Food Research International 66 (2014) 207–215

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

HPLC-DAD–MS/MS characterization of phenolic compounds in white wine stored without added sulfite

Sandra Pati [a,*], Pasquale Crupi [b,**], Ilaria Benucci [c], Donato Antonacci [b], Aldo Di Luccia [a], Marco Esti [c]

[a] Department of the Sciences of Agriculture, Food and Environment, University of Foggia, Via Napoli 25, 71121 Foggia, Italy
[b] CRA — Consiglio per la Ricerca e la sperimentazione in Agricoltura, Unità di ricerca per l'uva da tavola e la vitivinicoltura in ambiente mediterraneo, Via Casamassima 148, 70010 Turi, BA, Italy
[c] Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), Tuscia University, via S. Camillo de Lellis snc, 01100 Viterbo, Italy

ARTICLE INFO

Article history:
Received 27 June 2014
Accepted 21 September 2014
Available online 28 September 2014

Keywords:
No sulfite-added wine
Caffeic acid
Mass spectrometry
Phenolic compounds
Oxidation

Chemical compounds studied in this article:
trans-Caftaric acid (PubChem CID: 13887348)
Caffeic acid (PubChem CID: 689043)
trans-Coutaric acid (PubChem CID: 72551455)
trans-Fertaric acid (PubChem CID: 72551457)
p-Coumaric acid (PubChem CID: 637542)

ABSTRACT

The oxidation of dihydroxybenzene and hydroxycinnamate compounds generates unstable quinones which undergo further reactions to combine nucleophilic compounds. These reactions are limited when sulfur dioxide (SO_2) is used as wine preservative, as SO_2 plays an important role in reducing quinones back to their phenol form. In no sulfite-added wine, oxidation chemistry undoubtedly results changed. Therefore, in this work, the phenolic profile of a white wine without adding sulfites, after twelve-month storage, was investigated by HPLC-DAD-MS/MS and compared to a control stored with sulfur dioxide. A total of twenty-six phenolics were detected including hydroxycinnamate derivatives and minor compounds resulting from oxidation processes, i.e. oxidized caffeic acid dimers, whose structures were tentatively proposed on the basis of their UV, MS, and MS/MS typical fragmentation. The experimental evidence of these latter is described for the first time in wine stored without added sulfites, and it may contribute to get new insight in the field of wine oxidation processes.

1. Introduction

Phenolic compounds are secondary metabolites found in plant tissues; their study in food and especially in wine is of great interest, as they are directly related to wine quality attributes, such as color, astringency, and aging attitude (Li, Pan, Jin, & Duan, 2011). On the other hand, it is well documented that polyphenols are fruit and wine constituents that can be easily oxidized, thereby both antioxidant properties (Rice-Evans, Miller, & Paganga, 1996) and sensitiveness to oxidative browning (Gómez, Martínez, & Laencina, 1995; Wu, Dastmalchi, Long, & Kennelly, 2012) have been widely reported. Particularly the white wine, whose most abundant phenolic compounds are the hydroxycinnamic acids (Myers & Singleton, 1979), is susceptible to the oxidative browning due to enzymatic and/or non-enzymatic oxidation (Simpson, 1982).

Enzymatic browning (Li, Guo, & Wang, 2008) almost entirely occurs in grape must; when caftaric acid is oxidized to its corresponding quinone by polyphenoloxidases (PPO), the available glutathione (GSH) quickly reacts with the quinone forming the compound 2-S-glutathionylcaffeoyltartaric acid, also referred to as grape reaction product (GRP), which is not a suitable substrate for further oxidation (Cheynier, Trousdale, Singleton, Salgues, & Wylde, 1986). Therefore, the formation of GRP is believed to limit the must browning. Following glutathione depletion, the exceeded caftaric acid quinones are involved in coupled oxidation mechanisms in which the glutathionyl adduct or catechin can serve as reductant (Cheynier, Basire, & Rigaud, 1989). Conversely, non-enzymatic oxidation occurs in wine in the presence of transition metal ions and involves polyphenols with ortho-dihydroxybenzene or trihydroxybenzene moieties (Danilewicz, 2003; Waterhouse & Laurie, 2006). The quinones formed from polyphenols oxidation are unstable and may undergo further reactions combining nucleophilic compounds (i.e. phenols, thiols, amines) to produce colored dimers (Cheynier & da Silva, 1991; Kader, Irmouli, Zitouni, Nicolas, & Metche, 1999; Oliveira, Ferreira, De Freitas, & Silva, 2011). Subsequently, these products may rearrange to form new dihydroxybenzene moieties, which are more easily oxidized because they have lower redox potentials than their initial phenols (Singleton, 1987). These reactions have been widely investigated in model solutions by HPLC–UV–vis and HPLC–MS for structural identification (Antolovich et al., 2004; Arakawa, Yamaguchi, Hotta, Osakai, & Kimoto, 2004; Cilliers & Singleton, 1991; Fulcrand, Cheminat, Brouillard, & Cheynier, 1994; Nahrstedt et al., 1990; Pati,

* Corresponding author. Tel.: +39 0881 589123.
** Corresponding author. Tel.: +39 080 8915711.
E-mail addresses: sandra.pati@unifg.it (S. Pati), pasquale.crupi@entecra.it (P. Crupi).

http://dx.doi.org/10.1016/j.foodres.2014.09.017
0963-9969/

Losito, Palmisano, & Zambonin, 2006; Rompel et al., 1999; Sonni, Clark, Prenzler, Riponi, & Scollary, 2011; Tazaki, Taguchi, Hayashida, & Nabeta, 2001); nevertheless, specific brown products have not been well characterized in wine.

Sulfur dioxide (SO_2) is widely used as additive during the winemaking process (from must pressing to wine bottling) in order to protect wine (especially white wine) owing to its antimicrobial and antioxidant activities. SO_2 reacts with the oxygen reduced form (i.e. hydrogen peroxide) playing an important role in reducing quinones formed during oxidation process back to their phenol form; moreover, it inhibits tyrosinase and prevents the production of GRP, which will maintain a high level of free hydroxycinnamates with high browning potential (Danilewicz, Seccombe, & Whelan, 2008; Oliveira et al., 2011). However, low amounts of SO_2 are also naturally produced by yeast during wine fermentation.

It is worth noting that sulfites have been associated with the triggering of asthmatic responses in certain individuals, although studies addressing this issue in wine have been inconclusive as wine-induced asthmatic responses may be complex and may involve several cofactors (Vally & Thompson, 2003). However, the perception that sulfites may cause negative health effects (i.e. migraines and headaches) appears to be common (Costanigro, Appleby, & Menke, 2014). For this reason, in the last years, there is growing interest in winemaking without added sulfites that is becoming increasingly feasible due to technological improvements (Lustrato et al., 2006; Santos, Nunes, Saraiva, & Coimbra, 2012; Sonni, Cejudo Bastante, Chinnici, Natali, & Riponi, 2009). The reduced content of SO_2 in wine (due only to the naturally produced amount of yeast) likely changes the wine phenolic chemistry during storage.

The aim of this work was to characterize the phenolic profile of a white wine without added sulfites, after a one-year storage, compared with a control stored with sulfur dioxide, in order to highlight minor compounds resulting from oxidation processes. New phenolic structures have been hypothesized on the basis of combination of UV, MS, and MS/MS information.

2. Material and methods

2.1. Wine samples

Frascati Superiore white wine (30 hL), made from cv. Malvasia of Lazio (70%) and Trebbiano (30%) grapes (*Vitis vinifera* L.) harvested at the optimal technological maturity (21 °Brix) was elaborated in Capodarco winery (Grottaferrata, Rome in central Italy), following a reductive winemaking methodology. To minimize air contact and oxidation, all juice and wine transfers, during the whole winemaking process until bottling, were made by previous saturation of pipes and tanks with carbon dioxide.

After early morning picking, the grapes were quickly and carefully transported in 25 kg plastic bins to the winery. The mass was gently pressed with a dry ice (15 mm pellets) covering (3 kg 100 kg^{-1} of grape) and the free run juice was quickly clarified via nitrogen flotation using pectolytic enzyme preparation (3 mL 100 L^{-1}) and gelatin (15 mL 100 L^{-1}), as flocculants. Clear juice was then moved to fermentation tank under carbon dioxide blanket and, after thiamine (50 mg 100 L^{-1}) and ammonium phosphate (10 g 100 L^{-1}) supplementation, the alcoholic fermentation was quickly induced via inoculation of previously activated (12 h before) *Saccharomyces cerevisiae* starter (Lalvin D254 Yseo, Lallemand Italia) culture (20 g 100 L^{-1}). Fermentation, carried out in temperature-controlled stainless steel tank at 16 °C, lasted about 3 weeks with a nearly complete consumption of the reducing sugars (5 g L^{-1}). One week after the end of alcoholic fermentation, the wine was separated from the yeast and grape lees, and racked to clean tank to be further settled using a complex plant protein-based fining allergen free agent (35 g 100 L^{-1}), and granular sodium bentonite (30 g 100 L^{-1}). After cold stabilization, the wine was racked and filtered, previous saturation of pipes and tanks with carbon dioxide, at 3 months from vintage. Wine bottling was performed under isobaric condition; in one hundred bottles, used as control, metabisulfite (160 mg L^{-1}) was added before filling. Until analysis time (12 months after bottling), the wine was kept at normal cellar conditions (80% of relative humidity and 14 °C) in the dark, with the bottle lying down to keep the technical cork stopper moist.

2.2. Chemicals and analysis of the main oenological parameters

Formic acid and HPLC grade water were purchased from J.T. Baker (Deventer, Holland). LC–MS grade solvent acetonitrile was purchased from Riedel-de Haën (Steinheim, Germany). Gallic acid, (+)-catechin, (−)-epicatechin, tyrosol, hydroxytyrosol, *trans*-caffeic acid, *p*-coumaric acid, and ferulic acid were purchased from Extrasynthese (Genay, France).

The main oenological parameters were determined according to European Regulation (EEC No. 2676/1990) on five bottle samples, for each wine.

2.3. HPLC-DAD–MS/MS

As previously described (Crupi, Pichierri, Basile, & Antonacci, 2013), separation and identification of phenolic compounds were carried out using an HPLC 1100 (Agilent Technologies, Palo Alto, CA, U.S.A.) equipped with a model G1379A degasser, a model G1311A quaternary pump solvent delivery, a model G1316A column oven, a model G1315B DAD system and a model G2447A XCT-trap Plus mass detector (Agilent Technologies) coupled with a pneumatic nebulizer-assisted electrospray LC–MS interface. Briefly, after filtration through 0.45 μm pore size regenerated cellulose filters (VWR International, USA), wine samples were directly injected onto a reversed stationary phase column, Luna C18 (150 × 2 mm i.d., particle size 3 μm, Phenomenex, USA) using a model G1313A autosampler (Agilent Technologies). The following gradient system was used with water/formic acid (99:1, v/v) (solvent A) and acetonitrile (solvent B): 0 min, 2% B; 10 min, 13% B; 25 min, 15% B; 30 min, 22% B; 40 min 22% B; followed by washing and re-equilibrating the column. The column temperature was not controlled, the flow was maintained at 0.2 mL min^{-1} and the sample injection was 3 μL. The flow rate and the elution program were controlled by the LC ChemStation 3D software (Hewlett-Packard, USA). UV–vis detection wavelengths were set at 330 and 280 nm, and spectrophotometric spectra were registered from 250 to 450 nm.

Negative electrospray mode (ESI) was used for ionization of molecules with capillary voltage at 4000 V and skimmer voltage at 40 V. The nebulizer pressure was 30 psi and the nitrogen flow rate was 8 L min^{-1}. Temperature of drying gas was 350 °C. In the full scan mode, the monitored mass range was from *m/z* 100 to 1200 at a scan speed of 13,000 Da s^{-1}. MS/MS analysis was performed by using helium as the collision gas at a pressure of 4.6×10^{-6} mbar. Fragmentation spectra were obtained with an isolation width of 4.0 *m/z* for precursor ions and a fragmentation amplitude of 0.8 V. Tentative compound identification was achieved by combining different information: UV absorption maxima (λ_{max}), elution order, and mass spectra, which were compared with those from pure standards, when available, and interpreted with the help of structural models already hypothesized in the literature (Boselli, Minardi, Giomo, & Frega, 2006; Cilliers & Singleton, 1991; Crupi et al., 2013; Rompel et al., 1999).

3. Results and discussion

The main oenological parameters of no sulfite-added wine (NSW) were determined after a storage of twelve months, and compared with a control stored with sulfur dioxide (SW). Results reported in Table 1 revealed no statistical difference between the two samples as regards all oenological parameters with the exception of the absorbance at 420 nm (Abs_{420}). NSW showed Abs_{420} values higher than SW, which

Table 1
The main oenological parameters in no sulfite-added wine (NSW) and in sulfite-added wine (SW).
[a,b]Means in the same row followed by different letters differ significantly (one-way variance analysis, $p < 0.05$).

Oenological parameters	NSW	SW
pH	3.4 ± 0.1	3.4 ± 0.1
Alcohol concentration (v/v)	12.9 ± 0.2	13.0 ± 0.2
Reduced dry extract (g/L)	24 ± 2	23 ± 2
Total acidity (g/L tartaric acid)	5.16 ± 0.04	5.14 ± 0.03
Volatile acidity (g/L acetic acid)	0.54 ± 0.06	0.44 ± 0.05
Acetaldehyde mg/L	62 ± 4	60 ± 6
Total SO_2 (mg/L)	10^a ± 1	79^b ± 2
Free SO_2 (mg/L)	–	56 ± 2
Total phenols (mg/L catechin)	257 ± 10	250 ± 13
Abs_{420}	0.097^b ± 0.002	0.049^a ± 0.002

may be ascribed both to the bleaching effect of SO_2 and to possible oxidation processes. The two contributors cannot be separated; however, if some oxidation processes occurred, this occurred at a low level since no increased production of acetaldehyde was observed in NSW. Obviously, also SO_2 contents resulted different between NSW and SW, as in NSW sample yeasts naturally produced 10 mg L^{-1} of SO_2 and, in SW, SO_2 was added before bottle filling as preservative.

As regards the phenolic profile, numerous phenolic compounds were detected in both white wines after 12-month storage, with the exception of oxidative products only identified in NSW samples (Table 2). Peak number has been assigned in accordance with elution order in the chromatogram (Fig. 1). The use of both UV and MS detectors allowed to observe co-eluted peaks, whose UV spectral information could not be diagnostic; in these cases MS and MS/MS data (Table 2) were essential for proposing compound structures. A rigorous structural analysis by 2D-NMR was not attempted due to the very low abundance of the interesting peaks, which are difficult to isolate.

3.1. Non oxidative phenolic compounds

The largest number of the revealed compounds belongs to the group of hydroxycinnamate derivatives, namely *trans*-caffeic acid (peak **18**), *p*-coumaric acid (peak **23**), ferulic acid (peak **24**) together with *cis*- and *trans*-caftaric acids (peaks **8** and **12**), *cis*- and *trans*-coutaric acids (peaks **15** and **16**), *cis*- and *trans*-fertaric acids (peaks **19** and **21**). Their identification was achieved on the basis of UV–vis spectra together with MS fragmentation mainly yielding to the loss of CO_2 from the deprotonated molecule $[M–H]^-$ of hydroxycinnamic acids, and the loss of tartaric acid moiety from hydroxycinnamic ester. It is worth noting that peak **7**, showing $[M–H]^-$ at *m/z* 616, and UV spectrum with a maximum absorption at 330 nm, was attributed to 2-S-glutathionylcaftaric acid (GRP). Being a hydroxycinnamic ester, fragment ions were found at *m/z* 484 and 272, deriving from the typical loss of tartaric and 2-S-caftaric acid moieties, respectively. Also, the product ion at *m/z* 440, arising from the cleavage of the bond C═C – C═O on the 2-S-glutathionylcaftaric acid unit, was observed, in good agreement with the literature (Boselli et al., 2006). On the basis of its elution order and UV–vis spectrum, very similar to that of GRP, peak **2** could be ascribed to a GRP hydrolysis product in agreement with what Cejudo-Bastante, Pérez-Coello, and Hermosín-Gutiérrez (2010) reported about 12-months aged white wines; however the unavailability of suitable MS spectra did not allow further statements on the nature of this compound.

Peak **22** has been attributed to dihydrokaempferol 3-O-beta-D-glucoside, being UV, MS, and MS/MS information consistent with what found by Baderschneider and Winterhalter (2001). Two stilbenes

Table 2
Summary of peaks and MS information obtained by HPLC–UV–MS/MS of phenolic compounds in NSW and SW samples after a 12-month storage
[1]Identification confirmed by injection of reference compounds; SW, sulfite-added wine; NSW, no sulfite-added wine.
sh, shoulder; t_R, retention time; nd, not determined.

Peak	Compounds	SW	NSW	t_R (min)	UV λmax (nm)	$[M–H]^-$ (m/z)	MS/MS (m/z)
	Hydroxybenzoic acids and flavanols						
1	Gallic acid[1]	X	X	8.4	276	169	
14	Catechin[1]	X	X	18.8	280	289	245; 175
20	Epicatechin[1]	X	X	22.0	nd	289	
	Phenylethanoids						
3	Hydroxytyrosol[1]	X	X	14.5	280	153	123
11	Tyrosol[1]	X	X	17.8	nd	137	
	Flavonols and stilbenes						
22	Dihydrokaempferol 3-O-ß-D-glucoside	X	X	22.9	290	449	287
25	*trans*-Piceid	X	X	32.4	316	389	227
26	*cis*-Piceid	X	X	38.5	282	389	227
	Hydroxycinnamic acids						
2	GRP hydrolysis product	X	X	14.2	330	nd	nd
7	2-S-glutathionyl-caffeoyltartaric acid	X	X	15.6	330	616	484; 440; 272
8	*cis*-Caftaric acid	X	X	16.6	308	311	179; 149
12	*trans*-Caftaric acid	X	X	17.8	305sh, 328	311	179; 149
15	*cis*-Coutaric acid	X	X	20.2	290sh, 310	295	163; 149
16	*trans*-Coutaric acid	X	X	20.8	300sh, 314	295	163
18	*trans*-Caffeic acid[1]	X	X	21.1	300sh, 323	179	135
19	*cis*-Fertaric acid	X	X	22.0	282, 322	325	193; 149
21	*trans*-Fertaric acid	X	X	22.6	305sh, 328	325	193; 149
23	*p*-Coumaric acid[1]	X	X	27.6	302sh, 310	163	119
24	Ferulic acid[1]	X	X	31.2	302sh, 323	193	
	Oxidation products						
4	Dihydrodicaffeic acid lactone		X	14.6	270, 335	357	311; 293; 275
5 + 6	Hydroxy-caffeic acid dimer isomer		X	15.1	260, 315	373	305; 193; 178
9	Hydroxy-caffeic acid dimer isomer		X	17.4	nd	373	327; 305; 281
10	Acid 3-(4′,5′-*o*-quinone-caffeoyl) caffeic		X	17.7	nd	355	311; 283; 267; 241
13	Hydroxy-caffeic acid dimer isomer		X	18.0	nd	373	355; 327; 305; 175
17	Caffeic-(*o*-quinone-caffeic)-ether		X	20.9	nd	355	311; 217; 193

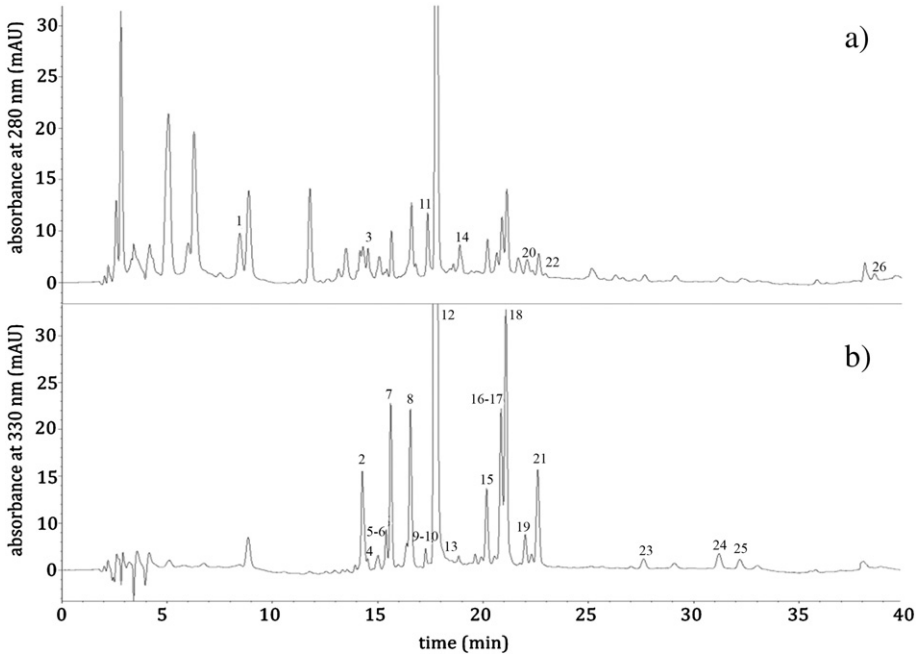

Fig. 1. HPLC-DAD trace of no sulfite-added white wine after a 12-month storage. a, detection at 280 nm; b, detection at 330 nm.

were also identified in the wine: the deprotonated molecule $[M–H]^-$ at *m/z* 389 together with its MS/MS principal fragment at *m/z* 227 in mass spectra of both peaks **25** and **26** suggest that they can be two piceids. Furthermore, on the basis of their UV–vis spectra and HPLC elution order, the two compounds can be distinguished as *trans*-piceid (peak **25**) and *cis*-piceid (peak **26**) (Crupi et al., 2013). Gallic acid (peak **1**) and flavanol monomers, namely (+)-catechin (peak **14**) and (−)-epicatechin (peak **20**), together with hydroxytyrosol (peak **3**) and tyrosol (peak **11**) were also detected and confirmed by means of reference standards.

3.2. Oxidation-derived phenolic compounds

Seven peaks (**4**–**6**, **9**, **10**, **13**, **17**) have been tentatively identified in NSW as oxidized phenolic compounds arising from coupling reactions (Fig. 1 and Table 2). These peaks were not observed in SW, suggesting that they were absent or were formed at not detectable concentrations. This could be explained by the presence of SO_2 acting as antioxidant, thus slowing down the oxidation processes.

To the best of our knowledge, this is the first time that these structures, elsewhere hypothesized and in some studies identified in caffeic acid model systems (Antolovich et al., 2004; Arakawa et al., 2004; Cilliers & Singleton, 1991; Fulcrand et al., 1994; Nahrstedt et al., 1990; Pati et al., 2006; Rompel et al., 1999; Tazaki et al., 2001), have been detected in wine. In addition, most of these studies were conducted at high pH and in the absence of ethanol, simulating reaction conditions rather different than wine matrix. The major dimer structures reported in the literature, showing a molecular weight of 358 Da, have been gathered in Fig. 2. A mechanism of reverse disproportionation has been postulated between catechol and *o*-quinone moieties generating semiquinone radicals, which, in the absence of other substrates such as SO_2, can randomly couple to form different isomers through C–C (type a structure), Cα–Cα (type b, f, g structures), ether (type c, d structures), and Cα–O (type e, h structures) links (Cilliers & Singleton, 1991; Nahrstedt et al., 1990; Tazaki et al., 2001). Also non-radical mechanisms have been proposed (Rompel et al., 1999; Singleton, 1987), such as condensation between *o*-quinones and the corresponding catechol through a Michael type 1,4-addition (type a structure), nucleophilic addition of hydroquinone double-bond after rearrangement to quinone methide with formation of cyclolignan derivatives (type b, f, g structures), or polymerization involving one or two catechol groups and the side chain double-bond with the generation of neolignans derivatives (type e, h structures) (Fig. 2).

Peak **4** revealed an ion at *m/z* 357, suggesting the presence of a caffeic acid dimer. The MS/MS fragmentation yielded the product ion at *m/z* 311, showing a loss of 46 Da; but, dimers reported in the literature (Arakawa et al., 2004; Pati et al., 2006), including type a and e structures, have shown a loss of 44 Da, which corresponds to CO_2 elimination from the carboxylic groups, being the resulting carboanion stabilized by conjugation. Additionally, fragmentation pattern of caffeicin-like structures (e, Fig. 2) have been showed to give the diagnostic ions at *m/z* 179 and 177, corresponding to a retro Diels-Alder rearrangement with the loss of a caffeic acid moiety. No further MS/MS study about dimer fragmentation has been described in the literature. Taking into account these considerations and the suggested structures (Fig. 2), the loss of 46 Da, corresponding to a HCOOH unit, could preferentially occur from b, f, and/or h-type dimers, in which it can be accomplished by the formation of a double bond on the adjacent carbon unit. Similar fragmentations, even though for different molecules, have been reported and can be explained in terms of an elimination mechanism driven by a hydrogen abstraction by the carboxylate anion (Couldwell, Thomas, Mitchell, Hulbert, & Blanksby, 2005). However, charge-remote fragmentation mechanisms could be possible, too (Cheng & Gross, 2000). Nevertheless, dimers of b and h-type structures can be excluded because, together with $[M\text{-}H\text{-}46]^-$, they should likely give rise also to $[M\text{-}H\text{-}44]^-$ fragments generating from the loss of the conjugated carboxylic acid unit (Arakawa et al., 2004; Couldwell et al., 2005; Pati et al., 2006), which instead were not observed in MS/MS spectrum of *m/z* 357 (Fig. 3). Therefore, a dihydrodicaffeic acid lactone (f-type dimer structure) has been proposed for peak **4**, whose fragmentation pattern (Fig. 4) would be consistent with typical eliminations (i.e. water loss and ring opening) that have been largely reported for similar compounds (Moss, Mao, Taylor, & Saucier, 2013). Furthermore, UV–vis spectrum of the compound **4** (inset, Fig. 3) resulted very similar to that reported by Rompel et al. (1999), apart for the observed bathochromic shift of around 10 nm of its absorption maximum and characteristic broad shoulder which are probably due to a positive solvatochromism effect, typically reported for analogous catechol-derivative compounds in polar solvents (Riedel & Spange, 2012). It is worth noting that the formation of

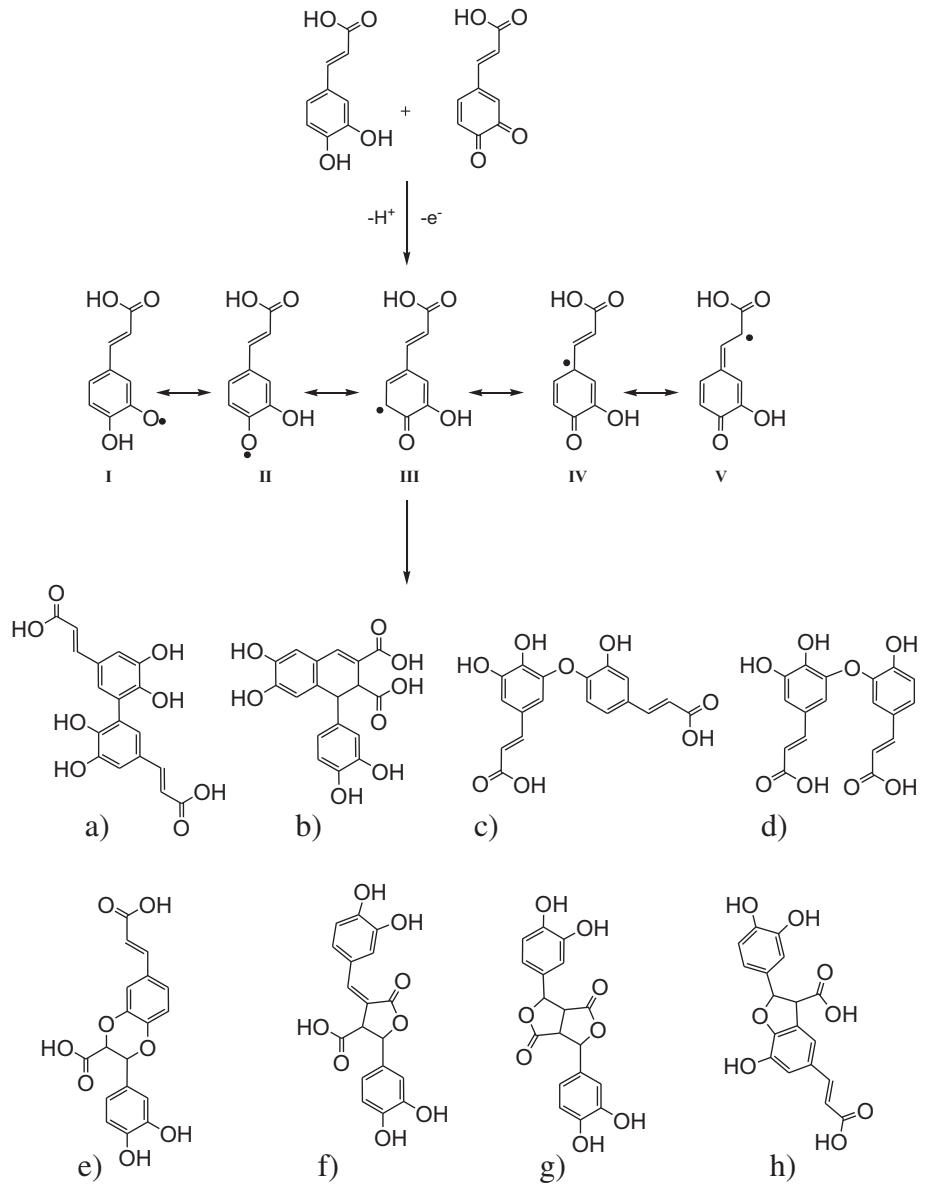

Fig. 2. Radical coupling of caffeic acid and caffeic acid quinone with the main dimer products (MW 358 Da) reported in the literature.

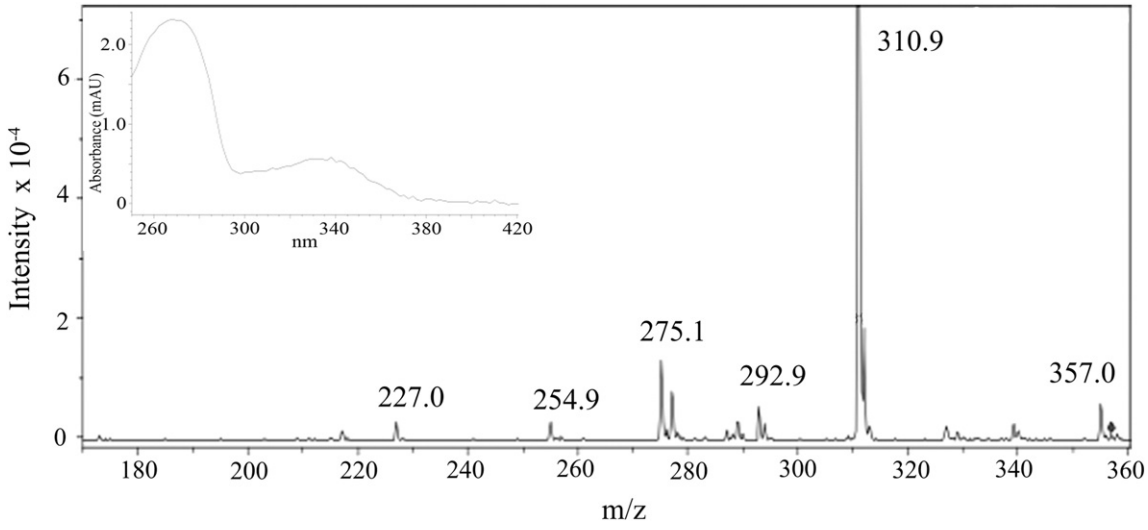

Fig. 3. MS/MS product ion spectrum of *m/z* 357 ion observed in the MS spectra for compound **4**. Inset: UV spectrum of peak **4**.

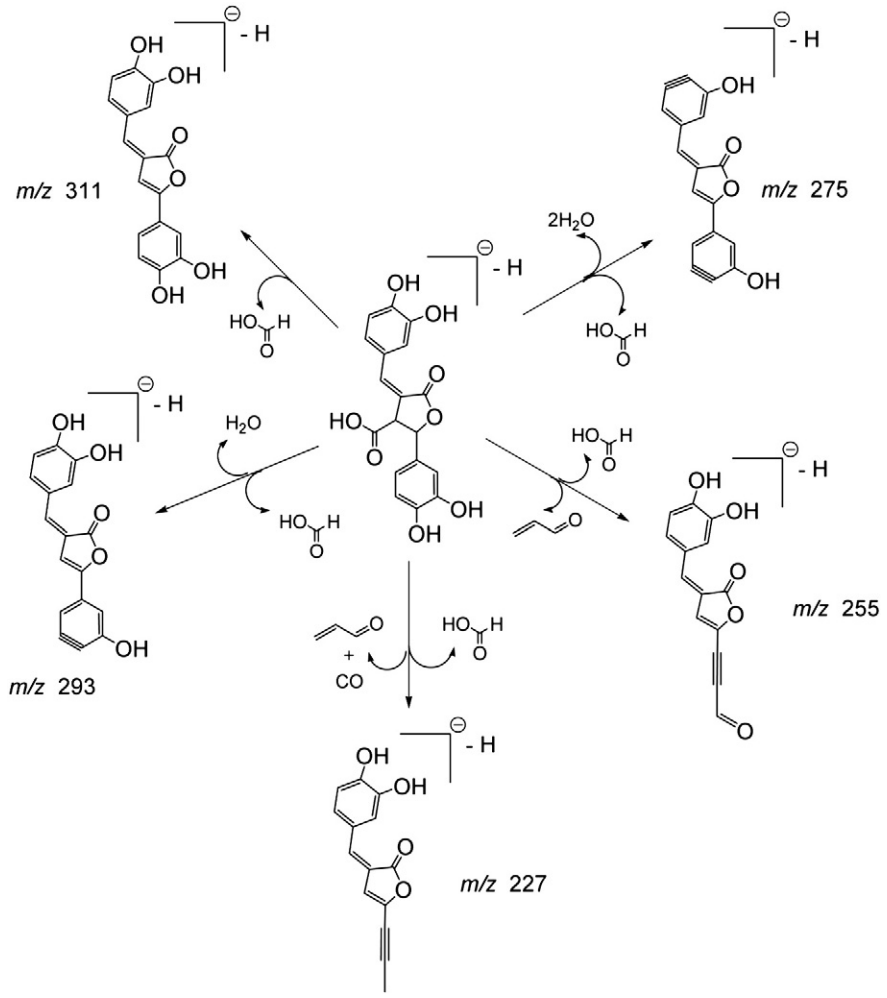

Fig. 4. MS/MS fragmentation pattern proposed for the ion at m/z 357 observed in the MS spectrum for compound **4**.

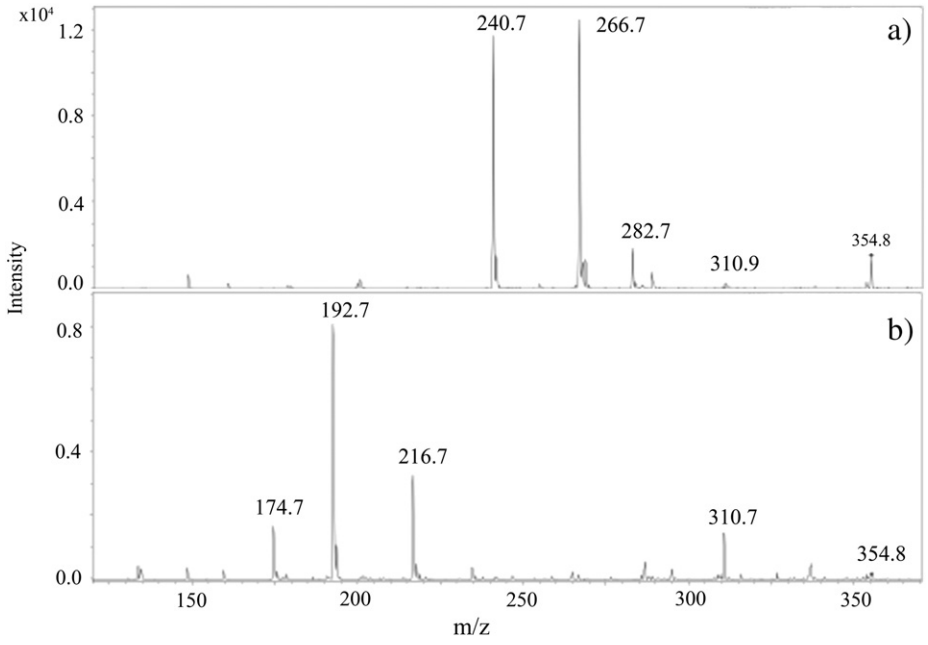

Fig. 5. MS/MS product ion spectra of *m/z* 355 ion observed in the MS spectra for compounds **10** (a) and **17** (b).

a f-type dimer structure has been previously proposed from Rompel et al. (1999) as result of enzymic oxidation of caffeic acid, and from Tazaki et al. (2001) as result of non-enzymic processes, both in model solutions.

Peaks **10** and **17**, showing $[M–H]^-$ ions at *m/z* 355 have been attributed to oxidized caffeic acid dimers; the different fragment ions of related MS/MS spectra (Fig. 5) revealed that they are not stereoisomers but structural isomers. As concerns peak **10**, the presence of *m/z* 311 and 267 ions arose from the loss of 44 (CO_2) and 88 (2 CO_2) Da, respectively, supporting the hypothesis of dimeric structures with two conjugated carboxylic units which favored the CO_2 eliminations (a, c, d, type structures, Fig. 2). Moreover, MS/MS spectra revealed a different fragmentation pattern with respect to the one reported for oxidized caffeic acid dimers with caffeicin-like structure, demonstrating that the two isomers are likely different from the oxidized caffeic acid dimers deriving from e-type structures (Arakawa et al., 2004). Product ions at *m/z* 283, consistent with the loss of hydroxycinnamic acid side chain, and at *m/z* 241, that can arise from both hydroxycinnamic acid side chain and CO_2 elimination, were also found (Fig. 5a) and can be explained by the fragmentation scheme sketched in Fig. 6a. The second isomer (peak **17**) showed a fragmentation yielding three different fragments, as depicted in Fig. 5b. Within the structures proposed in the literature (Fig. 2), the one reported in Fig. 6b seems to be able to give the observed fragments. Indeed, the ion at *m/z* 193 can be derived from the quinone of 3,4,5-trihydroxycinnamic acid, and the ion at *m/z* 217 could be generated from the ring cleavage. Moreover, the loss of

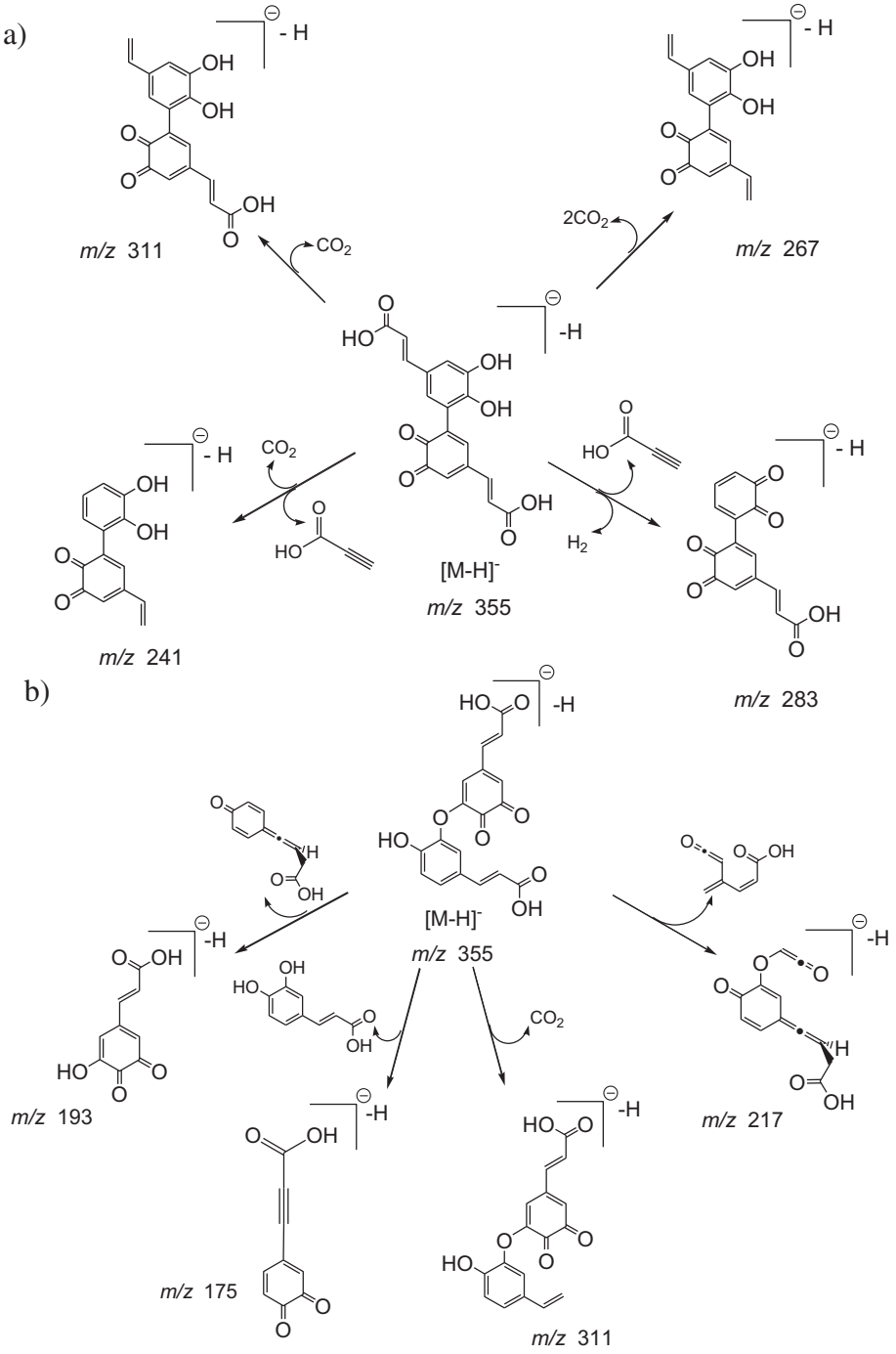

Fig. 6. MS/MS fragmentation patterns proposed for the ions at m/z 355 observed in the MS spectra for compounds **10** (a) and **17** (b).

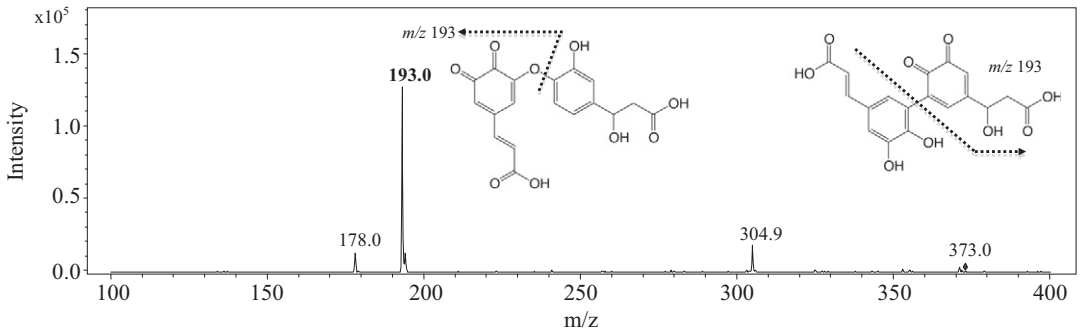

Fig. 7. MS/MS product ion spectrum of *m/z* 373 observed in the MS spectrum for compound **6**. Inset: suggested structures for *m/z* 193 ion.

one CO_2 unit (ion at *m/z* 311) and the loss of a caffeic acid moiety (ion at *m/z* 175) were also observed (Figs. 5b, 6b).

It is worth noting that the observed fragment ions enabled to exclude any alternative structures for the aforementioned isomers, such as dimeric adducts formed by hydrogen bonding; indeed, if the deprotonated molecules at *m/z* 355 were hydrogen-bonded complexes, the dissociation should occur preferentially to yield the ion at *m/z* 177.

Four peaks (**5**, **6**, **9**, and **13**) showed the deprotonated molecule at *m/z* 373 (S1 — Supporting Information). Peaks **5** and **6** exhibited a similar fragmentation pattern (Fig. 7): MS and MS/MS information suggested that in these compounds only one caffeic acid moiety was oxidized and with a hydroxyl group on the side chain. Something similar, although regarding oxidation processes of verbascoside, was reported in the literature (Mulinacci et al., 2005). Type a, c and d dimers (Fig. 2), in the oxidized form and with a hydroxy group can generate, after molecule rearrangement, the predominant fragments at *m/z* 193 proposed in the inset of Fig. 7. As regards peaks **9** (S2 — Supporting Information) and **13** (S3 — Supporting Information), the fragmentation of *m/z* 373 gave rise to different patterns, yielding the main ion at *m/z* 327, which could derive from the loss of a HCOOH unit, and other minor ions (Table 2), which could not be attributed.

It is worth noting that caftaric dimers, whose deprotonated ions should be at *m/z* 621, were not observed, suggesting that its hydrolysis, catalyzed by the acidic medium, to caffeic acid is likely more favored than its oxidation, as corroborated by the fact that organic esters are more quickly hydrolyzed rather than oxidized, in aqueous solutions (Mill, 1982). It appears, then, that caffeic acid was the compound predominantly involved in oxidation processes during storage. Moreover, although the same structures have been demonstrated to be formed in model solutions both via enzymic and nonenzymic pathways (Rompel et al., 1999; Tazaki et al., 2001), a nonenzymic mechanism should have been favored since enzymes have been likely removed and/or deactivated in the wine after clarification treatments. The wine matrix, the low pH and the compound concentration could be the main factors that have favored the detection of only some oxidation-derived phenolic compounds described in model systems (Antolovich et al., 2004; Arakawa et al., 2004; Cilliers & Singleton, 1991; Fulcrand et al., 1994; Nahrstedt et al., 1990; Pati et al., 2006; Rompel et al., 1999; Tazaki et al., 2001). The absence (or the presence at not detectable levels) of these oxidized compounds in SW samples suggested that their formation is favored in wines with a low content of SO_2, since oxidation in NSW is supposed to be faster. However, these results do not exclude that oxidized compounds could be formed also in sulfited wines stored over a longer period of time.

In conclusion, different phenolic compounds, such as hydroxycinnamates derivatives and oxidized phenolic compounds, present in a white wine without added sulfites have been investigated in this research. On the basis of UV, MS and MS/MS characteristics, the structures of hydroxyl and oxidized caffeic acid dimers were tentatively elucidated; to the best of our knowledge, this is the first report on the experimental evidence of these compounds in wines. The reported results have been obtained in a white wine without added sulfites, where the oxidation can occur faster, but similar processes cannot be excluded in sulfited wines stored over a longer period of time, where oxidation is slowed down by sulfur dioxide.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.foodres.2014.09.017.

References

Antolovich, M., Bedgood, D.R., Jr., Bishop, A.G., Jardine, D., Prenzler, P.D., & Robards, K. (2004). LC–MS investigation of oxidation products of phenolic antioxidants. *Journal of Agricultural and Food Chemistry*, *52*, 962–971.

Arakawa, R., Yamaguchi, M., Hotta, H., Osakai, T., & Kimoto, T. (2004). Product analysis of caffeic acid oxidation by on-line electrochemistry/electrospray ionization mass spectrometry. *Journal of the American Society for Mass Spectrometry*, *15*, 1228–1236.

Baderschneider, B., & Winterhalter, P. (2001). Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. *Journal of Agricultural and Food Chemistry*, *49*, 2788–2798.

Boselli, E., Minardi, M., Giomo, A., & Frega, N.G. (2006). Phenolic composition and quality of white d.o.c. wines from Marche (Italy). *Analytica Chimica Acta*, *563*, 93–100.

Cejudo-Bastante, M.J., Pérez-Coello, M.S., & Hermosín-Gutiérrez, I. (2010). Identification of new derivatives of 2-S-glutathionylcaftaric acid in aged white wines by HPLC-DAD-ESI-MSn. *Journal of Agricultural and Food Chemistry*, *58*, 11483–11492.

Cheng, C., & Gross, M.L. (2000). Applications and mechanisms of charge-remote fragmentation. *Mass Spectrometry Reviews*, *19*, 398–420.

Cheynier, V., Basire, N., & Rigaud, J. (1989). Mechanism of trans-caffeoyltartaric acid and catechin oxidation in model solutions containing grape polyphenoloxidase. *Journal of Agricultural and Food Chemistry*, *37*, 1069–1071.

Cheynier, V., & da Silva, J.M.R. (1991). Oxidation of grape procyanidins in model solutions containing trans-caffeoyltartaric acid and polyphenol oxidase. *Journal of Agricultural and Food Chemistry*, *39*, 1047–1049.

Cheynier, V., Trousdale, E.K., Singleton, V.L., Salgues, M., & Wylde, R. (1986). Characterization of 2-S-glutathionylcaftaric acid and its hydrolysis in relation to grape wines. *Journal of Agricultural and Food Chemistry*, *34*, 217–221.

Cilliers, J.J.L., & Singleton, V.L. (1991). Characterization of the products of nonenzymic autoxidative phenolic reactions in a caffeic acid model system. *Journal of Agricultural and Food Chemistry*, *39*, 1298–1303.

Costanigro, M., Appleby, C., & Menke, S.D. (2014). The wine headache: consumer perceptions of sulfites and willingness to pay for non-sulfited wines. *Food Quality and Preference*, *31*, 81–89.

Couldwell, A.M., Thomas, M.C., Mitchell, T.W., Hulbert, A.J., & Blanksby, S.J. (2005). Tandem mass spectrometry of deprotonated iodothyronines. *Rapid Communications in Mass Spectrometry*, *19*, 2295–2304.

Crupi, P., Pichierri, A., Basile, T., & Antonacci, D. (2013). Postharvest stilbenes and flavonoids enrichment of table grape cv Redglobe (*Vitis vinifera* L.) as affected by interactive UV-C exposure and storage conditions. *Food Chemistry*, *141*, 802–808.

Danilewicz, J.C. (2003). Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. *American Journal of Enology and Viticulture*, *54*, 73–85.

Danilewicz, J.C., Seccombe, J.T., & Whelan, J. (2008). Mechanism of interaction of polyphenols, oxygen and sulfur dioxide in model wine and wine. *American Journal of Enology and Viticulture*, *59*, 128–136.

Fulcrand, H., Cheminat, A., Brouillard, R., & Cheynier, V. (1994). Characterization of compounds obtained by chemical oxidation of caffeic acid in acidic conditions. *Phytochemistry*, *35*, 499–505.

Gómez, E., Martínez, A., & Laencina, J. (1995). Prevention of oxidative browning during wine storage. *Food Research International*, *28*, 213–217.

Kader, F., Irmouli, M., Zitouni, N., Nicolas, J., & Metche, M. (1999). Degradation of cyanidin 3-glucoside by caffeic acid o-quinone. Determination of the stoichiometry and characterization of the degradation products. *Journal of Agricultural and Food Chemistry*, *47*, 4625–4630.

Li, H., Guo, A., & Wang, H. (2008). Mechanisms of oxidative browning of wine. *Food Chemistry*, *108*, 1–13.

Li, Z., Pan, Q., Jin, Z., & Duan, C. (2011). Comparison on phenolic compounds in *Vitis vinifera* cv. Cabernet Sauvignon wines from five wine-growing regions in China. *Food Chemistry*, *125*, 77–83.

Lustrato, G., Alfano, G., Belli, C., Grazia, L., Iorizzo, M., & Ranalli, G. (2006). Scaling-up in industrial winemaking using low electric current as an alternative to sulfur dioxide addition. *Journal of Applied Microbiology*, *101*, 682–690.

Mill, T. (1982). Hydrolysis and oxidation processes in the environment. *Environmental Toxicology and Chemistry*, *1*, 135–141.

Moss, R., Mao, Q., Taylor, D., & Saucier, C. (2013). Investigation of monomeric and oligomeric wine stilbenoids in red wines by ultra-high-performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, *27*, 1815–1827.

Mulinacci, N., Innocenti, M., La Marca, G., Mercalli, E., Giaccherini, C., Romani, A., et al. (2005). Solid olive residues: Insight into their phenolic composition. *Journal of Agricultural and Food Chemistry*, *53*, 8963–8969.

Myers, T.E., & Singleton, V.L. (1979). The non flavonoid phenolic fraction of wine and its analysis. *American Journal of Enology and Viticulture*, *30*, 98–102.

Nahrstedt, A., Albrecht, M., Wray, V., Gumbinger, H.G., John, M., Witerhoff, H., et al. (1990). Structures of compounds with antigonadotropic activity obtained by in vitro oxidation of caffeic acid. *Planta Medica*, *56*, 395–398.

Oliveira, C.M., Ferreira, A.C.S., De Freitas, V., & Silva, A.M.S. (2011). Oxidation mechanisms occurring in wines. *Food Research International*, *44*, 1115–1126.

Pati, S., Losito, I., Palmisano, F., & Zambonin, P.G. (2006). Characterization of caffeic acid enzymatic oxidation by-products by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. *Journal of Chromatography A*, *1102*, 184–192.

Rice-Evans, C.A., Miller, N.J., & Paganga, G. (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine*, *20*, 933–956.

Riedel, F., & Spange, S. (2012). Solvatochromism of catechol derivatives –solute/solvent interactions. *Journal of Physical Organic Chemistry*, *25*, 1261–1268.

Rompel, A., Fisher, H., Meiwes, D., Büldt-Karentzopoulos, K., Magrini, A., Eicken, C., et al. (1999). Substrate specificity of catechol oxidase from *Lycopus europaeus* and characterization of the bioproducts of enzymic caffeic acid oxidation. *FEBS Letters*, *445*, 103–110.

Santos, M.C., Nunes, C., Saraiva, J.A., & Coimbra, M.A. (2012). Chemical and physical methodologies for the replacement/reduction of sulfur dioxide use during winemaking: Review of their potentialities and limitations. *European Food Research and Technology*, *234*, 1–12.

Simpson, R.F. (1982). Factors affecting oxidative browning of white wines. *Vitis*, *21*, 233–239.

Singleton, V.L. (1987). Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical implications. *American Journal of Enology and Viticulture*, *38*, 69–77.

Sonni, F., Cejudo Bastante, M.J., Chinnici, F., Natali, N., & Riponi, C. (2009). Replacement of sulfur dioxide by lysozyme and oenological tannins during fermentation: Influence on volatile composition of white wines. *Journal of the Science of Food and Agriculture*, *89*, 688–696.

Sonni, F., Clark, A.C., Prenzler, P.D., Riponi, C., & Scollary, G.R. (2011). Antioxidant action of glutathione and the ascorbic acid/glutathione pair in a model white wine. *Journal of Agricultural and Food Chemistry*, *59*, 3940–3949.

Tazaki, H., Taguchi, D., Hayashida, T., & Nabeta, K. (2001). Stable isotope-labeling studies on the oxidative coupling of caffeic acid via o-quinone. *Bioscience, Biotechnology, and Biochemistry*, *65*, 2613–2621.

Vally, H., & Thompson, P.J. (2003). Allergic and asthmatic reactions to alcoholic drinks. *Addiction Biology*, *8*, 3–11.

Waterhouse, A.L., & Laurie, V.F. (2006). Oxidation of wine phenolics: A critical evaluation and hypotheses. *American Journal of Enology and Viticulture*, *57*, 306–313.

Wu, S., Dastmalchi, K., Long, C., & Kennelly, E.J. (2012). Metabolite profiling of Jaboticaba (*Myrciaria cauliflora*) and other dark-colored fruit juices. *Journal of Agricultural and Food Chemistry*, *60*, 7513–7525.