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Potential use of new Colombian sources of betalains. Colorimetric study of red prickly pear (*Opuntia dillenii*) extracts under different technological conditions

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A B S T R A C T

A new source of betalains to be used as natural colorant (*Opuntia dillenii*) has been studied. The stability of *O. dillenii* extracts in different pHs and temperatures over time has been scrutinized. Our attention was focused on differential tristimulus colorimetry and betalain content related to the color, not previously conducted in conjunction in that raw material. On the basis of the results, cold storage conditions (4 °C) were optimal to maintain as possible the initial red color (h_{ab}), lightness (L^*) and betalain content of the *O. dillenii* extracts, regardless of pH. Highly-acidic extracts (pH 4) manifested a significantly ($p < 0.05$) lower colorant intensity (C^*_{ab}) and betalain concentration (around 25% and 35%, respectively), with a clearer tendency toward yellowish tonalities (values of h_{ab} from 45° to 90°) over time. Furthermore, visually perceptible color changes ($\Delta E^*_{ab} > 3$) were induced among very acid (pH 4) and low-acidic (pH 5 and 6) extracts at each temperature.

1. Introduction

Opuntia is a shrub from America and brought to Spain in the nineteenth century, point at which it became widespread to other Mediterranean zones, and even to Asiatic countries. It usually grows in semi-desert climate, with an optimal low pluviometry for its growth (150–200 mm median annual precipitation). This cactus is characterized by yellow flowers, red fruits and thick, flat and stiff stems. The genus *Opuntia* belongs to the family of Cactaceae, with the species *Opuntia-ficus indica* being the most widespread, consumed and investigated. However, there are several other species of *Opuntia,* among which stand out *O. dillenii* (Ker-Gawl) Haw.

Prickly pears of *O. dillenii* have a very intense red color and acidic taste, with a great number of seeds (Medina, Rodríguez, & Romero, 2007), and an average length and width around 4.0 and 3.2 cm, respectively (Touil, Chemkhi, & Zagrouba, 2010). Some studies about *O. dillenii* from different countries (Tunisia, Spain and Canada) have been reported on the basis of a general characterization: analysis of acidity, nitrogen or sugars (Touil et al., 2010), moisture, °Brix, total fiber, protein, fat, ash, pH, acidity, ascorbic acid, total phenolics and several minerals (Medina et al., 2007), and polysaccharides (Yang, Chen, Zhou, & Zhang, 2013). In addition, the analysis of antioxidant activity has been a widely studied parameter in this kind of cactus, both in the entire fruit or separately in pulp, peel and seeds (Chang, Hsieh, & Yen, 2008; Liu et al., 2009; Yang et al., 2013). These authors affirmed that *O. dillenii* had high values of antioxidant activity, above all seeds and peel. From a healthy point of view, Chang et al. (2008) established a relationship between the antioxidant activity of different parts of *O. dillenii* and the protective effect against low-density lipoprotein peroxidation. Also, in fact, this cactus has been commonly used as medicine to avoid inflammation, gonorrhea and ophthalmia, among others diseases (Touil et al., 2010).

The market is continuously changing and consumers are becoming more conscious about how the food and its ingredients could positively influence their health. In that way, the consumption of products with antioxidant and beneficial properties has recently increased. From an agro-alimentary point of view, the natural colorants are replacing the traditionally-consumed synthetic colorants for this purpose. As a consequence, new raw materials that could satisfy this target market have been found in flowers, fruits and vegetables. Among them, there are several species with a wide range of colors, which are due to the presence of different chemical families such as anthocyanins, chlorophylls and carotenoids. However, there is a group of colored compounds that was still poorly investigated, the so-called betalains.

Betalains are water-soluble compounds whose basic structure consists of a moiety of betalamic acid. Depending on the residue added, betalains are classified as: (i) red/purple betacyanins and (ii) yellow betaxanthins, when hydroxycinnamic acid derivatives or sugars, and amines or amino acids residues, respectively, are linked to betalamic

acid (Herbach, Stintzing, & Carle, 2006). Betalains are presented in a restricted number of families of the plant order Caryophyllales and of the genus *Amanita* of the Basidiomycetes (Waterman, 2007) and they have been studied in a large series of plants, vegetables and flowers (Azeredo, 2009; Kugler, Stintzing, & Carle, 2007; Nemzer et al., 2011; Pavokovi & Krsnik-Rasol, 2011). Among fruits, cactus pears have been widely studied (Castellanos-Santiago & Yahia, 2008; Stintzing, Schieber & Carle, 2002a, 2002b), taking *O. ficus-indica* the lead over the other kind of *Opuntia.* However, to the best of our knowledge, very scarce studies on betalains about other species of *Opuntia,* such as *O. dillenii*, have been reported.

Furthermore, despite such broad agro-alimentary implications as natural colorant, none of the research studies about colorimetry of *O. dillenii* has been found in bibliography either. In that way, the study was performed on *O. dillenii*, which wildly grows in the arid zone of Nariño, Colombia, and our interest was focused on the color characterization and betalain content. Moreover, not only a color characterization of *O. dillenii* is needed, but even the knowledge of its colorimetric behavior when it acts as colorant of other foodstuffs. Therefore, in order to establish the best commercial conditions of the natural colorant addition to other agro-alimentary foods, stability assays in different pHs and temperatures over time have been also carried out, which have not been previously attempted in that raw material.

2. Materials and methods

In order to reflect as possible with the technological conditions that *O. dillenii* may be involved when it is used as natural colorant, a monitoring of stabilization in different circumstances has been carried out. A colorimetry study and betalain content of *O. dillenii* extracts at three temperatures (4 °C, 20 °C and 80 °C) and pHs (4, 5 and 6) has been conducted over time (0 and 5 h, and 1, 2, 6, 8 and 12 days). The temperature of 80 °C has been applied only for one day in order to reproduce as possible real conditions. Those terms have been chosen in order to cover a wide range of foods (from vegetables to meat, for example) and diverse ways of food processing (refrigeration, room temperature or cooking).

2.1. Chemical and solvents

Sodium ascorbate was purchased from Panreac (Barcelona, Spain) and methanol of analytical grade from J. T. Baker (Baker, Mallinckrodt, Mexico). Hydrochloric acid and sodium hydroxide (J. T. Baker, Baker Mallinckrodt, Mexico) were used for adjusting pH.

2.2. Samples

Red prickly pear samples (*O. dillenii*) were collected in the village of Remolinos (Nariño, Colombia). A representative set of samples of up to a weight of about 2 kg was harvested, collecting mature fruits according to visual characteristics and similar size using a simple random sampling model of ten plants. After homogenization, only 1450 kg of fruits was taken into account, with an average weight of each fruit around 11 g. They were carefully washed and dried, and prickles were manually removed. Fruits were cut into small pieces (1 cm^2) and extracted with 1 L of methanol:water (60:40) for 24 h at 10 °C (maceration). After filtration, the organic solvent was evaporated at 35 °C using a rotary evaporator (Heidolph, Schwabach, Germany) and the re-dissolved with distilled water (relation 1 g/mL) was lyophilized (Labconco, MO, USA). Lyophilized samples were stored at 4 °C until their analysis.

2.3. Extract reconstitution

9.5 mL of methanol and water (50:50), containing 50 mM of sodium ascorbate, were added to 3 g of lyophilized extract. Solutions were then stirred at 225 rpm for 10 min in darkness, in order to avoid possible degradation by light. Afterwards, a centrifugation at 12,000 $\times g$ at 10 °C and 5 min was carried out, according to the method proposed by (Cejudo-Bastante, Hurtado, Mosquera, & Heredia, 2014). Supernatants were separated and, in order to achieve the complete dissolution of the lyophilized extract, the procedure was developed once more with the extraction solution and finally with 100% methanol. Later, the extract obtained was concentrated in vacuum (30 °C) and resuspended until 15 mL with purified water. All experiences were carried out in triplicate.

2.4. Colorimetric measurements

A Hewlett-Packard UV–vis HP8452 spectrophotometer (Palo Alto, CA) was employed to develop color measurements. The whole visible spectrum (380–770 nm) was recorded at constant intervals ($\Delta\lambda = 2$ nm) using 2 mm path length glass cells and distilled water as reference. The original software CromaLab© was used for determining the CIELAB parameters (Heredia, Álvarez, González-Miret, & Ramírez, 2004), following the Commission Internationale de L'Eclairage's recommendations (CIE, 2004): the CIE 1964 10° Standard Observer and the Standard Illuminant D65. Euclidean distance between two points in the three-dimensional space define by L^*, a^*, and b^* were used for calculating color differences (ΔE^*_{ab}): $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

2.5. Spectrophotometric quantification of betalains

The UV–vis spectra were recorded from 360–800 nm in order to develop a photometric quantification of betalains, according to the method proposed by (Svenson, Smallfield, Joyce, Sansom, & Perry, 2008). Maximum absorbance of 484 and 535 nm was reported to quantify betaxanthins and betacyanins, respectively. Betalain content (B) was developed by the following equation:

$$[B]\ (\mu g/g) = [1000(Abs)(D)(V)(MW)/(\varepsilon)(L)(W)]$$

where Abs is the value of maximum absorbance at 484 or 535 nm, D is the dilution factor, V is the final volume (mL) of the extracts, MW and ε are the molecular weight and the molar extinction coefficient of betanin (550 g/mol and 60,000 L/(mol cm) in H_2O) and indicaxanthin (308 g/mol and 48,000 L/(mol cm) in H_2O), the major betacyanin and betaxanthin presented in *Opuntia* sp., respectively, L is the path-length (0.2 cm) and W the dried weight of the sample (g). All measurements were carried out in triplicate.

2.6. Statistical analysis

Statistical analysis was carried out by using Statistica version 8.0 software (Statistica, 2007). Univariate analyses of variance (Tukey test and ANOVA) were applied to discriminate among the means of chemical data.

3. Results and discussion

The effect of temperature and pH over time on the color and betalain stabilization has been investigated. Several temperatures (4 °C, 20 °C and 80 °C) and pH values (pH 4, 5 and 6) have been selected in order to cover a wide range of processing and storage conditions, and acidities of raw materials where *O. dillenii* was added as colorant. In order to observe the evolution during storage and/or cooking, the three perspectives (CIELAB color parameters, tristimulus differential colorimetry and betalain content) have been jointly studied over time (0 and 5 h, and 1, 2, 6, 8 and 12 days). In order to have a better understanding, the effect of temperature and pH has been separately discussed. Relative standard deviations were always <10%.

3.1. Effect of temperature over time

3.1.1. Temperature and colorimetric characteristics

Regardless of pH, the major and significant ($p < 0.05$) differences on color caused by temperature were found in hue (h_{ab}) (Fig. 1). When *O. dillenii* extracts underwent cold storage (4 °C), the red color was maintained without significant differences ($p > 0.05$) at least for 2 days of treatment. When time passed, the variations of shade were significant ($p < 0.05$) but only of 15% among the beginning and after 12 days of storage. However, when extracts were kept at room temperature (20 °C) or after cooking (80 °C), a remarkable and significant ($p < 0.05$) increase of hue was noticed, highlighting a change of the typology of color from reddish to yellowish. That fact could be due to an accelerated hydrolysis phenomena catalyzed by temperature, since betanin (red) could produce betalamic acid (yellow-orange) and cyclo Dopa-5-*O*-glucoside (colorless) as intermediary products (Sanchez-Gonzalez, Jaime-Fonseca, San Martin-Martinez, & Zepeda, 2013). Significant differences ($p < 0.05$) were observed at each time-point after the two days of treatment among 4 °C and 20 °C, and with samples after only 5 h of cooking (80 °C) (Fig. 1). Therefore, regardless of the pH of the foodstuff to which the natural colorant *O. dillenii* would be incorporated, it is better to keep it under refrigeration in order to maintain as possible the initial red color.

Furthermore, an upward trend of the values of lightness over time was observed. From the sixth day of treatment, and regardless of pH, the extracts stored at room temperature (20 °C) developed a remarkable increase of lightness, being significantly higher ($p < 0.05$) in comparison with those conserved at 4 °C. Thus, cold conditions permitted a better conservation of the initial lightness of the extracts. It is remarkable that, for both hue (h_{ab}) and lightness (L^*), samples kept at 20 °C for 12 days showed similarly values than those heated at 80 °C in only 5 h. That fact evidenced that the color degradation of *O. dillenii* was accelerated by the thermal treatment, as affirmed (El Hosry, Auezova, Sakr, & Hajj-Moussa, 2009).

Moreover, temperature did not exerted a significant ($p > 0.05$) effect on chroma (C^*_{ab}) at each time-point neither in highly- nor low-acidic extracts, causing the same effect in terms of tinctorial strength to kept them at 20 °C or 4 °C. As expected, heating at 80 °C provoked a remarkable and significative ($p < 0.05$) increase (around 50%) of chroma values, turning towards a more saturated color.

The (a^*b^*)-color diagram exposes *O. dillenii* extracts at the different pHs and temperatures over time, and it has been represented in Fig. 2. All samples were situated in the first quadrant of the diagram, but very close to the second quadrant for the last points of the treatment. Regardless of pH, the natural tendency of *O. dillenii* colorant was directed to the enhancement of b^* and the diminution of a^* levels, and

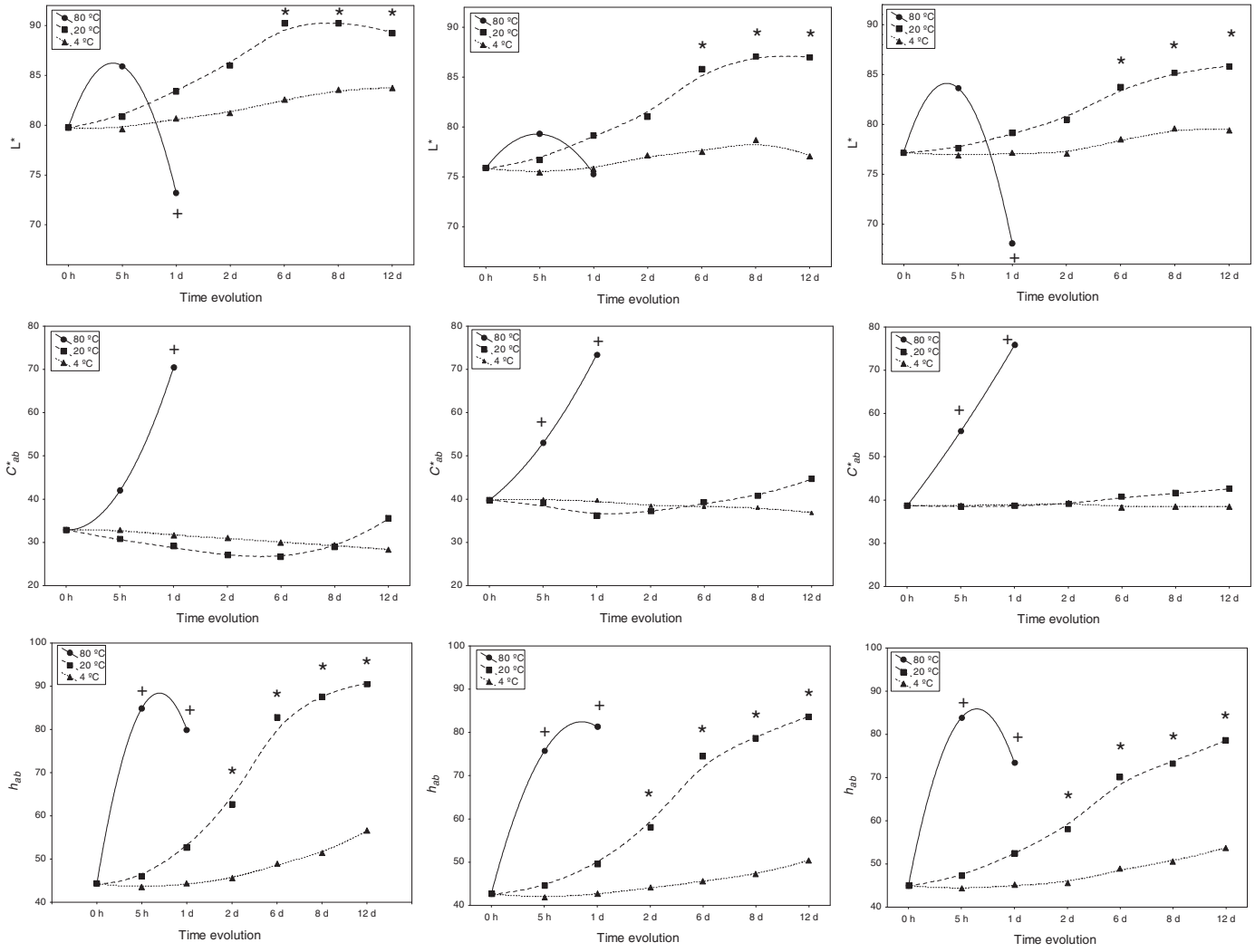

Fig. 1. Effect of temperature on CIELAB parameters of *Opuntia dillenii* extracts at different pHs over time. Significant differences ($p < 0.05$) according to the Tukey test among 4 °C and 20 °C (*), and among 80 °C and the rest of temperatures (+).

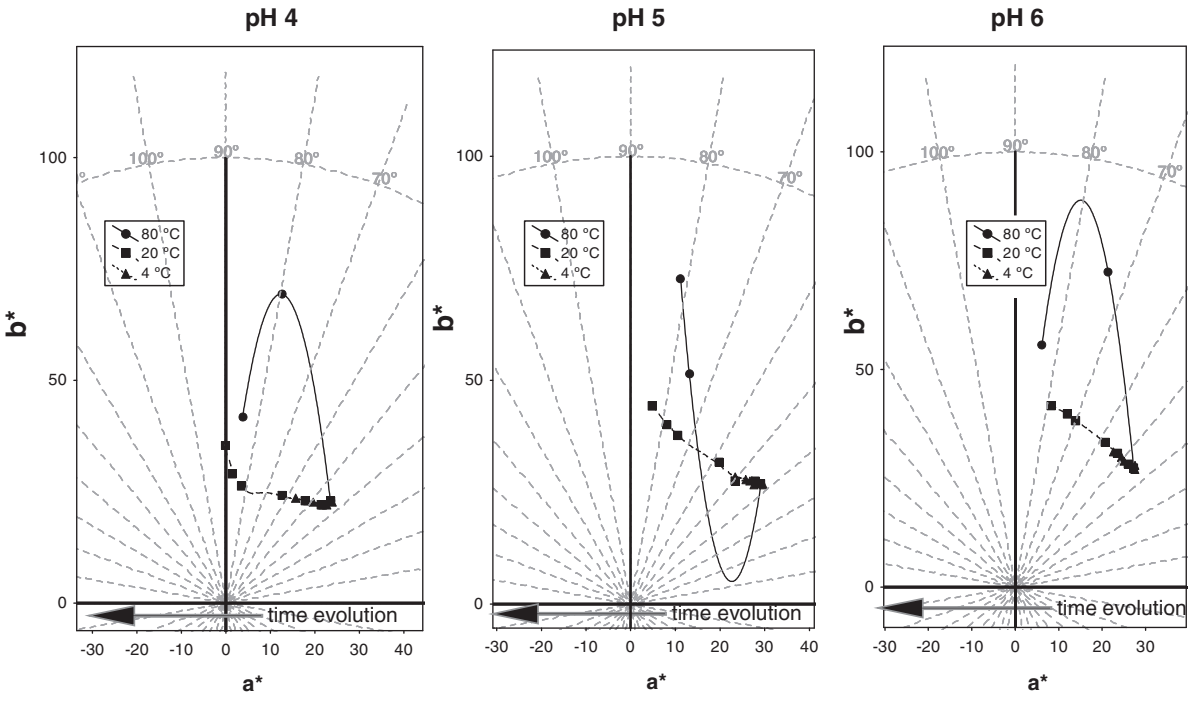

Fig. 2. Effect of temperature on CIELAB color space (a^*b^*)-plane of *Opuntia dillenii* extracts at different pHs over time.

it was more noticeable as temperature increased. Indeed, samples kept under refrigeration exhibited a scarce percentage of variation from the beginning to the end of the treatment, assuming similar values of b^* and a^* the samples under cold storage for 12 days and those conserved at room temperature for only 2 days. When extracts were cooked (80 °C), only 5 h is enough to situate samples in a similar location than after 12 days at 20 °C (Cai, Sun, & Corke, 1998). Therefore, the temperature supposed a catalyst of the color degradation of the *Opuntia* natural colorant towards more yellowish dyes. The color loss could probably due to dehydrogenation and decarboxylation reactions, resulting in the neobetanin (yellow) and 17-decarboxy-betanin (orange-red) compounds (Herbach, Stintzing, & Carle, 2004).

In conclusion, temperature exerted an important influence on the color of the *O. dillenii* extracts. For being used as natural colorant, and regardless of the pH of the foodstuff, it is better to keep them under refrigeration in order to maintain as possible the initial values of hue (h_{ab}) and lightness (L^*). So, *O. dillenii* extracts could be added to both highly-acidic (such as vegetables, salads, jams) as low-acid foods (meats, etc.) to improve their red color, provided that they were kept under cold conditions.

3.1.2. Temperature and differential colorimetry

With the aim of inquiring into the effect of temperature on color differences (ΔE^*_{ab}), only samples submitted to cold refrigeration and room temperature (4 °C and 20 °C) have been taken into account (Fig. 3). Obvious color differences occurred when samples submitted to 80 °C were considered (data not shown), due to their colorimetric characteristics that greatly differed from those obtained by the rest of temperatures.

Taking into account that the perceptible colorimetric differences to the human eyes have been established around three CIELAB units (Fernández-López et al., 2013; Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001), a visually appreciable effect on ΔE^*_{ab} took place when compared samples submitted to both temperatures at each pH, concluding that *O. dillenii* extracts suffered from thermolability. Those color differences were much more marked over time, even reaching values of 20 units between the beginning and the end of the treatment. The percentage of the quadratic increases of lightness, chroma and color shade to know the role of each color attribute respect $\Delta^2 E^*_{ab}$ was calculated (Gordillo et al., 2012). Hue and lightness ($\Delta^2 H$ and $\Delta^2 L$) actively contributed to the color differences, given the evolution of the color shade (towards yellowish tonalities) when samples were stored at room temperature.

3.1.3. Temperature and betalain content

Fig. 4 shows the characteristic spectrum of *O. dillenii* extracts, with maximum wavelengths around 483 and 535 nm, corresponding to the two groups of betalains studied (betaxanthins and betacyanins, respectively). By the measurement of the absorbance at those wavelengths, the content of betalains were also calculated (Fig. 5).

Over time, and considering each pH, the instability increased as temperature raised because a diminution of the absorbance at both wavelengths occurred (Fig. 4). Regarding temperature, while the spectra of the two maximum wavelengths were perfectly marked under refrigeration (even after 12 days of treatment), they became fading to turn into a flat line after 6 days of storage at 20 °C, and only 5 h at 80 °C. As a matter of fact, the effect of temperature was also observed in the time-sequence evolution (black and dotted lines, Fig. 4). Therefore, it must be affirmed that temperature accelerated the process of

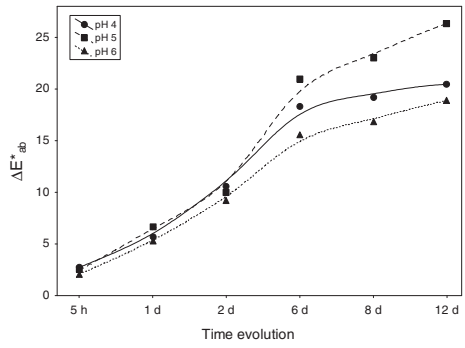

Fig. 3. Color variation (ΔE^*_{ab}) of *Opuntia dillenii* extracts between 20 °C and 4 °C at different pHs.

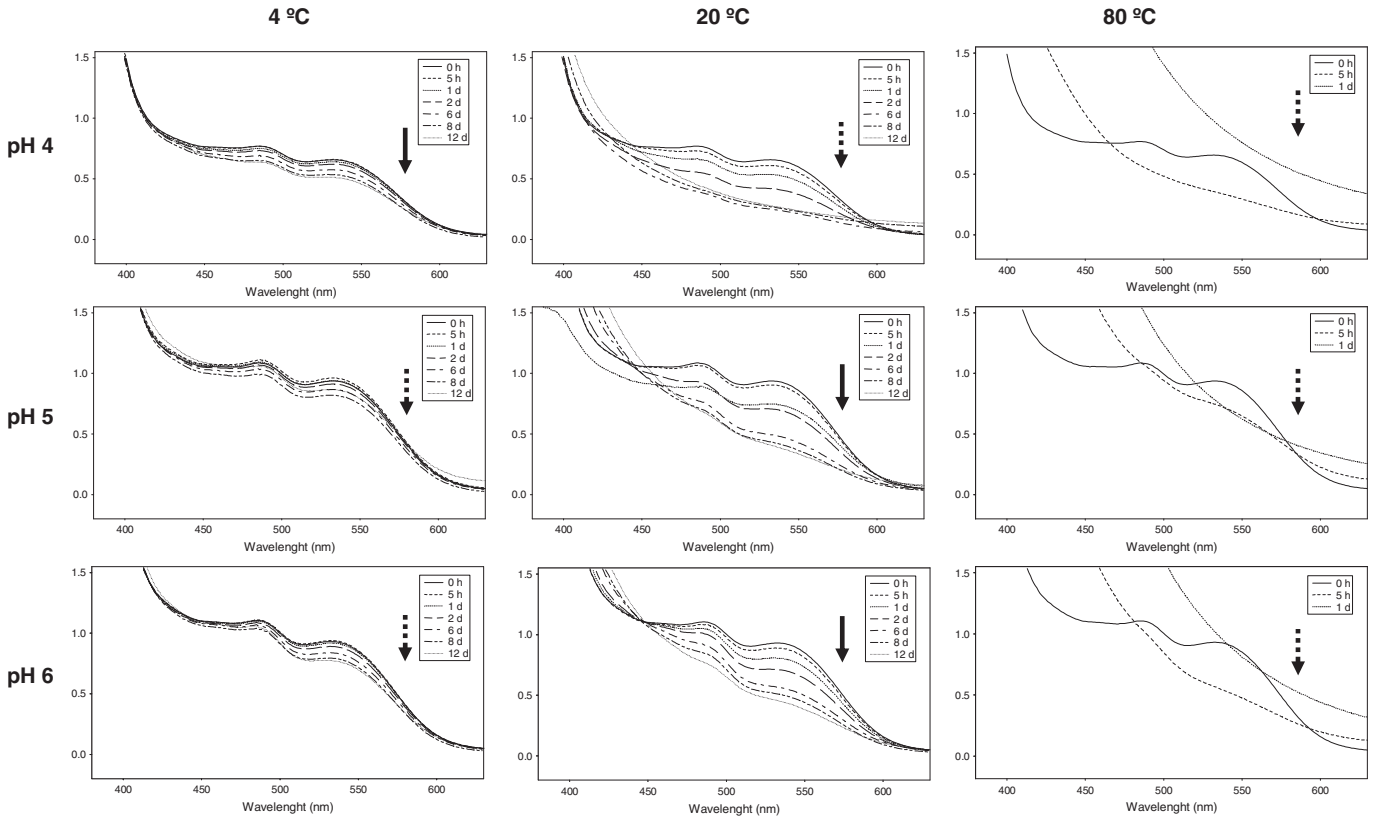

Fig. 4. Effect of the pH and temperature on the visible spectra (400–650 nm) of *Opuntia dillenii* extracts over time. Black line, descendent evolution over time; dotted line, descendent evolution over time except for the day 12.

spectra degradation, being *O. dillenii* a thermo sensitive raw material under those conditions. When samples were cooked (80 °C), the logical downward trend of absorbance values was not followed over time, but the absorbance at 420 nm was remarkably enhanced (dotted line, Fig. 4). That fact revealed a possible oxidation and/or accelerated browning, in agreement with El Hosry et al. (2009) and Fernández-Zurbano

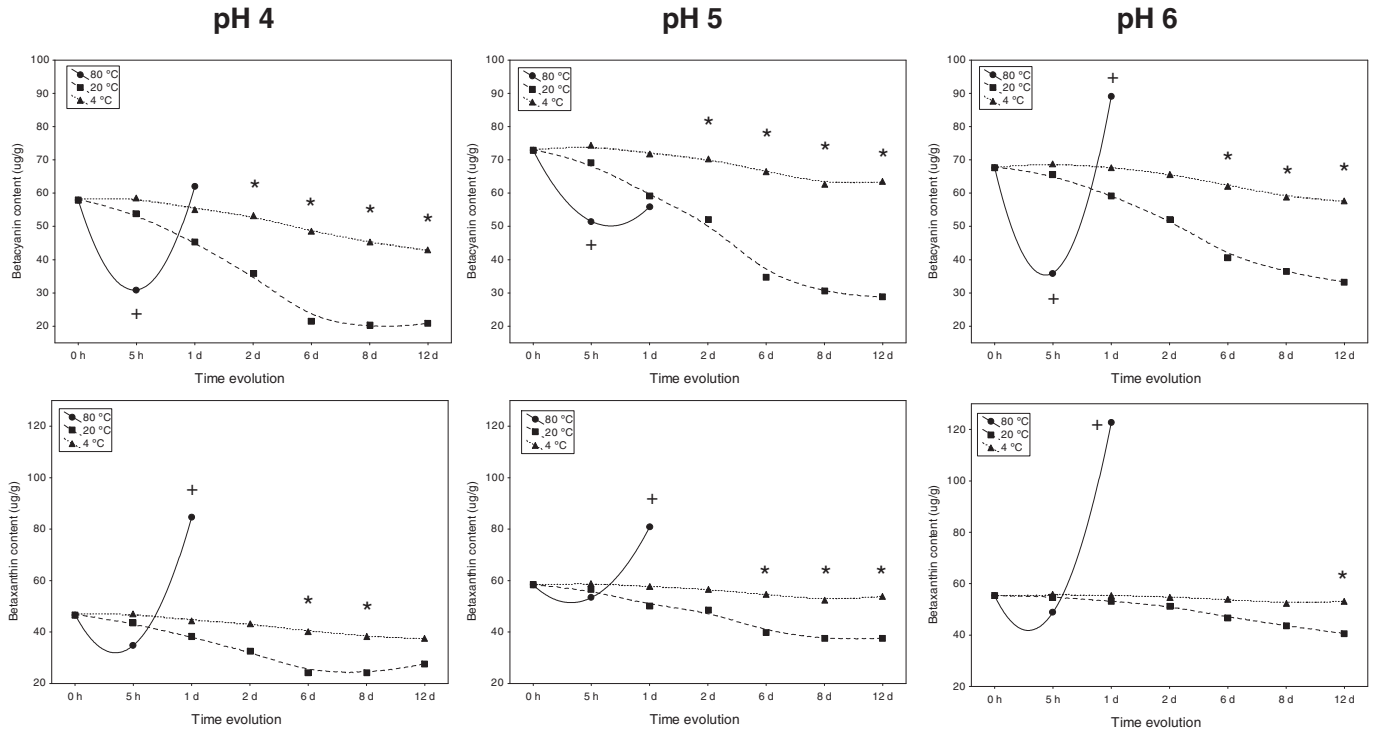

Fig. 5. Effect of temperature on betacyanin (a) and betaxanthin (b) contents of *Opuntia dillenii* extracts at different pHs over time. Significant differences ($p < 0.05$) according to the Tukey test among 4 °C and 20 °C (*), and among 80 °C and the rest of temperatures (+).

et al. (1995) who demonstrated that an oxidation of wine phenolics is derived from a severe thermal treatment.

With regard to betalains, their content at the beginning of the treatment of both betacyanins and betaxanthins was similar at each pH (60–70 μg/g and 50–60 μg/g, respectively). Similar values were also reported by Butera et al. (2002) in *O. ficus-indica* extracts, owing to the overlapping of betanin absorbance with the absorbance of indicaxanthin. In general, the amount of betalains of *O. dillenii* extracts decreased over time (Fig. 5), being the temperature an important factor on their pattern evolution. These results were in agreement with those obtained by Castellar et al. (2006) in other species of *Opuntia* sp.

The effect of temperature began to be significantly ($p < 0.05$) marked from the two days of treatment. Regardless pH, if extracts were conserved in the absence of cold storage, remarkable losses of betacyanin content (around 40–65%) were highlighted after the 12 days of treatment, versus the 11–25% observed at 4 °C. In the case of betaxanthins, the losses were situated around 30%. Moreover, the conservation under cold conditions (4 °C) made the extracts been maintained as possible the initial content of betalains (both betacyanins and betaxanthins), having significantly ($p < 0.05$) higher amounts in comparison with room storage conditions at each time-point (Fig. 5). When extracts were cooked (80 °C), it took only 5 h to reduce by 50% the concentration of betacyanins and betaxanthins. Similar results were found when three species of *O. indica* (red, yellow and white) were thermally submitted for one hour (Sanchez-Gonzalez et al., 2013). However, the fact that their content greatly and significantly ($p < 0.05$) increases after one day of treatment lead us to think that an accelerated browning occurred, in light of the great maximum absorption at 420 nm.

3.2. Effect of pH over time

3.2.1. pH and colorimetric characteristics

The effect of pH on several perspectives (CIELAB color parameters, tristimulus differential colorimetry and betalain content) has been also scrutinized.

At the beginning of the process (0 h) there were significant ($p < 0.05$) differences among pH values, above all in lightness and chroma (L^* and C^*_{ab}) (Fig. 6). Concretely, extracts adjusted to pH 4 exhibited a significantly ($p < 0.05$) lower color intensity (C^*_{ab}) and higher lightness (L^*) than the rest of pHs, being insignificant the differences on hue (h_{ab}) (Fig. 6).

Over time, and regardless of the temperature applied, it is highlighted the significant ($p < 0.05$) differences on chroma (C^*_{ab}) among samples. In that way, *O. dillenii* extracts at very acid pH displayed significantly ($p < 0.05$) the lowest values of chroma at each time-point. That fact means that it could be necessary to add more quantity of colorant to

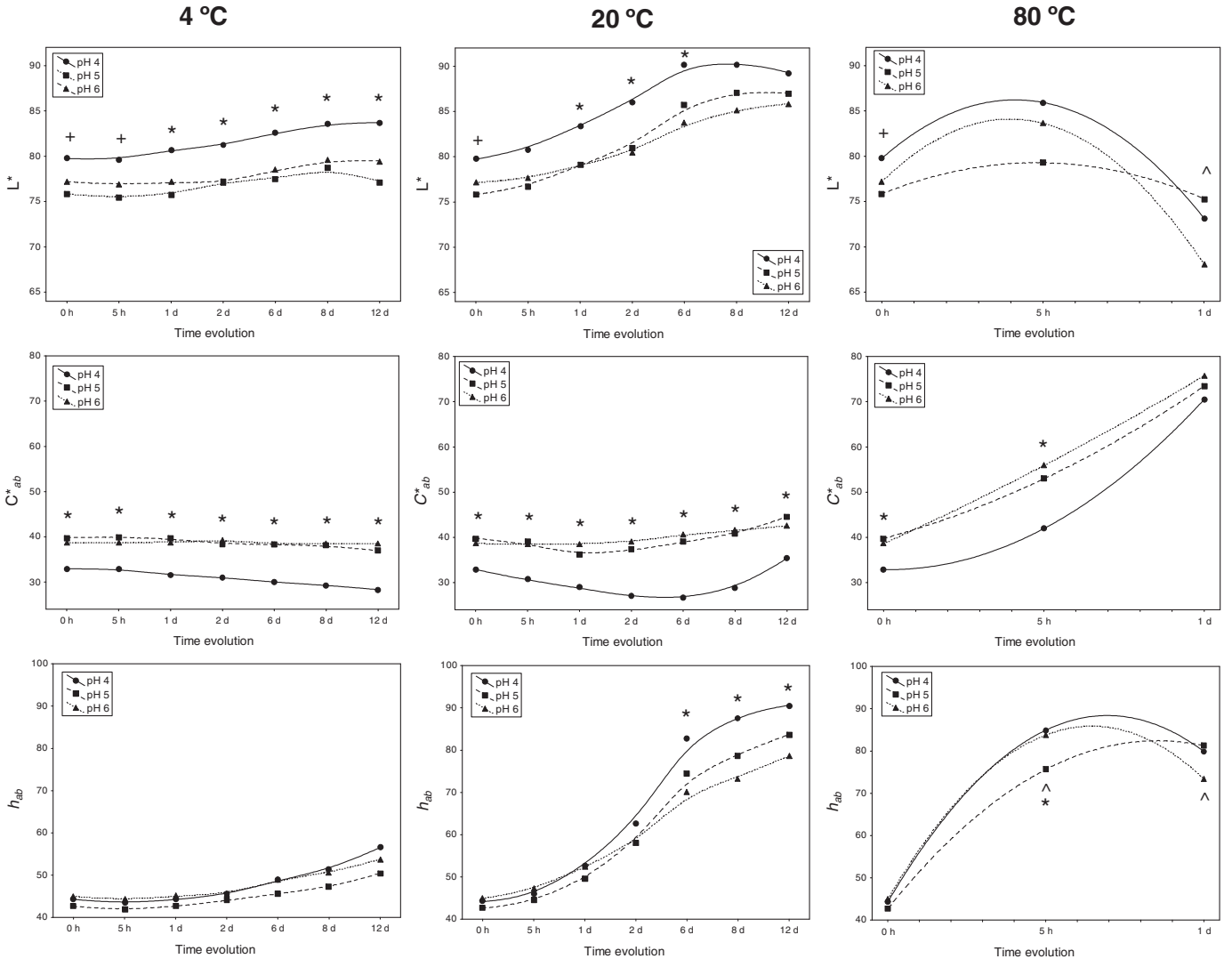

Fig. 6. Effect of pH on CIELAB parameters of *Opuntia dillenii* extracts at different temperature over time. Significant differences ($p < 0.05$) according to the Tukey test among pH 4 and the rest of pHs (*), among pH 4 and 5 (+), and among pH 5 and 6 (^).

reach a higher colorant intensity of the foodstuff. Similar results were obtained by Cejudo-Bastante et al. (2014) after developing stabilization assays on *Ullucus tuberosus*. Moreover, pH also exerted an important effect on lightness (L^*), with the significantly ($p < 0.05$) highest values of the highly-acidic extracts at each time-point. Furthermore, it is important to highlight that the aforementioned commented change toward yellowish tonality after room temperature storage was much more accentuated as pH decrease, contrarily to that found (Hurtado, Morales, González-Miret, Escudero-Gilete, & Heredia, 2009) when a pH stability assay was carried out in anthocyanin-rich tamarillo (*Solanum betaceum* Cav.). When samples were heated (80 °C), scarce variations according to pH were manifested among them.

Regarding the (a^*b^*)-color diagram, the effect of pH was more pronounced in the behavior of b^* (Fig. 7). Thus, at each time-point, highly-acidic extracts presented a significantly lower values of the component b^* of the color. That fact means that *O. dillenii* extracts became less yellow when they were added to acid food products.

Overall, *O. dillenii* extracts could be more suitable for being added to low-acidic foods (meats, for example) because the hue and color stability increased with pH. If it was needed to add extracts to more acidic food to promote red color, more quantity must be added to counteract the lower colorant intensity. However, the fact that highly-acidic *O. dillenii* extracts would be directed hopelessly towards yellowish dyes would be also exploited by colorant industry. That is to say that, the voluntary submission of *O. dillenii* extracts at 6 days of storage at room temperature or 5 h of heating would be used for complementing highly-acidic foods with yellow color (such as yogurts, fruit smoothies, ice creams, jams, to name a few).

3.2.2. pH and differential colorimetry

The pattern evolution of the color differences (ΔE^*_{ab}) among pHs over time at each temperature was showed in Fig. 8. As it can be seen, pH exerted an important effect in color differences. As $\Delta E^*_{ab} > 3$, it could be asserted that color differences were appreciable among highly- (pH 4) and low-acidic extracts (pH 5 and 6), whatever the temperature applied. So, it could be confirmed that it was pH 4 the acidity which established the limit from after color differences were visually appreciable. When compared among low-acidic extracts (pH 5 against pH 6), ΔE^*_{ab} was maintained over time in values below 3, indicating that they cannot be visually discriminated regardless of temperature (Fig. 8). Also visually discrimination upon heating was already found by Fernández-López et al. (2013) when the submitted temperature on several red pigment extracts was 50 °C.

Thereby, at both 4 °C and 20 °C, color differences were mainly quantitative ($\Delta^2 C$ and $\Delta^2 L$) and due to the different behavior of highly-acid extracts (pH 4), although qualitative differences ($\Delta^2 H$) also contributed in the last steps of the storage at room temperature (6–12 days). At 80 °C, there were hue and lightness ($\Delta^2 H$ and $\Delta^2 L$) the main contributors to color differences, although an important qualitative participation of chroma took also on special significance (data not shown).

3.2.3. pH and betalain content

pH exerted an influent effect on the maximum absorption at the two wavelengths (484 and 535 nm) for each temperature (Fig. 4). The time-sequence of the spectra of *O. dillenii* extracts followed a chronological descendent order until the 8 days of storage. Subsequently, the behavior depended on temperature.

At the beginning of the treatment, although without significant differences ($p > 0.05$), the most abundant absorptions of both wavelengths was attributed to extracts adjusted to pH 5 (in agreement with Sanchez-Gonzalez et al. (2013)), followed by pH 6 and pH 4 (Fig. 4). That fact was directly related to the concentration of betaxanthins and betacyanins, respectively, which were significantly ($p < 0.05$) higher in low-acidic foodstuffs (Fig. 9). Over time, the diminution of the absorbance at 484 and 535 nm between 0 h and 12 days was mitigated as pH increased, similarly to that observed by Castellar et al. (2003) in several species of *Opuntia* sp. and by Cejudo-Bastante et al. (2014) in other raw materials. That fact could be extrapolated to the betaxanthins' and betacyanins' content, respectively (Fig. 9), whose percentage of diminution over time in highly- (pH 4) and low-acidic foods (pH 6) ranged

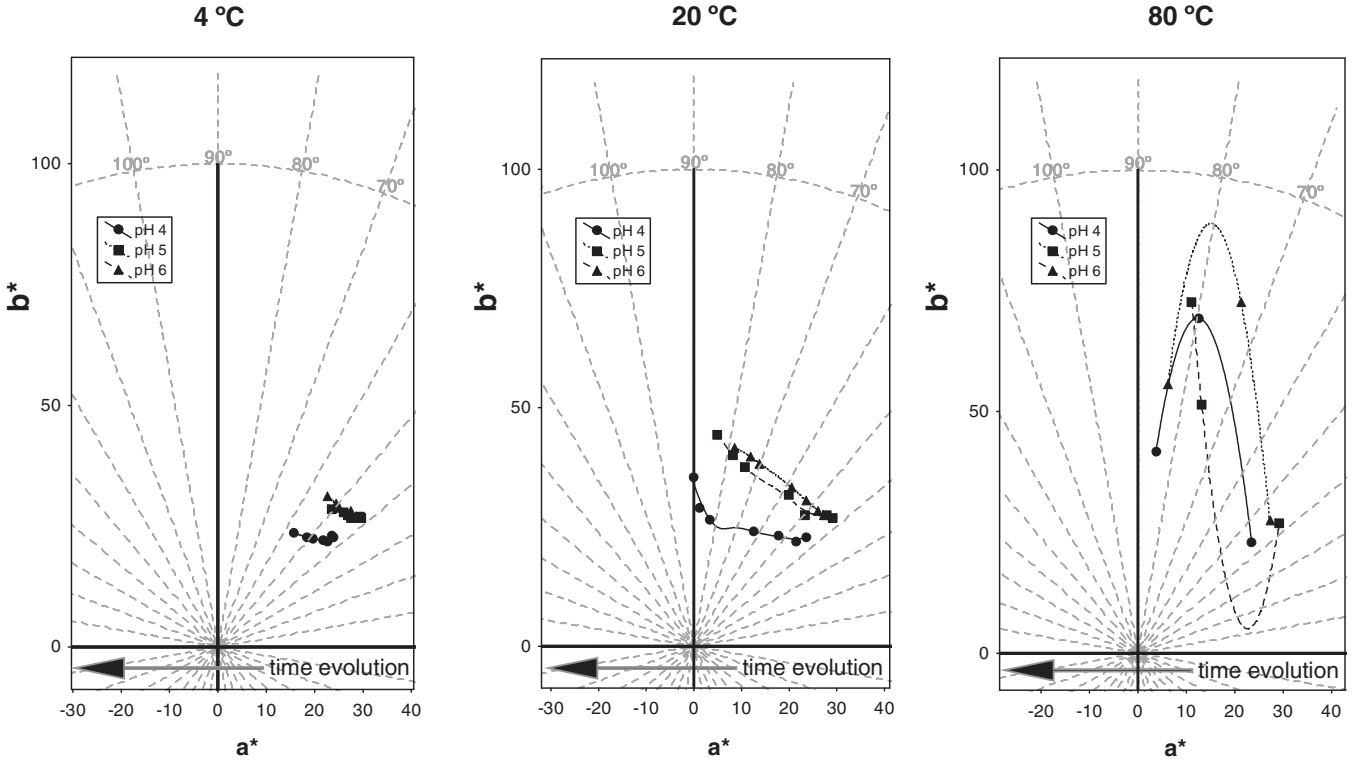

Fig. 7. Effect of pH on CIELAB color space (a^*b^*)-plane of *Opuntia dillenii* extracts at different temperature over time.

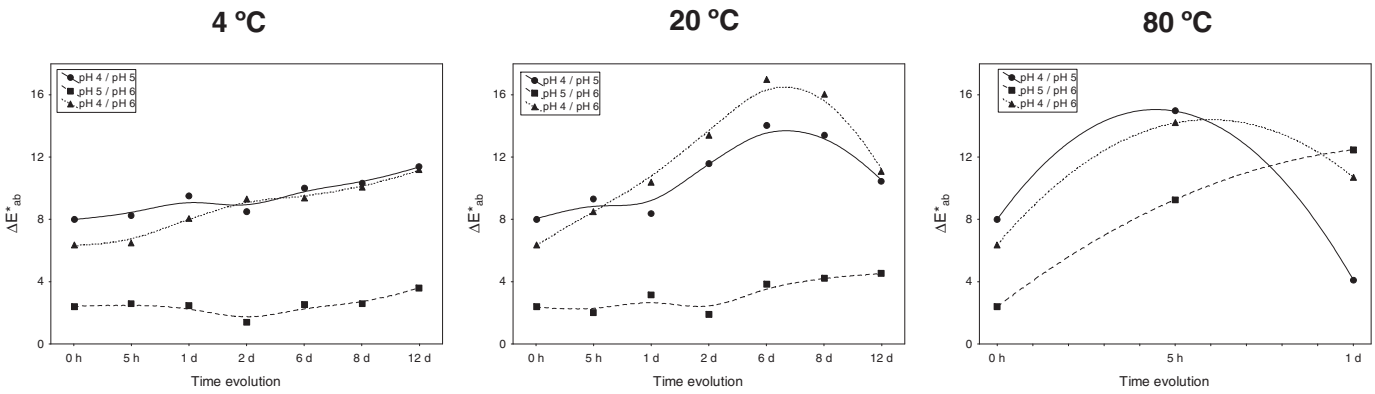

Fig. 8. Color variation (ΔE^*_{ab}) of *Opuntia dillenii* extracts among different pHs at each studied temperature.

between 20–40% and 4–25% in betaxanthins, and among 25–65% and 15–50% in betacyanins. Therefore, and regardless of temperature, the betalain stability of *O. dillenii* extracts depended on the pH, because betalains were most stable in low-acidic foods with significantly ($p < 0.05$) higher values at each time-sequence (Fig. 9). That results were contrary to that affirmed Cejudo-Bastante et al. (2014) in *U. tuberosus*.

Similarly, pH also showed a clear effect on the demeanor of heat-treated *O. dillenii* extracts (80 °C); the content of betacyanins greatly decreased in the samples adjusted to pH 4 and 6. However, samples with medium-acidity did not experiment remarkable changes on the content of both chemical families after 5 h of treatment, leading them in the most significantly abundant betalain content ($p < 0.05$).

4. Conclusions

It has been demonstrated that *O. dillenii* is a raw material suitable for being used as natural colorant in a wide range of pH products and under different processing and storage conditions. Hence, at least during two days of treatment, the red nuance of the foodstuff to which *O. dillenii* was added would be independent of their acidity. In the case of a long lasting storage, it would be the temperature which dictates the food tonality containing the *O. dillenii* colorant. Thus, *O. dillenii* extracts added to highly-acidic foods (vegetables- or fruit-based dishes) and kept at room temperature provided a significantly more yellowish dye and more lightness than the low-acidic foodstuffs. If samples were conserved under cold refrigeration, food products of diverse acidities would not have differences on the hue but it could be necessary to add higher quantity of extract to highly-acidic food to counteract their lower colorant intensity and betalain content. Even when food was cooked (80 °C) the colorant intensity provided by the highly-acidic extract would be lower. Finally, this research could be interesting because it has deepened not only in the occurrence of new natural sources as colorants, but also in how the extract might change the color and betalain content according to the nature of the product where it is added and to the different ways of processing. In addition, this study could be directly applicable not only to food industry, but also to other kind of trades in which the color, is the target market (such as

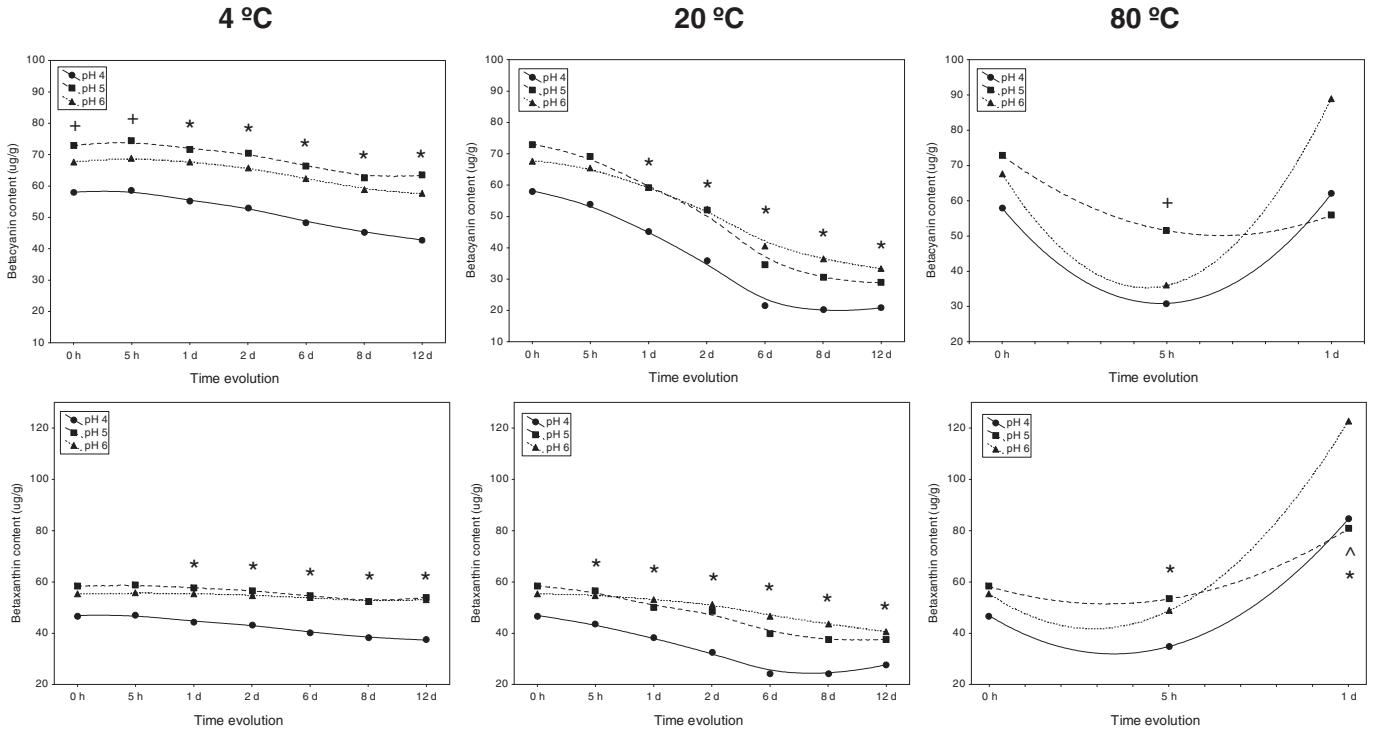

Fig. 9. Effect of pH on betacyanin (a) and betaxanthin (b) contents of *Opuntia dillenii* extracts at different temperatures over time. Significant differences ($p < 0.05$) according to the Tukey test among pH 4 and the rest of pHs (*), among pH 4 and 5 (+), and among pH 5 and 6 (^).

cosmetics, pharmaceutics, drugs and paints, among others). Although further researches are needed in relation to natural colorants, such as a chemical individual betalain characterization of scarcely studied sources, this study could be a great step forward to displace synthetic colorants.

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