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Monosaccharide anhydrides, new markers of toasted oak wood used for ageing wines and distillates

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ABSTRACT

In order to shed light on the thermal carbohydrate transformation chemistry, the non-volatile composition of natural and toasted oak wood samples of different origins were studied. GC–MS analysis revealed the presence of monosaccharide anhydrides (MAs) only in toasted oak wood samples. They were formed by the cellulose and hemicelluloses thermal degradation produced during the toasting process, and the content was dependent on the degree of toasting. The principal MAs formed in the oak wood toasting process were 1,6-anhydro-β-D-glucopyranose (levoglucosan), 1,6-anhydro-β-D-galactosane (galactosan) and 1,6-anhydro-β-D-mannopyranose (mannosan), with levoglucosan being the dominant MAs detected. Monosaccharide anhydrides can be considered specific products formed during the thermal treatment of wood; therefore they are proposed as markers of toasted wood and could be used to monitor and determine the intensity of toasting process.

1. Introduction

It is well known that *Quercus sp*. oak is by far the most widely wood used in the manufacture of barrels intended for aging wines and spirits. During this period of maturation, these beverages develop a distinctive aroma and flavour that are appreciated and well-valued by consumers.

A common practice used in cooperage and regarded as the most important technological step, is the toasting process. Throughout this procedure, the increase in temperature causes a modification in the physical structure, that is crucial in facilitating the shaping of the staves. However, the most important fact is that the chemical composition of the wood undergoes great changes due to the thermal degradation of oak wood polymers.

Oak wood is mainly composed of three large polymers: (i) cellulose (50%), which is a linear polymer made up of long-chain D-glucose monomers, (ii) hemicelluloses (20%) which is a mixture of polysaccharides derived from glucose, mannose, galactose, xylose, arabinose, 4-*O*-methylglucuronic acid and galacturonic acid, and (iii) the other large polymer is lignin (30%), a three-dimensional reticulated polymer of phenylpropane with units of guaiacyl (2-methoxyphenol) and syringyl (2,6-dimethoxyphenol) that are cross-linked by oxidation.

Due to the high temperature reached during the toasting process, the chemical bonds between polymers are disrupted and the hemicelluloses and lignin in particular are degraded since they are less structured than cellulose. Their degradation gives rise to a great number of new compounds which play an important role in the development of wine flavours (Singleton, 1995).

For cooperages, it is essential to control and check the degree of toasting applied to barrels, since it strongly the affects taste, body, aroma and flavours of aged beverages. A strong intensity of toasting may produce the appearance of burnt notes which are unpleasant for consumers. Therefore, the intensity of toasting has a considerable influence on the quality of the aged beverages.

Determining the toasting degree is problematic. Monitoring the change in concentrations of certain volatile compounds, such furfural, vanillin, guaiacol, has been used to determine and control the toasting degree (Chatonnet, Cutzach, Pons, & Dubourdieu, 1999). The problem with this method is that the heating of oak increases the concentrations of volatile compounds up to a certain level of toasting but, if toasting continues, concentrations tend to decrease due to the pyrolytic effect (Pérez-Coello, Sanz, & Cabezudo, 1997). Furthermore, the variability of these compounds in oak samples is another factor to take into account at the time of determining and controlling the toasting degree. Therefore, new compounds formed exclusively in the toasting process are better markers for toasting and its intensity determination.

The thermal carbohydrate degradation, of cellulose and hemicelluloses produces hexoses, pentoses and short chain polysaccharides during the toasting process. Previous papers have studied the odoriferous volatile compounds derived from parietal oak wood carbohydrates such as furfural, 5-methyl-furfural,

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5-hydroxy-methyl-furfural, furfuryl alcohol (Chatonnet, 1998; Nishimura & Matsuyama, 1989; Nishimura, Ohnishi, Masuda, Koga, & Matsuyama, 1983). During the toasting process, other volatile substances are also formed as a result of interaction between sugars and amino acid, known as Maillard reaction. These include, 2-hydroxy-3-methyl-2-cyclopenten1-one (cyclotene), 3-hydroxy-2-methyl-4H-pyran-4-one (maltol), 2,3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), 4-hydroxy-2,5-dimethyl-3(2H)-furan-3-one (furaneol) and 2,3-dihydro-5-hydroxy-6-methyl-4H-pyran-4-one (dihydromaltol) which contribute to the "toasty caramel" aroma of heated oak (Cutzach, Chatonnet, Henry, & Dubourdieu, 1997). 2,5-Furandicarbaldehyde, furylhydroxymethyl ketone and hydroxymaltol also have a "toasty caramel" odour, and their presence are due to Maillard reactions and direct pyrolysis of sugars (Cutzach, Chatonnet, Henry, & Dubourdieu, 1999). In addition, 2-furaldehyde and its derivatives 5-methoxy-2-furaldehyde and 5-methyl-2-furaldehyde, have also been described as products of the degradation of sugars during the wood toasting process (Goldberg, Hoffman, Yang, & Soleas, 1999; Ho, Hogg, & Silva, 1999). However, there has been no reported correlation between the formation of these compounds and the toasting level have been found.

In spite of numerous papers about the volatile oak wood compounds generated during the toasting process, the thermal degradation process of carbohydrates is not completely known. Studies related to the non-volatile compounds formed from thermal carbohydrate degradation have not been conducted until now.

In order to determine the thermal carbohydrate transformation chemistry, the non-volatile composition of natural and toasted oak wood was studied. MAs were identified in toasted oak wood as a consequence of thermal degradation of cellulose and hemicellulose produced during the toasting treatment. The relationship between the temperature used in the toasting process and the amount of these new compounds formed were evaluated. Their facility to pass from wood to wines and distillates, and their possible sensorial effects were also investigated.

2. Materials and methods

2.1. Reagents and standards

Methanol (chromatographic purity) was supplied by Scharlau Chemie S.A. (Barcelona, Spain), ethanol was obtained from Panreac Química S.A.U (Barcelona, Spain) and water was purified using a Mili-Q system (Millipore Corp., Billerica, MA). Internal standard, methyl-β-D-galactopyranoside, reference standard compounds and derivatization agents were supplied by Sigma–Aldrich (Madrid, Spain).

2.2. Samples

2.2.1. Oak wood samples

Ten samples of oak wood shavings (sized 2 cm × 1 cm × 0.1 cm) collected from non-toasted and toasted oak wood of different origins: American (*Quercus alba*), French and Hungarian (*Quercus petraea*) and Rumanian and Russian (*Quercus robur*) were obtained from Tonelería Magreñan S.L. (La Rioja, Spain). Initially, the oak woods were naturally seasoned in the open air and sections of the non-toasted oak woods received thermal treatment. Toasted samples were obtained over the same type of oak wood fire at a medium intensity level (45–50 min with the temperature of wood surface being 160–170 °C).

In order to study the thermal effect on non-volatile compounds, shavings of natural American oak wood (*Q. alba*) were toasted in a laboratory oven. Two batches of natural shavings were extended in a fine layer and toasted at different temperatures: 100, 150, 175, 200 and 250 °C for 50 min.

Prior to the extraction process, shavings of all samples were ground with a mechanical mill and sieved (size < 1 mm), in order to obtain a homogenous sawdust.

2.2.2. Assay of model wine and distillate solutions in contact with toasted oak wood

Fourteen grams of American toasted shavings supplied by Magreñan S.L. (La Rioja, Spain) were soaked for three weeks in a liter of hydroalcoholic solution: 12% ethanol (v/v) in the case of model wine, adjusted to pH 3.5 with tartaric acid, and 40% ethanol (v/v) for the model distillate solution. The solutions, performed in duplicate, were shaken daily and finally filtered through glass wool prior to analysis.

2.3. Accelerated solvent extraction (ASE)

Extraction of target compounds from wood shavings were carried out using a accelerated solvent extractor ASE 200 (Dionex Corp, Sunnyuale, CA) equipped with a solvent controller. Extractions were performed using the optimized method developed in a previous study by our group (Alañón, Ruiz-Matute, Martínez-Castro, Díaz-Maroto, & Pérez-Coello, 2009). Three hundred milligram of sawdust mixed with two grams of diatomaceous earth as a dispersant agent, were placed in inox extraction cells of 11 ml. Extractions were carried out using methanol at 90 °C. After the injection of solvent into the cell, a pressurised static extraction phase lasting 15 min was carried out under pressure, at 1500 psi. Between extractions, a rinse of the complete system was performed to avoid any carry-over.

2.4. Derivatization procedure

A derivatization step must be conducted before GC–MS analysis of sugars to improve their chromatographic responses. A two-step derivatization procedure (oximation and trimethylsilylation) was used (Sanz, Sanz, & Martínez-Castro, 2004).

An aliquot of 10 ml of the extract obtained by accelerated solvent extraction and 10 ml of hydroalcoholic model solution were mixed with 100 μl of methyl-β-D-galactopyranoside in 70% methanol (1.0 g l^{-1}) as an internal standard. The solvent was removed *in vacuo* at 40 °C. Then, the residue was derivatized by adding 350 μl of hydroxylamine chloride (2.5%) in pyridine and heated to 70 °C for 30 min. The oximes obtained in this step were silylated with 350 μl of hexamethyldisilazane and 35 μl of trifluoroacetic acid at 45 °C for 30 min (Brost & Lott, 1996). The reaction mixture was centrifuged at 3600*g* for 5 min at 5 °C (Li & Schumann, 1981). Supernatants containing the carbohydrate oximes were injected onto the GC column or stored at 4 °C prior to analysis.

2.5. Gas chromatographic–mass spectrometry analysis (GC–MS)

GC–MS analyses were performed with an Agilent Technologies HP-G 1800B, GCD System couple to a mass detector (Agilent Technologies, Palo Alto, CA, USA). Helium was the carrier gas, with a linear velocity of 33 cm s^{-1}. A HP-5 capillary column (5% phenylmethylpolysiloxane) stationary phase, (30 m, 0.25 mm i.d., 0.25 μm film thickness) was used. The oven temperature was held at 80 °C for 3 min and increased to 250 °C at 3 °C min^{-1}, then at 8 °C min^{-1} to 290 °C holding it for 30 min. The injector temperature was 300 °C. Solvent delay was set at 15 min and 1 μL was injected in split mode with a ratio of 1:18.

The temperatures of ion source and the transfer line were 170 °C and 280 °C, respectively. Positive ion electron impact

spectra were recorded at 70 eV ionisation energy in SCAN mode with a 35–450 amu mass acquisition range.

Chromatographic peaks were identified by comparing their retention index and mass spectra with those of control standards. The identification of compounds with no matching reference was carried out by comparison of their mass spectra and retention indices with those reported in the literature (Carlavilla, Villamiel, Martínez-Castro, & Moreno-Arribas, 2006; Sanz et al., 2004; Fabbri & Chiavari, 2001; Hsu, Cheng, Lee, & Ding, 2007). Semi-quantitative assessment of compounds was carried out by the internal standard method assuming a response factor equal to one.

2.6. Statistical analysis

One way analysis of variance (ANOVA) was utilised to determine whether there were significant differences amongst the samples. A regression analysis was carried out to obtain regression models. Both statistical processes were carried out using the SPSS 15.0 for Windows statistical package.

2.7. Sensory analysis of levoglucosan

A panel of five judges aged 25–50 years, experienced in the field of sensorial analysis were used for the taste evaluation. The sensory experiments were performed in a sensory room at 22–25 °C in two independent sessions. Levoglucosan was added to mineral water at different concentrations: 10, 20 and 30 mg l^{-1}. The judges tasted all of the solutions and they were asked to describe the sensations perceived and to evaluate their intensities on a scale ranging from 0 to 10 points.

3. Results and discussions

3.1. Qualitative and quantitative analysis of sugars in toasted and non-toasted oak wood samples

For determination of sugars present in wood samples, a previous derivatization procedure was employed prior to GC–MS analysis. After oximation and silylation reactions, each reducing sugar gave two chromatographic peaks corresponding to their per-*O*-trimethylsilylated syn- and anti-oxime derivatives. Non-reducing sugars gave per-*O*-trimethylsilyl derivatives, evident by the presence of a single chromatographic peak. Table 1 shows the spectral data of TMS derivatives of non volatile compounds studied.

The identification and concentration of the non-volatile compounds found in natural and toasted oak wood samples from different origins are given in Table 2. Chromatographic profiles of non-toasted samples showed similar spectra (Fig. 1a). The most abundant compound detected in oak wood samples was quercitol (1,3,4/2,5-cyclohexane-pentol). This compound, also called "acorn sugar", is a specific deoxyinositol of the *Quercus* species and is regarded as a good marker of wine ageing in oak woods (Carlavilla et al., 2006). Two compounds, whose identifications have not been possible, were also found and assigned as deoxyinositols due to the similarity of the mass spectra to that of quercitol. The presence of these unknown compounds has already been detected in other oak wood samples and published in a previous paper (Alañón et al., 2009). *Muco*-inositol, *chiro*-inositol, *scyll*o-inositol and *myo*-inositol were also found in the samples. These were identified by their characteristic mass fragments *m*/*z* 305 and 318. Inositols, present in the vegetable kingdom as components of plants, have been used as an indicator for the origin of honey

Table 1
Spectral data of trimethylsilylated-oximes and trimethylsilyl derivatives of non volatile compounds found in oak wood samples.

R.I.[a]	Peak[b]	*m*/*z*[c]	Assignment[d]	References[e]
1683	1	73, 147, 204, **217**	1,6-Anhydro-galactopyranose (galactosan)	T, M[f,g]
1699	2	**73**, 191, 217, 332	1,4-Anhydro-galactopyranose	T, M[f]
1703	3	73, 204, 217, 333	1,6-Anhydro-mannopyranose (mannosan)	T, M[f,g]
1712	4	73, **217**, 319	1,6-anhydro-galactofuranose	T, M[f]
1728	5	**73**, 204, 217, 333	1,6-Anhydro-glucopyranose (levoglucosan)	S, M[f,g]
1736	6	**73**, 204, 217, 333	1,4-Anhydro-mannopyranose	T, M[f]
1748	7	73, 147, **217**, 319	1,6-Anhydro-mannofuranose	T, M[f]
1758	8	**73**, 217, 307, 319	Arabitol	S
1763	9	73, 129, **217**, 319	1,6-Anhydro-glucofuranose	T, M[f]
1793	10	**73**, 147, 217, 307	Xylose	S
1801	10,11	**73**, 147, 217, 307	Xylose, arabinose	S
1802	11	**73**, 147, 217, 307	Arabinose	S
1830	12	73, **217**, 305, 318	Quercitol	T, M[h,i]
1913	13	73, **217**, 305, 318	Deoxy-inositol n.i.* 1	T
1946	14	**73**, 217, 305, 318	*muco*-inositol	S, M[h]
1969	15	**73**, 147, 205, 319	Mannitol	S
1986	16	73, 147, 217, 307	Fructose	S
1991	17	**73**, 217, 305, 318	*chiro*-inositol	S, M[i]
1994	16	**73**, 147, 217, 307	Fructose	S
2003	18	73, **217**, 305, 318	Deoxy-inositol n.i.* 2	T
2032	19	**73**, 147, 217, 319	Galactose	S
2045	20	**73**, 147, 205, 319	Glucose	S
2058	19,20	**73**, 147, 205, 319	Galactose, glucose	S
2069	21	**73**, 217, 305, 318	*scyllo*-inositol	T, M[i]
2124	22	73, **217**, 305, 318	*myo*-inositol	S, M[h,i]

[a] Retention index calculated on a HP-5 capillary column.
[b] A single chromatographic peak for non-reducing sugars and two chromatographic peaks for reducing sugars, corresponding to their oxime isomers E and Z.
[c] Characteristic ions in the mass spectrum (base peak in bold).
[d] All peaks are TMS derivatives of the given compounds.
[e] Identification based on: S, analysis of TMS standard compounds; M, compilation of data.
[f] Fabri and Chiavari (2001).
[g] Hsu et al. (2007).
[h] Sanz et al. (2004).
[i] Carlavilla et al. (2006); T, tentatively identified by mass spectra.
* n.i.: Not identified.

Table 2
Carbohydrate content (mg g^{-1}) of toasted and non-toasted oak wood from different provenances. Standard deviations in parentheses ($n = 2$).

Compound	American		French		Hungarian		Rumanian		Russian	
	Non-toasted	Toasted	Non-toasted	Toasted	Non-toasted	Toasted	Non-toasted	Toasted	Non-toasted	Toasted
1,6-Anhydro-galactopyranose (galactosan)	n.d	0.215(0.009)	n.d	0.422(0.046)	n.d	0.512(0.020)	n.d	0.438(0.006)	n.d	0.256(0.012)
1, 4-Anhydro-galactopyraonse	n.d	0.032(0.000)	n.d	0.053(0.003)	n.d	0.058(0.003)	n.d	0.073(0.004)	n.d	0.041(0.005)
1,6-Anhydro-mannopyranose (mannosan)	n.d	0.286(0.004)	n.d	0.148(0.007)	n.d	0.274(0.022)	n.d	0.202(0.017)	n.d	0.240(0.000)
1,6-Anhydro-galactofuranose	n.d	0.044(0.001)	n.d	0.092(0.010)	n.d	0.129 (0.010)	n.d	0.093(0.007)	n.d	0.067(0.003)
1,6-Anhydro-glucopyranose (levoglucosan)	0.010(0.005)	1.53(0.149)	0.001(0.000)	2.59(0.358)	0.030(0.006)	6.09(0.396)	0.006(0.001)	2.72(0.101)	0.014(0.003)	2.34(0.018)
1,4-Anhydro-mannopyranose	n.d	0.009(0.002)	n.d	0.005(0.002)	n.d	0.007(0.001)	n.d	0.005(0.001)	n.d	0.008(0.001)
1,6-Anhydro-mannofuranose	n.d	0.004(0.000)	n.d	0.004(0.000)	n.d	0.004(0.002)	n.d	0.003(0.001)	n.d	0.004(0.000)
Arabitol	0.023(0.002)	0.008(0.001)	0.018(0.000)	0.006(0.001)	0.041(0.011)	Tr	0.009(0.001)	0.004(0.001)	0.050(0.000)	0.009(0.002)
1,6-anhydro-glucofuranose	n.d	0.124(0.001)	n.d	0.245(0.029)	n.d	0.570(0.081)	n.d	0.180(0.013)	n.d	0.189(0.010)
Xylose and arabinose	1.27(0.110)	0.300(0.017)	1.45(0.037)	0.400(0.074)	1.46(0.015)	0.198(0.024)	0.819(0.025)	0.099(0.009)	0.813(0.039)	0.122(0.007)
Quercitol	4.19(0.110)	2.51(0.009)	1.52(0.077)	0.909(0.028)	1.88(0.063)	0.595(0.055)	3.32(0.095)	1.97(0.067)	3.17(0.197)	1.43(0.127)
Deoxy-inositol n.i. 1	0.088(0.001)	0.055(0.000)	0.043*(0.004)	0.028*(0.002)	0.071(0.004)	0.025(0.000)	0.079*(0.003)	0.046*(0.007)	0.086(0.003)	0.040(0.005)
Muco-inositol	0.076*(0.003)	0.080*(0.004)	0.077*(0.026)	0.055*(0.007)	0.138(0.009)	0.044(0.002)	0.093*(0.006)	0.055*(0.010)	0.112*(0.006)	0.055*(0.000)
Mannitol	0.011(0.000)	Tr	0.005(0.002)	Tr	0.015(0.001)	Tr	0.008(0.002)	Tr	0.007(0.001)	Tr
Fructose	0.469(0.035)	0.119(0.015)	0.298(0.054)	0.093(0.016)	0.522(0.034)	Tr	0.378(0.003)	0.032(0.007)	0.135(0.010)	0.027(0.004)
Chiro-inositol	0.021*(0.006)	0.021*(0.004)	0.035*(0.013)	0.024*(0.012)	0.027(0.002)	0.006(0.001)	0.022*(0.005)	0.016*(0.001)	0.028(0.001)	0.017(0.001)
Deoxy-inositol n.i. 2	0.201(0.006)	0.151(0.003)	0.153(0.003)	0.104(0.002)	0.202(0.009)	0.082(0.003)	0.221(0.006)	0.140(0.005)	0.291(0.008)	0.152(0.008)
Galactose and glucose	1.14(0.065)	0.281(0.030)	1.29(0.137)	0.323(0.055)	1.33(0.086)	0.119(0.013)	0.915(0.015)	0.092(0.025)	0.800(0.001)	0.074(0.012)
Scyllo-inositol	0.157*(0.001)	0.104*(0.008)	0.191*(0.023)	0.137*(0.002)	0.160(0.006)	0.059(0.010)	0.383(0.020)	0.214(0.010)	0.275(0.014)	0.147(0.020)
Myo-inositol	0.479(0.000)	0.339(0.010)	0.280*(0.013)	0.197*(0.004)	0.298(0.008)	0.126(0.008)	0.346(0.022)	0.203(0.011)	0.400(0.006)	0.209(0.009)

n.d.: Not detected; Tr: traces, concentration < 0.001 mg g^{-1}; n.i.: not identified.
* Not significant differences found between non-toasted and toasted oak wood samples from each provenance.

(Sanz et al., 2004; Sanz, Gonzalez, De Lorenzo, Sanz, & Martínez-Castro, 2005) to control the genuineness of the concentrated rectified grape must (Monetti, Versini, Dalpiaz, & Reniero, 1996) and to provide information on the quality and genuineness of fresh fruit juice (Sanz et al., 2004; Villamiel, Martínez-Castro, Olano, & Corzo, 1998). More recently, the presence of the seven inositols seems to be a good indicator for the *Quercus* species (Ruiz-Matute et al., in preparation).

In non-toasted oak samples, the identification of sugars such as arabitol, xylose, arabinose, mannitol, fructose, galactose and glucose have also been possible. Xylose and arabinose could not be quantified individually due to overlapping of the second xylose peak with the first arabinose chromatographic signal. Galactose and glucose were also quantified together, since one of the glucose chromatographic signals appeared to overlap a galactose peak, complicating their separate measurement.

The analysis of toasted oak wood samples revealed changes in the carbohydrate profile after toasting process (Fig. 1b). There was a general decrease in the inositol concentrations, although in some cases there was no significant difference between toasted and non-toasted oak wood samples (Table 2). The presence of quercitol was considerably reduced in all toasted samples, showing significant differences ($p < 0.05$) between toasted and non toasted oak wood. Therefore, although quercitol is the major inositol present in toasted oak wood samples, it is also the most prone inositol to thermal degradation.

Comparing the amounts of polyalcohols (arabitol and mannitol) and monosaccharides (xylose, arabinose, fructose, galactose and glucose) present in toasted samples with those found in non-toasted woods, it is possible to appreciate an important decrease in their concentrations. This fact can be attributed to the thermal degradation that these compounds seem to suffer during the toasting process.

The most striking difference found between toasted and non-toasted oak wood samples was the presence of new peaks (1–7 and 9) at the beginning of chromatogram (Fig. 1b). These peaks corresponded to non-reducing monosaccharides, which seem to have formed during the toasting process.

By mass spectroscopy analysis, it was possible to confirm that these compounds were MAs. The formation of these new compounds can be explained by the thermal degradation of cellulose and hemicelluloses, which is produced during the toasting step. This degradation implies the releasing of their monomers: glucose, mannose and galactose which, under anhydrous conditions, suffer an intramolecular dehydratation by loss of molecules of water originating from MAs (Paez, Martínez-Castro, & Olano, 1987). The presence of MAs in oak wood used in barrelmaking for wine and spirit ageing has not previously been reported. However, MAs have been proposed as specific indicators for the presence of cellulose in biomass burning and in atmospheric samples (Hsu et al., 2007; Nolte, Schuauer, Cass, & Simoneit, 2001; Simoneit et al., 1999).

The principal MAs formed in the toasting process of oak wood were 1,6-anhydro glucopyranose (levoglucosan), 1,6-anhydro galactopyranose (galactosan) and 1,6-anhydro mannopyranose (mannosan). These compounds indicate that anhydrosugars of six member rings are the main species formed during the thermal degradation of cellulose and hemicelluloses. Among then, levoglucosan was the dominant MA detected in all samples, and also the main non-volatile compound found in the majority of toasted samples. These derivatives of 1,6-anhydrohexopyranoses showed a common fragmentation pathway in their mass spectra profile (Fig. 2a). Two relative intense ions, m/z 217 and 204, were observed and were tentatively attributed to the loss of $C_6H_{13}O_3Si$ and $C_7H_{14}O_3Si$ from the molecular ion, respectively, while the relative abundant ion m/z 333 was tentatively attributed to the loss

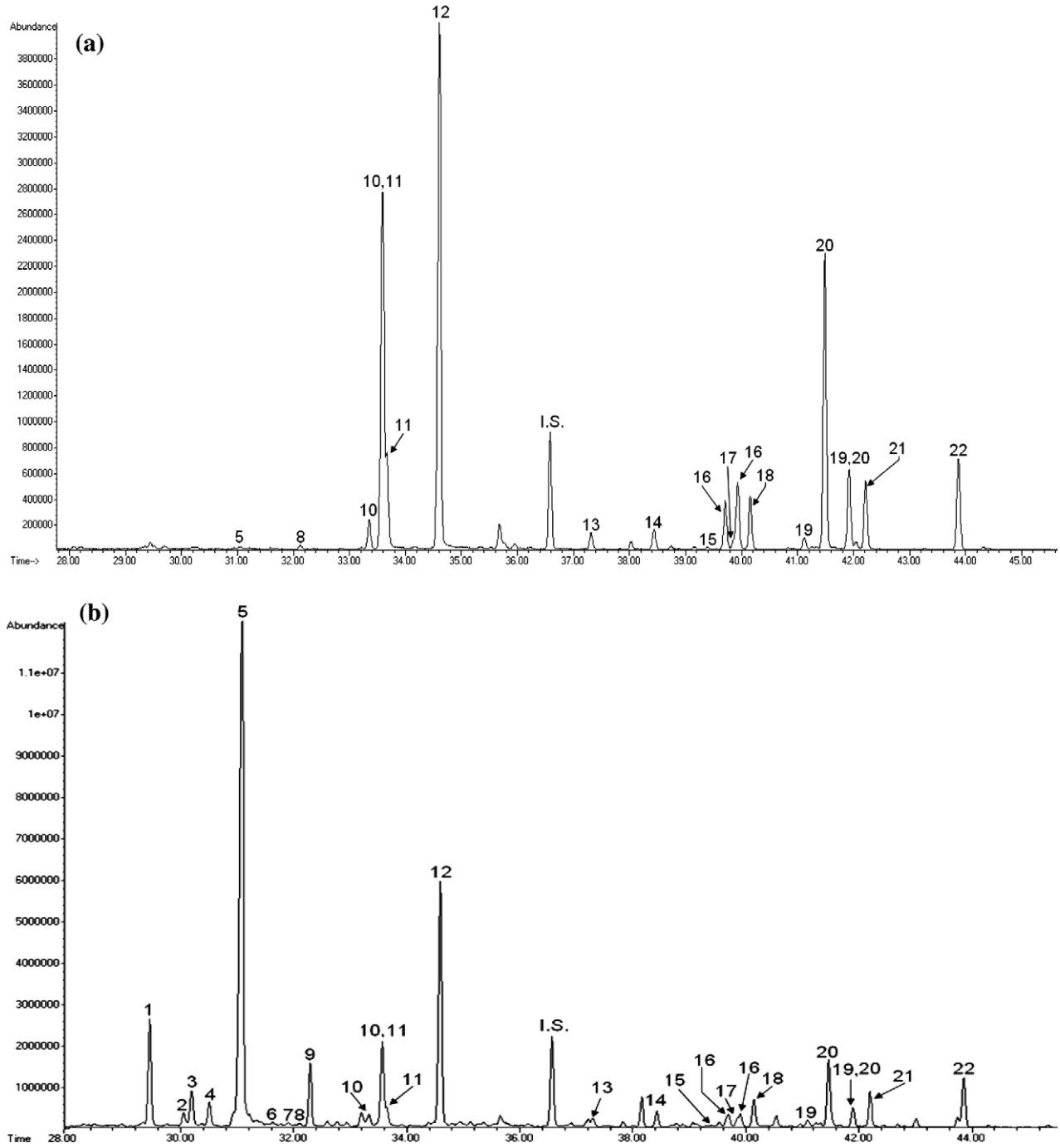

Fig. 1. Chromatogram profiles obtained by GC analysis of TMS derivatives of non toasted French oak wood (a) and toasted French oak wood (b).

of a C_2H_5O from the molecular ion (Hsu et al., 2007). All derivatives displayed ion at *m*/*z* 73 $[(CH_3)_3Si]^+$ which was characteristic of the TMS group.

Furthermore, small quantities of other anhydride derivatives were also determined in toasted samples. The presence of 1,6-anhydro glucofuranose, 1,6-anhydro galactofuranose and 1,6-anhydro mannofuranose were detected by the interpretation of fragmentation pattern of their mass spectra. They showed a base peak at *m*/*z* 217 and another relative small peak at *m*/*z* 319 (Fig. 2b). High levels of both these ions are characteristic of the mass spectra of sugars in the furanoside form (DeJongh et al., 1969). 1,4-anhydrohexopyranoses, 1,4-anhydrogalactopyranose and 1,4-anhydro-mannopyranose, were tentatively identified (Fig. 2c). Their assignments were based on the mass spectra found in another study (Hsu et al., 2007).

In samples of non-toasted oak wood, low concentrations of levoglucosan were also detected. This fact can be attributed to a slight dehydration suffered during the seasoning process of oak wood, during the milling and/or during the extraction process.

3.2. Effect of toasting degree of the oak wood

The effect of the toasting intensity on the amount of target non-volatile compounds were evaluated in a model experiment, paying special attention to the new compounds, monosaccharide anhydrides, formed in the toasting process. Chips of oak wood (*Q. alba*)

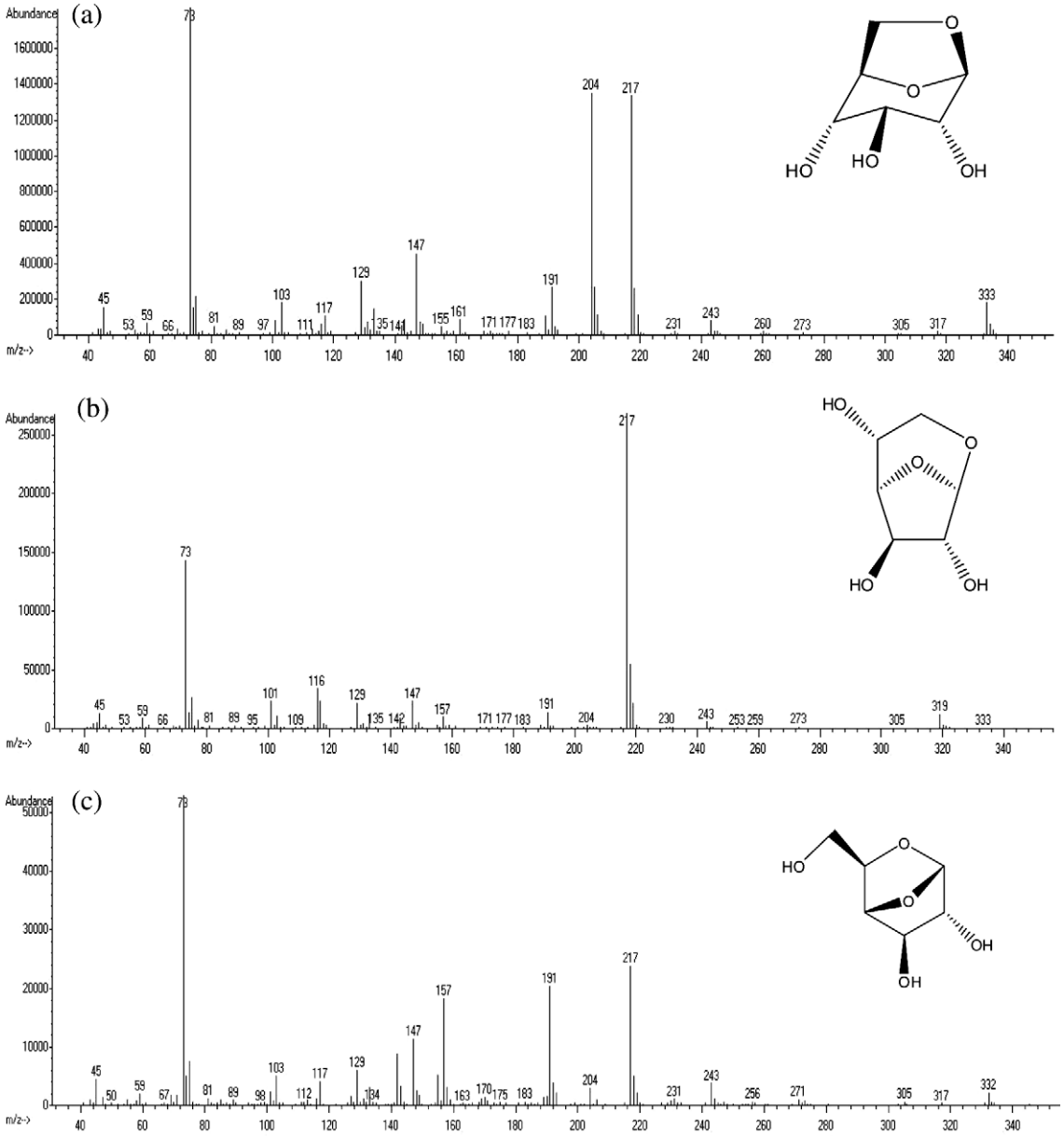

Fig. 2. Mass spectrum and structures of some monosaccharides anhydrides found: (a) 1,6-anydro-glucopyranose (levoglucosan), (b) 1,6-anhydro-glucofuranose, (c) 1,4-anhydro-galactopyranose.

were toasted in a laboratory oven at different temperatures (100, 150, 175, 200 and 250 °C) for 50 min. The evolution of monosaccharides, inositols and MAs content at different temperatures is shown in Table 3. The changes produced in the target compounds coincide with the results observed in oak wood samples. The concentration of monosaccharides and inositols decreased seriously according to the toasting temperature applied. This behaviour is attributed to the thermal degradation that these compounds suffer during the toasting process. Consequently, new compounds are formed by means of hydrolysis, oxidation, dehydration and pyrolysis reactions or as a result of interaction of sugars with an amino acid, known as Maillard reactions. With regards to MAs, as the intensity of toasting increased the content of these compounds also increased (Table 3). The most notable anhydrosugars formed were levoglucosan, mannosan and galactosan, with levoglucosan being the main specie produced in the toasting step. It is also remarkable that the main significant increase in MAs content was observed at 250 °C.

To determine if there was a correlation between the concentrations of some compounds and the temperature, a regression analysis was carried out. Quercitol showed a good regression model that fit very well to the linear regression curve (regression *r*-squared of 0.938) Fig. 3. This confirms that there is a linear relationship between the degradation of quercitol with the temperature.

In contrast, the content of levoglucosan showed a progressive increase up to 200 °C and then suffered a significant increase at 250 °C (Table 3). Therefore, the relationship between the concentration of levoglucosan with temperature displayed an exponential growth curve. Data of regression analysis confirmed this fact with a regression *r*-squared of 0.865. The exponential equation showed a slope of 0.018 and a constant of 0.005.

Based on the results obtained, the relationship between some non-volatile compounds and toasting degree became evident. Due to the exponential correlation between the formation of MAs with the temperature, MAs could be used to monitor and

Table 3
Evolution of carbohydrate content from American oak wood at different toasting intensities. Standard deviations in parentheses ($n = 2$).

Compounds	Carbohydrate content (mg g^{-1})					
	Non toasted	100 °C	150 °C	175 °C	200 °C	250 °C
1,6-Anhydro-galactopyranose (galactosan)	n.d.	0.003 (0.001)	0.009 (0.001)	0.024 (0.001)	0.115 (0.012)	0.128 (0.017)
1,4-Anhydro-galactopyranose	n.d.	n.d.	Tr	0.012 (0.000)	0.025 (0.004)	0.036(0.004)
1,6-Anhydro-mannopyranose (mannosan)	n.d.	Tr	0.011 (0.001)	0.013 (0.003)	0.041 (0.003)	0.204 (0.021)
1,6-Anhydro-galactofuranose	n.d.	n.d.	Tr	0.004 (0.001)	0.025 (0.003)	0.038(0.002)
1,6-Anhydro-glucopyranose (levoglucosan)	0.010 (0.001)	0.032 (0.007)	0.057 (0.003)	0.070 (0.008)	0.100 (0.003)	1.18 (0.040)
1,4-Anhydro-mannopyranose	n.d.	n.d.	n.d	Tr	0.001 (0.000)	0.008 (0.001)
1,6-Anydro-mannofuranose	n.d.	n.d.	n.d	Tr	0.001 (0.000)	0.002 (0.000)
Arabitol	0.073 (0.002)	0.079 (0.003)	0.075 (0.003)	0.062 (0.001)	0.012 (0.002)	Tr
1,6-Anhydro-glucofuranose	n.d.	n.d.	0.006 (0.000)	0.010 (0.001)	0.015 (0.002)	0.060 (0.013)
Xylose and arabinose	1.27 (0.110)	1.20 (0.055)	0.446 (0.019)	0.143 (0.014)	0.020 (0.002)	0.011 (0.002)
Quercitol	4.19 (0.110)	2.03 (0.034)	1.92 (0.014)	1.76 (0.002)	0.918 (0.052)	0.109 (0.009)
Deoxy-inositol n.i. 1	0.088 (0.001)	0.039 (0.003)	0.037 (0.002)	0.029 (0.003)	Tr	Tr
Muco-inositol	0.096 (0.003)	0.097 (0.001)	0.103 (0.006)	0.085 (0.012)	0.042 (0.004)	0.004 (0.001)
Mannitol	0.031 (0.000)	0.026 (0.003)	0.019 (0.001)	0.017 (0.000)	Tr	n.d
Fructose	0.469 (0.035)	0.210 (0.026)	0.099 (0.010)	0.037 (0.001)	0.007 (0.000)	Tr
Chiro-inositol	0.021 (0.006)	0.018 (0.002)	0.015 (0.003)	0.015 (0.000)	0.012 (0.001)	Tr
Deoxy-inositol n.i. 2	0.201 (0.006)	0.088 (0.002)	0.086 (0.002)	0.079 (0.001)	0.047 (0.002)	0.007 (0.002)
Galactose and glucose	1.14 (0.065)	0.750 (0.135)	0.505 (0.017)	0.235 (0.005)	0.015 (0.001)	0.011 (0.003)
Scyllo-inositol	0.177 (0.001)	0.174 (0.004)	0.177 (0.008)	0.159 (0.007)	0.110 (0.002)	0.025 (0.007)
Myo-inositol	0.479 (0.000)	0.306 (0.009)	0.275 (0.004)	0.265 (0.021)	0.142 (0.012)	0.024 (0.002)

Tr: traces, concentrations < 0.001 mg g^{-1}; n.d.: not detected; n.i.: not identified.

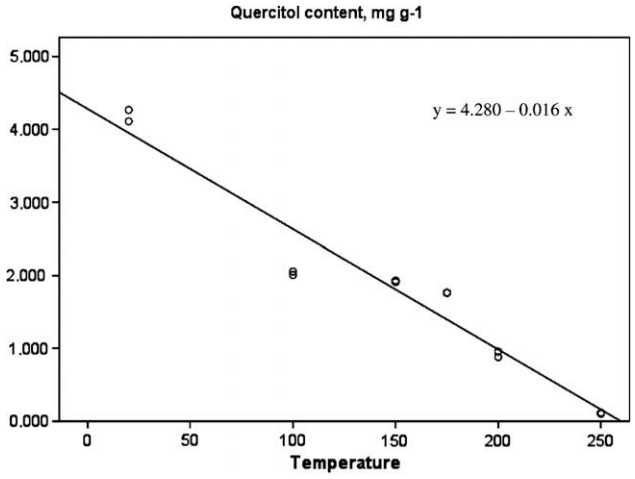

Fig. 3. Regression line of quercitol content versus temperature.

determine the toasting process at high temperatures. Furthermore, the linear degradation of quercitol could also be useful to monitor and determine the toasting intensity applied to barrels in the process step.

3.3. Analysis of model wine and distillate solutions in contact with toasted oak wood

The feasibility of MAs to pass from toasted oak wood to aged beverages was evaluated by means of a model experiment. Synthetic wine and distillate solutions were macerated with toasted oak wood (*Q. alba*). Results of wine and distillate analysis obtained at the end of ageing process are shown in Table 4. All MAs detected in toasted samples were capable of being released into both solutions. 1,6-anhydro glucopyranose (levoglucoan), 1,6-anhydro mannopyranose (mannosan) and 1,6-anhydro galactopyranose (galactosan) were the most representative anhydrides found in both solutions. It is interesting to note that levoglucosan was the main MA released by toasted oak wood. The rest of the non-volatile compounds studied were also detected in both synthetic solutions, with quercitol being the major compound detected.

Table 4
Non-volatile oak related compound concentration (mg l^{-1}) of model hydroalcoholic solutions macerated with toasted American oak chips. Standard deviations in parentheses ($n = 2$).

Compound	Model wine	Model spirit
1,6-Anhydro galactopyranose (galactosan)	2.62 (0.27)	2.88 (0.14)
1,4-Anhydro galactopyranose	0.39 (0.02)	0.49 (0.05)
1,6-Anhydro mannopyranose (mannosan)	3.21 (0.47)	3.34 (0.14)
1,6-Anhydro galactofuranose	0.45 (0.09)	0.43 (0.02)
1,6-Anhydro glucopyranose (levoglucosan)	18.1 (1.12)	18.2 (0.37)
1,4-Anhydro mannopyranose	0.08 (0.02)	0.08 (0.03)
1,6-Anhydro mannofuranose	0.05 (0.00)	0.06 (0.01)
Arabitol	0.25 (0.03)	0.27 (0.02)
1,6-Anhydro glucofuranose	1.44 (0.04)	1.41 (0.07)
Xylose and arabinose	5.56 (0.39)	5.05 (0.23)
Quercitol	41.7 (1.50)	41.9 (2.90)
Deoxy-inositol n.i. 1	1.10 (0.13)	1.72 (0.12)
Muco-inositol	0.55 (0.04)	0.68 (0.05)
Mannitol	Tr	Tr
Fructose	1.95 (0.00)	1.79 (0.01)
Chiro-inositol	0.22 (0.02)	0.32 (0.07)
Deoxy-inositol n.i. 2	2.22 (0.02)	2.09 (0.11)
Galactose and glucose	4.70 (0.16)	4.65 (0.49)
Scyllo-inositol	1.75 (0.24)	1.80 (0.12)
Myo-inositol	5.77 (0.69)	6.45 (0.82)

Tr: traces, concentration < 0.001 mg l^{-1}.
n.i.: Not identified.

These findings demonstrate that MAs, considered as specific components of toasted wood, can serve as a marker of the use of toasted wood in aged beverages.

3.4. Sensory evaluation of levoglucosan

Due to the high quantity of monosaccharide anhydrides found in model beverages, the sensorial properties of levoglucosan were evaluated. The sensorial evaluation of the rest of MAs were not possible because they are not commercially available.

Sensorial analysis revealed that the oral sensation imparted by this compound was clearly perceived in the majority of all solutions tasted. Panellists detected a slight sensation of sweetness (0.6 and 0.8 points on a scale of 10 points, in the solutions of 20 and 30 mg l^{-1}, respectively) except in solutions of 10 mg l^{-1} where

there was no sweetness detected. A moderate bitter character was also observed by judges in all the solutions tasted. The concentrations of 10, 20 and 30 mg l^{-1} were evaluated with 1.2, 3.1 and 4.6 points for each one. The detection of these two descriptors are in a good agreement with papers reported previously, whose perceived gustative properties seem to be common among the majority of anhydrosugars such as 1,6-anhydro mannopyranose (mannosan) and 1,6-anhydro galactopyranose (galctosan) (Birch & Lee, 1976; Lee & Birch, 1975). A moderate metallic taste was also perceived by panellists (2.6, 4.2 and 5 points for 10, 20 and 30 mg l^{-1} solutions) however, no references have been found that corroborate this fact.

4. Conclusions

This is the first research paper focused on the study of non-volatile compounds derived from thermal degradation of oak wood carbohydrates. The presence of new compounds, MAs, were detected in toasted oak wood samples whose formation is due to the thermal degradation of cellulose and hemicelluloses. The toasting intensity of wood reduced the amount of sugars and inositols at the same time that MAs were produced, a fact that could be used to monitor the toasting degree.

These MAs were identified not only in toasted wood samples but also in model wines and distillates aged with this type of wood. For that reason and due to their absence in natural woods, MAs are proposed as markers for toasted woods and as indicators of beverages aged with these types of woods.

The occurrence of these compounds in toasted woods used in barrelmaking and in beverages aged with toasted woods has not been reported until now. Furthermore, it was possible to draw preliminary conclusions about the organoleptic role of these substances; however more research about this latter aspect will be carried out in future investigations.

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