The effect of citric acid on stabilization of betanin solutions

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Elevated temperature is known to be the most crucial factor influencing betanin integrity during food processing and storage. Nevertheless, certain chelating agents or antioxidants, such as acetic acid, may act as stabilizers. Therefore, the effect of citric acid on the stability of betanin – basic betacyanin – was investigated during the heating experiments in water as well as 50% and 95% (v/v) aqueous-organic solutions of methanol, ethanol and acetonitrile.

The presence of citric acid in tested solutions affects betanin stability, especially in the 95 % solutions, in which the stabilizing effect of this compound is significantly higher than in aqueous solutions. However, some decrease of retention in 50% methanol was also observed. The main products of betanin thermal degradation in aqueous and aqueous-organic solutions were compounds characterized by absorption bands around the wavelength at 420 nm.

Keywords and phrases: betanin, betacyanins, citric acid, Beta vulgaris L.

Introduction

Betanin belongs to red-violet betacyanins, which together with yellow-orange betaxanthins, represent class of natural plant pigments – betalains. This pigment group provides color to many fruits and flowers of several plant families of the order *Caryophyllales* [1]. The main source of betanin is red beet root (*Beta vulgaris* L.), which is the most commonly encountered variety in North America, Central America, and Europe.

Betanin colorant (E-162) is used in food industry for coloring purposes. Moreover, betalains are reported to

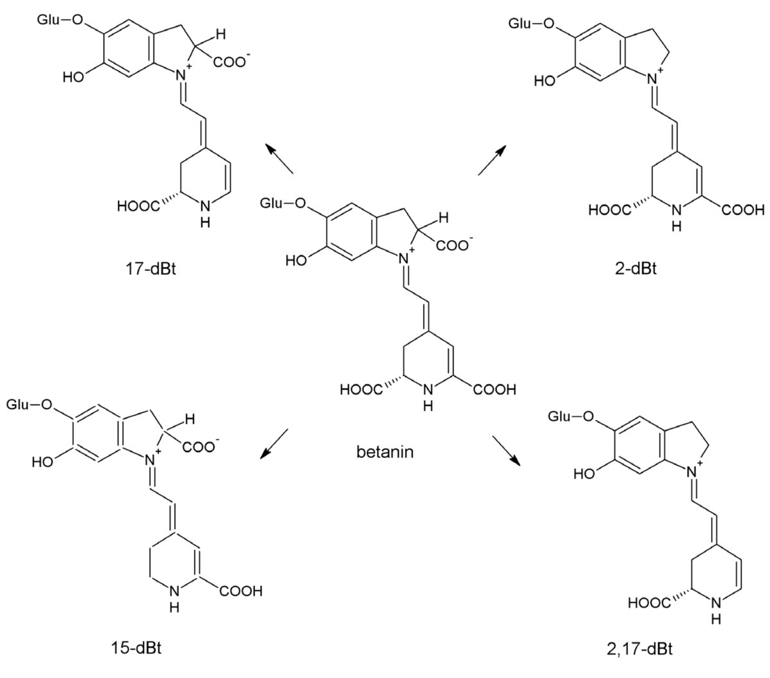

Fig. 1. The most common products of betanin decomposition (17-dBt – 17-decarboxy-betanin, 2-dBt – 2 decarboxy-betanin, 15-dBt – 15-decarboxy-betanin, 2,17-dBt – 2,17-decarboxy-betanin).

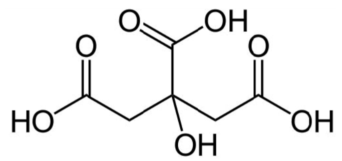

Fig. 2. Chemical structure of citric acid

exhibit antioxidant activity, as well as to have chemopreventive properties and anti-inflammatory effects, which predispose them to broad applications in both pharmaceutical and cosmetics industries. Interesting properties of betalains lead to rediscovery of coloring foodstuffs and replacement of synthetic colorants [2-3].

Temperature has the most significant influence on betanin stability during food manufacture or storage. Their degradation rate depends strongly on both temperature and heating period, and it increases with time [1]. However, simultaneous effect of other parameters, such as degrading factors or the addition of certain stabilizers, has to be taken into consideration. Betalain stability diminishes at pH conditions beyond pH range 3-8 and in the presence of oxygen. Previous studies proved that betalains exhibit high lability at the following temperature range: 50-80 ˚C [1, 4-6]. This has significance in food industry, where for food preservation a thermal treatment of the raw materials is performed.

On the other hand, many additives have positive effect on betalain integrity both in their natural matrix and purified pigment preparations. Supplementation with ascorbic and isoascorbic acids (or other antioxidants) improves betalain stability by oxygen elimination [7]. According to conducted studies, an optimal concentration of ascorbic acid ranges from 0.003% to 0.2-1% [1]. Moreover, citric acid was also found to be suitable for betanin stabilization, although at a lower activity than the above-mentioned antioxidants. Citric acid is a common food additive, which acts as a chelating agent, binding to metal ions. In the food industry, this natural conservant is generally applied as an acidifier or flavoring addition.

Very few studies provide information on application of citric acid to improve betalain stability at elevated temperatures, therefore, a set of thermal degradation experiments of betanin in aqueous as well as aqueous-organic solvents in the presence of this stabilizer was performed.

Material and methods

The solutions of betanin at a concentration of 1 mg/mL were prepared in water as well as 50% and 95% (v/v) aqueous-organic solutions of the following solvents: acetonitrile, methanol and ethanol, at pH range 3-8. For this purpose, acetate and phosphate buffers were used. Betanin solutions with and without citric acid were heated for 60-75 min at 85 ˚C in a water bath in a special deep 96-well plate. The concentration of citric acid in the tested solutions was 0.01% (w/v). Every 15 min from the start of the heating, samples were collected and analyzed by spectrophotomety and HPLC-DAD. In order to remove the solvents, samples were lyophilized dissolved in 200 μL of demineralized water before the analysis.

The spectrophotometric measurements were performed in a microplate reader (Inifnite M200, TECAN, Austria) in a wide range of the visible spectra.

For chromatographic analysis a Gynkotek HPLC system with UVD340U, Gynkotek HPLC pump Series P580 and thermostat (Gynkotek Separations, H.I. Ambacht, The Netherlands) was applied. The analytical column was a Luna C-18(2) 250x3 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

The spectrophotometric measurements enabled the determination of the pigment stability, which is expressed by "retention" – a percentage of pigment residue after heating time relative to its initial concentration. A comparison of pH-dependence of betanin retention obtained for all tested solutions with/without citric acid is shown in Figs. 3-9. The retention values depend on pH and the heating period as well as on the type of applied solvents. In general, a considerable decline of retention with heating time is observed.

The effect of citrate anion on betanin in aqueous (Fig. 3) as well as 50% (v/v) and 95% (v/v) ethanolic solutions (Fig. 4 and Fig. 7, respectively), is negligible. In ethanolic media, the only difference is shifting of optimal pH range of 1 unit towards more acidic pH-value after 60 minutes of thermal degradation. Moreover, the pigment retention values were almost at the same level at pH above 5.0 in the case of 50% ethanolic solutions with citric acid. Nevertheless, a minimal increase of the retention values was noticed in the initial stages of heating (15 minutes after the start of the experiment). The optimal pH of betanin stability in aqueous solutions with/without citric acic at pH 5-6 was noticed.

Surprisingly, the addition of citric acid caused reduction of betanin retention value in 50% (v/v) methanolic and 50% (v/v) acetonitrilic solutions (Fig. 5-6). In both the media, the pigment underwent rapid decomposition. After 75 minutes of reaction, betanin was almost completely decomposed.

In 95 % (v/v) methanolic solutions, betanin exhibits high lability during the whole heating experiments. A similar conclusion can be drawn for betanin solutions with ad-

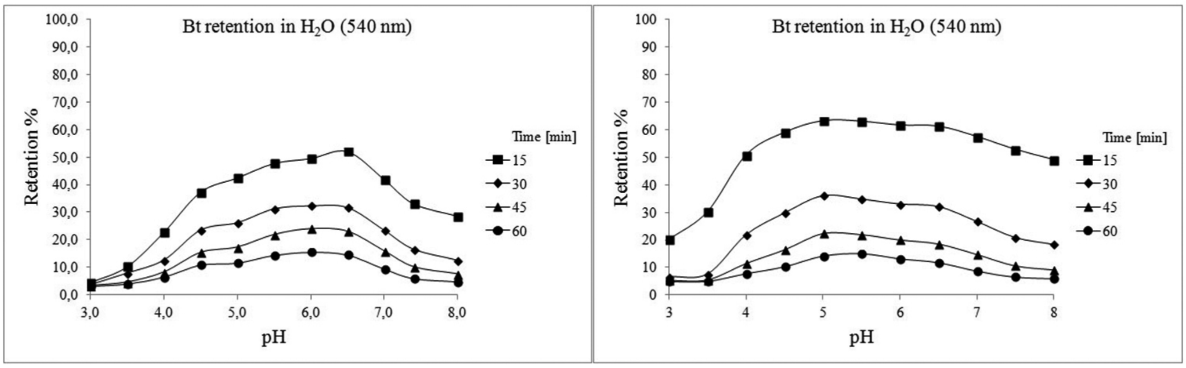

Fig. 3. The comparison of betanin retention in aqueous solutions without and with citric acid, respectively, depending on heating time and pH

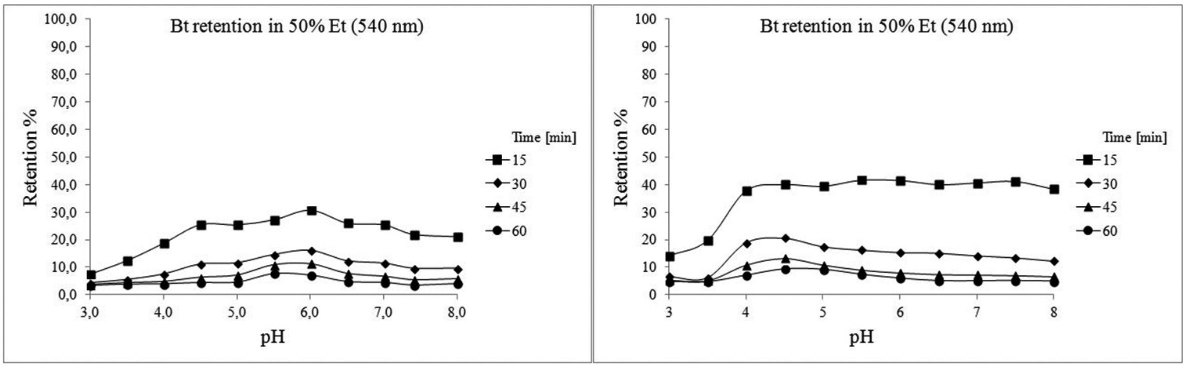

Fig. 4. The comparison of betanin retention in 50% ethanolic solutions without and with citric acid, respectively, depending on heating time and pH

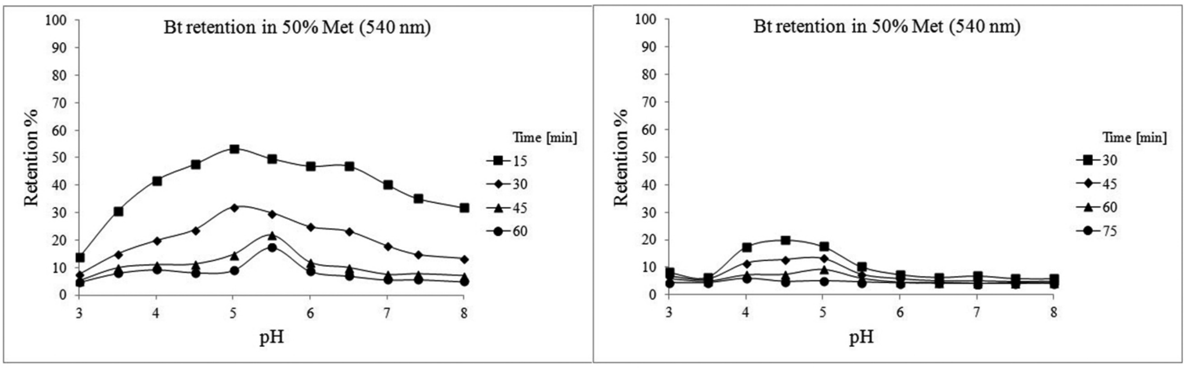

Fig. 5. The comparison of betanin retention in 50% methanolic solutions without and with citric acid, respectively, depending on heating time and pH

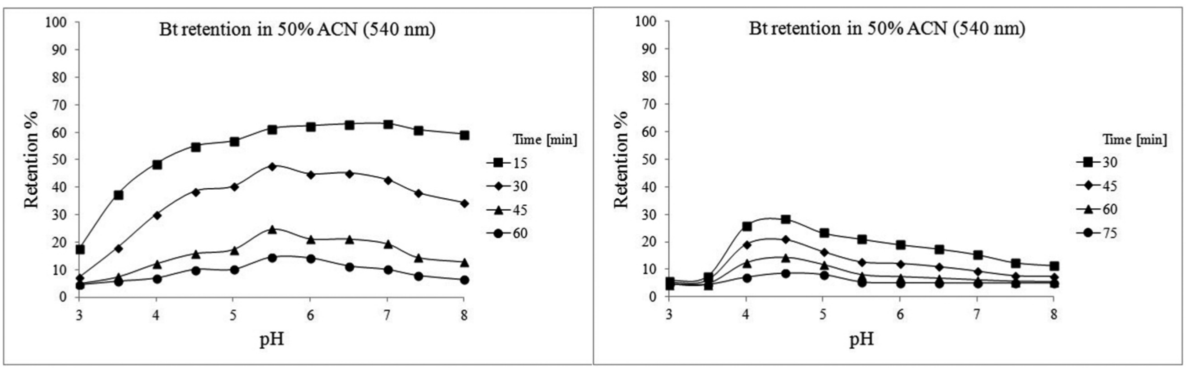

Fig. 6. The comparison of betanin retention in 50% acetonitrilic solutions without and with citric acid, respectively, depending on heating time and pH

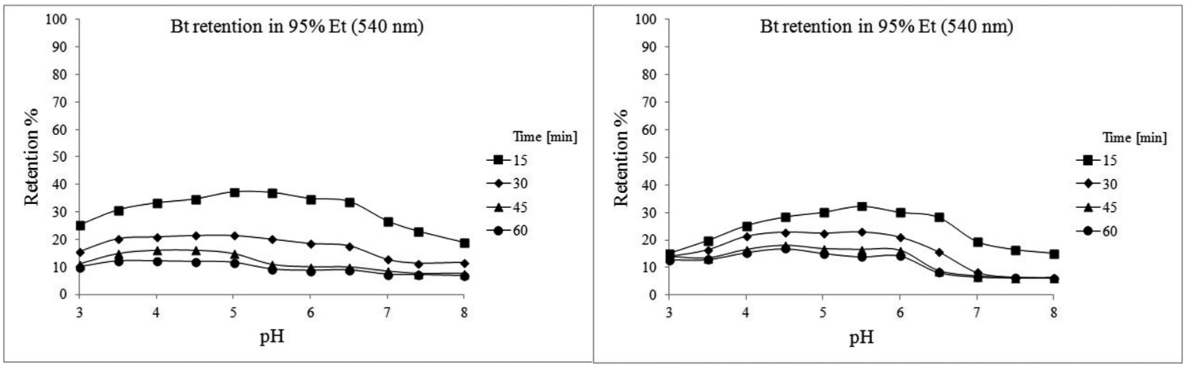

Fig. 7. The comparison of betanin retention in 95% ethanolic solutions without and with citric acid, respectively, depending on heating time and pH

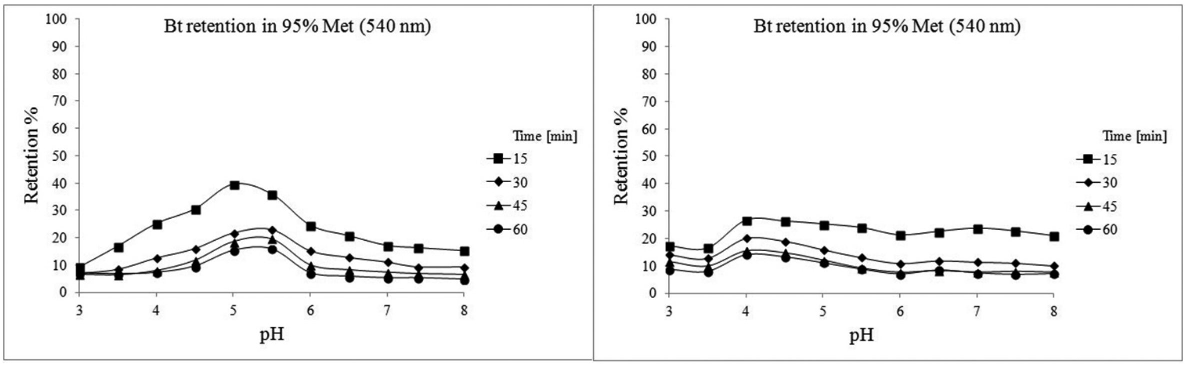

Fig. 8. The comparison of betanin retention in 95% methanolic solutions without and with citric acid, respectively, depending on heating time and pH

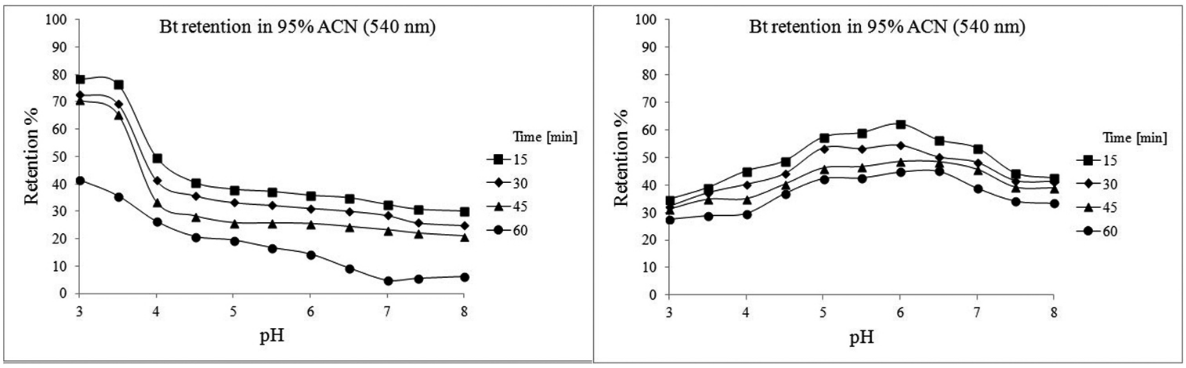

Fig. 9. The comparison of betanin retention in 95% acetonitrilic solutions without and with citric acid, respectively, depending on heating time and pH

dition of citric acid. However, after 60 minutes of heating, the pH of the greatest stability changed from 5.5 to 4.0 (Fig. 8).

In 95 % (v/v) acetonitrilic solutions, retention values range between ca. 25% and 38% (Fig. 9). During thermal degradation in experiments without citric acid, the greatest stability of betanin at extreme acidic pH was found. In 95% acetonitrile, the optimal pH range is shifted to higher values and varies between 5.0 and 6.5. In this media the most considerable protective influence of citrate anion on betanin stability is observed. The retention increased from 5% to 45% at pH 6. In general, pigment stability was improved above pH 4.

Compounds possessing absorption maxima at λ_{max} 420 nm appeared in higher quantities after prolonged heating in aqueous, as well as organic solutions (Fig. 10-11). The main groups of compounds possessing absorption maxima at λ_{max} ca. 420 nm are several derivatives with different decarboxylation and dehydrogenation levels, which will be identified by high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS).

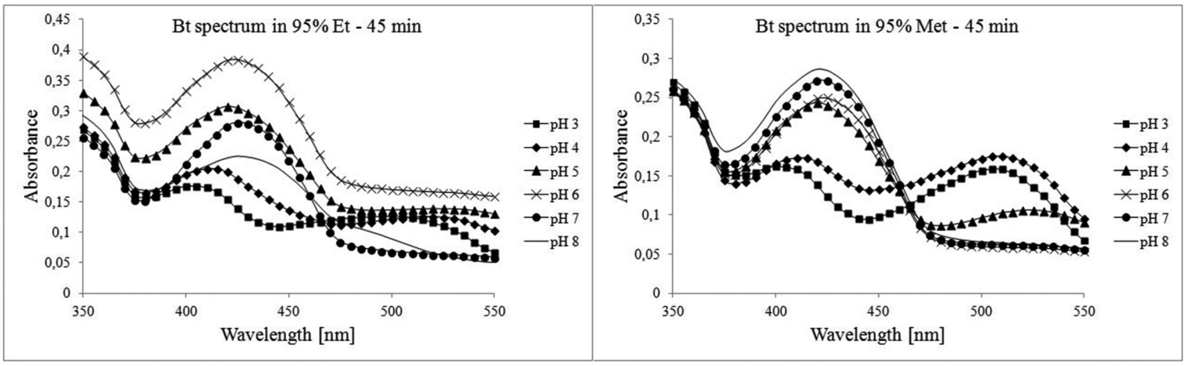

Fig. 10. Betanin spectra in 95% ethanolic and 95% methanolic solutions with citric acid, depending on heating time and pH

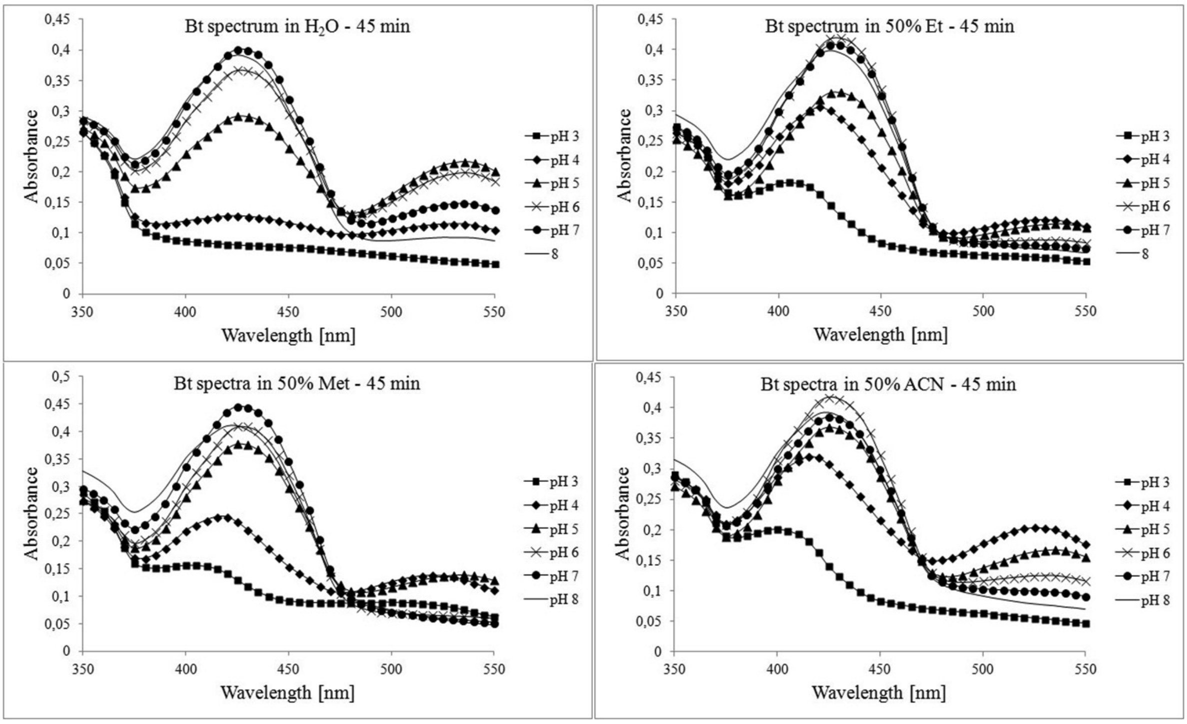

Fig. 11. Betanin spectra in different solutions with citric acid, depending on heating time and pH

Generally, absorption band of betanin at λ_{max} 538 nm is diminishing with increasing heating time in all media, whereas compounds absorbing at λ_{max} 420 appear in higher abundance. Additionally, further hypsochromic shift in more acidic media is observed.

In summary, the effect of citric acid addition depends on the concentration of the organic solvent. This stabilizing effect is the greatest in the 95% solvents, whereas the least impact was noticed in aqueous solutions.

Conclusions

The conducted studies confirmed betanin low stability in all the investigated solutions. Under the influence of the protective activity of citric acid, a slight increase of betanin retention is observed. The presence of citric acid in tested solutions affects betanin stability. Stabilizing action of citric acid is significantly higher in 95 % aqueous-organic solutions than in aqueous solutions. Surprisingly, some decrease of retention in 50% methanol was also observed. The main products of betanin thermal degradation in aqueous and aqueous-organic were characterized by absorption bands around the wavelength at 420 nm.

The results of these studies give knowledge about strategies for betanin stabilization. Citric acid is shown to enhance stability of betanin upon thermal treatment only in several cases. This stabilizing effect is the greatest in the 95% solvents. Addition of this stabilizing compound is less effective than EDTA or ascorbic acid, respectively.

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