



HAL
open science

Isolation of a new taste-active brandy tannin A: Structural elucidation, quantitation and sensory assessment

Delphine Winstel, Yoan Capello, Stéphane Quideau, Axel Marchal

► To cite this version:

Delphine Winstel, Yoan Capello, Stéphane Quideau, Axel Marchal. Isolation of a new taste-active brandy tannin A: Structural elucidation, quantitation and sensory assessment. *Food Chemistry*, 2022, 377, pp.1-10. 10.1016/j.foodchem.2021.131963 . hal-03565853

HAL Id: hal-03565853

<https://hal.inrae.fr/hal-03565853>

Submitted on 8 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Isolation of a new taste-active brandy tannin A: structural**
2 **elucidation, quantitation and sensory assessment**

3

4

5

6 Delphine Winstel¹, Yoan Capello², Stéphane Quideau^{2,3} and Axel Marchal^{1,*}

7

8

9

10 ¹ Univ. Bordeaux, Unité de recherche Œnologie, EA 4577, USC 1366 INRA, ISVV, 33882
11 Villenave d'Ornon Cedex, France.

12 ² Univ. Bordeaux, ISM (CNRS-UMR 5255), 351 cours de la Libération, 33405 Talence
13 Cedex, France.

14 ³ Institut Universitaire de France, 1 rue Descartes, 75231 Paris Cedex 05, France.

15 * Correspondence: axel.marchal@u-bordeaux.fr; Tel.: +33-557-575-867

16

17 **Highlights**

- 18 • Original approach to search for taste-active compounds in spirits.
- 19 • Identification of a new taste-active compound: brandy tannin A.
- 20 • Brandy tannin A is an oxidation product of vescalagin.
- 21 • Brandy tannin A is mostly present in cognacs.

22 **ABSTRACT**

23 Enjoying a glass of spirits can be one of the delights of life. While it is well known that their
24 taste improves during barrel aging, the molecular explanations of this phenomenon remain
25 largely unknown. The present work aimed at searching for taste-active compounds formed in
26 spirits during aging. An untargeted metabolomic approach using HRMS was applied on “eau-
27 de-vie” of cognac. A fractionation protocol was then performed on brandies to isolate a
28 targeted compound. By using HRMS and NMR, its structure was elucidated for the first time.
29 This new ellagitannin, called brandy tannin A, considerably increased the sweetness of spirits
30 at 2 mg/L. After development of an LC-HRMS quantitation method, it was assayed in various
31 spirits and was detected mainly in cognacs up to 7 mg/L. These findings demonstrate the
32 sensory contribution of this compound and more generally the relevance of combining
33 metabolomics and separative techniques to purify new taste-active compounds.

34

35 **Key words:** Ellagitannin; sweetness; taste-active compounds; quantitation; oak aging.

36 **1. Introduction**

37 Taste is a sense involved in the chemical detection of compounds likely to develop nutritional
38 or toxic properties. Conventionally, humans can distinguish five basic flavours: salty, sour,
39 bitter, sweet and umami. Many taste-active molecules, belonging to various chemical
40 families, have been identified in numerous plants (Kinghorn, 1987). The investigation of these
41 products in natural matrices such as foods or beverages is a major challenge for chemists.
42 Such studies are particularly relevant in oenology since they allow a better understanding of
43 the taste of wines and spirits.

44 In recent years, oenological research has enabled the molecular characterization of non-
45 volatile compounds involved in the perception of taste, as well as tactile sensations. To isolate
46 such sensory-active molecules, several strategies have been developed. First, inductive
47 fractionations guided by sensory analysis were implemented by using various separation
48 techniques (Frank et al., 2006; Marchal, Waffo-Téguo, et al., 2011). Another strategy was to
49 search for structural analogues to already known taste-active compounds on the basis of their
50 putative empirical formulas (Gammacurta et al., 2019; Marchal, Génin, et al., 2015). Then,
51 analogues were isolated following targeted purification with liquid chromatography-high
52 resolution mass spectrometry (LC-HRMS) screening and were tasted to determine their
53 sensory properties. More recently, a new method that combines untargeted and targeted
54 approaches in the search for new natural products was proposed and applied to spirits
55 (Winstel et al., 2021). However, the molecular determinants associated with sweet or bitter
56 taste have only been partially elucidated. These perceptions are linked in particular to the
57 presence of several compounds released during winemaking by grapes (Cretin et al., 2019;
58 Fayad et al., 2021), yeasts (Marchal, Marullo, et al., 2011) or by oak wood (Gammacurta et
59 al., 2019; Marchal, Cretin, et al., 2015; Saucier et al., 2006).

60 Used for a long time as shipping devices, oak barrels are now mainly used for producing
61 wines and spirits, owing to the physico-chemical and sensory changes they cause. The non-
62 volatile compounds associated with modifications of colour (Chassaing et al., 2010), tactile
63 sensations (Glabasnia & Hofmann, 2006) and taste (Marchal, Waffo-Téguo, et al., 2011) that
64 are consecutive to the aging of wines and spirits have been studied in recent decades.
65 Empirically, the observations of winemakers suggest an increase in sweetness of wines and
66 spirits during oak wood aging. This observation has been confirmed (Marchal et al., 2013),
67 interpreted at the molecular level, and was found to be due to the release of glucosylated and
68 galloylated triterpenoids called quercotriterpenosides (QTT) (Gammacurta et al., 2019;
69 Marchal, Waffo-Téguo, et al., 2011). On the contrary, excessive or inappropriate use of oak
70 wood can increase bitterness in wines and spirits. This phenomenon is mostly attributed to
71 polyphenols such as lignans (Marchal, Cretin, et al., 2015) and coumarins (Winstel et al.,
72 2020). Moreover, the ellagitannins of oak wood have been extensively studied for their
73 possible health effects (Auzanneau et al., 2012; Cardullo et al., 2020; Georgess et al., 2018),
74 as well as for their sensory properties (Chira et al., 2015).

75 Even if the bitter characteristics of the main ellagitannins have been suggested and their
76 gustatory detection threshold established (Glabasnia & Hofmann, 2006), the concentrations
77 observed in wines are significantly below these thresholds. In spirits, the main hydrolysable
78 tannins, such as vescalagin, castalagin, roburins A to E and grandinin, have been identified
79 (Gadrat et al., 2020; Puech et al., 1990). However, they seemed to be extracted from oak
80 wood into “eau-de-vie” from the beginning of barrel aging and were quickly degraded (Viriot
81 et al., 1993). Likewise and by comparison with the concentrations obtained in various spirits,
82 no clear correlation could be established between these compounds and the bitterness
83 sometimes perceived in oaked brandies (Gadrat et al., 2020; Glabasnia & Hofmann, 2006).
84 Since spirits are a highly complex matrix characterized by an ethanol concentration that is

85 usually between 36% to 55% (v/v), ellagitannins may be involved in various chemical
86 reactions, including hydrolysis, solvolysis and oxidation. For instance, Quideau *et al.* reported
87 that in an ethanol solution of vescalagin, the C-1 epimer of castalagin was converted to β -1-*O*-
88 ethylvescalagin (Quideau *et al.*, 2005). This ethanol adduct has been identified in wines
89 (Saucier *et al.*, 2006), but never in spirits. Moreover, Fujieda *et al.* discovered oxidation
90 products of castalagin, named whiskey tannins A and B, in Japanese whiskey (Fujieda *et al.*,
91 2008). Their structure suggested that they were formed by regioselective oxidation of the
92 pyrogallol ring linked at the glucose C-1 position of castalagin, and subsequent addition of
93 ethanol followed by a benzylic acid-type rearrangement. Therefore, spirits aging can lead to
94 the formation of new chemical species from oak extractables. A recent study provided
95 confirmation by showing that the diversity of compounds in aged spirits is greater than in the
96 wood itself (Roullier-Gall *et al.*, 2018). This finding suggests that non-volatiles of oaked
97 spirits are both native compounds released from wood and molecules newly formed during
98 aging. Further knowledge is needed to better understand how such changes in the chemical
99 composition of spirits are linked the overall improvement of their sensory quality during
100 aging.

101 To search for new taste-active compounds in spirits, the recently proposed combination of
102 untargeted and targeted approaches was implemented in the present work. First, untargeted
103 metabolomic profiling by HRMS was carried out on several “eau-de-vie” of cognac of
104 different vintages. Statistical analyses were performed to evaluate the overall structure of the
105 metabolomic data and to select compounds potentially newly formed in spirits. Then, a
106 targeted fractionation protocol, including liquid-liquid extractions, centrifugal partition
107 chromatography (CPC) and preparative-HPLC, allowed the isolation of new taste-active
108 compounds that can be further identified, characterized for their sensory properties, and
109 quantitated in spirits.

110 2. Materials and methods

111 2.1. Chemicals

112 HPLC grade solvents (acetonitrile, ethanol (EtOH), ethyl acetate (EtOAc), *n*-heptane, propan-
113 2-ol, methanol and butan-1-ol (BuOH) from VWR International, Pessac, France and methyl
114 *tert*-butyl ether (MTBE) from Acros Organics, Fisher Scientific, Illkirch, France) and
115 ultrapure water (Milli-Q purification system, Millipore, France) were used. LC–HRMS
116 chromatographic separations were performed with deionized ultrapure water, LC–MS grade
117 acetonitrile and formic acid (Optima, Fisher Chemical, Illkirch).

118 2.2. Samples

119 A commercial spirit (Cognac XO) and an oak wood extract (100 g/L), prepared in a hydro-
120 alcoholic solution (50:50 H₂O/EtOH) for three days, were screened by LC-HRMS.

121 For targeted compound isolation, a blend of “eau-de-vie” (EDV) of cognac aged in barrels for
122 19, 20 and 21 years, was used.

123 For quantitative analysis, 36 commercial spirits aged in oak wood were assayed. The second
124 set of spirits consisted of 9 vintages of EDV of cognac. The samples were not commercial
125 cognac but EDV still in barrels. They corresponded to a real aging kinetics, i.e. samples of the
126 same EDV were collected each year in the same barrel. The third set of spirits (Table S1,
127 Supplementary data) consisted of ten different vintages of EDV of cognac, still in barrels. The
128 samples came from the same distillery, had undergone similar aging conditions, and had been
129 matured in used barrels (350 L coarse-grained oak barrels). A sample was collected from five
130 different barrels for each year (except for 1970 and 1973 for which only four replicates were
131 available).

132 All spirits were provided by the House of Rémy-Martin. They were diluted with water by a
133 factor 5 and then filtered at 0.2 µm. This dilution factor was considered when calculating the
134 final concentration.

135 2.3. *LC-Analysis*

136 For the screening and quantitative analysis, the UHPLC appliance consisted of a Vanquish
137 system (Thermo Fisher Scientific, Les Ulis, France) with binary pumps, an autosampler and a
138 heated column compartment. For LC-HRMS analyses and quantitation, a Hypersil Gold C₁₈
139 column (100 × 2.1 mm, 1.9 μm, Thermo Fisher Scientific) was used as the stationary phase,
140 with water (Eluent A) and acetonitrile (Eluent B), containing both 0.1% of formic acid, as
141 mobile phases. The flow rate was set at 600 μL/min and the injection volume was 2 μL. The
142 temperature of the column chamber was set at 30°C in forced air mode. For screening
143 analysis, eluent B varied as follows: 0 min, 10%; 1.0 min, 10%; 5.0 min, 50%; 5.3 min, 98%;
144 6.0 min, 98%; 6.15 min, 10%; 7 min, 10%. For the quantitative analysis, eluent B varied as
145 follows: 0 min, 10%; 1.6 min, 10%; 5.3 min, 35%; 6.1 min, 98%; 7.1 min, 98%; 7.3 min,
146 10%; 8.3 min, 10%.

147 2.4. *HRMS*

148 For the screening and quantitative analysis, an Exactive Orbitrap mass spectrometer was
149 equipped with a heated electrospray ionization (HESI-II) probe (both from Thermo Fisher
150 Scientific). The ionization and spectrometric parameters were designed for each type of
151 analysis and are summarized in Table 1. Optimization of gas values, voltages and
152 temperatures applied for ionization and ion transfer was carried out in negative mode.

153 Detection of the targeted compound was based on the theoretical exact mass of its
154 deprotonated molecular ion ([M - H]⁻) and its retention time. Peak areas were determined by
155 automatic integration of extracted ion chromatograms (XIC) built in a 3-ppm window around
156 its exact mass. All data were processed using the Qual Browser and Quan Browser
157 applications of Xcalibur version 3.0.

158 2.5. *Metabolomic approach*

159 Untargeted metabolomic profiling by HRMS on several EDV of Cognac of different vintages
160 has already been described in a previous study (Winstel et al., 2021). The analysed samples
161 corresponded to the third test of spirits (Table S1, Supplementary data). For this approach, a
162 Q-Exactive Plus mass spectrometer with a HESI-II probe (Thermo Fisher Scientific) was used
163 and the HPLC appliance consisted of a Waters Acquity I-Class UPLC system (Waters,
164 Guyancourt, France). Optimization of gas values, voltages and temperatures applied for
165 ionization and ion transfer was carried out in negative mode (Table 1). After analysing the
166 spirits samples, Thermo RAW files were exported to the open-source software package
167 MZmine 2 (2.38 version) for data processing (Pluskal et al., 2010). All the statistical analyses
168 were carried out using the open-source software R Statistical (Foundation for Statistical
169 Computing, Vienna, Austria). Results were interpreted by one-way analysis of variance
170 (ANOVA), using *vintage* as factor. Principal Component Analysis (PCA) was performed on
171 normally distributed data. K-means clustering was also established for data classification
172 (package ClassDiscovery). Pearson correlations were then carried out ($\alpha < 0.05$, correlation
173 coefficient $r > 0.8$) and allowed the creation of groups of compounds with a similar evolution
174 according to the vintages.

175 2.6. Extraction and isolation

176 2.6.1. Liquid-liquid extractions

177 The blend of EDVs used for isolation was titrated around 64% vol. alc. It was evaporated to
178 dryness *in vacuo* to remove the ethanol. After evaporation and freeze-drying of four bottles of
179 750 mL of EDV, a dry extract of 7.35 g was obtained. To start the liquid-liquid extractions,
180 the extract was first solubilized in 900 mL of milli-Q water. This aqueous extract was washed
181 twice with 450 mL of *n*-heptane. This aqueous layer was then extracted successively with
182 MTBE (6×500 mL), EtOAc (5×800 mL) and with water-saturated BuOH (4×800 mL). The
183 combined organic layers were evaporated *in vacuo*, suspended in water, and freeze-dried

184 twice to obtain brownish powders of MTBE (1.8 g), EtOAc (1.3 g), BuOH (1.2 g) and
185 aqueous (3 g) pre-purified extracts. They were stored under air- and light-protective
186 conditions.

187 2.6.2. CPC fractionation

188 CPC was performed on a Spot prep II LC coupled with a SCPC-100+1000 (Armen
189 Instrument, Saint-Avé, France), both controlled by Armen Glider Prep V5.0 software. A 1 L
190 rotor was used. The solvent was pumped into the column by a 4-way quaternary high-pressure
191 gradient pump. The samples were introduced into the CPC column *via* an automatic high-
192 pressure injection valve. All the experiments were conducted at room temperature with UV
193 detection at 254 and 280 nm. Following the procedure described by Marchal *et al.* (Marchal,
194 Waffo-Téguo, et al., 2011), the selection of an appropriate biphasic system of solvents was
195 based on the study of the partition of extract compounds in both phases. Several systems were
196 tested, and the BuOH extract was fractionated using the ternary biphasic system
197 EtOAc/propan-2-ol/H₂O (3:1:3, v/v/v). Separation was carried out by one CPC run of 1.2 g
198 injection. Experiment was performed at 1200 rpm in ascending mode with a flow rate of 30
199 mL/min for 135 min for the elution phase and 50 mL/min for 40 min for the extrusion. The
200 Spot Prep fraction collector was set to 25 mL/min. Every 10 CPC tubes, 200 µL were taken,
201 evaporated, dissolved in 1 mL of H₂O/MeOH 90:10 (v/v), filtered and analysed by LC-
202 HRMS. Ten fractions, named F-I to F-X, were formed according to their similar
203 chromatographic profile, after being combined, evaporated *in vacuo*, suspended in water, and
204 freeze-dried.

205 2.6.3. Preparative liquid chromatography

206 Preparative HPLC analyses were performed using a Waters Prep 150 LC including a 2545
207 Quaternary Gradient Module, a 2489 UV/Visible detector (Waters). Final purification of
208 targeted compound 1 (TC1), which was present in the CPC fractions F-II (83.2 mg), F-III

209 (144.9 mg) and F-IV (62.8 mg), was achieved by preparative HPLC using columns chosen
210 after LC-HRMS tests. Separations were carried out using a Hypersil Gold C18 (20 mm × 250
211 mm, 5 μm, Thermo Fisher Scientific) equipped with a Hypersil Gold preparative C18 guard
212 cartridge (20 × 10 mm, 5 μm, Thermo Fisher Scientific). The mobile phase was a mixture of
213 ultrapure water (Eluent A) and acetonitrile (Eluent B), both containing 0.1% of formic acid.
214 The flow rate was set to 20 mL/min. Eluent B varied as follows: 0 min, 10%; 7.4 min, 10%;
215 44.2 min, 30%; 46.4 min, 98%; 59 min, 98%; 60 min, 10%; 66 min, 10%. Aliquots (20 mg) of
216 CPC fractions were dissolved in 400 μL of H₂O/MeOH 60:40 (v/v), 0.2 μm-filtered and
217 introduced manually into the system. UV detection was performed at 280 nm and
218 chromatographic peaks were collected manually just after the detector. The pure compound
219 solution was evaporated *in vacuo* to remove acetonitrile and freeze-dried to obtain a pale-
220 yellow amorphous powder (10.2 mg).

221 Brandy tannin A (TC1): pale-yellow amorphous powder; $[\alpha]_D^{25} - 77.8$ ($c = 0.1$, MeOH);
222 HRMS m/z 703.1143 $[M - H]^-$ (C₃₁H₂₇O₁₉⁻, 0.6 ppm); ¹H NMR [acetone-D₆/D₂O (9:1, v/v),
223 400 MHz] δ 1.09 (t, $J = 7.0$ Hz, 3H), 1.16 (t, $J = 7.1$ Hz, 3H), 3.47 (dq, $J = 9.3, 7.0$ Hz, 1H),
224 3.59 (dq, $J = 9.4, 7.0$ Hz, 1H), 3.77 (m, 1H), 3.86 (m, 1H), 4.18 (m, 2H), 4.28 (d, $J = 1.1$ Hz,
225 1H), 4.40 (dd, $J = 9.3, 6.7$ Hz, 1H), 4.90 (ddd, $J = 9.3, 4.3, 2.8$ Hz, 1H), 5.32 (d, $J = 6.7$ Hz,
226 1H), 5.40 (s, 1H), 5.48 (d, $J = 1.0$ Hz, 1H), 6.69 (s, 1H); ¹³C NMR [acetone-D₆/D₂O (9:1,
227 v/v), 100 MHz] δ 201.9, 170.1, 168.6, 168.0, 163.1, 157.9, 146.0, 145.5, 144.5 (2C), 142.6,
228 136.6, 135.4, 125.4, 125.0, 114.7, 113.5, 111.0, 108.1, 84.6, 80.3, 74.8, 72.7, 67.8 (2C), 65.2,
229 63.3, 61.9, 45.5, 15.3, 14.0 (Table 2 and Figure S6, supplementary data).

230 2.6.4. NMR experiments

231 NMR experiments were conducted on Bruker Avance II 400 and Avance III 600 NMR
232 spectrometers equipped with a 5 mm PA BBO and a 5 mm PA BBI probe, respectively. All
233 1D (proton, carbon, and DEPT-135) and 2D (COSY, HSQC, HMBC, and ROESY) spectra

234 were acquired at 298.15 K in a 9:1 (v/v) acetone-D₆/D₂O solvent mixture and were calibrated
235 using residual undeuterated acetone as an internal reference (¹H δ 2.05 ppm; ¹³C δ 29.8 ppm).
236 Proton, carbon, DEPT-135, COSY and HSQC spectra were obtained on the Bruker 400 MHz
237 spectrometer, and HMBC and ROESY spectra were obtained on the Bruker 600 MHz
238 spectrometer. The following abbreviations were used to describe the multiplicities: s = singlet,
239 d = doublet, t = triplet, q = quartet, m = multiplet. Data analysis was performed with Mnova
240 NMR version 14.2.0.

241 *2.7.Method validation for quantitation*

242 A stock solution of brandy tannin A (1 g/L) was prepared in methanol. One range of
243 calibration was prepared by successive dilutions of this solution in a non-oaked EDV adjusted
244 to 12% v/v with 0.1% of formic acid, in order to supply calibration samples (1 μg/L, 2 μg/L, 5
245 μg/L, 10 μg/L, 20 μg/L, 50 μg/L, 100 μg/L, 200 μg/L, 500 μg/L, 1 mg/L, 2 mg/L, 5 mg/L and
246 10 mg/L).

247 The validation method for quantitating brandy tannin A in spirits was performed by studying
248 linearity, sensitivity, specificity, intraday repeatability, and trueness. The LC-HRMS method
249 sensitivity was established using the approach described by De Paepe *et al.* (De Paepe *et al.*,
250 2013). Limit of detection (LOD) of a molecule is defined as the lowest concentration at which
251 a reliable and reproducible signal is observed. The signal must be different from a blank
252 performed under the same conditions. The lowest levels of the calibration curve (from 1 to 20
253 μg/L) were injected into five replicates. Limit of quantitation (LOQ) is defined as the lowest
254 concentration of the molecule that can be quantitatively determined by the method, with a
255 precision lower than 10% and an accuracy (recovery of back-calculated concentrations)
256 higher than 90%. The working range was based on the LOQ previously determined. A
257 calibration curve was determined by plotting the areas for each concentration level versus the
258 nominal concentration. Quadratic regression was used with a 1/x statistical weight. Linearity

259 was evaluated by correlation coefficient (R^2) and by deviations of each back-calculated
260 standard concentration from the nominal value. To determine intraday precision, five
261 replicates of three intermediate calibration solutions (10 $\mu\text{g/L}$, 200 $\mu\text{g/L}$ and 10 mg/L) were
262 injected, and the relative standard deviation (RSD%) was calculated. Trueness was checked
263 by calculating the recovery ratio (between measured and expected areas) from two samples of
264 EDV (EDV-1; EDV-2). They were chosen among the analysed samples and were spiked with
265 calibration solution corresponding to an addition of 20 $\mu\text{g/L}$, 200 $\mu\text{g/L}$ and 10 mg/L of brandy
266 tannin A. Interday repeatability was estimated by injections of the same two samples (10 $\mu\text{g/L}$
267 and 10 mg/L) for five successive days. Specificity was assessed by evaluating the mass
268 accuracy and retention time repeatability. These parameters were determined concomitantly
269 with the precision and trueness analysis described above.

270 *2.8. Sensory analysis*

271 Taste evaluation was performed in a dedicated room, at room temperature (around 20 °C)
272 (ISO 8589:2010, 2010) and with INAO normalized glass (ISO 3591:1977, 1977). Pure
273 compounds were tasted by five experts (four women, one man, aged from 24 to 54 years old)
274 in wine and spirits tasting, at 2 mg/L in demineralized water (eau de source de Montagne,
275 Laqueuille, France), as well as in a non-oaked EDV adjusted to 40% (v/v). Experts described
276 the gustatory perception (bitterness, sweetness, perception of burning and taste of fat) of the
277 targeted compound using the vocabulary of spirits tasting and were asked to evaluate the
278 intensity on a scale from 0 (not detectable) to 5 (strongly detectable). The panelists were
279 informed of the risks and nature of this study and were asked to give their consent to
280 participate in the sensory analyses. Even though the compound was purified from EDV of
281 cognac, the experts were advised to spit out the samples after tasting.

282

283 **3. Results and discussion**

284 3.1. Untargeted metabolomic analysis of spirits to select relevant compounds

285 An untargeted analysis by HRMS was achieved on a series of EDV of cognac of 10 different
286 vintages from 2015 to 1970 (Table S1, supplementary data), as described in a previous study
287 (Winstel et al., 2021). After the U-HPLC-HRMS analysis, the data were processed with the
288 use of MZmine 2 software. Thanks to these treatments, a peaklist of 42,120 negative ions was
289 obtained between m/z 100 to 1500, then filtered into a peaklist of 331 ions having an
290 associated data-dependent MS² spectrum. PCA of the data was carried out using the peak
291 areas of the 331 negative ions highlighted by the ANOVA. The vintage effect was clearly
292 significant on the first axis and the ANOVA showed that it was significant (p-value <0.05) for
293 321 compounds out of 331 (97%). The compounds were then divided into different groups
294 showing a similar trend to evolve according to the vintages, by using k-means clustering
295 followed by Pearson correlations. Of the 321 compounds detected in negative mode and
296 significantly influenced by the vintage, 298 were assigned to a group among the four created
297 (Figure S2, supplementary data). Groups 1, 3 and 4 represented 92% of the compounds,
298 whose concentrations were significantly influenced by the vintage and were generally more
299 abundant in older vintages. Group 2 was composed of 24 compounds whose contents
300 increased during 20 years of aging and then slowly decreased.

301 As in the previous study, the statistical groups (Figure S2, supplementary data) revealed the
302 presence of a wide diversity of molecules in these spirits. For most of them (274/298), the
303 contents were higher in aged spirits, while the opposite was observed for less than 8%. Such a
304 result could be explained by two phenomena: a continuous release of oak native compounds
305 during aging and/or the neoformation of molecules through chemical reactions involving oak
306 extractables. The aim of this study was to focus on compounds that were formed during spirits
307 aging, since they cannot be isolated by focusing on oak wood. Therefore, the peaklist obtained
308 by the MZmine analysis and the data from the statistical analysis were used to target new

309 natural products and attempt to purify them. The compounds of interest were selected
310 according to three criteria: a significant abundance of the targeted compound, a strong
311 increase in concentrations in old vintages, and a large gap in intensity between the
312 concentration in spirits and in oak wood extracts, which could suggest a neoformation rather
313 than an extraction.

314 First, the 298 compounds were classified in a table according to their intensity in the EDV of
315 cognac from 1979 to 2015 (data not shown). Then, they were screened by HRMS in the
316 analysed spirits and in oak wood extracts. Among all the chromatographic peaks, a compound
317 combining the previously defined criteria was observed and targeted for the rest of the study:
318 TC1 with a nominal mass of 703. Its concentration increased until 1995 and then slightly
319 decreased until 1970, while remaining abundant in the EDV of cognac. It was one of the 24
320 compounds present in group 2. XICs were built by targeting the negative ion at m/z 703.1143
321 in a 3-ppm window around its theoretical m/z . LC-HRMS screening revealed the presence of
322 TC1 only in spirits, which could be explained by a possible chemical reaction in the matrix
323 during aging (Figure S3, supplementary data). Consequently, its purification protocol was
324 carried out using the EDV of cognac.

325 *3.2. Isolation and Identification of TC1 in Spirits*

326 *3.2.1. Purification of TC1 from “eau-de-vie” of cognac*

327 Metabolomic profiling revealed that TC1 was present at higher levels in the EDV aged in
328 barrels for 20 years (Figure S4, supplementary data). Its purification was then carried out
329 from a blend of three EDVs aged in barrels for 19, 20 and 21 years, respectively. First, they
330 were evaporated to dryness to remove ethanol, which could interfere with subsequent
331 fractionation steps. After freeze-drying, the second step consisted of sequential liquid/liquid
332 extractions using MTBE, EtOAc and BuOH to obtain pre-purified extracts. TC1 was mainly
333 present in the BuOH extract, so this fraction was selected to continue the fractionation. The

334 resulting extract had a complex chromatographic profile with various peaks and co-elutions.
335 The use of the CPC was necessary to fractionate it and obtain a fraction enriched in
336 compound m/z 703. Preliminary tests showed that the ternary solvent system EtOAc/propan-
337 2-ol/H₂O (3:1:3, v/v/v) in ascending mode allowed the best partition of the sample. Since
338 many tubes were collected, fractions were constituted by grouping tubes together on the basis
339 of their LC-HRMS profiles. After solvent evaporation and freeze-drying, 10 fractions (noted
340 F-I to F-X) were obtained as powder in variable quantities. Fractions F-II, F-III and F-IV were
341 richer in TC1, so they were submitted to preparative HPLC with UV detection. A first
342 injection of 5 mg of each fraction revealed that the chromatograms exhibited a refined profile
343 with only a few peaks detected both in UV a 280 nm. Therefore, a suitable gradient was
344 chosen for each fraction and F-II, F-III and F-IV were fractionated by successive injections.
345 The peak corresponding to TC1 was collected manually just after UV detection for each
346 fraction to give 10.2 mg of a pale-yellow amorphous powder after acetonitrile removal and
347 freeze-drying.

348 3.2.2. *Structural elucidation of TC1*

349 The resolution, mass accuracy and stability offered by HRMS are particularly useful for the
350 determination of empirical formulas of unknown natural compounds. The HRMS spectrum of
351 TC1 exhibited a quasi-molecular $[M - H]^-$ ion at m/z 703.1143. Given the isotopic ratio
352 (around 35% abundance) and the experimental mass (with a delta of 0.6 ppm) of the
353 deprotonated ion, the empirical formula C₃₁H₂₈O₁₉ was assigned to TC1. To our knowledge,
354 no compound with this empirical formula has been described in the literature. To investigate
355 the nature and the sequence of the functional groups, fragmentation was performed on the
356 pure molecule by non-resonant activation in the higher collision dissociation (HCD) mode
357 with collision energy of 35 arbitrary units. The fragmentation of TC1 led to the formation of
358 many ions (Figure S5, supplementary data). The m/z 657.0731 ion, with the molecular

359 formula of $C_{29}H_{21}O_{18}^-$, corresponded to a species formed by the loss of a neutral group C_2H_6O
360 regarding the m/z 703.1143 ion. This group could correspond to a loss of ethanol. Likewise,
361 the negative m/z 639.0626 ion of the empirical formula $C_{29}H_{19}O_{17}^-$ corresponded to a
362 dehydration regarding the m/z 657.0731 ion. Furthermore, the fragmentation spectrum showed
363 the presence of a negative ion at m/z 523.0513 and could correspond to a species formed by
364 the loss of a $C_6H_{12}O_6$ group from the m/z 703.1143 ion, which is characteristic of a hexose. In
365 addition, an ion at m/z 169.0134 ($C_7H_5O_5^-$) was observed, which may correspond to a gallic
366 acid. The spectrum also exhibited an ion at m/z 300.9991 ($C_{14}H_5O_8^-$). This might reveal the
367 presence of the ellagic acid bislactone in the molecule or a structural unit from which this
368 bislactone could be derived. In addition, ions at m/z 249.0404 ($C_{12}H_9O_6^-$) and m/z 275.0195
369 ($C_{13}H_7O_7^-$) were detected. They may be 2,2',3,3',4,4'-hexahydroxybiphenyl and 3,4,8,9,10-
370 pentahydroxydibenzo[b,d]pyran-6-one, respectively. These latter three ions at m/z 301, 275
371 and 249 are generally characteristic fragments of the main C-glucosidic ellagitannins, such as
372 vescalagin and castalagin (Bowers et al., 2018). Therefore, by comparing the fragments
373 obtained with the data in the literature, TC1 could be a C-glucosidic ellagitannin (Engström et
374 al., 2015; Jourdes et al., 2011).

375
376 A full characterization by NMR was then carried out to identify the structure of TC1, which
377 was dissolved (5 mg) in a 9:1 (v/v) acetone- D_6 / D_2O solvent mixture (Figure S6,
378 supplementary data). The 1H NMR spectrum displayed only one aromatic signal resonating at
379 6.69 ppm, several signals in the downfield sector of the aliphatic chemical shift range between
380 about 3.5 and 5.5 ppm, which could be due to resonances of protons attached to sugar-type
381 oxygenated carbon atoms, and two diagnostic triplets just above 1 ppm, each integrating for
382 three protons. These two signals suggested the presence of two ethoxy units in the structure of
383 TC1, resulting from chemical transformations involving the spirit ethanol. The ^{13}C NMR

384 spectrum showed 29 distinct carbon resonances out of the 31 carbon atoms presumably
385 constituting TC1. The observation of two aliphatic carbon signals resonating at 14.0 and 15.3
386 ppm was in accordance with the presence of two ethyl groups, a finding further corroborated
387 by the attribution of three signals to (oxygenated) CH₂ carbon resonances at 61.9, 63.3 and
388 65.2 ppm in the DEPT-135 spectrum. One of these CH₂ signals could be attributed to the
389 carbon atom of the primary alcohol function of the glucosidic core of TC1 (*i.e.*, C6, Table 2).
390 The ¹³C NMR spectrum also displayed five signals resonating above 160 ppm, which could be
391 attributed to four carbonyl carbon atoms of ester functions (163.1, 168.0, 168.6 and 170.1
392 ppm), and a fifth much further downfield signal (201.9 ppm) to a ketone carbon atom.

393
394 Our hypothesis concerning the *C*-glucosidic ellagitannin nature of TC1 was then further
395 challenged by performing standard 2D NMR correlation analyses (*i.e.*, COSY, HSQC,
396 HMBC). The proton signals of the presumed open-chain glucosidic core were assigned on the
397 basis of ¹H-¹H COSY data, showing ³*J* correlations between H1 and H2 (weak), H3 and H4
398 (strong), H4 and H5 (strong), H5 and the H6's (strong). The latter two diastereotopic protons
399 H6a and H6b resonated at 3.77 and 3.86 ppm, whose signals overlapped that of the residual
400 undeuterated water solvent. The COSY data map also revealed the presence of an ethoxy
401 group through a correlation between the methyl protons at 1.16 ppm (t, *J* = 7.1 Hz) and
402 methylene protons at 4.18 ppm, and that of another ethoxy group through a correlation
403 between the methyl protons at 1.09 ppm (t, *J* = 7.0 Hz) and two signals of similar multiplicity
404 at 3.47 and 3.59 ppm (dq, *J* = 9.4, 7.0 Hz). These two signals, each integrating for one proton,
405 indicated that they emanate from diastereotopic methylene protons. The same type of signals
406 was also observed in the ¹H spectrum of the previously described β-1-*O*-ethylvescalagin
407 (Quideau et al., 2005). Moreover, no correlation was observed with the proton signal
408 resonating at 5.40 ppm. The signals of protonated carbon atoms could then be assigned on the

409 basis of ^1H - ^{13}C 1J HSQC data, which notably indicated that C1 and C4 of the glucosidic core
410 of TC1 would have the same chemical shift at 67.8 ppm. Finally, the analysis of the ^1H - ^{13}C
411 $^2J/^3J$ HMBC data map enabled us to determine the most likely structure of TC1. A 3J
412 correlation between C1 and the methylene H1" protons at 3.47 and 3.59 ppm confirmed the
413 presence of one of the two ethoxy groups on the open-chain glucosidic core, as in the case of
414 β -1-*O*-ethylvescalagin (Quideau et al., 2005). The corollary 3J correlation between H1 at 4.28
415 ppm and the methylene C1" at 65.2 ppm was also observed.

416 The other set of methylene H1'" protons resonating at 4.18 ppm were found to correlate with
417 the ester carbon atom at 170.1 ppm. Acylation of the hydroxy groups at C2, C3 and C5 of the
418 glucosidic core of TC1 by galloyl-derived units was evidenced by 3J correlations between H2,
419 H3, H5 and the carbonyl CI (163.1 ppm), CII (168.0 ppm), CIII (168.6 ppm), respectively.

420 However, the more upfield shift of the carbonyl CI cast doubt on the galloyl nature of the unit
421 bearing it. Moreover, several remaining carbon signals resonating at 201.9 ppm (ketonic),
422 157.9 and 142.6 ppm (olefinic), 84.6 and 45.5 (aliphatic) remained to be assigned. In fact, it is
423 the aforementioned single proton resonance at 5.40 ppm that was the keystone of this
424 structural determination, since this H5'_I proton, which is attached to the aliphatic C5'_I
425 resonating at 45.5 ppm (HSQC data), correlated with the ketonic C3'_I at 201.9 ppm, the
426 olefinic C1'_I and C2'_I at 142.6 and 157.9 ppm, and the tertiary alcoholic C4'_I at 84.6 ppm. In
427 addition, a 3J correlation between H5'_I and the ester carbonyl at 170.1 ppm was also observed.

428 The proximity of H5'_I with the galloyl-derived unit II was evidenced by 2J and 3J correlations
429 to C-1'_{II}, C-2'_{II}, and C-3'_{II}. Furthermore, the olefinic C1'_I showed a 3J correlation with H1 and
430 a surprisingly strong 4J correlation with H2 through the ester linkage, and the olefinic C2'_I
431 showed a 2J correlation with H1.

432 All these correlation data suggested that the unit bearing the upfield (α,β -unsaturated) ester
433 carbonyl CI resonating at 163.1 ppm was a cyclopentenone moiety. The position of the ketone

434 function was established by observing a 3J correlation between H1 and the carbonyl C3₁ at
435 201.9 ppm. The cyclopentenone nature of ring I was confirmed by comparing the chemical
436 shifts of its protons and carbons, and resonance correlations thereof, with those of the same
437 moiety in whiskey tannin A (Fujieda et al., 2008). In fact, our TC1 is an analogue of whiskey
438 tannin A, although it is likely not derived from castalagin but rather from its C1-epimer
439 vescalagin. The 3J coupling constant between H1 and H2 has a small value of about 1 Hz,
440 which implies a dihedral angle close to 90° between these two protons and is hence indicative
441 of its α -orientation at C1 (Quideau et al., 2005), whereas the same coupling constant in
442 whiskey tannin A has a value of 3.0 Hz (Fujieda et al., 2008). Furthermore, the ROESY
443 through-space correlation data map showed signals between H1 and H2, as well as H3, which
444 are also consistent with an α -orientation of H1 (Figure 6, supplementary data). The
445 configurations of the C4' and C5' centres of the cyclopentenone ring I could not be
446 unambiguously determined, but ROESY correlations between H5'₁ and the ethoxy protons of
447 the ester function at C4'₁ suggest that H5'₁ and this ester function are *syn*-oriented to one
448 another. The absence of through-space correlations between H5'₁ and H1 and/or H2 cannot be
449 used as a strong argument to confirm the β -orientation of H5'₁, especially since the correlation
450 between H5'₁ and the β -oriented H1 was also not observed in the NOESY data of whiskey
451 tannin A (Fujieda et al., 2008). Altogether, the interpretation of our NMR data and the
452 comparison with literature data on analogous compounds led us to propose the structure
453 displayed in Figure 1 and Table 2 for TC1, which we name brandy tannin A in reference to
454 the matrix in which it was identified for the first time.

455

456 Besides the configuration at C1, the other main difference between Tanaka's whiskey tannin
457 A and our brandy tannin A is the presence of an ethoxy group at this same C1 centre. The
458 formation of brandy tannin A likely begins with the installation of this ethoxy group onto a

459 starting vescalagin in the ethanol-rich brandy solution (Figure 1). Such a formation of the
460 resulting β -1-*O*-ethylvescalagin from vescalagin in ethanol was previously described as a
461 relatively fast, high-yielding and diastereoselective nucleophilic substitution reaction strictly
462 occurring with retention of configuration at C1 under standard solvolysis conditions (Quideau
463 et al., 2005). The second step of its formation is probably the oxidative dehydrogenation of
464 the galloyl-I group leading to the α -hydroxy-*ortho*-quinone **A**, which can then be subjected to
465 the nucleophilic addition of ethanol at its most electrophilic carbonyl group. The resulting
466 dienolic hemiketal **B** might then tautomerize to produce the enonic hemiketal **C**, which can
467 then undergo a ring contraction *via* a benzilic acid-type rearrangement that forges the C–C
468 bond between C3'1 and C4'1. Thus, this transformation gives rise to the formation of a
469 cyclopentenonic ethyl ester, as previously proposed for the formation of whiskey tannins
470 (Fujieda et al., 2008). Similar dehydrogenation-mediated contractions of ellagitannin galloyl
471 rings into cyclopentene rings have also been previously reported (Petit et al., 2013; Tanaka et
472 al., 1990; Wakamatsu et al., 2020). The proposed vescalagin-derived cyclopentenone is in fact
473 the β -1-*O*-ethyl ether analogue of whiskey tannin B, hereafter referred to as brandy tannin B
474 (Figure 1). This compound was not observed during our analyses, even though brandy tannin
475 A certainly derives from it. In the hydroalcoholic brandy solution, the solvolytic cleavage of
476 its hexahydroxydiphenoyl (HHDP) unit would slowly lead to the formation of brandy tannin
477 A (TC1).

478 3.2.3. *Gustatory properties of brandy tannin A*

479 Brandy tannin A was then dissolved in water and in a non-oaked EDV at 2 mg/L, and the taste
480 of each solution was characterized in comparison to the same water/EDV as a reference.
481 Quercotriterpenoside I was used as a sweetness standard since its sensory properties have
482 already been characterized (Marchal, Waffo-Téguo, et al., 2011). In water, brandy tannin A
483 exhibited a slight taste of fat, no sweetness, and no bitterness. On a 0–5 scale representing

484 relative taste of fat and sweetness intensity assessed as a consensus between the five panelists,
485 brandy tannin A scored 2/5 and 0/5, respectively, and QTT I was assessed as 0/5 and 3/5,
486 respectively. Brandy tannin A was also dissolved in non-oaked EDV to study its influence on
487 the taste balance of spirits. The control EDV was scored 0/5 for sweetness, bitterness and taste
488 of fat, but 5/5 for the perception of burning. As a reference, EDV spiked with QTT I (2 mg/L)
489 was described as sweeter (4/5) and less burning (2/5). Brandy tannin A also modified the taste
490 of the EDV by significantly decreasing the perception of burning (1/5) and by significantly
491 increasing that of sweetness (4/5).

492 The results suggested that brandy tannin A developed a taste of fat at 2 mg/L in water, which
493 modulated the perception of burning of the EDV of cognac and hence improved its overall
494 taste balance. Moreover, its taste intensity was close to that of QTT I, whose gustatory
495 detection threshold is relatively low for non-volatile compounds (i.e. 590 µg/L in wine
496 (Gammacurta et al., 2019), which is much lower than that of glucose, i.e., 4 g/L (Ribéreau-
497 Gayon et al., 2017).

498 Koga *et al.* found a positive correlation between the antioxidant activity and the aging time of
499 commercial whiskeys (Koga et al., 2007). In spirits, longer aging leads to a higher
500 concentration of phenols, especially ellagic and gallic acids and lyoniresinol (Koga et al.,
501 2007; Winstel & Marchal, 2019). These compounds play an important role in the taste of
502 whiskey thanks to ROS (Reactive Oxygen Scavenging) and SOD (Superoxide Dismutase)-
503 like activities (Koga et al., 2011). However, they have mostly been described as bitter
504 (Marchal, Cretin, et al., 2015; Purwayanti, 2013), so this could not explain why spirits are
505 known to improve during oak wood aging. Koga *et al.* also considered that there was a
506 component of spirits which had ROS activity that offered a comfortable aftertaste rather than
507 an unpleasant one (Koga et al., 2007). Thus, identification of brandy tannin A could provide a
508 better understanding of the taste balance of spirits aged for a long time in barrels.

509 *3.3. Development of an LC-HRMS method to assay brandy tannin A in spirits*

510 From a chemical point of view, spirits are complex matrices with thousands of molecules.
511 Consequently, specific powerful instruments are required to study their composition. Owing
512 to its mass measurement accuracy and its wide dynamic range, LC-HRMS appeared to be a
513 reliable technique to quantify brandy tannin A in spirits. To avoid strong matrix effects,
514 absolute quantitation was carried out by preparing calibration solutions of brandy tannin A in
515 a non-oaked EDV adjusted to 12% (v/v) with 0.1% of formic acid. In this study, LOD and
516 LOQ were established at 1 µg/L and 2 µg/L, respectively. A calibration curve was obtained
517 with a good correlation coefficient (R^2 of 0.999) for a range from 2 µg/L to 10 mg/L, this
518 validating the linearity of the method. Moreover, all the samples had concentrations that were
519 in the working range, which confirmed the relevance of the latter. The recovery of back-
520 calculated concentrations was higher than 90% at each method calibration level, thus
521 establishing the accuracy of the method. Intraday repeatability for each concentration was
522 lower than 4.2%. Interday repeatability was not as good at low concentrations (up to 16% at
523 10 µg/L) but efficient at 10 mg/L (<5%). To overcome this issue, all calibration solutions
524 were injected for each quantitative analysis of an unknown sample. Two spirits spiked with
525 stock solutions were also injected. Recovery ratios ranged from 94 to 105%, which is in
526 accordance with common specifications (Guidance for Industry, 2018). Consequently, these
527 results established the repeatability and the trueness of the method applied to spirits. Analysis
528 of the above samples revealed small variations in retention time (<0.02 min) and a mass
529 deviation lower than 0.9 ppm at various concentrations, guaranteeing the specificity of the
530 method. All these results validated the LC-HRMS method to quantitate brandy tannin A in
531 spirits (Table 3).

532 *3.4. Quantification of brandy tannin A in spirits*

533 *3.4.1. Evolution of brandy tannin A over 8 years*

534 Brandy tannin A was quantitated in samples of EDV of cognac of nine different vintages from
535 the same distillery (Table S7, Supplementary data). The samples were not commercial cognac
536 but EDV which have been aged in barrels since 2010. A sample was collected each year from
537 the same barrel from 2010 to 2018, so the 2011 sample corresponds to one year of aging in
538 barrels, the one of 2012 to 2 years and so on. Brandy tannin A was detected and quantitated in
539 all spirits at a concentration of 100 $\mu\text{g/L}$ for the sample aged for 1 year in barrels. This result
540 suggested that it was formed quite quickly after the beginning of aging. The contents of
541 brandy tannin A were higher in old vintages, reaching a concentration of 2 mg/L for the oldest
542 sample which had been aged in barrels for 8 years (Figure 2, A). Long barrel aging appeared
543 to promote the formation of brandy tannin A. Moreover, its reaction rate appeared to be
544 proportional to its concentration and could be compared to first-order kinetics.

545 *3.4.2. Content of brandy tannin A in various vintages of same spirits*

546 Brandy tannin A was also assayed in the samples of EDV of cognac previously used for
547 untargeted metabolomic analysis (Table S8, Supplementary data). The concentrations in
548 Figure 2 correspond to the mean values of the five replicates for the spirits from 2015 to 1990,
549 and of four replicates for the last two vintages (Figure 2, B). The measured values ranged
550 from 0.4 mg/L (2015) to 4.2 mg/L (1995). For each vintage, the coefficient of variation
551 between the replicates was relatively low (from 12.7% to 36.6%), so the heterogeneity
552 between barrels was not too high, except for the 2005 vintage (47.2%). The evolution of
553 concentrations for the vintages from 2015 to 2005 was consistent with that of the first series
554 of spirits. However, brandy tannin A concentrations seemed to follow a bell-shaped curve;
555 low in the 2015 sample (0.4 mg/L), maximal in the 1995 sample (4.2 mg/L) and lower in
556 older vintages (e.g. 0.7 mg/L for the 1970 vintage). These results were consistent with its
557 relative quantitation (Figure S4, Supplementary data) obtained by the untargeted metabolomic
558 approach, in which the same trend was observed. Even if this was not a strict kinetic study as

559 in the first series of EDV of cognac, this suggested its degradation with barrel aging.
560 However, this hypothesis needs to be studied more deeply, since the results could also have
561 been due to modifications of aging practices in the distillery or to changes in barrel supplies.

562 Moreover, six of the ten vintages had concentrations greater than 2 mg/L. Sensory
563 studies showed significant taste modifications at this concentration, thus demonstrating its
564 contribution to the taste balance of these spirits. Spirits are known to improve during oak
565 wood aging and brandy tannin A might play a key role in modulating their taste balance.

566 *3.4.3. Content of brandy tannin A in various commercial spirits*

567 Thirty-six commercial spirits were also analysed to measure the concentration of brandy
568 tannin A (Table S9, Supplementary data). It was detected in almost all cognacs at
569 concentrations ranging from 0.03 to 7.7 mg/L but also in two brandies, two whiskeys and one
570 rum, in smaller quantities (from 0.01 to 0.4 mg/L) (Figure 3). In addition, two Japanese
571 whiskeys (W-6 and W-7, Figure 3) were analysed since the whiskey tannins A and B have
572 already been purified from this kind of spirits (Fujieda et al., 2008). Results showed very low
573 levels of brandy tannin A (< 13 µg/L) in these spirits. The higher brandy tannin A
574 concentration in the C-7 sample could be due to the significant addition of “boisé” (aqueous
575 extract of oak wood chips) to this spirit, which is permitted by law for some brandies. The
576 differences in concentration between the other spirits could be due to the botanical origin of
577 the wood used for aging. Bourbons are aged in American oak barrels, while cognacs and
578 brandies are generally aged in French sessile or pedunculate oak barrels. In addition, this
579 result did not seem surprising since American oaks are known to have much lower
580 concentrations of ellagitannins than pedunculate oak (Chatonnet & Dubourdieu, 1988).
581 Additional studies will be necessary to validate this hypothesis. The influence of cooperage
582 parameters such as the botanical origin of oak wood on brandy tannin A concentrations could

583 be studied. A better control of this parameter could improve the monitoring of oak wood
584 aging and its sensory effect.

585 **4. Conclusion**

586 This study focused on discovering new taste-active compounds formed during spirits aging in
587 barrels. For this purpose, an untargeted metabolomic profiling by HRMS in negative mode
588 was performed on EDV of cognac from several vintages. After statistical analysis, TC1 was
589 found to be significantly more abundant in spirits than in oak wood, which could suggest its
590 neoformation. After the development of a fractionation protocol, brandy tannin A (*i.e.*, TC1)
591 was identified and purified from a blend of old EDVs of cognac. To our knowledge, its
592 identification, its presence in spirits, mostly in cognacs, and its sensory properties have never
593 been described until now. Moreover, its impact on the spirits taste balance was perceived
594 more strongly by decreasing the burning perception. By determining its gustatory detection
595 threshold, it might be possible to establish its influence during aging on the taste balance of
596 old spirits. It would also be interesting to measure its ROS activity to attest to its comfortable
597 aftertaste. Its concentrations in several EDV of cognac seemed to follow a bell-shaped curve,
598 suggesting the competition of two phenomena: its formation from a native oak precursor and
599 its degradation. In both cases, it will be necessary to clarify the chemical species involved, the
600 reaction mechanisms and the factors that could influence their evolution. The present findings
601 illustrate the efficiency of our novel method, which allowed the purification of a new
602 ellagitannin from highly complex mixtures. In future work, such a strategy could be used to
603 reveal new sensory-active products in natural matrices.

604 **Acknowledgements**

605 Delphine Winstel grant is funded by Remy-Martin and Seguin-Moreau. The authors are also
606 grateful to J.-C. Mathurin and L. Urruty for providing samples. We thank Dr. Ray Cooke for
607 proofreading the manuscript.

608

609 **Competing interest statement**

610 The authors declare no competing financial interest.

611

612 **Author contributions**

613 **Delphine Winstel:** Conceptualization, Methodology, Investigation, Validation, Writing –
614 Original Draft

615 **Yoan Capello:** Conceptualization, Validation, Writing – Original Draft

616 **Stéphane Quideau:** Conceptualization, Validation, Writing – Review and editing,
617 Visualization

618 **Axel Marchal:** Conceptualization, Validation, Writing – Review and editing, Supervision,
619 Funding acquisition

620

621 **References**

- 622 Auzanneau, C., Montaudon, D., Jacquet, R., Puyo, S., Pouységu, L., Deffieux, D.,
623 Elkaoukabi-Chaibi, A., De Giorgi, F., Ichas, F., Quideau, S., & Pourquier, P. (2012).
624 The Polyphenolic Ellagitannin Vescalagin Acts As a Preferential Catalytic Inhibitor of
625 the α Isoform of Human DNA Topoisomerase II. *Molecular Pharmacology*, 82(1),
626 134–141. <https://doi.org/10.1124/mol.111.077537>
- 627 Bowers, J. J., Gunawardena, H. P., Cornu, A., Narvekar, A. S., Richieu, A., Deffieux, D.,
628 Quideau, S., & Tharayil, N. (2018). Rapid Screening of Ellagitannins in Natural
629 Sources via Targeted Reporter Ion Triggered Tandem Mass Spectrometry. *Scientific*
630 *Reports*, 8(1), 1–10. <https://doi.org/10.1038/s41598-018-27708-3>
- 631 Cardullo, N., Muccilli, V., Pulvirenti, L., Cornu, A., Pouységu, L., Deffieux, D., Quideau, S.,
632 & Tringali, C. (2020). C-glucosidic ellagitannins and galloylated glucoses as potential
633 functional food ingredients with anti-diabetic properties: A study of α -glucosidase and
634 α -amylase inhibition. *Food Chemistry*, 313, 126099.
635 <https://doi.org/10.1016/j.foodchem.2019.126099>
- 636 Chassaing, S., Lefeuvre, D., Jacquet, R., Jourdes, M., Ducasse, L., Galland, S., Grelard, A.,
637 Saucier, C., Teissedre, P.-L., Dangles, O., & Quideau, S. (2010). Physicochemical
638 Studies of New Anthocyano-Ellagitannin Hybrid Pigments: About the Origin of the
639 Influence of Oak C-Glycosidic Ellagitannins on Wine Color. *European Journal of*
640 *Organic Chemistry*, 2010(1), 55–63. <https://doi.org/10.1002/ejoc.200901133>
- 641 Chatonnet, P., & Dubourdieu, D. (1988). Comparative Study of the Characteristics of
642 American White Oak (*Quercus alba*) and European Oak (*Quercus petraea* and *Q.*
643 *robur*) for Production of Barrels Used in Barrel Aging of Wines | American Journal of
644 Enology and Viticulture. *Am. J. Enol. Vitic.*, 49(1), 79–85.

645 Chira, K., Zeng, L., Le Floch, A., Péchamat, L., Jourdes, M., & Teissedre, P.-L. (2015).
646 Compositional and sensory characterization of grape proanthocyanidins and oak wood
647 ellagitannin. *Tetrahedron*, *71*(20), 2999–3006.
648 <https://doi.org/10.1016/j.tet.2015.02.018>

649 Cretin, B. N., Waffo-Teguo, P., Dubourdieu, D., & Marchal, A. (2019). Taste-guided isolation
650 of sweet-tasting compounds from grape seeds, structural elucidation and identification
651 in wines. *Food Chemistry*, *272*, 388–395.
652 <https://doi.org/10.1016/j.foodchem.2018.08.070>

653 De Paepe, D., Servaes, K., Noten, B., Diels, L., De Loose, M., Van Droogenbroeck, B., &
654 Voorspoels, S. (2013). An improved mass spectrometric method for identification and
655 quantification of phenolic compounds in apple fruits. *Food Chemistry*, *136*(2), 368–
656 375. <https://doi.org/10.1016/j.foodchem.2012.08.062>

657 Engström, M. T., Pälijärvi, M., & Salminen, J.-P. (2015). Rapid Fingerprint Analysis of Plant
658 Extracts for Ellagitannins, Gallic Acid, and Quinic Acid Derivatives and Quercetin-,
659 Kaempferol- and Myricetin-Based Flavonol Glycosides by UPLC-QqQ-MS/MS.
660 *Journal of Agricultural and Food Chemistry*, *63*(16), 4068–4079.
661 <https://doi.org/10.1021/acs.jafc.5b00595>

662 Fayad, S., Le Scanff, M., Waffo-Teguo, P., & Marchal, A. (2021). Understanding sweetness
663 of dry wines: First evidence of astilbin isomers in red wines and quantitation in a one-
664 century range of vintages. *Food Chemistry*, *352*, 129293.
665 <https://doi.org/10.1016/j.foodchem.2021.129293>

666 Frank, O., Zehentbauer, G., & Hofmann, T. (2006). Bioresponse-guided decomposition of
667 roast coffee beverage and identification of key bitter taste compounds. *European Food*
668 *Research and Technology*, *222*(5), 492. <https://doi.org/10.1007/s00217-005-0143-6>

669 Fujieda, M., Tanaka, T., Suwa, Y., Koshimizu, S., & Kouno, I. (2008). Isolation and Structure
670 of Whiskey Polyphenols Produced by Oxidation of Oak Wood Ellagitannins. *Journal*
671 *of Agricultural and Food Chemistry*, 56(16), 7305–7310.
672 <https://doi.org/10.1021/jf8012713>

673 Gadrat, M., Lavergne, J., Emo, C., Teissedre, P.-L., & Chira, K. (2020). Validation of a mass
674 spectrometry method to identify and quantify ellagitannins in oak wood and cognac
675 during aging in oak barrels. *Food Chemistry*, 128223.
676 <https://doi.org/10.1016/j.foodchem.2020.128223>

677 Gammacurta, M., Waffo-Teguo, P., Winstel, D., Cretin, B. N., Sindt, L., Dubourdieu, D., &
678 Marchal, A. (2019). Triterpenoids from *Quercus petraea*: Identification in Wines and
679 Spirits and Sensory Assessment. *Journal of Natural Products*, 82(2), 265–275.
680 <https://doi.org/10.1021/acs.jnatprod.8b00682>

681 Georgess, D., Spuul, P., Le Clainche, C., Le Nihouannen, D., Fremaux, I., Dakhli, T.,
682 Delannoy López, D. M., Deffieux, D., Jurdic, P., Quideau, S., & Génot, E. (2018).
683 Anti-osteoclastic effects of C-glucosidic ellagitannins mediated by actin perturbation.
684 *European Journal of Cell Biology*, 97(8), 533–545.
685 <https://doi.org/10.1016/j.ejcb.2018.09.003>

686 Glabasnia, A., & Hofmann, T. (2006). Sensory-Directed Identification of Taste-Active
687 Ellagitannins in American (*Quercus alba* L.) and European Oak Wood (*Quercus*
688 *robur* L.) and Quantitative Analysis in Bourbon Whiskey and Oak-Matured Red
689 Wines. *Journal of Agricultural and Food Chemistry*, 54(9), 3380–3390.
690 <https://doi.org/10.1021/jf052617b>

691 Guidance for Industry. (2018). Bioanalytical Method Validation. *U.S. Department of Health*
692 *and Human Services, Food and Drug Administration, Center for Drug Evaluation and*
693 *Research (CDER), Center for Veterinary Medicine (CVM).*

694 ISO 3591:1977. (1977). Analyse sensorielle—Appareillage—Verre à dégustation pour
695 l'analyse sensorielle des vins. In *Analyse sensorielle: Recueil, normes,*
696 *agroalimentaire*. <https://www.iso.org/fr/standard/9002.html>

697 ISO 8589:2010. (2010). Analyse sensorielle—Directives générales pour la conception de
698 locaux destinés à l'analyse. In *Analyse sensorielle: Recueil, normes, agroalimentaire.*
699 AFNOR. [https://www.boutique.afnor.org/norme/nf-en-iso-8589/analyse-sensorielle-](https://www.boutique.afnor.org/norme/nf-en-iso-8589/analyse-sensorielle-directives-generales-pour-la-conception-de-locaux-destines-a-l-analyse/article/784220/fa165403)
700 [directives-generales-pour-la-conception-de-locaux-destines-a-l-](https://www.boutique.afnor.org/norme/nf-en-iso-8589/analyse-sensorielle-directives-generales-pour-la-conception-de-locaux-destines-a-l-analyse/article/784220/fa165403)
701 [analyse/article/784220/fa165403](https://www.boutique.afnor.org/norme/nf-en-iso-8589/analyse-sensorielle-directives-generales-pour-la-conception-de-locaux-destines-a-l-analyse/article/784220/fa165403)

702 Jourdes, M., Michel, J., Saucier, C., Quideau, S., & Teissedre, P.-L. (2011). Identification,
703 amounts, and kinetics of extraction of C-glucosidic ellagitannins during wine aging in
704 oak barrels or in stainless steel tanks with oak chips. *Analytical and Bioanalytical*
705 *Chemistry*, *401*(5), 1531–1539. <https://doi.org/10.1007/s00216-011-4949-8>

706 Kinghorn, A. D. (1987). Biologically Active Compounds from Plants with Reputed Medicinal
707 and Sweetening Properties. *Journal of Natural Products*, *50*(6), 1009–1024.
708 <https://doi.org/10.1021/np50054a002>

709 Koga, K., Tachihara, S., Shirasaka, N., Yamada, Y., & Koshimizu, S.-I. (2011). Profile of
710 non-volatiles in whisky with regard to superoxide dismutase activity. *Journal of*
711 *Bioscience and Bioengineering*, *112*(2), 154–158.
712 <https://doi.org/10.1016/j.jbiosc.2011.04.002>

713 Koga, K., Taguchi, A., Koshimizu, S., Suwa, Y., Yamada, Y., Shirasaka, N., & Yoshizumi,
714 H. (2007). Reactive Oxygen Scavenging Activity of Matured Whiskey and Its Active
715 Polyphenols. *Journal of Food Science*, *72*(3), S212–S217.
716 <https://doi.org/10.1111/j.1750-3841.2007.00311.x>

717 Marchal, A., Cretin, B. N., Sindt, L., Waffo-Téguo, P., & Dubourdiou, D. (2015).
718 Contribution of oak lignans to wine taste: Chemical identification, sensory

719 characterization and quantification. *Tetrahedron*, 71(20), 3148–3156.
720 <https://doi.org/10.1016/j.tet.2014.07.090>

721 Marchal, A., Génin, E., Waffo-Téguo, P., Bibès, A., Da Costa, G., Mérillon, J.-M., &
722 Dubourdieu, D. (2015). Development of an analytical methodology using Fourier
723 transform mass spectrometry to discover new structural analogs of wine natural
724 sweeteners. *Analytica Chimica Acta*, 853, 425–434.
725 <https://doi.org/10.1016/j.aca.2014.10.039>

726 Marchal, A., Marullo, P., Moine, V., & Dubourdieu, D. (2011). Influence of Yeast
727 Macromolecules on Sweetness in Dry Wines: Role of the *Saccharomyces cerevisiae*
728 Protein Hsp12. *Journal of Agricultural and Food Chemistry*, 59(5), 2004–2010.
729 <https://doi.org/10.1021/jf103710x>

730 Marchal, A., Pons, A., Lavigne, V., & Dubourdieu, D. (2013). Contribution of oak wood
731 ageing to the sweet perception of dry wines: Effect of oak ageing on wine sweetness.
732 *Australian Journal of Grape and Wine Research*, 19(1), 11–19.
733 <https://doi.org/10.1111/ajgw.12013>

734 Marchal, A., Waffo-Téguo, P., Génin, E., Mérillon, J.-M., & Dubourdieu, D. (2011).
735 Identification of New Natural Sweet Compounds in Wine Using Centrifugal Partition
736 Chromatography–Gustatometry and Fourier Transform Mass Spectrometry. *Analytical*
737 *Chemistry*, 83(24), 9629–9637. <https://doi.org/10.1021/ac202499a>

738 Petit, E., Lefeuvre, D., Jacquet, R., Pouységu, L., Deffieux, D., & Quideau, S. (2013).
739 Remarkable Biomimetic Chemoselective Aerobic Oxidation of Flavano-Ellagitannins
740 Found in Oak-Aged Wine. *Angewandte Chemie International Edition*, 52(44), 11530–
741 11533. <https://doi.org/10.1002/anie.201305839>

742 Pluskal, T., Castillo, S., Villar-Briones, A., & Orešič, M. (2010). MZmine 2: Modular
743 framework for processing, visualizing, and analyzing mass spectrometry-based

744 molecular profile data. *BMC Bioinformatics*, 11(1), 395. <https://doi.org/10.1186/1471->
745 2105-11-395

746 Puech, J. L., Rabier, P., Bories-Azeau, J., Sarni, F., & Moutounet, M. (1990). Determination
747 of ellagitannins in extracts of oak wood and in distilled beverages matured in oak
748 barrels. *Journal - Association of Official Analytical Chemists*, 73(4), 498–501.

749 Purwayanti, S. (2013). Taste Compounds from Crude Extract of Bekkai Ian (*Albertia*
750 *papuana* Becc.). *Journal of Food and Nutrition Sciences*, 1(4), 33.
751 <https://doi.org/10.11648/j.jfns.20130104.11>

752 Quideau, S., Jourdes, M., Lefeuvre, D., Montaudon, D., Saucier, C., Glories, Y., Pardon, P.,
753 & Pourquier, P. (2005). The Chemistry of Wine Polyphenolic C-Glycosidic
754 Ellagitannins Targeting Human Topoisomerase II. *Chemistry - A European Journal*,
755 11(22), 6503–6513. <https://doi.org/10.1002/chem.200500428>

756 Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2017). *Traité*
757 *d'oenologie—Tome 2—7e éd. - Chimie du vin. Stabilisation et traitements*. Dunod.

758 Roullier-Gall, C., Signoret, J., Hemmler, D., Witting, M. A., Kanawati, B., Schäfer, B.,
759 Gougeon, R. D., & Schmitt-Kopplin, P. (2018). Usage of FT-ICR-MS Metabolomics
760 for Characterizing the Chemical Signatures of Barrel-Aged Whisky. *Frontiers in*
761 *Chemistry*, 6. <https://doi.org/10.3389/fchem.2018.00029>

762 Saucier, C., Jourdes, M., Glories, Y., & Quideau, S. (2006). Extraction, Detection, and
763 Quantification of Flavano-Ellagitannins and Ethylvescalagin in a Bordeaux Red Wine
764 Aged in Oak Barrels. *Journal of Agricultural and Food Chemistry*, 54(19), 7349–
765 7354. <https://doi.org/10.1021/jf061724i>

766 Tanaka, T., Nonaka, G., & Nishioka, I. (1990). Tannins and Related Compounds. C.:
767 Reaction of Dehydrohexahydroxydiphenic Acid Esters with Bases, and Its Application

768 to the Structure Determination of Pomegranate Tannins, Granatins A and B. *Chemical*
769 *& Pharmaceutical Bulletin*, 38(9), 2424–2428. <https://doi.org/10.1248/cpb.38.2424>

770 Viriot, C., Scalbert, A., Lapierre, C., & Moutounet, M. (1993). Ellagitannins and lignins in
771 aging of spirits in oak barrels. *Journal of Agricultural and Food Chemistry*, 41(11),
772 1872–1879.

773 Wakamatsu, H., Matsuo, Y., Omar, M., Saito, Y., Nishida, K., & Tanaka, T. (2020).
774 Oxidation of the Oak Ellagitannin, Vescalagin. *Journal of Natural Products*, 83(2),
775 413–421. <https://doi.org/10.1021/acs.jnatprod.9b00917>

776 Winstel, D., Bahammou, D., Albertin, W., Waffo-Téguo, P., & Marchal, A. (2021).
777 Untargeted LC–HRMS profiling followed by targeted fractionation to discover new
778 taste-active compounds in spirits. *Food Chemistry*, 359, 129825.
779 <https://doi.org/10.1016/j.foodchem.2021.129825>

780 Winstel, D., Gautier, E., & Marchal, A. (2020). Role of Oak Coumarins in the Taste of Wines
781 and Spirits: Identification, Quantitation, and Sensory Contribution through Perceptive
782 Interactions. *Journal of Agricultural and Food Chemistry*, acs.jafc.0c02619.
783 <https://doi.org/10.1021/acs.jafc.0c02619>

784 Winstel, D., & Marchal, A. (2019). Lignans in Spirits: Chemical Diversity, Quantification,
785 and Sensory Impact of (±)-Lyoniresinol. *Molecules*, 24(1), 117.
786 <https://doi.org/10.3390/molecules24010117>

787

788 **Abbreviations**

789 EDV : “eau-de-vie”

790 PCA: Principal component analysis

791 TC1: Targeted Compound 1

792 LOD: limit of detection

793 LOQ: limit of quantitation

794 **Appendix A. Supplementary data**

795 **Table S1:** Features of “eaux-de-vie” of cognac used for untargeted LC-HRMS approach.

796

797 **Figure S2:** Representation of different groups of compounds according to their evolution in
798 48 “eaux-de-vie” of cognac.

799

800 **Figure S3:** Negative LC-ESI-FTMS XIC of an oaked “eau-de-vie” of cognac (A, on the left),
801 an oak wood extract (B, on the right) corresponding to a negative ion at m/z 703.1143.

802

803 **Figure S4:** Evolution of TC1 in the “eau-de-vie” of cognac from 2015 to 1970. *Error bars*
804 represent standard deviation of different replicates.

805

806 **Figure S5:** HRMS spectrum of TC1 (with fragmentation 35 eV).

807

808 **Figure S6:** ^1H , ^{13}C , DEPT-135, COSY, HSQC, HMBC, ROESY NMR spectra and
809 correlation data map of brandy tannin A (TC1) in acetone- $\text{D}_6/\text{D}_2\text{O}$ (9:1, v/v) at 400 MHz and
810 600 MHz.

811

812 **Table S7:** Individual concentrations of brandy tannin A in 9 vintages of same spirit. All
813 concentrations expressed in (mg/L).

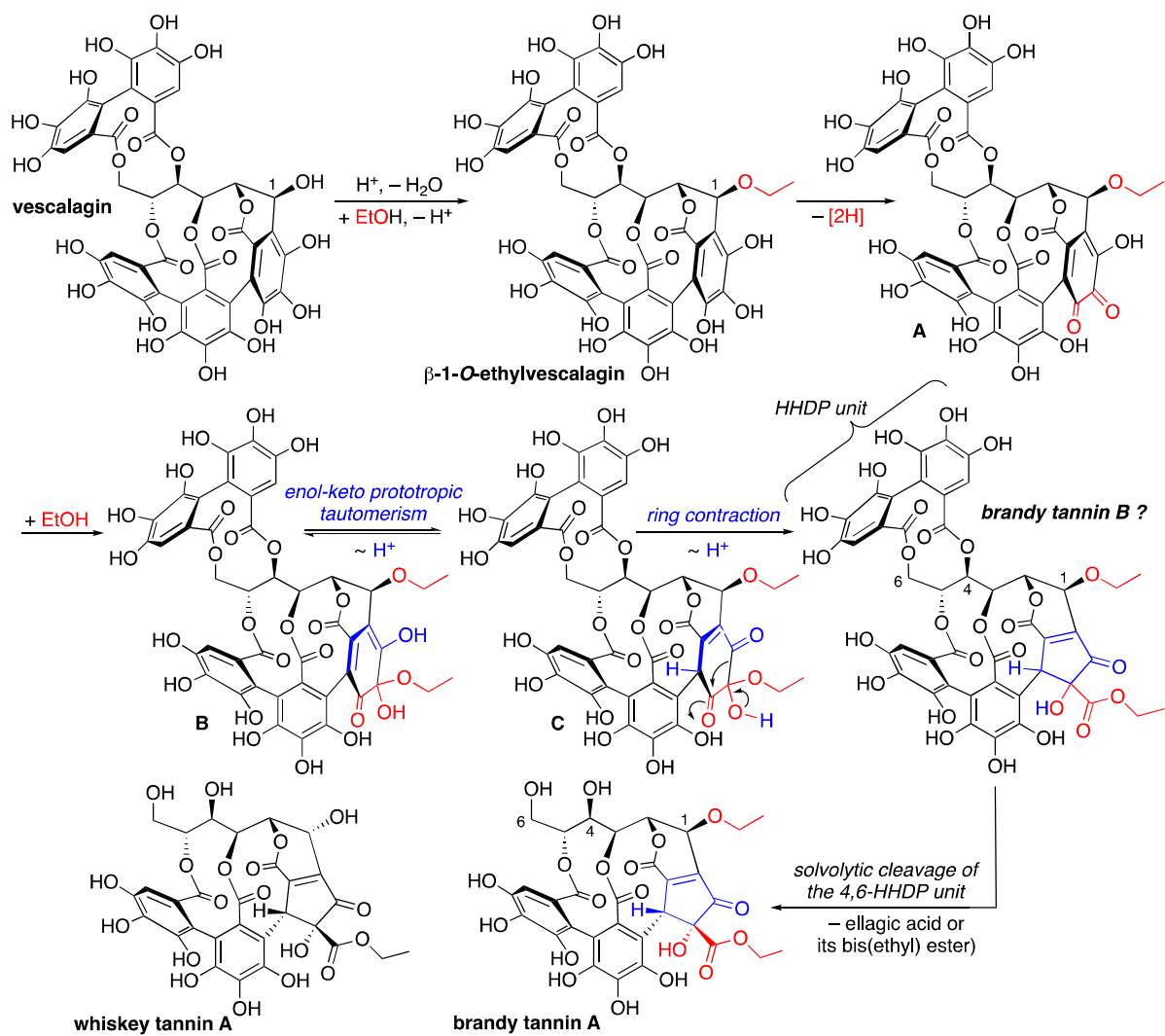
814

815 **Table S8:** Individual concentrations of brandy tannin A in 10 vintages of same spirit. All
816 concentrations expressed in (mg/L).

817

818 **Table S9:** Individual concentrations of brandy tannin A in 36 commercial spirits. All
819 concentrations expressed in (mg/L).

820



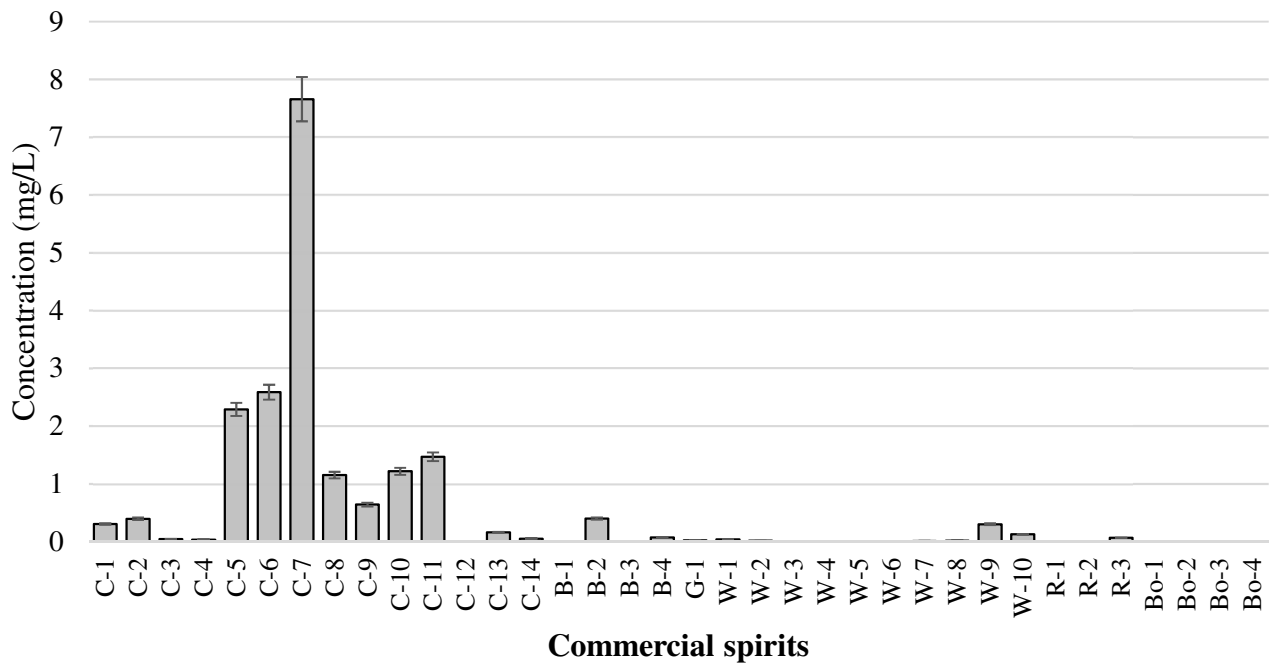
822

823 **Figure 1.** Mechanistic depiction of formation of brandy tannin A from vescalagin.

824



826 **Figure 2:** Concentrations of brandy tannin A over 8 years of aging in barrels (A) and in 10
 827 vintages of “eau-de-vie” of cognac from same distillery (B).



829

830 **Figure 3:** Concentrations of brandy tannin A in 36 commercial spirits (C: Cognac; B: Brandy;

831 G: Gin; W: Whisky; R: Rum; Bo: Bourbon).

832

833 **Table 1.** Ionization and spectrometric conditions for HRMS analyses.

| Ionization mode | Negative | | | |
|---------------------------------|--|----------------------|----------------------|-------------------------|
| Mass Spectrometer | Q-Exactive Plus | | Exactive | |
| Use | LC-MS ⁿ Metabolomic approach | | LC-HRMS Screening | LC-HRMS Quantitation |
| Mass scan | Full MS | dd-MS ² | Full MS | Full MS |
| Sheath gas flow ^a | 48 | | 70 | 60 |
| Auxiliary gas flow ^a | 11 | | 15 | 15 |
| Spare gas flow ^a | 2 | | 0 | 0 |
| HESI probe temperature | 300 °C | | 320 °C | 350 °C |
| Capillary temperature | 300 °C | | 350 °C | 300 °C |
| Electrospray voltage | - 3.3 kV | | - 3.5 kV | - 3.5 kV |
| S-lens RF level ^b | 55 | | - | - |
| Capillary voltage | - | | - 25 V | - 95 V |
| Tube lens voltage offset | - | | - 120 V | - 160 V |
| Skimmer voltage | - | | - 20 V | - 18 V |
| Mass range (in Th) | 100 - 1500 | 200 - 2000 | 200 - 1000 | 200 - 1000 |
| Resolution ^c | 35,000 | 17,500 | 25,000 | 10,000 |
| AGC value ^d | 10 ⁶ ions | 10 ⁵ ions | 10 ⁶ ions | 3.10 ⁶ ions |
| Maximum injection time | 60 ms | 50 ms | - | - |
| Fragmentation | - | 28 eV | - | - |

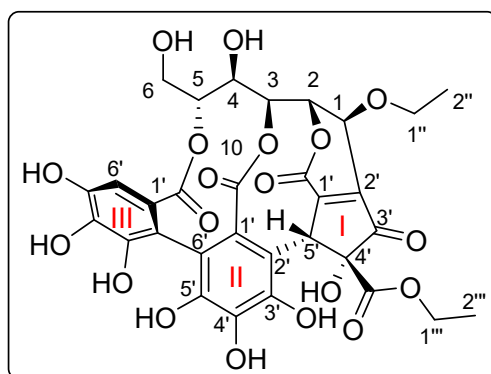
834 ^a Sheath gas, auxiliary gas and spare gas flows (all nitrogen) are expressed in arbitrary units

835 ^b S-lens RF level are expressed in arbitrary units

836 ^c Resolution $m/\Delta m$, fwhm at m/z 200 Th

837 ^d Automatic Gain Control

838

839 **Table 2.** ^1H and ^{13}C NMR signal assignments of brandy tannin A (TC1).840
841

| Position | δ_{H} ($J = \text{Hz}$) | δ_{C} | HSQC | HMBC / COSY |
|-----------------------|---|---------------------|----------------------|---|
| Glucose | | | | |
| 1 | 4.28 (<i>d</i> , 1.1) | 67.8 | C-1 | H-2, H-3, C-1'', C-2, C-3, C-1', C-2', C-3' |
| 2 | 5.48 (<i>d</i> , 1.0) | 80.3 | C-2 | H-1, H-3, C-1, C-1', C _I =O |
| 3 | 5.32 (<i>d</i> , 6.7) | 72.7 | C-3 | H-2, H-4, C-4, C _{II} =O |
| 4 | 4.40 (<i>dd</i> , 9.3, 6.7) | 67.8 | C-4 | H-3, H-5, C-6, C-3, C-5 |
| 5 | 4.90 (<i>ddd</i> , 9.3, 4.3, 2.8) | 74.8 | C-5 | H-3, H-4, H-6, C-4, C _{III} =O |
| 6 | 3.77 (<i>m</i>) 3.86 (<i>m</i>) | 61.9 | C-6 | H-5, C-4 |
| Cyclopentenone | | | | |
| 1' _I | | 142.6 | | H-1, H-2, H-5' _I |
| 2' _I | | 157.9 | | H-1, H-5' _I |
| 3' _I | | 201.9 | | H-1, H-5' _I |
| 4' _I | | 84.6 | | H-5' _I |
| 5' _I | 5.40 (<i>s</i>) | 45.5 | C-5' _I | C-1' _I , C-2' _I , C-3' _I , C-4' _I , C-1' _{II} , C-2' _{II} , C-3' _{II} , C=O _{Ester} |
| Aromatics | | | | |
| 1'' _{II} | | 125.0 | | H-5' _I |
| 1'' _{III} | | 125.4 | | |
| 2'' _{II} | | 111.0 | | H-5' _I |
| 2'' _{III} | | 113.5 | | H-6' _{III} |
| 3'' _{II} | | 146.0 | | H-5' _I |
| 3'' _{III} | | 144.5 | | H-6' _{III} |
| 4'' _{II} | | 135.4 | | |
| 4'' _{III} | | 136.6 | | H-6' _{III} |
| 5'' _{II} | | 144.5 | | H-5' _I |
| 5'' _{III} | | 145.5 | | H-6' _{III} |
| 6'' _{II} | | 114.7 | | |
| 6'' _{III} | 6.69 (<i>s</i>) | 108.1 | C-6'' _{III} | C-2'' _{III} , C-3'' _{III} , C- |

| Carbonyls | | | | |
|----------------------|--|-------|--------|---|
| C=O _{ester} | | 170.1 | | H-1'', H-5' _I |
| C _I =O | | 163.1 | | H-2 |
| C _{II} =O | | 168.0 | | H-3 |
| C _{III} =O | | 168.6 | | H-5, H-6' _{III} |
| Ethyl ether | | | | |
| 1'' | 3.47 (<i>dq</i> , 9.3, 7.0) 3.59 (<i>dq</i> , 9.4, 7.0) | 65.2 | C-1'' | H-2'', C-1, C-2'' |
| 2'' | 1.09 (<i>t</i> , 7.0) | 15.3 | C-2'' | H-1'', C-1'' |
| Ethyl ester | | | | |
| 1''' | 4.18 (<i>m</i>) | 63.3 | C-1''' | H-2''', C-2''', C=O _{ester} |
| 2''' | 1.16 (<i>t</i> , 7.1) | 14.0 | C-2''' | H-1''', C-1''' |

842

843

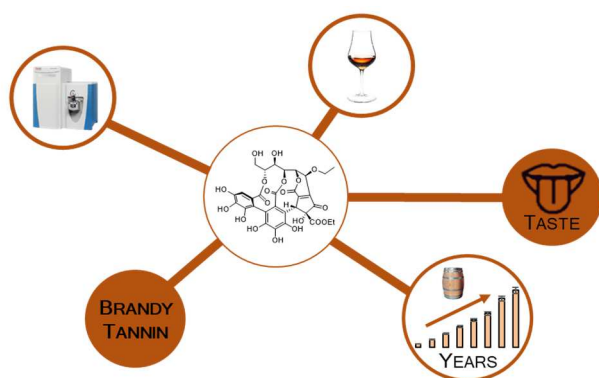
844 **Table 3:** Validation parameters for HRMS quantitation of brandy tannin A in spirits.
 845

| Parameters | Matrix - Spirits | | |
|-----------------------------------|---|---|------------------------------------|
| Sensitivity | LOD ($\mu\text{g/L}$) | LOQ ($\mu\text{g/L}$) | |
| | 1 | 2 | |
| Linearity and accuracy | Working range | R² | |
| | 2 $\mu\text{g/L}$ - 10 mg/L | 0.999 | |
| Specificity | tr variation | Mass accuracy | |
| | 0.02 min | 0.9 ppm | |
| | Intraday repeatability | | |
| | <i>10 $\mu\text{g/L}$</i> | <i>200 $\mu\text{g/L}$</i> | <i>10 mg/L</i> |
| | 4.2% | 2.7% | 2.4% |
| | Interday repeatability | | |
| Repeatability and trueness | <i>10 $\mu\text{g/L}$</i> | <i>10 mg/L</i> | |
| | 16.3% | 4.2% | |
| | Recovery | | |
| | <i>20 $\mu\text{g/L}$</i> | <i>200 $\mu\text{g/L}$</i> | <i>10 mg/L</i> |
| EDV-1 | 102% | 102% | 94% |
| EDV-2 | 105% | 100% | 94% |

846

847

848 **Graphical abstract**



849