Extraction of oak volatiles and ellagitannins compounds and sensory profile of wine aged with French winewoods. Implication of chemical composition on the final wine quality

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ABSTRACT
The evolution of volatile and non volatile compounds extracted from winewoods while being macerated for 12 months in Merlot wines was studied. Seven types of winewoods subjected to different toasting methods were used. Sensory analysis concerning vanilla, spicy, overall woody, astringency, bitterness and sweetness was conducted in parallel. Different rates of extraction have been observed, depending mainly on the origin of the compounds in the wood (toasting or present in natural wood) as well as on the watering process during toasting. The above differences are reflected by perceived sensory differences. Astringency and bitterness are related significantly to ellagitannin levels ($R = 0.828$, $p = 0.001$ for astringency and $R = 0.607$, $p = 0.005$ for bitterness). Judges perceived sweeter the wines that presented more lactones and vanillin levels. Spicy and vanilla descriptors are related to eugenol and vanillin as well as to other odorous chemicals. This study provides some insight into the implication of nonvolatile composition on astringency sensation as well as on bitterness and sweetness perception.

INTRODUCTION
Oak barrels have long been used in fine wine making, initially for easy product handling during production, storage and transport. Oak wood positive effects on wine development became appreciated, namely the ceding of pleasant aromas, and the regulation of red wine color. During aging in oak barrels, wines composition change because of the addition of phenolic compounds and other molecules extracted from the wood. Such compounds include lignins, hydrolysable and condensed tannins, gallic acid, ellagic acid, aromatic carboxylic acids, and various aldehydes. Ellagitannins (hydrolysable tannins) are among these substances. In oak heartwood they may represent 10% of the dry weight and are responsible for the high durability of this wood (Scalbert, et al., 1988).

Oak also contains a high level of volatile compounds that have a great impact on wood-matured wines aroma. The main volatile compounds susceptible to migration from oak wood to wine are the *cis* and *trans* isomers of *β*-methyl-*γ*- octalactone, furfural and its derived compounds, phenolic aldehydes such as vanillin and syringaldehyde, and volatile phenols such as eugenol, guaiacol, and ethyl- and vinylphenols. Their extraction of oak barrels depends mainly on the quantity of compounds that are potentially extractible on the contact time between wine and oak wood and on the wine composition. At present, alternatives to the oak barrel are being looked at to carry out the wine-ageing process. This practice recently was approved and legislated by the European Community (CE 2165/2005 and CE 1507/2006). Different shapes of oak wood pieces are used: chips, cubes or beans, powder, shavings or granulates, dominoes, and blocks or segments. Factors such as piece size, amount of added wood and contact time between wood and wine affect both sensory and chemical wine characteristics (Frangipane, et al., 2007) especially their wood-related volatile composition. Up to now, it does not seem very logical to establish an ageing period in barrels or with wood pieces through legislation. Hence, it would be important to know more about the influence of the wine composition in the process of the extraction of oak wood compounds. It is likely that a study of wine volatile and non volatile composition along with a tasting assessment would

be a more efficient method to establish the optimum contact time between wine and oak wood. Therefore, the objectives of this study were defining the chemical (ellagitannins and volatile composition) and sensory characteristics of wine treated with winewoods representing different toasting methods with the aim to monitor the extraction kinetic of the above compounds during 12 months. The toasting level impact on both volatile, non volatile compounds and sensory perception is studied in parallel. The relationship between the chemical composition and the sensory assessment of oak wood was also investigated.

MATERIALS AND METHODS

Wood Origin and Drying Conditions. The wood samples were constituted from two oak species (*Quercus robur and Quercus petraea*) from the same forest located in Center in France. The raw winewoods were stored for natural seasoning during 24 months in the Tonnellerie Nadalié (Ludon-Medoc, France) seasoning park. Then they were submitted to different toasting procedures according to the desired final product using oak fire.

Red wine aging in stainless steel tanks with winewoods. Merlot grapes were manually harvested at maturity in Bordeaux region in France at the end of September 2010. The same day, the grapes were crushed, and some SO_2 was added (5 g/hL) during the transfer of the must to 80-hL stainless steel tank. After alcoholic fermentation, the stainless steel temperature tank was maintained at 21°C in order to initiate spontaneously the malolactic fermentation, which lasted for 40 to 50 days. After malolactic fermentation, the red wine was transferred and kept in 2 hL stainless steel tanks for aging. Different types of winewoods (LT (Light Toast), MT (Medium Toast), MT+ (Medium Plus Toast), Noisette, Special) were added in separate stainless steel tanks for 12 months (2 ww/hL and 0.24m^2/ww). Table 1 shows the temperature and the toasting time of every winewood used. For MT, Noisette and Special the same toasting temperature is used. However in the case of Noisette, there is a prolongation on the toasting time, whereas in the case of Special, 30 minutes before the end of the toasting process a watering process takes place. For the purpose of our study, 2 tanks were used for every trial and a tank contained only wine, was used as control. During the year of aging in tanks with winewoods, each red wine was sampled at 1 month, 2 months, 3 months, 6 months, 9 months and 12 months, then the quantification of elagitannins and of aromatic compounds was performed by HPLC-UV and GC-MS analysis respectively. Sensory analysis was performed in parallel.

Table 1: Winewood characteristics

Winewood	Toasting Temperature (°C)	Toasting Time (hours)
LT	165	2.0
MT	180	3.0
MT+	190	3.5
Noisette	180	5.0
Special	180	3.0

Extraction of volatile compounds. Wine solutions were extracted with dichloromethane. 200µl of a solution of dodecan-1-ol as internal standard was added to 50 mL of samples. Three extractions were then carried out using 4, 2, and 2 mL of dichloromethane. The organic fractions were combined and dried on sodium sulfate anhydrous and then concentrated to 500 µL under a nitrogen stream. In all cases, the samples were analyzed in duplicate.

Gaz-Chromatography analysis. A simple and reliable GC method for quantitative determination of the volatile compounds arising from oak wood was used according to an adaptation of a previous method (Barbe, et al., 1996).

Red Wine Sample Preparation Prior to Total Ellagitannins Levels Determination. The red wine (50 mL) was evaporated under reduced pressure, and the resulting 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 (Chira, et al., 2013).

Total Ellagitannins Levels Determination HPLC-UV analysis. The total ellagitannins concentration was determined by the quantification of ellagic acid released during acidic hydrolysis (2 h at 100°C, 2NHCl in MeOH) as previously described (Michel, et al., 2011). Each sample was analyzed in triplicate, and each reaction mixture was subjected to HPLC-UV using a Lichrospher 100 RP 18 column, 250x4.6 mm, 5 μm. The mobile phases used were solvent A [H2O/H3PO4 (99,9/1)] and solvent B [methanol/H3PO4 (99,9/1)], 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.

Sensory Analysis. The sensory assessment was done by a committee of 20 expert judges from the Oenology faculty of Bordeaux. The judges were specially trained in the employment of scales and aroma descriptors according to ISO 8586-1 (2008). The attributes selected were grouped in two families: olfactive descriptors related to wood-wine interaction (vanilla, spicy, overall woody) and gustative descriptors (sweetness astringency and bitterness). The "overall woody" descriptor was chosen by tasters to describe all olfactory sensations brought about by the wood. Panellists attended 16 training sessions over a period of 2 months. The training period included a first general phase a second and a third, product-specific training phase (Chira et al., 2013). In every session the expert judges had to start with evaluation of orthonasal odor (first without moving the glass, then moving it gently) and then, after a short break they evaluated the perception.

Data Analysis. Statistical data analysis was performed using the analysis of variance (ANOVA) of Statistica V.7 software (Statsoft Inc.,Tulsa, OK). Tukey's HSD and Duncan's tests were used as comparison tests when samples were significantly different after ANOVA ($p < 0.05$) for chemical and sensory analysis, respectively. Pearson's correlation analysis was used to investigate relationships between chemical composition and sensory perception.

RESULTS AND DISCUSSION

Oak wood volatile composition. The following wood volatile compounds were studied: furanic aldehydes, furfural and 5-methylfurfural, the two isomers of methyl-γ-octalactone, *cis* and *trans* (commonly known as oak lactones or whiskey lactones); the volatile phenols guaiacol, eugenol, and isoeugenol; the aldehyde phenols and vanillin. The levels of wood volatile compounds extracted were quantitatively different depending on contact time and type of winewood (Figure 1). This Figure shows that furfural and 5-methylfurfural reached its maximum concentration either at 3 or 6 months of ageing. Thus in all samples furfural contents were exhausted after 12 months. Noisette has been toasted 2 hours more than MT and presented more furfural and methyl-furfural. Moreover, Special (oak wood is heating using medium temperature with watering), presented almost two times more methyl-furfural comparing to MT. Suggesting that along with the prolongation of toasting method, the watering process also has an important impact on furanic compounds. Concerning phenolic alcohols, the maximum extraction of guaiacol occurred during the first nine or twelve months (Figure 1) and the concentration of 4-methylguaiacol was less important throughout the period studied. Relating to eugenol content, an increase is observed during the maceration time for all the wines. Wines with LT staves presented the highest concentrations after 12 months. Both lactones increased linearly in concentration in wine during the oak maturation period (Figure 1), the rate of extraction increasing further from 6 to 9 months, meaning that once a portion of wood is wetted, dissolution of lactones occurs rapidly. Among phenolic aldehydes, vanillin considered to have the most important influence on wine aroma. Just as happens with the furanic aldehydes, for short ageing periods, vanillin accumulates in wine because at the beginning its extraction is high, due to the difference of concentration between the wine and the wood (Cerdán, et al., 2006). Its biggest concentration is found in the wine with Noisette winewoods (305.81μg/L). Similarly, to furanic compounds the prolongation of MT toasting increases vanillin content.

Oak wood Total Ellagitannins Concentration. The total ellagitannin level, expressed as milligrams per L of released ellagic acid of wine, revealed a large diversity of concentrations ranging from 6.31 to 26.07 mg of released ellagic acid/L of wine (Figure 2). Such differences were expected since ellagitannins undergo thermolytic degradation during the toasting process (Doussot, et al., 2002). Their extraction rates appeared to be faster during the first three months. Figure 2 shows that for almost all the wines a maximum extraction of ellagitannins is obtained after a 2 to 3 month time. In all wines, after 9 and 12 months of contact, the overall concentration of ellagitannins decreased with time. Particularly, after 12 months, a 10-20% decrease was observed on ellagitannins levels for wines with LT winewoods, a 30% loss was monitored for wines with Noisette, a 50-60% reduction was noticed for wines with MT, MT+ winewoods and finally the most important decrease of ~ 70% was noted for wines with Special winewoods. Thus not only the pyrolytic toasting stages diminish the quantity of these compounds but also the watering procedure. Moreover, wines with light toast winewoods extract not only more ellagitannins but also faster than the other samples. The above decreases during the maceration time can be attributed to the high reactivity of ellagitannins toward other wine constituents (Jordão, et al., 2008; Quideau, et al., 2005). In the first months, the red wine extracts ellagitannins at a rate faster than the rate of the condensation reactions between ellagitannin and the other nucleophilic wine constituents (e.g., catechin, epicatechin, and ethanol). Then, when most of the ellagitannins has been extracted from the first millimeter of the wood, the red wine solution needs to go deeper in the wood to extract more ellagitannins, consequently, at a slower rate.

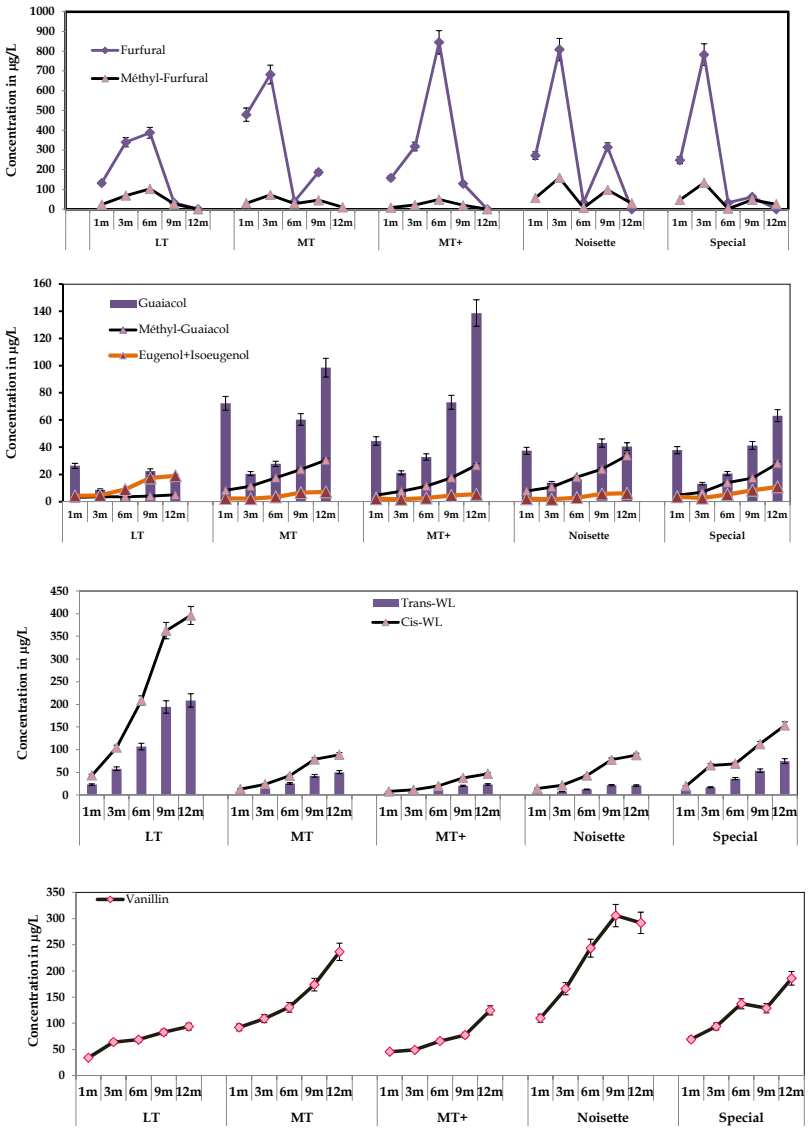

Figure 1 : Evolution of oak volatile concentration (µg/ L) in Merlot wine during twelve months (m=month)

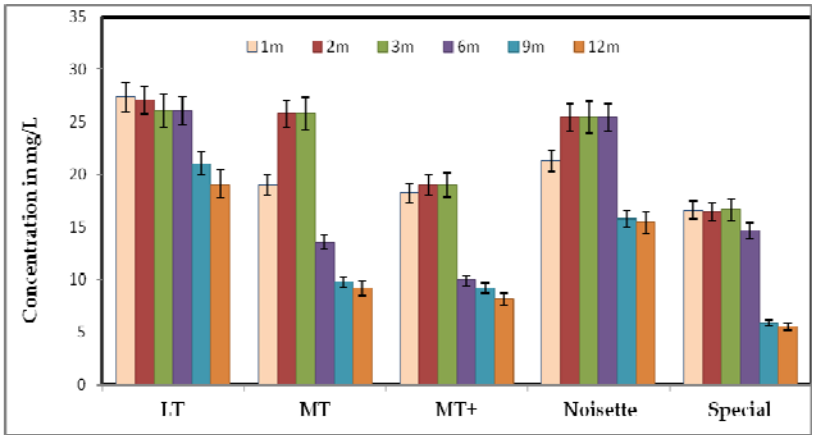

Figure 2 : Evolution of total ellagitannins during different contact times

Oak wood sensory evaluation. Figure 3 shows the average intensities of each gustatory and olfactory attributes during the different contact times of the control wine and those of the same wine treated with oak wood during twelve months. An attentive look on Figure 3 shows that wines treated with toasted oak winewoods become less astringent and bitter during the contact time. This reduction in the astringency sensation could be attributed to their lower ellagitannins levels as well as to chemical complex formation between wine tannins, polysaccharides and peptides brought out by the oak wood. For all the wines treated with toasted oak winewoods, it is observed that vanilla, spicy and woody characters along with sweetness build up. The woody aroma of wines with Special winewoods was more intense after two months of contact, afterwards it didn't intensify significantly. Therefore, judges found that woody, spicy and vanilla aromas don't fluctuate during the maceration time for wines treated with Special winewood. Thus, the watering process during toasting process influences not only the levels of hydrosoluble tannins but also wines sensory profile.

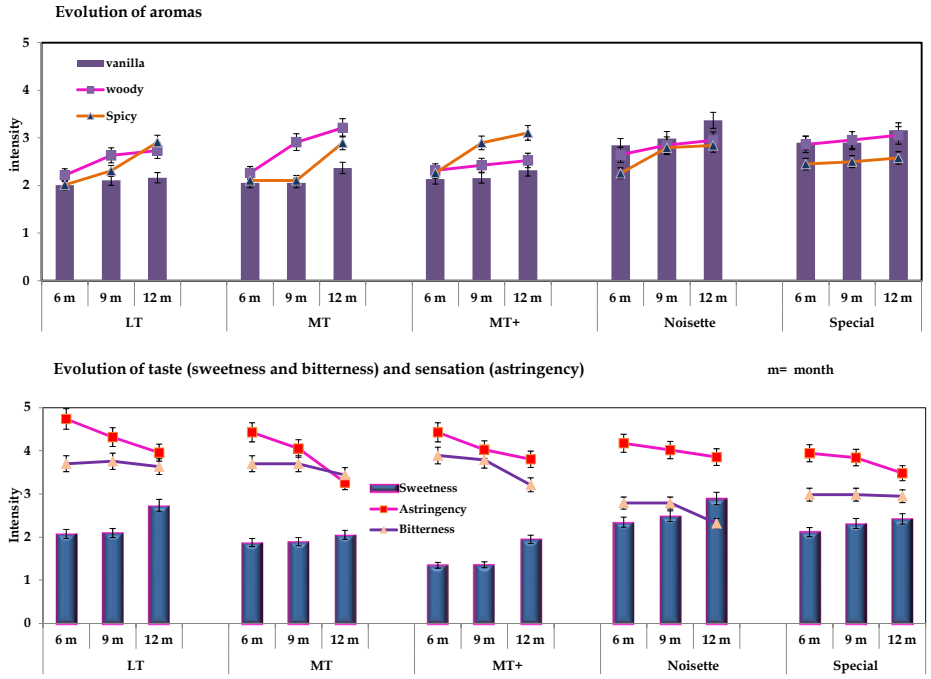

Figure 3 : Sensory evaluation of wine treated with different winewoods during different contact times

Correlations between sensory and chemical results. The Analysis of Variance revealed that the toasting method has a significant impact ($p < 0.05$) on chemical composition and sensory perception of oak wood extracts. Each sensory descriptor was correlated with the chemical concentration of the oak wood compounds of interest (Table 2). This procedure allowed us to measure the extent to which sensory and chemical variables are correlated. Based on the correlation analysis, astringency and bitterness intensify significantly with ellagitannins concentration ($R = 0.828$, $p = 0.001$ for astringency and $R = 0.607$, $p = 0.003$ for bitterness). The highest astringency and bitterness was perceived for wines with LT oak wood winewoods (4.98 for astringency and 4.67 for bitterness) containing 27.33 mg/L of released ellagic acid. The above established correlations permit to characterize tannins quality when ellagitannins levels are known. Additionally astringency and bitterness were more perceived in the wines with important levels of furanic compounds (Table 2).

Table 2 : Pearson Correlations between chemical and sensory data. Marked correlations are significant at p <0.05

	Vanilla	Woody	Spicy	Sweetness	Astringency	Bitterness
Elagitannins	-0,290 NS	-0,085 NS	-0,278 NS	-0,180 NS	0,828***	0,607***
Furfural	-0,352*	0,011 NS	-0,437*	-0,315 NS	0,430*	0,393*
Methyl Furfural	-0,034 NS	0,050 NS	-0,139 NS	0,114 NS	0,403*	0,264 NS
Trans-WL	0,351*	0,582**	0,388*	0,353*	0,166 NS	0,196 NS
Cis-WL	0,445*	0,636***	0,458**	0,537**	0,056 NS	0,024 NS
Ratio	0,502**	0,385*	0,417*	0,758***	-0,327 NS	-0,525**
Guaiacol	0,065 NS	-0,035 NS	0,025 NS	-0,158 NS	-0,431*	-0,207 NS
Methyl-Guaiacol	0,484*	0,396*	0,420*	0,467 NS	-0,658***	-0,546**
Eugenol	0,428*	0,636**	0,462**	0,314*	0,139 NS	0,154 NS
Syringol	0,028 NS	-0,108 NS	0,004 NS	-0,169 NS	-0,526**	-0,292 NS
Syringaldeyde	0,684 NS	0,691***	0,601***	0,547**	-0,382*	-0,373*
Vanillin	0,595***	0,657***	0,509**	0,551**	-0,212 NS	-0,279 NS

NS=non significant

On the other hand, wines with high contents of guaiacol, methyl-guaiacol, syringol and syringaldeyde were characterized less astringent. This observation is logical as toasting decreases ellagitannins and at the same time increases the concentration of volatile compounds that enhance the oak wood aroma. Judges perceived the sweetness perception more intense in wines with higher levels of lactones, eugenol and vanillin compounds. The levels of these compounds are correlated positively with the perceived intensity of vanilla aroma (Table 2). Vanillin is the principal marker of vanilla smell, lactones can be regarded as direct contributors and/or possible enhancers of this descriptor. Woody overall character is positively correlated to guaiacol, methyl-guaiacol, eugenol, syringaldeyde, lactones and vanillin levels, which is reasonable since oak wood sensation is complex and influenced by the presence of various odour-active wood extractives (García-Carpintero, et al., 2012). For example, whisky lactone is an attribute that accounts for a woody and coconut character while high concentrations of this compound are associated with wine with an intense vanilla perfume. Perceived spicy intensity is closely related to eugenol content, which is logical, since pure eugenol is described as clove-like. In our experiment, it is also linked positively to the presence of other odorous chemicals like lactones, vanillin, methyl guaiacol, suggesting that in a complex medium such as wine the above volatile compounds values may influence spicy aroma by means of additive, or synergistic effects. An important reduction in spicy and vanilla aroma takes place in wines with significant levels of furfural.

Conclusion. The results have shown that each oak winewood added unique and special characteristics to the same wine, and in addition each sample showed a different ability to extract the compounds (volatile and non-volatile). Different rates of extraction have been observed, depending mainly on the origin of the compounds in the wood (toasting or present in natural wood) as well as on the watering process during toasting. The above differences were reflected by perceived sensory differences. In general, volatile phenols, such as eugenol, guaiacol along with aldeydes phenols (vanillin) and lactones, showed an increasing tendency with increasing maceration time. Ellagitannins are extracted faster during the first three months, after six months an important decrease is observed. Wines with special winewoods presented the lowest ellagitannins concentrations in comparison with other wood toasting trials. Concerning sensory evaluation, oak wood contact time enhances vanilla, spicy, woody, characters and sweetness perception. For all the studied samples, with the exception of wines

with special winewoods, vanilla and spicy flavors amplify linearly during the storage time. Wines treated with special winewoods didn't show substantial changes in the evolution of aromas during maceration time. Moreover, wine storage with winewoods has a sweetening effect and in parallel decreases the astringency and bitterness sensation. Astringency and bitterness are related significantly to ellagitannin levels ($R = 0.828$, $p = 0.001$ for astringency and $R = 0.607$, $p = 0.005$ for bitterness). A model like this satisfactorily predicts the sensation intensity of both astringency and bitterness if ellagitannins levels are known. Additionally, relationships between volatile oak compounds and sensory perception are found. The above correlations obtained between sensory descriptive evaluation performed by a trained panel and wine chemical characterization resulted in a useful tool applicable to wine development.

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