

A study of the antioxidant capacity of oak wood used in wine ageing and the correlation with polyphenol composition

Article

Accepted Version

Alañón, M.E., Castro-Vázquez, L., Díaz-Maroto, M.C., Gordon, M. H. and Pérez-Coello, M.S. (2011) A study of the antioxidant capacity of oak wood used in wine ageing and the correlation with polyphenol composition. *Food Chemistry*, 128 (4). pp. 997-1002. ISSN 0308-8146 doi:
<https://doi.org/10.1016/j.foodchem.2011.04.005> Available at
<http://centaur.reading.ac.uk/20442/>

It is advisable to refer to the publisher's version if you intend to cite from the work.

To link to this article DOI: <http://dx.doi.org/10.1016/j.foodchem.2011.04.005>

Publisher: Elsevier

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1 **A STUDY OF THE ANTIOXIDANT CAPACITY OF OAK WOOD USED IN**
2 **WINE AGEING AND THE CORRELATION WITH POLYPHENOL**
3 **COMPOSITION**

4
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26 **Abstract**

27

28 The antioxidant capacity of oak wood used in the ageing of wine was studied by four
29 different methods: measurement of scavenging capacity against a given radical (ABTS,
30 DPPH), oxygen radical absorbance capacity (ORAC) and the ferric reducing antioxidant
31 power (FRAP). Although, the four methods tested gave comparable results for the
32 antioxidant capacity measured in oak wood extracts, the ORAC method gave results
33 with some differences from the other methods. Non-toasted oak wood samples
34 displayed more antioxidant power than toasted ones due to differences in the
35 polyphenol composition. A correlation analysis revealed that ellagitannins were the
36 compounds mainly responsible for the antioxidant capacity of oak wood. Some phenolic
37 acids, mainly gallic acid, also showed a significant correlation with antioxidant
38 capacity.

39

40 **Keywords:** Antioxidant capacity, oak wood, wine ageing, phenolic compounds.

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51 **1. Introduction**

52

53 In recent decades increasing interest in natural antioxidant present in the diet has
54 developed among consumers and the scientific community. Natural antioxidants seem
55 to play a very important role in reducing the concentration of free radicals, which are
56 harmful and highly reactive intermediates constantly produced due to numerous
57 biological reactions. Antioxidants prevent the oxidation process thanks to their capacity
58 for capturing, de-activating or repairing the damage caused by free radicals which are
59 implicated in the development of multiple diseases.

60

61 Epidemiological studies have indicated that frequent intake of natural dietary
62 antioxidants is associated with a lower risk of cardiovascular disease and cancer
63 (Renaud, Guegue, Schenker, & d'Houtaud, 1998; Kaur & Dapoor, 2001; Record,
64 Dreosti, & McInerney, 2001). Fruits, vegetables and all the foods and drinks derived
65 from these commodities are the main source of natural antioxidants due to their high
66 content of polyphenols. Wine has been one the most studied beverages due to its
67 verified antioxidant potential and health benefits attributable to its high content of
68 polyphenols, which are present in solution with good bioavailability (Renaud et al.,
69 1998; Tomera, 1999).

70

71 The ageing process is a common technological procedure used in winemaking
72 which seems to contribute to an increase in the antioxidant capacity of wines (Larrauri,
73 Sánchez-Moreno, Rupérez, & Saura-Calixto, 1999; Canas, Casanova, & Belchior, 2008;
74 Alonso, Castro, Rodríguez, Guillén, & Barroso, 2004) This is due to the important

75 amount of polyphenols which is extracted from the oak wood during contact with wine
76 during the ageing stage.

77

78 However, the estimation of antioxidant capacity contributed by oak wood is a
79 difficult task because wines are complicated mixtures rich in polyphenols and
80 oenological practices including sulphur dioxide addition (Manzocco, Mastrocloa, &
81 Nicoli, 1999), skin contact (Fuhrman, Volkova, Suraski, & Aviram, 2001), carbonic
82 maceration (Pellegrini, Simonetti, Gardana, Brema, Brighenti, & Pietta, 2000),
83 vinification conditions (Burns, Gardner, Matthews, Duthie, Lean, & Crozier, 2001) and
84 microoxygenation (Rivero-Pérez, González-Sanjosé, Muñiz, & Pérez-Magariño, 2008),
85 which can all influence the antioxidant capacity.

86

87 Therefore, despite the demonstrated increase in antioxidant capacity of aged
88 wines, there are no previous reports of the antioxidant capacity acquired from oak
89 wood. The toasting process is a crucial practice used in cooperage that causes deep
90 changes in oak wood chemical composition (Hale, McCafferty, Larmie, Newton, &
91 Swan, 1999) which could affect the antioxidant capacity of oak wood. Therefore, we
92 were interested in the study of antioxidant capacity taken up from oak wood used in
93 cooperage for aging wine and how the toasting process affects it.

94

95 Despite many analytical methods being available for assessing antioxidant
96 capacity *in vitro*, there are no approved standardised methods. Sometimes, this diversity
97 of methodologies used to evaluate natural antioxidants has led to widely conflicting
98 results that are extremely difficult to interpret (Frankel & Meyer, 2000). For instance,
99 Ou et al (Ou, Huang, Hampsch-Woodill, Glanagan, & Deemer, 2002) reported no

100 correlation of antioxidant activity between values determined by the FRAP (ferric
101 reducing antioxidant power) and ORAC (oxygen radical absorption capacity)
102 techniques among most of the 927 freeze-dried vegetable samples. They concluded that
103 the ORAC method is chemically more relevant to the activity of chain-breaking
104 antioxidants but only measures activity against peroxy radicals. In contrast the FRAP
105 assay estimates only the Fe III reducing activity, which is not necessarily the
106 antioxidant activity. Furthermore the FRAP assay has some drawbacks due to
107 interference, reaction kinetics, etc. Fernandez-Pachon, Villaño, García-Parilla, &
108 Troncoso (2004) determined that the antioxidant activity evaluated as the capacity to
109 quench radicals assessed by means of the ABTS (3-ethyl-benzothiazoline-6-sulfonic
110 acid) and DPPH (diphenyl-1-picrylhydrazyl) methods is 10 or 15-times higher for red
111 wines than for white ones. However, according to the ORAC assay, red wines are only
112 five-times more active than white wines.

113

114 These differences are mainly attributable to the different chemistry principles
115 underlying these methods. Other factors such as matrix type, hydrophilic/lipophilic
116 character of compounds and heterogeneity of substrates can also influence the results
117 obtained. Therefore, a valid evaluation of antioxidant capacity requires the use of
118 several methods with different mechanisms for inhibiting oxidation (Frankel et al.,
119 2000).

120

121 The aim of this work was to gain an insight into the validity of existing
122 methodologies for the evaluation of the antioxidant characteristics of oak wood.
123 Selected methods include the measurement of scavenging capacity against a given
124 radical (ABTS, DPPH), the oxygen radical absorbance capacity (ORAC) and the ferric

125 reducing antioxidant power (FRAP). They have been applied to aqueous alcoholic
126 extracts of samples of oak woods prepared to simulate the wine aging process. The
127 antioxidant capacity conferred by two types of oak woods, toasted and non toasted, of
128 different provenance was evaluated. In addition, the relationship between antioxidant
129 capacity and phenolic composition (phenolic acids and ellagitannins) has also been
130 considered in order to evaluate the contribution of each phenolic compound to
131 antioxidant capacity.

132

133 **2. Materials and methods**

134

135 *2.1. Samples*

136

137 Shavings from oak wood samples (sized 2 cm x 1 cm x 0.1 cm) collected with
138 different provenance (American, French, Hungarian, Rumanian and Russian) were
139 supplied by the cooperage Magreñan S.L. (La Rioja, Spain). Samples were naturally
140 seasoned in the open air and one section of each non-toasted oak wood sample, was
141 submitted to thermal treatment. Toasted samples received a medium intensity toasting
142 (45-50 min) with the temperature of the wood surface being: 160-170 °C.

143

144 *2.2. Extractions*

145

146 In order to investigate the extraction of oak wood compounds from wines during
147 a simulated aging process, fourteen grams of shavings of each wood sample were
148 soaked in a litre of synthetic wine model solution (12% ethanol v/v adjusted to pH 3.5
149 with tartaric acid). (Pérez-Coello, Sánchez, García, Gonzalez-Viñas, Sanz, & Cabezudo,

150 2000). The solutions, prepared in duplicate, were shaken daily and after three weeks,
151 filtered and kept refrigerated until their analyses.

152 *2.3. Total phenolic index*

153

154 The total phenol content of extracts was determined according to the Folin-
155 Ciocalteu procedure (Singleton, & Rossi, 1965). Deionised water (1.8 mL) was added to
156 0.2 mL of each extract. Folin-Ciocalteu reagent (0.2 mL) was then added and tubes
157 were shaken vigorously. After 3 minutes, 0.4 mL sodium carbonate solution (35 % w/v)
158 was added, along with 1.4 mL of deionised water. Samples were well mixed and left in
159 the dark for 1 hour. The absorbance was measured at 725 nm using a UV-vis
160 spectrophotometer (Lambda 5, Perkin-Elmer, Seer Green, UK)) and the results were
161 expressed in gallic acid equivalents, GAE, using a gallic acid standard curve (0-0.2
162 mg/mL). Extracts were further diluted if the absorbance value measured was above the
163 linear range of the standard curve.

164

165 *2.4. Antioxidant capacity determination*

166 *2.4.1. DPPH assay*

167

168 The DPPH assay was carried out according to the method of Brand-Williams,
169 Cuvelire, & Berset, (1995) where 1,1-diphenyl-2-picrylhydrazyl radical was used as a
170 stable radical. One hundred microliters of different dilutions of extracts were added to
171 3.9 mL of a 0.06 mM methanol DPPH radical solution. Methanol was used to adjust the
172 zero and the decrease in absorbance was measured at 515 nm every minute for 25
173 minutes in a UV-vis spectrophotometer (Helios, Thermo Spectronic, Cambridge, UK).
174 Only values between 20 and 80 % of the initial absorbance of the radical DPPH were

175 taken into consideration. Concentrations were calculated from a calibration curve in the
176 range between 0.1 and 0.8 mM Trolox. Results were expressed in μM Trolox
177 equivalents per milligram of oak wood.

178

179 2.4.2. ABTS assay

180

181 The method used was the ABTS \cdot^+ (radical cation) decolorisation assay (Re,
182 Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999). The assay is based on the
183 ability of an antioxidant compound to quench the ABTS \cdot^+ relative to that of a reference
184 antioxidant such as Trolox. A stock solution of ABTS \cdot^+ radical cation was prepared by
185 mixing ABTS solution and potassium persulfate solution at 7 mM and 2.45 mM final
186 concentration respectively. The mixture was maintained in the dark at room temperature
187 for 12-16 hours before use. The working ABTS \cdot^+ solution was produced by dilution in
188 ethanol (1:90 v/v) of the stock solution to achieve an absorbance value of 0.7 (\pm 0.02) at
189 734 nm. An aliquot of 20 μL of diluted extract was added to ABTS \cdot^+ working solution
190 (2 mL). For the blank and standard curve, 20 μL of ethanol or Trolox solution was used
191 respectively. Absorbance was measured by means of a UV-vis spectrophotometer
192 (Perkin Elmer Lambda 5) at 734 nm immediately after addition and rapid mixing ($A_{t=0}$)
193 and then every minute for 5 minutes. Readings at $t = 0$ min ($A_{t=0}$) and $t = 5$ min ($A_{t=5}$) of
194 reaction were used to calculate the percentage inhibition value for each extract.

195

196 A standard reference curve was constructed by plotting % inhibition value
197 against Trolox concentration (0-15 μM). The radical-scavenging capacity of extracts
198 was quantified as μmol of Trolox equivalent per milligram of oak wood.

199

200 2.4.3. FRAP assay

201

202 The FRAP assay was performed as previously described by Benzie and Strain
203 (Benzie, & Strain, 1999) with some modifications. This spectrophotometric assay
204 measures the ferric reducing ability of antioxidants. The experiment was conducted at
205 37 °C and pH 3.6. In the FRAP assay, reductants (“antioxidants”) present in the extract
206 reduce Fe (III)-tripyridyltriazine complex to the blue ferrous form, with an absorption
207 maximum at 593 nm. The assay was performed by means of automated microplate
208 reader (Tecan GENios Pro, (Tecan Ltd, Dorset, UK)) with 96-well plates. Reagents
209 included 300 mM acetate buffer pH 3.6; 40mM hydrochloric acid; 10 mM TPTZ
210 solution and 20mM ferric chloride solution. The working FRAP reagent was prepared
211 fresh on the day of analysis by mixing acetate buffer, TPTZ solution and ferric chloride
212 solutions in the ratio 10:1:1 and the mixture was incubated at 37 °C. Diluted extract (30
213 µL) and pre-warmed FRAP reagent (225 µL) were put into each well. The absorbance at
214 time zero and after 4 min was recorded at 593 nm. The calculated difference in
215 absorbance is proportional to the ferric reducing/antioxidant power of the extract. For
216 quantification, a calibration curve of Trolox was prepared with dilutions from 0 µM to
217 750 µM. The final results were expressed as µmol of Trolox equivalent per milligram of
218 oak wood.

219

220 2.4.4. ORAC assay

221

222 The method of the ORAC assay was adapted from Cao, & Prior (1999). The assay was
223 performed with an automated microplate reader and 96-well plates. The perimeter wells
224 were not used for samples, but they were filled with 250 µl of water to ensure all sample

225 wells were surrounded by full wells. Diluted extract (25 μ l) was pipetted into each well
226 and then fluorescein working solution (96 nM, 150 μ l) in phosphate buffer, pH 7.4 and
227 37 °C, was added to each sample. The plate was placed in a Genios spectrophotometer
228 (Tecan Ltd, Dorset, UK) and incubated for 10 minutes at 37 °C. The initial fluorescence
229 was recorded at an excitation wavelength of 485 nm and an emission wavelength of 535
230 nm. 2,2'-Azobis(2-amidopropane)di-hydrochloride (AAPH, 153 mM, 75 μ l) was then
231 added to each sample well and the fluorescence was measured immediately and every 5
232 minutes thereafter for 150 minutes. The procedure was repeated using solutions of
233 Trolox in the range 0 μ M to 100 μ M to prepare the calibration curve. The ORAC value
234 for each extract was calculated using a regression equation relating Trolox
235 concentration to the net area under the fluorescence decay curve (AUC). Results are
236 expressed as μ mol of Trolox equivalents per milligram of oak wood.

237

238 *2.5. HPLC-DAD-ESI-MSⁿ Analysis of Phenolic Acids.*

239

240 Standards of *p*-coumaric acid, gallic acid, ferulic acid, caffeic acid, vanillic acid,
241 protocatechuic acid and protocatechuic aldehyde were acquired from Sigma (St. Louis,
242 MO, USA) and sinapic acid, syringaldehyde, coniferaldehyde, sinapaldehyde, 4-
243 hydroxybenzoic acid, vanillin were provided by Fluka (Buchs, Switzerland).

244

245 Identification and quantification of low molecular weight phenolic compounds
246 in aqueous alcoholic extracts was achieved by HPLC analysis using an Agilent 1100
247 series system (Agilent, Waldbronn, Germany), equipped with a DAD photodiode
248 detector (G1315B) and a LC/MSD Trap VL (G2445C VL) electrospray ionisation mass
249 spectrometry (ESI/MSⁿ) system, both coupled to an Agilent Chem Station (version

250 B.01.03) for data processing. The aqueous alcoholic extracts (50 μ l), after filtration
251 (0.20 μ m, polyester membrane, Chromafil PET 20/25, Machery-Nagel, Düren,
252 Germany) were injected, in duplicate, onto a reversed-phase column Zorbax Eclipse
253 XDB-C18 (4.6 x 250 mm; 5 μ m particle; Agilent), with temperature control at 40 °C.
254 The solvents were water/formic acid (990:10 v/v) as solvent A; and MeOH/formic acid
255 (990:10 v/v) as solvent B. The flow rate was 0.70 mL min⁻¹. The linear gradient for
256 solvent B was as follows: 0 min, 5 %; 15 min, 35 %; 30 min, 43 %; 32 min, 100 %; 40
257 min, 5 %.

258

259 Components were quantified using the DAD chromatograms obtained at 280 nm
260 with external standard calibration curves. The identity of each compound was
261 established by comparing the retention time, UV-Vis spectra and mass spectra of the
262 peaks in every sample with those previously obtained by injection of standards. For
263 identification, ESI-MSⁿ was used, setting the following parameters: positive ion mode;
264 dry gas, N₂, 11 mL min⁻¹; drying temperature, 350 °C; nebulizer, 65 psi; capillary, -
265 2500 V; capillary exit offset, 70 V; skimmer 1, 20 V; skimmer 2, 6 V; and scan range,
266 50–1200 m/z.

267

268 Ellagitannins were analysed with a 500B HPLC chromatograph (Konik
269 Instrument, Spain) with a 7176-LC Rheodyne injection valve connected to a Konik UV-
270 Vis model 206 PHD diode-array detector. Components were detected at 325nm. The
271 column used was a reverse-phase C18 LiChrospher[®] 100 (Merck, Darmstadt) with
272 dimensions of 250 x 4 mm and a particle size of 5 μ m. Extracts (50 μ L) were injected
273 onto the HPLC system. The elution conditions were as follow: the flow rate was 1 mL
274 min⁻¹ and the temperature 25 °C. Two solvents were used for elution: A: MeOH/ H₃PO₄

275 (999:1 v/v) and B: H₂O/ H₃PO₄ (999:1 v/v). The gradient was: 0-8 % A in 0-70 min, 8-
276 50 % A in 70-80 min, 50-100 % A in 80-90 min (Jordão, Ricardo, & Laureano, 2007).
277 Quantitative results are expressed in mg g⁻¹ ellagic acid equivalents. Due to the lack of
278 commercial standards, chromatographic peaks were identified by comparing the
279 retention time and elution order with data described in the literature (Viriot, Scalbert,
280 Herve du Penhoat, Moutounet, 1994; Fernández de Simón, Cadahía, Conde, & García-
281 Vallejo, 1999), and by their spectral mass obtained by the HPLC-DAD-ESI-MSⁿ
282 instrument previously described.

283

284 *2.6. Statistical analysis*

285

286 Pearson's correlation coefficient, Student's t-test and Student-Newman-Keults
287 test were applied to data in order to identify statistically significant differences in
288 phenol composition and antioxidant capacity among non-toasted and toasted oak wood
289 samples. All statistical analysis was done using SPSS v. 17.0.

290

291 **3. Results and discussions**

292 *3.1. Antioxidant capacity and total phenol index*

293

294 Antioxidant capacity results expressed as μmol of Trolox equivalents per
295 milligram determined by several methods for different oak wood samples under study
296 are shown in Table 1. Antioxidant capacity determined by means of different assays,
297 DPPH, FRAP, ORAC and ABTS were measured four times to test the reproducibility of
298 the assays. In all determinations, the standard deviation (sd) was lower than 0.08. The

299 magnitude of the antioxidant capacity values depended on the method employed as a
300 consequence of different conditions, reaction mechanism and end-points considered.

301

302 Non-toasted oak wood extracts showed higher antioxidant capacity values than
303 those found for toasted oak wood extracts. This fact indicates that the toasting process
304 reduces the antioxidant capacity of oak wood.

305

306 Significant differences were found between toasted and non-toasted samples in
307 the total phenol index and antioxidant capacity values estimated by DPPH, FRAP and
308 ABTS assays. However this differentiation between the types of oak wood was less
309 noticeable when the ORAC method was used. Indeed, significant differences in the
310 antioxidant capacity values assessed by the ORAC assay for toasted and non-toasted
311 oak woods were not found according to the Student-Knewman-Keuls test (Table 1).
312 Similar behaviour also was observed by Fernández-Pachón et al. (2004) who obtained
313 lower differentiation between antioxidant capacity of red and white wines when the
314 ORAC method was employed.

315

316 The correlation between the antioxidant capacity values assessed by the DPPH,
317 FRAP, ORAC and ABTS assays was investigated (Table 2). The correlations between
318 the assays studied were highly positively ($0.69 < r < 0.97$, $P < 0.01$), indicating that the
319 four assays provided comparable values when they were used for estimating the
320 antioxidant capacity of oak wood. A high correlation between these techniques was also
321 found by other authors in sorghum and its products and in guava fruit extracts (Awika,
322 Rooney, Wu, Prior, & Cisneros-Zevallos, 2003; Thaipong, Boonprakob, Crosby,
323 Cisneros-Zeballos, & Hawkins-Byrne, 2006). However, the lowest correlation

324 coefficients were found for the ORAC assay. This fact can be attributable to the
325 different chemistry principle upon this method is based. The DPPH, FRAP and ABTS
326 methods are based on single electron transfer (SET) reaction. In these methods
327 antioxidants are oxidized by oxidants, such as a metal (Fe III) or a radical (DPPH[•] or
328 ABTS^{•+}). As a result, a single electron is transferred from the antioxidant molecule to
329 the oxidant. In contrast, the ORAC assay is based on a hydrogen atom transfer (HAT)
330 reaction after a peroxy radical ROO[•] has been generated in which this radical abstracts
331 a hydrogen atom from the antioxidant compounds. Better correlations were found
332 between assays based on the same chemistry principle. Furthermore, the ORAC assay
333 only measures the activity of chain-breaking antioxidants against peroxy radicals.
334 Therefore a relative difference in the ability of antioxidant compounds of oak wood in
335 the extracts to quench peroxy radicals and to reduce DPPH[•], ABTS^{•+}, and Fe (III) was
336 observed.

337

338 Table 1 also shows the total phenol index of the oak wood aqueous alcoholic
339 extracts. The differences between total phenol content were consistent with those found
340 in the antioxidant capacity values. Therefore, the correlation with values from the
341 antioxidant assays tested were also evaluated (Table 2). The total phenol index, TPI,
342 showed a highly positive correlation with antioxidant capacity determined by all assays
343 ($0.72 < r < 0.97$, $P < 0.01$), which indicates that TPI is an important contributor to
344 antioxidant capacity in oak wood extracts.

345

346 *3.2. Phenolic composition*

347

348 Table 3 shows the content of low molecular weight phenols in oak wood
349 aqueous alcoholic extracts. It was evident that the toasting process caused major
350 changes to the polyphenol composition of the oak wood samples studied. An increase in
351 the phenolic aldehydes including protocatechuic aldehyde, vanillin, syringaldehyde,
352 coniferaldehyde and sinapaldehyde and some phenolic acids like vanillic, ferrulic and
353 sinapic acids was observed. The increase of these compounds is attributable to the
354 thermal degradation of lignin in wood, which was suffered during the toasting process
355 (Hale et al., 1999). However, the content of *p*-coumaric acid was not significantly
356 affected by the toasting process.

357

358 Meanwhile, an increase in the ellagic acid content of toasted samples was also
359 observed. This change was due to the release of this compound by ellagitannins during
360 their thermal degradation (Viriot, Scalbert, Lapiere, & Moutounet, 1993). On the other
361 hand, other phenolic compounds, such as gallic, protocatechuic and caffeic acids and
362 scopoletin, were sensitive to thermal degradation causing a significant decrease in their
363 content in toasted samples.

364

365 Others compounds studied include ellagitannins which constitute a complex class
366 of polyphenols characterized by one or more hexahydroxydiphenoyl (HHDP) moieties
367 esterified to a sugar, usually glucose. Table 4 shows the ellagitannin content of the oak
368 wood samples studied. Four C-glucosidic ellagitannins monomers were detected
369 (vescalagin, castalagin, grandin, and roburin E) and also ellagitannin dimers (roburins
370 A-D). Among the ellagitannins detected, the ellagitannin monomers (gradinin,
371 vescalagina, roburin E and castalagin) were found in higher concentrations. Castalagin
372 was the main ellagitannin found in all samples. The toasting process had a great

373 influence on the ellagitannin composition of oak wood, since these compounds tended
374 to decrease due to thermal degradation during the toasting process. In all cases, toasted
375 oak samples showed significantly lower ellagitannins concentrations than non-toasted
376 samples.

377

378 *3.3. Correlation between antioxidant capacity and phenolic composition of oak wood.*

379

380 In order to determine the contribution of individual phenolic compounds to the
381 antioxidant capacity, the correlation between the antioxidant capacity estimated by the
382 four methods and the concentration all the phenolic compounds detected was
383 investigated (Table 5). The results obtained by the different methods were in good
384 agreement. No correlation was found between the antioxidant capacity and the
385 concentration of phenolic aldehydes (protocatecaldehyde, vanillin, coniferaldehyde and
386 sinapaldehyde). Therefore, it can be concluded that these types of compounds do not
387 make a amjor contribution to the antioxidant capacity of oak wood.

388

389 A significant correlation was found between antioxidant capacity and some
390 phenolic acids including gallic acid, protocatechuic acid, caffeic acid and *p*-coumaric
391 acid. This shows that these compounds can make a major contribution to the overall
392 antioxidant power of oak wood. The high antioxidant activity of gallic acid has been
393 demonstrated by others authors (Canas et al., 2008; Bakkalbase, Mente, & Artik, 2009).
394 Its antioxidant properties are a consequence of the three free phenolic hydroxyl groups
395 per molecule. However, no strong correlation between the rest of the phenolic acids and
396 antioxidant capacity was found.

397

398 Strong correlations were observed between the antioxidant capacity and the
399 concentration of all ellagitannins studied ($0.970 < r < 0.724$). Hence, ellagitannins, high
400 molecular weight polyphenols, are the compounds mainly responsible for the
401 antioxidant capacity of oak wood samples. This finding is consistent with reports by
402 others authors who analysed ellagitannins in different matrices (Bakkalbase et al., 2009;
403 Quideau, 2009). This activity is due to the structure of ellagitannins which is
404 characterized by the presence of several ortho hydroxyl substituents which exhibit a
405 higher ability to donate a hydrogen atom and to support the unpaired electron as
406 compared to low molecular weight phenolic compounds. Among the ellagitannins found
407 in oak wood samples, castalagin showed the highest correlation coefficient so it seems
408 to be the major contributor to antioxidant capacity, although further researches are
409 necessary to confirm this hypothesis.

410

411 **4. Conclusion**

412

413 On the basis of our results, it is concluded that the four methods tested (DPPH,
414 FRAP, ORAC and ABTS) gave comparable results for the antioxidant capacity of oak
415 wood extracts. However, results from the ORAC method were poorest in correlating
416 with the other methods. Furthermore the ORAC assay did not detect significant
417 differences in antioxidant capacity values between toasted and non-toasted oak wood
418 samples like the rest of the methods did.

419

420 This fact is attributed to the principle underlying the ORAC assay, which only
421 measures the activity of chain-breaking antioxidants against peroxy radicals.

422 Furthermore, the ORAC assay is based on a different mechanism compared with the
423 rest of the methods.

424

425 Therefore, due to different reactive oxygen species and differences in reaction
426 mechanisms, selecting a single method for antioxidant capacity evaluation is a rather
427 difficult task. The use of just one antioxidant capacity assay is oversimplified and thus
428 inappropriate. For that reason, it is recommended that comprehensive assays are
429 employed to elucidate a full profile of antioxidant activity against various reactive
430 oxygen species.

431

432 To the best of our knowledge, this is the first research that investigated the
433 antioxidant capacity of extracts from oak wood that can be conferred to aged wines and
434 how the toasting process can influence it. Non-toasted oak wood samples showed more
435 antioxidant power which was very closely correlated with the total polyphenolic content
436 of the samples. The main compounds responsible for the antioxidant capacity of extracts
437 from oak wood were some phenolic acids including gallic, protocatechuic, caffeic and
438 p-coumaric acids and also all the ellagitanins studied.

439

440 **Acknowledgments**

441

442 The authors are grateful to the Spanish Ministerio de Ciencia e Innovación for the award
443 of a doctoral grant to MEA and for its financial support towards the Project AGL2008-
444 04913-CO2-01/ALI.

445

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Table 1. Total phenol index and antioxidant capacity of oak wood determined by the DPPH, FRAP, ORAC and ABTS assays

Oak wood	TPI* n = 4	DPPH ^δ n = 4	FRAP ^δ n = 4	ORAC ^δ n = 4	ABTS ^δ n = 4
American	32.28 ^b ± 0.45	0.26 ^b ± 0.02	0.29 ^b ± 0.02	0.42 ^a ± 0.01	0.55 ^b ± 0.01
American toasted	22.52 ^a ± 0.95	0.17 ^a ± 0.01	0.18 ^a ± 0.01	0.38 ^a ± 0.02	0.39 ^a ± 0.01
French	50.95 ^b ± 1.58	0.45 ^b ± 0.05	0.45 ^b ± 0.03	0.46 ^a ± 0.02	0.97 ^b ± 0.06
French toasted	29.52 ^a ± 0.48	0.23 ^a ± 0.02	0.32 ^a ± 0.05	0.41 ^a ± 0.02	0.74 ^a ± 0.08
Hungarian	45.72 ^b ± 0.95	0.35 ^b ± 0.06	0.44 ^b ± 0.03	0.44 ^a ± 0.02	0.94 ^b ± 0.02
Hungarian toasted	22.90 ^a ± 1.28	0.14 ^a ± 0.02	0.15 ^a ± 0.03	0.37 ^a ± 0.01	0.49 ^a ± 0.05
Rumanian	25.30 ^b ± 0.44	0.18 ^b ± 0.03	0.20 ^b ± 0.01	0.39 ^a ± 0.04	0.44 ^b ± 0.02
Rumanian toasted	14.37 ^a ± 0.97	0.08 ^a ± 0.01	0.10 ^a ± 0.01	0.32 ^a ± 0.03	0.29 ^a ± 0.02
Russian	25.95 ^b ± 0.76	0.21 ^b ± 0.02	0.20 ^b ± 0.01	0.39 ^a ± 0.02	0.43 ^b ± 0.02
Russian toasted	14.41 ^a ± 0.39	0.08 ^a ± 0.01	0.08 ^a ± 0.00	0.33 ^a ± 0.03	0.25 ^a ± 0.00

579 *TPI: Total phenol index expressed as micrograms of gallic acid equivalents per
 580 milligram of oak wood
 581 ^δ Expressed as μmol of Trolox equivalents per milligram of oak wood
 582 Different superscript letters in the same column denote a significant difference among
 583 non-toasted and toasted samples according to the Student-Newman-Keuls test at P <
 584 0.05

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594 **Table 2.** Pearson's coefficient between different antioxidant capacity methods tested
595 and the total phenol index.

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	DPPH	FRAP	ORAC	ABTS
TPI	0.97*	0.96*	0.72*	0.95*
DPPH		0.92*	0.69*	0.90*
FRAP			0.73*	0.95*
ORAC				0.74*

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598 * Significant correlation $p < 0.01$ (bilateral)

599

600 **Table 3.** Concentrations of low molecular weight phenolic compounds expressed as $\mu\text{g g}^{-1}$ in toasted and non-toasted oak wood samples (n=2)

Compound	American oak wood		French oak wood		Hungarian oak wood		Rumanian oak wood		Russian oak wood	
	Natural	Toasted	Natural	Toasted	Natural	Toasted	Natural	Toasted	Natural	Toasted
Gallic acid	396.1±10.6	249.7±6.1	442.8±5.4	343.5±8.0	392.8±6.6	146.3±7.7	220.2*±0.7	195.5*±10.9	149.4±2.0	110.0±6.0
Protocatechuic acid	221.5±4.7	81.7±8.7	256.0±9.3	130.2±9.6	242.8±12.3	82.9±4.0	231.5±11.4	66.1±5.6	135.1±8.0	23.9±1.5
Protocatechuic aldehyde	nd	112.3±2.0	nd	116.0±5.9	9.9±1.8	32.4±4.2	nd	11.2±0.6	nd	34.8±4.7
Vanillic acid	91.6±8.5	129.9±3.2	87.1±14.8	140.2±5.2	70.8±0.7	96.8±1.9	45.7*±1.0	43.2*±0.3	39.6±2.7	70.6±9.4
Caffeic acid	101.0±2.6	26.4±0.9	89.5±1.1	18.3±1.1	36.6±2.0	4.6±0.5	46.9±2.1	6.1±0.5	46.0±0.9	7.5±0.5
Vanillin	106.4±10.8	210.2±3.7	94.7±10.2	204.0±10.1	76.7±7.7	192.0±0.1	45.6±2.7	133.5±1.1	37.0±1.8	208.9±9.9
Syringaldehyde	97.9±7.3	523.8±4.2	41.8±0.5	597.4±7.5	57.6±1.7	633.8±8.3	19.7±0.2	471.6±14.0	55.5±0.6	657.5±10.4
<i>p</i> -coumaric acid	27.5*±1.0	20.4*±1.8	31.5*±0.7	25.9*±3.3	32.7*±2.5	26.5*±0.4	18.8*±0.3	16.2*±0.7	20.5±0.8	15.6±0.6
Scopoletin	54.6±2.5	35.0±2.0	25.0*±3.0	21.1*±0.5	27.5±1.5	18.2±1.5	22.9±1.0	15.4±1.5	30.4±2.5	16.1±1.5
Ferulic acid	59.0±3.6	85.9±3.0	54.1±0.6	74.2±4.8	24.6*±1.5	29.9*±0.9	20.5±1.0	28.0±1.2	20.4±1.5	32.0±2.2
Sinapic acid	41.2±2.8	61.1±3.5	25.1±1.0	53.9±3.5	17.5±3.5	46.5±3.7	26.2±1.1	48.6±3.0	10.4±0.5	39.1±3.8
Coniferaldehyde	111.2±9.3	352.1±9.1	112.5±12.2	298.7±2.4	17.3±3.9	198.3±11.5	50.3±0.8	221.9±8.6	39.4±0.7	220.8±12.4
Sinapaldehyde	261.2±9.6	1112.2±19.4	305.7±8.5	1043.0±15.7	68.4±3.1	605.7±13.4	154.6±3.1	590.0±8.7	87.3±12.4	780.5±11.9
Ellagic acid	199.1±8.7	236.0±3.55	206.6±9.1	267.0±8.0	126.1±6.9	199.3±6.1	85.8±6.1	247.5±4.5	99.4±3.7	154.8±4.4

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602 * No differences were found between non-toasted and respectively toasted samples $p < 0.05$

603 **Table 4.** Concentrations of ellagitannins (expressed as $\mu\text{g g}^{-1}$ ellagic acid equivalents) in toasted and non-toasted oak wood samples (n=2).

Compound	American oak wood		French oak wood		Hungarian oak wood		Rumanian oak wood		Russian oak wood	
	Natural	Toasted	Natural	Toasted	Natural	Toasted	Natural	Toasted	Natural	Toasted
Roburin A	60.4±3.1	26.3±0.9	155.9±3.2	72.3±0.7	85.1±1.7	21.9±0.5	72.3±1.3	0.1±0.0	88.9±2.1	0.1±0.0
Roburin B	77.4±5.0	35.1±1.4	118.5±1.3	49.0±2.3	105.1±1.4	26.6±1.1	77.2±1.0	0.1±0.0	88.3±0.8	0.1±0.0
Roburin C	87.0±4.7	36.0±0.9	156.7±0.5	68.8±1.3	108.4±1.7	29.0±1.5	94.5±2.7	0.1±0.0	92.8±3.0	0.1±0.0
Grandinin	242.5±4.2	60.1±0.9	325.1±15.7	93.9±0.5	439.8±16.9	49.8±1.2	260.2±0.4	19.2±2.2	260.1±6.7	15.6±1.4
Roburin D	44.7±1.9	15.0±0.9	188.0±0.7	63.9±1.63	70.4±3.2	11.3±0.2	55.6±0.6	0.1±0.0	60.2±2.0	0.1±0.0
Vescalagin	349.8±3.2	106.9±0.1	1076.6±6.4	293.6±4.2	721.9±5.5	150.2±9.6	376.0±14.1	43.5±3.5	430.4±16.1	40.8±2.3
Roburin E	472.8±9.9	130.4±8.5	949.1±9.5	248.2±8.0	751.6±21.5	76.7±1.7	515.9±3.8	42.6±4.3	488.7±20.2	35.6±2.3
Castalagin	724.4±6.9	316.0±5.2	1995.0±128.7	902.7±18.0	1642.3±23.2	455.2±9.4	790.8±11.1	223.1±9.5	734.9±49.9	131.2±6.4

605 In all cases differences were found between non-toasted and respectively toasted samples $p < 0.05$

607 **Table 5.** Correlation matrix between antioxidant capacity methods tested and the
 608 concentration of each phenolic compound.

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Compound	DPPH	FRAP	ORAC	ABTS
Gallic acid	0.85*	0.90*	0.83*	0.84*
Protocatechuic acid	0.84*	0.82*	0.84*	0.71*
Protocatechuic aldehyde	-0.26	-0.12	-0.08	-0.06
Vanillic acid	0.14	0.24	0.31	0.29
Caffeic acid	0.69*	0.62*	0.73*	0.47
Vanillin	-0.47	-0.38	-0.38	-0.27
Syringaldehyde	-0.68*	-0.59*	-0.62*	-0.45
p-coumaric acid	0.84*	0.87	0.81*	0.91*
Scopoletin	0.32	0.33	0.44	0.14
Ferulic acid	0.14	0.23	0.32	0.18
Sinapic acid	-0.50	-0.38	-0.34	-0.33
Coniferaldehyde	-0.49	-0.41	-0.37	-0.34
Sinapaldehyde	-0.48	-0.38	-0.35	-0.31
Ellagic acid	-0.12	0.02	-0.09	0.04
Roburin A	0.90*	0.84*	0.87*	0.81*
Roburin B	0.90*	0.85*	0.88*	0.77*
Roburin C	0.92*	0.86*	0.91*	0.80*
Grandinin	0.85*	0.80*	0.76*	0.72*
Roburin D	0.89*	0.82*	0.83*	0.81*
Vescalagin	0.96*	0.88*	0.84*	0.86*
Roburin E	0.93*	0.86*	0.85*	0.80*
<i>Castalagin</i>	0.97*	0.94*	0.86*	0.94*

610

611 *Significant correlation at the 0.01 level (two-tailed).