Daniel Pambianchi

TANNINS & ANTHOCYANINS IN GRAPES & WINE

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ROCHESTER, NY
Objectives

REVIEW FUNDAMENTAL TANNIN & ANTHOCYANIN CHEMISTRY TO UNDERSTAND HOW THESE AND THE MANY OTHER WINE COMPONENTS INTERACT TO IMPACT QUALITY FROM VINE TO GLASS.
Agenda

- Overview of polyphenols
- Sources of tannins & anthocyanins
- Tannin & anthocyanin properties and reactivity
- Oxidation in wine
- Winemaking and impacts on phenolic extraction
- Oxygenation techniques to tame tannins and stabilize anthocyanins
Tannins & Anthocyanins

- Tannins – responsible for bitter taste and astringent sensation
- Anthocyanins – color pigments
- T-A interact and also react with other compounds to affect color, clarity, taste, mouthfeel, aging potential and overall wine stability and quality.
- Concentrations and extraction impacted by viticulture & winemaking.
- Belong to the class of polyphenols.
Polyphenols

- Complex compounds building on phenol groups.
- Highly reactive; involved in many types of simple & complex reactions.
- Known for their antioxidant power.

The simplest of phenols

Polyphenols: (a) catechin (tannin molecule), (b) anthocyanin
Polyphenols

- FLAVONOIDS
  - Anthocyanins
    - = Anthocyanidin + Glucose
  - Grape tannins
    - Flavanols
    - Condensed tannins (Proanthocyanins)
      - = Proanthocyanidin + Glucose
  - Flavonols (e.g. quercetin)
Polyphenols

- NONFLAVONOIDS
  - Phenolic acids (e.g. caftaric acid)
  - Phenolic aldehydes
    - Found in toasted oak
  - Volatile phenols
    - Found in toasted oak
    - Brett spoilage
  - Oak tannins (Hydrolyzable tannins)
  - Stilbenes (e.g. resveratrol)
Important Compounds

- **Quinones**
  - Dark yellow to brown-colored compounds resulting from the oxidation of phenolic acids.

- **Glutathione (GSH)**
  - A naturally occurring tripeptide with very high antioxidant power.

- **Grape Reaction Product (GRP)**
  - Quinone + GSH = *colorless* GRP

- **Ascorbic acid (Vitamin C)**
Antioxidant Power

+920 mV  Glutathione

+600–750 mV  Tannins

+282 mV  Ascorbic Acid

+170 mV  SO₂

Source: Laffort Product Literature

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Phenolics in Grapes

Phenolics in Grapes

SKIN
FLAVONOID
+ANTHOCYANINS
malvidin
delphinidin
cyanidin
petunidin
peonidin
+FLAVAN-3-OLS
catechins
epicatechins
+PROANTHOCYANINS
FLAVONOLS
quercetin
kaempferol
myricetin
FLAVANONOLS
dihydroquercitin
dihydrokaempferol
NONFLAVONOIDS
+PHENOLIC ACIDS
cinnamic acids

FLESH
NONFLAVONOIDS
+PHENOLIC ACIDS
benzoic acids
cinnamic acids
café-té acid
coutaric acid
fertaric acid
FLAVONOIDS
+PROANTHOCYANINS
+FLAVAN-3-OLS
catechins
epicatechins
FLAVANONOLS
dihydroquercitin
dihydrokaempferol

STEMS & RACHIS
NONFLAVONOIDS
+PHENOLIC ACIDS
benzoic acids
cinnamic acids
café-té acid
coutaric acid
fertaric acid
FLAVONOIDS
+PROANTHOCYANINS
+FLAVAN-3-OLS
catechins
epicatechins
FLAVANONOLS
dihydroquercitin
dihydrokaempferol

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## Distribution of Phenolics

<table>
<thead>
<tr>
<th></th>
<th>Pulp</th>
<th>Skin</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Trace</td>
<td>100–500</td>
<td>1000–6000</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>0</td>
<td>500–3000</td>
<td>0</td>
</tr>
<tr>
<td>Phenolic Acids</td>
<td>20–170</td>
<td>50–200</td>
<td>0</td>
</tr>
</tbody>
</table>

Typical mean concentration (mg/kg) of phenolic compounds in ripe grapes

Tannins

Condensed Tannins  Complex Tannins  Hydrolyzable Tannins

Gallotannins  Ellagitannins
Tannin Properties

- Extracted from grape skins & oak (BEST), seeds (NOT SO GOOD), & stems (WORST).
- Can self-condense or condense with ellagitannins or anthocyanins and impact color, color stability and mouthfeel.
- As the degree of polymerization increases, bitterness decreases, while astringency increases.
Tannin Properties

- Can cause pinking in white varietals.
- Bind with proteins to soften mouthfeel.
- A natural fining agent.
Tannins & Pinking Effect

- Colorless proanthocyanins can oxidize and discolor wine.
- Also affect flavor and freshness.
- Sauvignon Blanc & Viognier are white varieties at high risk of pinking in a warm dry vintage, when the skins have much higher levels of phenolics.

If this is a concern, it is possible to separate the pressings and then add PVPP to them before fermentation.
Color varies with pH.

Intramolecular reactions can give rise to many other types of anthocyanins that can affect color and color stability.

Can condense with tannins, including ellagittannins, and impact color & color stability.

Subject to bleaching effects when react with sulfite and/or water.

Metal ions can impact color through complexation reactions.
Anthocyanin Properties

- More stable when bound to glucose (anthocyanin = anthocyanidin + glucose)
- Less stable and more susceptible to oxidation at higher temperatures or when exposed to UV light.
Anthocyanins & pH

- **Carbinol pseudobase (colorless)**
- **Flavylium cation (red)**
- **Quinonoidal base (blue/violet)**
- **Anionic quinonoidal base (blue)**
- **Cis-chalcone (pale yellow)**
Anthocyanins & pH

Relative concentrations of flavylium ion, chalcone, carbinol & quinoidal malvidin-3-glucoside pigments vs pH

- flavylium cation
- carbinol
- chalcone
- quinoidal base

pH = 2.60
pH = 3.52
pH = 4.25

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Oxidation in Juice/Wine

- Tannins & anthocyanins act as natural antioxidants; reds are therefore better protected than whites.
- Polyphenols consume $\text{O}_2$, particularly in the presence of Fe/Cu metal ions or in the absence of $\text{SO}_2$.

Reaction is very slow, which explains the slow evolution of reds to an orange-brick color with time.
- The more $\text{O}_2$, the faster the oxidation & the shorter the aging potential.
Oxidation Mechanisms

PHENOLIC ACIDS

+ $O_2$  PPOs

OXIDIZED FORM

REDUCTANT (SO$_2$, AA)

POLYPHENOLS

BROWN-COLORED QUINONES

COUPLED OXIDATION

POLYPHENOL QUINONES

POLYPHENOL AGGREGATES

POLYPHENOLS

GRP QUINONES

GRAPE REACTION PRODUCT

+ GSH

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Autoxidation Reactions

Phenolic Extraction

- Phenolic ripeness
- Crushing & destemming
- Maceration & fermentation techniques
- Free vs. press run wine
Phenolic Ripeness

- Amount of phenolics in grapes
  - Varietal
  - Viticultural practices ↔ microclimate
  - Soil condition
  - Climatic conditions
# Crushing & Destemming

<table>
<thead>
<tr>
<th></th>
<th>Control (destemmed)</th>
<th>Non-destemmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing Sugars (g/L)</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Potential Alcohol (%alc/vol)</td>
<td>11.4</td>
<td>11.1</td>
</tr>
<tr>
<td>Total Acidity (g/L)</td>
<td>4.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Volatile Acidity (g/L)</td>
<td>0.70</td>
<td>0.75</td>
</tr>
<tr>
<td>Total Polyphenol Index</td>
<td>58</td>
<td>72</td>
</tr>
<tr>
<td>Tannins (g/L)</td>
<td>3.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Anthocyanins (g/L)</td>
<td>0.58</td>
<td>0.43</td>
</tr>
<tr>
<td>Color Intensity</td>
<td>1.7</td>
<td>1.3</td>
</tr>
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</table>

M/F techniques change the physical and chemical dynamics between the liquid & solid phases to hasten phenolic extraction, and extraction dynamics change as the must changes from an aqueous to a hydroalcoholic medium.
M/F Techniques

- Cold soak maceration
- Increase temperature
- Increase duration of skin contact
- Exogenous additions of macerating enzymes
- Increase skin-to-juice ratio
- Increase frequency of punch-downs
Maceration/Fermentation

- Anthocyanins – higher solubility in water
- Tannins – higher solubility in alcohol

Maceration/Fermentation

- *S. cerevisiae* yeast metabolism produces acetaldehyde and bisulfite.
- Acetaldehyde (and other yeast metabolism by-products) can:
  1. Cause anthocyanins to transform into complex anthocyananin molecules (e.g. pyranoanthocyanins),
  2. Bridge tannins & anthocyanins into more stable T-A complexes,

Both of which can shift color.
Maceration/Fermentation

- Bisulfite can bind with anthocyanins and block reactions to form *colorless* anthocyanin–sulfate compounds.
  - Similar reaction between water & anthocyanins; hence why amelioration is not always recommended.
- Choice of yeast strain very important, i.e. look at the relative amounts of by-products they generate.
- Yeast enzymes can hydrolyze anthocyanins into their less-stable anthocyanidins.
Barrel fermentation vs. barrel aging

- Acetaldehyde can bridge oak tannins to anthocyanins & "soften" tannins during fermentation.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Free</th>
<th>Press</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic Acids (not incl. caftaric acid)</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Caftaric acid</td>
<td>+</td>
<td>=</td>
</tr>
<tr>
<td>Glutathione (GSH)</td>
<td>+</td>
<td>=</td>
</tr>
<tr>
<td>Grape Reaction Product (GRP)</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>Flavanols</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>pH</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>Total Titratable Acidity (TA)</td>
<td>+</td>
<td>=</td>
</tr>
<tr>
<td>Total Soluble Solids (TSS)</td>
<td>=</td>
<td>+</td>
</tr>
</tbody>
</table>

Maceration/Fermentation

- Barrel fermentation vs. barrel aging
  - Acetaldehyde can bridge oak tannins to anthocyanins and “soften” tannins during fermentation.
Taming Tannins

- Hyperoxidation
- Macro-oxygenation
  - mg/L/hr or mg/L/day
- Micro-oxygenation (MOX)
  - mg/L/month
Hydrogenation

- **White** winemaking technique, recommended for high-polyphenol wines.
- **Unprotected must** (i.e. no SO$_2$) is subjected to great amounts of O$_2$ to hasten enzymatic oxidation of bitter-tasting, astringent polyphenols into colorless GRPs.
- Remove those polyphenols so they will not undergo chemical oxidation in wine.
- Increases wine’s resistance to oxidative effects during downstream processing & aging.
Hyperoxidation

- Improves wine’s sensory profile & color stability. Much debated! Some claim aroma loss due to oxidation.

- Must be performed prior to any clarification or counterfining operation, & before any SO$_2$ additions.
Flavonoids are much less soluble in juice than in EtOH, & so, as they oxidize & polymerize, they fall to the bottom of the tank. The dark brown precipitate is then racked, & the must is inoculated to initiate AF. During AF, there is further precipitation of brown pigments, which can be removed along with the lees by racking at the end of fermentation. Fining &/or mechanical filtration can be used to further clarify the wine.
Inject pure $O_2$ through a fine, sintered diffuser placed in-line with the transfer pipe while either pumping must from the crush vat to the holding tank or while recirculating must from the bottom valve of the holding tank to the top and over the must.

Alternatively, the diffuser can be immersed in the must & then injecting $O_2$ while stirring the must.

$T^\circ$ is not critical here, but recommended to perform the operation at cellar $T^\circ$, i.e. $13^\circC (55^\circF)$. 
Do *not* rack, clarify or sulfite the wine until *after* hyperoxidation is completed.

Filtered compressed air, free of contaminants & oil residues, can be used instead of pure O₂; but because the amount of gas—O₂ or air—required cannot be measured, use pure O₂ as it allows you to visually monitor the saturation point.

Submerge the gas hose into the must & then inject O₂ while stirring the must.
Hyperoxidation

- Stop the flow of $O_2$ when the must is saturated, which can be confirmed by degassing seen at the surface. Repeat after half an hour. *Don’t fret when you see the must turn a dark brown color; it means that hyperox is working.*

- Let the must cold settle for 12–24 hours, & then rack carefully to another tank.

- If the precipitate is not racked carefully & is allowed to redissolve into the must, the process will need to be restarted.
**Macro-oxygenation**

- **Red** winemaking technique.
- Involves vigorous splashing of wine during pump-over or racking operations.
- Softens astringent tannins & produce a softer wine approachable much younger, & to stabilize color.
- The high phenolic content & lower pH protect the wine from negative oxidation effects. *Not recommended for whites.*
- Also provides yeast cells with plenty of O₂ to ensure a good AF, & reduces H₂S production.
Macro-oxygenation

- By splashing wine
  - Vigorous splashing or by delestage (rack-&-return).
  - Some argue that wine is still too protected by CO$_2$ over the wine & therefore the wine does not absorb sufficient air.
Macro-oxygenation

- By injecting $O_2$ into wine
  - Uses a venturi attachment on the pump-over return hose (when returning wine to the top of the tank).

Source: http://www.micro-ox.com/ferm_macro.htm

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By injecting $O_2$ into wine (cont’d)

- The venturi attachment is a simple inverted-T connector that allows air to be drawn into the wine stream when the wine is being pumped over. A check valve may be used to prevent backflow.
Micro-oxygenation (MOX)

- A post-fermentation red winemaking technique.
- Used to inject miniscule amounts of $O_2$ into wine in controlled fashion in conjunction with oak adjuncts.
- Binds polyphenols & allows wine to develop its full potential of aromas & ageability.
- Reduces green, herbaceous aromas in Cabs much more rapidly than in conventional barrel aging, esp. in ‘green-prone’ Cabernet varieties.
Micro-oxygenation (MOX)

- Allows wine to develop gracefully
- Soften tannins
- Stabilizes phenols
- Improves color development
- Increases flavor complexity
- Curtails reductive sulfur off-odors
Micro-oxygenation (MOX)

- Emulates barrel aging; controlled & measurable oxygenation.
- Uses pure O₂ with oak adjuncts (gallotannins & ellagitannins).
- 1–3 mg/L/month.
Micro-oxygenation (MOX)

- *Gently* stir to distribute $\text{O}_2$; taste the wine to monitor development ... there should be no signs of color changing to orange or brown hues.
Home Winemaking Website & Blog

http://TechniquesInHomeWinemaking.com

http://TechniquesInHomeWinemaking.com/blog
References