

Impact of Caffeic Acid Addition on Phenolic Composition of Tempranillo Wines from Different Winemaking Techniques

José Luis Aleixandre-Tudó,[†] I. Álvarez,[†] Victoria Lizama,[†] María José García,[†] José Luis Aleixandre,^{*,†} and Wessel J. Du Toit[‡]

[†]Departamento de Tecnología de Alimentos, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

[‡]Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, Matieland, 7602 Stellenbosch, South Africa

ABSTRACT: The effect of prefermentative and postfermentative caffeic acid (CFA) addition, prefermentative cold maceration, and a simulation of the micro-oxygenation technique through acetaldehyde addition on the phenolic and color composition of Tempranillo wines was investigated. Cold soaking and dry ice addition were performed as prefermentative techniques. Wines were analyzed after the end of the malolactic fermentation and after 6 and 12 months' storage. The results showed an important effect in wines to which CFA had been added, suggesting intramolecular copigmentation reactions through direct interaction between anthocyanins and free phenolic acids, thereby increasing the acylated anthocyanin fraction with an increase in color stability. The higher concentration of total phenols and lower hue values in CFA-added wines also contributed to the stability of these compounds during storage. Prefermentative cold maceration was shown to be influenced by the vintage. Phenolic acids, the acylated anthocyanin fraction, and total phenolics showed higher values in CFA-added and acetaldehyde-added wines. No differences were found in color density between the control wines and both the prefermentative and postfermentative CFA-added wines. However, a higher anthocyanin polymeric fraction and higher acylated anthocyanins, phenolic acids, and total phenols were observed in the CFA-added wines. The implications of this for the color stability of Tempranillo are also discussed.

KEYWORDS: copigmentation, caffeic acid, dry ice, cold soak, acetaldehyde addition, Tempranillo

■ INTRODUCTION

The color of food products is an important quality parameter that can influence consumer behavior. Anthocyanins are natural pigments that are distributed widely in nature. Anthocyanins and their polymeric pigments, flavanols and their polymers, are phenolic compounds that can have a considerable impact on the sensory characteristics of a red wine. It is usually accepted that the color of red wine is mainly due to the anthocyanin composition of grapes, whereas the color of aged red wines is a consequence of their instability and reactivity.¹ These color changes result from the formation of more stable pigments during aging. Over the past few years, several groups of anthocyanin-derived pigments with different colors have been identified in red wine.²

An important factor in winemaking is that some of the initial red color of the must is lost during vinification. During the early stages of winemaking, the copigmentation phenomenon plays an important role in color stability. During copigmentation, loose associations are formed between the red compounds (anthocyanins) of the grape and other, mostly colorless, polyphenols, leading to hyperchromic and bathochromic shifts.^{3,4} The extent of these effects depends on many factors.^{3,5–8} Visually, the wine's color gets darker, or a change in color tonality is observed. This phenomenon contributes to the solubilization and conservation of the red color in young red wines, serving perhaps as a first step in the formation of more stable pigments during wine aging.

Caffeic acid (CFA) is a hydroxycinnamic acid product of caftaric acid hydrolysis, which may be induced by exposure of the grapes to sunlight.^{5,9} Cinnamic acids constitute the main acyl group in the structure of acylated anthocyanins. Several

studies have been published in which the effects of CFA addition, which thereby acts as copigment, in vitro and in vivo solutions have been investigated. The results show a great copigmentation effect when CFA interacts with anthocyanins in model systems^{9–14} and in wine.^{15–18} Several authors have mentioned that an increase in temperature or alcohol content diminishes the effect of copigmentation and that the copigment/pigment molar ratio increases.^{19–21} Moreover, Kunsági-Máté et al.²² suggest that copigmentation complexes become more stable over a critical alcohol concentration of 8% vol.

Prefermentative cold maceration, also known as cold soaking or cryomaceration, is increasingly being used by enologists worldwide to improve certain important quality characteristics of wines.^{23–25} During prefermentative extraction, polyphenols from the skins are extracted in the absence of ethanol.²⁵ The low temperatures required to obtain this effect can be achieved in different ways, with the addition of dry ice (solid carbon dioxide) being a common method.²⁶ When dry ice is added to the crushed grapes, the grape skin cells are broken and disorganized through freezing, which facilitates the extraction of aromatic and phenolic compounds.^{27,28} A prefermentative maceration phase can be considered to increase the contact between the skin phenolic compounds and the aqueous phase and to enhance copigmentation reactions.^{23,29,30}

Micro-oxygenation is a winemaking process in which small, controllable amounts of oxygen are introduced into red wines.

Received: June 20, 2013

Revised: September 26, 2013

Accepted: September 26, 2013

Published: September 26, 2013

The benefits claimed with this practice include stabilization of wine color, a softening of tannins, and a lessening of vegetative aromas.^{31,32} Acetaldehyde is thought to be formed from the oxidation of ethanol by peroxide or hydroxyl radicals. Acetaldehyde derived from ethanol oxidation can link anthocyanins and flavanols to form unstable (purple) ethyl-linked pigments, as well as more stable (orange) pyranoanthocyanin pigments.³⁴

The red cultivar Tempranillo is the most widely planted red grape cultivar in Spain, being present throughout the country. The aim of this work was to evaluate the effect of CFA addition on the color and phenolic composition of Tempranillo wines from Valencia (Spain). The effect of several winemaking techniques was also tested. Prefermentative cold maceration techniques, such as cold soak or dry ice addition, the effect of acetaldehyde addition, and a combination of these techniques with caffeic acid addition were also performed. Several authors have mentioned that an increase in copigmentation reactions takes place when copigments are added before fermentation.^{11,12,15,17,19,21,30} As far as we could determine, no information is available in the literature on the effect of in vivo postfermentative copigment additions or on the interaction between prefermentative maceration and micro-oxygenation techniques.

MATERIALS AND METHODS

Wine Samples. Tempranillo grapes were obtained from a commercial vineyard in the Utiel-Requena region of Valencia (Spain). Three consecutive vintages were harvested at commercial ripeness: 217 g/L of total sugar content, total acidity of 6.1 g/L (as tartaric acid measured by potentiometric titration), and potential alcohol of 12.8 in 2009; 232 g/L of total sugar content, total acidity of 5.9 g/L (as tartaric acid), and potential alcohol of 13.6 in 2010; and 215 g/L of total sugar content, total acidity of 6.3 g/L (as tartaric acid), and potential alcohol of 12.6 in 2011. The grapes were harvested manually and packed in 20 kg boxes, and the wines were produced at the experimental wine production center at the Universitat Politècnica de València (UPV). In each year the grapes were destemmed, crushed, mixed, and divided into closed 50 L stainless steel tanks. Sulfur dioxide was added at 100 mg/kg of potassium metabisulfite prior to the fermentation. The wine in all of the tanks was processed using traditional winemaking methods, with inoculation of commercial yeasts at 20 g/hL according to the supplier's recommendations (*Saccharomyces cerevisiae* strain EP 841, Agrovín, Spain). The temperature was controlled at 25 °C during the fermentation process. Temperature and density were measured daily. Manual punching down of the skins was carried out twice a day. After the completion of alcoholic fermentation, the skins were pressed, and the first 5 L of pressed wine was mixed with 25 L of free-run wine in all of the tanks. Malolactic fermentation (MLF) was induced by inoculation with *Oenococcus oeni* strain OE 104 (Agrovín, Spain) lactic acid bacteria and conducted at room temperature. Malic acid was measured colorimetrically by anion exchange column daily. When MLF was completed, sulfur dioxide was added to all of the wines at 5 g/hL. All of the vinifications were performed in triplicate. The bottled wines were then closed under cork.

Three different skin maceration methods were applied: no prefermentative maceration, known as traditional vinification (T-V); cold soak at 6–8 °C for 4 days (C-S); and dry ice addition (0–2 °C) and cold soak at 6–8 °C for 4 days (D-I) followed by traditional fermentation. Three different CFA treatments were also performed: control wines with no additions; 90 mg/L CFA added before fermentation; and 90 mg/L CFA added after fermentation (Fluka, Milwaukee, WI, USA). The wines were bottled after MLF. To reproduce the micro-oxygenation effect in the bottle, an acetaldehyde (ACS reagent ≥99.5%, Sigma-Aldrich) solution, equivalent to 3 mL of O₂/L of wine and per month was added to the wines through the cork

with a hypodermic syringe (AA). All treatments were done in triplicate. Figure 1 shows the experimental design carried out in the three consecutive years.

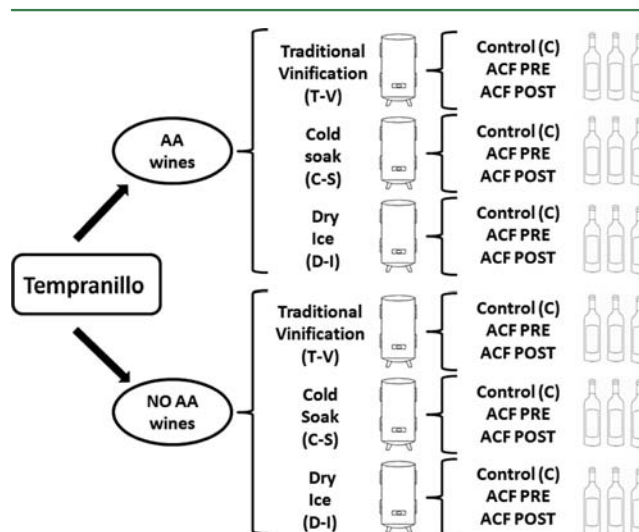


Figure 1. Experimental design.

Analytical Methods. The phenolic composition of the wines was determined with UV and visible spectrophotometry, using a UV–visible JASCO V-530 spectrophotometer, and with high-performance liquid chromatography (HPLC), using a JASCO MD-2010 Plus coupled with a diode array detector (DAD) (JASCO LC-Net II/ADC, Tokyo, Japan). Analytical methods were carried out at three time points: at the end of MLF (T0) after bottling and at 6 (T6) and 12 (T12) months of aging in bottle. Color intensity and hue were estimated using the analytical methods described by Glories.³³ The methylcellulose tannin precipitation assay was performed according to Sarneckis et al.³⁵ The contributions of the copigmented anthocyanins, noncopigmented free anthocyanins, and polymeric anthocyanins to the total wine color were determined according to the method of Boulton.³⁶ Individual phenolic compounds (phenolic acids, 3-flavanols, flavonols, main anthocyanidins, and acylated anthocyanins) were determined according to the method of Boido et al.³⁷ Total anthocyanins were estimated as the sum of anthocyanidins and acylated anthocyanins, whereas total phenols were calculated as the sum of the obtained individual phenolic compounds. The wine samples after centrifugation and filtration were injected directly into the HPLC (20 μL). Separation was carried out on a Gemini NX (Phenomenex, Torrance, CA, USA) 5 μm, 250 mm × 4.6 mm i.d. column at 40 °C. The solvents were trifluoroacetic acid at 0.1% (A) and acetonitrile (B). The elution gradient was as follows: 100% A (min 0); 90% A + 10% B (min 5); 85% A + 15% B (min 20); 82% A + 18% B (min 25); 65% A + 35% B (min 30). Individual chromatograms were extracted at 280 nm (3-flavanols and phenolic acids), 320 nm (hydroxycinnamic acids), 360 nm (flavonols), and 520 nm (anthocyanins). For quantification, calibration curves were obtained with commercially available standards (gallic acid (Fluka), (+)-catechin (Fluka), caffeic acid (Fluka), rutin (Sigma-Aldrich, St. Louis, MO, USA), and malvidin-3-glucoside (Sigma-Aldrich)).

Statistical Analysis. The data corresponding to the control and copigmented wines elaborated with different winemaking techniques were analyzed by analysis of variance (ANOVA) and principal component analysis (PCA). Statgraphics Plus 5.1 software was used for the statistical treatment of the data.

RESULTS AND DISCUSSION

Combined Effect of Prefermentative Maceration Techniques and Caffeic Acid Addition. The dry ice and cold soak wines often led to higher total anthocyanin

Table 1. Effect of Prefermentative Maceration Techniques and CFA Additions on the Color and Phenolic Profile of Tempranillo Wines from Three Vintages, with Mean and Standard Deviation Values of the Phenolic Parameters^a

		2009			2010			2011		
		control	CFA	control	CFA	control	CFA	control	CFA	
color density	T0	T-V	11.72 ± 0.36 b α	11.25 ± 0.33 c α	11.94 ± 0.45 α α	11.67 ± 0.2 α α	11.2 ± 0.51 α α	12.68 ± 0.49 α β		
		C-S	10.28 ± 0.13 α α	10.5 ± 0.13 b β	12.01 ± 0.29 α α	11.33 ± 0.82 α α	10.35 ± 0.42 α α	10.67 ± 0.45 α α		
		D-I	9.55 ± 0.08 α α	10.4 ± 0.12 α β	12.17 ± 0.12 α α	11.16 ± 1.21 α α	10.37 ± 0.46 α α	11.02 ± 0.37 α α		
	T6	T-V	11.81 ± 0.49 b α	11.61 ± 0.73 b α	13.09 ± 2.24 α α	13.66 ± 1.01 b α	11.77 ± 0.28 α α	12.3 ± 0.35 α α		
		C-S	11.1 ± 0.01 b α	11.82 ± 0.78 b α	12.14 ± 0.25 α α	12.64 ± 0.84 ab α	11.14 ± 0.57 α α	11.86 ± 0.49 α α		
		D-I	9.31 ± 0.11 α α	9.32 ± 0.79 α α	11.8 ± 0.73 α α	11.9 ± 0.94 α α	11.33 ± 0.41 α α	11.65 ± 0.23 α α		
	T12	T-V	11.54 ± 0.69 b α	11.46 ± 0.83 b α	11.41 ± 0.28 α α	12.47 ± 1.05 α α	11.99 ± 0.48 α α	12.63 ± 0.62 α α		
		C-S	12.22 ± 0.07 b α	11.46 ± 0.41 b α	11.67 ± 0.91 α α	12.34 ± 0.44 α α	10.84 ± 0.33 α α	11.85 ± 0.57 α α		
		D-I	9.65 ± 0.3 α α	9.76 ± 0.98 α α	10.84 ± 0.14 α α	11.58 ± 0.56 α α	10.54 ± 0.38 α α	10.87 ± 0.46 α α		
	hue	T0	T-V	68.81 ± 0.19 b α	68.54 ± 0.25 b α	59.26 ± 0.14 α α	60.1 ± 1.81 α α	72.34 ± 2.8 α α	64.05 ± 5.6 α α	
		C-S	67.75 ± 0.31 α α	67.38 ± 0.8 α α	58.63 ± 0.89 α α	60.33 ± 1.32 α α	72.6 ± 2.98 α α	65.84 ± 0.65 α α		
		D-I	70.79 ± 0.11 c β	69.15 ± 0.48 b α	61.33 ± 1.16 α β	59.27 ± 0.25 α α	73.02 ± 3.64 α α	64.53 ± 1.49 α α		
T6	T-V	70.58 ± 3.7 α α	70.07 ± 1.6 b α	71.43 ± 1.8 α β	68 ± 1.11 α α	76.9 ± 0.05 α β	69.57 ± 1.06 α α			
	C-S	68.59 ± 0.89 α α	66.68 ± 0.88 α α	68.91 ± 0.13 α α	67.96 ± 2.18 α α	76.6 ± 1.3 α β	67.98 ± 2.03 α α			
	D-I	73.68 ± 0.78 α α	69.47 ± 2.63 ab α	71.16 ± 1 α α	69.35 ± 2 α α	75.89 ± 1.46 α β	66.39 ± 0.97 α α			
T12	T-V	76.99 ± 0.68 b α	74.07 ± 2.17 b α	79.69 ± 1.97 α α	74.49 ± 4.02 α α	79.52 ± 0.42 α β	70.92 ± 1.07 α α			
	C-S	69.44 ± 3.11 α α	69.12 ± 2.14 α α	75.34 ± 1.29 α α	72.22 ± 2.03 α α	80.45 ± 2.76 α α	72.81 ± 6.22 α α			
	D-I	79.05 ± 2.23 b β	73.37 ± 1.94 b α	79.08 ± 0.74 α β	72.71 ± 2 α α	78.43 ± 0.67 α β	69.23 ± 1.61 α α			
% copigmented anthocyanins	T0	T-V	15.9 ± 2.03 α α	20.33 ± 3.55 α β	17.35 ± 3.35 α α	19.86 ± 2.5 α α	24.56 ± 4.34 α α	19.51 ± 7.47 α α		
	C-S	22.3 ± 1.32 b α	23.71 ± 0.68 α α	18.24 ± 1.57 α α	18.32 ± 3.33 α α	34.26 ± 3.38 b β	24.42 ± 4.98 α α			
	D-I	28.64 ± 0.83 c α	29.31 ± 0.75 b α	19.09 ± 2.99 α α	18.21 ± 2.3 α α	32.61 ± 2.59 b β	25.31 ± 5.29 α α			
T6	T-V	7.83 ± 4.59 α α	10.74 ± 5.01 α α	11.42 ± 1.18 α α	11.62 ± 2.94 α α	15.65 ± 1.98 α α	14.34 ± 1.93 α α			
	C-S	9.32 ± 4.08 α α	7.39 ± 4.99 α α	10.43 ± 3.2 α α	9.19 ± 2.9 α α	13.92 ± 1.19 α α	12.66 ± 2.92 α α			
	D-I	17.39 ± 3.34 b α	19.1 ± 4.56 b α	9.75 ± 1.89 α α	10.98 ± 3.27 α α	14.28 ± 2.03 α α	12.33 ± 2.14 α α			
T12	T-V	6.21 ± 3.04 α α	3.68 ± 1.94 α α	8.81 ± 2.38 α α	5.64 ± 3.66 α α	15.8 ± 3.44 α α	13.52 ± 4.18 α α			
	C-S	5.82 ± 2.12 α α	6.07 ± 3.59 b α	7.27 ± 2.82 α α	4.39 ± 2.85 α α	17.77 ± 1.46 α α	15.02 ± 2.91 α α			
	D-I	9.09 ± 0.61 b β	7.21 ± 0.55 c α	7.57 ± 1.25 α β	3.1 ± 1.93 α α	16.45 ± 2.31 α α	14.86 ± 2.81 α α			
% free anthocyanins	T0	T-V	52.96 ± 3.47 c β	45.63 ± 1.92 c α	53.47 ± 3.44 b β	47.37 ± 2.12 α α	48.44 ± 2.25 b α	44.93 ± 2.73 α α		
	C-S	45.77 ± 0.87 b β	41.79 ± 1.59 b α	48.43 ± 1.49 α α	48.85 ± 1.24 α α	40.11 ± 3.92 α α	40.6 ± 5.58 α α			
	D-I	40.36 ± 0.92 α β	36.94 ± 0.48 α α	47.15 ± 2.1 α α	48.46 ± 2.38 α α	42.51 ± 1.97 α α	40.35 ± 3.64 α α			
T6	T-V	54.56 ± 7.69 α α	50.1 ± 9.66 α α	39.61 ± 2.93 α α	37.3 ± 2.78 α α	40.79 ± 5.52 α α	37.53 ± 4.85 α α			
	C-S	54.33 ± 8.01 α α	48.24 ± 9.94 α α	44.94 ± 0.87 b β	39.55 ± 2.05 ab α	44.65 ± 2.07 α β	36.48 ± 2.54 α α			
	D-I	46.42 ± 4.12 α α	43.64 ± 3.04 α α	45.28 ± 1.07 b β	41.47 ± 2.35 b α	43.21 ± 0.96 α β	35.96 ± 3.06 α α			
T12	T-V	40.06 ± 0.76 α α	43 ± 4.16 b α	34.5 ± 3.2 ab β	24.49 ± 3.94 α α	37.7 ± 2.31 α β	29.27 ± 3.83 α α			
	C-S	40.77 ± 0.95 α α	37.14 ± 3.46 α α	31.79 ± 1.91 α α	29.75 ± 4.22 b α	36.5 ± 1.55 α β	26.6 ± 2.57 α α			
	D-I	47.01 ± 0.47 b α	44.79 ± 2.84 b α	36.84 ± 2.13 b β	30.7 ± 3.48 b α	35.82 ± 2.11 α β	27.43 ± 2.56 α α			

Table 1. continued

	2009			2010			2011		
		control		control		control		control	
		CFA	CFA	CFA	CFA	CFA	CFA	CFA	
% polymeric anthocyanins	T0	T-V	31.15 ± 2.46 a α	34.05 ± 1.85 a α	29.18 ± 1.17 a α	32.77 ± 0.58 a β	27 ± 2.28 a α	35.57 ± 4.86 a β	
		C-S	31.88 ± 0.33 a α	34.52 ± 0.97 a β	33.34 ± 0.71 b α	32.83 ± 2.22 a α	25.64 ± 0.78 a α	34.98 ± 0.88 a β	
		D-I	31.01 ± 0.41 a α	33.76 ± 0.41 a β	33.77 ± 0.99 b α	33.34 ± 0.35 a α	24.88 ± 1.64 a α	34.34 ± 2.09 a β	
	T6	T-V	37.61 ± 3.38 a α	39.16 ± 5.63 ab α	54.98 ± 10.42 a α	56.45 ± 5.47 a α	43.56 ± 7.2 a α	48.13 ± 5.04 a β	
		C-S	36.35 ± 4.41 a α	44.38 ± 6.19 b β	49.64 ± 3.11 a α	54.5 ± 4.58 a α	41.43 ± 1.77 a α	50.86 ± 2.44 a β	
		D-I	36.2 ± 1.36 a α	37.26 ± 3.24 a α	48.8 ± 3.89 a α	52.17 ± 4.58 a α	42.51 ± 3.26 a α	51.71 ± 2.31 a β	
	T12	T-V	53.74 ± 3.77 b α	53.32 ± 2.5 b α	54.43 ± 1.96 a α	69.87 ± 2.58 b β	46.5 ± 1.14 a α	57.22 ± 0.88 a β	
		C-S	53.41 ± 5.27 b α	56.8 ± 4.77 b α	60.94 ± 1.14 b α	66.11 ± 1.88 a β	45.73 ± 0.38 a α	58.39 ± 1.71 a β	
		D-I	43.9 ± 0.44 a α	48 ± 2.96 a β	55.59 ± 1.55 a α	66.2 ± 4.64 a β	47.73 ± 0.58 a α	57.71 ± 1.22 a β	
	phenolic acids (mg/L)	T0	T-V	123.9 ± 6.44 a α	165.49 ± 6.13 a β	140.47 ± 6.02 ab α	227.76 ± 19.31 a β	112.55 ± 4.42 a α	148.87 ± 4.8 a β
			C-S	129.31 ± 5.31 a α	182.31 ± 4.20 b β	147.12 ± 2.15 b α	222.08 ± 7.09 a β	117.52 ± 11.9 a α	179.63 ± 2.61 b β
			D-I	119.03 ± 1.26 a α	184.12 ± 10.71 b β	137.13 ± 4.47 a α	216.44 ± 4 a β	115.35 ± 8.16 a α	164.91 ± 6.46 b β
T6		T-V	100.11 ± 6.44 a α	145.34 ± 2.63 a β	129.96 ± 0.61 a α	192.78 ± 13.94 a β	79.68 ± 4.33 a α	102.65 ± 4.04 a β	
		C-S	103.68 ± 3.06 b α	153.26 ± 3.33 b β	131.32 ± 14.47 a α	192.06 ± 24.7 a β	80.21 ± 6.04 a α	109.97 ± 3.01 b β	
		D-I	97.23 ± 4.1 a α	152.11 ± 2.27 b β	122.78 ± 5.58 a α	182.35 ± 21.44 a β	81.37 ± 2.13 a α	115.26 ± 9.87 b β	
T12		T-V	87.43 ± 10.24 a α	131.16 ± 14.28 a β	114.19 ± 2.06 a α	145.48 ± 10.02 a β	51.95 ± 2.32 a α	69.14 ± 8.68 a β	
		C-S	88.21 ± 6.31 a α	129.18 ± 7.30 a β	120.59 ± 3.46 ab α	142.4 ± 14.69 a α	49.53 ± 6.89 a α	72.41 ± 7.01 a β	
		D-I	92.36 ± 2.09 a α	114.37 ± 13.21 a α	124.13 ± 0.45 b α	136.94 ± 5.4 a β	49.26 ± 4.95 a α	70.08 ± 3.64 a β	
acylated anthocyanins (mg/L)		T0	T-V	94.12 ± 5.25 a α	106.83 ± 13.82 a β	127.25 ± 5.93 a α	119.81 ± 7.48 a α	92.27 ± 3.69 a α	87.94 ± 8.73 a α
			C-S	95.23 ± 2.16 a α	102.77 ± 3.05 a β	129.99 ± 9.09 a α	157.41 ± 10.7 b β	112.47 ± 24.13 a α	86.28 ± 16.23 a α
			D-I	101.79 ± 0.83 a α	99.43 ± 6.12 a α	134.69 ± 4.28 a α	156.26 ± 7.11 b β	106.43 ± 7.21 a α	92.18 ± 6.46 a α
	T6	T-V	72.31 ± 2.18 a α	74.28 ± 3.6 a α	99.57 ± 0.62 a α	104.46 ± 3.92 a α	77.48 ± 2.85 a α	82.68 ± 0.9 a β	
		C-S	81.93 ± 7.42 b α	69.16 ± 4.19 a α	103.39 ± 1.52 b α	112.03 ± 11.74 ab α	92.77 ± 0.86 b α	88.53 ± 6.95 a α	
		D-I	76.84 ± 5.27 a α	83.44 ± 1.98 b β	110.3 ± 0.27 c α	120.93 ± 10.58 b α	81.23 ± 3.38 a α	89.9 ± 1.42 a β	
	T12	T-V	55.13 ± 5.47 a α	53.92 ± 2.86 a α	79.7 ± 3.65 a α	80 ± 2.87 a α	57.35 ± 3.68 a α	71.64 ± 1.87 a β	
		C-S	57.63 ± 8.71 a α	66.17 ± 2.16 b α	83.93 ± 3.99 a α	86.64 ± 3.45 b α	66.05 ± 0.68 a α	73.81 ± 1.69 a β	
		D-I	56.58 ± 6.14 a α	68.49 ± 4.28 b β	88.1 ± 0.08 a α	89.82 ± 1.6 b α	65.55 ± 4.27 a α	76.48 ± 4.93 a β	
	total anthocyanins (mg/L)	T0	T-V	717.29 ± 5.46 a α	731.76 ± 18.22 a α	764.74 ± 10.33 a β	742.82 ± 14.6 a α	612.67 ± 66.53 a α	685.89 ± 18.54 a α
			C-S	731.64 ± 1.97 ab α	706.26 ± 31.16 a α	780.53 ± 0.08 a α	978.31 ± 10.36 b β	620.11 ± 37.06 a α	747.13 ± 21.06 b β
			D-I	752.09 ± 8.46 b α	741.21 ± 6.52 a α	965.43 ± 14.6 b α	982.29 ± 1.44 b α	623.56 ± 19.42 a α	725.39 ± 13.59 b β
T6		T-V	509.7 ± 5.35 a α	494.45 ± 14.53 a α	575.65 ± 6.4 a α	583.93 ± 6.4 a α	429.33 ± 8.7 a α	410.8 ± 28.28 a α	
		C-S	562.09 ± 9.46 b β	511.29 ± 3.46 a α	589.83 ± 33.8 a α	592.74 ± 33.8 a α	488.42 ± 0.21 b α	431.93 ± 57.28 a α	
		D-I	553.32 ± 11.46 b α	559.16 ± 10.87 b α	639.21 ± 14.99 a α	624.02 ± 14.99 a α	489.45 ± 18.27 b α	467.97 ± 21.06 a α	
T12		T-V	371.27 ± 46.27 a α	351.82 ± 14.24 a α	418.44 ± 0.71 a α	444.64 ± 10.94 a α	247.87 ± 6.65 a α	275.09 ± 2.8 a β	
		C-S	409.83 ± 7.46 a α	412.7 ± 3.49 b α	431.46 ± 5.25 b α	435.35 ± 6.03 b α	294.02 ± 8.87 b α	291.3 ± 7.03 b α	
		D-I	404.75 ± 2.41 a α	420.36 ± 2.37 b β	447.45 ± 2.13 c α	444.67 ± 6.92 b α	295.52 ± 6.33 b α	296.75 ± 3.94 b α	

Table 1. continued

		2009		2010		2011	
		control	CFA	control	CFA	control	CFA
T0	T-V	1042.52 ± 6.51 a α	1089.83 ± 32.16 a α	1097.82 ± 28.52 a α	1185.77 ± 20.26 a β	940.28 ± 20.39 a α	1021.22 ± 34.81 a β
	C-S	1082.04 ± 16.26 b α	1101.31 ± 11.44 a α	1133.4 ± 16.51 a α	1428.13 ± 36.69 b β	954.18 ± 17.81 a α	1145.15 ± 29.73 b β
	D-I	1075.48 ± 4.23 b α	1124.32 ± 16.46 a β	1316.77 ± 12.3 b α	1427.29 ± 9.81 b β	949.66 ± 5.43 a α	1099.12 ± 28.41 b β
T6	T-V	762.72 ± 7.46 a α	784.32 ± 18.82 a α	835.73 ± 18.45 a α	913.46 ± 34.41 a β	680.79 ± 15.16 a α	678.08 ± 3.81 a α
	C-S	825.12 ± 11.45 b α	823.02 ± 4.51 b α	848.9 ± 12.66 a α	922.85 ± 15.25 a β	720.29 ± 13.19 b α	708.92 ± 25.12 a α
	D-I	800.19 ± 24.51 ab α	857.81 ± 13.5 b β	918.07 ± 9.08 b α	970.24 ± 52.8 b α	729.45 ± 9.93 b α	748.52 ± 2.4 b β
T12	T-V	563.98 ± 39.96 a α	595.54 ± 6.21 a α	625.14 ± 15.46 a α	681.93 ± 17.41 a β	418.28 ± 7.46 a α	461.26 ± 46.42 a α
	C-S	609.72 ± 8.53 a α	663.29 ± 10.48 b β	658.47 ± 8.36 b α	692.98 ± 36.59 a α	440.26 ± 5.16 b α	478.49 ± 14.52 a α
	D-I	601.97 ± 7.46 a α	636.57 ± 8.21 ab β	691.53 ± 23.19 b α	711.8 ± 30.9 a α	457.55 ± 13.13 b α	477.99 ± 9.58 a α

^aDifferent Roman (a–c) letters within the same column, parameter, and time of analysis (T0, T6, or T12) indicate significant differences ($p < 0.05$) between vinification techniques. Different Greek (α , β) letters within the same file, vinification technique (T-V, C-S, or D-I), and year (2009, 2010, or 2011) indicate significant differences ($p < 0.05$) between the control and CFA-added wines. T-V, traditional vinification; C-S, cold soak; D-I, dry ice; CFA, caffeic acid.

concentrations (Table 1). It seems that anthocyanin extraction took place through the prefermentative phase. In some cases, CFA addition and prefermentative maceration techniques also increased the total phenolic concentration. The extraction of anthocyanin and tannin is influenced by limited solubility, and this effect is supported by saturation behavior over time.³⁸ The results obtained showed an increase in the anthocyanins and total phenol content, although no effect was observed on the tannin fraction (data not shown). Thus, an increase in the tannin levels during the prefermentative step would not be expected, and we suggest that this is due to a lack of extraction and solubility in the absence of ethanol. As it has been previously reported, tannins could be extracted during the prefermentative step,³⁰ although the mentioned increase should be related to low molecular weight tannins, compounds which cannot be quantified by the MCP tannin assay because it has been proven that the methylcellulose polymers do not precipitate low molecular weight tannin material.³⁹ The rationale behind the use of these techniques is that aqueous extraction improves wine color. However, an improved extraction of anthocyanins would not be anticipated on the basis of the above-mentioned effects. Even if anthocyanin extraction increases, an increase in the polymeric pigments, which are compounds that have a long-term impact on color stability, will not be expected.

Generally speaking, lower color density values were observed in wines made with dry ice, whereas higher values correspond to traditional vinification. Higher prefermentative temperatures sometimes led to more wine color. Better color density values were also observed in CFA-added (CFA) wines after MLF in 2009. In the same vintage, lower hue values were observed in the cold-soaked wines throughout the aging period. Whereas cold soak appeared to be the technique with the lowest hue in 2009, dry ice and traditional vinification gave rise to similar values. Several authors have reported better color protection in prefermentative cold-macerated wines than in nonmacerated wines when both techniques were applied.^{24,25} On the other hand, lower, but not always significant, values were also observed in the hue of CFA wines.¹⁷

The color that accounted for the copigmented anthocyanins showed higher values in the prefermentative cold-macerated wines than in the control wines, to which no CFA was added. Dry ice additions led to the highest value, although this effect was observed only in the 2009 wines. Álvarez et al.⁴⁰ found higher copigmented anthocyanins in wines made from less mature grapes. However, the positive copigmentation effect observed in 2009 did not occur in 2010 and 2011, indicating the important role played by the vintage as well as the grape characteristics at the time of harvest in relation to the effectiveness of enological practices for improving wine color.^{41–43}

Schwarz et al.⁴⁴ pointed out that the direct reaction between anthocyanins and hydroxycinnamic acids is the only experimentally verified mechanism leading to the development of 4-vinylcatechol and 4-vinylsyringol pigments, as free vinylphenols have not been detected in wines due to the impossibility of generating these compounds via enzymatic decarboxylation.⁴⁵ The occurrence of these reactions could explain the inverse occurrence of the polymeric and free anthocyanin fractions in CFA-treated wines, in which pyranoanthocyanin formation during aging leads to a higher polymeric fraction. 4-Vinylguaiacol and 4-vinylcatechol adducts of other anthocyanins have most likely been generated in several studies on

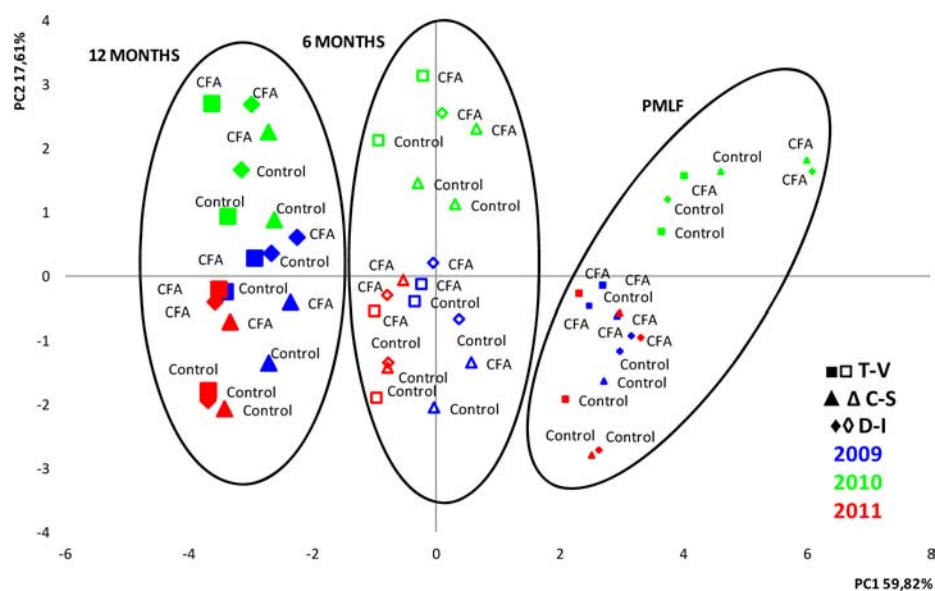


Figure 2. Principal component analysis of wines elaborated with prefermentative techniques and with caffeic acid added.

copigmentation in red wines^{16,17} and in model solutions.⁴⁶ However, the stabilization of color was interpreted solely by assuming the formation of noncovalent anthocyanin copigment complexes, so-called intermolecular copigmentation.

Pyranoanthocyanins have different UV–visible spectral characteristics, showing a hypsochromic shift in the visible spectrum. The higher total phenol concentration and lower hue values in CFA-added wines indicate that the presence of 4-substitutes on the anthocyanin molecule gives them better resistance to color bleaching by sulfur dioxide⁴⁷ and to pH variations,⁴⁸ thereby increasing the stability of these compounds during storage. In accordance with our results, Gauche et al.⁴⁹ suggested that the formation of pyranoanthocyanins with caffeic and ferulic acids does not lead to an increase in the color density, but that better color stability is observed. According to Monagas et al.⁵⁰ grape anthocyanins in Tempranillo wines showed 2-fold slower kinetics in anthocyanin disappearance than those in Graciano and Cabernet Sauvignon wines, which emphasizes that the chemical reactivity of grape anthocyanins is influenced by the grape variety, a factor that imposes the stability associated with the chemical structure of the anthocyanin form.

One of the main problems when copigments are added under *in vivo* conditions is their solubility. The higher phenolic acid concentrations observed for CFA and the prefermentative cooling techniques indicate an initial solubilization and later integration into the wine matrix. The main anthocyanins identified in *Vitis vinifera* spp. grapes and wines are the 3-*O*-glucosides, 3-*O*-acetyl glucosides and 3-*O*-*p*-coumaroyl glucosides, as well as the 3-*O*-caffeoyl glucosides of malvidin and peonidin.⁵⁰ Tempranillo grapes have been described by several authors as being a rich *p*-coumaroyl glucoside variety.^{51,52} This fact, along with caffeic acid addition, possibly increased the acylated anthocyanin concentrations after MLF in 2009 and 2010 and in the aged wine in 2011. CFA-treated wines showed higher phenolic acid values for the cold soak and dry ice wines, which suggests a positive effect when refrigeration techniques and copigment addition are combined. However, this effect was observed only in the 2009 and 2011 wines after MLF and after 6 months of bottle aging. An increase in both anthocyanin and

hydroxycinnamic acid derivatives during the prefermentative step could possibly explain this result. Koyama et al.²³ noted that cold soaking retarded the initial increase in all types of phenolics except hydroxycinnamates. Moreover, other authors have observed an increase in the anthocyanins and in the low molecular weight phenolic fraction in prefermentative cold-macerated wines.^{24,26,40}

PCA was performed on the autoscaled data (54 wine samples and 13 variables), and two principal components accounting for 77.42% of the total variance are presented in Figure 2. The sample set was composed using samples to which CFA was or was not added (control), elaborated with the three vinification techniques (T-V, C-S, and D-I) and from the three vintages (2009, 2010, and 2011). The evolution of the wines developed according to sampling stage, with wines after MLF located on the positive side of PC1, whereas at 6 and 12 months of aging they are located in the middle and on the negative side of the graph, respectively. A complete separation of samples into two groups was found according to vintage in PC2. The first group, composed of samples from 2010, is located in the positive part of PC2, whereas the second group, in the negative part, is made up of samples from 2009 and 2011. Finally, a CFA effect was also observed. The three consecutive vintages show CFA wines on the positive side of PC2. In 2010, these wines are located on the upper side, whereas the control wines are mostly in the middle. The same effect was observed in the 2009 and 2011 wines, but in this case the CFA treatments are located in the middle of PC2, whereas the control wines are on the negative side. These results highlight differences when wines are supplemented with caffeic acid. PCA showed that the time of sampling (after MLF, at 6 or 12 months) resulted in a greater explanation of the variance than prefermentative maceration techniques or CFA addition.

Combined Effect of Acetaldehyde Addition and Caffeic Acid Addition. In accordance with other authors,^{53–55} a higher color density was found in micro-oxygenated wines at 6 months in both the control and CFA wines, although it was significantly higher only for the CFA wines at 6 months of aging in 2011 (Table 2). Lower hue values, although not significant, were observed in the CFA

Table 2. Effect of Acetaldehyde and Caffeic Acid Addition on the Color and Phenolic Profile of Tempranillo Wines from Three Vintages, with Mean and Standard Deviation Values of the Phenolic Parameters^a

	2009			2010			2011		
	control	CFA		control	CFA		control	CFA	
		AA	no AA		AA	no AA		AA	no AA
color density	6 months	10.83 ± 1.48 a α	11.35 ± 1.4 a α	13.1 ± 1.36 a α	13.25 ± 1.17 a α	11.76 ± 0.3 a α	12.39 ± 0.24 b α		
	12 months	10.65 ± 1.11 a α	10.49 ± 1.31 a α	11.58 ± 0.35 a α	12.11 ± 0.97 a α	11.15 ± 0.59 a α	11.77 ± 0.43 a α		
hue	6 months	11.06 ± 1.21 a α	10.83 ± 0.8 a α	11.19 ± 0.36 a α	11.78 ± 0.74 a α	11.7 ± 0.89 a α	12.52 ± 0.77 a α		
	12 months	11.21 ± 1.54 a α	10.96 ± 1.41 a α	11.42 ± 0.81 a α	12.48 ± 0.7 a α	11.13 ± 0.74 a α	11.96 ± 0.56 a α		
% copigmented anthocyanins	6 months	70.47 ± 3.32 a α	68.42 ± 2.45 a α	70.34 ± 1.44 a α	69.14 ± 1.7 a α	77.23 ± 0.42 a α	69.47 ± 1.8 a α		
	12 months	71.42 ± 3 a α	69.06 ± 2.26 a α	70.66 ± 1.95 a α	67.75 ± 1.91 a α	76.27 ± 0.83 a β	68.07 ± 1.54 a α		
% free anthocyanins	6 months	74.06 ± 5.91 a α	71.1 ± 3.21 a α	77.7 ± 1.26 a α	73.73 ± 3.58 a α	80.81 ± 2.25 a α	74.07 ± 5.21 a α		
	12 months	76.26 ± 4.5 a α	73.27 ± 2.49 a α	78.37 ± 3.5 a β	72.55 ± 1.79 a α	79.16 ± 0.93 a β	69.65 ± 1.42 a α		
% polymeric anthocyanins	6 months	13.1 ± 3.61 a α	12.91 ± 5.04 a α	11.64 ± 1.55 a α	12.28 ± 1.96 b α	16.35 ± 1.84 a α	14.18 ± 2.79 a α		
	12 months	9.93 ± 7.25 a α	11.91 ± 8.48 a α	9.43 ± 2.21 a α	9.31 ± 2.82 a α	17.22 ± 1.87 a α	16.82 ± 1.43 b α		
% acylated anthocyanins	6 months	5.93 ± 5.39 a α	3.99 ± 2.24 a α	8.12 ± 1.87 a β	3.98 ± 2.94 a α	13.36 ± 3.23 a α	10.96 ± 2.23 a α		
	12 months	10.82 ± 2.2 a β	7.31 ± 3.51 a α	7.65 ± 2.56 a α	4.78 ± 3.06 a α	16.21 ± 0.87 a α	13.58 ± 4.28 a α		
tannins (g/L)	6 months	48.31 ± 2.97 a β	43.85 ± 3.47 a α	42.09 ± 3.84 a β	38.08 ± 2.89 a α	38.36 ± 5.16 a β	35.93 ± 4.61 a α		
	12 months	55.23 ± 9 a α	50.8 ± 10.29 b α	44.46 ± 2.08 a β	40.71 ± 2.47 b α	43.08 ± 1.8 a β	34.09 ± 2.72 a α		
% polymeric anthocyanins	6 months	41.81 ± 2.83 a α	41.47 ± 5.39 a α	34.21 ± 3.34 a β	26.3 ± 3.11 a α	38.11 ± 2.26 a β	28.63 ± 2.78 a α		
	12 months	42.08 ± 2.1 a α	41.82 ± 4.23 a α	34.55 ± 3.18 a α	30.32 ± 5.17 b α	36.09 ± 0.94 a β	27.24 ± 4.25 a α		
phenolic acids (mg/L)	6 months	38.6 ± 2.69 b α	43.24 ± 5 b α	56.17 ± 6.07 b α	58.22 ± 3.98 b α	45.29 ± 6.1 a α	49.9 ± 5.65 a β		
	12 months	34.84 ± 2.2 a α	37.29 ± 5.15 a α	46.11 ± 0.7 a α	50.28 ± 2.2 a β	39.7 ± 0.27 a α	49.09 ± 1.8 a β		
acylated anthocyanins (mg/L)	6 months	52.26 ± 8.08 a α	54.55 ± 5.41 a α	57.67 ± 1.82 a α	69.72 ± 2.46 b β	48.53 ± 1.11 a α	60.42 ± 1.05 a β		
	12 months	47.1 ± 4.08 a α	50.87 ± 3.99 a α	56.3 ± 4.41 a α	65.06 ± 2.99 a β	47.7 ± 0.39 a β	59.19 ± 1.59 a α		
phenolic acids (mg/L)	6 months	1.84 ± 0.15 a α	1.74 ± 0.12 a α	2.17 ± 0.18 a α	2.09 ± 0.15 a α	1.22 ± 0.48 a α	1.47 ± 0.12 a α		
	12 months	1.72 ± 0.1 a α	1.66 ± 0.14 a α	2.07 ± 0.11 a α	2.08 ± 0.21 a α	1.49 ± 0.05 a α	1.42 ± 0.17 a α		
acylated anthocyanins (mg/L)	6 months	1.96 ± 0.22 a α	1.96 ± 0.24 a α	2.8 ± 0.12 b α	2.66 ± 0.18 a α	1.63 ± 0.07 a α	1.7 ± 0.12 a α		
	12 months	2.11 ± 0.11 a α	2.12 ± 0.26 a α	2.43 ± 0.007 a α	2.61 ± 0.22 a α	1.83 ± 0.26 a α	1.74 ± 0.14 a α		
phenolic acids (mg/L)	6 months	103.42 ± 11.03 a α	147.78 ± 7.25 a β	119.65 ± 14.75 a α	170.69 ± 13.25 a β	76.28 ± 0.47 a α	105.39 ± 4.18 a β		
	12 months	119.29 ± 2.98 a α	169.06 ± 3.42 b β	136.38 ± 21.2 a α	205.84 ± 18.41 b β	83.61 ± 1.23 b α	107.24 ± 6.43 a β		
acylated anthocyanins (mg/L)	6 months	72.3 ± 3.21 a α	117.48 ± 7.28 a β	120.89 ± 4.56 a α	138.36 ± 6.14 a β	47.49 ± 4 a α	69.47 ± 7.17 a β		
	12 months	75.26 ± 5.49 a α	123.63 ± 1.37 a β	118.5 ± 5.96 a α	144.86 ± 13.2 a β	54 ± 0.57 a α	72.07 ± 8.7 a α		
acylated anthocyanins (mg/L)	6 months	92.16 ± 3.67 a α	99.71 ± 2.12 a β	104.86 ± 5.07 a α	114.77 ± 3.83 a β	86.43 ± 9.81 a α	85.68 ± 5.96 a α		
	12 months	89.26 ± 3.2 a α	96.34 ± 4.82 a β	103.98 ± 5.86 a α	111.23 ± 8.46 a α	83.81 ± 11.81 a α	85.53 ± 6.03 a α		
acylated anthocyanins (mg/L)	6 months	62.46 ± 5.16 a α	65.92 ± 4.21 a α	65.67 ± 3.06 a α	66.53 ± 4.05 a α	62.76 ± 3.97 a α	73.19 ± 2.59 a β		
	12 months	61.23 ± 6.13 a α	61.34 ± 4.86 a α	62.15 ± 5.56 a α	64.44 ± 5.9 a α	60.64 ± 8.34 a α	72.26 ± 1.5 a β		

Table 2. continued

		2009			2010			2011		
		control		CFA	control		CFA	control		CFA
		AA	no AA	AA	no AA	AA	no AA	AA	no AA	AA
total anthocyanins (mg/L)	6 months	494.43 ± 25.16 a α	514.98 ± 16.14 a α	524.48 ± 29.84 a α	504.59 ± 41.68 a α	462.02 ± 37.53 a α	419.8 ± 47.89 a α			
	12 months	AA	492.47 ± 28.13 a α	518.63 ± 44.89 a α	533.63 ± 35.37 a α	455.72 ± 46.03 a α	422.92 ± 45.59 a α			
		no AA	330.82 ± 16.26 a α	339.32 ± 9.31 a α	374.35 ± 15.02 a α	371.07 ± 13.82 a α	270.16 ± 24.88 a α	283.06 ± 11.42 a α		
	no AA	325.46 ± 14.14 a α	321.55 ± 16.28 a α	370.54 ± 14.21 a α	372.04 ± 17.58 a α	271.73 ± 40.39 a α	283.33 ± 10.11 a α			
total phenols (mg/L)	6 months	769.46 ± 28.76 a α	842.83 ± 16.82 a β	843.37 ± 35.43 a α	865.33 ± 28.56 a α	691.82 ± 20.17 a α	694.6 ± 9.74 a α			
	12 months	AA	799.65 ± 22.43 a α	835.38 ± 38.34 a α	801.68 ± 21.16 a α	907.58 ± 41.29 a β	709.25 ± 22.19 a α	692.4 ± 38.79 a α		
		no AA	522.65 ± 30.28 a α	571.39 ± 17.66 a β	616.44 ± 23.78 a α	624.37 ± 33.16 a α	416.94 ± 36.14 a α	471.11 ± 21.71 a α		
	no AA	523.7 ± 27.49 a α	573.7 ± 16.2 a β	614.46 ± 32.93 a α	641.73 ± 28.81 a α	441.59 ± 24.8 a α	466.62 ± 16.92 a α			

^aDifferent Roman (a–c) letters within the same column, time of analysis (6 or 12 months), and year (2009, 2010, or 2011) indicate significant differences ($p < 0.05$) between acetaldehyde addition treatments. Different Greek (α , β) letters within the same file, treatment (AA or no AA), and year (2009, 2010, or 2011) indicate significant differences ($p < 0.05$) between the control and CFA-added wines. AA, acetaldehyde addition; no AA, no acetaldehyde addition; CFA, caffeic acid.

wines in 2009, which indicates a positive effect of CFA addition on wine color. Higher, although not always statistically significant, noncopigmented free anthocyanin values were observed in the control wines. At 6 months of aging, non-acetaldehyde-added wines sometimes showed a higher free anthocyanin fraction, although this effect was not present at the end of storage. The polymeric anthocyanin fraction showed higher values for the acetaldehyde-added wines throughout the aging period in 2009 and 2010, although these differences were slightly smaller at 12 months. Higher, but not significant, tannin concentrations were found in the acetaldehyde-added wines at 6 months, whereas no effect was observed at the end of storage. Small quantities of oxygen seem to be involved in the formation of A-type vitisins,⁵⁶ whereas the acetaldehyde produced by the effect of oxygen on ethanol³⁴ is involved in the formation of ethyl-linked anthocyanins and tannin adducts, B-type vitisins, vinyl-flavanols, or vinyl-pyranoanthocyanins.⁵⁷ Therefore, whereas a decrease in the free noncopigmented anthocyanin fraction was observed, a higher polymeric fraction and tannin concentration were achieved with the acetaldehyde addition treatments.

Despite the effects observed, the effectiveness of the micro-oxygenation technique depends on several factors, the most important being the moment of application, the dose of oxygen introduced into the wine, and the phenolic characteristics of the wine. Typical dosage rates are relatively small, ranging from 2 to 90 mg O₂/L wine/month.⁵⁸ Micro-oxygenation requires relatively low concentrations of SO₂ to be effective.⁵⁹ Phenolic compounds are the main consumers of oxygen,⁶⁰ and it is clear that the initial phenol content of the wine must be important.³¹ Because the dosage rates and the phenolic content seem to be in accordance with the literature, the moment of application could be the key factor that explains the decrease in the micro-oxygenation effects observed at the end of storage. Geldenhuys et al.⁶¹ also found that the effects of micro-oxygenation on certain color and phenolic parameters in Pinotage red wine decrease over time.

Phenolic acids had higher values in the CFA-treated wines. CFA and acetaldehyde addition also showed a positive, but not always significant, effect on the acylated anthocyanin fraction. The results suggest the greater likelihood of these compounds to participate in complexation and polymerization reactions with other phenols through ethyl-linked products. Some authors have reported acylated anthocyanin–flavanol compounds in red wines.^{51,62} However, the ability of different anthocyanin molecules to react with other compounds via acetaldehyde-mediated reactions has not yet been reported, and further research would be needed. Although no differences were found for the total anthocyanins, increases in total phenols were sometimes observed in CFA-treated wines.

The PCA plot (68.70% of the total variability) in Figure 3 shows sample separation into two groups according to the aging period (6 or 12 months). The sample set was constructed using samples to which CFA was added or not (control) as well as where acetaldehyde was added or not (AA and no AA, respectively) from the three vintages (2009, 2010, and 2011). The 2010 wines are located in the higher part of the graph, in contrast to the 2009 and 2011 wines, which are positioned on the negative side. Even though there was not a complete separation between the acetaldehyde-added wines, a higher position was observed when each sample was compared to its non-acetaldehyde-added pair. This distribution of the data suggests differences when the technique was applied. On the

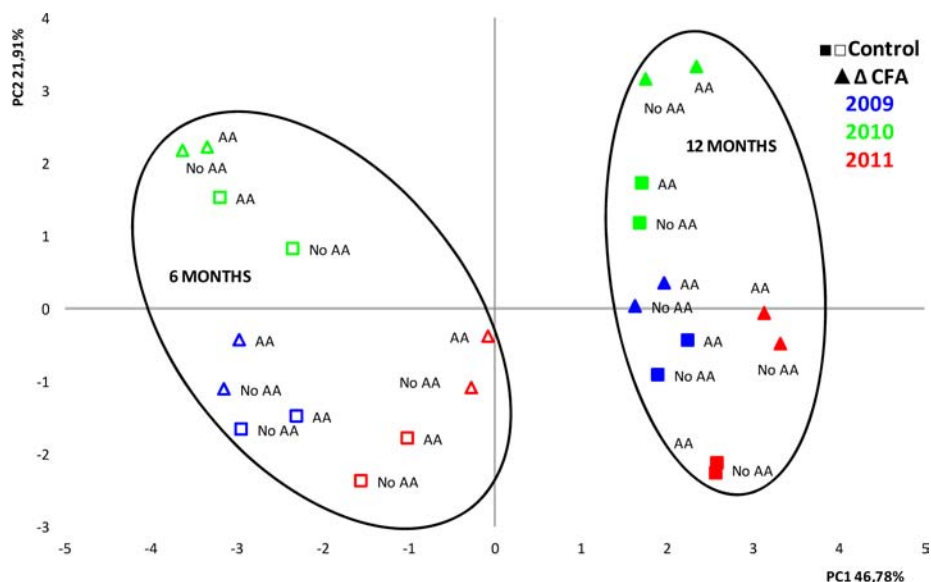


Figure 3. Principal component analysis of wines elaborated with AA and caffeic acid.

other hand, no special behavior was observed between the CFA and control wines. As we reported in Figure 2, the time of sampling (after 6 or 12 months) contributed more to the variance than the treatment (acetaldehyde and CFA addition).

Effect of Prefermentative and Postfermentative Addition. No significant differences were found for color density, although color values were higher in both the prefermentative and postfermentative CFA-added wines (Table 3). Lower hue values were found in the CFA-treated wines in 2010 and 2011. Postfermentative CFA-added wines showed nonsignificant higher copigmented fraction values regarding prefermentative addition at the end of storage (intermolecular copigmentation), even though at this point the control wines showed the highest copigmented percentage, although only significantly different from prefermentative CFA-added wines. Whereas several authors have mentioned that an increase in alcohol content diminishes the effect of copigmentation, Kunsági-Máté et al.²² suggest that much more stable copigmentation complexes form over a critical alcohol concentration. The 2010 and 2011 CFA-treated wines showed lower free anthocyanin fractions over the whole aging period. A higher polymeric pigment fraction was also observed in these wines. The results suggest that higher complexation and polymerization reactions occurred when CFA was added. Postfermentative CFA addition did not produce an increase in the intermolecular copigmentation reactions (% copigmented anthocyanins). The increased formation of new polymeric pigments during storage would explain the values observed in the wines to which CFA was added prefermentatively and postfermentatively. However, contrary to the results reported by several authors, no effect on copigmentation reactions was observed in this study when CFA was added. The previous rationale suggests that the Tempranillo variety can be classified as a rich anthocyanin-cofactor variety,^{15,50,52} because an increase in copigmentation reactions would not be anticipated.

The control wines had the lowest phenolic acid concentrations, and this trend was maintained throughout the 12 months' aging period. These compounds also occurred at higher concentrations when CFA was added before and after fermentation. No differences were noted in total anthocyanins, higher acylated anthocyanins, and total phenols when CFA was

added prefermentatively and postfermentatively. The results obtained (color density, hue, free and polymerized anthocyanins and phenolic acids) suggest that the effect of CFA addition is mainly due to intramolecular copigmentation reactions, in which diverse molecules are covalently linked to an anthocyanin chromophore, instead of intermolecular copigmentation reactions (% copigmented anthocyanins), in which compounds react through weak hydrophobic forces with anthocyanins.

A PC plot of these data can be seen in Figure 4. The two first principal components represent 78.27% of the total variability. The sample set was constructed using samples to which caffeic acid was added before (PRE) and after (POST) fermentation as well as those to which no addition was made (control) from the three vintages (2009, 2010, and 2011). A complete separation of samples into two groups was observed according to time in the bottle. The same vintage effect as reported previously was also found here. However, PCA did not allow for a separation between the CFA-added wines and the control wines. Each control wine shows a pair of wines (prefermentatively and postfermentatively added) with a slightly different position in the graph, which suggests some differences. The pair composed of prefermentative and postfermentative CFA-added wines has almost the same position, which indicates no differences between CFA addition before or after fermentation.

The addition of caffeic acid to Tempranillo red grapes or wine led to higher phenolic acid concentrations, which, together with the high *p*-coumaroyl glucosides presence in these grapes, increased the acylated anthocyanin fraction. This could have favored pyranoanthocyanin formation through the direct reaction of anthocyanins with free phenolic acids, thereby increasing the anthocyanin polymeric fraction, with an increase in color stability during storage. The results reported here indicate intramolecular copigmentation reactions via covalent linkages, which increase the stability of the new molecules during the aging period and thus have an impact on wine color. The higher total phenol concentration and lower hue values in the wines to which caffeic acid had been added also contributed to increasing the stability of these compounds during storage. Subsequent work could entail assessing the pyranoanthocyanin

Table 3. Effect of Prefermentative and Postfermentative Caffeic Acid Addition on the Color and Phenolic Profile of Tempranillo Wines from Three Vintages, with Mean and Standard Deviation Values of the Phenolic Parameters^a

			2009	2010	2011
color density	6 months	control	10.74 ± 1.17 a	12.34 ± 1.22 a	11.45 ± 0.52 a
		PRE	10.84 ± 1.75 a	12.74 ± 1.1 a	12.01 ± 0.32 a
		POST	11 ± 1.02 a	12.73 ± 1.27 a	12.15 ± 0.62 a
	12 months	control	11.14 ± 1.24 a	11.36 ± 0.63 a	11.41 ± 0.75 a
		PRE	11.19 ± 0.6 a	12.08 ± 0.87 a	12.23 ± 0.3 a
		POST	10.59 ± 1.43 a	12.18 ± 0.75 a	12.25 ± 1.02 a
hue	6 months	control	70.95 ± 2.88 a	70.5 ± 1.54 b	76.75 ± 0.77 b
		PRE	68.3 ± 2.58 a	69.22 ± 2.08 ab	68.05 ± 2.25 a
		POST	69.18 ± 2.06 a	67.65 ± 1.09 a	69.5 ± 0.66 a
	12 months	control	75.16 ± 4.85 a	78.39 ± 2.47 c	79.99 ± 1.7 b
		PRE	72.78 ± 3.92 a	75.11 ± 2.66 b	70.47 ± 2.17 a
		POST	71.59 ± 1.75 a	71.18 ± 0.7 a	73.26 ± 5.69 a
% copigmented anthocyanins	6 months	control	11.51 ± 5.71 a	10.53 ± 2.16 a	16.79 ± 1.78 a
		PRE	12.06 ± 5.62 a	11.2 ± 3.23 a	15.24 ± 2.91 a
		POST	12.75 ± 8.13 a	9.99 ± 2.49 a	15.77 ± 2.28 a
	12 months	control	8.37 ± 4.68 b	7.55 ± 2.16 b	14.79 ± 2.67 b
		PRE	5.15 ± 3.41 a	3.48 ± 1.75 a	10.77 ± 3.93 a
		POST	6.15 ± 3.34 ab	5.28 ± 3.68 ab	13.77 ± 2.58 ab
% free anthocyanins	6 months	control	51.77 ± 7.34 b	43.27 ± 3.19 b	40.72 ± 4.38 b
		PRE	44.9 ± 7.52 a	39.9 ± 1.72 a	33.2 ± 2.06 a
		POST	49.75 ± 8.65 ab	38.99 ± 3.75 a	36.82 ± 4.35 ab
	12 months	control	41.95 ± 2.38 ab	35.16 ± 2.72 b	37.1 ± 1.93 b
		PRE	44.2 ± 3.27 b	29.97 ± 4.57 a	28.73 ± 4.63 a
		POST	39.09 ± 4.7 a	26.65 ± 4.27 a	27.13 ± 1.56 a
% polymeric anthocyanins	6 months	control	36.72 ± 3.06 a	51.14 ± 6.67 a	42.5 ± 4.99 a
		PRE	43.03 ± 5.98 a	53.9 ± 4.65 a	51.57 ± 1.34 b
		POST	37.5 ± 4.29 a	54.85 ± 5.51 a	47.42 ± 4.89 b
	12 months	control	49.68 ± 6.67 a	56.39 ± 3.3 a	48.11 ± 0.89 a
		PRE	50.65 ± 5.12 ab	66.54 ± 4.19 b	60.5 ± 1.31 c
		POST	54.77 ± 4.15 b	68.24 ± 2.78 b	59.1 ± 1.29 b
tannins (g/L)	6 months	control	1.78 ± 0.14 b	2.12 ± 0.15 a	1.35 ± 0.35 a
		PRE	1.64 ± 0.15 a	2.06 ± 0.19 a	1.42 ± 0.18 a
		POST	1.76 ± 0.09 b	2.07 ± 0.18 a	1.46 ± 0.1 a
	12 months	control	2.03 ± 0.18 ab	2.57 ± 0.21 a	1.73 ± 0.21 a
		PRE	2.13 ± 0.26 b	2.69 ± 0.18 a	1.72 ± 0.16 a
		POST	1.95 ± 0.22 a	2.58 ± 0.21 a	1.72 ± 0.08 a
phenolic acids (mg/L)	6 months	control	98.32 ± 3.62 a	128.02 ± 18.73 a	79.94 ± 4.3 a
		PRE	107.4 ± 10.14 b	200.2 ± 21.97 b	105.26 ± 6.98 b
		POST	133.26 ± 8.93 c	177.92 ± 22.16 b	107.36 ± 3.06 b
	12 months	control	72.85 ± 5.12 a	119.03 ± 5.19 a	50.74 ± 4.42 a
		PRE	81.81 ± 5.95 b	137.41 ± 7.43 b	64.17 ± 2.93 b
		POST	95.88 ± 9.48 c	145.8 ± 11.82 b	77.38 ± 2.57 c
acylated anthocyanins (mg/L)	6 months	control	92.31 ± 14.63 a	104.42 ± 4.92 a	85.12 ± 8.99 a
		PRE	96.28 ± 7.21 a	107.5 ± 8.56 ab	82.21 ± 0.35 a
		POST	97.81 ± 3.85 a	117.44 ± 11.63 b	89 ± 6.41 a
	12 months	control	68.37 ± 3.16 a	83.34 ± 4.73 a	61.7 ± 5.47 a
		PRE	75.43 ± 5.38 b	85.87 ± 4.08 a	73.05 ± 0.77 b
		POST	79.29 ± 0.86 b	85.1 ± 6.06 a	72.4 ± 2.94 b

Table 3. continued

			2009	2010	2011
total anthocyanins (mg/L)	6 months	control	518.49 ± 38.42 a	601.56 ± 44.24 a	458.87 ± 34.48 b
		PRE	512.64 ± 29.71 a	592.61 ± 42.52 a	384.42 ± 3.39 a
		POST	512.05 ± 28.83 a	607.85 ± 37.53 a	458.3 ± 26.98 b
	12 months	control	378.28 ± 19.54 a	431.9 ± 14.73 a	270.95 ± 27.4 a
		PRE	374.56 ± 16.49 a	433.81 ± 9.61 a	281.12 ± 5.56 a
		POST	390.05 ± 12.94 a	429.3 ± 19.9 a	285.27 ± 13.79 a
total phenols (mg/L)	6 months	control	782.78 ± 36.28 a	895.02 ± 28.19 a	700.52 ± 9.37 b
		PRE	794.77 ± 35.16 a	975.93 ± 41.76 b	650.45 ± 28.62 a
		POST	816.65 ± 22.87 a	960.81 ± 17.94 b	736.53 ± 4.16 b
	12 months	control	563.62 ± 21.82 a	674.25 ± 26.77 a	429.26 ± 14.98 a
		PRE	574.68 ± 16.23 a	696.99 ± 12.36 a	459.19 ± 7.08 b
		POST	605.23 ± 7.46 b	691.11 ± 23.87 a	478.55 ± 26.19 b

^aDifferent Roman (a–c) letters within the same column indicate significant differences ($p < 0.05$). PRE, CFA added prefermentatively; POST, CFA added postfermentatively.

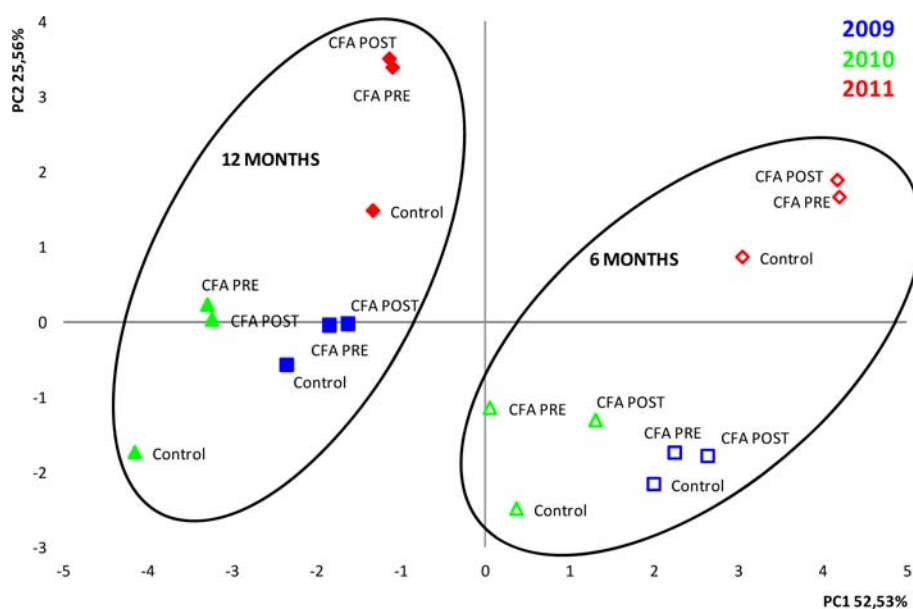


Figure 4. Principal component analysis of wines with caffeic acid added prefermentatively or postfermentatively.

development in Tempranillo wines under similar conditions using HPLC techniques.

Prefermentative maceration techniques did not show a significant effect, indicating the important role played by grape characteristics at the moment of harvest in the effectiveness of enological practices for improving wine color. In the case of Tempranillo, prefermentative techniques should not be considered by winemakers for color stabilization. On the other hand, acetaldehyde addition resulted in a positive effect on wine color. However, the combined effect when acetaldehyde and CFA were added was not very significant. Although few differences were observed between prefermentative and postfermentative caffeic acid additions, these treatments did lead to differences compared to the control treatments. The results suggest an increase in the copigmentation reactions when wines are supplemented with caffeic acid. However, the intramolecular copigmentation effect favored by caffeic acid addition does not allow for the establishment of the effect of alcohol content on copigmentation reactions.

AUTHOR INFORMATION

Corresponding Author

*(J.L.A-T.) E-mail: jaleixan@tal.upv.es.

Notes

The authors declare no competing financial interest.

REFERENCES

- (1) Somers, T. C. Polymeric nature of wine pigments. *Phytochemistry* **1971**, *10*, 2175–2186.
- (2) Berke, B.; de Freitas, V. A colorimetric study of oenin pigmented by procyanidins. *J. Sci. Food Agric.* **2007**, *87*, 260–265.
- (3) Dangles, O.; Saito, N.; Brouillard, R. Anthocyanin intramolecular copigment effect. *Phytochemistry* **1993**, *34*, 119–124.
- (4) Dimitric-Markovic, J. M.; Petranovic, N. A.; Baranac, J. M. The copigmentation effect of sinapic acid on malvidin: A spectroscopic investigation on colour enhancement. *J. Photochem. Photobiol.* **2005**, *78*, 223–228.
- (5) Asen, S.; Stewart, R. N.; Norris, K. H. Co-pigmentation of anthocyanins in plant tissues and its effect on color. *Phytochemistry* **1972**, *11* (3), 1139–1144.

- (6) Baranac, J. M.; Petranovic, N. A.; Dimitric-Markovic, J. M. Spectrophotometric study of anthocyanin copigmentation reactions. *J. Agric. Food Chem.* **1996**, *44*, 1333–1336.
- (7) Santos-Buelga, C.; Bravo-Haro, S.; Rivas-Gonzalo, J. C. Interactions between catechin and malvidin-3-monoglucoside in model solutions. *Z. Lebensm.-Unters. Forsch.* **1995**, *201*, 269–274.
- (8) Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *Am. J. Enol. Vitic.* **2001**, *52* (2), 67–80.
- (9) Castelluccio, C.; Paganga, G.; Melikian, N.; Bolwell, P. B.; Pridham, J.; Sampson, J. Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. *FEBS Lett.* **1995**, *368*, 188–192.
- (10) Dimitric-Markovic, J. M. D.; Petranovic, N. A.; Baranac, J. M. Spectrophotometric study of the copigmentation of malvin with caffeic and ferulic acids. *J. Agric. Food Chem.* **2000**, *48*, 5530–5536.
- (11) Dimitric-Markovic, J. M.; Ignjatovic, L. M.; Markovic, D. A.; Baranac, J. M. Antioxidant capabilities of some organic acids and their co-pigments with malvidin. Part I. *J. Electroanal. Chem.* **2003**, *553*, 169–175.
- (12) Dimitric-Markovic, J. M.; Ignjatovic, L. M.; Markovic, D. A.; Baranac, J. M. Antioxidant capabilities of some organic acids and their co-pigments with malvidin. Part II. *J. Electroanal. Chem.* **2003**, *553*, 177–182.
- (13) Marinova, E. M.; Yanishlieva, N. V. Antioxidant activity and mechanism of action of some phenolic acids at ambient and high temperatures. *Food Chem.* **2003**, *81*, 189–197.
- (14) Sroka, Z.; Cisowski, W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. *Food Chem. Toxicol.* **2003**, *41*, 753–758.
- (15) Schwarz, M.; Picazo-Bacete, J. J.; Winterhalter, P.; Hermosín-Gutiérrez, I. Effect of copigments and grape cultivar on the color of red wines fermented after the addition of copigments. *J. Agric. Food Chem.* **2005**, *53*, 8372–8381.
- (16) Darias-Martín, J.; Carrillo, M.; Díaz, E.; Boulton, R. B. Enhancement of wine colour by prefermentation addition of copigments. *Food Chem.* **2001**, *73*, 217–220.
- (17) Darias-Martín, J.; Martín, B.; Carrillo, M.; Lamuela, R.; Díaz, C.; Boulton, R. The effect of caffeic acid on the color of red wine. *J. Agric. Food Chem.* **2002**, *50* (7), 2062–2067.
- (18) Bloomfield, D. G.; Heatherbell, D. A.; Nikfardjam, M. S. Effect of *p*-coumaric acid on the color in red wine. *Mitt. Klosterneuburg* **2003**, *53*, 195–198.
- (19) Dangles, O.; Brouillard, R. A. Spectrophotometric method based on the anthocyanin copigmentation interaction and applied to the quantitative study of molecular complexes. *J. Chem. Soc., Perkin Trans. 2* **1992**, 247–257.
- (20) Ribereau-Gayon, P.; Pontallier, P.; Glories, Y. Some interpretations of colour changes in young red wines during their conservation. *J. Sci. Food Agric.* **1983**, *34*, 506–516.
- (21) Brouillard, R.; Wigand, M. C.; Dangles, O.; Cheminat, A. pH and solvent effects on the copigmentation of malvin with polyphenols, purine and pyrimidine derivatives. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1235.
- (22) Kunsági-Máté, S.; Kumar, A.; Sharma, P.; Kollar, L.; Nikfardjam, M. Effect of molecular environment on the formation kinetics of complexes of malvidin-3-O-glucoside with caffeic acid and catechin. *J. Phys. Chem. B* **2009**, *113*, 7468–7473.
- (23) Koyama, K.; Goto-Yamamoto, N.; Hashizume, K. Influence of maceration temperature in red wine vinification on extraction of phenolics from berry skins and seed of grape (*Vitis vinifera*). *Biosci., Biotechnol., Biochem.* **2007**, *71* (4), 958–965.
- (24) Gil-Muñoz, R.; Moreno-Pérez, A.; Vila-López, R.; Martínez-Cutillas, A.; Fernández-Fernández, J. L.; Gómez-Plaza, E. Influence of low temperature prefermentative techniques on chromatic and phenolic characteristics of Syrah and Cabernet sauvignon wines. *Eur. Food Res. Technol.* **2009**, *228*, 777–788.
- (25) Gómez-Míguez, M.; González-Miret, M.; Heredia, F. Evolution of color and anthocyanin composition of Syrah wines elaborated with pre-fermentative cold maceration. *J. Food Eng.* **2007**, *79*, 271–278.
- (26) Heredia, F. J.; Escudero-Gilete, M. L.; Hernanz, D.; Gordillo, B.; Meléndez-Martínez, A. J.; Vicario, I. M.; Gonzalez-Miret, M. L. Influence of the refrigeration technique on the color and phenolic composition of Syrah wines obtained with pre-fermentative cold maceration. *Food Chem.* **2010**, *118*, 377–383.
- (27) Álvarez, I.; García, M. A.; González, R.; Martín, P. *Avances en Ciencias y Técnicas Enológicas*; Itacyl y Gienol: Palencia, Spain, 2005.
- (28) Couasnon, M. Une nouvelle technique: La macération prefermentaire à froid – extraction à la niègè carbonique. *Rev. Oenol. Tech. Vitic. Oenol.* **1999**, 92–26.
- (29) Gordillo, B.; López-Infante, M. I.; Ramírez-Pérez, P.; González-Miret, M.; Heredia, F. Influence of prefermentative cold maceration on the color and anthocyanin copigmentation of organic tempranillo wines elaborated in warm climate. *J. Agric. Food Chem.* **2010**, *58*, 6797–6803.
- (30) Álvarez, I.; Alexandre, J. L.; García, M. J.; Lizama, V.; Alexandre-Tudó, J. L. Effect of the prefermentative addition of copigments on the polyphenolic composition of Tempranillo wines after malolactic fermentation. *Eur. Food Res. Technol.* **2009**, *228*, 201–210.
- (31) Du Toit, W. J.; Lisjak, K.; Marais, J.; Du Toit, M. The effect of micro-oxygenation on the phenolic composition, quality and aerobic wine-spoilage microorganisms of different South African red wines. *S. Afr. J. Enol. Vitic.* **2006**, *27*, 57–67.
- (32) McCord, J. Application of toasted oak and micro-oxygenation to ageing of Cabernet Sauvignon wines. *Aust. N. Z. Grapegrower Winemaker* **2003**, July, 43–53.
- (33) Glories, Y. La couleur des vins rouges 2^a parte: mesure, origine et interpretation. *Connaiss. Vigne Vin* **1984**, *18* (4), 253–271.
- (34) Fulcrand, H.; Atanasova, V.; Salas, E.; Cheynier, V. The fate of anthocyanins in wine. Are they determining factors? In *Red Wine Color. Revealing the Mysteries*; Waterhouse, A., Kennedy, J. A., Eds.; ACS Symposium Series 886; American Chemical Society: Washington, DC, 2004; pp 68–88.
- (35) Sarneckis, C. J.; Damberg, R. G.; Jones, P.; Mercurio, M.; Herderich, M. J.; Smith, P. A. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimized tool for grape and wine analysis. *Aust. J. Grape Wine Res.* **2006**, *12*, 39–49.
- (36) Boulton, R. B. Method for the assessment of copigmentation in red wines. ASEV 47th Annual Meeting, Reno, NV, 1996.
- (37) Boido, E.; Alcalde-Eon, C.; Carrau, F.; Dellacassa, E.; Rivas Gonzalo, J. C. Aging effect on the pigment composition and color of *Vitis vinifera* L. cv. Tannat wines. Contribution of the main pigment families to wine color. *J. Agric. Food Chem.* **2006**, *54*, 6692–6704.
- (38) Sacchi, K.; Bisson, L.; Adams, D. A review of the effect of winemaking techniques on phenolic interaction in red wines. *Am. J. Enol. Vitic.* **2005**, *53* (3), 197–206.
- (39) Mercurio, M. D.; Smith, P. A. Tannin quantification in red grapes and wine: comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency. *J. Agric. Food Chem.* **2008**, *56*, 5528–5537.
- (40) Álvarez, I.; Alexandre, J. L.; García, M. J.; Lizama, V. Impact of prefermentative maceration on the phenolic and volatile compounds in Monastrell red wines. *Anal. Chim. Acta* **2006**, *563*, 109–115.
- (41) Bautista-Ortín, A. B.; Fernández-Fernández, J.; López-Roca, J. M.; Gómez-Plaza, E. The effect of enological practices in anthocyanins phenolic compounds and wine color and their dependence on grape characteristics. *J. Food Compos. Anal.* **2007**, *20*, 546–552.
- (42) Canals, R.; Llaudy, M. C.; Valls, J.; Canals, J. M.; Zamora, F. Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.* **2005**, *53*, 4019–4025.
- (43) Kontoudakis, N.; Esteruelas, M.; Francesca Fort, F.; Canals, J. M.; De Freitas, V.; Zamora, F. Influence of the heterogeneity of grape

phenolic maturity on wine composition and quality. *Food Chem.* **2011**, *124*, 767–774.

(44) Schwarz, M.; Wabnitz, T. C. P.; Winterhalter, P. Pathway leading to the formation of anthocyanin-vinylphenol adducts and related pigments in red wines. *J. Agric. Food Chem.* **2003**, *51*, 3682–3687.

(45) Chatonnet, P.; Dubourdieu, D.; Boidron, J. N.; Lavigne, V. Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. *J. Sci. Food Agric.* **1993**, *62*, 191–202.

(46) Eiro, M. J.; Heinonen, M. Anthocyanin color behavior and stability during storage: effect of intermolecular copigmentation. *J. Agric. Food Chem.* **2002**, *50*, 7461–7466.

(47) Timberlake, C. F.; Bridle, P. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976**, *27*, 97–105.

(48) Sarni-Manchado, P.; Fulcrand, H.; Souquet, J. M.; Cheynier, V.; Moutounet, M. Stability and color of unreported wine anthocyanin-derived pigments. *J. Food Sci.* **1996**, *61*, 938–941.

(49) Gauche, C.; Da Silva-Malagoli, E.; Bordiguen-Luiz, M. Effect of pH on the copigmentation of anthocyanins from Cabernet Sauvignon grape extracts with organic acids. *Sci. Agric. (Piracicaba, Brazil)* **2010**, *67* (1), 41–46.

(50) Monagas, M.; Gómez-Cordovés, C.; Bartolomé, B. Evolution of the phenolic content of red wines from *Vitis vinifera* L. during aging in bottle. *Food Chem.* **2005**, *95*, 405–412.

(51) Monagas, M.; Núñez, V.; Bartolomé, B.; Gómez-Cordovés, C. Anthocyanin-derived pigments in Graciano, Tempranillo, and Cabernet Sauvignon wines produced in Spain. *Am. J. Enol. Vitic.* **2003**, *54* (3), 163–169.

(52) Núñez, V.; Monagas, M.; Gómez-Cordovés, C.; Bartolomé, B. *Vitis vinifera* L. cv. Graciano grapes characterized by its anthocyanin profile. *Postharvest Biol. Technol.* **2004**, 3169–3179.

(53) Gómez-Plaza, E.; Cano-López, M. A. A review on micro-oxygenation of red wines: claims, benefits and the underlying chemistry. *Food Chem.* **2011**, *125*, 1131–1140.

(54) Rayne, S.; Sheppard, S.; Di Bello, T.; Eggers, N. J. Chromatic characteristics and optically derived compositional descriptors of micro-oxygenated wines from *Vitis vinifera* cv. Merlot and Cabernet Sauvignon. *Food Bioprocess. Technol.* **2011**, *4*, 254–265.

(55) Wirth, J.; Morel-Salmi, C.; Souquet, J. M.; Dieval, J. B.; Aagaard, O.; Vidal, S.; Fulcrand, H.; Cheynier, V. The impact of oxygen exposure before and after bottling on the polyphenolic composition of red wines. *Food Chem.* **2010**, *123*, 107–116.

(56) Lee, D.; Swinny, E.; Asenstorfer, R.; Jones, G. Factors affecting the formation of redwine pigments. In *Red Wine Color. Revealing the Mysteries*; ACS Symposium Series 886; Waterhouse, A., Kennedy, J. A., Eds.; American Chemical Society: Washington, DC, 2004; pp 125–142.

(57) Oliveira, J. M.; Faria, M.; Sá, F.; Barros, F.; Araújo, I. M. C6-alcohols as varietal markers for assessment of wine origin. *Anal. Chim. Acta* **2006**, *563*, 300–309.

(58) Dykes, S. The effect of oxygen dosage rate on the chemical and sensory changes occurring during micro-oxygenation of New Zealand red wine. Ph.D. Thesis, The University of Auckland, New Zealand, 2007.

(59) Tao, J.; Dykes, S.; Kilmartin, P. Effect of SO₂ concentration on polyphenol development during red wine micro oxygenation. *J. Agric. Food Chem.* **2007**, *55*, 6104–6109.

(60) Devatine, A.; Chiciuc, I.; Poupot, C.; Miettton-Peuchot, M. Microoxygenation of wine in presence of dissolved carbon dioxide. *Chem. Eng. Sci.* **2007**, *62*, 4579–4588.

(61) Geldenhuys, L.; Oberholster, A.; Du Toit, W. J. Monitoring the effect of micro-oxygenation before malolactic fermentation on South African Pinotage red wine with different colour and phenolic analyses. *S. Afr. J. Enol. Vitic.* **2012**, *33*, 150–160.

(62) García-Marino, M.; Hernández-Hierro, J. M.; Rivas-González, J.; Escribano-Bailón, M. Color and pigment composition of red wines obtained from co-maceration of Tempranillo and Graciano varieties. *Anal. Chim. Acta* **2010**, *660*, 134–142.