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Evolution of pomegranate juice anthocyanins during the ripening of fruit of three clones: ME16, VA1 and BA1

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SUMMARY – Six anthocyanin pigments were found to be responsible for the red colour of pomegranate juice (cv 'Mollar de Elche', 'Valencia' and 'Borde de Albatera'). These were analysed quantitatively and qualitatively using HPLC 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 is an increase in juice pigmentation with fruit ripening. In the early fruit development stages, delphinidin 3,5-diglucoside was the main pigment, followed by cyanidin 3,5-diglucoside, while in the late stages of fruit development, the 3-glucoside of cyanidin and delphinidin increased considerably. The pelargonidin derivatives are always present in much smaller amounts, and were even difficult to quantify in some instances.

Key words: Pomegranate, anthocyanins, pigments, clones, ripening.

RESUME – *"Evolution des anthocyanines du jus de grenade pendant la maturation du fruit des trois clones : ME16, VA1 et BA1". Six pigments d'anthocyanines se sont avérés responsables de la couleur rouge du jus de grenade (cv 'Mollar de Elche', 'Valencia' et 'Borde de Albatera'). Ils ont été analysés quantitativement et qualitativement en utilisant HPLC et identifiés comme 3-glucoside et 3,5-diglucoside delphinidine, 3-glucoside et 3,5 diglucoside cyanidine, et 3-glucoside et 3,5-diglucoside pélargonidine. Généralement, il y a une augmentation de la pigmentation du jus avec la maturation du fruit. Dans les premiers stades de développement du fruit, 3,5-diglucoside delphinidine était le principal pigment, suivi par 3,5-diglucoside cyanidine, tandis que dans les derniers stades de développement du fruit, 3-glucoside de cyanidine et delphinidine augmentaient considérablement. Les dérivés de pélargonidine sont toujours présents en quantités bien moindres, et il était même difficile de les quantifier dans certaines instances.*

Mots-clés : *Grenade, anthocyanines, pigments, clones, mûrissement.*

Introduction

The pomegranate is one of the main sources of anthocyanins. One of the main characteristics of its quality is shown by the red colour of its seeds and its juice. The red colour depends on the concentration and type of anthocyanins contained in the fruit. Delphinidin derivatives give blue and purple shades, whereas pelargonidin is responsible for orangish red shades (Harborne, 1982).

Six anthocyanins were isolated and identified as being responsible for the colour of the pomegranate juice: delphinidin 3-glucoside and 3,5-diglucoside, cyanidin 3-glucoside and 3,5-diglucoside, and pelargonidin 3-glucoside and 3,5-diglucoside (Du *et al.*, 1975).

Previous studies on the anthocyanin evolution during the ripening process show that during the first stages of fruit development, the 3,5-diglucoside forms are the main pigments found in pomegranate juice, the main one of these being delphinidin; whereas in the more advanced stages of ripening the 3-glucoside forms stand out, and especially cyanidin.

The aim of this study is to identify, quantitatively and qualitatively, the anthocyanin content in the pomegranate juice of three clones: ME16, VA1 and BA1, cultivated in homogeneous conditions; and also to study anthocyanin evolution throughout the ripening process.

Materials and methods

The vegetable matter used is the fruit of clones ME16 (Mollar de Elche No. 16), VA1 (Valenciana) and BA1 (Borde de Albatera No. 1), taken from the experimental plot of the Escuela Politécnica Superior de Orihuela (Universidad Miguel Hernández), where they are cultivated in homogeneous conditions (Melgarejo, 1993).

Harvesting of the fruits began 26 weeks after the start of pomegranate flowering (19 August) and continued until week 34 (14 October), which is the normal ripening period for the pomegranate. The fruits were harvested at random from each of the four orientations of the tree and were immediately taken to the laboratory for analysis.

The pomegranates were peeled manually, and the seeds liquidised in a Moulinex liquefier (Turmix model). The resulting juice was centrifuged for 15 minutes at 14,000 rpm, immediately filtered through a 0.45 ❍m filter and the different anthocyanins were analysed using HPLC and cyanidin-3-rutinoside as an external standard.

An HP cromatograph (HP-1100 model) was used, with a C-18 column (12.5×0.4 cm; 5 ❍m particle size), using water + 5% formic acid (solvent A) and methanol (solvent B) as solvents. Injection flow 1 ml/minute. Detection at 520 nm. Under these conditions, the anthocyanins were determined with the following retention times: delphinidin 3,5-diglucoside (5.05 min), cyanidin 3,5-diglucoside (6.79 min), pelargonidin 3,5-diglucoside (8.25 min), delphinidin 3-glucoside (9.50 min), cyanidin 3-glucoside (11.29 min), pelargonidin 3-glucoside (13.14 min).

Results and discussion

Changes in the total content of anthocyanin pigment during development

The changes in the total content of anthocyanins has been followed in the pomegranate fruits of the three clones (ME16, VA1 and BA1) for 8 weeks during fruit development from an immature stage (mid-August, 10 cm diameter, 50 g weight) to commercially ripe fruits (mid-October, 20 cm diameter, 450 g weight).

The anthocyanin total is around 160 mg/l of juice for clone VA1, about 120 mg/l for clone ME16, and around 35 mg/l for clone BA1. These values are within the same range as for other pomegranate clones. 50-267 mg/kg of fresh arils for the 'Mollar' cultivar (Gil *et al*., 1995a; Artés *et al*., 1998), and 6-120 mg/l of juice in Tunecinas pomegranates (Gil *et al*., 1995b).

Most of the samples show some pigmentation in mid-August, with clone VA1 having greatest pigmentation in the first stages, whereas clone BA1 hardly shows any pigmentation at this stage (Fig. 1). In mid-October (commercial ripeness), the clone with most pigmentation was VA1, clone BA1 had very low pigmentation and clone ME16 showed intermediate pigmentation. The three clones did not show changes in pigment concentration during the first four weeks of fruit development, but then all three clones presented a rapid increase in anthocyanin concentration during the last four weeks of the experiment.

Changes in the individual content of anthocyanin pigments during development

During the first stages of ripening, 3,5-diglucoside are the major forms in pomegranate juice, and amongst these 3,5-delphinidin is the main one, followed by cyanidin 3,5-diglucoside; whereas at the stages closest to the optimum ripeness of the pomegranate, 3-glucoside forms are the main ones, and amongst these 3-cyanidin and delphinidin. Pelargonidin derivatives are always present, but in very small quantities.

In the 'Mollar' clone (ME16), delphinidin derivatives are constant during the different stages of fruit development, but there is a marked increase in cyanidin derivatives at the beginning and end of September, with these becoming the main constituent by mid-October (Fig. 2).

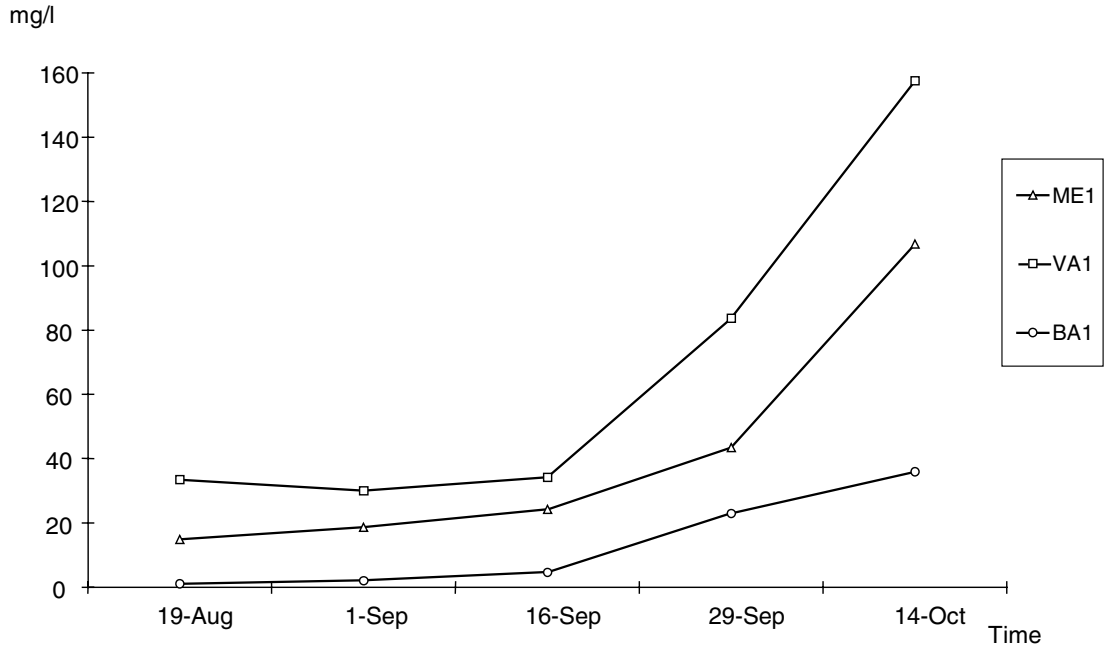

Fig. 1. Anthocyanin evolution in clones ME16, VA1 and BA1.

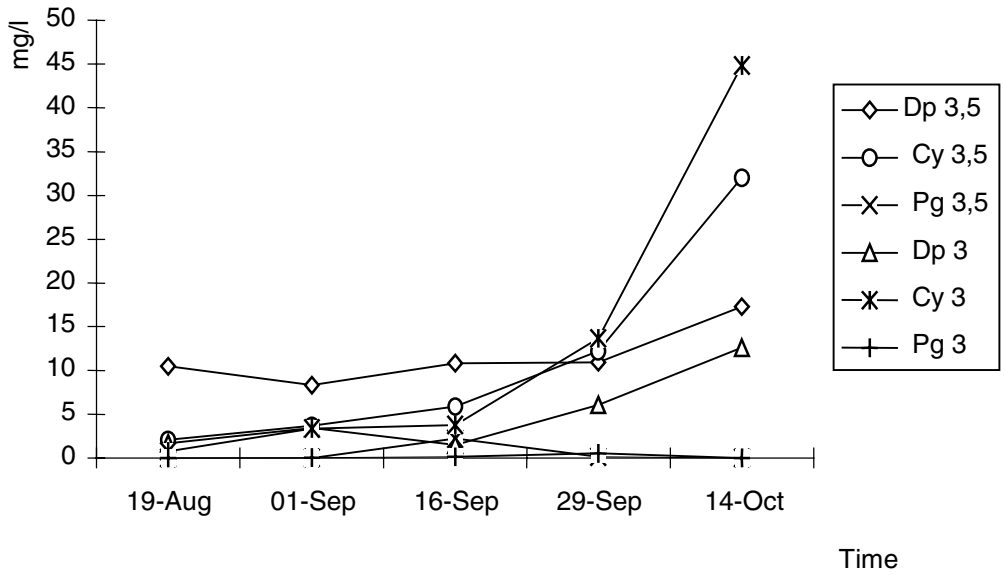

Fig. 2. Anthocyanin evolution in clone ME16.

Clone VA1 shows an increase in delphinidin derivatives during all stages of fruit development, and the increase in cyanidin derivatives is at earlier stages than for clone ME16 (mid-September) (Fig. 3).

Clone BA1 shows a different model from clones ME16 and VA1, with very low pigment biosynthesis in the early development stages, and with a later increase in cyanidin and delphinidin derivatives in mid-September (Fig. 4).

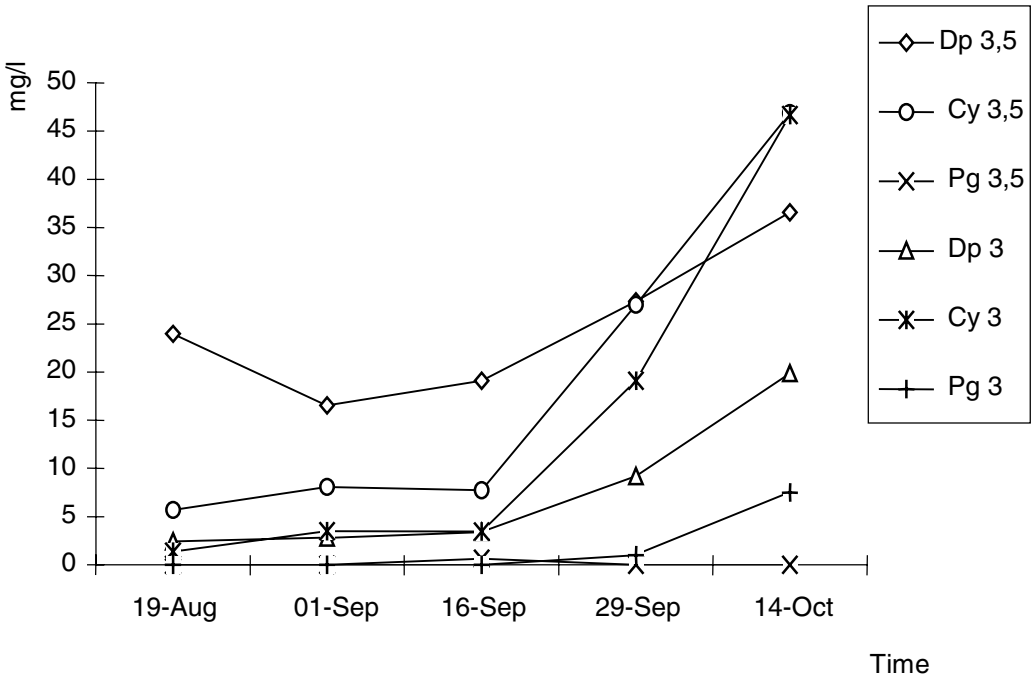

Fig. 3. Anthocyanin evolution in clone VA1.

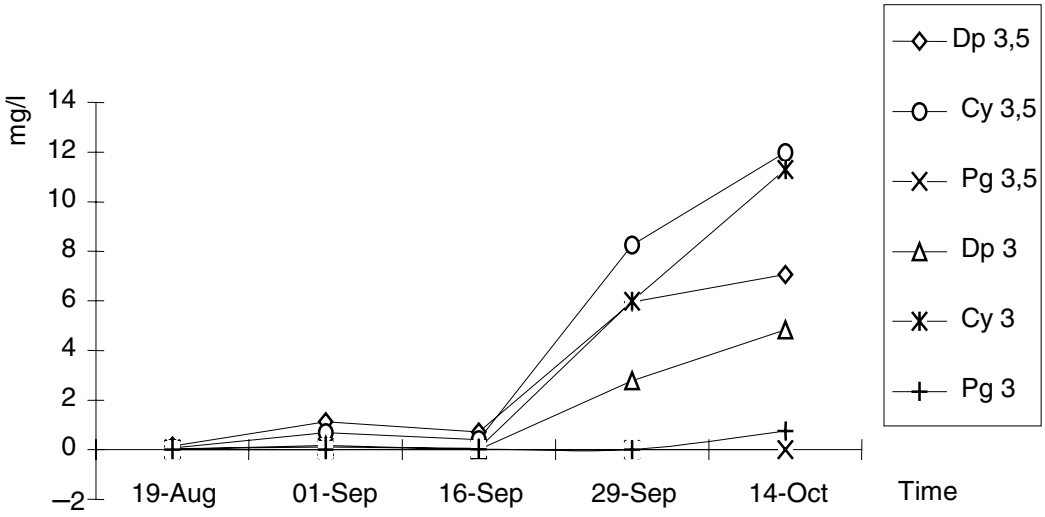

Fig. 4. Anthocyanin evolution in clone BA1.

References

Artés, F., Tudela, J.A. and Gil, M.I. (1998). Improving the keeping quality of pomegranate fruit by intermittent warming. *Z. Lebensm. Unters. Forsch.*, 207(4): 316-321.

Du, C.T., Wang, P.L. and Francis, F.J. (1975). Anthocyanins of pomegranate, *Punica granatum. J. Food Sci.,* 40: 417-418.

Gil, M.I., Artés, F. and Tomás-Barberán, F.A. (1996). Minimal processing and modified atmosphere packaging effects on pigmentation of pomegranate seeds. *J. Food Sci.*, 61: 161-164.

Gil, M.I., Cherif, J., Ayed, N., Artés, F. and Tomás-Barberán, F.A. (1995b). Influence of cultivar, maturity stage and geographical location on the juice pigmentation of Tunisian pomegranates. *Z. Lebensm. Unters. Forsch,* 201: 361-364.

Gil, M.I., García-Viguera, C., Artés, F. and Tomás-Barberán, F.A. (1995a). Changes in pomegranate pigmentation during ripening. *J. Sci. Food Agric*., 68: 77-81.

Harborne, J.B. (1982). *Introduction to Ecological Biochemistry.* Academic Press, London, UK.

Melgarejo, P. (1993). *Selección y tipificación varietal de granado (*Punica granatum *L*.). PhD Thesis, Universidad Politécnica de Valencia.