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COLORANT EXTRACTION FROM RED PRICKLY PEAR (*OPUNTIA LASIACANTHA*) FOR FOOD APPLICATION

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KEYWORDS

Betalain, betanin, spray drying, food colorant, red prickly pear.

ABSTRACT

The research on alternative natural colorants is of growing interest as substitutes for synthetic dyes in food. *Opuntia lasiacantha* Pfeiffer (red prickly pear) pigment was extracted from fruits of three different localities. The extracted pigment was identified as betanin by HPLC analysis comparing to the commercial beet red colorant E-162/1600. Color parameters and betanin content were also determined and no significant differences were found for the betanin content among the studied sites. Pigments extracted from fruits gathered from location L1 were spray dried using maltodextrin as coating material. After 24 weeks of storage color parameters and pigment retention were measured. Pigment retention was 86.2%±3.9 similar to the values found in the red beet pigment. The obtained results suggest that the colorant from red prickly pear can be considered a potential source of natural colorant.

INTRODUCTION

Betalains are natural pigments that are found in natural form in most families belonging to the Centroespermae order that includes the *cactaceae* family in which it is found the *Opuntia* genus (Yizhong *et al*., 2001a). The chemical structure of these pigments is derived from the betalamic acid and, depending on the united components to this structure, the yellow betaxanthins and the red-violet betacyanins will be present (Piattelli and Minale, 1964). The most-studied betalains are found in red beets (*Beta vulgaris*) which main betacyanins are betanin and isobetanin. Betalains stability is affected by temperature, pH, oxygen, light, and aqueous activity (Reynoso *et al*., 1997; Yizhong *et al.*, 1998; Yizhong *et al.*, 2001b).

In the food industry, there is a growing tendency to replace synthetic dyes by natural pigments, as the red beet betacyanins which are approved to be used as a food additive in the United States of America (No. 1600), and in the European Union (E-162); and commercially, they are exempt from batch certification and widely used in the world. (Castellar *et.al*., 2003).

Freeze-drying is the best method to dry pigments which are sensitive to high temperatures, similar to the aforementioned. Nevertheless, the freeze-drying is 30 to 50 times more expensive than the spray-drying (method usually chosen due to its economy and suitability). The spray-drying requires capsuling the pigments. In order to do this, maltodextrins are frequent to be used as encapsulating agents for sensitive ingredients, as flavors and dyes, by creating a wall around the pigment in order to isolate it from the environment protecting it from oxidation (Stephane *et al*., 1997).

The use of prickly pears as a source of betalains may be interesting since the plants of the *Opuntia* genus need minimal requirements from soil and water. This way, they may be a great alternative to agricultural economy in arid and semiarid regions (Castellar *et. al*., 2003).

The purpose to the present work was to obtain and to identify a pigment obtained from the red prickly pear, and their color stability study during storage.

MATERIALS AND METHODS

The species Opuntia lasiacantha Pfeiffer LS 6813 was chosen to be studied due to its production of red fruits. In the same way, three locations in the state of Hidalgo (México) were chosen because of their *O. lasiacantha* widespread groups and accessibility. One of these groups is located in San Salvador community, in which some fruits were collected from the plants identified as: LS-6813, FD-11, FD-12 and FD-13 (L1 Location). The second group is located in Pachuca City, in the surroundings of the University of Hidalgo, in which some fruits were collected from the plants: FD-01, FD-02, FD-03, FD-04 (L2). The third group is also located in Pachuca City, in the surroundings of the Healthy Institute in which some fruits were collected from the plants: FD-05, FD-06. FD-07, FD-08 and FD-09 (L3).

Among 10 and 20 fruits per plant were collected during July and August, 2002-2003.

Pigment extraction

The peeled fruits of each plant were homogenized with an equal amount of water. The mixture was heated for 5 minutes at 80°C and quickly cooled on ice bath until it reached a temperature of 8-10°C. Then, the extract was centrifuged at 3400 g and 4°C for 20 minutes in a Centra GP8R centrifuge (IEC, USA). The supernatant was stored at -20°C.

Betanin identification

Betanin identification was performed comparing by means of HPLC the pigment obtained from red prickly pear against a red beet's betanin commercial standard (No. 1600/E-162) provided by CHR Hansen de Mexico. The analyses were carried out by means of the equipment Perkin Elmer-200 series (USA), using an analytic column spheri-5 RP-C18, 5µm, 220 x 4.6mm i.d. (Perkin Elmer-USA). The following solvent and gradient system were used: A: 1.5% aq. H_3PO_4 (Merk de México); B: Acetonitrile (Merk de México); linear gradient from 100% A to 76% A in (A+B) within 40 minutes; the flow rate was 1 ml min^{-1}. The injection volume was 10µL, and the detection was performed at 536nm (Kobayashi *et al.,* 2000).

UV-VIS Analysis and Betanin quantization

The betanin content and the UV-Vis absorption spectra were determined on a lambda 40 UV-Vis spectrophotometer (Perkin Elmer, USA). The Betanin content was spectrophotometrically evaluated by the absorbance at 536 nm, using a molar extinction coefficient of 62 x 10^6 cm^2 mol^{-1} (Kobayashi *et. al*., 2000).

Colour parameters

Color parameters (CIELab) L*,a*,b*,C* and h° were determined by using the computer program CCC3, kindly provided by the University of La Rioja, Spain,, in order to calculate the tristimulus values and color coordinates parting from absorption spectrums. The samples were standardized by dilution to an

absorbance of 1.0 at λ_{max} ~535nm (Cai *et. al.*, 1998), performing an absorbance spectra from 380 to 780nm in a 1.0cm quartz cell.

Drying method

For the drying process, the extract from location L1 was homogenized with maltodextrin 10 ED (Equivalent Dextrose), provided by Industrializadora de Maiz (Mexico), which was used as protective agent adding 14% in order to adjust the solids final percentage to 22%. The feed mixture was spray-dried in a mini spray dryer B-191 (Büchi, Switzerland), under the following operating conditions: initial temperature 150°C, final temperature 90°C, feeding 14% (Cai and Corke, 2000). Freeze drying was conducted for comparison with spray drying. The feed mixture was frozen in liquid N_2 and freeze-dried in a freeze dryer Freezone 4.5 (Labconco, USA), for 24hrs.

Scanning electron microscopy (SEM)

The spray-dried pigment particles were observed in a SEM JSM-6300 (JEOL, Japan). The samples were coated with gold and observed to 2.5kV.

Pigment powder storage

Pigment powders were stored in crystal glasses sealed and stored at 25 ºC in absence of light for 24 weeks. Characteristics at zero storage time were analyzed within 1 day after drying. Storage stability was evaluated comparing their HPLC chromatogram and their absorption spectrum. The pigment retention percentage was calculated using this formula: (betanin content at 24wk. storage time) x 10^2 / (betanin content at zero storage time). Color parameters were determined by diluting 1g of dried pigment in 100mL of water using the aforesaid absorbance spectra and program.

Statistical analysis

Data were analyzed using the statistical program Statgraphics Plus (Statistical Graphics Corp., 1994-1999).

RESULTS AND DISCUSSION

The visible absorption spectrum (380-780nm), from the obtained extractions shows only one peak of maximum absorption at 536nm. This peak is a characteristic of the red-violet betalains group known as betacyanins which are optical active because they have two chiral carbons: C-2 and C-15. Due to the chiral center in C-15, betacyanins exist in two epimeric forms, One of the most widely studied is the betanin in red beet root and its epimer C-15, isobetanin (Kanner *et. al.*, 2001; Slawomir and Mizrahi, 2002).

The obtained colorant was compared under the same conditions in HPLC against a commercial beet-red standard (E-162/1600). Chromatograms are shown in Figure 1. In both chromatograms (beet-red colorant and prickly pear colorant), peaks 23.1 and 24.5 min corresponding to betanin and isobetanin are observed. The epimerization of betanin to isobetanin may be produced due to heat or acid when food containing betanin is heated since the balance of isobetanin to betanin grows up (Fennema, 2000).

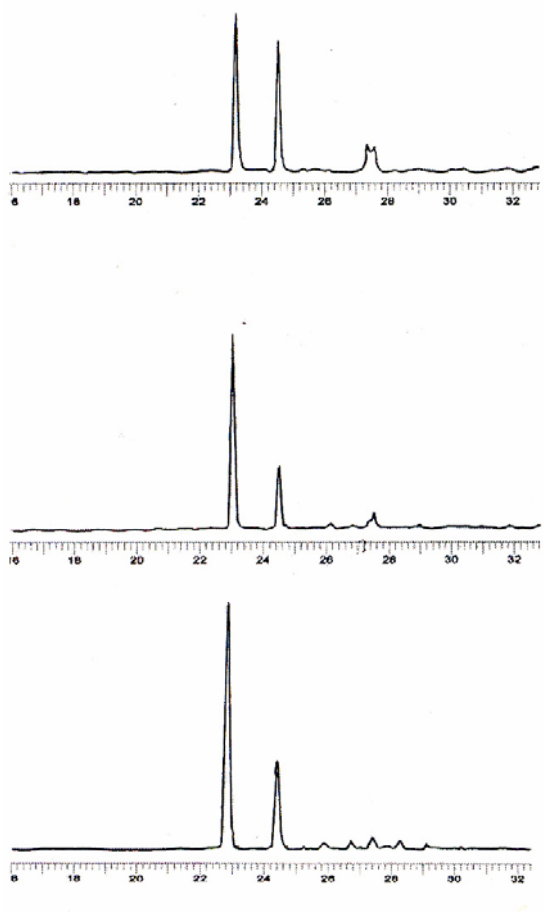

Figure 1. HPLC chromatograms (A) red beet colorant E162/1600; (B) red prickly pear colorant; (C) spray dried red prickly pear colorant.

The average betanin content in extracts from L1 location was 19.33±6.99mg $100g^{-1}$ having a variation coefficient of 36.16%; for L2 location 20.80±4.52mg $100g^{-1}$ having a variation coefficient of 21.73%; and for L3 location 27.70±3.18mg $100g^{-1}$ having a variation coefficient of 11.48. Hence, taking into account these results, no significant differences were observed related to the betanin content among the three collecting locations ($P>0.05$), influenced by a high variability in each zone according to the variation coefficients found.

In comparison with another authors, the report values were among 14 and 19 mg 100^{-1} in red fruits of *Opuntia ficus-indica* (Forni et. al. 1992; Fernandez-López and Almela, 2001), And 19.6mg $100g^{-1}$ of betanin in *O. undulata* (Castellar *et al.*, 2003). These values are minor in comparison with the results found in the current study. Higher betanin values (80mg $100g^{-1}$), have been reported in *O. stricta* (Castellar et al., 2003).

In other species of vegetables, as the red beet (Kanner et al., 2001), it is also reported a mayor betanin content (30-60 mg $100g^{-1}$). On the other hand, it is possible to increase the betalain content following a fermentation process (Reynoso *et al*., 1997), which could also be used to study our species.

Color parameters L*, a*, b*, C* and h*, of pigments solution, are shown in table 1. L* measures the sample's lightness; a* measures redness (+a*=red, -a*=green), meanwhile b* is related to yellowness (+b*=yellow, -b*=blue), and C* is the chroma or color purity; h° refers to the hue angle of tone and indicates the sample's color (0° or 360°=red, 90°=yellow, 180°=green, and 270°=blue). Pigments solutions at constant absorbance ($A_{\lambda max}$=1.0) gave L* values which varied within narrow limits (60.70-

62.80), indicating that the standardized solutions were similar in lightness so that the color characters of the pigment samples could be compared using the other tristimulus parameters (Cai *et al.,* 1998).

Table 1. Averages values for color parameters for extracts from O. lasiacantha, collected from different locations.

Location	a*	b*	L*	C*	h°
L1	56.53a	12.44b	60.7a	57.9ab	12.39b
L2	55.53a	4.47b	62.05a	55.74a	4.57b
L3	59.45b	-5.14a	62.80a	59.90b	-4.89a

†a, b. Different letters in the same column are significantly different (P<0.05).

The parameter a* resulted slightly big for pigments extracted from fruits in location L3, whereas the negative value of b* shows a combination of blue component and the value of C* indicates a more vivid color. These values together with angle h° value (-4.89, which is only negative in L3), indicate a slight tendency to purple color for the fruits in this location.

Extracts from locations L1 and L2 do not have significant differences among the values of their red component a*, which results inferior to the ones in L3; meanwhile, b* in L1 and L2 is in a positive value and it does not have significant differences, showing in comparison with L3, a yellow component. The same happens with the value in angle h°, which counts on positive values different from L3's extracts. L1 and L2 results are taken together as a tendency towards an orange-red color.

The differences in color among red prickly pears may be due apart from the red betanin pigment concentration, to another pigments concentration as indicaxanthin. Orange-yellow pigment is also present in red prickly pears but in less amount (Butera *et al.,* 2002),.

Fruits from location L1 were spray-dried and freeze-dried. No significant differences were found for visible absorption spectrums and color parameters between spray-dried pigment and the freeze-dried one. In addition, the visible absorption spectrums do not change at all after drying in comparison with the spectrum before drying, observing only one maximum absorption band at 536nm, which proved the pigment stability during the drying process.

The examination of scanning electron microscopy micrographs showed that the particle size of spray-dried pigment ranged from 5 to 50μm approximately; which is present in the form of agglomerates (Fig. 2A).

In relation to morphology, Cai and Corke (2000) report many less surface indentation and cracks in wall systems containing higher DE maltodextrins than in those containing lower DE maltodextrins. In our case, capsules containing 10 DE maltodextrins looked like smooth spheres (Fig. 2C) together with surface indentation and cracks in the wall system (Fig. 2B). In contrast, the freeze-dried pigment does not form agglomerates and it forms shapeless particles of different size and irregular form (Fig. 2D). The micrographs show the maltodextrin capsule effect when spray-dried.

Pure betacyanins pigments quickly absorb humidity from the environment due to their betacyanin molecules which contain lots of hydrophilic groups; and the maltodextrin, used as an agent to capsule, not only protects the pigment by allowing the drying process, but also helps to reduce betacyanins hygroscopicity by increasing their stability.

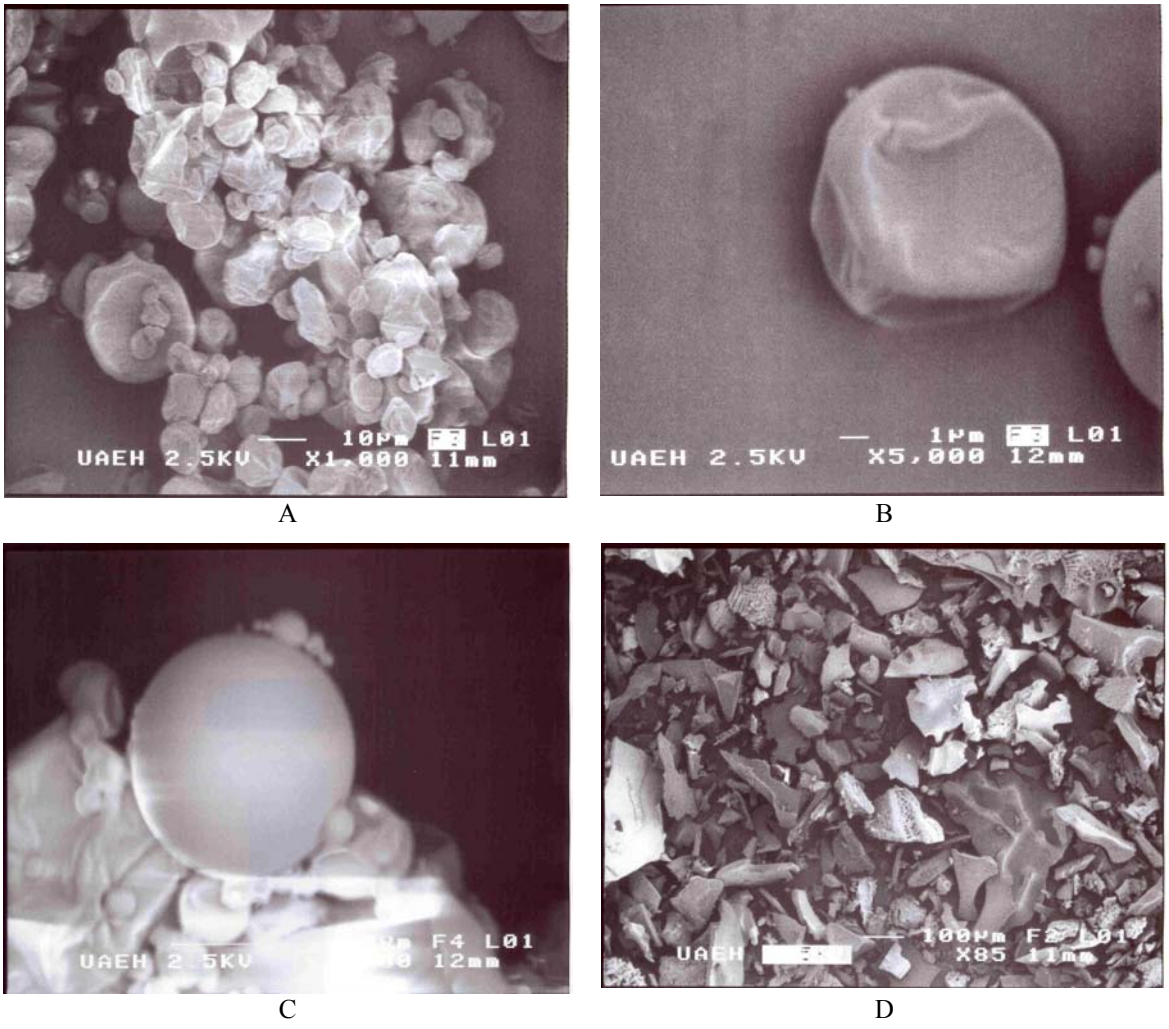

Figure 2. Micrographs of the microcapsules from the spray dried colorant; (A) agglomerated; (B) particle with surface indentation; (C) spherical particle with smooth wall; (D) freeze-dried colorant.

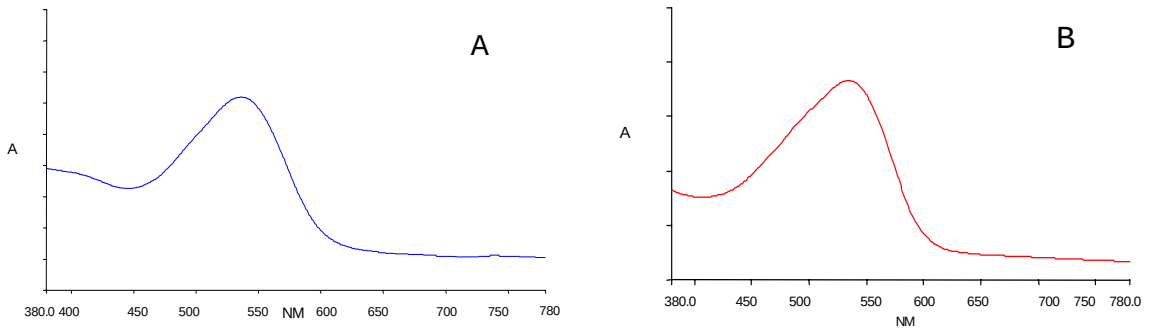

Figure 3. Visible absorption spectrum; (A) spray dried colorant at storage time t = 0 weeks; (B) storage time t = 24 weeks

The pigment stability during storage was evaluated after 24 weeks; the HPLC chromatogram (Fig. 1) of the spray-dried pigment, presents peaks at 23 and 24.5 min corresponding to betanin and isobetanin. In the chromatogram another non-identified small peaks are observed maybe due to the maltodextrin added in order to capsule. Furthermore, the visible spectroscopy chromatogram keeps showing only one peak at 536nm (Fig. 3), without showing any changes in comparison with t=0 storage.

The amount of betanin in powder after drying is 65.7mg $100g^{-1}$, which is less than the one reported to betacyanins extracted from another vegetable sources like the commercial colorant from beet-red E-162/1600 that reports 310mg $100g^{-1}$ (Cai and Corke, 2000). In case of pigment's retention after 24 weeks, it was 86.20±3.9% (100% corresponds to the amount of betanin in powder at zero storage time), being very close to the one reported for the commercial colorant from beet-red E-162/1600 at 84.3±1.4% (Cai and Corke, 2000). This reduction in the pigment content during storage is also observed in the color parameters at t=0 and t=24 weeks (Table 2), appreciating in them a reduction in the values of a* and C*, and indicating a decrease in the storage of red color intensity in the samples.

Table 2. Averages values for color parameters and betanin content for dry pigment at cero (t=0) and 24 weeks of storage (t=24)[†].

Storage time (weeks)	a*	b*	L*	C*	h°	Betanin content (mg/100g)
t=0	43.09b	-3.42b	66.70a	43.22b	-4.50b	65.70b
t=24	33.11a	-5.06a	69.40a	33.49a	-8.70a	56.63a

[†]a, b. Different letters in the same column are significantly different (P<0.05)

CONCLUSIONS

The fruits taken from the three studied locations did not show significant differences in the content of betanin but they did in their color parameters. Thus, the obtained color not only depends on the content of betanin but also on another present pigments. The extracted pigment to capsule with maltodextrin is stable to the spray-drying process and it remains stable by long periods in storage similarly to red beet betacyanins.

In summary, the results show that the *Opuntia lasiacantha* Pfeiffer could be a betanin source being useful as a natural colorant to the food industry. Nevertheless, it is required to study the economic feasibility in order to determine its potential as a natural colorant

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