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Impact of Must Replacement and Hot Pre-Fermentative Maceration on the Color of Uruguayan Tannat Red Wines

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Abstract: This research aimed to evaluate the impact of different options for winemaking on the color composition of Uruguayan Tannat red wines. The techniques evaluated were the substitution of ripe grape juice with immature grape juice and the heating of the crushed grapes before fermentation, called must replacement and hot pre-fermentative maceration, respectively. These procedures were proposed to reduce the alcohol content and increase the phenolic composition of the wine, according to the expected effects of climate change and current trends in consumer preferences. The investigation was made over three consecutive years (2016, 2017, and 2018). Both winemaking techniques allow the enhancement of the chromatic characteristics of wines via the modification of the phenolic composition. Additionally, such techniques allow the overcoming of the well-known limitations in the extractability of anthocyanins presented by the Tannat cultivar. Hot pre-fermentative maceration increases the proportion of the most oxidizable molecules delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, and petunidin-3-O-glucoside, suggesting heat inactivation of polyphenoloxidases enzymes. Must replacement and hot pre-fermentative maceration are technological alternatives that could significantly improve the intensity and chromatic characteristics of red wines.

Keywords: Tannat; must replacement; hot pre-fermentative maceration; wine color; wine composition

1. Introduction

The color of red wine is generally the first sensory property to be appreciated by consumers [1]. The limpidity and intensity of the wine color are responsible for the consumer's first opinion, which can also condition the sensory perception of other wine qualities, such as the aroma, taste, or mouthfeel [1,2]. Wines with little color, the presence of precipitates in the bottle, or with unexpected hue relative to their age can be a reason for an initial rejection [3].

Anthocyanins are the primary pigment responsible for the color of grapes and young red wines [4]. These compounds are synthesized by the secondary metabolism of the vine and are accumulated in grape skins during maturation [5]. In *Vitis vinifera* cultivars, grape anthocyanins are delphinidin, cyanidin, petunidin, peonidin, and malvidin monoglucosides, as well as acylated derivatives with acetic, p-coumaric, and caffeic acids. The composition of wine anthocyanins is determined by the

cultivar [6–8], the grape maturity state and the extractability of its components [9,10], and the maceration procedures used in winemaking [11–13]. The climatic conditions and therefore the year of each harvest are factors of great importance [9,10,14,15]. In traditional winemaking, only 40% of the anthocyanins of the grapes are transferred to the wine [4,16]. The limited extraction of anthocyanins is mainly due to the lack of permeability of cell walls and cytoplasmic membranes [17,18], because these compounds are in the skin, in the upper cellular layers of the hypodermis. The composition of cell walls is genetically determined and modifies the changes in the hardness of skin and seed tissues along with ripening. The extraction of anthocyanins and proanthocyanidins during winemaking depends on the grape variety [19,20]. The simultaneous development of maceration and alcoholic fermentation influence the extraction of polyphenols, because the ethanol content determines the disintegration of the vacuolar membranes and the walls of the skin cell [15]. Anthocyanins are compounds easily soluble in water and therefore are dissolved from the beginning of the maceration, independent of the ethanol concentrations [21].

However, wine color not only depends on the anthocyanin concentration [4,22]. Anthocyanins undergo structural transformations depending on the pH of the medium. They present a red color in an acid medium, acquire a violet color when approaching a neutral pH, and decrease the intensity of the color as the pH increases. Under very high pH conditions, anthocyanins are irreversibly destroyed. Further, during the making, conservation, and aging of wine, the formation of new compounds and their polymerization modify the red wine color and determine its stability [23]. These molecules are partially degraded due to hydrolysis or oxidation reactions [24,25], while other molecules participate in cyclo-addition reactions with metabolites produced by yeasts [26]. Other anthocyanins are condensed with catechins [27,28]. A significant fraction of the anthocyanins extracted from grape skins will be adsorbed by yeasts and will precipitate in the lees [29], whereas there is also a fixation of these compounds in the solid parts of the grapes [21].

In the last few decades, several alternative techniques of maceration have been proposed that allow a differentiated extraction of the phenolic and aromatic compounds of the grape to the wine to improve quality and aging potential [11,13,30]. Most of these techniques have had a substantial impact on the color of red wines [13,31]. More recently, some research groups have evaluated different winemaking techniques to regulate the ethanol content and pH of wines in response to the effect of global warming on the composition of grapes [32–34]. The results obtained with the application of these procedures have allowed the reduction of the ethanol content and pH of the wines, but the effects on the sensory characteristics, particularly on the color, have not been conclusive [32,33,35].

In Uruguay, Tannat is the most relevant red cultivar due to its adaptation to the country's eco-physiological conditions. The polyphenolic and anthocyanin richness of Tannat wines is related to the enological potential of their grapes. The grapes have a low extraction capacity of anthocyanins and lower proportions of malvidin and acetylated glycosides compared with other red cultivars, such as Cabernet Sauvignon and Merlot [30]. Consequently, the color stability of Tannat wines is lower than wines of other varieties [3,8], although they maintain the characteristic anthocyanin profile of the grape of origin for a specified period. Additionally, high interannual climate variability has been recorded during the ripening period, which strongly affects the composition of the grape. In particular, high temperatures during the ripening period cause a high accumulation of sugars and degradation of acidity [36] due to the consumption of malic acid [37] and alter the synthesis of polyphenols [38,39]. Thermal stress during the maturation period causes the degradation and inhibition of the accumulation of anthocyanins, compounds responsible for the color of grapes and red wines [38]. Currently, there is a growing concern of winemakers regarding having tools that allow regulation of the contents of ethanol and pH and the concentrations of phenolic compounds without causing detriment to the color of Tannat red wines. The intensity and hue of the color of Tannat red wines determine the target market and commercial value.

This research aims to study the impact of must replacement and hot pre-fermentative maceration in the color of Uruguayan Tannat red wines produced in three consecutive vintages. Both techniques have been proposed to obtain red wines with lower alcohol content and pH and higher phenolic

compound concentration [35]. Hot pre-fermentative maceration consists of the degradation of cellular structures, mainly of the grape skins, through the heating of the must before alcoholic fermentation at a temperature and a period variable [40]. These techniques increase the extraction of phenolic compounds. Moreover, must replacement consists of the substitution of a percentage of grape juice of very ripe grapes with the grape juice of unripe grapes before alcoholic fermentation to reduce the alcohol content and the pH of the wines [35].

2. Materials and Methods

2.1. Chemicals and Equipment

Methanol, acetonitrile, formic acid, and acetic acid were of HPLC grade (>99%) and purchased from Panreac (Barcelona, Spain). Acetaldehyde (>99.5%), ascorbic acid (>99%), and sodium acetate (>99%) were purchased from Sigma-Aldrich (Madrid, Spain). Absolute ethanol and hydrochloric acid (37%) were purchased from Panreac. Malvidin-3-O-glucoside chloride ($\geq 95\%$), was purchased from Extrasynthese (Genay, France). A Winescan TM Autosampler 79,000 infrared analyzer (Foss, USA) and Foss Integrator software version 154 (Foss, Denmark) were used to determine the alcohol content, total acidity, and pH of the wines. The HPLC analyses were performed using an Agilent 1200 series liquid chromatograph equipped with a G1315D diode array detector (DAD), a G1311A quaternary pump, a G1316A column oven, and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA). All the spectrophotometric measurements were performed using a Helios Alpha UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.2. Grapes and Wines

This research was carried out with grapes of Tannat *Vitis vinifera* L., Vitis International Variety Catalogue (VIVC) number 12,257 [41], in 2016, 2017, and 2018 vintages. The grapes were manually harvested from a commercial vineyard located in Canelones in the south of Uruguay.

At the beginning of veraison, 100 kg of Tannat grapes were harvested to obtain a must with high acidity and low sugar concentration. The grapes were crushed (Alfa 60 R crusher, Italcom, Piazzola Sul Brenta, Italy) and lightly pressed in a manual press to obtain 50 L of an unripe grape must. The grape must was immediately sulphited with 100 mg/L of $K_2S_2O_8$, settled overnight, packaged in a 50-L recipient, and conserved at 4 °C until use. When the grapes reached technological maturity, 120 kg of grapes were collected and randomly distributed into 12 lots of 10 kg. The grapes were destemmed and crushed (Alfa 60 R crusher, Italcom, Piazzola Sul Brenta, Italy), and the must was sulphited with 100 mg/L of $K_2S_2O_8$ and distributed in 12 polyethylene containers (each of 10-L capacity). The must containers were randomly divided into two groups of six containers each. Six containers were considered to be controls (original must—OM), whereas in the other six containers (must replacement—MR), 3 L of the original grape must were replaced with 3 L of unripe grapes must with the aim of decreasing sugar content and pH.

Next, three containers of each experimental group (OM and MR) were traditionally macerated (TM), whereas the other three were subjected to hot pre-fermentative maceration (HM) for 1 h at a temperature between 60 and 70 °C. The heating was carried out by transferring the pomace to 11-L stainless-steel tanks that were submerged in a hot water bath (at 80–90 °C). During warming, the pomace was homogenized manually. At the end of the heat treatment, the stainless-steel tanks were submerged in a cold water bath in order to refrigerate them to ambient temperature (around 26 °C). After that, the must was transferred to the original 10-L polyethylene containers. Thus, four experimental groups for each cultivar were obtained: control wine with traditional maceration (OM-TM), must replacement with reduced alcohol and pH in the wine obtained by traditional maceration (MR-TM), control wine with hot pre-fermentative maceration (OM-HM), and must replacement and hot pre-fermentative maceration (MR-HM) (Figure 1).

All the containers were inoculated with 200 mg/L of active dry yeast (*Saccharomyces cerevisiae* ex bayanus Natuferm 804; Oenobiotech, Paris, France) and were fermented in contact with the skins and seeds. During maceration, all the containers were manually pumped over once daily, followed by a

manual punching down of the cap to favor polyphenol extraction. The fermentation temperature ranged between 26 and 29 °C in the 2016 vintage, between 22 and 27 °C in the 2017 vintage, and between 25 and 29 °C in the 2018 vintage. After 7 days of maceration, the free-run wine was extracted by gravity, and the resting pomace was lightly pressed in a manual press. The free-run wine and the lightly pressed wine of each tank were blended and maintained in 5-L vessels at room temperature (18 ± 2 °C). The alcoholic fermentation was completed when the daily measurements of the must density were less than 998 g/L for three consecutive days. The wines were preserved in polyethylene containers of 5 L of capacity at laboratory room temperature (18 ± 2 °C), and once spontaneous malolactic fermentation was finished (around 35 days later), all the wines were stabilized with 100 mg/L of $K_2S_2O_8$ and 300 mg/L of lysozyme (Delvo®Zyme, Delft, the Netherlands). Finally, the wines were bottled and stored in a dark cellar at laboratory ambient temperature until analysis. The analyses started 2 months after bottling and ended 3 weeks later.

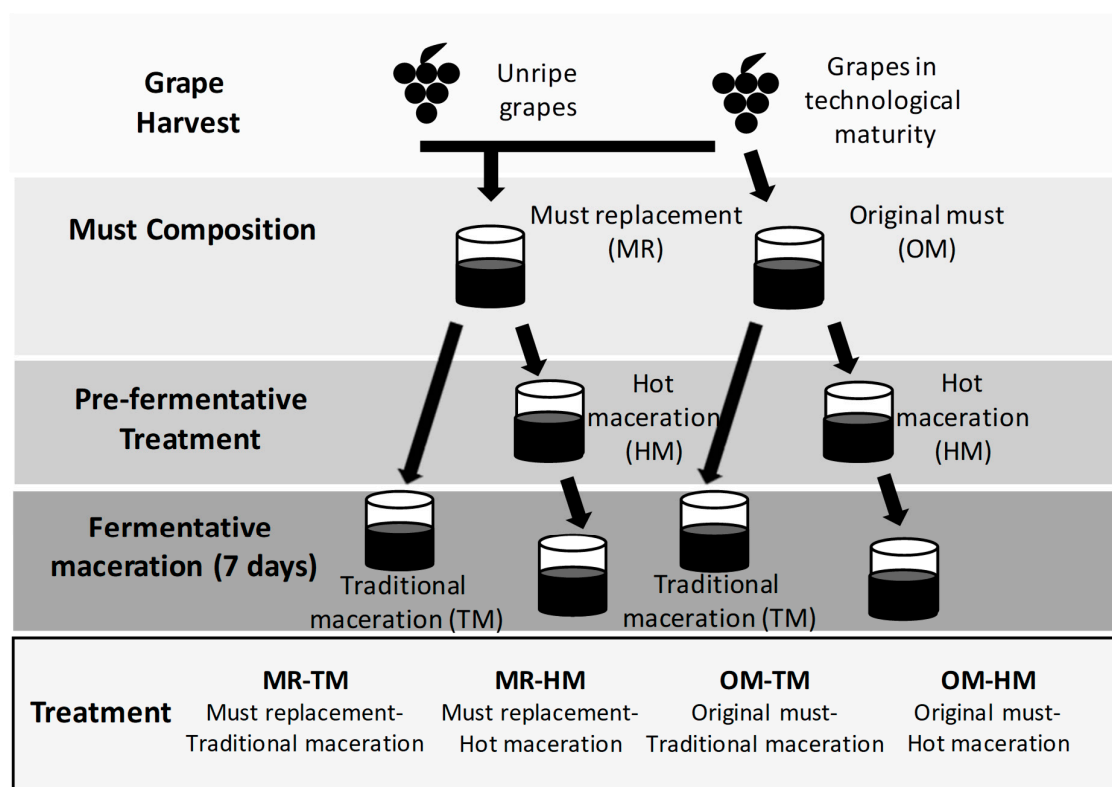


Figure 1. Diagram of the experimental design.

2.3. Standard Grape Juice and Wine Analysis

Analytical methods recommended by the International Organization of Vine and Wine [42] were used to determine the sugar concentration, pH, and titratable acidity of the grape juices. During the fermentation, the temperature and density of the must were monitored daily. The ethanol content, titratable acidity, pH, residual sugars, and volatile acidity of the wines were analyzed using an infrared analyzer Winescan TM Autosampler 79,000 (Foss, USA) and Foss Integrator software version 154 (Foss, Denmark).

2.4. Color Parameters

The color parameters were determined directly on wine samples placed in a 1-mm pathlength cuvette. Color intensity (CI) was estimated using the method proposed by Glories [43]. The CIELAB coordinates, lightness (L^*), chroma (C^*), hue (h^*), red-greenness (a^*), and yellow-blueness (b^*), were

determined according to the method described by Ayala et al. [44]. Thus, data processing was performed with MSCV software [45].

2.5. Spectrophotometric Analysis of Anthocyanins and Related Parameters

The total anthocyanin content of the grapes, their extractability, and their total phenolic index were determined, according to the procedure outlined by González-Neves et al. [46].

The polyphenolic composition was evaluated using classical spectrophotometric indices. The total polyphenols were determined using the Folin–Ciocalteu method, according to Singleton and Rossi [47], and their contents in the wines are expressed in mg of gallic acid per liter. The total pigment and anthocyanin content were analyzed using the technique described by Ribéreau-Gayon and Stonestreet [48], and they are expressed as mg of malvidin-3-glucoside equivalent (EMG) per liter. Catechins were quantified using the method proposed by Swain and Hillis [49], and their concentrations are expressed in mg of D-catechin per liter. Proanthocyanidins were determined according to Ribéreau-Gayon and Stonestreet [50], and their contents are expressed in mg of cyanidin chloride per liter of wine. The ionization index (which indicates the proportion of red-colored anthocyanins at wine pH) and the PVPP index (which indicates the proportion of anthocyanins combined with proanthocyanidins) were determined in line with the method described by Glories [43]. The copigmentation index was measured in accordance with the procedure outlined by Boulton [4].

2.6. HPLC Anthocyanidin Analysis

Reversed-phase HPLC analyses of the anthocyanidins were carried out by injecting 40 μ L of wine into an Agilent 1200 series liquid chromatographer (HPLC-DAD) and using an Agilent Zorbax Eclipse XDBC18, 4.6 \times 250 mm, 5- μ m column (Agilent Technologies). The solvents used were 10% aqueous formic acid (solvent A) and a mixture of 45% methanol, 45% water, and 10% formic acid (solvent B), following the method described by Valls [51]. Chromatograms were recorded at 530 nm, and anthocyanin standard curves were made using malvidin-3-O-glucoside chloride. Compounds were identified considering the relative retention times between the compounds and by recording their UV spectra with a diode array detector and comparing these with the UV spectra reported by Valls [51]. The five anthocyanidin-3-monoglucosides of wine (delphinidin, cyanidin, peonidin, petunidin, and malvidin) and their respective acetylated and p-coumarylated anthocyanins were quantified.

2.7. Statistical Analysis

All the data are expressed as the arithmetic average \pm standard deviation of three replicates. Multifactorial analysis of variance (MANOVA) was carried out with INFOSTAT [52] (version 2017, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina), and multiple comparisons between samples were performed by using the Hotelling test.

3. Results and Discussion

3.1. Fermentation Kinetics

Figure 2 shows the fermentation kinetics of the treatments evaluated according to the year of vintage. In the treatments with must replacement (MR-TM and MR-HM), the density was lower due to lower concentrations of sugars. Therefore, these musts finished alcoholic fermentation before the must without substitution and traditional maceration (OM-TM). These results were expected, because the sugar concentrations of the musts were low, and the level of alcohol generated did not affect the development of the yeasts, achieving a complete fermentation of the musts. Moreover, the musts produced by hot pre-fermentative maceration finished alcoholic fermentation before the traditional maceration musts. When a must is subjected to temperatures above 40 °C, the populations of lactic and acetic bacteria, as well as yeasts, disappear [53]. Additionally, the extraction of growth

factors during warming favors the subsequent development of inoculated yeasts [54], which explains the results obtained for this treatment. These results are more clearly observed for the wines produced from the 2016 and 2018 vintages, as the climatic conditions allowed the grape to reach a higher degree of maturity. On the contrary, in the vintage of 2017, the ripening stopped, so the harvested grapes were immature.

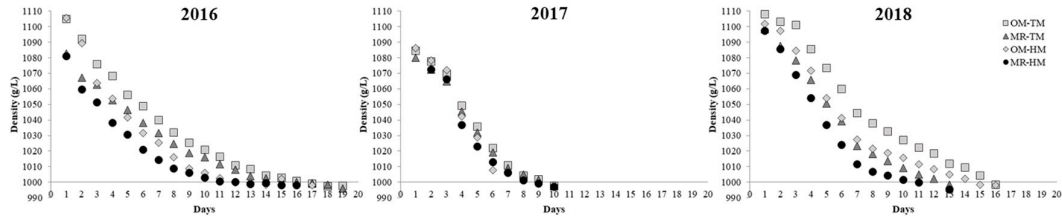


Figure 2. Fermentation kinetics of the treatments by the year of vintage. Average of three wines. OM-TM: original must and traditional maceration; MR-TM: must replacement and traditional maceration; OM-HM: original must and hot pre-fermentative maceration; MR-HM: must replacement and hot pre-fermentative maceration.

3.2. General Composition of Wines

Table 1 shows the effects of the year of vintage, must composition, and winemaking technique factors on the contents of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of wines.

The vintage factor expresses the average content of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of all the wines produced in the same vintage, regardless of the must composition and winemaking procedure. Wines produced from the 2018 vintage had the highest ethanol content, and those of the 2017 vintage had the lowest. The highest values of titratable acidity and pH were recorded in the wines produced in 2016 and the lowest in 2017. During the ripening of the grapes, the sugar concentration and the pH increased, whereas titratable acidity decreased. However, climatic conditions during the ripening determine the composition of the grape [14,41]. The ripeness conditions were different between vintages. The grapes harvested in 2016 and 2018 had better maturation conditions, with high concentrations of sugar and an optimum pH. In contrast, in 2017, grape maturation halted, resulting in lower concentrations of sugars and pH. The wines produced from the 2016 and 2017 vintages presented residual sugar concentrations lower than 2 g/L [53], whereas the 2018 wines presented a slightly higher value. These results may be related to a higher concentration of non-fermentable sugars in the 2018 vintage, because the grapes showed a high concentration of sugars. Another possibility may be that the high levels of alcohol generated during alcoholic fermentation affected the development of yeasts in the final stages of alcoholic fermentation [53]. The volatile acidity of the wines elaborated in the different vintages were expected according to the winemaking system used.

Table 1. General composition of the wines.

Factor Analyzed		Ethanol (% v/v)	Titratable Acidity (gH ₂ SO ₄ /L)	pH	Residual Sugars (g/L)	Volatile Acidity (gH ₂ SO ₄ /L)
Year of vintage (*)	2016	14.0 ± 0.1 ^b	4.30 ± 0.27 ^a	3.92 ± 0.16 ^a	1.47 ± 0.41 ^c	0.36 ± 0.07 ^b
	2017	11.2 ± 0.2 ^c	2.93 ± 0.05 ^c	3.86 ± 0.04 ^c	1.85 ± 0.21 ^b	0.43 ± 0.09 ^a
	2018	15.4 ± 0.2 ^a	3.85 ± 0.03 ^b	3.89 ± 0.09 ^b	2.44 ± 0.44 ^a	0.44 ± 0.07 ^a
Must composition (**)	OM	14.0 ± 0.1 ^a	3.51 ± 0.17 ^b	3.95 ± 0.09 ^a	2.07 ± 0.59 ^a	0.43 ± 0.09 ^a
	MR	13.0 ± 0.1 ^b	3.88 ± 0.06 ^a	3.83 ± 0.09 ^b	1.83 ± 0.39 ^a	0.39 ± 0.08 ^b
Maceration technique (***))	TM	13.3 ± 0.2 ^b	3.74 ± 0.19 ^a	3.87 ± 0.09 ^a	2.01 ± 0.55 ^a	0.47 ± 0.06 ^a
	HM	13.7 ± 0.1 ^a	3.64 ± 0.04 ^a	3.92 ± 0.09 ^a	1.89 ± 0.46 ^a	0.35 ± 0.05 ^b
	OM-TM	14.0 ± 0.2 ^a	3.61 ± 0.30 ^b	3.92 ± 0.09 ^b	2.30 ± 0.56 ^a	0.50 ± 0.06 ^a
Must composition - Maceration technique (****))	MR-TM	12.6 ± 0.2 ^c	3.87 ± 0.09 ^a	3.81 ± 0.12 ^d	1.72 ± 0.37 ^c	0.45 ± 0.06 ^b
	OM-HM	14.0 ± 0.1 ^a	3.40 ± 0.03 ^c	3.98 ± 0.08 ^a	1.84 ± 0.53 ^{bc}	0.36 ± 0.05 ^c
	MR-HM	13.4 ± 0.1 ^b	3.88 ± 0.04 ^a	3.85 ± 0.09 ^c	1.95 ± 0.39 ^b	0.33 ± 0.05 ^c

(*) Average of 12 wines \pm standard deviation regardless of the grape juice composition and the winemaking technique. (**) Average of the 18 wines \pm standard deviation regardless of the year of vintage and the winemaking technique. (***) Average of 18 wines \pm standard deviation regardless of the year of vintage and the grape juice composition. (****) Average of nine wines \pm standard deviation regardless of the year of vintage. Different letters indicate statistical differences ($p < 0,05$). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot pre-fermentative maceration.

The must composition factor expresses the average contents of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of all the wines produced with original must (OM) or must replacement (MR), independent of the vintage or the maceration technique. The MR wines had lower ethanol content and pH and higher titratable acidity than the OM wines. These results were expected, because the must replacement of the well-ripened grapes with the must of unripe grapes implicated a decrease in sugar content and pH and an increase of titratable acidity. These data agree with those obtained by Kontoudakis et al. [32] and Role et al. [33], who proposed a similar but different procedure. Kontoudakis et al. [39] proposed the simultaneous reduction of the ethanol content and the pH of the wine by mixing wines, one of them obtained with green grapes and the other with ripe grapes [32]. Moreover, Role et al. [33] proposed three alternative procedures to achieve alcohol reduction: (i) pre-fermentation addition of liquid derived from grape must (reverse osmosis byproduct); (ii) mixed fermentations with strains of *Starmerella bacillaris* and *Saccharomyces cerevisiae*; and (iii) dealcoholization of wine post-fermentation with a polypropylene membrane. In our research, the partial replacement of grape juice had a low impact on the chemical composition of the wines. The concentration of residual sugars in the wine was not affected by the must replacement, whereas the volatile acidity was slightly lower.

The maceration technique factor expresses the average contents of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of all the wines produced by traditional maceration or hot pre-fermentative maceration, without considering the initial must composition and the vintage. The HM wines presented higher ethanol content than the TM wines, without significant differences in the total acidity or pH. The highest levels of ethanol were observed in the HM wines. These results agree with those obtained by other authors [54,55] and could be explained by two factors, the first of which is due to how the hot pre-fermentation maceration was carried out. Weak evaporation of water could have occurred during the pre-fermentative stage, which may have contributed to the small concentration of all the compounds of the must, particularly the sugars. Second, a higher level of amino acids has been reported in thermovinified musts [54]. This increase in amino acids could contribute to improving ethanol yields [56]. However, the residual sugar concentrations of the wines were not affected by the winemaking technique, whereas the volatile acidity was slightly lower.

The must composition \times maceration technique factor expresses the average contents of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of all the wines produced with the original must and traditional maceration (OM-TM), must replacement and traditional maceration (MR-TM), original must and pre-fermentative hot pre-fermentative maceration (OM-HM), or must replacement and hot pre-fermentative maceration (MR-HM), regardless of the vintage. The ethanol content of the OM-TM and OM-HM wines was significantly higher than that of the MR-TM and MR-HM wines, which evidenced significant differences due to the maceration techniques used. In contrast, the ethanol content of the MR-HM wine was significantly higher than that of the MR-TM wine, probably because of the maceration technique described previously. As expected, the MR-TM and MR-HM wines presented the highest values of titratable acidity and the lowest pH values in comparison with the OM-TM and OM-HM wines. When analyzing the combination of both winemaking techniques, changes in pH were observed, associated with the initial composition of the must and the maceration technique. In this sense, it has been reported that wines developed via hot pre-fermentative maceration have shown higher pH values, because, during the pre-fermentative heating, the extraction of cations increases, which results in a rise in the pH mainly given by the salification of tartaric acid [57]. Additionally, the wines produced with must replacement and/or hot pre-fermentative maceration showed the lowest concentrations of residual sugars and lower values of volatile acidity.

3.3. Spectrophotometrical Phenolic Composition and Related Parameters

The phenolic composition of the wines was different according to the vintage (Table 2). Wines produced in 2016 were characterized by the highest concentrations of total polyphenols, anthocyanins, and proanthocyanidins, whereas the wines produced in 2017 presented the lowest values. The concentrations of catechins in the wines produced in 2018 were significantly higher than those in the wines produced in other vintages. The concentration of anthocyanins did not significantly differ from the wines produced in 2016, whereas the concentrations of total polyphenols and proanthocyanidins were intermediate (Table 2). These results indicate that the ripening stage of the grapes strongly determined the wine composition. Fourment et al. [58] reported that for the conditions of Uruguay, the interannual climate variability strongly modifies the composition of the grape, especially in the concentration of secondary metabolites.

Table 2. Polyphenolic composition of the wines.

Factor Analyzed		Total Polyphenol (mg/L)	Anthocyanins (mg/L)	Catechins (mg/L)	Proanthocyanidins (mg/L)
Year of vintage (*)	2016	2479 ± 252 ^a	1052 ± 156 ^a	1769 ± 455 ^b	4172 ± 714 ^a
	2017	1624 ± 68 ^c	614 ± 68 ^b	1420 ± 58 ^c	2690 ± 60 ^c
	2018	2140 ± 43 ^b	1165 ± 43 ^a	1883 ± 86 ^a	3260 ± 80 ^b
Must composition (**)	OM	2045 ± 140 ^a	960 ± 67 ^a	1667 ± 239 ^a	3397 ± 372 ^a
	MR	2117 ± 102 ^a	994 ± 73 ^a	1714 ± 160 ^a	3352 ± 197 ^a
Maceration technique (***)	TM	1784 ± 112 ^b	838 ± 69 ^b	1281 ± 215 ^b	2764 ± 261 ^b
	HM	2379 ± 129 ^a	1117 ± 71 ^a	2100 ± 184 ^a	3985 ± 308 ^a
	OM-TM	1821 ± 131 ^c	832 ± 69 ^c	1273 ± 268 ^b	2792 ± 352 ^b
Must composition- Maceration technique (****)	MR-TM	1747 ± 94 ^d	843 ± 69 ^c	1289 ± 161 ^b	2735 ± 170 ^b
	OM-HM	2345 ± 149 ^b	1088 ± 66 ^b	2061 ± 209 ^a	4001 ± 390 ^a
	MR-HM	2413 ± 109 ^a	1146 ± 77 ^a	2141 ± 159 ^a	3968 ± 225 ^a

(*) Average of 12 wines ± standard deviation regardless of the grape juice composition and the winemaking technique. (**) Average of the 18 wines ± standard deviation regardless of the year of vintage and the winemaking technique. (***) Average of 18 wines ± standard deviation regardless of the year of vintage and the grape juice composition. (****) Average of nine wines ± standard deviation regardless of the year of vintage. Different letters indicate statistical differences ($p < 0,05$). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot maceration.

Total polyphenols, anthocyanins, catechins, and proanthocyanidins of the MR wines did not differ significantly from those of the OM wines. The techniques proposed by Role et al. [33] to reduce the alcohol content of the wines reduced the concentration of highly polymerized flavonols without substantially modifying the concentration of anthocyanins. According to these authors, the lower ethanol concentration could be the extraction of high polymerized flavanols from the grapes during fermentation. Moreover, they suggest that although lower concentration of anthocyanins would be expected, because a portion of must was eliminated, this does not necessarily imply anthocyanin losses, because the replacement was done before maceration. With ripe berries, however, these red pigments are more easily extracted from the skins during the crushing process and the short time of skin contact, and therefore, the fraction removed could contain a considerable amount of anthocyanin [59]. This was not observed in our results. Meanwhile, Kontoudakis et al. [32] found that anthocyanins remained almost unchanged when the ethanol concentration was reduced by 3.0% *v/v* by replacing a part of the total volume of the grape juice with the same volume of a low-ethanol wine. These authors reported that proanthocyanidin was less abundant in the reduced alcohol wines than in the control wines.

In contrast, total polyphenols, anthocyanins, catechins, and proanthocyanidins of the HM wines were significantly higher than those of the TM wines (Table 2). These results agree with previous studies [30,40,57,60] and confirm that this technique is useful to improve polyphenol extraction, because pre-fermentative heating contributes to degrading the tissues of the skins, releasing these compounds into the must.

When we analyzed the joint effect of the grape juice composition and the maceration technique, it was observed that the wines produced by hot pre-fermentative maceration presented the highest concentrations of the different phenolic families evaluated. In particular, the HM-OM wines presented lower contents of total polyphenols and anthocyanins than the HM-MR wines, whereas no significant differences were observed in the concentrations of catechins and proanthocyanidins given by the initial composition of the must. Similar results were observed between the OM and MR wines made by traditional maceration. These results indicate that the combination of must replacement and hot pre-fermentative maceration increased the concentration of anthocyanins in wines, whereas the concentration of catechins and proanthocyanidins was affected only by this winemaking technique, as was discussed previously.

Table 3 shows the effects of the vintage, must composition, maceration technique, and the combination of must composition–maceration technique on the ionization, copigmentation, and PVPP indices. The ionization index represents the percentage of anthocyanins colored given the standard pH and free SO₂ concentration of the wine [4], the copigmentation index represents the percentage of color due to the copigmentation process [4], and the PVPP index measures the percentage of anthocyanins combined with proanthocyanidins [49]. These indices were different according to the vintage. These results could be explained by the effects of ripening conditions on the concentration and the relationship between the phenolic compounds that subsequently interact in the wine. Thus, the highest indices of ionization and PVPP were recorded in the 2016 vintage together with the highest concentrations of total polyphenols, anthocyanins, and proanthocyanidins, whereas the lowest values of these indices were recorded in the 2017 harvest. In the 2018 harvest, the highest value of the copigmentation index was probably associated with a higher concentration of catechins, whereas in the 2016 harvest, it was the lowest value.

Table 3. Color fractions of the wines.

Factor Analyzed		Ionization Index (%)	Copigmentation Index (%)	PVPP Index (%)
Year of vintage (*)	2016	33.9 ± 2.3 ^a	16.5 ± 3.7 ^c	45.2 ± 0.8 ^a
	2017	15.7 ± 2.4 ^c	17.8 ± 4.2 ^b	35.9 ± 0.8 ^c
	2018	17.7 ± 0.6 ^b	31.7 ± 3.1 ^a	40.0 ± 1.2 ^b
Must composition (**)	OM	20.1 ± 1.8 ^b	20.9 ± 3.2 ^b	38.2 ± 0.9 ^b
	MR	24.8 ± 1.7 ^a	23.1 ± 4.0 ^a	42.4 ± 0.9 ^a
Maceration technique (***)	TM	18.0 ± 2.1 ^b	18.4 ± 3.4 ^b	35.9 ± 0.9 ^b
	HM	26.9 ± 1.4 ^a	26.6 ± 3.9 ^a	44.7 ± 0.9 ^a
	OM-TM	16.0 ± 2.6 ^d	15.7 ± 3.0 ^c	35.2 ± 0.9 ^c
Must composition - Maceration technique (****)	MR-TM	20.0 ± 1.7 ^c	21.0 ± 3.9 ^b	36.6 ± 1.0 ^c
	OM-HM	24.2 ± 1.1 ^b	26.0 ± 3.4 ^a	41.3 ± 0.9 ^b
	MR-HM	29.6 ± 1.7 ^a	25.3 ± 4.3 ^a	48.2 ± 0.9 ^a

(*) Average of 12 wines ± standard deviation regardless of the grape juice composition and the winemaking technique. (**) Average of the 18 wines ± standard deviation regardless of the year of vintage and the winemaking technique. (***) Average of 18 wines ± standard deviation regardless of the year of vintage and the grape juice composition. (****) Average of nine wines ± standard deviation regardless of the year of vintage. Different letters indicate statistical differences ($p < 0.05$). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot maceration.

Nevertheless, an effect of the must replacement treatments on the different indices was found. The MR wines presented higher ionization, copigmentation, and PVPP indices. The color of red wine is the result of the concentration of ionized free anthocyanins and the interactions between these and other components of the wine that produce new pigments [22]. During the winemaking, the new pigment produced when anthocyanins combine with tannins is much less sensitive to bleaching by pH and SO₂, so the percentage of coloring increases [12,27].

This effect and the result obtained in the pH (Table 1) of the wines could explain the differences registered in both indices. Further, the HM wines presented higher values of all these indices than the TM wines (Table 4). This effect could be determined by the increase in the concentrations of anthocyanins, catechins, and proanthocyanidins registered in the wines produced with hot pre-

fermentative maceration, which could promote their interaction in the wine by increasing copigmentation and condensation between anthocyanins and tannins [24].

The wines presented significant differences in the evaluated indices given by the initial composition of the must and the winemaking technique with which they were developed. The OM-HM and RM-HM wines presented higher ionization, copigmentation, and PVPP indices than the OM-TM and RM-TM wines, but the highest values recorded were in the wines where pre-fermentative treatment was carried out on the must replacement. The anthocyanin, catechins, and proanthocyanidin contents of the HM wines were higher than those of the TM wines (Table 3). These results suggest that hot pre-fermentation maceration favors the reactions between anthocyanins and tannins, which suggests greater color stability over time, according to [61]. Moreover, when hot pre-fermentative maceration was carried out on the replaced grape juice, the values registered in the indices were substantially higher, suggesting that the combination of both techniques improves the stability of the wine color.

3.4. Wine Anthocyanin Composition

Figure 3a,b shows the average of the levels and profiles of the anthocyanin composition of the wines elaborated in the 2016, 2017, and 2018 vintages, according to treatment. As observed, total anthocyanin concentrations determined by HPLC-DAD were lower than the total anthocyanin concentrations measured by spectrophotometry. It should be considered that spectrophotometric analysis includes contributions from other pigments in the measurement and therefore overestimates the total anthocyanin concentration, whereas the HPLC-DAD analysis only detects free anthocyanins. In general, Tannat wines had a high non-acylated glucosides, delphinidin, and petunidin proportions and low acylated anthocyanin (acetylated and coumarylated) proportions, as has been previously reported [1,8].

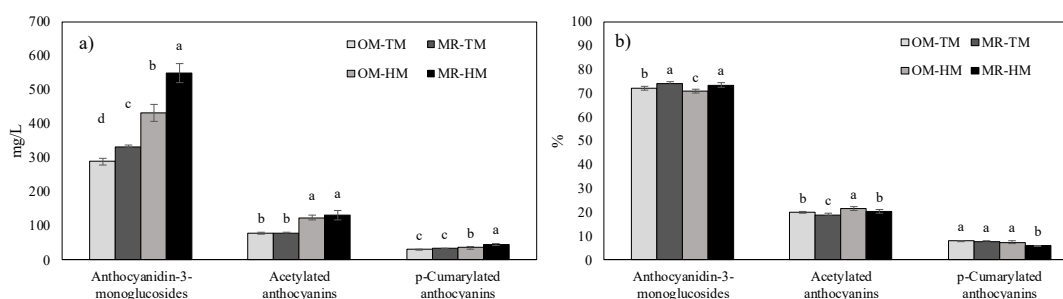


Figure 3. Concentration (a) and proportion (b) of anthocyanidin-3-monoglucosides, acetylated anthocyanins, and p-coumarylated anthocyanins. Average of nine wines \pm standard deviation. Different letters indicate statistical differences ($p < 0.05$). OM-TM: original must and traditional maceration; MR-TM: must replacement and traditional maceration; OM-HM: original must and hot pre-fermentative maceration; MR-HM: must replacement and hot pre-fermentative maceration.

Figure 3a shows the effect of the treatments evaluated on the concentration of monoglucosylated, acetylated, and coumarylated anthocyanins. Both must replacement and the hot pre-fermentative maceration contributed to increase the concentrations of monoglucosylated and p-coumarylated anthocyanins compared with those of the wine produced by original must followed by a traditional maceration. Instead, the concentration of acetylated anthocyanins was differentiated between wines only by the maceration technique used. These results confirm those obtained through spectrophotometric analysis. The must replacement seemed to increase the concentration of monoglucosides, probably because these wines had a lower pH, whereas the hot pre-fermentation maceration seemed to generate an increase in the monoglucosylated, acetylated, and p-coumarylated anthocyanins concentration. However, when analyzing the proportion of different anthocyanins, we observed that the differences between treatments were attenuated (Figure 3b).

In general, it was observed that in the wines produced from must replacement the percentage of monoglucosylated anthocyanins was lower, and the percentage of acetylated anthocyanins was higher compared with the wines produced from the original grape must. In this sense, it could be said that there was a modification in the proportion of the different anthocyanin forms that was more affected by the must replacement than by the hot pre-fermentative maceration. In a previous investigation where must replacement and hot pre-fermentative maceration were evaluated on the composition of Pinot Noir and Tannat wines produced from the 2016 vintage, a differential behavior was observed according to the cultivar [35]. The monoglucosylated anthocyanin concentration of Pinot Noir wines with must replacement was significantly lower in relation to that of the control wines, especially when they were subjected to hot pre-fermentation maceration. This behavior was explained because the lower pH caused by the substitution of must could favor the formation of other pigments at high temperatures. However, in the Tannat wines, the changes in monoglucosylated, acetylated, and p-coumarylated anthocyanin concentrations caused by the must replacement and the hot pre-fermentation maceration were different. In general, no significant effect of the must substitution was observed on the concentration of these anthocyanins, but its concentration was increased when hot pre-fermentative maceration was carried out. The results obtained in this research help to clarify the effect of both winemaking techniques, where must replacement and hot pre-fermentation maceration increase the concentrations of monoglucosylated, acetylated, and p-coumarylated anthocyanins in Tannat wines without modifying their proportions.

The average concentration of the different anthocyanin forms and the anthocyanin profile of wines produced in the 2016, 2017, and 2018 vintages are shown in Figure 4 a,b, respectively. As can be observed, the concentrations of the different anthocyanin forms of the wines were increased by the must replacement and the hot pre-fermentative maceration with the sole exception of petunidin-3-glucoside, whose concentration in the MR-TM wines did not differ from that in the OM-TM wines.

Wines produced by the combination of both techniques presented the highest concentrations of all anthocyanin forms independent of the composition of the must. It is known that pH and the ethanol content of the medium are factors that contribute to the extraction of the phenolic compounds during the fermentative maceration [24]. As seen in Figure 4b, the anthocyanin profile of the wines was modified by the winemaking techniques used. In general, must replacement and hot pre-fermentative maceration increased the percentages of delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, and peonidin-3-glucoside. In particular, the winemaking in which hot pre-fermentation maceration was carried out presented the highest values. In contrast, the percentage of malvidin-3-glucoside was lower in the OM-HM and MR-HM wines than in the MR-TM and OM-TM wines. As previously discussed, hot pre-fermentative maceration allows greater extraction of the anthocyanins by degrading the cellular structures of the skins [34]. The effect of hot pre-fermentation maceration was also observed in the anthocyanin profile of the wines where, in the three vintages, the HM wines had higher percentages of delphinidin, petunidin, and peonidin and a significantly lower percentage of malvidin than the TM wines. At this point, the results obtained in our research are contradictory, because it was shown that wines produced by hot pre-fermentation maceration had a higher percentage of less stable anthocyanidins and a lower percentage of the more stable anthocyanidins. As is known, malvidin is more resistant to thermal degradation than other anthocyanin forms [40], so the idea that hot pre-fermentation maceration affects malvidin more than the other anthocyanidins does not seem to be the correct explanation. On the other hand, it has been shown that pre-fermentative heating above 60 °C degrades polyphenoloxidases enzymes, which are responsible for the oxidation of phenolic compounds in the early stages of winemaking [24,60]. Because the adjacent hydroxyl groups of o-diphenols are sensitive to oxidation, the malvidin-3-O-glucoside and peonidin-3-O-glucoside that do not possess ortho-positioned hydroxyl groups are comparatively more resistant to oxidation than cyanidin-3-O-glucoside [22]. Therefore, it could be thought that the increase in the proportions of petunidin, delphinidin, and cyanidin occurred, because these forms were preserved from enzymatic oxidation during winemaking by hot pre-fermentation maceration.

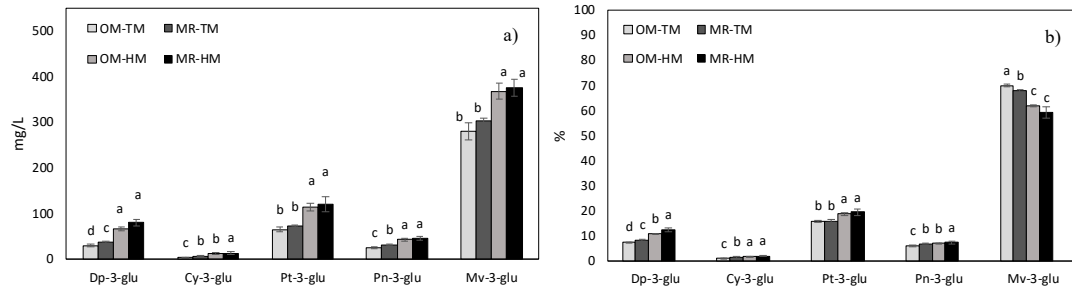


Figure 4. Concentration (a) and proportion (b) of different anthocyanidin forms. Average of nine wines \pm standard deviation. Different letters indicate statistical differences ($p < 0.05$). OM-TM: original must and traditional maceration; MR-TM: must replacement and traditional maceration; OM-HM: original must and hot pre-fermentative maceration; MR-HM: must replacement and hot pre-fermentative maceration.

3.5. Wine Color

Table 4 shows the chromatic parameters of the wines produced. The wines produced from the 2016 vintage were characterized by having the highest coloring intensity and the greatest hue, whereas those produced from the 2017 harvest presented the highest lightness and the lowest speed of coloring intensity, chroma, and hue. The wines produced during the 2018 vintage presented the highest chroma value with intermediate values of coloring intensity and hue. In general, the MR wines had a deeper red color, because the color intensity, chroma, and hue were significant higher and the lightness was significant lower than that of the OM wines, while the HM wines also had a deeper color than the TM wines due to the fact that the color intensity and the chroma were significantly higher and the lightness was significantly lower in the HM wines. No significant differences were observed due to the hot pre-fermentative maceration.

Table 4. Color of the wines.

Factor Analyzed		Color Intensity	Lightness (L*)	Chroma (C*)	Hue (h _{ab})
Year of vintage (*)	2016	32.5 \pm 1.4 ^a	31.5 \pm 1.2 ^b	45.0 \pm 1.0 ^b	348.1 \pm 1.6 ^a
	2017	16.0 \pm 0.5 ^c	60.5 \pm 1.5 ^a	28.1 \pm 1.5 ^c	10.6 \pm 1.3 ^c
	2018	24.2 \pm 0.5 ^b	25.5 \pm 0.9 ^c	53.1 \pm 0.8 ^a	11.8 \pm 0.5 ^b
Must composition (**)	OM	23.2 \pm 0.9 ^b	40.2 \pm 1.3 ^a	41.0 \pm 1.2 ^b	3.27 \pm 1.0 ^a
	MR	25.1 \pm 0.8 ^a	37.9 \pm 1.2 ^b	43.1 \pm 1.4 ^a	3.74 \pm 1.2 ^a
Maceration technique (***)	TM	20.4 \pm 0.7 ^b	44.8 \pm 1.2 ^a	41.4 \pm 1.3 ^b	4.66 \pm 0.8 ^a
	HM	27.9 \pm 0.9 ^a	33.3 \pm 1.3 ^b	42.7 \pm 1.3 ^a	2.35 \pm 1.4 ^a
	OM-TM	19.6 \pm 1.0 ^d	45.9 \pm 1.4 ^a	40.4 \pm 1.0 ^c	5.11 \pm 0.6 ^a
Must composition - Maceration technique (****)	MR-TM	21.2 \pm 0.4 ^c	43.8 \pm 0.9 ^b	42.6 \pm 1.7 ^b	4.21 \pm 0.9 ^a
	OM-HM	26.8 \pm 0.8 ^b	34.6 \pm 1.2 ^c	41.6 \pm 1.5 ^{bc}	1.43 \pm 1.4 ^c
	MR-HM	29.0 \pm 1.1 ^a	32.0 \pm 1.4 ^d	43.7 \pm 1.0 ^a	3.27 \pm 1.5 ^b

(*) Average of 12 wines \pm standard deviation regardless of the grape juice composition and the winemaking technique. (**) Average of the 18 wines \pm standard deviation regardless of the year of vintage and the winemaking technique. (***) Average of 18 wines \pm standard deviation regardless of the year of vintage and the grape juice composition. (****) Average of nine wines \pm standard deviation regardless of the year of vintage. Different letters indicate statistical differences ($p < 0.05$). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot maceration.

When analyzing the effect of the combination of the initial must composition and the maceration technique, it was observed that the MR-HM wines presented the highest intensity of color and chroma and the lowest lightness, whereas the OM-TM wines presented the lowest values. Meanwhile, the OM-HM wines presented a lower value of hue, which suggests that these wines are more bluish. For the other chromatic parameters, the RM-HM and OM-TM wines presented intermediate values.

The differences in the chromatic parameters of the wines were associated with the differences in the concentrations of phenolic compounds found, in particular, those of the anthocyanins; the pH of the wine and the percentage of ionized, copigmented, and polymerized anthocyanins were also different among the wines produced in different vintages and from different treatments, as was previously discussed. Furthermore, hot pre-fermentative maceration increasing the extraction of anthocyanins explains the differences in the color parameters. Other authors have previously described similar results [31]. Moreover, in this sense, the increase in the extraction of anthocyanins from the first stages of the maceration and the increase in the extraction of tannins allowed a greater association of these molecules, which has been reported as a determining factor to improve the color stabilization [12]. The results obtained in this investigation in the ionization, copigmentation, and PVPP indices support this theory.

While it is true that in a sensory evaluation, the chromatic characteristics of these wines can be challenging to differentiate, even for a panel of experts, it must be considered that the wines were evaluated two months after bottling. As is known, the color of the wine evolves during conservation, decreasing its coloring intensity and increasing its angle. The results obtained in this research suggest that wines made by both winemaking techniques could have a more stable color over time and, consequently, a greater potential for aging.

3.6. Multifactorial Analysis of Variance

Multifactorial analysis of the variance shows the effect of each factor and its interaction on the different components of the wines (Table 5). In general, it was verified that the year of vintage (Y), the composition of the grape must (M), the maceration techniques (T), and their interactions (Y×M, Y×T, M×T, Y×M×T) influenced differently the color and the concentration of the phenolic composition of the wine.

The results obtained in the ethanol content, pH, and titratable acidity of the wines seem logical, because the initial composition of the grape must (concentration of sugars, pH, and titratable acidity) at harvest was very different in the vintages due to the climatic conditions of maturation. In this sense, in the treatments where a must replacement for immature grape must was produced, the initial composition of the must, and therefore the wine, was also affected. Moreover, the maceration technique strongly influenced the ethanol content and the pH of the wines. The results obtained regarding the concentration of residual sugars and the volatile acid content of the wines corresponded to the initial composition of the grape and the conditions in which the alcoholic fermentation took place. The vintage and the maceration technique strongly influenced all the phenolic compounds and the ionization, copigmentation, and PVPP indices. Several authors have shown that the phenolic composition of a grape and a wine is determined by the maturation conditions of each year in particular [15]. Moreover, hot pre-fermentative maceration strongly degrades the cellular structures of the skins, extracting their content toward the grape juice and favoring the interaction between them, as mentioned above. The composition of the grape must influences significantly the concentrations of total polyphenols and anthocyanins and the ionization, copigmentation, and PVPP indices.

Table 5. Multifactorial analysis of variance.

	Year of Vintage (Y)	Must Composition (M)	Vinification Technique (V)	Y × M	Y × V	M × V	Y × M × V
Ethanol	5152.9 ***	939.6 ***	137.5 ***	61.8 ***	52.5 ***	131.1 ***	120.2 ***
Titratable acidity	185.5 ***	38.8 ***	2.93 *	25.1 ***	6.9 ***	3.3 *	3.9 **
pH	10.6 ***	101.0 ***	18.3 ***	41.9 ***	10.8 ***	0.4	21.9 ***
Reducing sugars	80.9 ***	9.1 **	2.2	8.0 **	4.6 **	43.4 ***	9.4 ***
Volatile acidity	21.5 ***	11.7 ***	193.1 ***	7.2 **	10.1 ***	0.2	17.8 ***
Total polyphenols	574.8 ***	11.7 ***	824.7 ***	11.9 ***	25.8 ***	0.1	2.9
Anthocyanins	1232.6 ***	10.8 ***	728.2 ***	14.4 ***	89.5 ***	5.11 **	10.1 ***
Catechins	92.4 ***	2.7	800.3 ***	9.5 ***	12.0 ***	1.2	3.0 *
Proanthocyanidins	193.6 ***	0.5	387.0 ***	2.2	0.2	0.1	0.4

Ionization index	248.7 ***	41.6 ***	149.9 ***	4.1 **	28.2 ***	0.9	3.6 **
Copigmentation index	690.4 ***	36.8 ***	385.1 ***	3.8 **	12.9 ***	66.1 ***	3.6 **
PVPP index	15.4 ***	9.33 ***	41.4 ***	1.6	28.8 ***	4.0 *	6.6 ***
Color intensity	1526.4 ***	60.9 ***	966.5 ***	7.6 *	29.5 ***	2.6	2.7
Lightness (L*)	5272.7 ***	65.0 ***	1519.0 ***	10.8 ***	9.6 ***	0.8	5.5 ***
Chroma (C*)	1180.2 ***	25.3 ***	8.0 ***	6.4 ***	94.7 ***	0.1	5.8 ***
Hue (h _{ab})	2160.5 ***	2.0	48.3 ***	5.8 ***	37.0 ***	17.0 ***	7.4 ***
Anthocyanidin-3-monoglucosides	566.1 ***	25.3 ***	364.3 ***	15.5 ***	53.6 ***	1.4	4.6 **
Acetylated anthocyanins	138.1 ***	1.2	231.5 ***	1.2	25.1 ***	1.3	0.1
p-Coumarylated anthocyanins	439.0 ***	16.3 ***	91.2 ***	22.7 ***	1.7	41.9 ***	3.8 **
Delphinidin-3-glucoside	219.3 ***	31.1 ***	531.8 ***	4.9 *	85.2 ***	3.6 *	11.3 ***
Cyanidin-3-glucoside	58.1 ***	2.4	71.2 ***	1.5	20.0 ***	3.7 *	2.6 *
Petunidin-3-glucoside	117.1 ***	3.3 *	158.0 ***	4.9 **	16.6 ***	0.1	0.6
Peonidin-3-glucoside	208.5 ***	12.9 ***	209.2 ***	0.5	12.3 ***	3.2 *	1.6
Malvidin-3-glucoside	623.8 ***	1.6	207.5 ***	8.4 ***	33.6 ***	7.7 ***	1.6

F values and statistical significance ($p < 0.001 = ***$; $p < 0.01 = **$; $p < 0.1 = *$). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot maceration.

As discussed above, the ethanol content and pH are factors that contribute to the extraction during fermentative maceration, but this effect was only observed in the concentrations of total polyphenols and anthocyanins. A strong interaction between YxT was detected for the phenolic compounds and the indices analyzed, except for the concentration of proanthocyanidins, which was not significant. The YxM interaction was not significant for the concentration of proanthocyanidins or for the PVPP index, whereas the MxT interaction was highly significant only for the anthocyanin concentration and the copigmentation index. The year of harvest and the technique of maceration strongly influenced the concentrations of the different anthocyanin forms, while the initial composition of the grape must only affected the concentrations of monoglucosylated anthocyanins, p-coumarylated, delphinidin-3-glucoside, and petunidin-3-glucoside. Again, a strong interaction was detected in the anthocyanin composition of the wines between the harvest year and the maceration technique (YxT), while the other interactions were significant in the concentrations of some anthocyanin forms.

As discussed earlier, the color of red wine results from the concentration of anthocyanins, their interactions with other phenolic compounds or metabolites of alcoholic fermentation, and the physical-chemical conditions of the medium in which these pigments are found. Therefore, any modification of these factors determines a change in the wine color. The year of vintage, the composition of the grape must, and the maceration technique had a strong impact on all the color parameters, with the only exception being the effect of the composition of the grape must on the hue (h_{ab}), which showed a lower significance. All the interactions were significant with respect to the chromatic parameters, except for the MxT interaction, which was only significant for the hue of the wine.

4. Conclusions

The must replacement of mature grape juice for immature grape juice and hot pre-fermentative maceration are technological alternatives to improve the color of Tannat red wines.

The effect of MR on the color and the general composition of wines is highly dependent on the composition of the grape. In contrast, HM improved the intensity and quality of the wine color by increasing the extraction of phenolic compounds and promoting condensation between anthocyanins and tannins, suggesting greater color stability. The results obtained in our research are relevant, because this winemaking technique allows us to mitigate the limitations in the extractability of anthocyanins presented by the Tannat cultivar. Moreover, this winemaking technique modified the anthocyanin profile of the wines in which a relative increase of the most oxidizable forms was obtained. Further studies should be focused on determining the effect of pre-fermentation heating on the degradation of oxidation enzymes and how that influences the phenolic profile of wines.

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