PROFILING BOURBONS AND AMERICAN WHISKEYS USING UHPLC/QTOF-MS

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Defining Whiskey

- Whiskey is a spirit produced by distilling grain based mashes; grains in common use include:
  - corn
  - rye
  - wheat
  - barley (malted or un-malted)

- Whiskeys are distilled using pot stills or in column or continuous stills

- Most whiskies are aged in wooden casks for some period of time, which is often legally defined
Whiskey types

• Bourbon whiskey
  • Minimum of 51% corn, is often higher
  • Must be aged in new charred American oak
  • 2 years of aging for straight whiskey
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• Blended whiskeys, craft distilled whiskeys
Whiskey profiling

• Confirmation of authenticity
  • Isotopic ratios for source of ethanol
  • Volatile profiles by GC/MS
  • Spectroscopic methods—near IR and mid-IR
  • UHPLC/QTOF

• Quality assurance/process improvement
  • Evaluation of raw materials
  • Impact of fermentation/distilling process
  • Evaluation of cooperage
  • Changes in whisky composition during aging
UHPLC/QTOF

- High performance liquid chromatography coupled to high resolution mass spectrometer
- The time of flight (TOF) mass spectrometer provides accurate mass data
- The quadrupole (Q) provides MS/MS capability
Profiling workflow

UHPLC separation & MS
- C18 Reverse phase
- Electrospray ionization
- Time of Flight MS

MassHunter Qualitative Analysis
- Finding compounds
- Isotope patterns
- Adducts

Mass Profiler Pro
- Define samples
- Align mass & RT
- Quality control & filters
- Statistical analysis
Whiskey samples

- 67 whiskeys covering a broad range of American whiskeys
  - 41 Bourbon whiskeys
  - 13 Rye whiskeys
  - 6 Tennessee whiskeys
  - 7 Other American whiskeys

- Commercial product purchased at retail stores and reputable online merchants
Whisky profiling

- Samples were run neat, in triplicate
- ESI, negative mode, 100-1100 m/z
- Sample order was randomized and all samples were run over a two day period
- Each sample was also analyzed in an untargeted MS/MS mode (20V collision energy)
Whiskey profiling

• The initial set of ~3000 entities (accurate mass & RT) across the samples was narrowed down by screening for:
  • Presence across replicates
  • Minimum abundance
  • ANOVA for significance

• A set of 40 entities was selected to differentiate across the whiskeys
  • An accurate mass database search of these masses found compounds in these chemical classes:
    • Wood related volatile phenols and polyphenols
    • Terpenes and related compounds
    • C6 and higher alcohols, esters and acids
3D Principal Component Analysis (PCA) of 67 American whiskeys

X axis: 43.5%   Y axis: 12.8%   Z axis: 10.9%
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American whiskeys—blended and craft whiskeys
3D PCA of 60 Bourbon, Rye and Tennessee whiskeys

X axis: 31.9%  Y axis: 15.4%  Z axis: 9.1%
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Craft or Rye only distillers
Profiling

- Profiling can be targeted or untargeted
  - If specific compounds of interest are known, these can be used for targeted profiling—e.g., use of oak aroma related volatiles for profiling barrels

- In many cases, profiling is a discovery process—it is used to find compounds to screen samples within a set

- It is not always necessary to identify the compounds which drive a profile
  - While the accurate mass data can be sufficient to provide a chemical formula, in many instances there are multiple compounds with the same formula and accurate mass
Identifying the entities used to differentiate these whiskeys

• Search Scripp’s Metlin Metabolomics and Tandem MS database
  • Accurate mass MS database
  • Has MS/MS data for an ever-increasing number of compounds

• Comparison with other published MS and MS/MS results in whiskeys, other spirits and oak barrels
  • Much of the published work is on volatile composition, but there is some data on semi volatile and non-volatile composition as well.
2D PCA of Bourbons only, averaged data by individual bourbons (37 entities)

Observations (axes F1 and F2: 50.35 %)
2D PCA of Bourbons only, averaged data by individual bourbons

Observations (axes F1 and F2: 50.35 %)

Increasing age of whiskey
Compounds associated with longer aging of the whiskey

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mass</th>
<th>RT</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esculetin</td>
<td>178.0265</td>
<td>2.66</td>
<td>Metlin MS/MS</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>302.0035</td>
<td>4.52</td>
<td>Metlin + standard</td>
</tr>
<tr>
<td>Octadecanoic acid, dihydroxy- or hydro-peroxy</td>
<td>346.2327</td>
<td>7.25</td>
<td>Metlin MS/MS</td>
</tr>
<tr>
<td>Unknown flavonoid</td>
<td>368.1229</td>
<td>5.39</td>
<td>Mass Hunter</td>
</tr>
<tr>
<td>Lyoniresinol</td>
<td>420.1718</td>
<td>4.14</td>
<td>MacNamara(^1)</td>
</tr>
<tr>
<td>Methylated Lyoniresinol</td>
<td>434.1526</td>
<td>4.96</td>
<td>MacNamara(^1)</td>
</tr>
<tr>
<td>Lyoniresinol xyloside</td>
<td>552.2156</td>
<td>4.36</td>
<td>MacNamara(^1)</td>
</tr>
</tbody>
</table>

\(^1\)McNamara, et al, LC/GC Europe, 2011
## Compounds associated with lesser aged whiskeys

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<tr>
<td>Octanoic acid</td>
<td>144.1097</td>
<td>7.30</td>
<td>Metlin MS/MS</td>
</tr>
<tr>
<td>Decanoic acid</td>
<td>172.1459</td>
<td>8.56</td>
<td>Metlin MS/MS</td>
</tr>
<tr>
<td>Coniferaldehyde</td>
<td>178.0502</td>
<td>4.08</td>
<td>Metlin MS/MS</td>
</tr>
<tr>
<td>Syringaldehyde</td>
<td>182.0548</td>
<td>3.53</td>
<td>Metlin MS/MS</td>
</tr>
<tr>
<td>Dodecanoic acid</td>
<td>200.1791</td>
<td>5.39</td>
<td>Metlin MS/MS</td>
</tr>
</tbody>
</table>

![Coniferaldehyde](https://via.placeholder.com/150)
Discriminant Analysis of Bourbons by Producer

Observations (axes F1 and F2: 78.53 %)

95% confidence ellipses
Associated compounds by producer

Compounds associated with producer #5
## Compounds associated with producer #5’s whiskeys

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<tr>
<td>Unknown</td>
<td>163.0376</td>
<td>4.08</td>
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<tr>
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¹McNamara, et al, LC/GC Europe, 2011
Conclusions

• UHPLC/QTOF-MS was used to profile 67 whiskeys from a broad range of origins and ages
  • Bourbon, Rye and Tennessee whiskeys were well differentiated from blended American whiskeys
  • Some Tennessee and some Rye whiskeys could be differentiated from Bourbons
  • Identification of compounds which drive these differentiations is ongoing, using MS/MS results to compare with database and previously published MS/MS data
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