Factors affecting betacyanin stability in juice of LD5 Red-fleshed dragon fruit (*Hylocereus polyrhizus*)

Anh H. T. Nguyen, Mai T. P. Phung, Trang T. T. Nguyen, Thuy T. P. Nguyen, Huan T. Phan, & Ngan N. T. Trinh*

Faculty of Food Science and Technology, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: September 20, 2018
Revised: November 11, 2018
Accepted: December 02, 2018

Keywords

Betacyanin
Binh Thuan dragon fruit
Colourant stability
LD5 variety
Fruit juice

***Corresponding author**

Trinh Ngoc Thao Ngan
Email: tntngan@hcmuaf.edu.vn

ABSTRACT

In Binh Thuan province (Vietnam), the red-fleshed dragon fruit (*Hylocereus polyrhizus*), concretely LD5 variety majorly grows and contains a large amount of betacyanin, a natural colourant that potentially applied to many products in the food industry. In this study, the processing factors possibly influencing the betacyanin stability in the red-fleshed dragon fruit juice were in turn investigated. The heating treatment included 2 factors: temperature (65, 75 and 85^0C) and heating time (10, 20 and 30 minutes); while the pH values ranged between 3.0 to 7.0 and the ascorbic acid addition varied in concentrations (0.1, 0.2, 0.3, 0.4 and 0.5% w/w). The processed fruit juice was stored in different packaging materials (plastic and glass) with and without light exposure for 5 weeks to monitor the retained betacyanin. The results showed that the betacyanin was remained with the highest proportion (0.84 ± 0.02) at 0.3% ascorbic acid addition, pH 4.0 and heat treatment at 65^0C for 10 minutes. In storage without light exposure, both plastic and glass packaging materials kept efficiently betacyanin in fruit juice. However, the glass material represented better efficiency in the betacyanin remaining than the plastic material did.

Cited as: Nguyen, A. H. T, Phung, M. T. P., Nguyen, T. T. T., Nguyen, T. T. P, Phan, H. T., & Trinh, N. N. T. (2018). Factors affecting betacyanin stability in juice of LD5 Red-fleshed dragon fruit (*Hylocereus polyrhizus*). *The Journal of Agriculture and Development* 17(6), 72-76.

1. Introduction

Dragon fruit belongs to the *Cactaceae* family that widely grown in several countries such as Taiwan, Vietnam and Malaysia (Nur' Aliaa et al., 2010). In Vietnam, it is one of the popular fruit-bearing trees with 2 available types: *Hylocereus undatus* (*H. undatus*) with pink skin and white-flesh; and *Hylocereus polyrhizus* (*H. polyrhizus*) with pink skin and red flesh. In recently, the red-fleshed dragon fruit has been extensively planted in many provinces such as Long An, Ninh Thuan, Tien Giang etc due to several benefits in terms of health, sensorial values, economic issues, etc. With more than 26,000 ha of growing dragon fruit, Binh Thuan Province has the largest area allocated to the fruit in the country, according to province's Agriculture and Rural Development Department. The dragon fruit planting area will predictably have increased to 28,000 ha with 750 000 tons of production by 2020 (Tran & Nguyen, 2017). The LD5 dragon fruit, a natural source of antioxidants and attractive colour betacyanin, mostly grows in Binh Thuan due to a high yield, good adaptation to the natural environment and (strong) resistance against diseases.

Betacyanin, made of water-soluble nitrogen-containing pigments (betalains), is a set compound contributing fundamentally to the colour of dragon fruit *H. polyrhizus* (Rebecca et al., 2010). It is unstable and easily degrades or breaks down into the degradation product such as cyclo-dopa 5-O-β-glucoside (colourless) and betalamic acid (bright yellow), leading to the discoloration of the pigments (Herbach et al., 2004). Several factors including heat, oxygen, light, pH and moisture are reported to have significant effects on the betacyanin stability (Woo et al., 2011). In addition, the discoloration rate of red colour from garambullo tree (*Myrtillocactus geometrizans*), from red-fleshed dragon fruits (*H. polyrhizus*) was also diminished by the addition of some antioxidants such as ascorbic acid, citric acid (Reynoso et al., 1997; Wong & Siow, 2015).

The aim of this study was to investigate the effects of heat treatment, pH and ascorbic acid concentration on the betacyanin stability. The effects of storage conditions including light exposure and packaging material were also studied within 5 weeks at ambient temperature. These results are the preliminary findings for the further application into fruit juice process, that potentially contributes to value addition for red-fleshed dragon fruits in Binh Thuan Province.

2. Materials and Methods

2.1. Sample preparation

Red-fleshed dragon fruits were collected from farmer households in Binh Thuan province with the similarity in terms of maturity, weight and without any defects and/or crushes. The fresh fruits were stored at cool temperature (15-20^{0}C) before use. Red-fleshed dragon fruits were washed, peeled before collecting pulp. The pulp then crushed directly to collect the dragon fruit. The juice was centrifuged at 3000 g for 10 minutes (Z206A – Hermle – German) and filtered through a filter paper (Whatman) to remove the solid parts. The filtrate was then obtained to carry out the betacyanin analysis.

2.2. Experiments

Completely randomized design was serially applied to investigate the effects of heating condition, pH, ascorbic acid concentration and storage condition. The research was carried out at Food Engineering Laboratory, Faculty of Food Science and Technology, Nong Lam University Ho Chi Minh City.

Effects of heating conditions: The filtered dragon fruit juices (12 mL) at natural pH in test tube were treated at various heating conditions including 65, 75 and 85^{0}C for 10, 20 and 30 minutes in water bath. The time was counted since the center temperature rose up to the required temperature using the thermometer. The heated juices then cooled down in ice water and subsequently were subjected to betacyanin analysis.

Effects of pH: The filtered juice samples (12 mL) were adjusted to pH 3.0; 4.0; 5.0; 6.0 and 7.0 using 1M HCl and 1M NaOH. All these samples were subjected to heat treatment at 650C for 10 minutes and cooled immediately in iced water. The betacyanin analysis was then carried out Effects of ascorbic acid addition: Different concentrations of ascorbic acid including 0 (control); 0.1; 0.2; 0.3; 0.4 and 0.5% (w/w) were added into the juices. All the juice samples were then adjusted to pH 4.0 by 1M HCl and 1M NaOH; subjected to heat treatment at 65^{0}C for 10 minutes and cooled down in iced water before betacyanin analysis.

Effects of storage conditions: The dragon fruit juices treated with the optimal conditions from previous experiments were kept in 2 types of test tubes: the glass test tubes and the plastic test tubes. The glass ones were considered as imitation of glass packaging material, while the other represented for plastic packaging material. The tubes covered with aluminum-foil were the samples stored in light prevention condition; while the tubes without aluminum-foil were the samples stored in light exposure condition. All the samples were stored for 5 weeks at ambient temperature (30 ± 2^{0}C). The betacyanin analysis was determined after treating with heat and every week to compare the proportion of betacyanin retained during storage.

2.3. Betacyanin analysis and retained betacyanin proportion

The Mcllvaine buffer (pH 6.5) prepared from 0.1M citric acid (30 mL) and 0.2M dibasic sodium phosphate (70 mL) was used to dilute the dragon fruit juice. The juice sample, diluted into 40 times by adding 0.1 mL of juice sample to 3.9 mL of Mcllvaine buffer solution, was analyzed with UV-vis spectrophotometer (Shimadzu 1240, Japan). The wavelength was 540 nm which was tested preliminarily from 537 nm to 600 nm to attain the maximum absorbance. The Mcllvaine buffer solution with 4.0 mL (without sample) was a blank.

The concentration of betacyanin (Bc) is expressed as the following equation:

$$\mathrm{Bc}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) = \frac{\mathrm{A} \times \mathrm{F} \times \ \mathrm{MW} \times 1000}{\varepsilon \times \mathrm{l}}$$

Where:

A: Absorption value at $\lambda = 540$ nm

F: Dilution factor

MW: Molecular weight of betacyanin (550 g/mol)

ε: Molar extinction coefficient of betacyanin (60,000 L/mole × cm)

l: path length of the cuvette (1 cm)

The retained betacyanin proportion was calculated by the ratio between the betacyanin content in the untreated juice and the betacyanin content in the juice treated by factors of experiment, as following:

$$\text{Retained betacyanin proportion} = \frac{\text{Bc}_1}{\text{Bc}_0}$$

Where:

Bc_0: the initial betacyanin content (e.i betacyanin in the untreated/natural juice)

Bc_1: the betacyanin content in the juices treated by the factors of experiments)

2.4. Statistical analysis

All experiments were carried out in triplicate. Calculation, tabulating and graphing of data were carried out using Microsoft Excel 2007 (Microsolft, USA). Statistical analysis was performed by using JMP software version 10.0 (SAS Institute Inc, USA). The difference was considered significant at $P < 0.05$.

3. Results and Discussion

3.1. Effects of heating treatment

The betacyanin content remained in the juice after treating with heat is shown in Figure 1. Generally, heat treatment obviously caused decrease of betacyanin and the temperature has a significant effect on the retained betacyanin in the juice. The higher temperature applied, the less retained content in the juice was recorded. The betacyanin content decreased from initial content of 204.11 ± 2.40 (mg/L) to 175.54 ± 5.39; 140.85 ± 10.79 and 114.31 ± 4.09 (mg/L) as heating at 65^0C, 75^0C and 85^0C for 10 minutes, respectively ($P < 0.05$). The similar trend was also found when the fruit juices were subjected to heat for 20 and 30 minutes. Otherwise, the time fairly affected on the remained betacyanin content, particularly at the small variation in time. For examples, the juice heated at 65^0C contained 175.54 ± 5.39; 158.64 ± 13.03 and 145.62 ± 10.48 (mg/L) corresponding to 10, 20 and 30 minutes, respectively. Interestingly, when the temperature increased up to 85^0C, the retained betacyanin content decreased dramatically as compared to the results at the other temperatures and did not depend on the heating time. According to Herbach et al. (2004), the heating could accelerate the degradation of betanlains (the main structure of betacyanin) to cyclo-dopa 5-O-β-glucoside (colourless) and betalamic acid (bright yellow) by the bond cleavage, leading to the discoloration of the pigments. The results were supported by research of Reshmi et al. (2012): the betacyanin in basella alba fruit was maintained at 0, 10 and 20^0C and decreased when the temperature heated up from 40, 50 and 60^0C Similarly, Wong & Siow (2015) reported the proportion of betacyanin retained decreased when the temperatures increased 65, 75 and 85^0C in the long heating time.

As a result, the heating condition at 65^0C for 10 minutes was the optimal condition to remain efficiently the betacyanin content in the juice.

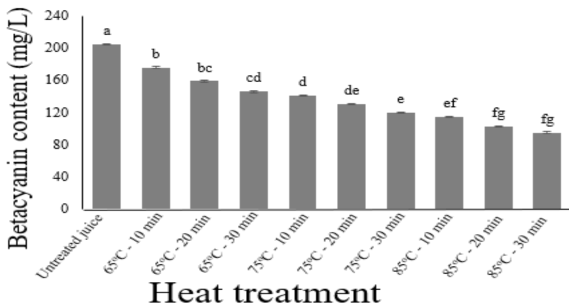

Figure 1. The betacyanin content in the different juice samples before and after heating.

3.2. Effects of pH

Figure 2 obviously represents the significant effects of pH on the betacyanin stability based on the results of betacyanin content in the juice samples and the retained betacyanin proportion. The betacyanin content of 204.03 ± 4.35 (mg/L) in the unheated juice decreased after heating to different amounts depending on the adjusted pH values. According to Reshmi et al. (2012), betacyanin was stable with red colour in neutral and slightly acidic media; on the contrary betacyanin was unstable and change colour from red into yellow in the alkaline medium at pH values upper 7.5. The acidic medium favored the connection between betalamic acid and cyclodopa-5-Oβ-glucoside to form betacyanin (Azeredo, 2009). The proportion of betacyanin retained was found to be the highest at pH 4.0 (with 0.87 ± 0.01 retained proportion of betacyanin) that was in consistent with the study of Herbach et al. (2007). But other researchers reported pH 5.0 was the optimal value to support the betacyanin stability (Tang & Norziah, 2010; Wong & Siow, 2015). The betacyanin content of pH 3.0 sample was the least at 118.98 ± 1.21 (mg/L) with 0.58 ± 0.01 retained proportion. It was explained by Azeredo (2009) that the CO_2 removal forms 17-decarboxybetanin with orange-red colour, or the dehydrogenation produces the neobetanin with yellow colour, leading to the pigment degradation. The pH 4.0 was the optimal for the betacyanin stability in the LD5 red-fleshed dragon juice.

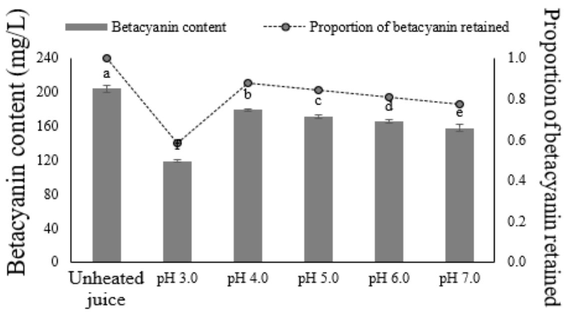

Figure 2. The betacyanin content and proportion of betacyanin retained in the juice samples after adjusting at different pH and heating.

3.3. Effects of ascorbic acid addition

Ascorbic acid has been well-known as an antioxidant due to its ability to combine with oxygen from surrounded environment – the main factor caused degradation of several sensitive compounds such as betacyanin (Attoe & von Elbe, 1982). Figure 3 shows the slightly positive effects of ascorbic acid on the betacyanin content remaining in the LD5 red-fleshed dragon fruit juice after processing. Generally, the betacyanin content in the unheated juice (203.79 ± 3.01 mg/L) reduced after heating process and in almost treatments the betacyanin proportion could be remained up to 75%. Without ascorbic acid and adding 0.1 and 0.2% ascorbic acid, the proportions of betacyanin remained were 0.80 ± 0.01; 0.79 ± 0.02 and 0.82 ± 0.01, respectively without any significant difference ($P > 0.05$). The highest retained betacyanin proportion was 0.84 ± 0.01 attained at 0.3% ascorbic acid addition with 171.18 ± 3.35 (mg/L) betacyanin content. Interestingly, the increment in concentration up to 0.4% ascorbic acid did not further increase the proportion of betacyanin retained (0.82 ± 0.01), while 0.5% ascorbic acid addition did not show any positive effect on the retained betacyanin proportion (0.78 ± 0.02). This result supported to the research of Wong & Siow (2015) that ascorbic acid efficiently remained the betacyanin at the concentrations less than 0.5% (w/w).

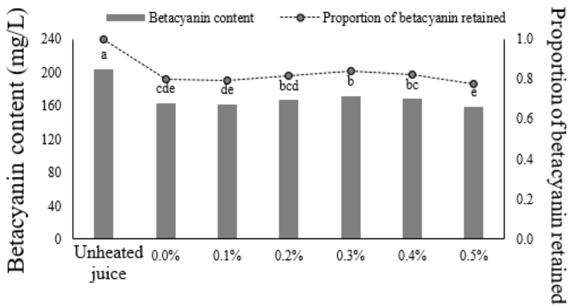

Figure 3. The betacyanin content and proportion of betacyanin retained in the juice samples after adding different ascorbic acid concentrations and heating.

3.4. Effects of storage conditions

Generally, the proportions of betacyanin retained decreased during the storage time for all the treatments with different rate (Figure 4). Obviously, during storage time, the juices in both plastic and glass test tubes in case of light exposure had the low retained betacyanin proportions and without any significant difference in results between these treatments in every week. The betacyanin content kept in the plastic tubes reduced down to almost half in the 1st week storage (0.57 ± 0.02) and continuously to 0.51 ± 0.03, 0.46 ± 0.03, 0.39 ± 0.03 and 0.33 ± 0.03 at the 2nd, 3rd, 4th and 5th week, respectively. Similar trend was found in the juice sample stored in the glass test tube in light exposure. Otherwise, the juice in case of without light exposure showed the better remained betacyanin proportion, especially as juice was kept in the glass packaging material. The juice in plastic tubes remained 0.63 ± 0.02 betacyanin proportion in the 1st week that slightly higher as compared to the formers; however, the proportion then in storage time was in the insignificant difference. The highest betacyanin proportions during storage were observed at the juice in glass test tubes with 0.90 ± 0.01 in the 1st week, 0.76 ± 0.03 in the 2nd week, 0.74 ± 0.04 in the 3rd week, 0.64 ± 0.02 in the 4th week and 0.56 ± 0.02 in the last week. These findings were in agreement with the research conducted by Wong & Siow (2015) who also studied about betacyanin stability in red-fleshed dragon fruit juice. According to Jackman & Smith (1996), the pigment molecules absorb ultraviolet and visible light that excite π electrons into the higher state (π^*), resulted in the decrease of molecular stability.

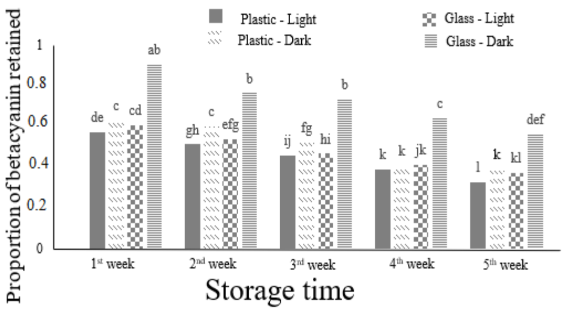

Figure 4. The retained proportion of betacyanin in the juice stored in different storage conditions during storage time.

4. Conclusions

The highest proportion of retained betacyanin was obtained from the dragon fruit juice that subjected to heat treatment at 65^{0}C for 10 minutes, pH 4.0 and 0.3% ascorbic acid addition. In storage, light exposure was an important factor that caused decrease of retained betacyanin in the fruit juice. In addition, the glass material represented better efficiency in the betacyanin remaining than the plastic material did.

Acknowledgement

This study was financially sponsored by Nong Lam University Ho Chi Minh City, Vietnam

References

Attoe, E. L., & Von Elbe, J. H. (1982). Degradation kinetics of betanin in solutions as influenced by oxygen. *Journal of Agricultural and Food Chemistry* 30(4), 708-712.

Azeredo, H. (2009). Betalains: properties, sources, applications, and stability – a review. *International Journal of Food Science & Technology* 44(12), 2365-2376.

Herbach, K. M., Maier, C., Stintzing, F. C., & Carle, R. (2007). Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice. *European Food Research and Technology* 224(5), 649-658.

Jackman, R. I., & Smith, J. L. (1996). Anthocyanin and Betalain. In: Henry, C. F. & Houghton J. D. (Eds.) *Natural food colorants* (244-309). London, UK: Blackie Academic and Professional.

Kapur, A., Hasković, A., Čopra-Janićijević, A., Klepo, L., Topčagić, A., Tahirović, I., & Sofić, E. (2012). Spectrophotometric analysis of total ascorbic acid content in various fruits and vegetables. *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina* 38(4), 39-42.

Nur'Aliaa, A. R., Siti Mazlina, M. K., Taip, F. S., & Liew Abdullah, A. G. (2010). Response surface optimization for clarification of white pitaya juice using a commercial enzyme. *Journal of Food Process Engineering* 33(2), 333-347.

Rebecca, O. P. S., Boyce, A. N., & Chandran, S. (2010). Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*). *African Journal of Biotechnology* 9(10), 1450-1454.

Reshmi, S. K., Aravindhan, K. M., & Suganyadavi, P. (2012). The effect of light, temperature, pH on stability of betacyanin pigments in basella alba fruit. *Asian Journal of Pharmaceutical and Clinical Research* 5(4), 107-110.

Reynoso, R., Garcia, F. A., Morales, D., & Gonzalez de Mejia, E. (1997). Stability of betalain pigments from a cactacea fruit. *Journal of Agricultural and Food Chemistry* 45(8), 2884-2889.

Tang, C. S., & Norziah, M. H. (2010). Stability of betacyanin pigments from red purple pitaya fruit (*Hylocereus polyrhizus*): influence of pH, temperature, metal ions and ascorbic acid. *Indonesian Journal of Chemistry* 7(3), 327-331.

Tran, T. T. H., & Nguyen, N. P. (2017). Economic efficiency of VIETGAP dragon fruit production in Ham Thuan district, Binh Thuan province – Current status and solutions. *Journal of Forestry Science and Technology* 4, 152-161.

Wong, Y. M., & Siow, L. F. (2015). Effects of heat, pH, antioxidant, agitation and light on betacyanin stability using red-fleshed dragon fruit (*Hylocereus polyrhizus*) juice and concentrate as models. *Journal of Food Science and Technology* 52(5), 3086-3092.

Woo, K. K., Ngou, F. H., Ngo, L. S., Soong, W. K., & Tang, P. Y. (2011). Stability of betalain pigment from red dragon fruit (*Hylocereus polyrhizus*). *American Journal of Food Technology* 6(2), 140-148.