



Betaxanthins and antioxidant capacity in *Stenocereus pruinosus*: Stability and use in food



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ABSTRACT

Betalains are important pigments for the food, pharmaceutical, and cosmetics industry. In the yellow *Stenocereus pruinosus* fruits (pitayas), total betalain concentration, Folin-Ciocalteu reduction capacity, and antiradical capacity per dry weight were 2345.9 $\mu\text{g g}^{-1}$, 7.3 mg gallic acid equivalents g^{-1} , and 48.8 $\mu\text{mol Trolox equivalent g}^{-1}$, respectively. The stability of betaxanthins, which represent 89% of total betalains in yellow pitayas, was evaluated over a range of pH, temperature, as well as in the presence of food additives. Maximum stability was observed at pH 6.6, and addition of ascorbic acid increased the half-life 1.8 times. Thermal stability at pH 6.48 \pm 0.05 was also evaluated from 50 °C to 80 °C, over which the activation energy for betaxanthin degradation was determined to be 66.2 kJ mol^{-1} . Model gelatin gummies and beverages were then prepared with pitaya juice or pulp, and pigment retention and color parameters were investigated during storage under various conditions. To match the yellow color of commercial products, gummies were supplemented with 4.6% w/w juice or pulp, and beverages were supplemented with 5% w/v juice, achieving H^a values of 69.0–86.2° and 64.6–87.1°, respectively. Results indicate that betaxanthins were more stable in gummies than in beverages, and that pigment retention increased when products were stored in the dark or at low temperatures. Also, different changes in color during storage were observed between gummies and beverages.

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1. Introduction

Color is an important factor in food acceptance by consumers, and is considered an indicator of quality (Azeredo, 2009). Consequently, worldwide demand for food colorants has increased to several thousand tons per year (Manchali, Murthy, Nagaraju, & Neelwarne, 2013). Food colorants include the synthetic azo-dyes Yellow 5 and Yellow 6 used in food, drugs, and cosmetics. However, consumers have become increasingly concerned about the possible harmful effects of synthetic colorants. As a result, the search has intensified for novel and natural pigments that could be used in food.

Betalains can be classified into two groups of pigments based on their chemical structure. The yellow betaxanthins and the red-violet betacyanins (Fig. 1) are immonium conjugates of betalamic acid with amino acids or amines and cyclo-DOPA, respectively (Delgado-Vargas, Jiménez, & Paredes-López, 2000). Fruits and vegetables present complex betaxanthin profiles, with >30 compounds having been detected by HPLC-DAD-MS (Khan & Giridhar, 2015). However, only a few

structures predominate in each source. Thus, yellow beetroot and yellow Swiss chard contain mainly miraxanthin V and vulgaxanthin I, whereas yellow-orange cactus pear contains muscaaurin VII and indicaxanthin (Kugler, Graneis, Stintzing, & Carle, 2007).

Betalains display anti-inflammatory, antifungal, and anticarcinogenic activities (Stintzing & Carle, 2004). In addition, the phenolic and amine groups confer these pigments with reducing and radical-stabilizing properties that make them potent antioxidants (Moreno, García-Viguera, Gil, & Gil-Izquierdo, 2008).

Betalains are advantageous over anthocyanins because of their superior stability as natural colorants at pH 4–7 (Swarna, Lokeswari, Smita, & Ravindhran, 2013).

The stability of betalain-type pigments is enhanced by high pigment concentration, high glycosylation and acylation levels, matrix components, low water activity, pH values between 4 and 7, low temperature, darkness, oxygen-free atmosphere, presence of glycosidase, chelating agents, antioxidants, and cyclodextrins (Khan, 2016).

Betaxanthins from plants of the genus *Opuntia* (Moßhammer, Rohe, Stintzing, & Carle, 2007), *Celosia* (Cai, Sun, Schliemann, & Corke, 2001), and *Ullucus* (Svenson, Smallfield, Joyce, Sansom, & Perry, 2008) have been extracted and characterized. The genus *Stenocereus*, whose fruits (pitayas) have red, purple, or yellow-orange pulp, is also a potential source of pigments. The plant is native to the American continent,

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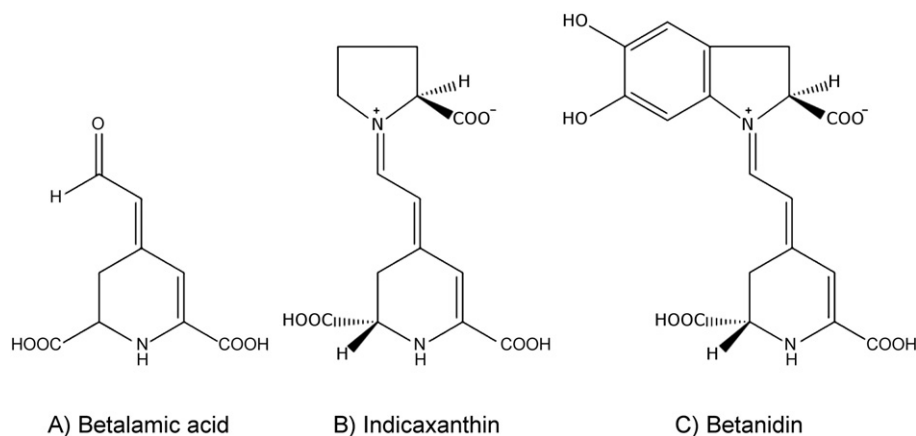


Fig. 1. A) Betalamic acid, chromophore of betalains. B) Indicaxanthin, example of betaxanthins. C) Betanidin, example of betacyanins.

with some species endemic to central Mexico (Parra, Pérez-Nasser, Lira, Pérez-Salicrup, & Casas, 2008). High acidity confers a pleasant sweet-sour taste to pitayas, and enhances microbiological stability (Pérez-Loredo, García-Ochoa, & Barragán-Huerta, 2016) as compared to cactus pear. The fruits are consumed fresh, their production in Mexico increased by >200% in the last decade, from 1680.2 tons in 2004 to 3884.9 tons in 2014, and the production value increased by 487% within the same period (SIAP, 2016). Therefore, the use of pitayas as a source of betaxanthin pigments could have important commercial repercussions in the future on the colorant market.

Betacyanins have been used as red colorants, owing to their deep tints, and functional properties (Delgado-Vargas et al., 2000). In contrast, betaxanthins are rarely used as yellow pigments, presumably because of limited supply. In addition, the stability of betaxanthins is not well-characterized.

Thus, stability studies may help turn pitaya pigments into quality products with a long shelf life, and thereby increase their commercial value. Hence, here we investigated the stability of pitaya betaxanthins in both, aqueous extracts and model food matrices (gummies and beverages) colored with yellow pitaya fruits.

2. Materials

2.1. Chemicals

Analytical-grade gallic acid, 2,2-azinobis-(3-ethylbenzothiazoline 6-sulphonic acid) (ABTS), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (México).

2.2. Plant material

S. prunosus fruits were collected from Santiago Tonahuiztla Puebla (18° 12' 5.35'' N, 97° 53' 50.75'' W) during harvest season in May 2015 (Fig. 2). The fruits were 126.8 to 295.3 g, with polar and equatorial diameters of 8.60 ± 0.75 and 6.52 ± 0.5 cm, respectively.

3. Methods

3.1. Fruit selection and preparation

Healthy fruits without spines were packed in Food Saver® plastic bags of 500 to 800 g whole fruit under vacuum, and stored at -20 °C. Prior to analysis, samples were first placed at 4 °C for 24 h, and peeled. The pulp was then sieved through a 710 - μm mesh to homogenize and eliminate seeds. Portions of 50 g of flesh were placed into plastic containers and stored for no longer than one week at -20 °C until analysis.

3.2. Preparation of extracts from *S. prunosus* fruits

Homogenized pulp (1 g) was mixed with 4 mL water in 10-mL screw-capped test tubes, vortexed (Vortex Mixer VM-300; Gemmy, Taipei, Taiwan) at 3150 rpm for 1 min, and centrifuged (Velocity 14R; Dynamica Scientific, Newport Pagnell, UK) at $10,576 \times g$ for 20 min. The supernatant was recovered, and remaining solids were extracted one more time in the same manner. Extracts were then pooled in a 10-mL volumetric flask (Pérez-Loredo et al., 2016), and analyzed for total betalains, betaxanthins, betacyanins, Folin-Ciocalteu (FC) reduction capacity, and antiradical capacity. Color parameters L^* , a^* , b^* , H° , and C^* were also determined.

3.3. Stability of *S. prunosus* betaxanthin

3.3.1. Effect of pH

Extracts were prepared as described using 0.1 M citrate-phosphate buffer with pH 2.4, 3.6, 4.3, 4.8, 5.4, 6.6, 7.4, and 7.5. Aliquots (4 mL) of each extract ($A\lambda_{483} \approx 1.2$) were placed in 10-mL test tubes, and incubated at 60 °C in the dark in a stirring water bath at 150 rpm. Samples were withdrawn at 0, 0.33, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 h, and immediately cooled to 20 °C. Betaxanthin content was measured at each time point, and the degradation rate constant and half-life ($t_{1/2}$) were calculated using a first-order kinetic model (Von Elbe, Maing, & Amundson, 1974). To calculate the percentage retained at the end of



Fig. 2. Fruits of *S. prunosus* from Puebla, México.

the experiment, initial and final FC reduction and antiradical capacities were determined.

3.3.2. Effect of temperature

Aliquots (4 mL) of extracts prepared at pH 6.6 ($A\lambda_{483} \approx 1.2$) were placed in 10-mL tubes, and incubated at 50, 60, 65, 70, 75, and 80 °C in the dark in a stirring water bath at 150 rpm. Samples were withdrawn at 0, 0.33, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 h, and immediately cooled to 20 °C. Betaxanthin content were measured at each time point, along with initial and final FC reduction and antiradical capacity as described. Degradation rate constant, and half-life using were calculated using a first-order kinetic model (Von Elbe et al., 1974). Finally, the activation energy was calculated using the Arrhenius equation (Sun, Ma, Xingqian, Kakuda, & Meng, 2010).

3.3.3. Effect of food additives

The average pH of 10 yellow-orange commercial beverages was determined to be 3.6 ± 0.25 . Thus, betaxanthin extracts were prepared using 0.1 M citrate-phosphate buffer pH 3.6, and mixed with 5 g L⁻¹ glucose or sucrose, or 1 g L⁻¹ citric or ascorbic acid (Mojhammer et al., 2007). Aliquots (4 mL, $A\lambda_{483} \approx 1.2$) were placed in test tubes, and immersed in the dark in a stirring water bath at 60 °C and 150 rpm. Samples were withdrawn at 0, 0.33, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 h, and the degradation rate constant and half-life were calculated as described, along with the percentage of retained FC reduction and antiradical capacity.

3.4. Use and stability in food products

Synthetic food colorants are mostly used in sugary products and beverages, usually to promote consumption, especially by children (Martins, Roriz, Morales, Barros, & Ferreira, 2016). Therefore, gummies and beverages were selected as model food products.

3.4.1. Selection of commercial products for color matching

Samples of yellow-orange commercial products, including eight gummies and 10 beverages, were obtained from a local market, and analyzed at 22 °C for color, pH, and water activity (Aqualab Series 3 TE, Pullman, WA, USA). Color was measured in terms of L*, a*, and b* values using a Color Flex EZ 45/0 colorimeter (CFEZ1005; Hunter Lab, Reston, Virginia, USA), with directional excluded specular geometry at 45/0° and reflectance measurements with D65 illumination and at a 10° observer angle against a white background. A hedonic test of 5 levels of preference was conducted with 50 untrained judges (18–25 years) to determine the color preferred by consumers for all commercial products. Each level was assigned a numeric value (from 1 = dislike to 5 = really like), which were added up after the application. The color with the highest evaluated score in each category was matched to laboratory-prepared model products using the appropriate concentration of *S. pruinosus* pulp or juice.

3.4.2. Model food products

To take advantage of all chemical compounds in the fruit, gummies and beverages were prepared using yellow pitaya juice or pulp instead of extracts. Solids obtained after homogenizing the fruit were used as pulp, and the supernatant recovered after centrifugation of the pulp at 10,576 × g for 20 min was used as juice. Gummies were formulated according to Gutiérrez-Zúñiga, Arriaga-Alba, Ordaz-Pichardo, Gutiérrez-Macías, and Barragán-Huerta (2014) with some modification, and consisted of 4.6, 6.9, or 11.5 g pulp or juice, 6.7 g gelatin, 25.5 g sucrose, 38 g glucose, 6.5 g glycerol, and 0.3 g citric acid, topped up with water to 100 g. Model beverages were formulated according to Dirby, Westgaard, and Stapelfeldt (2001) with some modification, and consisted of 20 g sucrose, 1 g citric acid, 0.14 g potassium benzoate, and pitaya juice (0, 1, 2, 3, 4, 5, 7, 10, or 20 g), topped up with water to 100 mL. Betaxanthin content and color were analyzed. The chromatic

parameters L*, a*, and b* were compared with the corresponding selected commercial product using Eq.(1), and the model product with the lowest ΔE was selected for betaxanthin stability assays.

$$\Delta E = \sqrt{(L^*_i - L^*_0)^2 + (a^*_i - a^*_0)^2 + (b^*_i - b^*_0)^2} \quad (1)$$

where ΔE is the color difference between model (L^*_i , a^*_i , and b^*_i) and commercial products (L^*_0 , a^*_0 , and b^*_0).

3.4.3. Betaxanthin stability in gummies

Pigmented gummies (3 g) were stored for 28 days in test tubes at 40 °C in the dark, and samples were collected at 0, 1, 2, 3, 8, 10, 14, 21, and 28 days. Each sample was mixed with 3 mL distilled water, ultrasonicated for 30 min at 40 °C, vortexed for 1 min at 3150 rpm, and then centrifuged at 10,576 × g for 10 min. The supernatant was used to determine betaxanthin concentration and color. Degradation rate constant and half-life were calculated as above using a first-order kinetic model.

3.4.4. Betaxanthin stability in beverages

Pigmented model beverages (25 mL) were stored for 28 days at 4 °C in the dark, or for 24 h at 25 °C with or without light (Fluorescent lamp FLC/CIRC/22/Daylight 20 watts). Pigment concentration and color were assessed at 0, 1, 2, 3, 4, 5, 6, 7, 9, 12, 15, 18, 23, and 28 days for samples stored at 4 °C, or at every hour for samples stored at 25 °C. Degradation rate constant and half-life were calculated as above using a first-order kinetic model.

3.5. Photometric quantification of betalain

Betaxanthin and betacyanin content were quantified by absorbance at 483 and 538 nm, respectively (Castellanos-Santiago & Yahia, 2008), using a UV-Vis spectrophotometer (DR5000; Hach, Mexico State, Mexico). Betaxanthin and betacyanin concentrations were calculated using Eq.(2), and summed to determine total betalains.

$$B = \frac{(A \times DF \times W \times V)}{(\epsilon \times P \times L)} * 1000 \quad (2)$$

where B represents betaxanthin and betacyanin content (μg g⁻¹) determined using indicaxanthin and betanin, respectively, as references. A is absorbance; DF is dilution factor; W is molecular weight: 550 g mol⁻¹ for betanin, and 308 g mol⁻¹ for indicaxanthin; V is extract volume (mL); ε is molar extinction coefficient (60,000 L. mol⁻¹ cm⁻¹ for betanin and 48,000 for indicaxanthin); P is the amount of sample (g); and L is the path length (1 cm).

Table 1

Main bioactive compounds and color parameters of the pulp in yellow *S. pruinosus* fruits.

Parameter	Value
Betaxanthins (μg indicaxanthin g ⁻¹)	2077.81 ± 53.83
Betacyanins (μg betanin g ⁻¹)	268.09 ± 19.21
Total Betalains (Bc + Bx, μg g ⁻¹)	2345.89 ± 68.55
FC-RC (mg GAE g ⁻¹)	7.29 ± 0.22
Antiradical capacity (μmol TE g ⁻¹)	48.80 ± 1.29
a*	33.60 ± 0.03
b*	28.21 ± 0.05
Lightness (L*)	17.90 ± 0.04
Hue angle (H°)	61.95 ± 0.04
Chroma (C*)	38.07 ± 0.04

FC-RC, Folin-Ciocalteu reduction capacity; GAE, gallic acid equivalents; TE, Trolox equivalents. Values are average of three measurements ± SD, and are normalized to g pulp dry weight.

Table 2
Degradation rate constants (k), half-life ($t_{1/2}$), and retention (%) of betaxanthin, and retention of the Folin-Ciocalteu reduction capacity (FC-RC) and antiradical capacity (AC) over a range of pH values and temperatures.

	Betaxanthins		R^2	Retention (%)	FC-RC retention (%)	AC retention (%)
	k (h^{-1})	$t_{1/2}$ (h)				
pH^a						
2.4	0.948 ± 0.017 ^a	0.73 ± 0.01 ^f	0.998	4.3 ± 0.0 ^g	87.1 ± 1.3 ^a	96.2 ± 1.2 ^a
3.6	0.567 ± 0.013 ^b	1.22 ± 0.05 ^e	0.995	13.2 ± 0.6 ^f	81.7 ± 0.8 ^b	94.8 ± 2.9 ^a
4.3	0.311 ± 0.002 ^c	2.23 ± 0.01 ^d	0.984	30.0 ± 0.6 ^e	77.3 ± 1.8 ^c	92.1 ± 2.8 ^a
4.8	0.186 ± 0.000 ^d	3.73 ± 0.00 ^c	0.991	49.1 ± 0.3 ^d	76.7 ± 1.3 ^{cd}	84.8 ± 1.6 ^b
5.4	0.123 ± 0.001 ^e	5.65 ± 0.06 ^b	0.997	62.1 ± 0.3 ^b	71.5 ± 2.0 ^{ef}	75.9 ± 1.9 ^c
6.6	0.094 ± 0.003 ^f	7.37 ± 0.26 ^a	0.965	66.9 ± 0.2 ^a	73.5 ± 1.3 ^{de}	76.9 ± 0.5 ^c
7.4	0.119 ± 0.000 ^e	5.82 ± 0.02 ^b	0.972	59.8 ± 0.2 ^c	68.6 ± 0.7 ^f	77.6 ± 1.9 ^c
T^b (°C)						
50.0	0.040 ± 0.001 ^f	17.41 ± 0.47 ^a	0.985	84.1 ± 0.6 ^a	95.9 ± 1.9 ^a	62.5 ± 1.4 ^c
60.0	0.086 ± 0.002 ^e	8.05 ± 0.21 ^b	0.963	70.7 ± 0.5 ^b	81.2 ± 0.7 ^b	77.4 ± 4.2 ^b
65.0	0.110 ± 0.001 ^d	6.28 ± 0.04 ^c	0.967	62.5 ± 0.2 ^c	70.3 ± 1.0 ^c	82.1 ± 2.3 ^b
70.0	0.171 ± 0.003 ^c	4.05 ± 0.08 ^d	0.989	49.3 ± 0.2 ^d	69.5 ± 0.3 ^c	89.2 ± 3.3 ^a
75.0	0.233 ± 0.002 ^b	2.98 ± 0.02 ^e	0.983	37.7 ± 0.1 ^e	64.6 ± 0.8 ^d	77.1 ± 1.8 ^b
80.0	0.325 ± 0.004 ^a	2.13 ± 0.04 ^f	0.984	25.6 ± 0.4 ^f	62.2 ± 0.7 ^d	77.9 ± 1.1 ^b

^aConditions: 4 h at 60 °C in the dark, and in 0.1 citrate-phosphate buffer. ^bConditions: 4 h in 0.1 M citrate-phosphate buffer pH 6.48 ± 0.05. Different letters represent significant differences between pH or temperature conditions ($P < 0.05$).

3.6. In vitro antioxidant activity

The FC assay has traditionally been used as a measure of total phenolics in vegetables and fruits. However, additional non-phenolic organic and inorganic substances in crude extracts may also react with the FC reagent and give elevated apparent phenolic concentrations. It has recently been suggested that, unless interference can be eliminated, the results obtained by this method should be mentioned as FC reduction capacity instead of total phenolics (Prior, Wu, & Schaich, 2005). Here, we applied the traditional FC method described for the determination of total phenolics; however, we use the term FC reduction capacity because the experiments were performed on crude extracts. The results (expressed as mg gallic acid equivalent per g sample) were compared with those reported in the literature for crude extracts.

3.6.1. Folin-Ciocalteu reduction capacity

Extracts (2 mL) were mixed with 2.25 mL 1:10 FC reagent, kept in the dark for 7 min, diluted with 2.25 mL 6% sodium carbonate, vortexed for 30 s at 3150 rpm, and incubated in the dark for 90 min at 20 °C. Finally, absorbance was measured at 765 nm. FC reduction capacity was quantified as mg gallic acid equivalents per g dried pulp (Li, Smith, & Hossain, 2006).

3.6.2. Antiradical capacity using the ABTS assay

The ABTS assay was performed as proposed by Re et al. (1999). Aqueous ABTS (7 mM) was mixed with 2.45 mM potassium persulfate, and incubated for 16 h in the dark to generate free radicals. ABTS^{•+} radical (1 mL) was diluted with 0.1 M phosphate buffer pH 7.4 to achieve absorbance of 0.7 ± 0.05 at 734 nm. A 100- μ L aliquot of pitaya extract was added to 1.9 mL of diluted ABTS^{•+} radical, vortexed for 10 s, and

kept in the dark for 7 min. The absorbance was measured at 734 nm, using as blank 100 μ L water similarly reacted with 1.9 mL ABTS^{•+} radical. Results are reported as μ mol Trolox equivalent per g dried pulp.

3.7. Statistical analysis

Results from three independent assays are reported as mean ± standard deviation. Means were compared by analysis of variance and Tukey's comparison test, with significance level $P < 0.05$. Data were processed and analyzed in Minitab 16 (Minitab Inc., State College, PA, USA).

4. Results and discussion

4.1. Color and main bioactive compounds of *S. pruinosa* pulp

The concentrations of bioactive compounds in yellow *S. pruinosa* fruits are listed in Table 1. We found that the pulp of *S. pruinosa* contained 2345.89 μ g of total betalains g^{-1} dry weight, with betaxanthins representing 89% of that value. Thus, the fruit is a potential source of natural yellow colorants with antioxidant properties. It should be noted that although betaxanthins are widely distributed, few plants naturally contain high concentrations of these pigments (Kugler et al., 2007).

Red beetroot (*Beta vulgaris*) is the major commercial source of betalain-type pigments; however, its appeal is limited by the presence of geosmin and pyrazines, which negatively impact sensorial acceptance, as well as elevated concentrations of nitrates, which are associated with formation of carcinogenic nitrosamines (Stintzing & Carle, 2004). Betaxanthin content in pitaya is lower than the one reported for red beet (5543.4 μ g g^{-1} DW), but similar to that of yellow beet

Table 3
Degradation rate constants (k), half-life ($t_{1/2}$), and retention (%) of betaxanthin, and retention of the Folin-Ciocalteu reduction capacity (FC-RC) and antiradical capacity (AC) in the presence of several additives commonly used in acidic foods.

Additive	Betaxanthins		R^2	Retention (%)	FC-RC retention (%)	AC retention (%)
	k (h^{-1})	$t_{1/2}$ (h)				
Without additive	0.520 ± 0.015 ^b	1.33 ± 0.04 ^b	0.995	13.5 ± 0.9 ^b	76.4 ± 0.4 ^a	87.4 ± 0.1 ^a
Glucose	0.578 ± 0.012 ^a	1.20 ± 0.02 ^c	0.997	10.5 ± 0.6 ^c	74.1 ± 0.4 ^b	87.8 ± 0.2 ^a
Sucrose	0.532 ± 0.005 ^b	1.30 ± 0.01 ^{bc}	0.996	12.6 ± 0.3 ^b	75.4 ± 0.4 ^{ab}	89.9 ± 1.5 ^a
Citric acid	0.519 ± 0.012 ^b	1.34 ± 0.03 ^b	0.996	13.6 ± 0.7 ^b	75.5 ± 0.7 ^a	87.5 ± 2.8 ^a
Ascorbic acid	0.282 ± 0.008 ^c	2.46 ± 0.07 ^a	0.989	31.5 ± 0.9 ^a	–	–

Conditions: Glucose or sucrose at 5 $g L^{-1}$, citric or ascorbic acid at 1 $g L^{-1}$; 4 h at 60 °C in 0.1 M citrate-phosphate buffer, pH 3.5. Different letters represent significant differences between additives ($P < 0.05$).

Table 4
Chromatic parameters of model food products and consumer-preferred commercial samples.

Product		Code	L*	a*	b*	C*	H°	ΔE		
Gelatin gummies	Commercial	YW2	32.8 ± 0.3	4.0 ± 0.1	19.1 ± 0.3	19.5 ± 0.2	78.1 ± 0.4	Reference		
		Control	31.1 ± 0.1	0.1 ± 0.0	9.2 ± 0.2	9.2 ± 0.2	89.5 ± 0.1	11.1 ± 0.5		
	Formulated	4.6P	30.9 ± 0.6	3.2 ± 0.1	12.8 ± 0.7	13.2 ± 0.7	76.1 ± 0.4	6.0 ± 0.2		
		6.9P	29.3 ± 0.1	4.5 ± 0.1	11.9 ± 0.1	12.3 ± 0.8	69.9 ± 0.9	8.0 ± 0.1		
		11.5P	25.9 ± 0.0	3.5 ± 0.1	10.8 ± 0.1	10.6 ± 1.3	73.2 ± 1.7	10.8 ± 0.1		
		4.6 J	30.1 ± 0.2	3.4 ± 0.2	15.2 ± 0.3	15.6 ± 0.3	76.9 ± 0.9	4.9 ± 0.2		
		6.9 J	28.1 ± 0.1	0.9 ± 0.0	11.2 ± 0.4	10.8 ± 0.8	85.7 ± 0.5	9.9 ± 0.1		
		11.5 J	27.0 ± 0.1	2.0 ± 0.1	11.3 ± 0.2	11.5 ± 0.2	79.7 ± 0.5	9.9 ± 0.1		
		Beverages	Commercial	CB2	57.8 ± 0.0	20.6 ± 0.1	72.5 ± 0.2	75.4 ± 0.1	74.1 ± 0.1	Reference
				Control	68.5 ± 0.0	-0.8 ± 0.0	5.3 ± 0.6	5.4 ± 0.6	81.0 ± 0.9	71.4 ± 0.5
Formulated	BJ01		64.4 ± 0.0	1.7 ± 0.0	32.6 ± 0.0	32.7 ± 0.0	87.1 ± 0.0	44.7 ± 0.0		
	BJ02		61.7 ± 0.0	4.6 ± 0.0	52.3 ± 0.0	52.5 ± 0.0	85.0 ± 0.0	26.1 ± 0.0		
	BJ03		59.9 ± 0.1	7.2 ± 0.0	63.7 ± 0.0	64.1 ± 0.0	83.6 ± 0.0	16.3 ± 0.0		
	BJ04		58.4 ± 0.0	9.8 ± 0.0	71.4 ± 0.0	72.1 ± 0.0	82.2 ± 0.0	10.9 ± 0.0		
	BJ05		57.1 ± 0.0	12.2 ± 0.1	75.8 ± 0.0	76.8 ± 0.0	80.9 ± 0.1	9.1 ± 0.0		
	BJ07		54.7 ± 0.0	16.6 ± 0.0	81.0 ± 0.1	82.7 ± 0.0	78.4 ± 0.0	9.9 ± 0.0		
BJ10	51.6 ± 0.0	22.3 ± 0.0	82.2 ± 0.1	85.2 ± 0.1	74.8 ± 0.0	11.6 ± 0.1				
BJ20	44.0 ± 0.0	35.2 ± 0.0	74.1 ± 0.0	82.1 ± 0.0	64.6 ± 0.0	20.2 ± 0.0				

Control, product without pigment; P, pulp; J, juice. The ΔE of a formulated product is relative to commercial samples YW2 (gummies) and CB2 (beverage). Numbers in the Code column are percentage of juice or pulp added.

(2286.9 μg g⁻¹ DW) (Kugler et al., 2007). Currently, the pigment market demands diversification, warranting the search for novel and alternative betalain sources, particularly those rich in betaxanthins. These are in limited supply and yellow pitaya from *S. pruinosa* represents a promising alternative.

FC reduction capacity in *S. pruinosa* was higher than reported for *S. stellatus*, *S. griseus*, and *Hylocereus polyrhizus* fruits, which contained from 3.7 to 5.8, 0.5 to 1.6, and 1.4 to 4.2 mg gallic acid equivalents g⁻¹ dry weight, respectively (Pérez-Loredo et al., 2016; García-Cruz, Salinas-Moreno, & Valle-Guadarrama, 2012; Woo, Ngou, Ngo, Soong, & Tang, 2011). In addition, the antiradical capacity of pitaya pulp was higher than the one reported for *Opuntia*, which contained 10.2–43.1 μmol Trolox equivalents g⁻¹ dry weight (Pérez-Loredo et al., 2016; Fernández-López, Angosto, Giménez, & León, 2013). Thus, *Stenocereus* fruits appear to be a rich source of beneficial antioxidants.

4.2. Stability of betaxanthin, FC reduction capacity, and antiradical capacity in *S. pruinosa*

4.2.1. pH stability

Results indicate that the degradation of betaxanthins followed first-order kinetics over a range of pH, with R² between 0.965 and 0.998 (Table 2). The pigments were most stable at pH 6.6. Similarly, pigment retention was highest between pH 6.0 and 7.0 for indicaxanthin from yellow *Opuntia* fruits (Gandía-Herrero, Jiménez-Atiéndar, Cabanes, García-Carmona, & Escribano, 2010). In contrast, the maximum stability at 60 °C for partially purified betaxanthins and crude extracts from *Celosia plumosa* was at pH 5.5, with half-lives of 9.6 and 12.9 h, respectively (Cai et al., 2001). It has been reported that the pH conferring maximum betalain stability could be influenced by temperature and oxygen concentration in the medium (Herbach, Stintzing, & Carle, 2006). Sanchez-Gonzalez, Jaime-Fonseca, San Martin-Martinez, and Zepeda (2013) reported that the pH giving maximum betalain stability depended on the food matrix as evinced by the combined effect of pH (3–7) and temperature (25–80 °C) on betalain stability of red beetroot and *Opuntia joconostle* fruit. Accordingly, at 25 °C betalain from *B. vulgaris* was most stable at pH 7, but at 60–80 °C optimum pH changed to 6.0. For *O. joconostle*, the dependence of pH on temperature was not significant ($P = 0.545$), and pH of maximum stability was 6.0 at all assayed temperatures.

The pH for maximum retention of betaxanthins from *S. pruinosa* differed from the acidic pH at which FC reduction and antiradical capacity were highest (Table 2). At such pH, betalains are susceptible to hydrolysis, releasing betalamic acid and amino compounds or *cyclo*-DOPA

derivatives, corresponding to betaxanthin or betacyanin structures respectively, which maintain high levels of antioxidant activity (Herbach et al., 2006). In addition, Maillard products with antioxidant activity can be produced by the reaction between free amino acids and sugars (Laroque et al., 2008).

4.2.2. Thermal stability

At pH 6.48 ± 0.05, the thermal degradation of betaxanthin followed first-order kinetics, with R² 0.963–0.989 (Table 2). Accordingly, the degradation reaction constants (k) increased with temperature. These results are consistent with Cai et al. (2001), who reported k values of 0.0197 to 0.97 h⁻¹ for *C. plumosa* betaxanthins. Similarly, retention of

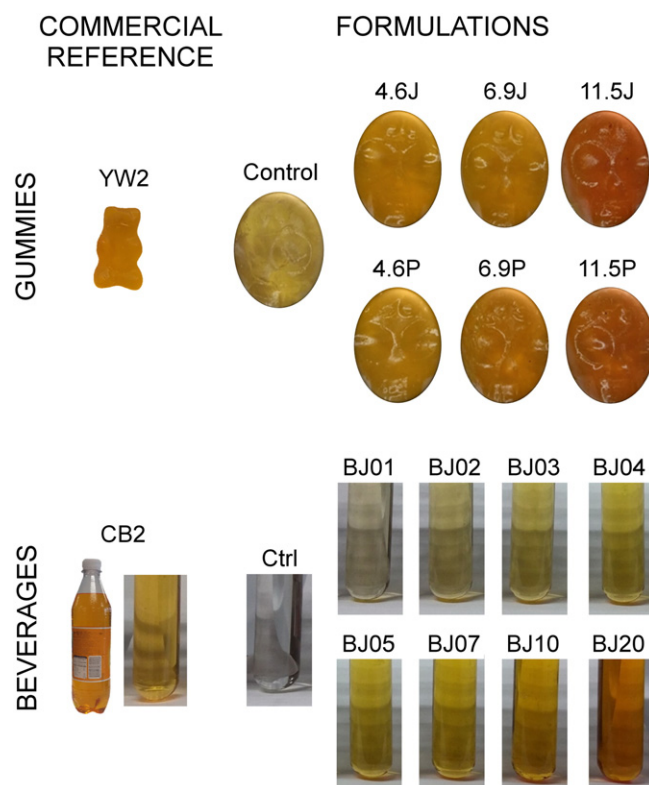


Fig. 3. Gelatin gummies and beverages prepared with various percentages of yellow pitaya pulp (P) and juice (J).

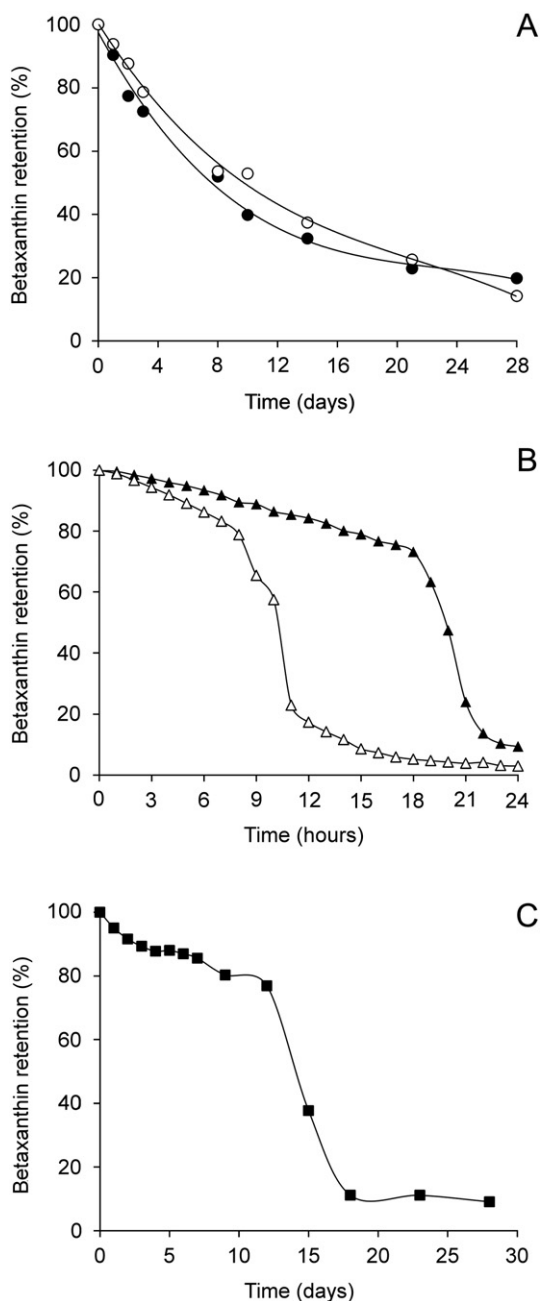


Fig. 4. Betaxanthin retention during storage. A) Gummies pigmented with pulp (●) or juice (○) and stored at 40 °C in the dark. B) Beverage with pH 3.5 and stored at 25 °C in darkness (▲) or exposed to light (△). C) Beverage with pH 3.5 and stored at 4 °C in darkness (■).

Table 5

Degradation rate constants (k) and half-life ($t_{1/2}$) of betaxanthin in gummies and beverages stored in various conditions.

Product		Storage conditions	Betaxanthins		
			k (day ⁻¹)	$t_{1/2}$ (days)	R^2
Gummy	Pulp	40 °C, darkness, 28 d	0.059 ± 0.004^e	11.73 ± 0.85^b	0.967
		40 °C, darkness, 28 d	0.067 ± 0.000^e	10.38 ± 0.05^b	0.995
Beverage	Juice	4 °C, darkness, 28 d	0.019 ± 0.003^e	36.99 ± 4.8^a	0.953
		I: 0–12 d II: 12–28 d	0.322 ± 0.001^d	2.15 ± 0.00^c	0.981
	25 °C, darkness, 24 h	I: 0–18 h II: 18–24 h	0.421 ± 0.014^d 8.896 ± 0.045^a	1.65 ± 0.05^c 0.08 ± 0.00^c	0.986 0.962
	25 °C, light, 24 h	I: 0–8 h II: 8–24 h	0.704 ± 0.017^c 4.823 ± 0.122^b	0.99 ± 0.02^c 0.14 ± 0.00^c	0.945 0.909

Stage I: From 100% to 75% or retention. Stage II: From 75% to the end of experiment. Different letters represent significant differences between products ($P < 0.05$).

FC reduction capacity decreased with temperature, possibly due to a decrease in ascorbic acid contained in pitaya extracts. In contrast, retention of antiradical capacity peaked at 70 °C (Table 2), presumably due to heat-induced formation of Maillard products and other compounds, which depends on the natural matrix and presents a possible biological function (Montes-Lora, Hurtado, Mosquera, Heredia, & Cejudo-Bastante, 2016). An increase of antiradical capacity with temperature has been reported elsewhere in *Opuntia ficus-indica* and *O. dillenii* (Aguirre-Joya, De la Garza-Toledo, Zugasti-Cruz, Belmares-Cerda, & Aguilar-Cristóbal, 2013; Montes-Lora et al., 2016).

Besides hydrolysis, isomerization of betaxanthin upon thermal exposure is also possible (Stintzing & Carle, 2004). Moreover, betacyanins are susceptible to decarboxylation, which produces more stable structures and maintains color, as well as dehydrogenation, which occurs due to aerobic oxidation and generates yellow neobetacyanins (Herbach et al., 2006).

We found that $\ln(k)$ was linearly correlated with the inverse of the temperature ($1/T$), with R^2 0.998. Accordingly, the activation energy for the degradation of betaxanthins from *S. prinosus* was calculated to be $66.25 \text{ kJ mol}^{-1}$, suggesting that these pigments are similarly stable as vulgaxanthin-I from *Beta vulgaris* ($68.19 \text{ kJ mol}^{-1}$, measured at 61.5–85.5 °C; Saguy, 1979), and yellow-orange pigments from *Opuntia ficus-indica* (65.1 kJ mol^{-1} , measured at pH 5 and 50–90 °C; Coskuner, Turker, Ekiz, Aksay, & Karababa, 2000). However, *S. prinosus* betaxanthins are more sensitive to temperature than other natural yellow pigments, such as lutein, riboflavin, curcumin, β -carotene, or yellow gardenia, for which activation energies are between 3.2 and 43.7 kJ mol^{-1} when measured from 30 to 90 °C (Giménez, Fernández-López, Angosto, & Obón, 2015).

4.2.3. Effect of food additives

Betaxanthins were degraded in the presence of various food additives according to first-order kinetics ($0.989 < R^2 < 0.997$), with degradation rate constants listed in Table 3. Except for ascorbic acid, which increased the half-life by 85%, additives did not significantly affect stability ($P > 0.05$). Indeed, organic acids such as citric, ascorbic, and isoascorbic acid have been reported to stabilize betaxanthins in aqueous extracts by isomerization, increasing regeneration rate, and enhancing resistance to degradation (Herbach et al., 2006). For instance, retention of betaxanthins from yellow-orange *Opuntia ficus-indica* fruit juice was highest at pH 6 in the presence of 0.1% citric acid (Moßhammer et al., 2007). In contrast, addition of citric acid did not have any effect ($P < 0.05$) on the stability of pigments and antioxidants in the current work.

4.3. Pigment and color stability in model food products

4.3.1. Selection of commercial products for color matching

The chroma and hue values for commercial samples of yellow gelatin gummies were 11.41 ± 1.1 to 19.7 ± 0.1 and 68.5 ± 0.1 to 87.6 ± 0.4 , respectively. For yellow beverages, the chroma and hue values were more disperse, and ranged from 21.38 ± 0.10 to 87.78 ± 0.31

and from 53.9 ± 0.1 to 87.8 ± 0.1 , respectively. The chromatic parameters for the commercial gummie (YW2) and beverage (CB2) most preferred by consumers are listed in Table 4.

4.3.2. Color properties of gummies and beverages pigmented with pitaya

The color parameters of gummies and beverages pigmented with *S. pruinosa* depended on the concentration of juice or pulp used (Fig. 3). In particular, the C^* values of formulated gelatin gummies pigmented

with juice and pulp ranged from 10.6 ± 0.9 to 15.6 ± 0.3 and from 10.6 ± 1.3 to 13.2 ± 0.7 , respectively. In beverages pigmented with juice, values were between 32.7 ± 0.0 and 85.2 ± 0.1 (Table 4). Accordingly, the formulated food products exhibited various shades of yellow-orange (Fig. 3).

Formulated gummies containing 4.6% pulp and 4.6% juice had the lowest ΔE (6 and 5, respectively) relative to the preferred commercial product. In contrast, beverages pigmented with 5% juice were most similar in color to the preferred commercial drink, with ΔE of 9. A $\Delta E < 3$ is desirable for color equalization, as consumers do not detect color differences within this value (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001). Even though pitaya displayed a yellow-orange color in formulated products, color matching to selected commercial products with $\Delta E < 3$ was not possible due to the fruit's chromatic properties. Therefore, the above formulated products, with the lowest ΔE , were used in pigment stability tests.

4.3.3. Betaxanthin and color stability in formulated gummies and beverages

The initial betaxanthin concentration in formulated products was $2.71 \pm 0.13 \mu\text{g g}^{-1}$ in gummies with 4.6% pulp, $2.94 \pm 0.05 \mu\text{g g}^{-1}$ in gummies with 4.6% juice, and $7.05 \pm 0.01 \mu\text{g mL}^{-1}$ in beverages with 5% juice. Betaxanthin retention during storage was dependent on light, temperature, water activity, and food matrix (Khan, 2016).

In particular, 50% of pigments were lost in gummies stored at 40°C for 11 days (Fig. 4A) but the same percentage was lost in beverages stored for only 20 h at 25°C (Fig. 4B). Moreover, degradation in beverages stored at 25°C was 2-fold higher in samples exposed to light than in samples kept in the dark (Fig. 4B). Similarly, degradation was 15 times higher in beverages stored at 25°C than in those at 4°C (Fig. 3C).

Differences in degradation kinetics were observed also between gummies and beverages. In particular, betaxanthin degradation followed first-order kinetics ($0.967 < R^2 < 0.995$) in gummies (Table 5), but occurred in two stages in model beverages, regardless of storage conditions (Fig. 4B, C). Indeed, betaxanthin retention decreased only gradually from 100% to 75% during stage I, disappearing rapidly then during stage II. Both degradation stages followed first-order kinetics (Villalobos-Castillejos, Cerezal-Mezquita, Hernández-De Jesús, & Barragán-Huerta, 2013), with R^2 between 0.909 and 0.986 (Table 5).

The biphasic behavior is probably due to the presence of natural-matrix compounds, such as antioxidants or co-pigments, which may enhance pigment stability and retard betaxanthin oxidation (Khan, 2016). Moreover, the apparent instability of betaxanthins in beverages is probably due to the effects of the food matrix and to water activity, which was higher in beverages ($a_w = 0.96 \pm 0.01$) than in gelatin gummies ($a_w = 0.75 \pm 0.01$). High water activity is known to promote pigment degradation (Oh, Shin, Kyungae, & Choe, 2013), whereas interactions with proteins may protect pigments from hydrolysis and oxidation.

The results are consistent with Cai et al. (2001), who reported that 73.3–77.5% of lyophilized betaxanthins from *Celosia argentea* were retained after 20 weeks at 4°C in model drinks with pH 5.5. However, only 9.8–18.7% of pigments were retained after 10 weeks at 22°C . Moreover, up to 30% of pigments from *Opuntia dillenii* extracts with pH 4.0–6.0 were lost at 20°C , although these pigments were stable at 4°C for 12 days (Cejudo-Bastante, Hurtado, & Heredia, 2015). Taken together, the data indicate that *S. pruinosa* is a suitable source of pigments that can be successfully used in beverages and gummies with acidic pH, and can be stabilized with low-temperature storage or with additives such as ascorbic acid.

Changes in the CIELAB color parameters a^* and b^* were also evaluated over time in model foods stored under different conditions (Fig. 5). Inspection of the (a^*b^*) color diagram indicated a similar decrease in b^* in both gummies and drinks, suggesting decolorization in all samples (Fig. 5).

Nevertheless, some remarkable differences in color changes were observed between gummies and beverages. In particular, gummies

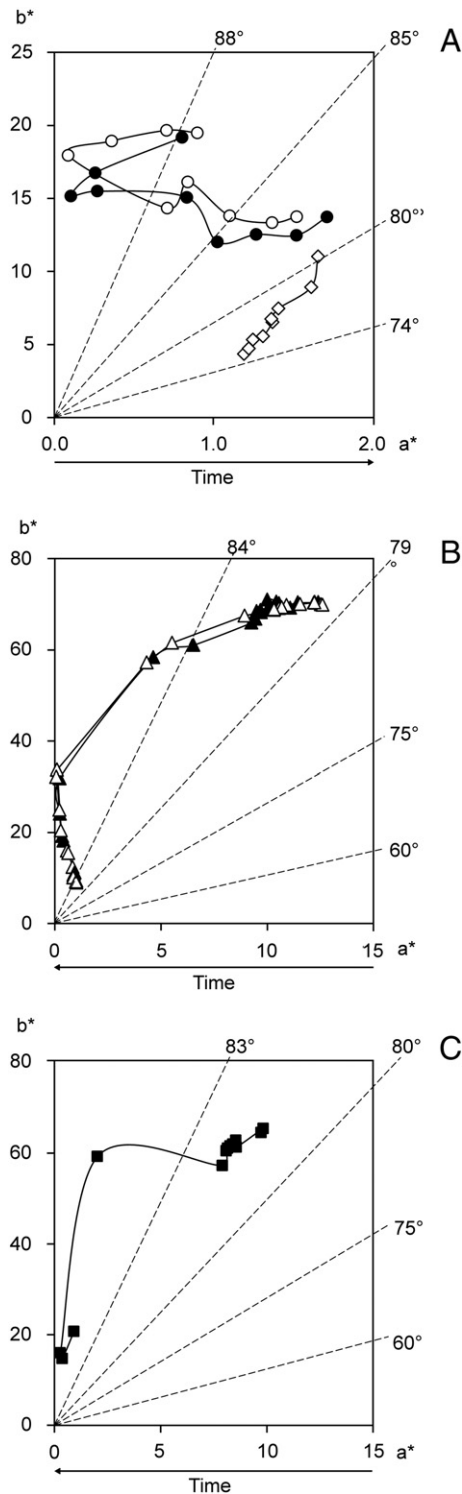


Fig. 5. CIELAB color space plot during storage. A) Gummies formulated with pulp (●) or juice (○) or without pitaya (◇) stored at 40°C for 28 d. B) Beverage stored for 24 h at 25°C with (△) or without (▲) light. C) Beverage stored for 28 d at 4°C (■).

pigmented with pitaya pulp and juice had H° and C^* values of 86.99–87.54° and 18.64–19.52 at day zero, respectively, indicating a bright and intense yellowish tone (Fig. 3A). On the contrary, un-pigmented control gummies (Fig. 3A) were pale yellow, with H 74.63° and C^* 4.51. Over time, the chromatic parameters decreased in gummies pigmented with pulp or juice, but increased in control samples, so that both had a similar color at the end of the experiment (Fig. 5A). The intensifying color in control samples may be due to heat-induced production of Maillard compounds. Similarly, beverages that were to be stored at 25 °C with and without light had initial H° and C^* values of 79.85–80.16° and 71.15–71.62 at day zero, respectively. Over time, hue angles increased to 90° while saturation diminished. Exposure to light during storage accelerated these color changes (Fig. 5B). On the contrary, samples that were to be stored at 4 °C had H° and C^* values of 81.43° and 65.28 at day zero, respectively, indicating a saturated bright yellow-orange color (Fig. 5C).

5. Conclusions

The fruits of *S. pruinosa* are an important source of betaxanthin, which is beneficial to human health. We have characterized for the first time the stability of betaxanthins from yellow *S. pruinosa* fruits when exposed to several stress factors. We found that the pH and thermal stability of these pigments were similar to those from fruits of other Cactaceae species. In particular, stability was highest at pH 6.6 for betaxanthins, whereas FC reduction and antiradical capacities were highest at pH 2.4. Importantly, we demonstrated that these betaxanthins could be used to match the yellow color of commercially available gelatin gummies and beverages. Notably, the betaxanthins were more stable in gummies than in beverages, with remarkable differences in pigment retention and color changes during storage. Thus, pigments from *S. pruinosa* fruits can substitute synthetic yellow pigments in manufactured food. These data may help develop healthy food products with a longer shelf life, and thereby increase the commercial value of pitayas. Besides the potential use of pitaya pigments in the food industry, data provided in this paper are of interest for the scientific community.

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