Introduction: Aroma Compounds

- Grape-derived – provide varietal distinction
- Yeast and fermentation-derived
  - Esters
  - Higher alcohols
  - Carbonyls
  - Volatile acids
  - Volatile phenols
  - Sulfur compounds
What is and Esters?

• Volatile molecule
• Characteristic fruity and floral aromas
• Esters are formed when an alcohol and acid react with each other
• Few esters formed in grapes
• Esters in wine - two origins:
  – Enzymatic esterification during fermentation
  – Chemical esterification during long-term storage
Ester Formation

• Esters can be formed enzymatically by both the plant and microbes

• Microbes
  – Yeast (Non-\textit{Saccharomyces} and \textit{Saccharomyces} yeast)
  – Lactic acid bacteria
  – Acetic acid bacteria

• But mainly produced by yeast (through lipid and acetyl-CoA metabolism)
Ester Formation

\[
R_1\text{-OH} + R_2\text{-C}~\text{CoA} \rightarrow R_1\text{-O-C-R}_2
\]

Alcohol function + Keto acid-Coenzyme A → Ester
Ester Classes

- Two main groups
  - Ethyl esters
  - Acetate esters

- Ethyl esters = EtOH + acid

- Acetate esters = acetate (derivative of acetic acid) + EtOH or complex alcohol from amino acid metabolism
Ester Classes

• Acetate esters
  – Ethyl acetate (*solvent-like aroma*)
  – Isoamyl acetate (*banana aroma*)
  – Isobutyl acetate (*fruit aroma*)
  – Phenyl ethyl acetate (*roses, honey*)

• Ethyl esters
  – Ethyl hexanoate (*aniseed, apple-like*)
  – Ethyl octanoate (*sour apple aroma*)
Acetate Ester Formation

• 2 Main factors influence acetate ester formation
  – Concentration of two substrates acetyl-CoA and fusel alcohol
  – Activity of enzyme responsible for formation and break down reactions

• Enzyme activity influenced by fermentation variables
  – Yeast
  – Composition of fermentation medium
  – Fermentation conditions
Acetate/Ethyl Ester Formation

- Fermentation composition and conditions
  - Total sugar content and optimal N₂ amount pos. influence
  - Amount of unsaturated fatty acids and O₂ neg. influence

- Ethyl ester formation
  - 1 Main factor
    - Conc. of precursors
  - Enzyme activity smaller role
    - Higher fermentation temp ↑ formation
    - C and N increase small effect

Acetate/Ethyl Ester Formation

- Acetate esters had similar profiles
- Max conc close to dryness
- Decrease 17 days after inoculation
- Then stable

Ethyl esters had similar profiles
Max conc. midpoint in fermentation
Decrease until dryness with 2\textsuperscript{nd} increase before declining

Ester Formation

- Esters synthesized by yeast from grape precursors
  - Ester formation also variety depended
- For example in Pinot noir characteristic aromas
  - Plum, strawberry, raspberry, blackcurrant and blackberry influenced by 4 esters
• Pinot noir characteristic esters

- Ethyl anthranilate
  - Sweet-fruity, grape-like odor

- Methyl anthranilate

- Ethyl cinnamate
  - Cinnamon-like, sweet-balsamic, sweet-fruity, plum, cherry

- 2,3-dihydrocinnamate
## Yeast Produced Esters

### Esters (fruity flavors)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wine (mg/L)</th>
<th>Threshold (mg/L)</th>
<th>Aroma descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>22.5-63.5</td>
<td>7.5*</td>
<td>Fruity, VA, nail polish</td>
</tr>
<tr>
<td>Isoamyl acetate</td>
<td>0.1-3.4</td>
<td>0.03*</td>
<td>Banana, pear</td>
</tr>
<tr>
<td>Isobutyl acetate</td>
<td>0.01-1.6</td>
<td>1.6***</td>
<td>Banana, fruity</td>
</tr>
<tr>
<td>2-Phenylethyl acetate</td>
<td>0-18.5</td>
<td>0.25*</td>
<td>Flowery, rose, fruity,</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>0-4.8</td>
<td>0.07**</td>
<td>Sweet, perfume</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>0.01-1.8</td>
<td>0.02*</td>
<td>Floral, fruity</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>0.03-3.4</td>
<td>0.05*</td>
<td>Green apple</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>0.05-3.8</td>
<td>0.02*</td>
<td>Sweet, soap</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>0-2.1</td>
<td>0.2****</td>
<td>Floral, soap</td>
</tr>
</tbody>
</table>

*10% ethanol, **wine, ***beer, ****synthetic wine

Swiegers et al., 2005 Austr. J. Grape Wine Res. 11: 139-173
Esterase activity of wine-associated bacteria not well understood

- Lactic acid bacteria show esterase activity
  - Ethyl esters ↑ with MLF
    - ethyl acetate (nail polish)
    - ethyl hexanoate (apple)
    - ethyl lactate (creamy, fruity, coconut)
    - ethyl octanoate (sweet soap)
  - Some others ↓ with MLF

![Ester Formation diagram]
Esters

- Increases and decreases in ester content
  - Suggest that esterases are both involved in the synthesis and hydrolysis of esters
  - This may increase or decrease wine quality depending on the ester metabolism

\[ \text{Ethyl hexanoate} \]
\[ \text{Ethyl octanoate} \]
\[ \text{Ethyl lactate} \]
Most prominent ester is ethyl acetate

- Acetic acid + ethanol = ethyl acetate
- Small quantity produced by yeast during fermentation
- Larger amounts produced by aerobic acetic bacteria during barrel aging
- Some possibly produced by lactic acid bacteria

Ethyl Acetate

\[ \text{O} \]

\[ \text{C} - \text{O} - \text{CH}_3 \]

ethyl acetate
Esters

• Ethyl acetate (nail polish, solvent, glue)
  – Aroma threshold 7.5 mg/L
  – Wine normal 22.5-63.5 mg/L, spoiled >150 mg/L
  – Fermentation temp, SO₂ levels, duration of MLF
  – Biggest influence is air, increased production under aerobic conditions

[Chemical structure of ethyl acetate]

ethyl acetate
• Generally loss upon barrel aging (volatilization and hydrolysis)
• Loss upon bottle aging (hydrolysis)
  – Accelerated with increase in temperature
• Most esters gone 6 months post-fermentation, depending on aging and temperature of aging

L. Bisson (2010) Production of Fermentation Floral and Ester Taints
Fermentation Taints

- Higher alcohols (fusel alcohols)
  - Secondary yeast metabolites and can have both positive and negative impacts on aroma

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<th>Threshold (mg/L)</th>
<th>Aroma descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propanol</td>
<td>9-68</td>
<td>500**</td>
<td>Fruity, sweet, pungent, harsh</td>
</tr>
<tr>
<td>2-methylpropanol</td>
<td>25.8-110</td>
<td>4</td>
<td>Fruity, wine-like</td>
</tr>
<tr>
<td>Butanol</td>
<td>.5-8.5</td>
<td>150*</td>
<td>Fusel, spirituous</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>9-174</td>
<td>40*</td>
<td>Fusel, spirituous</td>
</tr>
<tr>
<td>Isoamyl alcohol</td>
<td>6-490</td>
<td>30*</td>
<td>Harsh, nail polish</td>
</tr>
<tr>
<td>Hexanol</td>
<td>0.3-12</td>
<td>4*</td>
<td>Green, grass</td>
</tr>
<tr>
<td>2-Phenylethyl alcohol</td>
<td>4-197</td>
<td>10*</td>
<td>Floral, rose</td>
</tr>
</tbody>
</table>

*10% ethanol, **wine
Fusel alcohols

- < 300 mg/L add complexity (fruity characteristics)
- >400 mg/L (strong, pungent smell and taste)
- Different yeast strains contribute variable amounts of fusel alcohols
  - Non-*Saccharomyces* yeast – higher levels of fusel alcohols
Fusel alcohols

• Conc fusel alcohols produced:
  – Amount of precursor - amino acids
  – EtOH conc, fermentation temp, pH, must composition, amount of solids, skin contact time etc. influence conc of higher alcohols

From Linda Bisson: The Fusel Family
Fermentation Taints

• Carbonyl compounds
  – Acetaldehyde (bruised apple, sherry, nutty)
    • Sensory threshold of 100 mg/L, typical conc. in wine 10‐75 mg/L
    • Major intermediate in yeast fermentation
    • Increase over time due to oxidation of EtOH - due to aeration
    • Use of high conc of SO₂ can cause accumulation of acetaldehyde
    • Acetaldehyde in white wine is indication of oxidation
Fermentation Taints

- Volatile acids (500-1000 mg/L)
  - Acetic acid (90%)
    - High conc. vinegar-like aroma
    - Fault > 0.7-1.1 g/L depending on wine style
    - Production by *Saccharomyces cerevisiae* strains varies widely 0.1-2 mg/L
    - However, commercially used strains produce less than native strains

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\begin{align*}
  \text{CH}_3\text{COOH} \\
  \text{acetic acid}
\end{align*}
```
Volatile acids (VA)

- Acetic acid
  - Excess conc. largely the result of metabolism of EtOH by aerobic acetic acid bacteria
  - Small increase in VA with MLF
    - 2 possible pathways
    - Produced from res. sugar through heterolactic metabolism
    - First step in citric acid metabolism
Concluding remarks

• Main fermentation off-flavors
  • Neg. aromas associated with volatile esters
    • Nail polish, perfume, soapy, too intense fruity or floral
  • Other main fermentation taints
    • VA (vinegar-like aroma)
    • Oxidation (acetaldehyde – bruised apple, sherry, nutty)
Concluding remarks

• Most off-flavors can be minimized or prevented by
  • Using clean fruit
  • Sufficient nutrient, oxygen and temperature control during fermentation
  • Good winery sanitation and adequate SO₂ use
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