Fişa suspiciunii de plagiat / Sheet of plagiarism's suspicion

Opera suspicionată (OS)	Opera autentică (OA)
Suspicious work	**Authentic work**
OS: I.D. Nistor, N.D. Miron, A. Gradinaru, A.M. Dospinescu, Phenolic content of blends of Merlot with Pinot noir or Cabernet Sauvignon wines produced in Romania, In: Modelling and Optimization in the Machines Building Field (MOCM) 13, Vol.1, 2007, pp.220-227.	
OA: M.Monagas, V.Núñez, B.Bartolomé, C. Gómez-Cordovés, Phenolic Content of Wine Blends, In: Food Technol. Biotechnol. 44 (4), 2006, pp.507–513	

Incidența minimă a suspiciunii / Minimum incidence of suspicion	
p.220:18 - p.220:21	p.507:1s – p.507:11s;
p.220:22 - p.220:30	p.507:4d – p.507:12d; p.508:1s – p.508:9s
p.220:31 – p.221:10	p.508:10s – p.508: 50s
p.221: Table 1	p.508: Table 1
p.222:3 – p.223:9	p.509: 1s – p.509: 34d.

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PHENOLIC CONTENT OF BLENDS OF MERLOT WITH PINOT NOIR OR CABERNET SAUVIGNON WINES PRODUCED IN ROMANIA

I.D. NISTOR, N.D. MIRON, A. GRADINARU, A.M. DOSPINESCU

University of Bacau, Calea Marasesti, nr. 157, Bacau

Abstract: The effect of Pinot Noir (PN, Romanian valuable variety) 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 Merlot wines (a largely cultivated Romanian 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 according to the blend factor were observed for CAT and TA. The findings of this work scientifically confirm that, in terms of the phenolic content, Pinot Noir wines possess properties similar to Cabernet Sauvignon for blending with Merlot.

Keywords: Merlot, Pinot Noir, Cabernet Sauvignon, wine blends, phenolic compounds, ageing

1. INTRODUCTION

The wine blending or coupage 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 (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).*

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 factors also determine the pH of the wine. An acidic pH assures a higher proportion of anthocyanins in flavylium 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 Roumainian *Vitis vinifera* L. cv. Pinot Noir is a variety that has a very limited production (0,02 % of the total grape varieties grown) concentrated mainly in Muntenia and Oltenia regions, but is highly appreciated for providing distinctive elements (freshness, aroma and colour) to the blending of wines, specially of Merlot, a widely cultivated Romanian 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.

Classic polyphenol methods based on global spectrophotometric determinations are 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 Pinot Noir (PN) *vs.* Cabernet Sauvignon (CS) wines on the phenolic content of Merlot blends during the period from 4 to 23 months of bottle ageing that covers their commercial life, using analytical methods usually peformed 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.

2. MATERIALS AND METHODS

2.1. Materials

As materials we used vanillin and Folin-Ciocalteau reagent, malvidin-3-gluco-side and cyanidin and (+)-catechin and gallic acid, HPLC-grade acetone, methanol, acetic and formic acids.

2.2. Wines

2.2.1. Monovarietal wines

Monovarietal young red wines made from grapes of *Vitis vinifera* cv. Merlot, Pinot Noir and Cabernet Sauvignon grown in the same geographical area (Buzău, Romania) and harvested at their technological maturity 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 Romania as follows: 12 kg of grapes of each variety were destemmed, crushed and collected into 10-litre stainless-steel wine vats. Pilot scaled fermentations were performed with a yeast inoculum of 25 g/hl (80 % *Saccharomyces cerevisiae* yeast strain and 20 % *Saccharomyces bayanus* 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.

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

	Merlot	**Pinot Noir**	**Cabernet Sauvignon**
Grapes			
Vineyard Yield/(kg/ha)	8815	5093	6730
pH	3,9	3,6	3,7
Sugar/^{0}Brix	21,1	24,3	21,9
Wines			
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

2.2.2. Wine blends

Four wine blends were prepared taking Merlot as the base wine, and Pinot Noir (PN) and Cabernet Sauvignon (CS) wines as modifiers in volume proportions of 10 and 25 % each (M-GRA 90:10 and 75:25, M-CS 90:10 and 75:25). The wines were then filtered through SEITZ K250 filters (2.5-3.0 mm) and finally bottled after correcting the free SO_2 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.

2.3. Grapes

Two groups of 100 berries from *Vitis vinifera* L. cv. Merlot, Pinot Noir and Cabernent 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 °C under nitrogen for subsequent analysis.

2.4. Extraction of phenolic compounds from the solid parts of the grape

2.4.1. 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 °C. Distilled water was added to the sample up to 50 ml and kept at -18 °C under nitrogen until analysis. Each extraction procedure was carried out in duplicate.

2.4.2. Seeds

Grape seeds were ground using a coffee bean miller to a particle size of less than 50 mm. 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 °C. The supernatant was filtered and concentrated to 5 ml using a rotary evaporator under partial vacuum at 35 °C. Samples were then freeze-dried. Extraction was performed in duplicate. For the phenolic determinations, 0,02 g of the freeze-dried sample was reconstitued in 1 ml of methanol/water (50:50).

2.5. 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--Ciocalteau 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-Ciocalteau 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-Ga-yon 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 $FeSO_4$)

were incubated at 90 °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.

2.6. Sensorial analysis

The different blends and the base wine were evaluated by the trained taster panel of Romania 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 recomendations. 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.

2.7. Statistical analysis

ANOVA and linear regression analysis of the data were performed using the PC software package Statgraphics Plus 2.1.

3. RESULTS AND DISCUSSIONS

3.1. Phenolic content of wine blends during ageing in the bottle

The effect of blending a monovarietal Merlot wine (base wine) with either Pinot Noir or Cabernet Sauvignon wines on the content of total polyphenols (TP), total anthocyanins (TA), catechins (CAT) and proanthocyanidins (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-Ciocalteau 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 method (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 the their size and structural composition, could render different reaction yields with the Porter reagent *(30)*.

In relation to TA, both M-PN and M-CS (90:10 and 75:25, each) blends presented a lower TA concentration than the Merlot base wine (M) (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-flavanols), leading to the so called pyranoanthocyanins *(14,15,18,19,31,32)*. In addition, during wine ageing, anthocyanins may also undergo hydrolytic and degradation reactions in minor extension *(33)*. As reported by other authors

(34-36), 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.
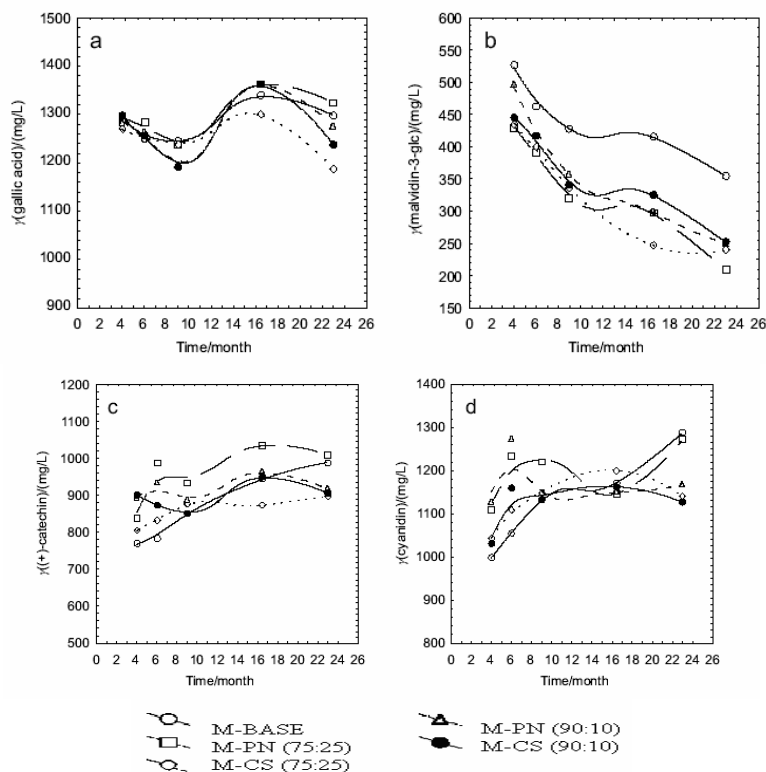

Fig. 1. Evolution of total phenols and of different groups of phenolic compounds during ageing in the bottle: (a) total polyphenols (TP), (b) total anthocyanins (TA), (c) catechins (CAT), (d) proanthocyanidins (PRO). The curves were fitted to the coordinate data according to the distance-weighted least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

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 $t_{1/x}=(lnx-ln(x-1))/k$, 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 (Pinot Noir 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 % (t1/4) 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 M-PN blends presented slightly higher *k* values than the M-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 M-PN blends (52 % for the 75:25 and 50 % for the 90:10) than for the M-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. Dissapearance rate of total anthocyanins in Merlot base wine and in the different blends

	$t_{1/4}$/month	$t_{1/2}$/month	k/(10^{-3}·month^{-1})	R^2
M-BASE	16.7	40.3	17.2	0.8797
M-PN 90:10	8.7	20.9	33.2	0.9486
M-CS 90:10	10.5	25.3	27.4	0.9247
M-PN 75:25	8.3	20.1	34.5	0.9407
M-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 M-CS (75:25) blend at 16,5 months, and of M-CS and TEM-PN (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 Merlot base wine. Since the vanillin reaction used for the CAT determination is specific of meta-dihydroxyphenyl 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 M-PN (90:10); 77,2 for M-CS (90:10); 79,7 for M-PN (75:25), and 89,5 for M-CS (75:25). Pinot Noir was judged to make a substantial improvement in the colour and structure of Merlot base wine, whereas Cabernet Sauvignon was recognized as improving the colour of Merlot as well as its aromatic complexity and flavour.

3.2. 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 Merlot, Pinot Noir and Cabernet Sauvignon. In the case of grape seeds, only TP, CAT and PRO are presented. Merlot monovarietal wine presented a slighlty higher TA concentration than Pinot Noir 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, Pinot Noir skins presented the highest TA content followed by Merlot 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.

Pinot Noir wine presented the highest content of CAT, followed very closely by Cabernet Sauvignon, and then much lower in Merlot (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 Pinot Noir and Cabernet Sauvignon wines, which were only slightly higher in comparison with Merlot wine. A similar sequence (PN>CS>M) 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, Merlot showing the highest CAT concentration followed by Cabernet Sauvignon skins, and then very low in the Pinot Noir variety. In terms of PRO, Merlot and Pinot Noir 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 *(37-38)*. 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 (M>CS>PN) in comparison with that of the skins (PN>CS>M) (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). Wine blending is therefore a practical way of increasing the flavanol content of Merlot wines. On the other hand, Pinot Noir seems an ideal variety for this purpose.

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

Monovarietal wine	Merlot	Pinot Noir	Cabernet Sauvignon
Wine			
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
Grape skins			
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±0.42	0.50±0.02	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
Grape seeds			
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)

4. CONCLUSIONS

In general, the effect of blending with Pinot Noir on the phenolic composition of Merlot 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 Pinot Noir and Cabernet Sauvignon grape seeds presented in comparison with Merlot, 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 M-PN and M-CS blends may partially explain their faster anthocyanin disappearance rate when compared to Merlot base wine. Finally, the findings of this work suggest that in terms of the phenolic content, Pinot Noir wine presents properties similar to Cabernet Sauvignon for the blends of Merlot.

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