Flavour Components of Whiskey. II. Ageing Changes in the High-Volatility Fraction

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The volatile compounds isolated from whiskey by fractional vacuum distillation were identified by two-dimensional capillary gas chromatography/mass spectrometry. Changing levels with ageing were quantified for the most abundant compounds by direct split injection of whiskeys on a gas chromatograph equipped with a flame ionisation detector. The ageing decreases in volatile sulfides were similarly determined using a sulfur chemiluminescence detector. Large volume headspace injection sufficiently reproduced the distillation enrichment to allow direct two-dimensional determination of similar ageing changes for other trace compounds. Seven compounds at µg/L and low mg/L levels were monitored and quantified.

Volatile compounds of low molecular weight can be powerful odorants with significant effect on sensory properties (Maarse, 1991). In whiskey the volatile compounds present after distillation are further modified during the ageing process in oak barrels. These changes are contributory to the accepted flavour improvement associated with maturation and their study is important for both commercial and scientific reasons.

Successful analysis of trace volatile compounds necessitates an approach which combines both enrichment of the volatiles and their isolation from other compounds (Jennings & Rapp, 1983; Maarse & Belz, 1985; Marsili, 1997). In this way subsequent chromatographic separation can be specifically tailored to the high-volatility range. Sample preparation techniques such as extraction, simple distillation, and simultaneous distillation-extraction simply act to isolate all compounds which can volatilise from an involatile matrix.

Preliminary isolation of volatiles from distilled spirits has been attempted in a number of ways. A preparative headspace approach has been described for aged cognac which used a seven-step tandem arrangement of porous polymer adsorption tubes to eliminate water (ter Heide, 1978). Ethanol vapour was retained by an additional diglycerol column. In a device coupling dynamic stripping with liquid-liquid extraction, an extract was obtained from wine showing a similar profile to static headspace analysis (Rapp & Knipser, 1980). A procedure for rum allowed the volatiles from a 1.5 L sample to diffuse at room temperature to a small flask cooled in a dry ice bath. After 36 h 0.33 mL of liquid was collected (Liebich *et al.*, 1970). A vacuum stripping approach to beer has been described in which the collected volatile fraction was further separated by a series of trap to trap fractionations at successively decreasing temperatures (Pickett *et al.*, 1976).

The above approaches are complicated in terms of equipment required and are time consuming. They also have not been generally used to monitor ageing changes in a sample series. In a previous paper a commercially available column distillation unit working under vacuum was described in which the volatiles from two litres of unaged or aged whiskey could be isolated in a convenient one-step operation as a discrete low boiling fraction (Mac Namara *et al.*, 2001). Using this approach the purpose of the present investigation was to identify and quantitatively monitor the changes in concentration of the highly volatile compounds of whiskey during ageing in heavy charred American oak wood barrels once used for the ageing of Bourbon. Efficient techniques for isolating and monitoring these compounds and their ageing changes are important commercially, as results can be used to assess the relative contribution of both different wood barrel types and wood barrels that have undergone a number of ageing cycles.

MATERIALS AND METHODS

Material

Whiskey, unaged and at three and six years old, was used for distillation and gas chromatographic investigation of the high volatility compounds. The unaged parent whiskey was at 65% vol/vol ethanol. The aged whiskeys were from standard once-used American bourbon barrels and were composites from similar casks at the same age. Natural evaporative loss of ethanol during ageing resulted in strengths of between 1 and 3% vol/vol lower than the unaged parent, depending on the age.

Sample preparation

The fractional distillation separation of whiskey used to isolate the high-volatility compounds has been described previously (MacNamara *et al.*, 2001). The high-volatility fraction 1 compounds from an unaged whiskey were analysed by two-dimensional gas chromatography (2D-GC) with mass spectrometric (MS) detection for compound identification. Quantitative changes in compounds that changed most were established for the various whiskeys without any sample pre-treatment. Separate procedures were used to quantify the different volatile groups in the whiskeys. Direct injection GC with flame ionisation (FID) detection was used for the quantitatively abundant compounds. A similar approach but with specific sulfur chemiluminescent

detection was used for volatile sulfur compounds. Large volume headspace injection with MS detection after 2D-GC was used for detection of other trace-level compounds.

Two-Dimensional Gas Chromatography

The 2D-GC system used for initial identification of the volatiles, and subsequent quantification after headspace injection, was constructed from two Hewlett-Packard 5890 Series 2 gas chromatographs and a Hewlett-Packard 5971 mass selective detector (Hewlett-Packard, Palo Alto, CA., USA). The columns were connected in the first oven through a heated interface line by a micro column switching device (Gerstel Gmbh, Mülheim, Germany) with a split connection to a monitor FID. The unwanted first column components were vented at the column-switching device by a mass flow controlled countercurrent flow. For transfer of a selected cut to the second column this flow is stopped for the duration of the transfer and the compounds of interest pass to the head of the second column, which is cooled by liquid nitrogen in the interface line. Rapid heating of the interface line "re-injects" the compounds for chromatography on the second column. All pressures before and after switching are quickly re-established by electronic proportional valves to give pulseless switching required for high-resolution capillary chromatography. A schematic of the system is presented in Fig. 1.

The pre-column separation was carried out on a polar CP-Wax 57 fused silica column (50 m x 0.32 mm i.d. x 1.17df, Chrompack, Middelburg, The Netherlands) using an oven temperature programme of 40°C (17 min) x 3°C/min to 200°C (10 min). The main column for separation of cuts transferred from the pre-column was an apolar Rtx-5 fused silica capillary (30 m x 0.32 mm i.d. x 3.0df, Restek, Bellefonte, PA., USA) with an oven temperature programme of –50°C (until after transfer of the selected cut) x 70°C/min to 60°C x 2°C/min to 80°C x 5°C/min to 250°C. Helium was used as carrier gas at 1 mL/min. All injections were in splitless mode to a programmed temperature vaporiser (PTV) (Gerstel Cis-3) equipped with a glass vigreux liner, which was heated immediately after injection according to the following programme, 40°C x 10°C/sec to 200°C. Temperature programmed retention indices were calculated after similar injection of a mixture of C6 to C10 alkanes. The MS detector after the main column was operated in scan mode, 25 to 200 amu, at 1600 EV. Three cuts (1-16 min, 15-20 min, 19-28 min) covering the elution of the fraction 1 volatiles on the pre-column were individually separated on the main column. The slight overlap was to ensure transfer of all compounds during the three consecutive analyses.

Headspace injection

This analysis consisted of five replicate 1 mL headspace injections from a vial of each whiskey reduced to 10% vol/vol ethanol. Injections were made to a PTV (Gerstel Cis-3) capable of being cooled to trap and enrich volatile compounds on the liner. The headspace unit was a multi-purpose sampler (Gerstel MPS) equipped with a 1 mL gas-tight syringe. Vial contents were thermostatted at 60°C for 10 min. The PTV liner was packed with 15-20 mg of 50-80 Porapak Q and held in place by two small plugs of deactivated glass wool to give a bed length of 4 cm. The injector had a split flow of 60 mL/min and was cooled to -75°C during injection using liquid nitrogen. After headspace injection the PTV changed to splitless mode for heated transfer of the enriched compounds to the pre-column and used the following programme, -75°C x 10°C/sec to 180°C, (10 min). Two-dimensional chromatography then proceeded as previously described, except that after the second column an additional micro crosspiece (Gerstel Gmbh) was installed for simultaneous MS and FID detection. The former was used for spectral confirmation of the compounds of interest, which were then quantified using the FID signal. For each whiskey headspace run two cuts (1–16 min and 16–30 min) covering the elution of the compounds to be quantified were consecutively separated on the main column after separate injections. Two compounds were quantified from cut 1 and five compounds from cut 2. Quantification was by external standardisation using pure compounds in 65% ethanol and three-point calibration curves. The individual compound solutions were reduced to 10% ethanol before headspace injection.

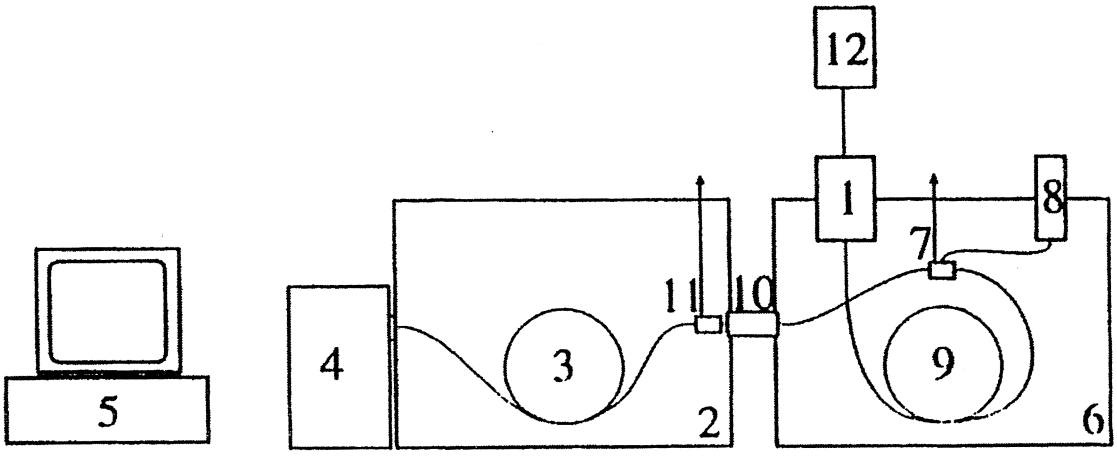

FIGURE 1

Two-dimensional GC configuration. 1: Programmed temperature vaporising injector. 2: Main GC. 3: Main column. 4: Mass selective detector. 5: PC Chemstation. 6: Pre-column GC. 7: Column switching device. 8: Monitor FID detector. 9: Pre-column. 10: Heated interface. 11: Liquid nitrogen trap. 12: Headspace injector.

Gas chromatography with flame ionisation detection

A Hewlett Packard 5880A GC was used for the direct determination of the most abundant volatile compounds in whiskeys of various ages. Separation was performed on a chemically bonded CP Wax 57 fused silica capillary column (50 m x 0.25 mm i.d. x 0.25 df, Chrompack). The injector port temperature was 200°C and the detector temperature 220°C. Hydrogen was used as carrier gas at 16 psi constant pressure to give a flow rate of 1.5 mL/min. The oven temperature was 40°C (5 min) x 5°C/min to 200°C (10 min). 1 µL of each sample was directly injected using a 1/50 split ratio. For compound quantification 4 – methyl-2-pentanol was used as internal standard with two levels of calibration using pure compounds (Fluka, Buchs, Switzerland) in an ethanol water solution.

Gas chromatography with sulfur chemiluminescent detection

A Hewlett Packard 5890 Series 2 GC equipped with a Sievers 350B sulfur chemiluminescence detector (Sievers Inc., Boulder, Colorado, USA) was used to determine dimethyl sulfide and dimethyl disulfide in aged and unaged whiskeys. Separation was performed on a chemically bonded CP Wax 57 thick film fused silica capillary column (50 m x 0.32 mm i.d. x 1.17 df, Chrompack). The injector was a PTV (Gerstel CIS 3), 40°C x 10°C/sec to 200°C. Helium was used as carrier gas at a flow rate of 1.5 mL/min and 1 µL of each whiskey was directly injected in splitless mode to a glass liner with a 1 min purge delay. The oven temperature was 40°C (2 min) x 3°C/min to 180°C (10 min). The detector was operated at 800°C using 8 mL/min oxygen and 100 mL/min hydrogen for plasma generation in the burner. Ozone for chemiluminescence of the resultant sulfur monoxide was generated from pure oxygen. Three-point calibration curves were obtained using pure compounds in 65% ethanol solutions. Ethyl methyl sulfide at a concentration of 41µg/L was used as internal standard and all compounds were Fluka Purum grade.

RESULTS AND DISCUSSION

Identification

Fig. 2 shows the monitor FID trace from injection of fraction 1 to the polar pre-column. The trace consisted of volatile compounds eluting before and just after the ethanol peak. The distillation had concentrated the volatiles to such a degree that resolution, even on this relatively thick film column with a phase ratio ß = 68, was poor. (The phase ratio of a wall-coated open tubular capillary column is a measurement of the "openness" of the tube and is a function of the inner tube radius and the liquid phase film thickness).

The selected cuts indicated in Fig. 2 cover the entire elution range on the first column.

Cuts slightly overlapped to ensure transfer of all components for separation on the main column. Figure 3 a, b, and c shows the MS total ion traces of these cuts after transfer, liquid nitrogen focusing and elution from the apolar thick film (phase ratio ß = 27) main column.

The two-dimensional chromatography on thick film columns with dissimilar phases provided substantial additional resolution and allowed detection of minor compounds overlapped by the ethanol and major volatiles on any single chromatographic phase (Cortes, 1990). Cut 1 transferred the compounds eluting up to the appearance of ethanol. Cuts 2 and 3 transferred compounds eluting under and on the tail of the ethanol peak. For these later cuts an MS solvent delay was used until after elution of ethanol. Since the apolar main column separated principally by molecular weight, all the species of interest could be detected after this solvent delay. The compounds were identified on the basis of their electron impact mass spectra using spectrum libraries (ten Noever de Brauw *et al.*, 1982), and spectra of authentic compounds as reference. The two-dimensional chromatography allowed a total of 28 compounds to be identified. These compounds with retention indices on the apolar column are listed in Table 1.

Quantification procedures

Two-dimensional fraction 1 screening on different whiskeys indicated those compounds whose concentrations changed most with ageing. Low boiling sulfides decreased while aldehydes and ethyl and acetate esters increased. Analytical procedures in turn were matched to the concentrations and functionality of the compounds of interest as follows:

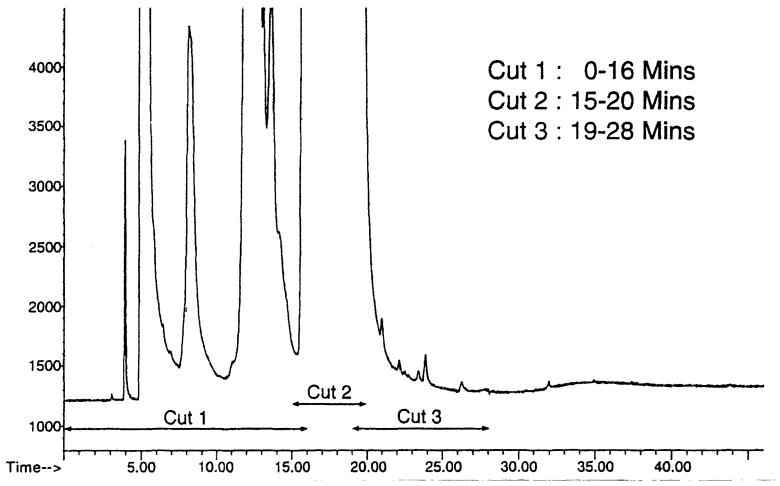

FIGURE 2

Thick film pre-column gas chromatogram of fraction 1 into three cuts for transfer to main apolar column.

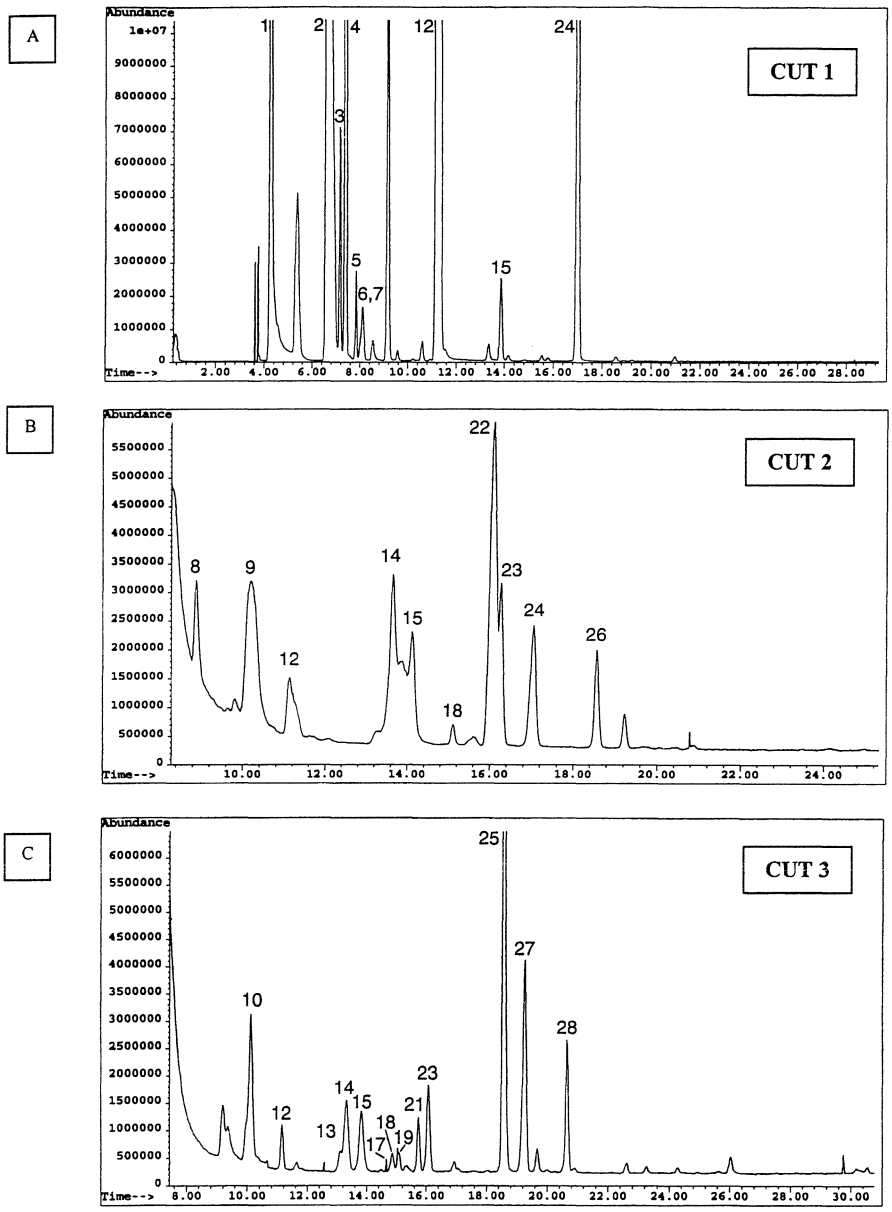

FIGURE 3

Fraction 1 cuts on apolar main column. A. Main column trace from 0 – 16 mins cut 1 on pre-column. (Fig.2). B. Main column trace from 15 – 20 mins cut 2 on pre-column.(Fig.2). C. Main column trace from 19 – 28 minscut 3 on pre-column.(Fig.2). Mass spectrometric detection. Peak identifications in Table 1. Conditions as in text.

- The abundant compounds acetaldehyde, acetaldehyde diethyl acetal and ethyl acetate were directly quantified in the whiskeys by split capillary GC-FID with internal standardisation.
- Dimethyl sulfide and dimethyl disulfide were similarly quantified, but using splitless capillary GC and sulfur chemiluminescence detection.

For remaining trace compounds some form of enrichment was necessary and in an initial attempt volatile internal standards were added to the whiskey before distillation to standardise recovery of volatiles into fraction 1. This was unsatisfactory because a different internal standard was needed for each consecutive cut, and main column co-elution of internal standards and compounds of interest was a problem. Substituting direct large volume headspace for the distillation enrichment was found to give adequate sensitivity for these compounds and allowed external standard quantification. Since only light volatiles were enriched by headspace, an advantage was that no higher boiling compounds from the whiskey were transferred to the precolumn. Cuts 2 and 3 from the fraction 1 qualitative investigation were collapsed into a single cut to fully recover each compound of interest for transfer to the main column.

Changes in major volatile compounds with ageing

The whiskeys in question came from a small traditional distillery, where uniformity of both the fermented product prior to distillation and the distilled unaged whiskey is well documented. The

TABLE 1

Compounds identified in the volatile fraction 1 after two-dimensional gas chromatography on dissimilar phases.

Peak no.	Compound	Retention Index[a]
1	Acetaldehyde	461
2	Ethanol	513
3	Acetone	522
4	Ethoxyethene	528
5	Ethyl formate	537
6	Dimethyl sulfide	539
7	Methyl acetate	543
8	Isobutyraldehyde	568
9	2,3 Butanedione	590
10	2-Butanone	597
11	3-Methyl furan	603
12	Ethyl acetate	610
13	2-Butenal	651
14	3-Methyl butanal	655
15	2-Methyl butanal	665
16	Formaldehyde diethyl acetal	670
17	Thiophene	679
18	2-Pentanone	688
19	2,4-Pentanedione	690
20	2-Ethyl furan	699
21	5-Methyl thioacetate	703
22	Ethyl propionate	710
23	Propyl acetate	714
24	Acetaldehyde diethyl acetal	725
25	Dimethyl disulfide	754
26	Ethyl isobutyrate	759
27	Isobutyl acetate	770
28	Ethyl butyrate	797

[a] Temperature Programmed Retention Indices

TABLE 2

Changes in major volatile compounds with ageing.

Compound[a]	Whiskey		
	0 years	3 years	6 years
Acetaldehyde	36	53	99
Ethyl acetate	148	411	523
Acetaldehyde diethyl acetal	61	101	158

[a] Amounts in mg/L at absolute alcohol.

TABLE 3

Changes in volatile sulfides with ageing.

Compound[a]	Whiskey		
	0 years	3 years	6 years
Dimethyl sulfide	446	29	Traces
Dimethyl disulfide	462	79	20

[a] Amounts in µg/L at absolute alcohol.

subsequent maturation process is also highly standardised. In view of this production uniformity the observed magnitude of the differences in concentrations of the major volatile compounds can in fact be attributed to the effect of maturation and not simply be regarded as normal fluctuations in the sample. Table 2 shows the levels of three major volatile compounds in whiskey samples of respectively 0,3 and 6 years old. These levels clearly show an increase with ageing for each of these compounds.

Similar increases in bourbon whiskey are recorded (Reazin *et al.*, 1976; Reazin, 1981) and the mechanism involved has been described by the same workers. By adding a small amount of radioactive ethanol to a whiskey at the start of ageing, they found over a 56-month period that this radioactivity is incorporated into acetaldehyde, ethyl acetate and acetic acid. The mechanism involves oxidation of ethanol by molecular oxygen to produce acetic acid via acetaldehyde. Excess ethanol combines with acetic acid to produce ethyl acetate, and with acetaldehyde to produce diethyl acetal (Reazin, 1981). The equilibrium between aldehydes and their acetals is important from the odour aspect (Perry, 1986). Aldehydes can be sour and pungent, while acetals are pleasant, fruity and contribute to the flavour of whiskey (Nykänen & Suomaleinen, 1983). The concentration of diethyl acetal produced during ageing is dependent on the ethanol strength and is significant down to 40% v/v ethanol (Perry, 1986). It has been pointed out that an important secondary effect of diethyl acetal is its corresponding contribution to a decrease in acetaldehyde (Simpson, 1979). Substantial data are available on the individual sensory contributions of these volatile compounds (Salo *et al.*, 1972; van der Merwe & van Wyk, 1981). Since the isolated fraction 1 from six-year-old whiskey was clearly less harsh than unaged or younger whiskey, the overall contribution with ageing is positive despite the negative effect of acetaldehyde increase.

Changes in volatile sulfides with ageing

Decreases in volatile sulfides were quantified for the same set of samples (Table 3).

The dimethyl sulfide (DMS) content of unaged whiskey was approximately 15-fold greater than in a whiskey aged for 3 years. This represents a non-linear decrease with most loss occurring in the first year. A similar amount of dimethyl disulfide (DMDS) in unaged whiskey reduced by ca. 83% over the first three years. Similar results have been reported for Japanese whiskey (Masuda & Nishimura, 1981). Natural evaporation is a factor in the decrease in these compounds, but oak wood is also necessary for their removal (Nishimura *et al.*, 1983). Wood hydrolysable tannins are implicated in removing sulfides. The mechanism postulated is that in aqueous medium the oxidation of gallic acid produces hydrogen peroxide, a very reactive molecule that can efficiently oxidise sulfides (Wildenradt & Singleton, 1974). Because of their characteristic unpleasant odours these alkyl sulfides play an important role in the flavour of alcoholic beverages. Sensory thresholds of 35 µg/L for DMS and 5-7 µg/L for DMDS are reported for a 3% ethanol matrix (Haboucha *et al.*, 1982). In white wine a threshold of 25 µg/L for DMS is reported (Park *et al.*, 1994) and another study quotes 20 µg/L for DMDS in 10%

vol/vol ethanol solution (Leppanen *et al.*, 1979). The levels from Table 3 indicated therefore that these compounds were contributory in all probability to the odour of unaged whiskey and that this negative contribution apparently decreased during ageing. One study estimates average concentrations of DMS and DMDS in commercial whiskey at between 2 and 10 times their odour thresholds (Philp, 1986).

Changes in minor volatiles with ageing

The combination of large volume cryogenic headspace injection with two-dimensional chromatography allowed resolution and detection of low amounts of trace volatile compounds. Results are tabulated in Table 4 for the same samples as before.

Porapak Q was chosen as adsorbent based on previous trapping results with this packing (Peppard, 1984; Tuan *et al.*, 1995). The material is slightly polar and tends to trap a wide range of compounds efficiently. The additional cryogenic cooling to -75°C ensured complete retention of all compounds of interest from the headspace vapour. Triplicate 5 mL headspace injections of the lowest calibration level for ethyl formate and formaldehyde diethyl acetal gave relative standard deviations of 4.8% and 4.6%, respectively. Figure 4 shows the external standard regression line for ethyl butyrate from 0.2 to 2.2 mg/L.

The higher levels of ethyl formate and formaldehyde diethyl acetal are analogous to those for the increases for the similar acetaldehyde by-products (Table 2). The same trend is observed for the other trace ethyl esters and acetates. The fruity odours of these compounds are considered important contributors to aroma. In the case of wine, acetates are considered more important than ethyl esters of fatty acids for intensity and quality of aroma (van der Merwe & van Wyk, 1981). The same is likely for whiskey because of the low sensory odour threshold values of these compounds (Salo, 1970). Some compound levels from Table 4 reached maximum levels after 3 years, while others such as ethyl butyrate appeared to have continued to increase with ageing. Whiskey with higher levels of butyric acid and a resultant sour note correlates with higher levels of ethyl butyrate (Carter-Tijmstra, 1986). Ethyl butyrate has been reported as having a threshold value of 0.15 mg/L in 9.4% grain spirit (Salo *et al.*, 1972) and 0.4 mg/L in beer (Meilgaard, 1975), and can easily be detected at levels above 0.5 mg/L in rectified alcohol (Chialva *et al.*, 1984). The possible contribution of the higher levels of these minor ethyl esters, acetates and acetals may be enhanced by higher levels of the major volatiles and lower levels of the alkyl sulfides.

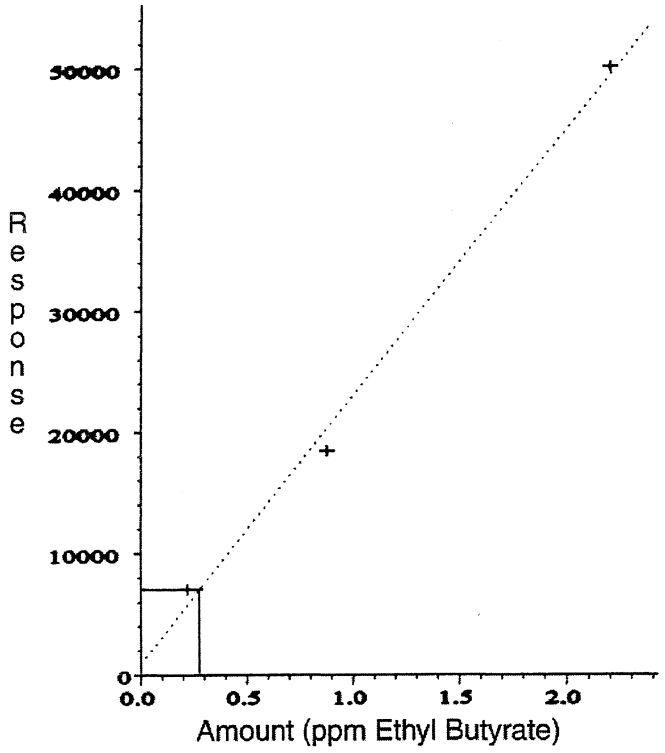

FIGURE 4

Main column calibration line for ethyl butyrate for 0.2 to 2.2 mg/L in 65% rectified ethanol. Direct large volume headspace injection to pre-column after reduction to 10% ethanol followed by two-dimensional GC-MS.

TABLE 4

Changes in minor volatile compounds with ageing.

Compound [a]	Whiskey		
	0 years	3 years	6 years
Ethyl formate [b]	0.33	2.62	9.10
Formaldehyde diethyl acetal [b]	0.11	0.17	0.45
Ethyl propionate [c]	0.77	1.28	1.24
Propyl acetate [c]	0.16	0.40	0.23
Ethyl isobutyrate [c]	0.17	0.25	0.33
Isobutyl acetate [c]	0.38	0.78	0.61
Ethyl butyrate [c]	0.55	0.86	2.20

[a] Amounts in mg/L at absolute alcohol.
[b] Precolumn cut from 1 to 16 min (Fig. 2).
[c] Precolumn cut from 16 to 30 min (Fig. 2).

CONCLUSIONS

High-volatility compounds and their changes with ageing in whiskey have been investigated. Substantial changes occurred with ageing. Compounds associated with the pathway for oxidation of ethanol increased, while sulfur compounds showed major decreases. The identity of a number of trace compounds has been confirmed and increases with ageing were established for a range of ethyl esters and acetates. These compounds are associated with fruity, pleasant notes and it is reasonable to associate their increase with improved flavour. Although the sensory significance of the observed changes in concentrations of the compounds in question has not been determined in this study, it would appear as though these changes in the light of reported threshold values might contribute significantly to the odour and quality of whiskey.

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