The Impact of Exogenous Tannin* & EXTENDED MACERATION in Red Wine Production

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Red Winemaking Overview

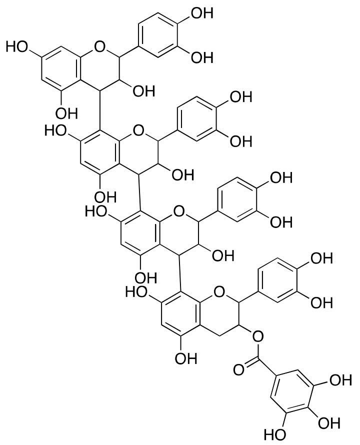
- Fermentation Temperature
- Skin and seed contact time
  - Extraction Techniques
    - **Extended Maceration**
    - **Prefermentation Juice Runoff**
    - Cold Soak
    - Must or Grape Freezing
    - Thermovinification
    - Carbonic Maceration
    - Enzyme Additions
- Oak Aging
  - Oak Alternatives
  - Micro-oxidation
- Amelioration
  - **Tannin Additions**

Tannins

- Importance
  - Sensory Attributes
    - Astringent
  - Antioxidant
  - Formation of Polymeric Pigments
- Condensed and Hydrolysable Tannins
  - Grapes contain Condensed in Skin, Seed, Stem
  - Oak Barrels contain Hydrolysable and Condensed (primarily Hydrolysable)
- Oenotannins
  - Extract dried into powder
  - Grapes, Barrels, Exotic Trees, Oak Galls

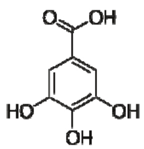
Grape Tannins

- Condensed Tannins
- Main Tannins found in Wine
- Origin
  - Skins (0.5-1.2 mg/berry)
  - Seeds (3.0-4.0 mg/berry)
  - Stems (?/racchis)

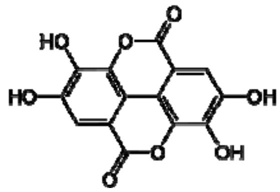


Oak Tannins

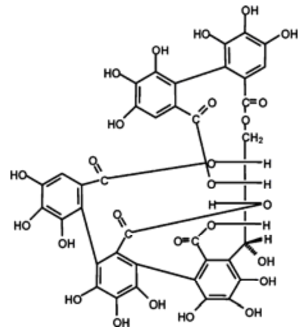
Gallic Acid



Ellagic Acid



Castalagin



- Ester linkages hydrolyze under acidic aqueous conditions of wine
- Castalagin may directly contribute to astringency of wine
- Syngeristic effect with wine tannins
  - Some evidence of direct impact

Oenological Tannins

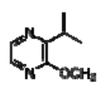
- Removal of Protein Haze
- Sulfide Removal
- Removal of Veggie Aroma
- Deactivation of Laccase Enzyme
- Sacrificial Tannins
- Color Stabilization
- Astringency Modification

Protein Haze Removal

- Addition of tannin to remove proteins
  - Primarily used for protein stabilizing beer
  - Potential use for white wine production
    - Condensed tannins favored
      - Cross-linking mechanism linear relationship
  - Potential added bitterness or aroma
- Research Scale Immobilized Tannins
  - Reaction with metals and proteins

Removal of Veggie Aroma

- Veggie Aroma Compound Spectrum
  - Pyrazines
  - Thiols (Cabbage Aroma)
  - C6 Aldehydes Hexanal (Fresh cut grass)



- Similar to use of oak chips?
  - Aroma cover up rather than removal
- Potential reaction thiol, aldehyde?

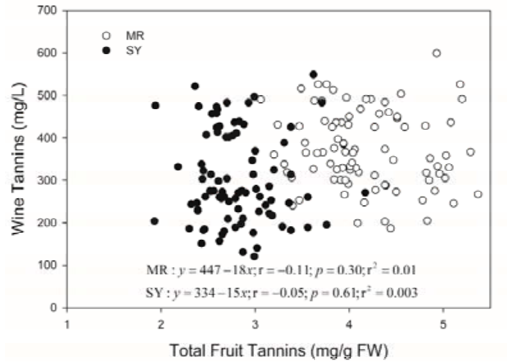
Removal of Sulfur Aroma

- Similar to Formation of GRP
  - PPO oxidized Caftaric Acid
  - GSH reacts with Quinone
- Oxidized Phenolic (Electrophile for Nucleophile (–SH))
- Requires Oxidized Phenolic
  - Role of Metals and Sulfur Dioxide
- No Evidence but seems possible

Laccase

- Tannins are well known enzyme inhibiters
  - Goldstein and Swain 1965
- Tannin Addition Friend or Foe?
- Impurity: monomeric phenolics (substrates for enzyme)
- Compete with Laccase for O_2?
  - Laccase affinity for oxygen is 0.16 -0.32 mg/L
  - Solubility of O_2 in water
    - 0°C – 15 mg/L O_2
    - 10°C – 11.4 mg/L O_2
    - 20°C – 9.1 mg/L O_2
    - 30°C- 7.7 mg/L O_2
- No evidence tannin addition actually works
- PPO?

Sacrificial Tannins



- Fruit & Wine Tannins
  - Poor correlation
- Grape Fining Agent
  - Cell walls, polysaccharides
- Tannin Sponge Theory
  - Must occupy all binding sites for tannins to escape
    - Simplistic Idea
- No thorough examination of early additions
  - Canuti et al. 2012 added grape and gallnut tannins to Sangiovese primarily observed color change

Polymeric Pigments

- Heterogeneous mixture
  - HSO_3^- resistance (partial)
  - pH color shift small
  - Possible reduction in astringency
- Anthocyanins react with multiple classes
  - Aldehydes
  - Keto-Acids (Pyruvate)
  - Tannins
- Some Data Supports Addition of Tannins
  - Primarily Excessive Additions
  - Small Additions provide temporary increase
    - Copigmentation or oxidation prevention?

Astringency

- Impact depends on target wine
  - Original amount of tannins
  - 100 mg/L added to 100 mg/L: 2-Fold Increase
  - 100 mg/L added to 1000 mg/L: 10% increase
- Additions of 200 mg/L – 400 mg/L
  - No improvement
    - Parker et al. 2007 (200 mg/L)
    - Bautista-Ortin et al. 2007 (400 mg/L)
- Change in Aroma Observed not Astringency
  - Parker et al. 2007
  - Diaz-Plaza et al. 2002

Purity: OIV CODEX

- Water extracts that are dried
  - Powder must be 98% water soluble
- International Oenological Codex
  - COEI-1-TANINS : 2009
- Not the most stringent set of rules
- Estimation of Total Phenolics in powder must be greater than 65% (gallic acid)
- Condensed tannins use (DMACH) 10 mg/g
- Ellagitannins use nitrous acid 20 mg/g (2%)
- Limits on yellow A_{420nm} and red color A_{520nm}
- Specific Definitions: Grape = 50 mg/g Catechin (5%)

Purity: Literature

- Discrepancies in labeling and content
- Lack of relationship between total phenolics and tannins
  - Obreque-Slier et al. 2009
- 12-48% of Total IRP is PPT (CE)
  - Harbertson et al. 2012
  - Keulder 2005 thesis
- Better purity than OIV requirements

Experimental

- Cabernet Sauvignon Wine from Columbia Valley WA
- Biotan by Laffort (Tanin VR Grape)
  - Grape Tannin: Information Confusing listed in units of non-flavonoid (coumaric acid)
  - Total phenolics > 65%
- Tanin Galacool by Laffort
  - Chestnut gall tannin
  - Hydrolysable tannins
  - Total phenolics > 80%
  - Used for deactivation of Laccase
- US: 150 mg/L Tannic Acid Addition is legal

Tannin Analysis

BIOTAN GRAPE TANIN

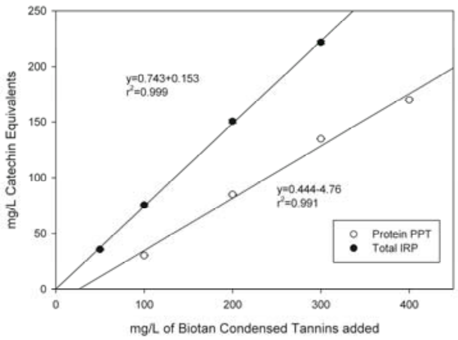


Figure 1.

Linear PPT: y=0.44x – 4.76

TANIN GALACOOL

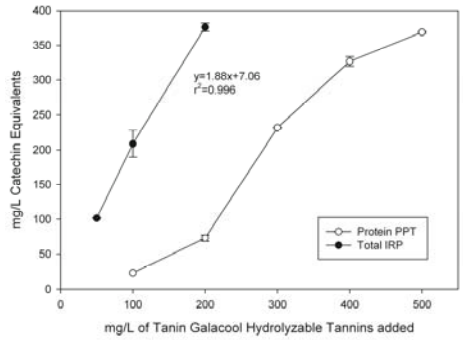
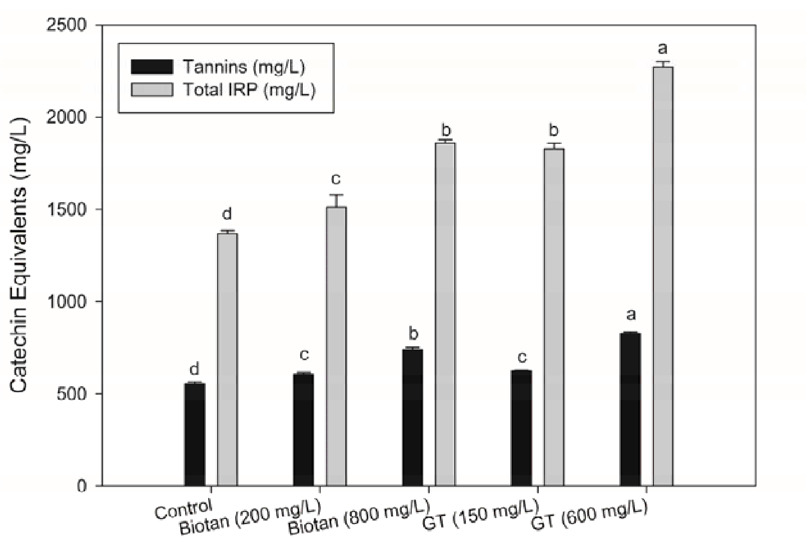


Figure 2.

Exponential PPT

Tannins and Total IRP



Predicted 85 and 350 mg/L Addition of Tannin for Biotan
Found 53 and 187 mg/L Addition for Biotan

Anthocyanins and Polymeric Pigments

Treatment	Anthocyanins (mg/L)	SPP (A_{520nm})	LPP (A_{520nm})
Control	330±3.5	1.75±0.06	1.99±0.04 ab
+ Biotan 200 mg/L	319±2.5	1.71±0.03	1.93±0.02 c
+ Biotan 800 mg/L	321±4.5	1.77±0.06	2.15±0.06 ab
+ GT 150 mg/L	322±3.5	1.71±0.02	2.03±0.08 abc
+ GT 600 mg/L	324±9.0	1.66±0.05	2.21±0.02 a

Sensory Evaluation

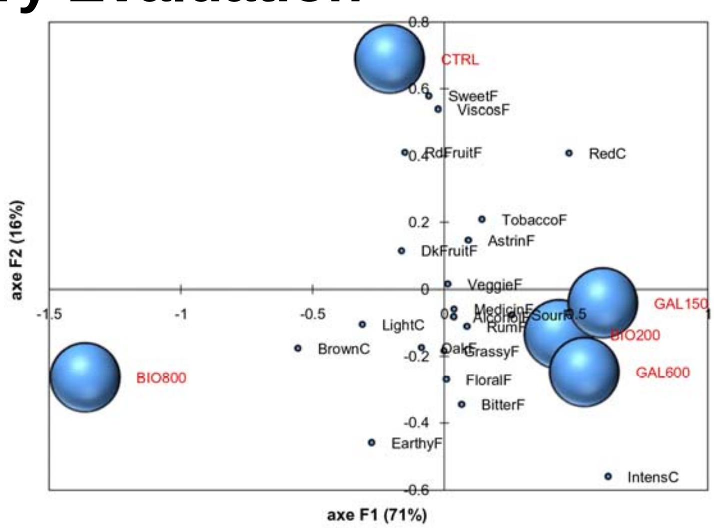


Fig. 4. Discriminant Analysis of the sensory data of the control wine (CTRL) and wines added with tannins (BIO200, BIO800, GAL150, GAL600).

Conclusions

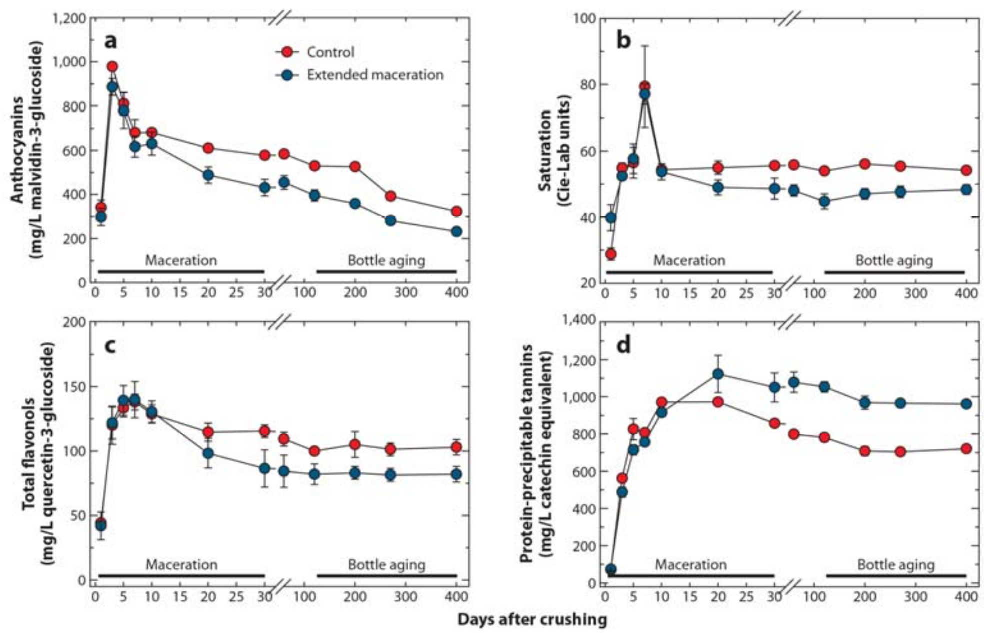
- Significant Tannin, LPP and Total IRP impacts
- Sensory Evaluation
  - Tannin Addition Wines Characterized
  - Primarily Negative Attributes
    - Brown Color, Bitter Flavor and Earthy Aroma
      - BIOTAN 800 mg/L most Earthy
    - Bitterness Change consistent with greater Total IRP
  - Lower concentration additions had no detrimental impacts but small improvement in phenolics

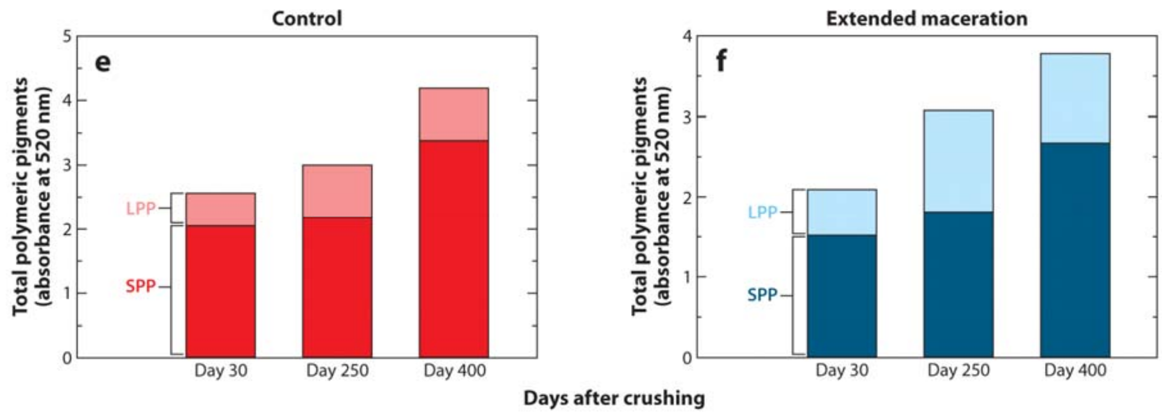
Comments

- Purity Needs to Improve
- Standard should be the same
  - Epicatechin or Catechin Eq. for Condensed Tannins
  - Gallic Acid for Hydrolysable Tannins
  - Use of Tannic Acid is confusing
    - Mixture of different compounds
- Legal amount allowable needs to change
- Many use tannins as flavorant
  - They come with “friends”
  - Threshold for odorants ng/L, µg/L
- New studies utilizing QTOF to evaluate all of the compounds that are actually present in tannin additions via GC and LC
- Nutritional Facts and Ingredient Listing on wines will be necessary soon
- Consumers will begin asking why things were added to their drink.
- What will wineries want to say?
  - All natural ingredients?
  - Magic?

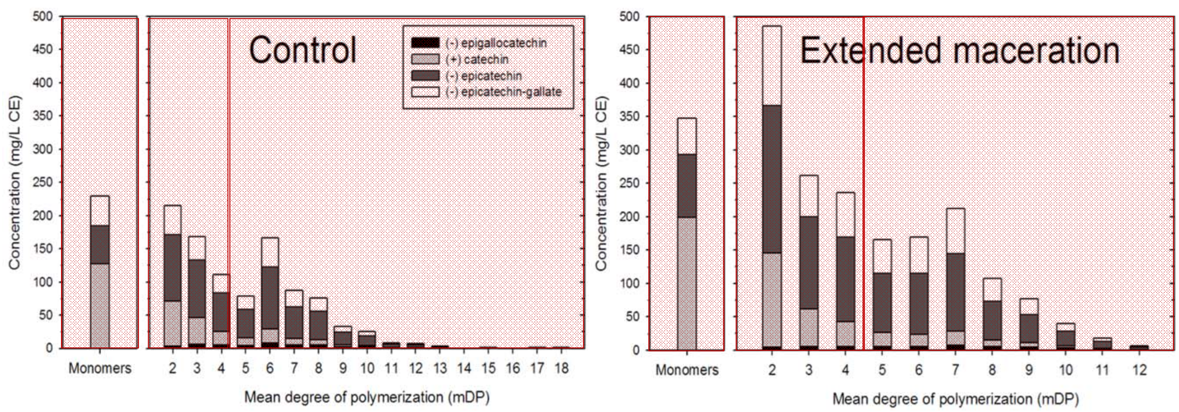
Extended Maceration

- Longer period of skin/seed contact (10-30 days)
  - We have ongoing experiment at 240 days
- Increases seed tannin content in wine
  - Increases in both bitterness and astringency
  - Change in amount and size of tannins
- Increases polymeric pigment
  - Loss of anthocyanins
  - Primarily due to increase in tannins
  - Wines tend to be less saturated, more brick red
- Risk of Oxidation
  - Use heavy inert gases (CO_2, Ar_2)





Impact on Tannin Size Distribution



$p < 0.0109$

Supplemental Table 10 Summary of the probability density distributions with the best goodness of fit (GOF) and main statistical parameters of each distribution. The distributions were fitted on the individual wines from an mDP 2 to mDP 18.

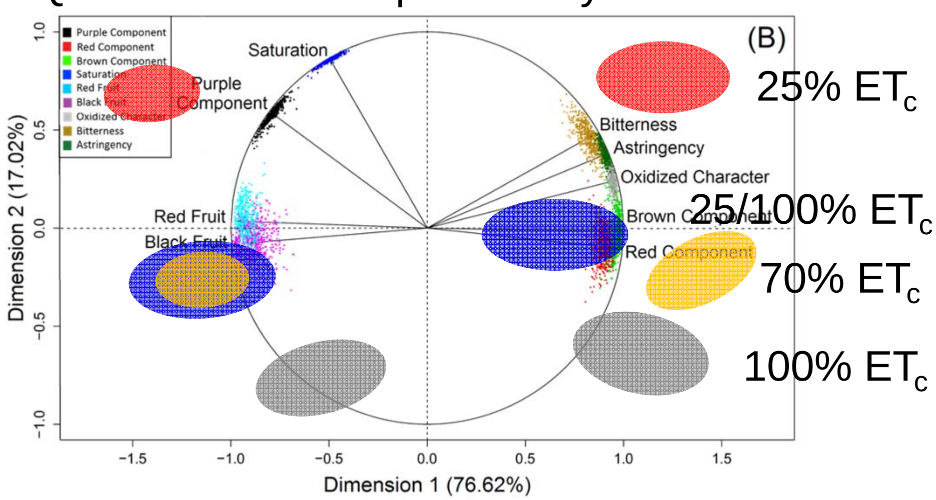
	Rayleigh distribution		Rice distribution		Weibull (3P) / Johnson SB	
Treatment	Distribution parameters	Summary statistics	Distribution parameters	Summary statistics	Distribution parameters	Summary statistics
Control	s=3.9063; GOF=0.1351	Mode=3.90; Mean=4.89; Variance=6.54; Std.Dev.=2.55; Skewness=0.63; Kurtosis=0.24	n=0.0022; s=3.9063; GOF=0.1351	Mean=4.89; Variance=6.54; Std.Dev.=2.55	a=0.8373; b=3.0022; g=2.0; GOF=0.1354	Mode=2.00; Mean=5.29; Variance=16.57; Std.Dev.=3.95; Skewness=2.62; Kurtosis=10.89
EM[a]	s=3.6977; GOF=0.1404	Mode=3.69; Mean=4.63; Variance=5.86; Std.Dev.=2.42; Skewness=0.63; Kurtosis=0.24	n=1.1459; s=3.6131; GOF=0.1408	Mean=4.64; Variance=5.87; Std.Dev.=2.42	g=0.68264; d=0.7324; l=10.35; x=1.1803; GOF=0.1409	NA[b]

[a]Extended maceration

[b]Not applicable

Effect of EM and RDI

Quantitative Descriptive Analysis 2011



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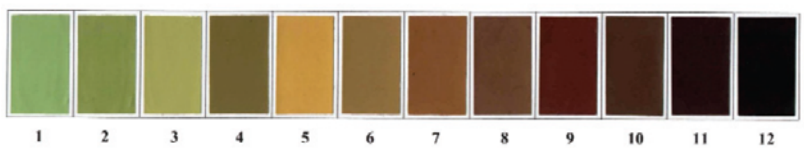
How does ripening impact seed tannin extraction?

- Seed Tannins
  - Harsh Astringency Owing to EC Gallate
  - Longer Ripening Mellows Astringency
- Browning of Seed Coincides with Less Tannins and Catechins

Australian Journal of Grape and Wine Research 11, 43–58, 2005

Figure 2. A colour chart indicating changes in grape seed coat colour during seed development and maturation.



Our Experiment

- How does Fruit Maturity Impact Tannin Extraction?
- Fruit Maturity
  - Ethanol Concentration
  - Extended Maceration
- 2-years of Fruit & Wine Data Collected
- 1-year of Sensory

EM, Ethanol and maturity

✓ Experiment II: Merlot 2011 & 2012

- Merlot (clone 3)

- Harvest dates: early: 9-22-2011 and 9-13-2012

  33 d. 34 d.

  late: 10-25-2011 and 10-17-2012

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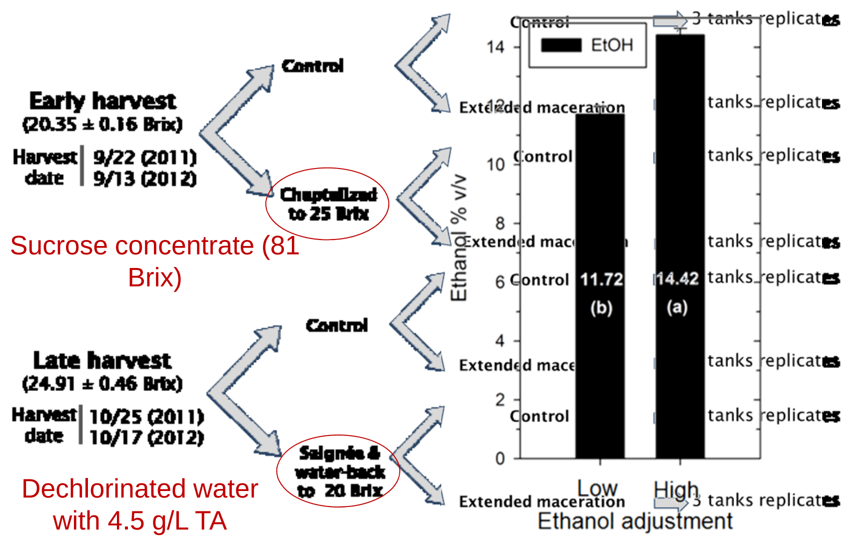
EM, Ethanol and maturity

✓ Experiment II: Merlot 2011 & 2012



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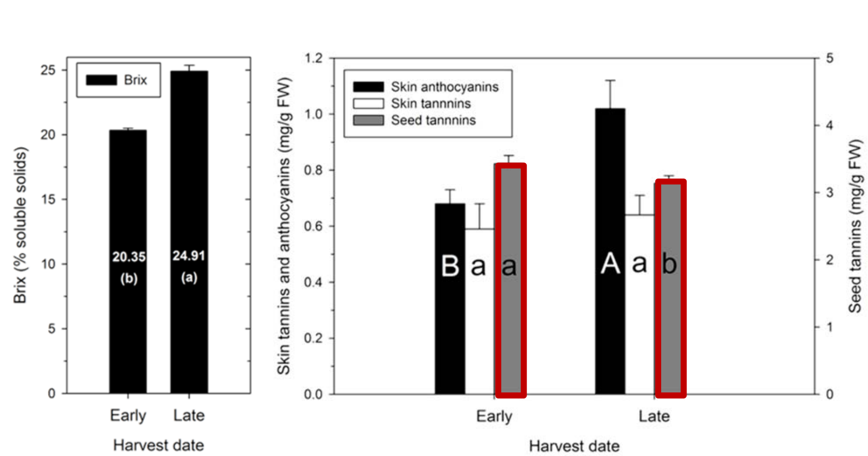
EM, Ethanol and maturity

- Experiment II: Merlot 2011 & 2012: Berry chemistry



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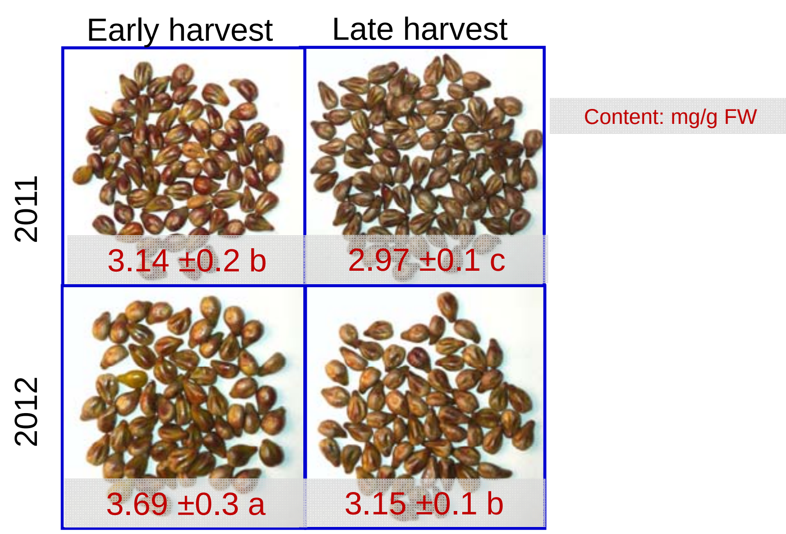
EM, Ethanol and maturity

- Experiment II: Merlot 2011 & 2012: Seed maturity



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ANOVA FACTOR	TREATMENT	ANTHOCYANINS (mg/L)	TANNINS (mg/L)
Season (S)	2011	386	390
	2012	365	539
	p-value	0.881	<0.0001
Maturity (M)	Early	259 b	473
	Late	492 a	456
	p-value	<0.0001	0.855
Maceration (W)	Control	416 a	373
	EM	335 b	558
	p-value	<0.0001	<0.0001
EtOH Adjust (EtOH)	Low 11.7%	370	438
	High 14.4%	381	491
	p-value	0.176	0.141
W x M		0.649	0.258
W x S		0.021	0.298
W x EtOH		0.874	0.899
W x M x S		<0.0001	<0.0001
W x M x S x EtOH		0.005	0.065

EM, Ethanol and maturity

✓ Experiment II: Merlot 2011 & 2012: Sensory analysis

- DA approach
- Trained panel (n = 11)
- Principal component analysis with confidence ellipses constructed using Hotelling's test for $p < 0.05$

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EM, Ethanol and maturity

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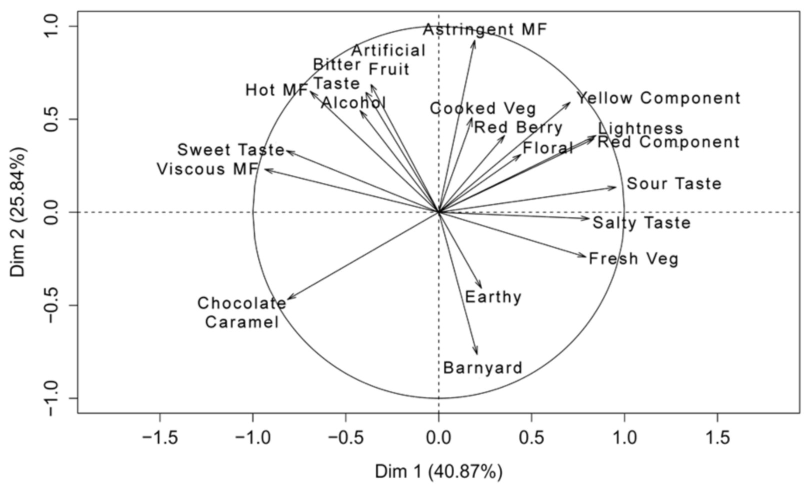

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EM, Ethanol and maturity

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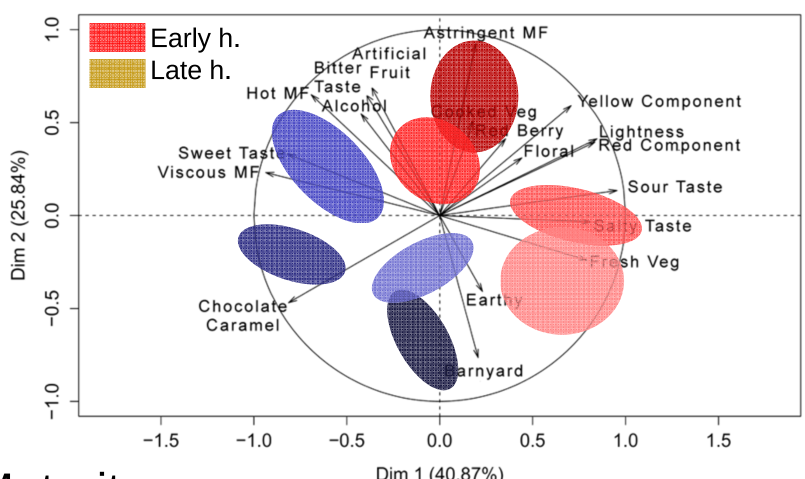

Maturity

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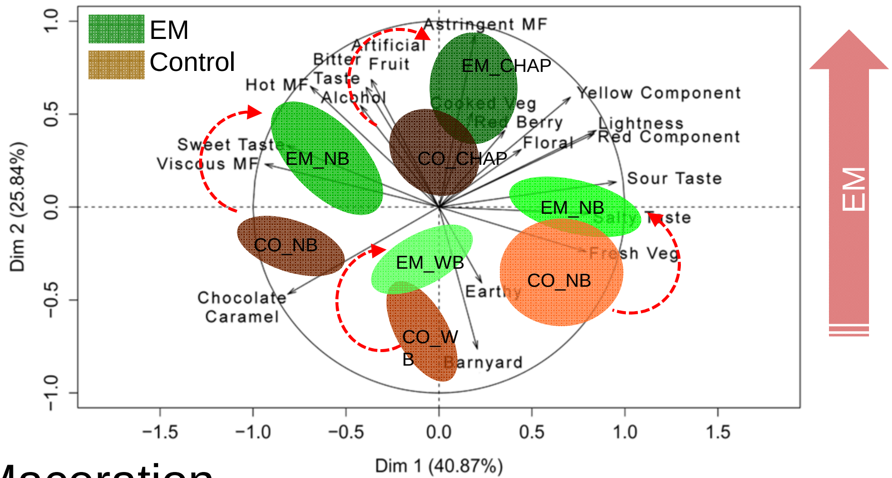

Maceration

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EM, Ethanol and maturity

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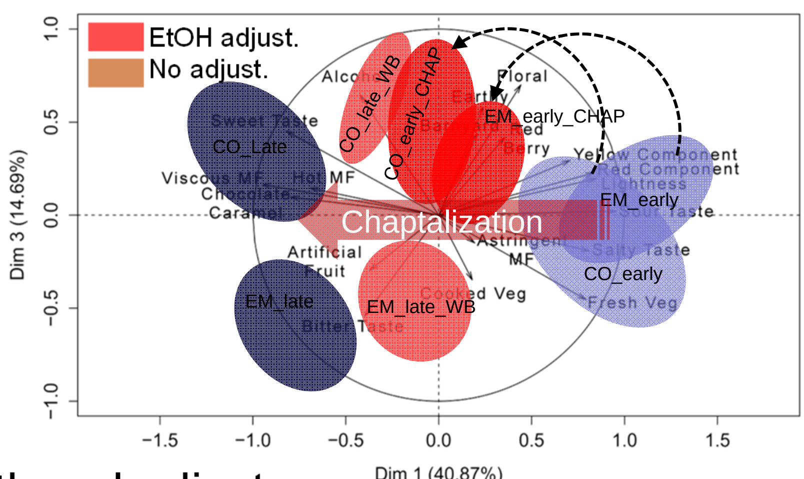

Ethanol adjust.

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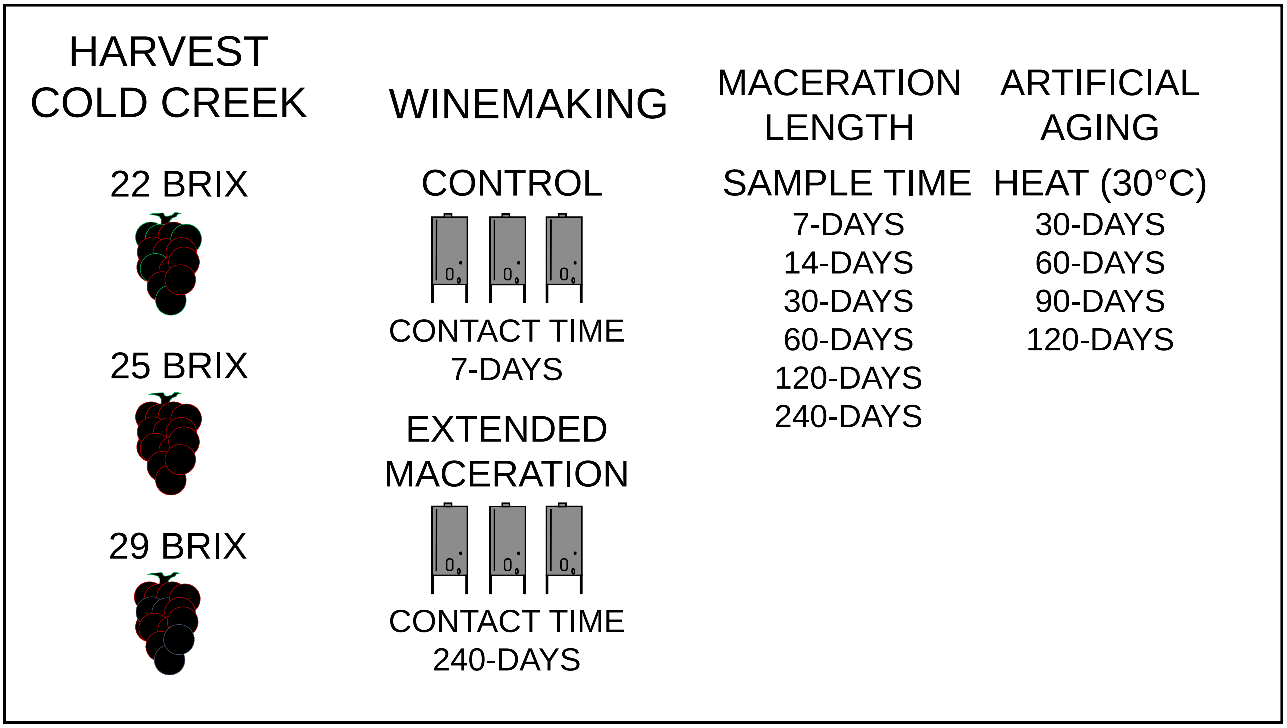
Take Home Messages

- Tannin extraction from seeds was unaffected by fruit maturation (30 days)
  - Same result was observed over 2-vintages
- Impact of ethanol difference on extraction was negligible
  - Ethanol impact on sensory was large
  - Drop in Veggie Characters
  - Result confirmed with follow up study published in 2017

- *Casassa, L.F., C.W. Beaver, M.S. Mireles, R.C. Larsen, H. Hopfer, H. Heymann, J.F. Harbertson. 2013. Influence of fruit maturity, maceration length, and ethanol amount on chemical and sensory properties of Merlot wines. Am. J. Enol. Vitic. 64:437-449.*

Open questions

- Most winemakers describe increase in astringency and then just after it reaches its peak a steady decline
- Almost an ephemeral moment?
- Or a moment of practicality?



Experimental Questions

- Does ripening or winemaking impact tannins more?
  - Can't control for EtOH here so that ? will be unanswered
- How are tannins changing during extended maceration?
  - How do they become more approachable?
  - Is this as a result of polymeric pigment formation?
  - Is this due to other changes in tannin structure?
- Do extended maceration wines age differently than normal red wines?
- How does length of extended maceration impact tannin content?
  - How long do you need to do it for?
- Many more questions of course…..
- ASEV this summer stay tuned.

Acknowledgments

- Thanks! Anita Oberholster
- Funding: Wine Research Advisory Committee, the Washington Wine Commission, the Washington Grape and Wine Research Program
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