

Volatile and semi-volatile components of oak wood chips analysed by Accelerated Solvent Extraction (ASE) coupled to gas chromatography–mass spectrometry (GC–MS)

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Received 31 March 2006; received in revised form 6 July 2006; accepted 6 July 2006

Abstract

Volatile and semi-volatile compounds of French and American oak wood used in wine and spirits ageing were extracted by an Accelerated Solvent Extractor (ASE) and analysed by gas chromatography–mass spectrometry. More than 90 compounds were isolated, characterised and quantified after being separated on two chromatographic columns with distinct polarity. The main oak wood components were quantitatively determined by using standard reference compounds. In addition, a number of compounds, mainly guaiacol and syringol derivatives, were detected and characterised. In particular, 10 compounds were tentatively identified as lignin dimers derivatives, whose presence in oak wood chips or barrels for wine and spirits ageing had not been previously described. Several of the characterised compounds enabled a distinction to be drawn between medium and high toasted wood chips, independently of their geographical origin.

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Keywords: Oak wood chips; Toasting; Volatile; Semi-volatile; Lignin dimers; Accelerated Solvent Extraction; ASE; Mass spectrometry

1. Introduction

Wood ageing is a common practice in the production of alcoholic beverages and one of key factors that influence their quality (Mosedale & Puech, 1998; Singleton, 1994). American or French oak wood species have traditionally been used for this purpose owing to both their mechanical properties and their extractable compounds, which can influence the composition of wines and distillates (Pérez-Coello, Sanz, & Cabezudo, 1999). One alternative to ageing alcoholic beverages and wine vinegars in oak barrels is to use oak chips (Mosedale & Puech, 1998; Morales, Benitez, & Troncoso, 2004; Pérez-Coello, Sánchez, García, Gonzá-

lez-Viñas, & Cabezudo, 2000), which are prepared according to traditional cooperage methods, including boiling in water and toasting.

Oak wood chemical composition mainly depends on the species, its provenience and the various treatments the wood undergoes in cooperage, such as seasoning and toasting (Cadahía, Fernández de Simón, & Jalocha, 2003; Cadahía, Muñoz, Fernández de Simón, & García-Vallejo, 2001; Chatonnet & Dubourdiou, 1998; Doussot, De Jeso, Quideau, & Pardon, 2002; Marco, Artajona, Larrechi, & Rius, 1994; Pérez-Prieto, López-Roca, Martínez-Cutillas, Pardo-Mínguez, & Gómez-Plaza, 2002). In particular, toasting has a significant influence on wood's chemical compounds, modifying both the quantity and the quality of the extractable substances in oak (Chatonnet, Cutzach, Pons, & Dubourdiou, 1999; Cutzach,

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Chatonnet, Henry, & Dubourdiou, 1997). Hence, extensive information about the volatile composition of oak barrel or chips would be of great interest to the wine industry. Toasting drastically enhances the gain in volatile compounds arising from the thermal degradation of oak wood (Chatonnet et al., 1999; Cutzach et al., 1997; Dousot et al., 2002). During this process, wood biopolymers such as lignin, polyosides and lipids are degraded by pyrolysis and thermolysis reactions, which induce a notable modification of the chemical composition of wood. Mainly volatile phenols, phenolic aldehydes, phenyl ketones and some phenyl alcohols are formed from lignin thermodegradation. In particular, high levels of mono and dimethoxylated phenols, benzoic and cinnamic aldehydes were identified in toasted wood. Heat degradation of polyosides leads to the production of furanic aldehydes, pentacyclic and hexacyclic ketones, and from wood lipids lactones are formed, which increase their concentration at the beginning of toasting, although they can be destroyed by a lengthy toasting process (Cadahía et al., 2003; Chatonnet et al., 1999; Giménez-Martínez, López-García de la Serrana, Villalón-Mir, Quesada-Granados, & López-Martínez, 1996).

The aim of this paper was to obtain additional information on the volatile composition of oak wood chips used for wine ageing, especially in relation to the toasting degree. Volatile and semi-volatile compounds present in French and American oak wood with different treatments (medium and high toasting) were isolated, characterised and quantified by Accelerated Solvent Extraction (ASE) coupled to gas chromatography–mass spectrometry.

2. Materials and methods

2.1. Reagents, standards and samples

Dichloromethane for gas chromatography was supplied by Merck (Darmstadt, Germany) and anhydrous Na_2SO_4 came from BDH Chemicals (Poole, UK). Internal standard (2-octanol) and reference standard compounds reported in Table 1 were purchased by Sigma-Aldrich (St. Louis, Missouri, USA).

The 15 oak wood chips of French and American origin used in this study were commercial samples supplied by several producers. Samples were characterised by different toasting treatments: “medium” ($n = 5$) and “high” toasting ($n = 10$). Toasting characteristics are protected by industry secret.

2.2. Accelerated Solvent Extraction (ASE)

Extraction from wood chips was carried out using an Accelerated Solvent Extractor ASE 200. Five grams of chips were weighted and placed in the inox extraction cell in the oven of the instrument. Extraction was carried out at the temperature of 150 °C using dichloromethane as

extraction solvent. After the injection of the solvent into the cell, a pressurised static extraction phase lasting 7 min was carried out (20 MPa), followed by a flow of fresh dichloromethane. After removal of the extracts (approx. 20 ml), they were added with Na_2SO_4 , filtered and added with internal standard (2-octanol). They were successively evaporated under nitrogen flow to a volume of approximately 2 ml.

2.3. GC–MS analysis

GC analyses were performed on an Agilent Technologies 6890 N Network gas chromatograph coupled to an Agilent Technologies 5973 Network quadrupole mass selective spectrometer and provided with a split/splitless injection port. Helium was the carrier gas, at a linear velocity of 38 cm/s. Compounds were separated on a HP-5MS capillary column (Hewlett-Packard, Avondale, PA, USA) and successively on a SPB-1 capillary column (Supelco Ltd., Bellefonte, PA, USA), both 30 m × 0.25 mm ID, 0.25 μm film thickness. Column temperature was held at 40 °C for 5 min and increased to 75 °C at 4 °C/min, then at 8 °C/min to 250 °C holding 10 min. The injector temperature was 250 °C, and samples (1 μl) were injected in the splitless mode.

The temperatures of the ion source and the transfer line were 175 and 280 °C, respectively. Positive ion electron impact mass spectra were recorded at 70 eV ionisation energy, 2 scan/s.

2.4. Identification and quantification of compounds

GC–MS analysis in the complete scanning mode (SCAN) in the 40–400 amu mass range was performed to allow the identification of compounds. Chromatographic peaks were identified by comparing their retention times and mass spectra with those of standards or those reported in the literature, or else by comparison of their mass spectra with those included in the Wiley 6 commercial library. Some compounds remained unidentified or only tentatively identified on the basis of their mass spectra.

Finally, all the detected compounds were characterised by their mass spectral fragmentation and by Kovat's retention indices determined on two distinct chromatographic capillary columns.

Quantitative assessment of compounds was carried out by the internal standard method. The main volatile compounds present in the samples were quantified by using the experimental relative response factors calculated by using standard reference compounds. Standard solutions in dichloromethane were prepared in the range 26–260 mg l⁻¹ (26, 80, 130, 200, 260 mg l⁻¹) and analysed in duplicate under the same conditions described for samples.

Relative concentrations of the remaining compounds with respect to the internal standard were calculated by using the representative ions described in Table 1.

Table 1
Characterisation and relative concentrations of volatile and semi-volatile compounds detected in oak wood chips

Compound	Ion ^a <i>m/z</i>	KI ^b	KI ^c	Mass spectral data ^d <i>m/z</i>	ID ^e	References ^f	MT ^g (<i>n</i> = 5)	HT ^h (<i>n</i> = 10)	<i>p</i> ⁱ
1 Acetic acid	43	602	–	43(100), 45(88), 60(62)	S, MS, RT	1, 2, 3	n.q.	n.q.	
2 Propanoic acid	74	668	620	74(100), 45(74), 73(70)	S, MS, RT	3	0.07	0.24	
3 Butanoic acid	60	827	790	60(100), 41(33), 43(31)	S, MS, RT	1	0.05	0.08	
4 2-Furancarboxyaldehyde (furfural)	96	831	801	96(100), 95(98)	S, MS, RT	1, 2, 3, 4, 5	12.3	14.6	
5 1-(2-Furanyl)-ethanone	95	911	880	95(100), 110(34)	MS ^j	2, 3	0.07	0.12	
6 Dihydro-2(3 <i>H</i>)furanone (γ -butyrolactone)	42	913	883	42(100), 28(93), 86(56), 56(41)	S, MS, RT	2, 3	–	0.03	
7 2 <i>H</i> -Pyran-2-one	68	945	–	39(100), 68(85), 96(66)	MS		0.03	0.09	
8 Benzaldehyde	105	957	925	77(100), 105(89), 106(91)	S, MS, RT	1, 2, 3	–	0.01	
9 5-Methylfurancarboxyaldehyde (5-methylfurfural)	110	963	929	110(100), 109(89), 53(47)	S, MS, RT	1, 2, 3, 5	1.45	1.59	
10 Hexanoic acid	60	982	891	60(100), 73(48)	S, MS, RT	1, 3, 4	0.06	0.06	
11 2-Octanol (I.S.)	45	1001	988	45(100), 55(38), 70(12)	IS	–	1.00	1.00	
12 Phenylacetaldehyde	91	1046	1004	91(100), 65(25), 120(16)	MS		0.06	0.02	
13 γ -Ethoxy-butylolactone	85	1070	–	57(100), 58(98), 85(79)	MS		0.09	0.17	
14 2-Furancarboxylic acid	95	1072	1065	112(100), 95(87)	MS	1	0.05	0.08	
15 Furandicarboxyaldehyde	124	1079	1024	124(100), 123(59), 95(50)	MS ^j	2, 6	0.51	0.49	
16 Furylhydroxymethylketone	95	1083	1054	95(100), 126(49)	MS ^j	6	0.36	0.27	
17 2-Methoxyphenol (guaiacol)	109	1090	1061	109(100), 124(87), 81(50)	S, MS, RT	1, 2, 3, 5	0.05	0.12	
18 3-Hydroxy-2-methyl-4 <i>H</i> -pyran-4-one (maltol)	126	1111	1074	126(100), 97(29), 71(25)	MS	2, 3	0.06	0.29	
19 2-Furanmethanol	98	1113	–	98(100), 81(60), 69(29)	S, MS, RT	2	0.05	0.08	
20 4-Methyl-2-methoxyphenol (methyl guaiacol)	138	1194	1164	138(100), 123(98) 95(33)	MS, RT ^k	2, 3, 5	0.05	0.17	<0.05
21 5-Hydroxymethylfurancarboxyaldehyde (5-hydroxymethylfurfural)	97	1215	1192	97(100), 126(72), 109(13)	S, MS, RT	1, 2, 3, 5	0.99	0.84	
22 2-Ethyl-3-hydroxy-4 <i>H</i> -pyran-4-one (ethylmaltol)	140	1238	1340	140(100), 97(26), 125(24)	MS		0.05	0.10	<0.05
23 4-Ethyl-2-methoxyphenol (ethylguaiacol)	137	1282	1253	137(100), 152(67)	S, MS, RT	2, 3	0.01	0.13	
24 <i>trans</i> -4-Methyl-5-butylidihydro-2(3 <i>H</i>)furanone (<i>trans</i> - β -methyl- γ -octalactone)	99	1294	1252	99(100), 71(28)	S, MS, RT	1, 2, 3, 4	0.88	0.58	
25 Tridecane	57	1300	1300	43(100), 57(86), 71(42)	S, MS, RT	1	0.06	0.15	<0.05
26 5-Hydroxy-2-methyl-4 <i>H</i> -pyran-4-one (allomaltol)	126	1310	1260	126(100), 69(24)	MS		0.09	0.29	
27 4-Vinyl-2-methoxyphenol (vinylguaiacol)	150	1317	1313	150(100), 135(73), 107(28)	MS, RT ^k	2, 3, 5	6.98	1.09	
28 <i>cis</i> -4-methyl-5-butylidihydro-2(3 <i>H</i>)furanone (<i>cis</i> - β -methyl- γ -octalactone)	99	1327	1280	99(100), 69(25), (71(23)	S, MS, RT	1, 2, 3, 4	1.57	2.42	
29 2,6-Dimethoxyphenol (syringol)	154	1354	1312	154(100), 139(47)	S, MS, RT	2, 3	0.44	1.19	<0.05
30 2-Methoxy-4-(2-propenyl)phenol (eugenol)	164	1361	1329	164(100), 149(30)	S, MS, RT	1, 2, 3, 4, 5	0.17	0.18	
31 4-Hydroxy-3-methoxybenzaldehyde (vanillin)	152	1401	1351	151(100), 152(95)	S, MS, RT	1, 2, 3, 4, 5	7.75	18.2	<0.05
32 <i>cis</i> - or <i>trans</i> -2-Methoxy-4-(1-propenyl)phenol (<i>cis</i> - or <i>trans</i> -Isoeugenol)	164	1438	1325	164(100), 149(29)	S, MS, RT	1, 2, 3, 4, 5	–	0.06	
33 4-Methyl-2,6-dimethoxyphenol (methylsyringol)	168	1450	1517	168(100), 153(45), 125(25)	MS, RT ^k	2, 3	0.22	1.01	<0.05
34 <i>cis</i> - or <i>trans</i> -2-Methoxy-4-(1-propenyl)phenol (<i>cis</i> - or <i>trans</i> -Isoeugenol)	164	1454	1417	164(100), 149(28)	S, MS, RT	7	0.43	0.46	
35 1-(4-Hydroxy-3-methoxyphenyl)acetaldehyde (homovanillin)	137	1464	1409	137(100), 166(37), 122(25)	MS ^l , RT	2, 5	4.61	7.34	<0.05
36 n.i.	162	1483	1441	162(100), 147(72)	MS	3	2.10	1.40	
37 1-(4-Hydroxy-3-methoxyphenyl)ethanone (acetovanillone)	151	1490	1442	151(100), 166(51), 123(20)	S, MS, RT	1, 2, 3, 5	0.54	1.69	<0.05
38 (4-Hydroxy-3-methoxyphenyl)propanone isomer	137	1537	1482	137(100), 180(24), 122(16)	MS ^l		1.13	3.21	<0.05
39 1,2-Dimethoxyphenyl-4-(1-propenyl)	149	1564	1508	149(100), 178(93)	MS, RT ^k	7	2.55	1.52	
40 4-Hydroxy-3-methoxy-benzoic acid (vanillic acid)	168	1566	1529	168(100), 153(63)	S, MS, RT	1,8	0.68	2.03	<0.05

41	1-(4-Hydroxy-3-methoxyphenyl)-2-propanone (vanillyl propan-2-one)	180	1571	1521	180(100), 165(36), 137(27)	MS, RT ^k	2, 4	0.78	2.13	<0.05
42	1-(4-Hydroxy-3-methoxyphenyl)propanal (vanillyl propanal)	137	1581	—	137(100), 180(59), 124(45), 151(25)	MS, RT ^k		0.15	0.46	<0.05
43	1-(4-Hydroxy-3-methoxyphenyl)propanone (propiovanillone)	151	1588	1541	151(100), 180(22), 123(17)	MS ^l	1, 2, 3, 4	0.15	0.18	
44	4,5-Dimethoxyphenyl-2-(2-propenyl)	178	1591	1536	178(100), 151(85), 163(6)	MS, RT ^k	7	0.63	0.46	
45	1-(4-Hydroxy-3-methoxyphenyl)butanone (butyrovanillone)	151	1593	1539	151(100), 123(17), 194(4)	MS ^l	2, 3	1.26	2.77	<0.05
46	4-Hydroxy-3-methoxy-benzoic acid, methyl ether (vanillyl methyl ether)	168	1603	—	168(100), 137(17), 194(4)	MS ^l	2	0.10	0.42	<0.05
47	2,6-Dimethoxy-4-(2-propenyl)phenol (allylsyringol)	194	1608	1563	194(100), 167(21)	MS ^p , RT ^k	2, 3, 5	0.13	0.44	<0.05
48	4-Hydroxy-3-methoxy-benzoic acid, ethyl ether (vanillyl ethyl ether)	137	1654	—	137(100), 182(44), 108(3)	MS ^l	2	1.34	2.11	
49	1,2-Dihydroxy-3,4-dimethoxybenzene	170	1659	—	170(100), 155(76), 127(57), 109(39)	MS		2.30	1.39	
50	4-Hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde)	181	1667	1605	182(100), 181(61)	S, MS, RT	1, 2, 3, 4, 8	12.4	68.2	<0.05
51	n.i.	181	1678	—	137(100), 180(79), 124(43)	—	—	0.48	0.32	
52	4-Hydroxy-3-methoxy-benzoic acid, methyl ester (methyl vanillate)	151	1687	1625	151(100), 182(21), 123(15)	MS ^m	1	1.29	3.01	
53	n.i.	192	1692	1641	192(100), 177(41)	—	—	0.28	0.38	
54	n.i.	192	1698	1643	192(100), 177(56)	—	—	0.17	0.30	
55	<i>cis</i> - or <i>trans</i> -2,6-Dimethoxy-4-(1-propenyl)phenol (<i>cis</i> - or <i>trans</i> -Propenylsyringol)	194	1702	1655	194(100), 131(87), 179(6)	MS ^m , RT ^k	4,5	0.50	0.26	
56	<i>cis</i> - or <i>trans</i> -2,6-Dimethoxy-4-(1-propenyl)phenol (<i>cis</i> - or <i>trans</i> -propenylsyringol)	194	1707	1632	194(100), 179(19), 131(15)	MS ^m , RT ^k	5	0.62	1.48	
57	4-Hydroxy-3-methoxy-benzoic acid, ethyl ester (ethyl vanillate)	167	1711	1648	167(100), 196(30), 123(8)	MS	2, 4	7.27	17.8	<0.05
58	4-Hydroxy-3-methoxy-benzeneacetic acid, methyl ester (methyl homovanillate)	137	1734	1670	137(100), 196(20), 122(13), 180(8)	MS, RT ^k		6.82	8.02	
59	1-(4-Hydroxy-3,5-dimethoxyphenyl)ethanone (acetosyringone)	181	1739	1684	181(100), 196(49), 153(11)	MS, RT ^k	2, 3, 5	0.73	2.35	<0.05
60	3-Methoxy-4-hydroxycinnamaldehyde (coniferaldehyde)	178	1744	1689	178(100), 135(41), 147(34)	S, MS, RT	1, 2, 3, 4, 8	13.00	20.6	<0.05
61	3-Methoxy-4-hydroxycinnamyl alcohol (coniferyl alcohol)	137	1749	1680	137(100), 180(71), 124(58)	S, MS, RT	4	17.1	9.49	<0.05
62	1-(4-Hydroxy-3,5-dimethoxyphenyl)-2-propanone (syringyl propan-2-one)	167	1779	1714	167(100), 210(21), 123(7)	MS, RT ^k	1, 2	1.49	8.21	<0.05
63	1,2,3-Trimethoxyphenyl-5-(2-propenyl)	208	1812	1747	208(100), 179(44), 165(22), 193(8)	MS ^m , RT ^k	7, <i>n</i>	1.28	1.79	
64	4-Hydroxy-3,5-dimethoxy-benzoic acid (syringic acid)	198	1819	1824	198(100), 183(30), 167(7)	S, MS, RT	8	1.55	10.1	<0.05
65	1-(4-Hydroxy-3,5-dimethoxyphenyl)propanone (propiosyringone)	167	1827	1863	167(100), 210(39), 182(33)	MS, RT ^k	2, 3, 4, 5	0.28	1.07	<0.05
66	1-(4-Hydroxy-3,5-dimethoxyphenyl)butanone (butyrosyringone)	181	1833	1769	181(100), 210(5), 224(5)	MS, RT ^k	7	2.29	8.07	<0.05
67	1,2,3-Trimethoxyphenyl-5-(1-propenyl)	181	1837	1773	208(100), 165(13), 180(10)	MS, RT ^k	7	0.81	1.31	<0.05
68	n.i.	222	1879	1821	222(100), 131(80), 179(44)	—	—	0.71	0.26	<0.05
69	4-Hydroxy-3,5-dimethoxy-benzoic acid, methyl ether (syringyl ethyl ether)	167	1892	1831	167(100), 212(53), 137(8)	MS		0.18	1.96	<0.05
70	3,5-Dimethoxy-4-hydroxy-dihydro cinnamyl alcohol (dihydrosynaptic alcohol)	167	1898	1843	167(100), 168(93), 212(77)	MS, RT ^k	5	0.92	2.23	
71	1-(4-Hydroxy-3,5-dimethoxyphenyl)propanal (syringyl propanal)	210	1921	1753	210(100), 167(91)	MS		0.61	0.63	
72	4-Hydroxy-3,5-dimethoxy-benzoic acid, methyl ester (methyl syringate)	181	1931	1863	181(100), 212(21), 153(9)	MS ^m		1.65	7.54	<0.05
73	1-(4-Hydroxy-3,5-dimethoxyphenyl)-2- or 3-butanone (syringyl 2- or 3-butanone)	224	1951	1889	224(100), 167(47), 181(41)	MS		0.48	0.35	
74	Hexadecanoic acid	256	1958	—	73(100), 129(57), 256(18)	S, MS, RT	1	0.14	0.10	

(continued on next page)

Table 1 (continued)

Compound	Ion ^a <i>m/z</i>	KI ^b	KI ^c	Mass spectral data ^d <i>m/z</i>	ID ^e	References ^f	MT ^g (<i>n</i> = 5)	HT ^h (<i>n</i> = 10)	<i>p</i> ⁱ	
75	Scopoletin	192	1963	1895	192(100), 177(49), 149(42), 164(6)	MS	8	0.27	0.63	
76	4-Hydroxy-3,5-dimethoxy-benzoic acid, ethyl ester (ethyl syringate)	167	1973	1901	167(100), 208(26), 226(20)	MS		9.56	11.2	
77	3,5-Dimethoxy-4-hydroxycinnamaldehyde (sinapic aldehyde)	208	1989	1919	208(100), 165(43), 137(32), 180(25)	MS ^{l,m} , RT	1, 2, 4, 5, 8	22.2	81.3	<0.05
78	3,5-Dimethoxy-4-hydroxycinnamyl alcohol (sinapic alcohol)	210	1998	1936	210(100), 167(84), 149(27), 181(15)	MS ^p	4,5	19.8	13.9	<0.05
79	n.i.	238	2021	1963	238(100), 161(27), 194(24)	–	–	1.13	0.64	
80	n.i.	224	2036	1965	224(100), 167(9), 195(24)	–	–	0.29	0.73	<0.05
81	4-Hydroxy-3,5-dimethoxy-benzeneacetic acid, methyl ester (methyl homosyringate)	226	2068	1999	181(100), 226(32), 153(11)	MS		0.97	1.18	
82	1,2,3-Trimethoxyphenyl-5-propenoic acid methyl ester	252	2124	2054	252(100), 209(35), 161(24)	MS ^m		0.17	0.21	
83	n.i.	302	2907	2964	302(100), 181(4), 198(3)	–		2.72	5.43	<0.05
84	Sterol	414	2994	–	414(100), 396(59), 255(33)	MS		0.31	0.24	

1: Pérez-Coello et al. (1998); 2: Cadahía et al. (2003); 3: Chatonnet et al. (1999); 4: Pérez-Coello et al. (1999); 5: Antonelli et al. (1996); 6: Cutzach et al. (1999); 7: detected in oak wood smoke by Guillén and Ibargoitia (1998); 8: Cadahía et al. (2001); 9: Matějček et al. (2005).

^a Ion used for quantification.

^b Kovat's index calculated on a HP-5 capillary column.

^c Kovat's index calculated on a DB-1 capillary column.

^d Percent amount of principal fragments.

^e Identification method.

^f References of previous identification of compounds in oak wood or in oak wood smoke.

^g Medium toasted samples, mean amount expressed as relative areas.

^h High toasted samples, mean amount expressed as relative areas.

ⁱ Significance of the difference between medium toasted and high toasted samples.

^j Mass spectrum interpretation according to Cutzach et al. (1999).

^k Tentatively identified by retention time according to Guillén and Ibargoitia (1998, 1999) and Guillén and Manzanos (2002).

^l Mass spectrum interpretation according to Cadahía et al. (2003).

^m Mass spectrum interpretation according to Klap et al. (1998); S: identified by comparison with standard compounds; MS: tentatively identified by mass spectra; RT: tentatively identified by retention time.

3. Results and discussion

The GC–MS analysis of the oak wood chips extracts revealed the presence of more than 90 compounds. Characterisation results are described in Table 1, and comprise the molecular structure attribution, the identification method employed, the mass spectral data and the linear retention indices calculated on two distinct chromatographic columns. The molecular structures of the extracted compounds were attributed by comparison with reference standard compounds or only by tentative manner, as reported in Table 1. In several cases, retention times and mass spectra could be compared with those reported in the literature, whereas other compounds were characterised only by interpreting their mass spectra and chromatographic behaviour.

The majority of the compounds extracted by ASE are those reported in the literature as constituents of oak wood (Antonelli, De Giacomo, Galletti, Carnacini, & Bocchini, 1996; Cadahía et al., 2003; Cadahía et al., 2001; Chatonnet et al., 1999; Cutzach, Chatonnet, Henry, & Dubourdiou, 1999; Pérez-Coello, Sanz, & Cabezudo, 1998; Pérez-Coello et al., 1999). Moreover, a number of compounds whose presence had not been mentioned before in oak wood chips or barrels, were tentatively identified in the present study (Table 1). However, some of these compounds had been previously described as components of oak wood smoke preparations (Guillén & Ibargoitia, 1998, 1999; Guillén & Manzanos, 2002). Furan, pyran, syringol, guaiacol, lactone, dimethoxyphenyl and trimethoxyphenyl derivatives were detected in the oak wood chips extracted by ASE. In particular, a wide number of guaiacol and syringol derivatives were detected and characterised. The high ratio of guaiacol and syringol species in toasted oak seems to be due to the structure of angiosperms lignin (Cadahía et al., 2003). The mass fragments of the two series of compounds were distinguished by a methoxy group with m/z 30. Almost the same alkyl, keto, alcohol, aldehyde, acid, ester and ether derivatives were detected as guaiacyl- and syringyl homologues. In addition to the guaiacyl- and syringyl-ethanone, propan-1-one, and propan-2-one derivatives, their propanal and butanone derivatives were tentatively identified for both the guaiacol and the syringol structures (Table 1). Likewise, ethyl ether, ethyl, and methyl ester derivatives of guaiacol and syringol were tentatively identified, as well as the methyl esters of homovanillic and homosyringic acids.

Moreover, dimethoxy- and trimethoxyphenyl- propenyl derivatives were also detected, whose presence had been documented in liquid wood smoke preparations (Guillén & Ibargoitia, 1998) but not in oak wood used for wine ageing.

Regarding the compounds deriving from sugars degradation, eight furan derivatives were present in the analysed extracts, including furandicarboxaldehyde and furylhydroxymethylketone, described in toasted oak by Cutzach et al. (1999). Furthermore, in addition to maltol, two com-

pounds tentatively identified as ethylmaltol and allomaltol were detected in oak wood chips extracts.

The extraction conditions were chosen in order to maximise the extraction of analytes, with the aim to obtain a profile of sample composition as complete as possible. The solvent extraction performed at high pressure and temperature facilitated the detection of a large number of both volatile and semi-volatile compounds, providing a detailed description of possible oak wood chips volatile and semivolatile constituents. According to Pollnitz, Pardon, Sykes, and Sefton (2004) the use of high temperatures in presence of polar solvents for the analysis of oak wood compounds may imply the risk of artefacts generation. Nevertheless, the nature of the compounds detected in the present study makes admissible their presence in this kind of samples, and the majority of them had been already detected by means of other extraction techniques (Table 1). Moreover, the concentrations observed for the main compounds (Table 3) were comparable with those already reported in toasted oak wood (Cadahía et al., 2003; Singleton, 1994).

3.1. Characterisation of lignin dimers

The presence of lignin dimers derivatives in oak wood chips and barrels used for wine and spirit ageing had not been reported until now. These compounds have been recently identified in liquid wood smoke flavourings (Guillén & Ibargoitia, 1998, 1999; Guillén & Manzanos, 2002), in wood smoke condensate (Edye & Richards, 1991) and in biodegraded wood tissues (Klap, Boon, Hemminga, & Van Soelen, 1998). Lignin dimers could result from lignin pyrolysis by phenyl, guaiacyl and syringyl monomers condensation or by incomplete lignin degradation (Guillén & Ibargoitia, 1999). As lignin dimers have never been isolated, their chemical and physical properties are largely unknown. However, as well as other lignin monomers and oligomers, some lignin dimers are characterised by a high antioxidant activity (Barclay, Xi, & Norris, 1997; Guillén & Manzanos, 2002), while no data are available on their organoleptic properties.

In the present work, compounds were detected showing retention times and mass spectra comparable to compounds previously identified as lignin dimers (Guillén & Ibargoitia, 1999; Guillén & Manzanos, 2002; Klap et al., 1998) (Table 2). The presence of these compounds documented in toasted oak wood derived products, such as liquid smoke, would corroborate the likelihood of the presence of lignin dimers in toasted oak chips samples, rather than their formation during the extraction process.

The lack of reference standard compounds and spectral data hampers the identification of these compounds. Chromatographic retention times were comparable to those observed in similar chromatographic conditions by Guillén and Ibargoitia (1999) for lignin dimers detected in smoke flavourings. According to other authors (Guillén & Ibargoitia, 1999; Klap et al., 1998), the interpretation of lignin

Table 2
Characterisation and quantification of the tentatively identified lignin dimers

Compound	Ion ^a <i>m/z</i>	KI ^b	KI ^c	ID ^d	Mean (area/IS)		<i>p</i> ^e	
					MT ^e (<i>n</i> = 5)	HT ^f (<i>n</i> = 10)		
85	3,3'-Dimethoxy-4,4'-dihydroxy-1,1'-biphenyl	246	2204	2123	MS ^h , RT ⁱ	0.001	0.028 ⁱ	<0.05
86	1,2-Diguaiacyl ethylene <i>cis</i> or <i>trans</i>	272	2315	2240	MS ^j	0.13	0.20k ⁱ	<0.05
87	1,2-disyringylethylene <i>cis</i> or <i>trans</i>	332	2356	2347	MS ^h , RT ⁱ	nq ^k	nq	
88	Lignin dimer (guaiacyl derivative dimer)	151 ^a , 274	2471	2370	MS ^h , RT ⁱ	0.027	0.112 ⁱ	<0.05
89	Lignin dimer (guaiacyl derivative dimer)	151 ^a , 274	2521	2418	MS ^j	0.031	0.204 ⁱ	<0.05
90	Lignin dimer (guaiacyl derivative dimer)	151 ^a , 274	2540	2435	MS ^j	0.080	0.362 ⁱ	<0.05
91	Lignin dimer (syringyl derivative dimer)	167, 280 ^a	2557	2450	MS ^h , RT ⁱ	0.099	0.306 ⁱ	<0.05
92	Lignin dimer (guaiacyl derivative dimer)	151 ^a , 288	2592	2498	MS ^h , RT ⁱ	0.136	0.428 ⁱ	<0.05
93	1,2-Diguaiacyl ethylene <i>cis</i> or <i>trans</i>	332	2655	2574	MS ^h , RT ⁱ	8.69	5.19	
94	1,2-Disyringylethylene <i>cis</i> or <i>trans</i>	272	2701	2642	MS ^j	4.62	6.27 ⁱ	<0.05

^a Ion used for quantification.

^b Kovat's index calculated on a HP-5 capillary column.

^c Kovat's index calculated on a DB-1 capillary column.

^d Identification method.

^e Medium toast samples, mean amount.

^f High toasting samples, mean amount.

^g Significance of the difference between medium toasted and high toasted samples.

^h Mass spectrum interpretation according to Guillén and Ibargoitia (1998, 1999) and Guillén and Manzanos (2002).

ⁱ Retention time according with Guillén and Ibargoitia (1998, 1999) and Guillén and Manzanos (2002).

^j Mass spectrum interpretation according to Klap et al. (1998).

^k Detected but not quantified (not present in all the samples).

dimers mass spectra was based on the molecular ion mass and on the presence of typical lignin monomers fragments. As can be observed in Table 2, ten compounds were tentatively identified as lignin dimers with different molecular structure. The mass spectra of these compounds are shown in Fig. 1. The compound whose base peak *m/z* 246 corresponds to the molecular ion (Table 2) should be a guaiacyl dimer with a biphenyl structure (Guillén & Ibargoitia, 1999). Four compounds with an ethylene-bis-phenyl structure were identified according to Klap et al. (1998). They correspond to the 1,2-diguaiacyl ethylene (*m/z* 272) and 1,2-disyringylethylene (*m/z* 332), hypothesising both *cis* and *trans* configurations (Table 2). Moreover, five compounds having the same base peak as some lignin monomers were detected. Four of them showed the fragment *m/z* 151, characteristic of guaiacyl derivatives, as main peak, and the fragments *m/z* 274 and 288 as molecular ion. It is noteworthy that the difference between the molecular ions and the base peak are 123 and 137, respectively, and these fragments agree with fragments of guaiacyl derivatives mass spectra. Finally, a compound with a syringyl-type fragmentation (*m/z* 167, 181) and molecular ion *m/z* 280 was determined.

A compound with the same molecular ion (*m/z* 302) of a guaiacyl-syringyl-ethylene structure (Klap et al., 1998) was detected (Table 1). Nevertheless, the high retention time and the mass fragmentation excluded the attribution of such a structure to this compound.

3.2. Quantitative determination of volatile compounds

Available reference compounds of the characteristic volatiles of toasted oak wood chips were used to calculate

their response factors. They are reported in Table 3, together with the correlation coefficient of the calibration function. The same table reports the concentrations of compounds expressed in µg per g of chips, the relative standard deviation and significant differences observed between the concentrations of compounds in medium and high toasted wood chips. Among the quantified compounds, in both of the groups of samples the most abundant resulted 2-furancarboxyaldehyde, vanillin, syringaldehyde, conifer-aldehyde and coniferyl alcohol, with concentrations in reasonable agreement with those found by other authors (Cadahía et al., 2003; Singleton, 1994). Compounds that significantly modified their concentration due to the toasting treatment were syringol, vanillin, acetovanillone, syringaldehyde, coniferaldehyde and vanillic acid, which incremented their amounts with toasting, according to previous results (Cadahía et al., 2003; Cadahía et al., 2001; Chatonnet, Boidron, & Pons, 1989; Giménez-Martínez et al., 1996). On the contrary, coniferyl alcohol concentration was lower in high toasted wood chips than in medium toasted wood chips, which is possibly due to oxidation reactions. As reported by Campbell, Sykes, Sefton, and Pollnitz (2005), the outcome of heating oak is a combined process of volatile formation and possible losses due to volatilisation and/or decomposition, including oxidation. Otherwise, oak lactones and eugenol were not drastically affected by the toasting grade, in accordance with Waterhouse and Towey (1994), Chatonnet et al. (1999) and Cadahía et al. (2003).

Most of the detected compounds were found to be related to the toasting, showing higher amounts in high toasted wood chips samples (Table 1). Means of relative areas and significance of the differences between high and

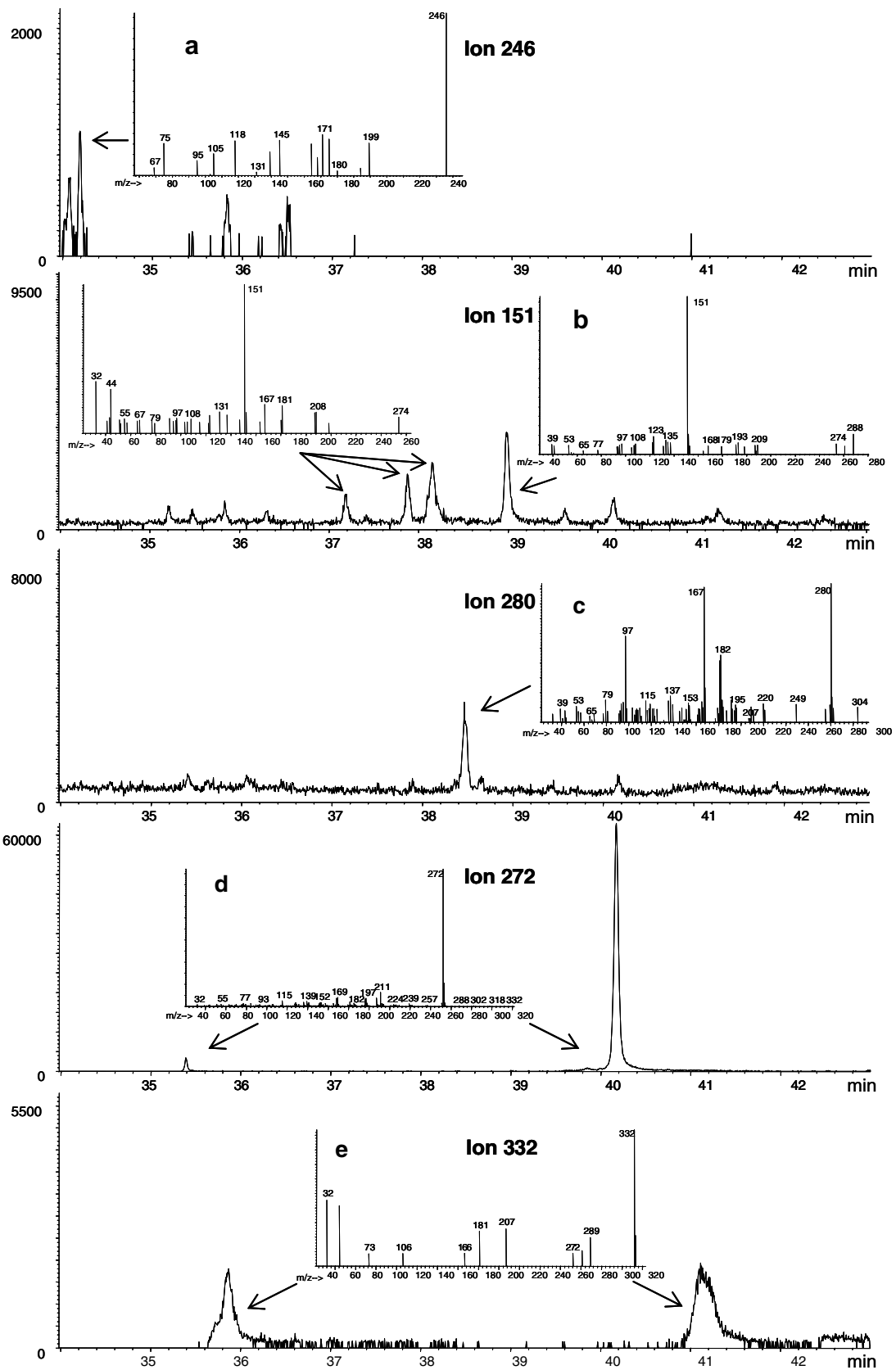


Fig. 1. Extracted ion chromatograms and mass spectra of the tentatively identified lignin dimers. Separation was carried out on a HP-5 capillary column. Mass spectra correspond to: (a) 3,3'-dimethoxy-4,4'-dihydroxy-1,1'-biphenyl; (b) guaiacyl derivative dimers; (c) syringyl derivative dimer; (d) 1,2-disyringylethylene *cis* or *trans*; (e) 1,2-diguaiacylethylene *cis* or *trans*.

Table 3
Quantitative data of some of the main volatile compounds found in medium and high toasted oak wood chips extracts

	RF ^a	<i>r</i> ^b	RSD% (<i>n</i> = 5) ^c	Mean concentration (µg g ⁻¹)		<i>p</i> ^f
				MT ^d (<i>n</i> = 5)	HT ^e (<i>n</i> = 10)	
2-Furancarboxyaldehyde	0.47	0.978	11.8	250.3	333.6	
γ-Butyrolactone	0.25	0.992	3.8	nq ^g	0.4	
5-Methylfurfural	0.42	0.995	3.7	34.2	48.4	
Guaiacol	1.14	0.984	4.9	nq	1.1	
5-Hydroxymethylfurfural	0.41	0.982	6.5	37.4	26.7	
Ethylguaiacol	1.90	0.989	4.2	nq	0.2	
<i>trans</i> -β-Methyl-γ-octalactone	0.55	0.985	5.9	17.4	13.4	
<i>cis</i> -β-Methyl-γ-octalactone	0.56	0.985	4.5	50.9	58.2	
Syringol	1.14	0.981	7.5	3.0	10.7	<0.05
Eugenol	1.84	0.988	4.2	2.0	2.4	
Vanillin	1.01	0.973	10.7	87.3	274.6	<0.05
Isoeugenol	1.44	0.973	7.3	3.2	5.5	
Vanillic acid	0.13	0.892	2.8	15.8	62.8	<0.05
Acetovanillone	1.28	0.974	13.6	2.5	12.0	<0.05
Syringaldehyde	1.12	0.969	7.2	120.3	756.4	<0.05
Coniferaldehyde	0.15	0.904	9.0	272.3	511.1	<0.05
Coniferyl alcohol	0.05	0.928	11.1	835.3 ^g	412.8	

^a Response factor; relative area in relation to concentration.

^b Correlation coefficient calculated with eight calibration points.

^c Relative standard deviation (%) calculated at 200 µg g⁻¹.

^d Medium toasted samples.

^e High toasted samples.

^f Significance of the difference between medium toasted and high toasted samples.

^g Not quantified.

medium toasting are reported in Table 1. Only a few compounds were found to be significantly ($p < 0.05$) lower in high toasted wood than in medium toasted wood. They were coniferyl alcohol, as mentioned above, and sinapyl alcohol, which were probably oxidised during the toasting treatment. Other unidentified compounds were present in higher amounts in medium toasted samples than in high toasted samples (Table 1).

Quantitative data of the tentatively identified lignin dimers (expressed as relative areas) were found to be influenced by the toasting (Table 2). Excepting a 1,2-diguaiacyl-ethylene isomer and a not quantified 1,2-disyringylethylene isomer, lignin dimers resulted significantly higher in high toasted oak wood chips than in medium toasted chips ($p < 0.05$). In like manner, the presence of these compounds documented in smoke flavouring preparations (Guillén & Ibargoitia, 1998, 1999; Guillén & Manzano, 2002) suggests the relation between their presence and the toasting treatment of oak wood.

These results allowed a distinction to be drawn between medium and high toasting wood chips, independently to their origin. These quantitative differences could be used to classify wood chips according to their toasting process.

Acknowledgements

This study was supported by the Generalitat de Catalunya, project 2005SGR00156, by the Comisión Interministerial de Ciencia y Tecnología (CICYT), project AGL2005-03451/ALI, and by the Secretaría de Estado de Educación

y Universidades (Ministerio de Educación y Ciencia) Ref. SB2003-0118.

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