Flavour Development in the Vineyard

Green Characters in Red Wine &
Tropical Characters in White Wine

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Background on Wine Aroma

Complex with ~ 800 volatile compounds identified to date

Can be derived from numerous sources:

(either chemically or by enzymatic reactions)

- Grape berry itself: **e.g. terpenoids, MP’s & C₆ compounds**
- Non volatile precursors in the grape being released during processing/storage: **e.g. varietal thiols & glycosides**
- Fermentation derived: **e.g. higher alcohols, ethyl esters & acetates**
- Oakwood contact: **e.g. oak lactones & vanillin**
- Oxidative & acid-catalysed reactions upon storage: **e.g. TDN & sotolon**
- Exogenous sources: **e.g. TCA**
Part 1: Known compounds involved in giving ‘green’ characters in wine

- **Methoxypyrazines**
  - **IBMP**, SBMP, IPMP

- **Sulfur compounds**
  - **DMS**, DES, DMDS
  - 2-Isobutylthiazole

- **C6 compounds**
  - *(Z)-3-Hexen-1-ol*
  - *(E)-2-Hexenal*
  - *(Z)-3-Hexenal*
  - Hexanal
  - 1-Hexanol
  - Hexyl esters

- 3-Isobutyl-2-methoxypyrazine (IBMP)

- Dimethyl sulfide (DMS)

- *(Z)-3-Hexen-1-ol* (cis-3-Hexen-1-ol)
Sensory impact of IBMP

- IBMP – capsicum, fresh green, asparagus, earthy; 2 ng/L threshold (extremely potent)

- Significant influence on wine aroma in white wine from 2 ng/L and red wine from 15 ng/L

- Found in wine up to
  - 50 ng/L in Sauvignon Blanc (Sth Af and NZ), 30 ng/L (Aus)
  - 56 ng/L in Cabernet Sauvignon (Aus)
  - 23 ng/L in Merlot (France)
  - 34 ng/L in Cab Franc (France)
Location of IBMP in grape berry

Within berry

\textbf{Skin: 95%}  
Seeds: 4%  
Pulp: <1%

Within cluster,

Stem accounts for \textasciitilde50\% of IBMP

Modulating factors - IBMP

- **Viticulture**
  - Associated with Cabernet Sauvignon, Merlot, Cabernet Franc, Sauvignon Blanc
  - Berry maturity (MPs Accumulate pre-veraison and degrade post-veraison)
  - Vigour, canopy density (Less vine vigor reduces MPs)
  - Climate (Warmer regions reduces MPs)
  - Fruit exposure (Better cluster exposure – Lowers MPs)
  - Skin contact during winemaking (Increases during fermentation whilst on skins)
  - Green stalks

![ IBMP Concentration Diagram ](image-url)
Bordeaux study
(van Leeuwen et al. 2004)

- Best vintages where water supply to vine from flowering to harvest was most limiting
- Either soil effect or seasonal effect or both
- Water deficit prior to veraison → early cessation of shoot growth

Acknowledgment: Peter Dry

Cessation of shoot growth by veraison

- Relationship between shoot vigour and concentration of methoxypyrazines (MP) in Cab Sauv fruit

- MP strongly correlated with pre-veraison shoot vigour
  - Independent of bunch exposure

(Jakso and Sacks (2010) Pract Winery and V’yard May/June 35-49, 73)

Acknowledgment:
Peter Dry
Modulating factors - IBMP

Winemaking
- Quantitative extraction early during fermentation
- IBMP not modified during winemaking
- Bentonite fining, oak contact, pectinases and micro-ox have no effect
- Activated charcoal may reduce IBMP but is not specific

Storage
- IBMP may be affected by packaging type
- Greatest decrease with bag in box, then synthetic, then screw cap
- Natural cork retained the most IBMP
- Typically very stable over time
IBMP level in grapes correlates closely with IBMP in wine

IBMP in wines vs. grapes for 16 small lot Cabernet franc fermentations

\[ y = 0.8079x - 0.4146 \]
\[ R^2 = 0.9614, \ p < 0.0001 \]

From Gavin Sacks, Cornell University

They have also shown that IBMP is stable in the bottle

*Ryona, Pan, Sacks (JAFC, 2009)*
Silicone tubing selectively removes IBMP

Generally, 50-90% reduction in MPs; no significant reduction of other wine volatiles (esters, fusel alcohols, most terpenoids, etc)

Fig. 2  Effects of pre-fermentation silicone treatment on A) IBMP in 2007 Cabernet Franc rosé without skin fermentation, and C) IBMP in 2008 Cabernet Franc red with skin fermentation

Reference: Treatment of grape juice or must with silicone reduces 3-alkyl-2-methoxypyrazine concentrations in resulting wines without altering fermentation volatiles. Food Research International Volume 47, Issue 1 2012 70 – 79, Imelda Ryona, Johannes Reinhardt, Gavin L. Sacks, http://dx.doi.org/10.1016/j.foodres.2012.01.012
Sensory impact of DMS

- DMS – vegetal, canned corn, canned tomato, asparagus, black currant; 25 μg/L threshold

- Higher concentrations lead to vegetal characters, lower levels can enhance berry/fruity notes

- Can be beneficial to wine aroma perhaps below 100 μg/L; highly matrix-dependent but sound wines are typically well below this level

- Found in wine up to
  - 185 μg/L in Chardonnay
  - 37 μg/L in Riesling
  - 118 μg/L in Sauvignon Blanc
  - 380 μg/L in Cabernet Sauvignon
  - 235 μg/L in Merlot
  - 756 μg/L in Shiraz
Modulating factors - DMS

- **Origin**
  - Methionine and S-methylmethionine (SMM) likely precursors

- **Viticulture**
  - Vine nutrient management and vineyard site affect amino acids
  - AA profiles vary greatly between grape varieties
  - Vintage differences also noticeable – similar pattern but variable concentrations
  - Shiraz potentially rich in precursors, Grenache not so

- **Winemaking**
  - Must nutrients and DAP addition
  - AA or SMM produced by yeasts
  - Yeast strain effects – *bayanus* vs *cerevisiae*
  - Wild and inoculated ferments
Sensory impact of *cis*-3-Hexen-1-ol

- *cis*-3-Hexen-1-ol – cut grass, herbaceous, leafy; 400 µg/L threshold

- Typically not found above threshold in most studies

- Found in wine up to
  - 650 µg/L in young red wines (highest in Tempranillo)
  - 800 µg/L in aged red wines
  - 75 µg/L in Gewurztraminer
  - 600 µg/L in some Italian and Spanish white wine varieties (Falanghina and Macabeo)
Modulating factors – *cis*-3-Hexen-1-ol

**Origin**
- Enzymatic formation via LOX pathway leads to C6 compounds

**Viticulture**
- Differs between varieties and during berry development (e.g. Riesling vs Cabernet Sauvignon)
- Highest at pre-veraison in line with unsaturated fatty acid levels – decline in linolenic acid with ripening
- Higher in skin (from press cake) than must at all ripening stages
Modulating factors – cis-3-Hexen-1-ol

Winemaking

- Time and temperature of skin contact – similar extraction from 15-28 °C with max after 10-15 h, continual increase during contact time at 10 °C after 25 h
- Relatively stable but SO₂ and enzymatic activity have effects – O₂ needed for formation
- Presence of vine leaves has minimal impact – large release from leaves crushed in air as opposed to in must
- Esterification to the acetate – from green (alcohol) to green/floral/fruity (ester)

Storage

- Not affected by storage in presence of oxygen
- Minimal change with storage on lees for up to seven months
- Unaffected by short-term oxidative storage in presence of phenolics
- Slow decline with storage for 210 days but no impact from different SO₂ levels
Summary

- Green flavours in wine are caused by a number of different compound classes, with vastly different potencies
- Compound origins are in the grape, often in precursor form
- Viticultural practices and harvesting decisions can impact on green flavours
- Green flavours may be desirable, adding complexity or typicity to wine styles
Part 2: Tropical Flavours - Varietal thiols

- Polyfunctional thiols are especially potent and have some of the lowest aroma thresholds of any food odorant.
- Varietal thiols are important impact odorants in some wines e.g. Sauvignon Blanc.

<table>
<thead>
<tr>
<th>Thiol</th>
<th>Perception threshold</th>
<th>Aroma</th>
<th>OAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-MMP</td>
<td>3 ng/L</td>
<td>blackcurrant box tree passionfruit</td>
<td>Up to 30</td>
</tr>
<tr>
<td>3-MH</td>
<td>60 ng/L</td>
<td>grapefruit passionfruit</td>
<td>Up to 210</td>
</tr>
<tr>
<td>3-MHA</td>
<td>4 ng/L</td>
<td>passion-fruit box tree sweaty</td>
<td>Up to 195</td>
</tr>
</tbody>
</table>

Darriet et al. Flavour Fragr. 1995, 10, 385-392
Tominaga et al. Flavour Fragr. 1998, 13, 159-162
Volatile thiol sensory descriptors

- Individual volatile thiols contribute *tropical* aromas to wine, 3-MH also *citrus* aroma
- Volatile thiol combinations had aromas of *tropical* & *cooked green vegetal* at both levels, and at high levels also *cat urine/sweaty*
- 4-MMP does not contribute any distinctive sensory properties at high levels
- At high concentrations 3-MHA is responsible for *cat urine/sweaty* aromas
## Grape varieties containing volatile thiol compounds

<table>
<thead>
<tr>
<th>White varieties</th>
<th>Red varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sauvignon Blanc</strong></td>
<td>Petit Manseng</td>
</tr>
<tr>
<td>Chardonnay</td>
<td>Pinot Blanc</td>
</tr>
<tr>
<td>Chenin Blanc</td>
<td>Pinot Gris</td>
</tr>
<tr>
<td>Colombard</td>
<td>Riesling</td>
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<tr>
<td>Gewürztraminer</td>
<td>Scheurebe</td>
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<tr>
<td>Gros Manseng</td>
<td>Semillon</td>
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<td>Koshu</td>
<td>Sylvaner</td>
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<tr>
<td>Maccabeo</td>
<td>Tokay</td>
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<tr>
<td>Muscat</td>
<td></td>
</tr>
<tr>
<td>Muscadet</td>
<td></td>
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<tr>
<td>Petit Arvine</td>
<td></td>
</tr>
</tbody>
</table>
Volatile thiol concentrations in SAB wines from around the world

4MMP

Country of origin

3MH

Country of origin

3MHA

Country of origin
Varietal thiol formation

- Optimise formation and maximise stability of varietal thiols
- Need to further understand precursor formation
  (Stress response: Kobayashi et al)
- Yeast plays a key role in thiol release into wine
- Need to understand relationship between precursors and free thiols

Other Intermediates

from grapes  winemaking
Modulation of volatile thiol precursors

- 3MH precursors are mainly found in the skins of grape berries
- 4MMP precursors are mainly found in the flesh of grape berries
HPLC-MS/MS analysis of precursors

- Amount of precursors measured in SAB juice:
  - Cys-3-MH: 21 – 55 µg/L
  - Glut-3-MH: 245 – 696 µg/L

- Also found precursors in other varieties (in the juice) generally:
  Sauvignon Blanc > Pinot Gris > Chardonnay > Riesling

Capone et al. JAFC 2010, 58, 1390-1395
Precursor and thiol studies

- Ripening
- Transportation / Holding
- Yeast Selection
Amount of 3-MH precursors during ripening

Capone et al. 2011, JAFC. 59: 4649-4658
Effect of transportation on precursor concentration

Analysed shortly after machine harvesting then ……..
Effect of transportation on precursors

Capone et al. 2011, JAFC. 59: 4659-4667
Storage of grape must increases ‘tropical’ precursor levels

Cysteine precursor
Glutathione precursor

3-MH precursor concentration (µg/L)

0 100 200 300 400 500 600

at harvest 12 h post-harvest
Yeast strains can release differing levels of volatile thiols

### A. 4MMP

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>VL3</th>
<th>NT116</th>
<th>QA23</th>
<th>X5</th>
<th>L2056</th>
<th>VIN7</th>
<th>VIN13</th>
</tr>
</thead>
</table>

### B. 3MH

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>VL3</th>
<th>NT116</th>
<th>QA23</th>
<th>X5</th>
<th>L2056</th>
<th>VIN7</th>
<th>VIN13</th>
</tr>
</thead>
</table>

### C. 3MHA

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>VL3</th>
<th>NT116</th>
<th>QA23</th>
<th>X5</th>
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Modified from Swiegers et al. (2009)
Further modulating factors for varietal thiols

• Higher fermentation temperatures increased volatile thiol levels (20°C compared to 13°C)
• 3MH decreased during malolactic fermentation and barrel ageing
• The addition of Sulfur dioxide stabilised 3MH and 4MMP levels in wine
• Cork closures decreased the levels of 3MH and 3MHA in wine
• 3MHA levels decreased dramatically within the first year of bottling
• Addition of Copper as a wine fining agent decreased volatile thiol levels
• In-mouth release of volatile thiol precursors by saliva bacteria
Current work – Varietal thiols in Chardonnay

- Predominance of varietal thiols in Commercial Australian Chardonnay
  - 106 Commercial bottles purchased

- Predominance of thiols in Chardonnay across Australia
  - 18 Sites selected from major wine growing areas
  - Standardised winemaking on each juice sample
  - Wines have just been bottled and awaiting:
    - Sensory analysis
    - Analysis of both thiol precursors in the juice and free thiol analysis in the finished wine
Concluding remarks

- 3-MH precursor and free 3-MH concentration can be significantly affected by
  - Ripening
  - Transportation / Storage of grapes
  - Harvest type: Machine versus Hand Picking
  - Yeast selection
Flavour optimisation – the future

- Be able to predict concentrations of volatiles from:
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