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Electrochemical Oxidation of Wine Polyphenols in the Presence of Sulfur Dioxide

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ABSTRACT: Electrochemical oxidation of three representative wine polyphenols (catechin, caffeic acid, and quercetin) in the presence of sulfur dioxide in a model wine solution (pH = 3.3) was investigated. The oxidation was undertaken using chronoamperometry at a rotating glassy carbon rod electrode, and the reaction products were characterized by HPLC-MS. The mechanism of electrochemical oxidation of polyphenols in the presence of sulfur dioxide was proposed to be an ECEC mechanism. The polyphenols first underwent a one-electron oxidation to a semiquinone radical, which can be reduced back to the original polyphenol by sulfur dioxide, or further oxidized to the quinone form. In the cases of caffeic acid and catechin, the quinone combined with sulfur dioxide and produced new derivatives. The quercetin quinone underwent further chemical transformations, producing several new compounds. The proposed mechanisms were confirmed by digital simulation of cyclic voltammograms.

KEYWORDS: *wine polyphenols, sulfur dioxide, electro-oxidation, cyclic voltammetry, digital simulation, oxidation mechanism*

INTRODUCTION

Oxidation reactions are a major problem for white wines.[1] These can lead to color browning, a loss of varietal aroma and flavor, and the development of bitterness. The chemistry of wine oxidation has been the subject of considerable research.[2] However, there are still many questions that remain to be answered regarding the mechanisms of nonenzymatic wine oxidation.[3]

Oxidation reactions in wine commence with the oxidation of phenolic compounds,[4] catalyzed by transition metal ions such as iron and copper.[5] The process is thought to initially result in the formation of a semiquinone radical,[5] which is further oxidized to the corresponding quinone. Polyphenols can also be oxidized by the hydroperoxyl radical,[6,7] resulting from oxygen reduction, also involving metal ions. Phenolics are good hydrogen donors, so hydroperoxide radicals are able to abstract protons from polyphenol hydroxyl groups and become reduced to hydrogen peroxide in the process. The overall mechanism of polyphenol oxidation to quinones catalyzed by metal ions is presented in Scheme 1.

In order to protect must and wine against oxidation, sulfur dioxide is used from pressing to bottling, especially for white wines. SO_2 prevents a wine from browning[9] and slows down the decrease of esters[10] and varietal thiols[11] during handling operations and storage. However, SO_2 is toxic[12] to some groups of people and may cause allergic reactions, such as headaches, abdominal pain, and dizziness. According to the FAO/WHO expert committee on Food Additives, the acceptable daily sulfite intake is 0.7 mg/kg body weight.[12] As a result, in recent years the trend has been to limit the use of SO_2,[13−15] and to look for replacements.[9] To find the most suitable compound that could replace or supplement sulfur dioxide, it is very important to understand the chemical roles of SO_2 during wine aging.

Several ways by which sulfur dioxide can lessen wine oxidation have been described in the literature. Some authors consider that sulfur dioxide can prevent oxidation reactions in wine by removing oxygen.[16] However, Danilewicz[5] questioned that the direct reaction of sulfur dioxide with oxygen can occur in wine, as this reaction is a radical chain process, which would be prevented by the radical scavenging activity of polyphenols. Further authors suggested that the main antioxidant function of sulfur dioxide is in reacting with hydrogen peroxide,[2,8] which is formed during the oxidation of polyphenols (Scheme 1). Another view is that an important role of sulfur dioxide, as an antioxidant in wine, is in its reaction with quinones,[17,18] by reducing these back to the original polyphenols or by adding to the quinones with the formation of a sulfonic acid derivative.[19,20] The extent to which sulfur dioxide interacts with a quinone by a 1,2-addition, and reverses it back to a polyphenol, or by 1,4-addition with the formation of the sulfonic acid, depends on the quinone itself. For example, it was shown that about 38% of the quinone derived from 4-methylcatechol added bisulfite to produce the sulfonic acid, while the rest of quinone was reduced back to the original phenolic.[17] By comparison, in the case of (+)-catechin in a wine model system, practically all of the quinones were reduced back to the original polyphenol.[18]

As wine polyphenols can be oxidized at a glassy carbon electrode,[21] electrochemical techniques can be applied to generate quinones in a controlled manner, and then study the interaction of these quinones with further wine components such as sulfur dioxide.[22] Previously, diagnostic criteria derived by Nicholson and Shain for various electrode mechanisms[23,24] have been applied to cyclic voltammetry experiments to study reactions between electrochemically generated quinones and different nucleophiles.[25−30] Mechanistic studies of reaction

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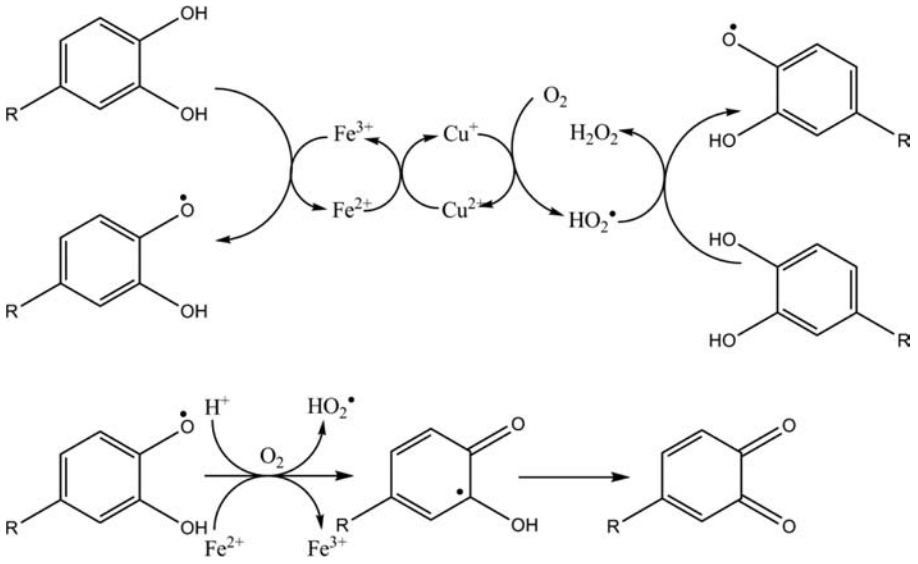
Scheme 1. General Scheme for the Oxidation of Phenolic Compounds Catalyzed by Metal Ions[1,8]



mechanisms for polyphenol oxidation in the presence or absence of SO_2 have been performed by digital simulation of cyclic voltammograms.[31,32]

In our previous paper,[22] cyclic voltammetry was employed to demonstrate how sulfur dioxide can interact with oxidized wine polyphenols. The current paper describes further experiments involving longer term electrolysis studies, to further investigate how electrochemically oxidized wine polyphenols interact with sulfur dioxide.

MATERIALS AND METHODS

Chemicals. Catechin, caffeic acid, quercetin, L-tartaric acid, and NaOH were purchased from Sigma-Aldrich. Other chemicals included $Na_2S_2O_5$ (Scharlau) and ethanol (Univar, Ajax Finechem). Ultrapure water (18.2 MΩ cm) was from a Millipore Milli-Q system and was used to prepare all of the solutions.

Chronopotentiometry. The electrochemical oxidation of the polyphenols was conducted by applying a constant current to solutions of polyphenols with or without sulfur dioxide present. For this purpose, a sealed three electrode cell was constructed, placing the working glassy carbon electrode and stainless steel counter electrode in direct contact with the test solution. A reference Ag/AgCl electrode was housed in a luggin capillary filled with model wine solution in order to protect the electrode from the build-up of phenolic compounds. Before each experiment the glassy carbon electrode was polished with sand paper (WET/DEY, 1200 grit) for 1 min, and then ultrasonic treatments were applied at 30 °C, first in ethanol and then in water, for 15 min in each solvent. For activating the glassy carbon electrode surface, the electrochemical cell was filled with model wine solution, and a constant potential of 1500 mV was applied for 60 min followed by a potential of −1000 mV for 1 min.

All test solutions were initially bubbled with nitrogen for 10 min to displace any dissolved oxygen. The working electrode was rotated at 1200 rpm using a Cilenco rotator (Boreham Wood) during the oxidation, in order to lessen the rate of build-up of oxidized polyphenolic species on the electrode surface, and therefore to extend the time before deactivation of the working electrode occurred. The electrolytic cell was thermostatted at 25 °C.

HPLC Analysis. The concentrations of catechin, caffeic acid, and quercetin were monitored during the electrochemical oxidation using reversed-phase high-performance liquid chromatography with a UV−vis detector (RP-HPLC-UV) as described previously.[33] The oxidation products were analyzed by HPLC-MS. HPLC-MS was conducted on a RP-HPLC (Dionex Ultimate 3000) equipped with a binary pump and connected in series to a micrOTOF-QII mass spectrometer (Hybrid Quadrupole, Bruker Daltonics, Madison, WI). The column and solvent gradient were the same as for the analytical HPLC. All mass spectrometric data were obtained in positive-ion mode. Nitrogen gas was used in the nebulizer, 4 bar, and for dry gas 9.5 L/min at 200 °C. Argon was used as the collision gas. The collision energy was set at 6 eV with ion energy at 1.4 eV, and the capillary was set at 3500 V, using an electrospray ionization source. Data acquisition and processing were performed using HyStar software.

SO_2 Analysis. The concentration of sulfur dioxide during the electrochemical oxidation was monitored using the aspiration method.[34]

Cyclic Voltammetry and Digital Simulations. Cyclic voltammograms were recorded using a BioLogic SP-300 potentiostat. The working electrode was a 3 mm glassy carbon disk electrode (MF-2012, surface area = 0.0707 cm^2) which was cleaned by polishing with 0.05 μm alumina powder (CF-1050) for 30 s, followed by 30 s ultrasonic bath treatments in Milli-Q water, between runs. A BAS Ag/AgCl reference electrode (+207 mV versus SHE) was used in conjunction with a platinum wire counter electrode.

The cyclic voltammograms were recorded straight after the glassy carbon electrode was inserted into the solution, as a consistent measurement procedure, given the occurrence of polyphenol adsorption on the electrode surface prior to the run. Background cyclic voltammograms were taken in the model wine solution and were recorded on the same day as the other samples, and were subtracted away from the cyclic voltammograms obtained for the polyphenols to allow the oxidation and reduction processes to be more clearly revealed. The cyclic voltammogram of each solution was taken from −100 mV to four different upper potential limits (from 450 to 650 mV) and at four different scan rates (from 25 to 200 mV/s). The temperature of the test solutions was thermostatted at 25 °C.

Digital simulation of the cyclic voltammograms was conducted using the DigiSim 3.03b simulation software.[35]

Chronoamperometry. In order to estimate the diffusion coefficients of the polyphenols and sulfur dioxide in solution, a constant potential was applied for different concentrations of the polyphenols (0.02−0.1 mM) and sulfur dioxide (0.05−2 mM) in a model wine solution (pH = 3.3) for 10 s. The same three electrode cell used for taking the cyclic voltammograms was employed in the chronoamperometric experiments. In the case of quercetin and catechin, the applied potential was 500 mV, while in case of caffeic acid and sulfur dioxide, the potential was set at 475 and 1000 mV, respectively. Diffusion coefficients (D_0's) were estimated from the formula[36]

$$Q = nFAD_0^{1/2}C_0t^{1/2}\pi^{-1/2} \quad (1)$$

where Q is the charge passed through the electrode, n is the number of electrons transferred per one molecule, F is Faraday's constant (96 495 C mol^{-1}), A is the electrode area (cm^2), C_0 is the bulk concentration, and t is time.

RESULTS AND DISCUSSION

Electrochemical Oxidation of Polyphenols in the Presence of Sulfur Dioxide. The oxidation of polyphenols in the presence of sulfur dioxide was first studied in an electrochemical cell, by applying a constant current of 400 μA/L to the solution of a polyphenol containing a catechol group and representing the main polyphenol types found in wines. The three polyphenols chosen were catechin (a flavan-3-ol), caffeic acid (a hydroxycinnamic acid), and quercetin (a flavonol), with and without sulfur dioxide present in the solution.

Electrochemical Oxidation of Polyphenols without Sulfur Dioxide. During the oxidation of 0.1 mM catechin, without rotation of the glassy carbon electrode, the potential steadily increased during the first 20 min of applied current, until it reached 1100 mV, after which the potential was stable (Figure 1, dotted curve i). Analysis of the solution composition by

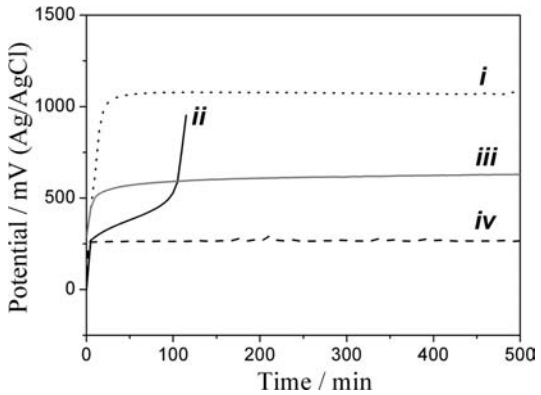


Figure 1. Chronopotentiometric curves during the oxidation of 0.1 mM catechin (black curves i to ii) and 0.4 mM sulfur dioxide (gray curve iii), dissolved in model wine solution (pH = 3.3) at glassy carbon electrode at 400 μA/L, without electrode rotation (black dotted curve i) and with electrode rotation for the remaining curves. The black dashed curve (iv) shows the potential during oxidation of 0.1 mM catechin in the presence of 0.4 mM SO_2.

HPLC showed no change in catechin concentration after 12 h of passing the constant current. However, according to Faraday's low of electrolysis,[36] the number of moles (N) in the reaction is given by

$$N = Q/Fn \quad (2)$$

where Q is charged passed in electrolysis, F is the Faraday's constant, and n is the number of electrons per oxidized molecule. If all the current went into catechin oxidation ($n = 2$), the passage of 17 C/L would lead to the consumption of 0.09 mM catechin. No measurable change in catechin concentration in this case might be explained by passivation of the electrode by a compact, adherent, and insulating organic/polymeric film, the formation of which has been observed previously at electrode surfaces.[37−39] The presence of organic oligomers or polymers may lead to the deactivation of the carbon electrode, and much of the current likely went directly into processes such as solvent oxidation at the higher potentials generated.

However, rotating of the working electrode led to the potential remaining between 300 and 400 mV for a much longer time than in the unstirred case, for up to 100 min (Figure 1, solid curve ii). During this time, the potential remained in the region in which catechin oxidation is expected,[40] but without the presence of a passivating layer of catechin oxidation products, which were instead driven away from the electrode by the action of electrode rotation. However, after 100 min of applying a constant current, passivation of the electrode still occurred (Figure 1, solid curve ii).

Rotation of the working electrode also slowed the deactivation processes during the oxidation of caffeic acid and quercetin (data not shown). Without electrode rotation, the potential rose sharply after 10 min in the case of quercetin, and after 200 min in the case of caffeic acid, while rotation of the working electrode led to the potential remaining between 300 and 400 mV for 30 and 400 min in the case of quercetin and caffeic acid, respectively. Therefore, rotation of the working electrode decreased the rate of polymer formation on the electrode surface, and allowed the electrode to remain in an active state for a longer time, compared to the case without electrode rotation. Hence, for further chronopotentiometric experiments, rotation of the carbon electrode was employed.

The concentrations of catechin, caffeic acid, and quercetin during oxidation at the electrode were monitored. The loss of polyphenols was only observed when the electrode potential was between 300 and 400 mV. The rate of polyphenol disappearance in model wine solution was equal to 2.2×10^{-9} M/s in the case of caffeic acid, 1.9×10^{-9} M/s in the case of catechin, and 2×10^{-9} M/s in the case of quercetin (Table 1). These results correspond to the value expected for a two-electron Faradaic oxidation for this applied current:

$$d[\text{polyphenol}]/dt = I/Fn = 2.1 \times 10^{-9}\ \text{M/s} \quad (3)$$

Table 1. Initial Rate of Polyphenol and Sulfur Dioxide Disappearance (M/s) in the Model Wine Solution During Chronopotentiometric Oxidation (I = 400 μA/L) at the Rotating Glassy Carbon Electrode

	reaction rate × 10^{-9} M/s		
compd	without SO_2	+ 0.4 mM SO_2	+1 mM SO_2
catechin	1.9 ± 0.2	0.6 ± 0.1[a]	
caffeic acid	2.2 ± 0.1	0.8 ± 0.1	
quercetin	1.7 ± 0.1	1.5 ± 0.2	1.2 ± 0.1

compd	without polyphenol	+ 0.1 mM catechin	+ 0.1 mM caffeic acid	+ 0.05 mM Quercetin
sulfur dioxide	3.9 ± 0.0	4.1 ± 0.0	3.8 ± 0.0	4.0 ± 0.0

[a]The decrease in catechin concentration started only after 4 h of reaction. Each experiment was performed in duplicate.

In the case of quercetin oxidation, two extra peaks, with adsorption maxima at 290 nm, appeared on the HPLC chromatograms of the reaction mixture (Figure 2c). The electrochemical oxidation of quercetin has been previously studied by several authors.[41−45] In all of these papers the appearance of several new HPLC peaks, with adsorption maxima around 290 nm, has been observed during the course of the electrochemical oxidation of quercetin. The following

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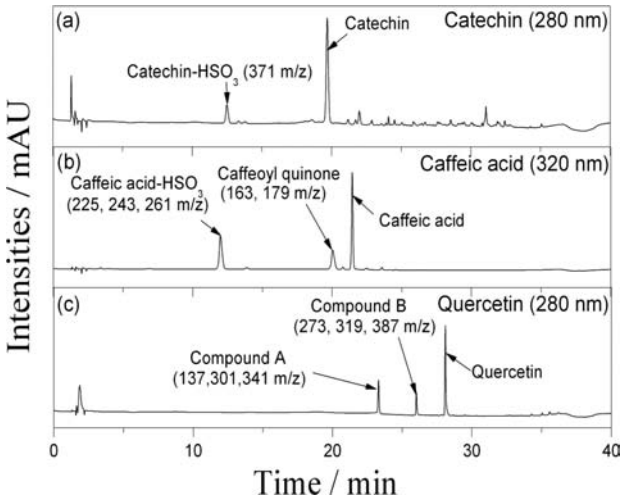


Figure 2. HPLC chromatogram of (a) 0.1 mM catechin with 0.4 mM SO_2, (b) 0.1 mM caffeic acid with 0.4 mM SO_2, and (c) 0.1 mM quercetin with 1 mM SO_2 diluted in model wine solution (pH = 3.3) after passing a constant current equal to 400 μA/L for 24 h. The peaks that appeared on the HPLC chromatogram of quercetin, with and without sulfur dioxide present, were the same.

mechanism for quercetin oxidation has been proposed (Scheme 2). Considering the mass spectra of peaks 1 and 2 (Figure 2c), these peaks can be assigned to compounds A and B, respectively.

Electrochemical Oxidation of Sulfur Dioxide. Oxidation of 0.4 mM sulfur dioxide in the model wine solution at a constant current of 400 μA/L led to potential values around 500−600 mV (Figure 1, gray curve iii), which corresponds to the potential at which SO_2 oxidation has been observed previously at a glassy carbon electrode in a model wine solution.[22] The decrease in the SO_2 concentration during chronopotentiometry in the model wine solution was monitored using the aspiration method (Table 1). The reaction rate of sulfur dioxide disappearance during oxidation was found to be equal to 3.9 $\times 10^{-9}$ M/s. Given that a current of 400 μA/L provides for 4.15 $\times 10^{-9}$ moles of electrons passed per liter, by Faraday's law of electrolysis (eq 2), this result implies that n was equal to 1. This is a surprising result, as the oxidation of one molecule of SO_2 at glassy carbon surface was considered to involve two electrons.[21,46]

In order to check that there were no chemical reactions occurring between SO_2 and the components of the model wine solution, giving rise to extra loss of sulfur dioxide, electrolysis was also conducted in ultrapure water. The same reaction rate for SO_2 loss was observed. Therefore, electrolysis of sulfur dioxide at the glassy carbon electrode is indeed a one-electron process. In the literature,[47,48] a one-electron oxidation of sulfur dioxide has been described for the oxidation of SO_2 at Pt and PbO_2 film electrodes:

$$H_2SO_3 \rightarrow H_2SO_3^+ + e^- \quad (4)$$

The first electrochemical step is followed by a chemical step during which dithionate is produced:

$$2H_2SO_3^+ \rightarrow H_2S_2O_6 + 2H^+ \quad (5)$$

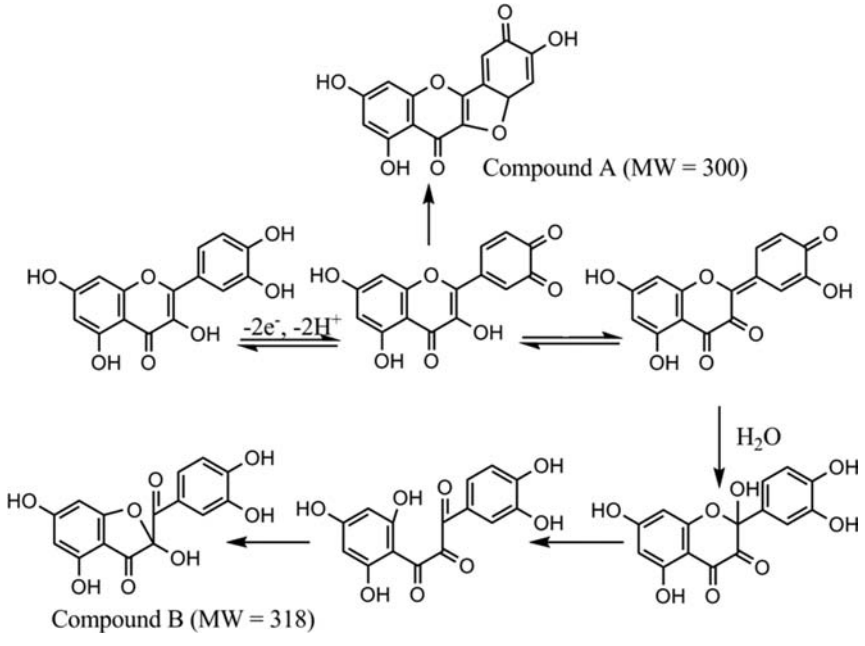
This reaction is suggested to be the rate determining step,[47] which in acidic medium is followed by a disproportionation step

$$H_2S_2O_6 \rightarrow SO_2 + H_2SO_4 \quad (6)$$

with recycling of SO_2.

Electrochemical Oxidation of Polyphenols in the Presence of Sulfur Dioxide. Addition of sulfur dioxide to the catechin solutions completely prevented electrode deactivation (Figure 1, black dashed curve iv), and the potential remained stable at 280 mV for almost 24 h. This potential matches that seen previously for catechin oxidation.[40] The same outcome was observed in the case of caffeic acid oxidation in the presence of sulfur dioxide, where the potential was stable at 250 mV and began to rise only after 21 h of reaction (data not shown). These results provide further evidence to support our previous

Scheme 2. Proposed Oxidation Mechanism of Quercetin[42−44]



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findings,[22] that sulfur dioxide interacts with oxidized polyphenols (quinones) by reducing them back to the original polyphenols, or by forming sulfur derivatives, thus avoiding the build-up of a polymeric film on the carbon surface from the coupling of oxidized polyphenols.

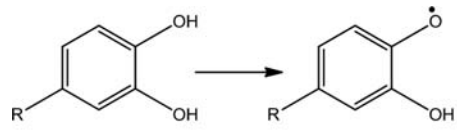
However, the presence of sulfur dioxide did not prevent deactivation of the electrode during quercetin oxidation, but instead only delayed electrode deactivation for a short period of time; without sulfur dioxide, the potential started to rise after 20 min of oxidation, while with SO_2 present, the potential was stable for about 40 min.

The concentrations of caffeic acid, catechin, and quercetin during electrochemical oxidation in the presence of sulfur dioxide were also monitored. The concentration of catechin was unchanged during the first four hours of anodic oxidation in the presence of 0.4 mM sulfur dioxide, and then decreased at a rate three times slower than for the anodic oxidation of catechin without sulfur dioxide present (Table 1). The concentrations of caffeic acid and quercetin started decreasing straight after the beginning of the experiment; however, the presence of SO_2 slowed the rate of oxidation of these polyphenols (Table 1). In the case of caffeic acid, the oxidation rate was about three times slower with 0.4 mM SO_2 present, while in case of quercetin the presence of 0.4 mM SO_2 only slightly decreased the rate of oxidation from 1.7 to 1.5 nM/s. An increase in the sulfur dioxide concentration to 1 mM further slowed the quercetin oxidation rate to 1.2 nM/s.

Therefore, the presence of SO_2 lowered the rate of polyphenol disappearance during anodic oxidation at a glassy carbon electrode (Table 1). These results indicate that sulfur dioxide reduced some of the oxidized polyphenols back to the original catechin, caffeic acid, and quercetin polyphenol forms. The remaining quinones might interact with sulfur dioxide with the production of sulfur dioxide derivatives, or might undergo further reactions such as polymerization with other quinones or polyphenols present.

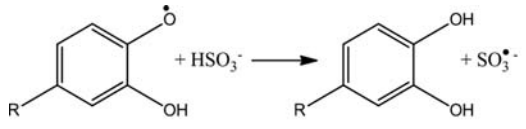
The rates of sulfur dioxide disappearance during the caffeic acid, catechin, and quercetin oxidation were twice higher than the rates for oxidation of the polyphenols without sulfur dioxide present (Table 1), indicating that during the oxidation of one polyphenol molecule two molecules of sulfur dioxide were consumed. Previously,[17] it was found that, under wine conditions, the disappearance of one molecule of O_2 resulted in the consumption of one molecule of polyphenol and two molecules of sulfur dioxide. The authors concluded that one molecule of SO_2 interacts with each quinone molecule, and the other reacts with hydrogen peroxide produced during the iron-catalyzed oxidation of polyphenols. However, in our case, there was no hydrogen peroxide produced, as the polyphenols were oxidized electrochemically. Moreover, in a recent paper,[2] Elias and Waterhouse showed that in order to completely inhibit the reaction between hydrogen peroxide and iron ions (the Fenton reaction), more than 64 mg/L of sulfur dioxide was needed. When the concentration of SO_2 in the model wine solution was less than 64 mg/L, the Fenton reaction was observed, which means that sulfur dioxide could not react with all of the hydrogen peroxide present in the solution. Therefore, there must be some other explanation why with catechin, caffeic acid, or quercetin the SO_2 molar ratio was equal to 1:2.

The first step of the anodic or iron-catalyzed oxidation of polyphenols is the formation of a semiquinone radical:[5,49,50]



(7)

Sulfur dioxide can probably reduce this semiquinone radical back to the original polyphenol, with the formation of a sulfite radical as a byproduct:



(8)

Under anaerobic wine conditions, two sulfite radicals interact with each other to produce dithionate[51,52]

$$2SO_3^{-\bullet} \rightarrow S_2O_6^{2-} \quad k_{4.30} = 1.8 \times 10^8 \text{ (pH = 4.3)} \qquad (9)$$

which then undergoes disproportionation to sulfur dioxide and sulfate by reaction 6. The reaction between the sulfite ion and phenoxy radicals was described previously,[53] where the authors studied the chemistry of sulfite and peroxomonosulfite radicals. The reaction constant of the reaction

$$PhO^{\bullet} + SO_3^{2-} \rightarrow SO_3^{-\bullet} + PhO^{-} \qquad (10)$$

at pH = 11.1 was estimated to be $k = 1 \times 10^7$.

The reaction of sulfite radicals with polyphenols has also been described in the literature;[53,54] however, the reaction constants of these reactions were smaller than the constant determined for reaction 9. For example, the reaction constant of a reaction between $SO_3^{-\bullet}$ and quercetin at pH 11.5 was estimated to be 2.5×10^8 M^{-1} s^{-1},[54] while $k_{4.30}$ at the same pH was found to be 0.95×10^9 M^{-1} s^{-1}.[52] Moreover, Elias et al.[55] studied free radical chemistry in oxidized wine and clearly showed that the sulfite radical cannot directly oxidize catechols.

The reaction products of the electrochemical oxidation of catechin, caffeic acid, and quercetin in the presence of sulfur dioxide were analyzed by HPLC-MS. A constant current of 400 μA/L was passed through the model wine solution of polyphenols with sulfur dioxide for 20 h, and afterward these solutions were injected onto the HPLC-MS system.

Only two relatively large peaks were observed on the HPLC (280 nm) chromatogram of the catechin−sulfur dioxide mixture after applying the constant current for 20 h (Figure 2a). The second peak corresponds to the catechin remaining in the solution after the reaction. The mass spectrum of the first peak at 12.8 min in positive LC-MS mode was 371 (Figure 2a), which is the mass of a sulfur derivative of catechin ($[C_{15}H_{13}O_6{-}HSO_3]H^+$).

The HPLC (320 nm) chromatogram of the caffeic acid−SO_2 mixture, after applying the constant current for 20 h, showed two relatively large peaks in addition to the remaining caffeic acid peak (Figure 2b). The first peak at 12.5 min corresponds to a sulfur derivative of caffeic acid ($C_9H_7O_4{-}HSO_3$), as one of the main mass ions of this peak was m/z = 261. The main mass ion of the second peak was at m/z = 179, which corresponds to the caffeic acid quinone. Masuda et al.[56] also reported the identification of a quinone derivative of methyl caffeate, which was produced by radical oxidation of methyl caffeate. In another paper,[57] the authors studied the stability kinetics of

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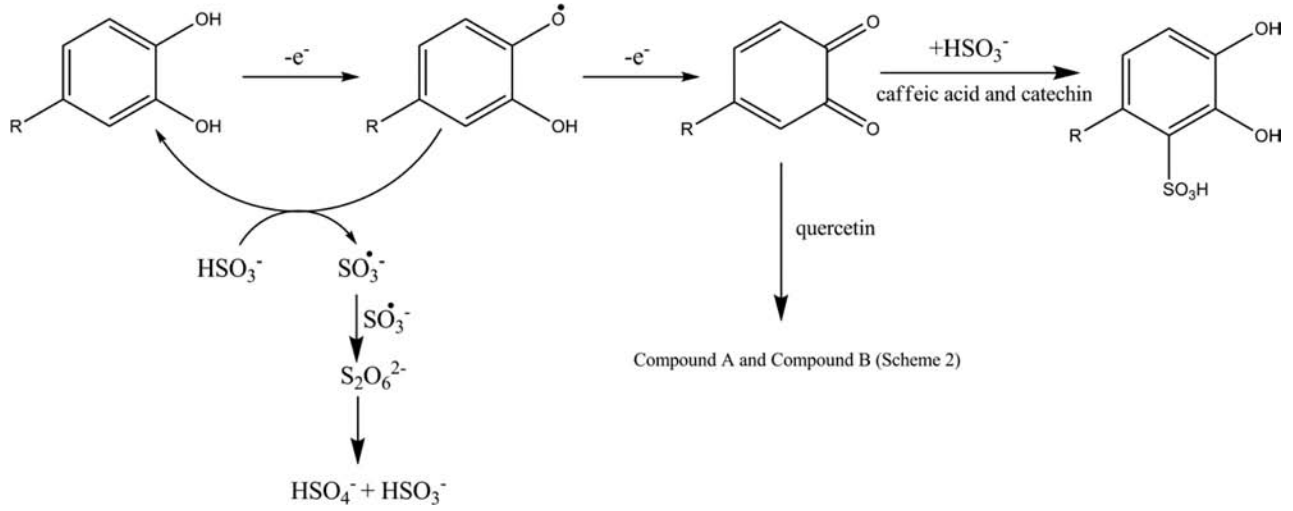
Scheme 3. Proposed Mechanisms for the Anodic Oxidation of Caffeic Acid, Catechin, and Quercetin in the Presence of Sulfur Dioxide



different *o*-quinones, and found that the half-life of the caffeic acid quinone at pH = 7.4 was 10.5 min.

In the case of quercetin oxidation in the presence of sulfur dioxide no new peaks were observed at 365 nm, which means that a sulfur dioxide adduct of quercetin was not produced in this case. Moreover, two new peaks appeared on the HPLC chromatogram at 280 nm, which were exactly the same peaks observed during electrochemical oxidation of quercetin without sulfur dioxide present (Figure 2c). Therefore, quercetin degradation (Scheme 2) occurred even in the presence of sulfur dioxide, although sulfur dioxide might lower the rate of degradation (Table 1).

Considering all of the above observations, the following mechanisms for catechin, caffeic acid, and quercetin electrochemical oxidation in the presence of sulfur dioxide can be proposed (Scheme 3).

Cyclic Voltammetry Studies of Polyphenol Kinetic Parameters. Cyclic voltammograms of caffeic acid, catechin, and quercetin in the absence (solid gray) and in the presence (black curve) of sulfur dioxide are presented in Figure 3. In the case of catechin and caffeic acid, the presence of sulfur dioxide increased the anodic peak and decreased the cathodic one, while in the case of quercetin the presence of SO_2 did not alter the anodic peak, but only decreased the cathodic one. The same results were obtained in our previous work[22] and are consistent with our proposed mechanism for the anodic oxidation of polyphenols in the presence of sulfur dioxide (Scheme 3). Sulfur dioxide interacts both with semiquinone radicals and with quinones to form the original polyphenol or a sulfur derivative of the polyphenol that can be further oxidized at the carbon electrode. This new phenolic species explains the observed increase in the anodic peak and decrease in cathodic peak seen for cyclic voltammograms of a mixture of polyphenols with SO_2 (Figure 3).

Digital simulation of cyclic voltammograms was used to evaluate the kinetic parameters of the reaction between oxidized polyphenols and sulfur dioxide.[35] The experimental cyclic voltammograms were compared with the theoretical ones using the DigiSim3.03b software, in order to confirm the reaction mechanisms, proposed above, and to evaluate thermodynamic and kinetic parameters for the electron transfer and chemical processes. The following ECEC mechanism was tested

$$P = P^{\bullet} + e,\ E^0_1,\ k_{s1}{}^0,\ \alpha_1$$

$$P^{\bullet} + HSO_3^- = P + SO_3^{\bullet},\ k_1$$

$$P^{\bullet} = Q + e,\ E^0_2,\ k_{s2}{}^0,\ \alpha_2$$

$$Q + HSO_3^- = P{-}HSO_3,\ k_2$$

$$P{-}HSO_3 = P{-}HSO_3^{\bullet} + e,\ k'_{s1},\ \alpha'_1$$

$$P{-}HSO_3^{\bullet} = Q{-}HSO_3 + e,\ k'_{s2},\ \alpha'_2$$

where P and $P^{\bullet}$ are polyphenol and semiquinone radicals, respectively, Q is a quinone, and $P{-}HSO_3$ is a polyphenol sulfur derivative. The 2-electron potential $E^0(P/Q)$ can be estimated from a cyclic voltammogram of each polyphenol as the midpoint potential between $E_{p,a}$ and $E_{p,c}$, and is related to the one electron couples by

$$2E^0(P/Q) = E^0(P/P^{\bullet}) + E^0(P^{\bullet}/Q) \quad (11)$$

In the case of quercetin oxidation, one more reaction was added to the digital simulation

$$Q = R,\ k_3$$

where R stands for new product(s) formed from the quercetin quinone (Scheme 2).

The simulation was carried out assuming semi-infinite diffusion and a planar geometry for the electrode. Diffusion coefficients, D_0's, were calculated by applying a constant potential to the solutions of polyphenols and sulfur dioxide.[36] Due to similarity in size of the electrode reagents, their diffusion coefficients, D_0's, were considered to be equal for P, $P^{\bullet}$, and Q, and also for HSO_3^- and SO_3.

First, in order to evaluate the parameters of electrode reactions, such as transfer coefficients (α), formal potentials (E^0), and heterogeneous rate constants (k_s), digital simulations were performed for cyclic voltammograms of catechin, caffeic, and quercetin without SO_2 present in the solution. Then, after the best fit of experimental cyclic voltammograms of individual polyphenols were obtained, the numbers obtained in these

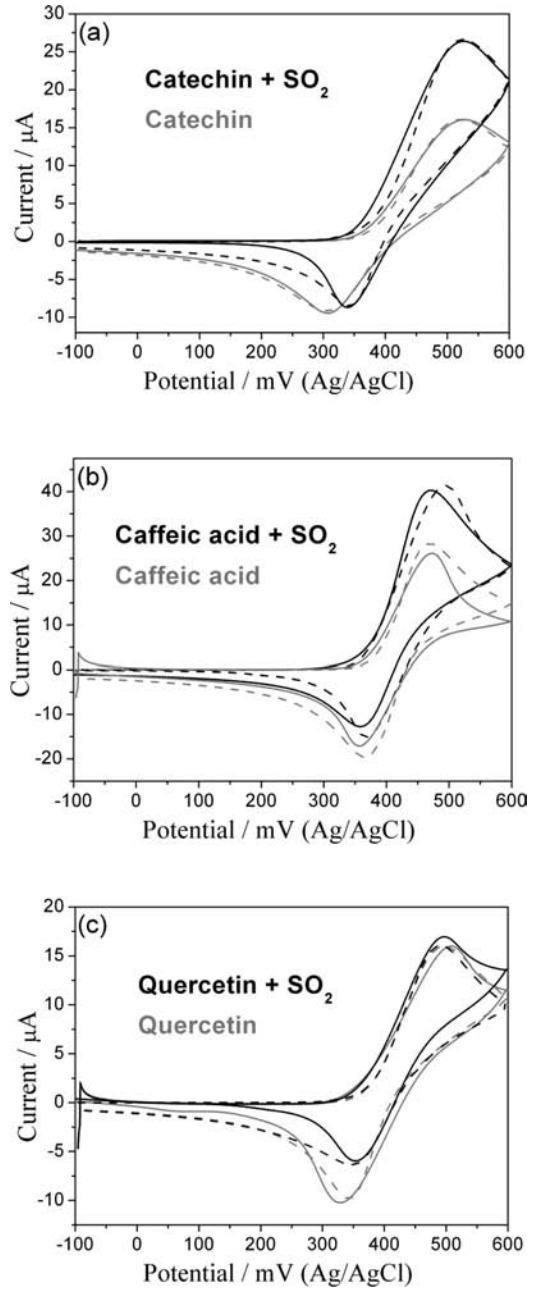


Figure 3. Digital simulation analysis of the cyclic voltammograms of (a) 1 mM catechin (gray curve) and 1 mM catechin with 0.5 mM SO_2 (black curve), (b) 1 mM caffeic acid (gray curve) and 1 mM caffeic acid with 0.5 mM SO_2 (black curve), and (c) 1 mM quercetin (gray curve) and 1 mM quercetin with 2 mM SO_2 (black curve) dissolved in model wine solution (pH = 3.3), T = 25 °C. Solid curves are experimental data (at 100 mV/s) obtained at a glassy carbon electrode, while dotted curves are simulated cyclic voltammograms. Fitting parameters are presented in Table 2.

simulations, such as formal potentials (E_0^1 and E_0^2), heterogeneous constants (k_s^1 and k_s^2), and transfer coefficients (α_1 and α_2), were used as a constants for fitting the cyclic voltammograms of polyphenols in the presence of sulfur dioxide.

Figure 3 illustrates a typical fit of simulations to experimental responses, and Table 2 lists the kinetic parameters obtained for the best fits. The fitting procedures were carried at four different scan rates, different substrate concentrations, and different upper switching potentials. Good agreement between theory and experiment was obtained in most cases (Figure 3), which means that proposed ECEC mechanism agrees with the experimental data.

Therefore, the protective role of sulfur dioxide consists of reducing the semiquinone radicals of caffeic acid, catechin, and quercetin back to the original polyphenols, and of combining with the caffeic acid and catechin quinones to produce new derivatives. The electrochemical methodology can now be used to assess the antioxidant action of additional small molecular antioxidants with oxidized polyphenols, compounds that can be considered as replacements or supplements for SO_2 in winemaking.

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Notes

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ABBREVIATIONS

HPLC-MS, high-performance liquid chromatography−mass spectrometry; Q, charge passed through the electrode; n, the number of electrons transferred per one molecule; F, Faraday's constant; A, the electrode area; C_0, the bulk concentration; D_0, diffusion coefficient; t, time; N, number of moles; P, polyphenol; P$^{\bullet}$, semiquinone radical; Q, quinone; E^0, formal potential; $E_{p,a}$, potential of anodic peak on cyclic voltammogram; $E_{p,c}$, potential of cathodic peak on cyclic voltammogram; R, new products formed from quercetin quinone; α, transfer coefficient, k_s, heterogeneous rate constant

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Table 2. Fitting Parameter Constants Obtained by Simulation for Cyclic Voltammograms of Polyphenols at a Glassy Carbon Electrode

polyphenol	$D_0 \times 10^6$	E_1^0, V	E_2^0, V	k_{s1}^0, cm/s	α_1	k_{s2}^0, cm/s	α_2	k^b, s^{-1}	k_1, M^{-1} s^{-1}	k_2, M^{-1} s^{-1}
catechin	2.6	0.46	0.33	0.0015 ± 0.0001	0.5	0.005 ± 0.003	0.55		10^7	$(4.1 \pm 1.4) \times 10^4$
caffeic acid	5.2	0.47	0.34	0.011 ± 0.004	0.55	0.015 ± 0.006	0.4		$(6 \pm 4) \times 10^6$	$(2 \pm 1) \times 10^4$
quercetin	2	0.51	0.29	0.02 ± 0.01	0.37	0.007 ± 0.001	0.5	0.06 ± 0.02	10^7	0

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