Flavour components of whiskey

by

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is all my own original work and that I have not previously in its entirety or in part submitted it at any University for a degree.

Signature: Date:

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SUMMARY

Aged distilled spirits such as whiskey are complex mixtures of flavour compounds in an ethanol-water matrix. The flavour compounds involved can have widely different volatility and relative amounts. Many of the organoleptic properties that make whiskey suitable for commercial sale have their origin in reactions occurring during the ageing process in oak wood barrels.

To investigate the complex changes that take place during spirit ageing a preparative fractional vacuum distillation process was developed. Both high and low volatility compounds could be individually isolated as fractions and free from both the ethanol matrix and the fermentation fusel alcohols. This allowed a range of sensory and analytical procedures to be conducted on these fractions, in particular to investigate changes occurring during ageing.

Gas chromatographic (GC) analysis of the low volatility fraction is complicated by the fact that both the compounds and their ethanol matrix have very similar chromatographic behaviour when separated simultaneously on standard chromatographic phases. Compound and matrix co-elution becomes a major problem and conditions for mass spectrometric (MS) investigation are disadvantageous. A two-dimensional GC configuration using dissimilar chromatographic phases was configured to overcome these limitations. Using this approach 27 compounds were separated and identified. Headspace injection was used to increase detection sensitivity. Changes with ageing for seven compounds present at very low levels were quantified. In addition changes in the most abundant compounds were quantified by standard split injection, and changes in trace level sulfur compounds by headspace injection with sulfur chemiluminescent detection (SCD). Increases of the concentrations of pleasant fruity ethyl esters and acetates were established. Volatile sulfides with generally objectionable aroma showed concomitant major decreases.

Appropriate techniques could also be applied to the low volatility compounds recovered from the whiskey water fraction. High temperature GC-MS analysis of an extract of the water fraction allowed the identification of 30 compounds. Three phenolic esters were identified in whiskey for the first time. These compounds were synthesised and shown to be contributory to desirable ageing flavour. Increases in concentrations of 16 oak derived compounds during

a 10 year ageing period were established. Several compounds increased significantly over this time period. Ratios of aromatic phenolic aldehydes, and changes in these ratios during ageing, were unique to the type of barrel used in these experiments. This suggests that the final sensory properties of aged whiskey may be more dependent on wood parameters than previously thought.

Preparative reverse phase High Pressure Liquid Chromatography (HPLC) with an ethanol water gradient was used to further fractionate an extract of the low volatility compounds. Subsequent analysis and sensory testing allowed a group separation of compounds with each group contributing characteristic attributes to the total flavour. One group contained the three new phenolic esters together with a number of other unidentified compounds. This group was found to be important for desirable ageing flavour that seems to develop slowly with time. Further studies in this area to understand the individual and synergistic contributions of the many facets of ageing chemistry will have important commercial implications.

OPSOMMING

Verouderde spiritus soos Whiskey is 'n komplekse mengsel van geurstowwe in 'n etanol-water oplossing. Die vlugtigheid van die geurstowwe asook die konsentrasies waarin hul aanwesig is, varieer aansienlik. Verskeie van die sintuiglike eienskappe wat kommersiële waarde aan whiskey verleen, het hul oorsprong in reaksies wat tydens die verouderingsproses in eikehoutvate plaasvind.

Ten einde die ingewikkelde veranderinge wat tydens die veroudering van spiritus plaasvind, te ondersoek, is 'n preparatiewe fraksionele vakuumdistillasieproses ontwikkel. Hoogs vlugtige en minder vlugtige verbindings kon geskei word in afsonderlike fraksies wat vry was van etanol en fuselalkohole. Dit het die sintuiglike en fisies-chemiese analises van die fraksies moontlik gemaak, veral om die veranderings wat tydens veroudering plaasvind, te ondersoek.

Gaschromatografiese (GC) analise van die fraksie met 'n lae vlugtigheid word gekompliseer deur die feit dat hierdie komponente en die etanol waarin dit opgelos is soortgelyke chromatografiese eienskappe toon wanneer hul gelyktydig op standaard gaschromatografie fases geskei word. Die gelyktydige eluering van dié komponente en etanol waarin hul opgelos is, skep 'n probleem wat nadelig vir massaspektrometriese (MS) analise is. Die beperkings is oorkom deur die gebruik van tweedimensionele GC en stasionêre fases met uiteenlopende eienskappe. Op dié wyse is 27 verbindings geskei en geïdentifiseer. Die veranderinge in konsentrasies tydens veroudering is vir sewe verbindings gekwantifiseer. Veranderinge in die konsentrasies van die verbindings teenwoordig in die hoogste konsentrasies is gekwantifiseer deur split-inspuitings, terwyl veranderinge in die spoor-konsentrasies van vlugtige swawelverbindings mbv dampfase-inspuitings en met swawel chemolumisensie deteksie (SCD) bepaal is. Toenames in die konsentrasies van die aangename vrugtige esters en asetate is bepaal. Vlugtige sulfiede met meesal onaanvaarbare aromas toon gelyktydige groot afnames.

Geskikte tegnieke is ook gebruik vir die herwinning van minder vlugtige verbindings met die waterfase van whiskey. Hoë temperatuur GC-MS analises van 'n ekstrak van die waterfase het die identifikasie van 30 komponente moontlik gemaak. Drie fenoliese esters is vir die eerste keer in whiskey gevind. Hierdie verbindings is gesintetiseer en hul bydrae tot die

gewenste verouderingsgeur is sintuiglik bevestig. Toenames in die konsentrasies van 16 eikehoutverwante verbindings gedurende 'n verouderingsperiode van 10 jaar is bepaal. 'n Betekenisvolle toename het voorgekom in die konsentrasies van verskeie van hierdie whiskey verbindings. Die verhoudings van aromatiese fenoliese aldehiede en die verandering in die verhoudings tydens veroudering was kenmerkend van die tipe eikehoutvat wat gebruik is. Dié bevinding dui daarop dat die fenole sintuiglike eienskappe van verouderde whiskey meer afhanklik mag wees van eikehout parameters as wat voorheen algemeen aanvaarbaar is.

Preparatiewe omgekeerde fase hoëdrukvloeistofchromatografie met etanol/water as 'n gradient elueermiddel is gebruik om 'n ekstrak van die minder vlugtige verbindings verder te fraksioneer. Verdere GC-, MS- en sintuiglike analise het die skeiding van groepe van verbindings waarvan elk kenmerkende bydraes tot die totale geur lewer, moontlik gemaak. Een groep het drie nuut geïdentifiseerde fenoliese esters, tesame met 'n aantal ongeïdentifiseerde verbindings, bevat. Daar is vasgestel dat hierdie groep 'n belangrike bydrae maak tot die gewenste geur wat klaarblyklik stadig tydens veroudering ontwikkel. Verdere ondersoeke in hierdie verband om die individuele en sinergistiese bydraes van verskeie fasette van die chemie van veroudering te verstaan, kan belangrike kommersiële implikasies hê.

CONTENTS

PAGE

CHAPTER 1

INTRODUCTION

The ageing or maturation of distilled spirits in oak barrels is a very important part of the entire production process. Unaged whiskey is generally raw in both aroma and taste, and the ageing process converts this immature spirit into a desirable high-value commercial product. Elucidation of these changes is therefore important both scientifically and commercially.

Since whiskey is distilled in pot stills, which have limited separation power, the intermediate immature spirit is still a complex mixture in an ethanol-water matrix. These compounds originate from the raw material and the various production stages of brewing, fermentation and distillation. A major difficulty therefore is in trying to clarify the chemical ageing changes against a background of compounds that do not change or change very little.

To investigate the changes that take place during spirit ageing a preparative fractional vacuum distillation process was developed. The design and operation of this unit is presented in Chapter 1, together with data on distribution and recovery of compounds. In the fractional distillation process both the flavor compounds and the matrix components separate according to volatility and azeotropic boiling points. High volatility compounds can be recovered as a small enriched fraction in ethanol. The ethanol matrix itself can be isolated in greater than 90% yield, giving a useful depletion of this neutral fraction. The fusel fraction, which changes little during ageing, is similarly concentrated and isolated. The majority of low volatility compounds originating from the wood, and their reaction products over time, remain in the water fraction, which can be analysed free of interfering fusel fraction compounds. Sensory testing in the absence of any extraneous solvent material can be carried out on both fully reconstituted whiskeys in comparison to their unfractionated parents, and on individual fraction reconstitutes from different samples. This technique was applied to the monitoring of ageing changes in whiskey during oak barrel maturation. Only fractions considered contributory to perceived ageing character need be considered and gas chromatographic analysis can be tailored to the volatility of the fractions.

Chapter 2 outlines appropriate techniques, which were developed for analysis of the high volatility compounds, together with results on their changes during ageing. These compounds

are associated with perceived flavour changes because of their volatility and generally low sensory threshold values. The most abundant compounds could be analysed by standard split capillary gas chromatography, as splitting conveniently reduces the amount of matrix ethanol transferred to the column. Sulfur compounds at very low levels were analysed by a combination of headspace injection and sulfur chemiluminescent detection. Headspace injection maximised sensitivity and specific chemiluminescent detection meant that large solvent peaks were essentially not detected. Volatile sulfides, with generally disagreeable aroma, were found to decrease with ageing time. For other trace compounds a two-dimensional gas chromatographic configuration using dissimilar phases was found necessary for separation and identification. Using multiple individual injections of the sample to the first column, with different cuts from each injection transferred to the main column for further resolution, it was possible to separate trace-level compounds from the main ethanol peak. Using these approaches the changes in concentration of the highly volatile compounds of whiskey during ageing were monitored.

Storage in oak barrels significantly improves the sensory properties of whiskey and the mechanisms involved range from extraction of wood components to reactions of these components with each other and with components of the distillate. In Chapter 3 an attempt is made to interpret some of these changes by preliminary isolation of the compounds in the water fraction in the vacuum fractional distillation process. Chromatography can again be tailored to the specific compounds in this fraction. In this case high temperature capillary gas chromatography after programmed temperature vaporization injection is useful for elution of high boiling compounds. After extraction of the relevant compounds from a water fraction high pressure liquid chromatography was used to further fractionate the flavour compounds. These fractions could in turn be analysed by high temperature GC to reveal further compounds previously masked by chromatographic overlapping. By using ethanol and water as eluting solvents for the liquid chromatographic separation, sensory information was also available from the resulting fractions. This integrated strategy was then applied to water fractions of the same whiskey at different ages to characterise both abundant and trace compounds formed during maturation.

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CHAPTER 2

FLAVOUR COMPONENTS OF WHISKEY. 1. DISTRIBUTION AND RECOVERY OF COMPOUNDS BY FRACTIONAL VACUUM DISTILLATION

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Condensed title: Vacuum distillation of whiskey

ABSTRACT

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 whiskeys. 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.

INTRODUCTION

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, Koenig & Bayer, 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 successfully analyse the very volatile compounds. 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, Burke & Conway, 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: Whiskeys were standard unpeated Irish Malt Whiskey and were obtained directly from warehouse at a cask strength of ca. 65% v/v. 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 litre 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 meter column was silver vacuum jacketed and packed with 3 mm glass Wilson helices. A vapour dividing reflux 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 was used to confirm that the unit was clean and ready for the next distillation.

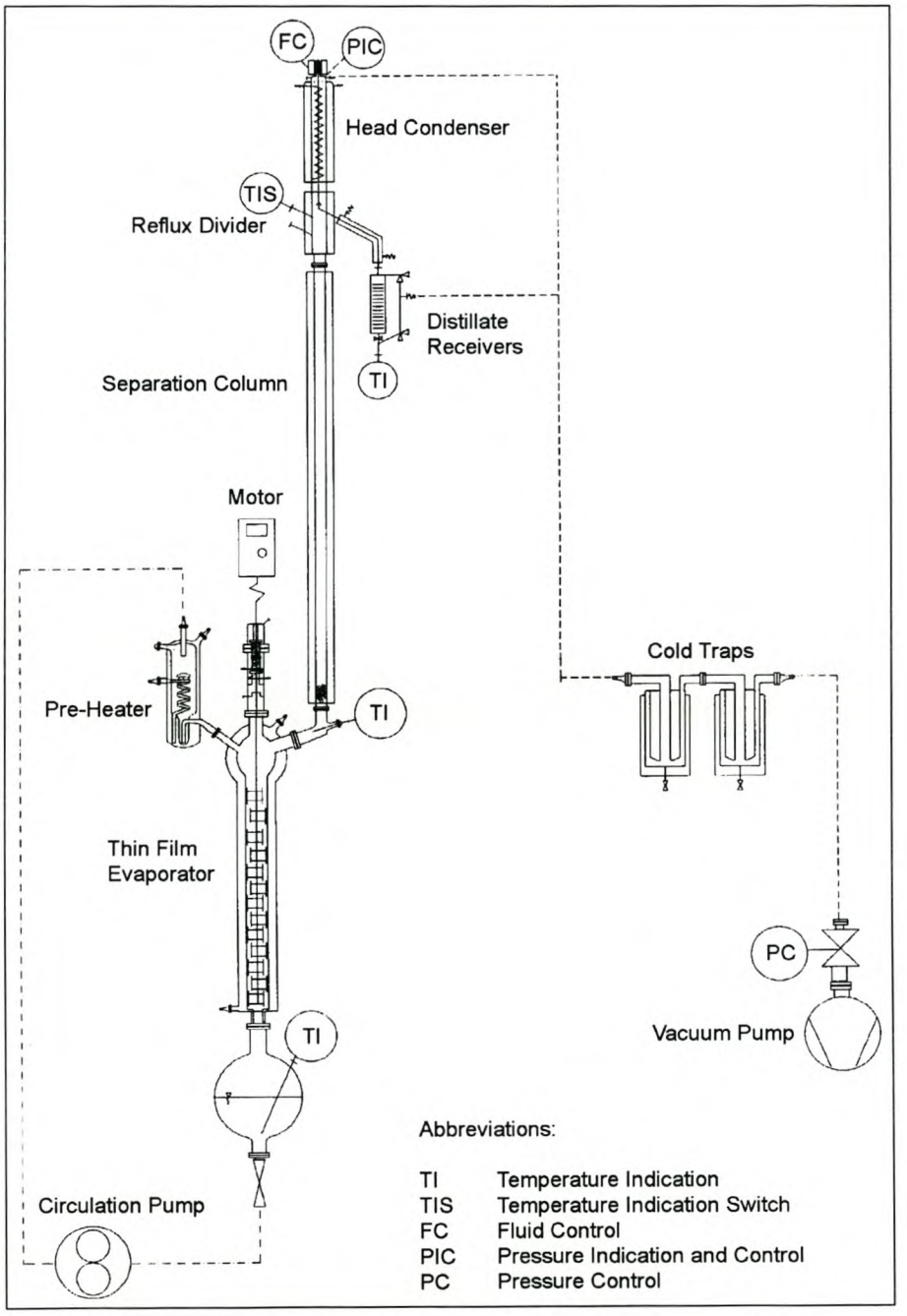

Figure 1. Apparatus for vacuum fractional distillation

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, Nakatsubo & Takayoshi, 1974). The MSD detector voltage was 1600 volts 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% v/v 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).

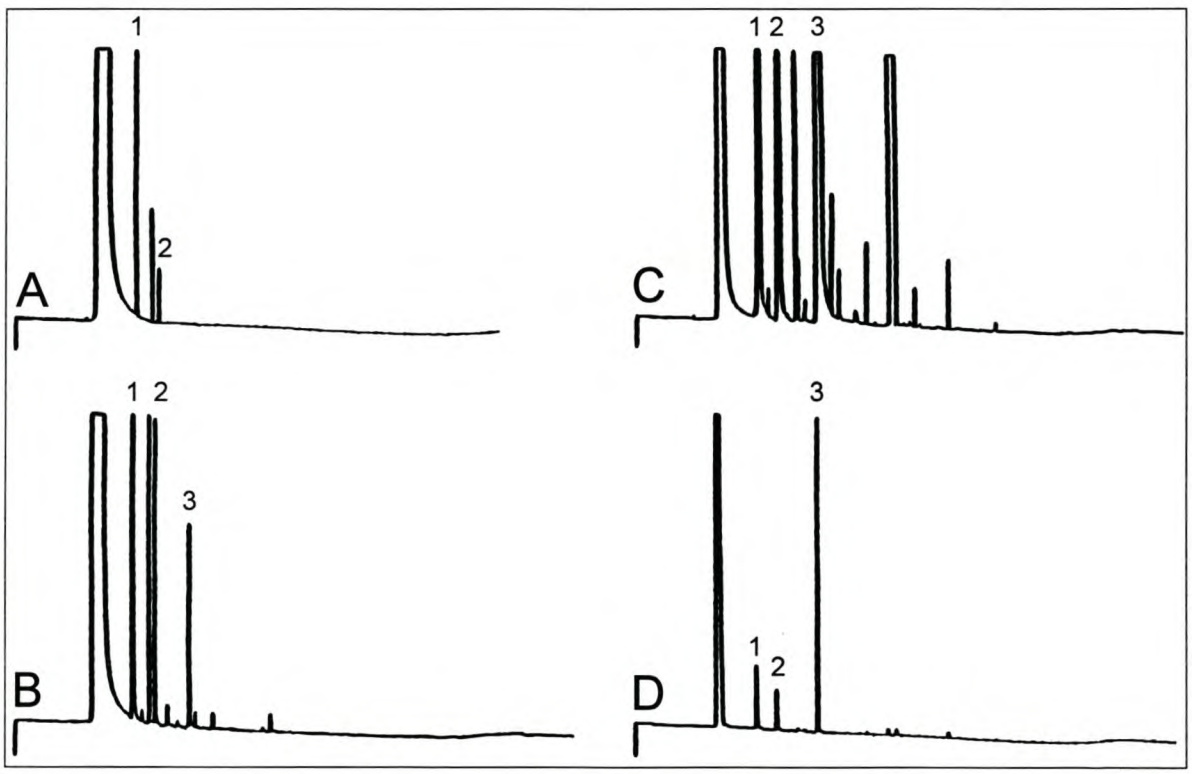

Figure 2. Gas chromotograms illustrating recovery of fusel alcohols during fraction 4 take-off.

A = start; D = finish. Peak identities: 1 = n-propanol; 2 = isobutanol; 3 = amyl alcohols. Conditions as in text.

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 contains all the colour of the original sample, most of the cis and trans-β-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, Reichlmayr-Lais & Kirchgessner, 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% v/v 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 whiskeys.

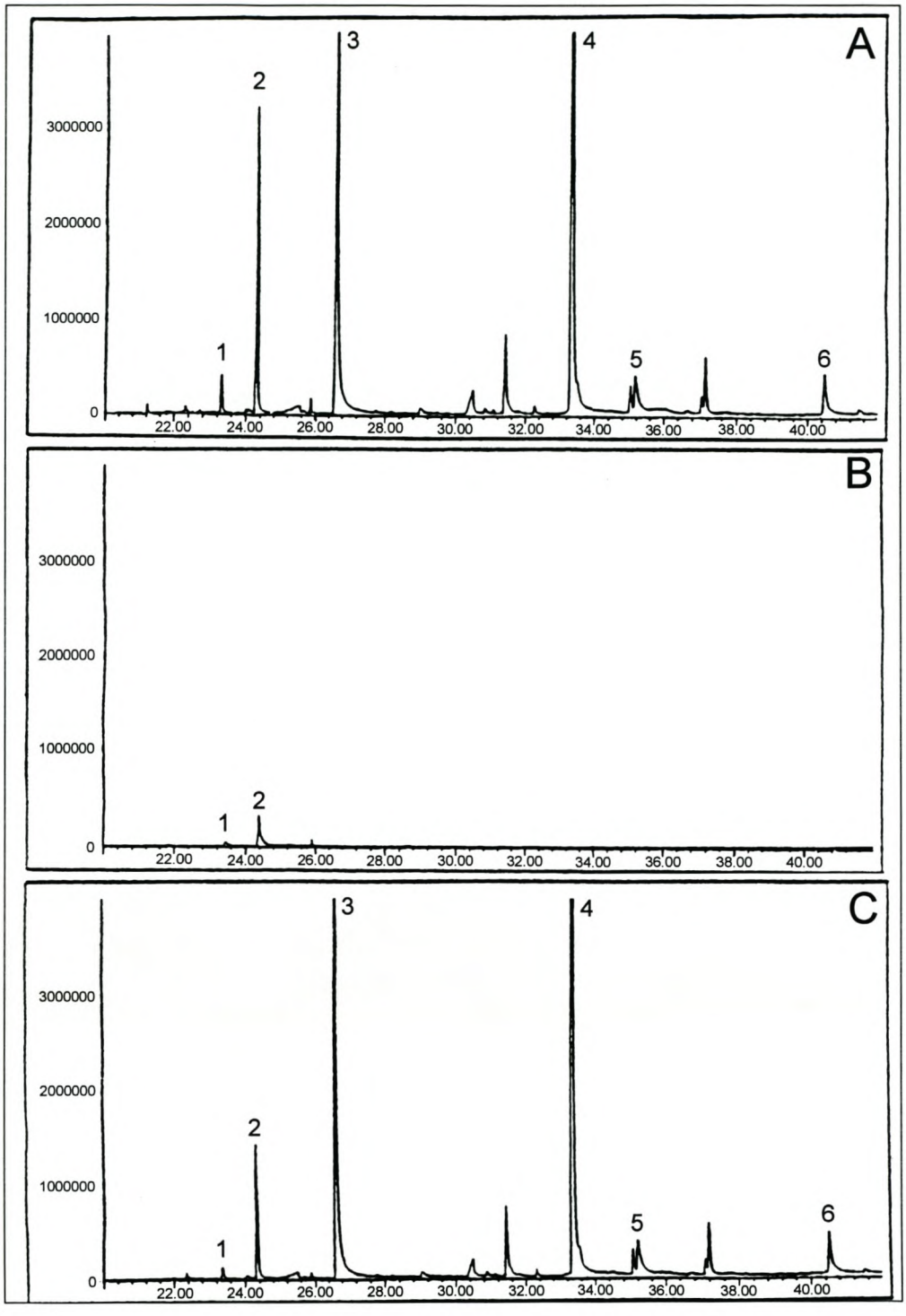

Figure 3. Reconstituted ion chromatograms for extracts of an original aged whiskey and its reconstituted fraction 4 and fraction 5.

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.

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).

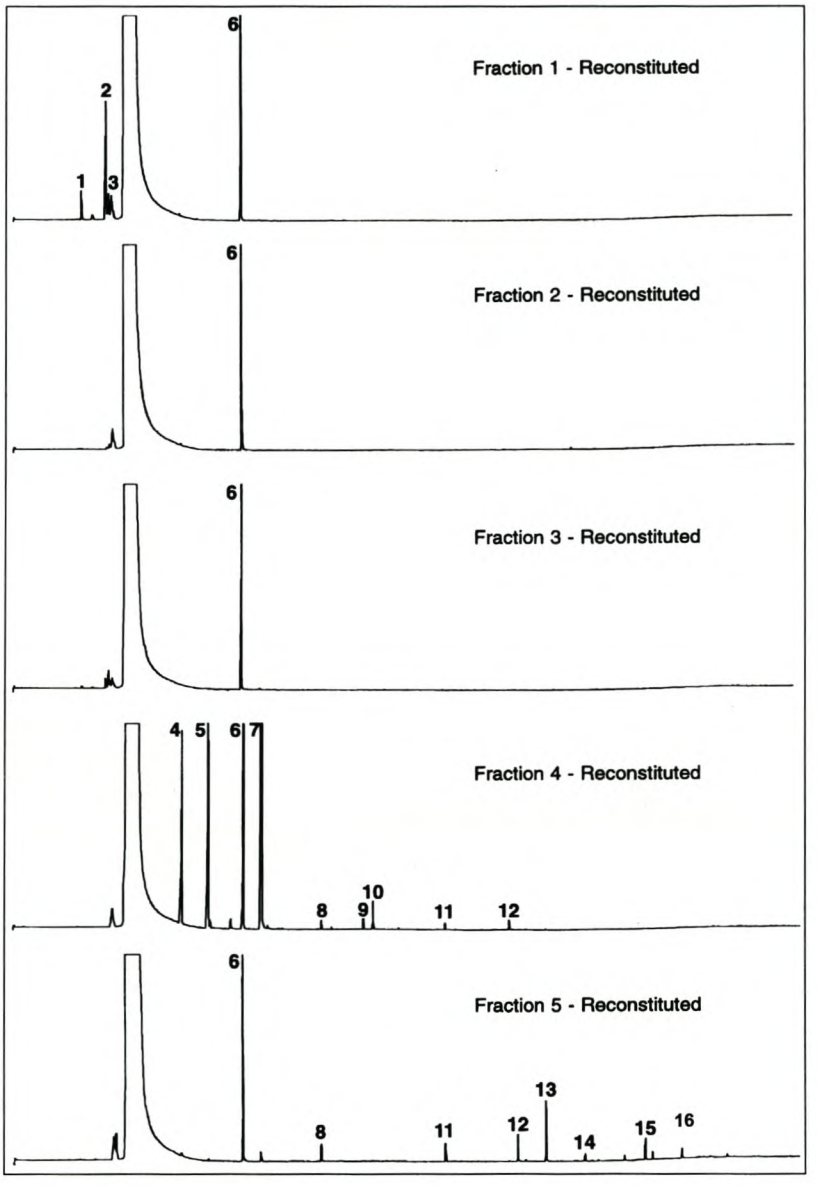

Figure 4. Comparative gas chromatographic profiles for individual fraction reconstitutes.

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.

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.

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	1108	1119	1118	6
Total Fusel Alcohols	1744	1763	1768	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)] **Amounts in mg/L absolute alcohol.**

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

In the case of aged whiskeys 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.

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

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, 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, Nykänen & Moring, 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 whiskeys, and ensured that the sensory changes observed were principally due to the ageing process.

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CHAPTER 3

FLAVOUR COMPONENTS OF WHISKEY. 2. AGEING CHANGES IN THE HIGH VOLATILITY FRACTION.

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Key words: Whiskey, volatiles, ageing, headspace, sulfur compounds, two dimensional chromatography.

Condensed title: Highly volatile whiskey fraction.

ABSTRACT

The volatile compounds isolated from whiskey by fractional vacuum distillation were identified by two-dimensional capillary gas chromatography/mass spectrometry (2D-GC-MS). 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 (FID). The ageing decreases in volatile sulfides were similarly determined using a sulfur chemiluminescence detector (SCD). 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.

INTRODUCTION

Volatile compounds of low molecular weight can be powerful odourants 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 litre sample to diffuse at room temperature to a small flask cooled in a dry ice bath. After 36 hours 0,33 ml of liquid was collected (Liebich, Koenig & Bayer, 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, Coates & Sharpe, 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 discreet low boiling fraction (MacNamara *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% v/v 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% v/v 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 previously been described (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 gas chromatography with flame ionisation 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 mass spectrometric detection after two-dimensional gas chromatography 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 flame ionisation detector. 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.

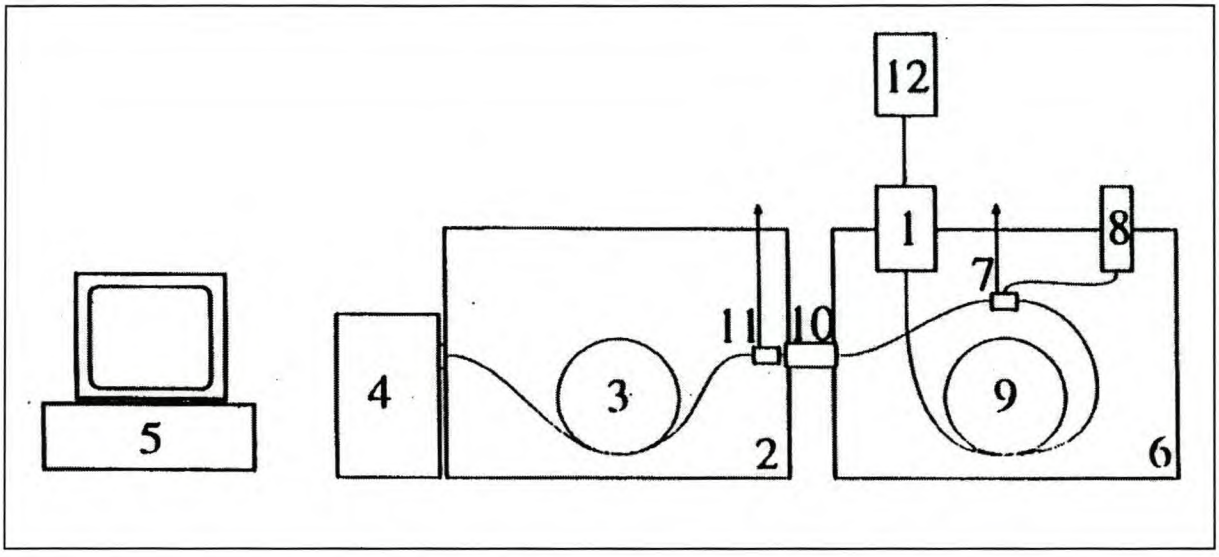

Figure 1. Two-dimensional GC configuration.

1: Programmed temperature vaporizing 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.

The pre-column separation was carried out on a polar CP-Wax 57 fused silica column (50m x 0,32 mm i.d. x 1,17 df, Chrompack, Middelburg, The Netherlands) using an oven temperature program of 40°C (17min) 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 (30m x 0,32 mm i.d. x 3,0 df, Restek, Bellefonte, PA., USA) with an oven temperature program 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 vaporising injector (Gerstel Cis-3) equipped with a glass vigreux liner, which was heated immediately after injection according to the following program, 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 mass selective 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% v/v ethanol. Injections were made to a programmed temperature vaporising (PTV) injector (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 liner had a split flow of 60ml/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 program, -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.

Gas chromatography with flame ionisation detection: A Hewlett Packard 5880A gas chromatograph 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 gas chromatograph 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 programmed temperature vaporiser (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 41 µg/L was used as internal standard and all compounds were Fluka Purum grade.

RESULTS AND DISCUSSION

Identification: Figure 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 phaseratio $\beta = 68$, 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.

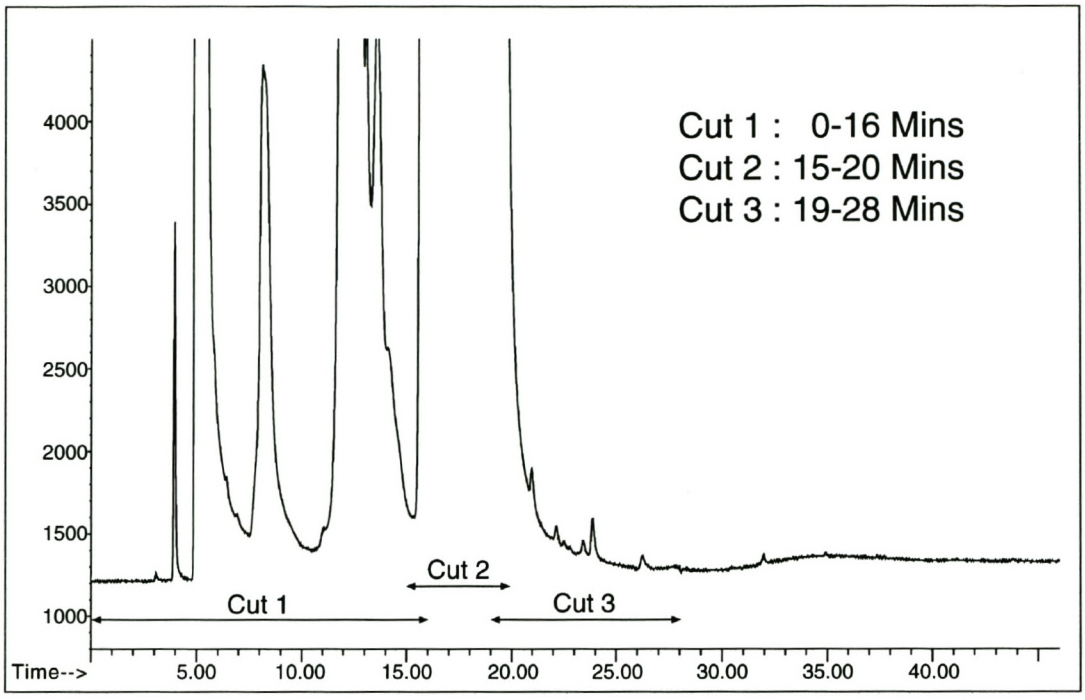

Figure 2. Thick film pre-column gas chromatogram of fraction 1 into three cuts for transfer to main apolar column.

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 $\beta = 27$) main column.

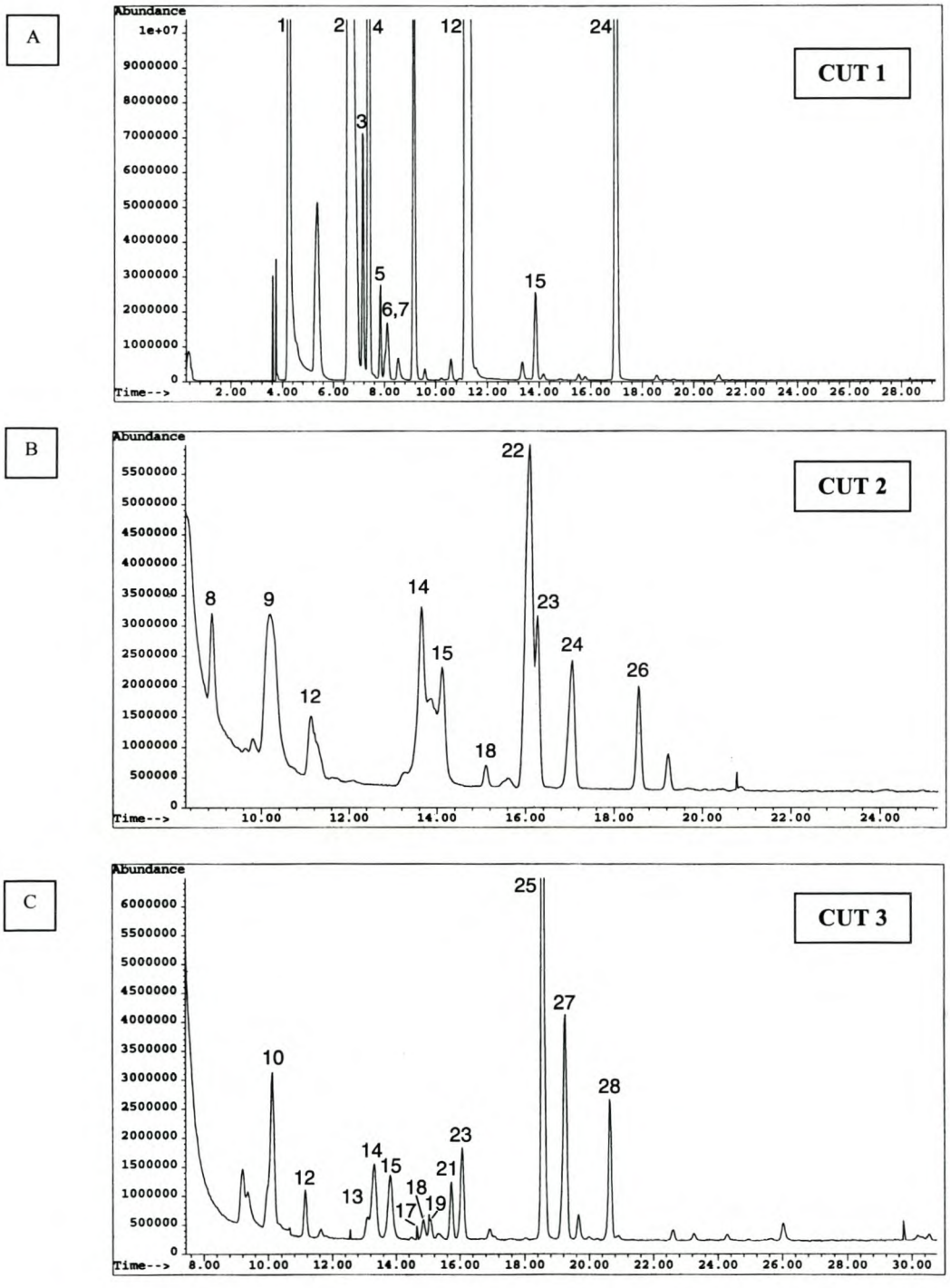

Figure 3. Fraction 1 cuts on apolar main column.

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 mins. cut 3 on pre-column (fig. 2)
Mass spectrometric detection. Peak identifications in table 1.

Quantification procedures: 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.

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**

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:

- 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 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.

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.**

Similar increases in bourbon whiskey are recorded (Reazin, 1981; Reazin *et al.*, 1976) 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 equilibria 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 dependant 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 is available on the individual sensory contributions of these volatile compounds (van der Merwe & van Wyk, 1981; Salo, Nykänen & Suomaleinen, 1972). 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).

Table 3. Changes in major 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 mg/L at absolute alcohol.**

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, Devreux & Masschelein, 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% v/v ethanol solution (Leppanen, Denslow & Ronkainen, 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 amount 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 efficiently trap

a wide range of compounds. 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.

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).**

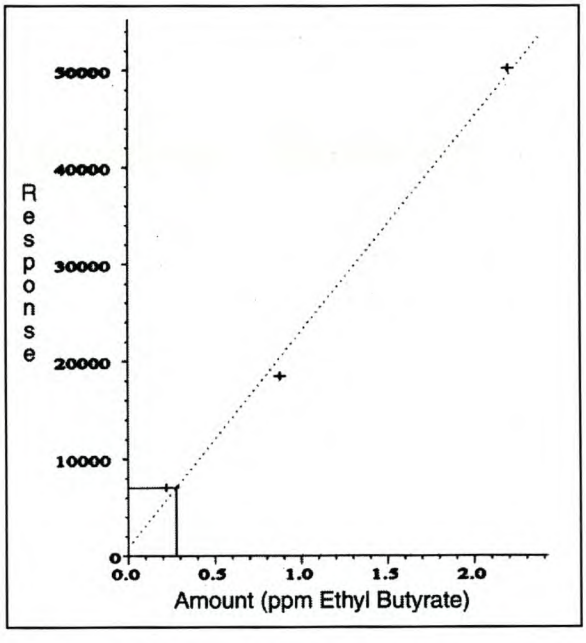

Figure 4. Main column calibration line for ethyl butyrate for 0,2 to 2,2 mg/L in 65% rectified ethanol. Direct large volume headspace injection to pre-column after reduction to 10% ethanol followed by two-dimensional GC-MS

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 correlate 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.

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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CHAPTER 4

FLAVOUR COMPONENTS OF WHISKEY.

3. AGEING CHANGES IN THE LOW VOLATILITY FRACTION.

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Key words: Whiskey, ageing, flavour compounds, wood lignin.

Condensed title: Whiskey ageing.

ABSTRACT

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 characterization 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.

INTRODUCTION

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.*, 2001 a). A similar approach was used to isolate the high volatility compounds from whiskey and to investigate their changes with ageing (MacNamara *et al.*, 2001 b). 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, Nykänen & Moring, 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 β-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 vaporization (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 principle wood originating compounds prior to GC-MS analyses. Thus it is believed that the above-integrated analytical strategy would allow the characterization 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% v/v 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 previously been described (MacNamara *et al*, 2001 a).

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 ten 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 254 nm and 2,0 AUFS. The column was a 250 mm x 10 mm Lichrospher RP-18 (Merck Gmbh, Darmstadt, Germany) with a 10 µm 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 20µl 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 volts and an ionization voltage of 70eV. The scan range was 25-400 amu, and spectra were acquired at 2 scans/sec. Helium was used as carrier gas at 1ml/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 colections. Ethyl homovanillate, ethyl syringate and ethyl homosyringate were synthesised as described later.

Simultaneous Mass Spectrometric and Flame Ionization 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 three-year-old whiskey at the level found for the ten-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% v/v.

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.

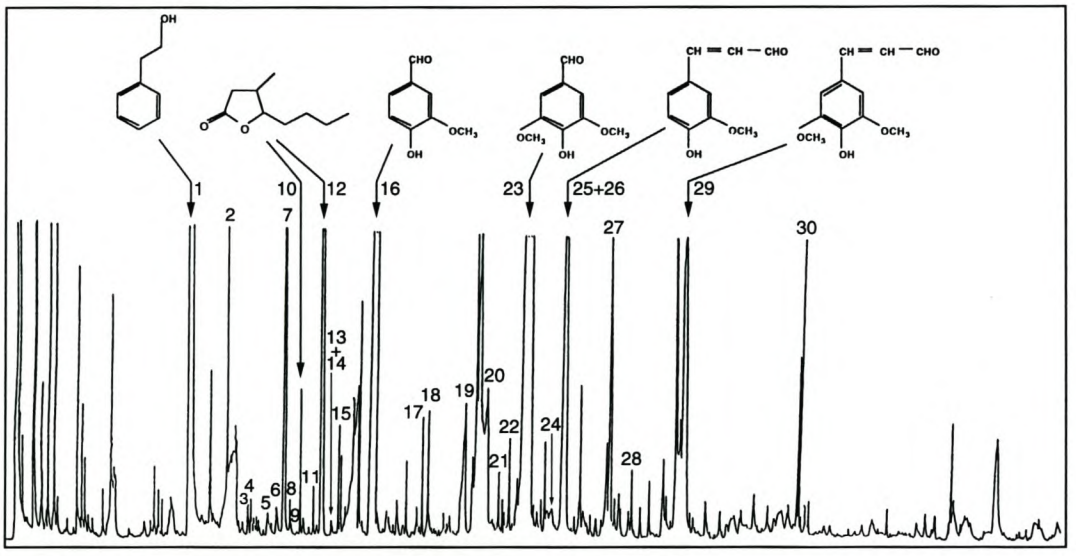

Figure 1. GC-MS trace on a high temperature apolar column of an extract from the fraction 5 of a 10 year old whiskey.

Peak identifications in Table 1.

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	β-Methyl-γ-octalactone, cis	1302
11	4-Vinyl guaicol	1330
12	β-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.**

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

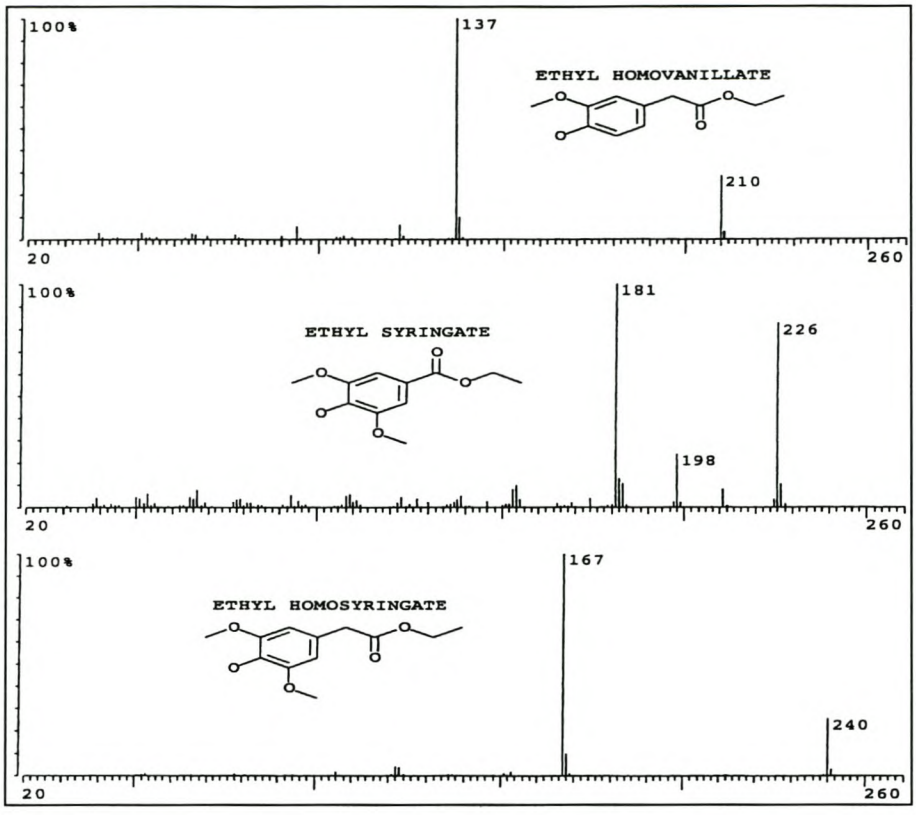

Figure 2. Mass spectra of new phenolic esters identified in whiskey.

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.

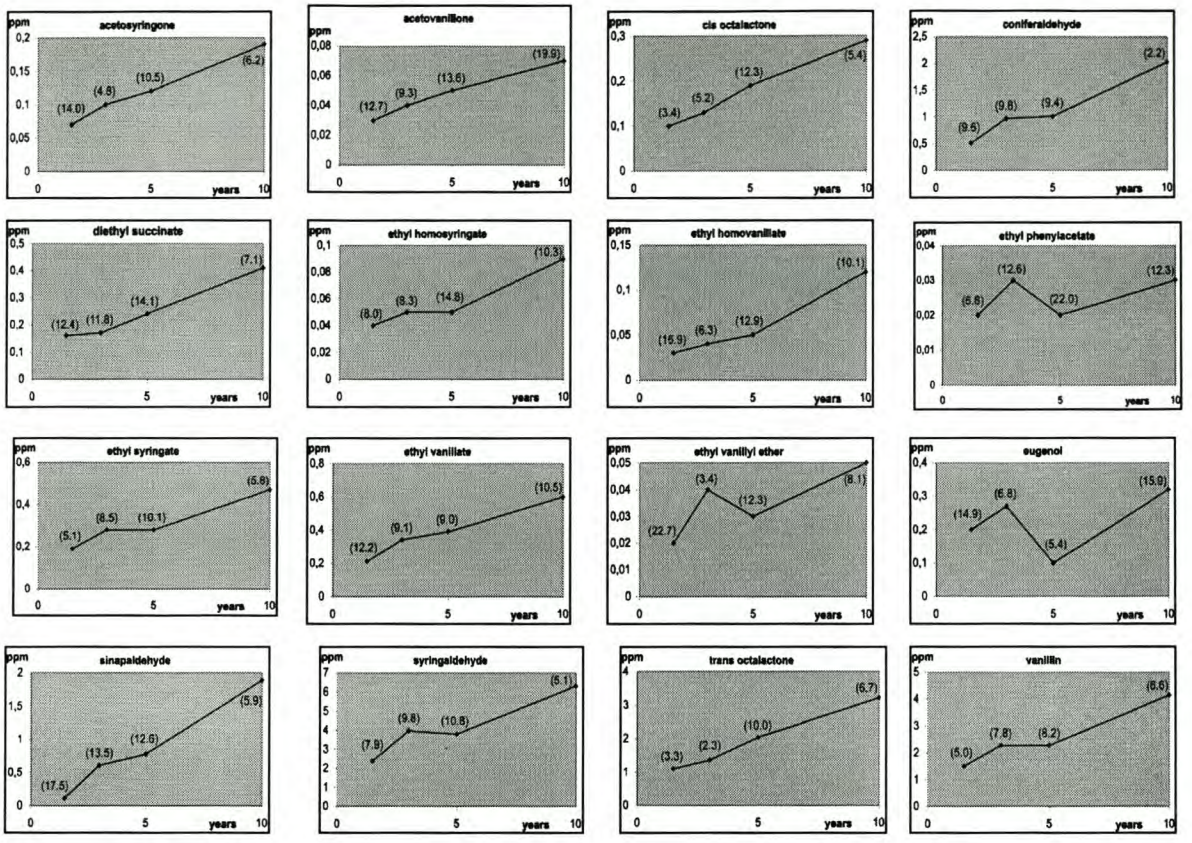

Figure 3. Increase in concentrations [1] of oak derived aroma compounds in whiskey during ageing [2].

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

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

These graphical data indicate that the concentrations of these compounds increased over time. The extraction of the *cis* and *trans* β-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 reuse 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, Paterson & Piggott, 1993). An important consequence is that barrels for reuse 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 (Deibner, Jouret & Puech, 1976; Puech *et al.*, 1977; Puech, Jouret & Deibner, 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 characterized 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, Nykänen & Moring, 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 β-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.

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

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 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 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 materialize 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, Jouret & Moutounet, 1973). Therefore, characteristic analytical profiles of aged distilled spirits must be interpreted in terms of the different variables of wood type, wood pretreatment, barrel history in the reusage 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.

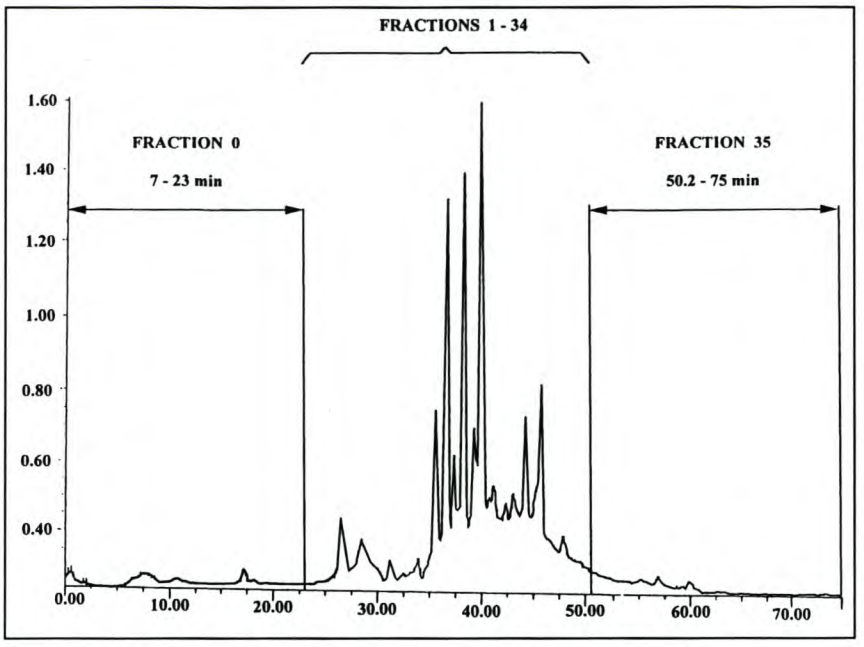

Figure 4 HPLC-UV[1)] trace of fraction 5 concentrated extract [2)].

[1)] Ethanol-water gradient. [2)] 36 cuts per injection as indicated.

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 compounds 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.

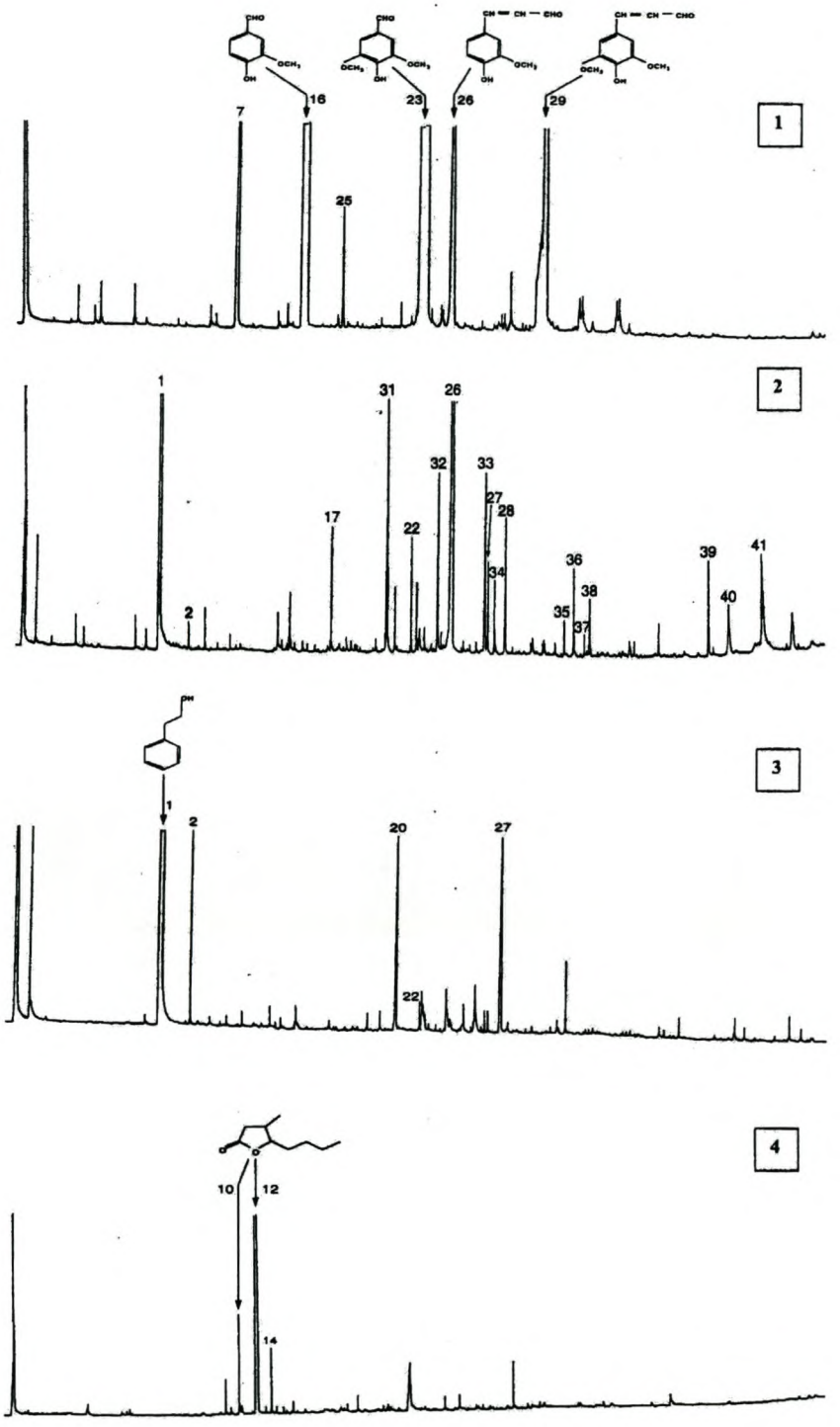

Figure 5 GC-MS traces[1-4)] of extracts of composites after preparative HPLC separation of concentrated fraction 5 from 10 years old whiskey.

[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.

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).

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-dimethoxy-phenyl)-ethyl acetate[b]	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.**

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-methyl-propiovanillone 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 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 have 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 syringyl-methylketone. 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, Jouret & Deibner, 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 summarizes the opinions of an experienced whiskey taste panel.

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.

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 assessor's 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 three year and ten-year-old whiskeys. Initially sixteen judges were used, but in an initial screening judges that were not able to detect the ten year old product or those that did not rate the ten year highest in maturation characteristics were excluded. Table 5 represents the results of the tasting after three months using the twelve remaining judges.

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	$72,69^{a}$

* **LSD (p = 0,05) = 10,08**

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

tion odour to some extent demonstrates that these esters are in 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, it's 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.

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CHAPTER 5

CONCLUSIONS

Preliminary vacuum fractional distillation followed by high-resolution capillary gas chromatography with mass spectrometric and sulfur chemiluminescent detection, permitted monitoring of changes in a range of compounds involved in the ageing of whiskey in oak barrels.

The fractional distillation gave a useful pre-separation of the whiskey. The distillation was at ambient temperature and therefore no thermal changes were induced. The fractions could be recovered and were available for sensory evaluation, since the separation was achieved in the natural matrix of the whiskey. Important low and high volatility compounds were isolated practically free from the whiskey ethanol and it's fusel compounds. Sensory and analytical data indicated good recovery of all compounds. Our work has shown that this technique is a very practical and suitable technique for the pre-separation of distilled spirits. Even though the separation is based on relative azeotropic volatility, it is still a volatility separation, and an important consequence is that the isolated fractions can be analysed by specific chromatographic techniques suited to their volatility.

Changes in the volatile compounds with ageing were analysed using capillary gas chromatographic techniques. These included specific sulfur detection, and two-dimensional heart cutting on serially coupled columns to overcome inherent limitations of solvent interference and compound co-elution. These techniques are relatively new and use modern developments in instrumental hardware and software. In our opinion they will become the next generation of routine laboratory techniques. Substantial changes in high volatility compounds occurred with ageing. Compounds associated with the pathway for oxidation of ethanol increased. Volatile sulfur compounds showed major decreases. Increases were also established for a range of ethyl esters and acetates, which are associated with fruity, pleasant notes. The observed changes in concentrations of the compounds in question and their generally low threshold values imply that these changes might contribute significantly to the odour of whiskey.

An important achievement of the vacuum fractional distillation process is the isolation in the water fraction of low volatility whiskey compounds. The majority of these compounds originate from the wood and many more develop during ageing through interdistillate reactions. Gas chromatographic analysis of these compounds is also facilitated by the prior removal of the complex fusel fraction. This allowed many compounds to be identified by GC-MS in an extract of a ten-year-old whiskey water fraction. Profiles of ageing changes could also be established for ageing of whiskey in oak barrels. Three phenolic esters were synthesised and found to be contributory to maturation intensity when added to young whiskeys. An important finding was unique ratios of phenolic aldehydes, which seem to be related to the particular barrel specification used for this study.

While it is recognized that the ageing flavour of whiskey is substantially influenced by oak wood maturation, there is evidence to suggest that these changes may be more related to wood type or it's pre-treatment than previously thought.