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Red Beetroot Thematic Issue

Thermal Decarboxylation of Betacyanins in Red Beet Betalain-Rich Extract

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Betalains are one of the most common groups of plant pigments found in nature, especially in red beetroot (*Beta vulgaris* L.) which is the main commercially exploited source of betalains produced in the form of concentrates or powders. This report presents results of thermal decarboxylation studies on betacyanins present in a specifically purified highly concentrated betalain-rich extract (BRE). The first tentative structures formed by decarboxylation of the main pigment present in BRE, betanin and its diastereomer, were established by means of liquid chromatography coupled to diode array detection and electrospray ionization tandem mass spectrometry (LC-DAD-ESI-MS/MS). In the extract, two new isomeric bidecarboxylated betanins were tentatively identified. A high rate of generation of 2-decarboxy-betanin/-isobetanin which are present in the BRE extract at very low level was observed, which was dependent on the starting concentration of the BRE substrate. The bidecarboxylated derivatives were generated at a higher rate mostly from 17-decarboxy-betanin/-isobetanin as well as 15-decarboxy-betanin by further decarboxylation at carbon C-2. Further studies will be performed to demonstrate if the decarboxylated betanins being products of heating *B. vulgaris* preparations can be used for various food applications with new health-promoting actions and colorant properties.

INTRODUCTION

Betalains are one of the most common groups of plant pigments found in nature, however, they are not as well studied as compared to other natural pigments such as anthocyanins, carotenoids, and chlorophylls [Stafford *et al.,* 1994]. Betalain pigments which are composed of red-violet betacyanins together with yellow-orange betaxanthins are mainly found in most families of the Caryophyllales order [Chhikara *et al.,* 2019]. In addition, both betacyanins and betaxanthins can occur in the same plant part, despite the difference in its coloration [Martins *et al.,* 2017]. These pigments are commercially recognizable as food colorants due to their non-toxic, non-carcinogenic, and non-poisonous nature [Esatbeyoglu *et al.,* 2015; Siervo *et al.,* 2013].

Betalains are stable at pH values ranging from 3 to 7 and suitable for dyeing low acidic and neutral foods. In addition, they may be stabilized by ascorbic acid. In contrast, anthocyanins are unsuitable for coloration of such foods as they are unstable at pH values over 3, in addition their degradation is facilitated by ascorbic acid. For that reason, utilization of betalain pigments instead of anthocyanins for coloring food with a high vitamin C content or of vitamin C-supplemented products seems to be more favorable. Due to their thermolability, betalains are also utilized to color low-temperature products [Azeredo *et al.,* 2008; Herbach *et al.,* 2007; Stintzing *et al.,* 2004].

Red beetroot (*Beta vulgaris* L.) is the main commercially exploited source of betalain pigments which are produced in the form of concentrates or powders [Ciriminna *et al.,* 2018]. The most abundant pigments present in red beet are betanin (red betacyanin) and vulgaxanthin I (yellow betaxanthin). Due to their satisfying nutritional value and disease-preventing effects, such extracts are regarded beneficial to human health and applied as food additives, colorants, and dietary supplements [Nemzer *et al.,* 2011]. They are also characterized by the best quality in terms of the color and its intensity. In addition, betanin is approved by the US FDA and European Union as a natural colorant used for coloring dairy products, cosmetics, and pharmaceuticals [Esatbeyoglu *et al.,* 2015]. Beetroot extracts are utilized to emphasize the redness of such products as tomato soups, sauces, pastes, desserts, jams, sweets, and jelly beans. They are also used to protect meat from discoloration and to extend its shelf-life [Chhikara *et al.,* 2019; Tang *et al.,* 2015].

Several studies have attributed a wide spectrum of bioactive properties to betalain pigments and betalain-rich extracts. They may serve as biologically active nutraceuticals

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with a wide variety of therapeutic, anti-carcinogenic, hepato-protective, and antitumor properties [Chhikara *et al.,* 2019; Vulić *et al.,* 2013]. It has also been shown that some of the betalain pigments exhibit even higher antioxidant activity in comparison to typical natural antioxidants such as ascorbic acid, rutin, and catechin [Cai *et al.,* 2006; Gandía-Herrero *et al.,* 2009]. The free radical-scavenging activity of betanin extracted from red beet measured in a TEAC assay at pH 7.4 and in a 1,1-diphenyl-2-picrylhydrazy (DPPH) assay is about 7.5- and 3.0-fold higher, respectively, than that of vitamin C, which is an effective natural antioxidant [Cai *et al.,* 2003; Gengatharan *et al.,* 2015]. Furthermore, studies with different cell lines have demonstrated that betalains exhibit a chemopreventive potential [Gandía-Herrero *et al.,* 2016]. *In vivo* anti-tumor formation activity in mouse skin has been demonstrated for *B. vulgaris* extracts. Results showed a significant decrease in the incidence and number of papillomas found in mice skin. In the same study, lung tumor formation was induced to mice, and inhibited by the oral administration of *B. vulgaris* extracts [Kapadia *et al.,* 2003]. Pure betanin was assayed, revealing a strong inhibition of the proliferation of melanoma cancer cells [Wu *et al.,* 2006]. Betanin from red beet also showed excellent growth inhibition of MCF-7 (breast), HCT-116 (colon), AGS (stomach), SF-268 (CNS), and NCI-H460 (lung) cancer cell lines with IC_{50} values of 162, 142, 158, 164, and 147 μg/mL, respectively [Reddy *et al.,* 2005]. Recently a novel betalain-rich extract/concentrate (BRE) was tested in a pilot clinical study that reported short-term treatment with BRE which improved the function and comfort of knee joints in individuals with knee distress [Pietrzkowski *et al.,* 2014]. The chemopreventive and strong antioxidant properties of betalains stimulate research on their new structures, derivatives, and especially their influence on health.

Recently, several detailed research have been published on new products of degradation of betacyanins present in preparations subjected to thermal processing, especially on decarboxylated derivatives as well as their influence on human health [Cai *et al.,* 2005; Stintzing *et al.,* 2004; Tesoriere *et al.,* 2005]. Such derivatives were obtained by heating the natural substrates, previously isolated in aqueous and alcoholic solutions [Wybraniec, 2005; Wybraniec & Mizrahi, 2005]. The research on betanidin decarboxylation in ethanolic solutions was performed by Dunkelblum *et al.* [1972] as well as by Minale & Piattelli [1965]. Additionally, thermal treatment of betanin in aqueous /alcoholic media was described by Altamirano *et al.* [1993] and by Simon *et al.* [1993] however, without structural studies. In the case of *B. vulgaris* L. juice, thermal treatment led to the formation of different mono- (17-decarboxy- and 2-decarboxy-), bi- (2,17-bidecarboxy-), and tri- (2,15,17-tridecarboxy-) decarboxylated betacyanins along with their diastereomers, and minor levels of 14,15-dehydrogenated (neo-) derivatives which were identified by LC-DAD-ESI-MS/MS [Herbach *et al.,* 2004, 2006; Wybraniec, 2005; Wybraniec & Mizrahi, 2005]. Due to lower polarity of decarboxylated derivatives, their retention times during HPLC analysis on the reversed phase are longer in contrast to their starting substrates. Furthermore, different mechanisms of decarboxylation influenced by the type of alcoholic or aqueous media were indicated based on different mono-decarboxylation products of betanin/isobetanin. Definitely faster degradation process, leading to the formation of double decarboxylation products, was observed in ethanolic solutions, which should be taken into account during analytical samples preparation [Wybraniec, 2005; Wybraniec & Mizrahi, 2005].

Degradation products of thermal processing of isomeric to betanin gomphrenin pigments present in *Basella alba* L. fruit juice were also identified and their tentative structures were established for the first time by LC-DAD-ESI-MS/MS and LCMS-IT-TOF. The research reports that the principal degradation products present in heated *B. alba* fruit juice were 2-, 17-, and 2,17-decarboxy-gomphrenins, their diastereomers, as well as minor levels of their 14,15-dehydrogenated derivatives (neo-derivatives). It was also noticed that the position of betanidin glucosylation at carbon C-5 or C-6 affected the chromatographic differences between betanin and gomphrenin derivatives. Due to various chromatographic properties as well as greater stability in relation to their corresponding betacyanins, the processed betacyanins arising in the process of thermal decarboxylation and dehydrogenation represent a very interesting research material suitable for wider applications [Kumorkiewicz *et al.,* 2017].

In this contribution, further studies on betacyanins thermal decarboxylation are reported, especially these searching for new structures formed by decarboxylation of the main pigments present in the BRE extract by means of liquid chromatography coupled to diode array detection and electrospray ionization tandem mass spectrometry (LC-DAD-ESI-MS/MS). Influence of different heating conditions on generation of decarboxylated betanins was investigated as well.

MATERIALS AND METHODS

Reagents

Formic acid, acetic acid, LC-MS grade methanol, and water were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Plant material

Betalain-rich extract (BRE) [Nemzer *et al.,* 2011] was obtained from FutureCeuticals, Inc. (Momence, IL, USA).

Heating experiments

BRE aqueous solutions (30 mL) were prepared at concentrations: 0.70, 0.50, 0.25, and 0.10 g/L (Table 1) and acidified with acetic acid (1.0 and 2.5 g/L). These samples were heated at 85°C in a water bath for 45 min. Aliquots (1 mL) of the heated samples were taken in one repetition for LC-DAD-ESI-MS/MS analysis every 15 min.

Chromatographic analysis in the LC-DAD-ESI-MS/MS system

An LCMS-8030 mass spectrometric system (Shimadzu, Kyoto, Japan) coupled to LC-20ADXR HPLC pumps controlled with LabSolutions software (Shimadzu) was used for the chromatographic and mass spectrometric analyses.

TABLE 1. Composition of aqueous solutions containing the betalain-rich extract (BRE) heated at 85°C.

Test No.	Sampling time (min)	Concentration of BRE (g/L)	Concentration of acetic acid (g/L)
H1	0, 15, 30, 45	0.70	2.5
H2	0, 15, 30, 45	0.50	2.5
H3	0, 15, 30, 45	0.25	2.5
H4	0, 15, 30, 45	0.10	2.5
H5	0, 15, 30, 45	0.70	1.0
H6	0, 15, 30, 45	0.50	1.0
H7	0, 15, 30, 45	0.25	1.0
H8	0, 15, 30, 45	0.10	1.0

The samples were eluted through a 150 mm × 4.6 mm i.d., 5.0 μm, Kinetex C18 chromatographic column preceded by a guard column of the same material (Phenomenex, Torrance, CA, USA). The injection volume was 20 μL, and the flow rate was 0.5 mL/min. The column was thermostated at 40°C. The analytes were separated using a gradient system as follows: 5% B in A (v/v) at 0 min; gradient to 70% B in A at 12 min, gradient to 20% B in A at 15 min, isocratic at 20% B in A till 19 min, with A – 2% aqueous formic acid (v/v), and B – methanol. Online UV/Vis spectra were acquired using the PDA (photodiode-array detection) mode.

The positive ion electrospray mass spectra (*m/z* range 100–2000) were recorded on the LC-MS system which was controlled with LabSolutions software (electrospray voltage 4.5 kV; capillary 250°C; sheath gas: N_2), recording total ion chromatograms, mass spectra and ion chromatograms in selected ion monitoring mode (SIM) as well as the fragmentation spectra. Argon was used as the collision gas for CID experiments. The relative collision energies for MS/MS analyses were set at -35 V.

RESULTS AND DISCUSSION

The LC-MS selected ion chromatograms present in Figure 1 depict a typical betacyanin and decarboxylated betacyanin profile in a betalain-rich extract/concentrate (BRE) before the heating experiments. The dominant presence of betanin (**1**) and its isoform (**1'**) with substantial participation of very well-separated 17-decarboxy-betanin/-isobetanin (**2/2'**) as well as 15-decarboxy-betanin (**3**) (Table 2) confirms results from the previous research [Nemzer *et al*., 2011]. Further inspection of chromatograms revealed small quantities of a slightly resolved pair of 2-decarboxy-betanin/-isobetanin (**5/5'**) (absorption maximum at λ_{max} 533 nm) and an unresolved pair of 2,17-bidecarboxy-betanin/-isobetanin (**6/6'**), similarly to the previous reports (λ_{max} 507 nm) [Nemzer *et al.*, 2011; Wybraniec, 2005]. For the identification, a series of already known decarboxylated betanin standards was used in the study [Wybraniec *et al.,* 2006].

Figure 2 presents the structures of the studied pigments (Table 2) and possible decarboxylation reactions starting from betanin.

Interestingly, except for very well-known betanin derivatives identified as 2,17-bidecarboxy-betanin/-isobetanin (**6/6'**), other two new isomeric bidecarboxylated betanins (peaks **4** and **7**) were detected in BRE which fitted to the reaction scheme (Figure 2). These compounds displayed absorption maxima at λ_{max} 494 and 532 nm, respectively, thus differing from the maximum for compound **6/6'** and pseudomolecular ions at *m/z* 463 (Table 2), clearly confirming a loss of two CO_2 moieties from the starting Bt/IBt (**1/1'**). Subsequent fragmentation to ions of *m/z* 301 confirmed the existence of a bidecarboxylated fragment of betanidin and suggested the formation of bidecarboxylated betanin /isobetanin. These compounds could presumably be assigned to 15,17-bidecarboxy-betanin (**4**) and 2,15-bidecarboxy-betanin (**7**) based on analogous retention differences between 17-dBt (**2**) and 2-dBt (**5**) (2-decarboxylated betacyanins are more retarded on the column than the 17-decarboxylated derivatives)

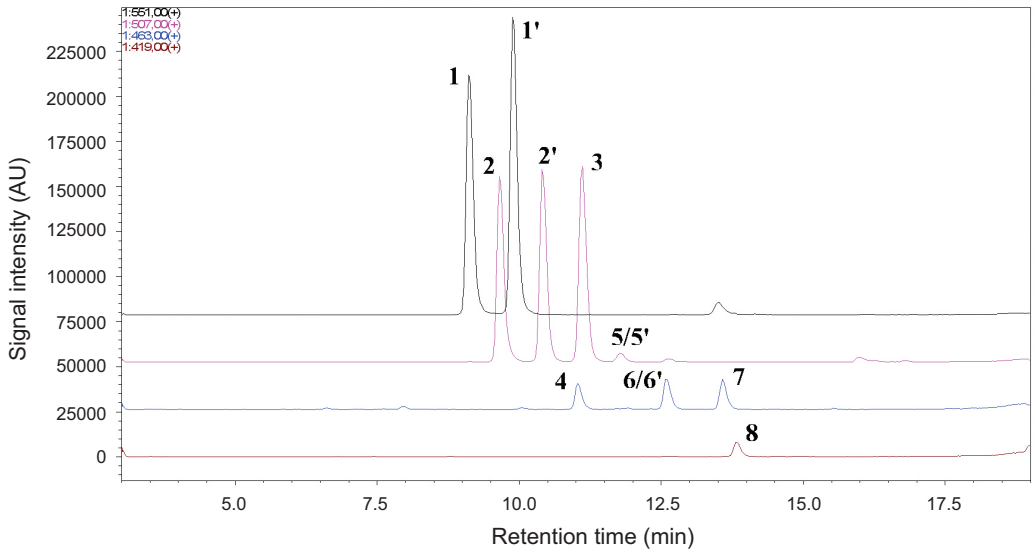

FIGURE 1. Chromatographic LC-MS traces of selected ions of betanin and its decarboxylated derivatives in the betalain-rich extract (BRE).

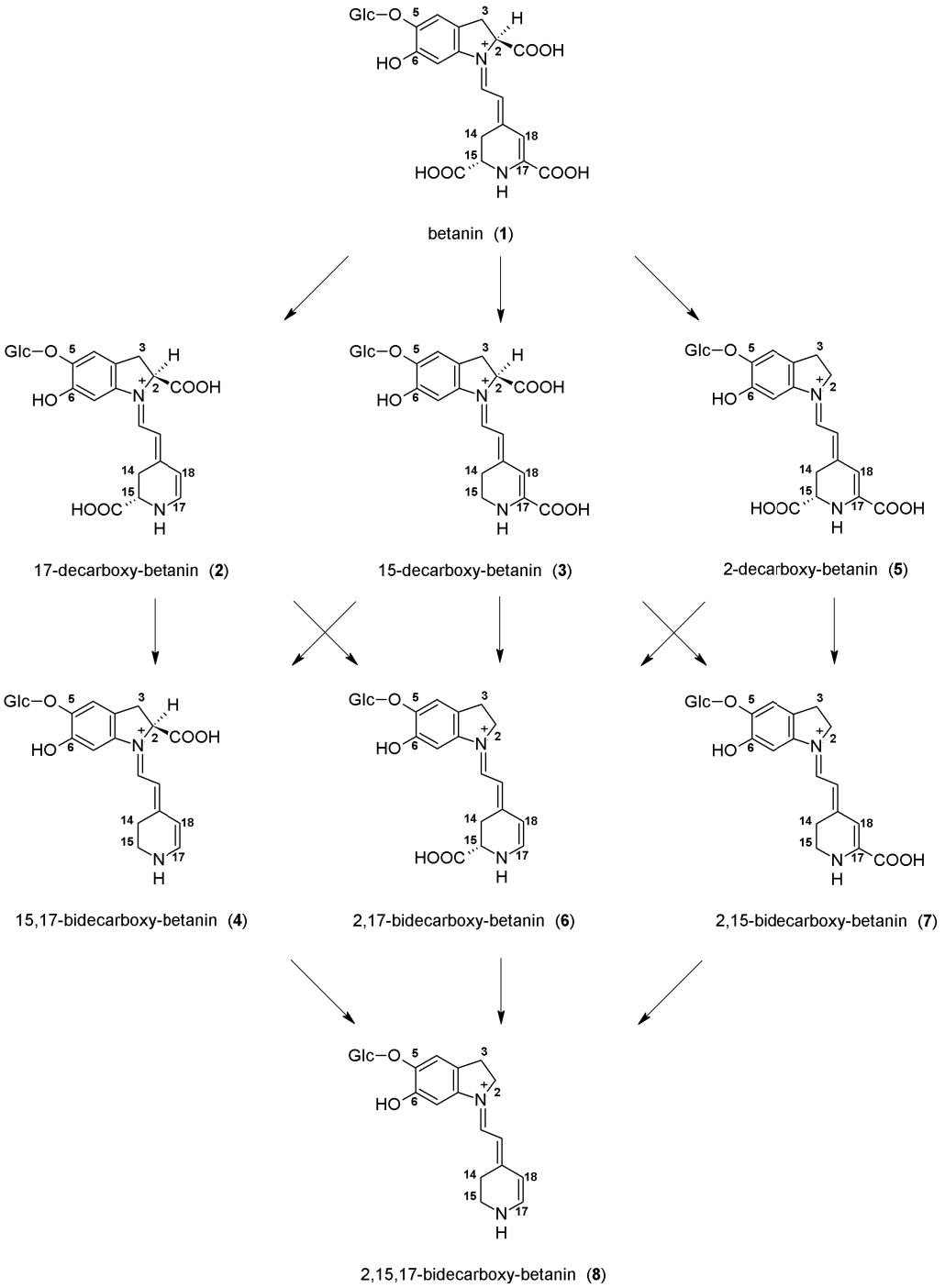

FIGURE 2. Chemical structures of detected betanin and its decarboxylated derivatives in the betalain-rich extract (BRE) before and after heating at 85°C for 15, 30 and 45 min. The reaction scheme of possible decarboxylation paths is also presented.

[Wybraniec, 2005]. The lack of the carboxyl moiety at carbon C-15 implicates the lack of the chirality at this position, therefore, only single forms of the pigments **4** and **7** are detected in the chromatograms, which supports their identification.

In contrast to the previous report [Nemzer *et al.*, 2011], the presence of 2,15,17-tridecarboxy-betanin (**8**) was acknowledged (Figure 2, Table 2). This compound displayed a pseudomolecular ion at *m/z* 419 during LC-MS analysis and an absorption maximum of λ_{max} 503 nm. Subsequent fragmentation experiments on the pseudomolecular ion at *m/z* 419 revealed fragmentation ions at *m/z* 257, which proved the existence of the tridecarboxylated fragment of betanidin. This conclusion was supported by the detection of only one chromatographic peak **8** in the HPLC system, which resulted from the loss of the chiral center at carbon C-15 of this compound. This confirmed the presence of a tridecarboxy-betanin for which the only possible structure can be predicted as 2,15,17-tridecarboxy-betanin similarly to the previous report based on the prolonged heating of *B. vulgaris* juice [Wybraniec, 2005].

The presence of some additional quantities of bi- and tridecarboxylated derivatives of betanin in the extract is pre-

TABLE 2. Chromatographic, spectrophotometric, and mass spectrometric data of the analyzed betanin-based decarboxylated betacyanins present in the betalain-rich extract (BRE) heated at 85°C for 15, 30, and 45 min.

No.	Compound name	Abbreviation	t_R	λ_{max}	*m/z*
			(min)	(nm)	$[M+H]^+$
1	betanin	Bt	9.2	536	551
2	17-decarboxy-betanin	17-dBt	9.7	505	507
1'	izobetanin	IBt	9.9	536	551
2'	17-decarboxy-isobetanin	17-dIBt	10.4	505	507
3	15-decarboxy-betanin	15-dBt	11.1	527	507
4	15,17-bidecarboxy-betanin	15,17-dBt	11.1	494	463
5/5'	2-decarboxy-betanin	2-dBt/-IBt	11.8	533	507
6/6'	2,17-bidecarboxy-betanin/isobetanin	2,17-dBt/-IBt	12.6	507	463
7	2,15-bidecarboxy-betanin	2,15-dBt	13.6	532	463
8	2,15,17-tridecarboxy-betanin	2,15,17-dBt	13.8	503	419

sumably a result of a deeper decarboxylation process which is inherent in the current preparation process of BRE. Nevertheless, further decarboxylation experiments performed in this study showed that the generation of these pigments can be due to the controlled thermal decarboxylation of Bt/IBt (**1/1'**) and especially mono-decarboxylated betanins (**2/2'**, **3** and **5/5'**).

The profiles of the main pigments in thermally-treated BRE were similar to the profiles of early heating products of *Beta vulgaris* L. root [Wybraniec, 2005]. The aqueous solutions acidified by acetic acid were heated for 45 min, and the temperature (85°C) of the heating process was high enough to enable monitoring changes in the compositions of the resulting mixtures within that time range. All the detected heating products (Figures 1–3) were less polar than their corresponding precursors. Similar experiments were performed also on betanin-based pigments, phyllocactin, and hylocerenin which released high quantities of mono-decarboxylated and especially bi-decarboxylated derivatives in aqueous or ethanolic solutions [Wybraniec & Mizrahi, 2005].

The experimental results (Table 3) obtained after 30 min of heating when betanin was almost completely degraded were presented graphically as a generation ratio (GR) which is the ratio between measured signals (chromatographic peak areas) for a selected compound after 30 min of heating and a reference before heating. Each reference was prepared for the defined concentration level (Table 1) of BRE (0.70, 0.50, 0.25, and 0.10 g/L). The GR index represents the tendency of a compound to be generated over being degraded, therefore, obviously only degradation was observed (very low GR values) for Bt/IBt (**1/1'**) (Table 3). Table 3 presents a full set of the results obtained for the sampling times of 15, 30, and 45 min.

In this study, the heating experiments performed with the higher concentration of acetic acid (2.5 g/L) in the dissolved extract sample revealed, first of all, a selective generation of 2-decarboxy-betanin/-isobetanin (**5/5'**) (Figure 3, Table 3) which are present in the BRE extract at a very low level. As a result, high levels of the GR index reaching the value of 20–40 after heating for 30 min (Figure 3) indicate mostly the generation of compounds **5/5'** and their low tendency to be degraded under the experimental conditions. This also means that the bidecarboxylated compounds (**6/6'** and **7**) generated at a higher rate (Table 3) according to the reaction scheme (Figure 2) are formed rather not from compounds **5/5'** but mostly from 17-dBt/-IBt (**2/2'**) as well as 15-dBt (**3**), respectively, by further decarboxylation at carbon C-2.

The diastereomeric pair of 2-decarboxy-betanin/-isobetanin (**5/5'**) can obviously be formed only from Bt/IBt (**1/1'**). The latter pigment was mostly degraded after 30 min of heating, especially at medium concentration levels (Figure 3, Table 3). Decreasing extract concentration from 0.7 to 0.1 g/L resulted in an increased signal ratio of compounds **5/5'** to **2/2'** or **5/5'** to **3**. This is also due to the further decarboxylation of 17-dBt/-IBt (**2/2'**) and 15-dBt (**3**) (Table 3) which are already present at high quantities in the BRE extract and are generated from betanin at a lower rate in these conditions. Similarly to betanin, pigments **2/2'** and **3** were degraded at the highest rate at BRE medium concentration levels (Table 3).

A previous report [Wybraniec, 2005] also presented data indicative of the preferential generation of 2-dBt/-IBt (**5/5'**) in aqueous acidic solutions of red beet extract in contrast to ethanolic solutions which enhanced the generation of 17-dBt/-IBt (**2/2'**). Our report more specifically defines conditions in which the formation of target derivatives occurs. According to the results, the most decisive is the concentration of the substrate (Bt/IBt (**1/1'**)). Another important factor is the concentration of acetic acid. Its lower concentration (1 g/L) increased the optimal substrate concentration (Table 3), which promoted the generation of 2-dBt/-IBt (**5/5'**).

The profile of bidecarboxylated betanins (**4**, **6/6'** and **7**) generated during the heating experiments was also dependent on the starting concentration of the BRE substrate (Table 3). Concentration of bidecarboxylated betanin (**4**) decreased after

TABLE 3. Results of the 15, 30 and 45 min heating of the betalain-rich extract (BRE) expressed as the generation ratio (GR) between measured signals (chromatographic peak areas) after heating and a reference before heating for betanin as well as its generated decarboxylated derivatives.

		Generation ratio (GR)							
		2.5 g/L acetic acid				1.0 g/L acetic acid			
Sample code:		H1	H2	H3	H4	H5	H6	H7	H8
BRE conc. (g/L):		0.70	0.50	0.25	0.10	0.70	0.50	0.25	0.10
No.	Compound	15 min							
1	Bt	0.087	0.017	0.077	0.39	0.46	0.10	0.020	0.22
2	17-dBt	0.58	0.17	0.38	0.85	0.68	0.46	0.15	0.39
1'	IBt	0.070	0.014	0.059	0.34	0.43	0.086	0.023	0.21
2'	17-dIBt	0.51	0.13	0.36	0.81	0.64	0.40	0.11	0.33
3	15-dBt	0.66	0.30	0.34	0.52	0.89	0.64	0.23	0.59
4	15,17-dBt	0.41	0.12	0.32	0.71	0.60	0.29	0.029	0.30
5/5'	2-dBt/-IBt	27.2	19.1	22.2	22.5	42.4	21.9	5.7	4.0
6/6'	2,17-dBt/-IBt	9.7	6.1	1.8	1.1	3.3	3.6	1.0	0.34
7	2,15-dBt	4.2	3.5	1.6	1.6	2.4	2.9	0.93	0.001
8	2,15,17-dBt	1.2	1.0	0.44	0.41	0.83	0.72	0.33	0.46
No.	Compound	30 min							
1	Bt	0.049	0.002	0.002	0.059	0.20	0.025	0.002	0.11
2	17-dBt	0.42	0.095	0.24	0.56	0.68	0.34	0.031	0.046
1'	IBt	0.039	0.002	0.002	0.045	0.20	0.019	0.002	0.071
2'	17-dIBt	0.36	0.061	0.21	0.54	0.65	0.26	0.019	0.087
3	15-dBt	0.52	0.22	0.28	0.39	0.80	0.54	0.11	0.41
4	15,17-dBt	0.28	0.056	0.18	0.42	0.55	0.22	0.010	0.13
5/5'	2-dBt/-IBt	22.6	14.8	18.6	19.5	36.4	16.5	1.8	1.4
6/6'	2,17-dBt/-IBt	11.7	8.3	2.7	1.7	4.8	6.1	2.2	0.30
7	2,15-dBt	6.0	5.5	2.4	2.5	3.3	3.9	1.9	0.001
8	2,15,17-dBt	1.6	1.3	0.7	0.8	1.1	1.1	0.55	0.77
No.	Compound	45 min							
1	Bt	0.011	0.001	0.001	0.021	0.043	0.006	0.001	0.023
2	17-dBt	0.29	0.085	0.12	0.43	0.45	0.21	0.014	0.015
1'	IBt	0.015	0.001	0.001	0.017	0.041	0.008	0.003	0.018
2'	17-dIBt	0.25	0.042	0.10	0.37	0.42	0.16	0.006	0.026
3	15-dBt	0.37	0.16	0.19	0.25	0.58	0.37	0.077	0.28
4	15,17-dBt	0.10	0.035	0.07	0.27	0.43	0.13	0.003	0.040
5/5'	2-dBt/-IBt	17.4	14.6	15.0	15.0	20.3	8.9	1.4	0.63
6/6'	2,17-dBt/-IBt	14.0	9.4	3.4	2.3	6.0	6.3	3.2	2.0
7	2,15-dBt	7.3	7.0	3.1	3.2	4.9	5.0	2.6	1.6
8	2,15,17-dBt	1.8	1.7	0.96	1.1	1.4	1.2	0.46	0.68

the heating, therefore, this pigment was rather not meaningfully generated. It is possible that its presence results only from a chemical process taking place during production of the BRE extract, but it cannot be formed by heating. A high concentration of BRE enhances the formation of 2,17-bidecarboxy--betanin/-isobetanin (**6/6'**) over 2,15-bidecarboxy-betanin **7** (Table 3). During the heating experiment, contents of compounds **6/6'** and **7** successively increased at all conditions (Table 3), however, at the low concentration of BRE, the latter pigment signal outweighed and this effect was more pronounced at the higher concentration of acetic acid (2.5 g/L). These differences can, presumably, be attributed to the matrix effect.

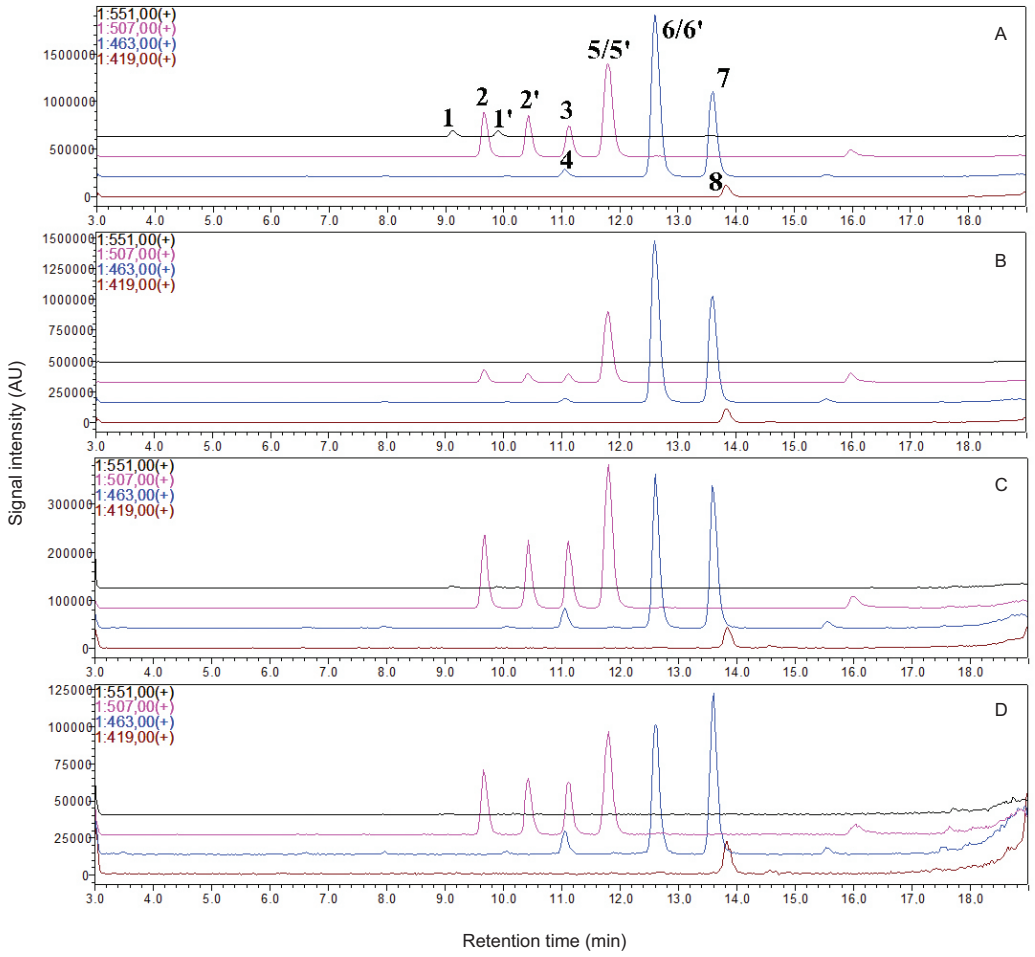

FIGURE 3. Chromatographic LC-MS traces of selected ions of betanin and its decarboxylated derivatives in the betalain-rich extract (BRE) after its heating at 85°C for 30 min. The experiments were performed for acetic acid concentration of 2.0 g/L and for BRE concentrations of 0.75 (A), 0.50 (B), 0.20 (C), and 0.10 g/L (D).

The presence of 2,15,17-tridecarboxy-betanin (**8**) in the reaction mixtures was strongly dependent on both the factors (Table 3) and acetic acid concentration increased enhanced the generation of pigment **8**, especially at the higher BRE concentration, however, during the heating experiment, the content of compound **8** increased successively at all conditions (Table 3).

CONCLUSION

This is the first report on the presence of new bidecarboxylated betanins in *B. vulgaris* extract as well as their generation by heating betanin/isobetanin and mono-decarboxylated betanins as the main ingredients of the extract mixture. Taking into account that especially 2,15-bidecarboxy-betanin can be present at higher quantities in the processed *B. vulgaris* juices and extracts, this compound – as an additional decarboxylated betanin – might have a strong influence on the bioactivities of *B. vulgaris* products, which was not considered before. Further studies will be performed to demonstrate if the decarboxylated betanins, which are degradation products of heated *B. vulgaris* preparations, can be used for various food applications with new health-promoting potentials and colorant properties.

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REFERENCES

1. Altamirano, R.C., Drdak, M., Simon, P., Rajniakova, A., Karovicova, J., Preclik, L. (1993). Thermal degradation of betanine in various water alcohol model systems. *Food Chemistry*, *46*(1), 73–75.
2. Azeredo, H.M.C. (2009). Betalains: properties, sources, applications, and stability – a review. *International Journal of Food Science & Technology*, *44*(12), 2365–2376.
3. Cai, Y.Z., Sun, M., Xing, J., Luo, Q., Corke, H. (2006). Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sciences*, *78*(25), 2872–2888.
4. Cai, Y., Sun, M., Corke, H. (2003). Antioxidant activity of betalains from plants of the amaranthaceae. *Journal of Agricultural and Food Chemistry*, *51*(8), 2288–2294.

5. Cai, Y., Sun, M., Corke, H. (2005). Characterization and application of betalain pigments from plants of the Amaranthaceae. *Trends in Food Science & Technology*, *16*(9), 370–376.
6. Ciriminna, R., Fidalgo, A., Danzi, C., Timpanaro, G., Ilharco, I.M., Pagliaro, M. (2018). Betanin: A Bioeconomy insight into a valued betacyanin. *ACS Sustainable Chemistry & Engineering*, *6*(3), 2860–2865.
7. Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., Panghal, A. (2019). Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry*, *272*, 192–200.
8. Dunkelblum, E., Miller, H.E., Dreiding, A.S. (1972). On the mechanism of decarboxylation of betanidine. A contribution to the interpretation of the biosynthesis of betalaines. *Helvetica Chimica Acta*, *55*(2), 642–648.
9. Esatbeyoglu, T., Wagner, A.E., Schini-Kerth, V.B., Rimbach, G. (2015). Betanin – a food colorant with biological activity. *Molecular Nutrition & Food Research*, *59*(1), SI, 36–47.
10. Gandía-Herrero, F., Escribano, J., Garcia-Carmona, F. (2016). Biological activities of plant pigments betalains. *Critical Reviews in Food Science and Nutrition*, *56*(6), 937–945.
11. Gandía-Herrero, F., Escribano, J., Garcia-Carmona, F. (2009). The role of phenolic hydroxy groups in the free radical scavenging activity of betalains. *Journal of Natural Products*, *72*(6), 1142–1146.
12. Gengatharan, A., Dykes, G.A., Choo, W.S. (2015). Betalains: Natural plant pigments with potential application in functional foods. *LWT – Food Science and Technology*, *64*(2), 645–649.
13. Herbach, K.M., Stintzing, F.C., Carle, R. (2004). Impact of thermal treatment on color and pigment pattern of red beet (*Beta vulgaris* L.) preparations. *Journal of Food Science*, *69*(6), C491-C498.
14. Herbach, K.M., Stintzing, F.C., Carle, R. (2006). Betalain stability and degradation-structural and chromatic aspects. *Journal of Food Science*, *71*(4), R41-R50.
15. Herbach, K.M., Maier, C., Stintzing, F., Carle, R. (2007). Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice. *European Food Research and Technology*, *224*(5), 649–658.
16. Kapadia, G.J., Azuine, M.A., Sridhar, R., Okuda, Y., Tsuruta, A., Ichiishi, E., Mukainake, T., Takasaki, M., Konoshima, T., Nishino, H., Tokuda, H. (2003). Chemoprevention of DMBA-induced UV-B promoted, NOR-1-induced TPA promoted skin carcinogenesis, and DEN-induced phenobarbital promoted liver tumors in mice by extract of beetroot. *Pharmacological Research*, *47*, 141–148.
17. Kumorkiewicz, A., Wybraniec, S. (2017). Thermal degradation of major gomphrenin pigments in the fruit juice of *Basella alba* L. (Malabar Spinach). *Journal of Agricultural and Food Chemistry*, *65*, 7500−7508.
18. Martins, N., Roriz, C.L., Morales, P., Barros, L., Ferreira, I.C.F.R. (2017). Coloring attributes of betalains: a key emphasis on stability and future applications. *Food & Function, 8*(4), 1357–1372.
19. Minale, L., Piattelli, S. (1965). Decarbossilazione termica dei betacianie delle betaxantine. *Rendiconto dell'Accademia delle Scienze Fisiche e Matematiche*, *32*, 165 (in Italian; English abstract).
20. Nemzer, B., Pietrzkowski, Z., Spórna, A., Stalica, P., Thresher, W., Michałowski, T., Wybraniec, S. (2011). Betalainic and nutritional profiles of pigment-enriched red beet root (*Beta vulgaris* L.) dried extracts. *Food Chemistry*, *127*, 42–53.
21. Pietrzkowski, Z., Argumedo, R., Shu, C., Nemzer, B., Wybraniec, S., Reyes-Izquierdo, T. (2014). Betalain-rich red beet concentrate improves reduced knee discomfort and joint function: a double blind, placebo-controlled pilot clinical study. *Nutrition and Dietary Supplements*, *6*, 9−13.
22. Reddy, M.K., Alexander-Lindo, R.L., Nair, M.G. (2005). Relative inhibition of lipid peroxidation, cyclooxygenase enzymes, and human tumor cell proliferation by natural food colors. *Journal of Agricultural and Food Chemistry*, *53*, 9268–9273.
23. Siervo, M., Lara, J., Ogbonmwan, I., Mathers, J.C. (2013). Inorganic nitrate and beetroot juice supplementation reduces blood pressure in adults: a systematic review and meta-analysis. *The Journal of Nutrition*, *143*, 818–826.
24. Simon, P., Drda'k, M., Cruz Altamirano, R. (1993). Influence of water activity on the stability of betanin in various water/alcohol model systems. *Food Chemistry*, *46*, 155–158.
25. Stafford, H.A. (1994). Anthocyanins and betalains: evolution of the mutually exclusive pathways. *Plant Science*, *101*, 91–98.
26. Stintzing, F.C., Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends in Food Science & Technology*, *15*, 19–38.
27. Tang, C.B., Zhang, W.G., Dai, C., Li, H.X., Xu, X.L., Zhou, G.H. (2015). Identification and quantification of adducts between oxidized rosmarinic acid and thiol compounds by UHPLC-LTQ-Orbitrap and MALDI-TOF/TOF tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, *63*, 902−911.
28. Tesoriere, L., Butera, D., Allegra, M., Fazzari, M., Livrea, M. (2005). Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to *ex vivo* induced oxidative hemolysis in humans. *Journal of Agricultural and Food Chemistry*, *53*, 1266–1270.
29. Wu, L.C., Hsu, H.W., Chen, Y.C., Chiu, C.C., Lin, Y.I., Ho, J.A. (2006). Antioxidant and antiproliferative activities of red pitaya. *Food Chemistry*, *95*, 319–327.
30. Vulić, J.J., Cebović, T.N., Canadanović, V.M., Cetković, G.S., Djilas, S.M., Canadanović-Brunet, J.M., Velićanski, A.S., Cvetković, D.D., Tumbas, V.T. (2013). Antiradical, antimicrobial and cytotoxic activities of commercial beetroot pomace. *Food & Function*, *4*, 713–721.
31. Wybraniec, S. (2005). Formation of decarboxylated betacyanins in heated purified betacyanin fractions from red beet root (*Beta vulgaris* L.) monitored by LC-MS/MS. *Journal of Agricultural and Food Chemistry*, *53*(9), 3483–3487.
32. Wybraniec, S., Mizrahi, Y. (2005). Generation of decarboxylated and dehydrogenated betacyanins in thermally treated purified fruit extract from purple pitaya (*Hylocereus polyrhizus*) monitored by LC-MS/MS. *Journal of Agricultural and Food Chemistry*, *53*(17), 6704–6712.
33. Wybraniec, S., Nowak-Wydra, B., Mizrahi, Y. (2006). ^{1}H and ^{13}C NMR spectroscopic structural elucidation of new decarboxylated betacyanins. *Tetrahedron Letters*, *47*(11), 1725–1728.

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