Potential use of new Colombian sources of betalains. Color stability of Ulluco (*Ullucus tuberosus*) extracts under different pH and thermal conditions

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ABSTRACT

The potential use of ulluco (*Ullucus tuberosus*) extracts to be added as natural colorant to other products has been studied. The stability of ulluco extracts at different pHs and temperatures over the time has been thoroughly conducted. Our attention was focused

Potential use of new Colombian sources of betalains. Color stability of Ulluco (*Ullucus tuberosus*) extracts under different pH and thermal conditions

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on the tristimulus colorimetry, differential colorimetry and betalains related to color. On the basis of the results, although ulluco extract adjusted to pH 4 showed a significantly lower color intensity (C^*_{ab} ~ 40), the stability of red hue (h_{ab}) over the time was higher. Betalain content showed the same trend, and lower values in high-acidic extracts have been reported. It could be also affirmed that visually appreciable color changes (ΔE^*_{ab} > 2) were induced when pH changed and different temperatures were applied. Despite of the vivid red color of the initial extracts, ulluco extracts added to low-acidic foodstuffs showed a tendency toward yellowish tonalities (values of h_{ab} from 45° to 80°), mitigated by using refrigeration storage conditions (h_{ab} values from 45° to 55°).

Keywords: Ulluco (*Ullucus tuberosus*), differential tristimulus colorimetry, betalains, stability.

ABSTRACT

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1. INTRODUCTION

Ulluco (*Ullucus tuberosus*) is a plant grown as a root and leaf vegetable normally cultivated 2800 m above sea level (Flores, Walker, Guimarães, Reis, & Vivanco, 2003). It is the sole species in the monotypic genus *Ullucus* belonging to *Basellaceae* family. The ulluco is one of the most widely grown and economically important root crops in the Andean region of South America, second only by potato. It is also known with the common name of *papa lisa* or *lisa*, but also by the regional names *melloco* (Ecuador), *olluco* (Peru), *chugua* (Colombia) or *ruba* (Venezuela), among others (King, 1989). Regarding the morphology, the ulluco' shape ranged between oblong and spherical, and it grows only a few inches long (normally between 2 and 15 cm). The skin clearly differs from the pulp (with a white/yellow color), whereas skin could have a large extent of colors such as yellow, orange, red, magenta and purple, and with or without freckles (Flores & Flores, 1997; Flores et al., 2003).

Nowadays, the importance of the healthy and natural food both in the manufacture or consumption is increasing. In this sense, the agro-alimentary, drug or cosmetic market is turning towards diversification, concretely over the use of natural products as colorants. 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 agro-alimentary products rich in colored compounds responsible of beneficial healthy properties (Cai, Sun, & Corke, 2003; Stintzing et al., 2005) but still less investigated, the so-called betalains.

Betalains are water-soluble compounds present in a restricted number of families of the plant order Caryophyllales and of the genus *Amarita* of the Basidiomycetes (Waterman, 2007). They are classified into two chemical groups, betacyanins and betaxanthins (Herbach, Stintzing, & Carle, 2006a), whose maximum absorption is situated around

540 nm and 480 nm, respectively (Azeredo, 2009; Strack, Vogt, & Schliemann, 2003). Betalains are widely distributed in a great variety of products. Although red beet (*Beta vulgaris* L.) is the most studied source of betalains (De Azeredo, Pereira, De Souza, Gouveia, & Mendes, 2009; Nemzer et al., 2011; Pavokovi & Krsnik-Rasol, 2011), this chemical family has been also studied in other raw materials. It is important to highlight the researches carried out in plants and flowers (*Gomphrena globosa* L. and *Bougainvillea* sp.) (Kugler, Stintzing, & Carle, 2007), fruits (pitaya, yellow beet and cactus pear (*Opuntia* sp.)) (Castellanos-Santiago & Yahia, 2008; Stintzing, Schieber, & Carle, 2002a, 2002b), and vegetables (Kugler, Stintzing, & Carle, 2004). However, up to present, scarce studies about betalains in tubers have been developed. That is the case of ulluco (*Ullucus tuberosus*), subject of this study. In that way, the studies of Campos et al. (2006) and Svenson, Smallfield, Joyce, Sansom, & Perry (2008) are two of the few research articles found about betalains in that raw material; concisely in different red and yellow varieties of the Andean tuber crop ulluco grown in New Zealand. Therefore, further researches in that way are needed.

In spite of the high consumption of this raw material in South America and its potential use as natural colorant, very scarcely scientific information about colorimetry of ulluco has been found. Busch et al. (2000) are the only authors that reported CIELAB parameters of fresh and cooked skin and flesh of ulluco cultivated in New Zealand in order to determine their sensorial evaluation. For this reason, not only a color characterization of ulluco is needed, but even the knowledge of its colorimetric behavior when it acts as colorant of other products. For example, it could be interesting to elucidate their stability in products with different pHs (from vegetables to meats), or at different temperatures (when cooked or kept refrigerated), and also over the time.

However, hitherto, any research article related those different analytical parameters have been found in bibliography for the raw material ulluco.

Therefore, the aim of this research was to know the potential of Ulluco (*Ullucus tuberosus*) cultivated in Colombia as natural colorant with a wide range of use in foods. This study is the first attempt to measure accurately the stability of betalains and color by applying differential tristimulus colorimetry over the time at different pHs and temperatures in this raw material. Hence, this study could provide with important commercial information about the optimal conditions of storage and technological treatments of ulluco extracts used as food colorant.

2. MATERIALS AND METHODS

An exhaustive follow-up of stabilization of Ulluco extracts in different conditions have been carried out, in order to reproduce possible situations that it could be found when this raw material was added to other food as natural colorant. A colorimetry study and betalain content of ulluco extracts at three pHs (pH 4, pH 5 and pH 6) and temperatures (4 ºC, 20 ºC and 80 ºC) has been conducted over the time (0 and 5 hours, and 1, 2, 6, 8 and 12 days). The selected values of pHs and temperatures have been assigned in order to cover a wide range of pH values of foods (from vegetables to meats) and processing (refrigeration, room temperature or cooking). Unlike the rest of the applied temperatures and with the aim of reflecting real conditions as possible, the application of 80 ºC has been scrutinized only during one day.

In order to a better understanding of the results, the effect of pH and temperature on color, color differences by differential colorimetry and total betalains of ulluco extracts have been separately developed and discussed.

2.1. Chemical and Solvents

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

2.2. Samples

Magenta ulluco samples were collected in the village of Catambuco, which is located at approximately 8 Km south of the city of San Juan de Pasto (Nariño, Colombia) and at 2820 meters above sea level. The average temperature of this zone is situated at around 12 ºC. The analyzed tuber, commonly known as Ulluco "Chincheño", was characterized by having a white pulp and a thin surface portion of magenta color-rich skin in pigments.

They were carefully washed, dried and manually peeled to separate the white pulp from the magenta peel. The peelings were cut into small pieces (1 cm^2) and extracted with 1 L of methanol:water (60:40) for 24 h at 10 ºC (maceration) (relation solution:skin, 2 mL/g of peel). After filtration, the organic solvent was evaporated at 35 ºC using a rotary evaporator (Heidolph, Schwabach, Germany) and the re-dissolved with distilled water (relation 1 g/mL) was lyophilized (Labconco, MO, USA). Lyophilized extracts were stored at 4 ºC until their analysis.

2.3. Extract reconstitution

Lyophilized extracts (3 g) were added to 9.5 mL of methanol:water (50:50) containing 50 mM of sodium ascorbate. Subsequently, solutions were stirred at 225 rpm for 10 min in darkness. Afterwards, samples were centrifuged at 12000 x *g* at 10 ºC for 5 min and supernatants were separated. 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 ºC) and resuspended until 15 mL with purified water. All experiences were carried out in duplicate.

2.4. Colorimetric measurements

In order to develop color measurements, a Hewlett-Packard UV-vis HP8453 spectrophotometer (Palo Alto, CA) was used. 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, corresponding to the natural daylight. CIELAB parameters were calculated: L^* (ranging from 0, black, to 100, white), and two color coordinates, a^* (which takes positive values for reddish colors and negative values for greenish ones) and b^* (positive for yellowish colors and negative for the bluish ones). From these coordinates, other color parameters are defined: the hue angle (h_{ab}) and the chroma (C^*_{ab}), as qualitative and quantitative attributes of color, respectively. 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

A photometric quantification of betalains was also carried out, following the method proposed by Svenson et al. (2008). The UV-vis spectra were recorded from 360-800 nm and all measurements were performed in duplicate. Two different absorbance at maxima were reported (484 nm and 535 nm) on the basis of the quantification of 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 histidine-betaxanthin (348 g/mol and 48,000 L/(mol cm) in H_2O), the major betacyanin and betaxanthin presented in Ulluco, respectively, L is the path-length (0.2 cm) and W the dried weight of the sample (g). All analyses were carried out in duplicate.

2.6. Statistical analysis

Statistical analysis was performed 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

3.1. Colorimetric characteristics

The evolution of CIELAB color parameters (L^*, C^*_{ab} and h_{ab}) of ulluco extracts at different pHs and temperatures was evaluated over the time (Figure 1). Moreover, in order to locate ulluco extracts in the CIELAB space, the (a^*b^*)-color diagram has been represented (Figure 2).

3.1.1. Effect of pH over the time on the colorimetric characteristics

The effect of pH over the time (different values of pH at each temperature) on the basis of colorimetric characteristics has been discussed.

It is highlighted that the behavior of the color evolution of the samples submitted to 80 ºC considerably differed from the rest of temperatures, and therefore, it will be separately discussed.

A clear impact of pH on CIELAB parameters was observed in ulluco initial extracts. The significant differences ($p < 0.05$) among samples according to pH were appreciated in chroma and hue (C^*_{ab} and h_{ab}), and lightness (L^*) (with higher and lower values as pH increased, respectively). However, different evolution pattern of each CIELAB parameters was observed over the time.

With regard to the chroma (C^*_{ab}), all ulluco extracts exposed to 20 ºC and 4 ºC followed the same evolution scheme, i.e., slightly decreases from the initial to the final point of the treatment (Figure 1). As can be seen, ulluco samples were grouped by pH, with higher values as pH increased. Different behavior was observed by Castellar, Obón, Alacid, & Fernández-López (2003), who demonstrated that, in a pH range of 3-7, *Opuntia stricta* extracts adjusted to pH 5 obtained the highest percentage of colorant capacity. Temperature did not affect chroma. Therefore, it would be the same to keep extracts at room temperature or under refrigeration, due to the pH exerted a more influent effect.

Similarly, hue (h_{ab}) values of samples adjusted to pH 4 were lower in comparison with the rest of pHs (4 ºC and 20 ºC). pH effect on hue regardless temperature was observed until two days of treatment. Although punctual significant variations of hue were initially found according to pH (between pH 4 and 6, $p < 0.05$), contrarily to that observed in cactus pears (Moßhammer, Stintzing, & Carle, 2005), pH 4 samples increasingly differed from the rest with significantly lower values of hue angle. As a consequence, the more acid ulluco extracts showed a much more reddish tonality since the first moment, and it was maintained practically without variations until two days regarding temperature.

On the basis of lightness (L^*), it adopted higher values in the first steps of the treatment (0-5 h) when acid extracts were considered, greatly differing samples at pH 4 from the

others. Thereafter, the pH did not exert any clear effect over the time evolution regardless temperature.

The behavior pathways of hue at 80 °C was similar to that commented by the other temperatures during the first five hours, but any pH effect was observed when the thermal treatment lasted one day.

The (*a*b**)-color diagram with ulluco extracts at the different pHs and temperatures over the time is represented in Figure 2. Although all samples were situated in the first quadrant of the diagram, as expected from their red color, it could be seen that different locations concerning pHs for each temperature were observed. The effect of the time provoked a continuously lower values of *a**, whereas the effect of pH was observed in the behavior of the values of *b**. As it can be observed, ulluco extracts added to low-acidic foods became more yellow despite of the applied temperature.

In summary, until the two days of treatment, if ulluco extract is added to low-pH foods (for example, vegetables), the tonality would be more reddish than the initial extracts (pH = 6.1). With the aim of counteracting the lower color intensity of these samples, previously commented, higher quantities of ulluco extracts might be added to the foodstuffs. However, if ulluco extract was added to foods with pH value over 6 (such as meats) its red original color could be converted into more yellowish. Thereafter, the color characteristics highly depended on the temperature, as affirmed other authors (Castellar et al., 2003; Stintzing & Carle, 2007). Important variations of hue towards yellow tonalities were produced when ulluco extract was heated at 80 ºC during 5 hours. When a very acid food containing ulluco extract as colorant was considered, a loss of color intensity and an augmentation of lightness were observed, without remarkable changes on these parameters in low acid foods.

3.1.2. Effect of temperature over the time on the colorimetric characteristics

The major differences caused by the temperature were observed in hue and after the two days of treatment (Figure 1). Thereafter, when ulluco extracts were maintained at 4 ºC, the variations of shade over the time were slight, contrarily to that observed at room temperature or after heating (even with an increase of 40º, reflecting a change in background color from red to yellow) (Figure 1). Hence, the temperature involved an important effect on the hue of foodstuffs containing ulluco extract as colorant. In fact, it is noticeable the enhancement of hue angles of ulluco extracts adjusted at pH 4 and kept at room temperature (increment of 50 º), initially with the lowest values of hue. Samples were also grouped by temperature when lightness was taking into account. Thus, regardless of pH, samples kept at 20 ºC showed significantly higher values of lightness in comparison with those conserved at 4 ºC, at least until 8 days of treatment. Afterward, samples at pH 4 and 5 kept at 20 ºC greatly diminished the lightness, sitting them close to those kept at 4 ºC.

Remarkable changes caused by temperature have been also observed in chroma, above all samples adjusted at pH 4. Thus, any chroma difference was observed among keeping low-acidic ulluco extracts (pH 5 and 6) at 20 ºC or 4 ºC, whereas the storage at room temperature of acidic ulluco extract (pH 4) provoked a significant ($p < 0.05$) lower values of color intensity in the following days after the day 2. This pointed to the fact that a decline of color purity during the thermal process in samples adjusted to pH 4 occurred, in agreement with Herbach, Stintzing, & Carle (2004b) when assays with heated pitaya were performed.

As expected, samples submitted to 80 ºC were significantly different, with higher intensity color and lower values of lightness, deeply changing the typology of color from reddish to yellowish, and producing a total degradation of the color as affirmed Herbach, Stintzing, & Carle (2004a). Similar findings observed Sadilova, Carle, &

Stintzing (2007), who studied anthocyanin product-rich such as elderberry, strawberry and black carrot submitted to heat, as well Fernández-López, Angosto, Giménez, & León (2013) who demonstrated that color variation patterns showed a direct relationship with the intensity of the thermal exposure when temperatures of 50 ºC, 70 ºC and 90 ºC were applied along six hours to several natural extract.

According to (*a*b**)-color diagram, the significant decrease of *a** values during the time were more notable at room temperature, and much faster at 80 ºC (Figure 2). As it can be observed, the thermal treatment accelerated the process of color change. That fact could be observed because only five hours were enough to locate samples exposed to 80 ºC in a similar position than those stored at room temperature during 12 days (Cai, Sun, & Corke, 1998). Moreover, that situation could be also observed when compared samples submitted to 20 ºC and 4 ºC, on the basis of the changes found in the component *b** of the color. In general, samples under room temperature during 2 days are similarly situated in the (*a*b**)-color diagram to those kept under refrigeration during 12 days, for each pH value.

This behavior suggests that when ulluco extract will be used as natural colorant, it is better to keep it under refrigeration in order to maintain as possible the initial values of hue and lightness, regardless of the pH of the foodstuff. When ulluco extract will be added to high-acidic foods, it is recommended to avoid room temperature in order to enhance the color intensity, fact that is not necessary in low-acid foods. In addition, the hue angles of both low- and high-acidic foods containing ulluco extract greatly changed during five hours of severe heating (80 ºC). When the thermal processing was more lasting, all CIELAB parameters were clearly modified, regardless of pH.

3.2. Differential colorimetry

3.2.1. Effect of pH over the time on differential colorimetry

Taking into account that, approximately, ΔE^*_{ab} of up to two CIELAB units indicates color differences appreciable to the human eyes (Fernández-Vázquez, Stinco, Hernanz, Heredia, & Vicario, 2013), it was confirmed that changes on pH led to color differences visually appreciable ($\Delta E^*_{ab} > 2$) (Figure 3a, b and c). The significantly major ΔE^*_{ab} were observed between the most diverse values of pH (4 and 6) regarding temperature, according to Tukey test ($p < 0.05$). The lower color differences, although visually appreciable, were observed among the pH 5 and 6 values.

Therefore, color differences among pairs of pHs were always visually appreciable and significant, above all among the most extreme pHs. As a consequence, ulluco extract would have an appreciable diverse coloration on the basis of the pH of the food where it was added. The evolution over the time of those color differences followed a different pattern depending on the temperature, and it was always clearly perceptible.

3.2.2. Effect of temperature over the time on differential colorimetry

The pattern evolution of the color differences over the time was different among temperatures (Figure 3a, b, c). At 4 ºC, ΔE^*_{ab} had an ascendant tendency from the initial point to the end of the treatment (Figure 3a). However, different evolution profile was followed when samples were submitted to 20 ºC and 80 ºC (Figure 3b, c). In general, in the latter cases, color differences when compared pairs of pH values increased until the middle of the treatment to notably decrease afterwards. In order to know the role of each color attribute respect $\Delta^2 E^*_{ab}$, the percentage of the quadratic increases of lightness, chroma and hue were calculated (Gordillo, Rodríguez-Pulido, Escudero-Gilete, González-Miret, & Heredia, 2012). In the first case, those differences were mainly qualitative and due to the behavior of hue in samples adjusted to pH 4 and stored at room temperature, and, in the second case, they were mainly quantitative and due to chroma (data not shown).

Taking into account that largest visually appreciable total color differences were observed (Fernández-Vázquez et al., 2013) when comparing samples heated at 80 ºC with the rest of temperatures (on the order of 30 units), only the comparison between 20 ºC and 4 ºC was only considered for discerning the effect of the temperature on color differences (ΔE^*_{ab}) (Figure 3d). First of all, temperature provoked a visually appreciable effect ($\Delta E^*_{ab} > 2$) on color differences of each pH, so total color differences confirmed the greater thermolability of ulluco extracts (Figure 3d). That fact was also observed by Fernández-López et al. (2013), when heating of several natural red pigment extracts at moderate temperature (50 ºC) was applied. Those color differences were increasingly more marked over the time, even reaching values of 23 units between the beginning and the end of the treatment, aggravating ΔE^*_{ab} in low-acid samples. However, it is highlighted that color differences of samples adjusted at pH 4 were practically maintained at low values virtually until the end of the treatment (8 days). Therefore, ulluco extract added as colorant to high-acid foods would be temperature-independent because stable color differences were observed. As a consequence, low pH-foods (such either vegetables- and fruit-based salads) could be conserved as under refrigeration or at room temperature. However, due to the continuously higher color differences when ulluco extracts were added to acid foods in a lesser extent (such as meats), they must be conserved at refrigeration conditions to postpone as possible the color degradation.

3.3. Betalain content

As it can be seen, ulluco samples showed a characteristic spectrum, with two maximum wavelengths around 484 and 535 nm (Figure 4). Those wavelengths correspond to the two groups of betalains (betaxanthins and betacyanins, respectively). After measuring absorbance, the content of each group of betalains were calculated at the different pHs

and temperatures. Figure 5 shows the amount of betaxanthins and betacyanins present in the ulluco extracts, which ranged between 30-100 µg/g. Those values were in agreement with Campos et al. (2006), who studied several species of Andean tuber crops.

3.3.1. Effect of pH over the time on betalains

In general, the values of absorbance of the two maximum wavelengths decreased for all samples as time progressed (Figure 4). This fact was in agreement with that observed in *Opuntia* species at different pHs (Castellar et al., 2003).

pH showed a clear effect on the absorption of the two maximum wavelengths, regardless of the temperature applied. In general, higher values of absorbance of each wavelength were measured as pH increased (Figure 4). Moreover, in general, the diminution of absorbance at 535 nm between 0 hours and 12 days was mitigated as pH decreased, contrarily to that observed at 480 nm (Figure 4). So, the red color was more stable in high-acid foods, contrarily to the yellow color, given the evolution of the hue in that conditions (Figure 1). Similar results were obtained by Hurtado, Morales, González-Miret, Escudero-Gilete, & Heredia (2009), when the skins of tamarillo (anthocyanin-rich natural food) was assayed across a pH range (2.0-8.7), concluding in a more stable red color under low acid conditions. It is noticeable that, contrarily to the samples that underwent refrigeration, the time-sequence evolution of the spectra of ulluco extracts kept at room temperature disappears as pH decreased, i.e., the instability of the color over the time appeared earlier as food pH was lower at 20 ºC. (Cai, Sun, Schliemann, & Corke, 2001) also found a parallel evolution in *Celosia argentea*, affirming that the minimum stability corresponded to high-acidic samples.

That fact could be extrapolated to the quantification of total betalains, both betaxanthins and betacyanins, when 480 and 535 nm was respectively considered (Figure 5). At the beginning of the treatment, significant differences ($p < 0.05$) on the content of

betacyanins were found among pH 4 and 5, and besides pH 4 and 6 on the betaxanthins concentration. The initial content of betalains was in accordance with the pH, i.e., ulluco extracts adjusted to pH 5 and 6 showed the highest amount of betalains. Same values of pH was ascertained as maximum colorant capacity in several species of *Opuntia* (Castellar et al., 2003). As time passed, any common effect according to pH was formed when considering 4 ºC and 20 ºC. In heat-treated ulluco extracts (80 ºC), the content of betacyanins and betaxanthins greatly decreased in the lowest pH values. As a consequence, samples adjusted at pH 6 did not show remarkable changes on the content of betalains after five hours of treatment, leading them in the most significant abundant betalain content ($p < 0.05$). All those differences disappeared when samples were heated during one day, so any pH effect was observed. Namely, red color was stable over the time in a large extent when ulluco extract was added to high-acid foodstuffs, regardless of the storage temperature, but contrarily to that happened when heating (80 ºC).

3.3.2. Effect of temperature over the time on betalains

At each pH value, the color instability over the time increased with the temperature (Figure 4), in agreement to that observed by Saguy (1979) and Cai et al. (1998), who affirmed that the degradation rate of betalains in model solutions or other raw materials accelerates with increasing temperature and heating period. The two maximum wavelengths initially observed were gently fading and becoming flat, being more remarkable when temperature increased. Upon heating, ulluco extract suffered a pronounced degree of degradation, being a thermosensitive raw material at those conditions. That blurring of the spectrum was observed since the first 5 hours, with values of absorbance even higher to those initial. That fact could be due to the possible formation of other compounds as a consequence of the thermal process (Maillard

reaction compounds) (Stintzing & Carle, 2007). These compounds could be characterized by decarboxylation of betacyanins at C-17 and/or C-2, and dehydrogenation at C-14/C-15, which could modify appearance and stability of the genuine pigments (Herbach et al., 2006).

With regard to betalain content, the effect of the temperature began to be observed after one or two days of treatment (Figure 5). In general, the amount of both betacyanins and betaxanthins maintained more or less constant over the time when samples were submitted to 4 ºC. However, remarkable losses (even until 50 %) happened when samples were kept at room temperature, above all at higher pHs. At 80 ºC, ulluco extracts only needed five hours to quickly drop the betalain content, above all pH adjusted to 5. (Herbach et al., 2004) brought similar results out when purple pitaya juice was heated during five hours. As previously commented, and judging by the diffused spectra at 80 ºC (Figure 4), the very high content of betalains at the end of treatment lead us to think that an accelerated browning occurred, in light of the great maximum absorption at 420 nm. In fact, Fernández-Zurbano et al. (1995) and El Hosry, Auezova, Sakr, & Hajj-Moussa (2009) already affirmed that an accelerate oxidation of wine phenolics occurred by thermal application. It could be remarkable that samples under refrigeration conditions were more stable from a betalain content standpoint, besides being samples with high content of them.

CONCLUSIONS

It can be concluded that ulluco extract is suitable for being added as natural colorant in a wide range of pH, so its application appears to be promising. A clear pH effect on color has been observed, with color differences visually perceptible among values of pH. When ulluco extract was added to high-acidic foodstuffs, the color intensity was lower but more stable over the time. Moreover, due to the content of betalains was also

significantly lower, high quantities of extract must be added to counteract this situation. When ulluco extract was added to low-acid agro-alimentary products, the chroma was higher but the initial red hue angle led towards yellowish shade. Temperature was also an important factor on the stability of ulluco extracts, mainly affecting to hue and betalain content. Finally, it must be more appropriate to add ulluco extract to high-acidic foodstuffs (vegetables- or fruit-based dishes), but its addition could be suitable for meat industry or other foods with pH values on the order of 6, by assuming that possible yellow tonalities could confer over the time. In conclusion, this research study could be useful for food industry, because the color and betalain variations that it could suffer according to the nature of the product where it is added have been deeply scrutinized. Although further researchers are needed in relation to natural colorants, this study not only could be a great step forward in the field of food, but also in cosmetics and drugs, among others, pushing synthetic colorants into the background.

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FIGURE CAPTIONS

Figure 1. Time evolution of CIELAB parameters of ulluco extracts submitted to different pHs and temperatures.

Figure 2. CIELAB color space (a^*b^*)-plane for ulluco extracts at different pHs and temperatures over the time.

Figure 3. Color variation (ΔE^*_{ab}) between different pHs (a, b, c) and temperatures (d) of ulluco extracts.

Figure 4. Effect of the pH and temperature on the visible spectra (400-650 nm) of ulluco extracts over the time. h, hours; d, days. Black line, descendent evolution over the time; dotted line, descendent evolution over the time except for the day 12.

Figure 5. Betacyanin (a) and betaxanthin (b) contents of ulluco extracts at different pHs and temperatures over the time. h, hours; d, days.

Figure 1

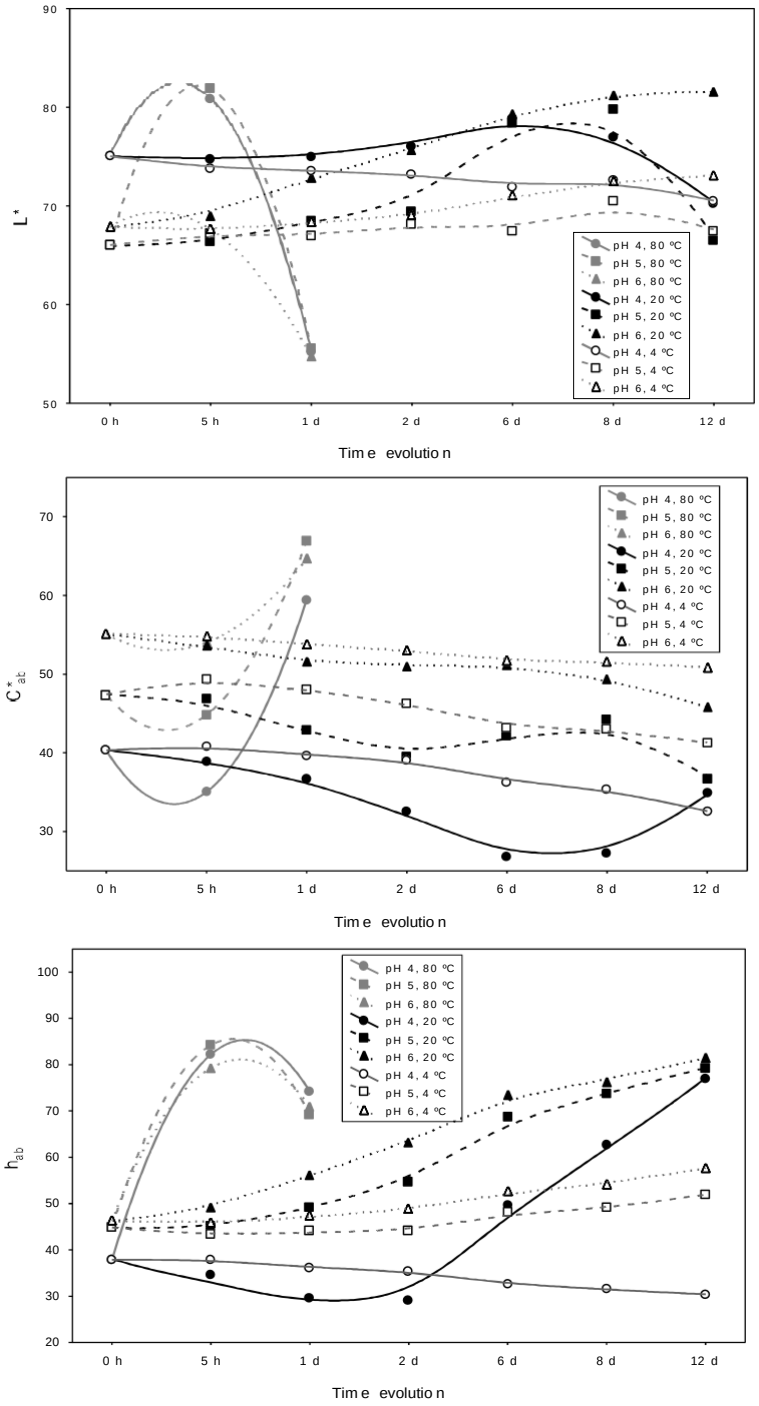

Figure 1.

Figure 2

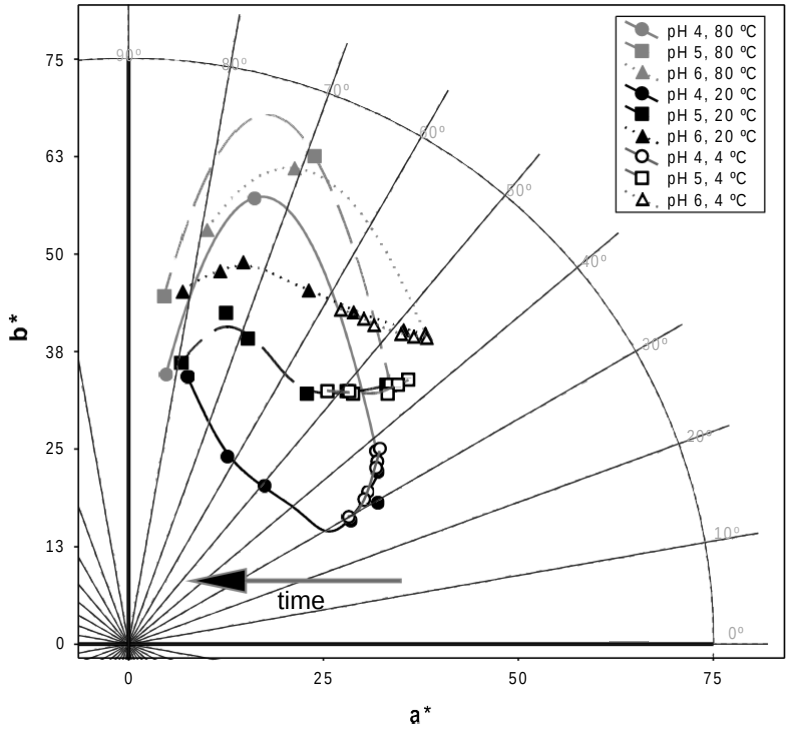

Figure 2.

Figure3

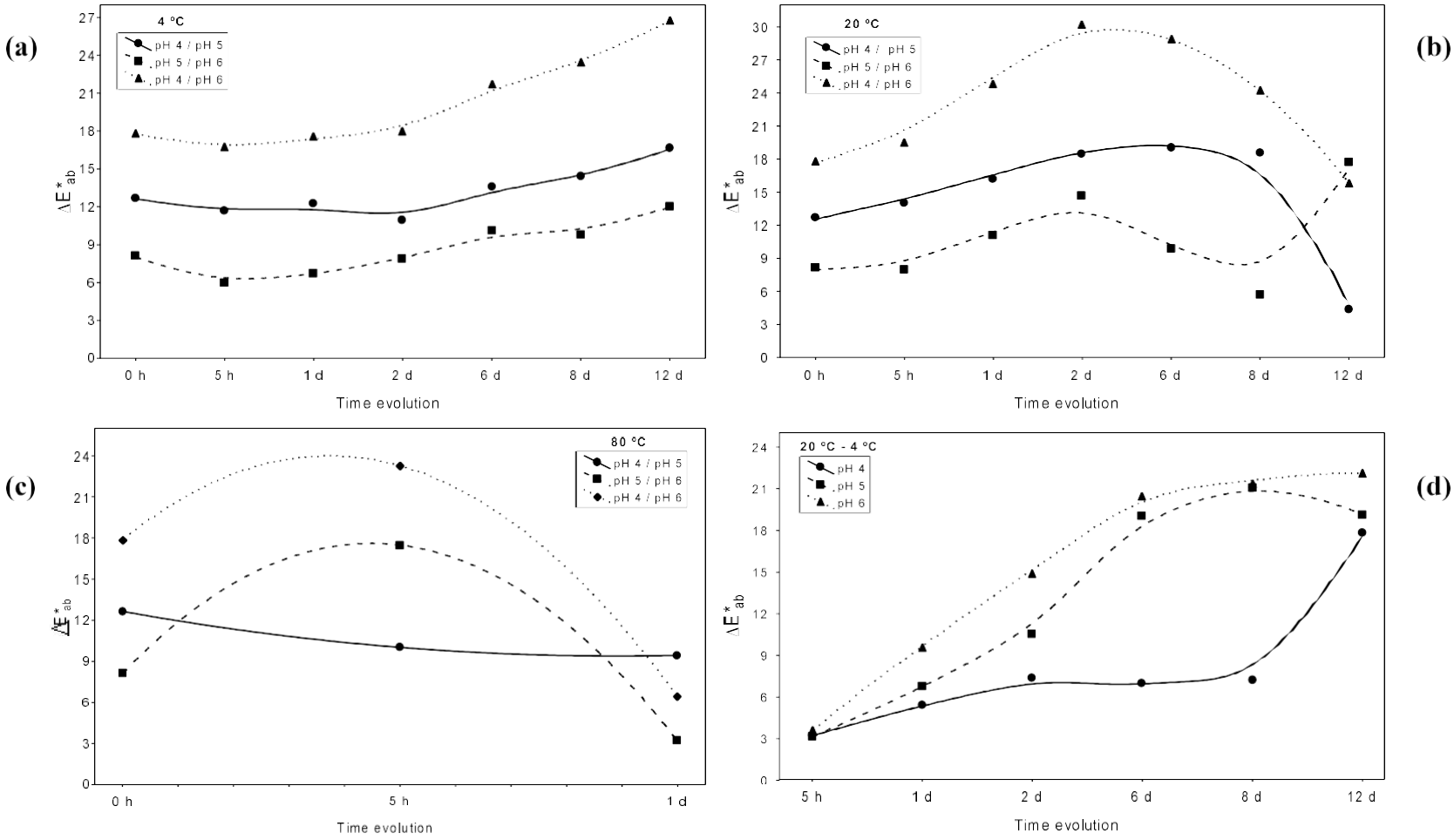

Figure 3.

Figure 4

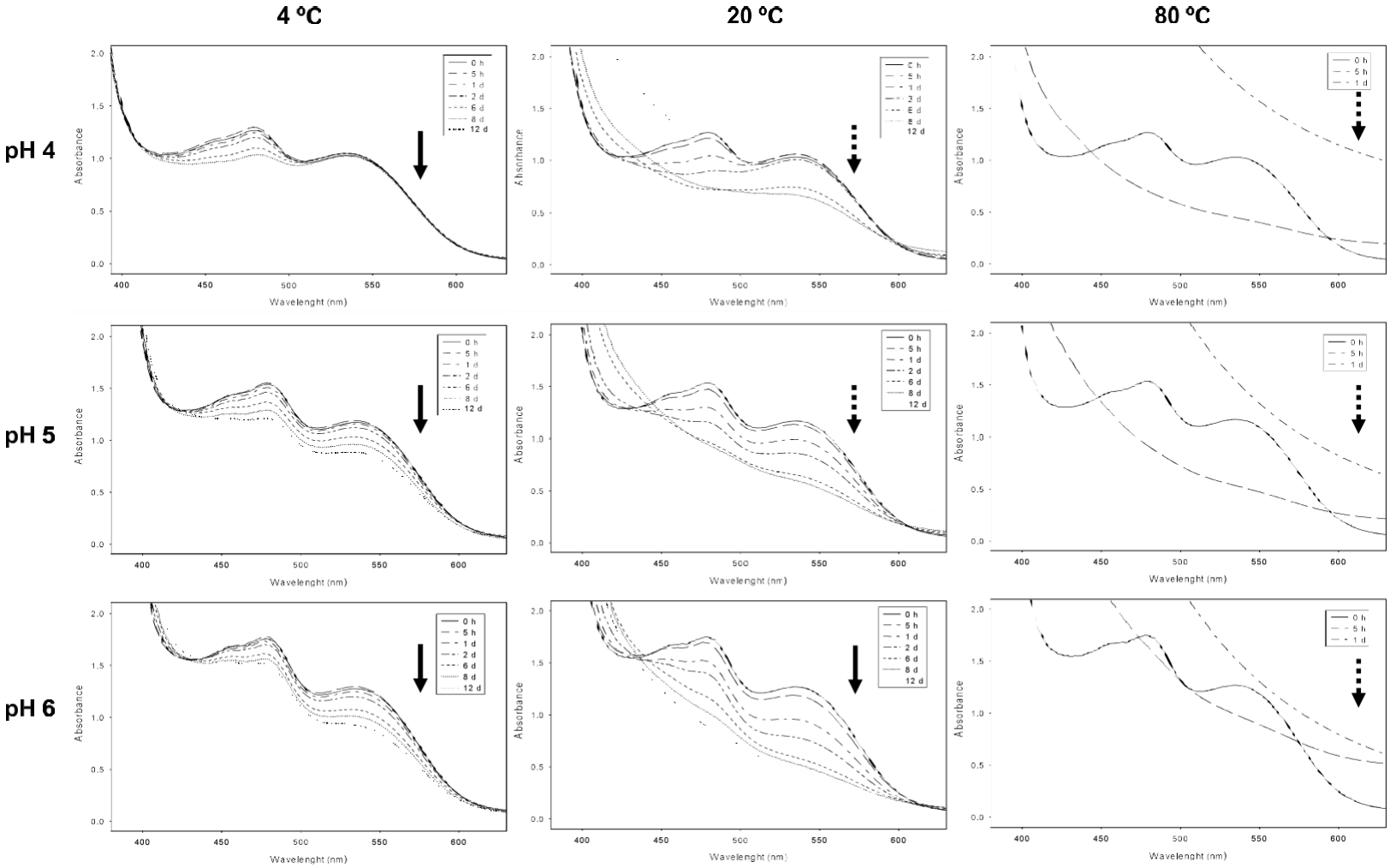

Figure 4.

Figure 5

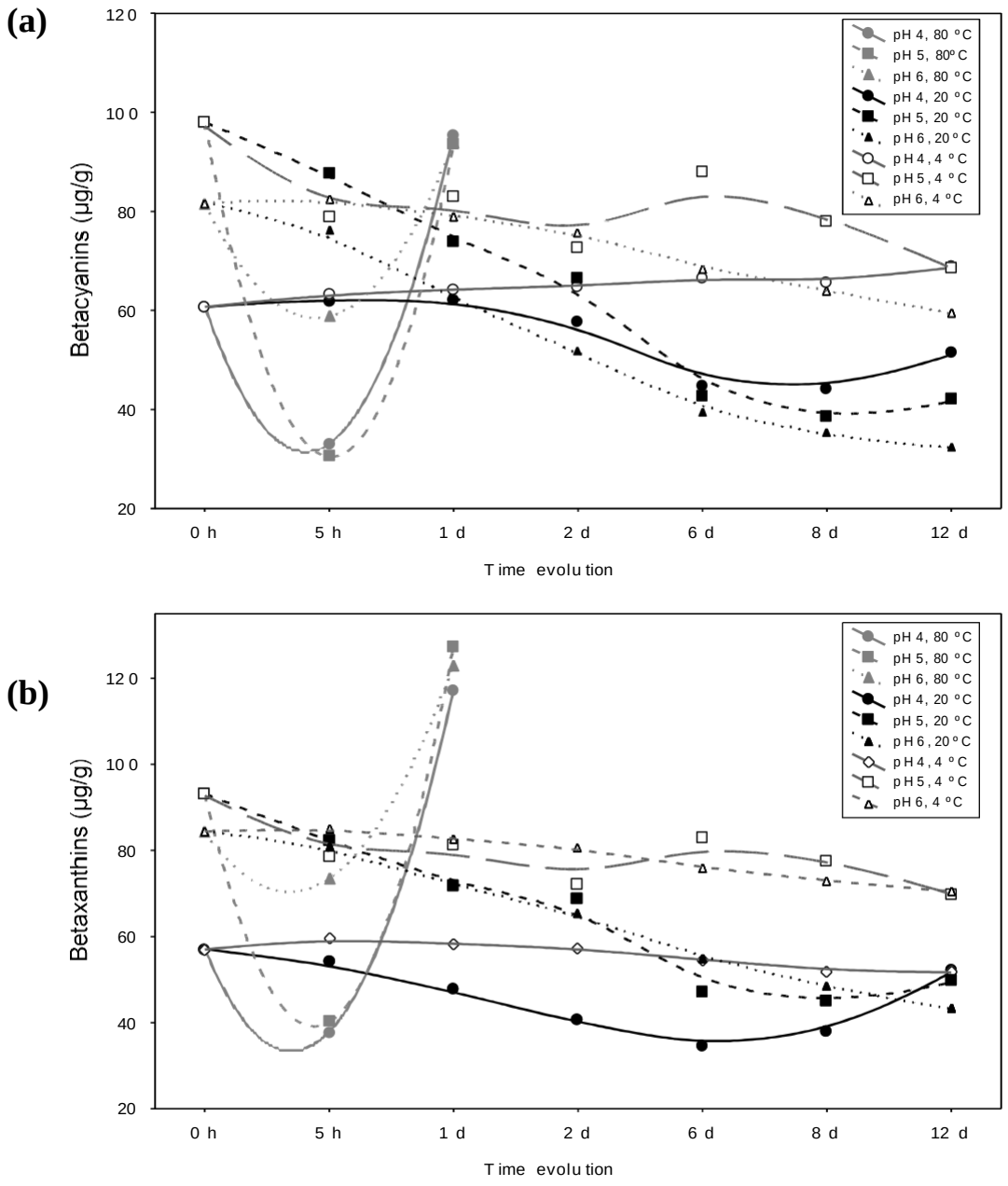

Figure 5.