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Colorant and antioxidant properties of red-purple pitahaya (*Hylocereus* sp.)

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RESUMEN ESPAÑOL, p. 12

Colorant and antioxidant properties of red-purple pitahaya (Hylocereus sp.).

Abstract — **Introduction**. Red-purple pitahaya (*Hylocereus* sp.) is a promising crop grown commercially in dry regions of Central America. Both its skin and flesh are characterized by being a glowing, deeply red-purple color. Materials and methods. The main physicochemical characteristics of three commercial cultivars of red pitahaya were assessed, including total phenolic compounds contents, total betacyanins, vitamin C and oxygen radical absorbance capacity (ORAC). Thermal stability of betacyanins at different temperature and pH was also assessed. Results and discussion. Pitahaya fruit has a low vitamin C content ranging from (116 to 171) $\mu g \cdot g^{-1}$ of fresh pulp without seeds, but it is rich in betacyanins [(0.32 to 0.41) mg·g⁻¹] and phenolic compounds [$(5.6 \text{ to } 6.8) \text{ } \mu\text{mol Eq gallic acid} \cdot \text{g}^{-1}$]; it has a high antioxidant ORAC value of (8.8 to 11.3) µmol Eq Trolox g⁻¹. Visible spectra of aqueous fruit extracts were very similar to that of pure betacyanin. Indeed, the characteristic color of juice diluted to 1% presents a high hue angle ($H^{\circ} = 350^{\circ} \pm 3$) and high chroma values ($C^{*} = 79 \pm 2$). Thermal stability of pitahaya betacyanin decreases with pH, but it remains compatible with industrial utilization as a colorant (half-time = 22.6 min at 90 °C at pH = 5 of the fruit) and was found to be very similar to that previously reported for beetroot. Conclusions. Pitahaya juice combines the functional properties of a natural food colorant with high antioxidant potency.

Central America / Nicaragua / Hylocereus / food colorants / betaine / antioxidants

Propriétés colorantes et antioxydantes de la pitahaya rouge (Hylocereus sp.).

Résumé — **Introduction**. La pitahaya rouge (*Hylocereus* sp.) est une culture pleine de promesses qui est exploitée commercialement dans des régions sèches de l'Amérique centrale. Sa peau tout comme sa chair se caractérisent par leur couleur d'un rouge profond. Matériel et méthodes. Les principales caractéristiques physico-chimiques des fruits de trois cultivars commerciaux de pitahaya rouge ont été évaluées, dont leur teneur totale en composés phénoliques, leur teneur totale en bêtacyanines et en vitamine C, ainsi que leur capacité d'absorbance du radical oxygène (ORAC). La stabilité thermique des bêtacyanines à différentes températures et pH a été également évaluée. Résultats et discussion. La pitahaya a une faible teneur en vitamine C comprise entre (116 et 171) µg·g⁻¹ de pulpe fraîche sans graines, mais elle est riche en bêtacyanine [(0,32 à 0,41) mg·g⁻¹] et en composés phénoliques [(5,6 à 6,8) µmol Eq d'acide gallique·g⁻¹]; elle a une valeur élevée en antioxydant ORAC : (8,8 à 11,3) µmol Eq Trolox·g⁻¹. Les spectres visibles des extraits aqueux de fruits ont été très semblables à celui de la bêtacyanine pure. En effet, la couleur caractéristique du jus dilué à 1 % a une forte tonalité (H° = 350° ± 3) et des valeurs élevées de chromas (C* = 79 ± 2). La stabilité thermique de la bêtacyanine de pitahaya diminue avec le pH, mais elle demeure compatible avec une utilisation industrielle comme colorant (demi période = 22,6 min à 90 °C au pH = 5 du fruit) et elle s'est révélée très semblable à celle précédemment rapportée pour les betteraves. **Conclusions**. Le jus de pitahaya combine les propriétés fonctionnelles d'un colorant alimentaire normal accompagné d'un pouvoir antioxydant élevé.

1. Introduction

Originating in Central America [1], Hylocereus sp., red-purple pitahaya, is nowadays grown commercially mainly in Central America, from northern Costa Rica to Nicaragua, and in Israel in greenhouses [2]. This redskinned fruit with red-purple flesh is commonly consumed in Central America as juice, often mixed with lemon juice to balance the low acidity. As it belongs to the Cactaceae family, its crassulacean acid metabolism allows its cultivation in areas suffering from drought and high atmospheric sulfur concentrations. Hence, the crop is grown mostly on the volcanic hillsides of Nicaragua and Costa Rica [1], areas that have very high poverty indices. Often representing the only developmental perspective for agriculture, this crop has very high social significance in these regions.

Currently, about 420 ha of red-purple pitahaya are grown in Nicaragua, corresponding to an estimated national production of 3000 t. In well-organized plantations, yields can reach 26 t·ha⁻¹. In 2003, two processing companies exported about 55 t of frozen pitahaya pulp with seeds to an essentially ethnic market in the USA. This market is growing as the juice is found to be very attractive for its glowing, deep red-purple color.

Recently, in response to consumer concern, the food industry is renewing its interest in replacing synthetic red dyes with natural plant colorants, now in high demand. Colored pitahayas are, therefore, highly promising. The pigments responsible for the pitahaya's deep red-purple color are betacyanins [3, 4]. In contrast to beetroot (Beta vulgaris L.) [5], no yellow betaxanthins have been detected – a technological advantage, as these latter compounds are less stable [6], degrading into a shade of brown under typical technological conditions [7]. Despite this, beetroot concentrates have been the only extensively used betalain source applied in the food industry until now [8]. However, beetroot preparations have further drawbacks such as an earthy flavor, high nitrate concentration, and may be highly contaminated with soilborne microorganisms, which requires extensive heating before use [9].

Cactus fruits thus have the potential to become an important alternative edible source of betalains [10].

Juice from red-purple-fleshed pitahayas may also be interesting for its antioxidant potential, as with other betalain-rich plants such as beetroot [11, 12], Amaranthus sp. [13], and prickly pear [14]. These fruits possess a reasonable antioxidant potential. Nowadays, increasing the consumption of food with high antioxidant capacity is a significant human health issue. Epidemiological studies have demonstrated that a strong relationship exists between high-antioxidant diets and low incidence of degenerative diseases [15]. Free radicals, resulting from oxidative stress, have been shown to damage DNA and living cells, inducing cell death, tissue injury, and the development of numerous diseases. Some phytochemicals from fruits and vegetables, and among them, betalains, can act as free radical scavengers and are readily bio-available [12]. This represents an additional positive argument for using natural colorants.

The characterization of the red-purple pitahaya's colorant and antioxidant properties is, therefore, an important issue for introducing this new crop into the market and responding to consumer demand for natural products.

2. Materials and methods

2.1. Plant materials

Three main cultivars of *Hylocereus* sp. are grown on a commercial scale in Central America: 'Cebra', 'Lisa', and 'Rosa'. We obtained fruits of these cultivars from the Masaya and Jinotepe commercial plantations of Nicaragua. Healthy fruits, free of injury, were randomly selected. They were cut longitudinally into halves and the pulp carefully detached from the pericarp with a spoon. The slurry, that is, the crude pitahaya juice, was filtered manually through a cloth sheet to remove seeds prior to assessment of yield and physicochemical characteristics

2.2. Analysis

Samples were analyzed for pH, titratable acidity and density, using standard methods [16]. Total soluble solids (TSS) content was measured with an Abbe refractometer (Atago Co., Ltd., Japan). Sucrose, fructose and glucose were also determined by HPLC, following Englyst and Cummings [17]. Viscosity was measured with a glass Oswald capillary viscosimeter on serum after centrifuging a 1% solution of raw juice and deionized water at 4000 g. A modified Folin-Ciocalteu assay [18] was used to determine total phenolic compounds of a juice extract, using gallic acid as standard and expressing the results as gallic acid equivalent (GAE) at 755 nm. Ascorbic acid and dehydroascorbic acid contents were assessed by HPLC, using the method as modified by Kacem et al. and Brause et al. [19, 20].

Red-purple pitahaya juice was filtered and then diluted to reach an acceptable maximum absorbance (A538 nm < 0.8) with McIlvaine's citric-phosphate buffer at pH = 5 and 1 M, and used to estimate betacyanin concentration from spectrophotometric data as described by Saguy et al. [21]. Absorbance values were computed at 10-nm intervals between (350 and 650) nm on a spectrophotometer (Shimadzu UV 240). Color was measured with a Hunter Lab DP 9000 colorimeter (2° standard observer angle and illuminant C), using a white tile as background to the sample. Color was expressed as L^* , a^* , b^* , hue angle [H° = arctan (b^*/a^*)], and chroma $[C = (a^{*2} + b^{*2})^{1/2}]$. Prior to color measurement, the juice was diluted with citric-phosphate buffer at pH = 5, at 1% (v/v). Other color measurements were also performed on juice diluted to reach an absorbance of $A_{538 \text{ nm}} = 1 \pm 0.1$. Thermal stability at different pH values was assessed by measuring residual total betacyanin in red-purple pitahava juice previously mixed with citric acid and potassium sorbate (1 g·L⁻¹) to avoid microorganism growth. Then, the juice was maintained in a thermostatic bath at given temperatures for different time periods. The rate constants of betacyanin degradation, half-time and activation energy were determined using a first-order reaction model, according to the reaction kinetic proposed by Huang and von Elbe [22].

2.3. Determination of antioxidant capacity

The antioxidant capacity of each sample was measured in terms of oxygen radical absorbing capacity (ORAC), using fluorescein as the peroxyl radical damage indicator, following the method described by Ou et al. [23]. All ORAC analyses were performed on a spectrofluorometer (Shimadzu RF-1501), featuring a xenon lamp. Excitation wavelength was fixed at 493 nm and emission at 515 nm. An aliquot of crude pitahaya juice (5 g) was macerated in an agitator at room temperature for 1 h with 20 mL of acetone/ water (50:50, v/v). The slurry was then centrifuged at 3000 g for 15 min and the supernatant used in the ORAC assay, using various dilution factors with 75 mM of phosphate buffer (pH = 7.4). A sample of 750μ L of the fruit extract was incubated for 15 min at 37 °C with 1.5 mL of a fluorescein solution at 8.16×10^{-5} M (Sigma, USA) directly in the fluorometer cell. At time (t), 0.750 uL of a 153 mM AAPH solution [2,2'-azobis(2-amidinopropane) dihydrochloride] (Wako International, USA) was added to give a final reaction volume of 3 mL. A first measurement of fluorescence (f_0) was done at 30 s and then at every minute (f_1 to f_T), keeping the fluorometer vessel in the thermostatic bath between measurements. The net area under the fluorescence decay curve (AUC) was expressed in minutes and calculated, using equation 1:

AUC = 0.5 +
$$f_1/f_0$$
 + f_2/f_0 + f_3/f_0 + f_4/f_0 + ... + f_T/f_0 .

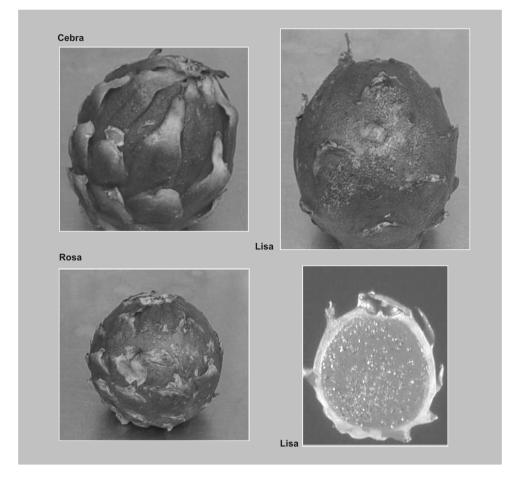
The AUC of a control conducted with 75 mM of phosphate buffer was deduced for all AUC obtained with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and samples. Before assessing fruit-extract samples, the linearity of a Trolox standard curve between (10 and 50) μ M was checked ($r^2 > 0.98$) and the slope (S) that had the best fit with equation 2 [S = (AUC_{Trolox} – AUC_{control}) / molarity_{Trolox}] was evaluated.

The ORAC value of fruit extracts was then expressed in µmol of Trolox equivalent per g, using equation 3:

$$ORAC = [(AUC_{sample} - AUC_{control}) / S] \times dilution factor.$$

Figure 1.

Main cultivars of *Hylocereus* sp. grown commercially in Central America (Nicaragua and Costa Rica) and a fruit of the cultivar Lisa cut longitudinally.



3. Results and discussion

3.1. Main characteristics

The three varieties studied can be distinguished externally by the shape of their fruits and scales (*figure 1*). Fruits from Cebra are the smallest and the most elongated, whereas fruits from Lisa are ovoid, and those from Rosa are rounder than the latter. The scales from Lisa fruits are poor; in Rosa, they are well separated and in Cebra, they are elongated. Even if production is well staggered throughout the rainy season (June to October in Nicaragua and Costa Rica), cv. Cebra is known to be early-maturing, Lisa intermediate, and Rosa late-maturing.

Differences between varieties can also be noted on the basis of the average weight of the fruits, number of seeds present in the pulp, and the viscosity of pulp mucilage (table I). Cultivar Lisa presents a high number of small seeds but this does not seem to affect juice recovery. Cultivar Rosa has the most viscous juice. Except for cv. Cebra, the varieties studied presented high industrial potential with a high juice extraction yield (> 60%). TSS contents were significantly higher for Lisa than for the other two varieties. For all the varieties, total titratable acidity was low and very similar. Cultivar Rosa tended to be more acid with a better sugar-to-acid ratio than the others, thus explaining why this variety is often most preferred by consumers. Even so, the varieties tested generally presented a low sugarto-acid ratio, giving a low sensorial quality, which is traditionally improved by blending pitahaya juice with an acid juice such as lemon juice. All varieties presented an absence of sucrose and a predominance of glucose over fructose.

Table I. Mean and standard deviation of main characteristics of three commercial Nicaraguan pitahaya cultivars: Cebra (n = 40 f 18 fruits), Rosa (n = 15 fruits).

Cultivars studied	s Length (cm)	Diameter (cm)	Weight (g)	Edible part (juice + seeds) %		•	,	Viscosity (juice 1%) (cP ^a)	•	Total titratable acids ^b (g·L ⁻¹)	e Total soluble solids % (w/w)	Glucose ^c Fri (g·L ⁻¹) (
Cebra	7.9 (0.7)	6.5 (0.4)	206 (30)	60 (17)	2604 (904)	55 (1)	12.0 (0.2)	1.18	4.3 (0.1)	2.40 (0.03)	7.1 (1)	54.0 (1.5) 7.
Lisa	7.8 (0.6)	7.1 (0.4)	235 (42)	68 (19)	5155 (1082)	62 (3)	11.6 (0.3)	1.16	4.7 (0.1)	2.50 (0.02)	10.7 (1)	45.0 (2.4) 4.
Rosa	7.6 (0.6)	7.6 (0.5)	245(65)	71 (35)	1875 (1346)	62 (1)	12.0 (0.1)	1.63	4.6 (0.3)	3.00 (0.02)	7.9 (1)	30.0 (1.4) 6.

^a cP = centipoise = $10^3 \text{ N} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$

^b Expressed as citric acid.

^c Mean and standard deviation for n = 3.

^d Calculated as [glucose + fructose / total titratable acids].

Table II.Oxygen radical absorbance capacity (ORAC), betacyanins, vitamin C and phenolic contents expressed per gram of fresh pulp without seeds of three pitahaya cultivars. Mean and standard deviation of four samples analyzed independently.

Cultivars	ORAC	Betacyanins (mg⋅g ⁻¹)		Total phenols			
studied	(µmol Trolox·g ⁻¹)		Ascorbic acid	Dehydro-ascorbic acid	Total vitamin C	(µmol GAE·g ⁻¹)	
Cebra	11.3 (1.4)	0.40 (0.02)	13 (1)	110 (2)	123 (3)	6.8 (0.2)	
Lisa	8.8 (1.9)	0.41 (0.02)	47 (2)	69 (5)	116 (7)	5.6 (0.1)	
Rosa	9.6 (0.5)	0.32 (0.05)	111 (5)	60 (5)	171 (10)	7.4 (0.2)	
GAE: gallic acid equivalent at 755 nm.							

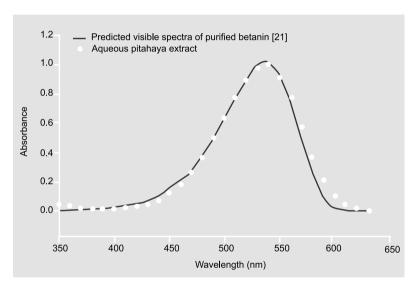


Figure 2.UV-vis absorption spectrum of red-purple pitahaya juice.

3.2. Antioxidant properties

The total antioxidant capacities of the juices prepared from the three cultivars of pitahaya ranged from (8.8 to 11.3) µmol Trolox·g⁻¹ of fresh pulp without seeds as assessed by the ORAC method (*table II*). The ORAC value of pitahaya juice is almost twice that measured by the same method for commercial apple and white grape juice and it is of the same magnitude as beetroot [24] and strawberry [25]. ORAC values were very similar for the three cultivars. Recent findings rank beetroot among the 10 most antioxidant vegetables, which means that red pitahaya can be considered as a potent antioxidant juice.

The concentration of betacyanins in pitahaya juice ranged between (0.32 and

0.41) mg·g⁻¹ of fresh pulp without seeds [$(2.5 \text{ to } 3.4) \text{ mg} \cdot \text{g}^{-1}$, dry weight basis], with the highest amount being found for cultivars Lisa and Cebra. Similar results have been found for Hylocereus sp. from Israel, with betacyanin contents ranging from (0.52 to 0.23) mg·g⁻¹ in fresh pulp ([3, 4]). Overall, the amount of betacyanins in red pitahaya juice is of the same magnitude as found in the flesh of commercial beetroot cultivars [26–28], but is slightly lower than the concentration reported in the inflorescences and leaves of various Amaranthus species [13]. Content of total phenolic compounds is high, being similar to that found in white grape, blueberry, apple, pear and plum [29]. Vitamin C content was the highest in cv. Rosa but it is still relatively low. If we consider that the ORAC activity of 1 umol of ascorbic acid is equivalent to 0.95 µmol of Trolox [23], the contribution of vitamin C to the total ORAC activity would be less than $0.5 \text{ }\mu\text{mol Trolox } \text{g}^{-1}$, on a fresh weight basis. Thus, the high ORAC value should be attributed essentially to the betacyanins, which would explain the very similar antioxidant capacity in beetroot and pitahaya.

3.3. Colorant properties

In addition to its high antioxidant capacity, red pitahaya juice can be used for its colorant properties in a similar way to beetroot. The UV-visible absorption spectra of pitahaya juice and of a pure beetroot betacyanin solution at the same concentration are very similar (*figure 2*). The betalains responsible for the pitahaya's red color correspond almost

Table III.Hunter colour transmission values for different dilutions of three pitahaya juices buffered at pH 5.

Solution	Cultivars	Hur	nter paran	neters	Hue angle (H°)	Chroma
	studied	L*	a*	b*		(C)
At $A_{538 \text{ nm}} = 1 \pm 0.05$	Cebra	55.4	58.5	-27.8	335	65
	Lisa	51.3	61.0	-27.0	337	72
	Rosa	59.0	50.4	-22.0	336	55
At 1% (v/v)	Cebra	32.0	78.5	-16.3	348	80
	Lisa	34.5	78.3	-13.4	350	79
	Rosa	30.3	76.0	-9.0	353	77

exclusively to betacyanins, as the dilute juice spectrum in the visible range shows a sole peak at about 538 nm, which accords with previous HPLC analyses of pitahaya coloring pigments [3, 4]. In contrast, beetroot extracts show a second absorption peak at about 480 nm, which is characteristic of yellow betaxanthins [5].

Consequently, pitahaya juice presents purer red-purple hues (table III) than beetroot juice, which presents more orange-red hues [6]. Indeed, the very high hue angle (H°) indicates that pitahaya juice has a more purple shade of red that is closer to that of some Amaranthus genotypes [13] than to that of beetroot. However, chroma (C), which expresses color purity or brilliance, is considerably higher for pitahaya juice than for Amaranthus extracts, even for the highly pigmented genotypes, and similar to beetroot, indicating a more vivid purple-red color. The high chroma of the juice diluted at 1% (v/v) correlates well with the betacyanin concentration found in the different cultivars. For example, cv. Rosa, which has the lowest betacyanin contents, also has the lowest chroma value. However, when the juices were compared at the same $A_{538 \text{ nm}}$ = 1 ± 0.05 and not on the same dilution level, significant differences for the chroma were found, probably because different proportions of betacyanins and their isoforms were present in the juice [30]. Very similar values of C and H° were found by Stintzing et al. [3] on a pitahaya juice diluted at $A_{538 \text{ nm}}$ =

 1 ± 0.1 , nonetheless, as measurements were made with an acidified juice (pH = 1), luminosity L^* was slightly higher, around 65 instead of values below 60 in our case.

To assess the potential of pitahaya juice as a source of natural colorant, the thermal stability of betacyanins was evaluated, using a standard commercial pitahaya juice obtained from a Nicaraguan processing plant. The juice at 11 g·100 g⁻¹ of TSS corresponded to a mixture of the different cultivars. The results obtained for crude juice and for juice acidified with citric acid were compared (table IV). The kinetics of thermal degradation of betacvanins in pitahaya can be described, using a first-order reaction model. Rate constants (k) and half-times were very similar to those calculated in beetroot [31, 32]. Optimal pH for stability was the natural pH for pitahaya juice (pH = 5). In the presence of oxygen, the optimum pH for red beet is pH = 5 to 6[33]. Activation energy (E_a) for the degradation of betacyanins decreases with pH, but remains within the range that allows its use in most foodstuffs undergoing normal thermal treatments. From a practical point of view, the results show that pitahaya juice acidified around pH = 4 can be easily pasteurized, with betacyanin losses being less than 10%. For instance, during pasteurization at 80 °C with 5 min holding time, only about 8% of the original betacyanin content was lost (data not shown). This performance can also be improved in the absence of oxygen; Huang and von Elbe showed [31]

Table IV.Thermal stability of betacyanins in pitahaya juices at natural and acidified pH regimes.

Pitahaya juice studied	рН	Temperature (°C)	Rate constant $k \times 10^{-3}$ (min ⁻¹)	Half-time $t_{1/2}$ (min)	Energy of activation (kcal·mol ⁻¹)
Natural	5.0 ± 0.1	50 70 80 85 90	1.7 16.6 24.1 25.0 30.6	407.7 41.9 28.7 27.7 22.6	17.0 - - - -
Acidified	4.0 ± 0.1	50 70 80 85 90	1.9 17.4 31.9 43.5 47.4	394.8 39.9 21.7 15.9 14.6	19.4 - - - -
	3.5 ± 0.1	50 70 80 85 90	1.9 39.0 91.6 134.9 172.4	357.3 17.8 7.6 5.1 3.6	26.8 - - - -

that the loss of betacyanins during thermal treatment is strongly influenced by the presence of oxygen. for use in multi-ingredient, tailor-made, fruit juices that combine functional properties such as color and high antioxidant capacity.

4. Conclusions

The Cactaceae family possesses the only fruits that are known to contain betacyanins. Red pitahaya juice can substitute beetroot colorant extracts in many applications for coloring foodstuffs and fruit-based products. If compared with beetroot, pitahaya juice contains about the same amount of betacyanins, but presents purer purple hues because of the absence of other betalains. Also, pitahaya betacyanins, compared with beetroot extracts, present a very similar thermal stability in a pH range of 3 to 5.

In addition to its quality as a natural colorant, pitahaya juice was characterized for its high hydrophilic total antioxidant capacity. The high betacyanin content of pitahaya seems to contribute significantly to this high antioxidant capacity, which is very similar to that of beetroot. Thanks to these qualities, pitahaya juice should represent a healthier substitute for synthetic colorants, especially

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Propiedades colorantes y antioxidantes de la pitahaya roja (Hylocereus sp.).

Resumen — **Introducción**. La pitahaya roja (*Hylocereus* sp.) es un cultivo muy prometedor que se explota comercialmente en las regiones secas de Centroamérica. Su piel y su pulpa se caracterizan por un color rojo intenso. Material y métodos. Se evaluaron las principales características fisicoquímicas de los frutos de tres cultivares comerciales de pitahaya roja: contenido total de compuestos fenólicos, contenido total de betacianinas y de vitamina C y capacidad de absorción del radical oxígeno (ORAC). Se evaluó también la estabilidad térmica de las betacianinas a diferentes temperaturas y pH. Resultados y discusión. La pitahaya tiene un bajo contenido de vitamina C, comprendido entre (116 y 171) µg·g⁻¹ de pulpa fresca sin semillas, pero es rica en betacianina [(0.32 a 0.41) mg·g⁻¹] y en compuestos fenólicos [(5.6 a 6.8) µmol Eq de ácido gálico g⁻¹]; tiene un alto valor antioxidante ORAC: (8.8 a 11.3) umol Eq Trolox g⁻¹. Los espectros visibles de los extractos acuosos fueron muy similares al de la betacianina pura. En efecto, el color característico del jugo diluido al 1% tiene una tonalidad intensa ($H^0 = 350^{\circ} \pm 3$) y elevados niveles de crominancia ($C^* = 79 \pm 2$). La estabilidad térmica de la betacianina de pitahaya disminuye con el pH, pero sigue siendo compatible con una utilización industrial como colorante (semiperíodo = 22.6 min a 90 °C al pH = 5 del fruto) y se reveló muy similar a la anteriormente descrita para las remolachas. Conclusiones. El jugo de pitahaya combina las propiedades funcionales de un colorante alimentario normal con un alto poder antioxidante.

America Central / Nicaragua / Hylocereus / colorantes alimentarios / betaina / antioxidantes