Replacement of SO_2 by plant phenolic concentrates to control oxidations in winemaking

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Abstract. In conventional winemaking, sulfites have long been used to control oxidation and spoilage microorganisms. However, the current wine trends and growing consumer health concerns have increased the need to seek alternatives to this preservative. In this context, the present study investigated plant-phenolic concentrates and explored their potential to replace the useful properties of SO_2 to control oxidations. The concentrates came from ten different plants and were provided by the company Biolethics Europe, from the Netherlands. Laccase activity control assays were performed using the syringaldazine chromogenic substratein must from botrytized grapes, and the oxygen consumption rate was measured using a non-invasive method based on luminescence in model wine solutions. Positive results were obtained in the two essays, which must be complemented with sensory analyzes and monitoring of the evolution of the wines.

1 Introduction

SO_2 is a chemical preservative used in wine production since the 17th century. It has assumed relevant importance throughout all the winemaking processes thanks to its chemical properties, such as antioxidant, antioxidasic and antiseptic in wines, as well as its biocidal properties for the conservation and disinfection of wine vessels. Despite its useful properties, it is considered a potentially toxic element and may cause adverse reactions for wine consumers and winemakers in quantities above 10 mg/L [1]. For this reason, international authorities have set sulfite's suggested daily intake limits as 0.7 mg/kg body weight [2]. Considering that a 60-80 kg consumer who drinks half a liter of wine can easily exceed the suggested limit (without considering the consumption of other foods containing SO_2), the WHO has recommended promoting research into alternative methods to reduce the use of SO_2 in food production, in addition to estimating that wine is one of the main contributors to SO_2 intake in adults who regularly consume wine [2].

Wine oxidation can be divided into enzymaticoxidation (by the action of oxidoreductase enzymes) and non-enzymatic oxidation (indirectly triggered by oxygen). Among the enzymes responsible for the enzymatic oxidation of phenolic compounds in juice, the most important are polyphenol oxidases, such as laccase [3].

The origin oflaccase in musts is due to the presence ofthe filamentary fungus *Botrytis cinerea*. This pathogen is present worldwide, especially in vineyards exposed to cold and wet conditions during the ripening period [4], and is responsible for substantial annual economic losses [5]. The oxidative enzymes laccases decompose anthocyanins and proanthocyanidins, decreasing the quality of the wine. These phenolic compounds contribute to essential characteristics of the structure of the palate, such as bitterness and astringency, as well as the color of red wines [4]. The decrease in quality in white wines is manifested by the loss of aromatic complexity, leading to oxidative aromas and browning of the color [6].The laccase enzyme in wine grapes presents some processing challenges due to its tolerance to high sulfur dioxide concentrations, wine and must pH range, alcohol, and fining agents [7]. It is estimated that sulphur dioxide addition in the amount of 50 mg/L causes a reduction in polyphenol oxidase activity in 75-90%, depending on the intensity of the infestation and the type of enzyme [3].

Non-enzymatic oxidations are also present in musts, but due to the slowness of the process, enzymatic oxidation plays a more important role [8]. However, non-enzymatic oxidations play a preponderant role during the storage and aging of wines, contrary to enzymatic oxidations.

In non-enzymatic oxidation, when the wine is exposed to O_2, the oxidation of metals such as Fe and Cu occurs, which are then reduced by oxidizing polyphenols to quinones. SO_2 slows Fe oxidation by removing H_2O_2 and reducing the oxidation products to their original form (e.g., o-quinonesto phenols) [9].

White wines are susceptible to oxidation due to the small amounts of phenolic compounds, while red wines contain higher concentrations of phenolic compounds. Therefore, they are easier to oxidize, but their high phenolic concentration (despite the oxidation) makes wines more resistant to oxygen. Therefore, contact with oxygen in the case of red wines is not as undesirable as in the case of white wines [3]. However, oxygen in the production of white wines is generally perceived as an undesirable factor. It causes adverse changes in the aroma, color, and taste of the wine [10]. Therefore, it is interesting to look at the antioxidant properties of wines. Various methods are available for this purpose.

Different studies have shown that the different tests to measure antioxidant capacity (TPC, DPPH, ABTS, and ORAC) show different results, which are sometimes contradictory [11-13]. The method developed for the oxygen consumption rate (OCR) measurement determines the direct oxygen consumption by the

different phenolic concentrates in a model wine solution which provides a direct reference value to estimate the actual capacity of the different antioxidants to protect wines against oxidation [11].

In searching for natural alternatives for the substitution or reduction of SO_2 in winemaking, plant phenolic extracts were evaluated by measuring the inhibition of laccase activity using the syringaldazine method. In addition, a second test was performed measuring the kinetics of the oxygen consumption ratios using the non-invasive luminescence method to determine the control capacity of non-enzymatic oxidative processes by plant phenolic concentrates.

2 Materials and methods

2.1 Laccase activity measurement

In the essays, 600 kg of whole bunches affected with Botrytis bunch rot (BBR) were selected, so the must came from grapes affected between 50% and 70% of BBR incidence, estimated visually and contrasted with reference primer according to the method used by Hill et al. [14]. The grape variety for the trials was Riesling, harvested manually during the 2021 season from the vineyards of the University of Geisenheim in the Rheingau region, Germany.

Botrytized must was vinified to monitor laccase activity and track the evolution of color, from musts to finished wines. A control treatment without additions, a treatment with SO_2, and a treatment with phenolic concentrates was vinified. All with three repetitions of 50 L each. Immediately after pressing, atwo liters sample of botrytized must was taken to the laboratory, where it was centrifuged at 5,000 rpm for five minutes. The basic must and wine analysis were recorded by Fourier transform infrared spectroscopy at the Geisenheim University Beverage Technology Laboratory, and the levels of infection correlated with the levels of gluconic acid present in the musts, as can be seen in Table 1. The analysis of basic parameters of the wines after alcoholic fermentation can be seen in Table 2.

Table 1. General must analysis. All the data are the mean ± SD of three replicates.

Density 20/20	pH	Total acid. g/L	Volatile acid g/L	Ethanol g/L	Gluconic acid g/L	Glycerin g/L
1,0845 ± 0,0	2,8 ± 0,05	11,65 ± 0,05	5,3 ± 0,0	0,1 ± 0,0	1,45 ± 0,05	1,4 ± 0,1

The must was separated into three treatments of three replicates of 200 ml each. A treatment without additions and another treatment with 60 mg/L of SO_2 added with Erbslöh's potassium bisulfite solution (Solution sulfureuse P15) in the must were used as controls to contrast the efficacy in deactivating the laccaseenzymatic activity with the phenolic treatments to which a dose of 2 ml/L of the phenolic concentrate was added.

Laccase activity measurements was carried out using the syringaldazine chromogenic substrate, according to the method described by Dubourdieu et al. [15], with the "Botrytest Kit", from the Laffort company. The musts to be tested were introduced into syringes containing PVPP for the removal of phenols, and 1 ml of must was collected in test tubes, to which 1.4 ml of buffer solution (sodium acetate 0.1 M, pH 5) and then 0.6 ml of "Botrytestlaccase" reagent (syringaldazine solution) was added. The tubes were mixed by shaking, and after three minutes, the laccase activity was determined by comparing the color developed with those of the colorimetric scale. Laccase activity was tested just after grape pressing, 24 hours after grape pressing, and also at the end of alcoholic fermentation. All analyses were performed in triplicate.

Table 2. General analysis of wines after alcoholic fermentation. All the data are the mean ±SD of three replicates. The letters represent the significant differences between the different treatments.

	Control	SO2	Phenols	Cont	SO2	Phen
Density 20/20	0,9944 ± 0,00	0,9944 ± 0,00	0,9944 ± 0,00	"a"	"a"	"a"
Alcohol g/L	93,80 ± 0,10	93,70 ± 0,10	95,90 ± 0,00	"a"	"a"	"b"
Extract g/L	24,90 ± 0,00	24,90 ± 0,00	25,70 ± 0,00	"a"	"a"	"b"
sugar-free extract g/L	24,10 ± 0,00	24,33 ± 0,15	24,80 ± 0,10	"a"	"a"	"b"
Fermentable sugar g/L	0,80 ± 0,00	0,60 ± 0,10	0,90 ± 0,10	"ab"	"a"	"b"
Glucose g/L	0,03 ± 0,06	0,00 ± 0,00	0,10 ± 0,00	"ab"	"a"	"b"
Fructose g/L	0,80 ± 0,00	0,60 ± 0,06	0,77 ± 0,10	"b"	"a"	"ab"
Total acidity g/L	9,90 ± 0,00	10,30 ± 0,00	10,30 ± 0,00	"a"	"b"	"b"
pH	2,80 ± 0,00	2,80 ± 0,00	2,80 ± 0,01	"a"	"a"	"a"
Tartaric acid g/L	4,60 ± 0,00	4,50 ± 0,00	4,90 ± 0,00	"b"	"a"	"c"
Malic acid g/L	3,50 ± 0,00	3,87 ± 0,06	3,70 ± 0,00	"a"	"c"	"b"
Lactic acid g/L	0,30 ± 0,00	0,30 ± 0,00	0,20 ± 0,00	"b"	"b"	"a"
Volatile acidity g/L	0,70 ± 0,01	0,70 ± 0,01	0,80 ± 0,01	"a"	"a"	"b"
Glycerin g/L	7,20 ± 0,00	6,60 ± 0,00	7,17 ± 0,06	"b"	"a"	"b"
Free So2 mg/L	0,00 ± 0,00	20,00 ± 0,58	0,00 ± 0,00	"a"	"b"	"a"
Total So2 mg/L	19,67 ± 0,58	102,00 ± 1,15	18,00 ± 0,00	"a"	"b"	"a"
Total phenol mg/L	151 ± 0,00	233 ± 1,00	274 ± 0,00	"a"	"b"	"c"

2.2 Color evolution

Vinifications were carried out to monitor the color evolution of musts affected by BBR. Upon arrival at the winery, at a temperature of 23°C, the grapes were destemmed and pressed, and the must was placed in a 600 L capacity accumulator tank for homogenization and then divided into three treatments, with three replicates of 50 L each. The must was then cold-clarified for 24 hours in a cold room at 8°C and subsequently inoculated with Oenoferm® Freddo yeast, fromErbslöh, for all treatments at a dose of 15 gr/hL.

The objective was to measure the efficacy of SO_2 and contrast it with the treatments enriched with phenols, having a control without additions, to monitor the evolution of color and organoleptic properties.

The control treatments were left without additions throughout the process. To the SO_2 treatment, 60 mg/L of SO_2 was added with Erbslöh's potassium bisulfite solution (Solution sulfureuse P15) in the must, and another 50 mg/L SO_2 once the alcoholic fermentation was finished and it was corrected to 30 mg/L of free SO_2 before bottling. Phenolic concentrates were supplied by the company Biolethics Europe, being added at doses of 2 ml/L in the must, 3 ml/L after alcoholic fermentation, and 1 ml/L before final filtration. Finally, before bottling, all the treatments were pre-filtered and filtered with K100 and EK depth filtration sheets, respectively, from the Pall company.

Once the fermentations were completed, the impact of BBT on the color of the wine was determined by measuring the absorbance at 420 nm (yellow) in the final wines and later after eight months in the bottle. Finally, the total color difference (ΔEab*) between samples was obtained using the CIELAB coordinates and was calculated using the following equation [16]:

$$\Delta Eab* = \sqrt{(L2* - L1*)^2 + (a2* - a1*)^2 + (b2* - b1*)^2} \quad (1)$$

The ΔEab* represents a measure of the difference between two colors. ΔEab* is used to determine whether the human eye can visually detect the difference between two samples. Generally, it is considered that the difference is visible to the human eye when ΔEab*>3 [16], and values above one reflect a difference that is noticeable for a trained observer [17].

The ΔEab* values were subjected to two-way ANOVA, one-way ANOVA, and Tukey's Honestly Significant Difference (HSD) for multiple comparisons (at $\alpha = 0.05$).

2.3 Antioxidant control

In this assay, two different formulations of plant-derived phenolic (A and B) were tested at two different doses (1 ml/L and 2 ml/L), which were supplemented by the Dutch company Biolethics Europe. Phenol-enriched treatments were contrasted with SO_2 treatments to which 50 mg/L of SO_2 was added through a 5% potassium bisulfite solution. The total concentration of phenolic substances was recorded through the Folin-Ciocalteu index.

The different formulations (A and B) subtly differ in the concentrations of the phenolic components in their preparation, and their difference is based on their use, formulation A being intended for white wines while formulation B is intended for red wines.

A pre-test was performed on model wine without additions, taken as a control to check the viability of the test conditions and the sealing of the bottles. The results verified the conditions by showing a flat linear behavior without consumption or an increase in oxygen (data not present in this report).

The OCR of phenol concentrates extracted from plants was measured using a non-invasive method based on luminescence, using the experimental design previously described by Navarro et al. [18] and Pascal et al. [11]. A model wine solution composed of ethanol (12% v/v) and tartaric acid (4 g/L) adjusted to pH = 3.5 with sodium hydroxide was used. In addition, this solution was enriched with 3 mg iron/L in the form of iron (III) chloride hexahydrate and 0.3 mg copper/L in the form of copper (II) sulfate pentahydrate. This model solution was used to prevent the natural phenols present in wines from competing for oxygen consumption in the tested treatments, as indicated by Pascal et al. [11].

The different doses of phenolic concentrates and potassium metabisulfite were placed in 220 ml clear glass bottles, which contained a pill (PreSens Precision Sensing GmbH, lot number: 210223-001) for non-invasive measurement of dissolved oxygen by luminescence (Nomasense TM O_2 Trace Oxygen Analyzer from PreSens Precision Sensing GmbH). The model wine solution was adjusted to a temperature of 20 ± 1 °C and saturated with oxygen with an air diffuser for ten minutes. The bottles were filled entirely with the model wine solution and immediately closed with a crown cap to minimize the headspace volume. The bottles were gently shaken to dissolve the phenolic concentrates and the SO_2 solution. Throughout the measurements, the bottles were kept at a constant temperature of 20 ± 0.5°C, submerged in a temperature-controlled water bath. The oxygen consumption rate was determined by periodically monitoring the oxygen levels in the bottles. All treatments were performed in triplicate.

2.4 Statistical analysis

All data are expressed as mean values ± standard deviation (SD). Statistical analyzes were performed using the statistical program R with the R Commander package. The two hypotheses of normality and homoscedasticity of the data were tested, for all parameters, using the Shapiro-Wilk test and the Levene test, respectively. When the populations were normally distributed and presented homogeneity in variance, parametric tests (ANOVA and Tukey) were used to detect significant differences at $p < 0.05$. When populations were not normally distributed or had heterogeneity in variance, nonparametric tests (Kruskal-Wallis and pairwise-Wilcox) were used. Differences were considered statistically significant at $p < 0.05$. The graphs were developed with the SigmaPlot program in its version 11.

3 Results and Discussion

3.1 Laccase activity measurement

Figure 1 shows the units of residual laccase activity in botrytized grape juice, without additions (control), with 60 mg/L of SO_2 and supplemented with 2ml/L of plant phenolic concentrates. The initial laccase activity of the grape must was 7.33 ± 1.15 unit/Laccase/ml, as observed in the control must, while the initial mean of the SO_2 treatment was 0.33 ± 0.58 unit/Laccase/ml, and the treatment with plant phenols it was 0.33 ± 0.58 unit/Laccase/ml. At 24 hours, the control must present values of 6.67 ± 1.15 unit/Laccase/ml, the treatment with average SO_2 was 0.0 ± 0.0 unit/Laccase/ml, and the treatment with plant phenols was 0.33 ± 0.58 unit/Laccase/ml.

Significant differences were recorded between the control treatments versus SO_2 and the phenolic concentrates, while there were no significant differences between the SO_2 treatments and the phenolic concentrates.

The supplementation with plant phenols caused a significant decrease in residual laccase activity compared to the control, showing results comparable to treatments with SO_2. These data confirm thatplant phenols significantly inhibited laccase activity at the dose of

2 ml/L, and it can be a viable alternative to avoid unwanted effects in wine production, avoiding or reducing the use of sulfur in musts affected by BBR.

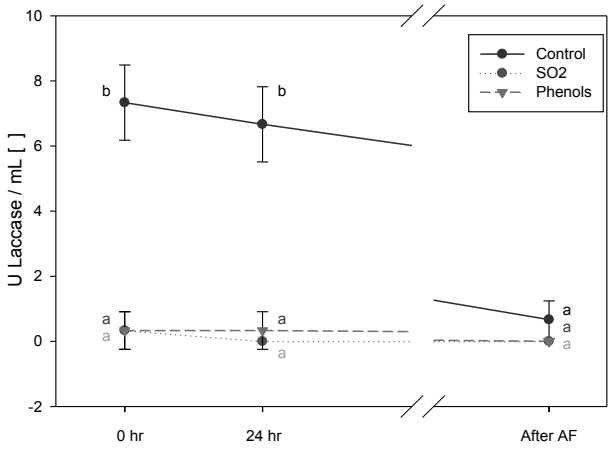

Figure 1. Inhibition effect of plant phenols on laccase activity, at 0 and 24 hours, and after alcoholic fermentation (AF). All the data are the mean ±SD of three replicates. The letters represent the significant differences between the different treatments.

3.2 Color evolution

The intensity changes of the yellow color at 420nm absorbance (A420) in the white musts after 24 hours are shown in Figue 2, where significant differences between all treatments can be seen. As expected, the A420 of the control was significantly higher when contracted with the SO_2 treatments and the phenolic concentrates. A clear browning of the must is appreciated, with values of 1.289 ± 0.003 A420 nm. The treatment with phenols showed the lowest values in musts with 0.629 ± 0.003, while the treatments with SO_2 obtained values of 0.784 ± 0.002.

At the end of the alcoholic fermentation, the evolution of the yellow component A420 was compared, which is shown in Figue 2. As can be seen, there were no significant differences between the treatments, which can be explained by the precipitation of oxidized phenols in the control treatments. The A420 values were 0.222 ± 0.007 for the control treatments, 0.185 ± 0.045 for the SO_2 treatments and 0.204 ± 0.010 for the phenol-enriched treatments.

After eight months, all treatments showed significant differences between them, as shown in Figure 3. The SO_2 treatments presented the lowest values; the treatments enriched with phenols showed intermediate values, while the control treatments showed the highest values, as was expected. The A420 values for control treatments were 0.186 ± 0.002, for the SO_2 treatments 0.173 ± 0.001 and those for the phenol-enriched treatments were 0.097 ± 0.004.

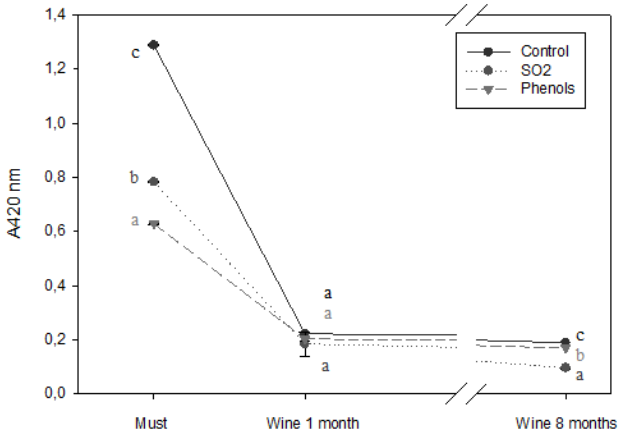

Figure 2. Influence of supplementation with phenol concentrates on changes in the yellow color component (absorbance at 420 nm) of must and wines made with Riesling grape juice affected by BBR. All the data are the mean ±SD of three replicates. The letters represent the significant differences between the different treatments.

Figure 3 shows the total color differences obtained in botrytized Riesling wines. In must, all comparisons showed significant differences between them. When comparing the control treatments with those enriched with phenols, an average ΔEab* of 26.24 ± 0.15 was obtained. The comparison between control treatments with SO_2 showed values of ΔEab*19.30 ± 0.09, while the comparison between treatments enriched with phenols and SO_2 showed values closer to each other, of ΔEab* 7.00 ± 0.12. All ΔEab* values in musts were higher than three, so the human eye notices substantial color differences between these treatments.

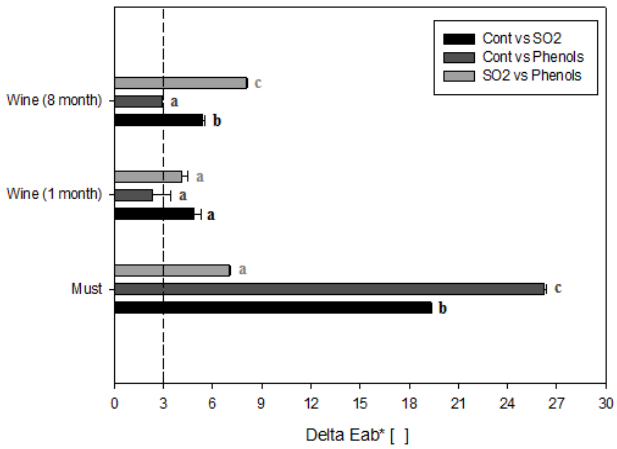

Figure 3. Impact of plant phenols added in botrytized must and wines on color visible to the human eye. All the data are the mean ±SD of three replicates. The letters represent the significant differences between the treatments.

The comparison of ΔEab* values in wines one month after the end of alcoholic fermentation showed values of 2.09 ± 1.46 when comparing the control treatments with those enriched with phenols, 4.85 ± 0.44 between control treatments and those with SO_2, and 4.15 ± 0.38 between the treatments with SO_2 and those enriched with phenols. Statistically, no significant differences were detected between the treatments.

Finally, at eight months, all treatments showed significant differences between them. However, the differences between the control treatments and those enriched with phenols are at the edge of the detection limit between them, with ΔEab* values of 2.91 ± 0.06. The differences between control treatments versus the SO_2 showed ΔEab* values of 5.38 ± 0.18, while the ΔEab* between the SO_2 and phenol-enriched treatments was 8.05 ± 0.08.

The results would indicate that the control treatments, although they present significant differences, are similar to those enriched with phenols after eight months in thebottle, this could be explained by the fact that over time the oxidized phenols could have a tendency to brown, and therefore the color evolution would be similar to the controls over time.

3.3 Antioxidant control

A test was performed in model wine, without any additions, taken as a control to check the viability of the test conditions and the seal of the bottles. The results verified the conditions by showing a flat linear behavior without consumption or an increase in oxygen (data not present in this report).

In the first trial with the formulation of phenols A, the model wine solution was saturated with 8.2 ± 0.08 mg/L of oxygen with an air diffuser for ten minutes. Table 3 show basic analyzes performed on the treatments at the beginning of the experiment to check the levels of phenolic concentrates (measured in total phenols) and total and free SO_2 levels.

Table 3. General analysis model wine solution, essay with phenolic concentrates A. All the data are the mean ± SD of three replicates. The letters represent the significant differences between the different treatments.

Treatment	Alcohol g/L	pH	Free So2 mg/L	Total So2 mg/L	Total phenols mg/L
Phenols A 1mL	93,3 ± 0,1 "a"	3,5 ± 0,0 "a"	4 ± 0,0 "a"	4 ± 1,2 "a"	30 ± 0,0
Phenols A 2mL	94,4 ± 0,1 "b"	3,5 ± 0,0 "a"	4 ± 0,0 "a"	3 ± 0,0 "a"	50 ± 0,0
SO2	93,1 ± 0,1 "a"	3,5 ± 0,0 "a"	44 ± 0,0 "b"	61 ± 0,0 "b"	<10

Figure 4 shows the kinetics of OCR, appreciating that the treatments with SO_2 gradually decrease through the first five days, while the treatments with phenols show an important consumption during the first 24 hours, to then stabilize and continue to decrease gradually. As expected, the doses of phenolic concentrates have an influence on OCR, with the highest dose of 2 ml/L showing the highest OCR, followed by the dose of 1 ml/L, showing greater efficiency at higher doses.

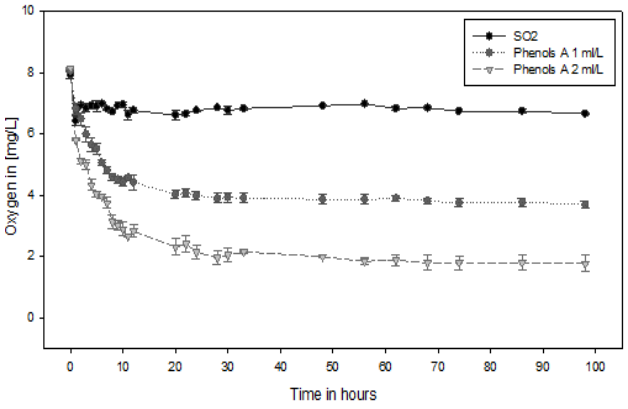

Figure 4. Kinetics of oxygen consumption for formulation A of phenolic concentrates at two different concentrations and treatments with SO_2 as control.

The repetitions with concentrations of 1 ml/L stabilize the OCR after the first 24 hours at values close to 4 mg/L of O_2, while the treatments with phenolic concentrations of 2 ml/L, after the first 24 hours, stabilize the OCR at values close to 2 mg/L of O_2, showing greater efficiency. These stabilization values will serve as a reference to be compared with the phenolic treatments treated with the phenolic formulation B.

As seen in Table 4, all treatments showed significant differences in OCR from the beginning of the essay. These differences correspond to the hour-by-hour evaluation of the different treatments.

Table 4. Summary of OCR per hour, according to different SO_2 treatments and formulation A of phenolic concentrates at two different concentrations. All data are the mean ± SD of three replicates. The letters represent the significant differences between the different treatments.

	SO2			Phenols 1ml			Phenols 2ml		
hr	O2 mg/L	OCR/hr		O2 mg/L	OCR/hr		O2 mg/L	OCR/hr	
0	7,94 ± 0,14	-	a	8,05 ± 0,03	-	b	8,14 ± 0,01	-	b
1	6,43 ± 0,16	1,51 ± 0,11	b	6,81 ± 0,19	1,24 ± 0,19	c	5,80 ± 0,04	2,34 ± 0,04	a
2	6,91 ± 0,01	-0,52 ± 0,16	c	6,48 ± 0,19	0,33 ± 0,03	b	5,11 ± 0,07	0,69 ± 0,07	a
3	6,84 ± 0,14	0,10 ± 0,14	c	5,99 ± 0,01	0,50 ± 0,05	b	5,00 ± 0,16	0,11 ± 0,09	a
4	6,91 ± 0,03	-0,07 ± 0,14	c	5,64 ± 0,22	0,34 ± 0,07	b	4,32 ± 0,18	0,68 ± 0,18	a
5	6,90 ± 0,19	0,02 ± 0,18	c	5,51 ± 0,19	0,13 ± 0,05	b	4,02 ± 0,10	0,30 ± 0,16	a
6	6,99 ± 0,03	-0,09 ± 0,16	c	5,06 ± 0,07	0,45 ± 0,19	b	3,96 ± 0,06	0,06 ± 0,06	a
7	6,80 ± 0,02	0,18 ± 0,04	c	4,80 ± 0,11	0,25 ± 0,09	b	3,74 ± 0,18	0,22 ± 0,22	a
8	6,72 ± 0,04	0,08 ± 0,05	c	4,60 ± 0,12	0,21 ± 0,03	b	3,12 ± 0,22	0,62 ± 0,23	a
9	6,93 ± 0,04	-0,02 ± 0,04	c	4,51 ± 0,13	0,09 ± 0,02	b	3,01 ± 0,16	0,11 ± 0,21	a
10	6,95 ± 0,07	-0,05 ± 0,08	c	4,42 ± 0,13	0,09 ± 0,10	b	2,89 ± 0,22	0,13 ± 0,32	a
11	6,63 ± 0,16	0,32 ± 0,12	c	4,69 ± 0,17	-0,27 ± 0,18	b	2,66 ± 0,02	0,23 ± 0,23	a
12	6,76 ± 0,10	-0,13 ± 0,08	c	4,41 ± 0,25	0,28 ± 0,08	b	2,82 ± 0,20	-0,16 ± 0,21	a
20	6,61 ± 0,17	0,15 ± 0,17	c	4,02 ± 0,13	0,39 ± 0,22	b	2,30 ± 0,26	0,52 ± 0,25	a
22	6,65 ± 0,05	-0,04 ± 0,12	c	4,06 ± 0,13	-0,04 ± 0,10	b	2,40 ± 0,26	-0,10 ± 0,06	a
24	6,76 ± 0,02	-0,11 ± 0,04	c	3,99 ± 0,14	0,08 ± 0,11	b	2,13 ± 0,26	0,27 ± 0,13	a
28	6,85 ± 0,06	-0,09 ± 0,05	c	3,90 ± 0,16	0,09 ± 0,07	b	2,00 ± 0,21	0,18 ± 0,02	a
30	6,77 ± 0,11	0,08 ± 0,06	c	3,92 ± 0,15	-0,02 ± 0,04	b	2,04 ± 0,21	-0,09 ± 0,13	a
33	6,83 ± 0,04	-0,06 ± 0,07	c	3,90 ± 0,16	0,02 ± 0,03	b	2,15 ± 0,06	-0,10 ± 0,26	a
48	6,92 ± 0,03	-0,09 ± 0,02	c	3,86 ± 0,16	0,04 ± 0,02	b	2,00 ± 0,04	0,15 ± 0,08	a
56	6,98 ± 0,03	-0,06 ± 0,01	c	3,86 ± 0,16	0,00 ± 0,01	b	1,84 ± 0,11	0,15 ± 0,08	a
62	6,83 ± 0,07	0,15 ± 0,05	c	3,89 ± 0,11	-0,04 ± 0,09	b	1,87 ± 0,18	-0,04 ± 0,07	a
68	6,85 ± 0,03	-0,02 ± 0,04	c	3,81 ± 0,07	0,08 ± 0,09	b	1,79 ± 0,24	0,09 ± 0,08	a
74	6,74 ± 0,04	0,11 ± 0,02	c	3,75 ± 0,12	0,06 ± 0,06	b	1,79 ± 0,23	-0,01 ± 0,02	a
86	6,75 ± 0,05	-0,01 ± 0,04	c	3,77 ± 0,13	-0,02 ± 0,01	b	1,79 ± 0,24	0,01 ± 0,03	a
98	6,66 ± 0,03	0,09 ± 0,02	c	3,68 ± 0,12	0,08 ± 0,03	b	1,76 ± 0,27	0,03 ± 0,27	a

Figure 5 shows the average daily OCR. As can be seen, significant oxygen consumption was recorded by the phenolic concentrates during the first 24 hours, to then stabilize until the end of the test, while the treatments with SO_2 show a slight, gradual, and stable decrease throughout the entire experiment.

Table 5. Oxygen consumption rate per hour. Total average values consider all recorded values (not indicated in the table). All data are the mean ±SD of three replicates.

	OCR/hr (mg/L O2)		
Time	**SO2**	**Phen 1ml**	**Phen 2ml**
First 12 hr	0,08 ± 0,44	0,30 ± 0,36	0,44 ± 0,65
First 24 hr	0,04 ± 0,43	0,27 ± 0,34	0,40 ± 0,60
First 48 hr	0,05 ± 0,40	0,22 ± 0,32	0,32 ± 0,56
Total Avarage	**0,05 ± 0,35**	**0,17 ± 0,29**	**0,26 ± 0,51**

For the second essay, with phenol formulation B, the model wine solution was saturated with 8.05 ± 0.06 mg/L oxygen with an air diffuser for ten minutes. Table 6 show basic analyzes performed on the treatments at the beginning of the experiment to check the levels of phenolic concentrates (measured in total phenols) and total and free SO_2 levels.

Table 6. General analysis model wine solution, essay with phenolic concentrates B. All the data are the mean ± SD of three replicates. The letters represent the significant differences between the different treatments.

Treatment	Alcohol g/L	pH	Free So2 mg/L	Total So2 mg/L	Total phenols mg/L
Phenols B 1mL	88,7 ± 0,0 "a"	3,5 ± 0,0 "a"	4 ± 0,0 "a"	5 ± 1,0 "a"	30 ± 0,0
Phenols B 2mL	90,2 ± 3,6 "a"	3,5 ± 0,0 "a"	4 ± 0,0 "a"	4 ± 0,6 "a"	50 ± 0,0
SO2	93,1 ± 0,1 "a"	3,5 ± 0,0 "a"	44 ± 0,0 "b"	61 ± 0,0 "b"	<10

As observed in Figure 5, the formulation of phenolic concentrates B, at doses of 1 ml and 2 ml, presented the same pattern of OCR kinetics as formulation A, seen previously. The treatments enriched with phenols showed a substantial oxygen consumption during the first 24 hours, to then stabilize during the rest of the trial. The treatments with SO_2, as in the previous case, showed a slow and progressive OCR during the experiment.

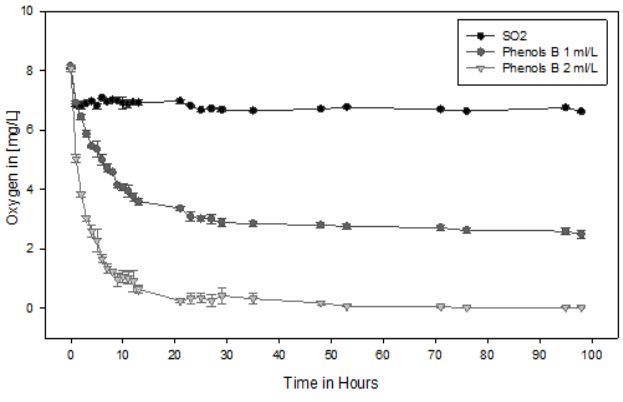

Figure 5. Kinetics of oxygen consumption for formulation A of phenolic concentrates at two different concentrations and treatments with SO_2 as control.

As in the essay with phenolic concentrates A, significant consumption of oxygen by phenolic concentrates B is observed during the first 24 hours to stabilize until the end of the test, while the SO_2 treatments showed a gradual and stable decrease throughout the experiment. Table 7 shows the OCR records for phenolic concentrates B.

Table 7. Summary of OCR per hour, according to different SO_2 treatments and formulation B of phenolic concentrates at two different concentrations. All data are the mean ±SD of three replicates. The letters represent the significant differences between the different treatments.

	SO2			Phenols 1ml			Phenols 2ml		
hr	O2 mg/L	OCR/hr		O2 mg/L	OCR/hr		O2 mg/L	OCR/hr	
0	8,11 ± 0,07	-	a	8,16 ± 0,04	-	a	8,07 ± 0,11	-	a
1	6,86 ± 0,05	1,26 ± 0,12	b	6,93 ± 0,04	1,23 ± 0,04	b	5,04 ± 0,13	3,03 ± 0,11	a
2	6,84 ± 0,13	0,02 ± 0,10	c	6,45 ± 0,10	0,47 ± 0,08	b	3,84 ± 0,10	1,20 ± 0,15	a
3	6,91 ± 0,03	-0,07 ± 0,15	c	5,88 ± 0,11	0,57 ± 0,07	b	3,03 ± 0,09	0,81 ± 0,02	a
4	6,96 ± 0,03	-0,05 ± 0,03	c	5,46 ± 0,05	0,42 ± 0,13	b	2,60 ± 0,19	0,43 ± 0,28	a
5	6,82 ± 0,09	0,14 ± 0,08	c	5,37 ± 0,27	0,22 ± 0,09	b	2,29 ± 0,36	0,31 ± 0,53	a
6	7,08 ± 0,01	-0,26 ± 0,09	c	6,00 ± 0,17	0,37 ± 0,13	b	1,66 ± 0,14	0,63 ± 0,35	a
7	6,96 ± 0,05	0,12 ± 0,06	c	4,70 ± 0,15	0,26 ± 0,19	b	1,35 ± 0,15	0,31 ± 0,07	a
8	7,01 ± 0,03	-0,05 ± 0,03	c	4,59 ± 0,03	0,15 ± 0,14	b	1,25 ± 0,07	0,10 ± 0,18	a
9	6,99 ± 0,04	0,02 ± 0,02	c	4,14 ± 0,07	0,45 ± 0,09	b	0,98 ± 0,22	0,27 ± 0,23	a
10	6,92 ± 0,21	0,07 ± 0,17	c	4,08 ± 0,13	0,05 ± 0,19	b	1,07 ± 0,18	-0,09 ± 0,19	a
11	6,84 ± 0,14	0,03 ± 0,08	c	3,94 ± 0,18	0,14 ± 0,09	b	1,02 ± 0,20	0,05 ± 0,36	a
12	6,94 ± 0,04	-0,04 ± 0,12	c	3,77 ± 0,13	0,18 ± 0,08	b	0,91 ± 0,20	0,11 ± 0,52	a
13	6,93 ± 0,09	0,00 ± 0,10	c	3,61 ± 0,12	0,16 ± 0,23	b	0,64 ± 0,13	0,27 ± 0,23	a
20	6,98 ± 0,07	-0,05 ± 0,08	c	3,38 ± 0,07	0,24 ± 0,18	b	0,26 ± 0,05	0,38 ± 0,17	a
24	6,82 ± 0,03	0,16 ± 0,05	c	3,08 ± 0,15	0,24 ± 0,09	b	0,35 ± 0,18	-0,09 ± 0,23	a
29	6,69 ± 0,07	0,03 ± 0,06	c	2,80 ± 0,14	0,11 ± 0,05	b	0,43 ± 0,27	-0,18 ± 0,45	a
35	6,66 ± 0,02	0,03 ± 0,09	c	2,85 ± 0,10	0,57 ± 0,08	b	0,32 ± 0,19	0,10 ± 0,24	a
48	6,71 ± 0,07	-0,06 ± 0,05	c	2,81 ± 0,09	0,05 ± 0,02	b	0,19 ± 0,06	0,14 ± 0,13	a
53	6,78 ± 0,02	-0,06 ± 0,03	c	2,76 ± 0,08	0,04 ± 0,01	b	0,07 ± 0,03	0,12 ± 0,06	a
71	6,69 ± 0,04	0,08 ± 0,04	c	2,71 ± 0,10	0,05 ± 0,04	b	0,06 ± 0,05	0,01 ± 0,05	a
76	6,64 ± 0,03	0,05 ± 0,07	c	2,62 ± 0,10	0,09 ± 0,02	b	0,02 ± 0,01	0,03 ± 0,04	a
95	6,75 ± 0,02	0,13 ± 0,01	c	2,60 ± 0,12	0,02 ± 0,02	b	0,04 ± 0,01	-0,02 ± 0,02	a
98	6,63 ± 0,04	0,13 ± 0,05	c	2,49 ± 0,12	0,11 ± 0,07	b	0,04 ± 0,01	0,00 ± 0,01	a

The stabilization values for phenolic concentrates B correspond to values close to 3 mg/L of O_2 for treatments with concentrations of 1 ml/L of phenols, and close to 0 mg/L of O_2 for treatments with concentrations of 2 ml/L of phenols, after 24 hours. This would indicate that concentrates B have higher antioxidant power than concentrates A, which have lower stabilization values than concentrates B.

4 Conclusion

It can be concluded that plant phenolic concentrates effectively inhibit laccase activity in Riesling musts affected by Botrytis. No differences in effectiveness were observed compared to the use of SO_2 at a dose of 60 mg/L, all treatments achieving an inhibition in very short periods of time. Supplementation with phenolic concentrates would mitigate the negative effect of oxidations caused by laccase. However, the evolution of color should be considered in the long term, and future sensory analyzes should complement the evaluation of the antioxidant protective effects of phenolic concentrates.

Regarding the antioxidant power evaluated for two different formulations of plant phenolic concentrates, it can be concluded that the tested formulations indeed have a high antioxidant level which was reflected in the kinetics of oxygen consumption. Of the two formulations tested, formulation B showed greater antioxidant power than formulation A, consuming oxygen to levels close to zero during the first day of the trial (at the highest concentrations tested).

The use of phenolic concentrates shows to be an interesting alternative in the effort to replace or reduce

the use of sulfites in wine production. This study should be complemented with sensory evaluations and monitoring of the evolution of wines produced with plant-derived phenolic concentrates to corroborate their properties.

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