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Impact of Concentration of Ellagitannins in Oak Wood on Their Levels and Organoleptic Influence in Red Wine

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Supporting Information

ABSTRACT: Some wood substances such as ellagitannins can be extracted during wine aging in oak barrels. The level of these hydrolyzable tannins in wine depends of some parameters of oak wood. Their impact on the organoleptic perception of red wine is poorly known. In our research, oak staves were classified in three different groups according to their level of ellagitannins estimated by NIRS (near infrared spectroscopy) online procedure (Oakscan). First, the ellagitannin level and composition were determine for each classified stave and an excellent correlation between the NIRS classification (low, medium and high potential level of ellagitannin) and the ellagitannin content estimated by HPLC−UV was found. Each different group of NIRS classified staves was then added to red wine during its aging in a stainless tank, and the extraction and evolution of the ellagitannins were monitored. A good correlation between the NIRS classification and the concentration of ellagitannins in red wine aging in contact with the classified staves was observed. The influence of levels of ellagitannins on the resulting wine perception was estimated by a trained judge's panel, and it reveals that the level of ellagitannins in wine has an impact on the roundness and amplitude of the red wine.

KEYWORDS: ellagitannins, oak wood, NIRS (near infrared spectroscopy), roundness, amplitude, red wine

INTRODUCTION

The tannins present in red wines play a major role in wine quality since they contribute to their taste and color properties.[1−4] The primary source of wine tannins is grape berry seeds and skins[5,6] which contain proanthocyanidins, also called condensed tannins, in their vacuoles and cell walls.[7] Another important source of tannins for wine is the oak heartwood used to make barrels in which red wines are aged.[8,9]

These tannins are called hydrolyzable tannins; this term refers to both ellagitannins and gallotannins.[10−12] Today, over 500 members of these gallic acid-derived polyphenolic natural products have been isolated from various plants and fully characterized.[10,11,13,14] Among this myriad of gallic acid metabolites, the *C*-glycosidic ellagitannins present a structural specificity of having a highly characteristic C−C linkage between the carbon-1 atom of an open-chain glucose core and the carbon-2′ atom of a galloyl-derived unit esterified to the 2-position of the glucose core. This C-1-linked galloyl-derived unit is either part of a terarylic nonahydroxyterphenoyl (NHTP) unit that is attached *via* three ester bonds to the 2-, 3- and 5-positions of the glucose core. The latter biarylic unit commonly given the acronym "HHBP" for hexahydroxybiphenoyl to signify the biarylic nature of this ellagitannin unit type.[15] Vescalagin (**2**) (Figure 1) and its C-1 epimer castalagin (**1**) are the first *C*-glycosidic ellagitannins that have been isolated and characterized thirty years ago from *Castanea* (chestnut) and *Quercus* (oak) woody species by Mayer and co-workers.[16−18] Six other NHTP-containing *C*-glycosidic ellagitannins were later isolated from Fagaceous *Quercus* and *Castanea* hardwood species, i.e., the lyxose/xylose-bearing monomers grandinin (**3**) and roburin E (**4**), the dimers roburins A (**5**) and D (**6**) and the lyxose/xylose-bearing dimers roburins B (**7**) and C (**8**).[19,20] Vescalagin (**2**) and castalagin (**1**) are largely predominant in the fagaceous woody species containing them. They represent between 40% and 60% by weight of the ellagitannins in *Quercus petraea* and *robur* heartwoods, which are the two main oak species used to make barrels along with the American species *Quercus alba*.[21−23] These molecules are soluble in hydroalcoholic environment; that is why they are gradually extracted by wine during its aging in oak barrels.[8] Moreover, the level of the ellagitannins in oak wood used to make barrels depends in part on the species, age, geographical origin, the forest management practices of the tree, the sampling position in the tree used[21,23−25] and the processing of wood in cooperage like the type and length of drying and toasting.[23,26] However, the two European oak species used to make barrels, *Quercus robur* and *Quercus petraea*, reveal a higher concentration of ellagitannins than *Quercus alba*.

Recently, there has been an increase of interest on ellagitannin level and evolution in wine since it has been shown that they are involved in several reactions with the other phenolic constituents of wine. It has been observed that ellagitannin affects red wine organoleptic properties such as color stability, astringency, and bitterness and also protects it against oxidation.[27−30] They also reveal important biological properties (antioxidant, anticancer,

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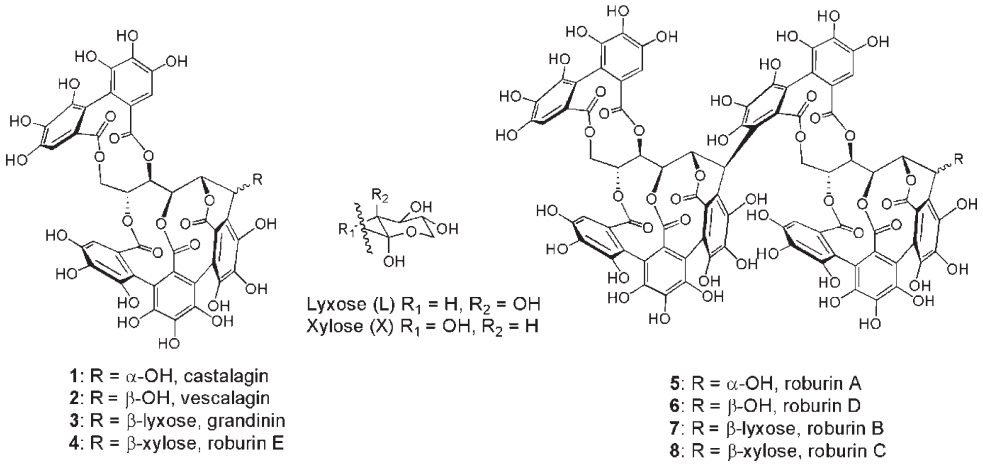

Figure 1. Structure of main monomeric ellagitannins vescalagin (**2**), castalagin (**1**), as well as the grandinin (**3**) and roburine A−E (**4**−**8**) isolated from *Castanea* (chestnut) and *Quercus* (oak) species.

anti-inflammatory, antibacterial and anti-HIV replication activities).[12,31−36] Due to their ability to precipitate proteins, in particular the salivary proteins, the level and composition of ellagitannins can affect the astringency of the wine.[27,28,37]

In this work, we decided to focus on the identification and quantification of the different ellagitannins in the wood and the wine as well as their influence on wine organoleptic properties. For this study, we used oak wood staves which were classified in three different groups according to their ellagitannin concentration estimated by a new NIRS procedure (Oakscan).[38] First, we determined by HPLC−UV−MS the ellagitannin level and composition in each oak stave in order to confirm the NIRS classification. In a second time, the three different groups of staves were added to red wine during its aging in stainless steel tanks and the ellagitannin extraction kinetics were monitored every month during four months. Finally the impact of ellagitannin level on the organoleptic properties of the obtained red wine was estimated by a trained judge's panel, and the results were treated by FIZZ software using a Newman test.

MATERIALS AND METHODS

General. Ellagic acid, formic acid (>95%), chlorhydric acid (37%) and acetic acid were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Orthophosphoric acid (85%) and methanol (HPLC quality) were purchased from VWR (Strasbourg, France). Acetone was purchased from Xilab (Atlantic labo, Bordeaux, France). Distilled water was performed with an ELGA system, and the Milli-Q (Millipore) water was prepared using a Sarterius-arium 611 system. Evaporations were conducted under reduced pressure at temperatures less than 40 °C. Methanol (HPLC quality) and Milli-Q (Millipore) water were used for HPLC−UV separations and analyses. Oak *C*-glucosidic ellagitannins such as castalagin (**1**), vescalagin (**2**), grandinin (**3**) and roburin A−E (**4**−**8**) were extracted from *Quercus robur* heartwood and purified as previously described.[15] Pure procyanidins were purchased from Extrasynthèse (Genay, France).

Wood Origin and Drying Conditions. The stave samples were constituted from two oak species (*Quercus robur* and *Quercus petraea*) from the same forest located in Vosges in France. The raw staves were stored for natural seasoning during 18 months in the Tonnellerie Radoux (Jonzac, France) seasoning park. The dried wood sampling procedure was as follows: during the mechanical processing of raw staves, the ending part of each stave was cut just before the NIRS (Oakscan) scanning and classification.[38] Then, we used these stave cutoffs as starting material for the determination of the ellagitannin concentration. The classification realized by NIRS was organized in three groups according to their polyphenol index (low potential (IP = 13 ± 2.6), medium potential (IP = 32 ± 7.2), high potential (IP = 50 ± 9.8)). Each stave (960 × 47 × 6.5 mm) was then submitted to an identical toasting procedure using an industrial convective oven; the temperature level and time correspond to a "moyenne plus" toasting level as usually used on the wood by the Tonnellerie Radoux.

Winemaking and Wine Aging. Cabernet sauvignon and merlot grapes were separately mechanically collected in October 2008 at maturity in the Aude region in France. The same day, the grapes were separately crushed, SO_2 was added (5 g/hL), and extraction enzyme (Kzym+, ICV, France) and yeast (ICV K1, Lallemand) were also added prior to their transfer to a separate stainless steel tank (40 hL). The alcoholic fermentation was processed at 25 °C. After the alcoholic fermentation, the malolactic fermentation was induced by inoculation with lactic acid bacteria (Viniflora CH16, Chr. Hansen) for 47 days in both cases. Then, in January 2009, cabernet sauvignon and merlot red wine were assembled (50/50, v/v) and aged in duplicate in the tanks of 3 hL with 9 staves (960 × 47 × 6.5 mm) selected as describe above. After 4 months of aging in contact with the oak staves, the wine was filtered, bottled and stored at 16 °C until sensory evaluations.

Wood Extraction Procedure. Each wood sample was first mechanically crushed with a Mill-FOSS Cyclotec-1093 grinder (particle size <0.6 mm) at room temperature and thereafter stored in the dark prior to processing. The obtained powder (4 g) was submitted to 6 solid/liquid extractions by acetone/water (70/30) during 17 min using an accelerated solvent extraction system (DIONEX ASE 350) with the following parameters: static time set at 8 min, temperature set at 60 °C and pressure set at 150 bar. All extracts were combined and evaporated under reduced pressure, and the obtained residue was redissolved in 20 mL of methanol prior to ellagitannin analysis.

Red Wine Sample Preparation Prior to Total Ellagitannin Level Determination. The red wine (50 mL) was evaporated under reduced pressure, and the resulting dark red viscous residue was dissolved in methanol (20 mL); then 4 mL of this mixture was loaded in the hydrolysis tubes for the determination of the total ellagitannin level.

Red Wine Sample Preparation Prior to Ellagitannin Composition Estimation. Red wine preparation was adapted from Saucier et al.[39] A sample of a red wine (30 mL) was evaporated under reduced pressure, and the resulting dark red viscous residue was dissolved in H_2O/CH_3COOH (996/4, 10 mL). This solution was loaded on a column (55 mm × 25 mm) that had been packed with TSK HW 50F

resin which had been previously swelled overnight in methanol and equilibrated with H_2O/CH_3COOH (996/4, 250 mL). After the sample was loaded on the column, the acidic aqueous solvent (50 mL) was first used to wash out tartaric acid and sugars. Then, an acidic hydromethanolic solvent [H_2O/MeOH/CH_3COOH (698/298/4), 100 mL] was used to elute an important amount of the nonellagic polyphenols. Finally the ellagitannin fraction was eluted using H_2O/acetone/CH_3COOH (298/698/4, 100 mL). This fraction was evaporated under reduced pressure to furnish a reddish light brown residue, which was dissolved in H_2O/CH_3COOH (996/4, 1 mL) and filtered (0.45 μm) prior to HPLC−UV−MS analysis.

Total Ellagitannin Concentration Determination in Wood and Wine. The total ellagitannin concentration was determined by the quantification of ellagic acid released during acidic hydrolysis (2 h at 100 °C, 2 N HCl in MeOH) as previously described by Peng et al.[40] Each sample was analyzed in triplicate, and each reaction mixture was subjected to HPLC−UV analysis using a Hewlett-Packard series 1100 and a 250 × 4.6 mm, 5 μm Lichrospher 100 RP 18 column. The mobile phases used were composed of solvent A [H_2O/H_3PO_4 (999/1)] and solvent B [methanol/H_3PO_4 (999/1)], and the gradient elution was 0−35% of B in 5 min, 35−45% of B in 25 min and 45−100% of B in 5 min. The flow rate was set at 1 mL/min with detection set at 370 nm.

Ellagitannin Composition Estimation Determination in Wood and Wine. The ellagitannin composition in wood was determined directly from the wood crude extract, whereas this composition was estimated from ellagitannin fraction obtained after column fractionation in case of the red wine. The equipment used for this analysis was a Thermo-Finnigan Surveyor HPLC system consisting of UV−vis detector (Surveyor PDA Plus), an autosampler (Surveyor autosampler Plus) and a quaternary pump (Surveyor LC pump Plus) and controlled by Xcalibur data treatment system. This HPLC system was also coupled to a Thermo-Finnigan LCQ Advantage spectrometer equipped with an ion trap mass analyzer. The electrospray ionization mass spectrometry detection was performed in negative ion mode with the following optimized parameters: capillary temperature 400 °C, capillary voltage −3 V, nebulizer gas flow 1.75 L/min, desolvation gas flow 1 L/min, and spray voltage 5 kV. These analyses were carried out in duplicate on a 250 × 4.6 mm, 5 μm Lichrospher 100 RP 18 column. The mobile phases used were solvent A [H_2O/HCOOH (996/4)] and solvent B [MeOH/HCOOH (996/4)] and gradient elution of 0−3% B in 5 min, 3−12% from 5 to 35 min and 12−100% from 35 to 40 min with a flow rate set at 1 mL/min and a detection wavelengths set at 280 nm. Each ellagitannin was separately identified by comparison of the UV spectra and mass spectra to purified standard. The quantification of each compound **1**−**8** was performed using external standard calibration curves at 280 nm in wood extracts or by using their molecular ion for the wine sample. The concentrations of castalagin (**1**), vescalagin (**2**), grandinin (**3**) and roburin A−E (**4**−**8**) were expressed as equivalents of vescalagin (**2**).

Others Parameters of the Wine. The alcoholic strength, concentration of CO_2, glucose/fructose sugar, total and volatile acidity, malic, lactic and tartric acids, the density, the pH and the TPI (total polyphenolic index) were obtained with a Winescan apparatus (FOSS-France). The total tannins and total polyphenols were determined by Bate−Smith[41] and Folin−Ciocalteu[42] procedures, respectively. The free anthocyanins were measured by discoloration method using SO_2.[43] The concentration of the flavanols, dimeric and oligomeric proanthocyanins were obtained by from the adapted Lamuela-Raventos et al. method[44] with a Thermo-Electron Surveyor HPLC system coupled to a fluorimetric detector[45] (see Supporting Information for detailed values).

Sensory Evaluation. All sensory analysis was performed by a panel of 10 trained judges, composed of 7 men and 3 women. All of the judges had extensive wine tasting experience (oenologist, wine researcher and wine salesperson). Preliminary evaluation of the wines during the aging indicated that differences among the wines were found in taste and in aroma.

Table 1. Ellagitannin Concentration in NIRS Classified Oak Staves

	concn			
	total ellagitannins[a]		8 native ellagitannins[b]	
sample no.	ellagitannins	mean ellagitannins	ellagitannins	mean ellagitannins
	Low Potential (IP = 13 ± 2.6)[c]			
48	5.46 ± 0.27		6.13 ± 0.31	
23	6.67 ± 0.28		5.93 ± 1.70	
32	9.64 ± 0.50		14.24 ± 1.66	
21	10.79 ± 0.12	9.67 ± 2.50	15.41 + 1.68	13.75 ± 5.86
33	10.86 ± 0.24		17.86 ± 0.73	
52	11.04 ± 0.55		12.92 ± 0.65	
19	13.20 ± 0.25		23.79 ± 2.45	
	Medium Potential (IP = 32 ± 7.2)[c]			
47	9.10 ± 0.75		13.36 ± 1.74	
84	12.97 ± 0.15		23.17 ± 1.13	
27	13.48 ± 0.35		17.03 ± 1.13	
77	13.73 ± 0.54	15.74 ± 4.78	16.41 +0.82	24.44 ± 10.62
82	16.88 ± 0.43		24.09 ± 1.21	
12	18.95 ± 0.76		29.84 ± 1.29	
65	25.09 ± 4.73		47.15 ± 2.08	
	High Potential (IP = 50 ± 9.8)[c]			
57	21.05 ± 1.05		34.70 ± 1.74	
54	21.24 ± 0.90		29.91 ± 7.06	
68	26.32 ± 0.39		31.31 ± 2.60	
88	26.59 ± 0.04	26.32 ± 3.78	46.97 + 3.73	41.73 ± 9.30
66	27.17 ± 0.40		45.00 ± 1.29	
87	29.68 ± 3.46		45.00 ± 1.29	
81	32.18 ± 2.04		45.00 ± 1.29	

[a] Ellagitannin concentration estimated by acidic hydrolysis and expressed as mg equivalent of released ellagic acid per g of dry wood. [b] Each ellagitannin **1**−**8** separately quantified by HPLC−UV−MS and expressed as mg equivalent of vescalagin per g of dry wood. [c] NIRS classification (Oakscan class).

Consequently, the evaluation was focused on profiling the wine aromas and flavors in the mouth. The formal evaluation consisted of a series of 4 wines and took place on a morning between 9:00 a.m. and 12:00 noon. The intensities of the descriptors (such as fruity intensity, woody intensity, roundness, amplitude, bitterness, astringency, wood and fruit balance) were rated on a discrete scale of 1 to 7; a score of 1 indicated that a descriptor was not perceived, and a score of 7 indicated a high intensity. The intensity level of each descriptor was then expressed as the mean value of all the judges from two different testing days. The samples were presented in randomly coded, clear, 125 mL glasses; distilled water was provided for rinsing between wines. All evaluations were conducted at 20 ± 1 °C under white lights in separate booths. When a judge had completed the evaluation of a series, those samples were removed and the next were introduced into the booth.

Data Analysis. Significant differences among wines and for each variable, for both analytical and sensorial data, were assessed by Newman test to study the effects of ellagitannin concentration on all the constituents measured in the wines. The results of this data were expressed as the arithmetic average for the 4 wines tasted 2 times by the 10 trained judges. These statistical analyses were performed using FIZZ treatment v2.41B.

RESULTS AND DISCUSSION

Ellagitannin Level and Composition in the NIRS Classified Oak Staves. The ellagitannin level and composition in each oak stave were determined by HPLC−UV and correlated with the NIRS (near infrared spectroscopy) classification in order to validate this nondestructive classification. In a first approach the total ellagitannin level was estimated by the determination of the amount of ellagic acid released after acidic hydrolysis. During this reaction each ellagitannin monomer or dimer released one molecule of ellagic acid. The total ellagitannin level, expressed as milligrams of released ellagic acid per gram of dry wood, revealed a large diversity of concentrations ranging from 5.46 to 32.18 mg of released ellagic acid/g of dry wood (Table 1). Such variations between different staves is not surprising since the ellagitannin concentrations in oak wood depend on the species of oak, the origin of the tree, the rain washing during the staves' seasoning, their degradation by micro-organisms, their chemical oxidation,[21,23−25,27,46] and the treatment during the processing of wood in cooperage.[9,23,26]

The total ellagitannin concentrations in staves classified in the low potential group (IP = 13 ± 2.6) ranged between 5.46 and 13.20 mg of ellagitannins/g of dry wood, whereas staves classified in the medium potential group (IP = 32 ± 7.2) revealed ellagitannin level between 9.10 and 25.09 mg/g of dry wood (Figure 2A). Finally, the third group, classified as the high potential group (IP = 50 ± 9.8), ranged between 21.05 and 32.18 mg of ellagitannins/g of dry wood (Table 1). Even if some small overlapping is observed between the low and medium and between the medium and high ellagitannin potential groups, overall there is a very good correlation between the ellagitannin concentration in each stave determined by acidic hydrolysis and their classification group estimated by the NIRS procedure as previously described.[38] This small overlapping is probably due to variability induced by the aromatic toasting step during the barrel production.

In order to have a better overview of the correlation between ellagitannin concentration and NIRS classification, the eight main ellagitannins **1**−**8** were separately quantified by HPLC−UV−MS in each studied stave. As previously observed for the total ellagitannin level estimated after acid hydrolysis, the sum of all the different ellagitannin concentrations shows a broad range between the lowest (i.e., 5.93 mg/g) and the highest (i.e., 57.30 mg/g) concentrations (Table 1). However, in this case also there is a good correlation between the level of the eight ellagitannins and the NIRS classification (Figure 2B). The low potential group (IP = 13 ± 2.6) of staves revealed a molecular total ellagitannin concentration ranging from 5.93 to 23.79 mg of ellagitannins/g of dry wood, whereas the medium potential group (IP = 32 ± 7.2) of staves ranged from 13.36 to 47.15 mg of ellagitannins/g of dry wood and the high potential group (IP = 50 ± 9.8) of staves ranged between 29.91 and 57.30 mg of ellagitannin/g of dry wood (Table 1). In this case too, there is a good correlation between the NIRS classification and the sum of each specific ellagitannin concentration quantified by HPLC−UV−MS (Figure 2B, see Supporting Information for detailed values). Overall the fact that such correlation between the NIRS classification and ellagitannin level estimated by two different procedures reveal that the NIRS classification procedure is a good, fast and efficient method to estimate and classified oak wood according to their ellagitannin level.

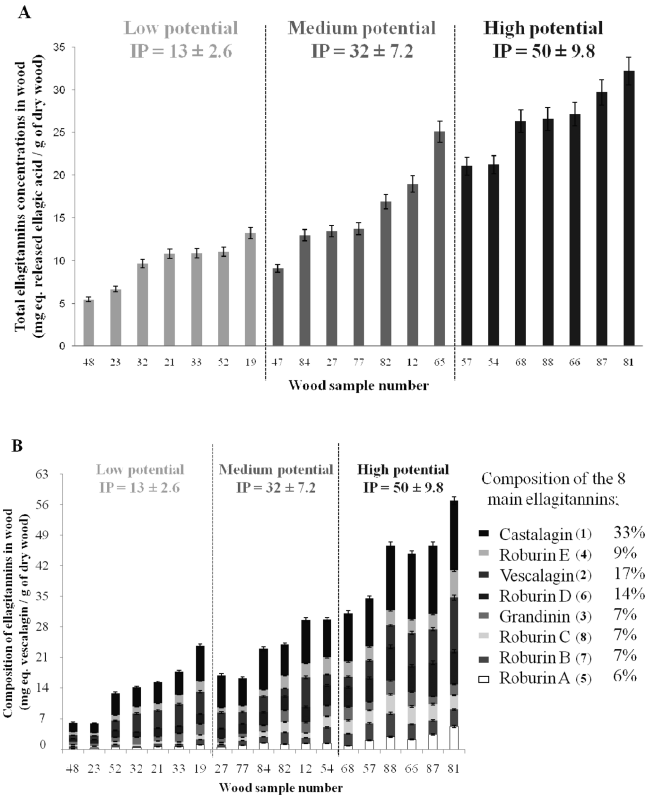

Figure 2. (A) Total ellagitannin concentrations in the wood and correlation with the NIRS oak classification and their IP determined. (B) Composition of the 8 main ellagitannins and correlation with the NIRS oak classification and their IP (see Supporting Information for detailed values).

Moreover, it appears that castalagin was always the main ellagitannin and represented around 33% of all ellagitannins. The average compositions of the eight main ellagitannins were found as follows: castalagin (∼33%), vescalagin (∼17%), roburin D (∼14%), roburin E (∼9%), grandinin (∼7%) roburin C (∼7%), roburin B (∼7%) and roburin A (∼6%) (Figure 2B). However, the composition in ellagitannins of each stave revealed some important differences, since castalagin, the main ellagitannin, can range from 28 to 43%. Similar differences were also observed for the other ellagitannins: vescalagin (7 to 29%), roburin D (9 to 17%), roburin E (7 to 13%), grandinin (4 to 11%), roburin C (4 to 10%), roburin B (4 to 12%) and roburin A (4 to 9%). This disparity in ellagitannin composition results from the oak trees' variability as previously observed;[21,23−25,27,46] however this difference in ellagitannin composition did not affect the NIRS classification since this variation was not correlated with the classification.

Ellagitannin Level and Composition in Red Wine Aged in Contact with the NIRS Classified Oak Staves. In order to verify if the difference in ellagitannin content in wood will have an influence on the ellagitannin concentration in wine aged in contact with this wood and also to check if the oak wood classified by the NIRS procedure can be correlated with the level of ellagitannins in red wine, each group of the classified oak staves was separately added to red wine in a 3 hL tank during four months. The 50/50 (v/v) cabernet sauvignon and merlot red wine had the following characteristics: an alcoholic strength of 13.89%, a pH of 3.47, a total acidity of 6.09 g equiv of tartaric

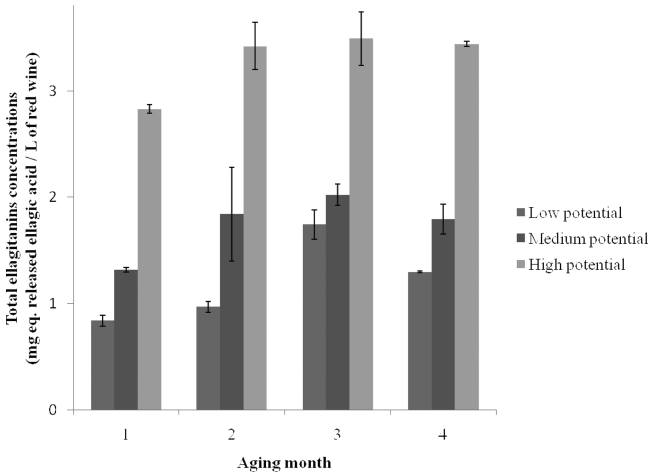

Figure 3. Total ellagitannins concentrations in the same French red wine according to their months of aging in contact with the NIRS classified oak staves.

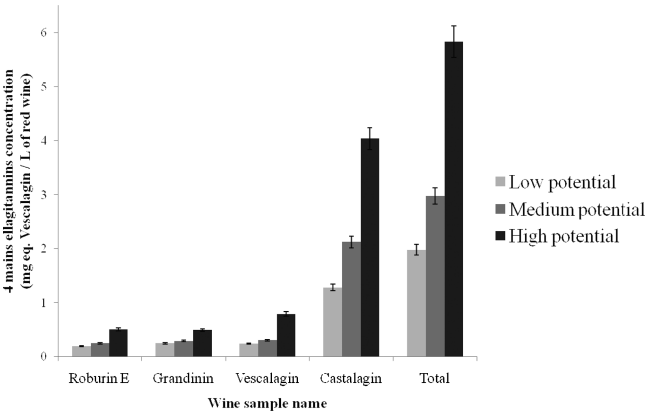

Figure 4. Native ellagitannin compositions in red wine after 4 months of aging according to their aging in contact with NIRS classified oak staves (see Supporting Information for detailed values).

acid/L, a volatile acidity of 0.43 g equiv if acetic acid/L, total polyphenols of 1787.3 mg equiv of gallic acid/L, total grape tannin concentration of 2.93 g/L and 241.30 mg/L of free anthocyanins (see Supporting Information for detailed values).

During its aging in contact with the NIRS classified oak staves, red wine extracted ellagitannins. Thus, the total ellagitannin concentration in the wine was estimated every month during four months (Figure 3), whereas the ellagitannin composition has been determined only after the fourth month of aging when the organoleptic properties of the red wine were estimated. In the red wine aged in contact with the NIRS classified oak staves, the total ellagitannin content estimated by acidic hydrolysis was low since the observed maximum level was 3.49 mg of released ellagic acid/L of wine. After four months of aging, the wine in contact with the staves classified as medium potential presented the medium ellagitannin concentration which was 1.38 times higher than the wine aged with low potential staves. In similar trends, the ellagitannin concentration in the wine aged in contact with the staves classified as high potential revealed an ellagitannin concentration almost two times higher. Thus as observed below with the ellagitannin level in wood, there is a good correlation between the NIRS classification and the concentration of ellagitannins in red wine aged in contact with the classified oak wood. Such correlation revealed that the NIRS oak classification procedure is an efficient technique to classify oak wood according to ellagitannin level prior to its use for red wine aging. Such classification will allow the wine maker to have a better control on the ellagitannin level in red wine. Moreover, it appears that the ellagitannin extraction kinetics by red wine were slightly influenced by the ellagitannin level in wood (Figure 3).

In the red wine aged in contact with the staves classified as low potential, the ellagitannin content maximum was observed after 3 months, and then this concentration decreased rapidly. By contrast, the others wines aged in contact with the staves classified as medium and high potential, the ellagitannin maximum concentration was observed after only 2 months of aging and then this concentration remained stable at three and four months of aging. In fact, during the first two months, the red wine hydroalcoholic solution extracts the ellagitannin at a rate faster than their evolution (oxidation and/or condensation with the wine constituents) rate resulting in an increase of their concentration. Then, when most the ellagitannins have been extracted from the first millimeters of the wood, the red wine solution needs to go deeper in the wood to extract more ellagitannins, thus the extraction rates decreased and the overall concentration of the ellagitannins remained stable. By contrast in the red wine aged in contact with the staves classified as low potential, most of the ellagitannins are extracted during the first several months.

Moreover, after four months of aging in contact with each NIRS classified oak stave group, a very strong correlation between the classification (low, medium, high) and the level of each specific ellagitannin extracted by the red wine during its aging was observed (Figure 4, see Supporting Information for detailed values). This means that the red wine aged in contact with the high ellagitannin potential stave presented the highest level of each specific ellagitannin. Moreover, differences in the ellagitannin composition between the wood and the wine appeared. In fact, a higher proportion for the castalagin was observed in the wine (68.97% ± 1.86%) than in the wood (49.73% ± 4.20%). Such difference is due to the fact that castalagin is more stable than vescalagin and also because castalagin is not involved in chemical reactions with other wine constituents.[12,47] It has been proved that castalagin does not react with flavanols or anthocyanins like vescalagin to form adducts such as acutissimin, epiacutissimin[48,49] or anthocyano-ellagitannins.[50]

Influence of Ellagitannin Contents on Red Wine Organoleptic Perception. After the characterization of ellagitannin concentration in the wood staves and in the wine aged in contact with these staves, the impact of the ellagitannin level on the organoleptic perception of the wine was investigated by sensory profile tasting. This evaluation focused on profiling the three red wines in terms of aromas and flavors in nose and mouth. First, the aromatic profile of each wine was estimated by the judges. The wine aged in contact with the staves classified as high potential of ellagitannins was described with a more intense woody aroma compare to the two other wines ($p < 0.001\%$), however this woody aroma did not have a negative impact on the fruity intensity. Moreover, the wine aged in contact with the staves classified as high potential, which was also the wine with the highest level of ellagitannins, was described as rounder ($p < 0.01\%$) by the trained judges. A similar trend was also observed for the wine amplitude (Figure 5). Moreover, it appeared that fruity aromas, astringency and bitterness were not positively or

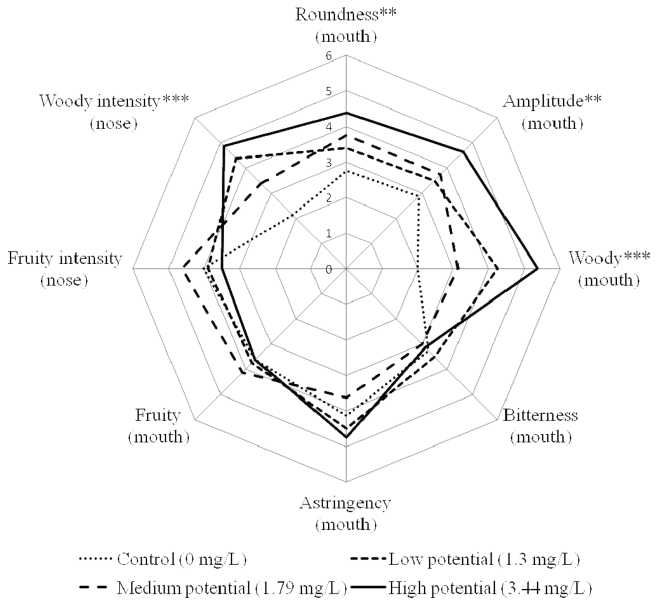

Figure 5. Mean sensory profiles of the red wines aged in contact with the different NIRS classified oak staves rated by the judges.

negatively impacted by the level of ellagitannins in wine, since no significant differences were noted. However, a trend between the ellagitannin concentration and the astringency intensity was observed by the judges. Wood derivative aromas were also significantly influenced by the classified oak staves: the red wine aged in contact with the staves classified as medium potential appeared to have the lowest intensity of these aromas compared to high potential, which revealed the highest aroma intensity.

In conclusion, this study shows that there is a good correlation in oak wood between the ellagitannin concentration determined by acidic hydrolysis or by molecular quantification and the NIRS (near infrared spectroscopy) classification. Moreover, a similar correlation between staves classified by the NIRS procedure and the level of ellagitannins found in red wine aged in contact with the classified staves was found. Overall, such correlations reveal that the NIRS oak classification procedure is an efficient technique to classify oak wood according to the ellagitannin level in wood, which is also directly related to the ellagitannin content of red wine aged in contact with the classified oak wood as shown in this study. Classifying oak wood prior to barrel formation will allow the wine maker to have better control of the ellagitannin level in their red wine and thus a better control of their red wine orgonoleptic properties.

Moreover, during the tasting of the red wine aged in contact with the different NIRS classified oak staves, it appears that ellagitannin concentration has a major impact on red wine organoleptic properties such as the roundness and amplitude, which were directly correlated with ellagitannin level. Furthermore, ellagitannin molecular analysis in the red wine confirms the higher stability of castalagin compared to the other ellagitannins.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Table S1, individual ellagitannin concentrations in the NIRS classified oak stave. Table S2, individual ellagitannin concentrations in the red wine sample after four months of aging in contact with the NIRS classified oak stave. Table S3, classical parameters on the wine samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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