
**STABILITY OF ANTHOCYANINS FROM *Rubus glaucus* Benth
AND *Solanum betaceum* Cav. dark-red strain AS AFFECTED
BY TEMPERATURE, STORAGE TIME AND WATER ACTIVITY**

**Efecto de la temperatura, almacenamiento y la actividad de agua
sobre la estabilidad de antocianinas de *Rubus glaucus* Benth
y *Solanum betaceum* Cav. dark-red strain**

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ABSTRACT

The stability of sprayed-dried microencapsulated anthocyanins from Andes berry (*Rubus glaucus*) and Tamarillo (*Solanum betaceum*), as affected by storage time, water activity (A_w) and temperature was compared. The fruits were osmotically dehydrated with ethanol and the anthocyanin extract was microencapsulated with maltodextrin DE 20 by spray drying. Half life of the anthocyanins; changes in color, total phenolics, and antioxidant activity of the powders, were analyzed during storage at two different temperatures (25 °C and 40 °C) and two A_w levels (0.20 and 0.35). A decrease in monomeric anthocyanin was observed in both samples. The half life of the Andes berry pigments ranged between 11 and 32 days while the half life of the tamarillo pigments ranged between 9 and 21 days. A darkening effect occurred in both samples as a result of storage time. The antioxidant activity decreased while the phenolic content increased with time. Antioxidant activity of Andes berry samples was highly correlated with anthocyanin content and total phenolic content while the antioxidant activity of tamarillo samples was highly correlated with total phenolic content. These results would be useful in developing applications for spray-dried anthocyanins as powdered food-grade colorants.

Key words: Microencapsulation, anthocyanin, stability, antioxidant activity.

RESUMEN

Se comparó el efecto del tiempo de almacenamiento, la temperatura y la actividad de agua (A_w) sobre la estabilidad de antocianinas microencapsuladas de Mora de Castilla (*Rubus glaucus*) y tamarillo (*Solanum betaceum*). Las frutas se sometieron a deshidratación

osmótica con etanol y el extracto antocianínico se microencapsuló con maltodextrina ED 20 por atomización. La vida media de las antocianinas; los cambios en color, fenoles totales y actividad antioxidante se analizaron durante el almacenamiento a dos temperaturas (25 °C y 40 °C) y dos niveles de Aw (0,20 y 0,35). Se observó una disminución de la antocianina monomérica en las dos muestras. La vida media de los pigmentos de mora de Castilla varió entre 11 y 32 días, mientras que la vida media de los pigmentos del tamarillo varió entre 9 y 21 días. Hubo oscurecimiento de las muestras como resultado del tiempo de almacenamiento. La actividad antioxidante de las dos muestras disminuyó, mientras que el contenido fenólico aumentó con el tiempo. La actividad antioxidante de las muestras de mora de Castilla presentó una alta correlación con los contenidos de antocianinas y fenoles totales mientras que la actividad antioxidante de las muestras de tamarillo se correlacionó con el contenido de fenoles totales. Estos resultados son útiles en el desarrollo de aplicaciones de antocianinas microencapsuladas como colorantes alimenticios.

Palabras clave: microencapsulación, antocianinas, estabilidad, actividad antioxidante.

INTRODUCTION

Color is an important quality factor when choosing a food product; for this reason, there is a high demand of colorants by the food industry. However, research has associated the consumption of synthetic food colorants with conditions such increasing levels of hyperactivity in children (McCann *et ál.*, 2007). As a result, natural plant colorants have been in high demand for the past decade with the purpose of replacing synthetic dyes such as FD&C red 40 and the banned FD&C red No 2. (Fabre *et ál.*, 1993).

Anthocyanins (ACNs) are highly colored plant pigments with high potential as natural colorants (Wrolstad, 2000). Besides the advantages ACNs present as food colorants, they are associated with health benefits such reduced risk of coronary heart disease, reduced risk of stroke, anticarcinogenic activity, anti-inflammatory effects, improved visual acuity, and improved cognitive behavior (Clifford, 2000; Prior, 2004). Nevertheless, limitations to the application of ACNs as natural colorants at a commercial scale are their low stability to processing, formulation and storage conditions (Cevallos-Casals and Cisneros-Zevallos, 2004) and the low availability of vegetable sources in seasonal locations. Concentration, temperature, pH, oxygen, enzymes, metal ions, ascorbic acid, and small changes in Aw contribute to destruction of the pigments (Mazza and Miniati, 1993; Garzón and Wrolstad, 2001). Microencapsulation of pigments with maltodextrins by spray drying is a technique currently investigated in the food industry with the aim to increase the shelf life of pigments (Dziezak, 1988; Dib Taxi *et ál.*, 2003; Ersus and Yurdagel, 2007). This technique protects food pigments from destructive changes and allows controlled release of the substances under specific conditions.

Andes berry (*Rubus glaucus* Benth), and tamarillo (*Solanum betaceum* Cav. dark-red strain) are fruits native to high tropical areas such as the country of Colombia, where they are harvested throughout the year. Both fruits are promising sources of natural dyes and antioxidants due to their high ACN content. Cyanidin 3-rutinoside (Cy-3-rut) is the major ACN in Andes berry (Garzón, 2009) while delphinidin 3-rutinoside (Dp-3-rut) is

the main ACN in tamarillo (Vera de Rosso and Mercadante, 2007).

During osmotic dehydration of Andes berry and tamarillo for production of healthy snacks, there is an important transfer of ACNs from the fruits to the osmotic solutions (Osorio *et al.*, 2007). As the solutions have been discarded so far, microencapsulation may be useful for utilization of this waste material and for obtaining ACNs as potential natural colorants. Accordingly, the main objective of this work was to find a potential added value for what currently constitutes a waste material. Andes berry and tamarillo ACNs were microencapsulated with maltodextrins by sprayed drying and their stability under two levels of water activity and temperature was compared. Further objectives of this work were to monitor changes in antioxidant activity and total phenolic content of the microencapsulated pigments and to determine the correlation between antioxidant activity and ACN concentration or phenolic content and during storage.

MATERIALS AND METHODS

REAGENTS

Sodium hydroxide, hydrochloric acid, sodium carbonate, calcium chloride, potassium persulfate, and Folin-Ciocalteu reagent were purchased from Merck® (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and gallic acid were from Sigma-Aldrich (St. Louis, MO). Sodium metabisulphite was acquired from Carlo Erba® (Rodano, Italia) and corn maltodextrin with a dextrose equivalent (ED) of 20 was purchased from Corn Products, Brazil.

SOURCE OF ANTHOCYANIN PIGMENTS

Fresh Andes berries and tamarillo were acquired from the local market in Bogotá, Colombia. The fruits were characterized according to color, size, soluble solids content (°Brix) using a digital refractometer Abbe II (Reichert-Jung, Leica Inc., Buffalo, N.Y., U.S.A.), and total titratable acidity (TA). TA was determined as g citric acid per 100 g fruit by titrating a sample of fruit extract with 0.1 N NaOH to a final pH of 8.2. Measurement of the pH was done with a Schott Geräte® pH meter, model CG820 (Mainz, Germany).

Monomeric ACN content was determined by the pH differential method (Giusti and Wrolstad, 2001) and expressed as mg of Cy-3-glu (molecular weight: 445.2 g/mol, ϵ : 26900) per 100 g of Andes berry and as mg of Dp-3-rut (molecular weight: 611.2g/mol, ϵ : 28800) per 100 g of tamarillo.

After characterization, the fruits were subjected to osmotic dehydration by mixing fruit and 96% ethanol in a 1:3 proportion in a sealed polyethylene to allow pigment extraction for 12 hours (Osorio *et al.*, 2007). Three consecutive cycles were applied by replacing the fruit.

PREPARATION OF MICROENCAPSULATED PIGMENT POWDERS BY SPRAY-DRYING

The ACNs obtained after ethanolic extractions were recovered and the ethanol was removed at 40 °C by using a Büchi rotoevaporator. Each pigment concentrate was mixed with corn maltodextrin ED 20 until reaching a 65 °Brix final solid content. The resultant

slurries were spray-dried in a rotary spray dryer Mobile Minor™ (Niro Inc. Columbia, MD, USA) operated at 230 °C inlet temperature and 150 °C outlet temperature maintaining a feed flow of 10 mL/min. The moisture content of the microencapsulated pigments was determined on an Ohaus water analyzer, model MB (New Jersey, USA) and the A_w was measured with a Novasina mj1 water activity analyzer (Switzerland).

SCANNING ELECTRON MICROSCOPY (SEM)

The morphology of the microcapsules was evaluated by scanning electronic microscopy (SEM) using a QUANTA 200 FEI, (Ontario, Canada) electronic microscope. The microcapsules were attached to SEM stubs using a sputter Balzers SDC-050 and coated with gold-palladium (Plasma deposition method) and examined at 30 kV and 4 Torr.

STORAGE OF MICROENCAPSULATED PIGMENTS

Microencapsulated pigment (1.5 g) was evenly distributed on an open plastic Petri dish. To control A_w , the samples were placed on sealed desiccators containing saturated CH_3COONa and CaCl_2 solutions to obtain A_w levels of 0.20 and 0.35, respectively (Labuza, 1984). The desiccators were placed in separate incubators model HM-19 (Jaelsa, Madrid, Spain) at 25 ± 1 °C and 40 ± 1 °C for 35 days in the dark. The samples were allowed to equilibrate with the constant humidity environment before taking the first measurement. Afterwards, samples were retrieved at 7, 14, 21 and 35 days for analysis. At the same time, control samples were stored in close plastic Petri dishes at -14 °C in the dark.

COLOR EVALUATION

CIE $L^* a^* b^*$ values of the powders were measured directly in the Petri dishes using a Minolta CM 508I colorimeter (Osaka, Japan). The operating conditions were: reflectance mode, specular component included, illuminant D_{65} and a 10° observer angle. Hue angle (h°) was calculated as $\arctan b^*/a^*$, and chroma or color intensity (C^*) as $(a^{*2} + b^{*2})^{1/2}$.

CHANGES IN ANTHOCYANIN CONTENT, PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY

To determine changes of the microencapsulated samples, 500 mg of powder were resolubilized in 0.01% HCl, stirred for 10 min and taken to a 25-mL final volume. All analyses were done on this stock solution.

ACN content was determined as described. Determination of color density ($A_{420\text{ nm}} - A_{700\text{ nm}}$) + ($A_{520\text{ nm}} - A_{700\text{ nm}}$) and % polymeric color [(polymeric color/color density)] X 100 was done (Giusti and Wrolstad, 2001). Results are reported on a 100 g of microencapsulated pigment basis.

The antioxidant activity was measured by the Trolox Equivalent Antioxidant Capacity (TEAC) assay activity (Re *et al.*, 1999). Absorbance of the samples at 734 nm was compared to that one of Trolox standards and the results were expressed in terms of mmoles Trolox equivalents (TE)/100 g powder.

Total phenolic content was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Absorbance at 765 nm of the samples and standards was determined. Results are expressed as mg gallic acid equivalents (GAE) /100 g powder.

STATISTICAL ANALYSIS

The experiment was carried out in duplicate. Multifactor analysis of variance (ANOVA) was applied for each microencapsulated pigment. Significant ($P < 0.05$) differences between means were identified using the least significant difference procedure (LSD). Linear regression analysis was applied to determine adequacy of the model describing kinetics of ACN degradation. Rate constants were calculated from slopes of the lines plotted, and half lives ($t_{1/2}$) were calculated from the equation: $t_{1/2} = \ln 0.5/k$, where k = rate constant.

Significant correlations between antioxidant activity and ACN concentration or total phenolic content were calculated by simple linear regression analysis. All analyses were performed with Statgraphics plus, version 2.1.

RESULTS

CHARACTERISTICS OF THE FRUIT

The compositional data of the fresh fruit is reported in table 1. Fresh Andes berry had a monomeric ACN content of 14.7 ± 0.89 mg/100 g fresh fruit, $15.2 \% \pm 0.02$ soluble solids and a pH of 4.59 ± 0.01 . Monomeric ACN content of tamarillo was 7.60 ± 1.23 mg/100 g fresh fruit. This fruit had $9.82 \% \pm 0.02$ soluble solids and a pH of 3.88 ± 0.01 .

Parameter	Andes berry	Tamarillo
² Titrateable acidity (%)	10.1 ± 0.05	1.62 ± 0.08
Soluble solid content (°Brix)	15.2 ± 0.02	9.82 ± 0.02
pH	4.59 ± 0.01	3.88 ± 0.01
³ Monomeric ACN (mg/100 g fw)	14.7 ± 0.89	7.60 ± 1.23

Table 1. Compositional analysis of Andes berry and tamarillo before anthocyanin extraction¹.

¹Values are means \pm standard error ($n = 2$). ²Expressed as g citric acid/100 g fruit. ³Expressed as mg cy-3-glu for Andes berry and as mg Dp-3-rut for tamarillo.

CHARACTERISTICS OF SPRAY DRIED MICROCAPSULES

The Andes berry spray dried ACNs had an average moisture content of 3.99 ± 0.43 , a A_w level of 0.35 ± 0.29 while the average moisture content and A_w of the tamarillo powder were 4.73 ± 0.27 and 0.45 ± 0.009 respectively.

SEM microphotographs of Andes berry powder are presented in figure 1A. The microcapsules showed spherical shapes with diameters ranging between $3.3 \mu\text{m}$ a $23.3 \mu\text{m}$. Some of the particles exhibited smooth surface, while others presented either porous surface of surface dents. As for the tamarillo samples (Fig. 1B), the microcapsule diameter ranged between $2.5 \mu\text{m}$ a $30.2 \mu\text{m}$ and the surface was either smooth or presented surface dents. The ACN content, total phenolic content and antioxidant activity of microcapsules immediately after spray drying are shown in table 2. The average ACN in Andes berry powder was 51.2 mg/100 g; this yield corresponded to approximately 3 times the concentration of total ACNs in fresh fruit. The ACN yield for tamarillo powder was 39.4 mg/100 g, which represents about 5 times the concentration of total ACNs in fresh fruit. The results indicated that the total phenolic content of Andes berry microcapsules (1271 mg GAE/100 g) was about two-fold higher than the phenolic content in tamarillo

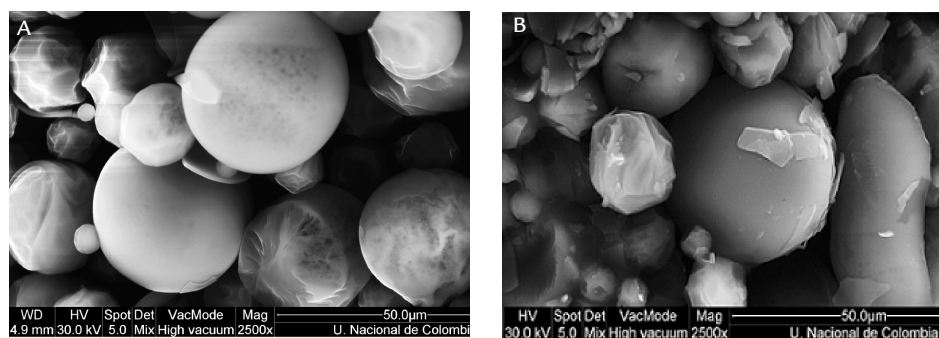


Figure 1. Scanning electron microscopic (SEM) image of microencapsulated anthocyanin pigments. (A) Andes berry, (B) Tamarillo.

microcapsules (602 mg GAE /100 g). The average antioxidant activity of Andes berry powders (6.81 mmoles TE/100 g) was close to the average antioxidant activity of tamarillo samples (6.09 mmoles TE/100 g).

Parameter	Andes berry	Tamarillo
³ Monomeric ACN (mg/100 g powder)	51.20 ± 0.34	39.4 ± 0.35
% polymeric color	4.92 ± 0.89	8.0 ± 0.09
Antioxidant activity (mmoles TE/100 g powder)	6.81 ± 0.15	6.09 ± 0.59
Total phenolics (mg GAE/100 g powder)	1271.00 ± 7.0	602.00 ± 5.0

Table 2. Anthocyanin content, total phenolic content, and antioxidant activity of microencapsulated pigments from Andes berry and tamarillo pigments after spray drying^{1,2}.

¹Values are means of three subsamples at each replicate of the experiment ± standard error, GAE = gallic acid equivalent, TE = trolox equivalents. ²Calculated based on a dilution of 500 mg of powder to a 25-mL final volume in 0.01% HCl. ³Expressed as mg cy-3-glu for Andes berry and as Dp-3-rut for tamarillo.

CHANGES IN ANTHOCYANINS DURING STORAGE

Figures 2A and 2B show changes in pigment concentration in Andes berry and tamarillo microcapsules during storage. Temperature, storage time and *A_w* significantly affected the monomeric ACN content in Andes berry samples while only storage time and temperature affected the monomeric ACN content in tamarillo samples ($P < 0.00001$). At 25 °C Andes berry samples showed a decrease of 54 % at 0.20 *A_w* and of 60 % at 0.35 *A_w*. The samples stored at 40 °C showed reductions of 66 % and 96 % for *A_w* levels of 0.20 and 0.35, respectively. In contrast, the decrease in the control sample was of 24 %. The tamarillo systems stored at 25 °C and 0.20 *A_w* showed a decrease of 74 % in the monomeric pigment while the samples stored at 25 °C and 0.35 *A_w* showed a reduction of 76 %. At 40 °C the decrease in monomeric pigment was of 94 % and 97 % at *A_w* levels of 0.20 and 0.35, respectively. The losses in the control samples were of 30.2%. The degradation of the Andes berry and tamarillo pigments followed first-order kinetics, as confirmed by linear regression analysis (p -values < 0.00001). As shown in table 3, Andes berry samples were significantly more stable than tamarillo samples (p -values < 0.05) at all storage conditions. The half life ($t_{1/2}$) of the Andes berry samples was significantly higher than the $t_{1/2}$ of the tamarillo samples ($p < 0.05$), and ranged between 11 and 32 days

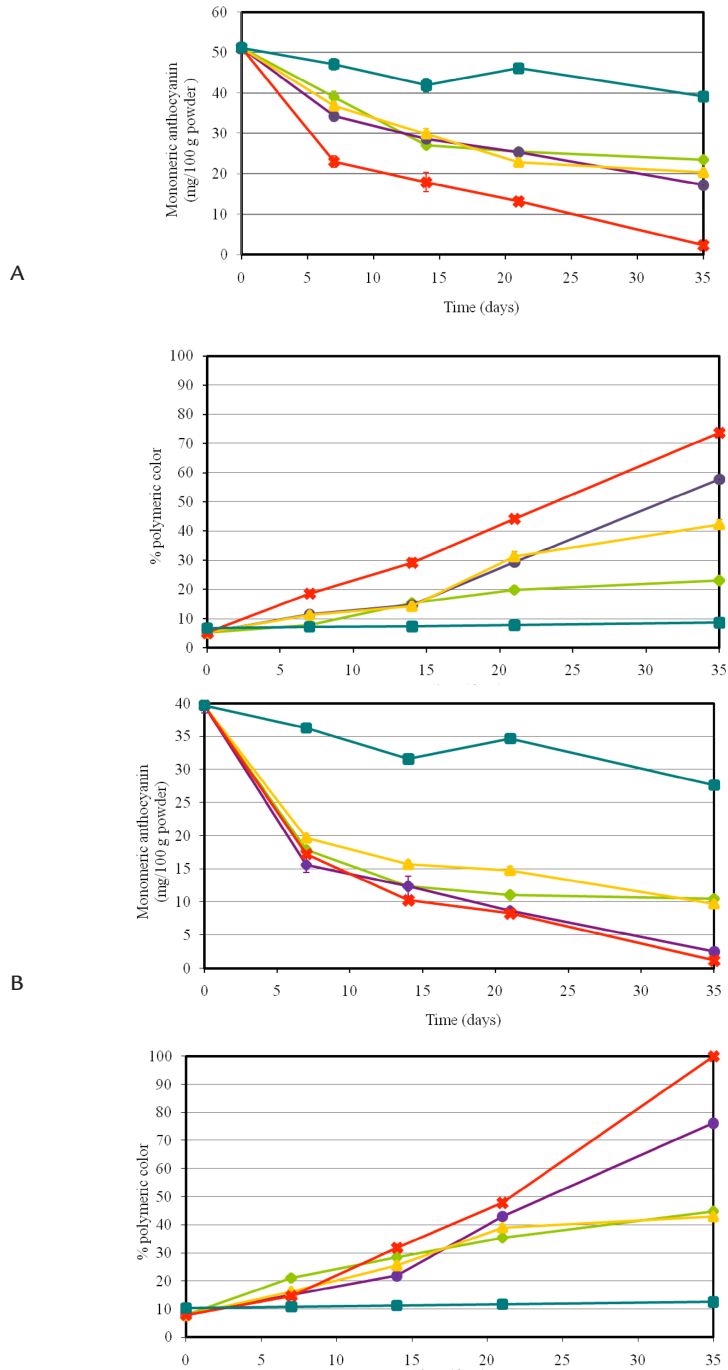


Figure 2A. Changes in anthocyanin content during storage of Andes berry microencapsulated pigments. ◆ T 25°C Aw 0.20, ▲ T 25°C Aw 0.35, ● T 40°C AW 0.20, × T 40°C AW 0.35, ■ Control. B. Changes in anthocyanin content during storage of tamarillo microencapsulated pigments. ◆ T 25 °C Aw 0.20, ▲ T 25 °C Aw 0.35, ● T 40 °C AW 0.20, × T 40 °C AW 0.35, ■ Control.

while the $t_{1/2}$ of the tamarillo microencapsulated samples ranged from 9 to 21. Due to the little degradation of the control samples stored at $-14\text{ }^{\circ}\text{C}$, the correlation coefficients found by linear regression were lower than those for the treatment samples. The estimated $t_{1/2}$ of the controls was of 99 days and 77 days for Andes berry and tamarillo, respectively.

Storage conditions	Andes berry					Tamarillo						
	r	β_0	β_1 Rate of degradation (days ⁻¹)	$t_{1/2}$ (days)	r	β_0	β_1 Rate of degradation (days ⁻¹)	$t_{1/2}$ (days)	r	β_0	β_1 Rate of degradation (days ⁻¹)	$t_{1/2}$ (days)
T 25 °C Aw 0.20	-0.866	-0.170 ± 0.040	-0.022 ± 0.002a	32 ± 0.5a	-0.820	-0.430 ± 0.076	-0.033 ± 0.004a	21 ± 0.3a				
T 25 °C Aw 0.35	-0.917	-0.147 ± 0.036	-0.026 ± 0.002b	27 ± 0.4b	-0.881	-0.310 ± 0.059	-0.035 ± 0.003a	20 ± 0.4b				
T 40 °C Aw 0.20	-0.921	-0.134 ± 0.038	-0.028 ± 0.002b	25 ± 0.4c	-0.942	-0.196 ± 0.083	-0.073 ± 0.004b	9 ± 0.3c				
T 40 °C Aw 0.35	-0.675	-0.218 ± 0.158	-0.061 ± 0.012c	11 ± 0.3d	-0.965	-0.155 ± 0.047	-0.073 ± 0.004b	9 ± 0.2c				
Control	-0.681	-0.060 ± 0.023	-0.007 ± 0.001d	99 ± 1.0e	-0.889	-0.035 ± 0.015	-0.009 ± 0.0008c	77 ± 0.9d				

Table 3. Linear regression parameters for first order kinetics degradation of microencapsulated Andes berry and tamarillo anthocyanins^{1,2}.

¹ Values are means ± standard error (n = 2)

² Means in a column followed by different letters are significantly different using Fisher's least significant difference at P < 0.05.

r = Pearson correlation coefficient

β_0 = slope

The degree of polymerization in the Andes berry powders was affected by storage time, temperature, and Aw ($p < 0.0001$; Fig. 4A) with the samples stored at $40\text{ }^{\circ}\text{C}$ and 0.35 Aw having the highest polymeric color levels and the samples stored at $25\text{ }^{\circ}\text{C}$ and 0.20 Aw having the lowest. Similar trends of polymer formation were obtained with tamarillo powders, but these were significantly affected by storage time and temperature only

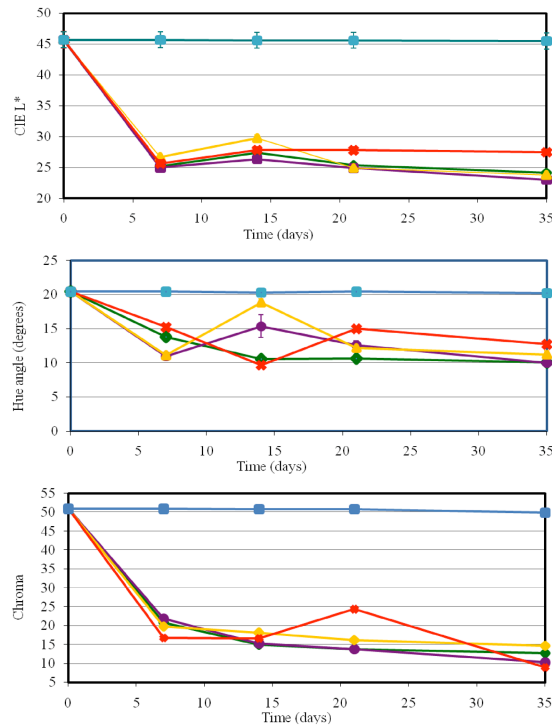


Figure 3. Changes in color during storage of Andes berry powders. ♦ T 25 °C Aw 0.20, ▲ T 25 °C Aw 0.35, ● T 40 °C AW 0.20, XT 40 °C AW 0.35, ■ Control.

($p < 0.0001$). Control powders stored at -14 °C did not show significant increase in polymeric color.

CHANGES IN COLOR DURING STORAGE

Figures 3 and 4 show variations in color parameters during storage of the microcapsules. Andes berry samples presented lower lightness ($L^* = 45.7 \pm 1.34$) at the beginning of the storage than tamarillo samples ($L^* = 58.7 \pm 0.03$). Storage time caused a significant decrease in L^* value in powders from both fruits ($P < 0.00001$), which indicates darkening of the samples. There was a decrease of 18-23 units for Andes berry samples as compared to a decrease of 33-38 units for tamarillo samples. Aw level also had a significant effect on L^* value of Andes berry pigments ($p = 0.01$). Samples stored at -14 °C did not present a significant change in L^* value.

Microencapsulated Andes berry pigments showed higher hue angle ($20.4^\circ \pm 0.05$) than tamarillo powders ($5.2^\circ \pm 0.10$). Although a^* and b^* values showed a significant change in response to storage time for both pigments ($p < 0.0001$), Andes berry samples showed a decrease in these parameters while tamarillo samples showed a decrease in a^* value and an increase in b^* value. Therefore, the hue of Andes berry samples decreased indicating an overall shift towards red while the hue of tamarillo samples increased causing a shift towards orange red.

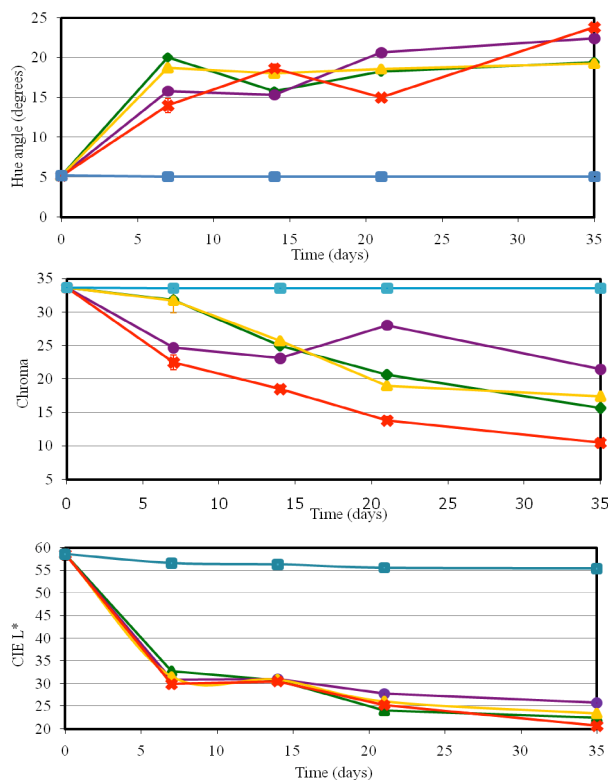


Figure 4. Changes in color during storage of tamarillo powders. ♦T 25 °C Aw 0.20, ▲T 25 °C Aw 0.35, ●T 40 °C AW 0.20, X T 40 °C AW 0.35, ■Control.

The initial color intensity of the Andes berry samples was higher ($C^* = 51 \pm 0.50$) than the tamarillo samples ($C^* = 34 \pm 0.01$). This color parameter was significantly affected by storage time ($p < 0.00001$) and Aw ($p = 0.0001$) in Andes berry powders and by storage time ($p < 0.00001$), temperature ($p = 0.0009$), and Aw ($p = 0.029$) in tamarillo systems. However, tamarillo microcapsules showed the lowest reduction in chroma during storage at all conditions. A maximum reduction of 23 units in chroma was observed at 40 °C and 0.35 Aw while Andes berry samples presented a maximum reduction of 42 units under the same conditions.

Low temperatures greatly improved color stability of the control samples, which did not present a significant change in any of the monitored color parameters.

CHANGES IN ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT

The antioxidant activity of the powders decreased significantly during storage ($P < 0.05$) (Table 4). The activity of tamarillo samples was affected only by storage time ($p < 0.0001$) and decreased between 63% and 70% with the higher decrease being for the samples stored at 40 °C and 0.35 Aw. The antioxidant activity of Andes berry samples decreased due to storage time ($p < 0.0001$) and Aw ($p = 0.0314$). The decrease ranged between 72 % and 85 % with the maximum decrease being for the samples stored at 25 °C and 0.20 Aw.

Microencapsulated Andes berry pigments showed higher total phenolic content (1272 ± 7.0 mg GAE/100 g) than tamarillo (602 ± 5.0 mg GAE/100 g). Storage time caused a significant increase on the total phenolic content in Andes berry systems ($p = 0.027$) and tamarillo systems ($p < 0.00001$). Such change was more pronounced in the tamarillo systems which increased 35 % when stored at 25 °C and 0.20 Aw and a 34 % when stored at 25 °C and 0.35 Aw. At 40 °C the increase was 38 % when Aw was 0.20 Aw while the increase was 40 % when the Aw was 0.35. Microencapsulated Andes berry pigments were additionally affected by temperature ($p = 0.022$); systems stored at 25 °C showed an increase of 17 % at 0.20 Aw and of 12 % at 0.35 Aw. At 40 °C the increase was of 22 % at 0.20 Aw and of 23 % at 0.35 Aw.

Storage Conditions	Storage Time (days)	Andes berry		Tamarillo	
		Antioxidant activity (mmoles TE/100 g powder)	Total phenolics (mg GAE/100g powder)	Antioxidant activity (mmoles TE/100 g powder)	Total phenolics (mg GAE/100g powder)
T 25 °C	0	6.80 ± 0.15a	1272 ± 7.0a	6.09 ± 0.59a	602 ± 5.0a
	7	4.50 ± 0.32b	1315 ± 12.0b	5.30 ± 1.02ab	631 ± 6.0a
	14	4.32 ± 0.47b	1408 ± 1.0c	4.56 ± 0.27ab	746 ± 23.0b
	21	2.12 ± 0.46c	1462 ± 3.0d	2.17 ± 0.03b	797 ± 2.0c
	35	1.01 ± 0.04c	1471 ± 10.0d	2.00 ± 0.18b	812 ± 3.0c
T 25 °C	0	6.80 ± 0.15a	1272 ± 7.0a	6.10 ± 0.59a	602 ± 5.0a
	7	5.61 ± 1.19b	1296 ± 5.0a	5.96 ± 0.93a	619 ± 2.0a
	14	2.71 ± 0.36c	1305 ± 24.0a	3.42 ± 0.44b	676 ± 11.0b
	21	1.66 ± 0.05c	1361 ± 8.0b	1.99 ± 0.37b	772 ± 5.0c
	35	1.74 ± 0.10c	1407 ± 12.0b	1.93 ± 0.11b	807 ± 6.0d
T 40 °C	0	6.80 ± 0.15a	1272 ± 7.0a	6.09 ± 0.59a	602 ± 5.0a
	7	5.77 ± 0.69a	1289 ± 12.0ab	5.33 ± 0.78ab	629 ± 8.0b
	14	3.41 ± 0.43b	1326 ± 7.0b	3.90 ± 0.42bc	661 ± 2.0c
	21	3.39 ± 0.62b	1514 ± 27.0c	1.18 ± 0.03cd	782 ± 3.0d
	35	1.88 ± 0.07c	1533 ± 8.0c	2.23 ± 0.24d	827 ± 6.0e
T 40 °C	0	6.80 ± 0.15a	1272 ± 7.0a	6.09 ± 0.59a	602 ± 5.0a
	7	3.62 ± 0.74b	1318 ± 7.0a	4.60 ± 0.04a	639 ± 2.0b
	14	3.37 ± 0.80bc	1466 ± 47.0b	2.90 ± 0.28b	704 ± 3.0c
	21	1.99 ± 0.30cd	1541 ± 10.0bc	1.95 ± 0.18b	802 ± 1.0d
	35	1.57 ± 0.01d	1562 ± 9.0c	1.84 ± 0.05b	843 ± 1.0e
Control -14 °C	0	6.80 ± 0.15a	1272 ± 7.0a	6.09 ± 0.59a	602 ± 5.0a
	7	6.73 ± 0.04a	1296 ± 5.0a	5.87 ± 0.91a	619 ± 2.0b
	14	6.66 ± 0.06a	1272 ± 7.0a	4.77 ± 0.09a	605 ± 2.0a
	21	5.41 ± 0.71a	1273 ± 8.0a	4.43 ± 0.19a	636 ± 1.0c
	35	4.05 ± 0.13a	1266 ± 1.0a	4.39 ± 0.22a	642 ± 1.0d

Table 4. Changes in antioxidant activity and total phenolic content of microencapsulated Andes berry and tamarillo anthocyanins^{1,2}.

¹Data are reported as mean ± standard error (n = 2)

²Different letters in the same column for the same storage conditions mean significant difference using Fisher's least significant difference at $P < 0.05$.

GAE = gallic acid equivalent; TE = Trolox equivalent.

CORRELATIONS BETWEEN ACNS OR TOTAL PHENOLICS AND ANTIOXIDANT ACTIVITY

The relationship between antioxidant activity and total monomeric ACN or total phenolic content of the powders is described by the correlation parameters found by regression analysis (Tables 5 and 6). There was a significant linear relationship between ACN concentration ($P < 0.05$), or total phenolic content ($P < 0.1$), and antioxidant activity for the Andes berry samples. The association between total phenolic content and antioxidant activity was higher for the tamarillo samples than the one for Andes berry samples.

Storage Conditions	Andes berry				Tamarillo			
	¹ model	r	p-value	r ²	¹ model	r	p-value	r ²
T 25 °C Aw 0.20	Y = -1.183 + 0.155 X (1.341) (0.039)	0.918	0.0276	0.844	Y = 1.920 + 0.115 X (1.191) (0.056)	0.766	0.1313	0.586
T 25 °C Aw 0.35	Y = -2.213 + 0.184 X (1.052) (0.031)	0.960	0.0092	0.922	Y = 1.119 + 0.139 X (1.417) (0.063)	0.785	0.1156	0.617
T 40 °C Aw 0.20	Y = -0.427 + 0.149 X (0.958) (0.029)	0.949	0.0139	0.899	Y = 1.923 + 0.115 X (1.015) (0.050)	0.800	0.1043	0.640
T 40 °C Aw 0.35	Y = 1.059 + 0.112 X (0.285) (0.011)	0.987	0.0018	0.974	Y = 1.443 + 0.125 X (0.480) (0.023)	0.951	0.0130	0.904

Table 5. Regression models and correlation coefficients between monomeric anthocyanin content and antioxidant activity in stored Andes berry and tamarillo pigments. r = Pearson correlation coefficient. p-value = significance levels of regression models. r² = coefficient of determination. ¹Model Y= antioxidant activity (mmoles TE/100 g powder), X = ACN concentration (mg ACN/100 g powder).

Storage Conditions	Andes berry				Tamarillo			
	¹ model	r	p-value	r ²	¹ model	r	p-value	r ²
T 25 °C Aw 0.20	Y = 32.712 - 0.021 X (6.133) (0.004)	-0.938	0.0183	0.880	Y = 17.103 - 0.0182 X (2.623) (0.004)	-0.945	0.0152	0.894
T 25 °C Aw 0.35	Y = 51.778 - 0.036 X (17.910) (0.014)	-0.840	0.0747	0.706	Y = 18.921 - 0.022 X (2.484) (0.004)	-0.962	0.0090	0.925
T 40 °C Aw 0.20	Y = 22.593 - 0.013 X (6.876) (0.005)	-0.840	0.0753	0.705	Y = 17.228 - 0.019 X (3.226) (0.005)	-0.925	0.0244	0.855
T 40 °C Aw 0.35	Y = 23.600 - 0.0140 X (5.873) (0.004)	-0.893	0.0414	0.797	Y = 16.091 - 0.018 X (2.169) (0.003)	-0.929	0.0098	0.920

Table 6. Regression models and correlation coefficients between total phenols content and antioxidant activity in stored Andes berry and tamarillo pigments. r = Pearson correlation coefficient. p-value = significance levels of regression models. r² = coefficient of determination. ¹Model Y= antioxidant activity (mmoles TE/100 g powder), X = total phenolics (GAE/100g powder). Values in parenthesis are standard errors of regression coefficients.

DISCUSSION

The monomeric ACN content of Andes berry was higher than the value reported by other authors (Osorio *et ál.*, 2007), who found a concentration of 6.28 ± 0.01 mg of ACN/100 g fresh fruit. Our experimental value is comparable to that one reported previously (Pantelidis *et ál.*, 2007) for other four blackberry cultivars (*Rubus fruticosus*; 14.5-17.5 mg of ACNs per 100 g fresh fruit). Regarding tamarillo, the ACN experimental value is in accordance with previous reports (Osorio *et ál.*, 2007) of 7.41 ± 4.05 mg/100 g fresh fruit.

SEM analysis showed that both the Andes berry and tamarillo particles were among the size range for microcapsules (0.2 µm-5000 µm; King, 1995). The presence of surface dents can be attributed to the shrinkage at the cooling process during high drying rates which results in solidification of the wall prior to expansion of the microcapsules (Sheu and Rosenberg, 1995). This characteristic has also been related to wall materials with high proportions of carbohydrates such maltodextrins (Sheu and Rosenberg, 1995) while particle porosity has been explained as the result of fast evaporation of solvent during drying (Freitas *et al.*, 2004).

The total phenolic content of Andes berry powders is close to the one reported for evergreen blackberry seeds (1460 mg GAE/100g) while the content for tamarillo powder is lower than the one found for whole evergreen blackberries (822 mg GAE/100g) (Siriwoharn and Wrolstad, 2004).

Although the monomeric ACN and total phenolic contents of Andes berry were higher than the one for tamarillo, the antioxidant activity of the powders obtained from both fruits was comparable. Fruits are identified as rich sources of antioxidants including polyphenols, carotenoids, and vitamins (McCarty, 2004). Therefore, components other than ACNs and other phenolics must be contributing to the antioxidant activity of tamarillo powders. Comparison of our results with the antioxidant activity of several fruits showed that the obtained powders have similar values to those reported for red grapes (6.84 mmol TE/100 g fw) and higher values than those for blueberry (3.95 mmol TE/100 g fw; Netzel, 2007).

Regarding changes in the microencapsulated ACNs during storage, other researchers observed similar kinetics during storage of microencapsulated purple carrot ACNs. Such pigments losses were of 30% at 25 °C and of 13 % at 4 °C after a storage period of 64 days (Ersus and Yurdagel, 2007). We hypothesize that one factor responsible for the higher stability of Andes berry powders is the higher ACN initial concentration. In experiments fortifying the ACN concentration in strawberry juice and concentrate with different pelargonidin derivatives, other authors confirmed that concentration of monomeric pigment increased stability regardless of the derivative (Garzón and Wrolstad, 2002).

A shift in hue value represented the main changes observed in color. Decreases in this parameter have been observed during storage of processed cherries, grapes and berries (García-Alonso *et al.*, 2003) while increases due to breakdown of ACNs have been reported during storage of strawberry juice and concentrate (Garzón and Wrolstad, 2002).

Although a decrease in antioxidant activity probably due to degradation of ACNs and vitamins was observed, there was an increase in total phenolic content. Such behavior has been reported for stored fruit products as the result of the compounds generated during oxidation that can react with the Folin Ciocalteu reagent (Klimczak *et al.*, 2007) and the result of hydrolytic reactions (Hatzidimitriou *et al.*, 2007; Zafrilla *et al.*, 2001). The significant linear relationship between ACN concentration ($P < 0.05$), or total phenolic content ($P < 0.1$), and antioxidant activity for the Andes berry samples suggest that both ACN content and phenolic content are good predictors of antioxidant activity of these microencapsulated pigments. This correlation between fruit ACNs and antioxidant activity has been observed by other researchers (Moyer *et al.*, 2002; Chaovanalikit and Wrolstad, 2004; Chaovanalikit *et al.*, 2004). The high association between total phenolic content and antioxidant activity of the tamarillo samples along with their lower and non-significant

correlation coefficients between ACN concentration and antioxidant activity of tamarillo powders confirm our conclusion about components other than ACNs contributing to the antioxidant activity of the tamarillo microencapsulated pigments.

CONCLUSIONS

Microencapsulation of Andes berry and tamarillo anthocyanins by spray drying proved to be a useful technique for obtaining potential natural food colorants from waste material of fruit osmodehydration.

Storage under 40 °C and 0.35 Aw caused the highest degradation rate in both systems under study. However, anthocyanins from Andes berry were more stable than anthocyanins from tamarillo.

The antioxidant activity of the microencapsulated pigments was affected during storage and the decrease in the activity was higher for the Andes berry pigments. In these samples the antioxidant activity was highly related to both the ACN content and the total phenolic content while the antioxidant activity of the tamarillo powders was highly related to the phenolic content, which increased during storage.

The color characteristics (hue angle shift towards red) and higher ACN stability of Andes berry powders during storage suggest that these microencapsulated ACNs have better potential as natural replacers of red artificial colorants as compared to tamarillo powders. These results would be useful in developing applications for spray-dried anthocyanin extracts as powdered food-grade colorants.

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