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SPECIAL ISSUE

Influence of matter other than grapes on quercetin evolution and wine polyphenols

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ABSTRACT

Matter other than grapes (MOG), such as leaves and stems, is increasingly present in mechanically harvested fruit and is known to affect wine quality. This study investigated how MOG levels below 3 % (*w/v*), which is the threshold considered acceptable for quality wine by industry standards, impact quercetin concentration and the broader phenolic composition of wine. Fermentations were conducted using both synthetic must and Merlot must, with the addition of 1 %, 2 %, and 3 % (*w/v*) grape leaves or stems. Results showed that grape leaves significantly increased the levels of quercetin derivatives, particularly quercetin-3-glucuronide and quercetin-3-glucoside, which may contribute to instability and precipitation during ageing. Grape stems, while contributing little to quercetin enrichment, markedly increased catechin and proanthocyanidin concentrations. Both leaves and stems affected fermentation kinetics and modified chemical parameters such as pH and total acidity. Colour measurements also varied depending on the type and amount of MOG added. This study provides quantitative data on how MOG levels below 3 % influence quercetin derivatives and other phenolic compounds during wine fermentation. The results highlight the importance of limiting MOG in harvested grapes to maintain wine composition and quality.

KEYWORDS: quercetin evolution, matter other than grape (MOG), grape leaves, grape stems, wine fermentation, polyphenols, Macrowine 2025

INTRODUCTION

Quercetin precipitation is a phenomenon that primarily occurs in red wines and, to a lesser extent, in white wines (Somers & Ziemelis, 1985; Ziemelis & Pickering, 1969). Quercetin is a flavonol naturally present in grapes. Under certain conditions, it becomes insoluble and forms needle-shaped crystals that cause turbidity and undesirable deposits (Gambuti *et al.*, 2020; Luciano *et al.*, 2024). This issue, which significantly impacts wine quality, has garnered considerable attention.

Factors influencing quercetin precipitation include the hydrolysis of quercetin glycosides during fermentation and ageing, which releases quercetin aglycone, a compound with low solubility in hydroalcoholic solutions, particularly at low temperatures (Lanati *et al.*, 2021; Lanati *et al.*, 2022; Li *et al.*, 2013; McDonald *et al.*, 1998). Supersaturation of quercetin aglycone, with concentrations exceeding 3 mg/L, also poses a significant risk of precipitation (Gambuti *et al.*, 2020; Srinivas *et al.*, 2010). Grape-related factors such as variety (*e.g.*, Sangiovese), sunlight exposure, and water stress influence the accumulation of quercetin and its precursors (Gambuti *et al.*, 2020; Ojeda *et al.*, 2002; Price *et al.*, 1995; Vendramin *et al.*, 2022). Moreover, copigmentation with anthocyanins initially increases quercetin solubility, but the instability of these complexes can lead to precipitation over time (Lanati *et al.*, 2022; Luciano *et al.*, 2024). Other contributing factors include low temperatures, prolonged storage, oxidation, and nucleation seeds, which further accelerate quercetin precipitation (Castellari *et al.*, 2000; Castellari *et al.*, 2001; Lanati *et al.*, 2022; Luciano *et al.*, 2024). The composition of wine, including pigments and other compounds, may enhance or inhibit quercetin solubility and crystal growth (Luciano *et al.*, 2024; Vendramin *et al.*, 2022).

A further factor that may exacerbate quercetin precipitation is the presence of matter other than grapes (MOG) with harvested fruit, which is an increasingly common issue due to the widespread use of mechanical harvesting (Lan *et al.*, 2022; Petrucci & Siegfried, 1976). MOG typically includes vine leaves, stems, petioles, and other extraneous plant material and, in machine-harvested grapes, can reach up to 5 % of the total fruit mass (Ward *et al.*, 2015). MOG alters the wine's composition by reducing ethanol levels while increasing pH, malic acid, total phenolics, and flavonoids. Additionally, MOG can absorb anthocyanins, thereby decreasing colour intensity (Arfelli *et al.*, 2010; Guerrini *et al.*, 2018; Huang *et al.*, 1988; Suriano *et al.*, 2016). From a sensory perspective, MOG, especially stems and leaves, can introduce green aromas such as green pepper notes, methoxypyrazines, and higher alcohols (Capone *et al.*, 2021; Kilmartin & Oberholster, 2022). While some of these compounds may contribute aromatic complexity, they can also result in off-flavours. Stems (3–7 % of cluster weight) are rich in proanthocyanidins, flavonoids, and tannins, which influence pH, acidity, and total phenolics, and are known to increase astringency. (Blackford *et al.*, 2021; Capone *et al.*, 2021; Guerrini *et al.*, 2018; Scanff & Marchal, 2024; Wimalasiri *et al.*, 2022). Leaves also contribute to green aromas and are a notable source of flavonols, including quercetin (Guerrini *et al.*, 2018; Lan *et al.*, 2022).

In certain cases, MOG is deliberately added during winemaking to enhance specific organoleptic characteristics. For example, astilbin in grape stems has been associated with sweetness perception (Scanff & Marchal, 2024), while rotundone, found in grape skins and stems, contributes spicy or peppery aromas in Austrian Grüner Veltliner wines (Philipp *et al.*, 2023). Additionally, grape varieties with naturally low levels of phenolics and tannins may be co-fermented with stems to improve structure and flavour (Suriano *et al.*, 2016). However, in most contexts, MOG is regarded as a contaminant. The Australian Society of Viticulture and Oenology (ASVO) has established that MOG levels exceeding 3 % are unacceptable for quality wine production, recognising their potential to negatively affect wine composition and sensory properties (Allan, 2003).

This study aimed to assess how sub-threshold MOG levels (1 %, 2 %, and 3 % *w/v*), considered acceptable under ASVO guidelines, influence quercetin concentration and polyphenol composition in wine. To achieve this, fermentations were carried out using both synthetic and Merlot must, with the addition of grape stems or leaves as MOG.

MATERIALS AND METHODS

1. Microvinification

Synthetic must and Merlot grapes were used as fermentation matrices to compare the effects of leaves and stems in the complex environment of natural grape juice fermentation versus a controlled, "interference-free" synthetic medium. The experimental design (Figure 1) included a control group with no addition of MOG and treatment groups supplemented with 1 %, 2 %, and 3 % (*w/v*) Merlot grape leaves or stems. Each treatment was conducted in triplicate under controlled conditions at 18 °C in the experimental winery of the University of Padova, monitoring fermentation kinetics through weight loss measurements and collecting daily samples to analyse quercetin and total polyphenol content.

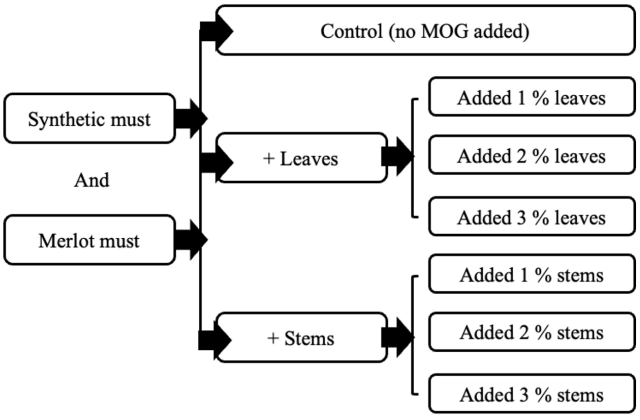


FIGURE 1. Experimental design.

The preparation of the synthetic must was based on the recipe by Delfini with minor modifications (Delfini & Formica, 2001). The concentrations of the synthetic must components are as follows: macroelements include 0.1 g/L of $CaCl_2$, 0.1 g/L of NaCl, 1 g/L of KH_2PO_4, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, and 3 g/L of tartaric acid. Microelements contain 200 mg/L of $NaMoO_4 \cdot 2H_2O$, 400 mg/L of $ZnSO_4 \cdot 7H_2O$, 500 mg/L of H_3BO_3, 40 mg/L of $CuSO_4 \cdot 5H_2O$, 100 mg/L of KI, and 400 mg/L of $MnSO_4 \cdot H_2O$. Iron was added as $FeCl_3 \cdot 6H_2O$ at a concentration of 0.4 mg/L. Additionally, vitamins include 400 mg/L of pyridoxine hydrochloride, 400 mg/L of thiamine hydrochloride, 2 g/L of inositol, 20 mg/L of biotin, 400 mg/L of calcium pantothenate, 400 mg/L of nicotinamide, and 200 mg/L of p-aminobenzoic acid. Finally, the variable components consist of 0.3 g/L of $(NH_4)_2SO_4$, 0.3 g/L of $(NH_4)_2HPO_4$, 200 g/L of glucose, 0.2 g/L of casein hydrolysate, and 2 g/L of malic acid.

The Merlot must was prepared from grapes manually harvested at the vineyard of the Cerletti Institute (Conegliano, Italy). Leaves and stems for treatments were also manually collected during the harvest. Fermentations were carried out in Erlenmeyer flasks with volumes of 200 mL for synthetic must and 400 mL for Merlot must. Grape leaves or stems were added at the beginning of the fermentation at concentrations of 1 %, 2 %, and 3 % (*w/v*), and remained in contact with the must throughout the entire alcoholic fermentation. Active dry yeast Inverno 1936 (*Saccharomyces cerevisiae*, EVER) was added at 20 g/hL without external sugar addition. Prior to fermentation, 200 mg/L of lysozyme (Enoconsul snc, Italy) was added to the must. All fermentations were conducted at a constant temperature of 18 °C. Table 1 shows the oenological parameters of the Merlot juice before fermentation.

TABLE 1. Oenological parameters of Merlot must.

Parameters (*n* = 3)	Merlot must
pH	3.650 ± 0.000
Total acidity (g/L)	5.250 ± 0.071
Acetaldehyde (mg/L)	9.500 ± 0.707
Acetic acid (g/L)	0.415 ± 0.001
Lactic acid (g/L)	0.038 ± 0.001
Malic acid (g/L)	1.882 ± 0.021
Brix %	25.075 ± 1.209
Total polyphenols (mg/L)	190.39 ± 14.08

2. Conventional oenological parameters

The pH was measured with a pH meter (model Accumet AB315, Fisher Scientific, Segrate, Italia). Total acidity was measured by the titrimetric method with NaOH and bromothymol blue. Free and total sulfur dioxide were determined by iodine titration in the presence of a starch indicator. Acetaldehyde, acetic acid, lactic acid, malic acid, and sugar concentrations were quantified using an enzymatic analyser (iMagic-M9, R-Biopharm, Milano, Italy). The alcohol content was determined using a Gibertini oenochemical distillation unit coupled with a MIA 2020 hydrostatic balance (Novate Milanese, Italy). Before analysis, must and wine samples were filtered through a 0.45 µm cellulose acetate syringe filter (Sartorius).

3. Quercetin extraction

One gram of Merlot leaves or stems was mixed with 12 mL of extraction buffer (70 % alcohol, 1 % formic acid) and homogenised using a high-speed homogeniser (FSH-2A, Vevor) until the plant material was completely homogenised. The mixture was shaken for 1-hour on a rotary mixer, centrifuged at 5000 g for 10-minutes, and the supernatant was collected. Six millilitres of extraction solution were added to the pellet, subjected to 30-minutes of ultrasonication, and centrifuged again to collect the supernatant. A further 6 ml of extraction solution was added to the pellet, and the mixture was shaken for 12-hours before a final centrifugation step to collect the supernatant. After extraction, all supernatants were pooled and the final volume of the extraction was adjusted to 25 mL. All steps were conducted at room temperature. Samples were stored at –20 °C until analysis.

4. Total polyphenols, catechin and total proanthocyanidins analysis

Total polyphenols were determined using the Folin–Ciocalteu method according to Singleton and Rossi (1965) and expressed as mg/L of gallic acid. The total proanthocyanidins content was measured with the butanol-hydrochloric acid method described by Bate-Smith (1975) and the standard calibration curve (range 0.125–2 g/L) was prepared with purified proanthocyanidin extracted from grape skins. Catechin quantification was performed using a colorimetric method described by Zironi *et al.* (1992) and expressed as mg/L of catechin. Samples were filtered through a 0.45 µm cellulose acetate filter (Sartorius) before analysis.

5. Quercetin quantification

The analysis of quercetin-3-glucuronide, glucuronide quercetin-3-glucoside and quercetin aglycone was performed on an HPLC (Shimadzu Nexera XR) equipped a Kinetex C18 column (4 × 150 mm, 5 µm, Phenomenex) and a UV detector with the diode array (Shimadzu SPD-M20A) set to 350 nm. A binary gradient consisting of solvent A (25 mM ammonium formate in water adjusted to pH 4.5 with formic acid) and solvent B (acetonitrile) was applied at a flow rate of 1 mL/min as follows: 10 % B was maintained for 5 minutes, then increased to 45 % until 20.00 min, followed by a gradient to 100 % B until 20.50 min, which was held for 5.00 min. The column was then reconditioned by returning to 10 % B and held until 30.00 min. Each sample of wine was filtered at 0.2 µm (cellulose acetate, Sartorius) and 10 µL was injected. Quantification was performed using a calibration curve from 0 to 200 mg/L of commercial standard for three molecules (quercetin-3-glucuronideglucuronide with purity ≥ 95 %, quercetin-3-glucoside with purity ≥ 98 %, and quercetin aglycone with purity ≥ 95 %). The calibration curves were prepared starting from stock solutions (10 mg/mL in 100 %

ethanol) and diluting them in 50 % methanol). All reagents were analytical grade and were purchased from Sigma (Milan, Italy). Wine samples collected during fermentation were filtered through a 0.45 µm cellulose acetate filter (Sartorius) prior to analysis.

6. Colour analysis

The CIELab coordinates of the wines were measured using an Ultrospec 2100 pro UV/Visible spectrophotometer (Euroclone, Milano, Italy) with 0.1 cm path-length quartz cuvettes. The transmittance was recorded from 380 to 780 nm in 1 nm intervals. Prior to analysis, wine samples were clarified using 0.45 µm filters. Colour coordinates were calculated from the spectra using the CIE method, following OIV guidelines (OIV, 2021), employing the CIE 1964 10° standard observer and D65 illuminant. Colour intensity (CI) was determined as the sum of absorbances at 420, 520, and 620 nm. Tonality (T) was calculated as the ratio of absorbances at 420 and 520 nm (Glories, 1984). Samples underwent filtration through a 0.45 µm cellulose acetate filter (Sartorius) to remove particulate matter before analysis.

7. Data analysis

To determine the statistical significance of differences between the results, data were analysed using analysis of variance (ANOVA), followed by Tukey's HSD test for multiple comparisons. Separate ANOVAs were conducted for treatments with varying amounts of grape leaves and treatments with varying amounts of grape stems, with both compared to the control, which consisted of samples without the addition of MOG. Statistical significance was set at $P < 0.05$. All analyses were performed using JMP Pro 17 software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

1. Fermentation dynamic

Figure 2 shows how grape leaves and stems affect fermentation in both synthetic must (A) and Merlot must (B). The higher weight loss observed in Merlot must is explained by the different initial volumes of fermentation (200 mL for synthetic must and 400 mL for Merlot must). In both cases, stems accelerated fermentation more than leaves, suggesting that stems provide additional nutrients, such as nitrogenous compounds, or structural advantages such as higher heat dissipation during fermentation (Blackford *et al.*, 2021). In synthetic must, the control exhibited the slowest fermentation, reaching 20.44 g by Day 10, while leaves progressively increased fermentation rates, with 3 % of leaves reaching 21.86 g. Stems had an even stronger effect, with 3 % stems achieving the highest weight loss of 23.65 g. Similarly, in Merlot must, the control reached 41.02 g by Day 10, while leaves accelerated fermentation, with 3 % leaves reaching 41.90 g, and stems producing the fastest fermentation, with 3 % stems reaching 42.35 g. Regardless of the volume difference, the trends observed were consistent, indicating that both leaves and stems enhance fermentation, with stems having a more pronounced impact in both musts.

2. Conventional oenological parameters

The addition of grape leaves and stems (MOG) at varying concentrations (1 %, 2 %, 3 % *w/v*) influenced key oenological parameters in both synthetic and Merlot musts, with distinct effects observed for leaves and stems. Relevant oenological parameters were measured at the end of fermentation and are shown in Table 2.

The addition of MOG, especially stems, can raise wine pH by releasing potassium, which precipitates tartaric acid as potassium tartrate (Guerrini *et al.*, 2018; Lan *et al.*, 2022). Consistent with this, Merlot musts containing 3 % stems were significantly different from the control, reaching a pH of 3.93. A similar pattern can be seen with musts containing 3 % leaves, which showed a tendency towards higher pH values, although not statistically significant. This tendency was confirmed in synthetic musts, where pH increased from 3.08 (control) to 3.32 (3 % stems) and 3.27 (3 % leaves).

The presence of MOG generally decreased total acidity during fermentation (Lan *et al.*, 2022). In synthetic musts, acidity declined with both treatments, more notably with stems. In Merlot musts, the lowest total acidity was observed in the 3 % stem treatment (6.50 g/L), which was not significantly different from the control (6.43 g/L). In contrast, the addition

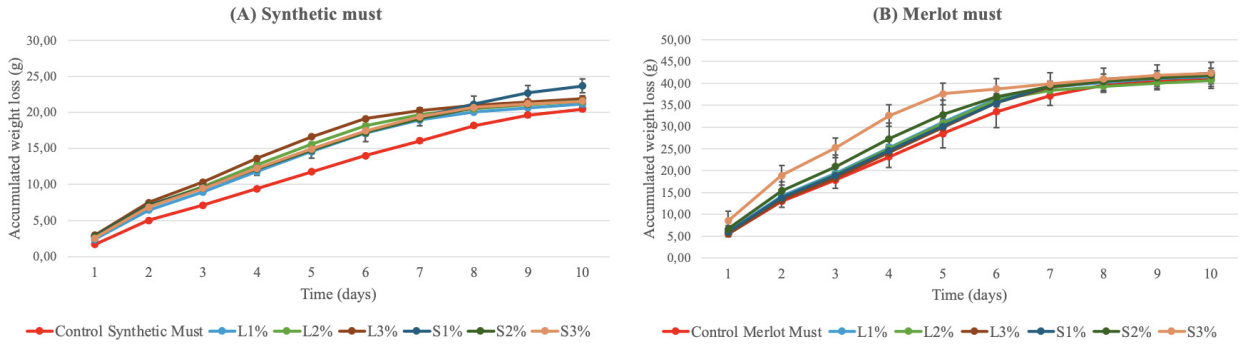


FIGURE 2. Weight loss during fermentation of synthetic (A) and Merlot musts (B) with varying quantities of grape leaves and stems (n = 3).

of 3 % leaves increased total acidity to 6.83 g/L, which was significantly higher than the control. Interestingly, this was not the case in the synthetic must, where the total acidity significantly decreased in all treatments. This probably indicates that MOG may have a different acid-releasing or buffering behaviour in simple (synthetic must) *vs* complex (real must) systems.

The concentration of malic acid tended to increase with MOG addition (Guerrini *et al.*, 2018; Lan *et al.*, 2022). In Merlot musts, malic acid reached 1.61 g/L at 3 % leaves, significantly higher than the control (1.34 g/L, $P < 0.05$), while 3 % stems yielded 1.52 g/L, also elevated but not statistically different from the control. In synthetic musts, leaves also led to an increase in malic acid from 0.94 g/L in the control to 1.24 g/L at 3 % leaves ($P < 0.05$), while stem treatments resulted in slightly lower malic acid levels, around 0.75 g/L at 3 %.

These observations reinforce the notion that leaves and stems exhibit different chemical behaviours during fermentation. Although MOG is generally associated with elevated polyphenol content (Guerrini *et al.*, 2018; Lan *et al.*, 2022), its effects on fundamental wine parameters such as pH, acidity, and malic acid also appear to be significant and type-dependent. Variability in response may also be influenced by grape variety, ripeness level, climate, and vinification conditions (Allan, 2003), which modulate compound extraction and chemical equilibria in the must.

3. Total polyphenols

The inclusion of MOG is known to increase the content of total phenols, flavonoids, and tannins in must and wine. This increase occurs because phenolic compounds, such as flavonoids and tannins, are transferred from MOG to wine during fermentation (Huang *et al.*, 1988; Lan *et al.*, 2022). Additionally, the inclusion of MOG can modify the specific polyphenol profile, increasing the concentration of non-anthocyanin flavonoids (Huang *et al.*, 1988). Stems release a variety of phenolic compounds, including phenolic acids (*e.g.*, gallic acid, syringic acid), ortho-diphenols, and flavonoids, and can, in some cases, contain higher concentrations of these compounds than the grapes themselves (Prusova *et al.*, 2020). The inclusion of stems during fermentation generally increases the concentration of polyphenols, enriching the wine's structural complexity and mouthfeel.

In the present study, the evolution of total polyphenols during fermentation varied depending on the type and concentration of grape material added. In synthetic must (Figures 3A–B), leaf treatments (L1–L3) exhibited a clear trend of increasing polyphenol content during the first six days, followed by stabilisation and a slight decline toward the end. Stem treatments (S1–S3) also showed an initial increase in polyphenol levels during the first six days, though at lower rates compared to leaves. In the final four days, polyphenol

TABLE 2. Oenological parameters of synthetic and Merlot musts with varying MOG after fermentation.

Samples (n = 3)	pH	Total acidity (g/L)	Alcohol (% v/v)	Acetaldehyde (mg/L)	Acetic acid (g/L)	Sacc/Glu/Fru (g/L)	L-Malic acid (g/L)
Synthetic control	3.08 ± 0.02$^{d/D}$	7.03 ± 0.06$^{a/B}$	9.50	12.00 ± 1.00^{b}	0.96 ± 0.01$^{a/B}$	2.20 ± 0.29$^{a/A}$	0.94 ± 0.01$^{c/A}$
Synthetic L1 %	3.18 ± 0.01^{c}	6.63 ± 0.06^{b}	10.20	15.33 ± 0.58^{a}	0.39 ± 0.04^{b}	0.88 ± 0.05^{b}	0.94 ± 0.02^{c}
Synthetic L2 %	3.22 ± 0.01^{b}	6.63 ± 0.06^{b}	9.99	16.33 ± 0.58^{a}	0.31 ± 0.02^{c}	0.85 ± 0.13b	1.07 ± 0.02^{b}
Synthetic L3 %	3.27 ± 0.01^{a}	6.60 ± 0.00^{b}	10.88	14.00 ± 1.73^{ab}	0.25 ± 0.01^{c}	0.72 ± 0.10^{b}	1.24 ± 0.06^{a}
Synthetic S1 %	3.17 ± 0.02^{C}	7.87 ± 0.23^{A}	9.97	11.00 ± 1.00	1.18 ± 0.11^{A}	1.03 ± 0.29^{B}	0.83 ± 0.04^{B}
Synthetic S2 %	3.27 ± 0.02^{B}	6.97 ± 0.06^{B}	9.39	11.67 ± 0.58	0.74 ± 0.05^{C}	0.81 ± 0.07^{B}	0.74 ± 0.00^{C}
Synthetic S3 %	3.32 ± 0.02^{A}	6.60 ± 0.00^{C}	11.30	11.00 ± 1.00	0.64 ± 0.06^{C}	0.86 ± 0.00^{B}	0.75 ± 0.02^{C}
Merlot control	3.73 ± 0.05^{B}	6.43 ± 0.06$^{c/B}$	12.97	10.67 ± 1.15$^{b/B}$	0.41 ± 0.06^{B}	1.04 ± 0.06	1.34 ± 0.01$^{b/AB}$
Merlot L1 %	3.73 ± 0.01	6.63 ± 0.06^{b}	12.47	13.00 ± 1.00^{ab}	0.42 ± 0.07	1.02 ± 0.03	1.43 ± 0.03^{ab}
Merlot L2 %	3.74 ± 0.01	6.73 ± 0.12^{ab}	12.93	15.33 ± 3.21^{ab}	0.42 ± 0.08	1.06 ± 0.07	1.54 ± 0.04^{ab}
Merlot L3 %	3.75 ± 0.01	6.83 ± 0.06^{a}	12.71	19.00 ± 3.00^{a}	0.45 ± 0.16	0.99 ± 0.19	1.61 ± 0.16^{a}
Merlot S1 %	3.75 ± 0.02^{B}	6.70 ± 0.26^{B}	12.95	11.50 ± 2.12^{B}	1.07 ± 0.11^{A}	0.95 ± 0.06	1.25 ± 0.12^{B}
Merlot S2 %	3.82 ± 0.07^{AB}	7.10 ± 0.00^{A}	12.64	17.50 ± 2.12^{A}	1.21 ± 0.05^{A}	1.02 ± 0.09	1.36 ± 0.11^{AB}
Merlot S3 %	3.93 ± 0.01^{A}	6.50 ± 0.00^{B}	11.70	15.50 ± 0.71^{AB}	0.96 ± 0.10^{A}	0.97 ± 0.05	1.52 ± 0.11^{A}

Different letters represent a significant difference in the Tukey HSD *post hoc* test. Statistically significant differences ($P < 0.05$) are indicated for leaves (a. b. c. d) and stems (A. B. C. D), where letters are absent, this indicates no significant difference among treatments. Alcohol content determinations were performed $n = 1$.

levels in stem-treated samples showed fluctuations and slight decreases, indicating less stability compared to leaf treatments.

In Merlot must (Figures 3C–D), the control started at 972.07 mg/L and peaked at 1564.82 mg/L on Day 10. Leaf treatments (L1–L3) demonstrated strong polyphenol release, with the highest values observed around Day 5, followed by stabilisation. Stem treatments (S1–S3) also increased polyphenol levels, peaking at 1933.39 mg/L in S3 on Day 10. The release of polyphenols from leaves and stems followed distinct trends during fermentation, with rapid increases in the early stages (Days 1–6) before stabilising or slightly declining toward the end. Leaves released polyphenols in larger quantities than stems, particularly at higher concentrations (L3 and S3).

In synthetic must, increases in polyphenol contents compared to the control were substantial, with 1 %, 2 %, and 3 % leaf additions resulting in 183.66 mg/L, 325.08 mg/L, and 613.51 mg/L increases, respectively, while 1 %, 2 %, and 3 % stem additions led to increases of 118.85 mg/L, 255.15 mg/L, and 401.89 mg/L. In Merlot must, the increases were more modest due to the higher baseline polyphenol levels. Leaves resulted in 64.42 mg/L, 272.54 mg/L, and 375.27 mg/L increases, while stems exhibited 116.08 mg/L, 186.33 mg/L, and 368.57 mg/L increases compared to the control. This highlights a greater influence of leaves in enhancing polyphenol concentrations, particularly in the synthetic medium. The study by Huang *et al.* (1988) observed that grape leaves, when included during fermentation, release significant amounts of phenolic compounds, including flavonoids and tannins. This results in an overall increase in the total phenolic content of wine. Treatments with higher leaf inclusion, such as 5 % leaves, demonstrated notably elevated levels of these compounds compared to other MOG components like stems, highlighting the substantial contribution of leaves to the phenolic profile during fermentation. The lower contribution of MOG to total polyphenols in Merlot must compared to synthetic one can be due to both a lower extraction caused by the buffering/saturating effect of grape polyphenols, and a higher complexation/precipitation with the grape components, such as proteins and polysaccharides.

4. Quercetin

Before fermentation, the quercetin content in grape leaves and stems was analysed to determine the levels of quercetin derivatives in these two materials. The concentrations represent the average of several leaves and stems collected from the vineyard during the Merlot grape harvest (Table 3). Merlot leaves showed significantly higher quercetin levels than stems, with quercetin-3-glucuronide being the most abundant compound at 6.24 mg/g fresh weight, compared to 0.80 mg/g in stems. Quercetin-3-glucoside in leaves was 2.60 mg/g, about half the concentration of quercetin-3-glucuronide, while stems contained only 0.27 mg/g. Leaves had no measurable quercetin aglycone (0.00 mg/g), whereas stems had a trace amount (0.04 mg/g). These results highlight grape leaves as a richer source of quercetin derivatives, dominated by quercetin-3-

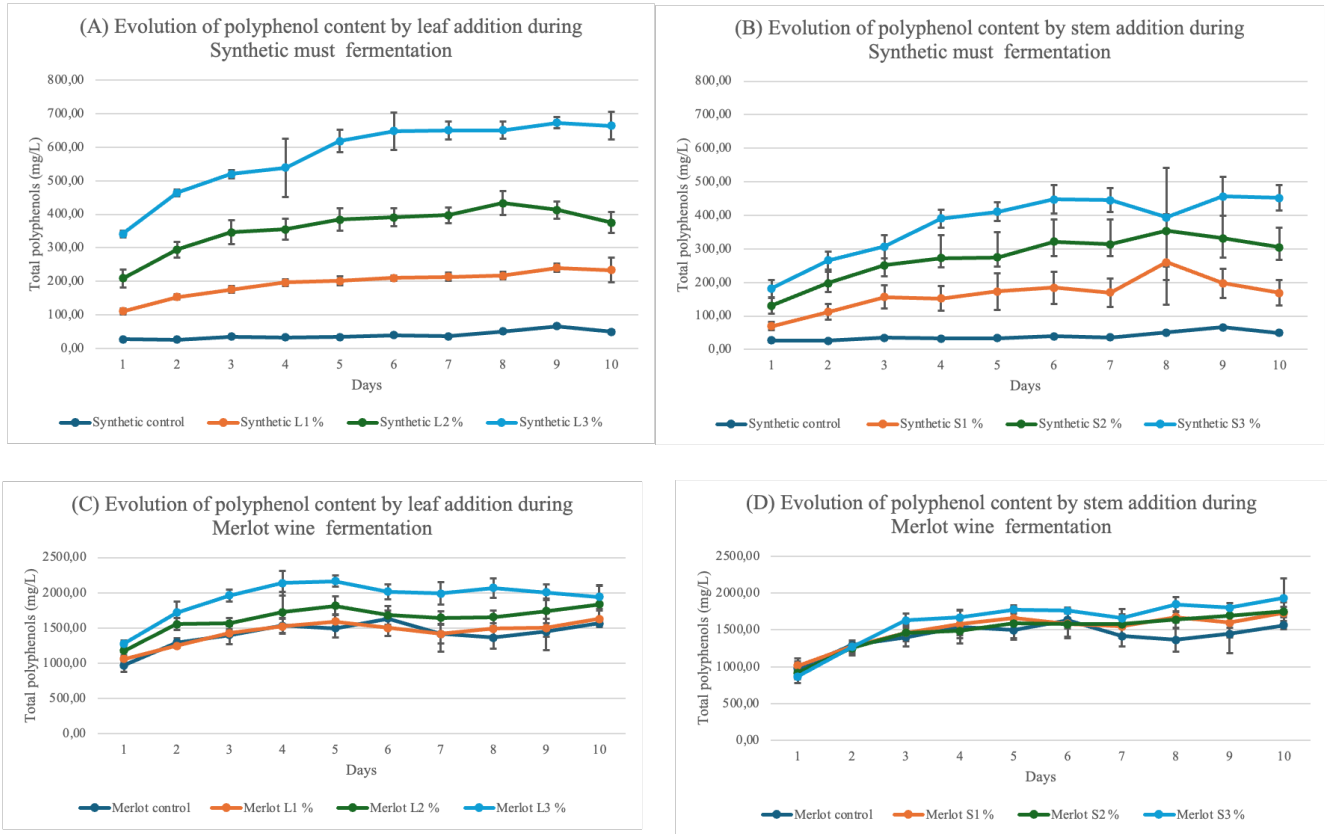


FIGURE 3. Total polyphenol evolution during fermentation of synthetic and Merlot musts with varying grape leaves or stems (n = 3).

glucuronide. The levels of quercetin, as well as other phenolic compounds, vary depending on the grape variety analysed. The leaves of Merlot leaves in the present study exhibit total quercetin derivative content comparable to that found in Aglianico leaves (2.00-2.89 mg/g of quercetin-3-glucuronide and 2.35 mg/g of quercetin-3-glucoside (Labanca *et al.*, 2020) Stems contained much lower quercetin levels, consistent with findings that stems generally have less quercetin than leaves or skins. In addition, the results confirmed that in stems, quercetin-3-glucuronide was much more abundant than quercetin-3-glucoside (Souquet *et al.*, 2000).

Regarding the effect of Merlot grape leaves on quercetin content during alcoholic fermentation (Figure 4), their addition to synthetic and Merlot must result in substantial increases in all quercetin derivatives. In synthetic must, quercetin-3-glucuronide peaked at 166.88 mg/L (3 % leaf treatment, Day 10), quercetin-3-glucoside at 62.97 mg/L (Day 7), and quercetin aglycone at 16.16 mg/L (Day 10). During the fermentation of the synthetic must, no decreases in quercetin-3-glucuronide and quercetin-3-glucoside levels were observed in the final days before the end of alcoholic fermentation; in general, their concentrations remained stable. Meanwhile, a continuous increase in quercetin aglycone was observed, which can be attributed to the simultaneous extraction of quercetin-3-glucuronide and quercetin-3-glucoside and their transformation into quercetin aglycone. In Merlot must, an increase in quercetin-3-glucuronide and quercetin-3-glucoside was observed during the first 6 to 7 days, followed by a slight decrease. These results align with our previous observations in Sangiovese grapes during fermentation (Liu *et al.*, 2024). However, the evolution of quercetin aglycone during the fermentation of Merlot must was less clear compared to its behaviour in synthetic must. Nonetheless, a general trend was observed, with an initial increase at the beginning of fermentation and a subsequent decrease toward the end. Additionally, the extraction of quercetin derivatives during Merlot must fermentation was lower than a synthetic must. For instance, with the addition of 3 % leaves during fermentation, the synthetic must released a maximum of 89 % of quercetin-3-glucuronide contained in leaves and 81 % of quercetin-3-glucoside. In contrast, the leaves released only 53 % of quercetin-3-glucuronide and 20 % of quercetin-3-glucoside in Merlot must. This indicates that, compared to synthetic must fermentation, the release of quercetin-3-glucuronide was nearly halved, and the release of quercetin-3-glucoside was reduced by fourfold in Merlot must. These differences highlight the influence of the more complex matrix of Merlot must, which may limit the efficiency of quercetin derivative release. Even so, these findings confirm that the inclusion of grape leaves during fermentation directly increases quercetin concentration in wine by releasing glycosylated derivatives, which can subsequently undergo enzymatic hydrolysis into free quercetin during fermentation and ageing. This hydrolysis is primarily catalysed by β-glucosidases produced by yeasts and other microorganisms, resulting in the formation of quercetin aglycone. The aglycone form is considerably less soluble and may precipitate from the wine, particularly during ageing. This transformation has been documented in several studies, which report a decline in glycosylated quercetin derivatives and a corresponding increase in quercetin aglycone throughout fermentation and wine maturation (Castillo-Muñoz *et al.*, 2009; Vendramin *et al.*, 2022; Wang *et al.*, 2016).

Regarding the effect of stems on quercetin content during fermentation (Figure 5), their contribution was minimal in both synthetic and Merlot must. In synthetic must, the 3 % stem treatment peaked at 1.03 mg/L for quercetin-3-glucuronide (Day 3), 0.60 mg/L for quercetin-3-glucoside (Day 5), and 2.33 mg/L for quercetin aglycone (Day 10). These values represented only 10 % and 22 % of the total quercetin-3-glucuronide and quercetin-3-glucoside content in stems, respectively, highlighting their lower extractability compared to leaves and their limited contribution to quercetin enrichment. A similar trend was observed in Merlot must, where the highest concentrations in the 3 % stem treatment were 17.98 mg/L for quercetin-3-glucuronide, 0.60 mg/L for quercetin-3-glucoside, and 4.01 mg/L for quercetin aglycone. While the absolute values were slightly higher in Merlot must, the overall impact of stems was negligible compared to leaves in both systems. These findings indicate that grape stems have a limited capacity to release quercetin derivatives during fermentation. Grape stems contain less quercetin, which may explain this observation (Table 3). And stems contribute to an overall increase in flavan-3-ols and may influence wine characteristics, such as herbaceous notes caused by methoxypyrazines (Capone *et al.*, 2021).

The comparison between leaves and stems underscores the higher ability of leaves to enrich quercetin content in both synthetic and Merlot must. In synthetic must, the 3 % leaf treatment produced quercetin-3-glucuronide levels over 278 times higher, quercetin-3-glucoside levels 188 times higher, and quercetin aglycone levels nearly 7 times higher than the 3 % stem treatment at the end of fermentation. A similar pattern was observed in Merlot must, where leaves consistently contributed higher concentrations of quercetin derivatives than stems. For example, quercetin-3-glucuronide in the 3 % leaf treatment was 117.80 mg/L compared to 17.98 mg/L in the 3 % stem treatment, while quercetin aglycone was 5.02 mg/L in leaves versus 4.01 mg/L in stems. The trend of leaves outperforming stems is even more pronounced in

TABLE 3. Quercetin and catechin in Merlot leaves and stems (mg/g fresh weight).

Samples	Quercetin-3-glucuronide	Quercetin-3-glucoside	Quercetin aglycone	Catechin
Leaves	6.24 ± 1.34	2.60 ± 0.95	0.00 ± 0.00	3.81 ± 0.09
Stems	0.80 ± 0.10	0.27 ± 0.01	0.04 ± 0.00	21.79 ± 0.33

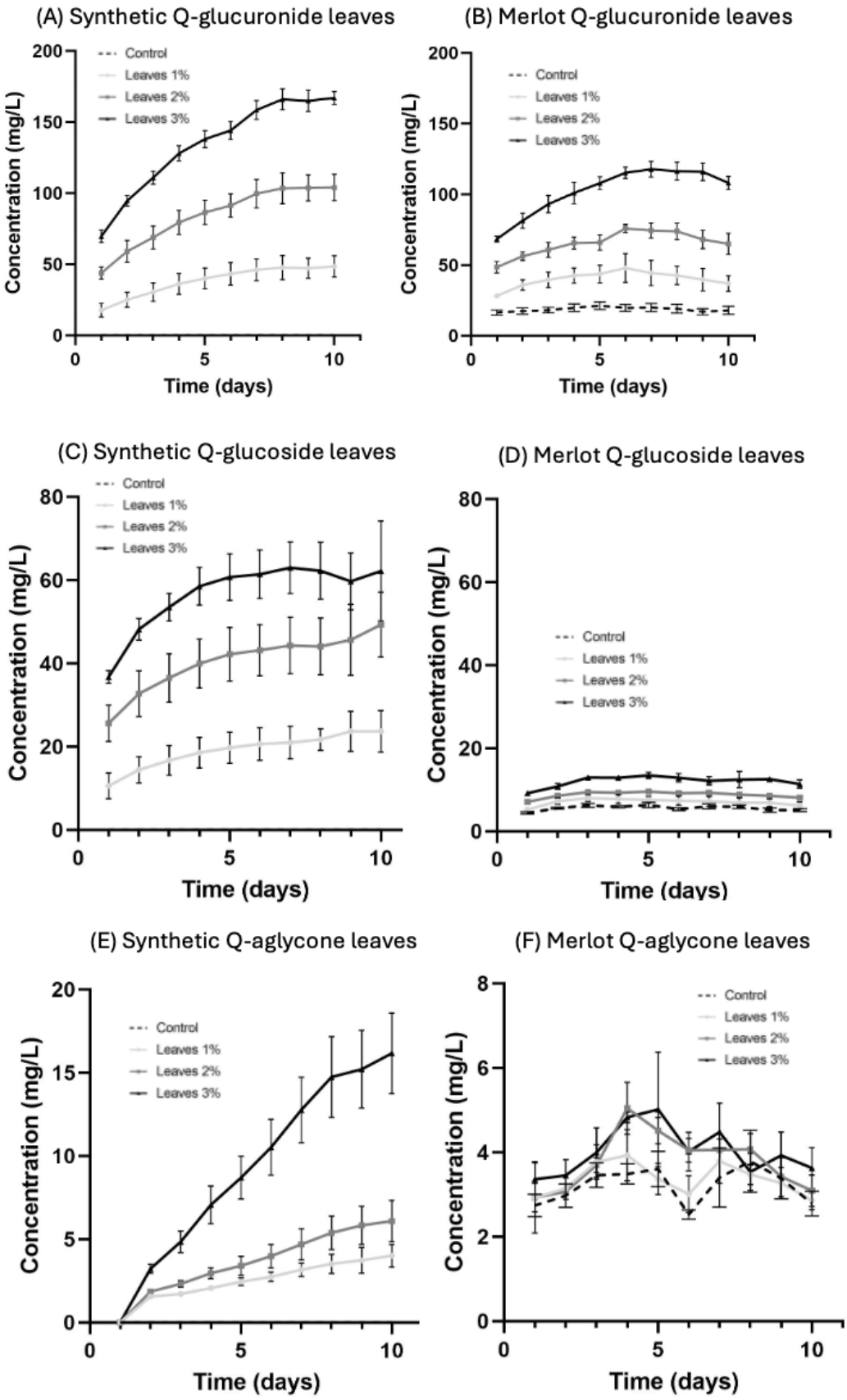


FIGURE 4. Quercetin evolution during fermentation in synthetic and merlot must with added leaves (n = 3).

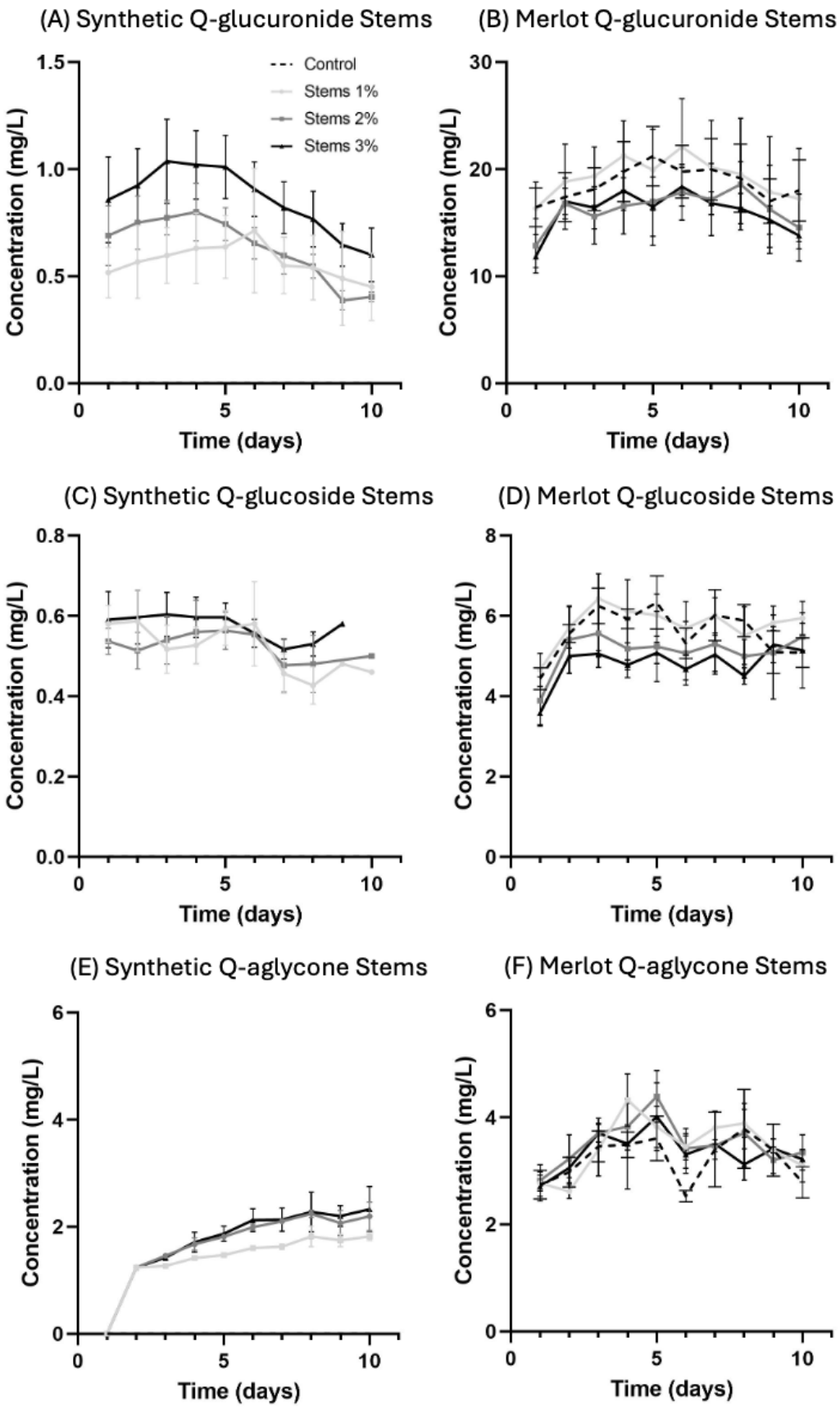


FIGURE 5. Quercetin evolution during fermentation in synthetic and merlot must with added stems (*n* = 3).

synthetic must due to the absence of interfering compounds, emphasising their potential for phenolic enrichment. In both systems, grape leaves substantially increase the levels of quercetin derivatives, while grape stems play a minor role.

The trend of quercetin evolution during fermentation can be summarised as follows: Quercetin-3-glucuronide increases rapidly during the early to mid-stages (Days 1–7), peaking on Day 7–10, and then declines due to enzymatic hydrolysis or interactions with other compounds. Quercetin-3-glucoside rises early (Days 1–5), peaking before quercetin-3-glucuronidee, and decreases as it is easily hydrolysed into quercetin aglycone. Quercetin aglycone increases steadily during the mid to late stages (Days 3–10) and stabilises or slightly declines by Day 10, likely due to complexation or precipitation. This evolution is more pronounced in synthetic must, where the absence of interfering compounds allows for clearer observation of these transformations.

5. Catechin

Catechin levels were analysed after 10 days of fermentation to complement polyphenol data (Table 4). In the synthetic must, the control exhibited a low catechin content (10.03 mg/L), with slight increases likely influenced by fermentation by-products. Catechin concentrations significantly increased with higher leaf concentrations ($P < 0.05$), showing a clear dose-dependent trend, particularly notable at 3 % leaf addition (+380 % *vs* control). Stem treatments showed a much stronger effect, with a +2096 % increase at 3 %, confirming their greater catechin-releasing capacity in the synthetic matrix. This aligns with findings from Pinot noir fermentation, where catechin levels increased from 228 mg/L in wines without added stems to 315 mg/L with whole bunch additions (Wimalasiri *et al.*, 2022). Analysis of fresh Merlot leaves and stems prior to fermentation supports these observations, with stems containing 21.79 ± 0.33 mg/g catechin, compared to only 3.81 ± 0.09 mg/g in leaves. In Merlot must, catechin concentrations increased by 10 % with 3 % leaf addition, but this change was not statistically significant. In contrast, 3 % stem additions led to a 14 % increase, which was statistically significant ($P < 0.05$), confirming the greater impact of stems on catechin enrichment in a more complex matrix. These findings confirm that mechanical harvesting, which increases the amount of matter other than grapes (MOG), such as stems, leaves, and petioles, can enhance the catechin content in wine. Mechanically harvested grapes have been shown to contain almost three times more phenolic compounds than hand-harvested grapes (Huang *et al.*, 1988). The addition of MOG, including stems, has been shown to increase the total phenolic, flavonoid, and tannin content in wine, including catechins (Huang *et al.*, 1988; Lan *et al.*, 2022). Stems are a natural source of tannins, which enrich wine by providing more structure and complexity (Suriano *et al.*, 2016). During maceration, contact with stems has been observed to enhance the levels of all flavan-3-ols, including catechins and oligomeric procyanidins (Lan *et al.*, 2022).

These results show that stems consistently contribute more catechins than leaves, likely due to their higher initial catechin content. This effect is more pronounced in the simpler synthetic matrix, where stems allow for clearer extraction, whereas the complex Merlot must matrix limits their relative impact.

TABLE 4. Catechin and total proanthocyanidin content in synthetic must and merlot must after fermentation with varying grape leaf or stem additions.

Samples (n = 3)	Catechin (mg/L)	Total proanthocyanidins (g/L)
Synthetic control	$10.03 \pm 0.01^{c/C}$	-
Synthetic L1 %	14.70 ± 0.07^{c}	-
Synthetic L2 %	23.31 ± 2.45^{b}	-
Synthetic L3 %	48.11 ± 0.71^{a}	-
Synthetic S1 %	25.38 ± 2.04^{C}	-
Synthetic S2 %	104.96 ± 33.95^{B}	-
Synthetic S3 %	220.21 ± 13.74^{A}	-
Merlot control	399.81 ± 10.61^{B}	$0.711 \pm 0.023^{b/B}$
Merlot L1 %	413.87 ± 0.32	0.753 ± 0.143^{b}
Merlot L2 %	421.28 ± 6.90	1.336 ± 0.140^{a}
Merlot L3 %	439.23 ± 17.82	1.705 ± 0.002^{a}
Merlot S1 %	431.52 ± 18.96^{AB}	1.162 ± 0.169^{AB}
Merlot S2 %	443.69 ± 5.89^{A}	1.233 ± 0.212^{AB}
Merlot S3 %	456.59 ± 1.07^{A}	1.689 ± 0.255^{A}

Different letters represent a significant difference in the Tukey HSD *post hoc* test. Statistically significant differences ($P < 0.05$) are indicated for leaves (a, b, c) and stems (A, B, C). where letters are absent. This indicates no significant difference among treatments.

6. Total proanthocyanidins

The quantification of total proanthocyanidins in Merlot musts with varying amounts of leaves or stems revealed their impact after fermentation (Table 4). Proanthocyanidins, key contributors to mouthfeel characteristics like astringency in red wines, increased with the addition of both leaves and stems. Proanthocyanidin concentrations significantly increased with higher leaf concentrations ($P < 0.05$), showing a clear dose-dependent trend. The most notable increase was observed at 3 % leaf addition, which resulted in a 140 % increase compared to the control. Stem treatments also produced a substantial rise in proanthocyanidin levels, with a 138 % increase at 3 %, confirming their comparable potential to enhance proanthocyanidin content during fermentation. While both leaves and stems enhanced proanthocyanidin content, leaves contributed slightly more at higher concentrations (2 % and 3 %). In the present study, higher levels of total proanthocyanidins were obtained in the treatment of grape leaves, highlighting their significant role in enhancing these compounds.

It is already known that the inclusion of leaves and stems during fermentation increases tannin levels by extracting phenolic compounds from these solid grape components (Huang *et al.*, 1988; Lan *et al.*, 2022). Stems are rich in proanthocyanidins and other phenolics, and their extraction depends on maceration time and ethanol concentration. Polar phenolics are extracted during the aqueous phase, while less polar compounds like proanthocyanidins are released as ethanol levels rise during fermentation (Ginjom *et al.*, 2011). Extended maceration with MOG further boosts phenolic extraction (Blackford *et al.*, 2021). Studies also show that wines fermented with stems have significantly higher tannin levels than those without, and higher stem percentages proportionally increase tannins (Wimalasiri *et al.*, 2022).

The results demonstrate that leaves and stems contribute to the enrichment of proanthocyanidins during fermentation, enhancing the tactile properties and complexity of red wines. Stems are confirmed to play an important role, but also grape leaves show substantial potential for proanthocyanidin enrichment, particularly at higher concentrations.

7. Colour

The addition of grape leaves influenced the colour parameters of Merlot must after fermentation (Table 5). Compared to the control (IC: 7.70), higher leaf concentrations (L2 % and L3 %) increased IC to 7.11 and 8.19, while lightness (L*) decreased to 22.8 in L3 %, corresponding to darker wines. Tonality (T) shifted toward redder hues, decreasing from 0.89 in the control to 0.84 in L3 %. Leaves also reduced redness (a*) and yellowness (b*), with a* dropping from 55.83 to 54.74 and b* from 40.22 to 36.98 at higher concentrations. As shown before, leaves increase wine's phenolic content by transferring flavonoids during fermentation. They may also influence colour tone, calculated as the ratio of absorbance at 420 nm (yellow) and 520 nm (red), shifting it toward reddish hues due to the contribution of non-anthocyanin flavonoids, as reported by (Guerrini *et al.*, 2018).

In contrast, grape stems consistently reduced IC, with S3 % showing the lowest value (6.10) compared to the control (7.70). This decrease may result from water released by stems, pH changes, or anthocyanin absorption (Blackford *et al.*, 2021). Lightness (L*) increased to 31.8 in S3 %, and tonality (T) rose to 1.00, indicating a shift toward yellower hues. Absorbance at 520 nm dropped from 3.67 (S1 %) to 2.71 (S3 %), suggesting dilution or stabilisation of red pigments.

Although statistical analysis (ANOVA) revealed no significant differences in colour parameters between the control and the different treatments with grape leaves or stems ($P > 0.05$), the comparison of average values highlights clear trends in how MOG addition influences wine colour. Leaves tended to enhance colour intensity and darken the wine, while stems reduced colour intensity and brightened the wine. Leaves also shifted tonality toward redder hues, likely due to their flavonoid contribution, whereas stems caused a shift toward yellow hues, possibly through dilution and pigment absorption mechanisms.

These findings are consistent with those reported by ASVO (Allan, 2003), which state that the addition of MOG at levels below 3 % (*w/v*) has an acceptable impact on wine quality. The present study confirms that, at these levels, MOG does not have a statistically significant effect on wine colour,

TABLE 5. The colour of Merlot must after fermentation with varying grape leaves and stems.

Samples (n = 3)	420 nm	520 nm	620 nm	IC	T	a*	b*	L*
Merlot control	3.28 ± 0.27	3.69 ± 0.53	0.73 ± 0.06	7.70 ± 0.86	0.89 ± 0.06	55.83 ± 1.38^{ab}	40.22 ± 2.17^{ab}	25.9 ± 2.6^{ab}
Merlot L1%	2.92 ± 0.15	3.01 ± 0.29	0.59 ± 0.07	6.51 ± 0.51	0.97 ± 0.05	58.10 ± 0.97^{a}	41.95 ± 0.43^{a}	29.7 ± 2.6^{a}
Merlot L2%	3.07 ± 0.02	3.38 ± 0.12	0.66 ± 0.03	7.11 ± 0.17	0.91 ± 0.03	57.41 ± 0.32^{ab}	41.07 ± 0.23^{ab}	26.9 ± 0.2^{ab}
Merlot L3%	3.38 ± 0.24	4.04 ± 0.56	0.77 ± 0.09	8.19 ± 0.90	0.84 ± 0.05	54.74 ± 1.65^{b}	36.98 ± 2.68^{b}	22.8 ± 2.3^{b}
Merlot S1%	3.14 ± 0.22	3.67 ± 0.57	0.70 ± 0.09	7.50 ± 0.88	0.86 ± 0.07	56.24 ± 1.86	40.67 ± 2.70	26.4 ± 3.2
Merlot S2%	2.71 ± 0.46	3.04 ± 0.77	0.60 ± 0.12	6.35 ± 1.34	0.90 ± 0.08	56.49 ± 1.10	40.20 ± 1.79	30.2 ± 5.3
Merlot S3%	2.72 ± 0.27	2.71 ± 0.30	0.66 ± 0.19	6.10 ± 0.74	1.00 ± 0.02	55.92 ± 0.92	41.43 ± 0.94	31.8 ± 1.1

Different letters represent a significant difference in the Tukey HSD *post hoc* test. Statistically significant differences ($P < 0.05$) are indicated for leaves (a, b) and stems (A, B). where letters are absent. This indicates no significant difference among treatments.

though a trend of influence was evident. Overall, leaves had a greater impact on enhancing and intensifying colour, whereas stems contributed more to brightness and tonality modulation. Thus, leaves influence wine colour more than stems in terms of intensity and phenolic enrichment.

CONCLUSION

Matter other than grapes (MOG) is generally regarded as an undesirable component in winemaking due to its capacity to negatively affect wine quality. This study demonstrates that even MOG levels below 3 % (*w/v*), considered acceptable for quality wine, also can significantly influence quercetin and other phenolic concentrations during fermentation.

Grape leaves were more effective in increasing quercetin concentrations, particularly glycosylated quercetin derivatives, which are precursors to the aglycone form known to precipitate during ageing. Stems contributed strongly to catechin and proanthocyanidin enrichment, potentially intensifying astringency and altering the mouthfeel of the wine. Both leaves and stems affected fermentation kinetics and modified key chemical parameters, including pH, acidity, and anthocyanin content, with implications for wine stability and sensory attributes.

A key contribution of this study is the detailed analysis of quercetin evolution in response to MOG addition, providing a mechanistic understanding of how harvest practices, particularly the degree of mechanical harvesting, can influence quercetin precipitation. The results offer practical guidance for managing MOG levels to preserve phenolic balance, improve wine quality, and minimise the risk of quercetin instability. Future research should explore the long-term stability of quercetin derivatives during wine ageing. Overall, these insights support more targeted winemaking strategies that consider vineyard and harvest decisions to reduce the risk of quercetin precipitation.

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