

## Phenolic Content of Blends of Tempranillo with Graciano or Cabernet Sauvignon Wines Produced in Spain

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### Summary

The effect of Graciano (GRA, Spanish valuable variety of limited production in Mediterranean countries) *vs.* Cabernet Sauvignon (CS, world-wide known French variety) on the phenolic content [total polyphenols (TP), total anthocyanins (TA), catechins (CAT) and proanthocyanidins (PRO)] of Tempranillo wines (TEM-BASE, a largely cultivated Spanish variety) was studied in blends prepared with 25 and 10 % of each variety after 4, 6, 9, 16.5 and 23 months of bottle ageing. Significant differences among wines (blends and base wine) according to the »blend« factor were observed for CAT and TA. Besides, although the evolution trend during wine ageing of different families of phenolic compounds studied was similar in the blends and base wine, different blends presented a faster anthocyanin disappearance kinetics than the base wine, probably due to their higher CAT content, which may favour the progress of certain anthocyanin condensation reactions during ageing in the bottle. This effect was slightly more pronounced in the TEM-GRA blends than in the TEM-CS ones. A further study of the phenolic composition of the monovarietal wines used for blending, as well as of the grapes (skins and seeds) from which these wines were elaborated, revealed that the blending effect on CAT could be associated with higher concentration of these compounds in Graciano and Cabernet Sauvignon grape seeds in comparison with Tempranillo. Finally, the findings of this work scientifically confirm that, in terms of the phenolic content, Graciano wines possess properties similar to Cabernet Sauvignon for blending with Tempranillo.

*Key words:* Graciano, Cabernet Sauvignon, Tempranillo, grape seeds, grape skins, wine blends, phenolic compounds, bottle ageing

### Introduction

The practice known as wine blending or »courage« is one of the most common steps but least studied aspects of the winemaking process. Wines are blended for improving their colour, taste, alcohol content, body or aroma, with the final aim of enhancing the product quality. Another objective of blending is to optimize the use of certain grape varieties and the final production costs (1,2). Most of the studies found in the literature related to wine blending are based on meeting certain desired sensorial characteristics mainly related to wine aroma compounds (3–5). Although enologists have learned

through years of work which grape varieties render the best blends, there is very little scientific information supporting this empiric knowledge.

Grape phenolic compounds contribute to the wine colour, flavour, astringency and bitterness (6–8), and are also responsible for the ageing behaviour of red wines (9,10). During red wine fermentation, phenolic compounds are transferred from the solid parts of the grapes (skins and seeds) into the wine (11). The most important factors influencing the concentration of these compounds in wine are the grape variety, the winemaking technology, and the maturation and ageing conditions. These

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factors also determine the pH of the wine. It is known that an acidic pH assures a higher proportion of anthocyanins in flavylum form and, therefore, a higher amount of highly reactive anthocyanins in electrophilic form. In addition, an acidic pH is highly important for promoting the C-C bond cleavage of procyanidins from which intermediate-sized carbocations are liberated to participate in anthocyanin-flavanol and flavanol-flavanol direct condensation reactions.

The study of grape varieties that could result in varietal and blended wines with special and original quality attributes is becoming of great interest. The Spanish *Vitis vinifera* L. cv. Graciano is a variety that has a very limited production (0.02 % of the total grape varieties grown) concentrated mainly in Navarra and La Rioja regions, but is highly appreciated for providing distinctive elements (freshness, aroma and colour) to the blending of wines, specially of Tempranillo, a widely cultivated Spanish variety used for monovarietal and blended wines. However, there is no scientific information supporting the blending attributes of Graciano, particularly related to the phenolic composition and therefore to the colour, in comparison with other well known varieties (*i.e.* Cabernet Sauvignon). In addition, considering that phenolic compounds, in particular anthocyanins and flavanols, participate in numerous chemical reactions during wine ageing, leading to colour and astringency changes (9,12–19), little is known about the changes that these compounds undergo in wine blends during ageing in the bottle (commercial life) with respect to those of the original base wines.

Although HPLC-DAD is usually employed for the detection and quantification of phenolic compounds, this technique is not affordable in wineries for routine polyphenol analysis. Classic polyphenol methods based on global spectrophotometric determinations are more practical for the control of vinification process and usually provide the information that wineries need to make decisions concerning product quality and process improvements (20–24). The aim of the present work was to evaluate the effect of Graciano (GRA) *vs.* Cabernet Sauvignon (CS) wines on the phenolic content of Tempranillo (TEM) blends during the period from 4 to 23 months of bottle ageing that covers their commercial life, using analytical methods usually performed in wineries. In order to understand possible effects of blending with each varietal wine (modifier), the phenolic composition of the monovarietal wines, as well as the grapes (skins and seeds) from which these wines were elaborated, was also studied.

## Materials and Methods

### Materials

Vanillin and Folin-Ciocalteu reagent were purchased from Merck (Darmstadt, Germany). Malvidin-3-glucoside and cyanidin were obtained from Extrasynthèse (Genay, France). (+)-Catechin and gallic acid were purchased from Sigma (USA). HPLC-grade acetone, methanol, acetic and formic acids were obtained from Fisher Scientific (Houston, TX, USA).

### Wines

#### Monovarietal wines

Monovarietal young red wines made from grapes of *Vitis vinifera* cv. Tempranillo, Graciano and Cabernet Sauvignon grown in the same geographical area (Olite, Spain) and harvested at their technological maturity (vintage 2000) were used for this study. The values corresponding to some characteristics of different grape varieties are presented in Table 1. Wines were elaborated at the Viticulture and Enology Station of Navarra (EVENA, Olite, Spain) as follows: 220 kg of grapes of each variety

Table 1. Vineyard yield and classical parameters determined in grapes and young wines

	Tempranillo	Graciano	Cabernet Sauvignon
<b>Grapes</b>			
Vineyard yield/(kg/ha)	8815	5093	6730
pH	3.9	3.6	3.7
sugar/°Brix	21.1	24.3	21.9
<b>Wines</b>			
pH	4.3	3.5	3.6
Alcohol/%	13.2	13.7	12.8
Density/(g/L)	0.993	0.990	0.992
Volatile acidity/(g/L)	0.34	0.19	0.18
Total acidity/(g/L)	4.0	5.7	5.9
Total dried extract/(g/L)	30.4	25.9	28.1

were destemmed, crushed and collected into 200-litre stainless-steel wine vats. Semi-industrial scaled fermentations were performed with a yeast inoculum of 25 g/hL (80 % EVENA *Saccharomyces cerevisiae* Na33 yeast strain and 20 % Lallemand *Saccharomyces bayanus* EC118 yeast strain) at a temperature up to 27 °C. The cap was punched down twice a day until it remained submerged during a 14-day maceration period. At the end of alcoholic fermentation, the wines were racked and stabilized for a period of one month at –2 °C. The values corresponding to some classical wine parameters of the young wines are shown in Table 1.

#### Wine blends

Four wine blends were prepared taking Tempranillo (TEM) as the base wine, and Graciano (GRA) and Cabernet Sauvignon (CS) wines as modifiers in volume proportions of 10 and 25 % each (TEM-GRA 90:10 and 75:25, TEM-CS 90:10 and 75:25). The wines were then filtered through SEITZ K250 filters (2.5–3.0 µm) (Sert Schenk Filter System GmbH, Bad Krevznach, Germany) and finally bottled after correcting the free SO<sub>2</sub> level to 30 mg/L. The different blends and the base wine were analyzed after 4, 6, 9, 16.5 and 23 months of bottling and storage at 13 °C and 80–85 % relative humidity. At each ageing time, two bottles from each wine were combined, homogenized and then analyzed. Wine analyses were performed in duplicate and the mean value was used for statistical analysis.

## Grapes

Two groups of 100 berries from *Vitis vinifera* L. cv. Tempranillo, Graciano and Cabernet Sauvignon used for elaborating the wines were randomly selected from the collected samples (5 kg) of each variety. The solid parts of the grape, skins and seeds, were manually separated, lyophilized and frozen at  $-18\text{ }^{\circ}\text{C}$  under nitrogen for subsequent analysis.

## Extraction of phenolic compounds from the solid parts of the grape

### Skins

Anthocyanin extracts were prepared by maceration of 2.5 g of milled skins in 50 mL of methanol/formic acid (volume ratio of 95:5) for 12 h, the coloured liquid was separated from the solid matrix and replaced with fresh solvent twice. The three combined solutions were concentrated in a rotary evaporator up to 25 mL, avoiding temperatures higher than  $35\text{ }^{\circ}\text{C}$ . Distilled water was added to the sample up to 50 mL and kept at  $-18\text{ }^{\circ}\text{C}$  under nitrogen until analysis. Each extraction procedure was carried out in duplicate.

### Seeds

Grape seeds were ground using a coffee bean miller to a particle size of less than  $50\text{ }\mu\text{m}$ . Lipids from the ground grape seeds (0.25 g) were removed by extracting twice with 40 mL of hexane for 15 min each time. The lipid-free solid was air-dried and the procyanidins were extracted with 40 mL of acetone/water/acetic acid (70:29.5:0.5). The mixture was vortexed and sonicated for 30 min and left at room temperature for 30 min. The extract was then centrifuged at 3000 rpm for 15 min at  $20\text{ }^{\circ}\text{C}$ . The supernatant was filtered (Whatman No. 1) and concentrated to 5 mL using a rotary evaporator under partial vacuum at  $35\text{ }^{\circ}\text{C}$ . Samples were then freeze-dried. Extraction was performed in duplicate. For the phenolic determinations, 0.02 g of the freeze-dried sample was reconstituted in 1 mL of methanol/water (50:50).

## Global phenolic determinations

Extracts from grape skins and seeds, and monovarietal and blended wines were assayed for total polyphenols (TP), total anthocyanins (TA), catechins (CAT) and proanthocyanidins (PRO).

TP were determined using the method of Singleton and Rossi (25), which is based on the oxidation of the hydroxyl groups of phenols in basic media by the Folin-Ciocalteu reagent (mixture of phosphotungstic and phosphomolybdic acids of yellow colour). Briefly, 0.1 mL of the sample (wine or extract), 0.5 mL of Folin-Ciocalteu reagent and 10 mL of sodium carbonate solution (75 g/L) were mixed and the volume made up to 25 mL with distilled water. After 1 h, the absorbance was measured at 750 nm against a blank prepared in the same way but without the addition of the reagent. Gallic acid was used as standard to construct the calibration curve. Analysis was performed in duplicate.

CAT was determined by the method of Swain and Hillis (26). In this assay, monomeric flavanols, and oligomeric and polymeric proanthocyanidins (tannins) react

with vanillin in acidic medium to yield chromophores absorbing at 500 nm. Briefly, 0.1 mL of the sample (wine or extract) and 2 mL of vanillin (1 % in 70 % chloride acid) were mixed and the volume made up to 10 mL with 70 % chloride acid. After 25 min, the absorbance was measured at 500 nm against a blank prepared in the same way but without vanillin. (+)-Catechin was used as standard to construct the calibration curve. Analysis was performed in duplicate.

PRO was determined as described by Ribéreau-Gayon and Stonestreet (27). The method is based on the acid-catalyzed oxidative cleavage of the C-C interflavanic bond of proanthocyanidins in butanol-HCl (Bate-Smith method). Briefly, 0.2 mL of the sample (wine or extract) and 20 mL of butanol/HCl (50:50) (0.54 mM  $\text{FeSO}_4$ ) were incubated at  $90\text{ }^{\circ}\text{C}$  for 1 h. After cooling, the mixture volume was made up to 25 mL with butanol-HCl mixture, and the absorbance was measured at 550 nm against a blank prepared in the same way but without heating. Cyanidin was used as standard to construct the calibration curve. Analysis was performed in duplicate.

TA was determined as described by Paronetto (28). The method was based on the pH structural-dependent property of anthocyanins. The difference in absorbance measured at 525 nm when the pH is changed from 3.5 (phosphate-citrate buffer) to 0.1 (1 M chloride acid) was taken as a measure of total anthocyanins. Considering that oligomeric and polymeric pigments are more resistant to pH changes than monomeric anthocyanins, the TA determination mostly included free monomeric or simple anthocyanins. Malvidin-3-glucoside was used as standard to construct the calibration curve. Analysis was performed in duplicate.

## Sensorial analysis

The different blends and the base wine were evaluated by the trained taster panel of EVENA (Spain) at the end of the ageing period (23 months), following the Office International de la Vigne et du Vin (OIV) (29) sensorial analysis tasting cards and recommendations. Visual attributes (fluency, transparency, hue and vivacity), smell attributes (cleanliness, intensity, fineness and harmony), taste attributes (cleanliness and intensity), and taste-smell attributes (body, harmony, persistence and final sensation) were considered in the evaluation.

## Statistical analysis

ANOVA and linear regression analysis of the data were performed using the PC software package Statgraphics Plus 2.1 (Graphics Software Systems, Rockville, MD, USA).

## Results and Discussion

### Phenolic content of wine blends during ageing in the bottle

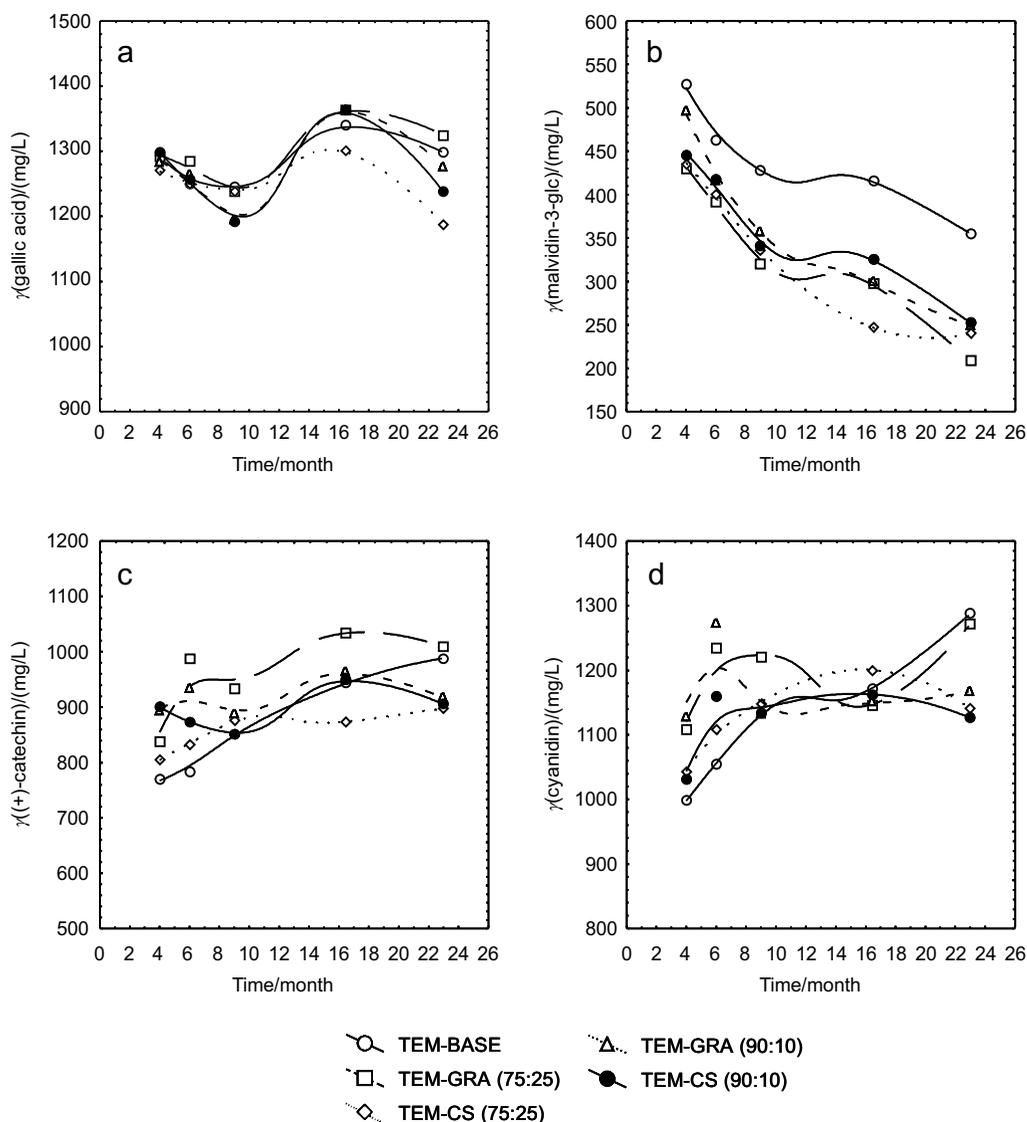
The effect of blending a monovarietal Tempranillo wine (base wine) with either Graciano or Cabernet Sauvignon wines on the content of total polyphenols (TP), total anthocyanins (TA), catechins (CAT) and proantho-

cyanidins (PRO) after 4, 6, 9, 16.5 and 23 months of ageing in the bottle is shown in Fig. 1.

A two-way ANOVA analysis indicated significant differences ( $p < 0.05$ ) among wines according to both time ( $p = 0.00$ ) and blend ( $p = 0.00$ ) factors for the content of TA and CAT. Results for these two determinations are discussed below. However, for TP and PRO significant differences ( $p < 0.05$ ) among wines were only presented in the function of the ageing time in the bottle, but not ( $p > 0.05$ ) in the function of the blend factor. TP registered an increase in concentration after 9 months of ageing followed by a decrease at 23 months of ageing in the bottle. These changes are possibly due to the transformation of phenolic compounds into condensed forms that possess slightly different chemical properties and reactivities towards the Folin-Ciocalteu reagent (25). In the case of PRO, their content increased, particularly during the first months of ageing, followed by a period (from 9 to 16.5 months) when fewer changes were registered. Considering the principle of this analytical me-

thod (the acid-catalysed oxidative cleavage of the C-C interflavanic bond of proanthocyanidins) (30), the overall profile of PRO could be the result of the formation of new phenolic condensation products and of the cleavage of polymeric proanthocyanidins during ageing, which, depending on their size and structural composition, could render different reaction yields with the Porter reagent (30).

In relation to TA, both TEM-GRA and TEM-CS (90:10 and 75:25, each) blends presented a lower TA concentration than the Tempranillo base wine (TEM) (Fig. 1b). During bottle ageing, a progressive decrease in the TA content of the different wines was registered. This behaviour is consistent with the participation of monomeric anthocyanins in numerous condensation reactions during bottle ageing, mainly including the direct or acetaldehyde-mediated condensation reactions with flavanols (9,12), as well as their combination with other small molecules possessing a polarizable double bond (*i.e.* pyruvic acid, acetaldehyde, vinylphenols and vinyl-



**Fig. 1.** Evolution of total phenols and of different groups of phenolic compounds during ageing in the bottle: (a) total polyphenols (TP), (b) total anthocyanins (TA), (c) catechins (CAT), (d) proanthocyanidins (PRO). The curves were fitted to the coordinate data according to the distance-weighted least square smoothing procedure

flavanols), leading to the so called pyranoanthocyanins (14,15,18,19,31–33). In addition, during wine ageing anthocyanins may also undergo hydrolytic and degradation reactions in minor extension (34). As reported by other authors (35–38), the anthocyanin decline followed the first order kinetic, which is defined by the equation  $\ln[A] = -kt + \ln[A]_0$ , where  $[A]$  is the pigment concentration (mg/L) and  $t$  is the time (months) of ageing in the bottle.

The reaction rate constant ( $k$ ) for TA was determined by calculating the slope of the curve  $\ln[A]$  vs.  $t$  by linear regression analysis. The reaction quarter-life ( $t_{1/4}$ ) and half-life ( $t_{1/2}$ ), corresponding to the time required for a 25 and a 50 % reduction of the initial anthocyanin concentration, respectively, were also calculated by the equation  $t_{1/x} = (\ln x - \ln(x-1))/k$ , being  $[A]_0/x$  the reduced concentration. The results of the kinetic study are presented in Table 2. Independently of the variety of the modifier (Graciano or Cabernet Sauvignon wine), anthocyanins in the blends presented an approximately 2-fold faster disappearance kinetic than in the base wine, as can be observed from the  $t_{1/4}$  and  $t_{1/2}$  values. The time required for a 25 % ( $t_{1/4}$ ) reduction of the initial anthocyanin concentration in the Tempranillo base wine was approximately of the order required for a 50 % ( $t_{1/2}$ ) reduction of the initial anthocyanin content in the blends (Table 2). The TEM-GRA blends presented slightly higher  $k$  values than the TEM-CS ones, especially in the case of the 90:10 blends. Finally, as expected from the kinetic data, losses in total anthocyanins registered after 23 months of ageing in the bottle were also higher for the TEM-GRA blends (52 % for the 75:25 and 50 % for the 90:10) than for the TEM-CS ones (45 % for the 75:25 one and 43 % for the 90:10) with the same proportion of modifier wine, being only 33 % in the base wine. Losses were slightly higher for the 75:25 blends than for the 90:10 ones, independently of the variety of the modifier wine employed.

Table 2. Dissappearance rate of total anthocyanins in Tempranillo base wine and in the different blends

	$t_{1/4}$ /month	$t_{1/2}$ /month	$k/(10^{-3}\cdot\text{month}^{-1})$	$R^2$
TEM-BASE	16.7	40.3	17.2	0.8797
TEM-GRA 90:10	8.7	20.9	33.2	0.9486
TEM-CS 90:10	10.5	25.3	27.4	0.9247
TEM-GRA 75:25	8.3	20.1	34.5	0.9407
TEM-CS 75:25	8.7	20.9	33.1	0.9213

$t_{1/4}$ : time required for a 25 % reduction of the initial anthocyanin concentration

$t_{1/2}$ : time required for a 50 % reduction of the initial anthocyanin concentration

$k$ : rate constant

Considering the CAT content, the blends presented higher values than the base wine, with the exception of TEM-CS (75:25) blend at 16.5 months, and of TEM-CS and TEM-GRA (90:10) blends at 23 months of ageing (Fig. 1c). A slight increase in the CAT concentration was registered during bottle ageing, particularly in the Tempranillo base wine. Since the vanillin reaction used for the CAT determination is specific of *meta*-dihydroxyph-

nyl moieties and thus of the free A-rings of monomeric flavanols and of the upper flavanol unit of proanthocyanidin oligomers and polymers (26), this evolution trend could be the result of the interflavanic bond cleavage of polymeric proanthocyanidins during ageing (13), generating smaller size molecules and thus increasing the concentration of available end units.

Finally, the wine blends and the base wine were evaluated by a trained taster panel at the end of the ageing period. The overall rating of the blends was higher than that of the base wine (67.2/100), in direct relation to the proportion of modifier wine employed: 77.2 for TEM-GRA (90:10); 77.2 for TEM-CS (90:10); 79.7 for TEM-GRA (75:25), and 89.5 for TEM-CS (75:25). Graciano was judged to make a substantial improvement in the colour and structure of Tempranillo base wine, whereas Cabernet Sauvignon was recognized as improving the colour of Tempranillo as well as its aromatic complexity and flavour.

#### *Phenolic content of monovarietal wines, and grape skins and seeds*

In order to explain lower TA and higher CAT contents of the blends in comparison with the base wine, different monovarietal wines as well as grapes (skins and seeds) from which these wines were elaborated were studied. Anthocyanins are mainly located in the grape skins, whereas flavanols are located in both, the skins and seeds. Table 3 shows the content of total polyphenols (TP), total anthocyanins (TA), catechins (CAT) and proanthocyanidins (PRO) in grapes and wines from Tempranillo, Graciano and Cabernet Sauvignon. In the case of grape seeds, only TP, CAT and PRO are presented. Tempranillo monovarietal wine presented a slightly higher TA concentration than Graciano and Cabernet Sauvignon wines (Table 3), which explains the lower TA content registered in the different blends in comparison with the base wine (Fig. 1). However, when comparing the TA content of the skins of the three grape varieties, Graciano skins presented the highest TA content followed by Tempranillo and then by Cabernet Sauvignon (Table 3). These differences between the TA of the skins and derived wines could be due to differences in the thickness of the skins among varieties, which may affect the anthocyanin extractability under normal maceration and fermentation conditions.

Graciano wine presented the highest content of CAT, followed very closely by Cabernet Sauvignon, and then much lower in Tempranillo (Table 3). These results are consistent with higher CAT values of the different blends (Fig. 1). No significant differences ( $p > 0.05$ ) were found in the PRO concentration between Graciano and Cabernet Sauvignon wines, which were only slightly higher in comparison with Tempranillo wine. A similar sequence (GRA > CS > TEM) to that presented for the CAT content of the wines was found for the CAT and PRO concentration of the grape seeds, although in the case of PRO differences found between varieties were smaller than in CAT, as observed in the wines (Table 3). However, a different situation was found for the grape skins, Tempranillo showing the highest CAT concentration followed by Cabernet Sauvignon skins, and then very low in the

Table 3. Phenolic content of the wine, grape skins and seeds

Monovarietal wine	Tempranillo	Graciano	Cabernet Sauvignon
<i>Wine</i>			
TP	1320±11a	1503±12c	1409±11b
TA	596±2b	565 ±10a	553±16a
CAT	829±29a	1262±38c	1092±38b
PRO	1044±63a	1316±25b	1237±43b
PRO/CAT	1.26	1.04	1.13
<i>Grape skins</i>			
TP	18.0±0.3b	16.2±0.3b	11.6±1.3a
TA	18.2±0.6b	22.1±0.4c	16.0±0.5a
CAT	8.1±0.42c	0.50±0.02a	2.05±0.08b
PRO	27.7±1.3b	25.4±0.6b	17.3±1.3a
PRO/CAT	3.42	50.80	8.44
<i>Grape seeds</i>			
TP	22.3±2.2a	28.5±4.6a	21.8±3.0a
CAT	54.6±4.1a	117±5c	76.3±2.6b
PRO	40.7±2.0a	57.2±0.5c	53.9±6.6b
PRO/CAT	0.75	0.49	0.71

Mean value ( $n=2$ )±standard deviation

Different letters in the same row indicate significant differences at  $p<0.05$

TP: total polyphenols (gallic acid, mg/L wine, mg/g seeds and skins), TA: total anthocyanins (malvidin-3-glc, mg/L wine, mg/g skins), CAT: catechins ((+)-catechin, mg/L wine, mg/g seeds and skins), PRO: proanthocyanidins (cyanidin, mg/L wine, mg/g seeds and skins)

Graciano variety. In terms of PRO, Tempranillo and Graciano skins showed similar levels and higher than Cabernet Sauvignon. Independently of the grape variety, the grape skins exhibited a lower CAT and PRO content than the seeds, in accordance with the results previously reported by other authors (39–42). The calculation of the PRO/CAT ratio in wines, skins and seeds in the three varieties also revealed similarities in the profile of wines and seeds (TEM>CS>GRA) in comparison with that of the skins (GRA>CS>TEM) (Table 3). Although flavanols from grape skins are more easily extracted than from seeds during maceration and fermentation (in the conditions used in this experiment), these findings strengthen the idea supported by other authors (11,42) that the seeds could largely contribute to the flavanol content of wines, if appropriate maceration time and fermentation temperature are applied, as in the case under study (14 days at 27 °C). Other authors (43,44) have also found that Tempranillo grape seeds and wines possess low flavanol content in comparison with other varieties. Wine blending is therefore a practical way of increasing the flavanol content of Tempranillo wines. On the other hand, Graciano seems an ideal variety for this purpose. Considering the phenolic potential of this Spanish grape variety, more effort should be devoted to increase its production and to optimize its phenolic extraction during winemaking.

## Conclusions

In general, the effect of blending with Graciano on the phenolic composition of Tempranillo base wine was similar to that of blending with Cabernet Sauvignon. Significant differences among wines (blends and base wine) according to the blend factor were observed for CAT and TA. In fact, it was found that this effect on the CAT concentration of the base wine most likely originated from the higher CAT content that Graciano and Cabernet Sauvignon grape seeds presented in comparison with Tempranillo, finally conditioning a higher CAT level in the monovarietal wines from which the blends were elaborated. Although the evolution trend of the different groups of phenolic compounds was very similar in the blends and base wine, the blends presented a faster anthocyanin disappearance kinetic than the base wine. Since CAT participate in anthocyanin condensation reactions, the higher content of these compounds in the TEM-GRA and TEM-CS blends may partially explain their faster anthocyanin disappearance rate when compared to Tempranillo base wine. Finally, the findings of this work suggest that in terms of the phenolic content, Graciano wine presents properties similar to Cabernet Sauvignon for the blends of Tempranillo. However, wine blends from the same varieties belonging to other production areas and vintages should be studied in order to confirm the results found in this work.

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