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Research Article

The Effects of Destemming/Crushing and Pressing Conditions in Rosé Wine Production

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Background and Aims. Rosé wines are becoming more popular, as is the demand for organic wines. However, there are few studies on the effects of operative choices on the quality and style of these wines. The paper aims to investigate the effects of the early stages of processing (destemming/crushing and pressing) on rosé wine characteristics. *Methods and Results*. A full factorial experiment was carried out on *Sangiovese*, considering two factors related to grape processing: the prepressing treatment (intact clusters or destemmed/crushed berries) and the pressing pressure. Sugars, ethanol, acidity, phenols, and color were measured in juices, while volatile compounds and sensory profiles were evaluated in wines. Destemming/crushing dramatically changed the composition of both juices and wines'; notably, acidity increased and floral and color intensities decreased in intact cluster samples. Furthermore, a clear interaction was found between prepressing treatment and pressure. Pressure, in turn, affected several acidity and color parameters. This could be related to the chemical composition and consistency of the different berry zone, which interact with the mechanical action of the destemmer/crusher and press. *Conclusions*. Processing choices affect all of the main qualitative features of rosé wine; they could be successfully used to decide the wine's style and minimize the use of additives and adjuvants. Significance of the study: in rosé production, the capability to modulate the operational protocols allows selecting of different wines from the same grape batch. This represents a useful tool to optimize grape processing according to the company's commercial priorities.

1. Introduction

Recently, rosé wine, also called *Rosato*, and has gained in popularity, especially among consumers looking for a new taste experience. The product has a wide range of styles, depending mainly on the grape variety, climate, and production method, which influence its color, sweetness, degree of sparkling, and flavor [1].

Rosé wine production lies halfway between that of red and white wines, as it starts life as red before becoming white, even though, since 2009, EU regulations permit the blending of white wines and red wines in specific regulated cases [2]. Several methods are widely used. The *Saignée* method consists in taking a fraction of red grape must during red wine fermentation, and draining the juice after a short maceration. Skin contact is another very common method. This consists of macerating grapes with their skins after crushing; the product is then drained or pressed in order to obtain a low-pigmented juice [1]. In the latter method, operative parameters and machine settings could be viewed as quality control mechanisms, as they can modify the characteristics of the juice and, consequently, the quality of the obtained wine.

Hence, the choices made by the winemaker can play a key role in the style of the obtained rosé wine. One of the most important phenomena that occurs after crushing is the

redistribution of grape components among the different fractions of the disrupted cluster. The skin, pulp, and seeds each have a different chemical composition. For instance, while the peripheral fraction of the grape (i.e., the skin) is very abundant in minerals, aromatic compounds, and phenols, the intermediate–central zone is rich in sugars and acids, whilst seeds mainly contain essential oils and catechins responsible for bitter and herbaceous notes [3].

The duration of skin contact or maceration is one of the main parameters affecting the color and chemical composition of the grape juice, since it allows several volatile and phenolic compounds to be distributed among solid and liquid fractions [4]. During rosé winemaking, a short skin contact time is needed, in order to extract volatile and antioxidant compounds [4–6] and, at the same time, to ensure sufficient juice pigmentation to enhance the color stability of the finished wine [7]. A too-short or too-long skin contact time can lead to poor extraction of anthocyanins and aromas or the collateral extraction of undesired molecules linked to astringent and bitter notes, respectively [7].

In addition to maceration time, the press type and/or its setting are other relevant parameters. Catania et al. [8] tested grape oxygen exposure during pressing under inert or aerobic conditions. The latter authors observed improved quality parameters for juice obtained using nitrogen, notably, a higher concentration of phenolic compounds and a reduction of volatile acidity. Similarly, Pons et al. [5] found that inert pressing improved the glutathione concentration in grape juice, while Day et al. [9] noted a modulating effect on the volatile, phenolic, and protein fractions of wine produced under controlled oxygen exposure conditions during pressing and juice handling.

Although pressure is a key factor affecting the migration of grape fraction components during pressing, few studies have investigated its effects on grape juice and wine. Yokotsuka [10] evaluated grape juice composition using different press types, with a gradual increase in pressure. The experiment tested three types of *Koshu* grapes: destemmed, 50% stemmed, and 100% stemmed. The author found that different types of presses exerted different mechanical actions on the grape fraction that, consequently, resulted in substantial differences in juice composition in terms of acidity, sugars, proteins, phenolic compounds, browning capacity, grape reaction product, caftaric acid, and glutathione. Similar results were reported as a function of increased pressure. Finally, Maggu et al. [4] pointed out a modulating effect of pressing pressure on varietal aroma compounds and the phenolic oxidation of *Sauvignon Blanc* juice.

However, to the best of our knowledge, there are no similar studies of rosé winemaking where even small variations in prefermentation conditions can have a major impact on the finished wine style. Our study addresses this gap in the literature. In a full factorial experiment, we test the influence of different pressure levels, applied using a pneumatic press, together with two grape preparation methods (direct pressing of intact clusters and pressing after destemming and crushing). Our overall goal was to assess how early processing operations could affect the characteristics of the obtained rosé wine.

2. Materials and Methods

2.1. Experimental Trials. Trials were conducted in three replicates at Podere dell'Anselmo (Montespertoli, Florence, Italy) using the company's cellar equipment. During each test, a pneumatic press (PST 16 AE 400 V 50 Hz 3PH, Škrlj d.o.o., Črniče, Slovenia) of 1000 kg nominal capacity was filled with 700 kg of *Sangiovese* grapes. Grapes were grown in Montespertoli (Florence, Italy) and were manually harvested the day before the trials, distributing them in perforated plastic boxes of approx. 50 L of volume. Then, they were treated with an aqueous solution of ascorbic acid and potassium metabisulphite, in a concentration of 50 mg and 70 mg per kg of grapes, respectively, and transported to the wine cellar for storage at 4°C in a refrigerated cell the night before pressing. The following morning, half of the grapes were pressed unstemmed, while the remaining half were pressed after destemming/crushing through a destemmer-crusher (R50, Mori Luigi Srl, San Casciano in Val di Pesa, Florence, Italy), placing the crushing rollers at the maximum allowable distance (i.e., 6.2 cm). The press operated at four pressures: 0.3, 0.6, 0.9, and 1.2 bars. Each pressure was applied in two consecutive cycles, where it was maintained for 5 min before releasing. Between the two blocks, the press was rotated three times with the membrane deflated. The juice resulting from each pressure test was collected separately and weighed. At the end of the pressing operation, the juices were merged according to the weighted proportions to simulate the juice obtained with different pressures. Specifically, the 0.3 bar fraction was not mixed, the 0.6 fraction was obtained by mixing the juice obtained at 0.3 bar with the juice obtained at 0.6 bar in appropriate proportions, and so on. We tested two methods to obtain juice: the intact cluster (IC) and the destemmed and crushed cluster (DCC) at four pressures. This resulted in eight coded samples (IC03, IC06, IC09, IC12, DCC03, DCC06, DCC09, and DCC12). Three replications were performed, for a final total of 24 samples.

Next, 10 kg of grape juice was sampled into 12 L glass vessels, which were placed in a refrigerated cell for three days at 4°C to allow settling of impurities (a turbidity value below 200 NTU). Then, 7.5 kg of the resulting clarified juice were transferred into another 12 L vessel for fermentation. After these samples reached ambient temperature (20°C), they were inoculated with 0.25 g/L of *Saccharomyces cerevisiae* yeast when the difference in temperature between the yeast and grape juice was less than 10°C. Yeast had previously been activated by mixing 2 g of commercial yeast (EC1118™ Organic, Lallemand Inc., Verona, Italy) with 3 g of nitrogen nutrients (Go-Ferm Protect, Lallemand Inc., Verona, Italy), and 3 g of sugar in 100 mL of water at 35°C. Fermentations were carried out at 20°C in a controlled temperature room, and fermentation kinetics (change in density) were monitored daily with a Babo must meter. Fermentation ended after 14 days; at this point, wine samples were stored for 48 h at 4°C to decant yeast lees and impurities. Finally, wines were immediately sampled for either physical or chemical analysis and bottled in 0.75 L dark glass bottles with plastic stoppers in preparation for the sensory evaluation. The wine bottles were stored in the dark and at room temperature, i.e., 20°C,

then, the sensory evaluation was performed after 1 month of storage. Sulfur dioxide was added neither to the juice nor to the wine.

2.2. Turbidity Measurements. Juice turbidity was measured before and after clarification, and at the end of settling with a Hach Ratio XR Turbidimeter (Hach, Loveland, CO, USA) in NTU mode. A 30 mL glass vial was filled with sample and sealed with a cap, which was then placed in the turbidimeter vessel chamber. Measurements were carried out 3 h after juice production, and after settling (3 days after juice production).

2.3. Fourier Transform Infrared (FTIR) Analysis. FTIR analyses were performed on both the grape juice samples (immediately after pressing) and the finished wine samples. Specifically, 50 mL of sample was added to a Falcon tube, and centrifuged at 6000 rpm (4467 ×g) × 10 min (HERMLE Z 206-A, Benchmark Scientific, Sayreville, NJ, USA) in order to remove impurities and optimize clarity. Then, 50 mL of the clarified fraction was collected into a Falcon tube that recovered the supernatant from multiple aliquots. FTIR measurements were carried out using a FOSS WineScan™ FLEX (FOSS, Hilleroed, Denmark) by directly sampling either the juice or the wine from the Falcon tube using the instrument's sampler probe. All measurements were performed in duplicate using the "grape" and "finished wine" programs for grape juice and wine samples, respectively. The final output value was expressed as the average of the two measurements. The following parameters were evaluated: ethanol, pH, total and volatile acidity, total and free sulfur dioxide, Abs_{420}, Abs_{520}, Abs_{620}, color intensity, color hue, glucose and fructose, reducing sugar, methanol, total anthocyanins, tartaric acid, malic acid, lactic acid, citric acid, gluconic acid, glycerol, potassium, yeast assimilable nitrogen, and total phenols.

CIELab coordinates were obtained using the following method. A transparent plastic cuvette was filled with the wine sample, placed against a white background. Then, a picture was taken in the presence of light, maintaining a fixed distance between the camera and the sample cuvette (approximately 30 cm). Next, the obtained picture was analyzed using ImageJ software, and L^*, a^* and b^* values were acquired through the Color Inspector 3D Plugin.

2.4. HS-SPME GC-MS Analysis. The volatile profile of wine samples was evaluated using head space solid phase microextraction coupled to gas chromatography and mass spectrometry (HS-SPME GC-MS) using an Agilent 7820 gas chromatograph and a 5977 MSD with electron ionization (Agilent, Santa Clara, CA, USA), and a 3-phase (Carboxen/PDMS/DVB) 75 μm–1 cm long fiber produced by Supelco (Sigma, Darmstadt, Germany).

Volatile organic compounds were identified and quantified using the method described in Guerrini et al. [11]. Briefly, samples were prepared by adding 1 mL of wine to 4 mL of distilled water, and 70 μl of internal standards that were either deuterium labelled, or not present in the test specimens, but with chemical similarities, namely: ethyl acetate D3, butanol D10 acetate D3, butanol D10, ethyl hexanoate D11, o-xylene D10, 5-methyl hexanol, butyl hexanoate D11, acetic acid D3, naphthalene D8, hexanoic acid D11, and 3,4-dimethyl phenol. ISTD mix was added to samples and calibration scales to normalize analyte areas. After 5 min of equilibrium, the fiber was exposed in the headspace of the sample vial to extract volatile organic compounds for 5 min at 60°C. A Gerstel MPS2 XL autosampler, equipped with a temperature-controlled agitated tray, was used for SPME extraction.

The chromatographic analysis was carried out using a J&W Innowax column (50 m, 0.2 mm, and i.d. 0.4 μm DF). Injection was carried out in splitless mode at 250°C. The oven temperature started at 40°C, which was held for 1 min; it was then increased to 60°C at 2 °C/min, then 150°C at 3°C/min, then 200C at 10°C/min, and finally, 260°C at 25°C/min. The final temperature was held for 6.6 min.

2.5. Sensory Analysis. Samples were evaluated using a sensory evaluation method known as descriptive analysis. Tasting was conducted at the Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence. The panel consisted of nine judges (seven male and two female) who were recruited from staff and students of viticulture and enology based on availability, familiarity, and experience with wine evaluation. All assessors were familiar with wine consumption, and were trained in advance by the panel leader, to recognize a subset of standard references [12–14] listed with their respective recipes in supporting information (Table SI). As regards the astringency attribute, the panelists were asked to score wines considering the intensity scale as the range for a medium-low astringent wine. Three training sessions were carried out on three different days, during which the panel also evaluated eight samples per session. Participation in this study was voluntary, and the assessors were not compensated monetarily.

Three tasting sessions were carried out to assess the three experimental replicates, respectively. Thus, for each session lasting approximately 20 mins, judges evaluated eight samples in random order, waiting 1 min between contiguous samples to rinse mouth with water. 40 mL of wine samples were served at room temperature (20°C) in coded alphanumerically tasting glasses covered with plastic discs. The judges were first asked to make preliminary olfactory exams of the odor descriptors perceived by the nose. Then, they were asked to sip the sample and rate the intensity of the following attributes: overall intensity, floral, fruity, alcohol, herbal, acidity, sweetness, bitterness, and astringency. The perceived intensity of each sensation was rated on a nine-point scale ranging from 1 (extremely weak) to 9 (extremely strong).

2.6. Statistical Analysis. A linear model was built to relate each of the measured wine features (dependent variables) with the tested operative factors. Grape preparation (intact clusters or destemmed and crushed clusters), was considered

as a fixed factor, while the applied pressure was considered as a random factor and tested for first and second order effects. If the second order term was not found to be statistically significant, it was dropped. The first order interaction between grape preparation and applied pressure was also tested. These linear models were tested with the analysis of variances (ANOVA) to assess the significance of the model's coefficients. The significance level was set at $p < 0.05$. If the second order term was not found to be statistically significant, it was dropped in the residual. For sensory data, the median score among judges was considered. Then, the linear model and the ANOVA were done as for the other tested factors.

A principal component analysis was run on statistically significant parameters related to sugars, ethanol, acids, phenols, color, and the panel assessment. Data were scaled and centered before the analysis was run.

3. Results

3.1. Yield, Turbidity, and Operative Parameters. The analysis showed that the method adopted to obtain the juice strongly affected yield at different pressures (Figure 1). The overall amount of juice obtained was slightly higher with the DCC method (average 69.6 kg/100 kg of grapes) than with the IC method (63.5 kg/kg w/w). There was little variability in yield at different pressures using the IC method: the minimum (18.4%) was obtained between 0 and 0.3 bar and the maximum (35.7%) between 0.3 and 0.6 bar. This was not the case, however, for the DCC method. Here, yield was highest (70.6%) at 0–0.3 bar due to grape crushing (i.e., the 32.7% was free run juice), while it was lowest (5.5%) between 0.9 and 1.2 bar. This difference in yield is mainly attributable to the destemming and crushing of grapes. In the DCC treatment, most of the juice was extracted with minimum pressure as the grape was already crushed. On the other hand, in the IC method, yield depended exclusively on grape tissue resistance, and thus, on the ripeness of the berries. Therefore, minimum pressure only extracted a small part of the juice, and a further pressure increase was required to reach an acceptable yield.

At 3 h after pressing, must obtained with the IC method showed significantly lower turbidity than must obtained with the DCC method. Trends were a function of the method (Figure 2 upper part); in the IC method, there was an almost linear increase in NTU with applied pressure, while in the DCC method, it initially increased, reaching a maximum at roughly 0.9 bar, then slightly decreased. The latter trend is consistent with the findings reported in Darias-Martín et al. [3]. Next, the obtained juices were analyzed and clarified according to the procedures described in the previous section. Juice turbidity after three days of cold settling dropped below the 200 NTU threshold defined by Ribéreau-Gayon et al. [15]. However, turbidity remained higher in DCC samples compared to IC samples (Figure 2 lower part). This is due to the smaller fraction of lees (with a higher surface/volume ratio), which are more prone to releasing herbaceous odor and bitter compounds during subsequent stages [15].

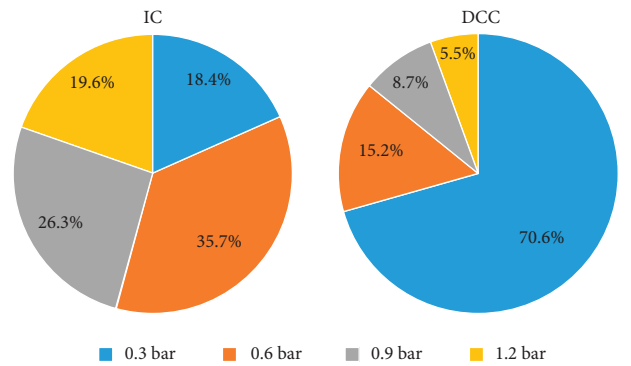

Figure 1: Juice extracted (average $n = 3$) at the different working pressures from intact clusters (IC), and after destemming and crushing (DCC).

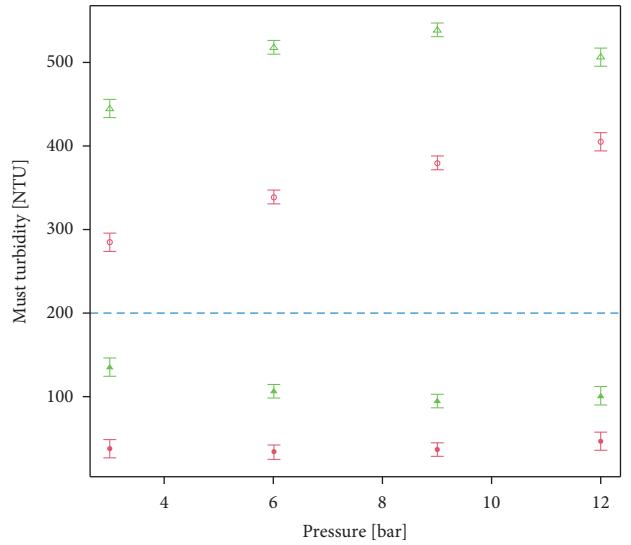

Figure 2: Juice turbidity (average $n = 3$) after 3 h of settling (top half, empty circles) and after 3 days of settling (bottom half, filled circles). Red circles represent IC juices, while green triangles show DCC juices. Error bars represent the standard error.

An assessment of the maximum equivalent diameter of the remaining lees is possible using Stoke's equation, and the geometry of the settling vessel. The maximum diameter is found as follows:

$$D = \sqrt{\frac{18\,\mu h}{t_s\left(\rho_p - \rho_j\right)g}}, \quad (1)$$

where D = the maximum equivalent diameter of suspended particles, μ = juice viscosity at 4°C (2.8 mPa*s–Friso 2018), h = height of the sedimentation vessel (0.3 m), t_s = settling time (250000 s), ρ_p and ρ_j = densities of particles and juice (1368 and 1118 kg/m^3, respectively–Friso 2018), and g = gravitational acceleration (9.81 m/s^2). The result of the calculation gives a maximum equivalent diameter of suspended particles of roughly 5 μm (surface/volume = $1.2 * 10^6$ m^{-1}). Thus, the DCC treatment produces a higher fraction of lees than the IC method, highlighting the severe effect of mechanical destemming and crushing processing on grapes.

3.2. Sugars and Ethanol. Total sugar content of the juice was significantly affected by the pressing method, the applied pressure, and their interaction (Figure 3, left plot, red circles). In the IC method, the sugar-concentration was highest in the IC03 fraction and decreased by about 5 g/L in the IC06–IC12 fractions. At the lower pressure, a small quantity of highly sugar-concentrated juice was extracted, which was then diluted by the addition of subsequent juice fractions obtained at higher pressures. This result was due to the greater sugar distribution in the central area of the berry [3]. On the other hand, in the DCC method sugar-concentration increased linearly from DCC03 to DCC12, with an average difference of 3 g/L (Figure 3, left plot, green triangles). Glucose and fructose concentrations were consistent with the general trend found for total sugars and confirmed earlier work, which shows that fructose, is slightly more concentrated than glucose [11].

Ethanol content in juices at the end of pressing was found to be zero in all fractions. As a result, spoil fermentations for any pressing treatment did not begin.

The basic composition of the finished wines is provided in supporting information (Table SII). After fermentation, all wines contained less than 1 g/L of sugars, with no significant difference due to either the pressing method or the applied pressure. Ethanol content was consistent with the findings for sugar in juices; however, it was significantly affected by the pressing method, the applied pressure, and their interaction. The highest ethanol content was found in the IC03 wine, which was around 0.5% higher than IC06–IC12 wines (Figure 3, right plot). In the IC method, ethanol content decreased as pressure increased. Conversely, in wines obtained using the DCC method, ethanol increased as applied pressure increased. Overall, ethanol content was high (above 14% v/v) in all of the produced samples (Figure 3, right plot).

3.3. Acids, pH, and Potassium. Juice pH was significantly higher in the DCC samples than the IC pressing. The difference was, on average, 0.1 pH units; hence, it cannot be considered negligible. pH rosé with the increase in pressure in DCC juices, while it remained stable in IC juices, indicating a significant interaction between the method and pressure. The former pH increase has been reported in the literature during pressing for white winemaking [10]; [3, 5], while change in pH during the pressing of intact clusters is poorly investigated. Only Yokotsuka [10] describes the pH of juice with different pressing techniques, but the reported experiment detected no differences between the destemmed system and systems with the re-addition of 50% or 100% stems.

Table 1 shows results for acidity. Consistent with pH results, total acidity was higher in juices obtained with the IC method than in those obtained with the DCC method. Furthermore, in the IC method, total acidity decreased as pressure increased. This trend may be due to the maximum extraction of acids at the beginning of pressing (IC03), followed by the extraction of more diluted fractions (from IC06 to IC12). Conversely, the DCC method is consistent with lower total acidity at the beginning of pressing, followed by fractions richer in acids as the pressure increases. At maximum pressure, total acidity values were roughly the same for both IC and DCC methods. Tartaric acid makes up most of the acids, and concentrations are consistent with total acidity results; content increased with pressure in DCC juices and decreased in IC juices. Citric acid content decreased as pressure increased in both IC and DCC methods. However, in IC juices, the decrease was more dramatic than in DCC juices. Malic acid increased with pressure in the IC method but decreased in the DCC method. These results show that the combination of method and applied pressure was able to change the juice acid profile, with a potential impact on the final rosé wine characteristics.

Potassium was higher in DCC juices and increased in both methods as pressure increased. Consistently, potassium concentrations were higher in DCC wines than in IC wines. Cation content only increased with pressure in DCC wines. At first glance, this result may appear counterintuitive since stems are rich in potassium [16]. Moreover, in IC samples, stems were present in the press, while in DCC they were removed. However, in IC, the maximum pressure of 1.2 bar was probably not high enough to compromise the stem integrity, causing potassium to escape. Conversely, the destemmer action in DCC could have damaged the stem, causing the potassium release and the pressure increase observed. The relationship between pH and potassium in juices is shown in Figure 4 ($p = 8 * 10^{-9}$, $R^2 = 0.79$). The presence of stems during pressing may negatively affect the extraction of potassium from the skins in the IC sample. In turn, potassium is responsible for the chelation of tartaric acid [5], followed by precipitation and, consequently, an increase in pH in DCC juices. Furthermore, Figure 4 clearly shows the significant effect of extraction pressure in the DCC samples for both pH and potassium.

Results for wine pH were consistent with those obtained for juice pH. Wines produced with the DCC method had a higher pH than those produced with the IC method. Furthermore, pH increased as pressure increased.

Consistent with trends for juices, total acidity was higher in IC wines (Table 1). Increased pressure decreased wine acidity slightly in DCC wines but significantly in IC wines (a significant interaction between method and pressure). The tartaric acid content in wines was consistent with the tartaric acid content in juices. A significant interaction between pressure and method was found, as tartaric acid content increased with pressure in DCC wines but decreased in IC wines. Finally, no significant difference was found for malic and citric acids in wines.

In general, two phenomena could occur simultaneously: (i) increased pressure favors the extraction of potassium from the skin and seeds, acidity falls, and pH increases in DCC samples and/or (ii) the presence of stems in the press (in the IC method) reduces the extraction effect linked to the pressing pressure. It may act like a damper, reaching the same final pressure slowly and consequently, making the action of the pressure gentler on the more fragile parts of the cluster (e.g., seeds).

3.4. Phenols and Color. Results obtained for color and phenols in juices and wines are shown in Table 2. A statistically significant interaction between pressing method

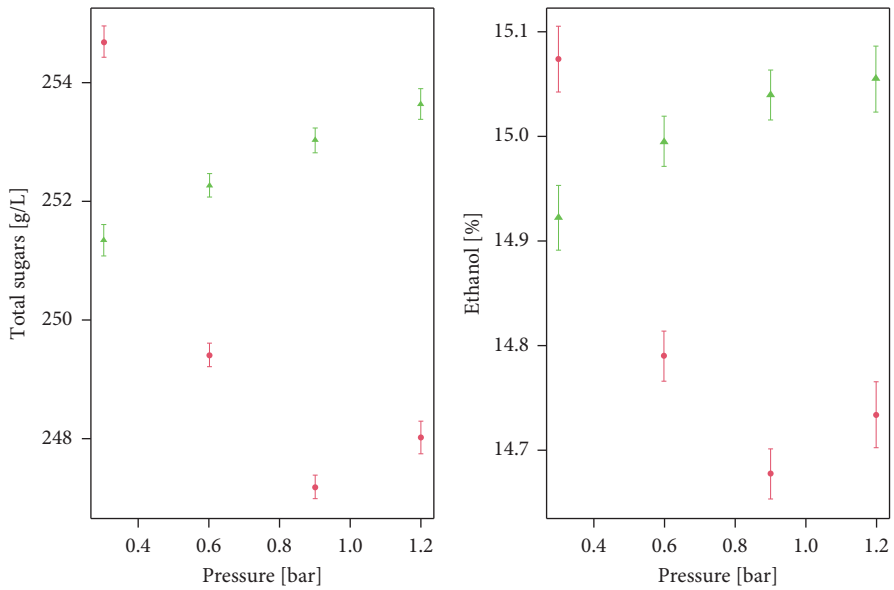

FIGURE 3: Total sugars (average $n = 3$) in juices (a), and ethanol percentage in wines (b) obtained with intact clusters (IC) and destemmed and crushed clusters (DCC) at different pressures. Green triangles represent DCC juices and wines, while red circles represent IC juices and wines. Error bars represent the standard error.

and pressure was found for anthocyanins and total polyphenols in juices. For both parameters, the DCC method resulted in high extraction, regardless of the pressure level. For the IC method, we observed the lowest anthocyanins and total polyphenol content in the IC03 condition, followed by a significant increase as the pressure increased, reaching a maximum at 0.9 bar. No further increase was found between IC09 and IC12. With respect to anthocyanins, at pressures higher than 0.9 bar, both DCC and IC samples had roughly the same content, while total polyphenols were consistently lower in the IC method compared to the DCC method.

After settling and fermentation, the obtained wines had lower anthocyanin and total polyphenols content than the respective juices. Differences for total polyphenols were negligible; while for anthocyanins the difference between IC and DCC methods was statistically significant (DCC wines had higher anthocyanins than IC wines).

The color of the resulting wines consistently changed due to the interaction between the pressing method and the applied pressure. At the lowest pressure, both IC and DCC methods had the same color intensity (no significant difference between IC03 and DCC03). The pressure increase caused an increase in color intensity in DCC wines, while no significant changes were found for IC wines. The increase in color intensity is related to the simultaneous increase in absorbance at the three considered wavelengths (420 nm, 520 nm, and 620 nm). The same trends were observed for these three variables considered individually. A significant interaction between pressing method and applied pressure was also found for wine hues (defined as the ratio OD 420/OD 520). In IC wines, hue increased with the applied pressure, reaching a maximum in IC09, then decreased. On the other hand, the hue of DCC wines was at its maximum in DCC03, and then, decreased with increased pressure. The increased pressure led to an increase in the yellowish tone (the 420 component) in IC wines, while it led to an increase in the reddish tone (the 520 component) in DCC wines. The increase of color intensity in DCC wines is consistent with the trend in DCC sugar concentration of the respective juices, thus, it can be assimilated to a different composition of the juices obtained through different grape treatment and pressing pressure, which removed the factor of grape ripeness level. According to Merrell and Harbertson [1]; the intensity of the color of wine is affected by the degree of ripeness of the grape and can evolve in a different way during aging as a function of sulfur dioxide levels.

Results for CIELab coordinates were consistent with OD measurements. Except for wines obtained at 0.3 bars, L^* was lower in DCC samples compared to IC samples. Furthermore, a decrease in L^* was found in DCC wines as a result of the pressure increase, while no significant changes were found in IC wines. On the a^* axis, the reddish tone significantly increased with pressure for DCC samples, while it slightly decreased for IC samples; b^* values were lower for IC samples than DCC samples. The values for IC samples were stable as pressure increased, while there was an increase in DCC samples. CIELab coordinates allow the calculation of color distances between samples, namely, ΔE. Distances greater than three ($\Delta E > 3$) are considered perceivable by humans [17]. Our results (Figure 5) show that IC09, IC12, and DCC03 can be considered as the same color, while DCC09 and DCC12 are quite similar in color since the distance among them is slightly above three. All of the other samples have a different color. This result demonstrates that physical methods such as pressure and destemming are able

Table 1: Acidity of the obtained juices and wines. The last 5 columns represent the results of the statistical analysis.

Parameter	DCC03	DCC06	DCC09	DCC12	IC03	IC06	IC09	IC12	GT	P	P^2	GTxP	$GTxP^2$
Juices													
Total acidity (g/L)	4.54 ± 0.03	4.52 ± 0.01	4.55 ± 0.01	4.62 ± 0.01	5.12 ± 0.13	5.09 ± 0.03	4.91 ± 0.02	4.74 ± 0.02	***	***	ns	***	**
Malic acid (g/L)	1.12 ± 0.07	1.08 ± 0.03	1.07 ± 0.02	1.01 ± 0.04	0.84 ± 0.05	1.11 ± 0.04	1.16 ± 0.07	1.13 ± 0.04	ns	**	***	***	**
Citric acid (g/L)	0.41 ± 0.01	0.40 ± 0.01	0.39 ± 0.01	0.38 ± 0.01	0.60 ± 0.01	0.47 ± 0.02	0.44 ± 0.02	0.42 ± 0.00	***	***	***	***	***
Tartaric acid (g/L)	5.26 ± 0.07	5.39 ± 0.09	5.54 ± 0.02	5.7 ± 0.03	5.81 ± 0.1	5.71 ± 0.08	5.54 ± 0.07	5.44 ± 0.03	***	ns	ns	***	ns
Wines													
Total acidity (g/L)	6.20 ± 0.05	6.05 ± 0.09	6.07 ± 0.08	6.06 ± 0.02	6.97 ± 0.07	6.45 ± 0.30	6.43 ± 0.06	6.29 ± 0.08	***	***	*	**	ns
Malic acid (g/L)	0.89 ± 0.02	0.90 ± 0.03	0.90 ± 0.01	0.91 ± 0.04	0.90 ± 0.05	0.86 ± 0.09	0.89 ± 0.05	0.88 ± 0.04	ns	ns	ns	ns	ns
Citric acid (g/L)	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.02	0.16 ± 0.01	0.17 ± 0.02	ns	ns	*	ns	ns
Tartaric acid (g/L)	3.00 ± 0.15	3.17 ± 0.14	3.19 ± 0.05	3.21 ± 0.17	3.28 ± 0.08	3.25 ± 0.12	3.19 ± 0.08	3.12 ± 0.04	ns	ns	ns	**	ns

Data are reported as average ± standard deviation of 3 replicates. Probabilities: ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. GT is the main effect of grape pretreatment, P is the main effect of pressure, P^2 is the main effect of squared pressure, GTxP is the interaction between grape treatment and pressure, and $GTxP^2$ is the interaction between grape treatment and squared pressure.

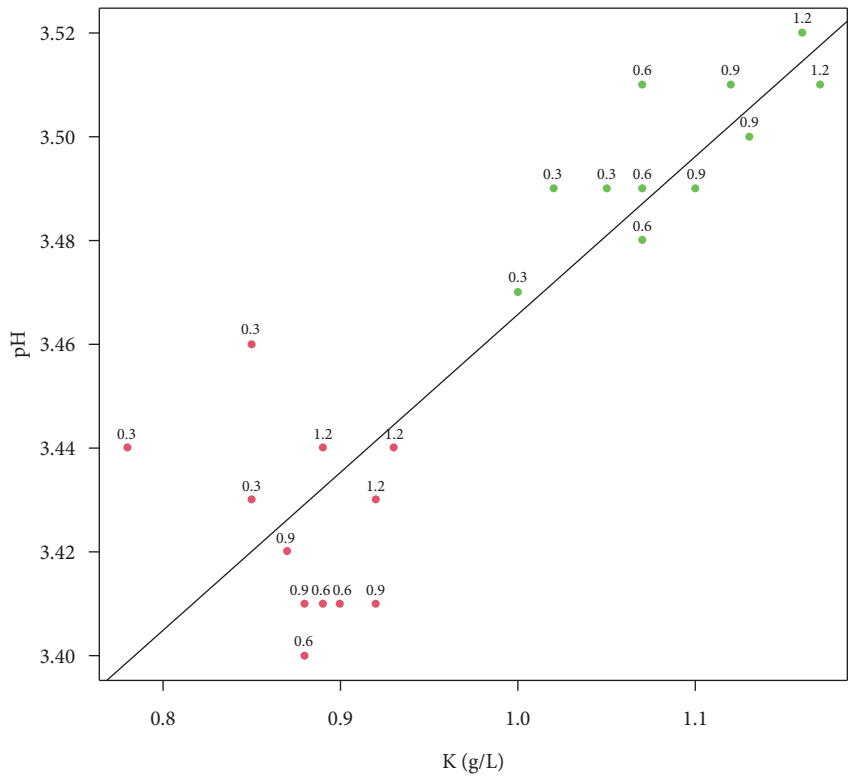

FIGURE 4: The linear relationship between extracted potassium and pH in juices ($p = 8 * 10^{-9}$, $R^2 = 0.79$). Red points represent the IC method, while green points represent the DCC method. Extraction pressure is reported above the points.

to define the color in rosé wines, avoiding the use of adjuvants or clarifying agents. Color is one of the main features of rosé wines, driving consumer choices [18, 19], and defining the wine's style [20]. Thus, it is important to identify points in the process where controls can be introduced to change the color, and quantify their impact on the finished wines.

3.5. Volatile Compounds and the Panel Test. Both pressure and destemming affected the volatile profile of the obtained wines (Table 3); in particular, a statistically significant difference was found for 21 of the analyzed compounds related to one of the main effects or their interaction. In 13 of the 21 compounds, the main effect of destemming/crushing was found; most (7 out of 13) were higher in DCC samples, while the remaining six were higher in IC samples. Specifically, p-cymene, α-terpineol, and 1-hexanol are considered to be useful indicators of the extraction of solids directly from grapes [11, 21], and these were higher in DCC wines. P-cymene is usually considered a positive terpene, associated with a balsamic-like aroma [22], while α-terpineol is a cyclic monoterpenoid related to the floral odor [21]. Their higher concentrations in DCC wines could be related to their extraction from the higher amount of small suspended solids that remained in DCC juices after settling.

Higher concentrations of 1-hexanol were consistently found in DCC wines. This lipoxygenase-related compound derives from C18 unsaturated fatty acids in grape seeds; although it is related to herbaceous notes in wines, at high concentrations it must be considered a negative compound [23]. Furthermore, 1-hexanol significantly increased with increased pressure. Its higher concentration in DCC wines could be related to the strong mechanical action of destemming and crushing, which leads to the disruption of the grape seed and enzymatic oxidation of the seed oil.

Another statistically significant compound derived from the lipoxygenase pathway was (Z)-3-hexen-1-ol. This increased with pressure, regardless of the destemming/crushing treatment. Like the previously-described compounds, it has been related to the herbaceous note [24].

Four compounds were found at higher concentrations in DCC wines, potentially due to microbial metabolism, namely, diacetyl, 1-propanol, isobutyric acid, isovaleric acid, whist acetoin, and isoamyl acetate were lower in DCC compared to IC wines. Alcohol 1-propanol is derived from glycerolipid metabolism, isobutyric, and isovaleric acids are derived from amino acid metabolism, diacetyl, and acetoin are compounds that derive from pyruvate in yeast and bacteria (e.g., acetoin is the reduction of diacetyl). No significant difference between IC and DCC samples was found for diacetyl at 0.3 bars, while at increased pressure, its concentration increased in DCC wines, but remained constant in IC wines. Conversely, two contributors to the floral note, namely, phenylethyl acetate and phenylethyl alcohol [25] were higher in IC wines than DCC wines, regardless of the effect of pressure. Furthermore, pressure enhanced the extraction of eugenol, a phenolic compound related to the spicy taste in wine.

Table 2: Color parameters in juices and wines. The last 5 columns represent the results of the statistical analysis.

Parameter	DCC03	DCC06	DCC09	DCC12	IC03	IC06	IC09	IC12	GT	P	P^2	GTxP	$GTxP^2$
Juices													
Anthocyanins (mg/L)	496.3 ± 17.4	519.3 ± 21.9	510.3 ± 3.1	513.7 ± 5.5	22.3 ± 28.0	387.7 ± 14.5	443.0 ± 7.0	475.3 ± 16.2	***	***	***	***	***
Total polyphenols (mg/L)	1250.0 ± 45.1	1330.0 ± 34.0	1428.3 ± 21.7	1412.7 ± 21.2	555.0 ± 67.4	1083.0 ± 67.4	1164.0 ± 27.2	1203.0 ± 30.0	***	***	***	***	***
Wines													
Anthocyanins (mg/L)	123.3 ± 5.7	120.0 ± 21.2	126.0 ± 16.0	133.0 ± 8.0	72.3 ± 16.1	67.3 ± 3.2	80.0 ± 9.5	95.7 ± 4.0	***	*	ns	ns	ns
Total polyphenols (mg/L)	371.0 ± 8.9	424.7 ± 46.5	458.0 ± 10.6	515.3 ± 12.5	506.7 ± 21.2	368.7 ± 13.6	380.3 ± 32.3	421.0 ± 14.4	***	*	ns	ns	ns
Color intensity	1.007 ± 0.038	1.403 ± 0.072	1.583 ± 0.012	1.727 ± 0.038	1.067 ± 0.032	0.857 ± 0.067	0.893 ± 0.015	1.020 ± 0.026	***	***	ns	***	***
Hue	2.108 ± 0.065	1.857 ± 0.003	1.695 ± 0.003	1.605 ± 0.003	1.851 ± 0.108	2.085 ± 0.054	2.145 ± 0.058	2.053 ± 0.007	***	***	*	***	***
CIELab L*	52 ± 1	50 ± 1	47 ± 0	45 ± 1	50 ± 2	54 ± 1	53 ± 1	51 ± 2	***	***	**	***	**
CIELab a*	23 ± 1	26 ± 1	30 ± 1	32 ± 1	28 ± 2	24 ± 1	24 ± 1	25 ± 1	***	***	**	***	***
CIELab b*	17 ± 1	21 ± 1	21 ± 1	23 ± 0	17 ± 1	15 ± 1	16 ± 1	17 ± 1	***	***	ns	***	**

Data are reported as average ± standard deviation of 3 replicates. Probabilities: ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. GT is the main effect of grape pretreatment, P is the main effect of pressure, P^2 is the main effect of squared pressure, GTxP is the interaction between grape treatment and pressure, and $GTxP^2$ is the interaction between grape treatment and squared pressure.

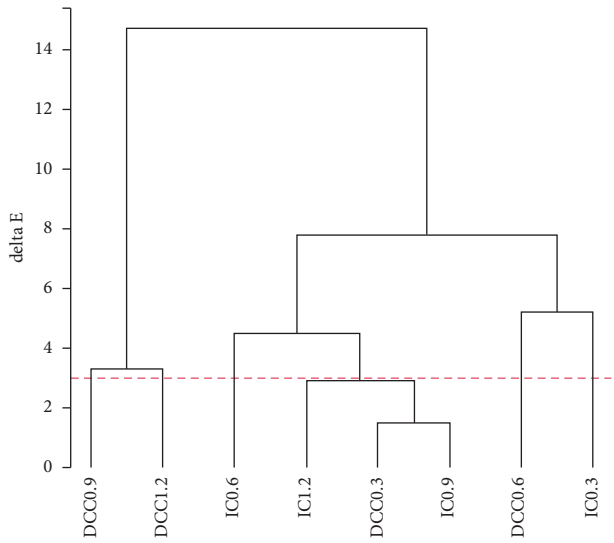

Figure 5: Distance between wine colors measured as ΔE. Distances above 3 (i.e., above the red dashed line) indicate visible differences [17].

Both DCC and IC methods impacted the final taste of the produced wines in terms of acid, astringency, floral, and fruity attributes. Consistent with the findings reported above for acids, pH, and potassium, IC wines were perceived as more acid than DCC wines by judges (Figure 6). Here again, a statistically significant interaction between destemming/crushing and pressure was found. IC wines increased in Astringency with increasing pressure. The softer treatment of pressing intact grapes reduced astringency in IC03 wines, and then, it increased as the mechanical action increased. Conversely, DCC grapes were exposed to strong mechanical action, and the wines that were perceived as most astringent were produced at 0.3 bar. Increased pressure and the release of more juice from the berries diluted the juice and decreased the initial sensation. Floral intensity was found to be higher in DCC wines than IC wines. Furthermore, in both methods floral intensity increased with pressure, reaching a maximum at 0.9 bar, before decreasing in the IC12 and DCC12 wines. Finally, a statistically significant difference was found at pressures above 0.9 bar for the fruity attribute. At high pressure, DCC wines were said to be significantly fruitier than IC wines.

3.6. Grouping Data by Principal Component Analysis. A principal component analysis (PCA) was run to visually inspect the data and identify clusters (Figure 7). The first latent variable (PC1), explaining 42.7% of total variance, was able to discriminate wines produced with IC and DCC methods. The only exception was DCC03 wines. Variables with the highest positive loadings were total acidity, hue, and astringency, which characterized ICC and DCC03 wines. Conversely, variables with the highest negative loadings were ethanol, pH, color intensity, anthocyanin, potassium, total polyphenols, and floral and fruity attributes, which characterized DCC06–DCC12 wines. Thus, in rosé wine production, the choice of working with intact clusters, or to de-stemming and crush grapes leads to wines with different characteristics, regardless of the applied pressure.

The second latent variable (PC2) explained 15.9% of the total variance. PC2 discriminated among the applied pressures. IC03 samples had negative values for PC2. As the pressure increased, values for IC samples increased, moving from the bottom to the top of the plot. Conversely, DCC03 samples had the highest scores among all DCC samples on PC2. As pressure increased, DCC scores decreased, and DCC samples moved from the top to the bottom of the plot. Variables with the highest PC2 positive loadings were pH, anthocyanins, and astringency, which increased as pressure increased in IC wines and decreased as pressure increased in DCC wines. Conversely, variables with the highest negative loadings on PC2 were total acidity, tartaric acid, and total polyphenols. These attributes increased with pressure in DCC wines but decreased in IC wines. Considering both PC1 and PC2, IC03, and DCC12 were most similar, although produced with different treatments, while IC12 and DCC03 were most different.

4. Discussion

To understand the results of this study, it is important to keep in mind the different cell wall consistencies and the different chemical composition of the different zones of a ripe grape berry, as presented in the work of Darias-Martín et al. [3]. The periphery of the berry is rich in polyphenols and mineral salts, and here the cell walls have the highest consistency. Conversely, the intermediate zone of the berry has the lowest consistency and is rich in sugars and tartaric acid. Finally, the central zone (i.e., the pulp around the seeds and the fibrovascular bundle) is rich in sugars and malic acid, and its consistency is midway between the periphery and the intermediate zone. Depending on the adopted grape processing method, these differences in consistency define the style of the obtained rosé wine.

In the IC method, the first juice release is rich in sugars and tartaric acid, from the intermediate zone of the *Sangiovese* berries. Then, as pressure increases, there is an increase in malic acid (from the inner zone), astringency, and hue (from the periphery). Conversely, grapes processed using the DCC method undergoes strong mechanical action, leading to the extraction of most of the juice at 0.3 bar. The produced juices had higher turbidity due to small solid particles. In this case, all of the zones of the berry were extracted simultaneously. Hence, observed differences for different pressures were due to the contact between the juice and solids, either in the press or during fermentation. Moreover, the interaction between the presence of stems and the pressure level during pressing affects the extraction of acids and potassium, and consequently, on juice pH. In terms of practical application, the results show the grape treatments can lead to obtaining wines with different pH, acidity, ethanol content, sensory profile and color as well, starting from the same grape, i.e., the same degree of ripeness. This also includes other oenological aspects linked to the addition of chemical additives and adjuvants, such as sulfur dioxide, in order to control the alcoholic fermentation and aging stages.

Table 3: Volatile organic compounds in wines found to be significantly different as a function of prepressing treatment and/or pressing pressure.

Compound	DCC03	DCC06	DCC09	DCC12	IC03	IC06	IC09	IC12	GT	P	P^2	GTxP	$GTxP^2$
1-propanol (mg/L)	2.27 ± 0.06	1.68 ± 0.25	1.77 ± 0.22	1.68 ± 0.23	1.80 ± 0.11	1.77 ± 0.20	1.54 ± 0.01	1.64 ± 0.13	*	**	*	ns	ns
1-propanol-2-methyl (mg/L)	51.8 ± 5.67	41.12 ± 2.21	41.39 ± 1.29	43.54 ± 3.80	43.83 ± 2.99	45.11 ± 7.01	43.45 ± 3.23	42.12 ± 2.73	ns	*	ns	ns	*
Isoamyl acetate	136.50 ± 23.18	53.89 ± 9.01	36.34 ± 6.00	102.8 ± 17.52	286.60 ± 58.41	234.00 ± 48.50	186.50 ± 29.06	176.00 ± 3.63	*	ns	ns	ns	ns
1-butanol (mg/L)	3.14 ± 0.18	3.24 ± 0.34	3.02 ± 0.12	3.19 ± 0.25	3.67 ± 0.30	3.23 ± 0.48	3.33 ± 0.17	2.95 ± 0.20	ns	*	ns	*	ns
1-butanol-3-methyl (mg/L)	396.20 ± 7.42	344 ± 34.14	359.30 ± 18.05	372.20 ± 14.06	371.80 ± 15.85	364.00 ± 29.54	372.50 ± 19.86	356.60 ± 4.82	ns	ns	ns	ns	*
Diacetyl (mg/L)	31.38 ± 2.99	54.20 ± 13.47	65.89 ± 18.60	84.63 ± 18.42	45.23 ± 14.49	39.11 ± 6.73	39.39 ± 9.51	40.46 ± 9.36	**	**	ns	***	ns
Acetoin (mg/L)	1.49 ± 2.51	1.38 ± 2.33	4.41 ± 6.83	2.05 ± 3.39	7.18 ± 5.38	7.66 ± 4.06	4.10 ± 4.58	3.12 ± 1.91	*	ns	ns	ns	ns
p-cymene (μg/L)	57.31 ± 97.20	44.48 ± 75.16	26.52 ± 42.85	90.49 ± 153.80	1.56 ± 0.13	1.34 ± 0.12	1.41 ± 0.11	1.37 ± 0.24	*	ns	ns	ns	ns
1-hexanol (mg/L)	2.18 ± 0.02	2.40 ± 0.16	2.50 ± 0.09	2.72 ± 0.32	1.26 ± 0.09	1.62 ± 0.09	1.84 ± 0.06	1.91 ± 0.06	***	***	ns	ns	ns
(Z)-3-hexenol (mg/L)	0.13 ± 0.00	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.16 ± 0.00	0.17 ± 0.01	ns	***	ns	ns	ns
Isobutyric acid (mg/L)	1.49 ± 0.55	1.48 ± 0.63	1.77 ± 0.85	1.69 ± 0.69	1.15 ± 0.09	1.25 ± 0.23	1.25 ± 0.18	1.27 ± 0.16	*	ns	ns	ns	ns
Ethyl decanoate (mg/L)	15.58 ± 6.39	19.59 ± 8.13	15.13 ± 0.88	38.96 ± 17.29	38.08 ± 3.74	55.91 ± 39.77	28.79 ± 11.18	26.93 ± 10.30	*	ns	ns	*	ns
Isovaleric acid (mg/L)	28.56 ± 12.50	35.73 ± 14.69	37.89 ± 17.78	38.68 ± 17.79	28.85 ± 3.61	25.42 ± 2.09	27.21 ± 2.04	24.77 ± 3.12	*	ns	ns	ns	ns
Diethyl succinate (mg/L)	2.65 ± 0.33	2.36 ± 1.00	2.45 ± 1.30	6.39 ± 3.45	5.55 ± 1.36	4.70 ± 1.79	4.32 ± 0.11	2.79 ± 1.12	ns	ns	ns	**	*
α-terpineol (mg/L)	0.13 ± 0.03	0.10 ± 0.02	0.11 ± 0.01	0.11 ± 0.03	0.09 ± 0.00	0.09 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	*	ns	ns	ns	ns
Phenylethyl acetate (mg/L)	19.90 ± 5.43	13.71 ± 7.60	12.79 ± 6.26	13.88 ± 6.83	24.65 ± 3.74	24.43 ± 8.43	25.44 ± 7.94	25.38 ± 3.42	***	ns	ns	ns	ns
Hexanoic acid (mg/L)	3.41 ± 0.53	4.30 ± 0.29	4.55 ± 0.35	4.79 ± 0.53	4.11 ± 0.73	4.87 ± 0.45	4.80 ± 0.34	4.28 ± 0.48	ns	**	*	*	ns
Phenylethyl alcohol (mg/L)	88.84 ± 18.56	83.85 ± 17.16	90.79 ± 23.42	97.61 ± 22.07	119.10 ± 16.73	102.50 ± 5.93	112.20 ± 4.57	98.00 ± 8.24	*	ns	ns	ns	ns
4-ethyl-guaiacol (mg/L)	0.02 ± 0.00	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.14 ± 0.10	0.02 ± 0.00	0.02 ± 0.00	ns	ns	*	ns	ns
Octanoic acid (mg/L)	6.48 ± 1.46	7.18 ± 1.60	7.56 ± 1.68	8.01 ± 2.20	8.15 ± 1.07	8.75 ± 0.48	9.63 ± 0.50	8.53 ± 0.90	*	ns	ns	ns	ns
Eugenol (mg/L)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0	ns	*	ns	ns	*

Data are reported as average ± standard deviation of 3 replicates. Probabilities: ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. GT is the main effect of grape pretreatment, P is the main effect of pressure, P^2 is the main effect of squared pressure, GTxP is the interaction between grape treatment and pressure, and $GTxP^2$ is the interaction between grape treatment and squared pressure.

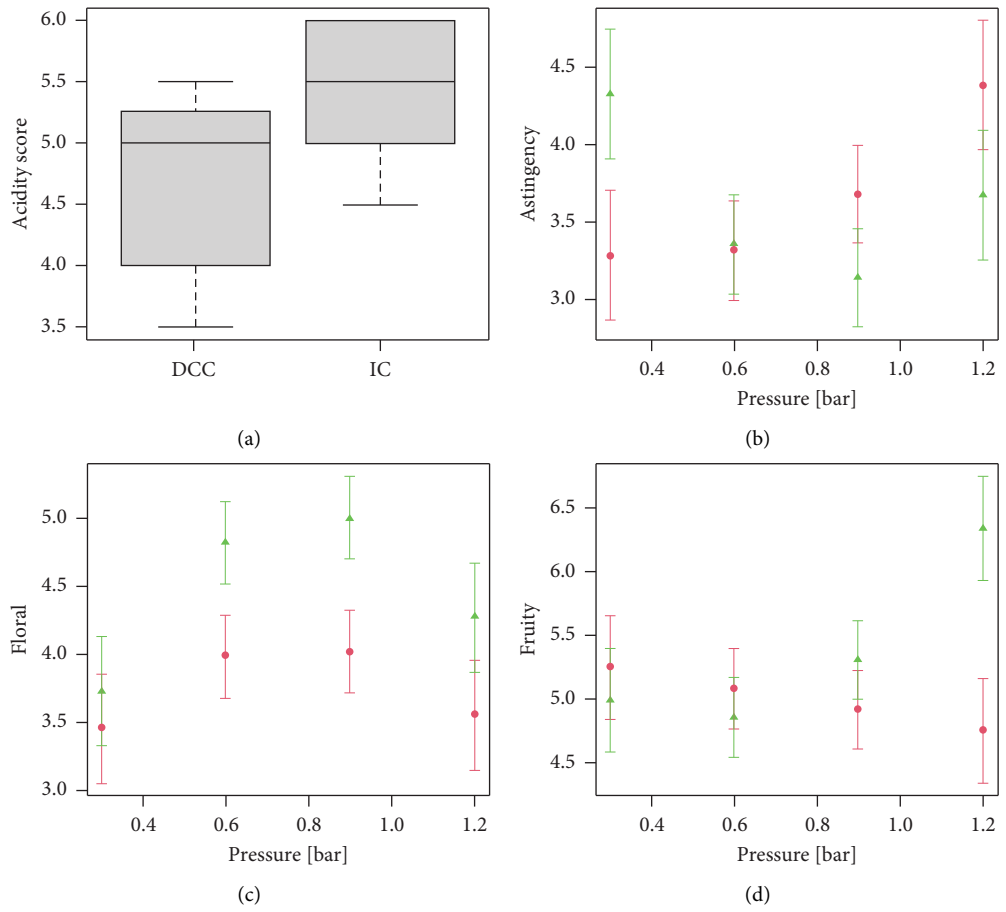

FIGURE 6: Statistically significant scores (average $n = 3$) for acidity (a), astringency (b), floral (c), and fruity (d) attributes from the sensory panel. Green triangles represent DCC wines, while red circles represent IC wines. Error bars represent the standard error.

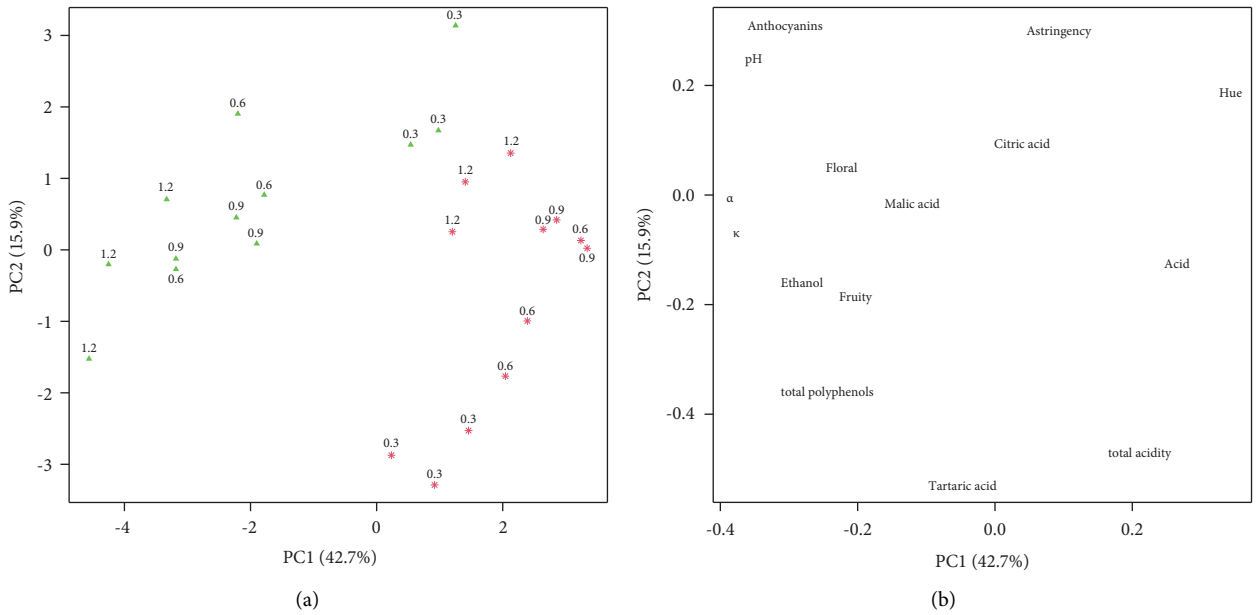

FIGURE 7: PCA plots (a) and loadings (b). Red points represent the IC method, while green points represent the DCC method. Extraction pressure is reported above the points.

5. Conclusions

Our experiment demonstrates that the choice of the operative protocol for rosé wine production deeply affects the characteristics of the obtained wines. Our results confirm what is already well-known in white wine production, namely, that the most important differences are related to the choice of working with intact clusters or after destemming and crushing. Our work also highlights that the applied pressure determines the final product's characteristics, notably, through a strong interaction with the destemming-crushing choice previously made. Overall, these mechanical strategies make it possible to change the main qualitative characteristics of rosé wine.

Moreover, we show that different combinations of crushing and pressure can change key features such as color, acidity, and astringency in wines. In oenology, several chemical additives or adjuvants are available to control these features. However, our findings indicate that it is possible to control the rosé-style using only physical and mechanical action; this dramatically reduces (or even avoids) the use of chemicals and makes the technique particularly interesting for organic wine producers. The choice of the operative protocol could also lead to the production of different wines from the same grape batch (e.g., a premium and a low-priced wine). Being able to control both yield and chemical composition could help all wine producers to optimize their income according to the company's commercial priorities.

Data Availability

The data used to support the findings are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All the authors have significantly contributed to the making of the research paper and they are in agreement with the manuscript.

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Supplementary Materials

Table SI: list of attributes and standard recipes used for sensory descriptive analysis and ANOVA table. Table SII: basic composition of wines obtained from different grape treatment and different pressing pressure. Data are reported as average ± standard deviation of 3 replicates. (*Supplementary Materials*)

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