Journal of Chromatography A, 1489 (2017) 51–57

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Separation of betacyanins from purple flowers of *Gomphrena globosa* L. by ion-pair high-speed counter-current chromatography☆

Aneta Spórna-Kucab*, Joanna Jagodzińska, Sławomir Wybraniec

Department of Analytical Chemistry, Institute C-1, Faculty of Chemical Engineering and Technology, Cracow University of Technology, ul. Warszawska 24, Cracow 31-155, Poland

ARTICLE INFO

Article history:
Received 28 September 2016
Received in revised form 20 January 2017
Accepted 24 January 2017
Available online 25 January 2017

Keywords:
Betacyanins
Ion-pair reagents
Counter-current chromatography
Gomphrena globosa L.

ABSTRACT

Betacyanins, known as antioxidants and chemopreventive natural compounds with colourful properties, were extracted from purple flowers of *Gomphrena globosa* L. belonging to the Amaranthaceae family and separated for the first time by ion-pair high-speed counter-current chromatography (HSCCC). The pigments were detected by LC-DAD-ESI-MS/MS technique. Separation of betacyanins (300 mg) by HSCCC was accomplished in four solvent systems: *tert*-butyl methyl ether – butanol – acetonitrile – water (0.7% and 1.0% HFBA – heptafluorobutyric acid – **system I** and **III**) and *tert*-butyl methyl ether – butanol – methanol – water (0.7% and 1.0% HFBA – **system II** and **IV**) (2:2:1:5, v/v/v/v) in the head-to-tail mode. The mobile phase (aqueous phase) was run at 2.0 ml/min and the column rotation speed was 860 rpm.

The applied systems enabled to study the influence of HFBA concentration as well as systems polarity on betacyanins separation. Comparison of the systems containing 0.7% HFBA (**systems I–II**) demonstrates that the replacement of acetonitrile by methanol increases the resolution (R_s) between all betacyanins and does not influence the retention of the stationary phase (S_f = 76%). Higher concentration of the acid in **systems III–IV** slightly decreases S_f to 71% in the systems with 1.0% HFBA. Comparison of the resolution values for betacyanins in the systems with 0.7% and 1.0% HFBA demonstrates that higher concentration of the acid improves the separation effectiveness for all betacyanins as a result of increasing of the chemical affinity of the pigments to the organic stationary phase in HSCCC. The **systems III–IV** with 1% HFBA are the most effective for the separation of all the studied betacyanins.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Betalains – red-violet betacyanins and yellow-orange betaxanthins – are natural plant pigments present in several members of the plant order Caryophyllales (e.g. *Beta vulgaris* L., *Bougainvillea glabra* Choisy, *Chenopodium rubrum* L., *Phytolacca americana* L. and *Gomphrena globosa* L.) and some higher fungi such as *Amanita muscaria* (L.) Lam [1–3]. The interest in betalains grows because they are substitutes for synthetic dyes and nutraceuticals due to their colouring properties and positive effects on health such as antioxidant and chemopreventive activities [4–8]. Application of betacyanins in industry as well as further research on their biological activities requires effective methods of their isolation and separation because their synthesis is very inconvenient [1,9].

Natural plant compounds including betacyanins are usually less stable beyond their natural matrix [5,10–13], therefore, obtaining of pure compounds is very tough and time-consuming. Betacyanins stability is affected by numerous factors, such as temperature, pH, oxygen environment and metal cations. It is known that betacyanins are stable in pH range 4–7 and decompose at higher temperature in the presence of metal cations and oxygen. It was reported that esterification of some betacyanins with aliphatic acids enhanced their stability [5,13].

Electrophoresis and chromatography had been the main techniques in betalains purification from a variety of sources [14]. Nowadays, betalains are most frequently purified by RP-HPLC as well as ion exchange and gel chromatography [15]. Only one study on betalains analytical separation realized by capillary zone electrophoresis was reported [16]. Purification and concentration of betalains was also realized by aqueous two-phase extraction (ATPE) but isolation of single compounds was not reported [15].

Nowadays, separation of betacyanins is mainly realized by traditional column preparative liquid chromatography with solid stationary phase considered as fast and efficient technique and

☆ Selected paper from the 9th International Counter-current Chromatography Conference (CCC 2016), 1–3 August 2016, Chicago, IL, USA.

* Corresponding author.
E-mail address: anetasporna@chemia.pk.edu.pl (A. Spórna-Kucab).

http://dx.doi.org/10.1016/j.chroma.2017.01.064
0021-9673/© 2017 Elsevier B.V. All rights reserved.

only recently counter-current chromatography has been applied for betacyanins. In traditional preparative liquid chromatography, pure fractions of betacyanins are obtained by several separation stages [3,6,7,11,12,14]. Application of counter-current chromatography (CCC) usually enables to limit separation stages for complex matrices, therefore, this technique is common for natural compounds [17–19]. A huge advantage of CCC is a lack of irreversible adsorption due to the presence of liquid stationary phase, therefore, all compounds are recovered after separation. Liquid stationary and mobile phases create two phase solvent system prepared by mixing of at least two solvents in a separatory funnel, therefore, modification of physico-chemical properties of phases is possible by changing solvent system composition. Variety of available solvent systems enables to separate different compounds without necessity of buying of new columns [19–21].

So far, isolation and purification of betacyanins by CCC have been realized in two types of solvent systems: highly polar solvent systems containing salts (ammonium sulfate or sodium chloride) [22–24] and systems with ion-pair reagents such as perfluorocarboxylic acids (trifluoroacetic acid – TFA and heptafluorobutyric acid – HFBA). The positively charged betacyanins create ion-pairs with negatively charged acid residues (TFA, HFBA) leading to decreasing of their polarity, therefore, effective separation of these highly polar compounds in solvent systems consisted of *tert*-butyl methyl ether – butanol – water – acetonitrile is possible and very effective for selected structures [23,25–30]. HFBA and TFA were shown to be useful volatile ion pairing reagents which significantly increase retention of selected ionic analytes [31]. The systems with TFA or HFBA were applied in separation of betacyanins in *Beta vulgaris* L. [23], *Hylocereus polyrhizus* (Weber) Britton. & Rose [25], *Phytolacca americana* L. [26] *Bougainvillea glabra* Choisy and *Iresine lindenii* Van Houtte [30]. The systems with salts are dedicated to highly polar compounds because application of high salt concentration enables salting out of lower aliphatic alcohols, therefore, the upper organic phase (e.g. butanol) becomes more polar [23,24]. The highly polar systems with salts are effective for separation of betacyanins from *Beta vulgaris* L. [23] and red *Gomphrena globosa* L. [29].

Gomphrena globosa L. species and its cultivars are widespread ornamental plants with orange, red or purple flowers containing different profiles and contents of betacyanins. Previous investigation [32] on betacyanins in flowers from red, purple and orange *Gomphrena globosa* L. revealed the highest concentration of betacyanins in fresh purple *Gomphrena globosa* L. flowers (556.8 mg/kg). Orange and red *Gomphrena globosa* L. cultivars contain 30.6 and 93.7 mg/kg of betacyanins, respectively. Moreover, previous research [29,32] of the betacyanins profiles in petals of *Gomphrena globosa* L. revealed the presence of highly polar pigments (mainly amaranthine) in red cultivar and less polar compounds (mainly gomphrenin I, gomphrenin II and gomphrenin III) in purple *Gomphrena globosa* L.

So far, red beet root has been the main source of betacyanins because the average amount of betalains in red beet root (*Beta vulgaris* L.) has been estimated as much as 1200 mg/kg [6]. The profile of betacyanins in *Beta vulgaris* L. consists of prebetanin, betanin, betanidin, neobetanin and their decarboxy-derivatives. High concentration (556.8 mg/kg) and different profile of betacyanins in the purple *Gomphrena globosa* L. flowers make this plant a valuable source of alternative betacyanins for further research and applications. The main purpose of this contribution was to study the isolation possibilities for betacyanins from purple-coloured flowers of *Gomphrena globosa* L. separated for the first time in ion-pair counter-current chromatography in solvent systems consisted of *tert*-butyl methyl ether – butanol – acetonitrile – water – HFBA as well as in modified systems in which acetonitrile was replaced by less polar methanol. In addition, the separation effectiveness of polar betacyanins in solvent systems containing different concentrations of HFBA acting as ion-pairing reagent was investigated. This is the first report on a successful isolation (fractionation) of betacyanins from purple *Gomphrena globosa* L flowers and is of special importance because hitherto betacyanins with 6-*O*-glucosylated position have never been purified in any CCC solvent system.

2. Experimental

2.1. Plant material

Purple flowers of fresh *Gomphrena globosa* L. were purchased from a local market in July 2015. The flowers were extracted according to the procedure described in paragraph 2.3.

2.2. Reagents

HPLC grade butanol (BuOH), *tert*-butyl methyl ether (TBME), methanol (MeOH), acetonitrile (ACN), acetone were obtained from Avantor Performance Materials Poland S.A. (Gliwice, Poland). HPLC-grade heptafluorobutyric acid (HFBA) and trifluoroacetic acid (TFA) were obtained from Merck (Darmstadt, Germany). Pure p.a. grade ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) and ascorbic acid (+) were obtained from Avantor Performance Materials Poland S.A. (Gliwice, Poland).

LC–MS grade methanol and formic acid (purity $\geq$ 98%) were obtained from Sigma-Aldrich (St. Louis, United States).

2.3. Crude pigment extract

Purple flowers of fresh *Gomphrena globosa* L. were ground in a blender (Zelmer, Rzeszów, Poland) and weighed before further extraction. Extraction of plants (600 g) by maceration with 10 l of 20% acetone (v/v) containing 25 ml of saturated EDTA and 25 ml of 10% (w/w) ascorbic acid were carried out for 30 min at room temperature.

A Bűchner funnel with a filter paper disk (Prat Dumas Filter Paper, Couze-St-Front, France) and a 2 cm bed of silica gel with 0.063–0.200 mm mesh (Merck, Darmstadt, Germany) under reduced pressure was used for filtration of the plant material from the extract. The eluates were concentrated by a rotary evaporator for further purification on a C18 cartridge. The C18 cartridge (Merck) was activated with 3 vol of 100% acetone and then rinsed with 3 vol of acidified water with TFA (pH 2). All fractions were eluted with 30% acetone and 2% formic acid. The eluates were again concentrated in a rotary evaporator and freeze-dried before the LC-DAD-ESI-MS/MS analysis and the HSCCC experiments. After the freeze-drying, the 16.3 g of the crude extract was obtained.

2.4. Apparatus

A semi-preparative AECS QuikPrep HSCCC *J-type* hydrodynamic chromatograph (London, United Kingdom) with 137 ml theoretical volume given by manufacturer, 121 ml measured total volume (one bobbin with PTFE tubbing for coil and one counterbalance), i.d. 2.0 mm and rotation speed of the coil ca. 860 rpm was used for betacyanins separation (Table 1) in four solvent systems (Table 2) in head-to-tail mode. The distance (revolution radius – *R*) of the holder axis of the coil to the central (solar) axis of the instrument was 7.0 cm. The inner β_r value was measured to be 0.3 from the equation $\beta_r = r/R$. In applied systems, the mobile phase (the aqueous phase) was pumped at a flow rate of 2.0 ml/min. The HSCCC system was connected to a K-501 pump, UV–vis detector and fraction collector Foxy Jr. from the Knauer company (Berlin, Germany).

The crude extract as well as all the HSCCC fractions were analysed by the LCMS-8030 (Schimadzu, Japan) mass spectrometric

Download English Version:

https://daneshyari.com/en/article/5135457

Download Persian Version:

https://daneshyari.com/article/5135457

Daneshyari.com