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Ellagitannins quantification in oak wood and cognac *eaux-de-vie*

Sourced from the research article "Validation of a Mass Spectrometry Method to Identify and Quantify Ellagitannins in Oak Wood and Cognac during Aging in Oak Barrels." (Food Chem., 2020)[1].

>>> Ellagitannins are the main extractible oak wood phenolic compounds. These compounds are responsible for high wood durability and they can contribute to wine and spirits organoleptic quality (colour, astringency and bitterness). Despite their importance, their presence and forms in distilled spirits are not well known. Thus, the aim of this study was to develop and validate a measurement methodology and to quantify Cognac oak wood ellagitannins. <<<

Due to their properties, oak barrels have been an integral part of wine and spirit production for a long time. They provide good thermal insulation, have remarkable impermeability, and mainly contribute to the organoleptic quality of wines and spirits. During aging in oak barrels, the composition of wines and spirits changes because of the release of wood compounds, which affect its organoleptic properties, such as aroma and colour, and even sensation and taste, such as astringency and bitterness[2]. Among these compounds, ellagitannins (hydrolysable tannins) are responsible for the high durability[3] of wood, they exhibit antioxidant activity[4] and have an impact on bitterness and sensation[5] of astringency.

Up to now, eight ellagitannins have been identified (Figure 1). These compounds have been widely studied in red wine[6], but very seldom in spirits, especially Cognac "eaux-de-vie". Due to their high reactivity and structural similarities, it is difficult to detect and quantify ellagitannins in spirits. Therefore, the aim of our study was to identify and quantify these compounds in oak wood and distilled spirits, using a rapid method and to validate it.

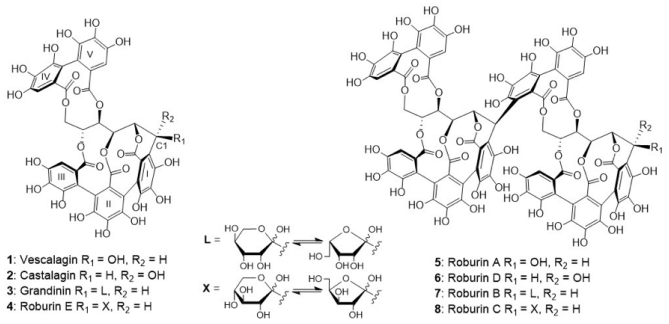

Figure 1. Chemical structures of ellagitannins 1-8. L and X correspond, to Lyxose and Xylose respectively (adapted from Gadrat *et al.*)[1].

■ Ellagitannin quantification method

The challenge of this work was to develop a simple and reproductible method to quantitate individual ellagitannins in oak wood and eau-de-vie with high selectivity. The high performance liquid chromatography-triple quadrupole LC-QQQ combination appeared to be a powerful technique for phenolic compounds[1].

To characterise ellagitannins in oak wood and Cognac eaux-de-vie, a mass spectrometry method was established. The analysis of the oak wood was carried out on stave samples stored outside for 36 months. For the Cognac "eaux-de-vie" analysis, 20 samples of eaux de vie which had aged for 6 months in oak barrels were used.

All concentrations were expressed in vescalagin equivalent. The quantification of individual ellagitanins was performed in the negative mode and they were identified from the following masses: (-)-vescalagin and (-)-castalagin, monomers and isomers with an identical m/z ratio of 933.0634 ($C_{41}H_{25}O_{26}^-$); (-)-grandinin and (-)-roburin E, glycosylated monomers with similar structures and an identical m/z ratio of 1065.1057 ($C_{46}H_{33}O_{30}^-$); likewise, roburin A and D, two dimers with an identical m/z ratio of 1849.1241 ($C_{82}H_{49}O_{51}^-$), but being bi-charged molecules, they are more easily observable in mass spectrometry at a m/z ratio of 924.5620; finally, roburin B and C, two glycosylated dimers with an identical m/z ratio of 1981.1664 ($C_{87}H_{57}O_{55}^-$), but like roburin A and D, they are bi-charged molecules, so they are more observable at a m/z ratio of 990.5831.

Observations were made for the eight oak wood ellagitannins in all samples, and all the concentrations were measured using the vescalagin calibration curve. Figure 2 shows the eight ellagitannins chromatograms according to their form and their retention time found in analysed oak wood extracts (Figure 2a) and in Cognac eau-de-vie (Figure 2b). The quantitation method was validated[1] in terms of LOD, LOQ, sensitivity, linearity in working range, intraday repeatability and intraday precision. To determine intraday precision, five replicates of two intermediate concentrations (2 mg/L and 20 mg/L) of the calibration curve were injected successively. Intraday repeatability (RSD) was < 2 % to 2 mg/L and < 1 % to 20 mg/L, thus ensuring a good repeatability of the method.

■ Ellagitannin determination in oak wood samples

To assay ellagitannins in oak wood, 9 staves from different positions on a wooden pallet were studied: 3 samples at the bottom, 3 in the middle and 3 at the top of the pallet[1]. All concentrations were expressed as milligrams of vescalagin equivalent per grams of wood on the basis of the dilution factor. Total individual ellagitannin concentrations ranged from 10.37 to 18.77 mg/g of vescalagin equivalents.

ANOVA statistical analysis showed that ellagitannin content depended on the wood position on the pallet. At $p < 0.05$,

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the content of total ellagitanins is significantly different according the stave position inside the pallet: the content increase from the top (11,27 mg/g of wood in vescalagin equivalent) to the bottom of the pallet (16.72 mg/g of wood in vescalagin equivalent) (Figure 3). This was also the case for castalagin and roburin D. For the other individual ellagitanins the differences observed were either less or not significant. This preliminary study helped to optimise the wood piece sampling to achieve good representativeness of ellagitannin concentrations in oak wood.

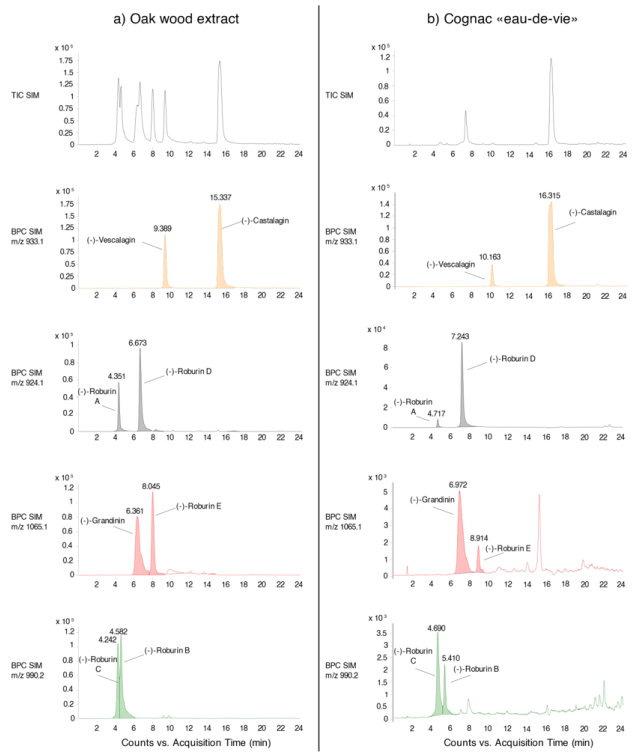

Figure 2. Negative LC-QQQ of a) oak wood extract and b) Cognac eau-de-vie corresponding to [M – H]- ions of ellagitannins (adapted from Gadrat *et al.* 2020[1]).

■ Ellagitannin determination in Eaux-de-vie samples

The method developed in this study was also applied to quantitate ellagitannins in Cognac. Twenty samples of young Cognac "eaux-de-vie" aged in barrels were analysed using four replicates for each "eau-de-vie". Ellagitannins were detected in all samples.

The results showed that young "eaux-de-vie" contained all eight ellagitannins. However, each individual ellagitannin had its own extraction pattern. Castalagin comprised around 40 to 70 % of the total ellagitannins extracted. This monomer is the majority ellagitannin in oak wood and is more stable than its isomer, vescalagin, due to the position of the hydroxyl on the carbon 1. The roburin D dimer and grandinin glycosylated monomer are the most extractible ellagitannins after castalagin. In general, the total ellagitannin concentration ranged from 1.9 to 9.3 mg/L in vescalagin equivalents. As individual ellagitannin concentrations in cognac had never been analysed before this study, the values observed here are the first to be reported for Cognac spirits. In brandies, the sum of vescalagin and castalagin has been reported to be between 9 to 12 mg/L of ellagitannins in gallic acid equivalents[7]. In a red French Cabernet Sauvignon wine, the sum of individual ellagitannin concentrations ranged from 0.6 to 15.5 mg/L in castalagin equivalents for six and twelve months of aging in oak barrels respectively.

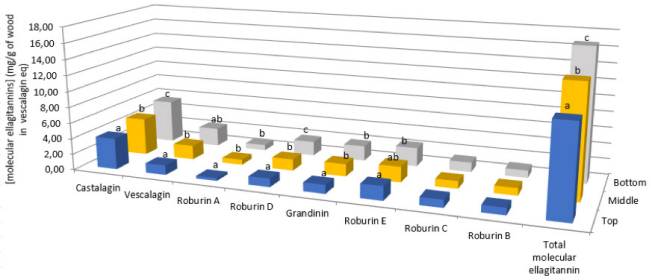

Figure 3. Content of the eight main ellagitannins of wood taken from staves at three different levels (low, middle, high) in the pallet; a-c shows the significant differences among the different positions ($p < 0.05$) (adapted from Gadrat *et al.*, 2020[1]).

Italian and American Cabernet Sauvignon wines presented a sum of 0.5 to 5.76 mg/L and 0.6 to 12.4 mg/L in castalagin equivalents after between six and twelve months of aging respectively. As in red wine, castalagin is the most abundant component in "eau-de-vie" and represents 68 to 79 % of the total ellagitannin fraction[8].

■ Conclusion

For the first time, oak ellagitannins in Cognac "eaux-de-vie" have been quantified with precision using a rapid and accurate spectrometry method. This validated method was successfully applied to the detection and quantification of the eight main oak ellagitannins in different samples of oak wood and "eaux-de-vie". ■

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