Flavour Components of Whiskey. I. Distribution and Recovery of Compounds by Fractional Vacuum Distillation

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Submitted for publication: December 2000
Accepted for publication: July 2001
Key words: Whiskey, vacuum distillation, flavour compounds, gas chromatography

A vacuum fractional distillation procedure is described for separating both the matrix components and flavour compounds of a whiskey into well-defined groups based on differences in azeotropic boiling points. The distillation was carried out at near ambient temperatures to accommodate both unaged and aged whiskies. Analytical and sensory data indicated good recovery of congeners. Individual fractions were reconstituted with ethanol and water to the original volume and strength dimensions of the whiskey. Undesirable thermal changes in the aged products were minimised by the low temperature fractionation and allowed changes in the flavour composition of whiskey due to maturation to be investigated for such unaged and aged reconstituted pairs.

Aged whiskey is a complex mixture of hundreds of flavour compounds in an ethanol water matrix. These compounds originate from the cereal raw material, the individual production stages of starch conversion, fermentation and distillation and the ageing process in oak barrels (Lyons & Rose, 1977; Lehtonen & Suomalainen, 1979; Nykänen & Nykänen, 1991). Analysis of the majority of the flavour compounds at their naturally occurring levels requires concentration and isolation techniques. Various approaches have been described and a general trend is to both isolate and concentrate specific compound groups (Maarse & Belz, 1985). An analysis of Jamaica Rum has been described (Liebich *et al.*, 1970) employing initial solvent extraction with subsequent acid and/or base manipulation for isolation of acids, phenols and lactones. Further preparative gas chromatography was used to isolate individual compounds for spectroscopic study. A more comprehensive general separation scheme for distilled spirits (ter Heide *et al.*, 1978; ter Heide, 1984) involves the above steps, but also subsequent fractional and short path distillation.

There are certain disadvantages to these approaches. When a sample is initially solvent extracted, it is not possible to analyse the very volatile compounds successfully. Additional headspace concentration techniques on the sample itself are necessary to recover these volatile compounds (ter Heide *et al.*, 1978). Extraction also makes sensory investigation more difficult because of residual solvent traces.

A different approach describes a semi-automated commercial apparatus employing vacuum column distillation to fractionate the actual sample (MacNamara *et al.*, 1989). Applied to whiskey this distillation gives the required compound separation and enrichment by taking advantage of both compound volatility and the azeotropic behaviour of ethanol and water with the secondary flavour compounds. The fractions obtained are in the original whiskey matrix only and will therefore be suitable for direct sensory evaluation. However, since they differ in volume and ethanol content an individual fraction reconstitution procedure is necessary to remove these variables. Gas chromatography with flame ionisation detection (GC-FID) is used to define the start and finish of fractions. Gas chromatography/mass spectrometry (GC-MS) is used to demonstrate the isolation of important compounds originating from wood into one specific fraction. Further GC analysis on the individual fraction reconstitutes and on a total reconstitute is employed to monitor the general distribution of flavour compounds in all of the fractions.

The aim of the present work is the extension of this approach to the monitoring of ageing changes in whiskey during oak barrel maturation. A major advantage is that only those fractions which are judged contributory to perceived ageing character need be considered. In addition the volatility fractionation offered by the process greatly simplifies the subsequent chromatographic analysis of these fractions.

MATERIALS AND METHODS

Whiskeys

The whiskeys were standard unpeated Irish malt whiskey and were obtained directly from the warehouse at a cask strength of ca. 65% vol/vol. These samples were at various ages and each sample was a composite of 12 aliquots from similar casks at the same age. Casks were standard once-used American bourbon barrels and composites were used to minimise any cask-to-cask variation. A 50 L sample of the original unaged standard malt whiskey had been retained for comparison purposes. Samples and their subsequent fractions from the distillation were either stored in a cold room at 4°C in Duran flasks with teflon-lined closures, or frozen in the case of fraction 5 with low ethanol content.

Distillation apparatus

Two-litre samples of whiskey were distilled in the apparatus shown in Fig. 1 (Normschliff, Wertheim, Germany).

Evaporation occurred by recirculating the sample through a thin film evaporator, which was heated by an external oil bath (not shown). The 1.2 m column was silver vacuum jacketed and packed with 3 mm glass Wilson helices. A vapour dividing reflux

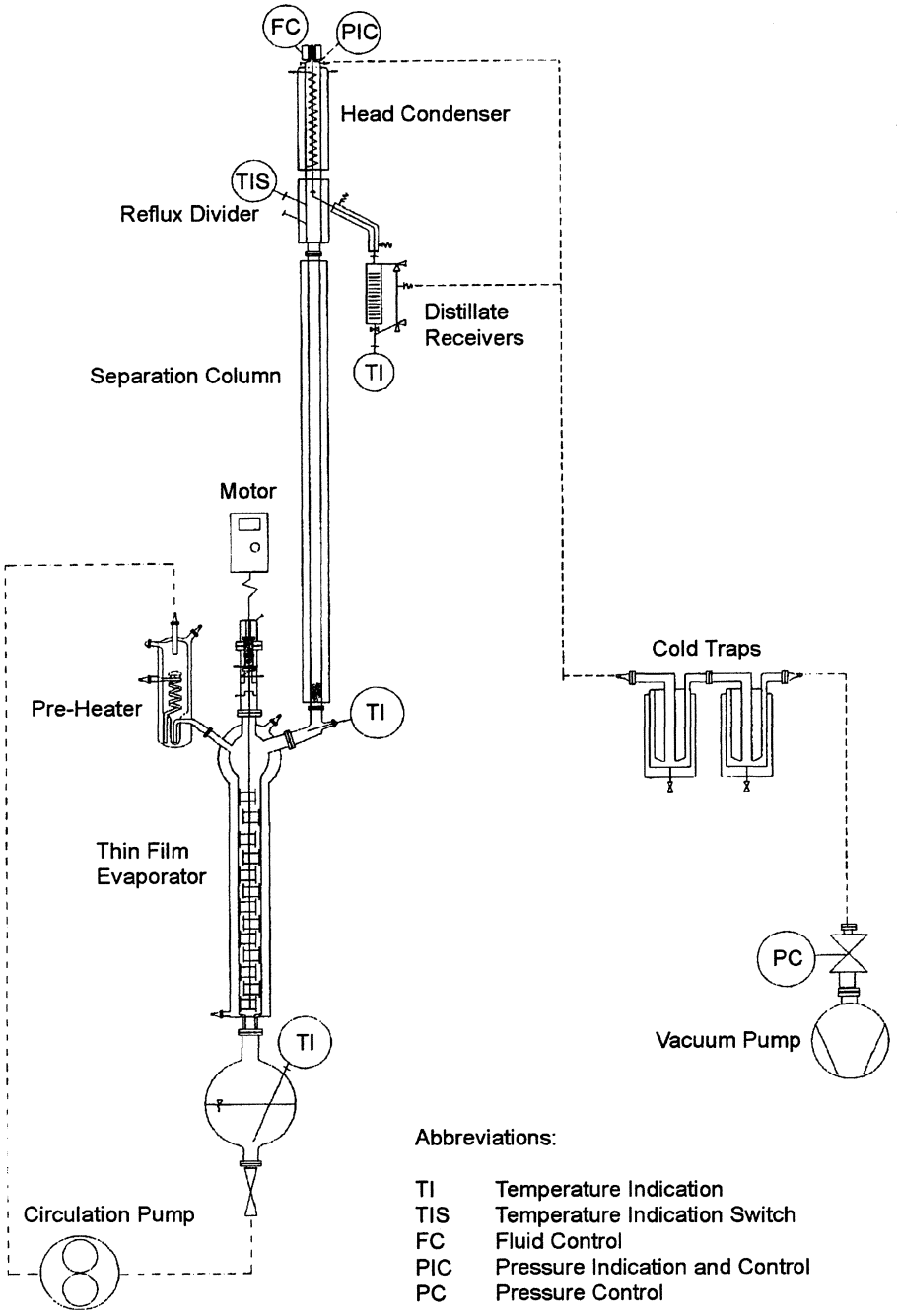

FIGURE 1
Apparatus for vacuum fractional distillation.

head was used between the column and head condenser. This divider led into a sidearm condenser and receiver and both head and sidearm condensers were cooled to -25°C by an external methanol bath (not shown).

Vacuum in the system was maintained at 80 mbar by a vacuum pump operating through a switchable three-way arrangement of cold traps. The traps were cooled with liquid nitrogen for recovery of the very volatile compounds. Electronic control units (not shown) operated through pressure and temperature sensors and allowed measurement and control of vacuum, reflux withdrawal ratio and temperatures in the plant. All materials in contact with the sample or its vapour were glass or PTFE and the sample circulation pump had stainless steel displacement heads. The distillation plant was cleaned between processing of different samples by similarly distilling two litres of rectified neutral 65% ethanol under total reflux for two hours, followed by withdrawal of 200 mL to clean the sidearm and receiver. Further rinsing with neutral 65% ethanol and subsequent sensory evaluation were used to confirm that the unit was clean and ready for the next distillation.

Gas chromatography-flame ionisation detection

A Hewlett-Packard 5880A gas chromatograph (Hewlett-Packard, Palo Alto, CA., USA) was used for the determination of the major

compounds in original whiskeys, fractions, subfractions, and total and individual reconstitutes. 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, Middelburg, The Netherlands).

The injection 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 about 1.5 mL/min. The oven temperature was 40°C (4 min.) x 5°C/min. to 200°C (10 min.). 1 µL of each sample was directly injected using a 1/50 split ratio (MacNamara, 1984). 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.

Sample preparation for gas chromatography-mass spectrometry

For profiling of the phenolic aldehyde and whiskey lactone distribution between the distillation fractions of an aged whiskey equal volumes of the samples were reduced to 10% ethanol using clean water (Milli-Q, Millipore Corporation, Bedford, MA., USA) and 250 mL aliquots were continuously extracted for 22 hours into a solvent mixture comprising 90% freon 11 and 10% dichloromethane (Burdick and Jackson grade) (Mandery, 1983). The freon was distilled immediately before use. After removal of the solvent in a Kuderna-Danish apparatus, the extract was recovered in 200 µL of ethanol.

Gas chromatography-mass spectrometry

The GC-MS analyses of the fraction extracts were performed on a Hewlett-Packard 5890 GC coupled to a 5971 mass selective detector. 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 mass spectrometer was operated in selected ion monitoring mode for the following time-programmed group of ions:

- Group 1, m/z 99 for cis and trans lactones.
- Group 2, m/z 151, 152 for vanillin.
- Group 3, m/z 181, 182 for syringaldehyde.
- Group 4, m/z 135, 177, 178 for coniferaldehyde.
- Group 5, m/z 165, 177, 180, 208 for sinapaldehyde.

The ions were selected from the mass spectra of authentic standards and published data (Nakamura *et al.*, 1974). The MSD detector voltage was 1600 with 100 msec dwell time per ion. The oven temperature was 60°C (1 min) x 5°C/min to 300°C. The injector was a programmed temperature vaporiser (PTV), 40°C x 10°C/sec to 300°C. Helium was used as carrier gas at a flow rate of 1 mL/min and 1 µL of extracts were injected at 1/50 split ratio into an empty deactivated vigreux glass liner.

Sensory testing

The integrity and recovery of fractionation was investigated by triangular sensory difference testing on both unaged and aged original whiskeys and their reconstitutes. Seven experienced whiskey tasters each evaluated three sets of three samples, reduced to 20% vol/vol immediately before tasting and presented in a coded random manner. Minimum correct judgements for significant difference at various levels were as per published Tables (Sensory Testing Methods, 1996). Similar difference testing was carried out on corresponding unaged and aged individual fraction reconstitutes to investigate their relative difference contributions.

RESULTS AND DISCUSSION

Fraction characteristics

Table 1 describes the set of fractions obtained from a typical distillation run.

TABLE 1

Fractions obtained from vacuum distillation of a 2-litre whiskey charge.

Fraction	Time (hours)	Volume (mL)	Ethanol % v/v
1	0 – 6[a]	3 – 5	98%
2	6 – 7[b]	50	98%
3	7 – 23[b]	1200	98%
4	23 – 24[b]	40	50%
5	24 – 26[c]	690	<1%

(a) Fraction 1 recovered from cold traps at -196°C.
(b) Fractions 2, 3 and 4 recovered from distillate receiver at 9:1 reflux ratio. Bulk of fraction 3 recovered overnight.
(c) Fraction 5 recovered as undistilled water fraction combined with residues of fraction 5 recovered from column packing and plant with rectified neutral ethanol.

The rationale for the five principal fractions can be understood in terms of compound and matrix volatility, together with reduced volatility due to azeotropic behaviour between the matrix components or between compounds and matrix components (Horsley, 1973).

Fraction 1 consisted of very volatile compounds that passed with a little ethanol through the head condenser and were recovered from the cold traps. Fractions 2 and 3 were essentially the azeotrope of ethanol and water (ca. 98% ethanol and 2% water at 80 mbar). Fraction 2 is a practical "buffer" fraction between fractions 1 and 3 and its function was to remove any last traces of volatile compounds that did not pass to the cold traps. The homogeneity of fraction 3 was reflected in a stable head temperature of 24°C during its entire removal. Its main advantage is to give a very useful isolation and depletion of the semi-neutral matrix as it contains ca. 60% of the total sample volume and ca. 92% of the total sample ethanol content. At the end of fraction 3 the ethanol content in the pot has practically been depleted. New higher boiling azeotropes of the remaining ethanol, water and less volatile flavour compounds (i.e. higher alcohols) now entered the column. The pot and column entry temperatures quickly rose to 41°C (boiling point of water at 80 mbar), indicating that this new fraction was essentially trapped in the column. As the remnants of fraction 3 were removed from the system, the head temperature in turn rose above 24°C. Fraction 4 was then removed during a head temperature increase from 24 to 41°C. Qualitative GC profiling was used to detect the start and finish of fraction 4 in terms of total recovery of higher alcohols (Fig. 2).

Fraction 5 was immediately recovered as the water residue from the distillation flask. This fraction contains remnants of fraction 4 compounds together with some lower volatility fermentation compounds, but in the case of an aged spirit it also con-

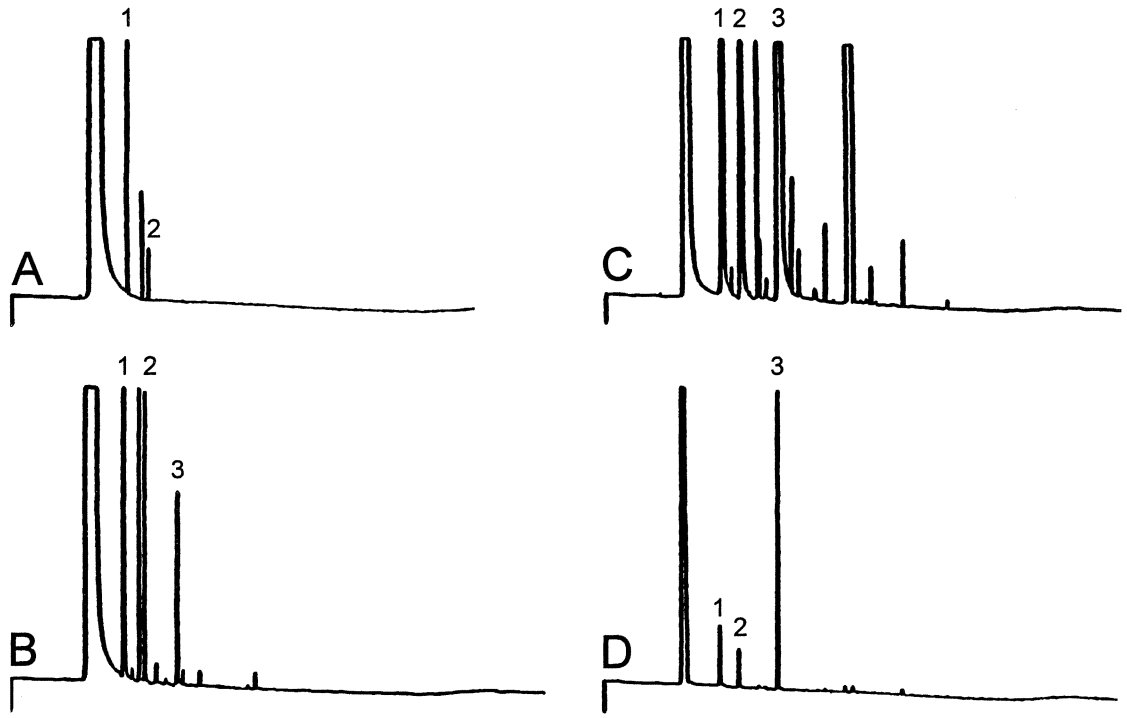

FIGURE 2

Gas chromotograms illustrating recovery of fusel alcohols during fraction 4 take-off.
A = start; D = finish. Peak identities: 1 = n-propanol; 2 = isobutanol; 3 = amyl alcohols. Conditions as in text.

tains all the colour of the original sample, most of the cis and trans-ß-methyl-γ-octalactones (whiskey lactones), and all the wood lignin-derived phenolics as represented by the four principal phenolic aldehydes (Fig. 3). The traces in Fig. 3 compare reconstructed ion chromatograms after selected ion monitoring for these specific compounds in an original whiskey, and fraction 4 and fraction 5 from the whiskey.

A slight partitioning of the whiskey lactones into fraction 4 was observed. This represents a balance between their preferred retention in fraction 5 and the objective of removing the entire higher alcohol content into fraction 4. Programmed temperature injection is particularly useful for capillary gas chromatography of these semi-volatile compounds. The technique avoids the well-known discrimination in the needle due to selective vaporisation of the solvent that occurs in hot split/splitless injectors (Eder *et al.*, 1991).

Total and fraction reconstitution

This procedure represented a total physical segmentation of the sample rather than a selective removal or enrichment of certain congeners. The first interesting procedure was therefore to compare a total reconstitution of the fractions (using proportional aliquots) with the original undistilled sample. Since the fractions differed greatly in volume and strength, a second interesting approach was the concept of individual fraction reconstitution. This consisted of using rectified neutral ethanol and/or water to dilute each fraction back to the original matrix dimensions of 2 litres at 65% vol/vol ethanol. If, by comparative testing of an undistilled whiskey and its total reconstitute, it can be shown that the integrity of the undistilled whiskey can be re-established in the total reconstitute, then all the flavour must be distributed within the fractions and two main productive approaches become available. Firstly, the relative contribution of individual fractions to the overall flavour of a sample can be assessed. Secondly, differences between similar fractions from different starting samples can be examined. This approach has been used to investigate maturation changes between new and aged whiskies.

Recovery and distribution of major congeners

The partitioning of certain compound groups between fractions has previously been mentioned (Figs 2, 3). An overall view of this trend in terms of the most abundant fermentation compounds can be obtained by comparing standard split capillary GC profiles of individual fraction reconstitutes (Fig. 4).

In Table 2 quantitative data for both recovery and distribution of major flavour compounds is presented for an original (undistilled) whiskey, its total reconstitute, and individual fraction 4 and 5 reconstitutes.

The partitioning of the entire fusel alcohol content into fraction 4 gives a significant advantage when monitoring maturation changes as the majority of lignin derived lactone and phenolic compounds partition into fraction 5 (Fig. 3).

Sensory assessment of reconstitutes

For both aged and unaged whiskies the panel repeatedly returned a non-significant difference for pairs of both unaged and aged originals and their total reconstitutes These data are presented in

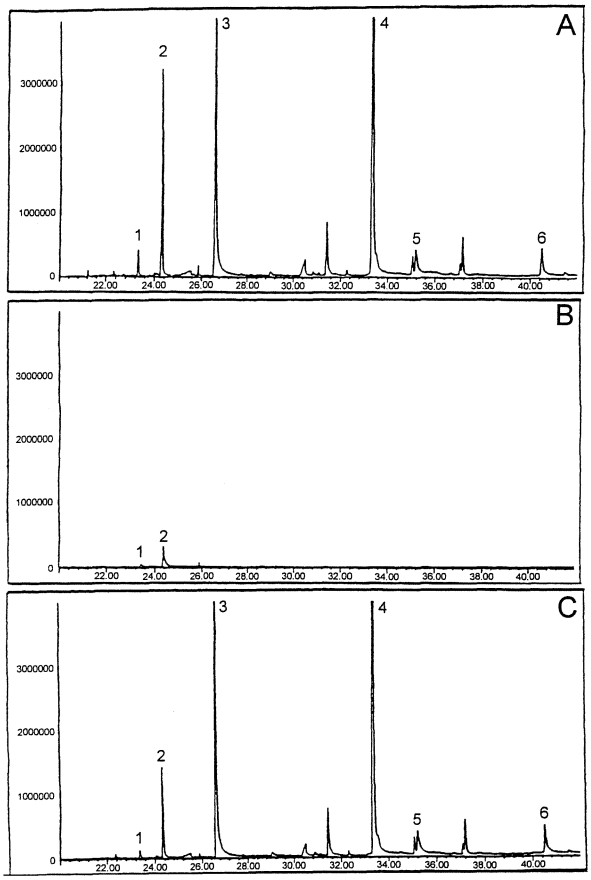

FIGURE 3

Reconstituted ion chromatograms for extracts of an original aged whiskey and its reconstituted fraction 4 and fraction 5. A = extract of original aged whiskey; B = extract of reconstituted fraction 4; C = extract of reconstituted fraction 5. Peak identities: 1 & 2 = whiskey lactones; 3 = vanillin; 4 = syringaldehyde; 5 = coniferaldehyde; 6 = sinapaldehyde. Conditions as in text.

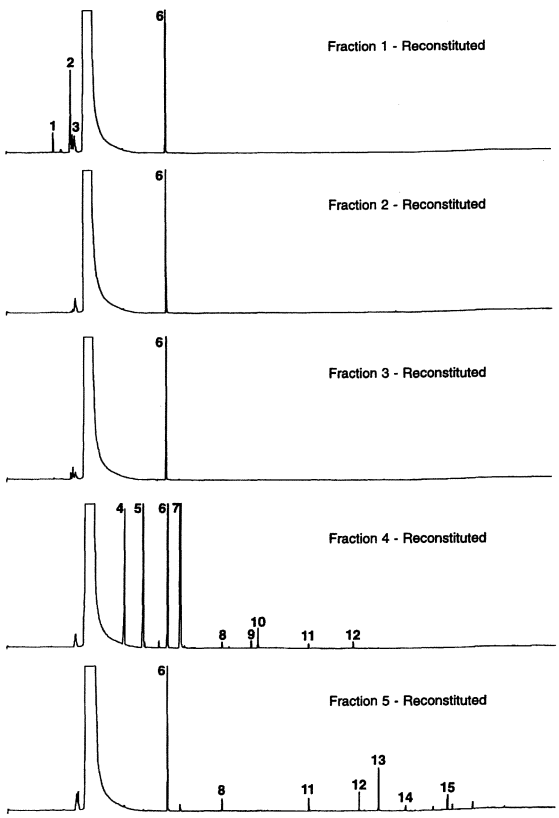

FIGURE 4

Comparative gas chromatographic profiles for individual fraction reconstitutes. Peak identities: 1 = acetaldehyde; 2 = ethyl acetate; 3 = diethyl acetal; 4 = n-propanol; 5 = isobutanol; 6 = 4-methyl-2-pentanol (internal standard); 7 = amyl alcohols; 8 = ethyl lactate; 9 = ethyl caprylate; 10 = furfural; 11 = ethyl caprate; 12 = phenyl ethyl acetate; 13 = ethyl laurate; 14 = 2- phenyl ethanol; 15 = ethyl myristate; 16 = ethyl palmitate. Conditions as in text.

TABLE 2

Recovery and distribution of major volatile compounds.

Compound [a]	Original Whiskey	Total Reconstitute	Fr. 4 Reconstitute	Fr. 5 Reconstitute
Acetaldehyde	31	21	–	–
Ethyl Acetate	149	126	–	–
Diethyl Acetal	53	44	–	–
Amyl Alcohols	1 108	1 119	1 118	6
Total Fusel Alcohols	1 744	1 763	1 768	8
Ethyl Lactate	40	44	14	29
Furfural	29	29	28	–
Ethyl Caprate	28	22	4	17
Ethyl Laurate	26	21	–	22
2-Phenyl Ethanol	30	37	–	35
Ethyl Myristate	7	5	–	5
Ethyl Palmitate	20	17	–	18

[a] All concentrations in mg/L absolute alcohol.

Table 3. It therefore appears as though virtually no sensorily detectable changes were introduced by the vacuum distillation of whiskey into five fractions.

In the case of aged whiskies that mature at ambient temperatures, the low temperature vacuum distillation is important to minimise possible thermal reactions. The sample has remained at ambient temperature for most of this process and only rises to 41°C for a short period to remove fraction 4.

The triangular sensory difference testing was extended to the corresponding pairs of unaged and aged individually reconstituted fractions in order to investigate difference contributions from the individual fractions. These results are also presented in Table 3 and show that significant differences are detected in all the corresponding unaged and aged pairs. Such differences were expected in the fraction 1 and 5 pairs based on the compound types isolated into these fractions. Fraction 1 contains volatile compounds and changes in these compounds are associated with a decrease in negative sulfur aroma and pungency, and an increase in sweetness (Reazin, 1981; Nishimura *et al.*, 1983; Nishimura & Matsuyama,

TABLE 3

Difference sensory analysis[1] of original and reconstituted whiskey samples and vacuum-distilled fractions of aged and unaged whiskeys.

Sample/Fraction Pair	Correct Identifications[2]	Significance
Unaged: Original vs total reconstitued sample	9	NS
Aged (1): Original vs total reconstituted sample	8	NS
Aged (2): Original vs total reconstituted sample	11	NS
Aged vs Unaged reconstituted fraction 1	15	***
Aged vs Unaged reconstituted fraction 2	16	***
Aged vs Unaged reconstituted fraction 3	13	**
Aged vs Unaged reconstituted fraction 4	15	***
Aged vs Unaged reconstituted fraction 5	17	***

[1] Triangular difference test

[2] Required correct identification for significance. (7 judges x 3 replications).

P > 95% (*): 12

P > 99% (**): 13

P > 99.9% (***): 15

1989). Fraction 5 isolates the lignin-derived maturation compounds and their flavour contribution has been extensively investigated both in actual spirit samples and in model ethanol/wood systems (Nykänen, 1984; Nykänen *et al.*, 1984; Maga, 1984; Maga, 1989). These changes are interrelated, as oak wood is necessary for the decrease in volatile sulfides (Nishimura *et al.*, 1983)

Fractions 2 and 3 were not investigated further due to their relative neutrality. Differences between the unaged and aged pairs could be due to acetal formation during ageing. Acetaldehyde increase during ageing leads to the possibility of acetals of higher alcohols appearing in aged fractions 2 and 3. In a previous study on an extract of aged Cognac the fusel fraction was also removed by distillation and judged to have limited organoleptic value (ter Heide *et al.*, 1978). Fraction 4 was therefore also excluded from further investigation. Since the compounds in fractions 1 and 5 have been particularly associated with flavour changes during ageing, it was decided to preferentially investigate the relative changes in these fractions which will be the subject of future papers.

CONCLUSIONS

A scheme has been described for routine fractionation of the most volatile and least volatile compounds in unaged and aged whiskeys from both the common ethanol and fusel matrix. The apparatus can be assembled from readily available commercial units. A high degree of automation in terms of temperature, vacuum control and fraction collection is possible. Low vacuum during the distillation avoids thermal changes in the case of aged whiskies and ensured that the sensory changes observed were principally due to the ageing process.

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