Chemical characterization, antioxidant properties and oxygen consumption rate of 36 commercial oenological tannins in a model wine solution

Adeline Vignault[a,b,c,d], Maria Reyes González-Centeno[a,b], Olga Pascual[c], Jordi Gombau[c], Michael Jourdes[a,b], Virginie Moine[d], Nerea Iturmendi[d], Juan Miquel Canals[c], Fernando Zamora[c] and Pierre-Louis Teissedre[a,b]

[a] Univ. Bordeaux, ISVV, EA 4577 Œnologie, 210 Chemin de Leysotte, 33140 Villenave d'Ornon, France

[b] INRA, ISVV, USC 1366 Œnologie, 210 Chemin de Leysotte, 33140 Villenave d'Ornon, France

[c] Departament de Bioquímica i Biotecnologia, Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili, C/Marcel.li Domingo s/n, 43007 Tarragona, Spain

[d] Laffort, 11 rue Aristide Bergès, 33270 Floirac, France

ABSTRACT

The chemical composition (CC), antioxidant capacity (AC) and oxygen consumption rate (OCR) of 36 different commercial tannins were measured. The CC was analyzed by total polyphenol index, Bate-Smith, methyl-cellulose, Folin-Ciocalteu, OIV official method and phloroglucinolisis. The AC was measured by different methods (ABTS, CUPRAC, DPPH, FRAP, ORAC) using Trolox as standard. The OCR was measured using a non-invasive method based on luminescence. The results indicate that it is possible to obtain differentiation between procyanidins/prodelphinidins, profisetinidins/prorobinetidins, gallotannins and ellagitannins by PCA based on their CC data. It is also possible to separate condensed from hydrolysable tannins by PCA based on their AC data. The results show that ellagitannins are the fastest oxygen consumers of the various oenological tannins, followed in descending order by condensed tannins and finally gallotannins. The combination of CC, AC and OCR analyses enable to classify tannins according to their effectiveness in protecting wines against oxidation.

Keywords: Oenological tannins, botanical origin, total phenolics, oxygen consumption rate, antioxidant capacity.

1. Introduction

There is in fact a wide range of oenological tannins in the market which differ in chemical structure (condensed and hydrolysable tannins), botanical origin (grape seed or grape skin, oak wood, exotic wood) and/or preparation process. These include hydrolysable tannins from nut galls, tara, oak and chestnut, and condensed tannins from grape seeds and skins and other plant sources such as quebracho, mimosa and acacia (Versari, du Toit & Parpinello, 2013).

Their use in winemaking has become common practice worldwide, but so far, they are only authorized by the International Organization of Vine and Wine (OIV) (OIV, 2015) to facilitate the fining of wines and musts. Nevertheless, they are also used for other purposes because of their interesting and varied properties. These properties, as demonstrated by various authors, can be classified into different groups: "impact on oxygen/metals", "impact on color/pigments", "protein interaction", "sensory/mouthfeel properties" and

37 “bacteriostatic effects”. The first group includes antioxidant capacity (protection of wine against oxidation)
38 (González-Centeno, Jourdes, Femenia, Simal, Rossello & Teissedre, 2012; Magalhães, Ramos, Reis &
39 Segundo, 2014), antioxidasic activity (anti-laccase activity) (Obradovic, Schulz & Oatey, 2005), the ability
40 to scavenge superoxide radicals (Farhadi, Esmaeilzadeh, Hatami, Forough & Molaie, 2016), the prevention
41 of oxidative damage mediated by Fenton-based reactions (Perez, Wei & Guo, 2009), the ability to chelate
42 iron (Karamac & Pegg, 2009) and the direct consumption of dissolved oxygen (Navarro, Kontoudakis,
43 Giordanengo, Gomez-Alonso, Garcia-Romero, Fort, Canals, Hermosin-Gutierrez & Zamora, 2016; Pascual,
44 Vignault, Gombau, Navarro, Gomez-Alonso, Garcia-Romero, Canals, Hermosin-Gutierrez, Teissedre &
45 Zamora, 2017). The second group includes color improvement and stabilization of red wines (Canuti,
46 Puccioni, Giovani, Salmi, Rosi & Bertuccioli, 2012; Trouillas, Sancho-Garcia, De Freitas, Gierschner,
47 Otyepka & Dangles, 2016), copigmentation effect (Neves, Spranger, Zhao, Leandro & Sun, 2010) and the
48 direct formation of new pigments (Versari et al., 2013). The third group is related to their ability to interact
49 with wine proteins and their use in preventing protein haze (Ribéreau-Gayon, Glories, Maujean, &
50 Dubourdieu, 2006) caused by over-fining when white wines are treated with gelatin (Mierczynska-Vasilev
51 & Smith, 2015). The fourth group involves their impact on sensory/mouthfeel properties. In this regard,
52 oenological tannins are used to improve wine structure and mouthfeel (astringency and bitterness) (Preys,
53 Mazerolles, Courcoux, Samson, Fischer, Hanafi, Bertrand & Cheynier, 2006) and to eliminate reduction
54 odors (Vivas, 2001). Finally, the bacteriostatic effects of oenological tannins (Lempereur, Blateyron,
55 Labarbe, Saucier, Kelebek & Glories, 2002) have been also described.
56 Tannins are usually classified into two families: hydrolysable and condensed tannins. Hydrolysable tannins
57 are classified into two sub-families, gallotannins and ellagitannins. Gallotannins are polymers formed by
58 esterification between D-glucose and gallic acid. Tannic acid is the commercial name for gallotannin extract
59 comprising mixtures of polygalloyl quinic acid ester or polygalloyl glucoses (Pascual et al., 2017). The
60 main sources of commercial gallotannins are nut galls and tara.
61 Ellagitannins are polymers of ellagic, gallic and/or hexahydroxidiphenic acids (Versari et al, 2013). To be
62 more precise, a nonahydroxyterphenoyl unit (NHTP) is esterified in positions 2, 3 and 5 with a C-glycosidic
63 bond, while an open-chain glucose is esterified in positions 4 and 6 with a hexahydroxydiphenoyl unit
64 (HHDP) forming the chemical structure of ellagitannins (Quideau, Varadinova, Karagiozova, Jourdes,
65 Pardon & Baudry, 2004). They constitute one of the most important families of tannins with many
66 biological features such as antioxidant capacity (Hosu, Cristea & Cimpoiu, 2014). The main sources of
67 commercial ellagitannins are oak and chestnut.
68 Condensed tannins, also known as proanthocyanidins, come from different botanical origins such as grapes,
69 quebracho, mimosa and acacia. They differ mainly as regards the monomer released after acidic cleavage,
70 the degree of polymerization (mDP), and their levels of galloylation and ramification (Versari et al., 2013).
71 Grape-skin tannins are composed of procyanidins and prodelphinidins because their acidic cleavage gives
72 cyanidin and delphinidin, whereas grape-seed tannins are composed only of procyanidins. Grape-skin
73 tannins have a high mDP and a low level of galloylation, while grape-seed tannins have a lower mDP and a
74 high level of galloylation (Souquet, Cheynier, Brossaud & Moutounet, 1996). Quebracho tannins are
75 profisetinidins, because their acidic cleavage gives fisetinidin, and they have a high level of ramification,
76 while mimosa tannins are prorobinetidins because they release robinetinidin (Celzard, Szczurek, Jana,

77 Fierro, Basso, Bourbigot, Stauber & Pizzi, 2015). Less is known about acacia tannins, but it seems they are
78 composed of a mixture of profisetinidins, prorobinetidins and prodelphinidins (Beng Hoong, Pizzi, Tahir &
79 Pasch, 2010). Condensed tannins as a whole are called proanthocyanidins.
80 The antioxidant capacity attributed to oenological tannins is probably one of the main reasons they are
81 widely used in wineries. It is generally accepted that they are very useful in protecting grape juice and wine
82 against oxidation and avoiding browning (Nichols-Orians, 1991; Versari et al., 2013). On this subject there
83 are quite a few references about the antioxidant properties of commercial tannins (Laghi, Parpinello, Del
84 Rio, Calani, Mattioli & Versari, 2010; Magalhães et al., 2014) using diverse antioxidant assays (ABTS,
85 CUPRAC, DPPH, FRAP, ORAC, . . .). However, Magalhães et al. (2014) have shown that different
86 antioxidant assays produce different and sometimes contradictory results. More recently, Pascual et al.
87 (2017) measured the oxygen consumption rate (OCR) of two hydrolysable tannins and three condensed
88 tannins.
89 Given the wide range of commercial tannins present in the market and their great chemical diversity, the
90 main goal of this research is to carry out an exhaustive study to determine their chemical characterization,
91 antioxidant properties and oxygen consumption rates using a large number of samples. A classification of
92 their efficiency according to their chemical composition is then proposed.

93 2. Materials and methods

94 *2.1. Chemicals and equipment*

95 All samples and standards were handled without exposure to light. 2,2'azino-bis(3-ethylbenzothiazoline-
96 6-sulfonic acid (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97% (Trolox),
97 2,2-azobis(2-methylpropionamidine) dihydrochloride (AAPH), gallic acid, copper (II) sulfate pentahy-
98 drate, iron (III) chloride hexahydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), copper (II) chloride dihy-
99 drate, neocuproine, Folin-Ciocalteu reagent, L(+)-tartaric acid, sodium hydroxide, sodium carbonate,
100 potassium persulfate, sodium phosphate monobasic, phosphate buffer solution, polyvinylpolypyrrolidone
101 (PVVP), methyl-cellulose, ascorbic acid, phloroglucinol, ammonium sulfate and ammonium acetate were
102 purchased from Sigma-Aldrich (St. Quentin, Fallavier, France). Fluorescein, sodium acetate and 2,4,6-
103 triazine-s-tripyridyl (TPTZ) were from Fluka Analytical (Munich, Germany). Sodium dihydrogen phos-
104 phate, disodium hydrogen phosphate, ethanol (HPLC grade) and methanol (HPLC grade) were supplied
105 by VWR Prolabo Chemicals (Fontenay-sur-Bois, France). Acetic acid (HPLC grade) and hydrochloric
106 acid (HPLC grade) were obtained from Fisher Scientific (Illkirch, France).
107 The equipment used was the following: a spectrophotometer UV-Vis Helios Alpha™ (Thermo Fisher
108 Scientific Inc., Waltman, MA, USA); an HPLC-UV Agilent 1200 series™ (Agilent Technologies, Santa
109 Clara, CA, USA); an Xterra RP18 (100 x 4.6 mm, 3.5 µm) column (Agilent Technologies, Santa Clara,
110 CA, USA); a 96-well microplate reader (FLUOstar Omega, BMG Labtech, Germany); a NOMASense™
111 O2 Trace (Nomacorc SA, Thimister-Clermont, Belgium); and a CB Standard Balance (COBOS, Barcelo-
112 na, Spain).

113 *2.2. Commercial tannins*

114 Thirty-six commercial tannins were considered in this study. Specifically, the following were analyzed: 17
115 proanthocyanidins comprising 9 procyanidins/prodelphinidins (3 from grapes, 4 from grape seeds and 2
116 from grape skin) and 8 profisetinidins/prorobinetidins (2 from acacia and 6 from quebracho), and 19
117 hydrolysable tannins comprising 8 gallotannins (4 from nut galls and 4 from tara) and 11 ellagitannins (8
118 from oak and 3 from chestnut). They were provided by eight different companies: Laffort (Floirac, France),
119 Agrovin (Ciudad Real, Spain), Sofralab (Magenta, France), Institut Oenologique de Champagne (IOC)
120 (Epernay, France), Esseco (Trecate Novara, Italy), AEB (Brescia, Italy), Erblsöh (Geisenheim, Germany)
121 and Vason (Verona, Italy).

122 *2.3. Determination of polyphenol and tannin contents*

123 All the oenological tannins were characterized using the analytical methods described below to determine
124 their richness. Solutions of 2 g/L of each tannin were prepared in a synthetic model wine solution (12 % v/v
125 ethanol, 4 g/L tartaric acid adjusted to pH 3.5 with sodium hydroxide). All the analyses were carried out at
126 least in triplicate.

127 *2.3.1. Total polyphenol index*

128 The total polyphenol index (TPI) was analyzed by measuring the 280 nm absorbance of a 1:100 dilution of
129 tannin solutions with a spectrophotometer, using a 10 mm quartz cuvette and multiplying the absorbance
130 value by 100 as described by Ribéreau-Gayon et al., (2006). The tannin richness (g of tannin/100 g of
131 commercial product) of the different oenological tannins was estimated by interpolating the TPI in two
132 different calibration curves according to tannin type and with regard to the original weight of the sample.
133 Figure 1 shows the A280nm calibration curves for (-)-epicatechin, gallic acid, ellagic acid and tannic acid.
134 These clearly indicate that gallic acid, ellagic acid and tannic acid have similar absorptivity coefficients
135 (expressed in $L.mg^{-1}.cm^{-1}$), whereas that of (-)-epicatechin is around four-fold lower. Proanthocyanidins
136 were therefore interpolated in a (-)-epicatechin calibration curve because this is the main subunit of
137 condensed tannins. In contrast, gallotannins are composed mainly of glucose and gallic acid and should
138 therefore be interpolated in a gallic acid calibration curve or, even better, a tannic acid calibration curve,
139 since this tannic acid is available at a high purity level. Ellagitannins, on the other hand, are composed
140 mainly of glucose and ellagic acid and consequently should be interpolated in an ellagic acid calibration
141 curve. The impossibility of obtaining commercial vescalagin or another pure ellagitannin in sufficient
142 quantity and the poor solubility of ellagic acid led us use tannic acid as standard for ellagitannins too.

143 *2.3.2. Folin-Ciocalteu assay*

144 The total phenolic content (TPC) was measured using the Folin-Ciocalteu reagent in accordance with the
145 method proposed by Lorrain, Ky, Pasquier, Jourdes, Guerin Dubrana, Geny, Rey, Donèche & Teissedre
146 (2012) using 96-well microplates. Stock solutions were diluted around 10 times to reach the interval of
147 sensitivity for the method. For the measurement, 20 µL of gallic acid standard solution (0.00625-0.2 mg/L)
148 or oenological tannin solution, 100 µL of diluted Folin-Ciocalteu reagent (1:10, v/v with distilled water) and
149 80 µL of 7.5% (w/v) Na_2CO_3 buffer were placed in each well. The reduction of phosphotungstate-

150 phosphomolybdate heteropoly acid salts at alkaline pH was measured at 760 nm and 25 °C after 30 min of
151 reaction. The reagent blank was obtained by the addition of 20 µL distilled water instead of sample. The
152 results are expressed as richness (g of gallic acid equivalents/100 g of commercial product).

153 *2.3.3. Bate-Smith assay*

154 All the oenological tannins were analyzed using an adaptation of the Bate-Smith method (Ribéreau-Gayon
155 & Stonestreet, 1966). Briefly, a fixed volume of oenological tannin solutions (20 µL), water (1.48 mL) and
156 hydrochloric acid (1.5 mL, 37%) were placed in two tubes. Tube A was then placed in an ice bath (0 °C)
157 while tube B was placed in warm bath (100 °C). After standing in their respective baths for 30 minutes, 600
158 µL of ethanol was added to the samples to stop the reaction. The proanthocyanidin concentration (g/L) was
159 obtained by multiplying the difference in absorbance at 550 nm (ΔA550) between tube B and tube A by
160 19.33, which is the absorptivity coefficient of cyanidin after the acidic cleavage of the condensed tannins
161 (Bate-Smith reaction). The results are expressed as richness (g of tannin/100 g of commercial product).

162 *2.3.4. Tannin analysis by the methyl-cellulose precipitation method*

163 All the oenological tannins were analyzed using the methyl-cellulose precipitation method (Sarneckis,
164 Dambergs, Jones, Mercurio, Herderich & Smith, 2006). Briefly, a fix volume of oenological tannin
165 solutions (125 µL for condensed tannins and 75 µL for ellagitannin and gallotannin) were placed in two
166 centrifuge tubes. Tube A received an addition of 1 mL methylcellulose solution (0.4 % w/v) and 1 mL
167 saturated ammonium sulfate solution, and the volume was made up to 5 mL. Tube B was prepared
168 similarly, but the added methyl cellulose solution was omitted and replaced with water. After standing at
169 room temperature for 20 minutes, the samples were centrifuged and absorbance measured at 280 nm. The
170 difference in absorbance (ΔA280) between tube B and tube A corresponds to the tannin content of the
171 sample. The tannin richness (g of tannin/100 g of commercial product) of the condensed tannins was
172 estimated by interpolating ΔA280 in a (-)-epicatechin calibration curve, while the hydrolysable tannin
173 richness was estimated using tannic acid as calibration standard, as described for the TPI analysis.

174 *2.3.5. Analysis of proanthocyanidins following acid catalysis with phloroglucinol*

175 Acid-catalyzed depolymerization of proanthocyanidins in the presence of an excess of phloroglucinol
176 (phloroglucinolisis method) was used to analyze the proanthocyanidin content, monomeric composition and
177 mDP of all the oenological condensed tannins (Kennedy & Jones, 2001). A 0.1 N solution of hydrochloric
178 acid in methanol containing 10 g/L ascorbic acid and 50 g/L phloroglucinol was prepared. 200 µL of
179 commercial tannin solution (10 g/L) and 200 µL of the previous solution were incubated at 50 °C for 20
180 min, then combined with 5 volumes of 40 mM aqueous sodium acetate to stop the reaction. Phloroglucinol
181 adducts were analyzed by reversed-phase HPLC using an Xterra RP18 (100 x 4.6 mm, 3.5 µm) column
182 protected by a guard column with the same characteristic. A binary gradient with mobile phases containing
183 1% v/v aqueous acetic acid (mobile phase A) and methanol (mobile phase B) was used. The elution
184 conditions were 1.0 mL/min; 5% B for 25 min; a linear gradient from 5 to 20% B in 20 min; a linear

185 gradient from 20 to 32% B in 15 min; and a linear gradient from 32 to 100% B in 2 min. The column was
186 then washed with 100% B for 5 min and reequilibrated with 5% B for 5 min before the next injection. The
187 volume injection was 20 µL and the eluting peaks were monitored at 280 nm. To calculate the apparent
188 mean degree of polymerization (mDP), the sum of all subunits (flavan-3-ol monomer and phloroglucinol
189 adduct, expressed in moles) was divided by the sum of all flavan-3-ol monomers (expressed in moles). The
190 richness (g of proanthocyanidin/100 g of commercial product) was calculated taking into account the sum
191 of all subunits (excluding the phloroglucinol portion in the phloroglucinol adducts) as regards the original
192 weight of the commercial product.

193 *2.3.6. Tannin analysis by the OIV method*

194 All the oenological tannins were analyzed by official OIV methods (OIV, 2015). First, a stock solution of
195 tannins (solution A) was prepared by adding 6 g of tannins to 950 mL of warm distilled water (~ 60-70 °C).
196 After 30 min at room temperature, the solution was cooled in a bath thermostated at around 20 °C and the
197 volume completed to 1 L. Total solids (TS) measurement was done by determining the dry weight of 25 mL
198 of solution A after complete evaporation in an aluminum dish. Soluble solids (SS) measurement was done
199 similarly with the supernatant of 25 mL of solution A after centrifuging (4000 rpm; 5 min) and filtering
200 (0.22 µm GSWP, Millipore, Tullagreen, Ireland) to eliminate possible insoluble substances. Finally, the
201 non-phenolic solids (NPS) were determined using an SPE column with polyvinylpolypyrrolidone (PVPP).
202 7.0 g of PVPP was rehydrated in the column with a 20% hydroalcoholic solution for 15 min, then placed on
203 the vacuum manifold. The column was activated by carrying out three washes (50 mL of ethanol (20% v/v),
204 50 mL of distilled water and 50 mL of solution A). 30 mL of solution A was then put at the top of the
205 column and the eluate was collected in a flask. This was stopped when the liquid reached the level of the
206 upper frit and 25 mL of the eluate was taken and transferred to an aluminum dish to determine the dry
207 weight. A blank measurement (BK) was also taken by doing the same as for the NPS but adding 30 mL of
208 distilled water instead of solution A. The tannin richness (% tannins) was estimated using the following
209 equation:

210 $$\%Tannins = (SS - NPS - BK)/TS * 100$$

211 *2.4. Determination of antioxidant capacity*

212 Antioxidant capacity was measured using 96-well microplates and five different methods: ABTS,
213 CUPRAC, DPPH, FRAP and ORAC. Each assay was performed following Gonzalez-Centeno et al., (2012).
214 Stock solutions (1.5 mg/10 mL) of oenological tannins were prepared in model wine solution. In the case of
215 the ORAC assay, a dilution of the stock solution of between 1/30 and 1/100 in deionized water was
216 necessary. Trolox, used as a standard, was also prepared in model wine solution (0.25-1.25 mM for the
217 ABTS, CUPRAC and FRAP assays; 0.1-1 mM for the DPPH assay and 40-1.5 µM for the ORAC assay).
218 Except for ORAC assay, a reagent blank was taken by adding 190 µL of working solution in each well.
219 Then 10 µL of deionized water, Trolox solution or oenological tannin solution was added to each well and
220 spectrophotometric measurements were performed at 25 °C after 30 min of reaction. Fluorometric ORAC

221 assay was performed at 37 °C and lasted 1.5 hours. All the results, expressed as mg of Trolox equivalents/g
222 tannin (dm), are the average of six determinations.

223 *2.4.1. The ABTS assay*

224 ABTS reagent was prepared by mixing in equal volumes a solution of $ABTS^{•+}$ (7 mM) and an aqueous
225 solution of persulfate potassium (2.45 mM) and then allowing it to react at room temperature in darkness for
226 12-16 h. After that, the working solution was diluted with deionized water to obtain an absorbance of 0.9 ±
227 0.01 units at 734 nm. The measurement was monitored at this wavelength.

228 *2.4.2. The CUPRAC assay*

229 The CUPRAC reagent was prepared by mixing equal volumes (1:1:1) of a Cu(II) aqueous solution (10
230 mM), a neocuproine solution in ethanol (7.5 mM) and ammonium acetate buffer (1 M, pH 7). The
231 measurement was monitored at 450 nm.

232 *2.4.3. The DPPH assay*

233 The DPPH reagent ($6. 10^{-5}$ M) was freshly prepared in methanol. The measurement was monitored at 515
234 nm.

235 *2.4.4. The FRAP assay*

236 FRAP reagent was prepared by mixing acetate sodium buffer solution (300 mM, pH 3.6), TPTZ (10 mM)
237 and iron (III) chloride hexahydrate aqueous solution (20 mM) at a ratio of 10:1:1. The measurement was
238 monitored at 593 nm.

239 *2.4.5. The ORAC assay*

240 The oxygen radical absorbance capacity assay was carried out following Dudonné, Vitrac, Coutière,
241 Woillez & Mérillon, 2009. All reagents were prepared in phosphate buffer (75 mM, pH 7.4). First of all 30
242 µL of deionized water, Trolox standard solution or oenological tannin solution was placed in each well. 180
243 µL of fluorescein (117 nM, final concentration) and 90 µL of AAPH (40 mM) were then added to each
244 well. Fluorescence was monitored at 485 (excitation) and 530 nm (emission). The area under the curve
245 (AUC) was calculated for each sample by integrating their relative fluorescence curves. The net AUC of the
246 oenological tannins was calculated by subtracting the AUC from the blank and then correlating with the
247 Trolox calibration curve.

248 *2.5. Determination of the oxygen consumption rate (OCR)*

249 The OCR was performed following the non-invasive luminescence technique described by Pascual et al,
250 (2017). Clear glass bottles (0.75 L) provided with a pill (PreSens PrecisionSensing GmbH, order code: SP-
251 PSt3-NAU-D5-CAF; batch number: 1203-01_PSt3-0828-01, Regensburg, Germany) to measure dissolved

252 oxygen were used. The pills were all placed at the middle of the bottle. The experiments were conducted in
253 a model wine solution composed of 12 % ethanol, 4 g/L tartaric acid and pH adjusted to 3.5. This was
254 supplemented with 3 mg Fe (III)/L in the form of iron (III) chloride hexahydrate, and 0.3 mg of copper/L in
255 the form of copper (II) sulfate pentahydrate to mimic as closely as possible real wine conditions. The model
256 wine solution was saturated with oxygen by bubbling with air for 10 min. The bottles with the inserted pill
257 were first filled with 20 mL of the model wine solution (control) or with the different oenological tannins
258 dissolved in 20 mL of model wine solution. Immediately afterwards, the bottles were filled with the
259 oxygen-saturated solution to almost overflow to minimize the volume of headspace. The bottles were
260 hermetically sealed and carefully shaken to fully homogenize. The oxygen measurements started 1 h later
261 and were performed every two days. Bottle temperature was maintained at 20 °C the whole time. Two
262 different experimental setups were performed: the first to evaluate the influence of the oenological tannin
263 doses and the second to evaluate the influence of the type of oenological tannin. All the samples were
264 analyzed in triplicate.

265 *2.5.1. Influence of the oenological tannin doses*

266 Four different doses of oenological tannins (25, 50, 75 and 100 g/hL) from the two usual botanical origins
267 (grape skin and oak) were used to evaluate the influence of the oenological tannin and to identify the best
268 dose for performing the next experiment. The concentrations used are somewhat higher than those usually
269 applied to must and wines under real winemaking conditions to shorten the experimentation time.

270 *2.5.2. Influence of the botanical origin of the oenological tannins*

271 Once the best oenological tannin dose was estimated, the influence of botanical origin on oxygen
272 consumption was studied. To this end the oxygen and tannin concentrations were fixed at 8 mg/L and 100
273 g/hL respectively. All the commercial tannins of various botanical origins (quebracho, tara, nut galls, oak,
274 chestnut, acacia, grape seeds and grape skins) were considered for this experiment.

275 *2.6. Statistical analysis*

276 All the chemical and physical data are expressed as mean values ± standard deviation. The statistical
277 analyses were carried out using the XLSTAT 2017 statistical package. The normality and homocedasticity
278 of the data were tested for all parameters by using the Shapiro-Wilk test and Levene's test respectively.
279 When populations were distributed normally and presented homogeneity in variance, parametric tests
280 (ANOVA and Tukey) were used to evaluate the existence and degree of significant differences. In contrast,
281 when populations were not distributed normally and/or presented heterogeneity in variance, non-parametric
282 tests (Kruskal-Wallis and pairwise-Wilcox) were used. Differences were considered to be statistically
283 significant at p-value < 0.05.

284 **3. Results and discussion**

285 Table 1 shows the chemical characterization of the 36 oenological tannins. The different tannins were
286 grouped in 2 families of condensed tannins: procyanidins/prodelphinidins (PC/PD) and
287 profisetinidins/prorobinetidins (PF/PR); and 2 families of hydrolysable tannins: gallotannins (GT) and
288 ellagitannins (ET). Each family was in turn classified according to different botanical origin. Richness was
289 expressed in all cases as a percentage (%w/w; g of tannin per 100 g of commercial product).
290
291 When tannin richness was calculated by the Folin-Ciocalteu method using gallic acid as the calibration
292 standard, the average richness of all the oenological tannins studied was 55.6 ± 2.2% with a minimal value
293 of 30.2% and a maximal value of 78.9%. However, no significant differences were found between the
294 richness of the different tannin families, which ranged from 47.8 ± 7.3% in the case of PF/PR tannins to
295 65.0 ± 6.9% for GT. Neither was any significant difference detected between the different botanical origins
296 of the tannins in each family. The lack of significance in the richness of any of the different tannin types is
297 probably due to the non-specificity of the Folin-Ciocalteu method, since this analytical procedure
298 determines not only tannins but other phenolic compounds as well (El Rayess, Barbar, Wilson & Bouajila,
299 2014).
300 The second method used for estimating tannin richness was TPI. According to the data, the average richness
301 of the tannins was somewhat lower than that obtained by the Folin-Ciocalteu method (41.2 ± 14.3 %). The
302 richness of the different tannins ranges from 16.1 ± 1.7 to 98.7 ± 4.6 %, explain the high standard deviation
303 and indicates the existence of a wide variability among oenological tannins. No significant differences were
304 found between the richness of the two families of condensed tannins (PC/PD: 56.1 ± 6.1%; PF/PR: 46.1 ±
305 24.9%) and gallotannins (GT: 46.9 ± 1.9%). However, the richness of the ellagitannins was significantly
306 lower (ET: 20.1 ± 0.6%). Within the PF/PR family, the richness of the quebracho tannins (63.7 ± 18.0%)
307 was significantly higher than that of the acacia tannins (28.5 ± 2.0%). In contrast, no significant differences
308 were found between the different botanical origins of the other tannin types. It would therefore appear that
309 the TPI method, using the different calibration curves according to tannin type, is more discriminating than
310 the Folin-Ciocalteu method even though it is not specifically for tannins but detects all types of phenolic
311 compounds.
312 The methyl-cellulose precipitation method was also used for determining the richness of the different
313 oenological tannins. In theory this method is more specific for tannin estimation that the previous two
314 (Sarneckis et al., 2006) since it only analyzes the phenolic compounds that precipitate with methylcellulose
315 rather than all those present in the commercial product. The average richness obtained for all the tannins by
316 this method was 50.7 ± 17.8%, an intermediate value between those obtained by the Folin-Ciocalteu and
317 TPI methods, with values ranging between 10.6 ± 2.0% and 110.1 ± 4.0%. This again confirms the wide
318 heterogeneity of the oenological tannins. Like with the TPI method, no significant differences were found
319 between the richness of the family of condensed tannins (PC/PD: 65.4 ± 7.9%; PF/PR: 54.9 ± 40.9%) and
320 gallotannins (GT: 68.8 ± 8.6%), while the richness of ellagitannins was significantly lower (ET: 13.7 ±
321 1.1%). Within each family of tannins, no significant differences were observed as regards botanical origin,
322 with the exception of the PF/PR tannins. The high standard deviation detected in this family was due to the
323 great differences observed between quebracho (83.8 ± 14.0%) and acacia tannins (26.0 ± 7.0%).

324 The Bate-Smith reaction is specific reaction for condensed tannins (El Rayess et al., 2014) and
325 consequently it makes no sense applying this methods to hydrolysable tannins. However, due to the great
326 heterogeneity of the commercial tannins, it was considered best to apply the reaction to check all the
327 commercial tannins because it is a simple way to discriminate the two tannin families. As expected, this
328 method provided significantly higher richness for condensed tannins (61.2 ± 23.1%) than for hydrolysable
329 tannins (4.3 ± 4.0%). The very low richness detected in hydrolysable tannins may be due to the fact that
330 these commercial tannins contain a very low proportion of proanthocyanidins. However, it is more likely to
331 be the result of other unspecific reactions that can cause browning under strong reaction conditions (100 ºC
332 for 30 minutes) like the Maillard reaction. Since the Bate-Smith reaction is followed by an increase in
333 absorbance at 550 nm, the appearance of brown pigments can lead to an overestimation. According to this
334 method the richness of PC/PD tannins was significantly higher than that of PF/PR (77.5 ± 7.0% and 44.9 ±
335 7.8% respectively). The lower richness of PF/PR tannins may be due to a lower real tannin content, but it
336 must be highlighted that the other methods detected no significant differences between PF/PR and PC/PD
337 tannins either. Another possible explanation could be that PF/PR are more resistant to acidic cleavage
338 (Schofield, Mbugua & Pell, 2001), thereby reducing the proportion of anthocyanins generated by the Bate-
339 Smith reaction.
340 The analysis of proanthocyanidins by acidic cleavage in the presence of an excess of phloroglucinol and the
341 subsequent HPLC analysis of the subunits obtained (phloroglucinolisis method) was applied only to the
342 condensed tannins. In fact, the method is specifically for these kinds of tannins and also because the Bate-
343 Smith reaction gave very low richness for all hydrolysable tannins. All the PC/PD family of tannins gave
344 rise to similar richness regardless of whether they came from seeds, skins or the whole berry, with an
345 average of 30.9 ± 0.5% for a range of values between 29.6 ± 0.3% and 31.7± 0.3%. In contrast, when this
346 method was applied to the PF/PR tannin family the results were below the detection limit. Other authors
347 have also reported that quebracho tannins could not be analyzed by acidic cleavage in the presence of
348 another nucleophile like toluene-α-thiol (Vivas, Nonier, Vivas de Gaulejac, Absalon, Bertrand & Mirabel,
349 2004; OIV, 2015). This together with the lower yield for these tannins obtained by the Bate-Smith reactions
350 indicates that profisetinidins and prorobinetidins are more resistant to acidic cleavage regardless of the
351 nucleophile.
352 Finally, the current OIV method was also applied to determine the richness of the different oenological
353 tannins. This method determines all phenolic compounds and not just specifically tannins since it is based
354 on the gravimetric measurement of all the phenolic compounds retained by a column of PVPP. The average
355 richness obtained by this method was 68.7 ± 10.2% with a minimal value of 37.1 ± 0.3% and a maximal
356 value of 87.5± 7.7%. No significant differences were found between condensed tannins (62.7 ± 8.0%) and
357 hydrolysable tannins (74.7 ± 10.1%). Overall, the richness of the PC/PD family of tannins was similar to
358 that of the PF/PR tannins. Moreover, no significant differences were found between the different sources
359 (grape, seeds or skins) in the case of PC/PD tannins. However, quebracho tannins have significantly higher
360 richness than acacia tannins (75.0 ± 6.1% and 39.0 ± 2.7% respectively) within the PF/PR family of tannins.
361 Of the hydrolysable tannins, gallotannins have significantly higher richness than ellagitannins (81.8 ± 4.5%
362 and 67.5± 2.0% respectively). No statistical differences were found among the different botanical origins in
363 either type of hydrolysable tannin.

364 Overall, these results are very complex because each analytical method supplies different results, which is
365 only to be expected given that each method has a different chemical basis. The Folin-Ciocalteu, TPI and
366 OIV methods are not in fact specifically for tannins because they analyze all types of phenolic compounds,
367 whereas the Bate-Smith reactions and phloroglucinolisis are specifically for condensed tannins (El Rayess
368 et al., 2014). The methyl-cellulose precipitation method, on the other hand, determines all types of tannins
369 because it measures the difference in absorbance at 280 nm before and after precipitating them with methyl-
370 cellulose (Sarneckis et al., 2006).
371
372 For these reasons, and to better understand the information provided by each of these analytical methods for
373 the different types of oenological tannins, a principal component analysis (PCA) was performed using those
374 parameters. Figure 2-A shows the plot of varimax-rotated PCA for the different oenological tannins. The
375 first component explains 46.41% of the variance and the second 28.94%, which means that the aggregate
376 variance explained by these two components was 75.35%. PCA enabled the different oenological tannins to
377 be separated with only two incorrect classifications (one GT was classified as ET and one ET as GT). PC1
378 placed ET on the left and PC/PD on the right, with GT and PF/PR being in the center. PC2 enabled GT and
379 PF/PR to be separated, locating GT above PF/PR. The loadings are presented as arrows, the length and
380 direction of which indicate the contribution made by both components. Hence the methyl-cellulose, TPI,
381 Bate-Smith and phloroglucinolisis methods contribute mainly to PC1, the OIV method to PC2 and the
382 Folin-Ciocalteu to both components. These data simply indicate that the samples placed more to the right
383 have higher richness with the methyl-cellulose, TPI, Bate-Smith and phloroglucinolisis methods and that
384 samples located further up have higher richness with the OIV method.
385
386 Table 2 shows the antioxidant capacity of the different oenological tannins assessed by different analytical
387 methods (ABTS, ORAC, FRAP, CUPRAC and DPPH). The aim of these analyses was to provide a way to
388 classify the different oenological tannins according to their antioxidant properties. In general terms, the
389 different antioxidant capacity tests produced different and sometimes contradictory results for the different
390 tannin families. Briefly, the ABTS and FRAP tests gave the highest values to both the hydrolysable tannins,
391 and these were followed in decreasing order by PC/PD and PF/PR. The CUPRAC test also gave the highest
392 value to ET but placed gallotannins in second position followed by PC/PD and PF/PR. No significant
393 differences were found among the different types of tannins when using the DPPH test, although the
394 antioxidant capacity of hydrolysable tannins tended to be higher than that of condensed tannins. Overall, the
395 ABTS, FRAP, CUPRAC and DPPH tests indicate that hydrolysable tannins have a higher antioxidant
396 capacity than condensed tannins, although these differences are not always significant. In contrast, the
397 ORAC test supplied contradictory results since it gave the highest antioxidant capacity to PF/PR tannins
398 followed by, in descending order, PC/PD tannins, GT and ET. Other authors (Magalhães et al., 2014,
399 Pascual et al., 2017) have obtained similar results, concluding that each antioxidant assay yields different
400 information of a complementary nature.
401
402 Figure 2-B shows the PCA applied to all the oenological tannins using the different antioxidant capacities.
403 The first component explains 51.56% of the variance and the second 26.24%, so the aggregate variance

404 explained by these two components totals 77.80%. The PC1 enables separation between hydrolysable
405 tannins (on the right) and condensed tannins (on the left) with only 5 incorrect classifications (3 CT were
406 classified as HT and 2 HT as CT). The PC1 simply indicates that HT have a higher antioxidant capacity
407 than CT according to the ABTS, CUPRAC, FRAP, DPPH. In contrast the PC2, which is explained mainly
408 by the ORAC test, did not improve the separation between tannins obtained with PC1. Consequently, it is
409 possible to distinguish between hydrolysable and condensed tannins according to their antioxidant
410 capacities, but not possible to differentiate between PC/PD and PF/PR or between GT and ET.
411
412 Our research team recently developed a method for measuring the oxygen consumption rate (OCR) of
413 different oenological tannins (Pascual et al., 2017) and suggested that oenological tannins should be
414 classified in terms of their effectiveness to consume oxygen and thus protect wine against oxidation. A
415 preliminary study was carried out with some representative samples to select the most suitable
416 concentration for all tannins given their wide variety. Figure 3 shows the OCR obtained for a condensed
417 tannin (procyanidin/prodelphinidins: skin 2) and a hydrolysable tannin (ellagitannin: oak 4). The results
418 indicate that in both tannin types the OCR was higher when the concentration was greater. In line with these
419 results, the highest concentration (100 g/hL) was selected for the following analysis so as to have higher
420 precision and shorten the measurement times.
421
422 Figure 4-A compares the OCRs of the different oenological tannins according to their botanical origin.
423 Overall the data show that chestnut tannin has the highest OCR, followed in decreasing order by oak,
424 acacia, grape skin, quebracho, grape, grape seed, tara and nutgall tannins. The differences were only
425 significant between chestnut and tara/nutgall tannins, probably because of the wide variability observed in
426 the richness of the different types of commercial tannins (see Table 1).
427
428 Figure 4-B shows the results obtained when these oenological tannins are grouped by family according to
429 chemical structure independently of botanical origin: procyanidins/prodelphinidins (PC/PD),
430 profisetinidins/prorobinetidins (PF/PR), gallotannins (GT) and ellagitannins (ET). These data clearly show
431 that ellagitannins are the most efficient at consuming oxygen (average OCR: 0.75 mg of O_2/L by day and by
432 g of product), followed in decreasing order by PF/PR tannins (average OCR: 0.50 mg of O_2 /L.day.g) and
433 PC/PD tannins (average OCR: 0.25 mg of O_2/L.day.g). Finally, gallotannins (GT) are the least effective
434 type for consuming oxygen (average OCR: 0.10 mg of O_2/L.day.g). Again, these differences are only
435 significant between ET and GT. Pascual et al. (2017) reported similar results using just one tannin from
436 each family type. This study confirms that ellagitannins are the most effective of the various oenological
437 tannins, followed in decreasing order by condensed tannins (PC/PD and PF/PR) and finally gallotannins in
438 terms of protecting the wine against chemical oxidation using a large sample of commercial products.
439
440 **4. Conclusions**

441 It can be concluded that the combination of chemical composition, antioxidant capacity and oxygen
442 consumption data makes it possible to classify the various families of oenological tannins and provides
443 useful information regarding their protective effect against wine oxidation. The different oenological

tannins can be classified using principal component analysis based on their chemical composition and using different analytical methods. It is also possible separate condensed tannins from hydrolysable tannins using principal component analysis based on their antioxidant capacities. In general terms, hydrolysable tannins have a higher antioxidant capacity than condensed tannins according to all the analytical methods with the exception of ORAC. In contrast, ellagitannins and gallotannins behave completely differently in terms of their ability to consume oxygen, with ellagitannins being the most effective and gallotannins the least. Condensed tannins were being always at an intermediate level. This study therefore confirms that ellagitannins are the most effective of the various oenological tannins, followed in decreasing order by condensed tannins (PC/PD and PF/PR) and finally gallotannins in terms of protecting the wine against chemical oxidation. Complementary studies are needed to determine the effect of the various oenological tannins in inhibiting polyphenol oxidases (tyrosinase and laccase) to prevent the enzymatic browning of musts and wines.

Acknowledgments

The authors are grateful to Laffort (Floirac, France) for their financial support for this research. They would also like to thank Oenoppia for their financial support and more specifically AEB, Agrovin, Erbslöh, Esseco, IOC, Laffort, Sofralab and Vason companies for participating in this project by providing the commercial tannins.

References

Canuti, V., Puccioni, S., Giovani, G., Salmi, M., Rosi, I., and Bertuccioli, M. (2012) Effect of Oenotannin Addition on the Composition of Sangiovese Wines from Grapes with Different Characteristics. *American Journal of Enology and Viticulture 63*, 220–231.

Celzard, A., Szczurek, A., Jana, P., Fierro, V., Basso, M.-C., Bourbigot, S., Stauber, M., and Pizzi, A. (2015) Latest progresses in the preparation of tannin-based cellular solids. *Journal of Cellular Plastics 51*, 89–102.

Dudonné, S., Vitrac, X., Coutière, P., Woillez, M., and Mérillon, J.-M. (2009) Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays. *Journal of Agricultural and Food Chemistry 57*, 1768–1774.

El Rayess, Y., Barbar, R., Wilson, E. A., and Bouajila, J. (2014) Analytical Methods for Wine Polyphenols Analysis and for Their Antioxidant Activity Evaluation. Nova Science Publishers: New York, NY, USA.

Farhadi, K., Esmaeilzadeh, F., Hatami, M., Forough, M., and Molaie, R. (2016) Determination of phenolic compounds content and antioxidant activity in skin, pulp, seed, cane and leaf of five native grape cultivars in West Azerbaijan province, Iran. *Food Chemistry 199*, 847–855.

González-Centeno, M. R., Jourdes, M., Femenia, A., Simal, S., Rosselló, C., and Teissedre, P.-L. (2012) Proanthocyanidin Composition and Antioxidant Potential of the Stem Winemaking Byproducts from 10 Different Grape Varieties (Vitis vinifera L.). *Journal of Agricultural and Food Chemistry 60*, 11850–11858.

Hoong, Y. B., Pizzi, A., Md. Tahir, P., and Pasch, H. (2010) Characterization of Acacia mangium polyflavonoid tannins by MALDI-TOF mass spectrometry and CP-MAS 13C NMR. *European Polymer Journal 46*, 1268–1277.

492 Hosu, A., Cristea, V.-M., and Cimpoiu, C. (2014) Analysis of total phenolic, flavonoids, anthocyanins
493 and tannins content in Romanian red wines: Prediction of antioxidant activities and classification of wines
494 using artificial neural networks. *Food Chemistry 150*, 113–118.
495
496 Karamać, M., and Pegg, R. B. (2009) Limitations of the Tetramethylmurexide Assay for Investigating the
497 Fe(II) Chelation Activity of Phenolic Compounds. *Journal of Agricultural and Food Chemistry 57*, 6425–
498 6431.
499
500 Kennedy, J. A., and Jones, G. P. (2001) Analysis of Proanthocyanidin Cleavage Products Following
501 Acid-Catalysis in the Presence of Excess Phloroglucinol. *Journal of Agricultural and Food Chemistry 49*,
502 1740–1746.
503
504 Laghi, L., Parpinello, G. P., Rio, D. D., Calani, L., Mattioli, A. U., and Versari, A. (2010) Fingerprint of
505 enological tannins by multiple techniques approach. *Food Chemistry 121*, 783–788.
506
507 Lempereur, V., Blayteyron, L., Labarbe, B., Saucier, C., Klebek, H., and et Glories, Y. (2002) Groupe
508 national de travail sur les tanins oenologiques: premiers résultats. *Revue Française d'Oenologie, Paris*
509 23–29.
510
511 Lorrain, B., Ky, I., Pasquier, G., Jourdes, M., Dubrana, L. G., GéNy, L., Rey, P., DonèChe, B., and
512 Teissedre, P.-L. (2012) Effect of Esca disease on the phenolic and sensory attributes of Cabernet
513 Sauvignon grapes, musts and wines: Esca effect on grapes and wines composition. *Australian Journal of*
514 *Grape and Wine Research 18*, 64–72.
515
516 Magalhaes, L. M., Ramos, I. I., Reis, S., and Segundo, M. A. (2014) Antioxidant profile of commercial
517 oenological tannins determined by multiple chemical assays. *ajgw 20*, 72–79.
518
519 Mierczynska-Vasilev, A., and Smith, P. A. (2015) Current state of knowledge and challenges in wine
520 clarification. *Australian Journal of Grape and Wine Research* 615–626.
521
522 Navarro, M., Kontoudakis, N., Giordanengo, T., Gómez-Alonso, S., García-Romero, E., Fort, F., Canals,
523 J. M., Hermosín-Gutíerrez, I., and Zamora, F. (2016) Oxygen consumption by oak chips in a model wine
524 solution; Influence of the botanical origin, toast level and ellagitannin content. *Food Chemistry 199*, 822–
525 827.
526
527 Neves, A. C., Spranger, M. I., Zhao, Y. Q., Leandro, M. C., and Sun, B. S. (2010) Effect of addition of
528 commercial grape seed tannins on phenolic composition, chromatic characteristics, and antioxidant
529 activity of red wine. *Journal of Agricultural and Food Chemistry* 11775–11782.
530
531 Nichols-Orians, C. (1991) Differential effects of condensed and hydrolyzable tannin on polyphenol
532 oxidase activity of attine symbiotic fungus. *Journal of chemical ecology 17*, 1811–1819.
533
534 Obradovic, D., Schulz, M., and Oatey, M. (2005) Addition of natural tannins to enhance the quality of red
535 wine. *Australian and New Zealand Grapegrower Winemaker* 52–54.
536
537 OIV. International Oenological Codex. COEI-1-TANINS: 2015.
538 http://www.oiv.int/public/medias/4093/e-coei-1-tanins.pdf
539
540 Pascual, O., Vignault, A., Gombau, J., Navarro, M., Gómez-Alonso, S., García-Romero, E., Canals, J. M.,
541 Hermosín-Gutíerrez, I., Teissedre, P.-L., and Zamora, F. (2017) Oxygen consumption rates by different
542 oenological tannins in a model wine solution. *Food Chemistry 234*, 26–32.
543
544 Perez, C. A., Wei, Y., and Guo, M. (2009) Iron-binding and anti-Fenton properties of baicalein and
545 baicalin. *Journal of Inorganic Biochemistry 103*, 326–332.
546
547 Preys, S., Mazerolles, G., Courcoux, P., Samson, A., Fischer, U., Hanafi, M., Bertrand, D., and Cheynier,
548 V. (2006) Relationship between polyphenolic composition and some sensory properties in red wines
549 using multiway analyses. *Analytica Chimica Acta 563*, 126–136.
550
551 Quideau, S., Varadinova, T., Karagiozova, D., Jourdes, M., Pardon, P., and Baudry, C. (2004) Main

552 structural and stereochemical aspects of the antiherpetic activity of nonahydroxyterphenoyl-containing C-
553 glycosidic ellagitannins. *Chemistry and Biodiversity* 247–258.
554
555 Ribéreau-Gayon, J., and Stonestreet, E. (1966) Dosage des tanins du vin rouge et determination de leur
556 structure. *Chim. Anal* 188–196.
557
558 Ribéreau-Gayon, P., Dubourdieu, D., and Donèche, B. (2006) Handbook of enology 2nd ed. John Wiley,
559 Chichester, West Sussex, England; Hoboken, NJ.
560
561 Sarneckis, C. J., Dambergs, R. G., Jones, P., Mercurio, M., Herderich, M. J., and Smith, P. A. (2006)
562 Quantification of condensed tannins by precipitation with methyl cellulose: development and validation
563 of an optimised tool for grape and wine analysis. *Australian Journal of Grape and Wine Research 12*, 39–
564 49.
565
566 Schofield, P., Mbugua, D. M., and Pell, A. N. (2001) Analysis of condensed tannins: a review. *Animal*
567 *Feed Science and Technology* 21–40.
568
569 Souquet, J.-M., Cheynier, V., Brossaud, F., and Moutounet, M. (1996) Polymeric proanthocyanidins from
570 grape skins. *Phytochemistry 43*, 509–512.
571
572 Trouillas, P., Sancho-García, J. C., De Freitas, V., Gierschner, J., Otyepka, M., and Dangles, O. (2016)
573 Stabilizing and Modulating Color by Copigmentation: Insights from Theory and Experiment. *Chemical*
574 *Reviews 116*, 4937–4982.
575
576 Versari, A., du Toit, W., and Parpinello, G. P. (2013) Oenological tannins: a review: Oenological tannins.
577 *Australian Journal of Grape and Wine Research 19*, 1–10.
578
579 Vivas, N. (2001) Les tanins oenologiques, d'hier à ajourd'hui: Une révolution discrète que nous devons
580 assimiler dans les pratiques de chais. *Revue Française d'Oenologie, Paris* 11–14.
581
582 Vivas, N., Nonier, M.-F., de Gaulejac, N. V., Absalon, C., Bertrand, A., and Mirabel, M. (2004) Differen-
583 tiation of proanthocyanidin tannins from seeds, skins and stems of grapes (Vitis vinifera) and heartwood
584 of Quebracho (Schinopsis balansae) by matrix-assisted laser desorption/ionization time-of-flight mass
585 spectrometry and thioacidolysis/liquid chromatography/electrospray ionization mass spectrometry. *Ana-*
586 *lytica Chimica Acta 513*, 247–256.