Protective activity of EDTA against betacyanins thermal degradation in *Hylocereus polyrhizus* juices

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**Keywords:** betacyanins; *Hylocereus polyrhizus*; natural pigments

**Abstract:** At elevated temperatures betalains forfeit their integrity. However, a presence of specific food stabilizers and natural matrix compounds exerts a positive effect on the maintenance of betalains. The positive effect of adding EDTA and matrix constituents on the stability of betalains was investigated during the heating experiments in aqueous solutions of *Hylocereus polyrhizus* juices. Betacyanin stability was determined by estimating its retention value – the percentage of the pigment residue after the experiment relative to its initial concentration before heating. A protective effect of the fruit flesh matrix is more significant at alkaline pH in pitaya samples without EDTA addition, as compared to the same samples with this potent chelating agent added. In the other case, the pigments are the most stable at a more acidic pH (pH 3 – 5).

1. Introduction

Nowadays, the application of natural food products instead of synthetic compounds has gained on importance. Consumers consciously choose natural products due to their higher health benefits, rejecting foods with artificial ingredients. Therefore, in the food industry, where product color is a very important quality determinant, strategies of generating new plant colorants are developed. In this light, demand for betalainic products is rediscovered (Herbach et al., 2004).

Betalains are nitrogenous water-soluble pigments, consisting of two major groups: violet betacyanins and yellow betaxanthins. Betacyanins are condensation products of betalamic acid with *cyclo*-Dopa (*cyclo*-3-(3,4-dihydroxyphenylalanine). The common precursor aglycon of all betacyanins is betanidin that can be glucosylated with one or two monosaccharides. Additionally, acylation of 5-*O*- or 6-*O*-glucosides, refers to 29 possibilities of betacyanin structures known to date. Moreover, their number is doubled due to stereoisomerism at C_{15}. Only neobetanin (14,15-dehydrobetanin) is

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devoid of the chiral center at C\textsubscript{15}. Nevertheless, most studies are focused on betanin (betanidin 5-\textit{O}-\textit{β}-glucoside) and its C\textsubscript{15} epimer sobetanin. Betaxanthins belong to the second very large group of this pigment class. They are condensation products of betalamic acid with amino acids or amines. The most common betaxanthins in nature are vulgaxanthin I (glutamine-betaxanthin) and indicaxanthin (proline-betaxanthin).

Most plants containing betalains belong to the order 	extit{Caryophyllales}. The occurrence of betalains was also proved in some higher fungi of genera within the 	extit{Basidiomycetes} such as: Amanita, Hygrocybe, and Hygrophorus. In the food industry, pigments from red beet root are commercially exploited (Strack et al., 2003). However, the discovery of cactus fruits and 	extit{Amaranthaceae} plants as a great pigment source renewed demand for betalains.

The red beet (\textit{Beta vulgaris} L.) is a popular source of betalains, which contain mainly betanin, and smaller quantities of vulgaxanthin I and vulgaxanthin II. The first one is intensively used in food industry for coloring purposes. Due to lower stability of betalains in comparison to artificial dyes, their use is limited to specific food products, such as low-acidic foods (dairy and meat products), fresh foods or foods that undergo no heat treatment (Stintzing and Carle, 2007). Fruits of cactus, such as purple pitaya (\textit{Hylocereus polyrhizus}) were found to be a new very promising betalain source. They may exhibit a white, red, or red-purple fruit pulp. Compared to the red beet, pitayas are devoid of high nitrate level or the earthy smell. In contrast to cactus pears (\textit{Opuntia} sp.), \textit{Hylocereus} fruits have higher betalain content, hence the smaller portions of coloring foodstuff are required to obtain the appropriate tinctorial strength. Besides betalain and its isomer, pitaya contain acylated structures such as phyllocactin (betanidin 5-\textit{O}-(6'-\textit{O}-malonyl)-\textit{β}-glucoside) and hylocerenin (betanidin 5-\textit{O}-(6'-\textit{O}-3"-hydroxy-3"-methylglutaryl)-\textit{β}-glucoside), together with their respective C-15 epimers. The acylated structures may be more stable, especially in the presence of matrix compounds (Herbach et al., 2005, 2007).

Fig. 1. Chemical structures of betanin, phyllocactin and hylocerenin – the main betacyanis in purple pitaya.

Betalains are sensitive to several specific factors for natural pigments. In this paper, we focused on the inferior impact of elevated temperatures and the protective
effect of matrix constituents, as well as the additive of potent chelating agent (EDTA, ethylenediaminetetraacetic acid) (Herbach et al., 2004).

Fig. 2. Chemical structure of EDTA – a potent chelating agent.

Betalains are most stable at broad pH range of 3 – 8, whereas more common anthocyanins do not exhibit unchanged tincture and do not retain their coloring power in these conditions. However, at elevated temperatures, betalains forfeit their integrity. In previous studies, significant decline of their stability, especially above 85°C was noticed (Czapski, 1985). However, the presence of specific food stabilizers and natural matrix compounds exerts positive effect on maintenance of betalains. Previous studies showed that matrix compounds such as pectin, guar gum, and locust bean gum enhance storage stability of red beet solutions, probably by lowering the $a_w$ (water activity) value (Herbach et al., 2006). Moreover, some studies proved that EDTA increases half-life time of betanin 1.5 times, probably by neutralizing the electrophilic centre (Herbach et al., 2006)).

Therefore, in this study, the positive effect of adding EDTA and matrix constituents on the stability of betalains was investigated during heating experiments in aqueous solutions of *Hylocereus polyrhizus* juices.

2. Materials and methods

**Plant material.** Freeze-dried *Hylocereus polyrhizus* fruit flesh powder was obtained from Ben-Gurion University of the Negev in Beer-Sheva (Israel). The purple pitaya juices were obtained by extraction of the plant material with demineralized water. The aqueous solutions were centrifuged and filtered to remove seeds and mucilage material. The solutions of *Hylocereus polyrhizus* juices were prepared at pH range 3 – 8. For this purpose, 25 mM acetate (3 – 5.5) and phosphate (6 – 8) buffers were used.

**Heating experiment.** The tested solutions for heating experiment were prepared on a special deep 96-well plate. The samples were heated to 85 °C in a water bath, for about 60 min until reaction products were formed. After every 10 – 20 min of thermal treatment, the samples were rapidly cooled and taken for spectrophotometric and HPLC-DAD (diode-array detection) analyzes. Analogous studies were performed with addition of EDTA, whose concentration in the tested solutions was 0.01 % (w/v).

**Spectrophotometric and HPLC analysis.** The spectrophotometric measurements were carried out in a microplate reader (Infinite M200, TECAN, Austria).
Tested samples were introduced into 300 µL wells of 96-well plates and the spectrophotometric measurements were performed at 25 °C in a wide range of the visible spectrum (350 – 550 nm).

All samples were analyzed with 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) 250 x 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.

3. Results and discussion

The main aim of the study was to determine betacyanins thermal stability in their natural matrices of *Hylocereus polyrhizus* juices. Additionally, at the same conditions, protective activity of potent chelating agent, EDTA, against betacyanin decomposition was investigated. The monitoring of the thermal degradation reaction progress was carried out in the microplate reader by spectrophotometric measurements.

Fig. 3 shows the initial spectra of betacyanins from *Hylocereus polyrhizus* before the start of thermal processing. In this source of pigments, betacyanins, such as betanin, phyllocactin and hylocerenin, exhibit absorption maxima at $\lambda_{\text{max}}$ ca. 540 nm.

![Fig. 3. The comparison of purple pitaya juices spectra with/without EDTA in aqueous solutions, depending on the pH.](image)

The spectra in Fig. 4 – 7 show the absorption bands of parent pigments and their reaction products obtained by thermal treatment. During the heating of the solutions of purple pitaya juices, formation of new derivatives of betacyanins is observed. The main degradation products, generated after prolonged heating of natural juices, are compounds possessing absorption maxima at $\lambda_{\text{max}}$ ca. 450 nm. These products were formed as the first ones after 20 min of the experiment in the samples containing no EDTA. A protective activity of this chelating agent has delayed the formation of betacyanin derivatives. The first significant changes in the composition of solution...
with EDTA occurred after 40 min.

Fig. 4. The comparison of purple pitaya juices spectra with/without EDTA in aqueous solutions after 10 min of heating, depending on the pH.

Fig. 5. The comparison of purple pitaya juices spectra with/without EDTA in aqueous solutions after 20 min of heating, depending on the pH.

Fig. 6. The comparison of purple pitaya juices spectra with/without EDTA in aqueous solutions after 40 min of heating, depending on the pH.

Compounds with absorption maxima at $\lambda_{\text{max}}$ 450 nm appeared at higher abundances after 60 min of heating, especially in solutions without EDTA. The largest band is observed at pH3. In general, absorption bands at $\lambda_{\text{max}}$ ca. 540 nm diminish with increasing heating time in all media. However, this decrease is the
most considerable at the extremely acidic pH in the solution without EDTA, and at pH 8 in the solution with the added chelating agent. Additionally, another hypsochromic shift of \( \lambda_{\text{max}} \) to 420 nm, typical for betalain degradation products, is not visible.

Fig. 7. Comparison of purple pitaya juices spectra with/without EDTA in aqueous solutions after 60 min of heating, depending on the pH.

The most common products of the reactions are decarboxylated and/or dehydrogenated derivatives of betanin, phyllocactin and hylocerenin, detected by LC-MS. Moreover, further multiple decarboxylation and/or dehydrogenation of compounds lead to a loss in red-violet color hue. The matrix of unpurified juice contains a complex mixture of betalains together with matrix constituents, thus determination of degradation paths for individual pigments is difficult. Hydrolysis of betanin results in a formation of betalamic acid and cyclo-dopa 5-\(O-\beta\)-glucoside. However, this process may be partly reversible after cooling.

In pitaya samples, the most considerable protective influence of matrix is observed at pH 8. In the spectra after 60 min of heating at pH 7 – 8, the main visible absorbance bands of betacyanins are the highest, indicating their greatest stability in these media. Additional large bands arising at 450 nm are observed at pH 3. However, adding EDTA shifts the pH range of the greatest stability of pigments towards the most acidic pH of the tested solutions.

Betacyanin stability was determined by estimation of a retention value – the percentage of pigment residue after the heating experiment relative to its initial concentration before the heating. A comparison of pH-dependence of retention obtained for all tested solutions is shown in Fig. 8. The retention values depend on the pH and the heating time. Generally, a considerable decline of retention with increasing heating time is noticed. The comparison of the matrix effect on pigment stability in aqueous solutions of purple pitaya with/without EDTA is presented in Fig. 8.

After 10 min of heating, the changes are not so spectacular. Except pH 3.0, retention values are almost at the same level in both cases. However, after prolonged heating, differences in retention values are more pronounced. After 20 min, a significant increase of retention is noticed, especially at more acidic pH. The retention value increases from about 45 % to 87 % at pH 3.0, after 20 min of heating. After this time, the improvement of pigment stability is less considerable with the increase of pH of the solutions. At pH 8.0, a slight retention increase is noticed from values of 70 % to 75 %. In the medium pH range, which is optimal for stability of beta-
cyanins, retention enhances by about 20%. A similar tendency after 40 and 60 min is observed.

Lowering the pH value in the solutions with added EDTA leads to an improvement of pigment stability after prolonged heating. In the presence of EDTA, the pigments are the most stable at pH 3 at the last stage of the experiment and the retention values remain slightly above 50% of the values obtained for purple pitaya solutions. In general, in all samples of purple pitaya with EDTA, retention is almost at the same level after 60 min (about 50%).

In general, EDTA exerts a very positive effect on the stability of betalains in their natural matrices of *H. polyrhizus* juices. However, the most significant activity is noticed at pH 3. Additionally, matrix constituents also exhibit protective action for stability of betalains. Previous studies on purified compounds, such as betanin, have shown their low stability in these conditions. After 60 min of heating at 85°C betanin was almost completely decomposed.

Interestingly, a protective effect of matrix is more significant at alkaline pH in pitaya samples without EDTA, as compared to the same samples with this chelating agent added. In the second case, the pigments are the most stable at more acidic pH (pH 3–5). The pigments from purple pitaya without EDTA are more stable in alkaline media. The pigments undergo rapid decomposition after 20 min of heating at pH 3–4. The retention values diminish from 80% to 45% at pH 3.0. During the experiments, the optimal pH of pigment stability in *H. polyrhizus* solutions at pH 8.0 was noticed.

In summary, the effect of matrix depends on the pH of the tested solutions. This stabilizing effect is the greatest at pH 8 in *H. polyrhizus* samples without EDTA, whereas in matrix with EDTA, the greatest stability is at pH 3.0. Under the influence of the protective activity of matrix, a significant increase of betacyanin retention is observed in comparison to the results of previous analogous studies on purified pigments.

4. Conclusion

The present studies confirmed the positive matrix effect in *Hylocereus polyrhizus* juices heated to 85°C. Moreover, EDTA supplementation exhibits a very positive effect on

![Figure 8](image-url)  
**Fig. 8.** Comparison of retention of heated *H. polyrhizus* juice with/without EDTA in aqueous solutions, depending on heating time and the pH.
the stability of betalains in their natural matrices of *H. polyrhizus* juices.

A protective effect of matrix is more significant at alkaline pH in pitaya samples without EDTA, as compared to the same samples with this potent chelating agent added. In the second case, the pigments are the most stable in more acidic pH (pH 3 – 5). The main degradation products generated after prolonged heating of natural juices are compounds possessing absorption maxima at $\lambda_{\text{max}}$ ca. 450 nm, especially at pH3 in solutions without EDTA.

The results of these studies confirm the protective activity of EDTA on the stability of betalains in their natural matrices of *H. polyrhizus* juices and show purple pitaya as a valuable source of betalains for their potential application in the food industry.

References


Herbach, K. M., C. Maier, F. C. Stintzing and R. Carle (2007), *Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice*, European Food Research and Technology.


