

Mass spectrometric detection of products of decarboxy-betanins UV-irradiation

Karolina Starzak*, Anna Skopińska, Dominika Szot, 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, *kstarzak@chemia.pk.edu.pl

Betalains are a group of natural plant pigments, responsible for red-violet colour of numerous flowers, fruits and roots. They commonly occur in order of Caryophyllales (Caryophyllales). Recent studies revealed great health promoting properties of these pigments, but their natural origin make them very labile under various physicochemical conditions. In the case of betanin degradation, many new, decarboxy-betanins are formed. Latest study showed that UV-irradiation had significant impact on betanin stability, however, no deeper studies were performed in this field. In order to know an impact of the process conditions on degradation of betanin and its decarboxylated derivatives, series of experiments with UV-photo-irradiation was performed in aqueous solutions as well as in various organic solvents at wide range of pH. The highest pigment stability was observed in aqueous solutions for betanin and 17-decarboxybetanin at pH 4.5–7, and for 2,17-didecarboxybetanin at low pH. In all investigated organic solutions, all of the studied compounds have been degraded. The LC-DAD-ESI-MS analysis identified new reaction products. The results showed that UV-photo-irradiation leads mainly to a formation of many new and various 14,15-dehydrobetanins.

Introduction

Betalains are a group of natural plant pigments, responsible for red-violet colour of numerous flowers, fruits and roots [1,2]. They commonly occur in order of Caryophyllales (*Caryophyllales*). Recent studies revealed great health promoting properties of these pigments, but their natural origin make them very labile under various physicochemical conditions [3-11]. In the case of betanin [Fig. 1] degradation, many new, decarboxylated betanins are formed.

According to the latest studies, light radiation [12] has a significant impact on decrease of betalains stability, however until now, there are no detailed studies in this area. Tendency of betalains to degrade under influence of the ra-

diation is a result of absorption of UV-Vis light. Irradiation of the chromophoric part of the betalainic molecule leads to light absorption, which causes excitation of the molecule and increasing its ground state to excited state. In addition, the molecule becomes more reactive.

Material and methods

Pigments solutions (1 mg/mL) were prepared in following solvents: water, 50% acetonitrile, 50% methanol, at pH in the range of 3-8, for which the acetic and phosphoric buffers were used. Photodegradation experiments were performed with immersion mercury lamp, which emits UV lights.

The related experiments were performed with the use of a microplate reader (Infinite M200, TECAN, Austria). Every time, 100 μ L of buffer and 100 μ L of a pigment and corresponding volume of water and the solvents were sequentially introduced into 2.5 mL reservoir wells corresponding to the 96-wells of the microplate reader. The finale pigment concentration was 1 mM in the 2 mL samples.

The reservoir plate was incubated in a water bath at a temperature 25 °C covered by a quartz glass plate and irradiated by UV lamp from the distance of 25 cm.

For chromatographic analysis, 200 μ L of each sample was collected and lyophilized in the case of the presence of the organic solvents. All lyophilized solutions were dissolved in 100 μ L of water and analyzed by HPLC-DAD and LC-DAD-MS.

A Gynkotek HPLC system with UVD170S, Gynkotek HPLC Pump Series P580 and thermostat (Gynkotek Sep-

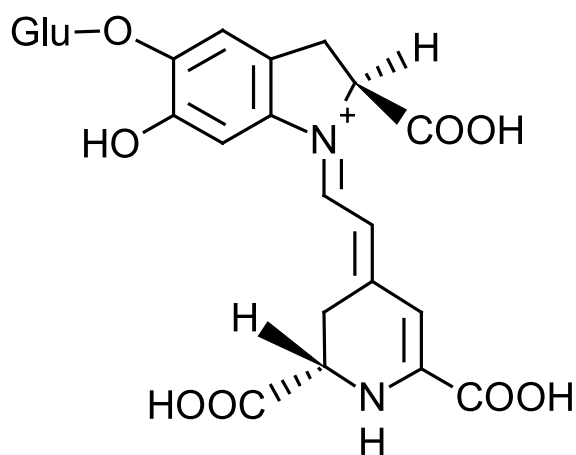


Figure 1. Structure of betanin

arations, H.I. Ambacht, The Netherlands) was used for the chromatographic analysis. For the data acquisition, the software package Chromeleon 4.32 (Gynkotek Separations) was used. For the on-line UV/Vis spectra acquisition, the detection was performed in the DAD (diode-array detection) mode. The column used was a 250 mm x 3 mm i.d., 5 μ m, Luna C18(2), with a 4 mm x 2 mm i.d. guard column of the same material (Phenomenex, Torrance, CA). The injection volume was 10 μ L, and the flow rate was 0.5 mL/min. The detection of analytes was performed typically at 538, 505, 480 and 440 nm. The column was thermostated at 35 °C. For the separation of the analytes, following gradient system was used: 3% (v/v) A with 97% (v/v) B at 0 min; gradient to 16% (v/v) A with 84% (v/v) B at 17 min, gradient to 50% (v/v) A with 50% (v/v) B at 30 min with A – acetonitrile, B – 2% formic acid.

The positive ion electrospray mass spectra were recorded on a ThermoFinnigan LCQ Advantage (electrospray voltage 4.5 kV; capillary 250 °C; sheath gas: N₂) coupled to ThermoFinnigan LC Surveyor pump utilizing the HPLC gradient Systems 1 and 2. The MS was controlled and total ion chromatograms and mass spectra were recorded using ThermoFinnigan Xcalibur software (San Jose, CA). Helium was used to improve trapping efficiency and as the collision gas for CID experiments. The relative collision energies for MS/MS analyses were set at 30% (according to a relative energy scale).

Results and discussion

The highest stability of the pigments was observed in aqueous solutions. In opposite to thermal degradation experiments, betanin and 17-decarboxybetanin turned out to be the most stable in aqueous solutions after 5 h of irradiation. 2,17-decarboxybetanin, usually the most stable pigment in thermo degradation tests, after UV radiation showed up to be the most labile in aqueous solutions (total degradation after 5 h).

Characteristic feature of all decarboxybetalains spectra after UV irradiation is lack of clear bands in visible range, which means that there are no new, colourful products.

After the reaction, all samples were analyzed by LC-DAD-ESI-MS. Tables 1-3 show detected products of UV irradiation experiments with decarboxybetalains in aqueous solutions. 17-decarboxybetanin turned up to be more stable at pH 4.0-7.5. In the case of these reactions, the main detected product was 2,17-decarboxyneobetain, especially in acidic samples, as a result of the pigment decarboxylation and dehydrogenation. At pH 3.5-6.5, as a product of decarboxylation, 2,17-decarboxybetanin was identified. In whole range of tested pH's, small amount of 17-decarboxyneobetain was formed. As a finale product, with basic chromophoric part, 2,15,17-decarboxy-2,3-dehydroneobetain is formed in the whole range of tested pH.

Table 1. Products formed after 2 hours of UV irradiation of 17-decarboxybetanin in aqueous solutions

No	Compound	m/z	pH										
			3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
			Peak area (*10 ⁻²)										
1	17-dBt	507	2000	2600	2800	2800	2800	2800	2800	2600	2400	2400	2400
2	17-dIBt	507	200	200	200	200	200	200	200	200	200	200	200
3	17-dNeoBt	505	6	6	4	10	7	10	7	8	10	15	13
4	2,17-dBt/2,17-dIBt	463	0	50	50	50	50	50	50	50	---	---	20
5	2,17-dNeoBt	461	400	280	160	150	170	220	140	80	24	34	30
6	2,17-dec-2,3-dHBt	461	---	---	---	---	---	---	---	---	---	---	---
7	2,17-dec-2,3-dHNeoBt	459	7	---	---	---	---	---	---	---	---	10	2
8	2,15,17-dNeoBt	417	---	---	---	---	---	---	---	---	---	---	---
9	2,15,17-dec-2,3-dHNeoBt	415	150	140	70	60	80	60	45	60	20	26	20

Table 2. Products formed after 2 hours of UV irradiation of 2-decarboxybetanin in aqueous solutions

No	Compound	m/z	pH										
			3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
			Peak area (*10 ⁻²)										
1	2-dBt	507	---	100	200	350	500	500	500	400	400	250	150
2	2-dNeoBt	505	---	---	---	---	---	---	---	---	---	---	---
3	2-dec-2,3-dHNeoBt	503	---	---	---	5	15	8	20	10	8	---	---
4	2,17-dBt/2,17-dIBt	463	---	---	6	20	10	8	6	---	---	---	---
5	2,17-dNeoBt	461	---	---	---	---	---	---	---	---	---	---	---
6	2,17-dec-2,3-dHBt	461	---	---	10	---	---	---	---	---	---	---	---
7	2,17-dec-2,3-dHNeoBt	459	18	36	26	18	15	11	7	5	10	10	---
8	2,15,17-dNeoBt	417	---	---	---	---	---	---	---	---	---	---	---
9	2,15,17-dec-2,3-dHNeoBt	415	---	4	---	---	---	---	---	---	---	---	---

Table 3. Products formed after 2 hours of UV irradiation of 2,17-decarboxybetanin in aqueous solutions

No	Compound	m/z	pH										
			3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
			Peak area (*10 ⁻²)										
1	2,17-dBt/2,17-dIBt	463	1300	1700	2000	2000	2000	2000	1800	1300	1300	1000	700
2	2,17-dNeoBt	461	130	150	100	60	100	70	60	45	25	15	8
3	2,17-dec-2,3-dHBt	461	----	----	10	15	20	----	----	----	----	----	----
4	2,17-dec-2,3-dHNeoBt	459	10	20	13	12	20	10	14	5	6	6	6
5	2,15,17-dNeoBt	417	120	80	80	60	80	80	95	46	35	30	20
6	2,15,17-dec-2,3-dHNeoBt	415	30	30	30	15	20	20	18	8	5	4	3

Table 4. Products formed after 2 hours of UV irradiation of 17-decarboxybetanin in methanolic solutions

No	Compound	m/z	pH										
			3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
			Peak area (*10 ⁻²)										
1	17-dBt	507	2600	3000	b.w	3600	3000	3000	3000	3000	3000	2600	2400
2	17-dIBt	507	200	200	b.w	200	200	200	200	200	200	200	200
3	17-dNeoBt	505	3	3	b.w	5	6	3	3	3	8	8	8
4	2,17-dBt/2,17-dIBt	463	10	10	b.w	10	10	10	10	10	10	10	10
5	2,17-dNeoBt	461	300	150	b.w	100	90	70	40	100	80	150	140
6	2,17-dec-2,3-dHBt	461	----	----	b.w	----	----	----	----	----	----	----	----
7	2,17-dec-2,3-dHNeoBt	459	13	15	b.w	8	8	8	6	5	8	16	12
8	2,15,17-dNeoBt	417	----	----	b.w	----	----	----	----	----	----	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	90	70	b.w	40	55	30	30	25	25	20	18

Table 5. Products formed after 2 hours of UV irradiation of 2-decarboxybetanin in methanolic solutions

No	Compound	m/z	pH										
			3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
			Peak area (*10 ⁻²)										
1	2-dBt	507	20	150	300	200	350	400	450	450	300	150	150
2	2-dNeoBt	505	----	----	----	----	----	----	----	----	----	----	----
3	2-dec-2,3-dHNeoBt	503	5	5	5	6	3	3	3	6	6	8	8
4	2,17-dBt/2,17-dIBt	463	4	6	16	10	20	6	12	6	6	2	---
5	2,17-dNeoBt	461	----	----	----	----	6	4	----	----	----	14	8
6	2,17-dec-2,3-dHBt	461	----	----	----	----	----	----	----	----	----	----	----
7	2,17-dec-2,3-dHNeoBt	459	11	11	6	6	6	6	2	2	4	4	4
8	2,15,17-dNeoBt	417	----	----	----	----	----	----	----	----	----	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	4	4	3	18	13	----	----	----	----	----	----

Irradiation of 2,17-decarboxyneobetatin leads to a formation of four basic products in the whole range of pH: 2,17-decarboxyneobetatin, 2,17-decarboxy-2,3-dehydroneobetatin, 2,15,17-dehydroneobetatin and 2,15,17-decarboxy-2,3-dehydroneobetatin. The biggest amounts of these compounds are formed in acidic media. In addition, at pH 4-5, 2,17-decarboxy-2,3-dehydrobetatin is formed.

The results of LC-DAD-ESI-MS analysis of methanolic samples are presented in Tables 4-6. The products profile is almost the same as in the case of aqueous solutions. An additional product is presumably 2,17-decarboxy-2,3-dehydroneobetatin, showed up in small amounts at whole range of pH.

Irradiation of 2-decarboxybetatin in methanolic solutions leads to a formation of the same compounds as in aqueous samples, however, they were also detected at low

pH values, the same as for generation of 2,15,17-decarboxy-2,3-dehydroneobetatin. Additionally, a small amount of 2,17-decarboxyneobetatin was formed at pH 5-8.

As a result of 2,17-decarboxybetatin irradiation in methanol, a fast degradation and a generation of small number of its products in comparison to the aqueous solutions is observed. The main detected products are: 2,17-decarboxy-2,3-dehydroneobetatin (pH 3-8) and 2,15,17-decarboxy-2,3-dehydroneobetatin (pH 3-5).

Tables 7-9 show results of LC-DAD-ESI-MS analysis of acetonitrile samples. Irradiation of 17-decarboxybetatin, the same as in the case of aqueous solutions, leads to a formation of 2,17-decarboxyneobetatin in the whole pH range as well as 2,15,17-decarboxy-2,3-dehydroneobetatin at pH 3-5.5. 2,17-decarboxybetatin was also detected, but at very small amounts, in the whole range of pH. Direct

product of dehydrogenation 7-decarboxyneobetainin occurred at pH 6.5-8. In contrast to aqueous solutions, there were huge amounts of 2,15,17-decarboxyneobetainin formed in the whole range of pH, same as for 2,17-decarboxy-2,3-dehydroneobetainin.

After irradiation of 2-decarboxybetainin in acetonitrile, we received small amounts of the same derivatives we got in water, however we could observe them also in acidic samples. Additionally we noticed 2,15,17-decarboxy-2,3-dehydroneobetainin at low pH and 2,17-decarboxyneobetainin at pH 7-8.

Table 6. Products formed after 2 hours of UV irradiation of 2,17-decarboxybetainin in methanolic solutions

No	Compound	m/z	pH										
			3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
			Peak area (*10 ⁻²)										
1	2,17-dBt/2,17-dIBt	463	4	6	16	10	20	6	12	6	6	2	---
2	2,17-dNeoBt	461	---	---	---	---	6	4	---	---	---	14	8
3	2,17-dec-2,3-dHBt	461	---	---	---	---	---	---	---	---	---	---	---
4	2,17-dec-2,3-dHNeoBt	459	11	11	6	6	6	6	2	2	4	4	4
5	2,15,17-dNeoBt	417	---	---	---	---	---	---	---	---	---	---	---
6	2,15,17-dec-2,3-dHNeoBt	415	4	4	3	18	13	---	---	---	---	---	---

Table 7. Products formed after 2 hours of UV irradiation of 17-decarboxybetainin in acetonitrile solutions.

No	Compound	m/z	pH										
			3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
			Peak area (*10 ⁻²)										
1	17-dBt	507	1000	2000	2600	3000	2600	2600	3000	2400	2200	1800	1400
2	17-dIBt	507	50	50	50	50	50	50	50	50	50	50	50
3	17-dNeoBt	505	---	---	---	---	---	---	---	2	6	6	6
4	2,17-dBt/2,17-dIBt	463	5	5	5	5	5	5	5	5	5	5	0
5	2,17-dNeoBt	461	360	200	110	130	180	110	80	90	200	280	260
6	2,17-dec-2,3-dHBt	461	---	---	---	---	---	---	---	---	---	---	---
7	2,17-dec-2,3-dHNeoBt	459	22	18	13	12	14	18	13	13	16	16	15
8	2,15,17-dNeoBt	417	110	50	30	20	70	35	25	18	18	15	15
9	2,15,17-dec-2,3-dHNeoBt	415	200	140	95	75	120	120	65	60	65	65	65

Table 8. Products formed after 2 hours of UV irradiation of 2-decarboxybetainin in acetonitrile solutions.

No	Compound	m/z	pH										
			3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
			Peak area (*10 ⁻²)										
1	2-dBt	507	---	10	150	150	250	350	500	250	150	100	40
2	2-dNeoBt	505	---	---	---	---	---	---	---	---	---	---	---
3	2-dec-2,3-dHNeoBt	503	---	---	4	6	6	6	8	7	8	10	14
4	2,17-dBt/2,17-dIBt	463	---	---	---	4	6	6	6	---	---	10	0
5	2,17-dNeoBt	461	5	---	---	---	---	---	---	---	18	20	22
6	2,17-dec-2,3-dHBt	461	---	---	---	---	---	---	---	---	---	---	---
7	2,17-dec-2,3-dHNeoBt	459	3	10	10	10	10	10	10	2	2	2	0
8	2,15,17-dNeoBt	417	---	---	---	---	---	---	---	---	---	---	---
9	2,15,17-dec-2,3-dHNeoBt	415	6	---	3	---	---	---	---	---	---	---	---

Table 9. Products formed after 2 hours of UV irradiation of 2,17-decarboxybetainin in acetonitrile solutions.

No	Compound	m/z	pH										
			3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
			Peak area (*10 ⁻²)										
1	2,17-dBt/2,17-dIBt	463	1000	1300	700	1500	1800	1400	1800	1800	1200	900	300
2	2,17-dNeoBt	461	55	55	30	30	90	60	55	38	100	60	180
3	2,17-dec-2,3-dHBt	461	---	---	---	---	---	---	---	---	---	---	---
4	2,17-dec-2,3-dHNeoBt	459	6	13	13	8	6	6	6	6	6	6	6
5	2,15,17-dNeoBt	417	2	6	10	8	8	12	12	7	---	---	---
6	2,15,17-dec-2,3-dHNeoBt	415	60	90	50	50	80	80	90	44	36	36	25

Irradiation of 2,17-decarboxybetanin also leads to a formation of four products in the whole range of pH: 2,17-decarboxyneobetanin, 2,17-decarboxy-2,3-dehydroneobetanin, 2,15,17-decarboxyneobetanin and 2,15,17-decarboxy-2,3-dehydroneobetanin, however, in different amounts. The differences are mainly in the case of 2,15,17-decarboxyneobetanin, formed in acetonitrile at very low quantity at pH 3-6.5 and higher amounts of 2,17-decarboxyneobetanin as well as 2,15,17-decarboxy-2,3-dehydroneobetanin generated at pH 7-8.

Conclusions

According to our studies, the UV irradiation has a detrimental effect on stability of decarboxylated betalain. In the presence of organic solvents, a faster decrease of stability was observed than in the case of aqueous samples. As a result of the reactions, many, new decarboxylated and dehydrogenated derivatives were formed. All new products were analyzed and tentatively identified by LC-DAD-ESI-MS. Obtained new compounds can be used in food or cosmetic industry as a new dyes, showing higher stability and resistance to light and irradiation.

References

- [1] D. Strack, T. Vogt, W. Schliemann, "Recent advances in betalain research", *Phytochemistry*, 2003, 62, 247-269.
- [2] G.A.F. Hendry, J.D. Houghton, "Natural Food Colorants", London, UK, Blackie Chapman & Hall, 1996.
- [3] Tesoriere, L.; Butera, D.; Allegra, M.; Fazzari, M.; Livrea, M. A. 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. *J. Agric. Food Chem.* 2005, 53, 1266-1270.
- [4] Martinez-Parra, J.; Munoz, R. Characterization of betacyanin oxidation catalyzed by a peroxidase from *Beta vulgaris* L. roots. *J. Agric. Food Chem.* 2001, 49, 4064-4068.
- [5] Kapadia, G. J.; Azuine, M. A.; Sridhar, R.; Okuda, Y.; Tsuruta, A.; Ichiishi, E.; Mukainake, T.; Takasaki, M.; Konoshima, T.; Nishino, H.; Tokuda, H. 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. *Pharmacol. Res.* 2003, 47, 141-148.
- [6] Kanner, J.; Harel, S.; Granit, R. Betalains - A new class of dietary cationized antioxidants. *J. Agric. Food Chem.* 2001, 49, 5178-5185.
- [7] Escribano, J.; Pedreño, M. A.; García-Carmona, F.; Muñoz, R. Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots. *Phytochem. Anal.* 1998, 9, 124-127.
- [8] Butera, D.; Tesoriere, L.; Di Gaudio, F.; Bongiorno, A.; Allegra, M.; Pintaudi, A. M.; Kohen, R.; Livrea, M. A. Antioxidant activities of Sicilian prickly pear (*Opuntia ficus-indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. *J. Agric. Food Chem.* 2002, 50, 6895-6901.
- [9] Pavlov, A.; Kovatcheva, P.; Georgiev, V.; Koleva, I.; Ilieva, M. Biosynthesis and radical scavenging activity of betalains during the cultivation of red beet (*Beta vulgaris*) hairy root cultures. *Z. Naturforsch. C.* 2002, 57, 640-644.
- [10] Cai, Y.; Sun, M.; Corke, H. Antioxidant activity of betalains from plants of the Amaranthaceae. *J. Agric. Food Chem.* 2003, 51, 2288-2294.
- [11] Gandía-Herrero, F.; Escribano, J.; García-Carmona, F. Structural implications on color, fluorescence, and antiradical activity in betalains. *Planta* 2010, 232, 449-460.
- [12] Skopińska A., Tuwalska D., Wybraniec S., Starzak K., Mitka K., Kowalski P., Szaleniec M. Spectrophotometric study on betanin photodegradation *Challenges of Modern Technology* 2012 vol.3 (No 4).