

## Article

# Fortification of White Wines with Antioxidants and Se: Impacts on Browning Development and Phenolic Content

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**Abstract:** The present study explores the efficiency of selenomethionine (Semeth), an organic form of Se, as an antioxidant compared with commonly used antioxidants (ascorbic acid, glutathione, and potassium metabisulfite) in preventing oxidative browning in Greek white wines (Malagouzia and Retsina). The experimental procedure involved an accelerated browning test conducted over 12 days at 55 °C, measurement of antioxidant activity values (using the Folin—Ciocalteu and the free radical diphenylpicrylhydrazyl (DPPH) methods), determination of free sulfhydryl groups using the Ellman’s method, and High-Performance Liquid Chromatographic analysis of selected phenolic compounds. Semeth consistently exhibited a preserving effect on total and free SO<sub>2</sub> content and antioxidant activity values of Malagouzia wines. Semeth also showed a protecting effect on free sulfhydryl groups (-SH), even higher than that of SO<sub>2</sub> suggesting that its role in maintaining wine color involves more mechanisms than just the prevention of SO<sub>2</sub> reduction. Moreover, Semeth demonstrated promising effects in preserving individual phenolic content, in particular (+)-catechin and ferulic acid, compared to the other antioxidant additions. Both browning rate constants and percentage color change values of Retsina were higher than the corresponding values of Malagouzia wines indicating greater susceptibility to browning. Browning development was dependent on the particular antioxidant added, with ascorbic acid being the least effective. The results of this study suggest that Semeth could be an important candidate for enhancing the oxidative stability of white wines, offering at the same time valuable information for optimizing antioxidant strategies in winemaking practices.

**Keywords:** accelerated browning; white wine; oxidation; antioxidants; selenomethionine; malagouzia; retsina; glutathione; SO<sub>2</sub>; phenolic compounds



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## 1. Introduction

Wine oxidation presents a significant challenge in both winemaking and bottling processes, potentially leading to undesirable alterations in organoleptic attributes of the final product [1]. In particular, white wines are more sensitive to oxidation, when they are exposed to oxygen contents exceeding the mean solubility threshold [2]. Detrimental effects of white wine oxidation include the loss of fresh fruity character, appearance of oxidized nuances, and color shifts towards brown hues [3].

The appearance of browning is closely related with wine phenolic composition and parameters which might influence their susceptibility to oxidation [4]. Individual phenolic compounds such as caffeic and gallic acids, (+)-catechin, and (–)-epicatechin play an important role in oxidative browning, with their concentrations ranging across different white wine varieties [5].

Both hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and iron ions, participate in the formation of hydroxyl radicals, via the Fenton reaction. The hydroxyl radicals, as oxidizing substrates, affecting various molecules prone to oxidation in the wine [6]. The target molecules within the wine

matrix include ethanol, tartaric acid, glycerin, sugars, and organic acids and their oxidation rate depends on their concentration. [7].

Phenolic compounds are important wine antioxidants acting on peroxide radicals converting them into hydroperoxides and consequently into non-radical substrates via the formation of intermediate compounds such as phenoxy-radicals. While the phenolic content of white wines is lower compared to reds, it is considered adequate to participate in oxidative reactions. Glyoxylic acid which is formed by the oxidation of tartaric acid in white wines is a reactive compound capable of undergoing various chemical reactions. When present, it may interact with phenolic compounds and potentially decreasing their content. [8]. In certain cases, phenolic compounds might as well extract  $H^+$  ions from polymers leading to a secondary cycle of oxidation [9].

Sulphur dioxide ( $SO_2$ ) is considered the most effective antioxidant in winemaking technologies for more than four centuries. It exerts its protective effect through two mechanisms: it is oxidized directly in place of wine's more oxidizable compounds, and inactivates indirectly the enzymes such as oxidases, preventing the initiation of oxidative reactions [10,11]. While sulfur dioxide ( $SO_2$ ) exhibits the highest efficacy as both an antioxidant and antimicrobial agent, there is currently a trend towards reducing or even substituting its use. This shift is motivated by concerns regarding its potential impact on human health and its association with allergic responses.

Alternative antioxidant compounds which might substitute  $SO_2$  in winemaking, include glutathione and ascorbic acid. Glutathione is a tripeptide naturally occurring in grapes formed by covalent bonds between the carboxyl group of glutamate, the amino group of cysteine and glycine [12]. It has been demonstrated that it is very efficient in protecting wine volatile compounds and preventing or decreasing browning development [13]. Ascorbic acid, also present in grapes although not in sufficient quantities in wine, may be utilized as an antioxidant compound. Its primary function is to catalyze the reduction of  $Fe^{3+}$  to  $Fe^{2+}$  [2]. Although several research studies have demonstrated a positive role in protecting the color of white wines during bottling, the existing literature is inconsistent regarding its effectiveness in preventing browning [14].

Selenium (Se) is an element possessing antioxidant properties, and it is naturally present in wine at trace concentrations (0.5–5  $\mu\text{g/L}$ ). American white grape varieties, contain higher Se contents (5  $\mu\text{g/kg}$ ) than their red counterparts (2  $\mu\text{g/kg}$ ) while in European varieties Selenium's concentration is higher in red [15]. Its antioxidant action is exerted through multiple protective mechanisms, including scavenging of reactive oxygen species (ROS), increasing glutathione peroxidase activity resulting in ROS and  $H_2O_2$  inactivation, and chelating metal ions such as Fe. [16].

Elementary, selenium can undergo reduction to the  $-2$  oxidation state (selenide,  $Se^{-2}$ ) or oxidation to the  $+4$  (selenite,  $SeO_3^{-2-}$ ) and  $+6$  (selenate,  $SeO_4^{2-}$ ) oxidation states. In organoselenium chemistry, selenium in the  $-2$  oxidation state predominates. Selenide serves as a crucial metabolite in animals and certain microorganisms, as it acts as the precursor of selenocysteine at the active centers of numerous selenoenzymes. Selenium compounds exhibit greater nucleophilicity and acidity compared to their sulfur counterparts [17].

Organic amino acids containing selenium, such as selenomethionine, offer antioxidant benefits by acting both as direct antioxidants and as a source of selenium for the synthesis of selenium-dependent antioxidant and repair proteins (e.g., glutathione peroxidases, thioredoxin reductases, methionine sulfoxide reductases) [17]. Selenoproteins play important roles in antioxidant defense, immune function, thyroid hormone metabolism, and DNA synthesis. Se is present in both wine and living organisms in its organic form, selenomethionine [18,19].

Selenomethionine's direct antioxidant effects result from the nucleophilic properties of the ionized selenol ( $RSe^-$ ), (prevailing over the neutral form at physiological pH levels) and the fast oxidation of selenomethionine. This results in higher rate constants for reacting with multiple oxidants compared to corresponding thiols/thioethers. Additionally, the

resulting oxidation products can be more readily and rapidly reversed by both enzyme and nonenzymatic reactions, making the antioxidant effects catalytic. Seleno amino acids may also bind to redox-active metal ions. It has been shown that the SeMet oxidation process is favoured at lower temperatures, which might have implications for the storage stability of selenomethionine at such conditions [20]. The inclusion of Se in the catalytic site of selenium-dependent antioxidant enzymes improves their kinetic properties and expands their catalytic activity against biological oxidants compared to sulfur-containing species [21].

It has been demonstrated that *Saccharomyces cerevisiae*, through their enzymatic activity, are able to convert selenium into selenomethionine [19,22]. Moreover, it has been shown that the addition of selenomethionine to wines led to increased antioxidant activity and phenolic content [23]. Additionally, it affected the enzymatic activities of several antioxidant enzymes including glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) [24]. In addition to its antioxidative activity, selenomethionine appears to exert a positive effect on yeast physiology, improving wine quality during alcoholic fermentation [25].

Greek wines are comparatively underrepresented in scientific research despite their scientific interest. Retsina, a unique white wine is exclusively produced in Greece, mainly in the Attica region from Savvatiano grape variety. It is made by pine tree resin, addition, during fermentation. Besides the aromatic enhancement of wines with characteristic pine aromas, resin also possesses antioxidant properties [26]. On the contrary, Malagouzia wines, characterized by their low phenolic content, fall within the group of more sensitive to oxidation wines requiring particular attention during winemaking and strong antioxidant protection strategies [27].

A recent study [28] investigated the potential benefits of supplementing white wine made from the Assyrtiko variety with inorganic selenium. The results were promising, indicating significant color protection and preservation of total SO<sub>2</sub>.

Due to the limited availability of relevant articles in current literature, there was an interest in further investigating the antioxidant role of selenium (Se) in white wine. This involved examining its impact on key wine parameters associated with the oxidation process. Selenomethionine (Semeth) was introduced into two distinct Greek white wines (Retsina and Malagouzia) to elucidate the role of the organic form of selenium in antioxidant protection. Comparative assessments were undertaken with conventional antioxidants such as glutathione and ascorbic acid, with the aim of gaining a deeper understanding of the role of wine additives and potentially reducing sulfur dioxide content.

## 2. Materials and Methods

### 2.1. Reagents

The following reagents were used in this study: selenomethionine (C<sub>5</sub>H<sub>11</sub>NO<sub>2</sub>Se) (99%), potassium metabisulfite (K<sub>2</sub>S<sub>2</sub>O<sub>5</sub>) (>98%), ascorbic acid (C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>) (>95%), glutathione (C<sub>10</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S) (>98%), sodium hydroxide (NaOH) (>98%), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) (98%), Folin-Ciocalteu reagent (98%) (C<sub>10</sub>H<sub>5</sub>NaO<sub>5</sub>S), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) (>99.5%), gallic acid (C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>) (98%), DPPH reagent (C<sub>18</sub>H<sub>12</sub>N<sub>5</sub>O<sub>6</sub>) (95%), methanol (>99.8%), Trolox reagent (C<sub>14</sub>H<sub>18</sub>O<sub>4</sub>) (97%), potassium phosphate (K<sub>2</sub>HPO<sub>4</sub>) (>98%), potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) (>99%), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), ethanol (>99.8%) and tartaric acid (99.5%). The reagents were supplied by Sigma–Aldrich (Darmstadt, Germany).

### 2.2. Samples

Two white wines produced in 2022 by Malagouzia and Savvatiano varieties (*Vitis vinifera* sp.) were used in this experiment. Retsina was made from Savvatiano variety by resin (of pine tree origin) addition during alcoholic fermentation. Both wines were commercial samples. All treatments and chemical analyses took place three months after bottling. Malagouzia displayed a total alcoholic strength of 11.5% vol, with a pH value of 3.39 and total acidity of 4.8 g/L (expressed as tartaric acid). Retsina, on the other hand, had

a total alcoholic strength of 11.5% vol, a pH of 3.29, and total acidity of 5.3 g/L (expressed as tartaric acid). Sulfur dioxide was added to both wines before the addition of antioxidants to reach a total concentration of 80 mg/L. Total and free sulfur dioxide measurements were conducted using the automatic titrator OENO 20 (Oeno Bio Sarl, Saint-Martin-le-Vieil, France) with the addition of H<sub>2</sub>SO<sub>4</sub> for free SO<sub>2</sub> and 5N NaOH and H<sub>2</sub>SO<sub>4</sub> for total SO<sub>2</sub>, according to the method outlined by the International Organization of Vine and Wine (OIV) [29].

Eleven samples were prepared for each wine: Control (C) without any addition, two samples with Glutathione (Glut) (10 mg/L and 20 mg/L), two samples with Selenomethionine (Se) (25 µg/L and 50 µg/L), two samples with ascorbic acid (Ascor) (100 mg/L and 200 mg/L), two samples with potassium metabisulfite (KMS) (final content of SO<sub>2</sub> 20 mg/L and 40 mg/L), one sample containing the minimum concentration of all antioxidants (10 mg/L Glut., 25 µg/L Se., 100 mg/L Ascor., and 20 mg/L SO<sub>2</sub>), and one sample containing the maximum concentration of all antioxidants (20 mg/L Glut., 50 µg/L Semeth., 200 mg/L Ascor., and 40 mg/L SO<sub>2</sub>). Details concerning sample composition are shown in Table 1.

**Table 1.** Addioxidant addition in wine samples used in this experiment.

Sample	Glutathione (mg/L)	Selenomethionine (µg/L)	Ascorbic Acid (mg/L)	KMS (mg/L)
C	-	-	-	-
Glut. Min	10	-	-	-
Glut. Max	20	-	-	-
Se Min	-	25	-	-
Se Max	-	50	-	-
Ascor. Min	-	-	100	-
Ascor. Max	-	-	200	-
SO <sub>2</sub> Min	-	-	-	20
SO <sub>2</sub> Max	-	-	-	40
All Min	10	25	100	20
All Max	20	50	200	40

C: Control, Glut. min: 10 mg/L Glutathione, Glut. max: 20 mg/L Glutathione, Se. Min: 25 µg/L Selenomethionine, Se. Max: 50 µg/L Selenomethionine, Ascor. min: 100 mg/L Ascorbic acid, Ascor. max: 200 mg/L Ascorbic acid, SO<sub>2</sub> min: 20 mg/L SO<sub>2</sub>, SO<sub>2</sub> max: 40 mg/L SO<sub>2</sub>, All min: 10 mg/L Glutathione, 25 µg/L Selenomethionine, 100 mg/L Ascorbic acid, 20 mg/L SO<sub>2</sub>, All max: 20 mg/L Glutathione, 50 µg/L Selenomethionine, 200 mg/L Ascorbic acid, 40 mg/L SO<sub>2</sub>.

### 2.3. Accelerated Browning Test

The accelerated browning tests were conducted following the modification [30] of the method developed by Singleton and Kramling [31], where the duration of the experiment was extended from 5 to 12 days, and the samples were not sprayed with N<sub>2</sub>. On day 0, 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 and placed in a water bath at 55 °C. Every 24 h for 12 days, samples were withdrawn for measurement of absorbance at 420 nm. After withdrawal, the samples were kept at room temperature (20 °C) and absorbance at 420 nm was measured using a spectrophotometer (UPLAB UV—VIS (STEROGLASS S.r.l. Strada Romano di Sopra, 2/C 06132 San Marino in Campo—Perugia, Italy). Browning (A420) was measured in triplicate against 12% *v/v* ethanol.

### 2.4. Determination of Total Phenols (TP), Antioxidant Ability (AA), Free Sulphydryl Groups (SH), High Performance Liquid Chromatography (HPLC)

Folin-Ciocalteu and DPPH methods were employed to measure the antioxidant activity of the wine samples. In glass tubes, 2 mL of deionized water was transferred in duplicate, followed by the addition of 50 µL sample, 250 µL Folin's reagent, 750 µL 20% Na<sub>2</sub>CO<sub>3</sub> solution, and 1950 µL of deionized water. The tubes were then left to stand for 30 min at room temperature to allow the chromophore to develop. After this incubation

period, the absorbance at 765 nm was measured. The results were expressed in equivalents of gallic acid (GAE) [32]. Antioxidant activity was measured using the free radical diphenylpicrylhydrazyl (DPPH) reagent. The samples were diluted 1:3 with deionized water. Following DPPH addition, two absorbance measurements at 515 nm took place with a 30-min interval between the first and the second. The results were expressed in equivalents of Trolox (mM) [33]. Total free sulfhydryl groups were determined using Ellman's method as described by Kontogeorgos and Roussis (2014) [34]. Free -SH groups were determined after the addition of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) which reacts with free thiols to form disulphide and 2-nitro-5-thiobenzoic acid. Results were expressed in equivalents of glutathione (GSH mg/L). HPLC analysis was performed using a reversed phase Waters Nova-Pak C18 (150 × 3.9 mm, 4 μm) column. All samples were filtered through a 0.22 μm syringe filter before injection. The injection volume was 20 μL and the flow rate 1 mL/min. Identification was achieved by comparing the retention times of the peaks with those of standard compounds, and by UV/VIS spectral data. All analyses were performed in triplicate and concentrations were expressed in milligrams per liter (mg/L) according to the method of Kallithraka et al. (2001) [35].

### 2.5. Statistical Analysis

Accelerated browning test for each variety, at 55 °C, with each antioxidant addition, were conducted in duplicate to ensure the validity of the experiment. Average values and standard deviations were calculated. Statistical analysis and data processing were performed using IBM SPSS Statistics 21.0. One-way analysis of variance (ANOVA) and the Tukey HSD method were used to determine statistically significant differences at a 5% significance level ( $p < 0.05$ ).

## 3. Results and Discussion

### 3.1. Accelerated Browning Test

The oxidative reactions in wine are influenced by various factors, with a primary dependency on the presence of OH radicals. Free radicals initiate the oxidation of phenolic components, setting off a sequence of reactions with unstable intermediates. Ultimately, this leads to the formation of brown-colored polymers, resulting in a noticeable change in the wine's color, commonly referred to as browning.

As evident from Table 2, the browning rate constants ( $k$ ) for both varieties range from 8.55 to 50.5. These values are in accordance with those reported in a previous study regarding oxidation of Greek white wines [36].

All samples of Malagouzia, regardless of the antioxidant addition, exhibited oxidation and subsequent browning development [36]. In more detail, SO<sub>2</sub>-treated samples showed the least browning development ( $k$ ), while those treated with ascorbic acid displayed the most pronounced effect, with both exhibiting statistically significant differences compared to the other treatments. Samples treated with selenomethionine and glutathione demonstrated a response similar to the control ( $k$ ), with a moderate level of browning. These findings are consistent with the results reported by Kanavouras et al. [36], who also conducted accelerated browning tests on Malagouzia samples, although the wines were maintained at a lower temperature.

The percentage changes in absorbance at 420 nm ( $\% \Delta A_{420}$ ) were in agreement with the calculated browning rate constants ( $k$ ) (Table 2). Notably, all samples exhibited  $\% \Delta A_{420}$  values exceeding 70%, indicating a tendency to oxidation [37]. The addition of both SO<sub>2</sub> contents resulted in the lower color change values ( $\% \Delta A_{420}$ ). Furthermore, no statistically significant differences were observed in the  $\% \Delta A_{420}$  values between samples treated with either Semeth or Glut, indicating the potential equivalence of both antioxidants, however less efficient than SO<sub>2</sub>.

**Table 2.** Percentage changes (day 12 and day 0) of absorbance values at 420 nm (% $\Delta A_{420}$ ) and browning rate constants (k).

Sample	% $\Delta A_{420}$	% $\Delta A_{420}$	k (Day <sup>-1</sup> ) * 10 <sup>-3</sup>	k (Day <sup>-1</sup> ) * 10 <sup>-3</sup>
	Malagouzia	Retsina	Malagouzia	Retsina
C	182.90 <sup>b</sup>	517.81 <sup>ab</sup>	10.60 <sup>ab</sup>	37.4 <sup>d</sup>
Semeth. Min	204.63 <sup>bc</sup>	577.17 <sup>bc</sup>	11.00 <sup>ab</sup>	38.1 <sup>d</sup>
Semeth. Max	205.01 <sup>bc</sup>	574.61 <sup>bc</sup>	10.70 <sup>ab</sup>	37.6 <sup>d</sup>
Glut Min	185.70 <sup>b</sup>	588.57 <sup>bc</sup>	10.20 <sup>ab</sup>	37.7 <sup>d</sup>
Glut Max	203.67 <sup>bc</sup>	552.66 <sup>b</sup>	11.35 <sup>b</sup>	36.9 <sup>cd</sup>
Ascor. Min	233.11 <sup>d</sup>	685.22 <sup>d</sup>	14.85 <sup>c</sup>	45.5 <sup>e</sup>
Ascor. Max	264.10 <sup>e</sup>	662.67 <sup>d</sup>	18.85 <sup>d</sup>	50.5 <sup>f</sup>
SO <sub>2</sub> Min	137.91 <sup>a</sup>	549.91 <sup>b</sup>	9.2 <sup>ab</sup>	34.9 <sup>bc</sup>
SO <sub>2</sub> Max	141.69 <sup>a</sup>	486.10 <sup>a</sup>	8.55 <sup>a</sup>	31.6 <sup>a</sup>
All Min	213.80 <sup>cd</sup>	602.40 <sup>c</sup>	14.45 <sup>c</sup>	44.1 <sup>c</sup>
All Max	205.55 <sup>bc</sup>	751.71 <sup>e</sup>	18.8 <sup>d</sup>	48.2 <sup>f</sup>

\* Different letter indicates statistically significant difference at 5% within each column. C: Control, Glut. min: 10 mg/L Glutathione, Glut. Max: 20 mg/L Glutathione, Se. Min: 25 µg/L Selenomethionine, Se. max: 50 µg/L Selenomethionine, Ascor. min: 100 mg/L Ascorbic acid, Ascor. max: 200 mg/L Ascorbic acid, SO<sub>2</sub> min: 20 mg/L SO<sub>2</sub>, SO<sub>2</sub> max: 40 mg/L SO<sub>2</sub>, All min: 10 mg/L Glutathione, 25 µg/L Selenomethionine, 100 mg/L Ascorbic acid, 20 mg/L SO<sub>2</sub>, All max: 20 mg/L Glutathione, 50 µg/L Selenomethionine, 200 mg/L Ascorbic acid, 40 mg/L SO<sub>2</sub>.

Retsina proved to be more sensitive to the onset of oxidative browning compared to Malagouzia. In contrast to a previous study where Savvatiانو demonstrated significantly lower values (k and % $\Delta A_{420}$ ), under the same accelerated oxidation conditions at 55 °C [36], it is crucial to take into account the impact of resin after heating, as it can enhance the browning process. The presence of pine resin derivatives in film-based food packaging materials speeded up the color changes showing a more marked yellow coloration which turned to black after 49 days [38]. Retsina demonstrated a substantial increase in absorbance at 420 nm starting from day 1, independent of the antioxidant treatment. This phenomenon may be attributed to the influence of resin, particularly after heating, which intensifies browning in wines [39]. SO<sub>2</sub>-treated samples exhibited the lowest values (k and % $\Delta A_{420}$ ) among all treatments. The values obtained for Se, and Glutathione treated samples, as well as the Control did not differ statistically.

The % $\Delta A_{420}$  values of all Retsina samples exceeded 70% and were nearly double than those of Malagouzia (Table 2). The maximum SO<sub>2</sub> addition resulted in the least color change (% $\Delta A_{420}$ ), followed by the control. However, Semeth, Glut, and minimum SO<sub>2</sub> additions did not exhibit statistically significant differences (% $\Delta A_{420}$ ). The results of browning rate constants (k) were also in line with the values of % $\Delta A_{420}$ . More specifically, ascorbic acid, along with all antioxidant-treated samples, demonstrated the highest k values, implying an augmentation of browning development.

In general, samples with SO<sub>2</sub> addition showed the lowest k values, confirming its efficacy as an antioxidant, while ascorbic acid seems to accelerate oxidation and browning development, under the conditions of the test. The mechanism of the reaction of ascorbic acid with oxygen is metal-ion mediated and results in the formation of dehydroascorbic acid and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) [40]. If SO<sub>2</sub> is simultaneously present, it reacts with H<sub>2</sub>O<sub>2</sub> to form sulphuric acid, while dehydroascorbic acid may reversibly bind sulphur dioxide and degrade to form a number of new compounds such as furoic acid and 3-hydroxy-2-pyrone. However, in case of excessive oxygen exposure, SO<sub>2</sub> is not sufficient to scavenge H<sub>2</sub>O<sub>2</sub> which results in the formation of hydroxyl radicals which in turn oxidize other components of wine. In this case, ascorbic acid could act as a pro-oxidant in wine resulting in browning development. It has been shown, that upon depletion of SO<sub>2</sub>, wines containing ascorbic acid, consumed higher amounts of oxygen leading to higher amounts of H<sub>2</sub>O<sub>2</sub> production and carbonyl degradation products [40]. In addition, dehydroascorbic acid degrades to form yellow pigmented compounds such as the xanthilium cations increasing thus the pool of color precursors in wine [40]. This is possibly the case in this study where heating wine

at 50 °C in the presence of oxygen reduced the availability of SO<sub>2</sub> to scavenge H<sub>2</sub>O<sub>2</sub> and dehydroascorbic acid resulting in browning.

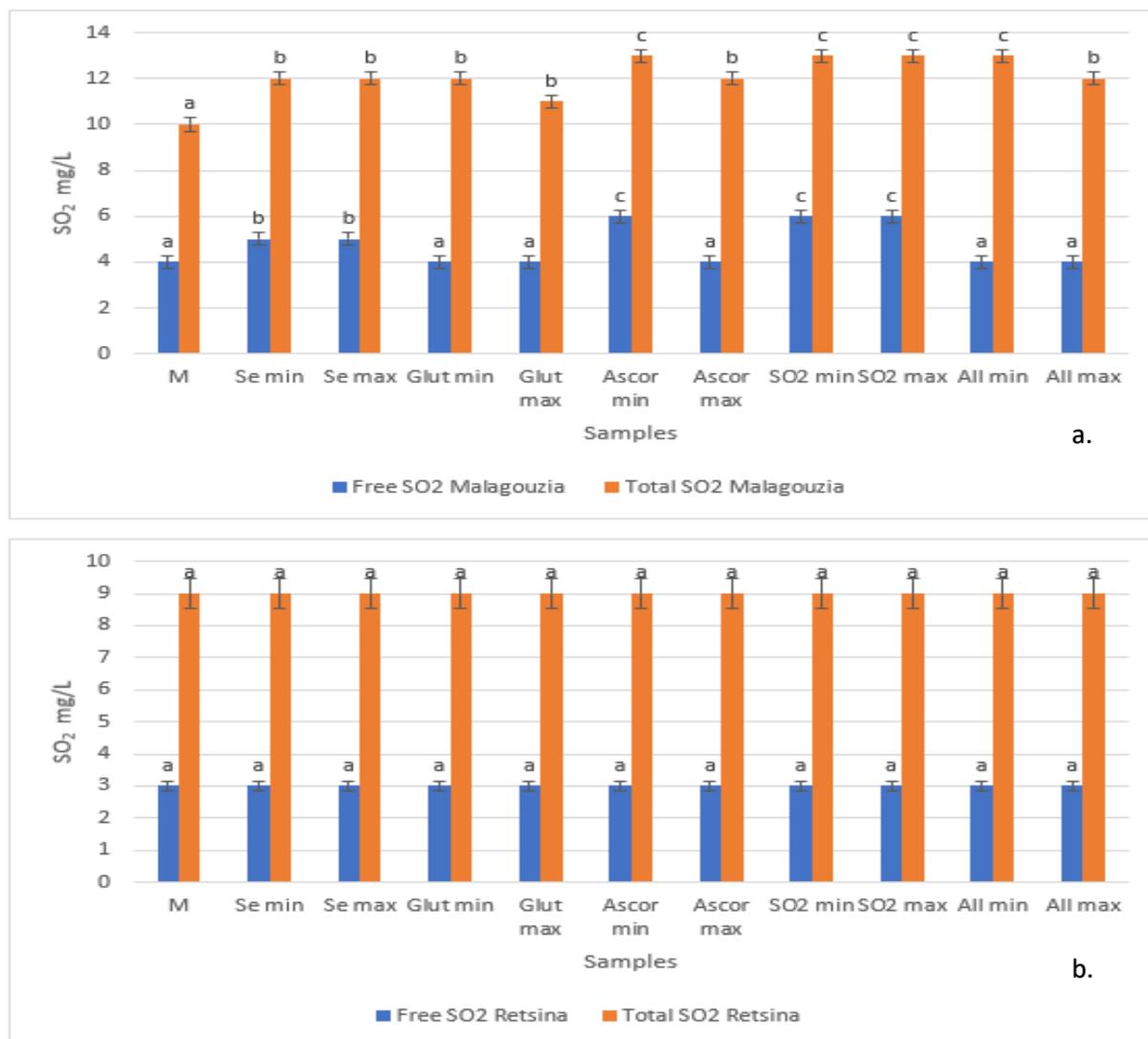
### 3.2. SO<sub>2</sub> Content (Total and Free)

The dissolved oxygen in wine could have a detrimental effect on white wine quality, unless an antioxidant is simultaneously present at sufficient concentration to scavenge the initially formed oxidation products. Sulfur dioxide is widely used for wine preservation, inhibiting both the non-enzymatic and enzymatic browning and preventing oxidation. In wine, SO<sub>2</sub> exists in two forms: the free and the bound. Molecular SO<sub>2</sub>, hydrogen sulfite and sulfite coexist in a pH-dependent equilibrium in the free form while the rest is bound mainly to carbonyl compounds. The molecular form exhibits antimicrobial properties, while the anionic form, which constitutes the larger percentage, offers antioxidant protection [41].

Regarding Malagouzia wines, by the 12th day of the experiment (Figure 1a), the SO<sub>2</sub> content of all samples examined was reduced. As it can be seen in Figure 1a, the total SO<sub>2</sub> concentration was significantly higher in the samples containing antioxidants in comparison with the control. An interesting observation was that the samples containing ascorbic acid were characterized by the highest SO<sub>2</sub> content. It has been shown that ascorbic acid has the ability of lowering total SO<sub>2</sub> depletion through the weak binding to dehydroascorbic acid and its degradation products as opposed with the irreversible oxidation and degradation of SO<sub>2</sub> to sulfate [40]. In the absence of ascorbic acid SO<sub>2</sub> is consumed at a higher rate after reaction with o-quinones and the middle ring of flavan-3-ols [42]. SO<sub>2</sub> content of Riesling wines was 33% higher when ascorbic acid was added in the wine in a study by Skouroumounis [43]. However, there is a critical ratio of ascorbic acid to total SO<sub>2</sub> (for a given amount of oxygen) where this protective effect can be observed. Ascorbic acid at higher concentration is not so efficient to maintain SO<sub>2</sub> content since the oxidation products (such as H<sub>2</sub>O<sub>2</sub>) are formed at a higher rate resulting in higher consumption of SO<sub>2</sub>. In this study, the higher ascorbic acid content was less efficient to protect SO<sub>2</sub> degradation compared to the lower content (Figure 1a).

The results concerning free SO<sub>2</sub> content were similar, showing the ability of all antioxidant compounds exhibiting a protective effect. The addition of the low concentration of ascorbic acid resulted in the highest protection of free SO<sub>2</sub>, in agreement with the results regarding total SO<sub>2</sub>. An interesting observation was that the addition of Semeth in wines resulted in higher preservation of free sulfur dioxide in comparison with the samples where Glut had been added (Figure 1a). The samples with both concentrations of Semeth contained significantly higher amounts of free SO<sub>2</sub> than the control. This is consistent with the findings of Vlachou et al. [28], where the addition of inorganic Se protected total SO<sub>2</sub> content of the wines heated at 50 °C. Possibly Se reduces the electron transfer reactions between flavonoids and quinones preserving SO<sub>2</sub> content of wines [44]. However, the organic form of Se (Semeth) seems to be more efficient antioxidant than the inorganic Se since it preserved both free and total SO<sub>2</sub> content in this study. Se in its inorganic form was able to prevent browning in white wines after heating at 35 °C but this effect was no longer observed when heating took place at 50 °C [28]. In this study only one temperature was studied (55 °C) where no protective effect on wine color was noticed.

An interesting observation concerning Retsina samples was that the addition of antioxidants had a similar effect on the SO<sub>2</sub> content (both free and total) of all samples studied. No significant differences were observed between the wine samples with the different antioxidant additions and the control. This was probably due to the presence of resin which was the detrimental factor that promoted oxidation and browning coloration. All antioxidant concentrations were probably below the thresholds required to efficiently scavenge the initial oxidation products and hence the differences between their action were not noticeable. However, the browning development in retsina wines appears to be more influenced by the specific antioxidant added, as indicated in prior results of this experiment (Table 2), rather than by variations in free and total SO<sub>2</sub> concentrations.



**Figure 1.** Free and total SO<sub>2</sub> content (mg/L) of Malagouzia (a) and Retsina (b) samples the 12th day of the experiment. Different letters indicate statistically significant difference at a 5% level within samples. Free and total SO<sub>2</sub> measurements were treated separately. C: Control, Glut. min: 10 mg/L Glutathione, Glut. max: 20 mg/L Glutathione, Se. min: 25 µg/L Selenomethionine, Se. max: 50 µg/L Selenomethionine, Ascor. min: 100 mg/L Ascorbic acid, Ascor. max: 200 mg/L Ascorbic acid, SO<sub>2</sub> min: 20 mg/L SO<sub>2</sub>, SO<sub>2</sub> max: 40 mg/L SO<sub>2</sub>, All min: 10 mg/L Glutathione, 25 µg/L Selenomethionine, 100 mg/L Ascorbic acid, 20 mg/L SO<sub>2</sub> All max: 20 mg/L Glutathione, 50 µg/L Selenomethionine, 200 mg/L Ascorbic acid, 40 mg/L SO<sub>2</sub>.

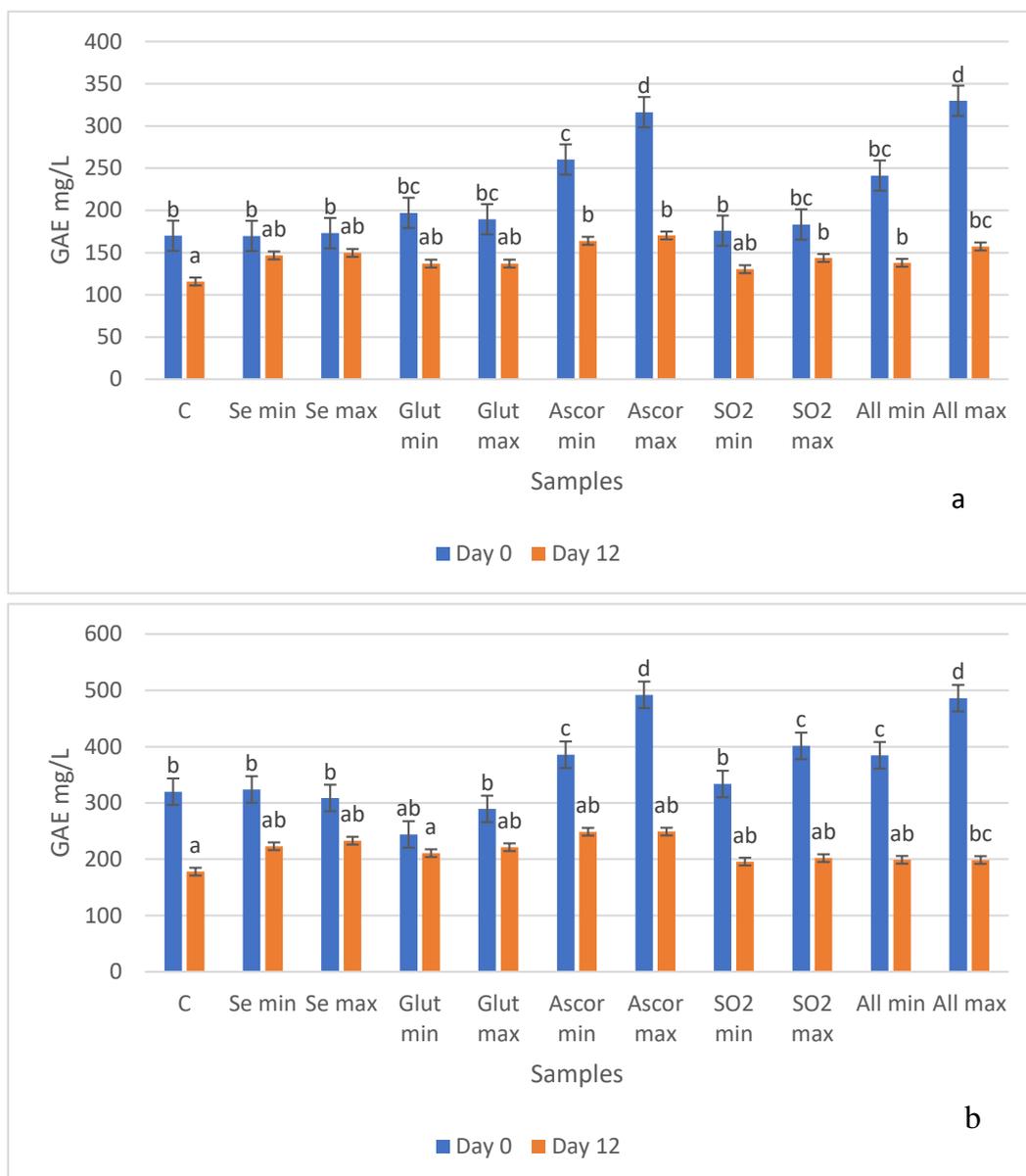
The elevated temperature (55 °C) employed in this experiment together with the presence of excess O<sub>2</sub> were the key factors leading to a decrease in the amount of both free and total SO<sub>2</sub> in all samples after 12 days of heating. The accelerated oxidative reactions of ethanol, glycerol and tartaric acid resulted in acetaldehyde, glyceraldehyde and glyoxylic acid formation respectively. The aldehyde moiety of these compounds can irreversibly bind to SO<sub>2</sub> [45] reducing their content.

### 3.3. Antioxidant Activity (Folin and DPPH Methods)

The measurement of total phenols of the wines using the Folin-Ciocalteu method is based on reduction of the reagent due to the oxidation of the phenolic hydroxyl groups [42]. At this point, it should be mentioned that methods which depend on redox reactions, such as the Folin-Ciocalteu method, do not measure the absolute content of phenolics in

wine. Various compounds which are simultaneous present and possess antioxidant activity such as ascorbic acid, thiols, redox-active metal ions, and nucleotide bases all reduce Folin reagent and have an influence on the final results. Therefore, the results obtained from this method will be regarded as antioxidant activity data.

In this study, the results obtained by this method, of all Malagouzia wine samples decreased by 40–50% after 12 days of heating in agreement with the results obtained by both Vlachou et al. [28] for Assyrtiko wines heated at 50 °C for 12 days and by Kallithraka et al. [46] for Greek rare cultivars after extended bottle ageing (Figure 2a). The samples containing ascorbic acid exhibited the highest initial value (mg gallic acid equivalents/L) probably due to its higher reactivity towards the reagent. However, after 12 days of heating these samples showed the highest percentage reduction. An interesting observation was that samples with Semeth exhibited the least decrease (from 0 to day 12) compared to the rest samples indicating a possible higher protection of Se over the wine oxidation.



**Figure 2.** Antioxidant activity (GAE) of Malagouzia (a) and Retsina wines (b) at day 0 and 12 of the experiment. Different letters indicate statistically significant difference at a 5% level within samples

The two wines were treated separately C: Control, Glut. min: 10 mg/L Glutathione, Glut. max: 20 mg/L Glutathione, Se. min: 25 µg/L Selenomethionine, Se. max: 50 µg/L Selenomethionine, Ascor. min: 100 mg/L Ascorbic acid, Ascor. max: 200 mg/L Ascorbic acid, SO<sub>2</sub> min: 20 mg/L SO<sub>2</sub>, SO<sub>2</sub> max: 40 mg/L SO<sub>2</sub> All min: 10 mg/L Glutathione, 25 µg/L Selenomethionine, 100 mg/L Ascorbic acid, 20 mg/L SO<sub>2</sub>, All max: 20 mg/L Glutathione, 50 µg/L Selenomethionine, 200 mg/L Ascorbic acid, 40 mg/L SO<sub>2</sub>.

The initial data obtained for Retsina were higher than that of Malagouzia which can be attributed to both the higher reactivity of resin towards the Folin-Ciocalteu reagent and to the higher phenolic content of the grape variety used for Retsina (Savvatiano). All Retsina samples exhibited a quantitatively higher decrease (50–60%) compared to Malagouzia (Figure 2b). After the heating period, the lowest value was measured for control wine while the highest for ascorbic acid samples. However, samples with ascorbic acid addition exhibited the highest decrease in agreement with the results obtained for Malagouzia (Figure 1a). Samples containing the max concentrations of Semeth and Glut preserved their antioxidant capacity more efficiently compared with the rest.

Generally, in both wine types there was a decrease in Folin-Ciocalteu values in agreement with other studies [47,48], with Retsina wines exhibiting a higher decrease implying that resin is more vulnerable to heating and its oxidation degradation products may accelerate oxidation. In both wine types Semeth was effective suggesting that it might be more protective against oxidation compared to the other antioxidants.

To demonstrate the effect of heating on wine antioxidant status, the DPPH method was employed to determine antioxidant activity values of the samples [48] (Figure 3a,b).

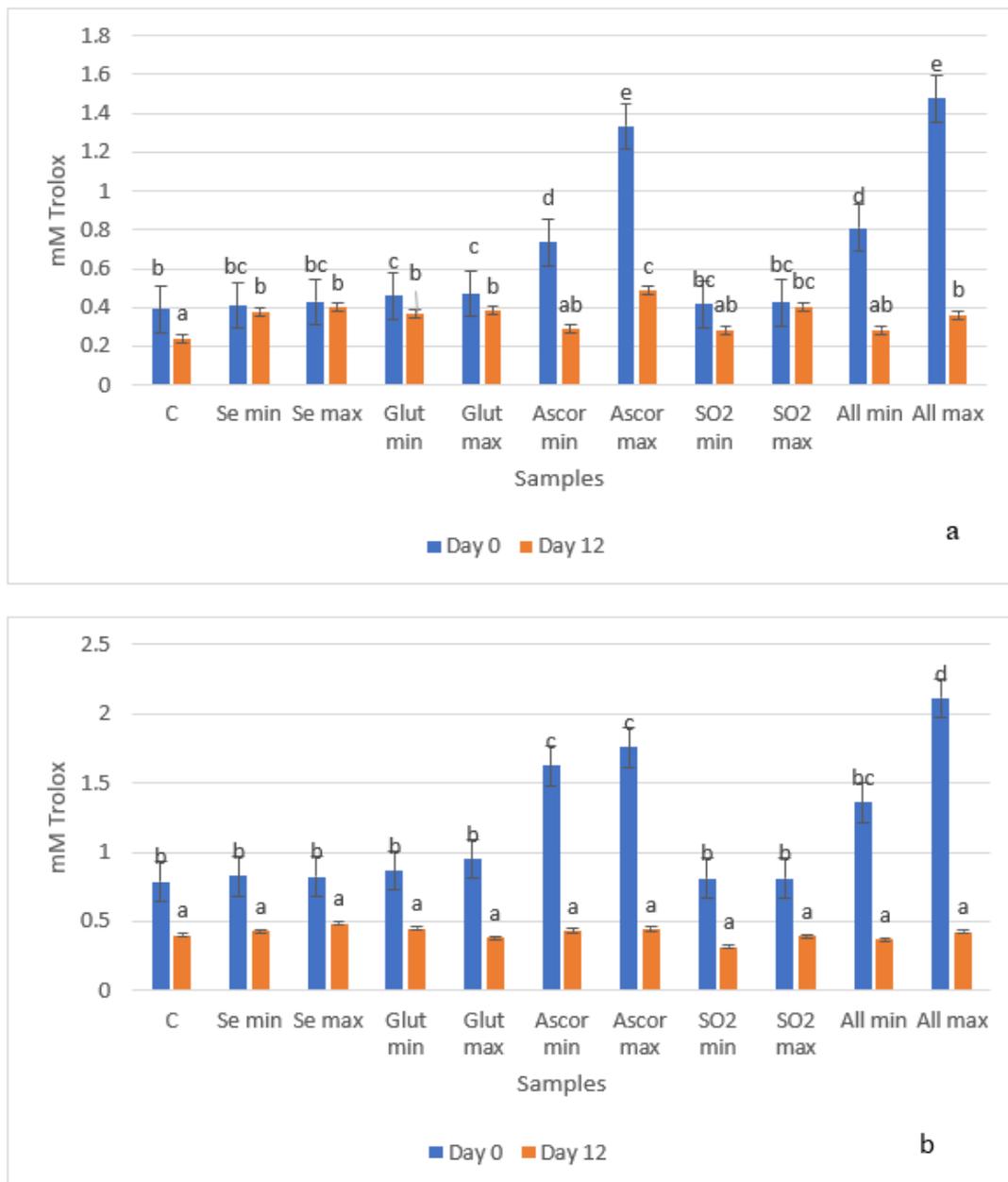
All samples showed a similar evolution of antioxidant activity values (AA) with heating at 50 °C consistent with the results obtained by Vlachou et al. [28] for Assyrtiko wines. AA values exhibited a decreasing trend indicating that the heat-treated samples had lower ability for free radical scavenging than the non-heated. In the case of Malagouzia, the addition of antioxidants had a different effect on AA of the samples. Although wines with ascorbic acid as well as samples containing 'all antioxidants' were those with the highest AA at day 0, they exhibited the highest percentage change at day 12. Semeth addition resulted in the less percentage change of AA values at both concentrations added indicating that it could exert a protective effect.

Retsina wines exhibited higher antioxidant activity than Malagouzia at day 0, which might be explained by the antioxidant effect of the resin [26] (Figure 3a,b). However, after heating, the AA of all samples decreased showing similar values with Malagouzia wines. The AA values of the wines with the different antioxidants did not differ statistically after heating. This might be attributed to the presence of resin oxidation products which probably exert a greater effect on free radical scavenging compared with the rest antioxidants.

The AA values are generally in agreement with the data obtained concerning total phenolic content (Figure 2a,b) measured by the Folin-Ciocalteu method. Since both spectrophotometric methods are based on oxidation-reduction reactions, where added antioxidants and wine phenolic compounds have an active participation, the same wine samples are expected to possess similar reactivities towards the two reagents.

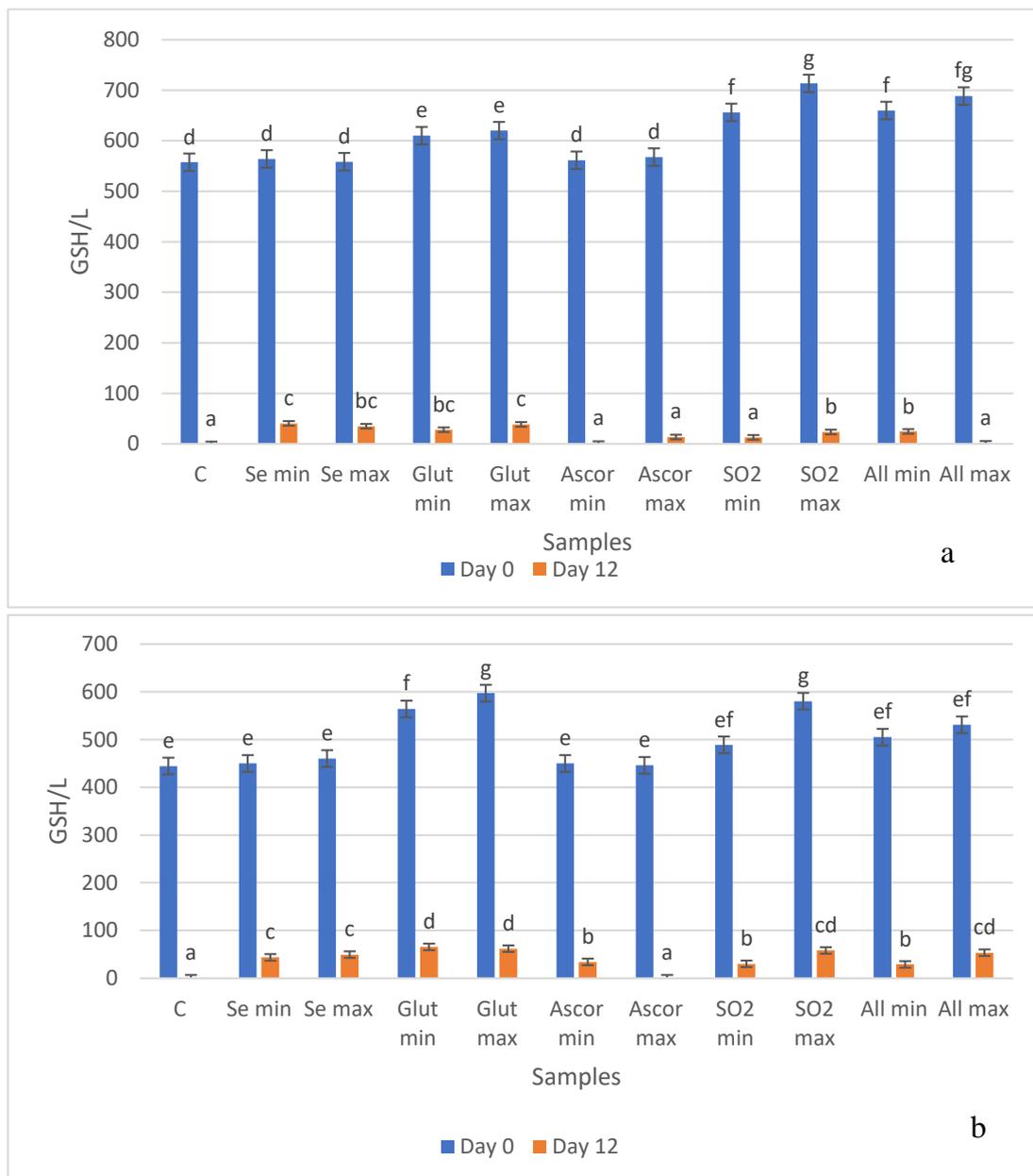
### 3.4. Free -SH Groups

It has been shown that compounds with free sulfhydryl groups (-SH) such as for example glutathione, cysteine and thiols in wine, could provide antioxidant protection, mainly preserving color and volatile compounds [1,34]. The sulfhydryl group within the molecules serve as a nucleophile, capable of replacing the electrophilic ring of o-quinones. Consequently, this process regenerates the dihydroxy ring, forming adducts that are less susceptible to oxidation. This mechanism effectively limits browning reactions.



**Figure 3.** Antioxidant activity (Trolox mM) of Malagouzia (a) and Retsina (b) samples. Different letters indicate statistically significant difference at a 5% level within samples. The two wines were treated separately. C: Control, Glut. min: 10 mg/L Glutathione, Glut. max: 20 mg/L Glutathione, Se. min: 25 µg/L Selenomethionine, Se. max: 50 µg/L Selenomethionine, Ascor. min: 100 mg/L Ascorbic acid, Ascor. max: 200 mg/L Ascorbic acid, SO<sub>2</sub> min: 20 mg/L SO<sub>2</sub>, SO<sub>2</sub> max: 40 mg/L SO<sub>2</sub>, All min: 10 mg/L Glutathione, 25 µg/L Selenomethionine, 100 mg/L Ascorbic acid, 20 mg/L SO<sub>2</sub>, All max: 20 mg/L Glutathione, 50 µg/L Selenomethionine, 200 mg/L Ascorbic acid, 40 mg/L SO<sub>2</sub>.

As expected, total free -SH content was higher in wine samples with Glut, both before and after heating (Figure 4a,b). Retsina wines were characterized by higher -SH content before heating in agreement with the results presented regarding Savvatiano wines by Kontogiorgos and Roussis [34]. However, after heating, Malagouzia wines contained higher contents of -SH than the respective Retsina samples probably due to the enhanced oxidation that resin has induced.



**Figure 4.** Total free -SH of Malagouzia (a) and Retsina (b) samples. Different letters indicate statistically significant difference at a 5% level within samples. The two wines were treated separately: C: Control, Glut. min: 10 mg/L Glutathione, Glut. max: 20 mg/L Glutathione, Se. min: 25 µg/L Selenomethionine, Se. max: 50 µg/L Selenomethionine, Ascor. min: 100 mg/L Ascorbic acid, Ascor. max: 200 mg/L Ascorbic acid, SO<sub>2</sub> min: 20 mg/L SO<sub>2</sub>, SO<sub>2</sub> max: 40 mg/L SO<sub>2</sub>, All min: 10 mg/L Glutathione, 25 µg/L Selenomethionine, 100 mg/L Ascorbic acid, 20 mg/L SO<sub>2</sub>, All max: 20 mg/L Glutathione, 50 µg/L Selenomethionine, 200 mg/L Ascorbic acid, 40 mg/L SO<sub>2</sub>.

An interesting observation in both Retsina and Malagouzia samples was that the addition of Selenomethionine exerted a protective effect on -SH content after heating, even more efficient than the SO<sub>2</sub> addition (Figure 4a,b). It is known that Se is a component of several antioxidant enzymes including glutathione peroxidase which scavenge the free radicals generated during oxidation [49]. It is thus possible that this protective effect on -SH content to be related with its glutathione peroxidase activity. Moreover, recent studies reported an enhanced radical scavenging activity of mulberry wine samples supplemented with Se compared with the controls [50].

In accordance with the findings reported by Voltea et al. [1], who observed that -SH groups could react with (+)-catechin, and thereby reducing the occurrence of browning, in this experiment, the samples with the highest concentrations of -SH, were those with the lowest browning constants.

### 3.5. Individual Phenolic Compounds Determined by HPLC (Flavanols and Hydroxycinnamic Acids)

The primary components in white wines that can participate in oxidation reactions include hydroxycinnamates and flavanols. Specifically, the oxidation of ortho-dihydroxyphenolic compounds, like (+)-catechin and (–)-epicatechin, as well as hydroxycinnamic acids, leads to the creation of yellow-brown compounds due to ortho-quinone polymerization [23]. Research on model wine solutions has validated the generation of two categories of yellow pigments: xanthylum salt pigments and ethyl-esters of xanthylum salts. These pigments exhibit visible absorption peaks at 440 and 460 nm, respectively, both originating from flavanol oxidation and polymerization [51]. With an absorption peak within the 400–500 nm range, these pigments directly contribute to the browning of white wine during the aging process. This reaction, and consequently the degree of browning, is accelerated in white wines by introducing transition metals such as iron and copper, which likely serve as catalysts for the formation of intermediate oxidation products [1,28].

In view of the important implication of both flavanols and hydroxycinnamates in browning reactions, it was considered of interest to determine their content before and after heating. In agreement with previous studies [28,48] regarding Greek wines, the concentration of (+)-catechin was less affected by heating compared with (–)-epicatechin and (–)-epigallocatechin gallate (EGCG) contents which were completely depleted.

Table 3 presents the % change in (+)-catechin concentration of Malagouzia and Retsina wines induced by heating.

**Table 3.** Percentage change of (+)-catechin content in Malagouzia and Retsina samples after 12 days of heating.

Samples	(+)–Catechin (% Reduction) *	
	Malagouzia	Retsina
C	100% <sup>c</sup>	100% <sup>c</sup>
Se min	53.3% <sup>b</sup>	54.8% <sup>a</sup>
Se max	50% <sup>b</sup>	61.9% <sup>a</sup>
Glut min	53.3% <sup>b</sup>	76.2% <sup>b</sup>
Glut max	26.6% <sup>a</sup>	78.6% <sup>b</sup>
Ascor min	90.0% <sup>a</sup>	238% <sup>d</sup>
Ascor max	96.7% <sup>c</sup>	238% <sup>d</sup>
SO <sub>2</sub> min	43.3% <sup>b</sup>	238% <sup>d</sup>
SO <sub>2</sub> max	50.0% <sup>b</sup>	238% <sup>d</sup>
All min	46.7% <sup>b</sup>	238% <sup>d</sup>
All max	26.7% <sup>a</sup>	238% <sup>d</sup>

\* Different letters indicate statistically significant difference at a 5% level within samples. The two wines were treated separately C: Control, Glut. min: 10 mg/L Glutathione, Glut. max: 20 mg/L Glutathione, Se. min: 25 µg/L Selenomethionine, Se. max: 50 µg/L Selenomethionine, Ascor. min: 100 mg/L Ascorbic acid, Ascor. max: 200 mg/L Ascorbic acid, SO<sub>2</sub> min: 20 mg/L SO<sub>2</sub>, SO<sub>2</sub> max: 40 mg/L SO<sub>2</sub>, All min: 10 mg/L Glutathione, 25 µg/L Selenomethionine, 100 mg/L Ascorbic acid, 20 mg/L SO<sub>2</sub>, All max: 20 mg/L Glutathione, 50 µg/L Selenomethionine, 200 mg/L Ascorbic acid, 40 mg/L SO<sub>2</sub>.

As it is shown in Table 3, (+)-catechin content of Malagouzia wine was less affected in comparison with Retsina samples. The addition of Semeth was as effective as the lower dose of Glut and SO<sub>2</sub> in preserving (+)-catechin content of Malagouzia wines in contrast with ascorbic acid which didn't offer any protection. The addition of both the max concentration of Glut and 'all the antioxidants' offered the highest protection.

Regarding Retsina wines, samples with Semeth exhibited the least % change in their (+)-catechin content. In samples containing ascorbic acid, SO<sub>2</sub>, and 'all antioxidants', (+)-catechin was completely depleted at the end of the heating period.

Flavanols in wine undergo oxidation, transforming into highly reactive quinones that actively engage in subsequent chemical reactions. These compounds can be mitigated by nucleophiles, such as the A-ring of flavonoids, preventing oxidation and the onset of browning. Alternatively, they may oxidize the B-ring (catechol ring) of flavanols through electron transfer, leading to the formation of flavonoid quinones, which serve as substrates for browning development. Ma and Waterhouse's [30] observations proposed a faster electron transfer compared to nucleophilic reactions in the model solution involving (+)-catechin and caffeic acid quinone, elucidating why flavanols contribute to browning in wine rather than delaying it.

It has been demonstrated that (–)-epicatechin and EGCG could be the key factors contributing to the development of browning in white wines following an accelerated browning test due to the higher oxidizability (lower oxidation potential) of (–)-epicatechin compared to its isomer, (+)-catechin [28]. The results of the present study confirm the previous observations since (+)-catechin content was less affected by heating compared to (–)-epicatechin and EGCG.

It appears that the reactions leading to the formation of brown compounds involve the oxidation of o-diphenols, with the oxidation of (–)-epicatechin and EGCG playing a crucial role. In particular, in the study of Vlachou et al. [28], EGCG emerged as the most affected compound after heating, likely attributable to its higher number of ortho-OH groups in the B ring compared to the other two studied flavanols [(+)-catechin and (–)-epicatechin]. Given the B ring's primary role in browning development, the observed reduction in EGCG is likely linked to an augmented formation of quinones, resulting in a more pronounced browning appearance. These findings are in agreement with the results presented in other studies [48,52] regarding both Greek and international wines.

As far as hydroxycinnamic acids are concerned, in Malagouzia samples there was a decrease in the content of tartaric acid esters (caftaric, coutaric and fertaric acids) with heating (Table 4). Consequently, the concentrations of caffeic acid were slightly increased (2–4%) () due to hydrolysis of caftaric acid as reported in the literature [42,53]. In agreement with the findings of Vlachou et al. [28] caftaric acid was the major hydroxycinnamic acid in Malagouzia wines and its content was the most affected by heating. This is probably attributed to both the formation of lower oxidation adducts with Glut [23] and the presence of two -OH groups in ortho position in its benzenic ring. Samples with ascorbic acid exhibited the largest decrease while the effect of Semeth and Glut was not significantly different than the control. Moreover, the addition of SO<sub>2</sub> was the most effective in preserving caftaric acid loss.

The contents of coutaric and fertaric acids were less affected by the oxidation induced by heating in agreement with Kallithraka et al. [48] and Vlachou et al. [28]. The higher concentration of Semeth and both contents of Glut were the most effective in preserving coutaric acid along with the addition of "all antioxidants". Regarding fertaric acid, the addition of Semeth and the higher Glut content proved to as effective as 'all antioxidants' resulting in less % change.

In Retsina the initial content of hydroxycinnamic acids was lower than the respective content of Malagouzia something that could be attributed to genetic differences among the two varieties [54]. After heating, all esters were completely depleted. The appearance of resin intermediate oxidation products probably serves as catalyst accelerating their oxidation. Another possible explanation is that new adducts are formed between hydroxycinnamic acids and resin degradation products which possibly can't be determined with the HPLC method employed in this study.

**Table 4.** Percentage change \* of hydroxycinnamic acid content in Malagouzia wines after 12 days of heating.

Samples	Caftaric Acid	Coutaric Acid	Fertaric Acid
C	100% <sup>c</sup>	100% <sup>c</sup>	100% <sup>c</sup>
Se min	112.1% <sup>c</sup>	78.6% <sup>bc</sup>	17.6% <sup>a</sup>
Se max	100% <sup>c</sup>	42.9% <sup>b</sup>	23.5% <sup>a</sup>
Glut min	96.9% <sup>b</sup>	50.0% <sup>b</sup>	52.9% <sup>b</sup>
Glut max	90.9% <sup>b</sup>	57.1% <sup>b</sup>	29.4% <sup>ab</sup>
Ascor min	127.3% <sup>d</sup>	78.6% <sup>bc</sup>	82.3% <sup>c</sup>
Ascor max	160.6% <sup>e</sup>	71.4% <sup>bc</sup>	88.2% <sup>c</sup>
SO <sub>2</sub> min	63.6% <sup>a</sup>	114.3% <sup>c</sup>	82.3% <sup>c</sup>
SO <sub>2</sub> max	63.6% <sup>a</sup>	107.1% <sup>c</sup>	64.7% <sup>bc</sup>
All min	93.9% <sup>b</sup>	14.2% <sup>a</sup>	17.6% <sup>a</sup>
All max	169.7% <sup>e</sup>	57.1% <sup>b</sup>	41.2% <sup>ab</sup>

\* Different letters indicate statistically significant difference at a 5% level within samples C: Control, Glut. min: 10 mg/L Glutathione, Glut. max: 20 mg/L Glutathione, Se. min: 25 µg/L Selenomethionine, Se. max: 50 µg/L Selenomethionine, Ascor. min: 100 mg/L Ascorbic acid, Ascor. max: 200 mg/L Ascorbic acid, SO<sub>2</sub> min: 20 mg/L SO<sub>2</sub>, SO<sub>2</sub> max: 40 mg/L SO<sub>2</sub>, All min: 10 mg/L Glutathione, 25 µg/L Selenomethionine, 100 mg/L Ascorbic acid, 20 mg/L SO<sub>2</sub>, All max: 20 mg/L Glutathione, 50 µg/L Selenomethionine, 200 mg/L Ascorbic acid, 40 mg/L SO<sub>2</sub>.

#### 4. Conclusions

In conclusion, the accelerated test was a helpful tool which provided important information concerning the oxidative reactions related to browning appearance in Malagouzia and Retsina wines under several antioxidant treatments. As expected, SO<sub>2</sub> was the most effective antioxidant in minimizing color change while ascorbic acid acted as a prooxidant accelerating oxidation and browning appearance. Retsina wines were more sensitive to oxidative browning that Malagouzia most probably due to the presence of resin.

Regarding preservation of SO<sub>2</sub> content, Semeth exhibited a higher protective effect of free SO<sub>2</sub> compared to glutathione in Malagouzia wines suggesting its potential as a future wine additive. The lower concentration of ascorbic acid resulted in increased contents of both free and total sulfur dioxide (SO<sub>2</sub>), whereas the higher concentration proved to be less efficient in protecting against degradation.

Antioxidant activity values showed a remarkable decrease with heating further supported the findings of this study. Additionally, Semeth demonstrated a high efficiency in preserving antioxidant activity. As expected, free sulfhydryl content (-SH) was higher in the wines with Glut addition, while Semeth exhibited the best protective effect even higher than that of SO<sub>2</sub> suggesting that Semeth's role in preserving wine from oxidation probably involves more mechanisms than just the prevention of SO<sub>2</sub> depletion. The results of this study also highlighted the effect of Se on the individual phenolic compounds emphasizing its protective role on (+)-catechin and fertaric acid. Among the flavanols studied, (+)-catechin was less affected by heating while (−)-epicatechin and EGCG were completely degraded by heating suggesting their important role in browning development. Retsina wines were found to be more susceptible to browning and oxidation irrespectively of the specific antioxidant added. This challenge caused by the presence of resin emphasizes the need for specific antioxidant strategies in resin-containing wines.

The different antioxidant additions displayed varying degrees of effectiveness in preventing oxidative damages in wine. Interestingly, Semeth emerged as an important antioxidant candidate for enhancing oxidative stability in white wines. However, deeper exploration of the mechanisms behind Semeth's activity and its potential synergies with the other wine antioxidants is necessary in order to provide valuable information for optimizing its application in winemaking practices. As the industry continues to search for sustainable and effective alternatives to SO<sub>2</sub>, Semeth seems to be a promising preservative for enhancing the overall quality and protection of white wines.

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