Thermal degradation of 17-decarboxy-betanin monitored by LC-MS

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A thermal stability study on 17-decarboxy-betanin depending on physicochemical process conditions was conducted.
17-decarboxy-betanin is one of betacyanin, natural origin pigments applied in food and pharmaceutical industry instead of artificial colorants. There is a need for searching of new non-toxic natural food components and this is a reason of increasing interest of betacyanins.
As most derivatives of betanin obtained by decarboxylation, 17-decarboxy-betanin tends to degrade in the presence of some factors such as increased temperature or other conditions of the reaction environment [1]. This subject was investigated in this study because the stability of the pigments is still a significant issue limiting their wide application.
The degradation of 17-decarboxy-betanin during heating in selected solutions: water as well as aqueous solutions of ethanol 50% (v/v), methanol 50% (v/v) and acetonitrile 50% (v/v) at pH in the range 3-8 was tested. As UV-Vis spectra indicate, 17-decarboxy-betanin tends to degrade mostly at pH 3, notwithstanding a type of solution. The products of degradation were identified by LC-DAD-ESI-MS. As a result of incubation at 85°C in different solutions, various mono-, bi- and tridecarboxylated as well as dehydrogenated derivatives were obtained. The dominant product of 17-decarboxy-betanin degradation is 2,15,17-tridecarboxy-2,3-dehydro-betanin.

Key words: 17-decarboxy-betanin , betacyanins, thermal degradation

Introduction

17-decarboxy-betanin (Fig. 1) is a derivative of betanin and belongs to betacyanins pigments. These pigments have been applied in food and pharmaceutical industry as colorants, but now more and more of their advantages is found. In addition, decarboxylated betacyanins generated by heating of red beet root preparations (*Beta vulgaris* L.) are non-toxic and their pro-health, both antioxidant and antitumoral activity was reported [2-4], therefore, a growing interest in betalains is noticed. However, this group of natural compounds is much less explored than carotenoids or anthocyanins. The main reason is low stability of betacyanins and tendency to degrade under the influence of elevated temperature, light, presence of several metal cations, oxidizing factors like enzymes or gaseous oxygen in air, solvents at very acidic or basic pH [5]. Degradation of betacyanins may lead to a discoloration which limits their applications in food industry for non-heated products. Therefore, widening the knowledge about improvement of their stability is an interesting and important topic.

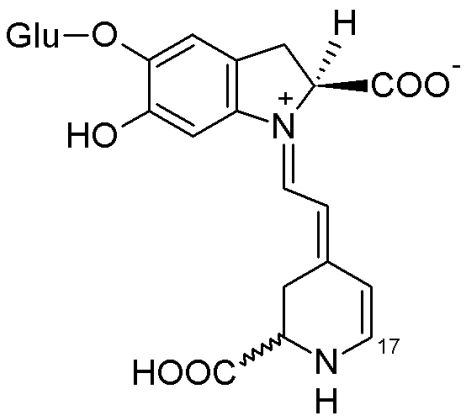

Fig. 1. Structural formula of 17-decarboxy-betanin

Thermal degradation is one of the crucial issues in food colorants due to interest of consumers in safe food components. Because of the fact that temperature is a common technological factor in food manufacturing, its influence has to be extensively studied. In previous reports, betalainic extracts from red beet root and purple pitaya were tested in this regard [1,6] and the main decarboxylated heating products were identified. However, the stabilizing effect of presence of natural plant matrix or addition of some chelating agents in comparison to pure pigments is significant [7,8]. Hence, this report includes investigation on thermo-degradation of isolated and purified 17-decarboxy-betanin in selected solutions, which were subjected to heating at 85 °C.

Experimental

Preparation of sample

The solutions of 17-decaboxy-betanin at a concentration of 1 mg/mL were prepared in four selected solvents: water as well as 50% (v/v) aqueous solutions of ethanol, methanol

and acetonitrile at pH 3-8. Acetate, phosphate and citrate buffers were used for the experiments to keep appropriate pH of the solutions. Obtained samples (2 mL) were heated at 85 °C for 60 min in a water bath. Then, these samples (200 µL) were collected after 0, 15, 30, 45 and 60 minutes of experiment, and subsequently were frozen and lyophilized. Afterwards, the samples were dissolved in 200 µL of demineralized water for further analysis.

UV-Vis assay

For the pigment stability tests, absorption at 505 nm of all collected samples (at 0, 15, 30, 45, 60 min) was measured with a use a microplate reader (Inifnite M200, TECAN, Austria). Calculated light-path for a volume of 200 µl was 0.53 cm. Additionally, UV-Vis spectra were collected after 45 minutes of experiment in order to observe possible new absorption bands, indicating degradation products.

HPLC-DAD

For the chromatographic analyses of the tested pigments and reaction products, a Gynkotek HPLC system with UVD340U, Gynkotek HPLC pump Series P580 and thermostat (Gynkotek Separations, H.I. Ambacht, The Netherlands) was used, and a column Luna C18(2) 250 x 3 mm i.d., 5 µm (Phenomenex, Torrance, CA, USA). was thermostated at 35°C. For the separation of analytes, the following gradient system was used: 3% A in B at 0 min, 16% A in B at 17 min and a gradient to 50% A in B at 30 min (A, acetonitrile; B, 2% formic acid in water). In each case, the injection volume was 10 µL, and the flow rate was 0.5 mL/min. A diode array detection system was applied for signal detection.

LC-DAD-ESI-MS

Analysis of the samples by LC-DAD-ESI-MS was performed on a mass spectrometer ThermoFinnigan LCQ Advantage (electrospray voltage 4.5 kV; capillary 250°C; sheath gas: N2). The MS was controlled, and total ion chromatograms and mass spectra were recorded using the ThermoFinnigan Xcalibur software (San Jose, CA). The relative collision energies for MS/MS analyses ranged 30% (according to a relative energy scale). Helium was used to improve trapping efficiency and as the collision gas for CID experiments.

Results and Discussion

A useful parameter for estimation of pigment stability is retention which is defined as a percentage ratio of absorption value of the compound in selected time of experiment to absorption value before heating (Formula 1). Because absorption is directly proportional to concentration, the stability of the pigment can be studied this way.

$$R = \frac{[A]_t}{[A]_0} \cdot 100\ \% \qquad (1)$$

where:
$[A]_t$ – pigment absorption in time of measurement [-],
$[A]_0$ – pigment absorption before heating experiment [-].

Figure 2 shows the results of the retention tests of heating 17-decarboxy-betanin solutions in different solvents and pH. The results indicate the highest stability of 17-decarboxy-betanin in water (Fig. 2a) even after 60 minutes of heating, whereas the lowest stability in ethanolic solutions (Fig. 2b). A degree of degradation of 17-decarboxy-betanin after 60 minutes in methanolic (Fig. 2c) and acetonitilic solutions (Fig. 2d) is comparable, but in the case of acetonitrilic solutions it is progressing slowly but gradually. Moreover, the initial (after 15 min) increased retention of 17-decarboxy-betanin in this solution is higher than in water (Fig. 2a), which could indicate on stabilizing effect of acetonitrile. In most cases, pH near 5.5 reveals a stabilizing impact. Although, the aqueous solutions at pH 6-7 are optimal for the pigment stability (Fig. 2a), pH 3 was proved to have detrimental influence on 17-decarboxy-betanin and caused fast degradation irrespectively of the type of used solvents.

For further retention results, the spectra collected after 45 minutes of heating were depicted in Fig. 3. Due to the fact that 17-decarboxy-betanin reveals absorption maximum at 505 nm, the presence of new absorption bands indicates on a formation of new degradation products.

A formation of new derivatives of 17-decarboxy-betanin, which are its degradation products can be observed in all the spectra in Fig. 3, especially in the range of wavelength 410-420 nm. As expected, higher absorption maxima of new derivatives are obtained in the case of aqueous-organic solutions than in water, particularly in ethanolic solutions. Interestingly, at pH 3, new absorption maxima at ca. 400 nm are observed.

In agreement with the retention results, the highest stability of 17-decarboxy-betanin in the mid-range of pH was confirmed by the highest absorption maxima of 17-decarboxy-betanin at 505 nm. The main products of the thermal degradation of 17-decarboxy-betanin were identified by the LC-DAD-ESI-MS technique (Tables 1-4).

Obtained degradation products were recognized as the following compounds: 17-decarboxy-neobetanin (17-dNeoBt, **3**); 2,17-bidecarboxy-betanin/-isobetanin (2,17-dBt/2,17-dIBt, **4**); 2,17-bidecarboxy-neobetanin (2,17-dNeoBt, **5**); 2,17-bidecarboxy-2,3-dehydro-betanin (2,17-dec-2,3-dHBt, **6**); 2,17-bidecarboxy-2,3-dehydro-neobetanin (2,17-dec-2,3-dHNeoBt, **7**); 2,15,17-tridecarboxy-neobetanin (2,15,17-dNeoBt, **8**); 2,15,17-tridecarboxy-2,3-dehydro-betanin (2,15,17-dec-2,3-dHNeoBt, **9**). In addition, during the heating, isomerization took place and a certain amount of 17-decarboxy-isobetanin was formed.

In acidic solutions, compounds **4**, **6**, **7** and **8** prevailed, whereas in solutions at pH 8, 17-dNeoBt (**3**) is the main product, which is nearly not formed at low pH. Degrada-

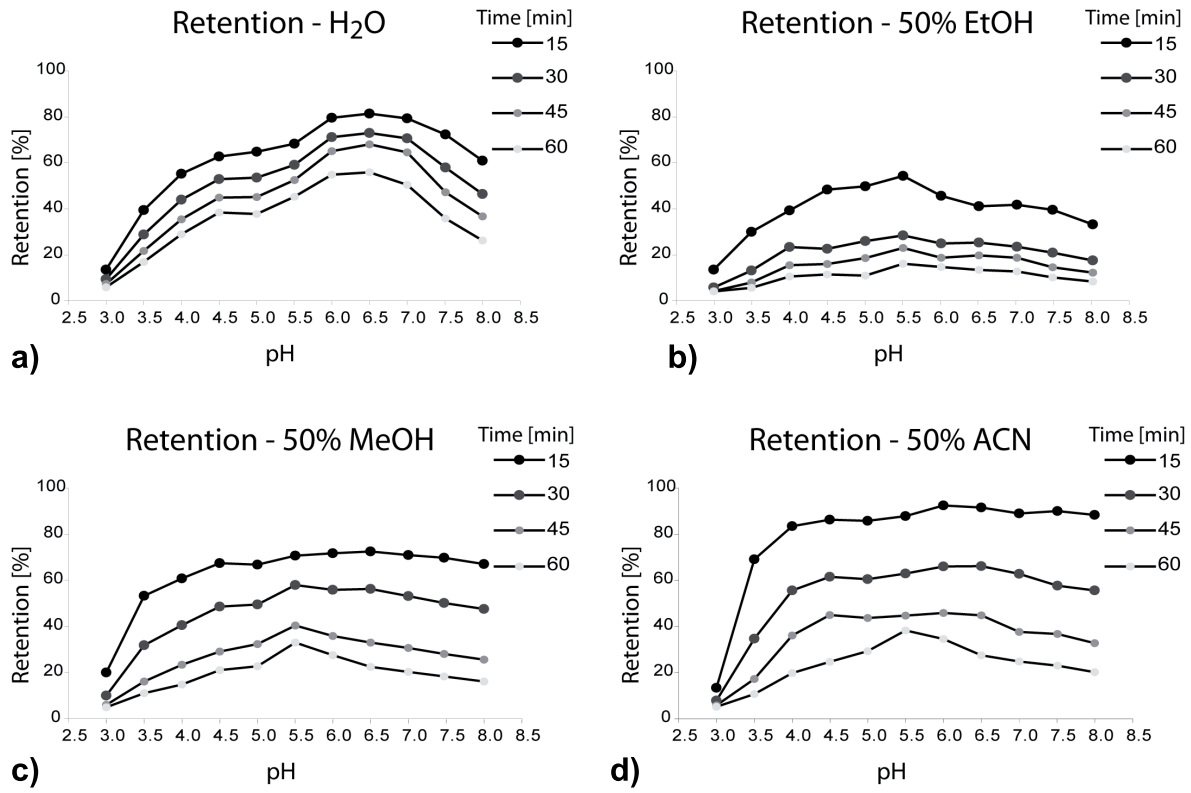

Fig. 2. Retention of 17-dekarboxy-betanin in heated aqueous (a), 50% ethanolic (b), 50% methanolic (c), 50% and acetonitrilic (d) solutions tested at different pH's

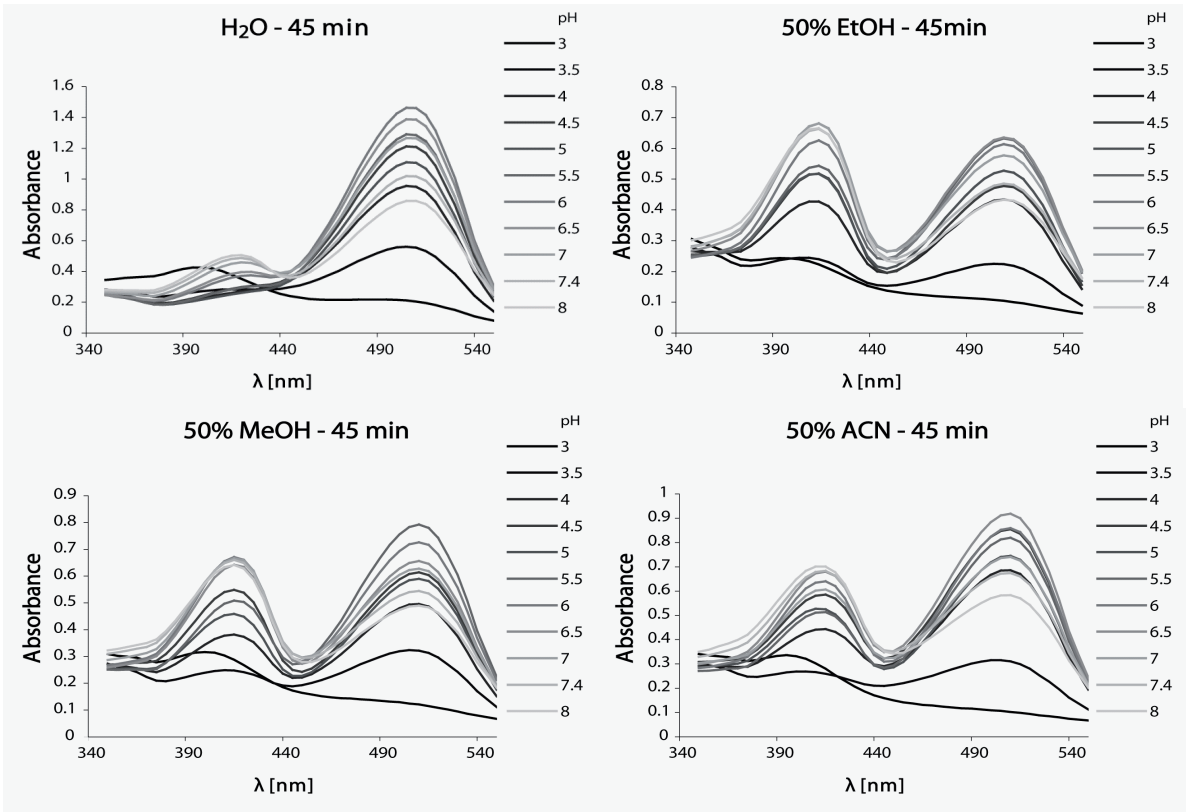

Fig. 3. Retention of 17-dekarboxy-betanin in heated aqueous (a), 50% ethanolic (b), 50% methanolic (c), 50% and acetonitrilic (d) solutions tested at different pH's

Table 1. Tentatively detected products of 17-decarboxy-betanin thermal degradation in aqueous solutions after 30 min heating at 85°C

No.	Identified 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	1100	3000	4000	4500	4500	4500	4500	4500	4500	4500	4000
2	17-dIBt	507	50	100	400	50	50	50	50	50	50	50	50
3	17-dNeoBt	505	---	8	14	30	20	20	140	200	400	550	550
4	2,17-dBt/2,17-dIBt	463	150	340	340	300	200	140	100	80	80	40	30
5	2,17-dNeoBt	461	40	50	50	30	10	----	----	----	----	----	----
6	2,17-dec-2,3-dHBt	461	700	700	400	60	300	40	----	----	----	----	15
7	2,17-dec-2,3-dHNeoBt	459	600	300	80	30	60	20	10	10	10	20	20
8	2,15,17-dNeoBt	417	260	360	260	110	100	30	----	----	----	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	1250	400	100	22	60	15	----	----	6	10	13

Table 2. Tentatively detected products of 17-decarboxy-betanin thermal degradation in 50% ethanolic solutions after 30 min heating at 85°C

No.	Identified 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	180	1200	2000	2000	2200	2400	3000	2600	2600	2600	2600
2	17-dIBt	507	20	100	200	200	200	200	400	400	400	400	600
3	17-dNeoBt	505	----	----	20	2	----	----	14	6	20	70	80
4	2,17-dBt/2,17-dIBt	463	60	75	100	100	170	130	120	85	60	30	26
5	2,17-dNeoBt	461	----	----	----	----	----	----	----	----	----	----	----
6	2,17-dec-2,3-dHBt	461	80	20	5	5	35	5	5	20	20	8	6
7	2,17-dec-2,3-dHNeoBt	459	40	7	10	6	4	6	8	8	11	15	20
8	2,15,17-dNeoBt	417	30	30	20	3	----	----	----	----	----	8	20
9	2,15,17-dec-2,3-dHNeoBt	415	440	170	55	2	10	10	----	3	3	8	8

Table 3. Tentatively detected products of 17-decarboxy-betanin thermal degradation in 50% methanolic solutions after 30 min heating at 85°C

No.	Identified 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	3600	4000	4000	4000	4500	4500	4500	4500	4500	3800
2	17-dIBt	507	100	200	200	200	200	200	500	500	700	1000	1400
3	17-dNeoBt	505	----	----	----	2	2	40	3	3	120	200	150
4	2,17-dBt/2,17-dIBt	463	500	700	700	600	600	600	500	400	360	400	180
5	2,17-dNeoBt	461	400	100	70	26	25	60	45	18	22	22	28
6	2,17-dec-2,3-dHBt	461	----	----	----	----	----	----	----	----	----	----	----
7	2,17-dec-2,3-dHNeoBt	459	18	18	18	----	----	14	12	11	16	45	50
8	2,15,17-dNeoBt	417	75	34	11	4	2	----	----	----	----	3	3
9	2,15,17-dec-2,3-dHNeoBt	415	400	55	30	4	4	6	6	2	3	30	30

tion of 17-decarboxy-betanin leads to a formation of **3** mostly in aqueous solutions. In ethanolic solutions, formation of 2,17-dNeoBt (**5**) is not observed. This compound is detected in acidic methanolic and acetonitrilic, as well as in aqueous solutions, but to a lesser degree than other derivatives. The absence of **6** in methanolic and acetonitrilic solutions was observed in contrast to aqueous at acidic pH. Likewise, the neo-derivatives of betanin **7**, **8** and **9**, were detected in all the tested solutions, but they dominate in acidic aqueous solutions.

The most frequently detected thermo-degradation product is **4**, especially at pH 3.5-6, and it is formed to a greater degree in 50% methanolic solutions.

Table 4. Tentatively detected products of 17-decarboxy-betanin thermal degradation in 50% acetonitrilic solutions after 30 minutes heating at 85°C

No.	Identified 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	1100	3400	4000	4000	4000	4000	4000	4000	3600	3000	3000
2	17-dIBt	507	200	200	200	200	200	200	200	200	200	200	200
3	17-dNeoBt	505	----	----	12	---	24	34	22	22	16	180	280
4	2,17-dBt/2,17-dIBt	463	300	380	400	500	500	450	450	300	180	160	160
5	2,17-dNeoBt	461	300	80	45	30	20	10	18	10	300	10	10
6	2,17-dec-2,3-dHBt	461	----	----	----	----	----	----	----	----	----	----	----
7	2,17-dec-2,3-dHNeoBt	459	20	5	5	5	5	5	11	10	45	36	80
8	2,15,17-dNeoBt	417	60	40	24	5	----	----	----	----	----	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	1100	80	36	13	13	6	----	2	7	30	36

Conclusions

Summarizing, the lowest stability of 17-decarboxy-betanin is indicated in solutions at pH 3 regardless of the organic solvent addition and the dominant product of the degradation is 2,15,17-tridecarboxy-2,3-dehydro-betanin. However, in the whole range of pH, 2,17-decarboxy-betanin and its isoform are the most often formed degradation products.

The detrimental impact of high acidic pH was confirmed by the retention tests and obtained spectra. In consequence, 17-decarboxy-betanin is not adequate for colouring of acidic food products. The ethanolic solutions were proved to decrease 17-decarboxy-betanin stability and thereby should not be used during manufacturing of food coloured by 17-decarboxy-betanin.

References

[1] Wybraniec S., Mizrahi Y. *Generation of Decarboxylated and Dehydrogenated Betacyanins in Thermally Treated Purified Fruit Extract from Purple Pitaya (Hylocereus polyrhizus) Monitored by LC-MS/MS*, J. Agric. Food Chem. 2005, 53; pp 6704-6712

[2] Moreno D. A., Gracía-Viguera C., Gil J. I., Gil-Izquierdo A., *Betalains in the era of global agri-food science, technology and nutrional health*, Phytochem Rev 2008, 7; pp 261-280

[3] 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; pp 124-127

[4] Kapadia G. J., Azuine M. A., Sridhar R., Yoko 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 (2); pp 141-148

[5] Herbach K. M., Stinzing F.C., Carle R., *Betalain Stability and Degradation Structural and Chromatic Aspects*, J. Food Sci., 2006, 71(4); pp 41- 50

[6] Wybraniec S., *Formation of Decarboxylated Betacyanins in Heated Purified betacyanin Fractions from Red Beet Root (Beta vulgaris L.) Monitored by LC-MS/MS*, J. Agric. Food Chem., 2005, 53; pp 3483-3487

[7] von Elbe J.H., Maing I.Y., Amundson C.H., *Colour stability of betanin*, J Food Sci.,1974, 39; pp 334–337

[8] Skopińska A., Starzak K., Wybraniec S., *Influence of EDTA on stabilization of decarboxylated betalains*, Challenges of Modern Technology, 2012, 3; pp 29-33