Analysis of Resveratrol in Wine by HPLC
Outline

- Introduction
- Resveratrol
  - Discovery
  - Biosynthesis
- HPLC separation
- Results
- Conclusion
Composition of flavoring, coloring and other characteristic ingredients in wine is largely dependent on make and storage.

White and red wine differ not only in color of the grapes, but also in the way they are produced.

For white wine, grapes are pressed and the resulting must is fermented into an alcoholic beverage “Mostgärung”.

To produce red wine the grapes are squashed to produce a slurry of grape juice, pulp and skin. During fermentation of this mash, the colorants are extracted into solution. Pressing takes place after fermentation “Maischegärung”.

Introduction
Only fermentation from mash can result in extraction of compounds from grape skin and seeds.

Therefore red wine contains a number of compounds that are not found in white wine.

Also after fermentation, the process of “aging” during the period of storage in a wooden cask results in distinct changes and a unique profile of over 1000 different compounds in each lot.
Apart from water and ethanol, red wine contains in average:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>1 %</td>
<td>Oily, sweetness</td>
</tr>
<tr>
<td>Organic acids</td>
<td>0.4 %</td>
<td>Acidity</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td></td>
<td>Red colour</td>
</tr>
<tr>
<td>Catechins</td>
<td>0.1 %</td>
<td>Bitterness (&gt; 20 mg/L), antioxidants</td>
</tr>
<tr>
<td>Tannins</td>
<td></td>
<td>Astringency, bitterness</td>
</tr>
<tr>
<td>Flavonols</td>
<td></td>
<td>antioxidants</td>
</tr>
<tr>
<td>Other compounds</td>
<td>0.5 %</td>
<td></td>
</tr>
</tbody>
</table>
Another molecule in red wine, however, has been the main focus of health benefits in recent years: Resveratrol.

It has been shown in studies that resveratrol, as well as having antioxidant properties, can help prevent high blood pressure (hypertension) in mice and also has anti-inflammatory effects. It’s also suspected to have anticancer, and chemopreventive abilities.
Resveratrol - Discovery

- Reseveratrol was first discovered by Japanese researcher Michio Takoaka and his group in 1939.
- They collected *veratum grandiflorum*, a stout, exotic and flowering plant from the Hokkaido Island. A phenolic compound was obtained by crystallization and the molecular formula was identified.
- The new compound was named RES (resorcinol family), VERATR (from veratum grandiflorum) OL (used to indicate hydroxyl groups)
Resveratrol - Biosynthesis

• Resveratrol is found in the skin of red grapes, peanuts, in many berries and a few other plants… but how does it get there?
• Resveratrol is derived from p-coumaric acid which is an intermediate in lignin production. The two key enzymes are Coenzyme A (CoA) Ligase (4CL) and Stilbene Synthase (STS)
• Resveratrol is produced in plants when they are exposed to stress such as UV-light, disease and pests.
HPLC separation

• Reversed phase HPLC is most appropriate to separate the cis- and trans isomers from each other.

• Fluorescent detection is used for high sensitivity (LOQ ≤ 0.015 mg/L\(^1\) vs. 0.3 mg/L\(^2\) using DAD)

• Wine sample complexity requires gradient optimization to resolve the trans-resveratrol peak from the rest of fluorescent stilbenes and polyphenols.

• Fast analysis using Nexera X2 UHPLC system equipped with PDA and RF-20 AXS Fluorescent detector.

\(^1\) J. Sep. Sci. 2007, 30, 669 – 672
\(^2\) J. Agric. Food Chem., Vol. 51, No. 18, 200
Method Development

Columnn: GIST C18, 100 x 2.1 mm, 2 µm

Mobile Phase:
  A: Water incl. 0.2 % Formic Acid
  B: Acetonitrile

GE Program
  0 min BCONC 20%
  4 min BCONC 50%
  4.01 min BCONC 20%

Flow rate: 0.6 ml/min

Temperature: 50 °C

PDA detection: 190 – 360 nm

Detection: RF-20AXS – Excitation λ = 300 nm Emission λ = 386 nm SENS = Med

Cell Temperature: 20 °C

Inj. Vol.: 2 µl
Results

- 17.5 mg /L trans-resveratrol standard – PDA detection 291 nm
Results

- 9 mg/L trans-resveratrol standard – RF - Ex:300nm  Em:386nm
Method Development

- Cell temperature affects sensitivity
- Improve baseline stability and eliminate the effect of temperature
Sample preparation
Sample preparation

1. Sample 5 mL
2. Adjusted to pH 2 by adding 0.1 mol/L HCl
3. Diethylether 5 mL
4. Mixing 5 min
5. Aqueous (lower) and Organic Phase (upper)
6. Evaporate to dryness
7. 50% Methanol 1 mL
8. Filtration (0.2 μm)
9. 1 μL injection
Results

- Wine sample, LL extracted – PDA detection 280 nm

trans-Resveratrol = 7.7 mg/L

cis-Resveratrol = 1.6 mg/L
Example: Filtered red wine
LL extraction vs filtered (0.2 µm) sample RF Signal

Data1: Shim-Wine-filtered_GE_.lcd Detector A: Ex: 300nm, Em: 386nm
Data2: Shim-Wine-LL-extract_2ul_IV_GE_002.lcd Detector A: Ex: 300nm, Em: 386nm
LL extraction vs filtered (0,2 µm) sample PDA signal
Conclusion

• The proposed method allows the determination of trans-resveratrol in wine in a one step procedure.
• Fluorescence detection is highly sensitive and allows for the detection of resveratrol in concentrations present in red wine.
• Animal studies suggest as much as 500 mg daily may be necessary to provide any health benefits, even if a 40 mg daily dose is sufficient, as suggested elsewhere, red wine contains at most 12 mg resveratrol per liter, you'd need to drink a little over 3 liters of wine daily to get that much resveratrol.
Cheers!