

ORIGINAL RESEARCH PAPER

IMPROVING THE ROSÉ WINES QUALITY THROUGH THE SAIGNÉE
TECHNIQUE

BOGDAN BOCĂNEALĂ¹, OANA EMILIA CONSTANTIN¹, CONSTANTIN CROITORU²,
IULIANA APRODU¹, NICOLETA STĂNCIUC¹, CLAUDIA MUREȘAN³, GABRIELA
RÂPEANU^{*1}

¹Faculty of Food Science and Engineering, Dunarea de Jos University of Galati, 111 Domnească Street, 800201,
Galati, Romania

²Academy of Agricultural and Forestry Sciences, 61 Marasti Blvd, Bucharest, Romania

³Faculty of Food Engineering, Tourism and Environmental Protection, Aurel Vlaicu University of Arad, 2 Elena
Dragoi Street, 310330, Arad, Romania

*Corresponding author: Gabriela.Rapeanu@ugal.ro

Received on 15 March 2025

Revised on 29 April 2025

Abstract

The saignée technique involves starting the vinification of black grapes using red wine technology, mentioning that, after a short period, a part of the must is vinified according to the rosé technology (white vinification). The technique was meant to differentiate it from other winemaking methods, highlighting the varietal characteristics of the grape varieties and the climate and soil. The study's objective was to test the Saignée technique for the most representative native grape variety, Fetească Neagră, in the pedoclimatic and cultural conditions of the southern wine region of Romania, the Danube Terraces Region. Both wines - obtained by direct pressing and by the Saignée technique - may withstand potential long-term aging, according to the results of the physicochemical studies, which also showed higher stability and age potential, total and volatile acidity, pH, and free and total SO₂ levels. In terms of color parameters, rosé wines showed a mixed behavior, reflecting characteristics typical of both red and white wine evolution. Sensory analysis showed that Saignée wine was usually better enjoyed, scoring higher in several categories: smell, taste, aroma, aftertaste, roundness, full-bodied, and general appreciation. The traditional rosé wine was valued for its beauty and freshness.

Keywords: rosé wine, saignée technique, grapes

Introduction

Rosé wines represent a category of wines gaining more importance on the market. In their case, they have significantly changed basic assumptions over the last few years. Although in the past rosé wines were regarded as low-quality wines, obtained

from grapes that had either not reached maturity or did not meet the desired level of quality for red winemaking (*e.g.* due to defects or diseases), today they are often considered to be wines on the same quality level as white and red wines. During the winemaking procedure, they are given even more attention than red wines. New pressing technologies have been devised to preserve the grapes' flavors and those derived during fermentation, prevent color oxidation, and allow vinification as quickly as possible (Baiano *et al.*, 2009; Baiano *et al.*, 2016). In their case, freshness is the most demanded characteristic.

The main challenge comes from balancing a high and relatively constant quality during the different years of grape production and highlighting as much as possible the climatic particularities, soil, and human component of the region from which the wines come, aspects included in *terroir* (Gonzaga *et al.*, 2022; Petriashvili *et al.*, 2023; Gaitán *et al.*, 2023; Bohnert and Martin, 2023). These are also important elements of differentiation in the market, the marketing component being increasingly considered in the construction of wine at the concept level, in a continuous exchange of information between producers and the market.

The old rosé wine producers are France (Languedoc-Roussillon – the largest rosé wine-producing region in the country, Provence, the Rhône Valley and the Loire Valley, Bordeaux, with wines made mainly from the Cabernet Sauvignon, Merlot, Cinsault, Mourvèdre, Malbec, Pinot Noir varieties, a special role being played by the Syrah and Grenache varieties), Spain (Tempranillo variety being known in the Rioja region) and Italy (Sangiovese variety) (Magrini *et al.*, 2016; Lereboullet *et al.*, 2013; Gaitán *et al.*, 2023). The new rosé wine producers are located in Australia and New Zealand; winemakers competed to offer expressiveness and uniqueness to these wines (Buican *et al.*, 2023). Probably the most representative style that has stood out throughout the world is that of the Provence style, which has as characteristics a very light, pale color, with a tint of pink or very light salmon pink, and which has fresh and fruity notes, having an increased acidity that gives these wines a characteristic freshness. Although these styles clearly show the positive evolution of the qualities of rosé wines and the possibilities for constant improvement, there are still consumers that consider them too simple compared to red wines, all the more so as there is a tendency to reduce the extraction of color and implicitly of some flavor components with less contact of the must with the skins. Customers from the more conventional segments can thus consider they're not expressive enough, particularly when compared with red wines that benefit from extended maceration periods (Wang and Spence, 2019; Mora *et al.*, 2021; Gonzaga *et al.*, 2022; Petriashvili *et al.*, 2023).

In this context, one of the techniques designed to differentiate itself from other winemaking styles on the market while highlighting the varietal characteristics of the grape varieties used and the soil climate, respectively, is the *saignée* technique, also known as *bleeding*. This involves starting the vinification of red grapes using red wine technology (destemming, respectively crushing, of the grapes and placing the crushed grapes in a vessel for maceration for 30 to 90 minutes). One part of the must (approx. 30%) is extracted and transferred to another vessel (bleeding-off), which is processed according to rosé technology (white winemaking). The *Saignée*

process may increase wine color and phenolic component extraction, improving the wine color stability and intensity (Wu *et al.*, 2017).

The Saignée technique has traditionally been applied with the main purpose of enhancing red wines, the rosé fraction resulting from the bleeding process being often treated as a secondary product with limited enological value. In this study, the focus shifts toward evaluating this rosé fraction as a primary product.

The objective of the present study was to evaluate the application of the Saignée technique as a enological approach, which could serve as a winemaking protocol for the obtaining of balanced rosé wines in terms of sugar/alcohol content, acidity, and pH wines with aromatic complexity and typicality, rich in polyphenols and which also meet the current requirements of consumers (light, fresh, easy to drink and easy to associate with gastronomic dishes). This approach also responds to sustainability and cost-efficiency considerations, by valorizing a rosé fraction traditionally regarded as a by-product, increasing overall yield through the simultaneous production of rosé and red wines from the same grape batch, and also minimizing the use of additional oenological inputs — all contributing to a more economically viable process while maintaining, or even enhancing, the organoleptic quality of the wines.

Materials and methods

Grapes

The grapes were *Vitis vinifera* subsp. *vinifera*, var. *Fetească Neagră* belonging to the harvest year 2022, from Danube Terraces region, Romania. Several microsamples were carried out to determine the optimum moment of harvesting for the winemaking process. Starting from a sugar level of 220 g/L, a series of microvinification trials were conducted to assess grape maturity and select the most suitable harvest point. The grapes were ultimately harvested at 240 g/L sugars with an acidity of 7-7.5 g/L tartaric acid, and a pH of 3.1-3.2 were harvested.

Chemicals

The experimental reagents and solvents utilized in this investigation were obtained from Sigma Aldrich (Germany).

Winemaking process

The grapes with 240 g/L sugars were sorted manually on a sorting belt (Bucher Vaslin Delta TBE, France) and after were destemmed (Boucher Vaslin Delta E, France) and crushed (Bucher Vaslin Delta F, France). The crushed grapes were sulphited with 100 g potassium metabisulfite/tonne of grapes. The crushed grapes were separated into two fractions. The first fraction used for preparing the P₁ sample was transferred for pressing to a pneumatic press (PREXA N, Puleo S.p.A., Italy), and the must was then transferred to a 7500 L capacity tank, where it was permanently under a protective nitrogen atmosphere to avoid any oxidation. The second fraction used for preparing the P₂ samples was placed in a roto maceration tank, where the must was in contact with the skins and crushed pulp for 90 minutes at a temperature of 4-5°C. After 90 minutes, a fraction of the must (15%) was

transferred to a 7500 L capacity tank (P₂). Both tanks were set to a temperature of 1-2°C to prevent the spontaneous alcoholic fermentation, and an enzymatic preparation with pectolytic activity (LAFAZYM™ 600 XL ICE, LAFFORT®, France) was added (1.25 mL/hL) to promote the must clarification.

When the musts reached a 50-100 NTU turbidity, they were transferred to other 7500 L tanks equipped with cooling jackets, set at 16-18°C. Both were inoculated with yeast cultures of *Saccharomyces cerevisiae* (ZYMAFLORE™ X5, LAFFORT®, France). When the musts reached a density of 1050 kg/m³, a dose of 20 g/hL bentonite (MICROCOL™ ALPHA, LAFFORT®, France) was added to promote the clarification of the young wines. When the fermentation stopped (for two days, there were no changes in the liquid density), the wines were transferred to two 5000L tanks where a dose of 50 g/hL of potassium metabisulfite was added.

Subsequently, for 3 weeks, the technological operation of battonage was carried out for both wines (P₁ and P₂), an operation by which, by stirring, the wine and the fine yeasts deposited on the bottom of the tanks are back in contact. After another week, a new dose of bentonite (50g/hL) was added to each tank, stored for 5 days, and then separated in another 5000L tank equipped with a cooling jacket and nitrogen protective atmosphere. Finally, the wines were filtered using a Cardboard plate filter (Cardboard plate press-filter KAPPA, Spadoni Meccanica SRL, Italy) and K200 (3.5-6 µm), K100 (1.25-3.25 µm), and EK (sterilizing plates – 0.4-0.6 µm) filter plates (Seitz® K Series Depth Filter Sheets, Pall Corporation, U.S.A.) made from cellulose, kieselgur and perlite. Just before the last filtration - sterilizing- the sulfur correction was performed using liquid SO₂ with 18% concentration (BISULFITE 18, LAFFORT®, France) to reach a free SO₂ concentration of 50 mg/L. The wines were then bottled in 0.75L glass bottles and stored for 12 months (P₁' and P₂') at a constant temperature of 15±0.5°C, at a relative humidity of max. 70%, in the dark. At the same time, a layer of wax was applied over the corks to prevent it from drying out and the transfer of moisture between the wine and the environment.

Chemical characterization of wines

The physicochemical determinations (alcohol content, residual sugar, pH, total and volatile acidity, free and total sulfur dioxide, and turbidity) were carried out according to OIV methods—Compendium of International Methods of Analysis of Musts and Wines (OIV-MA-INT-00-2020).

Determination of the color parameters of wines

Color intensity (CI) and hue were determined using the method developed and described by Glories (1984). The method is based on spectrophotometric determinations through readings at 420, 520, and 620 nm wavelengths. Other analyses were performed to determine the percentages of monomeric, polymeric, and copigmented pigments, and the total color was determined according to the method described by Mazza *et al.* (1999) by reading at 520 nm. Spectrophotometric determinations were performed by a Libra S22 UV-VIS spectrophotometer (Biochrom, Cambridge, UK). To determine the total color (A_{acet}), a volume of 20 µl of acetic aldehyde of 20% concentration (v/v) was added to 2 mL of wine; the sample

was left to rest for 45 minutes at room temperature before its absorbance was read. Acetaldehyde reacts with anthocyanins in wine, generating more intensely colored pigments and making it easier to measure color, making them more visible.

To determine the percentage of polymerized pigments (A^{SO_2}), 160 μ L of sulfur dioxide of concentration 6% (m/v) was added over 2 mL of wine; the absorbance was measured after 10 minutes. Sulfur dioxide is known for its ability to discolor monomeric pigments but does not affect polymerized pigments. This determination thus allows the differentiation of the polymerized pigments from the other color compounds in wine.

At the same time, the absorbance of the samples was read at 700 nm to eliminate the interference caused by turbidity. The wine samples were not previously diluted. A^{wine} represents the absorbance measured at 520 nm, used as a reference in the calculation of copigmented and monomeric anthocyanin fractions.

The color parameters were calculated as follows:

$$\text{Color intensity (CI)} = [(A_{420} - A_{700}) + (A_{520} - A_{700}) + (A_{620} - A_{700})] \quad (1)$$

$$\text{Tint} = [(A_{420} - A_{700}) / (A_{520} - A_{700})] \quad (2)$$

$$\begin{aligned} \text{\% copigmented pigment} = \\ [(A^{acet} - A_{700}) - (A^{wine} - A_{700}) \times 3] \times 100 / (A^{acet} - A_{700}) \end{aligned} \quad (3)$$

$$\begin{aligned} \text{\% monomeric anthocyanins} = \\ [(A^{wine} - A_{700}) \times 3 - (A^{SO_2} - A_{700})] \times 100 / (A^{acet} - A_{700}) \end{aligned} \quad (4)$$

$$\text{\% polymeric anthocyanins} = (A^{SO_2} - A_{700}) \times 100 / (A^{acet} - A_{700}) \quad (5)$$

$$\text{Total color} = A^{acet} - A_{700} \quad (6)$$

Color Determinations according to the CIELab method were performed with a Minolta Chromameter CR 410 (Konica Minolta, Osaka, Japan), where the following parameters were measured and then calculated: L^* , a^* , b^* , $c = \sqrt{a^2 + b^2}$, $h = \arctan(\frac{b}{a})$, and $\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$

The percentage of each color component (yellow, red, and violet) was calculated from the absorbance values corrected for turbidity by subtracting the absorbance at 700 nm, according to the method described by Glories (1984), as follows:

$$\text{\% Yellow} = (A_{420} - A_{700}) / CI \times 100 \quad (7)$$

$$\text{\% Red} = (A_{520} - A_{700}) / CI \times 100 \quad (8)$$

$$\text{\% Violet} = (A_{620} - A_{700}) / CI \times 100 \quad (9)$$

Sensory analysis

For hedonic analysis, a panel of 24 tasters, all trained and certified as professional tasters, had to taste, evaluate, and score each wine sample, using a scale from 1 (extremely unpleasant) to 9 (extremely pleasant) for several descriptors. The tasters were either from certain sectors of the wine industry (production, marketing, sales, distribution, HoReCa, etc.) or simply consumers passionate about wine, which

practically represented the final market. The analyzed descriptors were: appearance (visual component: color, clarity, brightness, etc.), smell (olfactory characteristics), taste (palate), aroma (the combination of smell and taste, aromas being perceived retronasally), aftertaste (“the end” of the wine), roundness, mouthfeel, freshness and global appreciation of the wine.

Statistical analysis

The results are presented as average values for the determinations made in duplicate, with the standard deviation indicated $\pm$. Statistical analysis was performed using Minitab software for Windows, version 22.1. To compare the differences between the mean values, the analysis of variance test (ANOVA) was used. The Tukey test was applied in cases where significant variations were observed, with a 95% confidence interval.

Results and discussion

Chemical composition of the wines

The results from the physicochemical analysis of the wine samples are presented in Table 1.

For freshly bottled wines, the alcoholic degree showed values of $13.36 \pm 0.24\%$ (v/v) for the P₁ sample (rosé wine obtained by direct pressing) and $13.28 \pm 0.18\%$ (v/v) for the P₂ sample (rosé wine obtained by the Saignée process). These values are considered acceptable, given the climatic and technological aspects involved and consumer expectations and related perceptions (Casassa *et al.*, 2013; Mora *et al.*, 2021; Petriashvili *et al.*, 2023).

Table 1. Chemical characterization of the wine samples produced through direct pressing and Saignée procedure, immediately after obtaining (P₁ and P₂, respectively) and after 1 years of storage (P₁' and P₂').

Samples	P ₁	P ₂	P ₁ '	P ₂ '
Alcoholic degree (% v/v)	13.36 ± 0.24^{aA}	13.28 ± 0.18^{aA}	13.36 ± 0.08^{aA}	13.27 ± 0.12^{aA}
Sugars (g/L)	4.52 ± 0.03^{aA}	4.78 ± 0.02^{bB}	4.44 ± 0.06^{aA}	4.72 ± 0.02^{bB}
pH	3.24 ± 0.01^{aA}	3.25 ± 0.01^{aA}	3.24 ± 0.01^{aA}	3.28 ± 0.02^{aA}
Total acidity (g/L tartaric acid)	6.34 ± 0.09^{aA}	6.00 ± 0.06^{aB}	6.28 ± 0.13^{aA}	5.89 ± 0.24^{bB}
Volatile aciditate (g/L acetic acid)	0.21 ± 0.02^{aA}	0.37 ± 0.03^{bB}	0.24 ± 0.01^{aA}	0.39 ± 0.03^{bB}
Free SO ₂ (mg/L)	50.4 ± 0.1^{aA}	47.3 ± 0.2^{aB}	48.9 ± 0.1^{bA}	46.1 ± 0.3^{bB}
Total SO ₂ (mg/L)	117.2 ± 0.3^{aA}	121.0 ± 0.9^{aB}	115.6 ± 0.5^{aA}	120.3 ± 1.2^{aB}
Turbidity (NTU)	0.50 ± 0.02^{aA}	0.66 ± 0.01^{aB}	0.52 ± 0.02^{aA}	0.70 ± 0.03^{aB}

In a row, lowercase superscript letters indicate significant differences among mean values corresponding to wine samples produced with different procedures at the same time, while different capital letters indicate differences among values corresponding to wines produced with the same procedure, at different moments of time, at $p < 0.05$.

The alcoholic concentrations were close to those reported by Medina-Plaza et al. (2024) for a rosé wine Grenache variety ($12.89 \pm 0.02\%$, v/v) from the region of Yolo County, California, the harvest year 2019 and those of a wine obtained from Pinot Noir ($14.20 \pm 0.03\%$, v/v) harvested from the region of Sonoma County, California, same year of harvest (Medina-Plaza et al., 2024)— This reflects a trend that has been observed since 2019, toward rosé wines with increasingly higher alcohol levels, often exceeding 14% v/v.

After one year of bottling, the values did not change significantly for both samples: $P_1' = 13.36 \pm 0.08\%$ v/v, and $P_2' = 13.27 \pm 0.12\%$, v/v ($p > 0.05$). This is not unusual, as the wines were bottled immediately, thus limiting the access to oxygen that could have led to possible acetic fermentation. The results align with others reported by Bai et al. (2023), Benucci (2020) and Lan et al. (2021).

The sugar content after bottling was 4.52 ± 0.03 g/L for P_1 , and 4.78 ± 0.02 g/L for P_2 . Correlating these values with those of acidity according to present legislation, the wines are included in the category of dry wines. No significant changes were found for P_1' and P_2' samples after one year of bottling ($p > 0.05$). The pH values for the two samples (3.24 ± 0.01 for P_1 ; 3.25 ± 0.01 for P_2) were perfectly normal, showing an optimal time for grape harvesting and proper development of technological processes (Benucci, 2020; Lan et al., 2021; Vicente et al., 2022; Medina-Plaza et al., 2024).

The total acidity of the wines showed values of 6.34 ± 0.09 and 6.00 ± 0.06 , respectively, expressed in g/L of tartaric acid. They indicate optimal harvesting at the right time before the accelerated decrease in grape acidity occurs (Casassa et al., 2013; Asproudi et al., 2018; Vicente et al., 2022; Cui et al., 2024). Values of 5.70 ± 0.08 g/L, 6.14 ± 0.03 g/L, and 6.415 ± 0.04 g/L were determined in similar studies on rosé wines of the Grenache, Pinot Noir, and Zinfandel varieties (Primitivo in it.) at the time of bottling (Medina-Plaza et al., 2024). Also, for the application of the Saignée process combined with prolonged maceration, values of 5.30 ± 0.18 g/L, 5.30 ± 0.13 g/L, and 5.75 ± 0.05 g/L for three Pinot Noir clones (Casassa et al., 2021) were reported. No significant changes were found for total acidity for P_1' and P_2' samples. The same evolution was found for volatile acidity with no significant changes after one year of bottling. This is probably due to the way of bottling (in 750 mL glass bottles, using nitrogen, corked, and waxed) (Chidi et al., 2018; Ubeda et al., 2022; Mazarrón and Cañas, 2023; Xi et al., 2024).

A low decrease in free and total SO_2 content was measured after one year of bottling ($p < 0.05$). The turbidity measured for samples P_1 and P_2 , was 0.50 ± 0.02 NTU and 0.66 ± 0.01 NTU, respectively, specific to clear wines. Considering the turbidity values of 0.52 ± 0.02 NTU and 0.70 ± 0.03 NTU measured for P_1' and P_2' , respectively after one year of storage, one can conclude that the wines were relatively stable.

Color parameters determination

The color index results, as shown in Figure 1, indicate that during aging, color intensity decreased while hue increased in both types of rosé wine (classic and Saignée). Simultaneously, the Saignée technique allowed obtaining wines with

higher color intensity values than the direct pressing technique and lower tint values in winemaking methods. This suggests a more substantial contribution of red pigments in the P2 sample as a result of maceration (Babincev *et al.*, 2016; Casassa *et al.*, 2013; Casassa *et al.*, 2021; Cheng and Watrelot, 2022; Gil *et al.*, 2019; Sacchi *et al.* 2005; Lukić *et al.*, 2017; Magrini *et al.*, 2016; Pantani *et al.* 2014), compared to the yellow ones, the latter playing a more important role in the case of the P₁ sample.

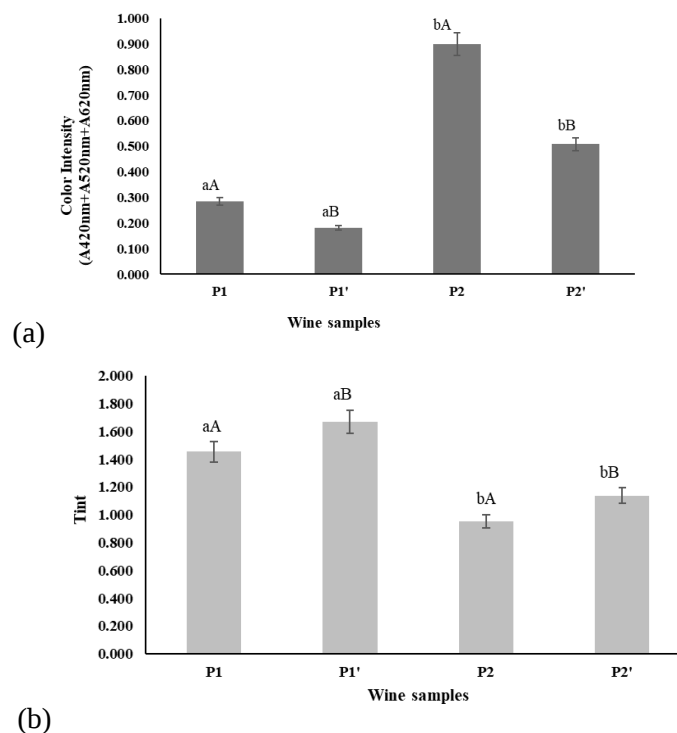


Figure 1. Color intensity (a) and tint (b) of wine samples at the time of bottling and after 12 months of storage.

On top of the bars, lowercase superscript letters indicate significant differences among mean values corresponding to wine samples produced with different procedures at the same time, while different capital letters indicate differences among values corresponding to wines produced with the same procedure, at different moments of time, at $p < 0.05$.

The evolution of these characteristics, the decrease in color intensity, and the increase in tint values indicate a normal evolution, also found in other studies in the literature (Stávek *et al.*, 2012; Babincev *et al.*, 2016; Benucci, 2020; de Oliveira *et al.*, 2024).

The percentages of yellow, red, and purple pigments that contribute to the total color of the 4 wine samples are presented in Figure 2. The data are correlated with those from the color intensity and tint. The yellow hue is more significant in the P₁ sample

(direct pressing) than in the P₂ sample (Saignée). In contrast to the P₁ sample, the P₂ sample exhibits a higher level of red contribution to the overall color composition, which is simultaneously visually more pronounced. This is a result of the technological process, as the flavonoids (flavones) responsible for the yellow color are present in both the skins and seeds, with the pulp being particularly abundant. The pulp is readily incorporated into the must after pressing, eliminating the need for prolonged and intensive maceration stages required for extraction. On the contrary, red pigments, represented by anthocyanins and anthocyanidins, are mainly located in grape skins, requiring technological steps to favor their extraction and solubilization in must and wine, respectively (Sacchi *et al.*, 2005; Stávek *et al.*, 2012; Casassa *et al.*, 2013; Garrido and Borges, 2013; Pantani *et al.*, 2014; Magrini *et al.*, 2016; Lukić *et al.*, 2017; Casassa *et al.*, 2021; Cheng and Watrelot, 2022).

Therefore, the increased proportion of red color in the P₂ sample is attributable to the bleeding procedure, which is defined by a brief maceration period during which the must was in contact with the skins of the crushed grapes. This process resulted in the partial release of the color components from the vacuoles of the cells in the skin into the must. Some of them also passed the P₁ sample spontaneously during the pressing operation, depending on its intensity and the chosen technological option, leading, together with the crushing operation, to the mechanical denaturation of the cells in the composition of the grape skins, but to a lesser extent in the absence of a maceration (Sacchi *et al.*, 2005; Casassa *et al.*, 2013; Garrido and Borges, 2013; Pantani *et al.*, 2014; Babincev *et al.*, 2016; Lukić *et al.*, 2017; Casassa *et al.*, 2021; Cheng and Watrelot, 2022).

The percentage of violet color is low in both samples (with higher values in the case of P₂ due to maceration), which is explained by the fact that it is generally correlated with extended maceration and pre-maceration operations, accompanied by fermentation on the barrel or even other processes, the most significant results being found in the application of thermal treatments or experimental ultrasonic treatments (Sacchi *et al.*, 2005; Casassa *et al.*, 2013; Garrido and Borges, 2013; Ghanem, 2014; Pantani *et al.*, 2014; Babincev *et al.*, 2016; Magrini *et al.*, 2016; Lukić *et al.*, 2017; Casassa *et al.*, 2021; Cheng and Watrelot, 2022; Gutiérrez *et al.*, 2023). The purple component is less frequently significant in rosé wines, although it is acknowledged that even a brief maceration, such as the Saignée procedure, increased its value.

In the case of the rosé wine sample processed by direct pressing (P₁), a higher decrease in the percentage of violet color is observed, from 5.26% to 3.30% after 12 months of storage. As mentioned before, the change in the color of rosé wines is a known aspect of practice and is a phenomenon often recorded. The wine thus goes from the various shades of pink, which can vary in diversity and intensity depending on the variety or varieties used, the conditions of the harvest year, and various technological aspects (e.g., rose, intense pink, candy pink, pale pink, etc.) to shades of yellow or orange (the color of onion peel, the color of the peach, the color of the salmon, etc.) and even brown when oxidation occurs.

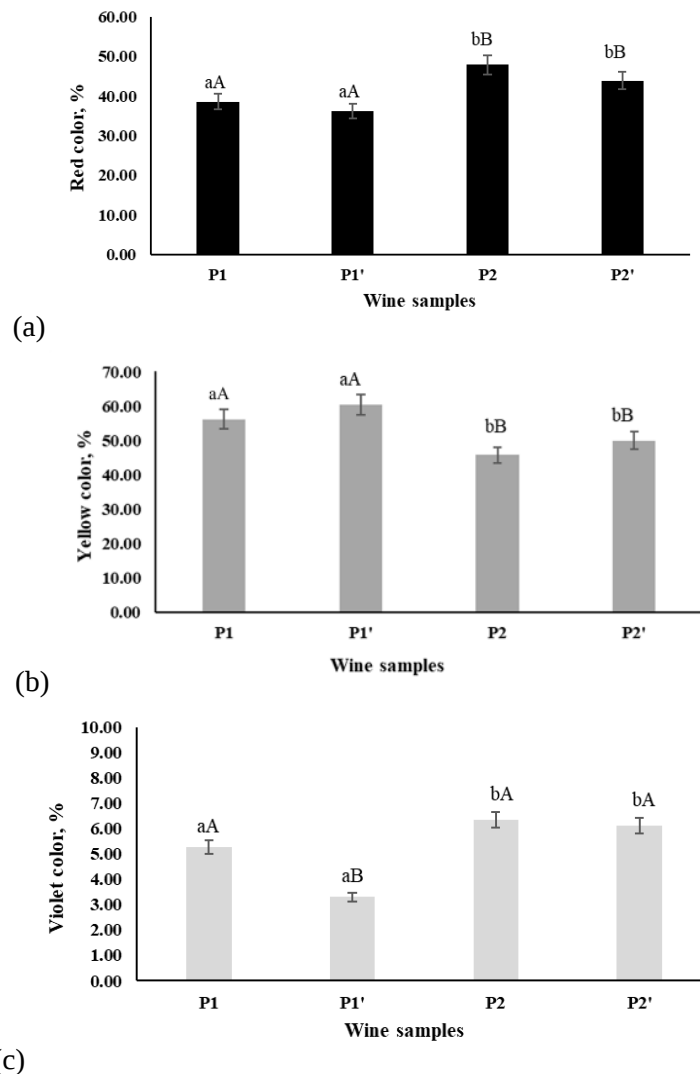


Figure 2. Evolution of percentage of yellow (a), red (b), and violet (c) colors during 12 months of storage.

On top of the bars, lowercase superscript letters indicate significant differences among mean values corresponding to wine samples produced with different procedures at the same time, while different capital letters indicate differences among values corresponding to wines produced with the same procedure, at different moments of time, at $p < 0.05$.

The percentages of monomeric and polymeric pigments, respectively, and the percentage of copigmentation are presented in Figure 3.

Thus, the P₂ sample, because of the bleeding procedure, which also involves a short-term contact between the must and the solid part after crushing the grapes (a short-term maceration), naturally presents higher concentrations of monomeric pigments than the P₁ sample, where the grapes were pressed directly.

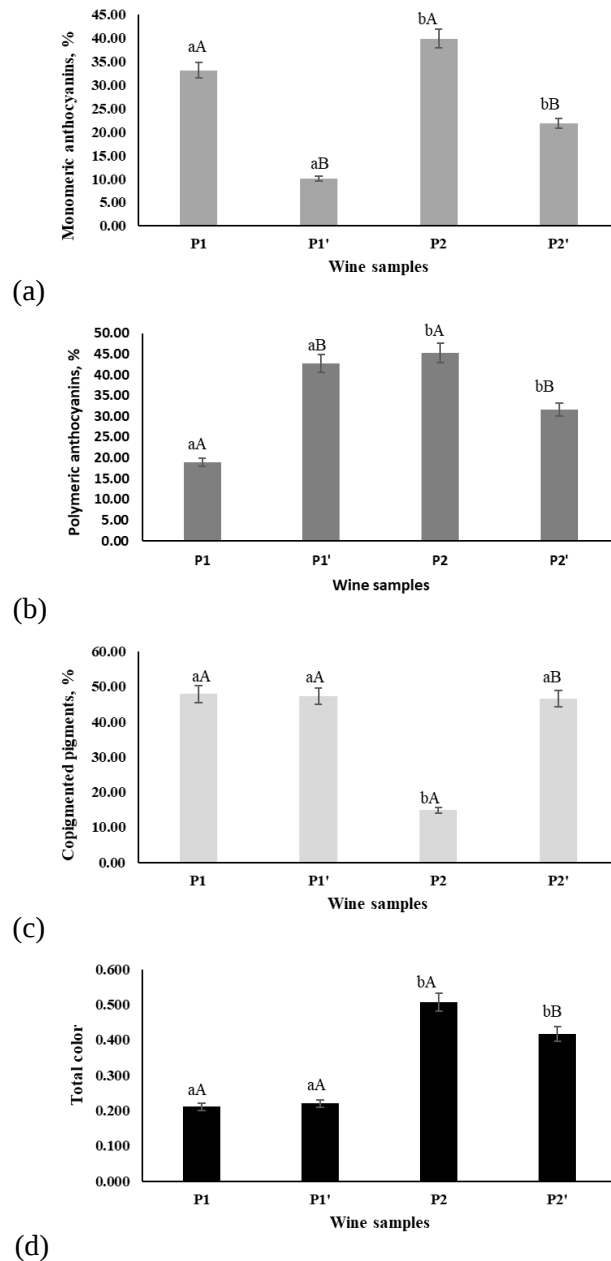


Figure 3. Percentages of monomeric (a) and polymeric (b) pigments, the percentage of copigmentation (c), and total color (d) of the wine samples.

On top of the bars, lowercase superscript letters indicate significant differences among mean values corresponding to wine samples produced with different procedures at the same time, while different capital letters indicate differences among values corresponding to wines produced with the same procedure, at different moments of time, at $p < 0.05$.

Since there was a short maceration period (average duration of 90 minutes), the difference is only about 6% (39.92% vs. 33.18%), but enough to be visible not only in terms of intensity but also in the contribution of colors-specific to young wine, with more intense notes of pink and light red (Figure 3a). The presence of purple anthocyanins was noted in the Fetească Neagră variety by the violet notes accompanying the intense pink or candy-pink color. These aspects were also taken into account during the sensory analysis.

The results regarding the evolution of these color parameters show a decrease in the percentages of monomeric pigments in the case of both wines, a normal phenomenon for their evolution during aging (Garrido and Borges, 2013; Babincev *et al.*, 2016; Gil *et al.*, 2019; Gutiérrez-Escobar *et al.*, 2021; Cheng and Watrelot, 2022), the compounds taking other forms, either by polymerization, becoming more stable, or by copigmentation, binding to other components in the wine.

Higher differences between samples are found in the case of the percentage of polymeric pigments, with the P₁ sample having a contribution of 18.96%. In contrast, the P₂ sample, produced by the Saignée technique, has one of 45.26% (Figure 3b). These values thus present major differences between the two wines, being favorable to the aging processes and the stability of the color of the wines in general. The P₁ sample is a common one for young rosé wines, in which, as was already mentioned, a significant initial value of monomeric anthocyanin compounds is normal, and their evolution in time leads to polymerization phenomena and an increase in the percentages of polymeric pigments. In the case of the Saignée wine P₂ sample, an advanced situation is observed, the technique leading, according to the results, to wines with more stable anthocyanin compounds (Borges, 2013; Babincev *et al.*, 2016; Garrido and Gil *et al.*, 2019; Gutiérrez-Escobar *et al.*, 2021).

In the case of P₁, the percentage of polymeric pigments increases from 18.96% to 42.73% as they age, resulting in a more stable form of the color compounds and a decrease in the monomeric ones (Figure 3b). From a chemical point of view, this occurs through the combinations that take place between different anthocyanins, as well as between them and proanthocyanidins (tannins), thus forming more stable compounds over time. These developments differ depending on the variety, the winemaking process, and the harvest year (Garrido and Borges 2013; Babincev *et al.* 2016; Gil *et al.* 2019; Gutiérrez-Escobar *et al.* 2021; Cheng and Watrelot 2022).

The percentage of polymeric pigments in the P₂ sample decreased from 45.26% to 31.65% (Figure 3b). This occurs concurrently with the increase in the degree of copigmentation, which rises from 14.82% to 46.52% (Figure 3c). Regarding the evolution of the percentages of copigmentation following aging, in the case of the P₁ sample obtained by the classic procedure, there is an insignificant decrease in the percentage of copigmentation from 47.87% to 47.27%. The intensity and total color do not change visibly; the changes produced are due exclusively to the change in the ratio between monomeric and polymeric pigments, in an evolution specific to most rosé wines produced at this time.

The total color determinations confirm these values and evolutions and the specific differences between rosé and red wines (Figure 3d). According to Figure 3d, a

different dynamic between the two wines was observed. At first, the sample P₂ exhibited higher values of the total color, which are correlated with the percentages of each color and the value of the color intensity. This is due to the brief maceration period, as anticipated. Sample P₂' exhibited a 17.5% reduction in total color. The P₁ sample values are approximately equivalent to those after 12 months of aging (P₁'). The results for the color of samples using the CIELab method are shown in Table 2. L* (brightness) parameter showed higher initial values for the P₁ sample (46.78±0.08) compared to the P₂ sample (39.50±0.04). This is mainly due to the higher content of red and purple pigments in the P₂ sample. Both wines presented higher values of L* parameter after 12 months of aging (47.92±0.47 for P₁', respectively 43.05±0.11 for P₂'), indicating a slight lightening of the color. This behavior contrasts with the commonly reported tendency of white and rosé wines to darken over time due to copigmentation, polymerization, and oxidative shifts in hue (Boulton, 2001; Cáceres-Mella *et al.*, 2014; Medina-Plaza *et al.*, 2024). However, in this case, the observed lightening may be attributed to pigment instability or partial discoloration during aging.

At the same time, the results are consistent with the behavior of red wines, which typically exhibit lightening and a decrease in color intensity during aging (de Guerrini *et al.*, 2019; Oliveira *et al.*, 2024). This duality is logical in the case of rosé wines, which can express characteristics from both white and red profiles depending on the pigment concentration. In the case of the Saignée wine, where pigment levels are higher, the behavior more closely resembles that of red wines, as these wines are intermediate between classical rosé and light red wines. Additionally, copigmentation and polymerization phenomena may contribute not only to color stabilization but also to partial pigment loss and decoloration over time (Garrido and Borges, 2013; Babincev *et al.*, 2016; Gutiérrez-Escobar *et al.*, 2021).

Table 2. Color parameters of wines (CIELab method).

Samples	P ₁	P ₁ '	P ₂	P ₂ '
L*	46.78±0.08 ^{aA}	47.92±0.47 ^{aB}	39.50±0.04 ^{bA}	43.05±0.11 ^{bB}
a*	2.63±0.04 ^{aA}	2.05±0.07 ^{aB}	11.18±0.12 ^{bA}	7.03±0.09 ^{bB}
b*	6.23±0.02 ^{aA}	8.25±0.94 ^{aB}	8.11±0.07 ^{bA}	8.37±0.05 ^{aA}
c*	6.76±0.04 ^{aA}	8.50±0.90 ^{aB}	13.81±0.14 ^{bA}	10.92±0.10 ^{bB}
h*	1.17±0.00 ^{aA}	1.33±0.03 ^{aB}	0.63±0.00 ^{bA}	0.87±0.00 ^{bB}
ΔE	-	2.46±0.60	-	5.46±0.02

In a row, lowercase superscript letters indicate significant differences among mean values corresponding to wine samples produced with different procedures at the same time, while different capital letters indicate differences among values corresponding to wines produced with the same procedure, at different moments of time, at p<0.05.

The values of a parameter representing the red and green color chromatic scale for the P₁ and P₂ samples were 2.63±0.04 and 11.18±0.12, respectively. The two wine

samples exhibit a propensity toward redness, as evidenced by the positive values. These values are directly correlated with the concentration of red anthocyanin compounds in wines, with the P₂ sample exhibiting a higher concentration (Ghanem, 2014; Bastianetto *et al.*, 2015; Khoo *et al.*, 2017; Martin *et al.*, 2017; Chen *et al.*, 2019; Bendokas *et al.*, 2020; Benbouguerra *et al.*, 2021). The parameter values of the two wines decrease to 2.05 ± 0.07 for the P₁ sample and 7.03 ± 0.09 for the P₂ sample after storage. There is a decrease in the red color in the case of the P₂ sample and an increase in the yellow color in the total color. Thus, the process cannot support the higher value of red pigments, but the values remain higher than those corresponding to rosé wines obtained by the classic process. This procedure could be improved by adding exogenous proanthocyanidins in a controlled manner from natural products marketed nowadays or by a short period of maturation in barriques, which would lead to an increase in the intake of tannins from its wood.

The evolution of the b* parameter is perfectly normal for white and rosé wines. Thus, the P₁ sample has a value of 8.25 ± 0.94 , and the P₂ sample has a close one of 8.37 ± 0.05 . Chroma c* was 6.76 ± 0.04 for the P₁ sample and 13.81 ± 0.14 for the P₂ sample. The P₂ sample exhibits a higher color saturation due to its higher pigment concentration, as evidenced by the disparity between the two wines. As a result of the storage, there are changes in the c* parameter, which significantly increases to the value of 8.50 ± 0.90 for the P₁ sample ($p < 0.05$).

Significant changes in h* values were measured for both samples from 1.7 ± 0.00 to 0.63 ± 0.00 (P₁) and from 1.33 ± 0.03 to 0.87 ± 0.00 (P₂). The calculated ΔE values were 2.46 ± 0.60 (P₁'), respectively 5.46 ± 0.02 (P₂'), P₁' sample having color stability after 12 months of storage.

Sensory analysis

The sensory analysis compares the classic rosé wine (P₁) with the Saignée wine (P₂) to ascertain their differences and how trained and nontrained consumers perceive the wine obtained by the Saignée technique. For the analysis, a scale from 1 to 9 was used, the value 1 corresponding to the level “extremely unpleasant,” and the value 9 being assigned to a wine qualified as “extremely pleasant.” The results are shown in Figure 4.

There were no significant visual differences between the two wine samples. This is normal since the wines differed only in terms of color (intensity, nuance, saturation, etc.), the other aspects such as clarity or brightness being influenced by other technological stages where there were no differences in terms of the way of working applied to obtain them (e.g., clarifying, filtering, etc.). The wines did not present visual defects, such as turbidity and any traces of oxidation. It seems that the rosé wine obtained by the direct pressing technique (P₁ sample) had a significantly higher score in freshness, being better modeled according to the specifics of the market, for other descriptors such as smell, taste, aroma, aftertaste, roundness, and body, P₂ sample wine is preferred to the P₁ sample.

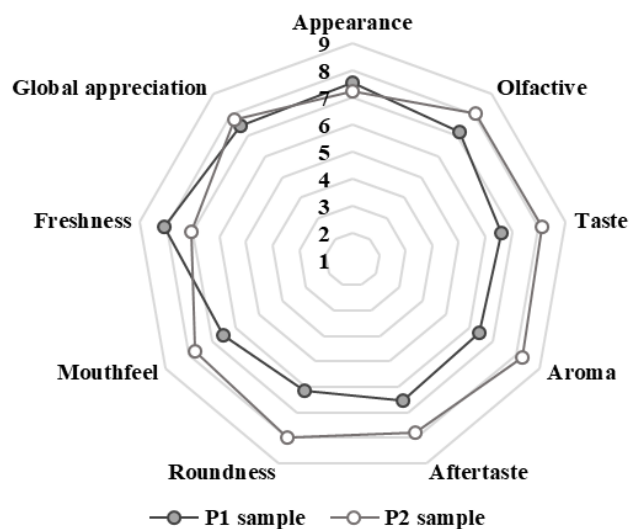


Figure 4. Results of sensory analysis of wine samples.

Figure 5 offers a comprehensive overview of the interrelations between the wine samples and attributes within a singular plane. As can be seen, the attributes found in the upper-right quadrant are represented by olfactive, mouthfeel, taste, aftertaste, and global appreciation, which have a positive contribution on axis F1. Other attributes such as aftertaste, taste, aroma, and roundness also positively influence axis F1. The F1 and F2 axes explained 87.92% of the total variance. It is visible that the P₂ sample was located close to these descriptors. PCA analysis revealed that the P₂ sample was the most prevalent in tasters' choices.

However, it was undoubtedly a success that the P₂ sample represents a novelty in this context (even if it has very old roots). It was so quickly accepted and even preferred in most cases to classic rosé wine. This is because of its sensory features, starting from the visual aspect, which remains deeply subjective, passing through the most important characteristics of smell, taste, and aroma and ending with the complex perceptions regarding the roundness and body of the wine.

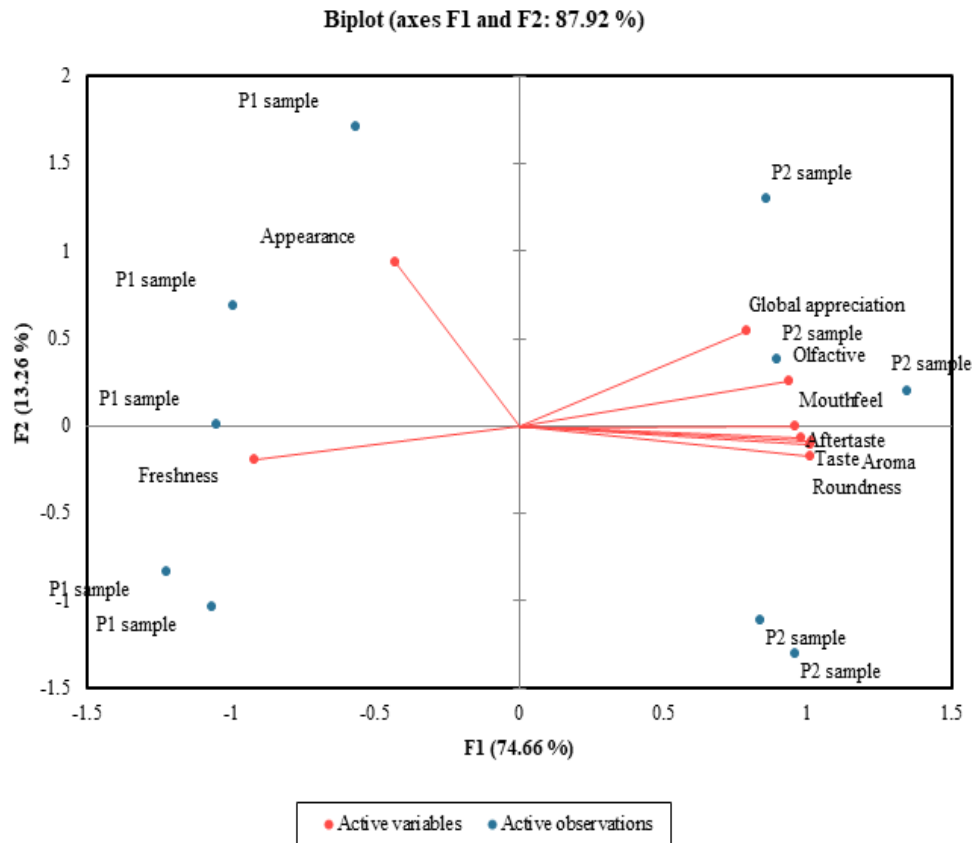


Figure 5. Correlation of sensory attributes by Principal Component Analysis (PCA).

Conclusions

The present study aimed to apply the Saignée winemaking technique, also known as *bleeding*, to improve the quality and acceptability of rosé wines in the Danube Terraces wine region. At the same time, it represents an attempt to combat the effects of climate change in viticulture and winemaking, such as sugar-acid imbalance, wine instability, and the inability to reach full aromatic and phenolic potential, by applying a technique that avoids significant use of oenological additives and does not entail major cost increases — thus supporting a more sustainable and integrative production strategy.

The values of the physicochemical analyses indicated increased stability of the wines and aging potential, the total and volatile acidity, the pH, and the free and total SO₂ levels, showing that both wines can sustain possible long-term aging.

Compared to the results reported in the literature, the rosé wines in this study exhibited color evolution patterns that incorporate characteristics typical of both red and white wines, confirming the intermediate nature of rosé wines in terms of

pigment behavior during aging. . The Saignée wine (P₂ sample) exhibited a threefold increase in color intensity due to the technological process named after the color aspects. The pigment intensity decreased in both wines after one year of maturation, particularly affecting the Saignée wine. In contrast, tint values undergo an opposite path, namely an increase in response to aging, a phenomenon prevalent in rosé wines. It is important to highlight that the hue exhibited higher values in the P₁ sample, which was subsequently determined to be a result of the higher percentages of yellow in the total color. All changes were correlated with percentages of monomeric and polymeric pigments and the percentage of copigmentation.

Hedonic analysis of samples led to the conclusion that Saignée wine was generally more appreciated, scoring additionally in several aspects, such as smell, taste, aroma, aftertaste, roundness, full-bodied, and general appreciation. The classic rosé wine was appreciated in terms of freshness and appearance.

These results suggest that, through the Saignée technique, the wine can better express the potential of the Fetească Neagră variety and the specific terroir of the Danube Terraces region, achieving a superior sensory profile compared to the classic rosé, and thereby supporting the overall objectives of the research.

Acknowledgments

The Integrated Center for Research, Expertise, and Technological Transfer in Food Industry is acknowledged for providing technical support.

References

- Asproudi, A., Ferrandino, A., Bonello, F., Vaudano, E., Pollon, M., Petrozziello, M. 2018. Key norisoprenoid compounds in wines from early-harvested grapes in view of climate change. *Food Chemistry*, **268**, 143–52.
- Babincev, L., Guresic, D.M., Simonovic, R.M. 2016. Spectrophotometric characterization of red wine color from the vineyard region of Metohia. *Journal of Agricultural Sciences, Belgrade*, **61**(3), 281–90.
- Bai, Y., Zhao, P., Du, Y., Lin, J., Han, F. 2023. Effect of postharvest grape dehydration on the phenolic composition of ‘Marselan’ rose wine during aging. *Journal of Food Composition and Analysis*, **123**, 105630.
- Baiano, A., Terracone, C., Gambacorta, G., La Notte, E. 2009. Phenolic content and antioxidant activity of Primitivo wine: comparison among winemaking technologies. *Journal of Food Science*, **74**(3), C258-C267.
- Baiano, A., Scrocco, C., Sepielli, G., Del Nobile, M. A. 2016. Wine processing: A critical review of physical, chemical, and sensory implications of innovative vinification procedures. *Critical reviews in food science and nutrition*, **56**(14), 2391-2407.
- Bastianetto, S., Ménard, C., Quirion, R. 2015. Neuroprotective action of resveratrol. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, **1852**(6), 1195–1201.
- Benbougerra, N., Hornedo-Ortega, R., Garcia, F., El Khawand, T., Saucier, C., Richard, T. 2021. Stilbenes in grape berries and wine and their potential role as anti-obesity agents: A review. *Trends in Food Science and Technology*, **112**, 362–81.
- Bendokas, V., Stanys, V., Mažeikienė, I., Trumbeckaitė, S., Baniene, R., Liobikas, J. 2020. Anthocyanins: From the field to the antioxidants in the body. *Antioxidants*, **9**(9), 1–16.

- Benucci, I. 2020. Impact of post-bottling storage conditions on colour and sensory profile of a rosé sparkling wine. *LWT*, **118**, 108732.
- Bohnert, G., Martin, B. 2023. Impacts of climate change induced drought and adaptation strategies in wine-growing in the Rhine Valley (France, Germany, Switzerland). *Total Environment Research Themes*, **8**, 100081.
- Boulton, R. 2001. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American journal of enology and viticulture*, **52**(2), 67-87.
- Buican, B.C., Colibaba, L.C., Luchian, C.E., Kallithraka, S., Cotea, V.V. 2023. "Orange" wine—The resurgence of an ancient winemaking technique: A review. *Agriculture*, **13**(9), 1750.
- Cáceres-Mella, A., Flores-Valdivia, D., Laurie, V. F., López-Solís, R., Peña-Neira, Á. 2014. Chemical and sensory effects of storing Sauvignon Blanc wine in colored bottles under artificial light. *Journal of Agricultural and Food Chemistry*, **62**(29), 7255-7262.
- Casassa, L.F., Vega-Osorno, A.A., Hernandez, J.P. 2021. Chemical and chromatic effects of saignée combined with extended maceration and microwaved stem addition on three Pinot Noir clones from the Central Coast of California. *Australian Journal of Grape and Wine Research*, **27**(4):540–52.
- Casassa, L.F., Beaver, C.W., Mireles, M., Larsen, R.C., Hopfer, H., Heymann, H., Harbertson, J.F. 2013. Influence of fruit maturity, maceration length, and ethanol amount on chemical and sensory properties of Merlot wines. *American Journal of Enology and Viticulture*, **64**(4), 437-449.
- Chen, S., Yi, Y., Xia, Y., Hong, Z., Zhang, Y., Shi, G., He, Z., Zhong, S. 2019. The influences of red wine in phenotypes of human cancer cells. *Gene*, **702**, 194–204.
- Cheng, Y., Watrelot, A.A. 2022. Effects of Saignée and bentonite treatment on phenolic compounds of Marquette red wines. *Molecules*, **27**(11), 3482.
- Chidi, B.S., Bauer, F.F., Rossouw, D. 2018. Organic acid metabolism and the impact of fermentation practices on wine acidity: a review. *Journal of Enology and Viticulture*, **39**(2), 3164.
- Cui, W., Wang, X., Han, S., Guo, W., Meng, N., Li, J., Sun, B., Zhang, X. 2024. Research progress of tartaric acid stabilization on wine characteristics. *Food Chemistry:X*, 101728.
- Gaitán, E., Pino-Otín, M.R. 2023. Using bioclimatic indicators to assess climate change impacts on the Spanish wine sector. *Atmospheric Research*, **286**, 106660.
- Ghanem, C., Hanna-Wakim, L., Nehme, N., Souchard, J.P., Taillandier, P., El Rayess, Y. 2014. Impact of winemaking techniques on phenolic compounds composition and content of wine., In: *Wine Phenolic Composition, Classification and Health Benefits*; El Rayess, Y., Ed, Nova Publishers.
- Garrido, J., Borges, F. 2013. Wine and grape polyphenols - A chemical perspective. *Food Research International*, **54**(2), 1844–58.
- Gil, M., Louazil, P., Iturmendi, N., Moine, V., Cheynier, V., Saucier, C. 2019. Effect of polyvinylpyrrolidone treatment on rosés wines during fermentation: Impact on color, polyphenols and thiol aromas. *Food Chemistry*, **295**, 493-498.
- Glories, Y. 1984. La couleur des vins rouges. *Conn. Vigne Vin*, **18**(4), 253-271.
- Gonzaga, L.S., Bastian, S.E., Capone, D.L., Danner, L., Jeffery, D.W. 2022. Consumer perspectives of wine typicity and impact of region information on the sensory perception of Cabernet Sauvignon wines. *Food Research International*, **152**, 110719.
- Guerrini, L., Pantani, O. L., Politi, S., Angeloni, G., Masella, P., Calamai, L., Parenti, A. 2019. Does bottle color protect red wine from photo-oxidation?. *Packaging Technology and Science*, **32**(5), 259-265.

- Gutiérrez, A.R., Portu, J., López, R., Garijo, P., González-Arenzana, L., Santamaría, P. 2023. Carbonic maceration vinification: A tool for wine alcohol reduction. *Food Chemistry*, **426**, 136558.
- Gutiérrez-Escobar, R., Aliaño-González, M. J., Cantos-Villar, E. 2021. Wine polyphenol content and its influence on wine quality and properties: A review. *Molecules*, **26**(3), 718.
- Khoo, H.E., Azlan, A., Tang, S.T., Lim, S.M. 2017. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food and Nutrition Research*, **61**(1), 1361779.
- Lan, H., Li, S., Yang, J., Li, J., Yuan, C., Guo, A. 2021. Effects of light exposure on chemical and sensory properties of storing Meili Rosé wine in colored bottles. *Food Chemistry*, **345**, 128854.
- Lereboullet, A.L., Beltrando, G., Bardsley, D.K. 2013. Socio-ecological adaptation to climate change: A comparative case study from the Mediterranean wine industry in France and Australia. *Agriculture, Ecosystems and Environment*, **164**, 273-285.
- Lukić, I., Budić-Leto, I., Bubola, M., Damijanić, K., Staver, M. 2017. Pre-fermentative cold maceration, saignée, and various thermal treatments as options for modulating volatile aroma and phenol profiles of red wine. *Food Chemistry*, **224**, 251-261.
- Magrini, A., Pantani, O.L., Biondi Bartolini, A., Stefanini, F.M. 2016. On prefermentative maceration techniques: statistical analysis of sensory descriptors in Sangiovese wine. *Biometrical Letters*, **53**(1), 1-20.
- Martin, M.A., Goya, L., Ramos, S. (2017). Protective effects of tea, red wine and cocoa in diabetes. Evidences from human studies. *Food and Chemical Toxicology*, **109**, 302-314.
- Mazarrón, F.R., Cañas, I. 2023. Hygrothermal conditions for the aging of red wine from experimental data. *LWT*, **173**, 114272
- Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., Ewert, B. 1999. Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. *Journal of agricultural and food chemistry*, **47**(10), 4009-4017.
- Medina-Plaza, C., DuBois, A., Tomasino, E., Oberholster, A. 2024. Effect of storing conditions (lighting, temperature and bottle color) on rosé wine attributes. *Food Chemistry*, **439**, 138032.
- Mora, M., de Matos, A.D., Vázquez-Araújo, L., Puente, V., Hernando, J., Chaya, C. 2021. Exploring young consumers' attitudes and emotions to sensory and physicochemical properties of different red wines. *Food Research International*, **143**, 110303.
- OIV-MA-INT-00-2020. Compendium of international methods of analysis of wines and musts; International Organisation of Vine and Wine Paris: Paris, France, 2020. <http://www.oiv.int/en/technical-standards-and-documents/methods-of-analysis/compendium-of-international-methods-of-analysis-of-wines-and-musts-2-vol>
- de Oliveira, J.B., Fialho, F.B., Laureano, O., de Castro, R., Pereira, G.E., Ricardo-da-Silva, J.M. 2024. Impact of aging on the physical-chemical and phenolic stability of tropical red wines from Brazil produced with grapes harvested in the summer season. *Journal of Food Composition and Analysis*, **125**, 105794.
- Pantani, O.L., Stefanini, F.M., Lozzi, I., Calamai, L., Biondi Bartolini, A., Di Blasi, S. 2014. Pre-maceration, saignée, and temperature affect the daily evolution of pigment extraction during vinification. COBRA Preprint Series. <https://biostats.bepress.com/cobra/art107>
- Petriashvili, A., Mach, J., Štěbeták, M., Prášilová, M., Svoboda, R., Navrátilová, M., Markéta Beranová, Kamila Veselá, Václav Hofman, Némec, O. 2023. The impact of climate change on the sustainability of wine production and the structure of its consumption in Czechia. *Heliyon*, **9**(7), e17882.

- Sacchi, K.L., Bisson, L.F., Adams, D.O. 2005. A review of the effect of winemaking techniques on phenolic extraction in red wines. *American Journal of Enology and Viticulture*, **56**(3), 197-206.
- Stávek, J., Papouskova, B., Balik, J., Bednar, P. 2012. Effect of storage conditions on various parameters of colour and the anthocyanin profile of rosé wines. *International Journal of Food Properties*, **15**(5), 1133-1147.
- Ubeda, C., Peña-Neira, Á., Cortiella, M.G. 2022. Combined effects of the vessel type and bottle closure during Chilean Sauvignon Blanc wine storage over its volatile profile. *Food Research International*, **156**, 111178.
- Vicente, J., Baran, Y., Navascués, E., Santos, A., Calderón, F., Marquina, D., Rauhut, D. Benito, S. 2022. Biological management of acidity in wine industry: A review. *International Journal of Food Microbiology*, **375**, 109726.
- Wang, Q.J., Spence, C. 2019. Is complexity worth paying for? Investigating the perception of wine complexity for single varietal and blended wines in consumers and experts. *Australian Journal of Grape and Wine Research*, **25**(2), 243-251.
- Wu, Y., Xing, K., Zhang, X., Wang, H., Wang, F., Wang, Y., Li, J. 2017. Effect of pre-fermentation saignée treatment on phenolic compound profile in wine made of Cabernet Sauvignon. *Journal of Food Biochemistry*, **41**(4), e12380.
- Xi, C., Zhang, J., Zhang, F., Liu, D., Cheng, W., Gao, F., Wang, P. 2024. Effect of postharvest grape dehydration on chemical composition, antioxidant activity and sensory characteristics of Marselan wines. *Food Chemistry:X*, **22**, 101503.