Identification and Determination of Betacyanins in Fruit Extracts of Melocactus Species

Katarzyna Sutor and Slawomir Wybraniec*

ABSTRACT: Betacyanin pigments were studied in edible fruits of four Melocactus species, M. violaceus Pfeiff., M. bahiensis (Britton & Rose) Luetzelb., M. amoenus (Hoffm.) Pfeiff., and M. curvispinus Pfeiff., by means of chromatographic and mass spectrometric techniques. The main pigment constituent, melocactin, endogenously present in the Melocactus species, was identified as betaninid-5-O-β-sophoroside betacyanin, previously known as “bougainvillein-r-1”. The highest total concentration of betacyanins was found in fruits of M. amoenus (~0.08 mg/g). Except for melocactin being the most abundant betacyanin (34.8–38.8%) in the analyzed species, a presence of its malonylated derivative, mammillarinin (15.2–19.9%), as well as more hydrophobic feruloylated and sinapoylde melocactins was confirmed by additional co-chromatographic experiments with authentic reference betacyanins. The acyl migration isomers of the malonylated betacyanins as well as a presence of S′-O-E-sinapoyl-2′-O-apiosyl-betanin (2.3–3.0%) found frequently in light-stressed cacti was also acknowledged.

KEYWORDS: Melocactus, Cactaceae, acylated betacyanins, betalains, melocactin, mammillarin, phyllocactin, acyl migration, bougainvillein-r-1

INTRODUCTION

Increasing consumer awareness and targeting of the market to nontoxic and natural additives for cosmetics or food touches upon issues related to the still insufficiently studied betalains. Compounds from this group are water-soluble dyes, containing nitrogen in their structures, red-violet betacyanins and yellow-orange betaxanthins, with similar structural properties and origins.1 Due to the high coefficient of molar extinction, their dyeing ability is competitive with that of synthetic dyes2 but also other natural pigments such as anthocyanins.3 Therefore, plants rich in betalains are not only regarded as valuable foods worldwide4 but also have wide potential for use in food chemistry and technology.5,6 Increasingly, attention is also paid to the valuable health-promoting properties of betalains.7 In vitro studies as well as animal models in vivo showed promising perspectives in the use of betalin antioxidant and anti-inflammatory properties in the case of chronic inflammation, liver diseases,8 arthritis,9 and even with diseases associated with cancer (e.g., skin, lung, liver, colorectal cancers).10 Attention is drawn also to the possible benefits of using betalains with drugs which can increase therapeutic effect and alleviate the toxic side of anticancer drugs.11

As emphasized by scientific sources, there is a need for a broader study of other betacyanins,12 which will not only contribute to a better understanding of the influence of structural factors on bioactivity and antioxidant properties but also help in their effective use in medicine or pharmacy. Betalains are synthesized by most plants of the order Caryophyllales wherein they replace anthocyanins3,13,14 and were also found in some genera of higher fungi such as the fly agaric Amanita muscaria L., Hygrocybe, and Hygrophorus.15 Other plant sources include species of the genus Bougainvillea in which a rich betacyanin profile was discovered16–18 as well as Basella alba L.,19 Gomphrena globosa L.,20 with gomphrenins, and the Amaranthus genus for which the occurrence of amaranthin is characteristic.21

The presence of betalains in cacti was widely confirmed in the genus Opuntia.22 Fresh juice from the fruits of Opuntia ficus-indica in studies on rats contributed to the reduction of the negative effects of ulcerative colitis and the inhibition of oxidative stress markers.23 The reduction of hepatic cholesterol taking place under the influence of the aforementioned extract was also indicated, which allowed maintenance of an appropriate cholesterol balance.24 In addition, research indicates an anti-inflammatory effect25 and the possibility of inhibiting the replication of some viruses.26

In fruits of Hylocereus polyrhizus, three major pigments were identified, betalin, phyllocactin, and hylocerenin,27,28 but also apioufaranosyl betacyanins and compounds containing sinapoyl moiety.29 Hylocereus plant extract also has the ability to lower total cholesterol in the body, which has also been tested in rats,30 and additionally has beneficial effects on the liver31 and on stabilization of blood glucose levels.32 The extracts of these plants also have strong antibacterial effects.33

Received: July 25, 2020
Revised: August 20, 2020
Accepted: September 15, 2020
Published: September 15, 2020
In a large number of *Mammillaria* species, the dominant one is 5-O-(6′-O-malonyl)-β-sophoroside (mammillarinin) isolated from their fruits. Additional betacyanins found in *Mammillaria* species are betanin, phyllocactin, betanidin 5-O-β-sophoroside, 2′-O-apiosyl-phyllocactin, and acyl migration derivatives. The health-promoting properties of *Mammillaria* plants have been less studied; however, these plants have also been proven to have a strong antibacterial effect and high antioxidant activity. There are also reports on the anticancer effects of plant extracts of *Opuntia*, *Hylocereus*, and *Mammillaria*.

This contribution reports on betacyanins in fruits of *Melocactus* species. According to the literature, it is a type of betalain-rich cactus that has not received enough attention so far and which can be a good source of the pigments. *Melocactus* is a genus of 30–40 cacti species found mainly in Southern and Central America which produce cephalia (Figures 1 and 2) during two distinct growth phases. The first or juvenile phase of growth is nonreproductive, and the plants look like normal globose cacti. The second or adult phase results in a radical change in appearance through the production of the cephalium, which is a mass of areoles that produces the reproductive structures. The cephalia grow slowly and persist for years, producing flowers and fruits each season. The fruits are conical fleshy berries, mostly magenta or red, and multiple-seeded.

*Melocactus bahiensis* (Britton & Rose) Luetzelb is very well-known for its fresh fruits in human diet. *Melocactus* species are still poorly studied food and medicinal plants. The only known bioactivities are the antibacterial as well as in vitro antiparasitic activities against *Trichomonas vaginalis* of *Melocactus zehntneri* aerial parts. Chemosprotective effect of the alkaloid extract of *Melocactus bellavistensis* against DMH-induced colon cancer in rats was reported. The edible fruits of *Melocactus* species contain betalains, but their structures are scarcely known.

## MATERIALS AND METHODS

### Plant Material

The fruits of *Melocactus violaceus* Pfeiff., *M. bahiensis* (Britton & Rose) Luetzelb, *Melocactus amoenus* (Hoffm.) Pfeiff., and *Melocactus curvispinus* Pfeiff. as well as Bougainvillea glabra Choisy bracts, *Mammillaria coronata* (Scheidweiler) fruits, *Schlumbergera × buckleyi* (T. Moore) Tjaden sunlight-stressed leaves, and *Portulaca oleracea* stems were obtained from the Botanical Garden of Jagiellonian University Institute of Botany (Cracow, Poland). *Hylocereus ocamponis* fruit peel extract was obtained from a previous study.

### Fast Betacyanin Screening in the Fruits

The betacyanins from fresh fruits of *Melocactus* species (kept at −18 °C after harvesting for a maximum of 2 h) (0.1–0.2 g) were extracted with 2 mL of 1% aqueous formic acid during 5 min of fruit grinding in a mortar, followed by centrifugation and immediate spectrophotometric as well as liquid chromatography–mass spectrometry (LC–MS) analysis without any purification. For the pigment profile representation in each species, a method of internal normalization of the chromatographic peaks derived from the MS signals was applied. For the measurement of a total concentration of the pigments, the extracts were analyzed by an Infinite 200 microplate reader (Tecan Austria GmbH, Grödig/Salzburg, Austria). The total concentration was expressed as milligrams of betanin equivalents per 100 g of fresh fruits. Quantification of betacyanins was evaluated by taking a molar extinction coefficient of $\varepsilon = 65,000 \text{ M}^{-1} \text{ cm}^{-1}$ at 536 nm for betanin in spectrophotometric calculations. Three samples per species were analyzed according to this procedure.

![Figure 1. A photograph of Melocactus amoenus (Hoffm.) Pfeiff.](image1)

![Figure 2. A photograph of Melocactus violaceus Pfeiff.](image2)
Table 1. Chromatographic, Spectrophotometric, and Mass Spectrometric Data of the Analyzed Betacyanin Pigments in Melocactus Fruit Extracts

| no. | compd                                   | R<sub>e</sub> [min] | λ<sub>max</sub> [nm] | m/z [M + H]<sup>+</sup> | m/z from MS/MS of [M + H]<sup>+</sup> |
|-----|----------------------------------------|---------------------|----------------------|------------------------|---------------------------------------|
| 1   | betanidin 5-O-β-glucoside (melocatin)  | 7.1                 | 537                  | 713                    | 551; 389; 343                         |
| 1'  | isobetanidin 5-O-β-glucoside (isomelocatin) | 7.8                 | 537                  | 713                    | 551; 389; 343                         |
| 2   | betanidin 5-O-β-glucoside (betanin)     | 8.4                 | 535                  | 551                    | 389                                   |
| 3   | 6'-O-malonyl-melocatin (mamillarinarin) | 9.0                 | 538                  | 799                    | 713; 637; 551; 389                     |
| 2'  | isobetanidin 5-O-β-glucoside (isobetanin) | 9.5                 | 535                  | 551                    | 389                                   |
| 4   | 4'-O-malonyl-melocatin                  | 9.7                 | 538                  | 799                    | 713; 637; 551; 389                     |
| 3'  | 6'-O-malonyl-isomelocatin (isomamillarinarin) | 9.7                 | 538                  | 799                    | 713; 637; 551; 389                     |
| 5   | betanidin 6-O-β-glucoside (gomphrenin)  | 10.0                | 539                  | 551                    | 389                                   |
| 4'  | 4'-O-malonyl-isomelocatin               | 10.4                | 539                  | 799                    | 713; 637; 551; 389                     |
| 5'  | isobetanidin 6-O-β-glucoside (isogomphrenin) | 10.8                | 539                  | 551                    | 389                                   |
| 6   | 6'-O-malonyl-betanin (phyllacotin)      | 10.8                | 537                  | 637                    | 619; 593; 551; 389                     |
| 7   | 4'-O-malonyl-betanin                    | 11.4                | 537                  | 637                    | 619; 593; 551; 389                     |
| 6'  | 6'-O-malonyl-isobetanin (isophyllacotin) | 11.8                | 537                  | 637                    | 619; 593; 551; 389                     |
| 8   | 4'-O-malonyl-isobetanin                 | 12.3                | 537                  | 637                    | 619; 593; 551; 389                     |
| 9   | feruloyl-dihexosyl-betanidin            | 15.2                | 536                  | 889                    | 727; 713; 551                         |
| 10  | sinapoyl-dihexosyl-betanidin            | 15.5                | 532                  | 919                    | 757; 713; 551                         |
| 11  | 5'-O-E-sinapoyl-2'-O-apiosyl-betanin    | 16.2                | 541                  | 889                    | 683; 551; 389                         |
| 10' | 5'-O-E-sinapoyl-2'-O-apiosyl-isobetanin | 16.5                | 541                  | 889                    | 683; 551; 389                         |
| 8'  | feruloyl-dihexosyl-isobetanidin         | 16.8                | 536                  | 889                    | 727; 713; 551                         |
| 9'  | sinapoyl-dihexosyl-isobetanidin         | 17.4                | 532                  | 919                    | 757; 713; 551                         |
| 11' | 6'-O-E-feruloyl-melocatin              | 18.2                | 532                  | 889                    | 727; 713; 551; 389                    |
| 11''| 6'-O-E-feruloyl-isomelocatin           | 18.9                | 532                  | 889                    | 727; 713; 551; 389                    |
| 12  | sinapoyl-dihexosyl-betanidin           | 19.0                | 533                  | 919                    | 757; 713; 551; 389                    |
| 12' | sinapoyl-dihexosyl-isobetanidin        | 19.5                | 533                  | 919                    | 757; 713; 551; 389                    |

* Tentatively identified.

**Pigment Extraction.** For chromatographic co-injection experiments, extraction of *Melocactus* fruits and samples containing authentic standards of betacyanins derived from *Ma. coronata* fruits in *H. ocamporius* fruit peel, as well as of *B. glabra* bracts and *P. oleracea* stems was separately performed three times with 30–200 mL of 80% of aqueous MeOH acidified with 5% formic acid (v/v) and subsequently filtered through a 0.2 μm i.d. pore size filter (Millipore, Bedford, MA). The extracts were concentrated using a rotary evaporator under reduced pressure at 25 °C and diluted with water before being freeze-dried.

**Pigment Purification.** Purification of the pigment extracts was performed mainly to obtain preconcentrated samples for high-resolution LC–MS ion trap time-of-flight (LC–MS-IT-TOF) experiments. The extracts were chromatographically concentrated by flash chromatography on Bioacrom cartridges (Agela Technologies, Newark, DE) filled with non-endcapped silica C18 sorbent (porosity of 60 Å and particle size of 40–60 μm). After rinsing with water, the betacyanin fractions were eluted with 50% aqueous methanol acidified with 5% formic acid (v/v). The eluates were pooled and concentrated using a rotary evaporator under reduced pressure at 25 °C and freeze-dried.

**Chromatographic Analysis by the Liquid Chromatography Diode Array–Electrospray Ionization Tandem Mass Spectrometry (LC–DAD–ESI–MS/MS) System.** For the chromatographic and mass spectrometric analyses, an LCMS-8030 mass spectrometric system (Shimadzu, Kyoto, Japan) coupled to LC-20ADXR HPLC pumps, an injector model SIL-20ACXR, and a PDA detector (photodiode array) model SPD-M20A, all controlled with LabSolutions software version 5.60 SP1 (Shimadzu), were used. The samples were eluted through a 250 mm × 4.6 mm i.d., 5.0 μm, ReproSIL C18 chromatographic column preceded by a guard column of the same material (Dr. Maisch GmbH, HPLC, Ammerbuch-Entringen, Germany). The injection volume was 20 μL, and the flow rate was 0.5 mL/min. The column was maintained at 40 °C by a thermostat. The separation of the analytes was performed using a binary gradient elution. For the separation, a gradient system that consisted of A, 4% formic acid in water, and B, pure methanol, was used as follows: 0 min, 3% B; increasing linearly to 12 min, 14% B; increasing linearly to 21 min, 15% B; increasing linearly to 30 min, 95% B. The full range PDA signals were recorded. Chromatograms were individually displayed at 540, 505, 480, and 440 nm. Positive ion electrospray mass spectra (ESI) were recorded on the LC–MS system which was controlled with LabSolutions software in selected ion monitoring mode (SIM) for registration of total ion chromatograms, mass spectra (as well as the fragmentation spectra), and ion chromatograms. The ionization electrospray source was operated in positive mode (ESI+) at an electrospray voltage of 4.5 kV and capillary temperature at 250 °C using N2 as a gas for the spray. Argon was used as the collision gas for the collision-induced dissociation (CID) experiments. The relative collision energies for MS/MS analyses were set by changing acceleration voltage at the collision cell rods in the range from −20 to −70 V.

**Chromatographic Analyses with Detection by the Liquid Chromatography–Mass Spectrometry Ion-Trap Time-of-Flight System (LC–MS-IT-TOF).** High-resolution mass spectra were recorded using an LC–MS-IT-TOF mass spectrometer (Shimadzu) equipped with an ESI source coupled to a Prominence HPLC (Shimadzu). Separation of the compounds was carried out with the gradient system as in the case of the LCMS-8030 mass spectrometer. Parameters of the LC–MS-IT-TOF spectrometer were set as follows: curved desolvation line (CDL) and heat block temperature, 230 °C; nebulizing gas flow rate, 1.5 L/min; and capillary voltage, 4.5 kV. All mass spectra, including fragmentation mass spectra, were recorded in the positive ion mode with mass range of 100–2000 Da and collision energy between 15% and 50% depending upon the compound's structure. The results of high-resolution mass spectrometry experiments (HRMS) were studied using the Formula Predictor within the LCMS Solution software. Only empirical formulas with a mass error below 5 ppm were considered relevant and recorded.
**RESULTS AND DISCUSSION**

Detection of Polar Betacyanins in Fruits of *Melocactus* Species. The first betacyanin identification in fruits of selected *Melocactus* species was performed by low-resolution LC-DAD–ESI-MS/MS. For this aim, a pigment profile in fruit extract of *Ma. coronata* was a first chromatographic approximation of the polar group of detected betacyanins. Comparison of characteristic retention times, wavelengths of absorption maxima as well as the mass-to-charge (m/z) signals of protonated molecular and fragmentation ions in positive mode with those of reference pigments derived from the *Ma. coronata* fruits was performed (Table 1). The chromatograms in Figure 3 depict typical betacyanin profiles in *M. amoenus* and *M. violaceus* fruit samples (Figures 1 and 2). The fresh extracts of the *Melocactus* species were analyzed without any purification aiming to reflect the real betacyanin profiles in the fruits.

The presence of the main known polar betacyanins [betanin 5-O-β-sophoroside 1 (melocactin), betanin 3, malonylated betanin 5-O-β-sophoroside (mammillarinin), and phyllocactin 7] as well as their 15R-isomers (Figures 3 and 4) was confirmed by their characteristic spectroscopic properties (Table 1) and coelution experiments with the authentic pigments from *Ma. coronata* fruits. 

Betanin 5-O-β-Sophoroside (Melocactin, Bougainvillein-r-I). The major betacyanin 1 showed a protonated molecular ion at m/z 713 and its daughter ion fragments at m/z 551 and 389 using positive ion mode LC–MS/MS (Table 1, Figures 3 and 4). The mass and the fragmentation pattern suggested the presence of a dihexose (713 – 389 = 2 × 162). During analysis by the high-resolution LC–MS-IT-TOF system, the protonated molecular ion was found at m/z 713.2055 (C30H37N2O18 calculated mass, 713.2036) confirming the conclusion that 1 is a dihexosyl of betanin (Table 2). Furthermore, the high polarity of the pigment reflected in a very low retention time indicated that no acylation (increasing retention time) can be taken into account instead of glycosylation in the pigment structure. Finally, comparison of the retention time of 1 with the authentic standard which was previously elucidated by NMR confirmed the presence of betanin 5-O-β-sophoroside. The standard was isolated from *H. polyrhizus* fruits in the previous study.

The pigments, betanin/isobetanin 5-O-β-sophoroside 1/1’, were present in all the tested *Melocactus* species at the highest level (Table 3, Figures 3 and 4) in contrast to *Mammillaria* species in which the dominant pigment was mainly mammillarinin. Pigment 1 was identified for the first time as “bougainvillein-r-I” in *Bougainvillea × buttiana* ("Mrs. Butt") violet bracts; however, it is not the main constituent of betacyanins determined in the bracts. Therefore, for the aim of simplicity in naming of betacyanins and because 1 is a pralimal pigment from which the other derivatives will be derived and discussed as well as because pigment 1 is an endogenous betacyanin present in *Melocactus* species at the dominant proportion, we propose to name betanin 5-O-β-sophoroside 1 as “melocactin”.

Malonylated Betanidin 5-O-β-Sophoroside (Mammillarinin) and Acyl Migration Derivative. The other betacyanins 3/3’ (Table 3, Figures 3 and 4) were detected by low-resolution LC–MS/MS at m/z 799 (Table 1) suggesting the presence of mammillarinin identified previously in *Mammillaria* species. This was confirmed by their daughter ion fragments at m/z 713, 637, 551, and 389 suggesting the presence of malonylated (799 – 713 = 86) dihexose (713 – 389 = 2 × 162). The position of the malonyl residue on the first hexose unit was suggested by the loss of one hexose (799 – 637 = 162). Further coelution experiments with the authentic standard previously structurally elucidated by NMR and derived from fruits of *Mammillaria* species as well as IT-TOF high-resolution experiments (obtained mass at...
Table 2. High-Resolution Mass Spectrometric Data Obtained by IT-TOF Analysis for Melocactin 1, Mammillarinin 3, and Their Fragmentation Ions as well as for Hydrophobic Acylated Betacyanins 8–12 Extracted from Fruits of Melocactus Species

| no. | fragmentation ions | molecular formula | [M + H]+ | [M + H]+ | predicted | error [mDa] | error [ppm] | MS5 ions |
|-----|-------------------|-----------------|----------|----------|-----------|------------|------------|----------|
| 1   | [M + H]+          | C₅₀H₅₀N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 2   | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 3   | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 4   | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 5   | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 6   | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 7   | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 8   | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 9   | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 10  | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 11  | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |
| 12  | [M + H]+          | C₄₀H₄₅N₂O₂₁     | 133.2895 | 133.2895 | 1.9       | 2.66       | 551; 389  |

“nl: neutral losses from the protonated molecular ions.

Table 3. Total Contents and Relative Concentrations of Betacyanins Identified in Four Melocactus Fruit Extracts (I, M. amoenus; II, M. violaceus; III, M. curvispinus; IV, M. bahiensis) by LC-DAD–MS Measurements

| no. | compd                  | percentage of the total peak area (%)a,b       | I      | II     | III    | IV     |
|-----|------------------------|-----------------------------------------------|--------|--------|--------|--------|
| 1   | melocactin             | 3.48 ± 0.22                                  | 15.2   | 17.8   | 17.8   | 17.8   |
| 2   | isomalocactin          | 0.3 ± 0.11                                   | 1.3    | 1.3    | 1.3    | 1.3    |
| 3   | betanin                | 5.0 ± 0.12                                   | 5.0    | 5.0    | 5.0    | 5.0    |
| 4   | mammillarinin          | 3.0 ± 0.12                                   | 3.0    | 3.0    | 3.0    | 3.0    |
| 5   | isobetanin             | 5.0 ± 0.12                                   | 5.0    | 5.0    | 5.0    | 5.0    |
| 6   | 4′-O-malonyl-melocactin| 1.0 ± 0.21                                   | 1.0    | 1.0    | 1.0    | 1.0    |
| 7   | isomalocactin          | 3.0 ± 0.12                                   | 3.0    | 3.0    | 3.0    | 3.0    |
| 8   | gomphrenin             | 1.0 ± 0.11                                   | 1.0    | 1.0    | 1.0    | 1.0    |
| 9   | 4′-O-malonyl-isomalocactin| 5.0 ± 0.22                         | 5.0    | 5.0    | 5.0    | 5.0    |
| 10  | isomalocactin          | 3.0 ± 0.12                                   | 3.0    | 3.0    | 3.0    | 3.0    |
| 11  | 4′-O-malonyl-betanin   | 1.0 ± 0.21                                   | 1.0    | 1.0    | 1.0    | 1.0    |
| 12  | 4′-O-malonyl-isobetanin| 5.0 ± 0.22                                   | 5.0    | 5.0    | 5.0    | 5.0    |

“Relative concentrations were expressed as percentage of the total peak area. Average of three measurements. a: nd: not detected. a: not quantified due to peak coelution. b: Tentatively identified. c: Tentatively identified. d: In betanin equivalents.

m/z 799.2061; C₃₃H₉₉N₂O₂₁ calculated mass, 799.2040 confirmed the presence of malonylated betanin/isobetanin 5-O-β-sophorosidase.

Along with 3′/3′, accompanying isomers previously chromatographically assigned as acyl migration products, betanin/isobetanin 5-O-(4′-O-malonyl)-β-sophoroside 4/ 4′, were also identified in the Melocactus samples (Tables 1 and 3, Figures 3 and 4) according to their retention times and coelution with the authentic standards derived from fruits of Mammillaria species as well as low- and high-resolution mass spectrometric experiments. As in the case of Mammillaria fruits, the characteristic profile of two pairs of structural isomers 3′/4 and 3′/4′ presented already in the extensive acyl migration study was confirmed here for the Melocactus fruits. For this aim, analyses of the 3′/4 and 3′/4′ standard mixtures obtained from a Mammillaria fruit extract were performed (data not shown) which enabled confirmation of retention times of 4′/4′. A similar pigment profile was reported recently for celosistrinatin (malonylated amaranthin) structural isomers extracted from a callus culture of Celosia cristata Linn. Detected protonated molecular ion at m/z 799 and its daughter ion fragments at m/z 713, 637, 551, and 389 was well as IT-TOF high-resolution experiments (obtained mass at m/z 799.2074; C₃₃H₉₉N₂O₂₁ calculated mass, 799.2040) confirmed the isomeric structures of 4′/4′.

In contrast to Mammillaria species, a presence of gomphrenins 5′/5′ (betanin/isobetanin 6-O-β-glucoside) in Melocacti is reported (Tables 1 and 3, Figure 3).
Furthermore, a presence of phyllocactin/isophyllocactin (6′-O-malonyl-betanin/isobetanin) 6/6′ as well as their acyl migration isomers,35,49,50 4′-O-malonyl-betanin/isobetanin 7/7′, is acknowledged in Melocacti only at very low concentration levels (Tables 1 and 3, Figures 3 and 4).

Detection of Hydrophobic Acylated Betacyanins Assisted by Co-chromatography with Reference Pigments. Pigments 1–7 constitute a group of relatively polar betacyanins in contrast to less polar acylated betacyanins 8–12 which were detected in the Melocactus species. These pigments were characterized by protonated molecular ions at m/z 889 and 919.

The acylated pigments 10/10′ detected at m/z 889 by low-resolution LC-DAD–MS were present in three Melocactus species: M. violaceus, M. bahiensis, and M. curvispinus (Tables 1 and 3, Figures 3 and 4). Daughter ion fragments of the pseudomolecular ion detected at m/z 683, 551, and 389 indicated a presence of sinapoyl (889 − 683 = 206) apiosylated betanin (683 − 551 = 132) which was previously identified in fruit peel of H. ocamponis47 and light-stressed stems of cacti.47 Some of the hydrophobic betacyanins accumulate in response to white and UV light presumably acting as photoprotectors in living tissues.47,50 The $\lambda_{\text{max}}$ 545 nm and coelution experiments with the authentic standards derived from fruit peel of H. ocamponis47 confirmed the pigment’s 10 assignment to 5′′-O-E-sinapoyl-2′-O-apiosyl-betanin. Subsequent high-resolution LC–MS-IT-TOF analysis (Table 2) confirmed the molecular formula of 10 (obtained mass at m/z 889.2531; C46H43N2O21 calculated mass, 889.2509). Interestingly, this pigment was not accompanied by a feruloylated analogue of 10 with m/z 859 which was frequently noticed in the cacti samples.29,47

Other acylated pigments 11/11′ detected at m/z 889 were present in all four studied Melocactus species and were characterized by low absorption maxima $\lambda_{\text{max}}$ (534 nm) (Tables 1 and 3, Figures 3 and 4). Subsequent high-resolution LC–MS-IT-TOF analysis (Table 2) confirmed the molecular formula of 11 (obtained mass at m/z 889.2478; C40H45N2O21 calculated mass, 889.2509). The fragmentation experiments of 11 (Figure 5) resulted in generation of the same ion fragments as for 8 (m/z 727, 713, and 551) suggesting a presence of feruloylated (889 − 713 = 176) dihexose (713 − 389 = 2 × 162). Co-injection experiments with isomeric pigments (m/z 889) derived from violet bracts of B. glabra indicated that the pigments 11/11′ were not gomphrenin-based betacyanins.18 Additionally, co-chromatography with other isomeric betacyanins (m/z 889) derived from P. oleracea stems excluded cellobiose-based pigments.38 Similar experiments applying isomeric pigments derived from roots of red beet (Beta vulgaris) confirmed the chromatographic identity of the pigments 11/11′. According to previous reports, the same pigments, betanidin/isobetanidin 6′-O-E-feruloyl-5-O-β-sophoroside, were most probably detected in colored Swiss chard petioles (B. vulgaris L. ssp. cicla [L.] Alef. Cv. Bright Lights)52 and UV-A light-irradiated ice plant (Mesembryanthemum crystallinum).33

Therefore, pigments 11/11′ can be tentatively assigned to 6′-O-E-feruloyl-melocactin based on LC–MS and co-injection experiments as well as assuming that the sugar moiety is sophorose present in the structure of the melocactin unit (Table 1, Figure 4).

Detection of Other Novel Hydrophobic Acylated Betacyanins. In M. amoenus, the most distinctive acylated hydrophobic pigments 8/8′ determined by low-resolution LC-DAD–MS were detected at m/z 889 with maxima of
absorption at $\lambda_{\text{max}}$ 536 nm (Tables 1 and 3, Figures 3 and 4). The fragmentation experiments of 8 resulting in detection of ion fragments at $m/z$ 727, 713, and 551 suggest a presence of feruloylated (889 – 713 = 176) dihexose (713 – 389 = 2 × 162). Detection of the fragment at $m/z$ 727 confirms that the acyl moiety is attached to the first hexosyl unit (889 – 727 = 162). Subsequent high-resolution LC–MS-IT-TOF analysis (Table 2) confirmed the molecular formula of 8 (obtained mass at $m/z$ 889.2489; C$_{40}$H$_{45}$N$_2$O$_{21}$ calculated mass, 889.2509).

The obtained low $\lambda_{\text{max}}$ for 8 indicates on 5-O-betaninid substitution by a sugar moiety and the attachment position of the feruloyl moiety at carbon C-6′ which is distant from the C-15 carboxylic group. Otherwise, the interactions between the carboxyl and feruloyl moieties would result in bathochromic shift of the $\lambda_{\text{max}}$. These results would suggest a structure similar to 11 but with isomeric feruloyl moiety configuration (cis). The elution order (Z isomer eluted earlier than the E isomer) might be proposed to be analogous to the elution order found for the E–Z stereoisomers of acylated gomphrenins$^{55}$ tentatively assigning pigment 8 to 6′-O-Z-feruloyl-melocactin assuming that the sugar unit is sophorose present in the structure of melocactin. However, the lack of additional data prevented more detailed structure elucidation; therefore, the structure of 8/8′ was tentatively determined as feruloyl-dihexosyl-betanin/isobetanin (Table 1, Figure 4).

Additional inspection of the chromatographic and spectrometric results revealed two isomeric pairs of pigments, 9/9′ and 12/12′ (Tables 1 and 3, Figures 3 and 4). An acylated pigment 9 detected at $m/z$ 919 only in M. amoenus extract exhibited low absorption maximum $\lambda_{\text{max}}$ (532 nm). The MS/MS fragmentation results obtained for 9 (ions at $m/z$ 757, 713, 551, and 389) suggest a presence of sinapoylated (919 – 713 = 206) dihexose (713 – 389 = 2 × 162). Detection of the fragment at $m/z$ 757 confirms that the acyl moiety is attached to the first hexosyl unit (919 – 757 = 162). Therefore, pigments 9/9′ can be tentatively assigned to sinapoyl-dihexosyl-betanin/isobetanin (Table 1, Figure 4). As in the case of 8, low $\lambda_{\text{max}}$ for 9 indicates on 5-O-betaninid substitution by a sugar moiety and the attachment position of the sinapoyl moiety at carbon C-6′ which is distant from the C-15 carboxylic group. Subsequent high-resolution LC–MS-IT-TOF analysis (Table 2) confirmed the molecular formula of 9 (obtained mass at $m/z$ 919.2642; C$_{40}$H$_{47}$N$_2$O$_{22}$ calculated mass, 919.2615). Acylated pigments 12/12′ detected at $m/z$ 919 in extracts of three Melocactus species, M. violaceus, M. bahiensis, and M. curvispinus, exhibited similar analytical properties as pigments 9/9′ (Tables 1 and 3, Figures 3 and 4): low absorption maximum $\lambda_{\text{max}}$ (534 nm), analogous MS/MS fragmentation results (ions at $m/z$ 757, 713, 551, and 389), and high-resolution LC–MS-IT-TOF results (Table 2) confirming the molecular formula of 12 (obtained mass at $m/z$ 919.2587; C$_{40}$H$_{47}$N$_2$O$_{22}$ calculated mass, 919.2615); therefore, it is possible that 12 is another isomer of sinapoyl dihexose present in the Melocactus species. As in the case of 9, low $\lambda_{\text{max}}$ for 12 indicates on 5-O-betaninid substitution by a sugar moiety and the attachment position of the sinapoyl moiety at carbon C-6′ which is distant from the C-15 carboxylic group. The lack of additional data prevented more detailed structure elucidation; therefore, the structure of 9/9′ was tentatively determined as sinapoyl-dihexosyl-betanin/isobetanin (Table 1, Figure 4).

Quantification of Betacyanins in the Fruits of Melocactus Species. The highest total concentration of betacyanins was found in fruits of M. amoenus (0.08 mg/g), and for the other species, M. violaceus, M. bahiensis, and M. curvispinus, it was in the range of 0.02–0.03 mg/g (Table 3). These concentration levels obtained for the species grown in a botanical garden are similar to concentrations determined in fruits of Mammillaria which is the largest genus of the Cactaceae family. In spite of the apparent similarity of the Melocactus fruits to fruits of Mammillaria species, their placement in the cephalium makes their origin exceptional, which was reflected in the analytical results of betacyanin profiles in the fruit samples (Figures 3 and 4).

Except for melocactin 1 being the most abundant betacyanin in the analyzed species (normalized concentration of 34.8–38.8%) exceeding by far the contribution from the other pigments (Table 3) except mammillarin 3 (15.2–19.9%), the other more hydrophobic pigments 8–12 constitute specific profiles based mostly on tentatively feruloyled and sinapoyled melocactin derivatives.

In fruits of M. violaceus, M. bahiensis, and M. curvispinus, these pigments contain trans-feruloyled derivative 11 at the highest proportion (normalized concentration of 2.9–3.4%). The presence of 5′-O-E-sinapoyl-2′-O-apisoyl-betanin 10 found frequently in light-stressed cacti$^{47}$ is also acknowledged (2.3–3.0%). Interestingly, the hydrophobic betacyanin profile for M. amoenus is completely different (Figures 3 and 4) and contains tentatively identified cis-feruloyled derivative 8 of melocactin at the highest proportion (0.8%). This interesting phenomenon is presumably a result of different metabolism in the much bigger fruits of M. amoenus influenced by light.

This contribution characterized for the first time the interesting betacyanin profiles in edible fruits of Melocactus species. Although detailed analysis of chemical structures of the pigments was not possible in all cases due to insufficient sample material, the co-chromatographic experiments with the known reference betacyanins supported identification of selected pigments. The structures of the acyl migration isomers of the malonylated betacyanins were confirmed by comparison with the extracts derived from Mammillaria species. In general, the polar pigments of high concentration are complemented in the Melocactus fruits by known as well as novel acylated betacyanins of higher hydrophobicity. The principal pigment, melocactin, appears as the main structural unit for most of the acylated derivatives in Melocactus species and together with the other betacyanins may be an important constituent of these chemopreventive compounds in the human diet.

### AUTHOR INFORMATION

**Corresponding Author**

Slawomir Wybraniec — Department of Analytical Chemistry, Cracow University of Technology, Cracow 31-155, Poland; orcid.org/0000-0002-1263-4188; Phone: +48-12-628-3074; Email: slawomir.wybraniec@pk.edu.pl, swybran@chemia.pk.edu.pl; Fax: +48-12-628-2036

**Author**

Katarzyna Sutor — Department of Analytical Chemistry, Cracow University of Technology, Cracow 31-155, Poland

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.0c04746
Funding
This research was financed by the Polish National Science Centre for years 2015–2018 (project no. UMO-2014/13/B/ST4/04854).

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the technical assistance of Mrs. Teresa Kołodzinska in the access to the plant samples in the Botanical Garden of Jagiellonian University Institute of Botany. The authors thank Beata Wielenśka Ph.D., eng. and Bartłomiej Fedorczyk M.Sc. from the Laboratory of Biologically Active Compounds (Warsaw University) for the excellent technical assistance with LC–MS-IT-TOF experiments.

REFERENCES
(1) Mabry, T. J.; Dreiding, A. S. The betalains. In Recent Advances in Phytochemistry; Mabry, T. J., Alston, R. E., Runckles, V. C., Eds.; Appleton: New York, 1968.
(2) Esquivel, P. Betalains. In Handbook on Natural Pigments in Food and Beverages: Industrial Applications for Improving Food Color; Carle, R., Schweiggert, R., Eds.; Woodhead Publishing: Cambridge, U.K., 2016.
(3) Stintzing, F. C.; Carle, R. Betalains. In Food Colors: Chemical and Functional Properties; Socaciu, C., Ed.; CRC Press: London, 2007.
(4) Sarbojeet, J. Nutraceutical and Functional Properties of Cactus (Opuntia ficus-indica) and its Utilization for Food Applications. J. Agric. Food Chem. 2015, 64, 1180.
(5) Hussain, A.; Sadiq, E.; Brown, L.; Ismail, P.; Rahmat, A. Effects of red-purple pitahaya (Hylocereus polyrhizus) peel powder as a fat replacer in ice cream. J. Food Process. Preserv. 2020, 44, e14420.
(6) Gengatharan, A.; Dykes, G. A.; Choo, W. S. Betalains: Natural plant pigments with potential application in functional foods. LWT - Food Sci. Technol. 2015, 64, 645–649.
(7) Belladji Slimen, I.; Najar, T.; Abderrabba, M. Chemical and Antioxidant Properties of Betalains. J. Agric. Food Chem. 2017, 65, 675–689.
(8) Vulic, J. J.; Cebovic, T. N.; Canadanovic-Brunet, J. M.; Cetkovic, G. S.; Canadanovic, V. M.; Djlas, S. M.; Tumbas Saponjac, V. T. In vivo and in vitro antioxidative effects of beetroot pomace extracts. J. Funct. Foods 2014, 6, 168–175.
(9) Pietrzkowski, Z.; Nemzer, B.; Spóra, A.; Stalica, P.; Tresher, W.; Keller, R.; Jimenez, R.; Michalowski, T.; Wybraniec, S. Influence of betalain-rich extract on reduction of discomfort associated with osteoarthritis. New Med. 2010, 1, 12–17.
(10) Hussain, A.; Sadiq, E.; Zia-Ul-Haq, Z. Betalains: Biomolecular Aspects; Springer International Publishing: London, 2018.
(11) Das, S.; Filippono, S. M.; Williams, D. S.; Das, A.; Kukreja, R. C. Beet root juice protects against doxorubicin toxicity in cardiomyocytes while enhancing apoptosis in breast cancer cells. Mol. Cell. Biochem. 2016, 421, 89–101.
(12) Chhikara, N.; Kushwaha, K.; Sharma, P.; Gat, Y.; Panghal, A. Bioactive compounds of beetroot and utilization in food processing industry: A critical review. Food Chem. 2019, 272, 192–200.
(13) Mabry, T. J. Selected Topics from Forty Years of Natural Products Research: Betalains to Flavonoids, Antiviral Proteins, and Neurotoxic Nonprotein Amino Acids. J. Nat. Prod. 2001, 64, 1596–1604.
(14) Brockington, S.; Yang, Y.; Gandia-Herrero, F.; Covshoff, S.; Hibberd, J. M.; Sage, R. F.; Wong, G. K. S.; Moore, M. J.; Smith, S. A. Lineage-specific gene radiations underlie the evolution of novel betalain pigmentation in Caryophyllales. New Phytol. 2015, 207, 1170–1180.
(15) Gill, M. Pigments of fungi (Macromycetes). Nat. Prod. Rep. 1994, 11, 67–90.
(16) Piattelli, M.; Imperato, F. Betacyanins from Bougainvillea glabra. Phytochemistry 1970, 9, 455–458.
(17) Piattelli, M.; Imperato, F. Pigments of Bougainvillea glabra. Phytochemistry 1970, 9, 2557–2560.
(18) Wybraniec, S.; Jerz, G.; Gebers, N.; Winterhalter, P. Ion-pair high speed countercurrent chromatography fractionation of a high-molecular weight variation of acyl-oligosaccharide linked betacyanins from purple bracts of Bougainvillea glabra. J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 2010, 878, 538–550.
(19) Glassgen, W. E.; Metzger, J. W.; Heuer, S.; Strack, D. Betacyanins from fruits of Basella rubra. Phytochemistry 1993, 33, 1525–1527.
(20) Heuer, S.; Wray, V.; Metzger, J. W.; Strack, D. Betacyanins from flowers of Physostegia virginiana. Phytochemistry 1992, 31, 1801–1807.
(21) Miguel, M. G. Betalains in Some Species of the Amaranthaceae Family: A Review. Antioxidants 2018, 7, 53.
(22) Minale, L.; Piattelli, M.; Nicolaus, A. Pigments of Centrospermae - IV. On the biogenesis of indicaxanthin and betanin in Opuntia ficus-indica mill. Phytochemistry 1965, 4, 593–597.
(23) Babitha, S.; Bindu, K.; Nageena, T.; Veerapur, V. P. Fresh Fruit Juice of Opuntia dillenii Haw. Attenuates Acetic Acid-Induces Ulcerative Colitis in Rats. J. Diet. Suppl. 2019, 16, 431–442.
(24) Milan-Noris, A. K.; Chavez-Santoscoy, R. O.; Olmos-Nakamura, A.; Gutierrez-Uribe, J. A.; Sema-Saldívar, S. O. An extract from prickly pear peel (Opuntia ficus-indica) affects cholesterol excretion and hepatic cholesterol levels in hamsters fed hyperlipidemic diets. Curr. Bioact. Compd. 2016, 12, 10–16.
(25) Park, E. H.; Kahng, J. H.; Lee, S. H.; Shin, K. H. An anti-inflammatory principle from cactus. Fitoterapia 2001, 72, 288–290.
(26) Hasse, C.; Feisterl, B.; Felker, P.; Heinrich, M.; Wypyszyk, S.; Butterweck, V.; Godard, M.; Pischel, I. Opuntia - Aactus crop and its many health benefits. NutraCos 2008, 9, 17–22.
(27) Wybraniec, S.; Platzner, I.; Gersh, S.; Gottlieb, H. E.; Haimberg, M.; Moglińczuk, M.; Mizrahi, Y. Betacyanins from cactus fruit Hylocereus polyrhizus. Phytochemistry 2001, 58, 1209–1212.
(28) Stintzing, F. C.; Schieber, A.; Carle, R. Betacyanins in fruits from red-purple pitaya, Hylocereus polyrhizus (Weber) Britton & Rose. Food Chem. 2002, 77, 101–106.
(29) Wybraniec, S.; Nowak-Wydra, B.; Mitka, K.; Kowalski, P.; Mizrahi, Y. Minor betalains in fruits of Hylocereus species. Phytochemistry 2007, 68, 251–259.
(30) Sani, H. A.; Baharoom, A.; Ahmad, M. A.; Ismail, I. L. Effectiveness of Hylocereus polyrhizus extract in decreasing serum lipids and liver MDA-TBAR Level in hypercholesterolemic rats. Sains Malaysiana 2009, 38, 271–279.
(31) Ramli, N. S.; Brown, L.; Ismail, P.; Rahmat, A. Effects of red pitaya juice supplementation on cardiovascular and hepatic changes in high-carbohydrate, high-fat diet-induced metabolic syndrome rats. BMC Complementary Altern. Med. 2014, 14, 189.
(32) Lugo-Radillo, A.; Delgado-Enciso, I.; Pena-Beltran, E. Betanidin significantly reduces blood glucose levels in BALB/c mice fed with an atherogenic diet. Nat. Prod. Bioprospect. 2012, 2, 154–155.
(33) Yong, Y. Y.; Dykes, G.; Lee, S. M.; Choo, W. S. Biofilm inhibiting activity of betacyanins from red pitahaya (Hylocereus polyrhizus) and red spinach (Amaranthus dubius) against Staphylococcus aureus and Pseudomonas aeruginosa biofilms. J. Appl. Microbiol. 2019, 126, 68–78.
(34) Pilbeam, J. Mammillaria; Nuffield Press: Oxford, U.K., 1999.
(35) Wybraniec, S.; Nowak-Wydra, B. Mammillaria: In A new Monolayered Betacyanin from Fruits of Mammillaria. J. Agric. Food Chem. 2007, 55, 8138–8143.
(36) Elansary, H. O.; Szopa, A.; Klimek-Szczukotowicz, M.; Jafernik, K.; Ekiert, H.; Mahmoud, E. A.; Barakat, A. A.; El-Ansary, D. O. Mammillaria species - Polyphenols Studies and Anti-Cancer, Anti-Oxidant, and Anti-Bacterial Activities. Molecules 2020, 25, 131.
(37) Dasasesamoh, R.; Youravong, W.; Wichienchot, S. Digestibility, fecal fermentation and anti-cancer of dragon fruit oligosaccharides. *Int. Food Res. J.* **2016**, *23*, 2581–2587.

(38) da Silva Barbosa, A.; Goedder, J. Q. D.; Woodrow, I. E.; de Andrade, A. P.; de Lucena, R.; de Souza, I. Elucidation of the betalainic chromoalkaloid profile of *Pilosocerus catingica* (Gurke) Byles & Rowlsey subsp. *Salvadoresii* (Werderm.) Zappi (*Cactaceae*) from Paraiba, Brazil. *Afr. J. Agric. Res.* **2017**, *12*, 1236–1243.

(39) Anderson, E. F. *The Cactus Family*, Timber Press: Portland, U.K., 2001.

(40) Zamith, L. R.; Cruz, D. D.; Richers, B. T. T. The effect of temperature on the germination of *Melocactus violaceus* Pfeiff. (*Cactaceae*), a threatened species in restinga sandy coastal plain of Brazil. *An. Acad. Bras. Cienc.* **2013**, *85*, 615–622.

(41) Trentin, D. D. S.; Giordani, R. B.; Zimmer, K. R.; da Silva, A. G.; da Silva, M. V.; Correia, M. T. D. S.; Baumvol, I. J. R.; Macedo, A. J. Potential of medicinal plants from the Brazilian semi-arid region (*Caatinga*) against *Staphylococcus* epidermidis planktonic and biofilm lifestyles. *J. Ethnopharmacol.* **2011**, *137*, 327–335.

(42) Frasson, A. P.; dos Santos, O.; Duarte, M.; da Silva Trentin, D.; Giordani, R. B.; da Silva, A. G.; da Silva, M. V.; Tasca, T.; Macedo, A. J. First report of anti-Trichomonas vaginalis activity of the medicinal plant *Polyscias decumbens* from the Brazilian semi-arid region, *Caatinga*. *Parasitol. Res.* **2012**, *110*, 2581–2587.

(43) Rios-Leon, K.; Fuertes-Ruoton, C.; Arroyo, J.; Ruiz, J. Chemoprotective effect of the alkaloid extract of *Melocactus bellavistensis* against colon cancer induced in rats using 1,2-dimethylhydrazine. *Rev. Peru. Med. Exp. Salud Publica* **2017**, *34*, 70–75.

(44) Reznik, H. Die Pigmente der Centrospermen als systematisches Element II. Untersuchungen über das Ionophorestische Verhalten. *Planta* **1957**, *49*, 406–434.

(45) de Lucena, C. M.; de Lucena, R. F. P.; Costa, G. M.; Carvalho, T. K. N.; da Silva Costa, G. M.; Alves, R. R.; Pereira, D. D.; da Silva Ribeiro, J. E.; Alves, C. A. B.; Quirino, Z. G. M.; Nunes, E. N. Use and knowledge of *Cactaceae* in Northeastern Brazil. *J. Ethnobiol. Ethnomed.* **2013**, *9*, 62.

(46) Schwartz, S. J.; von Elbe, J. H. Quantitative determination of individual betacyanin pigments by high-performance liquid chromatography. *J. Agric. Food Chem.* **1980**, *28*, 540–543.

(47) Wybraniec, S.; Stalica, P.; Spórna, A.; Mizrahi, Y. Profiles of betacyanins in epidermal layers of grafted and light-stressed cacti studied by LC-DAD-ESI-MS/MS. *J. Agric. Food Chem.* **2010**, *58*, 5347–5354.

(48) Imperato, F. Acylated betacyanins of *Portulaca oleracea*. *Phytochemistry* **1975**, *14*, 2091–2092.

(49) Wybraniec, S. Chromatographic investigation on acyl migration in betacyanins and their decarboxylated derivatives. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2008**, *861*, 40–47.

(50) Lystvan, K.; Kumorikiewicz, A.; Szneler, E.; Wybraniec, S. Study on betalains in *Celosia cristata* Linn. callus culture and identification of new malonylated amaranthins. *J. Agric. Food Chem.* **2018**, *66*, 3870–3879.

(51) Wendel, M.; Nizinski, S.; Tuwalska, D.; Starzak, K.; Szot, D.; Prukala, D.; Sikorski, M.; Wybraniec, S.; Burdzinski, G. Time-resolved spectroscopy of the singlet excited state of betanin in aqueous and alcoholic solutions. *Phys. Chem. Chem. Phys.* **2015**, *17*, 18152–18158.

(52) Kugler, F.; Stintzing, F. C.; Carle, R. Identification of betalains from petioles of differently colored Swiss chard (*Beta vulgaris* L. ssp. *cicla* [L.] Alef. Cv. Bright lights) by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 2975–2981.

(53) Vogt, T.; Ibdah, M.; Schmidt, J.; Wray, V.; Nimtz, M.; Strack, D. Light-induced betacyanin and flavonol accumulation in bladder cells of *Mesembryanthemum crystallinum*. *Phytochemistry* **1999**, *52*, 583–592.

(54) Schliemann, W.; Strack, D. Intramolecular stabilization of acylated betacyanins. *Phytochemistry* **1998**, *49*, 585–588.

(55) Kugler, F.; Stintzing, F. C.; Carle, R. Charakterisation of betalain patterns of differently coloured inflorescences from *Gomphrena globosa* L. and *Bougainvillaea* sp. by HPLC-DAD-ESI-MS*. *Anal. Bioanal. Chem.* **2007**, *387*, 637–648.