Scientific report
Manuscrit n° 110895 - ISBN 2-9509403-1-5
J. Sci. Tech. Tonnellerie, 1996, 2, 51-75
Printed in France

The heartwood ellagitannins of different oak (*Quercus* sp.) and chestnut species (*Castenea sativa* Mill.). Quantity analysis of red wines aging in barrels

Nicolas VIVAS[1*], Yves GLORIES[2],
Guy BOURGEOIS[3] et Christiane VITRY[3]

1 : Tonnellerie DEMPTOS détaché à la Faculté d'Œnologie, Université de Bordeaux II, 351 cours de la Libération, 33405 Talence (France) ;
2 : Laboratoire de chimie appliquée, Faculté d'Œnologie, Université de Bordeaux II ;
3 : Centre d'Étude Structurale et d'Analyse des Molécules Organiques (CESAMO), CNRS URA n°35, Université Bordeaux I, 351 cours de la Libération, 33405 Talence (France).

(Received after revision the 20th septembre 1995)

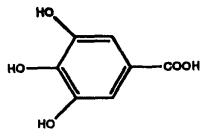
Summary : The heartwood of several species of oak and chestnut were analysed ; their content of total phenols in the dry extract were estimated, extracted by way of an acetone/water mix (7:3). Furthermore, we quantitatively analysed the ellagitannins using different specific methods : quantity analysis using a nitrous acid reagent, by acid degradation and by reversed phase HPLC quantity analysis. The study was carried out on three European oaks (*Q. robur*, *Q. petraea* and *Q. farnetto*), 3 oaks from Central and North America (respectively *Q. oocarpa*, *Q. alba* and *Q. stellata*) and a European chestnut (*C. sativa*). The proportions showed that the extractable phenols of diverse species is principally represented by ellagitannins. Vescalagin and castalagin were found in all the samples in majority proportions. The European species are characterised by the presence, in variable quantities, of the 4 monomer ellagitannins (vescalagin, castalagin, grandinin and roburin E) and 4 dimers (roburin A, D, B, and C).The American species had practically no dimers. The chestnut was distinguished by the absence of the dimer forms and pentolised ellagitannins. A comparison of the methods of quantitative analysis showed that the HPLC method consistently gave results inferior to the other two methods. We interpreted this as being due to the existence of ellagitannins of an unknown structure and by the presence of combinations of polysaccharides-ellagitannins which are determinable by reversed phase chromatography. Finally, the application of the quantitative analysis method, by acid degradation of red wines, enabled us to show the presence of ellagitannins in red wines matured in new barrels. The ellagitannins are easily soluble during the first months of maturing. But their participation in oxidation reactions causes them to degrade. In fact, the oxidised forms of ellagitannins do not release ellagic acid by way of acid hydrolysis. This explains the weak concentrations of ellagitannins found by some authors in wines and spirits.

Key words : *Fagacea*, *Quercus* sp., *Castenea sativa*, ellagitannins, quantity analysis, heartwood, barrels, red wines

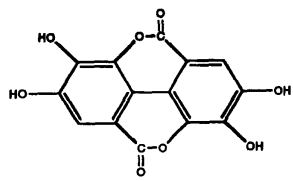
INTRODUCTION

Oaks and chestnuts belong to the angiosperm group and to the fagacea family. These two species have largely been used in cooperage for making barrels intended for transportation, conserving wines (TARANSAUD, 1976) and for the preparation of

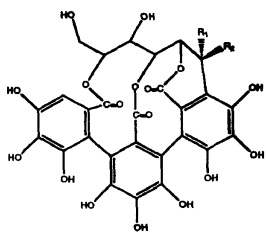
*author to whom the correspondence should be addressed



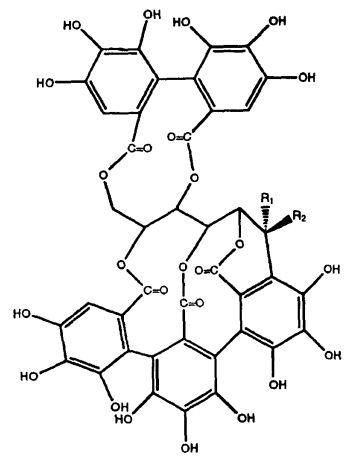
I - gallic acid



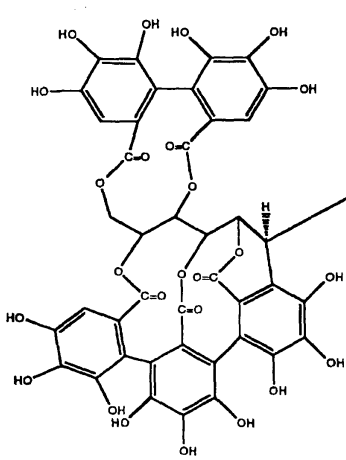
II - ellagic ac.



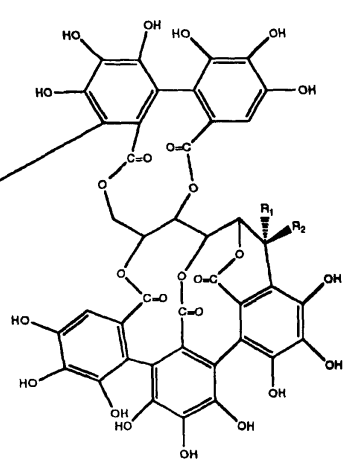
III - vescalin : R1 = H, R2 = OH
IV - castalin : R1 = OH, R2 = H



V - vescalagin : R1 = H, R2 = OH
VII - castalagin : R1 = OH, R2 = H
VII - granidin : R1 = H, R2 = Lyxose
VIII - roburin E : R1 = H, R2, Xylose



IX - roburin A : R1 = H, R2 = OH
X - roburin D : R1 = H, R2 = Lyxose



XI - roburin B : R1 = OH, R2 = H
XII - roburin C : R1 = H, R2, Xylose

Figure 1 - The structure of phenol acids (I, II) and of ellagitannins (III, XII) found in the extracts of heartwood from oaks and chestnut

oenological tannins (VIVAS *et al.*, 1993a). In Europe, it is essentially the *Quercus robur* and *Q. petraea* from France and the East of Europe, the *Q. farnetto* (*Q. conferta*) from Hungary, the *Q. alba* from the United States and the *Castenea sativa* from France which were principally used.(TARANSAUD, 1976 ; KELLER, 1992 ; VIVAS, 1995). Actually, only *Q. robur*, *Q. petraea* and *Q. alba* are still frequently worked in cooperage and the former two come from French forests and the latter from the east coast of the United States. Chestnut is only used rarely, even though it is still employed by certain cooperages either for making 100 % chestnut barrels, or mixed oak/chestnut barrels. In the future, the search for new sources of supply of quality wood is an important point to be aware of, for the dual objective of preserving our very sought after regions of supply, and furnishing the quality barrel makers so that the quality - price ratio remains reasonable.

The heartwood tannins of numerous species of oak (*Quercus* sp.) belong principally to the group of hydrolysable tannins (HASLAM, 1981). It includes the gallotannins and ellagitannins, released respectively by gallic acid and ellagic acid after acid hydrolysis. In the oak wood (*Q. petraea*, *Q. robur*) and the chestnut (*Castenea sativa*), we find for the most part two ellagitannins and particularly two isomers : vescalagin and castalagin (MAYER, 1971 ; SCALBERT *et al.*, 1988 ; VIRIOT *et al.*, 1994 ; VIVAS *et al.*, 1995a). More recently, the dimer ellagitannins and pentosylated forms have been described (NONAKA *et al.*, 1989 ; HERVÉ DU PENHOAT *et al.*, 1991a, HERVÉ DU PENHOAT *et al.*, 1991b), they are roburins A, B, C, D, E, and grandinin. These structures are shown in figure 1 and appear to be constituted of a linear glucosidic chain in which the OH is esterified by the carboxylic functions of the hexahydroxydiphenic and nonahydroxytriphenic groups. These different ellagitannins are hydrosoluble and solubilized rapidly in hydroalcoholic mediums such as wines and spirits (MOUTOUNET *et al.*, 1989 ; VIRIOT *et al.*, 1993). Their oxidizing potential (VIVAS and GLORIES, 1993, 1996) and their taste properties (POCOCK *et al.*, 1994) give them a real role in the process of aging red wines (VIVAS and GLORIES, 1993 ; SINGLETON, 1995) and spirits (PUECH, 1987 ; VIRIOT *et al.*, 1993) in oak barrels.

In the oak wood extracts and white wines conserved in wood, HPLC identification and global quantitative analysis of the ellagitannins by nitrous acid oxidation (BATE-SMITH, 1972) or after acid hydrolysis (PENG *et al.*, 1991) are relatively easy (MOUTOUNET *et al.*, 1989 ; SCALBERT *et al.*, 1990 ; PUECH *et al.* 1992). On the other hand, in red wines, rich in phenolic compounds, these different techniques were not applied. The literature do not give quantitative data about total ellagitannins present in red wines aging in barrels. The complexity of the medium makes all direct injection HPLC analysis impossible. All the same, the probable interference between the polyphenols of the wine and the Bate-Smith nitrous acid chemical reagent does not permit the application of direct colorimetric methods.

The object of this article is to recall the different methods of quantitative analysis of the ellagitannins and clarify a technique adapted to red wines. Finally, the methods are applicable to the study of the levels of ellagitannins of the different species of oak woods used or potentially usable in cooperage, as well as the quantities of ellagitannins present in red wines.

MATERIALS AND METHODS

I - MATERIALS

1 - Reference compounds

The gallic acid, the ellagic acid and the (+)catechin were supplied by Fluka™. The vescalagin and the castalagin were isolated and purified from the duramen of *Q. robur*, under conditions described by VIVAS *et al.* (1995). The different roburins (A-E), the grandinin and the pentagalloylglucose were kindly offered by SCALBERT (INA/INRA Thiverval-Grignon). The castalin and the pedunculagin were supplied by Nonaka (University of Kyushu, Japan). The seed extracts and the free anthocyans were prepared according to GLORIES (1978).

2 - Oak samples

The oak samples are made up of heartwood of trees ranging from 110 to 150 years of age, coming from homogeneous and appropriately maintained forest clumps. The chestnut heartwood came from isolated trees. Only the first quarter of the trunk, constituting in general the coopers wood, is used for the study.

The wood cut by chopping or sawing is naturally dried for 24 months. The different samples taken at random from wood piles (n = 10) are planed and then reduced to sawdust crushings in liquid nitrogen, before being strained so as to keep only the particles with a size less than 60 mesh. The samples are conserved after freeze-dried for analysis within a delay of 2 months. The different species studied are *Q. robur* (Limousin, France), *Q. petraea* (Allier, France), *Q. farnetto* (Hungary), *Q. alba* (Missouri, USA), *Q. stellata* (Missouri, USA), *Q. oocarpa* (Costa Rica), *C. sativa* (Dordogne, France).

3 - Wine samples

The analyses were performed on a Cabernet sauvignon red wine matured in new barrels (Allier, naturally dried for 18 months, medium toasted) for 2 years. The quantitative analyses are equally performed on a series of wines from various origins (Rioja-Spain, red wines from Tempranillo ; St-Émilion and Médoc-France, Merlot noir and Cabernet sauvignon wines).

II - EXTRACTION AND IDENTIFICATION OF OAK AND CHESTNUT ELLAGITANNINS

Different extraction solvents were compared : water, ethanol/water 5:5, ethanol, methanol, methanol/chloroform 8:2, acetone/water 7:3. The solvent having the greatest extractive strength is kept for the successive work. 1 g of sawdust (60 mesh) is extracted by 100 ml of solvent for 12 h at an ambient temperature of (20°C ± 2°C) on an agitation table (150 rpm). The extract obtained is then filtered through membranes 0.45 µm, freeze-dried and weighed.

100 µg of freeze-dried extract is used for LSIMS identification of the major ellagitannins. The spectra are performed with a VG-autospec EQ, with a Cesium gun, in negative mode, under the following conditions : calibration : cesium iodate salt of 200 to 1500 Da, matrix : thioglycerol, bombardment energy : 35 keV, 2µA, tempera-

ture < 40°C, sample solvent : anhydrous methanol.

A second aliquot fraction of the extract (1 mg) is solubilized with a minimum of methanol/water (6:4) for HPLC analysis coupled, through a flux divider, to a UV detector and a mass spectrometer « LSIMS » using the device perfected by VIVAS *et al.* (1995). The HPLC method of separation is described in the following paragraph. Some modifications have however been made, on the one hand, in order to ensure a LSIMS detection we must add to the elution solvents. 2 % of glycerol which acts as a matrix, and on the other hand, the injected volume is increased (100 µl) to improve the quality of the registered spectra.

III - SEPARATION AND HPLC QUANTITATIVE DETERMINATION OF THE ELLAGITANNINS

The chromatographic separation technique and the quantitative determination of the ellagitannins comply with the method perfected by SCALBERT *et al.* (1990). The wood extracts are analysed in the reversed phase on a HPLC Beckman TM range (pumps 126 and detector 167). The column is an Ultrasphere TM (250 x 4,6 mm, dp : 4 µm). The injection volume is 20 µl. The elution program is performed at a constant debit of 1 ml/min, passing from 0 % to 40 % of B in 40 min., and then rising to 100 % of B in 25 min (solvent A H_2O/H_3PO_4 990:1, solvent B : $MeOH/H_3PO_4$ 990:1). The detection is performed at λ 280 µm, the chromatographic purity of the peaks, and the UV spectra (220-230 nm) are realised using a diode bar spectrophotometer, this is managed by Gold 6™ software.

IV - QUANTITATIVE DETERMINATION OF THE TOTAL ELLAGITANNINS

1 - Estimating the levels of total phenolic composites

The total phenolic compounds of wood extract is performed either by the method using the Folin-Ciocalteu reagent or by measuring the absorption at D.O. 280 nm of the extracts diluted at 1/100e (VIVAS *et al.*, 1993). These two methods induced to comparable results but are non specific to the ellagitannins.

2 - The nitrous acid oxidation reaction

According to this method proposed by BATE-SMITH (1972), the esters from the hexahydroxydiphenic acid and the glucose are oxidised by the nitrous acid in nitrogen. The reaction brings about a blue coloration. One mixes with the dosing solution 1 ml of methanol and 160 µl of 6 % acetic acid, after which the oxygen is expelled by 10 minutes of nitrogen sparging, finally we add 160 µl of 6 % sodium nitrite followed by a brief sparging (1 min). The tube is hermetically sealed and the reaction develops within 60 min in a bain-marie at 30°C. The intensity of the colour which develops was measured at λ 600 nm. The results were estimated in mg/g or mg/l of equivalent castalagin (ε 600 nm : 983 g^{-1}).

2- Acid degradation

The proposed method was adapted to that perfected by PENG *et al.*, (1991). It is based on acid hydrolysis of the ellagitannins, in a bain-marie, followed by a HPLC quantity analysis of the free ellagic acid. 10 ml of wood extract or wine are evaporated dry (rotary evaporator, 40°C) in a hydrolysis tube fitted with a Teflon seal, the

contents are solubilized by 10 ml of methanol/hydrochloric acid 6N (8,4:1,6). We measured the ellagic acid present, and then, after two hours of acid hydrolysis (oil bath at 100°C), the created ellagic acid. The difference between the two values corresponds to the ellagitannins released by the ellagic acid. The HPLC quantitative determination was performed with the material described bellow (elution program : 0 to 100 % of B in 20 min, λ : 370 nm). The results are expressed in mg/g or mg/l of equivalent castalagin, bearing in mind that one mole of castalagin gives, under these conditions, one mole of ellagic acid (PENG *et al.*, 1991).

RESULTS

I- EXTRACTION AND IDENTIFICATION OF THE PRINCIPAL ELLAGITANNINS

Different solvents were tested so as to compare their dissolvent capacities of the extractable wood composites and in particular the phenolic composites. The results were identical for the oaks and chestnut studied ; the only ones presented are, by way of example, the results obtained for *Q. robur* (figure 2). The solvents were classified, according to the dry extract : acetone/water (7:3) ≥ water ≥ ethanol/water (5:5) ethanol = methanol methanol/chloroform (8:2) and total phenols : acetone/water (7:3) ethanol methanol ≥ ethanol/water (5:5) ≥ methanol/chloroform (8:2) water.

The water and the water acetone mix permitted the greatest isolation of extractable composites. Meanwhile, the solvent acetone/water enabled a maximum extraction,

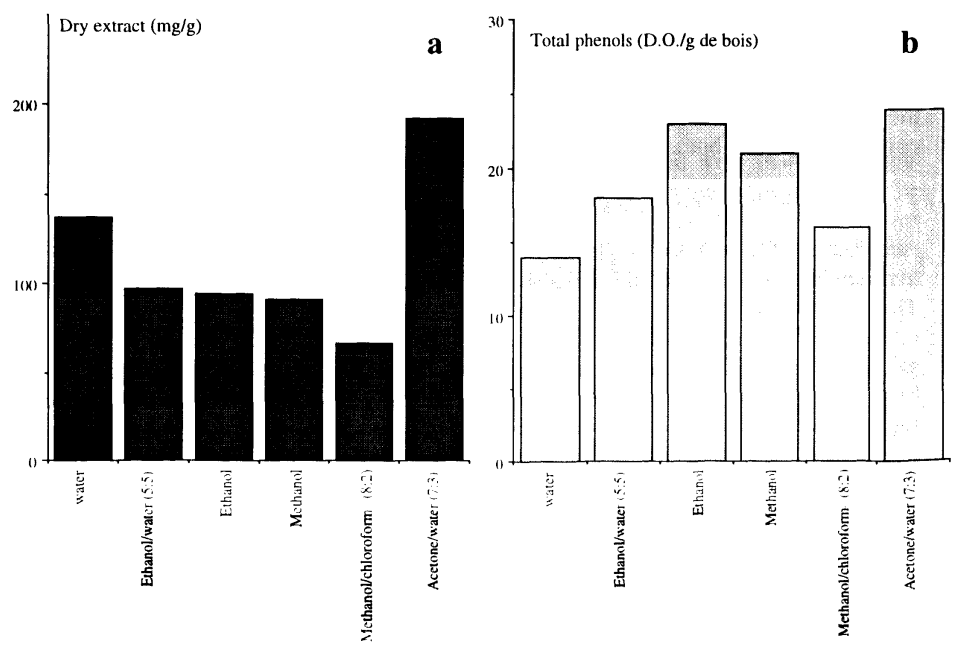


Figure 2 - Extraction of fixed composites (a) and total phenols (b) from the heartwood of *Q. robur* using different solvents

simultaneously, of soluble phenolic composites. The extraction from the wood samples using a hydroalcoholic solution with a composition close to that of wine (12 % vol. ethanol, 5 g/l tartaric acid, NaOH N qsp pH 3,5) gives values comparable to the water extractions (results not shown). The ethanol and methanol extracted few composites but contained the essentials of the extractable phenols. For the rest of the work, the acetone/water mix was retained.

The LSIMS analysis, performed directly on the extracts, enabled the identification of the major compounds of the species studied (table 1). In our samples, we constantly found phenol acids, gallic acid, ellagic acid and ellagitannins, vescalagin and castalagin. Meanwhile most of the ellagitannins are present in the mixture in feeble quantities and cannot be identified under the prescribed conditions. They take the form of pentosylated monomers and dimers, which have only been found with certitude in *Q. petraea*, *robur* and *Q. stellata* (pentosylated monomers) and in *Q. farnetto* (pentosylated dimers). The figure 3 has grouped together the LSIMS spectra of *Q. robur* and *C. sativa* ; the spectra of *Q. robur* and *Q. petraea* being identical, no matter which sample used. We notice, on the spectra, the presence of quasi- molecular peaks $[M-H]^-$ of the phenol acids and the ellagitannins cited in table 1, but equally the quasi molecular peak characteristic of vescalagin and castalagin (m/z : 632, $[M-H]^-$). These two ellagitannins are considered to be the products of vescalagin and castalagin hydrolysis (MOUTOUNET *et al.*, 1989 ; VIRIOT *et al.*, 1994). We found them in quantities large enough for detection in the water and ethanol/water extracts.

The coupling of HPLC/LSIMS enabled confirmation of the peak allocation, usually performed by comparing the UV spectra and the retention time (Tr) of the identifiable molecules with purely referential products. By way of example, the chromatogramme of an extract of *Q. robur* and the LSIMS spectra recorded at the apex of some peaks are grouped together in figure 4. The concentration of roburins B and C are insufficient for obtaining a clear spectrum. However weak the concentration, there appears, in the Tr of the roburin A and D a peak which is distinguished from the back ground noise at m/z : 1849 $[M-H]^-$. The presence of vescalagin and castalagin is confirmed in the aqueous extract. The appearance of the chromatogrammes of the analysed heartwood extracts reveal distinct differences. According to these profiles, we distinguish :

- the group of species presenting the 8 ellagitannins « Et » : *Q. robur*, *Q. petraea*, *Q. farnetto* ;
- the group of species presenting the monomers « Em » : *Q. oocarpa* ;
- the group species presenting vescalagin, castalagin and the vescalagin dimers « Ev » : *Q. stellata*, *Q. alba* and *C. sativa*.

The chromatographic profiles were identical for the samples coming from the same species. The observed qualitative differences thus for the most part have a varietal origin regarding the intraspecific heterogeneity.

II - QUANTITATIVE DETERMINATION OF THE ELLAGITANNINS IN THE HEARTWOOD EXTRACTS

1 - Comparison of the different methods of quantitative determination of the phenolic compounds and total ellagitannins

Table 1

Identification of the principal phenol acids and heartwood ellagitannins of the different species of oak and chestnut by negative mode LSIMS

Molécules	M_w^+	Structure++	Samples						
			Q. robur	*Q. petraea*	*Q. farnetto*	*Q. alba*	*Q. stellata*	*Q. oocarpa*	*C. sativa*
Phenol acids									
Gallic acid	170		+	+	+	+	+	+	+
Ellagic acid	302		+	+	+	+	+	+	+
Ellagitannins									
Monomers :									
Vescalagin	934	V	+	+	+	+	+	+	+
Castalagin	934	C	+	+	+	+	+	+	+
Grandinin	1066	Vl	+	+	nd	nd	+	+	nd
Roburin E	1066	Vx	+	+	nd	nd	+	+	nd
Dimers :									
Roburin A	1850	VV	+	+	nd	nd	nd	nd	nd
Roburin D	1850	VC	+	+	nd	nd	nd	nd	nd
Roburin B	1932	VVl	nd*	nd	+	nd	nd	nd	nd
Roburin C	1932	VVx	nd	nd	+	nd	nd	nd	nd

+molar mass determined by LSIMS - ++V : vescalagin - C : castalagin - l : lyxose - x : xylose - * nd : non detectable

Table 2

Comparison of methods of estimation of the content of ellagitannins

(The results are given in g of equivalent castalagin.)

Solution	Composition*	Quantity of product (g of weighted product)	Quantitive determination methods			
			FCI#	HPLC£	Nitrous ac.°	acid degradation§
1	V	1	0.8 (20 %)**	1	1.1 (10 %)	0.9 (10 %)
2	C	1.1	1.0 (10 %)	1.08 (2 %)	1.0 (9 %)	1.0 (9 %)
4	V+C (0.6:0.5 ; p:p)	1.2	1.1 (8.3 %)	1.12 (6.7 %)	1.1 (8 %)	1.1 (8 %)
5	V+C (0.5:0.5 ; p:p)	0.24	0.33 (37.5 %)	0.240 (0 %)	0.217 (9.5 %)	0.226 (6 %)

*V : vescalagin, C : castalagin - ** deviation compared to the quantity of weighed product - #FCI / Folin-Ciocalteu index. £ quantitative determination of the ellagitannins by inverted phase HPLC - quantitative determination of the ellagitannins using the BATE-SMITH nitrous acid method - § quantitative determination of the total ellagitannins by way of quantitative determination of the ellagic acid formed after acid hydrolysis.

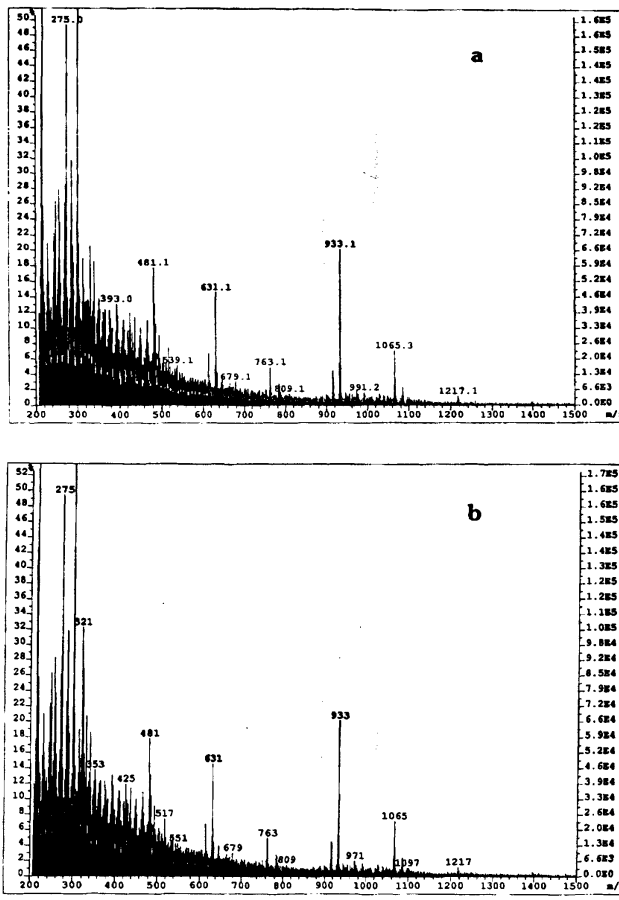


Figure 3 — LSIMS spectra of the acetone/water extracts of *Q. robur* (a) and *C. sativa* (b).
Spectra recorded in negative mode, between 200 and 1500 Da, thioglycerol matrix

The accepted methods of quantitative determination were applied to solutions of vescalagin and castalagin. In table 2, we notice that the different methods yield comparable results in relation to the weighed quantity of ellagitannins. The deviations concerning the real values remain inferior to 10 %. The HPLC method is the most precise.

The different methods were then used to estimate the content of soluble polyphenols and total ellagitannins of the oak and chestnut heartwood The results are grouped together in table 3. For each quantitative analysis performed for the 6 oaks and the chestnut, the deviations on average are relatively reduced enabling an appropriate differentiation of the species amongst them. *Q. robur* and especially *Q. farnetto* and *C. sativa* are the richest in dry extract and total phenols, whilst *Q. stellata*, *Q. oocarpa* and especially *Q. alba* are slightly deprived holding intermediary posi-

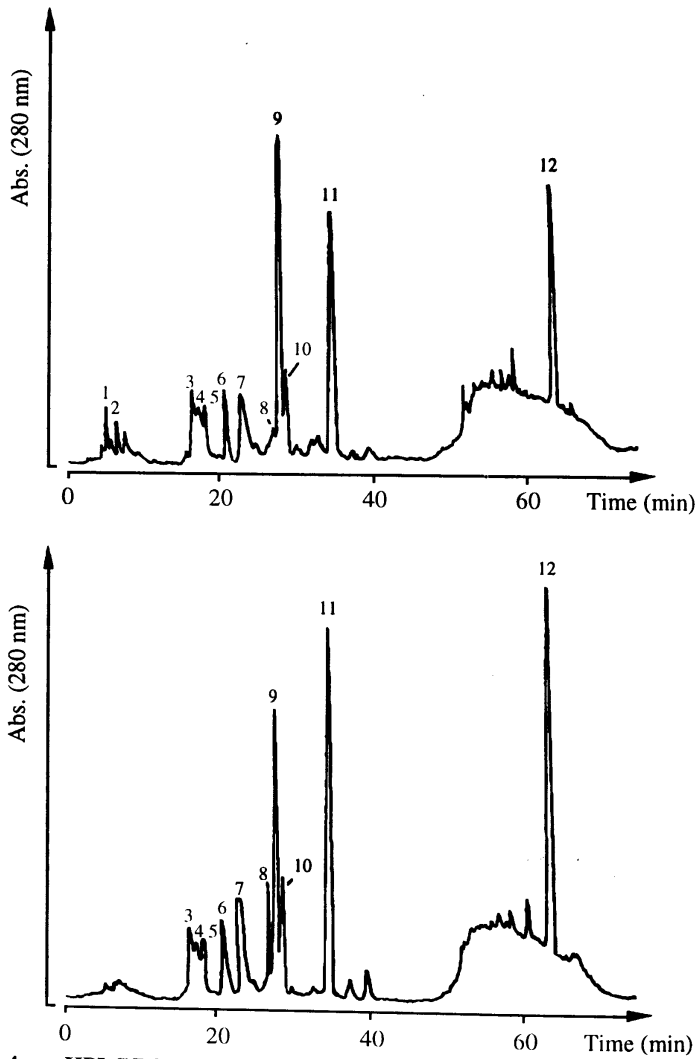
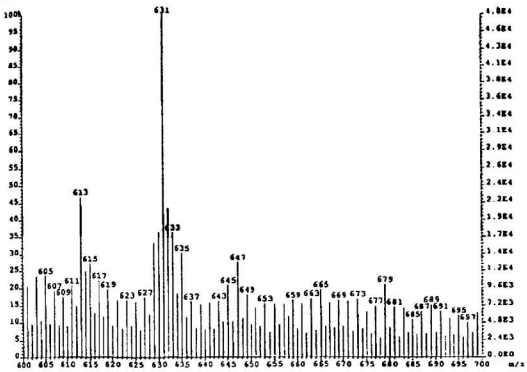


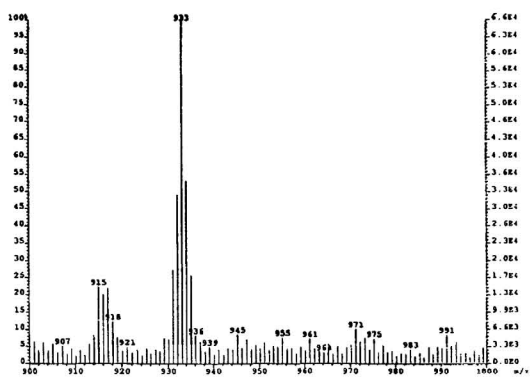
Figure 4a — HPLC/LSIMS coupling of an aqueous extract and acetone/water of *Q. robur*. Inverted phase HPLC chromatogram of *Q. Robur* extract with water (a) and with acetone/water (b)

(peak identification : 1 : vescalin - 2 : castalin - 3 : roburin A - 4 : roburin B - 5 : roburin C - 6 : gallic ac. 7 : grandinin - 8 : roburin D - 9 : vescalagin - 10 : roburin E - 11 : castalagin - 12 : ellagic ac.)

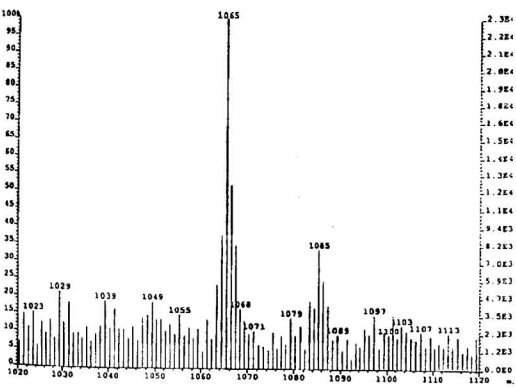
tions. We noticed, within our samples, that the European species have both a greater dry extract and soluble phenols content than the species from Central and North America. The HPLC method of determining the total ellagitannin yield results is inferior to methods using degrading acid or the nitrous acid reagent. For *Q. farnetto* and *Q. oocarpa*, the differences between HPLC and degrading acid represents 24 mg/g of wood, 16.5 mg/g for *C. sativa*, 17.5 mg/g for *Q. robur*, 15 mg/g for *Q. alba* and *Q. stellata*, and 13.5 mg/g for *Q. petraea*.



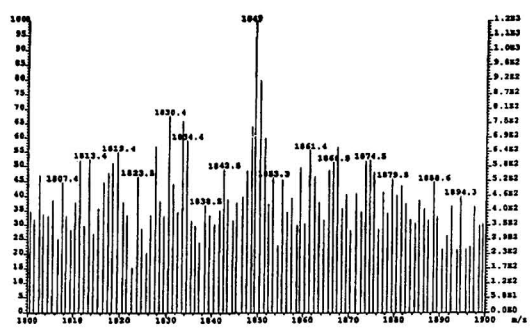
Vescalin (1), castalin (2)
$(M-H)^-$ = 631



Vescalagin (9), castalagin (11)
$(M-H)^-$ = 933



Grandinin (7), roburin E (10)
$(M-H)^-$ = 1065



Roburin A, roburin D (8)
$(M-H)^-$ = 1849

Figure 4b - HPLC/LSIMS coupling of an aqueous and acetone/water extract from *Q. robur*. LSIMS spectra of different chromatographic peaks.
(Spectra recorded in negative mode , corresponding to $(M-H)^-$, glycerol matrix

These deviations could be attributable to unknown structures. These molecules are probably the ellagitannins. The nitrous acid method yields results superior to those given by acid degradation, the deviations are however inferior (5 to 10 mg/g of wood). This might be explained by the existence of substances which interfere in the quantitative determination and increase the absorbency to 600 nm. The calculation of the ratio of total ellagitannins/dry extract (figure 6) enables us to notice that the acid degradation and nitrous acid reagent methods best represent the fraction of ellagitannins in the total extract. We observed that the dry extracts of *Q. alba* and *Q. stellata* are mostly composed of fractions of ellagitannins. In the other species, and in particular *Q. petraea* and *C. sativa*, in the process of characterisation the dry extracts hold a majority of non ellagitannin fractions.

The different accepted methods for determining the total ellagitannins of oak and chestnut heartwood extracts yield comparable values (figure 7) which are significantly correlated ($r > 0,97$; $p < 0,01$)

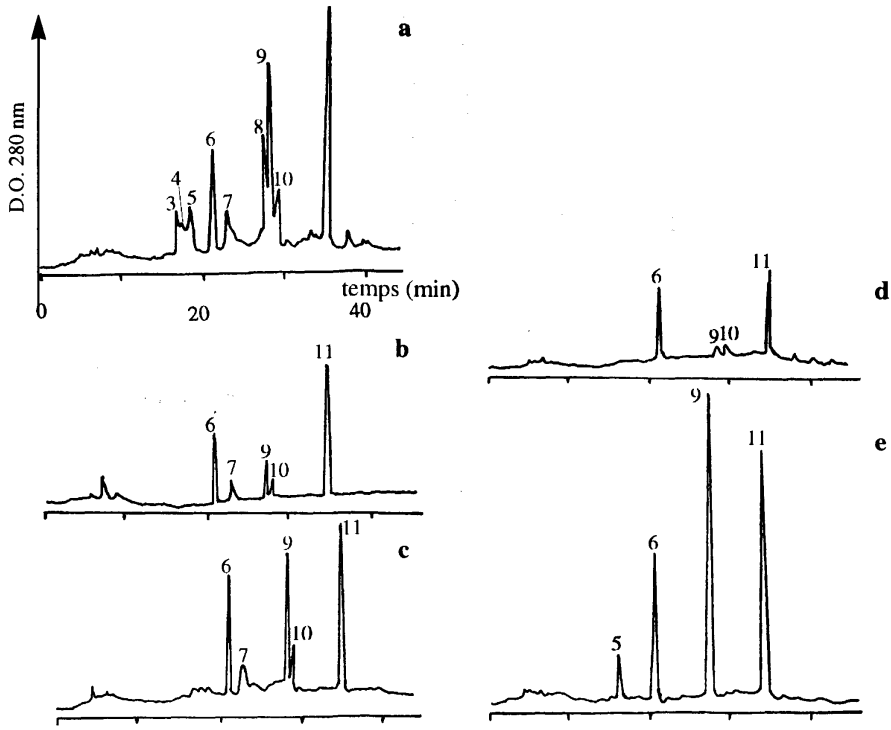


Figure 5 - Inverted phase HPLC chromatograms of acetone/water oak heartwood

Q. petraea (a), *Q. oocarpa* (b), *Q. stellata* (c), *Q. alba* (d) and of chestnut *C. sativa* (e)
(peak identification : 3 : roburin A - 4 : roburin B - 5 : roburin C - 6 : gallic acid
7 : grandinin - 8 : roburin D - 9 : vescalagin - 10 : roburin E - 11 : castalagin)

2 - Taxonomic application to the quantitative determination of phenol acids and the monomer and dimer ellagitannins

As previously noticed (figure 5), the chromatograms of the analysed extracts are sufficiently different from one species to another to constitute characteristic profiles. The quantification of gallic acid, ellagic acid and the known monomer and dimer ellagitannins revealed, in the same way, appreciable deviations between species. Only *Q. robur* and *Q. petraea* had a similar composition (table 4).

The three European species possess the described 4 monomer and 4 dimer ellagitannins (table 4). *Q. farnetto* is distinguished from the other two species by its greater content of pentolised ellagitannins (table 4 : 55 %, 22,5 %, 39 % respectively for *Q. farnetto*, *Q. robur* and *Q. petraea*), associated with a high content of pentosylated dimers, roburin B and C (figure 8). The total vescalagin/total castalagin from *Q. robur* and *Q. petraea* is in a ratio of approximately 2. It is 4,5 in *Q. farnetto*, for which the vescalagin represents the predominant ellagitannin.

The heartwood extracts from American species contain almost exclusively monomer ellagitannins (> 90 %). *C. sativa* presents the same characteristics with, what is more, a quasi absence of pentosylated molecules (figure 8).

J. Sci. Tech. Tonnellerie, 1996, 2
© Vigne et Vin Publications Internationales - Bordeaux (France)

Table 3

Estimation of the quantity of total phenolic composites and comparison of the different methods of quantitative determination of the ellagitannins from extracts of oak and chestnut heartwood

(The total ellagitannins are expressed in equivalent castalagin)

Species	Dry extract (mg/g of wood)	Total phenols		Total ellagitannins (mg/g of dried wood)		
		D.O. 280 nm	FCI+	HPLC++	Nitrous acid*	Acid degradation §
Quercus :						
Q. robur	118 ±5.7	27 ±2.5	24 ±3.5	87.5 ±11.6	112 ±13.5	105 ±12.5
Q. petraea	85 ±4.3	*17.5 ±3.2*	*15 ±2.7*	*39 ±9.5*	*56 ±9.6*	*52.5 ±10.3*
Q. farnetto	136 ±8.5	23.5 ±4.3	21 ±3.6	110 ±12.8	138.5 ±12.7	134 ±14.6
Q. alba	43 ±4.5	6 ±1.2	8.5 ±2.3	33 ±3.5	61 ±8.6	48 ±9.7
Q. stellata	64 ±8.5	11 ±0.8	12.5 ±1.4	67 ±10.7	98.5 ±10.8	91 ±11.4
Q. oocarpa	65.5 ±7.4	11 ±1.5	13.5 ±1.8	39 ±9.6	66 ±5.3	55.5 ±6.8
Castanea :						
C. sativa	137 ±12.7	31 ±5.8	27 ±6.8	44 ±7.4	57.5 ±8.2	51 ±6.5

+FCI Folin-Ciocalteu Index - ++HPLC quantitative determination of total ellagitannins - *BATE-SMITH (1972) nitrous acid reagent quantitative determination of total ellagitannins - §

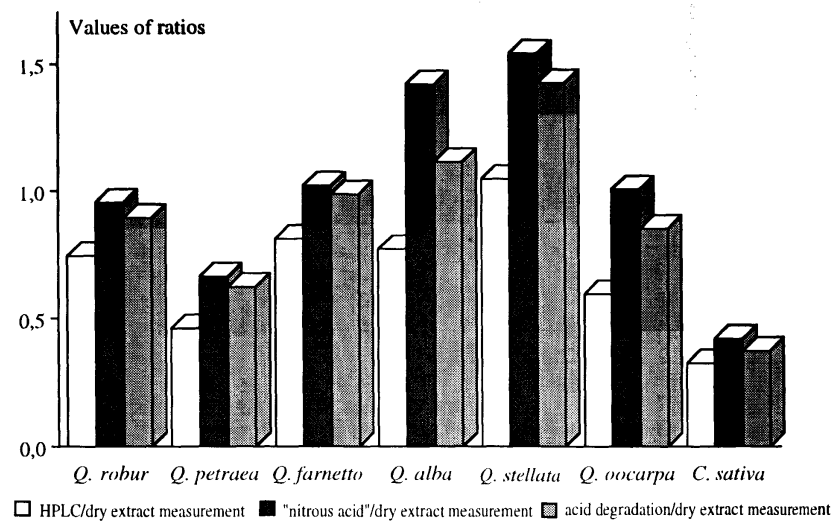


Figure 6 - Values of ratios between the different methods of quantitative determination of the ellagitannins and the dry extract of heartwood from species of oak and chestnut

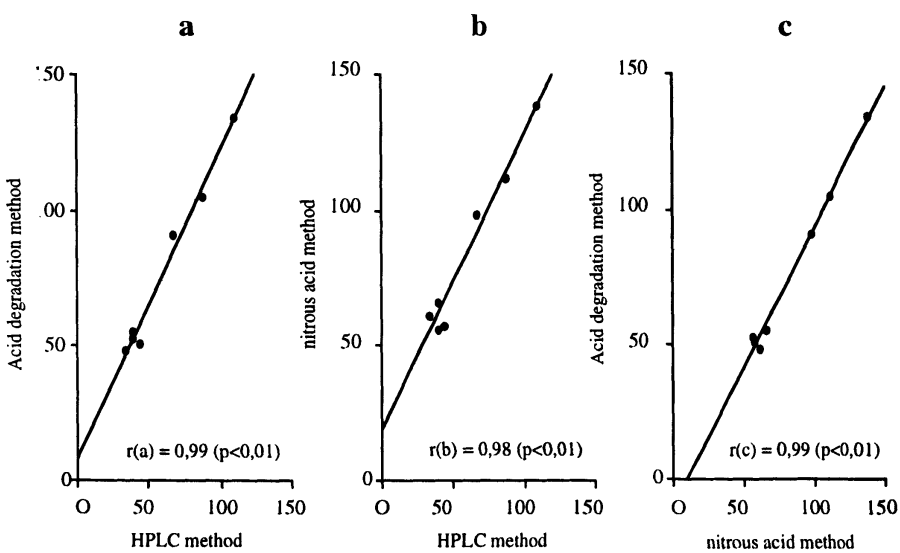


Figure 7 - Correlation between the quantitative analyses of total ellagitannins by HPLC and acid degradation (a), HPLC and the nitrous acid reagent (b) and the nitrous acid reagent and acid degradation (c).

The results are given in equivalent castalagin mg/g of wood.
Each point represents on average 10 analyses.

III - QUANTITATIVE DETERMINATION OF THE ELLAGITANNINS IN RED WINES MATURED IN BARRELS

1- Quantitative determination of the ellagitannins using the nitrous acid reagent (BATE-SMITH, 1972)

The method of quantitative determination of the ellagitannins by nitrous acid oxidation in nitrogen is very specific to the esters of hexahydroxydiphenic acid. We observe, in table 5, that in a hydroalcoholic medium, the procyanidins of the seeds and the monoglucosidic anthocyanins of the grape skins (*Vitis vinifera* c.v. Cabernet sauvignon) responded weakly to quantitative analysis. On the other hand, in red wines, due to the complexity of the medium and the presence of a large quantity of phenolic compounds, particularly condensed tannins, quantitative determinations are

Table 4

Phenol acids and ellagitannins of extracts from the heartwood of oaks and chestnut.

(ellagitannins and phenol acids in mg/g of wood)

	Q. robur	*Q. petraea*	*Q. farnetto*	*Q. alba*	*Q. stellata*	*Q. oocarpa*	*C. sativa*
Phenol acids							
gallic ac.	1.4	1.1	3.2	1.2	1.8	1.3	2.1
ellagic ac.	1.9	3.1	0.6	18.4	11.2	4.2	1.7
Ellagitannins							
Monomers :							
Vescalagin	26.7	8.7	15.7	3.8	16.3	6.5	22.6
Castalagin	30.1	12.4	14	26.4	29.6	23.7	17.4
Grandinin	8.5	5.8	5.2	tr.*	6.4	6.2	tr.
Roburin E	4	4.7	8.7	2.8	8.5	2.9	0
Dimers :							
Roburin A	8.7	2.4	12.2	tr.	1.9	0	3.8
Roburin D	2.3	0.4	5.2	0	0	0	0
Roburin B	3.6	2.6	26	tr.	2.3	0	tr.
Roburin C	3.5	2.1	21	tr.	1.9	0	0
% dimers	21	19	58.5	< 1	9	0	9
% monomers	79	81	41.5	> 99	91	100	91
% pentosylated ellagitannins**	22.5	39	55	9	28	23	< 1
Gallic ac./ellagic ac.	0.73	0.35	5.3	0.06	0.16	0.3	1.23
Free vescalagin/ free castalagin	0.88	0.7	1.1	0.14	0.55	0.27	1.3
Total vescalagin/ total castalagin#	1.7	2	4.6	0.26	1.25	0.65	1.5
Total ellagitannins/ ellagic ac.	46	12.6	183	1.8	59.8	9.3	25.8

*tr : traces - **pentosylated ellagitannins : grandinin + roburin E + roburin B + roburin C - # vescalagin and total castalagin = free molecules + pentosylated + in dimer form

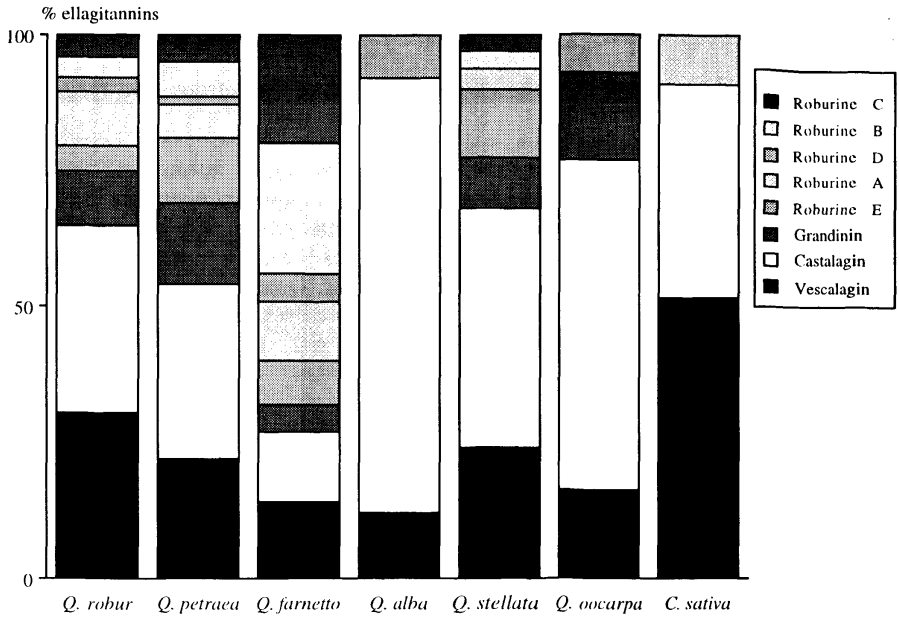


Figure 8 - Centesimal proportion of the HPLC determined monomer and dimer ellagitannins in the heartwood extracts of different analysed species

not applicable. The elimination of the anthocyanins by isoamylic alcohol (table 5) and of the procyanidins by ethyl acetate (results not shown) does not help to improve the quantitative determination results and makes it even more delicate. With white wines far less rich in phenols, the method can yield relatively satisfactory comparative results.

2- Quantitative determination of the total ellagitannins by acid degradation

This method of quantitative analysis is based on the release of ellagic acid during acid hydrolysis of ellagitannins. The ellagic acid formed can thereby be quantified by HPLC. There are many problems which need to be solved when adapting the method to red wines : the complex mixture constituting the wine needs a chromatographic gradient adapted to the separation of the ellagic acid and other phenols, and in a time compatible with the series analysis of the samples ; choose a specific wavelength which has a small molecular absorption, optimise the method of acid hydrolysis so as to obtain the maximum ellagic acid in a minimum delay ; finally, it is appropriate to choose a solvent in which the ellagic acid is highly soluble. The accepted conditions yield repeatable results and allow a satisfactory quantification of the total ellagitannins in solution in the red wines (cf. Materials and Methods).

The oak wood ellagitannins dissolve rapidly in wine kept in barrels (figure 9), since within 3 months, the maximal content is attained, after which they regularly diminish. The ellagic acid accumulates more rapidly during the first month, and then, increases very slowly during the entire maturation. (figure 9). Table 6 groups toge-

ther the quantitative analyses carried out on several red wines. The ellagitannins were measured in great quantities in young wines, especially during the first month of maturation. After some months in the barrel, the level of ellagitannins became low. Even if we do not find more ellagitannins in appreciable quantities in wines after some years of conservation in bottles, the ellagic acid, however, remains as a marker of the conservation of the wine in barrels. In effect, the ellagic acid is stable enough to be measured, even after several decades. These results suggest that the ellagitannins, in their native form, under go rapid transformations which do not allow them to give ellagic acid by hydrolysis.

DISCUSSION

The heartwood of oaks and chestnuts contain extractables which constitute 4 to 10 % of their dry weight and which are easily soluble using mixtures of ethanol/water, methanol/water (PENG *et al.*, 1991) and acetone/water. The greater majority of these extracts are soluble in water (SCALBERT et *al.*, 1989). Under our experimental condi-

Table 5
Influence of the phenolic composites of the grape and the wine on nitrous acid oxidation measurement value of the ellagitannins
($NaNO_2$: sodium nitrite 6 %, solution 1-7 : hydroalcoholic solution 12 % vol. EtOH, 5 g/l tartaric ac., NaOH N qsp pH = 3,5)

Solution	Composition	Total phenols (DO 280 nm)	D.O. 600 nm			Total ellagitanins (mg/l)
			water	$NaNO_2$	Δ DO	
1	*Q. robur* extract (1 g/l)*	14.5	0.07	0.78	0.71	774
2	Oligomers procyanidins (1 g/l)**	42.3	0.09	0.1	0.01	9.5
3	Solutions 1 + 2 (1:1)	19.1	0.07	0.86	0.79	750.5 (11 %)§
4	monoglucosidic anthocyanins (1 g/l)**	4.5	0.12	0.14	0.02	19
5	Solutions 1 + 4 (1:1)	19.3	0.15	0.88	0.73	693.5 (3 %)
6	Grape seed extract (1 g/l)**	43.8	0.12	0.1	—	—
7	Solutions 6 + 1 (1:1)	19.2	0.11	0.84	0.73	693.5 (3 %)
8	White wine (stainless steel tank)	6.8	0.05	0.15	0.1	95
9	Ellagitannins 8 + 1 g/l	22.2	0.1	0.89	0.79	750.5
10	Red wine (stainless steel tank)	28	0.48	0.8	0.32	304
11	Ellagitannins 10 + 1 g/l	38	0.61	1.67	0.66	627
12	Red wine treated with isoamylic alcohol#	22.4	0.4	0.6	0.2	190
13	Ellagitannins 12 + 1 g/l	36	0.35	1.51	1.16	1102

* Acetone/water extract (7:3) - ** Phenolic compounds isolated from the grape under the condition described by VIVAS *et al.*, 1994 - # Red wine extracted by twice its volume of isomylic alcohol RP - § Deviations in % in relation to solution 1

Table 6
Quantitative analysis of the ellagic acid of the total ellagitannins in different red wine samples
(the results are expressed in mg/l. The wines 1-5 ; 6 and 7 ; 8 and 9 are identical)

Samples	Origins	Vintage	Type of maturation	Ellagic ac.	Released ellagic ac.	Total ellagitannins
1	Spain (Rioja)	1993	Stainless steel tank (4 months)	0	0	0
2	"	"	Used barrels (4 months)	4.5	1.3	4
3	"	"	New oak barrels USA (4 months)	16.8	1.9	5.8
4	"	"	New oak barrels - Limousin Fr. (4 mois)	12.2	30.5	94.2
5	"	"	New oak barrels - Allier Fr. (4 mois)	8.3	21.6	66.7
6	France (Saint-Emilion)	1992	New oak barrels - Allier Fr. (2 mois)	4.6	38.7	119.8
7	"	"	New oak barrels - Allier Fr. (6 mois)	10.4	13	40.3
8	France (Médoc)	"	New oak barrels - Allier Fr. (6 mois)	7.8	15.4	47.5
9	"	"	New oak barrels - Allier Fr. (18 mois)	12.2	10.1	31.2
10	France (Médoc)	1970	New oak barrels - Fr.	7.3	0.9#	2.8#
11	France (?)	1880	Barrrels	5.6	0	0

#Estimation

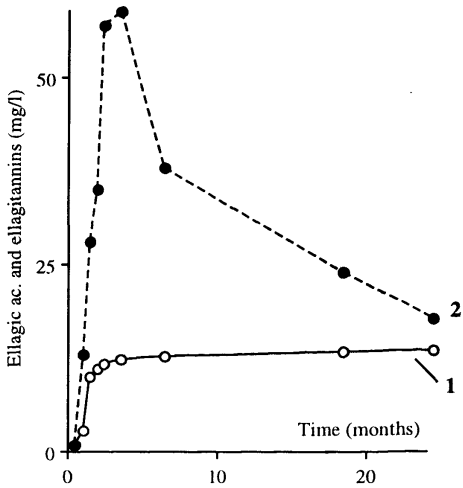


Figure 9 - Evolution of the content of ellagic acid (1) and total ellagitannins (2) of a wine during its maturation in a new barrel (*Q. petraea*, Allier, France)

tions, the mixture acetone/water (7:3) gave us both the greatest quantity of dry extract and the richest total phenols yield. Amongst the other solvents employed, the water gave a high level of dry extract, but few total phenols ; the ethanol/water (5:5), the ethanol, the methanol and the methanol/chloroform (8:2) gave few dry extracts but had greater concentrations of phenols. The mixture of acetone and water is recognised as being a good solvent for quantifying the total soluble fractions of heartwood. But the powder obtained did not contain ellagitannins only. Partially extractable by organic solvents, using the dry extract dissolved in water : 0,5 to 1 % in chloroform, 5 to 7 % in diethylic ether and 5 to 10 % in ethyl acetate. These fractions contain respectively the fatty acids and steroids, the phenol acids and catechol, the procyanidines (SCALBERT *et al.*, 1989 ; BAUCH *et al.*, 1991 ; VIRIOT *et al.*, 1993). A more detailed study is actually under way. A large part of the extract (17 to 23 %) is able to precipitate with the ethanol (water:ethanol 1:9), does not dialyse across membranes of 2000 and 3500 Da and is practically totally soluble in the DMSO. This is probably the influence of the polysaccharides associated with the phenolic composites (absorbed at 280 nm and reacting with the Folin-Ciocalteu solution). In total, the majority of the extract (60 to 70 %) contains ellagitannins.

Several methods of quantitative determination of the total ellagitannins using different principles have been tested on the extracts of oak and chestnut heartwood. The study was performed on 6 species of oak and 1 chestnut *Q. robur*, *Q. petraea*, *Q. farnetto* (Eastern Europe), *Q. alba*, *Q. stellata*, *Q. oocarpa* (North and Central America) and *C. sativa* (Europe). We compared the quantities determined by HPLC, by acid degradation followed by a HPLC estimation of the ellagic acid formed, and by nitrous acid in nitrogen oxidation of the hexahydroxydiphenic esters. Finally to enable comparison, all the results were expressed in equivalent castalagin. We noticed that the HPLC determination gave results inferior to the other two methods. The efficiency of the acid degradation is not exactly 1 mole of ellagic acid per mole

of monomers, it is in the region of 0,9, and 2 moles per mole of dimers (results not shown). Despite these, the greater quantity of ellagitannins measured using this method suggest the presence of other molecules capable of releasing ellagic acid through acid hydrolysis. They are probably structures akin to ellagitannins because they react in the same way as nitrous acid. This last mentioned method is recognised as being very specific to these types of molecules. The analyses of the ellagitannins and heartwood extracts, by inverse phase HPLC, are frequently affected by the presence of non-ellagitannin composites which complex easily with these molecules and interfere with quantitative analyses (SCALBERT *et al.*, 1990). Whilst splitting up the acetone/water extracts, we isolated an ethanol precipitate, almost probably of a polysaccharide nature, which released large quantities of ellagic acid by way of acid hydrolysis and reacted positively with the nitrous acid (results not shown). The different results of the quantitative determinations between the HPLC method and the other methods represent between 10 to 20 mg of ellagitannins/g of wood. They can be attributed to the water extractable combined forms of polysaccharides-ellagitannins. KLUMPERS *et al.* (1994) have isolated other DMSO soluble fractions which seem to be equally attributable to the ellagitannin forms combined with the polysaccharides more tightly retained in the cellular walls. Their DMSO solubility make them closer to hemicellulose (JOSELEAU, 1980).

The LSIMS technique as well as the HPLC/LSIMS coupling have been applied to the dry extracts of the different species selected for our study. These methods complete the techniques habitually applied for HPLC product identification (co-injection of purely referential products, comparison of their Tr and their UV spectra). In all the samples, we found ellagic acid indicating that the retained species have variable quantities of ellagitannins in their extracts (SCALBERT *et al.*, 1989). The vescalagin and the castalagin are systematically identified next to other monomer and dimer forms of ellagitannins. We also found, in the water extracts, vescalagin and castalagin (identified by LSIMS, m/z : 631 $[M-H]^-$ and its Tr compared to that of the referent castalagin). The presence of these molecules in the *Q. robur* and *C. sativa* heartwood extracts has already been reported by MAYER *et al.* (1967, 1971). SCALBERT *et al.* (1990) did not find these molecules. Their presence could be partially associated with the vescalagin and castalagin hydrolysis reactions during extraction. In fact, we have found these molecules uniquely in the water and the ethanol/water mixture (5:5) extracts. Recently, VIRIOT *et al.* (1994) suggested their presence in the heartwood of these same two species. According to the authors, the presence of vescalagin and castalagin is attributable to the hydrolysis reactions in the wood in the course of its ageing. They noticed an augmentation in the concentration of these two molecules and the ellagic acid commensurate with the age of the wood : the recent layer of sapwood and duramen were poor, but the older layers of duramen held significant quantities. Under our conditions, the analyses have been performed on samples representing all heartwood, we believe that the global content of vescalagin and castalagin of the sample has become too small to be measured by HPLC. Meanwhile the exact origin of these molecules is still uncertain and thus complementary investigations are necessary. The gallic acid and the ellagic acid are the principal simple phenols of low molecular weight coming from the heartwood extracts of oaks and chestnut. Even if the presence of ellagic acid is easily explained through hydrolysis of numerous wood ellagitannins, the origin of gallic acid is more questionable. We known that the common precursor of the ellagitannins is β-penta-

O-galloyl-D-glucose (OKUDA *et al.*, 1982 ; ADAMCZESKI *et al.*, 1992 ; VIRIOT *et al.*, 1994 ; VIVAS *et al.*, 1995a), but we did not find any in the wood extracts of oaks and chestnut. SCALBERT *et al.* (1988) identified penta-galloylglucose in leaf and shim tissue cultures ; on the other hand, they were not evident in the leaves, the bark and the wood of adult *Q. robur*s. In the leaves of the same species, SCALBERT and HASLAM (1987) reported the presence of divers hexahydroxydiphenic esters such as vescalagin, castalagin and pedunculagin, but no gallic acid ester. The origin of the gallic acid is thus still unresolved. Never the less we notice an augmentation of its content during the ageing of the uncut tree wood (VIRIOT *et al.*, 1994), which leads us to suppose, along with the authors, that it is derived from the hydrolysis of galloyl esters probably associated with the parietal composites of the cells (GARLAND *et al.*, 1985 ; SCALBERT *et al.*, 1988). In light of these different works, the presence of these gallotannins in the heartwood of oak is not to be excluded (SEIKEL *et al.*, 1971 ; VIVAS *et al.*, 1993a). In the particular case of the chestnut *C. sativa*, in accordance with the results of SALAGOITY *et al.* (1986), we have measured digallic acid in the aqueous heartwood extracts (VIVAS *et al.*, 1993b). The presence of this depside in the chestnut extract has been recently confirmed (VIVAS *et al.*, 1996). We can thus presume that part of the free gallic acid originates from digallic acid hydrolysis. The mechanism is easily performed in an enzymatic way (VIVAS *et al.*, 1991).

The quantitative determination of the monomer and dimer ellagitannins extracts of the species studied give chromatographic profiles which are sufficiently differentiated to allow a taxonomic application. The vescalagin and the castalagin are the principal ellagitannins in the extracts of all the heartwood samples. In the 3 European species of oak (*Q. robur*, *Q. petraea* and *Q. farnetto*), we find the 4 monomer ellagitannins (vescalagin, castalagin grandinin, and roburin E) and the 4 dimers (roburin A, roburin D, roburin B, roburin C). The American species (*Q. alba*, *Q. stellata* and *oocarpa*) are characterised by the quasi absence of dimers. *C. sativa* is distinguished by the exclusive presence of non - pentolised forms of monomers. The analytic results obtained for *Q. robur*, *Q. petraea* and *C. sativa* are in accordance with those of MAYER, 1971 ; SCALBERT *et al.*, (1986) : SCALBERT *et al.*, (1990) ; VIRIOT *et al.* (1994). For the other species, to our knowledge, no detailed study of their ellagitannin composition has been carried out. The greater reactive potential of vescalagin compared to castalagin (VIVAS *et al.*, 1995 and 1996) propitiates nuclear attacks on the C1-glucosidic of the vescalagin, explaining why the majority forms are dimers. The total vescalagin/total castalagin ratio is greater than 1, except in *Q. alba* and *Q. oocarpa* in which it is probable that other unknown dimeric forms are present. In comparison with the other species, *Q. farnetto* presents a best aptitude for dimerisation (58 % dimers, as opposed to 20 % for *Q. robur* and *Q. petraea* and < 10 % for the other species) and the formation of pentolised ellagitannins (55 %, as opposed to 0-40 % for the other species).

In the last part of the work, we measured the total ellagitannins found in red wines matured in barrels. Although quantitative determination by way of the nitrous acid reagent is very specific to the hexahydroxydiphenic groups, applying this method to red wines has revealed to be impossible. The existence of substances, unlike the anthocyans and procyanidines, interfere by reacting positively with the nitrous acid. The acid degradation applied to a testing sample of wine, in a hydrochloric acid/methanol mixture, released variable quantities of ellagic acid. The determined

quantities indicate that the ellagitannins solubilise during the first months of maturation, after which their content diminishes rapidly. The capacity of these molecules to oxidise easily (VIVAS and GLORIES, 1996) enable them to participate in the oxidation reducing reactions which occur during maturation in barrels (VIVAS and GLORIES, 1993). The presence of ellagitannins in white wines matured in barrels has already been reported by QUINN and SINGLETON (1985) after isolation of the ellagitannin fraction on a low pressure LH20™ column and by MOUTOUNET *et al.* (1989) using HPLC quantity analysis and acid hydrolysis. Meanwhile, the fact that only small quantities of total ellagitannins have been found in the wines (MOUTOUNET *et al.*, 1992) tends to show that barrels cede little. In fact, the determined quantity is the result of assessments of the solubilised and oxidised ellagitannins. The oxidised forms release only a little ellagic acid through acid hydrolysis whilst their absorbency is at O.D. 280 nm and their reactivity to the Folin-Ciocalteu reagent are in less affected proportions (KLUMPERS *et al.*, 1994). One part of the ellagitannins in solution in the wines are in forms combined with polysaccharides (DUBOURDIEU, 1992). In these conditions, they are more accessible to classic quantitative analyses.

CONCLUSION

The different oak species analysed in this study show that their content of extractables and total phenols are variable but comparable to the varieties of oak usually employed in cooperage. Among the total phenols, all the samples present a majority of ellagitannins. This property is important because it confers on the wood a better durability (HART et HILLIS, 1972) in comparison with other deprived species such as *Q. cerris* (LAVISCI *et al.*, 1991) which enables them to be used in cooperage.

A method of quantitative analysis of red wine total ellagitannins is also proposed. It enabled us to show, on the one hand, that the ellagitannins are very hydrosoluble and are soon found in the wines after some weeks in new barrels and, on the other hand, these molecules disappear when oxidation reactions occur in the wine in which they lie.

Acknowledgements *: We would like to thank, most graciously, Augustin SCALBERT (INRA - INA, Thivernal-Grignon) for his never ceasing interest regarding our works, as well as his frequent advise.*

BIBLIOGRAPHY

ADAMCZESKI M., HUANG J., KANG R., N.J.X., JABER H. and NAKATSU T., 1992. *in « Plant Polyphenols : Biogenesis, chemical properties and significance »*, Hemingway R.W. and Laks P.E. (eds.), Plenum press, New York.

BATE-SMITH E.C., 1972. Detection and determination of ellagitannins. *Phytochemistry*, **11**, 1153-1156.

BAUCH J., HUNDT H., WEIBMAN G., LANGE W. and KUBEL H., 1991. On the cause of yellow discoloration of oak heartwood (*Quercus* sect. *robur*) during drying. *Holzforschung*, **45**, 2, 79-85.

DUBOURDIEU D., 1992. Vinification des vins blancs secs en barriques. *in « Le bois et la qualité des vins et des eaux-de-vie »*, Guimberteau G. (ed.), Vigne et Vin

Publications Internationales, Martillac, 137-143.

GARLAND C.P., JAMES F.C., NELSON P.J. and WALLIS A.F., 1985. *in* « *Proceeding of International symposium in wood pulp chemistry* », Vancouver, Canada, **1**, 123-127.

GLORIES Y., 1978. Recherches sur la matière colorante des vins rouges. *Thèse doct. ès sciences*, Université de Bordeaux II.

HART J.H. and HILLIS W.E., 1972. Inhibition of wood-rotting fungi by ellagitannins in the heartwood of *Quercus alba*. *Phytopathology*, **62**, 620-626.

HASLAM E., 1981. Vegetable tannins. *in* « *The biochemistry of plants* », Conn E.E. (ed.), Academic press, New York.

HERVÉ du PENHOAT C.L.M., MICHON V.M.F., OHASSAN A., PENG S., SCALBERT A. and GAGE D., 1991a. Roburin A, a dimeric ellagitannin from heartwood of *Quercus robur*. *Phytochemistry*, **30**, 1, 329-332.

HERVÉ du PENHOAT C.L.M., MICHON V.M.F., PENG S., VIRIOT C., SCALBERT A. and GAGE D., 1991b. Structural elucidation of new dimeric ellagitannins from *Quercus robur* L., Roburins A-E. *J. Chem. Soc. Perkin Trans.*, **1**, 1653-1660.

JOSELEAU J.P., 1980. Les hémicellulose, *in* « *Les polymères végétaux. Polymères pariétaux et alimentaires non azotés* », Monties B. (ed.), Bordas, Paris, 87-121.

KELLER R., 1992. Les chênes dans le monde. Les chênes de tonnellerie en France : *Quercus petraea* et *robur*. *in* « *Le bois et la qualité des vins et des eaux-de-vie* », Guimberteau G. (ed.), Vigne et Vin Publications Internationales, Martillac, 7-24.

KLUMPERS J., SCALBERT A. and JANIN G., 1994. Ellagitannins in european oak wood : Polymerisation during wood ageing. *Phytochemistry*, **36**, 5, 1249-1252.

LAVISCI P., SCALBERT A., MASSON D. and JANIN G., 1991. Quality of Turkey oak (*Quercus cerris* L.) wood. *Holzforschung*, **45**, 4, 291-296.

MAYER V.W., GABBER W., RIESTER A., and KORGER H., 1967. Uber die Gerbstoffe aus dem Holz der Edelkastanie and der Eich. II- Die Isolierung von Castalagin, Vescalin, Castalin und Vescalin. *Lieb. Ann. Chem.*, **707**, 177-192.

MAYER V.W., SEITZ H., JOCHIMS J.C., SCHAUERTE K. and SCHILLINJ G., 1971. Uber die Gerbstoffe aus dem Holz der Edelkastanie and der Eich. *Lieb. Ann. Chem.*, **751**, 60-73.

MAYER V.W., 1971. Uber die Gerbstoffer aus dem Holz der Edelkastanie und Eich. *Das Leder*, **12**, 277-283.

MOUTOUNET M., RABIER PH., PUECH J.-L., VERETTE E. and BARILLERE J.-M., 1989. Analysis by HPLC of extractable substances in oak wood. Application to a chardonnay wine. *Sci. aliments*, **9**, 1, 35-51.

MOUTOUNET M., RABIER PH., SARNI F. et SCALBERT A., 1992. Les tanins du bois de chêne. Les conditions de leur présence dans les vins. *in* « *Le bois et la qualité des vins et des eaux-de-vie* », Guimberteau G. (ed.), Vigne et Vin Publications Internationales, Martillac, 75-79.

NONAKA G.I., ISHIMARU K., AZUMA R., ISHIMATSU M. and NISHIOKA I., 1989. *Chem. Pharma. Bull.*, **37**, 2071-2077.

OKUDA T., YOSHIDA T., HANATO T., YAZAKI K. and ASHIDA M., 1982. Phytochemistry, **21**, 2871-2875.

PENG S., SCALBERT A. and MONTIES B., 1991. Insoluble ellagitannins in *Castanea sativa* and *Quercus petraea* woods. *Phytochemistry*, **30**, 3, 775-778.

POCOCK K.F., SEFTON M.A. and WILLIAMS P.J., 1994. Taste thresholds of phenolic extract of french and american oak wood :

The influence of oak phenols on wine flavor. *Am. J. Enol. Vitic.*, **45**, 4, 429-434.

PUECH J.-L., RABIER P. and MOUTOUNET M., 1993. Dosage des tanins ellagiques sur des extraits de bois de chêne et des eaux-de-vie vieillies en fût. *in « Elaboration et connaissance des spiritueux »*, Cantagrel R. (ed.), Lavoisier (diffusion), Paris.

PUECH J.-L., 1987. Extraction of phenolic compounds from oak wood in model solution and evolution of aromatic aldehydes in wines aged in oak barrels. *Am. J. Enol. Vitic.*, **38**, 3, 236-238.

QUINN M.K. and SINGLETON V.L., 1985. Isolation and identification of ellagitannins from white oak wood and an estimation of their roles in wine. *Am. J. Enol. Vitic.*, **36**, 2, 148-155.

SALAGOITY-AUGUSTE M.-H., TRICARD C., MARSAL F. and SUDRAUD P., 1986. Preliminary investigation for the differentiation of enological tannins according to botanical origin : Determination of gallic acid and its derivatives. *Am. J. Enol. Vitic.*, **37**, 4, 301-303.

SCALBERT A. and HASLAM E., 1987. Polyphenols and chemical defence of the leaves of *Quercus robur*. *Phytochemistry*, **26**, 3191-3195.

SCALBERT A., MONTIES B. and FAVRE J.M., 1988. Polyphenols of *Quercus robur* : Adult tree and *in vitro* grown calli and shoots. *Phytochemistry*, **27**, 11, 3483-3488.

SCALBERT A., MONTIES B. and JANIN G., 1989. Tannins in wood : Comparison of different estimation methods. *J. Agric. Food Chem.*, **37**, 5, 1324-1329.

SCALBERT A., DUVAL L., PENG S., MONTIES B. and HERVE DU PENHOAT C.L.M., 1990. Polyphenols of *Quercus robur* L. II- Preparative isolation by low-pressure and high-pressure liquid chromatography of heartwood ellagitannins. *J. Chromatogr.*, **502**, 107-119.

SEIKEL M.K., HOSTETTLER F.D. and NIEMANN G.J., 1971. *Fagaceae* - Phenolic of *Quercus rubra* wood. *Phytochemistry*, **10**, 2249-2251.

SINGLETON V.L., 1995. Maturation of wines and spirits : Comparisons, facts, and hypotheses. *Am. J. Enol. Vitic.*, **46**, 1, 98-115.

TARANSAUD J., 1976. *Le livre de la tonnellerie*. La roue à livres diffusion (ed.), Paris.

VIRIOT C., SCALBERT A., LAPPIERRE C. and MOUTOUNET M., 1993. Ellagitannins and lignins in aging of spirits in oak barrels. *J. Agric. Food Chem.*, **41**, 1872-1879.

VIRIOT C., SCALBERT A., HERVÉ DU PENHOAT C.L.M. and MOUTOUNET M., 1994. Ellagitannins in woods of sessile oak and sweet chestnut. Dimerization and hydrolysis during wood ageing. *Phytochemistry*, **36**, 5, 1253-1260.

VIVAS N., GLORIES Y., DONECHE B., et GUEHO E., 1991. Observation sur la flore fongique du bois de chêne (*Quercus* sp.) au cours de son séchage naturel. *Ann. Sc. Nat. Bot.* (Paris), **13**, **11**, 4, 149-153.

VIVAS N. et GLORIES Y., 1993. Les phénomènes d'oxydoréduction liés à l'élevage en barrique des vins rouges : Aspects technologiques. *Rev. Fr. Œnol.*, **33**, 142, 33-38.

Vivas N., CHAUVET S., SUDRAUD P. et GLORIES Y., 1993a. Techniques de contrôle et d'évaluation de la qualité des tanins œnologiques. *Ann. Fals. Exp. Chim.*, **86**, 919, 215-222.

VIVAS N., CHAUVET S., Glories Y. et SUDRAUD P., 1993b. Caractérisation et définition des préparations commerciales de tanins œnologiques. *Ind. Agric. Alim.*, **110**, 10, 705-713.

VIVAS N., BELLEMERE L., LONVAUD-FUNEL A., GLORIES Y. et AUGUSTIN M., 1994. Etude sur la fermentation malolactique des vins rouges en barriques et en cuves. I° partie. *Rev. Fr. Œnol.*, **34**, 149, 37-42.

VIVAS N., 1995. The notion of grain in cooperage. *J. Sci. Tech. Tonnellerie*, **1**, 17-48.

VIVAS N., LAGUERRE M., GLORIES Y., BOURGEOIS G. and VITRY C., 1995. Structure simulation of two ellagitannins from *Quercus robur* L. *Phytochemistry*, 39, 5, 1193-1199.

VIVAS N., LAGUERRE M. et GLORIES Y., 1996. Structure et propriétés physico-chimiques des deux principaux ellagitanins du bois de chêne : Vescalagine et castalagine. *in* « *Œnologie 95* », Lonvaud-Funel A. (ed.), Lavoisier, Paris.

VIVAS N. and GLORIES Y., 1996. Role of oak wood ellagitannins in the oxidation process of red wines during aging. *Am. J. Enol. Vitic.*, **47**, 1.

VIVAS N., BOURGEOIS G., VITRY C., DE FREITAS V. and GLORIES Y., 1996. Study on the composition of commercial tannins extracts by liquid secondary ion mass spectrometry (LSIMS). *J. Sci. Food Agric.* (accepted for publication).