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

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Abstract

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

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

1. Introduction

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

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

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

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

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

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

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

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

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

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

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

2. Materials and methods

2.1. Samples

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

2.2. Extractions

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

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

2.3. Total phenolic index

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

2.4. Antioxidant capacity determination

2.4.1. DPPH assay

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

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

2.4.2. ABTS assay

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

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

2.4.3. FRAP assay

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

2.4.4. ORAC assay

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

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

2.5. HPLC-DAD-ESI-MS^n Analysis of Phenolic Acids.

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

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

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

Components were quantified using the DAD chromatograms obtained at 280 nm with external standard calibration curves. The identity of each compound was established by comparing the retention time, UV-Vis spectra and mass spectra of the peaks in every sample with those previously obtained by injection of standards. For identification, ESI-MS^n was used, setting the following parameters: positive ion mode; dry gas, N_2, 11 mL min^{-1}; drying temperature, 350 °C; nebulizer, 65 psi; capillary, -2500 V; capillary exit offset, 70 V; skimmer 1, 20 V; skimmer 2, 6 V; and scan range, 50–1200 m/z.

Ellagitannins were analysed with a 500B HPLC chromatograph (Konik Instrument, Spain) with a 7176-LC Rheodyne injection valve connected to a Konik UV-Vis model 206 PHD diode-array detector. Components were detected at 325nm. The column used was a reverse-phase C18 LiChrospher® 100 (Merck, Darmstadt) with dimensions of 250 x 4 mm and a particle size of 5 µm. Extracts (50 µL) were injected onto the HPLC system. The elution conditions were as follow: the flow rate was 1 mL min^{-1} and the temperature 25 ºC. Two solvents were used for elution: A: MeOH/ H_3PO_4

(999:1 v/v) and B: H_2O/ H_3PO_4 (999:1 v/v). The gradient was: 0-8 % A in 0-70 min, 8-50 % A in 70-80 min, 50-100 % A in 80-90 min (Jordão, Ricardo, & Laureano, 2007). Quantitative results are expressed in mg g^{-1} ellagic acid equivalents. Due to the lack of commercial standards, chromatographic peaks were identified by comparing the retention time and elution order with data described in the literature (Viriot, Scalbert, Herve du Penhoat, Moutounet, 1994; Fernández de Simón, Cadahía, Conde, & García-Vallejo, 1999), and by their spectral mass obtained by the HPLC-DAD-ESI-MS^n instrument previously described.

2.6. Statistical analysis

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

3. Results and discussions

3.1. Antioxidant capacity and total phenol index

Antioxidant capacity results expressed as μmol of Trolox equivalents per milligram determined by several methods for different oak wood samples under study are shown in Table 1. Antioxidant capacity determined by means of different assays, DPPH, FRAP, ORAC and ABTS were measured four times to test the reproducibility of 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 DDPH,
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

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

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

3.2. Phenolic composition

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

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

Others compounds studied include ellagitanins which constitute a complex class of polyphenols characterized by one or more hexahydroxydiphenoyl (HHDP) moieties esterified to a sugar, usually glucose. Table 4 shows the ellagitannin content of the oak wood samples studied. Four C-glucosidic ellagitannins monomers were detected (vescalagin, castalagin, grandin, and roburin E) and also ellagitannin dimers (roburins A-D). Among the ellagitannins detected, the ellagitannin monomers (gradinin, vescalagina, roburin E and castalagin) were found in higher concentrations. Castalagin 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

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

4. Conclusion

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

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

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

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

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

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576 **Table 1.** Total phenol index and antioxidant capacity of oak wood determined by the

577 DPPH, FRAP, ORAC and ABTS assays

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

579 *TPI: Total phenol index expressed as micrograms of gallic acid equivalents per

580 milligram of oak wood

581 ð 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 $P <$

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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)

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600 **Table 3**. Concentrations of low molecular weight phenolic compounds expressed as μg 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
p-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 μg g^{-1} ellagic acid equivalents) in toasted and non-toasted oak wood samples (n=2).

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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 $p < 0.05$

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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*
Castalagin	0.97*	0.94*	0.86*	0.94*

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