Flavour Components of Whiskey. III. Ageing Changes in the Low-Volatility Fraction

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The low-volatility wood-originating compounds isolated from whiskey by vacuum fractional distillation were analysed by high-resolution gas chromatography and mass spectrometry (GC-MS). Three phenolic esters previously unreported in whiskey were identified and confirmed by synthesis. Formation profiles for sixteen compounds were established in whiskeys aged for periods from 1.5 to 10 years in second-fill heavy-charred American Bourbon barrels. These profiles indicated significant increases for several compounds, especially in the older whiskeys. Ratios of aromatic phenolic aldehydes, and similar ratio changes during ageing, were different from reported data relating to other wood types and treatments. Further preparative separation by high-pressure liquid chromatography (HPLC) of the wood fraction followed by GC-MS allowed retention and mass spectral characterisation of additional compounds originating from wood. Sensory investigation indicated different and unique contributions from the HPLC cuts. Spiking of the three phenolic esters into a young whiskey gave a detectable increase in maturation intensity.

Freshly distilled whiskey is colourless with a pungent aroma and harsh taste. The practice of storage in oak casks modifies and significantly improves the sensory properties of the product. Maturation of distilled spirits in oak barrels takes place slowly and therefore over many years. The mechanisms involved in this barrel contribution include direct extraction of wood components, decomposition of wood components, and reaction of wood components both with each other and with components of the distillate (Nishimura & Matsuyama, 1989). Some of these reactions occur in the already complex matrix of the unaged whiskey with resultant difficulties for analysis of the new compounds produced and related subsequent changes.

The approach of this work was to attempt to interpret some of these complex changes by first isolating the relevant low-volatility compounds as a distinct fraction from the whiskey (MacNamara et al., 2001a). A similar approach was used to isolate the high-volatility compounds from whiskey and to investigate their changes with ageing (MacNamara et al., 2001b). In both cases the vacuum fractional distillation procedure separates either the high- or low-volatility compounds free from both the dominant ethanol and the complex fusel compounds. This allowed subsequent chromatography to be tailored to the specific compounds in each fraction.

When the low-volatility compounds of interest are isolated in this way, the increases in concentration of dominant and trace compounds can be measured for natural barrel-aged whiskey. A different approach towards the identification of oak wood aroma compounds involved the extraction of such compounds from oak wood chips and shavings in model solutions. In one study over one hundred compounds were identified from the steam distillate of methanol extracts of white oak shavings (Nishimura et al., 1983). Extraction of volatile and non-volatile compounds by 60% ethanol from oak hardwood shavings was also investigated (Nykänen, et al., 1984). Maximum extraction occurred after three months and, with the aid of subsequent analysis, carbohydrates and a range of carboxylic acids were identified.

In both of these studies the presence of ß-methyl-y-octalactone was not reported even though the isomers of this compound had previously been identified in spirits stored in oak casks (Suomalainen & Nykänen, 1970). The cis and trans isomers were also shown to be major constituents of oak wood (Masuda & Nishimura, 1971) and subsequent work confirmed the presence of these compounds in spirits stored in oak wood (Nishimura & Masuda, 1971; Guymon &Crowell, 1972). Organoleptic thresholds of both isomers have been established in 30% alcohol solution and a positive correlation has been established by a scale method, involving ranking for aroma and taste evaluation, between desirable aged flavour and lactone content for ten commercial whiskies (Otsuka et al., 1974). Other studies have shown that production of lactones is substantially enhanced by thermal oxidation of lipid precursors during charring or toasting of wood (Maga, 1989), and no such treatment was indicated in both of the previously mentioned studies where lactones were not reported. Therefore care must be taken with data from model solution experiments, as they may not fully represent the natural ageing process in barrels. Isolating the wood compounds by vacuum fractional distillation from barrel whiskey at different ages as was proposed for this study allows a more accurate and authentic representation of the chemical changes to be established.

High-pressure liquid chromatography (HPLC) is usually the technique of choice for analysing the low-volatility compounds produced during ageing (Lehtonen, 1984). However, since it


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offers limited resolution and suffers from lack of a routine universal detector, high-resolution gas chromatography – mass spectrometry (GC-MS) was selected as a better alternative to analyse the isolated lower-volatility flavour compounds in aged whiskey. In addition, programmed temperature vapourisation (PTV) followed by chromatography on a stable high-temperature column was selected for the elution of low-volatility compounds previously not amenable to gas chromatography. Despite the limitations of HPLC, it still appears very useful as a technique to segregate the principal wood-originating compounds prior to GC-MS analyses. Thus it is believed that the above integrated analytical strategy would allow the characterisation of both abundant and trace compounds formed during ageing. Such analysis of premium whiskeys aged for long periods of time in order to develop significant maturation flavour should permit a better understanding of compound development during maturation and may allow the achievement of greater effects in less time with important implications for production costs.

**MATERIALS AND METHODS**

**Material**

Whiskey at 1.5, 3, 5 and 10 years old was used for both GC-MS investigations of low-volatility compounds and formation profiles for selected compounds over the full time range. The 10-year-old sample was also used for additional GC-MS analysis after a further preparative chromatographic procedure. All samples were from standard once-used American Bourbon barrels and at strengths with those volatile and fermentation compounds that partition with ethanol/water gradient was used starting from 10% ethanol and increasing at 1.5% ethanol/min to 100% ethanol. A further period of 15 min at 100% ethanol was used to clean the column. Thirty injections were made using the concentrate from 500 mL of the 10-year-old fraction 5. The injection volume was 20 μL per run with 36 fractions per run collected on a time basis.

**Gas chromatography-mass spectrometry**

The GC-MS analyses of the 10-year-old fraction 5 concentrate and similar concentrates of its HPLC composites were performed on a Hewlett-Packard 5890 GC coupled to a 5971 Mass Selective Detector (Hewlett-Packard, Palo Alto, CA, USA). The column used was a chemically bonded XT15 fused silica capillary (50 m x 0.25 mm i.d. x 0.25 df, Restek, Bellefonte, PA, USA) directly interfaced to the ion source of the mass selective detector. The oven temperature was programmed from 60°C at 2°C/min to 300°C, where it was held for 10 min. Linear temperature programmed retention indices were calculated using the same conditions after injection of a mixture of C9 to C26 alkanes. The mass selective detector was operated in scan mode at a detector setting of 1600 V and an ionisation voltage of 70eV. The scan range was 25-400amu, and spectra were acquired at 2 scans/sec. Helium was used as carrier gas at 1 mL/min. 1 μL of each sample was injected in splitless mode using a programmed temperature injector (CIS-3, Gerstel Gmbh) with an empty deactivated vigreux glass liner. The injector temperature was programmed from 40°C at 10°C/sec to 300°C. The splitless time was 1 min. Mass spectra and retention indices of authentic compounds were used for identification. Compounds were either purchased (Sigma-Aldrich, Poole, Dorset, UK) or were available from internal collections. Ethyl homovanilllate, ethyl syringate and ethyl homosyringate were synthesised as described later.

**Simultaneous mass spectrometric and flame ionisation detection**

The MS and FID analyses on the triplicate fraction 5 concentrates at various ages were performed using the same GC-MS conditions as above, but with a split injection of 1/10 to ensure resolution of all compounds for quantification. At the column exit a micro crosspiece (Gerstel Gmbh) with individual fused silica segments to MS and FID was used to achieve the simultaneous detection. Quantification was obtained from the FID signal with spectral confirmation from the MS signal.

**Synthesis of phenolic esters**

Ethyl syringate and ethyl homovanilllate were synthesised from the corresponding commercially available acids by esterification with p-toluene sulfonic acid in the presence of an excess of ethanol. Homosyringic acid was synthesised via a rhodanine complex from syringaldehyde (Fischer & Hibbert, 1947; Tanner & Osman, 1987) and esterified as above. The following IR, NMR and MS data are in agreement with the proposed structures.

**Ethyl homovanilllate**

GC data: non polar index: 1645 (on XTI-I), polar index: 2721 (on FFAP).

Spectroscopic data:- 1H-n.m.r. (400MHz) δ (CDCl3): 1.23 (3H, t, -OH), 1.25 (3H, t, -OCH3-CH2), J=7.4Hz), 3.5 (2H, s, -CH2-), 3.83 (3H, s, -OCH3), 4.12 (2H, q, -OCH2-CH3, J=7.4Hz), 5.73 (1H, s, -OH), 6.74 (1H,dd, 6-H, J=2, 8.36 Hz), 6.78 (1H,d, 2-H, J=2Hz), 6.83 (1H,
study. In this regard, for Bourbon in heavy charred new barrels, maximum amounts of phenolic aldehydes will be immediately released into the spirit from degraded lignin beneath the heavy char layer, and their relative ratios could be different from phenolic aldehydes produced in once-used Bourbon barrels by the slower acidic ethanolysis mechanism. This also agrees with the substantial differences, both in absolute levels of phenolic aldehydes and in the vanillin/coniferaldehyde and syringaldehyde/sinapaldehyde ratios reported for uncharred wood soaked in 60% ethanol, in comparison to similarly treated charred wood (Nishimura et al., 1983). In the Cognac study the wood type was also different and initial charring of the wood was not employed. An additional complicating factor is that the Cognac was initially matured for one year in new oak, and then transferred to used casks for further ageing (Puech et al., 1984). In a separate study on Armagnac in Limousin oak the increase in the ratios of vanillin to coniferaldehyde and syringaldehyde to sinapaldehyde did not materialise until after fifteen years of ageing (Puech, 1981). This is not in agreement with the previously mentioned Cognac study, where a regular decrease over fifty years was presented. However, the Armagnac results are in agreement with data presented here and may imply that if whiskey is left sufficiently long in cask, such a similar increase in these ratios may occur. Normal commercial whiskey is not usually matured for more than twelve years.

Relative levels and ratios of the aromatic aldehydes at various stages of ageing were also clearly different in a comparison of aged Armagnac and Rum (Puech et al., 1977). In this case the additional factor of climatic condition was cited, in addition to different wood type and pretreatment. In rum-producing countries warehouses are generally heated during winter to produce an average temperature of 20°C to 25°C (Kervegant, 1946), and this temperature increase will cause an acceleration in oxidation reactions (Mourgues et al., 1973). Therefore, characteristic analytical profiles of aged distilled spirits must be interpreted in terms of the different variables of wood type, wood pre-treatment, barrel history in the re-usage cycle, and the climatic conditions for storage during maturation. There is a possibility here for commercial producers to use such profiles to aid authentication of their own products in the market place.

**HPLC separation of fractions**

Separation of the fraction 5 extract from the 10-year-old whiskey according to the HPLC procedure previously described is represented in Fig. 4.

Thirty-six fractions were collected per run, comprising an initial zero fraction, thirty-four fractions during elution of compounds and a final fraction. The opinion of experienced whiskey tasters was that the zero and final fractions had little sensory interest and these were excluded from further investigation.

Small aliquots of the intermediate thirty-four fractions were then analysed by GC-MS and based on these results the fractions were combined into four composite fractions in order to achieve the maximum segregation of the dominant 2-phenyl ethanol, whiskey lactones and the four phenolic aldehydes. After extraction and GC-MS analysis these composites give the traces in Fig. 5.

From this figure it is clear that the phenolic aldehydes, 2-phenyl ethanol and the whiskey lactones were substantially segregated into separate composites, allowing cleaner mass spectra of the minor components.

Preparative HPLC has also been used previously for concentrating flavour compounds from distilled spirits (Piggott et al., 1992). However, this study simply involved initial dilution of 200 mL of the spirit to 5% ethanol followed by pumping of the diluted solution through the HPLC column to enrich flavour com-

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**FIGURE 4**

HPLC-UV 1) trace of fraction 5 concentrated extract 2). 1) Ethanol-water gradient. 2) 36 cuts per injection as indicated.

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![FIGURE 4](image-url)

HPLC-UV trace of fraction 5 concentrated extract. Ethanol-water gradient. 36 cuts per injection as indicated.
### Table 4: Sensory Characterization of Phenolic Esters in Whiskey Aroma

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ret. (a) Index</th>
<th>Mass Spectral Data (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propiovanillone (^{(b)})</td>
<td>1609</td>
<td>151(100), 180(55, (M^+)), 123(49), 108(25), 52(17), 65(16), 77(13), 51(10)</td>
</tr>
<tr>
<td>Homosyringyl ethyl ether (^{(b)})</td>
<td>1714</td>
<td>167(100), 168(57), 212(47, (M^+)), 123(23), 153(20), 95(15), 107(13), 77(12), 53(11)</td>
</tr>
<tr>
<td>Propiosyringone (^{(b)})</td>
<td>1850</td>
<td>181(100), 210(43, (M^+)), 182(20), 153(18), 67(13), 108(13), 123(12), 138(10)</td>
</tr>
<tr>
<td>Butyl vanillate (^{(b)}) (principal loss of m/z 73)</td>
<td>1874</td>
<td>151(100), 123(17), 152(11), 149(10), 224(4, (M^+))</td>
</tr>
<tr>
<td>2-Ethoxy-(4-hydroxy-3,5-dimethoxy-phenyl)-ethyl acetate (^{(b)})</td>
<td>2035</td>
<td>211(100), 123(42), 95(16), 212(12), 140(10), 155(10), 167(9), 284(9, (M^+))</td>
</tr>
<tr>
<td>3-Ethoxy-3(4-hydroxy-3-methoxy phenyl) methyl propanoate (^{(b)})</td>
<td>2064</td>
<td>181(100), 182(18), 153(14), 67(11), 123(10), 108(10), 254(9, (M^+))</td>
</tr>
<tr>
<td>Vanillic acid derivative</td>
<td>2093</td>
<td>151(100), 207(11), 123(10), 152(9), 252(6, (M^+))</td>
</tr>
<tr>
<td>Possible isomer of peak 36 (principal loss of m/z 73)</td>
<td>2108</td>
<td>181(100), 182(16), 154(21), 179(15), 153(12), 254(9, (M^+))</td>
</tr>
<tr>
<td>Syringic acid derivative</td>
<td>2493</td>
<td>182(100), 85(96), 167(85), 181(72), 81(54), 83(40), 57(26), 154(25), 168(25), 237(17), 310(11, (M^+))</td>
</tr>
<tr>
<td>Vanillic acid derivative</td>
<td>2567</td>
<td>151(100), 123(18), 274(11, (M^+)), 108(9), 152(8), 243(6)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2694</td>
<td>272(100, (M^+)), 211(24), 168(20), 136(19), 197(17), 273(17), 207(15)</td>
</tr>
</tbody>
</table>

(a) Programmed retention indices.
(b) Mole in brackets. Suggested molecular ion is the highest mass detected in the electron impact mass spectrum.

...
Description of HPLC composites by an experienced whiskey panel.

TABLE 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite 1</td>
<td>Sweet, woody aroma.</td>
</tr>
<tr>
<td>HPLC Cuts 1 – 20</td>
<td>Strong vanillin note.</td>
</tr>
<tr>
<td></td>
<td>Dull wood taste.</td>
</tr>
<tr>
<td>Composite 2</td>
<td>Spicy delicate aroma. Intense</td>
</tr>
<tr>
<td>HPLC Cuts 20 – 25</td>
<td>taste characteristics similar</td>
</tr>
<tr>
<td></td>
<td>to well-aged whiskey.</td>
</tr>
<tr>
<td>Composite 3</td>
<td>Rose-like aroma.</td>
</tr>
<tr>
<td>HPLC Cuts 26 – 30</td>
<td>Also fatty ester type notes.</td>
</tr>
<tr>
<td></td>
<td>Fatty bland taste.</td>
</tr>
<tr>
<td>Composite 4</td>
<td>Intense sweet coconut aroma.</td>
</tr>
<tr>
<td>HPLC Cuts 31 – 34</td>
<td>Little taste.</td>
</tr>
</tbody>
</table>

Maturation intensity rankings on young whiskey, young whiskey after spiking and old whiskey.

TABLE 5

<table>
<thead>
<tr>
<th>Sample</th>
<th>Maturation intensity (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Year-Old Whiskey</td>
<td>38,85*</td>
</tr>
<tr>
<td>3-Year-Old Whiskey+ Level 1 Spike</td>
<td>46,54bc</td>
</tr>
<tr>
<td>3-Year-Old Whiskey + Level 2 Spike</td>
<td>49,54b</td>
</tr>
<tr>
<td>10-Year-Old Whiskey</td>
<td>72,69c</td>
</tr>
</tbody>
</table>

* LSD (p = 0.05) = 10.08.

fact making a contribution to the odour intensity, although not significantly at the lower level of spiking.

Although these esters may contribute significantly to the aroma intensity of aged whiskey, this contribution should also be evaluated together with several other aroma impact components previously reported and also found in this study.

CONCLUSIONS

Vacuum fractional distillation followed by GC-MC analysis allowed construction of practical profiles of age changes during maturation of whiskey in second-fill heavy-charred Bourbon oak barrels. There is evidence to suggest that these aging patterns may be related to wood type, its pre-treatment and fill history. Ratios of certain aromatic phenolic aldehydes were different from similar published data relating to other wood types and other treatments. Ratios of syringyl and guaiacyl phenolic aldehydes decreased rather than increased over ten years of ageing. These observations are fundamentally linked to a unique balance of extraction mechanisms, which in turn is related to the wood type and fill history of the barrel. An appreciation of the relative contribution of these maturation parameters can be used to investigate and improve the ageing flavour of whiskey.

A combination of vacuum fractional distillation and preparative HPLC allowed the maturation flavour of whiskey to be segregated into composites. This approach isolated a unique group of compounds, free from the known dominant aroma-contributing components, and these compounds were shown to be partially significant for maturation character.

LITERATURE CITED


Chatonnet, P. & Dubourdieu, D., 1998. Comparative study of the characteristics of American white oak (Quercus alba) and European oak (Quercus petraea and Quercus robur) for production of barrels used in barrel ageing of wines. Am. J. Enol. Vitic. 49, 79-85


