Oak barrel tannin and toasting temperature: Eﬀects on red wine condensed tannin chemistry

# A R T I C L E I N F O

*Keywords:* Ellagitannins Oak wood

Flavan-3-ol polymers Tannin activity Molecular mass

# A B S T R A C T

Red wine is known to derive speciﬁc aromas and to have its color enhanced by oak wood compounds, but the eﬀect of oak barrels on wine tannin chemistry is not well known. Cabernet Sauvignon wines were aged from 8 to 12 months in oak barrels made with three diﬀerent ellagitannin levels (Tannin Potential, TP) and two toasting levels. Condensed tannin composition, activity and ellagitannin contents have been characterized using HPLC- DAD and protein precipitation. Red wines aged in high TP barrels contained more ellagitannins, condensed tannins with lower mass and lower pigmented tannins percentage than in low TP barrels. Red wines aged in high-temperature toasted barrels contained less ellagitannins, condensed tannins with higher mass and higher pigmented tannins percentage than in low-temperature toasted barrels. Combined eﬀects of ellagitannin, toasting levels and aging have been identiﬁed. The selection of barrels for ellagitannin content and toasting temperature can be used to manage red wine development.

1. Introduction

Red wine quality is usually deﬁned as the sensory perception of ﬁnished wine in the mouth. Condensed tannins are the most important macromolecules which inﬂuence the bitterness/mouthfeel perception of red wine ([Vidal et al., 2003](#_bookmark43)). They are oligomeric and polymeric forms of ﬂavan-3-ols ((−)-epicatechin, (+)-catechin, (−)-epigalloca- techin and (−)-epicatechin-3-*O*-gallate), linked mainly by C4eC8 lin- kages. Depending on the degree of polymerization, average number of constitutive units, condensed tannins can interact more or less with salivary proteins and induce an astringent mouthfeel ([Poncet-Legrand](#_bookmark37)

astringency and mouthfeel ([Glabasnia & Hofmann, 2006, 2007; Michel,](#_bookmark19) [Jourdes, Giordanengo, Mourey, & Teissedre, 2012; Mosedale, Puech, &](#_bookmark19) [Feuillat, 1999](#_bookmark19)). These are hydrolyzable tannins, characterized by one or more hexahydroxydiphenoyl (HHDP) moieties esteriﬁed with a sugar, typically glucose ([Michel et al., 2011](#_bookmark35)). Ellagitannins are easily ex- tractable in red wine during aging as they are soluble in hydroalcoholic solution. Due to their structure, such as the presence of the galloyl functional groups, it has been proposed that they are involved in the oxidation process by being the ﬁrst compound in red wine to be oxi- dized. Indeed, the oxidation of the pyrogalloyl ring at the glucose C1 position of the castalagin lead to a cyclopentenone moiety, forming

[et al., 2010](#_bookmark37)). This tannin perception can vary by the tannin structure or

their behavior in solution, caused by exterior parameters such as oxi- dation reactions ([Poncet-Legrand et al., 2010](#_bookmark37)) and interactions with other macromolecules ([Carvalho et al., 2006](#_bookmark11)).

For thirty years, research work devoted to the study of wood mo- lecules giving nuances and ﬂavors to wine has identiﬁed a number of impact compounds. Among aromatic compounds released by wood, the *trans-* and *cis-*whisky lactone, vanillin, eugenol and isoeugenol ([Prida &](#_bookmark38) [Chatonnet, 2010; Spillman, Sefton, & Gawel, 2004](#_bookmark38)); 5-hydro- xymethylfurfural, which contributes to wine's toasty character, and furfural and 5-methylfurfural, involved in the reduction of the fruity character ([Prida & Chatonnet, 2010](#_bookmark38)). However, ellagitannins, the major non-volatile extractives from oak, also play a major role with regard to

[Koshimizu, & Kouno, 2008](#_bookmark17)). They are also known to be highly reactive with other ﬂavonoids by reacting with condensed tannins through condensation reactions to form ﬂavano-ellagitannin products ([Ishimaru](#_bookmark23) [et al., 1988; Saucier, Jourdes, Glories, & Quideau, 2006](#_bookmark23)) and with an- thocyanins to stabilize red wine color ([Dumitriu, Lerma, Cotea, Zam](#_bookmark16)ﬁ[r,](#_bookmark16) [& Peinado, 2016](#_bookmark16)), such as 1-deoxyvescalagin-malvidin. The levels of these compounds in wines after aging depend on many parameters including raw material (wood) content, and toasting. When hydrolyzed from the glucose, HHDP spontaneously lactonizes to ellagic acid in aqueous solution, and these reactions can be used to simplify the quantiﬁcation of ellagitannins ([Mämmelä, Savolainen, Lindroos,](#_bookmark28) [Kangas, & Vartiainen, 2000](#_bookmark28)).

Regarding the raw material, the variability in chemical composition of oak wood is now well known; both between trees ([Doussot, De Jéso,](#_bookmark15) [Quideau, & Pardon, 2002; Michel et al., 2012; Snakkers, Nepveu,](#_bookmark15) [Guilley, & Cantagrel, 2000](#_bookmark15)) and within the same tree ([Masson,](#_bookmark29) [Moutounet, & Puech, 1995; Mosedale, Charrier, Crouch, Janin, & Savill,](#_bookmark29) [1996](#_bookmark29)), as well as between the resulting barrel ([Towey & Waterhouse,](#_bookmark42) [1996](#_bookmark42)). Recent studies based on the classiﬁcation of cooperage oak ac- cording to its ellagitannin levels conﬁrmed a high variability in levels of these compounds ([Michel et al., 2013](#_bookmark34)), and demonstrated the inﬂuence of the tannins on red wine oral sensation. Wood ellagitannins have been observed to be related to red wine astringency perception either by direct interaction with salivary proteins, or by combination with con- densed tannins that lead to synergized interaction with salivary pro- teins ([Chira & Teissedre, 2015](#_bookmark14)). In addition, some major changes in wood chemical composition occur during toasting. While thermal de- gradation of wood macromolecules generates many of the important oak aroma compounds (furanics, phenolic aldehydes, and phenols), the ellagitannins are also aﬀected, and are partially degraded by heavy toasting conditions (temperature, duration and level of wood moisture) ([Chira & Teissedre, 2013; Fernández de Simón, Cadahía, del Álamo, &](#_bookmark13) [Nevares, 2010](#_bookmark13)). By changing the thermal proﬁle, it is therefore possible to modulate the aromatic and taste impact of the barrels ([Chira &](#_bookmark14) [Teissedre, 2015](#_bookmark14)).

However, the interactions between oak barrel ellagitannin compo-

sition on condensed tannins properties have not been studied yet de- spite the impact of ellagitannins and condensed tannin structure on red wine mouthfeel perception ([Harbertson, Parpinello, Heymann, &](#_bookmark21) [Downey, 2012; Rinaldi, Gambuti, Moine-Ledoux, & Moio, 2010](#_bookmark21)). Here, two red wines with diﬀerent tannin concentration were aged for 8–12

months in oak barrels made with staves of three diﬀerent ellagitannin

levels and toasted at two temperature levels, and the resulting wines were evaluated for tannin concentrations, molecular weight, and ac- tivity, and the formation of wine pigments.

1. Material and methods
   1. *Reagent and chemicals*

All solvents were HPLC grade. Acetonitrile, methanol, acetic acid, L- (+) ascorbic acid, hydrochloric acid, lithium chloride, *o*-phosphoric acid, *N,N-*dimethylformamide, and anhydrous sodium acetate were purchased from VWR International (Radnor, PA). Phloroglucinol, (−)-epicatechin, (+)-catechin hydrate and ellagic acid were purchased from Sigma-Aldrich (St. Louis, MO). Procyanidin B1 and (−)-epica- techin-3-*O*-gallate were purchased from Extrasynthèse (Lyon, France).

* 1. *Oak barrel characteristics*

The commercial barrels (225 L) were made from woods coming from French forests (90%) and neighboring countries (10% mainly Germany) that were naturally seasoned for 30 months.

* + 1. *Oak wood sorting methodology according to its tannin potential (TP)* Wood classiﬁcation according to its ellagitannin content was per- formed by near-infrared spectroscopy (NIRS) using a technique based on the use of an acousto-optic tunable ﬁlter (AOTF, Brimrose, USA) ([Michel et al., 2011](#_bookmark35)). After machining, the untoasted staves were ﬁrst being intended to gather spectral data and then ellagitannins total level analyzed through ellagic acid dosage in HPLC-DAD after extraction and hydrolysis in acidic medium (method described in 2.3.1.). The cali- bration was performed from a partial least squares (PLS) regression after selecting the most discriminant spectral zones. The correlation coeﬃcient between spectral measurement and HPLC dosage of total ellagitannins (0.89) shows the performance of the model used. The classiﬁcation from the NIRS method was used to sort the staves into three groups of tannin potential (TP) i.e. the ellagitannin content in

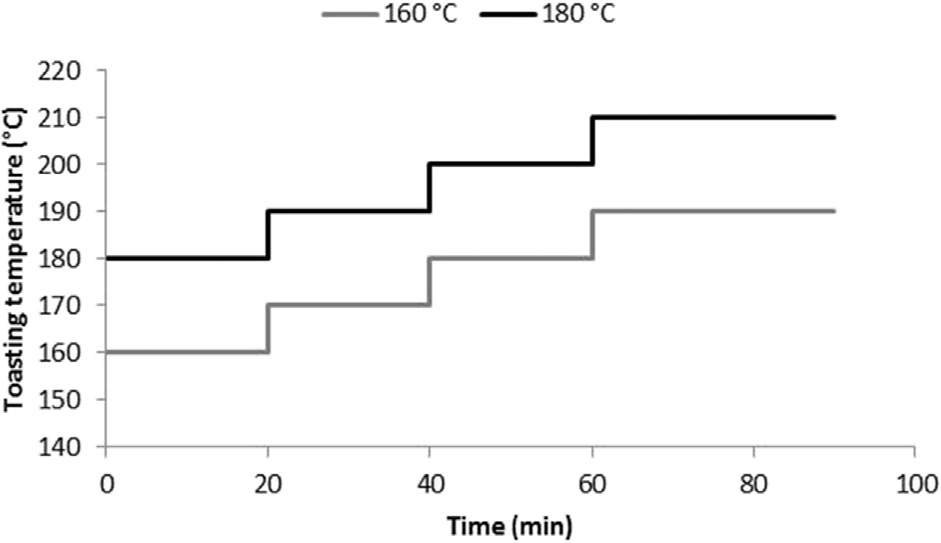


Fig. 1. Toasting temperature versus time of oak barrel toasted at 160 and 180 °C as initial temperature.

untoasted wood: Low or LTP < 4000, Medium or MTP from 4001 to 6000 and High or HTP from 6001 to 8000 μg of ellagic acid equivalent/ g of dry wood. The thickness of staves was of 22 mm and the porosity of staves was ﬁne grain (visual classiﬁcation: < 2 mm) for LTP and MTP

barrels and medium grain (visual classiﬁcation: 2–3 mm) for HTP bar- rels. The bottoms of the barrels contained the same tannin potential but

were not toasted.

* + 1. *Wood toasting methodology*

Toasting was made after a 4 min automated steam bending, which allows obtaining a neutral white barrel inside. Toasting, controlled by computer, was performed using radiant heat rather than direct contact with ﬂame. The toasting pot was fed regularly with fuel (100% oak pellets) by an auger. The barrel, placed on a turntable, rotates around a double cone that covers the ﬁre and channels the heat source, during the entire toasting phase. An infrared sensor, performing measurements on the internal surface of the shell, provides temperature control, with a heating accuracy of ± 3 °C. In our study, all barrels (LTP, MTP and HTP) underwent a gradual toasting (G) with a temperature increase of 10 °C every 20 min ([Fig. 1](#_bookmark3)), and two initial temperatures were com- pared (160 and 180 °C).

* 1. *Red wine winemaking and characterization*

Red wines were made by two Californian wineries (B and CR) from 2015 Cabernet Sauvignon grapes of Napa Valley. The alcoholic fer- mentation was made using Zymaﬂore F15 and D254 yeast strains in B and CR wines, respectively. Spontaneous malolactic fermentations oc- curred after yeast fermentation was complete. Sulfur dioxide, 60 mg/L, was added to the wines prior to barreling and three wine samples of each winery were analyzed. The wine from each winery was barreled in 18 barrels (225 L) (triplicate of 6 modalities). Barrels were topped every month (with the same wine coming from another barrel), racked every three months and samples were analyzed each time. B wines were aged for 12 months in 18 barrels (triplicate of 6 modalities) and CR wines were aged for 8 months in 18 barrels (triplicate of 6 modalities) ([Fig. 2](#_bookmark4)). The cellar humidity was around 80%. pH, ethanol content and ti- tratable acidity were determined using a Winescan FT120 (FOSS, Eden Prairie, Minnesota, USA) for each modality and replicate, and the average and standard deviation of the eighteen wines (from 18 barrels: triplicates of 6 barrel modalities) was used to compare these parameters between wineries.

Prior to barreling, B and CR wines contained 15.4 and 14.8% (v/v)

of ethanol, had a pH of 3.9 and 3.7, and a titratable acidity of 5.3 and

4.9 g/L, respectively. Surprisingly, after 12 and 8 months of aging of B and CR wines, the pH values had signiﬁcantly decreased down to 3.7 and 3.6 respectively. The ethanol content had slightly decreased in B wines to 15.2% v/v and increased in CR wines (14.9% v/v). Titratable acidity increased with aging up to 6.0 and 5.9 in B and CR wines

porated using a Centrivap cold trap at 37 °C, then dissolved in 1 mL methanol. After addition of 1 mL 2 N hydrochloric acid in methanol, the acidic hydrolysis was carried out at 100 °C for 2 h. Samples were cooled down in ice prior to injecting 20 μL and analysis by HPLC-DAD. The 1100 Agilent HPLC system was composed of a degasser, an auto-

sampler, a quaternary pump and a diode array detector, controlled by Chemstation software. For analysis, a reversed-phase 250 × 4 mm, 5 μm, RP-18 Lichrospher® 100 column was used. The mobile phases used were composed of solvent A (water: phosphoric acid; 999:1, v/v) and solvent B (methanol: phosphoric acid; 999:1, v/v), with the fol-

lowing elution gradient (time in min (%B)): 0 (0), 5 (35), 30 (45), 35

(100), 37 (0), 45 (0). The ﬂow rate was set at 0.76 mL/min at 35 °C with detection set at 370 nm (maximum absorbance of ellagic acid). Wine ellagitannin concentration is expressed in mg/L ellagic acid equiva- lents.

* + 1. *Condensed tannin activity*

The HPLC method for measuring the tannin activity (enthalpy of interaction) of wine tannins has been described ([Watrelot, Schulz, &](#_bookmark44) [Kennedy, 2017; Yacco, Watrelot, & Kennedy, 2016](#_bookmark44)). Brieﬂy, the HPLC

method used a polystyrene divinylbenzene reversed-phase column (PLRP-S, 2.1 × 50 mm, 100 Å, 3 μm, Agilent Technologies, Santa Clara, CA) protected with a guard column (PRP-1, 3 × 8 mm, Hamilton

Company, Reno, NV), with a DAD detection at 280 nm. The mobile phases consisted of 1.5% (w/w) *ortho*-phosphoric acid in water (180 mM, mobile phase A) and 20% (v/v) mobile phase A in acetoni- trile (mobile phase B) with a ﬂow rate of 0.3 mL/min. The linear gra- dient was as follows (time in min (%B)): 0 (14), 12.6 (34), 12.6–13.3

(34), 15.05 (70), 15.05–16.8 (70), 19.6 (14), and 19.6–28 (14). For

elucidation of tannin activity samples were run at four diﬀerent tem- peratures, from 25 °C to 40 °C in 5 °C increments, in order to obtain a linear van't Hoﬀ plot. All temperatures were converted into degrees Kelvin and the chromatograms were monitored at 280 nm. For calcu- lations, all chromatograms were normalized, subtracting the back- ground signal of the water injection. A baseline was drawn at 0 mAUs and cut near 5 min, at the beginning of (−)-epicatechin standard elu- tion at 30 °C. The chromatogram was then cut at 16.8 min (change of gradient), corresponding to partial tannin (TanninP). The alternative response factor *kalt* was calculated as follows (1), where TanninT is total tannin and TanninP is partial tannin.

*RT R*

ΔH° and ΔS° are the speciﬁc enthalpy and entropy of interaction, re- spectively, and R is the universal gas constant. The tannin activity (speciﬁc enthalpy ΔH°) was calculated from the slope (R2 of 0.99). The speciﬁc entropy of interaction was always negative and negligible in the

calculation of the tannin activity.

* + 1. *Protein-precipitable tannin concentration*

The tannin concentration was determined using a protein pre- cipitation method as previously described by [Harbertson, Kennedy, and](#_bookmark20) [Adams (2002)](#_bookmark20) and [Kennedy, Ferrier, Harbertson, & Gachons, (2006)](#_bookmark25) The concentration of tannins is expressed in (+)-catechin equivalent.

* + 1. *Condensed tannin composition and mDP*

Constitutive subunits as well as the mean degree of polymerization (mDP) of condensed tannins were characterized by HPLC-DAD at 280 nm after an acid-catalysis in an excess of phloroglucinol as pre- viously described ([Kennedy & Jones, 2001](#_bookmark26)). Brieﬂy, tannins extracted from wine by solid phase extraction (HyperSep C18, 1 g, 6 mL cartridge, ThermoFisher scientiﬁc) were dissolved in methanol and were added to the phloroglucinol reagent solution (0.1 N hydrochloric acid in me- thanol, containing 50 g/L phloroglucinol and 10 g/L ascorbic acid)

(1:1, v/v) and maintained at 50 °C for 20 min. The reaction was ar- rested by addition of 500 μL of cold 40 mM aqueous sodium acetate to 100 μL of samples, prior to injection of 20 μL to an HPLC. The HPLC

system consisted of two Chromolith RP-18e (100 × 4.6 mm) columns connected in series and protected by a guard column containing the same material. The binary gradient run consisted of mobile phase A (1% v/v aqueous acetic acid) and mobile phase B (1% v/v acetic acid in acetonitrile). The column ﬂow rate was 1.5 mL/min and with a column temperature of 30 °C. The gradient was as follows: time in min (%B): 0 (3), 8 (3), 28 (18), 28.02 (80), 32 (80), 32.02 (3), 40 (3). Detection of

eluting peaks was made using a diode array detector (DAD) at 280 nm. For the calculations of the subunit composition, calibration curves of terminal subunits were carried out on (+)-catechin, (−)-epicatechin and (−)-epicatechin-3-*O*-gallate standards. Calibration curves of (−)-epicatechin-phloroglucinol product were carried out on procya- nidin B1 standard after acid-catalysis reaction and were used for ex- tension subunit characterization. The mDP was calculated by the sum of all constitutive subunit (in moles) divided by the sum of all terminal

subunits (in moles).

* + 1. *Mass and pigmentation of tannins by gel permeation*

The size distribution of wine tannins was determined using the previously described method ([Kennedy & Taylor, 2003](#_bookmark27)) after few modiﬁcations. Brieﬂy, the HPLC method used two PLgel columns in series (300 × 7.5 mm, 5 μm, 500 Å and 300 × 7.5 mm, 5 μm, 103 Å, Agilent Technologies, Santa Clara, CA) protected with a guard column

(PLgel, 50 × 7.5 mm, 5 μm, Agilent Technologies, Santa Clara, CA), with a DAD detection at 280 nm and 520 nm. The mobile phase con-

sisted of 6.3 g lithium chloride in 1% (v/v) acetic acid, 5% (v/v) water adjusted to 1 L with *N*,*N*-dimethylformamide with a ﬂow rate of 1 mL/ min 20 μL of sample was injected and previously isolated and puriﬁed pre-veraison grape skin tannin fractions were used as size calibration curve (previously characterized by the acid-catalysis with an excess of

phloroglucinol). The percentage of pigmented tannins was determined based on the ratio of the area under the curve at 520 nm to the area under the curve at 280 nm (Supporting Information).

* 1. *Statistical analysis*

Experiments were performed in triplicate: red wine was aged in three barrels for each modality and analysis was performed on each barrel, so the results are expressed as the mean and the standard de- viation of experimental replicates and all statistical analysis were made using XLstat software for Microsoft Excel®. The diﬀerences between means of categories with a conﬁdence interval of 95% were analyzed using a Fisher (LSD) test. A multivariate analysis of variance (MANOVA) using toasting temperature, ellagitannin content and aging time as factors was analyzed to determine the eﬀect of these individual factors and their interactions on the ellagitannin content, condensed tannin concentration and activity, molecular mass, pigmented tannins and mDP. Principal component analysis (PCA) was performed on the correlation matrix of the averaged data set at the end of aging (8 months for CR wines and 12 months for B wines).

1. Results and discussion
   1. *Ellagitannins in red wines*

Ellagitannins are released from wood into the hydroalcoholic medium of red wine. In agreement with [Michel et al. (2012)](#_bookmark33) ellagi- tannin concentration increased in red wine with aging in barrel ([Table 1](#_bookmark6)). After 3 months of aging the average ellagitannin con- centration (4.1 mg/L ellagic acid equivalents) quickly increased at the same rate for wines B and CR, and then a slower increase was observed between 3 and 5 months. This was consistent with a fast extraction of ellagitannin from the outer layer of staves immediately after wine contact, and then slower extraction afterwards ([Michel et al., 2011](#_bookmark35)). After 5 months of aging, the average ellagitannin concentration in- creased faster in CR wines compared to B wines and was much higher at the end of aging (15.7 mg/L ellagic acid equivalents at 8 months in CR wines versus 11.6 mg/L ellagic acid equivalents at 12 months in B wines). As ellagitannin are easily extractable from wood into hydro- alcoholic solution, the level of ethanol would have an eﬀect on the speed of extraction. In B wines, the ethanol content slightly decreased with aging in contrast to CR wines which might explain that ellagi- tannin extraction in B wines become slower than in CR wines. Low toasting temperature (LTP-160, MTP-160, HTP-160) resulted in higher ellagitannin concentration (in ellagic acid equivalents) in both wines compared to wine aged in high-toast temperature barrels (LTP-180, MTP-180, HTP-180) ([Table 1](#_bookmark6)). It was in agreement with previous re- sults ([Chira & Teissedre, 2015](#_bookmark14)) which was explained by the thermolytic degradation of ellagitannins, hemicelluloses and lignins of wood. In accordance with [Michel et al. (2012)](#_bookmark33) wines aged in HTP barrels con- tained signiﬁcantly higher ellagitannin concentrations than in MTP and

LTP barrels at each time of aging ([Table 1](#_bookmark6)), being twice as high in HTP wines compared to LTP wines. In both wines, the ellagitannin con- centration (in ellagic acid equivalents) was the lowest in LTP-180 and the highest in HTP-160. In accordance with these results, multivariate analysis of variance ([Table 2](#_bookmark7)) showed that the ellagitannin concentra- tion in red wine is dependent on the ellagitannin content in wood, aging time, and toasting temperature, in spite of a limited signiﬁcance in the B wines. In the latter wine, only the interaction of ellagitannin and aging time were signiﬁcant versus the ellagitannin concentration in wine. In CR wines, ellagitannin concentration was signiﬁcantly de- pendent on the interactions of the three factors (toasting temperature, ellagitannin content in wood and aging time).