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Preliminary study on the evolution of oak barrels *C*-glucosidic ellagitannins in wine model solution

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ABSTRACT

Oak wood has a significant impact on the chemical composition of wine, influencing its organoleptic properties such as aroma, structure, astringency, bitterness, and colour. Among the key extractable non-volatile polyphenols are ellagitannins, which can undergo oxidation and condensation reactions due to their multiple ortho-hydroxyl groups. Although these compounds are extracted from oak into wine, their high reactivity often results in lower concentrations in the final product.

To investigate the compounds transferred from oak to wine, a model wine solution was aged for one year in two barrels with different toasting levels: a light-plus toasting barrel (LT+AAS) and a medium-plus toasting barrel (MT+AAS). Samples were collected monthly and analysed by liquid chromatography coupled with mass spectrometry (HPLC-QQQ-MS and HPLC-TOF-MS).

Results showed significant compositional changes over time, including the formation of oxidation and hydrolysis products. Monomeric ellagitannins, such as castalagin, increased sharply within the first eight months – from 29.83 mg/L to 115.98 mg/L in the LT+AAS barrel – while dimer concentrations rose more gradually and stabilised around the sixth month, with roburin D reaching about 15.25 mg/L. Several derivative products of *C*-glucosidic ellagitannins, including whiskey tannins and brandy tannins, were also identified and progressively accumulated during ageing. Differences between barrels suggest that wood toasting level influences the formation of these ellagitannin-derived products. These results provide new insights into the transformation pathways of *C*-glucosidic ellagitannins and the formation of whiskey and brandy tannins during barrel ageing.

KEYWORDS: oak wood, ellagitannins, ageing wine, organoleptic impact

INTRODUCTION

Ageing wine in wood barrels is a long-established practice that plays a crucial role in shaping high-quality wines. Different woods have been used for wine ageing, but oak has become the dominant choice. The robustness, flexibility, and relative impermeability of oak wood explain its widespread use in wine ageing (del Alamo-Sanza & Nevares, 2018).

Oak barrel ageing enhances wine quality by enabling the gradual release of wood-derived compounds into the wine matrix, where they interact and integrate with the wine components in the barrel. These interactions influence colour, aromatic, and gustatory parameters that make wines so distinctive. Among these compounds, ellagitannins, which are hydrolysable tannins, are of particular interest due to their significant reactivity and ability to modify the sensory and chemical profile of wine (Chira & Teissedre, 2013b; Gadrat *et al.*, 2022a). The *C*-glucosidic ellagitannins are a subclass of ellagitannins with a distinct structure, a C-C bond is formed between the carbon C-1 of the open-chain glucose core linked to the 4,6-hexahydroxydiphenoyl (HHDP) unit and the carbon C-2 of the 2,3,5-nonahydroxyterphenoyl (NHTP) unit (Jourdes *et al.*, 2009; Quideau *et al.*, 2005) (Figure S1).

This family of polyphenolic compounds includes molecules such as vescalagin and castalagin, which are found in higher concentrations in oak wood (Puech *et al*., 1999). *C*-glucosidic ellagitannins also exist in more complex forms, including derivatives like grandinin, roburin E, and dimeric roburins (roburins A, D, B and C) (Chira *et al*., 2020; Hervé Du Penhoat *et al.*, 1991). These compounds are involved in oxidation, condensation, and hydrolysis reactions, mainly due to the hydroxyl groups present in ellagitannins, leading to a gradual decrease in their concentrations. In wine, oxidation reactions between ellagitannins and ethanol can occur during barrel ageing. For instance, vescalagin can be oxidised to form β-1-*O*-ethylvescalagin in wine (Quideau *et al*., 2005). Over time, this compound can undergo hydrolysis to produce β-1-*O*-ethylvescalin. In the same matrix, vescalagin and castalagin can respectively give rise to vescalin and castalin by hydrolysis reaction (Figure S2). In previous work about spirits, when toasting takes place, castalagin and vescalagin are oxidised and reduced, respectively (Glabasnia & Hofmann, 2007). In another study, two *C*-glucosidic ellagitannins were isolated and characterised for the first time from French oak wood after heating under laboratory conditions. These two new derivatives were detected with $[M–H]^-$ ion peaks at *m/z* 1055.0631 (compound A) and *m/z* 1011.0756 (compound B) (Chira & Teissedre, 2015). Other new evolution-derived ellagitannins, known as whiskey tannins, have been isolated from Japanese whiskey (Fujieda *et al*., 2008). Whiskey tannin B, which exhibits a quasi-molecular ion with a nominal mass of *m/z* 977 ($[M–H]^-$), is thought to arise from a regioselective oxidation of the pyrogallol ring attached to the C-1 glucose of castalagin, followed by ethanol addition and subsequent cyclisation rearrangement. Whiskey tannin A, with a molecular ion at *m/z* 675 ($[M–H]^-$), is believed to be the hydrolysis product of whiskey tannin B. It results from the loss of the upper HHDP (hexahydroxydiphenoyl) unit, which undergoes bislactonisation to form ellagic acid (Fujieda *et al.*, 2008). Structures and mechanisms are shown in Figure S3.

Several studies have investigated the extraction kinetics of ellagitannins from oak wood into wine or model wine solutions, showing that their release is influenced by multiple factors such as wood species, toasting level, contact time, and storage conditions. In general, ellagitannin extraction follows a rapid initial phase followed by a slower evolution over time.

This trend has been confirmed in both white and red wines, demonstrating this behaviour in white wine over short contact time (Marinov *et al.*, 1997). Over a one-hour extraction time, the kinetics of ellagitannin concentrations followed a square root-type behaviour, consistent with a diffusion-controlled process, with a progressive increase in ellagitannin concentration in the wine over time. Other studies have also reported similar kinetic patterns in red wines over a one-year extraction period (Chira & Teissedre, 2013a; González-Centeno *et al*., 2016). While González-Centeno *et al.* (2016) observed a continuous increase in ellagitannin concentrations over time, Chira and Teissedre (2013a), using a more detailed sampling design, reported a rapid increase up to three months, followed by a stabilisation phase and a slight decrease after nine months.

Additionally, model wine studies have also confirmed this general behaviour, characterised by a fast initial extraction phase followed by a more gradual evolution (Chira & Teissedre, 2013b). In addition to contact time, solution parameters such as temperature, ethanol content, and pH significantly affect both the extraction and evolution of individual ellagitannins (Jordão *et al.*, 2005). Indeed, higher temperatures and ethanol levels enhance extraction, whereas pH mainly affects their stability by promoting hydrolysis and transformation reactions over time. These findings highlight the complexity of ellagitannin behaviour in wine matrices and the value of controlled model systems to better isolate and understand these effects.

Moreover, differences in extractability related to wood botanical origin and toasting level have been widely documented (García-Estévez *et al*., 2015; González-Centeno *et al.*, 2016; González-Centeno *et al.*, 2017; González-Centeno *et al.*, 2019; Michel *et al.*, 2011), reinforcing the key role of these factors in wood-wine interactions. However, despite these advances, the combined effect of toasting intensity on both the extraction kinetics and the transformation pathways of individual ellagitannins over extended ageing periods remains insufficiently characterised.

More recently, the evolution of *C*-glucosidic ellagitannins and their derivatives were analysed over 18 months following their kinetics and chemical transformations in spirits (Fernandes *et al*., 2022; Gadrat *et al*., 2022b). The same research team identified other compounds during ageing, including the well-known ellagitannins mentioned above, as well as some of their derivatives with the discovery of the brandy tannin B, found for the first time in Cognac (Gadrat *et al*., 2022a). The formation mechanisms of these compounds are detailed

in Figure S4. In water/ethanol solutions simulating Cognac conditions, concentrations of castalagin and vescalagin decrease over time. This decrease is more pronounced at 70 % (*v/v*) than at 40 % (*v/v*), especially for vescalagin, which is less stable than its isomer castalagin. The above findings suggest that these compounds are either rapidly degraded or undergo chemical transformations over time.

Wine, like spirits, constitutes a complex matrix, complicating the identification and quantification of ellagitannin derivatives. To overcome this challenge, model wine solutions are often employed as simplified systems, providing a controlled environment. These solutions mimic the hydroalcoholic environment of wine while excluding unwanted factors such as microbial activity and natural wine constituents. Despite numerous studies in different matrices, the fate of ellagitannin derivatives in wine matrices remains underexplored due to analytical complexity (Chira & Teissedre, 2015; Gadrat *et al.*, 2022a; Quideau *et al.*, 2005; Tanaka *et al.*, 2011). Indeed, much of the existing literature emphasises the initial stages of extraction, leaving questions about long-term transformations unanswered (García-Estévez *et al.*, 2015). Thanks to the use of model wine solutions, researchers gain clearer insights for studying the interactions between oak and wine (Quinn & Singleton, 1985). To date, only one study has evaluated the evolution of ellagitannins during 12 months of ageing in a wine model solution contained in oak barrels. This study focused exclusively on the monomeric ellagitannins vescalagin and castalagin (Moutounet *et al.*, 1989).

In this context, the present study aims to provide a detailed investigation of the evolution of *C*-glucosidic ellagitannins and their transformation products in a model wine solution over one year of ageing, with particular emphasis on the influence of oak toasting intensity. By combining kinetic monitoring and compound-specific analysis, this work seeks to improve the understanding of how toasting modulates both the extraction dynamics and the stability of ellagitannins, ultimately contributing to a better control of wine ageing processes.

MATERIALS AND METHODS

1. Chemicals

For sample preparation, analytical reagent-grade ethanol was purchased from VWR International (Pessac, France). The methanol and formic acid used for chromatographic separation were LC-MS grade and purchased from Fisher Chemicals (Strasbourg, France). Sodium hydroxide (NaOH) was also bought from Fischer Chemicals (Strasbourg, France). Trifluoroacetic (TFA) and tartaric acid were acquired from Sigma-Aldrich (Milan, Italy). Ultrapure water (Milli-Q purification system, Millipore, France) was used. For the calibration curve, vescalagin standard was extracted from oak chips (*Quercus robur/Quercus petraea*) and further isolated using semi-preparative chromatography.

2. Isolation of vescalagin by semi-preparative HPLC

Vescalagin purification was adapted from a previously reported method (Chira *et al.*, 2020). Vescalagin was extracted from untoasted oak chips (*Quercus robur/Quercus petraea*), to preserve the initial levels of ellagitannins, using an acetone:water (70:30) solution and purified by semi-preparative HPLC on a Varian 25 system. Chromatographic separation was performed on a Prontosil C18 column (250 × 8 mm, 5 µm, Metrohm, France), with UV detection at 280 nm. The mobile phases consisted of (A) water and (B) methanol, both containing 0.025 % TFA. The flow rate was set at 2 mL/min, and elution followed a gradient program as follows: 1 to 3 % from 1-25 min; 3 to 100 % from 26 to 36 min, 100 % from 37 to 42 min, 100 to 0 % from 43 to 45 min, and 0 % from 46 to 48 min. The injection volume was 200 µL. The data obtained were analysed by LC Open Lab.

3. Experimental design

In order to create a wine model solution that best represented the wine matrix, several parameters were taken into account, including the alcohol content, set at 12.5 % ethanol, and the pH, set at 3.5. Tartaric acid was added to the solution at a concentration of 5 g/L, and the pH was adjusted using sodium hydroxide (NaOH). This solution was then transferred into 225 L oak barrels (*Quercus robur/Quercus petraea*) supplied by Nadalié cooperage (Ludon-Médoc, France). These oak species are commonly used in cooperage and are often blended to achieve specific sensory and compositional profiles. The barrels underwent two different toasting levels: light-plus toasting with double watering (LT+AAS) and medium-plus toasting with double watering (MT+AAS), according to the cooperage specifications. The watering process consists of moistening the inner surface of the barrel, before the end of the toasting process, with water while heat is still applied, enhancing the thermal degradation and leaching of ellagitannins.

Ageing was carried out in a temperature-controlled cellar at 12 °C and a relative humidity of 75 %. For each experimental condition, one first-use barrel was employed. Although no biological replicates were included, this experimental design was intended as a preliminary study to specifically assess the effect of toasting on ellagitannin evolution while minimising variability related to other barrel parameters.

All barrels were produced by the same cooperage from the same oak species and differed only in the toasting protocol. Samples were collected monthly over a one-year period, resulting in twelve samples per barrel.

Details of temperatures and toasting times are presented in Table 1.

4. Quantification of ellagitannins

To investigate the evolution of the eight oak-derived *C*-glucosidic ellagitannins over 12 months of ageing and to monitor the formation of their derived forms quantification was conducted in vescalagin equivalents. The previous step

TABLE 1. Toasting conditions applied to the oak barrels used in this study.

Toasting name	Notation	Toasting time (min)	Toasting temperature (°C)
Light-plus toasting with watering	LT+AAS	56	52 ± 3
Medium-plus toasting with watering	MT+AAS	68	62 ± 3

of vescalagin purification enabled the preparation of a stock solution at a concentration of 1 g/L, using a solvent mixture of 50 % methanol and 50 % water, containing 1 % formic acid. A range of calibration was prepared by successive dilutions of this stock solution in the same solvent to supply calibration samples (0.1 to 100 mg/L). Detection of vescalagin was based on the theoretical exact mass of the most intense ion, the fragment ion at *m/z* 933.1, and its retention time at 13.9 min. Based on the studies aforementioned, a reference list of compounds was established, including their retention times, molecular ions, and characteristic fragmentations. This compilation, presented in Table S1, served as the analytical foundation for the targeted search of ellagitannin derivatives in our model wine solution.

5. LC-HRMS analyses

The collected samples were injected into HPLC-QQQ (Liquid Chromatography coupled with Triple Quadropole Mass Spectrometry) using the chromatographic and spectrometric parameters described in this session. The method was carried out following the methodology described by Gadrat *et al*. (2021), with slight modifications. All concentrations were expressed in mg/L of vescalagin equivalents.

5.1 HPLC-QQQ-MS

5.1.1 High Performance Liquid Chromatography (HPLC)

High Performance Liquid chromatography (HPLC) separations were performed using an Agilent 1200 Infinity series (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector (DAD). The column was a Kinetex column (150 × 3 mm, 2.6 µm particle size, Phenomenex, Le Pecq Cedex, France), used with acidified water at 0.1 % formic acid as eluent A and acidified methanol containing 0.1 % formic acid as eluent B. For HPLC-DAD injections, the flow rate was set at 400 µL/min, and the injection volume was 10 µL. The gradient of solvent B was as follows: 1 to 2 % from 0 to 2 min; 2 to 3 % from 2 to 5 min, 3 to 3.5 % from 5 to 6 min, 3.5 to 4 % from 6 to 7 min, 4 to 4.5 % from 7 to 8 min, 4.5 to 5 % from 8 to 10 min, 5 to 10 % from 10 to 16 min, 10 to 15 % from 16 to 19 min, 15 to 20 % from 19 to 22 min, 20 to 98 % from 22 to 35 min, and 98 % from 35 to 40 min and then the HPLC column was equilibrated for 5 min using the initial conditions before the next injection. The DAD signals were carried out at 280 nm and 250 nm wavelengths.

5.1.2 Triple quadrupole-Mass Spectrometry (QQQ-MS) analyses

For the ellagitannin quantification, a 6460 Triple Quadrupole mass spectrometer equipped with a heated electrospray ionisation probe (both from Agilent Technologies, Waldbronn, Germany), connected to the HPLC system was used. The calibration of the mass analyser was carried out each week using an ESI-L Low Concentration Tuning Mix (Agilent Technologies, Waldbronn, Germany). To perform targeted screening, the ionisation and spectrometric parameters were optimised in negative mode: gas temperature and flow were 350 °C and 5 L/min, respectively; sheath gas temperature and flow were 250 °C and 10 L/min, respectively; capillary voltage was 4500 V and the cell accelerator voltage was 4 V. All data were processed using MassHunter Qualitative Analysis software (Agilent Technologies, Waldbronn, Germany).

5.2 UPLC-TOF-MS

5.2.1 Ultra Performance Liquid Chromatography (UPLC)

The UPLC-TOF-MS system used was an Agilent 1290 Infinity (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump, a thermostatic column compartment, an autosampler module and a diode-array detector. The column was an Eclipse Plus C18 column (2.1 × 100 mm, 1.8 µm). ForUPLC-DAD injections, the flow rate was set at 300 µL/min, and the injection volume was 20 µL. The same solvent system used for HPLC–QQQ was employed, consisting of acidified water with 0.1 % formic acid as solvent A and acidified methanol containing 0.1 % formic acid as solvent B. The gradient of solvent B was as follows: 1 to 3 % from 0 to 5 min; 3 to 8 % from 5 to 7 min, 8 to 12.5 % from 7 to 25 min, 12.5 to 15 % from 25 to 30 min, 15 to 20 % from 30 to 35 min, 20 to 99 % from 35 to 40 min, 99 % from 40 to 42 min and then the UPLC column was equilibrated for 4 min using the initial conditions before the next injection. The DAD signals were carried out at 280 nm and 250 nm wavelengths.

5.2.2 Time-of-Flight Mass Spectrometry (TOF-MS)

The UPLC system was connected to an Electrospray Ionization – Time-of-Flight Mass Spectrometry (ESI-TOF-MS Agilent 6530 Accurate Mass). To perform targeted screening, mass acquisitions were made for 42 min in negative ionisation mode. The method was settled with the following parameters: gas temperature and flow were 300 °C and 9 L/min, respectively; sheath gas temperature and flow were 350 °C and 11 L/min, respectively; capillary voltage was 3500 V; the auxiliary gas at 18, and the sweep gas at 0 (arbitrary units); capillary voltage and the skimmer voltage were set at 3500 and 65 V, respectively. A mass range of 100–3000 amu was acquired in full scan MS mode. The collision energy was adjusted according to the compound, ranging from 15 to 30 eV (or 10–60 % in normalised CE units). Data were processed using MassHunter Qualitative Analysis software (Agilent Technologies, Waldbronn, Germany).

6. Statistical analyses

Statistical analyses were performed using R (version 4.4.3). For each time point and experimental modality, a single barrel sample was analysed in triplicate. Means and standard deviations were calculated from these triplicate measurements using the dplyr package. ANOVA tests were followed by Tukey's HSD post hoc tests, which were applied to determine significant differences between time points and treatments based on analytical replicates ($p < 0.05$). As no biological replication at the barrel level was available, the statistical analyses reflect only analytical variability.

RESULTS AND DISCUSSION

1. Evolution of *C*-glucosidic ellagitannins

As previously described, the kinetics of a wine model solution were monitored over a one-year period to evaluate the extraction potential of oak wood. First, the concentrations of the eight main *C*-glucosidic ellagitannins were determined monthly in both light-plus (LT+AAS) and medium-plus toasting barrels (MT+AAS) (Figures 1 and 2). Monthly sampling and analysis provided a detailed profile of compound evolution throughout the study, with only a minor gap in month 8 due to a sampling issue.

In both barrels, the temporal evolution of ellagitannin concentrations seems to follow general trends. Castalagin appeared to be the predominant compound regardless of the ageing stage, reaching a maximum concentration of 116 mg/L for LT+AAS and approximately 75 mg/L for MT+AAS barrel. These levels exceeded those of vescalagin (17.98 mg/L in LT+AAS) and grandinin (14.13 mg/L in MT+AAS), indicating that castalagin was likely the most abundant ellagitannin measured. These results are consistent with previous findings reported in studies on the evolution of *C*-glucosidic ellagitannins in the Cognac (Gadrat *et al*., 2022b) and wine model solutions matrices (Chira & Teissedre, 2013b; García-Estévez *et al*., 2015; Moutounet *et al.*, 1989). The lower concentration of vescalagin compared to castalagin indicating that castalagin is the more stable of the two isomers (Jourdes *et al.*, 2013). This difference in stability can be explained by its respective chemical structures (Figure S3). Vescalagin is more prone to degradation than castalagin due to the position of its hydroxyl group (Puech *et al.*, 1999; Vivas *et al*., 2004).

Furthermore, these observations align with studies in model wine solutions showing that extraction and transformation kinetics are influenced by both ageing time and barrel toasting, highlighting the importance of these factors in modulating ellagitannin levels during ageing.

The monthly data suggested that the maximum concentration for most compounds was reached between the seventh and ninth months of ageing. However, castalagin and roburin A reached their maxima later in LT+AAS (11 and 10 months, respectively), whereas grandinin reached its maximum earlier in MT+AAS (6 months). The concentrations of monomers (castalagin, vescalagin, grandinin and roburin E) decreased over time, indicating degradation reactions such as oxidation or hydrolysis, whereas the concentrations of dimers (roburins A, B, C, and D) remained stable, reflecting low chemical reactivity under the experimental conditions. Most ellagitannins show significant differences between sampling months ($p < 0.05$), with exceptions for roburin B and C in the LT+AAS barrel, and for grandinin, roburin A and B in the MT+AAS barrel. The results of post hoc analysis are represented by the letters shown on the bar charts. All corresponding statistical data are also reported in Table S2.

Overall, higher concentrations of ellagitannins tended to be measured in the LT+AAS barrel compared to the MT+AAS barrel. This trend was consistent across all identified compounds, indicating that lighter toasting preserves a greater proportion of extractable compounds. For example, vescalagin reached 20.01 mg/L in LT+AAS compared to 11.38 mg/L in MT+AAS at seven months. This enhanced extraction in LT+AAS wood is likely due to both the preservation of ellagitannins and the structural modifications of the wood during toasting. Light toasting induces limited thermal degradation while maintaining the integrity of extractable compounds. In contrast, more intense toasting leads to partial degradation and transformation of ellagitannins, reducing their availability for extraction. Additionally, excessive heat treatment may alter the wood structure and contribute to changes in compound extractability (Chira & Teissedre, 2015; Matricardi & Waterhouse, 1999).

2. Evolution of the ellagitannin derivatives

Mass spectrometry analyses also demonstrated the presence of ellagitannin derivatives in both barrels (Figures 3 and 4).

A substitution product of vescalagin, β-1-*O*-ethylvescalagin, along with two oxidation products, whiskey tannins B1 and B2, and five hydrolysis products, whiskey tannins A1 and A2, castalin, vescalin, and brandy tannin A, were identified.

The evolution profiles of ellagitannin derivatives differed markedly between the two barrels. Indeed, in the LT+AAS barrel, seven derivatives were identified, compared with only six in the MT+AAS barrel (Figures 3 and 4). Moreover, the compounds differed from one barrel to another, indicating that the toasting level influences the evolution and stability of the ellagitannins. Some derivatives, such as β-1-*O*-ethylvescalagin, were detected as early as the first month of ageing. This early appearance is consistent with the immediate availability of ethanol in the model wine solution, promoting adduct formation from the initial stages of ageing. Notably, castalin and vescalin, the hydrolysed forms of castalagin and vescalagin, were also detected from the early months of ageing in both barrels. Conversely, some compounds were only detected at a later stage of the ageing process. Similarly, brandy tannins A appeared at month 9 only in MT+AAS barrels. The lighter toasting in LT+AAS preserves more of the thermolabile monomers, allowing sufficient precursors for hydrolysis, whereas the more intense and prolonged toasting in MT+AAS results in partial

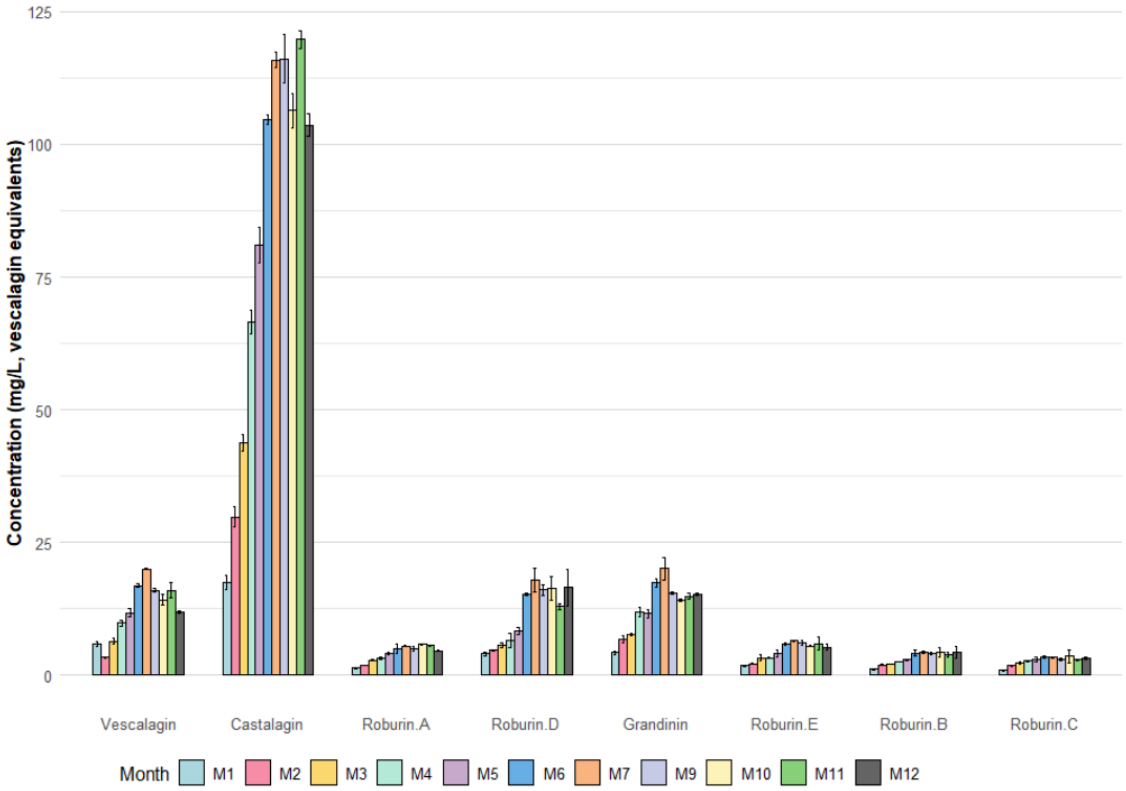


FIGURE 1. Evolution of the concentrations of *C*-glucosidic ellagitannins over a year in light-plus toasting barrel (LT+AAS). Concentrations are expressed as vescalagin equivalents. Error bars represent triplicate analytical determinations (mean ± SD).

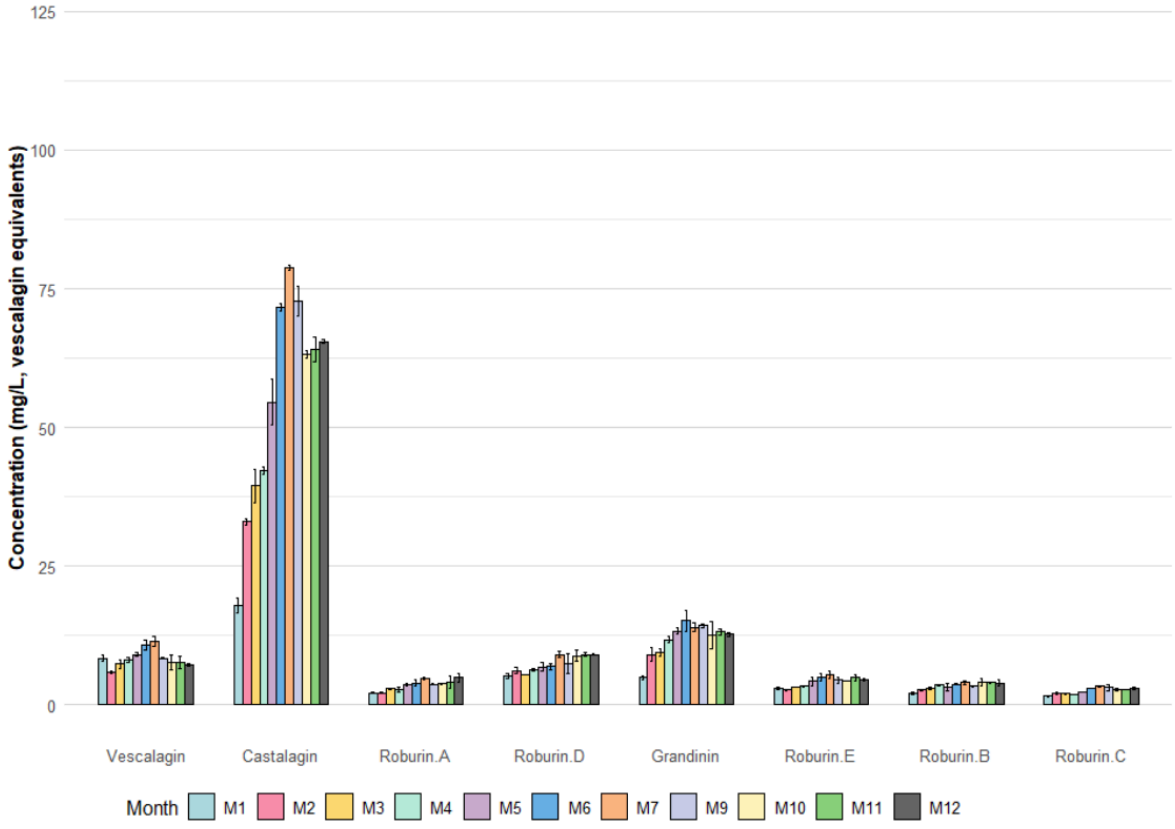


FIGURE 2. Evolution of the concentration of *C*-glucosidic ellagitannins over a year in medium-plus toasting barrel (MT+AAS). Concentrations are expressed as vescalagin equivalents. Error bars represent triplicate analytical determinations (mean ± SD).

degradation of ellagitannins, limiting the formation of these hydrolysed derivatives. Similarly, the delayed appearance of brandy tannin A can be attributed to the slower extraction and transformation kinetics of ellagitannin precursors under higher-temperature toasting conditions.

Oxidative derivatives, namely whiskey tannins B1 and B2, respectively coming from vescalagin and castalagin, were detected exclusively in the LT+AAS barrel. These compounds then underwent hydrolysis to form whiskey tannins A1 and A2, which were present in both barrels. The absence of B1 and B2 in MT+AAS, despite the presence of their hydrolysis products (A1 and A2), suggests either complete hydrolysis or thermal degradation due to the higher toasting level, in agreement with previous reports on ellagitannin thermal lability (González-Centeno *et al*., 2016; Navarro *et al*., 2016). Notably, brandy tannin, an oxidation product of vescalagin found recently in spirits (Gadrat *et al*., 2022a), was detected in the MT+AAS barrel and appeared later than previously reported (month 9 versus rapid formation from the onset in other studies). This divergence likely reflects matrix-dependent formation and highlights the influence of toasting intensity, hydrolysis, and thermal-oxidative transformations on the dynamics of oak-derived ellagitannin derivatives during ageing.

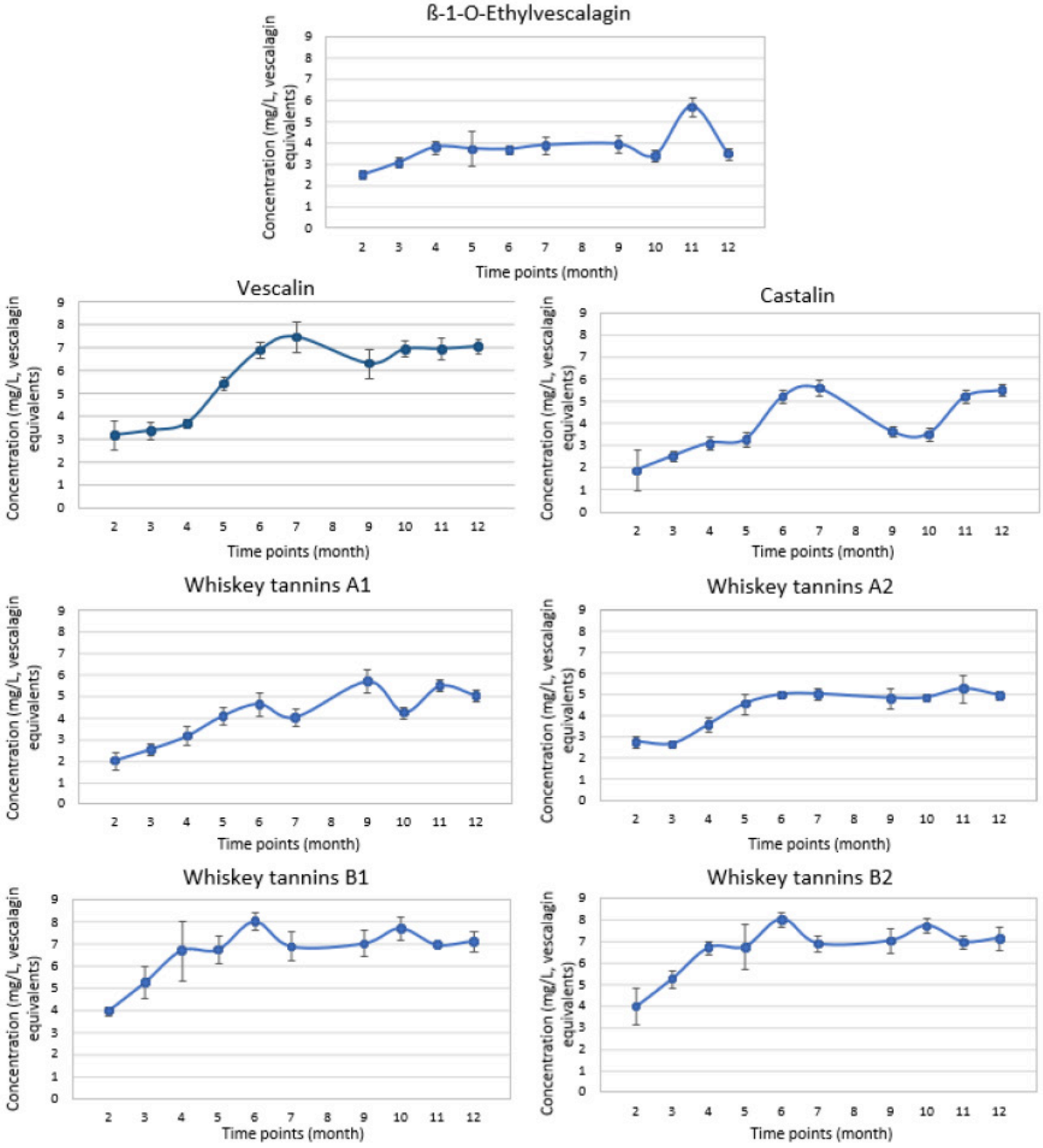


FIGURE 3. Evolution of the concentration of *C*-glucosidic ellagitannin derivatives over the course of a year in light-plus toasting barrels (LT+AAS). Concentrations are expressed as vescalagin equivalents. Error bars represent triplicate analytical determinations (mean ± SD).

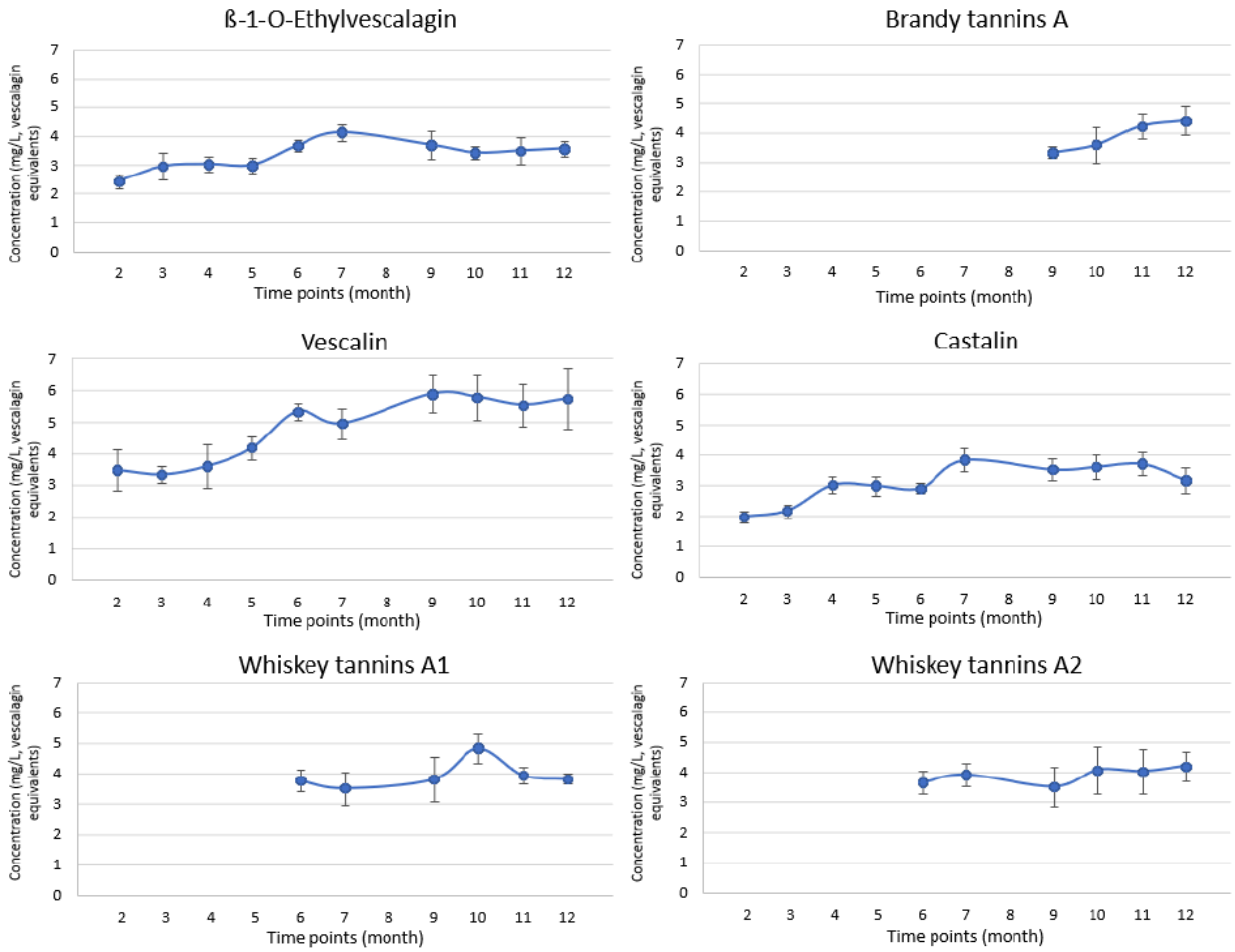


FIGURE 4. Evolution of the concentration of *C*-glucosidic ellagitannin derivatives over a year in medium-plus toasting barrels (MT+AAS). Concentrations are expressed as vescalagin equivalents. Error bars represent triplicate analytical determinations (mean ± SD).

For Figures 3 to 6, the significant differences between months during the ageing period are summarised in Table S2.

To simplify the results and highlight the dominant transformation mechanisms, the identified ellagitannin derivatives were subsequently classified according to their reaction type. Based on the structures and origins of the compounds, the derivatives were grouped into three major reaction pathways: ethanol adduct formation (substitution products, represented by β-1-O-ethylvescalagin), oxidation (including whiskey tannins B1 and B2), and hydrolysis (including vescalin, castalin, whiskey tannins A1 and A2, and brandy tannin A) (Figures 5 and 6).

The cumulative evolution of *C*-glucosidic ellagitannin derivatives over the 12-month ageing period is illustrated by the combined trends of the individual compounds, highlighting the predominance of hydrolysis products in both barrels.

The predominance of hydrolysis products can be explained by the intrinsic reactivity of *C*-glucosidic ellagitannins in a hydroalcoholic and acidic medium. While oxidative derivatives and ethanol adducts require specific conditions, such as sufficient oxygen availability, redox, ethanol content, and pH and occur at lower levels, ester bonds within ellagitannins are readily cleaved, leading to the rapid and sustained accumulation of hydrolysed products such as castalin, vescalin, and whiskey tannins A1 and A2. As a result, hydrolysis represents the dominant transformation pathway during the 12-month ageing period in both barrels.

Moreover, Figure 5 shows the evolution of *C*-glucosidic ellagitannin derivatives in the LT+AAS barrel over 12 months. Hydrolysis products (castalin, vescalin, whiskey tannins A1 and A2) accumulated rapidly and dominated throughout the ageing period. Oxidative products increased between months 1 and 6 before stabilising, while adducts showed a gradual rise but remained consistently lower than the other classes.

Regarding the MT+AAS barrel (Figure 6), oxidative products (whiskey tannins B1 and B2) were entirely absent, whereas their hydrolysis products (A1 and A2) were detected. This pattern suggests either complete conversion of B1 and B2 into A1 and A2 or thermal degradation during the higher-temperature MT+AAS toasting, highlighting the strong influence of toast intensity on ellagitannin transformation pathways.

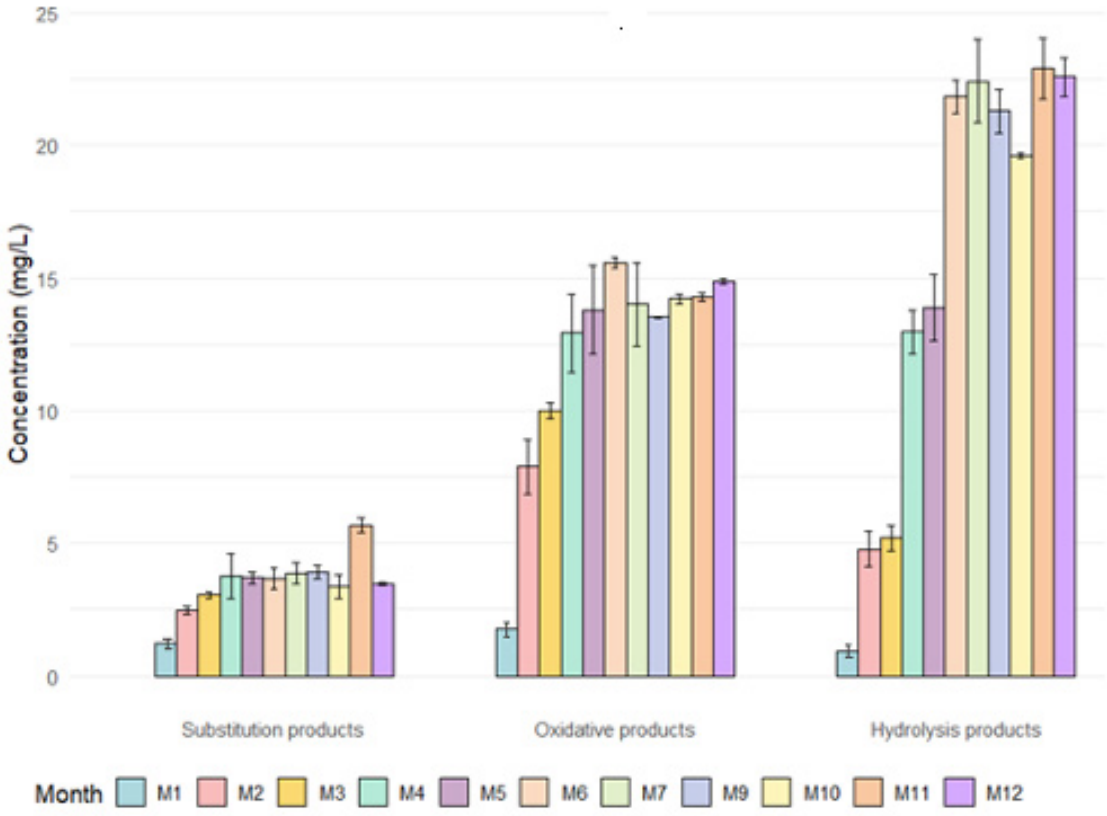


FIGURE 5. Evolution of the concentration of *C*-glucosidic ellagitannin derivatives classified by types of reactions over a year in light-plus toasting barrels (LT+AAS). Concentrations are expressed as vescalagin equivalents. Error bars represent triplicate analytical determinations (mean ± SD).

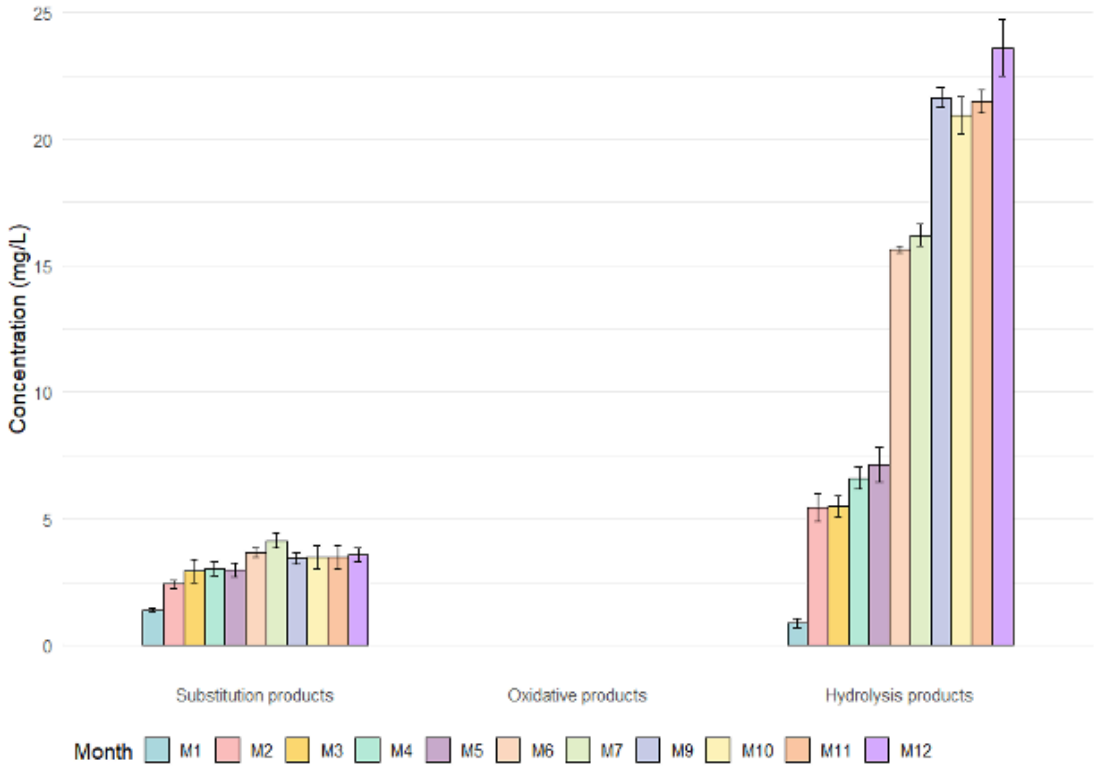


FIGURE 6. Evolution of the concentration of *C*-glucosidic ellagitannin derivatives classified by types of reactions over a year in medium-plus toasting barrels (MT+AAS). Concentrations are expressed as vescalagin equivalents. Error bars represent triplicate analytical determinations (mean ± SD).

3. Comparison of toasting levels

Comparative analysis of LT+AAS and MT+AAS confirmed that lighter toasting resulted in higher concentrations of both ellagitannins and their transformation products. Overall, ellagitannin levels were generally higher in LT+AAS barrels compared to MT+AAS, highlighting the influence of toasting intensity on both extraction and transformation processes, supporting previous observations for *C*-glucosidic ellagitannins (Cadahía *et al*., 2001; Chira *et al.*, 2020; González-Centeno *et al.*, 2016).

Ellagitannins undergo dynamic chemical transformations during ageing. Oxidation and hydrolysis occur at different stages, leading to a balanced distribution of *C*-glucosidic ellagitannins and their derived compounds by the end of ageing (12 months). Detailed concentrations of ellagitannin classes and comparisons between toasting levels are provided in the Table S3. A two-factor ANOVA performed on compound concentrations at month 12 showed significant differences between the classes of compounds, with monomers being logically the most abundant, followed by dimers and hydrolysis products. Post-hoc analysis confirmed that in LT+AAS barrels, the levels of hydrolysis and oxidation products were comparable to those of dimers and glycosylated monomers. Given their concentrations and the known influence of ellagitannins on the organoleptic properties of wines, both primary and derived ellagitannins likely contribute substantially to the wine's sensory profile.

CONCLUSION

This work presents a preliminary investigation into the temporal evolution of *C*-glucosidic ellagitannins and their transformation products in a wine model solution aged in oak barrels with different toasting intensities (LT+AAS and MT+AAS). The present data suggest that lighter-plus toasting (LT+AAS) may favour both the extraction and apparent stability of ellagitannins, including thermally sensitive compounds, compared to medium-plus toasting (MT+AAS). These observations highlight the influence of barrel toasting on the evolution and persistence of ellagitannins during ageing. Whiskey tannins B1 and B2 and their hydrolysis products A1 and A2 were detected in a wine model solution, whereas these compounds had previously only been reported in high-ethanol spirits. This finding suggests that *C*-glucosidic ellagitannins can undergo similar transformation pathways in hydroalcoholic wine-like systems. The relatively high concentrations of hydrolysis products indicate that these derivatives may contribute significantly to the chemical composition and potentially to the organoleptic properties of aged wines.

These findings provide new insight into the transformation pathways of ellagitannins under controlled ageing conditions. Future work will aim to confirm the presence of these derivatives in real wine matrices and to evaluate their potential sensory contribution through targeted tasting trials. Further experiments using food-grade ethanol may also allow direct organoleptic assessment of isolated ellagitannins and their derivatives. Overall, this study highlights the role of barrel toasting in modulating ellagitannin transformations and provides support for further investigation of their sensory relevance in wine ageing.

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