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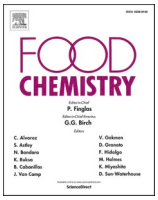

Characterization of the color parameters and monomeric phenolic composition of 'Tempranillo' and 'Graciano' wines made by carbonic maceration

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ABSTRACT

The current study aims to gather information about the color-related characteristics and the monomeric phenolic composition of carbonic maceration (CM) wines. The study was conducted over two vintages, with two different grape varieties. Color-related general parameters and wine monomeric phenolic composition were determined in both free-run and press CM fractions and were compared to conventional winemaking (CW). Overall, wines made by CM had lower phenolic content and color intensity. The analysis of wine detailed phenolic composition showed that CM wines were characterized by low contents of anthocyanins and flavonols. In contrast, free-run wine obtained by CM had the greatest concentration of flavanols and hydroxycinnamic acids, probably due to the presence of stems during the fermentation. In conclusion, vinification by CM leads to important differences in wine phenolic composition and, as consequence, on wine color properties. These differences could play an important role in wine sensorial properties and wine aging potential.

1. Introduction

Phenolic compounds form a heterogeneous group of secondary metabolites which are divided according to their structure in non-flavonoids (i.e. phenolic acids, hydrolysable tannins, and stilbenes) and flavonoids (i.e. anthocyanins, flavonols, flavanols). These compounds, together with wine aroma compounds, are the major responsible for grape and wine quality, determining important organoleptic properties like wine color, taste, and mouthfeel properties (Ferrero del Teso et al., 2022), contributing to wine quality perception (Sáenz-Navajas et al., 2010). In addition, due to their antioxidant properties, these compounds are known to play a key role in the beneficial health properties related to the moderate consumption of wine. Therefore, a considerable amount of literature has been published on studying their biological activities, like anticarcinogenic, antidiabetic, cardioprotective or neuroprotective (Nemzer et al., 2022), which could depend on the gut microbiota composition (Nash et al., 2018). Wine phenolic composition is greatly influenced by many factors, including vineyard factors, wine elaboration and wine storage (Gutiérrez-Escobar et al., 2021).

There are several factors that could influence wine phenolic composition during wine elaboration, which basically depend on the vinification methodology. In this respect, the vinification methodology known as carbonic maceration (CM) can have a significant impact on the phenolic composition of wine. CM involves the process whereby the grapes are subjected to anaerobic conditions and berries undergo a self-fermentation before yeast and malolactic fermentation (Tesniere & Flanzy, 2011). To carry out CM, grapes must be harvested with minimal breakage. In detail, the intact grape clusters, without destemming or crushing, are placed into tanks and kept under a carbon dioxide atmosphere. Under these anaerobic conditions, intracellular fermentation occurs inside the whole grapes triggering various chemical and physicochemical processes, including ethanol production, malic acid degradation, pectolytic and proteolytic phenomena, the formation of volatile compounds and the diffusion of phenolic compounds from the skin to the pulp (Tesniere & Flanzy, 2011). Via these processes, grape berries, at a certain moment, split open and release their juice to the bottom of the tank, where it is fermented by yeasts. After this first phase, racking is done by drawing off a free-run, partly fermented wine, and the grapes that remain whole are pressed releasing a higher-density press-wine. In CM, press-wine is of higher organoleptic quality than that of free-run, because it is derived from grapes that have undergone intracellular fermentation for a longer time (Tesniere & Flanzy, 2011). Then, a second phase begins when both wines, mixed or separated, complete their

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alcoholic fermentation by yeast action and the malolactic fermentation by lactic acid bacteria.

CM process leads to obtain wines with specific and distinctive properties. Wines obtained by CM are considered to be lighter and fruity and to be ideally suited for drinking during their first year. CM wines have been characterized to have, in general, lower density, dry extract, fixed acidity and phenolic compounds concentrations than wines obtained from crushed grapes (Ribéreau-Gayon et al., 2006). In this respect, this vinification methodology seems more appropriate when used with highly tannic grape varieties or with acidic grapes (Ribéreau-Gayon et al., 2006). In addition, CM produces wines with a unique fruity aroma, which seems positive for neutral varieties (Jackson, 2008). In this respect, bibliography has shown that wines made by CM have singular aroma composition. Spranger et al. (2004) showed that benzyl alcohol and ethyl lactate were increased by CM. Ayestarán et al. (2019) showed that CM led to obtain white wines with higher odor activity value (OAV), characterized by higher content of alcohols and carbonyl compounds which were associated with higher aromatic intensity and ripe fruit descriptors. In agree with this, Zhang et al. (2019) reported that CM wine had higher concentration of volatile compounds, especially esters and terpenes, as well as higher OAV.

Nowadays, this traditional vinification system is fairly widespread for the production of red young wines, especially in the Spanish Rioja Qualified Designation of Origin (D.O.Ca. Rioja), one of the most important winemaking regions in Spain, as well as in other known wine regions, like Beaujolais (France). Although CM wines are recognized as high quality young wines, recent works that describe their physicochemical and microbiological characteristics are scarce and contradictory (Sacchi et al., 2005). The contradictory results could be due to a wide range of causes such as the grape variety or the vintage (González-Arenzana et al., 2020; Ribéreau-Gayon et al., 2006; Santamaría et al., 2022). Especially, very little is currently known about the impact of this vinification methodology on the detailed wine phenolic composition. Additionally, no works have been found considering multiple vintages and grape varieties.

Consequently, the objective of this study was to gather information about the color characteristics of CM wines and to study in depth the influence of this winemaking system on the wine monomeric phenolic composition. For this purpose, the study was conducted over two consecutive vintages (2019 and 2020) with two different grape varieties ('Tempranillo', a very versatile variety from an oenological point of view; and 'Graciano', with a marked acidity and high polyphenolic content). It is hoped that this study will generate fresh insight into the characterization of the phenolic composition of wines made by CM by considering different grape varieties and vintages and by performing UHPLC-MS analysis.

2. Materials and methods

2.1. Chemicals and reagents

Caffeic acid, *p*-coumaric acid, ferulic acid, gallic acid, (+)-catechin, *trans*-resveratrol, and *trans*-piceid were purchased from Sigma-Aldrich (St. Louis, MO, USA). *trans*-Caftaric acid and quercetin-3-*O*-glucuronide were purchased from Biopurify Phytochemicals (Chengdou, China). Malvidin-3-*O*-glucoside was purchased from Extrasynthese (Genay, France). Formic acid (LC-MS grade) was purchased from VWR International (Radnor, PE, USA). Acetonitrile (LC-MS) and methanol (LC-MS grade) were purchased from Scharlab (Barcelona, Spain). Water was Milli-Q quality (Millipore Corporation, Burlington, MA, USA).

2.2. Experimental layout and vinification

This study was conducted during two consecutive vintages (2019 and 2020) on two different *Vitis vinifera* L. grape varieties: 'Tempranillo' (VIVC 12350) and 'Graciano' (VIVC 4935).

Twelve vinifications were carried out each year in 300 kg stainless steel tanks, six for each variety. Three tanks were vinified by conventional winemaking (CW) and the other three by carbonic maceration (CM) (n = 3).

At CW, grapes were destemmed, crushed, sulfited at a dose of 40 mg L^{-1} SO_2, and placed in each tank. The alcoholic fermentation took place spontaneously. The density and temperature of the liquid was measured daily after pumping over. The tanks were devatted and pressed when the relative density of the liquid was 1,000. The end of the alcoholic fermentation was determined by measuring reducing sugars (<2.5 g L^{-1}). Then, the wines were drawn off from the lees and placed into 100 L stainless steel vats. The malolactic fermentations developed spontaneously. At the end of process, the wines were sulfited at a dose of 40 mg L^{-1} of SO_2.

At CM, before the distribution of the bunches into the tanks, 30 kg of grapes were crushed and sulfited at a dose of 40 mg L^{-1} of SO_2. These grapes were not destemmed and were added in each tank in order to simulate the breakage that occurs in industrial tanks during filling. After this, the bunches were carefully introduced in a proportion of 270 kg $tank^{-1}$. The stainless steel tanks were subjected to anaerobiosis by adding carbon dioxide besides the one generated by the fermentation of the bottom must. The level of carbon dioxide inside the tanks, the temperature of the bottom must and the temperature of the bunch mass were checked daily. The alcoholic fermentation was developed spontaneously. The tanks were devatted when the relative density of the bottom must was 1,000, and two fractions from each tank were obtained: free-run liquid in the tank, and liquid obtained by pressing the solid mass with a pneumatic press. The time of intracellular fermentation/maceration was 11 days for Tempranillo and 7 days for Graciano at the first year and 5 days for both varieties at the second year. Subsequently, the two fractions obtained (free-run and press) were maintained separately and completed both alcoholic and malolactic fermentations spontaneously. At the end of the malolactic fermentation, the wines (CM free-run wine and CM press wine) were sulfited at a dose of 40 mg L^{-1} of SO_2. Once the malolactic fermentation was finished, aliquots of each wine were frozen and stored at −20 °C until the analyses of phenolic compounds were carried out.

2.3. Determination of wine color-related physicochemical parameters

Wine color properties were characterized by measuring hue, color intensity (CI), and CIELab parameters according to the International Organisation of Vine and Wine (2020). Total phenolics were determined as total polyphenol index (TPI) by spectrophotometric absorbance at 280 nm after previous dilution of samples (Ribéreau-Gayon & Stonestreet, 1965). Folin-Ciocalteu index was determined with an automated clinical chemistry analyzer (Miura One, TDI, Spain). Polymerization index was calculated according to Ruiz (1999). Wine total antioxidant activity was determined according to the DPPH method as described by Nixdorf and Hermosín-Gutiérrez (2010). The color fractions due to free anthocyanins, copigmented anthocyanins and polymeric pigments were obtained according to Boulton (1996) following the methodology described by Heras-Roger et al. (2016).

2.4. Determination of wine low molecular weight phenolic compounds by UHPLC-QqQ-MS/MS

2.4.1. Sample preparation

Wine samples were stored at −20 °C after the alcoholic fermentation. Just before UHPLC analyses, wine samples were defrost and centrifuged. After centrifugation, samples were filtered with LLG Syringe Filters SPHEROS, PTFE, 0.22 μm pore size (LLG Labware, Meckenheim, Germany).

2.4.2. Analysis of phenolic compounds by UHPLC/QqQ-MS/MS

Wine phenolic compounds were analyzed by using a Shimadzu

Nexera (Shimadzu Corporation, Kyoto, Japan) chromatograph, equipped with a 3200QTRAP® triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA), with atmospheric pressure ionization source (ESI and APCI). The analytical column used was a Waters Acquity BEH C18 (100 × 2.1 mm, 1.7 μm) equipped with a VanGuard pre-column Acquity BEH C18 (5 × 2.1 mm, 1.7 μm) from Waters (Milford, MA, USA). Mobile phase solvents were Milli-Q water, LC/MS grade acetonitrile and LC/MS grade formic acid.

Flow rate was established in both methods at 0.45 mL min^{-1}, and 2.5 μL of wine samples were injected. The autosampler and oven temperatures were respectively 7 °C and 40 °C. For the analysis of the anthocyanins, the mobile phase was 2 % formic acid in water (eluent A), and 2 % formic acid in acetonitrile (eluent B). The elution gradient was: 0–0.5 min, 1 % B isocratic; 0.5–1.5 min, 1–8 % B; 1.5–4 min, 8 % B isocratic; 4–5 min, 8–12 % B; 5–5.5 min, 12 % B isocratic; 5.5–6 min, 12–14 % B; 6–7 min, 14 % B isocratic; 7–9 min, 14–22 % B; 9–12 min, 22–30 % B; 12–13.5 min, 30–90 % B; 13.5–14.5 min, 90 % B isocratic; 14.5–15 min, 90–1 % B; 15–18 min, 1 % B isocratic.

The mobile phase for the analysis of the rest of the non-anthocyanin phenolic compounds was 0.1 % formic acid in water (eluent A), and 0.1 % formic acid in acetonitrile (eluent B). The elution gradient was the same as for anthocyanins.

Tandem MS analyses were carried out on a 3200QTRAP triple quadrupole mass spectrometer (AB Sciex) equipped with an electrospray ionization source (ESI Turbo V™ Source).

Ionization was achieved using the electrospray (ESI) interface operating in the positive mode $[M-H]^+$ for the analysis of anthocyanins, and in the negative mode $[M-H]^-$ for the rest of the phenolic compounds. The data was acquired through multiple reaction monitoring (MRM). The ionization source parameters were an ion spray voltage of ± 4.5 kV, the source temperature was 700 °C and the gas pressures were curtain gas 50 psi; GS1 50 psi and GS2 60 psi). Nitrogen (>99.99 % purity, degasified liquid nitrogen from a tank, Air Liquide, Paris, France) was used as the source and collision gases. The dwell time established for each transition was optimized through the chromatogram with the Scheduled MRM tool by means of the retention time, MRM detection window of 60 s and a target scan time of 0.75 s. Data acquisition was carried out with the Analyst® 1.6.2 software (AB Sciex).

Some of the anthocyanins and non-colored phenolic compounds were quantified using the calibration curves of their corresponding pure commercial standards. The other compounds were tentatively quantified using the calibration curves of standards with similar chemical structures: *p*-coumaric acid for coutaric acid, ferulic acid for fertaric acid, quercetin-3-*O*-glucuronide for flavonols, catechin por flavanols, resveratrol for viniferins and piceatanols, piceid for astringins, malvidin-3-*O*-glucoside for anthocyanins. All samples were injected two times: without dilution and diluted 10 times with a solution of Milli-Q water/ethanol (80:20, v/v).

Concentrations in wine samples were expressed as milligrams per liter of wine (mg L^{-1}).

2.5. Statistical analysis

The statistical procedure was carried out with IBM SPSS Statistics for Windows, version (Armonk, NY, USA). Multifactor analysis and post-hoc Tuckey's multiple range test ($p \leq 0.05$) were performed to determine the statistically significant differences for each parameter between type of wines (n = 12) and grape varieties (n = 18), as well as between vintages (n = 18). A canonical discriminant analysis was performed to discriminate samples using variables (monomeric wine phenolic compounds) with predefined groups (wine types).

3. Results and discussion

3.1. Color-related physicochemical parameters

Table 1 shows the results from the analyses of wine color-related physiochemical parameters.

Comparing both grape varieties, wines made from Graciano were deeper in color, with higher polymerization index, total anthocyanin content, and color intensity and lower hue, with a greater contribution of copigmented anthocyanins. Tempranillo wines showed higher Folin-Ciocalteu index and total antioxidant activity, probably due to the greater amount of wine tannins. In addition, Tempranillo wine showed higher values for all CIELab parameters and a greater contribution of free anthocyanins and polyermic pigments. There were not important differences between vintages, although 2019 was characterized by higher Folin-Ciocalteu and polymerization indexes, antioxidant activity and higher contribution of polymeric pigments to wine color. 2020 had higher tannin content, L*and a* values, and a greater contribution of copigments to the wine color.

Regarding the influence of CM on the wine color-related parameters, wine made by CW was characterized by the highest polyphenolic content, obtaining higher TPI, Folin-Ciocalteu index, polymerization index, total anthocyanins and tannins content. In contrast, the CM press wine had the lowest concentration of phenolic compounds, showing lower values for the abovementioned parameters when compared to the free-run fraction. In addition, press CM wine had also the lowest total antioxidant activity.

Although some conflicting results can be found in literature (Sacchi et al., 2005), our study, which was carried out in two vintages with two grape varieties, agrees with most of the previous studies that showed that CM wine has lower concentrations of phenolic compounds (Gómez-Míguez & Heredia, 2004; Spranger et al., 2004), although it greatly depends in factors such as temperature (Flanzy et al., 1987) or maceration time (Pace et al., 2014).

Regarding wine color, CM press wine was characterized by the lowest color intensity and the highest hue, while CW led to the highest color intensity and the lowest hue. CIELAB parameters were higher in CM press wine when compared to CW wine. Therefore, CM press wine was lighter (L*), with a greater contribution of redness (a*), yellowness (b*), with more vivid color (C*) and higher hue angle (H*). CM free-run wine had more vivid color (C*), higher hue angle (H*) and greater contribution of redness (a*) than CW wine. Taking into consideration CIELAB parameters, colorimetric differences between wines was calculated (ΔE*) and results showed that wines could be distinguished by human eye, as ΔE* was of 5.21 and 13.48 when CW was compared to free run and press CM wine, respectively, while ΔE* between both CM fractions was 8.44. Finally, both fractions obtained by CM showed a greater contribution of free-anthocyanins to the wine color, while CW wine was characterized by a higher contribution of copigmented anthocyanins. In addition, color due to polymeric pigments was of greater importance in CM press wine, followed by free-run and CW wine.

Therefore, our work confirms previous studies that described that CM wine has less color intensity and lower concentration of anthocyanins (Castillo-Sánchez et al., 2006; Gómez-Míguez & Heredia, 2004; González-Lázaro et al., 2020; Spranger et al., 2004), especially the fraction obtained from the press juice (Ribéreau-Gayon et al., 2006). Moreover, these wines usually have higher values of a*, b*, and C* so they are considered to have a more vivid color than CW wine (Gómez-Míguez & Heredia, 2004; Zhang et al., 2019). Nonetheless, there are contradictory results in literature regarding the rest of the CIELAB parameters. In this respect, our work agrees well with Gómez-Míguez and Heredia (2004) on the higher values of L* in CM but Zhang et al. (2019) reported no differences in this parameter in the case of 'Muscat Hamburg' wine.

Finally, our work also indicates that copigmentation is not an important phenomenon in CM wine. Besides, although polymeric

Table 1
Multivariate analyses of variance of wine color-related general parameters between types of wine (conventional winemaking wine (CW) and carbonic maceration free-run and press wines), cultivars (Tempranillo and Graciano) and vintages.

	Type of wine			Cultivar		Vintage	
	CW	Free-run	Press	Tempranillo	Graciano	2019	2020
Total polyphenol index (TPI)	47.30c	40.15b	30.45a	38.30a	40.29b	39.82	38.77
Folin-Ciocalteu index[b]	1668c	1521b	1127a	1536b	1342a	1485b	1392a
Polymerization index	2.41c	1.75b	1.45a	1.48a	2.27b	2.11b	1.63a
Total anthocyanins (mg L^{-1})	705.9c	456.5b	385.9a	491.7a	540.5b	511.0	521.2
Total tannins (g L^{-1})	1.81c	1.54b	1.18a	1.62b	1.40a	1.41a	1.61b
Total antioxidant activity[c]	5.70b	5.87b	4.37a	5.72b	4.90a	11.51b	9.56a
Color intensity	14.24c	9.84b	7.53a	7.42a	13.65b	0.574	0.564
Hue	0.530a	0.573b	0.604c	0.657b	0.481a	5.21	5.41
L* (CIELab units)	12.87a	15.60a	21.62b	18.75b	14.64a	15.23a	18.16b
C* (CIELab units)	48.51a	52.47b	58.47c	55.38b	50.92a	52.00	54.30
H* (CIELab units)	24.51a	27.43b	27.91b	27.56b	25.67a	26.56	26.67
a* (CIELab units)	43.52a	46.51b	51.54c	49.06b	45.32a	46.01a	48.36b
b* (CIELab units)	20.97a	24.25ab	27.37b	25.63b	22.77a	23.90	24.50
$X_{Free\ anthocyanin}$	45.28a	50.28b	52.76b	51.56b	47.31a	50.32	48.56
$X_{Copigmentation}$	40.39b	33.67a	29.65a	32.10a	37.04b	32.33a	36.81b
$X_{Polymeric\ pigment}$	14.33a	16.05b	17.60c	16.34b	15.65a	17.35b	14.63a

[a]For each parameter, different letters (a-c) indicate significant differences between types of wine, cultivars or vintages at the 95% confidence level.
[b]as mg of gallic acid equivalents per liter.
[c]as mmol of Trollox equivalents per liter.

pigments display a greater contribution to CM wine color, the color of CW wines is more stable and has a higher polymerization index, so CM wine seems less suitable for aging.

3.2. Wine anthocyanin composition

The results of the UHPLC analyses of wine anthocyanins are shown in Table 2. Overall, malvidin-type anthocyanins were found to be the most

Table 2
Multivariate analyses of variance of wine anthocyanins (mg/L) between types of wine (conventional winemaking wine (CW) and carbonic maceration free-run and press wines) and cultivars (Tempranillo and Graciano) and vintages.

	Type of wine			Cultivar		Vintage	
	CW	Free-run	Press	Tempranillo	Graciano	2019	2020
Dp-3-glc	61,26c	29,38b	19,22a	39,87b	33,37a	42.98b	30.25a
Cn-3-glc	5,20c	3,31b	0,98a	2,83a	3,49b	3.44b	2.88a
Pt-3-glc	83,65c	42,32b	35,86a	61,38b	46,51a	53,77	54,12
Pn-3-glc	50.60c	29,51b	19.15a	17,45a	48,72b	35,50b	30,67a
Mv-3-glc	307,5c	199,9b	180,3a	222,4a	236,0b	192,3a	266,2b
Dp-3-acglc	6,52c	3,01b	2,61a	3,85a	4,24b	3,53a	4,56b
Cn-3-acglc	1,87c	1,03b	0,77a	1,00a	1,44b	0,97a	1,47b
Pt-3-acglc	10,70c	4,85b	3,92a	5,91a	7,08b	5,74a	7,24b
Pn-3-acglc	17.79c	12.20b	8.80a	4,45a	21,42b	7,36a	18,51b
Mv-3-acglc	91,24c	52.67b	46,67a	39,48a	87,58b	62,38	64,68
Dp-3-*cis*-cmglc	0,20c	0,15b	0,09a	0,21b	0,09a	0,00a	0,30b
Dp-3-*trans*-cmglc	5,40c	2,76b	2,19a	4,88b	2,03a	2,69a	4,21b
Cn-3-*cis*-cmglc	0,09c	0,06b	0,04a	0,08b	0,05a	0,00a	0,13b
Cn-3-*trans*-cmglc	4,20c	1,97b	1,50a	3,11b	2,02a	2,09a	3,04b
Pt-3-*cis*-cmglc	0,24c	0,17b	0,09a	0,23b	0,11a	0,00a	0,33b
Pt-3-*trans*-cmglc	5,49c	2,80b	2,20a	4,60b	2,39a	2,68a	4,31b
Pn-3-*cis*-cmglc	0.54c	0,35b	0,41a	0,19a	0,54b	0,00a	0,74b
Pn-3-*trans*-cmglc	10,26c	6,18b	4,71a	3,64a	10,46b	5,12a	8,98b
Mv-3-*cis*-cmglc	1,72c	1,24b	0,75a	1,00a	1,50b	0,00a	2,47b
Mv-3-*trans*-cmglc	19,34b	14,33b	12,05a	14,93	15,55	13,29a	17,19b
Mv-3-cfglc	1,55b	0,41a	0,50a	0,64a	1,00b	1,11b	0,53a
∑ non-acylated	508,2c	304,5b	255,5a	344,0a	368,1b	328,0a	384,1b
∑ acetylated	128,1c	73,8b	62,7a	54,7a	121,8b	79,98a	96,45b
∑ coumaroylated	47,48c	30,03b	23,83a	32,86	34,70	25,87a	41,69b
∑ acylated	177,2c	104,2b	87,1a	88,2a	157,4b	107,0a	138,7b
∑ delfinidins	73,38c	35,30b	24,10a	48,80b	39,73a	49,20b	39,32a
∑ cyanidins	11,36c	6.37b	3,29a	7,02	7,00	6,50a	7,51b
∑ petunidins	100,1c	50,14b	42,07a	72,12b	56,08a	62,19a	66,00b
∑ peonidins	79,19c	48,25b	32,87a	25,73a	81,14b	47,98a	58,89b
∑ malvidins	421,3c	268,6b	240,2a	278,5a	341,6b	269,1a	351,0b
Total anthocyanins	685,3c	408,6b	342,6a	432,2a	525,5b	434,9a	522,8b
Vitisin A	0,83c	0,44b	0,29a	0,43a	0,61b	0,59b	0,44a
Vitisin B	3,12	4,37	3,39	1,34a	5,91b	3,54	3,71
Vitisins	3,94	4,80	3,68	1,77a	6,52b	4,14	4,15

[a]For each parameter, different letters (a-c) indicate significant differences between types of wine, cultivars or vintages at the 95% confidence level.
[b]Nomenclature abbreviations: Dp, delphinidin; Cn, cyanidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; glc, glucoside; acglc, acetylglucoside; cmglc, p-coumaroylglucoside; cfglc, caffeoylglucoside.

abundant anthocyanins in wines from both grape varieties (Tempranillo and Graciano), accounting for around the 65 % of the total anthocyanin content. The comparison between the two grape varieties showed that Tempranillo wines were characterized by a higher amount of petunidin-type anthocyanins, while Graciano showed a higher concentration of peonidin-type anthocyanins, which is in agreement with previous studies (Portu, López, Santamaría, et al., 2018). In addition, wines made from Graciano grape variety had higher anthocyanin content than those made from Tempranillo, both acylated and non-acylated, as well as higher amount of pyranoanthocyanins. Moreover, Graciano anthocyanin composition was characterized by a higher degree of acylation (30 %) when compared to Tempranillo (20 %). Comparing the two vintages, 2020 had higher amount of anthocyanins than 2019.

As it can be seen from Table 2, wines made by CW were characterized by higher amounts of anthocyanins in comparison with wines made by CM (both free-run and press wines), in accordance with the results obtained by spectrophotometric determinations (Table 1). In this respect, wines made by CW showed significantly higher amounts of non-acylated and acylated anthocyanins (both coumaroylated and acetylated). Moreover, the sum of all types of anthocyanins (delphinidins, cyanidins, petunidins, peonidins and malvidins) was greater in CW wines compared to CM wines. Comparing the fractions obtained by CM, free-run wine was richer in all the anthocyanin compounds than press wine. Regarding pyranoanthocyanins, differences were not so noticeable and total pyranoanthocyanins amount remained similar between the three kind of wines.

Therefore, our results suggest that vinification by CM led to lower amount of anthocyanins and less-colored wines when compared to CW, in accordance with previous studies that showed that CM wine is usually less colored. In this respect, several authors (Gómez-Míguez & Heredia, 2004; Spranger et al., 2004; Sun et al., 2001) found that carbonic maceration led to wines with lower anthocyanin content. Castillo-Sánchez et al. (2006) also found lower amount of anthocyanins in CM wines immediately following vinification. However, the latter authors observed that this kind of wine underwent less color degradation than traditional fermentation on skins, so differences were negligible over time.

In contrast, a few studies slightly differ from the previous works. González-Lázaro et al. (2020) studied the effect of CM on red sparkling wines of Tempranillo grape variety. In this case, press wine obtained by CM showed less amount of non-acylated anthocyanins but, in contrast, coumaryl-glucoside anthocyanins were higher. Partly in agreement with this study, González-Arenzana et al. (2020) observed higher amounts of total coumaroylated and total acylated anthocyanins in CM commercial wines obtained in the D.O.Ca. Rioja region. However, regarding the latter work, it should be noted that it was performed with commercial CM wines, so press and free-run fractions were probably mixed.

Regarding pyranoanthocyanins, it could be surprising that, despite the huge differences on wine monomeric anthocyanins, no differences were observed between wines in total pyranoanthocyanin content. Despite the fact that wine made by CW showed higher amount of vitisin A, no differences were observed in vitisin B. This result could be explained by the higher amount of acetaldehyde coming from anaerobic metabolism, which has been suggested as a specific feature of wines produced by CM, and could result in a greater proportion of B-type pyranoanthocyanins (Chinnici et al., 2009). In agreement with this, González-Arenzana et al. (2020) found that the concentrations of vitisins A and B were considerably increased by CM when they analyzed commercial CM and CW wines from D.O.Ca. Rioja of the same vintage (2017).

Table 3
Multivariate analyses of variance of wine flavonols (mg/L) between types of wine (conventional winemaking wine (CW) and carbonic maceration free-run and press wines) and cultivars (Tempranillo and Graciano) and vintages.

	Type of wine			Cultivar		Vintage	
	CW	Free-run	Press	Tempranillo	Graciano	2019	2020
M−3−gal	0,08c	0,03a	0,06b	0.10b	0.02a	0.07b	0.05a
M−3−glc	1,28b	0,62a	1,15b	1.61b	0.43a	1.13b	0.90a
M−3−glcU	4,27c	1,43a	2,08b	3.56b	1.63a	3.07b	2.11a
Myricetin	5,35c	3,20b	1,61b	3.14a	3.63b	1.33a	5.44b
∑ myricetins	10,97b	5,28a	4,90a	8.40b	5.70a	5.60a	8.50b
Q-3-gal	0,40c	0,18a	0,32b	0.58b	0.03a	0.30	0.30
Q-3-glc	0,28a	0,17a	0,62b	0.71b	0.00a	0.54b	0.17a
Q-3-glcU	8,53c	3,62a	5,24b	6.45b	5.14a	6.47b	5.12a
Q-3-rut	0,08b	0,03a	0,03a	0.09b	0.00a	0.05	0.05
Quercetin	1,53c	1,16b	0,58a	0.54a	1.64b	0.31a	1.88b
∑ quercetins	10,83c	5.16a	6.80b	8.38b	6.81a	7.67	7.52
L-3-gal	0.03b	0.01a	0.03b	0.03b	0.02a	0.04b	0.01a
L-3-glc	1.55b	0.67a	1.60b	1.87b	0.68a	1.09a	1.46b
Laricitrin	0.43c	0.23b	0.12a	0.12a	0.41b	0.18a	0.35b
∑ laricitrins	2.01c	0.91a	1.75b	2.02b	1.10a	1.30a	1.81b
K-3-gal	0.07b	0.03a	0.09b	0.13b	0.00a	0.07b	0.05a
K-3-glcU	0.12	0.12	0.11	0.20b	0.03a	0.11a	0.12b
K-3-rut	0.02b	0.01a	0.01a	0.02b	0.01a	0.00a	0.02b
Kaempferol	0.05b	0.03a	0.01a	0.01a	0.05b	0.00a	0.06b
∑ kaempferols	0.26b	0.20a	0.24ab	0.38b	0.09a	0.20a	0.26b
I-3-gal	0.02b	0.00a	0.03c	0.03b	0.01a	0.02	0.02
I-3-glc	0.17b	0.06a	0.31c	0.31b	0.05a	0.21b	0.15a
I-3-glcU	0.05b	0.02a	0.04b	0.02a	0.06b	0.03a	0.05b
I-3-rut	0.59c	0.44b	0.34a	0.68b	0.23a	0.06a	0.85b
Isorhamnetin	0.01b	0.01a	0.00a	0.00a	0.01b	0.00a	0.01b
∑ isorhamnetins	0.83c	0.53a	0.72b	1.02b	0.37a	0.32a	1.07b
S-3-gal	0.02c	0.01a	0.02b	0.00a	0.03b	0.04b	0.00a
S-3-glc	4.30b	3.20a	3.25a	1.98a	5.18b	2.95a	4.21b
Syringetin	0.20b	0.14a	0.13a	0.13a	0.18b	0.10a	0.21b
∑ syringetins	4.52b	3.35a	3.39a	2.12a	5.39b	3.08a	4.42b
Total flavonols	29.43c	15.42a	17.80b	22.30b	19.46a	18.18a	23.59b

[a]For each parameter, different letters (a-c) indicate significant differences between types of wine, cultivars or vintages at the 95% confidence level.
[b]Nomenclature abbreviations: M, myricetin, Q, quercetin; L, laricitrin; K, kaempferol; I, isorhamnetin; S, syringetin; glcU, glucuronide; gal, galactoside; glc, glucoside; rut, rutinoside.

3.3. Wine flavonol composition

Results from the UHPLC analysis of detailed wine flavonol composition are outlined in Table 3. Comparing both grape varieties, Tempranillo wines showed higher amount of this kind of phenolic compounds, in particular regarding myricetin-, quercetin-, laricitrin-, kaempferol- and isorhamnetin-type flavonols. Only syringetin-type flavonols were found in greater amount in wines made from Graciano. In both grape varieties, myricetin- and quercetin-type flavonols were the most abundant, accounting together for 75 % of total flavonol content in Tempranillo and 65 % in Graciano. Finally, as for the vintages, 2020 was characterized by higher amounts of flavanols than 2019 as it was seen for anthocyanins.

Comparing the three types of wine, as seen for anthocyanins, wines made by CW were richer in this kind of compounds. In this respect, CW wines showed the highest amount of flavonols and higher values of the sum of all types of flavonols. Comparing the two fractions obtained by CM, press wine had in general higher amount of flavonols than free-run wine, especially regarding quercetin-, laricitrin- and isorhamnetin-type flavonols.

Up to now, far too little attention has been paid to the effect of CM on wine flavonol composition. In this respect, we have only found two studies that addressed this question (González-Lázaro et al., 2020; Pellegrini et al., 2000). Pellegrini et al. (2000) only identified three flavonols compounds: free-quercetin and myricetin, which were higher in CW wine, as well as quercetin-3-*O*-glucuronide, which was observed in CM wine. However, the generalizability of these results is subject to certain limitations since there were only two replicates and it was conducted just at one vintage. Recently, González-Lázaro et al. (2020) provided new insights into the effect of CM on flavonols compounds. These authors found a lower content of total flavonols in red sparkling CM wines when compared to CW, although they did not show results on the detailed flavonol composition.

Despite only two works were found in the literature, our results seem to be in agreement with them in the sense that CM decreases flavonol content when compared to CW, especially regarding the fraction obtained by free-run.

3.4. Wine flavanol composition

Results from the UHPLC analysis of wine flavanol compounds are shown in Table 4. Catechin was the compound found in highest concentration in wine samples. Comparing the two grape varieties, wines made from Graciano grape variety showed a higher concentration of this type of compounds. In addition, 2020 had higher flavanols than 2019.

The free-run wine obtained by CM led to highest content of catechin, gallocatechin, and procyanidins B1 and B3. Wines obtained by CW led to the highest content of epicatechin, epigallocatechin, and procyanidins B2 and T. Press wine obtained by CM had the lowest amount of every compound when compared to the other two types of wine. As for the total content of flavanol compounds, CM free-run wine had the highest amount of these compounds while CM press wine had the lowest.

The bibliography has shown contradictory results regarding the influence of CM on this family of phenolic compounds. On the one hand, most of authors have shown that CM decreases flavanol monomers concentration when compared to CW (Castillo-Sánchez et al., 2006; González-Lázaro et al., 2020; Pellegrini et al., 2000). On the other hand, Sun et al. (2001) observed that catechin and nongalloylated procyanidin contents in CM wine were much higher than in CW wine, since grape stems are an important source of both monomeric and polymeric

Table 4
Multivariate analyses of variance of wine flavanols, phenolic acids and stilbenes (mg/L) between types of wine (conventional winemaking wine (CW) and carbonic maceration free-run and press wines) and cultivars (Tempranillo and Graciano) and vintages.

	Type of wine			Cultivar		Vintage	
	CW	Free-run	Press	Tempranillo	Graciano	2019	2020
Flavanols							
Catechin	12,79b	20,58c	9,25a	11,39a	17,03b	9,27a	19,15b
Epicatechin	8,18c	4,51b	2,91a	2,95a	7,45b	3,76a	6,64b
Gallocatechin	4,10b	5,30c	3,07a	3,60a	4,71b	2,95a	5,36b
Epigallocatechin	3,68c	2,79b	2,23a	2,20a	3,61b	3,11b	2,69a
Procyanidin B1	9,52b	13,84c	7,18a	7,84a	12,52b	11,16b	9,20a
Procyanidin B2	5,25c	2,58b	1,43a	1,80a	4,37b	3,56b	2,61a
Procyanidin B3	1,08b	1,44c	0,67a	0,91a	1,22b	0,99a	1,13b
Procyanidin T	0,06c	0,04b	0,02a	0,03	0,04	0,04	0,04
Total	44,69b	51,08c	26,80a	30,74a	50,97b	34,86a	46,85b
Hydroxybenzoic acid							
Gallic acid	16,28c	13,59b	7,43a	10,66a	14,21b	14,09b	10,78a
Hydroxycinnamic acids							
Caftaric acid	23,22b	29,09c	16,77a	25,08b	20,97a	30,57b	15,48a
Coutaric acid	24,84b	28,05c	16,67a	29,67b	16,70a	23,87	22,50
Fertaric acid	2,33b	2,78c	1,91a	2,67b	2,01a	1,92a	2,76b
Caffeic acid	0,95	0,93	1,47	1,64b	0,59a	0,52a	1,71b
p-Coumaric acid	6,52	9,94	6,32	6,98	8,20	4,57a	10,62b
Ferulic acid	0,12	0,14	0,15	0,16b	0,11a	0,12a	0,15b
Total	57,98b	70,92c	43,28a	66,2b	48,60a	61,56b	53,22a
Stilbenes							
trans+*cis*-Resveratrol	2,61b	1,17a	3,39c	1,11a	3,67b	1,20a	3,58b
trans+*cis*-Piceid	19,52b	13,49a	17,49ab	6,88a	26,78b	22,58b	11,09a
ε-Viniferin	0,31c	0,17b	0,12a	0,06a	0,34b	0,07a	0,33b
Ω-Viniferin	0,19c	0,11b	0,06a	0,07a	0,18b	0,09a	0,16b
Piceatannol	0,24b	0,18a	0,13a	0,06a	0,31b	0,05a	0,31b
Astringin	0,76b	0,50a	1,12c	0,47a	1,12b	1,31b	0,28a
Total	23,63b	15,61a	22,32b	8,65a	32,40b	25,30b	15,74a

[a]For each parameter, different letters (a-c) indicate significant differences between types of wine, cultivars or vintages at the 95% confidence level.

flavanols. Spranger et al. (2004) observed higher content of catechin in CM wine, but lower contents of epicatechin and non-galloylated procyanidins.

Therefore, based on the bibliography, CM generally decreases flavanol compounds content, but those found in great amounts in grape stems, like catechin (Souquet et al., 2000), could be increased depending on variables such as variety, ethanol concentration or grape maturity (González-Lázaro et al., 2020). Our results, based on two vintages and two different grape varieties, suggest that flavanol concentration depends on the CM fraction, so free-run CM wine is richer in these compounds than press CM wine, which especially lacks these compounds. This is probably due to the fact that press CM wine is not in contact with grape stems due to the intracellular fermentation, and that maceration occurs with low presence of alcohol.

3.5. Wine non-flavonoid composition

Table 4 shows the results of the analysis of non-flavonoid compounds. Overall, Tempranillo grape variety was characterized by a higher content of hydroxycinnamic acids and lower content of stilbenes than Graciano, which correlates well with previous references (Portu, López, Ewald, et al., 2018). In this respect, it is noteworthy that Graciano is a potential source of stilbene compounds, with potential antioxidant properties. When comparing both vintages, 2019 had in general higher concentration of total non-flavonoid compounds than 2020, which is in contrast to the results shown for flavonoids (i.e. anthocyanins, flavonols and flavanols).

Significant differences were found between the three types of wine regarding the tartaric esters of hydroxycinnamic acids, while no differences were observed in the non-esterified compounds. CM free-run wine was the most abundant in these compounds, followed by CW wine, while CM press wine had the lowest content of hydroxycinnamic acids. Therefore, the behavior was similar to the one described for flavanols and it is probably caused by the fact that free-run wine had more contact with grape stems (Souquet et al., 2000).

We have found no references on the effect of CM on hydroxycinnamic acid composition in red wines, but there is a previous study that evaluated CM in red sparkling wine made from Tempranillo premature grapes (González-Lázaro et al., 2020), finding that CM increased the total content of hydroxycinnamic acids. In addition, Gao et al. (2012) found that non-flavonoids compounds, including caffeic, coumaric, and ferulic acid, were found in higher contents in CM wines of blackberry. These previous studies suggest that CM could favor the extraction of hydrocynnamic acids during vinification. Our results, however, show that this fact depends on the fraction obtained by CM and on the specific compound, affecting mainly the tartrate esters.

Evaluating CM process on wine stilbene composition (Table 4), it can be observed that CW wine had the highest content of piceid, viniferins, and piceatannol, while CM press wine had the highest content of resveratrol and astringin. CM free-run wine showed the lowest amount of total stilbenes. We have only found one study (Clare et al., 2004) that investigated the influence of CM on the concentration of *cis*- and *trans*-resveratrol, and resveratrol glucoside isomers in Cabernet Sauvignon, finding that CM resulted in no detectable levels of stilbenes when compared to CW. According to our results, stilbene content depends on the fraction obtained by CM, so CM press wine is more abundant in these compounds when compared to CM free-run wine, although it is similar in the total amount when compared to CW.

3.6. Canonical discriminant analysis on wine monomeric phenolic composition

To classify the wines produced by the two types of vinification (CW and CM), a discriminant analysis was performed on the basis of their monomeric phenolic composition (Fig. 1). Function 1 explained 56.5 % of variance while function 2 explained 43.5 % of variance. Therefore, cumulative variance explained by the two functions was 100 %. The variables that contributed mostly to function 1 were petunidin-3-*O*-acetylglucoside, procyanidin B2, and quercetin-3-*O*-rutinoside, while malvidin-3-*O*-*trans*-coumaroyl-glucoside and procyanidin B3 contributed negatively. To function 2, isorhamnetin-3-*O*-glucoside, epigallocatechin, malvidin-3-*O*-*trans*-coumaroyl-glucoside contributed positively, while delphinidin-3-*O*-glucoside and procyanidin B3 contributed negatively. The two discriminant functions separated perfectly the wine samples and correctly classified 100 % of the samples (n = 36). In this sense, function 1 separated CW wine from wines made by CM, while function 2 clearly separated CM press wine from CM free-run wine.

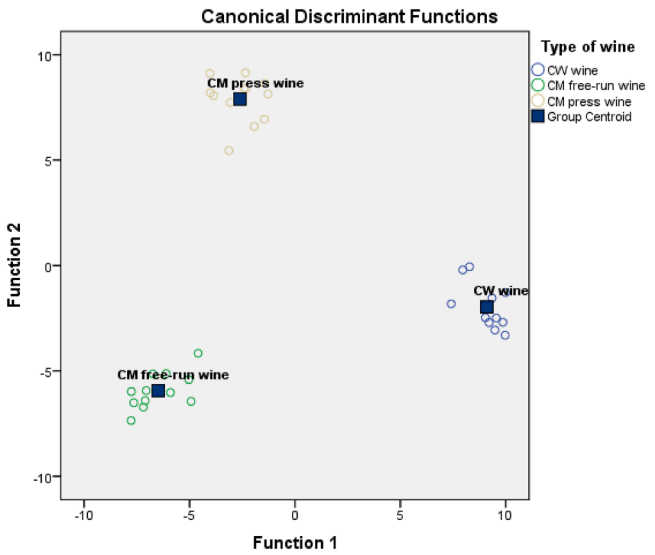

Fig. 1. Classification by canonical discriminant analysis of wine made by conventional winemaking (CW wine) and wines made by carbonic maceration (CM free-run wine and CM press wine) on the basis of their monomeric phenolic composition.

4. Conclusions

To our knowledge, the present study is the first report on the characterization of the detailed phenolic composition and color-related parameters of CM wines considering multiple variables (vintages and two red grape varieties: Tempranillo and Graciano). The results showed that there are important differences between wines made by CW and those made by CM, which in addition showed big differences between press and free-run wines. Therefore, wines made by CM showed lower concentrations of phenolic compounds and were less colored than CW wine. Regarding their monomeric phenolic composition, CM wines were characterized by low contents of anthocyanins and flavonols, resulting in poor-colored wines. However, free-run wine obtained by CM showed the greatest content in flavanols and hydroxycinnamic acids, and the lowest in stilbenes, while CM press wine showed the opposite trend. Moreover, wines were clearly discriminated according to their phenolic composition. The significant differences on wine phenolic composition could have important implications on wine sensorial properties and wine aging potential.

CRediT authorship contribution statement

Javier Portu: Conceptualization, Investigation. **Ana Rosa Gutiérrez-Viguera:** Conceptualization, Investigation. **Lucía González-Arenzana:** Investigation. **Pilar Santamaria:** Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ayestarán, B., Martínez-Lapuente, L., Guadalupe, Z., Canals, C., Adell, E., & Vilanova, M. (2019). Effect of the winemaking process on the volatile composition and aromatic profile of Tempranillo Blanco wines. *Food Chemistry, 276*, 187–194. https://doi.org/10.1016/j.foodchem.2018.10.013

Boulton, R. B. (1996). Methods for the assessment of copigmentation in red wines. *47Th Annual Meeting of the American Society for Enology and Viticulture*.

Castillo-Sánchez, J. J., Mejuto, J. C., Garrido, J., & García-Falcón, S. (2006). Influence of wine-making protocol and fining agents on the evolution of the anthocyanin content, colour and general organoleptic quality of Vinhão wines. *Food Chemistry, 97*(1), 130–136. https://doi.org/10.1016/j.foodchem.2005.03.030

Chinnici, F., Sonni, F., Natali, N., Galassi, S., & Riponi, C. (2009). Colour features and pigment composition of Italian carbonic macerated red wines. *Food Chemistry*, *113* (2), 651–657. Scopus. 10.1016/j.foodchem.2008.07.055.

Clare, S. S., Skurray, G., & Shalliker, R. A. (2004). Effect of pomace-contacting method on the concentration of cis- and trans-resveratrol and resveratrol glucoside isomers in wine. *American Journal of Enology and Viticulture, 55*(4), 401–406.

Ferrero del Teso, S., Suárez, A., Ferreira, C., Perenzoni, D., Arapitsas, P., Mattivi, F., … Sáenz-Navajas, M.-P. (2022). Modeling grape taste and mouthfeel from chemical composition. *Food Chemistry, 371*. https://doi.org/10.1016/j.foodchem.2021.131168

Flanzy, C., Flanzy, M., & Benard, P. (1987). La Vinification par Maceration Carbonique, *INRA*.

Gao, J., Xi, Z., Zhang, J., Guo, Z., Chen, T., Fang, Y., … Liu, J. (2012). Influence of Fermentation Method on Phenolics, Antioxidant Capacity, and Volatiles in Blackberry Wines. *Analytical Letters, 45*(17), 2603–2622. https://doi.org/10.1080/00032719.2012.696223

Gómez-Míguez, M., & Heredia, F. J. (2004). Effect of the maceration technique on the relationships between anthocyanin composition and objective color of Syrah wines. *Journal of Agricultural and Food Chemistry, 52*(16), 5117–5123. https://doi.org/10.1021/jf049570z

González-Arenzana, L., Santamaría, R., Escribano-Viana, R., Portu, J., Garijo, P., López-Alfaro, I., … Gutiérrez, A. R. (2020). Influence of the carbonic maceration winemaking method on the physicochemical, colour, aromatic and microbiological features of tempranillo red wines. *Food Chemistry, 319*. https://doi.org/10.1016/j.foodchem.2020.126569

González-Lázaro, M., Martínez-Lapuente, L., Guadalupe, Z., Ayestaran, B., Bueno-Herrera, M., López de la Cuesta, P., & Pérez-Magariño, S. (2020). Evaluation of grape ripeness, carbonic maceration and pectolytic enzymes to improve the chemical and sensory quality of red sparkling wines. *Journal of the Science of Food and Agriculture, 100*(6), 2618–2629. https://doi.org/10.1002/jsfa.10291

Gutiérrez-Escobar, R., Fernández-Marín, M. I., Richard, T., Fernández-Morales, A., Carbú, M., Cebrian-Tarancón, C., … Cantos-Villar, E. (2021). Development and characterization of a pure stilbene extract from grapevine shoots for use as a preservative in wine. *Food Control, 121*. https://doi.org/10.1016/j.foodcont.2020.107684

Heras-Roger, J., Alonso-Alonso, O., Gallo-Montesdeoca, A., Díaz-Romero, C., & Darias-Martín, J. (2016). Influence of copigmentation and phenolic composition on wine color. *Journal of Food Science and Technology, 53*(6), 2540–2547. https://doi.org/10.1007/s13197-016-2210-3

International Organisation of Vine and Wine. (2020). *Compendium of International Methods of Wine and Must Analysis*. OIV.

Jackson, R. S. (2008). Specific and Distinctive Wine Styles. In R. S. Jackson (Ed.), *Wine Science: Principles and Applications* (pp. 520–576). Academic Press.

Nash, V., Ranadheera, C. S., Georgousopoulou, E. N., Mellor, D. D., Panagiotakos, D. B., McKune, A. J., … Naumovski, N. (2018). The effects of grape and red wine polyphenols on gut microbiota – A systematic review. *Food Research International, 113*, 277–287. https://doi.org/10.1016/j.foodres.2018.07.019

Nemzer, B., Kalita, D., Yashin, A. Y., & Yashin, Y. I. (2022). Chemical Composition and Polyphenolic Compounds of Red Wines: Their Antioxidant Activities and Effects on Human Health—A Review. *Beverages, 8*(1). https://doi.org/10.3390/beverages8010001

Nixdorf, S. L., & Hermosín-Gutiérrez, I. (2010). Brazilian red wines made from the hybrid grape cultivar Isabel: Phenolic composition and antioxidant capacity. *Analytica Chimica Acta, 659*(1–2), 208–215. https://doi.org/10.1016/j.aca.2009.11.058

Pace, C., Giacosa, S., Torchio, F., Río Segade, S., Cagnasso, E., & Rolle, L. (2014). Extraction kinetics of anthocyanins from skin to pulp during carbonic maceration of winegrape berries with different ripeness levels. *Food Chemistry, 165*, 77–84. https://doi.org/10.1016/j.foodchem.2014.05.070

Pellegrini, N., Simonetti, P., Gardana, C., Brenna, O., Brighenti, F., & Pietta, P. (2000). Polyphenol content and total antioxidant activity of Vini novelli (young red wines). *Journal of Agricultural and Food Chemistry, 48*(3), 732–735. https://doi.org/10.1021/jf990251v

Portu, J., López, R., Ewald, P., Santamaría, P., Winterhalter, P., & Garde-Cerdán, T. (2018). Evaluation of Grenache, Graciano and Tempranillo grape stilbene content after field applications of elicitors and nitrogen compounds. *Journal of the Science of Food and Agriculture, 98*(5). https://doi.org/10.1002/jsfa.8662

Portu, J., López, R., Santamaría, P., & Garde-Cerdán, T. (2018). Methyl jasmonate treatment to increase grape and wine phenolic content in Tempranillo and Graciano varieties during two growing seasons. *Scientia Horticulturae, 240*. https://doi.org/10.1016/j.scienta.2018.06.019

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2006). *Handbook of Enology* (Vol. 1, 2nd ed.). John Wiley & Sons, Ltd (Chapter 12).

Ribéreau-Gayon, P., & Stonestreet, E. (1965). Determination of anthocyanins in red wine. *Bulletin de la Societe Chimique de France, 9*, 2649–2652.

Ruiz, M. (1999). *La Crianza del Vino Tinto desde la Perspectiva Vitícola*. AMV Ediciones.

Sacchi, K. L., Bisson, L. F., & Adams, D. O. (2005). A review of the effect of winemaking techniques on phenolic extraction in red wines. *American Journal of Enology and Viticulture, 56*(3), 197–206.

Sáenz-Navajas, M.-P., Tao, Y.-S., Dizy, M., Ferreira, V., & Fernández-Zurbano, P. (2010). Relationship between nonvolatile composition and sensory properties of premium Spanish red wines and their correlation to quality perception. *Journal of Agricultural and Food Chemistry, 58*(23), 12407–12416. https://doi.org/10.1021/jf102546f

Santamaría, P., González-Arenzana, L., Escribano-Viana, R., Garijo, P., López, R., Sanz, S., & Gutiérrez, A. R. (2022). Difficulties Associated with Small-Scale Production of Carbonic Maceration Wines. *Fermentation, 8*(1). https://doi.org/10.3390/fermentation8010027

Souquet, J.-M., Labarbe, B., Le Guernevé, C., Cheynier, V., & Moutounet, M. (2000). Phenolic composition of grape stems. *Journal of Agricultural and Food Chemistry, 48* (4), 1076–1080. https://doi.org/10.1021/jf991171u

Spranger, M. I., Clímaco, M. C., Sun, B., Eiriz, N., Fortunato, C., Nunes, A., … Belchior, A. P. (2004). Differentiation of red winemaking technologies by phenolic and volatile composition. *Analytica Chimica Acta, 513*(1), 151–161. https://doi.org/10.1016/j.aca.2004.01.023

Sun, B., Spranger, I., Roque-do-Vale, F., Leandro, C., & Belchior, P. (2001). Effect of different winemaking technologies on phenolic composition in Tinta Miúda red wines. *Journal of Agricultural and Food Chemistry, 49*(12), 5809–5816. https://doi.org/10.1021/jf010661v

Tesniere, C., & Flanzy, C. (2011). Chapter 1 - Carbonic Maceration Wines: Characteristics and Winemaking Process. In R. S. Jackson (Ed.), *Advances in Food and Nutrition Research* (Vol. 63, pp. 1–15). Academic Press.

Zhang, Y.-S., Du, G., Gao, Y.-T., Wang, L.-W., Meng, D., Li, B.-J., … Guan, W.-Q. (2019). The effect of carbonic maceration during winemaking on the color, aroma and sensory properties of 'Muscat Hamburg' wine. *Molecules, 24*(17). https://doi.org/10.3390/molecules24173120