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Comparison of monomeric anthocyanins and colour stability of fresh, concentrate and freeze-dried encapsulated cherry juice stored at 38°C

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Abstract.
BACKGROUND: Anthocyanins are natural colorants and bioactive compounds widely distributed among vegetables and fruits. Unfortunately, in fruit juices stored at room temperature monomeric anthocyanins easily convert to colourless compounds and subsequently to insoluble brown pigments.
OBJECTIVE: The object of present study is to compare the stability of monomeric anthocyanins and colour changes during storage temperature at 38°C of pasteurized cherry juice, concentrate and freeze-dried juice.
METHODS: Colour changes of, pasteurized cherry juice (18.7 ºBrix), concentrate (61 ºBrix) and juice freeze-dried with addition of maltodextrin and arabic gum as encapsulating agents, were evaluated by CIELab parameters, a^*, b^* and, L^*. Anthocyanins content was measured by pH-differential method and total phenolics were determined by Folin-Ciocalteu method. The glass transition temperature (T_g) of the freeze-dried matrix was determined.
RESULTS: It was found the stability of monomeric anthocyanins and colour retention during storage at 38ºC was significantly superior in the freeze-dried encapsulated juice powder than in liquid cherry juices.
CONCLUSIONS: This is attributed to the protective encapsulation of the anthocyanins in the amorphous matrix of maltodextrin/arabic gum of reduced water activity.

Keywords: Anthocyanins, colour, stability, cherry juice, concentrate, freeze-dried, glass transition

1. Introduction

Anthocyanins are bioactive compounds present in many fruits, vegetables and their products. They are natural colorants widely distributed in fruit and vegetables; notably cherries and berries [1]. In addition, anthocyanins have multiple biological roles, e.g. antioxidant activity, anti-inflammatory action, inhibition of blood platelet aggregation and antimicrobial activity, and prevention of cholesterol-induced atherosclerosis [1, 2].

Anthocyanins are the basis for the red, blue and purple colours of fruits, vegetables and their products. Unfortunately, under normal processing and storage at room temperature monomeric anthocyanins transform relatively easy to colourless compounds and subsequently to insoluble brown pigments [3]. Since anthocyanins impart a characteristic

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colour to fruits and vegetables they have a considerable impact on consumer sensory acceptance due to perception of inferior product quality [1].

Several studies indicate anthocyanin stability is not merely a function of temperature but is also influenced by intrinsic properties of the product and process conditions. Patras et al. [4] reviewed some important aspects of anthocyanin degradation during thermal processing; conclusions regarding the mechanisms and kinetics of anthocyanin degradation during heat treatment were postulated.

Cherry juice is a good source of anthocyanins, but its stability during heat processing or accelerated storage temperature suffers from the same drawbacks as those from other fruit juices [5]. Arslan [6] studied the effects of degradation preventive agents on storage stability of anthocyanins in sour cherry concentrate. It was concluded the duration and temperature of storage influence anthocyanin stability in cherry juice.

The appearance of a food product is one of the most important quality characteristics for consumer acceptance. As for red fruit–based products such as cherry juice, attractive colour should remain relatively constant throughout shelf life [1]. However, as mentioned above colour stability of cherry juice stored at ambient temperature is a difficult task because of rapid anthocyanin degradation; even at refrigeration temperature of about 4ºC [6].

The aim of this research was to study the stability of monomeric anthocyanins and color changes during 38ºC storage of fresh (18.7 ºBrix), concentrate (61 ºBrix) and freeze-dried encapsulated pasteurized cherry juice, to determine the most satisfactory procedure to improve anthocyanin and red colour retention.Temperature of 38°C was selected because it is usually recommended for accelerated shelf life studies of foods to be marketed at ambient temperature [7].

2. Materials and methods

2.1. Reagents

Maltodextrin Dextrose Equivalent 10 (MD_{10}) from Productos de Maíz S.A., Buenos Aires, Argentina and Arabic Gum from Gelfix S.A., Buenos Aires, Argentina, were used for encapsulation of freeze dried cherry juice.

Ethanol and chlorhydric acid used as solvents for juice extraction were from Biopack, Buenos Aires, Argentina.

Folin–Ciocalteu reagent was purchased from Merck KgaA Darmstadt, Germany. Gallic acid used for phenolic standard curve was obtained from Anedra, Buenos Aires, Argentina.

2.2. Samples

Lapins Dark cherry fruit from Tunuyan, Mendoza (Argentina), was provided by Rio Alara S.A. The fruit was frozen at –20°C until processing.

2.2.1. Juice

Frozen cherries were thawed in a water bath at 20°C, blanched (87°C, 3 min), destemmed and pitted, and processed in a blender with mesh to obtain the juice. Ascorbic (0.25 g/kg) and citric (4 g/kg) acids were then added; the juice was fractionated and sealed in hermetic plastic bags 50 g each, pasteurized (90°C, 5 min), cooled and stored until used for the study.

2.2.2. Concentrate

A fraction of pasteurized juice described above (18.7 °Brix) was evaporated at 40°C in vacuum at 25 mbar in a Laborota Heidolf (Schwabach, Germany) rotary evaporator. The product obtained (61 °Brix) was re-pasteurized, fractionated in sterile and hermetic flasks 10 g each and used for storage studies. This low temperature concentration did not alter anthocyanins content of juice.

2.2.3. Freeze drying and encapsulation procedure

Pasteurized cherry juice (18.7 ºBrix) was mixed with Maltodextrin DE_{10} and Arabic Gum (80:20) at a ratio of 25% of encapsulating agents, poured onto an aluminium tray and frozen at –20°C during 24 h. Then it was freeze

dried at room temperature ($22 \pm 3^{\circ}C$) in a FIC-LI-I-E300-CRT freeze dryer (Buenos Aires, Argentina) operated with a freezing plate and condenser at –40°C and a vacuum of 100 μm Hg during 40 h. Freeze drying did not alter monomeric anthocyanin content of cherry juice. The freeze-dried amorphous carbohydrate microstructure was milled to obtain a powder which was fractionated in sterile, hermetic flasks (5 g) and stored.

2.3. Storage conditions

Samples were stored in darkness at 38°C for 33 days (fresh juice) and for 60 days (concentrate and freeze-dried); two samples of juice, concentrate and encapsulated freeze dried juice were removed at selected times to take measurements.

2.4. Juice extract

Five grams of juice were extracted twice in 20 ml ethanol: HCl 0.1 N (85:15). The pellets with no detectable residual content of phenolics, were eliminated by centrifugation and the supernatants were mixed and utilized for measurements of total phenolics, monomeric anthocyanins and colour. Samples of concentrate and freeze dried juice were previously reconstituted with water to their original weights.

2.5. Methods

2.5.1. Physicochemical properties

Total soluble solids content was evaluated with a manual refractometer Atago N2 (Tokyo, Japan). pH was measured at 25°C using a Hanna HI 8424 instrument (Hanna Instruments Inc., Woonsocket, RI, USA).

Water activity was determined using an electronic dew-point water activity meter Aqualab TE (Decagon Devices, Pullman, WA). The equipment was calibrated with saturated salt solutions in the water activity range of interest [8].

Moisture content was performed on 1.5 g of powder in an oven at 90°C up to constant weight.

Glass transition temperature (T_g) of cherry juice freeze dried with maltodextrin + arabic gum was determined by differential scanning calorimetry (DSC) using a DSC822e 104 Mettler Toledo calorimeter (Schwerzenbach, Switzerland). The instrument was calibrated with indium (156.6°C), lead (327.5°C) and zinc (419.6°C). Masurements were performed at a heating rate of 10°C / min. Hermetically sealed 40 μL medium pressure pans were used, (an empty pan served as a reference). Thermograms were then evaluated using Mettler Stare program.

2.5.2. Total phenolics

Total phenolics (TP) were determined on the juice extract using the Folin– Ciocalteu method according to Waterhouse [9]. Sample absorbance at 765 nm (PG Instruments T60U UV-Vis spectrophotometer, Leicestershire, United Kingdom) was measured, and phenolic concentrations were expressed as gallic acid equivalent (GAE) in mg/100 g of product, calculated by means of a standard curve of gallic acid.

2.5.3. Monomeric anthocyanin content

Monomeric anthocyanin content of juice extracts was determined by pH differential method [10]. Absorbances were read at 510 and 700 nm, and its content was calculated as cyanidin-3-glucoside in mg/100 g of product (MW: 449.2 g mol^{-1} and ε: 26 900 L $cm^{-1}mol^{-1}$). Monomeric anthocyanin retention (%) was relative to the initial content considered as 100 %.

2.5.4. Colour measurements

Cherry juice color was analyzed using a Minolta Spectrophotometer CM-600d (Konica Minolta Observer), with D65 illuminant and an observer angle of 2°. The colour measurement was obtained by placing 0.4 g of juice or reconstituted juice for concentrate and freeze dried samples, in plastic white containers. CIELab parameters (CIE 1976 L^* a^* b^*) were L^* for lightness, a^* for redness and b^* for yellowness. Calculations of C^* $((a^{*2} + b^{*2})^{1/2})$ for chroma and h°(arctan b^*/a^*) for hue angle were made. Total colour difference was calculated as

$\Delta E^* = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2\ ]^{½}$ and expressed the magnitude of difference between 0 and 33 days of juice storage. The instrument was standardized with a white tile ($L^* = 91.10$, $a^* = 1.12$ and $b^* = 1.26$).

2.6. Data analysis

Replicate bottles of each juice product were analyzed at indicated time of storage. All the parameters studied were determined at least by duplicate, and the average was reported. Colour parameters during storing were analyzed applying one-way ANOVA and Student-Newman-Keuls test for multiple means comparisons, using Infostat v.2009 (Universidad Nacional de Córdoba, Argentina). Pearson's correlations between colour parameter a^* and monomeric anthocyanin content were made.

3. Results and discussion

Table 1 shows physicochemical characteristics of fresh (18.7 ºBrix), concentrate (61 ºBrix) and freeze-dried encapsulated cherry juice ($a_w = 0.10$ and 3.8 ± 0.1 % moisture content). Total phenolics and anthocyanin contents in fresh juice are within range reported in literature [11].

Figure 1 compares monomeric anthocyanin stability in the liquid juices (fresh and 61 ºBrix concentrate) and in the encapsulated cherry juice powder during storage at 38ºC. It can be seen that in fresh and concentrate juices anthocyanin retention decreases rapidly during storage while in the freeze dried powder is much more stable. At 33 days of storage the % anthocyanin retention in the liquid juices (both fresh and concentrate) fell to 11% while it remained at around 90% in the powder.

The poor stability of anthocyanins in the liquid juices stored at 38ºC is in agreement with literature data, as reviewed in Table 2 which shows the half-life of monomeric anthocyanin degradation ($t_{1/2}$ = time to reduce concentration to 50 % of its initial value) in various fruit juices (mainly berries) stored at near 38ºC. Half-life times ranged between 2.1 days to 31.5 days highlighting the limited stability of monomeric anthocyanins [12–20].

Table 1
Physico-chemical characteristics of cherry juices used in this study

	Total soluble solids (°Brix)	pH	a_w	Total phenolics content (mg/100 g)	Monomeric anthocyanins (mg/100 g)
Fresh juice	18.7 ± 0.1	3.71 ± 0.01	0.973 ± 0.001	159 ± 11	23.5 ± 0.2
Juice Concentrate	61.0 ± 0.1	3.90 ± 0.01	0.824 ± 0.001	459 ± 20	86.2 ± 2.9
Freeze-dried encapsulated juice			0.100 ± 0.001	392 ± 30	67.5 ± 4.0

Values are means ± standard errors.

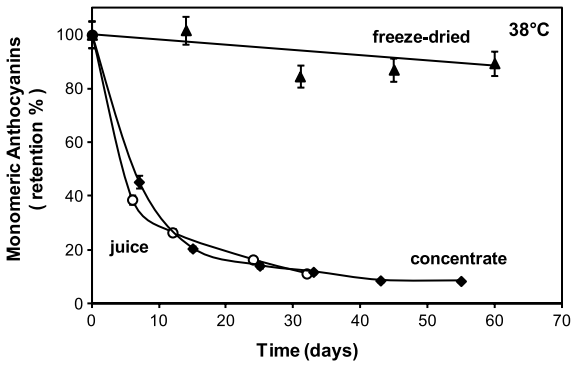

Fig. 1. Comparison of monomeric anthocyanins retention in: ▲ freeze dried encapsulated ($a_w = 0.10$), ♦ concentrate (61 °Brix) and ○ fresh (18.7 °Brix) cherry juices stored at 38°C.

Table 2
Half-life of monomeric anthocyanin degradation ($t_{1/2}$) in various (fresh and concentrate) fruit juices (mostly berries) stored at near 38ºC

Fruit juice	°Brix	pH	Storage Temperature (°C)	Previous thermal treatment	t ½ (days)	Reference
Elderberry	36.8	3.6	40	Pasteurized at 80°C	27.7	Busso Casati et al., 2015
Pomegranate	13.7	3.2	37	Pasteurized at 93°C	25.4	Alighourchi et al, 2009
Bloor orange	45	3.4	37	Concentrated at 80°C in Rotavapor	2.1	Kirca et al., 2003
Bloor orange	69	3.4	37	Concentrated at 80°C in Rotavapor	3.1	Kirca et al., 2003
Black carrot in various juices	9.9–26.2	3.0–3.9	37	Pasteurized at 85 °C	12–16	Kirca et al., 2006
Black carrot	30	4.3	37	Pasteurized at 85°C	28.7	Kirca et al., 2007
Black carrot	45	4.3	37	Pasteurized at 85°C	31.5	Kirca et al., 2007
Black carrot	64	4.3	37	Pasteurized at 85°C	28	Kirca et al., 2007
Blackberry	8.9	2.9	37	Pasteurized at 85°C	11.7	Wang and Xu, 2007
Blackberry	65	2.9	37	Pasteurized at 85°C	9.4	Wang and Xu, 2007
Sour cherry	45	3.2	37	Pasteurized	14	Cemeroglu et al., 1994
Sour cherry	71	3.1	37	Pasteurized	11	Cemeroglu et al., 1994
Agraz (*Vaccinium meridionale sw)*	2.5	3	37	Pasteurized at 85°C	4.9	Martínez Zambrano et al., 2011
Agraz (*Vaccinium meridionale sw.)*	19.5	2.6	37	Pasteurized at 85°C	21	Martínez Zambrano et al., 2011
Model blackcurrant juice	11	3.4	40	Fruits blanched in boiling water 2 min	9.4	Harbourne et al., 2008

Cherry juice was encapsulated by freeze drying with a mixture of encapsulating agents (maltodextrin and arabic gum) to protect anthocyanins in an amorphous glassy matrix [21, 22]. Cherry juice has a high monosaccharides content; main sugars are fructose and glucose (with small amounts of sucrose). It is well known these sugars (mainly fructose) have very low glass transition temperatures, T_g [23]. Thus, one may expect structural collapse in the amorphous structure if cherry juice is attempted to freeze dried alone. For this reason encapsulating agents (maltodextrin and arabic gum) were used to increase the resulting glass transition temperature; thus improving its physical properties [24].

Figure 2 shows the DSC thermogram for encapsulated cherry juice at a water activity of 0.10; the onset, midpoint and endpoint glass transition temperature values are indicated. Although a glass transition seems to be apparent in the thermogram, the witdth of the glass transition (difference between the end and onset values) is large (over 20ºC) suggesting that in this case T_g is actually a transition region, rather than a specific temperature. Since the convention is to report a single temperature, the midpoint glass transition temperature (35.5ºC) was taken as most representative of the thermal transition in freeze-dried encapsulated cherry juice [25]. Since the midpoint glass transition temperature (35.5ºC) is close to the storage temperature (38ºC) one may explain the stability of anthocyanins (Fig. 1) due to the existence of a *cuasi* glassy state. It is known that physical changes in an amorphous matrix are time dependent being a function of (T-Tg), where T is the storage temperature. In present conditions this difference (38 – 35.5ºC) is very small and the system would behave as in the glassy state, at least for the time-scale of present work (60 days) [24]. As observed in Fig. 3 visual examination of the dry cherry powder after 60 days storage at 38ºC revealed absence of collapse/caking therefore confirming a glassy-like behavior.

Laine et al. [26] reported that a polyphenol-rich raspberry extract was stabilized by freeze-drying with maltodextrins as material coating, and found the freeze-dried particles were stable over long periods providing to polyphenols an effective protection against the oxidation phenomenon during their storage. Estupiñan et al. [27] found that addition of maltodextrin DE_{20} improved the color and stability of antioxidants in freeze-dried powders from Andes berry

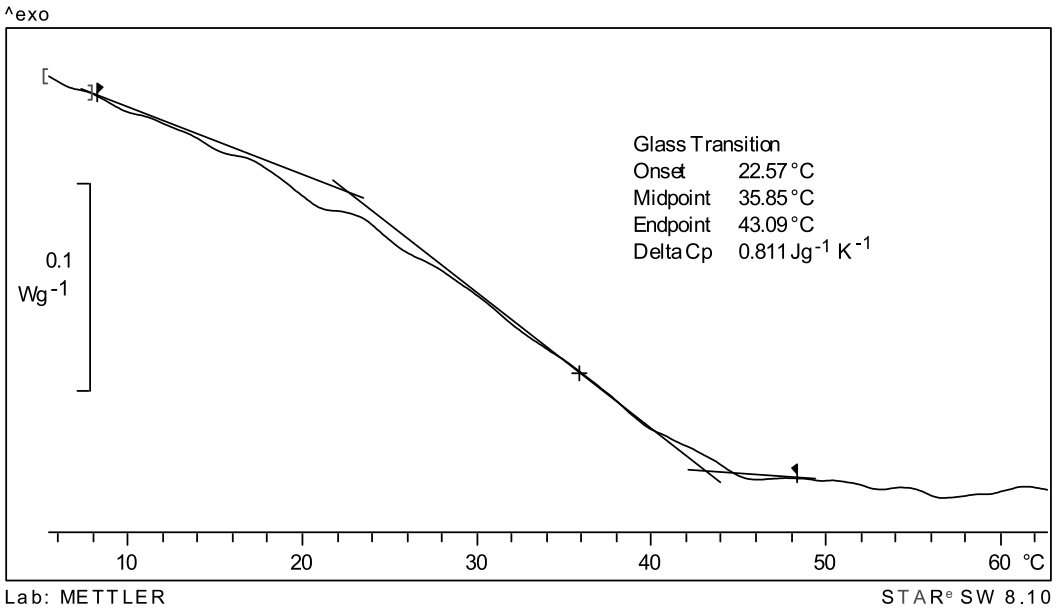

Fig. 2. DSC thermogram for cherry juice freeze-dried encapsulated in a maltodextrin/arabic gum matrix of $a_w = 0.10$.

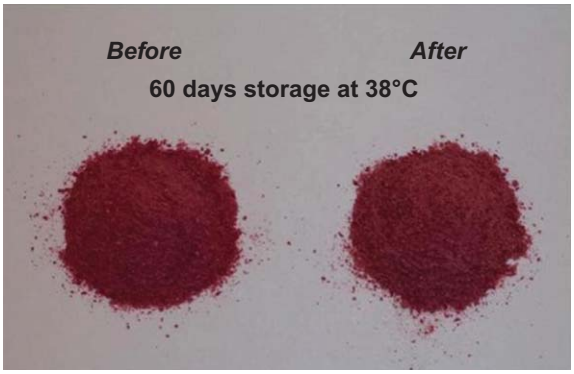

Fig. 3. Encapsulated cherry juice powder ($a_w = 0.10$) before and after 60 days storage at 38ºC – *left side* : before storage; *right side* : after storage.

during storage. Osorio et al. [28] also reported that the stability of anthocyanins was enhanced by spray drying encapsulation in maltodextrin and/or arabic gum matrixes.

The attractive red colour of cherry juice plays such a vital role in consumer sensory acceptance; therefore, it is highly desirable it should remain relatively constant throughout shelf life. Figure 4 shows colour parameter a* (redness) as a function of storage time for liquid juices (fresh and concentrate) and encapsulated juice of low water activity. It can be seen that parameter a* decreases rapidly from the beginning of storage for both liquid cherry juices but remained more or less constant for the encapsulated cherry juice. These results resemble those showed in previous Fig. 2, which compared the monomeric anthocyanin stability of liquid juices (regular and 61 ºBrix concentrate) and encapsulated juice, suggesting that the loss of anthocyanins is associated to loss of characteristic red colour. For fresh and concentrate juices a high correlation between parameter a* and anthocyanin content during storage was found, being $R = 0.94$ and 0.95 respectively ($P < 0.05$).

Figure 5 shows the changes in all colour parameters: a* (redness), b* (yellowness), L* (lightness), as well as calculated purity (C*) and hue angle (h°) values, for liquid juices and encapsulated juice before and after 33 days

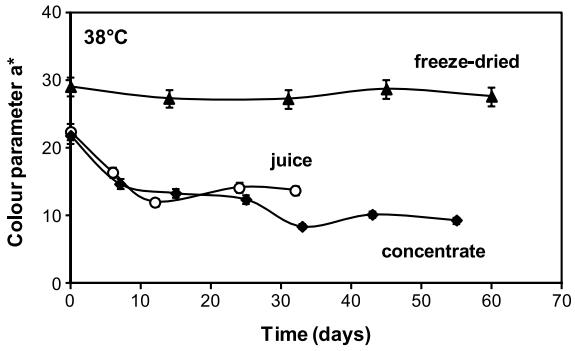

Fig. 4. Comparison of a* (redness) colour parameter of: ▲ freeze dried encapsulated ($a_w = 0.10$), ♦ concentrate (61 °Brix) and ○ fresh (18.7 °Brix) cherry juices stored at 38°C.

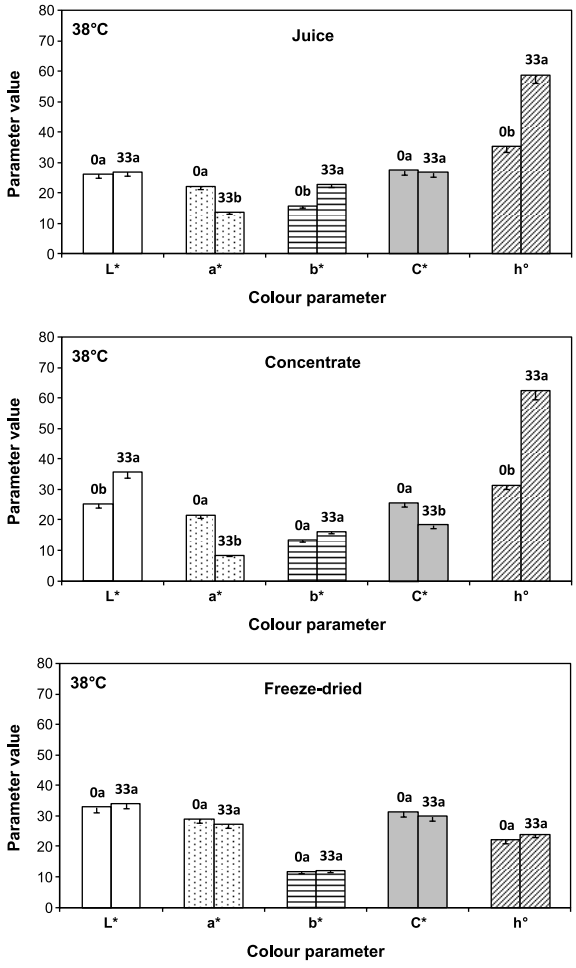

Fig. 5. Comparison of all colour parameters (L*, a*, b*, C*, h°) in freeze dried encapsulated, concentrate (61 ºBx) and fresh (18.7 ºBx) cherry juice at 0 and 33 days stored at 38°C. Different letters above the data bars indicate that colour parameter differed between storage time, $P < 0.05$, Student-Newman-Keuls test.

storage at 38ºC. Overall, it can be observed that colour parameters experimented significant modifications in the liquid juices (18.7 and 61 ºBrix) after 33 days at 38ºC, with decreased a* and increased b* resulting in a higher tone (h°) value and in a brownish juice. For the encapsulated juice the changes were much less important. Total colour difference (ΔE^*) values were 11.2 ± 0.1, 18.6 ± 0.5 and 2.2 ± 0.3 for fresh, concentrate and freeze dried juice respectively, indicating more noticeable visual changes in colour during storage in liquid systems; these changes being more relevant for concentrate juice. Particularly in this case significant increase of lightness (L^*) and decline of saturation (C^*) were observed; and this may be attributed to non-enzymatic browning reactions which are known to proceed faster at intermediate water activity [29]. Changes in L^* and C^* values produce fading of colour that could reflect decolourization of anthocyanins and a shift from vivid to a duller colour respectively. Similar results were obtained in strawberry puree storing 8 weeks at 25°C as a consequence of processing and storage [30].

4. Conclusions

In addition to contributing to bioactive properties, attractive colour is one of the most important sensory characteristics of cherry juice. However, in pasteurized liquid juices (fresh and concentrate) both anthocyanin content and red colour are unstable and susceptible to degradation during storage at 38ºC. On the contrary, the encapsulated cherry juice powder of low water activity, exhibited a good stability of both anthocyanins and colour parameters.

Maintaining anthocyanin content as well as a stable red colour in cherry juice during storage at room temperature is problematic; thus juice encapsulation in a dried matrix of low water activity could be used as a strategy for product stabilization.

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