# THE EFFECT OF CLUSTER THINNING ON PHENOLIC MATURITY OF *VITIS VINIFERA* CV. XINOMAVRO GRAPES

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## Abstract

**Aims**: The purpose of this study is to determine the effect of cluster thinning at veraison stage on berry phenolic maturity of the red Hellenic *Vitis vinifera* grape variety « Xinomavro » and more precisely on the anthocyanin content. It is noted that this variety is relatively poor in anthocyanins, but it has a high level of astringency.

**Methods and results**: This study was conducted in four experimental vine-plots within a vineyard located in the VQPRD area of Naoussa and more specifically at Giannakoxori Imathia (Northern Greece). Cluster removal was done manually on three vine-plots (the 4th one being the control) leaving one cluster per sprout (shoot). The sampling was started almost a month before grape-harvest and was repeated 2, 3 or 4 times during the ripening, depending on the vine-plot, with an interval of 3 to 7 days between each sampling. A total of 17 berry samples, coming from the vineyard mentioned above, were studied. The total phenols were assessed by UV spectrophotometry and the concentration of phenolic acids and catechin of seeds was determined by UV-HPLC.

**Conclusions and significance of the study**: The concentration of total anthocyanins during grape ripening in vine-plots that had been thinned out was increased compared to the control vine-plot. This increase varied from 52 to 89% for the study plots and 10% for the control plot. The skin tannin content was increased by 56.2 to 114% for plots that had been thinned out and only by 17.7% for the control plot. In contrary, the seed tannin content decreased during ripening by 27.2 to 50.6% compared to 23.5% for the control plot. As for phenolic acids and catechin in seeds, we conclude that catechin was the most abundant compound. Consequently, cluster thinning appeared to have contributed to the increase of the total phenolic content of the specific « Xinomavro » Greek variety and more precisely of the anthocyanins.

**Key words**: phenolic maturity, cluster thinning, phenolic acids, catechin, grape seeds, HPLC

## Résumé

**Objectifs**: Le but de cette étude vise à déterminer l'effet de la « vendange verte » des raisins, au stade de véraison, sur la maturité phénolique des baies de raisins du cépage hellénique rouge « Xinomavro » (*Vitis vinifera*) et plus précisément sur leur contenu en anthocyanes. Il est à noter que ce cépage est relativement pauvre en anthocyanes, mais il a un niveau élevé d'astringence.

**Méthodes et résultats**: Quatre parcelles expérimentales du vignoble étudié, situé dans la zone viticole VQPRD de Naoussa et plus précisément à Giannakoxori of Imathia (Grèce du Nord), ont été choisies pour les besoins de notre recherche. La « vendange verte » de raisins a été effectuée manuellement sur trois parcelles du vignoble (la 4e étant témoin) ne laissant qu'une seule grappe par sarment. L'échantillonnage a commencé un mois environ avant la récolte de raisins et a été répété 2, 3 ou 4 fois pendant la période de maturité, selon la parcelle de la vigne, avec un intervalle de 3 à 7 jours entre chaque échantillonnage. Dix-sept échantillons, provenant du vignoble susmentionné, ont été étudiés. Les phénols totaux ont été évalués par spectrophotométrie-UV et la concentration des acides phénoliques et de la catéchine de pépins a été déterminée par HPLC-UV.

**Conclusions et importance de l'étude** : La concentration des anthocyanes totaux au cours de la maturation des raisins, dans les parcelles où la « vendange verte » a été appliquée, a augmenté par rapport à la parcelle témoin. Cette augmentation varie de 52 à 89% pour les parcelles étudiées au lieu de 10% pour la parcelle témoin. La teneur des tanins des pellicules des baies de raisins a augmenté, au cours de leur maturation, de 56.2 à 114% pour les parcelles qui ont subit la « vendange verte » et seulement 17.7% pour le témoin. Contrairement à cette observation, la teneur des tanins des pépins a baissé au cours de la maturation de raisins de 27.2 à 50.6% au lieu de 23.5% pour le témoin. En ce qui concerne les acides phénoliques et la catéchine, présents dans les pépins, nous concluons que la catéchine était le composé le plus abondant. Par conséquent, la « vendange verte » des raisins semble avoir contribué à l'augmentation du potentiel phénolique des baies de raisins du cépage « Xinomavro » et plus précisément des anthocyanes.

**Mots clés** : maturité phénolique, « vendange verte », acides phénoliques, catéchine, pépins de raisins, HPLC

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## INTRODUCTION

During veraison, chlorophyll is broken down and pigments start to be formed (Stavrakas, 1999). The formation of pigments in *Vitis vinifera* species takes place in the cells of the internal skin layer called hypoderma (Soufleros, 2000). Flavonoides are the only pigments of white grape varieties, whereas anthocyanins are mostly responsible for the colour of red grape varieties. The anthocyanin composition has been studied in order to identify a variety or to prove the geographical origin of grapes (Berente *et al*., 2000; Kallithraka *et al*., 2001; Nunez *et al*., 2003). Xinomavro is a variety with medium anthocyanin potential (Lourakou-Dragonas, 1998). However, the anthocyanin content is dependent upon the region in which the grapes have been cultivated (Faustino *et al*., 2000). The most frequent anthocyanins naturally occurring in various species of Vitis are the glycosides of cyanidin, peonidin, delphinidin, petunidin and malvidin. Tannins start to be formed before veraison; therefore, at this stage, half the quantity of total phenols has already been formed (Stavrakas, 1999). The greatest amount of tannins exists in seeds (65%); an important amount exists in stems (22%), whereas in skin and pulp the concentration is 12% and 1%, respectively. Phenolic acids, which are divided into benzoics and cinnamics, are not free but they are found in a complex form (pyranoanthocyanins) with anthocyanins (Soufleros, 2000; Schwarz *et al*., 2004).

Phenolic acids (gallic, syringic, p-hydroxybenzoic, vanillic and p-coumaric) and catechin are formed during grape maturation by various reactions of oxidation, reduction, hydrolysis and methylation; however, the mechanisms are not yet perfectly understood (Kourakou-Dragonas, 1998).

The study of phenolic acids is of great importance because it is based on the fact that they have antiseptic properties, they can be used as food preservatives and they have antibacterial action (Soufleros, 2000). Moreover, they take part in the formation of flavonoids as well as non-flavonoid compounds (stilbenes). p-Coumaric acid is one of the most important phenolic acids in wine, due to the fact that it is the key metabolite in the pathway of phenylalanine ammonia lyase, which is an enzyme that participates in the formation of flavonoids and stilbenes. It has been found that the concentration of p-coumaric acid in grape berries increases during stress periods (Kilinc and Kalkan, 2003).

In grape skins there are also non-flavonoid compounds, such as resveratrol (Jeandet *et al*., 1991). Resveratrol protects the grapes from fungal infections (e.g., *Botrytis cinerea* Pers). It is also one of the phytoalexins, a class of plant antibiotics that are formed during stress or environmental conditions (Baptista *et al*., 2001).

The concentration of phenolic compounds in grapes depends on various factors, such as variety and region of cultivation, soil and climate conditions, viticulture techniques, fertilization, history of infections, as well as on the use of substances like ethylene.

Many winegrowers use polyphenols as a maturity indicator (as well as the weight of grape, the amount of sugars, the acidity) for determining the appropriate harvest-time (Perez-Magarino and Gonzalez-San Jose, 2004).

The purpose of this study is to determine the effect of grape cluster thinning at veraison stage on berry phenolic maturity of the red Hellenic *Vitis vinifera* grape variety Xinomavro and more precisely on the anthocyanin content. It is noted that this variety is relatively poor in anthocyanins, but it has a high level of astringency.

## MATERIALS AND METHODS

### 1. Preparation of samples and standards

In the present study, two 200-berry samples of Xinomavro grapes per sampling date were collected as described below (see 3a), so as to determine the various phenolic and colour markers. The sampling was started almost a month before grape-harvest and was repeated 2, 3 or 4 times during the maturation period, depending on the vine-plot (Table 1).

**Table 1. Samplings during grape maturation in 2003**

| | | Number and date of samplings | | | | |
|---|---|---|---|---|---|---|
| **Vine-plot** | **Treatment** | **1st** | **2nd** | **3rd** | **4th** | **5th** |
| 1 (14)* | Control | 10-9-2003 | - | 22-9-2003 | - | 29-9-2003 |
| 2 (16)* | Grape removal 50% | 10-9-2003 | 17-9-2003 | 22-9-2003 | 25-9-2003 | - |
| 3 (17)* | " | 10-9-2003 | 17-9-2003 | 22-9-2003 | 25-9-2003 | 29-9-2003 |
| 4 (18)* | " | 10-9-2003 | 17-9-2003 | 22-9-2003 | 25-9-2003 | 29-9-2003 |

* Numbers determinant the vine-plots in the vineyard


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A total of 17 samples were studied. The samples were obtained from a vineyard located in the viticultural zone of the Naoussa VQPRD, Giannakoxori Imathia (Northern Greece), which extends to the Vermio Mountain slopes at an altitude of 280-330 m. Vine spacing was 1.20 m x 1.50 m within row and between rows, respectively. The sample was representative, as it came from both sides of the vineyard, those sunny and those shaded, according to a completely randomised experimental design. Four plots were used. The vine-plot number 1 was the control sample without cluster-thinning treatment, whereas the other three vine-plots were the study samples with cluster thinning treatment (removal of 50% of the clusters) at veraison stage. The cluster removal was done manually and one cluster was left per sprout (shoot). Xinomavro was grafted onto 110R rootstock in the first, third and forth vine-plot, while the second vine-plot was planted with Xinomavro grafted onto 41 B rootstock. The soil was loam-clay with pH 7.5 in vine-plots 1, 2 and 4, whereas in vine-plot 3 the soil was sandy-loam with pH 6-6.5.

The following method of Guendez *et al.* (2005) was applied for the determination of phenolic acids and catechin of seeds. Two (2) g of seeds, coming from a seed sample obtained from 200 berries, were ground in a mortar with 8 ml of ethyl acetate (p.a. grade) as solvent. The extract was centrifuged at 6000 rpm for 5 minutes at -4 °C. The procedure was done in triplicate. The centrifuged clear extracts were mixed and dried in a rotary vacuum evaporator at a temperature of ≤ 40 °C. The dry extract was resuspended in 8 ml methanol (p.a. grade) containing 5% (v/v) perchloric acid (p.a. grade). Before analysis, the solution was filtered through 0.45 $\mu$m filter membranes (Alltech Associates Inc., Deerfield, IL, USA). Quantification was accomplished using commercial standards such as syringic and vanillic acids (Fluka Biochemika, Switzerland), and gallic, p-coumaric, ellagic, p-hydroxybenzoic acids and catechin (Sigma Chemical Co, Germany).

## 2. Apparatus

The Universal E1M6 (204), Sigma 3K30, Sigma 3E1 and Rotavapor R-3000 centrifuges were used for the preparation of the samples. A Hitachi U1100 spectrophotometer was used for all UV absorbance measurements. For the determination of phenolic acids and catechin, an apparatus of high performance liquid chromatography (HPLC), consisting of two Marathon IV (State College, PA, USA) pumps, and a UV-Vis detector L-4250 (Merck Hitachi) were used. An Adsorbosphere XL $C_{18}$, 250 mm x 4.6 mm I.D. (Alltech Associates Inc., Deerfield, IL, USA) column was used. The filling-material of the column was silica gel-$SiO_2$, with 5 $\mu$m spherical granules and 90A size pores. The precolumn was Kromasil $C_{18}$ 5U of 4.6 mm internal diameter and 7.5 mm length (Alltech Associates Inc., Deerfield, IL, USA). The ChromQuest chromatography software (Thermo Quest Inc., San Jose, CA, USA) was used for data storage and integration.

## 3. Determination of parameters

a. Total and extracted anthocyanins

The determination of anthocyanins was performed according to the method of Ribéreau-Gayon and Stonestreet (1965), that is, the sulphur decolourisation method: anthocyanins react with $HSO_3^-$ giving colourless compounds and they are transformed in the coloured form of flavyl by oxidation. For the extraction of anthocyanins, the Glories method (1991, 2001) has been employed.

For the absorbance measurements of total and extracted anthocyanins, a Hitachi U1100 Spectrophotometer and glass cuvettes (1 cm light path) were used. HCL N/10, HCL 2% (v/v), solution of tartaric acid 5% (w/v) pH = 3.2 and ethanol containing 0.1% (v/v) concentrated HCl were used as solvents.

Two samples of 200 grape berries were taken at each sampling date. The first one was processed immediately and the density of must was measured. The second one was processed in a mixer and two parts of pulp were collected (50 g each). The first part was mixed with an equal volume of HCL N/10 (pH=1) and the second one was mixed with an equal volume of tartaric acid solution (pH =3.2), coded as $pH_1$ and $pH_{3.2}$, respectively. Both solutions were shaken, left for four hours and then filtered through glass wool. One ml of the filtered $pH_1$ solution was mixed with 1 ml of ethanol (acidified with 0.1% (v/v) concentrated HCL) and 20 ml of HCL 2% (v/v). This solution was split equally into two test tubes A and B. In test tube A, 4 ml distilled water were added and in test

**Table 2. Gradient elution program and column clean up protocol**

| Time (min) | Solvent A (% v/v) | Solvent B (% v/v) | Flow (ml/min) |
|---|---|---|---|
| 0 | 100 | 0 | 1 |
| 30 | 85 | 15 | 1 |
| 40 | 78 | 22 | 1 |
| 50 | 0 | 100 | 1 |
| 65 | 0 | 100 | 1 |
| 70 | 100 | 0 | 1 |


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tube B, 4 ml $NaHSO_3$ 15% (w/v) were added. After 20 minutes, absorbance at 520 nm was measured in both test tubes A and B (D.O.A and D.O.B, respectively). Distilled water was used as blank.

Calculation

The concentration of total anthocyanins measured in mg/l of must is given by the following equation: ApH1 (mg glycoside of malvidin/l of must) = (D.O.A - D.O.B)*875

For the measurement of extracted anthocyanins in mg/l of must, the same procedure is followed in filtered solution ($pH_{3.2}$):

$ApH_{3.2}$ (mg glycoside of malvidin/l of must) = (D.O.A – D.O.B)*875

b. Coefficient extraction of anthocyanins (EA%)

The coefficient extraction of anthocyanins is given by the following equation:

$$EA\ (\%) = \frac{(A\ pH_1 - A\ pH_{3.2})}{A\ pH_1} * 100$$

c. Concentration of skin (dpell) and seed tannins (dTpep)

The concentration of skin tannins (dpell) and seed tannins (dTpep) in g/l of must, according to the modification for Xinomavro by Tavernaraki (1999), is calculated from the following equations:

$$dpell = \frac{(A\ pH_{3.2} * 60)}{1000} \qquad dTpep = D.O._{280} - dpell$$

d. Contribution of seed tannins to total phenolic content (in percentage) (MP%)

The contribution of seed tannins to total phenolic content, according to the modification for Xinomavro by Tavernaraki (1999), is calculated from the following equation:

$$MP\% = \frac{[D.O._{280} - (A\ pH_{3.2} * 60)] * 100}{D.O._{280}}$$

e. Total phenol marker (TPM)

For the absorbance measurements of total phenols, a Hitachi U1100 Spectrophotometer and quartz cuvettes (1 cm light path) were used.

The wine-must $pH_{3.2}$ was filtered and diluted to 1/100 with distilled water and the total phenolic content was given by the absorbance measurement at 280 nm ($D.O._{280}$). The absorbance was measured at 280 nm, because the characteristic benzoic rings of phenolic compounds of must and wine show maximum ultraviolet absorbance at 280 nm.

Calculation

$$TPM = D.O._{280} * 100$$

## 4. Qualitative and quantitative determination of phenolic acids and catechin of seeds

The identification of the phenolic acids and catechin of seeds of Xinomavro grapes was based on the comparison of the retention time of the compounds of the sample with that of the standard compounds. The integration of peak areas, which correspond to the determined compounds, was done by the Chrom & Spec Chromatography Data System Version 1.52b (Ampersand International, Inc. 2000) program.

The mobile phase consisted of two different solvents, prepared daily:

Solvent A: Perchloric acid (p.a. grade) 0.6% v/v (70-72%) in « twice distilled water » (milli-Q water),

**Table 3. Control of linearity of method and coefficients of correlation**

| | Area corresponding to different dilutions of standard solution | | | | Equation | Coefficient of correlation (R) |
|---|---|---|---|---|---|---|
| | $X_1$ 5% | $X_2$ 10% | $X_3$ 20% | $X_4$ 80% | | |
| Gallic acid | 38,927 | 124,887 | 250,105 | 2626,533 | y=0,166x+46,294 | 0,994 |
| p-Hydroxybenzoic acid | 25,548 | 93,108 | 188,524 | 2025,900 | y=0,2864x+62,946 | 0,994 |
| Catechin | 4,581 | 20,520 | 33,763 | 466,728 | y=0,7692x+42,678 | 0,992 |
| Vanillic acid | 6,642 | 55,844 | 105,046 | 1203,218 | y=0,24x+32,742 | 0,994 |
| Syringic acid | 102,088 | 309,906 | 498,747 | 5188,968 | y=0,1409x+72,628 | 0,993 |
| p-Coumaric acid | 48,630 | 114,966 | 162,543 | 1678,030 | y=0,0877x+13,567 | 0,992 |
| Ellagic acid | 18,907 | 32,132 | 41,554 | 252,039 | y=0,3113x+1,9287 | 0,995 |


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Solvent B: Methanol 100% for chromatography use.

The chromatography program that was applied is a modification of the program suggested by Guendez *et al.* (2005), so as to achieve the best separation of components examined (Table 2).

The quantitative determination of phenolic compounds was done based on curves of reference compounds that resulted from the standard solutions of different concentrations, after validation of the method.

a. Validation of method

a1. Linearity of method

The linearity of the curves of reference compounds was tested using four different concentrations (5%, 10%, 20%, 80%) of the standard solution of the seven acids: Gallic, p-hydroxybenzoic, vanillic, syringic, p-coumaric, ellagic and catechin. The equations that describe the curves of the standard solution of the phenolic compounds and the coefficient of correlation (R) of each equation are shown in table 3. The coefficient of correlation was ranging from 0.992 to 0.995.

a2. Repeatability and reproducibility of method

Repeatability of the method was controlled by five consecutive injections of the same sample during a day. The coefficients of variance for the concentrations (mg/l) of compounds ranged from 1.4 to 10.4 (Table 4). Accuracy and reproducibility of the method between days were controlled by injecting the same standard solution three times over a period of 30 days (Table 5). The calculated concentrations of the individual compounds showed coefficients of variance from 8.1 to 14.5.

## RESULTS AND DISCUSSION

The results for total and extracted anthocyanins ($ApH_1$ and $ApH_{3,2}$, respectively), coefficient extraction of anthocyanins (EA %), concentration of skin and seed tannins (dpell and dTpep, respectively), the contribution (percentage) of seed tannins to total phenolic content (MP%) and the total phenol marker (TPM) are demonstrated in table 6. Cluster-thinned vine-plots showed an increase in concentration of grape anthocyanins during maturation, compared to the control plot (no thinning). It was also observed that the concentration of anthocyanins was increasing in all four samples until September 22, after which a decrease was noticed, with the exception of vine-plot 4, which demonstrated an increase in concentration until the end of the sampling period.

**Table 4. Repeatability of method during the same day**

| Compound | Injection | | | | | Average | Standard deviation | Coefficient of variance (%) |
|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th | | | |
| | Concentrations (mg/l) | | | | | | | |
| Gallic acid | 84,51 | 86,30 | 84,65 | 87,39 | 85,18 | 85,61 | 1,22 | 1,4 |
| p-Hydroxybenzoic acid | 4,64 | 4,47 | 4,13 | 4,91 | 4,95 | 4,62 | 0,34 | 7,3 |
| Catechin | 44,91 | 48,37 | 47,91 | 49,18 | 47,57 | 47,59 | 1,61 | 3,4 |
| Vanillic acid | 25,71 | 27,03 | 26,23 | 27,11 | 26,73 | 26,56 | 0,59 | 2,2 |
| Syringic acid | 45,58 | 46,68 | 45,32 | 47,50 | 46,40 | 46,30 | 0,88 | 1,9 |
| p-Coumaric acid | 13,26 | 12,99 | 12,88 | 13,82 | 13,20 | 13,23 | 0,36 | 2,8 |
| Ellagic acid | 30,94 | 32,51 | 25,64 | 27,88 | 32,79 | 29,95 | 3,10 | 10,4 |

**Table 5. Reproducibility of method on three different days over a 30-day period by injecting the same standard solution of 10% components**

| Compound | Injection | | | | | Average | Standard deviation | Coefficient of variance (%) |
|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th | | | |
| | Concentrations (mg/l) | | | | | | | |
| Gallic acid | 84,51 | 86,30 | 84,65 | 87,39 | 85,18 | 85,61 | 1,22 | 1,4 |
| p-Hydroxybenzoic acid | 4,64 | 4,47 | 4,13 | 4,91 | 4,95 | 4,62 | 0,34 | 7,3 |
| Catechin | 44,91 | 48,37 | 47,91 | 49,18 | 47,57 | 47,59 | 1,61 | 3,4 |
| Vanillic acid | 25,71 | 27,03 | 26,23 | 27,11 | 26,73 | 26,56 | 0,59 | 2,2 |
| Syringic acid | 45,58 | 46,68 | 45,32 | 47,50 | 46,40 | 46,30 | 0,88 | 1,9 |
| p-Coumaric acid | 13,26 | 12,99 | 12,88 | 13,82 | 13,20 | 13,23 | 0,36 | 2,8 |
| Ellagic acid | 30,94 | 32,51 | 25,64 | 27,88 | 32,79 | 29,95 | 3,10 | 10,4 |


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**Table 6. Evolution of grape phenolic maturity in cv. Xinomavro**

| Vine-plot | Sampling date | Total anthocyanins $ApH_1$(*) | Extracted anthocyanins $ApH_{3.2}$(*) | EA%(*) | dpell(*) | DTpep(*) | MP%(*) | TPM(*) (A280) |
|---|---|---|---|---|---|---|---|---|
| 1 No cluster removal (control) | 10/9/2003 | 193,6 | 103,2 | 46,7 | 6,2 | 17,9 | 74,3 | 24,1 |
| | 22/9/2003 | 214,7 | 122,4 | 43,0 | 7,3 | 15,6 | 68,0 | 22,9 |
| | 29/9/2003 | 191,8 | 118,0 | 38,5 | 7,1 | 13,7 | 65,9 | 20,8 |
| 2 Grape removal | 10/9/2003 | 163,7 | 89,2 | 45,5 | 5,4 | 17,2 | 76,3 | 22,6 |
| | 17/9/2003 | 265,8 | 146,2 | 45,0 | 8,8 | 13,7 | 61,0 | 22,5 |
| | 22/9/2003 | 309,8 | 172,8 | 44,2 | 10,4 | 12,1 | 53,8 | 22,5 |
| | 25/9/2003 | 309,8 | 172,5 | 44,3 | 10,4 | 12,0 | 53,7 | 22,4 |
| 3 Grape removal | 10/9/2003 | 230,6 | 122,2 | 47,0 | 7,3 | 18,0 | 71,1 | 25,3 |
| | 17/9/2003 | 339,7 | 180,4 | 46,9 | 10,8 | 14,9 | 57,9 | 25,7 |
| | 22/9/2003 | 349,9 | 194,2 | 44,5 | 11,7 | 13,7 | 54,0 | 25,4 |
| | 25/9/2003 | 322,1 | 180,4 | 44,0 | 10,8 | 13,3 | 55,1 | 24,1 |
| | 29/9/2003 | 323,8 | 190,7 | 41,1 | 11,4 | 13,1 | 53,4 | 24,5 |
| 4 Grape removal | 10/9/2003 | 151,4 | 94,6 | 37,5 | 5,7 | 16,2 | 74,1 | 21,9 |
| | 17/9/2003 | 255,2 | 166,4 | 34,8 | 10,0 | 9,8 | 49,5 | 19,8 |
| | 22/9/2003 | 257,0 | 176,0 | 31,5 | 10,6 | 9,2 | 46,6 | 19,8 |
| | 25/9/2003 | 272,8 | 188,0 | 31,1 | 11,3 | 8,0 | 41,5 | 19,3 |
| | 29/9/2003 | 295,7 | 204,0 | 31,0 | 12,2 | 8,0 | 39,5 | 20,2 |

(*) $ApH_1$: Total anthocyanins (mg glycoside of malvidin/l of must); $ApH_{3.2}$: Extracted anthocyanins (mg glycoside of malvidin/l of must); EA%: Coefficient Extraction of Anthocyanins; dpell: Skin tannins (g/l of must); dTpep: Seed tannins (g/l of must); MP%: Contribution-percentage of seed tannins to total phenolic content; TPM: Total phenol marker.

In conclusion in the present study, the concentration evolution of total anthocyanins during maturation showed that it increased to a maximum level in cluster-thinned plants and then decreased (Figure 1). More precisely, the concentration of total anthocyanins in vine-plot 1 (control) was increased from 193.6 mg on September 10 to 214.7 mg on September 22 (i.e., a 10% increase). During the same period, the anthocyanin concentration was increased from 163.7 mg to 309.8 mg in vine-plot 2 (i.e., an 89% increase) and from 230.6 mg to 349.9 mg in vine-plot 3 (i.e., an increase of approximately 52%). In vine-plot 4, the concentration of total anthocyanins continually increased from 151.4 mg, at the beginning, to 295.7 mg at the end, that is, about 78% increase.

Similar results were obtained by Sakellari (2004). According to this study, cluster thinning in Xinomavro variety at the veraison stage resulted in the increase of grape anthocyanin concentration. Other researchers also studied the effect of cluster thinning on the concentration of anthocyanins and obtained similar results. Guidoni *et al*. (2002) observed an 18% increase of the concentrations of anthocyanins in Nebbiolo variety after removal of 50% of grapes, compared to the non cluster-thinned plants. This increase occurred, probably due to the fact that the antagonism between grapes was diminished when one cluster is left per sprout. As a result, this cluster becomes the only receiver of photosynthetic products (Stavrakas, 1999).

In conclusion, the effect of thinning on anthocyanins depends, beyond the other factors, on the extent and the thinning time. All these factors are probably the cause of observable differences in the effect of thinning on anthocyanins. As shown in figure 1, anthocyanins from vine-plot 2 reached their highest level and stabilized earlier than the other vine-plots. This may be because of the different rootstock that had been used. Indeed, the 41B rootstock, which was used in this vine-plot, is of middle vivacity, compared to the 110R rootstock that was used in the other vine-plots, and contributes to early maturity (Stavrakas, 1998). In addition, vine-plot 3 had the highest content of anthocyanins. The soil in this plot is characterised as sandy-loam, which is more « floaty »

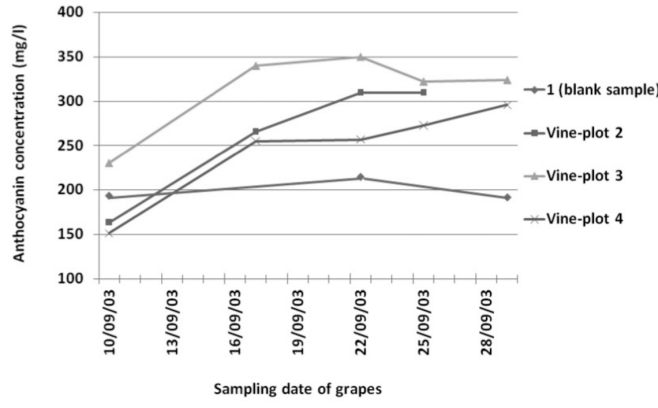



**Figure 1 - Evolution of concentration of total anthocyanins in mg/l of must, based on sampling date of grapes.**


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**Table 7. Concentration of phenolic acids and catechin in the seeds of Xinomavro variety during maturation and under the influence of cluster thinning (mg/100 g of seeds)**

| Vine-plot | Sampling date | Gallic acid | Syringic acid | p-Hydroxy-benzoic acid | Vanillic acid | p-Coumaric acid | Catechin |
|---|---|---|---|---|---|---|---|
| 1 Control | 10/9/03 | 5,1 | 18,1 | 5,0 | 1,8 | 9,5 | 113,4 |
| | 22/9/03 | 3,9 | 10,5 | 3,6 | 2,0 | 1,1 | 75,5 |
| | 29/9/03 | 6,3 | 22,5 | 3,0 | 1,4 | 3,3 | 42,3 |
| 2 Cluster thinning | 10/9/03 | 2,9 | 7,8 | 3,4 | 1,7 | 3,2 | 86,8 |
| | 17/9/03 | 4,4 | 17,0 | 4,7 | 1,7 | 2,7 | 44,8 |
| | 22/9/03 | 3,7 | 11,4 | 3,8 | 1,9 | 1,2 | 83,7 |
| | 25/9/03 | 4,9 | 20,7 | 4,6 | 1,5 | 2,7 | 26,3 |
| 3 Cluster thinning | 10/9/03 | 5,1 | 13,2 | 4,9 | 1,4 | 2,4 | 80,0 |
| | 17/9/03 | 3,5 | 9,7 | 4,0 | 1,4 | 3,8 | 33,1 |
| | 22/9/03 | 6,1 | 17,7 | 2,8 | 2,2 | 10,9 | 103,4 |
| | 25/9/03 | 5,9 | 20,1 | 5,1 | 2,2 | 9,7 | 92,4 |
| | 29/9/03 | 5,2 | 15,5 | 4,8 | 1,2 | 1,8 | 76,5 |
| 4 Cluster thinning | 10/9/03 | 2,3 | 4,9 | 3,2 | 2,0 | 1,5 | 67,3 |
| | 17/9/03 | 4,3 | 14,9 | 4,5 | 1,4 | 1,2 | 58,4 |
| | 22/9/03 | 4,5 | 7,0 | 3,1 | 1,8 | 1,3 | 67,3 |
| | 25/9/03 | 4,9 | 17,5 | 2,7 | 1,4 | 1,9 | 17,0 |
| | 29/9/03 | 6,3 | 23,0 | 4,0 | 1,5 | 1,2 | 11,6 |

than the loam-clay in the other plots. A loam-clay soil delays grape maturity by extending the vegetative period and, as a result, delays anthocyanin formation (Stavrakas, 1999).

In contrast to the anthocyanin content that was increasing during maturation, the coefficient of anthocyanin extraction (EA %) was decreasing .This is reasonable, as the value of extraction depends on skin thickness. Anthocyanins are more easily extracted from grape varieties with thin skins, because skins are destroyed more easily. Gonzalez-Neves *et al*. (2004) reported that the coefficient of anthocyanin extraction decreases with increasing grape ripeness, as the difference between total and extracted anthocyanins is decreased when decomposition of cell wall and skin membrane is increased.

Skin tannin concentration (dpell) increased during maturation by 56.2 to 114% for vine-plots that had been thinned out and only by 17.7% for the control vine-plot (Table 6). However, in some cases, a decrease was observed before maturity. Concerning seed tannins (dTpep), the decrease was obvious during maturation (Table 1). This decrease is a possible result of the formation of complexes of tannins with other cell compounds (Bogs *et al*., 2005).

The contribution of tannins to total phenolic content (MP %) was reduced during grape maturation (Table 1). The MP coefficient is a maturity marker of seeds and its decrease during the maturation process shows the small percentage of contribution of seed tannins to the tannins of wine.

The total phenolic content was reduced as maturation progressed (Table 6).

Table 7 shows the concentrations of phenolic compounds (gallic acid, syringic acid, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid and catechin) that were found in grape seeds of Xinomavro during maturation in the control and cluster thinned vine-plots, by the use of HPLC.

Table 7 shows that the most abundant of these compounds was catechin, whereas the less abundant was vanillic acid. The greatest concentration of catechin appeared in the vine-plot in which no cluster thinning had been done. Overall, catechin values ranged from 11.6 to 113.4mg/100g, being within the limits reported by other researchers (Fuleki and da Silva, 1997). Similarly, other researchers as Mateus *et al*. (2001) have found that catechin is the principal compound in seeds of Touriga Nacional and Touriga Francesa varieties. Furthermore, Ramey *et al.* (1986) came to the same result after studying the phenolic composition of seeds during the maturation of Grenache, Ugni blanc, Carignan and Maccabeo grape varieties. Our results show that catechin was significantly reduced in the control sample until the end of the maturation period, while in the cluster thinned vine-plots there was an increase until mid-maturation and afterwards the concentration was reduced. Mateus *et al*. (2001) mention that the increase in the concentration of catechin of seeds, before the final stage of maturity, is related to the variety, as well as to the height of the vinery and the climate. Increased moisture and temperature favour the increase of catechin concentration. Kennedy *et al*. (2000) agree with this ascertainment related to the catechin increase during maturation of grapes of the Syrah variety.


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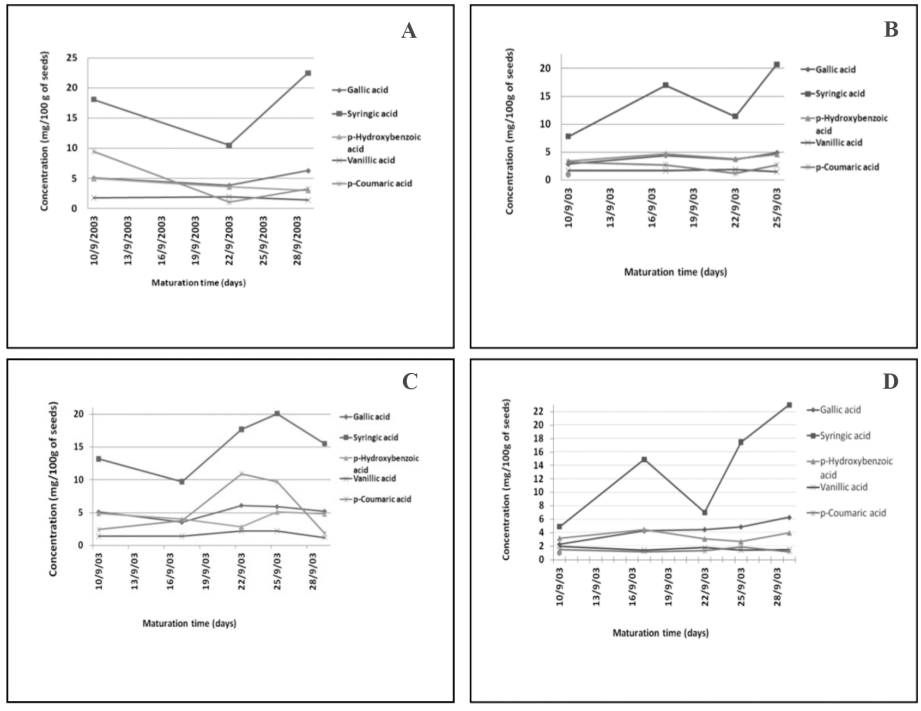



**Figure 2 - A: Concentration of phenolic acids in blank sample**
**B: Concentration of phenolic acids in vine-plot 2**
**C: Concentration of phenolic acids in vine-plot 3**
**D: Concentration of phenolic acids in vine-plot 4**

**Table 8. Concentrations of gallic acid and catechin in seeds of various grape varieties (mg/100g of seeds)**

| Varieties | Gallic acid | Catechin | References |
|---|---|---|---|
| Muscadine | 99,00 | 12,0 | Yilmaz and Toledo, 2004 |
| Chardonnay | 15,00 | 358,0 | " |
| Merlot | 10,00 | 127,0 | " |
| Xinomavro | 0,65 | 36,7 | Guendez *et al.,* 2005 |

The concentration of gallic acid and syringic acid clearly increased during grape maturation; that of vanillic acid and p-coumaric acid decreased, while p-hydroxybenzoic acid concentration fluctuated (Figures 2a, 2b, 2c and 2d)

The above phenolic acids have been studied by very few researchers in grape seeds. More precisely, only some studies for the gallic acid were found in the literature. The concentrations of gallic acid and catechin in seeds of various varieties are shown in Table 8.

In the present study the average value of the concentration of gallic acid was 5.7 mg/100 g of seeds.

## CONCLUSIONS

During grape ripening of Xinomavro variety, the concentration of anthocyanins was highest in cluster-thinned (by 50%) vine-plots. In general, in the majority of samples the concentration increased until September 22 followed by a decrease or stabilization of the concentration.

The vine-plot 3 shows a different trend in the process of phenolic maturity. After the peak level, total and extracted anthocyanins decreased to minor values and then increased again. On the other hand, the coefficient of anthocyanin extraction was reduced during grape maturation. Skin tannin concentrations were increased, whereas seed tannin concentrations were decreased during maturation. The contribution of tannins to total phenolic content (MP%) was reduced during grape maturation. Total phenol content progressively decreased during ripening.

As for the phenolic acids and catechin in seeds, we conclude that catechin was the most abundant compound


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in seeds of the Xinomavro variety and its concentration fell within the limits reported by other researchers.

Furthermore, gallic and syringic acids were shown to be the most abundant acids in seeds followed by p-hydroxybenzoic and p-coumaric acids.

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