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Microencapsulation by Spray-Drying of Anthocyanin Pigments from Corozo (*Bactris guineensis*) Fruit

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The anthocyanins of *Bactris guineensis* fruit were isolated with the aid of high-speed countercurrent chromatography (HSCCC) and preparative HPLC, and their chemical structures were elucidated by using spectroscopic methods. Among the identified pigments, cyanidin-3-rutinoside and cyanidin-3-glucoside were characterized as major constituents (87.9%). Peonidin-3-rutinoside, peonidin-3-glucoside, cyanidin-3-(6-*O*-malonyl)glucoside, and cyanidin-3-sambubioside were present in minor amounts. Four anthocyanin ethanolic extracts (AEEs) were obtained by osmotic dehydration and Soxhlet extraction and physicochemically characterized. The composition of anthocyanins was monitored by HPLC-PDA. The extracts with the highest anthocyanin content were subjected to the spray-drying process with maltodextrin. The so-obtained spray-dried powders were analyzed by scanning electron microscopy (SEM) and found to consist of spherical particles <50 μm in size. The anthocyanin composition was similar to that of the fruit. The microencapsulated powders were analyzed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), revealing that they are quite stable until 100 °C. Storage stability tests of microcapsules showed that the release of anthocyanin pigments follows pseudo-first-order kinetics and that the process rate is increased by temperature and humidity. The most suitable conditions for storage were below 37 °C and <76% relative humidity, respectively.

KEYWORDS: Corozo (*Bactris guineensis*); anthocyanins; microencapsulation; spray-drying

INTRODUCTION

Bactris guineensis (Arecaceae), commonly known as corozo, corozo de lata, uvita de lata, or coyol, is a wild palm, which grows up to almost 15 feet in warm regions of Central/South America, between 200 and 1000 m above sea level. The purple-black fruits, hanging in clusters of 50−100, are ovoid in shape and about 2.5−3 cm in size (**Figure 1**). The sour pulp has normally a single seed with a hard, thin shell and a white kernel. This tropical fruit is found in the northern part of Colombia, where it is used to prepare juices or alcoholic drinks. To the best of our knowledge there are no reports about the chemical composition of this species.

The bright red or purple colors in fruits are in many cases due to the presence of anthocyanins (*1*). These secondary plant metabolites consist of C_{15} heterocyclic nucleus (2-phenylbenzopyrilium cation or anthocyanidin) bearing at least one sugar residue. The sugar moiety may be further esterified by aliphatic and/or aromatic organic acids, thus adding to the structural diversity of this important group of plant pigments. The six most frequently observed anthocyanidins in plants are cyanidin, delphinidin, pelargonidin, malvidin, peonidin, and petunidin (*2*).

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At present, there is a growing demand for exotic fruits and fruit-derived products because of their novel sensory properties and health-promoting attributes (*3*−*7*). Additionally, the interest of the food industry in natural colorants has increased significantly over the past years, mainly due to consumer preferences as well as safety issues (*8*). The use of artificial food colors and other food additives has been associated by consumers to toxicity issues; thus, it has been suggested that their consumption affects the behavior of children (*9*). The anthocyanin pigments are harmless and exhibit good water solubility, which makes them an important group of natural water-soluble colorants (*10*). Moreover, the use of these compounds as potential natural colorants has been recently increased due to the possibility to improve their stability (*11*, *12*).

Despite the importance of fruits as a source of natural food colorants, transformation of fruit juices into dry powders still constitutes a key challenge due to their high sugar and acid contents. A strategy to overcome this problem is the use of spray-drying technique. Spray-drying is a unit operation by which a liquid product is atomized in a hot gas current to instantaneously form a stable powder. The initial liquid feeding into the sprayer can be a solution, emulsion, or suspension. Depending on the starting fruit-derived material and operating conditions, spray-drying produces fine powders (10−50 μm) or large-size particles

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Figure 1. Corozo (*Bactris guineensis*) fruit, pulp, and seeds.

(2−3 mm) (*13*). Thus, several attempts in this field have been successful and recently published (*14*−*16*).

Fruits are highly perishable, which often hampers their handling and trade. The development of fruit-derived value-added products is a suitable strategy to overcome some of these problems. The present investigation is part of our current research on the development of natural color- and aroma-enriched microencapsulates from fruits (*17*, *18*). The main purpose of this work was the study of the chemical composition of the anthocyanin fraction from *B. guineensis* fruit and the establishment of a process of generating stable pigment-enriched microencapsulates.

MATERIALS AND METHODS

Plant Material. *B. guineensis* fruits were purchased at local markets in Montería (Córdoba, Colombia). They were frozen (−20 °C) and transported in freezing conditions the same day to Bogota by air freight for their analysis. Fully ripe fruits, characterized by a pH of 3.0 ± 0.2, an acidity content of 2.8 ± 0.5 (expressed as grams of citric acid per 100 g of fruit), and a sugar-to-water ratio of 14.0 ± 0.2 °Brix, were selected. A voucher specimen (COL 509133) was identified and deposited at the Instituto de Ciencias Naturales, Universidad Nacional de Colombia.

Solvents and Reagents. All solvents (Merck, analytical grade) were redistilled before use. For LC analyses, acetonitrile and formic acid were purchased from Merck (Darmstadt, Germany), and water was deionized using a Milli-Q water purification system (Milli-Q, Bedford, MA). For LC-MS analyses, acetonitrile, water, and formic acid were supplied by Honeywell Burdick and Jackson (Muskegon, MI). Trifluoroacetic acid (TFA) was obtained from Sigma-Aldrich (Munich, Germany), and deuterated solvents for NMR analyses were purchased from Deutero (Kastellaun, Germany).

Extraction, Purification, and Isolation of Anthocyanins. The frozen fruits (8 kg) of *B. guineensis* without seeds were extracted with methanol/acetic acid (19:1, v/v) at room temperature overnight. The solvent was removed under vacuum, and the residue was lyophilized. Lyophilized extract (176 g) was fractionated according to the method published by Degenhardt et al. (*19*). Portions of lyophilized extract (44 g) were diluted with 50 mL of water and passed through an Amberlite XAD-7 column. The column was washed with water, and the elution of anthocyanins was carried out with 1 L of a mixture of methanol/acetic acid (19:1, v/v). The eluate was concentrated under vacuum, and the residue was freeze-dried to obtain 18.1 g of an anthocyanin-rich extract (ARE).

The ARE (4.7 g) was fractionated by the all-liquid chromatographic technique of HSCCC using a CCC-1000 high-speed countercurrent chromatograph (Pharma-Tech Research Corp., Baltimore, MD) equipped with three coils connected in series (inner diameter of tubing = 2.6 mm, total volume approximately 850 mL). The solvent system, a mixture of TBME/*n*-butanol/acetonitrile/water (2:2.1:5, v/v/v/v, acidified with 0.1% TFA), was delivered with a Biotronik BT 3020 HPLC pump (Jasco, Gross-Umstadt, Germany). Revolution speed was set to 1000 rpm. The less dense layer was always used as the stationary phase, and the flow rate of the mobile phase was 4.0 mL/min. XAD-7 extract (4.7 g) was dissolved and injected into the system via a 20 mL sample loop. Elution was monitored at 520 nm by a Knauer UV−vis detector, and chromatograms were recorded with a Knauer L250E plotter (Knauer, Berlin, Germany). Seventy fractions (16 mL each one) were collected with a Super Frac fraction collector (Pharmacia LKB, Bromma, Sweden) and pooled as follows: FI (32−36), FII (38−41), FIII (43−47), FIV (51−54), FV (56−60), FVI (62−69), and FVII (stationary phase).

Table 1. Chromatographic and Spectral Data of the Anthocyanin-Rich Extract from Corozo (*Bactris guineensis*) Fruit

compd[a]	fraction (HSCCC)	t_R[b] (min) (HPLC)	[M$^+$] (*m*/*z*) HRMS	MS/MS (*m*/*z*)	content[c] (%)
1	FII	20.07	581.1467	287, 449	1.8
2	FVI	21.48	449.1045	287	15.7
3	FIII	23.09	595.1631	287, 449	72.2
4	FVI	29.90	463.1161	301	1.1
5	FIII	30.35	609.1779	301, 463	5.1
6	FVII	31.56	535.1091	287	4.1

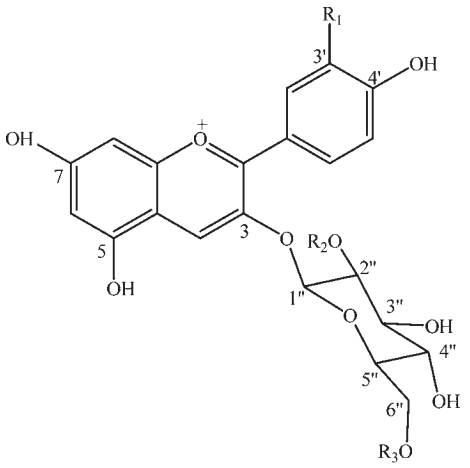
[a] For numbers of compounds cf. **Figure 2**. [b] Retention time in Luna RP-18 column (flow rate 0.5 mL/min). [c] Relative anthocyanin amount.

High-Performance Liquid Chromatography (HPLC). The composition of anthocyanins in ARE and each fraction was monitored by HPLC-PDA using a Jasco PU-980 Intelligent HPLC pump, a DG-980-50 3-Line degasser, an LG-980-02 ternary gradient unit, and a MD-1510 multiwavelength detector (Jasco). Sample was injected via a Rheodyne 7175 injection valve (Techlab, Erkerode, Germany) equipped with a 20 μL sample loop. Chromatograms were recorded with Jasco−Borwin chromatography software version 1.50. Separations were carried out on a Luna RP-18 column (250 × 4.6 mm, 5 μm, Phenomenex, Aschaffenburg, Germany). Solvent system was a mixture of water/formic acid/acetonitrile (87/10/3, v/v/v, solvent A; 40/10/50, v/v/v, solvent B), and the flow rate was 0.5 mL/min. A linear gradient from 6 to 20% B at 0−20 min, from 20 to 40% B at 20−35 min, from 40 to 60% B at 35−40 min, and from 60 to 90% B at 40−45 min was used. Six anthocyanins, **1** (7.5 mg), **2** (9.2 mg), **3** (59.4 mg), **4** (2 mg), **5** (3.2 mg), and **6** (3.3 mg), were detected and purified by preparative HPLC (**Table 1**), using a Knauer HPLC pump 64 and a Knauer UV−vis detector. Samples were injected via a Rheodyne 7175 injection valve (Techlab, Erkerode, Germany) equipped with a 400 μL sample loop on a Luna RP-18 column (250 × 10 mm, 5 μm, Phenomenex). The solvent system was an isocratic mixture of 90% solvent A and 10% solvent B at flow rate of 5.0 mL/min. Purified anthocyanins from *B. guineensis* were identified on the basis of their retention times, HPLC-ESI-MS^n analyses, and ^{1}H and ^{13}C NMR spectral data.

High-Performance Liquid Chromatography−Electrospray Ionization Multiple Mass Spectrometry (HPLC-ESI-MS^n). HPLC-MS analyses of fractions and purified anthocyanins were performed on a Bruker Esquire LC-MS system (Bruker Daltonik, Bremen, Germany). The HPLC system consisted of a System 1100 binary pump G1312A (Agilent, Böblingen, Germany) and a Lichrograph L-4000 UV−vis detector (Merck Hitachi, Tokyo, Japan). UV chromatograms were recorded with a Chromatopac C-R6A integrator (Shimadzu, Kyoto, Japan); the LC part of the system was controlled by ChemStation version A.06.01, and MS data were processed by Esquire NT 4.0 software (Bruker Daltonik). MS parameters were as follows: positive mode; capillary −2500 V; end plate offset, −500 V; capillary exit, 70 V; skim, 120 V; skim, 210 V; dry gas, 325 °C; gas flow, 11 L/min; nebulizer, 60 psi.

For high-resolution electrospray mass spectrometry (HRESIMS) of pure compounds, a Shimadzu liquid chromatograph−ion trap−time of flight mass spectrometer (LC-MS-IT-TOF, Kyoto, Japan) was used. Pure compounds (**1**−**6**) were dissolved in acetonitrile/water/formic acid (2:1:0.004, v/v/v) and at a concentration of 0.5 mg/mL. The sample solutions were injected directly into the system. The MS/MS parameters were as follows: positive mode; CDL temperature, 200 °C; heating block, 200 °C; detector voltage, 1.55 kV; flow rate, 1.5 L/min; ion accumulation, 20 ms; scan range, *m*/*z* 200−1600. The energy of the collision gas (argon) was fixed at 15%. LC-MS Solution software was used for data collection and analysis.

Proton and Carbon Magnetic Resonance (NMR). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 spectrometer (Bruker Biospin, Rheinstetten, Germany) at 300.13 and 75.49 MHz, respectively.



	R_1	R_2	R_3
1	OH	xylosyl	H
2	OH	H	H
3	OH	H	rhamnosyl
4	OCH_3	H	H
5	OCH_3	H	rhamnosyl
6	OH	H	malonyl

Figure 2. Chemical structures of the anthocyanins identified in *B. guineensis* fruit: cyanidin-3-sambubioside (**1**), cyanidin-3-glucoside (**2**), cyanidin-3-rutinoside (**3**), peonidin-3-glucoside (**4**), peonidin-3-rutinoside (**5**), and cyanidin-3-(6-*O*-malonyl)glucoside (**6**).

Anthocyanins were dissolved in a mixture of methanol-d_4/TFA-d_1 (19:1, v/v). Data were processed by WIN-NMR software version 6.1.0.0.

Spectroscopic Data. In this section the spectral data of compounds **1**−**7** are reported. Numbering of compounds is given in **Figure 2**.

Cyanidin-3-O-sambubioside (***1***)*:* HRESIMS 581.1467 [calcd for $C_{26}H_{29}O_{15}$ $(M)^+$, 581.1501].; ESI-MS/MS data, see **Table 1**; ^{1}H and ^{13}C NMR data, in agreement with those published by Johansen et al. (*20*).

Cyanidin-3-O-β-glucopyranoside (***2***)*:* HRESIMS 449.1045 [calcd for $C_{21}H_{21}O_{11}$ $(M)^+$, 449.1078]; ESI-MS/MS data, see **Table 1**; ^{1}H NMR [300 MHz, CD_3OD/CF_3CO_2D, 19:1, v/v) δ 9.02 (1 H br s, 4-H), 8.25 (1H, dd, J = 8.8, 2.3 Hz, 6′-H), 8.04 (1H, d, J = 2.3 Hz, 2′-H), 7.02 (1H, d, J = 8.7 Hz, 5′-H), 6.89 (1H, dd, J = 1.9, 0.7 Hz, 8-H), 6.66 (1H, d, J = 1.9 Hz, 6-H), 5.24 (1H, d, J = 7.7 Hz, 1″-H), 3.92 (1H, dd, J = 12.1, 2.2 Hz, 6a″-H), 3.71 (1H, dd, J = 12.1, 5.8 Hz, 6b″-H), 3.65 (1H, m, 2″-H), 3.56 (1H, ddd, J = 9.6, 5.9, 2.1 Hz, 5″-H), 3.54 (1H, t, J = 8.9 Hz, 3″-H), 3.44 (1H, dd, J = 9.5, 8.7 Hz, 4″-H); ^{13}C NMR data, in agreement with those published in the literature (*21*).

Cyanidin-3-O-rutinoside (***3***)*:* HRESIMS 595.1631 [calcd for $C_{27}H_{31}O_{15}$ $(M)^+$, 595.1663]; ESI-MS/MS data are in **Table 1**; ^{1}H NMR [300 MHz, CD_3OD-CF_3CO_2D, 19:1, v/v) δ 8.93 (1 H, br s, 4-H), 8.25 (1H, dd, J = 8.7, 1.7 Hz, 6′-H), 8.07 (1H, d, J = 1.8 Hz, 2′-H), 7.01 (1H, d, J = 8.8 Hz, 5′-H), 6.88 (1H, br s, 8-H), 6.67 (1H, br s, 6-H), 5.27 (1H, d, J = 7.0 Hz, 1″-H), 4.65 (1H, br s, 1‴-H), 4.06 (1H, dd, J = 10.7, 1.6 Hz, 6b″-H), 3.79 (1H, m, 2‴-H), 3.69 (1H, dd, J = 8.8, 3.7 Hz, 3‴-H), 3.62 (1H, dd, J = 10.1, 3.6 Hz, 6a″-H), 3.41 (1H, t, J = 9.1 Hz, 3″-H), 3.20−3.65 (5H, m, 2″-H, 4″H, 5″-H, 4‴-H, 5‴-H), 1.16 (3H, d, J = 6.2 Hz, 6‴-H_3); ^{13}C NMR data, in agreement with those published by Fossen and Øvstedal (*22*).

Peonidin-3-glucoside (***4***)*:* HRESIMS 463.1161 [calcd for $C_{22}H_{23}O_{11}$ $(M)^+$, 463.1240]; ESI-MS/MS data, see **Table 1**.

Peonidin-3-rutinoside (***5***)*:* HRESIMS 609.1779 [calcd for $C_{28}H_{33}O_{15}$ $(M)^+$, 609.1819]; ESI-MS/MS data, see **Table 1**; ^{1}H NMR [300 MHz, CD_3OD/CF_3CO_2D, 19:1, v/v) δ 8.99 (1 H, s, 4-H), 8.28 (1H, dd, J = 8.7, 2.1 Hz, 6′-H), 8.23 (1H, d, J = 2.1 Hz, 2′-H), 7.08 (1H, d, J = 8.7 Hz, 5′-H), 6.94 (1H, d, J = 1.5 Hz, 8-H), 6.68 (1H, d, J = 1.8 Hz, 6-H), 5.29 (1H, d, J = 7.6 Hz, 1″-H), 4.65 (1H, br s, 1‴-H), 4.05 (1H, dd, J = 10.7, 1.6 Hz, 6b″-H), 4.02 (3H, br s, OCH_3), 3.78 (1H, dd, J = 8.8, 1.4 Hz, 2‴-H), 3.69 (1H, dd, J = 8.8, 2.9 Hz, 3‴-H), 3.62 (1H, dd, J = 10.9, 3.4 Hz, 6a″-H), 3.48−3.76 (4H, m, 4″-H, 5″-H, 4‴-H, 5‴-H), 3.40 (1H, t, J = 9.1 Hz, 3″-H), 3.20−3.40 (1H, m, 2″-H), 1.15 (3H, d, J = 6.2 Hz, 6‴-H_3); ^{13}C NMR data, in agreement with those published by Fossen and Øvstedal (*22*).

Cyanidin-3-O-(6″-malonyl)glucopyranoside (***6***)*:* HRESIMS 535.1091 [calcd for $C_{24}H_{23}O_{14}$ $(M)^+$, 535.1088]; ESI-MS/MS data, see **Table 1**; ^{1}H data, in agreement with those published in the literature (*22*).

Preparation and Characterization of Anthocyanin Ethanolic Extracts (AEEs). Ethanolic extracts of anthocyanins to be spray-dried were prepared in triplicate by two methods: osmotic dehydration (*23*) and Soxhlet extraction. For the first one, the fruits (ca. 500 g) were thawed, washed, and put in polyethylene bags (20 × 30 cm) to allow the dehydration process. Two different osmotic agents, ethanol and ethanol/citric acid (19:1, v/v), were separately added to the bags in a ratio of 1:3 (fruit/extraction solvent, w/w) at 18 °C and then put under continuous stirring (80 rpm) during 4 h to get OE and OC extracts, respectively. Additionally, two more extracts were obtained by Soxhlet extraction with ethanol and ethanol/citric acid (19:1, v/v) during 2 h (ratio fruit/solvent 1:3, w/w) to get SE and SC extracts, respectively. For comparison, the fruit juice (FJ) was obtained by boiling of whole fruits in water (ratio 1:3) during 1 h.

For the AEEs the following physicochemical parameters were measured: pH (Schott CG820 pH-meter), density, and soluble solids (°Brix) at 20 °C (Abbe Atago 8682 refractometer). In addition, anthocyanin content and color measurement were performed. All treatments were evaluated in triplicates from three independent experiments done in each case.

The composition of anthocyanins in each AEE was monitored by HPLC-PDA (Merck-Hitachi) using an L-6200A intelligent pump, an interface D-6000, and a Merck-Hitachi L-4500 multiwavelength detector. Samples were injected via a Rheodyne injection valve equipped with a 50 μL sample loop. Separations were carried out on a Luna RP-18 5 μm column (250 × 4.6 mm i.d., Phenomenex). The same gradient described above was used, but the flow rate was set at 0.8 mL/min.

Microencapsulation of *B. guineensis* Anthocyanins. The AEEs (OC and SC) were concentrated until one-fourth of initial volume, separately combined with corn maltodextrin DE 19−20 in a ratio of 1/1 (w/w), and stirred until homogeneity. For each case, 1.2 L of feed mixture was prepared. The feed mixtures were separately spray-dried using a Niro Mobile Minor 2682 spray-dryer, operated at an air inlet temperature of 120 °C, an outlet temperature of 80 °C, and a feed flow rate of 10 mL/min. During the drying process, the temperature of the feed mixture was 18 °C. In this way, the microencapsulated anthocyanin powders (MOC and MSC) were obtained. The anthocyanin composition of those microencapsulates (1 g/mL) was evaluated by HPLC-PDA as described above for the AEEs.

Moisture Content. Moisture content of the powder was determined gravimetrically by drying in an oven at 105 °C until constant weight (*24*).

Particle Morphology. The morphology of the microcapsules was evaluated using a scanning electronic microscope JEOL T 120 model (Tokyo, Japan). Samples were coated with gold−palladium under vacuum (1.2×10^{-5} Torr) before examination. SEM was operated at 30 kV. A Nikon Eclipse E-600 phase contrast microscope was also used to analyze the microencapsulated anthocyanins obtained by spray-drying.

Thermal Analysis. Thermogravimetric measurements of microencapsulated anthocyanins (10 mg) and maltodextrin (encapsulating agent) were carried out with a Thermogravimetric Analyzer TGA 2050 (TA Instruments; accuracy, ±0.1%; resolution, 0.2 μg) coupled with a Dupont 990 thermal analyzer. The powders were also analyzed by using a differential scanning calorimeter (DSC 2910, TA Instruments). TA Instruments software (Universal V2.5H) was used for data collection and for determination of temperatures from DSC curves. The apparatus was calibrated with high-purity indium. The experiments were performed under a nitrogen flow. Samples (3 mg) of microencapsulates and maltodextrin were heated from 0 to 200 °C in aluminum crucibles with a linear heating rate of 10 °C/min. As a reference material, an empty aluminum crucible was used each time.

Color Measurements. Visible absorption spectra of AEEs were recorded between 380 and 770 nm on a HP 8452A spectrophotometer, using a 5 mm path length glass cell. To obtain tristimulus values, the weighed-ordinated method (constant intervals, $\Delta\lambda$ = 2 nm) was applied, with the CIE standard illuminant D_{65} and standard observer (10° visual field)

considered as reference. The CIELAB parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) were determined by using CromaLab software (*25*). The color of the microcapsules was determined by diffuse reflectance, using a Hunterlab Labscan XE colorimeter (1.00 in diameter light pass, nominal standardization), and CIE $L^*a^*b^*$ coordinates (D_{65}, 10°) were obtained.

Total Anthocyanin Content. The concentration of anthocyanins in the AEEs of *B. guineensis* and microencapsulates was determined by the spectrophotometric pH-differential method (*26*). Dilutions were prepared in 0.025 M potassium chloride and in 0.4 M sodium acetate, adjusted to pH 1.0 and 4.5 with HCl, respectively. The absorbance of each dilution was measured at 520 and 700 nm against a distilled water blank using a HP 8452A spectrophotometer. The total monomeric anthocyanin content was calculated as cyanidin-3-glucoside equivalents (in mg) per gram of fruit or solid (ε value of cyanidin-3-glucoside dissolved in 0.1% HCl in methanol was 26900 L cm^{-1} mol^{-1}, and the molar mass was 449.2 g mol^{-1}) (*26*).

Storage Stability Test. The *B. guineensis* microencapsulated anthocyanins, MOC and MSC, were stored at controlled temperature and humidity and in the absence of light for 40 days. Samples of 3 g of each powder were transferred to closed low-density polyethylene bags (5 cm × 5 cm). The experimental design was a $2^2 \times 3 \times 8$ complete factorial in a random design. The independent variables considered were the humidity (76 and 95%), temperature (18, 50, and 70 °C), and time of storage (5, 10, 15, 20, 25, 30, 35, and 40 days). The samples were placed on sealed desiccators containing saturated NaCl and $Na_2HPO_4 \cdot 12H_2O$ solutions to obtain humidity values of 76 ± 2 and 95 ± 2%, respectively. The humidity was measured with a thermo-Hygrometer (Extech Instruments). The dependent variable was the anthocyanin content, determined by pH-differential method on solutions of 1 g of solid/1.5 mL of water, in triplicate, removing samples every 5 days. Taking into account previous results (*15*), the first-order kinetic model was assumed for the release of anthocyanins encapsulated in maltodextrin during storage. To determine the release rate constant (k_{obsd}), the $\ln(C_0/C_t)$ was plotted against time (days), and a linear regression analysis was used, following eq 1

$$\ln(C_0/C_t) = kt \quad (1)$$

where k is the slope, C_0 is the initial anthocyanin content (as mg of cy-3-glu equiv/g of solid), C_t is the anthocyanin content at a specific time, and t is time (days). Half-life times ($t_{1/2}$) were calculated from eq 2

$$t_{1/2} = \ln(2)/k \quad (2)$$

where k is the rate constant.

Statistical Analyses. Significant differences between treatments were determined by using analysis of variance (ANOVA). Data were analyzed using a SAS package (SAS Institute 1999). Means were compared using Duncan's multiple-range test ($P < 0.05$). Significant correlations between anthocyanin content and phenolic content or antioxidant activity were calculated by simple linear regression analysis.

RESULTS AND DISCUSSION

Anthocyanins from Corozo (*Bactris guineensis*) Fruit. The freeze-dried aqueous extract of *B. guineensis* fruits was cleaned up by adsorption on Amberlite XAD-7 resin, and the ARE, which mainly contains the polyphenolic substances including the target pigments, was analyzed by HPLC-PDA and HPLC-ESI-MS^n. These analyses showed that the pigment composition of *B. guineensis* is a mixture of two major and four minor anthocyanins. To clearly identify these pigments by NMR and MS, the ARE was fractionated by HSCCC. This gentle all-liquid chromatographic technique takes place in a multilayer coil that is made by wrapping an inert Teflon tubing around a holder in multiple layers (*21*) and allows preparative separations up to 2 g per run without loss or degradation of compounds. Thus, this preliminary purification step yielded seven fractions (FI−FVII), including a stationary phase. The anthocyanin composition of each fraction was then evaluated by HPLC-PDA and HPLC-ESI-MS^n. These results as well as the relative composition of the extract calculated from peak areas obtained at 520 nm are presented in **Table 1**. HPLC-ESI-MS^n analysis provided useful information about the aglycone structure of the anthocyanins. Electrospray ionization mass spectrometry analysis of anthocyanins produced the intact molecular ions $[M]^+$, which were selected for second ionization (MS/MS) to get the fragments corresponding to anthocyanidin. The difference between molecular ions and anthocyanidin fragments provides information about sugar moiety; thus, the loss of 162 or 308 u suggests the presence of one hexose and one pentose, and the loss of 132 suggests the presence of a pentose. For the unequivocal identification of compounds, fractions II, III, IV, VI, and VII were subjected to preparative HPLC to get pure anthocyanins for NMR analyses.

From fraction II the minor pigment **1** was isolated and identified as cyanidin-3-sambubioside by comparison of the mass spectral and chromatographic data with those of a sample isolated in our laboratory from elderberry (*Sambucus nigra* L.) (*21*). From fraction III the anthocyanins **3** and **5** were isolated, and the ^{1}H NMR spectra of these pigments showed similar signals, except for the presence of a three-proton singlet at δ 4.02 (OCH_3) in the spectra of **5**, which is in agreement with the presence of peonidin as aglycone in this compound. The presence of rhamnose in the sugar moiety of these compounds was evident by the methyl signal at δ 1.16. These analyses allowed assignment of the structure of compound **3** as cyanidin-3-rutinoside, the major constituent of ARE, and that of **5** as peonidin-3-rutinoside. From fraction VI, compounds **2** and **4** were isolated. Compound **2** was identified as cyanidin-3-glucoside, a common anthocyanin found in fruits (*1*). The structure of the minor anthocyanin **4** was proposed to be peonidin-3-glucoside and confirmed by HPLC co-injection with a sample isolated from purple corn (*Zea mays*) (*21*). Finally, from the HSCCC stationary phase (FVII) compound **6** was purified and identified as cyanidin-3-*O*-(6″-malonyl)glucopyranoside. During MS/MS analysis, the molecular ion of **6** produced an ion at 287 (cyanidin) by loss of a hexose moiety (m/z 162) and a malonyl group (m/z 86). The presence of a malonyl group was confirmed by the ^{1}H NMR data (δ 3.35, s). Structures of anthocyanins **1**−**6** are shown in **Figure 2**. It is important to point out that this is the first chemical study of the pigment composition of this fruit species.

Microencapsulation of Anthocyanins from *B. guineensis* Fruit. Corozo fruits were used as a raw material to obtain anthocyanin-enriched extracts to produce natural colorants by spray-drying microencapsulation. Four ethanolic extracts were obtained, and their physicochemical properties were determined and compared to those of fresh juice (**Table 2**). In general, the anthocyanin content in the extracts was higher compared to the aqueous fruit juice. More specifically, the anthocyanin content was higher in the AEEs obtained with ethanol/citric acid (OC and SC) compared to ethanol (OE and SE). The effect of heat during the extraction of AEEs was advantageous to increasing the anthocyanin content in the AEEs.

The data obtained from the measurement of color parameters in the five AEEs were located in the first quadrant of a^*,b^* and showed similar values. The hue value ranged from 14 to 33°, and it is remarkable that the OC extract exhibited a deeper red color in agreement with a lower hue value. The anthocyanin composition of AEEs was analyzed by HPLC-PDA, revealing anthocyanin profiles qualitatively similar to the ARE, even in the samples obtained by Soxhlet extraction; thus, the anthocyanin enrichment in these solutions was confirmed. Usually, the effect of heat has been associated with the degradation of anthocyanins (*27*). However, the results obtained for *B. guineensis* indicate a positive effect of the thermal treatment for the extraction rate of these compounds.

Because OC and SC ethanolic extracts showed the highest anthocyanin content, they were selected for their transformation

Table 2. Physicochemical Characteristics of AEEs and Microencapsulated Anthocyanins of Corozo (*Bactris guineensis*) Fruit

					color parameters				
sample[a]	pH[b]	°Brix[b]	density (g/mL)[b]	anthocyanin content[c]	L^*	a^*	b^*	C^*_{ab}	h^*_{ab}
AEEs									
FJ	3.5 ± 0.2	20.3 ± 1.0	1.04 ± 0.02	0.80 ± 0.01d	42	54	32	63	31
OE	4.6 ± 0.2	22.4 ± 1.2	0.85 ± 0.02	0.85 ± 0.01e	44	62	32	70	27
OC	4.3 ± 0.2	23.0 ± 1.4	0.91 ± 0.03	1.34 ± 0.01f	39	70	18	72	14
SE	4.7 ± 0.2	22.5 ± 1.3	0.88 ± 0.02	1.10 ± 0.01g	41	61	36	70	31
SC	4.3 ± 0.1	23.4 ± 1.2	0.87 ± 0.01	1.48 ± 0.01h	37	61	40	73	33
microencapsulated anthocyanins									
MOC	2.2 ± 0.1	36.3 ± 1.2	1.1 ± 0.2	1.18 ± 0.01i	50	48	14	65	17
MSC	2.2 ± 0.1	39.4 ± 1.4	1.1 ± 0.2	1.31 ± 0.02j	47	47	16	69	19

[a] For description of samples see Materials and Methods. [b] For microencapsulated anthocyanins, these measurements were performed on a solution (1 g of solid/1.5 mL of water). Values are expressed as means ± SE (n = 3). [c] Expressed as mg of cy-3-glu equiv/g of fruit for AREs or g of solid for microencapsulated anthocyanins. Values are expressed as means ± SE (n = 3). Values with different letters (d−j) in a column are statistically different ($P \leq 0.05$) by ANOVA.

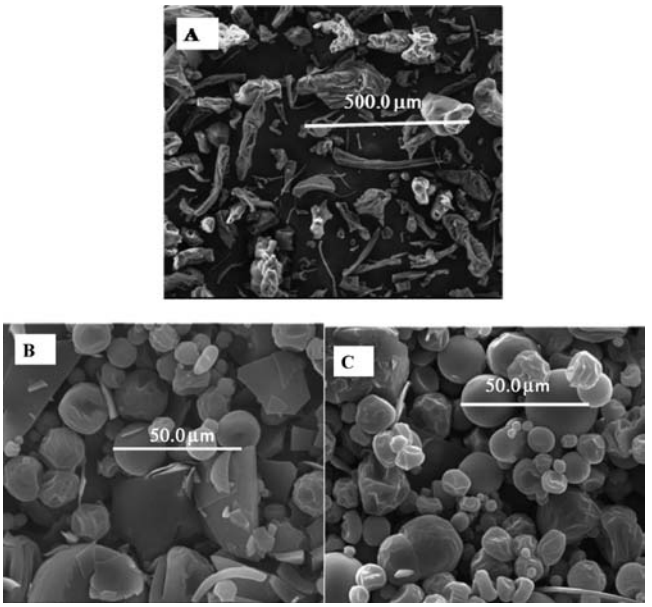


Figure 3. Scanning electron micrographs of maltodextrin (**A**) and microcapsules MOC (**B**) and MSC (**C**).

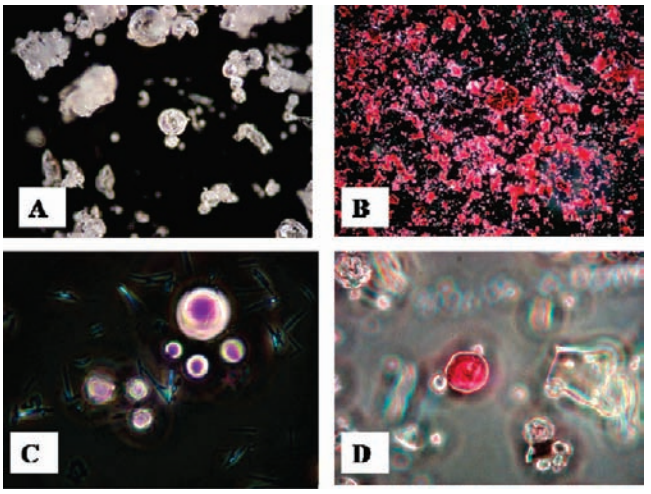


Figure 4. Representative microphotographs (100×) of maltodextrin (**A**), AEE (**B**), and microcapsules MOC (**C**) and MSC (**D**).

into powders (MOC and MSC, respectively) by using the spray-drying technique. The moisture contents of the two solids were quite similar, 10.0 ± 0.3 for MOC and 10.7 ± 0.2 for MSC, expressed as percentage on dry basis. After measurement of their physicochemical characteristics (**Table 2**), it was found that MSC solid exhibited higher anthocyanin content than MOC, in agreement with the results obtained for the corresponding AEEs. The same behavior was found for the values of total phenolics and antioxidant activity. Additionally, it was confirmed by HPLC-PDA that the anthocyanin composition in the microcapsules was similar to that of the fruit without processing.

The morphological characterization of microcapsules, MOC and MSC, was performed by SEM (**Figure 3**). The formation of microcapsules after the spray-drying process was confirmed by comparison with a microphotograph of the encapsulating agent (maltodextrin) before the process. It is clear that this material changed from an irregular to mostly spherical shape with some dented surfaces. The solids were obtained as fine powders with a particle size ranging between 10 and 30 μm. The formation of these dents on some microcapsules has been attributed to the fast water evaporation and consequent contraction of the particles during the drying process or throughout the vacuum applied during the SEM analysis (*28*, *29*). Smooth spheres are desirable for the stability of encapsulated ingredients and also for the controlled release. This concept is one of the main purposes of microencapsulation of food ingredients because it can improve the effectiveness of food additives (*30*).

The MOC and MSC powders were also analyzed by phase contrast optic microscopy to confirm the effectiveness of the coating of *B. guineensis* pigments after the microencapsulation process. As can be seen in **Figure 4C**,**D**, the spherical microcapsules were structurally composed of a red core (anthocyanin) surrounded by a thin layer of white encapsulating agent (maltodextrin). However, other microcapsules in which the pigment was adsorbed on the surface of maltodextrin were also observed. By comparison with the microphotographs of maltodextrin (**Figure 4A**) and AEE (**Figure 4B**), the changes of these starting materials after the spray-drying process was evident.

To evaluate the physicochemical changes of *B. guineensis* microencapsulated anthocyanins by the effect of temperature, MOC and MCS powders were subjected to thermal analysis by TGA and DSC. The thermograms obtained from the two samples (**Figure 5A**) showed a similar pattern, which can be divided into three stages. At the first stage there was no thermal activity detected up to 30 °C, but after this temperature a weight loss was seen until 100 °C, attributable to thermal desorption of volatiles and surface water evaporation (*31*). The second stage can be related to the loss of internal water in the particles between 100 and 180 °C with a weight loss of about 7%. The third stage, after 180 °C, in which the largest weight loss was seen (70% of initial value), is believed to be maltodextrin

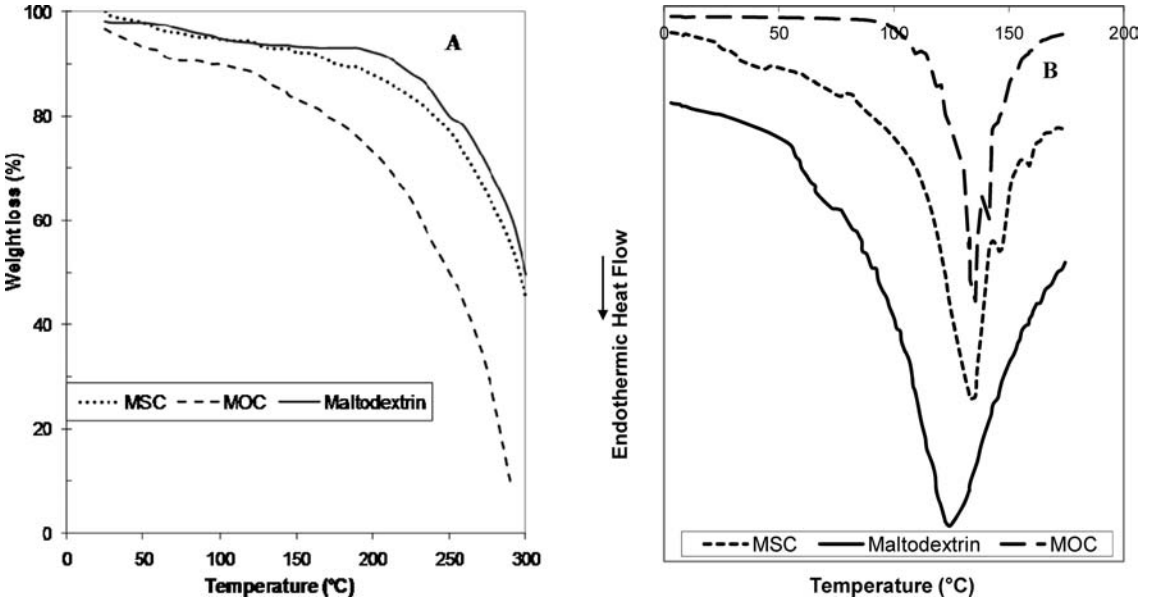


Figure 5. Thermogravimetric analysis (**A**) and DSC thermograms of maltodextrin and microencapsulated anthocyanins of *B. guineensis* fruit (**B**).

Table 3. Anthocyanin Content of the *B. guineensis* Microencapsulated Anthocyanins during Storage at Different Humidity and Temperature Conditions

	anthocyanin content[a]					
	18 °C		50 °C		70 °C	
storage time (days)	76% RH	95% RH	76% RH	95% RH	76% RH	95% RH
			MOC			
0	1.192 ± 0.006	1.179 ± 0.007	1.183 ± 0.004	1.196 ± 0.005	1.185 ± 0.001	1.192 ± 0.003
5	1.183 ± 0.003	1.169 ± 0.003	1.174 ± 0.003	1.167 ± 0.006	1.131 ± 0.002	1.112 ± 0.004
10	1.170 ± 0.002	1.142 ± 0.004	1.161 ± 0.003	1.108 ± 0.005	1.106 ± 0.002	1.015 ± 0.004
15	1.142 ± 0.004	1.119 ± 0.007	1.142 ± 0.002	1.053 ± 0.002	1.030 ± 0.005	1.002 ± 0.056
20	1.119 ± 0.004	1.088 ± 0.005	1.115 ± 0.004	1.014 ± 0.004	0.994 ± 0.006	0.847 ± 0.004
25	1.096 ± 0.006	1.050 ± 0.005	1.077 ± 0.008	0.995 ± 0.005	0.939 ± 0.003	0.785 ± 0.004
30	1.072 ± 0.005	1.018 ± 0.007	1.056 ± 0.005	0.939 ± 0.002	0.891 ± 0.006	0.720 ± 0.005
35	1.037 ± 0.005	0.983 ± 0.009	1.026 ± 0.006	0.904 ± 0.004	0.810 ± 0.004	0.652 ± 0.004
40	1.020 ± 0.004	0.969 ± 0.006	0.955 ± 0.004	0.849 ± 0.003	0.740 ± 0.004	0.519 ± 0.007
			MSC			
0	1.297 ± 0.003	1.294 ± 0.004	1.296 ± 0.007	1.292 ± 0.006	1.307 ± 0.004	1.300 ± 0.003
5	1.292 ± 0.005	1.272 ± 0.004	1.283 ± 0.007	1.277 ± 0.003	1.292 ± 0.004	1.258 ± 0.004
10	1.267 ± 0.007	1.259 ± 0.005	1.242 ± 0.003	1.233 ± 0.005	1.228 ± 0.004	1.220 ± 0.007
15	1.248 ± 0.003	1.217 ± 0.004	1.210 ± 0.004	1.164 ± 0.007	1.195 ± 0.007	1.192 ± 0.005
20	1.233 ± 0.003	1.195 ± 0.005	1.202 ± 0.005	1.158 ± 0.003	1.148 ± 0.004	1.117 ± 0.006
25	1.219 ± 0.004	1.180 ± 0.003	1.184 ± 0.003	1.149 ± 0.003	1.129 ± 0.006	1.057 ± 0.004
30	1.210 ± 0.002	1.158 ± 0.002	1.173 ± 0.002	1.132 ± 0.003	1.087 ± 0.005	0.981 ± 0.004
35	1.201 ± 0.002	1.149 ± 0.003	1.157 ± 0.002	1.103 ± 0.005	1.049 ± 0.005	0.905 ± 0.004
40	1.194 ± 0.003	1.140 ± 0.003	1.145 ± 0.002	1.076 ± 0.004	0.996 ± 0.004	0.767 ± 0.012

[a] Values are expressed as mg of cy-3-glu equiv/g of extract, means ±SD (n = 3).

thermal degradation. This process is completed at a temperature of around 370 °C with a maximum interval of change around 280 °C, which is close to the melting point of maltodextrin (240 °C). Although MOC and MSC powders follow the general behavior of encapsulating agent (maltodextrin), it is possible to find differences by the effect of anthocyanins. The weight loss in MOC was more pronounced than in MSC. Thus, from the curves of **Figure 5A**, water losses of 8 and 11% for MSC and MOC were calculated at 105 °C, respectively. These results suggest that MSC solid should be more thermally stable than MOC and that the water activity of these microcapsules is low.

Under DSC studies carried out at inert atmosphere, the microcapsules showed similar thermograms, confirming that there is no thermal phenomenon until 30 °C. In the temperature range of 30−100 °C a slight flow of heat was seen, explained by desorption of volatile compounds and evaporation of surface water adsorbed on the material. In the next stage, from 100 to 170 °C, a complex endothermic peak was observed. This can be explained by the energy required to evaporate the internal water in the powders. In general, it is possible to conclude that MSC and MOC microencapsulates are quite stable until 100 °C, the typical food industry sterilization temperature.

Storage Stability Evaluation of *B. guineensis* Microencapsulated Anthocyanins. A storage stability test to evaluate the effect of temperature and humidity was performed with the MSC and MOC powders. On the basis of the fact that microencapsulated anthocyanin

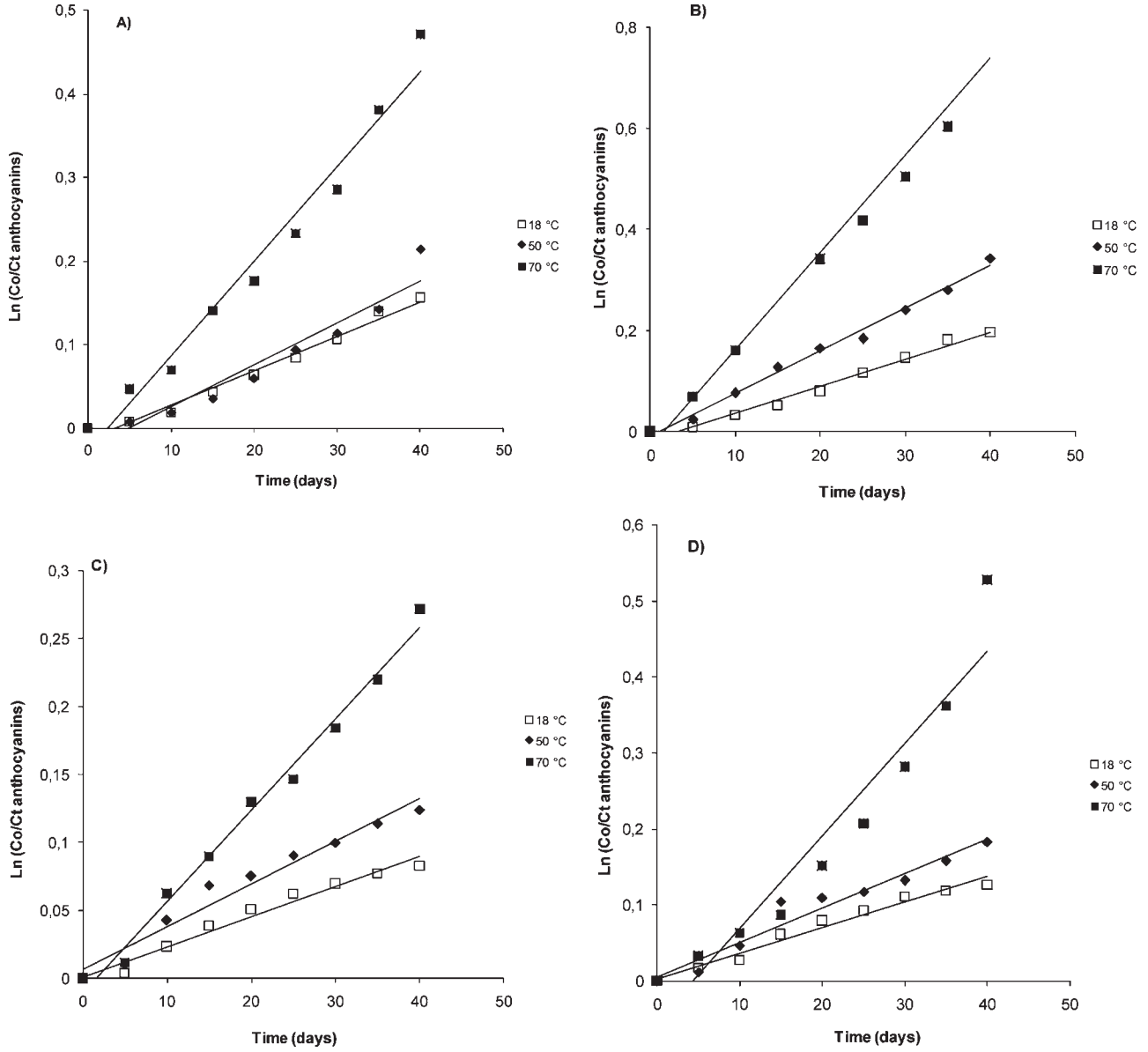


Figure 6. First-order kinetic plots for changes in anthocyanin content during storage of *B. guineensis* microencapsulated anthocyanins: MOC (76% RH) (**A**); MOC (95% RH) (**B**); MSC (76% RH) (**C**); MSC (95% RH) (**D**).

powders come from a drying process, the humidity is a critical factor for preserving the quality of these products during storage. For the experimental design, the results of thermal analysis were taking into account. The samples were placed in polyethylene bags and exposed to different humidity and temperature conditions, evaluating the anthocyanin content for a period of 40 days (**Table 3**). The obtained data reveal that the degradation of anthocyanins is higher at a relative humidity of 95% compared to 76% and is increased by temperature at both values of humidity. It was apparent that the microcapsules retain the macrostructure at temperatures above 50 °C but suffer a color change from red to brown. This fact can be explained by the oxidation of anthocyanins under oxygen, which produce colorless or brown products due to the formation of chalcones or coumarins, respectively (*32*).

Previous works on microencapsulated anthocyanin degradation showed that this reaction followed a pseudo-first-order kinetics (*15*). Therefore, this model was assumed to evaluate the data of **Table 3**, and the results are plotted as $\ln(C_0/C_t)$ versus t in **Figure 6**. In general, an increase in storage temperature led to an increase in rate constants of anthocyanin degradation (**Table 4**). The rate constants of anthocyanin degradation at 18 and 37 °C did not show any significant difference (data no shown). In addition, the degradation kinetics of microencapsulated anthocyanins was largely affected by the humidity of the environment, showing a gradual increase in the rate with increasing water activity. From **Figure 6** it is possible to find differences in the degradation kinetics of MOC and MSC. It seems that MOC powders at 50 and 70 °C undergo degradation with two slopes; the first one could be attributable to the release of anthocyanin adsorbed on the surface of microcapsules and the second one to the internal release due to the loss of structure. In contrast, MSC powder is more stable because it showed at 18 and 50 °C a first slope, and then the degradation rate tends to decrease (the curve tends to be concave to the X-axis), which is in agreement with our statement proposed from thermal analysis of microcapsules. With these results, it is feasible that thermal treatment of *B. guineensis* anthocyanins allows their stabilization by the development of any reaction. One of these phenomena is the copigmentation, widely seen in plant tissues and their aqueous extracts. Molecules acting as copigments, such as flavonoids, alkaloids, and organic acids,

Table 4. Anthocyanin Degradation Rate Constants of *B. guineensis* Microencapsulated Anthocyanins during Storage at Different Humidity and Temperature Conditions

sample	treatment (temperature/humidity)	k_{obsd} (days^{-1})	correlation coefficient (r)	half-life period $t_{1/2}$ (days)
MOC	18 °C/76% RH	0.004	0.990	173.3
	18 °C/95% RH	0.005	0.992	138.6
	50 °C/76% RH	0.005	0.963	138.6
	50 °C/95% RH	0.008	0.996	86.6
	70 °C/76% RH	0.011	0.986	63.0
	70 °C/95% RH	0.019	0.987	36.5
MSC	18 °C/76% RH	0.002	0.986	346.6
	18 °C/95% RH	0.003	0.987	231.0
	50 °C/76% RH	0.003	0.981	231.0
	50 °C/95% RH	0.004	0.975	173.3
	70 °C/76% RH	0.006	0.995	115.5
	70 °C/95% RH	0.012	0.960	57.8

usually have no color by themselves, but when they are present in an anthocyanin solution, they enhance the stability of the color (*33*). The above results suggest that the more suitable conditions for the storage of packaged *B. guineensis* microencapsulated anthocyanins are low relative humidity (76%) and temperatures of < 37 °C.

In conclusion, the anthocyanin composition of *B. guineensis* fruit was characterized, and the anthocyanins were successfully coated with maltodextrin by spray-drying. Two powders were obtained with a high recovery of these pigments, showing great stability under different temperature and humidity conditions of storage. The most remarkable result is the thermal stability of *B. guineensis* anthocyanins, which is an advantage for their use in the food industry. On the basis of these results a more widespread cultivation of *B. guineensis* plants, a wild tropical species, can be recommended because this fruit is a valuable source of natural food colorants.

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