

Identifying New Volatile Compounds in Toasted Oak

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Toasting wood to be used in barrels for aging wine produces a great number of volatile and odiferous compounds. Three new volatile odorous compounds in toasted oak were identified. Analysis by high-performance gas chromatography of toasted oak extracts, combined with olfactory detection, enabled various chromatographic peaks with these specific aromas to be isolated. These same odors were simultaneously studied by heating glucose both with and without proline and phenylalanine. Aromatic compounds of interest were identified thanks to a combination of gas chromatography and both mass and infrared spectrometry. An analysis RMN was also used. Hydroxymaltol, 2,5-furanedicarbaldehyde, and furylhydroxymethyl ketone have been detected in extract of toasted oak wood. These molecules may be formed by direct pyrolysis of sugar or Maillard reactions. The acetylformoine was not detected in extract of toasted oak wood, whereas it was detected in heated extracts of various sugars and sugars mixtures with amino acids.

Keywords: Oak wood; hydroxymaltol; 2,5-furanedicarbaldehyde; furylhydroxymethyl ketone; acetylformoine

INTRODUCTION

Heat is used in barrel production to facilitate bending the staves into shape. The structure and composition of the wood change according to the intensity of the heat and the length of time it is applied (Chatonnet, 1989a,b, 1995). New volatile, odorous compounds develop. "Toasty-caramel" aromas play an important role in assessing toasting quality. However, until recently, the volatile compounds responsible for these characteristic odors were not very well-known. The identification of several new molecules with a "toasty-caramel" aroma in toasted oak has been described in a previous paper (Cutzach et al., 1997).

In this work, we identify the new volatile, odorous compounds in toasted oak that are likely to contribute to the aroma of barrel-aged wines.

MATERIALS AND METHODS

Extraction of the Toasted Wood. The oak, from central France, corresponded to the type of wood most widely used for aging fine wines (*Quercus petraea*). Sawdust was collected from staves toasted for 20 min (200 °C on the surface, 110 °C at 5 min depth) in the traditional way over a wood fire (Chatonnet et al., 1989a). Samples of toasted wood (50 g/L) were extracted directly by dichloromethane (24 h at 20 °C, magnetic stirring 700 rpm). The organic phase thus obtained was dried over anhydrous sodium sulfate then cold concentrated to 2 mL under nitrogen.

Gas Chromatography and Olfactory Detection. Sensory analysis of the extracts thus obtained was carried out

through a combination of gas chromatography and olfactory detection (GC-OD). Three μ L was injected in splitless mode (division quotient = 70, closing time 30 s, injector temperature 230 °C) on a polyethylene glycol capillary column chemically grafted made of fused silica (20M Carbowax, 50m \times 0.25 mm; 0.25 μ ; BP-20, SGE). The vector gas was helium N55 (18 psi). The chromatographic temperature was programmed from 45 to 230 °C, at the rate of 3 °C/min, with a final isotherm of 30 min. A SGE ODO-1TM system was used.

Gas Chromatography–Mass Spectrometry. The volatile compounds extracted were identified by gas chromatography–mass spectrometry (GC–MS) under the following operating conditions. A Carbowax 20M column (50 m \times 0.25 mm; 0.25 μ ; BP-20, SGE) was installed on a chromatograph (HP5890) coupled to a quadrupole mass spectrometer (MSD HP5970b and 5972) operating by electron impact (ionization energy, 70 eV; source temperature: 250 °C). A 2 μ L sample was injected in splitless mode (split ratio 70, closed time 30 s, injector temperature 230 °C) on a capillary column of fused silica bonded with polyethylene glycol (Carbowax 20M, 50m \times 0.25 mm; 0.25 μ ; BP-20, SGE). The carried gas was helium N55 (18 psi). The chromatograph oven was programmed from 45 to 230 °C, at a rate of 3 °C/min, with a final isotherm of 30 min. Each compound was identified by its mass spectrum and retention index compared to those of control compounds, if available, or to data in relevant literature.

Gas Chromatography–Infrared Spectrophotometry. The organic extracts of volatile compounds were also analyzed by gas chromatography coupled with a Fourier transform infrared spectrophotometer (GC–FTIR) HP5965B. The chromatography conditions were as described above.

Experimental Production of Certain Odorous Compounds. The following were heated in an oil bath at 180 °C for 10 min: 180 mg of D-glucose (1 mM) and 90 mg of proline (0.78 mM), or 90 mg phenylalanine (0.55 mM), in an SVL test tube (Cutzach et al., 1997). Glucose and fructose were also directly pyrolyzed by heating them under the same conditions but without any amino acids.

Organic Synthesis of Substances not Commercially Available. Both 3,5-dihydroxy-2-methyl-4(*H*)-pyran-4-one (hydroxymaltol) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-py-

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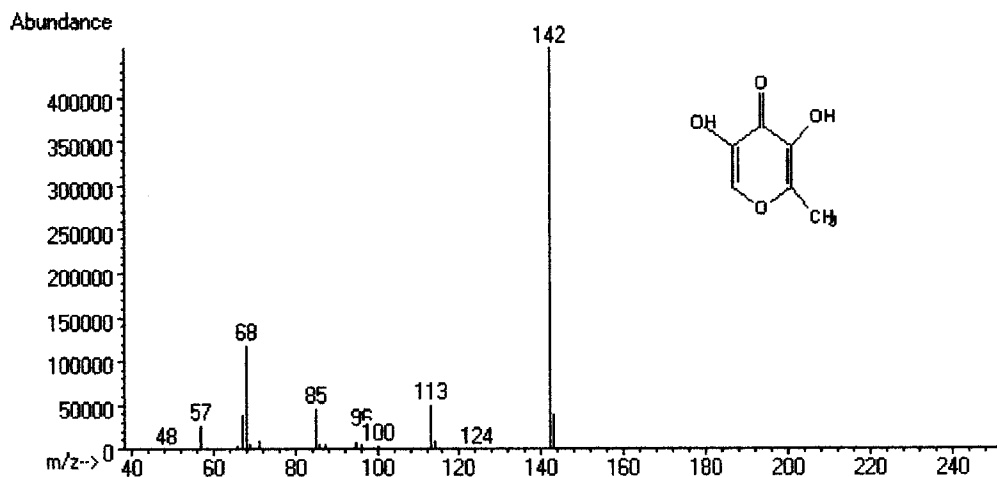


Figure 1. Mass spectra of 3,5-dihydroxy-2-methyl-4H-pyran-4-one.

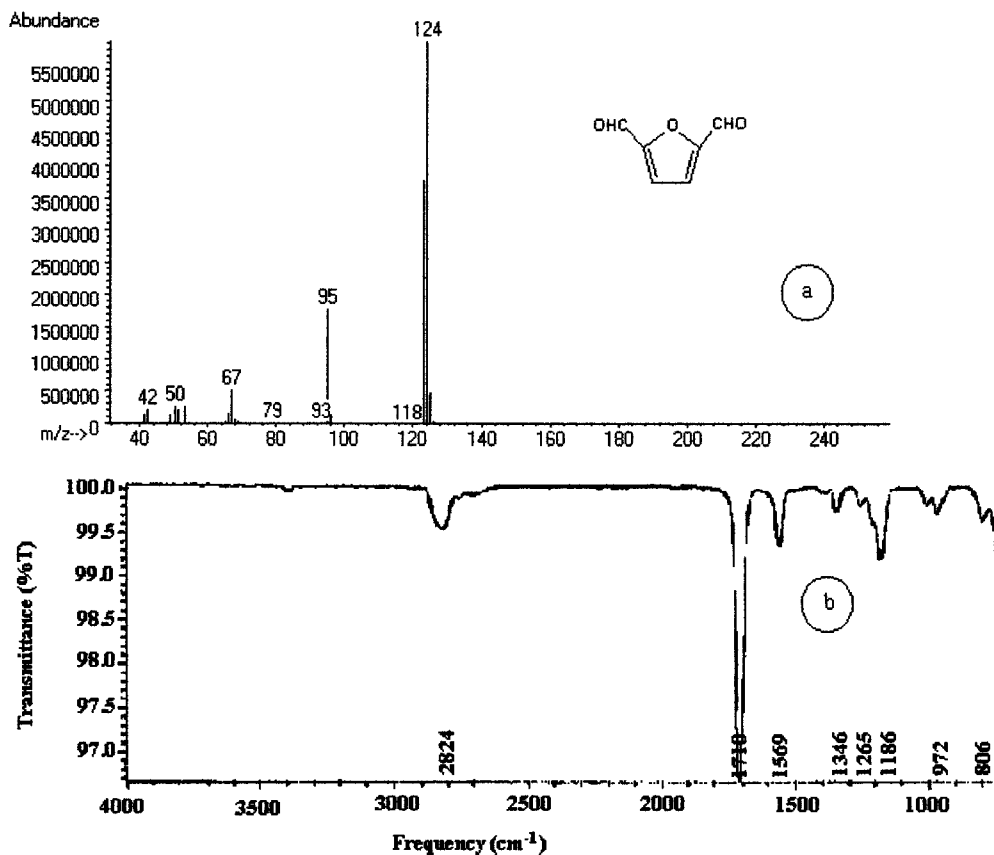


Figure 2. Mass (a) and infrared (b) spectra of 2,5-furandicarboxaldehyde.

ran-4-one (DDMP) were synthesized according to the method described by Van den Ouweland and Peer (1970), with slight modifications. A solution of 60 g anhydrous glucose (0.33 mol) in 250 mL ethanol was brought to reflux. A solution of 28.3 g piperidine in 50 mL ethanol was added, one drop at a time; the mixture was then maintained at for 2 h. Following the addition of 30.6 g mercaptoacetic acid dissolved in 50 mL ethanol to the solution over 60 min, the mixture was maintained at reflux for 24 h. Ethanol was then evaporated, and the residue dissolved in 50 mL water. The solution was continuously extracted with diethyl ether for 20 h. The ether was evaporated and the viscous, oily residue was distilled in a vacuum. Distillation at 120–150 °C produced 2.6 g of the fraction. The yield of this synthesis process is no more than 4%. The fraction collected in this way was purified by column chromatography, using 1 g of the distillate on a polyamide column ($l = 60$ cm, $d = 1$ cm, Machery and Nagel SC6 polyamide, 12 g), with a 50:50 ether–pentane mixture as the

eluant. The fractions were analyzed by TLC, using a 97:3 diethyl ether–methanol mixture as the eluant, then revealed using UV or FeCl_3 (blue coloring). The solid obtained by this method was recrystallized in an ether–pentane mix. Purification of the raw product produced 12% hydroxymaltol mixed with 65% of DDMP. The molecular structure is determined by RMN analysis. ^1H RMN (CDCl_3): 2.16 (3H, s); 4.04 (1H, q); 4.38 (1H, q); 4.45 (1H, q).

Furylhydroxymethyl ketone (hydroxymethylfuryl ketone) was synthesized using the method developed by Moriarty et al., (1992). The acetylfurane was converted into hydroxymethylfuryl ketone by direct α -hydroxylation using bis(trifluoroacetoxy)iodobenzene, in the presence of trifluoroacetic acid, in an acetonitrile–water 1:1 mixture. The synthetic product was isolated by chromatography on silica with a mixture of diethyl ether and pentane (70:30) as eluant. Chromatography on silica of the raw product gave an 82% pure compound, while the remaining 18% corresponded to the solvent (olfactory

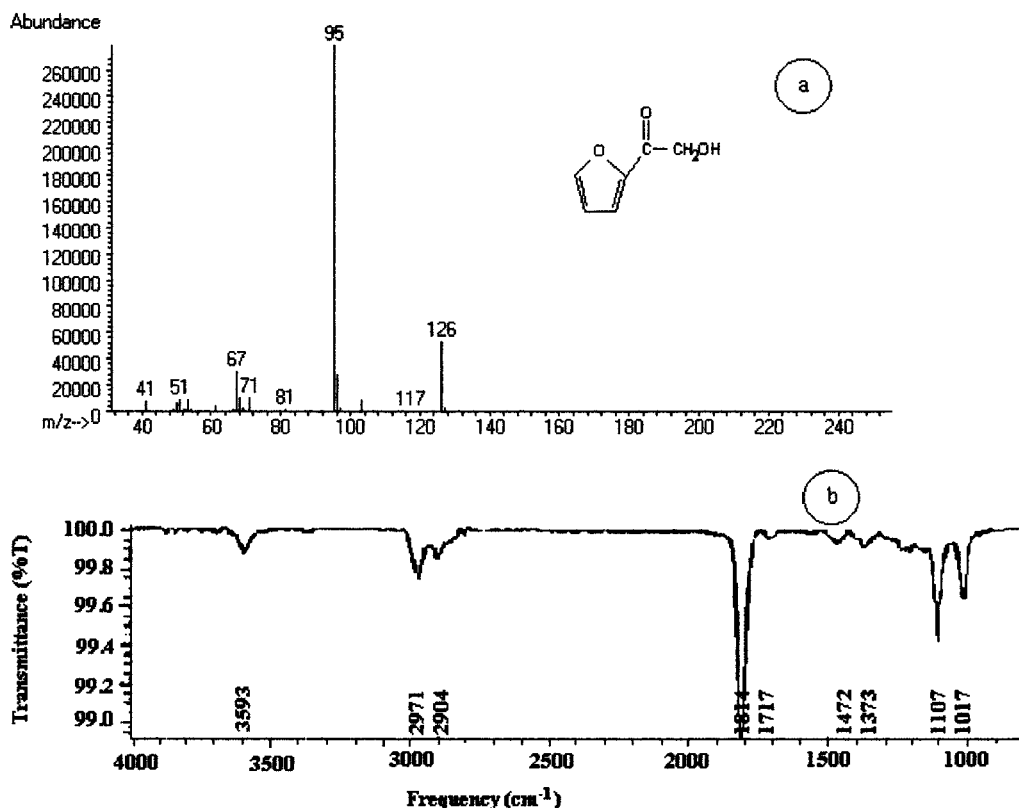


Figure 3. Mass (a) and infrared (b) spectra of furylhydroxymethyl ketone.

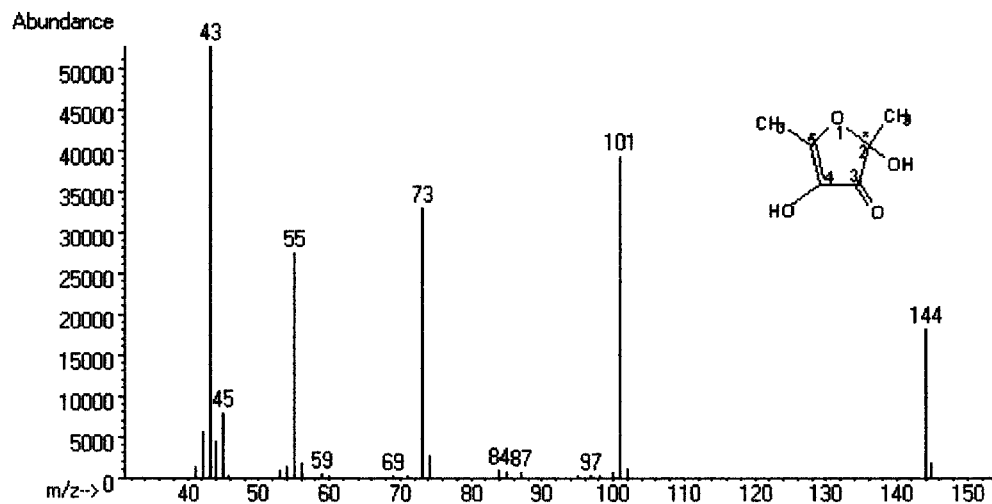


Figure 4. Mass spectra of 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one.

detection GC-OD). The molecular structure is determined by RMN analysis. ^1H RMN (CDCl_3): 3.26 (1H, s, OH); 4.75 (2H, s, CH_2); 6.62 (1H, m, CH); 7.3 (1H, m, CH); 7.64 (1H, m, CH). However, a coupling between CH_2 and OH may be noticed.

2,5-Furanedicarbaldehyde was synthesized using the method developed by Haryati (1992) from d-fructose. Chromatography on silica of the raw product gave an 85% pure compound, while the remaining 15% corresponded to the solvent (olfactory detection GC-OD). ^1H RMN (CDCl_3): 7.34 (2H, s); 9.87 (2H, s).

RESULTS AND DISCUSSIONS

Identifying certain compounds present in extracts from toasted oak wood. Direct extraction by dichloromethane was sufficient to identify the majority of the molecules already mentioned in the literature (Cutzach et al., 1997). In addition to previously identi-

fied compounds (Chatonnet et al., 1989b, 1991; Cutzach et al., 1997), three new molecules were identified in extracts from toasted oak wood: 2,5-furanedicarbaldehyde, furylhydroxymethyl ketone, and finally hydroxymaltol.

Hydroxymaltol (Kim and Baltes, 1996), identified as one of the major compounds produced when a sugar and an amino acid are heated together, had not previously been identified in toasted wood extracts (Cutzach et al., 1997), as it is only present in trace amounts. This compound corresponded to the chromatographic peak correlated to aromatic zone ZO 6 (retention index: 2238/BP-20) mentioned by Cutzach et al., (1997) (Figure 1).

Both 2,5-furanedicarbaldehyde and furylhydroxymethyl ketone have been identified by gas chromatography and olfactory detection (GC-OD) as being respon-

sible for slightly "honeyed" or "rancid" aromatic zones (Cutzach et al., 1998). These compounds were known as possible derivatives of Maillard reactions. In fact, reactions between glucose and phenylalanine when they are heated together produce large quantities of 2,5-furandicarbaldehyde and furylhydroxymethyl ketone (Baltes et al., 1988). Furthermore, 2,5-furandicarbaldehyde and furylhydroxymethyl ketone are also major components in the aroma of honey (Graddon et al., 1979). These two compounds were formally identified by their mass spectrum, retention index (1985 and 2009, respectively, on a BP-20 polar column), and GC-FTIR (Figures 2 and 3). Identification was confirmed by co-injection with standard reference compounds obtained by organic synthesis.

These molecules were both present in toasted wood: 2,5-furandicarbaldehyde was more abundant, while furylhydroxymethyl ketone was usually present only in trace amounts. Both of these molecules may also be identified in the pyrolysis of glucose. According to Baltes and Mevissen (1988), the Maillard reactions produced by heating the glucose-proline mixture, or in particular glucose-phenylalanine, give much higher concentrations. No 2,5-furandicarbaldehyde was found when fructose was pyrolyzed alone.

Hydroxymaltol, 2,5-furandicarbaldehyde, and furylhydroxymethyl ketone have also recently been identified in extracts of old sweet fortified wines (vins doux naturels), such as Banyuls, Rivesaltes, and Port (Cutzach et al., 1998). Hydroxymaltol and furylhydroxymethyl ketone, when pure, had a "honey" and "toasty-caramel" odor detected after combined gas chromatography and olfactory detection. Nevertheless, 2,5-furandicarbaldehyde had a "honey" odor detected after combined gas chromatography and olfactory detection, whereas the pure compound had a "cheese" odor.

Confirmation of the Identification of a Molecule with a "Toasty" Character Produced by Pyrolysis of Glucose and by Heating the Proline-Glucose Mixture. The chromatographic peak corresponding to odorous zone ZO 7 in the heated glucose-proline mixture (Cutzach et al., 1997) had not previously been formally identified. Its fragmentation by electron impact had led to the supposition that it had a molecular weight of 144. With the help of bibliographical research (Ledl et al., 1984; Schieberle, 1992; Kim and Baltes, 1996; D'Arcy et al., 1997), its electron impact mass spectrum and retention index have led to the formal identification of this compound as 2,4-dihydroxy-2,5-dimethyl-3(2*H*)-furanone, or acetylformoïne (Figure 4). This compound was also present in extracts of old sweet fortified wines (vins doux naturels) (Cutzach et al., 1998), as well as pyrolyses of glucose and fructose, but was mainly formed when a sugar was heated with an amino acid. Acetylformoïne has not always been identified in toasted wood extracts. It seems, therefore, that the conditions under which oak wood is toasted are not conducive to the formation of this molecule. This compound was probably broken down by dehydration and reduction into furaneol (4-hydroxy-2,5-dimethyl-3(2*H*)-furanone) (Ledl et al., 1984; Schieberle, 1992; Kim et al., 1996). Furthermore, acetylformoïne, and certain other aliphatic isomers, may be formed by the breakdown of 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) on heating and can coexist in a keto-enolic balance (Kim and Baltes,

1996). This product, when pure, has a "toasty-caramel" odor detected after combined gas chromatography and olfactory detection.

CONCLUSION

We confirm the presence of acetylformoïne in heated extracts of various sugars and sugar mixtures with amino acids. This molecule was not detected in extracts of toasted oak wood.

Both 2,5-furandicarbaldehyde and furylhydroxymethyl ketone were identified in toasted oak for the first time. These molecules may be formed by direct pyrolysis of glucose. The pyrolysis of fructose does not result in the formation of 2,5-furandicarbaldehyde. These two molecules were also present when sugars and amino acids were heated together, and in particular in the glucose-phenylalanine mixture.

Hydroxymaltol is only identifiable in trace amounts in toasted wood.

The presence of these compounds in oak from barrels toasted according to cooperage techniques may be attributable to Maillard reactions and pyrolysis of sugars.

Future research will focus on developing a sufficiently sensitive method for measuring concentrations of these compounds and determining their perception thresholds in wine. These data will make it possible to draw conclusions concerning the organoleptic impact of these substances and the influence of toasting intensity.

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