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Evolution of juice anthocyanins during ripening of new selected pomegranate (*Punica granatum*) clones

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Abstract For five new clones of pomegranate, cultivated under homogeneous conditions, changes in juice anthocyanin contents during ripening were studied. Six anthocyanin pigments were found to be responsible for the red color of pomegranate juice. These were quantitatively and qualitatively analyzed by high-performance liquid chromatography and identified as delphinidin 3-glucoside and 3,5-diglucoside, cyanidin 3-glucoside and 3,5-diglucoside and pelargonidin 3-glucoside and 3,5-diglucoside. Generally, there was an increase in juice pigmentation during fruit ripening. In the early fruit-ripening stages, delphinidin 3,5-diglucoside was the main pigment, followed by cyanidin 3,5-diglucoside, while in the later stages, the monoglucoside derivatives cyanidin 3-glucoside and delphinidin 3-glucoside increased considerably. The pelargonidin derivatives were always present in small amounts.

Key words Pomegranate · *Punica granatum* · Punicaceae · Anthocyanins · Pigments · Clones · Ripening · Fruit quality

Introduction

Pomegranate fruits are very rich in anthocyanin pigments [1]. One of the most important quality characteristics of the pomegranate is the red pigmentation of its seeds and juice. This red color depends on anthocyanin concentration and on the chemical structure of the individual anthocyanin [2]. Delphinidin derivatives are responsible for blue and violet hues, while pelargonidin is related to red–orange colors [3, 4].

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Previous studies have shown that six anthocyanins are found in the juice of pomegranates, namely delphinidin 3-glucoside and 3,5-diglucoside, cyanidin 3-glucoside and 3,5-diglucoside, and pelargonidin 3-glucoside and 3,5-diglucoside [1]. The peel contains only the pelargonidin and cyanidin derivatives, and no delphinidin is detected [5].

Anthocyanin content and evolution during the development and postharvest storage of pomegranates have been studied previously [1]. During the first steps of development the 3,5-diglucosides are the main pigments present in the juice, with delphinidin glycosides being the main pigment type, while during the optimum harvesting time, the 3-glucosides are the main pigments and the cyanidin derivatives are the predominant compounds.

The aim of the present work was to evaluate and quantify the anthocyanin content of five different pomegranate clones with distinctive quality characteristics (acidity, color stability, etc.), and to follow the changes in juice pigmentation, both qualitatively and quantitatively, during the development of the fruit. The effect of fruit location in the tree was also studied.

Materials and methods

Pomegranates. Fruits of the clones ME16, VA1, PTO8, BA1 and MA2 were obtained from the experimental field collection of pomegranate trees in the EPS Orihuela, Universidad Miguel Hernández, where they are cultivated under homogeneous conditions [6]. Fruits were harvested starting after 26 weeks of flower set (19 August) and ending after 34 weeks of flower set (14 October), which is the normal period for the development and maturation of pomegranate fruits. Fruits were harvested at random in any of the four tree orientations (north, south, east and west) and immediately transported to the laboratory for analysis.

Juice extraction and anthocyanin analysis. The pomegranate fruits were peeled by hand and the seeds were liquefied using a Moulinex turmix machine (Barcelona). The juice obtained was centrifuged for 15 min at 14 000 rpm, and filtered through 0.45 μm filters for high-performance liquid chromatography (HPLC) analysis. The HPLC analysis and the individual antho-

cyanin identification and quantification were performed as reported previously [1]. In this case the anthocyanins were quantified using an external standard of cyanidin 3-rutinoside. The HPLC analysis was performed on a Hewlet-Packard chromatograph model HP-1100, with a C-18 column (12.5 × 0.4 cm, 5 μm particle size), using as solvents water + 5% formic acid (solvent A) and HPLC grade methanol (solvent B). Elution was achieved with a gradient starting with 15% B in A, to reach 35% B at 15 min, then isocratic until 20 min, with a solvent flow rate of 1 ml/min and detection with a diode array detector. Chromatograms were recorded at 520 nm for quantification.

Results and discussion

Changes in anthocyanin pigmentation in the different pomegranate clones

Five pomegranate clones were selected paying attention to their quality characteristics, i.e. pH of the juice and acidity (Table 1). Three Mollar sweet cultivars, with soft stones and low acidity, were selected (MA2, VA1, ME16) as being representative of this group. They have juices with pH values between 4 and 4.5, and titratable acidity around 0.25 (Table 1). These are the most suitable for commercialization as fresh fruit. Sour–sweet cultivars (PTO8) have reasonable quality features (soft stones) and intermediate acidity. The sour cultivars have juices with very low pH values (below 3) and high acidity (above 2), contain acetic acid as one of the organic acids present, which is a characteristic of this group, and have higher pigment stability (BA1).

The anthocyanin content and the individual anthocyanins in the juice of the selected clones were studied by HPLC to detect chemical differences that could be related to the differences found in pigment stability during juice extraction and processing. The analyses revealed that all clones have a common anthocyanin profile when harvested at commercial maturity, characterized by the six pigments that had previously been identified in other cultivars. The highest anthocyanin content was that observed for clone VA1, (close to 160 mg anthocyanin per liter of juice), followed by those of the two Mollar clones (MA2, ME16), which contained more than 100 mg/l. The sour–sweet cultivar PTO8 had intermediate values, while the very acid clone BA1 only reached 30 mg/l.

It is interesting that the clone containing the more stable anthocyanins was BA1, which because of its poor anthocyanin content should be the least stable one. However, the low pH of this clone juice means that its anthocyanins are the most stable since anthocyanins are stabilized at lower pH values [3].

Table 1 Quality characteristics of the different pomegranate clones studied; values are the mean of two harvest seasons at commercial maturity (1996 and 1997) Maturity index $= \frac{SS}{Acidity}$; Acidity = Total acids contents

Clone	Type	pH	SS (°Brix)	Acidity (%)	Maturity index
MA2	Sweet	4.42	16.51	0.23	71.78
ME16	Sweet	4.09	15.20	0.26	58.46
VA1	Sweet	4.01	14.78	0.27	54.74
PTO8	Sour–sweet	3.98	13.48	0.29	46.48
BA1	Sour	2.89	15.46	2.03	7.61

Changes in total anthocyanin pigmentation with development

The changes in total anthocyanin pigmentation were followed in pomegranate fruits of the different clones during 8 weeks of development from immature fruits (mid August, 10 cm diameter, 50 g weight) to commercially mature fruits (mid October, 20 cm diameter, 450 g weight).

Total anthocyanins reached around 160 mg/l in cultivar VA1, with values between 90 and 130 mg/l for the other cultivars, with the exception of cultivar BA1, which reached only 35 mg/l. These values are in the same range as that reported for other pomegranate cultivars, i.e. 50–267 mg/kg fresh weight of arils for Spanish Mollar cultivars [1, 7], 6–120 mg/l in Tunisian pomegranates [8], and 185 mg/l in minimally processed pomegranate seeds [9]. This value was around 200 mg/l in the Wonderful pomegranate [2], and an increase in anthocyanin pigmentation has been described in this cultivar during postharvest refrigerated storage (up to 350 mg/l) [2].

The majority of samples collected in mid August had some pigmentation, with cultivar VA1 being the most pigmented at this early stage, while variety BA1 showed no pigmentation at all (Fig. 1). The pigmentation of VA1 increased fivefold at harvest and this increased from 7- to 10-fold for the other varieties. At commercial harvest (mid October), the most pigmented variety was VA1, while the most acid cultivar BA1 showed the lowest pigmentation, and the Mollar cultivars MA2 and ME16 showed intermediate pigment contents. During the first 4 weeks of fruit development, cultivars BA1, ME16 and VA1 did not show any pigment concentration changes, then the anthocyanin con-

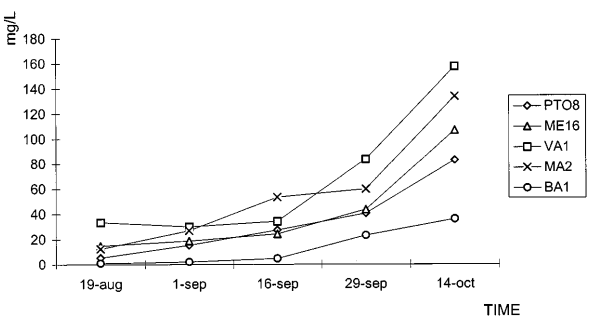

Fig. 1 Evolution of total anthocyanins with the development of different pomegranate clones

centration icreased rapidly during the last 4 weeks of the experiment. Varieties MA2 and PTO8, however, showed an anthocyanin increase from the start of sample collection.

Changes in individual anthocyanins with development

When the biosynthesis of individual anthocyanins was followed during fruit development, some similarities were observed for all cultivars. In the early development stages, delphinidin 3,5-diglucoside was the main pigment, followed by cyanidin 3,5-diglucoside, while in the later stages of fruit development, the 3-glucosides of cyanidin and delphinidin increased considerably. The pelargonidin derivatives are always present in much smaller amounts, and were difficult to quantify in some instances. These results are consistent with previously published data for the Mollar cultivar [1].

In the Mollar sweet cultivars (ME16, MA2), the delphinidin derivative content remained constant during the different stages of development, but a remarkable increase in cyanidin derivatives was seen, starting at the end of September, and these were the main constituents in the mid October sample (Fig. 2). Cultivars VA1 and PTO8 showed biosynthetic behavior different from that of the Mollar cultivars, since their delphinidin glycoside content increased during all the stages of fruit development, and the increase in cyanidin derivatives started earlier than in the Mollar cultivars (mid September) (Fig. 3).

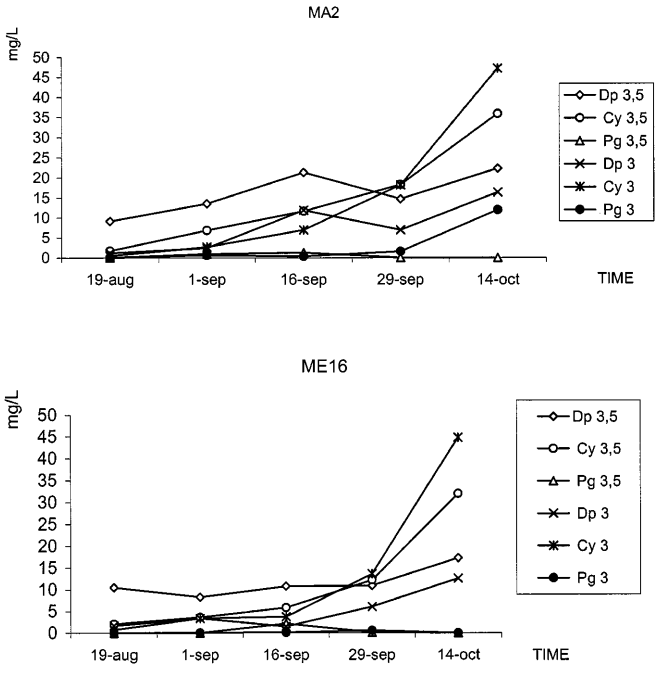

Fig. 2 Evolution of individual anthocyanin pigments in Mollar pomegranate clones (MA2, ME16). *Dp 3,5* Delphinidin 3,5-diglucoside; *Cy 3,5* cyanidin 3,5-diglucoside; *Pg 3,5* pelargonidin 3,5-diglucoside; *Dp 3* delphinidin 3-glucoside; *Cy 3* cyanidin 3-glucoside; *Pg 3* pelargonidin 3-glucoside

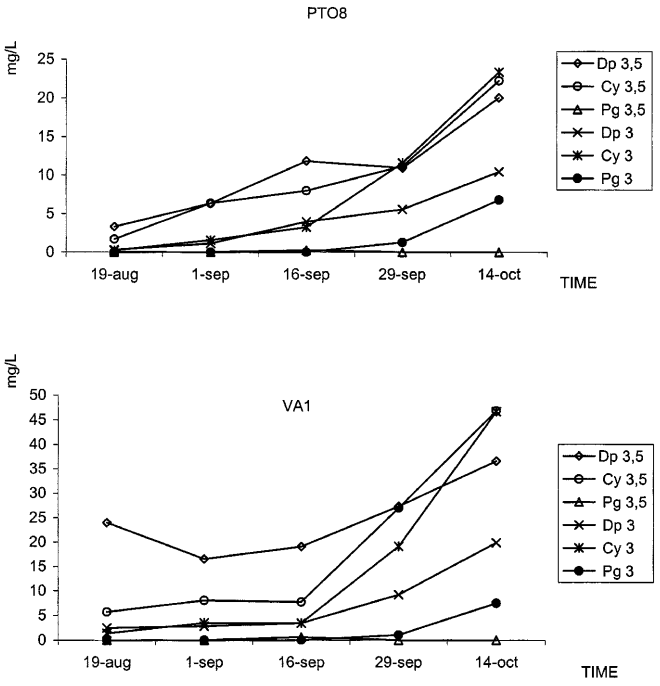

Fig. 3 Evolution of individual anthocyanin pigments in PTO8 and VA1 pomegranate clones. For abbreviations, see Fig. 2

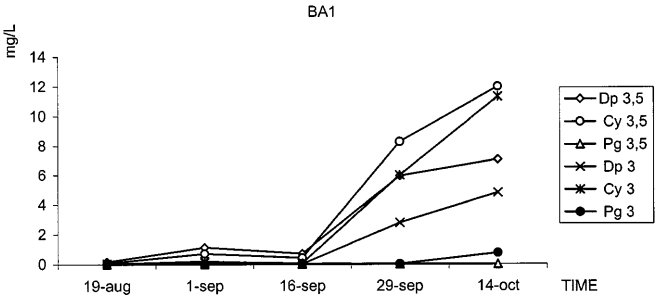

Fig. 4 Evolution of individual anthocyanin pigments in BA1 pomegranate. For abbreviations, see Fig. 2

The sour cultivar BA1 showed a different pattern again, with very little pigment biosynthesis in the early stages, then increased cyanidin and delphinidin derivatives in mid September (Fig. 4). These differences in the relative content of delphinidin and cyanidin derivatives, and in the presence of 3,5-diglucosides or 3-glucosides, influence the pomegranate juice color. The hue depends on the relative concentrations of delphinidin derivatives (bluish hues), cyanidin derivatives (violet hues) and pelargonidin derivatives (orange and scarlet hues). In addition besides has importance in pigment stability, since 3,5-diglucosides are more stable than the 3-glucosides, and the delphinidin derivatives are less stable (more easily oxidized) than the corresponding cyanidin or pelargonidin derivatives.

These results are similar to those previously reported for Mollar cultivars, in which it was shown that the amount of 3,5-diglucosides was higher than that of 3-glucosides during the first fruit development stages,

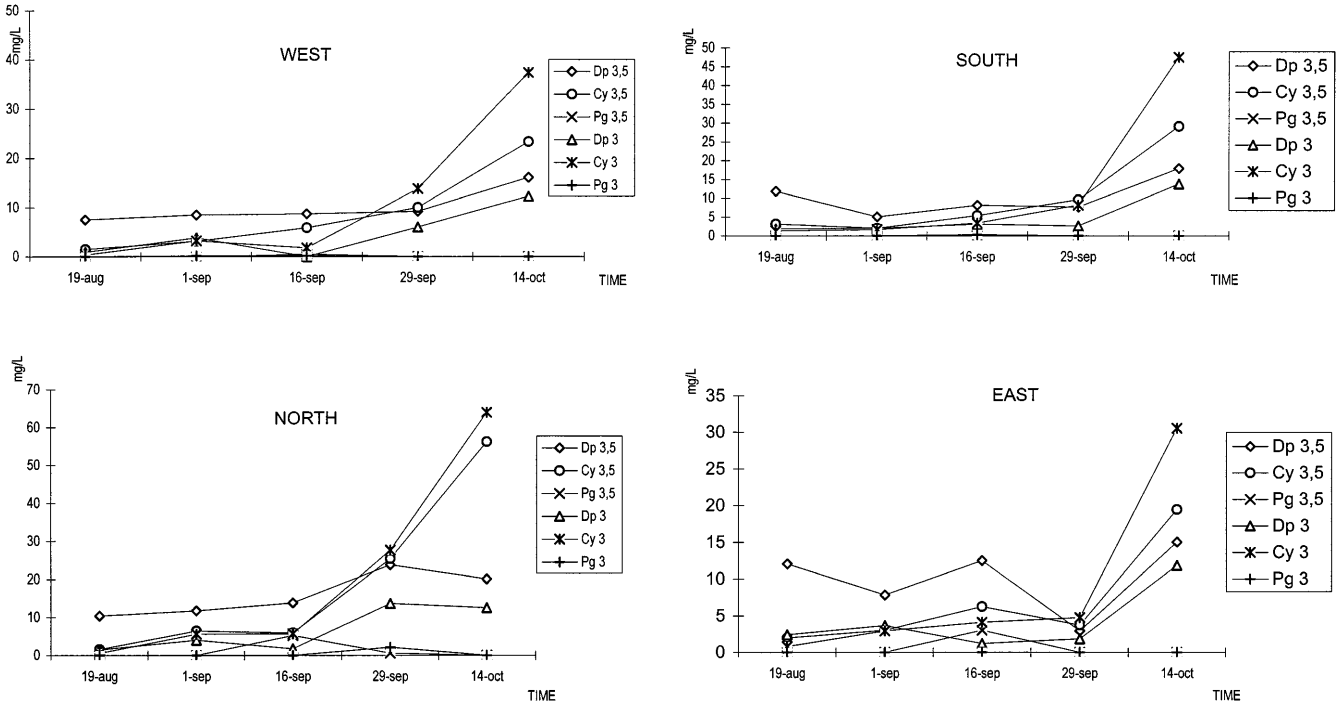

Fig. 5 Evolution of individual anthocyanin biosynthesis in pomegranates located in different tree orientations (clone ME16). For abbreviations, see Fig. 2

and that in early maturation stages the amount of delphinidin glucosides was higher than that of the cyanidin glycosides, while in the later maturity stages the cyanidin glycosides were the main pigments of the fruit juice, and the 3-glucosides reached similar or higher concentrations than the 3,5-diglucosides [1].

Effect of fruit localization in the tree

In general, those fruits located in the north orientation of the trees showed an earlier increase in anthocyanin pigmentation (Fig. 5). In the case of cultivar ME16, only those fruits located in the northern side of the tree showed a significant increase in juice pigments during September (Fig. 5). This could be explained by the lower temperatures reached during the night in those fruits facing the cold north wind.

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