

## A Process of Recovery of a Natural Yellow Colourant from *Opuntia* Fruits

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### Summary

This paper focuses on the development of a processing scheme to obtain a water-soluble natural yellow colourant from *Opuntia* fruits for application in food, paying special attention to the extraction procedure. Edible yellow *Opuntia* fruits grown in Murcia (Spain) were homogenized and extracted with ethanol, water, or ethanol/water (60:40) solvents. Pigment extract was chemically characterized and individual pigments were identified by high-performance liquid chromatography (HPLC), UV-VIS spectrophotometry, and electrospray ionization mass spectroscopy. A sequential extraction procedure was determined as follows: homogenization, stirring, centrifugation, filtration and concentration. The highest betaxanthin content per kg of fresh pulp (0.68 mmol of indicaxanthin) was obtained from the hydroalcoholic extract, and moreover, this solvent minimized the presence of mucilage and pectins, which are substances not desired in dye extracts. A concentrated pigment extract was obtained under rotary evaporation at 35 °C and reduced pressure (6 kPa), with a betaxanthin concentration (expressed as indicaxanthin) of 0.27 g/L, and CIELAB values of  $L^*=92.7$ ,  $a=-0.8$  and  $b^*=68.5$ . The individual pigment HPLC analysis with photodiode array and mass spectral detection revealed that proline-betaxanthin (indicaxanthin) was clearly dominant, while all other betalains were present in comparatively low quantities. The pigment stability was checked at 4 and 25 °C. Kinetics analyses indicate that the betaxanthin degradation pattern can be approximated as pseudo-first-order kinetics.

*Key words:* *Opuntia* sp., betalains, betaxanthins, indicaxanthin, natural food colourant

### Introduction

Every food designer knows that consumers judge a product not only by its flavour, but by its appearance as well. Colour mainly defines the aesthetic value of food, predetermines consumer's expectation and modulates appetite (1). One important class of ingredients exists solely to enhance the appearance of what we eat: food colours. Colour is probably the most important factor in the acceptance of food products because it is used as an indicator of quality. In the last years synthetic colourants are increasingly being perceived as undesirable or harmful by consumers (2,3), and there is growing interest in the development of natural colourants for use in the food

industry, which has been encouraged by a strong consumer demand for natural products. Natural colours are commonly used in the food industry and this natural trend has become of increasing importance among consumers. The market for natural food colourants is estimated at US\$ 500 million, and is growing by 10 % per year. Food and drink manufacturers need a full range of natural colours to suit all applications, and this demand provides the opportunity for innovation and research in new sources of natural colourants (4,5).

Cactaceae is considered the most promising family among betalain-bearing plants to be used as a source of betaxanthins. *Opuntia* fruits are widely consumed in Central and Southern America, Australia, South Africa,

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and the whole Mediterranean area. These plants may be found as spontaneous vegetation and grow in all of the semiarid countries, although cultivated cactus pears also reach the market (6). The main interest in *Opuntia* fruits is attributed to the betalain pigments due to the strong demand of the food industry for colourants obtained from natural sources (7–9), taking into account the interest of consumers for naturally derived products, associated with an image of health and quality.

Cactus pear fruits offer different colours based on betalains, which cover a wide spectrum from yellow to purple. Betalains are water-soluble, nitrogen-containing plant pigments of the order Caryophyllales. They comprise the yellow betaxanthins, and the red-purple betacyanins, both of which have betalamic acid in their basic structure, whose chromophore is a 1,7-diazaheptamethinium system, while they differ mainly in the radicals bonded to the main structure (10,11). Their colour is due to the conjugation of a substituted aromatic nucleus to the diaza system, which shifts the absorption maximum from around 535 nm in betacyanins to near 480 nm in betaxanthins (10). In cactus pear fruits (*Opuntia* sp.), these pigments are responsible for the purple, red, orange and yellow colours. Betalains are recognized as natural food colourants (12), and in contrast to other natural pigments, their appearance is maintained over a wide pH range (from 4 to 7). This property makes them ideal for colouring low acid foodstuff. Until now, only red beet (*Beta vulgaris*) has been used as a source of betalains, and other sources have not been considered for industrial use, despite the fact that several studies have been made concerning their presence and their stability in *Opuntia* fruits (13–15). Recently, there has been increasing interest in betalains since some studies have pointed to their possible antioxidant effects (16–18). If these investigations are confirmed, the added commercial value of these pigments will be increased due to the growing use of antioxidants in the food industry, not only for their usefulness in preservation but also because of their beneficial effects on human health.

The aim of this work is to establish a sequential process to obtain a concentrated betaxanthin extract from *Opuntia* fruits to be used as natural food colourant. A processing scheme was proposed paying special attention to the extraction procedure and to the chemical characterization and stability of the dye extract.

## Materials and Methods

### Plant material

Yellow-skinned cactus pear fruits (*Opuntia ficus-indica* L.) were harvested in Murcia (Spain) when the characteristic mature skin colour became manifest. The fruits of similar ripening degree were selected in order to have sample uniformity. The fruits were hand picked, separated into peel and pulp tissues, weighed, homogenized in a T25 basic ULTRA-TURRAX® (Ika-Werke, Staufen, Germany), frozen and stored at –25 °C until use.

### Chemicals

Ethanol and acetonitrile were of HPLC grade and were purchased from Labscan (Dublin, Ireland). Water

was purified in a Milli-Q water purification system from Millipore (Bedford, MA, USA). All other chemicals employed were of analytical grade.

### Pigment extraction

Homogenized pulp was magnetically stirred for 20 min in darkness using a fruit/solvent ratio of 1:5 (by mass per volume). Water, ethanol and ethanol/water (60:40 by volume) were used as solvents. After stirring, the samples were centrifuged at 15 000×g at 10 °C for 10 min in a Z383K Hermle centrifuge (Wehingen, Germany) to remove the vegetal tissue residue. Supernatants were concentrated using a vacuum Büchi Rotavapor R-200 (Flawil, Switzerland). Temperature was controlled at 35 °C and vacuum at 6 kPa. Ethanol was completely removed after the concentration process.

### Spectrophotometric analysis

The visible spectra (380–780 nm) of the extracts were recorded using an Agilent 8453 UV-visible spectrophotometer (Waldbronn, Germany). Betaxanthin content was expressed as indicaxanthin (molar mass  $M=308$  g/mol), applying a molar absorption coefficient  $\epsilon=48\,000$  L/(mol·cm) (19). Concentration of individual betaxanthins was calculated by multiplying the spectrophotometrically assessed value with the relative chromatogram area of the particular betaxanthin at 470 nm.

### Chemical analysis

Soluble solid content (°Brix) was measured using a Carl Zeiss (Tokyo, Japan) refractometer at 20 °C. Acidity was determined by titration with 0.1 M NaOH and expressed as mmol of citric acid per kg. The pH was measured with a Crison micropH 2000 pH meter (Madrid, Spain). Water content was determined using a Karl Fischer titrator DL38, Mettler (Toledo, Spain). All measurements were performed in triplicate.

### HPLC analysis

Pigment analyses were performed in a Waters modular liquid chromatographic system (Waters, Milford, MA, USA) equipped with two M510 pumps, a 717 plus autosampler and an M996 photodiode array (PDA) detector. HPLC was run by Millennium 2010 Chromatography Manager data system. An Atlantis dC<sub>18</sub> 5  $\mu$ m, 25 cm×4.6 mm i.d., column (Waters) was used. Elution was carried out following the method previously proposed (20), using a gradient between 175 mM acetic acid in water and 175 mM acetic acid in acetonitrile as mobile phase. The flow rate was 1 mL/min. Betaxanthins were monitored at 470 nm.

### Betaxanthin stability

Storage stability studies of the concentrated pigment extract were performed at 25 and 4 °C in the dark. Samples were withdrawn at different time intervals and analyzed spectrophotometrically. Betaxanthin content was determined in triplicate for each sample. Storage stability was expressed in terms of half-life ( $t_{1/2}$ ), the values of which were determined by calculating the ratio of betaxanthin content in the initial sample ( $B_0$ ) and in the

samples maintained at 4 or 25 °C during a determined period of time ( $B_t$ ). The natural logarithm of  $B_0/B_t$  ratio was plotted against the time (days) of storage. The slope of the graph through the origin obtained by connecting the data points was equated with  $k$ , from which the half-life values ( $t_{1/2} = \ln 2/k$ ) were deduced.

### Colour measurements

Based on the absorption measurements covering the range from 380 to 780 nm, objective colour (CIELAB) was calculated. The values ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and  $h^\circ$ ) were obtained using standard illuminant  $D_{65}$  and a  $10^\circ$  observer angle, according to the following expressions:

$$L^* = 116 \cdot (Y/Y_n)^{1/3} - 16 \quad /1/$$

$$a^* = 500 \cdot [(X/X_n)^{1/3} - (Y/Y_n)^{1/3}] \quad /2/$$

$$b^* = 200 \cdot [(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}] \quad /3/$$

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad /4/$$

$$h^\circ = \arctan(b^*/a^*) \quad /5/$$

where  $X_n$ ,  $Y_n$  and  $Z_n$  are the values of  $X$ ,  $Y$  and  $Z$  for illuminant  $D_{65}$  ( $X_n=95.0247$ ,  $Y_n=100.0000$ ,  $Z_n=108.8314$ ). If any of the ratios  $X/X_n$ ,  $Y/Y_n$  or  $Z/Z_n$  was equal to or less than 0.008856, it was replaced by  $(77.878 \cdot F + 16)$ , where  $F$  is  $X/X_n$ ,  $Y/Y_n$  or  $Z/Z_n$  as the case may be. Chromaticity ( $C^*$ ) and hue angle ( $h^\circ$ ) were calculated from  $a^*$  and  $b^*$  values. On the chromatic circle,  $h^\circ$  values are stepped from 0 to 360° (purplish-red) across a continuously fading hue circle, the other reference values of which are 90° (yellow), 180° (bluish-green) and 270° (or -90°) (blue).

## Results and Discussion

The design of a new industrial process requires an appropriate approach to consider the different aspects from which to assess the situation, which is a key factor in achieving success. The first steps of the processing of *Opuntia* fruits include the selection, classification, washing and conditioning (glochid elimination and pulp separation). After that, the plant material is ready for pigment extraction. Initially, the pulp is homogenized at high speed in a blender and before the addition of the solvent, the mixture is magnetically stirred to facilitate pigment extraction to the liquid phase. Three different solvents were used: water, ethanol and ethanol/water 60:40 (by volume). The selection of the most appropriate solvent is very important in order to obtain a highly pigmented extract, minimizing the presence of pectins and mucilage, which are found at high concentrations in cactus pear fruits. By means of centrifugation and subse-

quent filtration, the plant residue, mainly composed of pectins, cellulose and mucilage, is discarded, and a clarified pigment extract is obtained. Table 1 shows the main chemical characteristics of the extracts depending on the solvent used. The pH value was around 7.0 in the three extracts, similar to other pH values (5.3–7.1) reported for *Opuntia ficus-indica* fruits (21), confirming the *Opuntia* fruits as low-acid food. The refractive index, which depends on the presence of sugars, showed low variability among extracts, and density ranged from 0.85 to 1.00, showing the lowest value for the ethanolic extract. As regards the pigment content, the hydroalcoholic solvent extracted the highest level of betaxanthins (0.68 mmol of indicaxanthin per kg of fresh pulp) and therefore was chosen as the most efficient because it can recover the pigments completely from the pulp without co-extracting polysaccharides and other alcohol-insoluble solids. This simplifies further purification of the pigment extract. The level of betaxanthins obtained here is higher than that reported for Italian cactus pear (16), and is similar to the content found in some Mexican cultivars (22).

In order to obtain a highly pigmented betaxanthin extract to be used as commercial food colourant, a 21-fold concentrated extract was obtained under rotary evaporation at 35 °C and reduced pressure (6 kPa). It is important to offer a concentrated product to reduce water activity, thus increasing the stability of the pigments, minimizing the risk of microbial contamination and extending the shelf life of the colourant. Apart from that, transport and storage are made easier. Table 2 summarizes the results of analyses performed on the concentrated betaxanthin extract. It was found that its high colour strength was remarkable, and that the titratable acidity (10.90 mmol of citric acid per kg) is lower than that reported for *Opuntia ficus-indica* concentrated juice, which ranged between 158 and 53 mmol of citric acid per kg (21,23). Density (1.04 g/mL) was lower than previously reported for concentrated juice (1.29 g/mL) (23). These differences could be attributed to the use of ethanol in the extraction solvent, which leads to the reduction of sugars in the extract.

At this point, a natural yellow dye extract from *Opuntia* fruits was obtained. The flow diagram describing the overall extraction process, from the raw material to obtaining the dye extract, is shown in Fig. 1. The extraction process comprises the following stages of fruit conditioning: selection, classification, washing, glochid elimination and pulp separation, after which pigment extraction and concentration are performed.

The by-products from betaxanthin extraction include the ectodermis of *Opuntia* fruits, the pellet resulting from

Table 1. Chemical characteristics of pigment extracts

Extraction solvent	pH	Refractive index	Total soluble solids °Brix	$\rho$ g/mL	Betaxanthins mmol of indicaxanthin
water	6.89±0.03	1.371±0.002	12.1±0.1	1.00±0.01	0.59±0.01
ethanol	7.48±0.04	1.371±0.001	10.8±0.2	0.85±0.02	0.56±0.02
ethanol/water (60:40 by volume)	7.29±0.04	1.363±0.001	12.0±0.2	0.93±0.02	0.68±0.02

Values are expressed as the mean±S.D. of three determinations

Table 2. Chemical characterization of the concentrated betaxanthin extract from *Opuntia* fruits

Parameter	Value
pH	5.83±0.03
Titrateable acidity/(mmol of citric acid per kg)	10.90±2.50
$\rho$ /(g/mL)	1.04±0.04
Total soluble solids/°Brix	37.60±0.90
Colour strength ( $A_{480\text{ nm}}$ ) (1 % by volume solution)	0.18±0.02
$\gamma$ (betaxanthins)/(g/L)	0.27±0.01

Values are expressed as the mean±standard deviation (S.D.) of three determinations

the centrifugation of pigment extract, and the ethanol-water mixture obtained after the rotary evaporation of the initial pigment extract. The ectodermis constitutes 42–48 % of the whole fruit, and the main compounds in the ectodermis include cellulose, glucose and proteins. The ectodermis presents a potential use as biosorbent, which could serve as a cost effective means of treating the effluents charged with toxic heavy metals (24,25). The pellet resulting from centrifugation represents 10–12 % of the extracted pulp, and it mainly consists of pectins, mucilage and the seeds typical of these fruits. Both the polysaccharides and the seeds might also be used in the food industry. Recently seeds have been used as a source of high quality edible oil (26). The solvent obtained in the concentration process can be recycled in order to minimize the use of ethanol, and thus reduce the volume of disposable waste and decrease waste treatment costs.

The HPLC analysis of the betaxanthin extract (Fig. 2) revealed the presence of a main peak, with a maximum absorbance at 479 nm and an  $m/z$  ratio of  $[M+H]^+=309$ , which was identified as indicaxanthin (proline-betaxanthin). All other betaxanthins were present in comparatively low quantities. The yellow indicaxanthin is reported in the bibliography as the major betaxanthin of the cactus pear fruits (20,27).

To check the storage stability, two assays were performed at 4 and 25 °C, respectively. The kinetics of degradation of betaxanthin pigments was monitored over the storage period, and rate constants and half-life values of reactions were determined. Fig. 3 shows the behaviour

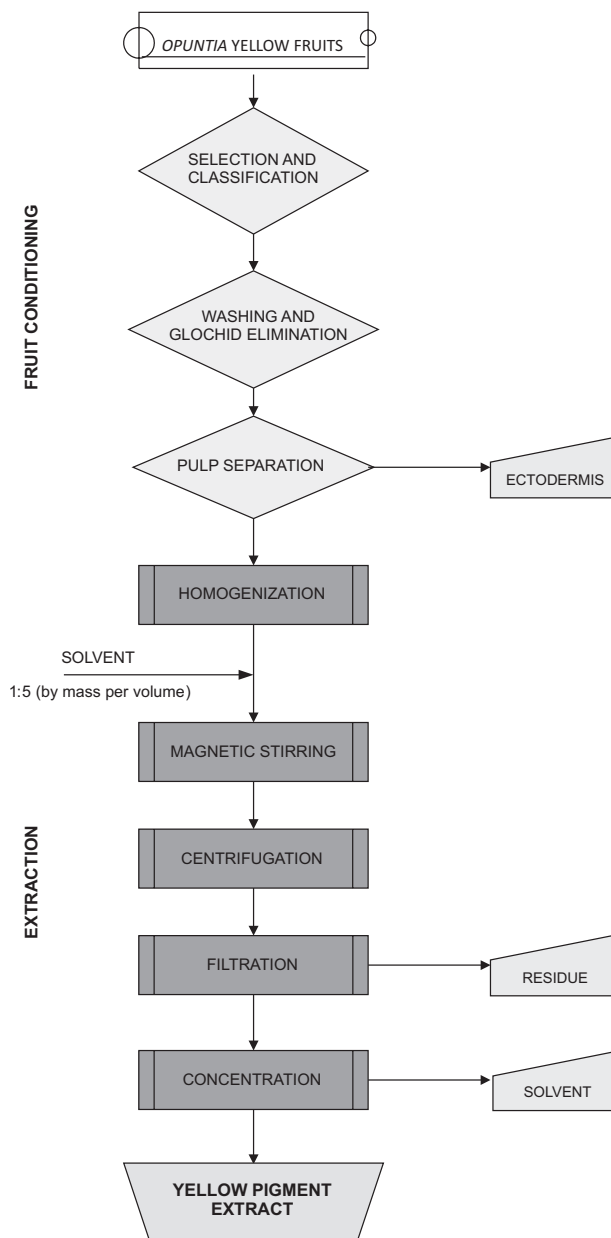


Fig. 1. Flowchart representing the extraction process

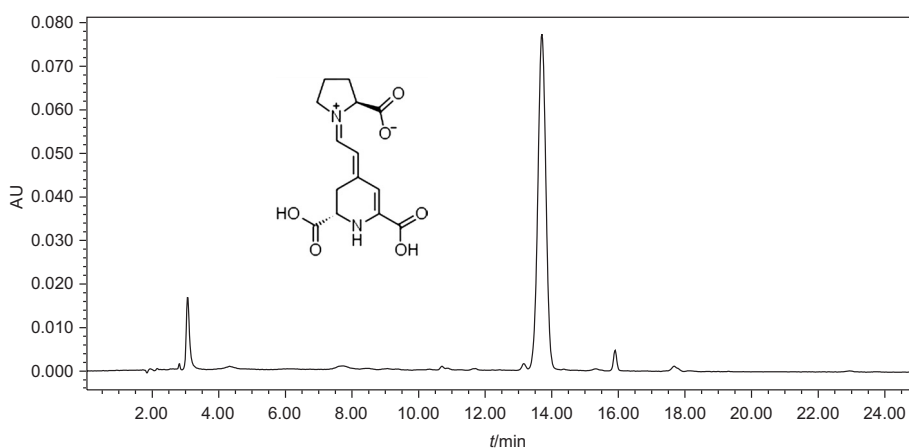


Fig. 2. Chromatogram ( $A_{479\text{ nm}}$ ) obtained from the HPLC analysis of the pigment extract from *Opuntia* fruits and chemical structure of indicaxanthin ( $t_R=13.8\text{ min}$ )



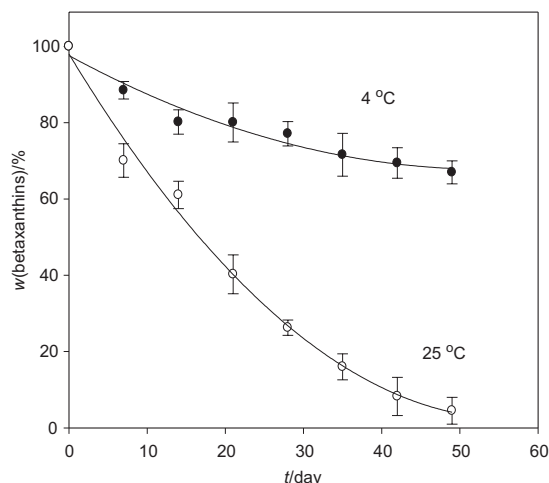


Fig. 3. Storage stability in the dark of the yellow pigment extract from *Opuntia* fruits

of betaxanthin content during degradation. First order kinetics can be observed, with half-life values ( $t_{1/2}$ ) of 80 and 15 days at 4 and 25 °C, respectively. These data are lower than those of betacyanin extracts under the same storage conditions (13). It must be said that these half-life values correspond to crude extracts, and they can be increased by means of spray drying with encapsulating materials as maltodextrins, which were found to be effective in protecting pigment extracts from oxidation and photodegradation processes (28,29).

With the CIELAB parameters, the colour of plant extracts can be measured precisely, determined unambiguously, and differences to a colour standard can be quantified. Thus, CIELAB colour measurements are used extensively in the food industry to assess chromatic properties, and are gaining greater use as descriptors for colour specification by food manufacturers. The results of the colour measurements (CIELAB parameters) for pigment extracts are shown in Table 3. High lightness in both extracts ( $L^*=88.24$  vs.  $L^*=92.72$ ) can be observed, the hue angle was quite similar ( $h^\circ=3.04$  vs.  $h^\circ=-0.66$ ), while the chromaticity was higher in the initial pigment extract ( $C^*=96.67$ ) than in the concentrated extract ( $C^*=68.49$ ).

Table 3. Colour analysis of yellow pigment extracts

Colour parameter	Initial pigment extract	Concentrated pigment extract
$a^*$	5.13	-0.78
$b^*$	96.54	68.49
$L^*$	88.24	92.72
$C^*$	96.67	68.49
$h^\circ$	3.04	-0.66

## Conclusion

*Opuntia* fruits can be used as a source of betaxanthins, natural water-soluble yellow pigments, which can be used as food colourant. In this paper, a simple and

reliable betaxanthin extraction process was established, which provides a concentrated natural yellow extract of high colour strength. HPLC analysis of the pigment extract revealed only one major betaxanthin identified as indicaxanthin. Although the pigment extract did not exhibit promising results in storage stability, encapsulated concentrated pigment can be an excellent natural colourant for use in the food industry. Therefore, concentrated betaxanthin extract from *Opuntia* fruits, with its pleasant taste and flavour, could offer new opportunities as a natural yellow colourant for the food industry.

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