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Isolation and identification of pelargonidin 3-glucoside in mangosteen pericarp

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ABSTRACT

In the present study, we have identified pelargonidin 3-glucoside, along with two known anthocyanin; cyanidin 3-sophoroside and cyanidin 3-glucoside, from acidified, methanolic extract of mangosteen pericarp. The compounds were separated by preparative HPLC after purification by partition against ethyl acetate and Amberlite XAD-7. The structures of the compounds were confirmed by high performance liquid chromatography (HPLC), UV–Vis absorption spectra, high-resolution electrospray mass spectrometry and 1D, 2D nuclear magnetic resonance (NMR) spectroscopy. This new pigment family adds to the growing body of data supporting the use of natural colourants in food. Cyanidin 3-sophoroside was the major anthocyanin detected in large amount (76.1%), followed by cyanidin 3-glucoside (13.4%) and pelargonidin 3-glucoside (6.2%).

1. Introduction

Anthocyanins have recently received increasing attention as natural colourants in food systems, as against synthetic ones. Synthetic dyes commonly used in the food industry have been suspected to cause adverse behavioural and neurological effects (McCann et al., 2007). Thus, new sources of pigments, with high colourant power, stability and low cost, make anthocyanins attractive natural colourants. The substituting groups on the anthocyanins mainly influence reactivity and colour by changing the electron distribution on the molecule.

Garcinia mangostana L. (mangosteen) is a tropical tree belonging to the Guttiferae family. The fruit has a dark purple pericarp that is rich in bioactive secondary metabolites, including anthocyanins, oligomeric proanthocyanins and xanthones (Fu, Loo, Chia, & Huang, 2007). The edible portion of the fruit comprises only about 25% of the total volume, whereas the remainder is tough, bitter pericarp, 6–10 mm in diameter, which exudes a yellow resin. As the fruit ripens the colour of the mangosteen pericarp changes from green to reddish-brown to purple-black. The purple colour of the mangosteen pericarp is reported due to be mainly anthocyanins. The anthocyanin of mangosteen fruits is underexplored. To the best of our knowledge there are limited data on mangosteen anthocyanins (Du & Francis, 1977; Palapol et al., 2009) which are identified as cyanidin 3-sophoroside, cyanidin 3-glucoside and cyanidin-glucosidepentoside, mainly by thin-layer chromatography (TLC), and HPLC/MS.

Various analytical methods have been reported for profiling anthocyanin content of fruits. The common approach includes a combination of liquid chromatography-based separation with light absorbance-based quantification (Aaby, Wrolstad, Ekeberg, & Skrede, 2007; Kahle et al., 2006), usually at 520 nm wavelength, and nuclear magnetic resonance (NMR) spectroscopy for structural identification. The most abundant anthocyanins in the edible parts of plants are cyanidin, followed by pelargonidin, peonidin, delphinidin, petunidin, and malvidin (Hager, Howard, Prior, & Brownmiller, 2008).

The major objective of the present study was to investigate the presence of anthocyanin pigments in mangosteen pericarp and to elucidate the chemical structures by NMR spectroscopy and HPLC with electrospray ionisation mass spectrometry (ESI-MS). We have now identified pelargonidin 3-glucoside for the first time in mangosteen pericarp.

2. Methods and materials

2.1. Chemicals and fruit material

All solvents were of HPLC grade and purchased from Merck (Mumbai, India), Amberlite XAD-7 resin was from Sigma–Aldrich (St. Louis, MO, USA) and C-18 Sep-Pak cartridges were purchased from Waters Corporation (Milford, MA, USA).

Mangosteen fruits (*G. mangostana* L.) were purchased from a commercial orchard (Tamil Nadu, India). The pericarps were removed from the fruit flesh with a stainless steel knife, immediately

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lyophilised, and finely ground to powder with a hammer mill with a sieve having apertures of 0.685 mm (Cadmach machinery Pvt. Ltd., Ahmedabad, India).

2.2. Isolation and identification of anthocyanins

The mangosteen pericarp powder (80 g) was extracted with 0.01% (v/v) hydrochloric acid (HCl) in 500 ml of methanol. The solution obtained from the extraction was filtered through Whatman No. 1 paper, and concentrated (5 g) under reduced pressure, purified by partition against an equal volume (four times) of hexane and ethyl acetate (to remove polyphenolics) and applied onto an Amberlite XAD-7 column (50 × 2.0 cm). The non-aromatic compounds were removed with the use of an Amberlite XAD-7 column. The column was washed with 250 ml of water, and the anthocyanins adsorbed to the column were eluted with 250 ml of methanol containing of 0.01% (v/v) HCl. The eluate was concentrated under reduced pressure at 25 °C. The obtained eluate was then passed through a C-18 Sep-Pak cartridge, previously activated with methanol, followed by 0.01% HCl (v/v) in deionised water. Anthocyanins and polyphenolics were adsorbed onto the Sep-Pak column, while sugars, acids and other water-soluble compounds were removed by washing the Sep-Pak cartridge with 0.01% (v/v) HCl in deionised water. The anthocyanins were recovered with methanol containing 0.01% HCl (v/v). The acidified methanol fraction was evaporated (120 mg) using a rotary evaporator at 30 °C (Buchi RE-111, Switerzerland).

2.3. Colour measurements

The changes in colour of the anthocyanin systems were determined from the CIELAB parameters, using a Hunter colourimeter (Hunterlab D25 A-9, USA) equipped with the light source D65 and observation angle of 10°. Using the parameters L^*(lightness), a^* (red) and b^* (yellow), the values for C^* (chroma), h (hue angle) and ΔE^* (total colour difference) were calculated using Eqs. (1)–(3).

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

$$h = \arctan(b^*/a^*) \quad (2)$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

2.4. Monomeric anthocyanin content

The total monomeric anthocyanin content was measured by the pH-differential method (Giusti & Wrolstad, 2000). A Shimadzu UV–Vis spectrophotometer (Shimadzu, Japan, Model UV-160A) was used to measure absorbance at 510 and 700 nm. Monomeric anthocyanins were calculated as cyanidin-3-glucoside equivalents, using the molar absorption coefficient of 26,900 l cm^{-1} mol^{-1}, and a molar mass of 449 g mol^{-1}.

2.5. Characterisation of anthocyanins

The individual anthocyanins were separated using preparative HPLC, and PDA detector (Shimadzu LC-10, Japan) equipped with Varian Pursuit XRs (250 × 21.1 mm, id., 10 μm particle size). All of the peak fractions were collected for several cycles. Before injection, samples were filtered through 0.45 μm nylon membrane filters (Fisher Scientific, Fair Lawn, New Jersey). Two solvent systems were used for elution: A = 5% formic acid in water and B = formic acid–water–acetonitrile (1:4:5, v/v). The elution profile consisted of a linear gradient from 12% B for 0.06 min, 30% B for 16 min, followed by 100% B for 20.5–22 min and 12% B for 25–35 min. The flow rate was 5 ml/min for 40 min, and aliquots of individual peaks were pooled and concentrated using a lyophiliser (ThermoLab, UK), separately, and subjected to NMR spectroscopy. The UV–Vis absorption spectra were recorded on-line during HPLC analysis, over the wavelength range of 200–600 nm.

Mass spectra were acquired using Ultima ESI-Q-TOF (Micromass, UK Limited) with electrospray ionisation in positive mode (ESP+). The following ion optics were used: capillary voltage: 3.50 kV; cone: 100, source temperature: 120 °C, desolvation temperature: 300 °C, cone gas flow 50 l/h, desolvation gas: 500 l/h. The mass scan range was from 250 to 1000 m/z, scan speed 1000 amu/s.

All of the individual anthocyanin peaks were analysed separately by ^{1}H (500.18 MHz) and ^{13}C (125.78 MHz) NMR on a Bruker Avance 500 instrument (Bruker Biospin, Fallanden, Switzerland). Sample (15 mg) was dissolved in 0.5 ml of deuterated methanol (CD_3OD) for recording the spectra. The one bond proton carbon shift correlations were established by heteronuclear single quantum coherence transfer (HSQCT). Chemical shifts were presented in δ (parts per million) and the coupling constants (J) in Hertz, respectively.

2.6. Acid hydrolysis of anthocyanins

Five millilitres of 2 M HCl were added to the solution of the purified anthocyanins (0.5 ml) in a screw-cap test tube, flushed with nitrogen and capped. The hydrolysis was carried out for 1 h at 100 °C and then the solution was immediately cooled in an ice bath, as described by Chaovanalikit, Thompson, and Wrolstad (2004). The hydrolysate was purified by using a C-18 Sep-Pak cartridge.

2.7. Alkaline hydrolysis of anthocyanins

Alkaline hydrolysis was done according to the method of Chaovanalikit et al. (2004). An aliquot of the purified anthocyanins (0.5 ml) was dissolved with 5 ml of 10% (w/v) KOH in a screw-cap test tube. The tube was flushed with nitrogen and capped. The pigments were hydrolysed for 8 min in the dark at room temperature. Then, the solution obtained was neutralised with 2 M HCl and the hydrolysate was purified by using a C-18 Sep-Pak cartridge.

3. Results and discussion

3.1. General

In order to obtain structural information, the compounds were identified by 2D-HSQCT NMR data, mass spectra (MS) fragmentation pattern and UV–Vis spectra. A brief structural elucidation of the compound 3 identified it as cyanidin 3-sophoroside and compound 5 as cyanidin 3-glucoside. Similarly, the NMR data for compounds 4 corresponded to pelargonidin 3-glucoside. Pelargonidin 3-glucoside has not been previously reported in mangosteen. The ^{1}H and ^{13}C NMR spectra (**P3**–**P5**) are provided in Table 1.

Fig. 1 shows a typical HPLC profile of mangosteen pericarp anthocyanins, which were obtained from a mixed sample of cultivars. As can be seen, five peaks are present in the chromatogram. The minor peaks 1 and 2, having an area percentage of less than 3.2% could not be identified. The three major anthocyanins, corresponding to peaks 3–5, represented about 76.1%, 6.2% and 13.4%, respectively, of the total peak area revealed at 530 nm. Alkaline hydrolysis of the pigments produced a chromatographic profile similar to that shown in Fig. 1, and a low absorbance in the