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Extraction of ellagitannins from oak wood of model casks

by

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S u m m a r y : Eight experimental model casks were constructed using wood from four oak trees and filled with a 12 % ethanol solution for 200 days. The concentration of ellagitannins was subsequently measured in the solutions and in the inner and outer faces of the cask wood. Only a low proportion of the total ellagitannins was extracted from the wood, and this proportion varied significantly between both different casks and between the eight ellagitannins measured. The two most abundant ellagitannins, castalagin and vescalagin, were the least easily extracted. The concentration of ellagitannins was much lower in the solutions than expected from calculations based on the difference between the inner and outer faces of the wood. This suggests that significant degradation of ellagitannins occurs subsequent to their extraction into solution.

K e y w o r d s : oak wood, ellagitannins, casks, extractives.

Introduction

In the heartwood of European oak wood (*Quercus robur* and *Q. petraea*) ellagitannins make up to 10 % of the dry weight. Eight different ellagitannins have been identified in oak wood (Mayer *et al.* 1967; Nonaka *et al.* 1989, 1990; Hervé du Penhoat *et al.* 1991) with the two most abundant compounds being the stereoisomers, vescalagin and castalagin. The remaining ellagitannins are either dimers of these compounds or differ by the presence of a pentose substituent. In most heartwood samples the majority of these tannins is extractable by aqueous alcoholic solutions. The concentration of vescalagin and castalagin extracted by organic solvents from wood, however, decreases as the age of the heartwood increases (Peng *et al.* 1993). Viriot *et al.* (1994) attributed this to a decline in solubility due to polymerization of tannins during ageing. These insoluble tannins are considered to give rise to the higher level of ellagic acid that is formed in older heartwood if the wood residue is heated with hydrochloric acid.

Recent studies (Klumpers *et al.* 1994; Masson *et al.* 1995; Mosedale *et al.* 1996) have examined the variation in the composition and concentration of ellagitannins extracted from oak chips or powder. These studies have generally emphasised the high yet variable concentration of soluble heartwood ellagitannins. However, the significance of such results to the maturation of wines and spirits in oak casks is uncertain, as other factors, in addition to the extractable concentration of ellagitannnins in the wood, are of importance. Despite the general abundance of soluble ellagitannins in oak wood, the concentration in cask-matured wines is low (Moutounet *et al.* 1989). The low stability of ellagitannins in alcoholic solutions, their possible complexation with other wine constituents such as proteins and the treatment of the wood during cooperage may all explain the low content in wines. The role of wood anatomy has rarely been considered despite speculation on its importance (Singleton 1974; Mosedale 1995) in determining the environment of maturation, the permeability of casks and the availability of extractives.

This paper examines the results of a recent trial using model casks where the concentration of different tannins extracted by a 12 % ethanol solution was compared with subsequent measurement of remaining concentrations of these tannins in the inner and outer faces of the cask wood. Finally the results are placed in context with other studies on ellagitannins and their role in wines.

Materials and methods

Experimental half-litre models (see Figure) were constructed using PVC and two 9 mm thick oak wood discs, to allow the extraction of oak wood constituents from solid wood under conditions similar to those found in full size wine casks. These model casks are fully described by Feuillat *et al.* (1994). For this study 8 model casks were constructed, 2 from the wood of each of 4 trees between 117 and 200 years old, 3 *Q. robur* and one *Q. petraea*. To identify tree species during the autumn we used the morphological criteria described by Grandjean and Sigaud (1987), namely bark shape, branch architecture and peduncle length. The wood used for the construction of the casks was dried but not heated.

Before being used to construct the model casks, the following anatomical properties of each heartwood sample disc were measured:

- Mean ring width and earlywood width in mm;
- The relative infradensity and total porosity. Infradensity was measured following the method described by Polge (1966). Total porosity is a measure of the proportion of empty space in the total volume of the sample and was calculated from the infradensity according to the formulae presented in Siau (1984);
- The frequency of tylosis blocking earlywood vessels was estimated by penetration through samples in the axial direction by light from a fibre optic lamp. The samples were divided into 3 classes: 1 = numerous vessels with no tylosis, 2 = partial tylosis, 3 = tylosis blocking all vessels.

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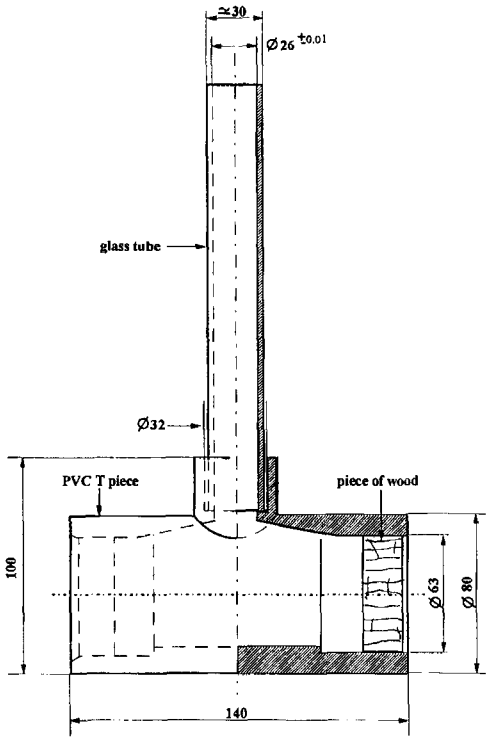

Figure: Experimental half-litre model, developed to allow the extraction of oak wood constituents under conditions similar to those found in full size wine casks (after FEUILLAT *et al.* 1994).

The number per cm and mean width in mm of multiseriate rays crossing the middle of the transversal section (30 mm) of each sample were measured using an image analysis system.

The casks were filled with a 12 % ethanol solution, adjusted to pH 3.4 by tartaric acid, and stored for 200 d at 15 °C and 90 % relative humidity. After storage the solutions were transferred into bottles under air-free conditions and stored in the dark at 4 °C for future analysis. Loss through impregnation and evaporation from model casks was monitored and is described by FEUILLAT *et al.* (1994). At the end of the trial, wood from the internal and external faces of each disc was removed down to a depth of 2 mm. Shavings were ground down to particle sizes of less than 0.5 mm and then extracted in an acetone-water (7:3) solution.

The concentration of a number of phenolic compounds in the 12 % ethanol solutions and in the wood extracts were measured. The concentration of ellagitannins, and the related compounds castalin and vescalin, were measured by HPLC using the method described by MASSON *et al.* (1995). The concentration of ellagic acid was measured by HPLC before and after hydrolysis of the solutions by heating with hydrochloric acid to give the free, total and combined concentrations (PUECH *et al.* 1990). Total phenols were measured by the Folin-Ciocalteau method (SINGLETON and ROSSI 1965).

Results and discussion

Greater losses, due to impregnation of the wood and evaporation, were observed for the experimental models than were found in true wine casks. Losses due to impregnation averaged 7.85 cm^3 while 32.1 cm^3 was lost due to evaporation, representing almost 10 % of the solution volume. The measurements of impregnation suggest that extraction is theoretically possible up to a depth of 5 mm into the wood.

Ellagitannin extraction: Tab. 1 shows the mean concentration of each compound extracted from the internal and external faces of the wood and in the 12 % ethanol solution at the end of the 200-day trial. The coefficients of variance indicate that the concentration of most compounds varied more among the ethanol solutions stored in different models than between extracts from either the inner or outer faces of the oak discs. The relative difference in concentration of each ellagitannin between the internal and external faces was also calculated. If one assumes that the concentration of extractives was homogenous throughout the oak discs, before exposure to the model solution, this value may be treated as the percentage of each ellagitannin extracted from the inner cask wood during the 200-day experiment.

A two-way analysis of variance on the percentage data confirmed that they varied not only between different experimental models but also between the 8 ellagitannins.

The difference of 25 % in total ellagitannin concentration between the internal and the external faces of the wood discs is similar to the difference in ethanol-soluble phenols extracted from the inner face of American oak staves used for wine maturation (HENDERSON, in SINGLETON 1995). While a 12 % ethanol solution is less efficient than the acetone-water solution used to extract remaining ellagitannins, unpublished results indicate that the acetone solution is at most only 50 % more efficient in extracting soluble phenols from oak chips. Therefore, the main reason for the low percentage of soluble ellagitannins extracted from the wood by the weak ethanol solution is the slower extraction taking place from a solid wood surface compared to the extraction from wood powder. The significant difference between casks in the percentage of tannins extracted suggests that wood structure influences extraction. However, no distinct correlations were found

Table 1

Mean concentrations (mg/g) of compounds extracted from the external and the internal faces of the wood from 8 experimental models, the concentration (mg/l) in cask solutions and percentage coefficients of variance (*%CV*). NA: not measured

Compound	External	*%CV*	Internal	*%CV*	Solution	*%CV*
Roburin A	1.44	*45.67*	0.89	*50.85*	4.49	*51.60*
Roburin B	2.85	*28.77*	1.88	*29.74*	5.58	*66.43*
Roburin C	2.89	*24.96*	1.87	*24.30*	4.52	*70.50*
Grandinin	4.18	*27.67*	2.83	*34.71*	6.44	*56.47*
Roburin D	4.44	*24.40*	3.10	*30.61*	11.31	*65.06*
Vescalagin	6.67	*68.21*	5.06	*68.33*	15.10	*69.60*
Roburin E	5.11	*29.84*	3.49	*37.69*	9.12	*36.24*
Castalagin	12.96	*33.09*	10.84	*39.06*	21.55	*48.93*
Total ellagitannins	40.54	*31.44*	29.96	*37.07*	78.12	*43.73*
Vescalin	0.74	*50.51*	0.49	*49.72*	5.05	*97.9*
Castalin	0.61	*55.45*	0.43	*55.43*	. 2.99	*78.45.*
Ellagic acid (comb)	29.89	*19.17*	24.23	*18.06*	. *NA*	*NA.*
Ellagic acid (free)	3.91	*24.91*	4.75	*31.68*	*NA*	*NA*
Ellagic acid (total)	33.80	*16.62*	28.98	*17.07*	. *NA*	*NA.*

between the percentage of total ellagitannins extracted and anatomical properties, despite wide variations of these characteristics, e.g. density varying from 475 to 640 kg/m^3.

The proportion of ellagitannins extracted from the wood also varied significantly between individual tannins. Tab. 2 shows that vescalagin and castalagin were the least extractable of the ellagitannins. These results support those of previous studies which have examined the kinetics of ellagitannin extraction by using a flow-through reactor which minimized re-polymerization, condensation and post-hydrolysis reactions (PUECH *et al.* 1994 a, b). The extraction of ellagitannins using this method followed a hyperbolic curve over time. Studies of the extraction from oak wood chips using white wine were carried out at both, 130 and 25 °C. At the higher temperature 2.18 times more vescalagin and castalagin was extracted, compared to 1.33 - 1.73 times more of the other ellagitannins (PUECH *et al.* 1994 b). Summarized, these results suggest that either the molecules of castalagin and vescalagin are less accessible or extractable than the other ellagitannins. MASSON *et al.* (1994) found that while the concentration of ellagitannins varied between different wood tissues, the composition remained relatively constant. Therefore, it is unlikely that the lower amounts of vescalagin and castalagin extracted from the wood can be explained by differences in their accessibility to the aqueous ethanol solution. Alternatively, vescalagin and castalagin may be more closely associated with cell wall components and therefore less easily extracted into solution.

While less vescalagin and castalagin was extracted from the wood, both compounds were still the dominant ellagitannins in both, the internal face and the model wine solution. If the concentration in the model wine solution is determined solely by the amount extracted from the wood, one would expect it to be closely correlated with the absolute difference in concentration between the inner and outer wood surfaces. However, there is at best only a weak correlation between the two sets of results.

The amount of wood (W in g) corresponding to the 2 mm exposed to the solution can be calculated from the surface area (S) of the two discs and the density at 15 % moisture content (D_{15}) calculated from the infradensity as described by JOLY and MORE-CHEVALIER (1980):

$$W = 2\ S\ D_{15}$$

The amount of ellagitannins extracted into solution (E in mg/l) can subsequently be estimated from the volume of ethanol solution V at the end of extraction (0.39 l)

Table 2

Percentage of ellagitannins extracted from the wood of 8 casks. Means, standard errors and least square groupings after analysis of variance

Ellagitannin	Mean (n=8)	Standard error	LSD - grouping			
Roburin A	39.1 %	2.33	1			
Roburin C	34.4 %	3.34	1			
Roburin B	33.3 %	2.89	1	2		
Grandinin	32.8 %	3.44		2		
Roburin E	32.5 %	3.19		2		
Roburin D	30.2 %	3.42		2		
Vescalagin	23.5 %	1.44			3	
Castalagin	17.2 %	3.37				4

and the difference (o-i) of total ellagitannins concentrations in the outer (o) and inner (i) surfaces of the wood disc:

$$E = W\ (o\text{-}i)\ /\ V$$

The results of these calculations are given in Tab. 3. Comparing the calculated values with the observed concentration in solution, one finds that the observed concentration of ellagitannins is between 2 and 8 times lower than expected. Furthermore, these calculations, which take into account only the first 2 mm of cask wood, underestimate the amount of ellagitannins extracted. Therefore, these results suggest that the depletion of ellagitannins in the internal face of the wood is not simply due to extraction and/or that the ellagitannins have degraded or reacted in solution after extraction.

Table 3

The concentration and difference (mg/g) in total ellagitannins extracted from the inner and outer surfaces of wood samples, the concentration (mg/l) in cask solutions and the expected concentration (mg/l) derived from measurements of wood infradensity (kg/m^3) and the difference between inner and outer faces

Tree*	[Outer]	[Inner]	Difference (o - i)	[Solution]	Infra-density (I)	[Expected]
Q.r 1 a	50.49	36.23	14.26	**103.91**	529	**303**
Q.r 1 b	54.30	43.11	11.19	**102.20**	471	**210**
Q.p 1 a	33.05	21.60	11.45	**52.17**	614	**287**
Q.p 1 b	33.69	20.29	13.41	**25.28**	573	**311**
Q.p 2 a	26.89	22.25	4.64	**55.89**	534	**100**
Q.p 2 b	30.47	23.23	7.24	**103.35**	616	**182**
Q.p 3 a	61.02	48.69	12.33	**122.63**	640	**323**
Q.p 3 b	34.37	24.28	10.09	**59.50**	575	**234**

*** Q.r: *Quercus robur*; Q.p: *Quercus petraea*; 1, 2, 3: trees; a, b: staves.**

Other compounds: In one half of the samples the level of free ellagic acid was considerably higher in the internal than in the external face (Tab. 1). The total ellagic acid content, after the hydrolysis of ellagitannins, showed, as would be expected, a pattern similar to that found for the ellagitannins, although the difference between the two faces was less pronounced. The higher content of ellagic acid in the internal faces of many samples is most easily explained by the hydrolytic breakdown of ellagitannins, or related compounds, at the solution-wood interface, with not all of the relatively insoluble ellagic acid being immediately extracted into solution. This same effect may at least partly explain the low concentrations of ellagitannins found in the ethanol solutions.

Vescalin and castalin are also products of the hydrolysis of ellagitannins. However, both showed similar results to those of the ellagitannins; the low concentration in the ethanol solutions and extracts from the inner face of the wood does not suggest that significant hydrolysis of ellagitannins has taken place.

Conclusions

The results reported emphasize the complexity of the cask environment. The experimental models approach the conditions of maturation more closely than extractions from powder or chips and allow better replication and monitoring of conditions than is possible with full-size

cask trials. The study was carried out with unheated wood and therefore does not account for the effects of heating staves which in many circumstances is likely to be the main factor explaining the low concentration of ellagitannins in wines (MOUTOUNET *et al.* 1995). The results indicate that only a proportion of the potentially extractable tannins is released into the solution and that this proportion differs both, between wood samples and between different ellagitannins. This is most likely explained by the varying permeability of wood and the varying solubility of ellagitannins.

The concentration of ellagitannins in the model wine solution was lower than expected, this suggests that additional reactions occur after extraction or that there is transformation of ellagitannins in the inner face of the wood without extraction into the solution.

The use of a simple 12 % ethanol solution instead of wine prevents reactions between the tannins and wine constituents, such as complexation with proteins. It has been suggested that ellagitannins may be more stable in a real wine than in the ethanol solution as other wine components may oxidize more readily than the ellagitannins (MOUTOUNET *et al.* 1992; SINGLETON 1995). However, VIVAS and GLORIES (1996) recently proposed that ellagitannins act as a buffer against oxidation of other wine constitutents, during the ageing process of wines, due to their more rapid and easy oxidation. The experimental models described here, despite the greater loss of solution due to evaporation, allow less penetration into the solution by oxygen than occurs in a full size wine cask. The lack of elevated levels of vescalin or castalin in either the inner wood face or the solution does not suggest that significant hydrolysis has occurred unless the products have undergone subsequent reactions. Whatever the cause the results indicate that even in the relatively simple environment of the model casks there is no simple relation between the ellagitannin content of the wood and the resulting concentration in solution.

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