

# Spectrophotometric study on betanin photodegradation

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The most commonly occurring betacyanin plant pigment in nature is betanin (5-*O*-glucoside of betanidin). Its main source is red beet root (*Beta vulgaris* L.), used for production of food colorants on a commercial scale [1].

Due to photo-lability of betacyanins, an effect of UV-irradiation on betanin degradation in various solutions containing organic solvents was investigated. Organic solvents are used frequently in many stages of pigment preparation or isolation, therefore, knowledge of betalain stability and degradation characteristics in these solutions is essential.

Pigment solutions at a concentration of 1 mg/ml were prepared in different solvents: water as well as aqueous solutions of 50% (v/v) acetonitrile, 50% (v/v) methanol or 50% (v/v) ethanol, at the pH range 3–8. Acetate, phosphate and citrate buffers were used for the experiments.

As a result of the pigment photo-decomposition, numerous decarboxylated and dehydrogenated derivatives were formed. Spectrophotometric studies enabled determination of the pigment retention (percentage of the pigment residue relative to its initial concentration after decomposition time). The studies demonstrated betanin high lability in all solvents, not only at extreme pH values, but also in the middle pH range which, in general, provides higher stability of betalains.

**Keywords and phrases:** betanin, betacyanins, photodegradation, *Beta vulgaris* L.

## Introduction

Red beet (*Beta vulgaris* L.) roots are a source of natural pigments — betalains, containing of red-violet betacyanins and yellow-orange betaxanthins. Nevertheless, they are also present in majority of plants belonging to the *Caryophyllales* order, as well as cactus fruits, which may be an alternative to red beet preparations. Especially betacyanins are the object of increased interest, because of their use for production of food colorants on a commercial scale. Additionally, the previous studies reported a superior stability of betacyanins in comparison to betaxanthins in specific conditions at both room and elevated temperatures. During thermal treatment, the half-life value of betanin was 11 times higher compared to vulgaxanthin I, the basic betaxanthin in red beet [2].

Betalains are reported to exhibit preventive anti-carcinogenic properties [1, 3]. They constitute a pro-

pective group of compounds, not only for food, but also pharmaceutical or cosmetic industries. Summarizing, their potential benefits for human health are invaluable. The most commonly occurring betacyanin pigment in nature is betanin (5-*O*-glucoside of betanidin) (Fig. 1), therefore, most studies are focused on this important pigment [1, 4].

Certain external factors such as light or temperature must be controlled during food manufacture, because of their detrimental effect on betalain stability. According to previous reports, UV irradiation has a significant impact on reducing of the betalains stability [5–8]. So far, detailed studies on pigments stability during irradiation were not conducted. Betalains tendency of degradation under the irradiation is a result of absorption of light in the UV and visible range. Light incident on the chromophore structure (1,7-diazaheptamethinium resonance system) is absorbed, leading to excitation of

electrons of chromophore from the ground state to a more energetic state. The result is a higher reactivity and decrease of the activation energy of the molecule [4].

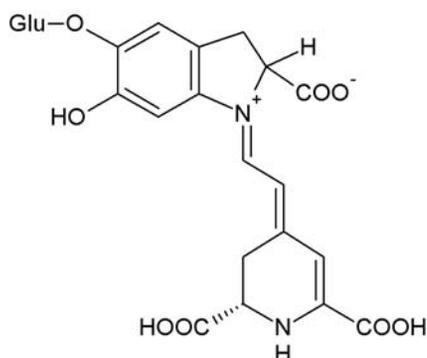


Fig. 1. Chemical structure of betanin — the most common betacyanin in nature.

Methanolic, ethanolic and acetonitrilic solvents are used frequently in many stages of pigment preparation or isolation, therefore, knowledge of betalain stability and degradation characteristics in these media is essential. For example, some previous studies have demonstrated the lowest stability of betanin in ethanolic media.

Due to photo-lability of betacyanins, an effect of UV-irradiation on betanin degradation in various solvents was investigated. Moreover, an investigation of the effect of medium, in which reaction takes place, on betanin degradation was performed at varying pH.

## Material and methods

Pigment solutions at a concentration of 1 mg/mL were prepared in the following solvents: water as well as aqueous solutions of 50% (v/v) acetonitrile, 50% (v/v) methanol and 50% (v/v) ethanol, at the pH range 3–8. Acetate, phosphate and citrate buffers were used for the experiments.

The photodegradation study was performed by using of an immersion mercury lamp, emitting radiation at the ultraviolet wavelength range (mostly at a wavelength of 254 nm). Into 2 mL wells of a 96-well of liquid reservoir used for preparation of samples for microplate readers, 100  $\mu$ L of buffer, 100  $\mu$ L of pigment and the appropriate volumes of organic solvents and water were introduced each time to obtain the requested solvent compositions. The target concentration of the pigments in a volume of 2 mL was 1 mM.

The reservoirs of the samples were inserted in a water bath at 25°C, covered with a quartz glass and irradiated by the mercury lamp from the top. Kinetic measurements were carried out by collecting of 200  $\mu$ L samples every 20–40 min during the several hours of UV-irradiation. The samples of tested solutions of betanin were analyzed by spectrophotometry in 96-well plates of a microplate reader. Collected samples were introduced into 300  $\mu$ L wells of 96-well plates microplate reader (Infinite M200, TECAN, Austria). The determined optical path for a volume of 200  $\mu$ L was 0.53 cm. Afterwards, a spectrophotometric measurement of spectra of the

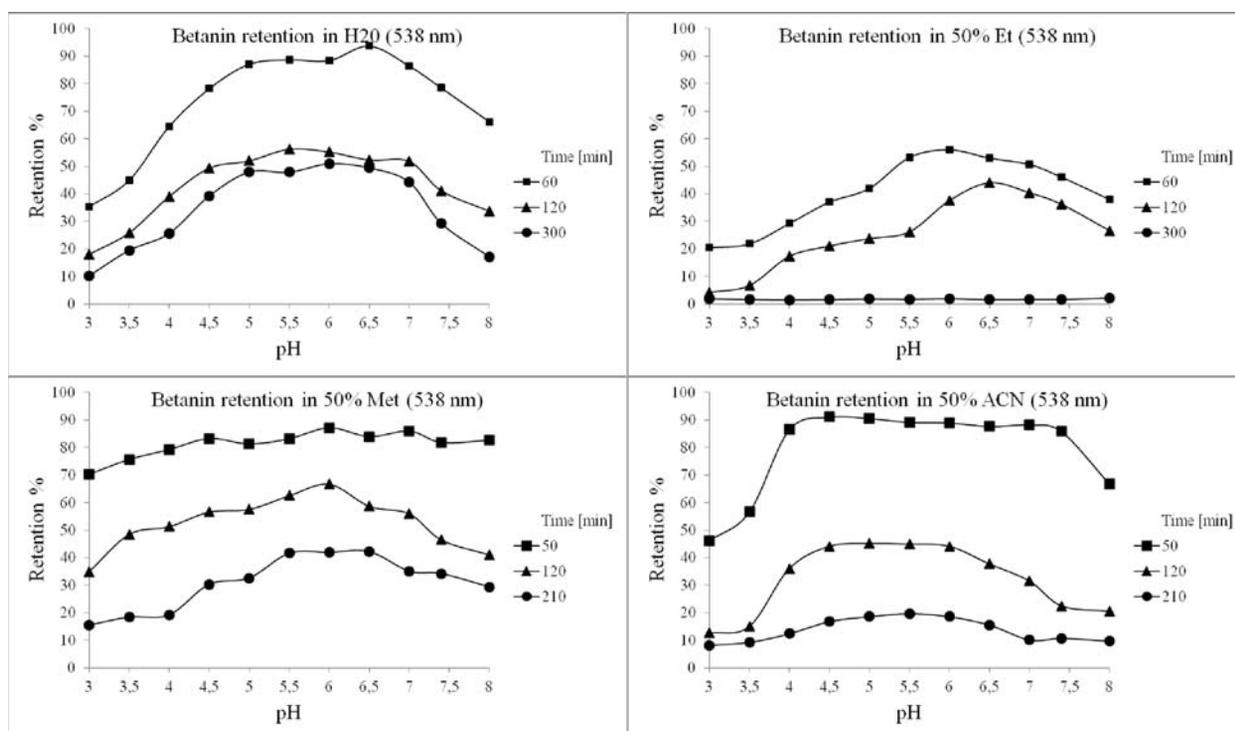


Fig. 2. Betanin retention in selected solutions at varying pH.

samples at 25°C was performed. The spectrophotometric monitoring was performed in a wide range of the visible spectrum.

200 µL samples were subjected to evaporation in a lyophilizer in the case of the presence of the solvents. Lyophilized solutions were dissolved in 100 µL of demineralized water and analyzed by HPLC-DAD. A Gynkotek HPLC system with UVD340U, Gynkotek HPLC pump Series P580 and thermostat (Gynkotek Separations, H.I. Ambacht, The Netherlands) was used for the chromatographic analysis. The analytical column was used a Luna C-18(2) 250×3 mm I.D., 5 µm (Phenomenex, Torrance, CA, USA). 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). The injection volume was 10 µL and the flow rate of 0.5 mL/min was applied. Detection was performed by diode array (DAD). The column was thermostated at 35°C.

Analysis of the tested samples by LC-DAD-ESI-MS was performed on a mass spectrometer Agilent 1100 VL LC-MS (electrospray voltage 3.5 kV; capillary temp. 220°C; sheath gas: N<sub>2</sub>) coupled to an Agilent LC pump and DAD detector (Agilent Technologies, Palo Alto, CA). The MS was controlled and total ion chromatograms and mass spectra were recorded using Chemstation software (Agilent Technologies, Palo Alto, CA).

## Results and Discussion

The spectrophotometric studies enabled determination of the pigment retention (percentage of the pigment residue relative to its initial concentration after decomposition time). The results of investigation of the effect of UV irradiation on the degradation of betanin expressed as betanin retention (%), depending on pH and UV-irradiation time are demonstrated in Fig. 2.

The photodegradation of betanin depends on the irradiation time and type of solvent as well as pH. As a result, it was found that the greatest stability of the pigment was observed in aqueous solutions, especially at pH range 4.5–7.0. In comparison to previous studies, in which the effect of Vis-irradiation on betanin stability was performed, the optimal pH range for pigment stability under influence of Vis-irradiation was found at 4.5–5.0. Moreover, in the organic solvents, the superior stability of betanin was observed at higher pH. In ethanolic solutions, betanin exhibits the highest lability.

The most interesting qualitative effects after photodegradation of pigment were observed in the ethanolic solutions. The chromatographic analysis showed the formation of new degradation products. In ethanolic

media, betanin is least stable and after 5 hours of irradiation is almost completely decomposed at all pH-values. The optimal pH range in these media ranges from 6.0 to 7.5.

In acetonitrile, the superior betanin stability is observed at pH 4.5–6.5 and in methanol, at the slightly higher pH-values (pH 5.5–6.5). Stability of betanin in water is similar to 17-decarboxybetanin (17-dBt) (Fig. 3), one of the main decarboxylated derivatives. The optimal pH for both compounds in water is similar. The study on the stability of the betanin derivatives was also conducted. Identification of their degradation products can expand the range of betalains potential applications. 2,17-bidecarboxy-betanin (2,17-dBt) (Fig. 4), usually the most stable pigment in tests of thermodegradation, was the most labile compound during UV-irradiation in water (almost complete degradation after 5 hours).

Initial spectrum of betanin in water before the start of irradiation is shown in Fig. 5. Betanin, the most common betacyanin in the nature, possess absorption maximum at  $\lambda_{\max}$  538 nm.

A characteristic feature of the obtained spectra for betanin UV irradiated solutions (Fig. 6) is a lack of clear bands in the visible range, indicating on the formation of large quantities of colored reaction products having

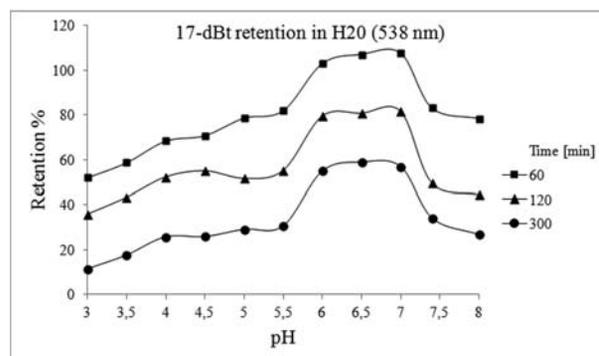


Fig. 3. 17-decarboxy-betanin retention in aqueous solutions.

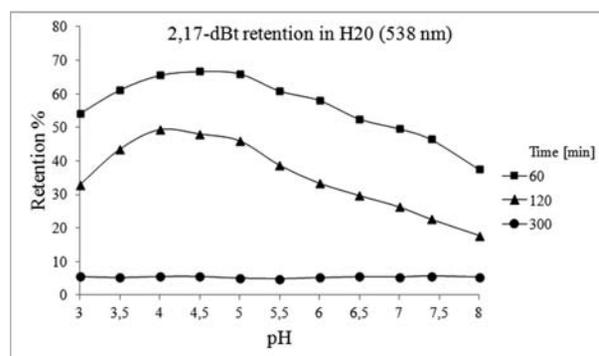


Fig. 4. 2,17-bidecarboxy-betanin retention in aqueous solutions.

in their structure chromophoric basic skeleton. Only for betanin in ethanolic solutions, bands with maximum absorption at a wavelength ca. 440 nm are formed.

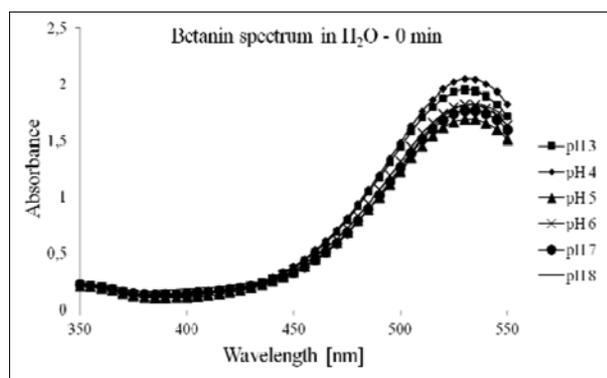


Fig. 5. Betanin spectra in water before the start of UV-irradiation.

The tentative identification of the reaction products was performed by chromatography with tandem mass spectrometry (LC-MS/MS) and diode array (LC-DAD) detection. In our previous studies on betanin photo-decomposition in buffered aqueous or organic solutions during Vis-irradiation, numerous decarboxylated and dehydrogenated derivatives were formed. The dominant decarboxylation products were 17-decarboxybetanin, 2-decarboxybetanin and 2,17-bidecarboxy-betanin. In this study, the detection of these derivatives was also expected. The main decarboxylated derivatives of betanin formed in water after 120 min of UV-irradiation are demonstrated in Table 1.

The significant quantities of 17-dBt were generated in whole pH range (3–8). This compound exhibits substantial stability at pH 3.5–7.5 in aqueous solutions. The formation of small amounts of 15-dBt as well as 2-dBt at pH 3.5–8.0 is noticed. However, the latter

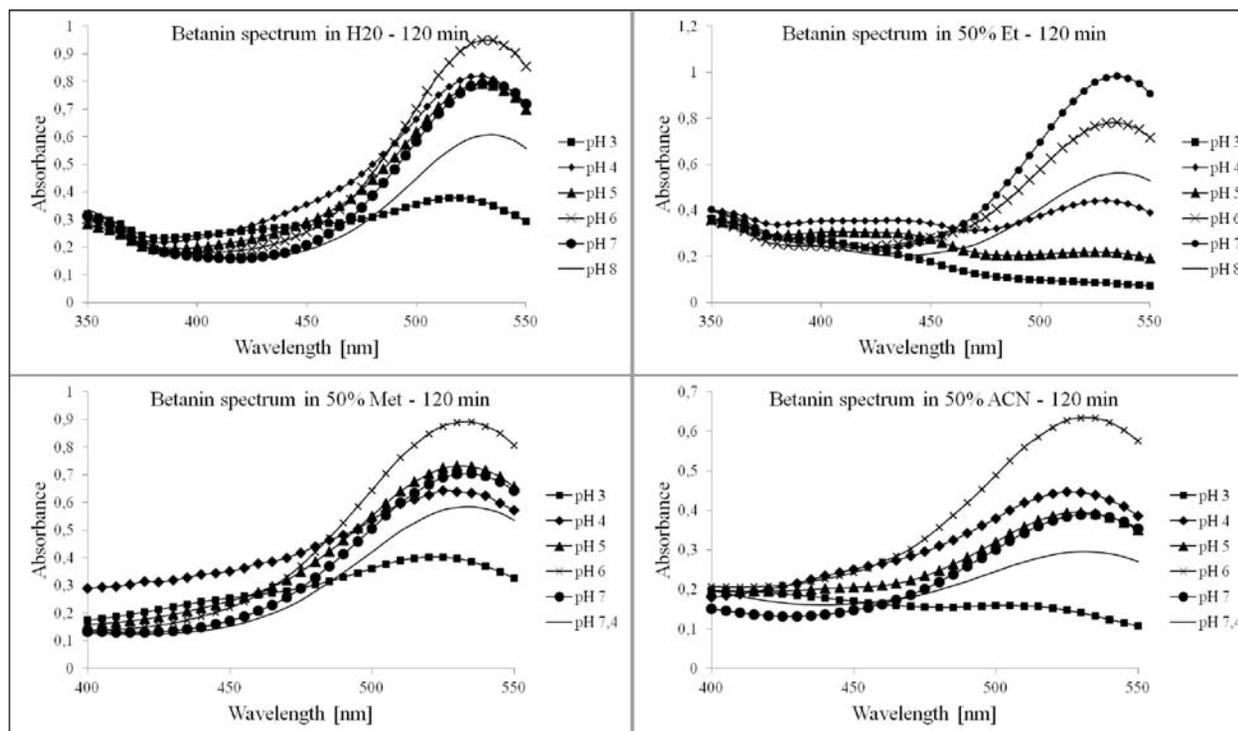


Fig. 6. Betanin spectra in different solutions after 120 min of UV-irradiation.

Table 1. The main decarboxylated products of betanin degradation formed during 120 min UV-irradiation.

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 ( $\times 10^{-2}$ )													
1	Bt	551	340	700	1300	1500	1500	1500	1500	1200	1500	1500	1400
2	lBt	551	20	100	200	300	300	400	400	200	300	300	300
4	17-dBt	507	350	500	550	650	550	600	650	500	600	550	450
5	17-dlBt	507	50	100	100	100	100	100	100	100	100	100	100
6	15-dBt	507	0	50	50	50	50	50	50	50	50	50	50
7	2-dBt	507	0	50	50	70	70	70	50	50	50	50	50

appears especially at pH 4.5–5.5. Moreover, dehydrogenated derivatives were also detected. Among them — neobetanin — appeared in small quantities at pH 3–8, but in the higher concentrations at alkaline pH.

## Conclusions

The spectrophotometric studies enabled determination of betanin stability expressed as the pigment retention. The studies demonstrated betanin high lability in all solvents, not only at extreme pH values, but also in the middle pH range which, in general, provides higher stability of betalains.

As a result of the pigment photo-decomposition in buffered aqueous or organic solutions, numerous decarboxylated and dehydrogenated derivatives were formed. The application of chromatography with tandem mass spectrometry (LC-MS/MS) and diode array (LC-DAD) detection enables the tentative identification of decarboxylated and dehydrogenated derivatives of the pigment. The main identified compound in the aqueous solutions was 17-decarboxy-betanin.

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