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ORIGINAL RESEARCH ARTICLE

The aroma of toasted oak wood (*Quercus petraea*): from sensory analysis to molecular characterisation

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ABSTRACT

The chemical complexity of the aroma compounds developed by oak wood during the toasting process is known to contribute to the quality of wines and spirits after barrel ageing. Molecular characterisation of toasted oak wood has increased steadily in recent years, using the traditional olfactory-guided approach by gas chromatography coupled with olfactometry and mass spectrometry (GC-O-MS); however, few studies have focused on its sensory characterisation. In this study, a sensory characterisation of oak wood (*Quercus petraea*) was first carried out during toasting in order to identify any descriptors that had not yet been characterised from a molecular point of view. Thus, in the second part of this work, we were able to identify volatile compounds associated with the aroma of toasted oak wood.

Based on previous work in oenology, the sensory characterisation of oak wood was carried out in three stages: identification, structuring and description of a sensory space. Data processing of the 215 descriptors generated by the panel of experts revealed six descriptors specific to the aroma of oak wood throughout its toasting (*i.e.*, fresh wood, fresh green, sweet, roasted, spicy and smoky). The results were represented graphically in the form of an aroma wheel, which was used to highlight the descriptors that had not yet been characterised from a molecular point of view. Thus, two volatile compounds were identified: thymoquinone (pencil and cedar odour) and verbenone (fresh, menthol and tealeaf odour). Their evolution in oak wood during toasting and their distribution in different wood species used in oenology (sessile oak, pedunculate oak, American oak, Caucasian oak, acacia and chestnut) were studied. Their sensory impact was studied by assessing their olfactory detection threshold in a model wine solution. The thresholds were evaluated at 49 ng/L for thymoquinone and 193 µg/L for verbenone.

KEYWORDS: oak wood, toasting process, sensory space, aroma wheel, sensory lexicon, molecular markers

INTRODUCTION

Oak barrel aging is recognised as an important step in the production of quality wines and spirits. Various wood essences have been used over time to make barrels, but nowadays oak wood (*Quercus sp.*) including sessile oak (*Quercus petraea* or *sessilis*), pedunculate oak (*Quercus robur*) and white oak (*Quercus alba*) are used almost exclusively. During barrel aging, oak wood influences the aroma, the taste and even the colour of the alcoholic beverage (Boidron *et al.*, 1988; Maga, 1989; Marchal *et al.*, 2011). The toasting process plays an important role in the development of the sensory identity of barrels. In recent decades, many studies have been conducted on the molecular characterisation of oak wood to explain the olfactory contribution of oak barrel aging (Chatonnet and Dubourdieu, 1991; Sauvageot and Feuillat, 1999). Based on literature data, we recently reported the identification of 340 volatile compounds in oak wood species selected by coopers (toasted or not) for the wine and spirits industry (Courregelongue *et al.*, 2022). These volatile compounds were found to belong to several chemical families including phenols, terpenoids, aldehydes, carboxylic acids, benzene derivatives, esters, furans, ketones, pyrazines, alcohols, C₁₃-norisoprenoïds, lactones, pyranes, pyrroles and others like quinolines. The sensory properties of some of them were established, allowing us to succinctly describe oak wood aroma.

Beyond the impact of the toasting process on the mechanical properties of oak wood, coopers use it to modulate its aroma. When oak wood is toasted, it develops complex notes reminiscent of coconut and vanilla (Chatonnet, 1995), as well as toasted, roasted and caramel nuances (Cutzach *et al.*, 1997; Cutzach *et al.*, 1999). At a higher toasting intensity, spicy and smoky notes appear (Chatonnet, 1995). On the other hand, in certain circumstances, oak wood can develop off-flavours such as sawdust aroma (Chatonnet and Dubourdieu, 1998), rancid butter aroma (Shinkaruk *et al.*, 2019) and cork aroma (Chatonnet *et al.*, 2010b). Despite this body of knowledge, few authors have studied the aroma of oak wood by sensory approaches. In a pioneering study, Sauvageot *et al.* (2002) studied the aroma of seasoned oak wood in relation to its botanical and geographic origin. This initial work on the sensory characterisation of oak wood mentioned some of the attributes used to describe and discriminate seasoned oak wood samples (*e.g.*, coconut, vanilla, fresh wood, hay, cloves and medicinal descriptors). However, no data was yet available on the sensory characterisation of toasted oak wood.

According to previous work in oenology, the sensory characterisation of a product calls upon the notion of sensory space. The characterisation of a sensory space can be divided into three phases (Barbe *et al.*, 2021; Picard *et al.*, 2015). The first step is to identify a sensory concept. The notion of sensory concept can be defined as the mental representation of the characteristics of a food product category (Ballester *et al.*, 2008). To ensure that a product category has a sensory concept, it is important to note how these products can be imagined and described by a panel.

A free association task is one of the most common qualitative methods used to identify individual mental representations and to study sensory concepts (Ares *et al.*, 2008). The panel writes down words or expressions spontaneously to describe a specific product (Langlois *et al.*, 2011; Picard *et al.*, 2015). This free association task can be complemented by a ranking test to assess the importance of the words or expressions generated (Parr *et al.*, 2011). It is often accompanied by a semantic analysis to remove auxiliary terms cited by few participants, and to categorise synonyms or words linked to the same lexical field (Symoneaux *et al.*, 2012). Finally, citation frequency is evaluated to identify the most relevant words or expressions (Symoneaux *et al.*, 2012). Statistical treatments such as hierarchical cluster analysis (HCA) can then be applied to interpret the results.

Once the existence of a sensory concept has been demonstrated, it can be evaluated and confirmed perceptually. This involves structuring the sensory space by highlighting the sensory boundaries between the samples (Ballester *et al.*, 2008). A sorting task is usually carried out in combination with verbalisation tasks to understand the categorisation strategy of participants, as has been widely the case in the sensory study of white and red wines (Ballester *et al.*, 2005; Campo *et al.*, 2008).

Finally, once the sensory space is defined, it must be described. Descriptive analyses are mainly applied when a detailed description of the sensory attributes of a product is required or when the sensory properties of different products need to be compared (Barbe *et al.*, 2021). In oenology, conventional profiling is usually used to describe the sensory properties of wines based on intensity ratings of descriptors. Conventional profiling comprises three main steps: the first crucial step is to train the panel, the second is to monitor participant performance, and the third is the evaluation of the samples by the panel. In the latter step, the participants were asked to rate the intensity of each descriptor on a structured or unstructured scale. Data analysis could thus be performed using ANOVA applied to intensity ratings.

Using this methodology, we studied the aromatic diversity of toasted oak wood in order to identify the gaps in knowledge in terms of its molecular characterisation, and thus the responsible volatile compounds. Given the value associated with the production of high-quality barrels with a specific aromatic fingerprint, it is crucial to improve our knowledge of the characterisation of the perceived sensory properties of toasted oak wood.

MATERIALS AND METHODS

1. Chemical and reference compounds

Ethanol (LC grade, LiChroSolv) was from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Dichloromethane (HPLC grade, HiPerSolv Chromanorm) was from VWR Prolabo (Fontenay-sous-Bois, France). Sodium sulphate (Na₂SO₄ > 99.5 %) was purchased from Fisher Scientific (Loughborough, United Kingdom). Tartaric acid (> 99.7 %)

and 3-octanol (97 %) were supplied by Sigma-Aldrich (St-Quentin-Fallavier, France). The reference compound thymoquinone (98 %) was from Santa Cruz Biotechnology (Santa Cruz, CA), and verbenone (94 %) from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Ultrapure water (Milli-Q, resistivity = 18.2 MΩ cm, Millipore, Saint-Quentin-en-Yvelines, France) was used.

2. Oak wood samples

2.1. Free association task and sensory approach

The oak wood samples were from Seguin-Moreau cooperage (Merpins, France) and used in Experiments 1 and 2. Three staves (*Quercus petraea*, 95 cm × 5 cm × 1.8 cm, outdoor season for 24 months) were cut into pieces of wood (5 cm × 5 cm × 1.8 cm) following the industrial protocol of the cooper. The pieces were blended, divided in four batches and heated in a convection oven at different temperatures: 160 °C (light toasting, LT), 180 °C (medium toasting, MT) and 240 °C (high toasting, HT) for 30 min corresponding to the temperature ranges selected to produce commercial chips. A control comprising a non-toasted oak wood sample (NT) was also studied. Then each piece was ground into chips (~1 × cm). Pictures of the samples were taken and included in the online form to illustrate the impact of toasting for the purposes of the mental representation experiment (Figure S1). The oak samples were used for the sensory experiments.

2.2. Quantification approach

2.2.1. Study of the toasting impact

Oak wood selection was similar to that carried out in the sensory experiment part, and the toasting conditions ranges were extended: 160, 180, 200, 220 and 240 °C for 30 min. A non-toasted oak wood control sample (NT) was also studied. Then each piece was ground into small chips (~1 × ~0.3 cm). To take into account the natural intra-variability, the experiment was conducted separately on three biological replicates of staves.

2.2.2. Study of the wood species impact

The selected wood samples were from Seguin-Moreau cooperage (Merpins, France). Six different wood species were studied: sessile oak (*Q. petraea*, SES), pedunculate oak (*Q. robur*, PED), Acacia tree (*Robinia pseudoacacia*) and Chestnut tree (*Castanea sativa*), American oak (*Q. alba*, AME), Caucasian oak (*Q. sp.*, CAU). The staves of each wood species (95 cm × 5 cm × 1.8 cm, outdoor season for 18 months) were transformed into pieces of wood (5 cm × 5 cm × 1.8 cm), then each piece was ground into small chips (~1 × ~0.3 cm). To limit natural intra-variability, the experiment was conducted on five biological replicates of staves.

3. Sensory analysis techniques

3.1. Descriptor generation and sensory space study (Experiment 1)

A free association task was carried out to identify individual mental representations associated with the aroma balance

of oak wood samples (*Q. petraea*) according to the toasting intensity. This task drew on the internal panel's expertise of the company regarding the aroma of toasted oak wood. Thus, an internal panel composed of 13 panellists (2 women and 11 men, age range 36-58 and years of experience 3-20). They were all volunteers involved in the production of oak or in sales, and they were selected for their interest in the aromatic balance of oak wood. We asked the panellists to write down the descriptors that come to mind when describing oak wood aroma, depending on the toasting intensity.

This work was carried out four years ago during the pandemic (containment period); for this reason, the free association task was adapted and carried out via an online questionnaire (Google Form). The participants were asked to draw on their own expertise in order to state, "what descriptors they would use to describe the aroma of oak wood" according to a specific toasting intensity. They were asked to generate at least four aroma descriptors per treatment. As it was an in-house exercise, the time dedicated to this was not controlled. However, we asked the jury not to modify the results once the form was filled. Each toasting intensity was represented by a picture of oak wood chips toasted at different temperature-time couples: non-toasted (NT), light toasting (LT, 160 °C for 30 min), medium toasting (MT, 180 °C for 30 min) and high toasting (HT, 240 °C for 30 min) [Figure S2].

3.2. Conventional sensory profiling (Experiment 2)

Conventional sensory profiling was performed to confirm the relevance of the descriptors for the aroma of toasted oak wood generated in the previous step based on the representation of the sensorial image of this product. An experienced panel from the research unit periodically trained to evaluate the sensory balance of wine aged or not in oak wood (Pelonnier-Magimel *et al.*, 2020) was selected to perform this task. It was composed of 10 panellists (9 women and 1 man, age range 25-40). We then asked to rate the intensity of seven descriptors on a structured scale (0 to 10) for different oak wood samples. These descriptors were selected on the basis of their ability to explain the complex aroma balance of oak after maturation and after toasting. In practice, oak wood chips (2 g) were placed in black glasses coded with a random three-digit number. All glasses were simultaneously submitted to each taster with a specified randomised order. To avoid the influence of the colour of the chips during olfactory analysis, the black ISO glasses were covered with perforated aluminium foil.

3.3. Olfactory detection threshold

The evaluation of the olfactory detection threshold was performed by a panel of 15 people with previous experience in sensory analysis (oenology unit staff). It took place in a temperature-controlled room maintained at 20 ± 1 °C and equipped with individual booths. Glasses were coded with a random three-digit number and filled with 25 mL of liquid. The composition of the model wine solution was bi-distilled ethanol 12 % vol., L-(+)-tartaric acid 5g/L, pH 3.5 (NaOH, 1 M). The detection threshold of the aroma compound was determined in a model solution with an

ascending procedure using the three-alternative forced-choice presentation method (3-AFC). For each compound, a first session was designed to evaluate its detection threshold range. Next, six increasing concentrations of each compound were presented to a panel in three sessions in order to find the best concentration range. Each session lasted approximately 10 min per assessor. The concentrations ranged from 6.25 to 200 ng/L for thymoquinone, and from 12.5 to 400 µg/L for verbenone.

The olfactory detection threshold corresponds to the minimum concentration below which 50 % of the tasters statistically failed to recognise the difference from the control, based on the analysis of the concentration/response function; *i.e.*, the sigmoid curve (NF ISO 13301, 2002).

4. Quantification approach (Experiment 3)

In this part, the oak wood samples were used for the study of the distribution of thymoquinone and verbenone.

4.1. Extraction protocol

Wood chips (100 g/L) were kept in 12 % hydroalcoholic solution for 24 hours under stirring (200 RPM) and at room temperature in an airtight amber flask. Ammonium sulphate (5 g), 3-octanol (internal standard IS, 10 µL at 100 mg/L in ethanol), ultrapure water (9 mL) and finally oak wood solution (1 mL) were added to a 20 mL amber vial, which was then sealed with poly(tetrafluoroethylene) (PTFE)-lined caps (Chromoptic, Villejust, France).

A 50/30 µm (divinylbenzene/carboxen/polydimethylsiloxane) [DVB/CAR/PDMS] Stableflex 23 Ga SPME fibre (Supelco, Bellefonte) was used for sample extraction. Using a combi PAL autosampler (CTC Analytics, Zwingen, Switzerland), the vials containing the prepared samples were initially incubated for 5 min at 50 °C, followed by sample extraction for 30 min at an agitation speed of 450 RPM (5 seconds on, 2 seconds off).

4.2. GC-ToFMS analysis

The fibre was desorbed into the injection port (230 °C) of a 7890B GC System (Agilent Technologies Inc., Palo Alto, CA, USA) for 2 min. The injector was initially set to splitless mode (closure time: 1 min), after which a split flow of 50 mL/min was used. The GC was coupled to a Pegasus BT 4D time-of-flight mass spectrometer (LECO, Saint-Joseph, MI, USA). Separation was performed on a non-polar DB-5ms capillary column (50 m × 0.22 mm × 0.25 µm, Agilent) coupled to a mid-polar Rxi-17Sil MS capillary column (2 m × 0.15 mm × 0.15 µm, Restek). The carrier gas was helium (Messer, France), 6.0 grade, with a flow rate of 1 mL/min. The GC oven was programmed from 55 °C (1 min) to 250 °C at 4 °C/min, held for 1 min. The transfer line was set at 260 °C and the source temperature at 250 °C. Ionisation was carried out by electron impact with an ionisation energy of 70 eV. Acquisition was carried out in scan mode (*m/z* 45–450). Quantification was performed on *m/z* 164 and 107 for thymoquinone and verbenone, respectively.

The linearity of the method was determined by analysing oak wood solution spiked with six increasing concentrations (until 1 µg/L) of thymoquinone and verbenone. The coefficient of determination (R^2) for each curve was higher than 0.990 (Table S1). The detection and quantification limits corresponded to the concentrations obtained for a signal-to-noise ratio equal to 3 and 10, respectively. The limit of detections were evaluated at 11.9 and 4.7 ng/L, and the limit of quantifications at 39.7 and 15.7 ng/L in hydroalcoholic oak wood solutions for thymoquinone and verbenone, respectively. Repeatability of the method was determined after four analysis of 12 % vol. solution spiked at 200 ng/L, for both compounds.

5. Statistical analysis

SigmaPlot 15.0 (Systat) software was used for graphic resolution and determination of olfactory detection thresholds. Statistical analyses were performed with XLSTAT software (version 2021.4.1). Citation frequency results were ranked to identify the most relevant term for each oak wood treatment. Only terms cited by a minimum of 2 judges (15 % of the panel, corresponding to a citation frequency > 0.015), were considered for subsequent statistical analysis and correspondence analysis (CA). Sensory scores were mean centred and standardised to compare sensory evaluation results from different panellists. The differences between the data sets (sensory and quantitation experiments) were determined using an analysis of variance (ANOVA) followed by a Tukey *post hoc* test when the application conditions were validated. The normality of the distribution was tested using the Shapiro-Wilk test; otherwise, the non-parametric Kruskal-Wallis test followed by the Steel-Dwass-Critchlow-Fligner and Dunn *post hoc* tests (pairwise comparison) were applied. For all tests, the α risk was set at 5 %.

RESULTS

1. Sensory characterisation of oak wood aroma

The sensory characterisation of a product calls upon the notion of sensory space, which involves three steps: identification, structuring and description. We used this methodology to study the sensory space of toasted oak wood.

1.1. Identification of a sensory space (Experiment 1)

The sensory concept of toasted oak wood can be compared to the mental representation of the sensory properties of oak wood during its toasting. To do this, a panel of experts performed a free association task to generate descriptors from pictures of oak wood chips toasted at different intensities (Figure S1).

In total, two hundred and fifteen descriptors were generated by the internal panel for four toasting intensities. To interpret these data, a semantic analysis was performed consisting in removing spelling mistakes, cancelling auxiliary terms and categorising synonyms or words linked to the same lexical field. For example, the descriptors ‘spices’ and ‘spicy’ were gathered in one unique descriptor ‘spicy’. This semantic

analysis allowed us to reduce the number of descriptors to eighty-three. Based on these descriptors, their citation frequencies were calculated for each toasting intensity (Figure S2). This approach revealed descriptors that are relevant and specific to a toasting treatment. Each toasting intensity has between two and four main descriptors ($f > 0.08$): ‘fresh’, ‘fresh wood’, and ‘sawdust’ for the non-toasted sample (NT), ‘vanilla’ and ‘fresh’ for the light toasted sample (LT), ‘vanilla’, ‘pastry/brioche’, ‘caramel’ and ‘toasted bread’ for the medium toasted sample (MT) and ‘smoky’, ‘coffee’ and ‘spicy’ for the high toasted sample (HT). The main descriptors evolve depending on toasting intensity. In addition, there are descriptors with a weaker citation frequency. These results illustrate the aromatic diversity and complexity of toasted oak wood, evidencing the existence of a sensory concept of toasted oak wood.

1.2. Structuring the sensory space

1.2.1. Categorisation of samples

To highlight any categorisation of the samples, a statistical sorting using a correspondence analysis (CA) was applied to

the citation frequencies ($Cf > 0.015$). This analysis measures the intensity of a correspondence between two qualitative variables (samples and descriptors in our case). The CA map in two dimensions is shown in Figure 2. The total inertia of the map was explained at 82.26 % by the two first axis 1 (50.42 %) and 2 (31.84 %). From a graphical point of view, the sequence follows a circular arch (*i.e.*, like the well-known horseshoe effect in correspondence analysis). Such arch-shaped configuration appears when CA is applied to data involving a linear gradient. The diagram illustrates the distribution of the different samples along these two axes and their correlation with a specific lexical field. For example, the NT sample was characterised by ‘dusty’, ‘mushroom’, ‘sawdust’, ‘fresh wood’ and ‘green’ descriptors, while the HT sample was characterised by ‘toasted’, ‘lard/bacon’, ‘cloves’ and ‘smoky/ashes’ descriptors. In this sense, this representation and projection on the first axis illustrates that non-toasted and heavily toasted oak woods are aromatically opposed. We also evidenced a sensory continuum associated with toasting intensity (axis 1). These results demonstrate the impact of toasting on the distribution of the descriptors.

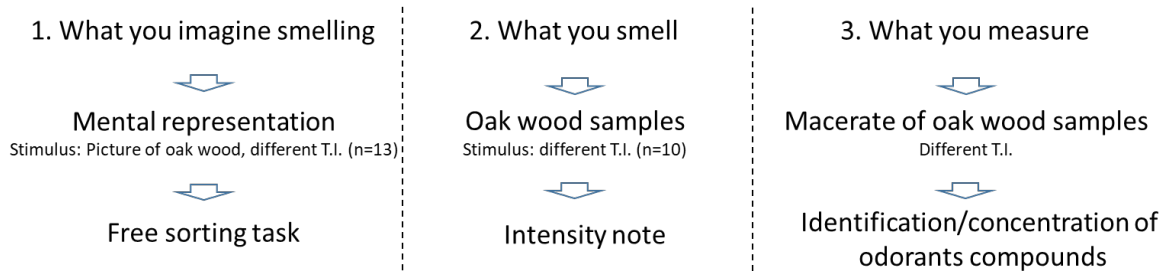


FIGURE 1. Representation of the approach broken down into three experiments (including sensory and analytical approaches) for the evaluation of the impact of toasting intensity (T.I.) on the aroma of oak wood (*Q. petraea*).

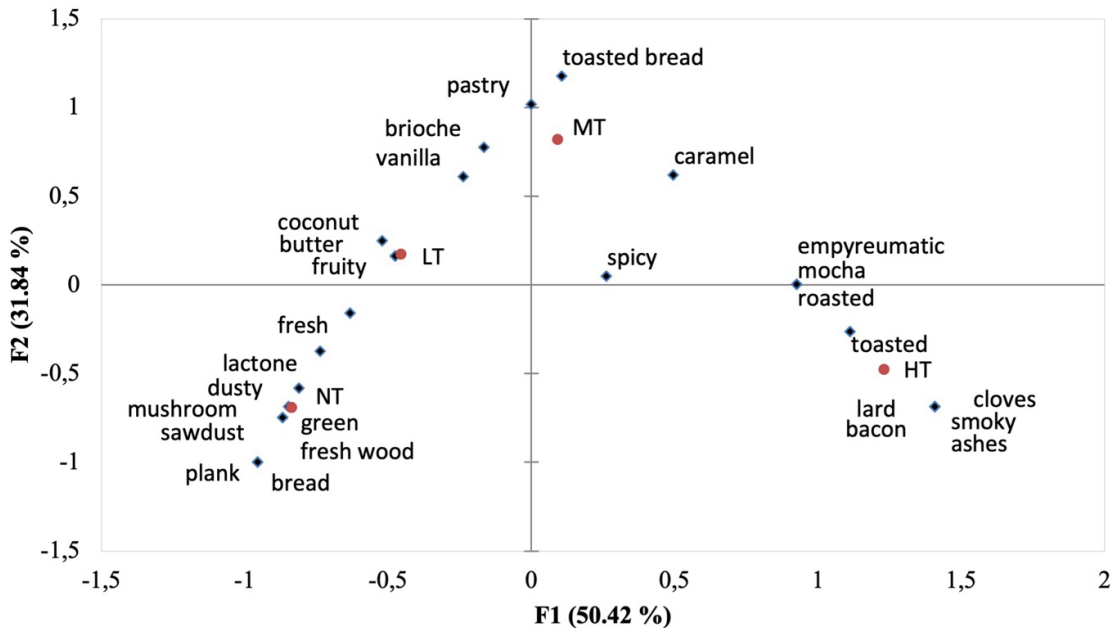


FIGURE 2. Projection of descriptors and samples in the correspondence analysis map (CA). Data set was obtained from citation frequencies ($Cf > 0.015$) of eighty-three descriptors generated by the expert panel depending on toasting intensity: NT = non-toasted; LT = light toasting; MT = medium toasting and HT = high toasting. ($n = 13$)

TABLE 1. Impact of toasting on intensity of descriptors (n = 10)

Samples	Descriptors ¹													
	Fresh wood		Fresh green		Dry green		Sweet		Roasted		Spicy		Smoky	
NT	7.4	b	3.0	b	3.8	-	3.6	ab	1.1	a	2.3	a	1.0	a
LT	5.2	b	1.6	ab	3.8	-	5.7	b	5.3	b	4.3	b	3.3	b
MT	6.6	b	2.6	ab	5.3	-	4.2	b	3.1	ab	2.8	ab	1.8	ab
HT	1.7	a	1.1	a	3.8	-	1.7	a	4.4	ab	5.5	b	9.6	c
p-value	***		**		ns		***		**		**		***	

¹average intensity; p-value < 0.05, *; < 0.01, **; < 0.001 ***; ns = not significant

1.2.2. Classification of descriptors (Experiment 2)

Unlike the previous sorting task, the classification of the descriptors was based on a consensus approach (Deneulin and Pfister, 2013). The purpose was to group the descriptors by sensory family to obtain a unique descriptor. They were grouped together according to their olfactory similarity. For example, the descriptors ‘spicy’, ‘cloves’, and ‘pepper’ were grouped in the sensory family defined by the unique descriptor ‘spicy’. By applying this approach to the eighty-three descriptors generated by the panel, seven sensory families were obtained: ‘fresh wood’, ‘fresh green’, ‘dry green’, ‘sweet’, ‘roasted’, ‘spicy’ and ‘smoky’. Details of the classification are given in Table S2.

1.2.3. Description of sensory space

Once the sensory space of the toasted oak wood had been evidenced and defined, it had to be described. Conventional profiling was carried out on oak wood samples toasted at different intensities (non-toasted, NT; 160 °C, 30 min, LT; 180 °C, 30 min, MT and 240 °C, 30 min, HT). An expert panel was asked to smell the oak wood chips and to rate the intensity of each previously defined descriptor (Table S1) using a structured scale for each sample of toasted oak wood.

The panellists’ consensus was assessed by applying principal component analysis (PCA) to their ratings. This analysis allows the agreement between panellists to be

assessed (Stone *et al.*, 1974). On the PCA, 65.74 % of the total variance was explained by axes 1 (48.92 %) and 2 (16.82 %) [Figure S3]. The distribution of the panellists to the right of axis 1 on this PCA demonstrates their consensus and validate the selection of the panelist. A consensus between panellists is important, as this demonstrates their ability to correctly assess the intensity of the descriptors (even without prior training), which has a direct impact on the quality and interpretation of the results.

Table 1 shows the average intensity of each descriptor for each toasting intensity. A non-parametric statistical test (Kruskal-Wallis) followed by a multiple comparison analysis (Steel-Dwass-Critchlow-Fligner) were applied to the average intensity of each descriptor at each toasting intensity (Table 1).

The results revealed the impact of toasting intensity on all descriptors, except for the descriptor ‘dry green’ (p-value > 0.05). As temperature increased (NT to HT), the intensity of the descriptors ‘fresh wood’ and ‘fresh green’ decreased, while the intensity of the descriptors ‘spicy’ and ‘smoky’ increased. In addition, the maximal intensity of the descriptors ‘sweet’ and ‘roasted’ was reached at intermediate temperatures (LT and MT samples).

The same non-parametric test was applied to the same dataset to explain the contribution of each descriptor

TABLE 2. Average intensity of each sensory descriptors according to the toasting intensity of oak wood (n = 10).

Descriptors	Samples ¹							
	NT		LT		MT		HT	
Fresh wood	7.4	b	5.2	ab	6.6	b	1.7	a
Fresh green	3	b	1.6	a	2.6	ab	1.1	abc
Dry green	3.8	ab	3.8	ab	5.3	b	3.8	abc
Sweet	3.6	ab	5.7	b	4.2	ab	1.7	ab
Roasted	2.3	a	4.3	ab	2.8	ab	5.5	bc
Spicy	1.1	a	5.3	ab	3.1	ab	4.4	cd
Smoky	1	a	3.3	a	1.8	a	9.6	d
p-value	****		***		*		****	

¹average intensity; p-value < 0.05, *; < 0.01, **; < 0.001, ***; < 0.0001 ****; ns = not significant

according to the sample studied (Table 2). The sample NT was characterised by the descriptors ‘fresh wood’ and ‘fresh green’ with a significantly higher intensity, while the sample HT was mainly characterised by the descriptor ‘smoky’. In samples toasted at intermediate temperatures (LT-MT), the distribution of the descriptors was more complex, illustrating their greater aromatic complexity. Despite this complexity, it was possible to discriminate these samples using these seven descriptors.

The application of sorting tasks and statistical treatments allowed us to define a sensory space for toasted oak wood and to describe it well by conventional profiling, demonstrating the relevance of the descriptors generated and thereafter selected in this work to describe the aromatic diversity of toasted oak wood. The results obtained by sensory analysis were consistent with the observations of the professionals and illustrate the aromatic complexity of oak wood when it is toasted. From these results, we tried to create a practical tool to describe and categorise the aromatic complexity of oak wood.

1.3. A practical tool: the oak wood aroma wheel

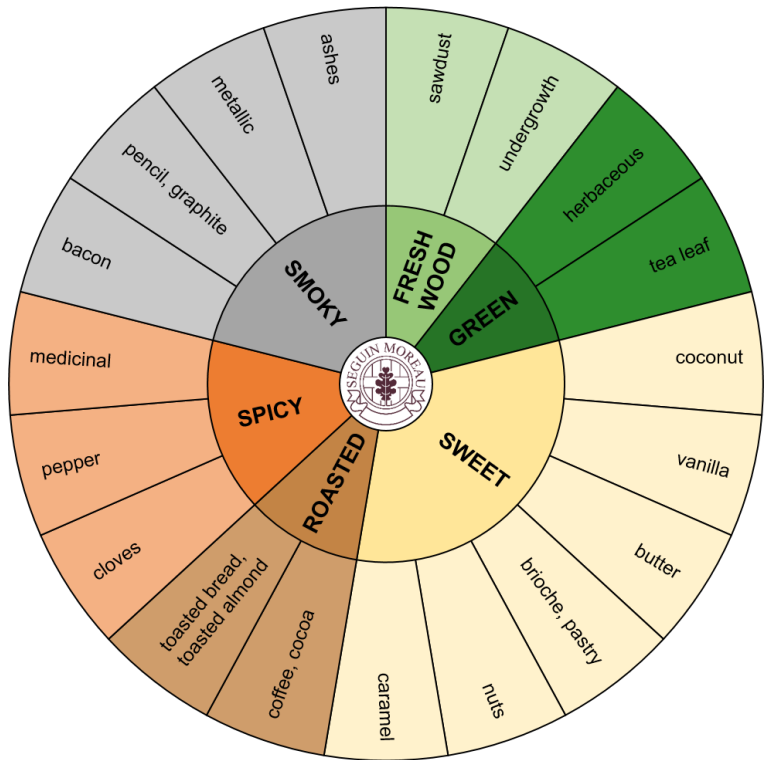
A literature review of the sensory characterisation of other food products revealed that the aroma wheel is the most common visual representation of the aromatic palette of products, such as foods and beverages. For example, there are already aroma wheels for wine (Noble *et al.*, 1987), Cognac (Lurton *et al.*, 2012), whisky (Lee *et al.*, 2001; Wishart, 2009), beer (Schmelzle, 2009), coffee (Spencer *et al.*, 2016), cheese (Bérodier *et al.*, 1997) and rum (Ickes *et al.*, 2017). An aroma wheel provides a common standardised lexicon to facilitate the sensory characterisation of a product.

Here, we produced an aroma wheel for oak wood (Figure 3) using the results of the impact of toasting (Table 1 and Table 2). We retained six main sensory families out of seven. Since the dry green descriptor was not directly impacted by toasting, we grouped it with the fresh green descriptor to create a single green sensory family. Therefore, the inner circle of the wheel is divided into six different colours and describes the main sensory families. The outer circle subdivides the six main sensory families into nineteen sub-classes. These sub-classes include all the descriptors generated and classified during the consensus approach (Table S1). Moreover, their arrangement in the aroma wheel, from the fresh wood family to the smoky family, illustrates the aromatic evolution of oak wood aroma during the toasting process. Several sensory families coexist at the same toasting intensity.

2. Molecular characterisation of oak wood aroma

In a previous paper (Courregelongue *et al.*, 2022), we reported on the methodology used to identify volatile compounds responsible for the aroma of toasted oak wood using gas chromatography coupled to olfactometry and time of flight mass spectrometry (GC-O-TOF-MS) followed by purification with semi-preparative HPLC. Based on this methodology and by comparison with literature data, we described odorous zones (OZ) associated with thymoquinone (LRI 1250 on DB5 capillary column) reminiscent of pencil odour and verbenone (LRI 1200 on DB5 capillary column) with a fresh, minty, tealeaf odour (Courregelongue, 2021). These two compounds have been identified by Ghadiriasli *et al.* (2018) in another oak wood species not used in oenology, the

FIGURE 3. Toasted oak wood aroma wheel.



Hungarian oak wood (*Q. frainetto*). The co-injection of the commercial compounds confirmed their identification in a toasted oak wood (*Q. petraea*) organic extract by GC-MS (Figure S4 and S5).

Thymoquinone was first recently identified by Schreiner *et al.* (2017) and described as a compound characteristic of the pencil odour in cedar wood (*Calocedrus decurrens*). In perfumery, the odour of cedar wood is defined as “an exact replica of the smell of pencil”. It is described as dry, green and resinous. Verbenone has already been found in the essential oils of many plants, such as rosemary essential oil, where it is described as a characteristic aroma compound (Pieracci *et al.*, 2021). This compound, highly prized by the cosmetic and food industries for its sensory properties, has also been the subject of numerous studies, which demonstrated its biosynthesis from α - and β -Pinenes (Bicas *et al.*, 2009; Sales *et al.*, 2018; Vespermann *et al.*, 2017). We evaluated the olfactory detection threshold of thymoquinone at 49 ng/L and that of verbenone at 193 μ g/L in a model wine solution ($n = 15$, Table 3).

2.1. Impact of toasting on thymoquinone and verbenone distribution in oak wood (*Q. petraea*)

The experimental protocol involved six temperature-time couples (Figure 4). We observed a significant impact of toasting on the concentrations of verbenone only (p -value < 0.05), which was the highest in non-toasted (NT) oak wood reaching 1.21 ng/g of wood (on average). Then, with the application of a heat treatment its concentration dropped to 0.4 ng/g of

wood (on average). Thymoquinone concentrations were not significantly impacted by heat treatment ($p = 0.074$). This can be explained by the concentrations found in the wood being close to the detection limit of our method (0.12 ng/g of wood). However, there is a trend towards the formation of this compound at 200 °C and above. Its maximum average concentration reaches 0.52 ng/g of wood at 220 °C.

Verbenone is mainly found in non-toasted oak wood while thymoquinone seems to be mainly found in medium/highly heated wood. This observation is consistent with the results obtained in the previous sensory part. In fact, on the aroma wheel, the descriptors “fresh wood, fresh green, tealeaf” were used to describe the aroma of lightly or non-toasted oak wood, whereas the descriptor “pencil” was cited to describe the aroma of highly toasted oak wood (Figure 3).

2.2. Distribution of thymoquinone and verbenone depending on wood species

To go further, we looked at their distribution in other wood species used by coopers (Figure 5). We quantified thymoquinone and verbenone in hydroalcoholic extract of sessile oak (*Q. petraea*), pedunculate oak (*Q. robur*), American oak (*Q. alba*), Caucasian oak (*Q. sp.*), acacia tree (*Robinia pseudoacacia*) and chestnut tree (*Castanea sativa*).

We show that there is a wide inter- and intra-species distribution. Sessile oak has the highest thymoquinone content, reaching on average 122 ng/L in the hydroalcoholic extract. It differs significantly from acacia, chestnut and

TABLE 3. Descriptors and olfactory detection threshold (ODT) of thymoquinone and verbenone evaluated in a model wine solution ($n = 15$).

Compound	Descriptors	ODT
Thymoquinone	Pencil, cedar wood	49 ng/L
Verbenone	Fresh, minty, tealeaf	193 μ g/L

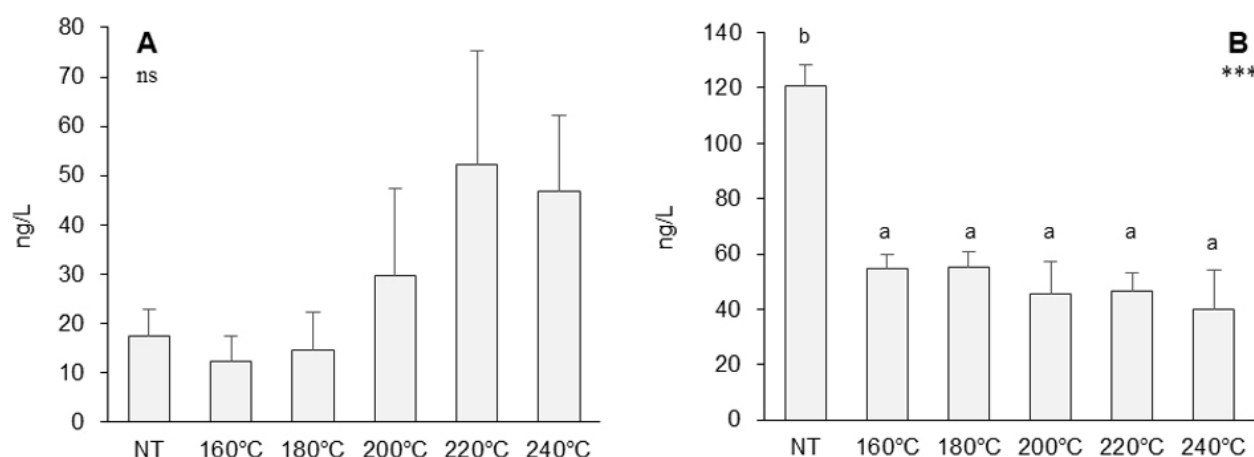


FIGURE 4. Evolution of thymoquinone (A) and verbenone (B) concentrations in oak wood hydroalcoholic extracts (*Q. petraea*) at different toasting intensities ($n = 3$, ns: not significant, p -value < 0.001 ***; different letter correspond to significant differences according to heat treatment).

pedunculate oak, which contain only trace concentrations. The extracts of Caucasian and American oak show intermediate concentrations (35–48 ng/L). By contrast, sessile oak has the lowest verbenone content (32 ng/L). Conversely, Caucasian oak has the highest verbenone concentrations, with an average concentration of 391 ng/L, followed by chestnut (217 ng/L). It seems that the wood species determines thymoquinone and verbenone composition. Therefore, after toasting, it is possible to obtain barrels or alternatives with different compositions.

This study illustrates the importance of mastering the choice of species when making a barrel, for example. This is not the first time that volatile compounds have been found to be specific to an oak species. Prida and Puech (2006) have identified volatile compounds, such as whisky lactone, eugenol, and vanillin, which can help discriminating between species and origins of oak wood.

DISCUSSION

Sensory analysis must follow precise protocols and uphold certain norms [e.g., ISO 13299 (2016), general guidelines for the development of a sensory profile]. In the literature, the sensory characterisation of a product calls upon the notion of sensory space. Previous studies have explored the sensory concepts of Chardonnay wine typicity (Ballester *et al.*, 2005), vins-de-garde typicity (Langlois *et al.*, 2011), wine complexity (Parr *et al.*, 2011) and the ageing bouquet of Bordeaux red wines (Picard *et al.*, 2015). To our knowledge, these notions have never been explored in cooperage. Apart from the work of Sauvageot *et al.* (2002) on the aroma of seasoned oak wood, no study has attempted the sensory characterisation of the aroma of oak wood using sensory analysis methodology. For this reason, we sought to identify and define the sensory concept of toasted oak wood.

To this end, a lexical analysis was performed by a panel of experts followed by statistical treatments to highlight descriptors used to characterise the aroma of oak wood during its toasting. As expected, the descriptors ‘fresh wood’, ‘sawdust’, ‘vanilla’, ‘caramel’, ‘smoky’ and ‘spicy’ were among the most cited descriptors, but other less usual descriptors were also used (e.g., ‘pastry’, ‘brioche’, ‘bacon’) (Figure S2). Some of them have already been reported in a sensory study of seasoned oak wood, such as ‘fresh wood’, ‘vanilla’ and ‘spicy’ (expressed as ‘cloves’) (Sauvageot *et al.*, 2002), as well as in the sensory study of hydro-alcoholic extracts of oak wood (fresh, seasoned and toasted), such as ‘sawdust’, ‘vanilla’, ‘caramel’ and ‘spicy’ (Francis *et al.*, 1992; Mosedale and Ford, 1996). We found similarities with those studies regarding the less cited descriptors ($f < 0.075$): ‘green’, ‘coconut’, ‘butter’, ‘sweet’, ‘nut’ and ‘burnt’.

The number of descriptors generated highlights the complexity of oak wood aroma and the importance of creating a practical tool to help study, communicate, and assess the quality of the sensory properties of toasted oak wood. Its development was made possible by performing conventional profiling using the seven main descriptors defined above on toasted oak wood samples, followed by statistical treatments coupled with literature data (Figure 3).

To go further, we took the possible association between a descriptor and a known oak wood volatile compound into account. Indeed, 340 volatile compounds have been identified to date in oak wood and some were reported as having an odour (Courregelongue *et al.*, 2022). Importantly, the present work focused on the sensory characterisation of high-quality barrels. For this reason, only volatile compounds associated with quality aroma are reported. Some studies sought to identify the volatile compounds responsible for off-flavours, such as 2-methoxy-3,5-dimethylpyrazine, which is responsible for a corky odour (Chatonnet *et al.*, 2010b), 2,4,6-trichloroanisole (TCA), which gives a musty odour (Chatonnet *et al.*, 2010a)

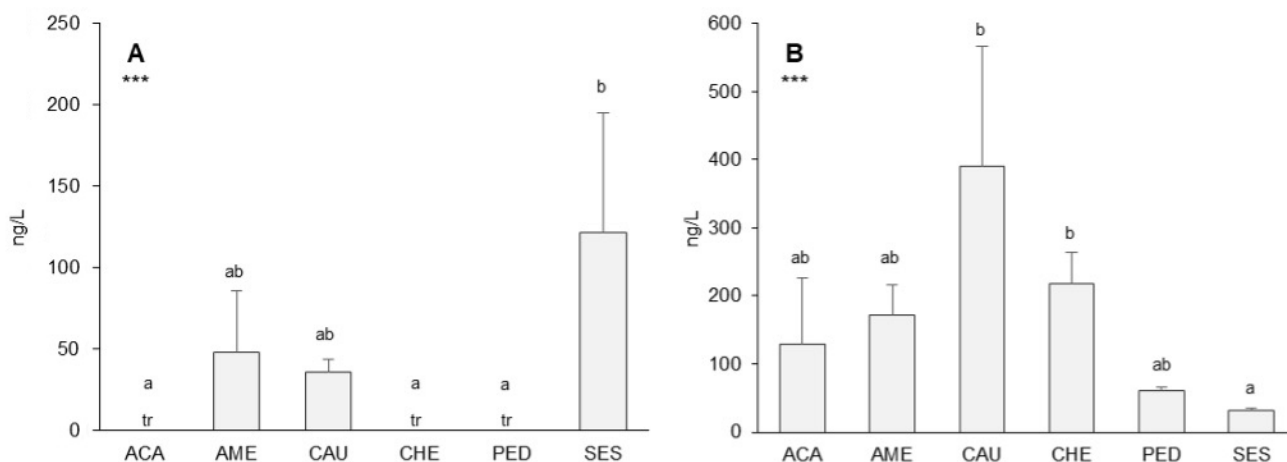


FIGURE 5. Distribution of thymoquinone (A) and verbenone (B) in different hydroalcoholic extracts of seasoned oak wood species: ACA (*R. pseudoacacia*), AME (*Q. alba*), CAU (Caucasian oak), CHE (*C. sativa*), PED (*Q. robur*) and SES (*Q. petraea*) ($n = 5$, tr: trace amounts, p -value < 0.05 *, < 0.01 **, < 0.001 ***, different letter correspond to significant differences according to wood species).

and some dialkylpyrazines, which give a rancid butter odour (Shinkaruk *et al.*, 2019), but they are not within the scope of this study. Table 4 shows the volatile compounds identified in oak wood and known to be responsible for some of the aroma wheel descriptors (Figure 3).

1. The “fresh wood” and “green” notes

Following the aroma wheel from the non-toasted notes (green side) to the highest toasted notes (grey side), (*E*)-2-nonenal was identified as being responsible for the sawdust aroma of non-toasted oak wood (Chatonnet and Dubourdieu, 1998). This compound is considered an off-flavour and its behaviour during toasting has been reported to comprise a significant formation step that precedes its degradation [220 °C] (Courregelongue *et al.*, 2022); therefore, its contribution to oak wood aroma can be modulated by the toasting process. 1-octen-3-one and (*Z*)-3-hexenal were identified by GC-O-MS in oak wood as being responsible for mushroom and herbaceous odorous zones, respectively (Ghadiriasli *et al.*, 2018, Ghadiriasli *et al.*, 2021). However, to our knowledge, no volatile compounds have been associated with the tealeaf aroma of non-toasted oak wood. Further work is needed to identify this volatile compound and understand its origin and its contribution to toasted oak wood aroma.

2. The “sweet” notes

Regarding the volatile compounds responsible for the sweet aroma of toasted oak wood, whisky lactone with coconut notes, which is present in two isomeric forms (*cis* and *trans*), is one of the main volatile compounds released by oak wood (Chatonnet, 1995). Another major volatile compound released by oak wood is vanillin, which is responsible for the vanilla aroma (Chatonnet *et al.*, 1991). Butter aroma is generally correlated with the presence of 2,3-butanedione (diacetyl) in wine (Bartowsky and Henschke, 2004). It has also been identified by GC-O-MS in various oenological wood species (cherry, ash) including oak wood as being responsible for a buttery odorous zone (Culleré *et al.*, 2013), but its contribution to the buttery aroma of oak wood has not been demonstrated. More recently, (2*E*,4*E*,6*Z*)-nonatrienal was identified as being responsible for the brioche and pastry aroma of toasted oak wood (Courregelongue *et al.*, 2022). This new aldehyde is the first molecular interpretation of the descriptor ‘brioche’ cited by the panellists (Experiment 1). The nutty aroma of toasted oak wood has already been studied and is associated with the presence of some N-heterocyclic compounds such as 2,6-dimethylpyrazine (Picard *et al.*, 2019) and 1-methylpyrrole-2-carboxaldehyde (Gammacurta *et al.*, 2021). As for the caramel aroma of toasted oak wood, maltol is one of the various volatile compounds responsible for this aroma (Cutzach *et al.*, 1997, Cutzach *et al.*, 1999).

3. The “spicy” and “roasted” notes

The roasted aroma of toasted oak wood is well documented and various volatile compounds may be associated with it. For example, 2-furfurylthiol is known to be responsible for its strong roasted coffee aroma (Tominaga *et al.*, 2000), and

furfural (Chatonnet *et al.*, 1999) and 2-ethyl-3-methylpyrazine (Picard *et al.*, 2019) as responsible for its toasted almond aroma. As for the spicy aroma of toasted oak wood, it is subdivided into cloves contributed by eugenol (Aiken and Noble, 1984), pepper whose direct link with rotundone (despite its identification) remains to be demonstrated (Genthner-Kreger and Cadwallader, 2021) and medicinal contributed by *o*-cresol (Fernández de Simón *et al.*, 2010) and quinoline (Picard *et al.*, 2019).

4. The “smoky” notes

Finally, the smoky character of toasted oak wood can be explained by the presence of guaiacol and syringol, and their derivatives, which are responsible for smoky/ashes notes (Chatonnet, 1995). Recently, a new aldehyde, *trans*-4,5-epoxy-(*E*)-2-decenal, was found to be responsible for the metallic aroma developed by toasted oak wood (Courregelongue *et al.*, 2022). To our knowledge, however, the bacon aroma of toasted oak wood has not been characterised from a molecular point of view. As with the tealeaf aroma, further work is needed to identify the volatile compound responsible and understand its origin and contribution to toasted oak wood aroma.

5. The missing part

This initial sensory work highlighted the descriptors that still need to be characterised from a molecular point of view, with the tealeaf descriptor attracting our attention. Based on previous laboratory studies and data from the literature, we identified thymoquinone as being associated with a pencil and cedar odour, and verbenone with a fresh, minty, tealeaf odour (Table 3). The evaluation of their olfactory detection threshold allows us to attribute a greater impact of thymoquinone (ODT = 49 ng/L) to the oak wood aroma compared to verbenone (ODT = 193 µg/L).

We developed a quantification method to study their distribution in wood samples. Verbenone was found in greater concentrations in non-toasted oak than in toasted oak, where it is degraded. For thymoquinone, even though the concentrations were not significantly different, the trend is reversed, which is consistent with the intensity of the descriptors “pencil” and “graphite” cited to describe the strongly toasted oak wood aroma (Table 1). These identifications will provide new insights into the characterisation of the aroma of oak wood for oenological use. It highlights the aromatic diversity of oak woods. In addition, the quantitation experiments carried out in hydroalcoholic solution showed that verbenone does not contribute to wine aroma, while the exact sensory contribution of thymoquinone needs further investigation.

6. From the aroma of oak wood to the aroma of wines and spirits

Furthermore, when comparing the descriptors used to describe the aroma of oak wood with those used to describe the aroma of wines and spirits aged in its contact, we found many of them to be similar. For example, the descriptors ‘coconut’, ‘vanilla’, ‘spicy’, ‘smoky’, ‘toasty’, ‘vegetative fresh and dried’ (for the green aroma), ‘caramel’ and

‘nutty’ are widely used to characterise the aroma of white and red wines (Bosso *et al.*, 2008; Cameleyre *et al.*, 2020; Perez-Prieto *et al.*, 2003). These descriptors are also found in the aroma wheels for beer, whisky and rum when describing the contribution of oak wood to these beverages, and in the aroma wheel for coffee when describing the aromas of roasting (Spencer *et al.*, 2016).

These similarities illustrate the link between the aroma of toasted oak and beverages aged in contact with it, and thus confirm the relevance of the descriptors generated in this work.

Beyond the release of compounds involved in particular nuances of the aroma of wines and spirits, perceptual interaction phenomena can occur and modulate the contribution of oak wood to the overall aroma. Indeed, it has been shown that some volatile compounds responsible for oak wood aroma can modulate the fruity aroma of red wines through perceptual interactions. Such is the case for 2-furfurylthiol (roasted) and whisky lactone (coconut), which have a masking effect on the perception of fruity

notes, thus reducing the fresh and red-berry-fruit character of red wines (Cameleyre *et al.*, 2020). Thus, the sensory and molecular interpretation of the contribution of barrel ageing to the aroma of wines and spirits is a more complex task than simply extrapolating our knowledge of oak wood aroma.

CONCLUSION

Despite the importance of oak barrel aging for producing quality wines and spirits, knowledge on the aroma of toasted oak wood is lacking. This work sought to fill this knowledge gap by combining sensory and analytical approaches. The results allowed us to produce standardised and precise lexicon for the characterisation of the sensory space of toasted oak wood, and thus to propose a practical tool in the form of an aroma wheel. This aroma wheel may be used in the cooperage industry, and more widely in oenology, for the quality assessment of the sensory properties of oak wood used to make barrels or alternatives, such as staves, chips and sticks. In addition, this sensory work made it possible to highlight descriptors still not characterised from

TABLE 4. Main aroma compounds identified in oak wood.

Oak wood descriptors	Aroma compounds	References
Sawdust	(E)-2-nonenal	(Chatonnet and Dubourdieu, 1998)
Undergrowth	1-octen-3-one	(Chatonnet and Dubourdieu, 1998)
Herbaceous	(Z)-3-hexenal	(Ghadiriasli <i>et al.</i> , 2021)
Coconut	cis/trans-β-methyl-γ-octalactone (cis/trans-whisky lactone)	(Chatonnet, 1995)
Vanilla	vanillin	(Chatonnet <i>et al.</i> , 1991)
Butter	2,3-butanedione	(Culleré <i>et al.</i> , 2013)
Brioche, pastry	(2E,4E,6Z)-nonatrienal	(Courregelongue <i>et al.</i> , 2022)
Nuts	2,6-dimethylpyrazine	(Picard <i>et al.</i> , 2019)
	1-methylpyrrole-2-carboxaldehyde	(Gammacurta <i>et al.</i> , 2021)
Caramel	maltol	(Cutzach <i>et al.</i> , 1997, 1999)
Coffee, cocoa	2-furfurylthiol	(Tominaga <i>et al.</i> , 2000)
	furfural	(Chatonnet <i>et al.</i> , 1999)
Toasted bread/almond	2-ethyl-3-methylpyrazine	(Picard <i>et al.</i> , 2019)
Cloves	eugenol	(Aiken and Noble, 1984)
Medicinal	α-cresol	(Fernández de Simón <i>et al.</i> , 2010)
	quinoline	(Picard <i>et al.</i> , 2019)
Metallic	trans-4,5-epoxy-(E)-2-decenal	(Courregelongue <i>et al.</i> , 2022)
Ashes	guaiacol	(Chatonnet, 1995)
	syringol	(Chatonnet, 1995)

a molecular point of view. The combination of sensory and analytical approaches enabled identification of two volatile compounds in oak wood (*Q. petraea*): thymoquinone with the characteristic smell of pencil and cedar wood, and verbenone with a fresh, menthol and tealeaf odour although the sensory impact of thymoquinone on oak wood aroma appears to be more pronounced than that of verbenone. This contributes to increasing our knowledge of the molecular characteristics of oak wood aroma.

The association of a descriptor with a volatile compound, and vice versa, can facilitate learning and therefore tend to standardise the lexicon used by professionals when describing the sensory properties of toasted oak wood. This would allow oenologists, winemakers, industrial stakeholders, students, and the public to ‘speak the same language’ when discussing the aroma of toasted oak wood, as we await the advent of tools resulting from the fine molecular characterisation of oak wood.

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