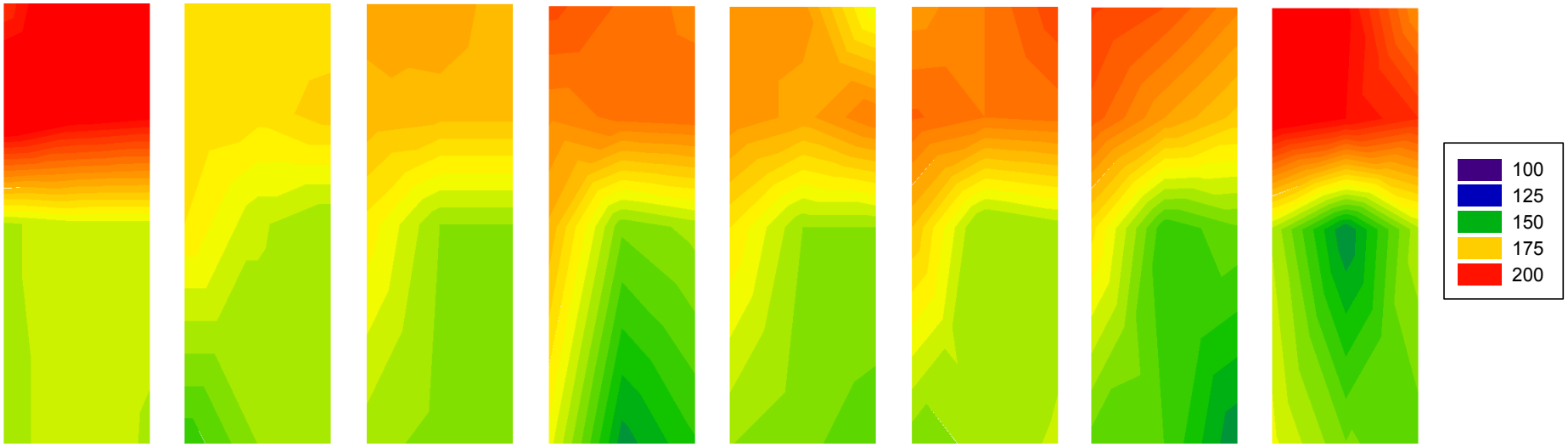


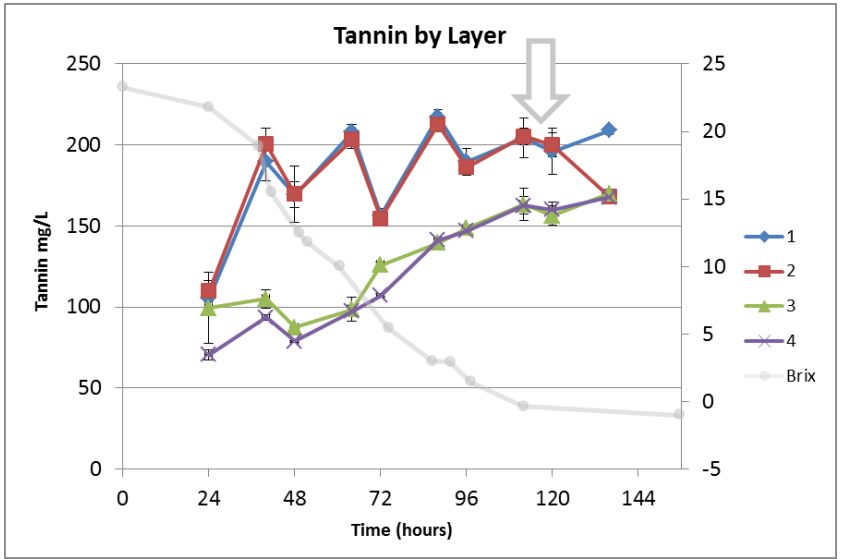
Tannin Before and After A Pump-Over (Day 3)



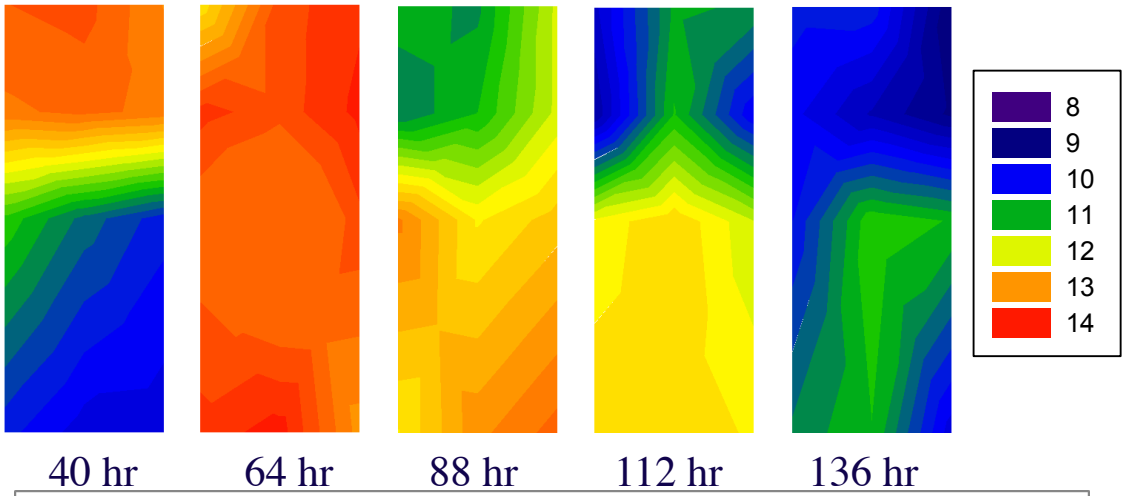


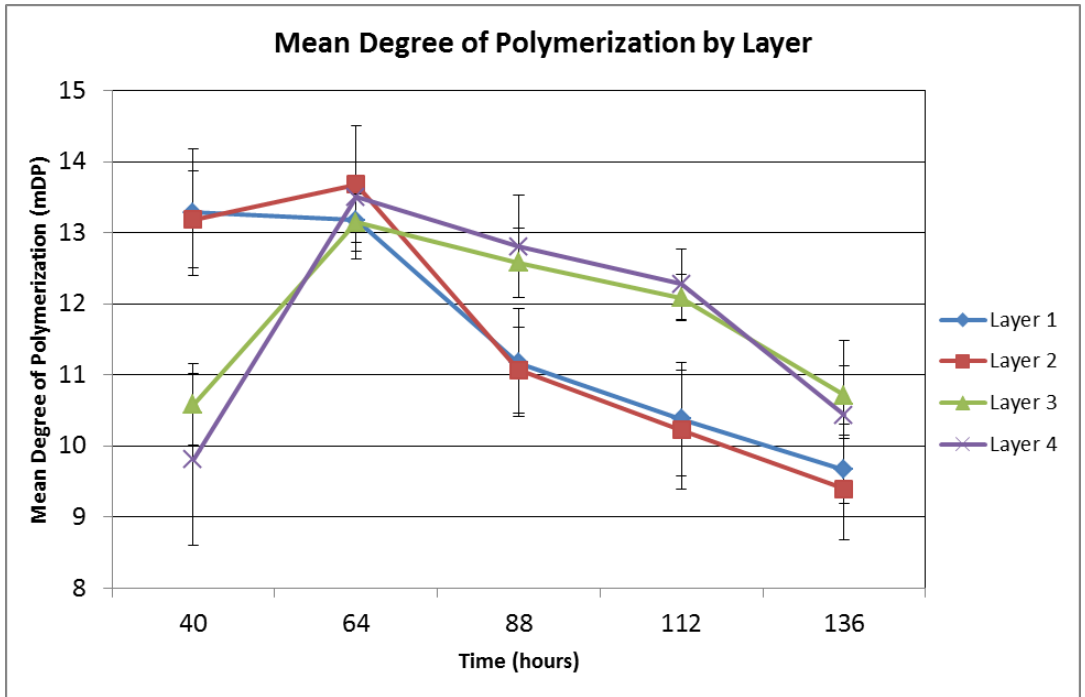
Tannin Before and After A Pump-Over (Day 5)





Mean Degree of Polymerization (mDP)

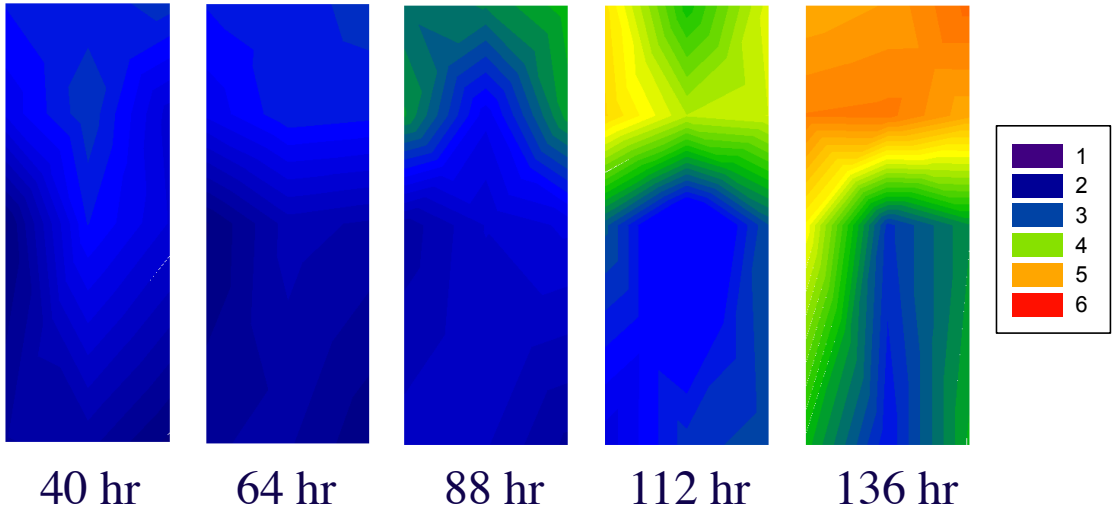


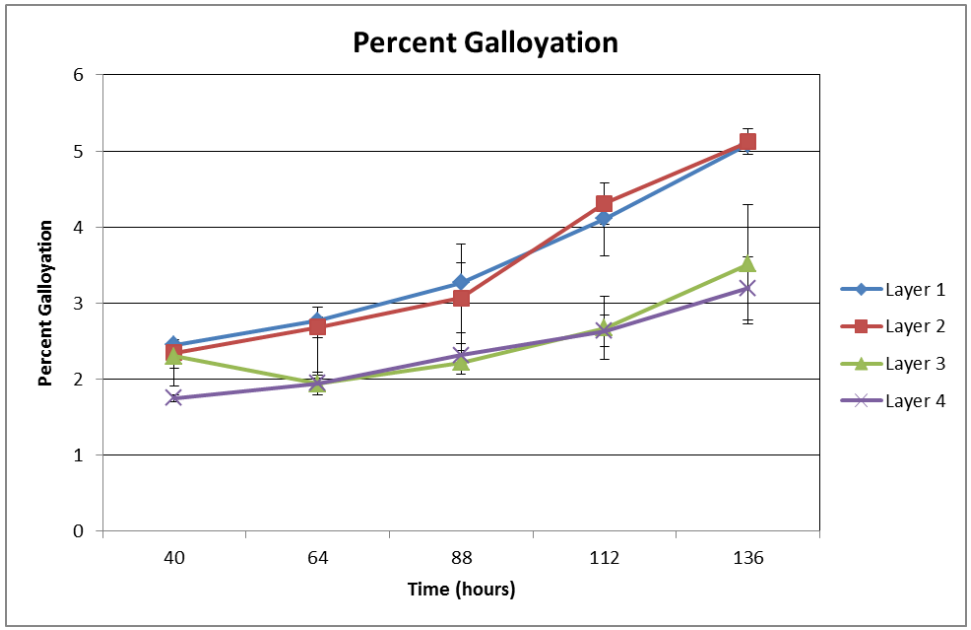


2 possible explanations:

1. Skin vs. seed tannin contribution
   - Initially, the larger skin tannins are extracted in the cap
   - Later in the fermentation, the smaller seed tannins are extracted, bringing the mDP down
2. Readsorption of larger tannins to the skin cell wall material
   - Larger tannin molecules have more reaction sites for hydrogen bonding

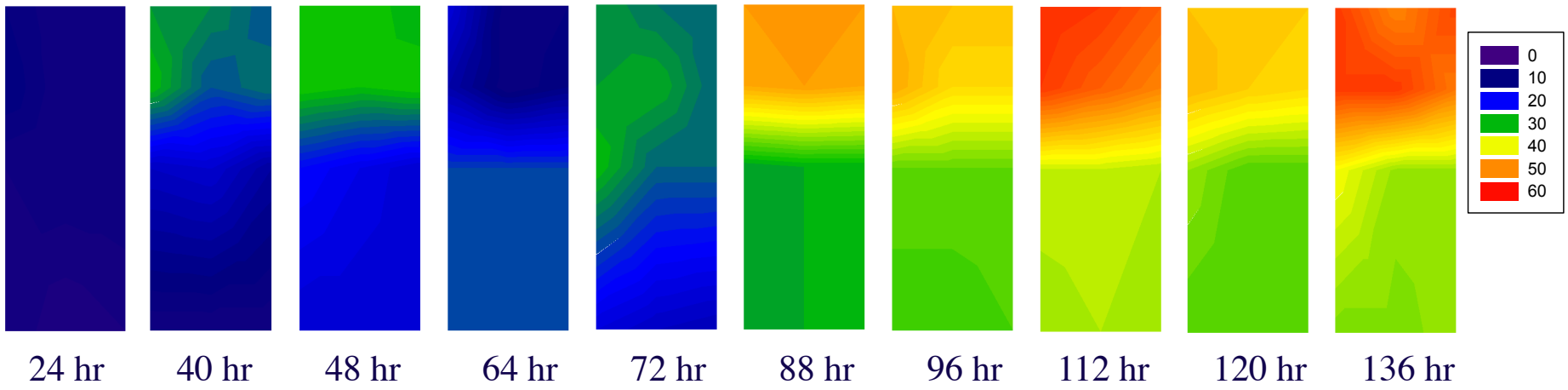
Percent Galloylation

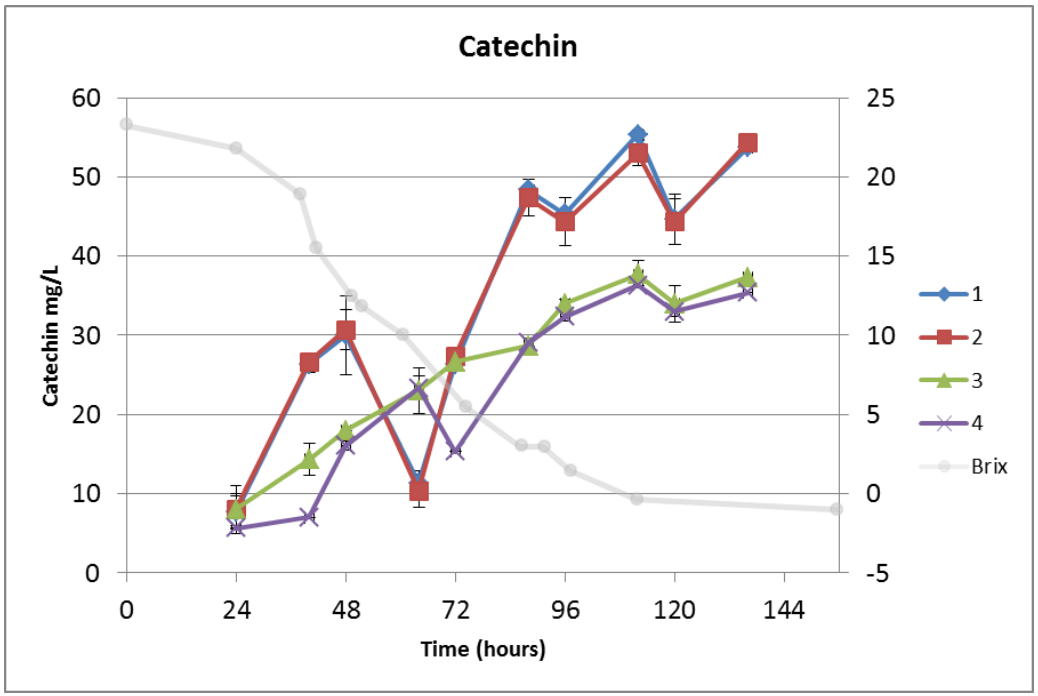




- Percent galloyation is the percentage of ECG subunits of tannins (determinged with phloroglucinolysis)
- % galloyation is greater in the seeds than the skins
- Increasing percent galloylation in the cap suggests increased extraction of tannins from the seeds

Phenolic Results - Catechin





- **Increases from ~8 mg/L to ~55 mg/L of catechin in the cap**
- **Relatively late to extract, could be a marker for seed extraction**

Learnings from gradient studies

- Gradients in phenolics do exist
- Skin extraction is early, seed extraction is late
- Early cap samples may indicate final tannin level—needs more work.
- Examining gradients in more detail may allow us to understand extraction at a more fundamental level

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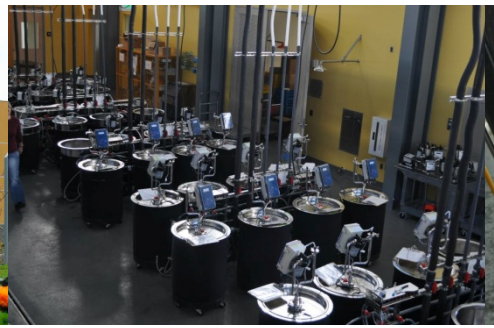



Effects of Pumpover Volume and Frequency

Driving innovation in grape growing and winemaking

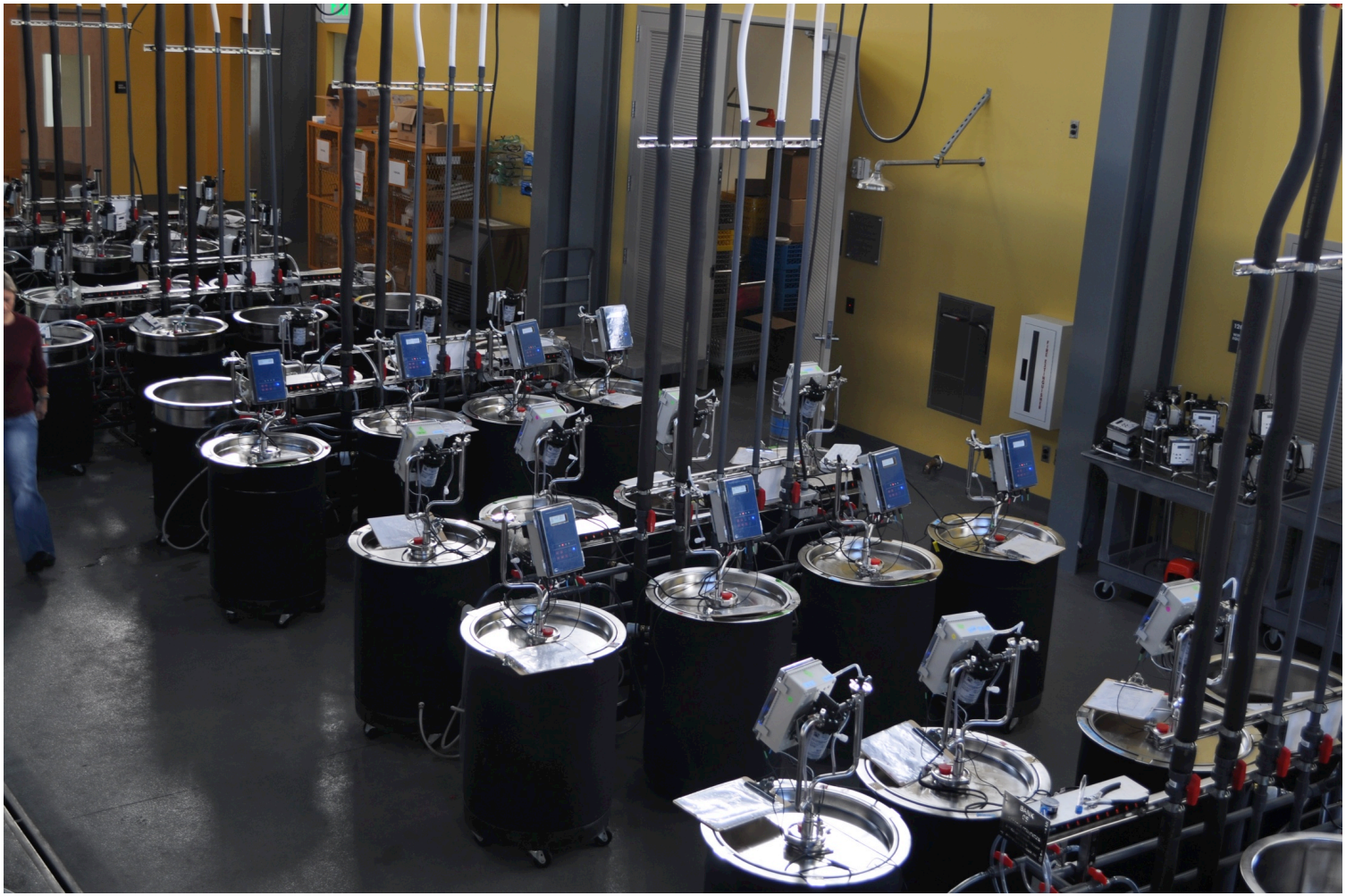
Fermentation treatments (2012)

Set	Treatment	Liquid Temp	Cap Temp	Cap Management Regime
A	Control (0.5)	25°C	n/a	PO 0.5 volume, 2/day
	Control (1)	25°C	n/a	PO 1 volume, 2/day
	Control (2)	25°C	n/a	PO 2 volumes, 2/day
B	1	20	20	As needed to maintain temp
	2	25	25	
	3	30	30	
	4	35	35	
C	1	20	25	As needed to maintain temp
	2	25	30	
	3	30	35	

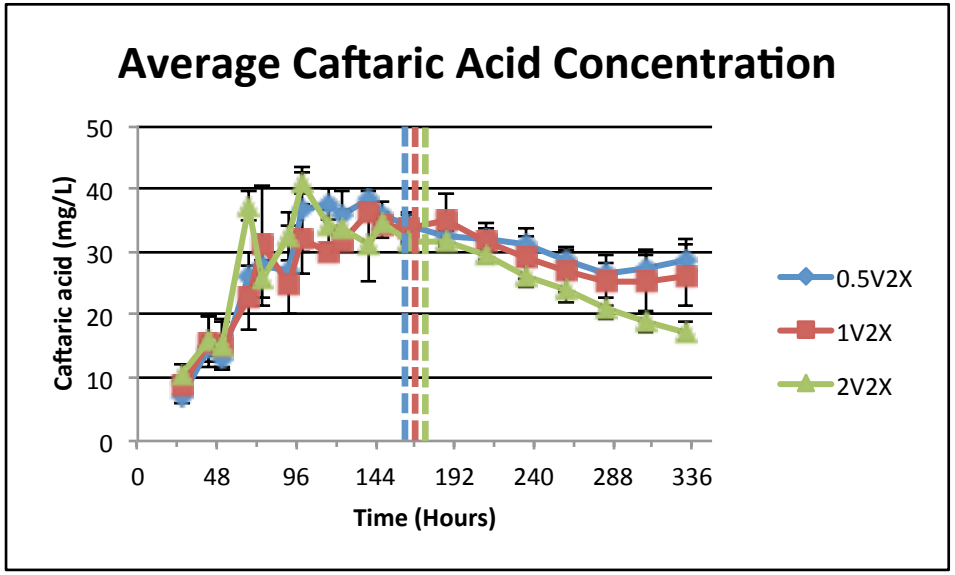
Wine production

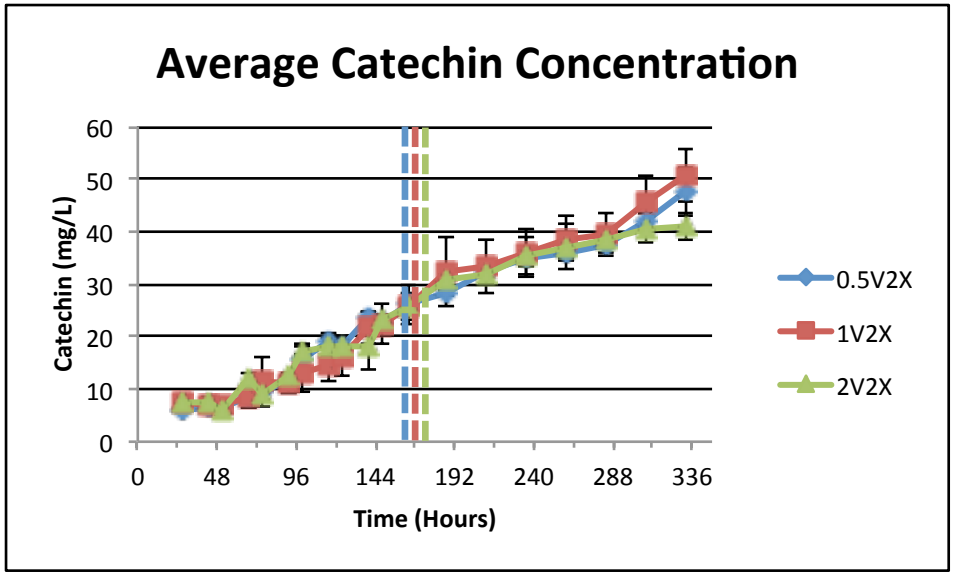
- Grapes: 2012 Cabernet Sauvignon from Lodi, CA
  - 24.3°Brix
  - pH = 3.85
  - T.A. = 3.8 g/L (adjusted to 5.97 g/L)
- YAN adjusted to 300 ppm, addition of 50 ppm SO_2
- Inoculated with *S. cerevisiae* strain Lalvin D254
- Pressed after 14 days – 7-9 days extended maceration
- Sampling: AM & PM till dry, then AM only
- Fermentations performed in triplicate using Cypress/UC Davis Research Fermentors (TJs)

Using the TJ Fermentors

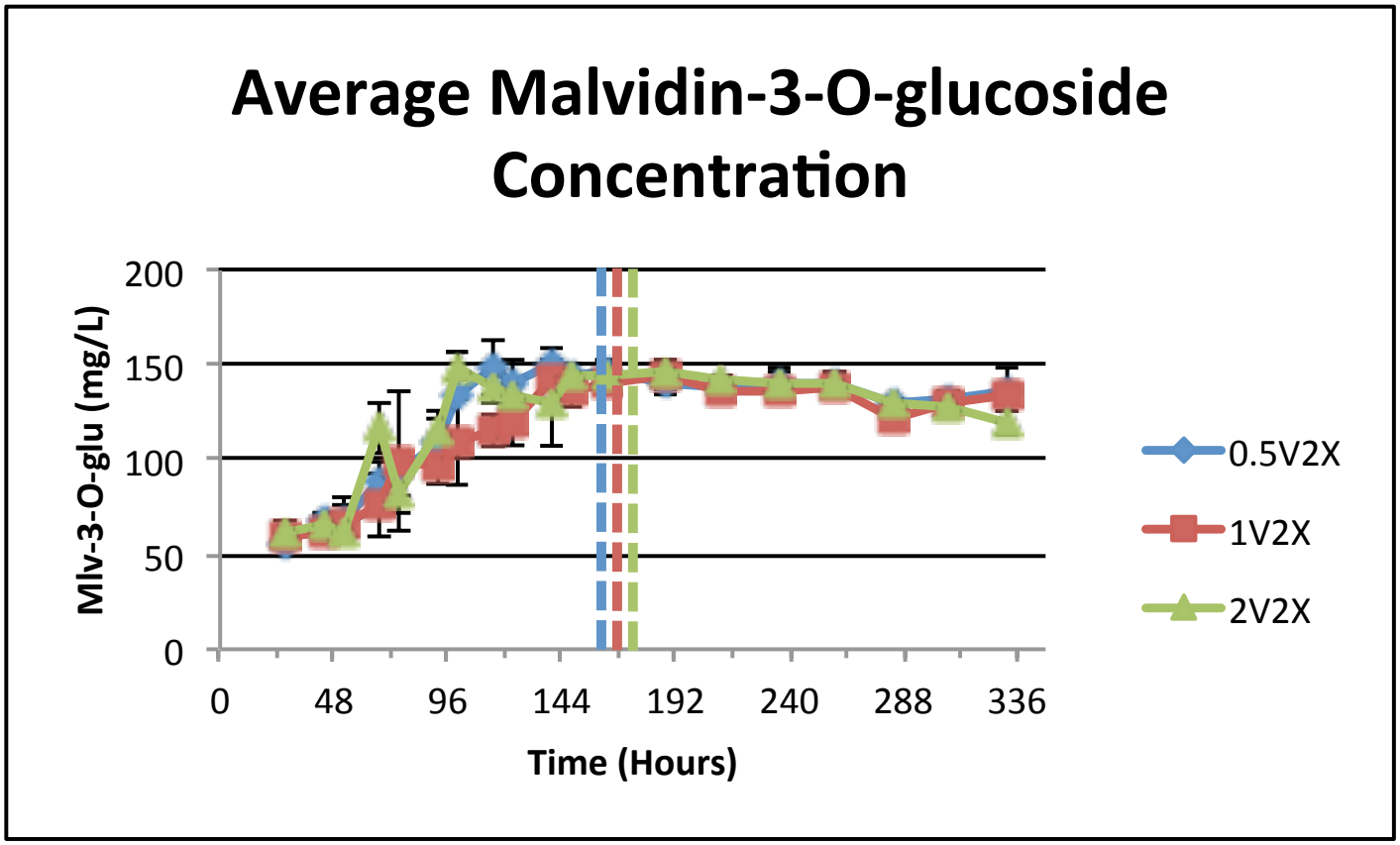


Effect of Pumpover Volume





Effect of Pumpover Volume

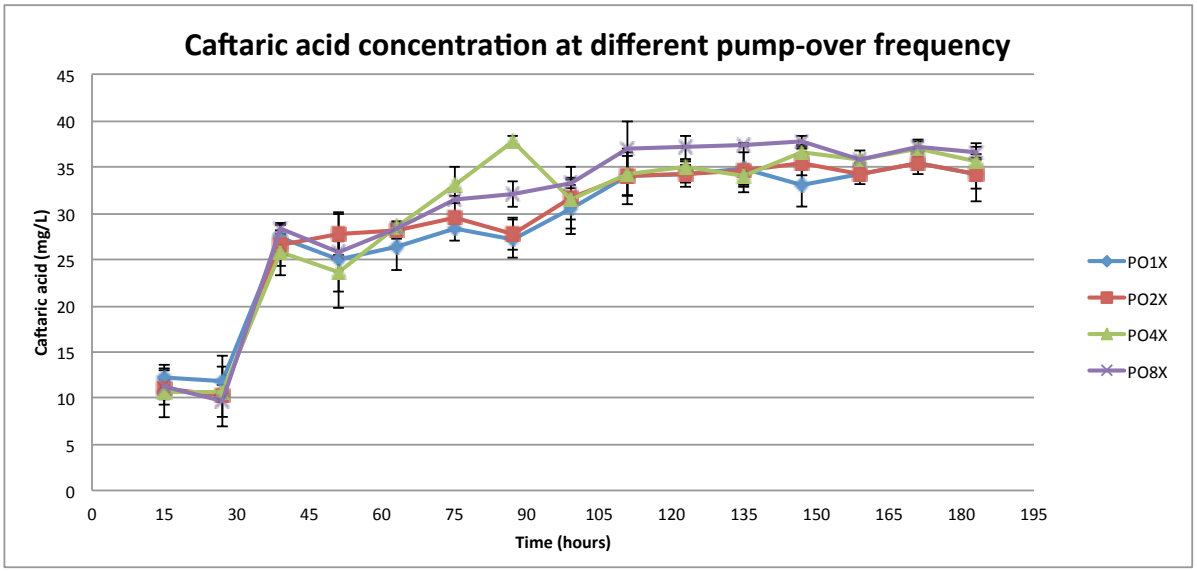


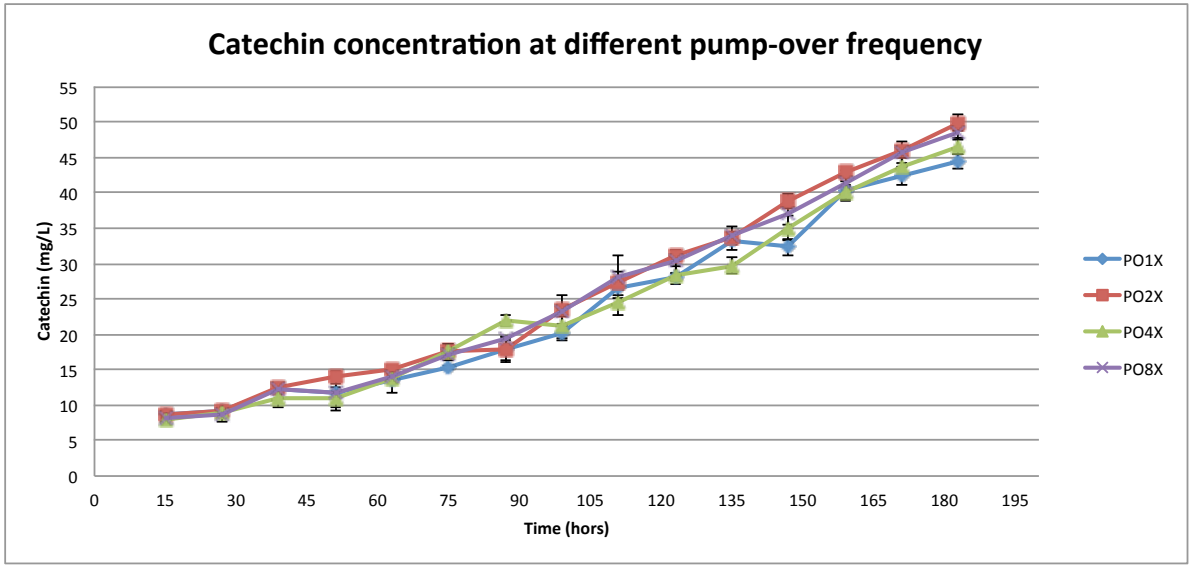
Examining pumpover frequency

Set	Treatment	Liquid Temp	Cap Temp	Cap Management Regime
A	1	25	NC	1/2 vol 8x per day
	2	25	NC	1 vol 4x per day
	3	25	NC	2 vol 2x per day
	4	25	NC	4 vol 1x per day
B	1	1 day Cold Soak, 25	NC	2 vol 2x per day
	2	4 day Cold Soak, 25	NC	2 vol 2x per day
	3	7 day Cold Soak, 25	NC	2 vol 2x per day
	4	10 day Cold Soak, 25	NC	2 vol 2x per day

For 2013, pressed all batches at 8 days.

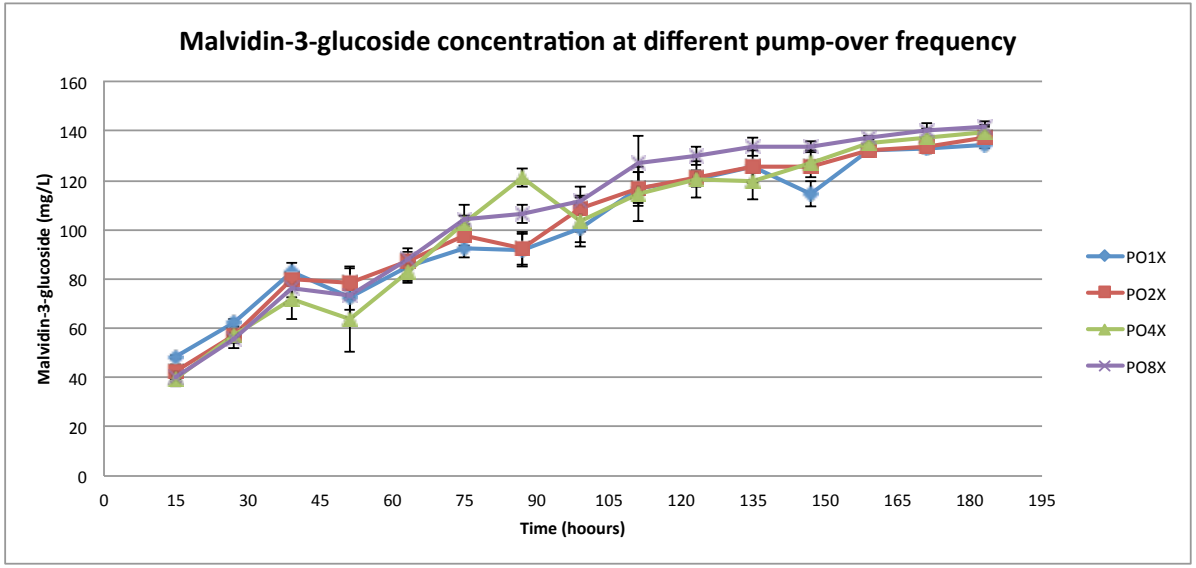
Pumpover frequency does not affect phenolic extraction

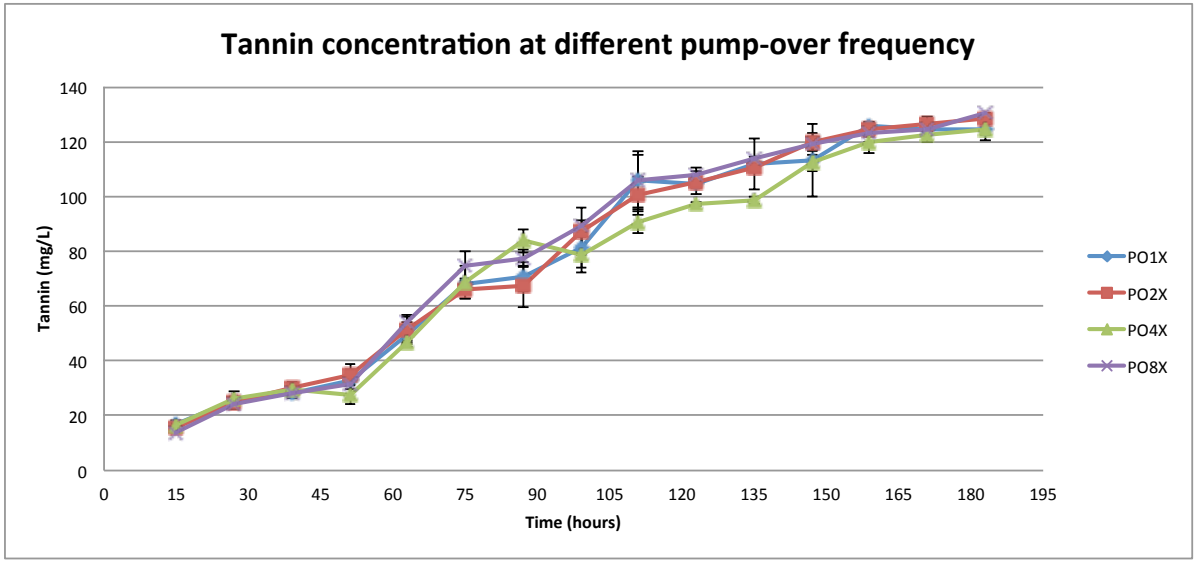




RP-HPLC

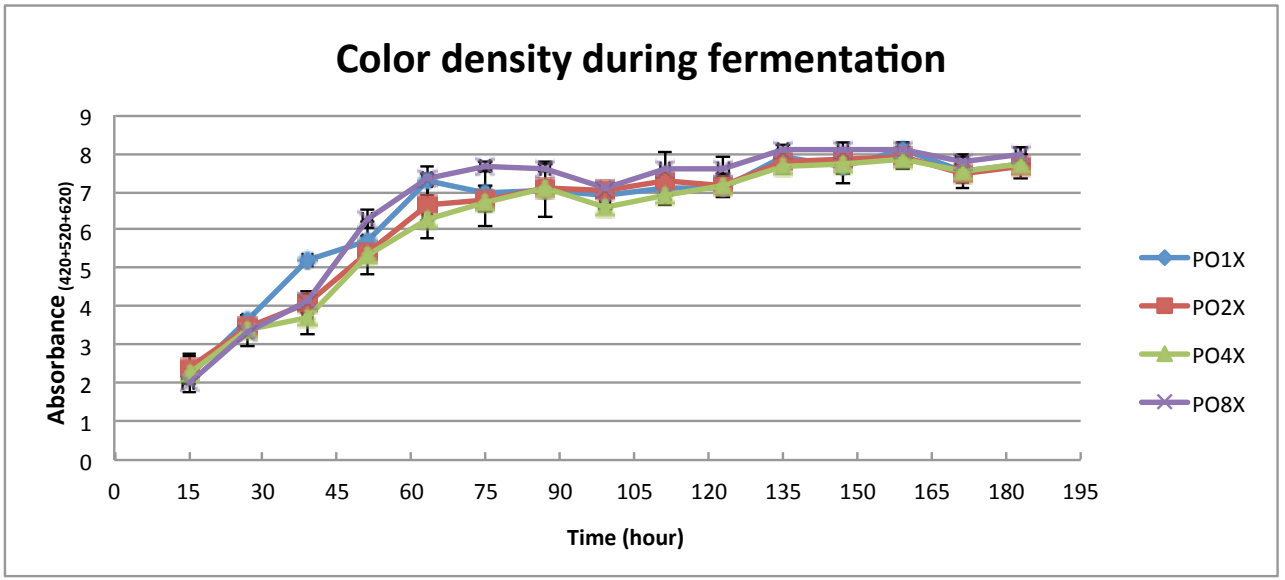
Pumpover frequency does not affect phenolic extraction

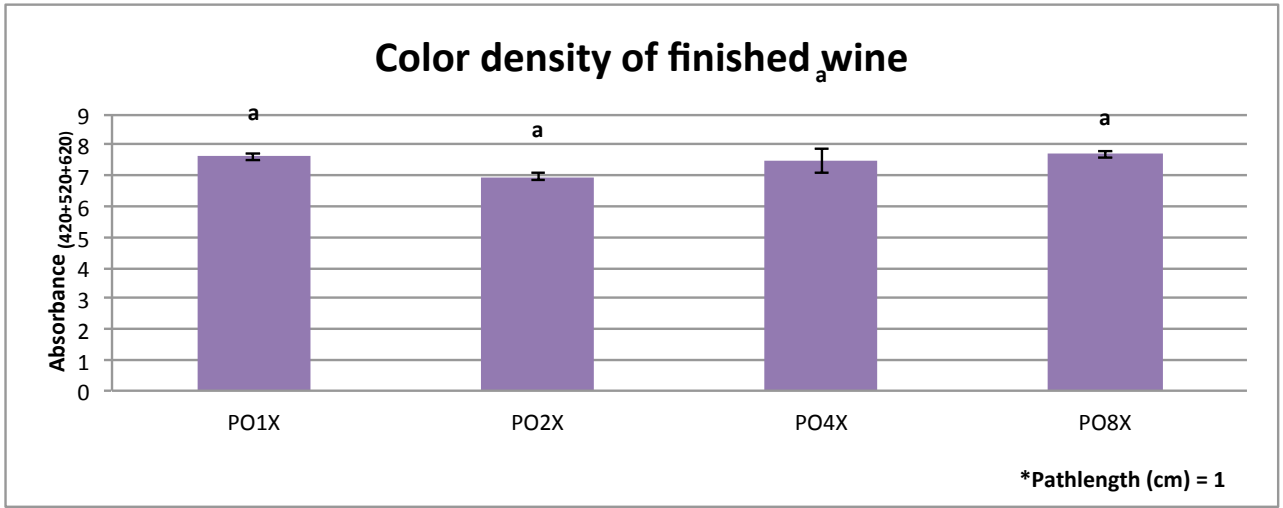




RP-HPLC

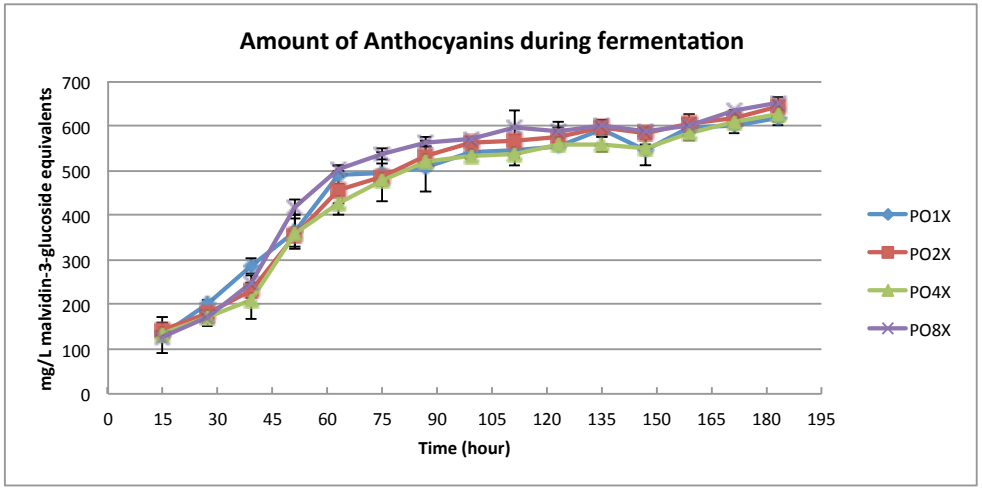
No change in color

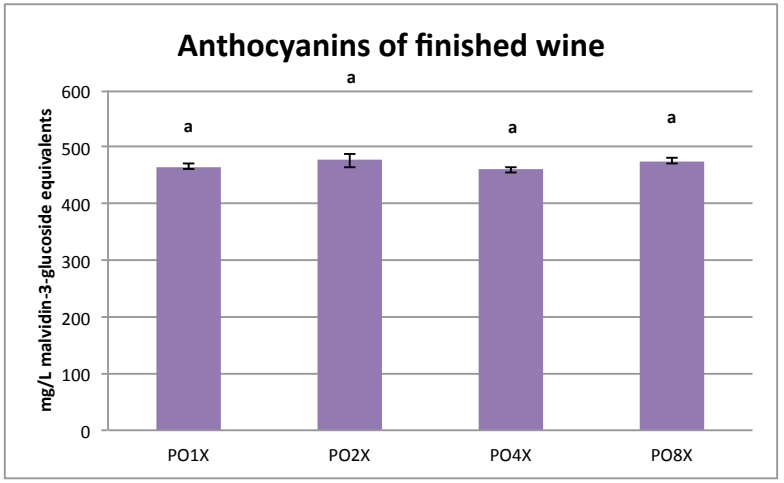


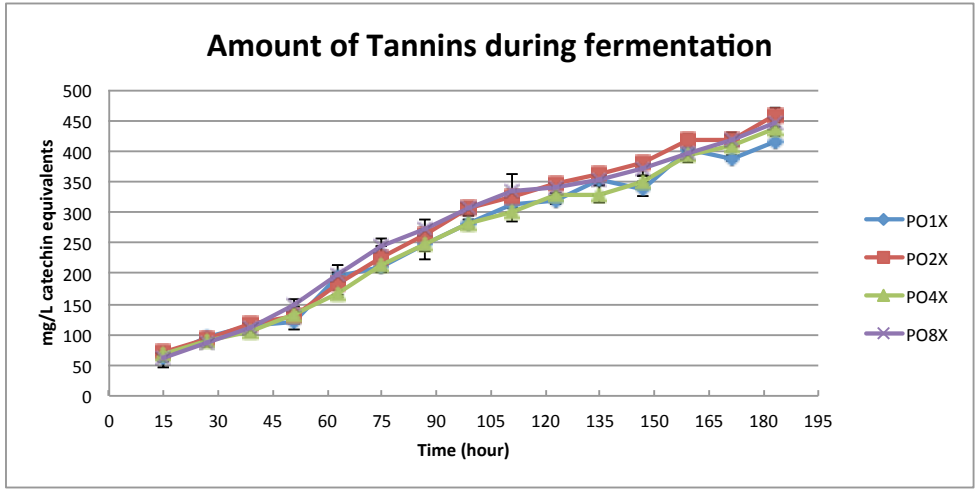


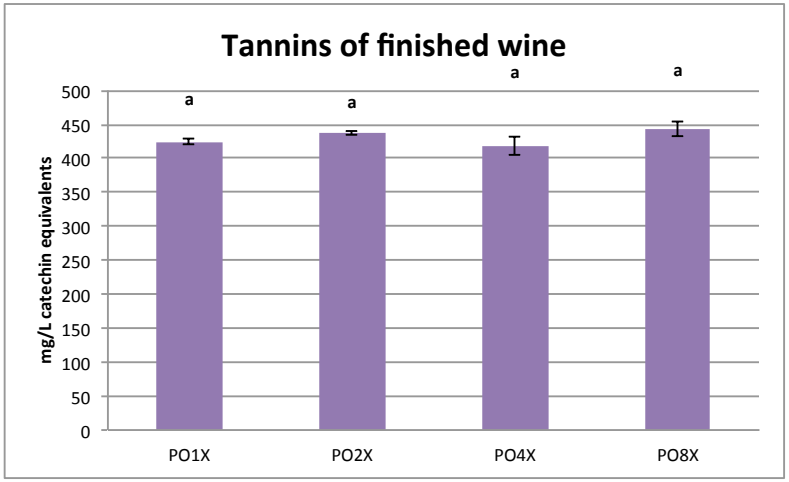
UV-VIS

No effect on anthocyanin or tannin extraction

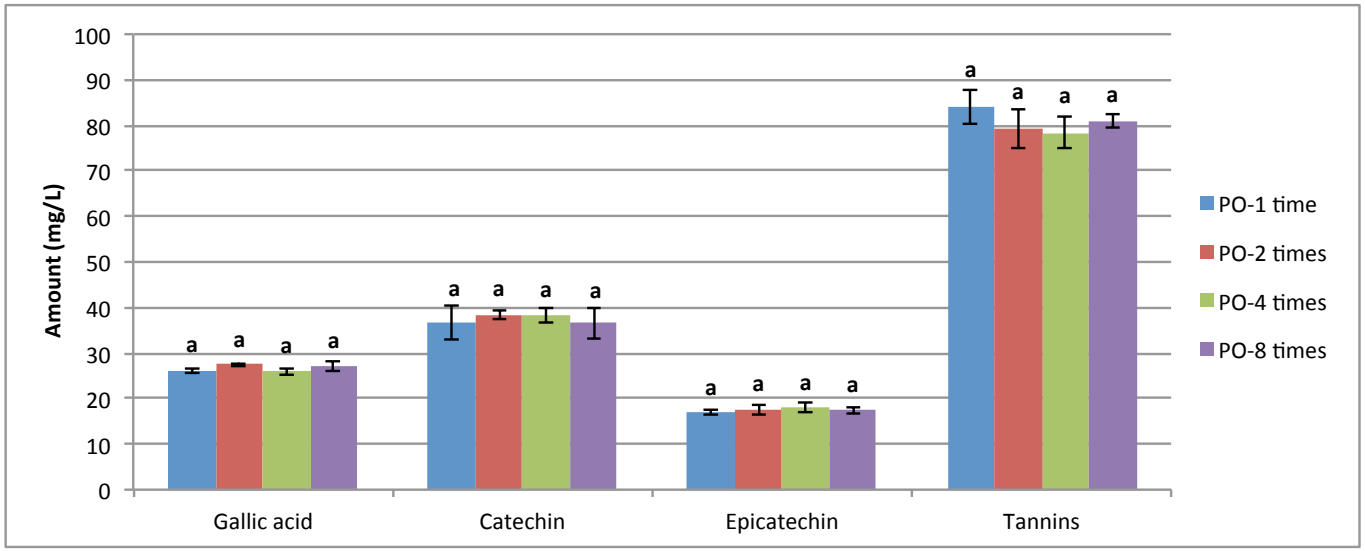


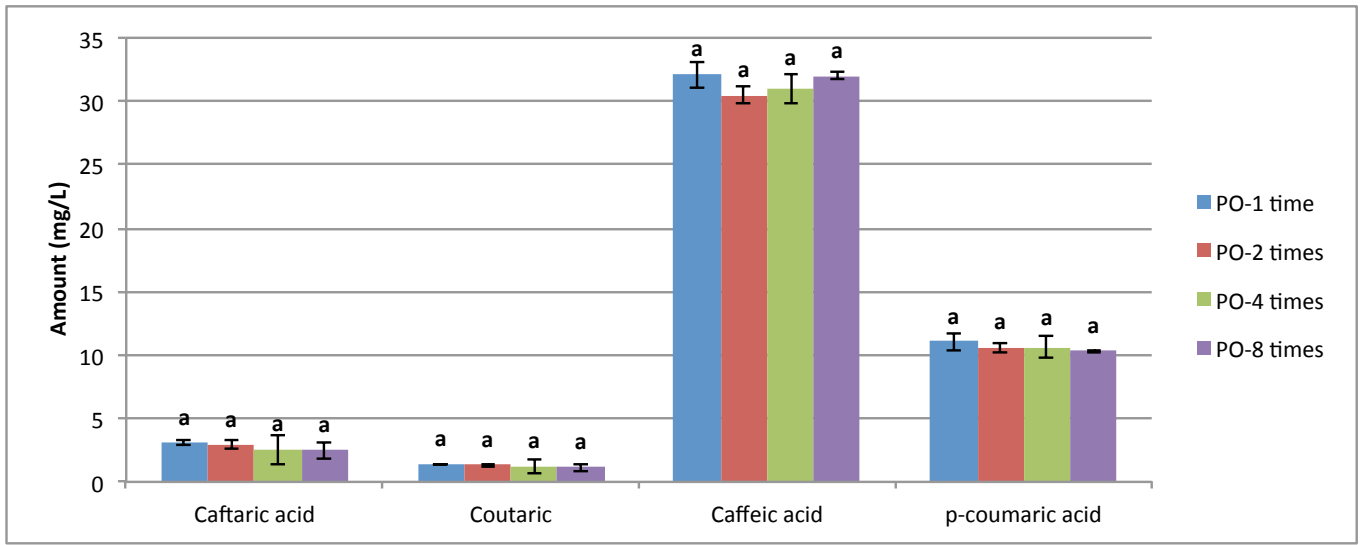






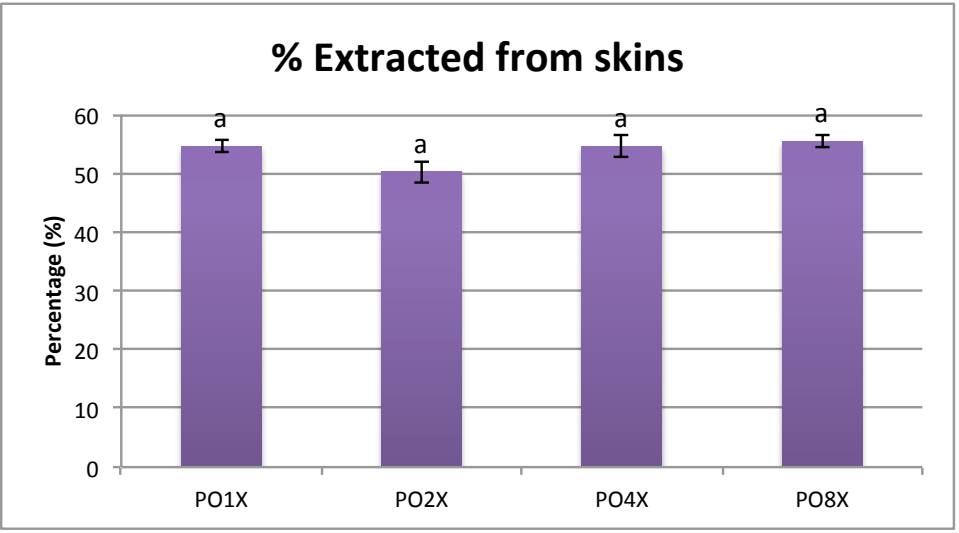
No effect on phenolic profile of finished wine

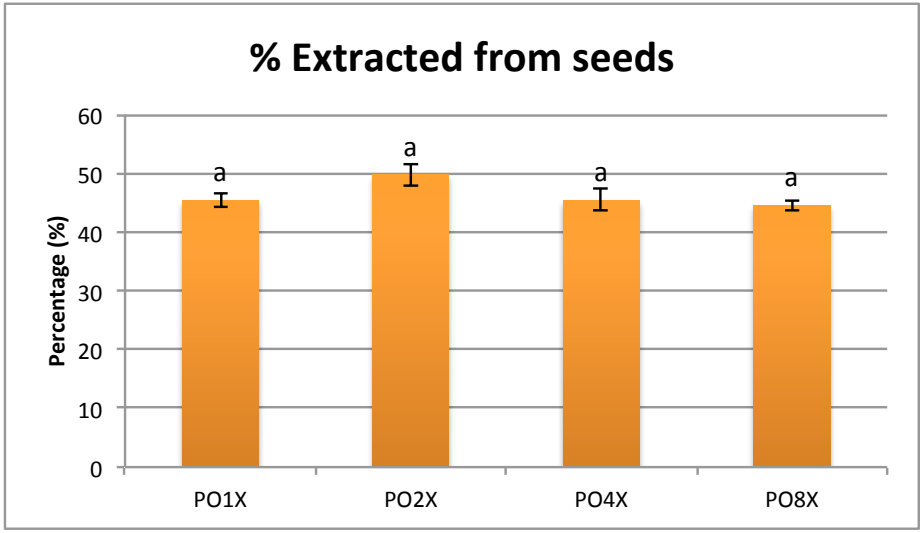




RP-HPLC

No effect on origin of tannins





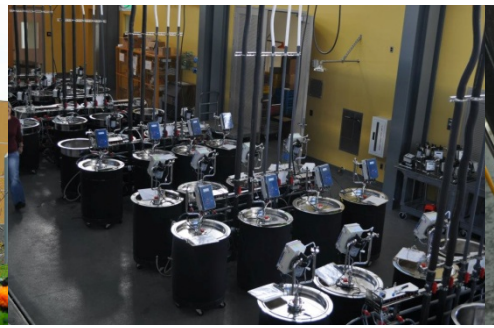
Phloroglucinolysis

Learnings from pumpover studies

- **Pumpover volume does not make a difference in phenolic extraction (for volumes tested)**
- **Pumpover frequency does not make a difference in phenolic extraction**

UC DAVIS
UNIVERSITY OF CALIFORNIA





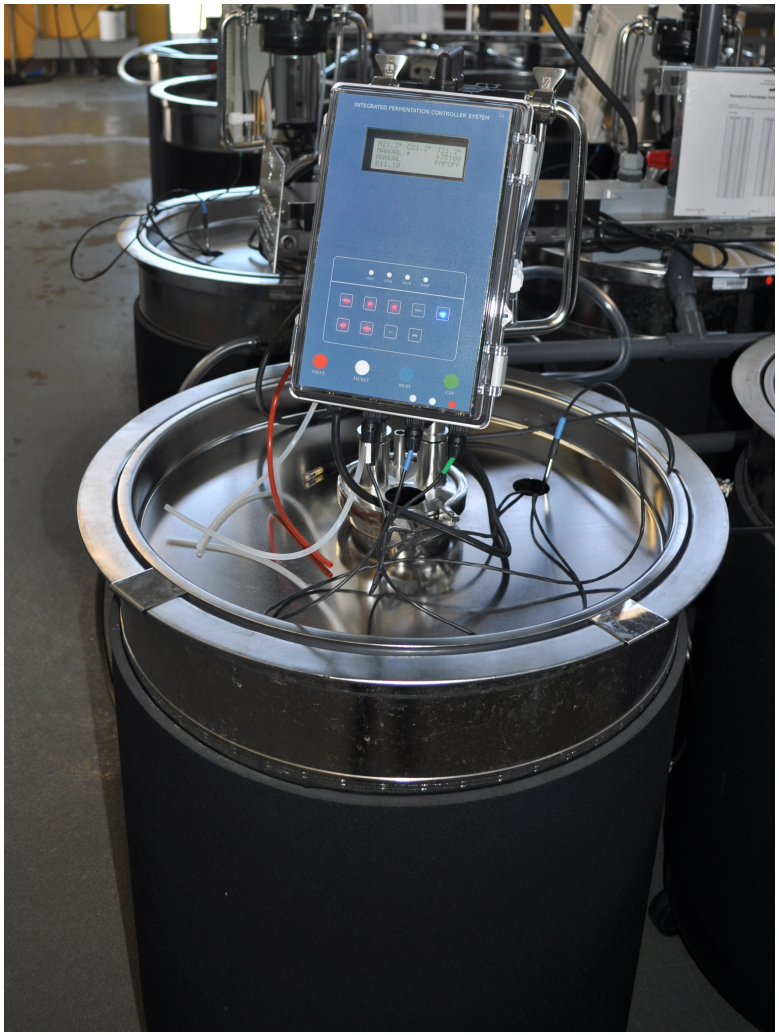

Effects of Cap and Must Temperature on Phenolic Extraction

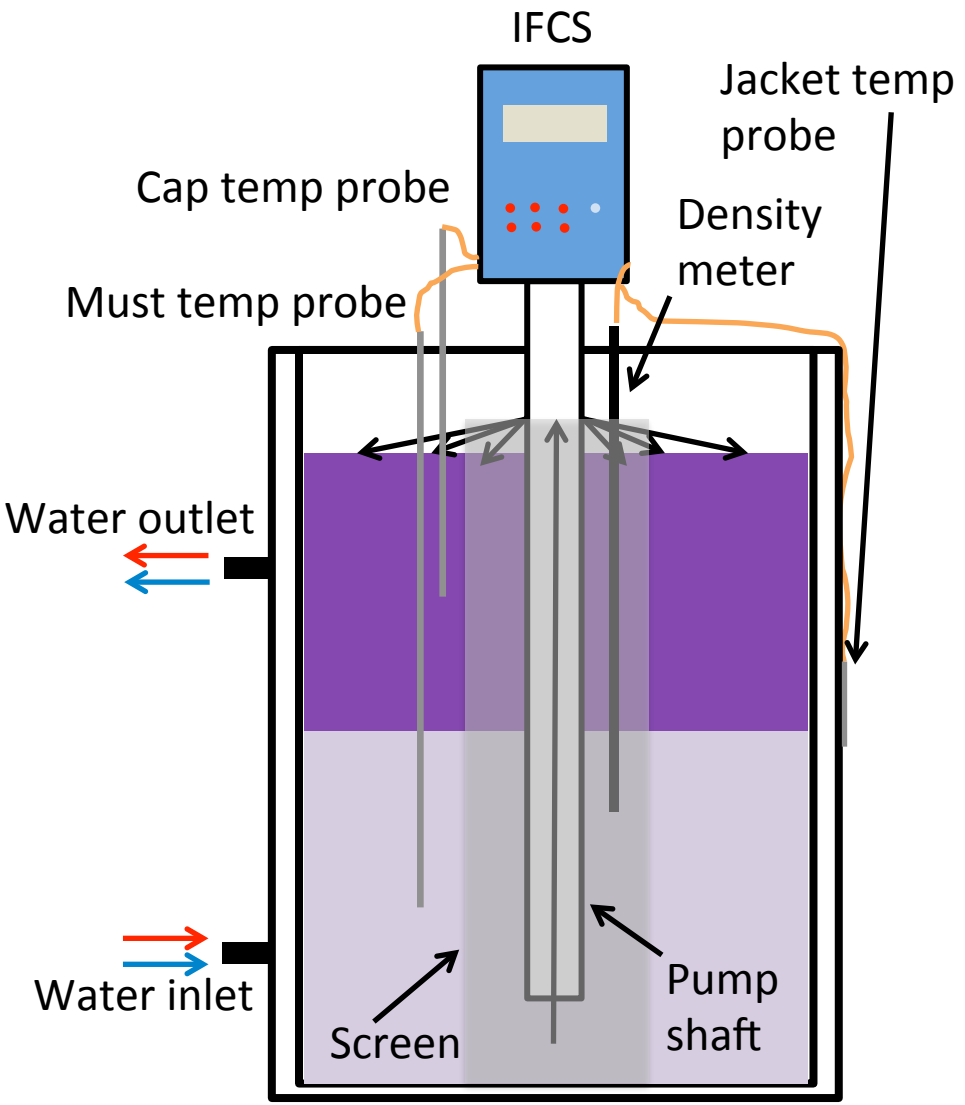
Driving innovation in grape growing and winemaking

Fermentation treatments (2012)

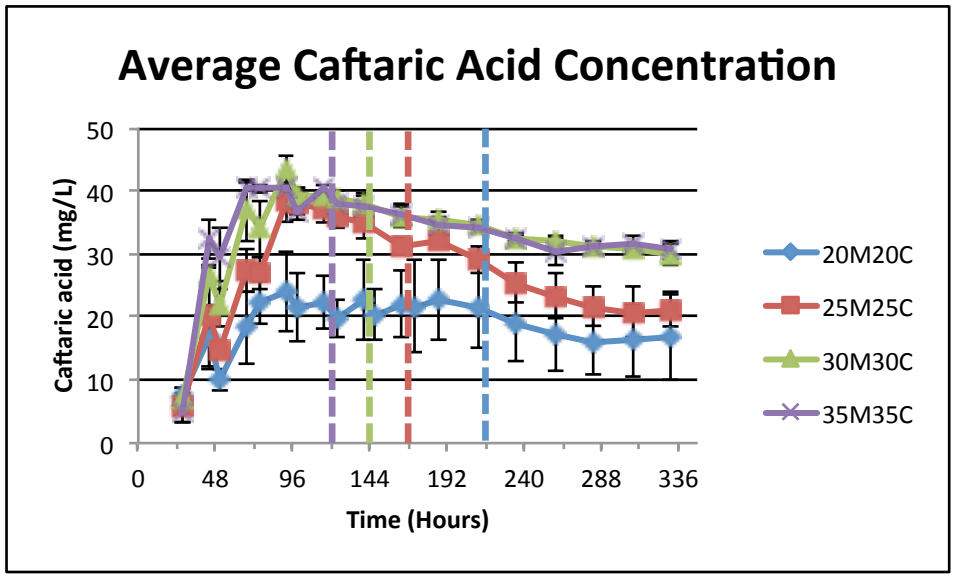
Set	Treatment	Liquid Temp	Cap Temp	Cap Management Regime
A	Control (0.5)	25°C	n/a	PO 0.5 volume, 2/day
	Control (1)	25°C	n/a	PO 1 volume, 2/day
	Control (2)	25°C	n/a	PO 2 volumes, 2/day
B	1	20	20	As needed to maintain temp
	2	25	25	
	3	30	30	
	4	35	35	
C	1	20	25	As needed to maintain temp
	2	25	30	
	3	30	35	

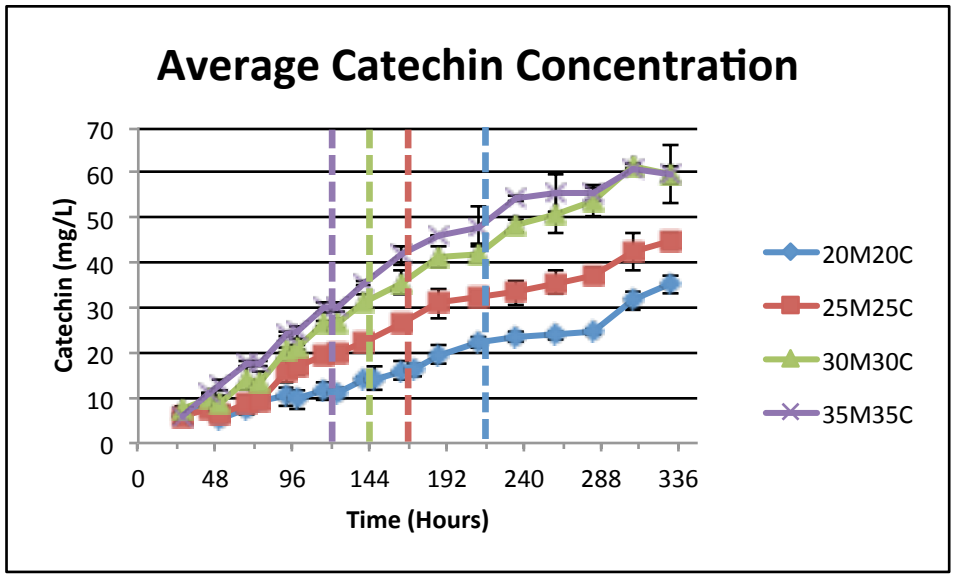
TJ Fermentors



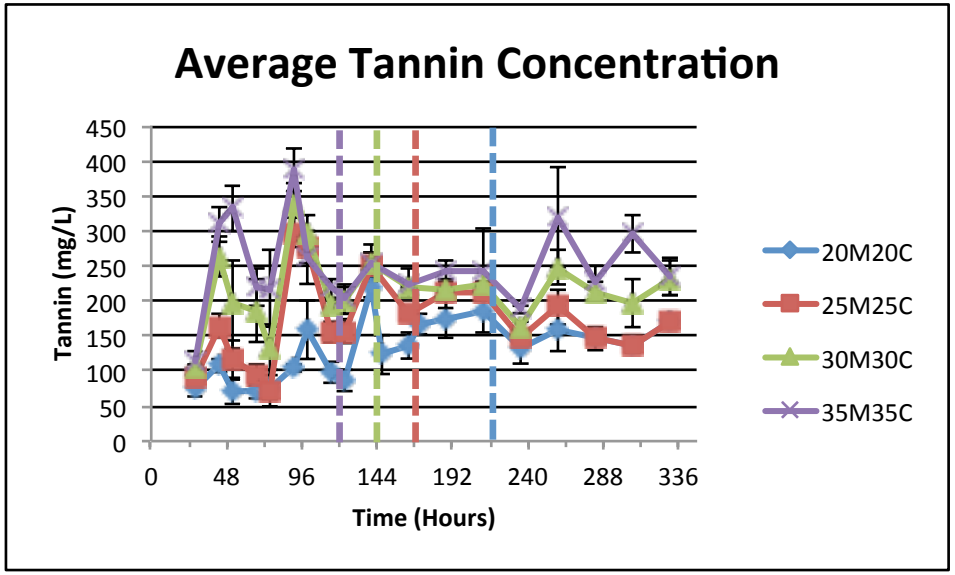


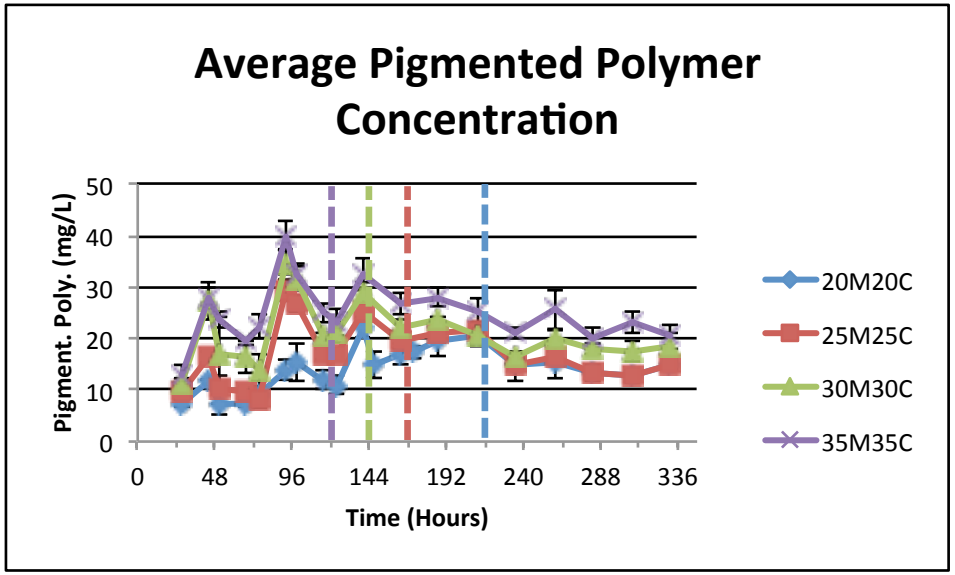
Effect of Cap and Liquid Temperature



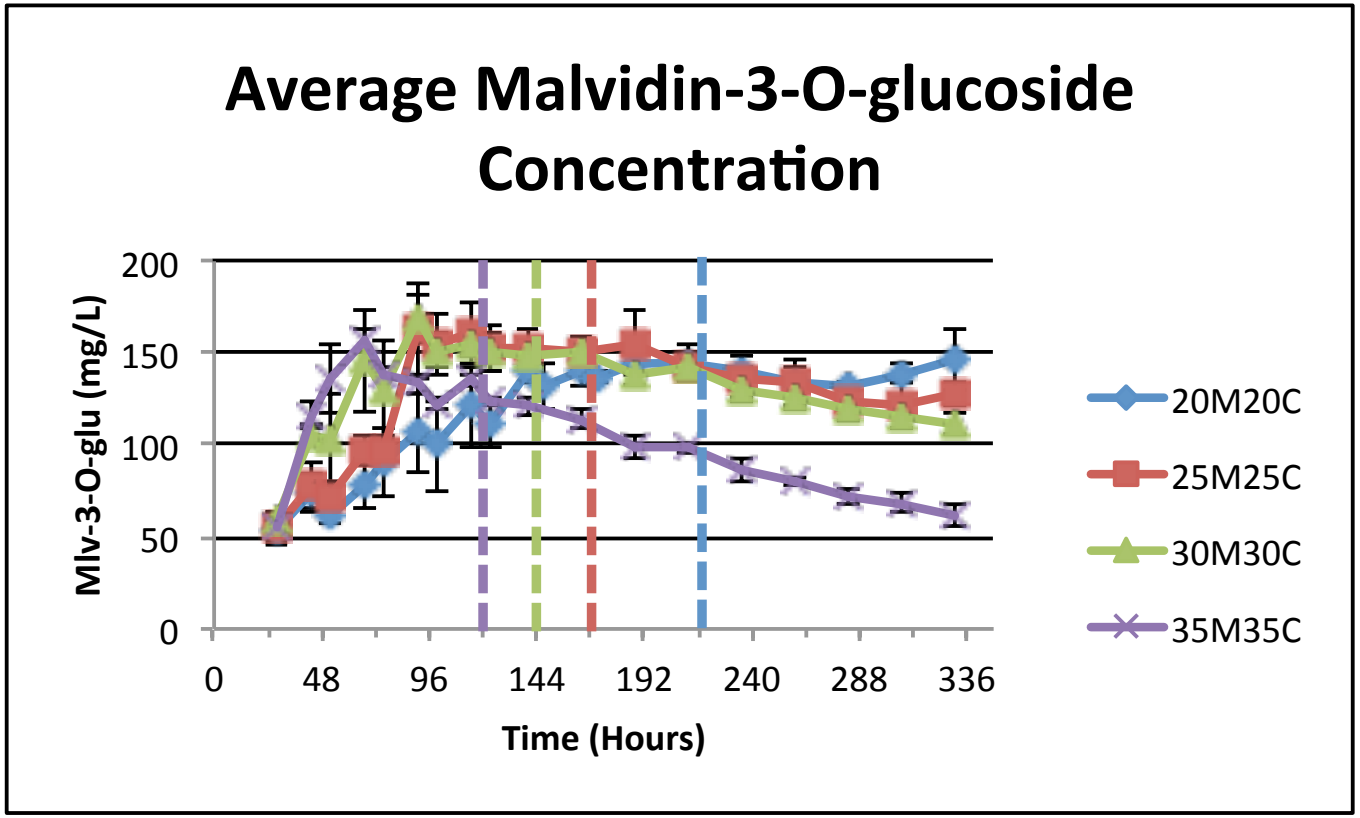


The effect of cap and liquid temperature

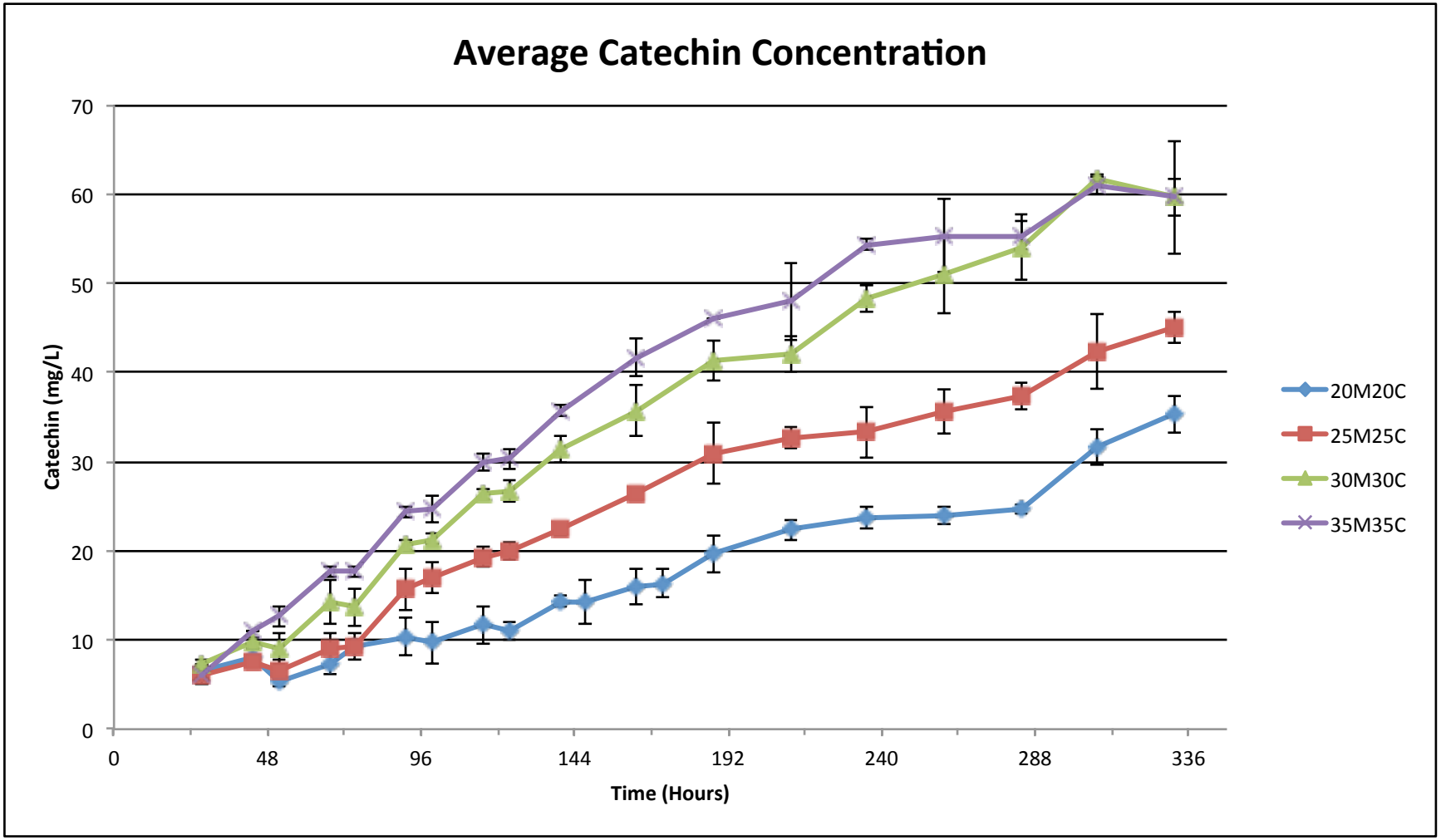




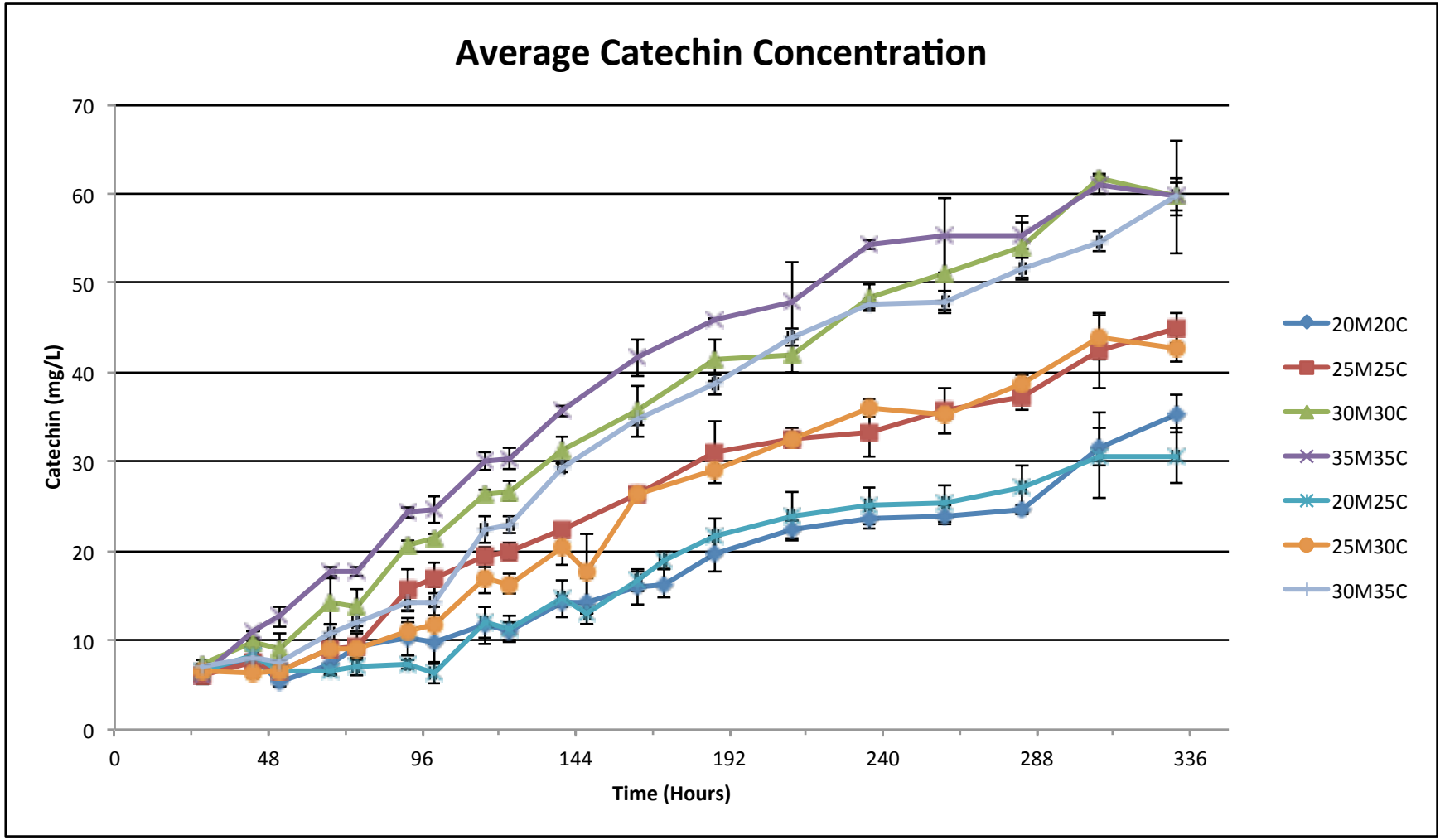
The effect of cap and liquid temperature



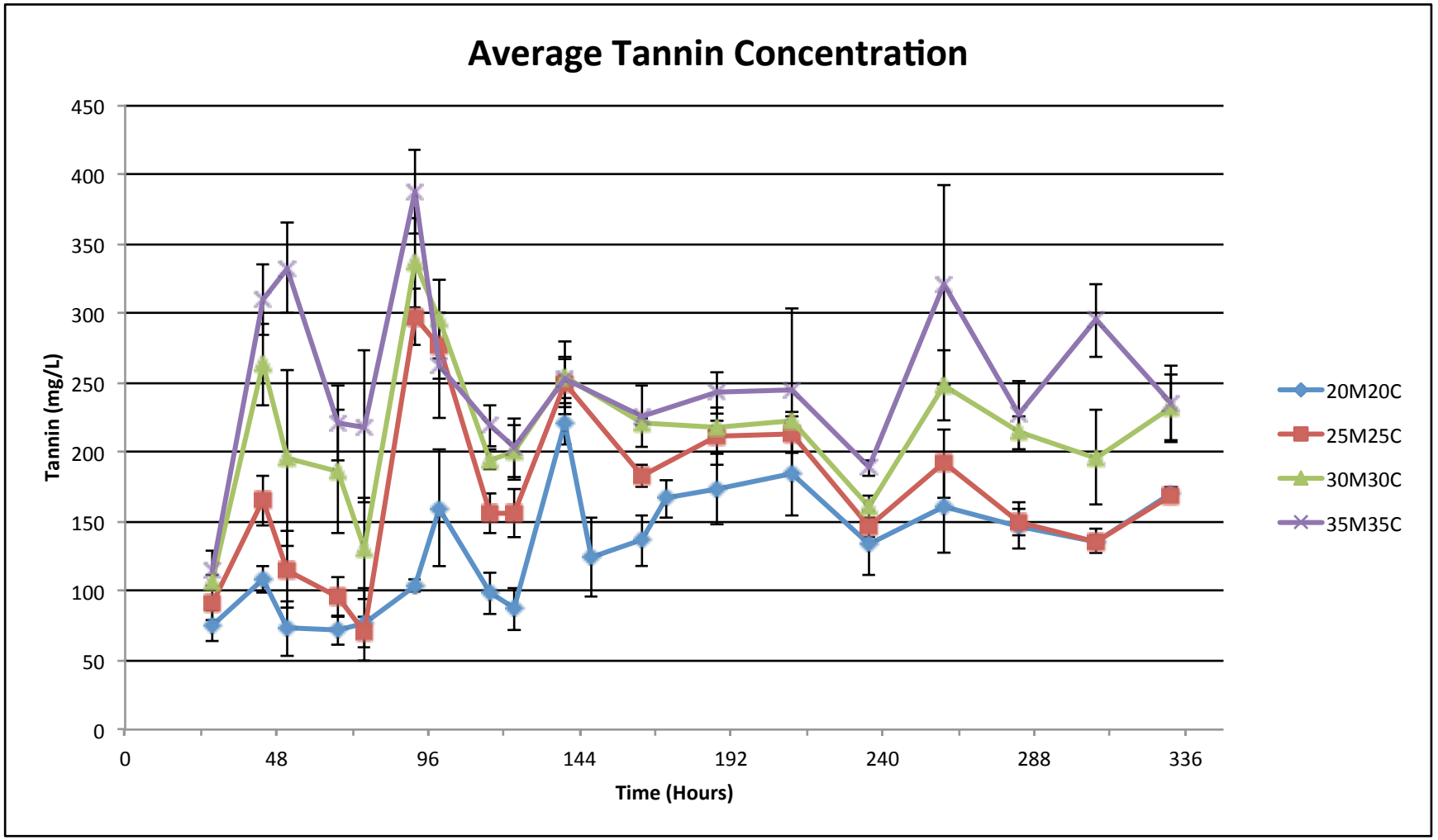
Is the main driver for extraction the liquid temperature or cap temperature?



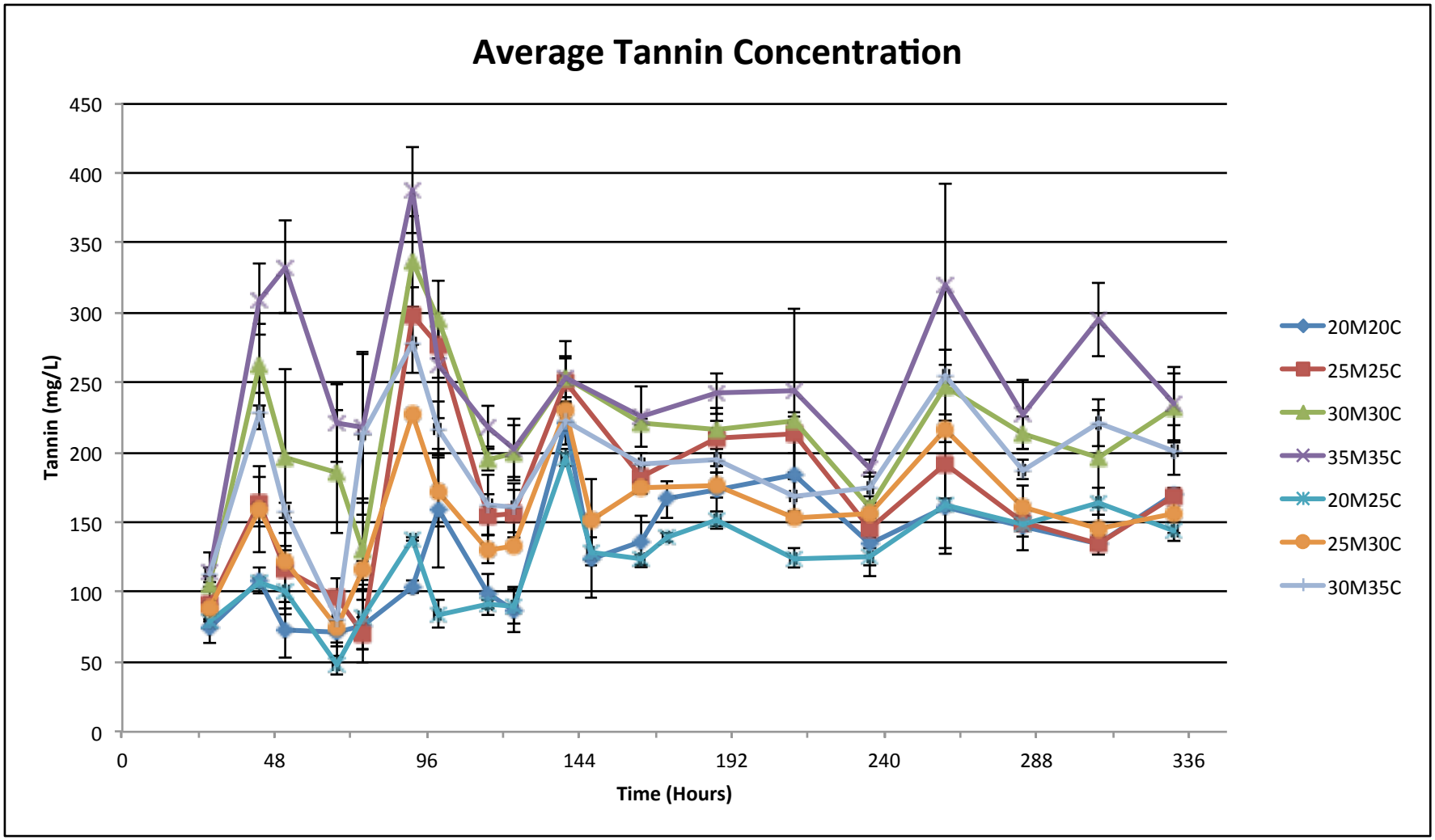
Is the main driver for extraction the liquid temperature or cap temperature?

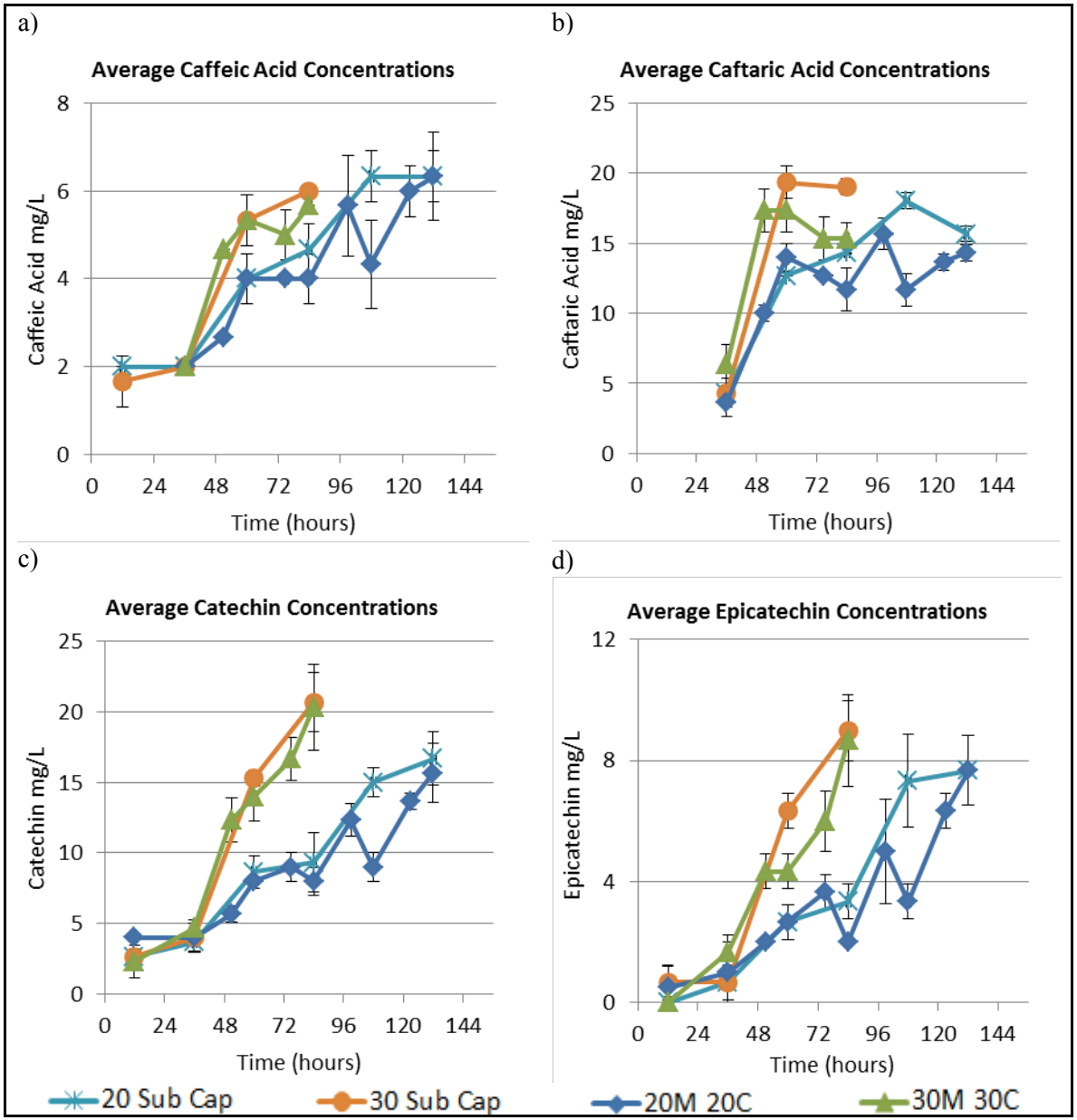


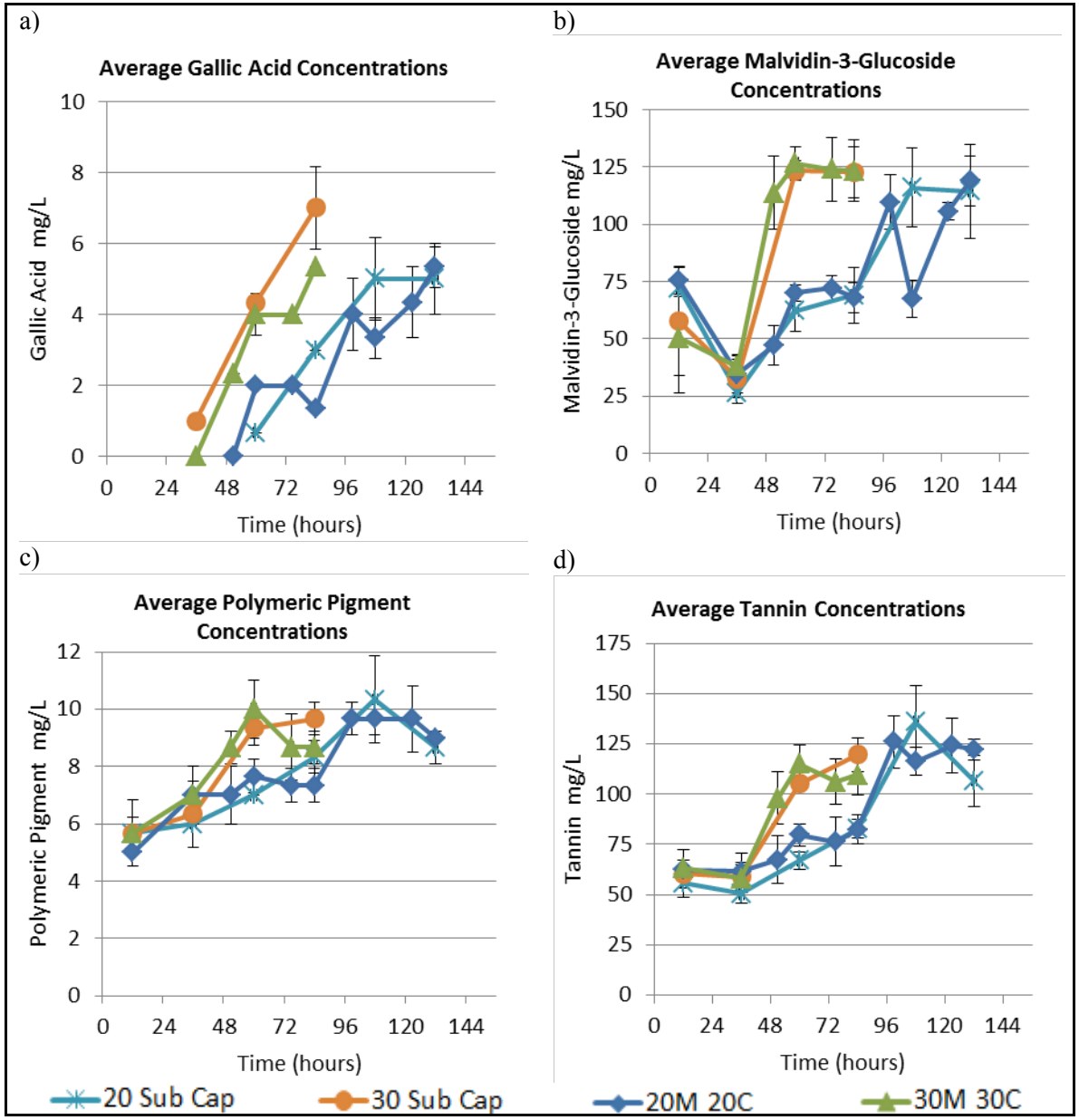
Liquid temperature or cap temperature?



Liquid temperature or cap temerpature?




a)
Average Caffeic Acid Concentrations
Caffeic Acid mg/L
Time (hours)
b)
Average Caftaric Acid Concentrations
Caftaric Acid mg/L
Time (hours)
c)
Average Catechin Concentrations
Catechin mg/L
Time (hours)
d)
Average Epicatechin Concentrations
Epicatechin mg/L
Time (hours)
20 Sub Cap
30 Sub Cap
20M 20C
30M 30C


a)
Average Gallic Acid Concentrations
Gallic Acid mg/L
Time (hours)
b)
Average Malvidin-3-Glucoside Concentrations
Malvidin-3-Glucoside mg/L
Time (hours)
c)
Average Polymeric Pigment Concentrations
Polymeric Pigment mg/L
Time (hours)
d)
Average Tannin Concentrations
Tannin mg/L
Time (hours)
20 Sub Cap
30 Sub Cap
20M 20C
30M 30C

Learnings from cap and liquid temperature studies

- Temperature affects how rapidly skins are extracted (not necessarily how much is extracted)
- Temperature definitely affects seed extraction
- Liquid temperature seems to be more important than cap temperature for extraction

Summary

- Chemical gradients were observed for a number of important compounds
- Extraction of compounds located in the skin appears to occur early in fermentation
  - Could early cap tannin be a marker for final tannin levels?
- Extraction of compounds located in the seeds appears to occur later in the fermentation and is more temperature dependent
- Liquid temperature seems to have more effect than pumpover volume on extraction

Understanding the mechanism of extraction will allow better manipulation of phenolic profiles

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- The Ebeler Lab
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