

Solubility of Quercetin in Wines

Donato Lanati¹, Patrizia Cascio¹, Matteo Pollon², Onofrio Corona², Dora Marchi^{1*}

(1) Enosis s.r.l., 11043, Fubine (AL), Italy

(2) Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy

Submitted for publication: May 2022

Accepted for publication: September 2022

Keywords: Solubility of quercetin, hydroalcoholic buffer solution, red wines, white wines

Quercetin solubility at 18°C and 0°C was determined in a hydroalcoholic buffer solution with a pH of 3.20 and in four Italian wines to study the formation mechanism of quercetin precipitate in wines. The wines selected were Barbera 2018, for its typically high content of bisulphite bleachable pigments, red Cirò 2014, for its typically high content of flavonoids, Sangiovese 2014, for the presence of quercetin deposits in the bottle, and white Cirò 2018, for the absence of red pigments. All the samples were spiked with 30 mg/L quercetin. The amount of quercetin solubilised at 18°C and 0°C in the hydroalcoholic buffer was much lower than in the wines, while that solubilised in Barbera was much higher compared to red Cirò, Sangiovese and white Cirò. Solubilised quercetin was lower in all wine samples stored at 0°C than in those stored at 18°C. The pigment composition of the three red wines examined suggests that the over-solubility of quercetin could be due to the formation of soluble co-pigmentation complexes between quercetin and monomer anthocyanins and/or bisulphate-bleachable flavanol-anthocyanin pigments. A positive correlation between quercetin solubility and bleachable pigment was noted: the richer the wine in bleachable pigments, the higher the solubility of quercetin. Quercetin haze formation appeared due to the release of quercetin from co-pigmentation complexes during wine maturation and storage, as its counterpart, anthocyanins, form non-bleachable pigments or are degraded in hydrolytic or oxidation reactions. Quercetin in aged red wines seems to reach a content similar to that of white wine spiked with quercetin. Nevertheless, the quercetin content of aged red wines in which a quercetin haze has been found could be lower than that when added to white wine, due to its degradation probably being induced by oxidation reactions. Finally, the solubilised quercetin content in white Cirò, which was higher than the hydroalcoholic buffer solution, suggests that there may be different substances in wines than pigments that prevent the growth of quercetin crystals. However, their nature was not determined in this study.

INTRODUCTION

Quercetin is a phenolic compound which that belongs to the class of flavonols whose glycosides are located in the skins of white and red grapes (Cheynier & Rigaud, 1986; Mattivi *et al.*, 2006; Makris *et al.*, 2006; Castillo-Muñoz *et al.*, 2007). Although to a lesser extent, white grapes also contain glycosylated derivatives of kaempferol and myricetin; while those of kaempferol, myricetin, isoramentin, laricitrin and syringetin are found in coloured grapes (Mattivi *et al.*, 2006; Castillo-Muñoz *et al.*, 2007). Quantitatively, quercetin is generally the most important flavonol in red and white grape skins. In some red grapes, however, the content of myricetin-3-glucoside can exceed that of any of the quercetin glycosides (Squadrito *et al.*, 2007). Flavonol biosynthesis begins shortly before véraison and can continue until the end of maturation (Keller & Hrazdina, 1998; Downey *et al.*, 2003; Castellarin *et al.*, 2007). It is affected by the level of exposure of the clusters to direct sunlight and could occur in response to and as a defence mechanism against UV-A rays. In shaded bunches it proceeds slowly or to a limited extent (Price *et al.*,

1995; Haselgrove *et al.*, 2000; Downey *et al.*, 2004; Cortell & Kennedy, 2006), while it is promoted in bunches directly exposed to sunlight (Downey *et al.*, 2004).

In white winemaking, the diffusion of flavonol glycosides from grape skin to the must is usually very little for the short contact time between the must and the grape skin. The content of quercetin glycosides is also very poor in must obtained from correctly cryo-macerated crushed white grapes (use of pre-cooled grapes, soft crushing, limited moving of crushed grapes, no SO₂). In red winemaking, the flavonols spread from the skin cells to the must during grape crushing and fermentative maceration. The reaction of the hydrolysis of flavonol glycosides with the production of individual aglycons starts at the same time. The rate at which this reaction occurs suggests that it is catalysed by enzymes. However, further studies are needed to elucidate its mechanism. Due to these hydrolytic reactions, quercetin has also been found in young red wines, sometimes reaching a higher level than expected on the basis of its solubility in wine: 3 mg/L to

*Corresponding author: E-mail address: direzione@enosis.it

4 mg/L (Somers & Ziemelis, 1985), around 5 mg/L (Boulton, 2001), and 3 mg/L (Gambuti *et al.*, 2020).

Despite this over-solubility, in the last few years quercetin haze has been found in some bottles of aged Sangiovese red wines after quite long periods since the end of wine maturation (Lanati *et al.*, 2014; Gambuti *et al.*, 2020). It does not deal with a new phenomenon, as quercetin deposits have also been found in a red wine in the past (Ziemelis & Pickering, 1969), as well as in a white one (Somers & Ziemelis, 1985). More recently, quercetin precipitate has been found in wines from some other cultivars and terroirs in both hemispheres (Marchi *et al.*, 2019), probably due to the current changes in climate. Even though it has been proven that the risk of formation of a quercetin haze decreases in wines that have undergone contact with oxygen (micro-oxygenation, maturation in the barrel, racking) (Lanati *et al.*, 2014; Gambuti *et al.*, 2020) due to the high antioxidant power of this compound, quercetin over-solubility and the mechanism of its precipitation in wines have to be rationalised. In order to clarify these problems, in this work we have studied the influence of the nature and content of wine pigments on the solubility of quercetin in wines of different ages and phenolic composition.

MATERIALS AND METHODS

Standards and reagents

Quercetin, myricetin and β -glucosidase were purchased from Sigma-Aldrich (Milan, Italy); and sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$), NaOH, NaCl, citric acid, ortho-phosphoric acid, methanol LiChrosolv and ethanol 96% v/v from Merck (Milan, Italy).

Composition of solutions

Quercetin solution was prepared as follows: 3 g/L of quercetin in methanol. Hydroalcoholic solution was prepared by placing 5 g/L of tartaric acid and 22.2 mL/L of 1 M NaOH in deionised water, and 125 mL/L of 95% to 96% ethanol was added. The final pH of the buffer was adjusted beforehand to reach a final volume with NaOH 1 M or HCl 1 M to 3.2.

Chemical analysis

Determination of flavonols in wine

Quercetin, myricetin and their glucoside and glucuronide derivatives have been determined by HPLC, as described by Lanati *et al.* (2021); briefly: 10 mL of wine was added to 0.5 g NaCl and extracted three times with 20 mL ethyl acetate. The solvent was evaporated under vacuum and the residue was dissolved in 2 mL of a solution composed of 60% 10^{-3} M H_3PO_4 in water and 40% methanol. Finally, the sample was filtered through a 0.45 μm membrane filter and injected for HPLC.

A Perkin-Elmer HPLC-DAD model Series 200 was used, equipped with a guard column LiChrospher® 100 RP-18 (5 μm) (LiChroCart® 4-4, Merck, Milan, Italy) and a column LiChrospher® 100 RP-18 (5 μm) (LiChroCart® 250-4, Merck, Milan, Italy).

Two solvents were used for the chromatographic elution: H_3PO_4 10^{-3} M in H_2O (solvent A); and methanol (solvent B). The gradient of analysis for the latter solvent was: 0% for 0 min, 5% for 5 min, 10% for 10 min, 30% for 25 min, 60%

for 40 min, 100% for 50 min, 100% for 60 min, and 5% for 65 min, with a solvent flow of 0.8 mL/min. The volume of injection was 20 μL and the wavelength of the diode array was fixed at 360 nm.

Quercetin and myricetin have been identified according to their chromatographic retention times and UV spectra in comparison with those of authentic standards; quercetin-3-glucoside and myricetin-3-glucoside have been identified from the disappearance of single peaks and an increase in the corresponding aglycons in the HPLC chromatogram, after treatment with β -glucosidase and 1 h incubation at 35°C (Vrhovsek *et al.*, 2004) of the extracts prepared as above, deprived of solvent and dissolved in citrate-phosphate pH 5.0 buffer; quercetin-3-glucuronide and myricetin-3-glucuronide have been identified from their UV spectra (similar to the ones of their glucoside derivatives) and the HPLC profile reported for the grape flavonols by Castillo-Muñoz *et al.* (2007).

Wines employed for quercetin solubility tests

The selection of the wine typologies employed for our experiment was done based on different chemical compositions that were needed to test our hypothesis and based on susceptibility to quercetin precipitation. Specifically, three types of wines in which the precipitation of quercetin has never been identified, namely Barbera (from the 2018 vintage), which is rich in anthocyanins and poor in flavanols (Cagnasso *et al.*, 2008), red Cirò (Gaglioppo-based wine from the 2014 vintage), which is poor in anthocyanins and rich in flavanols (Bosso *et al.*, 2019), and white Cirò (Greco bianco-based wine from the 2018 vintage), in which no anthocyanins are present, and then one typology of wine in which quercetin precipitation has been identified, namely Sangiovese (from the 2014 vintage).

Different levels of anthocyanins were chosen to test the influence of these substances on the solubility of quercetin in wine, and different ages and levels of flavanols were chosen to have variability in the polymerisation level of the colorant matter.

We chose a specific Sangiovese that has previously undergone precipitation of quercetin and in which we determined the profile of the flavonols both before precipitation (October 2017) and at the time of the event itself (March 2020).

Determination of quercetin solubility in pH 3.20 buffer solution and in wines

Procedure: 1 mL of 3 g/L quercetin solution in methanol was placed in 100 mL volumetric flasks and brought to 100 mL with pH 3.20 buffer solution or with red or white wines. After stirring, the samples (three for any trial) were stored at 18°C for three days in 100 mL dark glass bottles. Other samples of the same wines and buffer solution with 30 mg/L of quercetin added were stored at 0°C for three days. Thereafter, all the samples were centrifuged for 15 min at 4 000 rpm and the liquid phases were recovered and analysed following the methods reported in this section. The controls were the wines and buffer solution to which quercetin was not added; they were stored under the same conditions as the samples to which quercetin was added. The precipitates at 18°C and

0°C were solubilised in 10 mL of methanol and, when necessary, diluted with methanol : water at 1 : 1. After recording the absorption spectrum of the methanolic solutions of precipitates from 230 to 400 nm, A_{371} was determined by spectrophotometry. In the liquid phases (buffer solution and wines deprived of the precipitates), A_{371} was determined and converted to $A_{371, 10\text{mm}}$. In the control and the wines deprived of the precipitates, A_{420} , A_{520} and total anthocyanins, total flavonoids and dAl + dTA and dTAT (Glories, 1984) were also determined.

Determination of total anthocyanins, total flavonoids, A_{420}/A_{520} , $A_{420} + A_{520}$, dTAT% and d'TAT%

Total anthocyanins and total flavonoids were determined by spectrophotometry after wine dilution with ethanol:H₂O:conc.HCl 70:30:1 (ethanol-HCl) (Corona *et al.*, 2015) in which the λ_{max} of trisubstituted monomeric anthocyanins was 540 nm. Furthermore, small flavanol-anthocyanin polymers (Salas *et al.*, 2004; Fulcrand *et al.*, 2006) and flavanol-ethylidene-anthocyanin pigments (Atanasova *et al.*, 2002) were included in the total anthocyanins. According to this method, the A_{540} of the wine diluted in ethanol-HCl is expressed in mg/L malvidin-3-glucoside equivalents.

A_{420} and A_{520} were determined directly in the wine and expressed as $A_{420, 10\text{mm}}$ and $A_{520, 10\text{mm}}$. From these values it was possible to calculate A_{420}/A_{520} and $(A_{420, 10\text{mm}} + A_{520, 10\text{mm}})$. dTAT% and d'TAT% were determined respectively according to Glories (1984) and modified as reported by Corona *et al.* (2015). dTAT% and d'TAT% are the percentage fractions of A_{520} due to the non-bisulphite-bleachable pigments in wine and in wine diluted with a solution of HCl respectively.

Determination of dTAT% - In brief: 10 mL of wine and 2 mL 30% Na₂S₂O₅ in H₂O were placed in a 25 mL test tube. After stirring and 15 min of standing, the A_{520} of the sample was determined and expressed as $A_{1, 520, 10\text{mm}}$.

$A_{520, 10\text{mm}} = \text{dAl} + \text{dTA} + \text{dTAT}$ (A_{520} of the pigments in coloured form at wine pH, determined in non-diluted wine)

$A_{1, 520, 10\text{mm}} \times 1.2 = \text{dTAT}$ (A_{520} of non-bisulphite-bleachable pigments in coloured form at wine pH)

$A_{520, 10\text{mm}} - (A_{1, 520, 10\text{mm}} \times 1.2) = \text{dAl} + \text{dTA}$ (A_{520} of bisulphite-bleachable pigments in coloured form at wine pH)

$\text{dTAT\%} = [(A_{1, 520, 10\text{mm}} \times 1.2)/A_{520, 10\text{mm}}] \times 100$

dAl = A_{520} of free and co-pigmented monomeric anthocyanins in coloured form at wine pH

dTA = A_{520} of free and co-pigmented polymeric flavanol-anthocyanins in coloured form at wine pH

Dilution factor = 1.2

Determination of d'TAT% - In brief: 1 mL wine, 1 mL 96% ethanol:HCl conc. 99.9:0.1 v:v and 20 mL conc. HCl:H₂O 2:98 v:v were placed in a 50 mL flask. 10 ml of wine diluted in this manner were placed in two 25 mL test tubes, and 4 mL H₂O and 4 mL 15% Na₂S₂O₅ were added to each of the two respectively. After 15 min standing, the A'_{520} of the two samples was determined and these values were expressed as $A'_{1, 10\text{mm}}$ and $A'_{2, 10\text{mm}}$ respectively.

$A'_{1, 10\text{mm}} \times 30.8 = \text{d'Al} + \text{d'TA} + \text{d'TAT}$ (A'_{520} of total wine pigments in coloured form in wine acidified with HCl)

$A'_{2, 10\text{mm}} \times 30.8 = \text{d'TAT}$ (A'_{520} of non-bisulphite-bleachable pigments in coloured form in wine acidified with HCl)

$(A'_{1, 10\text{mm}} - A'_{2, 10\text{mm}}) \times 30.8 = \text{d'Al} + \text{d'TA}$ (A'_{520} of bisulphite-bleachable pigments in coloured form in wine acidified with HCl)

$\text{d'TAT\%} = (A'_{2, 10\text{mm}}/A'_{1, 10\text{mm}}) \times 100$

Dilution factor = 30.8

All the spectrophotometric determinations were made using an Agilent 8453 UV-Vis (Agilent Technologies, Santa Clara, CA, USA) spectrophotometer.

Statistical analysis

Statistical analyses were performed using the statistical software package SPSS (version 13.0, SPSS Inc., Chicago, IL, USA). The data analyses of the wines were processed using analysis of variance (ANOVA). To establish statistical differences by one-way analysis of variance (ANOVA), the Tukey b-test for $p < 0.05$ was used.

Linear regressions and Student's t test were performed with R version 4.0.3.

RESULTS AND DISCUSSION

Composition of Barbera 2018, Cirò 2014 and Sangiovese 2014 red wines

The data reported in Table 1 show that the red wines used in this study for the evaluation of quercetin solubility differed in terms of a few technological parameters (mainly ethanol % v:v and titratable acidity). The total flavonoid contents (monomeric and polymeric anthocyanins, flavanols) (Corona *et al.*, 2015) of Barbera 2018 and Sangiovese 2014 were less than that of red Cirò 2014 (Table 2). This result was expected for Barbera, but not for Sangiovese, whose grapes usually are poor (Cagnasso *et al.*, 2008) and rich (Benucci *et al.*, 2018) in flavanols respectively. Different from total flavonoids, the total anthocyanin content of Barbera was much higher than that of red Cirò (made from

TABLE 1

Base composition of wines used for determination of quercetin solubility

	Red Barbera 2018	Red Cirò 2014	Red Sangiovese 2014	White Cirò 2018
Ethanol % (v:v)	14.36 ± 0.16	13.21 ± 0.14	12.89 ± 0.13	11.96 ± 0.12
pH	3.41 ± 0.04	3.65 ± 0.05	3.46 ± 0.04	3.54 ± 0.04
Titratable acidity g/L*	6.40 ± 0.06	4.90 ± 0.05	5.20 ± 0.07	5.10 ± 0.06

*as tartaric acid

TABLE 2
Phenolic composition and colour parameters of red wines employed for determination of quercetin solubility

Wines	Red Barbera 2018		Red Barbera 2018 + Quercetin ^φ		Red Cirò 2014		Red Cirò 2014 + Quercetin ^φ		Red Sangiovese 2014		Red Sangiovese 2014 + Quercetin ^φ	
	Control	18°C	0°C	18°C	Control	18°C	0°C	18°C	Control	18°C	0°C	18°C
Total anthocyanins mg/L ^γ	298 ± 1.24 a	297 ± 1.53 a	299 ± 0.058 a	296 ± 1.53 a	71 ± 1.01 b	1680 ± 1576 ± 18.2 a	70 ± 1.34 b	69 ± 1.15 b	68 ± 1.58 a	68 ± 1.15 a	66 ± 1.83 a	68 ± 1.53 a
Total flavonoids mg/L ^δ	1174 ± 9.41 b	1157 ± 10.20 b	1072 ± 9.81 a	1046 ± 20.78 a	14.56 b	14.56 b	1576 ± 18.2 a	1562 ± 21.36 a	1278 ± 8.59 b	1278 ± 10.56 b	1568 ± 14.43 a	1242 ± 9.24 a
A _{371,10nm}	8.02 ± 0.00 a	8.38 ± 0.02 a	9.76 ± 0.31 b	9.98 ± 0.33 b	7.07 ± 0.02 b	7.07 ± 0.02 b	6.35 ± 0.11 a	8.12 ± 0.05 c	5.53 ± 0.03 b	5.43 ± 0.02 a	7.06 ± 0.04 b	5.82 ± 0.04 c
A _{420 10nm}	3.65 ± 0.00 a	3.65 ± 0.02 b	3.60 ± 0.01 b	3.62 ± 0.03 ab	2.49 ± 0.02 c	2.49 ± 0.02 c	2.38 ± 0.04 a	2.47 ± 0.01 bc	1.65 ± 0.01 b	1.65 ± 0.02 b	2.42 ± 0.01 ab	1.15 ± 0.02 b
A _{520 10nm}	6.33 ± 0.00 c	6.29 ± 0.03 b	6.00 ± 0.02 a	6.20 ± 0.06 b	2.08 ± 0.01 a	2.08 ± 0.01 a	2.16 ± 0.04 b	2.03 ± 0.01 a	1.02 ± 0.01 c	1.02 ± 0.01 b	2.04 ± 0.01 a	0.94 ± 0.01 b
A _{420 10nm} + A _{520 10nm}	9.97 ± 0.01 c	9.94 ± 0.05 bc	9.60 ± 0.02 a	9.82 ± 0.08 b	4.57 ± 0.01 b	4.57 ± 0.01 b	4.54 ± 0.06 b	4.50 ± 0.01 ab	2.18 ± 0.01 c	2.18 ± 0.01 c	4.46 ± 0.01 a	2.09 ± 0.02 b
A ₄₂₀ /A ₅₂₀	0.58 ± 0.00 a	0.58 ± 0.00 ab	0.60 ± 0.00 c	0.58 ± 0.00 b	1.20 ± 0.01 b	1.20 ± 0.01 b	1.10 ± 0.02 a	1.21 ± 0.00 b	1.15 ± 0.00 a	1.15 ± 0.00 a	1.18 ± 0.01 b	1.22 ± 0.02 c
dTAT _{10nm}	1.46 ± 0.09 C	1.46 ± 0.07 C	1.46 ± 0.07 C	1.46 ± 0.07 C	0.99 ± 0.05 B	0.99 ± 0.05 B	0.99 ± 0.05 B	0.99 ± 0.05 B	0.48 ± 0.02 A	0.48 ± 0.02 A	0.48 ± 0.02 A	0.48 ± 0.02 A
(dAI + dTA) _{10nm}	4.87 ± 0.07 C	4.87 ± 0.07 C	4.87 ± 0.07 C	4.87 ± 0.07 C	1.09 ± 0.05 B	1.09 ± 0.05 B	1.09 ± 0.05 B	1.09 ± 0.05 B	0.54 ± 0.02 A	0.54 ± 0.02 A	0.54 ± 0.02 A	0.54 ± 0.02 A
dTAT%	23 ± 1.18 A	23 ± 1.18 A	23 ± 1.18 A	23 ± 1.18 A	49 ± 1.46 B	49 ± 1.46 B	49 ± 1.46 B	49 ± 1.46 B	47 ± 2.12 B	47 ± 2.12 B	47 ± 2.12 B	47 ± 2.12 B
d*TAT %	12 ± 1.43 A	12 ± 1.43 A	12 ± 1.43 A	12 ± 1.43 A	36 ± 1.22 C	36 ± 1.22 C	36 ± 1.22 C	36 ± 1.22 C	31 ± 1.13 B	31 ± 1.13 B	31 ± 1.13 B	31 ± 1.13 B

^γ as malvidin-3-glucoside

^δ as (+)-catechin

^φ 30 mg/L

Different small letters (a, b, c) indicate significant differences between wines, while different capital letters (A, B, C) indicate significant differences between control wines. Tukey's HSD test, p < 0.05

Gaglioppo grapes rich in flavanols) and Sangiovese. This result was expected, as Barbera grapes usually are richer in anthocyanins than Gaglioppo and Sangiovese (Mattivi *et al.*, 2006). Moreover, trisubstituted anthocyanins are found in Barbera and Sangiovese grapes, while those in Gaglioppo are disubstituted (Mattivi *et al.*, 2006). In contrast to Barbera, the percentage of disubstituted anthocyanins in Sangiovese grapes could be high (Mattivi *et al.*, 2006) and can prevail over trisubstituted ones, in particular because of climatic conditions (Rinaldi *et al.*, 2021). Trisubstituted anthocyanins dominate in wines made with grapes from the three cultivars in question (Squadrito *et al.*, 2010).

The fact that λ_{\max} in the VIS region of Barbera diluted with ethanol:HCl (see Materials and Methods) was 540 nm and the λ_{\max} of red Cirò and Sangiovese diluted with ethanol:HCl was about 520 nm also suggests that monomeric anthocyanins, small anthocyanin-flavanol polymers, their co-pigmented forms and probably flavanol-ethylidene-anthocyanins prevail among the pigments of Barbera, while other classes of pigments were found in red Cirò and Sangiovese. Some of the latter may derive from anthocyanins (e.g. pyranoanthocyanins, anthocyanin-flavanol pigments, flavanol-anthocyanin pigments (De Freitas & Mateus, 2011; He *et al.*, 2012a, 2012b), others would be related to flavanols (xanthylum ions and brown polymers (Es Safi *et al.*, 2000)), and most of them would not be bisulphite-bleachable. Among the non-bleachable pigments there are also flavanol-ethylidene-anthocyanins (Escribano-Bailón *et al.*, 2001). On the other hand, the monomeric anthocyanins, the small flavanol-anthocyanin polymers and their co-pigmented forms are bleachable (Escribano-Bailón *et al.*, 2001).

The dTAT% (see Materials and Methods) of red Cirò 2014 and Sangiovese 2014 (49% and 47% respectively) shows that non-bleachable pigments are responsible for a large part of their A_{520} . The contribution of non-bleachable pigments to A_{520} of Barbera 2018 was much lower (dTAT% = 23%). The same conclusion can be drawn from the d'TAT% values of the wines diluted with HCl (Table 2). Barbera was richer than red Cirò and Sangiovese and red Cirò richer than Sangiovese in the bleachable and non-bleachable pigments contributing to the wines' A_{520} , as shown by the absolute values of dAl + dTA (4.870, 1.078 and 0.538 respectively) and dTAT (1.460, 0.997 and 0.477 respectively) (Table 2). Barbera therefore had young wine characteristics, while red Cirò and Sangiovese had characteristics of wines with a middle chemical age that had not achieved phenolic stability (the state in which most pigments are not bleachable, and in which the values of dTAT% and d'TAT% tend to coincide) (Di Stefano, personal communication, 2021). The dTAT% and d'TAT% values, and the A_{420}/A_{520} ratio (1.20 in red Cirò, 1.16 in Sangiovese), suggest that a significant part of the A_{520} of the wines from 2014 was due to pyranoanthocyanins ($\lambda_{\max} \cong 500$ nm) and/or to other non-bleachable pigments derived from anthocyanins and/or flavanols. Otherwise, $A_{420}/A_{520} = 0.58$ in Barbera suggests that monomeric anthocyanins and other bleachable pigments contribution to A_{520} wine value was prevalent. Nevertheless, the high absolute contribution of non-bleachable pigments and the $\lambda_{\max} = 540$ nm of the wine diluted with ethanol:HCl suggest that the non-bleachable pigments of Barbera could be flavanol-

ethylidene-anthocyanins. Although differences were evident between the red Cirò/Sangiovese and Barbera wines, the differences between red Cirò and Sangiovese only concern the values of A_{420} , A_{520} , A_{371} , $A_{420} + A_{520}$, dTAT, dAl + dTA, and the total flavonoid contents. All other colour parameters are practically the same: pH, dTAT%, d'TAT% and A_{420}/A_{520} .

Solubility of quercetin in pH 3.20 hydroalcoholic buffer solution and in Barbera 2018, red Cirò 2014, Sangiovese 2014 and white Cirò 2018 wines

To determine quercetin solubility in hydroalcoholic buffer solution in Barbera 2018, red Cirò 2014, Sangiovese 2014 and white Cirò 2018, 30 mg/L of quercetin was added to them and, after the precipitation of the over-soluble concentration, we established the value of interest. We chose this amount of quercetin because it is much higher than that found by Gambuti *et al.* (2020) and the literature reported therein. The 30 mg/L quercetin solution in methanol:H₂O 1:1 v:v added to the hydroalcoholic solution and the wines showed $\lambda_{\max} = 371$ nm and $A_{371, 10\text{mm}} = 2.415$ a.u.

Three days after the spike of quercetin, all the samples showed a precipitate. Subsequently to the removal of these crystals, we analysed the model solution and wines, and these results are reported in Table 3.

Starting from the $A_{371, 10\text{mm}}$ measured in the hydroalcoholic model solution, a quercetin solubility of 1.64 mg/L at 18°C and 1.16 mg/L at 0°C (Table 3) was calculated. The quercetin solubility found in this study for hydroalcoholic solutions seems to be in accord with that reported by Gambuti *et al.* (2020) for a similar solution to which 30 mg/L of quercetin had been added before being stored at 20°C for 15 days. Also, the solubilised quercetin in Barbera, red Cirò, Sangiovese and white Cirò was calculated using the values of $\Delta A_{371, 10\text{mm}}$ (Tables 2 and 3). Barbera treated solubilised 24.3 mg/L of quercetin (Table 3). If we add this value (24.3 mg/L) to the amount of quercetin already present in the control wine (3.5 mg/L, Table 4), the solubility of quercetin in this specific Barbera at 18°C becomes 27.8 mg/L. In the same wine, to which 30 mg/L quercetin has been added before storage at 0°C for three days, the solubilised quercetin was 19.4 mg/L. This decrease in quercetin content is the result of the relationship between the solubility of a solid and temperature.

The red Cirò control contained 3.0 mg/L of quercetin (Table 4), while our experiments at 18°C showed a solubilisation of 13.0 mg/L and the 0°C trial showed 9.6 mg/L of solubilised quercetin (Table 3). By adding these values to the original content of quercetin in red Cirò, the solubility under these conditions become 16 mg/L at 18°C and 12.6 at 0°C. Regarding the white Cirò, we did not find any presence of quercetin before the addition of 30 mg/L of the same flavanol, which means that the solubilised quercetin could be regarded as equal to the solubility. In our study, we observed a solubilisation of quercetin of 7.30 mg/L at 18°C and 5.51 mg/L at 0°C. Regarding the solubilisation in Sangiovese wine, we found 3.5 mg/L of quercetin for the 18°C trial and 2.4 mg/L quercetin for the 0°C trial (Table 3). If the contents of quercetin solubilised are added to the Sangiovese control concentration (5.3 mg/L, March 2020),

TABLE 3
Quercetin solubilised in pH 3.20 hydroalcoholic tartaric buffer solution, red Barbera 2018, red Cirò 2014, red Sangiovese 2014 and white Cirò 2018 wines spiked with 30 mg/L quercetin, calculated from $\Delta A_{371,10\text{mm}}$ (mg/L)

Tartaric buffer / wines	pH 3.20 tartaric buffer		Red Barbera 2018		Red Cirò 2014		Red Sangiovese 2014		White Cirò 2018	
	18°C	0°C	18°C	0°C	18°C	0°C	18°C	0°C	18°C	0°C
$\Delta A_{371,10\text{mm}}$	0.13 ± 0.02	0.09 ± 0.01	1.96 ± 0.00	1.56 ± 0.25	1.05 ± 0.05	0.77 ± 0.04	0.29 ± 0.05	0.20 ± 0.06	0.59 ± 0.04	0.45 ± 0.06
Solubilised quercetin	1.64 ± 0.19	1.16 ± 0.12	24.3 ± 0.00	19.4 ± 0.66	13.0 ± 0.63	9.55 ± 0.47	3.51 ± 0.61	2.81 ± 0.05	7.30 ± 0.53	5.51 ± 0.72

λ_{max} of the solution of 30 mg/L quercetin in methanol:H₂O 1:1 (v:v) was 371 nm and $A_{371,10\text{mm}} = 2.415 \pm 0.05$ u.a.

Quercetin solubilised in samples of the pH 3.20 hydroalcoholic buffer spiked with 30 mg/L quercetin, calculated from the analysis of the precipitate, was: 1.95 ± 0.12 at 18°C and 1.21 ± 0.16 at 0°C

TABLE 4

Quercetin, myricetin and some of their derivatives found in control red wines employed for the determination of quercetin solubility (as quercetin equivalents mg/L)

Wines	Red Sangiovese 2014		Red Barbera 2018		Red Cirò 2014		Red Barbera 2018	
	October 2017	March 2020	March 2020	March 2020	March 2020	March 2020	March 2020	March 2020
Myricetin glucuronide	0.8	0.7	0.3	0.2	0.3	0.2	0.3	0.2
Myricetin glucoside	3.0	2.0	0.4	0.1	0.4	0.1	0.4	0.1
Quercetin glucuronide	6.0	5.1	2.4	0.6	2.4	0.6	2.4	0.6
Quercetin glucoside	2.9	1.6	0.7	0.1	0.7	0.1	0.7	0.1
Myricetin	4.4	3.5	2.4	3.7	2.4	3.7	2.4	3.7
Quercetin	11.3	5.3	3.0	3.5	3.0	3.5	3.0	3.5
Quercetin precipitate		4.8						

the solubility of quercetin in this specific wine determined at 18°C is 8.8 mg/L and 7.7 mg/L at 0°C.

This result shows that the solubility of quercetin in Sangiovese wine in March 2020 (8.8 mg/L) was higher than the content found in the wine (5.3 mg/L). The amount of quercetin found in the wine in October 2017 (11.3 mg/L, Table 4) probably was also lower than its solubility at that date, which suggests that the quercetin content of the 2014 Sangiovese was even higher before the start of the ageing process. The decrease in the solubility of quercetin over time has recently been reported by Gambuti *et al.* (2020). As reported above, a quercetin precipitate in the bottle of Sangiovese wine selected for this study has been observed since October 2017 (amount not determined). In the bottle sampled on March 2020, 4.8 mg of precipitated quercetin was found – lower than that estimated from the decrease of quercetin in solution in the period between the two analyses (6 mg/L), plus the quercetin theoretically produced by the hydrolysis of its main derivatives (2.2 mg/L) and the quercetin previously precipitated (not determined). This could be due to the variability between bottles (not determined), or to the fact that part of the quercetin released by the hypothetical soluble complexes in which it was involved had undergone degradation reactions, due to quercetin's considerable tendency to be oxidised (Danilewicz, 2003).

Phenolic compounds are the major candidates that form with quercetin soluble complexes from which they could be released, as their structure undergoes profound changes during the maturation and storage of wine. The influence of other compounds whose changes could be significant during the ageing and storage of wine (e.g. volatile compounds) appears unlikely, as no interaction has been reported between them and quercetin. The same can be said for the other wine compounds, as they remain virtually unchanged over time and cannot influence the evolution of quercetin solubility. Given that the phenolic composition of Sangiovese 2014 was not determined in October 2017, but only in March 2020, it is not possible to directly assess the influence of the evolution of these compounds on quercetin solubility. However, some information can be obtained from the comparison of phenolic compositions determined in Sangiovese 2014, Cirò 2014 and Barbera 2018 in March 2020, in which very different amounts of the added quercetin were solubilised. The composition of the three red wines in question (Table 2) suggests that the quercetin amount solubilised in them probably did not depend on the flavanol content. In fact, although the total flavonoids in red Cirò and Sangiovese were higher than and similar to those in Barbera respectively (Table 2), the solubility of quercetin in them was lower. The presence of a higher anthocyanin content in Barbera compared to red Cirò and Sangiovese (Table 2) also suggests that the higher solubility of quercetin in Barbera could be due to its high content in the compounds that form with quercetin-soluble complexes (co-pigmentation complexes) (Baranac *et al.*, 1997). At the same time, because red Cirò and Sangiovese are poor in monomer anthocyanins, their quercetin over-solubility compared to the hydroalcoholic buffer solution could be due to polymeric pigments and/or other unidentified substances.

The fact that Barbera was much richer than red Cirò and

Sangiovese in anthocyanins and other bisulphite-bleachable pigments, as is evident from dAl + dTA (Table 2), suggests that it was the absolute content of these compounds that affected the amount of quercetin solubilised. On the other hand, there is a positive correlation between dAl + dTA and quercetin solubility.

It can be seen from Fig. 1a and 1b and Table 5 and Table 6 that there is a significant positive linear relationship between the concentration of bleachable pigments (monomeric anthocyanins and small polymers of the colouring matter) and quercetin solubility in wines.

At each temperature, both the intercepts and the slopes were significantly different from zero (Table 5 and Table 6), which suggests (in the case of the intercept previously explained) that there is dependence between the solubility of quercetin and bleachable pigments, while in the case of the intercept, as it was already clear earlier that a certain solubility of quercetin occurs at a zero value of bleachable pigments.

If the bleachable pigments are the only compounds capable of increasing the solubility of quercetin in wines, we would expect that the intercept values obtained in our linear models would be similar to the solubility values determined in the model solutions, as happens in the case of white Cirò, as the model solution, as in the case of the wine just mentioned, is devoid of bleachable pigments both in the monomeric and polymeric form. However, observing the solubility values in Table 7, the solubility of quercetin in the model solution is lower than that in white Cirò, and occurs consistently for both temperatures at which the determinations were done.

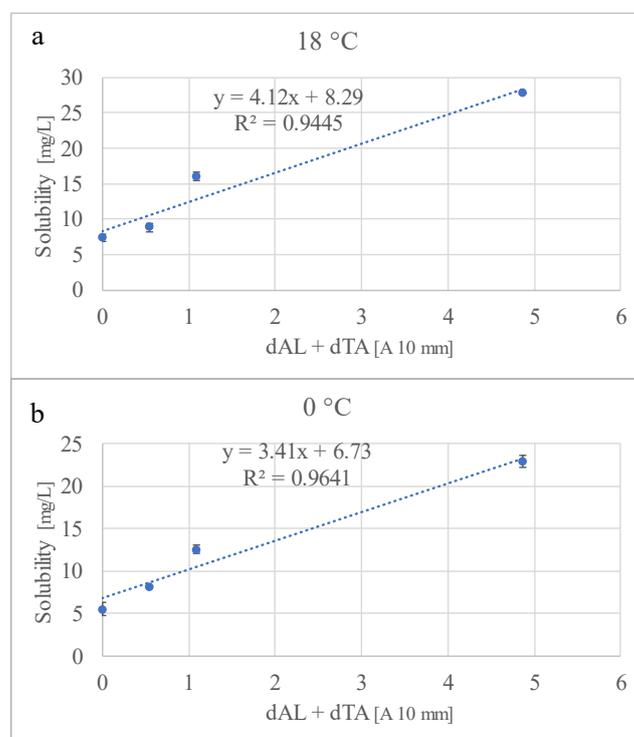


FIGURE 1

Linear regression between bleachable pigments (dAL plus dTA) and quercetin solubility in wines

These differences are also strongly supported statistically by Student's t test ($p(t) < 0.001$). This indicates that there are other substances capable of increasing the solubility of quercetin in wines besides bleachable pigments.

The exponential trend of the line that fits with the experimental data (Table 5 and Table 6) shows that the higher the content of bleachable pigments (proportional to $dAl + dTA$), the higher the solubility of quercetin. An influence from the non-bleachable pigments appears unlikely, as their absolute amount in Barbera (the wine richer wine in solubilised quercetin) was not much higher than that in red Cirò ($dTAT_{10mm}$ 1.46 a.u. and 0.99 a.u. respectively), while the absolute content of bleachable pigments was much higher ($dAl + dTA$ 4.87 a.u. and 1.09 a.u. respectively). Sangiovese, in which the solubility of quercetin was lower, represents a separate case, as it was poorer in both total flavonoids and pigments than Barbera and red Cirò (Table 2). However, the content of bleachable pigments found in red Cirò and Sangiovese ($dAl + dTA$ 1.09 a.u. and 0.54 a.u. respectively) could be due their quercetin over-solubility compared to the hydroalcoholic solution, which did not contain pigments. The difference between bleachable pigment contents ($dAl +$

dTA 1.09 a.u. and 0.54 a.u. respectively) could also explain the difference in quercetin solubilised in red Cirò and Sangiovese. Indeed, the quercetin decrease in Sangiovese from 2017 to 2020 (Table 4) could be due to the decrease in bleachable pigments and the increase in non-bleachable ones during this time.

The decrease in bleachable pigments and the increase in non-bleachable ones in red wine is supported by the literature. The absence of quercetin precipitate in red Cirò and its presence in Sangiovese wines could be due to their initial content of quercetin and quercetin glycosides, which is generally much higher in Sangiovese (Gambuti *et al.*, 2020) than in Cirò (see Mattivi *et al.* (2006) for the flavonol content of grapes). On the other hand, the above results prove that at least some of the quercetin that can exist in solution in a wine depends on the wine content of bleachable pigments that form with quercetin-soluble co-pigmentation complexes. The evolution of anthocyanins and other bleachable pigments to produce non-bleachable pigments during wine ageing and storage is due the release of quercetin from soluble co-pigmentation complexes and its precipitation.

TABLE 5

Linear regression between bleachable pigments and quercetin solubility coefficients and their statistics in wines at 18°C

18°C	Coefficient	Std. error	Sign.	R ²
Intercept	8.29	1.77	*	0.9445
Slope	4.12	0.7052	*	

Std. error = standard error of the coefficient; Sign. = difference from zero significance of Student's t test; sign. = level of significance of t test : ns.: $p(t) > 0.05$; *: $0.01 \leq p(t) < 0.05$; **: $0.001 \leq p(t) < 0.01$; ***: $p(t) < 0.001$; R² = coefficient of determination of the linear model

TABLE 6

Linear regression between bleachable pigments and quercetin solubility coefficients and their statistics in wines at 0°C

0°C	Coefficient	Std. error	Sign.	R ²
Intercept	6.73	1.17	*	0.9641
Slope	3.41	0.46	*	

Std. error = standard error of the coefficient; Sign. = difference from zero significance of Student's t test; sign. = level of significance of t test : ns.: $p(t) > 0.05$; *: $0.01 \leq p(t) < 0.05$; **: $0.001 \leq p(t) < 0.01$; ***: $p(t) < 0.001$; R² = coefficient of determination of the linear model.

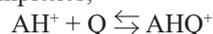
TABLE 7

Student's t test results applied in the comparison of quercetin solubility in white Cirò and in the model solution

Statistic	Solubility at 18°C	Solubility at 0°C
pH 3.20 tartaric buffer	1.64 ± 0.19	1.16 ± 0.12
White Cirò	7.30 ± 0.53	5.51 ± 0.72
t value	17.41	10.32
p. (t)	$6.39 \cdot 10^{-5}$	$4.97 \cdot 10^{-4}$
Sign.	***	***

t value = Student's t value; p. (t) = integral from right on the t distribution corresponding to the t value; sign. = level of significance of t test; ns.: $p(t) > 0.05$; *: $0.01 \leq p(t) < 0.05$; **: $0.001 \leq p(t) < 0.01$; ***: $p(t) < 0.001$. Data are reported as mean ± standard deviation of three repetitions

If the soluble complexes in which quercetin is involved are co-pigmentation complexes,



and/or



can be the relationships that bind quercetin (Q), flavylum (AH⁺) or quinonoid (A) monomers and small polymer flavanol-anthocyanin and co-pigmentation complexes (AHQ⁺, AQ) (Brouillard *et al.*, 1991). These equations also show that, as the anthocyanins decrease (polymerisation, degradation, other reactions), the equilibrium shifts to the left, with quercetin release that can precipitate if its content exceeds its solubility in that particular wine, or degraded if it is involved in oxidation reactions. Therefore, the quercetin decrease from 11.3 mg/L to 5.3 mg/L in Sangiovese 2014 from October 2017 to March 2020 could be due to its precipitation and/or degradation after its release from the co-pigmentation complexes. The solubilisation in March 2020 of 3.5 mg/L of added quercetin in Sangiovese 2014 also suggests that, besides precipitating, part of the quercetin in solution in wine in October 2017 (11.3 mg/L) and that produced by hydrolysis of its glycosylated derivatives, undergoes degradation reactions, reaching a lower content (5.3 mg/L) than what the wine was able to solubilise (8.8 mg/L) at that date. On the other hand, the comparison between flavonol composition in October 2017 and at the end of March 2020 (Table 4) allows the observation that the hydrolysis of flavonol glycosides continued over time (theoretical quercetin produced 2.2 mg/L), albeit slowly, and was not yet complete in March 2020. In accordance with Spayd *et al.* (2002), the hydrolysis rate of quercetin-3-glucoside could be higher than for quercetin-3-glucuronide.

The $\Delta A_{371, 10\text{mm}}$ for the white Cirò 2018 samples spiked with 30 mg/L quercetin and the white Cirò 2018 control, some of which was stored at 18°C and some at 0°C for three days, were 0.59 a.u. and 0.45 a.u. respectively (Table 3). From these values, a quercetin solubility of 7.3 mg/L at 18°C and 5.5 mg/L at 0°C was calculated (Table 3), and these results show that the amount of solubilised quercetin in white Cirò 2018, both at 18°C and at 0°C, was lower than quercetin solubility in Barbera 2018 and red Cirò 2014. This is quite similar to that of Sangiovese 2014, but higher than in the buffer solution at pH 3.20. However, one cannot exclude that substances other than polyphenols influence the solubilised quercetin. These values also suggest that quercetin solubility could reach about 7 mg/L at 18°C in wines in which there are no anthocyanins, or that most of them were transformed into non-bleachable polymeric pigments. However, as demonstrated by the 2014 Sangiovese, in aged red wines that were initially rich in quercetin yet contained bleachable pigments, and in which a higher content than 7 mg/L was expected, a smaller amount could be present as some of it involved in oxidation reactions might have been degraded.

It is interesting to note that the addition of quercetin to Barbera, red Cirò and Sangiovese induced an increase in A_{371} , but not in A_{520} (at 18°C and 0°C) compared to their respective controls (Table 2), unlike the expected hyperchromic effect induced by co-pigmentation complexes formation between pigments and solubilised quercetin. In contrast, a small decrease was found, probably due to co-precipitation

of some class of wine pigments with the added quercetin, or to a shift of the λ_{max} towards higher values (bathochromic effect, not determined). The lack of hyperchromic effect could be due to the fact that it was evident with co-pigment/anthocyanin ratios higher than those in this work (Baranac *et al.*, 1997). Furthermore, in order to observe a hyperchromic effect of the co-pigmentation complexes in which small flavanol-anthocyanin polymers are involved, a much higher co-pigment/pigment ratio is required (Salas *et al.*, 2004).

CONCLUSIONS

The data presented in this work shows that the quercetin solubilised in three red wines (Barbera 2018, red Cirò 2014, Sangiovese 2014) reaches much higher concentrations than in a pH 3.20 buffer solution containing 12% v:v ethanol, and that the over-solubility of quercetin depends on the wines' contents of monomeric and bisulphite-bleachable polymeric pigments. Quercetin over-solubility was also observed in white Cirò 2018, a white wine examined in this work. In this wine, about 7 mg/L had been solubilised at 18°C, which probably also represents the limit of quercetin that can be found in solution in red wines in which most of the monomeric anthocyanins and their bleachable polymeric derivatives have been transformed into non-bleachable pigments. The decrease in the quercetin content found in wines over time could be due to the evolution of anthocyanins and bleachable polymeric pigments, and to oxidative degradation reactions in which it is involved (probably the case in the Sangiovese 2014).

To explain the quercetin over-solubility in red wines rich in monomeric anthocyanins and their bleachable polymeric pigments (as in the case of Barbera 2018), we propose that it is the formation of soluble co-pigmentation complexes between quercetin and anthocyanins (as of 2014) that could prevent quercetin precipitation. As monomeric anthocyanins and bleachable polymeric pigments bound to quercetin in co-pigmentation complexes evolve into other molecular structures (e.g. non-bleachable pigments), part of the quercetin released by the dissociation of complexes could precipitate if it exceeds its solubility level in wine (which depends on the wine pigment composition), part could be degraded, probably through oxidation reactions, and part could remain in solution if its solubility level in wines is not reached. This mechanism seems to be proven by what was observed in Sangiovese 2014, which in March 2020 was poor in monomeric anthocyanins and bleachable polymeric pigments, with a precipitate of quercetin in the bottles. The fact that the content of quercetin was 5.3 mg/L in March 2020 (in October 2017 it was 11.3 mg/L), were solubilised 3.5 mg/L of the added quercetin (30 mg/L), proves that quercetin released from the hypothetical co-pigmentation complexes in part precipitated and in part was degraded. Its content in the wine therefore was lower than its actual solubility.

The higher quantity of solubilised quercetin in white Cirò 2018 (in which anthocyanins and related pigments were absent) compared to the hydroalcoholic buffer at pH 3.20 suggests that non-phenolic substances probably also prevent the formation of quercetin crystals. The limit of this work lies in the fact that their nature remains unidentified. On the other hand, the refrigeration of wines in which there are no

pigments, but only hypothetical substances that would inhibit the growth of its crystals (e.g. white wines), shows that quercetin precipitation may also be due to quercetin solubility decreasing with a decrease in temperature.

LITERATURE CITED

- Atanasova, V., Fulcrand, H., Le Guernevé, C., Cheynier, V. & Moutounet, M., 2002. Structure of a new dimeric acetaldehyde malvidin 3-glucoside condensation product. *Tetrahedron Lett.* 43(35), 6151-6153.
- Baranac, J.M., Petranović, N.A. & Dimitrić-Marković, J.M., 1997. Spectrophotometric study of anthocyan copigmentation reactions. 2. Malvin and the nonglycosidized flavone quercetin. *J. Agric. Food Chem.* 45(5), 1694-1697.
- Benucci, I., Cerreti, M., Liburdi, K., Nardi, T., Vagnoli, P., Ortiz-Julien, A. & Esti M., 2018. Pre-fermentative cold maceration in presence of non-*Saccharomyces* strains: Evolution of chromatic characteristics of Sangiovese red wine elaborated by sequential inoculation. *Food Res. Int.* 107, 257-266.
- Bosso, A., Guaita, M., Panero, L., Motta, S., Petrozziello, M., Tsolakakis, C. & Sansone, L., 2019. Chemical and sensory characteristics of Gaglioppo wines obtained from grapes grown under two different water regimes. *BIO Web of Conferences, Proc. 42nd World Congress Vine Wine 02008*, 15.
- Boulton, R., 2001. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *Am. J. Enol. Vitic.* 52, 67-87.
- Brouillard, R., Wigand, M.-C., Dangles, O. & Cheminat, A., 1991. pH and solvent effect on the copigmentation reaction of malvin with polyphenols, purine and pyrimidine derivatives. *J. Chem. Soc., Perkin Trans. 2* 8(1991), 1235-1241.
- Cagnasso, E., Rolle, L., Caudana, A. & Gerbi, V., 2008. Relationship between grape phenolic maturity and red wine phenolic composition. *Ital. J. Food Sci.* 3(20), 365-380.
- Castellarin, S.D., Matthews, M.A., Di Gasparo, G. & Gambetta, G.A., 2007. Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227, 101-112.
- Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E. & Hermosin-Gutiérrez, I., 2007. Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *J. Agric. Food Chem.* 55(3), 992-1002.
- Cheynier, V. & Rigaud, J., 1986. HPLC separation and characterization of flavonols in the skins of *Vitis vinifera* var. Cinsault. *Am. J. Enol. Vitic.* 37(4), 248-252.
- Corona, O., Squadrito, M., Vento, G., Tirelli, A. & Di Stefano, R., 2015. Over-evaluation of total flavonoids in grape skin extracts containing sulphur dioxide. *Food Chem.* 172, 537-542.
- Cortell, J.M. & Kennedy, J.A., 2006. Effect of shading on the accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot noir and extraction in a model system. *J. Agric. Food Chem.* 54(22), 8510-8520.
- Danilewicz, J.C., 2003. Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. *Am. J. Enol. Vitic.* 54(2), 73-85.
- De Freitas, V. & Mateus, N., 2011. Formation of pyranoanthocyanins in red wines: A new and diverse class of anthocyanin derivatives. *Anal. Bioanal. Chem.* 401, 1463-1473.
- Downey, M.O., Harvey, J.S. & Robinson, S.P., 2003. Synthesis of flavonols and expression of flavonol synthase genes in the developing grape berries of Shiraz and Chardonnay (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 9(2), 110-121.
- Downey, M.O., Harvey, J.S. & Robinson S.P., 2004. The effect of bunch shading on berry development and flavonoid accumulation on Shiraz grapes. *Aus. J. Grape Wine Res.* 10(1), 55-73.
- Escribano-Bailón, T., Álvarez-García, M., Rivas-Gonzalo, J.C., Heredia, F.J. & Santos-Buelga, C., 2001. Color and stability of pigments derived from the acetaldehyde-mediated condensation between malvidin-3-O-glucoside and (+)-catechin. *J. Agric. Food Chem.* 49(3), 1213-1217.
- Es-Safi, N.-E., Le Guernevé, C., Fulcrand, H., Cheynier, V. & Moutounet, M., 2000. Xanthylum salts formation involved in wine colour changes. *Int. J. Food Sci. Technol.* 35(1), 63-74.
- Fulcrand, H., Dueñas, M., Salas, E. & Cheynier, V., 2006. Phenolic reactions during winemaking and aging. *Am. J. Enol. Vitic.* 57(3), 289-297.
- Gambuti, A., Picariello, L., Rinaldi, A., Forino, M., Blaiotta, G., Moine, V. & Moio, L., 2020. New insights into the formation of precipitates of quercetin in Sangiovese wines. *J. Food Sci. Tech.* 57(7), 2602-2611.
- Glories, Y., 1984. La couleur des vins rouges. 2^e parti e: mesure, origine et interpretation. *J. Int. Sci. Vigne Vin* 4(18), 253-271.
- Haselgrove, L., Botting, D., Van Heeswijck, R., Høj, P.B., Dry, P.R., Ford, C. & Land, P.G.I., 2000. Canopy microclimate and berry composition: The effect of bunch exposure on the composition of *Vitis vinifera* L cv. Shiraz grape berries. *Aus. J. Grape Wine Res.* 6(2), 141-149.
- He, F., Liang, N.-N., Mu, L., Pan, Q.-H., Wang, J., Reeves, M.J. & Duan, C.-Q., 2012a. Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their color expression. *Molecules* 17(2), 1571-1601.
- He, F., Liang, N.-N., Mu, L., Pan, Q.-H., Wang, J., Reeves, M.J. & Duan, C.-Q., 2012b. Anthocyanins and their variation in red wines II. Anthocyanin derived pigments and their color evolution. *Molecules* 17(2), 1483-1519.
- Keller, M. & Hrazdina, G., 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. *Am. J. Enol. Vitic.* 49(3), 341-349.
- Lanati, D., Cascio, P., Pollon, M., Corona, O. & Marchi, D., 2021. Effect of leaf removal and ripening stage on the content of quercetin glycosides in Sangiovese grapes. *OENO One* 55(4), 71-81.
- Lanati, D., Marchi, D. & Cascio, P., 2014. Precipitati di quercetina nei vini. In *Proc. 37th World Congress Vine Wine and 12th Gen. Ass. OIV*, 06007, 5.
- Makris, D.P., Kallithraka, S. & Kefalas, P., 2006. Flavonols in grapes, grape products and wines: Burden, profile and influential parameters. *J. Food Compos. Anal.* 19(5), 396-404.
- Marchi, D., Lanati, D., Cascio, P. & Mazza, G., 2019. Influenza della sfogliatura sulla sintesi della quercetina in Sangiovese. Ulteriori acquisizioni sui precipitati di quercetina nei vini. *BIO Web of Conferences, Proc. 42nd World Congress Vine Wine 02010*, 15.
- Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M. & Velasco, R., 2006. Metabolite profiling of grape: Flavonols and anthocyanins. *J. Agric. Food Chem.* 54, 7692-7702.
- Price, S.F., Breen, P.J., Valladao, M. & Watson, B.T., 1995. Cluster sun exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* 4, 187-194.
- Rinaldi, A., Picariello, L., Soares, S., Brandão, E., De Freitas, V., Moio, L. & Gambuti, A., 2021. Effect of oxidation on color parameters, tannins, and sensory characteristics of Sangiovese wines. *Eur. Food Res. Technol.* 247(12), 2977-2991.
- Salas, E., Le Guernevé, C., Fulcrand, H., Poncet-Legrand, C. & Cheynier, V., 2004. Structure determination and color properties of a newly synthesized direct-linked flavanol-anthocyanin dimer. *Tetrahedron Lett.* 45(47), 8725-8729.
- Somers, T.C. & Ziemelis, G., 1985. Flavonol haze in white wines. *Vitis* 24, 43-50.

- Spayd, S.E., Tarara, J.M., Mee, D.L. & Ferguson, J.C., 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* 53(3), 171-182.
- Squadrito, M., Corona, O., Ansaldi, G. & Di Stefano, R., 2007. Relazioni fra percorsi biosintetici degli HCTA, dei flavonoli e degli antociani nella buccia dell'uva. *Riv. Vitic. Enol.* 60(3), 59-70.
- Squadrito, M., Corona, O., Ansaldi, G. & Di Stefano, R., 2010. Evolution of anthocyanin profile from grape to wine. *J. Int. Sci. Vigne Vin* 44(3), 167-177.
- Vrhovsek, U., Rigo, A., Tonon D. & Mattivi F., 2004. Quantitation of poly phenols in different apple varieties. *J. Agric. Food Chem.* 52, 6532-6538.
- Ziemelis, G. & Pickering, J., 1969. Precipitation of flavonols in a dry red table wine. *Chem. Ind.* 49, 1781-1782.