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Influence of Prefermentative Cold Maceration on the Color and Anthocyanic Copigmentation of Organic Tempranillo Wines Elaborated in a Warm Climate

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The stabilization of red wine color by the copigmentation phenomenon is a crucial process that does not always proceed favorably under natural conditions during the first stages of vinification. The impact of the prefermentative cold maceration technique on the phenolic composition and magnitude of the copigmentation level of organic Tempranillo wines elaborated in a warm climate have been studied as an enological alternative to the traditional maceration for obtaining highly colored wines. Tristimulus colorimetry was applied to study the color of wines during vinification, and a high-performance liquid chromatography (HPLC) procedure was used for the analysis of phenolic compounds. Spectrophotometric and colorimetric analyses were also performed to evaluate the copigmentation level of the wines. Significant chemical and color differences were found depending on the maceration technique applied. Prefermentative cold macerated wines were richer in those compounds accounting directly for the color of red wine (anthocyanins) and those involved in anthocyanin stabilization through copigmentation reactions (phenols), which was in accordance with the higher copigmentation degree and darker, more saturated and vivid bluish colors. The evaluation of the copigmentation based on colorimetric parameters in the CIELAB color space showed that prefermentative cold maceration caused greater effectiveness of copigmentation than traditional maceration since it induces more important and hence more easily perceptible color changes.

KEYWORDS: Color; organic red wine; warm climate; copigmentation

INTRODUCTION

The color of red wine is one of its most important quality parameters, which significantly determines the sensorial evaluation. Generally, it is the first characteristic perceived, and therefore, it plays a key role in the decision-making process of the consumer, who usually tends to prefer wines having deep color and hue (*1*). Phenolic compounds, which are responsible for wine color, are extracted from the skin and seeds of grapes and diffuse into the must and wine during the maceration step of the wine making process. While anthocyanins are the pigment accounting directly for the color of red wine, flavonols and hydroxycinnamic acid derivatives are involved in the stabilization of anthocyanins through copigmentation reactions (*2*). In this sense, the color of wines is determined first of all by the pigment content of the grapes and second by the pigments and copigments formed during vinification because the last ones exert an important influence on the higher or lower stability of color during aging.

In warm regions, the production of high quality red wines with high and stable color is greatly limited due to the stressful climate conditions that do not enable the grapes to reach optimum phenolic maturity at harvest (*3*, *4*). The most likely reason for this fact is that wines made from grapes low in pigments and cofactors are not able to form much copigmentation in the first steps of the wine making process (*2*); therefore, their color stabilization might not occur correctly. However, the concentrations and stability of wine anthocyanins can be affected by several factors, including viticultural practices and vinification techniques. In this sense, the elaboration of organic wines, based on an organic viticulture, constitutes an interesting strategy to improve the phenolic potential of red grapes in these areas since organic vineyards usually have higher natural resistance to the weather inclemency. Additionally, since no fungicides are used, microbes are more abundant, which lead to an increase in the synthesis of phenolic compounds acting as antioxidants (*5*).

Regarding the vinification technique, one of the most significant advances in warm climate vinifications has been the application of low maceration temperatures (5−15 °C) prior to fermentation, known as prefermentative cold maceration or cold soak. In the case of red wine production, this novel vinification technique was designed as an alternative to the traditional maceration for increasing the extraction of pigments, tannins, and aromas from the grape skins to the wine, which consequently improves some important quality characteristics of wines such as color and aroma (*6*−*10*). Although the application of prefermentative low temperature techniques implies an important investment in

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technology, one of the main advantages of this vinification practice compared to traditional vinification is the rapid cooling down of the must, which inhibits the activity of some enzymes, such as polyphenol oxidase, and microorganism development, such as acetic bacteria. In this sense, both the aroma compounds and the anthocyanic pigments extracted become protected from oxidative reactions in a nontoxic way, avoiding excessive traditional chemical treatments, which may produce losses of pigments, as well as potential health problems (*10*−*13*). In fact, the positive repercussions of prefermentative cold maceration on the final color and flavor of Syrah wines produced in Andalusia (southwest of Spain) have been already confirmed (*14*, *15*). However, there is no evidence of the application of the prefermentative cold maceration to organic wines as a useful technique to increase the extraction of anthocyanins and other phenolic compounds as well as the safety of the product, environment, and consumer.

Thus, the main objective of this work was to study the influence of prefermentative cold maceration on the phenolic composition and magnitude of copigmentation of organic wines elaborated from Tempranillo grapes grown in a warm climate and its effect on color quality.

The Tempranillo grape variety was selected because it is one of the most important red grape cultivars grown in Spain, with more than 75000 ha planted. This variety has been described as an excellent and robust grape, easy to cultivate with low vulnerability to diseases. It is defined as a neutral cultivar with subtle aroma and flavor that produces wines with intense fruity, spicy, and woody aromas (*16*). In recent years, it has been planted in warm climates vineyards, and its wines are becoming increasingly popular with consumers.

MATERIALS AND METHODS

Wine Making Protocol. Vinification was carried out on 350 kg of the *Vitis vinifera* var. Tempranillo, grown in an organic vineyard belonging to the Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), located in southeastern Spain (warm climate). Organic grapes were treated with natural pesticide such as sulfur and pheromones, allowed by organic agriculture. The grapes were harvested (2006 vintage) at optimum maturity (density, 12°Bé; total acidity, 5.76 g/L; and pH, 3.47), in good sanitary conditions, placed in 15 kg plastic boxes, and transported to an experimental wine-production center. Then, the grapes were destemmed and crushed, and the must was homogenized and distributed into 50 L stainless-steel tanks (pilot scale) so that each tank contained about 42−43 kg of must.

Alcoholic fermentation was induced by inoculation with *Saccharomyces cerevisiae* selected yeast (71D, 20 mg/Hl, 25 °C, Agrovin, Spain). To guarantee the development of malolactic fermentation, selected *Oenococcus oeni* lactic acid bacteria (VINIFERM Oe 104, Agrovin, Spain) were inoculated at the rate of 10 mg/L (after rehydration of cells in warm sterile water at 30 °C for 30 min) at the end of alcoholic fermentation. Two variants of maceration treatment, in four replicates for each one ($n = 4$), were performed.

Traditional Maceration (*TM*). Fermentation occurred at controlled temperature (25 °C). Fermentation caps were punched down once a day during the on-skin maceration period, which lasted 6 days (fermentative alcoholic maceration). After this, the mash was drawn off to remove the skins and other solid parts, and the free-run musts were left to finish malolactic fermentation, which occurred after 15 days. When fermentative processes were finished, the wines were racked and stored in 50 L stainless steel tanks and bottled 30 days later.

Cold Prefermentative Maceration (*CM*). The whole process consisted of two stages: a first stage of 8 days of prefermentative cold maceration (between 5 and 8 °C), followed by 6 days of traditional maceration (between 20 and 25 °C). Cold maceration was carried out controlling the skin contact time and temperature by using an industrial refrigeration system, consisting of a refrigeration unit (REVINSA mod. minifrico C-18, Arganda Del Rey, Madrid) for the recirculation of refrigerant liquid (water/glycerol at 2−7 °C) through cooling water jackets to keep low temperatures. After the cold maceration period was completed, the temperature of the tanks was rapidly brought to 20 °C to allow the starting of alcoholic fermentation. After this, the vinification process was carried out in the same conditions as those for the control wines, as previously explained.

Table 1. Conventional Analytical Data[a] of the Final Red Wines

analytical data	CM[b]	TM[b]
ethanol (% v/v)	12.10	11.74
pH	3.70	3.57
total acidity (g/L as tartaric acid)	4.30	4.47
volatile acidity (g/L as acetic acid)	0.60	0.50
reducing sugars (g/L)	1.88	1.75
malic acid (g/L)	0.063	0.046
total sulfur dioxide (ppm)	35.80	34.75
free sulfur dioxide (ppm)	9.00	7.75
K^+ (ppm)	2	1

[a] Average values of replicate. [b] Abbreviations: CM, cold macerated wines; TM, traditional macerated wines.

In this study, must and wine samples (100 mL) were taken at five different moments along the vinification process: (1) at the beginning of fermentative maceration period, (2) during the alcoholic fermentation, (3) at the end of alcoholic fermentation, just after skin removal, (4) at the end of malolactic fermentation, and (5) at the moment of bottling. These sampling moments were specifically selected since they correspond to different vital periods for wine color: from 1 to 3, during the maceration phase, when anthocyanins and other phenolic compounds are extracted from grape skins and transferred to the must; and from 4 to 5, during the first few months of storage since the short shelf life of red wines produced in warm climates requires careful control of their color characteristics, especially when the main mechanism of the stabilization of color (copigmentation phenomenon) occurs.

Oenological Parameters. The conventional oenological parameters (**Table 1**) were performed according to the Official Methods established by European Union (*17*).

HPLC Analysis of Anthocyanins. High-performance liquid chromatography was applied to the anthocyanin determination by direct injection of the samples, previously filtered through a 0.45 μm Nylon filter (E0034, ANALISIS VINICOS, Spain), in an Agilent 1100 chromatographic system equipped with a quaternary pump, an UV−vis diode-array detector, an automatic injector, and ChemStation software (Palo Alto, CA). All analyses were made in triplicate. The anthocyanin identification was performed following the method described by Heredia et al (*15*). Anthocyanins were separated using a Zorbax C18 column (250 × 4.6 mm, 5 μm particle size) maintained at 38 °C. Acetonitrile−formic acid−water (3:10:87) as solvent A and acetonitrile−formic acid−water (50:10:40) as solvent B were used. The elution profile was as follows: 0−10 min 94% A−6% B; 10−15 min 70% A−30% B; 15−25 min 60% A−40% B; 25−35 min 55% A−45% B; 35−40 min 50% A−50% B; 40−42 min 40% A−60% B; 42−43 min 94% A−6% B. The flow rate was 0.8 mL/min, and the injection volume was 50 μL. UV−Vis spectra were recorded from 200 to 800 nm with a bandwidth of 2.0 nm. The quantification was made at 525 nm by comparing the areas and the retention times with the malvidin 3-glucoside standard, and anthocyanin concentration was expressed as mg/L.

Total anthocyanins (TA), sum of nonacylated anthocyanins (sum_gl), sum of acetylglucosides (sum_ac) and sum of coumarylglucosides (sum_cm) were also calculated.

Copigmented and Polymerized Anthocyanin Determination. The contribution of copigmented anthocyanins (% CA), free anthocyanins (% FA), and polymeric pigments (% PP) to the total wine color at pH 3.6 were determined following the method proposed by Boulton (*18*). Wine samples were first adjusted to pH 3.6.

Colorimetric Measurements. The whole visible spectrum (380−770 nm) was recorded at constant intervals ($\Delta\lambda = 2$ nm) with a Hewlett-Packard UV−vis HP8452 spectrophotometer (Palo Alto, CA), using 2 mm path length glass cells and distilled water as a reference. The CIELAB parameters (L^*, a^*, b^*, C^*_{ab}, and h_{ab}) and the CIELUV saturation (s^*_{uv}) were determined by using the original software CromaLab (*19*), following

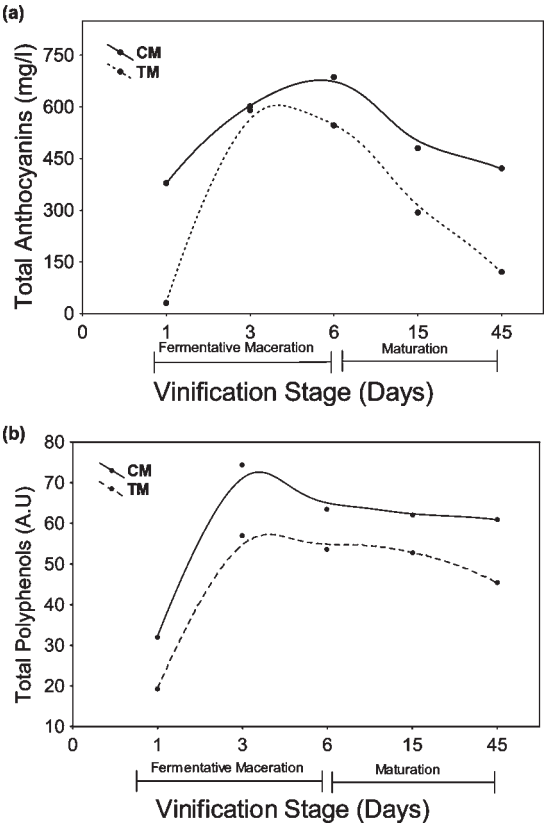

Figure 1. Total anthocyanin (**a**) and total polyphenol (TP) (**b**) evolution during vinification in cold macerated wines and traditional macerated wines.

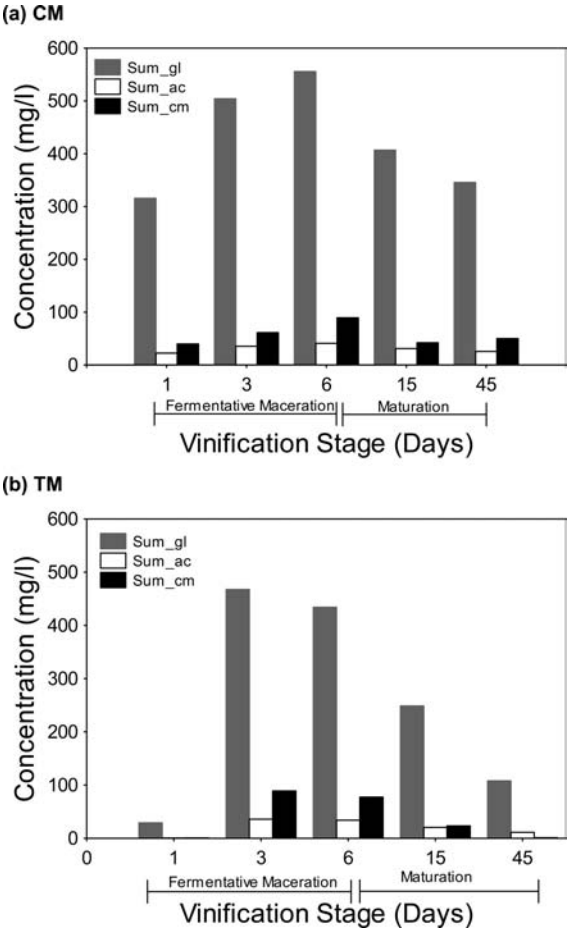

Figure 2. Evolution of the anthocyanin fractions (mg/L) during vinification for both cold macerated (**a**) and traditional macerated (**b**) wines.

the Commission Internationale de L'Eclariage's recommendations (*20*): the 10° Standard Observer and the Standard Illuminant D65. Saturation (s^*_{uv}) was included in the colorimetric analysis because it is considered the best correlation for the visually perceived saturation, and CIELAB space cannot define a similar correlation (*21*).

Color differences (ΔE^*_{ab}) were calculated as the Euclidean distance between two points in the three-dimensional space defined by L^*, a^*, and b^*: $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Statistical Analysis. Significant differences among wines and for each variable were assessed by analysis of variance (ANOVA) using the Statistica, version 8.0, software (*22*).

RESULTS AND DISCUSSION

Pigment Evolution. Independent of the maceration treatment, the two Tempranillo wines showed the same chromatographic profile; but quantitatively, the results showed that prefermentative cold maceration had a positive effect on the extraction and evolution of phenolic compounds during vinification.

Considering total anthocyanin (TA) and total polyphenol (TP) evolution in the two vinification protocols (**Figure 1**), it can be observed that along the whole alcoholic fermentation period (1−6 days), cold macerated wines had higher levels of phenolic compounds than traditional wines. The effectiveness of cold prefermentative treatment on the magnitude of pigment extraction was confirmed since prior to the beginning of the alcoholic fermentation, the anthocyanin content extracted represented 55% of the total extraction. As a consequence, prerefrigerated musts started the alcoholic fermentation with significantly higher phenolic content regarding traditional wines (TA = 378.16 ± 27.54 mg/L versus 30.82 ± 17.75 mg/L; TP = 32.06 ± 4.10 versus 19.37 ± 0.35).

It is also noteworthy that the wine making protocol influenced the evolution pattern of the different anthocyanin fractions resulting in clear differences regarding their stability (**Figure 2**).

In cold macerated wines, anthocyanins underwent a progressive increase during the whole fermentative maceration period, reaching their maximum just before skin removal (sum_gl = 555.73 ± 40.67 mg/L; sum_ac = 41.10 ± 4.17 mg/L; and sum_cm = 89.46 ± 18.30 mg/L). Notwithstanding, in traditional vinification, the rate of extraction increases rapidly during the first days of maceration because there was no previous contact between skins and must. The anthocyanin concentration reached a maximum on the third day of maceration (sum_gl = 467.63 ± 55.28 mg/L; sum_ac = 36.02 ± 7.78 mg/L; and sum_cm = 89.06 ± 10.52 mg/L), but after that, a slight decrease in all anthocyanin fractions was experimented. This meant a final loss of 7%, 5%, and 13%, respectively, which had important consequences over the pigment content (TA) of both wines at the end of the extraction process, being 20.5% higher in cold macerated wines (686.29 ± 19.03 mg/L versus 546.44 ± 25.09 mg/L). The statistical differences found in total anthocyanic content among the wines were due especially to monoglucosides, with higher level of significance ($p < 0.01$) for malvidin (384.68 ± 22.80 versus 311.44 ± 23.77 mg/L), delphinidin (61.16 ± 7.84 versus 40.28 ± 3.60 mg/L), and petunidin (81.00 ± 8.12 versus 56.64 ± 4.21 mg/L).

On the contrary, TP evolution was similar in the two wines (**Figure 1**). Although cold maceration induced higher extraction than traditional maceration, no significant difference was found among the two vinification treatments just before skin removal (TP = 63.45 ± 6.32 versus 53.72 ± 2.59). This result is according to previous studies on other varieties wines (*14*, *23*), confirming that phenolic extraction is not more influenced by the low temperature maceration technique as anthocyanin extraction is. Apparently,

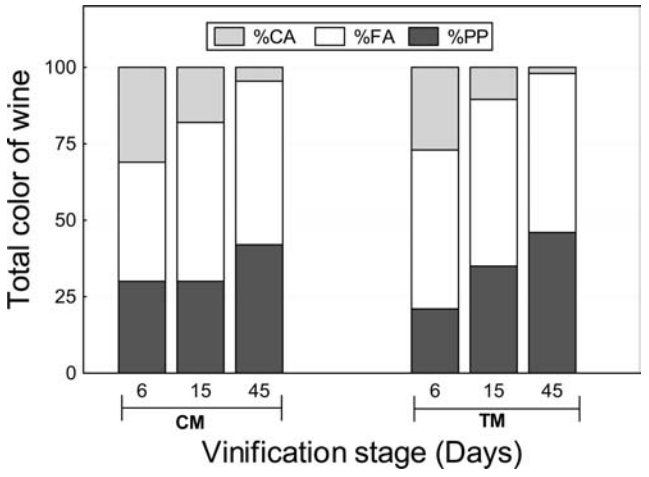

Figure 3. Evolution of the copigmented anthocyanins (% CA), free anthocyanins (% FA), and polymeric pigment (PP) during the two vinification techniques.

this observation might affect the future stability of wine color, but from a sensorial perspective, this effect can be considered positive because excessive phenolic extraction could make the wine too astringent and affect its global quality (*24*).

As expected, the concentration of monomeric anthocyanins decreased from skin removal to the moment of bottling in both kinds of wines studied, being especially remarkable for traditional macerated wines (77% versus 38% in cold macerated wines) (**Figure 1**). These observations confirm that red wines produced in warm climate regions easily suffer a considerable loss of pigment during the first stage of vinification, especially by traditional vinification. In this sense, the application of prefermentative cold maceration represented a useful oenological alternative to prevent an excessive pigment loss, improving the global quality of these wines. At the moment of bottling, both types of wines could be statistically differentiated regarding their chemical composition. Cold macerated wines showed the highest total anthocyanin content (421.55 ± 36.59 versus 120.83 ± 15.30 mg/L in traditional wines). An interesting observation is that the cryomaceration technique seemed to protect to a larger extent the presence of methylated anthocyanins (malvidin = 248.18 ± 10.46 mg/L versus 81.52 ± 15.58 mg/L; petunidin = 46.55 ± 4.78 mg/L versus 11.48 ± 2.38 mg/L; peonidin = 14.17 ± 3.34 mg/L versus 4.25 ± 0.64 mg/L) and acylated anthocyanins (sum_cm = 50.07 ± 9.98 mg/L versus 1.28 ± 0.70 mg/L) whose chemical characteristics have a great effect on the copigmentation phenomenon (*25*, *26*).

Copigmentation and Polymerization Evolution. At the end of the maceration period, the high anthocyanic and total polyphenol content reached by low temperature treatment caused CM wines to have a higher grade of copigmentation and polymerization than TM wines (31% versus 27% and 30% versus 21%, respectively) (**Figure 3**). Despite of the pigment degradation, at the end of malolactic fermentation, the prerefrigerated wines showed the highest percent copigmentation values (18% versus 10.5% in traditional macerated wines). The highest copigmentation degree observed in cold macerated wines is evidence that they presented a better cofactor/pigment ratio than traditional wines (*2*) probably due to the lower loss of pigment experienced.

During the last stage of the maturation phase (15−45 days), an important decrease of % copigmentation (% CA) was produced, which was almost zero at the moment of bottling (2−4%). The gradual formation of new and more stable pigments during this period in the two kinds of wines was confirmed by a notable increase of bisulphite-stable color (% PP), which was higher in traditional macerated wines (46% versus 42%, in cold macerated wines) (**Figure 4**).

Colorimetric Evolution. The evolution of the CIELAB (L^*, C^*_{ab}, and h_{ab}) and CIELUV (s^*_{uv}) psychometric color parameters in the course of the two wine making vinifications is shown in **Figure 4**. Color extraction was different for each maceration treatment but was coherent with pigment extraction.

In cold macerated wines, all of the colorimetric parameters evolve constantly in a positive way during the whole period of maceration indicating a positive effect not only on the color density but also on the color stability. Specifically, the lightness, L^*, of the initial must decreased by 13%, while chroma, C^*_{ab}, hue angle, h_{ab}, and saturation, s^*_{uv}, increased by 49%, 1°, and 56%, respectively.

In traditional macerated wines, the most color extraction was produced on the third day of fermentative maceration ($L^* = 76.17 \pm 3.32$, $C^*_{ab} = 35.22 \pm 4.74$ CIELAB units, and $s^*_{uv} = 0.67 \pm 0.11$ CIELUV units) coinciding with the maximum pigment extraction (TA = 590.03 ± 40.03 mg/L). However, the loss of pigments observed between the third and the sixth day of maceration (7% as total anthocyanins) resulted in a fall of color (L^* and h_{ab} increased by 10% and 3°, while chroma, C^*_{ab}, and saturation, s^*_{uv}, decreased by 36% and 42%). Consequently, TM wines tended to show lower chromatic stability from maceration period than CM wines. The most likely reason for this fact could be a dual effect of the temperature with competing contributions: (i) it enhances the solubility of most species but especially those cofactors that have limited solubility, enhancing the pool of copigmented pigments; (ii) thermodynamically, it favors the dissociation of copigmented forms and causes loss of color (*2*, *27*). On cooling treatments, the lower temperatures of the must inhibits the activity of some enzymes such as polyphenol oxidase and microorganism development, which avoid anthocyanin degradation favoring the color stability even when temperatures reach normal fermentation values (*11*, *28*).

Comparing the color coordinates obtained at the end of alcoholic fermentation, significant differences were found for all color parameters among the two kinds of wines, revealing that the application of prefermentative low temperature induced higher color extraction than traditional maceration ($L^* = 73.59 \pm 3.05$ versus 82.15 ± 1.35 CIELAB units, $C^*_{ab} = 33.18 \pm 5.61$ versus 22.49 ± 2.07 CIELAB units, and $h_{ab} = -9.25° \pm 0.59$ versus −6.45° ± 0.8, respectively).

Different causes have been attributable to the numerous anthocyanin transformations along the course of the first months following the maceration period, including the partial elimination by precipitation/adsorption by lactic bacteria, and also the stabilization by progressive displacement of copigmentation complexes into polymeric pigments (*29*, *30*). In this sense, the polymerization process was not the main cause of a decrease in anthocyanins during the malolactic fermentation period (6−15 days) because both types of wines experienced a notable loss of color with respect to skin removal ($L^* = 76.07$ CIELAB units and $s^*_{uv} = 0.52$ CIELUV units in cold macerated wines; and $L^* = 84.74$ CIELAB units and $s^*_{uv} = 0.31$ CIELUV units in traditional macerated wines).

At the moment of bottling, although higher grade of polymerization was obtained in traditional macerated wines, lighter and less saturated wines were finally obtained by this technique ($L^* = 83.52$ CIELAB units and $s^*_{uv} = 0.29$ CIELUV units versus 76.36 and 0.44 CIELAB and CIELUV units, respectively), the difference among them being statistically significant (< 0.05). In the two types of wines, between 15 and 45 days, it can be observed that chroma (C^*_{ab}) and saturation (s^*_{uv}) modifications were more variable, which showed a slight tendency to decrease, while lightness (L^*) remained practically constant. Obviously, browning

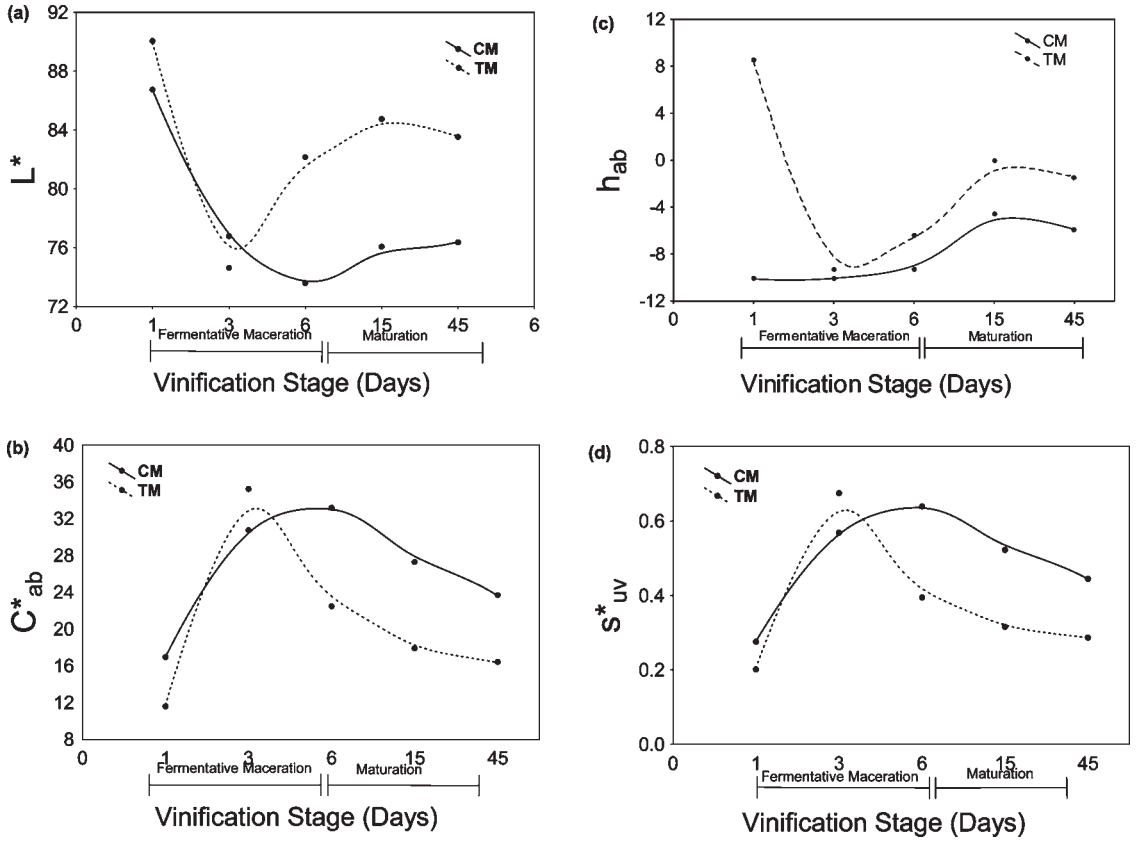

Figure 4. Evolution of color parameters: (**a**) L^* (lightness), (**b**) C^*_{ab} (chroma), (**c**) h_{ab} (hue angle), and (**d**) s^*_{uv} (saturation) during vinification. Cold macerated versus traditional macerated wines.

and precipitation of oligomeric and polymeric pigments also exist in red wines, which partially explain why anthocyanin polymerization led to wines with less color intensity and purity in late stages of vinification (*31*, *32*).

With respect to the qualitative aspect of color (h_{ab}), both wines exhibited an increase of hue toward 0° (from bright bluish-red to red hues) from skin removal to bottling, showing a clear reduction of the blue component of the red color. These changes in color characteristics reflect the progressive displacement of copigmentation complexes and free anthocyanins by more stable polymeric pigments (*33*). However, the hue evolution was less intense for cold macerated wines, keeping their bluer tonalities for a longer time than the traditional macerated wines ($h_{ab} = -5.92°$ versus $-1.48°$, respectively). The higher amount in bluish forms of anthocyanins (malvidin, petunidin, and delphinidin) and the higher degree of copigmentation could explain this finding (*34*).

The mean color difference calculated between the final wines was 9.56 CIELAB units. Considering that ΔE^*_{ab} of up to 3 CIELAB units indicates differences in color perceptible to the human eyes (*35*), it is concluded that a noticeable influence of the maceration technique on the color of wine existed. The differences of lightness (ΔL^*), chroma (ΔC^*_{ab}), and hue (Δh_{ab}) calculated among them showed that the color differences were more quantitative than qualitative ($\Delta L^* = -7.16$, $\Delta C^*_{ab} = 6.03$, and $\Delta h_{ab} = -3.98$ CIELAB units) and reveal that prefermentative cold maceration yields more darker, more intense, and with more bluish color wines than those submitted to traditional maceration.

These results are in agreement with those of Gómez-Míguez et al. (*14*) and Heredia et al. (*15*) for Syrah wines, but it should be taken into consideration that the effectiveness of the cryogenic technique used is largely determined by the way to achieve the temperature fall (freezing of grapes, dry ice, cold-maceration, etc.), as well as the time and intensity of prefermentative maceration or the grape variety used in vinification (*9*, *10*, *36*−*38*).

Colorimetric Study of Copigmentation: CIELAB Color Space. The colorimetric implications of the copigmentation phenomenon on the total color of wine has been evaluated by tristimulus colorimetry since the entire visible spectrum (380−770 nm) must be considered to obtain an integral definition of color as well as to quantify the color difference that this phenomenon implies (*39*). In this study, the wine color with copigmentation effect was obtained from the absorbance spectrum of the wines after eliminating the SO_2 effect by means of the addition of acetaldehyde in excess. The wine color without copigmentation effect was reconstituted from the absorbance spectrum of the wine sample after diluting 20 times with wine-like solution (pH 3.6) and multiplying by the dilution factor. That dilution leads to the dissociation of the copigment complex, while the contributions of the free anthocyanins and the polymeric pigments remain (*40*).

The chromatic parameters (L^*, C^*_{ab}, and h_{ab}) of cold and traditional macerated wines with and without the copigmentation effect are summarized in **Table 2**. The results obtained show that the copigmentation phenomenon provokes important changes in the wine color independent of the vinification protocol. Quantitatively, when copigmentation complexes were dissociated, the wines presented higher values of lightness (L^* increased by 3%) and lower values of chroma (C^*_{ab} decreased by 10%). Regarding the qualitative component of color, the wines showed higher values of the hue angle (h_{ab} increase in 7.14°). According to Castañeda-Ovando et al (*41*), the formation of the charger-transfer complex associated with the copigmentation phenomenon causes changes in the spectral properties of the molecules in the flavilium ion, increasing the absorption intensity (hiperchromic effect) and its maximum absorption wavelength (batochromic

Table 2. Color Parameters of Copigmented and No-Copigmented Wines in Cold Macerated and Control Tempranillo Wines[a]

		copigmented wines	no-copigmented wines
CM	L^*	76.20 ± 0.72 a	80.48 ± 1.71 a
	C^*_{ab}	26.68 ± 0.85 a	22.32 ± 0.93 b
	h_{ab}	−3.88 ± 0.42 a	9.24 ± 2.01 b
TM	L^*	83.22 ± 1.02 a	84.23 ± 3.24 a
	C^*_{ab}	20.12 ± 1.08 a	19.62 ± 1.73 a
	h_{ab}	0.15 ± 1.08 a	8.76 ± 0.05 a

[a] Different letters within the same row mean significant differences ($p < 0.05$).

Table 3. Color Differences (ΔE^*_{ab}) Associated with the Copigmentation Phenomenon in Cold Macerated and Traditional Macerated Wines

	cold macerated wines	traditional macerated wines
ΔE^*_{ab}	8.25	3.19
ΔL^*	−4.28	−1.01
ΔC^*_{ab}	+4.36	+0.50
Δh_{ab}	−12.62	−8.61

effect). From a sensory perspective, these findings mean that for the same wine, as a consequence of the existence of copigmentation, the color changes notably in terms of luminosity, chromaticity, and intensity. Specifically, cold maceration contributed to the typical chromatic characteristics of young red wine, described as a vivid color with a red-blue hue.

The color difference (ΔE^*_{ab}) calculated between the wines with and without the copigmentation effect for both maceration techniques were higher than 3 units CIELAB, hence visually relevant (**Table 3**). However, ΔE^*_{ab} was more marked in cold macerated wines (8.25 versus 3.19 CIELAB units, in traditional macerated wines), which was consistent with the higher values obtained regarding the magnitude of copigmentation. Thus, cold prefermentative maceration caused greater effectiveness in the copigmentation phenomenon than the traditional maceration since it induced more important and hence more easily perceptible color changes.

In an attempt to understand the significance of these changes, the lightness, chroma, and hue differences (ΔL^*, ΔC^*_{ab}, and Δh_{ab}) were also calculated (**Table 3**). It was verified that color differences in cold macerated wines were due to both quantitative and qualitative changes ($\Delta L^* = -4.28$, $\Delta C^*_{ab} = +4.36$, and $\Delta h_{ab} = -12.62$), while in traditional macerated wines, they were basically qualitative ($\Delta L^* = -1.01$, $\Delta C^*_{ab} = +0.50$, and $\Delta h_{ab} = -8.61$).

As a summary, it might be stated that from a global chemical perspective, the results indicate that the maturation stage starts from different phenolic and colorimetric characteristics of the wines depending on the maceration technique previously applied. In this sense, cold macerated wines were richer in the compounds that account directly for the color of red wine (anthocyanins) and those that are involved in the stabilization of anthocyanins through copigmentation reactions (polyphenols); therefore, cold prefermentative maceration increased not only the extraction of anthocyanins but also their initial stability. For these reasons, a priori, cold macerated wines presented the most desirable chemical and sensorial characteristics that are nowadays required to produce high quality wines, especially in terms of their color.

LITERATURE CITED

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