American Journal of

# Food Technology

ISSN 1557-4571

Academic
Journals Inc.

www.academicjournals.com

American Journal of Food Technology 6 (2): 140-148, 2011
ISSN 1557-4571 / DOI: 10.3923/ajft.2011.140.148


# Stability of Betalain Pigment from Red Dragon Fruit (*Hylocereus polyrhizus*)

K.K. Woo, F.H. Ngou, L.S. Ngo, W.K. Soong and P.Y. Tang
Department of Bioscience and Chemistry, Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Jalan Genting Kelang, 53300 Setapak, Kuala Lumpur, Malaysia

*Corresponding Author: K.K. Woo, Department of Bioscience and Chemistry, Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Jalan Genting Kelang, 53300 Setapak, Kuala Lumpur, Malaysia Tel: +603 410 79802 Fax: +603 410 79803*

## ABSTRACT

Betalain pigment from red dragon fruit (*Hylocereus polyrhizus*) or known as purple pitaya was extracted and the stability was evaluated. Fruits were homogenized with ethanol to separate pectic substances. The fruit extract obtained demonstrated absorbance peaks at 230 and 537 nm under UV/Vis spectrophotometric analysis. Absorbance peak at 537 indicated the presence of betacyanin. The changes of betacyanin intensity due to storage condition, light, temperature, pH and additives were monitored for three weeks with a UV/Vis spectrophotometer at 537 nm. Results revealed light as the major factor of betalain pigment degradation. Refrigeration storage (4°C) condition without light exposure managed to preserve the colour of fruit juice up to 3 weeks.

**Key words:** Purple pitaya, betacyanin, betanin, natural colourant

## INTRODUCTION

Colours play an important role in enhancing the aesthetic appeal of food products. Owing to some food processing procedures such as heat treatment, pH changes, light exposure and storage condition, many food products suffered colour loss. Previously, synthetic food colourants are incorporated to recover colour loss and to enhance the appearance of food products. Unfortunately, some synthetic colourants such as tartrazine (E102) and sudan red which has prolong history in food industry has been reported to be heath hazardous lately (Sasaki *et al.*, 2002; Ahlstrom *et al.*, 2005). Although, the side effects of some synthetic colourants remained controversial, this further alarmed the consumers about the safety of synthetic colourant. In addition, the tightening government restrictions are some reasons persuade the food industries to search for alternative colours. As a replacement colouring agent, it must at least fulfilled two major concerns in which it should be free of side effects and harmless to public health. Natural pigments from biological sources came into consideration especially pigment extracted from plant, fungi, bacteria, algae and insect. Some major categories of plant pigments include betalain, anthocyanins and other flavonoids, carotenoids and chlorophylls. Among these categories of pigment, anthocyanin earned the most attention due to its colour and antioxidant properties (Stintzing *et al.*, 2002a; Awika *et al.*, 2004). Although betalain is the five most widely used colourants in the food industries (Jackman and Smith, 1996), research involved in betalain has not been as thorough as compare to its counter parts. Betalain is a class of natural pigments comprising yellow betaxanthins and betacyanins. The distribution of betalain in nature is limited to confined plant species of *Caryophylalles*, red beet

(Chenopodaceae) and certain fungi such as fly-agaric mushroom (*Amanita muscaria*) (Leathers *et al*., 1992; Strack *et al*., 1993). Several edible sources of betalain are red and yellow beetroot (*Beta vulgaris* L. sp. *vulgaris*, Chenopodiaceae), coloured Swiss chard (*Beta vulgaris* L. sp. *cicla*, Chenopodiaceae), grain or leafy amaranth (*Amaranthus* sp., Amaranthaceae) and cactus fruits (Cactaceae) (Strack *et al*., 1993; Cai *et al*., 1998; Kugler *et al*., 2004).

Red beetroot is the major commercially exploited betalain crop. It is approved to be used in food industries as food additive in United State (Title 21 of Code of Federal Regulations, 21 CFR 73.40) and in European Union (E162). Betalain obtained from red beetroot is exempted from batch certification. To date, it is widely used as natural pigment in food industries especially ice creams and yoghurt. However, beetroot pigments have some major drawbacks in which its earthy odour caused by geosmin is not favourable among some consumers (Murray *et al*., 1975). Most of the natural pigments are highly instable and this has limited their application especially in foods undergoing thermal treatment (Cevallos-Casals and Cisneros-Zevallos, 2004). Betalain has been reported as sensitive to heat, pH, light, moisture and oxygen (Von Elbe, 1975; Saguy, 1979; Saguy *et al*., 1978, 1984; Attoe and von Elbe, 1981; Cohen and Saguy, 1983; Huang and von Elbe, 1987). Therefore, the pigment is only suitable to be added in food with short shelf lives. Alternatives natural food colourant with higher stability towards the above mentioned factors are indeed necessary to facilitate the food industries. Several reports on cactus fruits (Stintzing *et al*., 2001) and Amaranthaceae plants (Cai *et al*., 2005) as the potential source of natural food colourant provide a gate way to the alternative source of red beet betalain. A study conducted by Obon *et al*. (2009) demonstrated promising results with applying the spray dried *Opuntia stricta* fruit juice to yoghurt and soft drink. The observation showed that the colour in the product maintained a vivid red-purple tonality even after one month of refrigeration storage.

Several extraction methods have been development to ease the extraction of betalain pigment form various fruit. Stintzing *et al*. (2002b) reported the extraction method used for betalain from purple pitaya (*Hylocereus polyrhizus*). On the other hand, a method applying preparative ion-pair high-speed counter current chromatography (IP-HSCCC) has been reported recently (Wybraniec *et al*., 2009; Jerz *et al*., 2008). This method was tested on purple pitaya and *Phytolacca americana* (Phytolaccaceae).

Purple pitaya is well known as red dragon fruit in Malaysia due to its deep bluish red appearance. The report mentioned that vitamin C added up to one per cent demonstrated a promising observation in stabilizing the pigment. Therefore, betalain extracted from purple pitaya or red dragon fruit as an alternative source of red beetroot betalain is optimistic. In the present study, locally cultivated red dragon fruit was chosen as the source of betalain. Storage stability of red dragon fruit betalain in different treatments and storage conditions was observed.

## MATERIALS AND METHODS

**Materials:** Red dragon fruit was purchased from the local market. Fresh fruit was stored at 4°C before used.

**Methods:** The study of pigment stability of red dragon fruit extract was conducted in between 2008 to 2009.

**Extraction and purification of red dragon fruit:** Red dragon fruit was first separated from the pericarps and homogenized with 95% (v/v) ethanol by the ratio of 1:2 for 15 min. This step was to separate pectic substances. Subsequently the fruit pulp was sieved with gauze and centrifuged at 10,000 x g for 20 min to remove precipitates. Supernatant was then evaporated with a rotary evaporator (Buchi, Switzerland) at 160 Pa, 40°C until one forth from the initial volume. The fruit extract was used for further work.

**Treatments:** The fruit extract was subjected to various treatments including thermal treatment, pH, antioxidant additives and light exposure. Colour changes of the treated samples were monitored from week 0-3 with a UV/Vis spectrophotometer (Hitachi U-1800, Japan).

**(i) Additional of antioxidant:** Ascorbic acid was added into the fruit extract with the concentrations of 0.1% (w/v), 0.5% (w/v) and 1.0% (w/v). Fruit extract incorporated with ascorbic acid were then subjected to pH adjustment. Control was prepared without addition of ascorbic acid.

**(ii) pH treatment:** Samples added with different percentages of ascorbic acid were subjected to pH adjustment using 1 M HCl or 1M NaOH. Each set of samples in section (i) was adjusted to pH 3.0, pH 5.0 (original pH of red dragon fruit) and pH 7.0, respectively. These samples were further treated at different temperature.

**(iii) Heat treatment:** Samples obtained from section (ii) were treated at 3 different temperatures, respectively, 25, 50 and 85°C. Each set of the sample treatment was finally kept at 4°C, 25°C in dark and 25°C with light exposure.

**Spectrophotometer analysis:** Colour changes of the treated samples were monitored weekly at 537 nm with a UV/Vis spectrophotometer from week 0 to 3.

**Statistical analysis:** All measurements were done in triplicate. Analyses of Variance (ANOVA) were conducted by using SPSS Version 13.0 for Windows (SPSS, Chicago, IL, USA). Turkey tests were performed to test the significant differences between the mean values for treatments ($p<0.05$).

## RESULTS

**Extraction of pigment:** Freshly extracted and concentrated red dragon fruit juice was scanned through with a spectrophotometer between the wavelengths of 200 to 700 nm. The absorption spectrum showed two major absorption peaks corresponded to 230 and 537 nm (Fig. 1). Absorbance peaks at 230 and 537 nm is characteristic absorption for red violet betalain group, betacyanin (Sánchez *et al*., 2006).

**Storage stability of pigment:** Samples stored at 25°C with light exposure suffered almost 50% colour loss after first week of storage (Fig. 2). However, sample protected from light managed to retain approximately 70% of the betacyanin pigment until week 1 (Fig. 2) even though samples were stored at 25°C. Conversely, all samples stored at 25°C (dark and light) lost about 70 to 90% of the betacyanin pigment, respectively at the end of week 3. In contrary, sample stored at low temperature (4°C) observed no significant colour changes until the third storage week.

The influence of heat treatment to the degradation of red colour pigment of red dragon fruit is shown in Fig. 3. The 85°C pre-heat treated samples showed colour degradation up to 30% at the initial stage of storage. However, the colour managed to sustain until week two for dark storage at 4°C. Low temperature storage has successfully maintained the red purple hue of most samples to week 3 for both 50°C pre-heat treated and control samples. Samples pre-treated at 50°C and continuously kept in dark at 4°C showed a similar observation as compared to the control samples (Fig. 3). All the samples which kept in 25°C experienced colour loss from 70 to 90 %, respectively at the end of week 3 (Fig. 3).

In terms of pH treatment, all samples stored in 4°C are relatively stable except samples adjusted to pH 3.0, which colour was slightly lighter after pH adjustment. No significant colour degradation was observed within 3 weeks storage except for sample adjusted to pH 5.0 in dark storage. Samples

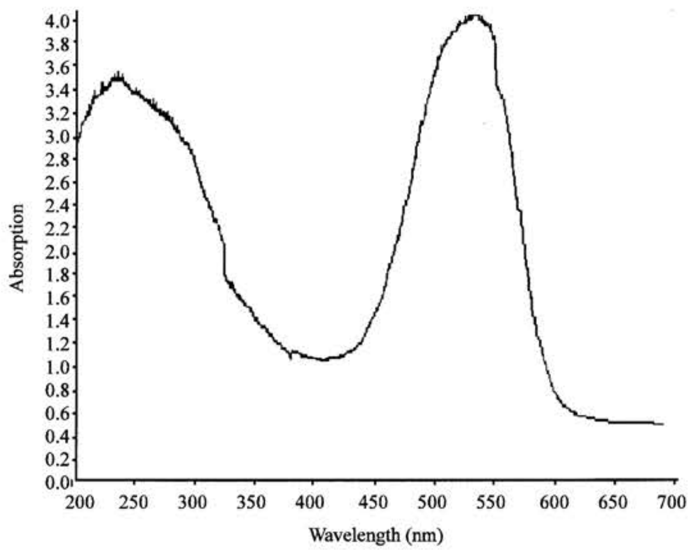


Fig. 1: Absorption spectrum of freshly extracted red dragon fruit pigment

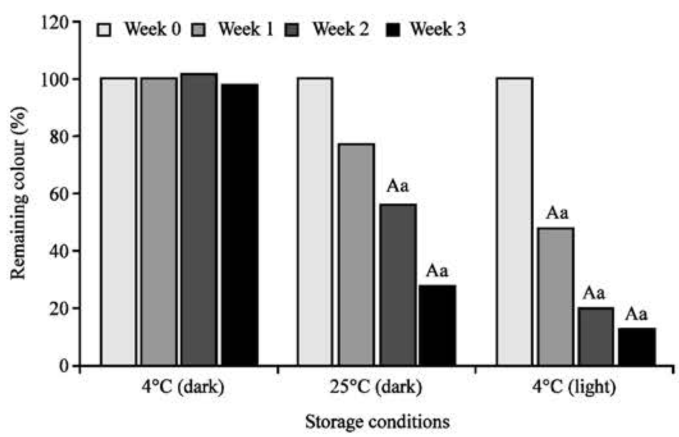


Fig. 2: The effect of storage conditions on the stability of red dragon fruit (*Hylocereus polyrhizus*) pigment. A: Statistical difference between treatment with respect to 4°C dark storage ($p<0.05$); a: Statistical difference between storage time within the same treatment with respect to 4°C dark storage ($p<0.05$)

stored at room temperature (25°C) exhibited significant colour changes after first week (Fig. 4). The samples experienced 80% to almost 90% colour lost from the initial absorbance at the end of

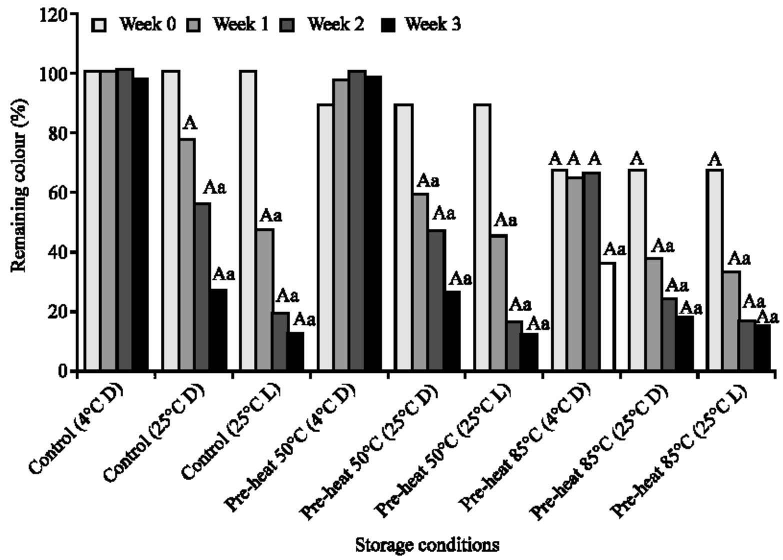


Fig. 3: The effect of pre-heat treatment on the stability of red dragon fruit pigment during storage. 4°C D, 4°C dark storage; 25°C D, 25°C dark storage; 25°C L, 25°C storage with light exposure; A: Statistical difference between treatment with respect to 4°C dark storage ($p<0.05$); a: Statistical difference between storage time within the same treatment with respect to 4°C dark storage ($p<0.05$)

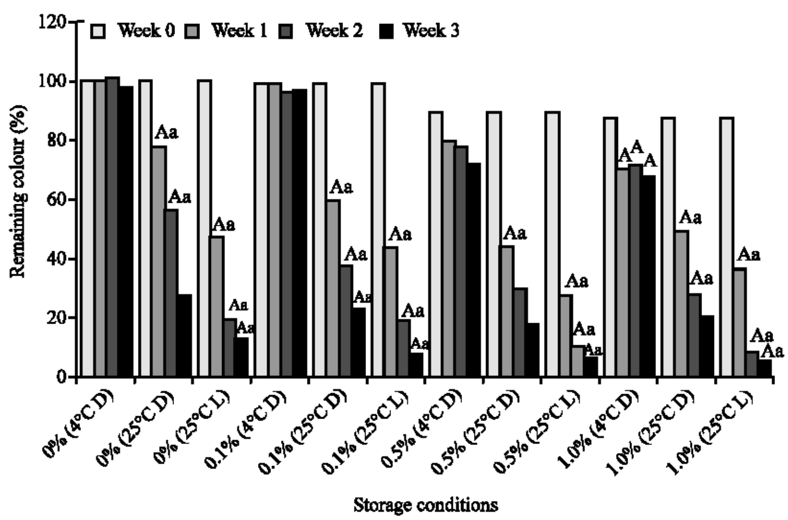


Fig. 4: Effect of pH on the stability of red dragon fruit during storage. 4°C D, 4°C dark storage; 25°C D, 25°C dark storage; 25°C L, 25°C storage with light exposure; A: Statistical difference between treatment with respect to 4°C dark storage ($p<0.05$); a: Statistical difference between storage time within the same treatment with respect to 4°C dark storage ($p<0.05$)

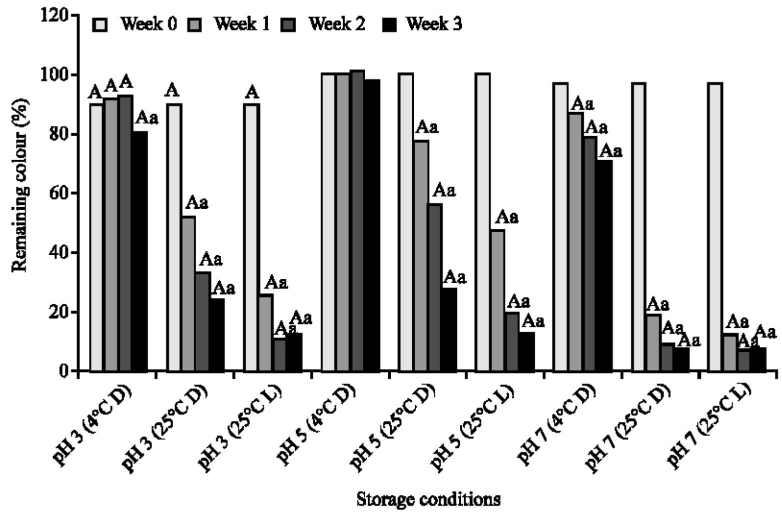


Fig. 5: Colour degradation pattern during storage for red dragon fruit pigment treated with ascorbic acid ranging from 0 to 1.0%. 4°C D, 4°C dark storage; 25°C D, 25°C dark storage; 25°C L, 25°C storage with light exposure; A: Statistical difference between treatment with respect to 4°C dark storage ($p<0.05$); a: Statistical difference between storage time within the same treatment with respect to 4°C dark storage ($p<0.05$)

storage. Samples with original pH of red dragon fruit (pH 5.0) seemed to have a slightly better colour retention among the three storage conditions. Adjustment of pH before storage did not express much significant effect in preserving colour degradation. The colour degraded in approximately 25% from the initial reading after 3 weeks storage in the dark at 4°C for sample adjusted to pH 7.0. In contrast, sample at pH 3.0 managed to maintain 80% of the colour at the end of week 3. The results indicated that betacyanin seems to be in favour to acidic pH region. The influence of pH towards colour degradation is shown in Fig. 4.

In this present study, ascorbic acid was added in the extracted juice in various concentrations from 0.1 to 1.0%. As shown in Fig. 5, addition of ascorbic acid to the concentration of 0.1% managed to preserve the colour in all dark storage conditions. Unfortunately, 0.1% ascorbic acid was not able to stop colour degradation in sample exposed to light. Ascorbic acid added to 0.5% on the other hand did not offer promising observation except for 4°C dark storage. The results were contradictory to the reported data by Reynoso *et al.* (1997). In the report, significant colour degradation of the red beet sample was observed after 2 days for sample without ascorbic acid. According to Reynoso *et al.* (1997), 1% ascorbic acid managed to preserve the red hue of red beet pigment. Therefore, suggesting that colour degradation in the present study might be due to factor besides oxidation.

## DISCUSSION

Red dragon fruit extract demonstrated the maximum peak at 230 and 537 nm, respectively indicated the presence of betalain compound. The absorbance peak at 537 nm is due to both the optical active chiral carbons on the C-2 and C-15 position (Kanner *et al.*, 2001; Slawomir and Mizrahi, 2002). On the other hand, absorbance peak between 470-480 nm, which is the characteristic peak for betaxanthin (Reynoso *et al.*, 1997), was not observed in the extracted juice confirmed the lack of betaxanthin in red dragon fruit. This result indicated that the compound responsible for the purple appearance of the fruit is betacyanin.

Generally, betalain stability was influence greatly by light. It has been reported to deteriorate betalain stability (Von Elbe *et al.*, 1974; Bilyk *et al.*, 1981; Cai *et al.*, 1998; Herbach *et al.*, 2007). The effect of light, UV and visible light, towards betalain stability is due to the excitation of electron of the betalain chromophores to a more energetic state, led to higher reactivity or lowered activation energy of the molecule (Jackman and Smith, 1996). It has been reported that light and oxygen caused betalain degradation by 15.6 and 14.6%, respectively (Von Elbe *et al.*, 1974). The observation from the present study indicated that the colour suffer severe lost as compared to the reported results (Von Elbe *et al.*, 1974). On the other hand, red dragon fruit extract demonstrated negative impact to betalain stability upon heat treatment. The nature of betalain towards high temperature has limited the application to food industry.

The lost of red purple tone observed during storage explained that betacyanin in red dragon fruit is similar to betacyanin from other sources, which is sensitive towards light and storage temperature. Therefore, the degradation of betacyanin in red dragon fruit might follow the mechanism explained by Huang and von Elbe (1987). According to their report, the primary steps of betalain degradation due to temperature are the nucleophilic attack by water at the C-11 position on betanin molecule. This step resulted cyclodopa-5-O-glycoside and betalamic acid. These compounds will undergo Schiff base condensation which leads to betanin regeneration at low temperature condition. In contrast, regeneration reaction could be irreversible for the pigment in high temperature because betalamic acid is heat liable, it might undergo aldol condensation or participate in Maillard reactions. Thus, colour lost in samples stored at 25°C colour were relative higher as compare to sample stored at 4°C.

It has been reported that betalain are stable between pH ranging from pH 3.0 to 7.0 (Stintzing and Carle, 2004). Betalain are readily to be degraded beyond this range. However, presence of oxygen and elevation temperature might slightly shift the optimum pH. Colour degradation observed at pH 7.0 in the present study might be due to hydrolytic cleavage of aldimine bond, which yield colourless cyclo-Dopa-5-O-**β**-glucoside (Schwartz and von Elbe, 1983; Herbach *et al.*, 2006a).

The effect of additive towards colour degradation of natural fruit pigments has been reported. Supplement such as antioxidants or the commonly used ascorbic and isoascorbic acid from the concentration of 0.1-1.0% showed some promising results in stabilizing red dragon fruit betacyanins (Pasch and von Elbe, 1979; Herbach *et al.*, 2006b).

According to Reynoso *et al.* (1997), 1% ascorbic acid managed to preserve the red hue of red beet pigment. Therefore, suggesting that colour degradation in the present study might be due to factor besides oxidation.

## CONCLUSION

As conclusion, pH, temperature and light have great impact to the colour degradation of betalain pigment in extracted red dragon fruit juice during storage. In the present study, light has been the major factor of colour deterioration. Exposure of light caused colour lost up to 50% after one week storage in room temperature. Pre-heat treatment of red dragon fruit juice before storage did not show significant effect in preventing colour degradation. In fact, high temperature (85°C) led to almost 30% of colour lost in the initial stage of storage. Sample subjected to heat treatment up to 50°C slightly reduced the juice colour. The treatment failed to preserve the colour of red dragon fruit for all storage in room temperature. Ascorbic acid as well, did not exhibit promising results in red dragon fruit juice stored in room temperature. The best condition in preserving betalain pigment in red dragon fruit juice is pH 5.0 at 4°C. Sunlight should be totally avoided to preserve the colour of the pigment. The next challenge of the red dragon fruit pigment is to incorporate it into food products. The stability of the pigment in the food products will lead us to the answer whether the pigment is an alternative to red beet betalain.

## REFERENCES

Ahlstrom, L.H., C.S. Eskilsson and E. Bjorklund, 2005. Determination of banned azo dyes in consumer goods. Trends Anal. Chem., 24: 49-56.

Attoe, E.L. and J.H. von Elbe, 1981. Photochemical degradation of betanine and selected anthocyanins. J. Food Sci., 46: 1934-1937.

Awika, J.M., L.W. Rooney and R.D. Waniska, 2004. Anthocyanins from black sorghum and their antioxidant properties. Food Chem., 90: 293-301.

Bilyk, A., M.A. Kolodij and G.M. Sapers, 1981. Stabilization of red beet pigment with isoascorbic acid. J. Food Sci., 46: 1616-1617.

Cai, Y.Z., M. Sun and H. Corke, 1998. Colorant properties and stability of *Amaranthus* betacyanin pigments. J. Agric. Food Chem., 46: 4491-4495.

Cai, Y.Z., M. Sun and H. Corke, 2005. Characterization and application of betalain pigments from plants of the *Amaranthaceae*. Trends Food Sci. Technol., 16: 370-376.

Cevallos-Casals, B.A. and L. Cisneros-Zevallos, 2004. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. Food Chem., 86: 69-77.

Cohen, E. and I. Saguy, 1983. Effect of water activity and moisture content on the stability of beet powder pigments. J. Food. Sci., 48: 703-707.

Herbach, K.M., F.C. Stintzing and R. Carle, 2006a. Betalain stability and degradation **β**_ Structural and chromatic aspects. J. Food Sci., 71: R41-R50.

Herbach, K.M., M. Rohe, F.C. Stintzing and R. Carle, 2006b. Structural and chromatic stability of purple pitaya (*Hylocereus polyrhizus* [Weber] Britton and Rose) betacyanins as affected by the juice matrix and selected additives. Food Res. Int., 39: 667-677.

Herbach, K.M., C. Maier, F.C. Stintzing and R. Carle, 2007. Effect of processing and storage on juice color and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice. Eur. Food Res. Technol., 224: 649-658.

Huang, S.A. and J.H. von Elbe, 1987. Effect of pH on the degradation and regeneration of betanine. J. Food Sci., 52: 1689-1693.

Jackman, R.I. and J.L. Smith, 1996. Anthocynins and Betalain. In: Natural Food Colorants, Hendry, C.F. and J.D. Houghton (Eds.). Blackie Academic and Professional, London, pp: 244-309.

Jerz, G., T. Skotzki, K. Fiege, P. Winterhalter and S. Wybraniec, 2008. Separation of betalains from berries o f *Phytolacca americana* by ion-pair high-speed counter-current chromatography. J. Chromatogr. A, 1190: 63-73.

Kanner, J., S. Harel and R. Granit, 2001. Betalain-a new class of dietary cationized antioxidants. J. Agric. Food Chem., 49: 5178-5185.

Kugler, F., F.C. Stintzing and R. Carle, 2004. Identification of betalain from petioles of differently colored Swiss chard (*Beta vulgaris* L. ssp. cicla, [L.] Alef. cv. Bright lights) by high-performance liquid chromatography-electrospray ionization mass spectrometry. J. Agric. Food Chem., 52: 2975-2981.

Leathers, R.R., C. Davin and J.P. Zryd, 1992. Betalain producing cell cultures of *Beta vulgaris* L. var. *Bikores monogerm* (red beet). *In Vitro* Cell. Dev. Biol., 28P: 39-45.

Murray, K.E., P.A. Bannister and R.G. Buttery, 1975. Geosmin: An important volatile constituent of beetroot (*Beta vulgaris*). Chem. Ind., 15: 974-975.

Obon, J.M., M.R. Castellar, M. Alacid and J.A. Fernandez-Lopez, 2009. Production of a red-purple food colorant from *Opuntia stricta* fruits by spray drying and its application in food model systems. J. Food Eng., 90: 471-479.

Pasch, J.H. and J.H. von Elbe, 1979. Betalain stability in buffered solutions containing organic acids, metal cations, antioxidants, or sequestrants. J. Food Sci., 44: 72-75.

Reynoso, R., F.A. Garcia, D. Morales and E.G. de Mejia, 1997. Stability of betalain pigments from *Cactacea* fruit. J. Agric. Food Chem., 45: 2884-2889.

Saguy, I., 1979. Thermostability of red beet pigments (betanine and vulgaxanthin-I): Influence of pH and temperature. J. Food. Sci., 44: 1554-1555.

Saguy, I., I.J. Kopelman and S. Mizrahi, 1978. Thermal kinetic degradation of betanin and betalamic acid. J. Agric. Food Chem., 26: 360-362.

Saguy, I., M. Godlman, A. Bord and E. Cohen, 1984. Effect of oxygen retained on beet powder on the stability of betanine and vulgaxanthine I. J. Food Sci., 49: 99-101.

Sasaki, Y.F., S. Kawaguchi, A. Kamaya, M. Ohshita and K. Kabasawa *et al*., 2002. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat. Res./Gen. Toxicol. Envrion. Mutagenesis, 519: 103-119.

Schwartz, S.J. and J.H. von Elbe, 1983. Identification of betanin degradation products. Zeitschrift Lebensmitteluntersuchung Forschung A, 176: 448-453.

Slawomir, W. and Y. Mizrahi, 2002. Fruit flesh betacyanin pigments in *Hylocerus Cacti*. J. Agric. Food. Chem., 50: 6086-6089.

Stintzing, F.C. and R. Carle, 2004. Functional properties of antocyanins and betalain in plants, food and human nutrition. Trends Food Sci. Technol., 15: 19-38.

Stintzing, F.C., A. Schieber and R. Carle, 2001. Phytochemical and nutritional significance of cactus pear. Eur. Food Res. Technol., 212: 396-407.

Stintzing, F.C., A. Schieber and R. Carle, 2002a. Identification of betalain from yellow beet (*Beta vulgaris* L.) and cactus pear [*Opuntia ficus-indica* (L.) Mill.] by high-performance liquid chromatography-electrospray ionization mass spectrometry. J. Agric. Food Chem., 50: 2302-2307.

Stintzing, F.C., A.S. Stintzing, R. Carle, B. Frei and R.E. Wrolstad, 2002b. Color and Antioxidant properties of cyanidin-based anthocyanin pigments. J. Agric. Food Chem., 50: 6172-6181.

Strack, D., W. Steglich and V. Wray, 1993. Betalain. In: Methods in Plant Biochemistry, Dey, P.M. and J.B. Harbone (Eds.). Academic Press Ltd., London, pp: 421-451.

Sánchez, F.D., E.M.S. Lopez, S.F. Kerstupp, R.V. Ibarra and L. Scheinvar, 2006. Colorant extraction from red prickly pear (*Opuntia lasiacantha*) for food application. Electron. J. Environ. Agric. Food Chem., 5: 1330-1337.

Von Elbe, J.H., 1975. Stability of betalain as food colors. Food Technol., 29: 42-46.

Von Elbe, J.H., I.Y. Maing and C.H. Amundson, 1974. Colour stability of betanin. J. Food Sci., 39: 334-337.

Wybraniec, S., P. Stalica, G. Jerz, B. Klose and N. Gebers *et al*., 2009. Separation of polar betalain pigments from cacti fruits of *Hylocereus polyrhizus* by ion-pair high-speed countercurrent chromatography. J. Chromatogr. A, 1216: 6890-6899.