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The presence of sulfonated oligomeric and polymeric procyanidins in red wines impacts the estimated mean degree of polymerisation of condensed tannins by phloroglucinolysis

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ABSTRACT

The calculation of proanthocyanidin mean Degree of Polymerisation (mDP) is a relevant analytical tool for gaining insight into the characteristics of wine. Despite its limitations, it remains an effective method for studying the polymeric forms of proanthocyanidins. Recently, sulfonated monomeric and dimeric flavan-3-ols have been detected in wine, and their main mechanism of formation suggested the formation of even larger sulfonated analogues. This work aimed to explore if aged wines contain sulfonated polymeric proanthocyanidins and how these compounds might impact the calculation of mDP. For this purpose, an experiment based on 4 single cultivar red wines (Sangiovese, Nerello Mascalese, Sagrantino, and Nebbiolo), four levels of SO_2 addition (0, 30, 50, and 100 ppm), and two storage conditions (Control and Room Temperature) was realised. The results showed that the epicatechin 4β-sulfonate released after phloroglucinolysis is 10 to 352 times higher than the free homologue, indicating the presence of oligomeric/polymeric sulfonated procyanidins in wine. Incorporating the quantified terminal unit of epicatechin 4β-sulfonate into mDP calculations resulted in a reduction of mDP values by 1 to 36 %. This overestimation of mDP values increases with wine ageing or storage at elevated temperatures.

KEYWORDS: proanthocyanidins, sulfur dioxide, phloroglucinolysis, wine ageing, LC-MS/MS, *Vitis vinifera*

INTRODUCTION

One of the most remarkable characteristics of proanthocyanidins, a widespread secondary metabolite group, ubiquitous in food, plants, and beverages, is their structural diversity and high complexity. Oligomeric and polymeric proanthocyanidins, also known as condensed tannins, are composed of extension and terminal flavan-3-ol subunits linked by interflavonoid bonds, and their polymerisation in grape skin can reach up to 10 to 70 units (Labarbe *et al.*, 1999; Dubourdieu *et al.*, 2006). Each monomeric flavan-3-ol subunit can have differences in the hydroxyl group position or number, also due to the presence of two chiral centres at C2 and C3, and it is possible to find diastereomers for a single flavan-3-ol subunit (Waterhouse *et al.*, 2016; Rousserie *et al.*, 2019; Bonaldo *et al.*, 2020). Moreover, the flavan-3-ol units can be covalently linked by C-C for B-type (C4-C8 or C4-C6) or bound by C-O-C for A-type (C2-O-C7 or C2-O-C5) forming oligomeric and polymeric structures (Waterhouse *et al.*, 2016; Rousserie *et al.*, 2019; Garrido-Bañuelos, 2021), and can be present in wine as ethyl-bridged derivatives (Drinkine, 2007), crown/cyclic tannins (Longo *et al.*, 2018; Longo *et al.*, 2019), and sulfonated proanthocyanidins (Arapitsas *et al.*, 2014; Mattivi *et al.*, 2015; Ma *et al.*, 2018).

The apparent proanthocyanidin mean degree of polymerisation (mDP) decreases during wine storage and ageing (McRae *et al.*, 2012; Ma *et al.*, 2018), presumably as a consequence of the slow acidic cleavage of the direct interflavanic bonds of oligomeric and polymeric proanthocyanidins at acidic conditions which may lead to decrease the proanthocyanidin content in red wines (Mattivi *et al.*, 2015; Ma *et al.*, 2018; Arapitsas *et al.*, 2018; Devi *et al.*, 2025). The acid-catalysed cleavage leads to the formation of free monomers and carbocations, which later can take part in several reactions of further depolymerisation, rearrangements, nucleophilic addition, anthocyanin-tannin formation, amongst others (Waterhouse *et al.*, 2016; Ma *et al.*, 2018; Bonaldo *et al.*, 2020; Ontañon *et al.*, 2020) (Figure 1).

One of the most recently discovered reactions in wine is the procyanidin sulfonation, whose main reaction mechanism involves the nucleophilic attack of the HSO_3^- to the C4 carbocation formed by the release of the extension units after the acidic interflavanic cleavage of the polymeric procyanidins (Mattivi *et al.*, 2015; Ma *et al.*, 2018; Bonaldo *et al.*, 2020; Devi *et al.*, 2025). This mechanism is promoted by elevated storage temperature and acidic pH. Between the fully characterised products of this reaction are epicatechin 4β-sulfonate, catechin 4β-sulfonate, epigallocatechin 4β-sulfonate, and procyanidin B2 sulfonate (Arapitsas *et al.*, 2014; Arapitsas *et al.*, 2018; Mattivi *et al.*, 2015; Bonaldo *et al.*, 2020; Ma *et al.*, 2018). These findings indicate the presence of several oligomeric proanthocyanidins with their terminal unit sulfonated in aged wines (Figure 1), and, therefore, wine might contain more sulfonated proanthocyanidins than just the monomeric and dimeric ones (Arapitsas *et al.*, 2014; Mattivi *et al.*, 2015; Arapitsas *et al.*, 2018; Ma *et al.*, 2018; Bonaldo *et al.*, 2020; Devi *et al.*, 2025). In other words, the measurable monomers and dimers could represent just the tip of the iceberg.

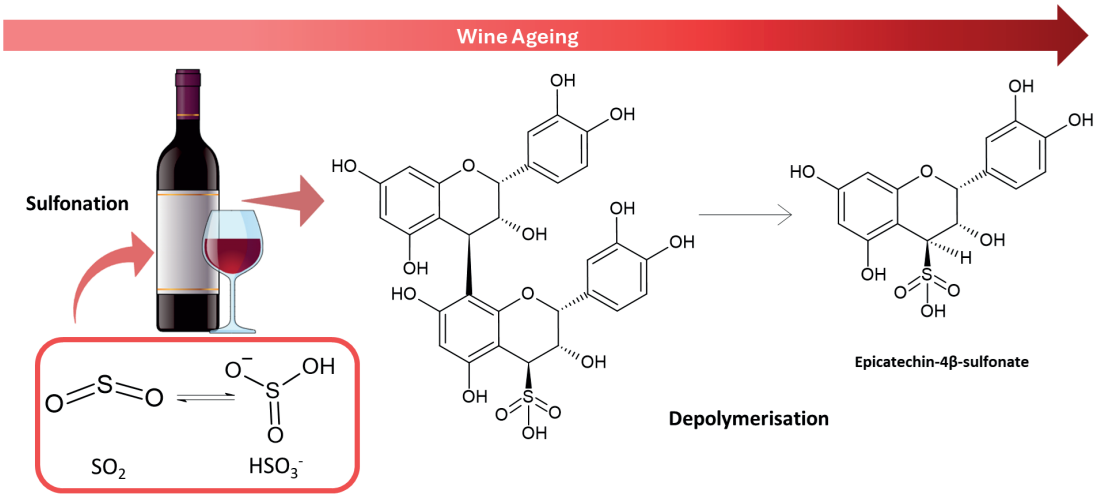

FIGURE 1. Schematic representation of procyanidin sulfonation to produce epicatechin 4β-sulfonate during wine ageing.

The knowledge about the type and number of the building blocks that construct the oligomeric and polymeric proanthocyanidin present in wine can provide us with relevant information about several wine quality features, such as ageing potential, colour stabilisation, flavour, and mouthfeel properties (Dubourdieu *et al.*, 2006; Drinkine, 2007; Tesseidre & Jourdes, 2013; Waterhouse *et al.*, 2016). Indeed, proanthocyanidins can be involved in dry sensation, astringent mouthfeel sensation, and bitter wine perception (Tesseidre & Jourdes, 2013; Piombino *et al.*, 2020; Ferrero del Teso, 2022; Paissoni *et al.*, 2023). Regardless of the employed methodology for condensed tannin characterisation

(degradative or non-degradative), in wine chemistry, the determination of mDP is used as an analytical tool to explain wine sensory character (Tesseidre & Jourdes, 2013; Ma *et al.*, 2018; Ferrero del Teso, 2022), and/or as a chemical descriptor to compare wines by region, variety, and terroir (Quaglieri *et al.*, 2017; Petropoulos *et al.*, 2017; Arapitsas *et al.*, 2022). Amongst the degradative characterisation methods, phloroglucinolysis (Kennedy & Jones, 2001) stands out as one of the most widely used. The methodology consists of a chemical depolymerisation reaction by acidic cleavage of interflavonoid bonds in the presence of phloroglucinol as a trapping nucleophile (Figure S1). According to the methodology, the procyanidin upper or extension units react with phloroglucinol and are measured as phloroglucinol adducts, while the terminal units are released and measured as free monomers. Despite the drawbacks (recalcitrancy of the A-type, impossibility of knowing the sequence of flavan-3-ol units), phloroglucinolysis, provides relevant information as the degree of galloylation (DG), the determination of the proportion among the constitutive units after reaction and an estimate of the mDP (Kennedy & Jones, 2001; Arapitsas *et al.*, 2021).

Taking into consideration the need to have methods that enable the accurate and detailed analysis of the polymeric proanthocyanidins, the main aim of this study was to evaluate the impact of the sulfonated flavan-3-ols terminal units on the precision of the mDP measurement, since this is still unknown. To get a wider image, the secondary aim of the study was to evaluate if the storage condition and the level of SO_2 addition may influence the concentration of the sulfonated procyanidins and the mDP.

MATERIALS AND METHODS

1. Chemicals

Catechin hydrate, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate, and catechin gallate, phloroglucinol, ascorbic acid, sodium acetate were purchased from Sigma-Aldrich; procyanidin B1 and B2 from Extrasynthese; methanol, acetonitrile, and formic acid LC-MS grade were purchased from Fluka; hydrochloric acid from Merck, while water was purified by the Milli-Q water purification system. Epicatechin-4β-sulfonate was isolated as described in Arapitsas *et al.* (2018), and epicatechin 4-phloroglucinol, epigallocatechin 4-phloroglucinol, and epicatechin gallate 4-phloroglucinol as reported in Arapitsas *et al.* (2021).

2. Wine samples and experimental design

The sample set included 12 wines of the vintage 2016, sampled during the beginning of 2017, from four single cultivar red wines (3 Nebbiolo from Piedmont, 3 Nerello Mascalese from Sicily, 3 Sangiovese from Tuscany, and 3 Sagrantino from Umbria). Each wine was produced by a different winery according to the winemaking protocol of the winery, further details about wine samples are described in Arapitsas *et al.* (2022), and basic oenological analysis is included in Table S1. Then, at the time zero of this experiment (February 2018, about one year after the initial sampling) the volume of each wine was divided into four portions, where different amounts of sodium metabisulfite were added to achieve four levels of SO_2 addition (0, 30, 50, and 100 mg/L) to create 48 samples. Two 10 mL amber vials from each sample were filled, up to the edges to avoid triggering oxygen-dependent reactions, giving a total of 96 wine samples, where one vial of each wine was stored for a two-year period at 8 °C (Controlled Temperature or CT), and one wine was stored in the laboratory at room temperature around 20 °C (RT).

3. Phloroglucinolysis

The sample preparation was done at the end of the storage experiment, about two years after time zero in February 2020, according to a validated protocol (Arapitsas *et al.*, 2021). The wine samples were prepared using a C18-SPE cartridge (Waters, Milford, MA, USA) which was previously activated with methanol (5 mL) and therebefore conditioned with H_2O (10 mL). Three mL of wine diluted with 15 mL of Milli-Q H_2O were poured into the C18-SPE cartridge for solid phase extraction (SPE), and the cartridge was washed with 20 mL of water. For the elution step, 10 mL of methanol was used. The eluent was evaporated under pressure to dryness at 25 °C, reconstituted in 1 mL of methanol, then filtered (0.22 µm PTFE) and stored in certified amber vials at –20 °C before analysis. The phloroglucinol reagent was prepared with 0.2 N of HCl in methanol with 20 g/L of ascorbic acid and 100 g/L of phloroglucinol. For the phloroglucinolysis reaction, 100 µL of purified wine was added to 100 µL of phloroglucinol reagent, the reaction was carried out at 50 °C, 600 rpm for 3-minutes (Thermomixer comfort, Eppendorf). The reaction was stopped using 1 mL of aqueous sodium acetate (40 mM) and then the sample was filtered (0.22 µm PTFE) in amber vials for further analysis.

4. UPLC-MS/MS analysis

The UPLC-MS/MS analysis was performed according to the new methodology proposed by Arapitsas *et al.* (2021). The analysis was performed using a Waters Acquity UHPLC system (Milford, MA, USA) coupled with Waters Xevo TQMS (Milford, MA, USA) instrument with an electrospray source (ESI), and the column used was an Acquity HSS T3 column (1.8 µm, 150 mm × 2.1 mm) (Milford, MA, USA).

For each sample, the quantification of catechin, epicatechin, procyanidin B1, procyanidin B2, gallocatechin, epigallocatechin, epicatechin gallate, catechin gallate was performed one time after the SPE step and before the phloroglucinol reaction (4 times diluted in methanol: H_2O (50:50)) and one time after the phloroglucinolysis reaction. The quantification of epicatechin-4β-sulfonate was performed before SPE and after the phloroglucinolysis reaction.

5. Data analysis and visualisation

Data processing was carried out using Waters MassLynx version 4.2 and TargetLynx software (Waters, Milford, MA). One-way ANOVA with post-hoc Tukey's Honest Significant Difference (HSD) statistical analysis was performed using

SPSS V19 (IBM Statistics), where hypotheses with p-values less than 0.05 were considered statistically significant.

For the calculation of the mean degree of polymerisation (mDP) the formulas previously reported were applied (Arapitsas *et al.*, 2021) and included the subtraction of the monomeric flavan-3-ols concentrations quantified before the phloroglucinolysis reaction.

RESULTS AND DISCUSSION

The sample set of the present study was based on 96 wine samples, which originated from 4 single cultivar red wines (Nebbiolo, Nerello Mascalese, Sangiovese, and Sagrantino) produced at industrial scale and sampled from the tank and differ by the level of SO_2 addition at the time zero of the experiment and by the storage conditions (CT versus RT). For the analysis of the wines a protocol that included the phloroglucinolysis of the samples was initially used, and then their analysis was performed with a sensitive, specific, and fast UPLC-MS/MS method, previously published (Bonaldo *et al.*, 2020; Arapitsas *et al.*, 2021; Arapitsas *et al.*, 2022).

According to the phloroglucinolysis protocol, the interflavanic bond of the polymeric proanthocyanidins is cleaved under acid conditions, releasing the terminal units as monomers and forming C4 phloroglucinol adducts of the extension units. Each sample is analysed by the same chromatographic method (UPLC-MS/MS in our case) before and after the reaction (Arapitsas *et al.*, 2021). The method used enabled the absolute quantification of the monomeric flavan-3-ols (catechin, epicatechin, gallocatechin, epigallocatechin, and catechin gallate) including epicatechin 4β-sulfonate, the dimeric procyanidin B1 and B2, and products of the reaction between phloroglucinol and epicatechin, epigallocatechin and epicatechin gallate. Then the data are used to calculate the mDP, the type and concentration of the terminal and upper sub-units, and the average degree of galloylation. The main novelty of this work was the simultaneous quantification of epicatechin 4β-sulfonate together with the above-described flavan-3-ols, both before and after the phloroglucinolysis.

The full data set, along with the statistical analysis of each sample, can be found in Tables S2 and S3. Although not the primary aim of the study, significant differences were observed between cultivars. To achieve more realistic and reliable results, the 96 samples mentioned above were derived from 12 different wines produced on an industrial scale. Overall, the different levels of SO_2 addition (0, 30, 50, and 100 mg/L) did not have a significant effect on the results. However, for Nebbiolo, gallocatechin, epicatechin-4-phloroglucinol, and epigallocatechin-4-phloroglucinol, as well as for Sangiovese, epicatechin 4β-sulfonate (free and terminal) and procyanidin B1 showed statistically significant differences with respect to the SO_2 addition (see Table S2 for specific values). On the other hand, high temperature had a major impact on most of the data, which will be discussed in detail in the following paragraphs.

1. Monomeric and dimeric flavan-3-ols

The average concentration of free monomeric flavan-3-ols, except for catechin gallate, during ageing at room temperature was approximately half compared to ageing at the controlled temperature (Figures 2A and 2B, Table S3). For instance, catechin and epicatechin were reduced by approximately 52 % and 63 %, respectively, when considering all cultivars, with Sangiovese wines experiencing the greatest reduction (–74 % for catechin and –80 % for epicatechin). Similarly, the results for monomeric flavan-3-ols were consistently and significantly lower, except for catechin gallate, which was not affected by the temperature of storage (Table S3). The degradation of the two dimeric procyanidins, measured prior to phloroglucinolysis, was even more pronounced at higher temperatures, with a loss of about 70 %. In the case of Sangiovese wines, procyanidin B2 was almost completely consumed (96 %).

Regarding epicatechin 4β-sulfonate, Nerello Mascalese wines had approximately five times more than Nebbiolo and Sagrantino wines, and 50 times more than Sangiovese wines before phloroglucinolysis. This finding is consistent with the existing literature, which suggests that Nerello Mascalese is richer in epicatechin 4β-sulfonate compared to other single Italian cultivar wines (Arapitsas *et al.*, 2020). Although higher temperatures appeared to promote the formation of epicatechin 4β-sulfonate in our experiment, the difference was statistically significant only for Sagrantino wines, possibly due to the reaction time during storage time and to the high variability of the sample set. In terms of mean values, Nebbiolo and Sagrantino wines nearly doubled their content of epicatechin 4β-sulfonate under room temperature conditions. Conversely, the different levels of SO_2 were statistically significant in relation to the concentration of epicatechin 4-sulfonate solely for the Sangiovese groups (Figure 2E and Table S3). The quantified epicatechin 4β-sulfonate at the samples with no SO_2 addition can be explained by the fact that the twelve initial wines were two years old when the experiment described here started, and SO_2 was added during their winemaking process.

The decrease in concentrations of the terminal units of the monomeric flavan-3-ols (catechin and epicatechin) after phloroglucinolysis was statistically significant for wines stored at higher temperatures. When considering all the wines, the concentrations of catechin, epicatechin, and the total terminal units were approximately halved (–46 %, –55 %, and –35 %, respectively). However, Nerello Mascalese wines showed a smaller decrease in concentrations (–24 % for catechin, –23 % for epicatechin, and –13 % for the total terminal units). Catechin gallate was less influenced by the temperature compared to the other terminal units (Figures 2C and 2D, Table S3).

The terminal units of epicatechin 4β-sulfonate, thus the one derived from oligomeric and polymeric procyanidins didn't show any statistically significant difference considering the temperature of storage, and their behaviour was similar to the one observed for the free epicatechin 4β-sulfonate too.

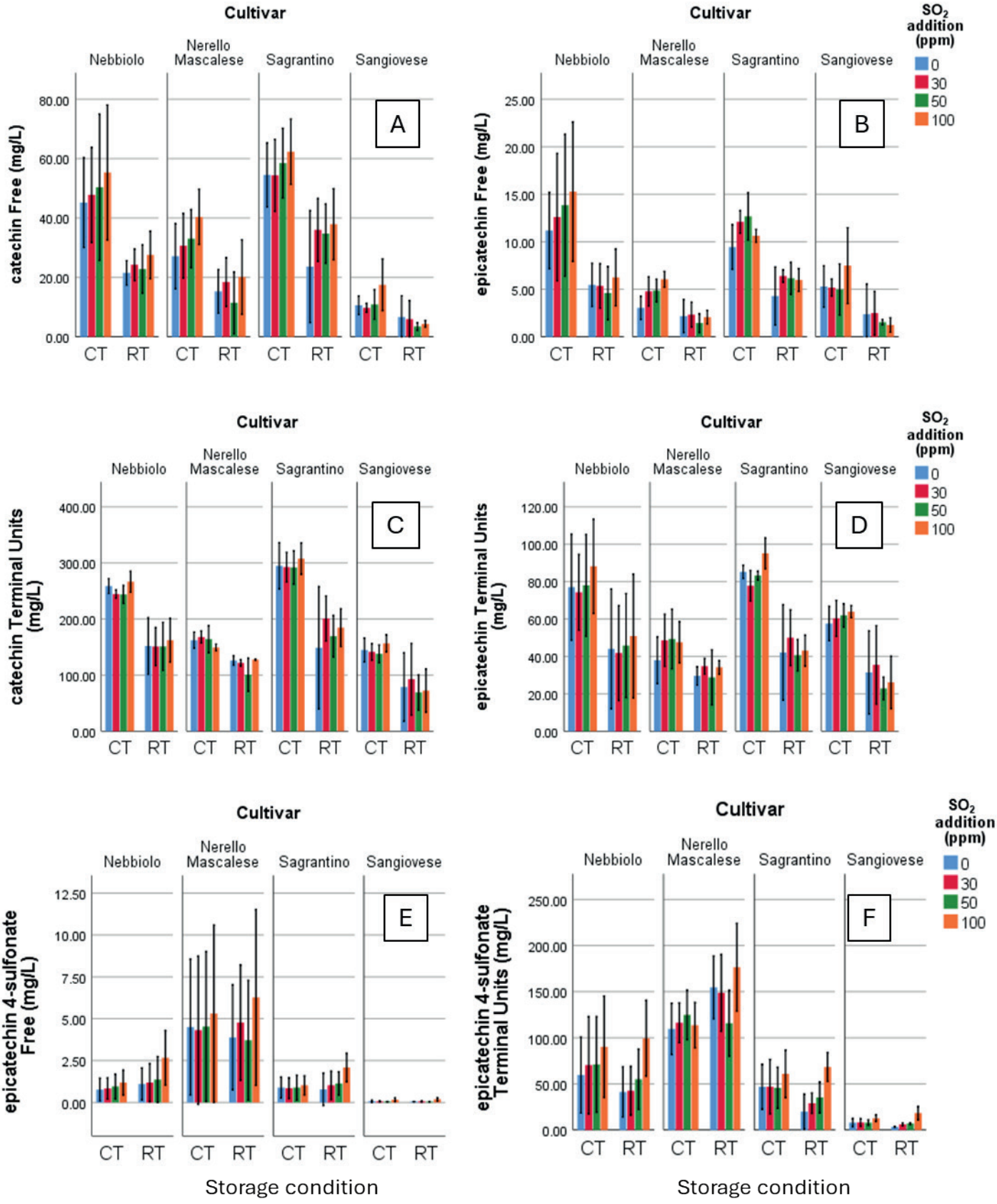

FIGURE 2. LC-MS/MS quantification results, for each wine cultivar and under the different experimental conditions (storage temperature and SO_2 addition). CT: Controlled Temperature and RT: Room Temperature. Standard deviation bars include the biological variability of three different wines of the same cultivar.

Thus, Nerello Mascalese demonstrated the highest concentrations followed by Nebbiolo, then Sagrantino, and finally Sangiovese (Figure 2F and Table S3). It was very interesting to notice that while for most monomers the concentration of the terminal units was about 5 to 8 times higher than the corresponding free unit, and especially for epicatechin it was between 5 and 15 times higher (only for catechin gallate was about the double), the terminal units of epicatechin 4β-sulfonate were about 27 times (Nerello Mascalese) to 103 times (Sangiovese) higher than the corresponding free analogue. At the same time, the percentage mean of epicatechin 4β-sulfonate on the free monomers was about 2.5 % considering all the wines stored at CT and 5.3 % for the wines stored at RT, its average percentage of the terminal units was 11.9 % for the wines stored at CT and 17.5 % for the RT storage. In detail, if only the wines stored at room temperature are considered, epicatechin 4β-sulfonate represented on average 17 % of the terminal units in Nebbiolo, 37 % on Nerello Mascalese, 11 % on Sagrantino and 6 % for Sangiovese. These findings suggest that wines should contain an important amount of various oligomeric and polymeric sulfonated procyanidins in the terminal unit, which currently are not taken into consideration due to the lack of commercial availability of the sulfonated standards. Given that other sulfonated flavan-3-ols, such as epigallocatechin and epicatechin gallate, have also been identified in wine alongside epicatechin 4β-sulfonate (Bonaldo *et al.*, 2020; Ma *et al.*, 2018; Devi *et al.*, 2025), but were not included in our study, the presence of polymeric sulfonated proanthocyanidins (including both procyanidin and prodelphinidin) is likely much higher.

In light of these results, we wondered if the oligomeric and polymeric sulfonated tannins could influence the mDP values of wine procyanidins.

2. Procyanidins mean degree of polymerisation

To assess the impact of sulfonated procyanidins across the different wine varieties examined in this study, the mDP was calculated twice for each wine sample: first, excluding the contribution of epicatechin-4β-sulfonate, and then including the measured epicatechin-4β-sulfonate before and after phloroglucinolysis.

When the mDP calculation did not include epicatechin-4β-sulfonate, all four cultivars showed similar values, with an mDP of around 5. Neither the addition of SO_2, nor the storage temperature had a significant impact on the mDP, except in one case. The 12 Sangiovese wines stored at room temperature had a significantly lower mDP of 4.42 ± 0.47 compared to the wines stored at control temperatures (5.40 ± 0.25), indicating that tannin depolymerisation was more pronounced in Sangiovese wines stored at higher temperatures (Figure 3A, Tables S3 and S4).

However, when epicatechin-4β-sulfonate was taken into consideration, the storage temperature had a significant effect on all wines except Nebbiolo, with Sangiovese wines again showing the largest difference in mDP (5.28 ± 0.20 *versus* 4.20 ± 0.43) (Figure 3B, Tables S3 and S4).

The outputs of the study clearly demonstrated that epicatechin-4β-sulfonate has a great impact on the calculation of mDP, consistently resulting in lower values (Figure 4, Tables S3 and S4). Thus, if epicatechin-4β-sulfonate is not considered, the mDP values are overestimated, which is reasonable since, as a terminal unit, it influences the denominator or the fraction. The absolute error of the mDP values, if epicatechin-4β-sulfonate is not quantified and used in the calculation, varied from –0.03 to –1.93, with the percentage being from –1 to –36 % (Figure S4B). As demonstrated in Figure 4A and Table S4, the error was statistically significant

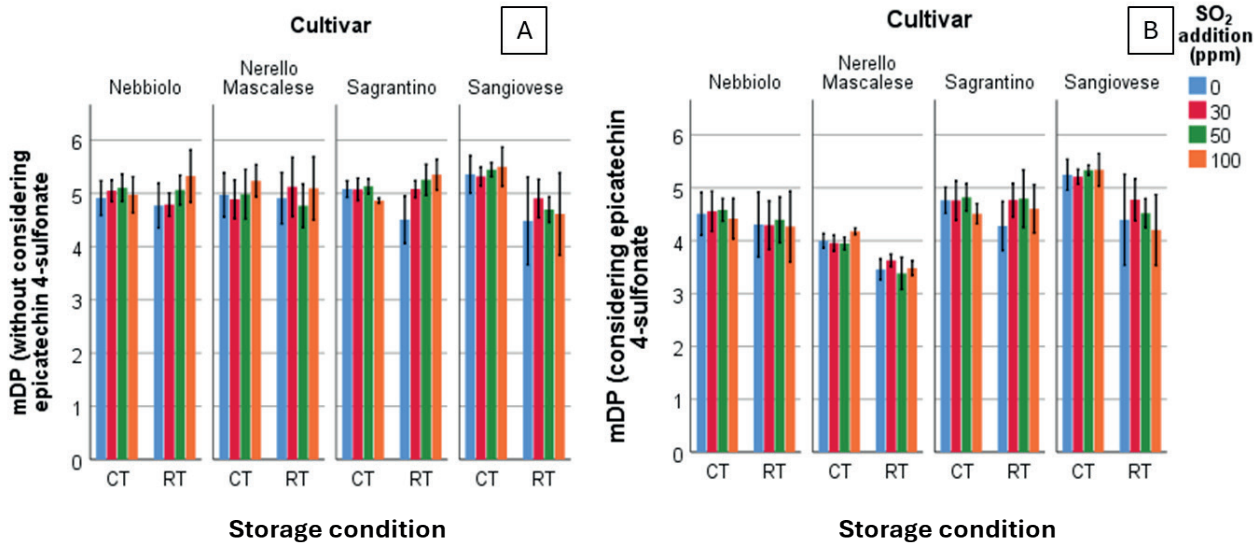

FIGURE 3. mDP without (A) and by considering (B) epicatechin 4-sulfonate on the calculations, for each wine cultivar and under the different experimental conditions (storage temperature and SO_2 addition). CT: Controlled Temperature and RT: Room Temperature. Standard deviation bars visualise the (biological) variability of three different wines of the same cultivar.

in most cases, with Sangiovese wines being the only exception. A plausible explanation is the stronger effect of the storage temperature in this variety. Sangiovese wines showed the smallest differences, while Nerello Mascalese had the largest (Table S3). As the free and terminal unit epicatechin 4β-sulfonate levels were significantly affected by storage conditions, this factor also led to statistically significant changes in the mDP values. On average, if we consider all the 96 wines of the study (Sangiovese included), the overestimation on the mDP calculation was (12 % ± 9), the major effect was found on Nerello Mascalese (24 % ± 7), followed by Nebbiolo (12 % ± 6) and Sagrantino (8 % ± 4). As shown in Figure 4, the error for only the wines stored at room temperature was higher, for example, for the "RT" Nerello Mascalese the error was 28 % ± 6 and for the "CT" 20 % ± 4.

The phloroglucinolysis protocol used in this study did not account for ethylidene-bridged flavan-3-ol compounds, ethyl-bridged tannin-anthocyanin complexes formed through condensation, or cyclic procyanidins. However, Drinkine *et al.* (2007) calculated that ethylidene-bridged flavan-3-ol constitutes less than 1.3 % of interflavonoid linkages, so the error due to these compounds should be small. With regard to ethyl-bridged tannin-anthocyanins, it has been suggested that they resist depolymerisation by acidic cleavage during thiolysis (Salas *et al.*, 2004), but there is no available information on their stability under phloroglucinolysis conditions. Zeng *et al.* (2019) demonstrated that crown procyanidins, which can reach concentrations of 30 to 50 mg/L in red wine (Jouin *et al.*, 2022), after prolonged phloroglucinolysis produced only phloroglucinol adducts and not free terminal units, therefor the impact to the mDP calculation should be almost insignificant. Consequently, while a minimal bias on mDP attributable to non-typical linkages introduced by phloroglucinolysis is anticipated, further research is required to ascertain their influence on wine mDP.

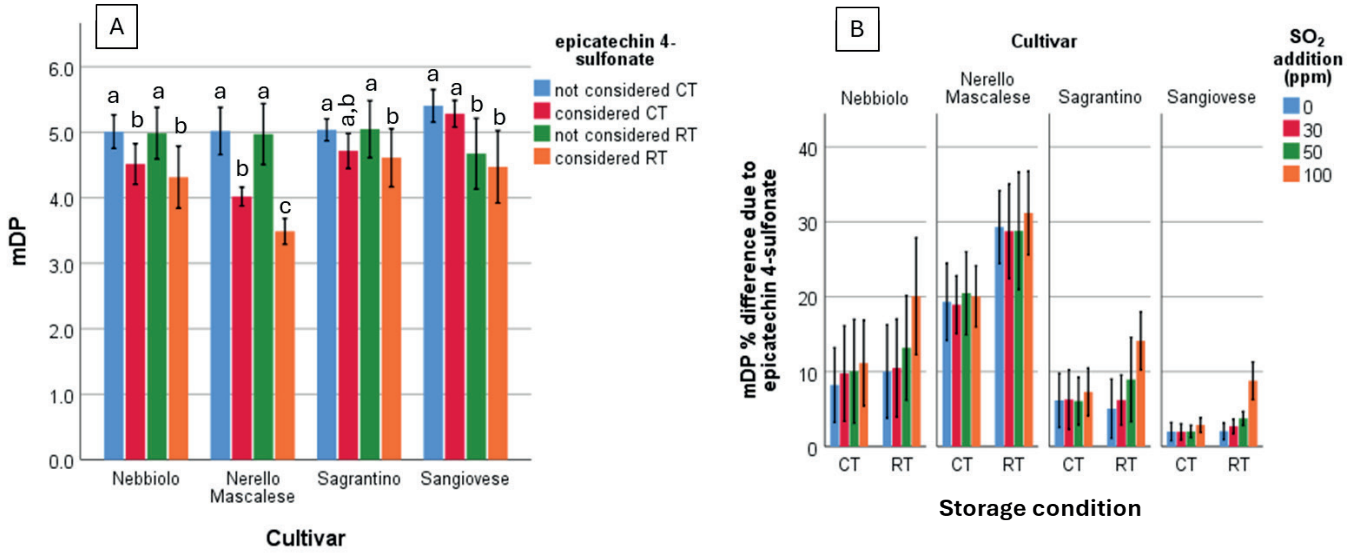

FIGURE 4. (A) mDP values with and without considering epicatechin 4-sulfonate on the calculations, for each wine cultivar and under the different storage conditions, and (B) the % error of the mDP if epicatechin 4-sulfonate is not considered on the calculation under the various experimental condition. CT: Controlled Temperature and RT: Room Temperature. Standard deviation bars include (A) the biological variability of 12 different wines of the same cultivar, and (B) of three wines. Small letters in (A) indicate the results of the ANOVA analysis, where means that do not share a letter are significantly different (significance value below 0.05).

Proanthocyanidins represent an important class of polyphenolic compounds, as they indirectly and directly influence wine sensory parameters. The length of their polymerisation, reflected in the mDP, is an important feature that helps wine scientists and winemakers understand wine quality. Accurate mDP measurement is essential to characterise these complex oligomers and polymers, providing valuable insights into wine sensory attributes and their evolution. Conversely, inaccuracies in mDP determination can lead to misinterpretations of wine astringency, bitterness, and ageing behaviour. Therefore, identifying factors that contribute to more accurate mDP measurements is relevant for both wine scientists and winemakers.

It has been discovered that as the wine ages, and due to the favourable acidic conditions, the interflavanic C-C links of their polymeric forms are slowly cleaved and the products of this cleavage take part in several reactions, including the C4 sulfonation that delivers sulfonated tannins (Figure 1). The acidic cleavage is promoted by the temperature, and wine storage at higher temperatures has higher amounts of sulfonated monomeric and dimeric flavan-3-ols. Lately, it has been demonstrated that flavan-3-ol sulfonation, a normal ageing wine reaction that leads oligomeric and monomeric sulfonated products (Devi *et al.*, 2025), is favoured if the wine is stored under anoxic conditions since the lack of acetaldehyde directs the nucleophile HSO_3^- to react with

the carbocation formed during the tannin depolymerisation (Ontañon *et al.*, 2020).

Storing wine at temperatures higher than the optimum is a method often used by researchers to accelerate wine ageing and to obtain possible projections of the future wine composition. In addition, it has been demonstrated that as wine ages, the amount of procyanidin sulfonation products increases (Arapitsas *et al.*, 2018; Ma *et al.*, 2018; Devi *et al.*, 2025), not only due to the nucleophilic attack of -HSO_3 to the cleaved flavanic carbocations, but primarily due to the high stability of this C-S link (Tachtalidou *et al.*, 2024). Therefore, if we assume that the two different storage temperatures in the present study represent two different ageing time points of the same wine sample set, we can conclude that as the wine ages (or as the wine is stored at high temperature for longer periods) the greatest is the impact of the sulfonated monomeric and polymeric proanthocyanidins to the mDP measurement.

CONCLUSION

This study examined the impact of proanthocyanidins sulfonation on mDP calculation in wine. By phloroglucinolysis methodology to release terminal units, we analysed epicatechin-4β-sulfonate levels and inferred the presence of significant proportions of oligomeric and polymeric proanthocyanidins sulfonated in addition to monomeric and dimeric sulfonated. The levels of the sulfonated polymeric and oligomeric forms are cultivar and storage temperature dependent.

The results indicated that as the wine ages, the number of sulfonated proanthocyanidins increases and therefore they become more and more critical on the correct calculation of the proanthocyanidins mDP. According to the wine samples subject of the study, if the terminal epicatechin 4β-sulfonate is not taken into consideration, the error on the mDP determination can achieve 36 %, with an average error of 12 %. In addition, the error always leads to an overestimation of the mDP value. Considering the solubility of the sulfonated proanthocyanidins and that other flavan-3-ols terminal units could be sulfonated too (epigallocatechin, epicatechin gallate, etc.), which were not quantified in this study, the error on the mDP estimation could be even larger. In other words, the expected presence of sulfonated oligomeric and polymeric proanthocyanidins in red wines is not negligible. Further research is needed to fully understand the sensory implications of these changes, and how chemical and/or physical parameters are influencing the level of this the impact of sulfonated proanthocyanidins in mDP.

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