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A Bioprotection Strategy of Musts to Limit Sulfiting Process During Winemaking

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SHORT COMMUNICATION

Abstract
In the wine industry, sulfur dioxide (SO_2) has an important role, as an antioxidant, and antiseptic additive. Lately, manufacturers of products specific to wine production technology are developing biological protocols for treating musts and wines using different types of yeasts. The purpose of the research is to study the impact of replacing SO_2 in the first phase of the winemaking process with biological methods on the flavonoids, polyphenols, and antioxidant potential in Fetească neagră wine. The red wine from Fetească neagră, 2021 harvest - Dealu Mare Vineyard was made through two methods: treated with SO_2 6% (25 mg/L) and treated with Primaflora VR Bio (7 g/hL). The IC50 value for the variant treated with Primaflora VR BIO was 722 mg/mL and the variant treated with SO_2 recorded a 724 mg/ml, the differences being non-significant. Primaflora VR Bio is an effective and promising alternative in terms of the quality of oenological and biochemical parameters, and a basic variant for ecological, biological wines with a low sulfite content.

Keywords: Fetească neagră, SO_2, polyphenols, Primaflora VR Bio, wine

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INTRODUCTION
Bioprotection in the wine sector is a strategy for protecting grape musts that have been used for a few years now. In the wine industry, sulfur dioxide (SO_2) has an important role, as an antioxidant, and antiseptic additive (Santos et al., 2023). SO_2 is also responsible for different allergic reactions, and for this reason, it is of great interest for its replacement with alternative additives or technologies (Puyo et al., 2023). Bioprotection is intended to be a partial or total alternative to the use of sulfites. Lately, manufacturers of products specific to wine production technology are developing biological protocols for treating musts and wines using different types of yeasts. The principle of bioprotection consists in providing, from the harvest, on the grapes or on the grape must, yeast biomass, which, by its action, will limit the development of the native microbial flora and consequently avoid microbiological alterations at the early stages of the winemaking process. Most often, the biomasses studied are selected strains of non-*Saccharomyces* such as *Torulaspora delbrueckii* or *Metschnikowia pulcherrima*, but the *Saccharomyces cerevisiae* species can also be used (Hervé et al., 2023). Numerous field trials have already demonstrated the effectiveness of bioprotection in oenological conditions

(Windholtz et al., 2021). However, there is still little information on mechanisms that bioprotection uses to limit the development of indigenous flora. In literature, numerous interactions have been identified in the oenological context (Zilelidou and Nisiotou, 2021), but none have been studied in the bioprotection context, leaving many possibilities unexplored. In the European Community, Commission Regulation (EC) No 934/2019 formalizes the limits laid down by the International Organization of Vine and Wine (OIV). Currently, the maximum levels are, respectively 150 mg/L for red wine, 100 mg/L for organic red wine and 70 mg/L for certain biodynamic certifications. Finally, for wines produced without any added sulfites, the limit for total SO_2 has been established at 10 mg/L, taking into account the small amount of this compound produced by yeast during alcoholic fermentation (OIV, 2023). Moreover, the cost of bioprotection is still high today, compared with the cost of sulfiting. In fact, bioprotection yeast preparations cost more than active dry yeasts (ADYs), starters of alcoholic fermentation. The development of production and stabilization strategies better adapted for these NS yeasts should make it possible to reduce the cost of the bioprotection strategy in the future.

The purpose of the research was to study the impact of replacing SO_2 in the first phase of the winemaking process with biological methods on the content of flavonoids and total polyphenols, and on the antioxidant potential of Fetească neagră ecological wine

MATERIALS AND METHODS

The red wine from Fetească neagră, 2021 harvest – SERVE Winery, Ceptura Winegrowing Center – Dealu Mare Vineyard was obtained by two variants: treated with PrimaFlor VR Bio (7 g/hl) from Oenolia – AEB France FN PF VR VI (after destemming) and treated with SO_2 6% (25 mg/L) from Milfachim FN SO2 VII (after destemming) Figure 1. For both variants, Zymaflore Xpure – selected non-Saccharomyces yeast was used, according to the producer indication.

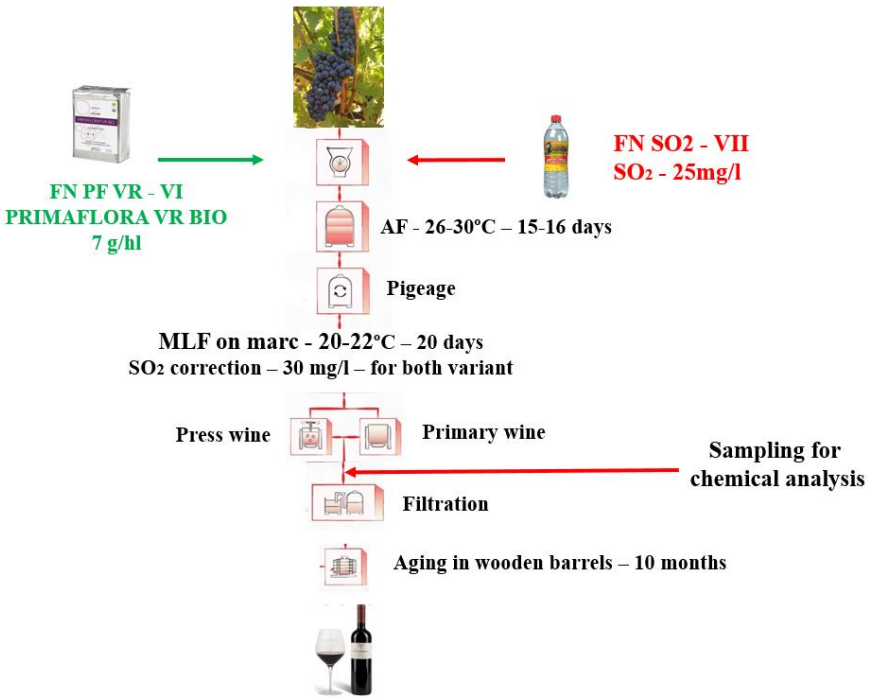

Figure 1. Technological scheme for experimental variants
(AF – alcoholoc fermentation; MLF – malolactic fermentation)

Primaflora VR BIO is a complex biological formulation which aims to provide microbiological protection for red and rosé musts. It contains Saccharomyces and non-Saccharomyces yeast strains, *Metschnikowia pulcherrima* (selected in collaboration with the University Institute of Vine and Wine (IUVV) University of Burgundy). Research methods consisted of must and oenological parameters. The Ripper method, a type of iodometric titration, is used to determine the content of free, combined, and total sulfur dioxide (SO_2) in the sample (Bora et al., 2018; OIV, 2023). The total flavonoid content was quantified by a colorimetric assay according to Kim et al., 2003. The results are expressed as milligrams of gallic acid equivalents (GAE) per liter of wine (mg GAE/L). The Folin-Ciocâlteu method is employed for total polyphenol content as it measures the reducing capacity of polyphenols in the sample against a molybdenum compound in the reagent. The results are expressed as milligrams of gallic acid equivalents per liter of sample (mg GAE/L). Antioxidant activity method utilizes the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical

scavenging assay**.** The DPPH assay is a common method for measuring antioxidant activity. It involves preparing a DPPH solution in methanol (typically with an absorbance around 0.973 at 517 nm). To perform the assay, a test tube containing 3 mL of this DPPH solution is mixed with 100 µL (microliters) of the leaf extract being analyzed. A control sample containing only DPPH solution is often prepared alongside the test samples. After incubating the mixtures in darkness for 30 minutes, the absorbance is measured at 517 nm. A specific formula is then used to calculate the percentage of antioxidants or free radical scavenging activity (RSA) based on the absorbance change.

$$\% \text{ of antioxidant activity} = [(Ac \times As) \times Ac] \times 100,$$

where: Ac—Control reaction absorbance; As—Testing specimen absorbance.

By monitoring the change in the optical density (absorbance) of DPPH radicals, the antioxidant potential of the sample is evaluated (Filimon et al., 2016; Tartian et al., 2017; Filimon et al., 2023).

Statistical analysis was performed by Excell Program. ANOVA (one-way analysis of variance) and Tukey test were used to compare differences between samples, when $p < 0.05$, it was regarded as significant.

RESULTS AND DISCUSSIONS

Based on the results shown in Table 1, grape harvest of Fetească neagră was suitable to obtain a wine with a high alcohol degree, usually for Dealu Mare Vineyard climatic conditions. As it may be seen in Table 1, the sugar content in grapes at harvest was 244 g/Lm a total acidity of 4.2 g/L sulphuric acid and a oH of 3.27. The alcohol content was of 14.1 (% v/v) for FN VR VI and of 14.3 (% v/v) for FN SO_2 VII Table 2.

Table 1. Basic parameters of grape must of Fetească neagră grapes on harvest date – October 13rd, 2021

Sugar (g/L)	Total acidity (g/L sulphuric acid)	pH
244	4.2	3.27

In Table 2 are present the result regarding oenological parameters of both experimental variants. Concerning sulfite concentrations in wines, the SO_2 concentrations (free and total) were different between conditions, with higher valueas for variant II – 35 mg/L free SO2 and, 59 mg/L total SO_2, respectively. Our results could be compared with those obtained by Puyo *et al*., 2023, for rose wines with higher values for both SO_2 free and total, for BPS variant (bioprotection and SO_2 addition) – 27.25 mg/L total SO_2 and 6.70 mg/L free SO_2 compared with BP (bioprotection) – 22.58 mg/L total SO_2 and 7.00 mg/L free SO_2.

Table 2. The physico-chemical analysis results

Variants	Alcohol content (% v./v.)	Total flavonoids mg GAE/L	Total polyphenols mg GAE/ L	Antioxidant activity IC 50 mg/L	Free SO_2 mg/L	Total SO_2 mg/L
FN PFVR (V I)	14.1±0.1 a	367±12.1 a	1498±96.2 a	722±56.1 a	20±1.3 b	38±1.2 b
FN SO2 (V II)	14.3±0.2 a	345±10.2 b	1501±87.3 a	724±76.2 a	35±1.6 a	59±1.3 a

Note: All concentrations are listed with average value and standard deviation. Different letters are significantly different between the samples ($p < 0.05$); GAE stands for (gallic acid equivalent)

The analyses of wines after MLF demonstrated that the addition of the bio-protection strain or sulphites during pre-fermentation stage did not impact the oenological parameters at the end of MLF, same result being recorded also by the Simonin et al., 2020 for Pinot noir wines using the bio-protection strain of *M. pulcherrima* MCR 24 (AEB group– France), the *S. cerevisiae* strain Levulia PNR (AEB group –France) and the *Oenococcus oeni* strain VP41 (Lallemand –France). Regarding flavonoids content, it is observed that FN PFVR VI accumulated a total of flavonoids of 367 mg GAE/L, with +3% more, compared to the FN SO_2 VII - 345 mg GAE/L. Variant FN PFVR V I accumulated less total polyphenols 1498 mg GAE/L, compared to FN SO2 VII - 1501 mg GAE/L, the differences being insignificant Table 2. The activity and antioxidant potential of wine is correlated with the content in total polyphenols (Tekos et al., 2023). The FN PFVR VI variant obtained 722 mg/mL and the variant FN SO2 VII recorded an antioxidant activity of 724 mg/mL, the differences being insignificant. Some studies by Yao et al., 2023 shows that the phenolic and anthocyanins have significant difference between bioprotected and others types of protection for Rara neagră

wines, with values of phenolic substance and total anthocyannins of 1312 mg/L and 492 mg/L, respectively for bioprotected wine, and of 994 mg/L and 189 mg/L, respectively for other wines. In our study, chemical analyses showed similar concentrations of polyphenols in both conditions. These results indicated that the total polyphenols concentration in wines was not significantly influenced by bioprotection, similar results being obtaning also by Puyo et al., 2023.

CONCLUSIONS

The bioprotection of musts and grapes represents a strategy for limiting sulfite addition during winemaking. This experiment carried out with the constraints of winemaking in the cellar led to a preliminary conclusion that the action of bioprotection could be an alternative to sulfites in pre-fermentative steps. Primaflora VR Bio represents an effective and promising alternative in terms of the quality of oenological and biochemical parameters and a basic variant for ecological, biological wines with a low sulfite content.

Author Contributions: S.I.B., C.I.B. and A.B. Conceived and designed the analysis; A.C.B. Collected the data; D.D. Contributed data and analysis tools; A.B. and F.D.B. Performed the analysis; A.C. Wrote the paper.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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