# ANTHOCYANINS AND TANNINS IN FOUR GRAPE VARIETIES (*VITIS VINIFERA* L.) EVOLUTION OF THEIR CONTENT AND EXTRACTABILITY

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**Abstract**

**Aims**: The knowledge of parameters as the quantity of anthocyanins and tannins present during grape maturation, their evolution during the ripening period and extractability data could improve the management of red wine fermentation and help predict the color of wines.

**Methods and results**: Grapes from *Vitis vinifera* var. Cabernet Sauvignon, Syrah, Merlot and Monastrell were harvested in 2002, 2003 and 2004 from a commercial vineyards and their physico-chemical characteristics together with the anthocyanin and tannin evolution during ripening were followed. The results showed that differences were found in the evolution of these compounds due to variety and year.

**Conclusions**: Some of the studied parameters seemed to be clearly related to variety (berry weight and extractability index) and others seemed to be clearly affected by soil and edaphoclimatic conditions. The high extractability index in Monastrell reflects the difficulty involved in extracting their anthocyanins. The short maturation period observed for Merlot grapes and the high seed tannin index could lead to excessively astringent wines.

**Significance and impact of the study**: This study demonstrate that the ripening period of some varieties (Merlot) in very warm conditions is too short, promoting the rapid accumulation of sugar in the pulp and an incomplete seed maturation that may lead to excessively astringent wines. Other varieties, e.g. Monastrell, show longer maturation period that usually permits pulp and seed maturity to be reached at the moment of harvest. The extractability index seems to be closely related to the grape variety and this is also an important fact to take into account when planning a vinification process.

**Key words**: anthocyanins, tannins, phenolic maturity, extractability, grapes

**Résumé**

**Objectifs** : La connaissance de certains paramètres tels que la quantité d'anthocyanes et de tannins, leur évolution pendant la période de maturation et leur extractibilité peuvent permettre d'améliorer la conduite de la vinification et aider à prévoir la couleur des vins obtenus.

**Méthodes et résultats** : les caractéristiques physico-chimiques et l'évolution d'anthocyanes et de tannins ont été étudiés pendant la maturation de cépages Cabernet-Sauvignon, Merlot, Syrah et Mourvèdre pendant trois années, 2002, 2003 et 2004. Les résultats ont montré des différences dans l'évolution de ces composés en fonction du cépage et de l'année de vendange.

**Conclusions** : On a démontré que certains paramètres étudiés (taille des baies et indice d'extractabilité) sont en rapport avec la variété, et d'autres dépendent du sol et des conditions climatiques. L'indice d'extractabilité élevé pour la varieté Mourvèdre reflète les problèmes dus à l'extraction des anthocyanes de la pellicule. La période de maturation très courte des raisins Merlot, accompagnée par un indice élevé de maturation des pépins pourrait entraîner, dans les vins, une astringence excessive.

**Signification et impact de l'étude** : Cette étude montre que la période de maturation de certains cépages (Merlot) est très courte en période de forte chaleur, ce qui mène à une accumulation rapide des sucres dans la pulpe. Ceci est accompagné notamment par une maturation incomplète des pépins, avec une forte concentration en tannins, ce qui peut entraîner dans les vins une astringence excessive. D'autres cépages, comme le Mourvèdre, montrent une période de maturation plus longue, ce qui permet d'obtenir la maturation dans la pulpe et les pépins au moment de la vendange. L'extractabilité dépend fortement du cépage et c'est un facteur à prendre en compte pour la planification de la vinification.

**Mots-clés** : anthocyanes, tannins, maturité phénolique, extractabilité, raisins

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## INTRODUCTION

Anthocyanins and tannins are among the most important phenolic compounds responsible for colour and astringency of wines. These compounds accumulate primarily in the skins and seeds of grape berries and are extracted into the must and wine during pomace contact.

Anthocyanins are only located in the skin. Within the cells they are located in the vacuoles, in free, non-complexing form (AMRANI JOUTEI and GLORIES, 1995; HRAZDINA and MOSKOWITZ, 1980). The concentration of anthocyanins varies with varieties (AROZARENA *et al.*, 2002; CACHO *et al.*, 1992; FERNANDEZ-LOPEZ *et al.*, 1995; GARCIA-BENEYTEZ *et al.*, 2002; GARCIA-BENEYTEZ *et al.*, 2003; REVILLA *et al.*, 2001), maturity (ESTEBAN *et al.*, 2001; KENNEDY *et al.*, 2002; ROGGERO *et al.*, 1986; RYAN and REVILLA, 2003), seasonal conditions (CACHO *et al.*, 1992; ESTEBAN *et al.*, 1999), production area (MATEUS *et al.*, 2001) and viticultural practices (DE LA HERA ORTS *et al.*, 2005; GUIDONI *et al.*, 2002).

Grape tannins are a very diverse set of biomolecules, varying in size from dimers and trimers up to oligomers with more than 30 units (ADAMS, 2006). Tannins are located in both skins and seeds, although the average size of skin tannins is much greater than that of seed tannins and skin tannins contain epigallocatechin subunits, whereas seed tannins generally do not (ADAMS, 2006). Tannins have been less studied than anthocyanins and less is known about the relationship between varieties, climatic conditions, production practices and their concentration in grapes.

Phenolic extractability is also an important issue. Grapes that are high in phenols do not necessarily produce wines that are also rich in phenolic compounds and it has been also reported that maximum extractability does not always coincide with the maximum content of anthocyanins in the skins (BAUTISTA-ORTIN *et al.*, 2006).

Furthering our knowledge of parameters such as the quantity of these anthocyanins and tannins, their evolution during the ripening period and the extractability data of the studied grapes could improve the management of red wine fermentation and more accurately predict the color of wines. Based on this premise, we studied the evolution of anthocyanins, tannins and the extractability index in four well known varieties in the climatic conditions of SE Spain.

## MATERIAL AND METHODS

Grapes from *Vitis vinifera* var. Cabernet-Sauvignon, Syrah, Merlot and Monastrell were harvested in 2002, 2003 and 2004 from a commercial vineyard located in Jumilla (S.E. Spain). Another sample of Monastrell grapes was also harvested from a commercial vineyard located in Bullas (S.E. Spain).

### 1. Physico-chemical determinations in grapes

Total soluble solids (°Brix) were measured using an Abbé-type refractometer. Phenolic maturity (or extractability index) was calculated according to the method described by GLORIES and AUGUSTIN (1993) and SAINT-CRICQ *et al.* (1998), macerating the grapes for four hours at two different pH values (3.6 and 1). The original pH 3.2 solution of the original methods was changed for one of pH 3.6, which is more suited to the pH of the musts from our region. The anthocyanin contents of the two solutions (ApH1 and ApH3.6) were then chemically assayed by measuring the absorbance of the samples at pH 1. The extractability index was calculated as follows:

$$\text{Extractability index} = \frac{(ApH1 - ApH3.6)}{ApH1} * 100$$

### 2. Anthocyanin monoglucosides in berry skins

Grapes were peeled with the help of a scalpel, and the skins were stored at -20 °C until analysis. 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 methanolic extracts were filtered through 0.45 µm, and analyzed by HPLC (ROMERO-CASCALES *et al.*, 2005).

### 3. Tannin analysis

Seed and skin tannin concentrations were evaluated using a protein precipitation assay. The method is based on the precipitation of tannins from a solution containing BSA (bovin serum albumin), redisolving the resultant precipitate and then determining the amount of tannin by reaction with ferric chloride, yielding a colored product that can be quantified by its absorbance at 510 nm. Sample preparation and the protein precipitation assay were conducted as described by HARBERTSON *et al.* (2002).

### 4. Statistical data treatment

Significant differences among varieties and growing seasons were assessed with analysis of variance (ANOVA). This statistical analysis was performed using Statgraphics 5.0 Plus.

## RESULTS AND DISCUSSION

The evolution of the physico-chemical data of the grapes during maturation as well as their anthocyanin and tannin content and extractability index were studied during three growing seasons. The climatic data of these three seasons showed very different characteristics (table 1). Year 2002 was a rather cool year with substantial rainfall during the ripening period, especially in the Jumilla area. Year 2003 was characterized by very high temperatures that lasted for several weeks and, in Jumilla, 2003 was also characterized for being very dry. Year 2004 was also a very warm year, although not to the same extent as 2003, with levels of rainfall slightly higher.

Although all the different parameters were studied and followed the three growing seasons, the trend of evolution was very similar, therefore only the evolution of the different parameters during 2003 is shown in the figures. Figure 1 shows the evolution of berry size and sugar content. The size of grapes is of fundamental importance for the quality of the subsequent wines. Anthocyanins and other compounds of interest are synthesized in the skin. Therefore, berry size is considered a quality factor since the most important compounds liberated during vinification are found in the skin and a larger berry weight will result in a lower skin-to-flesh ratio as a result of a dilution effect. Berry weight was significantly higher in the Monastrell grapes and particularly in those from the Bullas area (Monastrell-B), reaching 170 g per 100 berries. The smallest berries were found in Cabernet-Sauvignon grapes. As regards the evolution of this parameter during the last stages of ripening, Cabernet-Sauvignon, Syrah and Merlot grapes always increased their berry weight. Monastrell, especially Monastrell-B, reached their maximum weight very soon during ripening, after which the weight remained stable or even, occasionally, decreased, probably due to water losses in the later stages of ripening. Cabernet-Sauvignon, Syrah, Merlot and Monastrell from Jumilla area (Monastrell-J) presented a maximum berry weight lower than 1.6 g, being this size, and according to some authors, optimum for red wine vinification (REYERO *et al.*, 1999). Only Monastrell-B grapes were larger than this. These authors, and coincident with DE LA HERA *et al.* (2005) indicated that large berries are directly related to a dilution of those compounds closely related with the quality of the berry.

### 1. Soluble solids

The criteria traditionally used to determine grape maturity are based on sugar content, which is normally measured in °Brix. High values of °Brix indicate a high degree of ripeness, At the moment of veraison the grapes presented a sugar content between 10-15 ºBrix (Figure 1). As maturation advanced, the sugar content kept increasing and, at the moment of harvest, the grapes from the different varieties reached high sugar levels, and this fact may be closely related to the high temperature reached during the ripening period. Cabernet-Sauvignon, and especially Syrah and Merlot showed a shorter ripening period than Monastrell, which has a particularly long ripening period. The short ripening period of Syrah and Merlot could be detrimental for wine quality since pulp maturity may be reached some time before seed maturity, and immature and astringent seeds may then participate in the winemaking process.

### 2. Anthocyanins

Total anthocyanin levels (the sum of all the anthocyanins detected by HPLC, as shown in figure 2) reflected a continous synthesis for all the grape varieties

**Table 1 - Climatic data during berry development and ripening (from June to October) in the three years of study**

| | Temperature (°C) | | | | Rainfall (mm) |
|---|---|---|---|---|---|
| | Mean | Max | Min | Abs max | Total |
| **2002** | | | | | |
| **Jumilla** | 22.9 | 25.9 | 18.0 | 37.6 (July) | 146.0 |
| **Bullas** | 21.9 | 24.7 | 17.7 | 37.5 (August) | 62.0 |
| **2003** | | | | | |
| **Jumilla** | 24.5 | 27.8 | 20.8 | 41.1 (July) | 33.0 |
| **Bullas** | 23.1 | 26.3 | 19.2 | 40.5 (July) | 72.1 |
| **2004** | | | | | |
| **Jumilla** | 24.3 | 28.9 | 19.8 | 42.8 (July) | 43.6 |
| **Bulla** | 22.5 | 27.2 | 17.5 | 42.2 (July) | 84.1 |

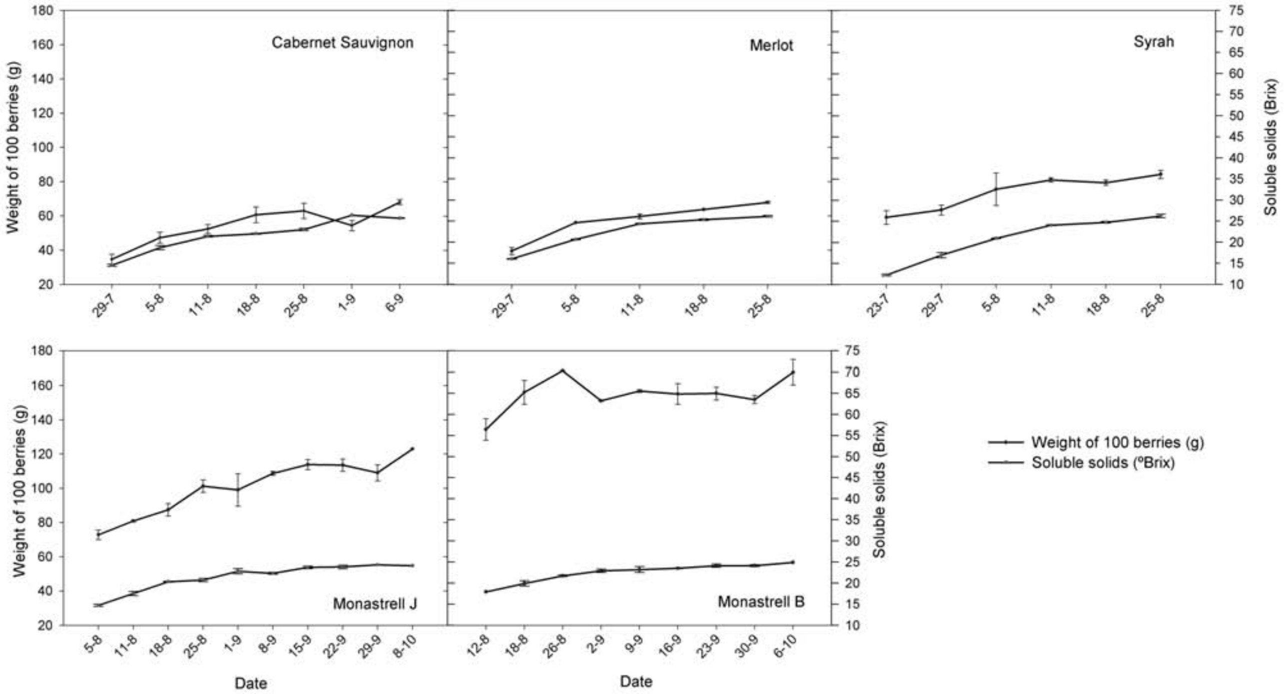


**Figure 1 - Changes in the weight of 100 berries and evolution of soluble solids during the ripening period of the different varieties**

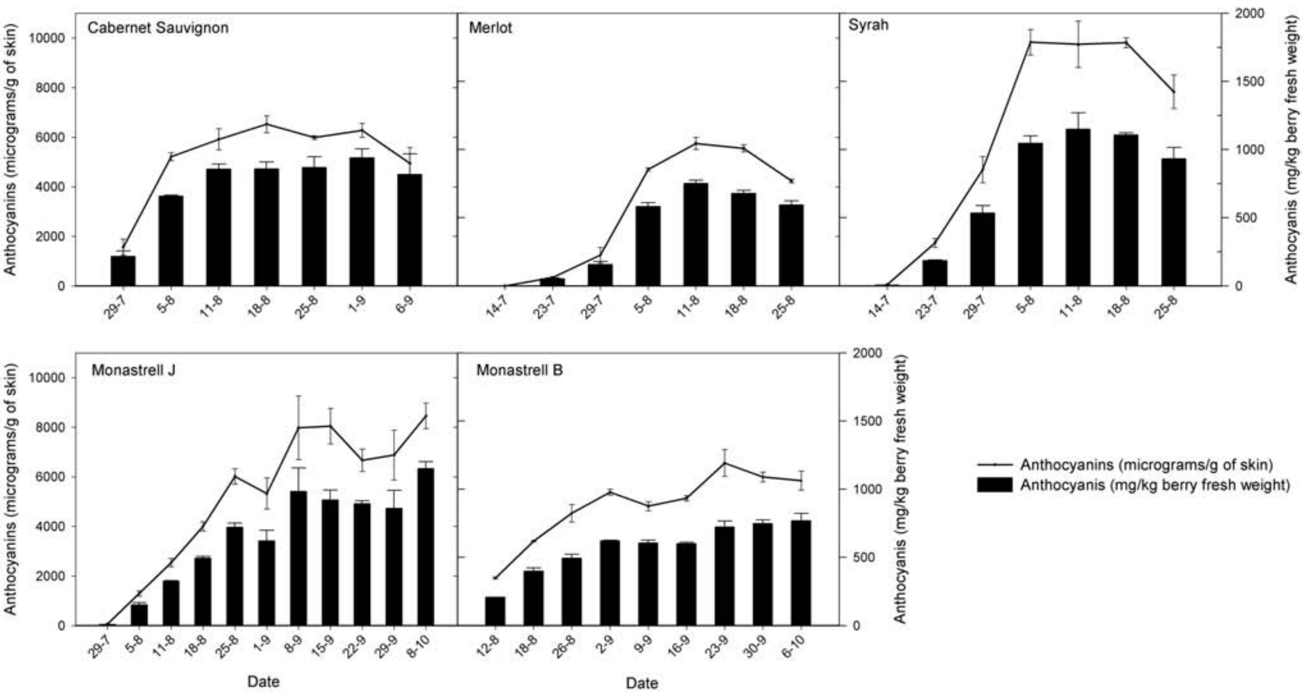


**Figure 2 - Evolution of anthocyanins in the skin (expressed as µg/g of skin and mg/kg of grapes ) during the ripening period of the different varieties.**

studied during the first weeks and a slight decrease at the end of ripening in Cabernet Sauvignon, Merlot and Syrah. The grape color appeared very fast after veraison except for Merlot.

In Cabernet-Sauvignon, Merlot and Syrah grapes, the anthocyanin concentration increased very quickly during the first weeks, the maximum value being reached at middle of August. Synthesis in Monastrell grapes was slower, as corresponds to a longer ripening period, the maximum concentration being reached a month later. After reaching a maximum, the anthocyanin concentration decreased in all varieties. A similar decline in the anthocyanin content of grapes during the period of over-ripening was reported by RIBÉREAU-GAYON and GLORIES (1980). FOURNAND *et al.* (2006) suggested that this decrease could be due to the conversion of free anthocyanins into polymeric pigments, since no differences were observed in their studies in total red pigments. The presence of such polymeric compounds in berry skins has previously been reported by other authors (VIDAL *et al.*, 2004).

The results, expressed either as µg/g of skin or mg/kg of grapes, show that Merlot grapes presented the lowest values (5700 µg/g of skin). Monastrell-J reached higher values than Monastrell-B, demonstrating the importance of soil and climate characteristics on anthocyanin accumulation.

**3. Extractability index**

The extractability index (also known as phenolic maturity index) was evaluated according to the method first described by GLORIES and AUGUSTIN (1993), macerating the grapes for four hours at two different pH values (3.6 and 1). The results are shown in figure 3. This method is based on the assumption that at pH 1, there is a complete disorganization of the vacuolar membrane which would facilitate the release of phenolic compounds. When the pH of the macerating solution is 3.6, the natural degradation of the cells is respected, a situation similar to that occurring during maceration during winemaking. The extractability is good when the difference between these two results is low. The quantity of anthocyanins was higher when the macerating solution was at pH 1, as expected, and the evolution was similar to that of total anthocyanins in the skin.

There was a continuous increase in the values of anthocyanins at pH 1 in all varieties, reaching a maximum and then a decrease in some of them was observed. The highest values were found for Syrah and Monastrell-J, which showed values higher that 700 mg/L. Contrary to our results, GONZÁLEZ *et al.* (2001) found irregular values in ApH1 for Listan Nero grapes.

The evolution of anthocyanins at pH 3.6 was similar to that of anthocyanins at pH 1 although the relationship

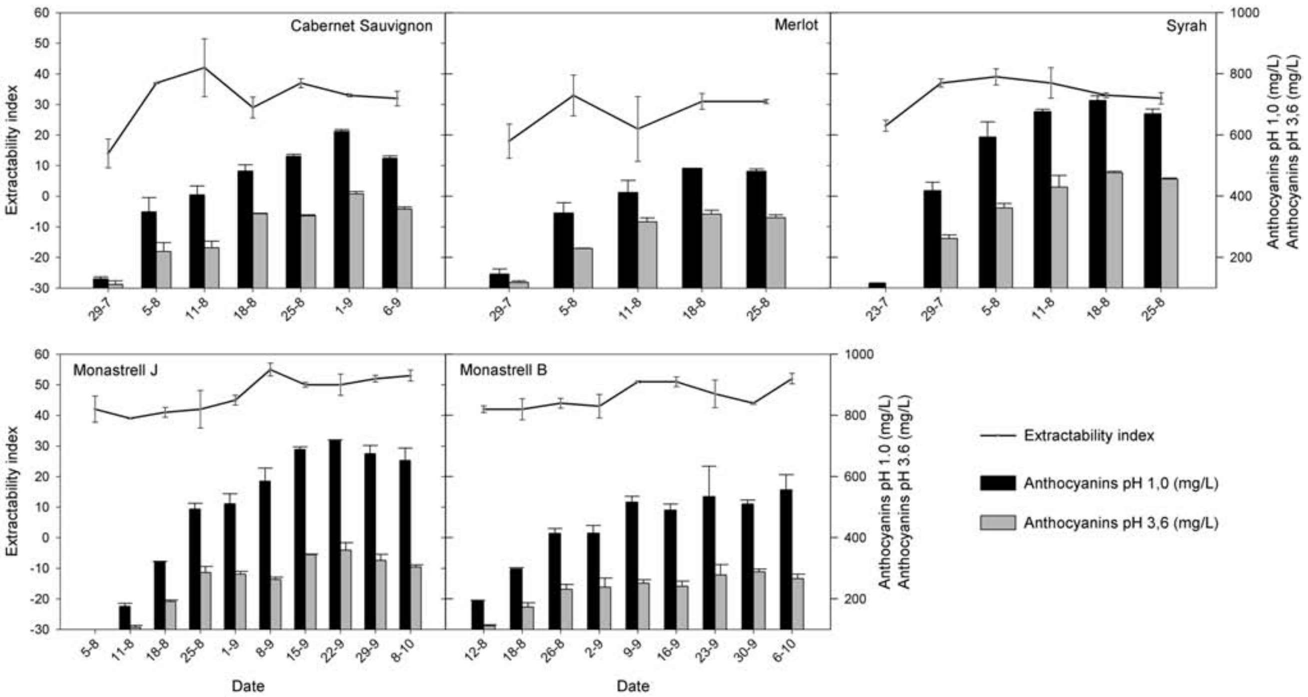


**Figure 3 - Evolution of the concentration of anthocyanins extracted in a solution at pH 1 and pH 3,6 and the extractability index**


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between both values differed according to the variety studied. In Cabernet-Sauvignon, Merlot and Syrah grapes, anthocyanin values at pH 3.6 were high, reflecting a high quantity of easily extracted anthocyanins. For Monastrell grapes, anthocyanins levels at pH 3.6 were less than half those at pH 1, indicating that anthocyanin accumulation in the skins was greater than the quantity extracted during vinification. Although their respective anthocyanin concentrations differed, similar values of extractability were found in Monastrell-J and Monastrell-B, which may indicate a clear variety effect in the easiness of anthocyanin extraction from skins.

Some authors have stated that the extractability index decreases during ripening (GLORIES, 1999). In our study, only a small decrease was observed for Cabernet Sauvignon, Merlot and Syrah, while Monastrell grapes presented high and steady values (around 50 %) from the third sampling date. A recent study on the extractability of phenolic compounds in Cabernet-Sauvignon grapes (FOURNAND *et al.*, 2006) found that the extraction yield of red pigments remained constant whatever the pulp sugar content, finding that about 70 % of total red pigments were extracted (that could be compared with an extractability index of 30, similar to our results).

## 4. Evolution of skin and seed tannins during maturation

Analysis of individual tannins is difficult because of the large number of chemical structures. Fortunately, for many practical applications, it is not essential to know the amount of individual tannins, but only the total amount of tannins (HARBERTSON *et al.*, 2002). It has been stated that skin tannins differ from seed tannins in the presence of prodelphinidins, their higher degree of polymerisation and a lower proportion of galloylated subunits (PEYROT and KENNEDY, 2003). Skin tannins have been reported to increase in size during the later stages of ripening and to undergo reactions with pectins and anthocyanins, which may affect the mouthfeel and texture of red wines as well as colour stability (KENNEDY *et al.*, 2001). Due to the analytical method used, our results only reflect quantitative changes and do not address qualitative changes. It can be considered as an index of tannins that precipitate with BSA, rather than the total quantity of tannins. The results showed that skin tannins in the different varieties diminished during ripening (Figure 4). Some authors have suggested that this decrease is due to reduced extractability as a result of tannin being bound to other cellular components (KENNEDY *et al.*, 2001; SAINT-CRICQ *et al.*, 1997). The largest decrease was found during the first weeks of maturation, except in Syrah, where an increase during the first weeks was found.

At the beginning of ripening, the highest values of skin BSA-precipitable tannins were found in Cabernet-Sauvignon and Monastrell-J but Cabernet-Sauvignon showed an important fall in this value along the ripening process. At the end of ripening, Monastrell-J and Monastrell-B showed the highest quantities of this skin tannin index.

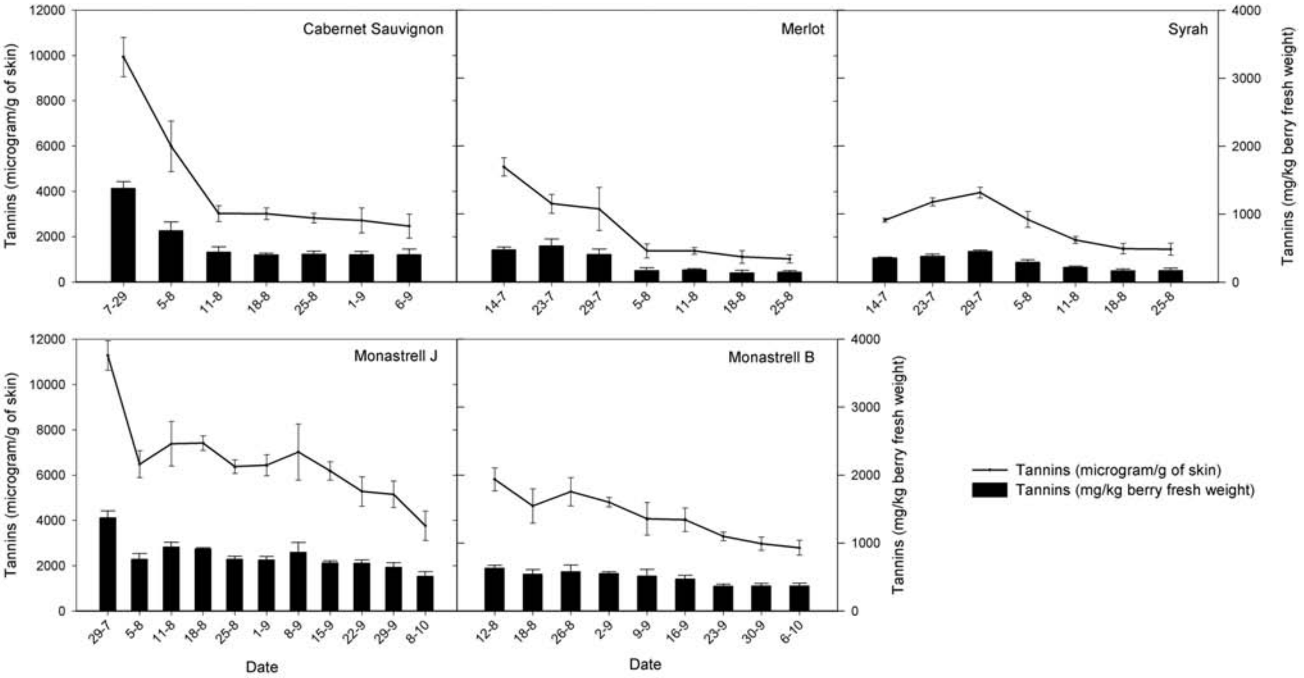


**Figure 4 - Evolution of tannins in the skin (expressed as µg/g of skin and mg/kg of grapes) during the ripening period of the different varieties**

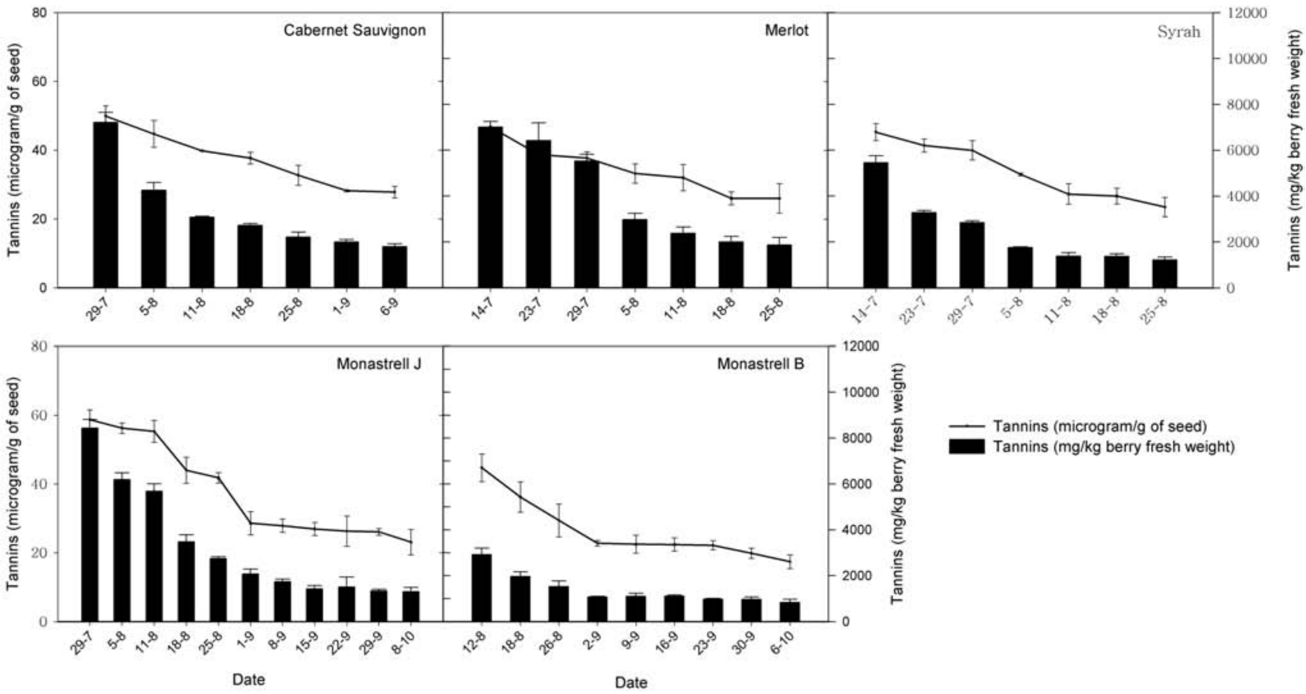


**Figure 5 - Evolution of tannins in the seeds (expressed as µg/g of seeds and mg/kg of grapes) during the ripening period of the different varieties**

When the results were expressed as mg/kg of grapes, we observed that tannins remained stable after the third week after veraison. These findings are consistent with those of HARBERTSON *et al.* (2002) but contrary to those of FOURNAND *et al.* (2006), who did not find any changes in these compounds, although differences may be due to the different methodology used to measure grape tannins. The highest values were found in Cabernet-Sauvignon and Monastrell. The levels found in Monastrell-J almost doubled those in Monastrell-B at the beginning of ripening; Merlot and Syrah showed the lowest values.

The seeds play an important role during red winemaking, contributing flavan-3-ols and procyanidins, important sensory components that provide red wine with bitterness and astringency but which also facilitate anthocyanin stabilization. DOWNEY *et al.* (2003) stated that the bulk of tannin synthesis occurred after fruit set and was complete five weeks before veraison. Concordant with this, the tannin index in seeds at veraison was high (Figure 5), and decreased during ripening. A general decline in seed tannin has been observed using several analytical methods and may reflect their covalent attachment to the insoluble matrix of the seed, rendering them unavailable for extraction during winemaking (ADAMS, 2006; SAINT-CRICQ *et al.*, 1997).

The seed tannins showed a similar evolution pattern for the different varieties during ripening. When expressed as mg/g of seeds, tannins decreased during ripening, Monastrell showing the greatest decrease. Monastrell-J showed the highest values during the first weeks of sampling and Monastrell-B the lowest. This differences observed in the same variety cultivated in two different vineyards were also detected by other authors in Pinot noir, when grapes from two different vineyards were analyzed, the differences in seed tannin content reaching up to 64 % (DOWNEY *et al.*, 2004). At the moment of harvest, Merlot presented the highest values and Monastrell-B the lowest. Merlot showed hardly any decrease in seed tannins, probably due to the short ripening period that promoted rapid pulp maturation and incomplete seed maturation.

When the results were expressed as mg/kg of fresh weight, the effect of the berry size could be observed. At the beginning of ripening Monastrell-J again presented the highest value and Monastrell-B the lowest. At the moment of harvest Cabernet-Sauvignon and Merlot showed the highest values. Seed tannins were more than three times the value of skin tannins at harvest. Similarly, HARTBERTSON *et al.* (2002) also found that seed tannins doubled skin tannins in Syrah. Seed tannins are also very important to wine quality since they participate in stabilizing wine color. The correlation between the seed tannin content and the wine tannin content was demonstrated in the studies of ROMERO-CASCALES *et al.* (2005), which showed that seed tannin was highly correlated with wine tannins ($r^2$=0.9) and total phenolic content ($r^2$=0.71).

**Table 2 - Two-way analysis of variance of the physico-chemical characteristics of the grapes at the moment of harvest according to variety and year**

| Effect | | Weight of 100 berries (g) | ºBrix | Total anthocyanins (mg/kg) | ApH1 | ApH3.6 | E% | Skin tannins (mg/kg) | Seed tannins (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|
| Variety | CS | 68.5a | 25.4a | 1244.0 d | 661.6 b | 411.0 c | 36.7 a | 632.5 c | 2031.7 c |
| | Merlot | 80.5a | 25.0a | 895.9 bc | 504.0 a | 331.8 b | 34.0 a | 290.9 a | 2139.3 c |
| | Syrah | 94.3a | 24.6a | 1090.9 cd | 626.9 ab | 430.8 c | 30.6 a | 357.5 ab | 1318.5 ab |
| | Mon-J | 175.8a | 24.9a | 841.4 bc | 627.6 ab | 335.3 b | 45.9 b | 557.0 c | 1398.0 b |
| | Mon-B | 187.5b | 24.7a | 539.2 a | 597.7 ab | 276.7 a | 52.5 b | 446.1 b | 1080.7 a |
| Year | 2002 | 131.3b | 24.3a | 993.6 b | 647.6 a | 370.1 a | 42.4 a | 339.9 a | 1719.3 b |
| | 2003 | 102.4a | 25.4b | 899.7 a | 576.6 a | 342.7 a | 40.0 a | 300.6 a | 1324.5 a |
| | 2004 | 129.3b | 25.0ab | 882.5 a | 586.5 a | 358.5 a | 37.4 a | 660.6 b | 1570.4 b |

The data of the grapes at the moment of harvest for the three years of study are shown in table 2, which represents a two way analysis of variance using variety and year as factors. Berry weight was significantly higher in the Monastrell grapes and particularly in the Monastrell from the Bullas area (Monastrell-B), reaching a mean value of 187.5 g per 100 berries. The smallest berries were found in Cabernet-Sauvignon grapes. No significant differences were found in ºBrix. Total anthocyanin content was higher in Cabernet-Sauvignon grapes as well as the anthocyanins extracted at pH 1 and pH 3.6. The highest value of the extractability index was found in Monastrell grapes. These values indicate that Monastrell grapes will need long maceration periods and frequent pumping over to extract their anthocyanins. Monastrell from Jumilla area and Cabernet-Sauvignon showed the highest values for skin tannins but Monastrell, together with Syrah grapes showed the lowest values for seed tannins.

As regards the effect of the climatic year, the highest berry weights were recorded in 2002 and 2004 and the lowest ºBrix in 2002 due to the lower temperatures and higher rainfall that year. The smaller berry weight in 2003 was related to the heat stress the grapes suffered during ripening. In the same way, anthocyanin and seed tannin synthesis were also affected by the high temperatures during ripening in 2003. It must be pointed out that the values for the extractability index were not affected by the climatic year, suggesting that it is a very stable value, closely linked to the varietal characteristics of the grapes.

## CONCLUSION

Among the studied parameters, some of them seemed to be clearly related to variety and others seemed to be clearly affected by soil and edaphoclimatic conditions. In this way, berry weight and the extractability index are closely related to variety, whereas the anthocyanin content and tannin content, although related to variety, showed also differences between the same variety cultivated in different areas and in the different growing seasons. Substantial differences in the length of the ripening period were also observed between varieties. In very warm conditions, such as those of SE Spain, the maturation period of some varieties, e.g. Merlot, is too short, promoting the rapid accumulation of sugar in the pulp and an incomplete seed maturation that may lead to excessively astringent wines. This is why other varieties, e.g. Monastrell, are more suited to this climatic conditions, because their longer maturation period usually permits pulp and seed maturity to be reached at the moment of harvest. But when processing this variety, it should be taken into account that their anthocyanins are not easily extracted and methods for promoting this extraction should be used.

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