International Food Research Journal 18(4): 1437-1448 (2011)

Influence of enzyme treatment on bioactive compounds and colour stability of betacyanin in flesh and peel of red dragon fruit *Hylocereus polyrhizus* (Weber) Britton and Rose

Kunnika, S. and *Pranee, A.

Department of Food Technology, Faculty of Science, Chulalongkorn University
Bangkok, Thailand

Abstract: The role of commercial pectinase enzyme, Pectinex® Ultra SP-L (10,292 PGU/ml) was studied with respect to the degradation of polysaccharide structure in middle lamella releasing bioactive compounds such as antioxidant, phenolic, total flanovoid and betacyanin in structural cell of flesh and peel of red dragon fruit. The effects of acid, salt, sugar and hydrocolloids on colour stability of betacyanins was also studied. The results showed that samples with enzymatic hydrolysis of the highest reducing sugar content was 70.56 in flesh and 44.54 mg glucose/g FM in peel containing higher bioactive compounds than other stages significantly ($p<0.05$). Their antioxidant activities measured by DPPH method were 3 and 8 times higher than undegraded samples (control) (1.05, 2.71 µg FM/µg DPPH), and those determined by ABTS method were 4 and 7 times higher than control (1,029.60, 815.03 µg Trolox equivalents/g fresh mass (FM)). Their total phenolic contents were 2 and 3 times higher than control (1,049.18, 561.76 mg gallic acid equivalents/100 g FM), and total flavonoid contents were 5 and 7 times higher than control (1,310.10, 220.28 mg catechin equivalents/100 g FM). Moreover, their betacyanin contents have increased from 15.53 to 45.66 (flesh) and 14.27 to 61.65 mg/100 g FM (peel). The soluble dietary fiber has increased from 0.90 to 1.63(flesh) and 1.93 to 3.53(peel) g/100 g FM. The prebiotic activity scores from using *L. acidophilus* La5 were 0.15 and 0.12 for flesh and peel, respectively, and those from *B. lactis* Bb12 were 0.34 and 0.29. Furthermore, it was found that all samples of flesh and peel of red dragon fruit composed of the same type of betacyanin which was betanin. Colour stability of betacyanin from the enzyme degrading samples was compared with those from non-enzymatic treatment. The results showed that food-grade acids with high pK_a brought about the highest stability of a^* color in flesh and peel (76.02, 78.46). While, the higher salt concentration decreased color stability (28.05, 27.09), and the addition of sugar affected to higher color stability (61.46, 60.13). The outcome from hydrocolloid adding showed that carageenan could increase the stability of betacyanin color (60.76, 60.03). The optimum condition to result the strongest red color was the temperature which is not higher than 25˚C, pH range of 4-6, and the light exposure of not more than 2 d. Moreover, the samples degraded by enzyme had more betacyanin content which leaded to get redder color stability with every studied factor than ones without enzymatic degradation. Therefore, red dragon fruit's flesh and peel extracted by enzyme can be a good material to be developed as food colorant with bioactive compound and to be used instead of synthetic agent in the future.

Keywords: Red dragon, betacyanin, pectinase, bioactive compounds, prebiotic, antioxidant activities

Introduction

Red dragon fruit/ Pitahaya/ Pitaya/ Strawberry pear, with the scientific name of *Hylocereus polyrhizus* in family of Cactaceae, is originated in Tropical Rain Forest area of Mexico (Wu *et al*., 2006). The main physical character is oval shape with red flesh and peel. The main coloring agent is betacyanin, the same compound found in beetroot, with property of solubility in water and alcohol solvent. The stability is in the range of pH 3-7 (Wybraniec *et al*., 2001; Stintzing and Carle, 2004; Wybraniec *et al*., 2006). The color does not change reversibly in the same way as anthocyanins do to pH and exhibit a tinctorial strength up to three times higher than the anthocyanin (Harivaindaran *et al*., 2008). Betacyanins can be classified by their chemical structures into four kinds: betanin-type, amaranthin-type, gomphrenin-type and bouginvillein-type (Cai *et al*., 2005). For red dragon fruit, the main chemical structure of betacyanin is betanin (5-*O*-glucosides) (Stintzing *et al*., 2002; Wybraniec *et al*., 2006). Nowadays, the purple-red color of betacyanin can be extracted from beetroot and used as food colorant for industry scale to get more attention for the health issue of consumers. The natural colorants can be alternative; however, there are some limitations such as high price, ease of degradation by some factors like pH, temperature, oxygen, light, water activity, ionic compounds such as metal ion and sulphur dioxide. Stintzing and Carle (2007) reported that beetroot color shows greatest stability at pH 4.5. At pH 7 and above, the betacyanin can be degraded rapidly and color is not significantly affected by pH change in the range of 3-7. In very

*Corresponding author.
Email: *Pranee.a@chula.ac.th*
Tel: +66(0)2 218 5530; Fax: +66(0)2 254 4314

acidic conditions, the color will become more blue-violet as the red anionic form is converted to the violet cation. In alkaline condition, the color will rapidly become yellow-brown due to the loss of betanin. Furthermore, the effect of temperature is clear on betacyanin stability. The increased temperatures are associated with high degradation rate; due to betanin degradation produces betalamic acid and cyclodopa-5-*O*-glycoside (Delgado-Vargas and Paredes-López, 2000). For the factor of light, it was reported that betacyanin from *Amaranthus* has stability under daylight condition lower than in darkness due to light excites the electrons of the double bonds, which causes a higher degradation (Cai *et al*., 1998). Moreover, red dragon fruit is also composed of the main bioactive compounds such as antioxidant in group of flavonoid, phenolic and others. The dietary fiber found in red dragon fruit can be divided into 2 types which are soluble dietary fiber like mucilage and pectin as well as insoluble dietary fiber (Phebe *et al*., 2009; Stintzing and Carle, 2007). From the prominent character of color, bioactive compound and dietary fiber, it can be said that red dragon fruit has potential to be developed as colorant in thick liquid form with the properties of bioactive compound and dietary fiber, being useful and providing good function for food product more than just basic nutrition. Furthermore, the enzymatic degradation in middle lamella layer of tissue is biological method which is safe. Moreover, the chemical released from tissue can remain the natural form with higher concentration. A related report is from Mobhammer *et al*. (2005) who did research in enzymatic extraction of betacyanin from cactus pear. The color appearance of cactus pear juice was measured and it was found that enzymatic extraction brought about the highest color density and higher than water extraction. Furthermore, there was a report of enzymatic extraction in the other pigment like lycopene. It was found that extraction with enzyme yielded 206% higher lycopene content from tomato peel than one without enzyme (Choudhari and Ananthanarayan, 2007).

This research was about extraction of bioactive compounds from red dragon fruit flesh and peel such as purple-red colorant of betacyanin, phenolic compound and other antioxidant by commercial pectinase enzyme to degrade tissue in middle lamella layer without fiber removal process. Moreover, the stability of betacyanin and prebiotic activity was studied compared with inulin and glucose to produce extract with natural form of chemical but higher concentration, leading to be used as food colorant and texturizer instead of synthetic chemical for adding value in agriculture raw material and applying in food industry in the future.

Material and Methods

Sample preparation and enzyme treatment

Red dragon fruit from a farm in Prajeenburi, Thailand, which was harvested 45-50 d after blooming, was used. The fruit was separated into two parts: flesh and peel. The pretreatment condition of heating at 85°C for 3 min together with adding ascorbic acid concentration of 0.2 and 0.1% (w/w) was suitable to inhibit the browning reaction in flesh and peel, respectively. The samples were degraded by commercial pectinase enzyme (Pectinex® Ultra SP-L, Novozymes Switzerland AG, Dittengen, Switzerland) produced from *Aspergillus aculeatus* with enzyme activity of 10,292 PGU/ml. The enzymatic reaction batch was done in 250 ml amble glass bottle, control at 30±5°C with stirring at 150 rpm. The enzyme concentration was varied into 10 levels between 0-10.0% (v/w), and the degradation time was varied into 9 levels between 0-6 h. The enzyme reaction was stopped by heating at 95±5°C for 5 min. Then, the degradation efficiency was measured by the derived amount of reducing sugar using the method of Nelson (1944). All chemicals and solvents used in this experiment were analytical grade, produced by Sigma Chemical Co., Ltd (St,Louis, MO,USA) and Sigma Aldrich Co., Ltd (Steinheim, Germany).

Determination of antioxidant activities

Samples were prepared by the method modified from Maisuthisakul *et al*. (2007). The flesh or peel samples (60 g) was mixed with 300 ml of 95% ethanol and set aside in the dark at 25°C for 4.5 h with continue stirring of 100 rpm. Then it was filtered through Whatman No. 1 to remove residue. The ethanol in extracted clear liquid has evaporated by Rotary vacuum evaporator at 75°C, kept in air-tight amber bottles and stored at -15°C until analysis.

The antioxidant activities of the extracts were evaluated using the DPPH and ABTS assays. The DPPH radical-scavenging activity assay was adjusted from the method of Masuda *et al*. (1999) and Maisuthisakul *et al*. (2007). The extracts were diluted by methanol in different concentrations, and then 4.9 ml of each dilution was mixed with 100 µl of 5 mM DPPH in methanol, and set aside at room temperature for 30 min. The absorbance of sample containing DPPH (A_1), diluted sample without adding DPPH (A_s), and DPPH solution (A_0) was measured at 517 nm using a spectrophotometer and methanol as blank. The decreased content of DPPH radical-scavenging activity in each concentration of sample

extract could be calculated by the formula:

$$\text{DPPH radical-scavenging activity (\%)} = \frac{[A_0 - (A_1 - A_s)]}{A_0} \times 100$$

The decreasing content of DPPH radical-scavenging activity was plotted against the extract concentration from red dragon fruit flesh and peel to determine the amount of extract capable to decrease DPPH radical concentration by 50% (EC_{50}). The unit of antioxidant activity was defined as $1/EC_{50}$.

For ABTS assay derived from the method of Thaipong *et al*. (2006), the working solutions contained 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution were prepared. Then mixing the two stock solutions in equal quantities and standing in room temperature for 12 h. The solution was then diluted by mixing with methanol in ratio of working solutions: methanol as 1:30 to obtain an absorbance of 1.1±0.02 units at 734 nm using methanol as blank. The extracts from different concentrations (150 µl) were allowed to react with 2850 µl of the derived ABTS solution for 2 h in a dark condition at room temperature, and then the absorbance was measured at 734 nm (A_{734}) and compared with the standard curve of 100-500 µM Trolox. The results were expressed in µg Trolox equivalents (TE)/mg FM.

Determination of total phenolic

Total phenolic content was determined by Folin-Ciocalteau colorimetry following Marinova *et al*. (2005).The extracts from red dragon fruit flesh and peel were prepared as described in the determination of antioxidant activities. The extract (1 ml) was mixed with 9 ml of deionised water and 1 ml of Folin-Ciocalteu's phenol reagent in 25 ml volumetric flask, keeping at room temperature for 5 min, 10 ml of 7% Na_2CO_3 solution was added, adjusted the volume with deionised water, then mixed again and stood at room temperature for 90 min. The absorbance value of the resulted solution was measured at 750 nm wavelength using deionised water as blank. The phenolic content was calculated from standard curve that was made from gallic acid as representative of phenolic compounds. The values were expressed as mg gallic acid equivalent (GAE)/100 g FM.

Determination of total flavonoid

Total flavonoid content was measured by following Zhishen *et al*. (1999). The extracts from red dragon fruit flesh and peel were prepared, then mixed 1 ml of sample extracts with 4 ml of deionised water in 10 ml volumetric flask, 0.3 ml of 5% $NaNO_2$ was added and stood for 5 min, then mixed with 0.3 ml of 10% $AlCl_3$ and stood for 6 min, after that mixed with 2 ml of 1 M NaOH, and adjusted the volume with deionised water. The absorbance value of the obtained solution was measured at 510 nm wavelength using deionised water as blank. The flavonoid content was calculated from standard curve that was made from catechin as representative of flavonoid compounds. The values were expressed as mg catechin equivalent (CE)/100 g FM.

Determination of dietary fiber

Total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) contents were analyzed according to the standard AOAC methods (AOAC, 1995).

Prebiotic activity determinations

The prebiotic activity of the sample was determined using a modification of the method developed by Huebner *et al*. (2007).

Bacterial strains: L. acidophilus *La5 and* B. lactis *Bb12*

L. acidophilus La5 and *B. lactis* Bb12 were cultivated on MRS agar and incubated at 37°C for 24–48 h with atmosphere condition for *L. acidophilus* La5 but anaerobic condition in anaerobic jar for *B. lactis* Bb12. Then, a single colony from each plate was transferred into 10 ml of MRS broth and incubated at 37°C for 24 h at ambient atmosphere.

E. coli was prepared by cultivation on Tryptic Soy Agar (TSA) and incubated at 37°C for 24–48 h in ambient atmosphere. A single colony was transferred into 10 ml of Tryptic Soy Broth (TSB) and incubated at 37°C for 24 h in ambient atmosphere. Then 1% (v/v) of prepared *E. coli* from TSB was transferred into 10 ml of Minimal Medium broth and incubated at 37°C for 24 h in ambient atmosphere.

Prebiotic activity assay

The 1% (v/v) of prepared *L. acidophilus* La5 and *B. lactis* Bb12 cultures was transferred into MRS Broth with 1% (w/v) glucose or 1% (w/v) inulin or 1% (w/v) samples. The 1% (v/v) of prepared *E. coli* culture was transferred into Minimal Medium broth with 1% (w/v) glucose or 1% (w/v) inulin or 1% (w/v) samples and incubated at 37°C in ambient atmosphere. After 0 and 24 h of incubation, samples were enumerated on MRS agar for *L. acidophilus* La5 and *B. lactis* Bb12 and on TSA for *E. coli*, and the prebiotic activity score was determined as the following equation:

$$= \frac{\text{The different of colony amount (log cfu ml}^{-1}\text{) of probiotic at 0 and 24 h in prebiotic}}{\text{The different of colony amount (log cfu ml}^{-1}\text{) of probiotic at 0 and 24 h in glucose}}$$

$$- \frac{\text{The different of colony amount (log cfu ml}^{-1}\text{) of } \textit{E. coli} \text{ at 0 and 24 h in prebiotic}}{\text{The different of colony amount (log cfu ml}^{-1}\text{) of } \textit{E. coli} \text{ at 0 and 24 h in glucose}}$$

Betacyanins content

The extraction extract was prepared according to the method modified from Wu *et al*. (2006). The flesh and peel of red dragon fruit samples (20 g) were mixed with 40 ml of 95% ethanol, blended for 5 min and filtered through Whatman® No 1 to remove the residue. The filtered liquid was centrifuged at 12000 rpm under -4°C for 5 min, then evaporated to remove ethanol by rotary vacuum evaporator at 25°C for 35 min. The extract was kept in amble glass bottle with lid at -15°C for total betacyanin content analysis with spectrophotometer.

Total betacyanins content determination was done according to Wu *et al*. (2006). The extract from flesh and peel of red dragon fruit was prepared, diluted with 0.05 M phosphate buffer, pH 6.5 in the ratio of flesh and peel samples: 0.05 M phosphate buffer, pH 6.5 as 1: 30 and 1 : 24, respectively. The absorbance was measured by spectrophotometer using 0.05 M phosphate buffer, pH 6.5 as blank until the absorbance of the solution measured at 538 nm was 0.4 – 0.5± 0.02. The betacyanin content of flesh and peel was calculated by formula:

$$\text{Betacyanins content (mg/100g of fresh mass)} = A_{538}(MW)V(DF) \times 100 /(ELW)$$

Where A_{538} = the absorbance at 538 nm(λ_{max}), L(path length) = 1.0 cm, DF = dilution factor, V = extract volume (ml), W = fresh weight of extracting material (g), for betanin E(mean molar absorptivity) = 6.5 x 10^4 L/mol cm in H_2O and MW = 550.

Chromatographic determination of betacyanins

The analysis of thin layer chromatography was followed Delgano-Vargas and Paredes-Lo'pez (2000) by using isopropanol-ethanol-distilled water-acetic acid, 6:7:6:1 v/v as mobile phase and aluminium silica gel. The Rf of betacyanin in samples was compared with standard betacyanin to extract betacyanin in sample for further analysis by High performance Liquid Chromatography (HPLC).

Then, the samples of flesh and peel of red dragon fruit from TLC were filtered via Whatman® No 42 and adjusted the volume by 95% ethanol until 50 ml volume. After that, the extract was injected for analysis by HPLC according to Stintzing *et al*. (2007) using C_{18}- column (250x4.6 mm i.d.).

Stability of betacyanin

The stability of betacyanin color was monitored by measuring a^* value using the color analysis system of CIE $L^*a^*b^*$, L^* = 0(black); L^* = 100(white), a^*(-a^* = green; +a^* = red), b^* (-b^* = blue; +b^* = yellow) and the light source as D65. Minolta colorimeter (Minolta Spectrophotometer CR 300 was used to determine L^*, a^*, b^*.

Influence of acid, salt, sugar and hydrocolloids

The solutions of acetic acid, ascorbic1 acid and citric acid were prepared at concentration of 0.2 M, adjusted to pH 3.9 by 0.1 N NaOH. The salt solution was prepared by 1-5% sodium chloride. The solutions of glucose, fructose and sorbitol were prepared at concentration of 7%, while hydrocolloid (corn starch, xanthan gum and carrageenan) solution was prepared at concentration of 0.5%. Then 1 L of each prepared solution was added into 1 g red dragon fruit sample, kept at room temperature in the dark condition for 7 d, then compared the result with control set without adding acid, salt and hydrocolloid.

Determination of pH stability profile

The solutions with different pH values were prepared using 0.1 M citric acid and 0.2 M Na_2HPO_4 by adding into deionised water until pH reached 2-6. Then 10 g of red dragon fruit samples were added into 30 ml of each solution, kept at room temperature in the dark condition for 7 d, then analyzed the results by comparing color value change in range of studied pH values.

Determination of temperature stability profile

10 g of red dragon fruit samples were mixed with 30 ml of deionised water, heated until 40, 60, 80 and 100°C, cooled down, and the results were compared with control at 25°C.

Light exposure

10 g of red dragon fruit samples were mixed in 30 ml of deionised water, set aside for light exposure (fluorescence constant irradiance of 6 Wm^{-2}) at 10 h/day for 7 d, and the results were compared with control (day 0).

Statistical analysis

The obtained experimental data were analyzed statistically for analysis of variance and mean values at the significance of $p \leq 0.05$ by Duncan's new multiple range test and T-test using SPSS version 11.5, USA.

All experiments were replicated three times.

Results and Discussion

Enzymatic treatments and bioactive compounds

The degradation of polysaccharide structure in middle lamella with commercial pectinase enzyme, Pectinex® Ultra SP-L, was studied with respect to release bioactive compounds in structural cell of flesh and peel of red dragon fruit such as antioxidant, phenolic, total flanovoid and betacyanin. It was found that the increased concentration and longer degradation time had affected to polysaccharide degradation rate in both flesh and peel by showing higher reducing sugar (RS) significantly ($p \leq 0.05$). It could be explained that Pectinex Ultra SP-L degraded the glycosyl bonds of pectin, cellulose and hemicellulose at the cell wall of the fruit tissue, leading to get higher reducing group (Grohmann and Baldwin, 1992; Mutlu *et al*., 1999). Monitoring the quantity of bioactive compounds after enzymatic degradation of polysaccharide structure was done by selecting the samples degraded as presented in reducing sugar content of 25.61-70.56 in flesh and 20.35- 44.54 mg glucose/g FM in peel to compare with control set (undegraded samples). The results were reported according to significant difference ($p<0.05$) of enzymatic hydrolysis stages and using code for red dragon fruit flesh as F0 (control)(RS=22.39), F1(RS=25.61), F2(RS=58.54) and F3(RS=70.56) and peel as P0 (control) (RS=10.63), P1(RS=20.35), P2(RS=32.73) and P3(RS=44.54).

Antioxidant activities

Antioxidant activities in the flesh and peel of red dragon fruit determined by DPPH and ABTS method were tending to increase according to Table 1 and 2.The highest hydrolysis stage of polysaccharide structure showed higher antioxidant activity than other stages significantly ($p<0.05$) in both flesh and peel samples. Samples with the highest hydrolysis stage (F3, P3) had antioxidant activities determined by DPPH method as 3 and 8 times higher than control (1.05, 2.71 µg FM/µg DPPH), while the ABTS method showed 4 and 7 times higher than control (1.029, 815.03 µg Trolox equivalents/g FM). The EC_{50} values at the highest hydrolysis stage of flesh and peel were 1.05 and 2.71 µg FM/µg DPPH, respectively. It can be seen that the enzymatic extraction gave higher antioxidant activity than methanol extraction as reported by Tachakittirungrod *et al*. (2007) who found that the antioxidant in guava leaves extracted by enzyme showed activity 3 times higher than ones extracted by methanol.

Total phenolics and total flavonoids

The analysis of total phenolic and flavonoid revealed that samples with the highest hydrolysis stage (F3, P3) had total phenolic content of 2 and 3 times higher than control (1049.18, 561.76 mg gallic acid equivalents/100 g FM) and total flavonoid content of 5 and 7 times higher than control (1310.10, 220.28 mg catechin equivalents/100 g FM) as shown in Table 1 and 2. This was related with the report of Versari *et al.* (1997) which revealed that the extraction of ellagic acid and quercetin, phenolic compounds in strawberry and raspberry, by commercial enzyme brought about higher compound amount after the enzymatic hydrolysis of polysaccharide structure. Furthermore, there were findings of increasing phenolic and flavonoid contents after enzymatic hydrolysis in olive oil (Najafian *et al*., 2009), pine leaves (Lin *et al*., 2009), and pigconpea leaves (Fu *et al*., 2008). It can be explained that enzyme hydrolyzed plant cell wall, leading to more release of extract and phenolic compound out of cell.

Enzymatic treatments and yield of dietary fiber, prebiotics and betacyanin

Dietary fiber

Table 3 revealed that total dietary fiber contents in F0 and P0 compared with F3 and P3 were not different in all samples. The soluble dietary fiber (SDF) content in F3 and P3 increased from 0.90 to 1.63 (increase 81%) and from 1.93 to 3.53 g/100 g FM (increase 83%), respectively. The increase of SDF in peel sample was 100% higher than flesh one due to the effect of many enzyme types in Pectinex Ultra SP-L® to hydrolyze pectin in red dragon fruit flesh and peel. The pectin esterase (PE) had function to remove methyl groups out of pectin compounds, while polygalacturonase (PG) can hydrolyze glysocidic bond between galacturonic acids of pectin compound, leading to more polarity of extract. Moreover, pectin in insoluble form was changed to soluble form because of this (Hubbermann *et al*., 2006) relating with the experiment in ripe bael fruit degraded by enzyme (Charoensiddhi and Anprung, 2010).

Prebiotic activity score

Prebiotic compounds, bioactive compounds such as inulin, oligofructrose or other types of carbohydrate, involved in supporting the growth of healthy bacteria or probiotics, *L. acidophilus* La5 and *B. lactis* Bb12. The comparison of both microbial cell amounts after growing in media with other types of prebiotic (inulin), media with red dragon fruit total

Table 1. Bioactive compounds and antioxidant activity of the various samples of flesh of red dragon fruit

Functional substances/ RS	Hydrolysate level			
	F0 (RS = 22.39±0.65 mg glucose/g FM)	F1 (RS = 25.61±0.09 mg glucose/g FM)	F2 (RS = 58.54±0.02 mg glucose/g FM)	F3 (RS = 70.56±0.19 mg glucose/g FM)
DPPH (EC_{50}: µg FM [A]/ µg DPPH)	3.27±0.05	2.03±0.04	1.33±0.57	1.05±0.33
ABTS (µg TE [B]/g FM)	332.14±0.21	840.78±0.01	1,001.21±0.13	1,029.60±0.67
Total phenolics (mg GAE[C]/100g FM)	480.47±0.01	693.39±0.04	1,043.33±0.57	1,049.18±0.33
Total flavonoids (mg CE[D]/100g FM)	288.27±0.04	1,094±0.01	1,281.21±0.13	1,310.10±0.67

Each value represents a mean ± standard deviation.
Means,with the different letter in the row are significantly difference (p<0.05)
RS = reducing sugars, [A]FM= fresh mass, [B]TE=Trolox equivalents , [C]GAE=gallic acid equivalents , [D]CE=catechin equivalents

Table 2. Bioactive compounds and antioxidant activity of the various samples of peels of red dragon fruit

Functional substances/ RS	Hydrolysate level			
	P0 (RS = 10.63±0.99 mg glucose/g FM)	P1 (RS = 20.35±0.09 mg glucose/g FM)	P2 (RS = 32.73±0.02 mg glucose/g FM)	P3 (RS = 44.54±0.65 mg glucose/g FM)
DPPH (EC_{50}: µg FM [A]/ µg DPPH)	20.88±0.02	3.11±1.09	2.03±0.19	2.71±0.02
ABTS (µg TE [B]/g FM)	110.41±0.06	596.70±0.52	754.28±0.19	815.03±0.22
Total phenolics (mg GAE[C]/100g FM)	191.24±0.05	324.63±1.09	379.37±0.19	561.76±0.02
Total flavonoids (mg CE[D]/100g FM)	32.63±0.03	143.70±0.52	175.28±0.19	220.28±0.22

Each value represents a mean ± standard deviation.
Means,with the different letter in the row are significantly difference (p<0.05)
RS = reducing sugars, [A]FM= fresh mass, [B]TE=Trolox equivalents , [C]GAE=gallic acid equivalents , [D]CE=catechin equivalents

Table 3. Total dietary fiber of the various samples of flesh and peel of red dragon fruit

Total dietary fiber (g / 100g FM)	Flesh		Peel	
	F0 (RS = 22.39±0.65 mg glucose/g FM)	F3 (RS = 70.56±0.19 mg glucose/g FM)	P0 (RS = 10.63±0.99 mg glucose/g FM)	P3 (RS = 44.54±0.65 mg glucose/g FM)
Total dietary fiber	2.50 ±0.06 b	2.61 ± 0.03 b	3.62 ±0.01 a	3.72 ± 0.05 a
- Soluble dietary fiber	0.90 ±0.02 d	1.63 ± 0.01 c	1.93 ±0.05 b	3.53 ± 0.03 a
- Insoluble dietary fiber	1.67 ±0.03 a	0.98± 0.01 b	1.69 ±0.07 a	0.19 ± 0.08 c

Each value represents a mean ± standard deviation.
Means,with the different letter in the row are significantly difference (p<0.05)
FM = fresh mass

dietary fiber of the various samples of flesh and peel of red dragon fruit samples F0, F3, P0 and P3, and the media without prebiotic (glucose) for 24 h as shown in Table 4, it was found that the amount of *L. acidophilus* La5 and *B. lactis* Bb12 grown in media with inulin and sample F3 were higher than other conditions significantly (p<0.05). Moreover, the amount of pathogen (*E. coli*) grown in media without prebiotic (glucose) was higher than one in media with all types of prebiotic significantly (p<0.05). The data in Table 4 were calculated for prebiotic activity score as shown in Figure 1, it was found that p rebiotic activity scores of all studied prebiotic types affect to the increase of *B. lactis* Bb12 amount more than one of *L. acidophilus* La5. Therefore, it can be indicated that both strains showed the differences in the metabolic capacity and transport systems of the particular prebiotic for bacterial growth (Huebner *et al*., 2007). It can be noticed that prebiotic activity scores in all samples degraded by enzyme were higher than ones without enzymatic hydrolysis significantly (p<0.05) considering from both probiotic types growth.

Betacyanin

The betacyanin content in F3 and P3 as shown in Figure 2 increased from 15.53 to 45.66 and from 14.27 to 61.65 mg/100 g FM, respectively. The content in peel was 1.35 times higher than flesh because cellulase acted with cellulose presented in the primary wall beneath the first layer of middle lamella of the plant cell wall. Primary wall consisted of a rigid skeleton of cellulose embedded in a gel-like matrix composed of pectic compounds, hemi-cellulose and glycoprotein. The enzyme broke down of cellulose into glucose, cellobiose and higher glucose polymers. The extraction by pectinase enzyme presented in some reports that it can increase lycopene content in tomato peel from 96.30 to 138.67 µg/g or 1.43 times of control (Choudhari and Ananthanarayan, 2007). It can be explained that enzyme hydrolyzed tissue in middle lamella and released pigment in the natural form.

From the analysis of component and betacyanin content in F3 and P3 of red dragon fruit by HPLC method as shown in Figure 3 and Table 5, it was

Table 4. The increase of cell density between time 0 and 24 h for bacterial cultures grown in various carbohydrates

Bacterial culture	Cell density [$\log_{10}$(cfu/ml)]					
			Flesh		Peel	
	Glucose	Inulin	F0	F3	P0	P3
L. acidophilus La5	1.99±0.01 c	2.23±0.01 a	2.18±0.03 b	2.25±0.02 a	2.01±0.02 c	2.17±0.04 b
B. lactis Bb12	2.01±0.06 d	2.66±0.03 a	2.51±0.02 b	2.65±0.04 a	2.27±0.05 c	2.53±0.03 b
E. coli ATCC 29922	2.03±0.02 a	1.71±0.05 e	1.97±0.04 c	1.99±0.03 b	1.81±0.02 d	1.96±0.01 c

Each value represents a mean ± standard deviation.
Means,with the different letter in the row are significantly difference (p<0.05)

Table 5. High-performance liquid chromatography-diode-array detection (HPLC-DAD) and liquid-chromatography-mass spectrometry (LC-MS) data of betacyanins of flesh(F3) and peel(P3) of red dragon fruit

Peak number	Name/proposed name (trivial name)	Retention Time(min)	HPLC-DAD λ_{max} (nm)
1	Cyclo-dopa 5-*O*-ß-glucoside	7.4	285
2	Betanidin 5-*O*-ß-glucoside(betanin)	20.6	538
3	17-Decarboxybetanin	22.1	505
4	Betalamic acid	23.0	410
5	Isobetanidin 5-*O*-ß-glucoside(isobetanin)	24.2	538
6	17-Decarboxyisobetanin	26.1	505
7	Betanidin 5-*O*-(6'-O-malonyl)-ß-glucoside (phyllocatin)	28.3	538
8	Betanidin 5-*O*-(6'-O-3-hydroxybutyryl)- ß-glucoside	30.6	535
9	15-Decarboxybetanin	31.1	538
10	Betanidin 5-*O*-(6'-O-3-hydroxy-3-methyl-glutaryl)- ß-glucoside (hylocerenin)	31.6	538
11	Isobetanidin 5-*O*-(6'-O-malonyl)- ß-glucoside (isophyllocactin)	33.2	538
12	Neobetanin, decarboxylated	33.3	454
13	Neobetanidin 5-*O*-ß-glucoside (neobetanin)	36.1	487
14	Isobetanidin 5-*O*-(6'-O-3-hydroxy-3-methyl-glutaryl)- ß-glucoside(isohylocerenin)	37.0	536

found that they had the same 7 main component of betacyanin as Betanidin 5-*O*-ß-glucoside(betanin) (peak2), 17-Decarboxyisobetanin(peak6),Betanidin 5-*O*-(6'-O-malonyl)-ß-glucoside(phyllocatin) (peak7), Betanidin5-*O*-(6'-O-3-hydroxybutyryl)-ß-glucoside(peak8),Betanidin5-*O*-(6'-O-3-hydroxy-3-methyl-glutaryl)-ß-glucoside (hylocerenin)(peak 10), Isobetanidin 5-*O*-(6'-O-malonyl)-ß-glucoside (isophyllocactin)(peak 11) and Isobetanidin 5-*O*-(6'-O-3-hydroxy-3-methyl-glutaryl)-ß-glucoside(isohylocerenin)(peak 14). These 7 compounds were the same betacyanin component found in red dragon fruit from Israel as reported by Herbach *et al*. (2006).

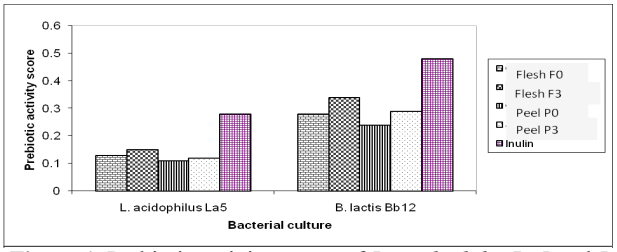

Figure 1. Prebiotic activity scores of *L. acidophilus* La5 and *B. lactis* Bb12 grown in various carbohydrate sources

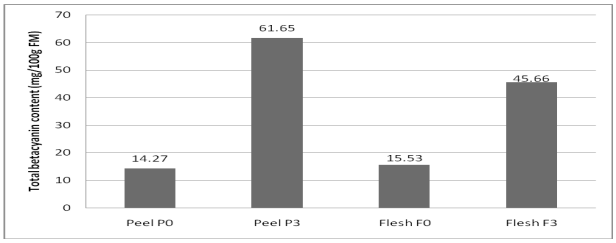

Figure 2. Total betacyanin content of the various flesh and peel samples of red dragon fruit

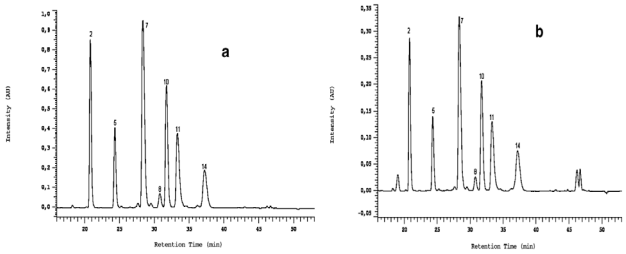

Figure 3. HPLC chromatographs of betacyanins of flesh (a) and peel (b) of red dragon fruit

Influence of sugars, salts, acids and hydrocolloids on the colour stability of betacyanin

Sugars

From the analysis of betacyanin color stability by consideration of a^* value in flesh (F0 and F3) and peel (P0 and P3) of red dragon fruit by adding sugar such as glucose, fructose and sorbitol compared with no sugar adding samples after 7 d storage, it was found that a^* values in all 4 samples (F0, F3, P0 and P3) with sugar addition were higher than control without sugar. While a^* values in enzymatic degraded samples (P3 and F3) were higher than those in non-enzymatic degraded ones (P0 and F0) significantly ($p \leq 0.05$) as shown in Figure 4. It can be seen that addition of glucose, fructose and sorbitol in peel samples (P0, P3) and flesh samples (F0, F3) can remain the betacyanin color stability as the results of higher a^* values in the range of 6-9 and 4-13%, respectively. Comparison of the sugar types showed that sorbitol brought the best stability. It can be explained that adding sugar can decrease water activity with the

combination between sugar molecule and water. The color of betacyanin showed high stability at low water activities (pigment stability decreased one order of magnitude when water activity was increased). In fact, the reaction of betacyanin degradation involved in water. This phenomenon had been collaborated by water-glucose system where degradation was reduced by decreasing water activity (Delgado-Vargas and Paredes-López, 2000). Furthermore, it was found that glucose addition into red dragon fruit samples degraded by enzyme (F3) can increase the highest a^* value from 53.12 to 55.59, relating with a study of betacyanin red color increase in beetroot with glucose addition in which the a^* value increased from 35.53 to 44.41(Pedreno and Escribano, 2001).

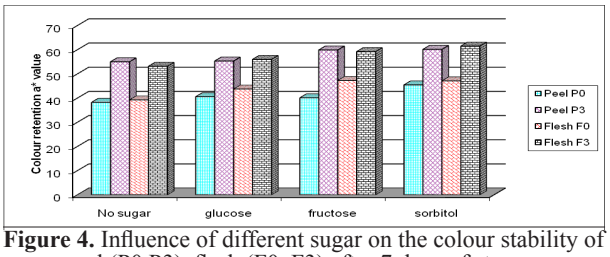

Figure 4. Influence of different sugar on the colour stability of peel (P0,P3) flesh (F0, F3) after 7 days of storage

Salts

By monitoring the effect of sodium chloride addition with concentration range of 1 - 5% by weight on color stability of betacyanin in flesh (F0 and F3) and peel (P0 and P3) of red dragon fruit with the consideration of a^* value, it was found that sodium ion in sodium chloride can decrease a^* values in all studied samples, while a^* values in enzymatic degraded samples (P3 and F3) were higher than those in non-enzymatic degraded ones (P0 and F0) significantly ($p \leq 0.05$) as shown in Figure 5. It can be explained that the cations of salt (Na^+) can accelerate betanin, the main structure of betacyanin, to be regenerated into two products, betalamic acid and cyclodopa-5-Oglycoside, in unstable form (Henry, 1992). Furthermore, a report about the effect of sodium chloride on color stability of other pigments such as anthocyanin in elderberry and black currant showed that red color value of anthocyanin decreased 32%, 42% and 55% of control (60%) at sodium chloride concentration of 1%, 3% and 5%, respectively (Hubbermann *et al*., 2006].

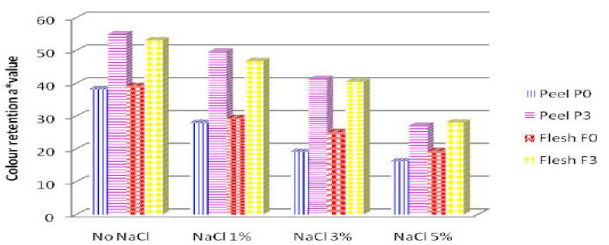

Figure 5. Influence of different NaCl concentration on the colour stabilityof peel(P0,P3) flesh (F0, F3) after 7 days of storage

Food acids

From the analysis of betacyanin color stability in flesh (F0 and F3) and peel (P0 and P3) of red dragon fruit by adding acids such as acetic acid, citric acid and ascorbic acid comparing with no acid adding samples after 7 d storage by considering of a^* value, it was found that a^* values in all 4 samples (F0, F3, P0 and P3) with acid adding were higher than control without acid. While a^* values in enzymatic degraded samples (P3 and F3) were higher than those in non-enzymatic degraded ones (P0 and F0) significantly ($p \leq 0.05$) as shown in Figure 6. Comparing the effect of acid types on a^* values in all samples showed that acetic acid brought about the best stability which in peel samples (P0, P3) and flesh samples (F0, F3) the a^* values increased 1.5, 1.6, 1.4 and 1.7 times higher than control, respectively. It can be explained that the hydration was slower in systems containing high pK_a (pK_a value acetic acid : 4.76) but can be accelerated by other acids with lower pK_a such as citric acid with pK_a value of 3.09 and ascorbic acid with pK_a value of 2.98, resulting in faster color loss. In the experiment, cation of acetic acid was at the C-11 position, the carbon atom adjacent to the quaternary amino nitrogen. Therefore, the formation of betanin with water was slower in system, decreasing the degradation into betalamic acid and cyclodopa-5-Oglycoside or unstable form (Cai *et al*.,1998). Furthermore, the effect of 0.2 M ascorbic acid (pH 3.9) on red dragon fruit samples degraded by enzyme (F3) brought about the increase of a^* value from 53.12 to 61.97, relating with a study of betacyanin red color increase in beetroot with the same adding of ascorbic acid concentration which the a^* value increased from 35.48 to 55.23 (Delgado-Vargas and Paredes-López, 2000).

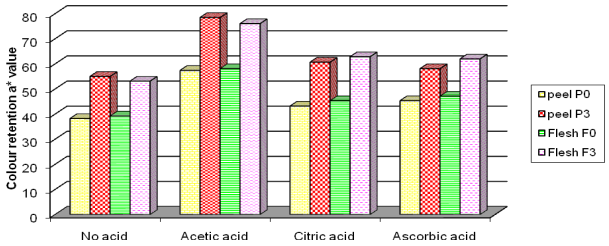

Figure 6. Influence of different food – grade acids (0.2 M, pH 3.9) on the colour stabilityof peel (P0, P3), flesh (F0, F3) after 7 days of storage

Hydrocolloids

By monitoring the effect of hydrocolloids (corn starch, xanthan gum and carrageenan) on color stability of betacyanin in flesh (F0 and F3) and peel (P0 and P3) of red dragon fruit with the consideration of a^* value compared with no hydrocolloids adding samples after 7 d storage, it was found that a^*

values in all 4 samples (F0, F3, P0 and P3) with hydrocolloids addition were higher than control without hydrocolloids. While a* values in enzymatic degraded samples (P3 and F3) were higher than those in non-enzymatic degraded ones (P0 and F0) significantly ($p \leq 0.05$) as shown in Figure 7. Comparison of the effect of hydrocolloids types on a* values in all samples showed that carrageenan brought about better stability than xanthan gum and corn starch. It can be explained that the addition of hydrocolloids leaded to the association of carboxylic group of the colloids with betacyanin, preventing the formation with water which can cause the betacyanin degradation, so leading to preserve the color stabilization (Azeredo *et al*., 2007). Furthermore, it was found that addition of carrageenan into red dragon fruit samples degraded by enzyme (F3) can increase a* value from 53.12 to 60.76, relating with a study of betacyanin red color increased in cactus pear with carrageenan addition in which a* value increased from 35.53 to 39.08 (Mobhammer *et al*., 2005).

Influence of pH, temperature and light on the colour stability of betacyanin

pH stability profile

By monitoring the effect of pH in range of 2- 6 on color stability of betacyanin in flesh (F0 and F3) and peel (P0 and P3) of red dragon fruit after 7 d storage with the consideration of a* value, it was found that peel samples of red dragon fruit (P3) had betacyanin red color values at pH 2, 3, 4, 5 and 6 as 43.34, 45.45, 53.19, 55.26 and 55.76 which were higher than other samples at the same pH levels significantly ($p \leq 0.05$). Furthermore, the optimum pH stability of all samples was in the same range of 4-6 as shown in Figure 8, showing the betacyanin in samples degraded by enzyme was in the same form of natural betacyanin (P0 and F0). Moreover, it was the similar pH range with best betacyanin color stability in red dragon fruit from Israel (pH 4.5-5.5) but with wider range than one in red dragon fruit from Taiwan (pH 5) and in beetroot (pH 5.5) according to Pedreno and Escribano (2001), Wu (2006) and Stintzing and Carle (2007).

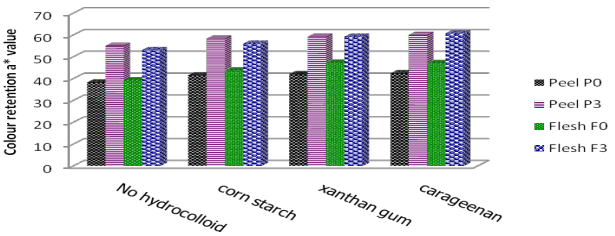

Figure 7. Influence of different hydrocolloids on the colour stability of peel (P0,P3), flesh (F0,F3) after 7days of storage

Temperature stability profile

By monitoring the effect of temperature (25, 40, 60, 80 and 100˚C) on color stability of betacyanin in flesh (F0 and F3) and peel (P0 and P3) of red dragon fruit with the consideration of a* value, it was found that the temperature increase in range of 25 - 100˚C affected to the decreasing a* value in all studied samples as shown in Figure 9. It can be explained that increased temperature can accelerate betanin, the main structure of betacyanin, to be regenerated into two products, betalamic acid and cyclodopa-5-Oglycoside, in unstable form (Herbach *et al*., 2004). For the consideation of betacyanin color stability in a* values, it was found that enzymatic degraded samples (P3 and F3) had higher color stability than non-enzymatic degraded ones (P0 and F0) in all temperature significantly ($p \leq 0.05$) due to the fact that betacyanin density of enzymatic degraded samples (P3 and F3) was higher than non-enzymatic degraded ones (P0 and F0), resulting in lower water activity than F0 and P0 samples. The low water activity can help to be lower the betacyanin degradation (Delgado-Vargas and Paredes-López, 2000). By considering the betacyanin stability at 100˚C, it was found that red dragon fruit with enzymatic degradation (P3 and F3) had lower a* values as 25.16 and 23.71, respectively. This process is likely to the loss of red color value of betacyanin from Malaysian red dragon fruit from 38.46 to 18.23 (Harivaindaran *et al*., 2008).

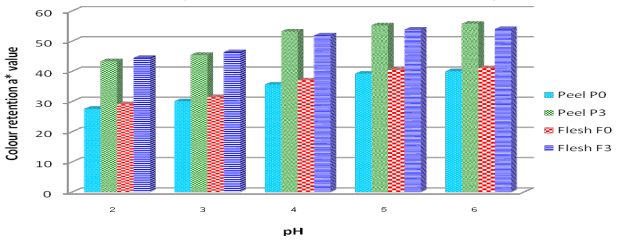

Figure 8. Influence of different pH on the colour stability of peel (P0, P3) flesh (F0,F3) after 7 days of storage

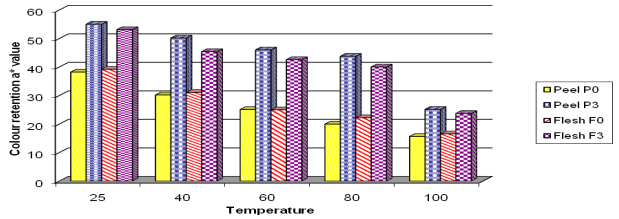

Figure 9. Influence of different temperature (˚C) on the colour stability of peel (P0,P3) flesh (F0, F3)

Light exposure

By monitoring the effect of light exposure (fluorescence constant irradiance of 6 Wm^{-2}) on color stability of betacyanin in flesh (F0 and F3) and peel (P0 and P3) of red dragon fruit with the consideration of a* value compared with control after 7 d storage, it was found that all samples getting light exposure had lower a* value significantly ($p \leq 0.05$). It can be

explained that light affected the electron of double bonds in betacyanin molecules to be in excited stage, resulting the higher degradation of betacyanin than dark condition (Cai *et al*.,1998). By considering betacyanin color stability in a* values, it was found that enzymatic degraded samples (P3 and F3) had higher color stability than non-enzymatic degraded ones (P0 and F0) due to betacyanin density of enzymatic degraded samples (P3 and F3) which was higher than non-enzymatic degraded ones (P0 and F0), the same reason explain about temperature effect. Furthermore, it was found that red dragon fruit with enzymatic degradation (P3 and F3) and keeping for 7 d light exposure had lower betacyanin red color values as 24.43 and 25.37, respectively. This might be different from red color value of betacyanin left in red dragon fruit juice (-8.31) which kept in the same condition (Herbach *et al*., 2004).

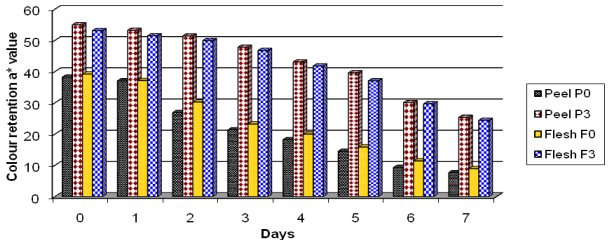

Figure 10. Influence of kept in 10 h of light exposure daily for 7 days on the colour stability Peel (P0,P3), Flesh (F0,F3)

Conclusion

Commercial pectinase enzyme, Pectinex® Ultra SP-L, was applied for the sake of degradation of polysaccharide structure in red dragon fruit flesh and peel until the reducing sugar content increased from 25.61 to 70.56 in flesh and from 20.35 to 44.54 mg glucose/g FM in peel. It can be concluded that the pulp had more bioactive compound than shell, except for the soluble fiber content. Furthermore, the analysis of prebiotic activity score of prebiotics in both pulp and shell samples showed the similar abilities in supporting the increase of both strains amount. The betacyanin content in peel was 1.35 times higher than flesh (flesh = 45.66, peel = 61.65 mg/100 g FM). Furthermore, it was found that all samples of flesh and peel of red dragon fruit composed of the same type of betacyanin which was betanin. The betacyanin stabilities of samples degraded by enzyme were compared with those from non-enzymatic treatment. The results showed that food-grade acids with high pKa brought about the highest stability of a* color in flesh and peel with enzymatic degradation. This was because of the lower betacyanin hydration, leading to decrease the structure degradation into betalamic acid and cyclodopa-5-Oglycoside with unstable form. While, the higher salt concentration decreased color stability due to the cations of salt (Na^+) can accelerate betanin oxidation, bringing about chemicals in unstable form. The addition of sugar affected to get higher color stability since sugar can decrease the water activity of system, leading to get the lower betacyanin hydration. The outcome from hydrocolloid addition showed that carageenan can increase the stability of betacyanin color as the addition of hydrocolloids leaded to the association of carboxylic group of the colloids with betacyanin, preventing the formation with water which can cause the betacyanin degradation, which in turn lead to preserve the color stabilization. The rest of hydrocolloids also increase the colour stability of betacyanin. The study of temperature, pH and light effects on betacyanin stability showed that the optimum condition for the highest red color was the temperature which is not higher than 25^0C, pH in range of 4-6, and the light exposure of not more than 2 d. Moreover, the samples degraded by enzyme had more betacyanin density which leaded to get higher stability with every studied factor (sugar, acid, salt, hydrocolloid, temperature, pH and light) than ones without enzymatic degradation.

Therefore, red dragon fruit's flesh and peel extracted by enzyme can be a good source of bioactive compounds such as phenolic, flavonoid, soluble dietary fiber, prebiotic and other antioxidant. Moreover, it is suitable to be developed as food colorant with bioactive compounds and to replace synthetic agents in the future.

Acknowledgements

The authors are grateful for the financial support by the Integration Project: Innovations for the improvement of food safety and food quality for new world economy, government research budget and Graduate School Chulalongkorn University, Thailand.

References

AOAC. 1995. Official Methods of Analysis of the AOAC International. Association of Official Analytical Chemists. Washington, D. C.

Azeredo, H. M. C., Santos, A. N., Souza, A. C. R., Mendes, K. C. B., and Andrade, M. I. R. 2007. Betacyanin stability during processing and strage of a microencapsulated red beetroot extract. American Journal of Food Technology 2: 307-312.

Cai, Y., Sun, M. and Corke, H. 1998. Colorant properties and stability of *Amaranthus* betacyanin pigments. Journal of Agricultural and Food Chemistry 46: 4491-4495.

Cai, Y., Sun, M. and Corke, H. 2005. Characterization and application of betalain pigment from plants of the Amaranthaceae. Trends in Food Science and Technology 16: 370-376.

Charoensiddhi, S. and Anprung, P. 2010. Characterization of bael fruit (*Aegle marmelos* (L.) Correa) hydrolysate as affected by enzyme treatment. Journal of Food Biochemistry 34 : 1249-1267.

Choudhari, S. M. and Ananthanarayan, L. 2007. Enzyme aided extraction of lycopene from tomato tissues. Food Chemistry 102: 77-81.

Delgado-Vargas, F. and Paredes-López, O. 2000. Betacyanins and phenolic compounds from *Beta vulgaris* L roots. Food Chemistry 58(3): 255-258.

Fu, Y.-J., Liu, W., Zu, Y.-G., Tong, M.-H., Li, S.-M., Yan, M.-M., Efferth, T. and Luo, H. 2008. Enzyme assisted extraction of luteolin and apigenin from pigeonpea [*Cajanus cajan* (L.) Millsp.]leaves. Food Chemistry 111: 508-512.

Grohmann, K. and Baldwin, E. A. 1992. Hydrolysis of orange peel with pectinase and cellulase enzymes. Biotechnology Letters 14(12): 1169-1174.

Harivaindaran, K. V. Rebecca, O. P. S. and Chandran, S. 2008. Study of optimal temperature, pH and stability of dragon fruit (*Hylocereus polyrhizus*) peel for use as potential natural colorant. Pakistan Journal of Biological Sciences 11(18): 2259-2263.

Henry, B. S. 1992. Natural Food Colours. In Hendry, G. A. F. and Houghton, J. D. (Eds.), Natural Food Colorants pp. 39-77. Blackie, Glassgow.

Herbach, K. M., Stintzing, F. C. and Carle, R. 2004. Thermal degradation of betacyanins in juice from purple pitaya (*Hylocereus polyrhizus* (Weber)Britton & Rose) monitored by high-performance liquid chromatography-tanden mass spectrometric analyses. European Food Reserarch Technology 219:377-385.

Herbach, K. M., Rohe, M., Stintzing, F. C. and Carle, R. 2006. Structural and chromatic stability of purple pitaya (*Hylocereus polyrhizus*) betacyanins as affected by the juice matrix and selected additives. Food Research International 39: 667-677.

Hubbermann, E. M., Heins, A., Stockmann, H. and Schwarz, K. 2006. Influence of acids, salt, sugars and hydrocolloids on the colour stability of anthocyanin rich black currant and elderberry concentrates. European Food Research and Technology 223: 83-90.

Huebner, J., Wehling, R. L. and Hutkins, R. W. 2007. Functional activity of commercial prebiotics. International Dairy Journal 17: 770-775.

Lin, S.-C., Chang, C.-M. J. and Deng, T.-S. 2009. Enzymatic hot pressurized fluids extraction of polyphenolics from *Pinus taiwanesis* and *Pinus morrisonicola*. Journal of the Taiwan Institute of Chemical Engineers 40: 136-142.

Masuda, T., Yonemori, S., Oyama, Y., Takeda, Y.,Tanaka, T., Andoh, T., Shinobara, A. and Nakata,M. 1999. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. Journal of Agricultural and Food Chemistry 47: 1749-1754.

Maisuthisakul, P., Suttajit, M. and Pongsawatmanit, R., 2007, Assessment of phenolics content and free radical-scavenging capacity of some Thai indigenous plants, Food Chemistry 100: 1409-1418.

Marinova, D., Ribarova, F. and Atanassova, M. 2005. Total phenolics and total flavonoids in Bulgarian fruits qand Vegetables, Journal of the University of Chemical Technology and Metallurgy 40(3): 255-260.

Mobhammer, M. R., Stintzing, F. C. and Carle, R. 2005. Development of a process for the production of a betalain-based colouring foodstuff from cactus pear. Innovative Food Science and Emerging Technologies 6: 221-231.

Mutlu, M., Sariglo, K., Demir, N., Ercan, M. T. and Acar, J. 1999. The use of commercial pectinase in fruit juice industry.PartI: viscosimetric determination of enzyme activity. Journal of Food Engineering 14: 147-150.

Najafian, L., Ghodsvali, A., Haddad Khodaparast, M. H. and Diosady, L. L. 2009. Aqueous extraction of virgin olive oil using industrial enzymes. Food Research International 42: 171-175.

Nelson, N., 1944, Determination of glucose. Journal Biological and Chemistry 153: 375-380.

Pedreno, M. and Escribano, J. 2001. Correlation between antiradical activity and stability of betanine from *Beta vulgaris* L roots under different pH, temperature and loght condition. Fournal of Science of Food and Agriculture 81: 627-631.

Phebe, D., Chew, M. K., Suraini, A. A., Lai, O. M. and Janna, O. A. 2009. Red-fleshed pitaya (*Hylocereus polyrhizus*) fruit colour and betacyanin content depend on maturity. International Food Research Journal 16: 233-242.

Stintzing, F. C., Schieber, A. and Carle, R. 2002. Evaluation of colour properties and chemical quality parameters of cactus juices. European Food Research and Technology 216: 303-311.

Stintzing, F. C. and Carle, R. 2004. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends in Food Science and Technology 15: 19-38.

Stintzing, F. C. and Carle, R. 2007. Betalain-emerging prospects for food scientists. Trends in Food Science and Technology 18: 514-525.

Tachakittirungrod, S., Okonogi, S. and Chowwanapoonpohn, S. 2007. Study on antioxidant activity of certain plants in Thailand: mechanism of antioxidant action of guava leaf extract. Food Chemistry 103: 381-388.

Thaipong, K., Boonprakob, U., Crosby, K., Luis, C. Z. and Byrne, D. H. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19: 669-675.

Versari, A., Biesenbruch, S., Barbanti, D., Farnell, P. J. and Galassi, S. 1997. Effects of pectolytic enzymes on selected phenolic compounds in strawberry and raspberry juices. Food Research International 30(10): 811-817.

Wu. L., Hsu. H., Chen, Y., Chiu, C., Lin, Y. and Ho. A.

2006. Antioxidant and antiproliferative activities of red pitaya, Food Chemistry 2: 319-327.

Wybraniec, S., Platzner, I., Geresh, S., Gottlieb, H. E., Haimberg, M., Mogilnitzki, M. and Mizrahi, Y. 2001. Betacyanins from vine cactus *Hylocereus polyrhizus*. Phytochemistry 58: 1209-1212.

Wybraniec, S. 2006. Effect of tetraalkylammonium salts on retention of betacyanins and decarboxylated betacyanins in ion-pair reversed-phase high-performance liqud chromatography. Journal of Chromatography A 1127: 70-75.

Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64: 555-559.