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-γ-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 whiskeys (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

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 vaporisation (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 between 60% and 65% vol/vol ethanol, depending on the natural evaporation loss during ageing. These samples at various ages were composites of twelve aliquots from similar casks at the same age.

Sample preparation

The general whiskey vacuum fractional distillation separation has been described previously (MacNamara *et al.*, 2001a).

Essentially, the distillation removes the matrix ethanol together with those volatile and fermentation compounds that partition into the first four fractions, leaving the compounds of interest in an aqueous fraction 5. Two 250 mL aliquots of fraction 5 from the 10-year-old whiskey were each continually extracted overnight with 60 mL of Freon 11/Dichloromethane (90%/10%). The organic layers were bulked and subsequently concentrated in a Kuderna Danish apparatus to 1 mL. This extract was further fractionated by preparative HPLC and the fractions obtained were assembled into composites, re-extracted as above and concentrated for GC-MS analysis. Triplicate 50 mL portions of the whiskey fraction 5 at the different ages were similarly extracted and concentrated after addition of 6 ppm 2, 3, 4-trimethoxy benzaldehyde as internal standard. These extracts were analysed by simultaneous GC-MS and GC-FID to quantitatively determine concentration increases of the selected compounds with time. For quantification the area ratio of each peak of interest to the internal standard at the different ages was used to give amounts relative to the known added amount of the internal standard.

Preparative high pressure liquid chromatography

The apparatus was a Waters Maxima 820 (Waters Corporation, Milford, MA., USA) with gradient capability and an SM400 multi UV/VIS detector set at 254nm and 2.0 AUFS. The column was a 250 mm x 10 mm Lichrospher RP-18 (Merck Gmbh, Darmstadt, Germany) with a 10 mm particle size. An 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 XTI5 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 homovanillate, 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 homovanillate 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 homovanillate

GC data: non polar index: 1645 (on XTI-5), polar index: 2721 (on FFAP).

Spectroscopic data:- ^{1}H-n.m.r. (400MHz) δ ($CDCl_3$): 1.23 (3H, t, -$OCH_2\underline{CH_3}$, J=7.4Hz), 3.5 (2H, s, -CH_2-), 3.83 (3H, s, -OCH_3), 4.12 (2H, q, -$O\underline{CH_2}CH_3$, 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,

d, 5-H, J=8.36Hz).-^{13}C-n.m.r. δ: 14.07, 40.90, 55.76, 60.74, 111.68, 114.31. 121.9, 125.77, 144.65, 146.42, 171.9.

I.R. KBr disc: 3300, 1700, 1600, 1130, 1040. cm^{-1}.

MS (70ev): 137 (100), 210 (28.5, M$^+$), 138 (9.8), 122 (6.6), 94 (6.0), 211 (3.7), 51 (3.1), 39 (3.0), 65 (2.8), 77 (2.4), 66 (2.3), 123 (1.5).

Ethyl syringate

GC data: non polar index: 1840 (on XTI-5), polar index: 3020 (on FFAP).

Spectroscopic data:-^{1}H-n.m.r. (400MHz) δ ($CDCl_3$):1.34 (3H, t, -$OCH_2\underline{CH_3}$, J=7.4Hz), 3.88 (6H, s, 2x-OCH_3), 4.31 (2H, q, -$O\underline{CH_2}CH_3$, J=7.4Hz), 6.03(1H, s, -OH), 7.27 (2H, s, 2-H, 6-H). - ^{13}C-n.m.r. δ: 14.3, 56.3, 60.85, 106.48, 121.26, 139.05, 146.51, 166.31.

I.R. KBr disc: 3350, 1700, 1620, 760 cm^{-1}.

MS (70ev):181 (100), 226 (82.8, M$^+$), 198 (23.8), 182 (13.0), 183 (10.7), 227 (10.5), 154 (10.0), 211 (8.2), 153 (8.1), 67 (7.9), 53 (6.2), 139 (5.3).

Ethyl homosyringate

GC data: non polar index: 1886 (on XTI-5), polar index: 3076 (on FFAP).

Spectroscopic data: ^{1}H-n.m.r. (400MHz) δ ($CDCl_3$): 1.23 (3H, t, -$OCH_2\underline{CH_3}$, J=7.4Hz), 3.5 (2H, s, -CH_2-), 3.80 (6H, s, 2x-OCH_3), 4.10 (2H, q, -$O\underline{CH_2}CH_3$, J=7.4Hz), 5.75 (1H, s, -OH), 6.50 (2H, s, aromatic). -^{13}C-n.m.r. δ: 171.7, 146.8, 133.7, 124.9, 105.8, 60.8, 56.1, 41.4, 14.1.

I.R. KBr disc: 3451, 1730, 1609, 755 cm^{-1}.

MS (70ev): 167 (100), 240 (25.6, M$^+$),168 (9.8), 122 (4.3), 123 (4.0), 241 (3.1),153 (1.8), 106 (1.8), 151 (1.4), 53 (1.3), 169 (1.1), 78 (0.9).

Sensory assessment of HPLC composites

These samples were adjusted to 20% ethanol and duplicates were presented in a random order to five experienced judges. They were requested to describe the aroma and taste of each composite in terms of general whiskey terminology to which they were acquainted. No panel training was done, as the judges were experts who were accustomed to using similar terminology.

Sensory Investigation of phenolic esters

A panel of 12 judges, all familiar with sensory evaluation of aged spirit samples, was used. The three esters were added together to a 3-year-old whiskey at the level found for the 10-year-old whiskey, and double this level. These levels were 0.12 mg/L and 0.24 mg/L for ethyl homovanillate, 0.5 mg/L and 1.0 mg/L for ethyl syringate, and 0.1 mg/L and 0.2 mg/L for ethyl homosyringate. The samples were supplied in random order and the judges were asked to indicate the intensity of the maturation character on a 150 mm unstructured line scale with indications for "none" and "intense" at the ends. All samples were judged at an alcohol strength of 23% vol/vol.

RESULTS AND DISCUSSION

Identification of compounds in aged fraction 5.

Figure 1 shows the GC-MS trace of the 10-year-old whiskey after extraction and concentration.

Dominating compounds in this extract are the 2-phenyl ethanol, the isomeric methyl octalactones and four phenolic aldehydes. Table 1 details the compounds identified together with retention indices on the apolar XTI5 capillary column.

2-Phenyl ethanol is a fermentation compound, but because of its relatively high boiling point and non-azeotropic behaviour with ethanol, it does not partition into the earlier distillation fractions with the other higher alcohols. Use of programmed temperature vaporisation and a low-bleed high-temperature apolar column allows GC determination of relatively high boiling polar compounds (Guntert *et al.*, 1986). Ethyl homovanillate, ethyl syringate and ethyl homosyringate were identified for the first time in aged whiskey. Confirmation was obtained by comparison of retention times and mass spectral data of the compounds in fraction 5 with synthesised standards. Ethyl syringate was previously identified as a reaction product in a model experiment involving storage of lignin-related compounds in 60% ethanol for

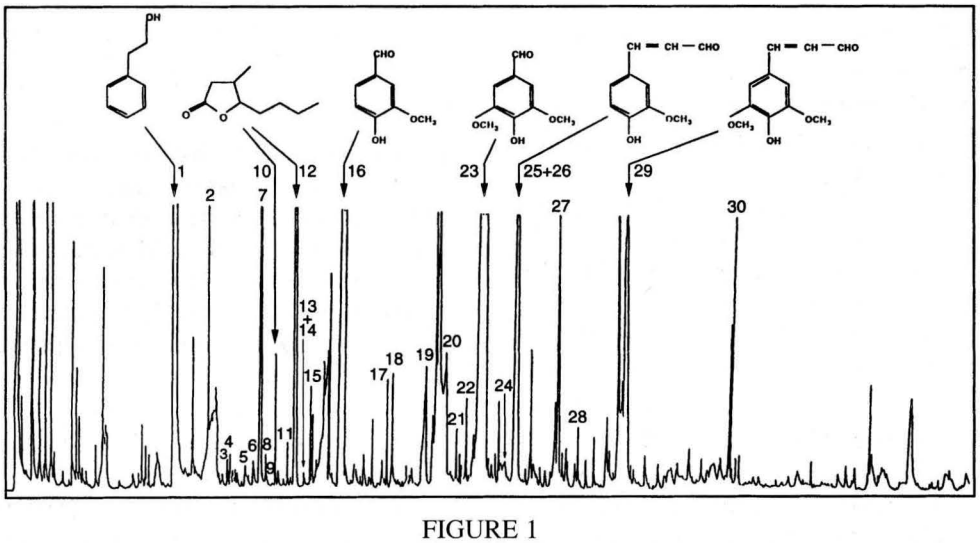

FIGURE 1

GC-MS trace on a high temperature apolar column of an extract from the fraction 5 of a 10-year-old whiskey. Peak identifications in Table 1. Conditions as in text.

TABLE 1

Compounds identified in aged whiskey fraction 5 after vacuum fractional distillation.

Peak No.	Compound	Retention Index on Xti5[a]
1	2-Phenyl ethanol	1122
2	Diethyl succinate	1186
3	Ethyl octanoate	1197
4	4-Methylguaicol	1208
5	4-Ethylphenyl acetate	1255
6	2-Phenylethyl acetate	1268
7	Diethyl malate	1271
8	Pentanedioic acid diethyl ester	1281
9	Ethyl nonanoate	1296
10	ß-Methyl-γ-octalactone, *cis*	1302
11	4-Vinyl guaicol	1330
12	ß-Methyl-γ-octalactone, *trans*	1339
13	2,6-Dimethoxyphenol	1362
14	Eugenol	1378
15	Ethyl decanoate	1396
16	Vanillin	1398
17	Ethyl vanillyl ether	1464
18	Acetovanillone	1484
19	Ethyl-9-oxononanoate	1510
20	Ethyl vanillate	1587
21	4-Allyl-2, 6-dimethoxyphenol	1615
22	Ethyl homovanillate	1645
23	Syringaldehyde	1661
24	Nonanedioic acid diethyl ester	1689
25	Acetosyringone	1720
26	Coniferaldehyde	1730
27	Ethyl syringate	1840
28	Ethyl homosyringate	1886
29	Sinapaldehyde	1979
30	Ethyl hexadecanoate	2005

[a] Temperature programmed retention indices.

4 years under an oxygen headspace (Nishimura *et al.*, 1983). Figure 2 shows structures and mass spectra for these compounds.

Formation profiles of wood originating compounds

Increases in the concentrations of 16 compounds from Table 1, originating directly from wood or its lignin breakdown, were monitored over 10 years of ageing. Triplicate assays were performed on fraction 5 from the composite whiskeys at 1.5, 3, 5 and 10 years old. The mean amount for each compound at the different ages, relative to the known amount of added internal standard, is outlined in graphical form in Fig.3. For each data point the % standard deviation from the triplicate analyses is also indicated.

These graphical data indicate that the concentrations of these compounds increased over time. The extraction of the *cis* and *trans* ß-methyl-γ-octalactone is nearly linear and this agrees with similar data for a model wood/alcohol system (Maga, 1989). The lactones have been found to be correlated to positive assessment of whiskey quality (Otsuka *et al.*,1974), and the flavour is described as sweet, woody and coconut-like. Eugenol is characteristic of oak-matured products and imparts a clove-like flavour (Masuda & Nishimura, 1971; Mosedale & Puech, 1998).

Maturation of distilled spirits in oak barrels is a complex process and much work has been carried out to elucidate the various mechanisms involved (Reazin,1981; Nishimura *et al.*, 1983). In the present study the whiskey was matured for ten years in second-fill heavy-charred American oak barrels used for ageing of Bourbon. This particular combination of wood type, treatment and barrel history will directly influence the amounts and relative levels of the compounds produced and, therefore, the ageing flavour of the product. Charring produces aerobic and anaerobic pyrolysis reactions in which the oak lignin is degraded in the layer immediately under the charcoal, releasing flavour compounds such as vanillin into the spirit (Philp, 1989; Singleton, 1995). In contrast toasting induces less burning and involves more darkening of the wood rather than pyrolitic or thermal degradation. The isomeric methyl octalactones are present in unheated oak wood but charring can significantly increase the amounts formed from thermal oxidation of precursors in the wood (Otsuka *et al.*, 1974; Maga, 1989).

In a study involving oak cask staves from charred Bourbon barrels it was shown that, with successive re-use for spirit maturation, the maxima for phenolic aldehydes and lactones were shifted after the first maturation from 5 mm below the char to the char layer. This suggests migration of these compounds from the interior to the spirit (Conner *et al.*, 1993). An important consequence is that barrels for re-use will provide correspondingly decreasing amounts of these compounds as successive maturation cycles will deplete the wood of aromatic aldehydes and lactones. In this respect use of second-fill heavy-charred barrels represents an intermediate treatment situation between charring and toasting. The amounts and ratios of the compounds produced will be different from either new charred or toasted wood and this is reflected in the patterns in Fig. 3. While in this study similar formation profiles have not been established for whiskey from new heavy-charred American oak barrels, literature data for whiskey from such casks indicates substantially higher amounts of aromatic phenolic aldehydes in comparison to second-fill equivalents (Baldwin *et al.*, 1967).

Mechanisms for production of these aldehydes have been postulated (Baldwin *et al.*, 1967; Guymon & Crowell, 1972; Reazin, 1981). In a study involving aqueous ethanol extraction of charred or toasted oak chips over twelve days, it was shown that toasting or charring produces aromatic aldehydes from lignin (Nishimura *et al.*, 1983). In similar treatment of the uncharred oak chips none or only trace amounts of these compounds were detected. On the other hand, the uncharred oak chips did have positive levels of these compounds after six months of storage, and this means that an additional mechanism unrelated to charring is in operation for production of these compounds. This procedure involves initial production of a complex of lignin and ethanol in which ethanol acts as both a solvent and a reactant, and the mild acidic hydrolysis of this complex to produce the aromatic phenolic compounds is termed ethanolysis (Puech *et al.*,1977). This ethanol lignin compound has been isolated and found to increase with whiskey ageing (Reazin, 1981). This differs fundamentally from classical ethanolysis in which oak wood is treated with boiling absolute alcohol for 48 hours in the presence of 2-3% hydrochloric acid

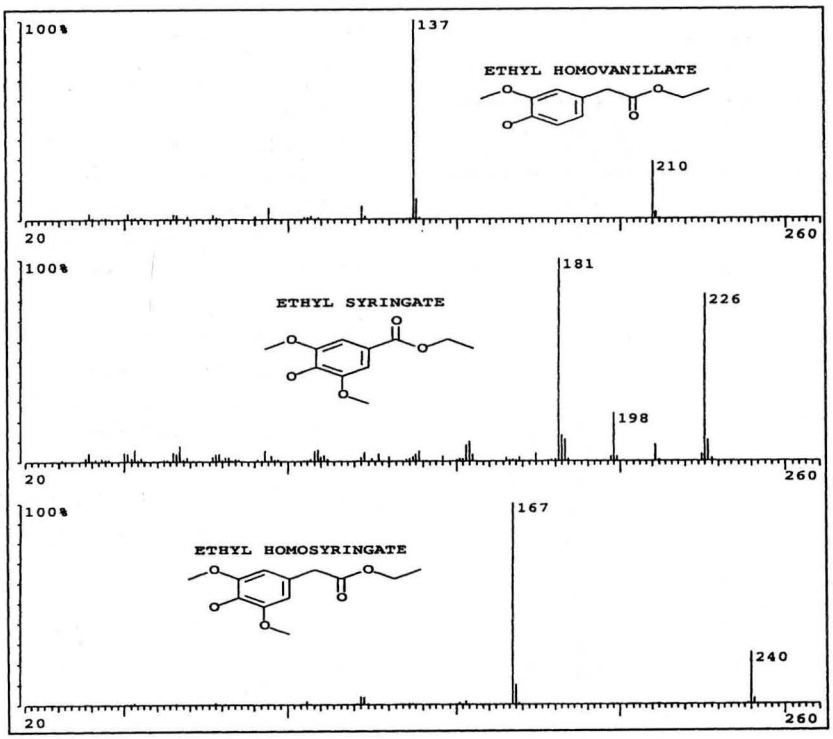

FIGURE 2
Mass spectra of new phenolic esters identified in whiskey.

(Deibner, *et al.*, 1976; Puech *et al.*, 1977; Puech, *et al.*, 1978). Therefore compounds produced during maturation can result from contributions from both charring and ethanolysis. All whiskeys are matured in charred casks, whether new or used, and will therefore be characterised by higher levels of pyrolysis products and lignin degradation compounds than are formed in Cognac and other brandies that are stored in casks subjected to less intense heating (Sarni *et al.*, 1990). In the case of whiskey from a previously used charred cask, acidic ethanolysis is thought to be the major route for formation of lignin breakdown products (Nishimura *et al.*, 1983). In the same study (Nishimura *et al.*, 1983), which involved soaking of differently treated wood in 60% ethanol, levels of aromatic phenolic compounds were much lower in the uncharred wood sample, and very different ratios of aromatic aldehydes were found depending on whether the wood was charred (lignin pyrolysis mechanism) or uncharred (ethanolysis mechanism). The ratio of syringaldehyde/vanillin remained constant, but for the charred wood the ratio of syringaldehyde/sinapaldehyde was 66% lower, and that of vanillin/coniferaldehyde 80% lower, in comparison to the uncharred wood. This again supports the suggestion that whiskey from a second-fill barrel will have an ageing flavor which will be a balance between pyrolysis and acidic ethanolysis reactions. A study involving extraction of oak hardwood shavings by 60% ethanol allowed identification of a range of carbohydrates and carboxylic acids (Nykänen, *et al.*, 1984). Neither the isomeric methyl octalactones or aromatic phenolics were reported and this is most likely due to the lack of any wood charring or toasting.

Wood species is also an important variable for the ageing flavour of distilled spirits (Chatonnet & Dubourdieu, 1998). American white oak (*Quercus alba*) contains higher quantities of the *cis* and *trans* isomers of ß-methyl-γ-octalactone and lower quantities of extractable polyphenols than either sessile oak (*Quercus petreae*) or pendunculate oak (*Quercus robur*), the two most commonly used European species (Mosedale, 1995). Even among the European species studies have shown that *Quercus petraea* has levels of methyl octalactone similar to American oak, while *Quercus robur* has high levels of ellagitannins and very low levels of octalactone (Mosedale, 1995; Chatonnet & Dubourdieu, 1998). Cognac and Armagnac are matured almost almost exclusively in Limousin oak, which is predominately *Quercus robur*. The whiskey used in this study was aged in once-used American oak Bourbon barrels.

An explanation for the rate of increase in concentrations of some compounds in Fig. 3 could be reactions subsequent to extraction as was suggested for such compounds present in an aged solution of 60% ethanol and lignin-related compounds (Nishimura *et al.*, 1983). In the case of esters (ethyl vanillate, ethyl syringate, etc) an interpretation can be initial solubilisation of the corresponding acid directly from the wood followed by esterification in the ethanol solution. Amounts of the cinnamic aldehydes are also much lower than amounts of vanillin and syringaldehyde. This is in agreement with other studies (Puech, 1981), but contradicts reports on Russian brandy (Skourikhin & Efimov, 1968).

Table 2 details the ratio of syringaldehyde to vanillin and the ratios of the syringyl type compounds syringaldehyde and sinapaldehyde, and the guaiacyl compounds vanillin and coniferaldehyde over the ten years of ageing.

The ratio syringaldehyde/vanillin varied between approximately 1.5 and 1.7 over ten years, and this is similar to a range of 1.6 to 2.0 found in aged Cognac over fifty years (Puech *et al.*, 1984). The

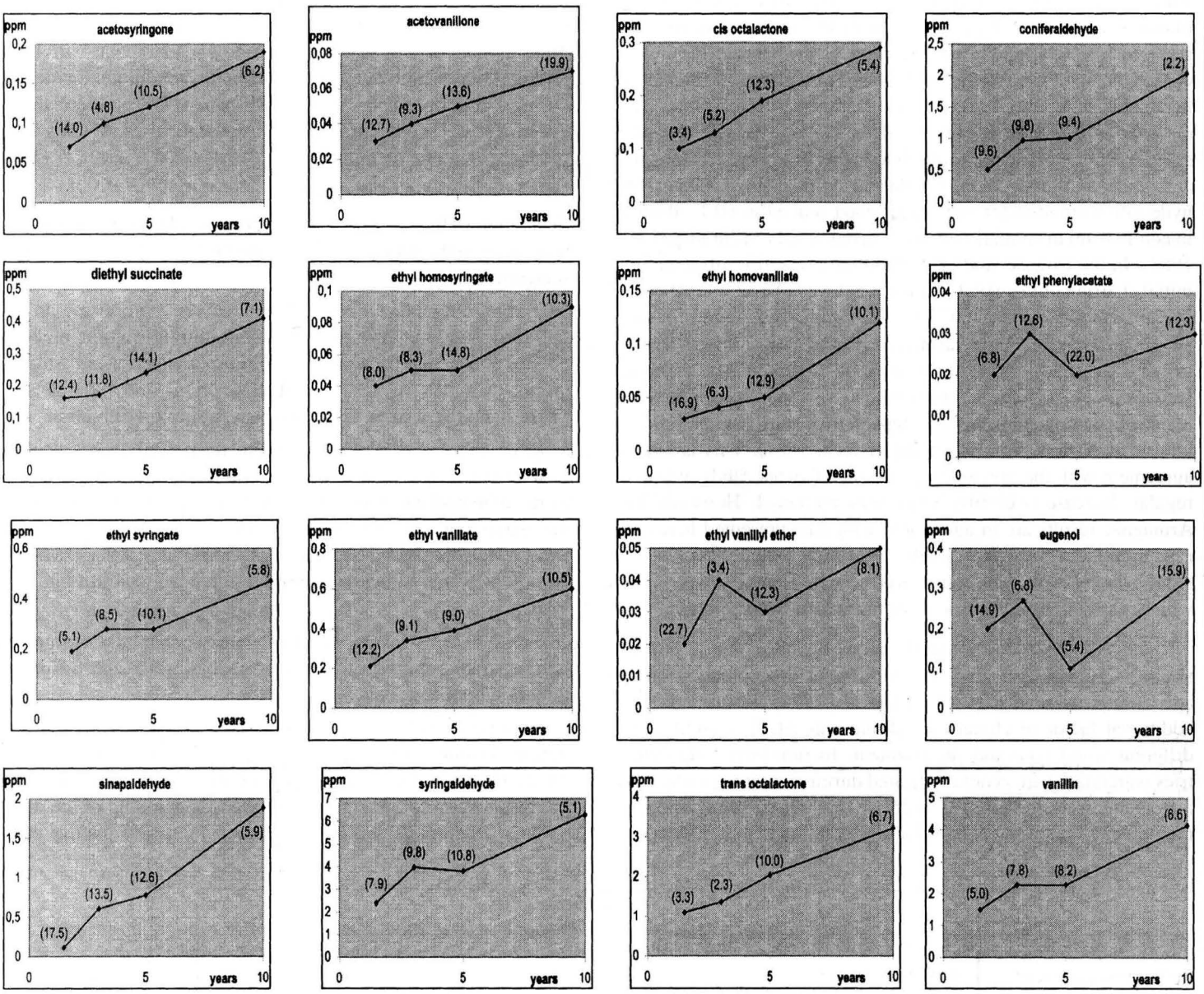

FIGURE 3

Increase in concentrations[1)] of oak-derived aroma compounds in whiskey during ageing[2)].

[1)] Average of triplicate analysis relative to an internal standard. Percentage standard deviation indicated for each average amount.

[2)]Each sample represents a composite of 12 similarly distilled and aged whiskeys.

sharp decrease in the syringaldehyde/sinapaldehyde ratio from 1.5 years to 3 years can be attributed to a much higher relative increase in the sinapaldehyde level over the same period. The ratios syringaldehyde/sinapaldehyde and vanillin/coniferaldehyde have been reported to increase with ageing in both Bourbon (Nishimura *et al.*, 1983), and Cognac (Puech *et al.*, 1984), and this has been attributed to oxidation of the cinnamic double bond in coniferaldehyde and sinapaldehyde with conversion to vanillin and syringaldehyde, respectively. This trend has not been observed in this study and the relevant ratios decrease regularly over the time of the study rather than increase. This could be partly due to a unique balance of compound extraction mechanisms in operation for the particular once-used barrels employed for the present

TABLE 2

Ratios of some aromatic aldehydes in whiskey over ten years of ageing in once-used Bourbon barrels.

Compound	Age			
	1.5 years	3 years	5 years	10 years
Syringaldehyde/ Vanillin	1.6	1.7	1.7	1.5
Syringaldehyde/ Sinapaldehyde	21.7	6.5	4.9	3.3
Vanillin/ Coniferaldehyde	3.0	2.3	2.2	2.0

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 comparasion 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-

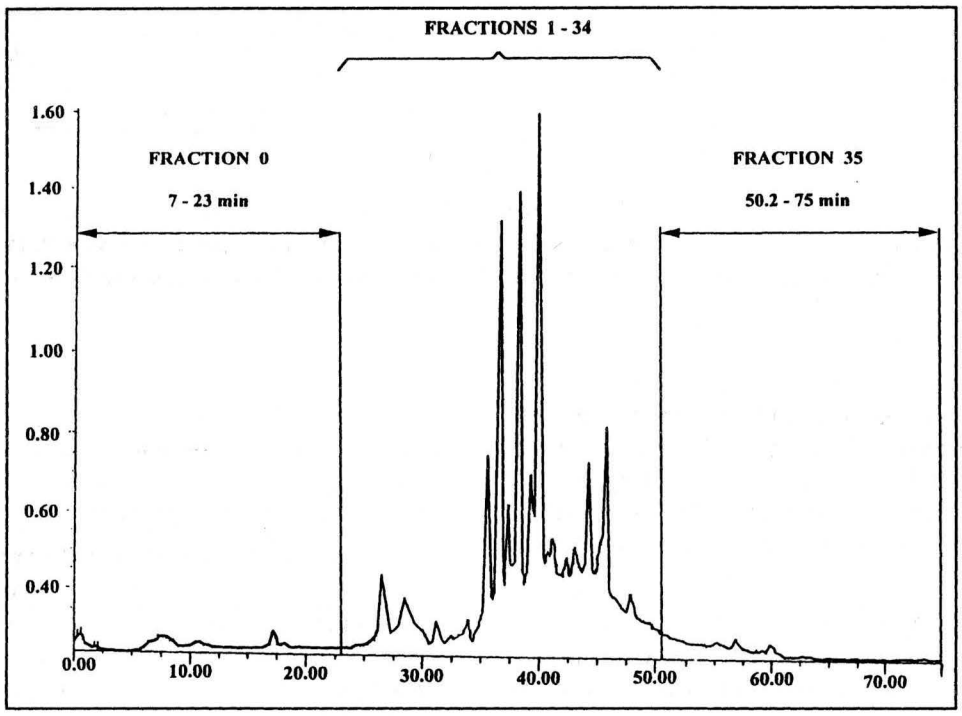

FIGURE 4

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

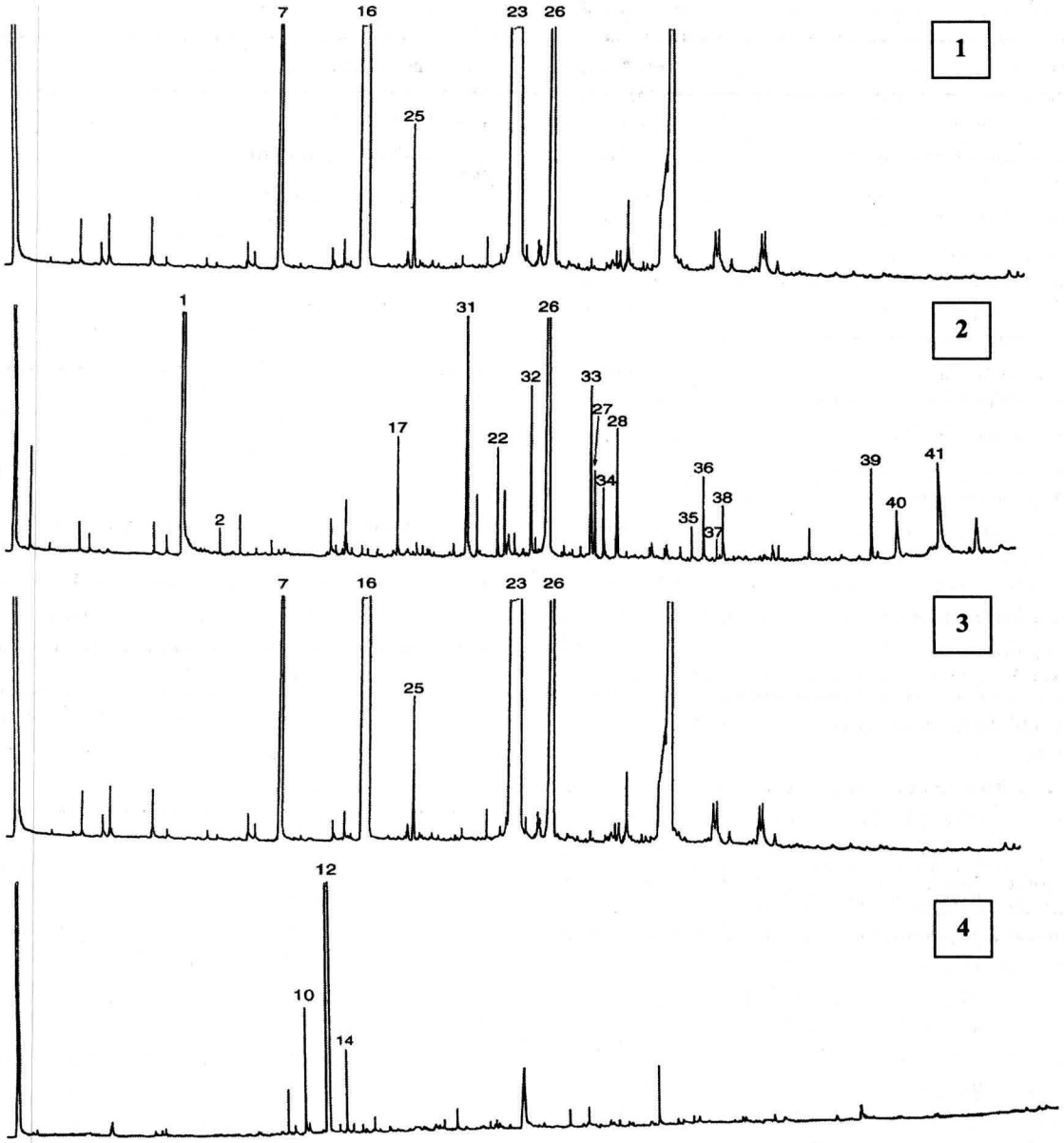

FIGURE 5

GC-MS traces [1–4] of extracts of composites after preparative HPLC separation of concentrated fraction 5 from 10-year-old whiskey. [1] HPLC cuts 1-20; [2] HPLC cuts 21-25; [3] HPLC cuts 26-30; [4] HPLC cuts 31-34. Peak identification in Tables 1 and 3.

pounds by reverse phase polarity trapping. This was followed by a gradient elution analysis to separate the flavour compounds. This approach suffers from the disadvantage that no pre-separation of compounds (e.g. higher alcohols) is used to simplify subsequent chromatography and may not offer sufficient concentration and enrichment for detection of trace levels of compounds associated with ageing character.

Additional compounds found in composites

The subsequent GC-MS analysis of the separate composite extracts allowed the additional compounds in Table 3 to be characterised (peaks 31 to 41 in Fig. 5).

Many of these compounds have major mass spectral ions at m/z 151 and/or m/z 181, which represent the molecular ions of vanillin and syringaldehyde, respectively. This indicates that they all probably have as their origin lignin breakdown pathways and associated reactions over time. Many also share similar fragmentation patterns. Propiovanillone and 2-methylpropiovanillone have previously been reported as constituents in oak matured wine (Etievant, 1981 ; Guntert *et al.*, 1986). Propiovanillone was also identified after direct extraction of oak wood chips (Nishimura *et al.*, 1983). The tentative structures for peaks 35 and 36 both have a principal loss of m/z 73 similar to butyl vanillate. Peaks 36 and 38 are probably isomers as both have almost identical mass spectra. Since the mass spectral data for the unidentified compounds in Table 3 and Fig. 5 indicate compounds from lignin breakdown, it is reasonable to assume that they also

TABLE 3

Additional compounds found in aged whiskey after distillation and preparative liquid chromatography.

Peak no.	Compound	Ret.[a] Index	Mass Spectral Data [c]
31	Propiovanillone [b]	1609	151(100), 180(55, M^+), 123(49), 108(25), 52(17), 65(16), 77(13), 51(10)
32	Homosyringyl ethyl ether [b]	1714	167(100), 168(57), 212(47, M^+), 123(23), 153(20), 95(15), 107(13), 77(12), 53(11)
33	Propiosyringone [b]	1850	181(100), 210(43, M^+), 182(20), 153(18), 67(13), 108(13), 123(12), 138(10)
34	Butyl vanillate [b] (principal loss of m/z 73)	1874	151(100), 123(17), 152(11), 149(10), 224(4, M^+)
35	2-Ethoxy-(4 hydroxy-3,5 [b] dimethoxy-phenyl)-ethyl acetate	2035	211(100), 123(42), 95(16), 212(12), 140(10), 155(10), 167(9), 284(9, M^+)
36	3-Ethoxy-3(4-hydroxy-3-methoxy phenyl) methyl propanoate [b]	2064	181(100), 182(18), 153(14), 67(11), 123(10), 108(10), 254(9, M^+)
37	Vanillic acid derivative	2093	151(100), 207(11), 123(10), 152(9), 252(6, M^+)
38	Possible isomer of peak 36 (principal loss of m/z 73)	2108	181(100), 182(16), 154(21), 179(15), 153(12), 254(9, M^+)
39	Syringic acid derivative	2493	182(100), 85(96), 167(85), 181(72), 81(54), 83(40), 57(26), 154(25), 168(25), 237(17), 310(11, M^+)
40	Vanillic acid derivative	2567	151(100), 123(18), 274(11, M^+), 108(9), 152(8), 243(6)
41	Unknown	2694	272(100, M^+), 211(24), 168(20), 136(19), 197(17), 273(17), 207(15)

[a] Temperature programmed retention indices.

[b] Tentative structure.

[c] Relative abundance in brackets. Suggested molecular ion is the highest mass detected in the electron impact mass spectrum.

increase with time either through extraction from the wood or subsequent reactions in the aqueous ethanol medium.

A series of similar compounds not found in this study has been produced either by heating of oak wood with absolute alcohol in the presence of hydrochloric acid (Puech, 1984), or after pyrolysis of plant and forage material (Ralph & Hatfield, 1991). Examples are 2-hydroxypropiosyringone, vanilloylmethylketone, α-ethoxypropiovanillone and syringylmethylketone. However, the first procedure, as was discussed earlier, constitutes classical ethanolysis, which represents an extreme treatment in comparison to the mild acidic ethanolysis which occurs during natural spirit maturation and would be expected to produce different wood chemical breakdown pathways (Puech, *et al.*, 1978). Pyrolysis of plant material represents a situation more similar to heavy-charred new Bourbon casks than to the once-used casks used in the present study. In new charred barrels the main mechanism for production of aromatic compounds is thermal degradation of the wood, whereas in the once-used variety mild acidic ethanolysis is probably the dominant route (Nishimura *et al.*, 1983).

Sensory investigation of sub-fractions

Since an ethanol-water gradient was used for the HPLC separation of the fraction 5 extract, it was possible to examine the resulting composites for aroma and taste. Table 4 summarises the opinions of an experienced whiskey taste panel.

The compound types that have been partitioned into the different composites generally support the descriptions. The phenolic aldehydes, phenyl ethyl alcohol, and the isomeric lactones were partitioned into composites 1, 3 and 4, respectively. The sensory characteristics of these compounds are well documented and were reflected in the assessors' comments. None, or only trace amounts, of these dominant aroma-contributing compounds partitioned into composite 2, and subsequently allowed the indicated positive maturation characteristics to be assigned to composite 2 without interference or masking from other compounds.

Effect of phenolic esters on young whiskey aroma

The interesting composite 2 contained the three phenolic esters in addition to the compounds described in Table 3. Therefore it was decided to investigate the effect of addition of these three esters to a young whiskey to determine if maturation character increased.. Control samples were the original 3-year- and 10-year-old whiskeys. Initially sixteen judges were used, but in an initial screening judges who were not able to detect the 10-year-old product or those who did not rate the 10-year-old highest in maturation characteristics were excluded. Table 5 represents the results of the tasting after three months using the twelve remaining judges.

The maturation intensity of the 3-year-old whiskey, as well as the same sample spiked with two levels of the three phenolic esters (amounts found in the 10-year-old whiskey and double this level), were ranked significantly lower than that of the 10-year-old whiskey. This illustrates that these three esters, even at double the level found in a 10-year-old whiskey, did not account for the higher maturation odour intensity of the 10-year-old product. However, at double the level found in the 10-year-old whiskey, they caused a significant increase in the maturation odour intensity of the 3-year-old whiskey. This intensification of the maturation odour to some extent demonstrates that these esters are in

TABLE 4

Description of HPLC composites by an experienced whiskey panel.

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

TABLE 5

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

Sample	Maturation intensity (Mean)
3-Year-Old Whiskey	38,85^{c}
3-Year-Old Whiskey+ Level 1 Spike	46,54^{bc}
3-Year-Old Whiskey + Level 2 Spike	49,54^{b}
10-Year-Old Whiskey	72,69^{c}

* 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 ageing changes during maturation of whiskey in second-fill heavy-charred Bourbon oak barrels. There is evidence to suggest that these ageing 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

Baldwin, S., Black, R., Andreasen, A. & Adams, S., 1967. Aromatic congener formation in maturation of alcoholic distillates. J. Agr. Food Chem. 15, 381 –385.

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

Conner, J., Paterson, P. & Piggott, J., 1993. Changes in wood extractives from oak cask staves through maturation of Scotch malt whiskey. J. Sci. Food Agric. 62, 169-174.

Deibner, L., Jouret, C. & Puech, J-L., 1976. Substances phénoliques des eaux-de-vie d'Armagnac.1.La lignine d'extraction et les produits de sa dégradation. Indust. Alim. Agric. 93, 401-414.

Etievant, P., 1981. Volatile phenol determination in wine. J. Agric. Food Chem. 29, 65-67

Fischer, H. & Hibbert, J., 1947. Studies on lignin and related compounds 1xxx111 – Synthesis of 3-hydroxy-1-(4-hydroxy-3 methoxyphenyl)-2- propanone. J. Am. Chem. Soc. 69, 1208 – 1210.

Guntert, M., Rapp, A., Takeoka, G. & Jennings, W., 1986. HRGC and HRGC-MS applied to wine constituents of lower volatility. Z. Lebensm. Unters. Forsch. 182, 200 – 204.

Guymon, J. & Crowell, E., 1972. GC-Separated brandy components derived from French and American oaks. Am. J. Enol. Vitic. 23, 114-120.

Kervegant, D., 1946. Rhums et eaux-de-vie de canne. Les Editions du Golfe. Vannes.

Lehtonen, P., 1984. Liquid chromatographic determination of phenolic aldehydes from distilled alcoholic beverages. In: Nykänen, L. and Lehtonen, P. (eds). Proceedings of the Alko Symposium on flavour research of alcoholic beverages, June 1984, Helsinki. Foundation for Biotechnical and Industrial Fermentation Research. pp. 121 – 130.

Macnamara, K., Van Wyk, C.J., Augustyn, O.P.H. & Rapp, A., 2001a. Flavour components of whiskey. I. Distribution and recovery of compounds by fractional vacuum distillation. S. Afr. J. Enol. Vitic. 22, 69-74.

Macnamara, K., Van Wyk, C.J., Augustyn, O.P.H. & Rapp, A., 2001b. Flavour components of whiskey. II. Ageing changes in the high volatility fraction. S. Afr. J. Enol. Vitic. 22, 75-81.

Maga, J., 1989. Formation and extraction of cis-and trans-b-methyl-g-octalactone from *Quercus alba*. In: Piggott, J. and Paterson, A. (eds). Flavour of Distilled Beverages. Ellis Horwood, Chichester, U.K. pp. 171 – 176.

Masuda, M. & Nishimura, K., 1971. Branched nonalactones from some *Quercus* Species. Phytochemistry 10, 1401 – 1402.

Mourgues, J., Jouret, C. & Moutounet, M., 1973. Détermination du taux d'oxygène dissous et du potentiel oxydo-réducteur des eaux-de vie d'Armagnac au cours de leur maturation. Ann. Technol. Agric. INRA 22, 75-90.

Mosedale, J., 1995. Effects of oak wood on the maturation of alcoholic beverages with particular reference to Whisky. Forestry 68, 203-230.

Mosedale, J. & Puech, J-L., 1998. Wood maturation of distilled beverages. Trends in Food Sci. and Technol. 9, 95-101

Nishimura, K. Masuda, M., 1971. Minor constituents of whiskey fusel oils. 1. Basic, phenolic and lactonic compounds. J. Food Sci. 36, 819 – 822.

Nishimura, K. & Matsuyama, R., 1989. Maturation and maturation chemistry. In: Piggott, J., Sharpe, R., Duncan, R. (eds). The Science and technology of whiskies. Longman, London. pp. 235 – 263.

Nishimura, K., Ohnishi, M., Masuda, M., Koga, K. & Matsuyama, R., 1983. Reactions of wood components during maturation. In: Piggott, J. (ed). Flavour of Distilled Beverages. Ellis Horwood, Chichester. UK. pp. 241 – 255.

Nykänen, L., Nykänen, I. & Moring, M., 1984. Aroma compounds dissolved from oak chips by alcohol. In: Adda, J. (ed). Progress in flavour research. Proceedings of the 4th Weurman flavour research symposium, May 1984, Dourdan, France. Elsevier, Amsterdam. pp. 339 – 346.

Otsuka, K., Zenibayashi, Y., Itoh, M. & Totsuka, A., 1974. Presence and significance of two diastereomers of ß-methyl-γ-octalactone in aged distilled liquors. Agr. Biol. Chem. 38, 485 – 490.

Philp, J., 1989. Cask quality and warehouse conditions. In: Piggott J., Sharpe, R., Duncan, R. (eds). The Science and technology of whiskies. Longman, London. pp. 264 – 294.

Piggott, J., Clyne, J., Patterson, A. & Conner, J., 1992. Preparative HPLC as a tool for concentrating flavour compounds from distilled beverages. In: Cantagrel, R. (ed). 1st Symposium Scientifique International de Cognac. Lavoisier – Tec and Doc., Paris. pp. 464 – 467.

Puech, J-L., Jouret, C., Deibner, L. & Alibert, G., 1977. Substances phénoliques des eaux de vie d'Armagnac et de Rhum. 2. Produits de la dégradation de la lignine: Les aldéhydes et les acides aromatiques. Indust. Alim.. Agric. 94, 483 – 493.

Puech, J-L., Jouret, C. & Deibner, L., 1978. Substances phénoliques des eaux-de-vie d'Armagnac 3. Sur la présence des éthoxyles dans la lignine d'extraction et les produits de sa dégradation. Indust. Alim. Agric. 95, 13-21.

Puech, J-L., 1981. Extraction and evolution of lignin products in Armagnac matured in oak. Am. J. Enol. Vitic. 32, 111-114.

Puech, J-L., 1984. Characteristics of oak wood and biochemical aspects of Armagnac ageing. Am. J. Enol. Vitic. 35, 77 – 81.

Puech, J-L., Leauté, R., Clot, G., Nomdedeu, L. & Mondiés, H., 1984. Evolution de divers constituants volatils et phénoliques des eaux de vie de cognac au cours de leur vieillissement. Sciences des Aliments 4, 65 – 80.

Ralph, J. & Hatfield, R., 1991. Pyrolysis GC-MS characterisation of forage materials. J. Agric. Food Chem. 39, 1426 – 1437.

Reazin, G., 1981. Chemical mechanisms of whiskey maturation. Am. J. Enol. Vitic. 32, 283-289.

Sarni, F., Moutounet, M., Puech, J-L. & Rabier, P., 1990. Effect of heat treatment of oak wood extractable compounds. Holzforschung 44, 461-466.

Skourikhin, J. & Efimov, B., 1968. Sur le mécanisme de la dégradation de la lignine dans l'alcohol de Cognac. Vinodel. Vinograd. 28, 8 – 12.

Singleton, V., 1995. Maturation of wines and spirits: Comparisons, Facts, and Hypotheses. Am. J. Enol. Vitic. 46, 98-115.

Suomalainen, H. & Nykänen, L., 1970. Investigations on the aroma of alcoholic beverages. Naeringsmiddelindustrien 23, 15 – 30.

Tanner, D. & Osman, J., 1987. Oxidative decarboxylation. On the mechanism of the potassium persulfate promoted decarboxylation reaction. J. Org. Chem. 52, 4689 – 4693.