IDENTIFICATION AND QUANTIFICATION OF *C*-GLUCOSIDIC ELLAGITANNINS AND THEIR DERIVATIVE IN RED WINE AGED IN OAK BARRELS[•]

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1. INTRODUCTION

The *C*-glycosidic ellagitannins constitute a subclass of hydrolyzable tannins, whose present remarkable structural diversity, today over 500 members of this family of gallic acid-derived polyphenolic natural products have been isolated from various plants and fully characterized (Okuda *et al.*, 1995; Okuda, 2005; Quideau, Feldman, 1996). Castalagin and its C-1 epimer vescalagin are the first *C*-glycosidic ellagitannins that have been isolated and characterized after their isolation thirty years ago in *Castanea* (chestnut) and *Quercus* (oak) woody species from the *Fagaceae* family (Mayer *et al.*, 1976). Six other NHTP-containing *C*-glycosidic ellagitannins were later isolated from fagaceous *Quercus* and *Castanea* hardwood species, *i.e.*, the dimers roburins A and D and the lyxose/xylose-bearing monomers grandinin and roburin E and dimers roburins B and C (Hervé du Penhoat *et al.*, 1991). Among these eight typical *C*-glycosidic ellagitannins, vescalagin and castalagin largely predominate in the fagaceous woody species containing them, representing for example between 40 % and about 60 % by weight of this group of *C*-glycosidic ellagitannins in *Quercus petraea* and *robur* heartwoods (Fernández de Simón *et al.*, 1999). The presence of *C*-glycosidic ellagitannins in a large variety of beverages such as wines and spirits results from their presence in oak heartwood used to make barrels in which those beverages are stored or aged. During this storage or aging periods, the contact between the wood and theses hydro-alcoholic beverages (~12 % of alcohol for wine) results in an extraction of the *C*-glycosidic ellagitannins presented in the wood as well as some others small phenols such as vanillin. Once in the wine, the *C*-glycosidic ellagitannins are slowly but continuously transformed through condensation, hydrolysis, and oxidation reactions.

Moreover, the *C*-glycosidic ellagitannins sub-class also includes so-called complex tannins also known as flavano-ellagitannins which are hybrid tannins with a *C*-glycosidic ellagitannin moieties derived such as vescalagin, and a flavan-3-ol moieties such as catechin or epicatechin. In these complex tannins, both moieties are connected via *C–C* linkage between the carbon-1 of the *C*-glucosidic ellagitannin moieties and either the

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carbon-8 or carbon-6 of the A ring of the flavan-3-ol unit. Acutissimin A and B are the first catechin/vescalagin-based complex tannins that have been isolated and characterized in the bark of *Quercus acutissima* (Ishimaru *et al.*, 1987). Therefore, the formation of flavano-ellagitannins in red wines aged in oak barrels could be expected since these red wines contained flavan-3-ol (deriving from grape seed and skin) and *C*-glycosidic ellagitannins (extracted from oak wood).

In order to answer this assumption, we first achieved the hemisynthesis of flavano-ellagitannins in a very high yield between vescalagin and flavan-3-ols (catechin/epicatechin) in organic acidic media as well as in a wine model solution. Then using the flavano-ellagitannins obtained by hemisynthesis as standard, we developed a new efficient detection and quantification procedure for the *C*-glycosidic ellagitannins as well as flavano-ellagitannins in order to monitor their extractions, evolutions and formations during different wine ageing conditions (oak barrels or stainless steel vats with oak chips) wine ageing.

2. MATERIALS AND METHODS.

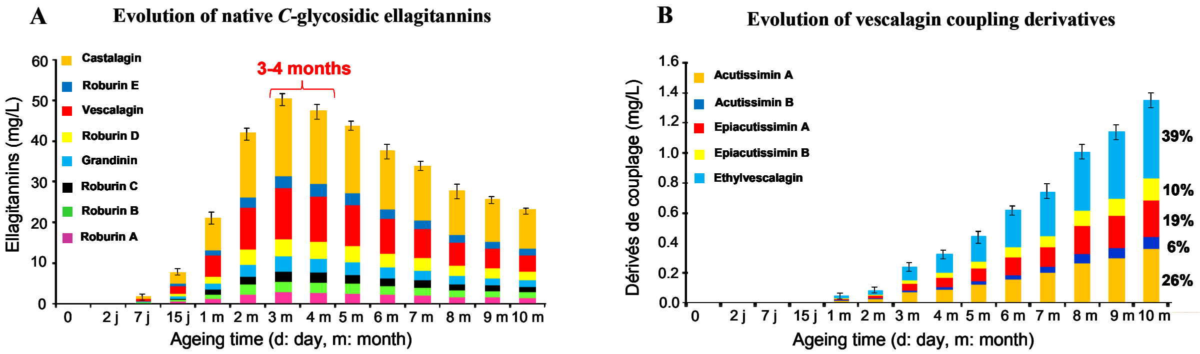
The hemisynthesis of the flavano-ellagitannins (acutissimin A/B and epiacutissimin A/B) as well as the β-1-*O*-ethylvescalagin were achieved in a very high yield between vescalagin and catechin, epicatechin and ethanol respectively, in acidic media (1.5 % v/v TFA/THF) at 60 °C over a period of 7 h (Quideau *et al.*, 2003; 2005).

The detection and quantification of the native *C*-glycosidic ellagitannins as well as the vescalagin coupling derivative (flavano-ellagitannins and β-1-*O*-ethylvescalagin) were performed by HPLC-UV-MS. Several wines obtained from different grape types ('Merlot', 'Cabernet sauvignon') and with different ageing conditions (oak barrels or stainless steel vats with oak chips) were used during this study. In order to perform a proper quantification of these five vescalagin derivatives and of the native *C*-glycosidic ellagitannins, calibration curves were established using the pure flavano-ellagitannins as well as β-1-*O*-ethylvescalagin obtained by hemisynthesis and with chlorogenic acid as internal standard (Saucier *et al*., 2006).

3. RESULTS AND DISCUSSION

Taken together the fact that wine aged in the oak barrels extracts *C*-glycosidic ellagitannins as well as the fact that wine (especially red wine) contains a large amount of flavan-3-ols such as catechin and epicatechin with a concentration about 115 to 190 mg L^{-1} and 80 mg L^{-1}, respectively (Cabanis *et al.*, 1998; Carando, Teissedre, 1999), the presence in red wine aged in oak barrel of acutissimins A and B, of epiacutissimins A and B as well as of β-1-*O*-ethylvescalagin was investigated since from a chemical perspective wine can simply be considered as a slightly acidic hydro-alcoholic solution (i.e., ~12 % of alcohol and pH~3–4).

It appears that during wine ageing (fig. 1), the concentration in native *C*-glycosidic ellagitannins increased regularly to reach its maximum at 3 to 4 months if aged in oak barrels and 1.5 to 2 months if aged in stainless steel vats with oak chips (data not shown). Then, in both cases, these concentrations slightly decreased with time. Moreover, during the first several months, the *C*-glycosidic ellagitannins composition (ratio between each native *C*-glycosidic ellagitannins) was similar to the *C*-glycosidic ellagitannins composition observed in oak wood used (barrels or chips). Thus, during these first months the composition of native *C*-glycosidic ellagitannins is influenced only by their extraction from oak by the red wine. Then, after several months following the decrease of the concentration of native *C*-glycosidic ellagitannins, the composition of the native *C*-glycosidic ellagitannins changes with time. For example the concentration of vescalagin diminishes faster than the concentration of castalagin. This behavior difference is mainly due to the specific reactivity of vescalagin in wine and especially to the formation of the flavano-ellagitannins (acutissimins and epiacutissimins) as well as β-1-*O*-ethylvescalagin since the concentration of these vescalagin-coupling derivatives were detectable even after 3-4 months of ageing. Then the concentration of these vescalagin-coupling derivatives increased regularly during the studied wine ageing period.



A Evolution of native *C*-glycosidic ellagitannins

B Evolution of vescalagin coupling derivatives

Fig. 1 - Evolution of native *C*-glycosidic ellagitannins concentration (A) and of the vescalagin-coupling derivative concentration (B) in a red wine aged in oak barrels.

4. CONCLUSIONS

Using our knowledge regarding the efficient hemisynthesis of the flavano-ellagitannins (acutissimins and epiacutissimins) in organic and in wine model solutions as well the hemisynthesis of the β-1-*O*-ethylvescalagin, we were able to monitor the evolution of the native *C*-glycosidic ellagitannins in a red wine aged in oak barrels or in stainless steel vats with oak chips. In our wine ageing conditions, the concentration in native *C*-glycosidic ellagitannins increased regularly to reach its maximum at 3 to 4 months and then slowly decreased during the rest of the ageing in barrels. During this study we were also able to monitor for the first time the formation and accumulation of some vescalagin-coupling derivatives such as the flavano-ellagitannins (acutissimins and epiacutissimins) and β-1-*O*-ethylvescalagin during red wine ageing.

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Abstract

The *C*-glycosidic ellagitannins constitute a subclass of hydrolyzable tannins of remarkable structural diversity. In this work we first achieved the hemisynthesis of flavano-ellagitannins, then we used them to develop a new efficient detection and quantification procedure for the *C*-glycosidic ellagitannins as well as flavano-ellagitannins. The presence in red wine aged in oak barrel of acutissimins A and B, of epiacutissimins A and B as well as of β-1-*O*-ethylvescalagin was investigated. We were able to monitor for the first time the formation and accumulation of these compounds during wine ageing. The concentration of total ellagitannins first increased then decreased, the single compounds showing specific changes during ageing.

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