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High Anthocyanin Level of Grape Hybrids from Monastrell and Their Wines

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Abstract

The Mediterranean area of Southern Europe, where Monastrell is the main variety planted will suffer the consequences of climate change due to a decrease in rainfall, accompanied by an increase in temperatures. In attempting to solve this problem one strategy could consist of breeding programs using hybrids obtained by crosses between Monastrell vines and other premium varieties such as Cabernet Sauvignon or Syrah.

In this work twelve hybrids from Monastrell, harvested at different moments, were characterized from the point of view of their anthocyanin content in order to select "new varieties" able to adapt to new climatological conditions. The results showed how all the hybrids analyzed and their corresponding wines provided a higher total anthocyanin content during the two seasons studied. Their profile was also modified, increasing the percentage of acetylated anthocyanin content and decreasing the percentage of dihydroxylated monoglucoside anthocyanins both in grapes and wines.

Keywords: Anthocyanin; hybrids; Monastrell; climate change; wine; grapes

Practical application: These results will have a big socio-economic impact, helping to boost the profitability of the crop and increase the yield and quality of both grape and the wines, by providing new varieties that are better adapted to the edaphoclimatic conditions of the area studied.

Introduction

In recent years, several papers have been published concerning the effects of climate change on grapevine [1, 2, 3] and on berry and wine quality. One such consequence is the earlier harvest time induced by the effect of warming on phenology, which will potentially reduce berry quality as a result of a greater loss of volatiles and greater water loss. Therefore, harvest dates need to be considered carefully since they are based on subjective evaluations of optimum fruit composition with a view to wine quality, whose definition is also subject to individual interpretation and trends, and may also depend on commercial targets, market constraints, processing capacity and other factors [4].

Some short term strategies may include the use of different crop management practices such as different irrigation systems, sunscreens for leaf protection, etc. On the other hand, some long term measures should after be considered such as varietal selection (one alternative could be the use of breeding programs) and land allocation changes [5]. Finally, changes in enological practices may represent another tool to obtain positive effects on wine quality [6].

Color is one of the most important attributes in red wines, and the principal sources of the red color in wines are the anthocyanins or their derivatives that are extracted or formed during the vinification process [7]. The concentration and composition of anthocyanins is affected by environmental factors such as temperature, exposure to light, and water availability [8]. An upward shift in seasonal temperature will dramatically shift the growing season, thereby changing the normal pattern of grape development toward an earlier onset of flowering, veraison and harvest. It is known that low temperatures (14/9 ºC day/night) are not conducive to high anthocyanin concentrations [9], and temperatures in excess of 30 ºC also lead to lower anthocyanin synthesis [8, 10, 11]. Therefore, in warm climates, grape berry temperature may frequently reach levels that inhibit the formation of anthocyanins and hence reduce grape color [12]. Besides absolute anthocyanin levels, compositional changes have also been described, with warm seasons associated with the increased formation of malvidin, petunidin, and delphinidin coumaroyl derivatives. However, Tarara, et al. [8] found that delphinidin, cyanidin, petunidin and peonidin- based anthocyanins decreased in sun-exposed Merlot berries, and while malvidin derivatives remained unaffected.

Other effects of climatic change on grape chemistry include increased pH values due to lower acid concentrations (especially malic acid), and lower anthocyanin and methoxypyrazine levels. This can favor oxidative reactions and may affect to the formation of the colorless hemiketal anthocyanin form, reducing wine color in young red wines [13].

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The proportion and amount of each anthocyanidin is influenced greatly by cultivar type and viticultural conditions. Although concentrations vary widely, it is commonly accepted that the anthocyanin profile of a given cultivar is closely linked to its genetic inheritance. Although environmental factors may have some influence [14, 15] this profile, or the relationship between some of the different anthocyanins, could be used to classify varieties. This obviously influences both the hue and the color stability, which are directly affected by the hydroxylation and methylation pattern of the B ring of the anthocyanidins. Blueness is enhanced by increases in free hydroxyl groups, whereas redness intensifies with increasing methylation of these hydroxyl groups as indicated by peak wavelengths. Malvidin is also the reddest individual anthocyanidin, while cyanidin, delphinidin and petunidin, which contain an o-diphenol structure on the B ring are more sensitive to oxidation. By contrast, neither malvidin nor peonidin possesses ortho-positioned hydroxyl groups, which explains their comparatively high resistance to oxidation [16].

Monastrell is a very late variety as regard both bud-breaking and ripening season, and is well adapted to the agro-ecological conditions of southeastern Spain. However although it has a high phenolic composition, its thick skin hinders extraction during winemaking [17]. While innovation in agriculture in general is commonly based on the development of new varieties, in wine grape viticulture, innovation has tended to be based on improvements in agronomic techniques or on the development of new enological technologies. This lack of genetic breeding programs is mainly due to the fact that only a reduced number of clones of a few varieties are authorized by the different Origin Appellations [18]. However, for all the reasons mentioned above it is necessary to obtain new varieties by means of intraspecific crosses, using Monastrell as the parental, although any new varieties must show a good adaptation to local agroecological and climatic conditions in order to have a high anthocyanin content with a high degree of extractability.

Finally, the main interest in of these compounds and their derivatives in grapes and wines are related with their potential beneficial effects on human health, although some researchers have cast doubts on their bioavailability. Such benefits include free radical scavenging and antioxidant activity, antimicrobial and antiviral activity, the prevention of cardiovascular disease, a protective effect against hepatic damage and disease, and anticancer and antimutagenic activities [19, 20, 21]. Therefore, if hybrids with high levels of these compounds can be obtained, especially if their bioactive compound content is superior to that of traditional varieties, they will be considered to show a high potential for providing human health benefits.

For the reasons mentioned above, the objectives of the present study were to investigate the parental effect on the content and profile of anthocyanins in various progenies and to estimate the heritability of anthocyanins in twelve hybrids. The potential influence of harvesting time was also investigated in two successive years in order to check the possible adaptation of these new vegetal materials to the impact of climate change.

Material and methods

Plant materials

A collection of 12 plants resulting from crosses of Monastrell x Syrah (4 plants) and Monastrell x Cabernet Sauvignon (8 plants) was used in this study. The study was conducted in a 1 ha experimental vineyard located in Bullas (Murcia, SE Spain). The parental (Monastrell) was planted in 1997, whereas the seeds for the interspecific hybrids were planted between 2010 and 2014 (Table 1). The training system was a bilateral cordon trellised to a three-wire vertical system and drip irrigation was applied. Planting density was 2.5 m between rows and 1.25 m between vines. Two two-bud spurs (4 nodes) were left at pruning time. Grapes were harvested from mid-August until the end of September, when they reached optimum ripening, with a total soluble content between 22.1 and 26.2 ºBrix (Table 1) and total acidity of between 3.0 and 5.2 mg/L tartaric acid (Table 1). They were sampled during two seasons (2015 and 2016), taking around 300 berries each time, which were kept frozen (-20ºC) until their analysis in triplicate.

Vinifications

Wines were made using 90 kg of grapes from each hybrid and the Monastrell variety. After cooling to 10 ºC, the grapes were crushed and destemmed and then 8 g of SO2/100 kg of grapes was added. The crushed grapes were distributed in thirteen 50 L tanks. Total acidity was corrected to 5.5 g/L and selected yeasts were added (10 g of dry yeast/100 kg of grapes; Laffort, DSM, Servian, France). All the vinifications were conducted at 25±1°C. During the pomace contact period (10 days), the cap was punched down twice a day and the temperature and must density were recorded. Wines were analyzed at the end of alcoholic fermentation in triplicate.

Analysis of anthocyanins in grapes and wines

Grapes were peeled with a scalpel, and the skins were stored at -20°C until analysis. Samples (2 g) were immersed in methanol (40 ml) in hermetically closed tubes and placed on a stirring plate at 150 rpm and 25°C. After 2 h, the methanolic extracts were filtered (0.45 μm) and analyzed by high-performance liquid chromatography (HPLC) according to Bautista-Ortín, et al. [22] and identified according to Gil-Muñoz, et al. [23]

Statistical data treatment

Significant differences among grapes and wines and for each variable were assessed by analysis of variance (ANOVA). Multivariable analysis of variance (MANOVA) was performed to study the effects of the harvest time and season on the anthocyanin parameters. Both analyses were performed using Statgraphics 5.0 Plus software (Statistical Graphics Corp., Rockville, MD, USA). The Duncan test was used to separate the means ($p < 0.05$) when the ANOVA and MANOVA tests were significant.

Results and discussion

Anthocyanin composition of Monastrell and hybrids grapes

The results for the individual anthocyanin composition of the hybrids and Monastrell are shown in Table 2 and 3. During the two seasons studied (2015 and 2016), all the hybrids had much higher anthocyanin concentrations than their parental although the concentrations were higher during the second season (2016). The appearance of a large number of hybrids in which the anthocyanin concentration is not within the range of concentration of their parental phenotypes is called transgressive segregation [24]. In our case, the segregation of a given trait was manifested in one direction (all hybrids obtained higher anthocyanin concentrations than the parental). For both vintages, the highest values were obtained in the hybrid MSy10. These results were significant for both grapes and wines, as anthocyanins in red grapes play an important role in protection against fungal and bacterial infections, have strong antioxidant properties, and contribute to the sensory and organoleptic characteristics [12].

Table 1: Physicochemical characteristics of the vegetal material.

Plantation Year		Harvest	º Brix		Total acidity*		Berry Size (g)	
		2015	2016	2015	2016	2015	2016	
MSy38	2010	Mid-August	23.4	24.9	3.4	4.1	1.05	0.84
MSy10	2012	Mid-August	24.7	25.8	4.5	4.5	1.35	0.94
MSy12	2010	End of August	24.4	22.3	3.0	3.2	1.22	1.08
MCS18	2014	End of August	24.7	24.0	3.7	4.1	1.25	0.96
MCS16	2010	Beginning September	26	25.4	4.5	5.2	0.96	0.87
MSy104	2010	Beginning September	22.2	24.5	3.0	3.6	1.32	1.20
MCS111	2012	Beginning September	24.4	26.2	5.0	4.7	0.88	0.73
MCS79	2014	Beginning September	22.1	24.8	4.8	4.9	0.97	0.87
MCS59	2010	End of September	23.6	26.3	3.5	4.3	1.22	0.92
MCS80	2010	End of September	25.2	24.5	3.1	3.3	1.14	1.07
MCS85	2012	End of September	23.2	23.8	3.6	4.5	1.30	1.06
MCS98	2012	End of September	24.6	25.8	4.5	4.1	1.39	1.27
Monastrell	2003	End of September	25.8	24.5	3.3	3.0	1.68	1.49

*expressed as mg/L tartaric acid

As regards individual anthocyanin compounds analyzed in the different plants, it could be observed that for all trihydroxylated anthocyanins (delphinidin, petunidin and malvidin-3-O-glucoside) the levels recorded in the crosses were higher than in the Monastrell grapes during both seasons studied. The range obtained for delphinidin-3-glucoside varied [83-284 mg/kg] in 2015 and [125 -579 mg/Kg] in 2016; for petunidin-3-O-glucoside the range varied [94-333 mg/kg] in 2015 and [184-398 mg/kg] in 2016, Finally, for malvidin-3-O-glucoside the results were the following, [537-804 mg/kg] in 2015 and [583-1360 mg/kg] during 2016. As occurs in all *Vitis viniferas,* malvidin-3-O-glucoside was the predominant anthocyanin in all the plants studied, but at harvest the levels recorded in the hybrids were much higher than those obtained by their parental. For all three compounds the results for Monastrell grapes were much lower during both years studied, with the values obtained in the parental being between one to six fold less.

By contrast, In lower case of dihydroxylated anthocyanins, the results differed, and higher values of cyanidin-3-glucoside were obtained in Monastrell in both seasons analyzed, except in the case of MCS16 and MSy10, which showed high levels in 2015. Monastrell grapes are also characterized by a relatively large concentration of dihydroxylated anthocyanins, as demonstrated in other studies [25,26]. As regards peonidin-3-glucoside, the results differed between vintages, although in general the concentration was higher in Monastrell grapes. In some hybrids the concentrations were higher - for example in MSy10 in 2015 or MCS16 in 2016. The high levels of these compounds in Monastrell and the hybrid grapes mentioned were due to the lower activity of the enzymes that control the formation of trihydroxylated anthocyanins in this variety [17].

In *Vitis vinifera* the presence of acylated anthocyanins (acetyl, coumaryl and to a lesser extended, caffeoyl) is to be expected. The profile of Monastrell is characterized by a comparatively high quantity of monoglucosides but a comparatively low presence of acylated anthocyanins, as was observed by Ortega-Régules, et al. [26] in Monastrell grapes from two different localizations (Bullas and Jumilla). Some other grape species, such as Muscadine grapes (*V. rotunidfolia*) do not accumulate acylated anthocyanins [27]. The interest in obtaining hybrids which higher levels of acylated compounds is due to the fact that these compounds are very important for wine color since they participate in intramolecular copigmentation processes [28].

With respect to acetylated anthocyanins, delphinidin, petunidin, and malvidin-3-acetylglucoside levels were also higher than in Monastrell for both seasons studied. As occurred for monoglucosides, a general increase in all acetylated anthcoyanins was obtained in hybrid grapes except for cyandin-3-acetylglucoside, whose values were lower in MSy104 than in Monastrell in 2015 and in MCS59, MCS85 and MCS98 in 2016. Also, values similar to those of Monastrell were observed for petunidin-3-acetylmonoglucoside in MCS59 and MCS85 in 2015. In a comparison of different varieties, Romero-Cascales, et al. [28] found that Monastrell grapes from two different locations (Jumilla and Bullas) had the lowest proportion of acylated anthocyanins, as also was observed by García-Beneytez, et al. [29] but in Cabernet Sauvignon and Syrah grapes had the highest percentages of acylated anthocyanins. With regard to coumarate anthocyanins, the results were variable for most of the compounds analyzed, although the highest concentration was obtained for malvidin-3-coumarylglucoside in both seasons. During 2015 the highest values were obtained in MSy10 and during 2016 in MSy104.

The anthocyanin percentages found in Monastrell grape and its hybrids are illustrated in (Figure 1). As can be observed the differences between Monastrell and the hybrid grapes mainly

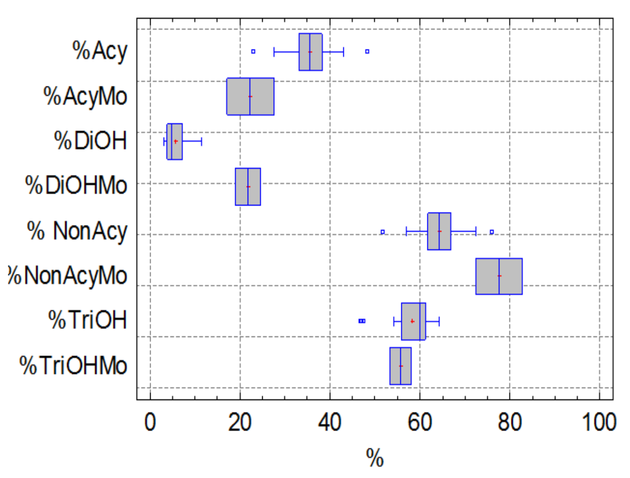

Figure 1: Box and whisker plot showing the percentage distribution of the different anthocyanin types in grapes from Monastrell and its hybrids

Abbreviations: Acy: Acylated anthocyanins; DiOH: Di-hydroxylated anthocyanins; NonAcy: Non-acylated anthocyanins; TriOH: Tri-hydroxylated anthocyanins: Mo: Monastrell.

concerned the accumulation of acylated and dihydroxylated anthocyanins. The boxes represented in Figure 1 enclose the middle 50% of the data where the median is drawn as a vertical line inside the box. The results showed that the profile of Monastrell hybrids was modified compared with its parental. The percentage of acylated anthocyanins was higher in the hybrids (36%) than in the parental (22%). With regards to the non-acylated anthocyanins, the levels were lower in the hybrids (64%) and in the dihydroxylated anthocyanins (5.7%) compared with the levels obtained in Monastrell grapes (78% for non-acylated and 22% for dihydroxylated anthocyanins). Finally, the percentage of trihydroxylated was also higher in the hybrids (58.2%) than in Monastrell (55.7%) grapes.

In conclusion, all hybrids presented higher anthocyanin content and most of them also a higher percentage of trihydroxylated anthocyanins and acylation, which could provide a more intense pigmentation. These new characteristics are interesting from an enological point of view since they could lead to wines with a much improved and more stable colour.

The anthocyanin composition of wines made from Monastrell and hybrids

The concentration of individual anthocyanins in the studied wines is shown in (Table 3). The typical concentrations of free anthocyanins in full-bodied young red wines is around 500 mg/L, but may in some cases be higher than 2,000 mg/L [30]. In our case, total anthocyanins in Monastrell wines reached 392.8 mg/L in 2015 and 689.9 mg/L in 2016. For the rest of the wines elaborated with the corresponding hybrids, the values were ranged from 799.5 to 2206.4 mg/L during 2015 and from 1636.3 to 2210.2 mg/L in 2016. Malvidin derivatives were the predominant anthocyanin in all wines, a situation common in several red wines, in which it forms the basis of their color. The total anthocyanin contents of the wines produced in each year were very different. Control wines produced in 2016 had significantly higher anthocyanin content than those produced in 2015, because the productivity was much lower in 2016 than 2015 due to climatological factors (data not shown; during 2016 the mean temperature was higher and rainfall was less than in 2015). The findings can also be explained by the smaller size of the berries in 2016, which facilitates the extraction of anthocyanins [31] (Table 1).

With regards to individual compositions, (Table 2) points to high trihydroxylated anthocyanin concentrations in the hybrids for both seasons, except cyanidin-3-glucoside whose values were similar in wines from MSy104, MCS98 and Monastrell grapes. The levels were also higher during 2016 than 2015. During the first season MSy10 provided the highest values, as occurred in the grapes, and during the second season the hybrid MCS111 reached higher values for all monoglucosides, except malvidin-3-glucoside, for which MSy10 found the highest values.

Table 2: Anthocyanin profile in grapes at harvest during two seasons.

Abbreviations: Dp: Delphinidin; Cy: Cyanidin; Pt: Petunidin; Pn: Peonidin; Mv: Malvidin; Gl: Glucoside; Ac acetyl glucoside; Cm: coumaryl glucosides; Cfglc: caffeate glucoside. [a]Different letters within the same column indicate significant differences according to Duncan's test (p > 0.05).

	Dp-3Gl	Cy-3Gl	Pt-3Gl	Pn-3Gl	Mv3Gl	DpAc	CyAc	PtAc	PnAc	MvAc	MvCum Cis	DpCum	Mv3cf	CyCum	PetCum	PnCum	MvCumTrans	Total
									GRAPE 2015									
Monastrell	32.2 a	41.2 e	52.6 a	62.1 .	205.5 a	1.5 a	2.2 ab	3.4 a	3.6 a	18.6 a	3.7 a	5.3 a	6.3 abc	6.3 c	12.7 a	13.4 abc	71.0 a	543.4 a
MCS16	244.2 ef	70.0 g	244.9 e	146.6 e	621.2 bcd	42.2 ef	12.2 i	56.2 f	29.2 f	168.4 b	3.7 a	18.3 de	3.6 a	10.8 e	30.3 b	31.3 e	130.3 b	1869.7 bc
MC59	125.9 cd	18.0 abc	121.5 bc	92.6 d	709.9 cde	19.1 bc	2.7 abc	25.3 bc	16.6 cd	237.4 cd	6.0 cd	6.7 ab	4.4 abc	2.8 a	13.1 a	19.4 cd	148.6 bc	1572.1 b
MCS80	184.0 d	17.0 abc	119.4 bc	66.2bc	756.1 de	34.0 de	3.2 bcd	35.2 cd	15.4 cd	298.3 e	6.5 cde	14.0 cd	7.9 cd	5.2 bc	17.9 a	14.2 abc	187.6 c	1786.6 bc
MSy104	83.4 bc	8.5 a	94.5 b	74.3 cd	645.2 bcd	13.0 b	1.6 a	22.6 b	19.4 de	254.4 de	8.7 f	23.2 ef	5.0 abc	5.6 c	41.9 cd	48.6 g	395.7 e	1756.8 bc
MSy38	85.3 b	25.0 cd	117.6 bc	136.3 e	537.9 b	17.2 b	4.0 cd	31.0 bcd	23.3 e	178.7 bc	9.1 f	24.8 f	13.4 ef	10.2 de	46.6 d	39.6 d	273.8 d	1582.1 b
MCS111	233.1 e	21.6 bcd	147.7 cd	32.5 a	656.4 bcde	43.3 f	5.7 ef	42.4 e	10.3 b	266.8 de	3.4 a	7.5 ab	4.2 ab	2.7 a	11.4 a	7.7 a	130.1 b	1615.5 b
MCS85	224.9 e	20.8 de	158.4 d	33.5 a	697.4 cde	45.6 f	6.1 f	42.3 e	11.0 b	278.8 de	3.8 ab	7.9 ab	4.5 abc	2.7 a	12.0 a	8.2 a	136.5 b	1697.1 bc
MCS98	283.9 f	31.3 de	213.6 e	69.4 bcd	750.2 de	59.2 g	8.0 g	63.4 f	21.2 e	261.0 de	5.9 c	28.0 f	7.4 bcd	8.7 d	35.2 bc	18.3 bcd	194.4 c	2068.6 c
MSy10	282.3 f	55.9 f	333.4 f	180.5 f	1039.1 f	58.7 g	10.0 h	88.6 g	37.8 g	360.0 f	11.2 g	41.8 g	14.3 f	16.7 f	74.6 e	47.8 g	379.9 e	3046.7 d
MSy12	91.1 bc	23.6 cd	123.4 bc	139.0 e	567.3 bc	18.9 bc	4.2 de	31.7 bcd	23.1 e	178.5 bc	8.2 ef	26.6 f	14.8 f	10.3 de	48.7 d	40.2 f	288.1 d	1646.5 b
MCS18	157.0cd	14.9 abc	104.4 b	49.4 abc	581.4 bc	30.1 d	3.8 cd	33.9 cde	16.5 cd	288.2 de	5.8 bc	10.8 bc	6.5 abc	3.9 ab	15.0 a	11.6 ab	144.8 bc	1481.7 b
MCS79	147.1cd	14.8 abc	169.9 d	45.2 ab	804.4 e	26.0 cd	3.9 cd	38.5 e	13.7 bc	248.7 de	7.9 def	17.6 d	10.4 de	6.2 c	32.9 b	21.4 d	251.5 d	1866.1 bc
									GRAPE 2016									
Monastrell	108.1 b	125.9 e	124.8 bc	153.5 hi	422.1 b	3.2 a	3.1 ab	5.3 a	4.8 a	22.4 a	2.6 dfg	2.6 f	2.6 a	14.0 def	19.2 abc	18.7 bc	86.3 ab	1127.4 abc
MCS16	433.7 e	85.5 d	415.0 f	179.5 i	1143.3 d	63.2 g	10.5 f	88.1 gh	34.0 f	306.3 d	2.3 cde	2.5 bc	47.3 d	16.9 fg	47.9 fg	32.5 ef	208.2 c	3117.7 f
MC59	25.9 a	8.2 a	22.3 a	18.8 a	77.6 a	12.7 ab	3.1 ab	18.3 ab	5.5 a	101.6 b	2.1 bcd	3.5 de	5.4 a	22.5 i	36.1 de	29.3 de	135.6 ab	641.2 a
MCS80	318.5 d	32.2 bc	184.2 cd	106.5 ef	1018.0 cd	50.0 f	4.8 bcd	40.3 d	24.9 de	420.2 e	2.8 efg	2.2 bc	24.4 c	8.9 b	25.4 bcd	24.6 cd	219.3 c	2519.1 e
MSy104	292.6 d	21.2 e	274.4 e	134.2 fgh	150.6 e	51.9 fg	5.3 cd	61.3 f	31.5 f	516.8 f	4.1 h	4.5 e	94.7 g	18.5 gh	87.7 i	62.4 h	366.1 e	3802.7 g
MSy38P52	149.8 bc	20.4 d	198.6 d	109.9 efg	922.9 cd	22.9 bcd	4.1 abcd	43.6 de	22.0 cd	267.8 d	3.2 fg	4.1 e	63.1 e	14.3 e	64.5 h	40.3 g	634.4 g	2319.0 e
MCS111	579.5 f	70.9 f	398.5 f	152.7 hi	1059.9 cd	81.5 h	8.2 e	78.0 g	26.3 e	298.8 d	1.7 abc	1.6 ab	82.8 f	20.4 hi	73.1 h	38.7 fg	289.8 d	3275.6 fg
MCS85	139.9 bc	16.7 b	86.6 b	20.4 a	378.7 a	19.9 bc	2.7 a	20.5 bc	5.0 a	210.4 c	1.4 a	1.1 a	9.7 ab	3.3 a	7.9 a	5.4 a	78.5 a	952.8 ab
MCS98	169.5 bc	12.4 a	128.0 bc	32.2 ab	467.5 b	29.3 cde	3.2 ab	30.9 bcd	6.6 cd	181.1 c	1.5 ab	0.9 a	28.0 c	5.8 a	25.8 bc	11.8 ab	146.7 b	1271.6 bc
MSy10	367.6 de	36.3 c	416.2 f	94.9 de	1360.3 e	59.2 fg	5.5 d	99.9 h	20.7 cd	429.9 e	3.4 g	2.3 bc	77.4 f	15.6 ef	87.6 i	32.5 ef	427.6 f	3538.0 fg
MSy38`2	200.3 c	34.9 bc	237.8 de	140.0 fgh	930.7 cd	33.0 de	4.1 abcd	56.3 ef	24.8 de	281.3 d	3.3 fg	4.1 e	55.3 de	14.4 ef	53.2 g	39.0 fg	283.5 d	2397.5 e
MCS18	209.1 c	25.8 abc	122.2 bc	66.3 cd	583.7 b	36.1 e	3.4 abc	34.7 cd	19.5 c	296.1 d	1.4 a	1.1 a	19.8 bc	5.2 a	14.5 ab	12.9 b	138.9 b	1591.0 cd
MCS79	179.5 bc	20.3 abc	204.9 d	54.7 bc	900.7 c	22.2 bcd	4.6 abcd	38.9 d	13.3 b	272.9 d	3.2 fg	2.9 cd	43.6 d	11.4 bc	39.2 ef	22.8 cd	252.3 cd	2088.3 de

As occurred in grapes, the concentration of acylated anthocyanins was generally higher in the wines made from the hybrids. Acylated anthocyanins are very important for wine color since they participate in intramolecular copigmentation processes, thus increasing the color [32]. Acetylated anthocyanins obtained higher values in all cases excepted for petunidin-3-acetyl glucoside in MSy38, MSy12 and MCS18 during the first season. With respect to coumarates, the values were higher in some hybrids and lower in others.

Although in our study hybrid wines were very pigmented, it is not always the case that highly colored grapes necessarily produce highly colored wines, which may be related to the ease with which anthocyanins are extracted from grape skins into musts [28]. It has been stated that the anthocyanin fingerprint only partially reflects the anthocyanin fingerprint of fresh grapes [29], the wines usually containing a higher proportion of malvidin-3-glucoside than grapes. However, the monoglucoside composition alone may not permit any clear conclusions on a wine's chromatic characteristics, as some anthocyanins could have been extracted from the skins and been polymerized, thus contributing to wine color [28]. The extraction of anthocyanins from the grape into the wine is essentially a diffusion process, and the rate and extent of extraction is influenced by the grape anthocyanin concentration, the composition of berry cell-walls and processing methods. The structural properties of the cell walls of the different varieties may determine the mechanical resistance, the texture and the ease of processing berries [33]. In the case of Monastrell, is known that their cell walls are genetically characterized by a more rigid structure, which would hinder anthocyanin extraction. In the case of the hybrids, some of them would have inherited these properties because although the wines obtained were highly pigmented, the concentration of anthocyanins in the grape was much higher, which means that the process of extraction was diminished. Ryan and Revilla et al. [34] studied the cell wall of some hybrids from Monastrell x Cabernet Sauvignon and noted that they have different carbohydrate and total sugar compositions which might affect the extraction process of anthocyanin compounds during winemaking.

The anthocyanin percentages found in Monastrell and their hybrids are shown in (Figure 2). The percentages obtained for hybrids and Monastrell wines were very similar to those obtained in grapes, except in two ways. In the first place, the percentage of dihydroxylated anthocyanins in Monastrell wines was still higher than that obtained by the hybrids, but the difference between them were less pronounced compared to the values found in grapes. In second place, the percentage of trihydroxylated

Table 3: Anthocyanin profile in wines at the end of alcoholic fermentation during two seasons

	Dp-3Gl	Cy-3Gl	Pt-3Gl	Pn-3Gl	Mv3Gl	DpAc	CyAc	PtAc	PnAc	MvAc/Dfcum	Peocf	Cycf/Cum	Mv3CumCis	PetCum	PnCum	MvCumTrans	Total
									WINE 2015								
Monastrell	15.4 a	8.6 b	36.4 a	30.1 c	203.9 a	2.6 a	2.0 a	1.5 a	7.4 a	16.9 a	4.9 b	8.6 bc	2.9 ab	2.1 bc	6.8 bc	37.0 d	392.8 a
MCS16	60.0 f	19.4 f	90.8 h	49.9 e	293.1 c	51.9 i	28.9 c	14.2 b	25.9 b	71.2 b	9.0 e	10.6 cd	5.4 c	7.2 d	6.7 b	22.0 a	807.6 c
MC59	64.1 g	15.9 e	81.1 f	53.9 f	510.3 h	43.9 g	12.1 b	27.4 c	31.0 d	152.5 e	11.8 f	11.9 de	8.3 de	8.5 d	10.2 d	53.0 f	1150. e
MCS80	79.0 h	16.5 e	68.9 e	45.0 d	502.0 h	51.8 i	18.5 b	31.9 d	44.6 f	177.1 g	13.7 g	12.8 de	11.5 f	13.0 e	9.4 cd	67.5 g	1243.5 g
MSy104	34.7 b	8.2 b	54.1 c	44.5 d	421.5 f	36.3 f	11.6 b	24.5 c	36.7 e	152.6 e	16.2 h	18.5 f	9.9 ef	7.4 d	16.2 f	67.2 g	1010.7 d
MSy38	54.3 e	12.2 cd	90.0 h	88.9 h	572.7 i	12.8 b	25.7 c	1.1 a	34.0 de	165.7 f	7.3 d	14.5 e	3.1 ab	0.1 a	20.6 g	77.1 h	1185. 3 f
MCS111	186.2 i	21.3 g	66.8 g	66.8 g	370.7 e	54.4 j	18.4 b	26.5 c	35.8 e	84.9 c	11.4 f	10.3 bc	7.5 d	12.4 e	9.9 d	33.0 c	999.2 d
MCS85	90.0 j	19.3 f	30.9 c	30.9 c	475.7 g	55.6 j	13.4 b	34.6 d	35.4 de	146.7 d	12.5 f	11.7 d	11.4 f	13.8 e	8.3 bcd	47.6 e	1180.7 ef
MCS98	48.6 c	5.1 a	12.9 a	12.9 a	224.4 b	23.3 c	17.1 b	31.1 a	14.2 b	71.2 b	1.4 a	1.1 a	1.0 a	3.3 c	3.3 a	26.6 b	516.8 b
MSy10	166.2 e	19.2 h	124.8 k	124.8 k	907.3 k	46.6 h	66.5 d	2.0 a	62.7 g	322.1 i	12.3 f	30.4 g	3.7 bc	1.3 ab	31.2 h	165.5 j	2206.4 i
MSy12	52.1 d	12.9 d	95.5 i	95.5 i	558.4 i	13.0 b	25.6 c	1.1 a	35.3 de	161.6 f	4.0 b	12.8 de	2.0 ab	0.4 a	21.4 g	69.5 g	1160.9 ef
MCS18	113.4 k	12.3 cd	44.6 d	44.6 d	611.9 j	31.5 e	29.1 c	5.5 a	34.4 de	285.9 h	6.1 c	7.9 b	4.0 bc	2.2 bc	13.6 e	89.6 i	1397.9 h
MCS79	32.9 b	11.1 c	19.8 b	19.8 b	343.0 d	26.8 d	15.6 b	23.6 c	25.9 c	89.4 c	12.0 f	10.7 cd	9.4 def	13.5 e	8.1 bcd	38.6 d	799.5 c
									WINE 2016								
Monastrell	41.3 a	16.8 a	76.5 a	42.4 a	346.2 a	3.4 a	9.0 a	11.1 a	8.6 a	27.4 a	8.0 a	5.1 a	12.5 d	7.9 abcd	7.9 ab	51.0 a	689.9 a
MCS16	152.7 e	42.2 g	184.0 f	95.0 h	591.0 b	65.6 f	24.8 cd	58.7 d	53.4 c	169.4 b	13.2 bc	24.3 f	7.9 a	6.2 bcde	15.0 bcd	73.8 b	1636.3 b
MC59	182.7 g	46.7 i	179.2 f	121.0 j	669.5 d	60.2 d	25.7 cd	49.1 b	53.3 c	248.7 d	15.4 cd	21.1 e	10.0 abcd	4.9 abac	17.6 cde	98.1 cd	1851.8de
MCS80	154.5 e	28.8 e	124.1 b	76.9 e	699.9 e	62.8 e	26.6 cd	48.8 b	64.2 e	271.8 efg	16.8 b	20.6 e	11.1 bcd	8.6 de	18.4 cde	101.3 d	1803.6 cd
MSy104	112.5 c	22.4 c	147.5 c	88.6 g	879.9 i	66.1 f	27.6 d	64.8 e	69.2 f	294.3 g	29.1 g	33.4 h	8.1 a	1.8 a	26.1 e	113.9 e	2051.7 g
MSy38P52	106.2 b	19.4 b	164.7 e	81.0 f	748.4 g	4.6 a	24.9 cd	59.3 c	59.2 d	255.7 de	17.6 d	13.3 c	26.6 e	8.3 cde	12.3 bc	90.6 cd	1712.0 bc
MCS111	229.0 i	44.5 h	217.6 h	103.0 i	709.5 e	91.7 i	27.4 d	73.1 f	80.6 g	216.9 c	25.0 f	39.5 i	11.3 cd	9.4 e	21.1 cde	117.0 ef	2088.5 g
MCS85	220.1 h	33.9 f	180.2 f	48.8 b	789.6 h	71.2 g	24.0 cd	61.6 de	6.6 a	319.1 h	17.9 d	18.5 de	8.4 ab	8.1 cde	2.5 a	131.6 g	1931.1 ef
MCS98	179.5 g	28.6 e	178.3 f	49.9 b	728.2 f	80.9 h	36.5 e	82.8 g	66.2 ef	284.3 fg	15.8 d	9.4 b	9.2 abc	34.0 f	3.1 a	149.7 h	1982.6 fg
MSy10	168.5 g	21.1 bc	251.5 i	69.6 d	953.5 k	7.6 b	18.3 b	87.7 h	28.8 b	338.5 d	23.3 ef	12.9 c	33.6 f	8.0 cde	12.3 bc	101.4 d	2210.2 h
MSy38`2	101.4 b	24.7 d	155.4 d	94.1 h	729.5 f	48.0 c	15.8 b	52.3 bc	28.9 b	201.44 c	11.3 b	30.3 g	7.6 a	4.5 ab	22.9 de	87.1 c	1692.7 bc
MCS18	173.9 f	28.1 e	128.1 b	71.1 d	635.2 c	65.9 f	23.1 c	50.1 b	56.5 cd	286.6 fg	11.3 b	16.4 d	8.6 ab	7.7 bcde	14.7 bcd	94.4 cd	1729.7 bc
MCS79	133.7 d	26.0 d	198.4 g	59.2 c	907.8 j	52.8 c	17. 4 b	54.0 c	60.0 d	265.1 def	22.3 e	31.0 gh	9.8 abcd	5.9 bcd	14.5 bcd	125.8 fg	2038.1 fg

Abbreviations: Dp: Delphinidin; Cy: Cyanidin; Pt: Petunidin; Pn: Peonidin; Mv: Malvidin; Gl: Glucoside; Ac acetyl glucoside; Cm: coumaryl glucosides; Cfglc: caffeate glucoside. aDifferent letters within the same column indicate significant differences according to Duncan's test (p > 0.05).

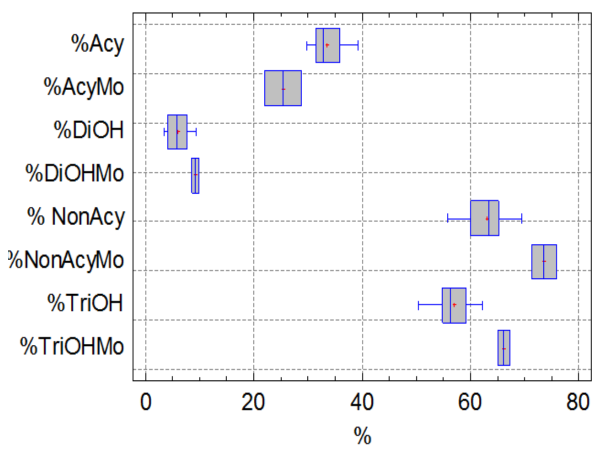

Figure 2: Box and whisker plot showing the distribution of percentage of the different anthocyanin types in wines from Monastrell and its hybrids.
Abbreviations: Acy: Acylated anthocyanins; DiOH: Di-hydroxylated anthocyanins; NonAcy: Non-acylated anthocyanins; TriOH: Tri-hydroxylated anthocyanins: Mo: Monastrell.

anthocyanins was greater in Monastrell wines than in the wines from the hybrids. This may have been due to intramolecular or intermolecular interactions of anthocyanins with themselves or with other organic chemicals, especially phenolic compounds, such as self-association and copigmentation, can further enhance their color expression [37] or to the formation of pyranoanthocyanis, such as vitisin A (malvidin-3-glucoside+ pyruvic acid) or vitisin B (malvidin-3-glucoside+acetaldehyde) during alcoholic fermentation.

When studying grapes for winemaking, it is not only the quantity of anthocyanins that is important. The hydroxylation pattern of the B-ring of anthocyanins is one of the main structural features of flavonoids and is also an important determinant of their coloration, stability and antioxidant capacity. Trihydroxylated anthocyanins (delphinidin, petunidin and malvidin-3-glucosides) are more stable in wines than dihydroxylated ones (cyanidin and peonidin-3-glucosides) [38]. Those with orto-di- phenolic groups (cyanidin, delphinidin and petunidin) have an enhanced susceptibility to oxidation. Methoxylated anthocyanins are also more stable. The same applies to acylated anthocyanins, since their esterification of anthocyanins promotes intramolecular aggregation or stacking, which protects the oxonium ion from decomposition [32]. Adaptation to climate change by using intraspecific hybrids.

To study separately the effects of harvest time and season, a MANOVA was applied. As can be observed in (Table 4), if a multivariable statistical analysis is made, no significant differences can be found between any of the percentages of the different anthocyanin types with respect to the harvest time and with respect to the season. Although harvest time was a source of variation in the total anthocyanin content, this difference was only observed between the results obtained for mid-August and the results obtained at the end of September. But these differences could be attributed to the fact that the concentration of anthocyanins may decrease slightly just before harvest [36] and/or during over-ripening, although climatological factors can also influence the results obtained.

Therefore, the challenge is to find well adapted hybrids (with good productivity and long ripening period) with high quality grapes (small berry size, a not excessively low acid content and a high anthocyanin content) [17]. Our hybrids were all characterized for having a smaller berry size, the same or a higher acidity and a much higher anthocyanin concentration both in grapes and wines than in the corresponding Monastrell grapes and wines. In addition, their anthocyanin profile was much improved, so the acylated and trihydroxylated anthocyanin percentage were superior to that of Monastrell. This suggests that the hybrids obtained by means of intraspecific crosses between Monastrell and other varieties may be a useful tool for alleviating the effect caused by earlier grape ripening and, in consequence, earlier harvest time on the quality of the grapes.

Table 4: Two-way ANOVA showing means separation of anthocyanin composition from the hybrids and Monastrell grapes and wines.

	Total anthocyanins (mg/L)	% Acylated	% Non-Acylated	% Di-OH	% Tri-OH
	Harvest Time (HT)				
Mid-August	2230.0 b	35.3 a	63.8 a	6.6 a	57.2 a
End of August	1637.2 a	36.1 a	62.9 a	6.7 a	56.2 a
Beginning September	1926.5 ab	34.5 a	63.2 a	6.1 a	57.3 a
End of September	1498.1 a	33.6 a	64.3 a	4.9 a	58.6 a
	Year (Y)				
2015	1538.6 a	35.6 a	62.9 a	6.5 a	56.3 a
2016	2107.3 b	34.2 a	64.1 a	5.6 a	58.3 a
	Interactions				
HT x Y	ns	ns	ns	ns	ns

Conclusion

Breeding programs of grapes intended for vinification are of priority importance in the present circumstances because new varieties adapted to climate change need to be identified. The anthocyanin composition and profile of the hybrids studied pointed to a higher concentration of total and trihydroxylated anthocyanins, and of acylated anthocyanins than observed in the parental grapes and wines. Besides, the selected hybrids corresponded to different harvest times, which make them useful for alleviating the effects caused by climate change, such as the overcoming of ripening grapes and the time of harvest.

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References

1. Neethling E, Barbeau G, Bonnefoy C, Quénol H. Change in climate and berry composition for grapevine varieties cultivated in the Loire Valley. Climate Research. 2012;53:89–101.
2. Dalu JD, Baldi M, Dalla Marta A, Orlandini S, Maracchi G, Dalu G, et al. Mediterranean climate patterns and wine quality in north and central Italy. International Journal of Biometeorology. 2013;57(5):729–742.
3. Leibar U, Aizpurua A, Unamunzaga O, Pascual I, Morales F. How will climate change influence grapevine cv. Tempranillo photosynthesis under different soil textures?. Photosynthesis Research. 2015;124(2):199–215.
4. Mira de Orduña R. Climate change associated effects on grape and wine quality and production. Food Research International. 2010;43(7):1844–1855.
5. Fraga H, Malheiro AC, Moutinho-Pereira J, Santos JA. An overview of climate change impacts on European viticulture. Food Energy Security. 2013;1(2):94–110.
6. Lobell DB, Field CB, Cahill KN, Bonfils C. Impacts of future climate change on California perennial crop yields: model projections with climate and crop uncertainties. Agricultural and Forest Meteorology. 2006;141(2-4):208–218.
7. Wrolstad RE, Durst RW, Lee J. Tracking color and pigment changes in anthocyanin products. Trends in Food Science & Technology. 2005;16(9):423–428.
8. Tarara JM, Lee J, Spayd SE, Scagel CF. Berry temperature and solar radiation alter acylation, proportion and concentration of anthocyanin in Merlot grapes. American Journal of Enology and Viticulture. 2008;59:235-247.

9. Comber B. Influence of temperature on composition and quality of grapes. Acta Horticuturae. 1987;206:23-35.
10. Buttrose MS, Hale CR, Kliewer WM. Effect of temperature on composition of 'Cabernet Sauvignon' berries. American Journal of Enology and Viticulture. 1971;22:71–75.
11. Spayd SE, Tarara JM, Mee DL, Ferguson JC. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. American Journal of Enology and Viticulture. 2002;53:171-182.
12. Doshi P, Adsule P, Banerjee K, Oulkar D. Phenolic compounds, antioxidant activity and insulin tropic effect of extracts prepared from grape (*Vitis vinifera* L.) by products. Journal of Food Science and Technology. 2015;52(1):181–190.
13. Ribereau-Gayon P, Dubourdieu D, Donèche B, Lonvaud-Funel A. Handbook of enology. I: The microbiology of wine and vinification. 1998;410-419.
14. Cacho J, Fernandez P, Ferreira V, Castells JE. Evolution of five anthocyanins-3-glucosides in the skin of the Tempranillo, Moristel, and Garnacha grape varieties and influence of climatological variables. American Journal of Enology and Viticulture. 1992;43:244–248.
15. Mori K, Goto-Yamamoto N, Kitayama M, Hashizume K. Loss of anthocyanins in red-wine grape under high temperature. Journal of Experimental Botany. 2007;58(8):1935–1945.
16. Jackson RS. Wine Science Principle and Applications (3rd ed.). 2008;287-295.
17. Gómez-Plaza E, Gil-Muñoz R, Hernández-Jiménez A, López-Roca JM, Ortega-Regules A, Martínez-Cutillas A. Studies on the anthocyanin profile of Vitis Vinifera intraspecific hybrids (Monastrell × Cabernet Sauvignon). European Food Research and Technology. 2008;227(2):479–484.
18. Hernández-Jiménez A, Gómez-Plaza E, Martínez-Cutillas A, Kennedy J. Grape Skin and Seed Proanthocyanidins from Monastrell x Syrah grapes. Journal of Agricultural and Food Chemistry. 2009;57(22):10798–10803.
19. Koide T, Kamei H, Hashimoto Y, Kojima T, Hasegawa M. Antitumor effect of hydrolyzed anthocyanin from grape rinds and red rice. Cancer Biotherapy Radiopharmaceuticals. 1996;11(4):273-277.
20. Jing P, Bomser JA, Schwartz SJ, He J, Magnuson BA, Giusti MM. Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth. Journal of Agriculture and Food Chemistry. 2008;56(20):9391-9398.
21. De Pascual-Teresa S, Sanchez-Ballesta MT. Anthocyanins: from plant to health. Phytochemistry Reviews. 2008;7(2):281-299.
22. Bautista-Ortín AB, Martínez-Cutillas A, Ros-García JM, López-Roca JM, Gómez-Plaza E. Improving color extraction and stability in red wines: The use of maceration enzymes and enological tannins. International Journal of Food Science and Technology. 2005;10:287-295.
23. Gil-Muñoz R, Bautista-Ortín AB, Ruiz-García Y, Fernández-Fernández JI, Gómez Plaza E. Improving phenolic and chromatic characteristics of Monastrell, Merlot and Syrah wines by using methyl jasmonate and benzothiadiazole. Oeno One. 2017;1:17-27.
24. Hernández-Jiménez A, Gil-Muñoz R, Ruiz-García Y, López-Roca JM, Martinez-Cutillas A, Gómez-Plaza E. Evaluating the polyphenol profile in three segregating grape (*Vitis vinifera* L.) populations. Journal of Analytical Methods in Chemistry. 2013.
25. Ruiz-García Y, Romero-Cascales I, Gil-Muñoz R, Fernández-Fernández JI, López-Roca JM, Gómez-Plaza E. Improving grape phenolic content and wine chromatic characteristics through the use of two different elicitors: Methyl jasmonate versus benzothiadiazole. Journal of Agricultural and Food Chemistry. 2012;60(5):1283–1290.
26. Ortega-Regules A, Romero-Cascales I, Ros-García JM, López-Roca JM, Gómez-Plaza E. A first approach towards the relationship between grape skin cell-wall composition and anthocyanin extractability. Analytica Chimica Acta. 2006;563(1-2):26–32.
27. Goldy RG, Maness EP, Stiles HD, Clark JR, Wilson MA. Pigment quantity and quality characteristics of some native *V. rotundifolia Michx*. American Journal of Enology and Viticulture. 1989;40:253–258.
28. Romero-Cascales I, Ortega-Regules A, López- Roca JM, Fernández-Fernández JI, Gómez-Plaza E. Differences in anthocyanin extractability from grapes to wines according to variety. American Journal of Enology and Viticulture. 2005;56:212-219.
29. García-Beneytez E, Revilla E, Cabello F. Anthocyanin pattern of several red grape cultivars and wines made from them. European Food Research and Technology. 2002;215(1):32-37.
30. Mulinacci N, Santamaria AR, Giaccherini C, Innocenti M, Valletta A, Ciolfi G, et al. Anthocyanins and flavan-3-ols from grapes and wines of *Vitis vinifera* cv. Cesanese d'Affile. Natural Product Research. 2008;22(12):1033–1039.
31. Ortega-Regules A, Romero-Cascales I, Ros-García JM, et al. Anthocyanin and tannins in four grape varieties (*Vitis Vinifera* L.). Evolution on their content and extractability. Journal International des Sciences de la Vigne et du Vin. 2008;42(3):147–156.
32. Sarni P, Fulcrand H, Souillol V, Souquet JM, Cheynier V. Mechanisms of anthocyanin degradation in grape must like model solutions. Journal of the Science of Food and Agriculture. 1995;69(3):385-391.
33. Hannah L, Roehrdanz PR, Ikegami M, Shepard AV, Shaw MR, Tabor G, et al. Climate change, wine, and conservation. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(17):6907-6912.
34. Ryan JM, Revilla E. Anthocyanin composition of Cabernet sauvignon and Tempranillo grapes at different stages of ripening. Journal of Agricultural and Food Chemistry. 2003;51(11):3372–3378.
35. Downey MO, Dokoozlian NK, Krstic MP. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. American Journal of Enology and Viticulture. 2006;57(6):257-268.
36. González-Manzano S, Santos-Buelga C, Dueñas M, Rivas-Gonzalo JC, Escribano-Bailón T. Colour implications of self-association processes of wine anthocyanins. European Food Research and Technology. 2008;226(3):483–490.

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