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Antioxidant Activity, Total Phenolics Content, Anthocyanin, and Color Stability of Isotonic Model Beverages Colored with Andes Berry (*Rubus glaucus* Benth) Anthocyanin Powder

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Abstract

The stability of anthocyanin (ACN) freeze-dried powders from Andes berry (*Rubus glaucus* Benth) as affected by storage, addition of maltodextrin as a carrier agent, and illumination was evaluated in isotonic model beverages. The ethanolic ACN extract was freeze dried with and without maltodextrin DE 20. Isotonic model beverages were colored with freeze-dried ACN powder (FDA), freeze-dried ACN powder with maltodextrin (MFDA), and red nr 40. Beverages were stored in the dark and under the effect of illumination. Half life of the ACNs, changes in color, total phenolics content (TPC), and antioxidant activity were analyzed for 71 d. Addition of maltodextrin and absence of light stabilized the color of beverages and improved ACN and TPC stability during storage. The antioxidant activity of the beverages was higher when they were colored with MFDA and highly correlated with ACN content. There was no correlation between antioxidant activity and TPC. It is concluded that addition of maltodextrin DE 20 as a carrier agent during freeze-drying improves the color and stability of nutraceutical antioxidants present in Andes berry extract. This suggests a protective enclosing of ACNs within a maltodextrin matrix with a resulting powder that could serve as a supplement or additive to naturally color and to enhance the antioxidant capacity of isotonic beverages.

Keywords

anthocyanin; antioxidant activity; beverages; color stability; freeze-drying

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, industry and costumer preferences trend toward using natural colorants in formulating new products (Francis 1989; Fabre and others 1993). As a result, natural plant colorants have been in high demand for the past decade with the purpose of providing an alternative to synthetic dyes, such as FD&C red 40 and the banned FD&C red nr 2. (Fabre and others 1993).

Anthocyanins (ACNs) are highly colored plant pigments with high potential as natural colorants (Wrolstad 2000, 2004). Besides, their potential advantages as food colorants, ACNs are associated with health benefits such as reduced risk of coronary heart disease, reduced risk of stroke, anticarcinogen activity, anti-inflammatory effects, improved visual acuity, and improved cognitive behavior (Clifford 2000; Prior 2004). Nevertheless, the application of ACNs as natural colorants at a commercial scale is limited because of the low availability of vegetable sources in seasonal locations and their relatively low stability to processing, formulation, and storage conditions (Cevallos-Casals and Cisneros-Zevallos 2004). Temperature, pH, oxygen, concentration, enzymes, metal ions, and ascorbic acid contribute to destruction of ACNs (Mazza and Miniati 1993). Changes in monomeric ACN due to relatively narrow *Aw* ranges in different food matrixes have also been reported (Garzón and Wrolstad 2001).

In the natural colorant field, production of ACN powders is an alternative technique for promoting easier handling, increasing solubility and extending shelf life, bioavailability, and processing stability (Dziezak 1988; Chandra and others 1993; Dib Taxi and others 2003; Ersus and Yurdagel 2007; Duangmal and others 2008; Laine and others 2008).

Spray drying is the most commonly applied method from liquid to solid conversion of natural colorants. However, this technique presents limitations with respect to other conversion processes such as freeze-drying. Loss of volatiles, oxidation reactions, and water phase transitions can occur during spray drying (Jacquot and Perneti 2004). In addition, care must be taken with regard to the inlet and outlet temperatures, since excessively high temperatures can lead to degradation of some product components such as ACNs and phenolics (Serris and Biliaderis 2001).

Maltodextrins are hydrolyzed products of starch. They are widely used as carrier agents for obtaining food dried powders. They have the ability to form amorphous glassy matrices produced by removing the dispersing medium by means of evaporation, freezing, or rapid cooling of carbohydrate melts (Ross 1995). The glassy matrix formed by carbohydrates results in a kind of solid network, which is assumed to hold up by hydrogen bonds between carbohydrate chains (Kilburn and others 2005). Enclosing of sensitive food components within the matrix results in their protection against outward adverse conditions, increase their stability, and promote controlled release (Ré 1998).

Andes berry (*Rubus glaucus* Benth) is a climbing perennial shrub belonging to the *Rosaceae* family and native to the Andes in northern South America, where it grows year round. The fruit is a promising source of natural dyes and bioactive compounds due to their high ACN content, total phenolics content (TPC), and antioxidant activity (Garzón and others 2009). In Colombia, the yearly production of Andes berry fruit is around 80.000 tons. A considerable portion of this crop is processed into natural juices, marmalades, and jellies. However, around 30% of the harvested product is discarded due to difficulties in postharvest conservation and high perishability of the fruit. These byproducts may be a potential source of natural colorants, nutraceuticals, and natural antioxidants.

Previous research shows that osmotic dehydration and subsequent spray drying of Andes berry ACNs allows utilization of waste material from this fruit and presents a potential for obtaining ACNs as natural colorants and nutraceuticals (Olaya and others 2009). Accordingly, the main objective of this work was to find a potential added value for what currently constitutes a waste material. Another object was to evaluate the use of maltodextrin as a carrier agent on the functional properties and stability of Andes berry powder produced by freeze-drying.

Andes berry ACNs were freeze dried with and without maltodextrins and used as natural colorant in isotonic model beverages. Changes in color, ACNs, TPC, and antioxidant activity were evaluated as a function of maltodextrin addition and illumination during storage.

Materials and Methods

Reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium carbonate, potassium persulphate, and Folin–Ciocalteu reagent were purchased from Merck® (Darmstadt, Germany). Gallic acid, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) and TPTZ (2,4,6-tripyridyl-s-triazine) were from Sigma Aldrich (St. Louis, Mo., U.S.A.). Methanol and liquids along with FeCl₃·6H₂O were purchased from Fisher Scientific Co. (Fair Lawn, N.J., U.S.A.). 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 ACN pigments

Fresh Andes berries were acquired from the local market in Bogotá, Colombia. The fruits were selected according to color (uniform dark red), uniform size (diameter between 23 and 26 mm), soluble solids content (°Brix) ranging between 5.5 and 7.5°, and total titratable acidity (TA) ranging between 3.1% and 3.4% malic acid (ICONTEC 1997). Soluble solids content was measured using a digital refractometer Abbe II (Reichert-Jung, Leica Inc., Buffalo, N.Y., U.S.A.), while TA was determined 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 Gerate® pH meter, model CG820 (Mainz, Germany).

After selection, the fruits were subjected to osmotic dehydration with 96% ethanol for 12 h according to the method described by Olaya and others (2009). In brief, 1 kg of fruit was subjected to extraction with 3.8 L of solvent as follows: the total amount of fruit was divided into 3 equal portions and each portion was replaced by fresh fruit every 4 h. This replacement ensured enrichment of the solvent with ACNs.

Three different lots of fruit were extracted as described above to obtain 3 replications of the experiment.

Determination of monomeric ACN

Monomeric ACN pigment content was determined on the extract by the pH differential method as described by Giusti and Wrolstad (2001). Absorbencies were read at 510 and 700 nm. For comparison purposes, pigment content was calculated as cyanidin 3-glucoside using an extinction coefficient (ϵ) of 26900 L/cm-mol and molecular weight of 449.2. Measurements were replicated 3 times with means being reported.

Preparation of freeze-dried ACN powder

The ACNs obtained after ethanolic extractions were recovered and the ethanol was removed at 40 °C by using a Büchi rotoevaporator. To obtain MFDA, corn maltodextrin DE 20 at a concentration of 3 g/100 mL extract was added to the extract and the mixture was stirred for 30 min. The sample was subjected to freeze-drying under 0.05 hPa vacuum for 15 h following the technique described by Duangmal and others 2008. Freeze-drying of the extract without maltodextrin was performed under the same conditions to obtain a control (FDA).

Preparation of isotonic beverage model solutions

Isotonic model beverages were made in McIlvaine buffer at pH 2.6 adding the freeze-dried pigment to obtain an absorbance of 1, corresponding to 20 mg ACN/l extract. Sucrose was added at 10% w/v and sodium chloride at 0.11% w/v (Maughan and Murray 2001). For preservation purposes potassium sorbate and sodium benzoate were added at 0.1% w/w. Samples were allowed to equilibrate with the buffer for 12 h at room temperature. Then, aliquots were transferred to 12-mL vials, screwcapped, pasteurized at 90 °C for 1 min, and subsequently stored. The effect of microencapsulation and daylight was evaluated through time (after 12 h and 8, 29, 41, 53, 62.5, and 71 d) at 16 °C.

Color evaluation

Transmittance of the model systems was determined every 10 nm from 400 to 700 nm using a Shimadzu UV visible spectrophotometer, model UV 160 U (Kyoto, Japan), using 1-cm disposable cells. The weighted ordinate method was applied for calculation of CIE tristimulus values X, Y, and Z of the pigments (Hunter and Harold 1987). CIE $L^* a^* b^*$ h_0 and C values were calculated from the X, Y, and Z tristimulus values using the following equations: $L^* = 116 (Y/Y_n)^{1/3} - 16$, $a^* = 500 [(X/X_n)^{1/3} - (Y/Y_n)^{1/3}]$, $b^* = 200 [(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}]$. Where $X_n = 94.825$, $Y_n = 100.00$, $Z_n = 107.399$ using the D_{65} illuminant and 10° observer angle (Hutchings 1999). Hue angle (h_0) was calculated as $\arctan b^*/a^*$, and chroma or color intensity (C^*) as $(a^{*2} + b^{*2})^{1/2}$. Total color difference between 2 samples at the initial and final time of storage was obtained by the equation: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Changes in ACN profile

High-performance liquid chromatography (HPLC)-DAD analysis of ACNs was carried out on a Waters 2695 gradient HPLC separation module (Waters Corp., Milford, Mass., U.S.A.) equipped with an autoinjector and a 996 photodiode array detector (PDA). Chromatographic separations were performed on 2 C18 Symmetry columns connected in series (75 mm × 4.6 mm i.d., 3.5 μm) (Waters Corp.). The mobile phase consisted of 5% (v/v) formic acid in water (solvent A) and 5% (v/v) formic acid in acetonitrile (solvent B) at a flow rate of 1.0 mL/min. The gradient condition was 0 to 20 min 20% B and 20 to 30 min 20% to 58% B. Absorbance spectra were recorded every 1.2 nm from 200 to 700 nm.

Peak identification of ACNs was performed by diode array spectral characteristics and comparison of retention times with literature (Garzón and others 2009). All analyses were done in triplicate.

Changes in phenolic content

Total phenolics were determined as gallic acid equivalents (GAE)/L using the method described by Waterhouse (2001). A 20-μL sample aliquot of extract or gallic acid standard (50 to 500 mg/L) was mixed with 1.58 mL water followed by 100 μL Folin–Ciocalteu's reagent. After vortexing and incubating at room temperature for 8 min, 300 μL of 20% aqueous sodium carbonate solution were added. Samples were vortexed and held at room temperature for 2 h. Absorbance of the blue-color solution was recorded at 765 nm on a Shimadzu UV visible spectrophotometer, model UV 160 U, using 1-cm disposable cells. All measurements were replicated 4 times.

Determination of antioxidant activity

Antioxidant activity was determined by the trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) assays. The TEAC assay, which determines ABTS radical cation scavenging activity was performed according to the method described by Re and others (1999). $ABTS^{+\cdot}$ was produced by mixing ABTS stock solution (7 mM in

water) with 2.45-mM potassium persulphate (final concentration). The solution was held at room temperature in the dark for 16 h before use. Once the radical was formed, the absorbance at 734 nm was adjusted to 0.7 by dilution with 95% ethanol. Fresh ABTS^{•+} solution was prepared for each analysis. ABTS^{•+} (1 mL) was added to 10-μL sample and the reaction mixture was allowed to stand at 30 °C for 6 min and the absorbance at 734 nm was immediately recorded. The percentage decrease of the absorbance at 734 nm was calculated by the formula $I = [(AB - AA)/AB] \times 100$; where: I = ABTS^{•+} inhibition %; AB = absorbancy of a blank sample (t = 0 min); AA = absorbency of a tested extract solution at the end of the reaction.

Standard curves using trolox (0 to 0.25 mM) were run with each set of extracts. The reaction of the samples was followed until it reached the plateau. ABTS values were expressed as μmoles Trolox equivalents (TE)/mL.

The FRAP assay was performed according to Benzie and Strain (1999). FRAP reagent was prepared by mixing 2.5-mL TPTZ solution (10 mM in 40 mM HCl), 25 mL acetate buffer (300 mM, pH 3.6), and 2.5 mL of FeCl₃·6H₂O solution (20 mM). After 4-min incubation at 37 °C, the reagent was used as a blank by determining the absorbance at 593 nm. FRAP solution (900 μL) was added to 90 μL of distilled water and 30 μL of standards or extracts. In addition to standard curves using trolox, ferrous sulphate standards (0 to 500 μM) were run with each set of extracts. The reaction of the samples was followed until it reached the plateau. Final results were expressed as μmoles ferric iron (Fe³⁺) reduced/mL or as μmoles TE/mL. All measurements were replicated 4 times.

Statistical analysis

For statistical analysis, multifactor analysis of variance was applied. Significant ($P < 0.05$) differences between means were identified using the least significant difference procedure.

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 TPC were calculated by simple linear regression analysis. All analyses were performed with Statgraphics plus, version 2.1 (Manugistics Inc., Rockville, Md., U.S.A.).

Results and Discussion

Changes in color during storage

Color parameters of the model beverages at the beginning and end of the storage are reported in Table 1. Once the buffer systems equilibrated, there was no significant difference in lightness among the model beverages whether they were colored with MFDA, FDA, or red nr 40. After 71 d of storage, L^* was affected by illumination ($P = 0.0001$) for all samples and addition of maltodextrin ($P = 0.002$) for beverages colored with ACNs. All samples presented a significant increase in this parameter ($P < 0.00001$). Increase in lightness of the sample colored with FDA and stored under light was of 9 units, which was significantly higher than the increase of the other samples. On the other hand, samples colored with MFDA and stored in the dark presented an increase of 3.4 units in lightness. This value is close to the change observed in samples colored with red nr 40 and stored under light (2.5 units). Increases in L^* value in model systems colored with purple corn ACNs (Cevallos-Casals and Cisneros-Zevallos 2004), roselle ACNs (Duangmal and others 2008), and red

cabbage (Walkowiak-Tomczak and Czapski 2007) have been interpreted as indication of discoloration due to ACN degradation.

The color intensity (C^*) of the samples colored with red nr 40 was significantly higher than that one of the samples colored with Andes berry ACNs before storage. In general, this color parameter presented a significant drop as affected by storage time ($P < 0.00001$) for all samples, illumination ($P = 0.0001$) and maltodextrin addition ($P = 0.0009$) for systems colored with ACNs. Conversely, systems colored with red nr 40 and stored in the absence of light presented an increase in chroma and the lowest change in this parameter (1.9 units) followed by systems colored with MFDA and stored in the absence of light (7.7 units). A maximum reduction of 23.6 units in chroma was observed in samples colored with FDA stored in the presence of light.

Hue angle values indicated that samples colored with red nr 40 were significantly more yellowish at the beginning of the storage than samples colored with ACNs. These values decreased significantly during storage of all samples except the ones colored with red nr 40 and the ones colored with MFDA stored in the dark, which denotes an overall shift toward red. Although they were significant differences in hue among all the samples at the end of the storage, h_o was below 1.0 for all samples indicating a stable red tone.

Changes in L^* , h_o , and C^* are in accordance with results reported on model systems colored with purple corn ACNs (Cevallos-Casals and Cisneros-Zevallos 2004), roselle ACNs (Duangmal and others 2008), and red cabbage (Walkowiak-Tomczak and Czapski 2007).

There was a significant effect of illumination over the total color difference (ΔE^*) between the beginning and the end of the storage for all samples ($P < 0.05$). The total color difference, which is calculated from changes of the 3 components L^* , a^* , and b^* , was higher than 1 in all samples, which is the threshold assumed as a basis for a color difference noticed by the human eye (Gonnet 1998). The total color difference was higher in model beverages colored with FDA stored under the effect of illumination. The smallest change in ΔE^* was found in model beverages colored with red nr 40 stored in the absence of light followed by samples colored with MFDA and stored in the dark.

In addition to illumination, there was a significant effect of maltodextrin addition over ΔE^* for samples colored with ACNs ($P < 0.05$), between the beginning and the end of the 71-d storage period. The total color differences were bigger for the samples colored with FDA and stored under light, which presented a ΔE^* value of 25.1 ± 4.2 . Conversely, beverages colored with MFDA and stored in the dark showed a total color difference of 8.9 ± 2.7 . For that reason, addition of maltodextrin as a carrier agent evidently stabilized the color of these beverages. In samples colored with FDA, there was a similar stabilizing effect observed when they were stored in the dark. These last samples did not reveal any statistically significant difference in ΔE^* when compared to beverages colored with red nr 40 and stored under light.

Changes in ACN content and ACN profile

Figure 1 shows changes in monomeric ACN concentration during storage. Maltodextrin addition and illumination significantly affected the monomeric ACN content ($P < 0.00001$). Samples colored with MFDA showed a decrease of 33% in the monomeric pigment when stored in the absence of light and of 55% when stored in the presence of light. The samples colored with FDA showed reductions of monomeric ACN of 39% and 63% when stored in the absence and presence of light, respectively. Similar results have been observed when in maltodextrin has been used as a carrier agent to obtain elderberry ACN powders (Bronnum-Hansen and Flink 1985), ACN pigments, extracted from tart cherries (Chandra and others

1993), carrot ACNs (Ersus and Yurdagel 2007), and roselle powder (Duangmal and others 2008). These authors explained that complexing of the flavylium cation form of the ACNs with dextrans prevented their transformation to other less stable forms and that maltodextrin had an additional stabilizing effect due to its ability to reduce reactant mobility. Likewise, Tonon and others (2010) observed higher ACN degradation in açai juice powder obtained without the addition of maltodextrin as compared to açai juice powder, when maltodextrins had been used as a carrier agent. This observation was explained as the result of greater contact of the juice powder with oxygen when maltodextrin was not added.

Degradation of Andes berry pigments followed 1st-order kinetics, as confirmed by linear regression analysis (P -values < 0.00001). Half life ($t_{1/2}$) of the ACNs in model beverages colored with MFDA ranged between 27 ± 0.4 and 32 ± 0.5 d. This time was significantly higher ($P < 0.05$) than the $t_{1/2}$ of ACNs in model beverages colored with FDA and stored under light, which was 11 ± 0.3 d (Table 2).

ACN profiles were monitored at the beginning and end of the storage study. The representative HPLC profile of the ACNs in the model beverages at the beginning of the storage is presented in Figure 2A. Figure 2B to 2E correspond to the profiles of ACNs in model beverages at the end of the storage. Identity of the peaks 1 and 2 (cyanidin 3-sambubioside, cyanidin 3-glucoside), 3 (cyanidin 3-xylorutinoside), 4 (cyanidin 3-rutinoside), 5 (pelargonidin 3-glucoside), and 6 (pelargonidin 3-rutinoside) has been established previously by Garzón and others (2009). These authors reported that peaks 1 and 2 could not be resolved by HPLC but were detected by mass spectrometry.

Changes in relative peak area during storage are reported in Figure 3. At day 0, 27.9% of the total peak area was represented by peaks 1 and 2. Peak 3 represented 20.6%, peak 4 accounted for 49.3%, while peaks 5 and 6 represented 1.1% each one. In general, relative areas of peaks 1 and 2 and 4 and 5 decreased significantly ($P < 0.05$), while relative areas of peaks 3 and 6 showed a significant increase ($P < 0.05$). Exceptions were found when the samples were colored with MFDA and stored in the absence of light, which can be attributed to the protective effect of the maltodextrin matrix and lack of illumination.

It has been reported that cleavage of glycosidic bonds between sugar moieties attached to the ACN aglycon is a stepwise process (Giusti and Wrolstad 1996). Accordingly, we hypothesize that the observed decrease in area of peaks 1 and 2 is due to cleavage of the glucose moiety attached to the cyanidin aglycone (peak 2) with a subsequent hydrolysis of the glycosidic bond between the xylose and glucose moieties of the sambubioside attached to cyanidin (peak 1). Similarly, decrease of peak 4 suggests hydrolysis of the glycosidic bond between rhamnose and glucose to yield the correspondent cyanidin monoglucoside, while decrease in peak 5 can be explained by cleavage of the monoglucoside glucose attached to the aglycone. The described hydrolysis reactions seem to explain the proportional increases in cyanidin 3-xylorutinoside and pelargonidin 3-rutinoside over time.

Interestingly, while area of peak 4 decreased, area of peak 6 increased, showing higher stability of pelargonidin 3-rutinoside over cyanidin 3-rutinoside. The relative ACN stability is related to their chemical structure. Stability studies of selected ACNs with mono, di, and trihydroxylated B rings, under simulated (*in vitro*) physiological conditions suggest lower stability of the pigments with increased ACN B-ring hydroxylation (Woodward and others 2009). In addition, cyanidin derivatives, which have 2 ortho phenolic groups in the B ring are oxidized via a coupled oxidation mechanism with o-quinones, while ACNs with single phenolic substitution in the B ring such as pelargonidin form adducts with the quinone. The coupled oxidation reaction occurs at a faster rate (Sarni and others 1995).

Changes in phenolic content and antioxidant activity

TPC was affected by maltodextrin addition ($P = 0.022$), illumination ($P = 0.042$), and storage time ($P = 0.027$). Changes were erratic during storage with an overall significant decrease at the end of the storage period in all beverages except those colored with MFDA and stored in the absence of light. Similar fluctuating behavior has been observed during storage of berry juices and orange juice (Klimczak and others 2007; Piljac-Žegarac and others 2009) and partially explained as the transformation of phenolic compounds by reactions of deglycosylation, demethylation, and demethoxylation produced by acid hydrolysis (Hatano and others 1989). These resulting phenolics interact with the Folin–Ciocalteu reagent, which measures both free and bound phenolics (Singleton and others 1999). Furthermore, the Folin–Ciocalteu reagent reacts not only with phenolic compounds but also with other reducing compounds such as carotenoids, amino acids, sugars, and vitamin C (Vinson and others 2001), which may result in overestimation of TPC.

Losses in TPC were more pronounced in the beverages colored with FDA. Decreases of 28% and 33% were observed, when they were stored under light in the absence of light, respectively. Changes in beverages colored with MFDA indicated that the TPC were not appreciably changed. A decrease of 10.4% with a final TPC of 82.7 ± 3.1 mg GAE/L was found on samples stored in the dark and of 3.5% with a final TPC content of 86.7 ± 7.5 mg GAE/L when stored under light. These values are comparable to the TPC of orange juice (70 ± 10 mg GAE/L) and iced green tea (80 ± 10 mg GAE/L), which are considered very good source of antioxidants (Seeram and others 2008).

Our observations agree with results reported by Laine and others (2008). These authors tested the effect of adding maltodextrin as a carrier for freeze-drying of cloudberry phenolics upon stability during storage. They observed an overall decrease in TPC, which was explained as the result of hydrolysis, oxidation, and polymerization reactions. However, storage stability of phenolics increased when they added maltodextrins. Likewise, studies by Zhang and others (2006) on the use of maltodextrins for obtaining phenolics powders from grape seed showed stability improvement upon maltodextrin addition. Later, Tonon and others (2009) reported high polyphenolic retention after storage at 40 °C for 15 d, when maltodextrin was used as a carrying agent to obtain açai powder.

Although decrease in TPC was erratic, in general, the lowest change in antioxidant capacity went hand in hand with lower decrease in TPC. Evaluation of antioxidant activity indicated that in general beverages colored with MFDA had the highest initial antioxidant value after the initial equilibration 0.5-d period and maintained the highest antioxidant capacity throughout the storage period. In general, the antioxidant activity of the model beverages dropped significantly during storage ($P < 0.05$) and was affected by storage time, maltodextrin addition, and illumination (P -values < 0.0001) (Table 3).

Among beverages, the ones colored with MFDA and stored in the dark showed the strongest Fe^{+3} reducing capacity with a value of 2.26 ± 0.02 $\mu\text{moles TE/mL}$ or 2.52 ± 0.13 $\mu\text{moles Fe}^{+3}$ reduced/mL followed by the ones colored with MFDA and stored under illumination with a TE value of 1.59 ± 0.21 $\mu\text{moles TE/mL}$ or 2.41 ± 0.21 $\mu\text{moles Fe}^{+3}$ reduced/mL. This Fe^{+3} reducing value is similar to the one for cranberry juice (2.7 ± 0.5 $\mu\text{moles Fe}^{+3}$ reduced/mL) and higher than the one for orange juice (1.5 ± 0.2 $\mu\text{moles Fe}^{+3}$ reduced/mL) as reported by Seeram and others (2008). Systems colored with FDA stored in the dark had a TE value of 1.52 ± 0.15 $\mu\text{moles TE/mL}$ or 1.74 ± 0.12 $\mu\text{moles Fe}^{+3}$ reduced/mL, while samples stored under illumination presented values of 1.40 ± 0.11 $\mu\text{moles TE/mL}$ or 1.66 ± 0.06 $\mu\text{moles Fe}^{+3}$ reduced/mL.

Beverages colored with MFDA also had the highest initial TEAC value after the initial equilibration 0.5-d period and maintained it throughout the storage period. After 71 d, the TEAC value for beverages colored with MFDA and stored in the dark was 0.53 ± 0.00 $\mu\text{moles TE/mL}$, while systems stored under illumination remained with 0.46 ± 0.02 $\mu\text{moles TE/mL}$. On the other hand, beverages colored with FDA stored under light had the lowest initial TEAC (0.58 ± 0.01 $\mu\text{moles TE/mL}$) and maintained the lowest value throughout the 71-d storage period with a final TEAC value of 0.36 ± 0.01 $\mu\text{moles TE/mL}$.

Our findings agree with results reported by Tonon and others (2009) who demonstrated that açai pulp samples freeze dried with addition of maltodextrin exhibited TEAC activity preservation during storage at 40 °C during 15 d. Likewise, Tonon and others (2010) observed that when maltodextrin was used as a carrier agent to obtain açai juice powder, there was pigment protection and high antioxidant activity, for all the storage conditions studied. The authors attributed these results to the protective effect of the carrier agent on the powder functional properties.

Since the ABTS method measures capacity to scavenge ABTS radicals by electron transfer, this behavior suggest that samples colored with MFDA and stored in the dark had better scavenging activity. Plumb and others (1998) have explained the decrease in radical-scavenging capacity as the result of steric hindrance, which reduces the availability of hydroxyl groups in reaction with the ABTS radicals. Such hindrance results form increased molecular complexity after ACN polymerization reactions.

Correlations between ACNs or total phenolics and antioxidant activity

The relationship between antioxidant activity and total monomeric ACN or TPC of the beverages is described by the correlation parameters found by regression analysis (Table 4 and 5). There was a high correlation between antioxidant activity and ACN content in all samples ($P < 0.05$) suggesting that ACN content is a good predictor of antioxidant activity of these beverages. This correlation has been observed by authors such as Moyer and others (2002) and Chaovanalikit and others (2004a, 2004b) in diverse small fruits: *Vaccinium*, *Rubus*, and *Ribes*.

Although the final TPC was in line with antioxidant activity, there was no correlation between the TPC evaluated by the Folin–Ciocalteu method and the antioxidant activity of our samples measured with both antioxidant methods. This might be attributable to the nonlinear decrease behavior of total phenolics during storage. Amakura and others (2000) and Piljac-Žegarac and others (2009) observed no correlation between TPC and radical-scavenging activity in 4/9 berry species and berry juices, respectively. Likewise, weak correlation between the TPC content of fruits and the total antioxidant activity measured by ferric reducing antioxidant power assay has been reported by Imeh and Khokhar (2002). These authors attributed the lack of correlation to low selectivity of the Folin–Ciocalteu reagent, since it determines both free and bound phenolics.

Conclusions

Addition of maltodextrin as carrier agent for obtaining Andes berry ACNs powder by freeze-drying proved to be a useful technique to increase stability of color, ACNs, and bioactivity during storage. Given the antioxidant potential and health benefits of ACNs and other phenolics present in the extract, the powder could be used as an alternative for potential utilization of Andes berry waste material. These results would be useful in developing applications for Andes berry ACN extracts as powdered food-grade colorants and nutraceutical ingredient, especially in red-colored isotonic beverages.

References

- Amakura Y, Umino Y, Tsuji S, Tonogai Y. Influence of jam processing on the radical scavenging activity and phenolic content in berries. *J Agric Food Chem.* 2000; 48:6292–6297. [PubMed: 11312801]
- Benzie IFF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Method Enzymol.* 1999; 299:15–27.
- Bronnum-Hansen K, Flink JM. Anthocyanin colorants from elderberry (*Sambucus nigra*). Process considerations for production of a freeze dried product. *J Food Technol.* 1985; 20:713–723.
- Cevallos-Casals B, Cisneros-Zevallos L. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. *Food Chem.* 2004; 86:69–77.
- Chandra A, Nair MG, Lezzoni AF. Isolation and stabilization of anthocyanins from tart cherries (*Prunus cerasus* L.). *J Agric Food Chem.* 1993; 41(7):1062–1065.
- Chaovanalikit A, Wrolstad RE. Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *J. Food Sci.* 2004a; 69(1):FCT67–FCT72.
- Chaovanalikit A, Thompson MM, Wrolstad RE. Characterization and quantification of anthocyanins and polyphenolics in blue honeysuckle (*Lonicera caerulea* L.). *J Agric Food Chem.* 2004b; 52:848–852. [PubMed: 14969540]
- Clifford MN. Anthocyanins. Nature, occurrence and dietary burden. *J Sci Food Agric.* 2000; 80:1063–1072.
- Dib Taxi C, De Menezes H, Santos A, Grosso C. Study of the microencapsulation of camu-camu (*Myrciaria dubia*) juice. *J Microencapsulation.* 2003; 20:443–448. [PubMed: 12851044]
- Duangmal K, Saicheua B, Sueprasarn S. Color evaluation of freeze-dried roselle extract as a natural food colorant in a model system of a drink. *Food Sci and Technol.* 2008; 41:1437–1445.
- Dziezak J. Microencapsulation and encapsulated ingredients. *Food Technol.* 1988; 42:13–151.
- Ersus S, Yurdagel U. Microencapsulation of anthocyanin pigments of black carrot (*Daucuscarota* L.) by spray drier. *J Food Eng.* 2007; 80:805–812.
- Fabre CE, Santerre AL, Loret MO, Baberian R, Pareilleux A, Goma G, Blanc PJ. Production and food applications of the red pigments of *Monascus ruber*. *J Food Sci.* 1993; 58:1099–1111.
- Francis F. Food colourants: anthocyanins. *Crit Rev Food Sci.* 1989; 28:273–314.
- Garzón GA, Wrolstad RE. The stability of pelargonidin-based anthocyanins at varying water activity. *Food Chem.* 2001; 75:185–196.
- Garzón GA, Riedl KM, Schwartz SJ. Determination of anthocyanins, total phenolic content, and antioxidant activity in Andes berry (*Rubus glaucus* Benth). *J Food Sci.* 2009; 74(3):C227–C232. [PubMed: 19397707]
- Giusti MM, Wrolstad RE. Characterization of red radish anthocyanins. *J Food Sci.* 1996; 61:322–326.
- Giusti, MM.; Wrolstad, RE. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Wrolstad, RE.; Acree, TE.; An, H.; Decker, EA.; Penner, MH.; Reid, DS.; Schwartz, SJ.; Shoemaker, CF.; Sporns, P., editors. *Current protocols in food analytical chemistry.* New York, N.Y.: John Wiley and Sons, Inc.; 2001. p. F1.2.1-F1.2.9.
- Gonnet JF. Color effects of co-pigmentation of anthocyanins revisited - 1. A colorimetric definition using the CIELAB scale. *Food Chem.* 1998; 63:409–415.
- Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, Yoshida T, Okuda T. Effects of the interaction of tannins with coexisting substances. VI. Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem Pharm Bull.* 1989; 37:2016–2021.
- Hunter, RS.; Harold, RW. The measurement of appearance. 2nd ed.. New York: John Wiley and Sons; 1987.
- Hutchings, JB. Food color and appearance. Gaithersburg, Md: Aspen Publishers, Inc.; 1999.
- Instituto Colombiano de Normas Técnicas ICONTEC. Norma Técnica Colombiana NTC 4106. Frutas Frescas: Mora de Castilla; 1997. Available from: <http://cenicafe.org>. [Accessed Sept 17, 2010]

- Imeh U, Khokhar S. Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. *J Agric Food Chem*. 2002; 50:6301–6306. [PubMed: 12381107]
- Jacquot, M.; Perneti, M. Fundamentals of cell immobilisation biotechnology. Dordrecht, the Netherlands: Kluwer Academic Publisher; 2004. Spray coating and drying processes.
- Kilburn D, Claude J, Schweizer T, Alam A, Ubbink J. Carbohydrate polymers in amorphous states: an integrated thermodynamic and nanostructural investigation. *Biomacro-molecules*. 2005; 6:864–879.
- Klimczak I, Małecka M, Szlachta M, Gliszczynska-Swiglo A. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *J Food Compos Anal*. 2007; 20:313–322.
- Laine P, Kylli P, Heinonen M, Kirsi J. Storage stability of microencapsulated Cloudberry (*Rubus chamaemorus*) phenolics. *J Agric Food Chem*. 2008; 56:11251–11261. [PubMed: 18989975]
- Mazza, G.; Miniati, E. Anthocyanins in fruits, vegetables and grains. Boca Raton, Fla: CRC Press, Inc.; 1993.
- Maughan, R.J.; Murray, R. Sports drinks: basic science and practical aspects. Boca Raton, Fla: CRC Press, Inc.; 2001.
- Moyer RA, Hummer KE, Finn C, Frei B, Wrolstad RE. Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: vaccinium Rubus, and Ribes. *J Agric Food Chem*. 2002; 50:519–525. [PubMed: 11804523]
- Olaya CM, Castaño MP, Garzón GA. Effect of temperature and water activity on the stability of microencapsulated anthocyanins extracted from Andes berry (*Rubus glaucus*) and Tamarillo (*Solanum betaceum*). *Acta Biol Col*. 2009; 14:143–158.
- Piljac-Žegarac J, Valek L, Martinez S, Belš ak A. Fluctuations in the phenolic content and antioxidant capacity of dark fruit juices in refrigerated storage. *Food Chem*. 2009; 113:394–400.
- Plumb GW, De Pascual T, Santos-Buelga S, Cheynier C, Williamson VG. Antioxidant properties of catechins and proanthocyanidins: effect of polymerization, galloylation and glycosylation. *Free Radical Res*. 1998; 29:351–358. [PubMed: 9860050]
- Prior, RL. Absorption and metabolism of anthocyanins: potential health effects. In: Meskin, MS.; Bidlack, WR.; Davies, AJ.; Lewis, DS.; Randolph, RK., editors. *Phytochemicals: mechanisms of action*. Boca Raton, Fla: CRC Press, Inc.; 2004.
- Ré MI. Microencapsulation by spray drying. *DryingTech*. 1998; 16:1195–1236.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol Med*. 1999; 26:1231–1233. [PubMed: 10381194]
- Ross, YH. Phase transitions in foods. San Diego, Calif: Academic Press; 1995.
- Sarni P, Fulcrand H, Souillol V, Souquet J-M, Cheynier V. Mechanisms of anthocyanin degradation in grape must-like model solutions. *J Sci Food Agric*. 1995; 69:385–391.
- Seeram NP, Aviram Y, Henning SM, Feng L, Dreher M, Heber D. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *J Agric Food Chem*. 2008; 56:1415–1422. [PubMed: 18220345]
- Serris GS, Biliaderis CG. Degradation kinetics of beetroot pigment encapsulated in polymeric matrices. *J Sci Food and Agric*. 2001; 81:691–700.
- Singleton V, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymol*. 1999; 299:152–175.
- Tonon RV 1, Brabet C, Pallet D, Brat P, Hubinger MD. Physicochemical and morphological characterisation of açai (*Euterpe oleracea* Mart.) powder produced with different carrier agents. *Food Res Int*. 2009; 44:1950–1958.
- Tonon RV, Brabet C, Hubinger MD. Anthocyanin stability and antioxidant activity of spray-dried açai (*Euterpe oleracea* Mart.) juice produced with different carrier agents. *Food Res Int*. 2010; 43:907–914.
- Vinson JA, Su X, Zubik L, Bose P. Phenol antioxidant quantity and quality in foods: fruits. *J Agric Food Chem*. 2001; 49:5315–5321. [PubMed: 11714322]

- Walkowiak-Tomczak D, Czapski J. Colour changes of a preparation from red cabbage during storage in a model system. *Food Chem.* 2007; 104:709–714.
- Waterhouse, L. Determination of total phenolics. In: Wrolstad, RE.; Acree, TE.; An, H.; Decker, EA.; Penner, MH.; Reid, DS.; Schwartz, SJ.; Shoemaker, CF.; Sporns, P., editors. *Current protocols in food analytical chemistry*. New York, N.Y.: JohnWiley and Sons, Inc.; 2001. p. 46-470.
- Woodward G, Kroon P, Cassidy A, Kay C. Anthocyanin stability and recovery: implications for the analysis of clinical and experimental samples. *J Agric Food Chem.* 2009; 57:5271–5278. [PubMed: 19435353]
- Wrolstad, RE. Anthocyanins. In: Lauro, GJ.; Francis, FJ., editors. *Natural food colorants*. New York: Marcel Dekker, Inc.; 2000.
- Wrolstad RE. Anthocyanin pigments-bioactivity and coloring properties. *J Food Sci.* 2004; 69:C419–C425.
- Zhang L, Mou D, Du Y. Procyanidins: extraction and micro-encapsulation. *J Sci Food Agric.* 2006; 87:2192–2219.

Figure 1.

Changes in anthocyanin content during storage of isotonic model beverages. ◆ colored with freeze-dried anthocyanins with maltodextrin, stored under light. ▲ colored with freeze-dried anthocyanins with maltodextrin, stored in the dark. ● colored with freeze-dried anthocyanins, stored in the dark. ■ colored with freeze-dried anthocyanins, stored under light.

Figure 2.

(A) Representative HPLC profile of individual ACNs of model beverages colored with Andes berry pigment A. Beverages at the beginning of the storage. (B) Beverages colored with freeze-dried anthocyanins with maltodextrin, stored in the dark. (C) Beverages colored with freeze-dried anthocyanins with maltodextrin stored under light. (D) Beverages colored with freeze-dried anthocyanins, stored in the dark. (E) Beverages colored with freeze-dried anthocyanins, stored under light.

Figure 3.

Changes in peak area during storage of model beverages. *Significantly different from initial storage profile ($P < 0.05$). Pigment/illumination conditions: MFDA D = freeze-dried anthocyanins with maltodextrin stored in the dark; MFDA I = freeze-dried anthocyanins with maltodextrin stored under illumination; FDA D = freeze-dried anthocyanins, stored in the dark; FDA I = freeze-dried anthocyanins, stored under illumination.

Changes in CIE parameters for isotonic model beverages colored with Andes berry anthocyanins and red nr 40 during storage.^{1,2,3}

Table 1

Pigment/ illumination condition	Days	Lightness (L*)	Chroma (C*)	Hue angle (h°)	Color difference (ΔE*)
FDA I	0	77.1 ± 2.4 ^a	53.0 ± 4.0 ^a	0.21 ± 0.03 ^a	
	71	86.1 ± 0.3 ^{bA}	29.4 ± 0.2 ^{bA}	0.19 ± 0.01 ^{aA}	25.1 ± 4.2 ^a
FDA D	0	77.6 ± 3.3 ^a	54.2 ± 3.1 ^a	0.21 ± 0.02 ^a	
	71	81.2 ± 0.7 ^{bB}	43.7 ± 0.5 ^{bB}	0.14 ± 0.00 ^{bB}	11.6 ± 3.4 ^b
MFDA I	0	75.2 ± 1.3 ^a	54.2 ± 2.1 ^a	0.21 ± 0.01 ^a	
	71	79.5 ± 1.6 ^{bC}	37.8 ± 0.4 ^{bC}	0.11 ± 0.00 ^{bC}	19.0 ± 2.0 ^c
MFDA D	0	75.4 ± 0.1 ^a	53.7 ± 2.3 ^a	0.21 ± 0.02 ^a	
	71	78.8 ± 0.5 ^{bC}	46.0 ± 0.3 ^{bD}	0.16 ± 0.00 ^{bD}	8.9 ± 2.7 ^b
Red nr 40 I	0	76.8 ± 2.5 ^a	60.0 ± 2.1 ^a	0.29 ± 0.02 ^a	
	71	79.3 ± 0.4 ^{bC}	51.1 ± 0.2 ^{bE}	0.19 ± 0.00 ^{bE}	11.4 ± 1.0 ^b
Red nr 40 D	0	76.6 ± 1.5 ^a	59.3 ± 0.0 ^a	0.31 ± 0.02 ^a	
	71	78.4 ± 0.3 ^{bC}	61.2 ± 0.3 ^{bF}	0.30 ± 0.01 ^{aF}	5.6 ± 3.1 ^d

¹ Values are means ± standard deviation (*n* = 3).

² Means in a column within the same treatment followed by different lowercase letters are significantly different using Fisher's least significant difference at *P* < 0.05.

³ Means in a column among different treatments followed by different uppercase letters are significantly different using Fisher's least significant difference at *P* < 0.05.

FDA I = beverage colored with freeze-dried anthocyanin stored under illumination.

FDA D = beverage colored with freeze-dried anthocyanin stored in the dark.

MFDA I = beverage colored with freeze-dried anthocyanin with maltodextrin stored under illumination.

MFDA D = beverage colored with freeze-dried anthocyanin with maltodextrin stored in the dark.

Table 2

Linear regression parameters for 1st-order kinetics degradation of freeze-dried Andes berry anthocyanins in isotonic model beverages.^{1,2}

Pigment/ illumination condition	r	β_0	β_1 rate of degradation (days ⁻¹)	$t_{1/2}$ (d)
MFDA D	-0.985	0.021 ± 0.002^a	-0.058 ± 0.004^a	32.0 ± 0.5^a
FDA D	-0.974	-0.013 ± 0.014^b	-0.140 ± 0.007^b	25.0 ± 0.4^b
MFDA I	-0.978	0.072 ± 0.041^c	-0.072 ± 0.004^c	27.0 ± 0.4^c
FDA I	-0.983	-0.010 ± 0.014^b	-0.184 ± 0.011^d	11.0 ± 0.3^d

¹ Values are means $\pm$ standard error ($n = 3$).

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 = intercept.

β_1 = slope.

FDA I = beverage colored with freeze-dried anthocyanin stored under illumination.

FDA D = beverage colored with freeze-dried anthocyanin stored in the dark.

MFDA I = beverage colored with freeze-dried anthocyanin with maltodextrin stored under illumination.

MFDA D = beverage colored with freeze-dried anthocyanin with maltodextrin stored in the dark.

Table 3

Changes in antioxidant activity and total phenolic content of isotonic model beverages colored with Andes berry anthocyanins.^{1,2}

Pigment/ illumination condition	d	FRAP (μ moles TE/mL)	FRAP (μ moles Fe ³⁺ /mL)	TEAC (μ moles TE/mL)	Total phenolics (mg GAE/L)
MFDA I	0.5	5.95 $\pm$ 0.08 ^a	6.17 $\pm$ 0.18 ^a	0.99 $\pm$ 0.05 ^a	89.8 $\pm$ 3.7 ^a
	8.0	5.36 $\pm$ 0.09 ^b	5.62 $\pm$ 0.08 ^b	0.84 $\pm$ 0.09 ^b	73.6 $\pm$ 9.1 ^{ab}
	29.0	3.93 $\pm$ 0.21 ^c	5.14 $\pm$ 0.30 ^c	0.57 $\pm$ 0.00 ^c	92.1 $\pm$ 6.7 ^{ab}
	41.0	2.54 $\pm$ 0.55 ^d	3.76 $\pm$ 0.22 ^d	0.53 $\pm$ 0.00 ^{cd}	84.6 $\pm$ 2.4 ^{bc}
	53.0	2.74 $\pm$ 0.20 ^d	2.73 $\pm$ 0.02 ^e	0.52 $\pm$ 0.01 ^{cd}	75.2 $\pm$ 4.6 ^{bc}
	62.5	2.46 $\pm$ 0.08 ^d	2.65 $\pm$ 0.17 ^e	0.48 $\pm$ 0.02 ^{cd}	67.9 $\pm$ 5.2 ^c
	71.0	2.26 $\pm$ 0.02 ^{ad}	2.52 $\pm$ 0.13 ^e	0.46 $\pm$ 0.02 ^d	86.7 $\pm$ 7.5 ^c
MFDA D	0.5	5.67 $\pm$ 0.18 ^a	6.25 $\pm$ 0.03 ^a	0.90 $\pm$ 0.00 ^a	92.3 $\pm$ 7.2 ^{ac}
	8.0	4.66 $\pm$ 0.09 ^b	6.25 $\pm$ 0.10 ^a	0.90 $\pm$ 0.00 ^a	66.2 $\pm$ 3.0 ^b
	29.0	3.81 $\pm$ 0.43 ^c	5.24 $\pm$ 0.24 ^b	0.66 $\pm$ 0.00 ^b	95.1 $\pm$ 2.3 ^a
	41.0	2.05 $\pm$ 0.29 ^d	3.63 $\pm$ 0.69 ^c	0.61 $\pm$ 0.00 ^c	89.8 $\pm$ 3.9 ^{ac}
	53.0	1.86 $\pm$ 0.20 ^d	3.95 $\pm$ 0.23 ^c	0.61 $\pm$ 0.00 ^c	77.6 $\pm$ 4.9 ^d
	62.5	1.71 $\pm$ 0.18 ^d	2.74 $\pm$ 0.09 ^d	0.56 $\pm$ 0.00 ^d	85.4 $\pm$ 1.1 ^{cd}
	71.0	1.59 $\pm$ 0.21 ^d	2.41 $\pm$ 0.21 ^d	0.53 $\pm$ 0.00 ^e	82.7 $\pm$ 3.1 ^{cd}
FDA I	0.5	5.03 $\pm$ 0.34 ^a	5.12 $\pm$ 0.19 ^a	0.58 $\pm$ 0.01 ^a	94.3 $\pm$ 10.1 ^{abc}
	8.0	4.65 $\pm$ 0.04 ^a	5.51 $\pm$ 0.16 ^a	0.55 $\pm$ 0.01 ^a	65.4 $\pm$ 8.5 ^b
	29.0	2.55 $\pm$ 0.42 ^b	2.53 $\pm$ 0.18 ^b	0.51 $\pm$ 0.06 ^a	80.8 $\pm$ 3.5 ^{ab}
	41.0	2.20 $\pm$ 0.73 ^{bc}	2.30 $\pm$ 0.15 ^b	0.47 $\pm$ 0.04 ^b	80.3 $\pm$ 4.0 ^{abc}
	53.0	1.85 $\pm$ 0.15 ^{cd}	2.49 $\pm$ 0.35 ^b	0.42 $\pm$ 0.02 ^{bc}	71.3 $\pm$ 7.3 ^c
	62.5	1.57 $\pm$ 1.78 ^{da}	1.78 $\pm$ 0.22 ^c	0.39 $\pm$ 0.00 ^c	61.1 $\pm$ 8.66 ^c
	71.0	1.40 $\pm$ 0.11 ^d	1.66 $\pm$ 0.06 ^c	0.36 $\pm$ 0.01 ^c	68.2 $\pm$ 1.7 ^c
FDA D	0.5	5.75 $\pm$ 0.34 ^a	6.01 $\pm$ 0.18 ^a	0.87 $\pm$ 0.11 ^a	101.4 $\pm$ 14.9 ^a
	8.0	4.70 $\pm$ 0.35 ^b	5.49 $\pm$ 0.30 ^a	0.81 $\pm$ 0.00 ^a	69.6 $\pm$ 6.2 ^{bd}
	29.0	2.29 $\pm$ 0.21 ^{cd}	4.56 $\pm$ 0.45 ^b	0.51 $\pm$ 0.00 ^b	94.0 $\pm$ 8.3 ^{ac}
	41.0	1.90 $\pm$ 0.04 ^{cd}	2.95 $\pm$ 0.45 ^c	0.46 $\pm$ 0.11 ^{bc}	77.7 $\pm$ 7.7 ^{bcd}
	53.0	1.80 $\pm$ 0.19 ^{cd}	2.82 $\pm$ 0.20 ^c	0.44 $\pm$ 0.00 ^{bc}	70.2 $\pm$ 2.2 ^{bd}

Pigment/ illumination condition	d	FRAP (μ moles TE/mL)	FRAP (μ moles Fe ²⁺ /mL)	TEAC (μ moles TE/mL)	Total phenolics (mg GAE/L)
	62.5	1.58 $\pm$ 0.06 ^d	1.80 $\pm$ 0.04 ^d	0.42 $\pm$ 0.00 ^{bc}	87.6 $\pm$ 3.1 ^{abc}
	71.0	1.52 $\pm$ 0.15 ^d	1.74 $\pm$ 0.12 ^d	0.40 $\pm$ 0.00 ^c	67.8 $\pm$ 3.7 ^d

¹ Data are reported as mean $\pm$ standard deviation ($n = 3$).

² Different 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.

FDA I = beverage colored with freeze-dried anthocyanin stored under illumination.

FDA D = beverage colored with freeze-dried anthocyanin stored in the dark.

MFDA I = beverage colored with freeze-dried anthocyanin with maltodextrin stored under illumination.

MFDA D = beverage colored with freeze-dried anthocyanin with maltodextrin stored in the dark.

Table 4

Regression models and correlation coefficients between monomeric anthocyanin content and antioxidant activity of isotonic model beverages colored with Andes berry anthocyanins.¹

Antioxidant assay	Pigment/illumination condition	Model ²	<i>r</i>	<i>P</i> -value
FRAP (μmoles TE/mL)	MFDA I	$Y = -2.296 + 0.383 X (0.501) (0.035)$	0.954	0.000
	MFDA D	$Y = -5.861 - 0.569 X (1.175) (0.070)$	0.920	0.000
	FDA I	$Y = -0.660 + 0.274X (0.440) (0.034)$	0.951	0.000
	FDA D	$Y = -5.115 + 0.524 X (1.185) (0.077)$	0.890	0.000
FRAP (μmoles Fe ⁺³ /mL)	MFDA I	$Y = -0.944 + 0.359 X (0.424) (0.029)$	0.963	0.000
	MFDA D	$Y = -5.397 + 0.585 X (1.372) (0.082)$	0.900	0.000
	FDA I	$Y = -0.457 + 0.283 X (0.554) (0.042)$	0.890	0.000
	FDA D	$Y = -4.810 + 0.558 X (0.938) (0.061)$	0.935	0.000
ABTS (μmoles TE/mL)	MFDA I	$Y = -0.020 + 0.046 X (0.080) (0.005)$	0.925	0.000
	MFDA D	$Y = -0.342 + 0.062 X (0.097) (0.006)$	0.950	0.000
	FDA I	$Y = 0.282 + 0.015 X (0.027) (0.002)$	0.908	0.000
	FDA D	$Y = -0.388 + 0.625 X (0.117) (0.008)$	0.921	0.000

¹ Data are reported as mean ± standard error (*n* = 3).

² Model Y = antioxidant activity; X = ACN concentration (mg ACN/mL).

r = Pearson correlation coefficient.

P-value = significance levels of regression models.

Values in parenthesis are standard errors of regression coefficients.

FDA I = beverage colored with freeze-dried anthocyanin stored under illumination.

FDA D = beverage colored with freeze-dried anthocyanin stored in the dark.

MFDA I = beverage colored with freeze-dried anthocyanin with maltodextrin stored under illumination.

MFDA D = beverage colored with freeze-dried anthocyanin with maltodextrin stored in the dark.

Table 5

Regression models and correlation coefficients between total phenols content and antioxidant activity of isotonic model beverages colored with Andes berry anthocyanins.¹

Antioxidant assay	Pigment/illumination condition	Model ²	<i>r</i>	<i>P</i> -value
FRAP (μmoles TE/mL)	MFDA I	$Y = -0.753 + 0.047 X$ (0.047) (0.045)	0.288	0.318
	MFDA D	$Y = -4.847 - 0.014 X$ (3.527) (0.042)	-0.099	0.735
	FDA I	$Y = -0.952 + 0.050 X$ (2.330) (0.031)	0.178	0.0133
	FDA D	$Y = -0.673 + 0.043 X$ (2.612) (0.032)	0.362	0.204
FRAP (μmoles Fe ⁺³ /mL)	MFDA I	$Y = -0.676 + 0.058 X$ (3.302) (0.040)	0.386	0.1723
	MFDA D	$Y = 5.820 - 0.017 X$ (3.752) (0.044)	-0.113	0.027
	FDA I	$Y = 0.172 + 0.039 X$ (2.601) (0.035)	0.308	0.283
	FDA D	$Y = -0.536 + 0.051 X$ (2.572) (0.031)	0.428	0.127
ABTS (μmoles TE/mL)	MFDA I	$Y = -0.020 + 0.046 X$ (0.080) (0.005)	0.925	0.000
	MFDA D	$Y = 0.939 - 0.003 X$ (0.369) (0.004)	-0.199	0.039
	FDA I	$Y = 0.190 + 0.004 X$ (0.112) (0.002)	0.571	0.033
	FDA D	$Y = 0.142 + 0.005 X$ (0.300) (0.004)	0.375	0.1866

¹ Data are reported as mean ± standard error (*n* = 3).

² Model *Y* = antioxidant activity; *X* = total phenolics (GAE/L).

r = Pearson correlation coefficient.

P-value = significance levels of regression models.

Values in parenthesis are standard errors of regression coefficient.

FDA I = beverage colored with freeze-dried anthocyanin stored under illumination.

FDA D = beverage colored with freeze-dried anthocyanin stored in the dark.

MFDA I = beverage colored with freeze-dried anthocyanin with maltodextrin stored under illumination.

MFDA D = beverage colored with freeze-dried anthocyanin with maltodextrin stored in the dark.