applied sciences

MDPI

Article

Browning Development and Antioxidant Compounds in White Wines after Selenium, Iron, and Peroxide Addition

Eftihia Vlahou [1], Stefania Christofi [1], Ioannis G. Roussis [2] and Stamatina Kallithraka [1,*]

[1] Laboratory of Enology and Alcoholic Drinks, Department of Food Science and Human Nutrition, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece; eftihia.vl@gmail.com (E.V.); christofistefania@gmail.com (S.C.)
[2] Laboratory of Food Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece; iroussis@uoi.gr
* Correspondence: stamatina@aua.gr

Abstract: The effect of oxidation on the organoleptic properties of white wines mostly involves increased browning color, loss of the fruity aromas, and appearance of unpleasant odors. Browning, however, is known to be related with polyphenol oxidation and therefore it may be delayed by the presence of antioxidants such as selenium (Se) and SO_2. On the other hand, the presence of oxidants such as metal ions and H_2O_2 can accelerate browning and oxidation phenomena. The browning capacity, the phenolic composition (both total and individual contents of flavanols and hydroxycinnamic acids), the antioxidant activity, and the SO_2 content of Assyrtiko white wines were studied after the addition of Fe^{2+} and H_2O_2 and Se at two temperatures, employing an accelerated test. Browning was approached from a kinetic point of view, and the study was focused on the implication of oxidants and antioxidants on browning rate, paying particular attention to the content of major redox-active polyphenols, including substances with an o-diphenol feature, such as flavanols and hydroxycinnamic acids. The results showed that after the addition of oxidants it was possible to significantly accelerate the rate of browning development (up to 4.7 and six times) depending on the temperature and the concentration of the added compounds. The presence of Se protected wine color and preserved total SO_2 at 35 °C, while at 50 °C, these effects were not observed. Total flavanol content decreased upon heating, while total hydroxycinnamic content showed a slight increase. Similarly, the content of the individual phenolic compounds (with the exception of caffeic acid and (+)-catechin at 35 °C) was decreased with oxidant addition, while Se addition was not adequate to prevent or even promote their oxidation.

Keywords: accelerated browning test; wine oxidation; polyphenolic compounds; sulfur dioxide; browning rate; flavanols; hydroxycinnamic acids; antioxidant compounds

Citation: Vlahou, E.; Christofi, S.; Roussis, I.G.; Kallithraka, S. Browning Development and Antioxidant Compounds in White Wines after Selenium, Iron, and Peroxide Addition. *Appl. Sci.* **2022**, *12*, 3834. https://doi.org/10.3390/app12083834

Academic Editor: Maria Kanellaki

Received: 4 March 2022
Accepted: 6 April 2022
Published: 11 April 2022

1. Introduction

White wines are particularly sensitive to oxidative spoilage which may take place during the various winemaking and storage stages as a result of oxygen exposure [1]. It is due to a complex series of oxidation reactions with detrimental effects on wine organoleptic properties including increase of browning, loss of fresh fruity character, appearance of oxidized notes described as honey, woody, cooked vegetables and farm-like, and increase in bitterness [2]. In particular, the browning phenomenon may compromise the shelf life of bottled wines since the development of browning is accompanied by both an increase of color intensity and a decrease of brightness [3]. However, some white wines may derive organoleptic benefits from oxidation which is often accompanied by a development of a characteristic aroma in the bottle. In particular, for sherries and sweet fortified wines (such as white Ports or Rivesaltes), browning can be perceived as a positive aspect.

Color change appears at the initial stages of winemaking, and it is mainly attributed to enzymatic reactions [4] catalyzed by the action of polyphenol oxidase. After alcoholic

fermentation, non-enzymatic oxidation is mainly responsible for the development of the browning, which progressively dominates in white wine color [5,6].

The most important chemical groups of polyphenols in white wines, both in terms of abundance and the capability to be involved in redox reactions, are flavanols and hydroxycinnamic acids. Oxidation of ortho-dihydroxyphenolic compounds (including (+)-catechin, (−)-epicatechin, caffeic, and other hydroxycinnamates) results to the formation of ortho-quinones which take part in polymerization processes and the development of brown products [7]. Studies involving wine model solutions confirmed the formation of two types of brown compounds, xanthylium salts and ethyl-ester of xanthylium salts, both derived from oxidation and polymerization of flavanols [8]. With an absorption maximum of 440 and 460 nm, respectively, these pigments directly contribute to white wine browning during storage and ageing.

These oxidation reactions lead to the formation of H_2O_2 which, in association with ferrous ions (Fe^{2+}), may lead to the formation of reactive oxygen species (such as hydroxyl radicals and hydroxide ions) through a reaction widely known as Fenton [9]. Hydroxyl radical is considered one of the most powerful nonselective oxidants having the ability to oxidize almost any substance in wine. The oxidation products, mainly ketones and aldehydes, are nucleophiles and are considered important compounds in color development [10]. Compounds such as sulfur dioxide, ascorbic acid, and glutathione, through their action as quinone reductants and/or scavengers, are considered important in increasing wine resistance to oxidation [11].

The browning capacity of the white wines is routinely measured by a simple test which directly measures the impact of elevated temperature on brown pigment formation. This test was initially developed by Singleton and Kramling [12] and was modified later by other researchers [13,14] by excluding the bentonite addition step. Wine samples are subjected to heating at a constant temperature (approximately at 50 °C), and the browning development is followed by measuring the absorbance at 420 nm (A_{420}) over a period of 12 days. This test permits the study of the browning development of wines within a reasonable time period, although the endpoint (where the maximum browning is attained) is chosen arbitrarily [14]. It is well-known that the kinetics of oxidation reactions are directly dependent on temperature and that the rate of browning development in white wines shows an exponential increase with temperature, having an activation energy between 66.4 and 74.6 kJ $mole^{-1}$ [15,16].

Browning of white wines mainly follows zero-order kinetics [1,14,17]. Because browning is mainly associated with polyphenol oxidation, oxidizable substrates such as o-diphenols and hydroxycinnamic acids are implicated to a certain degree in the oxidation mechanism, and many studies have shown that the formation of quinones has a significant contribution [16].

Selenium (Se) is naturally present in wines [18] and can be found in concentrations ranging from 2 to 5 μg/L [19]. It has been shown that it exhibits antioxidant activity by several antioxidant mechanisms including scavenging of reactive oxygen species (ROS), glutathione peroxidase activity, and metal-binding interactions [20]. There are several works regarding selenium tolerance of a diversity of wine-related yeasts as well as the effect on their antioxidant response mechanisms. Different concentrations of Se have been evaluated, and the data have shown that high doses of selenium might result in oxidative stress in yeasts, thereby increasing the process of lipid peroxidation. In addition, selenium might increase the activity of antioxidant enzymes, including glutathione peroxidase and glutathione reductase [21–23]. These results are considered as first indications of the possibility to use pre-enriched yeast cells as a selenium supplement in wine making. However, there is a lack of relevant studies focusing on the effect of selenium addition on wine chemical composition and brown color development.

Browning development in white wines is of high nutritional and technological importance due to its influence on the sensory characters and antioxidant activity of the wines. For this reason, wineries should be equipped with a fast and simple method which could

provide information on browning susceptibility of white wines and allow for suitable technological interventions. The investigation presented herein was undertaken in order to explore the possibility of further accelerating the duration of the browning test already used, after the addition of Fe^{2+} and H_2O_2 in white wines. Since both the presence of metal ions (such as Fe^{2+}) and H_2O_2 is known to increase browning rate [24,25], it was anticipated that their simultaneous addition in white wines would further reduce the time needed for the completion of the browning test. There are few published works regarding the study of browning development in Greek wines [14,26,27] using the accelerated browning test, however, none of them were undertaken in the simultaneous presence of Fe^{2+} and H_2O_2.

It was also of interest to monitor the polyphenolic composition, since these compounds are mostly implicated in the oxidation phenomena, and to evaluate the effect of the addition of an antioxidant substance which could offer protection through various antioxidant mechanisms, such as Se. For this purpose, two temperatures were selected for maintaining the samples during the study, 35 °C and 50 °C, the first resembling transportation temperatures of wines during summer in Mediterranean countries such as Greece, while the second is commonly employed during accelerating browning studies [28,29]. The outcomes of such a study would be of practical interest to the wine sector which is seeking rapid and economical methods which allow fast decision-making procedures in order to optimize the shelf life of their products.

2. Materials and Methods

2.1. Reagents

The reagents employed to carry out the experimental procedures were the following: $FeCl_2 \cdot 4\ H_2O$, H_2O_2, elemental Se, K_2HPO_4 and KH_2PO_4, Folin–Ciocalteu, DPPH (2,2-diphenyl-1-picrylhydrazyl), p-dimethylaminocinnamaldehyde (DMACA), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), glutathione, Trolox, Na_2CO_3, HCl, H_2SO_4, NaOH, (+)-catechin, gallic acid, caffeic acid, tartaric acid, methanol (≥99.8%), and ethanol (≥99.8%). All the reagents were purchased from Sigma-Aldrich (Darmstadt, Germany).

2.2. Samples

The white wine used in this experiment was made from a native Greek *V. vinifera* sp., Assyrtiko (harvest of 2020). The total SO_2 level was set to 80 mg/L prior to bottling. The initial concentration of ethanol was 13.5%vol, reducing sugar content was 0.64 g/L, pH 3.3, total and volatile acidity had values of 6.1 and 0.5 g/L (expressed as tartaric and acetic acid, respectively). The classic parameters of wines together with their free and total SO_2 contents were determined according to the methods of the International Organization of Vine and Wine (OIV) [30]. The contents of total and free SO_2 were measured with an automatic titrator ENO 20 (TDI S.L., Spain). The reagents used for SO_2 analysis were two different solutions, sulfuric acid (H_2SO_4) at 1/3 and sodium hydroxide (NaOH) 5N. The samples with $Fe^{2+,}$ were prepared by direct addition of $FeCl_2 \cdot 4\ H_2O$ to wine at two concentrations (0.1 and 0.25 mM) along with H_2O_2 (0.63 and 1.157 mM, respectively) according to Voltea et al. [25]. Se-enriched wines were prepared with the addition of Se directly into wine to rich final contents of 1.5 and 3.0 mg/L. Another two wine samples were prepared by the simultaneous addition of the high and low concentrations of Fe^{2+}, H_2O_2, and Se. The samples prepared for this experiment are shown in Table 1.

2.3. Accelerated Browning Test

The method employed to evaluate browning capacity [26] was a modification of that published by Singleton and Kramling [12]. Filtered wine samples (20 mL) were placed in 30 mL screw-cap glass vials with metal caps (7.5 cm length, 2.1 cm internal diameter). Samples were subjected to heating at two constant temperatures of 50.0 ± 0.2 °C and 35 ± 0.2 °C, respectively, in a water bath in obscurity. For each sample, 12 vials were placed into the water bath, one for each day of analysis (the experiment lasted for twelve days in

total). One out of the 12 vials of each sample was withdrawn at 24 h intervals over a period of twelve days, and browning (A_{420}) was measured in triplicate against 12% v/v ethanol.

Table 1. Wine samples used in this experiment.

Sample Code	FeII (mM)	H_2O_2 (mM)	Se (mg/L)
M	_	_	_
Fe min	0.10	0.63	_
Fe max	0.025	0.157	_
Se min	_	_	1.5
Se max	_	_	3.0
All min	0.10	0.63	1.5
All max	0.025	0.157	3.0

2.4. HPLC Determination of Individual Phenolic Compounds

The individual polyphenolic constituents were determined by HPLC. Separation was performed on a reversed phase Waters Nova-Pak C18 (150 × 3.9 mm, 4 µm) column with a 20 µL injection volume at a flow rate of 1 mL/min. Identification was achieved by comparing the retention times of the detected peaks with those of standard compounds, and by UV/VIS spectral data. All analyses were performed in triplicate. The chromatographic conditions are described in Kyraleou et al. [31].

2.5. Determination of Total Phenols (TP), Flavanols (TF), Hydroxycinnamic Contents (HC), Free Sulfhydryl Groups, and Antioxidant Activity (AA)

Total polyphenol concentration was determined with the Folin–Ciocalteau assay, with the microscale protocol previously reported [32]. Results were expressed as mg/L gallic acid equivalents (GAE). For the determination of the total flavanols, the p-dimethylaminocinnamaldehyde (DMACA) method was employed [33]. The mixture appropriately diluted in methanol wine and DMACA (0.1% in 1N HCl in methanol) was left to react for 10 min, and the absorbance at 640 nm was then measured against a blank without DMACA. The results are expressed as (+)-catechin equivalents (mg/L (+)-catechin). The absorbance at 320 nm was used to estimate the total hydroxycinnamic acid content [33]. The results are expressed as caffeic acid equivalents (mg/L). Ellman's method was adapted to wine samples for the determination of total -SH groups [34]. Free–SH groups were determined after the addition of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) which reacted with free thiols to form disulphide and 2-nitro-5-thiobenzoic acid. The results are expressed as glutathione equivalents (mg/L). For the determination of antiradical activity, assays were performed employing a DPPH stable radical [35]. The results are expressed as Trolox equivalents (TE mg/L).

2.6. Statistical Analysis

All determinations were run in triplicate, and values were averaged. The standard deviation (S.D.) was also calculated. Data were subjected to one-way analysis of variance (ANOVA), using Statistica V.7 software (Statsoft Inc., Tulsa, OK, USA). Comparison of mean values was performed using Tukey's HSD test when samples were significantly different by ANOVA ($p < 0.05$).

3. Results and Discussion

3.1. Browning Rates

The accelerated browning test is a reliable test commonly employed for evaluating the browning development of white wines [14,26]. The absorbance at 420 nm (A_{420}) could be considered as a browning index, since it measures the concentration of oxidation pigments and displays a linear increase with time (zero order reaction). The reaction rate constants (k) (A_{420} xdays^{-1}) of browning development were calculated from the slope of the regression lines, obtained after plotting A_{420} as a function of time according to

Sioumis et al. [14]. Table 2 presents k-values and percentages of changes in browning (absorbance at 420 nm) calculated for all samples at 35 and 50 °C after 12 days. As it can be seen, the calculated k values ranged from 1.0 to 45.5. These values fell within the range obtained from previous studies [14,26] concerning Greek wines. The wines that showed the lower k values were the control and those that contained Se (both high and low concentrations). In practice, these samples would develop brown color later than the other samples analyzed in this experiment.

Table 2. Browning rate constants (k) and percentage changes in absorbance at 420 nm (A_{420}).

Sample Code	k (day^{-1}) × 10^{-3} 35 °C	k (day^{-1}) × 10^{-3} 50 °C	%ΔA_{420} 35 °C	%ΔA_{420} 50 °C
M	1.0 [a]	7.5 [a]	10.37 [a]	97.70 [a]
Fe min	1.4 [b]	15.8 [b]	27.76 [b]	205.15 [b]
Fe max	4.7 [c]	43.8 [c]	162.21 [d]	312.04 [c]
Se min	–	8.8 [a]	–	107.73 [a]
Se max	–	7.7 [a]	–	101.17 [a]
All min	1.6 [b]	16.6 [b]	25.10 [b]	220.20 [b]
All max	5.1 [c]	45.5 [c]	136.8 [c]	338.74 [c]

[a–c] Different letters at each column indicate statistically significant differences ($p < 0.05$).

As far as the authors are aware, this is the first time that Se addition was studied in relation with browning development in wines. It was very interesting to note that at 35 °C, wine color did not change after 12 days of heating, while at 50 °C, the rate of browning development was not different than that of the control wine. However, at both temperatures, the simultaneous addition of Se with Fe^{2+} and H_2O_2 resulted in increased k rates. It is possible that Se might have been able to offer protection from oxidation at lower temperatures and in the absence of metals. However, when temperature was increased, and in the presence of oxidant compounds, the inhibition of oxidation was not observed any longer. This might have been probably due to increased contents of ROS which were generated by the increased temperature and were further enhanced by the presence of Fe^{2+} and H_2O_2. Transition metals such as Fe play a key role in ROS production since they react with hydrogen peroxide to generate a hydroxyl radical [20]. The added Se content in wine was probably not adequate to exert its antioxidant properties though ROS scavenging and metal-binding mechanisms.

On the other hand, the wines with the higher k values were those made by the addition of Fe^{2+} and H_2O_2. Browning rate was increased 1.4 and 4.7 times at 35 °C for the low and high concentrations of Fe^{2+} and H_2O_2 while at 50 °C, browning occurred about two and six times faster, respectively. Se did not affect significantly browning rates when it was added simultaneously with Fe^{2+} and H_2O_2. Hydrogen peroxide, which is formed during oxygen reduction, in the presence of Fe^{2+} can be converted to hydroxyl radicals by the Fenton reaction. These radicals together with hydrogen peroxide act as nonselective and strong oxidants, oxidizing most of the wine components [10]. In addition, quinones may be produced by direct oxidation by Fe^{2+} at the acidic pH of wine, leading to condensation reactions and further development of brown color [36]. Similar acceleration of browning development after Fe^{2+} and H_2O_2 addition was observed by Patrianakou and Roussis [24] in Chardonnay wines during storage for 40 days and nine months, respectively. However, they measured the browning index and not the rate of browning development in their study.

As it is shown in Table 2, the obtained values were strongly dependent on both the temperature and the presence of Fe^{2+} and H_2O_2. The higher the temperature and the Fe^{2+} and H_2O_2 content, the higher the k value. As expected, the higher temperature (50 °C) resulted in higher browning rates in agreement with other published data [26,37]. It has been shown that parameters such as light and temperature could have a strong influence on wine color through hydrolysis, oxidation, and condensation reactions [38]. The higher temperature employed (50 °C) for the browning test increased the rate of the reactions

involved, thus reducing the time needed for color change. The browning rate of the control wine was increased 7.5 times along with the temperature increase from 35 to 50 °C, while that of the samples containing the high and low concentrations of Fe^{2+} and H_2O_2 showed an increase of about 10- and 11-fold respectively. Similar results were observed by Ricci et al. [1] during their study which involved six Italian wines. During accelerated ageing at higher temperatures, the browning rate showed an Arrhenius-type dependence from temperature allowing to estimate the activation energies of the reaction. There are only a few publications regarding the effect of temperature on browning rate of wine, although the practical importance is very high. Boulton [39] provided data showing that the browning rate of a white wine increased 2.9 times along with a temperature increase from 10 to 20 °C. When temperature reached 40 °C, the rate of browning development occurred 20.7 times faster. These values were calculated using the Arrhenius equation assuming pseudo-first-order kinetics with respect to the depletion of dissolved oxygen.

The addition of Fe^{2+} and H_2O_2 resulted in reaching higher A_{420} values in a shorter time. Regarding ΔA_{420} of control wine stored at 35 °C, the % change (Table 2) was increased almost 1.7- and 16-fold after the addition of the low and high concentration of Fe^{2+} and H_2O_2, respectively. At 50 °C, the increase in A_{420} was more profound for all samples studied, including the control. However, the addition of Fe^{2+} and H_2O_2 resulted in two- and three-fold percentage increases in the absorbance at 420 nm. Oxidation in wine is a complex reaction with numerous steps, and browning development is determined by the rate-limiting step. In the case of higher temperature, it is possible that oxygen depletion, which occurred faster, was the critical parameter determining browning.

3.2. SO_2 Content

Sulfur dioxide is the most common additive that is widely used for wine preservation, inhibiting both the enzymatic and non-enzymatic browning during production and storage [7]. It acts as a multifaceted antiseptic and reducing agent that offers protection against oxidation. Temperature has a dual effect on the SO_2 content of wine since it affects both its equilibrium and the kinetics of the oxidative reactions in which it takes part as an inhibitor [16]. Concerning equilibrium, the higher the temperature, the greater the fraction of sulfur dioxide in the most active molecular form. However, as it was shown in this study, oxidation kinetics were accelerated, resulting in higher amounts of reactive oxygen species (ROS).

As it can be seen in Figure 1, the addition of Fe^{2+} and H_2O_2 resulted in significantly lower total SO_2 contents in the wines at 50 °C compared with that of the control. Similar observations were made at 35 °C with the exception of the wines containing low concentrations of Fe^{2+} and H_2O_2, where the differences with the control were not significant. It has been shown that in the presence of catalysts such as metal ions, it binds with dissolved oxygen and thus protects wines from chemical intense oxidations and consequently from losses of their phenolic and volatile contents [7,33].

An interesting observation was that the addition of Se in wines resulted in preservation of total SO_2 content. At both temperatures, the samples with Se contained significantly higher amounts of SO_2 than that of the control. However, when Se was added in the presence of Fe^{2+} and H_2O_2, the protection was not adequate to preserve total SO_2 content. Regarding free SO_2 content at 35 °C, the control and the wines with the addition of Se contained the highest amounts. However, at 50 °C, all samples contained significantly lower amounts of SO_2 than that of the control, even those with the Se addition. This is in agreement with the findings presented previously in this study, where Se at 35 °C prevented browning development in wines. Se probably protects wine color by preserving SO_2 content which in turn offers antioxidant protection to wine.

Ma and Waterhouse [40] reported that the reaction rates of nucleophilic molecules such as SO_2 with quinones are faster than those of electron transfer of flavonoids (where the B ring is implicated) and by this way, sulfur dioxide can prevent the browning due to quinone reactions in wine. It is thus possible that Se offered protection by reducing the

electron transfer reactions between quinones and flavonoids, preserving total SO_2 content of wines. However, acetaldehyde formation due to ethanol oxidation, which was enhanced by the higher temperature and the presence of metals, probably resulted in binding SO_2, thus reducing its free form [41].

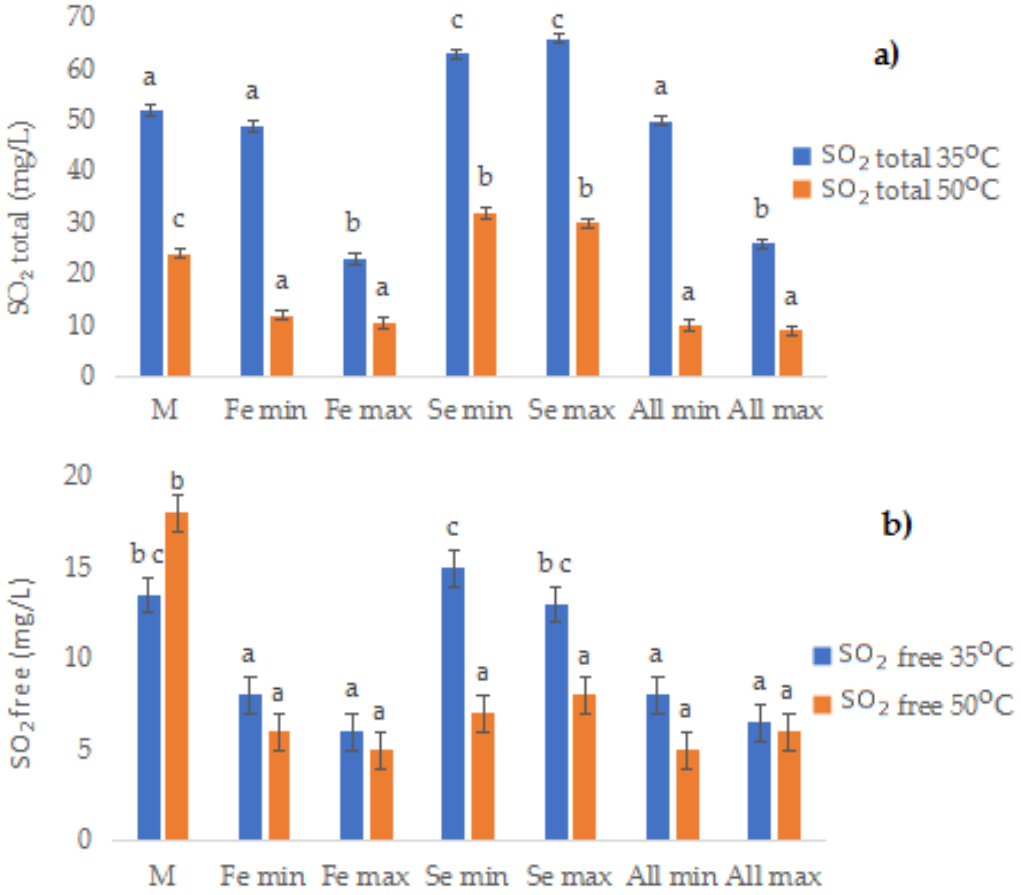

Figure 1. Total (**a**) and free (**b**) SO_2 content (mg/L) of the samples after 12 days of heating at 35 and 50 °C. Different letters at each temperature studied indicate statistically significant differences ($p < 0.05$). Sample codes are described in Table 1.

3.3. Free -SH Groups

Glutathione is a tripeptide which can be found naturally present in grapes and wines. This substance is directly related with the process of oxidation where it plays an important role as an antioxidant. The sulfhydryl group in its molecule acts as a nucleophile able to substitute the electrophilic ring of o-quinones and to regenerate thus the di-hydroxy ring. In this way, the adducts which are formed are no longer prone to oxidation, limiting browning reactions [34,42].

As it can be seen in Figure 2a,b, the addition of Fe^{2+} and H_2O_2 resulted in significantly lower total -SH contents (expressed as mg/L glutathione) in the wines at both 35 and 50 °C compared with that of the control due its implication in the oxidation reactions. This reduction was much more prevalent at the higher temperature. Santor et al. [42] observed a decrease in GSH content during biological ageing of sparkling wines and a correlation with lower percentages of browning. Its relation with (+)-catechin indicated that GSH might act by preventing the formation of (+)-catechin polymers which contribute to browning.

Interestingly, GSH content of the samples containing Se did not follow the same trend and after 12 days of accelerated ageing was similar to that of the control.

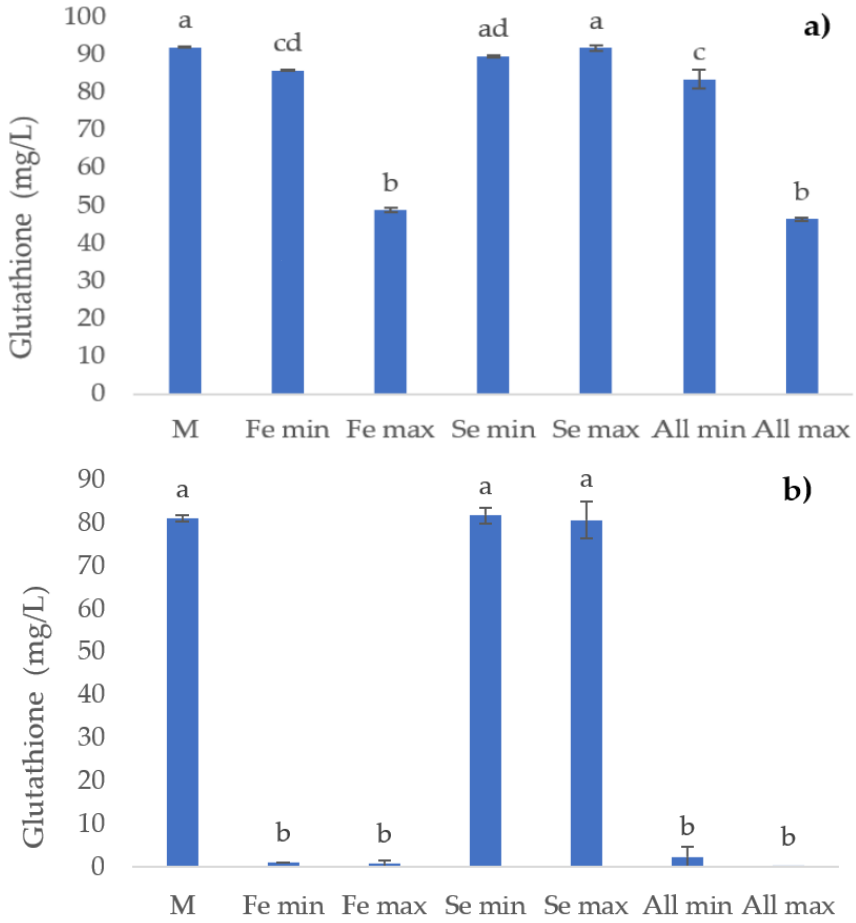

Figure 2. Total sulfhydryl groups (expressed as mg GSH/L) of samples after 12 days of heating at 35 (**a**) and 50 °C (**b**). Different letters at each temperature studied indicate statistically significant differences ($p < 0.05$). Sample codes are described in Table 1.

3.4. Antioxidant Activity, Total Phenols

To demonstrate whether and to what extent browning development affects the antioxidant status of white wines, antioxidant activity (AR) was measured during wine heating for twelve days (Figure 3a,b). AR showed a slight increase during heating at 35 °C, while at 50 °C, the opposite was observed. The existing data on the evolution of wine antioxidant activity with time is rather conflicting. Several studies [1,43,44] concluded that AR decreases or remains stable with biological or accelerated ageing, whereas others [26,33,42] stated that heat-treated or aged wines have higher ability for free radical scavenging than the non-aged do. These conflicting results reflect the different behavior of wines made by different varieties or winemaking technologies and the inconsistency induced by the different methods employed for AR determination.

The decrease in the free form of SO_2 content which was more pronounced at 50 °C (Figure 1) might be responsible for this different trend observed in AR values of the wines heated at two different temperatures.

In addition, after 12 days of heating at both temperatures, the AR of the samples did not show any significant differences. One exception was the antioxidant activity of the control sample at 35 °C, which was significantly higher than the respective values of the rest of the samples. Although the chemical composition of the samples studied in this experiment might differ after the heating period, the similar AR values observed might be attributed to the different condensation products which were formed and which display antioxidant activity sometimes even higher than that of the original monomeric compounds [26,45].

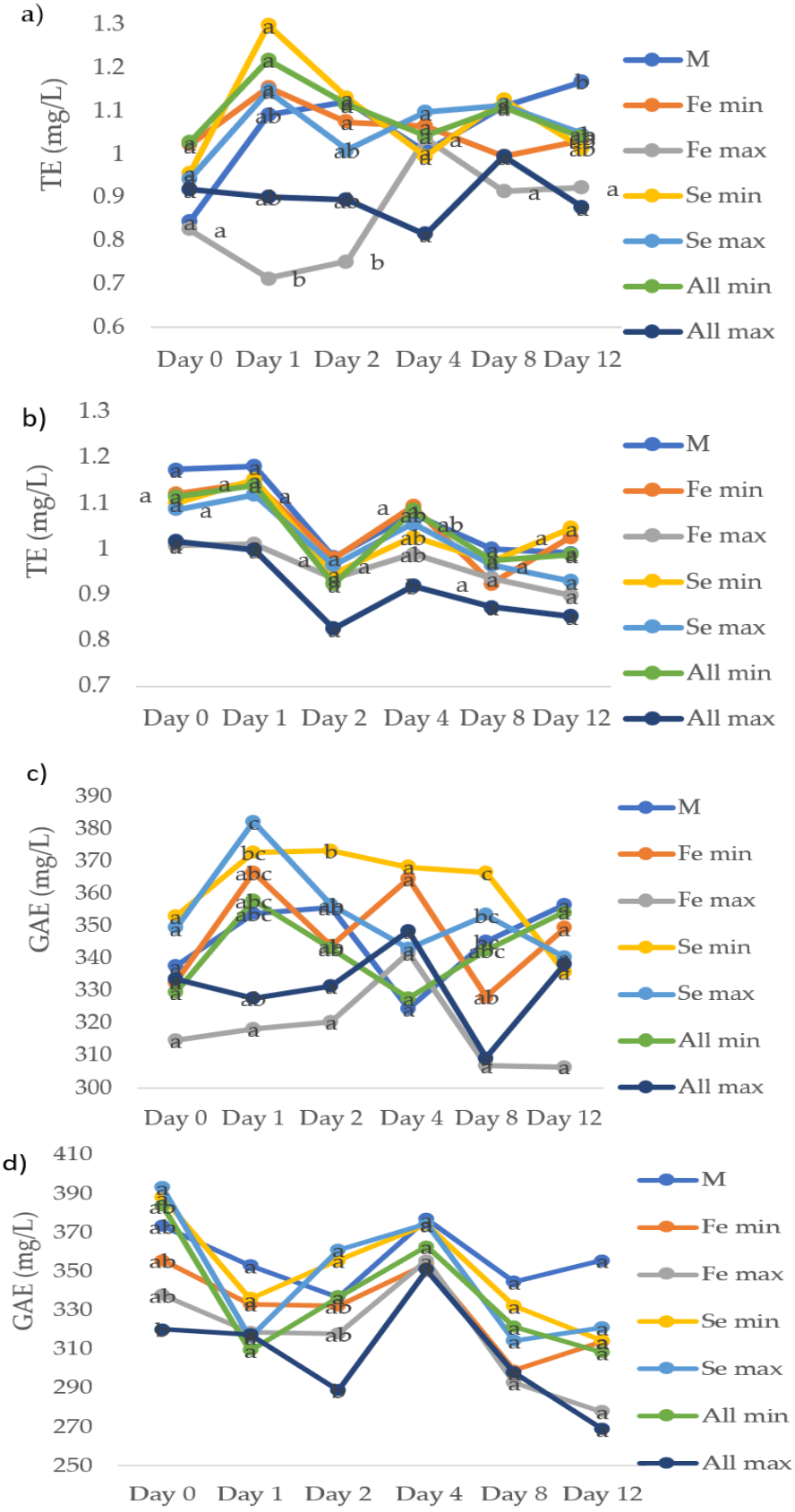

Figure 3. Change of antioxidant activity (TE mg/L) (**a**,**b**) and total phenols (**c**,**d**) GAE (mg/L) of the samples during 12 days of heating at 35 and 50 °C, respectively. Different letters at each temperature studied indicate statistically significant differences ($p < 0.05$). Sample codes are described in Table 1.

These results are in agreement with the data obtained concerning total phenols measured by the Folin–Ciocalteau method (Figure 3c,d). No significant differences were observed between the samples after the 12 days of heating at both temperatures. This spectrophotometric method is based on the oxidation of the phenolic hydroxyl groups with a simultaneous reduction of the Folin–Ciocalteu reagent [46]. The absence of differences in total phenolic content among the samples may possibly be attributed to the transformation of phenols into more condensed forms that possessed similar chemical properties and reactivity towards the reagent.

3.5. Total Flavanol and Hydroxycinnamic Content

Non-enzymatic oxidation of wine is favored by the oxidation of phenols containing a catechol (ortho-hydroxybenzene) or a galloyl (1,2,3-trihydroxybenzene) moiety in their molecules. Flavanols such as catechin and epicatechin, caffeic acid and its esters, and gallic acid are the most readily oxidized wine components. These compounds are oxidized to quinones which are unstable and may undergo further reaction, leading to the formation of brown products [5]. Given the importance of these groups of compounds in browning development, it was of interest to monitor their evolution during the accelerated test and to have a better understanding of their role in oxidation of white wines.

Regarding total flavanol (TF) content, there was a decrease with time at both temperatures studied (Figure 4a,b). This is in agreement with other studies, where similar results were obtained [16,26,38,47]. Within the flavonoid class of white wines, flavanols are the most sensitive compounds to heat, and there is substantial evidence that they may have an active role in the development of brownish shades [14,16]. Numerous studies have shown a positive correlation between flavanol content and browning degree of white wines, confirming their implication in the oxidation process [26,27,48].

After 12 days of heating, the highest statistically significant reduction in FC was observed in the samples which contained the highest concentration of Fe^{2+} and H_2O_2 (Figure 4a,b). This observation was irrespective of the temperature and the presence of Se. In addition, at 50 °C, the presence of the low contents of Fe^{2+} and H_2O_2 resulted in significantly lower TF contents than those in the control, however, still higher than that of the samples which contained high concentrations of the oxidants. It seems, therefore, that the crucial factor for browning development was the presence of iron and hydrogen peroxide, and the addition of antioxidants such as Se might not be able to inhibit the initiation of the oxidation process.

Figure 4c,d shows the evolution of total hydroxycinnamic content (HC) during heating time. As it can be observed, there was no clear trend regarding concentration change. In samples with absence or low contents of Fe^{2+} and H_2O_2, an increase of HC after the heating period was noted at both temperatures. This might have been probably due to the acceleration of the hydrolyzation reactions which took place during heating. These results are in agreement with the findings of other studies where the concentrations of hydroxycinnamic acids increased significantly with time [26,49]. However, in the case of the samples with high Fe^{2+} and H_2O_2 contents, HC content did not show a similar increase and was significantly lower than that of the rest of the samples. The abundance of the strong oxidants probably resulted in condensation reactions involving these compounds. In addition, it was interesting to note that in samples with high contents of oxidants, a sharp decrease of HC content was observed (at day 1 at 50 °C and at days 1 and 4 at 35 °C). This decrease might have been due to oxidation of these compounds, since together with flavanols they are considered the most susceptible to oxidation due to the catechol moiety in their structure [5]. However, afterwards, their content was increased gradually, probably due to the simultaneous presence of flavanols which are the primary oxidation substrates and probably more actively participated in the oxidation process, thus limiting oxidation of other compounds. The presence of Se at 35 °C seems to have retarded the initiation of this process, since the highest decrease in HC was observed after four days, while in the sample without Se addition, this occurred much faster (day 1).

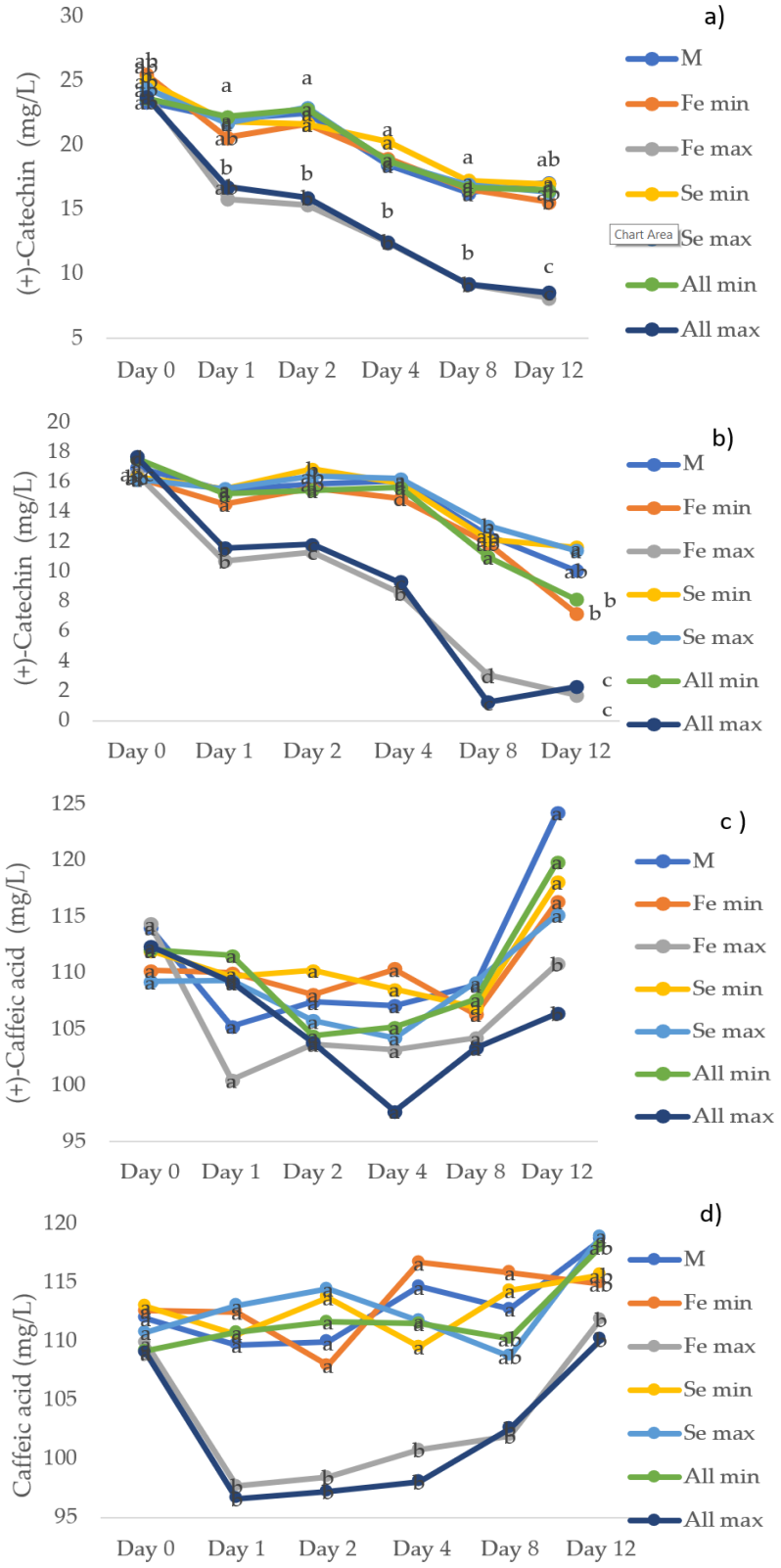

Figure 4. Change of total flavanol (mg/L (+)-catechin) (**a**,**b**) and total hydroxycinnamic (mg/L caffeic acid) (**c**,**d**) contents of the samples during 12 days of heating at 35 and 50 °C, respectively. Different letters at each temperature studied indicate statistically significant differences ($p < 0.05$). Sample codes are described in Table 1.

3.6. Individual Polyphenolic Compounds Determined by HPLC

In agreement with a previous study concerning browning development in Greek white wines [26], heating significantly increased the concentrations of the hydroxycinnaminc acids while slightly decreasing the contents of their tartaric esters, something that is also in line with the results presented previously regarding HC. Gallic acid content was increased by the browning test, (+)-catechin concentration was not significantly affected, while (−)-epicatechin and EGCG concentrations were decreased.

Table 3 presents (+)-catechin, (−)-epicatechin, and (−)-epigallocatechin-3-O-gallate (EGCG) contents (mg/L) of the samples after 12 days of heating at 35 and 50 °C. At 35 °C, (+)-catechin content of the samples did not show any statistically significant differences (with the exception of the sample that contained high concentrations of oxidants and Se) while (−)-epicatechin content was significantly reduced in the sample that contained the maximum concentration of all added compounds. EGCG was the most affected compound by the presence of Fe^{2+} and H_2O_2, since at high concentration of oxidants, its content was significantly reduced.

Table 3. Change of (+)-catechin, (−)-epicatechin, and (−) epigallocatechin-3–0-gallate concentration * (mg/L) of the samples during 12 days of heating at 35 and 50 °C.

Samples	(+)-Catechin	(−)-Epicatechin	(−)-Epigallocatechin-3-*0*-Gallate
Samples at 35 °C			
M	7.34 ^{ab} ± 0.01	2.56 ^{a} ± 0.01	1.34 ^{ab} ± 0.06
Fe min	8.42 ^{a} ± 0.45	2.25 ^{a} ± 0.12	1.26 ^{ab} ± 0.04
Fe max	7.87 ^{a} ± 0.27	1.90 ^{ab} ± 0.02	0.91 ^{b} ± 0.01
Se min	8.84 ^{a} ± 0.42	2.12 ^{a} ± 0.02	1.41 ^{a} ± 0.01
Se max	7.80 ^{a} ± 0.58	2.17 ^{a} ± 0.10	1.39 ^{a} ± 0.03
All min	8.58 ^{a} ± 0.00	1.81 ^{ab} ± 0.02	1.30 ^{a} ± 0.03
All max	5.29 ^{b} ± 0.01	1.69 ^{b} ± 0.06	0.88 ^{b} ± 0.02
Samples at 50 °C			
M	7.81 ^{a} ± 0.16	1.96 ^{a} ± 0.01	1.24 ^{a} ± 0.01
Fe min	5.30 ^{b} ± 0.37	1.81 ^{ab} ± 0.07	1.38 ^{a} ± 0.01
Fe max	5.27 ^{b} ± 0.16	1.36 ^{b} ± 0.03	0.71 ^{b} ± 0.02
Se min	7.02 ^{a} ± 0.58	1.56 ^{ab} ± 0.05	1.34 ^{a} ± 0.01
Se max	6.88 ^{ab} ± 0.02	1.56 ^{ab} ± 0.01	1.29 ^{a} ± 0.00
All min	4.39 ^{c} ± 0.03	1.34 ^{b} ± 0.01	1.28 ^{a} ± 0.03
All max	3.59 ^{c} ± 0.08	1.37 ^{b} ± 0.10	0.80 ^{b} ± 0.02

* Different letters at each column indicate statistically significant differences ($p < 0.05$).

At 50 °C, the differences in flavanol contents were more pronounced. The high concentrations of Fe^{2+} and H_2O_2 resulted in significantly lower contents of all three compounds in the wine samples, while the low concentration of the oxidants reduced significantly only the content of (+)-catechin. The flavanol content of the samples with Se did not differ significantly from that of the control. This observation is in agreement with the results presented in Figure 4a,b regarding total flavanol content of the samples. However, when Se was simultaneously present with oxidants (at both low and high contents), it resulted in significant reductions in the contents of (+)-catechin and (−)-epicatechin. It has been shown that depending on the dose, selenium might contribute to oxidation of thiols and cysteine residues to reduce GSH, generating ROS, thus promoting oxidation [22]. It was therefore possible when Se was present in a highly oxidizable medium to induce oxidative modifications of proteins, thus promoting oxidation.

These results are in line with previous works [26,40,41,48,50] where browning development was related to flavanol content of wines. Wine phenolic compounds are oxidized and converted to quinones which are very reactive species, and they participate in further chemical reactions. They could either by quenched by nucleophiles, such as the A-ring

of flavonoids, and prevent oxidation and browning development, or oxidize the B-ring (catechol ring) of flavanols though electron transfer, leading to flavonoid quinones, substrates of browning development. Ma and Waterhouse [40] observed a much faster electron transfer than nucleophilic reaction between (+)-catechin and caffeic acid quinone in a model solution, explaining the reason why flavanols in wine contribute to browning rather than delay it. In this study, EGCG was the most affected compound at 35 °C, probably due to the higher number of ortho –OH groups in its B-ring, compared with the other two flavanols studied. Since the B-ring is mainly responsible for browning development, the observed reduction of this compound was probably connected with increased formation of quinones and hence more intense browning appearance. However, at 50 °C, all three flavanols studied showed a similar trend probably due to the higher temperature at which the samples were subjected and the acceleration of their oxidation reactions and browning rate. Previous studies have shown that (−)-epicatechin content decreases faster than (+)-catechin concentration under oxidizing conditions [26,51], while others [52] reported that (+)-catechin is most sensitive to oxidation. This trend was not observed in the present study, since the amount of both compounds showed a similar reduction in the presence of oxidants.

Gallic acid content at 35 °C was also reduced significantly in samples containing high amounts of Fe^{2+} and H_2O_2, while at 50 °C, both contents of the oxidants resulted in significant differences compared with those of the control (Table 4). Since this compound contains three –OH groups in its phenol ring, it participates in oxidation reactions by similar mechanisms with flavanols [5].

Table 4. Change of caftraric, coutaric, fertaric, caffeic, ferulic, and p-coumaric concentrations * (mg/L) of the samples during 12 days of heating at 35 and 50 °C.

Samples	Caftaric Acid	Coutaric Acid	Fertaric Acid	Caffeic Acid	Ferulic Acid	p-Coumaric Acid	Gallic Acid
Samples at 35 °C *							
M	62.26 ^{ab} ± 0.10	6.69 ^{b} ± 0.60	7.86 ^{a} ± 0.60	2.62 ^{a} ± 0.01	0.38 ^{a} ± 0.00	0.34 ^{a} ± 0.00	4.43 ^{a} ± 0.00
Fe min	60.43 ^{b} ±1.10	7.07 ^{ab} ± 0.52	7.68 ^{a} ± 0.2	3.05 ^{b} ± 0.13	0.35 ^{ab} ± 0.09	0.32 ^{a} ± 0.01	4.13 ^{a} ± 0.05
Fe max	51.78 ^{c} ± 0.96	6.53 ^{b} ± 0.43	6.84 ^{b} ± 0.26	2.64 ^{a} ± 0.06	0.30 ^{b} ± 0.01	0.22 ^{b} ± 0.02	1.93 ^{b} ± 0.02
Se min	63.18 ^{ab} ±2.67	7.17 ^{ab} ± 0.48	7.77 ^{a} ± 0.15	2.82 ^{a} ± 0.01	0.33 ^{ab} ± 0.00	0.32 ^{a} ± 0.01	4.23 ^{a} ± 0.11
Se max	66.17 ^{a} ±4.51	7.60 ^{a} ± 0.01	7.83 ^{a} ± 0.23	2.96 ^{ab} ± 0.02	0.34 ^{ab} ± 0.01	0.36 ^{a} ± 0.01	4.47 ^{a} ± 0.02
All min	64.92 ^{ab} ±4.08	7.60 ^{b} ± 0.12	7.25 ^{a} ± 0.25	3.19 ^{b} ± 0.26	0.36 ^{ab} ± 0.02	0.34 ^{a} ± 0.02	4.35 ^{a} ± 0.06
All max	52.03 ^{c} ±1.52	6.43 ^{b} ± 0.01	6.02 ^{b} ± 0.14	2.90 ^{ab} ± 0.16	0.26 ^{c} ± 0.00	0.27 ^{b} ± 0.00	2.03 ^{b} ± 0.02
Samples at 50 °C							
M	64.48 ^{a} ±1.23	8.61 ^{a} ± 0.02	7.58 ^{a} ± 0.02	2.57 ^{ab} ± 0.01	0.42 ^{a} ± 0.00	0.50 ^{a} ± 0.01	4.45 ^{a} ± 0.08
Fe min	63.97 ^{a} ±1.42	8.27 ^{a} ± 0.01	7.32 ^{a} ± 0.02	2.58 ^{ab} ± 0.02	0.39 ^{ab} ± 0.01	0.47 ^{ab} ± 0.03	3.55 ^{b} ± 0.10
Fe max	46.46 ^{b} ± 0.31	6.89 ^{b} ± 0.06	5.73 ^{c} ± 0.02	2.37 ^{b} ± 0.08	0.26 ^{c} ± 0.02	0.44 ^{ab} ± 0.00	2.23 ^{c} ± 0.05
Se min	64.74 ^{a} ±1.59	8.60 ^{a} ± 0.04	7.46 ^{a} ± 0.08	2.51 ^{ab} ± 0.03	0.45 ^{a} ± 0.01	0.52 ^{a} ± 0.01	4.07 ^{a} ± 0.01
Se max	64.16 ^{a} ± 1.44	8.42 ^{a} ± 0.06	7.40 ^{a} ± 0.09	2.63 ^{a} ± 0.04	0.36 ^{b} ± 0.00	0.51 ^{a} ± 0.00	4.14 ^{a} ± 0.06
All min	44.95 ^{b} ± 0.15	6.66 ^{b} ± 0.10	6.69 ^{b} ± 0.03	2.28 ^{b} ± 0.03	0.27 ^{c} ± 0.00	0.43 ^{ab} ± 0.01	3.71 ^{b} ± 0.01
All max	37.85 ^{c} ± 0.28	7.44 ^{c} ± 0.14	5.54 ^{c} ± 0.02	2.71 ^{a} ± 0.03	0.29 ^{c} ± 0.04	0.35 ^{b} ± 0.003	2.11 ^{c} ± 0.01

*: Different letters at each column indicate statistically significant differences ($p < 0.05$).

Regarding the hydroxycinnamic content of the samples (Table 4), the following compounds were determined: trans-caftaric, coutaric, fertaric, trans-caffeic, ferulic, and p-coumaric acid. Among these, trans-caffeic acid and its ester with tartaric acid (trans-caftaric acid) possess a "catechol" moiety in their chemical structures. In agreement with other studies [7,42,53] trans-caftaric acid was the major HC acid determined in white wine samples, and its content was considerably reduced during oxidation. This observation might be due to its participation in the oxidation reactions, including the formation of lower oxidation adducts with GSH [54] and it is linked to the presence of two ortho-OH groups to the benzenic ring in its structure. In addition, the contents of fertaric, coutaric, p-coumaric, and ferulic acids were affected less by the oxidation procedure, in agreement with Pati et al. [7].

Caffeic acid content at 35 °C, was significantly increased in the samples with the addition of the low concentration of oxidants, confirming the hydrolysis of caftaric acid during heating as reported in the literature [7,42]. At 35 °C, the high concentration of Fe^{2+} and H_2O_2 in the wine resulted in significantly lower amounts of all HC with the exception of trans-caffeic acid. It is possible that trans-caffeic acid content remained stable during heating even at the presence of high concentrations of the oxidizing substances due to the liberation of free caffeic acid from the hydrolysis of its ester forms. The initial content in wine may have been involved in the formation of adducts and the appearance of brown pigments [52]. The samples containing Se contained similar amounts of HC with the control wine.

At 50 °C, all HC showed a significant decrease after the addition of the high concentrations of Fe^{2+} and H_2O_2 (Table 4). An interesting observation was that the addition of low content of oxidants did not result in statistically different contents compared with those of the control, however, when Se was simultaneously present, a significant decrease was noted for most of the HC studied (with the exception of p-coumaric acid), in agreement with previous results concerning flavanols. Regarding samples containing high contents of oxidants, it is possible that a similar action might have occurred, however, it may have been masked by the detrimental and more intense effects of oxidants and temperature.

4. Conclusions

The quality of white wines can be seriously affected by high temperatures during transportation and storage. Browning development is a serious undesirable color alteration which is mainly attributed to the oxidation of polyphenols. It is therefore necessary to develop a rapid method to measure the susceptibility of wines to browning that will provide valuable information to winemakers, allowing thus appropriate interventions. The addition of oxidants (Fe^{2+} and H_2O_2) in the wine samples could significantly accelerate the rate of browning development (up to 4.7 and six times) depending on the temperature and the concentration of the added compounds, allowing thus faster determination of the oxidation stability of wines in comparison with the accelerated browning test that is commonly used today, requiring 12 days. This study was the first one demonstrating that the change of wine color could be prevented by the addition of Se, which could also preserve total SO_2 content at 35 °C. However, at higher temperatures such as 50 °C, these protective effects were not observed any longer. Flavanols and hydroxycinnamic acids participated actively in oxidation reactions, since their total contents were significantly altered upon heating. Similarly, the content of the individual phenolic compounds (with the exception of caffeic acid and (+)-catechin at 35 °C) was decreased with oxidant addition, while the presence of Se was not adequate to prevent color change or, in several cases, it even enhanced their oxidation.

Author Contributions: Conceptualization, S.K. and I.G.R.; methodology, E.V., S.C., S.K. and I.G.R.; software, E.V. and S.C.; validation, E.V., S.C., I.G.R. and S.K.; formal analysis, E.V. and S.C.; investigation, E.V., S.C. and S.K.; resources, E.V., S.C. and S.K.; data curation, E.V. and S.K.; writing—original draft preparation, E.V., I.G.R. and S.C.; writing—review and editing, E.V., I.G.R. and S.C.; visualization, I.G.R. and S.K.; supervision, E.V. and S.C.; project administration, E.V. and S.C.; funding acquisition, S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author (pending privacy and ethical considerations).

Conflicts of Interest: The authors declare no conflict of interests.

References

1. Ricci, A.; Parpinello, G.P.; Versari, A. Modelling the evolution of oxidative browning during storage of white wines: Effects of packaging and closures. *Int. J. Food Sci. Technol.* **2017**, *52*, 472–479. [CrossRef]
2. Silva Ferreira, A.C.; Hogg, T.; Guedes de Pinho, P. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **2003**, *51*, 1377–1381. [CrossRef] [PubMed]
3. El Hosry, L.; Auezova, L.; Sakr, A.; Hajj-Moussa, E. Browning susceptibility of white wine and antioxidant effect of glutathione. *Int. J. Food Sci. Technol.* **2009**, *44*, 2459–2463. [CrossRef]
4. Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. Protecting juice from oxidation. In *Handbook of Enology: The Microbiology of Wine and Vinifications*, 2nd ed.; John Wiley & Sons, Ltd.: Chichester, UK, 2006; Volume 1, pp. 417–421.
5. Oliveira, C.M.; Silva Ferreira, A.C.; De Freitas, V.; Silva, A.M.S. Oxidation mechanisms occurring in wines. *Food Res. Int.* **2011**, *44*, 1115–1126. [CrossRef]
6. Pati, S.; Crupi, P.; Benucci, I.; Antonacci, D.; Di Luccia, A.; Esti, M. HPLC-DAD-MS/MS characterization of phenolic compounds in white wine stored without added sulfite. *Food Res. Int.* **2014**, *66*, 207–215. [CrossRef]
7. Pati, S.; Crupi, P.; Savastano, M.L.; Benucci, I.; Esti, M. Evolution of phenolic and volatile compounds during bottle storage of a white wine without added sulfite. *J. Sci. Food Agric.* **2020**, *100*, 775–784. [CrossRef] [PubMed]
8. Es-Safi, N.-E.; Le Guernevé, C.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Xanthylium salts formation involved in wine colour changes. *Int. J. Food Sci. Technol.* **2000**, *35*, 63–74. [CrossRef]
9. Waterhouse, A.L.; Laurie, V.F. Oxidation of Wine Phenolics: A Critical Evaluation and Hypotheses. *Am. J. Enol. Vitic.* **2006**, *57*, 306–313.
10. Li, H.; Guo, A.; Wang, H. Mechanisms of oxidative browning of wine. *Food Chem.* **2008**, *108*, 1–13. [CrossRef]
11. Ugliano, M.; Kwiatkowski, M.; Vidal, S.; Capone, D.; Siebert, T.; Dieval, J.-B.; Aagaard, O.; Waters, E.J. Evolution of 3-Mercaptohexanol, Hydrogen Sulfide, and Methyl Mercaptan during Bottle Storage of Sauvignon blanc Wines. Effect of Glutathione, Copper, Oxygen Exposure, and Closure-Derived Oxygen. *J. Agric. Food Chem.* **2011**, *59*, 2564–2572. [CrossRef] [PubMed]
12. Singleton, V.L.; Kramlinga, T.E. Browning of White Wines and an Accelerated Test for Browning Capacity. *Am. J. Enol. Vitic.* **1976**, *27*, 157–160.
13. Gonzales Cartagena, L.; Perez-Zuniga, F.J.; Bravo Abad, F. Interactions of Some Environmental and Chemical Parameters Affecting the Colour Attributes of Wine. *Am. J. Enol. Vitic.* **1994**, *45*, 43–48.
14. Sioumis, N.; Kallithraka, S.; Makris, D.P.; Kefalas, P. Kinetics of browning onset in white wines: Influence of principal redox-active polyphenols and impact on the reducing capacity. *Food Chem.* **2006**, *94*, 98–104. [CrossRef]
15. Boulton, R.B.; Singleton, V.L.; Bisson, L.F.; Kunkee, R.E. Oxidation and Browning. In *Principles and Practices of Winemaking*; Springer: New York, NY, USA, 2013; Volume 10E, pp. 406–414.
16. Scrimgeour, N.; Nordestgaard, S.; Lloyd, N.D.R.; Wilkes, E.N. Exploring the effect of elevated storage temperature on wine composition. *Aust. J. Grape Wine Res.* **2015**, *21*, 713–722. [CrossRef]
17. Serra-Cayuela, A.; Jourdes, M.; Riu-Aumatell, M.; Buxaderas, S.; Teissedre, P.-L.; López-Tamames, E. Kinetics of Browning, Phenolics, and 5-Hydroxymethylfurfural in Commercial Sparkling Wines. *J. Agric. Food Chem.* **2014**, *62*, 1159–1166. [CrossRef]
18. Witkowska, A.M.; Zujko, M.E.; Borawska, M.H.; Socha, K. Antioxidant Properties and Selenium Content of Wines. *Pol. J. Environ. Stud.* **2006**, *15*, 208–211.
19. Frías, S.; Díaz, C.; Conde, J.E.; Pérez Trujillo, J.P. Selenium and mercury concentrations in sweet and dry bottled wines from the Canary Islands, Spain. *Food Addit. Contam.* **2003**, *20*, 237–240. [CrossRef]
20. Battin, E.E.; Brumaghim, J.L. Antioxidant Activity of Sulfur and Selenium: A Review of Reactive Oxygen Species Scavenging, Glutathione Peroxidase, and Metal-Binding Antioxidant Mechanisms. *Cell Biochem. Biophys.* **2009**, *55*, 1–23. [CrossRef]
21. Assuncao, M.; Martins, L.L.; Mourato, M.P.; Baleiras-Couto, M.M. Effect of selenium on growth and antioxidant enzyme activities of wine related yeasts. *World J. Microbiol. Biotechnol.* **2015**, *31*, 1899–1906. [CrossRef]
22. Talbi, W.; Ghazouani, T.; Braconi, D.; Ben Abdallah, R.; Raboudi, T.; Santucci, A.; Fattouch, S. Effects of selenium on oxidative damage and antioxidant enzymes of eukaryotic cells: Wine Saccharomyces cerevisiae. *J. Appl. Microbiol.* **2018**, *126*, 555–566. [CrossRef]
23. Fontanella, M.C.; D'Amato, R.; Regni, L.; Proietti, P.; Beone, G.M.; Businelli, D. Selenium speciation profiles in biofortified sangiovese wine. *J. Trace Elem. Med. Biol.* **2017**, *43*, 87–92. [CrossRef] [PubMed]
24. Patrianakou, M.; Roussis, I.G. Decrease of Wine Volatile Aroma Esters by Oxidation. *S. Afr. J. Enol. Vitic.* **2013**, *34*, 241–245. [CrossRef]
25. Voltea, S.; Karabagias, I.K.; Roussis, I.G. Use of Fe (II) and H_2O_2 along with heating for the estimation of the browning susceptibility of white wine. *J. Appl. Sci.* **2022**. *submitted*.
26. Kallithraka, S.; Salacha, M.I.; Tzourou, I. Changes in phenolic composition and antioxidant activity of white wine during bottle storage: Accelerated browning test versus bottle storage. *Food Chem.* **2009**, *113*, 500–505. [CrossRef]
27. Salacha, M.-I.; Kallithraka, S.; Tzourou, I. Browning of white wines: Correlation with antioxidant characteristics, total polyphenolic composition and flavanol content. *Int. J. Food Sci. Technol.* **2008**, *43*, 1073–1077. [CrossRef]

28. Robinson, A.L.; Mueller, M.; Heymann, H.; Ebeler, S.E.; Boss, P.K.; Solomon, P.S.; Trengove, R.D. Effect of simulated shipping conditions on sensory attributes and volatile composition of commercial white and red wines. *Am. J. Enol. Vitic.* **2010**, *61*, 337–347. Available online: https://www.ajevonline.org/content/61/3/337 (accessed on 5 April 2022).
29. Walther, A.K.; Durner, D.; Fischer, U. Impact of temperature during bulk shipping on the chemical composition and sensory profile of a Chardonnay wine. *Am. J. Enol. Vitic.* **2018**, *69*, 247–257. [CrossRef]
30. International Organization of Vine and Wine (OIV). *Compendium of International Methods of Wine and Must Analysis*; OIV: Paris, France, 2020; Volume 1, 770p. Available online: https://www.oiv.int/public/medias/7372/oiv-compendium-volume-1-2020.pdf (accessed on 15 January 2022).
31. Kyraleou, M.; Kallithraka, S.; Chira, K.; Tzanakouli, E.; Ligas, I.; Kotseridis, Y. Differentiation of Wines Treated with Wood Chips Based on Their Phenolic Content, Volatile Composition, and Sensory Parameters. *J. Food Sci.* **2015**, *80*, 2701–2710. [CrossRef]
32. Arnous, A.; Makris, D.P.; Kefalas, P. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *J. Agric. Food Chem.* **2001**, *49*, 5736–5742. [CrossRef]
33. Roussis, I.G.; Lambropoulos, I.; Tzimas, P.; Gkoulioti, A.; Marinos, V.; Tsoupeis, D.; Boutaris, I. Antioxidant activities of some Greek wines and wine phenolic extracts. *J. Food Comp. Anal.* **2008**, *21*, 614–621. [CrossRef]
34. Kontogeorgos, N.; Roussis, I.G. Total free sulphydryls of several white and red wines. *S. Afr. J. Enol. Vitic.* **2014**, *35*, 125–127. [CrossRef]
35. Psarra, E.; Makris, D.P.; Kallithraka, S.; Kefalas, P. Evaluation of the antiradical and reducing properties of selected Greek white wines: Correlation with polyphenolic composition. *J. Sci. Food Agric.* **2002**, *82*, 1014–1020. [CrossRef]
36. Guo, A.; Kontoudakis, N.; Scollary, G.R.; Clark, A.C. Production and Isomeric Distribution of Xanthylium Cation Pigments and Their Precursors in Wine-like Conditions: Impact of Cu(II), Fe(II), Fe(III), Mn(II), Zn(II), and Al(III). *J. Agric. Food Chem.* **2017**, *65*, 2414–2425. [CrossRef]
37. Kanavouras, A.; Coutelieris, F.A.; Karanika, E.; Kotseridis, Y.; Kallithraka, S. Colour change of bottled white wines as a quality indicator. *OENO One* **2020**, *54*, 543–551. [CrossRef]
38. Celotti, E.; Lazaridis, G.; Figelj, J.; Scutaru, Y.; Natolino, A. Comparison of a Rapid Light-Induced and Forced Test to Study the Oxidative Stability of White Wines. *Molecules* **2022**, *27*, 326. [CrossRef]
39. Boulton, R. A Method for the Assessment of Copigmentation in Red Wines. *Am. J. Enol. Vitic.* **1996**, *47*, 346.
40. Ma, L.; Waterhouse, A.L. Flavanols react preferentially with quinones through an electron transfer reaction, stimulating rather than preventing wine browning. *Anal. Chim. Acta* **2018**, *1039*, 162–171. [CrossRef]
41. Cucciniello, R.; Forino, M.; Picariello, L.; Coppola, F.; Moio, L.; Gambuti, A. How acetaldehyde reacts with low molecular weight phenolics in white and red wines. *Eur. Food Res. Technol.* **2021**, *247*, 2935–2944. [CrossRef]
42. Sartor, S.; Burin, V.M.; Ferreira-Lima, N.E.; Caliari, V.; Bordignon-Luiz, M.T. Polyphenolic Profiling, Browning, and Glutathione Content of Sparkling Wines Produced with Nontraditional Grape Varieties: Indicator of Quality during the Biological Aging. *J. Food Sci.* **2019**, *84*, 3546–3554. [CrossRef]
43. Landrault, N.; Poucheret, P.; Ravel, P.; Gasc, F.; Cros, G.; Teissedre, P.-L. Antioxidant Capacities and Phenolics Levels of French Wines from Different Varieties and Vintages. *J. Agric. Food Chem.* **2001**, *49*, 3341–3348. [CrossRef]
44. De Beer, D.; Joubert, E.; Gelderblom, W.C.A.; Manley, M. Changes in phenolic composition and antioxidant activity of Pinotage, Cabernet Sauvignon, Chardonay and Chenin blanc wines during bottle ageing. *S. Afr. J. Enol. Vitic.* **2005**, *26*, 6–15.
45. Roginsky, V.; de Beer, D.; Harbertson, J.F.; Kilmartin, P.A.; Barsukova, T.; Adams, D.O. The antioxidant activity of Californian red wines does not correlate with wine age. *J. Sci. Food Agric.* **2006**, *86*, 834–840. [CrossRef]
46. Kallithraka, S.; Kotseridis, Y.; Kyraleou, M.; Proxenia, N.; Tsakiris, A.; Karapetrou, G. Analytical phenolic composition and sensory assessment of selected rare Greek cultivars after extended bottle ageing. *J. Sci. Food Agric.* **2015**, *95*, 2353. [CrossRef] [PubMed]
47. Castellanos, E.R.; Jofre, V.P.; Fanzone, M.L.; Assof, M.V.; Catania, A.A.; Diaz-Sambueza, A.M.; Heredia, F.J.; Mercado, L.A. Effect of different closure types and storage temperatures on the color and sensory characteristics development of Argentinian Torrontes Riojano white wines aged in bottles. *Food Control* **2021**, *130*, 108343. [CrossRef]
48. Bührle, F.; Gohl, A.; Weber, F. Impact of Xanthylium Derivatives on the Color of White Wine. *Molecules* **2017**, *22*, 1376. [CrossRef]
49. Recamales, A.F.; Sayago, A.; Gonzalez-Miret, M.L.; Hernanz, D. The effect of storage conditions on the phenolic composition and colour of white wine. *Food Res. Int.* **2006**, *39*, 220–229. [CrossRef]
50. Milat, A.M.; Boban, M.; Teissedre, P.-L.; Šešelja-Perišin, A.; Jurić, D.; Skroza, D.; Generalić-Mekinić, I.; Ljubenkov, I.; Volarević, J.; Rasines-Perea, Z.; et al. Effects of oxidation and browning of macerated white wine on its antioxidant and direct vasodilatory activity. *J. Funct. Foods* **2019**, *59*, 138–147. [CrossRef]
51. Di Lecce, G.; Boselli, E.; D'Ignazi, G.; Frega, N.G. Evolution of phenolics and glutathione in Verdicchio wine obtained with maceration under reductive conditions. *LWT-Food Sci. Technol.* **2013**, *53*, 54–60. [CrossRef]
52. Webber, V.; Dutra, S.V.; Spinelli, F.R.; Carnieli, G.J.; Cardozo, A.; Vanderlinde, R. Effect of glutathione during bottle storage of sparkling wine. *Food Chem.* **2017**, *216*, 254–259. [CrossRef]

53. Serra-Cayuela, A.; Aguilera-Curiel, M.A.; Riu-Aumatell, M.; Buxaderas, S.; López-Tamames, E. Browning during biological aging and commercial storage of Cava sparkling wine and the use of 5-HMF as a quality marker. *Food Res. Int.* **2013**, *53*, 226–231. [CrossRef]
54. Ferreira-Lima, N.E.; Burin, V.M.; Caliari, V.; Bordignon-Luiz, M.T. Impact of Pressing Conditions on the Phenolic Composition, Radical Scavenging Activity and Glutathione Content of Brazilian Vitis vinifera White Wines and Evolution During Bottle Ageing. *Food Bioprocess Technol.* **2016**, *9*, 944–957. [CrossRef]