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ANTHOCYANINS FROM POMEGRANATE (*Punica granatum* L.)

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The anthocyanin content in the fruits from three cultivars of *Punica granatum* L. (asseria, mollar and negral) was studied. Six anthocyanins were isolated from the red seeds of pomegranate fruits using a combination of Sephadex LH-20 column chromatography and reverse-phase semi-preparative HPLC. On the basis of chromatographic and spectroscopic techniques, they were identified as the 3-monoglucosides and 3,5-diglucosides of delphinidin, cyanidin and pelargonidin. Total anthocyanin content in fruits of the different cultivars varied in order mollar > negral > asseria. The relative order of aglycone occurrence was cyanidin > delphinidin >> pelargonidin. The 3-monoglucoside and 3,5-diglucoside anthocyanin derivatives occurred in similar amounts. The asseria cultivar was distinct in that it showed to contain more 3,5-diglucosides than 3-monoglucosides, and mollar contained twice as much pelargonidin derivatives than other cultivars. The anthocyanin extracts from fruits of *Punica granatum* L. could be a suitable source of natural food colorants.

KEYWORDS: *Punica granatum* L., Punicaceae, anthocyanins, natural food colorants.

INTRODUCTION

Pomegranate is an important commercial fruit in Mediterranean countries. The red seeds of the fruit can be consumed directly or as juice, which can also be used in beverages for jellies, flavouring and colouring agents (Al-Maiman and Ahmad, 2002). Portugal produced 668 tons pomegranate fruit in 1999 (Portuguese Fruits and Vegetables Yearbook, 2000/01). The purpose of this work was to investigate the anthocyanin content of three pomegranate cultivars grown in Portugal.

EXPERIMENTAL

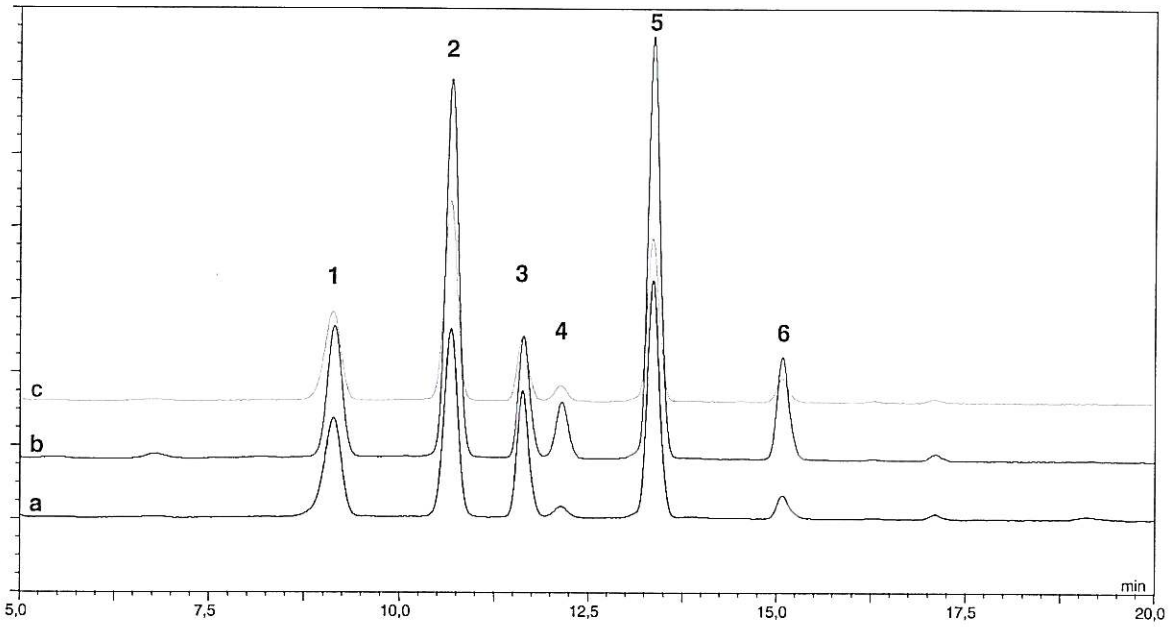
Samples from three pomegranate cultivars (*Punica granatum* L., var asseria, mollar and negral) grown in Algarve, southern Portugal, were obtained from Direcção Regional de Agricultura do Algarve. Fruits from an unknown cultivar were purchased from MARL (Mercado Abastecedor da Região de Lisboa).

For analytical purposes, 10 g of red seeds from pomegranate cultivars asseria, mollar and negral were extracted with 20 mL of methanol acidified with 0.1% TFA, filtered and analysed by HPLC-DAD. For preparative purposes, fruits purchased at MARL (21 Kg) were pealed and the red seeds (11.9 Kg) were extracted overnight with 12 L of a refrigerated methanolic solution acidified with 0.1% TFA. The crude extract was filtered and concentrated under vacuum in a rotary evaporator equipped with a water bath at 25º C. The concentrated extract (0.5 L) was partitioned against hexane and ethyl acetate. The partitioned extract was adsorbed onto a column packed with Amberlite XAD-7 resin and washed with water. Anthocyanins were eluted with a methanolic and aqueous solution acidified with 0.1% TFA, where the methanol content increased from 20 to 100%. The eluate was collected and concentrated under reduced pressure as before. The concentrated extract was fractionated by column chromatography in a Amersham Biosciences column (1000 x 50 mm) packed with Sephadex LH-20. The mobile phase was delivered at 5 $mL.min^{-1}$ and consisted of a methanolic and aqueous solution acidified with 0.1% TFA, where the methanol content increased from 40 to 60%. The separated fractions were collected, concentrated and analysed by HPLC-DAD on a reverse-phase column Luna C18(2), 3μ, 150 x 4.6 mm. Fractions where pigments were not fully purified were further submitted to semi-preparative HPLC on the same column. Mobile phase consisted of solvents A (Water: Formic acid, 9:1) and B (Water:Formic acid:Methanol, 4:1:5), delivered at 0.6 $mL.min^{-1}$ in a linear gradient mode from 10 to 100%B in 30 min. A Diode Array Detector was used. Quantification was based on averaged detection between 500-540 nm with 1 nm step interval.

NMR experiments were run on a Bruker 400 AMX instrument equipped with a 5 mm inverse detection probe, using the deuteriomethyl ^{13}C and the residual ^{1}H signal of the solvent (CF_3CO_2D-CD_3OD; 5:95 v/v) as secondary references (δ 49.0 and 3.4 from TMS, respectively).

RESULTS AND DISCUSSION

Six anthocyanins were isolated and identified from red seeds of pomegranate fruit. The HPLC-DAD (detection at 520±20 nm) analysis of pomegranate cultivars: a) asseria, b) molar, and c) negral. Pigment identification: 1) delphinidin 3,5-diglucoside, 2) cyanidin 3,5-diglucoside, 3) delphinidin 3-glucoside, 4) pelargonidin 3,5-diglucoside, 5) cyanidin 3-glucoside, and 6) pelargonidin 3-glucoside.



The anthocyanin content of pomegranate cultivars was determined by HPLC analysis. Mollar and negral contained 207% and 127% more anthocyanins than asseria, respectively. The occurrence of aglycones ranked in order cyanidin > delphinidin >> pelargonidin. The content in 3,5-diglucosides was similar to that of 3-monoglucosides, except in asseria.

	Asseria	Mollar	Negral
Total anthocyanin	30.64	63.69	38.83
Delphinidins	30.19	21.88	35.52
Cyanidins	63.16	65.51	59.90
Pelargonidins	6.65	12.60	4.58
3,5-diglucosides	59.07	48.92	48.73
3-glucosides	40.93	51.08	51.27

CONCLUSIONS

Six anthocyanins isolated from the red seeds of pomegranate fruits were identified as the 3,5-diglucoside and 3-monoglucoside derivatives of delphinidin, cyanidin and pelargonidin. The anthocyanin content in three pomegranate cultivars (asseria, molar and negral) was estimated by HPLC-DAD. Those cultivars were found to be distinguishable on the basis of their anthocyanin profile.

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