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Influence of pressing conditions on the chemical composition, oxygen consumption rate, and tyrosinase activity of the grape must

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ABSTRACT

Pressing is a key step in white winemaking that directly influences juice yield, chemical composition, and the oxidative stability of grape must. Among commonly used techniques, direct pressing (DP) and pressing after crushing (PaC) differ in their mechanical treatment and impact on must quality. This study compares these two methods. Our results show that PaC achieved a higher juice yield and greater mechanisation efficiency but also produced musts with higher turbidity, increased phenolic content, lower potential ethanol levels, lower titratable acidity, and greater oxidability. Specifically, PaC musts showed higher concentrations of hydroxycinnamic acids and a faster oxygen consumption rate despite exhibiting lower tyrosinase activity than DP musts. The results suggest that substrate availability plays a more critical role in must oxidation than enzyme activity alone.

KEYWORDS: grape pressing, grape berry must, enzymatic browning, oxygen consumption, tyrosinase activity



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INTRODUCTION

Grape pressing is a critical step in winemaking, particularly for white and sparkling wines, as it determines key qualitative attributes of both the must and the final product (Del Fresno *et al.*, 2021; Shanshiashvili *et al.*, 2024). The fractionation of grape must during pressing significantly influences parameters such as titratable acidity, pH, phenolic compounds concentration, coarseness, herbaceous character, colour and the oxidation level of both the grape must and the final wine (Godinot, 1718; Blanck & Valade, 1989; Hardy, 1990; Ferreira-Lima *et al.*, 2016; Jégou *et al.*, 2017; Marchal *et al.*, 2022a, Marchal *et al.*, 2024a). In sparkling wine production, this step is particularly significant due to its impact on the foaming properties of the base wine (Maujean *et al.*, 1990; Poinaut, 1991), which are closely linked to variations in protein content throughout the pressing cycle (Marchal *et al.*, 2019).

A full pressing cycle consists of alternating pressure increases and decreases, along with periodic pomace breakups, which result in notable changes in must composition throughout the process (Jégou *et al.*, 2017; Marchal *et al.*, 2022a; Marchal *et al.*, 2022b). Among the types of presses available, pneumatic presses and, to a lesser extent, traditional vertical presses historically used in the Champagne region (Godinot, 1718), are those most widely used for high-quality sparkling wine production. Both systems allow for a gradual build-up of pressure, which helps preserve the integrity of the pomace and limits the extraction of phenolic compounds from the skins (Ribéreau-Gayon *et al.*, 2006a; Jackson, 2008). As a result, a larger proportion of the press juice can be retained for use in premium wines.

Grapes may be pressed as whole clusters or subjected to crushing, and occasionally destemming, prior to pressing. Direct pressing of intact bunches minimises maceration, a critical factor in the production of Blanc de Noirs, where limiting colour extraction is essential. This method is mandatory in Champagne, where red varieties such as Pinot noir and Pinot meunier dominate, to avoid excessive anthocyanin extraction. Crushing before pressing facilitates faster drainage during filling and can increase press capacity by 30–50 % while also accelerating the pressing itself. However, it generally results in a more turbid must, slightly lowers the yield of clarified juice after settling, and may increase oxidative potential due to greater oxygen dissolution and exposure of internal grape tissues (Lukić *et al.*, 2019). Destemming is typically discouraged, as stems aid drainage and expedite pressing (Ribéreau-Gayon *et al.*, 2006a; Guerrini *et al.*, 2022). In mechanically harvested fruit, destemming may be advisable to avoid foreign matter that could damage press membranes (Hendrickson *et al.*, 2016). Nevertheless, mechanical harvesting is generally unsuitable for high-quality sparkling wine production, as it compromises berry integrity and complicates must fractionation while increasing the risk of oxidation.

The type of harvest, transport conditions, press type, and pressing parameters can significantly influence the extraction

and oxidation of phenolic compounds, thereby affecting wine quality in multiple ways. Oxidation levels in grape must are determined by oxygen exposure, phenolic content, polyphenol oxidase (PPO) activity, and the presence or absence of PPO inhibitors such as sulfur dioxide, ascorbic acid, glutathione, and oenological tannins. Sulfur dioxide, in particular, is widely employed in winemaking due to its dual role as a potent PPO inhibitor and antimicrobial agent (Ribéreau-Gayon *et al.*, 2006a; Queiroz *et al.*, 2008; Bustamante *et al.*, 2024).

Two polyphenol oxidases are involved in the oxidation of phenolic compounds in grape must: tyrosinase (EC 1.14.18.1), which is naturally present in grapes, and laccase (EC 1.10.3.2), which occurs only in grapes infected by *Botrytis cinerea* (Oliveira *et al.*, 2011). These enzymes catalyse the oxidation of ortho-diphenols into ortho-diquinones, which then undergo polymerisation to form melanins. While the initial diquinones are colourless, the melanins formed through subsequent chemical reactions exhibit a yellow to brown colouration. This process contributes to browning in white wines and colour degradation in red wines. Blanc de Noirs sparkling wines are particularly susceptible to enzymatic oxidation due to their low anthocyanin content. Glutathione, a naturally occurring compound in grape must, can react with diquinones to form 2-S-glutathionylcaftaric acid, also known as grape reaction product (GRP). GRP is colourless and inhibits the polymerisation of diquinones into melanins, thereby mitigating browning in wine (Cheynier *et al.*, 1990; Singleton & Kramling, 1976; du Toit *et al.*, 2006; Kritzing *et al.*, 2013).

Numerous studies have explored how press type and pressing conditions influence grape must composition and, ultimately, wine quality (Kinzer & Schreier, 1980; Yokotsuka, 1990; Somers & Pocock, 1991; Cheynier *et al.*, 1993; Darias-Martínez *et al.*, 2004; Leblanc *et al.*, 2008; Pate *et al.*, 2010; Ferreira-Lima *et al.*, 2016; Jégou *et al.*, 2017; Guerrini *et al.*, 2022). In particular, increasing attention has been paid to understanding how pressing methods and must fractionation affect must oxidability. Some studies have examined the impact of pressing on oxidation processes (Cheynier *et al.*, 1993; Ferreira-Lima *et al.*, 2016; Catania *et al.*, 2019; Day *et al.*, 2019; Marchal *et al.*, 2024b), while others have focused on changes in reduced glutathione concentration, a compound known for its antioxidant and anti-browning properties, under different pressing conditions (Patel *et al.*, 2010; Motta *et al.*, 2014; Pons *et al.*, 2015; Fracassetti & Tirelli, 2015). However, to the best of our knowledge, no prior research has investigated how pressing parameters influence tyrosinase activity and oxygen consumption across the various juice fractions. The aim of this study is to evaluate the effects of pressing conditions on oxygen consumption rate and tyrosinase activity in grape must.

MATERIALS AND METHODS

1. Chemicals

Polyvinylpolypyrrolidone (PVPP), iron (III) chloride hexahydrate (FeCl₃·7H₂O, purity ≥ 99.5 %), D-glucose (purity ≥ 99.5 %) and fructose (purity ≥ 99.5 %) were

purchased from Sigma-Aldrich (Madrid, Spain). Caftaric acid (purity $\geq 99.9\%$) was purchased from Biosynth S.R.O. (Bratislava, Slovakia). Sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$, purity $\geq 99.5\%$), L-(+)-tartaric acid (purity $\geq 99.5\%$), sodium hydroxide (purity $\geq 99.5\%$), copper (II) sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, purity $\geq 99.5\%$), acetonitrile (purity $\geq 99\%$), formic acid (purity $\geq 99\%$), and methanol (purity $\geq 99\%$) were purchased from Panreac (Barcelona, Spain).

2. Grape harvest

Parellada grapes were harvested manually from the vineyard of Universitat Rovira i Virgili (Mas dels Frares, Constantí, Tarragona: 41° 08' 44.1" N 1° 11' 51.0" E) during the 2024 vintage, ensuring optimal maturity (22.3 °Brix; titratable acidity 5.2 g of tartaric acid/L) and avoiding grapes affected by grey rot. The harvested grapes were transported to the winery for further oenological practices.

3. Pressing

3.1. Direct pressing

A total of 570 kg of whole grape bunches were directly loaded into a pneumatic press (M5, Marzola, Navarrete, Spain). Six consecutive pressing cycles were performed until the extraction yield obtained in a cycle was below 0.05 L/kg. The pressure ramp across the different cycles was as follows: C1: 0.2 bar; C2: 0.4 bar; C3: 0.6 bar; C4: 0.8 bar; C5: 1.0 bar; and C6: 1.6 bar. The volume of must collected during each cycle was quantitatively measured. A 1 L sample was taken from each pressing cycle in 1-L Pyrex bottles previously filled with argon to minimise the oxidation rate, and the must was immediately saturated with argon by direct bubbling.

3.2. Pre-pressing crushing

A total of 785 kg of whole grapes were crushed prior to pressing using a destemmer (Delta E2, Bucher Vaslin, Chalonnes-sur-Loire, France). The crushed grapes were then loaded into a pneumatic press (M5, Marzola, Navarrete, Spain) and pressed in six consecutive cycles until the extraction yield obtained in a cycle was below 0.05 L/kg. The pressure ramp across the different cycles was similar to that used for direct pressing. The must obtained by free-run draining, as well as that collected during each of the six pressing cycles, was quantitatively measured. A 1 L sample was taken from the free-run draining and from each pressing cycle following the methodology described above.

4. Analysis of general parameters

Turbidity was measured for each sample using a turbidimeter (2100N IS Turbidimeter; HACH, Loveland, CO, USA). Potential ethanol content, titratable acidity and pH were determined according to the methods recommended by the International Organisation of Vine and Wine (OIV, 2023). The total phenolic index (TPI) was determined by measuring absorbance at 280 nm, expressed as absorbance units (Ribéreau-Gayon *et al.*, 2006b). L-malic was measured enzymatically using the procedure determined for the Y-15 Autoanalyser (Biosystems, Barcelona, Spain).

5. Measurement of oxygen consumption

Oxygen consumption for each sample was monitored in triplicate using a luminescence-based oximeter (Nomasense™ O2 Trace Oxygen Analyzer, Nomacore, Thimister Clermont, Belgium) and 66 mL glass flasks equipped with an oxygen sensor spot (PreSens Precision Sensing GmbH, Regensburg, Germany), according to the methodology described by Diéval *et al.* (2011). Samples were diluted five-fold in a synthetic buffer to ensure accurate measurement of oxygen consumption, since undiluted must consumes oxygen rapidly, making reliable determination almost impossible. The synthetic buffer contained 100 g/L D-glucose, 100 g/L fructose, 4 g/L tartaric acid, 3 mg/L iron in the form of iron (III) chloride hexahydrate, and 0.3 mg/L copper in the form of copper (II) sulphate pentahydrate. The pH was adjusted to 3.5 by adding NaOH 1M. The diluted must was saturated in dissolved oxygen (roughly 8 mg/L) by vigorous shaking, and the dissolved oxygen concentration was measured periodically until asymptotic behaviour was observed or values below 1 mg/L were reached. Samples were then supplemented with 50 mg/L SO_2 to prevent further colour evolution.

The oxygen consumption rate at time zero (OCRT0) was determined to compare the oxidation rate of the samples using the following mathematical model: oxygen consumption kinetics were fitted to a quadratic polynomial model, and the first derivative at time zero was calculated to obtain OCRT0 according to the method proposed by Giménez *et al.* (2023).

6. Measurement of tyrosinase activity

Tyrosinase activity was assessed using the method previously established by our research group (García-Roldán *et al.*, 2025). The procedure can be summarised as follows: must samples obtained from each pressing cycle were discoloured by centrifugation and percolation through PVPP columns until a Total Polyphenol Index (TPI) lower than 2 units was achieved, following the methodology reported by Ribéreau-Gayon *et al.* (2006b). Absorbance was measured at 280 nm in a 10 mm optical path length quartz cuvette. Practically, 1.8 mL of decolourised grape must were placed in 10 mm optical path length spectrophotometer microcuvettes. The decolourised must was supplemented with caftaric acid at a concentration of 2 mM, which is known to be sufficient to saturate the enzyme (García-Roldán *et al.*, 2025). The total volume was adjusted to 2 mL with distilled water. After manual agitation to ensure homogenisation and oxygen saturation, absorbance at 420 nm was periodically measured to monitor the formation of brown pigments until asymptotic behaviour was observed, using a UV-Vis Helios Alpha™ spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The experiments were performed in triplicate at 25 °C, and tyrosinase activity was calculated from the reaction rate of the linear increasing phase.

7. Colour determination

Colour was determined by direct measurement of absorbance at 420 nm, which indicates the intensity of browning, and

by calculating the CIEL*a*b* coordinates following the methodology described by Ayala *et al.* (1997). The total colour difference between samples (ΔE_{ab}^*) was calculated as the Euclidean distance between two points in the CIEL*a*b* colour space, using the following formula:

$$\Delta E_{ab}^* = \sqrt{(L^*1-L^*2)^2 + (a^*1-a^*2)^2 + (b^*1-b^*2)^2}$$

where L^* is lightness, a^* is the green-red component, and b^* is the blue-yellow component. ΔE_{ab}^* equal to or greater than three units means perceptible colour differences (Martínez *et al.*, 2001). Every experiment was performed in triplicate.

8. Quantification of hydroxycinnamic acid by HPLC-DAD

Hydroxycinnamic acids and the grape reaction product (GRP) were determined by reversed-phase HPLC-DAD, following the method described by Lago-Vanzela *et al.* (2011). An Agilent Series 1200 HPLC system (Agilent, Waldbronn, Germany) equipped with a diode array detector (DAD, G1315D) and an Agilent Chem Station (version B.01.03) were used. The samples were filtered through a 0.20 μ m polyester membrane (Chromafil PET 20/25, Machery-Nagel, Düren, Germany), and 20 μ L of the filtered sample was injected into a Zorbax Eclipse XDB-C18 column (4.6 $\times$ 150 mm). The mobile phase consisted of solvent A, composed of water:formic acid:acetonitrile at 88.5:8.5:3 volume ratios; solvent B, composed of water:formic acid:acetonitrile at 41.5:8.5:50 volume ratios; and solvent C, composed of water:formic acid:methanol at 1.5:8.5:90 volume ratios. The flow rate was set at 0.19 mL/min, and the following gradient was applied: 0–37 min, 96 % A and 4 % B; 37–51 min, 70 % A, 17 % B, and 13 % C; 51–57 min, 50 % A, 30 % B, and 20 % C; and 57–64 min, 0 % A, 50 % B, and 50 % C. Detection was carried out by measuring absorbance at 320 nm, and quantification was conducted using caftaric acid as an external standard. Compounds were identified by their retention times and UV absorption spectra. All experiments were performed in triplicate to ensure reproducibility.

9. Quantification of potassium and calcium

Calcium and potassium concentrations in the wines were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Pasvanka *et al.*, 2021; Šelih *et al.*, 2014), according to the method described by Sarlo *et al.* (2024). The samples were first diluted at a 1:3 ratio using 1 % (v/v) HNO_3 containing 10 μ g/L of indium as an internal standard. This acidic dilution ensured stable sample storage by preserving elemental composition over time and reducing the risk of mineral precipitation or adsorption onto the walls of metal-free tubes. Prior to analysis, a second dilution (1:5) with 1 % (v/v) HNO_3 was performed, yielding a final dilution factor of 1:15. This final dilution was optimised to minimise matrix effects (Catarino *et al.*, 2006). This simple sample preparation approach provided an optimal compromise between user-friendliness, analytical accuracy, and precision (Godshaw *et al.*, 2017).

The diluted wine samples were analysed using an Agilent 7850 single quadrupole ICP-MS system equipped with an integrated SPS 4 autosampler for automated sample introduction and a Micromist nebuliser. The collision cell was set to helium mode for all elements. The operating conditions are summarised in Table 1.

TABLE 1. Operating conditions of the ICP-MS instrument.

RF power	1,550 W
Sample depth	7 mm
Plasma Ar gas flow rate	15 L/min
Auxiliary Ar gas flow rate	0.9 L/min
Nebulizer Ar gas flow rate	1.05 L/min
Stabilization time	30 sec
Collision He gas flow rate	5 mL/min
Integration time per isotope	600 ms

A semi-quantitative (SQ) analysis was applied to determine the elemental composition of the wine. The SQ approach was based on two-point semi-calibration using a 28-element standard and a 1 % HNO_3 solution as a blank. As a fast and robust method, the semi-quantitative approach is particularly well-suited to the analysis of large sample series intended for subsequent statistical processing. Only the following elements were used for semi-quantification, each at a concentration of 20 μ g/L: Al, Ag, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, Tl, V, and Zn. The concentrations of the remaining elements were interpolated from those included in the calibration, using response factors that took into account their isotopic mass, isotopic abundance, and ionisation energy. Among all detected elements, only ^{39}K and ^{43}Ca concentrations were retained for interpretation, given their relevance to the analytical scope of the study. The potential of the other detected elements will be explored in a future study.

10. Statistical analysis

Results are expressed as mean values $\pm$ standard deviation of three analytical replicates. Given that only a single experimental replicate was performed for each condition due to the complexity of the experimental design, three analytical replicates were conducted for each experimental group. Under these circumstances, differences in physicochemical parameter values between experimental groups were considered relevant when they exceeded the variability observed among the three analytical replicates because the differences were greater than the analytical variability of the method. To compare the general oenological parameters, a weighted average was calculated based on the extraction yield of each pressing cycle relative to the overall extraction yield principal component analysis (PCA) were performed using IBM SPSS Statistics 19 software (International Business Machines S.A.).

RESULTS AND DISCUSSION

1. General oenological parameters

Table 2 presents the grape weight used for each pressing method, along with the corresponding volumes of must obtained during each pressing cycle. In the case of direct pressing, 570 kg of grapes were processed, whereas pressing after crushing required 785 kg. This difference is primarily attributed to the operational characteristics of the press. In direct pressing, intact grape clusters are loaded without prior crushing or destemming, resulting in minimal juice release before the start of pressing. Conversely, when grapes are crushed prior to pressing, juice begins to drain during the filling stage, necessitating a larger quantity of grapes to ensure optimal press operation.

The pressing yield results reveal notable differences between the two systems. Direct pressing (DP) of 570 kg of grapes produced 330.2 L of juice, corresponding to an extraction yield of 57.9 % (w/v). In contrast, pressing after crushing (PaC), applied to 785 kg of grapes, yielded 521.0 L of juice, with a higher extraction efficiency of 66.4 % (w/v). These findings indicate that PaC not only increases total juice volume but also improves extraction efficiency relative to grape weight. This improvement is likely due to the disruption of berry structure during crushing, which facilitates juice release and reduces pressing resistance. Overall, the data support the conclusion

that pressing after crushing offers a more effective approach for maximising juice recovery in winemaking.

The number of pressing cycles influences the origin of the extracted juice within the grape. In the initial stages, the must is primarily derived from the mesocarp, the intermediate zone of the berries. As pressing progresses, the central and peripheral regions begin to release juice, leading to greater extraction and solubilisation of skin compounds during the later cycles (Possner & Kliever, 1985; Sabir *et al.*, 2010; Shanshiashvili *et al.*, 2024).

Figure 1 shows the evolution of the general parameters as a function of the pressing type and number of pressing cycles.

Figure 1A shows that more limpid musts are obtained by direct pressing, with no relevant variations from the second cycle onward. Turbidity in the first cycle of direct pressing is markedly high due to the greater extraction of solids, followed by a substantial decline in the subsequent cycles. From the second cycle onward, turbidity levels remain relatively stable and comparable across cycles. In pressing after crushing, the free run and first fraction are more limpid, while turbidity increases as more compounds from the skin and seeds are extracted. Due to the practical difficulty of working with the full volume of pressed must from each method, a weighted average of the turbidity values across all pressing cycles was calculated. This approach allowed estimation of the overall

TABLE 2. Pressing extraction.

Pressing conditions	Grape weight (kg)	Extraction yield	C ₀	C ₁	C ₂	C ₃	C ₄	C ₅	C ₆	Total pressing process
DP	570	Vol (L)	-	50.0	55.0	55.0	70.1	70.1	30.0	330.2
		Vol (% w/v)	-	8.8	9.7	9.7	12.3	12.3	5.3	57.3
PaC	785	Vol (L)	100.1	100.5	90.3	109.9	50.1	50.1	20.0	521.0
		Vol (% w/v)	12.8	12.8	11.5	14.0	6.4	6.4	2.6	66.4

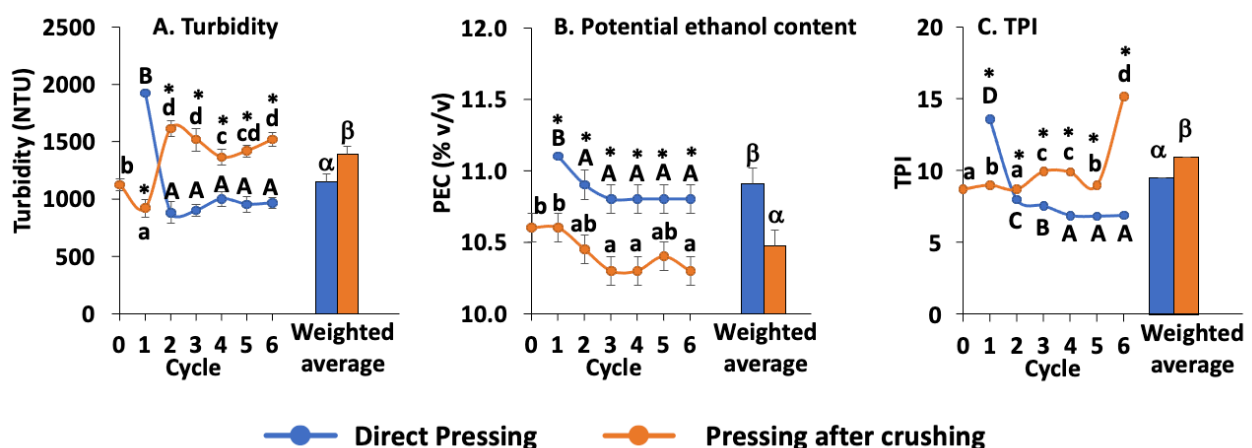


FIGURE 1. General parameters.

Results are expressed as mean $\pm$ standard deviation of three analytical replicates. PEC: Potential ethanol content expressed in percentage (% v/v), TPI: Total Phenolic Index. The presence of an asterisk (*) indicates the existence of a relevant difference between the same cycle of the two pressing conditions. Different letters indicate relevant differences between experimental conditions. Capital letters: comparison among cycles of direct pressing; lowercase letters: comparison among cycles of pressing after crushing; Greek letters: comparison between weighted averages of both pressing conditions.

turbidity representative of the total must obtained from each pressing system. A similar approach was applied to the other analytical parameters. The results show that the turbidity of the grape must obtained by PaC was relevantly higher than that obtained by DP. This higher turbidity implies a greater content of solid particles, which is likely to result in a greater loss of must after settling.

Figure 1B shows the potential ethanol content (PEC) of the various fractions. Under both pressing conditions, the PEC exhibited a marked tendency to decrease progressively as the pressing cycles advanced. This decline in PEC over successive pressing cycles is probably due to the fact that riper grapes release their contents more readily than less mature berries. Moreover, must from the intermediate zone (mesocarp) of the grape berry, which is known to contain higher sugar concentrations (Shanshiashvili *et al.*, 2024), is extracted more easily than must from the central or peripheral regions. As pressing progresses, juice is increasingly drawn from these latter zones, which are less rich in sugars, resulting in a gradual reduction in PEC. Notably, PEC values in the must obtained through DP were consistently and relevantly higher than those obtained through PaC across all pressing cycles. Consequently, the weighted average PEC of the total must produced by DP was slightly higher than that of PaC. These differences between the two pressing types can be attributed to the fact that, in DP, the juice comes primarily from the pulp of whole, uncrushed grapes. By pressing in this way, most of the must originates from the riper berries and mainly from the intermediate zone, which, as previously discussed, contains higher sugar concentration. Additionally, the different extraction yields of the two pressing systems may also contribute to this effect: 0.66 L/kg were obtained by pressing after crushing, whereas direct pressing produced only 0.58 L/kg. The lower yield of DP limits the contribution of juice from less ripe berries and from other parts of the

grape that are less rich in sugars, thus reducing dilution and resulting in a higher sugar content.

Figure 1C shows the Total Polyphenol Index (TPI) across the different cycles for both pressing conditions. In the case of direct pressing (DP), the initial TPI was very high but showed a decreasing trend as the pressing cycles progressed. In contrast, the TPI of the must drained without pressing (C0) and from the first cycle (C1) was relevantly lower than that of the first cycle of DP. However, as the pressing process advanced, the TPI of the different PaC cycles tended to increase, especially in the final cycle, becoming relevantly higher than the corresponding DP cycles. The higher TPI observed in the first cycle of direct grape pressing is probably due to the immediate release of polyphenols from the outer layers of the grape pulp and skin, which are the most exposed during the initial mechanical pressure. Although DP is relatively gentle compared to PaC, the first cycle still disrupts the cell walls of the most accessible grape tissues, especially those with higher maturity. These early extracts tend to be richer in polyphenolic compounds, particularly since no substantial dilution from less concentrated inner tissues or subsequent extraction steps has yet occurred. In any case, the extrapolation to obtain the weighted average of the total extracted must indicates that the TPI of the PaC must is relevantly higher than of the DP must. These results confirm the expected greater polyphenol extraction when the grape berries are crushed prior to pressing.

2. Acidity parameters

Figure 2 shows the evolution of titratable acidity, L-malic acid content, and pH among pressing cycles for both pressing conditions.

As shown in Figure 2A, titratable acidity decreases with successive pressing cycles in both pressing conditions, probably because the juice from the first fractions is

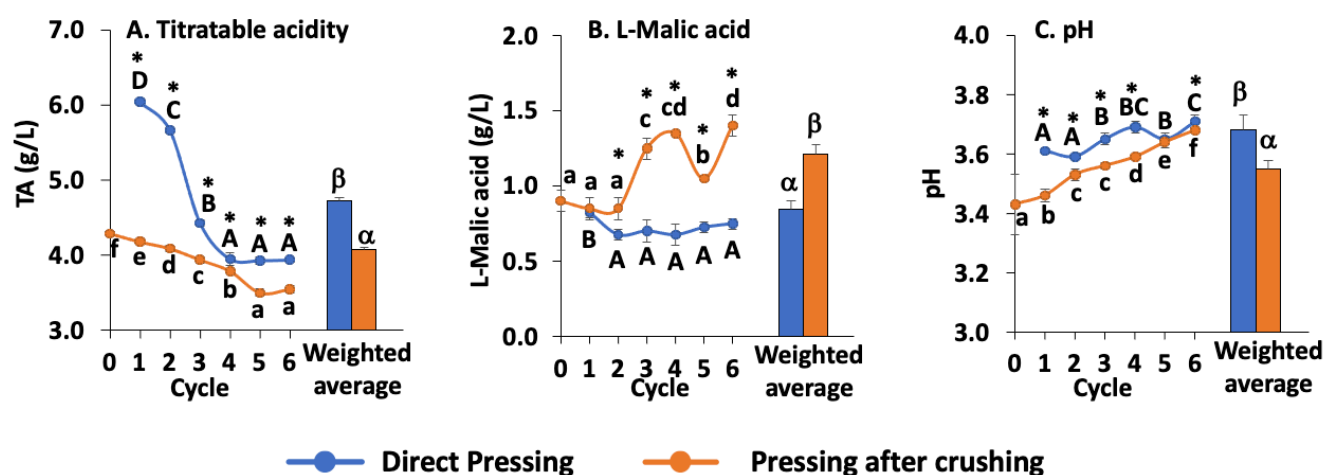


FIGURE 2. Acidity parameters.

Results are expressed as mean $\pm$ standard deviation of three analytical replicates. TA: titratable acidity expressed as g of tartaric acid/L. The presence of an asterisk (*) indicates the existence of relevant difference between the same cycle of the two pressing conditions. Different letters indicate relevant differences between experimental conditions. Capital letters: comparison among cycles of direct pressing; lowercase letters: comparison among cycles of pressing after crushing; Greek letters: comparison between weighted averages of both pressing conditions.

mainly extracted from the mesocarp, the zone of the grape that contains higher concentrations of organic acids (Ribéreau-Gayon *et al.*, 2006a), particularly tartaric acid (Shanshiashvili *et al.*, 2024). As the pressing process advances, grape must is increasingly extracted from the peripheral and central regions of the berry, which are comparatively poorer in acids. Consequently, titratable acidity tends to decrease in the later stages of pressing. Comparing the weighted average values of DP with PaC, higher titratable acidity is observed for DP, probably due to the greater extraction of skin components in PaC. Minerals, especially potassium, extracted from the skins can neutralise acids present in the must, leading to a lower titratable acidity (Boulton, 1980a).

As shown in Figure 2B, the L-malic acid content exhibited different behaviours under the two pressing conditions. In DP, a slight decrease was observed between the first pressing cycle and the subsequent cycles. In contrast, under PaC conditions, the concentration of L-malic acid clearly tended to increase starting from the third pressing cycle. This different behaviour may be attributed to the fact that, in PaC, grape must is more readily extracted from less ripe grapes, which contain higher levels of L-malic acid, as well as from the central and peripheral part of the berry, which also have elevated L-malic acid content (Shanshiashvili *et al.*, 2024). In any case, the weighted average value of L-malic acid concentration of the overall content of L-malic acid is higher for PaC than for DP. Given the higher total acidity observed in the DP, these data suggest that the concentration of tartaric acid is likely to be higher in musts obtained by DP than in those obtained by PaC.

Figure 2C illustrates the evolution of must pH throughout the pressing process under both conditions. In both cases, the pH tends to increase as pressing progresses. However, the increase in pH across the pressing cycles is more pronounced under PaC than under DP. This rise in pH may be correlated with the decrease in titratable acidity and the increase in potassium concentration, as discussed in the following

section. Across the pressing cycles, titratable acidity appears to decrease more under DP than under PaC.

Conversely, potassium concentration appears to increase more under PaC than under DP. Interestingly, and somewhat unexpectedly, the pH of the must obtained under DP was slightly but relevantly higher in almost all cycles than that of the must produced under PaC. This may be due to the well-known positive correlation between sugar and potassium accumulation during grape berry maturation (Rogiers *et al.*, 2017). Accordingly, riper grapes are likely to be pressed during the earlier pressing cycles in DP, which probably contain higher potassium content. This difference is also reflected in the final pH of the corresponding musts, as indicated by the weighted averages.

3. Potassium and calcium

Figure 3 shows the evolution of potassium and calcium contents under both pressing conditions. Potassium content (Figure 3A) was initially very high under both pressing conditions, before decreasing between the first and second pressing cycles. Under DP, potassium concentration remained relatively stable in the subsequent cycles, with a slight but relevant increase observed after the fourth cycle. In contrast, potassium concentration in PaC must showed a marked increase beginning after the third cycle. Taken together, these data indicate that the weighted average potassium concentration was relevantly higher in the must obtained from DP than that obtained from PaC. Surprisingly, these differences were not reflected in the pH values, as the must obtained through DP exhibited both higher potassium concentration and higher pH. However, it should be noted that potassium is not the only factor that influences pH (Boulton, 1980b).

The evolution of calcium concentration (Figure 3B) exhibited markedly different trends under the two pressing conditions. Under DP, calcium levels were initially very high during the first cycles, followed by a gradual decrease, whereas

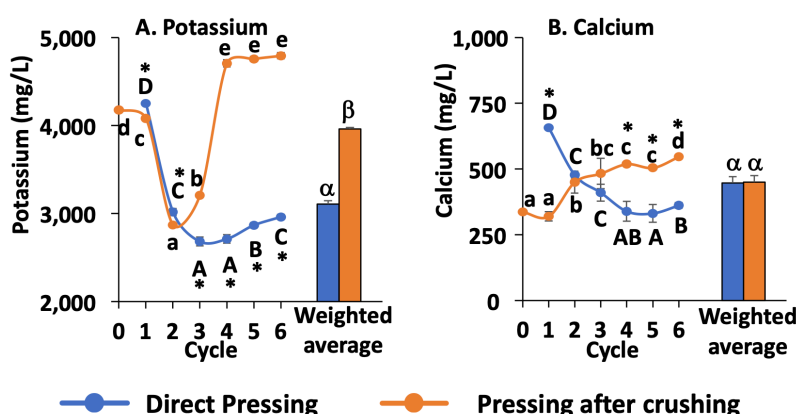


FIGURE 3. Potassium and calcium.

Results are expressed as mean $\pm$ standard deviation of three analytical replicates. The presence of an asterisk (*) indicates the existence of relevant difference between the same cycle of the two pressing conditions. Different letters indicate relevant differences between experimental conditions. Capital letters: comparison among cycles of direct pressing; lowercase letters: comparison among cycles of pressing after crushing; Greek letters: comparison between weighted averages of both pressing conditions.

in PaC must, the opposite trend was observed. Calcium is located in the peripheral zone of the grape and is therefore expected to increase with pressing cycles, as observed under PaC. The opposite trend in DP may be attributed to reduced disruption of berry skin cells. In any case, the final calcium concentration in the whole grape must (weighted average) was similar under both pressing conditions.

4. Colour parameters

Colour parameters are presented in Figure 4. In DP, the intensity of the yellow colour (A_{420} and b^*) showed minimal variation throughout the pressing process. Although initially high during the first cycle, it decreased slightly in the second cycle before experiencing a modest increase in the subsequent cycles. In contrast, both parameters were initially very low in PaC, followed by a relevant increase throughout the pressing process. This phenomenon may be explained by greater extraction of phenolic compounds, as evidenced by the TPI (Figure 1C), along with increased oxidation of the phenolics present in the must throughout the pressing cycles, which subsequently leads to the formation of brown pigments and an increase in these colour parameters. (Motta *et al.*, 2014; Queiroz *et al.*, 2008). However, the intensity of the yellow colour in the final whole must (weighted average) was similar for both pressing conditions, indicating that the pressing method does not influence the wine's final yellow intensity.

In DP, lightness (L^*) exhibited only minor variations throughout the pressing process, with a slightly but relevantly lower value observed during the first cycle compared to the subsequent cycles. In contrast, L^* was initially very high under PaC, followed by a relevant decrease as pressing progressed. Nevertheless, differences in the weighted average L^* values of the musts from the two pressing conditions were not relevant, suggesting that the overall impact of the pressing method on this colour parameter was minimal.

The green-red component of the colour (a^*) of the must obtained under DP was initially higher than that obtained under PaC. However, in DP, a^* remained largely stable after a slight but relevant increase between the first and second cycles. In contrast, for the must obtained under PaC conditions, a^* increased relevantly as the pressing process progressed.

Finally, the weighted averages of the CIEL a^*b^* coordinates were used to calculate the overall colour difference (ΔE_{ab}^*) between the musts from the two pressing conditions. The resulting value, 1.89, was clearly below the perceptibility threshold of 3 units, indicating that the difference is not discernible to the human eye. This finding suggests that, for white grapes, the pressing condition does not noticeably influence the final colour of the grape must.

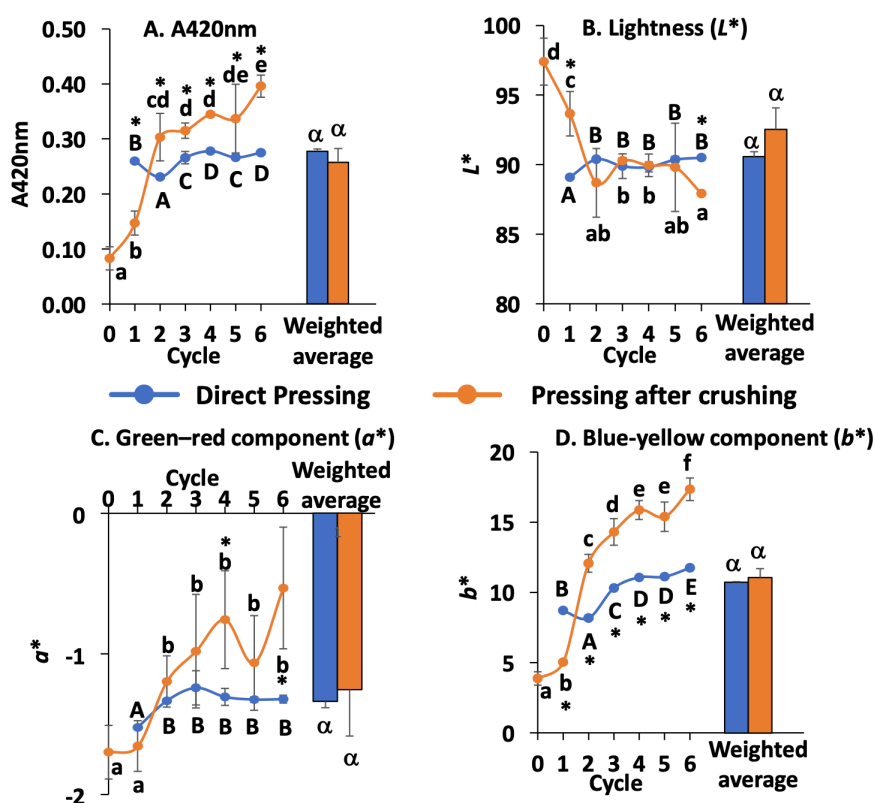


FIGURE 4. Colour parameters.

Results are expressed as mean $\pm$ standard deviation of three analytical replicates. The presence of an asterisk (*) indicates the existence of relevant difference between the same cycle of the two pressing conditions. Different letters indicate relevant differences between experimental conditions. Capital letters: comparison among cycles of direct pressing; lowercase letters: comparison among cycles of pressing after crushing; Greek letters: comparison between weighted averages of both pressing conditions.

5. Hydroxycinnamic acids

Figure 5 shows the concentrations of various hydroxycinnamic acids (HCA) and grape reaction product (GRP).

Overall, all hydroxycinnamic acids generally tended to decrease throughout the pressing process, a trend that was also observed for GRP. The decline in hydroxycinnamic acids can be attributed to progressive oxidation occurring over time during the pressing process, as previously reported (Darias-Martin *et al.*, 2004; Guerrini *et al.*, 2022; Motta *et al.*, 2014; Shanshiashvili *et al.*, 2024; Tochev *et al.*, 2022). This oxidative degradation appears to outweigh any potential increased extraction of hydroxycinnamic acids from later pressing cycles that could otherwise be expected due to greater grape tissue disruption and enhanced release from the skins (Rihák *et al.*, 2023). Notably, the intensity of the yellow colour (Figure 4), which serves as an indicator of oxidation, generally exhibited the opposite pattern.

Regarding pressing conditions, the grape must from the initial cycles of DP exhibited slightly but relevantly higher concentrations of caftaric acid (Figure 5A) and total hydroxycinnamic acids (Figure 5D), whereas fertaric acid (Figure 5C) followed the opposite trend. No substantial differences were observed in the levels of coutaric acid (Figure 5B) between pressing conditions. Taken together, these results indicate that the weighted average concentration of total hydroxycinnamic acids was slightly but relevantly

higher in the must obtained under PaC, suggesting a somewhat greater overall extraction of these compounds under this pressing condition.

As shown in Figure 5E, GRP levels also tended to decrease as the pressing process progressed under both conditions. GRP is formed through the conjugation of glutathione, either naturally present in grapes or added during winemaking, with the *o*-diquinone derivative generated via enzymatic oxidation of caftaric acid (Oliveira *et al.*, 2011). The progressive decline in GRP concentrations may therefore be attributed to the depletion of available glutathione over time. Once glutathione reserves are exhausted, further GRP formation is no longer possible, which may explain the observed decrease. Notably, GRP concentrations in grape must from the second, third, fourth, and fifth cycles of DP were slightly but relevantly higher than those observed under PaC. In contrast, the sixth cycle exhibited the opposite pattern, with higher GRP levels observed in the PaC must. Despite variations observed across individual pressing cycles, this study reports for the first time that the final weighted-average GRP content was slightly but relevantly higher in grape must obtained from PaC.

6. Tyrosinase activity

Tyrosinase is the main polyphenol oxidase in white grape must and is responsible for the enzymatic browning (Oliveira *et al.*, 2011). As this enzyme is mainly located in the chloroplasts of grape skins (Uddin Zaidi *et al.*, 2014),

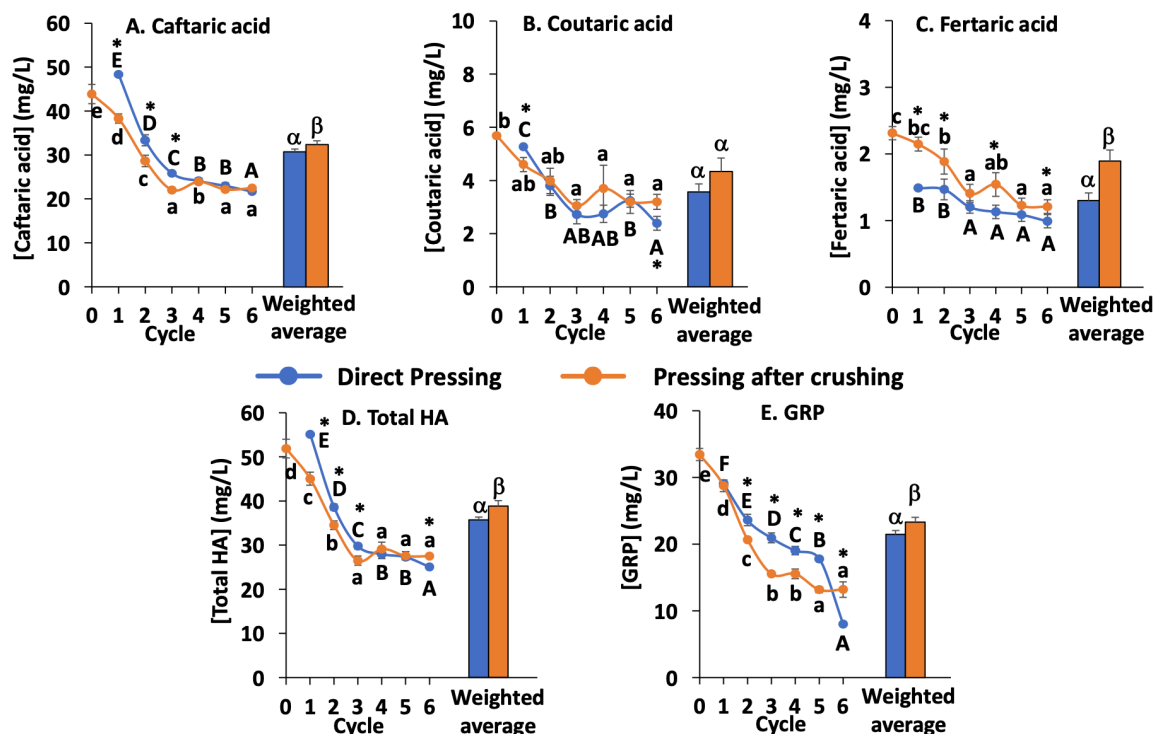


FIGURE 5. Hydroxycinnamic acids and GRP.

Results are expressed as mean (expressed as caftaric acid) $\pm$ standard deviation of three analytical replicates. DP: direct pressing, PaC: Pressing after crushing, GRP: Grape Reaction Product, HA: Hydroxycinnamic acids. The presence of an asterisk (*) indicates the existence of relevant difference between the same cycle of the two pressing conditions. Different letters indicate relevant differences between experimental conditions. Capital letters: comparison among cycles of direct pressing; lowercase letters: comparison among cycles of pressing after crushing; Greek letters: comparison between weighted averages of both pressing conditions.

apparent tyrosinase activity is expected to increase with greater skin maceration. As shown in Figure 6, tyrosinase activity increases across pressing cycles, probably due to the distribution and release dynamics of the enzyme tyrosinase during pressing.

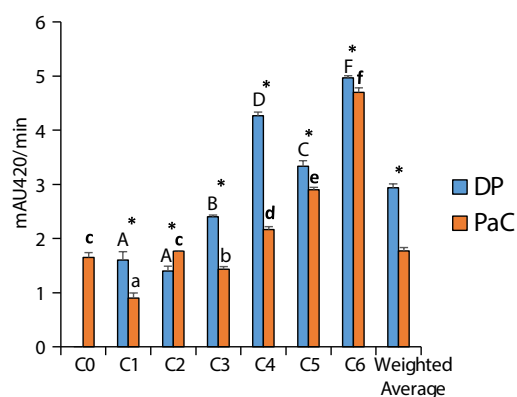


FIGURE 6. Tyrosinase activity.

Results are expressed as mean $\pm$ standard deviation of three analytical replicates. DP: Direct press; PaC: Press after crushing; Cn: Indicates the number (n) of pressing cycle. The presence of an asterisk (*) indicates the existence of relevant difference between the same cycle of the two pressing conditions. Different letters indicate relevant differences between experimental conditions. Capital letters: comparison among cycles of direct pressing; lowercase letters: comparison among cycles of pressing after crushing; Greek letters: comparison between weighted averages of both pressing conditions.

In the early pressing cycles, a substantial proportion of the enzyme remains bound within the grape skins, resulting in lower measurable tyrosinase activity in the initial juice fractions. As the pressing process advances, grape tissues undergo greater mechanical disruption, leading to increased release of intracellular enzymes and higher tyrosinase activity in the later fractions.

Interestingly, tyrosinase activity levels were consistently higher in must from most DP cycles than in must from PaC, even though DP pressing conditions are theoretically milder.

Consequently, the weighted-average tyrosinase activity was relevantly higher in DP must, which suggests a theoretical greater oxidative potential in musts produced under DP conditions. This difference may be due to the higher pH observed in DP must in comparison with PaC, since tyrosinase activity is strongly influenced by pH (García-Roldán *et al.*, 2025).

7. Oxygen consumption

Figure 7 presents the oxygen consumption rate at time 0 (OCRT0) for the various pressing cycles under both pressing conditions. In both cases, oxygen consumption exhibited a clear tendency to decrease as pressing progressed, likely reflecting the parallel decline in hydroxycinnamic acid concentrations (Figure 5) and highlighting the close relationship between oxygen consumption and the availability of these primary substrates for tyrosinase activity.

Except for the first cycle, oxygen consumption was consistently higher in the PaC cycles than in the corresponding DP cycles, leading to a relevantly greater weighted-average oxygen consumption across the must obtained via PaC. This phenomenon may also be explained by the somewhat higher concentration of hydroxycinnamic acids found in PaC musts. These observations suggest that oxygen consumption is more closely linked to the availability of tyrosinase substrates than to the enzyme's activity itself.

8. Principal component analysis

Figure 8 presents the varimax-rotated principal component analysis, showing that the two components explain an aggregate variance of 66.28 %. The parameters studied are represented by arrows whose length and direction indicate each component's contribution.

The main parameters driving this separation are potassium, calcium, TPI and L-malic acid, represented by the most vertical arrows. As discussed earlier, potassium, calcium and polyphenols are primarily located in the grape skins. Consequently, greater skin maceration during pressing after

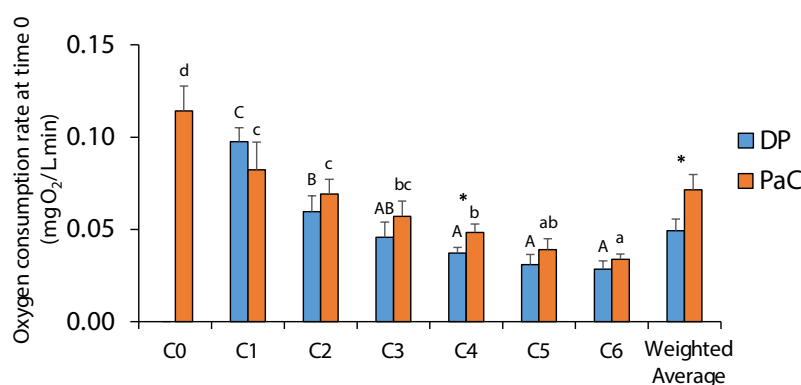


FIGURE 7. Oxygen consumption rate at time 0.

Results are expressed as mean $\pm$ standard deviation of three analytical replicates. DP: Direct press; PaC: Press after crushing; Cn: Indicates the number (n) of the pressing cycle. The presence of an asterisk (*) indicates the existence of relevant difference between the same cycle of the two pressing conditions. Different letters indicate relevant differences between experimental conditions. Capital letters: comparison among cycles of direct pressing; lowercase letters: comparison among cycles of pressing after crushing; Greek letters: comparison between weighted averages of both pressing conditions.

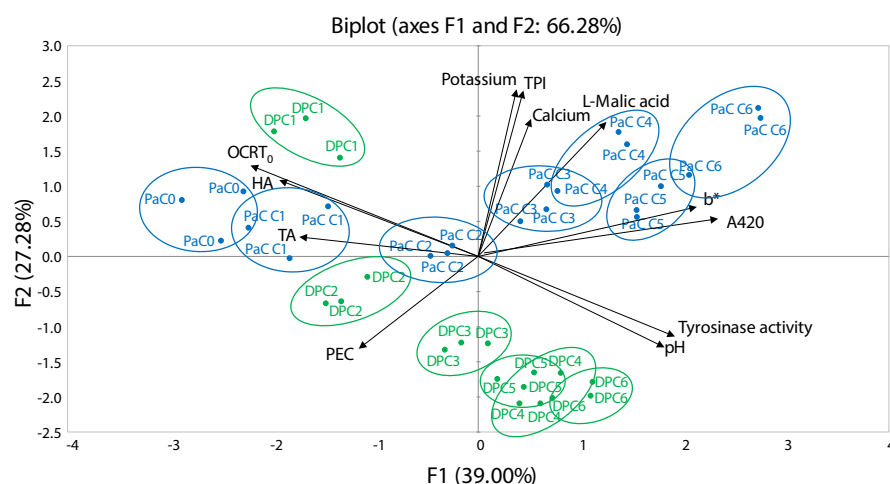


FIGURE 8. Plot of varimax-rotated principal components analysis.

DP: Direct press; PaC: Press after crushing; the number after DP or PaC indicates the number of the pressing cycle. PEC: Potential ethanol content; TA: Titratable acidity; TPI: Total Polyphenol Index; A_{420} : Absorbance at 420 nm; HA: Hydroxycinnamic acids; OCRTO: Oxygen consumption rate.

crushing results in higher concentrations of these compounds. Additionally, the vector for L-malic acid points upward, whereas the vector for potential ethanol content points downward, suggesting that pressing after crushing facilitates greater extraction of must from less mature grapes.

The F1 axis separates the pressing cycles for both conditions. The first cycle is positioned on the left of the plot, with subsequent pressing cycles progressively shifting to the right, reflecting the sequential nature of the pressing process. The main factors driving this trend include oxidative deterioration, tyrosinase activity, the oxygen consumption rate, hydroxycinnamic acid concentration, titratable acidity, pH, A_{420} and b^* . As discussed in previous sections, as pressing progresses, tyrosinase activity and pH increase, whereas the oxygen consumption rate, hydroxycinnamic acids concentration, and titratable acidity decrease. In parallel, yellow colour intensity (A_{420} and b^*) increases. These coordinated changes enable the samples to be distinguished according to the stage of the pressing cycles.

CONCLUSIONS

This study demonstrates the relevant influence of pressing conditions on the yield, chemical composition, and oxidative behaviour of grape must. Pressing after crushing (PaC) provides higher juice yields, faster processing speeds, and greater mechanisation of the process compared to direct pressing (DP). Overall, pressing after crushing (PaC) yields produces musts with higher turbidity, lower potential ethanol content, higher Total Phenolic Index (TPI), lower titratable acidity, higher concentrations of L-malic acid, lower pH, and increased potassium levels relative to those obtained through DP. No relevant differences were observed in calcium levels or yellow colour intensity between the two pressing methods. Moreover, PaC must exhibited higher hydroxycinnamic acids concentration, higher oxygen consumption rates, and lower tyrosinase activity than DP must. These findings suggest that the oxygen consumption rate is more closely associated with

the concentration of available substrates than with tyrosinase activity *per se*. Taken together, these results indicate that must obtained via PaC may have greater oxidative potential than must obtained via DP, despite both pressing conditions initially producing musts with similar initial yellow colour intensity.

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