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Impact of Adding White Pomace to Red Grapes on the Phenolic Composition and Color Stability of Syrah Wines from a Warm Climate

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ABSTRACT: The influence of the fermentative addition of Pedro Ximenez grape pomace (PXGP, white variety) on the phenolic composition and color of Syrah red wines from a warm climate was studied. Changes on phenolic composition (HPLC), copigmentation/polymerization (spectrophotometry), and color (tristimulus colorimetry) allowed differences among the maceration treatments to be established. PXGP additions at the rates studied increased the extraction of total phenolics, phenolic acids, and monomeric flavanols. However, the effect on the anthocyanins, copigmentation, and polymerization depended on the doses applied, with important consequences on the color. PXGP addition at 10% led to wines with higher polymerization, more stable colors, and bluish hues. in contrast, perceptibly lighter and less intense wines were obtained with PXGP addition at 20%. Thus, the use of white grape byproducts as wine additives at appropriate levels (10% w/w) could improve the phenolic potential of red young wines from a warm climate, contributing to preserve their color characteristic.

KEYWORDS: *white grape pomace, phenolic composition, copigmentation, red wine color, warm climate*

INTRODUCTION

The chemical stabilization of pigments in wine is one of the main research areas of enology. Studying and controlling both agronomic and enological factors that determine their content and evolution during vinification and aging is a major objective to produce quality wines, especially in terms of their color.[1,2]

The stability and quality of wine color is related to its phenolic composition, mainly native anthocyanins extracted from red grapes during maceration and their copigmented and polymeric products developed in subsequent stages of vinification.[2] From the first steps of the winemaking process, grape anthocyanins are present basically in their monomeric forms and are relatively protected because a high proportion of them are participating in noncovalent associations among themselves or with other wine constituents (copigments or cofactors) by means of copigmentation complexes.[3] Nevertheless, anthocyanins are highly reactive pigments and quickly undergo chemical transformations causing qualitative and quantitative changes in the wine color.[4] Some reactions involving anthocyanins, such as oxidation, hydration, or adsorption, result in their degradation with the consequent loss of color; but others such as polymerization are responsible for color stabilization.

The anthocyanin stabilization by copigmentation and polymerization mechanisms is highly dependent on the concentration and nature of other colorless phenols also extracted from grape skins and seeds during maceration.[5] In comparison with other wine constituents, the copigmenting capability of flavonoids or some hydroxycinnamic acids is stronger because their planar spatial structure confers to them a better aptitude to interact with anthocyanins.[6] In this way, they provide better protection to anthocyanins against discoloration and favor their incorporation into more stable polymeric structures (anthocyanin-derived pigments). In addition, colorless phenolics also contribute to color stability because they can act as effective oxidation substrates, which partially avoid undesirable color changes of anthocyanins due to browning/oxidation.

In this sense, insufficient levels of such phenolic compounds in grapes (pigments and copigments) make it difficult to obtain red wines with intense and stable color because the possibility to form copigmentation reactions is limited,[7] as typically occurs in warm climate regions due to a lack of adequate phenolic maturity of red grapes at ripeness.[8] Nevertheless, it is known that the concentration of the components involved in the copigmentation reactions can be modified by applying specific vinification techniques, which influence the stability and intensity of the final wine color. The external addition of phenolic copigments (as pure components or extracts from different natural sources) to the musts prior to, during, or after fermentation has been commonly employed to equilibrate the phenolic composition of red wines and improve their color characteristics in most cases.[9−14]

Among others, skins and seeds from white grapes are considered a good source of nonanthocyanic phenolics (catechin, procyanidins, quercetin glycosides, etc.) with enological interest for their use in cofermentations with red grape musts, especially when red grapes do not present a good balance between the concentrations of anthocyanins and

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copigmentation cofactors.[15,16] However, to date there are scarce studies about the addition of these natural sources during red winemaking, probably because of the difficulty of performing this process due to the different harvesting dates for white and red grapes. Some of these studies have revealed that the effects on the final quality of the wine are different depending on the winemaking stage and duration of the application and the dose applied as well as the particular phenolic potential of the grapes in the mixture.[7,17−19] Thus, further investigations are needed to better understand the contribution of these incorporated compounds to wine color and color stability.

Generally, white grapes are harvested several weeks before red grapes. Nevertheless, in some Mediterranean regions (such as Andalucía, southwestern Spain, warm climate) red and white grape varieties with similar ripening periods are usually harvested at the same time. In this case, skins and seeds from white grape pomace are available just at the moment they are needed to be added to red winemaking. This winemaking technique may be therefore interesting as an alternative to traditional maceration to improve the quality of red wines from a warm climate as well as to exploit the white winemaking residues.

Therefore, the main objective of this work is to study the impact of adding supplementary amounts of skins and seeds from white grape pomace (var. Pedro Ximenez) during the fermentative maceration on young Syrah wines from a warm climate, especially in terms of their phenolic composition and color stability.

MATERIALS AND METHODS

Vinification Protocols and Samples. *Vitis vinifera* var. Syrah (red grape) and Pedro Ximenez (white grape) grown in the Montilla-Moriles Designation of Origin (Cordoba, southeastern Spain), with the typical climatological conditions of warm climate regions, were used in this study. These cultivars were selected because they have similar periods of ripeness (end of August) and represent the main and more extensively cultivated red and white grape varieties in the region.

Pedro Ximenez grape pomace (PXGP), the main organic vinification byproduct from Pedro Ximenez grapes, was used in the vinification experiments to ferment with Syrah grapes.

About 40 kg of industrially pressed PXGP was provided by La Unión Winery Coop. (Cordoba, Spain). It was collected from the 2012 vintage after Pedro Ximenez grapes were processed for winemaking at optimum technological maturity (density = 13.6 °Baumé). As usual, in traditional white vinifications the grapes are destemmed, so it is assumed that the pomace generated after vinification contains only some stems, which were manually separated. Therefore, the raw material used in the vinification experiments was composed by a mixture of skins and seeds.

Syrah grapes were harvested in 2012 vintage at optimum technological maturity (density = 13.1 °Baumé; total acidity = 5.21 g/L; and pH, 3.61) and good sanitary conditions. About 360 kg of grapes was manually harvested, placed in 15 kg plastic boxes, and transported to an experimental wine production center belonging to the Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA, Cordoba, Spain).

Nine vinifications were carried out on a pilot scale using 50 L stainless steel tanks. For each vinification, 40 kg of Syrah grapes was processed. Grapes were destemmed and crushed, and the must with solid parts was homogenized and distributed into tanks for maceration. Three types of Syrah wines were elaborated, in three replicates for each one (n = 3), with the following mixtures of Syrah grapes and PXGP: (a) one monovarietal wine elaborate with 100% Syrah grapes (SY); as control; (b) two wines containing the same amount of Syrah grapes and additional 10 and 20% of PXGP (w/w as grapes). Thus, to elaborate PXGP 10% wines, 4 kg of PXGP was added to 40 kg of Syrah grapes in each replicate. In the case of PXGP 20% wines, 8 kg of PXGP was added to 40 kg of Syrah grapes for each replicate. An adequate homogenization among PXGP and Syrah grapes was assured with a gradual addition at crushing.

An identical red winemaking procedure was used for all assays. Enological treatments were adjusted at the same levels for all of the assays: 60 mg/L total sulfur dioxide and 7 g/L of total titratable acidity by adding tartaric acid. Fermentative alcoholic maceration was induced by inoculating *Saccharomyces cerevisiae* selected yeast (TTA, 30 g/hL, 25 °C, Agrovin, Spain) and occurred at controlled temperature (20−25 °C). Fermentation caps were punched down once a day during the on-skin maceration period, which lasted 14 days. After this, the mash was drawn off to remove the skins and other solid parts, and the free-run musts were left to finish the fermentation under the same conditions. To guarantee the development of malolactic fermentation, selected *Oenococcus oeni* lactic acid bacteria (VINIFERM Oe 104, UFC/mL > 10^6; Agrovin, Spain) were inoculated at the rate of 10 mL/hL at the end of alcoholic fermentation. When fermentative processes were finished, the wines were racked in 15 L stainless steel tanks and held for 2 weeks at 4 °C. They were bottled 30 days later and stored at 10−15 °C until analysis.

Must and wine samples (100 mL) were taken at the initial point or grape crushing (I), at the middle of the fermentative alcoholic maceration (FAM), just after the skin removal (SR), at the end of malolactic fermentation (MLF), and 1, 2, 3, 4, and 5 months after fermentative processes were finished (bottled wines).

Enological Parameters. The conventional enological parameters of wines (Table 1) were evaluated according to the official methods established by the European Union.[20]

Table 1. Conventional Analytical Data (Means ± SD, n = 3) of Final Red Wines

analytical data	maceration treatment[a]		
	SY	PXGP 10%	PXGP 20%
reducing sugars (g/L)	1.12 ± 0.13	1.90 ± 0.06	1.65 ± 0.11
malic acid (g/L)	<0.01	<0.01	<0.01
alcohol degree (% v/v)	12.90 ± 0.18	13.20 ± 0.12	13.10 ± 0.12
volatile acidity (g/L as acetic acid)	0.55 ± 0.09	0.57 ± 0.03	0.57 ± 0.03
total acidity (g/L as tartaric acid)	5.85 ± 0.18	5.57 ± 0.04	5.59 ± 0.04
pH	3.33 ± 0.07	3.37 ± 0.03	3.29 ± 0.03
free sulfur dioxide (mg/L)	<10	<10	<10
total sulfur dioxide (mg/L)	44.33 ± 1.25	45.31 ± 6.6	50.56 ± 7.4

[a]SY, 100% Syrah grapes; PXGP 10% and PXGP 20%, fermentation of Syrah grapes with PXGP at 10 and 20% (w/w), respectively.

Copigmented and Polymerized Anthocyanin Determination. The contribution of copigmented anthocyanins to the total wine color at pH 3.6 (% copigmented anthocyanins) and the degree of anthocyanin polymerization (% polymeric pigments) were determined following the method proposed by Boulton.[21] The wine sample pH values were first adjusted to 3.6 using 1 M NaOH or HCl.

Total Phenolic Content. Total phenolic content was determined using a modification of the Folin−Ciocalteu method.[22] Absorbance was measured at 765 nm, and the results were expressed as milligrams of gallic acid per liter (mg GAE/L).

Phenolic Compound Analysis by HPLC-DAD. An Agilent 1200 chromatographic system equipped with a quaternary pump, an UV−vis diode-array detector, an automatic injector, and ChemStation software (Palo Alto, CA, USA) was used for the HPLC separation, identification, and quantification of phenolic compounds. Prior to direct injection, the samples were filtered through a 0.45 μm nylon filter (E0034, Análisis Vínicos, Spain). All analyses were made in triplicate.

Table 2. Mean Values and Standard Deviations of the Anthocyanin Compounds (Milligrams per Liter) and Percentages of Copigmentation and Polymerization of Wines (n = 3), at Skin Removal (SR) and after 5 Months of Stabilization

		maceration treatment[a]		
	stage	SY	PXGP 10%	PXGP 20%
delphinidin-3-glucoside	SR	72.27 a ± 5.82	52.03 b ± 6.51	42.99 b ± 0.44
	5 months	0.23 a ± 0.02	4.86 b ± 0.86	nd
cyanidin-3-glucoside	SR	nd	nd	nd
	5 months	nd	nd	nd
petunidin-3-glucoside	SR	63.14 a ± 2.12	67.96 a ± 2.30	50.31 b ± 1.54
	5 months	4.01 a ± 1.87	10.51 b ± 1.10	1.88 a ± 0.79
peonidin-3-glucoside	SR	178.43 a ± 4.35	131.34 b ± 11.67	112.41 b ± 4.43
	5 months	39.18 a ± 2.43	37.06 a ± 0.88	13.99 b ± 1.02
malvidin-3-glucoside	SR	547.87 a ± 25.18	414.49 b ± 15.48	373.91 b ± 13.49
	5 months	214.61 a ± 11.63	201.52 a ± 4.73	155.23 b ± 2.49
petunidin-3-acetyl-glucoside	SR	43.17 a ± 1.90	29.34 b ± 4.39	33.60 b ± 0.99
	5 months	1.31 a ± 0.23	2.24 b ± 0.99	nd ±
peonidin-3-acetyl-glucoside	SR	46.72 a ± 6.16	28.57 b ± 0.57	21.39 b ± 1.47
	5 months	8.59 a ± 1.44	3.25 b ± 0.66	nd ±
malvidin-3-acetyl-glucoside	SR	310.46 a ± 12.40	244.20 b ± 6.03	225.32 b ± 6.43
	5 months	148.92 a ± 4.23	136.70 b ± 1.57	115.92 c ± 3.78
petunidin-3-*p*-coumaroyl-glucoside	SR	23.52 a ± 10.96	16.36 a ± 1.94	17.10 a ± 2.01
	5 months	5.27 ± 2.36	nd	nd
peonidin-3-*p*-coumaroyl -glucoside	SR	16.72 a ± 2.31	9.88 b ± 1.51	7.41 b ± 0.61
	5 months	nd	nd	nd
malvidin-3-*p*-coumaroyl -glucoside	SR	225.46 a ± 30.82	202.99 a ± 14.03	203.66 a ± 12.36
	5 months	26.51 a ± 3.03	35.18 b ± 1.51	11.43 b ± 2.78
sum of glucoside derivatives	SR	861.71 a ± 34.64	665.82 b ± 33.77	597.63 c ± 19.91
	5 months	258.04 a ± 16.22	253.97 a ± 8.37	171.11 b ± 2.20
sum of acetate derivatives	SR	400.36 a ± 19.46	302.12 b ± 3.92	280.32 b ± 6.90
	5 months	158.82 a ± 6.72	142.20 b ± 0.25	115.92 c ± 0.41
sum of *p*-coumaric derivatives	SR	265.40 a ± 43.99	229.23 a ± 15.80	228.17 a ± 14.98
	5 months	31.79 a ± 2.33	35.19 a ± 1.51	11.43 b ± 2.87
% copigmented anthocyanins (%CA)	SR	21.82 a ± 1.02	26.69 a ± 0.56	34.46 b ± 1.47
	5 months	13.56 a ± 0.23	16.06 b ± 0.25	29.51 c ± 1.02
% polymeric pigments (%PP)	SR	35.33 a ± 2.51	41.30 b ± 1.21	45.04 b ± 0.50
	5 months	66.71 a ± 0.67	70.67 a ± 1.03	54.06 b ± 0.44

[a]SY, 100% Syrah grapes; PXGP 10% and PSGP 20%, fermentation of Syrah grapes with PXGP at 10 and 20% (w/w), respectively. Different letters in the same row indicate significant differences (p < 0.05). nd, not detected.

The anthocyanin identification was carried out following the method described in Heredia et al.[23] 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 2.0 nm bandwidth. The quantification was made at 525 nm by comparing the areas and retention times with those of the malvidin-3-glucoside standard, and anthocyanin concentration was expressed as milligrams per liter. Sums of glucosides, acetates, and *p*-coumaric derivatives were estimated by summing the content of each member identified by HPLC, respectively, and the sum of anthocyanins was obtained by summing the content of all anthocyanin compounds identified.

The identification of the noncolored phenolic compounds (low molecular weight) was carried out following the method described in Gordillo et al.[14] Individual phenolic compounds were separated using a Zorbax C18 column (250 × 4.6 mm, 5 μm particle size) maintained at 40 °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 flow rate was 0.63 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 280, 320, and 360 nm by comparing the areas and the retention times with the gallic acid, caffeic acid, (+)-catechin, and quercetin standards, respectively. Phenolic compound concentration was expressed as milligrams per liter. Sums of phenolic acids, monomeric flavanols, and flavonols were also estimated by summing the content of each member identified by HPLC, respectively.

Colorimetric Analysis. Color measurements were made with a Hewlett-Packard UV−vis HP8453 spectrophotometer (Palo Alto, CA, USA), using 0.2 cm path length glass cells and distilled water as reference. The whole visible spectrum (380−770 nm) was recorded (λ = 2 nm), considering the Illuminant D65 and 10° Observer as references. Wine samples were centrifuged (4190*g*, 5 min), and the supernatants were filtered through Millipore-AP20 filters (Bedford, MA, USA) prior to the spectrophotometric analysis.

The CIELAB parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) were determined by using the original software CromaLab,[24] following the recommendations of the Commission Internationale de L'Eclairage.[25] The L^* value is the vertical axis and defines the lightness, the property according to which each color can be considered as equivalent to a member of the

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Table 3. Mean Values and Standard Deviations of Noncolored Monomeric Phenols and Total Phenolics of Wines ($n = 3$), at Skin Removal (SR) and after 5 Months of Stabilization

	stage	maceration treatment[a]		
		SY	PXGP 10%	PXGP 20%
phenolic acids (mg/L)				
gallic acid	SR	174.28 a ± 8.77	227.14 b ± 9.36	244.00 b ± 12.62
	5 months	149.85 a ± 7.68	195.43 b ± 4.18	214.34 b ± 20.31
GRP[b]	SR	23.47 a ± 3.69	22.43 a ± 2.53	22.27 a ± 1.88
	5 months	17.49 a ± 1.58	17.28 a ± 1.26	16.60 a ± 1.80
trans-caftaric acid	SR	21.57 a ± 1.72	16.37 b ± 0.52	16.62 b ± 1.96
	5 months	20.37 a ± 1.58	15.38 b ± 0.73	14.41 b ± 1.92
trans-coutaric acid	SR	11.62 a ± 1.23	9028 b ± 0.28	9.07 b ± 0.78
	5 months	11.41 a ± 1.45	8.84 b ± 0.39	8.75 b ± 0.77
syringic acid	SR	1.77 a ± 0.16	4.63 b ± 1.22	7.85 c ± 0.50
	5 months	2.13 a ± 1.10	5.05 a ± 4.04	5.48 a ± 0.19
flavan-3-ols (mg/L)				
(+)-catechin	SR	16.14 a ± 1.74	21.37 b ± 0.40	23.69 b ± 1.23
	5 months	15.32 a ± 0.71	18.29 b ± 0.37	20.91 c ± 1.25
(−)-epicatechin	SR	13.81 a ± 1.24	12.96 a ± 0.29	12.80 a ± 1.70
	5 months	12.90 a ± 0.79	12.92 a ± 0.39	12.57 a ± 1.16
flavonols (mg/L)				
myricetin-3-glucuronide	SR	0.81 a ± 0.11	0.67 ab ±0.02	0.57 b ± 0.18
	5 months	0.71 a ± 0.12	0.61 a ± 0.10	0.09 a ± 0.03
myricetin-3-glucoside	SR	14.95 a ± 0.11	12.15 b ± 0.12	10.36 b ± 0.95
	5 months	10.03 a ± 0.72	8.34 a ± 0.94	8.46 a ± 0.28
quercetin-3-glucuronide	SR	10.75 a ± 1.01	14.56 b ± 0.27	16.65 a ± 0.52
	5 months	7.79 a ± 0.29	11.32 b ± 0.39	11.95 b ± 0.06
quercetin-3-glucoside	SR	12.82 a ± 0.96	14.65 a ± 0.19	14.08 a ± 0.52
	5 months	7.13 a ± 0.68	9.33 b ± 0.27	9.54 b ± 0.15
laricitrin-3-glucoside	SR	8.47 a ± 1.02	7.18 b ± 0.08	3.70 c ± 0.19
	5 months	3.72 a ± 0.70	3.15 ab ±0.23	2.57 b ± 0.10
kaempferol-3-glucoside	SR	2.08 a ± 0.28	0.58 b ± 0.01	1.29 c ± 0.14
	5 months	nd	0.12 b ± 0.03	0.63 c ± 0.01
isorhamnetin-3-glucoside	SR	4.69 a ± 0.44	1.66 b ± 0.10	1.29 b ± 0.06
	5 months	1.39 a ± 0.10	1.17 b ± 0.05	0.90 c ± 0.03
syringetin-3-glucoside	SR	nd	3.47 b ± 0.08	2.65 c ± 0.26
	5 months	3.46 a ± 0.25	2.70 b ± 0.22	1.99 c ± 0.07
total phenolics (Folin−Ciocalteu) (mg GAE/L)	SR	2269.8 a ± 49.01	2416.6 a ± 105.79	2779.2 b ± 135.35
	5 months	2036.6 a ± 75.34	2278.2 b ± 93.10	2346.0 b ± 62.91

[a]SY, 100% Syrah grapes; PXGP 10% and PXGP 20%, fermentation of Syrah grapes with PXGP at 10 and 20% (w/w), respectively. Different letters in the same row indicate significant differences ($p < 0.05$). nd, not detected. [b]GRP, grape reaction product (2-*S*-glutathionyl-caftaric acid).

gray scale, between black and white, taking values within the range of 0−100, respectively. The a^* and b^* values represent the chromaticity scalar coordinates, which in turn represent opponent red−green and blue−yellow scales.

From L^*, a^*, and b^*, other parameters are defined, such as hue (h_{ab}) and chroma (C^*_{ab}). Hue angle (h_{ab}) is the attribute according to which colors have been traditionally defined as red, green, etc. On the other hand, the chroma (C^*_{ab}) is the attribute that allows each hue to be determined by its degree of difference in comparison to a gray color with the same lightness. Moreover, these colorimetric parameters can be distinguished as quantitative or qualitative color attributes as they indicate a quantitative contribution to color (L^* and C^*_{ab}) or qualitative one (h_{ab}).[26]

Also, the color differences (ΔE^*_{ab}) were calculated between the samples to state the implications of the maceration treatments on the color of the final wines, as well as to assess the color stability. It was 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. All statistical analyses were performed using Statistica v.8.0 software.[27] Univariate analysis of variance (ANOVA) was applied using the general linear model program to establish whether mean values of the physicochemical data obtained in each studied point differed significantly among the three types of wines (SY, PXGP 10% and PXGP 20%). The means values of each set of samples ($n = 3$) were compared by the Tukey test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Enological Parameters. No statistically significant difference was found among control wines and wines fermented with additional amounts of PXGP for any enological parameter analyzed. These results indicate that the general wine composition was not affected by the maceration treatment applied (Table 1).

The low values found in wines for the reducing sugars and malic acid concentration (<2.0 g/L and <0.01 mg/L, respectively) denoted the correct development of fermentative

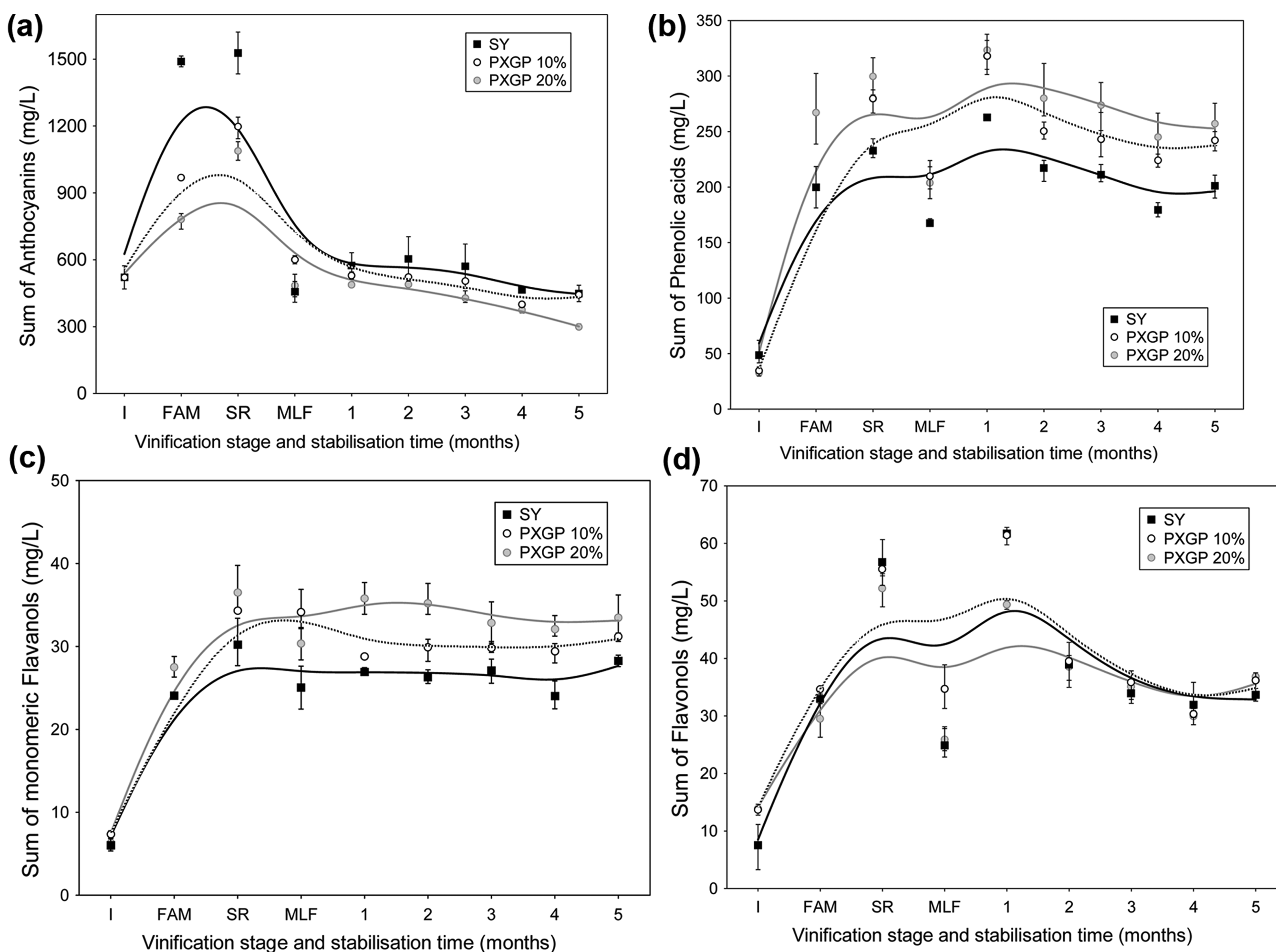

Figure 1. Evolution of the main phenolic families (mg/L ± SD, $n = 3$) in control wines (SY) and wines fermented with PXGP (10 and 20%) during the vinification process: (a) sum of anthocyanins; (b) sum of phenolic acids; (c) sum of monomeric flavanols; (d) sum of flavonols.

processes. The alcohol degree reached when fermentative processes were finished (between 12.9 and 13.2% v/v) was in accordance with the sugar concentration of Syrah grapes at ripeness. The good preservation state of wines was confirmed by the low values of the volatile acidity (around 0.5 g/L). As expected, all finished wines showed very similar values of total acidity (between 5.6 and 5.8 g/L as tartaric acid), pH (around 3.3 units), and free and total sulfur dioxide contents (<10 and between 44.3 and 50.6 mg/L, respectively) because these enological parameters were adjusted at the same levels for all assays.

Phenolic Composition and Changes during Vinification. Twenty-five monomeric phenolic compounds were identified and quantified in wines by HPLC. The comparative chromatographic analysis of samples revealed that control and fermented wines with PXGP did not differ in their phenolic profile in qualitative terms. It included several anthocyanin pigments and colorless phenols belonging to diverse phenolic families: 11 anthocyanins (including nonacylated, acetylated, and *p*-coumaroylated derivatives), 5 phenolic acids (gallic, GRP, *trans*-caftaric, *trans*-coutaric, and syringic), 2 monomeric flavanols ((+)-catechin and (−)-epicatechin), and 8 flavonols (myricetin-3-glucuronide, myricetin-3-glucoside, quercetin-3-glucuronide, quercetin-3-glucoside, laricitrin-3-glucoside, kaempferol-3-glucoside, isorhamnetin-3-glucoside, and syringetin-3-glucoside).

Table 2 summarizes the concentrations for the mentioned anthocyanins (mg/L) together with the percentage of copigmentation and polymerization of wines (mean ± SD, $n = 3$), showing the statistical differences among treatments at the end of the fermentative maceration (skin removal) and after 5 months of stabilization. In the same way, Table 3 summarizes the information relative to the colorless monomeric phenols (mg/L) and the total phenolic content (as mg GAE/L).

Results showed that the addition of PXGP at the beginning of fermentation causes a significant impact on the concentration of the most individual compounds identified and on the physicochemical transformations in which they are involved (copigmentation and polymerization). However, the effect was not the same for the different phenolic families and depended on the proportions of PXGP added. An accurate evaluation of the changes on the total levels of anthocyanins, phenolic acids, monomeric flavanols, and flavonols during the whole process of vinification allowed the stages at which the applied treatments had a greater influence on the chemical quality of wines to be established (Figure 1).

During the period of fermentative maceration, all wines elaborated with supplementary amounts of PXGP had lower levels of anthocyanins than wines elaborated by traditional maceration, although they were richer in phenolic acids and monomeric flavanols. The effect on the flavonols was quite different because additions of 10% PXGP had no significant

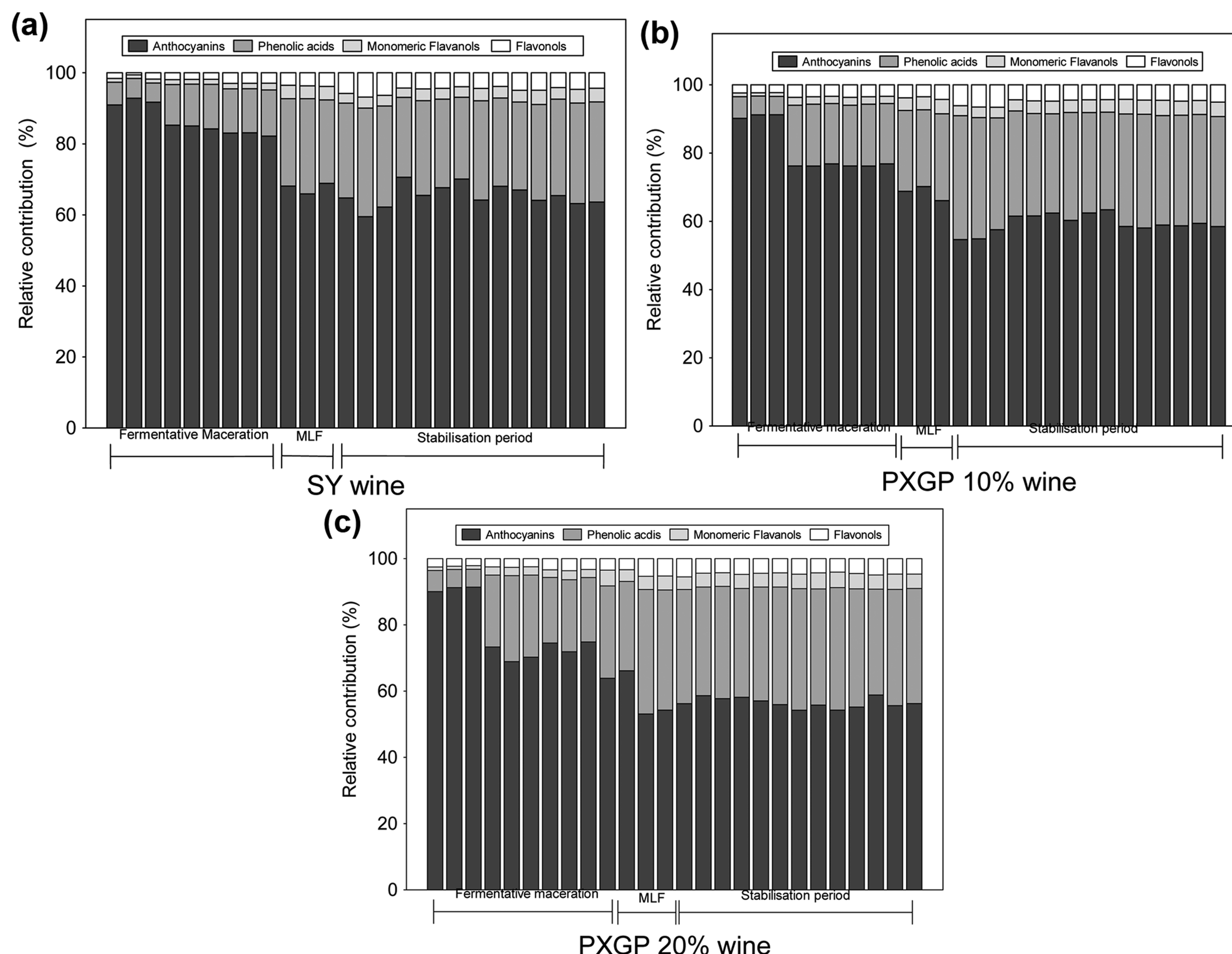

Figure 2. Relative proportion of the different phenolic families during the vinification process, according to the maceration treatment applied: (a) Syrah wines, traditional red wine vinification; (b) PXGP 10% wines, fermentative addition of PXGP at 10% (w/w); (c) PXGP 20% wines, fermentative addition of PXGP at 20%.

effect on the global content, but at higher proportions (20%) it was slightly reduced. Analogous chemical behavior was found by other authors in similar vinification experiments because two competitive effects are expected to occur simultaneously: (1) incorporation of the phenolics contained in the solid parts of grapes enhances the pool of copigmenting cofactors in the wine matrix; and (2) anthocyanin adsorption to the extra amounts of skin and seeds causes loss of color.[9,15,17]

In our winemaking conditions, significantly higher contents of gallic and syringic acids, (+)-catequin, quercetin-3-glucuronide, quercetin-3-glucoside, and syringentin-3-glucoside were found in PXGP wines at the end of the skin contact period, the effect being stronger when 20% was added (Table 3). Thus, adding PXGP at the rates studied favored the enrichment of Syrah wines in specific colorless phenolics, especially those cofactors having limited solubility.[28] Most of these compounds have been described as good copigments,[6] which support the higher grade of copigmentation reached by PXGP wines at skin removal (%CA = 34 and 27 versus 22% in PXGP 20%, PXGP 10%, and control wines).

On the other hand, the lower anthocyanin or flavonol content (only in the case of PXGP 20% wines) suggested the partial adsorption of pigments and even other phenolic compounds by extra amounts of skins and seeds, which could be especially drastic if too high proportions are added to the fermentation mash,[7] as occurred with PXGP at 20%.

Nevertheless, although the initial pigment content clearly differed for the treatments, the differences among them tended to diminish as the stabilization period proceeded (Figure 1a). A gradual decrease of total monomeric anthocyanins after skin removal was observed in the three wines, being more intense for control and PXGP 20% wines. As a consequence, the final values of total anthocyanins were almost the same order of magnitude for control and PXGP 10% wines (448.7 and 431.3 mg/L, respectively), whereas PXGP 20% wines showed the significantly lowest content (298.7 mg/L). Moreover, 10% PXGP addition seemed to protect to a larger extent the presence of bluish forms of anthocyanins (delphinidin-3-glucoside and petunidin-3-glucoside) and some acylated derivatives having chemical characteristics that great affect copigmentation (petunidin-3-acteyl-glucoside and malvidin-3-*p*-coumaroyl-glucoside), because significantly higher contents were found in the final wines.

With regard to the three groups of colorless phenols, wines elaborated with the addition of PXGP maintained the highest levels of phenolic acids (257.8 and 241.9 versus 201.3 mg/L, in 20%, 10%, and control wines, respectively), monomeric flavanols (33.5 and 31.2 versus 28.2 mg/L, respectively), and flavonols (36.2 and 36.1 versus 33.6 mg/L, respectively), and also the final total phenolic content (2346.0 and 2278.2 versus 2036.6 mg/L, respectively).

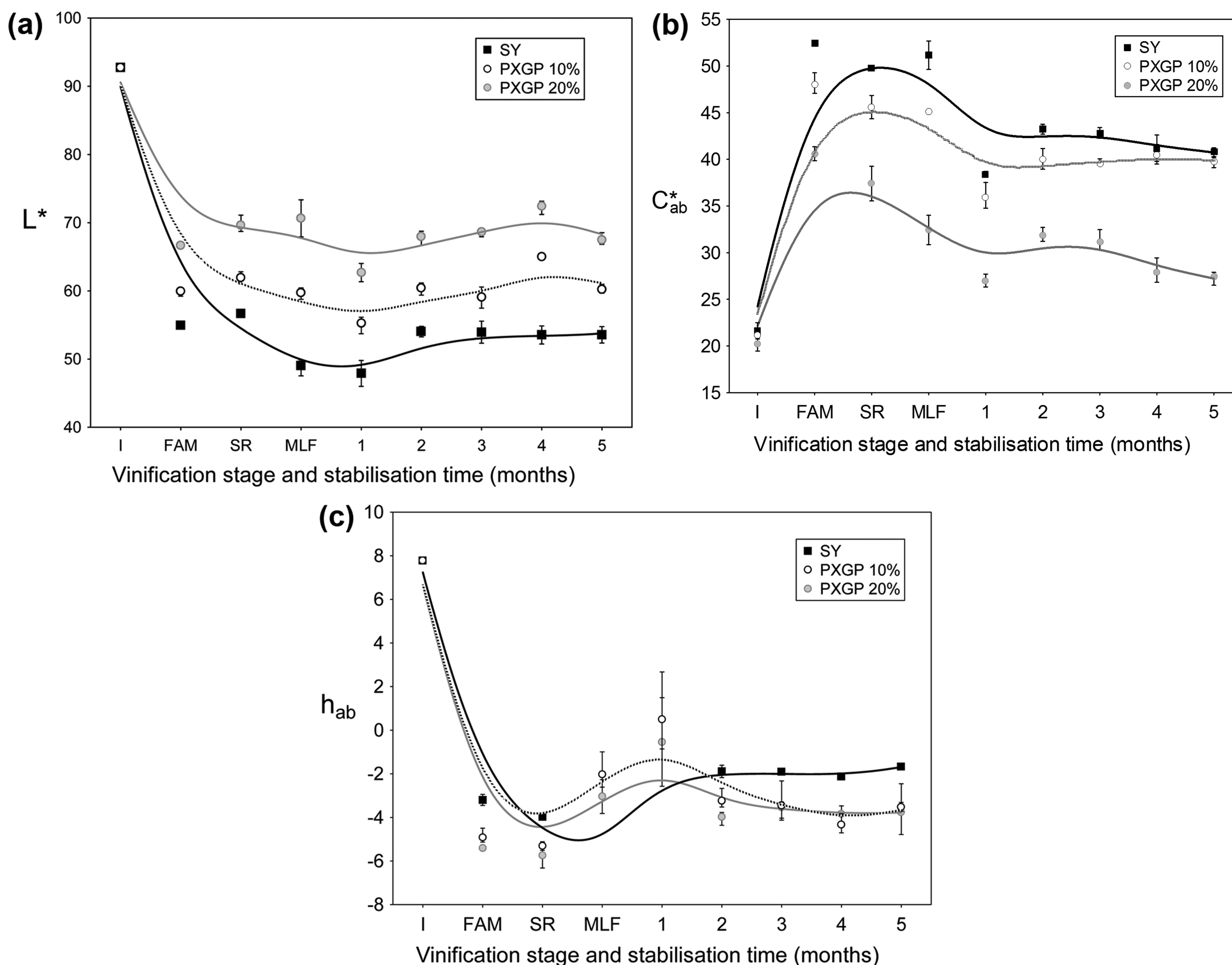

Figure 3. Changes in the color parameters (means ± SD, $n = 3$) for control wines (SY) and wines fermented with PXGP (10 and 20%) during the vinification process: (a) L^*, lightness; (b) C^*_{ab}, chroma; (c) h_{ab}, hue angle.

Table 4. Mean Values and Standard Deviations of Lightness (L^*), Chroma (C^*_{ab}), and Hue (h_{ab}) of Wines ($n = 3$), at Skin Removal (SR) and after 5 Months of Stabilization

		maceration treatment[a]		
	stage	SY	PXGP 10%	PXGP 20%
L^*	SR	56.29 a ± 0.18	61.92 b ± 0.89	69.63 c ± 1.29
	5 months	53.81 a ± 1.44	60.24 b ± 0.67	67.70 c ± 0.72
C^*_{ab}	SR	49.46 a ± 0.12	46.30 b 1.26	35.23 c ± 1.86
	5 months	41.22 a ± 0.91	40.49 a ± 1.06	27.40 b ± 0.78
h_{ab}	SR	−3.98 a ± 0.11	−4.56 b ± 0.9	−5.73 b ± 0.52
	5 months	−1.66 a ± 0.13	−3.17 a ± 0.63	−3.76 b ± 1.19

[a]SY, 100% Syrah grapes; PXGP 10% and PXGP 20%, fermentation of Syrah grapes with PXGP at 10 and 20% (w/w), respectively. Different letters in the same row indicate significant differences ($p < 0.05$).

Furthermore, the contribution of each group of pigments to the total color (copigmented and polymeric pigments) evolved differently depending on the maceration applied, which seemed to be related to the relative proportions of the different phenolic families in each wine (Figure 2). Adding 10% PXGP led to wines with the highest degree of polymerization (%PP = 70.6), meaning a higher proportion of more stable pigments than control wines (%PP = 66.7). Probably, the lower content of pigments in PXGP 10% wines at skin removal could be balanced by the relative highest proportion of copigments extracted in comparison with control wines, resulting in a better pigment/copigment ratio and higher chemical stability. On the contrary, the PXGP 20% wines reached the lowest proportion of polymeric pigments (%PP = 54.1%). This fact shows the difficulty of those wines to convert the earlier copigmentation complexes into more stable pigments despite having important amounts of copigments. Most likely, the significantly lower content of anthocyanins and flavonols from the first steps of vinification was insufficient to achieve higher chemical stability during stabilization.[2,29]

Color Characteristics and Changes during Vinification. The evolution of CIELAB color parameters (L^*, C^*_{ab}, and h_{ab} CIELAB parameters) during the winemaking processes is shown in Figure 3, where the mean value and standard deviation for these variables are included for each set of samples.

As determined from the whole set of data, red wine color evolved in a similar way both in control and in PXGP wines. A net increase of chroma (C^*_{ab}) and decreases of lightness (L^*) and hue (h_{ab}) due to the pigment extraction characterized the fermentative maceration, whereas all of the parameters became gradually stable after the skin contact period because of the stabilization processes.

As can be seen, the maceration treatments applied induced significant differences in the color characteristics of wines and their stability depending on the doses of PXGP added. As expected, the higher color extraction corresponded to control wines, which showed the lowest values of lightness (L^*) and highest values of chroma (C^*_{ab}) at skin removal, indicating darker and more saturated color than PXGP wines (Table 4). These results were consistent with the higher content of anthocyanins in control wines during the fermentative maceration period, which reflects the better balance between the extraction and adsorption of pigments with respect to wines elaborated with higher amounts of pomace than are naturally found in a traditional red wine vinification.

However, it is interesting to note how the particular changes during the stabilization in the phenolic fractions influenced the final color of wines (Table 4), in both quantitative (C^*_{ab} and L^*) and qualitative (h_{ab}) terms.[26] At the end of the stabilization period, the addition of PXGP at 10% led to wines with comparable content in pigments relative to wines elaborated by traditional maceration, but with higher proportions of bluish anthocyanins, copigments, and polymeric pigments, which resulted in lighter wines but with comparable quantity of color ($L^* = 60.24 \pm 0.67$ versus 53.81 ± 1.44 u; $C^*_{ab} = 40.49 \pm 1.06$ versus 41.22 ± 0.91 u) and more notably bluish hues (negative values of hue; $h_{ab} = -3.17° \pm 0.63$ versus $-1.66° \pm 0.13$, in PXGP 10% and control wines, respectively).

The effect on these analytical parameters was the opposite when 20% of pomace from PXGP was added, which can be attributable to the lower phenolic potential during the whole process of vinification. In this case, it was related to significantly lighter wines with less saturated color ($L^* = 67.70 \pm 0.72$ u; $C^*_{ab} = 27.40 \pm 0.78$ u). In terms of total color, the highest color difference values (ΔE^*_{ab}) were found between control wine and 20% PXGP wine (14.38 u) and the least one between control wine and 10% PXGP wines (3.93 u), although in all cases they were visually perceptible ($\Delta E^*_{ab} > 3.0$ CIELAB units).[30]

The assessment of the color differences (ΔE^*_{ab}) occurring from the skin removal to the end of the stabilization period (5 months) allowed evaluation of the color stability of each wine. The lowest values of color difference were obtained for PXGP 10% wines compared to control and PXGP 20% wines (ΔE^*_{ab} = 6.36 versus 9.53 and 8.81 u, respectively), indicating lower color variation and, thus, higher color stability. As can be observed in Figure 3b,c, the chroma decreased and the hue increased toward 0° (redness color) in all wines. This observation means that the color variation that took place during stabilization was due to a decrease of the quantity of color and bluish hue of wines (lower values of C^*_{ab} and higher values of hue). Specifically, these chromatic modifications were less intense in PXGP 10% wines ($\Delta C^*_{ab} = -5.7$ versus -7.7 and -8.6 u; $\Delta h_{ab} = +1.39°$ versus $+1.97°$ and $+2.37°$ in 10%, 20%, and control wines, respectively). Chemically, this may be explained by the lower loss of monomeric anthocyanins and the higher content in more stable pigments in PXGP 10% wines as a result of the phenolic compounds extracted from PXGP, which favored a better pigmet/copigment ratio and a more effective stabilization of wine color. Thus, it was confirmed that the addition of limited amounts (10%) of skins and seeds of PXGP during fermentation led to wines with more stable color that kept their vivid bluish tonalities for a longer time, and these characteristics are highly appreciated in young red wines. In the case of the higher proportions tested (20%), the adsorption of pigments during fermentation seems to have a stronger effect on the color than the copigmentation (color stabilization) due to the cofactors extracted from PXGP, as reported in the literature.[15]

On the basis of the results, it has been proved that the addition of a limited amount of skins and seeds from white grape pomace during the fermentative step of the winemaking process represents an interesting enological practice to improve the phenolic potential and color characteristic of young Syrah wines from a warm climate. The effectiveness of the fermentative addition was demonstrated by the increase of the concentration of several colorless phenolic compounds (copigments) and intermolecular copigmentation reactions in wines. Potential benefits observed in Syrah wines include greater chemical complexity and higher development of polymerization, as well as better color characteristics and color stability. However, the global effect clearly depends on the proportions of white skins and seeds applied. When too high a proportion of pressed grape pomace (20% w/w) is added to the fermentation mash, it may damage wine quality due to a higher adsorption of phenolic compounds during maceration (pigments and copigments), resulting in a net loss in color in final wines. Thus, not all proportions are acceptable to obtain phenolic and color enhancement effects.

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Notes

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