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in

Melgarejo P. (ed.), Martínez-Nicolás J.J. (ed.), Martínez-Tomé J. (ed.).  
Production, processing and marketing of pomegranate in the Mediterranean region:  
Advances in research and technology

Zaragoza : CIHEAM

Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 42

2000

pages 93-97

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=600257>

To cite this article / Pour citer cet article

Legua P., Melgarejo P., Martínez M., Hernández F. **Evolution of anthocyanin content of four pomegranate cultivars (*Punica granatum* L.) during fruit development.** In : Melgarejo P. (ed.), Martínez-Nicolás J.J. (ed.), Martínez-Tomé J. (ed.). *Production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology.* Zaragoza : CIHEAM, 2000. p. 93-97 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 42)



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## Evolution of anthocyanin content of four pomegranate cultivars (*Punica granatum* L.) during fruit development

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**SUMMARY** – Anthocyanins are responsible for the external and interior colour of the pomegranate and the red colour is considered by consumers to be one of the main quality parameters. The aim of the present work is the analysis and quantification of the anthocyanin pigments in pomegranate juice during fruit development and ripening. The cultivars studied are ME5, ME17, MO6 and MA4. First, fruits were labelled in the same phenological stage at four orientations. Then anthocyanins were analysed quantitatively and qualitatively using HPLC. Anthocyanin evolution was studied in different tree orientations and results were subjected to a statistical analysis. A rise in amount of pigment was observed during fruit development and juice colour changed from colourless to dark red. During the earlier stages the diglycoside form was predominant (particularly 3,5-delphinidin) in all cultivars. However, during the ripening stages the predominant compounds were monoglycosides.

**Key words:** Pomegranate, *Punica granatum* (Punicaceae), anthocyanins, pigments, ripening.

**RESUME** – "Evolution de la teneur en anthocyanines de quatre cultivars de grenadier (*Punica granatum* L.) pendant le développement du fruit". Les anthocyanines sont responsables de la couleur externe et interne de la grenade et la couleur rouge est considérée par les consommateurs comme l'un des principaux paramètres de qualité. Le but de la présente étude est l'analyse et la quantification des pigments d'anthocyanines dans le jus de grenade pendant le développement du fruit et son mûrissement. Les cultivars étudiés sont ME5, ME17, MO6 et MA4. D'abord, les fruits ont été étiquetés au même stade phénologique avec quatre orientations. Ensuite les anthocyanines ont été analysées quantitativement et qualitativement en utilisant HPLC. L'évolution des anthocyanines a été étudiée dans différentes orientations de l'arbre et les résultats ont été soumis à une analyse statistique. Une augmentation de la quantité de pigment a été observée pendant le développement du fruit et la couleur du jus a changé passant d'incolore à rouge sombre. Pendant les premiers stades la forme diglycoside était prédominante (particulièrement 3,5-delphinidine) chez tous les cultivars. Cependant, lors des stades de mûrissement, les composés prédominants étaient les monoglycosides.

**Mots-clés :** Grenade, *Punica granatum* (Punicaceae), anthocyanines, pigments, mûrissement.

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### Introduction

The red colour of the fruit and the seeds of the pomegranate is one of the most highly appreciated features by the consumer "a priori" (Melgarejo, 1997), which is why fruits with this appearance have a higher acceptance. Anthocyanins are responsible for the red colour of the pomegranate seeds. Six anthocyanins have been described in this fruit: 3,5 diglycoside derivatives and 3 delphinidin, cyanidin and pelargonidin glycosides (Du *et al.*, 1975; Kiventsov and Arendt, 1986; Gil *et al.*, 1995).

Knowledge on the development of the interior and exterior colour is of great interest for the harvest season, once the standard index of maturity has been reached. Colour development does not only depend on the date, but also the orientation, position of the fruit on the tree as well as topographical and other factors.

Knowledge of the correlation between the interior and exterior colour of the fruits will allow a colorimetric maturity index to be established that would be of commercial interest. Furthermore, if the development of colour at different orientations is known, it will be possible to draw up plantation planning strategies and to programme the harvest of the earlier fruits.

The present work studies the evolution of the anthocyanin content at different orientations of the tree in order to determine the influence that this orientation has on the synthesis of these compounds

as well as the changes during maturation that may help to define an index of commercial maturity based on the external colour.

## Material and methods

The plant material used was seed from fruits of the ME5, ME17, MO6 and MA4 pomegranate clones. These clones are grown in homogeneous conditions in the existing experimental plot at the Higher Polytechnical School of Orihuela (University Miguel Hernández). The fruits of these varieties were characterized as sweet and presented good organoleptic characteristics, being classified as recommendable and highly recommendable, both for their agronomic and commercial interest and for the red colour of their seeds (Melgarejo, 1993).

Following harvest, the kernels were removed from each fruit by hand. The mixture of seeds was homogenized by taking a sample and extracting the juice with a liquefier (Moulinex). The juice was centrifuged for 20 minutes at 14,000 rpm using a Selecta centrifuge, model 540. The juice extracted was frozen in vials and kept at -20°C until the time of analysis.

The analysis of anthocyanins was carried out using a high-resolution liquid chromatograph (HPLC) from Hewlett Packard, model 1100 using a C18 column (12.5 x 0.4 cm, with a particle size of 5 µm). The mobile phase used was of water and formic acid (19:1) as solution A and methanol as solution B. Determinations were made with a wavelength of 520 nm and an injection flow of 1 ml/min.

The colour of the juice before freezing samples was measured with a Minolta photocolourimeter model CR 300.

## Results and discussion

The anthocyanins responsible for the pigmentation of pomegranate seeds were isolated and identified as 3,5-diglycoside delphinidin (Dp 3,5) and 3-glycoside (Dp 3), cyanidin 3,5-diglycoside (Cy 3,5) and 3-glycoside (Cy 3) and pelargonidin 3,5-diglycoside (Pg 3,5) and 3-glycoside (Pg 3) (Du *et al.*, 1975). Thus, the derivatives of pelargonidin are responsible for the orange and red colours. The derivatives of cyanidin are responsible for red and crimson and delphinidin derivatives for violet and blue (Harborne, 1982). However, the same anthocyanins can give rise to different colours depending on factors such as pH, concentration and copigmentation (Brouillard, 1988).

The six previous anthocyanins were analysed and the results obtained can be observed in the following tables and figures.

### Evolution of anthocyanin content over time

From the analysis of the total anthocyanin content (Table 1), a gradual increase is observed in the pigmentation during the maturation of fruit (Ben-Arie *et al.*, 1984; Shulman *et al.*, 1984). This increase in colouring occurs more slowly during the first weeks of maturation whilst from mid-October onwards, there is a rapid rise in all clones studied except ME5. This sharply increases its colouring at the end of September, reaching the maturity index earlier as far as anthocyanins are concerned.

These results coincide with those obtained by Gil *et al.* (1996) studying fruits from the Mollar population grown in similar climatic conditions to our own.

Table 1. Evolution of the total anthocyanin content

	1/09/97	15/09/97	29/09/97	13/10/97	27/10/97	10/11/97	Mean
ME5	33.38	56.84	57.71	186.75	164.54	186.52	114.29
ME17	17.69	17.81	53.45	85.48	139.48	215.79	88.58
MO6	30.05	38.12	58.97	80.95	129.43	271.66	101.53
MA4	56.31	23.26	46.90	65.61	91.97	241.88	87.65

As can be observed in Table 1, the total content of anthocyanins ranged between 17.69 and 1.66 mg of anthocyanins/l of juice, lying within the range of results obtained upon the analysis of fruits from the Mollar cultivar (Gil *et al.*, 1996).

Figure 1 shows the evolution of the mono and diglycoside forms of anthocyanin.

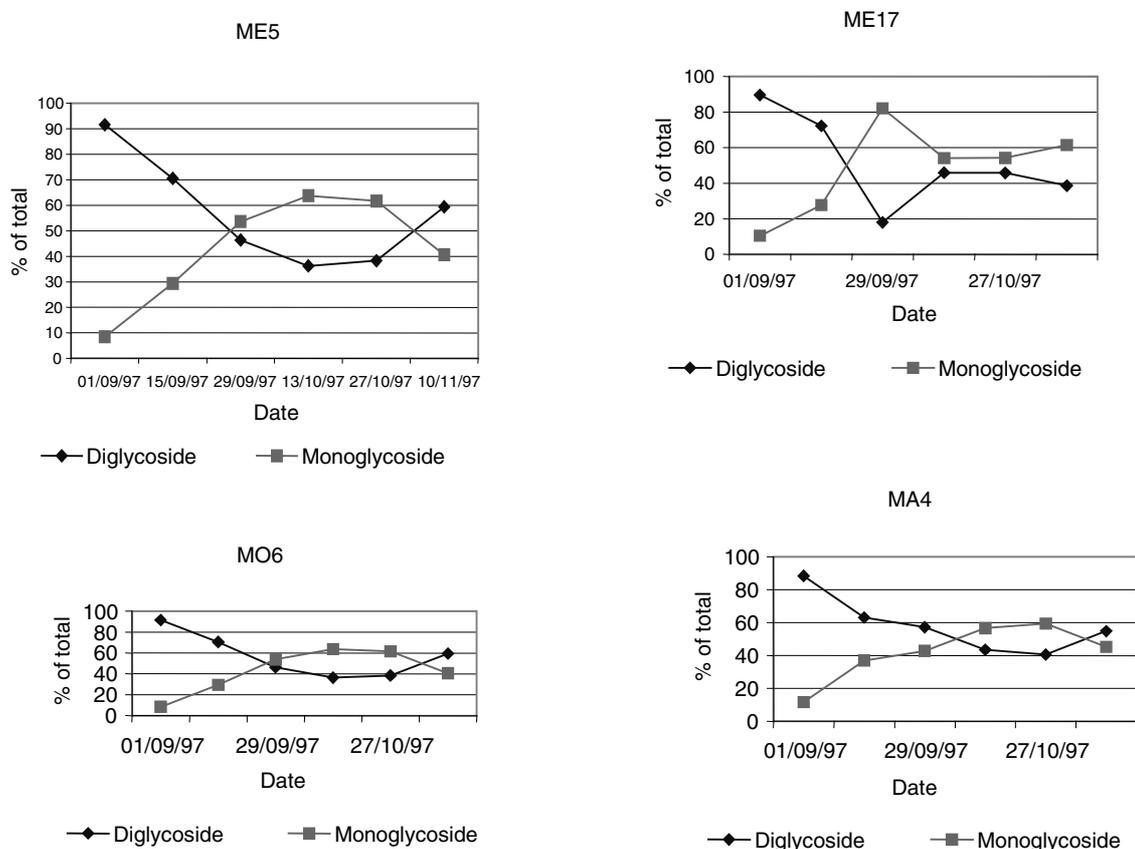


Fig. 1. Evolution of mono and diglycoside forms in anthocyanins.

The graphs show that until the end of September, the diglycoside forms are dominant and from that date onwards, the monoglycoside forms are dominant in the ME5, ME17 and MO6 clones. However, in the MA4 clone the diglycosides are the prevalent form until the beginning of October.

In studies carried out previously on the same variety in similar conditions, it has also been observed that during the first stages of maturation, the quantity of 3,5-diglycosides was greater than that of 3-glycosides.

The evolution of the different anthocyanins throughout maturation can be observed in Fig. 2, in which each anthocyanin is represented as a 100% of their total content.

This graph shows how during the first stages of maturation the most important pigments are delphinidin 3,5 and cyanidin 3,5. However, as maturity increases, one can see how the quantity of monoglycoside anthocyanins increases, becoming quantitatively more important at the end of maturation than the diglycosides. These results coincide with a previous study on the same cultivar in which the most important anthocyanins during the first stage of maturation were cyanidin and delphinidin 3,5 (Gil *et al.*, 1996).

### Evolution of the anthocyanin content according to orientation

Table 2 shows that the average anthocyanin content is higher in northerly exposures, followed by

easterly, westerly and finally southerly. This indicates that the accumulation of these compounds is higher on the coldest sides of the tree. These results have not been discussed as no previous publication has been found distinguishing the accumulation of anthocyanins according to orientation.

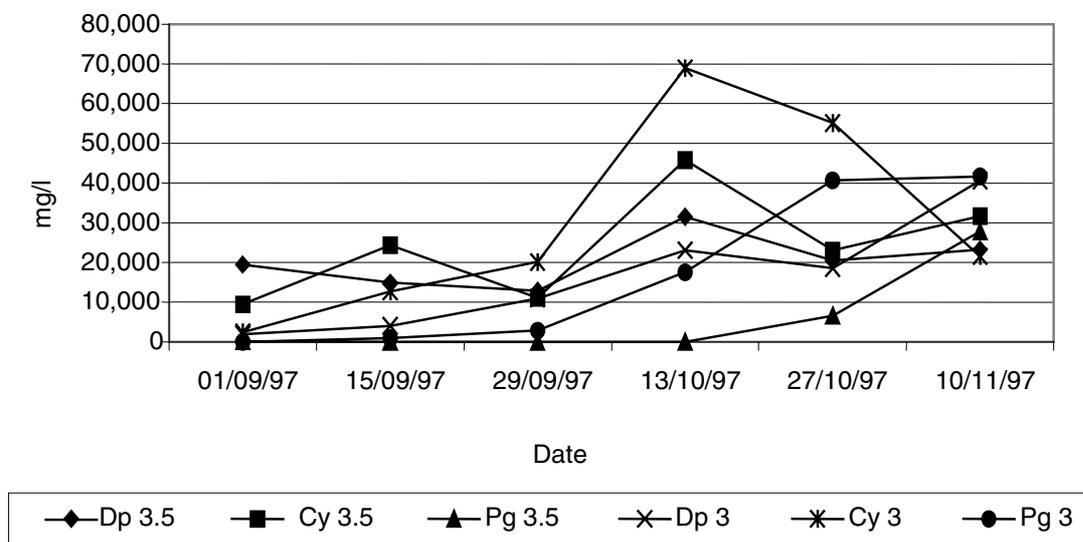


Fig. 2. Evolution of the content of different anthocyanins.

Table 2. Mean anthocyanin content according to orientation

	North	South	East	West	Mean
ME5	136.74	95.31	114.63	112.24	114.73
ME17	102.21	87.71	81.21	83.19	88.58
MO6	112.56	93.04	102.51	98.01	101.53
MA4	107.16	65.77	105.17	78.52	89.15

### Evolution of the juice colour during maturation

The changes in colouring associated to maturation were measured using *Lab* values (Table 3). In accordance with previous studies, it can be observed that the *L* and *b* values do not show significant changes from one week to another. However, for the *a* values, a gradual increase can be observed as ripening progresses, anthocyanin content increases and hence so does the colour red.

Table 3. *Lab* values for pomegranate juice during maturation

Date	ME5			ME17			MO6			MA4		
	<i>L</i>	<i>a</i>	<i>b</i>									
15/09/97	31.51	2.70	-1.90	33.19	0.31	-0.24	32.56	1.45	-0.93	32.56	1.45	-0.93
29/09/97	28.80	3.69	-1.64	31.09	2.16	-1.28	29.14	2.88	-1.37	29.14	2.88	-1.37
13/10/97	30.97	6.02	-1.37	32.51	4.14	-1.37	30.12	4.39	-1.58	30.12	4.39	-1.58
27/10/97	27.68	4.90	-1.23	29.63	5.32	-1.35	25.86	5.69	-1.09	25.86	5.69	-1.09
10/11/97	27.00	5.82	-0.78	27.34	5.76	-0.86	27.68	4.83	-0.90	27.68	4.83	-0.90

## Conclusions

(i) The anthocyanin content in pomegranate juice increases with ripening, which occurs more slowly during the first stages.

(ii) Until the end of September the diglycoside forms are predominant in all clones, and from then onwards it is the monoglycoside forms that dominate.

(iii) In the harvest season, the predominant anthocyanins are delphinidin 3,5 and cyanidin 3,5.

(iv) A significant accumulation of anthocyanins is observed on the northern face in comparison to the others. The southern face presents the highest accumulation.

(v) In the evolution of the colour measured with *Lab* values, an increase in the *a* value is observed, corresponding to an increase of the red colour.

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