Studies on the storage stability of fermented red dragon fruit (*Hylocereus polyrhizus*) drink

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Abstract The objective of this work was to study the effect of storage temperatures and duration on the stability of fermented red dragon fruit drink (FRDFD) on its betacyanins content, physicochemical and microbiological qualities (BPM) and determining sensory acceptability. Results showed that both storage temperatures and duration have a significant effect on betacyanins content and physicochemical properties of FRDFD. Aerobic mesophilic and yeast and mold counts were lower than 1×10^3 CFU/mL for FRDFD stored at both temperatures. The loss of betanin (16.53–13.93 g/L) at 4 °C was 15.73% with no significant changes in physicochemical properties from week two onwards compared to 56.32% (16.53–7.22 g/L) of betanin loss at 25 °C. At week eight, FRDFD stored at 4 °C still contained 13.93 g/L betanin with a pH value of

3.46, suggested its potential as a functional drink which is sensory acceptable (mean score > 80% using hedonic test) among consumers.

Keywords Fermentation · Betanin · Betacyanins · Antioxidant · Bioactive phytochemicals

Introduction

Over past decades, development of foods that promote health and well-being is one of the key research priorities of the food industry. This has led to increased production and consumption of foods containing ingredients that benefit for health and may modulate various physiological functions; something that beyond nutritional needs, which are recognized as functional foods [1, 2]. One of the emerging functional food products introduced in recent years is the fermented red dragon fruit drink [3].

Red dragon fruit or pitaya (*Hylocereus polyrhizus*) is a tropical fruit having high demand in the international market due to its exotic appearance and health properties [4]. Betacyanins has been identified as the main contributing pigment for its deep purple colored. There are at least seven known betacyanins identified from this fruit, namely: betanin, isobetanin, phyllocactin, isophyllocactin, betanidin, isobetanidin and hylocerenin [5]. Betanin is the most widely studied betacyanin which demonstrated various potential health benefits, included free radical scavenging activity of reactive oxygen species, protection of low density lipoprotein against oxidation, prevention of DNA-damage, induction of antioxidant, gene regulatory activity and anti-inflammatory activity [6–8].

Despite having the potential benefits, there are two critical areas need to be addressed which are the short shelf

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life of the fruit and low bioavailability of betanin [9, 10]. Fresh red dragon fruit can only last a maximum of 14 days at 10 °C and 5 days at room temperature, which is relatively short compared to other fruits [3]. Therefore, it is desirable to process fresh red dragon fruit into fermented red dragon fruit drink in order to improve its product shelf life and marketability. Fermentation technology not only could impart desirable flavor attribute, a study showed that antioxidant potential could be enhanced too [11]. This is further supported when Foong et al. [3] reported 1.42% of betanin in fermented red dragon fruit drink compared to 0.23–0.39% of betacyanins in fruit pulps of red dragon fruit stated in studies done by Wybraniec et al. [12] and Stintzing et al. [13].

Regardless of benefits brought upon by fermentation, the stability of betanin faces challenges imposed by various factors, namely: temperature, water activity, oxygen, light, and pH [14]. Therefore, the present work was conducted in order to evaluate the storage stability of fermented red dragon fruit drink in relation to betacyanins content, physicochemical, and microbiological qualities during 8 weeks of storage under refrigeration temperature (4 °C) and ambient temperature (25 °C).

Materials and methods

Reagents

Chemical reagents used in this study were betanin (TCI, Tokyo, Japan), methanol HPLC grade (Merck, Darmstadt, Germany), orthophosphoric acid (R&M Chemical, Essex, UK), Milli-Q system (Millipore, Missouri, USA) deionized water and ultra-pure water was used throughout this study.

Raw materials

Red dragon fruits (RDF) with an average weight of 0.4–0.6 kg were purchased from a local market (Aeon, Kuala Lumpur, Malaysia). The selected fruits were all at commercial maturity level; without damage, insect and foreign matter. White refined cane sugar (Gula Prai) was purchased from local markets. RDF was rinsed with tap water to remove dirt and residues followed by air dried. Then, the skin of RDF was peeled while the flesh was cut into pieces with each having an average thickness of 5 mm [3].

Fermentation

Fermentation of RDF was carried out in 2 L stainless steel fermentation tank according to Foong et al. [3] with slight modifications. Red dragon fruits (0.4–0.6 kg) were cut into

pieces and arranged layer by layer alternately with sugar in a ratio of 5:1. Then, fermentation tank was closed tightly and stored for 8 weeks in a clean cabinet at 25 °C. The fermented drink from the tank was strained, pasteurized at 75 °C for 15 s and stored in individual amber bottles at – 20 °C until further analysis.

Storage studies

Pasteurized fermented red dragon fruit drinks (FRDFD) were stored at refrigeration temperature (4 °C) and ambient temperature (25 °C) for 8 weeks. Samples were analyzed at regular intervals of 2 weeks.

Betacyanins analysis

Betacyanins quantification was done according to Foong et al. [3] with slight modifications. An Agilent 1200 series HPLC system fitted with G1322A degasser, C1325B injector, G1329A column oven and G1315D diode array detector (DAD) was employed. Working solutions of betanin standard (TCI, Tokyo, Japan) which consists of betanin and isobetanin (2, 4, 6, 8 and 10 g/L) was prepared. Each sample was analyzed in triplicate.

Ethanol analysis

The determination of ethanol content was done according to Foong et al. [3] with slight modifications. The alcohol content of sample was determined using Agilent A7890 gas chromatography (Agilent Ins, California, USA), equipped with a computer containing integrator software (ChemStation), a 30 m DB-Wax capillary column (250 µm id, film thickness: 0.25 µm) and a flame ionization detector (FID) (H₂: 30 mL/min and air: 400 mL/min). The flow rate of nitrogen gas was set at 25 mL/min while the temperature at injector port and the detector were set at 250 °C, and split injection (97.5:1 split ratios) was used (0.1 µL for each injection). Oven temperature was controlled with a temperature elevation program during analysis, which was initially set at 85 °C for 7 min, increased to 250 °C at a rate of 100 °C/min, with a total determination time of 9 min.

The sample was first diluted 5 times with ultra-pure water and filtered into each vial using nylon syringe filter of pore size 0.45 µm prior to injection into gas chromatography (GC) through automated injection method. All of the samples were assayed in triplicate.

pH analysis

The pH of samples was measured using pH meter (Mettler Toledo, Greifensee, Switzerland). Measurements were

done in triplicate and average reading was obtained for each sample.

Total soluble solids (TSS)

Hand-held refractometer with detection Brix° range of 0–32 Brix° (Fisher Scientific, New Hampshire, USA) was used to determine the TSS of samples [3].

Water activity

The water activity of samples was measured at 25 °C using an electronic dew-point water activity meter, AquaLab Pre (Decagon Devices, Washington, USA). For each determination, three replicates were obtained and the average readings were reported [15].

Microbiological analysis

Samples were serially diluted with sterile 0.1% peptone water and plated into microbiological media by the spread plate technique in duplicate. Aerobic mesophilic counts were determined using plate count agar (PCA) while yeast and molds counts were determined using potato dextrose agar (PDA) added with 10% tartaric acid. Plates were incubated inverted for aerobic mesophilic bacteria at 37 °C for 48 h while for yeasts and molds at 25 °C for 5 days [3, 16].

Hedonic test

One hundred panelists were randomly invited to take part in the test. Five samples were prepared (original, 1:1 dilution, 1:2 dilution, 1:3 dilution and 1:4 dilution) from FRDFD stored at 4 °C. The samples were presented at 4 ± 1 °C in individual booths illuminated with white light under controlled humidity and temperature (25 °C), serving 15 mL per sample to each panelist. Panelists were instructed to drink water between samples to cleanse the palate. They evaluated the samples using a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely. Appearance, color, taste, aroma and overall acceptance tests of the samples were accomplished [17]. This experiment protocol was given ethical clearance by the Medical Research and Ethics Committee, Ministry of Health Malaysia with approval code NMRR-17-2666-38694.

Statistical analysis

All experiment results were analyzed using Statistical Package for Social Science software (SPSS version 23.0). All numerical data were expressed as mean $\pm$ standard deviations of triplicate measurements of replicate

fermentations. One-way analysis of variance (ANOVA) with Tukey's test was used to determine significant differences ($p < 0.05$) between means.

Results and discussion

Betacyanins analysis

Figure 1 showed the effect of storage at 4 and 25 °C on the stability of betanin content in FRDFD. The betanin content in FRDFD stored at 4 °C was relatively stable in the drink up to 8 weeks storage, showing less than 20% degradation, from 16.53 to 13.93 g/L. As expected, betanin degradation was accelerated at a higher temperature (25 °C). Figure 1 showed that there has been a steep decrease in betanin content throughout the storage period at 25 °C. The degradation of betanin in FRDFD stored at ambient temperature was more than 50%, from 16.53 to 7.22 g/L after 8 weeks storage. Betanin is heat labile and hence degradation was accelerated when FRDFD was stored at ambient temperature as shown in the present study. This was in consistent with results reported by Woo et al. [18] whereby color lost in red dragon fruit extracts stored at 25 °C were relatively higher as compared to extracts stored at 4 °C. There was a strong possibility that the observed degradation in betanin was influenced by water activity, which caused betanin susceptible to aldimine bond cleavage. The water activity of FRDFD in the present study was observed to be higher than 0.90 throughout the storage period (Table 1). To improve betanin stability, reduction of water activity value to below 0.63 was suggested [19]. This was presented in a study done by Siow and Wong [20] where red dragon fruit concentrate with lower water activity (0.832) retained betacyanins better compared to juice with higher water activity (0.983). Pearson correlation coefficient was computed to access the relationship between storage duration and betanin content in FRDFD stored at different temperatures. There was a significant ($p < 0.05$) strong negative correlation between storage duration and betanin content in FRDFD stored at 25 and 4 °C, $r = -0.990$, and $r = -0.965$ respectively. This indicated that 98% (0.990^2) and 93% (0.965^2) of the variation in betanin content of FRDFD at 25 and 4 °C was explained by storage duration, respectively.

On the other hand, an increment of isobetanin was observed in FRDFD stored at both temperatures, as shown in Fig. 1, week 8 at 4 °C and week 4 at 25 °C. However, the percentage of increment was higher at 4 °C (9.26%) than at ambient temperature (2.65%). This was probably due to regeneration mechanism of betacyanins encouraged by acidic condition [21]. This is possible as long as the basic building blocks are present to reform the betacyanin

Fig. 1 Effect of storage at 4 and 25 °C on stability of betanin and isobetanin content in fermented red dragon fruit drink. Values are presented as mean $\pm$ SD, SD is less than 0.1 (n = 3). Values marked with the different lower case letters (a–e) are significantly ($p < 0.05$) different

Table 1 Effect of storage duration at 4 and 25 °C on physicochemical properties of fermented red dragon fruit drink

Storage duration (weeks)	Ethanol (%)		pH		Water activity (a_w)		TSS (Brix°)	
	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
0	1.72 $\pm$ 0.04 ^a	1.72 $\pm$ 0.04 ^c	3.13 $\pm$ 0.01 ^b	3.13 $\pm$ 0.01 ^d	0.949 $\pm$ 0.01 ^a	0.949 $\pm$ 0.01 ^a	28.7 $\pm$ 0.12 ^a	28.7 $\pm$ 0.12 ^a
2	0.93 $\pm$ 0.06 ^b	1.07 $\pm$ 0.06 ^d	3.47 $\pm$ 0.02 ^a	3.43 $\pm$ 0.02 ^a	0.947 $\pm$ 0.01 ^a	0.944 $\pm$ 0.01 ^a	28.6 $\pm$ 0.11 ^a	28.2 $\pm$ 0.06 ^b
4	0.83 $\pm$ 0.03 ^c	1.71 $\pm$ 0.03 ^c	3.42 $\pm$ 0.02 ^a	3.32 $\pm$ 0.02 ^b	0.948 $\pm$ 0.01 ^a	0.947 $\pm$ 0.01 ^a	28.4 $\pm$ 0.15 ^a	26.8 $\pm$ 0.06 ^c
6	0.84 $\pm$ 0.02 ^c	2.48 $\pm$ 0.03 ^b	3.44 $\pm$ 0.03 ^a	3.26 $\pm$ 0.01 ^c	0.948 $\pm$ 0.01 ^a	0.945 $\pm$ 0.01 ^a	28.6 $\pm$ 0.15 ^a	25.3 $\pm$ 0.12 ^d
8	0.81 $\pm$ 0.02 ^c	2.86 $\pm$ 0.03 ^a	3.46 $\pm$ 0.01 ^a	3.23 $\pm$ 0.02 ^c	0.952 $\pm$ 0.01 ^a	0.947 $\pm$ 0.01 ^a	28.7 $\pm$ 0.12 ^a	25.1 $\pm$ 0.12 ^d

Data are expressed as mean $\pm$ SD values (n = 3). Mean value with different superscript in each column differs significantly ($p < 0.05$)

chromophores as stated in a study done by Herbach et al. [22]. Similarly, there was a significant ($p < 0.05$) strong negative correlation between storage duration and isobetanin content in FRDFD stored at 4 and 25 °C, $r = -0.944$, and $r = -0.720$ respectively. This indicated that 89% (0.944^2) and 52% (0.720^2) of the variation in isobetanin content of FRDFD at 25 and 4 °C was explained by storage duration. This indicated that increases in storage duration were correlated with decreases in betacyanin content in FRDFD.

Comparing to the study done by Siow and Wong [20], fermentation technology has successfully produced a suitable and stable environment to preserve the betacyanin content during storage. In the present study, the retention proportion of betacyanin (betanin and isobetanin) in FRDFD stored at 25 °C was 0.50, which was higher than the retention proportion of betacyanin of red dragon fruit juice (0.35) at 25 °C as presented in the paper. This comparison showed that at ambient temperature, betacyanin was more stable in fermented drink compared to fresh

juice. This indicated that fermentation technique used in present study is a good approach as well as an important processing technique that could nutritionally and functionally enhance the quality of fresh produce apart from increasing the shelf life significantly. In addition, quantification of betacyanin content using spectrophotometer might lead to overestimation of betanin content in the sample due to different types of betacyanin that present in red dragon fruit [23]. Therefore, direct comparison of results in the study done by Siow and Wong [20] with present study was not suitable.

Physicochemical analysis

Table 1 showed that there was no significant effect of storage temperatures and duration on water activity of FRDFD. Table 1 showed that there has been a significant decrease in ethanol content from week 0 to week 4, then remained fairly constant until week 8. In contrast, Table 2 revealed that there has been a marked increase in the

Table 2 Effect of storage at 4 and 25 °C on microbiological qualities of fermented red dragon fruit drink

Storage duration (weeks)	Microbiological qualities (CFU/mL)			
	4 °C		25 °C	
	TPC	YM	TPC	YM
0	N.D	N.D	N.D	N.D
2	N.D	N.D	1.4×10^2	2.4×10^2
4	1.7×10^2	3.2×10^2	3.7×10^2	4.8×10^2
6	6.3×10^2	5.2×10^2	7.5×10^2	7.2×10^2
8	7.8×10^2	6.7×10^2	9.2×10^2	8.9×10^2

CFU colonies forming unit, TPC total plate count, YM yeast and mould, N.D not detected

ethanol content of the drink throughout the storage period at 25 °C. Table 1 showed that pH value of the drink at stored at 4 °C experienced a significant increase from week 0 to week 2, then remained constant until the end of storage period. On the other hand, FRDFD stored at 25 °C experienced slight rise and fall in pH throughout the study but generally, there has been a marked increase in week 8. Table 1 showed that the Brix value was fairly stable for drink stored at 4 °C whereas Table 1 showed that there has been a gradual decline in the Brix from 28.7° to 25.1° for drink stored at 25 °C throughout the 8 weeks storage.

The decrease in ethanol content during storage at 4 °C and rise in pH at both storage temperatures implicated that ethanol and acids have been consumed for certain reaction, such as esterification process. It was suggested that esterification of betanin with aliphatic acids, as well as aromatic acids, stabilize them by decreasing the susceptibility of the compound for hydrolytic attachment [19, 24]. Besides, betanin and ethanol could react to form betacyanin ethyl-ester by esterification of the three carboxylic groups in betanin structure [25]. These reactions were likely to explain the decrease in ethanol content and rise in pH of FRDFD. Even so, it was observed that the pH of FRDFD stored at 25 °C was unstable compared to those stored at 4 °C. This inconsistency may be due to the kinetic energy of molecules increases with temperature, which allow them to cross the activation energy barrier easier to react such as esterification reaction when stored at 25 °C [26]. In addition, esterification is a reversible process and thus the pH is likely to fluctuate throughout the storage period at 25 °C.

Nonetheless, the increase in ethanol content at the end of storage at 25 °C was probably due to fermentation initiated by microbes. This was further supported when the Brix of FRDFD stored at 25 °C gradually decline across the storage period. The findings of Brix value across storage period were in accordance to a study done by Abbo et al. [16] on storage stability of soursop juice whereby a decrease in Brix was observed in juice stored at ambient temperature. The stability of Brix of FRDFD stored at 4 °C could be due to inactivity of microbes at low temperature, which

inhibited further fermentation that will result in sugar reduction in the drink during storage [16]. As a final point, overall results showed that FRDFD stored at 4 °C has fairly stable physicochemical properties from week 2 onwards and thus storage at 4 °C is recommended.

Microbiological analysis

Microbiological analysis revealed that the drink after pasteurization and during 8 weeks of storage at 4 and 25 °C showed a total number of aerobic mesophilic bacteria, mold and yeasts lower than 1×10^3 CFU/mL (Table 2). The investigated product was fell under the satisfactory level of microbiological standards for ready-to-eat food, which is less than 10^4 CFU/mL of sample [27]. This was attributed to the bacteriostatic or bacteriocidal activity exhibited by pasteurization treatment, low pH, the presence of ethanol, carbon dioxide and organic acids formed during fermentation [3, 28]. This analysis confirmed that the investigated product is microbiologically safe for consumption as a beverage intended for dilution.

Hedonic test

Table 3 showed the appearance, color, taste, aroma and overall acceptance test score of FRDFD with different dilutions. Dilution had an effect on consumer acceptance with maximum overall acceptance was observed for FRDFD with 1:2 dilution (mean score of 86.33% on the 9-point hedonic scale). Higher dilution ratio resulted in lower consumer acceptance whereby FRDFD with 1:3 and 1:4 dilution presented mean scores of 77.22 and 67.56%, respectively.

Highest and lowest appearance and color score were recorded for the drink with 1:1 and 1:4 dilution respectively. Both appearance and color were depended on the red hue of FRDFD. The low score in appearance and color recorded for 1:4 dilution may be due to the loss in intensity of red hue of FRDFD. In term of taste attribute, 1:2 dilution recorded the highest mean score of 88.11% and was

Table 3 Appearance, colour, taste, aroma and overall acceptance of fermented red dragon fruit drinks stored at 4 °C with different dilution

Samples	Appearance ^a (%)	Colour ^a (%)	Taste ^a (%)	Aroma ^a (%)	Overall ^a (%)
Original	87.11 ± 1.55 ^{bc}	87.44 ± 1.66 ^b	83.67 ± 1.99 ^b	76.89 ± 1.90 ^b	84.67 ± 1.61 ^b
1:1	88.22 ± 1.31 ^b	89.22 ± 1.31 ^b	87.11 ± 1.40 ^b	76.67 ± 1.51 ^b	85.56 ± 1.28 ^b
1:2	85.11 ± 1.18 ^c	86.89 ± 1.17 ^b	88.11 ± 1.25 ^c	76.78 ± 1.73 ^b	86.33 ± 1.20 ^b
1:3	86.44 ± 1.34 ^{bc}	86.67 ± 1.41 ^b	73.56 ± 1.63 ^d	73.56 ± 1.37 ^c	77.22 ± 1.44 ^c
1:4	80.56 ± 1.50 ^d	81.67 ± 1.41 ^c	62.78 ± 1.79 ^e	71.44 ± 1.99 ^c	67.56 ± 1.72 ^d

^aEvaluated on a 9-point hedonic scale from 1 = dislike extremely to 9 = like extremely, expressed as percentage

^{b–e}Values followed by different superscript letters differ according to the Tukey test ($p < 0.05$)

significantly different from other samples. The significantly low score recorded in 1:4 dilution sample could be due to the bland taste after diluted 5 times with water. The findings were in accordance with results reported in a study done by Siow and Wong [20]. The study showed that panelists preferred sweeter drink reconstituted from red dragon fruit concentrate compared to fresh, grassy-smell like red dragon fruit juice. Results revealed that the aroma of all samples was like slightly (< 80%) by the panelists as shown in Table 3. The generally low score recorded in aroma attribute was probably due to the distinct aroma found in fermented fruit drinks. As a final point, sensory results showed that FRDFD with 1:2 dilution was preferable to consumers in terms of overall acceptance score (86.33%).

To conclude, the experimental results revealed that there was more than 80% of betanin retained in fermented red dragon fruit drink stored at 4 °C for a storage duration of 8 weeks. The content of betanin at the end of the storage at 4 °C was 13.93 g/L. Despite degradation of betacyanins after 8 weeks storage, the drink can still be considered a good source of bioactive compound betanin which is sensory acceptable (> 80% mean score with the hedonic test) among consumers. Further studies on other methods such as the application of spray drying technique to improve the storage stability of the drink should be conducted.

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References

- Granato D, Branco GF, Nazzaro F, Cruz AG, Faria JAF. Functional foods and non-dairy probiotic food development: trends, concepts, and products. *Comprehensive Reviews in Food Science and Food Safety* 9: 292–302 (2010)
- Mohammad Nor NAA, Mohd Masdek NRN, Sulaiman NH. Functional food business potential analysis in Malaysia, Thailand, Indonesia and the Philippines. *Economic and Technology Management Review* 11b: 99–110 (2016)
- Foong JH, Hon WM, Ho CW. Bioactive compounds determination in fermented liquid dragon fruit (*Hylocereus polyrhizus*). *Borneo Science* 31: 38–56 (2012)
- Rebecca OPS, Boyce AN, Chandran S. Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*). *African Journal of Biotechnology* 9: 1450–1454 (2010)
- Rebecca OPS, Zuliana R, Boyce AN, Chandran S. Determining pigment extraction efficiency and pigment stability of dragon fruit. *Journal of Biological Sciences* 8: 1174–1180 (2008)
- Esatbeyoglu T, Wagner AE, Motafakkerazad R, Nakajima Y, Matsugo S, Rimbach G. Free radical scavenging and antioxidant activity of betanin: Electron spin resonance spectroscopy studies and studies in cultured cells. *Food and Chemical Toxicology* 73: 119–126 (2014)
- Krajka-kuz V, Paluszczak J, Szaefer H, Baer-dubowska W. Betanin, a beetroot component, induces nuclear factor erythroid-2-related factor 2-mediated expression of detoxifying/antioxidant enzymes in human liver cell lines. *British Journal of Nutrition* 110: 2138–2149 (2013)
- Tsai P, Hsiao S, Chaung H, Hong C, Wang C. The LDL-cholesterol-lowering effects of nano-particled Djulis Grains. In: *International Conference on Bioscience, Biochemistry and Bioinformatics*. 26 February, Singapore. IACSIT Press, Singapore (2011)
- Kanner J, Harel S, Granit R. Betalains: A new class of dietary cationized antioxidants. *Journal of Agricultural and Food Chemistry* 49: 5178–5185 (2001)
- Tesorieri L, Tesorieri L, Allegra M, Butera D, Livrea MA. Absorption, excretion, and distribution of dietary antioxidant betalains in LDLs: Potential health effects of betalains in humans. *The American Journal of Clinical Nutrition* 80: 941–945 (2004)
- Adetuyi FO, Ibrahim TA. Effect of fermentation time on the phenolic, flavonoid and vitamin C contents and antioxidant activities of Okra (*Abelmoschus esculentus*) seeds. *Nigerian Food Journal* 32: 125–137 (2014)
- Wybraniec S, Platzner I, Geresh S, Gottlieb HE, Haimberg M, Mogilnitski M, Mizrahi Y. Betacyanins from vine cactus *Hylocereus polyrhizus*. *Phytochemistry* 58: 1209–1212 (2001)
- Stintzing FC, Schieber A, Carle R. Betacyanins in fruits from red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose. *Food Chemistry* 77: 101–106 (2002)
- Nestora S, Merlier F, Prost E, Haupt K, Rossi C, Tse BSB. Solid-phase extraction of betanin and isobetanin from beetroot extracts using a dipicolinic acid molecularly imprinted polymer. *Journal of Chromatography A* 1465: 47–54 (2016)
- Zamora MC, Chirife J. Determination of water activity change due to crystallization in honeys from Argentina. *Food Control* 17: 59–64 (2006)
- Abbo ES, Olurin TO, Odeyemi G. Studies on the storage stability of soursop (*Annona muricata* L.) juice. *African Journal of Biotechnology* 5: 1808–1812 (2006)

17. Castro WF, Cruz AG, Bisinotto MS, Guerreiro LMR, Faria JAF, Bolini HMA, Cunha RL, Deliza R. Development of probiotic dairy beverages: Rheological properties and application of mathematical model in sensory evaluation. *Journal of Dairy Science* 96: 16–25 (2013)
18. Woo KK, Ngou FH, Ngo LS, Soong WK, Tang PY. Stability of betalain pigment from red dragon fruit (*Hylocereus polyrhizus*). *American Journal of Food Technology* 6: 140–148 (2011)
19. Azeredo HMC. Betalains: properties, sources, applications, and stability - a review. *International Journal of Food Science and Technology* 44: 2365–2376 (2009)
20. Siow LF, Wong YM. Effect of juice concentration on storage stability, betacyanin degradation kinetics, and sensory acceptance of red-fleshed dragon fruit (*Hylocereus polyrhizus*) juice. *International Journal of Food Properties*, 20: 623–632 (2017)
21. Wong YM, Siow LF. Effects on 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: 3086–3092 (2015)
22. Herbach KM, Stintzing FC, Carle R. Betalain stability and degradation – structural and chromatic aspects. *Journal of Food Science* 71: 41–50 (2006)
23. Slimen IB, Najjar T, Abderrabba M. Chemical and antioxidant properties of betalains. *Journal of Agricultural and Food Chemistry*, 65: 675–689 (2017)
24. Khan MI. Plant betalains: Safety, antioxidant activity, clinical efficacy and bioavailability. *Comprehensive Reviews in Food Science and Food Safety* 15: 316–330 (2016)
25. Tuwalska D, Starzak K, Szot D, Wybraniec S, Winterhalter P, Jerz G. Semi-synthesis of red beet betacyanin ethyl-esters by esterification. *Challenges of Modern Technology* 5(3): 27–31 (2014)
26. Boekel MAJS. Kinetic modelling of food quality: a critical review. *Comprehensive Reviews in Food Science and Food Safety* 7: 144–158 (2008)
27. Centre for Food Safety. Microbiological Guidelines for Food (2014). Available from: http://www.cfs.gov.hk/english/food_leg/files/food_leg_Microbiological_Guidelines_for_Food_e.pdf. Accessed April 27 2017
28. Heredia N, Wesley I, Garcia JS. Microbiologically safe foods. Wiley, New Jersey. pp. 291–294 (2009)