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Correlation of Microbiological Stability with Redox Processes in White Wines

Gheorghe Duca[1], Rodica Sturza[2*], Natalia Vladei[2], Ecaterina Covaci[2]

[1]Research Centre of Physical and Inorganic Chemistry, State University of Moldova, Chisinau, Republic of Moldova
[2]Department of Oenology and Chemistry, Faculty of Food Technology, Technical University of Moldova, Chisinau, Republic of Moldova
Email: *rodica.sturza@chim.utm.md

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Abstract

In this paper, the authors analyzed the correlation between the microbiological stability of white wines and the content of sulfur dioxide, which influences the main redox processes that take place in the technological stages of the wine. The consecutive, parallel and spontaneous development of several redox processes and their impact on the quality, microbiological and crystalline stability of white wines were examined. The reduction of additive and subtractive technological interventions, of the amounts of adjuvants (sulphurous anhydride) is essential for the production of organic wines.

Keywords

White Wines, Acetobacter, Sulfur Dioxide, Redox Processes, Oxygen

1. Introduction

Wine is a complex system capable of undergoing many different compositional changes during its formation and storage, especially white wine. Undesirable changes in white wine after post-fermentation mostly involve microbiological instability, non-enzymatic and oxidative browning [1].

Sulfur dioxide, introduced into wine products, can be present in four free forms: molecular SO_2 as a solubilized gas, the bisulfite ion (HSO_3^-) and the sulfite ion (SO_3^{2-}), as well as in several combined forms stable and unstable [2]. The molecular form (SO_2) is responsible for the antimicrobial activity in wine products, inhibiting the activity of oenological yeasts approximately 20 times more effectively than bisulfite and 500 times that of bacteria, as well as it is responsible for the unpleasant, pungent smell of sulfur dioxide [3].

The bisulfite form (HSO_3^-) is predominant in musts and wines, inactivating

the action of polyphenol oxidases. The content of sulfite (SO_3^{2-}) at wine pH is minimal, but it reacts directly with oxygen and hydrogen peroxide and, consequently, has some antioxidant capacity [4]. The last form of combined SO_2 does not have antioxidant and antioxidative activity, its antimicrobial effects being much lower, which is why the combination of sulfur dioxide with the constitutive compounds of musts and wines practically implies the loss of its beneficial effects of oenological interest [4]. **Table 1** summarizes the global effects of the 4 forms of sulfur dioxide.

Traditionally, sulfur dioxide is the most common additive used in winemaking for decades. The importance of SO_2 is emphasized due to its antiseptic and antioxidant properties. The wine industry has a big issue regarding reducing or eliminating SO_2 especially in the production of organic wines [6]. Moreover, it can have negative effects on human health. Regulatory acts concerning sulfite content in wines have encouraged the wine industry to use alternative methods and, consequently, to reduce the SO_2 content in wines [7]. Besides, excessive administration of SO_2 in winemaking process leads to organoleptic changes of final product.

Organic wines production involves utilization of yeast strains characterized by the absence or the reduced production of sulfur compounds. In these wines, the lack of SO_2 requires a limited contact with oxygen, which leads to the negative effect of H_2S on the odorant profile of wines [8]. Although many of the alternatives to SO_2 presented remarkable efficacy, nowadays no other physical technique or additive can offer antioxidant and antimicrobial action as it is done by sulfur dioxide [6].

The purpose of the study is to elucidate the influence of sulfur dioxide forms on the microbiological stability of Chardonnay and Feteasca Regală dry white wines within the Oenological Research Center from the Technical University of Moldova (TUM). The studied microorganisms' species were from the category

Table 1. The antioxidant, antimicrobial and organoleptic properties of sulfur dioxide in wine and must depending on the chemical nature of the existing forms (adaptation according to [5]).

Selective capacity	Molecular SO_2	The bisulfite ion (HSO_3^-)	The sulfite ion (SO_3^{2-})	Combined form
Fungicidal	++	Lightly	–	–
Bactericide	++	Lightly	–	Lightly
Antioxidant	++	+	Lightly	–
Antioxidative	+	++	–	–
Redox potential	+	+	Lightly	–
Combination with acetic aldehyde	+	+	+	+
Taste influence	Pungent smell, taste of sulfur	Odorless	Odorless	Odorless

of oenological yeasts (genus *Saccharomyces*), contaminating yeasts (genus *Brettanomyces* and *Saccharomycodes*), sporophyllous bacteria (genus *Acetobacter* and *Streptococcus*) and molds (genus *Aspergillus* and *Penicillium*).

2. Materials and Methods

The grape varieties Chardonnay and Feteasca Regală, harvested 2021, were processed in the micro vinification section of the Oenology and Chemistry Department of the TUM using the classical method of dry white wines production [9]. Dynamic studies were carried out in order to determine the microbiological and oxidative stability of the experimental samples: the dry white wine Chardonnay was tested in 2 pH conditions (native sample - 3.22 and adjusted to 2.8 by acidification with tartaric acid) and the addition of SO_2 technological doses between 20 and 200 mg/L [10] [11].

Within the Oenological Research Center 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 [12] [13].

The content of sulfur anhydride forms was determined by 2 reference methods OIV-MA-AS323-04A and OIV-MA-AS323-04B [14]. The microbiological stability of dry white wine samples was achieved by determining the total number of germs (TNG) using the Koch method [15]. In order to accomplish the microbiological tests, the following media were used: MRS Agar for the detection of lactic bacteria (*Lactobacillus*), GYC for the detection of acetic bacteria (*Acetobacter*), Lauryl Tryptose for molds detection and Sabouraud for the detection of pathogenic and non-pathogenic fungi. The thermostating of the Petri dishes with the examined samples was carried out at 27˚C ± 2˚C for 3 - 5 days) [16]. The results of the studied wine microbial load were compared with the limit values described in the legislative and normative documents [17].

The oxidative stability of the studied samples was focused on the identification of the experimental wines color parameters by the CIELab method (color tristimulus values X, Y, Z, represented in Cartesian or cylindrical coordinates). The chromatic parameters were identified and calculated according to the OIV-MA-AS2-11 method [12] using visual sensation characteristics such as clarity, tonality, saturation, brightness, hue [18]. CIELab is the complex wine color determination model used to describe colors in the visible spectrum which allows detecting the largest number of color shades that can be distinguished by the human eye. The CIELab system characterizes color variations through the colorimetric coordinates L*, a* and b*. The vertical axis marked L* sets in the range 0 ÷ 100 (0 - completely opaque and 100 - completely transparent), the parameter "+a*" the red shade, "–a*" the green shade, the characteristic "+b*" the yellow shade, "–b*" the blue shade [19].

The sensory analysis of the experimental samples was carried out by a group of 14 tasters, which provided the description of the olfactory, gustatory and tac-

tile profile. Each sensory descriptor was scored by points between 0 (least felt) and 5 (most felt) and text descriptors regarding the quality or defects of the analyzed wines [20].

Experimental data were statistically processed by using Microsoft Office Excel 2007 in order to determine the mean values along standard error. Using a significance level of $p < 0.05$, the ANOVA and PCA statistical tests were used to analyze multiple variances in accordance with the Pearson test [21].

3. Results and Discussion

Experimental dry white wines obtained from Feteasca Regală and Chardonnay local grape varieties processed in the micro winery section at the Department of Oenology and Chemistry of TUM were submitted to physico-chemical analyzes and the obtained results are included in **Table 2**.

The results of microbiological studies on studied samples MRS Agar, GYC, Malt Extract Agar and Sabouraud Glucose Agar media are included in **Figure 1** and **Table 3**.

Following the microbiological studies at SO_2 doses up to 75 mg/L, bacterial species belonging to the group of lactic acid bacteria, with spherical (cocci) and cylindrical (bacilli) morphological forms were identified (**Figure 1**). These technological treatments did not provide sufficient antimicrobial protection to the wine samples, thus most of the Petri dishes were crowded with yeasts, bacteria

Table 2. Physical-chemical indices of the dry white wine samples, harvest 2021, Centru region.

Parameter	Type of Wine	
	Chardonnay dry white wine	Feteasca Regală dry white wine
Active acidity, -native pH -adjusted pH	 3.22 ± 0.01 2.84 ± 0.01	 3.12 ± 0.01 -
Alcohol by volume, % vol.	12.46 ± 0.25	12.18 ± 0.21
Mass concentration of residual sugar, g/L	3.54 ± 0.12	3.48 ± 0.18
Mass concentration of titratable acids, g/L tartaric acid	5.86 ± 0.52	6.92 ± 0.44
Mass concentration of volatile acids, g/L acetic acid	0.62 ± 0.06	0.48 ± 0.10
Turbidity, NTU	12.29 ± 0.08	14.18 ± 0.12
Organoleptic characteristics	Clear wine, without extraneous odors, velvety with aromas of cheese, floral and green apples nuances	Clear wine, with yellow-green hues, without extraneous odors, aromas of wildflowers and ripe apricots.

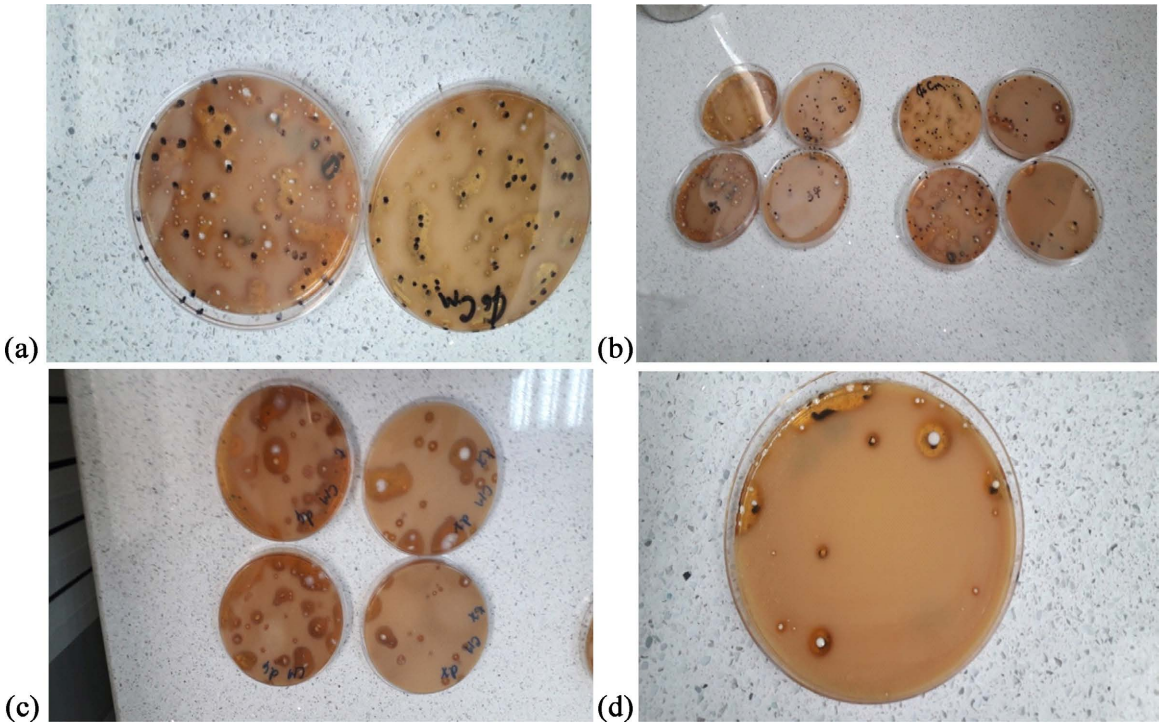


Figure 1. Dynamics of the examined samples microbiological state evolution: (a) Total SO_2 35 mg/L; (b) Total SO_2 75 mg/L; (c) Total SO_2 150 mg/L and (d) Total SO_2 200 mg/L.

Table 3. Value of microbiological tests of dry white wine samples examined at 20˚C at different concentrations of sulfur dioxide.

The sample	Active acidity, pH	Concentration of SO_2, mg/L			The microbiological result established by liquid seeding on different culture media, colonies
		total	free	active	
Chardonnay dry white wine, native	3.22 ± 0.01	20	4	1.23	4 - yeasts, 6 - bacteria and 2 - molds
		35	8	2.05	4 - yeasts, 4 - bacteria and 2 - molds
		75	2. 3	4.40	3 - yeasts, 0 - bacteria and 2 - molds
		125	45	7.33	3 - yeasts, 0 - bacteria and 2 - molds
		150	63	8.8	2 - yeasts, 0 – bacteria and 1 – mold
		200	86	17.72	not detected
Chardonnay dry white wine, acidified	2.84 ± 0.01	20	4	2.17	4 - yeasts, 6 - bacteria and 2 - molds
		35	8	5.15	4 - yeasts, 0 - bacteria and 2 - molds
		75	2. 3	11.04	3 - yeasts, 0 - bacteria and 2 - molds
		125	45	18.40	1 - yeasts, 0 - bacteria and 1 - mold
		150	63	22.08	not detected
		200	86	29.45	not detected

and molds (>120 UFC/mL). In these experimental samples, a deposit was found at the bottom of the storage vessel, changes in color (oxidation) and a medium disturbance of the clarity with a slight release of CO_2 was observed. In the samples treated with only 75 mg/L SO_2, yeast colonies were selectively identified, meanwhile in the other samples treated with augmented doses of SO_2, these co-

lonies were observed within the limits of the technological requirements (1 to 3 colonies), the Petri dishes being free of mold and characteristic bacteria.

The microbial load of the samples indicated that the doses of 20 - 35 mg/L (**Table 3**) influence negatively the multiplication of the existing microorganisms in the wine but do not ensure a lasting microbiological stability.

In the case of samples treated with 75 and 125 mg/L SO_2, wine characteristic yeasts and micromycetic species were identified, but other categories of microorganisms (bacteria) did not develop. The clarity of these wines was described as good, with characteristic smell and no extraneous odors.

For experimental samples treated with maximum doses of 150 and 200 mg/L SO_2, there was no evidence of the excessive presence of yeasts, nor of other categories of characteristic microorganisms present in the studied samples. Chardonnay wines presented a color specific to the studied varieties (straw-yellow), clear, characterized by fine aromas specific to the variety and without extraneous odors. This aspect is due to the synergistic action between free sulfur dioxide and its active form, confirming the ability to inhibit microorganisms [22] [23].

The results shown in **Figure 1** and **Table 3** denote the bactericidal effect of sulfur dioxide in studied white wines. In samples with active sulfur dioxide content above 4 - 5 mg/L, lactic and acetic bacteria did not develop, which proves the increased sensitivity of bacteria to this factor. Oenological yeasts preserve their vitality at concentrations of active sulfur dioxide higher than 8.8 mg/L, defining themselves as quite resistant [24] and confirmed by the negative dependence of the multiplication of microorganisms on variable doses of active SO_2 (**Table 4**).

According to Pearson coefficients, there are direct correlations between microbiological variables and the administered treatments. High positive correlations were obtained between the forms of sulfur anhydride (r = 0.850) and the species of molds and yeasts (r = 0.961) present in the study wines.

The antimicrobial effect of SO_2 forms on the microflora of wines was reconfirmed by the strongly negative correlation and linear dependencies (r = −0.9031 ÷ −0.934). Technologically, the microbiological stability increases with the increase of the active form of SO_2, a more significant effect was evident in the species of

Table 4. Correlation level of microbiological stability according to the forms of sulfur dioxide present in the study samples.

Study variables		2 forms		The type of microorganisms		
		Free	Active	Yeasts	Bacteria	Mold
SO_2 forms	Free	1.0000	0.8495	−0.9309	−0.6337	−0.9031
	Active	0.8495	1.0000	−0.9339	−0.5859	−0.9602
The type of found microorganisms	Yeasts	−0.9309	−0.9339	1.0000	0.5898	0.9610
	Bacteria	−0.6337	−0.5859	0.5898	1.0000	0.4438
	Mold	−0.9031	−0.9602	0.9610	0.4438	1.0000

molds (r = −0.9602) and yeasts (r = −0.94). Namely, the positive correlation between the species of microorganisms highlighted in the experimental study, with r values between 0.444 and 0.9610, explains the synergy of coexistence of yeasts, bacteria and characteristic molds of wine.

Therefore, it can be seen that the most resistant oenological microorganisms to SO_2 were wine bacteria and molds, for the destruction of which high concentrations of active sulfur dioxide within the limits of 17 - 19 mg/L are required.

Dynamically, the active sulfur anhydride form (HSO_3^-) in the studied wines reveal a 2 times higher content of this form at a lower pH of the wine, but at the same content of the free form. Significant values of statistical probability (sigma < 0.05), reveal the rejection of the null hypothesis, so with a probability of 95% it can be stated that there are significant statistical links between study variables, and the results obtained after the PCA and Anova tests described values of F (Anova ratio) significantly higher than the criterion, thereby the results obtained are significant.

Therefore, the optimal use of sulfur dioxide requires checking wine active acidity (pH), as well as carrying out procedures to reduce the oxidative factors. Key milestones in wine sulfiting could be considered:

- Addition of at least 50 - 75 mg/L of total SO_2 at the crushing-de-stemming stage to limit the redox activity;
- After pressing, at the must clarification stage, administration up to the level of 75 - 100 mg/L of total SO_2;
- Sulfiting of young wine with doses up to 150 mg/L of total SO_2 in order to preserve the microbiological and oxidative stability of the wine.

The intensity of the evaluated sensory descriptors of studied wines is shown in Figure 2. The organoleptic characteristics of the experimental samples, notably the floral and fruity descriptors (green apple, ripe fruits, wildflowers, etc.) of the samples treated with maximum doses of SO_2 were more expressive compared to those treated with less SO_2. The wines treated with the highest amount of SO_2 (150 mg/L) stood out for their "clean", balanced profile, showing up for a better stability over time.

Along with the increase of the of administered treatments doses of SO_2 represented in Figure 2, are noticeable characteristic odors of ripe fruits, grapefruit, green apple and lactic, moderate astringency and acidity, discreet notes of lemon and spices. Regarding the oxidized character and the color shading, their augmentation is observed in the case of samples treated with SO_2 at the minimal dose of 35 mg/L.

Regarding the influence of the SO_2 treatment dose on the taste perception of the analyzed wines, it was concluded that at doses of 35 mg/L the wines were characterized by dull, flat and oxidation notes. In the samples treated with doses of 150 mg/L, 4 from 14 tasters mentioned the presence of a slight metallic and pungent taste (which could be due to the particular sensitivity of individuals). Optimal tasting results were obtained for wines treated with 75 mg/L SO_2.

To summarize, the most organoleptically appreciated experimental samples

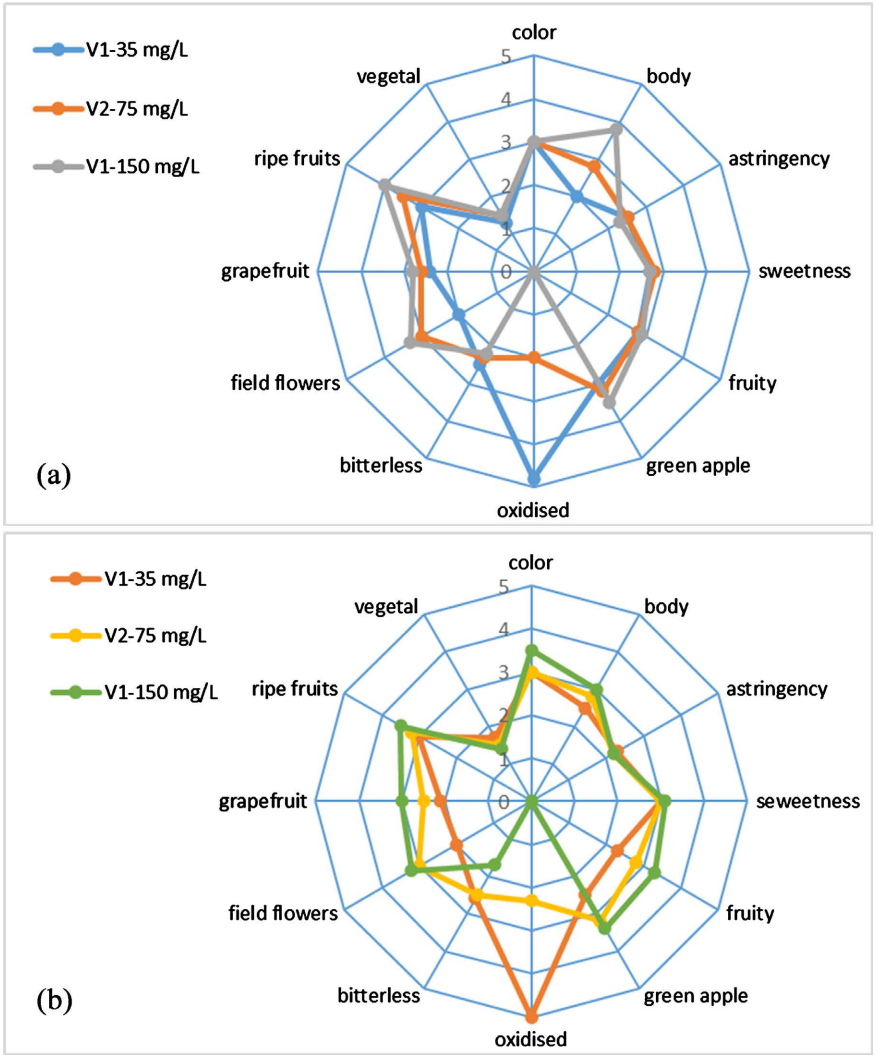


Figure 2. Diagram of the sensory evaluation with varied doses of SO_2: (a) Chardonnay dry white wine; (b) Feteasca Regală dry white wine.

were those treated with the highest dose, followed by those with 75 mg/L SO_2. The organoleptic characteristics were highlighted by a complexity of aromas specific to the variety from which the wine was produced and by a clean, harmonious profile, balanced between fruit and floral aromas, with a slightly freshness due to the acidity.

From the sensorial point of view, the impact was confirmed by the treatments showing significant influence on the analyzed sensory indicators: oxidized, taste and aftertaste, color, body, except for vegetal, sweet and bitter descriptors. The Pearson correlation coefficients for the sensory analysis showed positive values between the pairs of descriptors: green apple - ripe fruits, vegetal - grapefruit, green apple - wildflowers and wildflowers - vegetal.

The experimental oxidative stability of the wine samples obtained by the CIE-Lab method is presented in Table 5, and the statistical analysis indicated an increase in the brightness parameter (L*) proportional to the level of sulfur dioxide

Table 5. The value of the chromatic parameters of the samples, assessed by CIELab.

The sample	Brightness, L*	Colorimetric coordinates		Chromaticity, C	Color intensity, Ic	Color hue, Nc
		a*	b*			
Dry white wine Chardonnay, 35 mg/L SO_2	78.7 ± 0.03	8.25 ± 0.02	21.8 ± 0.2	22.4 ± 0.17	0.84 ± 0.09	1.94 ± 0.16
Dry white wine Chardonnay, 75 mg/L SO_2	97.64 ± 0.13	0.67 ± 0.09	6.22 ± 0.11	6.28 ± 0.18	0.17 ± 0.07	2.53 ± 0.12
Dry white wine Chardonnay, 150 mg/L SO_2	98.5 ± 0.19	0.24 ± 0.05	5.19 ± 0.16	5.31 ± 0.16	0.13 ± 0.02	3.61 ± 0.16
Feteasca Regală dry white wine, 35 mg/L SO_2	77.9 ± 0.27	11.70 ± 0.11	23.44 ± 0.24	20.01 ± 0.11	1.05 ± 0.09	1.44 ± 0.18
Feteasca Regală dry white wine, 75 mg/L SO_2	96.4 ± 0.19	0.93 ± 0.05	6.35 ± 0.03	6.13 ± 0.20	0.19 ± 0.06	2.23 ± 0.09
Feteasca Regală dry white wine, 150 mg/L SO_2	98.5 ± 0.11	0.17 ± 0.09	5.33 ± 0.20	4.87 ± 0.20	0.11 ± 0.03	3.34 ± 0.05

administered in the study groups.

Regarding the wine samples treated with 35 mg/L SO_2, the average value of a* parameter presented the highest intensity, being downward with the increase in sulfur dioxide concentration from 35 to 150 mg/L. Thus, it can be concluded that the wines do not have a specific color, slightly greenish in this case with minimal SO_2 content, the expectations being for the a* parameter to present negative values.

The intensity of the color Ic varied according to the degree of oxidation of the samples, the lightest version being stable from the chromatic point of view, and the sample with the darkest color (brick) being oxidized in the samples with minimal doses of SO_2.

Likewise, the b* parameter presented values inversely proportional to the administered level of sulfur dioxide, which, from the statistical point of view, suggests the intensity of the wine samples yellow shade at positive values with a significant level sig. < 0.05. Thus, for the group of variants treated with 75 mg/L sulfur dioxide an average value was obtained, almost 4 times lower for the samples with 35 mg/L sulfur dioxide addition and slightly higher compared to the samples with 150 mg/L of sulfur dioxide. For the chromatic indicator L*, there is a considerable increase of the value with the increase of the concentration of administered sulfur dioxide (from 79 for the variants with 35 mg/L of sulfur dioxide up to 98.5 for those with 150 mg/L sulfur dioxide).

Consequently, the obtained values for the a* parameter were much lower in the samples treated with higher amounts of sulfur dioxide, which lead to a good stability of the wines treated with SO_2.

In the case of the b* parameter, the average values decreased inversely proportional to the level of sulfur dioxide administered. Instead, the chromatic indicators C, color hue Nc and intensity Ic varied according to the dose of sulfur dioxide administered. This decreasing trend of the chromatic indicators C (on average from 21 to 5 units) and intensity Ic (on average from 0.9 to 0.4 units) is observed in both samples, nevertheless the increase of color hue Nc is proportional to the upward trend of the SO_2 dose reaching a maximum of 3.5 units.

For comparison, the color of the white wines varied directly with the admi-

nistered doses of SO_2 (Table 6), appearing brick shades when treated with 35 mg/L SO_2, an insufficient technological dose to ensure the antioxidant protection of the wines over a longer period of time. This could be explained by the formation of highly reactive *ortho*-quinone from grape juice polyphenols when enzymatic oxidation occurred, because of an insufficient dose for antioxidant function of SO_2 [25].

The samples with 75 and 150 mg/L SO_2 treatment are free from oxidation and manifest antioxidize effects directly proportional to the increase of doses, which could be explained by binding the dissolved oxygen from the must which leads to decrease of the redox potential and, respectively, inhibition effect on the polyphenol oxidase activity [24].

The results obtained by simulating the wine color highlight significant differences between the analyzed chromatic parameters and their direct correlation with the degree of oxidation of the studied wines (Table 6 and Table 7).

Anova analysis results attest that the experimental results obtained on the 2 batches of wine are comparable and homogeneous in terms of parameter variance. Thus, for all chromatic parameters, the sigma value is lower than 0.05 and the F value is clearly higher than the criterion one.

4. Conclusions and Recommendations

The processing of grapes of the 2022 harvest from the Center region under the micro vinification conditions at the Department of Oenology and Chemistry, as well as the realization of a controlled alcoholic fermentation allowed the elucidation

Table 6. Color hues in the case of color simulation of experimental wines.

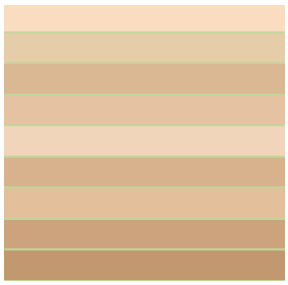
Dry white wine, 35 mg/L SO_2	Dry white wine, 75 mg/L SO_2	Dry white wine, 150 mg/L SO_2
		

Table 7. Chromatic parameters of the experimental samples Anova analysis results.

Chromatic parameters	Sum of squares, SS	Degrees of freedom, df	Mean square, MS	Anova report, F	Sigma, P-values	Fcrit
L	333.12	2	166.56	647.08	3.86857E−54	3.0977
a*	111465.05	5	22293.01	86608.48	4.6437E−164	2.3157
b*	3719.62	10	371.96	1445.08	8.71014E−95	1.9376
Total	115540.96	107				

of the antimicrobial effect of sulfur dioxide in 2 batches of qualitative dry white wine.

The bactericidal effect of sulfur dioxide in wine is achieved from a content of the active form of a maximum of 5 mg/L and the most resistant oenological microorganisms are molds, for the destruction of which high concentrations of active sulfur dioxide of over 17.6 mg/L are required. The decontaminating effect of sulfur dioxide solutions in wine at moderate concentrations of 150 mg/L can be considerably amplified by acidifying the wines to a pH of 2.8 units.

At lower concentrations of molecular sulfur dioxide yeasts may remain viable regardless of contact time. The most resistant oenological microorganisms are molds, for the destruction of which high concentrations of molecular sulfur dioxide are needed—more than 35 mg/L. At lower concentrations of molecular sulfur dioxide molds may remain viable regardless of contact time.

The statistical analysis of the study's chromatic parameter values pointed out an increase of the L* parameter proportional to the added level of sulfur dioxide, the other chromatic parameters described decreasing values with increasing sulfur dioxide concentration. Thus, it can be concluded that the wines do not have a specific color, being characterized by a slightly greenish shade at contents of at least 75 mg/L of total SO_2. Therefore, in order to assure stability of color quality parameters, the wines should be treated at least with 75 mg/L of total SO_2.

The intensity of the color varied according to the oxidation degree of the samples, the lightest version being stable from a chromatic and microbiological point of view, and the darker sample, with a brick color, being oxidized and with an increased microbiological load. Also, the b* parameter showed values inversely proportional to the level of sulfur dioxide administered.

Analyzing the data from the statistical point of view, it was found that the principal components analysis method can be applied to the results of the analysis of volatile compounds from the experimental samples, thus highlighting the existing correlations between the studied variables, at a confidence interval of 95%. According to these data, high positive correlations were obtained between the species of yeasts-molds-bacteria and negative between the active and total forms of sulfur dioxide with respect to the wine's characteristic microorganisms.

Furthermore, following the microbiological analyzes of the study samples, the best results were revealed by using maximum concentrations of 150 mg/L SO_2 and certainly 75 mg/L, so the wines presented microbiological stability, crystalline clarity, and specific color of the varieties from which were obtained, free from oxidation and refermentation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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