1 # Identification of two new taste-active compounds in oak
2 # wood: structural elucidation of potential *β*-methyl-*γ*-
3 # octalactone precursors and quantification in spirits

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ABSTRACT

Barrel aging is a crucial stage that influences the taste of wines and spirits, particularly increasing their sweetness and bitterness. This increase is caused by non-volatile compounds released from oak wood. To search for such molecules, we performed a taste-guided inductive fractionation protocol using several analytical techniques. By using HRMS and NMR, two new galloylated derivatives were elucidated. Their enzymatic hydrolysis revealed the formation of *β*-methyl-*γ*-octalactone, indicating that they are potential precursors. The taste properties of these isomers revealed a sweet and bitter taste for P-WL-1 and P-WL-2, respectively. An LC-HRMS quantification method was performed to evaluate the influence of ageing parameters such as botanical origin and toasting process on their concentrations. Several spirits were also analysed to confirm their presence in this matrix. These results improve the understanding of the molecular markers responsible for the taste of beverages.

Key words: taste-active compounds; oak wood; quantification; lactone; precursors.

INTRODUCTION

The quality of wines and spirits, as defined by the pleasure they give to the taster, depends on the complexity and harmony of their aromas and tastes[1]. This quality is perceived through the sensory detection of various chemical components, followed by the neurophysiological and cognitive processing of these stimuli[2].

Oak wood has been used for centuries in oenology and the elaboration of wines and spirits since it significantly enhances the profiles of these beverages. Oak barrels are traditionally crafted from species such as *Quercus petraea* (sessile oak), *Quercus robur* (pedunculate oak), and *Quercus alba* (American white oak), which play a key role in their organoleptic properties. Numerous studies have focused on the identification and quantification of aromatic substances imparted to wines and spirits by barrel aging. A number of volatile molecules contributing to the woody character of wines are now known[3]. These key compounds include vanillin (vanilla note), volatile phenols like eugenol (clove note), guaiacol (smoky aroma) and *trans*- and *cis*-β-methyl-γ-octalactone (known as whisky-lactone), which imparts a coconut scent[4]. Cooperage parameters, such as the botanical and geographical origin of the oak and wood aging conditions, are known to influence the concentration of these main molecules[5–8]. For instance, oak lactones are released in significant quantities through the seasoning and toasting of staves[9]. Thanks to pyrolysis and the enzymatic activity that occurs during barrel production, these lactones can result from glycoconjugated derivatives such as β-D-glucopyranoside (glucoside) and galloyl-β-D-glucopyranoside (galloyl glucoside) of 3-methyl-4-hydroxyoctanoic acid [10–12].

These compounds are not directly involved in the taste of wines and spirits. In fact, in a tasting of oak wood extract, the precursor galloyl glucoside was described as tasteless by Marchal *et al.*[13]. However, winemakers often assert that oak barrels aging imparts a sweetening effect, a phenomenon similarly observed in the maturation and overall quality improvement of spirits.

Plants are known naturally to contain taste-active molecules, which mainly exhibit sweetness but may also express bitterness. This suggests that taste-active compounds may be present in oak wood and that they are released into wines and spirits during the aging process. The recent discovery of quercotriterpenosides, i.e. galloylated and glucosylated triterpenoids from oak wood, has shown that they are involved in the perception of the sweetness perceived during maturation[13]. On the other side of the taste balance, lyoniresinol, an oak lignan, is a phenolic compound known for its bitter properties[14]. The sensory importance of bitter coumarins has also been highlighted in recent studies on wines and spirits, emphasizing their perceptual interactions[15]. Despite significant progress, however, the molecular mechanisms underlying taste modifications due to oak aging remain partially unexplored.

The quest for taste-influencing compounds has driven the development of various analytical methods[16–19]. Notably, inductive techniques incorporating taste-guided fractionation protocols have been applied to wines and spirits. This approach implemented in our laboratory[13,20,21] hinges on selecting the appropriate raw extract and the accuracy of sensory analysis. Moreover, access to effective analytical techniques is essential for purifying and identifying trace active molecules in a complex matrix, as they are often isolated on the basis of their biological activity rather than their chemical structure. Centrifugal partition chromatography (CPC) stands out for its ability to fractionate complex extracts without irreversible adsorption to the stationary phase, which means that large quantities of samples can be injected.

This study aimed to deepen our understanding of how oak wood influences the taste profiles of aged wines and spirits by identifying and quantifying new taste-active compounds, sweet or bitter, in sessile oak wood. Advanced extraction and chromatographic techniques, such as liquid-liquid extraction, CPC, Preparative-HPLC, HRMS and NMR, were used to elucidate the structural characteristics of these compounds. Beyond the gain in fundamental knowledge, such studies could have practical implications and lead to recommendations for coopers, winemakers

76 and the food and beverage industry. We decided to investigate the effect of various cooperage
77 parameters on the concentration of new purified compounds, providing insights into how
78 specific barrel treatments can be optimized to enhance the desired sensory properties in the final
79 product.

80 **MATERIALS AND METHODS**

81 **Chemicals.** Ultrapure water (produced by the Milli-Q purification system, Millipore,
82 France) along with HPLC-grade solvents including acetonitrile, ethanol, ethyl acetate, heptane,
83 methanol, and 1-butanol (all sourced from VWR International, Pessac, France) were employed
84 for sample preparation and compound purification. Methyl *tert*-butyl ether (MTBE) was
85 obtained from Scharlab S.L (Barcelona, Spain). Chromatographic separations using LC-HRMS
86 were conducted with LC-MS grade acetonitrile, deionized ultrapure water, and formic acid
87 (Optima, Fisher Chemical, Illkirch, France). The enzyme Rapidase® AR 2000 (DSM
88 FoodSpecialties, Delft, Netherlands) was utilized for enzymatic hydrolysis.

89 **Plant Material and Samples.** Oak wood used for screening and purification was
90 provided by the Seguin-Moreau cooperage company (Merpins, France). The wood was
91 collected in April 2017 from a batch of staves designated for barrel production. The botanical
92 species was identified as Quercus petraea using the method outlined by Marchal et al. [22]. The
93 staves underwent an air-drying process for two years, following the standard cooperage
94 procedures. To avoid the impact of toasting, untoasted wood (UW) was selected for the
95 purification protocol. The staves were subsequently ground into sawdust by the barrel
96 manufacturer (Seguin Moreau, Merpins, France).

97 For quantitative analysis, the influence of oak wood species on purified compounds was
98 determined on oak wood extracts (n = 25 for sessile oak wood, n = 25 for American oak wood).
99 Subsequently, the influence of toasting on the content of purified compounds was studied; 25

100 staves of each species were prepared by the cooperage and provided with untoasted wood (n =
101 25 for each species), corresponding to the outside of the staves, and toasted wood (n = 25 for
102 each species), corresponding to oak wood toasted at 180°C for 30 min and collected on the
103 inside of the staves. Oak wood extracts were macerated in a hydro-ethanolic solution (50/50,
104 v/v) at 50 g/L for three days. The samples were then diluted five times with ultrapure water and
105 filtered at 0.2 µm before injection.

106 Twenty-eight commercial oaked spirits were also assayed (including 12 cognacs, 4 grape
107 brandies, 3 rums, 5 whiskies and 4 bourbons). The second set of spirits consisted of 9 vintages
108 of eau-de-vie (EDV) of cognac. The samples were not commercial cognac but EDV still in
109 barrels. They corresponded to a real aging kinetics, i.e. samples of the same EDV were collected
110 each year in the same barrel. The spirits samples were reduced to approx. 8% alcohol with
111 ultrapure water and then filtered at 0.2 µm. The final concentration was calculated by applying
112 the dilution factor.

113 **Extraction and Isolation.** The oak wood material (600 g) was macerated in 6 L of
114 H_2O/EtOH solution (50:50; v/v) at room temperature for two weeks, under an inert atmosphere
115 and light-protected. Sawdust and particles were removed by filtration (0.45 µm). A pneumatic
116 press was then used for 15 minutes at 6 bar. The solution with dissolved wood compounds (5.5
117 L) was concentrated under vacuum by evaporating ethanol and partially removing water. The
118 resulting aqueous layer (900 mL) was washed twice with 450 mL of *n*-heptane. This aqueous
119 phase was then subjected to extractions using MTBE (6×500 mL), ethyl acetate (EtOAc)
120 (5×800 mL) and water-saturated butan-1-ol (BuOH) (4×800 mL). Previously, water-saturated
121 butanol was prepared by mixing butanol and ultrapure water in a decanting funnel. The upper
122 part was retained for the liquid-liquid extraction of the oak wood. The combined organic layers
123 were evaporated *in vacuo*, suspended in water, and freeze-dried to obtain powders of MTBE

124 (1.8 g), EtOAc (1.6 g), BuOH (3.6 g) and aqueous (14.6 g) pre-purified extracts. They were
125 stored under air- and light-protective conditions.

126 Centrifugal Partition Chromatography (CPC) was carried out using Spot prep II LC coupled
127 with a SCPC-100+1000 (Armen Instrument, Saint-Avé, France), both managed by Armen
128 Glider Prep V5.0 software. A 1 L rotor was used. The appropriate biphasic solvent system was
129 selected based on the partition behaviour of the extract compounds between the two phases, as
130 described by Marchal et al.[13]. The partition coefficient, *Kd*, for various compounds was
131 calculated as the ratio of the solute area in each phase. Based on these findings, different
132 systems were tested, and the MTBE extract was fractionated using the Arizona solvent systems
133 H (*n*-heptane/EtOAc/MeOH/H_2O, 1:3:1:3 v/v) and then by setting up a gradient on the Arizona-
134 G system (*n*-heptane/EtOAc/MeOH/H_2O, 1:4:1:4 v/v). Separation was conducted in a single
135 CPC run of 1.8 g injection. Experiment was carried out in ascending mode at 1200 rpm with a
136 flow rate of 30 mL/min for 145 min for the elution phase and 50 mL/min for 45 min for the
137 extrusion. The Spot Prep fraction collector was set to 25 mL/min. Every 10 CPC tubes, an
138 aliquot (200 µL) was sampled, evaporated, dissolved in 1 mL of H_2O/MeOH 95:5 and analysed
139 by LC-HRMS. Ten fractions, labelled F-I to F-X, were constituted on the basis of their similar
140 chromatographic profile, after being combined, evaporated *in vacuo*, suspended in water, and
141 freeze-dried.

142 The preparative HPLC equipment was a Waters Prep 150 LC, which included a 2545
143 Quaternary Gradient Module, a 2489 UV/Visible detector and a 2424 ELSD detector (Waters,
144 Guyancourt, France). CPC fraction F-V (146.7 mg) and CPC fraction F-VI (180.0 mg) were
145 subjected to preparative HPLC using columns selected by LC-HRMS tests. Separation was
146 carried out on a SunFire Prep C18 OBD (19 mm × 250 mm, 5 µm particle size, Waters)
147 equipped with a SunFire preparative C18 guard cartridge (19 × 10 mm, 5 µm particle size,
148 Waters). The mobile phase consisted of ultrapure water with 0.1% of formic acid (Eluent A)

149 and acetonitrile with 0.1% of formic acid (Eluent B). The flow rate was maintained at 20
150 mL/min. For CPC fraction F-V, the gradient of eluent B was as follows: 0 min, 25%; 5.9 min,
151 25%; 32 min, 30%; 39 min, 98%; 44.2 min, 98%; 45.3 min, 25%; 52 min, 25%. For CPC
152 fraction F-VI, eluent B varied as follows: 0 min, 25%; 13.3 min, 25%; 35.4 min, 35%; 39 min,
153 98%; 44.2 min, 98%; 45.3 min, 25%; 52 min, 25%. Aliquots (20 mg) of CPC fractions were
154 dissolved in 400 µL of a 60:40 H_2O/MeOH solution, 0.2 µm-filtered and manually injected into
155 the system. UV detection was performed at 280 nm and chromatographic peaks were collected
156 immediately after the detector. Samples and pure compounds resulting from successive
157 injections were combined, evaporated *in vacuo* to remove solvents, and freeze-dried twice to
158 obtain white amorphous powder.

159 **LC-MS Analyses.** For both screening and quantitative analysis, the HPLC setup
160 included an HTC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and an Accela
161 U-HPLC system equipped with quaternary pumps. Separation *via* liquid chromatography was
162 carried out on a C18 column (Hypersil Gold 2.1 mm × 100 mm, 1.9 µm particle size, Thermo
163 Fisher Scientific). The mobile phases for screening analysis were water containing 0.1% of
164 formic acid (A) and acetonitrile with 0.1% of formic acid (B). The flow rate was set to 600
165 µL/min and eluent B varied as follows: 0 min, 20%; 1.0 min, 20%; 5.0 min, 50%; 5.3 min, 98%;
166 6.0 min, 98%; 6.15 min, 20%; 7 min, 20%. For quantitative analysis, the same column, flow
167 rate and solvents were used, but the eluent B gradient was adjusted as follows: 0 min, 10%; 1.6
168 min, 10%; 6.3 min, 40%; 7.0 min, 98%; 8.0 min, 98%; 8.1 min, 10%; 9.3 min, 10%. The
169 injection volume for each method was 5 µL.

170 **HRMS.** An Exactive Orbitrap mass spectrometer equipped with a heated electrospray
171 ionization (HESI II) probe (both from Thermo Fisher Scientific, Les Ulis, France) was used.
172 The mass analyzer was calibrated weekly using Pierce®ESI Negative Ion Calibration solutions
173 (Thermo Fisher Scientific). The parameters for ionization and mass spectrometry differed

174 between screening and quantitative analysis. Voltage settings, gas flow rates, and temperatures
175 for ion transfer and ionization were optimized in negative mode. Table 1 provides a summary
176 of the key main parameters for both methods.

177 For screening and quantitative analysis, detection of each compound was based on the
178 theoretical exact mass of its deprotonated molecular ion ($[M - H]^-$). Peak areas were
179 determined by automatic integration of extracted ion chromatograms built in a 3-ppm window
180 around the exact mass of the $C_{29}H_{35}O_{16}^-$ ion. Quan and Qual Browser applications of Xcalibur
181 (version 3.0, Thermo Fisher Scientific) were used to process all the data.

182 **Method Validation for Quantitation.** A stock solution of each compound (1 g/L) was
183 prepared in methanol. A calibration series was prepared by successive dilutions of this solution
184 in ultrapure water to provide calibration samples (1 µg/L, 2 µg/L, 5 µg/L, 10 µg/L, 20 µg/L, 50
185 µg/L, 100 µg/L, 200 µg/L, 500 µg/L, 1 mg/L, 2 mg/L, 5 mg/L and 10 mg/L).
186 The validation procedure for the quantification of P-WL-1 and P-WL-2 in oak wood extracts
187 and spirits was carried out by investigating linearity, sensitivity, specificity, intra-day
188 repeatability and trueness. The sensitivity of the LC-HRMS method was determined using the
189 approach described by De Paepe et al.[23]. The instrumental detection limit (IDL) for a molecule
190 is defined as the lowest concentration at which a consistent and reproducible signal is
191 detectable, distinguishing it from a blank measured under identical conditions. To determine
192 the IDL, the lowest levels of the calibration curve (ranging from 1 to 20 µg/L) were injected
193 into five replicates. The IDL is identified as the lowest concentration that achieves a precision
194 of less than 10% and an accuracy (recovery of back-calculated concentrations) of more than
195 90%. The instrumental quantitation limit (IQL) is set at twice the IDL. The limits of detection
196 (LOD) and quantitation (LOQ) were determined considering the concentration of the oak wood
197 macerate (50 g/L of dry extract) and the 5-fold dilution factor used during sample preparation.
198 The working range was based on the previously determined IQL. A calibration curve was

199 created by plotting the areas for each concentration level versus the nominal concentration.
200 Quadratic regression was used with a 1/x statistical weight. Linearity was evaluated by the
201 correlation coefficient (R^2) and by the deviations of each back-calculated standard
202 concentration from the nominal value. For intraday repeatability, five replicates of three
203 intermediate calibration solutions (10 µg/L, 200 µg/L and 10 mg/L) were injected, and the
204 relative standard deviation (RSD%) was calculated. Trueness was evaluated by calculating the
205 recovery ratio (measured versus expected areas) from two samples (EDV; Oak wood extract),
206 which were spiked with calibration solution corresponding to the addition of 100 µg/L, 1 mg/L
207 and 10 mg/L of P-WL-1 and P-WL-2. Interday repeatability was assessed by injecting the same
208 two samples (10 µg/L and 10 mg/L) over five consecutive days. Specificity was estimated by
209 checking the accuracy of mass measurements and retention time consistency. These parameters
210 were determined in parallel with the analysis of precision and trueness described above.

211 **Sensory Analysis.** Taste evaluations were conducted in a specialized room under
212 natural daylight at approximately 20°C, using standardized glass. To prepare the solutions to
213 be tasted, the concentrations were calculated in proportion to the quantities obtained for each
214 fraction. After weighing, each extract was dissolved in 100 µL of ethanol and then diluted in
215 50 mL of a 12% vol. alc. hydro-alcoholic solution obtained by diluting a red wine distillate at
216 36% vol. alc. with pure and demineralized water (eau de source de Montagne, Laqueuille,
217 France). Five wine-tasting experts assessed the taste characteristics (bitterness, sourness,
218 sweetness) of the fractions using wine-tasting terminology, rating the intensity on a scale from
219 0 (not detectable) to 5 (highly detectable). Pure compounds were also evaluated at a
220 concentration of 5 mg/L in this hydro-ethanolic solution, as well as in a non-oaked "eau-de-
221 vie". All sensory procedures involving human participants adhered to the Helsinki Declaration
222 or equivalent ethical standards. The tasters were informed about the study's nature and risks and
223 gave their consent to participate. Despite the use of fractions and pure compounds derived from

224 commercial oak wood for cooperage, panellists were instructed to spit out the samples after
225 tasting and were assured of the analysis's safety and confidentiality. They were also informed
226 that they could choose to participate or withdraw at any time. No specific ethical approval for
227 the human sensory study was required by the institution where the study took place.

228 **Structural Characterization. *NMR.*** NMR experiments were performed on a Bruker
229 UltraShield® System Avance 600 NMR, spectrometer (^{1}H at 600.27 MHz and ^{13}C at 150.95
230 MHz) fitted with a 5 mm TXI probe. All 1D (proton) and 2D (^{1}H-^{1}H COSY, ^{1}H-^{1}H ROESY,
231 ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC) spectra were acquired at 300 K in methanol-d_4, which gave
232 the solvent signal (^{1}H δ 3.31; ^{13}C δ 49.00) as reference. The following abbreviations were used
233 to describe the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Data
234 analysis was performed with the NMRnotebook software (NMRTEC, Illkirch-Graffenstaden,
235 France).

236 Potential *β*-methyl-*γ*-octalactone precursors 1, (3*S*,4*S*)-3-methyl-4-*O*-(3'-*O*-galloyl)-(6'-*O*-
237 galloyl)-*β*-D-glucopyranosyloctanoic acid (639-1, P-WL-1): white amorphous powder (6.5
238 mg); $[\alpha]_D^{25} - 2.3$ (c = 0.1, MeOH); HRMS *m/z* 639.1935 $[M - H]^-$ ($C_{29}H_{35}O_{16}^-$, 0.1 ppm); ^{1}H
239 NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table 2.

240 Potential *β*-methyl-*γ*-octalactone precursors 2, (3*S*,4*S*)-3-methyl-4-*O*-(4'-*O*-galloyl)-(6'-*O*-
241 galloyl)-*β*-D-glucopyranosyloctanoic acid (639-2, P-WL-2): white amorphous powder (4.5
242 mg); $[\alpha]_D^{25} - 7.1$ (c = 0.1, MeOH); HRMS *m/z* 639.1935 $[M - H]^-$ ($C_{29}H_{35}O_{16}^-$, 0.1 ppm); ^{1}H
243 NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table 2.

244 ***Optical rotation.*** Optical rotation was recorded using a JASCO P-2000 polarimeter,
245 with the sodium emission wavelength set to 589 nm and the temperature maintained at 25 °C.
246 The pure compound was dissolved in MeOH, and the final reading was obtained by averaging
247 10 measurements, each lasting 10 seconds.

248 **Enzymatic Hydrolysis.** Two separate vials were filled with 0.2 mg of each purified
249 compound. To each white powder, 2 mL of sodium acetate buffer (pH 5.0, 10 mM) and 200 µL
250 of a glycosidase-rich enzyme preparation (AR2000, Delft, The Netherlands; 70 mg/mL in
251 sodium acetate buffer) were added. The mixtures were incubated for 24 hours at 37°C. The two
252 samples were prepared for GC-MS analysis as follows. After 24 h, 1 mL of the previously
253 prepared solution was removed and replaced with 1 mL of sodium acetate buffer, 10 µL of
254 internal standard (octan-3-ol at 11.75 mg/L) and 2 mL of dichloromethane. The liquid-liquid
255 extraction was accelerated by vortexing for 1 min and was then centrifuged at 5000 rpm for 5
256 min. After separation of the two phases, the aqueous phase was discarded, and the organic phase
257 was dried with anhydrous sodium sulphate and concentrated under a nitrogen stream to
258 approximately 200 µL.

259 **GC-MS analysis.** An Agilent Technologies Intuvo 9000 gas chromatograph coupled to
260 a mass spectrometer was used to quantify *cis* and *trans* whisky-lactone. Two µL samples of the
261 organic extract were injected in splitless mode (injector temperature, 240°C) onto a DB-
262 HeavyWax column (30 m × 0.25 mm, 0.25 mm, Agilent, France). The carrier gas was helium
263 N60 (Air Liquide, France) at a flow rate of 1 mL/min. The oven was programmed at 45°C for
264 the first minute, heated to 230°C at 10°C/min, and maintained at this temperature for 15 minutes
265 until the end of the 34.5-minute run. The ion source was set at 230°C. The mass spectrometer
266 was operated in electron ionization mode at 70 eV in selected ion monitoring (SIM) mode. The
267 ions monitored were *m/z* 99, 114 and 156.

268 A stock solution of whisky lactone (*cis* and *trans*) was prepared at 540 mg/L in the
269 mixture used for sample preparation. A calibration series was then carried out by successive
270 dilutions of this stock solution. The following concentrations were obtained 108, 54, 27, 13.5,
271 6.75, 3.38, 1.69, 0.84 and 0.42 mg/L. Quantitation was performed by adding an internal

standard at 11.75 mg/L (octan-3-ol). A quadratic calibration curve through the origin was obtained with a good correlation coefficient ($R^2 > 0.999$).

Statistical Analysis. Statistical analyses were performed with XL-STAT version 2019.1.1.56334 (Addinsoft, Paris, France). Results were interpreted using the Kruskal-Wallis test followed by Dunn's paired test.

RESULTS AND DISCUSSION

Isolation of Taste-Active Compounds from Sessile Oak Wood Extracts The solid-liquid extraction of *Quercus petraea* sawdust was carried out using a hydro-ethanolic solution to resemble cellar conditions. The molecules extracted in this solution are similar to those released in wine or brandy aged in oak barrels. This guarantees *a priori* the safety of the fractions tasted. Sequential liquid-liquid extractions, whose advantage lay in the choice of solvents, were the first step of the fractionation protocol. After ethanol removal, Untoasted Wood (UW) was pre-purified successively with solvents, from the least to the more polar. Four extracts were subsequently collected, corresponding to MTBE, EtOAc, BuOH extracts and the aqueous phase. After double freeze-drying to remove all traces of solvent, the samples were tasted and their bitterness/sweetness/astringency intensities were evaluated. The sensory analysis of the pre-purified extracts showed a clear fractionation of the taste. The astringency and bitterness perceived in the BuOH extract and aqueous phase were rather expected due to the presence of various oak ellagitannins[24]. The sweetness perceived in the MTBE and EtOAc fractions could be explained by sweet triterpenes already identified in the laboratory[13,25]. Despite these hypotheses, many taste perceptions remained unexplained from a molecular point of view. To discover new taste-active compounds, the fraction described as the sweetest and most chemically complex, i.e. the MTBE fraction, was separated a second time by centrifugal partition chromatography.

296 For this fractionation, systems with relatively low polarity, i.e. from the first third of the
297 ARIZONA range, were tested. The best partition of the MTBE extract was obtained using a
298 gradient from Arizona-H to Arizona-G. When the experiment ended, the tubes were analysed
299 using LC-HRMS and grouped according to their composition. Thus, 10 fractions (labelled F-I
300 to F-X) were obtained as powders in variable amounts after solvent evaporation and freeze-
301 drying (Supplementary data, Figure S1). Tasting revealed different tastes varying from one CPC
302 fraction to another. Among these, bitterness was perceived, which was not the case when the
303 pre-purified MTBE extract was tasted. Six fractions were considered relevant for their
304 sweetness or bitterness (Supplementary data, Figure S1).

305 LC-HRMS revealed that the first fractions contained quercotriterpenosides, described as
306 sweet[13]. In the hope of finding new taste-active compounds, fractions F-V and F-VI were
307 studied. As they showed a chromatographic profile with few peaks, they were directly
308 submitted to preparative HPLC with UV and ELSD detections.

309 For each fraction, an initial injection of 5 mg revealed that the preparative LC chromatograms
310 displayed a well-defined profile, with only a few peaks being detected in both ELSD and UV
311 at 280 nm (Supplementary Data, Figure S3). Appropriate gradients were therefore selected for
312 fractionation by successive injections. The main peaks were collected manually immediately
313 after the UV detector. After solvent removal and freeze-drying, white amorphous powders were
314 obtained. Finally, 6.5 mg of compound *m/z* 639-1 from F-V and 4.5 mg of compound *m/z* 639-
315 2 from F-VI were purified.

316 **Structural Characterization of Compounds Isolated from Sessile Oak Wood Extracts**

317 *HRMS and NMR Characterization.* The *m/z* 639 isomers showed identical mass spectra
318 (Supplementary date, Figure S4), with a deprotonated ion $[M-H]^-$ at *m/z* 639.1935
319 corresponding to a molecule with an empirical formula of $C_{29}H_{36}O_{16}$ (within 0.1 ppm of the

320 theoretical value). The negative ion *m/z* 487.1826, with empirical formula $C_{22}H_{31}O_{12}^{-}$,
321 corresponded to a species formed by the loss of a neutral group $C_7H_4O_4$ characteristic of a
322 galloyl group. Similarly, the negative fragment ion *m/z* 335.1678, with molecular formula
323 $C_{15}H_{27}O_8^{-}$, corresponded to a loss of $C_{14}H_8O_8$ from the ion *m/z* 639.1935. These results
324 suggested the presence of two galloyl groups in the structure of the molecule. In addition, the
325 fragmentation spectrum showed the presence of species *m/z* 331.0587 ($C_{13}H_{15}O_{10}^{-}$) and *m/z*
326 313.0573 ($C_{13}H_{13}O_9^{-}$), characteristic of a hexose gallate group. The *m/z* 639.1935 isomers could
327 therefore contain two galloyl groups and one hexosyl group, the latter being attached to at least
328 one of the two gallates. The presence of ions *m/z* 487.1826 and *m/z* 335.1678 suggested that the
329 sugar was not in a terminal position. These functional groups could be attached to a molecule
330 with the molecular formula $C_9H_{18}O_3$. HRMS data can be compared with De Rosso and
331 collaborators [26].

332 The major fragment ion, *m/z* 487.1826, may present the same structure as the 6'-*O*-gallate
333 derivative of (3*S*,4*S*)-4-*β*-D-glucopyranosyloxy-3-methyloctanoic acid (P-W), a precursor of
334 whisky lactone first identified in the wood of *Platycarya strobilacea* [27]. Experimental and
335 bibliographic NMR data confirmed this hypothesis [11]. Compounds *m/z* 639-1 and *m/z* 639-2
336 exhibit the same proton chemical shifts for the signals corresponding to P-W. Thus, the
337 molecule *m/z* 639.1935 could be composed of this moiety with an additional galloyl. To our
338 knowledge, there is no known galloyl derivative of this molecule in oak wood. However, two
339 compounds with the same molecular formula were identified by Maeda and collaborators in the
340 genus *Platycarya* [28].

341 One- and two-dimensional NMR data were analyzed to verify that the purified compounds were
342 the same as those reported in the publication. For the molecule *m/z* 639-1, two protons resonated
343 as singletons at 7.10 and 7.13 ppm. The same singletons were observed at 7.10 and 7.08 ppm
344 for molecule *m/z* 639-2. They corresponded to the protons of the two galloyl groups. Contrary

345 to the article by Maeda *et al.*, the COSY and HMBC spectra did not suggest that the galloyl
346 groups were linked. Furthermore, three chemical shifts belonging to the zone of aromatic
347 compounds were observed in that article (two doublets at 7.48 and 7.37 ppm and a singlet at
348 7.26 ppm) compared to two here. The COSY and ROESY spectra were analysed to determine
349 the position of the second galloyl in these molecules (Supplementary data, Figure S5). For both
350 molecules, one signal was different on the hexose compared to the P-W. One of the protons
351 resonated as a triplet at 5.1 ppm for *m/z* 639-1 and at 5.04 ppm for *m/z* 639-2. Conversely, for
352 P-W, the signals of the protons present on the hexose appeared in the spectrum between 3.20
353 and 4.40 ppm in the form of multiplets or doublets of doublets. It is therefore possible that the
354 proton resonating around 5 ppm was deshielded by the presence of the gallate. This hypothesis
355 was confirmed by analysing the HMBC spectrum. The proton at 5 ppm had a resonant carbon
356 at 167 ppm, characteristic of an ester. The difference between *m/z* 639-1 and *m/z* 639-2 was due
357 to the position of the second gallate on the hexose. COSY was used to ascertain that the gallate
358 was attached to the C-3' carbon of the hexose for compound *m/z* 639-1 and to the C-4' carbon
359 for *m/z* 639-2. These two compounds are regioisomers, differing by the position of the gallate
360 group on the sugar. The δ ^{13}C values were assigned using HSQC and HMBC spectra
361 (Supplementary data, Figure S5) and are presented in Table 2. So, P-WL-1 was identified as
362 (3*S*,4*S*)-3-methyl-4-*O*-(3'-*O*-galloyl)-(6'-*O*-galloyl)-*β*-D-glucopyranosyloctanoic acid and P-
363 WL-2 was identified as (3*S*,4*S*)-3-methyl-4-*O*-(4'-*O*-galloyl)-(6'-*O*-galloyl)-*β*-D-
364 glucopyranosyloctanoic acid. The chemical structures of these two compounds are shown in
365 Figure 1.

366 In the publication of Maeda *et al.*, no chemical shift was assigned to the protons present on
367 glucose at the H-3' and H-4' positions. Furthermore, the authors did not mention the
368 interpretation of their HMBC and COSY spectra to determine where the second galloyl group
369 might bind to P-WL. A comparison with their data was therefore not relevant.

370 To our knowledge, these compounds have never been described in the literature. In addition,
371 the specific optical rotation of these compounds was measured and found to be $[\alpha]^{25}$ = -2.3 (*c*
372 0.1, MeOH) for *m/z* 639-1 and $[\alpha]^{25}$ = -7.1 (*c* 0.1, MeOH) for *m/z* 639-2. The compounds
373 appeared to be present with a slight excess of the levorotatory form. However, considering the
374 precision error of the measurement and the values obtained, it is likely that they were present
375 in the form of a racemic mixture.

376 *Enzymatic hydrolysis of P-WL-1 and P-WL-2.* In their paper, Maeda *et al.* suggested that the
377 isolated *m/z* 639 compounds were whisky lactone precursors [28]. To determine whether our
378 purified compounds *m/z* 639-1 and *m/z* 639-2 could be potential whisky lactone precursors,
379 their enzymatic hydrolysis was performed using the methodology developed by Slaghenaufi *et*
380 *al.* [29].

381 Enzymatic hydrolysis is a chemical reaction catalyzed by hydrolase-type enzymes that
382 necessarily involves a water molecule, resulting in the cleavage of a compound. The AR 2000
383 product has numerous enzymatic activities, particularly of the *β*-glucosidase type [30].
384 Glucosidase plays a key role in releasing aromatic compounds from glycosidic precursors found
385 in juice, must and wine [31,32]. In practice, the product used will transform the non-volatile
386 precursors into free volatile compounds likely to exhibit olfactory properties.

387 The identification and quantification of whisky lactone potentially formed by enzymatic
388 hydrolysis was performed using gas chromatography-mass spectrometry (GC-MS). Samples
389 after enzymatic hydrolysis were injected. Analysis revealed the presence of *trans*- and *cis*-*β*-
390 methyl-*γ*-octalactone with retention times of 14.34 min (LRI_{DB-WAX} 1893) and 15.07 min
391 (LRI_{DB-WAX} 1951), respectively (Supplementary data, Figure S6). The chromatographic peak
392 was assigned by comparison with literature data [33]. Results confirmed the previous hypotheses.

393 In summary, the purified compounds *m/z* 639-1 and *m/z* 639-2 are indeed whisky-lactone
394 precursors that we named P-WL-1 and P-WL-2, respectively.

395 In a previous study on the quantification of P-W in oak wood, the authors showed that it was
396 the main precursor of whisky lactone [34]. Its release occurs during wood maturation under the
397 action of microorganisms [10]. The same phenomenon could occur for our precursors. In addition,
398 a higher concentration was observed for *cis*-WL than for *trans*-WL (Supplementary data, Figure
399 S6). The ratio between these two stereoisomers was calculated from the areas and corresponded
400 to 96.5% for *cis*-WL and 3.5% for *trans*-WL. The same trend was observed for both samples.
401 In view of this preliminary result, it is possible that, for this enzyme, P-WL-1 and P-WL-2
402 released more *cis* than *trans*-WL. Further studies are needed to validate these hypotheses.

403 In addition, an oak aged white wine and an oak-aged spirit were screened by LC-HRMS. The
404 presence of the two precursors was detected only in the oak-aged spirit, which appeared to be
405 consistent with their release from oak wood with a higher alcohol content. In addition, unlike
406 spirits, wine generally undergoes microbiological processes during aging. These processes may
407 involve glucosidase activity, which can promote the degradation of glycosylated precursors
408 (Supplementary data, Figure S2 and S7).

409 **Gustatory Properties of Isolated Compounds.** A panel of five expert tasters evaluated each
410 compound individually at a concentration of 5 mg/L in a hydro-alcoholic solution. If the taste
411 of bitterness, sweetness, saltiness, or sourness in the spiked solution differed from that of the
412 blank hydro-alcoholic solution, its intensity was rated on a scale from 1 to 5. Despite the
413 compositional similarities of P-WL-1 and P-WL-2, tasting in hydroalcoholic solution revealed
414 completely different tastes. Compound P-WL-1 was characterized as sweet (3/5) and P-WL-2
415 as bitter (2/5). These compounds may have contributed to the taste of their original fraction.
416 This is particularly the case for fraction F-VI; its profile and the majority of compounds it

417 contained suggested that P-WL-2 could be the main contributor to its taste. Therefore, the
418 purification of the fractions of interest revealed taste-active molecules.

419 In a non-oaked EDV, P-WL-1 and P-WL-2 were dissolved at 5 mg/L to study their influence
420 on the taste balance of spirits. Each solution was characterized in comparison to the same "eau-
421 de-vie" as a reference. Quercotriterpenoside I (QTT I) was used as a sweetness standard since
422 its sensory properties have already been characterized [13]. The control EDV was scored 0/5 for
423 sweetness and bitterness but 5/5 for the perception of burning. As a reference, EDV spiked with
424 QTT I (5 mg/L) was described as sweeter (4/5) and less burning (1/5). P-WL-1 also modified
425 the taste of the EDV by decreasing the perception of burning (2/5) and increasing that of
426 sweetness (3/5). In contrast, P-WL-2 changed the taste of the spirits by increasing the perception
427 of bitterness (3/5) and decreasing that of burning (2/5). The precursors seemed to have less
428 influence than QTT I at the same concentrations in the spirits matrix. However, sensory analysis
429 revealed an interesting structure-activity relationship, particularly showing that the position of
430 the gallate in the molecule strongly affected the taste properties.

431 **Validation of a LC-HRMS Method to Quantitate Taste-Active Compounds in Oak Wood
432 and Spirits.** A robust and reliable assay method was needed to measure the content of purified
433 compounds in oak extracts and EDV. We used LC-HRMS because of the wide dynamic range
434 and accuracy of mass measurement provided by the Orbitrap analyser.

435 The chromatographic parameters for quantification were fine-tuned to improve the separation
436 of the two precursors. In addition, the spectrometric settings were adjusted to improve detection
437 sensitivity. Optimization of gas flows, voltages, and temperatures for ionization and ion transfer
438 was performed in negative mode, using the signal intensities of *m/z* 639.1935 as a reference
439 (Table 1). To minimize strong matrix effects, absolute quantification was achieved by preparing
440 calibration solutions of these two compounds in the same liquid mixture used for macerating

441 oak extracts. In our work, the instrumental detection limit (IDL) was set at 1 µg/L for each
442 compound, while the instrumental quantitation limit (IQL) was determined to be twice the IDL
443 (2 µg/L). For oak wood extracts, limits of detection (LOD) and quantitation (LOQ) were re-
444 calculated in µg/g based on the dilution factor (5) used during sample preparation (50 g/L). A
445 quadratic calibration curve (using 1/x statistical weighting) was obtained for P-WL-1 and P-
446 WL-2, with an excellent coefficient of determination ($R^2 = 0.999$) across a range from 2 µg/L
447 to 10 mg/L. The recovery of back-calculated concentrations exceeded 90% at all calibration
448 levels, indicating adequate accuracy. Intraday repeatability for each concentration and for each
449 compound was lower than 9.4%, which complied with the standard specifications. For each
450 quantitative analysis of a sample or series of samples, a new calibration range was injected to
451 improve accuracy. Additionally, a spirit and an oak wood extract spiked with stock solutions
452 were analysed, yielding recovery ratios between 85 and 111%, which is consistent with
453 common specifications[35]. Consequently, these results demonstrated the trueness and the
454 repeatability of the method applied to the two matrices. Data processing revealed minimal
455 variations in retention times (<0.04 min) and mass deviations under 2.5 ppm for both precursors
456 at various concentrations, thus ensuring method specificity. Overall, these results validated the
457 LC-HRMS method for quantifying P-WL-1 and P-WL-2 in oak wood extracts and spirits (Table
458 3).

459 **Quantitation of P-WL-1 and P-WL-2 in Various Spirits.** *Content of P-WL-1 and P-WL-2 in*
460 *Commercial Spirits.* The results of the assay are shown in Figure 2A for the two whisky-lactone
461 precursors. Contrary to wines, some spirits contained these compounds in a range from 0 µg/L
462 to 64.6 µg/L (Supplementary data, Table S8). This observation was consistent with the results
463 obtained using the oak wood purification protocol. In fact, in a more alcoholic solution, these
464 compounds w ere extracted to a greater extent. Furthermore, the differences in concentration
465 observed between the EDV could be due to the botanical origin of the wood used for barrel

466 aging. French oak is commonly used to produce cognacs and brandies, while American oak is
467 preferred for the aging of bourbons. Rum (R-3), with P-WL-1 and P-WL-2 concentrations of
468 64.5 µg/L and 42.7 µg/L respectively, was also matured in American white oak barrels
469 previously used for American whisky. This observation may be related to the significantly
470 higher levels of whisky-lactone in American oak [36]. Surprisingly, the levels of these compounds
471 in bourbons are lower than in most of the cognacs analysed. These differences could therefore
472 be due to different ageing times from one EDV to another. This hypothesis cannot be verified
473 as this information was not provided.

474 *Evolution of P-WL-1 and P-WL-2 over 8 years.* P-WL-1 and P-WL-2 were quantitated in
475 samples of EDV of cognac of nine different vintages from the same distillery (Supplementary
476 data, Table S9). The samples were not commercial cognac but EDV which had been aged in
477 barrels since 2010. A sample was collected each year from the same barrel from 2010 to 2018,
478 so the 2011 sample corresponds to one year of aging in barrels, the one of 2012 to two years
479 and so on. The two precursors were detected and quantitated in all the EDV at concentrations
480 ranging from 10 µg/L for the sample aged in barrels for one year, to 18 µg/L for the sample
481 aged for eight years. These concentrations are consistent with the results obtained for the
482 commercial spirits. The two precursors appear to be extracted at the same time, in the same
483 concentrations, and remain stable during barrel aging (Figure 2B). Long barrel aging appeared
484 to promote the formation of these two compounds.

485 **Quantitation of P-WL-1 and P-WL-2 in Oak Wood Extracts.** Winemakers generally strive
486 to produce wines and spirits with more sweetness than bitterness so as not to compromise the
487 taste balance of these matrices. This issue is important in the beverage industry. Previous studies
488 have shown that the concentration of oak molecules released into wine and spirits varies
489 considerably according to cooperage parameters, such as differences in the oak species and the
490 degree of toasting[5,37–39]. Given the different tastes of the two precursors, we investigated

491 whether these oenological parameters could have a significant influence on their content in oak
492 wood.

493 *Influence of Oak Species on P-WL-1 and P-WL-2 Contents in Oak Wood.* Quantitation of
494 commercial spirits suggested that a difference in the botanical origin of the oak wood could
495 have an influence on the concentration of these precursors. To validate these observations,
496 different extracts of sessile oak and American white oak were analysed. The results showed that
497 P-WL-1 and P-WL-2 were detected and quantified in the 25 extracts of sessile oak wood and
498 in the 25 extracts of American oak wood. The concentration of P-WL-1 in oak wood was 25.1
499 and 15.8 µg/g on average for American oak and sessile oak samples, respectively (Figure 3A).
500 In addition, the concentration of P-WL-2 was 14.2 and 15.8 µg/g on average for American oak
501 and sessile oak samples, respectively (Figure 3A). Wilkinson and colleagues found levels of P-
502 W in the order of 100 to 280 µg/g, i.e. 10 times higher than those found for P-WL-1 and P-WL-
503 2. This difference shows that, in contrast to other precursors such as P-W, P-WL is in the
504 minority in oak wood [40]. A Kruskal-Wallis test was performed on the data obtained after
505 quantification of the pure compounds to determine whether a significant difference could be
506 observed between the two oak species. The results showed that no molecule was influenced by
507 the species (*p-value* > 0.05), despite significant differences in average content. These results do
508 not follow the same trend as those in the literature on whisky lactones, which shows that
509 American oak generally contains higher concentrations of whisky lactone than sessile oak [41]. It
510 might appear that the differences were not significant, due to the high degree of inter-individual
511 disparity within each species (Supplementary data, Table S10). Such inter-individual variation
512 has already been observed in both species for other compounds such as ellagitannins[8].

513 However, for American oak wood, significant results were observed between the two precursors
514 (*p-value* < 0.001). Higher levels of P-WL-1 were obtained compared to P-WL-2 (Figure 3A),
515 unlike sessile oak where no difference was found. Further analysis is required to determine

516 whether there is a relationship between the concentration of P-WL-1 and P-WL-2 in American
517 oak wood, as has been demonstrated for *cis*- and *trans*-whisky lactone [36,42].

518 *Influence of Wood Toasting Temperature on P-WL-1 and P-WL-2 Contents.* The influence of
519 barrel toasting was also studied. The two precursors were quantitated in all the samples of this
520 series and the same trends were noted. For both oak species, toasting of the staves appeared to
521 decrease P-WL-1 and P-WL-2 levels significantly at the 0.1% threshold (Kruskal-Wallis test).
522 The levels of whisky lactone precursors, which have already been identified in the literature
523 [10,11,27], seemed to evolve in the same way as P-WL-1 and P-WL-2 under the influence of
524 toasting. Wilkinson et al. quantified P-W and 3-methyl-4-*O*-(6′-*O*-*α*-L-rhamnosyl)-*β*-D-
525 glucopyranosyloctanoic acid in oak wood extracts at different temperatures and observed their
526 thermal degradation after toasting at 200°C for 30 min [40]. The TW modality corresponds to the
527 latter parameter. Conversely, *cis*- and *trans*-whisky lactone concentrations increase
528 significantly with wood toasting [38,43]. It has been shown that whisky lactone can be produced
529 from its precursors during stave toasting. Temperature and duration of toasting play a key role
530 in this process [40]. It is therefore possible that P-WL-1 and P-WL-2 behave in the same way,
531 these precursors releasing whisky lactone when degraded during stave heating. Furthermore,
532 toasting does not change the ratio between the two precursors, neither for American oak nor for
533 sessile oak. This ratio could serve as an indicator to determine the type of oak used to age a
534 wine or spirit.

535 This study aimed at establishing a purification protocol to isolate taste-active fractions from
536 oak wood extract using a gustatometry-guided inductive approach. The technique revealed
537 several fractions with bitter and/or sweet taste and led to the isolation of taste-active
538 compounds. After NMR structural elucidation, two new galloylated derivatives were
539 discovered. Moreover, their enzymatic hydrolysis revealed the formation of *β*-methyl-*γ*-
540 octalactone, indicating potential precursors. Sensory analysis highlighted differences in taste

541 depending on the chemical structure of the precursors and in particular the position of the gallate
542 in the molecule. P-WL-1 is sweet whereas P-WL-2 is bitter. Their presence in spirits was also
543 validated and their impact on the taste balance of spirits was perceived more strongly by
544 decreasing the burning perception. The determination of their gustatory detection threshold will
545 be necessary to establish their real effect in this matrix. An LC-HRMS quantitation method was
546 used to study the influence of oenological parameters on the concentration of these precursors
547 in oak wood extracts. Their better control could improve the monitoring of oak wood aging and
548 its sensory effects, leading to recommendations for coopers and winemakers. In comparison
549 with literature data, purified precursors are in the minority in oak wood compared with other
550 precursors such as P-W. Moreover, the results showed that they are not influenced by the
551 botanical origin of the wood. However, for *Quercus alba*, significant results were observed
552 between the two precursors, with higher levels of P-WL-1 compared to P-WL-2. Toasting of
553 the staves also led to a significant decrease in the content of these precursors, but without
554 changing their ratio. Further studies will be necessary for a better interpretation of this. As the
555 molecular understanding of the taste balance of wines and spirits remains partial, this work
556 must continue, in particular to clarify the relative contribution of the identified compounds to
557 the overall balance. Overall, the present findings provide a better understanding of the
558 molecular markers responsible for the taste of beverages such as spirits.

559

560 **SUPPORTING INFORMATION**

561 **This material is available free of charge via the Internet at http://pubs.acs.org.**

562 **Table S1:** Gustatogram of the pre-purified extracts and of the 10 fractions obtained after CPC
563 fractionation of the MTBE extract (with obtained masses).

564 **Figure S2**: Negative LC-ESI-HRMS XIC of pre-purified MTBE oak wood extract (UW_MTBE)
565 corresponding to negative ion at *m/z* 639.1935.

566 **Figure S3:** Negative LC-ESI-HRMS chromatograms of the F-V and F-VI fractions of pre-purified
567 MTBE oak wood extract (UW_MTBE).

568 **Figure S4**: HRMS spectrum of P-WL-1 and P-WL-2 (with fragmentation 30 eV).

569 **Figure S5**: ^{1}H NMR, 2D COSY, 2D ROESY, 2D HSQC, 2D HMBC (CD_3OD) spectra of P-WL-1 and
570 P-WL-2.

571 **Figure S6:** GC-MS chromatograms corresponding to the whisky lactone standard (left) and the
572 enzymatic hydrolysis of *m/z* 639-1 (middle) and *m/z* 639-2 (right); trans-WL (t_R = 14.34 min) and cis-
573 WL (t_R = 15.07 min).

574 **Figure S7:** Negative LC-ESI-FTMS XIC of oak wood extract (A) and an oaked eau-de-vie of cognac
575 (B) corresponding to a negative ion at *m/z* 639.1935.

576 **Table S8:** Individual concentrations of P-WL-1 and P-WL-2 in 28 commercial spirits. All
577 concentrations expressed in (µg/L).

578 **Table S9**: Individual concentrations of P-WL-1 and P-WL-2 in 9 vintages of same spirit. All
579 concentrations expressed in (µg/L).

580 **Table S10**: Individual concentrations of P-WL-1 and P-WL-2 in 100 samples of oak wood (*Quercus*
581 *alba* and *Quercus petraea* with two levels of toasting). All concentrations expressed in (µg/g of
582 wood).

ABBREVIATIONS USED

COSY: Correlated Spectroscopy

EDV: eau-de-vie

HMBC: Heteronuclear Multiple Bond Coherence

HSQC: Heteronuclear Single Quantum Coherence

IDL: instrumental detection limit

IQL: instrumental quantitation limit

LOD: limit of detection

LOQ: limit of quantitation

QTT I: Quercotriterpenoside I

ROESY: Rotating-frame nuclear Overhauser Effect correlation Spectroscopy

TW: Toasted Wood

UW: Untoasted Wood

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762

763 **List of Figures**

764 **Figure 1**: Chemical structures of P-WL-1 (*m/z* 639-1) and P-WL-2 (*m/z* 639-2).

765 **Figure 2**: Concentrations of P-WL-1 (in beige) and P-WL-2 (in red) in 28 commercial spirits
766 (A) (C: Cognac; B: Brandy; G: Gin; W: Whisky; R: Rum; Bo: Bourbon) and in Cognac samples
767 aged over 8 years in French oak barrels (B). *Error bars* represent the precision of the method.

768 **Figure 3:** Influence of oak species (right) and toasting process (left) on P-WL-1 (in beige) and
769 P-WL-2 (in red) content. Significant: *** *p-values* < 0.001; "a" and "b" represent significant
770 groups; UW: untoasted wood; TW: toasted wood. *Error bars* represent standard deviation of
771 different replicates.

772

Table 1: Ionization and spectrometric conditions of HRMS analyses

Mass Spectrometer	**Exactive**	
Ionization mode	**Negative**	
Use	LC-HRMS Screening	LC-HRMS Quantitation
Mass scan	Full MS	Full MS
Sheath gas flow [a]	70	60
Auxiliary gas flow [a]	15	15
Spare gas flow [a]	0	0
HESI probe temperature	320 °C	360 °C
Capillary temperature	350 °C	330 °C
Electrospray voltage	- 3.5 kV	- 3.5 kV
Capillary voltage	- 25 V	- 92.5 V
Tube lens voltage offset	- 120 V	- 170 V
Skimmer voltage	- 20 V	- 40 V
Mass range (in Th)	100 - 1500	100 - 1000
Resolution [b]	25,000	10,000
AGC value [c]	10^6 ions	3.10^6 ions

[a] Sheath gas and auxiliary gas flows (both nitrogen) expressed in arbitrary units

[b] Resolution $m/\Delta m$, fwhm at m/z 200 Th

[c] Automatic Gain Control

778 **Table 2:** ^{1}H and ^{13}C NMR signal assignments of P-WL-1 (*m/z* 639-1) and P-WL-2 (*m/z* 639-
779 2)

	P-WL-1		**P-WL-2**	
Position	$\delta_{H\ (J\ =\ Hz)}$	δ_C	$\delta_{H\ (J\ =\ Hz)}$	δ_C
Carbon chain				
1	/	177.26	/	178.5
2	2.14 *m* 2.56 *d* (15.1; 6.0)	37.16	2.14 *dd* (14.5; 7.6) 2.55 *dd* (14.6; 6.5)	33.04
3	2.22 *m*	32.81	2.23 *m*	33.17
4	3.63 *m*	82.33	3.65 *m*	82.15
5	1.43 *m* 1.50 *m*	30.56	1.42 *m* 1.51 *m*	30.68
6	1.22 *m* 1.31 *m*	27.11	1.22 *m* 1.30 *m*	27.11
7	1.12 *m* 1.18 *m*	21.8	1.12 *m* 1.18 *m*	21.8
8	0.74 *t* (7.2)	12.34	0.74 *t* (7.3)	12.34
9	0.93 *d* (6.8)	13.09	0.94 *d* (7.0)	13.26
Glucopyranosyl				
1'	4.51 *d* (7.8)	102.55	4.51 *d* (8)	102.77
2'	3.45 *dd* (9.4; 8.1)	72.7	3.30 *m*	74.3
3'	**5.1 *t* (9.2)**	**77.6**	3.73 *t* (9.6)	74.4
4'	3.69 *m*	69.6	**5.04 *t* (10.0)**	**71.3**
5'	3.71 *m*	73.6	3.86 *m*	71.4
6'	4.45 *dd* (11.8; 6.4 Hz) 4.50 *dd* (11.6; 2.2 Hz)	63.08	4.26 *m* 4.25 *m*	62.76
Galloyl 1				
1''	/	120.12	/	119.52
2''	7.13 *s*	108.8	7.10 *s*	108.63
3''	/	138.4	/	138.52
4''	/	144.8	/	145.12
5''	/	144.8	/	145.12
6''	7.13 *s*	108.8	7.10 *s*	108.63
7''	/	166.8	/	166.75
Galloyl 2				
1'''	/	119.75	/	119.52
2'''	7.10 *s*	108.7	7.08 *s*	108.63
3'''	/	138.5	/	138.52
4'''	/	144.8	/	145.12
5'''	/	144.8	/	145.12
6'''	7.10 *s*	108.7	7.08 *s*	108.63
7'''	/	167.06	/	166.75

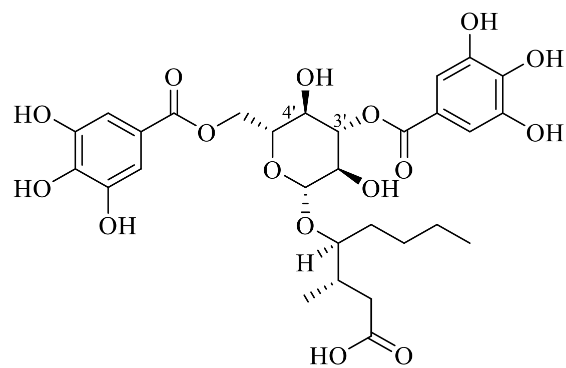
780

781

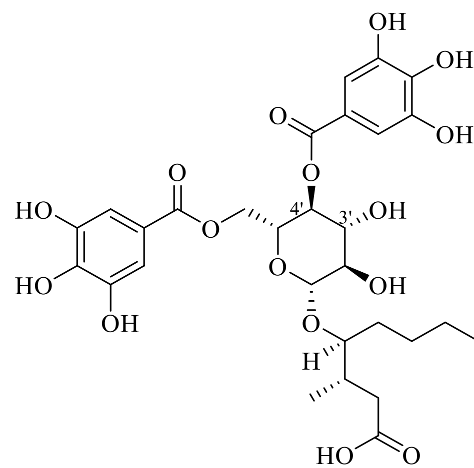
Table 3: Validation parameters for HRMS quantitation of P-WL-1 (*m/z* 639-1) and P-WL-2 (*m/z* 639-2).

Matrix	**Sensitivity**				**Linearity and Accuracy**		**Specificity**		**Repeatability and Trueness**								
	IDL (µg/L)	IQL (µg/L)	LOD (µg/g)	LOQ (µg/g)	Working range	R^2	t_R variation (min)	Mass accuracy (ppm)	Intraday repeatability			Recovery *EDV*			Recovery *Oak wood extract*		
									10 µg/L	*200 µg/L*	*10 mg/L*	*100 µg/L*	*1 mg/L*	*10 mg/L*	*100 µg/L*	*1 mg/L*	*10 mg/L*
EDV	1	2	-	-	2 µg/L – 10 mg/L	0.999	0.03	2.5	5.5%	7.4%	6.2%	85%	98%	103%	110%	98%	102%
Oak wood extract	1	2	0.1	0.2													
EDV	1	2	-	-	2 µg/L – 10 mg/L	0.9991	0.04	1.2	9.4%	5.9%	8.2%	100%	102%	107%	111%	104%	104%
Oak wood extract	1	2	0.1	0.2													

Figure 1.

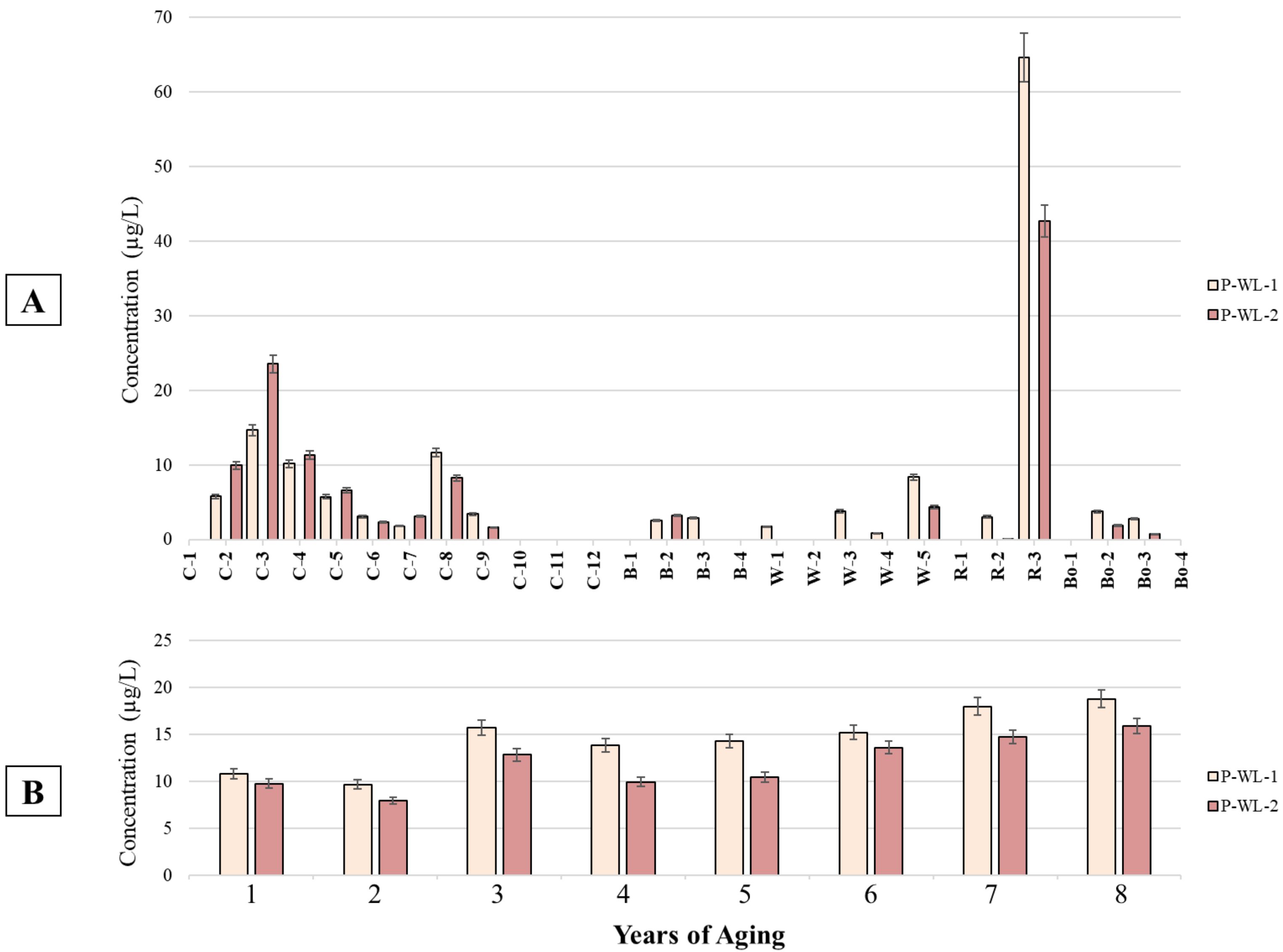


P-WL-1



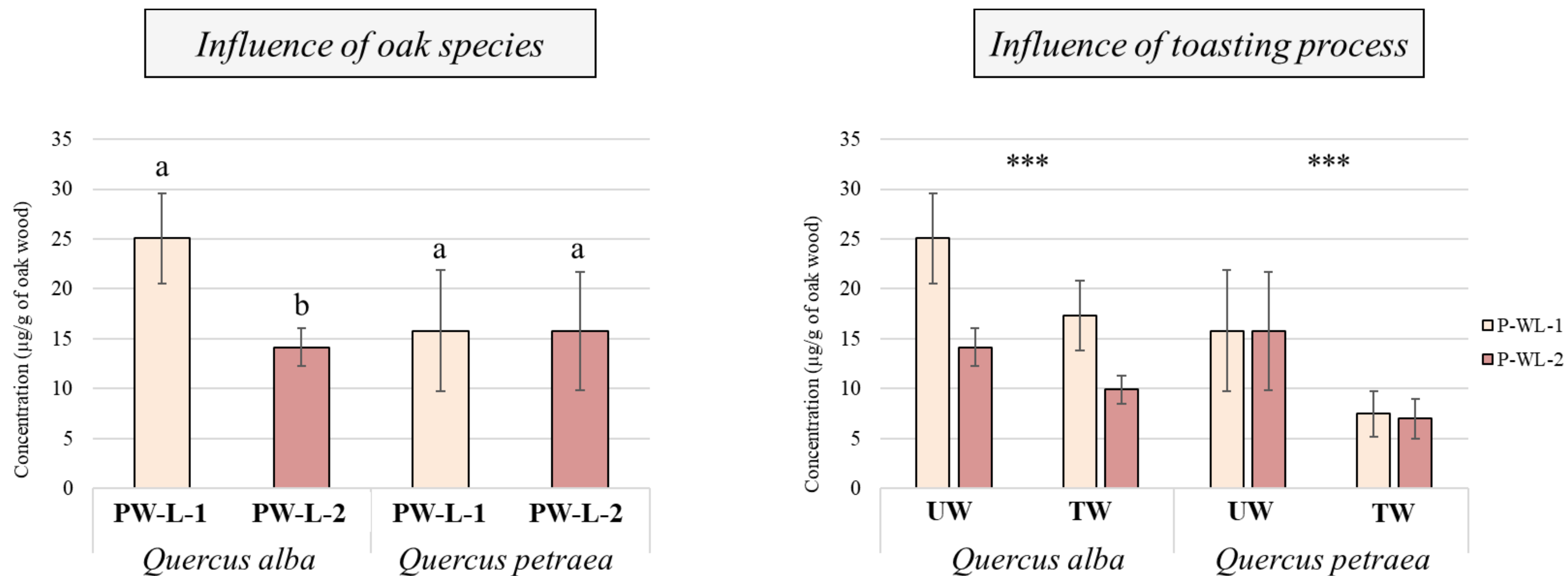
P-WL-2

788 **Figure 2**.



789

790 **Figure 3.**



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793 **TOC Graphic**

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