The Effect of Ascorbic Acid Supplementation on Betacyanin Stability in Purple Pitaya *(Hylocereus polyrhizus)* Juice

Anna Skopińska, Dominika Szot, Karolina Starzak, Yosef Mizrahi[b], Sławomir Wybraniec

Cracow University of Technology, Faculty of Chemical Engineering and Technology
Department of Analytical Chemistry,
Warszawska 24, 31-155 Cracow, Poland
e-mail: askopin@chemia.pk.edu.pl
[b] Ben Gurion University of the Negev Department of Life Sciences,
The Institutes for Applied Research,
Post Office Box 653, 84105 Beer-Sheva, Israel

Current studies on betalains are focused on searching of new plant sources of these pigments. In this light, purple pitaya (*Hylocereus polyrhizus*) is a valuable batalain-containing fruit. Betalains are known to be sensitive at elevated temperatures conditions. Nevertheless, addition of specific food stabilizers (EDTA, ascorbic acid, citric acid) as well as natural matrix compounds may exert stabilizing effect on maintenance of pigments. Therefore, in presented study, the stability of betalains in *Hylocereus polyrhizus* juices with protective addition of ascorbic acid at 85°C was examined. Spectrophotometric analyses enable monitoring of reaction during heating of solutions. The hypsochromic shift of λmax to 460 nm is observed in more acidic media without ascorbic acid, suggesting the generation of new reaction products. These absorption bands in solutions with ascorbic acid are not detected, indicating on retaining of the basic structure of betalain chromophore. Generally, pigment retention is diminishing with prolonged heating and depends on pH-values of tested solutions. The addition of ascorbic acid significantly affects the betalain stability. After 60 min of heating, more than 60% of the initial pigment is retained at pH 3-4 in samples of purple pitaya with ascorbic acid. In summary, betacyanins in purple pitaya juice are shown to exhibit proper heat stability, when are stabilized with ascorbic acid. However, a protective effect of natural juice matrix and ascorbic acid is more significant at acidic pH.

Keywords: betalains, betacyanins, *Hylocereus polyrhizus*, thermal treatment of natural pigments, ascorbic acid

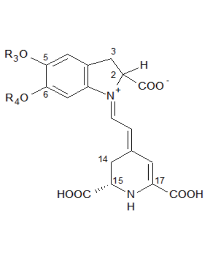
Introduction

Nowadays, new plant-derived colourants are in high demand in the food industry due to their valuable properties. Besides, consumers more consciously choose natural foodstuffs, thus nontoxic and intensely pigmented plant products are increasingly displacing artificial dyes. However, not only natural colour is important quality feature, some health benefits trigger interest in betalains [1]. They possess antioxidative properties due to their ability to inhibit lipid oxidation and peroxidation. Moreover, their chemopreventive and anti-inflammatory effects are reported.

Among betalains, red-violet betacyanins exhibit greater stability than yellow-orange betaxanthins, thus most studies are focused on the former group of pigments. So far, only red beet is especially suitable for colouring purposes. Betanin (betanidin 5-O-β-glucoside) from this source is particularly used in cooled or low acid food, e. g. dairy products, whereas more popular anthocyanin pigments at these conditions are colourless [2]. Nevertheless, red beet possesses significant nitrate level and betalains are sensitive to several factors, thus their application is restricted. Particularly, elevated temperatures influence betalain decomposition, leading to obtaining of new reaction products. Unfortunately, betanin also may decompose into cyclo-dopa 5-O-β-glucoside and betalamic acid, causing a discolouration of solution. However, pigment can be regenerated at low temperatures. Moreover, some popular chelating agent and antioxidant may act as stabilizers and inhibit betalain degaradation [1]. Supplementation with ascorbic acid has been described to exhibit positive effect on betalain stability by oxygen removal. This antioxidant ameliorates regeneration of pigments, and also averts their decomposition upon heating.

New studies are focused on searching of new betalain-containing plants. In this light, fruits of cacti, mainly from Hylocereus family are in the circles of scientific interest. In comparison to most cactus fruits, purple pitayas (Hylocereus polyrhizus) are devoid of glochids and posses higher betalain contents [3, 4]. Purple pitaya contains betanin and its C15 isomer isobetanin. Other constituents are acylated structures such as phyllocactin (betanidin 5-O-(6´-O-malonyl)-β-glucoside) and hylocerenin (betanidin 5-O-(6´-O-3´´-hydroxy-3´´-methylglutaryl)-β-glucoside) and their respective C15 stereoisomers (Tab. 1). Acylation may improve stability of pigment structure, thus presence of these derivatives is interest for potential application of purple

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

	Substitution pattern		Trivial name
	R3	R4	
	*β*-glucose	H	Betanin
	6′-*O*-(malonyl)-β-glucose	H	Phyllocactin
	6′-*O*-(3′′-hydroxy-3′′-methylglutaryl)-*β*-glucose	H	Hylocerenin

pitaya juice in food industry. Moreover, matrix substances included in the juice stabilize the structure of betalains. Therefore, in this study, the stability of betalains in Hylocereus polyrhizus juices with addition of ascorbic acid at 85˚C was examined.

Material and methods

Plant material

Freeze-dried purple pitaya fruit flesh powder was received from Ben-Gurion University of the Negev in Beer-Sheva (Israel). The plant material was extracted with demineralized water, centrifuged and filtered to preparation of the aqueous solutions of purple pitaya juices. The solutions were prepared in 25 mM acetic and phosphoric buffers at pH 3-5 and 6-8, respectively.

Thermal degradation

The solutions of H. polyrhizus juices at pH range 3-8 were heated at 85˚C in a water bath. In the second part of experiment, the tested solutions were supplemented with 0.01% (w/v) acsorbic acid to delay betacyanin degradation during the thermal treatment. The experiment was carried out for 60 min. After every 10-20 min of thermal processing, the samples were cooled and taken for spectrophotometric and HPLC-DAD (diode-array detection) analyses.

Spectrophotometric and HPLC analysis

All spectrophotometric analyses were performed using a microplate reader (Inifnite M200, TECAN, Austria). The measurements were made at 25 ˚C in a wide range of the visible spectrum (350-550 nm) in order to reaction monitoring. HPLC analysis of purple pitaya betalains was carried out with a Gynkotek HPLC system with UVD340U, Gynkotek HPLC pump Series P580 and thermostat (Gynkotek Separations, H.I. Ambacht, the Netherlands). 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 major goal of presented study was an estimation of betacyanin stability in their natural sources such as matrices of Hylocereus polurhizus juices upon thermal treatment. Additionally, the stabilizing effect of ascorbic acid 0.01% (w/v) on betacyanins at these conditions was examined.

Spectrophotometric analyses enable monitoring of reaction during heating of solutions at 85°C. In Fig. 1, the initial spectra before the start of heating process are shown. Betacyanins from purple pitaya, such as betanin, phyllocactin and hylocerenin, exhibit absorption maxima at λmax ca. 540 nm. Betanin, the basic compound also in red beet root, together with its C-15 diastereomer – isobetanin is the most common betacyanin in the nature. Additional constituents in these fruits are acylated structures such as phyllocactin (betanidin 5-O-(6′-O-malonyl)-β-glucoside) and hylocerenin (betanidin 5-O-(6′-O-3′′-hydroxy-3′′-methylglutaryl)-β-glucoside) which may enhance purple pitaya coloration capabilities. Moreover, some degradation products of acylated pigments, present in purple pitaya were reported to exhibit absorption maxima similar to their precursors and can enhance pigment stability [5].

In general, betacyanin absorption band at αmax 540 nm is diminishing with increasing heating time in all media

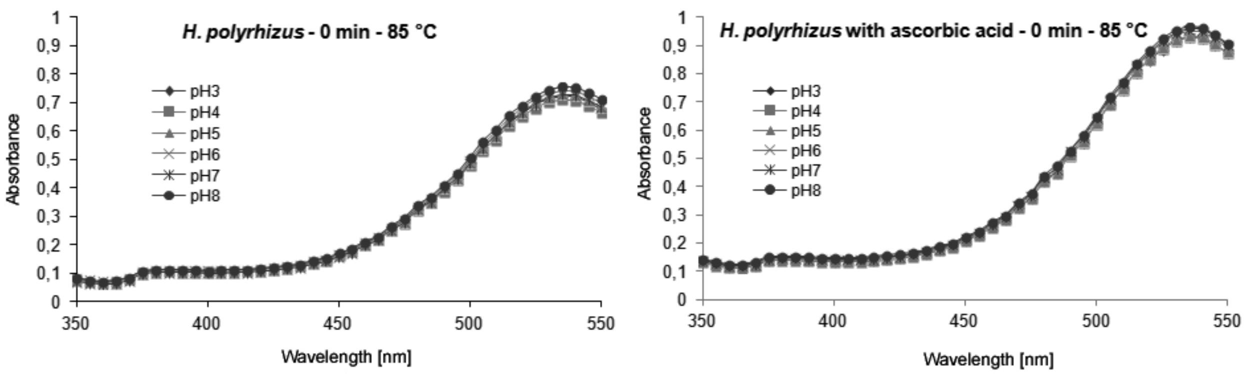


Fig. 1. The comparison of purple pitaya juices spectra with/without ascorbic acid in aqueous solutions before heating, depending on pH

(Figs. 2-5). However, these changes are much faster in solutions containing no ascorbic acid. Additionally, hypsochromic shift of λmax to 460 nm is observed in more acidic media without ascorbic acid. After 60 min of heating in solutions without ascorbic acid, especially at pH 3, compounds possessing absorption maxima at λmax 460 nm appear at higher abundances. It indicates on generation of reaction products, such as decarboxylated and dehydrogenated betacyanins. These absorption bands in solutions with ascorbic acid are not detected, indicating on retaining of the basic structure of betalain chromophore.

Decrease of absorption bands at λmax ca. 540 nm is the most considerable at the extreme pH-values in both cases. After 60 minutes, this change is the most significant at pH 3-4 in the solution without ascorbic acid and at pH 8 in the solution with the added antioxidant. At these conditions, betacyanin was the most converted into degradation products, such as decarboxylated and/or dehydrogenated derivatives of betanin, phyllocactin and hyllocerenin. The highest absorbance band of betacyanins in solutions without addition of the stabilizer is at pH 8. In presence of the antioxidant, trend is reverse. Hence, it may be concluded that the addition of asorbic acid shifts the pH range of the

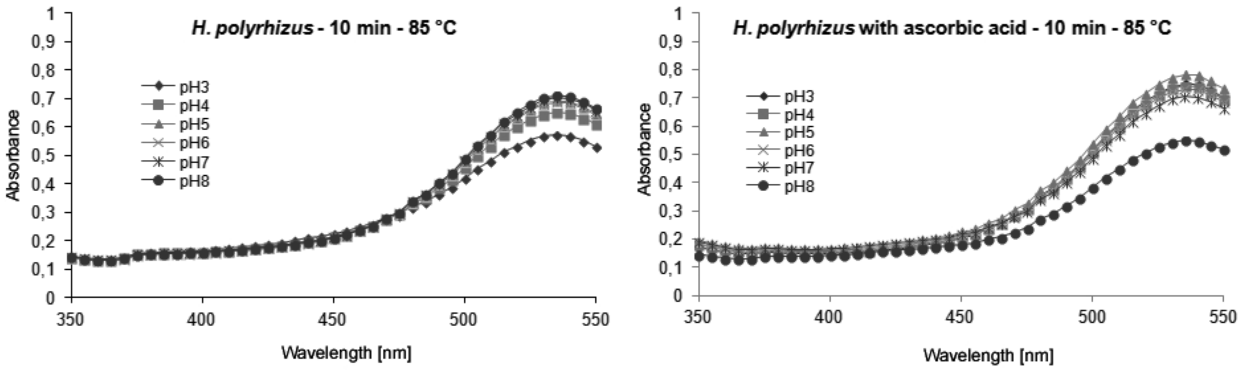


Fig.2. The comparison of purple pitaya juices spectra with/without ascorbic acid in aqueous solutions after 10 min of heating, depending on pH

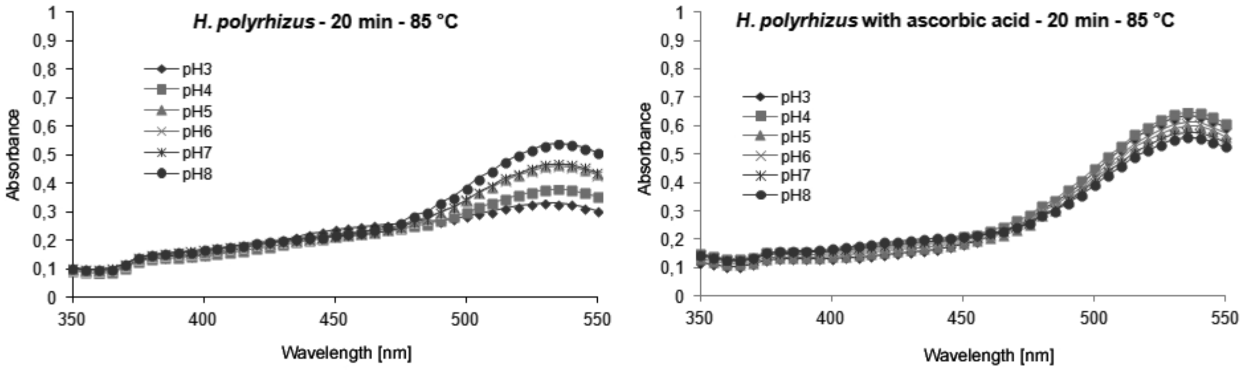


Fig.3. The comparison of purple pitaya juices spectra with/without ascorbic acid in aqueous solutions after 20 min of heating, depending on pH

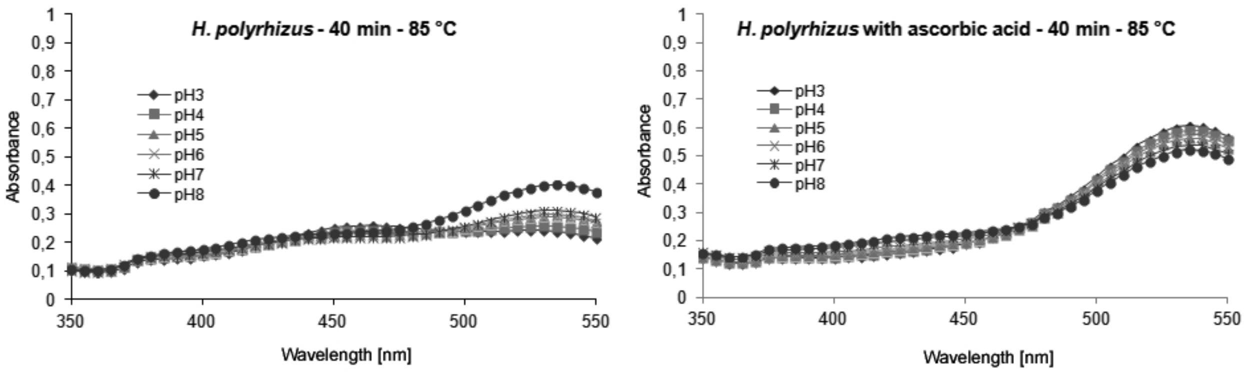


Fig.4. The comparison of purple pitaya juices spectra with/without ascorbic acid in aqueous solutions after 40 min of heating, depending on pH

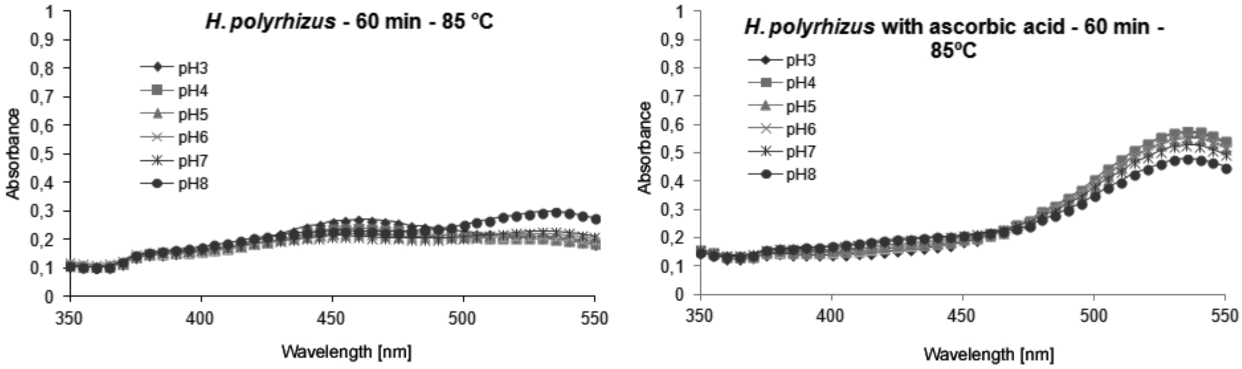


Fig.5. The comparison of purple pitaya juices spectra with/without ascorbic acid in aqueous solutions after 60 min of heating, depending on pH

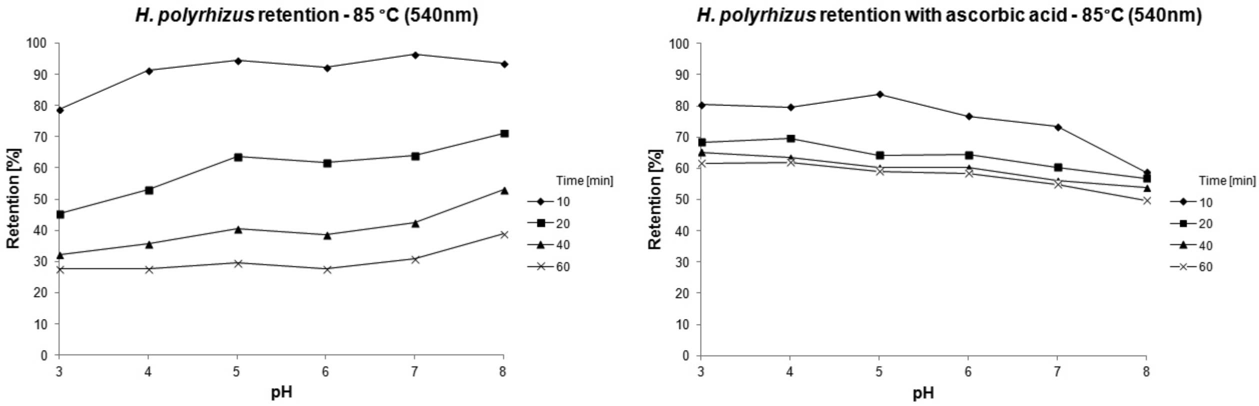


Fig. 6. The comparison of betacyanin retention in aqueous solutions without and with ascorbic acid, respectively, depending on heating time and pH

greatest stability of pigments towards the most acidic pH of the tested solutions.

Betacyanin retention during thermal treatment of purple pitaya fruits was monitored by spectrophotometry. Betacyanin retention after specified heating time was calculated as a percentage of pigment residue relative to its initial concentration. Spectrophotometric data for estimation of retention values was obtained at 540 nm throughout the heating process.

The addition of ascorbic acid significantly affects the betacyanin stability. Similar results of retention in both cases were obtained only after 10 minutes at pH 3. In other steps of process, the results deviate significantly from each other. Generally, pigment retention is decreasing with prolonged heating and depends on pH-values of solutions. After 60 min of heating, more than 60% of the initial pigment is retained at pH 3-4 in samples of purple pitaya with ascorbic acid. The retention value increases from ca. 28% to 61% at pH 3.0. Less spectacular changes at pH 8 are observed with 11% increase of the pigment retention. However, in each tested case, supplementation with 0.01% (w/v) ascorbic acid was reported to delay betacyanin degradation during thermal treatment of purple pitaya juice. It indicates on apparently higher betacyanin stability as compared to the samples without added antioxidant.

In summary, the effect of ascorbic acid addition depends on the pH of the tested solutions. This stabilizing effect is greatest in the acid pH, whereas the least impact is noticed in alkaline solutions.

Conclusions

Thermal stability of purple pitaya betacyanins is superior after the addition of 0.01% (w/v) ascorbic acid. Supplementation with the antioxidant was found to inhibit partly the thermal damage. In summary, betacyanins in purple pitaya juice are shown to exhibit proper heat stability, especially when are stabilized with ascorbic acid. However, a protective effect of natural juices matrix and ascorbic acid is more significant at acidic pH. The results of this study render purple pitaya as a valuable source for a red violet colouring foodstuff.

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