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Comparative study of the aromatic profile of different kinds of wine cork stoppers

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ABSTRACT

The sensory properties and aromatic composition of macerates (in wine-like medium) of different types of cork stoppers (five synthetic and three natural corks) have been determined. After the sensory description, extracts of the macerates were obtained using a customary head space technique and analyzed by semiquantitative gas chromatography–olfactometry (GC–O). The active odourants present in the macerates were determined using this technique and ranked by their potential importance.

From the sensory point of view, the eight samples were grouped into two categories with distinctive sensory properties. The first category was formed by non-synthetic stoppers and was described using the terms "sweet, toasted, sweet wood, and flowery-muscat". Remarkably, all these sensory terms are normally used in wine tasting. The second category consisted of synthetic samples. This category included a sample with a clear rubber aroma and two samples with a cork/mushroom aromatic note.

The results of GC–O confirmed the sensory study. Non-synthetic samples had complex profiles of 10–20 aromatic compounds, all well known natural components of healthy wine. In contrast, the GC–O profiles of the synthetic stoppers were extremely simple and consisted of few odourants. The rubber aroma may be due to *m*-cresol and an unknown odourant with RI 1758 (in DB-WAX column), while the cork/mushroom note was caused by a single odourant that elutes in a DB-WAX column at RI 1223 (identified as 1-hepten-3-one).

1. Introduction

Must taint, traditionally known as cork taint, is one of the most unpleasant organoleptic defects of wine and one of the biggest problems in winemaking and cork stopper production today. Chloroanisoles, especially 2,4,6-trichloroanisole (TCA), are the main compounds responsible for this defect. Other volatile organic compounds, such as 1-octen-3-ol, 1-octen-3-one (mushroom aroma), guaiacol (smoky, phenolic aroma), geosmin (molecule identified as responsible for "earthy" aroma by Darriet, Pons, Lamy, and Dubourdieu (2000), 2-methylisoborneol (earthy aroma), 2-methoxy-3,5-dimethylpyrazine (musty aroma) (Simpson, Capone, & Sefton, 2004) and, more recently, 2,4,6-tribromoanisole (Chatonnet, Bonnet, Boutou, & Labadie, 2004) have been reported as being responsible for cork taint (Amon, Vandepeer, & Simpson, 1989). This organoleptic defect usually is associated with a musty, mouldy aroma and taste (Simpson & Veitch, 1993).

In recent years, methods for quantifying some of the molecules responsible for off-flavour called "cork taint" or "corkiness" in wines and corks have increased considerably. Most of the procedures proposed are aimed at determining the chloroanisole family (overall TCA) using several procedures: liquid–liquid extraction (Buser, Zanier, & Tanner, 1982; Chatonnet, Labadie, & Boutou, 2003; Pena-Neira et al., 2000; Pollnitz, Pardon, Liacopolus, Skouroumounis, & Sefton, 1996), solid phase extraction (SPE) (Insa, Antico, & Ferreira, 2005; Sanvicens, Moore, Guilbaullt, & Marco, 2006; Soleas, Yan, Seaver, & Goldberg, 2002), solid phase microextraction (SPME or HS-SPME) (Carasek, Cudjoe, & Pawliszyn, 2007; Ezquerro & Tena, 2005; Gomez-Ariza, Garcia-Barrera, Lorenzo, & Beltran, 2006; Lizarraga, Irigoyen, Belsue, & Gonzalez-Penas, 2004; Pizarro, Perez-del-Notario, & Gonzalez-Saiz, 2007; Riu, Mestres, Busto, & Guasch, 2006), stir bar sorptive extraction (SBSE) (Callejon, Troncoso, & Morales, 2007; Zalacain, Alonso, Lorenzo, Iniguez, & Salinas, 2004), dynamic headspace method (DHS) (Boudaoud & Eveleigh, 2003; Gomez-Ariza, Garcia-Barrera, & Lorenzo, 2004) and supercritical fluid extraction (SFE) (Taylor, Young, Butzke, & Ebeler, 2000).

Chloroanisole determination is not conclusive, so Mazzoleni et al. and Rocha et al. analyze by GC–MS the volatile compounds of corks extracted using a simultaneous distillation-extraction process (SDE) (Rocha, Delgadillo, & Correia, 1996) or a dynamic headspace technique with adsorption on a polymer material (Mazzoleni, Caldentey, Careri, Mangia, & Colagrande, 1994). Both papers offer an extensive list of volatile compounds. The major compounds were aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, alkanes, aromatic compounds, cycloalkanes, furans, terpens and chloride compounds.

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Most publications have studied only natural cork stoppers, except that of Soleas et al. (2002), who have investigated the role of trichlorocompounds in different kinds of cork: composite, synthetic and natural corks.

Other authors (Boutou & Chatonnet, 2007; Vieira, Rocha, & Silvestre, 2007) have developed analytical methods for the determination of other odourants responsible for "corkiness" aside from the chloroanisoles, such as 1-octen-3-ol, geosmin, 2-methylisoborneol and others in wine and cork samples.

The information obtained by GC–MS analysis is interesting, but not complete, because it is necessary to evaluate which compounds present are odour-active compounds. In order to rank the odourants and locate those aromatically most important, olfactometric study (GC–O) is necessary. Few papers have undertaken olfactometric studies of stopper samples. Only Moio et al. studied two natural cork samples by olfactometry: a normal sample and a sample with "corky" defect. They concluded that the only molecule responsible for this sensorial defect is already known, TCA (Moio et al., 1998).

Rocha et al. also characterised cork odours using an electronic aroma sensing system (Rocha, Delgadillo, Correia, Barros, & Wells, 1998). This electronic system quickly discriminates between an acceptable aroma and unacceptable taint. However, it does not identify the nature of the odourants responsible for the taint.

The aim of this study was to examine the aromatic profiles of different types of cork stoppers (synthetic and natural) by sensory and olfactometry analysis. The two major contributions of this study are: In first place, a complete profile of the odour-active volatile constituents of synthetic and non-synthetic cork stoppers (which is a useful tool for classifying odourants by their aromatic importance and that will allow us to determining those that may be responsible for sensorial differences between wine-bottle stopper macerates). In second place, a comparative study of the aromatic profiles of different types of cork stoppers.

2. Materials and methods

2.1. Reagents and standards

Dichloromethane of HPLC quality was from Fisher Scientific (Loughborough, UK), absolute ethanol (ACS quality) and tartaric acid were purchased from Panreac (Barcelona, Spain) and pure water was obtained from a Milli-Q purification system (Millipore, USA). LiChrolut EN resins and polypropylene cartridges were obtained from Merck (Darmstadt, Germany). The chemical standards were supplied by Aldrich (Gillingham, UK), Fluka (Buchs, Switzerland), Sigma (St. Louis, MO, USA), Lancaster (Strasbourg, France), PolyScience (Niles, USA), Chemservice (West Chester, PA, USA), Interchim (Monlucon, France), International Express Service (Allauch, France) and Firmenich (Geneva, Switzerland).

2.2. Cork material

Eight cork stoppers were chosen for this study: five synthetic stoppers (S1, S2, S3, S4 and S5) and three natural cork stoppers. Two of them wre stoppers processed using different purification methods: stopper NC-P1 was treated with a clean up process with supercritical CO_2 (procedure I) and stopper NC-P2 was treated with a clean up process with steam distillation (procedure II). The third natural cork was not processed.

2.3. Maceration process

The conditions of maceration were set in accordance with Directive 93/8/EEC, which establishes mandatory guidelines for verifying the migration of components of materials and plastic objects matter that come into contact with food products. Based on this directive, analyses were made in an aqueous solution containing 15% ethanol as simulant. Tartaric acid, 5 g/L, was added and pH was adjusted to 3.2 to achieve conditions as close as possible to wine. Following these guidelines, 20 stoppers were submerged in an 800 mL volume of simulant solution for each sample. Maceration time and temperature were 10 days and 40 °C.

2.4. Sensory analysis

The sensory panel consisted of six women and two men, 20–40 years of age, all of them belonging to the laboratory staff and with a long experience in sensory analysis. Five specific one-hour training sessions were carried out before the panel has evaluated the macerates. In the first training session, judges generated descriptive terms for the eight cork samples. In the second and third sessions, different aroma standards were presented and discussed by the panel. Seven terms were selected through these discussions and used for further descriptive analysis: synthetic/rubber, alcoholic/pungent, sweet/matured fruit, toasted, sweet wood, cork/mushroom and flowery/muscat. In the fourth and fifth training sessions, panellists scored the intensity of each attribute using a 4-point scale (0 = not detected, 1 = weak, hardly recognisable note, 2 = clear but not intense note, 3 = intense note). After the training period, samples were evaluated in two formal sessions (four samples per session). In all cases, macerates (20 mL at 20 °C) were presented in coded, black, tulip-shaped wine glasses covered by glass Petri dishes. Samples were presented in random order. The data processed was a mixture of intensity and frequency of detection (which we labelled as "modified frequency" – MF), which was calculated with the formula proposed by Dravnieks (1985).

$$\mathrm{MF}\ (\%) = \sqrt{F(\%)I(\%)}$$

where F (%) was the detection frequency of an aromatic attribute expressed as a percentage and I (%) was the average intensity expressed as a percentage of the maximum intensity.

Data treatment: Descriptive analysis data was analyzed by chi-square test (χ^2) on the modified frequency of detection data. Statistical analysis of the sensory data was carried out by principal components (PCA).

2.5. Gas chromatography–olfactometry (GC–O)

Preparation of extracts: The volatiles of the macerates were collected using a purge-and-trap system following the headspace strategy proposed by Campo, Ferreira, Escudero, and Cacho (2005). The trap was formed by a standard polypropylene SPE tube (0.8 cm internal diameter, 3 mL internal volume) packed with 400 mg of LiChrolut EN resins. Such resins were selected because of their excellent ability to extract aroma compounds (Lopez, Aznar, Cacho, & Ferreira, 2002). The bed was washed with 20 mL of dichloromethane and dried by letting air pass through (negative pressure of 0.6 bar, 10 min). The tube was placed on the top of a bubbler flask containing 80 mL of macerate solution. The mixture was continuously stirred with a magnetic stir bar and kept at a constant temperature of 37 °C by immersion in a water bath. A controlled stream of nitrogen (100 mL/min) was passed through the sample during 200 min. Volatile constituents released in the headspace were trapped in the cartridge containing the sorbent and were further eluted with 3.2 mL of dichloromethane. The extract was kept at −30 °C for 2 h to eliminate any water content by freezing and further decantation. After this, the extract was concentrated under a stream of pure N_2 to a final volume of 200 μL.

GC–O analysis: Sniffings were carried out in a Thermo 8000 series GC equipped with a FID and a sniffing port (ODO-1 from SGE) connected by a flow splitter to the column exit. The column was

a DB-WAX from J&W (Folsom, CA, USA), 30 m × 0.32 mm I.D., with 0.5 μm film thickness and was preceded by a 3 m × 0.32 mm I.D. uncoated (deactivated, intermediate polarity) precolumn from Supelco (Bellefonte, PA, USA). The carrier was H_2 at 3 mL/min. One microlitre was injected in splitless mode, being 1 min the splitless time. Injector and detector were both kept at 250 °C. The temperature program was as follows: 40 °C for 2 min, then raised at 12 °C/min up to 105 °C and at 6 °C/min up to 220 °C and finally was held at 220 °C for 20 min. To prevent condensation of high-boiling compounds on the sniffing port, this was heated sequentially using a laboratory-made rheostat.

A panel of six judges carried out the sniffings of the six extracts chosen to represent all the samples. Sniffing time was approximately 30 min and each judge carried out one session per day. The panellists were asked to rate the intensity of the eluted odour using 7-point category scale (0 = not detected; 1 = weak, hardly recognisable odour; 2 = clear but no intense odour, 3 = intense odour), half values being allowed. The quantitative ability of this technique has been already proved (Ferreira, Pet'ka, Aznar, & Cacho, 2003). The odourants were identified by comparison of their odours and their retention index (calculated in DB-WAX and DB5 columns) and when it has been possible by comparison of their chromatographic retention properties and MS spectra with those of pure reference compounds.

Data treatment: A χ^2 test on the frequency of detection (MF%) data was performed to detect significant differences between the GC–O scores of a given odourant in different macerate samples.

1-hepten-3-one identification as responsible for "mushroom" odour of some synthetic samples.

2.6. Multidimensional gas chromatography

Analyses were performed using a multidimensional gas chromatograph from Varian (Walnut Creek, CA, USA), consisting of two independent gas chromatographs interconnected by a thermoregulated transfer line kept at 200 °C.

Chromatograph 1: The first chromatograph was a CP 3800 model equipped with a 1079 PTV injector, flame ionisation detection (FID) system, and olfactometric port (ODO-II from SGE, Ringwood, Australia), both of which were connected by a flow splitter to the column exit to enable simultaneous FID and sniffing monitoring of the effluent from the first column. This GC was retrofitted with a Deans pressure-driven switching valve (Valco Instruments, Houston, TX, USA), which makes it possible to selectively transfer heart cuts eluting from the first column directly into the analytical column placed in the second chromatograph. The carrier gas (He) was delivered at a constant pressure of 30 psi. During the two first minutes of each run, an auxiliary He flow (Deans valve) was maintained at 15 psi, then raised to 20 psi. The column was a DB-WAX (polyethylene glycol) from J&W (Folsom, CA, USA), 30 m × 0.32 mm ID with 0.50 μm film thickness. An uncoated, deactivated fused silica column (30 m × 0.32 mm ID) from Supelco (Bellefonte, PA, USA) was used as interface between the Deans switching valve and the FID and ODO detectors. The oven temperature program was 40 °C during 2 min, then raised by 12 °C min^{-1} to 105 °C, followed by 6 °C min^{-1} to 220 °C, and finally held at this temperature for 40 min. The FID was kept at 300 °C.

Chromatograph 2: The chromatograph was a CP 3800 model coupled to an ion trap mass spectrometric-detector (Saturn 2200). The system was equipped with a CO_2 cryotrapping unit and an olfactometric port (ODO-II from SGE) at the end of the column, so that simultaneous sniffing monitoring and MS scanning was possible. A make up flow was diverted through a flow splitter placed at the end of the column and a flow restrictor was placed between the flow splitter and MS detector. The column was a Factor Four VF-5MS (polymethylsiloxane-5% diphenyl) from Varian (30 m × 0.32 mm ID, 1-μm film thickness). The column was connected directly to the Deans valve placed in the first chromatograph via the thermostated transfer line. The first centimetres of this column in the second GC crossed the cryofocusing unit (CO_2) and the end of the column was linked to a splitter connected to both the MS and ODO detectors. Two minutes after the heart-cutting, CO_2 flow was removed at the same time that the temperature program (4 °C min^{-1} up to 200 °C and then 100 °C min^{-1} up to 300 °C) of the second oven was activated. MS parameters were: transfer line at 170 °C; ion trap at 150 °C, and trap emission current 30 μA. The global run time was recorded in full scan mode (45–250 m/z mass range). FID and MS data were recorded and processed with Workstation 6.30 software equipped with NIST 98 (US National Institute of Standards and Technology) MS library (NIST, Gaithersburg, MD, USA).

Programmable injector conditions: The insert, with 3.4 mm ID, was filled with ~50 mg of silane-treated glass wool (Supelco, Bellefonte, PA, USA). Large-volume injections (50 μL) were optimised for extracts in the solvent dichloromethane. The injection was carried out in the solvent split mode. The initial injector temperature was 40 °C. The split valve was closed after 0.4 min of solvent evaporation and the injector was then heated to 250 °C at 200 °C min^{-1}. After 3 min, the split valve was opened again (split ratio = 20). The different injection parameters were carefully optimised and the performance of the system was measured to ensure complete analyte transfer and good retention time reproducibility.

Delay-time and heart-cutting interval: For an odourant with the retention time "ts" in the first column (in minutes), the heart-cutting interval was determined to be [ts − 0.9] ± 0.15 min, 0.9 being the delay-time and 0.15 min being the interval required to guarantee quantitative transfer of the analyte from the first to the second column.

2.7. GC–NCI–MS analysis

These analyses were carried out by using the instruments and injection method described in the literature (Mateo-Vivaracho, Cacho, & Ferreira, 2007).

2.8. 1-Hepten-3-one derivatisation

This analysis was carried out using the derivatisation strategy developed for the quantification of 1-octen-3-one in wine (Cullere, Cacho, & Ferreira, 2006).

3. Results and discussion

3.1. Sensory analysis

The aroma of eight different macerates was described by the sensory panel using seven different aroma descriptors. Results of the sensory analysis are shown in Table 1. The χ^2 test revealed that all terms, except alcoholic/pungent, varied significantly among different samples ($p = 0.05$). The profile obtained for synthetic stoppers was consistently much less aromatic and simpler than the profile of the non-synthetic stoppers (Table 1), which allowed us to separate two groups (Fig. 1). This figure shows the principal components analysis (PCA) constructed from the sensorial data (expressed as modified frequencies) of the eight samples. The synthetic samples all had clearly negative values on the axis corresponding to PC1, whereas the three non-synthetic samples had positive values. Within the synthetic sample group, S4 and S5 were noteworthy due to the high intensity of the cork/mushroom note.

Table 1
Sensory evaluation of all the macerates (modified frecuency percentage (MF%))

	Synthetic corks					Natural cork		
	S1	S2	S3	S4	S5	NC-P1	NC-P2	NC
*Synthetic/rubber	50	7	10	9	7	0	0	0
Alcoholic/pungent	59	71	78	52	59	32	31	31
*Sweet/matured fruit	7	13	7	5	13	59	61	68
*Toasted	22	0	7	14	7	47	66	59
*Sweet wood	29	22	18	14	7	55	64	66
*Cork/mushroom	23	5	20	68	74	13	25	25
*Flowary/muscat	41	41	38	29	32	79	85	88

* Aromatic descriptors that differ significantly ($p = 0.05$) – test χ^2.

Four of the seven descriptors evaluated were perceived in one or more of the synthetic samples with a modified frequency of more than 30%. Two of these aromatic descriptors were notable: synthetic/rubber (detected in the S1 sample with MF 50%) and cork/mushroom (detected in the S4 and S5 samples with MF 68% and 74%, respectively). These notes are negative (undesirable). The χ^2 test revealed that both terms (synthetic/rubber and cork/mushroom) varied significantly between the five synthetic macerates (data not shown). In contrast, these negative notes were not detected in any of the non-synthetic samples at modified frequencies of more than 30%.

Non-synthetic samples also exhibited more aromatic complexity. Five aromatic notes scored with intensities equivalent to MF > 30%: sweet/matured fruit, alcoholic, toasted, sweet wood and flowery/muscat. These descriptors were responsible for aromas of a positive nature, contributing pleasant notes that are commonly present in healthy wines.

The difference in the aromatic complexity of these two groups may explain, in part, why the alcoholic/pungent note was perceived in the group of synthetic samples as much more intense. This accentuated note may be due to the ethanol content of the macerates (15%). Although all the samples had the same ethanol content, in the case of composite and natural cork stoppers the impact of the alcohol note may have been masked by the strong aromatic notes found (sweet, floral and others). This hypothesis was reinforced by conducting a sensorial test (triangular test) to compare the macerate obtained from synthetic sample S3 (which has shown a high score in alcoholic note, MF 78%) with a wine simulant (a 15% water/ethanol mixture containing 5 g/L tartaric acid and with the pH adjusted to 3.2 with 1 M NaOH). The result disclosed no significant differences between the two samples.

3.2. Gas chromatography–olfactometry

After the sensorial evaluation, an olfactometric study was made of 6 of the 8 initial stoppers. The samples discarded were S2 and S4, which had almost identical profiles to samples S3 and S5, respectively.

The GC–O experiment was carried out on extracts obtained in a dynamic headspace system. This strategy allow us to make it possible obtain simpler and cleaner olfactograms than those obtained in previous studies, in which extracts were obtained by Solid Phase Extraction (Cullere, Escudero, Cacho, & Ferreira, 2004; Lopez, Ortin, Perez-Trujillo, Cacho, & Ferreira, 2003). In this case, the recorded GC–O signal has been evaluated in terms of frequency of detection of an odourant. For gain in simplicity, those odourants not reaching a maximum GC–O score of 30% in any of the macerates were not considered. After this operation, the number of odourants was reduced to 23, as shown in Table 2. The semiquantitative olfactometric results and retention indexes (RI) of the odourants in the polar column (DB-WAX) and apolar column (DB5) are presented in this table, together with the descriptors used by the sniffers, component identity and score (modified frequency: maximum 100) for each odourant in each of the six samples studied. Among the odourants perceived that reached a modified frequency $\geqslant$30% in at least one sample were ethylic esters (ethyl isobutyrate, ethyl butyrate, ethyl 2-methylbutyrate), non-ethylic esters (propyl acetate, butyl acetate), terpens (linalool, α-terpineol, borneol), phenolic compounds (guaiacol and *m*-cresol, vanillates) and carbonyl compounds (diacetyl, octanal, 1-hepten-3-one and 1-octen-3-one).

Almost all the odourants listed in Table 2 were identified. Three remained unidentified despite using strategies like multidimensional chromatography (GC–O–GC–O–MS), which is generally very useful in these cases. One of these unknown odourants (RI DB-WAX 1758) is likely to be the major component of the synthetic/rubber note characteristic of synthetic sample S1.

The following conclusions were drawn from the content of Table 2:

(1) Synthetic samples all had very simple profiles that were dominated by one or two odourants and contained four or five secondary aromatic molecules. In contrast, non-synthetic samples had much more complex profiles in which there was no clearly dominant odourant and more than 14 odourants with MF > 30% were present.
(2) The odourants of non-synthetic samples all were aromatic molecules found naturally in healthy wine. They can be grouped according to their biochemical origin into the fol-

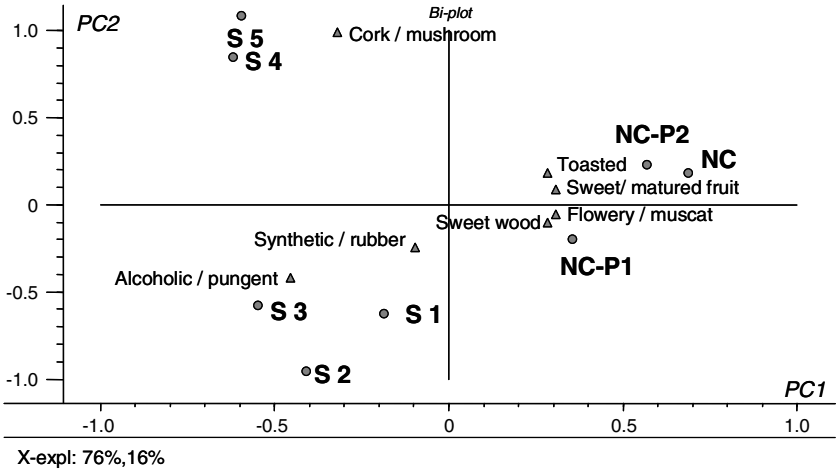

Fig. 1. Score plot of principal component analysis (PCA) applied to sensory data from eight macerates.

Table 2
Odourants found in the six macerates studied: gas chromatographic retention data, olfactory description, chemical identity and modified frequency percentage (MF (%))

LRI DB-WAX	LRI DB-5	Descriptor	Identity	S1	S3	S5	NC-P1	NC-P2	NC
955	<800	Fruity, strawberry	*Ethyl isobutyrate (2)	22	0	55	39	85	85
984	<800	Alcoholic	Propyl acetate (2)	39	22	27	25	12	25
1000	<800	Buttery, cream	Diacetyl (2)	0	0	0	0	57	0
1056	801	Fruity	Ethyl butyrate (2)	0	0	31	0	25	31
1069	849	Fruity, green apple	Ethyl 2-methylbutyrate (2)	0	0	22	0	39	33
1100	816	Grass	Butyl acetate (2)	0	0	0	33	41	0
1109	<800	Flowery, anise	Isobutanol (2)	0	0	0	0	43	0
1150	875	Fruity, anise	3-Methylbutyl acetate (2)	17	0	0	24	22	31
1181	854	Fruity, anise	Ethyl isovalerate (2)	0	0	0	31	0	0
1223	824	Mushroom	***ni**	0	0	97	0	0	0
1237	–	Fruity, anise	*ni	0	0	67	0	0	0
1318	1006	Lemon	*Octanal (2)	0	10	0	10	76	10
1323	975	Mushroom	1-Octen-3-one (2)	22	26	26	10	38	35
1565	1100	Flowery/muscat	Linalool (1)	0	0	0	0	22	33
1741	1195	Anise	α-Terpineol (1)	0	0	0	22	31	61
1748	1162	Camphor, anise	*Borneol (1)	0	0	0	0	0	42
1758	–	Synthetic, rubber	ni	43	0	0	0	0	0
1890	1089	Phenolic, spicy	*Guaiacol (1)	0	0	0	0	47	70
2123	1070	Bitumen, leather	*m*-Cresol (2)	57	22	0	0	29	0
2510	–	Dry herb, strong	ni	0	42	0	0	0	0
2630	1410	Vanillin	Vanillin (1)	0	0	0	44	46	37
2649	1500	Vanillin	Methyl vanillate (1)	0	0	0	0	33	38
2666	1560	Flowery, vanillin	Ethyl vanillate (1)	0	0	0	31	0	34

(1) Identification based on coincidence of gas chromatographic retention on both columns and mass spectrometric data with those of the pure compounds available in the lab.
(2) Identification based on coincidence of chromatographic retention on both columns and on the similarity of odours. The compounds did not produce any clear signal in the mass spectrometer because of its low concentration.
ni: unknown compound.
ni: initially unknown compound, but identified such as 1-hepten-3-one in this paper.
* Odourants in which olfatometric intensities differ significantly (95%) – χ^2 test.

lowing categories: aromas related to amino acid synthesis (ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl isovalerate; isobutanol and 3-methylbutyl acetate); aromas formed during fatty acid synthesis (ethyl butyrate and butyl acetate); phenolic compounds (guaiacol, cresol, vanillin, methyl and ethyl vanillate); terpens (α-terpineol, linalool, borneol) and derivatives of fatty acid degradation (1-octen-3-one, octanal and, perhaps, diacetyl). The natural cork purified by procedure I (NC-P1) was the simplest and had the fewest aromas of the three non-synthetic samples. It contained only five odourants with a score of more than 30% (ethyl isobutyrate, butyl acetate, ethyl isovalerate, vanillin and ethyl vanillate) and it was virtually free of terpens, guaiacol and 1-octen-3-one. The natural cork sample purified by procedure II (NC-P2) differed from the other two in its high diacetyl and octanal content. This suggests that these components may be formed during this process, probably as a result of fatty acid degradation. In view of these results, this purification process seems to reduce the terpen and guaiacol content with respect to the natural sample, but not as much as in process I. No reduction in 1-octen-3-one was observed.

(3) In contrast, the most important odourants of the synthetic samples are not usually found in healthy wine. Moreover, these odours can be responsible of undesirable aromas. The rubber note of sample S1 can be attributed to the presence of *m*-cresol and an unidentified compound with RI 1758 and the descriptors "synthetic/rubber". The cork/mushroom note characteristic of sample S5 should be attributed to the potent odourant RI 1223, with a clear mushroom aroma, which occurred with the highest frequency (97%). As will be seen below, this aromatic compound was identified as 1-hepten-3-one.

(4) The most discriminant compounds (by χ^2 tests) were ethyl isobutyrate, 1-hepten-3-one, octanal, borneol, guaiacol and an unknown compound with RI (DB-WAX) 1237.

(5) Finally it should be emphasised that 2,4,6-trichloroanisole (TCA) and 2,4,6-tribromoanisole (TBA) were not detected in any of the eight stopper samples.

3.3. 1-Hepten-3-one identification

The aromatic molecule 1-hepten-3-one was identified as responsible for the mushroom note perceived with a high modified frequency (97%) in the olfactometric study of one of the synthetic samples (S5). This identification was difficult because when extracts of the macerate with this note (S5) were obtained by different procedures (SPE, SPME, HS-SPE) and injected in the dual system (GC–O–GC–O–MS), no chromatographic signal was elicited after the respective capture. Nonetheless, the mushroom note was clearly and intensely perceptible in the two chromatographic columns. The only useful finding of this system was the retention indexes of this odourant in both the polar (DB-WAX) and apolar (DB5) columns. The indexes of the unknown compound were 1223 and 824, respectively. According to these values, the most likely candidate was 1-hepten-3-one, but the hypothesis had to be confirmed. The macerate was subjected to the analytical protocol designed for the determination of 1-octen-3-one after previous derivatisation with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) (Cullere et al., 2006). This reaction yielded two isomers of the resulting pentaflorobenzyl oxime, *E* and *Z*. Based on the structures of these derivatives (Fig. 2a and b), masses 181, 126 and 197 and 110 were postulated as important masses in the case of 1-hepten-3-one. An extract of the macerate bearing this mushroom note, previously derivatised, was injected in a GC–MS system. After acquisition in electron impact mode, a peak bearing all the masses characteristic of the derivative 1-hepten-3-one-PFBHA was located. Its mass spectrum is given in Fig. 3.

In any case, since the spectrum obtained was not absolutely clean, we decided to inject the extract of the macerate in a GC–NCI–MS system to test this hypothesis. We first obtained the 1-oc-

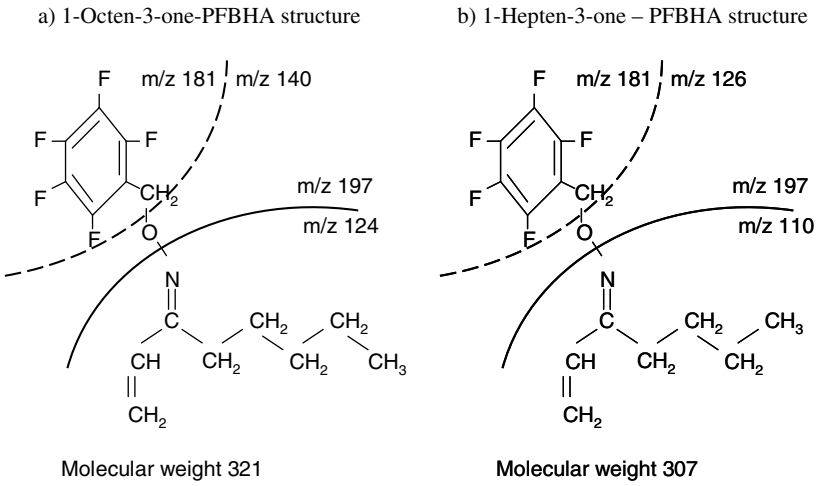

Fig. 2. Pentafluorobenzyl oxime structures resulting from the reaction between 1-octen-3-one and 1-hepten-3-one with PFBHA. The most probable *m*/*z* fragments in GC–MS–EI are shown.

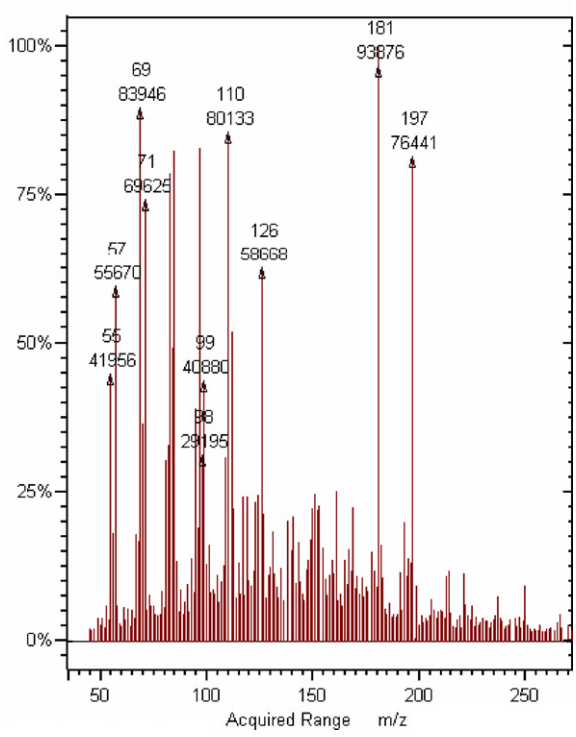

Fig. 3. Possible GC–MS spectrum (acquired in electron impact mode) of the derivative formed by 1-hepten-3-one-PFBHA found in the extract of macerate S5.

ten-3-one-PFBHA spectrum resulting from this system in negative-ion ionisation mode and used it as reference. Regarding these characteristic masses of 1-octen-3-one-PFBHA oxime acquired in NCI mode, mass 281 results from the loss of two HF molecules, while mass 301 results from the loss of one HF molecule. Others characteristic masses were 140 and 248. This spectral information was used to deduce as characteristic the masses 126 and 248, among others, obtained by the negative-ion chemical ionisation mode from the derivative formed by 1-hepten-3-one-PFBHA. Next, the previously derivatised extract of the problem macerate was injected by negative-ion chemical ionisation mode and SIM. The two isomers (*Z*/*E*) corresponding to the derivative 1-hepten-3-one-PFBHA were located in the resulting chromatogram. This confirmed the hypothesis that the compound that we had been seeking so assiduously in the mushroom note of some of the macerates analyzed was 1-hepten-3-one.

4. Conclusions

It has been demonstrated that all kinds of cork stoppers studied (natural and synthetic), are able to release into a wine-like medium the necessary amount of aroma compounds to impart characteristic aroma nuances to this medium. The aroma profiles (sensory and olfactometric) of synthetic corks are extremely simple. This aromatic simplicity of the synthetic stoppers was not a favourable quality because the most outstanding notes in some of these samples were negative aromas (e.g., rubber or mushroom). The only practically neutral macerates were S2 and S3 stoppers. While the odourant detected by GC–O with a rubber aroma (RI 1758) could not be identified, the mushroom aroma was attributed satisfactorily to 1-hepten-3-one. In contrast, none of the negative aromatic notes were detected in any samples of natural cork stoppers, despite their greater aromatic complexity. The aroma of non-synthetic stoppers was defined by sensory descriptors that were close to, or exactly the same as, those usually found in wine tasting: sweet/fruity, toasted, sweet wood and floral/muscat. The aroma profiles of the three samples (natural cork purified by procedure I, natural cork purified by procedure II and natural sample without purification treatment) were very similar, being the only difference that the sensory scores of one of the natural cork purified (NC-P1) were the lowest in most cases.

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