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Determination of anthocyanins in blood orange juices by HPLC analysis

Luigi Mondello [a,*], Antonella Cotroneo [a], Giacinto Errante [a], Giovanni Dugo [a], Paola Dugo [b]

[a] *Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università di Messina, viale Annunziata, 98168 Messina, Italy*
[b] *Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze, Università di Messina, salita Sperone 31, 98166 Messina, Italy*

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Abstract

Determination of anthocyanins in fresh and concentrated juices can be a parameter for the assessment of authenticity and quality of blood orange juices. This work reports an HPLC/UV-Vis method developed for quantitative determination of anthocyanins in blood orange juices, by using a calibration curve obtained for standard cyanidin-3-glucoside. Samples analysed have been obtained from fruits of different trees (one for each of the varieties: 'Moro', 'Tarocco', 'Sanguinello' and 'Sanguinello nocellare') harvested about every 15 days during the 1998 productive season. Seasonal variation has been also evaluated. HPLC results were compared with spectrophotometric measurements, using a calibration curve obtained for cyanidin-3-glucoside solutions. The two methods showed good agreement, but the results obtained greately differed with the data reported in the literature.

Keywords: Blood orange juice; Anthocyanins; HPLC; Spectrophotometric analysis

1. Introduction

Anthocyanins are a group of naturally occurring phenolic compounds responsible for the colour of many plants, flowers and fruits [1].

Recently, this group of compounds have gained a great importance because of their different pharmacological activities that have been demonstrated [2–13].

Literature does not report any data on the toxicity of anthocyanins. However, their safety has been extensively demonstrated by the large consumption of food products that contain anthocyanins, such as bilberries, grapes or wine. Hiterto the use of anthocyanins as food colour is limited, since they have a number of drawbacks, such as sensitivity to bleaching by sulfur dioxide and limited colouring capability at pH values above 3.5 [14].

Blood oranges (cultivar Moro, Tarocco and Sanguinello) are a characteristic product of East-

* Corresponding author. Fax: +39-90-6766532.
E-mail address: lmondello@pharma.unime.it (L. Mondello)

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ern Sicily, and represent a natural source of anthocyanins. Several studies carried out on this product have shown that cyanidin-3-glucoside is the main component of the fraction [15,16]. Recently, Maccarone et al. [17] identified other major anthocyanins of blood orange juice as cyanidin-3(6″-malonyl)-β-glucoside. This compound was previously identified as cyanidin-3-ramnoside [18]. The other minor pigments of the fraction are still unknown.

The concentration of anthocyanins can be dependent on genetic, and physiological factors, on the ripening of the fruits, on the soil and climate characteristics. The quantitation of this components can be useful for both the assessment of the commercial value of the product, and to determine the daily intake of these compounds, in relation with the possible pharmacological actions that they may have. Therefore, the determination of anthocyanin content in fresh and concentrated juices can represent a parameter for authenticity and quality control of the product. Many methods have been developed, mainly based on spectrophotometric measurements [19–21].

The difficulty to compare results obtained with different methods, and the lack of a reliable method to obtain certain and reproducible quantitative data render necessary the development of a simple and reproducible method for the quantitative analysis of anthocyanins in blood orange juice.

In this work, the quantitative determination of anthocyanins in blood orange juices has been determined following two different procedures: spectrophotometric analysis and HPLC-UV analysis.

2. Experimental

2.1. Samples

The analyses have been carried out on 25 samples of blood orange juice prepared in laboratory from fruits harvested during the productive season 1997–1998. Fruits were harvested about every 2 weeks from January to May from trees of cultivar Sanguinello, Sanguinello Nocellare, Moro and Tarocco. Orange juices were obtained by hand-squeezing about 10 kg of oranges per time point immediately after their harvest, and the juices were stored at −18°C until analysis.

The determination of the total amount of anthocyanins has been carried out by spectrophotometric analysis and by HPLC analysis.

2.2. Spectrophotometric analysis

Spectrophotometric analyses were performed under the following conditions: 10 ml of juice were filtered through glass wool, and the pulp washed with 90 ml of a EtOH/HCl mixture previously prepared mixing 79.7 ml of anhydrous ethyl alcohol with 20.3 ml of HCl (37%). The absorbance has been measured at 535 nm, by an UV-Vis Hitachi U-2000 spectrophotometer, using 1 cm cells.

The calibration curve has been obtained by measuring absorbance of standard solutions of pure cyanidin-3-glucoside chloride (99.5% purity grade tested by HPLC, Extrasynthese, Genay, France).

2.3. HPLC method

The HPLC analyses were carried out under the following procedure: 5 ml of juice have been filtered trough glass wool and pulp has been washed with 5 ml of distilled water. 20 μl of this solution have been injected in HPLC and analysed under these conditions: Shimadzu LC10-Avp system equipped with an auto-sampler, an UV-Vis detector and a data acquisition system Shimadzu Class VP-5 (Shimadzu, Milan, Italy). Column: Restek Pinnacle ODS, 250 × 4.6 mm, 5 μm particle (Superchrom, Milan, Italy), thermostatted at 40°C; elution with a binary high pressure gradient at a flow rate of 1 ml/min. Solvent A, H_2O:HCOOH, 9/1; solvent B, H_2O:HCOOH:CH_3CN, 4/1/5. The percentage of solvent B increased linearly, after an initial hold of 1 min, from 12 to 30% in 25 min; then, to 100% in additional 9 min the column was then reconditioned with the initial eluent for about 20 min.

Detection was by absorbance at 518 nm × 1AUFS.

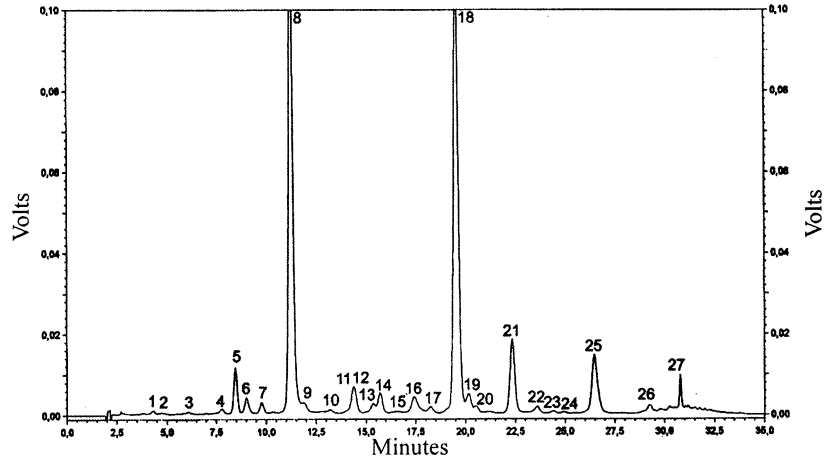

Fig. 1. HPLC chromatogram of the anthocyanin fraction of a blood orange juice of the 'Moro' cultivar.

3. Results

Fig. 1 reports the HPLC chromatogram of the anthocyanins fraction of a Moro juice. As can be seen, 27 components belonging to the anthocyanin family have been detected. Peak marked with number 8 corresponds to cyanidin-3-glucoside, while peak marked with number 18 corresponds to cyanidin-3-(6″-malonyl)-glucoside. Three injections of the same sample have been carried out to evaluate the robustness of the method. For all the 27 components, the RSD% of the area response was typically less than 5%, while for retention times RSD% was less than 1%. The reproducibility of the sample preparation has been checked by injecting three times the same sample freshly prepared each time. The reproducibility of the area response was typically 3–4%.

For the quantitative analysis of anthocyanins, a calibration curve has been obtained by injection of different concentration of cyanidin-3-glucoside standard. These curve was used to obtain quantitative data for all the analysed samples. The areas of the 27 peaks were summed and measured against cyanidin-3-glucoside. These results are shown in Table 1. As can be seen, the cultivar Moro contains the highest amount of anthocyanins, with a maximum value of 217 ppm at the first half of April; cultivar Tarocco shows the highest value of 133 ppm in the first decade of

Table 1
Amount of anthocyanins in the analysed orange juices

Juice	Date	Anthocyanins (ppm)
Sanguinello A1	23/01/98	14
Sanguinello A2	13/02/98	21
Sanguinello A3	10/03/98	37
Sanguinello A4	23/03/98	5
Sanguinello A6	15/04/98	2
Sanguinello A7	15/05/98	3
Sanguinello B1	23/01/98	18
Sanguinello B2	16/02/98	56
Sanguinello B3	10/03/98	54
Sanguinello B4	23/03/98	52
Sanguinello B5	30/03/98	49
Tarocco C1	23/01/98	41
Tarocco C2	16/02/98	88
Tarocco C3	10/03/98	133
Tarocco C4	23/03/98	46
Tarocco C5	30/03/98	61
Tarocco C6	15/04/98	54
Tarocco C7	15/05/98	76
Moro D1	23/01/98	135
Moro D2	13/02/98	172
Moro D3	10/03/98	181
Moro D4	23/03/98	176
Moro D5	30/03/98	215
Moro D6	15/04/98	217
Moro D7	15/05/98	128

Table 2
Content of anthocyanins in Moro orange juices produced in Sicily (ppm)

	Our results	Di Giacomo et al. [21]	Rapisarda et al. [19]	Russo and Galoppini [22]
Min	128	497	48	700
Max	217	1038	197	2200

March; cultivar Sanguinello and Sanguinello Nocellare show always the lowest values of anthocyanins: Sanguinello reaches its maximum value at 37 ppm in the first half of March, while Sanguinello Nocellare in the second decade of February (56 ppm). As can be seen, each cultivar show a characteristic seasonal variation of the content of anthocyanins.

The content of anthocyanins in Sanguinello oranges increases from the end of January to the first half of March, then drastically decreases showing very low values (2–3 ppm) by the end of the season; oranges of cultivar Sanguinello Nocellare show the highest value at the beginning of February, and this value remains approximately constant until the end of March. For juices obtained from Tarocco oranges the content of anthocyanins increases from the end of January, reaching its maximum value during the first decade of March, then decreases to the values of the beginning of the season. Samples obtained from Moro oranges show always the highest content of anthocyanins. The maximum value is reached between the end of March and the first half of April.

Data obtained by the HPLC method were compared with those obtained by spectrophotometric analysis. Results were in good agreement.

Table 2 reports the data obtained in this work compared with those previously reported in literature, for oranges of cv Moro produced in Sicily. As can be seen, our values agree with those obtained by Rapisarda et al. [19]; values reported by Di Giacomo et al. [21] and by Russo and Galoppini [22] are sensibly higher than those obtained by our method. These differences are mainly due to the calibration method used.

These results show the need to have a high pure compound to be used as standard for the preparation of a calibration curve. The quantitative composition of anthocyanins present in blood orange juices represents a good parameter for the characterisation of the product and may be universally determined with a simple and reproducible method.

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