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ARTICLE

Stabilization of the Bioactive Pigment of *Opuntia* Fruits through Maltodextrin Encapsulation

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Betalains are water-soluble, nitrogen-containing pigments of growing interest in the food industry. They are present in most plants belonging to the order Caryophyllales, where they fulfill the role of anthocyanins, and are divided into two groups: violet betacyanins and yellow betaxanthins. They are bioactive molecules that account for health-promoting properties, recently described for cactus pears (*Opuntia*). In this work, the characteristic betalain of cactus pears, indicaxanthin, is obtained purely, and its stability is highly promoted by its encapsulation in a maltodextrin matrix. A suitable spray-drying procedure for encapsulation is described, and a bright yellow powder is obtained. The stability is analyzed under different conditions. In the absence of light, pure encapsulated pigment can be stored at 20 °C for months without appreciable loss of the bioactive substance and color variation. Furthermore, free radical scavenging and antioxidant properties of the pigment are studied under the $ABTS^{\bullet+}$ radical and ferric reducing antioxidant power assays, in the presence and in the absence of maltodextrins. The stabilization of pure betalain pigments may boost the use of these bioactive and natural coloring molecules.

KEYWORDS: Indicaxanthin; betalain; encapsulation; stability; *Opuntia*; antiradical

INTRODUCTION

Betalains are water-soluble, nitrogen-containing pigments of growing interest in the food industry. They are present in most plants belonging to the order Caryophyllales, where they fulfill the role of anthocyanins (*1*), and are divided into two groups: betacyanins and betaxanthins (*2*, *3*). Betacyanins are violet in color and contain a *cyclo*-DOPA residue (usually glycosilated), while betaxanthins present yellow coloration and may contain different amino acids. Betalains bestow bright colors to flowers of a great variety of plant genera, like *Mirabilis* (*4*, *5*) and *Portulaca* (*6*, *7*), but they are also present in edible sources like beet root (*Beta vulgaris*) (*8*, *9*) and cactus pear (*Opuntia*) (*10*, *11*).

Betalains have been described as powerful antioxidants and free radical scavengers. The first studies that demonstrated a radical scavenging capacity in betalains were carried out with pigments extracted from beet root (*15*). Later, the activity of betalains present in other sources was investigated (*14*, *16*, *17*). Betalains also inhibit the peroxidation of linoleic acid and the oxidation of LDL (low-density lipoproteins) (*18*). In model animals (mice), the inhibition of skin and liver tumor formation has been demonstrated with low concentrations of dietary pigments (*19*). In humans, plasma concentrations of betalains after their ingestion are sufficient to promote their incorporation into the LDL and red cells, which are then protected from oxidative damage and hemolysis (*20*).

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In recent years, evidence has accumulated to support the health-promoting potential of betalain-containing foods, particularly cactus pears (*12*). The consumption of the fruits of *Opuntia* is involved in maintaining the body's redox balance, decreasing oxidative damage, and improving antioxidant status in humans (*13*). Betalains present in the fruits have been identified as important antioxidant components that are likely to account for the activities observed (*14*).

In cactus pears, the characteristic betalain is indicaxanthin, a betaxanthin derived from the amino acid proline first isolated from *Opuntia ficus indica* fruits (*10*). Indicaxanthin is responsible for the yellow and orange shades of the fruits and combines with the violet betacyanin betanin (*21*) to bestow reddish colors, depending on the cultivar (*22*). Betanin is the best-known betalain and exhibits a high free radical scavenging capacity (*23*). Because it is also the main pigment found in red beet roots, betanin is the main coloring component present in the E-162 food color additive. This is a natural additive derived from red beet root extracts and commercially available as a deep violet pasteurized liquid or powder. It is used by the food industry to give colors from pink to violet to foods and beverages (*24*). An alternative betalain-based coloring additive has been proposed using *Opuntia* fruits extracts (*25*), making it possible to obtain the extract in the form of a red-purple powder (*26*, *27*). However, probably due to stability concerns (*28*), no attempt has been made to encapsulate a pure betalain or to obtain a yellow colorant based on betaxanthins.

In this work, indicaxanthin stability is highly improved by its encapsulation in a maltodextrin matrix. A suitable spray-drying

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procedure for encapsulation is described to obtain a bright yellow powder containing a single pigment. The stability is analyzed under different conditions, and indicaxanthin free radical scavenging and antioxidant properties are studied in depth. The stabilization of pure betalain pigments may boost the use of these natural bioactive and coloring molecules in the food industry and promote their application in the pharmaceutical and cosmetic areas.

MATERIALS AND METHODS

Chemicals and Reagents. Red beet juice concentrate (B-50-WS) was purchased from CHR Hansen (Madrid, Spain). Maltodextrins (Paselli SA2) were from Probelte, S.A. (Murcia, Spain). Other chemicals and reagents were obtained from Sigma (Madrid, Spain). Solvents were from Merck (Spain). High-performance liquid chromatography (HPLC)-grade methanol was purchased from Labscan Ltd. (Dublin, Ireland). Distilled water was purified using a Milli-Q system (Millipore, Bedford, MA).

HPLC Analysis. A Shimadzu ELSD—LT II apparatus (Kyoto, Japan) equipped with a SPD-M20A photodiode array detector (PDA) was used for analytical HPLC separations. Reverse phase chromatography was performed with a Kromasil C_8 5 μm column (150 mm × 4.6 mm). Gradients were formed with solvents A and B. Solvent A was H_2O with 0.05% TFA, and solvent B was composed of methanol with 0.05% TFA. A linear gradient was performed over 21 min from 5 to 35% B. The flow rate was 1 mL/min, operated at 25 °C. The injection volume was 20 μL. Elutions were monitored at 405 (betalamic acid), 480 (betaxanthin), and 536 nm (betacyanin). Analyses were performed in duplicate.

Spectroscopy. A Jasco V-630 spectrophotometer (Jasco Corp., Tokyo, Japan), attached to a Tectron thermostatic bath (JP Selecta, Barcelona, Spain), was used for absorbance spectroscopy. The indicaxanthin concentration was evaluated taking a molar extinction coefficient of $\varepsilon = 48000$ M^{-1} cm^{-1} at 485 nm (*29*, *30*). Measurements were made in water at 25 °C.

***Opuntia* Fruits Pigment Extraction.** Edible yellow and violet *Opuntia* fruits were collected from naturally growing plants in Lorca (Murcia, South-Eastern Spain). Plants were growing side by side under the same conditions. Once the thorns had been removed, the fruits were peeled, and the epidermal layer was separated (*22*), thus obtaining three different fractions: flesh, peel, and epidermal layer. The last of these was carefully washed with distilled water and dried with absorbent paper. Pigments were extracted in ice cold 10 mM sodium phosphate buffer, pH 6.0, containing 10 mM ascorbic acid (AA) in a Polytron homogenizer (Kinematica AG, Switzerland) (10 s, 2 pulses, at medium speed). The homogenate was filtered through nylon cloth and centrifuged at 120000*g* for 40 min. The supernatant was used for pigments analysis under the HPLC system described above. Analyses were performed in triplicate. Standard indicaxanthin, betanin, and betanidin were obtained as described previously (*7*). Standard pigments were characterized spectrophotometrically and chromatographically and by electrospray ionization mass spectrometry (ESI-MS) (*31*). Pigments from extracts samples and standards had the same retention times (coelution analyses were performed) and had superimposed spectra.

Semisynthesis of Indicaxanthin. Indicaxanthin was obtained as an immonium condensation product of betalamic acid with proline. Synthesis was carried out following a method described previously by Gandia-Herrero et al. (*31*) and by Wyler et al. (*32*) In short, red beet juice concentrate was filtered through a 10 KDa membrane in an ultrafiltration step (QuixStand System, GE Healthcare). Betanin purified from this filtered solution was used as the starting material. Basic hydrolysis (pH 11.4) of betanin released betalamic acid, which was then condensed with proline after reaching pH 5.0. The corresponding betaxanthin was obtained, revealed by a characteristic deep yellow color (the betaxanthin maximum wavelength is $\lambda_m = 485$ nm). The whole process was carried out under nitrogen atmosphere. Once synthesis had been achieved, a C-18 solid phase extraction step was performed, and an automated system was used for pigment purification. Thirty-five milliliter C-18 cartridges (Waters, Milford, MA) were conditioned with 70 mL of acetone followed by 70 mL of purified water. Salts were removed by rinsing the column with 10 mM sodium phosphate buffer, pH 6.0, at 4 °C. Indicaxanthin was eluted with acetone and then concentrated to dryness under vacuum. The residue was redissolved in water for further use or stored at −80 °C.

Purification of Indicaxanthin. Anionic exchange chromatography of indicaxanthin was performed in an Äkta purifier apparatus (General Electric Healthcare, Milwaukee, WI). The equipment was operated via a PC using Unikorn software version 3.00. Elutions were monitored at 280, 480, and 536 nm. The solvents used were 10 mM sodium phosphate buffer, pH 6.0 (solvent A), and 10 mM sodium phosphate buffer, pH 6.0, with 2 M NaCl (solvent B). A 25 mm × 16 mm, 5 mL Q-Sepharose Fast Flow column (cross-linked agarose with quaternary ammonium as an exchanger group, 90 μm particle size) purchased from General Electric Healthcare was used. After sample injection, the elution process was as follows: 0% B from 0.0 to 35 mL; after a washing step, a linear gradient was performed over 45 mL from 0 to 16% B, with 2 mL fractions being collected. The injection volume was 10 mL, and the flow rate was 1.0 mL min^{-1}. Salts were removed by solid phase extraction using C-18 cartridges as described.

Spray Drying. Maltodextrin (20% w/v) was dissolved in purified indicaxanthin solution (1 mM) under vigorous vortexing. Particles were prepared in a Büchi B-290 mini Spray Dryer. Four inlet air temperatures were investigated 120, 140, 160, and 210 °C. Liquid feed was 2.5 mL/min, atomization air flow was 246 L/h, and the drying air flow was 36 m^3/h. The particles were separated from the drying air by a cyclone. The humidity percentage of the powders obtained was determined with a Kern MLS electronic moisture analyzer (Balingen, Germany). Powders were stored over silica gel in a desiccator at 4 °C until analysis. The indicaxanthin content in maltodextrin powders was evaluated by dissolving a weighted amount of material in 10 mM sodium phosphate buffer, pH 6.0, and further spectrophotometrical and HPLC analysis. The pigment stability (%) was calculated as: [(μg indicaxanthin/mg powder) final/(μg indicaxanthin/mg powder) initial] × 100. The CIELAB coordinates, L^* (lightness), a^* (red-green), and b^* (yellow-blue), of the maltodextrin encapsulated betaxanthin were determined using a JASCO V-650 spectrophotometer equipped with an ISV-722 integrating sphere (Jasco Corp., Tokyo, Japan).

Free Radical Scavenging Activity. The antiradical capacity of indicaxanthin was evaluated by following its effect on stable free radical $ABTS^{\bullet+}$ [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]. The decolorizing activity on $ABTS^{\bullet+}$ solutions was monitored spectrophotometrically at $\lambda = 414$ nm (*15*). The $ABTS^{\bullet+}$ radical was prepared from 2 mM ABTS through peroxidase activity (88 units/L commercial horseradish peroxidase type VI, obtained from Sigma) in the presence of H_2O_2 (45 μM), in 12 mM sodium acetate buffer, pH 5.0. The reactive was then diluted by 2/3 with the addition of samples, carrying out the reactions in 53 mM sodium phosphate buffer, pH 7.0. Other conditions are specified in the text. Measurements of 96-well plates were performed at $t = 0$ and after 24 h incubations at 20 °C in a Synergy HT plate reader (Bio-Tek Instruments, Winooski, United States). The TEAC (Trolox equivalent antioxidant activity) was evalualted as the concentration of Trolox with the equivalent antioxidant activity of a 1 mM concentration of the experimental substance. All experiments were performed in triplicate, and mean values and standard deviations were plotted. The final volume was 300 μL (calculated path length = 0.87 cm). The detector linearity under the assay conditions was confirmed ($r = 0.999$). Data analysis was carried out by using linear regression fitting using Sigma Plot Scientific Graphing for Windows version 8.0 (2001; SPSS, Chicago, IL). In each case, errors associated with the results provided were calculated on the basis of the residual standard deviation around the regression line.

Antioxidant Capacity. The antioxidant activity of indicaxanthin was characterized by means of the reduction of Fe(III) to Fe(II). The method described by Benzie and Strain (*33*) was used to evaluate the ferric reducing antioxidant power (FRAP). Briefly, $FeCl_3$ solutions at a final concentration of 1.48 mM, in 223 mM sodium acetate buffer, pH 3.6, were used. Fe(III) reduction to Fe(II) was observed through the addition of the reagent 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) at a final concentration of 741 μM, which is able to yield a colored complex with Fe(II). The reduction reaction was monitored spectrophotometrically at $\lambda = 593$ nm in a Synergy HT plate reader (Bio-Tek Instruments). All measurements were performed in triplicate, and mean values and standard deviations were plotted. Data analysis was carried out as indicated above.

Indicaxanthin Stability. *Stability of Soluble Indicaxanthin.* The stability of soluble indicaxanthin was analyzed at 20 °C and at different pH values, either in the absence or in the presence of light (light intensity = 2520 lm/m^2). The assay medium contained the pigment (200 μM) in 50 mM sodium acetate buffer (pH 4−5) or 50 mM sodium phosphate

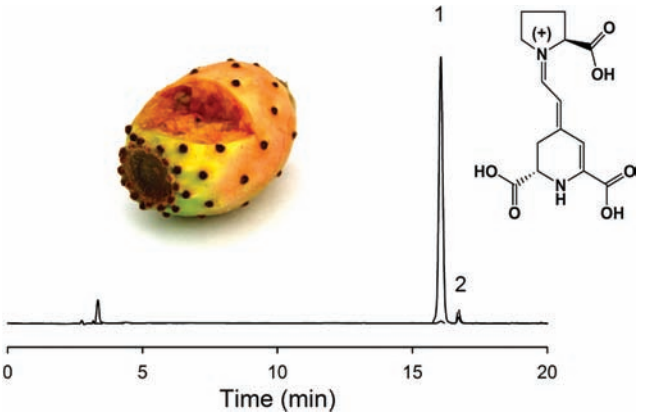

Figure 1. Chromatogram obtained for the HPLC analysis of flesh from yellow prickly pear: 480 (—) and 536 nm (- - -). Twenty microliters of an extract solution containing 550 μM indicaxanthin was injected. Peak 1 corresponds to indicaxanthin, and peak 2 corresponds to betanin. The full scale is 0.35 absorbance units. Insets: picture showing a yellow fruit and structure for indicaxanthin.

buffer (pH 6−8). Aliquots corresponding to the different pH values were taken at different times and analyzed by HPLC, as previously described, to follow the degradation of the betaxanthin. Each aliquot contained the betaxanthin, at an initial concentration of 50 μM in 150 mM sodium phosphate buffer, pH 6.0.

Stability of Maltodextrin Encapsulated Indicaxanthin. The powder containing the encapsulated pigment was stored at −20, 4, and 20 °C in the dark, and it was also stored at 20 °C in the presence of light (light intensity = 2520 lm/m^2). Aliquots were taken at different times and dissolved in 10 mM sodium phosphate buffer, pH 6.0, to an initial concentration of 340 μM for HPLC analysis.

Scanning Electron Microscopy (SEM). Particle morphology was evaluated by SEM. Powders were attached to pieces of double-sided adhesive tape mounted on SEM stubs, coated with gold under vacuum using a SEM coating System Biorad-Polaron Division, and examined with a JEOL JSM-6100 (Jeol, Tokyo, Japan) scanning electron microscope operated at 15 kV.

RESULTS AND DISCUSSION

Pigment Analysis in *Opuntia* Fruits. The existence of betaxanthins in the genus *Opuntia* has been known since the early description of these pigments by Piattelli et al. (*10*). Recent studies have shown the major presence of the pigment indicaxanthin and detected other minor novel betaxanthins (*11*). Violet fruits of *Opuntia* plants contain the betacyanin betanin in varying amounts (*22*). In this study, two different lines of prickly pear fruits of deep yellow and violet colors were used to confirm and quantify the presence of indicaxanthin in this edible fruit. The chromatogram obtained for the yellow fruit flesh analysis is shown in **Figure 1**. As can be seen, two major peaks appear, with peak 1 corresponding to indicaxanthin (structure shown in **Figure 1** inset). The identity was determined by retention time and absorbance spectrum and confirmed by coelution with semisynthetic indicaxanthin standard. The pigment content was calculated to be above 0.14 mg/g fresh weight in the flesh fraction, which is the edible part. Betanin was only present at 0.001 mg/g fresh weight. A similar result was found for the yellow fruit peel. According to HPLC analysis, coloration of violet fruits is due to the joint presence of indicaxanthin and the betacyanin betanin (betanidin-5-*O*-β-glucoside), although trace amounts of betanidin were also detected for the flesh and peel. **Table 1** summarizes the pigment content detected in the *Opuntia* fruits.

Indicaxanthin was obtained pure by condensation between betalamic acid and the amino acid proline (*32*). The sample was

Table 1. Pigment Content in Yellow and Violet Phenotypes of *Opuntia* Fruits[a]

	yellow			violet		
	flesh	peel	epidermis	flesh	peel	epidermis
indicaxanthin	145.3 ± 3.5	146.2 ± 3.5	12.1 ± 3.1	54.1 ± 2.8	40.2 ± 2.5	16.0 ± 3.4
betanin	1.2 ± 0.5	3.5 ± 0.7	2.0 ± 0.7	34.1 ± 1.2	88.4 ± 2.9	35.1 ± 1.9
betanidin	ND[b]	ND	ND	traces	traces	ND

[a] All data are shown in μg/g fresh weight. [b] Not detected.

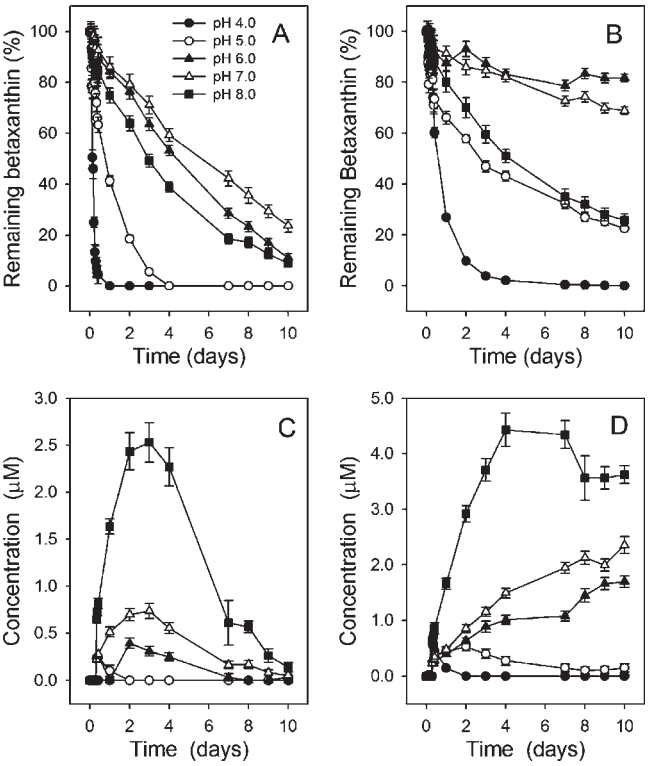

Figure 2. Percentage of the remaining betaxanthin as a function of the incubation time of indicaxanthin at 20 °C in the presence (**A**) or in the absence (**B**) of light at different pH values. Betalamic acid concentration in the presence (**C**) or in the absence (**D**) of light under the same conditions.

applied to a C-18 cartridge to remove salts and then used for anionic exchange chromatography purification in an automated system (FPLC). This protocol rendered indicaxanthin free from the amino acid and without *cyclo*-DOPA-glucoside, which is able to reverse the reaction to the formation of betanin. Proline was unable to interact with the column under the working conditions and was totally washed out as an unbound fraction. Afterward, a C-18 cartridge was used to remove the sodium chloride. The purity was confirmed by HPLC analysis with PDA detection.

Indicaxanthin Stability. Studies on the chemical stability of betalains have focused mainly on betanin (*34*) and betanidin (*35*). Huang and von Elbe (*34*) evaluated the influence of pH on the stability of betanin. Degradation kinetics of betanidin were analyzed, under different conditions of pH and ionic strength, demonstrating that pH values over 6.0 and high ionic strength reduced the pigment stability (*35*). Although data have been reported for other pigments, such as amaranthin (*36*), to our knowledge, kinetic studies on the degradation of indicaxanthin have not been carried out.

The stability of soluble indicaxanthin was analyzed at 20 °C and at different pH values, either in the presence (**Figure 2A**) or in the absence (**Figure 2B**) of light. As can be seen, the presence of light increased the degradation of indicaxanthin for all of the pH

Table 2. Values of Indicaxanthin Initial Degradation Rates in the Presence and Absence of Light and at Different pH Conditions[a]

	pH				
degradation rate (μM/h)	4	5	6	7	8
light	31.6 ± 2.6	6.8 ± 2.6	2.3 ± 1.0 c	0.7 ± 0.9 c	3.5 ± 0.9
darkness	4.5 ± 1.6 a	5.2 ± 2.4 a	1.5 ± 1.7 b	1.3 ± 0.6 b	1.8 ± 1.1 b

[a] For letters, according to stadistical analysis (*t* test, $0.2 < P < 0.9$), data are not significantly different.

values assayed. The data obtained, at each pH, during the first 6 h of the experiment could be adjusted to first order kinetics. The corresponding degradation rates were calculated from the slope of the linear regression fittings (**Table 2**). These results indicate that the stability of the pigment was lower at the pH values of 4, 5, and 8. The presence of light decreased the stability of the betaxanthin significantly at these pH values. As can be seen, the pigment was more stable at pH 6 and 7. This stability was similar in the presence and absence of light at short time (6 h), as shown by the degradation rates. However, **Figure 2A**,**B** clearly evidence that the exposure of indicaxanthin to the presence of light over a long period of time (240 h) considerably diminished the stability of the pigment, even at these pH values. Therefore, we can conclude that the betaxanthin was more stable in the absence of light and at pH range between 6 and 7.

To analyze if betalamic acid was a degradation product from indicaxanthin, its concentration was determined by HPLC (**Figure 2C**,**D**). The concentration of betalamic acid could only be evaluated at pH values higher than 5, due to the instability of betalamic acid at acidic pH values, being most stable at pH 8. The results obtained showed that the formation of betalamic increased as the pigment concentration decreased, reaching a maximum after 4 days. After that moment, there is a decrease in the betalamic acid concentration due to its instability (*34*). This betalamic acid concentration, produced as a result of the betaxanthin degradation, accounted for slightly less than 1% of the initial betaxanthin concentration (50 μM). In addition, the betalamic acid presence was higher in the absence of light (4 μM) (**Figure 2D**) than in its presence (2.5 μM) (**Figure 2C**).

Maltodextrin Encapsulation of Indicaxanthin. Spray drying of indicaxanthin was carried out using 20% (w/v) maltodextrin. The inlet air temperature was varied to optimize the spray drying conditions. The indicaxanthin encapsulation yields were in the range from 93 to 90% using 120, 140, 160, and 210 °C intlet temperatures. The humidity percentage of the yellow powders decreased from 8 (w/w) to 4% (w/w) with the increase of the different intlet temperatures. In **Figure 3**, SEM microphotographs of the powders at the end of the drying process for the three best drying conditions are shown. When drying at low temperatures (120 °C; **Figure 3A**), a greater degree of shrinkage is observed than when drying at higher temperatures (140 and 160 °C; **Figure 3B**,**C**, respectively). Mechanisms involved in shrinkage and deformation are, in general, more pronounced when drying at low temperatures since water diffusion is slower, allowing more time for structures to deform, shrink, and collapse (*37*, *38*). It is also possible to observe that particles tend to inflate, form a crust, and break when dried at high temperatures. These phenomena have been related to rapid evaporation and to high pressures generated inside the particles (*37*). Taking into account the stability of the indicaxanthin microencapsulated and the SEM images, the optimal inlet temperature for spray drying was considered 140 °C, and a bright yellow powder was obtained (**Figure 3D**).

Free Radical Scavenging Activity of Indicaxanthin. The free radical scavenging activity of a molecule can be evaluated by its

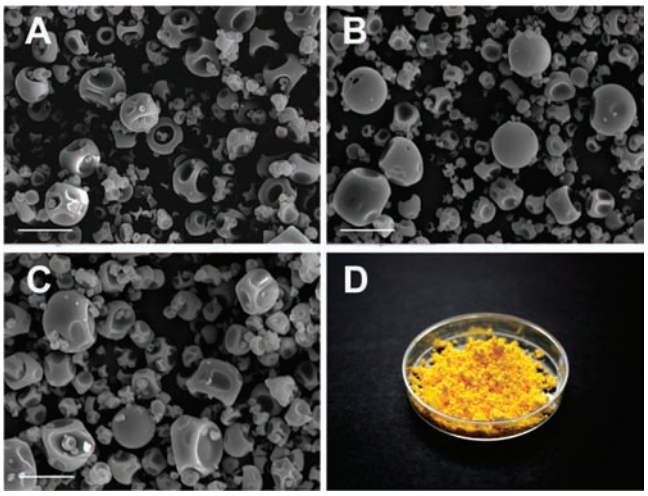

Figure 3. Scanning electron microscope images for maltodextrin-encapsulated indicaxanthin powders. Samples were obtained under the conditions specified in the Materials and Methods at the drying inlet temperatures of 120 (**A**), 140 (**B**), and 160 °C (**C**). The scale bar is 10 μm in all cases. Panel **D** shows a macroscopic picture for the final sample obtained at 140 °C.

effect on stable colored solutions of radical $ABTS^{\bullet+}$ (*39*). The assay is based on monitoring the decrease in the absorbance of the radical solution. The activity of the indicaxanthin was compared with that of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a potent antiradical, water-soluble derivative of vitamin E (*39*). **Figure 4A** shows the radical depletion dependence on betaxanthin concentration as compared to Trolox. The betaxanthin was added either as a soluble pigment or as a maltodextrin-encapsulated powder. When increasing concentrations of the betaxanthin or Trolox were added to the $ABTS^{\bullet+}$ radical mixture, a linear decrease was observed in the removal of the radical. As can be seen, the indicaxanthin exhibits higher free radical scavenging capacity than Trolox. This activity was retained by the pigment encapsulated in a maltodextrin matrix. When maltodextrins, without pigment bound, were added to the reaction medium, a slight decrease in absorbance was obtained; this is probably caused by the presence of hydroxyl groups at the matrix surface.

The TEAC value of soluble indicaxanthin, as calculated from the slopes of **Figure 4A**, is 2.7 ± 0.3 mM. For spray-dried indicaxanthin, the same contribution of the matrix as commented above can be seen. The TEAC value is of particular relevance if it is compared to potent known antiradical compounds, such as resveratrol (TEAC = 2.7 mM) (*40*). In addition, it is in strong contrast to studies where the lack of an aromatic ring system or of hydroxy groups is linked to no radical scavenging capacity at all (*41*).

Thus, in betaxanthins and in agreement with previous results (*42*), there is an "intrinsic activity" not linked to the presence of hydroxy groups or aromaticity in the pigment structure, which might be associated with the common electronic resonance system supported between the two nitrogen atoms and be general to all betalains. In other studies, free radical scavenging activity has also been reported for betaxanthins derived from glutamic acid (*15*) and tryptophan (*17*).

Effect of pH on the Free Radical Scavenging Activity of Indicaxanthin. The pH was varied in the range of 3.5−8.5 in the assay medium to evaluate its effect on the activity of indicaxanthin. Sodium acetate was used as a buffer for pH values ranging from 3.5 to 5.5 and sodium phosphate for 5.5 to 8.5. No difference was obtained for the activity measured for both buffers at pH 5.5.

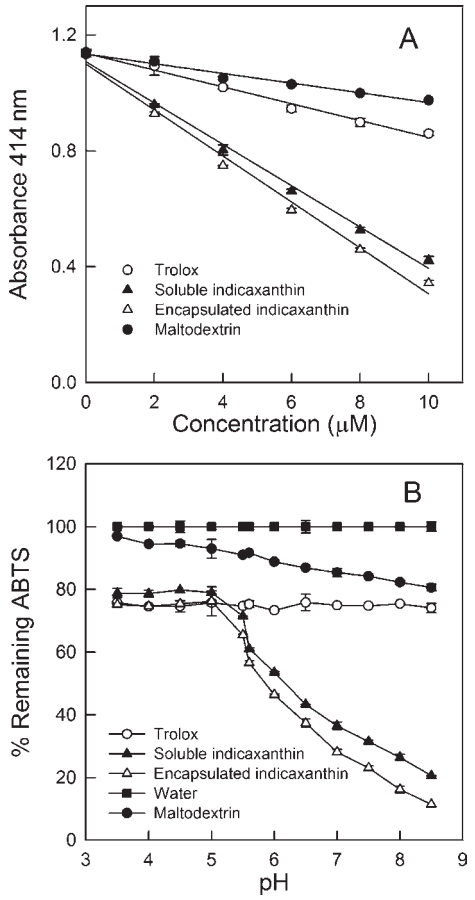

Figure 4. (**A**) $ABTS^{\bullet+}$ radical depletion dependence on indicaxanthin concentration as compared with Trolox. $ABTS^{\bullet+}$ was used at a concentration 47 μM. The reaction was followed spectrophotometrically at λ = 414 nm, at pH 7.0. For clarification, maltodextrin control is included within the same graph. It was used at the same concentration present in the maltodextrin-encapsulated indicaxanthin: 0.46 (2 μM indicaxanthin), 0.92 (4 μM indicaxanthin), 1.38 (6 μM indicaxanthin), 1.84 (8 μM indicaxanthin), and 2.30 mg/mL (10 μM indicaxanthin). (**B**) Indicaxanthin free radical scavenging activity dependence on pH. $ABTS^{\bullet+}$ radical initial concentration was 47 μM. Indicaxanthin and Trolox were added at a final concentration of 10 μM. For maltodextrin-encapsulated indicaxanthin and maltodextrin control, the final maltodextrin concentration was 2.30 mg/mL.

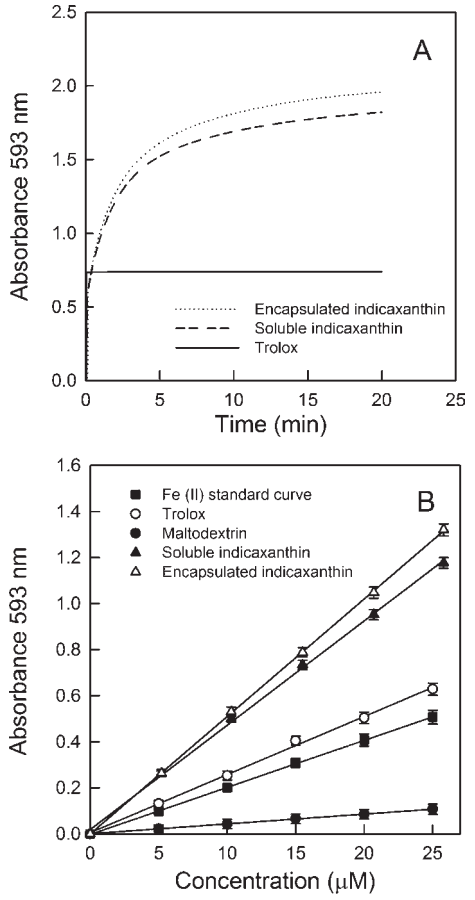

Figure 5. (**A**) FRAP reaction kinetics with individual antioxidants (25 μM); rate of increase in absorbance at 593 nm for maltodextrin-encapsulated indicaxanthin, soluble indicaxanthin, and Trolox. The maltodextrin final concentration was 5.75 mg/mL. (**B**) Antioxidant power of indicaxanthin through its ferric reducing ability and standard curve. The effect was evaluated spectrophotometrically at λ = 593 nm. The Fe(III) initial concentration was 1.48 mM.. Maltodextrin control is included within the same graph. It was used at the same concentration present in the maltodextrin-encapsulated indicaxanthin: 1.15 (5 μM indicaxanthin), 2.30 (10 μM indicaxanthin), 3.44 (15 μM indicaxanthin), 4.60 (20 μM indicaxanthin), and 5.75 mg/mL (25 μM indicaxanthin).

Figure 4B shows how at pH values above 5.5 there is a significant increase in the free radical scavenging activity for both soluble and encapsulated indicaxanthin. At pH values below 5.5, there is a basal activity. The behavior for the pigments in this range is analogous to that found for Trolox, removing around 20% of the initial radical.

The pH dependence on the measured activity suggests the existence of a protonation equilibrium in the pigment molecules. A similar effect of pH on the radical scavenging activity of betaxanthins was found not only for different betaxanthins (*42*) but also for betanin, which also has very high radical scavenging activity (*23*), indicating that this is due to the common electronic resonance system and is related to the deprotonation of the NH group present in all betalains. In the same manner, for flavonoids, deprotonation generates a phenolate anion, which is a better electron donor and a more active antioxidant (*43*, *44*).

Antioxidant Activity of Indicaxanthin. The antioxidant activity of betaxanthins was characterized by their capacity to directly reduce Fe(III) to Fe(II). The FRAP assay was used (*33*), monitoring the reduction reaction spectrophotometrically at λ = 593 nm.

Figure 5A shows the increase in absorbance at 593 nm for both soluble and encapsulated pigments as compared to that of Trolox. The reaction with indicaxanthin is slower than for Trolox, reaching a plateau after 15 min. Therefore, an increase in absorbance at 593 nm after 16 min of reaction was routinely chosen to measure the antioxidant activity.

Figure 5B shows the dose−response lines for the antioxidants assayed. Solutions of known concentrations of iron Fe(II) ($FeSO_4$) were used to set up a calibration curve. This figure shows the signals obtained for the ferric reduction by the soluble and spray-dried indicaxanthin assayed as well as for Trolox, as compared to the absorbance obtained for the calibration curve.

For comparative purposes, the Trolox molar equivalence in the reduction of Fe(III) was determined. For soluble indicaxanthin, it is 1.64 ± 0.04 mM, and a small contribution of the matrix can be seen for spray-dried indicaxanthin. Therefore, indicaxanthin presented a higher ferric reducing activity than Trolox.

Therefore, indicaxanthin presents high antioxidant and free radical scavenging activities. This may be general to all betalains, which contain a similar electronic resonance system (*42*).

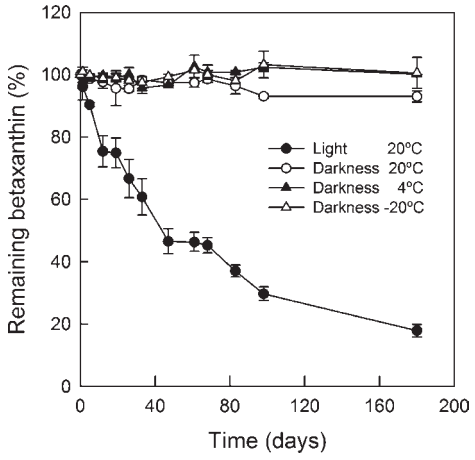

Figure 6. Stability analysis of maltodextrin-encapsulated indicaxanthin is shown at different temperatures and light conditions.

Table 3. Maltodextrin-Encapsulated Indicaxanthin Color Analysis (CIELAB)

	maltodextrin-encapsulated indicaxanthin		
	initial	6 months—light	6 months—dark
a^*	5.56	−0.84	4.79
b^*	25.81	24.95	26.09
L^*	85.99	90.3	86.44
H°	77.843	−88.072	79.597
C^*	26.4020	24.964	26.526
ΔE^*		7.76	0.93

In addition, both activities are preserved when indicaxanthin is encapsulated in a maltodextrin matrix.

Stability of Maltodextrin-Encapsulated Indicaxanthin. The stability of maltodextrin-encapsulated indicaxanthin was also studied to verify if this procedure improved the resistance of the pigment to degradation. **Figure 6** shows the results found when the stability of indicaxanthin, obtained as a yellow powder by encapsulation, was studied at different temperatures. This figure indicates that encapsulation strongly increased the stability of the pigment and that the pigment remained stable for months in the absence of light at different temperatures (−20, 4, and 20 °C). As compared to the results obtained for the pigment in the soluble form (**Figure 2**), these data show that the encapsulation of indicaxanthin in a maltodextrin matrix greatly improves the stability of this pigment. In the presence of light, the degradation of the pigment increased when it was kept at 20 °C.

The results of the color measurement for maltodextrin-encapsulated indicaxanthin after 6 months at 20 °C in the absence or presence of light are shown in **Table 3**. The L^* value measures the lightness of the sample, a^* measures the red color, while b^* measures the yellow color. The hue angle measures the property of the color and is the ratio of a^* and b^* [hue = tan − 1(b^*/a^*)]. Chroma (C^*) indicates the color intensity or saturation [chroma = ($a^*2 + b^*2$)1/2]. The total color difference (ΔE^*), a single value that takes into account the differences between L^*, a^*, and b^* of the sample and standard was also studied $\{\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}\}$. In the presence of light, the a^* and b^* values decreased, especially the a^* parameter, which reaches negative values. This contributed to the changes in hue angle and chroma (**Table 3**). Overall, the lightness of the powders increased, and the chroma of the powders decreased. This implied that the color of the powders has become lighter after 6 months in the presence of light. The change of hue angle is linked to the destruction of indicaxanthin, as discussed above. However, the color parameters did not change noticeably after storage for the same period in the absence of light. ΔE^* values showed the overall difference between the initial sample and the samples stored in the absence or the presence of light. The color of maltodextrin-encapsulated indicaxanthin is significantly changed after storage in the presence of light, and it is not significantly changed in the absence of light. This result confirms the high stability of the yellow powders under these conditions.

This paper demonstrates the high degree of stability achievable for betalains under encapsulation and the maintenance of the antiradical properties. It constitutes the first case of a pure encapsulated betalain, so providing a yellow colorant.

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