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The role of oak wood in the mint and floral notes of whisky: identification of common terpenoids by aromatic fractionation

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Abstract

Understanding the development of whisky aroma during maturation in oak casks provides a rational basis for improvements in product quality. While oak wood is an important contributor to the spicy, toasted, and smoky aromatic notes, limited studies have focused on its role in the floral and mint notes of matured whiskies. However, achieving consistent flavour profiles remains difficult since the odorant molecules are mostly present in low concentration. A semi-preparative ultra-high performance liquid chromatography (UHPLC) method was applied to whisky and oak wood extracts and used to isolate fractions characterised by floral and mint aromas. To investigate the contribution of terpenoids in floral and fresh fragrances, a similar analytical process was applied to solutions of targeted reference compounds. This approach, coupled with gas-chromatography/mass-spectrometry analysis, indicated the presence of terpenoids in the fractions of interest together with selective separation according to chemical structure (i.e., monoterpenes, monoterpenols, ketone monoterpenes, and C13-norisoprenoids). This approach resulted in the detection of, ten and seven new terpenoids in whisky and oak wood. To the best of our knowledge, piperitone, mintlactones, and β-damascone are reported for the first time in whisky and oak wood. Even though raw material, fermentation and distillation processes largely contribute to the presence of terpenoids in new make spirit, this study provides evidence that oak wood enhances floral and mint notes in whisky through chemical and sensory interactions taking place during maturation.

Keywords:
whisky maturation, oak wood, floral and mint aromas, terpenoids

Introduction

Floral, fruity, and woody notes are part of the sensory space that determines the organoleptic quality of fine whiskies (Lee et al. 2001; Wishart 2009). Floral aromas are in high demand in the beverage industry since they are considered as fragrant and delicate (Broom 2014; Jackson 2017). Therefore, cooperages seek to improve the whisky bouquet developed during maturation in oak cask (Nonier et al. 2004; Sefton et al. 1990). Even though numerous aroma active compounds have been found in oak wood (Ghadiriasli et al. 2018), the literature tends to focus on spicy, coconut and smoky flavours of matured whisky (Conner et al. 1993; Piggott and Conner 2003) and little on oak wood derived compounds contributed floral and mint aromas.

In wine, a large variety of monoterpenoid compounds have been identified, including monoterpenols, ketone monoterpenes, and monoterpenes possessing cyclic structures (Mateo and Jimenez 2000; Rocha et al. 2007), as well as C13-norisoprenoids (Black et al. 2015). These have been studied for their contribution to the floral, fruity (citrus), and sweet sensory characteristics of Muscat, Riesling, and Gewurztraminer grape varieties (Rapp 1998; Ribereau-Gayon et al. 1975; Strauss et al. 1986). Recently, p-menthane derivatives such as piperitone and mintlactones were identified in red wine as potent contributors of fresh minty notes (Picard et al. 2016; Picard et al. 2017). Although these compounds stimulate a wide spectrum of aromas - mostly perceived as very pleasant and subtle - there has been little attention to these molecular markers in whisky and oak wood. Specifically, nothing has been reported on the overlapping perception of floral aroma in whisky and oak, with regard to the corresponding molecular profile.

Whisky is a complex matrix from which it is difficult to discriminate a specific aromatic character. The method commonly used for such studies consists of obtaining an aromatic extract in an organic solvent, which is then analysed by gas chromatography–olfactometry (Boothroyd et al. 2014; Poisson and Schieberle 2008). However, due to the palette of aromatic notes in whisky extracts - over 1000 volatile compounds have been identified (Nykänen 1986) - it is difficult to characterise the compounds responsible for specific aromatic properties. Modern sensory science conducted on wine or whisky has suggested the use of High-Pressure Liquid Chromatography (HPLC) preparative method as a relevant tool for isolating targeted aromas in specific fractions (Ferreira et al. 1999; Pineau et al. 2009). The collected fractions can be described by direct olfaction without the problems of toxic solvents since they are obtained after elution of water/ethanol mixtures. This has enabled the identification of compounds responsible for aromatic notes (Picard et al. 2016; Stamatopoulous et al. 2013).

This work characterises new molecular determinant(s) involved in the floral and mint whisky aromas in whisky and those extracted from oak during cask maturation. A three-step analytical approach was used, combining (i) whisky and oak extract fractionation, (ii) sensory descriptive analysis of aromatic fractions, and (iii) GC-MS analyses on fractions of interest. In order to facilitate data interpretation and chemical identification, a targeted pool of terpenoids was studied, and whisky/oak aromatic fractions were analysed in parallel with their homologs from analytical molecular standards.

Materials and methods

Chemicals

Dichloromethane (purity 99.9%) was from Merck (Darmstadt, Germany) and absolute ethanol (99.9 %) from VWR Chemicals (Fontenay- sous-Bois, France). Ultrapure water (18.2 M Ω.cm) was obtained from a Milli-Q purification system (Millipore, Saint-Quentin-en-Yvelines, France), and sodium sulphate (> 99%) was from Sigma-Aldrich (Fontenay-sous-bois, France). All the reference compounds were provided by Sigma-Aldrich (Fontenay-sous-bois, France). Their characteristics (i.e., name, CAS number, purity, aromatic and structural specificities) are listed in Table 1. The use of some enantiomeric

Table 1.

Specificities and physicochemical properties of analytical standards and previous identification in whisky (W) or oak wood (OW).

n°	Compound	CAS number	Purity (%)	Aroma description	Molecular mass (g/moL)	Boiling point (°C) [a]	Specific ions for mass spectrometry detection (*m/z*) [b]	Occurrence in
Monoterpenes								
1	*α*-pinene	7785-70-8	98	herbal, resin-like, fresh	136.2	155	77/ **93**/105/121/136	OW (Ghadiriasli et al, 2018, 2021)
2	*β*-pinene	19902-08-0	99	woody, pine, mint, camphor, resin-like	136.2	166	**93**/121/136	OW (Ghadiriasli et al, 2018)
3	*α*-terpinene	99-86-5	90	woody, citrus, spicy, fresh	136.2	175	**91**/93/105/121/136	OW (Burlacu et al, 2020)
4	terpinolene	586-62-9	90	woody, citrus, pine, floral	136.2	186	79/91/**93**/121/236	OW (Burlacu et al, 2020)
5	limonene	5989-27-5	97	citrus, lemon and orange peel	136.2	177	**68**/93/136	W (Maarse,1983) OW (Ghadiriasli et al, 2018, 2021)
Monoterpenols								
6	*α*-terpineol	98-55-5	90	flowery, citrus, lilac, woody, pine	154.2	219	93/121/**136**/154	W (Nishimura et al, 1983) OW (Ghadiriasli et al, 2018, 2021)
7	*α*-ionol	25312-34-9	90	floral, violet, iris, woody	194.3	127	95/109/123/**138**/194	W (Câmara et al, 2007)
8	1,8-cineole	470-82-6	99	eucalyptus, herbal, camphor	154.2	176	81/108/139/**154**	OW (Ghadiriasli et al, 2018, 2021)
9	geraniol	106-24-1	97	flowery, rose, citrus	154.2	227	**69**/123/154	W (Oller-Ruiz et al, 2017) OW (Ghadiriasli et al, 2018, 2021)
10	linalool	78-70-6	97	flowery, rose, citrus, woody	154.2	197	**71**/93/121/154	W (Câmara et al, 2007) OW (Ghadiriasli et al, 2018, 2021)
11	nerol	106-25-2	97	flowery, rose, hyacinth, magnolia	154.2	226	**69**/93/121/139/154	OW (Ghadiriasli et al, 2018, 2021)
12	borneol	464-45-9	99	woody, pine, camphor	154.2	211	**95**/110/139/154	W (Nishimura et al,1983) OW (Ghadiriasli et al, 2018, 2021)
13	*β*-citronellol	106-22-9	95	flowery, citrus, citronella, rose	156.3	221	69/81/**95**/123/138/156	W (Gonzalez-Robles, 2018) OW (Ghadiriasli et al., 2018, 2021)
14	thymol	89-83-8	99	woody, spicy, camphor, herbal	150.2	232	91/**135**/150	W (Masuda et al, 1966) OW (Ghadiriasli et al, 2018, 2021)
Ketone monoterpenes and *p*-menthane lactones								
15	carvone	6485-40-1	99	mint, herbal, spicy	150.2	231	54/**82**/93/108/150	OW (Ghadiriasli et al, 2018, 2021)
16	menthone	10458-14-7	97	peppermint	154.2	207	69/139/**112**/154	W (Rodrigues et al, 2008)
17	piperitone	89-81-6	92	peppermint, spicy, herbal, fresh	152.2	233	82/95/**110**/137/152	-
18	pulegone	89-82-7	90	peppermint, herbal, camphor	152.2	224	81/109/137/**152**	W (Rodrigues et al, 2008)
19	mintlactones	13341-72-5	99	mint, coconut	166.2	304	109/123/137/**166**	-
C13-norisoprenoids								
20	*α*-ionone	127-41-3	90	flowery, violet, raspberry	192.3	258	93/109/**121**/136/192	W (LaRoe and Shipley, 1970)
21	*β*-ionone	14901-07-6	96	flowery, violet, berry, woody	192.3	271	**177**/192	W (LaRoe and Shipley, 1970) OW (Nonier et al, 2004)
22	*β*-damascenone	23696-85-7	98	cooked apple, rose, woody	190.3	275	105/121/175/**190**	W (Câmara et al, 2007) OW (Ghadiriasli et al, 2018, 2021)
23	*β*-damascone	23726-91-2	90	flowery, menthol, rose, currant, fruity	192.3	200	69/123/**177**/192	-

[a] obtained at 760 mm Hg. [b] Quantifier ions are in bold. (-): not previously identified

pure standards (i.e. (+)-(R)-α-pinene **1**, (+)-(R)-β-pinene **2**, (+)-(R)-limonene **5**, (-)-(S)-borneol **12**, (-)-(R)-carvone **15**, and (+)-(R)-pulegone **16**) is based solely on their availability from commercial sources and the analytical results presented here do not prejudge either the enantiomeric nature nor the corresponding relative abundance of the enantiomeric species detected in the samples. The mintlactones **19** reference standard corresponded to a mixture containing the two diastereoisomers (-) -(6R,7aR)-mintlactone **19a** and (+) -(6R,7aS)-isomintlactone **19b** at a ratio 20:1. Deuterated internal standards α-terpineol-d3 (99.8%) and carvone-d3 (99.3%) were purchased from Cluzeau Info Labo (Sainte-Foy-La-Grande, France).

Whisky and oak samples

The analyses were conducted on whisky and a wood solution. The whisky sample was taken from a commercial three-year old Scotch single malt whisky (46% ABV), exclusively matured in a new French *Q. petraea* oak cask from Jupilles forest (France) provided by Demptos cooperage (Saint-Caprais-de-Bordeaux, France). The wood had a thin and regular grain typical of sessile oak (*Quercus petraea*), was air-seasoned for 24 months, and used to prepare 225 L Bordeaux style casks. It was submitted to a medium toast (170-180°C) by heating over a variety of braziers according to the cooper's skills. The toasted oak sample (10 g) was taken by scrapping the wood from the inner side of the cask. The sample was extracted in 200 mL hydroalcoholic solution (50% ethanol) over 12 h at room temperature with magnetic stirring and was then filtered. To minimise the formation of an emulsion during the liquid extraction from an elevated ethanol concentration, 50 mL of whisky or wood solution was diluted in Milli-Q water to a final volume of 100 mL with an ethanol content equal or below 25% (v/v).

Analytical standards

Four stock solutions of analytical compounds were prepared at 1g/L in ethanol. Reference compounds were mixed according to chemical family (i.e. solution of monoterpenes: compounds **1** to **5**; monoterpenols: compounds **6** to **14**; ketone monoterpenes and p-menthane lactones: compounds **15** to **19**; C13-norisoprenoids: compounds **20** to **23**, Table 1). Stock solutions of the standards were stored in darkness at 4°C for one month. Prior to solvent extraction, stock solutions were diluted in hydroalcoholic solution (100 mL at 20% ABV) to obtain a final concentration at 50 mg/L.

Aromatic extraction

Each sample (whisky, wood solution, reference compounds) was extracted three times at room temperature (20°C) using 10, 5, and 5 mL of dichloromethane with magnetic stirring (500 rpm) each for 5 min. The organic phases were separated, collected, dried over sodium sulphate, and concentrated using rotary evaporation to 250 µL raw extract.

Aromatic raw extract fractionation

Reverse-phase liquid chromatography was performed on each extract using a Syncronis C18 column (250 × 10 mm internal diameter, (ID.), 5 µm, Thermo Fisher Scientific, CA, USA) with a guard column of the same phase. The Ultimate 3000 semipreparative HPLC system was from Dionex, ThermoFisher Scientific (CA, USA). The procedure was based on the method by Lytra et al (2012) with slight modifications. Chromatographic conditions were as follows: flow rate, 2 mL/min; injection volume, 250 µL; gradient, eluent A, microfiltered water, eluent B, ethanol; 0–2 min, 0% B, 2–50 min, 0–45% B. Fifty fractions of 2 mL exhibiting various aromas were obtained in dilute alcohol solution before their subsequent sensory characterisation. A diode-array detector in the 200–400 nm range was used for the identification of reference compounds in the fractions of standard solutions.

Selection of fractions by sensory analysis

Three panellists were selected based on their availability, sensory acuity, and ability to recognise the aromas of the sought after descriptors. They were also familiar with the aromas commonly found in whisky and oak matrices and were experienced in sensory descriptive studies. They were invited to freely describe the aromas of the 50 fractions

obtained from whisky and oak samples using their own vocabulary. The aim of this phase was to identify and select the fractions most representative of aromatic fractions bearing floral notes prior to their chemical analysis.

Aromatic fraction re-extraction

Fractions of interest were pooled, diluted in ultrapure water to obtain 20% ethanol (v/v) and re-extracted three times with 5 mL dichloromethane (stirring for 5 minutes at 700 rpm). Organic layers were combined, dried over sodium sulphate, and concentrated by rotary evaporation to a final volume of 250 µL before GC-MS analyses.

Gas chromatography–mass spectrometry

GC-MS analyses were carried out using a Trace GC Ultra system (Thermo Fisher Scientific, CA, USA) coupled to an ISQ quadrupole mass spectrometer, equipped with a Triplus autosampler (Thermo Fisher Scientific). 2µL organic extract of selected fractions was injected on an OptimaWax plus polar capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness, Macherey-Nagel, Düren, Germany). The carrier gas was helium N55 at a constant flow of 1.2 mL/min. The oven temperature was raised from 40 (1 min) to 260°C at a rate of 10°C/min (final isotherm for 7 min). The ion source was set at 200°C and the transfer line between GC and MS at 250°C. MS identification was carried out in electron ionisation (70 eV), either in SCAN mode for fractions from standard solutions or in SIM mode for fraction from whisky and oak solution samples. Detection was based both on the retention time of each reference compound and the selection of specific ions (Table 1). Linear Retention Indices (LRI) for reference compounds were obtained by simultaneous injection of samples and a series of alkanes (C8–C40; Sigma- Aldrich, St Quentin Fallavier, France), according to the procedure described by Kovats (1958). To confirm the identification of these compounds in oak and whisky matrices, the same analytical procedure was also performed on an Optima 5-MS apolar capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness: Macherey-Nagel, Düren, Germany).

Preliminary quantification of terpenoids in whisky and oak wood

To obtain an initial quantification of compounds **1**–**23** in both whisky and oak wood, 20 µL of the internal standards solution (mixture of α-terpineol-d3 and carvone-d3, 100 mg/L in alcoholic solution) were added to a 200 mL whisky or oak wood solution, diluted to 20% ABV. The liquid extraction was performed as described above and 2 µL of the final raw extracts were injected on the polar capillary column, using the same analytical conditions as the identification procedure. Compounds 1-23 were quantified in SIM mode with specific MS fragment ions (Table 1) using either α-terpineol-d3 (for monoterpenes and monoterpenols), or carvone-d3 (for ketones monoterpenes, p-menthane lactones and C13-norisoprenoids) as internal standard. The internal standards were identified with ions at m/z 62, 93, 124, and 139 for α-terpineol-d3; m/z 56, 84, 95, 110, and 154 for carvone-d3. Based on the best measured signal-to-noise ratio, the ions chosen for quantification were those at m/z 62 and 84 for α-terpineol-d3 and carvone-d3, respectively.

Calibration curves were prepared in 100 mL dilute alcohol solution (20% ABV), using the extraction procedure described above. Concentrations ranged from 0.2 to 100 µg/L, and 10 µL of internal standards solution (mixture of α-terpineol-d3 and carvone-d3, 100 mg/L in alcoholic solution) were added. The calibration curves were plotted as the relative peak areas (analyte versus internal standard) as a function of concentration. The functions were linear over these concentration ranges, with coefficients of determination (R^2) above 0.99 for all the compounds. Limit of Detection (LOD) and Quantification (LOQ) were obtained from the calibration line, at low concentrations and using the following formula: LOD = 3Sa/b and LOQ = 10Sa/b, where Sa is the standard deviation of the response, and b the slope of the calibration curve (Shrivastava et al, 2011).

Results and discussion

Sensory description of UHPLC fractions from whisky and oak wood

The assessors used direct olfaction to assess the aromatic characteristics of the 50 fractions extracted from whisky or oak wood. Different kind of odours were detected in 27 fractions while no odour was perceived in the remaining 23 (i.e., fractions 1 to 17 and 45 to 50). Some fractions with a similar aromatic description between whisky and oak wood were reported (Table 2). Fractions 18 and 19 presented strong vanilla, coconut and smoky notes. Both whisky and oak wood (fractions 23, 24, 25 and 29) were characterised by spicy, smoky and woody notes. These results were not surprising considering the broad impact of maturation in wood casks on the spirit aromatic profile (Slaghenaufi et al, 2016). The fractionation of some reference compounds by nose, UV, and MS detection highlighted the presence of whisky lactones in fractions 18-19 and 24-25, guaiacol in 18-19, 4-vinylguaiacol in 22-23, 4-methylguaiacol and eugenol in 24, and megastigmatrienone (tabanone) in fractions 29-30.

Floral and minty notes were found in 13 fractions from whisky and 14 from oak wood (Table 2). Interestingly, 11 floral and mint fractions overlapped between the two matrices. This common aromatic feature made it possible to underline the role of oak wood in the overall floral and mint perception of whisky, suggesting a direct extraction of chemical compounds related to this kind of aromatic notes from the wood into the whisky during the maturation process.

Preliminary study on key fractions of terpenoid standard solutions

Several reference compounds were fractionated using the same analytical procedure as for whisky and oak samples. It was postulated that, if reference compounds were detected in the aromatic fractions of interest, they were probably also present in the same fractions collected from whisky and oak. Further, this strategy may provide information concerning the chemical structures involved in the fractions of interest. Twenty-three terpenoids were chosen according to their known contribution to the floral and mint character of whisky or oak wood (Table 1). They belonged to monoterpene, monoterpenol, ketone monoterpene, and C13-norisoprenoid chemical families. Although little attention has been paid to these compounds in whisky and oak matrices, their influence in the aromatic expression of wine during the vinification and storage processes has been described (Black et al. 2015; Mateo and Jimenez 2000; Mendes-Pinto 2009). Moreover, piperitone **17** and mintlactone **19** were not previously identified in either whisky or oak. However, their role in the freshness and mint aromas of fine aged red wines has been reported (Picard et al. 2017; Picard et al. 2016). These compounds are generated from the metabolism of limonene biotransformation, which was studied in mint species (Mahmoud and Croteau 2003) and recently in wine (Picard et al. 2018). Considering that limonene and other secondary metabolites (carvone **15**, menthone **16**, pulegone **18**) were previously reported in whisky and/or oak wood (Ghadiriasli et al. 2018; 2021; Rodrigues et al. 2008), it may be assumed that piperitone **17** and mintlactones **19** are also present.

The selection of fractions from the standard solutions was assessed by sensory analysis and UHPLC-UV detection. The selected fractions were then analysed by GC-MS in SCAN mode to confirm the presence of targeted compounds. The results showed the reference compounds to be eluted according to their chemical properties, with p-menthane lactones from fractions 20 to 22, ketone monoterpenes from 25 to 32, monoterpenols from 30 to 32 and 35 to 36, C13-norisoprenoids from 33 to 35, and monoterpenes in fractions 43 to 44 (Table 3). Moreover, a comparison of fractions from reference standards with their counterparts in whisky and oak samples showed that fractions exhibiting floral and mint aromas in whisky and oak matched those reference compounds. These findings suggest that both whisky and oak targeted fractions present similar chemical features. Therefore, terpenoids should be considered as potentially important contributors to floral and mint aromas. The identification of a specific elution order according to the nature of the terpenoids also provided insight to enable the chemical characterisation of selected fractions.

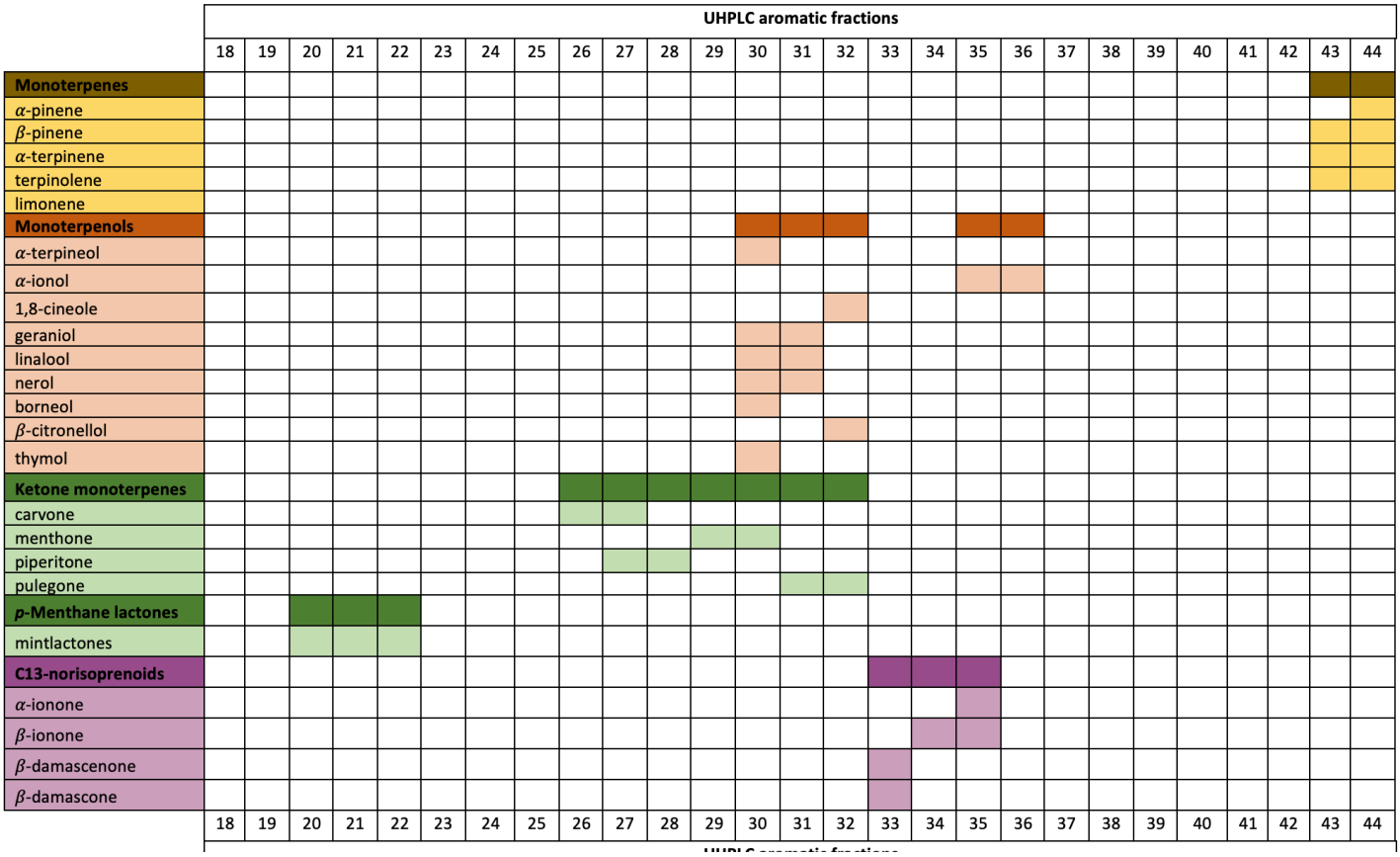
Table 2.

Olfactive description of HPLC aromatic fractions from whisky and oak wood. Fractions where no aromas were detected (1 to 17, 45 to 50) are omitted.

Aromatic Faction	Whisky	Oak
18	vanilla, higher alcohols	vanilla, smoky
19	fruity, metallic, coconut	coconut, smoky, vanilla
20	**light floral**, herbal, vegetal	milky, herbal, **mint**
21	fruity**, floral**, vanilla	**floral**, sweet
22	**floral,** sweet	**floral,** herbal, smoky, spicy
23	**floral**, smoky	woody, spicy
24	coconut, fresh wood, fruity	woody, leather
25	coconut, fruity	woody, **floral, rose**
26	**floral, citrus**	**fresh, floral, white flowers, jasmine**
27	**floral, fresh, menthol**	**floral, fresh**
28	**fresh, menthol**, fruity, **camphor**, vegetal	**floral, mint,** spicy
29	fruity, woody, spicy	spicy, cloves
30	fruity, sweet	fresh, pastry, sweet
31	fruity, spicy	**rose, fresh**
32	fruity, peach, pencil-like, metallic	pencil-like
33	**fresh, mint**, cooked apple	pencil-like
34	fresh, citrus	**floral, rose**
35	fresh	rubber-like
36	**floral**, citrus	**floral**, milky
37	fruity, vegetal, nuts	fresh
38	nuts, rancio	spicy
39	nuts, hazelnut	-
40	rancio	fresh
41	**floral,** citrus	**floral**
42	**floral,** citrus, **fresh**	**floral**
43	**floral**, citrus, **fresh**	**floral**
44	**floral,** lemon, **fresh**	**floral, pine,** woody

Table 3.

Order of elution of aroma compounds in the HPLC aromatic fractions from standard solutions (detection by UV-DAD and confirmation by MS).



Identification of new terpenoid derivatives in whisky and oak wood

Key aromatic fractions obtained from whisky and oak wood were combined in different pools for GC-MS analysis: fractions 20-22, 26-28, 29-33, 33-36, and 43-44. Each aromatic fraction pool was analysed in SIM mode using specific ions of the targeted molecules (Table 1). The presence of terpenoids previously identified in whisky or in oak was validated by comparison of the retention times and the mass spectra with the reference compounds (data not shown). Specific attention was paid to compounds that have not previously been detected in the two matrices. These included in whisky, α-pinene **1**, β-pinene **2**, α-terpinene **3**, terpinolene **4**, 1,8-cineole **8**, nerol **11**, and carvone **15**; oak wood, α-ionol **7**, menthone **16**, pulegone **18**, and α-ionone **20**; in both whisky and oak wood, piperitone **17**, mintlactones **19**, and β-damascone **23** (Tables 1 and 4). GC–MS analyses of the different aromatic pooled fractions from whisky or oak wood were conducted in SIM mode, while specifically monitoring for fragment ions of each targeted compound at the corresponding m/z values. Chromatographic peaks were clearly detected at the specific Linear Retention Indices on polar and apolar columns. The agreement in LRI was also confirmed by overloading the corresponding fractions with the pure reference compounds. In addition to the polar and non-polar LRI conformities (Table 4), comparison of the mass spectral characteristics for the compounds detected either in whisky or oak fractions with those obtained for the reference compounds confirmed the presence of 10 and seven new isoprenoid derivatives in whisky and oak wood. Total Ion Chromatograms (TIC) obtained from oak wood for α-ionol **7** and mintlactones **19** (mintlactone **19a** and isomintlactone **19b**), or whisky for β-damascone **23**, are reported in Figures 1, 2, and 3. Given the importance of terpenoids in wine, any contribution from ex-wine casks on the profile of whisky terpenoids can be ruled out as a new oak cask were used in this work for whisky maturation.

Determination of the quantitative terpenoid profile in whisky and oak wood

Terpenoids were quantified in the whisky and oak samples (Table 5). In both samples, α-pinene **1** and limonene **5** were the most abundant monoterpenes (1.36 and 1.03 µg/L in whisky, 66 and 27 ng/g in oak wood). The terpenoid family with the highest concentrations in whisky were the monoterpenols, with α-terpineol **6** (36.4 µg/L) and linalool **10** (45.2 µg/L) found at the highest concentrations and representing 82% of the monoterpenols. Also present were geraniol **9** (14.2 µg/L), nerol **11** (11.0 µg/L) and β-citronellol **13** (11.2 µg/L) (Table 5). In oak wood, borneol **12** was present at highest concentrations (243 ng/g), followed by other monoterpenols. Despite the semi-quantitative data for monoterpenols and monoterpenes in whisky (Câmara et al. 2007) or in oak, (Jordao et al. 2006) this is the first time that these compounds have been quantified. Although identified, β-pinene **2**, α-terpinene **3**, α-terpineol **6** and 1,8-cineole **8** could not be quantified in oak and whisky as the concentrations were below the LOQ.

Several optimisations were required to improve the sensitivity of the analytical method. Within the ketone monoterpenes and p-menthane lactones series, piperitone **17** and mintlactones **19** were the most representative in both matrices. This was not surprising as piperitone **17** and mintlactones **19** are end-products of the limonene biotransformation pathway, while the other compounds are considered as intermediates (Mahmoud and Croteau 2003; Frerot et al. 2002). To our knowledge, this is the first time that such compounds were quantified in both matrices. Interestingly, most of the concentrations in whisky were up to several µg/L, and significantly higher than in wine (Picard et al. 2018). The ratio of mintlactone to isomintlactone was around 1:15 and 1:4 in whisky and oak respectively, opposed to the ratios reported in peppermint oil (10:1) and in wine (14:1) (Picard et al. 2017; Takahashi et al. 1980).

Monoterpenoids are important secondary metabolites released by plants and are diverse chemically (Dudareva et al. 2006). Other than their odour activity, these compounds exert multiple functions, including defence against insect attack

Figure 1.

GC–MS chromatogram of pooled fractions (33-36) obtained from oak extract on an OptimaWax-Plus capillary column. Identification of α-ionol **7** based on mass spectrometry data acquired in SIM mode on the four main fragment ions (m/z 194, 138, 123, and 95) and mass spectra of the corresponding reference compound obtained in SCAN mode (only molecular ion at m/z 194 is shown).

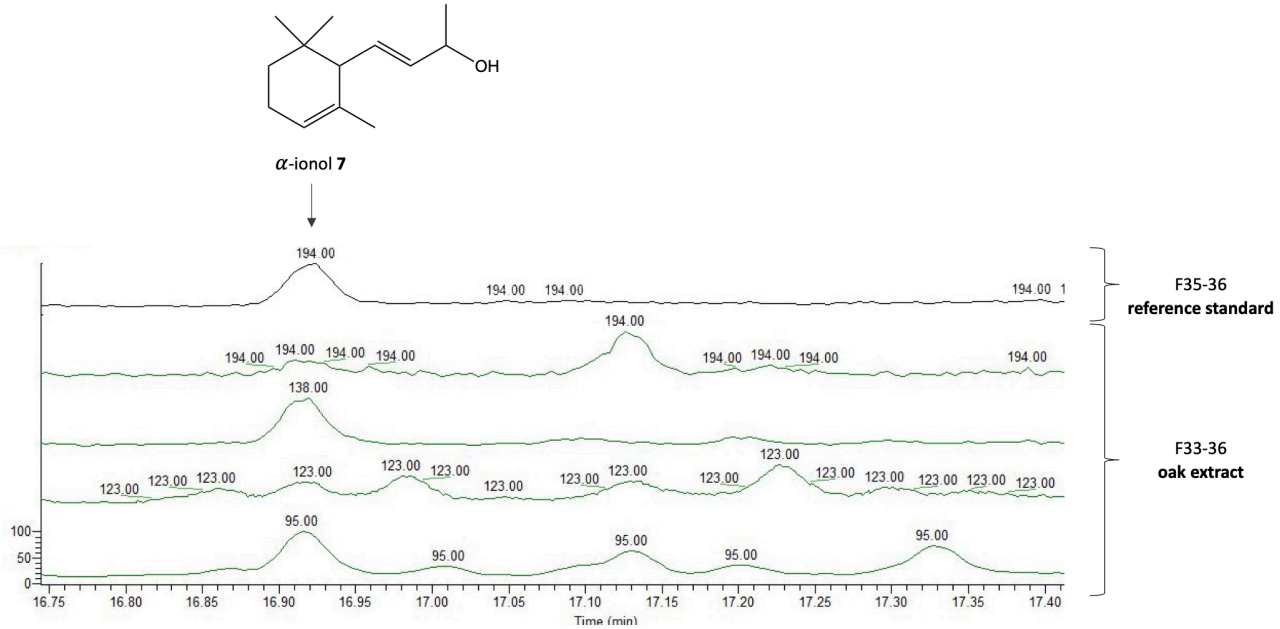


Figure 2.

GC–MS chromatogram of pooled fractions (20-22) from oak extract on an OptimaWax-Plus capillary column. Identification of mintlactone **19a** and isomintlactone **19b** based on mass spectrometry data acquired in SIM mode on the five main fragment ions (m/z 166, 137, 109, 95, and 81) and mass spectra of the corresponding reference compounds obtained in SCAN mode (only molecular ion at m/z 166 is shown).

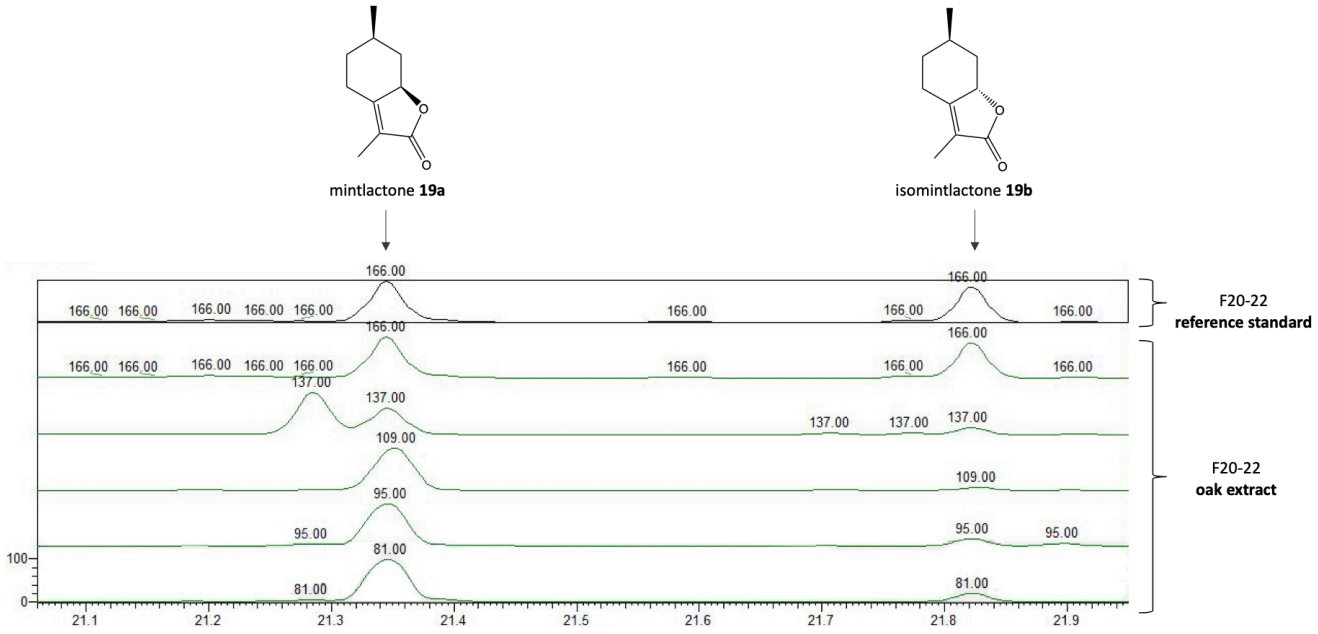


Figure 3.

GC–MS chromatogram of pooled fractions (33-35) obtained from whisky extract on an OptimaWax-Plus capillary column. Identification of β-damascone **23** based on mass spectrometry data acquired in SIM mode on the three main fragment ions (m/z 192,177, and 69) and mass spectra of the corresponding reference compound obtained in SCAN mode (only molecular ion at m/z 192 is shown).

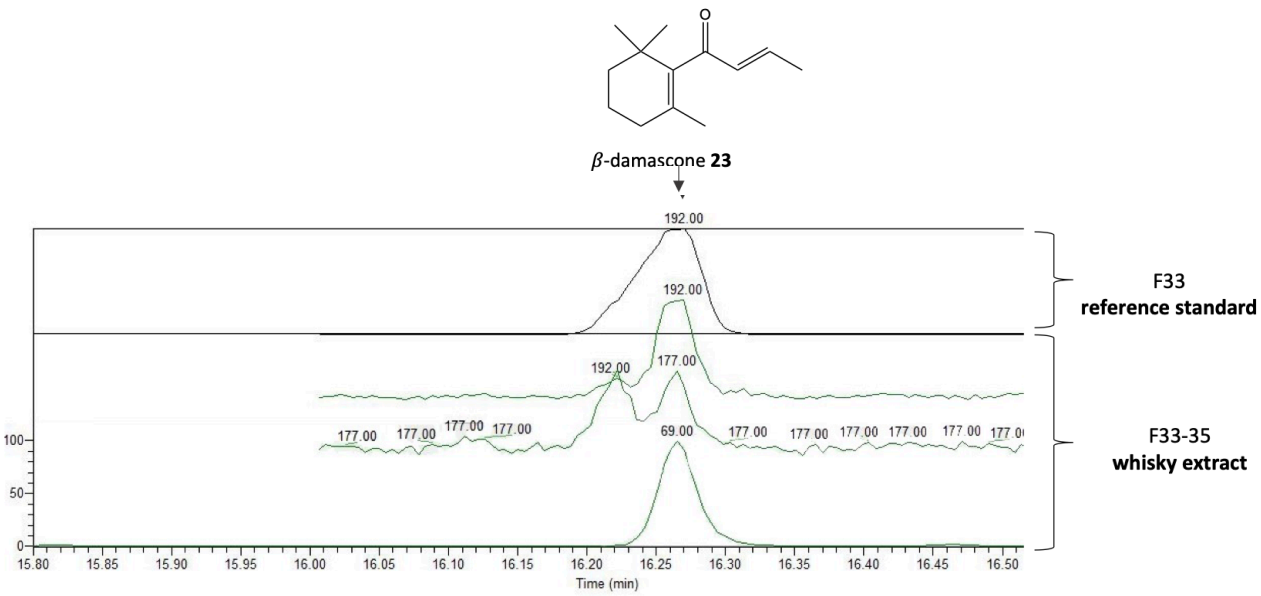


Table 4.

Linear Retention Indices (LRI) of new terpenoids identified in whisky and oak wood on polar (OptimaWax-Plus) or apolar (Optima 5-MS).

	Pool of aromatic fractions	Compound	LRI on polar column*	LRI on apolar column*	LRI literature	
					polar	*apolar*
WHISKY	20-22	mintlactones **19**	2355 and 2405	1527 and 1551	2368 and 2422[a]	1522 and 1546[a]
	26-28	carvone **15**	1768	1263	1715[b]	1242[c]
		piperitone **17**	1746	1267	1739[b]	1251[c]
		1,8-cineole **8**	1217	1040	1214[d]	1030[e]
	29-33	nerol **11**	1789	1215	1753[d]	1228[f]
		β-damascone **23**	1827	1420	n.a	1409[f]
		α-pinene **1**	1011	943	1027[g]	939[f]
	43-44	β-pinene **2**	1119	992	1113[g]	980[f]
		α-terpinene **3**	1187	1030	1223[h]	1018[f]
		terpinolene **4**	1288	1094	1283[i]	1088[f]
OAK	20-22	mintlactones **19**	2357 and 2409	1527 and 1551	2368 and 2422[a]	1522 and 1546[a]
	26-28	piperitone **17**	1747	1267	1739[b]	1251[c]
	29-32	menthone **16**	1478	1160	1470[b]	1150[j]
		pulegone **18**	1681	1246	1662[b]	1237[c]
		α-ionol **7**	1892	1390	1923[k]	n.a
	33-36	α-ionone **20**	1861	1436	n.a	1426[c]
		β-damascone **23**	1831	1419	n.a	1409[f]

* Reference compound LRIs were superimposable on the detected peaks; n.a: not available

[a] on polar BP21 and apolar HP-5MS columns (Picard et al, 2017); [b]on polar Carbowax column (Davies, 1990); [c] on apolar HP5-MS column (Pino et al, 2005); [d] on polar DB-Wax column (Nishimura, 1995); [e] on apolar HP5 column (Jordan et al, 2002); [f]on apolar DB5 column (Adams, 2007); [g] on polar DB-Wax column (Högnadóttir and Roussef, 2003); [h] on polar Supelcowax-column (Schieberle and Grosch, 1988); [i] on polar DB-Wax column (Khan et al, 2012);); [j] on apolar HP5 column (Leffingwell and Alford, 2005); [k] on polar Stilwax column (Klesk, 2004)

Table 5.

Concentration of terpenoids in whisky and oak wood samples.

n°	Compound	Analytical parameter				Concentration	
		R^2	slope	LOD (ng/L)	LOQ (ng/L)	whisky (ng/L)	oak wood (ng/g) [a]
Monoterpenes						**2742**	**100**
1	*α*-pinene	0.998	0.341	162	539	1 360	66
2	*β*-pinene	0.996	0.356	342	1140	<LOQ	<LOQ
3	*α*-terpinene	0.993	0.358	336	1120	<LOQ	<LOQ
4	terpinolene	0.990	0.345	53	177	350	7
5	limonene	0.994	0.307	47	158	1 032	27
Monoterpenols						**125 663**	**540**
6	*α*-terpineol	0.998	0.138	280	940	36 378	<LOQ
7	*α*-ionol	0.991	0.012	120	430	452	35
8	1,8-cineole	0.999	0.110	53	177	<LOQ	<LOQ
9	geraniol	0.994	1.198	175	585	14 180	58
10	linalool	0.993	0.389	234	782	45 237	37
11	nerol	0.994	0.614	305	1018	11 016	66
12	borneol	0.994	1.039	188	629	6 397	243
13	*β*-citronellol	0.995	0.183	86	316	11 226	13
14	thymol	0.996	0.976	187	559	677	88
Ketone monoterpenes and *p*-menthane lactones						**43 550**	**617**
15	carvone	0.993	0.033	284	946	8 300	82
16	menthone	0.991	0.405	74	215	500	5
17	piperitone	0.992	0.445	264	882	19 200	195
18	pulegone	0.999	0.280	191	639	1 400	14
19a	mintlactone	0.998	0.227	174	715	914	67
19b	isomintlactone	0.992	0.079	45	149	13 237	254
C13-norisoprenoids						**4 567**	**146**
20	*α*-ionone	0.995	0.546	204	681	1 010	29
21	*β*-ionone	0.995	0.579	201	672	920	108
22	*β*-damascenone	0.990	0.058	71	253	2287	5
23	*β*-damascone	0.995	0.327	62	206	350	4

[a] concentration in oak wood sample were firstly determined in the liquid extract in ug/L, and then converted in ng/g of dry wood.

(Michelozzi 1999). They are formed from the association of isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP), which are then converted to geranyl pyrophosphate (GPP). Terpene synthases, key enzymes in terpenoids biosynthesis, then catalyse rearrangements and cyclisation producing a wide variety of monoterpene end-products (Black et al. 2015). Oak *Quercus* species are one of the strongest emitters of monoterpenoids (Loreto et al. 2002), and several terpene synthase genes coding for these enzymes have been identified (Creyaufmüller et al. 2018; Loreto et al. 2009). Despite this they do not contribute to aroma directly. Monoterpenoid glycosides identified in plants have been recognised as important aroma precursors since they release the volatile aglycone via enzymatic and acid hydrolysis. Glycosylation of monoterpenoids in grape berries and their transformation during the wine making has been extensively studied (Black et al. 2015; Hjelmeland et al. 2015; Mateo and Jimenez 2000). Interestingly, glycosidic bound volatiles were also found in oak wood (Nonier et al. 2005), suggesting the presence of monoterpenoids in their conjugated and odourless form.

During bottle aging of wine, oxidation, hydrolysis, and chemical rearrangements in acidic conditions occur simultaneously, leading to changes in the terpenoids profile. For example, the conversion of linalool and nerol into α-terpineol, terpinolene and limonene has been reported (Marais 1983; Rapp and Mandery 1986; Slaghenaufi et al. 2018).

Other studies on tequila products reported the concentration of linalool, α-terpineol, and β-citronellol increased during cask maturation (González-Robles et al. 2016). Moreover, the synthetic mechanism of ketone monoterpenes and *p*-menthane lactones from limonene in peppermint integrates successive oxydo-reduction steps (Mahmoud and Croteau 2003). Given the occurrence of terpenoids in oak wood, either in free or bound forms, its porosity (Vivas et al. 2019), decrease in pH over time and influence of the redox state on whisky aroma (Reazin 1981). Further investigations are required to study the formation of these volatile compounds during maturation. The concentration of C13-norisoprenoids in whisky ranged from 330 ng/L for β-damascone **23** to 2.3 µg/L for β-damascenone **22**. Only β-damascenone has previously been quantified (at similar levels) in whisky (Poisson and Schieberle 2008). In oak, β-ionone was the most abundant among the norisoprenoids (108 ng/g of dry wood: Table 5). Although some of these compounds have previously been detected in trace amounts in oak (Sefton et al. 1990), no quantitative data has been published. C13-norisoprenoids are formed as biodegradation products of carotenoids initially in oak (Masson et al. 1997). The proposed mechanisms for the formation of norisoprenoids from the parent carotenoids include two types of reactions: (i) enzymic, catalysed by a dioxygenase enzyme or a breakdown of stored glycosides by glycosidase enzymes, and (ii) non-enzymic, involving one or several steps of carotenoid degradation, stimulated by light, oxygen, temperature, and acid hydrolysis (Mendes-Pinto 2009; Typ et al. 1999).

Differences in the profile of terpenoids were observed in whisky and oak, with some compounds found only in whisky (α-terpineol **6**) or present at higher concentrations than in oak. Although the cask contributes to an enrichment of whisky by direct extraction from oak, these findings suggest that the terpenoid concentrations in whisky not only reflect maturation but are also modulated by upstream parameters, including the raw material (Buśko et al. 2010) and the fermentation and distillation processes (Esteban-Decloux et al. 2022; Hock et al. 1984).

From a sensory standpoint, the complexity of aroma in alcoholic beverages results from the synergy between aroma compounds and, depending on their chemical structure, their concentration, and ethanol content. It has previously been demonstrated that terpenoids interact such that threshold of the mixture is lower than those of individual terpenoids (Ribéreau-Gayon et al. 1975). The enhancement of fruity aroma by C13-norisoprenoids (β-damascenone and β-ionone) was also seen in wine, suggesting interactions with esters (Escudero et al. 2007). Similarly, sensory experiments in a Cognac model solution, containing a mix of monoterpenoids at 40% ABV, showed that the supplementation of β-damascenone accentuated the perception of floral aromas (Thibaud et al. 2020).

This finding confirmed that sensory synergism is perceived even at high ethanol content. Accordingly, similar behaviour in a whisky matrix could be expected and sensory analyses are required to identify the organoleptic impact of these newly identified compounds. Based on these preliminary quantitative results, further analyses should be performed on whisky and oak wood samples to define the contribution of terpenoids to the floral and mint notes of the whisky bouquet developed during maturation.

Conclusions

The fractionation methodology used in this study showed the presence of similar floral and mint notes in whisky and oak wood. It also provided complementary information about the related molecular profiling, with the identification of new terpenoid derivatives in whisky and/or oak wood. These findings provide new evidence that oak wood contributes to the persistence of freshness perceived in mature whisky over time. Its role as a potential source of floral and mint aroma compounds in whisky during maturation requires confirmation. Given the complexity of the formation of terpenoids as well as the number of parameters involved (light, oxygen, temperature, water, pH), this study provides stimulation for optimisation of barrel making as well as whisky maturation.

Conflict of interest

The authors declare no competing financial interest.

Author contributions

Magali Picard: conceptualisation, methodology, formal analysis, validation, investigation, supervision, writing (original draft, review and editing), project administration.
Clara Oulieu: investigation, formal analysis, data curation.
Marie-Françoise Nonier: writing (review and editing).
Nathalie Vivas: resources, writing (review and editing).
Nicolas Vivas: investigation, project administration, writing (review and editing).

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