

Influence of EDTA on stabilization of decarboxylated betalains

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Betalains are known to be very sensitive to several factors, including higher temperature, therefore, the studies on their stability are essential for the food industry.

In order to determine the stability of betanin derivatives during thermal treatment, a set of degradation experiments was performed, including the protective influence of EDTA as a chelating agent. The pigments were dissolved in aqueous solutions of acetonitrile, methanol and ethanol.

The studies on thermal degradation of the pigments confirmed their high lability in all investigated solvents. Under the influence of protective activity of EDTA, the rate of the degradation of the decarboxylated betalains was substantially decreased. The analysis of obtained absorption spectra of the degradation products indicated that under the influence of EDTA, the products retained the basic chromophoric skeleton.

Keywords and phrases: betacyanins, decarboxylation, *Beta vulgaris* L., EDTA.

Introduction

Betalains, a group of N-heterocyclic water-soluble compounds, are a very prospective object of studies due to their non-toxic and colorant properties for the food industry. Moreover, anti-inflammatory effects of these pigments and their potential antioxidant or anticarcinogenic activities were also reported [1]. These numerous health benefits in the human body make them also very attractive for pharmacy as well as cosmetic industries.

Betalains, consisting of betacyanins and betaxanthins, are formed in most plants of the order *Caryophyllales* [1–3]. They are affected by inferior stability due to their sensitivity to natural pigment-specific and external factors, such as temperature, light or oxygen. Nevertheless, protective action of some antioxidants and chelating agents on betalains was also proved [4–5].

The most restrictive factor in the widespread use of natural pigments is usually their thermolability which has the most crucial effect on the pigments integrity during food processing and storage [5–7].

The important factor leading to the betalains degradation and the discoloration of the modified products is also the complexation of metal ions by these

pigments. However, even without complexation process, probably in a presence of metal ions, the pigments are decomposed. A noteworthy example of this phenomenon is effect of Fe^{2+} and Fe^{3+} ions, which in contrary to Cu^{2+} ions do not form complexes with betanin, nevertheless, they significantly increase of the pigment losses [14]. This effect can be reversed by an addition of EDTA (ethylenediaminetetraacetic) (Fig. 1), potent chelating agent, which prevents metal-catalyzed betalain degradation by pigment stabilization and complex formation with the metal ions. Previous studies reported that complexation of metal ions by EDTA decreases

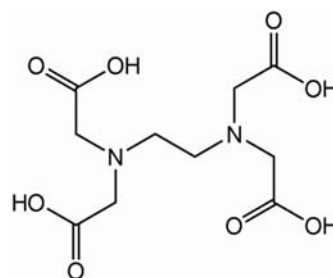


Fig. 1. Chemical structure of EDTA — the potent chelating agent.

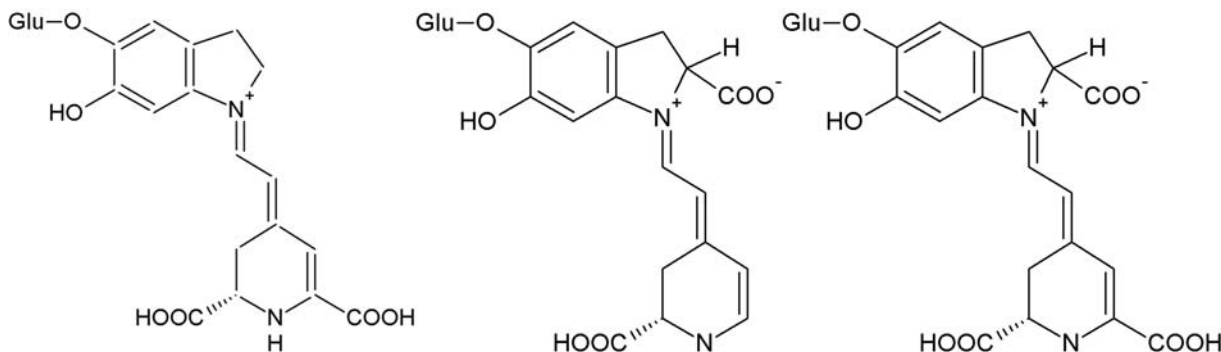


Fig. 2. The chemical structures of tested decarboxylated derivatives of betanin: 2-decarboxy-betanin, 17-decarboxy-betanin and 2,17-bidecarboxy-betanin.

the losses of betanin [7–13]. Moreover, increase of its half-time by ca. 1.5 times was observed [11]. Addition of EDTA to betalain solutions before introduction of metal salts prevents the complexes formation and the pigment decomposition. While the addition of EDTA to betalain solutions after the complexes formation causes their decomposition, it also returns the initial color of the solutions. The later is possible, if the pigment has not been previously decomposed. Additionally, acidification of solutions to pH 4 decomposes the generated complexes in the case of their formation in alkaline media. The acidification of the solutions restores the free pigments. However, in the case of heavy metal salts which cause the pigments degradation in acidic media, the only way of the stabilization is chelating [14].

The studies on the stability of betalains have revealed their degradation pathways. Moreover, new decarboxylated and dehydrogenated structures of betalains were identified. Decarboxylation may occur at C2, C15, and C17 of betacyanins. Previous studies reported numerous betalain degradation products, which retain their attractive color and even they are often more stable than the corresponding betalains [2–5]. Therefore, their identification and determination of their stability is crucial for their applications. Additionally, elaboration strategies for betalain stabilization should increase demand on this pigment class.

In order to determine the stability of following decarboxylated betacyanins: 17-decarboxy-betanin (17-dBt), 2-decarboxy-betanin (2-dBt) and 2,17-bidecarboxy-betanin (2,17-dBt) (Fig. 2) during thermal treatment, a set of degradation experiments was performed, including the influence of EDTA as a chelating agent.

Material and methods

The solutions of decarboxylated derivatives of betanin at a concentration of 1 mg/mL were prepared in selected

solvents: water as well as aqueous solutions of 50% (v/v) acetonitrile, 50% (v/v) methanol and 50% (v/v) ethanol, at pH range 3–8. Acetate, phosphate and citrate buffers were used for the experiments. The concentration of EDTA in the tested solutions was 0.01% (w/v). Heating of 2 mL samples was carried out mostly for 45–60 min at 85°C in a water bath. Every 10–15 min from the start of the experiment, 200 µL samples were collected, frozen and lyophilized in the case of the presence of the solvents. Afterwards, the samples were dissolved in 200 µL of demineralized water for the HPLC-DAD as well as spectrophotometric analysis.

The pigments solutions were analyzed by spectrophotometry in a microplate reader (Infinite M200, TECAN, Austria). The spectrophotometric measurements were performed in a wide range of the visible spectra and at the values near the maxima absorption of the pigments.

For the chromatographic analyses of the starting compound concentrations as well as for controlling of the isolation process of the tested pigments, a Gynkotech HPLC system with UVD340U, Gynkotech HPLC pump Series P580 and thermostat (Gynkotech Separations, H.I. Ambacht, The Netherlands) was applied. 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). In each case, the injection volume was 10 µL, and the flow rate of 0.5 mL/min was applied. Detection was generally performed with a DAD (diode array detection) system at 538, 505, 480 and 310 nm, respectively. The column was thermostated at 35°C.

Results and Discussion

In the Fig. 3–5 the pigments retention (percentage of pigment residue after heating time relative to its initial concentration) is shown obtained for aqueous solutions

of decarboxylated derivatives of betanin with and without EDTA. The retention values depend on pH and the heating time.

The addition of protective chelating agent to the tested solutions increases of the pigments stability. The previous studies revealed the increase of betanin stability under the influence of EDTA, especially in acidic media [14]. Similar conclusions can be stated for the solutions of decarboxylated derivatives.

The optimal pH range for 17-decarboxy-betanin stability in water is 6–7 (Fig. 3). After 60 min of heating, the retention diminishes to 56% at pH 6.5. This compound is the most labile at extreme pH-values,

especially at strongly acidic pH when the retention decreases to 6%. As a result of the EDTA protective activity, the significant increase of 17-dBt retention is noticed. Moreover, the optimal pH range for the stability of the compound is broader (4.5–7) and even at extreme pH-values, such as 3.0 and 8.0, the retention after 60 min is 55% and 64%, respectively. Increase of 17-decarboxy-betanin retention in alkaline media causes strong flattening of the retention curves.

In the case of 2-decarboxy-betanin (Fig. 4), the effect of EDTA is higher at pH 3 where the retention increases from 9% to 63%. At the pH of the greatest compound stability (4.5–6.0), the increase of retention is noticed

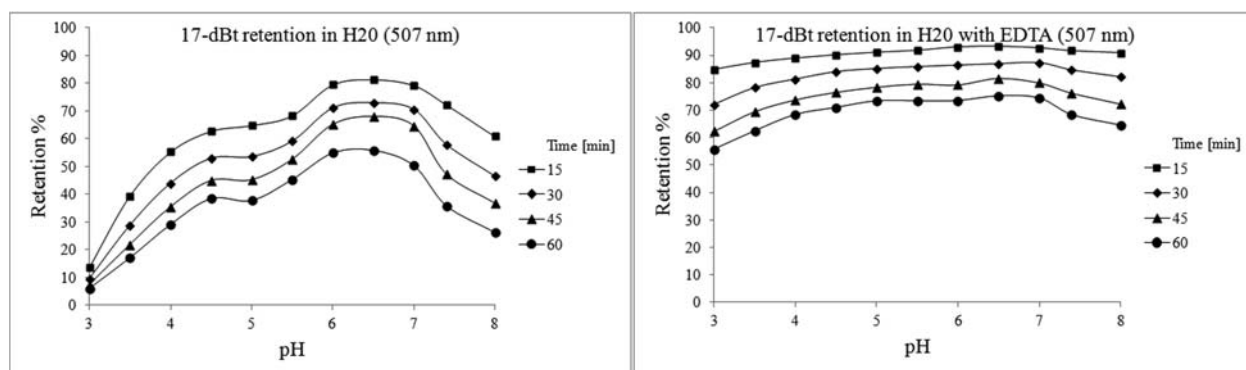


Fig. 3. 17-decarboxy-betanin retention in aqueous solutions, depending on heating time and pH.

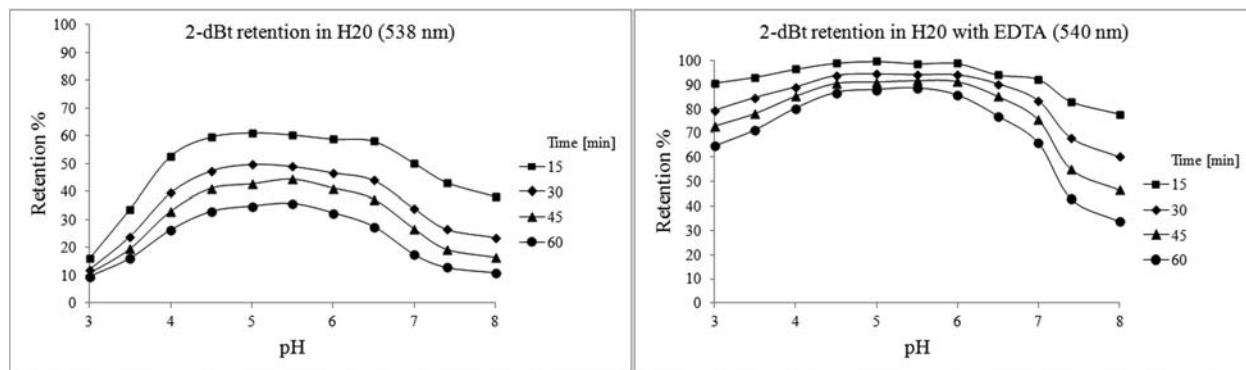


Fig. 4. 2-decarboxy-betanin retention in aqueous solutions, depending on heating time and pH.

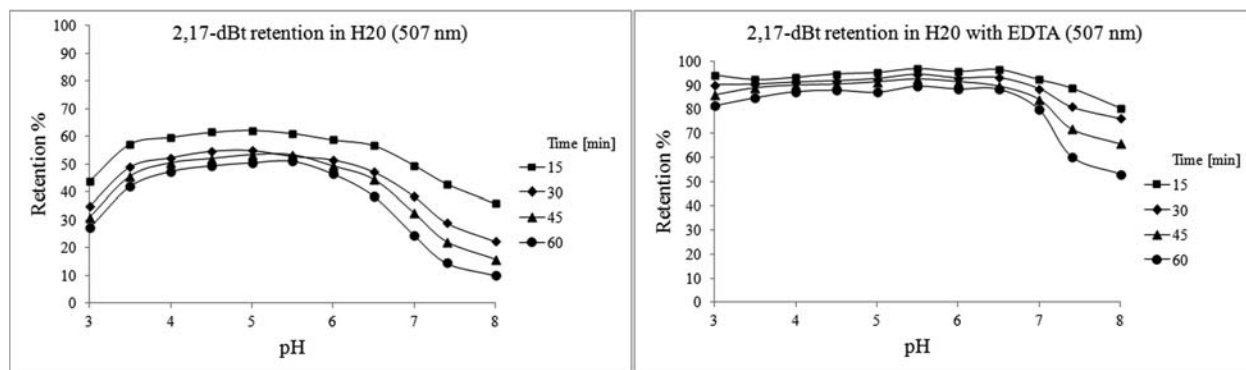


Fig. 5. 2,17-bidecarboxy-betanin retention in aqueous solutions, depending on heating time and pH.

from 30–35% to ca. 85%. At pH 8.0, the lowest rise of retention is observed (from 10% to 33%).

For 2,17-bidecarboxy-betanin (Fig. 5), much more significant increase of retention (to 80–87%) is noticed in a wide acidic pH range (3.0–6.5) under the influence of the chelating agent than at pH 8 (from 10% to 53%)

Under the influence of the protective activity of EDTA, the rate of the degradation of the decarboxylated betalains was substantially decreased. However, this effect is lower in alkaline media.

In the Fig. 6–8, the spectra obtained for the solutions of decarboxylated derivatives of betanin in water with/without EDTA after 45 min of the heating are

demonstrated. The main products of thermodegradation are compounds with the maximum light absorption λ_{\max} ca. 420 nm which appear especially at higher pH-values (6–8). These bands indicate on the generating multiple dehydrogenated and decarboxylated derivatives of betanin. After prolonged heating of solutions, increase of absorbance at the wavelengths between ca. 400–440 nm is observed. Moreover, a decrease of absorbance at ca. 507 nm for 17-dBt and 2,17-dBt, as well as at ca. 540 nm for 2-dBt during the further reaction steps are noticed. The largest bands at 420 nm are observed for 2-dBt, indicating on the highest lability of the compound among the tested derivatives.

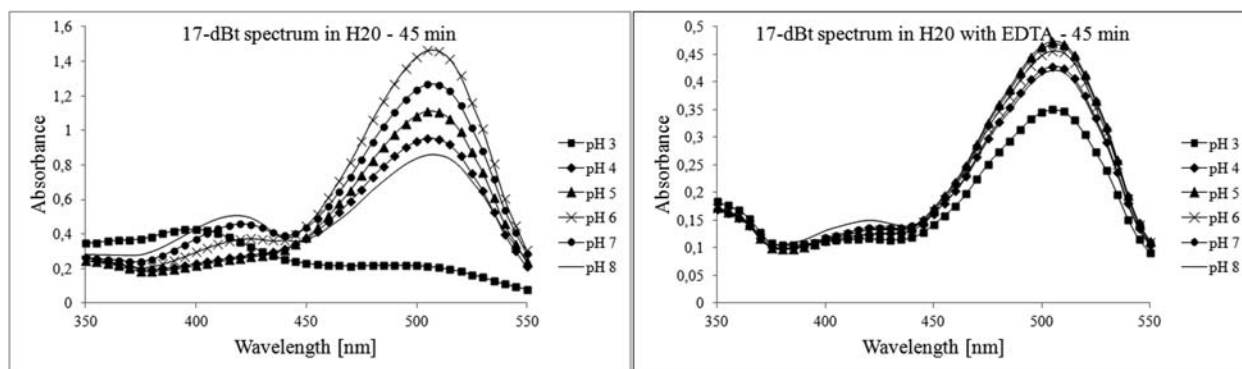


Fig. 6. 17-decarboxy-betanin spectra in aqueous solutions without and with EDTA, depending on heating time and pH.

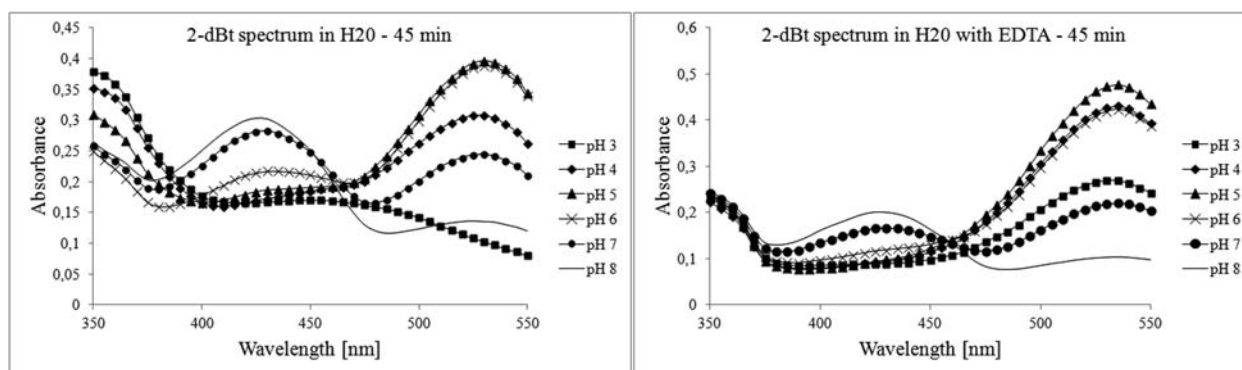


Fig. 7. 2-decarboxy-betanin spectra in aqueous solutions without and with EDTA at 85°C at different pH.

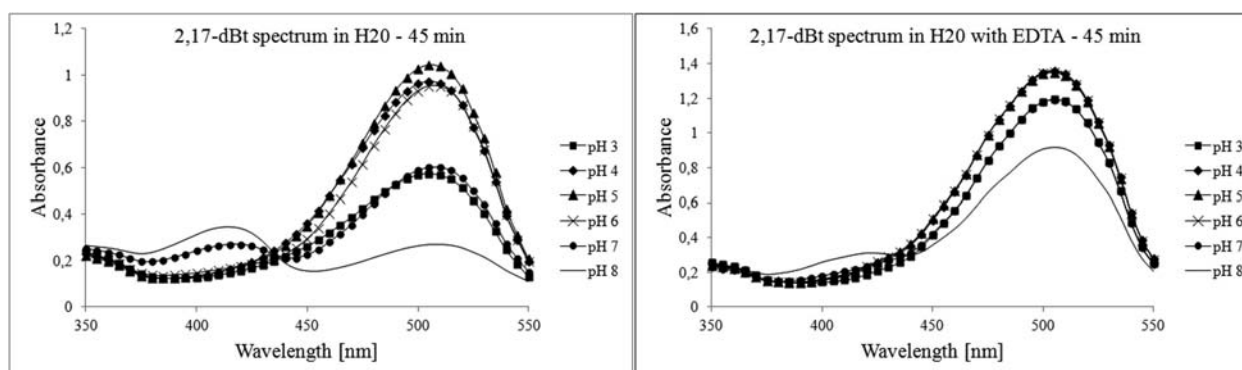


Fig. 8. 2,17-bidecarboxy-betanin spectra in aqueous solutions without and with EDTA at 85°C at different pH.

Significant changes in the visible spectra are observable in the case of the EDTA presence. The formation of much lower absorption bands at the wavelength ca. 400–440 nm (especially at pH 8) than in the case of the solutions which are not protected by a chelating agent is noticed. In aqueous solutions of 17-dBt at pH 3, additional absorption maximum occurs at ca. 400 nm, however, it completely disappears at the presence of EDTA. 2,17-bidecarboxy-betanin is the most stable compound, however, stabilization by EDTA improves its stability and the band at λ_{\max} 420 nm is observable only at pH 8.

Similar conclusions can be drawn for most of the organic solutions. More significant diminishing of the concentrations of tested derivatives was observed. Especially, fast degradation of the pigments occurs in ethanolic media. However, the protective influence of EDTA on stabilization of decarboxylated derivatives was also noticed.

Conclusions

The studies on thermal degradation of the pigments confirmed their high lability in all the investigated solvents. Under the influence of the protective activity of EDTA, the rate of the degradation of the decarboxylated betalains was substantially decreased. The analysis of obtained absorption spectra of the degradation products indicated that under the influence of EDTA, the products presumably retained the basic chromophoric skeleton. The identification of the thermodegradation products will be performed by chromatography with tandem mass spectrometry (LC-MS/MS) and diode array (LC-DAD) detection.

Subsequent investigation should reveal an effect of citric acid as another chelating agent on thermal stability solutions of betanin and its decarboxylated derivatives.

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