THE EFFECT OF GRAPE RIPENING STAGE ON RED WINE COLOR

EFFET DE LA MATURATION SUR LA COULEUR DES VINS ROUGES

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Abstract : The physico-chemical and chromatic characteristics of grapes (*Vitis vinifera* L. cv. Monastrell) harvested at six different degree of ripeness (from August 16 to October 24, 2002) and that of the wines obtained from these grapes have been studied. The grape anthocyanins content (mg/kg of berry fresh weight) was maximum in those grapes harvested on September 11 and 16 (804.1 and 822.6 mg/kg, respectively) and decreased for grapes harvested in October. However, the results showed that the grapes with the highest anthocyanin concentration did not lead to the highest colored wines. The wines elaborated from grapes harvested on October 16 (671.9 mg of anthocyanins per kg of berry fresh weight) had the best chromatic characteristics and better withstood aging in the bottle; the extent of cell wall degradation in overly matured grapes probably facilitated the extraction of phenolic compounds from skins. However, the chromatic quality of wines made from grapes harvested one week later (October 24, the most mature grapes) was lower than that from October 16, with lower color intensity (13 % lower in the wine elaborated from grapes harvested in October 24) and a percentage of yellow color 6 % higher in this wine.

Résumé : Les caractéristiques physico-chimiques et chromatiques de raisins (*Vitis vinifera* L. cv Monastrell), récoltés à six différentes périodes des vendanges (du 16 août au 24 octobre 2002) ainsi que celles des vins obtenus à partir de ces raisins, ont été étudiées. La concentration en anthocyanes a été maximale dans les grapes de raisin cueillies le 11 et 16 septembre (804.1 et 822.6 mg/kg), puis décroissante dans celles récoltées en octobre.
Cependant, les résultats démontrent que les grappes de raisin, dont la teneur en anthocyane était la plus importante, n'ont pas produit les vins les plus colorés. Les vins élaborés à partir de raisins récoltés le 16 octobre (671.9 mg des anthocyanes par kg de raisin) donnent les meilleures caractéristiques de couleur ainsi qu'une meilleure conservation en bouteilles. L'extension du phénomène de dégradation des parois cellulaires, qui se produit dans le raisin très mûr, facilite probablement l'extraction des composés phénoliques contenu dans les pellicules des baies de raisin. Néanmoins, la qualité chromatique des vins obtenus à partir du raisin cueilli une semaine plus tard (24 octobre, le raisin le plus mûr), a été plus petite que celle obtenue à partir du raisin récolté le 16 octobre, c'est-à-dire que l'intensité de couleur a été moindre (13 % en moins) et le pourcentage de la composante jaune de la couleur du vin plus élevé (6 %).

Key words : grape, wine, maturation, color, anthocyanins, harvest date

Mots clés : raisin, vin, maturation, couleur, anthocyanes, date de récolte

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J. Int. Sci. Vigne Vin, 2006, **40**, n°1, 15-24

INTRODUCTION

Red wine color and its stability is associated with the concentration of phenolic compounds in the grapes and the resulting musts. Harvesting winegrapes at their optimal levels of maturity is the first step in producing high quality wines. Determining the best time to harvest requires both experience and a careful assessment of winegrape maturity. Typical chemical analyses for determining winegrape maturity include monitoring the sugar content, titratable acidity and pH. However, grape maturity cannot be based solely on these routine chemical analyses because the development of optimal flavor and of phenolic compounds does not always coincide with specific levels of sugar, acidity or pH (WATSON, 2003).

Anthocyanins and tannins, and flavonols in a lesser extend, are among the most important flavonoid compounds responsible for the color and astringency of red wines. These compounds accumulate primarily in the skin and seeds of the grape berry and are extracted into the must and wine during alcoholic fermentation and pomace contact.

Grape skins rich in flavonoid compounds, especially anthocyanins, are usually associated with optimum maturity. However, grapes that are high in flavonoids do not necessarily produce wines that are also rich in phenolic compounds (ZOECKLEIN, 2002). Grape maturity is associated with changes in the skin and pulp cell walls, due to the action of pectolytic enzymes which hydrolyze cell wall pectins, making them permeable to the changes that occur in vinification (AMRANI JOUTEI and GLORIES, 1995a; AMRANI JOUTEI and GLORIES, 1995b; LECAS and BRILLOUET, 1994). Therefore, the maximum extractability of phenolic compounds does not always coincide with their maximum content in the grape skins (SAINT-CRICQ *et al*., 1998a) since the extractability depends on the extent of degradation of grape skin cell walls, the barrier for the diffusion of phenolic compounds. To this effect, we have studied the influence of harvesting grapes at different times (and therefore, with different anthocyanin concentration) on the color of the resulting wines. The aim was to obtain maximum color extraction in wines after the maceration step and to check the stability of this color after an aging period.

MATERIAL AND METHODS

Grapes of *Vitis vinifera* var. Monastrell (also known as Mourvèdre) were collected from a commercial vineyard in Jumilla (S.E. Spain), in 2002. The vines were 10 years old, head trained, cultivated without irrigation and grafted onto 161-49 rootstock. Veraison occurred around 30 July, 2002. Grapes were harvested at six different dates (August 16, September 11, September 16, September 18, October 16, and October 24) in 20 kg boxes and transported to the winery. Upon arrival at the winery, five to six berries from different parts of the cluster and from different clusters (ca. 300 g) were sampled (in triplicate) for the physico-chemical analyses. Another triplicate sample of grape berries was kept frozen (-24 °C) until the analysis of skin anthocyanins.

I - WINEMAKING PROCESS

Grapes were crushed and destemmed and then sodium metabisulfite was added (80 mg of SO_2/kg of grapes). The crushed grapes from each harvest date were distributed into three stainless steel containers (15 liters each). Titratable acidity was corrected with tartaric acid to 5.5 g/L (or 6.5 g/L if must pH values were over 3.8, as occurred in the October samples) and yeasts were added (Fermirouge, Gist Brocades, 1 g of dry yeast/10 kg of grapes, previous rehydratation of the dry yeasts).

Maceration lasted 15 days. Throughout the pomace contact period, the cap was punched down twice a day and the temperature and specific gravity were recorded (figure 1). After maceration, the wines were pressed at 1.5 bar in a 75 L tank membrane press. Free-run and pressed wines were combined and stored at room temperature. One month later, the wines were racked. Malolactic fermentation occurred spontaneously and when it was finished, the wines were racked again and sulfur dioxide was added (50 mg/L). In February, all the wines were cold stabilized (-3 °C) for one month and bottled.

II - PHYSICO-CHEMICAL DETERMINATION OF GRAPES

All reagents used in these analyses were of analytical grade. Total soluble solids (°Brix) were measured

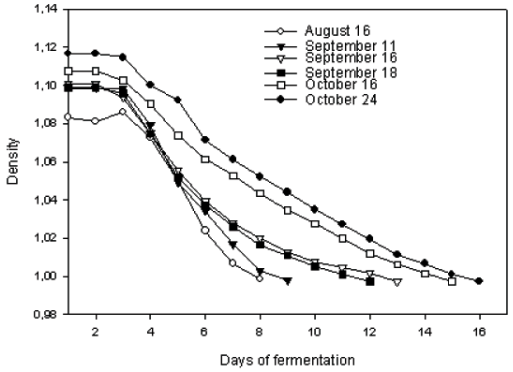

Figure 1 - Changes in the specific gravity of fermenting wines made from grapes harvested at six different dates in 2002 (each point represents mean values of three vinifications)

Changements de la gravité spécifique de la fermentation des vins élaborés avec des vins provenant de raisins récoltés à six dates différentes (en 2002). (Chaque point représente la moyenne de trois vinifications.)

using an Atago hand-refractometer (Atago, Japan). Titratable acidity and pH were measured using an automatic titrator (Metrohm, Switzerland). Titration was made with 0.1 N NaOH.

The phenolic maturity index (also known as extractability assay) was calculated according to the method described by SAINT-CRICQ *et al*. (1998b), with a slight modification. The grapes were homogenized and macerated for four hours at two different pH values (3.6 and 1.0), while the original method (SAINT-CRICQ *et al*, 1998b) used pH 3.2. A pH of 3.6 has proven more appropriate for wines made from *Vitis vinifera* cv. Monastrell grown in the Jumilla region of south east Spain. The anthocyanin contents of the two solutions (ApH 1.0 and ApH 3.6) were then chemically assayed by measuring the absorbance at 520 nm of the samples at pH 1 and pH 3.6. The phenolic maturity index was calculated as follows (SAINT-CRICQ DE GAULEJAC *et al*., 1998b):

$$\text{Phenolic maturity index (\%)} = \frac{ApH\ 1 - ApH\ 3.6}{ApH\ 1} \times 100$$

II - PHYSICO-CHEMICAL DETERMINATIONS IN WINES

Enological variables (ethanol content, pH, and SO_2) were determined according to the OIV Official Methods (O.I.V., 1990)

Color intensity (CI) represents the amount of wine color and was calculated as the sum of absorbance at 620 nm, 520 nm and 420 nm (GLORIES, 1984), and hue as the ratio between absorbance at 420 nm and absorbance at 520 nm (SUDRAUD, 1958). Other variables calculated were red (%R), yellow (%Y) and blue (%B) percentages, according to GLORIES (1984). Also, CIELab parameters were determined by measuring the transmittance of the wine every 10 mm from 380 nm to 770 nm, using the D65 illuminant and a 10° observer. L* (a measure of lightness), a* (a measure of redness) and b* (a measure of yellowness) parameters were determined. All the absorbance measurements were made in a Helios Alpha spectrophotometer (Thermospectronic, USA) with 0.2 cm path length glass cells. The spectrophotometer has the necessary software to calculate the CIELab parameters directly.

Monomeric anthocyanins (mg/L) and polymeric anthocyanins (mg/L) were calculated by fractionation using C18 Sep-pak cartridges, according the method of HO *et al.* (2001). A 0.5 mL aliquot of wine was passed through a C18 Sep-Pak cartridge that had been preconditioned by sequentially passing 5 mL of methanol and phosphate buffer pH 7 dropwise. Monomeric anthocyanins (fraction I) were removed by passing 10 mL of 16 % acetonitrile (pH 2) through the cartridge, and polymeric anthocyanins (fraction II) were eluted with 10 mL of methanol. Each fraction was then measured at 520 nm.

The methods described by RIBÉREAU-GAYON *et al.* (1998) were used for the determination of total tannins, total phenols and the HCl index. Total tannins were determined spectrophotometrically after their transformation into cyanidin in a strongly acid medium (12N HCl) and the solubilization of the newly formed red compounds with 95 % ethanol. Total phenols (A280) were determined by measuring the optical density at 280 nm. The HCl index, which represents the tannin polymerization level, was determined by measuring the absorbance of the wine (diluted 1:50) at 280 nm before and after the precipitation of condensed tannins in a strongly acid medium (12N HCl).

III - HPLC ANALYSIS OF GRAPE SKIN ANTHOCYANINS

Triplicate samples of frozen grapes were peeled with the help of a sharp knife. Skin samples (5 g) were immersed in methanol (50 mL) in hermetically closed tubes and placed on a stirring plate at 150 rpm and 25 °C. After 14 hours, the extract aliquots were filtered through nylon filters (0.45 µm, Scharlab, Barcelona, Spain) and analyzed by HPLC.

The HPLC analyses were performed on a Waters 2690 liquid chromatograph (Waters, USA), equipped with a Waters 996 diode array detector and a Licrochart RP-18 column (Merck, Germany), 25 x 0.4 cm, 5 µm particle size, using as solvents water plus 4.5 % formic acid (solvent A) and HPLC grade acetonitrile (solvent B) at a flow rate of 1.5 mL min^{-1}, at room temperature. Elution was performed with a gradient starting with 10 % B to reach 15 % B at 25 min, 21 % B at 65 min, and then became isocratic for 3 min. Chromatograms were recorded at 520 nm. Identification of the compounds has been described previously (REVILLA *et al*., 1999). Anthocyanins (the 3-monoglucosides of delphinidin, cyaniding, petunidin, peonidin and malvidin, together with their acetyl and coumaryl derivatives) were quantified at 520 nm as malvidin-3-glucoside equivalents, using malvidin-3-glucoside chloride as external standard (Extrasynthèse, France)

IV - STATISTICAL DATA TREATMENT

Significant differences among wines and for each variable were assessed with analysis of variance (ANOVA). This statistical analysis, together with a principal component analysis, were performed using Statgraphics 2.0 Plus (Statistical Graphics Corp., USA).

Table I - Physico-chemical characteristics of the grapes at the moment of harvest (n=3)
Caractéristiques physiques et chimiques des raisins au moment de la récolte (n=3)

Date of harvest	°Brix	Weight of 100 berries (g)	Titrable acidity*	pH
August 16	20.2a	167.1a	7.8c	3.32a
September 11	23.4c	167.2a	5.7b	3.51b
September 16	23.5c	172.4ab	5.7b	3.50b
September 18	22.4b	188.2c	5.5b	3.47b
October 16	25.8d	182.5bc	4.3a	3.78c
October 24	26.4d	182.3bc	4.2a	3.85c

Different letters in the same column mean significant differences at p>95 % according to a LSD test ; * expressed as g/L of tartaric acid

Table II - Concentration of the individual anthocyanins (expressed as mg/kg berries)
Concentration des anthocyanes individuelles (exprimée en mg/kg de raisins)

Date of harvest	Del-3-G	Cian-3-G	Pet-3-G	Pn-3-G	Mal-3-G	Del-3-G Coum	Cyan-3-G Coum	Pet-3-G Coum	Pn-3-G Coum	Mal-3-G Coum	Total(1)
August 16	92.4d	61.3a	102.2c	67.2a	303.1bc	18.3bc	8.6a	9.1b	7.7a	42.3ab	712.5bc
September 11	90.5d	88.2cd	104.6c	104.7c	329.9c	19.5c	10.8b	9.8b	11.8b	52.2b	804.1cd
September 16	97.1d	94.7d	105.1c	105.1c	310.1bc	17.1bc	9.9ab	9.3b	11.1b	44.2ab	822.6d
September 18	78.8c	77.4bc	89.7bc	88.9b	270.8b	16.3bc	9.1ab	8.9b	11.2b	45.1b	697.1b
October 16	67.3b	69.8ab	84.0b	95.3bc	265.2b	15.7ab	9.3ab	8.8ab	11.3b	44.8ab	671.9ab
October 24	52.4a	71.4ab	66.3a	94.5bc	213.4a	12.5a	8.6a	6.9a	11.2b	34.8a	572.4a

Abbreviations: Del-3-G: delphinidin-3-glucoside, Cyan-3-G: Cyanidin-3-glucoside, Pet-3-G: Petunidin-3-glucoside; Pn-3-G: Peonidin-3-glucoside, Mal-3-G: Malvidin-3-glucoside, Coum: coumarates ; Different letters in the same column mean significant differences at p>95 % according to a LSD test (n=3)
1:Represents the sum of all the anthocyanins quantified by HPLC-UV, using malvidin-3-glucoside as external standard

RESULTS AND DISCUSSION

The physico-chemical parameters of the grapes at each harvest date are shown in table I. The criteria traditionally used to determine grape maturity are based on the sugar content, which can be expressed in °Brix units. High values of °Brix indicate a high degree of ripeness, reaching maximum values of 25-26 °Brix in the late stages of ripening. Our first grape sample was harvested at 20.2 °Brix. As maturation advanced, the sugar content kept increasing. Heavy rain fell on September 16, after sampling the grapes, and one lot of grapes was also harvested two days later (September 18) to assess the effect of the rainfall on grape quality. The dilution effect of the rain lowered the sugar content. However, sugar content increased again in the last two sampling dates, reaching 26.4 °Brix on the last sampling date. However, the experience has shown that sugar alone may not be a fully adequate criterion for harvesting winegrapes, and other factors such as acidity, berry weight, anthocyanins, tannins and total phenols, are also important factors in deciding the timing of harvest.

Berry weight was almost stable on the first three sampling dates, increasing after the rain and then stabilizing again, although with a slight decrease, probably because of water loss due to evaporation in the berries (MCCARTHY, 1999).

Titratable acidity and pH are also of great importance for grape juice and wine stability, and both parameters are commonly used as indicators of quality. As can be seen, small differences in pH reflect large changes in titratable acidity, as also stated by ILAND (1987). ESTEBAN *et al.* (2002) found that pH increased linearly with berry ripening while titratable acidity decreased exponentially. The pH values found in the October samples were high. It is known that a must pH level above 3.6 increases the activity of detrimental microorganisms, lowers the color intensity of red wines, binds more SO_2, reduces free SO_2 and adversely affect the ability of a wine to age (JACKSON and LOMBARD, 1993). The pH of the last two musts (those from October samples) were greater than pH 3.6, thus it was necessary to adjust the pH during winemaking by addition of tartaric acid.

The color of grapes is of fundamental importance for the quality of red wines. Table II shows the concentration of individual anthocyanins in the skin of *Vitis vinifera* cv. Monastrell at the different harvesting dates. We found for Monastrell grapes that malvidin-3-glucoside represented

the largest proportion of all the anthocyanins and that non-acylated glucosides represented 87-89 % and acylated derivatives 11-13 % of the different compounds, results coincident with those of FERNANDEZ-LOPEZ *et al.* (1999) and GARCÍA-BENEYTEZ *et al.* (2002).

The grapes harvested in August already had a high concentration of anthocyanins. As FERNANDEZ-LOPEZ *et al.* (1998) stated, the pigmentation of Monastrell grapes develops quickly and in the second week after veraison the color is already blue-black. Also, DOWNEY *et al.* (2004) found that the bulk of anthocyanin accumulation in Shiraz grapes occurred three to four weeks following veraison.

Grapes harvested in September 16 presented the highest concentration of total anthocyanins, although with no significant differences with the grapes harvested in September 11. The effect of the rainfall is clearly observed in the grapes harvested on September 18, with a significantly decrease in total anthocyanins. The lowest values were found in the grapes from the last sampling date.

The results of the phenolic maturity index (phenolic extractability assay) are shown in figure 2. This method is based on the assumption that at pH 1.0 there is a complete disruption of the vacuolar membrane which facilitates the liberation of phenolic compounds. When the pH of the macerating solution is 3.6, the natural degradation of the cells is respected and this situation is similar to that occurring during maceration at winemaking (GLORIES and SAUCIER, 2000). The values for this index are usually comprise between 70 and 20, as reported by RIBÉREAU-GAYON *et al.* (1998). The extractability is considered good when the concentration of the anthocyanins extracted with the pH 1.0 solution is high and the difference between anthocyanins extracted at pH 1.0 and 3.6 is small and therefore, the phenolic maturity index is low.

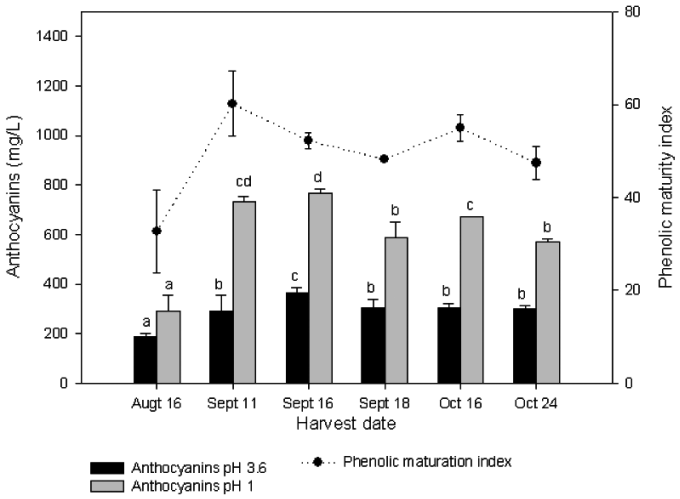

Figure 2 - Values of total anthocyanins at pH 1 and pH 3.6 , phenolic maturity index in the grapes at the different harvest dates

(Error bars represent standard deviation. Different letters above the bars representing the same parameter means significant differences at p>95 % according to a LSD test, n=3)

Valeurs des anthocyanes totales à pH 1 et à pH 3,6 et de la maturité phénolique dans les raisins récoltés à différentes dates

As it can be observed in the figure, the quantity of anthocyanins was always higher when the macerating solution was at pH 1.0, as expected, and their evolution was similar to that found for total anthocyanins in the berries (mg/kg of berries), although the lowest ApH 1 value was found on August 16 and not on the last sampling date, like in the case of anthocyanin measured by HPLC.

The smallest difference between ApH 1 and ApH 3.6 was found in the August 16, September 18 and October 24 samples, and a clear evolution was not found during ripening, since the value increased from the sample harvested on September 18 to the sample harvested on October 16.

Despite the fact that other authors have found an almost steady decrease in the phenolic maturity index as ripening advanced (RIBÉREAU GAYON *et al.*, 1998; SAINT-CRICQ DE GAULEJAC *et al.*, 1998b; ZAMORA, 2002), we observed no clear pattern, coinciding with the observations of GONZÁLEZ-NEVES et al. (2002), who also found that there was no predetermined relationship between the phenolic maturity index and technological maturity. Other authors, have criticized this extractability assay since when extracting at pH 1, the liquid phase has a pH>1 because must is a buffered medium, and probably not all the anthocyanins are extracted (HERMOSÍN *et al.*, 2002).

The results showed that the highest total anthocyanin content (mg/kg of berries) was obtained in grapes harvested in September 11 and 16 and applying the method described by SAINT-CRIQ *et al.* (1998b), the maximum extractability could be found on August 16, September 18 and October 24 (lowest values of phenolic maturity index), although in these cases, the potential anthocyanins that could be extractable (ApH1) were low, especially in the grapes harvested in August 16. Now, we turned our attention to seeing whether these results (anthocyanin concentration in grapes and phenolic maturity index) correlated with the color characteristics of the wines obtained.

Table III shows the chemical and chromatic characteristics of the different wines at the end of alcoholic fermentation and when the wines achieved their malolactic fermentation. As expected, the lowest ethanol percen-

J. Int. Sci. Vigne Vin, 2006, **40**, n°1, 15-24

Table III - Enological and chromatic characteristics of the wines obtained from grapes harvested at different ripening stages

Caractéristiques œnologiques et chromatiques des vins provenant de raisins récoltés à différentes dates (n=3)

Date of harvest	%EtOH	pH	SO_2 F/T	CI	Hue	%Y	%R	%B	A280	MA (mg/L)	PA (mg/L)	L*	a*	b*
Wines after maceration and alcoholic fermentation														
August 16	11.2a	3.5a	9/39	12.72a	0.50d	29.0b	61.2b	9.8b	48.85a	152.6a	69.2a	12.1cd	42.9bc	20.6cd
September 11	13.6b	3.6b	9/47	14.36b	0.45a	28.4a	62.7c	8.8a	51.4ab	207.5b	81.1b	12.7d	44.4c	21.8d
September 16	13.8c	3.6b	9/49	15.53d	0.47bc	29.2b	61.5b	9.3a	58.4bc	227.2c	92.8d	11.3bc	43.1bc	19.5bc
September 18	13.5b	3.6b	10/50	15.26cd	0.46ab	28.8ab	62.0bc	9.2a	55.8abc	228.1c	89.6cd	11.6bcd	43.3c	20.0bcd
October 16	14.5d	3.5a	10/28	17.24e	0.48c	29.9c	61.3b	8.8a	62.9c	247.6d	96.4d	9.6a	40.4a	16.6a
October 24	15.9e	3.5a	10/21	15.07c	0.53d	31.7d	59.0a	9.3a	60.8c	206.0b	84.8bc	10.7ab	41.6ab	18.4ab
Wines after malolactic fermentation														
August 16	11.2a	3.4a	22/89	7.6a	0.61c	33.6bc	54.8a	11.6d	35.6a	113.4a	68.9a	19.8b	51.3b	28.1c
September 11	13.6b	3.5b	15/79	14.7c	0.53a	30.5a	57.6bc	11.9d	47.0b	139.3b	113.8c	8.6a	39.4a	14.8a
September 16	13.8b	3.5b	25/90	9.8b	0.55ab	32.3ab	57.9cd	9.8ab	48.1bc	202.7c	78.3a	17.0b	49.3b	28.5c
September 18	13.6b	3.6b	20/93	9.3ab	0.59bc	33.5bc	56.5abc	10.0b	50.6c	215.3c	76.1a	17.6b	50.2b	29.4c
October 16	14.6c	3.4a	18/71	14.9c	0.52a	31.2a	59.5d	9.2a	63.1d	200.7c	107.4c	11.2a	42.4a	19.3b
October 24	15.9d	3.4a	18/47	13.3c	0.62c	34.6c	55.8ab	9.5ab	61.2d	137.2b	94.5b	12.2a	43.5a	21.1b

Abbreviations: %EtOH: percentage of alcohol, SO_2F/T: mg/L SO_2 content (free and total) CI: color intensity, %Y, %R, %B: percentages of yellow, red and blue in wine color, A280: absorbance at 280 nm, estimation of total phenols; MA: monomeric anthocyanins, PA: polymeric anthocyanins.
Different letters in the same column mean significant differences at p>95 % according to a LSD test, n=3

tage was found in the wines elaborated from grapes harvested on August 16 and the highest percentage in those wines elaborated from grapes harvested on October 24, which reached values around 16 %. The values of pH were very similar for all the wines, since must acidity was corrected at the beginning of winemaking.

The chromatic characteristics of the wines after alcoholic fermentation showed that wines from the grapes harvested on October 16 had the highest total phenol content, expressed as absorbance at 280 nm (A280), monomeric, polymeric anthocyanin content and thus the highest color intensity, being the darkest wines (lowest L* value) with the lowest hue. Grapes harvested on September 16, which had high skin anthocyanin concentration (mg/kg of berries) and anthocyanin values, both in the solutions at pH 1.0 and pH 3.6, resulted in wines with lower total phenol content, lower anthocyanin content and lower color intensity than those obtained from grapes harvested on October 16. Coincident with our results, SIMS and BATES (1994) and PEREZ-MAGARIÑO and GONZALEZ-SAN JOSÉ (2004) found that wines from grapes harvested one to two weeks after what is considered full mature grapes (full color and flavor development) had higher overall color intensity, and total anthocyanin and total phenol content.

The rain that occurred between September 16 and 18 did not affect the wine color characteristics. It can be seen then, that the flavonoid compounds measured in the grapes did not correlate with their content in wines, because other factors should have been involved. Probably, the higher permeability of the cell walls in the more mature samples led to greater extraction of anthocyanins and other phenolic compounds. The softening of the cell walls and solubilization of the pectins from these cell walls increase with ripening (BARNAVON *et al*., 2001; NUNAN *et al.*, 1998; NUNAN *et al.*, 2001), facilitating anthocyanin extraction (AMRANI JOUTEI and GLORIES, 1994). In addition, the possible effect of the higher ethanol content in wines made from the more mature grapes should not be overlooked, since it may help to skin and seeds degradation, and this can not be exactly evaluated using the phenolic maturity index assay. The higher alcohol concentration of wines made from grapes harvested in October may have enhanced tannins extraction especially from seeds, leading to a higher phenol content, as shown by the A280.

Wines were again analyzed after malolactic fermentation, observing a decrease in color intensity. Such decrease occurred in all the wines, especially in these made from grapes harvested on August, and on September 16 and September 18. One exception was the wine made from grapes harvested on September 11, in which, despite the decrease in monomeric anthocyanins (those eluting from the C18 Sep-pak cartridge with 16 % acetonitrile pH 2 and that mainly comprise the mono-

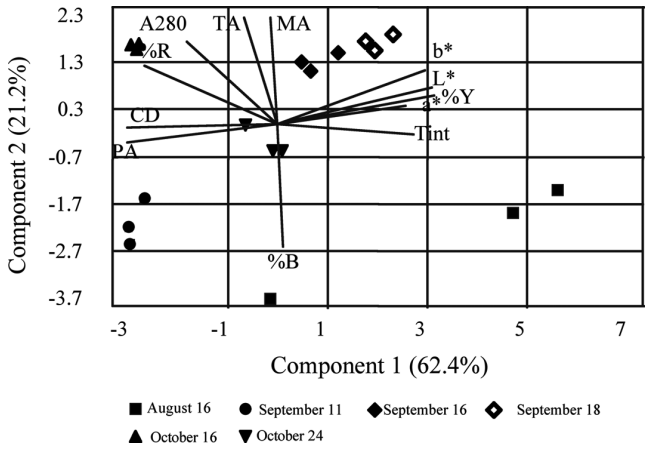

Figure 3 - Distribution of wines (after malolactic fermentation) in the two dimensional coordinate system defined by the first two principal components (the percentage of variance explained by each principal component is showed between brackets)

(Abbreviations: CI: color intensity, MA: monomeric anthocyanins, PA: polymeric anthocyanins, A280: total phenol content, %R: percentage of red color, %Y: percentage of yellow color, %B: percentage of blue color)

Distribution des vins (après fermentation malolactique) dans un système bidimensionnel défini par les deux premiers principaux composés (le pourcentage de la variance expliqué par chaque composé principal est noté entre parenthèses)

glucosides and acetyl and coumaryl derivatives of delphinidin, cyaniding, petunidin, peonidin and malvidin), the polymeric anthocyanins (those eluting from the C18 Sep-pak cartridge with methanol and that mainly comprise anthocyanin derivatives such as pyranoanthocyanins and anthocyanin and tannin adducts) increased and the color intensity did not decrease, showing values similar to wines from the grapes harvested in October 16. In looking for an explanation to this fact, we found that high quantities of acetaldehyde had been formed in these wines at the end of alcoholic fermentation (26, 42, 35, 36, 24, and 18 mg/L of acetaldehyde, measured with an enzymatic test, in the wines from August 16, September 11, 16, 18, and October 16 and 24 grapes, respectively). Perhaps a greater degree of oxygenation occurred in these wines, promoting a higher formation of acetaldehyde (LIU and PILONE, 2000), therefore, increasing the polymeric anthocyanin formation and the blue percentage of the wine color (DALLAS *et al.*, 1996). Acetaldehyde has been shown to increase spectral color and anthocyanin-tannin polymerization in red wines (SIMS and MORRIS, 1986), with no increase in browning (DALLAS *et al.*, 1996) and that is coincident with our results. DALLAS *et al.* (1996) reported that the interaction between anthocyanin and acetaldehyde led to the formation of new compounds that showed a violet color. From our results, we can see how the percentage of blue color was higher in wines obtained from September 11 grapes.

Our experiment therefore produced two wines with similar color intensity after malolactic fermentation but with different chromatic characteristics, one having less A280 and monomeric anthocyanins but a high percentage of blue color and polymeric anthocyanins. Multivariate analysis has proven its usefulness in the detection of the variables which best differentiate wine samples. When representing the wines in the plane defined by the two components obtained after performing a principal component analysis (figure 3), it can be seen how the wines from grapes harvested on September 11 and October 16 are clearly separated, the variables responsible for the sample distribution being % blue (higher in wines from grapes harvested on September 11) and A280, %red and monomeric anthocyanins (higher in wines from grapes harvested in October 16).

The next question to be addressed was whether the wines maintain their characteristics with aging, so we decided to study the evolution of chromatic characteristics during a period of eight months after bottling. We decided to eliminate wines made from the first harvested grapes and from the last harvested grapes, the first due to its low color intensity and the last because of its high yellow percentage, low aromatic intensity and high ethanol content.

ZAMORA (2003) stated that the phenolic composition of a young wine greatly influences the future evolution of wine color during aging. He gives, as orientative values, that total anthocyanins should be higher than 800 mg/L, total tannins should be higher than 3 g/L and the A280 should be higher than 60, with a minimum value of 40. At the moment of bottling, the wine from grapes harvested on October 16, with the highest anthocyanin content, A280 and tannin content (table IV) was considered to be the wine that should best withstand aging. At that moment, wines from the grapes harvested on September 11 still showed a high polymeric anthocyanin content and a high polymerization level of tannins, as reflected in the HCl index. After four months in bottle, the color intensity started to decrease in the wine from grapes harvested on September 11 and increased in that from October 16. After eight months of aging, the decrease observed in polymerized compounds was greater in the wine from grapes harvested on September 11, perhaps due to the formation of phenolic polymers large enough to precipitate (a decrease in HCl index was also obserbed). LIU and PILONE (2000) also reported that acetaldehyde may cause turbidity and deposits, presumably due to the precipitation of large anthocyanin-tannin complexes.

Table IV - Chromatic characteristics of the wines (after cold stabilization and bottling) obtained from grapes harvested at different ripening stages

Caractéristiques chromatiques des vins (après stabilisation par le froid et embouteillage) provenant de raisins récoltés à différents stades de maturité

	CI	Hue	%Y	%R	%B	A280	MA (mg/L)	PA (mg/L)	L*	TT (g/L)	HCl index
Date of harvest	Wine characteristics at bottling (t=0 months)										
September 11	14.5b	0.56a	30.5a	54.2a	15.3b	47.4a	61.3a	140.2c	7.1a	2.1a	48.2b
September 16	11.2a	0.56a	32.3b	54.4b	10.3a	52.7c	129.5b	88.9a	14.9bc	2.5b	30.3ba
September 18	10.9a	0.55a	31.8ab	57.9c	10.3a	50.3b	121.4b	89.1a	15.4c	2.1a	31.7a
October 16	15.6b	0.56a	32.3b	57.4c	10.3a	61.6c	125.3b	116.8b	10.0ab	3.7c	33.3a
Date of harvest	Wine characteristics at bottling (t=4 months)										
September 11	13.5b	0.58a	31.4a	53.6a	15.0b	48.9a	65.6a	150.4b	7.8a	1.8a	45.7b
September 16	11.5a	0.59b	33.0b	55.6b	11.3a	54.3b	145.3c	110.4a	13.0b	2.1b	35.5a
September 18	11.5a	0.57a	32.4b	56.2b	11.4a	51.7b	127.8bc	105.4a	12.8b	1.9ab	38.4a
October 16	16.7c	0.58a	32.6b	55.7b	11.6a	63.0c	111.1b	159.7b	7.3a	3.6c	36.7a
Date of harvest	Wine characteristics at bottling (t=8 months)										
September 11	12.45b	0.6a	32.1a	53.0a	14.8b	47.6a	70.5a	133.7b	8.8a	1.8a	39.5c
September 16	10.6a	0.6a	34.2c	54.1b	11.6	52.8b	127.8c	101.1a	13.7b	2.3b	32.1a
September 18	10.9a	0.6a	33.2b	54.7c	12.0a	51.2b	113.9c	105.8a	12.9b	2.1b	34.3ab
October 16	15.5c	0.6a	33.1b	54.6c	12.2a	64.1c	91.5b	150.6c	7.8a	3.8c	37.4bc

Different letters in the same column means significant differences at p>95 % according to a LSD test, n=3
Abbreviations: CI: color intensity, %Y, %R, %B: percentages of yellow, red and blue in wine color, A280: absorbance at 280 nm, estimation of total phenols; MA: monomeric anthocyanins; PA: polymeric anthocyanins; TT: total tannins

The principal component analysis of all the four wines during the aging period (figure 4) showed that all the wines decreased their values in PC1 as bottling time increased (decreasing L*, %red, HCl index and monomeric anthocyanins), except the wine from grapes harvested on September 11, that showed a decrease in PC2 values during aging (decreasing color intensity, A280, and polymeric anthocyanins and increasing in hue). The wines from October 16 had the highest values in PC2 (high color intensity, polymeric anthocyanins and A280), showing a stable behavior during aging.

Evidently, it is not only grape characteristics that influence wine color, but also enological factors such as the acetaldehyde content (without forgetting very important enological factors such as total acidity, pH, temperature, oxygen, etc.). The high level of acetaldehyde in the wine from grapes harvested on September 11 increased its color intensity almost up to values similar to those of the wines from grapes harvested on October 16 but with a phenolic content (low A280, low tannins and low total anthocyanins concentration) that could not guarantee the color stability shown by the latter wines.

We conclude that the wine made from grapes harvested on October 16 had the best color characteristics and was the most promising wine, despite the fact that the original grapes had a lower anthocyanin concentration and similar phenolic maturity index to those harvested on September 16. This fact shows that the anthocyanin content of grapes does not necessarily correlate with wine color and that the phenolic maturity index is not a useful tool for predicting the best harvesting date although it can be useful for predicting some of the chromatic wine parameters and for planning the fermentation process according to the characteristics of the grapes at the moment of harvest and the desired wine (ROMERO-CASCALES *et al*., 2005).

Therefore, it is difficult to propose criteria to decide when grapes are ready for harvest. The results show the convenience of waiting until berry anthocyanins start to diminish after reaching their maximum levels to obtain highly colored wines, since the degradation of cell walls will facilitate anthocyanin extraction. However, viticulturists and winemakers have to face other problems in warm viticultural regions, where the rapid accumulation of sugars in hot years might impose a premature harvest, since any delay would result in high alcohol levels. The

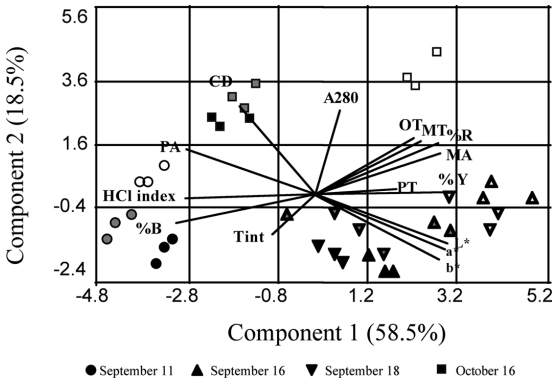

Figure 4 - Distribution of wines (during bottle aging) in the two dimensional coordinate system defined by the first two principal components (the percentage of variance explained by each principal component is showed between brackets)

(Abbreviations: CI: color intensity, MA: monomeric anthocyanins, PA: polymeric anthocyanins, A280: total phenol content, TT: total tannis, %R: percentage of red color, %Y: percentage of yellow color, %B: percentage of blue color, open symbols: t=0, grey symbols: t=4 months; black symbols: t=8 months)

Distribution des vins (pendant le vieillissement en bouteille) dans un système bidimensionnel défini par les deux premiers principaux composés (le pourcentage de la variance expliqué par chaque composé principal est noté entre parenthèses)

decision has to be taken on a year to year basis, looking for a slight overmaturation, but also bearing in mind the sugar content to avoid an excessively high alcohol level in the wines, which may be not adequate for wine tasting and consumption.

Acknowledgements : This work was made possible by financial assistance of the Ministerio de Ciencia y Tecnología, Project VIN00-028-C2-1 and the cooperation and assistance of the winery Julia Roch e Hijos.

REFERENCES

AMRANI JOUTEI K. and GLORIES Y., 1994. Étude en conditions modèles de l'extractibilité des composés phénoliques des pellicules et des pepins de raisin rouges. *J. Int. Vigne Vin*, **28**, 303-317.

AMRANI JOUTEI K. and GLORIES Y., 1995a. Tanins et anthocyanes: localisation dans la baie de raisin et mode d'extraction. *Rev. Fr. Oenol.*, **153**, 28-31.

AMRANI JOUTEI K. and GLORIES Y., 1995b. Localization and extractability of tannins and anthocyanins of grape skins. *In: Œnologie l995*. Ed. A. Lonvaud-Funel Ed. Lavoisier, Paris, pp. 119-123.

BARNAVON L., DOCO T., TERRIER N., AGEORGES A., ROMIEU C. and PELLERIN P., 2001. Involvement of pectin methyl-esterase during the ripening of grape berries: partial cDNA isolation, transcript expression and changes in the degree of methyl-esterification of cell wall pectins. *Phytochemistry*, **58**, 693-701.

DALLAS C., RICARDO DA SILVA J. M. and LAUREANO O., 1996. Products formed in model wine solutions involving anthocyanins, procyanidin B2 and acetaldehyde. *J. Agric. Food Chem.*, **44**, 2402-2407.

DOWNEY M., HARVEY J. and ROBINSON S., 2004. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.*, **10**, 55-73.

ESTEBAN M. A., VILLANUEVA M. J. and LISSARRAGUE J. R., 2002. Relationships between different berry components in Tempranillo (*Vitis vinifera* L.) grapes from irrigated and non-irrigated vines during ripening. *J. Sci. Food Agric.*, **82**, 136-146.

FERNANDEZ-LOPEZ J. A., ALMELA L., MUÑOZ J. A., HIDALGO V. and CARREÑO J., 1998. Dependence between colour and individual anthocyanin content in ripening grapes. *Food Res. Int.*, **31**, 667-672.

FERNANDEZ-LOPEZ J. A., HIDALGO V., ALMELA L. and LOPEZ-ROCA J. M., 1992. Quantitative changes in anthocyanin pigments of *Vitis Vinifera* c.v. Monastrell during maturation. *J. Sci. Food Agric.*, **58**, 153-155.

GARCÍA-BENEYTEZ E., REVILLA E. and CABELLO F.,2002. Anthocyanin pattern of several red grape cultivars and wines made from them. *Eur. Food Res. Technol.*, **215**, 32-37.

GLORIES Y., 1984. La couleur des vins rouges:1º partie "les équilibres des anthocyanes et des tanins. *Connaissance Vigne Vin*, **18**, 195-217.

GLORIES Y. and SAUCIER C., 2000. Tannin evolution from grape to wine. Effects on wine taste. 353-355. *Proceedings of the ASEV 50th Anniversary Annual Meeting*, Seattle, Davis, pp. 353-355.

GONZALEZ-NEVES G., GIL G. and FERRER M., 2002. Effect of different vineyard treatments on the phenolic contents in Tannat (*Vitis vinifera* L.) grapes and their respective wines. *Food Sci. Technol. Int.*, **8**, 315-321.

HERMOSÍN I., BLANCO F. J. and CASTRO J. M., 2002. Evolución de la madurez fenólica en uvas tintas de las variedades Cencibel (Tempranillo) y Cabernet Sauvignon. *Tecnología de Vino*, **8**, 37-47.

HO P., DA CONCEIÇÃO M., SILVA M. and HOGG T. A., 2001. Changes in the colour and phenolic composition during the early stages of maturation of port in wood, stainless steel and glass. *J. Sci. Food Agric.*, **81**, 1269-1280.

ILAND P., 1987. Interpretation of acidity parameters in grapes and wine. *Austr. Grapegr. Winemaker*, 280, 81-85

JACKSON D. I. and LOMBARD P. B., 1993. Environmental and management practices affecting grape composition and wine quality, a review. *Am. J. Enol. Vitic.*, **44**, 409-430.

LECAS M. and BRILLOUET J. M., 1994. Cell wall composition of grape berry skins. *Phytochemistry*, **35**, 1241-1243.

LIU S. Q. and PILONE G. J., 2000. An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. *Int. Food Sci. Tech.*, **35**, 49-61.

MCCARTHY M., 1999. Weight loss from ripening grapevines (*Vitis vinifera* L. Shiraz). *Aust. J. Grape Wine Res.*, **5**, 10-16.

NUNAN K. J., DAVIES C., ROBINSON S. P. and FINCHER G. B., 2001. Expression patterns of cell wall-modifying enzymes during grape berry development. *Planta*, **214**, 257-264.

NUNAN K. J. SIMS, I. M., BACIC A. and ROBINSON S. P., 1998. Changes in cell wall composition during ripening of grape berries. *Plant Physiol*., **118**, 783-792.

O.I.V., 1990. *Recueil des méthodes internationales d'analyse des vins et des moûts.* Office International de la Vigne et du Vin, Paris.

PÉREZ-MAGARIÑO S. and GONZÁLEZ-SAN JOSÉ M.L., 2004. Evolution of flavanols, anthocyanins and their derivatives during the aging of red wines, elaborated from grapes harvested at different stages of ripening. *J. Agric. Food Chem*., **52**, 1181-1189.

REVILLA I., PEREZ-MAGARIÑO S., GONZALEZ-SAN JOSE M.L., and BELTRAN S., 1999. Identification of anthocyanin derivatives in grape skin extracts and red wines by liquid chromatography with diode array and mass spectrometric detection. *J. Chromatography*, **847**, 83-90.

RIBÉREAU GAYON P., GLORIES Y., MAUJEAN A. and DUBOURDIEU D., 1998. *Traité d'Œnologie.2. Chimie du vin. Stabilisation et traitements.* Ed. Dunod, Paris.

ROMERO-CASCALES I, ORTEGA-REGULES A, LÓPEZ-ROCA J.M., FERNÁNDEZ-FERNÁNDEZ J.I., GÓMEZ-PLAZA E., 2005. Differences in anthocyanin extractability from grapes to wines according to variety. *Am. J. Enol. Vitic.*, **56**, 212-219.

SAINT-CRICQ DE GAULEJAC N., VIVAS N. and GLORIES Y., 1998a. Maturation phénolique des raisins rouges et relation avec la qualité des vins. Comparaison des cépages Merlot et Tempranillo. *Prog. Agric. Vitic.*, **115**, 306-318.

SAINT-CRICQ DE GAULEJAC N., VIVAS N. and GLORIES Y., 1998b. Maturité phénolique: définition et contrôle. *Rev. Fr. Œnol*., **173**, 22-25.

SIMS C. and MORRIS J., 1986. Effects of acetaldehyde and tannins on the color and chemical age of red muscadine wine. *Am. J. Enol. Vitic.*, **37**, 163-165.

SIMS C. A. and BATES R. P., 1994. Effects of skin fermentation time on the phenols, anthocyanins, ellagic acid sediment, and sensory characteristics of red *Vitis rotundifolia* wine. *Am. J. Enol. Vitic*., **45**, 56-62.

SUDRAUD P., 1958. Interpretation des courbes d'absortion des vins rouges. *Ann. Tech. Agric*., **7**, 203-208.

WATSON B., 2003. Evaluation of winegrape maturity. *In: Oregon Viticulture*. Ed. E. Hellman, Oregon State University Press, Corvallis, pp. 235-245.

ZAMORA F., 2002. La madurez fenólica, un tema abierto. *Enologos*, **18**, 24-28.

ZAMORA F., 2003. *Elaboración y crianza del vino tinto: Aspectos científicos y prácticos*. AMV Ediciones, Madrid.

ZOECKLEIN B., 2002. Red wine phenolic management. Enology notes number 61. http://www.fst.vt.edu/extension/enology/contentextenologynotes61.html.

Manuscrit reçu le 26 juillet 2005 ; accepté pour publication, après modifications le 18 janvier 2006