Carbohydrate and Nitrogenated Compounds in Liquid Smoke Flavorings

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Commercial smoke flavorings were extracted with dichloromethane and the remaining aqueous phase was evaporated at room temperature; the residues obtained were dissolved in methanol and studied by gas chromatography/mass spectrometry. The composition of these residues was totally different from that of the dichloromethane extracts, constituting a small number of compounds that were also detected in the dichloromethane extract, as well as a large number of compounds not described before as components of either smoke for food smoking or smoke flavorings. Among those compounds not previously described there are some furan, pyran, and phenolic derivatives, as well as some pyridine and carbohydrate derivatives. The main component of these fractions is 1,6-anhydro-β-D-glucopyranose, or levoglucosan. Likewise, aqueous residues of liquid smoke flavorings, prepared at a laboratory scale from beech, vine shoots, thyme, and sage, were studied in the same way. These contained compounds of the same groups cited above, but showed clear differences. The aqueous residue of beech smoke flavoring was the most similar to that of the commercial smoke flavorings. The aqueous residues of vine shoots, thyme, and sage smoke flavorings contained a lower number of furan, pyran, and carbohydrate derivatives and a higher number of nitrogenated derivatives. Instead of levoglucosan, the main component was an unidentified compound, present in all samples, included in the carbohydrate derivatives group. In the samples studied, the influence of the vegetal source on the composition of the aqueous fraction has been shown. In the future, attention must be paid to the functionality of these smoke components.

Keywords: *Smoke flavoring; aqueous fraction; composition; gas chromatography/mass spectrometry; carbohydrate derivatives; nitrogenated compounds*

INTRODUCTION

Nowadays, smoking of foodstuffs is frequently carried out by using smoke flavorings; these are complex mixtures formed, in general terms, by a liquid or solid matrix in which smoke components are retained (*1*−*4*). Smoking modifies the organoleptic properties of foods, including flavor, texture, and color, and also affects shelf life; these changes in foods are caused, among other factors, by functions that the smoke components may perform. For these reasons it is important to know in detail the composition of smoke flavorings, as their capacity to develop certain properties in foods depends on the nature and concentration of all the components.

The compounds reported in smoke and in smoke flavorings until the end of the 1980s have been gathered in several reviews by different authors (*5*−*7*). They are very numerous and show different functional groups: among them there are acids; alcohols; aldehydes; ketones; esters; furan and pyran derivatives having alcoholic, ketonic, and aldehydic functional groups; lactones; phenolic derivatives having several functional groups; ethers; hydrocarbons; and some nitrogenated derivatives (*1*−*3*, *7*−*11*).

It is very difficult to know the concrete functionality of each group of smoke components in the smoking process because synergistic effects can also occur. In general terms, phenol derivatives have been considered the primary contributors to smoke aroma and also as being responsible for antioxidant and antimicrobial activity (*6*, *7*, *12*−*17*). Furan and pyran derivatives soften the heavy aromas associated with phenolic compounds (*14*, *18*), and if they, in addition, have carbonylic groups, also contribute to producing changes in the texture and color of smoked foods.

Aldehydes and ketones have been considered of great influence in the development of texture, color, and aroma, and some of them have also been considered responsible for antimicrobial activity; their role in the development of texture and color has been associated with reactions with amine groups of the food proteins, similar to Maillard reactions. In general, the aroma of the compounds of this group has been described as caramel or burnt sugar (*14*, *19*). In the same way, lactones, acids, and alcohol derivatives are responsible for sensory properties and some of them can also contribute to antimicrobial activity (*13*, *15*, *16*).

Other smoke components, such as esters and nitrogenated derivatives, usually present in smoke in smaller concentrations than those mentioned above, are also important contributors to the global smoke aroma. Until now, no functionality has been attributed to components such as alkyl−aryl ethers or to hydrocarbons.

Recently lignans, or lignin dimers and trimers, have been detected in smoke flavorings (*4*, *20*). Compounds of this group have been shown to have very strong antioxidant abilities (*21*−*23*) and in addition, some of

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10.1021/jf000760t CCC: $20.00
Published on Web 04/18/2001

them have also been considered important for the sensory properties of aged alcoholic beverages (*24*).

All the above-mentioned compounds have been isolated from smoke flavoring preparations by means of organic solvents. However, it has been observed that the residual aqueous phase after extraction with an organic solvent has a dark color, showing the presence of smoke components. This aqueous phase was studied in order to determine whether it contains any compounds not described before as smoke flavoring components. At first, the study was carried out on commercial smoke flavorings, and later it was extended to smoke flavorings obtained in the laboratory to determine whether these were similar to the commercial products, but also to evaluate the influence of the wood used on the composition of the aqueous residue.

Gas chromatography/mass spectrometry is a technique that is able to separate complex mixtures and, at the same time, afford information on the nature of its components; but it requires the mixture to be dissolved in an organic solvent. For this reason, the aqueous phase, left after the extraction of the smoke flavoring with the organic solvent, was evaporated at room temperature to eliminate the water. The residue was dissolved in a very polar organic solvent, such as methanol, to study its composition using the technique cited above. Obviously, any compounds present in the initial aqueous mixture having volatility similar to or higher than that of water will also be lost by evaporation, and the concentration of others with volatilities lower than that of water will also be affected. Despite this, the study has detected some groups of compounds not previously described as components of smoke for food smoking which may play an important role in the process. Mass spectral data of some unidentified components are also reported.

MATERIALS AND METHODS

Samples. The subject samples of this study were two aqueous commercial smoke flavorings named A1 and A2, and four smoke flavorings obtained, at laboratory scale, from beech wood (*Fagus sylvatica*), vine shoots (*Vitis vinifera*), and the aerial parts of thyme (*Thymus vulgaris*) and sage (*Salvia lavandulifolia*), named A3, A4, A5, and A6, respectively.

Obtention of the Smoke Flavorings A3, A4, A5, and A6. The different vegetal materials were air-dried and ground in a Restch DR 15/40 mill. They were then sieved to yield a particle size of ≤2 mm powder. The pyrolytic process was carried out in a laboratory round-bottom flask smoke-generator made of quartz, by means of a rheostat-controlled heating mantle. The temperature of the process was measured with a Crison thermometer 639K positioned in the center of the charge. The resulting smoke was filtered through a glass wool filter to eliminate solid particles, and collected by condensation, solution, and/or dispersion in distilled water. The aqueous liquid smoke obtained was again filtered through a paper filter.

Extraction of the Smoke Flavorings. Five milliliters of the six smoke flavorings (2 commercial smoke flavorings and 4 smoke flavorings obtained in the laboratory) were exhaustively extracted with dichloromethane by liquid−liquid extraction, in several steps, as in previous studies (*1*, *4*, *9*). Two fractions were obtained: first, the fraction soluble in dichloromethane containing carbonylic, carboxylic, phenolic, and all those compounds previously considered responsible for the smoke functionality; and second, the aqueous fraction. The water was evaporated and the residue obtained was dissolved in methanol and studied by gas chromatography/mass spectrometry (GC/MS). Replicate experiments were performed to obtain the subject residues. The aqueous residue yields were determined by weight.

Study of the Residue Components. Study of the components was carried out by means of GC/MS by using a Hewlett-Packard gas chromatograph, model 6890 series II, equipped with a mass spectrometer selective detector 5973, and a Hewlett-Packard Vectra Pentium computer. A fused-silica capillary column (60 m long, 0.25 mm i.d., 0.25 μm thickness), coated with a nonpolar stationary phase (Hewlett-Packard 5, cross-linked 5% phenyl methyl silicone) was used. The temperature program began at 50 °C (0.5 min) with an increase of 5 °C min^{-1} up to 300 °C (10 min). Helium was used as the gas carrier with a 1 mL/min flow. Injector and detector temperatures were 250 and 280 °C, respectively. The injection technique used was split with a split ratio 1:10, and the injection volume was 1 μL. Mass spectra were recorded at an ionization energy of 70 eV.

Components were identified by their retention times, by their mass spectra, by comparing their mass spectra with those in a commercial library (Wiley 138k, Mass Spectral Database, 1990) and with others found in the literature, and, in some cases, by using standards as in previous studies (*1*, *4*, *9*); however, some components remained unidentified and others were tentatively identified. All standard compounds used (available from Aldrich, Fluka, and Sigma) are asterisked in Table 1.

Because of the sample being a very complex mixture in which many of the components remain unidentified and others are not available commercially, the quantification was carried out in a semiquantitative way by determining % peak areas. Replicate chromatographic analyses of each sample were performed.

RESULTS AND DISCUSSION

Initially, aqueous residues of the A1 and A2 commercial smoke flavorings were studied. Their yields were 108 g/kg of flavoring A1, and 2 g/kg of flavoring A2. Figure1, panels a and b, show the total ion chromatograms of these samples. It can be observed that these chromatograms are quite similar with two components in high proportions.

Table 1 gives the components of both samples, grouped in families by their nature, together with their area percentages. A total of 59 compounds was detected in the aqueous A1 flavoring residue, and 62 were detected in the aqueous A2 flavoring residue. Quantification of many of the compounds was not possible because of peak overlapping, and they are indicated in Table 1 with "nq". For unidentified compounds, the main mass fragments of their mass spectra are also given; although the number of unidentified compounds is high, the nature of some of them has been tentatively deduced from their mass spectra, their assignation to specific compounds being very difficult with the available data.

It might be thought that the flavoring smoke components were partitioned during the extraction process between the aqueous and the organic phase in function of their partition coefficients and that both phases would contain the same components in different proportions. Table 1 shows that the A1 and A2 aqueous residues contain, in small proportions, some of the components previously detected in the organic phase (*1*, *3*) and described by other authors (*5*−*7*), as well as compounds not reported before in smoke or in smoke flavorings. These latter compounds are marked with the symbol § in Table 1. Many of the main detected compounds are common in both samples and have been included in a reduced number of groups, such as furan and pyran derivatives; phenolic derivatives and related, nitrogenated compounds; and carbohydrate derivatives and related.

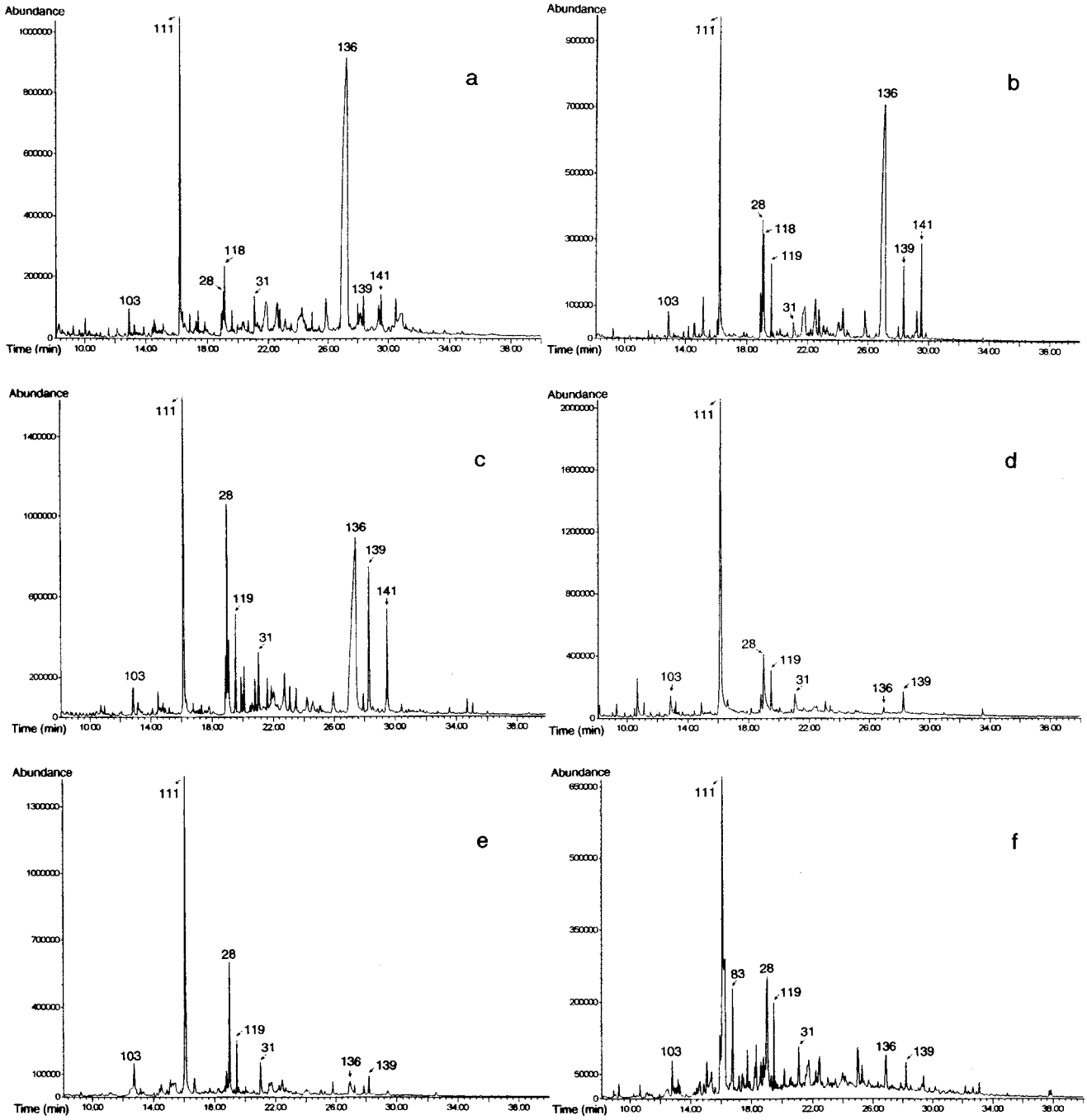

Figure 1. Total ion chromatograms of the residues of the smoke flavorings: (a) A1, (b) A2, (c) A3, (d) A4, (e) A5, and (f) A6.

The group of furan and pyran derivatives is formed, in A1 and A2 aqueous residues, by twenty components in very small proportions, from which fifteen are described for the first time as smoke flavoring components. These have their origins in cellulose and hemicellulose pyrolysis. There is controversy about the nature of compound number 14; this compound was first identified as 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one (*25*−*28*) and afterward as 1,5-anhydro-4-deoxypent-1-en-3-ulose (*29*, *30*). It is considered to be a characteristic marker of the thermal degradation of xylan and has been detected in the thermal degradation of carbohydrates such as cellulose (*25*, *31*). This compound is generated in high proportions during the degradation of hard woods rich in pentosans, and its mass spectrum ions $m/z = 58$ and 114 are considered as recognizable markers in extracts of good quality whisky aged in oak casks (*32*) together with those of 5-hydroxymethyl-2-furancarboxaldehyde (compound number **22** in Table 1). The impossibility of identifying the compound number **14** in the present work is because none of the cited compounds are available commercially.

Twelve phenolic derivatives, also in small proportions, have been detected in A1 and A2 aqueous residues, of which four have been described for the first time as components of smoke for food smoking and of smoke flavorings. Within this group, diphenol derivatives such as 1,2- and 1,4-dihydroxybenzene (compounds number **28** and **31** respectively) are in the highest proportions; both compounds have great antioxidant activity. Most of the components of this group are present in higher proportions in the organic phase than in the aqueous residue except diphenol derivatives.

Table 1. Identified and Unidentified Components in the Aqueous Residue of the A1, A2, A3, A4, A5, and A6 Smoke Flavorings, Together with Their Relative Proportions in % Peak Area

no.		compound[a] (mass spectral data)	A1	A2	A3	A4	A5	A6
		number and % of total furan and pyran derivatives	(14)*0.4*	(15)*1.6*	(17)*2.2*	(8)*5.5*	(6)*2.1*	(4)*nq*
1	§	2,5-furandione	nq[b]	--	nq	--	--	--
2		2-furancarboxaldehyde (furfural)*	tr[c]	tr	tr	tr	tr	--
3		2-furanmethanol*	tr	tr	tr	tr	tr	--
4	§	2,5-dimethoxytetrahydrofuran (or isomer)	nq	--	tr	--	--	--
5	§	dimethoxytetrahydrofuran	nq	--	tr	--	--	--
6	§	dihydro-2(3H)-furanone	nq	tr	--	4.0	tr	tr
7		2(5H)furanone (γ-crotonolactone)*	--	tr	--	--	--	--
8	§	2,5-dihydro-5-methylfuran-2-one (or isomer)	tr	--	--	--	--	--
9	§	2,3-dihydro-5-methylfuran-2-one (or isomer)	nq	tr	tr	tr	--	--
10	§	3-methyl-2,5-furandione	nq	tr	nq	tr	--	--
11	§	2,5-dimethyl-3(2H)-furanone	--	tr	tr	--	--	--
12	§	5-methyl-dihydro-2(3H)-furanone	--	--	--	--	tr	tr
13	§	hydroxy-dihydro-pyranone	tr	--	tr	--	--	--
14	§	4-hydroxy-5,6-dihydro-(2H)-pyran-2-one (?)	0.4	tr	nq	--	--	--
15	§	dihydro-2,5-furandione	--	tr	--	--	--	--
16	§	2-furancarboxylic acid	--	tr	nq	--	--	--
17	§	tetrahydro-2H-pyran-2-one	--	--	--	--	tr	tr
18		4-methyl-2(3H)-dihydrofuranone (or isomer)	--	tr	--	1.5	--	--
19	§	tetrahydro-2-furanmethanol	nq	1.6	1.0	--	2.1	nq
20		3-hydroxy-2-methyl-4H-pyran-4-one (maltol)*	--	--	nq	--	--	--
21	§	3,5-dihydroxy-2-methyl-4H-pyran-4-one (or isomer)	--	--	tr	--	--	--
22		5-hydroxymethyl-2-furancarboxaldehyde*	nq	tr	1.2	tr	--	--
23	§	1,3-isobenzofurandione	tr	tr	nq	--	--	--
24	§	3,4-dihydro-2H-1-benzopyran-2-one (3,4-dihydrocoumarin)*	--	--	--	tr	--	--
25	§	4-methyl-1,3-isobenzofurandione	--	tr	nq	--	--	--
		number and % of total phenolic derivatives and related	(8)*2.7*	(7)*4.9*	(20)*16.1*	(13)*17.6*	(7)*16.2*	(4)*14.5*
26	§	1,2-dimethylbenzene (or isomer)	--	--	nq	tr	--	--
27		2,3-dihydroxybenzaldehyde	tr	--	--	--	--	--
28		1,2-dihydroxybenzene (pyrocatechol)*	1.6	3.8	8.1	12.0	12.5	11.0
29		3-methyl-1,2-dihydroxybenzene (3-methylpyrocatechol)*	tr	--	1.3	--	tr	--
30		methyl-dihydroxybenzene	--	--	nq	--	--	--
31	§	1,4-dihydroxybenzene (hydroquinone)	1.1	1.1	3.2	3.4	3.7	2.6
32		3-methoxy-1,2-dihydroxybenzene (3-methoxypyrocatechol)*	--	tr	nq	nq	nq	--
33		4-methyl-1,2-dihydroxybenzene (4-methylpyrocatechol)*	nq	--	2.1	tr	nq	--
34	§	2,3,5,6-tetramethylphenol (or isomer)	--	--	--	--	tr	--
35	§	2-methyl-1,4-dihydroxybenzene (2-methylhydroquinone) (or isomer)	nq	--	1.4	1.5	--	--
36		2,6-dimethoxyphenol (syringol)*	--	tr	nq	tr	--	--
37		4-ethyl-1,2-dihydroxybenzene (4-ethylpyrocatechol) (or isomer)	--	--	nq	tr	--	--
38	§	1-(3-hydroxyphenyl)-ethanone	--	--	nq	--	--	--
39		4-hydroxy-3-methoxybenzaldehyde (vanillin)*	--	--	nq	--	--	--
40	§	methyl-4-methoxyphenol	nq	tr	--	--	--	0.9
41		4-methyl-2,6-dimethoxyphenol (4-methylsyringol)*	--	--	nq	--	--	--
42		4-(1-propenyl)-2-methoxyphenol (*cis*-propenylguaiacol)*	--	--	--	tr	--	--
43		4-ethyl-2,6-dimethoxyphenol (4-ethylsyringol)	--	--	nq	nq	--	--
44	§	1-(4-hydroxy-3-methoxyphenyl)-2-propanone (2-propiovanillone)	--	--	nq	tr	--	--
45		4-(2-propenyl)-2,6-dimethoxyphenol (4-allylsyringol)*	--	--	tr	--	--	--
46		4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde)*	--	tr	nq	--	--	--
47		4-(1-propenyl)-2,6-dimethoxyphenol (*cis*-propenylsyringol)	--	--	--	tr	--	--
48		1-(4-hydroxy-3,5-dimethoxyphenyl)-ethanone (acetosyringone)*	tr	--	nq	--	--	--
49	§	4-hydroxy-3-methoxyphenylhydroxyacetic acid (vanillomandelic acid)	--	--	--	--	tr	--
50	§	1-(4-hydroxy-3,5-dimethoxyphenyl)-2-propanone (2-propiosyringone)	--	tr	nq	0.7	--	--
51	§	4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid)*	--	--	nq	--	--	--
52	§	2,2′-methylene-bis[6-(1,1-dimethylethyl)−4-methylphenol] (or isomer)	--	--	--	--	--	tr
		number and % of total nitrogenated compounds	(6)*tr*	(10)*0.6*	(5)*nq*	(20)*6.2*	(29)*6.2*	(26)*29.2*
53	§	2-methylpyridine*	tr	tr	--	0.5	tr	--
54	§	3-methylpyridine*	tr	tr	tr	1.2	tr	tr
55	§	2,6-dimethylpyridine*	--	tr	tr	0.4	tr	--
56	§	2-ethylpyridine*	tr	--	--	0.7	--	--
57	§	3-methyl-1H-pyrazole (or isomer)	--	--	--	--	tr	tr
58	§	ethenylpyrazine	--	--	--	--	--	tr
59	§	2,5-dimethylpyridine*	tr	tr	--	1.1	tr	--
60	§	2-ethenylpyridine (or isomer)	--	--	--	--	tr	tr
61	§	2,3-dimethylpyridine*	tr	tr	tr	tr	tr	--
62	§	3-ethylpyridine*	--	--	--	tr	tr	tr

Table 1 (Continued)

no.		compound[a] (mass spectral data)	A1	A2	A3	A4	A5	A6
63	§	4-ethylpyridine*	--	--	--	tr	tr	tr
64	§	3,5-dimethylpyridine*	--	--	tr	tr	tr	--
65	§	2,4,6-trimethylpyridine*	--	--	--	tr	nq	--
66	§	1,3-dimethyl-1H-pyrazole (or isomer)	--	--	--	--	tr	tr
67	§	3-methoxypyridine*	tr	tr	nq	0.8	0.6	0.5
68	§	dimethylpyridine	--	--	--	tr	tr	--
69	§	2,3,6-trimethylpyridine (or isomer)	--	--	--	tr	tr	--
70	§	1,3-dimethyl-1H-pyrazole (or isomer)	--	--	--	--	tr	tr
71	§	2-ethyl-6-methylpyridine (or isomer)	--	--	--	tr	tr	--
72	§	2-ethenyl-6-methylpyrazine (or isomer)	--	--	--	--	--	tr
73	§	5-ethyl-2-methylpyridine*	--	--	--	tr	--	--
74	§	dimethyl-1H-pyrazole	--	--	--	--	tr	--
75	§	1-methyl-2-pyrrolidinone (or isomer)	--	--	--	--	--	tr
76	§	2-pyrrolidinone (or isomer)	--	--	--	--	nq	2.3
77	§	1,3,5-trimethyl-1H-pyrazole (or isomer)	--	--	--	--	--	tr
78	§	2-ethyl-3,5-dimethylpyridine (or isomer)	--	--	--	--	nq	--
79	§	1,3,5-trimethyl-1H-pyrazole (or isomer)	--	--	--	--	--	tr
80	§	trimethylpyridine	--	--	--	--	tr	--
81	§	3-pyridinol*	--	0.6	--	tr	nq	9.0
82	§	2-methyl-3-pyridinol*	--	tr	--	1.5	2.7	nq
83	§	2,5-pyrrolidinedione (or isomer)	--	tr	--	--	nq	6.3
84	§	methyl-pyridinol	--	tr	--	--	--	--
85	§	3-methyl-2,5-pyrrolidinedione (or isomer)	--	--	--	tr	tr	0.9
86	§	methyl-pyridinol	--	--	--	tr	1.4	2.3
87	§	1,3-dimethyl-2(1H)-pyridinone (or isomer)	--	--	--	--	1.5	2.4
88	§	methyl-pyridinol	--	--	--	tr	tr	2.7
89	§	6-ethyl-3-pyridinol (or isomer)	--	--	--	--	--	1.6
90	§	2,5-dimethyl-1-propylpyrrole (or isomer)	--	--	--	--	--	tr
91	§	ethyl-pyridinol	--	--	--	--	--	tr
92	§	1-(1-cyclopenten-1-yl)-pyrrolidine (or isomer)	--	--	--	--	tr	1.2
93	§	1-(1-cyclohexen-1-yl)-pyrrolidine (or isomer)	--	--	--	--	--	tr
		number and % of total carbohydrate derivatives and related	(31)*76.7*	(30)*89.2*	(25)*51.6*	(16)*65.9*	(28)*64.6*	(19)*46.0*
94		2-cyclopenten-1-one	--	--	--	nq	--	--
95	§	methyl 2-butenoate	--	tr	tr	tr	--	--
96	§	ethyleneglycol monoacetate*	tr	--	--	--	--	--
97	§	2-hexanol (or isomer)	--	nq	nq	--	--	--
98		1-acetoxy-propan-2-one	--	--	--	--	tr	--
99		unidentified (86, 74, 61, 43(100))	0.7	--	tr	--	--	--
100		2-methyl-2-cyclopenten-1-one*	tr	--	--	--	--	--
101		unidentified (111, 89, 73(100), 58, 44)	nq	--	tr	--	--	--
102	§	1,2,3-propanetriol (glycerol)	--	tr	--	--	nq	tr
103		unidentified (102, 57(100), 44)	0.9	1.4	0.7	3.6	7.8	1.9
104		unidentified (85, 72, 57(100), 43)	nq	tr	nq	tr	tr	tr
105		3-methyl-1,2-cyclopentanedione (cyclotene)*	tr	--	--	--	--	--
106		unidentified (115, 99, 86, 71, 56, 43(100))	nq	0.5	tr	--	tr	--
107		unidentified (147, 131, 115, 102, 87, 71, 57, 43(100))	nq	--	tr	--	--	--
108		unidentified (116, 85, 99, 70, 57(100), 44)	--	tr	1.0	nq	0.5	--
109		4-oxo-pentanoic acid (levulinic acid)*	tr	nq	nq	--	2.0	--
110	§	1,2,3-propanetriol 1-acetate	--	--	--	--	tr	3.8
111		unidentified (95, 71, 57, 44(100))	10.0	14.4	4.7	51.2	36.9	24.6
112		unidentified (128, 103, 86, 74, 71, 43(100))	nq	--	1.1	--	--	--
113		unidentified (128, 85(100), 57, 43)	nq	tr	tr	tr	--	--
114		unidentified (147, 131, 115, 99, 87, 71, 57, 43(100))	nq	--	tr	--	--	tr
115		unidentified (115, 103, 99, 86, 73, 57(100), 43)	--	--	--	--	nq	1.7
116		unidentified (114, 98(100), 82, 74, 55, 42)	--	--	--	tr	0.8	--
117		unidentified (85(100), 69, 57, 43)	nq	1.6	--	2.8	2.1	nq
118		unidentified (89, 71(100), 57, 43)	2.4	4.1	2.8	--	--	--
119	§	1,4:3,6-dianhydro-α-D-glucopyranose (144, 114, 98, 86, 69(100), 60, 57, 41)	nq	2.2	3.0	4.7	3.4	3.0
120		unidentified (128, 110, 97, 86(100), 69, 58, 43)	nq	tr	--	--	0.8	--
121		unidentified (145, 115, 97, 86, 73(100), 55, 41)	nq	0.6	--	--	--	--
122		unidentified (97, 81, 73, 71(100), 69, 55, 43)	--	--	1.4	1.1	0.6	--
123		unidentified (124, 102, 86, 73, 57, 45(100))	--	1.4	--	--	nq	--
124		unidentified (102, 87, 73, 57, 45(100))	nq	3.2	--	--	tr	nq
125		unidentified (102, 84, 73, 55, 45(100))	--	tr	--	--	--	1.0
126		unidentified (103, 86, 73, 57, 45(100))	2.7	4.0	--	--	2.4	2.7
127		unidentified (102, 82, 73, 57(100), 43)	nq	1.4	2.0	--	tr	--
128		unidentified (132, 114, 102, 84, 71, 57, 45(100))	--	1.0	--	--	--	tr
129		unidentified (133, 115, 101, 87(100), 73, 69, 61, 55, 45)	--	1.0	--	--	--	--
130	§	1,6-anhydro-α-D-galactopyranose (144, 126, 98, 73, 60(100), 57, 43)	nq	2.2	nq	--	nq	nq
131		unidentified (128, 114, 99, 84, 72, 55(100), 43)	0.6	--	--	--	--	--
132		unidentified (86, 73, 70, 61, 43(100))	--	--	--	--	tr	1.5
133		unidentified (86, 73, 70, 61, 43(100))	--	--	--	tr	tr	1.3

Table 1 (Continued)

no.		compound[a] (mass spectral data)	A1	A2	A3	A4	A5	A6
134		unidentified (168, 142, 127, 109, 85, 69, 43(100))	--	--	--	--	1.1	--
135	§	1,6-anhydro-*β*-D-mannopyranose (144, 116, 98, 73, 60(100), 57, 43)	2.6	2.0	1.7	--	--	--
136	§	1,6-anhydro-*β*-D-glucopyranose (levoglucosan)* (144, 115, 98, 85, 73, 60(100), 57, 43)	51.0	40.5	24.6	tr	3.0	3.1
137	§	*p*-menthane-1,2,8-triol	--	--	--	--	0.5	--
138		unidentified (164, 136, 122(100), 107, 94, 79, 66, 55, 44)	--	nq	nq	nq	0.7	--
139		unidentified (186, 115, 98, 85, 73, 60, 43(100))	1.3	2.8	5.1	2.5	1.4	1.4
140	§	1,6-anhydro-*β*-D-glucofuranose (115, 103, 98, 85, 73(100), 69, 61, 60, 57, 44, 43)	1.9	1.5	--	--	--	--
141		unidentified (144, 115, 99, 73, 60, 43(100))	1.0	3.4	3.5	tr	0.6	tr
142	§	1,6-anhydro-α-D-galactofuranose (115, 98, 85, 73(100), 69, 61, 57, 44)	1.6	--	--	--	--	--
143	§	1-deoxy-inositol (or isomer) (128, 102, 73(100), 60, 43)	tr	tr	--	--	--	--
144	§	levoglucosan isomer (144, 115, 98, 89, 85, 73, 60(100), 57, 43)	--	--	nq	--	--	--

[a] Asterisked compounds were acquired commercially and used as model compounds for the identification. Compounds indicated with the symbol § were detected for the first time in smoke or smoke flavorings (*7*). [b] nq, compound not quantified because its separation was not adequate. [c] tr, compounds in very small proportion.

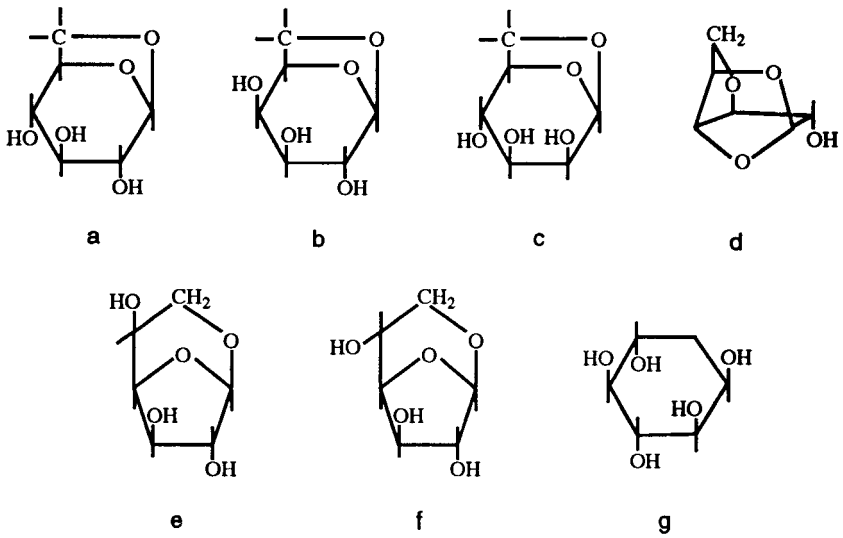

Figure 2. Structures of (a) 1,6-anhydro-*β*-D-glucopyranose or levoglucosan (no. **136**); (b) 1,6-anhydro-α-D-galactopyranose (no. **130**); (c) 1,6-anhydro-*β*-D-mannopyranose (no. **135**); (d) 1,4:3,6-dianhydro-α-D-glucopyranose (no. **119**); (e) 1,6-anhydro-*β*-D-glucofuranose (no. **140**); (f) 1,6-anhydro-α-D-galactofuranose (no. **142**); and (g) 1-deoxy-inositol (no. **143**).

In addition to the above-mentioned compounds, eleven pyridine derivatives, in very small proportions, have been detected in both samples. These compounds have never been reported as smoke or smoke flavorings components; however, a small number of them have been detected in the basic fraction of white oak wood partially charred with fire for manufacturing casks (*7*). These compounds could be formed during the thermal degradation of nitrogenated derivatives of wood such as alkaloids (*33*). They play an important role in coffee, tea, and cocoa flavors, with organoleptic notes described as green, bitter, toasted, and burnt (*34*). Some of these compounds, such as 3-pyridinol (**81**) and 2-methyl-3-pyridinol (**82**) have structures similar to that of vitamin B6 or pyridoxal.

Finally, a numerous group of compounds probably generated from the thermal degradation of carbohydrates has also been detected in both aqueous residues A1 and A2 (*31, 35*−*41*). This group, named "carbohydrate derivatives and related" in Table 1, includes compounds showing different functional groups such as ketones, esters, alcohols, acids, and anhydro-sugars, and a large number of unidentified components. These latter have been included in this group for several reasons: their mass spectra contain the essential fragmentations of different types of anhydro-sugars originated from wood pyrolysis (*26*, *27*); many of them show as base peaks $m/z = 43$ and 57 characteristic of a numerous group of unidentified compounds generated in cellulose pyrolysis (*31*); and, the average mass spectrum of the mixture of products coming from glucose pyrolysis at 540 °C shows as main fragments $m/z = 43$, 57, 60, 69, 73, 85, 97, 115, 126, 144, and 162 (*38*) which coincide with most of the base peaks and with the mass fragments of these cited unidentified compounds.

This group contains the main components of both samples. Noteworthy is the high proportion of anhydro-sugars, especially of 1,6-anhydro-*β*-D-glucopyranose or levoglucosan (compound **136**, Fig. 2a) whose proportion in residue A1 reaches 51% and in residue A2 reaches 40%. Other anhydro-sugars in smaller proportions are 1,6-anhydro-α-D-galactopyranose (**130**, Figure 2b); 1,6-anhydro-*β*-D-mannopyranose (**135**, Figure 2c); 1,4:3,6-dianhydro-α-D-glucopyranose (**119**, Figure 2d); 1,6-anhydro-*β*-D-glucofuranose (**140**, Figure 2e); 1,6-anhydro-α-D-galactofuranose (**142**, Figure 2f); and 1-deoxy-

inositol or isomer (**143**, Figure 2g). Tentative identification of these latter compounds has been based on mass spectral data given by other authors (*25*−*27*).

Even though it is the first time that these compounds have been described as smoke flavoring components some of them have been found in carbohydrate pyrolysis studies. So, both levoglucosan and its isomer in *furanose* form, 1,6-anhydro-β-D-glucofuranose, have been found as main components in cellulose-derived tar, generated in cellulose pyrolysis (*7*, *31*, *35*−*37*, *39*−*43*). In the same way, 1,6-anhydro-α-D-galactopyranose, 1,6-anhydro-α-D-galactofuranose, 1,6-anhydro-β-D-mannopyranose, and 1,4:3,6-dianhydro-α-D-glucopyranose have been detected in the thermal degradation of wood polysaccharides (*26*, *27*, *31*) and of microcrystalline cellulose (*25*).

In relation to foods, it is known that levoglucosan is produced, in low proportions, in the heating at 100 °C of glucose syrup (*44*); likewise an anhydroglucopyranose, probably levoglucosan, has been recently identified as the main component in pyrograms of different lignocellulosic materials and of oak and chestnut woods used to manufacture casks for wine aging (*28*, *30*), so the presence of this compound in aged alcoholic beverages cannot be disregarded. In the same way, levoglucosan has been found in human urine and its presence has been commonly associated with diet (*45*). Recently it has been proved that levoglucosan is able to form complexes with metals (*46*, *47*); this is an interesting property for eliminating toxic metals from the human body.

As far as we know, the presence of anhydro-sugars has not been reported until now in the food smoking context, for this reason its functionality in the smoking process should be subject of study in the future.

To know the degree of similarity between aqueous residues of commercial smoke flavorings A1 and A2, of unknown manufacture, and aqueous residues of smoke flavorings obtained in the laboratory, the study of these latter was carried out. In this context, aqueous residues of smoke flavorings obtained from beech wood, A3, vine shoots, A4, and from the aerial parts of thyme, A5, and sage, A6, were studied. The yield of each one of these aqueous residues, after smoke flavoring extraction with dichloromethane and subsequent water evaporation, was 1482 mg/100 g of beech sawdust, 637 mg/100 g of vine shoots sawdust, 837 mg/100 g of thyme sawdust, and 602 mg/100 g of sage sawdust.

Figures 1c, 1d, 1e, and 1f show the total ion chromatograms of the aqueous residues of the smoke flavorings A3, A4, A5, and A6 respectively, and Table 1 gives the proportions of their components.

The aqueous residue of beech smoke flavoring A3 contains compounds which are of a similar nature and number to those found in the aqueous residues of the commercial smoke flavorings previously mentioned. Among them there are numerous furan and pyran derivatives, in very low proportions; numerous phenol derivatives in considerable proportions, especially dihydroxybenzene derivatives; a reduced number of pyridine derivatives in very low proportion; and an appreciable number of carbohydrate derivatives, in high proportions, among which the main component is levoglucosan, just as in residues A1 and A2. These results confirm that wood smoke and smoke flavorings from wood smoke contain anhydro-sugars unless these are eliminated in the manufacture process.

The aqueous residue of vine shoots smoke flavoring A4 has a lower number of components than the aqueous residue of beech. This sample is constituted by a reduced number of furan and pyran derivatives, in low proportions and of the same nature as those detected in the samples above, except 3,4-dihydrocoumarin which is only present in this sample; a considerable number of phenol derivatives, among which dihydroxybenzene derivatives are the main components; a fairly large number of nitrogenated compounds, basically pyridine derivatives, some of them in appreciable proportions; a considerable number of compounds included in the group of carbohydrate derivatives, among which the main component, rather than levoglucosan, is the unidentified compound number **111**. The most important characteristics of this residue are the high proportion of dihydroxybenzene derivatives, the large number of nitrogenated derivatives, and the virtual absence of levoglucosan.

The aqueous residues of thyme and sage smoke flavorings, A5 and A6, respectively, are characterized by a reduced number of furan, pyran, and phenol derivatives, some of these latter in considerable proportions, especially dihydroxybenzene derivatives; a large number of nitrogenated compounds, identified as pyridine, pyrazole, and pyrrolidine derivatives, in appreciable proportions, especially in the sage residue; and finally, a considerable number of carbohydrate derivatives among which the main component, as in the vine shoot flavoring residue, is again the unidentified compound number **111**.

In conclusion, the aqueous residues of smoke flavorings are formed by a reduced number of compounds already detected in the organic phase, which remain in the aqueous phase in function of its partition coefficients, and a large number of compounds which are not detected in the organic phase of the same smoke flavoring, and have not been described before as components of smoke for food smoking or of smoke flavorings. Among these latter there are compounds of the group of furan and pyran derivatives in higher number in the commercial and beech smoke flavorings than in vine shoots, thyme, and sage smoke flavorings. There are also phenol derivatives, more numerous in beech and vine shoots flavorings than in the others. Nitrogenated compounds such as pyridine, pyrazole, and pyrrolidine derivatives are present in higher numbers and proportions in the vine shoots, thyme, and sage smoke flavorings, especially in the latter. Finally, carbohydrate derivatives and other related unidentified compounds constitute the largest group in the six samples; among these levoglucosan and the unidentified compound number **111** are the main components, the first in commercial and beech smoke flavorings and the second in vine shoots, thyme, and sage smoke flavorings. The functionality of these compounds in the smoking process should be the subject of attention in future studies. In addition, it has been shown that smoke and smoke flavorings obtained from different vegetal sources differ not only in nature and proportion of the compounds extractable with organic solvents, but also in nature and proportion of those compounds that remain in the aqueous phase, which generally have a great ability to establish hydrogen bondings with water. From these results it is evident that a complete characterization of smoke flavorings requires the study not only of their fractions extractable with organic solvents, but also of their aqueous residues.

LITERATURE CITED

(1) Guillén, M. D.; Manzanos, M. J.; Zabala, L. Study of a Commercial Liquid Smoke Flavoring by Means of Gas Chromatography/Mass Spectrometry and Fourier Transform Infrared Spectroscopy. *J. Agric. Food Chem*. **1995**, *43* (2), 463−468.

(2) Guillén, M. D.; Manzanos, M. J.; Ibargoitia, M. L. Ahumado de alimentos. Preparación, aplicación, métodos de estudio y composición de aromas de humo. *Alimentaria* **1996**, XXXIV, *274*, 45−53.

(3) Guillén, M. D.; Manzanos, M. J. Study of the components of an aqueous smoke flavouring by means of Fourier Transform Infrared Spectroscopy and Gas Chromatography with Mass Spectrometry and Flame Ionization Detectors. *Adv. Food Sci*. **1996**, *18* (3/4), 121−127.

(4) Guillén, M. D.; Ibargoitia, M. L. New Components with Potential Antioxidant and Organoleptic Properties, Detected for the First Time in Liquid Smoke Flavoring Preparations. *J. Agric. Food Chem*. **1998**, *46* (4), 1276−1285.

(5) Knowles, M. E.; Gilbert, J.; McWeeny, D. J. Phenols in Smoked Cured Meats. Phenolic Composition of Commercial Liquid Smoke Preparations and Derived Bacon. *J. Sci. Food Agric.* **1975**, *26*, 189−196.

(6) Tóth, L.; Potthast, K. Chemical Aspects of Smoking of Meat and Meat Products. *Adv. Food Res.* **1984**, *29*, 87−158.

(7) Maga, J. A. *Smoke in Food Processing*; CRC Press: Boca Raton, FL, 1988.

(8) Guillén, M. D.; Ibargoitia, M. L. Aqueous Liquid Smoke Flavoring Preparations from Vine Shoots and Beech Wood. *J. Sci. Food Agric.* **1996**, *72*, 104−110.

(9) Guillén, M. D.; Manzanos, M. J. Characterization of the components of a salty smoke flavouring preparation. *Food Chem*. **1997**, *58* (1/2), 97−102.

(10) Guillén, M. D.; Manzanos, M. J. Smoke and Liquid Smoke. Study of an Aqueous Smoke Flavouring from the Aromatic Plant *T. vulgaris* L. *J. Sci. Food Agric.* **1999**, *79*, 1267−1274.

(11) Guillén, M. D.; Manzanos, M. J. Extractable components of the aerial parts of *Salvia lavandulifolia* and the composition of the liquid smoke flavoring obtained from them. *J. Agric. Food Chem*. **1999**, *47* (8), 3016−3027.

(12) Daun, H. Antioxidative properties of selected smoked meat products. In *Proceedings of the 15th European Meeting of Meat Research Workers*, Helsinki, Finland, 1969; pp 274−276.

(13) Zaitsev, V.; Kizeretter, I.; LaGunov, L.; Makarova, T.; Minder, L.; Podsevalov, V. Fish Curing and Processing. *J. Fish. Res. Board Can.* **1973**, *30* (5), 631−636.

(14) Kim, K.; Kurata, T.; Fujimaki, M. Identification of Flavor Constituents in Carbonyl, Non-carbonyl, Neutral and Basic Fractions of Aqueous Smoke Condensates. *Agric. Biol. Chem*. **1974**, *38*, 53−63.

(15) Olsen, C. Z. Smoke flavoring and its bacteriological and antioxidative effects. *Acta Aliment. Pol*. **1977**, *3* (3), 313−324.

(16) Wendorff, W. L. Antioxidant and bacteriostatic properties of liquid smoke. In *Proceedings of Smoke Symposium*; Red Arrow Products Co.: Manitowoc, WI, 1981; pp 73−87.

(17) Faith, N. G.; Yousef, A. E.; Luchansky, J. B. Inhibition of *Listeria monocytogenes* by liquid smoke and isoeugenol, a phenolic component found in smoke. *J. Food Safety* **1992**, *12*, 303−314.

(18) Radecki, A.; Grzybowski, J.; Halkiewicz, J.; Lamparczyk, H. Isolation and identification of some components of the lower-boiling fraction of a commercial smoke flavoring. *Acta Aliment. Pol*. **1977**, *3*, 203−207.

(19) Baltes, W.; Wittkoswki, R.; Söchtig, I.; Block, H.; Tóth, L. Ingredients of smoke and smoke flavour preparation. In *The Quality of Food and Beverages*; Charalambous, G., Inglett, G., Eds.; Academic Press: New York, 1981; pp 1−19.

(20) Guillén, M. D.; Ibargoitia, M. L. GC/MS analysis of lignin monomers, dimers and trimers in liquid smoke flavourings. *J. Sci. Food Agric.* **1999**, *79*, 1889−1903.

(21) Ayres, D. C.; Loike, J. D. *Lignans: Chemical, biological and clinical properties*. Part of the Chemistry and Pharmacology of Natural Products series; Cambridge University Press: Cambridge, UK, 1990.

(22) Lu, H.; Liu, G. T. Anti-oxidant activity of dibenzocyclooctene lignans isolated from *Schisandraceae*. *Planta Med*. **1992**, *58*, 311−313.

(23) Barclay, L. C. R.; Xi, F.; Norris, J. Q. Antioxidant properties of phenolic lignin model compounds. *J. Wood Chem. Technol*. **1997**, *17*, 73−90.

(24) Viriot, C.; Scalbert, A.; Lapierre, C.; Moutounet, M. Ellagitannins and lignins in aging of spirits in oak barrels. *J. Agric. Food Chem*. **1993**, *41*, 1872−1879.

(25) Pouwels, A. D.; Eijkel, G. B.; Boon, J. J. Curie-point Pyrolysis−Capillary Gas Chromatography−High-Resolution Mass Spectrometry of Microcrystalline Cellulose. *J. Anal. Appl. Pyrolysis* **1989**, *14*, 237−280.

(26) Faix, O.; Fortmann, I.; Bremer, J.; Meier, D. Thermal degradation products of wood. Gas chromatographic separation and mass spectrometric characterization of polysaccharide derived products. *Holz Roh- Werkst.* **1991**, *49*, 213-219.

(27) Faix, O.; Fortmann, I.; Bremer, J.; Meier, D. Thermal degradation products of wood. A collection of electron-impact (EI) mass spectra of polysaccharide derived products. *Holz Roh- Werkst*. **1991**, *49*, 299−304.

(28) Galleti, G. C.; Bocchini, P. Pyrolysis/Gas Chromatography/Mass Spectrometry of Lignocellulose. *Rapid Commun. Mass Spectrom*. **1995**, *9*, 815−826.

(29) Ponder, G. R.; Richards, G. N. Thermal synthesis and pyrolysis of a xylan. *Carbohydr. Res*. **1991**, *218*, 143−155.

(30) Galleti, G. C.; Carnacini, A.; Bocchini, P.; Antonelli, A. Chemical Composition of Wood Casks for Wine Aging as Determined by Pyrolysis/GC/MS. *Rapid Commun. Mass Spectrom*. **1995**, *9*, 1331−1334.

(31) Jakab, E.; Liu, K.; Meuzelaar, H. L. C. Thermal Decomposition of Wood and Cellulose in the Presence of Solvent Vapors. *Ind. Eng. Chem. Res.* **1997**, *36*, 2087−2095.

(32) Reid, K. J. G.; Swan, J. S.; Gutteridge, C. S. Assessment of Scotch whisky quality by pyrolysis-mass spectrometry and the subsequent correlation of quality with the oak wood cask. *J. Anal. Appl. Pyrolysis* **1993**, *25*, 49−62.

(33) Viani, R.; Horman, Y. Thermal behaviour of trigonelline. *J. Food Sci.* **1974**, *39*, 1216−1217.

(34) Flament, Y. Coffee, cocoa, and tea. *Food Rev. Int*. **1989**, *5*, 317−414.

(35) McKenzie, L. M.; Hao, W. M.; Richards, G. N.; Ward, D. E. Quantification of major components emitted from smoldering combustion of wood. *Atmos. Environ*. **1994**, *28*, 3285−3292.

(36) Hirata, T. Kinetics of Pyrolysis of Wood and Cellulose. *Mokuzai-Gakkaishi* **1995**, *41*, 879−886.

(37) Soares, S.; Camino, G.; Levchik, S. Comparative Study of the Thermal Decomposition of Pure Cellulose and Pulp Paper. *Polym. Degradat. Stabil.* **1995**, *49*, 275−283.

(38) Evans, R. J.; Wang, D.; Agblevor, F. A.; Chum, H. L.; Baldwin, S. D. Mass spectrometric studies of the thermal decomposition of carbohydrates using ^{13}C-labeled cellulose and glucose. *Carbohydr. Res*. **1996**, *281*, 219−235.

(39) Milosavljevic, Y.; Oja, V.; Sunberg, E. M. Thermal Effects in Cellulose Pyrolysis: Relationship to Char Formation Processes. *Ind. Eng. Chem. Res.* **1996**, *35*, 653−662.

(40) Simkovic, I.; Francis, B. A.; Reeves, J. B. Pyrolysis−gas chromatography−mass spectrometry analysis of starch-based ion-exchangers. *J. Anal. Appl. Pyrolysis* **1997**, *43*, 145−155.

(41) Minowa, T.; Fang, Z.; Ogi, T.; Várhegyi, G. Decomposition of Cellulose and Glucose in Hot-Compressed Water under Catalyst-Free Conditions. *J. Chem. Eng. Jpn.* **1998**, *31* (1), 131−134.
(42) Alén, R.; Kuoppala, E.; Oesch, P. Formation of the main degradation compound groups from wood and its components during pyrolysis. *J. Anal. Appl. Pyrolysis* **1996**, *36*, 137−148.
(43) Simoneit, B. R. T.; Schauer, J. J.; Nolte, C. G.; Oros, D. R.; Elias, V. O.; Fraser, M. P.; Rogge, W. F.; Cass, G. R. Levoglucosan, a tracer for cellulose in biomass burning and atmospheric particles. *Atmos. Environ.* **1999**, *33*, 173−182.
(44) Belitz, H. D.; Grosch, W. *Food Chemistry*; Springer-Verlag: Berlin, Germany, 1999; Chapter 4, Carbohydrates.
(45) Dorland, L.; Wadman, S. K.; Fabery de Jonge, H.; Ketting, D. 1,6-Anhydro-β-D-glucopyranose (β-glucosan), a constituent of human urine. *Clin. Chim. Acta* **1986**, *159*, 11−16.
(46) Gack, C.; Klufers, P. A homoleptic cuprate(II) complex with deprotonated 1,6-anhydro-β-glucose (levoglucosan) ligands. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1996**, *52*, 2972−2975.
(47) Ahlrichs, R.; Ballauff, M.; Eichkorn, K.; Hanemann, O.; Kettenbach, G.; Klufers, P. Polyol metal complex, part 30- Aqueous ethylenediamine dihydroxo palladium(II): A coordinating agent for low-and high-molecular weight carbohydrates. *Chem.− Eur. J.* **1998**, *4*, 835−844.

Received for review June 21, 2000. Revised manuscript received December 28, 2000. Accepted February 15, 2001. This work has been supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT, ALI97-1095) and by the Universidad del País Vasco (UPV-101.123-EB087/99).

JF000760T