

# Reactions of decarboxylated betanins under influence of halogen light

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Betalains as natural plant pigments are very sensitive to several factors, including light, therefore, studies on stability of their derivatives which retain their attractive color, are crucial for their potential application in pharmaceutical, food or cosmetic industries. An effect of irradiation by halogen light on reactivity of main decarboxylated derivatives of betacyanins (2-decarboxy-betanin, 17-decarboxy-betanin and 2,17-decarboxy-betanin) was investigated in aqueous and organic-aqueous solutions: 50% (v/v) acetonitrile, 50% (v/v) methanol and 50% (v/v) ethanol, at pH range 3-8. The double decarboxylation of betanin (at C-2 and C-17 position of carbon) significantly increases the stability of this pigment in the acidic media. As a result of pigments reactions induced by light, some betacyanin derivatives with different decarboxylation and dehydrogenation levels were identified by chromatography with tandem mass spectrometry (LC-MS/MS) and diode array detection (LC-DAD).

**Key words and phrases:** betanin, decarboxylated betacyanins, photolability, mass spectrometry

## Introduction

Betalains are water-soluble plant pigments. They are found in certain families of the order *Caryophyllales*. Betalain pigments can be divided into two major structural subgroups, betacyanins and betaxanthins. In general, betacyanins appear red-violet and absorb in the 535-550 nm range [1]. The most common betacyanin in nature is betanin, which is present at high concentration in red beet root (*Beta vulgaris* L.).

In plant tissues, betalains are located within the cell vacuoles. They strongly absorb light energy in the ultraviolet (UV) and visible (Vis) range which is associated with electron excitation of the betalain chromophore to a more energetic state. This phenomenon leads to higher reactivity or lowered activation energy of the molecule, which causes its light-induced degradation [2]. Betalains should be kept

in the absence of light irradiation during food processing and storage. Hence, products packed in transparent materials, should be tested under illumination conditions. Although betalain stability depends on numerous parameters, which are specific for natural pigments, the demand for this pigment class is constantly increasing. This growing interest is due not only to their applications for coloring purposes in the food industry, but also induced by their pro-healthy properties, such as antioxidant activity. It may have a big influence on the pharmaceutical industry. Moreover, the betanin derivatives (modified betalains), generated during thermal decarboxylation and dehydrogenation, may be very interesting research material due to their various coloring properties and their greater stability in comparison to parent betacyanins [3].

Deleterious impact of light on betalains can be monitored at temperatures below 25 °C. Above this temperature,

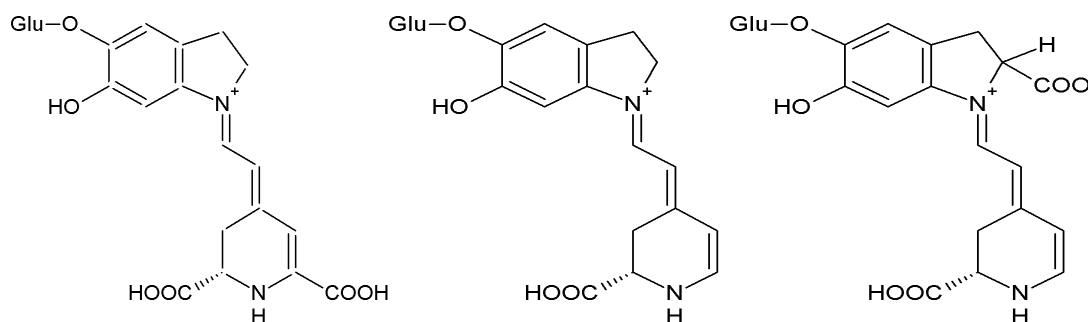


Fig. 1. The chemical structures of decarboxylated derivatives of betanin: 2-decarboxy-betanin, 17-decarboxy-betanin and 2,17-bidecarboxy-betanin

the effect of irradiation cannot be measured, because it is overlapped by thermal degradation. Betalains degrade rapidly in the presence of oxygen, as well as below pH 3 and above pH 8, which are pH values beyond the range of optimal stability of betalains [4]. Light and oxygen cause a decomposition of betanin by 15.6% and 14.6%, respectively, whereas in the presence of both of these factors, an increase of pigment degradation by 28.6% is observed [5]. Since a similar stability during storage of the pigments in light and in darkness was obtained, the effect of light under anaerobic conditions was found to be irrelevant [4]. On the other hand, the addition of 0.1% isoascorbic acid and 1.0% ascorbic acid to red beet and purple pitaya juices inhibits decomposition of betanin during their storage [6].

Due to a photo-lability of betacyanins, an effect of Vis-irradiation on reactivity of betanin derivatives (17-decarboxy-betanin (17-dBt), 2-decarboxy-betanin (2-dBt), 2,17-decarboxy-betanin (2,17-dBt)) in methanolic, ethanolic and acetonitrilic solvents was investigated. Identification of generated decarboxylated and dehydrogenated derivatives of betanin was conducted by chromatography with tandem mass spectrometry (LC-MS/MS) and diode array detection (LC-DAD).

## Material and methods

17-dBt, 2-dBt and 2,17-dBt solutions at a concentration of 1 mg/mL were prepared in various solvents: water and aqueous solutions of 50% (v/v) acetonitrile, 50% (v/v) methanol and 50% (v/v) ethanol, at pH range 3-8. Acetate and phosphate buffers were used for preparation of tested samples.

The study of pigments reactivity induced by light was conducted by the use of a halogen lamp (Philips PAR38 120 W), which emits radiation at a visible wavelength range. The reservoirs of the samples were inserted in a water bath at 25 °C, covered with a glass and irradiated from the top by the halogen lamp. Kinetic measurements were carried out by samples collected every few days during the 8.5-day exposure to halogen lamp. The samples of tested solutions

were analyzed by spectrophotometry in 96-well plates of a microplate reader (Inifinite M200, TECAN, Austria).

A Gynkotek HPLC system with UVD340U, Gynkotek HPLC pump Series P580 and thermostat (Gynkotek Separations, H.I. Ambacht, The Netherlands) was applied for chromatographic analysis. The analytical column was used a Luna C-18(2) 250x3 mm I.D., 5 μm (Phenomenex, Torrance, CA, USA).

Analysis of the resulting 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: N<sub>2</sub>). The MS was controlled, and total ion chromatograms and mass spectra were recorded using the ThermoFinnigan Xcalibur software (San Jose, CA).

## Results and Discussion

The results of LC-DAD-ESI-MS analysis are demonstrated in Tab. 1-8. Numerous products of 17-dBt, 2-dBt and 2,17-dBt photoreaction in water and aqueous 50% organic solutions were identified by applying the above-mentioned techniques.

After 6.5 days of Vis-irradiation of 2-decarboxy-betanin, the following compounds were generated: 2-decarboxy-2,3-dehydro-neobetanin (2-dec-2,3-dHNeoBt) at pH 4.5-8 and 2,17-decarboxy-betanin (2,17-dBt) mainly at pH 8, as well as at pH 3-6. The other compounds are the following: 2,17-decarboxy-2,3-dehydro-neobetanin (2,17-dec-2,3-dHNeoBt) and 2,17-decarboxy-2,3-dehydro-betanin (2,17-dec-2,3-dHBt); the latter one occurs only in acidic media. The above-mentioned compounds have also formed during previous UV-irradiation of 2-dBt experiments. The only difference is the lack of 2-decarboxy-neobetanin (2-dNeoBt), which was generated as a product of a single dehydrogenation of 2-dBt.

The results of 17-decarboxy-betanin irradiation by halogen light are similar to the results obtained for the UV lamp. In the largest quantities, 2,17-decarboxy-neobetanin (2,17-dNeoBt) is formed, particularly in acidic media. In contrast to the irradiation of 2-dBt, 2,17-dBt as a result of

Tab. 1. The main products of 2-decarboxy-betanin degradation formed in aqueous solutions after 6.5 days of Vis-irradiation

No.	Compound	<i>m/z</i>	<i>m/z from MS/MS</i>	pH					
				3.0	4.0	5.0	6.0	7.0	8.0
				Peak area (*10 <sup>-2</sup> )					
1	2-dBt	507	345	75	1400	1600	1300	750	700
2	2-dNeoBt	505	343; 299; 255	----	----	----	----	----	----
3	2-dec-2,3-dHNeoBt	503	341; 297; 253	----	1	6	6	6	6
4	2,17-dBt/2,17-dIBt	463	301	14	12	8	3	----	55
5	2,17-dNeoBt	461	299; 255	----	----	----	----	----	5
6	2,17-dec-2,3-dHBt	461	299; 255	24	70	14	7	----	----
7	2,17-dec-2,3-dHNeoBt	459	297; 253	20	8	12	6	6	8
8	2,15,17-dNeoBt	417	255	----	----	----	----	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	253	----	----	----	----	----	18

Tab. 2. The main products of 17-decarboxy-betanin degradation formed in aqueous solutions after 6.5 days of Vis-irradiation

No.	Compound	<i>m/z</i>	<i>m/z from MS/MS</i>	pH					
				3.0	4.0	5.0	6.0	7.0	8.0
				Peak area (*10 <sup>-2</sup> )					
1	17-dBt	507	345	3000	3500	3500	3000	3000	3000
2	17-dIBt	507	345	350	370	330	270	260	220
3	17-dNeoBt	505	343; 299; 255	75	90	60	65	130	130
4	2,17-dBt/2,17-dIBt	463	301	240	180	140	60	55	45
5	2,17-dNeoBt	461	299; 255	450	220	45	40	50	50
6	2,17-dec-2,3-dHBt	461	299; 255	50	20	----	----	----	----
7	2,17-dec-2,3-dHNeoBt	459	297; 253	170	90	35	50	150	140
8	2,15,17-dNeoBt	417	255	45	12	----	----	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	253	70	28	10	5	11	30

Tab. 3. The main products of 2,17-decarboxy-betanin degradation formed in aqueous solutions after 6.5 days of Vis-irradiation

No.	Compound	<i>m/z</i>	<i>m/z from MS/MS</i>	pH					
				3.0	4.0	5.0	6.0	7.0	8.0
				Peak area (*10 <sup>-2</sup> )					
1	2,17-dBt/2,17-dIBt	463	301	2800	3000	2600	2000	1400	1400
2	2,17-dNeoBt	461	299; 255	60	35	35	30	13	13
3	2,17-dec-2,3-dHBt	461	299; 255	30	30	30	10	8	---
4	2,17-dec-2,3-dHNeoBt	459	297; 253	40	45	55	60	60	75
5	2,15,17-dNeoBt	417	255	45	20	13	----	----	----
6	2,15,17-dec-2,3-dHNeoBt	415	253	30	20	40	40	36	36

Tab. 4. The main products of 17-decarboxy-betanin degradation formed in 50% ethanolic solutions after 6.5 days of Vis-irradiation

No.	Compound	<i>m/z</i>	<i>m/z from MS/MS</i>	pH					
				3.0	4.0	5.0	6.0	7.0	8.0
				Peak area (*10 <sup>-2</sup> )					
1	17-dBt	507	345	1800	2600	2600	2600	2000	2000
2	17-dIBt	507	345	1800	260	260	260	1800	1800
3	17-dNeoBt	505	343; 299; 255	50	150	300	400	300	180
4	2,17-dBt/2,17-dIBt	463	301	200	180	130	180	60	34
5	2,17-dNeoBt	461	299; 255	380	260	40	40	20	20
6	2,17-dec-2,3-dHBt	461	299; 255	40	20	10	10	----	----
7	2,17-dec-2,3-dHNeoBt	459	297; 253	900	160	75	90	90	100
8	2,15,17-dNeoBt	417	255	30	30	8	2	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	253	300	80	45	20	55	65

Tab. 5. The main products of 2-decarboxy-betanin degradation formed in 50% ethanolic solutions after 6.5 days of Vis-irradiation

No.	Compound	<i>m/z</i>	<i>m/z from MS/MS</i>	pH					
				3.0	4.0	5.0	6.0	7.0	8.0
				Peak area (*10 <sup>-2</sup> )					
1	2-dBt	507	345	----	1300	1400	1500	750	600
2	2-dNeoBt	505	343; 299; 255	----	13	24	36	6	---
3	2-dec-2,3-dHNeoBt	503	341; 297; 253	----	3	11	20	25	60
4	2,17-dBt/2,17-dIBt	463	301	5	20	6	----	----	----
5	2,17-dNeoBt	461	299; 255	----	----	----	----	----	----
6	2,17-dec-2,3-dHBt	461	299; 255	----	240	140	35	10	----
7	2,17-dec-2,3-dHNeoBt	459	297; 253	6	16	20	20	12	10
8	2,15,17-dNeoBt	417	255	----	----	----	----	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	253	----	----	----	----	----	----

Tab. 6. The main products of 2,17-decarboxy-betanin degradation formed in 50% ethanolic solutions after 6.5 days of Vis-irradiation

No.	Compound	<i>m/z</i>	<i>m/z from MS/MS</i>	pH					
				3.0	4.0	5.0	6.0	7.0	8.0
				Peak area (*10 <sup>-2</sup> )					
1	2,17-dBt/2,17-dIBt	463	301	4000	4000	3600	3000	1500	1500
2	2,17-dNeoBt	461	299; 255	65	50	20	20	20	20
3	2,17-dec-2,3-dHBt	461	299; 255	20	80	110	110	45	5
4	2,17-dec-2,3-dHNeoBt	459	297; 253	90	70	90	75	130	180
5	2,15,17-dNeoBt	417	255	55	55	55	8	2	0
6	2,15,17-dec-2,3-dHNeoBt	415	253	60	55	55	28	65	60

Tab. 7. The main products of 17-decarboxy-betanin degradation formed in 50% acetonitrilic solutions after 6 days of Vis-irradiation

No.	Compound	<i>m/z</i>	<i>m/z from MS/MS</i>	pH					
				3.0	4.0	5.0	6.0	7.0	8.0
				Peak area (*10 <sup>-2</sup> )					
1	17-dBt	507	345	3500	4000	4000	5000	5000	5000
2	17-dIBt	507	345	500	500	500	500	500	500
3	17-dNeoBt	505	343; 299; 255	65	85	180	180	180	180
4	2,17-dBt/2,17-dIBt	463	301	180	300	140	90	40	15
5	2,17-dNeoBt	461	299; 255	550	170	80	110	85	45
6	2,17-dec-2,3-dHBt	461	299; 255	40	30	20	----	----	----
7	2,17-dec-2,3-dHNeoBt	459	297; 253	1400	340	300	85	200	280
8	2,15,17-dNeoBt	417	255	180	30	15	----	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	253	900	180	180	32	45	55

Tab. 8. The main products of 2-decarboxy-betanin degradation formed in 50% acetonitrilic solutions after 6 days of Vis-irradiation

No.	Compound	<i>m/z</i>	<i>m/z from MS/MS</i>	pH					
				3.0	4.0	5.0	6.0	7.0	8.0
				Peak area (*10 <sup>-2</sup> )					
1	2-dBt	507	345	70	300	800	800	200	200
2	2-dNeoBt	505	343; 299; 255	5	----	----	----	----	----
3	2-dec-2,3-dHNeoBt	503	341; 297; 253	30	18	18	22	26	150
4	2,17-dBt/2,17-dIBt	463	301	2600	50	30	45	35	1400
5	2,17-dNeoBt	461	299; 255	50	20	----	----	----	34
6	2,17-dec-2,3-dHBt	461	299; 255	200	40	55	4	----	4
7	2,17-dec-2,3-dHNeoBt	459	297; 253	140	60	45	24	10	240
8	2,15,17-dNeoBt	417	255	140	5	----	----	----	----
9	2,15,17-dec-2,3-dHNeoBt	415	253	70	20	----	----	----	24

17-dBt decarboxylation is generated in higher abundance at pH 3.5-5.0. The direct product of 17-dBt dehydrogenation is 17-decarboxy-neobetatin (17-dNeoBt), which appears at the whole pH range, however its largest quantities can be found at alkaline pH. The final product, 2,15,17-decarboxy-2,3-dehydro-neobetatin (2,15,17-dec-2,3-dHNeoBt), also occurs at the whole pH range, nevertheless the highest concentrations of this compound were noticed at pH 3-4.

Irradiation of 2,17-decarboxy-betanin leads to a formation of four main products, which were also generated during experiments with UV lamp, however in smaller amounts.

Their highest concentration is noticed in acidic media. Additionally, 2,17-decarboxy-2,3-dehydro-betanin (2,17-dec-2,3-dHBt) appears at a broader pH range (3-7).

In 50% ethanolic solutions of 17-dBt, the compound profile is similar to the profile obtained in aqueous media. In large quantities, 2,17-decarboxy-neobetatin (2,17-dNeoBt) is formed, especially in acidic media, as well as 2,17-dBt. The product of 17-dBt dehydrogenation (17-dNeoBt) is generated at the whole pH range, especially 5-7.

The final product of reactions induced by light is 2,15,17-dec-2,3-dHNeoBt, which is also formed at the whole pH range, but in the highest concentration at pH 3.

In contrast to the results obtained for aqueous solutions at pH 3, the main product of 17-dBt irradiation by Vis lamp is 2,17-dec-2,3-dHNeoBt.

Exposure to halogen light of 2-dBt in ethanolic solutions leads to a generation of the same compounds which have occurred in water. Furthermore, the formation of 2-decarboxy-neobetainin (2-dNeoBt) at pH 4-7 was noticed, being a rare compound among betalains degradation products. At the largest concentrations, 2,17-dec-2,3-dHBt is formed at pH 4-5, while 2,15,17-dec-2,3-dHNeoBt and 2,17-dNeoBt is not formed.

Compound profiles during irradiation of 2,17-decarboxy-betanin in ethanol is similar to the profile in water. Generation of the following compounds is observed at low concentrations: 2,17-dNeoBt, 2,17-dec-2,3-dHBt, 2,17-dec-2,3-dHNeoBt, 2,15,17-dNeoBt, as well as 2,15,17-dec-2,3-dHNeoBt.

Interestingly, more derivatives of 17-decarboxylated betanin were found in the 50% acetonitrilic solutions in comparison to the aqueous solutions. 2,17-dec-2,3-dHNeoBt and 2,15,17-dec-2,3-dHNeoBt are formed at the highest quantities as well as 2,17-dNeoBt is formed especially in

acidic media. 17-dNeoBt occurs mainly at the pH range 5-8.

Reactions of 2-dBt in acetonitrilic solutions also lead to a generation of a complex mixture of derivatives. Considerable quantities of 2,17-decarboxy-betanin are noticed, especially at pH 3 and 8. Substantial abundances of 2,17-dec-2,3-dHBt and 2,17-dec-2,3-dHNeoBt were obtained in acidic media. A generation of smaller amounts of other derivatives is also observed.

Compound profiles obtained in methanolic solutions were similar to the profiles in acetonitrilic solvents. Recent studies established nonenzymatic oxidation mechanisms in betanin as well as its decarboxylated and dehydrogenated derivatives [7]. The mechanism of decarboxylation and dehydrogenation of betalains induced by halogen light is still unknown. Presumably, these reactions are also affected by oxidation.

Initial spectra of betanin derivatives in water before the start of irradiation are shown in the Fig. 2. 2-decarboxy-betanin possess absorption maxima at  $\lambda_{\max}$  ca. 540 nm, whereas 17-decarboxy-betanin, as well as 2,17-bidecarboxy-betanin have  $\lambda_{\max}$  at ca. 505 nm.

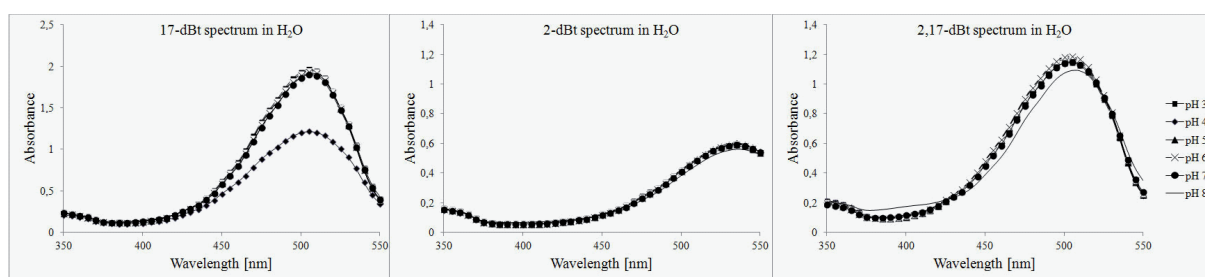


Fig. 2. Initial spectra of decarboxylated derivatives of betanin in water before the start of Vis-irradiation

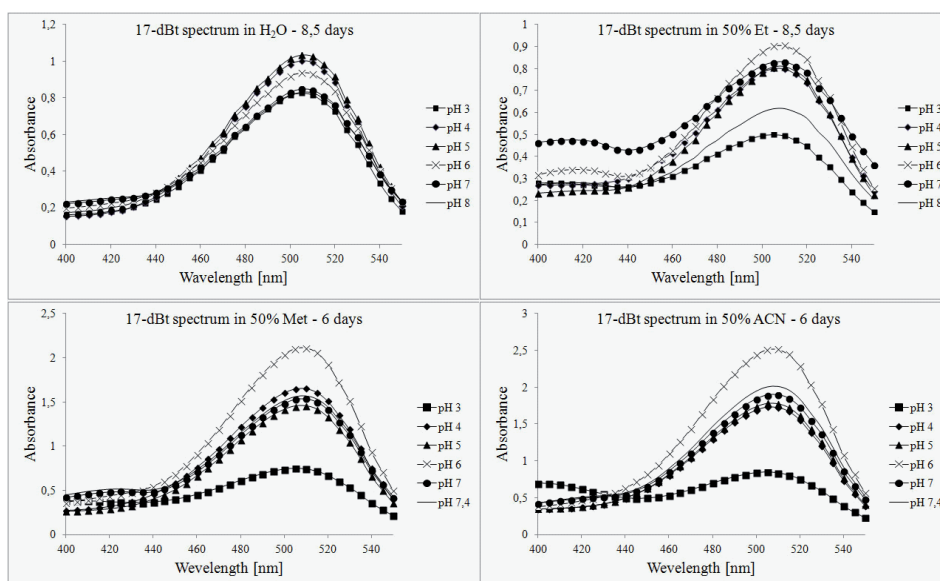


Fig. 3. 2-decarboxy-betanin spectra in different solutions after Vis-irradiation.

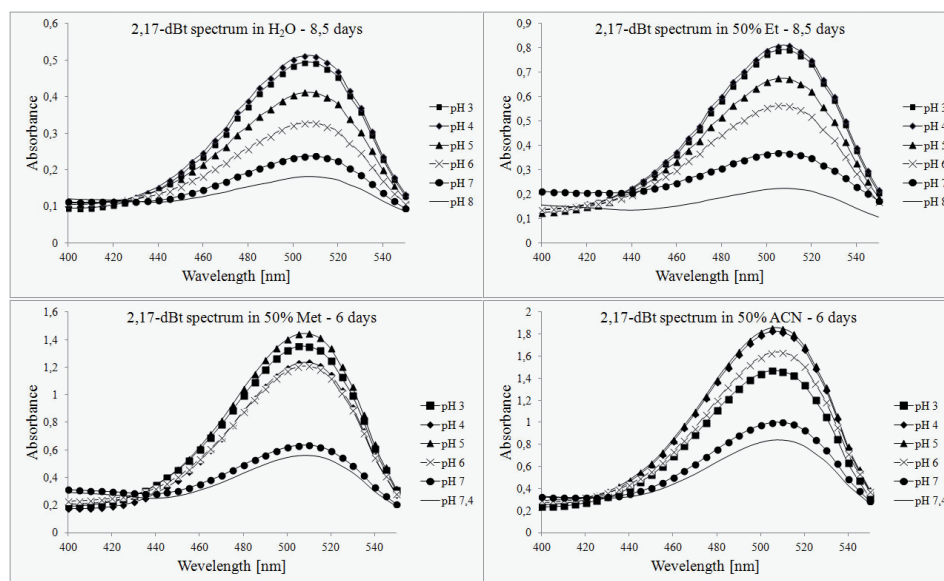


Fig. 4. 17-decarboxy-betain spectra in different solutions after Vis-irradiation

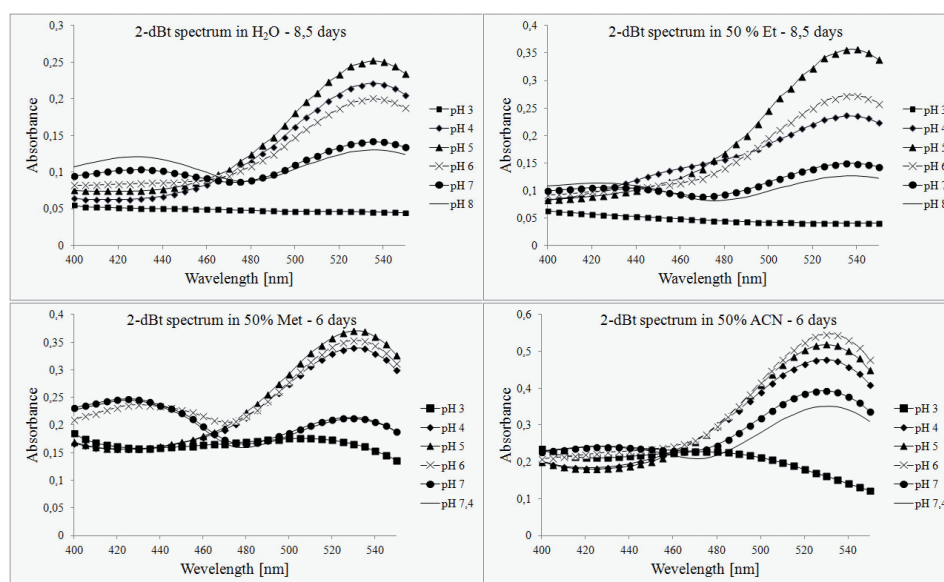


Fig. 5. 2,17-decarboxy-betain spectra in different solutions after Vis-irradiation

The main degradation products, generated after halogen light exposure of 2-dBt solutions, are compounds possessing absorption maxima at  $\lambda_{\max}$  ca. 420 nm at pH 4-8 (Fig. 3). In strongly acidic media, the initial absorption maxima occurred at a wavelength ca. 505 nm, and then almost completely disappeared, due to the decomposition into structures much smaller than betain derivatives. Similar results were obtained for betain solutions, for which Vis-irradiation experiments were recently conducted.

The rate of degradation of 17-dBt and 2,17-dBt was inconsiderable at broad pH range (Fig. 4-5). Compounds absorbing at  $\lambda_{\max}$  ca. 405 nm are formed during experiment, however absorbance values at their absorption maxima are small.

## Conclusions

The effect of the solvents on the degree and direction of betain derivatives reaction depends on pH of the solutions and Vis-irradiation time. 2-decarboxy-betain is the most reactive among all compounds at pH 3, particularly in aqueous and ethanolic solutions. The double decarboxylation of betain (at carbon C-2 and C-17) significantly increases the stability of this compound in the acidic media during Vis-irradiation experiment. A relatively high stability of 17-decarboxy-betain in aqueous samples was observed at the whole pH range.

As a result of pigments reactions induced by light, numerous betacyanin derivatives with different decarboxyla-

tion and dehydrogenation levels were identified by chromatography with tandem mass spectrometry (LC-MS/MS) and diode array detection (LC-DAD).

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