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Analysis of volatile components of oak wood by solvent extraction and direct thermal desorption–gas chromatography–mass spectrometry

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Abstract

Volatile compounds with important sensory properties (*cis*- and *trans*-β-methyl-γ-octalactone, eugenol, vanillin and syringaldehyde) present in oak woods have been studied. Two analytical methods have been compared: GC–MS analysis of wood hydroalcoholic extracts, and direct thermal desorption (DTD)–GC–MS in the selective ion monitoring mode. Although the quantitative results obtained from the two methods were comparable, the DTD based method is of considerable interest since woods of different origins can be discriminated rapidly from the ratio of the two oak lactone isomer concentrations, using a small (20 mg) sample size. © 1997 Elsevier Science B.V.

Keywords: Wood; Wine; Lactones; Eugenol; Vanillin; Syringaldehyde

1. Introduction

Most casks used to age wines and spirits (brandy, cognac, whisky, rum) are made of oak wood. The species most commonly used for wood are the American oak (a member of the white oak group, 45% being *Quercus alba*) and the French oak species (*Quercus robur* and *Quercus pedunculata*, followed by *Quercus sessilis*). There are two good treatises on the composition of oak wood and the substances leaching from the wood into wines or distillates [1,2].

The amount of components released by the wood during ageing will depend upon the species of wood, cask size, individual cask history (including manufacture, charring and initial conditioning and the number of times it was previously used) and warehouse conditions, although wood type and wood treatment are the main factors [3].

Most of the researchers who have investigated different types of oak wood have used an extraction step with organic solvents, hydro–alcoholic solutions and even wines or spirits, in contact with wood chips [4,5]. The extracts are usually analysed by GC (often with MS detection) or by HPLC [6]. Of the many volatile compounds identified in the extracts prepared under those conditions, some are quite important on account of their sensory properties and low threshold values [7].

Oak lactones (*cis*- and *trans*-β-methyl-γ-octalactone) in white wines aged in the wood were first described by Kepner et al. [8]. Günter and Mosandl [9] analysed the *cis* and *trans* configurations of these lactones and reported that 77% were in the *cis* configuration and 23% in the *trans* configuration. Detection thresholds for the lactones have been

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calculated for whisky [10] and wine [11] and established by sniffing [12]. Both types of lactones are present in Rioja white wines and some red wines in concentrations close to the threshold value and contribute to overall bouquet.

Even though their threshold values are higher than those for the lactones, synergic effects enable eugenol and the phenolic aldehyde derivatives of lignin leached out of the wood by ethanolysis (vanillin and syringaldehyde), to be detected in the aromas of wines and spirits that have been in contact with wood [13].

The wood is heated when bending the staves to make the casks, increasing phenolic aldehyde concentrations through pyrolysis of the lignin [14,15]. Heating and charring the wood does not seem to affect the ratio between the two oak lactone isomers [16] and consequently this ratio has been used as an index value for identifying woods of different species and different geographical origins. American white oak contains more *cis* isomer than French *Quercus robur* [17] and *Quercus sessilis* [18]. Variability among trees of the same species or region and differences in the treatments applied to the casks must be taken into account when differentiating between species, and the sampling method employed is of great importance. Towey and Waterhouse [19] reported variabilities of 30% in the chemical composition of oak casks in the same lot when four casks per lot were sampled, dropping to 10% when thirty-nine casks were sampled.

The present study has employed two different methods to analyse five volatile components with important sensory properties (*cis* and *trans*-β-methyl-γ-octalactone, eugenol, vanillin and syringaldehyde) present in four types of oak wood. The methods were: (a) steeping of oak chips in a hydro-alcoholic solution followed by dichloromethane extraction and GC–MS analysis; (b) direct thermal desorption (DTD) fractionation followed by GC–MS analysis in selective ion monitoring (SIM) mode. Direct thermal desorption has been successfully employed in the analysis by GC–MS of the volatile compounds in aromatic plants [20], but to date it has not been applied to wood analysis. Thermal treatment of samples in DTD systems can also be used to simulate the heating conditions employed in cask stave processing.

2. Experimental

2.1. Wood samples

Three samples of French oak (from Allier, Vosges and central France) and one sample of American oak were available, in the form of wood chips: they were ground prior to DTD fractionation.

2.2. Solvent extraction of volatile components from the wood

7 g of wood chips were steeped in darkness in 300 ml of a 12% (v/v) hydro–alcoholic solution (pH= 3.6) for six months. After that time the samples were filtered, and 50 μl of a γ-hexalactone solution (0.25 g/l) were added to 50 ml of filtrate as internal standard. A single extraction was performed using 8 ml of dichloromethane. The extract was concentrated to a final volume of approximately 200 μl under a nitrogen atmosphere.

2.3. GC and MS conditions for solvent extraction analysis

Sample extracts were analysed using a Model HP-5890 gas chromatograph equipped with a Model HP-5971A mass detector (Hewlett-Packard, Palo Alto, CA, USA). The column was a 50 m×0.25 mm, 0.25 μm SPB-1 methyl silicone column (Supelco, Bellefonte, PA, USA). Column temperature was 80°C (3 min)—3°C/min—250°C (40 min). Helium was used as carrier gas at 28 cm s^{-1}. Injection (1 μl) was carried out in the splitless mode (1 min, injector temperature 250°C). Transfer line temperature was 280°C. Mass spectra were recorded in the scan mode at 70 eV (1 scan s^{-1}, source temperature 180°C).

2.4. Direct thermal desorption (DTD) of wood samples

The ATD-400 is an automatic thermal desorption system developed by Perkin-Elmer (Norwalk, CT, USA). Volatile compounds trapped in a solid support are desorbed and injected onto a chromatographic column. Samples with a low water content can be directly introduced in the desorption system [20].

An amount of 20–125 mg of ground wood was placed together with the internal standard (0.741 μg of γ-hexalactone) in the desorption tube, plugged at both ends with silanized glass wool. The desorption tube was heated to the selected temperature (180 to 250°C range) for the selected time. The volatile substances were desorbed in a stream of helium and collected into a cold trap (−30°C) packed with Tenax TA (60–80 mesh, Supelco). The trap was quickly heated to 300°C and the volatile compounds transferred to the chromatographic column through a line heated to 225°C. Other experimental conditions are detailed in [20].

2.5. GC and MS conditions for DTD based analysis

A Fisons Model GC 8000 gas chromatograph connected to a Model MD 800 mass detector was used for GC analysis, with a laboratory-made 25 m×0.2 mm I.D. SE-54 column with a film thickness of 0.2 μm. The oven temperature program was 60°C (3 min)—6°C/min—250°C (40 min). Transfer line temperature was 225°C. Helium was the carrier gas at 30 cm s^{-1}.

Ionisation was carried out by electron impact (EI mode) with an electron energy of 70 eV. Mass spectral data and retention times were determined for standard compounds. Selected ions for SIM were: internal standard (γ-hexalactone), *m/z* 85; *cis*- and *trans*-β-methyl-γ-octalactones, *m/z* 99; eugenol, *m/z* 164; vanillin, *m/z* 152; syringaldehyde, *m/z* 182.

Relative ion abundance and high *m/z* value were the requirements used in choosing the ions.

3. Results and discussion

3.1. GC–MS analysis of extracts

Chromatographic analysis of the hydroalcoholic oak-wood extract using MS in the full scan mode identified more than 40 different compounds (Fig. 1 for an Allier sample), including the most relevant from a sensory standpoint: the two oak lactones, eugenol, vanillin and syringaldehyde. Semi-quantitative data were calculated from total ion current (TIC) peak areas, supposing the same response for the determined compounds and the standard. Table 1 shows the contents (in μg/g of wood) of those substances in the four types of wood considered.

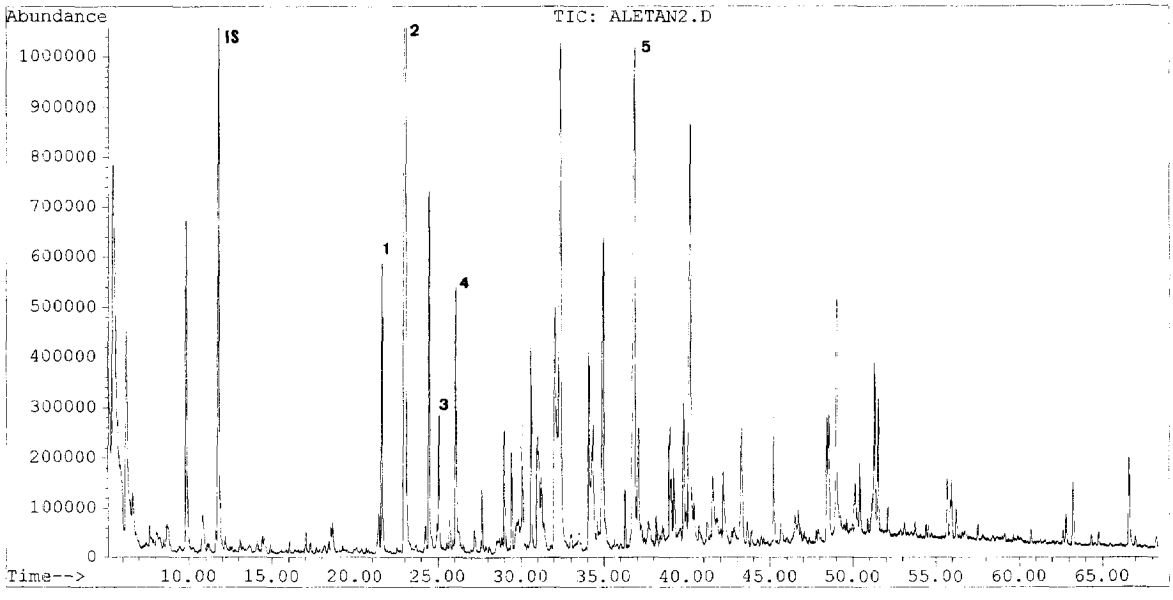

Fig. 1. Total ion current profile of the hydro–alcoholic extract of a French oak wood (Allier) analysed by GC–MS. I.S.=internal standard; 1=*trans*-β-methyl-γ-octalactone; 2=*cis*-β-methyl-γ-octalactone; 3=eugenol; 4=vanillin; 5=syringaldehyde.

Table 1
Volatile compounds (*cis*- and *trans*-β-methyl-γ-octalactone, eugenol, vanillin and syringaldehyde, μg/g of wood) in a hydroalcoholic extract of American and French (Allier, Vosges and central France) oak woods analysed by GC–MS

Compound	American oak	French oak		
		Allier	Centre of France	Vosges
trans-β-Methyl-γ-octalactone	4.07	4.82	22.58	11.77
cis-β-Methyl-γ-octalactone	37.02	27.82	25.36	44.51
Eugenol	1.93	2.46	5.14	2.78
Vanillin	9.84	4.82	5.46	8.99
Syringaldehyde	23.97	14.12	13.16	22.68

3.2. DTD–GC–MS analysis

An amount of 125 mg of French Allier oak wood were placed in the desorption tube of the DTD system at 180°C for 15 min, and analysed by DTD–GC–MS in the conditions described in Section 2.5, using a total split ratio of 1:60. Fig. 2 presents the resulting complete chromatogram (TIC trace).

cis- and *trans*-β-Methyl-γ-octalactone, eugenol, vanillin and syringaldehyde, which were present in very small quantities, were identified by comparing their mass spectral characteristic peaks and retention

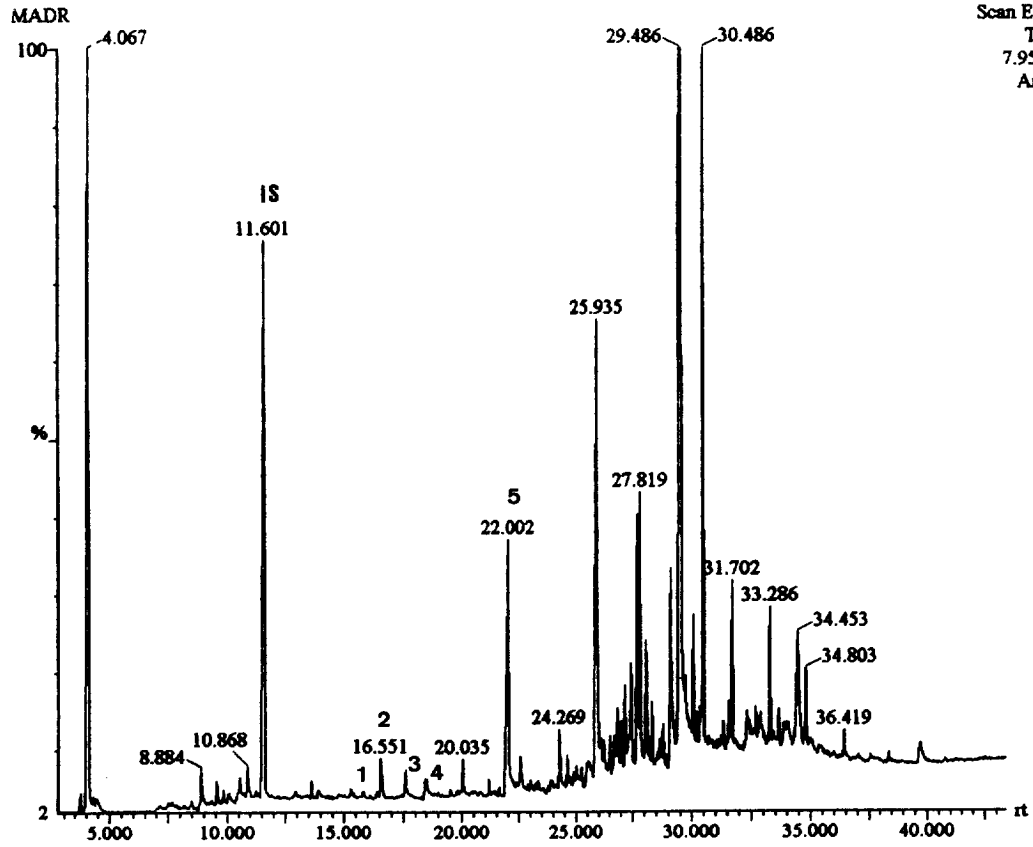

Fig. 2. Total ion current profile of volatiles from a French oak wood (Allier) analysed by DTD–GC–MS. I.S.=internal standard; 1=*trans*-β-methyl-γ-octalactone; 2=*cis*-β-methyl-γ-octalactone; 3=eugenol; 4=vanillin; 5=syringaldehyde.

Table 2
Analytical results (peak area/peak area of internal standard) for *cis*- and *trans*-β-methyl-γ-octalactone, eugenol, vanillin and syringaldehyde desorbed at 180°C using different desorption times

Minutes	*trans*-β-Methyl-γ-octalactone	*cis*-β-Methyl-γ-octalactone	Vanillin	Eugenol	Syringaldehyde
10	0.079	0.714	0.789	0.043	0.978
20	0.012	0.116	0.102	0.008	0.116
30	0.004	0.060	0.031	0.003	0.032
Total	0.095	0.890	0.922	0.053	1.126

times with those of standard compounds. A series of larger peaks for medium and long-chain hydrocarbons, fatty acids and esters appeared at the end of the chromatogram. These less volatile substances are partially retained in the less heated region of the DTD and may interfere with subsequent injections. To avoid this problem it was considered necessary to reduce the amount of sample, which in turn would reduce the peak size for the compounds of interest. The SIM mode makes it possible to lower the amount of sample to 20 mg, in spite of the high split ratio, while increasing sensitivity and decreasing noise by recording only the selected fragments of the mass spectra for the substances being studied. Detection limits (calculated for a 5:1 signal-to-noise ratio) ranged from 0.25 ng for syringaldehyde to 0.05 ng for eugenol and β-methyl-γ-octalactones.

3.3. Effect of desorption time and temperature

The effect of temperature on the extraction of the selected components was tested by desorbing American oak at 180°C, 200°C, 220°C and 240°C for different desorption times. An initial desorption lasting 10 min was carried out, and each of the components analysed was quantified. After a blank run, desorption of the same sample was repeated for a further 10 min, and so on successively in 10-min increments up to a total desorption time of 60 min, the objective being to ascertain the influence of desorption time and temperature on the extraction yields.

Tables 2–5 present the quantities desorbed by 10 min interval relative to the internal standard for each of the components considered. At a desorption temperature of 180°C, a desorption time of 10 min sufficed to desorb between 80 and 90% of the total quantity desorbed in 30 min, while no significant amount was extracted after a 30 min desorption time.

When desorption temperatures were higher than 180°C, the amount extracted increased for all the studied compounds. However, in the first 10 min only 50–70% of total amount was extracted and an hour or more was required to achieve complete desorption.

The incomplete desorption of all the substances at

Table 3
Analytical results (peak area/peak area of internal standard) for *cis*- and *trans*-β-methyl-γ-octalactone, eugenol, vanillin and syringaldehyde desorbed at 200°C using different desorption times

Minutes	*trans*-β-Methyl-γ-octalactone	*cis*-β-Methyl-γ-octalactone	Vanillin	Eugenol	Syringaldehyde
10	0.224	2.260	1.427	0.199	0.440
20	0.050	0.460	0.173	0.043	0.087
30	0.019	0.176	0.044	0.017	0.021
40	0.041	0.403	0.066	0.032	0.008
50	0.030	0.291	0.057	0.031	0.010
60	0.025	0.241	0.040	0.027	0.009
Total	0.388	3.831	1.807	0.349	0.575

Table 4
Analytical results (peak area/peak area of internal standard) for *cis*- and *trans*-β-methyl-γ-octalactone, eugenol, vanillin and syringaldehyde desorbed at 220°C using different desorption times

Minutes	*trans*-β-Methyl-γ-octalactone	*cis*-β-Methyl-γ-octalactone	Vanillin	Eugenol	Syringaldehyde
10	0.333	3.235	2.557	1.484	1.689
20	0.136	1.299	0.654	1.099	0.539
30	0.096	0.821	0.407	0.769	0.494
40	0.031	0.313	0.145	0.298	0.189
50	0.018	0.194	0.079	0.177	0.052
60	0.003	0.044	0.019	0.051	0.024
Total	0.618	5.908	3.861	3.876	2.986

high temperatures appears to be attributable to a pyrolytic effect. Pyrolysis of the non-volatile components present in the sample (lignin, cellulose, etc.) resulting in the formation of volatile substances has been investigated earlier [7,21]. In fact, the insides of casks are charred to achieve this effect, thereby augmenting the amounts of substances that can be leached from the wood. Therefore, different desorption temperatures can be employed to model the effect of charring of the wood on the amount of extractable volatile substances.

The internal standard added to the samples was completely desorbed in the first 10 min period: complete desorption in the first 10 min also occurred when the substances analysed were placed directly in the desorption tube in the form of standard compounds.

3.4. Method reproducibility

After establishing the influence of desorption time and temperature in the analysis of the oak lactones, eugenol, vanillin and syringaldehyde, a study was performed to verify the reproducibility of the method. A 15 min desorption time and a 180°C desorption temperature were selected in order to minimize sample pyrolisis, although recovery was not complete.

The ratio between the two isomers of β-methyl-γ-octalactone was selected as the test variable, since it has been considered the most important criterion to characterize oak wood samples of different origin. Certain workers have suggested using the ratio between the two oak lactone isomers as an indicator to distinguish between different wood types, because the ratio remains constant even when the wood has been subjected to different heat treatments [16,18].

Ten successive analyses of the lactones were performed in two different sample types, American oak and one of the French oaks (Allier). The results appear in Table 6.

The method afforded a good level of reproducibil-

Table 5
Analytical results (peak area/peak area of internal standard) for *cis*- and *trans*-β-methyl-γ-octalactone, eugenol, vanillin and syringaldehyde desorbed at 240°C using different desorption times

Minutes	*trans*-β-Methyl-γ-octalactone	*cis*-β-Methyl-γ-octalactone	Vanillin	Eugenol	Syringaldehyde
10	0.193	1.348	1.799	2.193	3.073
20	0.035	0.225	0.328	0.734	0.773
30	0.015	0.100	0.175	0.395	0.449
40	0.009	0.059	0.119	0.288	0.308
50	0.009	0.069	0.156	0.312	0.337
60	0.007	0.053	0.110	0.259	0.295
Total	0.268	1.855	2.689	4.181	5.235

Table 6
Distribution of the *cis*-β-methyl-γ-octalactone/*trans*-β-methyl-γ-octalactone concentration ratio values for ten American and French (Allier) oak woods

Kind of oak	Maximum	Minimum	Mean	Standard deviation
American	10.5	9.1	9.67	0.45
French (Allier)	6.1	5.6	5.92	0.19

ity and proved to be suitable for comparing wood samples. DTD–GC–MS(SIM) analysis of woods using the method described provides a rapid means of obtaining this ratio using small sample quantities (20 mg).

3.5. Application of the method in the analysis of the oak lactones of wood samples from different sources

DTD based analyses were also performed to detect the presence of the two lactone isomers in samples of French oak from Vosges and central France. Table 7 presents the ratio values for the lactone isomers in the four wood samples and also the results obtained for duplicate GC–MS analysis of the hydro–alcoholic extracts for the same wood types.

The ratio values obtained by the two methods were quite similar, demonstrating that the desorption temperature employed in the DTD analysis (180°C) was suitable and that the method was comparable to the extraction of the lactones using hydro–alcoholic solutions.

The differences between samples of different origin were easily appreciable, corroborating the usefulness of this ratio as an index value for differentiating between types of oak wood. The lactone isomer ratio values also showed good agreement with the values published by other workers for these types of wood [17,18,22].

Table 7
cis-β-Methyl-γ-octalactone/*trans*-β-methyl-γ-octalactone concentration ratio values in the four American and French oak woods studied, determined by DTD–GC–MS(SIM) and by GC–MS of the hydro–alcoholic extracts

Method of analysis	American oak	French oak		
		Allier	Centre of France	Vosges
DTD–GC–MS(SIM)	9.67	5.92	1.09	3.78
GC–MS	9.02	5.83	0.99	4.14

4. Conclusions

Volatile components in oak wood are present as a very complex mixture but in low concentrations. GC–MS analysis requires preparatory fractionation by means of extraction or distillation using 1 to 10 g of sample. The proposed method, based on direct thermal desorption, eliminates the need for such preliminary fractionation step and reduces the quantity of sample required to 20 mg; the necessary sensitivity is achieved by employing mass spectrometry in SIM mode.

Reproducibility of the results obtained was comparable to that of fractionation-based techniques, making the method appropriate for use in characterizing wood samples and for discriminating between samples of different origin on the basis of the chemical composition of the samples. The method present as advantages a smaller sample size and a reduced analysis time. Strict control of desorption temperatures can be used to model the wood charring process with on-line analysis of the desorbed components.

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