JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Color Properties and Stability of Betacyanins from *Opuntia* Fruits

ROSARIO CASTELLAR, JOSÉ M. OBÓN, MERCEDES ALACID, AND JOSÉ A. FERNÁNDEZ-LÓPEZ*

Department of Chemical Engineering, Higher Technical School of Agronomical Engineering, Polytechnic University of Cartagena, Paseo Alfonso XIII 44, E-30203 Cartagena (Murcia), Spain

The colorant properties of pigments from *Opuntia stricta*, *Opuntia undulata*, and *Opuntia ficus-indica* fruits were studied. The pigments were extracted with different solvents and identified by high-performance liquid chromatography. On the basis of their visible light spectra, the pigments were identified as betalains. In *O. undulata* and *O. ficus-indica* fruits, both betacyanins and betaxantins were identified, while in *O. stricta* fruits only betacyanins (betanin and isobetanin) were detected. *O. stricta* fruits showed the highest betacyanin content (80 mg/100 g fresh fruit). The thermal stability of the pigment extracts was dependent on the pH, with the maximum stability being at pH 5, as expected for betacyanins. At this value and a storage temperature of 4 °C, a deactivation half-life time of more than 1 year, with no added stabilizers, was determined. According to these studies, cactus pears from *O. stricta* may well be considered as a potential source of natural red colorants.

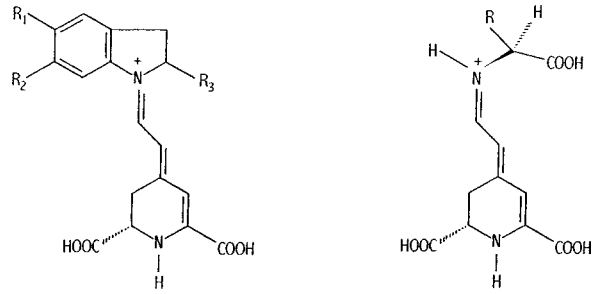
KEYWORDS: ***Opuntia*; betalains; betacyanins; color stability; food colorants**

INTRODUCTION

Betalains are natural pigments of chemotaxonomical significance typically associated with plants of the order Caryophyllales (*1*). The chemical structure of these pigments derives from betalamic acid and, depending on the components bonded to the main structure, betacyanins or betaxanthins arise, the former when the group is 3,4-dihydroxyphenylalanine (DOPA), which may or may not be glycosylated, and the latter if the conjugation partners are amino acids or derived amines (**Figure 1**).

Natural red pigments from plants are of growing interest as substitutes for synthetic red dyes in the food and pharmaceutical industry (*2*). Red colorants from the red beet root (*Beta vulgaris* L.) are approved additives for use in foods in the United States (Title 21 of the Code of Federal Regulations, 21 CFR 73.40) and in the European Union (E-162), and commercially, they are exempt from batch certification.

Much effort was put into identifying and quantifying the pigments responsible for the color of red beet root as well as into studying the main factors affecting its color deterioration. The main pigments present are red-purple betacyanins with betanin contributing to 75−95% of the red color and yellow betaxanthins with vulgaxanthin-I contributing to 95% of the yellow color (*3*−*5*). The stability of betalains is affected by temperature, pH, oxygen, light, and water activity (*6*−*11*). As temperature is the main parameter influencing color stability, special care was taken to study the effect of this parameter. It was found that thermal degradation of the pigments followed first-order reaction kinetics and was dependent on pH (*12*, *13*). The maximum thermostability of beet red juice was observed at pH 5.8 (*7*).



betacyanins betaxanthins

Figure 1. Chemical structure of betacyanins and betaxanthins.

Studies have shown that degraded betanin can be partially regenerated after thermal treatment with the aid of certain additives (*14*, *15*). An important additional feature of betanin when used as a natural colorant is its antioxidant capacity (*16*).

However, red beet preparations are obtained from roots and unfavorable flavor characteristics may affect their commercial use. Alternatives to red beet pigments are betacyanins from the genus *Amaranthus* (*17*), and some *Cactaceae* species such as *Myrtillocactus geometrizans* (*18*), *Hylocereus polyrhizus* (*19*, *20*), or *Opuntia ficus-indica* (*21*−*28*).

The use of cactus pears as a betalain source is an interesting possibility because they are highly flavored and show better nutritional properties than red beet root (*26*). In addition,

* To whom correspondence should be addressed. Tel: 34 968325549. Fax: 34 968325555. E-mail: josea.fernandez@upct.es.

10.1021/jf021045h CCC: $25.00
Published on Web 03/21/2003

Opuntia species have minimal soil and water requirements and may be regarded as an alternative for the agricultural economy of arid and semiarid regions. Thus, the aim of this study was to evaluate the feasibility of using betalain extracts from the fruits of different *Opuntia* species as natural colorants. Furthermore, special interest has been paid to the thermal and storage stability of betacyanins from cactus pears in order to determine the optimum conditions for their use and commercialization.

MATERIALS AND METHODS

Plant Material. Three species of *Opuntia* with fruits of red-violet color were selected for this study: *Opuntia stricta* Haw., *Opuntia undulata* Griff., and *O. ficus-indica* L. Mill. Mature cactus pears grown in the region of Murcia (Spain) were harvested between May and August 2001. The °Brix of the fruits ranged between 12.2 (*O. stricta*) and 13.6 (*O. undulata*). After they were harvested, they were carefully washed with water to remove the glochids before pigment extraction and then homogenized without peeling or removing the seeds.

Pigment Extraction. For extraction, the homogenate was stirred for 20 min in darkness using a 1:5 (w/v) ratio of fruit:solvent. Water, ethanol:water 80:20 (v/v), and 100 mM pH 5.5 citrate-phosphate buffer were used as solvents. After they were stirred, the samples were centrifuged at 15 000*g* and 10 °C for 10 min in a Z383K Hermle centrifuge (Wehingen, Germany) to remove the vegetal tissue residue. Supernatants were filtered through a 0.45 μm nylon Lida filter (Kenosha, WI), and the extracts obtained were analyzed spectrophotometrically and by high-performance liquid chromatography (HPLC).

Spectrophotometric Analyses. The visible spectra (350−650 nm) of the extracts were recorded using an Ati-Unicam UV2 spectrophotometer (Ati-Unicam, Cambridge, U.K.). The red pigment content, defined as the amount of pigment (mg) per 100 g of fresh fruit, was referred to betanin and determined by using the extinction coefficient of betanin at 535 nm ($E^{1\%}_{1cm} = 1120$) (*5*).

HPLC Analyses. A Waters modular liquid chromatographic system (Waters, Milford, MA) equipped with two M510 pumps, a M996 photodiode array detector (PDA), and a Rheodyne (Cotati, CA) model 7125 injector with a sample loop of 20 μL were used, along with the Millenium 2010 Chromatography Manager data system. A Spherisorb ODS-2 (Teknokroma, Barcelona, Spain), 5 μm, 25 cm × 4.6 mm i.d. column was used, and elution was carried out following the method previously proposed (*21*), using a gradient between 175 mM acetic acid in water and 175 mM acetic acid in acetonitrile as the mobile phase. The flow rate was 1 mL min^{-1}. Betacyanin pigments were monitored at 535 nm.

Storage and Thermal Stability. Both storage and thermal stability studies were performed using 100 mM citrate-phosphate buffers at pH values of 3, 4, 5, 6, and 7. Thermal stability was assayed at 50 and 90 °C and storage at 22 and 4 °C. Samples were withdrawn at different time intervals and analyzed spectrophotometrically and by HPLC. Pigment content and color retention were determined in triplicate for each sample. The thermal stability was expressed in terms of half-life time ($t_{1/2}$) calculated assuming first-order deactivation kinetics and using regression analysis of ln (% color retention) vs temperature exposition time.

Chemicals. Acetonitrile and ethanol were of HPLC grade. Acetic acid, sodium citrate, and sodium phosphate were of analytical reagent grade. All solvents were purchased from Lab Scan (Dublin, Ireland), except acetic acid, which was from Panreac (Spain). Water was purified in a Milli-Q water purification system from Millipore (Bedford, MA).

RESULTS AND DISCUSSION

Fruit Characteristics and Pigment Extraction. The physical properties of the fruits investigated were directly influenced by the species. The average sizes of the fruits were as follows: *O. stricta*, 2.5 cm × 6 cm; *O. undulata*, 5 cm × 9 cm; and *O. ficus-indica*, 4.5 cm × 8 cm. Other differences included peel thickness and seed number. *O. undulata* and *O. ficus-indica* fruits had a thick peel, while fruits from *O. stricta* had a very thin peel and the number of seeds was lower than in the other two species studied. These characteristics can make pigment extraction easier since neither peel nor seeds need be removed.

Table 1. Betacyanin Content (mg of Betanin/100 g Fresh Fruit) in Extracts from *Opuntia* Fruits[a]

solvent	betacyanin content (mg/100 g fresh fruit)		
	O. stricta	*O. undulata*	*O. ficus-indica*
water	80.1 ± 0.12	19.6 ± 0.09	15.2 ± 0.14
ethanol/water (80:20)	79.0 ± 0.11	19.4 ± 0.12	14.3 ± 0.08
citrate-phosphate buffer pH 5.5	67.0 ± 0.15	18.5 ± 0.10	14.5 ± 0.17

[a] Mean ± standard deviation from triplicate samples.

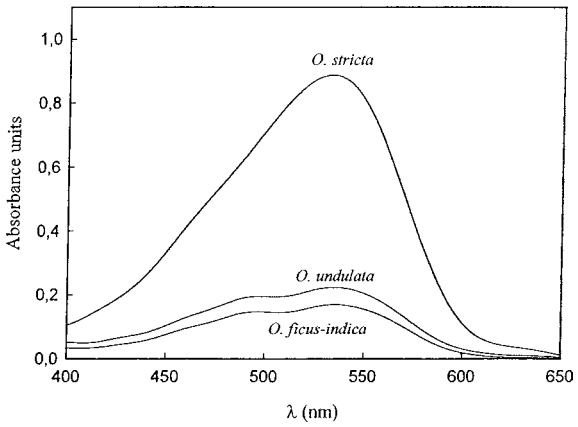


Figure 2. Visible spectra of *Opuntia* pigment extracts.

In investigations involving cactus pears, the peel and the seeds are usually removed manually (*18*, *24*, *27*). Although this procedure may give a higher pigment yield, it involves time-consuming manipulation. We, on the other hand, used a nonpeeling procedure to facilitate plant material manipulation, bearing in mind any future industrial extraction process.

Table 1 shows the results obtained for betacyanin extraction using the three solvents applied. In all cases, water extracted the highest level of pigments. As can be seen, *O. stricta* fruits showed the highest pigment content (80 mg/100 g fresh fruit), which was four times higher than that of *O. undulata* (20 mg/100 g fresh fruit) and around five times higher than in *O. ficus-indica* (15 mg/100 g fresh fruit). Although the extraction procedure used may have led to a lower pigment yield, the values obtained were high and, in the case of *O. ficus-indica*, comparable to other values reported so far (19 mg/100 g (*21*) and 14 mg/100 g (*23*)). Of great interest was the high amount of betacyanin found in *O. stricta* fruits. This was much higher than in the other *Opuntia* fruits studied (*21*, *23*) and even higher than that showed by some commercial red beets exploited for their purple color (40−60 mg/100 g (*4*), 71−77 mg/100 g (*14*)). *O. undulata* and *O. ficus-indica* extracts showed differences of around 5.5−6%, depending on the solvent used, while in *O. stricta* fruits these differences increased to around 16.5% (**Table 1**). In accordance with these results, water was chosen as the solvent for further experiments and all subsequent studies on chemical stability were performed in *O. stricta* fruits.

The pH of the aqueous extract of the pigments was 3.8 for *O. stricta* fruits, while for *O. undulata* and *O. ficus-indica* fruits the values were 6.2 and 5.9, respectively. It is clear then that there is a marked difference between cactus pears from *O. stricta* with respect to the other two species.

The visible absorption spectra (400−650 nm) of these extracts are reported in **Figure 2**, which illustrates the different pigment composition of the three extracts. While the *O. stricta* extract

Table 2. Effect of pH on the Colorant Capacity at 535 nm of *O. stricta* Extracts

pH	colorant capacity (%)	pH	colorant capacity (%)
3	96.8	6	98.8
4	98.7	7	96.4
5	100.0		

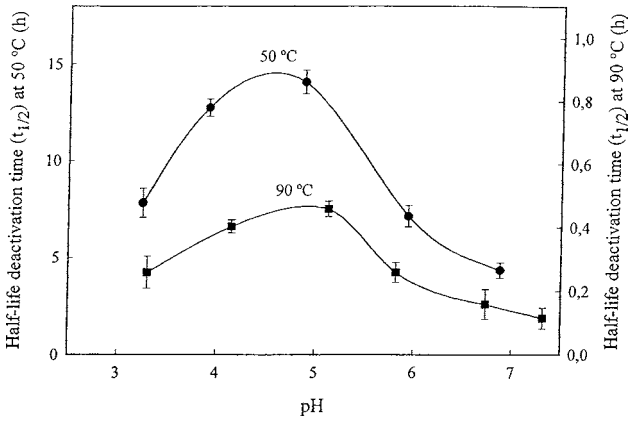


Figure 3. Influence of pH on the thermal stability of betacyanins from *O. stricta*.

had one peak at 535 nm, the extract from *O. undulata* had an additional peak at 484 nm and that of *O. ficus-indica* showed both peaks and an additional shoulder at about 465 nm. The peaks at around 460−480 nm are due to the presence of betaxanthins, with a yellow-orange color, while maximum absorbance at 535 nm corresponds to the presence of red betacyanins.

The spectral properties of the extracts were confirmed by HPLC analyses. In *O. stricta* extracts, two peaks (identified as betanin and isobetanin) were detected. In *O. undulata* betanin, isobetanin and the yellow pigment, indicaxanthin, were established as the main components, while in *O. ficus-indica* only betanin and indicaxanthin were detected (*29*).

Therefore, cactus pears from *O. stricta* have two important characteristics for red pigment production: First, this species has a very high pigment content, which is easily extracted in water, and second, *O. stricta* only contains the red pigments betanin and isobetanin, which are the compounds present in the additive, red beet (E-162). These characteristics make *O. stricta* a promising source of betacyanin pigments, which would be suitable for both food and pharmacological applications.

Effect of pH on the Thermal Stability of *O. stricta* Extracts. To ascertain the effect of pH on the colorant capacity of *O. stricta*, aqueous extracts at different pH values (3, 4, 5, 6, and 7) were obtained in citrate-phosphate buffers. The colorant capacity, expressed as a percentage of the maximum absorbance at 535 nm, is shown in **Table 2**. The maximum color was obtained at pH 5, with a slight decrease being observed at higher and lower values.

The thermal stability at different pH values (3, 4, 5, 6, and 7) was also studied at 50 and 90 °C (**Figure 3**). As expected, the extracts were more stable at 50 rather than at 90 °C, and in both cases, the highest stability was observed between pH 4 and pH 5. These results are similar to those reported for red beet extracts, which also are thermosensitive and very stable at around pH 5 (*6*, *7*, *9*, *13*).

The thermal degradation of betacyanins followed a first-order reaction kinetic and was dependent on pH. The half-life ($t_{1/2}$) at pH 5 was 30 min at 90 °C and 14 h at 50 °C. A first-order degradation kinetic for purified betanin under aerobic conditions has been previously reported (*12*, *13*). The $t_{1/2}$ value for betanin from *Beta vulgaris* roots described by Huang and von Elbe (*12*) of 22.6 min at 90 °C and pH 5 was lower than what we obtained in extracts from *O. stricta* fruits. Our data indicate higher stability than those obtained by other authors who reported a $t_{1/2}$ of 18.5 min for a red beet extract at 85.5 °C and pH 5.2 (*8*). Some components present in the extracts of cactus pears could act as pigment stabilizers, which would explain the behavior observed.

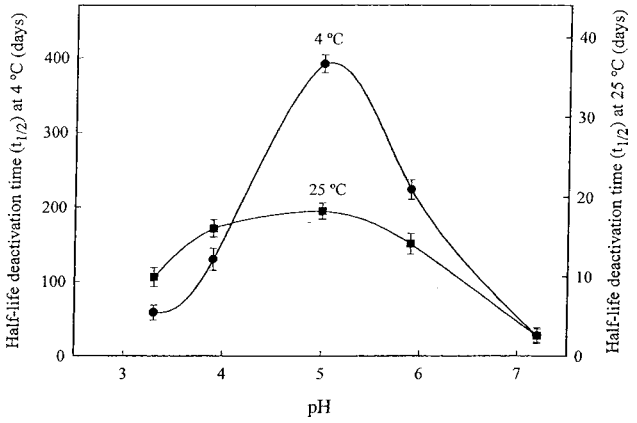


Figure 4. Influence of pH on the storage stability of betacyanins from *O. stricta*.

It has been reported that pigments from red beet extracts can be regenerated after heating (*12*, *14*). To confirm this, two extracts from *O. stricta* fruits at pH 3 and 7, respectively, were incubated for 4 and 6 h at 50 °C, with a color retention of 80 and 68%, respectively, at pH 3, and of 55 and 40%, respectively, at pH 7. To study regeneration, the samples were kept at 25 and 4 °C for 21 h, after which they were spectrophotometrically analyzed; no significant color regeneration was observed (data not shown).

Storage Stability of *O. stricta* Extracts. Storage stability was studied at different pH values (3, 4, 5, 6, and 7) and at 4 and 25 °C. The half-life times obtained are reported in **Figure 4**. The pigment extracts showed a very high storage stability, which was maximum at pH 5 and 4 °C. A $t_{1/2}$ of 11 days has been reported for red beet extract at 25 °C and pH 5 (*6*). In contrast, the same value reached 19 days in the present work and 392 days when the incubation temperature was 4 °C. The greater stability of betalains from *Cactaceae* fruits as compared with betalains from red beet, in general, is of great interest.

These results, together with the fact that betalains have no toxic effect on humans (*30*) and therefore represent a safe natural alternative to some synthetic colorants that are currently in use, would confirm *O. stricta* fruits as a promising source of betacyanin pigments for the food and pharmaceutical industry, not only for the high pigment concentration they contain but also for the high stability of their aqueous extracts.

Analyses of the Degradation Products from the Thermal Deactivation Experiments in *O. stricta* Extracts. To study the degradation products in our extracts, three assays at 50 °C and pH 3, 5, and 7 were performed. Samples were withdrawn at different time intervals and spectrophotometric and HPLC−PDA analyses were performed.

The visible absorption spectra of these samples are shown in **Figure 5**. Initially, only one peak at 535 nm could be detected, due to the exclusive presence of betanin and isobetanin. These compounds were progressively degraded, while absorbance at

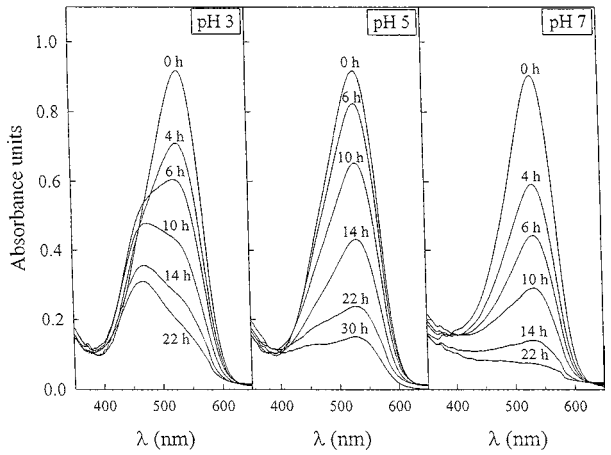


Figure 5. Influence of pH and temperature (50 °C) on the visible spectra of *O. stricta* pigments. The different lines correspond to the spectra after exposure times between 0 and 22 or 30 h.

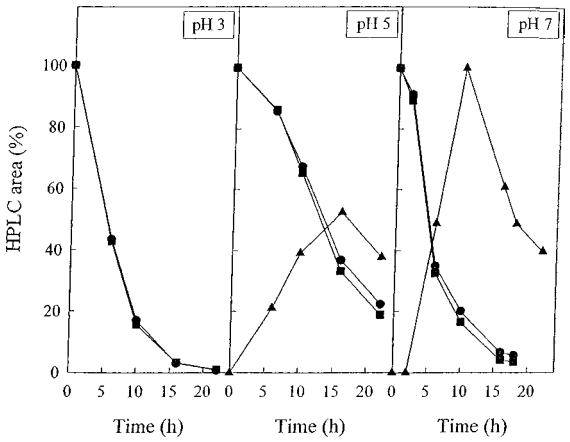


Figure 6. Time course of the content of betanin (●), isobetanin (■), and betalamic acid (▲) in *O. stricta* extracts after incubation (50 °C) at pH 3, 5, and 7.

535 nm decreased at the same time. At pH 7, a small shoulder was observed with maximum absorbance close to 410 nm, as a result of the degradation process. At pH 5, a new shoulder in the region between 455 and 465 nm could be detected, which was more intense in samples incubated for longer times (higher than 22 h). At pH 3, no shoulders were observed, while a peak in the 455−465 nm region was clearly depicted, especially in samples incubated from more than 6 h.

HPLC−PDA analyses of these samples pointed to betanin and isobetanin as the two main pigments. **Figure 6** shows the time course of these compounds with time for each pH value. Similar degradation rates were observed for both betacyanins. At pH 7, a compound with a maximum absorbance wavelength of 410 nm, which could be assumed to be betalamic acid, was detected. The content of this compound, which is highly probable to be betalamic acid, is presented in **Figure 6** as percent area as compared with the maximum value obtained. This degradation product of betanin and isobetanin could not be monitored at pH 3, but still at pH 5 and even better at pH 7. Degradation of betacyanins into betalamic acid and cyclo-DOPA-glycoside at pH 3 is stronger as compared to pH 5 and more slowly at pH 7. This fact would confirm that this degradation product would be betalamic acid, which is most stable at pH 9 (*31*), so its detection would be easier at pH 7 than at lower pH values. At pH 5 and at pH 7, this compound degraded after reaching a maximum value.

HPLC−PDA analyses of samples at pH 3 revealed the presence of two new degradation compounds absorbing at 457 and 535 nm. Future studies are required to ascertain their structures.

LITERATURE CITED

(1) Piattelli, M. The betalains: structure, biosynthesis and chemical taxonomy. In *The Biochemistry of Plants: A Comprehensive Treatise*; Conn, E. E., Ed.; Academic Press: New York, 1981; Vol. 7, pp 557−575.

(2) Wissgott, U.; Bortlik, K. Prospects for new natural food colorants. *Trends Food Sci. Technol*. **1996**, *7*, 298−302.

(3) Delgado-Vargas, F.; Jiménez, A. R.; Paredes-López, O. Natural pigments: Carotenoids, anthocyanins, and betains − Characteristics, biosynthesis, processing, and stability. *Crit. Rev. Food Sci. Nutr*. **2000**, *40*, 173−289.

(4) Sobkowska, E.; Czapski, J.; Kaczmarek, R. Red table beet pigments as food colorant. *Int. Food Ingredients* **1991**, *3*, 24−28.

(5) Kujala, T.; Loponen, J.; Pihlaja, K. Betalains and phenolics in red beet root (*Beta vulgaris*) peel extracts: extraction and characterization. *Z. Naturforsch*. **2001**, *56c*, 343−348.

(6) Sapers, G. M.; Hornstein, J. S. Varietal differences in colorant properties and stability of red beet pigments. *J. Food Sci.* **1979**, *44*, 1245−1248.

(7) Saguy, I. Thermostability of red beet pigments (betanin and vulgaxanthin-I): influence of pH and temperature. *J. Food Sci*. **1979**, *44*, 1554−1555.

(8) Saguy, I.; Kopelman, I. J.; Mizrahi, S. Thermal kinetic degradation of betanin and betalamic acid. *J. Agric. Food Chem*. **1978**, *26*, 360−362.

(9) von Elbe, J. H.; Maing, I. Y.; Amundson, C. H. Color stability of betanin. *J. Food Sci*. **1974**, *39*, 334−337.

(10) Saguy, I.; Goldman, M.; Bord, A.; Cohen, E. Effect of oxygen retained on beet powder on the stability of betanine and vulgaxanthine I. *J. Food Sci*. **1984**, *49*, 99−101.

(11) Simon, P.; Drdak, M.; Altamirano, R. C. Influence of water activity on the stability of betanin in various water alcohol model systems. *Food Chem*. **1993**, *46*, 155−158.

(12) Huang, A. S.; von Elbe, J. H. Kinetics of the degradation and regeneration of betanine. *J. Food Sci*. **1985**, *50*, 1115−1129.

(13) Huang, A. S.; von Elbe, J. H. Effect of pH on the degradation and regeneration of betanine. *J. Food Sci*. **1987**, *52*, 1689−1693.

(14) von Elbe, J. H.; Schwartz, S. J.; Hildebrand, B. E. Loss and regeneration of betacyanin pigments during processing of red beets. *J. Food Sci*. **1981**, *46*, 1713−1715.

(15) Han, D.; Kim, S. J.; Kim, S. H.; Kim, D. M. Repeated regeneration of degraded red beet juice pigments in the presence of antioxidants. *J. Food Sci*. **1998**, *63*, 69−72.

(16) Pedreño, M. A.; Escribano, J. Correlation between antiradical activity and stability of betanin from *Beta vulgaris* L. roots under different pH, temperature and light conditions. *J. Sci. Food Agric*. **2001**, *81*, 627−631.

(17) Cai, Y. Z.; Sun, M.; Corke, H. Colorant properties and stability of *Amaranthus* betacyanin pigments. *J. Agric. Food Chem*. **1998**, *46*, 4491−4495.

(18) Reynoso, R., García, F. A.; Morales, D.; González de Mejía, E. Stability of betalain pigments from a cactacea fruit. *J. Agric. Food Chem*. **1997**, *45*, 2884−2889.

(19) Stintzing, F. C.; Schieber, A.; Carle, R. Betacyanins in fruits of deep-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose. *Food Chem*. **2002**, *77*, 101−106, 517.

(20) Wybraniec, S.; Platzner, I.; Geresh, S.; Gottlieb, H. E.; Haimberg, M.; Mogilnitzki, M.; Mizrahi, Y. Betacyanins from vine cactus *Hylocereus polyrhizus*. *Phytochemistry* **2001**, *58*, 1209−1212.

(21) Fernández-López, J. A.; Almela, L. Application of high-performance liquid chromatography to the characterization of the betalain pigments in prickly pear fruits. *J. Chromatogr. A* **2001**, *913*, 415−420.

(22) Stintzing, F. C.; Schieber, A.; Carle, R. Identification of betalains of 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.* **2002**, *50*, 2302−2307.

(23) Forni, E.; Polesello, A.; Montefiori, D.; Maestrelli A. High-performance liquid chromatographic analysis of the pigments of blood-red prickly pear (*Opuntia ficus-indica*). *J. Chromatogr*. **1992**, *593*, 177−183.

(24) Merin, U.; Gagel, S.; Popel, G.; Bernstein, S.; Rosenthal, I. Thermal-degradation kinetics of prickly pear-fruit red pigment. *J. Food Sci*. **1987**, *52*, 485−486.

(25) Sáenz, C.; Sepúlveda, E.; Araya, E.; Calvo, C. Colour changes in concentrated juices of prickly pear (*Opuntia ficus-indica*) during storage at different temperatures. *Lebensm. Wiss. Technol*. **1993**, *26*, 417−421.

(26) Stintzing, F. C.; Schieber, A.; Carle, R. Phytochemical and nutritional significance of cactus pear. *Eur. Food Res. Technol*. **2001**, *212*, 396−407.

(27) Turker, N.; Coçkunes, Y.; Ekiz, H. I.; Aksay, S.; Karabada, E. The effects of fermentation on the thermostability of the yellow-orange pigments extracted from cactus-pear (*Opuntia ficus-indica*). *Eur. Food Res. Technol*. **2001**, *212*, 213−216.

(28) Sawaya, W. N.; Khatchadourian, H. A.; Safi, W. M.; Al-Muhammad, H. M. Chemical characterization of prickly pear pulp, *Opuntia ficus-indica*, and the manufacturing of prickly pear jam. *J. Food Technol*. **1983**, *18*, 183−193.

(29) Fernández-López, J. A.; Castellar, R.; Obón, J. M.; Almela, L. Screening and mass-spectral confirmation of betalains in cactus pears. *Chromatographia* **2002**, *56*, 591−595.

(30) Böhm, H.; Rink, E. Betalains. In *Cell Culture and Somatic Cell Genetics of Plants*; Academic Press: New York, 1988; pp 449−463.

(31) Kimler, L.; Larson, R. A.; Messenger, L.; Moore, J. B.; Mabry, T. J. Betalamic acid, a new naturally ocurring pigment. *Chem. Commun*. **1971**, *21*, 1329−1330.

Received for review October 15, 2002. Revised manuscript received January 21, 2003. Accepted January 27, 2003. This research was partly funded by the "Ministerio de Ciencia y Tecnología" from Spain, Project AGL2000-0497.

JF021045H