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SPECIAL ISSUE

# Screening of Italian red wines for quercetin precipitation risk index

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## ABSTRACT

Quercetin Q, a phenolic compound released from grape skins during red wine maceration, has been identified as a source of instability in bottled wines, particularly Sangiovese, due to its crystallisation. This phenomenon represents an economic challenge for producers and affects the clarity of wine and consumer perception. Recent studies have shown that anthocyanins, probably due to co-pigmentation, increase the solubility of Q, thereby reducing the risk of crystallisation. Based on these results, this study aimed to validate these findings in real red wines and to better understand the factors influencing the risk of Q precipitation. Thirty-two monovarietal Italian red wines from the same harvest, which differed significantly in phenolic composition, were analysed focusing on the quantification of Q-glycosides (Q-Gs) and aglycones in the wine bulk and the sediment when present at different time points. In 30 out of 32 wines, hydrolysis of Q-Gs occurred during the first year of ageing, but only in 2/3 of the wines analysed, Q remained soluble in solution. The remaining wines behaved like saturated solutions as Q was detected in insoluble precipitate: however, even after aging, no clear precipitation threshold could be determined. Partial least squares (PLS) regression showed that the most influential risk categories for Q precipitation were pH and the monomeric anthocyanin/flavanol ratio. In addition, the Pearson correlation analysis demonstrated that not only anthocyanins but also tannins help to make Q more soluble. Our results contribute to a deeper understanding of the factors influencing quercetin stability in red wines, with implications for producers seeking to mitigate precipitation risks.

**KEYWORDS:** quercetin, tannins, anthocyanins, risk index, red wines, Macrowine 2025

## INTRODUCTION

Quercetin (Q), a phenolic compound released from grape skins during red wine maceration, has been identified as a potential source of instability in bottled red wines, particularly in varieties such as Sangiovese (Lanati *et al.*, 2014). In grapes, Q is synthesised as glycosides in which sugar molecules are attached to the aglycone (Makris *et al.*, 2006). During the early stages of vinification, or later during wine ageing, enzymatic or acidic hydrolysis of these glycosides occurs, releasing the free aglycone into the medium. The aglycone is significantly less soluble than the glycosides in the hydroalcoholic matrix of the wine, increasing the likelihood of precipitation and sediment formation. The easy formation of crystals is thermodynamically favoured due to two main phenomena: Q molecules aggregate in π-stacking configurations and a network of intermolecular hydrogen bonding is formed among them (Filip *et al.*, 2013; Campo & Corral, 2022). The precipitation of Q can result in sedimentary deposits of the typical needle-like yellow crystals (Patel *et al.*, 2012), which negatively affect the visual clarity and perceived quality of the wine (Lanati *et al.*, 2014; Gambuti *et al.*, 2020a). However, other wine-coloured precipitates could contain Q due to the co-crystallisation of this molecule with pigmented wine components (Li *et al.*, 2022).

The content of Q and its glycosides in wine is strongly influenced by grape variety (Mattivi *et al.*, 2006), climatic conditions (Price *et al.*, 1995; McDonald *et al.*, 1998), winemaking practices applied during vinification (Gambuti *et al.*, 2004) and later during ageing (Fang *et al.*, 2007; Picariello *et al.*, 2023). There is no defined solubility range for Q in red wines in the literature even if Somers and Ziemelis (1985) defined a critical solubility threshold of 5 mg/L. This owes to the fact that Q solubility is strongly influenced by several factors, including pH, temperature, ethanol content, and the phenolic composition of the wine. In addition, it is important to consider the glycoside content in grapes and wines and factors impacting their hydrolysis such as time of ageing, temperature, and pH (Monagas *et al.*, 2005a). The pH of wine (usually between 3 and 4) influences molecular interactions of Q. At this acidic pH, the pH is well below the pKa values for the two successive deprotonation steps of its hydroxyl groups: 7.03 ($pKa^2$) and 9.15 ($pKa^2$) in water/ethanol mixtures (Bhatia *et al.*, 2022), so Q exists predominantly in its fully protonated, neutral form. Furthermore, the lower the pH of the wine, the greater the proportion of anthocyanins as flavylium cations, and the more effectively the positively charged flavylium cation can participate in non-covalent interactions, such as π-π stacking with Q, which is the main reason beyond the copigmentation phenomenon (Boulton *et al.*, 2001; Forino *et al.*, 2020). Therefore, the acidic pH not only ensured the fully protonated form of Q but also promoted its co-pigmentation interactions with anthocyanins. As wine contains Q-glycosides, pH is also a determinant for the rate and yield of hydrolysis of Q-Gs. Considering the importance of the red wine matrix, a previous study investigated the role of co-pigmentation in improving the solubility of Q (Lanati *et al.*, 2022). The interaction between Q and anthocyanins appears to stabilise Q in solution, reducing its tendency to precipitate. This finding was confirmed by Luciano *et al.* (2024) who showed that higher concentrations of anthocyanins in the model solution corresponded to higher concentrations of Q in the solution, however, these interactions are not stable underlining the importance of time in these kinds of molecular interactions. A positive correlation between Q solubility and bleachable pigments of red wine was also noted by Lanati *et al.* (2022) highlighting that compounds derived from red wine ageing under oxidative conditions (Coppola *et al.*, 2021) can have an impact on Q solubility.

Since wine represents a highly complex and dynamic chemical environment, factors such as pH, ethanol concentration, and the presence of other polyphenols such as tannins may also influence Q precipitation, it is necessary to evaluate the effect of wine composition on Q solubility and its evolution over time in real wines.

This study aims to investigate how the chemical composition of red wine can influence the behaviour of Q in solution over time. To this end, different red wines, mainly representative of Italian grape varieties with different phenolic compositions, were analysed. In addition, the study aims to define the main risk factors for predicting flavonol instability in red wine.

## MATERIALS AND METHODS

### 1. Wine samples

In this study, to explore a broad range of red wine matrices influencing Q solubility, different grape cultivars (16 Italian; 1 French) cultivated in various regions of Italy in 2023, were used to produce 100% single-cultivar wines used in the study. These wines were sourced from several wine producers. The wine set consisted of eight Aglianico (A1, A2, A3, A4, A5, A6, A7, A8 from Campania), three Barbera (B1, B2 from Campania; B3 from Piemonte), three Camaiola (C1, C2, C3 from Campania), one Casavecchia (CV from Campania), one Dolcetto (DP from Piemonte), three Merlot (M1, M2, M3 from Campania), one Montepulciano (MP from Puglia), one Nebbiolo (NEP from Piemonte), one Negroamaro (NEG from Puglia), one Nero d'Avola (NA from Puglia), one Nero di Troia (NT from Puglia), one Pallagrello Nero (PN from Campania), one Piedirosso (PD from Campania), one Primitivo (PP from Puglia), three Sangiovese (S1 from Puglia; S2 from Emilia-Romagna; S3 from Tuscany), one Susumaniello (SS from Puglia), and one Teroldego (TT from Trentino). All wines were fermented in stainless steel vats at a commercial scale and sampled before malolactic fermentation (MLF) and wood ageing (4 months after harvest). Each sample was preserved with $SO_2$ and bottled. Bottles were sealed with Select Green 100 corks (Nomacorc, Rivesaltes, France) and stored at a constant cellar temperature (16 ± 2 °C) until analysis (after 8 months of bottle ageing). The oenological parameters of the selected wines are reported in Table S1. Due to the diversity of grape varieties and vinification conditions, oenological parameters

varied among the samples. For the experiment two sampling times were considered: time 0 and time 1, corresponding to the moment of bottling and after 8 months of bottle ageing, respectively. Before HPLC analysis, all samples were filtered using polyvinylidene difluoride PVDF filters 0.45 µm (Durapore, Millipore, Ireland) membrane.

## 2. Wine sediment collection

To determine the factors influencing Q precipitation, it is necessary to evaluate the amount of Q in solution (Qsol) and, in sediment when present (Qsed). We evaluated both parameters at time 0 (4 months after vinification) and one year after harvest (after another 8 months of bottle ageing under control conditions). After 8 months of bottle storage, 350 mL of each wine was poured into a funnel and passed through a filter 2.0 µm pore size, hydrophilic glass fibre with a resin binder, 47 mm diameter (Millipore, Ireland) which retained the particulate matter. The filtrate was collected in a filter flask using a vacuum pump. The filters were dried in an oven at 37 °C for seven days. The material retained on each filter was carefully scraped, weighed, and dissolved in 10 mL of methanol to determine its content in flavonols. Although these conditions do not completely prevent degradation of Q or hydrolysis of glycosides, a study by Bhatia *et al.* (2022) on the effect of pH and temperature on Q degradation kinetics suggests that degradation should be limited to 37 °C.

## 3. Chemicals

All solvents were of HPLC quality and all chemicals were of analytical grade (> 99 %). Water (ultrapure), formic acid (95 %), acetonitrile (99.9 %), and methanol (99.9 %) were from Sigma-Aldrich (Milan, Italy). Commercial standards of quercetin dihydrate (> 99 %), quercetin-3-rhamnoside (also known as quercitrin) (> 98.5 %), quercetin-3-rutinoside (also known as rutin) (> 99 %), quercetin-3-glucuronide (> 99 %), quercetin-3-galactoside (also known as hyperoside) (> 98 %) and quercetin-3-glucoside (also known as isoquercitrin) were obtained from Extrasynthese (Genay, France). Sample filtration was carried out using 0.45 µm PVDF Durapore membrane filters (Millipore, Ireland). Malvidin-3-*O*-glucoside was purchased from Extrasynthese (Lyon, France). Bovine serum albumin, triethanolamine (> 99 %), iron (III) chloride, ethanol, acetic acid (≥ 99.5 %), and vanillin (ReagentPlus > 99 %) were purchased from Sigma-Aldrich (Milan, Italy). Aqueous solutions were prepared with Milli-Q water from Millipore (Bedford, MA, USA). Sodium decylsulfate was from Bioshop Canada, Burlington.

## 4. Wine main compositional parameters

The main compositional parameters of wines, reducing sugars, titratable acidity, volatile acidity, alcohol sulphur dioxide, and malic acid were determined following the procedure outlined in the OIV Compendium of International Methods of Wine and Must Analysis 2017.

## 5. Spectrophotometric analyses

Spectrophotometric analysis was conducted using a Jenway 7305 Spectrophotometer supplied by Exacta Labcenter SpA (San Prospero (MO) Italy). The colour intensity (CI) was determined as the sum of A420 nm, A520 nm, and A620 nm and the hue as the A420 nm/A520 nm ratio. Total anthocyanins, tannins reactive to bovine serum albumin (BSA), short polymeric pigments (SPP) large polymeric pigments (LPP), sum of polymeric pigments, and iron-reactive phenolic compounds were quantified using the Harbertson–Adams assay (Harbertson *et al.*, 2003). Vanillin reactive flavans (VRF) were determined as described by Gambuti *et al.* (2018). All analyses were performed in triplicate for each sample.

## 6. HPLC-DAD analysis of Q and Q-Gs

HPLC separation, identification, and quantification of flavonols were performed using an HPLC Agilent 1260 Infinity II LC apparatus (Santa Clara, USA) equipped with a binary pump (G7112B), an integrated two-channel degasser unit, and a UV–visible detector (G7114A). Data collection and analyses were performed through the software OpenLAB CDS ChemStation Edition (Agilent Technologies, Santa Clara, USA). The method described by Yang *et al.* (2017) was used with slight modifications. The chromatographic column used was a Poroshell 120 EC-C18 column (250 × 4.6 mm, 2.7 µm) with a pre-column UHPLC Guard 3PK (4.6 × 5 mm, 2.7 µm). An amount of 10 µL of either calibration standards or wine was injected into the column. All the samples were filtered through 0.45 µm PVDF Durapore membrane filters (Millipore, Ireland) into glass vials and immediately injected into the HPLC system. The mobile phase was set at a flow rate of 0.500 mL $min^{-1}$ and consisted of (solvent A) 0.3 % acetic acid in ultrapure water–acetonitrile mixture (8:1 v/v); and (solvent B) 0.3 % acetic acid in ultrapure water–acetonitrile (4:5 v/v). The following gradient was used: 0–45 min from 0 to 35 % B, 45–50 min from 35 to 100 % B, 50–55 min from 100 to 100 % B, 55–58 min from 100 to 0 % B, 58–64 min from 0 to 0 % B. The detection wavelength was set at 360 nm. A stock mixed standard solution (SMSS) was prepared by mixing the following flavonols in methanol to give a concentration of 100 mg/L for each of them: quercetin dihydrate (> 99 %), quercetin-3-rhamnoside (also known as quercitrin) (> 98.5 %), quercetin-3-rutinoside (also known as rutin) (> 99 %), quercetin-3-glucuronide (> 99 %), quercetin-3-galactoside (also known as hyperoside) (> 98 %) and quercetin-3-glucoside (also known as isoquercitrin). Calibration standards were prepared by pipetting SMSS into glass volumetric flasks and making up to volume with methanol to obtain six mixed standard solutions (MSS) for calibration. Calibration curves obtained by injecting MSS were characterised by a correlation coefficient ($r^2$) > 0.9991. The linearity range of the calibration curves is given in Table S2. Three analytical replicates were carried out for each sample. The sum of Q glycosides Q-Gs was calculated as the sum in mg/L of each glycoside.

### 7. HPLC-DAD analysis of anthocyanins

A Shimadzu LC10 ADVP system (Shimadzu Italy, Milan, Italy) with an SCL-10AVP system controller, two LC-10ADVP pumps, an SPD-M10AVP detector, and a model 7725 injection system (Rheodyne, Cotati, CA, USA) was employed for the separation and quantification of anthocyanins in red wines, following the procedure outlined in the OIV Compendium of International Methods of Wine and Must Analysis 2017. The analysis used a Waters Spherisorb column (250 × 4.6 mm, 4 µm particle size) with an attached precolumn. Calibration standards or wine samples (50 µL) were injected into the column. Monomeric anthocyanins were identified by comparing HPLC-DAD and LC-MS/MS data, as detailed in a prior study (Forino *et al.*, 2022). A stock standard solution (SMSS) was prepared by adding the malvidin-3-*O*-glucoside standard in methanol to give a concentration of 100 mg/L. Calibration standards were prepared by pipetting SMSS into glass volumetric flasks and making up to volume with methanol to obtain eight mixed standard solutions (MSS) for calibration; the calibration curve was plotted for malvidin-3-*O*-glucoside from the peak area, and the concentration was expressed as mg/L of malvidin-3-*O*-glucoside. Three analytical replicates were carried out for each sample.

### 8. HPLC-DAD analysis of acetaldehyde

The determination of acetaldehyde was carried out using HPLC, following the method described by Han *et al.* (2015). The HPLC analysis employed a SHIMADZU LC10 ADVP system (Shimadzu Italy, Milan) comprising an SCL-10AVP system controller, two LC-10ADVP pumps, an SPD-M10AVP detector, an SIL-20HTA autosampler, and a Waters Spherisorb column (250 × 4.6 mm, 4 µm particle size). Sample derivatisation was performed directly in glass vials successively adding the reagents as follows: 100 µL of wine sample or standard solution filtered at 0.45 µm PVDF Durapore membrane filters (Millipore, Ireland), 20 µL of freshly prepared sulfur dioxide solution (1120 mg/L), 20 µL of sulfuric acid (Carlo Erba reagent 96 %, 25 %), 140 µL of derivatisation reagent, 2,4-dinitrophenylhydrazine (Aldrich Chemistry, 2 g/L). The mixture was allowed to react for 15 minutes at 65 °C, then cooled to room temperature. Chromatographic conditions included a sample injection volume of 50 µL, a flow rate of 0.75 mL/min, a column temperature of 35 °C, and mobile phases consisting of (A) 0.5 % formic acid (Sigma-Aldrich, 95 %) in Milli-Q water (Sigma-Aldrich) and (B) acetonitrile (Sigma-Aldrich, 99.9 %). A gradient elution was applied with the following protocol: 35 % B to 60 % B (t = 8 min), 60 % B to 90 % B (t = 13 min), 90 % B to 95 % B (t = 15 min, 2-min hold), and 95 % B to 35 % B (t = 17 min, 4-min hold), for a total runtime of 21 minutes. Calibration was achieved using a derivatised acetaldehyde standard. All analyses were performed in triplicate for each sample.

### 9. Statistical analysis

One-way analysis of variance (ANOVA) was applied. Fisher's least significant differences procedure was used to discriminate among the means of the variables for all parameters determined with the assumption of variance homogeneity. Elaborations were carried out using the XLSTAT software (XLSTAT 2017). Partial least square (PLS) regression was carried out using the PLS module of the XLSTAT software (Addinsoft, 2009) to predict the precipitation of Q from the data collected. Data on the phenolic composition of wines were submitted to multivariate analysis (principal component analysis) performed using XLSTAT-Pro 7.5.3 (Addinsoft). The PCA biplot is used to effectively visualise the relationships between different wine samples based on their phenolic composition and colour.

## RESULTS

### 1. Phenolic composition of red wines

Grapes are rich in a variety of phenolic compounds distributed in their skins, pulp, and seeds, many of which are partially extracted during the winemaking process. In this study, to have a wide range of possible red wine matrices affecting Q solubility, Italian wines produced from 16 different autochthonous red cultivars and 3 Merlot wines were considered and characterised for their phenolic composition (Table S3). The wines differed in their phenolic composition, some being richer in tannins, such as CV, A6, S2, M1, M2, and NT, and others richer in anthocyanins (A1, C2, C3, A4).

To group the samples and highlight the key variables responsible for these separations, a Principal Component Analysis (PCA) was performed to gain further insight into the main parameters differentiating the red wines. The PCA (Figure 1) was performed on the data set (32 samples and 5 variables) to allow the identification of patterns and differences between samples based on their phenolic profiles. Two principal components were extracted, explaining a total of 77.27 % of the total variance. According to the variable loadings, the tannin/anthocyanin ratio emerged as the dominant contributor to the first principal component (PC1) followed by Q and tannins, conversely, monomeric anthocyanins have a negative correlation with PC1; colour intensity and monomeric anthocyanins were the most influential variables in the second principal component (PC2). The graph shows a relatively homogeneous distribution of the wines but with several outliers (C3; PD; NEP; M2). Two different groups of wines were identified. One group is characterised by a strong association with monomeric anthocyanins and colour intensity (C1; C2; A1; B2; A2; A4; A3; A5; B1; A7; SS; PN; DP; TT; B3); the second group is defined mainly by tannins and Q, indicating a higher concentration of these compounds (M1; M3; A8; NA; CV; MP; NEG; PP; S1; S2; S3; NT). Camaiola, Barbera, and Teroldego samples are mainly characterised by the anthocyanin content and colour intensity, S1, S2, and S3 by their Q content, as expected from the previous literature (Arapitsas *et al.*, 2020). The samples with the highest values of monomeric anthocyanins were displayed in the negative part of the X-axis, while the samples with the lowest content of monomeric anthocyanins and tannins were in the lower part of the scatterplot.

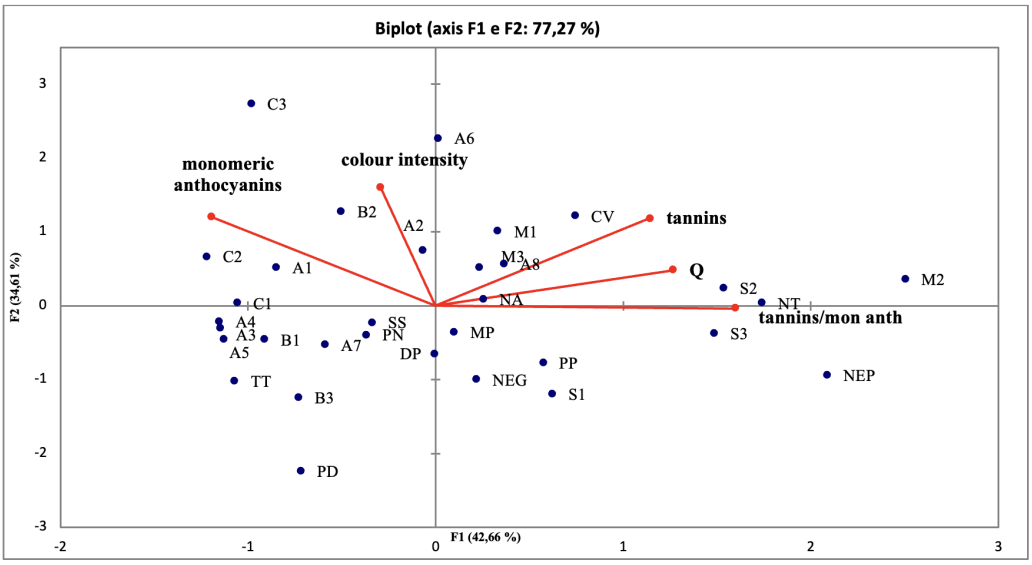



**FIGURE 1.** PCA score biplot of the first two PCs of the data set of monomeric anthocyanins, colour intensity, Q, tannins, and tannins/monomeric anthocyanins ratio in experimental wines.

## 2. Evolution of experimental wines over time

### 2.1. Content of Q and Q-Gs

The amount of Q in wine depends on several factors, including grape variety, winemaking practices, and environmental conditions. In addition, the literature points to significant variability in Q levels, not only between wines made from different grape varieties (Mattivi *et al.*, 2006) but also between wines made from the same grape variety (Buiarelli *et al.*, 2018; Gambuti *et al.*, 2020b). To avoid potential differences due to ageing and storage practices, all samples were taken directly from the tank, without prior contact with wood, and bottled in the laboratory under the same conditions.

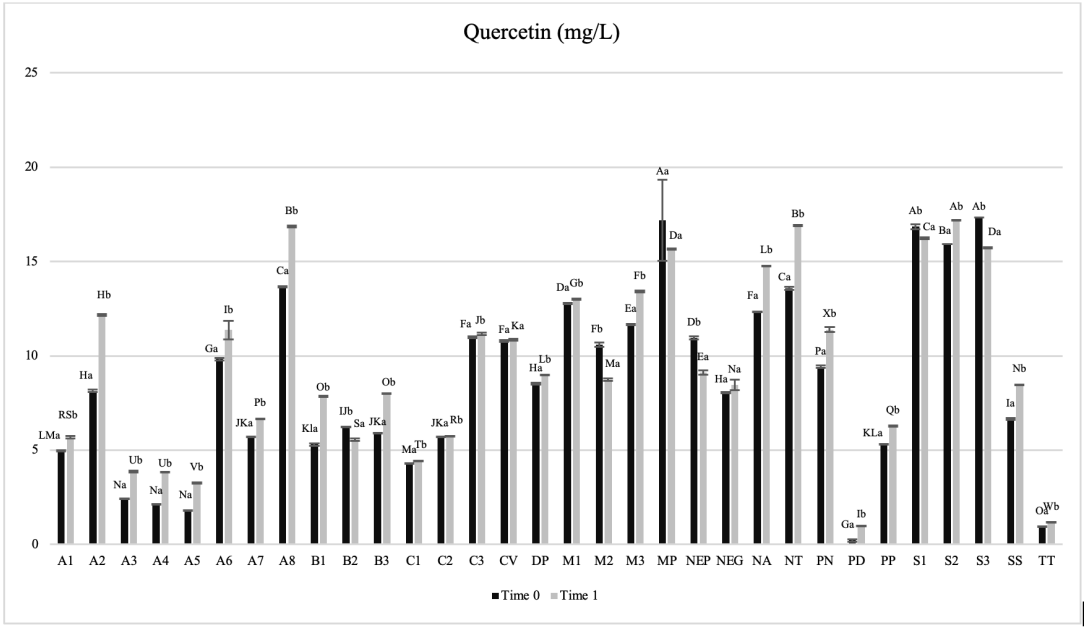



**FIGURE 2.** Quercetin content (mg/L) in experimental wines at time 0 and time 1. Different capital letters indicate significant differences ($p < 0.005$) among samples at the same time. Different lower-case letters indicate significant differences ($p < 0.005$) over time.

Figure 2 shows the Q content in all the experimental wines at time 0 and after 8 months of bottle storage. Before bottling (0 time), a wide range in Q content was observed in all the wines: values ranged between 0.22 and 17.3 mg/L; surprisingly, in 25 samples the content of Q in solution exceeded 5 mg/L, which corresponds to the proposed critical solubility threshold (Somers & Ziemelis, 1985). At time 0 no precipitate was observed in any of the samples, which means that Q is soluble in all wines. The high Q values in almost all wines with respect to previous studies (Buiarelli *et al.*, 2018; Lanati *et al.*, 2022) can be attributed to the fact that Q biosynthesis is strongly influenced especially

by solar radiation (Price *et al.*, 1995; Azuma *et al.*, 2012). In particular, the 2023 vintage was characterised by hot weather conditions (ISPRA).

As expected, the three Sangiovese samples had the highest Q content at time 0, with values ranging between (15.9–17.3 mg/L). MP is the second one in terms of Q abundance (17.2 mg/L). Moreover, A8, NT, M1, NA, and M3 show high levels of Q in solution. The data on Sangiovese and Nero d'Avola (NA) wines align with the findings of Simonetti *et al.* (2022), who quantified Q and Q-Gs levels in various Italian wines and identified Nero d'Avola and Sangiovese as the richest in Q and its analogues. PD is the sample with the lowest Q values (0.22 mg/L) followed by TT (1 mg/L). The lower amount of Q in these wines compared to others agrees with the results obtained by Gambuti *et al.* (2004) and Mattivi *et al.* (2006).

Regarding the effect of time on Q concentration, different behaviours were observed. Except for the CV that showed no variation in Q content over time, the samples can be divided into two groups: the first group, comprising the majority of the samples, showed an increase in Q content after 8 months of storage. This increase can be attributed to the hydrolysis of Q-Gs (Somers & Ziemelis, 1985). In contrast, in samples B2, M2, and S3, the Q content was lower than at time 0. In these cases, the decrease in Q concentration could be due to precipitation or reactions of oxidation and other phenolic compounds (Luciano *et al.*, 2024, Gambuti *et al.*, 2020a). It should also be noted that the wines analysed in this study were produced in the same year as the experiment, which may have further contributed to precipitation phenomena due to the general instability of wines. In young wines, tartaric precipitation is a common occurrence, as potassium bitartrate crystallises out of solution under certain conditions (Boulton *et al.*, 1999). Similarly, anthocyanin precipitation linked to colour instability may take place during the early stages of wine ageing. Both tartaric and anthocyanin precipitations could have caused the co-precipitation of quercetin, as these compounds can aggregate and settle together (Boulton *et al.*, 1999).

In addition, when assessing the risk of Q precipitation, it is important to consider the Q-Gs content, as they can hydrolyse releasing free Q (aglycone), and thus, the glycosides represent an additional risk factor.

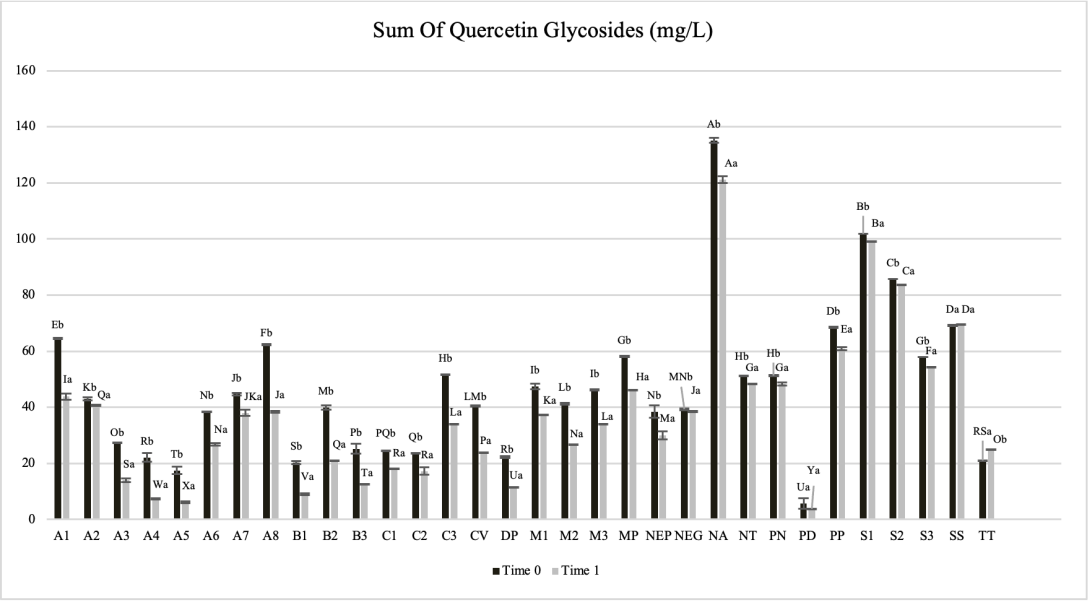



**FIGURE 3.** Content of Sum of Quercetin Glycosides (mg/L) in experimental wines at time 0 and time 1. Different upper-case letters indicate significant differences ($p < 0.005$) among samples at the same time. Different lower-case letters indicate significant differences ($p < 0.005$) over time.

Regarding the content of Q glycosides, at time 0 all the samples show levels of Q-glycosides comprised between 5.8 and 135.2 mg/L (Figure 3), the highest value was detected for NA followed by Sangiovese wines S1 and S2 confirmed to be the wines richest in Q-Gs (Gambuti *et al.*, 2020b); PD shows the lowest content of Q-Gs (5.8 mg/L).

Almost all wine samples exhibit a noticeable decrease in Q-Gs content over the storage, but the decrease varied among wines, and, for two samples (PD; SS) stable Q-Gs levels were detected. This observation is not surprising as the rate and extent of de-glycosylation are influenced by the specific structure of the flavonoid, the orientation of the bond between the sugar moiety and Q (α- and β-glycosidic bonds) (Zhang *et al.*, 2012), as well as the chemical-physical properties of the medium (pH, temperature, reaction time) (Dziadas & Jeleń, 2016).

## 2.2 Content of Anthocyanins

To evaluate the behaviour of Q in solution in terms of solubility, it is essential to consider another important phenolic fraction: anthocyanins. It is well known in the literature that Q is an efficient copigment of anthocyanins due to its extended π-conjugation (Trouillas *et al.*, 2016).

This efficiency is due to its ability to favour π-π stacking interactions and hydrogen bonding with anthocyanins (Waterhouse *et al.*, 2024). These interactions could help Q to stay in solution without precipitation (Lanati *et al.*, 2022; Luciano *et al.*, 2024).

Figure 4 shows the anthocyanin content determined with HPLC-DAD of all samples at different times. There is a significant variability in anthocyanin content already at time 0.

Two Camaiola and six Aglianico wines show the highest level of monomeric anthocyanins. PD and M2 show the lowest content of anthocyanins. Sangiovese wines have a low content of anthocyanins, as expected (Mattivi *et al.*, 1990).

From time 0 to time 1 there is a significant decrease in anthocyanins in all samples, this result is expected as it is well known that during the early stages of wine ageing, monomeric anthocyanins undergo various reactions with other phenolic compounds giving structures with high molecular weight (Fulcrand *et al.*, 2006). These reactions are crucial for the stabilisation of wine colour. Specifically, anthocyanins interact with tannins and flavanols to form polymeric pigments, which are more stable than their monomeric counterparts (Boulton *et al.*, 2001). Additionally, monomeric anthocyanins can undergo degradation due to oxidative processes (Verma *et al.*, 2023; Monagas *et al.*, 2005b). Given the substantial loss of monomeric anthocyanins observed across all the wines following bottle ageing, a proportionate decrease in Q solubility was expected, assuming monomeric anthocyanins were the sole determinants of the retention capacity of the wine matrix with respect to preventing Q crystallisation. However, the Q concentration detected in the aged wines (Figure 2) and the observed formation of deposits showed that the relationship between monomeric anthocyanins and Q solubility was not the only phenomenon explaining the observed outcomes.

## 3. Factors Influencing Precipitation Risks

Before bottling, the wines did not show any sediment, while after 8 months of bottle ageing some wines showed sediment (A7; B2; C1; C2; C3; CV; M1; M3; MP) and for all of them Q was found in the extract obtained from the sediment. The wines were then divided into two groups based on the presence or absence of Q sediment and the Spearman rank correlation test, a non-parametric test that measures the strength of association between two variables, was used to examine the associations between Qsol and Qsed, and the initial chemical parameters of red wines. To optimise the test, only the variables highly correlated with Qsol and Qsed were selected for correlation analyses, as shown in Figures 5 and 6.

As expected, a strong correlation was found between Qsol and the initial values of Q and Q-Gs in red wines. No significant correlation was observed between the Qsol concentrations and base parameters pH and ethanol content as well as with the content of anthocyanins. A moderate correlation was found between Qsol and Flavans, Fe-Phenols, Tannins, and the ratios Tannins/mon anth and Flavans/mon anth. The lack of a significant effect of anthocyanins was surprising, as previous research suggested that these compounds help to maintain Q in solution (Luciano *et al.*, 2024). However, in previous studies no other phenolic compounds were considered and, the effect of anthocyanins was significant but unstable. Considering that in this study the solubility of Q in real wines was considered together with all parameters

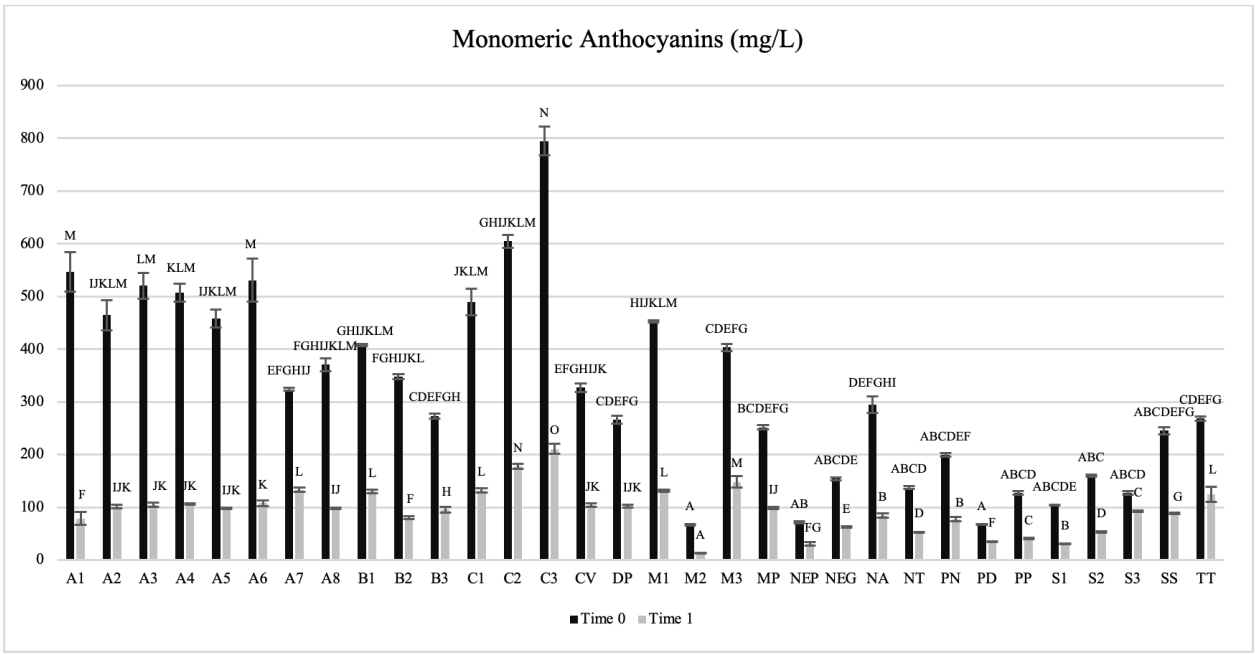



**FIGURE 4.** Content of monomeric anthocyanins (mg/L) in experimental wines at time 0 and time 1. Different uppercase letters indicate significant differences ($p < 0.005$) among samples at the same time. For each wine, statistically significant differences ($p < 0.005$) were observed at the two different time points considered.

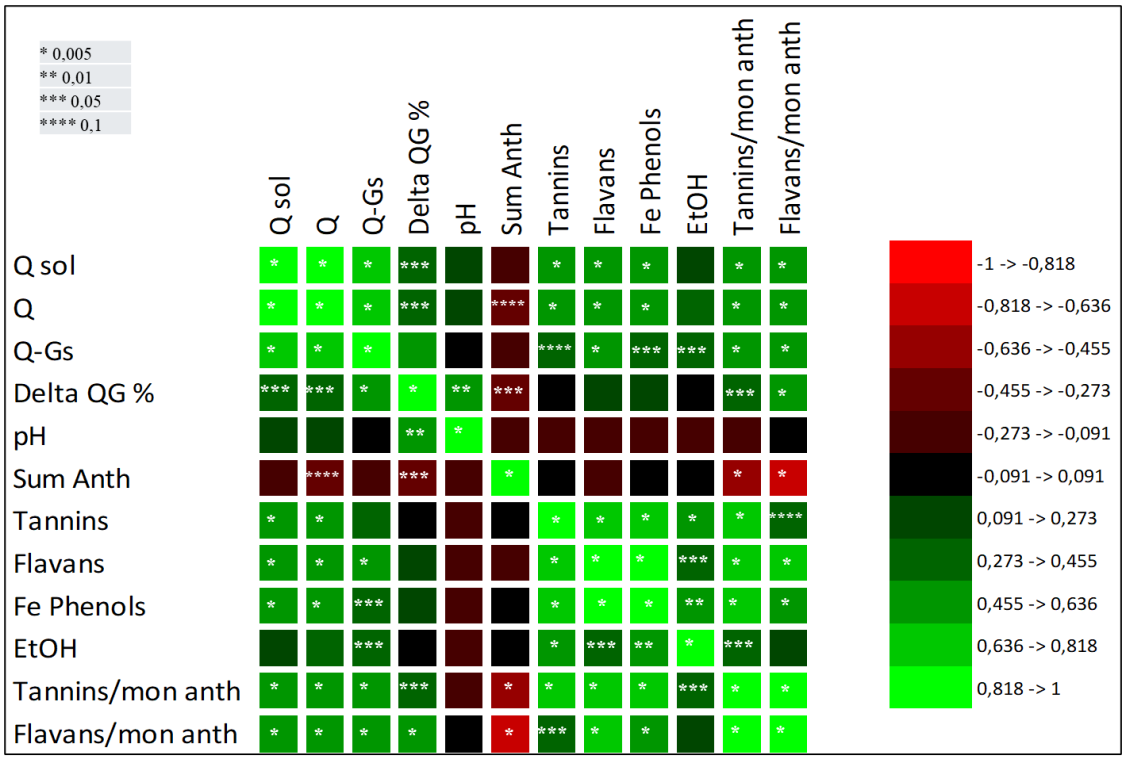



**FIGURE 5.** Correlation between Qsol and parameters of stable red wines (not showing any sediment after 8 months) before ageing (Spearman test: ****$p$ level < 0.1; ***$p$ level < 0.05; **$p$ level < 0.01; *$p$ level < 0.005).

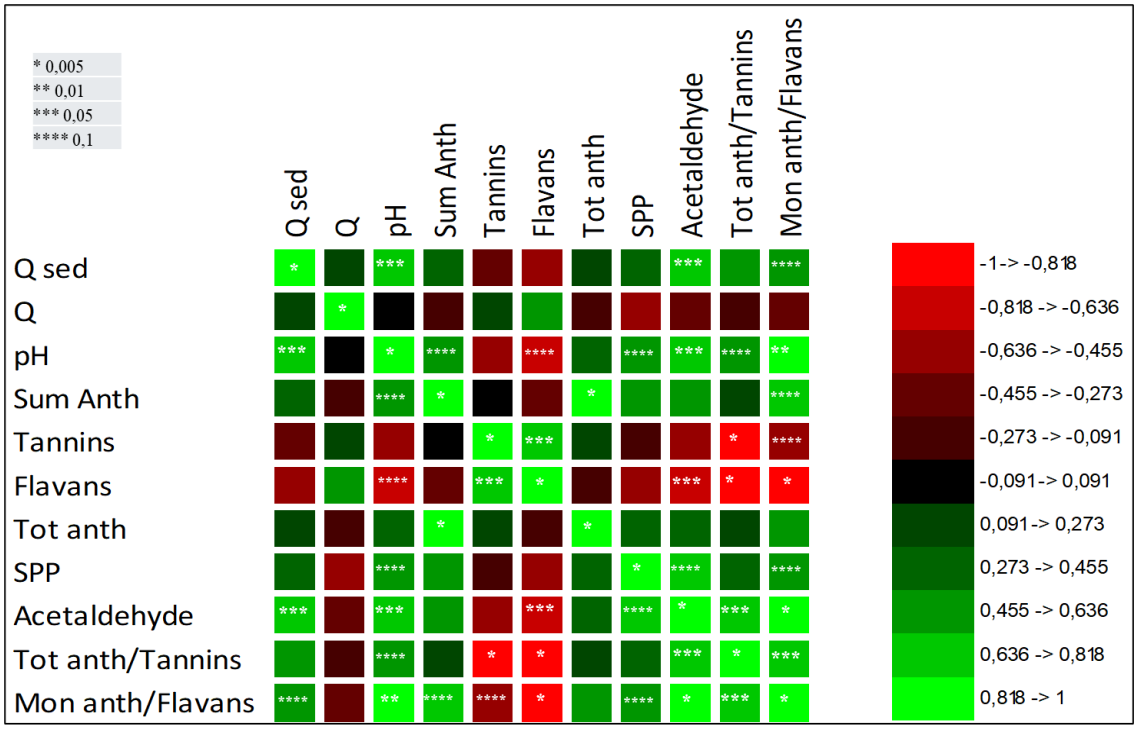



**FIGURE 6.** Correlation between Qsed and parameters of unstable red wines (showing a sediment after 8 months) before ageing (Spearman test: ****$p$ level < 0.1; ***$p$ level < 0.05; **$p$ level < 0.01; *$p$ level < 0.005).

characterising initial wines (Table S1 and Table S3), the results obtained are more indicative of real conditions. To try to explain the data obtained, it is necessary to consider the crystallisation process. It is known that some molecules in solutions can influence crystal growth rates by changing the properties of the solution and the equilibrium saturation concentration. In addition, the properties of the adsorption layer at the crystal-solution interface can be altered, affecting the further integration of growth units (Mullin, 2001). Factors that retard crystallisation include the presence of certain wine components that can be complex with the nucleation seeds and inhibit crystal growth. This is the case for potassium bitartrate crystallisation in red wine: phenolic compounds can be adsorbed on the nucleation seeds and crystal growth is more limited than in white wines (Balakian & Berg, 1968; Waterhouse *et al.*, 2024). Other studies have shown that the addition of tannic acid can completely prevent the formation of calcium carbonate crystals (Hadfi *et al.*, 2021). It is possible that similar mechanisms could explain the role of tannins on Q solubility. However, specific experiments on Q crystallisation in wine, in the presence or absence of tannins, could help to better understand these phenomena.

Instead, Qsed (Figure 6) was strongly correlated with pH, acetaldehyde content, and the mon antho/flavan ratio.

To try to explain the risk of precipitation of Q a satisfactory PLS model explaining Qsed was built considering the parameters of wines in which the formation of Q deposits was observed. The model was the following:

Qsed=–82.21726+0.91232*Q+22.87731*pH–0.01396*Sum Anth –0.00012*Tannins-0.00165*VRF-0.02377*Tot anth+1.21015*SPP+0.48721*$CH_3CHO$+6.54675*Tot Anth/Tannins-0,87133*mon anth/VRF

The model with 4 PCs explained 79.8 % of the original variance and indicated that the strongest factors impacting the content of Qsed are pH, SPP, acetaldehyde, and Tot Anth/Tannins. Instead Qsed is negatively correlated to the sum of monomeric anthocyanins, tannins, flavans, and total anthocyanins determined by Harbertson assay (Harbertson *et al.*, 2003).

Results for the pH effect could appear in contrast with Fusina *et al.* (2022) who showed that by increasing the pH water solubility of Q increases but, this is true for pH higher than 8. Given the acid-base properties of Q and pKa of its functional groups (Chebotarev & Snigur, 2015), it is not likely that there is a direct significant effect on solubility in the pH range of wine. On the other hand, pH has a strong influence on the percentage of flavylium cations in red wine (Peterson & Waterhouse, 2016). It is therefore not excluded that the low percentage of flavylium ions at higher pH values may limit the co-pigmentation and stacking reactions that help Q to be more soluble in wines (Boulton *et al.*, 2001). It is also possible that at a higher pH, by increasing the risk of crystallisation of potassium bitartrate, a possible co-crystallisation with Q is also favoured.

Regarding the positive correlation with acetaldehyde, it should be noted that acetaldehyde is one of the most important products of wine oxidation and it is a strong electrophile reacting with anthocyanins and flavanols giving condensation and polymerisation reactions (Peterson & Waterhouse, 2016). In a previous experiment, when red wines were subjected to controlled oxygenation, a positive correlation was observed between acetaldehyde levels and the initial anthocyanin/tannin ratio of red wines (Picariello *et al.*, 2023), confirming the significant positive correlation also found in the present study. In addition, the formation of insoluble products after acetaldehyde addition to red wine has been observed (Coppola *et al.*, 2021). Therefore, it is likely that co-precipitation of insoluble polymerised pigments and Q occurred in red wines with higher acetaldehyde levels. In fact, it is known that a higher anth/flavanol ratio favours the formation of lacquer-like pigmented deposits (Waters *et al.*, 1994) composed of a phenolic polymer of anthocyanins, procyanidins and protein (Cosme *et al.*, 2021).

## CONCLUSIONS

The analysis obtained in this study on real wines revealed that numerous factors are involved in the solubility of Q and, of these, the phenolic richness of the wines, especially in terms of tannins rather than anthocyanins, is the most important.

Regarding the risk of Q precipitation, PLS analysis indicates that at higher pH the risk is more pronounced. Although up to now Q-precipitation has been an issue related to a few varieties and could represent a rather marginal problem, climate change determines a higher exposure of grapes to solar radiation, and the consequent increased Q-Gs synthesis in all grapes, could determine a higher occurrence of Q instability in future wines with greater economic losses for this field.

Further microscopic and chemical analyses could help to understand the nature of Q in the deposit to continue searching for strategies to stabilise wines.

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