ABSTRACT

Keywords: Fetească Regală, Cabernet Sauvignon, sulfur dioxide, amino acids, biogenic amines, anthocyanins, radical scavenging activity, volatile compounds

The purpose of the study "CONTRIBUTIONS TO THE DIVERSIFICATION OF WINE VARIETIES WITHOUT ADDED SULFITES FROM THE PANCIU VINEYARD" is the production, monitoring of evolution, and quality of Cabernet Sauvignon and Fetească regală wines without added sulfur dioxide, vinified in various technological variants specific to the type of wine produced. In this context, the adaptation of four technological variants covering the most important categories from the perspective of interest and technological and scientific updates in the field of viticulture and oenology was pursued.

The key points of the study are focused on the main stages of winemaking adapted to the substitution of sulfur dioxide by using non-*Saccharomyces* species such as *Pichia Kluyveri* in the initial stage of grape treatment, as well as perfecting antioxidant protection methods with the use of oenological products that have proven to be as effective in protection as SO_2.

Monitoring was carried out in a longitudinal study with transversal stages where complementary data were collected from different samples (transversal), and then the results were followed over time (longitudinal). Finally, representative samples were subjected to quality and performance evaluations.

Optimizing winemaking procedures

It is essential to note that for sulfite-free wines, the treatment of grapes against oxidation consisted of using *Pichia kluyveri*, while for wines with sulfur dioxide, the treatment consisted of using potassium metabisulfite, ascorbic acid, and gallic tannin.

An important stage was antioxidant protection and preservation. Antioxidant protection was carried out in two different steps. For wines with added sulfur dioxide, after the completion of fermentation, antioxidant protection was mainly achieved with the addition of ascorbic acid at doses of 20 mg/L and free sulfur dioxide solutions equivalent to 40 mg/L free SO_2. All wines required acidity adjustments with lactic acid at doses equivalent to 6.1-6.3 g tartaric acid/L.

Other stages applied in winemaking methods were tartaric stabilization using carboxymethylcellulose, arabic gum, or potassium polyaspartate, and clarification was performed using bentonite, colloidal silica, or fish glue, with doses according to quality technical specifications, but optimized for each type of wine.

The correlation between determined amino acids and biogenic amines under conditions of additional malolactic fermentation aimed to evaluate the specific biogenic amines in wines in relation to the corresponding amino acids by determining direct correlations during the wine maturation period.

Red wines (Cabernet Sauvignon) had significantly higher levels of biogenic amines at (523 µmol/L) in total content, followed by rosé wines Cabernet Sauvignon Rosé (452 µmol/L), Fetească Regală (386.5 µmol/L), and finally Fetească Regală frizzante (302.3 µmol/L).

Decarboxylation is the main source of biogenic amine production under the activity of decarboxylase enzymes. Glutamine and arginine are involved in the production of putrescine and spermidine through decarboxylation/ deamination mechanisms.

Clarifying agents used, such as bentonite and gelatin, affected the amino acid levels in wine to different extents. Chelation with components of the clarifying agents was confirmed, showing significant variations in comparative values between raw and conditioned wines.

As a conclusion of the study, an explanation of the correlations is that pH influences decarboxylase enzymes through catalytic activation. The pH levels of the wines ranging between 3.0 and 4.0 were not favorable.

From previous data, the absence of SO_2 did not influence the differential stability of the samples, so a classification of the distribution from the perspective of malolactic and alcoholic fermentation was performed using principal component analysis (PCA).

Evaluation of anthocyanin levels in the context of wines vinified using methods that do not involve the use of SO_2.

The objective of this study is to evaluate the chemical substances and sensory impact of two commercial non-Saccharomyces yeast products used for bio protection.

The pH was important for the stability of the wines and was maintained between 3.1 and 3.3 throughout the winemaking process. During the monitoring period, a change in pH occurred due to the variation in organic acids, likely caused by the precipitation of tartaric acid, considered unstable.

The impact of antioxidant substances was verified according to variations during the maturation of the wine. In the case of Cabernet Sauvignon, color change was more intense than in Cabernet Sauvignon Rosé.

For the rosé samples with added glutathione precursors and gallic tannin, notable implications include the decrease in petunidine ($r=-0.60$), but there was a positive influence on the flavone content ($r=0.74$, $p<0.05$). Glutathione precursors had directly proportional effects between delphinine and total polyphenol content ($r=0.60$) and flavones ($r=0.90$).

Linear discriminant analysis determined the differentiation between rosé and red wines based on the maceration period (red versus rosé) and the inclusion of additional malolactic fermentation in rosé wines.

Monitoring the evolution of changes in antioxidant state parameters

The effect of SO_2 on wine color was not considered exhaustive, mainly focusing on its well-known role in anthocyanin discoloration. Thus, the aim of this study was to evaluate the wine color based on the presence of SO_2 throughout the winemaking process and during maturation. In addition to CIELab analyses, spectrophotometric measurements of color intensity (CI) were also conducted. All samples were measured in triplicate for these analyses.

The evaluation of the overall color hues of the wines was performed using CIELab Color Space software, which incorporates combinations of three values: L* for perceptual lightness and chromaticity a* and b* for the four unique human vision colors: red, green, blue, and yellow.

Cluster diagrams created with Color distance software provided an objective comparative tool for color analysis. Each cluster diagram highlighted the hue differences between wines considering the presence of SO_2 and the evolution during the monitoring period. The color distance score was calculated and evaluated for each type of wine.

Different hue difference values were observed for each variety, confirmed by multivariate tests. Possible oxidation reactions might explain color changes in white wines towards a darker shade.

Evaluation of antioxidant capacity evolution trends

The antioxidant properties of phenolic compounds in wines are evaluated by their capacity to inhibit stable organic radicals. This assessment is based on the wine's or hydro alcoholic solutions ability to neutralize free radicals.

For each dilution tested, the reaction kinetics were indicated through reaction curves. The plateau concentration was calculated based on a linear response-plateau model function.

Multifactorial analysis using wine type - treatment ($+SO_2$ and $-SO_2$) and variety (CS, CSR, FR, FRF) as comparison indicators revealed that, for Cabernet Sauvignon (CS) and Cabernet Sauvignon Rosé (CSR) samples, there was no significant difference between the two types of wine treatments.

Regarding antioxidant capacity, considering levels of phenolic compounds, flavones, and tannins, the mean levels of the studied compounds grouped by $+SO_2$/$-SO_2$ criteria revealed no significant differences, even when considering the monitoring period and stability of these compounds in various wine types.

There were significant differences within each variety depending on the type of wine and antioxidant treatments. These treatments showed similarities in the mixtures introduced, through tannins specific to each wine type (CS, CSR, FR, or FRF). Correlation coefficients were similar between the two technologies, with comparable variations. For IFC/flavones, correlation coefficients were r=0.80 ($-SO_2$) and r=0.88 ($+SO_2$). A similar relationship occurred for IFC/tannins (r=0.95) ($-SO_2$), which was maintained for $+SO_2$ samples (r=0.98).

Thus, we can conclude that the antioxidant activity of wines is more related to the type of individual phenolic compounds found in wines rather than the total phenol content.

Effect of added sulfur dioxide levels on the aromatic characteristics of Panciu vineyard wines

The study utilized various alcohols, carbonyl compounds, and esters: acetaldehyde, methanol, butan-1-ol, butan-2-ol, propan-1-ol, 2-methyl-propan-1-ol, 2-methyl-pentan-2-ol, pentan-1-ol, isoamyl acetate, and ethyl lactate.

The presence of similar concentrations of acetaldehyde in both sulfite-treated and sulfite-free samples confirmed the absence of excess free carbonyl compounds, which did not inhibit yeast fermentation.

To explain the positive evolution of acetaldehyde, the self-oxidation of ethyl alcohol, which can be catalyzed by the oxidation of ascorbic acid to dehydroascorbic acid in wines treated with ascorbic acid, was considered.

Ethyl lactate (ETL) had varying concentration values depending on the included varieties. Higher values compared to other esters can be explained by malolactic fermentation in sulfite-free and red wines, with acidity correction achieved using lactic acid.

Notably, methanol development exceeded tolerance limits to 130 mg/L and 5 mg/L, respectively, potentially having a slightly negative impact on olfactory properties.

By correlating these aspects in principal component analysis, multiple groups were highlighted based on similarities in the distribution of involved variables.

Visual descriptors tracked variations indicating potential changes in appearance due to oxidation processes. Olfactory parameters were selected to define sensory typicity, classified into primary, secondary, and tertiary aroma categories. Gustatory parameters evaluated sweetness, astringency, bitterness, persistence, and corresponding aroma intensity. The significance of values obtained for different sensory parameters was tested using ANOVA analysis. Secondary technology considered malolactic fermentation, dry winemaking, and the use of tannins with antioxidant characteristics.

In color evaluation, it was observed that sulfite-free samples had higher hue values anticipating color changes characteristic of oxidation processes.

Sulfite-treated wine samples had stronger fruit notes across all varieties with added sulfites (Cabernet Sauvignon rosé), (Fetească regală), and (Fetească regală Frizzante). Although floral note values were lower, they were not statistically

significant compared to sulfite-free samples. Results indicated that tannin-treated samples had slightly higher evaluations than SO_2-treated samples.

Sensory evaluation indicated that the timing and method of malolactic fermentation inoculation (MLB) significantly affected the taste and aroma of wines. Co-inoculated wines had higher levels of fruity, fresh, and floral notes than wines using sequential malolactic fermentation. The spontaneous process was perceived as producing wines with more buttery and bitter aroma.