

DYNAMICS OF MICROBIOLOGICAL AND OXIDATIVE STABILITY OF WHITE WINES DURING THE TECHNOLOGICAL PROCESS

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Abstract: Oxidation and the capacity for sufficient aging potential of white wines are constant problems for winemakers worldwide. The article analyses the correlation between the microbiological and oxidative stability of white wines and the main redox processes occurring in the technological stages of the wines. In micro-winery TUM conditions, parallel and spontaneous development of several redox processes and their impact on the quality, microbiological and oxidative stability of white wines were examined. As a result, the developed technological scheme has managed to provide a solution that is effective in properly controlling the oxidation process and preserving the white wine quality.

Keywords: *iron and copper ions, oxygen, quality, redox processes, white wines*

INTRODUCTION

Oxygen has the potential to initially positively influence wine and later, to destroy its aroma and bitterness. The influence of oxygen on wine quality and aroma depends on various factors, such as: type of wine, type of grapes, quality of grapes, temperature, technological conditions, etc. [1, 2]. For example, fruity and fresh notes of rosé wines appear when the wine is enriched with oxygen are very appreciated by consumers, while white wines can be negatively affected by small amounts of oxygen and red wines, due to their richness in phenolic compounds, can resist oxidation for longer time [3].

Redox processes are an inevitable and complex aspect of the winemaking process. Numerous substances, such as polyphenols and quinones, reduced glutathione form (GSH) and oxidized glutathione form (GSSG), endiol and diketones, acetoin and diacetyl, anthocyanins and reduced anthocyanins, etc., form redox couples in grapes, must and wine [4]. These substances' influence on the final product is determined by their participation in the general transformations that occur during the wine's life and ultimately become responsible for its quality [5].

Elias and Waterhouse proposed an oxidation mechanism based on the Fenton reaction, also called the chemical oxidation of wine, which results in a semi-quinone radical, which is further oxidized to quinone [6]. In this Fenton reaction, the H_2O_2 molecule is reduced to a more reactive hydroxyl radical ($\text{OH}^\cdot$), starting the cyclic chain of radical reactions. This reaction is directly mediated by the iron metal (Figure 1) [7].

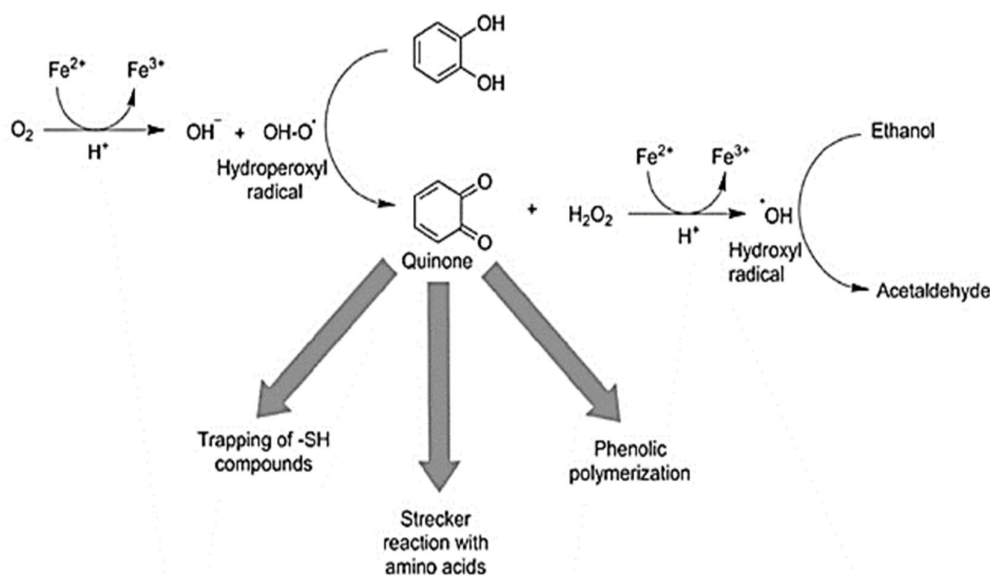


Figure 1. Formation of o-quinones and hydrogen peroxide and subsequent oxidation of ethanol to form acetaldehyde and other reactions in the wine matrix

In the same reaction, ferrous ion II (Fe^{2+}) is converted to ferric ion III (Fe^{3+}). The Fe^{3+} ion being highly reactive reduces the ethanol molecule to acetaldehyde [8].

The transition metal pairs $\text{Fe}^{3+}/\text{Fe}^{2+}$ and $\text{Cu}^{2+}/\text{Cu}^+$, which are found naturally in wines and many enzymes play a crucial role in oxidative-reducing chemical transformations during the winemaking process, performing their chemical oxidation (Figure 2) [9, 10].

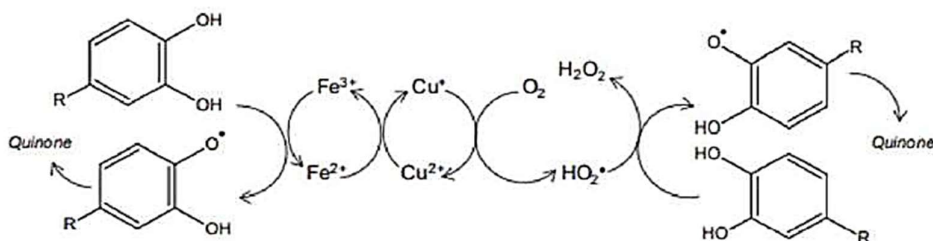


Figure 2. Iron and copper ions' catalytic role in the conversion of catechols to quinones (adapted after [7])

During this process of non-enzymatic oxidation of must and wine, oxidative processes are favored by the oxidation of polyphenols containing an ortho-dihydroxy-benzene group (catechol ring) or a 1,2,3-trihydroxy-benzene group (galloyl group), such as (+)- catechin or (-)-epicatechin, gallocatechin, gallic acid and its esters, as well as caffeic acid, which are the most easily oxidized wine constituents [7, 11]. These substrates are sequentially oxidized into semi-quinone and benzo-quinone radicals, while oxygen is reduced to hydrogen peroxide, and the whole process is mediated by the redox cycle of $\text{Fe}^{3+}/\text{Fe}^{2+}$ and $\text{Cu}^{2+}/\text{Cu}^{+}$ [8].

Knowing that, the physico-chemical characteristics and quality of the final product are primarily determined by the presence of phenolic compounds and minerals in grapes, must, and wine [12]. The purpose of the study is to establish the microbiological and oxidative stability of 2 white wines (Muscat Polsky and Traminer grape varieties) during the technological process within the Oenological Research Center (ORC) from the Technical University of Moldova (TUM).

MATERIALS AND METHODS

The grape varieties Muscat Polsky and Traminer, harvested in 2023 (Figure 3), from the Javgur central wine region of the Republic of Moldova, were processed in the micro-winery section of the Oenology and Chemistry Department of the TUM using the classical method of dry white wines production.

The grapes were de-stemmed and pressed and the resulting juice was treated with metabisulfite in doses of $50 \text{ mg}\cdot\text{L}^{-1}$, a pectinolytic enzyme of $0.06 \text{ mL}\cdot\text{L}^{-1}$, and maintained at 2°C for 3 days for clarification. Then, musts were inoculated with *Saccharomyces cerevisiae* oenological dry yeast ($0.3 \text{ g}\cdot\text{L}^{-1}$). For the oxidative stability, in 6 samples of must, 1 liter each-one, exogenous doses of copper (Cu II) and iron ions (Fe II) were administered, respectively: S_0 - control sample, S_1 - content of Cu II ions $3 \text{ mg}\cdot\text{L}^{-1}$, S_2 - content of Cu II ions $6 \text{ mg}\cdot\text{L}^{-1}$, S_3 - Fe II ion content $5 \text{ mg}\cdot\text{L}^{-1}$, S_4 - Fe II ion content $10 \text{ mg}\cdot\text{L}^{-1}$, S_5 - Fe II ion content $13 \text{ mg}\cdot\text{L}^{-1}$ and S_6 - Fe II ion content $15 \text{ mg}\cdot\text{L}^{-1}$ and Cu $6 \text{ mg}\cdot\text{L}^{-1}$, respectively. For all the samples, the alcoholic fermentations were conducted at $16 \div 18^\circ\text{C}$ for 10 days. After this period, the obtained wines were racked, decanted, corrected to $50 \text{ mg}\cdot\text{L}^{-1}$ free SO_2 and preserved at $10 \div 12^\circ\text{C}$.

Dynamic studies were carried out to determine the microbiological [13] and oxidative stability of the experimental samples: the experimental white musts were tested in 2

conditions (native sample, exogenous administration of Fe^{2+} ions in limits 5 to 15 $\text{mg}\cdot\text{L}^{-1}$ and Cu^{2+} ions within the range $3 \div 8 \text{ mg}\cdot\text{L}^{-1}$) [14].

The experimental samples have been filtered through the filter of 0.45 μm for spectrophotometric investigations (absorption spectra, phenolic compounds, the test of oxidation behavior/POM-test, etc.) [15]. The spectrophotometric analyses have been done at single beam spectrophotometer PG T80 (PG Instruments, UK) of TUM Oenological Research Center.



Figure 3. Experimentally processed grapes of ampelographic varieties:
a - Traminer and b - Muscat Polsky

Within the ORC of TUM were determined the physico-chemical and quality indices of grapes and raw wines produced by modern analysis methods recommended in national and international standards OIV [16]. The examination of the experimental must and wine samples for colloidal and metallic stability was carried out based on the specialist methodical indication of the ORC [14]. The POM-Test was performed according to Scutaru, Iu., this test appreciates the oxidation behavior of white wines in the presence of oxidant H_2O_2 3 % for 60 minutes at 60°C, as changes in optical absorption at 420 nm in a 1 cm cuvette. Calculations are performed using the following formula [17]:

$$POM_{test}(\%) = 100 * \frac{A_{420}(\text{wine and H}_2\text{O}_2) - A_{420}(\text{wine})}{A_{420}(\text{wine})} \quad (1)$$

Experimental data were statistically processed by using Microsoft Office Excel 2010 to determine the mean values along the standard error. Using a significance level of $p < 0.05$, the ANOVA and PCA statistical tests were used to analyze multiple variances following the Pearson test [18].

RESULTS AND DISCUSSION

Experimental grapes samples and dry white wines obtained from studied processed in the micro-winery section at the Department of Oenology and Chemistry of TUM were submitted to physico-chemical analyses and the obtained results are included in Table 1.

Table 1. *Physicochemical indices of experimental must and wine samples, harvest 2023*

Parameter	Type of grapes	
	Muscat Polsky	Traminer
Musts		
Active acidity (pH)	3.51 ± 0.01	3.75 ± 0.01
Mass concentration of sugar [g·L ⁻¹]	210 ± 5	223 ± 5
Mass concentration of titratable acid [g·L ⁻¹ tartaric acid]	5.81 ± 0.22	6.62 ± 0.34
Mass concentration of aminoacids [g·L ⁻¹]	172.84 ± 2.16	278.46 ± 3.10
Turbidity, NTU	22.49 ± 1.02	28.68 ± 1.65
POM-test [%]	0.25 ± 0.12	0.57 ± 0.08
Wines		
Active acidity pH	3.27 ± 0.01	3.42 ± 0.01
Mass concentration of residual sugar [g·L ⁻¹]	2.24 ± 1.15	4.10 ± 1.63
Alcool by volum [% vol.]	12.24 ± 0.65	12.96 ± 0.84
Total dry extract content [g·L ⁻¹]	20.6 ± 0.2	21.8 ± 0.2
Mass concentration of volatile acids [g·L ⁻¹ acetic acid]	0.48 ± 0.08	0.60 ± 0.1
Mass concentration of titratable acids [g·L ⁻¹ tartaric acid]	5.21 ± 0.22	5.62 ± 0.34
POM-test [%]	0.15 ± 0.02	0.38 ± 0.02
Content of SO ₂ , free/total forms [mg·L ⁻¹]	21/74 ± 5	13/64 ± 5
Total phenolic compounds [mg·L ⁻¹]	142 ± 8.5	162 ± 6.2
Color intensity (AU), A ₄₂₀	0.146 ± 0.02	0.186 ± 0.02
Contents of total iron ions [mg·L ⁻¹]	1.7 ± 0.1	0.48 ± 0.04
Contents of copper ions [mg·L ⁻¹]	0.86 ± 0.12	0.66 ± 0.02
Organoleptic characteristics	Clear dry wine, without strange odors, citric fruits with aromas of floral and tree fruit nuances, complete taste, rich and full.	Clear dry wine, without strange odors, with yellow-green hues, lime-tree odor, and honey-like/dried fruit, complete taste, rich and full.
Total quality score, points	84	82

According to data in Table 1, the Traminer grape varieties present highest physicochemical indices than the second-studied Muscat grape varieties. The pH index in the must varies in the interval of 3.5 and 3.75 units, in the final wines it decreased by 0.2 - 0.33 units. The concentration of titratable acidity of the must and final wines is within the permissible limits for dry white wines (4 - 8 g·L⁻¹ tartaric acid) and allows the preservation of freshness and fruitfulness of study samples. The pH values of the examined wines are 3.27 to 3.42 which is characteristic for dry white wines. The sensory properties of wines obtained from varieties of Traminer and Muscat grapes are characteristic: wines have good clarity, with a color characteristic of white dry wines. The aroma and taste of wines are appropriate for each variety and correspond to the

highest quality wines.

The results of microbiological studies on grapes studied samples are included in Figure 4.

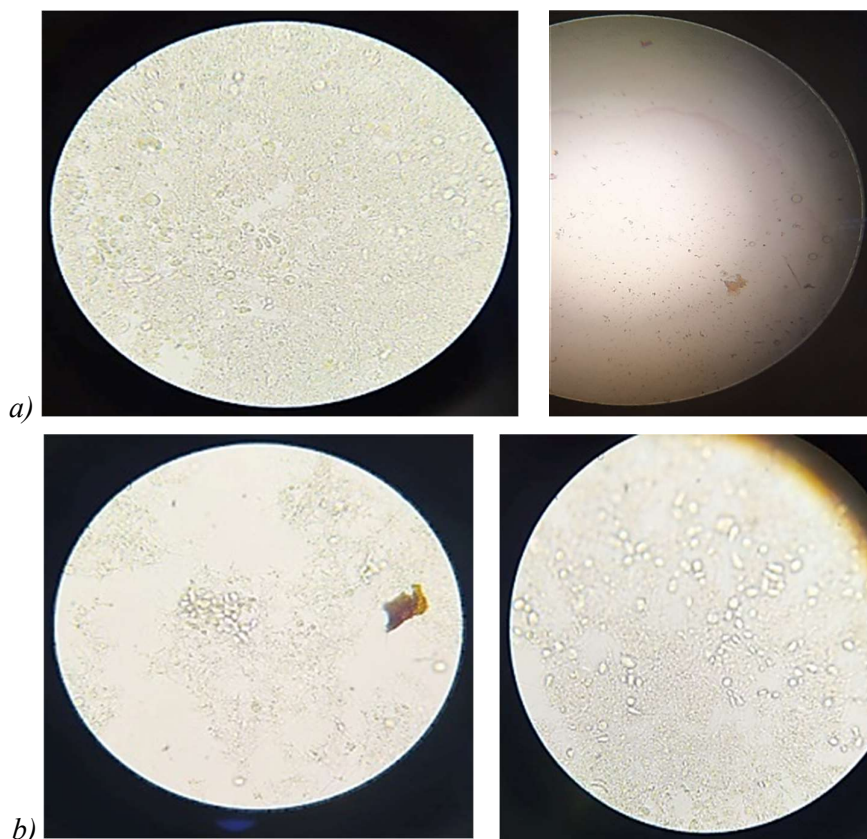


Figure 4. Photo image of grape microflora testing results:
a - Muscat and b - Traminer

Based on the microflora of the grape samples studied (Figure 4) were identified: yeasts of the *Pichia* species (oval or slightly elliptical cells with dimensions of $3.0 - 4.5 \times 3.5 - 7.2 \mu\text{m}$), *Zygosaccharomyces* (cells from harmful microflora), *Saccharomyces cerevisiae*, *Saccharomyces ludwigii* (large oval-shaped cells $3 - 8 \times 18 - 34 \mu\text{m}$).

In conclusion, the microbiological state of the grapes is of an average level, with specific and unwanted microflora species. The adjustment of the technological doses of SO_2 allows for ensuring the appropriate microbiological and oxidative stability of the future must fermentation environment, thus adjusting the physico-chemical and organoleptic parameters to the organoleptic requirements and standards, as well as to the quality and harmlessness norms of the finished product [19, 20].

The influence of the doses of iron and copper metal ions on the general appearance of the studied wines, as well as the dynamics of their content during the technological process are shown in Figures 5 - 7.

The concentration of iron and copper ions can variably influence the oxidative stability of wine: in small amounts, iron and copper are important as metabolism catalysts and enzyme activators. While at major contents they can cause instability, such as the cases

of ferric tannate, ferric phosphate and cupric spoilage/copper case [21], which subsequently change even the sensory characteristics and affect the overall quality of the wine and are linked to protein instability [22].

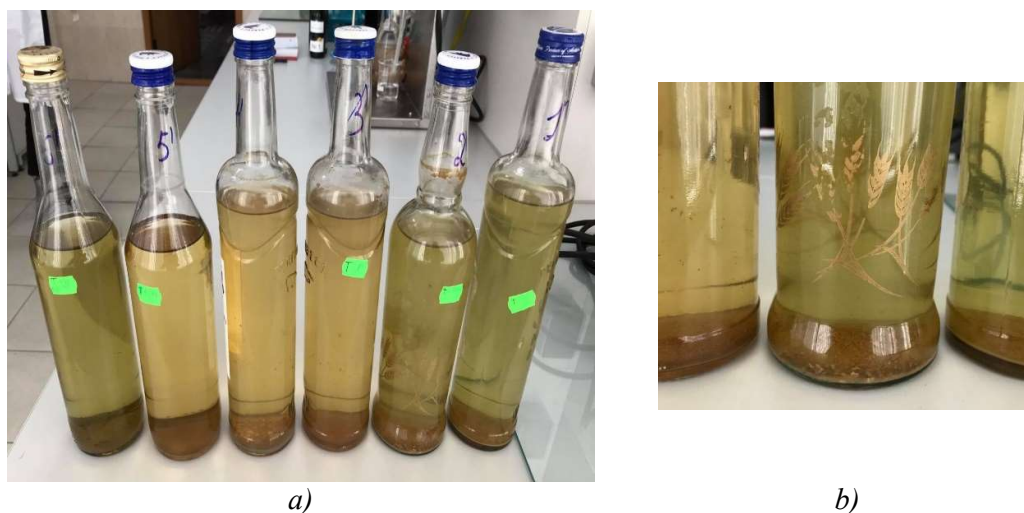


Figure 5. Photo image of the study samples, from right to left the metal ion content increases: a - S_1 to S_6 and b - the metallic sediment in the bottles

In the samples with higher ion content studied (Figure 5), the color intensity was proportional to the residual metal concentration, the excess of Fe ions directly affects the organoleptic characteristics, changing the color of the wine. Additionally, traces of crystals were observed at the bottom of the bottle (S_1 , S_2 and S_6), and their shapes differ from case to case. Iron and copper cations play an important role in the oxidation processes of wine, an effect visible to the naked eye.

The results of the elemental analysis of the metal content of the grape juice are presented in Figure 6, from which it can be seen that the samples in which no copper additions were made (S_3 to S_5) have a constant copper concentration of $0.86 \pm 0.12 \text{ mg} \cdot \text{L}^{-1}$ (all values are shown mean $\pm$ standard deviation in triplication). The reduced copper treatment (S_1) averaged $3.61 \pm 0.09 \text{ mg} \cdot \text{L}^{-1}$, and the increased copper treatments (S_2) averaged $6.52 \pm 0.03 \text{ mg} \cdot \text{L}^{-1}$ copper, which is consistent with the effective additions, including the error of the determination method. From the analysis of exogenous iron concentrations in the Muscat study samples, it can again be seen that the control sample (S_0) and the treatments without iron addition (S_1 and S_2) show very close results of 1.7 with $0.15 \pm 0.05 \text{ mg} \cdot \text{L}^{-1}$ deviation.

Treatment 3 (S_3) was the test with exogenous iron in the minimum content, briefly representing $6.63 \pm 0.02 \text{ mg} \cdot \text{L}^{-1}$ of iron. The highest iron additions were made in treatments 4, 5, and 6, which were found to be $9.11 \pm 0.03 \text{ mg} \cdot \text{L}^{-1}$, $11.8 \pm 0.038 \text{ mg} \cdot \text{L}^{-1}$ and $13.64 \pm 0.06 \text{ mg} \cdot \text{L}^{-1}$, respectively of iron. The results confirm that the addition of metal ions to the grape must or juice resulted in total concentrations as expected and no significant precipitation of the metals in the medium occurred before measuring their total concentrations.

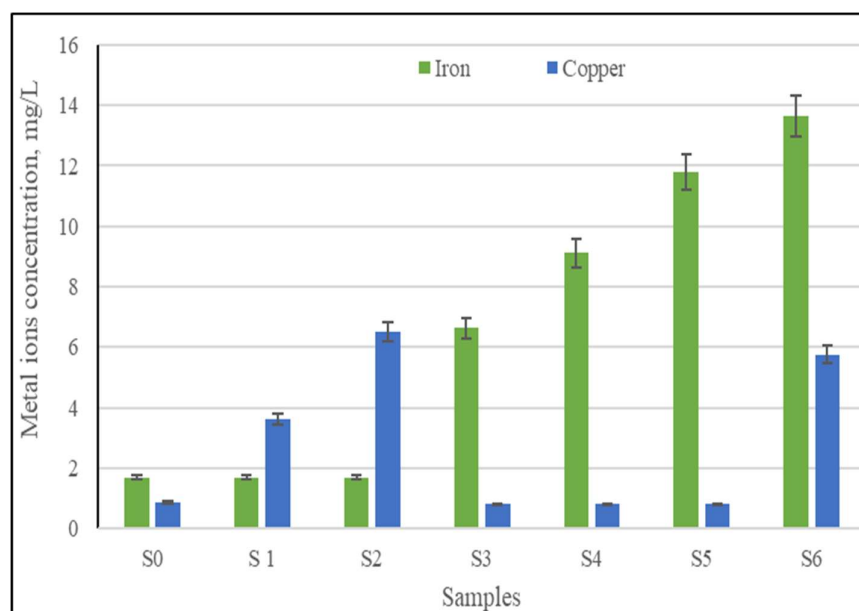


Figure 6. The content of metals (Cu II and Iron II ions) after administration in Muscat must samples after exogenous administration

Iron participates in the initiation of oxidation reactions, of the transformation of oxygen into the reactive form, thus, its concentrations indicate the oxidation of the constituent phenolic substances in the wine – contributing to the sediment formed at the bottom of the bottle (Figure 5 (b)).

Moreover, the test results aligned with findings regarding the impact of copper and iron concentrations on oxidative stability. Wines with added copper and iron showed differing POM-Test results, suggesting that these metals influence the oxidative processes and stability of the wines.

In Figure 7, metal concentrations detected in wine samples after alcoholic fermentation vary significantly for Cu^{2+} and Fe^{2+} ions.

According to the results of Figure 7, in the treatments without iron addition (S_0 , S_1 and S_2) the total concentrations were almost identical and significantly lower than the other three trials where metal additions were made (S_3 to S_6). The first three treatments were found to have a mean total iron concentration of $1.58 \pm 0.02 \text{ mg} \cdot \text{L}^{-1}$. Despite these low concentrations, a difference in species results is noted when comparing control S_0 with S_1 and S_2 .

Dynamically, the copper content after alcoholic fermentation must participate in a major proportion to the redox processes during the alcoholic fermentation, thus the value of the exogenously added copper content is found in wine in a proportion of maxim 14.7 %, being precipitated in 86.7 - 92 % of its initial content in must/juice. For the iron species, this is achieved with a maximum of $2.1 \text{ g} \cdot \text{L}^{-1}$ at S_6 and a minimum of $0.12 \text{ g} \cdot \text{L}^{-1}$ at S_0 to S_3 . The diminution percentage of iron content in the wine samples is within the limits of $0.4 \div 15 \%$ compared to the initial value in the must/juice.

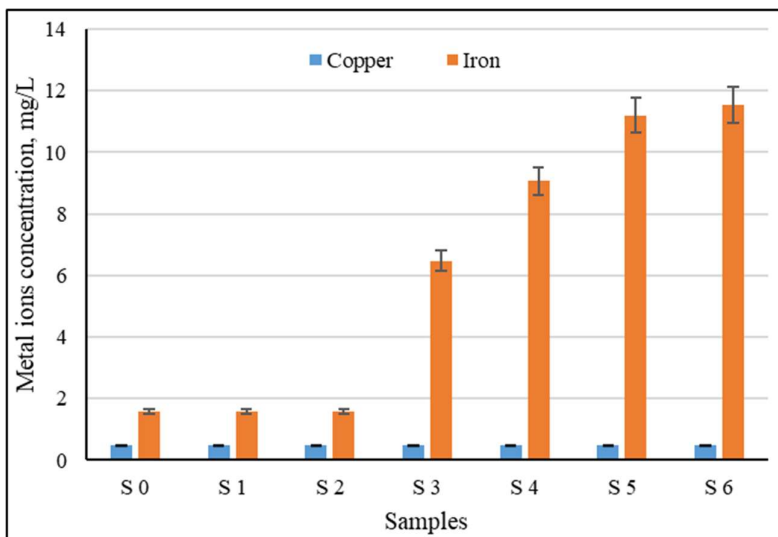


Figure 7. The content of metals (Cu II and Iron II ions) in the experimental Muscat wine samples after the alcoholic fermentation of the samples prepared according to Figure 6

Revealing the results, it was observed that, regardless of the amount of copper added to the grape must/juice, after alcoholic fermentation its concentrations decreased significantly (by more than 80 %). The opposite trend was found for iron, as nearly 80 % of all administered iron was quantified in the final wine product.

This effect is attributed to the fact that the administration of exogenous iron and copper ions changes the proportion of hydrophilic species, the residual and cationic forms of the studied metals. Based on the results, it can be asserted that the cationic form of copper is predominant in all experimental treatments and that the differences in copper additions made to grape juice did not significantly influence the distribution of copper species for all samples.

It could be hypothesized that either copper or iron additions to grape must/juice result in a decrease in the proportion of hydrophobic iron species and an increase in the proportion of cationic species. In the samples without the initial addition of copper and iron (S₀), the residual form of iron was predominant because this content remained constant until the final product after alcoholic fermentation. In samples S₃ ÷ S₅ of Muscat wines, the Fe²⁺ forms were found in higher concentration, which denotes a reduced rather state of the wine, in which there are still substances capable to reduce Fe (III) to Fe (II), which can generate free radicals when interacting with oxygenated water (H₂O₂, formed by reactions illustrated in figures 1 and 2), forming oxidizable substances (oxygenated water, in small quantities, is formed and consumed in wines continuously, with the involvement of O₂, then they become wine and endogenous enzymatic or inorganic catalysts).

In the study, the POM-Test enabled to quantify the degree of oxidative stability by monitoring the changes in specific phenolic compounds and other redox-active substances in the wines. The results indicated that wines with higher concentrations of phenolic compounds exhibited greater resistance to oxidation, as reflected in lower POM-Test values. This correlation underscores the crucial role of phenolics in maintaining the quality and longevity of the wines.

By employing the POM-Test, were able to establish a clear relationship between the chemical composition of the wines and their oxidative behavior, providing valuable insights into optimizing winemaking practices for enhanced wine quality and stability.

CONCLUSIONS

The results of the presented experimental and applied study generate aspects concerning the microbiological, oxidative stability and metal species involved in the redox process of wine. The oxygen participation rates in the redox process of wine showed a good correlation with the total copper concentration in wine samples. However, a stronger correlation was found between oxygen participation rates and residual copper species (within limits of $80 \div 92 \%$). A similar correlation was found for iron for both total and residual concentrations versus oxygen participation rates, although not as strong as for copper.

Dissolved oxygen in large quantities reacts directly with wine constituents such as phenolic compounds and SO_2 , but these reactions are thermodynamically unfeasible for oxygen as a di-radical. Ferric iron (Fe^{3+}) participates as a catalyst in this process to overcome the activation energy of the electron reduction stage of the oxidation process, and copper, in turn, catalyzes the reduction of Fe^{3+} to Fe^{2+} . Oxidation reactions proceed with the further formation of a hydroperoxyl radical ($\text{OOH}\cdot$), which is a precursor for other reactive oxygen species. At wine pH and the presence of ferrous iron Fe^{2+} , $\text{OOH}\cdot$ can further react to peroxide anion and hydrogen peroxide (H_2O_2). In the presence of transition metals (such as, Fe, Cu), another possible step is the formation of a hydroxyl radical ($\text{OH}\cdot$), a hydroxide ion (HO^-), and water from H_2O_2 via the Fenton reaction (Figure 1). This OH appears to be highly reactive toward many wine compounds. Various radicals formed in these reactions can react directly with phenolic compounds and are stronger oxidants than oxygen itself. Due to the excess presence of iron and copper, the oxidation of phenolic compounds is maximal in must or wine, and their absence from redox processes slows this process to a minimum.

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