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Betaxanthin-rich extract from cactus pear fruits as yellow water-soluble colorant with potential application in foods.

José A. Fernández-López[1], María J. Roca, José M. Angosto, José M. Obón

Department of Chemical and Environmental Engineering, Technical University of Cartagena (UPCT), Paseo Alfonso XIII 52, E-30203 Cartagena, Murcia, Spain

Abstract. Cactus pear (*Opuntia ficus-indica*) fruit juice is a source of betaxanthin pigments which can be used as a natural yellow food colorant. The HPLC chromatographic pigment pattern corresponding to the betaxanthin-rich extract revealed the presence of four betaxanthins, of which indicaxanthin (proline-betaxanthin) accounts for around 85%. A betaxanthin-rich water-soluble food colorant from cactus pears fruits was produced by spray-drying microencapsulation using maltodextrin as a wall material. The resulting powder was characterized by scanning electron microscopy, and its apparent color was analyzed by spectrometry. The stability of the microcapsules was examined at +20, +4 and −20ºC in the dark during 6 months of storage. The degradation of betaxanthins was delayed by microencapsulation and their colorant stability increased at lower temperatures. The potential application of the colorant microcapsules was successfully assessed in two food model systems: a yogurt and a soft-drink. Both foods presented an attractive pale yellow color. Pigment retention and color parameters were investigated during storage under controlled conditions. Slight changes in the pigment retention, in both model systems, pointed to excellent preservation in the dark, even after 28 days at 4ºC. However, the presence of light contributed to betaxanthin deterioration. Spray-drying microencapsulation succeeds in reducing volumen of the pigment extract and be easy in storage and delivery of the powders. It is proved to be a suitable process that can be recommended for stabilizing betaxanthins from cactus pears to be used as water-soluble natural colorants in foods.

[1] Corresponding author, e-mail address: josea.fernandez@upct.es
Telephone number: 34 968325549; Fax number: 34 968325555

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Introduction

In response to social media, focus groups and consumer surveys, the food industry is adjusting its products to meet the concerns of consumers as regards the food and beverages they acquire. Indeed, it is becoming increasingly important to meet consumers expectations for natural and healthy products. Hence, the search for new plant-derived colorants is of fundamental interest for the food industry [1-2]. The color of a food or beverage plays a huge role in the success of a product because color not only makes a product more attractive, but can affect how consumers perceive its taste. Major food industry initiatives include eliminating synthetic coloring and flavorings and replacing synthetic preservatives with natural ones [3]. The use of natural coloring extracts in food and beverages has been increasing in recent years, a development based on both technological improvements and market trends. The appearance of food is considered as important as the taste. Colors, no doubt, enhance the look and quality perception of food, but the health conscious movement is prompting more demand for natural colouring extracts [3]. Nowadays, fruit coloring or vegetable extracts are the most popular way to achieve the desired color in foods because their application does not require any certification [4-5], both of them are healthy and help food to look appetizing. However, unlike colors, natural coloring extracts are multifunctional as they combine color, flavor and natural antioxidants to create a well-rounded food enhancing product. In addition, these natural extracts may contain other bioactive ingredients that will also be incorporated into the food, thus increasing its added functional value.

It has been demonstrated that betacyanins impart an attractive strawberry color to ice creams, dairy products, jams and jellies as well as sugar confectionery products which are not subjected to heavy heat treatment [4,6]. For their part, betaxanthins impart a yellow color, and preliminary studies have suggested that they could be used as food additives, stabilised by the addition of ascorbic and citric acids [1]. Cactus betalains have increasingly attracted interest as a source of water-soluble pigment preparations [7-8]. However, a key factor to consider when these pigments are used as bioactive colorants in foods is their stability. In this respect, the stabilization of betalains could be improved using microencapsulation

technologies, such as spray-drying, to ensure their bioavailability [2,7,9]. This technology is also used to change liquid solutions to powders, which are easier to handle.

Betalains are water-soluble nitrogen-containing pigments [10-11] which include the red-purple betacyanins and the yellow-orange betaxanthins [12]. They are immonium conjugates of betalamic acid with *cyclo*-DOPA (betacyanins) and aminoacids or amines (betaxanthins), whose chromophore is a 1,7-diazaheptamethinium system [13]. They represent one of the most important natural pigment families. Betalains possess high antioxidant and free radical scavenging activities which have been described in plant extracts and purified pigments [14-15], and due to these characteristics they are considered as bioactive pigments [16].

Despite their coloring capacity, water solubility and bioactive characteristics, betaxanthin extracts are rarely used as potential natural colorants in foods, because of limited supply. Thus, the aim of this work was to encapsulate the yellow pigment extract from *Opuntia ficus-indica* fruits by spray-drying to investigate the pigment stability in two model food matrices (a yogurt and a soft drink) colored with a betaxanthin-rich extract from cactus pear fruits.

Materials and methods

Betaxanthin-rich extract preparation and analysis

Opuntia ficus-indica fruits (yellow pulp) were obtained from a plantation located in the municipality of Alhama de Murcia, province of Murcia (Spain). Cactus pear fruits were manually peeled and the pulp was homogenised with an Ultraturrax Ika Labortechnik (T25, Staufen, Germany). The homogenised pulp was centrifuged for 10 min at 15,000 *x g* in a Hermle centrifuge (Z383K, Wehingen, Germany) refrigerated at 5 ºC, and supernatant was used as betaxantin-rich extract.

Acidity, pH, moisture content and total soluble solids (ºBrix) were determined according to AOAC methods [17]. The total phenolic content was determined according to the Folin-Ciocalteu method [15]. The results were expressed as gallic acid equivalents (GAE) based on a calibration curve (0-100 μg/mL). For the UV/VIS spectrophotometric quantification of total betaxanthins a molar extinction coefficient (ε) of 48000 M^{-1} cm^{-1} at 480 nm was used, and a molar extinction coefficient (ε) of 54000 M^{-1} cm^{-1} at 535 nm for betacyanins [11].

Individual betalain pigments were analyzed chromatographically according to the methods previously described [11]. Betaxanthins were monitored at 480 nm and betacyanins at 535 nm. Identification was carried out by an HPLC-MS system.

Positive ion electrospray ionization (ESI) mass spectra were recorded on a quadrupole Waters ZQ instrument coupled to an HPLC system, model Alliance (Waters, Milford, USA) [18].

Color measurements were performed using a Minolta CM-508i spectrophotometer-colorimeter (Minolta, Osaka, Japan) to obtain the CIELAB parameters (L^*, C^* and h^o) using the 10° standard observer and illuminant D65 (corresponding to daylight). Euclidean distance between two points in the three-dimensional CIELab space was used to calculate total color differences (ΔE^*) [2].

Free radical scavenging activity of the betaxanthin-rich extract was evaluated by the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ methods. The $ABTS^{\bullet+}$ was generated in the reaction medium (3 mL) containing 400 μM ABTS, 39 μM H_2O_2 and 50 nM horseradish peroxidase in 100 mM phosphate-citrate buffer pH 4.5. TROLOX (0-40 μM) was used as reference [19]. The $DPPH^{\bullet}$ assay contained 150 μL of pigment extract and 2.85 mL of 150 μM DPPH dissolved in methanol [20]. TROLOX (0-40 μM) was also used as reference. The results of both methods are expressed as TROLOX equivalent activity (TEAC).

Spray-drying process

Spray-drying was used for microencapsulation of the betaxanthin-rich extract. Maltodextrin, (Glucidex 6D, from Roquette, Lestrem, France) was used as wall substance. Maltodextrin was dissolved in distilled water (15% w/v) under vigorous vortexing. In each batch, 100 mL of the betaxanthin-rich extract (15 ºBrix) were combined with maltodextrin solution (1:1 v/v) with constant stirring. The resultant solution (200 mL) was thermostated at 20 ºC and fed to a Büchi Mini Spray Dryer (B-290, Flawil, Switzerland) with the inlet air temperature at 160 ºC, and the outlet air temperature kept at 75 ºC. Liquid feed was 5 mL/min, atomization air flow was 0.45 m^3/h, and the drying air flow was 36 m^3/h. The nozzle internal diameter was 0.7 mm and a glass cylinder (50 x 15 cm) was used as drying chamber [8]. The particles were separated from the drying air by a cyclone.

Scanning electron microscopy (SEM)

Particle morphology was evaluated by SEM. Spray-drying powder was fixed onto SEM stubs using a two-side adhesive tape. The surface was sputered for 120 seconds and coated with a thin layer of gold and examined with a Hitachi S-3500N scanning electron microscope (Hitachi, Tokyo, Japan) operated at 5 kV with the lens at 7 mm.

Formulation of model food products

A yoghurt and a soft-drink were used to assess the colorant properties of the betaxanthin-rich extract in model food products. White fat-free yogurt purchased from a local supermarket, betaxanthin-rich extract powder from cactus pear fruits (15 g/kg) and sucrose (20 g/kg) were mixed under aseptic conditions in sterile flasks. The pH value of the model yogurt was 4.4. The soft-drink was formulated with water solutions of citric acid (1.52 g/L), potasium sorbate (0.18 g/L), sodium benzoate (0.14 g/L) and ascorbic acid (0.02 g/L) according to Dyrby and coworkers [21]. Betaxanthin-rich extract powder (50 g/L) was added as colorant and sucrose (86 g/L) was added as sweetener. The final pH of the soft drink was 3.2. After preparation, the soft-drink was filtered aseptically through 0.2 µm sterile filter and kept in sterile flasks.

Storage stability analysis

The stability of the betaxanthin-rich extract was analyzed at 20ºC in the absence of light. Aliquots were taken at different times for 30 days and spectrophotometrically analyzed to follow the degradation of the betaxanthin pigments. The storage stability studies of the betaxanthin-rich extract powders were performed maintaining samples (2.0 g) in Petri dishes (2.0 cm diameter) in the absence of light, at constant temperature (20ºC, 4ºC, −20ºC) in a desiccator filled with anhydrous silica gel. Samples were spectrophotometrically analyzed over a period of 6 months. The yoghurt and soft-drink were maintained in the absence of light under refrigeration (4ºC). In both cases, samples were withdrawn twice a week and analyzed spectrophotometrically.

Statistical analysis

The mean values, standard deviations and analyses of variance (ANOVA) were determined with Minitab statistical software, version 15.0 (Minitab Inc., State College, Pennsylvania, USA).

Results and discussion

This investigation was performed with cactus pear fruits from a second late bloom, induced by the manual removal of the first flowers by the middle of June. This practice improves fruit quality, providing a strongly colored juicy pulp, larger size and lower number of seeds, for harvest late in the season, between mid-October and late November [22].

Betaxanthin-rich extract characterization

Table 1 shows the physicochemical characteristics of the betaxanthin-rich extract obtained from the yellow pulp of the *Opuntia ficus-indica* fruits used in this investigation. As related to fruit ripeness, the results were in good agreement with data from Italian, Mexican and Chilean cultivars. The acidity of the fruits was quite low (0.022 %), and because of the extremely low content of organic acids, the pH of the fruit extract is high (6.58). The high soluble solid content (13.9 ºBrix) ensures that cactus pear fruits have an attractive tasty juice. A highly desirable balance between sweetness and acidity is shown, similar to those reported by other authors, who reported the acidity to be 0.02-0.15 % (expressed as citric acid) [8, 22] with a pH of 5.3-7.1 [9] and total soluble solids ranging between 12 and 17 ºBrix [7, 23]. The total phenolic content (220 mg GAE/100 g) was higher than those reported in *Opuntia* fruits by Chávez-Santoscoy and co-workers of 17-23 mg GAE/100 g [24] and by Díaz-Medina and co-workers of 45-117 mg GAE/100 g [25]. The betalain content (betaxanthins and betacyanins) was in accordance with previous reports [11], and is in the same order as the values found by other authors in cactus pear yellow fruits [1]. The betaxanthin-rich extract exhibited a notable free-radical scavenging activity, both against $ABTS^{\bullet+}$ and against $DPPH^{\bullet}$ radicals, similar to that reported previously in Sicilian cactus pears [26], and Chilean cactus pears [7]. These values, joined to the total phenolics content, would confirm a high level of antioxidant activity, which would be beneficial in terms of protecting human health.

Betaxanthin and color analysis

The HPLC chromatographic pigment pattern corresponding to the betaxanthin-rich extract monitored at 480 nm is shown in Figure 1. The isogram of each peak is also displayed. The characterization of each peak was performed on the basis of UV/VIS and mass spectrometry, as well as by comparison with previously reported retention and absorption characteristics of betalains [27-28]. The chromatogram revealed the presence of four betaxanthins (peaks 1, 2, 3 and 5) and one betacyanin (peak 4). The main pigment, which accounted more than 85% of the total, was indicaxanthin (proline-betaxanthin), which is the typical compound of *Opuntia* fruits [1, 23]. Others betaxanthins were also identified, as muscaaurine VII (histidine-betaxanthin), vulgaxanthin I (glutamine-betaxanthin), and methionine-betaxanthin, all of them previously detected in cactus pear fruits and shown to be genuine betaxanthins of betalainic plants [29]. Also detected, but

at lower level, was betanin (peak 4), the main betacyanin pigment. Although cactus pear fruits with yellow pulp were selected to prepare the betaxanthin-rich extract, betanin often accompanies the predominant betaxanthins [30].

The chromatic parameters corresponding to the betaxanthin-rich extract (Table 1) confirmed its attractive pale yellow color, which, together with its water-soluble character, means it is in high demand in the natural food market.

SEM of the maltodextrin-encapsulated betaxanthin-rich extract

The SEM micrographs of the spray-dried microcapsules are shown in Fig. 2. These images point to the succesful microencapsulation of the betaxanthin-rich extract using maltodextrin (Glucidex 6D) as a carrier, with an encapsulation efficency of around 71% . The spherical and non-agglomerated particles with smooth surfaces and uniform appearance suggest a uniform drying process. The spherical shape of the particles allows powder to flow better because there is no surface roughness and it does not form agglomerates. This morphology also reflects the positive tendency of maltodextrin to yield microcapsules to protect sensitive substances from light, moisture or oxidation, and confirms its physicochemical capability for encapsulating pigments and other bioactive molecules by spray-drying [2, 8, 31].

Stability of the maltodextrin-encapsulated betaxanthin-rich extract

Maltodextrin-encapsulated betaxanthin extract was studied in order to ascertain whether the encapsulation process increased pigment stability, by keeping its colorant properties intact, over a longer period of time. Figure 3 presents the results when the stability of maltodextrin-encapsulated powder was analyzed at three different temperatures, +20, +4 and −20ºC in dark conditions. As can be seen, maltodextrin encapsulation contributed greatly to the stability of the betaxanthin pigments, which remained stable for several months at temperatures of +4 and −20ºC. When the maltodextrin-encapsulated powder was kept at +20ºC for 6 months, only 60% of the betaxanthin pigments were not degraded.

The results obtained when the stability of the liquid extract was analyzed revealed that this extract degrades more easily. When it was stored at +20ºC in the absence of light, 47% of its colorant capacity was degraded in only 30 days (Table 3), while under the same conditions only 6% of the maltodextrin-encapsulated pigment extract was broken down.

Betaxanthins are considered very thermolabile compounds that deteriorate quickly during storage, with color loss following first order kinetics [32]. To improve their stability it is necessary to use encapsulation technologies such as spray-drying and storage temperature below 4ºC to ensure their proper preservation and subsequent bioavailability.

Pigment and color stability in model food products

To test the coloring ability of the maltodextrin-encapsulated powder, two food model systems were prepared: a yogurt and a soft-drink, both of them are shown in Online Resource 1. Two sets of triplicate yogurt samples were prepared and the powder colorant was added so that the color was similar to that of a lemon flavored yogurt (L^*=87.4, C^*=19.6, h^*=100.6). The pigmented model yogurts (125 g) were stored for 28 days at 4ºC in the dark or in the presence of light (fluorescent lamp FC/22/T5 daylight 22 watts) to register color alterations. Aliquots were taken at 0, 3, 6, 9, 12, 15, 18, 21 and 28 days to analyze betaxanthin concentration, pH and color modifications. The changes in pigment retention are shown in Fig. 3, and point to an excellent preservation in the absence of light (95.1%), even after 28 days at 4ºC. It is evident that light contributes strongly to betaxanthin deterioration (40.3%). In addition, changes in the CIELab parameters of the model yogurt were monitored during the sampling period. Table 3 shows that the hue angle (h^o), lightness (L^*) and intensity (C^*) were similar in the initial sample and after 28 days storage at 4ºC in the dark, so that the total color difference (ΔE^*) was only 1.0 CIELab units. Exposure to light during storage accelerated these color changes, and, in this case, the ΔE^* increased to 10.1 units. It is should be noted that for ΔE^*values below 3.0 consumers are not able to detect color differences [33]. The pH of the yogurt did not change during the 4 weeks of experimentation (4.4±0.2).

A soft-drink was selected as second food model system. Two sets of triplicate soft-drinks were prepared and maltodextrin-encapsulated betaxanthin extract was added to reach a final absorbance of 0.80±0.02 at 480 nm and CIELab parameters of L^*=36.5, C^*=11.9, h^o=103.7. The samples were maintained in refrigerated storage (4ºC) for 28 days in the absence and presence of light and aliquots were taken to evaluate pigment and color deterioration. In the samples stored for 28 days in the dark the betaxanthin loss was 9.8%, but in the presence of light the loss increased to 48.9% (Fig 3). As can be seen, the level of betaxanthin deterioration in the soft-drink was higher than in the model yogurt, both in the presence of light and in complete darkness. This can be explained by the fact that some of the yogurt components must play a protective effect towards the pigments, slowing down their deterioration [34]. Differences in color appearance were also observed between soft-drink stored in the dark and that stored in the presence of light. Changes in lightness (L^*) from 36.5 to 29.9 (light) or 34.2 (dark) were observed, while hue angle (h^o) varied from 103.7 degrees to 101.5 (light) or 102.3 (dark). As expected,

total color difference (ΔE^*) was higher (7.9 units) in the soft-drink stored in light conditions compared with the 2.3 units of color difference in the sample maintained in the dark (Table 4).

The present investigation confirms that the maltodextrin-encapsulated betaxanthin-rich extract from cactus pear fruits can be used as natural yellow colorant in yogurts and soft-drinks stored under refrigeration and formulated to be consumed in 3-4 weeks, extending our knowledge about the potential application of betaxanthins as water-soluble yellow colorant in foods.

Conclusions

Attractive water-soluble natural yellow colors are in great demand in the competitive food and beverage industry. Color stability in model foods was greatly dependent on storage temperature, increasing stability at lower temperatures. It should be emphasized the effect of spray-drying microencapsulation in enhancement of betaxanthin stability. Betaxanthins from cactus pear fruits can be considered a promising colorant with multiple possibilities in low temperature storage and non-transparent packaged foods. Worthy of note is the bioactivity of the betaxanthin pigments with proven antioxidant effects. This study represents a step towards the diversification of colorants used to replace synthetic colorants by naturally-occurring ones.

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Table 1
Physicochemical characterization of the betaxanthin-rich extract from cactus pear fruits

Parameter	*Value*
pH	6.6 ± 0.1
Acidity (% citric acid)	0.022 ± 0.003
Moisture content (%)	86.6 ± 0.5
Total soluble solids (ºBrix)	13.9 ± 0.2
Color parameters	
*L** (darkness-lightness) (0, 100)	86.07 ± 0.09
*C** (chroma)	98.09 ± 0.08
h^o (hue angle, 0º red, 90º yellow, 180º green, 270º blue)	86.59 ± 0.02
Betaxanthins (mg indicaxanthin / 100 g)	27.5 ± 1.6
Betacyanins (mg betanin / 100 g)	12.6 ± 0.9
Total phenolics compounds (mg GAE / 100 g)	220.1 ± 3.3
Free radical scavenging activity (ABTS) (μmol TEAC / g)	5.9 ± 0.8
Free radical scavenging activity (DPPH) (μmol TEAC / g)	4.8 ± 0.4

Values are means ± standard deviations of three replicates.

Table 2. Pigments detected in the betaxanthin-rich extract from *Opuntia* fruits.

Peak[†]	*Name*	t_R *(min)* [‡]	λ_{max} *(nm)*[*]	*m/z* $[M+H]^+$ *(daughter ions)*
1	Muscaaurine VII (histidine-betaxanthin)	4.63	476	349 (215, 124)
2	Vulgaxanthin I (glutamine-betaxanthin)	6.11	472	340 (323)
3	Indicaxanthin (proline-betaxanthin)	10.40	480	309, (263, 129)
4	Betanine	12.53	535	551 (389)
5	methionine-betaxanthin	14.01	477	343 (299)

[†]peak assignment refers to Fig. 2; [‡]retention time refers to Fig. 2; *absorption maxima.

Table 3. Stability analysis of the betaxanthin-rich extracts in dark conditions.

LIQUID EXTRACT			
DAYS	REMAINING BETAXANTHINS (%)		
	20 °C		
2	99.5±2.8[a]		
5	98.0±2.8[a]		
10	87.0±2.7[b]		
15	79.0±3.0[c]		
20	75.0±3.1[cd]		
25	69.9±2.5[d]		
30	55.1±2.3[e]		
ENCAPSULATED POWDERS			
DAYS	REMAINING BETAXANTHINS (%)		
	20 °C	4 °C	−20 °C
15	95.7±2.8[a]	98.0±1.8[a]	99.7±1.6[a]
30	94.4±2.7[a]	96.6±1.9[a]	99.5±1.4[a]
60	88.6±2.6[b]	95.8±1.8[a]	99.2±1.4[a]
90	75.1±3.3[c]	95.3±1.6[a]	99.1±1.3[a]
120	70.1±3.0[cd]	94.5±1.3[ab]	98.5±1.2[a]
150	60.6±2.0[e]	93.7±1.3[b]	96.9±1.4[ab]
180	58.9±4.0[e]	91.6±2.0[b]	96.6±1.2[b]

Values are means ± standard deviations of three replicates. Superscripts with different letters in same column indicate significant differences ($P \leq 0.05$).

Table 4. Color analysis (CIELab parameters) for model food products.

	YOGURT		
	Initial	28 days (dark)	28 days (light)
L^*	87.41±0.09[b]	87.13±0.07[c]	88.64±0.10[a]
C^*	19.57±0.09[a]	18.85±0.12[b]	9.47±0.10[c]
h^o	100.60±0.08[b]	98.97±0.10[c]	101.01±0.11[a]
ΔE^*	−−	0.95±0.08[b]	10.10±0.10[a]
	SOFT-DRINK		
	Initial	28 days (dark)	28 days (light)
L^*	36.52±0.06[a]	34.20±0.08[b]	29.89±0.09[c]
C^*	11.85±0.09[b]	12.19±0.11[a]	7.57±0.10[c]
h^o	103.67±0.09[a]	102.41±0.09[b]	101.67±0.11[c]
ΔE^*	−−	2.36±0.13[b]	7.90±0.15[a]

Values are means ± standard deviations of three replicates. Superscripts with different letters in same file indicate significant differences ($P \leq 0.05$).

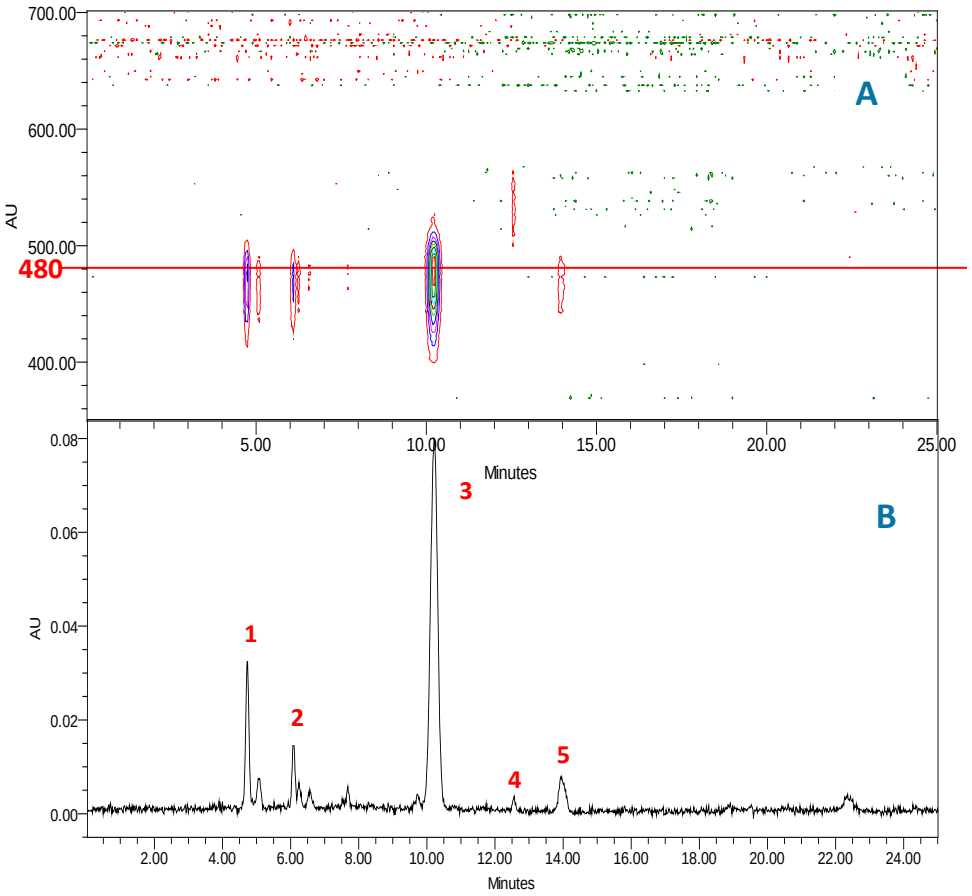

Fig. 1.

HPLC chromatographic pattern corresponding to the betaxanthin-rich extract from cactus pear fruits.

A. Contour-plots between 350 and 700 nm; B. Chromatogram at 480 nm.

(AU=absorbance units).
(Peak assignment: 1-muscaaurine VII; 2-vulgaxanthin I; 3-indicaxanthin; 4-betanine; 5-methionine-betaxanthin)

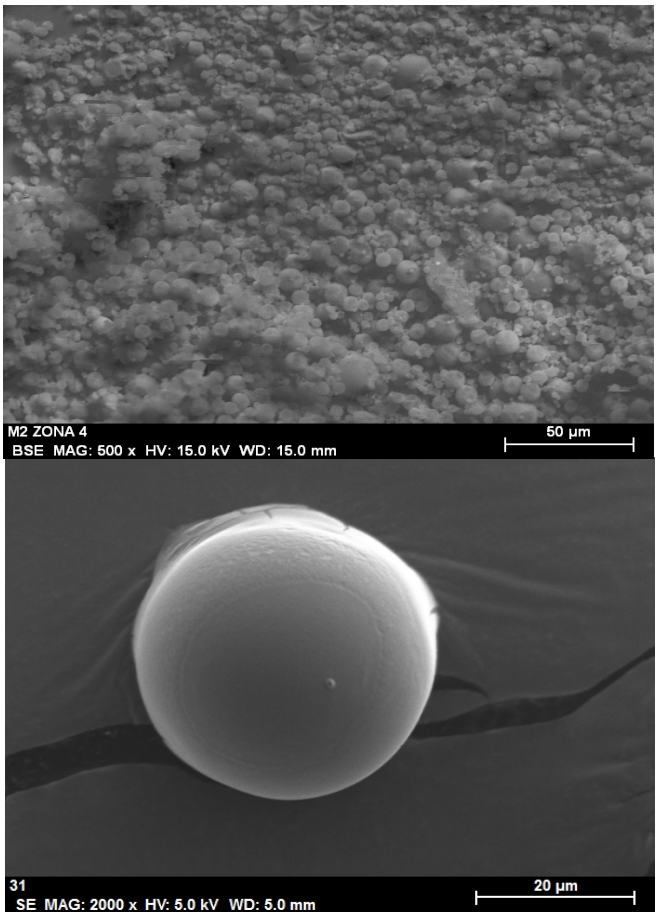

Fig. 2.

Scanning electron microscopic photographs of spray-dried microcapsules.

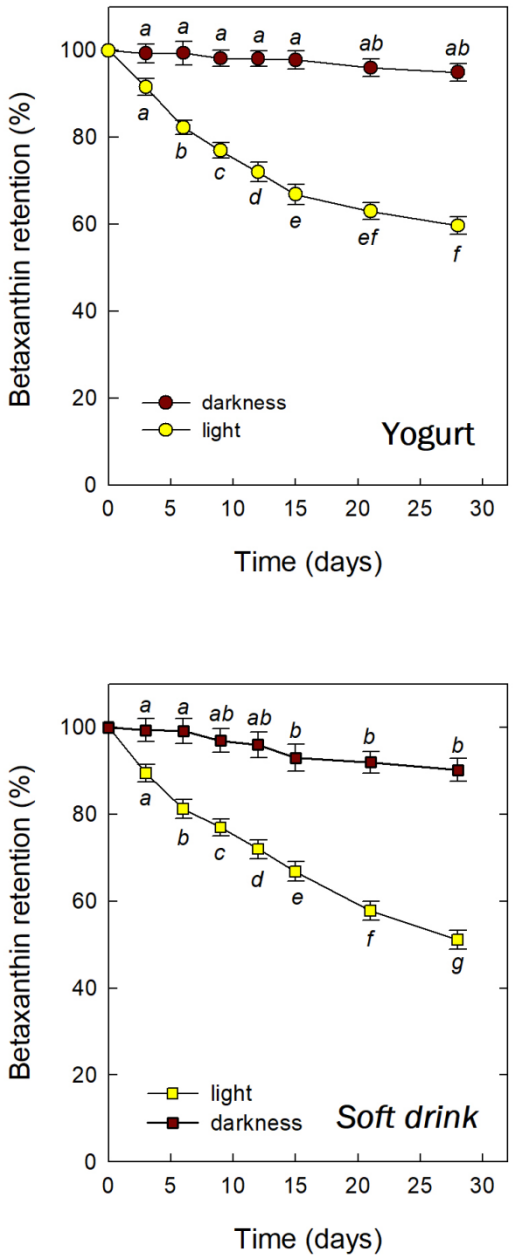

Fig. 3.

Betaxanthin retention in the model foods studied during storage at 4 ºC.

Values are means ± standard deviations of three replicates. Different letters in the same data series indicate significant differences ($P \leq 0.05$).