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Encapsulation of the Most Potent Antioxidant Betalains in Edible Matrixes as Powders of Different Colors

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ABSTRACT: Betalains are plant pigments with high antioxidant and free radical scavenging activities. While basal activity exists in all betalains, the dihydroxylated molecules present the highest TEAC values of the family of compounds. However, their lability limits possible applications. This work reports the encapsulation of the most active pigments, the yellow miraxanthin V and the violet betanidin in edible matrixes of chitosan and maltodextrin. An appropriate spray-drying procedure is described, with an inlet air temperature of 140 °C. The resulting particles were characterized by scanning electron microscopy, and powder color was analyzed by spectrophotometry using an integrating sphere. Stability of the bioactive compounds was followed by high-performance liquid chromatography, and it was highly promoted by encapsulation, with limited pigment loss after six months' storage. Particles retained the antioxidant and antiradical activities of the soluble pigments measured under the FRAP and ABTS radical assays. A combination of miraxanthin V and betanidin in variable proportions provides a bright palette of encapsulated powders of different colors suitable for food applications.

KEYWORDS: *antiradical, betalain, bioactive, encapsulation, stabilization*

INTRODUCTION

Betalains are nitrogen-containing natural pigments that provide bright coloration to fruits, flowers, and roots of plants belonging to the order Caryophyllales.[1] They are divided into two groups: the violet betacyanins, with absorbance spectra centered at wavelengths around λ_m = 536 nm, and the yellow betaxanthins, with absorbance spectra centered at wavelengths around λ_m = 480 nm. Both groups share betalamic acid as the structural and chromophoric unit, which is condensed with *cyclo*-DOPA in the betacyanins and with amines and amino acids in the betaxanthins.[2] Betalains fulfill the role played by anthocyanins in other plants, and the two families are mutually exclusive.[3,4] Betalains are water-soluble and possess high antioxidant and free radical scavenging activities that have been described for plant extracts and purified pigments.[5−7] These activities support the recently discovered chemopreventive potential of betalains against different types of cancer.[8−10] Betalains have been reported to inhibit the formation of tumors in mice *in vivo*, with very low concentrations of dietary pigments, thus demonstrating a strong health-promoting potential.[11−13]

Among the edible sources of betalains, the roots of red beet (*Beta vulgaris*) and the fruits of the cactus *Opuntia ficus-indica* are especially relevant in the diet.[14−16] Other edible sources are the fruits of the cacti *Hylocereus polyrhizus*, Swiss chard (*Beta vulgaris*), the tubers from *Ullucus tuberosus*, and the berries from *Rivina humilis*.[10,17−20] The betalain-containing extracts from the roots of *B. vulgaris* are used by the food industry as a natural colorant under code 73.40 in the 21 CFR section of the Food and Drug Administration (FDA) in the USA and under code E-162 in the European Union. This colorant is used to give pink to violet colors to different foods and beverages.[21−23] In recent years new colorants derived from *Opuntia* fruits have also been proposed.[24−27] Betalains are also present in nonedible parts of plants, such as bracts, stems, leaves, and flowers.[28−31] The presence of betalains in the latter is of particular importance due to the formation of colored and fluorescent patterns and its possible role in the attraction of animals for pollination.[32]

Structure−activity relationships have been studied for betalains' antioxidant and free radical scavenging activities. There is an "intrinsic activity" common to all betalains, which is supported by the betalamic acid moiety of the structure, although this can be modulated by different structural factors.[33] The presence of aromatic cycles in resonance with the main electron resonance system implies an enhancement of the antiradical activity. If this is done to form indoline-like substructures, like those present in betacyanins, the enhancement is higher.[2] The presence of hydroxyl groups in the molecule increases the antioxidant and antiradical capacity of the pigment in both betaxanthins and betacyanins. An increase in terms of Trolox equivalence from 2.5 TEAC units (Trolox equivalent antioxidant capacity) to 4.0 for one and to 5.8 TEAC units for two hydroxyl groups has been characterized.[6,34] The last value is higher than those found for other well-known antioxidants such as epigallocatechin gallate (EGCG) in green tea.[35,36]

Despite their coloring capacity and superior antiradical activity, the dihydroxylated betalains have not been considered by the food industry as potential additives. This is in part due to their instability, which prevents long-term storage. In this work, the pigments with the strongest antiradical capacity, mirax-

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anthin V (betaxanthin) and betanidin (betacyanin), shown in Figure 1, are used as model dihydroxylated pigments to study

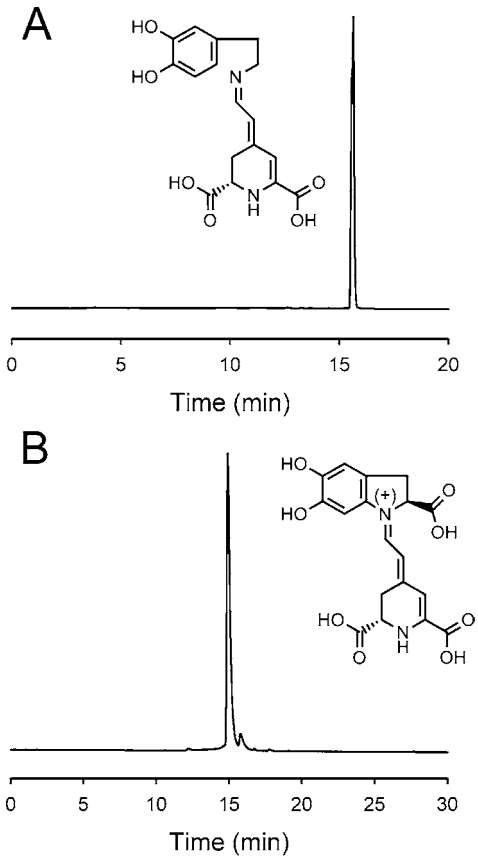

Figure 1. (A) Chromatographic profile for the individual analysis of purified miraxanthin V. Elution was followed at 480 nm. Full scale is A = 0.3 absorbance units. A 20 μL amount of pure pigment solution was injected with a concentration of 64 μM. Inset: Miraxanthin V structure. (B) Chromatographic profile of purified betanidin. Elution was followed at 542 nm. Full scale is A = 0.2 absorbance units. Injection volume = 10 μL, concentration = 286 μM. Inset: betanidin structure.

their possible encapsulation and its effect on stability and color. A palette of encapsulated powders containing these active betalains in different proportions is proposed for food applications.

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 (Madrid, Spain). HPLC-grade acetonitrile was purchased from Labscan Ltd. (Dublin, Ireland). Distilled water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

Plant Material and Betalains Preparation. *Lampranthus productus* plants with violet flowers were grown by the authors in Murcia (Spain). Flower samples were carefully collected, and the petals were removed and washed. Pigments were extracted in 10 mM sodium acetate buffer pH 5.0, containing 10 mM ascorbic acid (AA) in a Polytron homogenizer (Kinematica AG, Switzerland) (5 s, 2 pulses, at medium speed). The homogenate was filtered through nylon cloth and centrifuged at 120000g for 40 min. The supernatant was then filtered through Centriplus YM-10 membranes (Millipore) to remove proteins, and the filtrate was used for further betanidin purification. The whole process was carried out at 4 °C. Miraxanthin V was obtained as the condensation product of dopamine with betalamic acid obtained from *Beta vulgaris* roots. The process was carried out following a method described previously.[37,38] Briefly, red beet juice concentrate was filtered by a 10 kDa ultrafiltration step (QuixStand System, General Electric Healthcare, Milwaukee, WI, USA). Betanin purified from this filtered solution was used as starting material. Basic hydrolysis (pH 11.4) of betanin released betalamic acid, which was then condensed with dopamine after reaching pH 5.0. The betaxanthin miraxanthin V was obtained, accompanied by a color change from pale yellow (betalamic acid, λ_m = 424 nm) to deep yellow (dopamine betaxanthin, λ_m = 474 nm). The whole process was carried out in a nitrogen atmosphere. For pigment purification, a C-18 solid phase extraction step was performed. C-18 cartridges (35 mL, Waters, Milford, MA, USA) were conditioned with 70 mL of ethanol followed by 70 mL of sodium phosphate buffer (10 mM, pH 6). Miraxanthin V was eluted with ethanol and then concentrated to dryness under vacuum. The residue was stored at −80 °C for further purification by anionic exchange chromatography.

Anionic Exchange Chromatography Purification. Anionic exchange chromatography of miraxanthin V and betanidin was performed in an Äkta Prime Plus purifier apparatus (General Electric Healthcare).[31] The solvents used for the betaxanthin purification 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). The columns used were 25 × 16 mm, 5 mL Q-Sepharose Fast Flow (cross-linked agarose with quaternary ammonium as an exchanger group, 90 μm particle size) and were purchased from General Electric Healthcare. Three columns were connected in series in order to scale up the purification process. After sample injection, the elution process was as follows: 0% B from 0.0 to 127 mL; after washing, a linear gradient was performed over 135 mL from 0% B to 16% B, with collection of 6 mL fractions. Injection volume was 50 mL and the flow rate was 4.5 mL min^{-1}. Salts were removed using a C-18 cartridge as described above. The solvents used for betanidin purification were 10 mM sodium acetate buffer, pH 5.0 (solvent A), and 10 mM sodium acetate buffer, pH 5.0, with 2 M NaCl (solvent B). An analogous process to that used for miraxanthin V purification was used with only slight differences. After sample injection, the elution process was as follows: 0% B from 0.0 to 137 mL; after washing, a linear gradient was performed over 210 mL from 0% B to 35% B.

Spectroscopy. A Jasco V-630 spectrophotometer (Jasco Corporation, Tokyo, Japan), attached to a Tectron thermostatic bath (JP Selecta, Barcelona, Spain), was used for absorbance spectroscopy. For the quantification of betalains, pigment concentration was evaluated taking a molar extinction coefficient of ε = 48 000 M^{-1} cm^{-1} at 480 nm for miraxanthin V and ε = 54 000 M^{-1} cm^{-1} at 536 nm for betanidin.[39,40] Measurements were made in water at 25 °C.

HPLC Analysis. A Shimadzu LC-20AD apparatus (Kyoto, Japan) equipped with a SPD-M20A photodiode array detector (PDA) was used for analytical HPLC separations. Reversed phase chromatography was performed with a 250 × 4.6 mm Luna C-18(2) column packed with 5 μm particles (Phenomenex, Torrance, CA, USA). Gradients were formed with two solvents, A and B. Solvent A was H_2O with 0.05% trifluoroacetic acid (TFA); solvent B was acetonitrile with 0.05% TFA. A linear gradient was performed from 5% B to 35% B for 21 and 35 min for miraxanthin V and betanidin analysis, respectively.[31,33] The flow rate was 1 mL min^{-1}, operated at 25 °C. Injection volume was 20 μL for miraxanthin V and 10 μL for betanidin. Chitosan-containing samples were diluted, and water washings were performed between runs in order to minimize the undesirable effects of the mucoadhesive properties of chitosan. These include interaction with the column's stationary phase and a significant increase in the column pressure.

Spray Drying. Maltodextrin was dissolved in purified pigment solutions (20% w/v) under vigorous vortexing. Chitosan (1% w/v) was dissolved in acetic acid (1% v/v). Particles were prepared in a Büchi B-290 mini spray dryer (Büchi Labortechnik AG, Flawil, Switzerland). Inlet air temperature was 140 °C, and the outlet air temperature was kept at 75 °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.[27] The particles were separated from the drying air by a cyclone.

Color Assessment. Color determinations of the encapsulated pigments were made at 25 °C using a JASCO V-650 spectrophotometer equipped with an ISV-722 integrating sphere (Jasco Corporation, Tokyo, Japan). The color parameters corresponding to the uniform CIELAB space (L^*, a^*, b^*, C^*, and h°) were directly obtained from the apparatus software Spectra Manager version 2.07.[41]

Free Radical Scavenging Activity. The antiradical capacity of dopamine−betaxanthin and betanidin was evaluated by following their effect on stable free radical $ABTS^{\bullet+}$ [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)]. Decolorizing activity on $ABTS^{\bullet+}$ solutions was monitored spectrophotometrically at λ = 414 nm.[5] $ABTS^{\bullet+}$ 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 NaOAc buffer, pH 5.0. The reactive portion was then diluted by two-thirds with the addition of samples, and the reactions were carried out in 53 mM sodium phosphate buffer, pH 7.0. Other conditions are specified in the text. Measurements of 96-well plates were performed after 24 h incubations at 20 °C, in a Synergy HT plate reader (Bio-Tek Instruments, Winooski, VT, USA). All experiments were performed in triplicate, and mean values and standard deviations were plotted. Final volume was 300 μL (calculated path length = 0.87 cm). Detector linearity under the assay conditions was confirmed (r = 0.999). Data analysis was carried out using linear regression fitting under Sigma Plot Scientific Graphing for Windows version 8.0 (2001; SPSS, Chicago, IL, USA). In each case, errors associated with the results were calculated on the basis of the residual standard deviation around the regression line.

Antioxidant Capacity. The antioxidant activity of the pigments was characterized by means of the reduction of Fe(III) to Fe(II). The method described to evaluate the ferric reducing antioxidant power (FRAP) was employed.[42] 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 by adding the reagent 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) at a final concentration of 741 μM, which yields a colored complex with Fe(II). The reduction reaction was monitored spectrophotometrically at λ = 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 using linear regression fitting under Sigma Plot Scientific Graphing for Windows version 8.0. In each case, errors associated with the results were calculated on the basis of the residual standard deviation around the regression line.

Betalains Stability. *Stability of Soluble Miraxanthin V.* Stability of soluble miraxanthin V was analyzed at 20 °C at different pH values, both in the absence and in the presence of light (light intensity = 50 μmol/m^2·s). The assay medium contained the pigment (initial concentration of 75 μM) in 20 mM sodium acetate buffer (pH range 4.0−5.5) or 20 mM sodium phosphate buffer (pH range 5.5−8.0). Aliquots corresponding to the different pH values were taken at different times and analyzed by HPLC to follow the degradation of the betaxanthin.[27]

Stability of Encapsulated Pigments. The powders containing the encapsulated miraxanthin V or betanidin were stored at 20 °C, both in the absence and in the presence of light (light intensity = 50 μmol/m^2·s), at 4 °C and at −20 °C. Aliquots were taken at different times and dissolved in water (betaxanthin) or 10 mM sodium acetate buffer, pH 5.0 (betanidin), to an initial concentration of 300 μM for HPLC analysis.[27] When chitosan was used for encapsulation, the aliquots taken at different times were dissolved in 1% acetic acid to a concentration of 6 μM for HPLC study in order to reduce the viscosity of the sample. Due to the rheological properties of chitosan, water washings between runs were carried out in the HPLC system. This was the best way to minimize pressure problems in the chromatographic system and column caused by chitosan.

Scanning Electron Microscopy (SEM). Particle morphology was evaluated by scanning electron microscopy. Powders were attached to pieces of double-sided adhesive tape mounted on SEM stubs, coated with gold under vacuum using a SEM coating system by Bio-Rad Polaron Division, and examined with a JEOL JSM-6100 (Jeol, Tokyo, Japan) scanning electron microscope operated at 15 kV. Images were analyzed using the open source image analysis software ImageJ (National Institutes of Health, NIH, USA). Briefly, images were calibrated, and after that, an interactive measurement of the spheres was carried out. Micrographs were obtained with an Inca Oxford Image capture system (Oxford Instruments, Abingdon, UK).

RESULTS AND DISCUSSION

Pigment Purification and Stability. Miraxanthin V and betanidin were purified by anionic exchange chromatography and solid phase extraction. Purity was confirmed by HPLC analysis with PDA detection (Figure 1), and identity was confirmed by retention time and absorbance spectrum comparison with known standards.[38,43]

Studies on the chemical stability of betalains have focused mainly on betanin.[44] However, the stability of betanidin has been assessed previously,[43] and the results indicate that the compound was stable in solution in the pH range 3.0 to 6.0. Above this range, betanidin experiences a quick degradation that is stronger at higher pH values. Betanidin degradation is accompanied by a loss of color. These observations were supported by degradation kinetics studies.[43] Although data have also been reported for other pigments such as amaranthin or indicaxanthin, to our knowledge, kinetic studies on the degradation of miraxanthin V have not been carried out.[27,45]

Stability of soluble miraxanthin V was analyzed at different pH values, either in the presence (Figure 2A) or in the absence

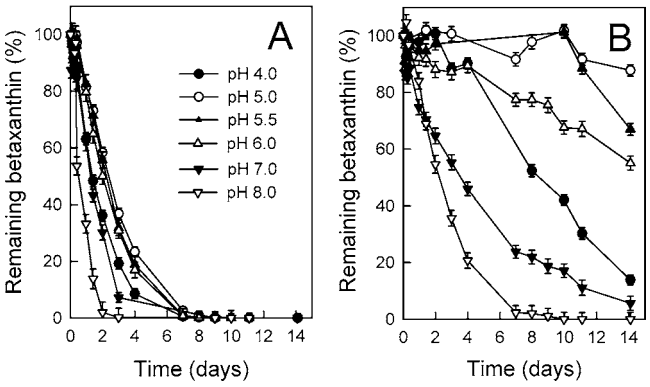

Figure 2. Percentage of remaining miraxanthin V as a function of the incubation time at 20 °C in the presence (A) or in the absence (B) of light at different pH values.

(Figure 2B) of light. As can be seen, the presence of light considerably decreased the stability of the pigment at all the pH values assayed. The data obtained at each pH during the first days of the experiment could be adjusted to first-order kinetics. The corresponding degradation rates were calculated from the slope of the linear regression fittings, as can be seen in Table 1. These results evaluate the higher degradation experienced by miraxanthin V in the presence of light. In addition, the stability of the pigment was lower at pH values of 4.0, 7.0, and 8.0 than at pH values between 5.0 and 6.0, with the highest value of the degradation rate being determined at pH 8.0. This behavior was found in both the presence and absence of light, probably due to the oxidation of the *o*-hydroxyl groups at a slightly basic pH. It can be concluded that miraxanthin V is stable in the absence of light and in a reduced pH range between 5.0 and 6.0. Outside these parameters degradation occurs. These results are

Table 1. Values for Dopamine−Betaxanthin Degradation Rates in the Presence and Absence of Light under Different pH Conditions[a]

	pH					
degr. rate (μM/h)	4.0	5.0	5.5	6.0	7.0	8.0
light	0.92 ± 0.04	0.57 ± 0.02	0.58 ± 0.02	0.61 ± 0.02	0.97 ± 0.07	1.8 ± 0.19
darkness	0.167 ± 0.009	0.012 ± 0.007	0.0195 ± 0.008	0.073 ± 0.005	0.378 ± 0.041	0.637 ± 0.035

[a]Dependent samples' Student's *t*-test statistical analysis was successfully applied (p = 0.0009).

comparable to those obtained for betanidin, where pH values above 6.0 implied quick pigment degradation.[43]

Encapsulation of Pure Pigments. In order to explore the possibility of the stabilization of betanidin and miraxanthin V, a spray-drying process of both compounds was carried out separately. Two encapsulating edible matrixes—chitosan and maltodextrin—were used in this experiment. The process was aimed at obtaining violet or yellow powders containing a single pigment, betanidin or miraxanthin V, respectively.

The inlet air temperature for spray drying was set at 140 °C, according to previous results obtained for the encapsulation of the pigment indicaxanthin.[27] This implied a compromise between the efficiency of the drying process and the thermal stability of the compounds. Figure 3 shows SEM microphotographs of the powders obtained for maltodextrin and chitosan encapsulation of miraxanthin V at the end of the drying process. The same results were obtained for maltodextrin encapsulation of betanidin. Particle analysis and sphericity were analogous to those obtained for miraxanthin V encapsulation, thus indicating that the morphology of the powders depends on the matrix and encapsulation conditions and not on the bioactive pigment. Analysis of the particle morphology reveals that maltodextrin spheres possess a mean particle diameter of 10.7 μm. Shrinkage was observed during the drying process and affected particle size. Those particles that collapsed during the drying process possessed a mean diameter of 5.8 μm. This affected the particle sphericity, which was reduced from Ψ = 1.0 to Ψ = 0.3, as determined from the mean surface area of the particles. The matrix change from maltodextrin to chitosan implied a considerable reduction in particle size, estimating a mean diameter of 3.1 μm. The morphology of the final particles relates to the chosen matrix, but it is also affected by the drying conditions, associated with shrinkage, inflation, and particle size.[46] Although matrixes of chitosan are currently receiving a great deal of interest for their biocompatibility and biodegradability, the use of this compound presents technical difficulties deriving from its mucoadhesive properties. Chitosan solutions are viscous and difficult to handle. In addition, it presents low solubility (1% in acetic acid 1%) compared to maltodextrin (20% in aqueous solutions). Taking into account these considerations, maltodextrin was preferred for the encapsulation of both betalains, and it is a more suitable candidate for application in the food industry.

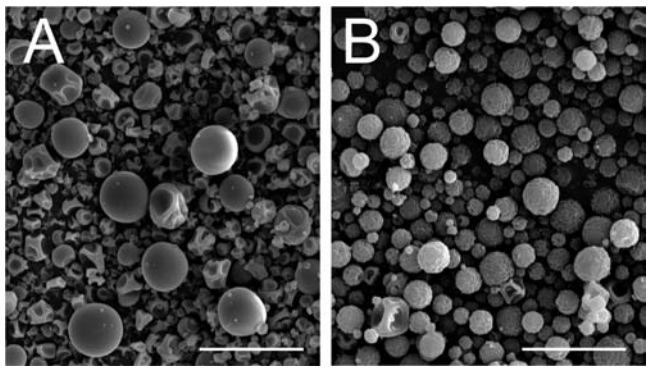

Figure 3. Scanning electron microscope images for encapsulated miraxanthin V powders. The matrixes used were (A) maltodextrin and (B) chitosan. Scale bars are 25 and 12.5 μm, respectively.

Free Radical Scavenging Activity of Encapsulated Pigments. Betanidin and miraxanthin V exhibit a high free radical scavenging capacity at much lower concentrations than Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a potent antiradical, water-soluble derivative of vitamin E used as standard. Figure 4A shows the dependence of the radical depletion on betalains' concentration compared to Trolox. The pigments were added either as soluble pigments or as powders obtained from their spray-drying treatment with maltodextrins. When increasing concentrations of the betalains or Trolox were added to the ABTS$^{\bullet+}$ radical mixture, a linear effect was observed on the removal of the radical. This activity was conserved by the pigments encapsulated in a maltodextrin matrix. When maltodextrins, without encapsulated pigment, were added to the reaction medium, a slight decrease in absorbance was obtained, probably due to the presence of hydroxyl groups on the matrix surface.

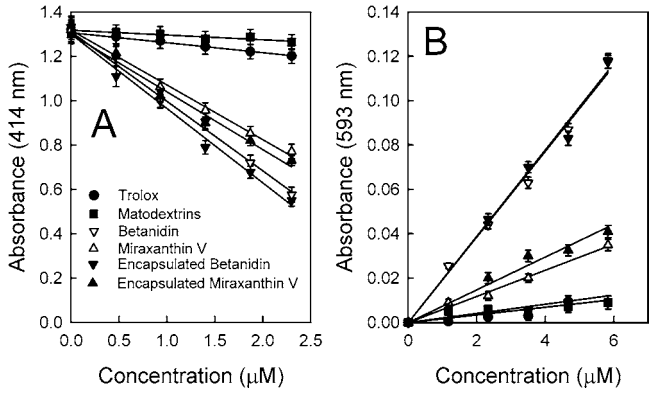

Figure 4. (A) ABTS$^{\bullet+}$ radical depletion dependence on betalains' concentration compared with Trolox. ABTS$^{\bullet+}$ was used at a concentration a 47 μM. The reaction was followed spectrophotometrically at λ = 414 nm, at pH 7.0. (B) Antioxidant power of the pigments through its ferric reducing ability. The effect was evaluated spectrophotometrically at λ = 593 nm. For clarification, maltodextrin control is included within the same graph. It was used at the same concentration present in the maltodextrin-encapsulated pigment.

The TEAC values of soluble miraxanthin V and betanidin, as determined from the slopes in this work (Figure 4A), were 5.5 ± 0.5 and 7.0 ± 0.6, respectively. These results are in accordance with previously reported data, where miraxanthin V was reported to possess the highest radical scavenging activity of the betaxanthins assayed and betanidin was the betalain with

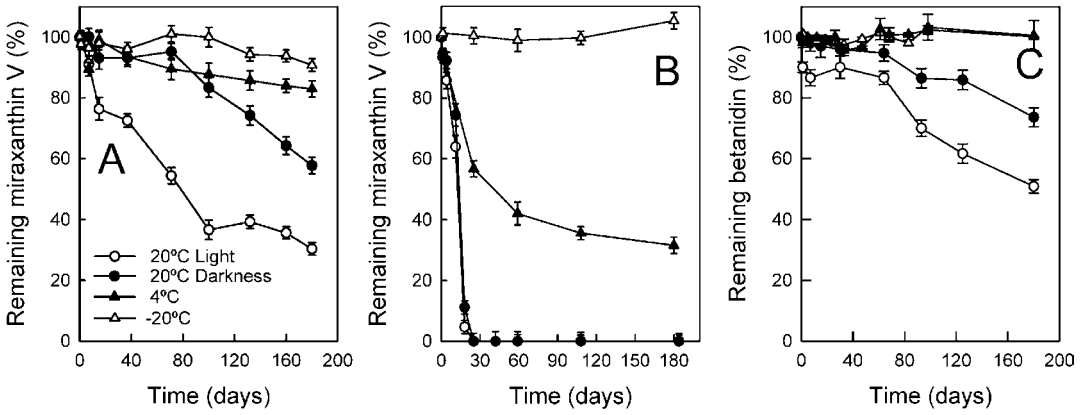

Figure 5. Stability analysis of encapsulated betalains at different temperatures and light conditions. (A) Maltodextrin-encapsulated miraxanthin V. (B) Chitosan-encapsulated miraxanthin V. (C) Maltodextrin-encapsulated betanidin.

the most potent antiradical activity.[2,34] For spray-dried miraxanthin V and betanidin, TEAC values were determined as 5.8 ± 0.6 and 7.5 ± 0.7, respectively. An analogous contribution of the maltodextrin matrix to the radical depletion, and thus to the TEAC value, can be seen in both cases (Figure 4A). The contribution was estimated by incubating ABTS with maltodextrin in the same proportion used for the encapsulated pigments, and a TEAC value of 0.5 ± 0.1 was determined for the maltodextrin control.

Antioxidant Activity of Encapsulated Pigments. The antioxidant activity of the pigments was characterized by their capacity to directly reduce Fe(III) to Fe(II). The FRAP assay was used, monitoring the reduction reaction spectrophotometrically at λ = 593 nm.[42] Figure 4B shows the signals obtained for the reduction of Fe(III) by betanidin and miraxanthin V, both in the soluble and encapsulated forms. For comparative purposes, these signals were compared to that obtained by Trolox, and the Trolox molar equivalence in the ferric reduction assay was determined. For soluble miraxanthin V it was 3.2 ± 1.1, and for the encapsulated compound it was 3.8 ± 1.4. As occurred with the ABTS radical assay, the difference between these values might be due to the contribution of maltodextrin. Trolox molar equivalence of maltodextrin was determined as 0.7 ± 0.3 in a control measurement using the same proportion of powders. In the case of miraxanthin V, the Trolox equivalence shown for the FRAP assay can be compared with the TEAC value obtained for the ABTS assay at the acidic conditions used in the FRAP assay (pH 3.6).[34] As regards betanidin, its antioxidant capacity was evaluated for the first time through the FRAP assay. The values obtained for the soluble and encapsulated pigments were 10.5 ± 3.5 and 10.6 ± 3.6, respectively. These values are in accordance with the behavior of betanidin shown under the ABTS assay at acidic pH values.[2] As expected, miraxanthin V and betanidin pigments presented a much higher ferric reducing activity than that of the vitamin E analog Trolox.

The two betalains assayed present high antioxidant and free radical scavenging activities. These activities are general to all betalains, and in the case of the assayed pigments they are highly enhanced by the presence of the catecholic substructure.[34] The absence of hydroxyl groups in other families of compounds implies the total lack of free radical or antioxidant activities, as is the case of the flavonoids *trans*-chalcone, flavone, flavanone, and isoflavone.[47] Both activities were preserved when miraxanthin V and betanidin were encapsulated.

Stability of Maltodextrin- and Chitosan-Encapsulated Betalains. Maltodextrin-encapsulated miraxanthin V was studied in order to ascertain whether this procedure increased pigment stability under different conditions. Figure 5 shows the results when the stability of the betaxanthin, obtained as a yellow powder by encapsulation, was studied at three different temperatures in both maltodextrin and chitosan matrixes. Stability was evaluated by dissolving powder samples and analyzing the pigment content by HPLC. Maltodextrin encapsulation strongly increased the stability of the pigment, which remained stable for months in the absence of light, at temperatures of −20 and 4 °C. When miraxanthin V was kept at 20 °C without light, approximately 60% of the pigment remained stable for six months. However, degradation of the pigment was increased considerably when it was kept at 20 °C in the presence of light, with only 30% of the initial betaxanthin amount remaining. The data clearly show that encapsulation of miraxanthin V in a maltodextrin matrix greatly improves the stability of this dihydroxylated pigment. When chitosan was used as matrix to encapsulate the betaxanthin (Figure 5B), the pigment also remained stable for six months when stored at −20 °C in the absence of light, but only 40% of the pigment was not degraded at 4 °C without light. The degradation rate of chitosan-encapsulated pigment sharply increased at 20 °C both in the presence and in the absence of light, and the pigment had completely degraded in one month.

Betanidin is a very labile molecule that degrades quickly under working and storage conditions.[43−45,48] The stability of this important metabolite has been evaluated for the first time in the form of maltodextrin-encapsulated powders. The stability of the encapsulated pigment was analyzed at 20 °C in the presence and in the absence of light. As can be seen in Figure 5C, almost 75% of the pigment remained stable after six months of treatment as long as light exposure was avoided. Exposure to light considerably reduced pigment stability, reaching 50% of the initial pigment amount. When the temperature was reduced to 4 and −20 °C in the absence of light, the pigment was much more stable and no appreciable loss of the initial pigment was detected after a period of six months. Although the time frame used in other studies was shorter, these results can be compared with previous observations on different pigments. When anthocyanin stability was analyzed after encapsulation in maltodextrin powders, it

Table 2. Color Analysis (CIELAB Parameters) for Maltodextrin-Encapsulated Miraxanthin V and Betanidin

	initial		6 months, light		6 months, dark	
	miraxanthin	betanidin	miraxanthin	betanidin	miraxanthin	betanidin
a^*	−3.41	35.87	−4.62	26.55	−3.6	27.04
b^*	45.57	−28.27	26.65	−13.76	40.51	−20.47
L^*	90.04	54.30	92.66	61.41	89.29	57.38
h°	94.27	321.76	99.97	332.60	95.08	322.87
C^*	45.69	45.67	26.66	29.91	40.67	33.91
ΔE^*			19.14	18.65	5.12	12.18

was observed that two months' storage in the absence of light reduced the pigment content by a 33% at 25 °C, while an 11% pigment loss was determined at 4 °C.[49,50] The stability of anthocyanins was lower than that shown for miraxanthin V and betanidin. The presence of light also accelerated anthocyanin degradation. Analogous results were obtained for a less similar pigment, as lycopene encapsulated in a starch matrix.[51] Storage for a period of 2.5 months resulted in a loss of 33−40% of the initial pigment at 25 °C and a loss of 17−22% at 10 °C.

For both betalains assayed spray-drying encapsulation in maltodextrin is a very useful technique to improve the shelf life of these bioactive betalains and make them suitable for industrial applications.

Encapsulated Pigments Color Analysis. The color of the powders containing the encapsulated pigments was evaluated by spectrophotometry using an integrating sphere. Data were collected immediately after powders were obtained and after six months of storage. In the case of miraxanthin V, CIELAB parameters of the initial measurements were analogous for maltodextrin (Table 2) and for chitosan (Table 3) matrixes. In

Table 3. Chitosan-Encapsulated Miraxanthin V Color Analysis (CIELAB Parameters)

	initial	6 months, light	6 months, dark
a^*	−2.64	−0.95	−1.58
b^*	46.57	5.54	26.93
L^*	87.96	92.67	90.49
h°	93.24	99.70	93.36
C^*	46.65	5.63	26.98
ΔE^*		41.33	19.83

both cases, the color (a^* and b^*), its intensity (C^*), and the lightness (L^*) of the solid sample were analogous. Thus, the appearance of the spray-dried sample was not dependent on the chosen matrix. The results for color evaluation for the maltodextrin-encapsulated miraxanthin V and betanidin after six months at 20 °C in the absence and presence of light are shown in Table 2. In the presence of light the a^* and b^* values decreased, thus indicating a loss in the red and yellow color of betanidin and betaxanthin, respectively. This contributed to the changes in hue angle (h°) and chroma (C^*). Overall, in the presence of light, the hue angle and the L^* value of the powders increased, but the chroma decreased. These changes of color parameters implied that the color of the powders become lighter after six months' storage under light, pointing to the decrease in the betalain content, as discussed in the previous section. However, the color parameters did not change in a noticeable way after storage for the same period of time at the same temperature in the absence of light. ΔE^* values show the overall difference between the initial sample and the samples stored under both conditions. The color of the maltodextrin-encapsulated betalains was not significantly changed in the absence of light. In contrast, for maltodextrin-encapsulated anthocyanins color degradation from the initial pink color to brown at the end of a two-month storage period at 25 °C has been described.[49]

The results of this section reveal the great stability of the maltodextrin-encapsulated betalains as demonstrated by HPLC, maintaining the bright yellow and purple appearance of the powders stored under these conditions. In the case of miraxanthin V encapsulated in chitosan (Table 3), a higher degree of color degradation was observed, in agreement with the lower values for the color and intensity, b^* and C^*, parameters.

Co-encapsulation of Miraxanthin V and Betanidin. Purified betalains were co-encapsulated for the first time in an edible matrix to provide a bright palette of powders of different colors. The two bioactive pigments were combined in solution at different relative concentrations and then submitted to spray drying. Figure 6 shows pictures of the two pure pigments

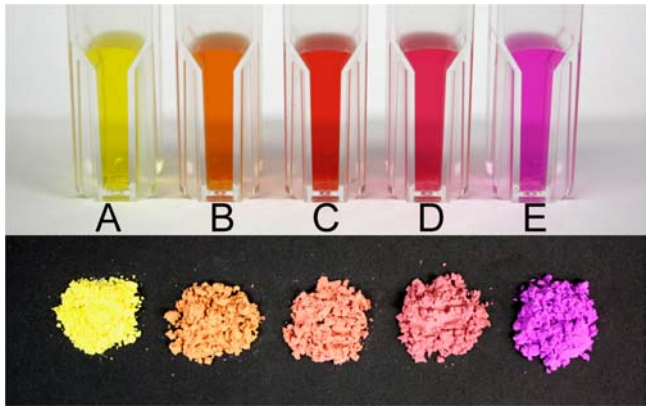

Figure 6. Macroscopic pictures of combinations of miraxanthin V and betanidin in the indicated proportions, both in soluble form and as maltodextrin-encapsulated powders. The proportion of both pigments ranged from 100% miraxanthin V (A) to 100% betanidin (E); intermediate percentages were (B) 75% miraxanthin V, (C) 63% miraxanthin V, and (D) 50% miraxanthin V.

miraxanthin V and betanidin individually and the colors obtained after their combination in the indicated proportions in both the soluble form and as maltodextrin-encapsulated powders. As can be seen, as betanidin concentration increases in relation to miraxanthin V, the tonality of the mixture tends to a reddish tone until a violet shade is obtained. The changes in the appearance of the maltodextrin-encapsulated samples were quantified by the results of color measurements in the integrating sphere (Table 4). As the betaxanthin proportion is reduced in the mixture, the value of the b^* parameter, which measures the yellow color, decreases and, at the same time, the

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Table 4. Color Analysis (CIELAB Parameters) for Co-encapsulated Miraxanthin V and Betanidin in Maltodextrin

	miraxanthin V content				
	100%	75%	63%	50%	0%
a^*	−3.41	15.20	19.59	22.70	35.87
b^*	45.57	18.13	10.25	3.60	−28.27
L^*	90.04	66.43	62.38	58.22	54.30
h°	94.27	50.03	27.61	9.00	321.76
C^*	45.69	23.66	22.11	22.98	45.67

value of the a^* parameter increases due to the higher betanidin content. The L^* parameter decreases when the contribution of betanidin is higher in the pigment mixture, indicating a reduction in the clarity of the powders. The change of the hue angle (h°) is also linked to the lower proportion of betaxanthin and therefore to the change of color from yellowish to reddish.

Co-encapsulated miraxanthin V and betanidin were assayed for antioxidant and free radical scavenging activities using the FRAP and $ABTS^{\bullet+}$ radical assays. As expected, both activities were maintained. TEAC values calculated from the slopes of the $ABTS^{\bullet+}$ free radical scavenging assay were 6.8 ± 0.6 (50% miraxanthin V content), 6.4 ± 0.6 (63% miraxanthin V), and 6.3 ± 0.7 (75% miraxanthin V). Calculated TEAC values obtained from the FRAP assay were 7.5 ± 2.9 (50% miraxanthin V), 6.1 ± 2.0 (63% miraxanthin V), and 5.5 ± 1.9 (75% miraxanthin). The mixtures presented intermediate properties among those of pure miraxanthin V and betanidin. Although it is possible that the joint presence of both pigments could promote the co-reduction of the $ABTS^{\bullet+}$ radical and Fe(III), no synergistic effect was detected. The stability of co-encapsulated pigments in maltodextrin was evaluated after storage at −20 °C for six months. Color analysis parameters for the powders after this time are shown in Table 5 and revealed a limited change with respect to the values of the initial samples (Table 4). Not only the color (a^*, b^*, h°) but also its intensity (C^*) and the lightness (L^*) of the solid sample were maintained. The parameter ΔE^* shows the overall change during the storage time at −20 °C. As can be seen, it was not significantly varied among the assayed pigment proportions, demonstrating that co-encapsulation and storage at −20 °C efficiently preserves the bioactive pigments. These data were supported by HPLC analysis of the stored samples. Miraxanthin V content experienced a slight reduction in the maltodextrin matrix stored at −20 °C that was below 4% in all cases, while betanidin remained stable with a variation of 3% with respect to the initial conditions.

Table 5. Color Analysis (CIELAB Parameters) for Co-encapsulated Miraxanthin V and Betanidin in Maltodextrin after Six Months' Storage at −20 °C (Darkness)

	miraxanthin V content				
	100%	75%	63%	50%	0%
a^*	−3.67	14.70	19.13	21.37	33.82
b^*	44.10	17.50	9.30	3.21	−26.53
L^*	89.50	67.02	63.40	59.25	55.16
h°	94.76	49.88	25.94	8.55	321.89
C^*	44.25	22.85	21.27	21.61	42.98
ΔE^{*a}	1.59	1.00	1.47	1.73	2.82

[a] ΔE^* accounts for the color variation calculated with the differences between L^*, a^*, and b^* of the stored samples and the initial conditions as shown in Table 4.

Co-encapsulation of purified bioactive pigments can be tailored to fit specific color requirements of products for the pharmaceutical, cosmetic, and food industries. The molecules encapsulated will retain their bioactive properties while remaining stable for months, as demonstrated above.

The present work demonstrates the high degree of stability achievable under encapsulation for miraxanthin V and betanidin, two dihydroxylated betalains that in spite of presenting the highest TEAC values are also the most labile members of this family of compounds. Different temperatures and light−darkness conditions were assayed in long-term experiments, demonstrating a limited pigment loss after six months' storage. In addition, it has been demonstrated that encapsulation in edible matrixes of chitosan and maltodextrin preserves the antioxidant and antiradical activities of the soluble compounds. This constitutes the first case of successful encapsulation of the most active plant pigments betalains. The combination of purified miraxanthin V and betanidin in different proportions allows the generation of a range of pigments of different colors, characterized in the uniform CIELAB space, that can be stabilized by encapsulation and that are suitable for food applications.

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Notes

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