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Characterization and bioaccessibility assessment of bioactive compounds from camu-camu (*Myrciaria dubia*) powders and their food applications

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ABSTRACT

Camu-camu (*Myrciaria dubia*) is a tropical fruit known for its content of bioactive compounds. This study aimed to evaluate physicochemically, morphologically, andsensorialpowders from camu-camu obtained by spray-drying at two inlet temperatures (150 °C and 180 °C) with three encapsulating agents (maltodextrin, whey protein and a 50:50 mixture of both) and by freeze-drying of whole fruit. The use of maltodextrin protected bet anthocyanins (cyanidin-3-glucoside (C3G) and delphinidin-3-glucoside (D3G)), but whey protein showed a better protective effect on ascorbic and malic acids. These facts were confirmed during the storage stability test, finding that relative humidity is a critical variable in preserving the bioactive compounds of camu-camu powders. The powders with the highest content of bioactive compounds were added to a yogurt and a white grape juice, and then sensory evaluated. The bioaccessibility studies in gastric and intestinal phases showed better recovery percentages of bioactive compounds in camu-camu powders (up to 60.8 %) and beverages (up to 90 %) for C3G, D3G, ascorbic acid, and malic acid than in the fruit juice. Dehydration of camu-camu (*M. dubia*) is a strategy to increase the bioactive compounds stability, modulate the fruit sensory properties, and improve their bioavailability after incorporation in food matrices.

1. Introduction

Camu-camu (*Myrciaria dubia* (Kunth) McVaugh) is a tropical fruit with biofunctional properties exhibiting antioxidant, anti-hyperglycemic, and anti-obesity activities (Conceição et al., 2020; García-Chacón et al., 2022), among others. Despite its bioactive compounds, *M. dubia* consumption is limited because it is highly perishable and has been described as too acidic and astringent after harvesting (Cunha-Santos et al., 2019). The food industry is interested in technological processes or strategies that allow the consumption of camu-camu products or use them as food ingredients. In this sense, microencapsulation is a good alternative for increasing the shelf-life of this fruit (Ozkan et al., 2019).

Over the drying process, wall materials should protect the bioactive compounds and, during storage, improve stability against oxygen, moisture, temperature, and light. Different substances can be used as encapsulating agents; for example, whey protein (WP) exhibits technological properties such as gel formation, emulsification, increase of solubility, and film formation, providing stability to the bioactive compounds present in the fruits and mask off-flavors (Feng et al., 2021); maltodextrin (MD) is widely used as an encapsulating agent because it changes its linear configuration for a circular one forming spherical-shape particles and allowing better microencapsulation. Besides, it is economical and increases the solubility of bioactive compounds in the final food matrix (Ozkan et al., 2019).

During the development of functional foods, assessing the effect of processing on the bioactive compounds and their absorption in the human body is essential. The *in vitro* gastrointestinal digestion process provides a low-cost method that simulates gastric and intestinal body fluids, mimicking physiological conditions. This procedure allows for the identification of the nutrients released and their availability for subsequent absorption, ensuring the bioaccessibility of bioactive compounds. The recovery levels of phytochemicals can be related to the *in vivo* expression of bioactivity (Minekus et al., 2014).

M. dubia fruit is a source of different chemical compounds with bioactive properties. The fruit epicarp contains cyanidin-3-glucoside as

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the major anthocyanin pigment, followed by delphinidin-3-glucoside (Zanatta et al., 2005). Anthocyanins are natural colorants that exhibit antidiabetic, anticancer, and anti-inflammatory effects and prevent cardiovascular and neurodegenerative diseases (Fernandes et al., 2020). *M. dubia* fruit is also recognized for having a high vitamin C content; depending on the geographical origin, the amount in pulp was reported between 18.05 ± 0.66 and 38.37 ± 2.13 g/100 g dry weight, and for peel between 6.60 ± 0.28 and 15.72 ± 0.41 g/100 g dry weight (Cunha-Santos et al., 2019). Ascorbic acid is used as a natural additive to avoid oxidation since it acts as a free radical scavenger and is involved in various physiological processes such as immune stimulation, prevention of mucosal bleeding, and reduction of blood glucose (Cunha-Santos et al., 2019). Malic acid reported in camu-camu fruit (Zapata & Dufour, 1993) is a four-carbon dicarboxylic acid mainly found in nature as the *L*-stereoisomer. The food industry employs it as an additive for acidity regulation and flavour booster (Santana Andrade et al., 2022). Malic acid is a bioactive compound that has shown *in vitro* anticancer, hepatoprotective, and antihyperglycemic activities (Alakolanga et al., 2015). Thus, anthocyanins, ascorbic acid, and malic acid were selected as targets for this study.

The aim of this work was to develop powders from camu-camu fruit (pulp and peel) by freeze-drying (lyophilization) and spray-drying techniques that preserved their bioactive compounds. The physicochemical, morphological, and sensory properties of microencapsulates were evaluated, as well as their bioactive compound content (anthocyanins, vitamin C, and malic acid). Additionally, the bioaccessibility of bioactive compounds was studied after *in vitro* gastrointestinal digestion process with the aim of developing functional foods. Finally, the powders with the best functional properties were incorporated into two food beverages (commercial yogurt and white grape juice), and their bioactive compound content and sensory properties were evaluated.

2. Materials and methods

2.1. Chemicals

Food grade maltodextrin (MD) (esterification grade 18:20, Casa Químicos SAS, Colombia) and whey protein (WP), powdered and pasteurized, with 12 % protein content (Nabot, Colombia) were used as encapsulating agents for the spray-drying microencapsulation. For obtaining ethanolic fruit extracts, 96 % food-grade ethanol was used (Laboratorio Bioalcohol, Colombia), and 96 % ethanol (PanReac AppliChem ITW Reagents, Barcelona, Spain) was used for the final moisture determination of the samples. For the identification and quantitation of malic acid and ascorbic acid in the samples, 0.05 M sulfuric acid H_2SO_4 (PanReac AppliChem ITW Reagents, Barcelona, Spain) and Milli-Q water were used for extraction and chromatographic method. Ascorbic acid standard (*L*-ascorbic acid, PanReac AppliChem ITW Reagents, Barcelona, Spain), and *DL*-malic acid standard (Labkem, Barcelona, Spain) were used for calibration purposes. Likewise, anthocyanins were extracted using RG methanol and hydrochloric acid at 32 % purity (VWR Chemicals, France). The identification and quantification chromatographic method of anthocyanins used HPLC-grade methanol, HPLC-grade acetonitrile, and 99 % LC-MS formic acid (VWR Chemicals, United Kingdom). The anthocyanin standards were delphinidin-3-glucoside (D3G) and cyanidin-3-glucoside (C3G) (Extrasynthese, Genay Cedex, France). Finally, saturated salts of potassium nitrate (KNO_3) (PanReac AppliChem ITW Reagents, USA), sodium chloride (NaCl) (HoneyWell, Fluka, Germany), and magnesium chloride ($MgCl_2$) (Merck, Darmstadt, Germany) were used during the stability test. For *in vitro* gastrointestinal digestion process, the pH of the samples was adjusted using sodium hydroxide (J.T. Barker, Deventer, The Netherlands) and 32 % hydrochloric acid (VWR Chemicals, Pennsylvania, United States). Porcine pepsin (P7012), porcine pancreatin (P7545), and bile salts (B8756) (Sigma Aldrich, Steinheim, Germany) were used in the digestive process. For the preparation of gastric fluids, ultrapure water from the Milli-Q system, sodium chloride (Honeywell, Fluka, Germany), potassium chloride (Panreac, Barcelona, Spain), and monopotassium phosphate (Sigma Aldrich, Steinheim, Germany) were used. Likewise, sodium bicarbonate and magnesium chloride hexahydrate (Panreac, Barcelona, Spain), ammonium carbonate, and calcium chloride dihydrate (Sigma Aldrich, Steinheim, Germany) were used for gastrointestinal fluids.

2.2. Fruit samples

Camu-camu (*M. dubia*) fruits were purchased at the local markets of Florencia (Caquetá, Colombia) and adequately transported to Bogotá (Colombia) for analysis. Fully ripe fruits (pH 2.43 ± 0.03, soluble solid content of 7.38 ± 0.18° Brix, and acidity content of 2.20 ± 0.85 % citric acid) were selected, and the seeds were manually removed (García-Chacón et al., 2022). A moisture determination balance (AMB 50, Adam Equipment, USA) was used to determine the moisture content of the fruit without seeds (88.98 ± 1.76 %).

2.3. Preparation of camu-camu powders

2.3.1. Freeze-drying

Whole camu-camu fruits (628 g) were frozen at −4 °C before and during lyophilization in a Beta 1–8 LDplus freeze dryer (CHRIST, Germany) for 48 h, with a main drying phase of 40 h at 1.0 mbar and −20 °C. The final drying phase lasted 8 h at 0.001 mbar and −76 °C, considering the initial moisture of fresh fruit (García et al., 2018). After lyophilization, seeds were manually removed from the fruits; peel and pulp were homogenized together using a grinder (A11 basic, IKA, Staufen, Germany), obtaining a fine powder consisting of lyophilized camu-camu fruit (58 g, LCC).

2.4. 2. Spray-drying

Microencapsulation experiments were carried out in an SD-6 spray-dryer (Labplant, London, UK), using similar conditions as previously published by García et al. (2018). An ethanolic extraction was performed on camu-camu fruit (pulp and peel) to obtain the highest content of bioactive compounds. For that purpose, seeds were manually removed, and the fresh fruits were homogenized using a blender to get juice (FCC). Then, ethanol was added to the mixture (3:1 w/w, ethanol: FCC, by three times) following the procedure reported by Osorio et al. (2010). The extract was homogenized, filtered, and the solvent removed under vacuum to be spray-dried.

The relation fruit: encapsulating agent was 3:2 (w/w) to have enough bioactive compounds (based on previous experiments). A 2 × 3 factorial arrangement experimental design was carried out using 2 inlet temperatures (150 °C and 180 °C), and 3 encapsulating agents (MD, WP, and MD: WP 1:1) to get six microencapsulates. Before encapsulation, feeding mixtures were homogenized in a blender at 18 °C and kept in darkness for 24 h. The outlet temperatures were 78 °C and 83 °C, for the two inlet temperatures (150 °C and 180 °C), respectively. The six samples were: those obtained with maltodextrin as encapsulating agent at 150 and 180 °C (MD150 and MD180, respectively); those with whey protein as encapsulating agent at 150 and 180 °C (WP150 and WP180, respectively); and those with a 1:1 mixture of MD:WP at both temperatures (MDWP150 and MDWP180, respectively).

2.5. Characterization of powders

pH Value, soluble solids content, and water activity (Aw) were determined following the methodology of García et al. (2018). For this purpose, 1.0 g of each powder was reconstituted in 10 mL of distilled water. The moisture content (%) of LCC and microencapsulates was measured using a moisture determination balance (AMB 50, Adam Equipment, USA), placing 1.0 g of each powder at a temperature of

100 °C, until a constant weight value was obtained. Moisture content was calculated by the difference between the initial weight and the final weight obtained from the drying process expressed as a percentage. All measurements were performed in triplicate for each treatment.

Encapsulating agent and camu-camu microencapsulate morphologies were evaluated by Scanning Electron Microscopy (SEM). The samples were metalized with gold and observed in a Vega3 SB microscope (Tescan, Czech Republic). The particle size was determined using the ImageJ® program (Java 1.8.0, NIH, USA). The values obtained were statistically analyzed through a frequency distribution of the values represented in a histogram, determining the dispersion of the particle size distribution.

Colour parameters of camu-camu powders were measured following the methodology of García et al. (2018). For stability purposes, the DigiEye® imaging system was used because it is specially designed to evaluate the appearance and colour measurements according to CIE guidelines. DigiEye consists of a dome lightning booth with two D_{65} standard illuminant emulators, a reflex camera, and a computer that controls the entire equipment. The output images contain in their pixels the $L^*a^*b^*$ colour coordinates. Four petri dishes of camu-camu were measured simultaneously in each capture. The segmentation, processing of images, the extraction of the colorimetric information, and the colour differences calculations expressed as ΔE^*_{ab} were carried out with an algorithm developed *ad-hoc* under Matlab R2021b (Rodríguez-Pulido et al., 2021).

2.6. UHPLC-PDA analyses of bioactive compounds

Anthocyanins were identified and quantified by UHPLC-PDA. For this analysis, 30 mg of each sample were weighed into a 2 mL Eppendorf tube, added with 500 μL of 1.0 % HCl solution in methanol, and homogenized in a vortex (IKA, Genius 3, Germany) for 40 s, followed by 5 min of constant agitation with a magnetic stirrer. Then samples were centrifuged in a 5430R centrifuge (Eppendorf, Germany) for 5 min at 10 °C and 10,000 rpm, and this process was repeated until a colorless supernatant was obtained. All supernatants were pooled in a single container, the solvent was removed up to 200 μL (Concentrator Plus, Eppendorf, Germany), and then dissolved in 1 mL of acidified water (0.1 % with HCl). All samples were filtered through a 0.45 μm syringe filter before the HPLC-PDA analysis, which was performed following the methodology of Fernández-Lara et al. (2015). The respective compounds were identified by adding known concentrations of the standards; retention time and UV spectrum were also compared. Quantification of anthocyanins was calculated from the peak areas recorded at λ 520 nm using a analytical curve purchase standard of cyanidin-3-glucoside (0.5–1000 mg/L), expressing the results in mg C3G/100 g sample.

Ascorbic acid and malic acid contents were also determined by UHPLC-PDA analyses. Approximately 250 mg of powders were dissolved in 5 mL of Milli-Q water and 1 mL of H_2SO_4 0.05 M solution until obtaining a homogeneous mixture. The mixture was filtered and quantitatively brought to 10 mL final volume with Milli-Q water. All samples were filtered through a 0.45 μm syringe filter before UHPLC-DAD analysis in an Agilent 1290 Infinity Chromatographic System equipped with a quaternary pump, a UV–Vis diode array detector, binary pump, autosampler, and ChemStation OpenLAB CDS software (Agilent Technologies, Palo Alto, CA, USA). The acids were identified using a Zorbax RRHD Eclipse Plus C18 column (2.1 × 50 mm, 1.8 μm particle size) at 25 °C. An isocratic separation method was used for 5 min using H_2SO_4 0.05 M as mobile phase (0.5 mL/min flow, 0.2 injection volume). The UV–Vis spectra were recorded from λ 190 to 800 nm (2.0 nm bandwidth), with detection at λ 254 nm for ascorbic acid and 210 nm for malic acid (Romero Rodríguez et al., 1992). For identification purposes, standards of acids were added in a known concentration and analyzed in the HPLC system; retention time and UV spectrum were also compared. The quantification was done with a analytical curve of each acid (0.0125–2.000 mg/L), and results were expressed in mg acid/100 g sample.

2.7. Storage stability test

The stability of four camu-camu powders (LLC, MD150, MDWP150, and MDWP180) was assessed at three relative humidities (33 %, 75 %, and 95 %) following the modified protocol of Osorio et al. (2010). In the dark, 3.0 g of each sample were placed in 5 cm diameter Petri dishes and stored at constant temperature (20 ± 2 °C) in desiccators with 200 mL of saturated solutions of KNO_3, NaCl, and $MgCl_2$ to obtain constant relative humidity values of 95 ± 2 %, 75 ± 2 %, and 33 ± 2 %, respectively. During equilibration, the humidity was measured using a HI-93640 thermohygrometer (Hanna Instruments, Portugal) over 31 days of storage. The retention of malic acid, ascorbic acid, and anthocyanins was determined as a percentage of the initial concentration (time = 0), expressed as mg of compound/100 g of sample. The kinetic of loss/degradation of compounds *vs.* time after storage was assessed assuming a first-order kinetic model. The half-life time ($t_{1/2}$) was also determined from equation (1):

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

2.8. Food beverages with camu-camu powders

Two beverage types were selected to incorporate the camu-camu powders: A commercial liquid yogurt (Y), natural flavor, skimmed and sweetened with brown cane sugar (DIA, Spain), and a commercial white grape juice (G), (Greip, Pepsico Company, Spain). Based on the physicochemical characterization, four powders were selected to apply on the beverage matrices: LLC, MD150, MDWP150, and MDWP180. Yogurt (Y) was used for LCC at a dose of 0.5 % (YLCC), and MD150, MDWP150, and MDWP180 at 15 % (YMD150, YMDWP150, and YMDWP180, respectively). Similarly, grape juice (G) was used for LCC at a dose of 0.5 % (GLCC) and the other three at 15 % (GMD150, GMDWP150, and GMDWP180). All samples were stirred for 5 min until homogeneous beverages were obtained and refrigerated until analysis.

2.9. Sensory analysis of beverages

The beverages were sensory evaluated through a descriptive evaluation performed by a 17-judge trained panel (11 women and 6 men, from 20 to 59 years old). Each panelist received four samples, and two blanks (FCC and the corresponding food matrix) were presented to panelists in two different sessions (one for yogurt and one for grape juice). Samples were served randomly at 18 °C and under white light in clear cups (30 mL volume) labeled with a three-digit code. Judges were asked to assess different parameters on a 0–10 scale for colour (0 = white, 10 = pink, for yogurt; 0 = slightly yellow, 10 = red, for grape juices, sweetness (0 = slightly, 10 = strong, for both), acidity (0 = slightly, 10 = strong, for both), and acceptance (0 = slightly liked, 10 = strongly liked) considering the global balance.

2.10. In vitro simulated gastrointestinal digestion

A static *in vitro* gastrointestinal digestion procedure was performed to simulate the physiological conditions in the upper digestive tract according to Minekus et al. (2014), with minor modifications implemented by Stinco et al. (2020). The content of anthocyanins, ascorbic acid, and malic acid was evaluated in the camu-camu juice (FCC), four selected powders (LCC, MD150, MDWP150, and MDWP180), and four beverages (YLCC, YMDWP180, GLCC, GMD150) during *in vitro* gastrointestinal digestion.

During the overall digestion process, aliquots of 500 μL were taken at the end of each digestive phase for further sampling procedure. For that purpose, four simulation phases were considered. (1) *Non-digested simulation*: samples with no-digestion process (original samples). Solid

samples were reconstituted in 5 mL of simulated salivary fluid (SSF) before the next simulation. (2) *Gastric phase simulation:* initial 3.0 mL of simulated gastric fluid (SGF) and porcine pepsin solution (2000 U/mL in SGF) was used. Digests were centrifuged at 2000 rpm for 10 min, and the supernatant (filtered through a 0.45 μm nylon membrane filter) was taken as the gastric sample. (3) and (4) *Duodenal phase simulation:* 6.0 mL of simulated duodenal fluid (SDF) were added to the digests from the gastric phase. After centrifugation at 4000 rpm, the supernatant was filtered through a 0.45 μm nylon membrane and taken as the IN digest (3); and the aqueous phase for epithelium bioaccessibility (filtered through a 0.22 μm nylon membrane) was taken as the OUT sample (4). Each sample was frozen (-20 °C), and all analyses were performed in quadruplicate.

2.11. Bioaccessibility

Bioaccessibility of anthocyanins (D3G and C3G), ascorbic acid, and malic acid (in all the samples mentioned in Section 2.9) was calculated as a recovery percentage, considering the ratio between the concentration of the bioactive compound in the digest and in the non-digested sample (FCC). Bioaccessibility was calculated for every gastrointestinal phase, although the intestinal OUT fraction was considered the final step for measuring compounds in the sample.

2.12. Statistical analysis

All experiments were performed in triplicate (despite those of bioaccessibility), presenting the data as the mean and standard deviation of the three independent experiments. The statistical analysis was performed by analysis of variance (ANOVA), and statistically significant differences ($p < 0.05$) were determined using the Tukey multiple comparison test. Statistical analyses were performed using Statistica v 12 (StatSoft) and InfoStat (Version 2020) softwares.

3. Results and discussion

3.1. Characterization of camu-camu powders

The physicochemical characterization of camu-camu powders is shown in Table 1. The lyophilized fruit showed a pH value (2.62 ± 0.10) close to that of the fresh fruit (2.43 ± 0.03), which is more acidic than exhibited by other Myrtaceae fruits; for instance, the pH of *Psidium guajava* ranged between 4.0 and 5.2, of *Myrciaria cauliflora ca.* 4.5, and of *Myrciaria jaboticaba ca.* 4.1, when they are fully ripe (Roquim Alezandro et al., 2013). Reconstituted microencapsulates showed significant differences in pH values related to the encapsulating agent ($p < 0.05$), showing the presence of whey protein (pH 6.23 ± 0.07) increased the pH significantly in comparison to the fruit and those microencapsulates only obtained with maltodextrin as the encapsulating agent. No significant differences in °Brix values were observed for the microencapsulates obtained by spray-drying, considering they were reconstituted in water before the measurements.

The initial moisture contents of powders were below 7 % for all treatments; as was expected, the microencapsulates obtained at 180 °C inlet temperature showed lower moisture content values than those obtained at 150 °C. According to the encapsulating agent, powders containing whey protein exhibited higher moisture values than the ones with maltodextrin. Previous studies have reported that whey protein presented higher moisture values since proteins in their amorphous state have a greater water-holding capacity (Tontul & Topuz, 2017). Likewise, the Aw values were less than 0.245 in all camu-camu powders, within the range reported in the literature (0.06–0.27) (Figueiredo et al., 2020). Also, it is below the value necessary to avoid the growth and proliferation of microorganisms (≥0.600), generation of off-flavors, and loss of significant aroma, flavour, and colour related-compounds (Ozkan et al., 2019).

Colour measurements of camu-camu powders showed that the LCC lightness value (47.86 ± 1.77) was lower than microencapsulates (*ca* > 80.00) related to a darkener colour due to the absence of any encapsulating agent. Additionally, no significant differences ($p < 0.05$) were found in lightness between the different microencapsulation treatments related to inlet temperatures or encapsulating agents. The colorimetric parameters presented positive values (located in the first quadrant $+a^*$, $+b^*$), as shown in Table 1. LCC and microencapsulates with 100 % MD, presented higher values of a^*, indicating samples with redder tones, while samples WP150 and WP180 showed higher b^* values suggesting yellowish tones due to the influence of the colorimetric parameters of WP as the encapsulated agent (data not shown). Moreover, samples in which WP was used presented a decrease in chroma (C^*_{ab}) compared to samples that only contained MD. The lowest *hue* values were found in the samples with 100 %WP; also, the presence of WP as an encapsulating agent, showed significant differences among *hue* values (yellowish tones) due to the inlet temperature. This fact suggests that WP directly influenced the colour of spray-dried samples (color yellow-brownish instead of red); it suggests that there were not an effective encapsulation of anthocyanins and flavonoids (compounds responsible for the colour of solutions of microencapsulates), and likely they were only adsorbed on the surface, such exposed to the thermal impact (Villacrez et al., 2014).

The morphologic characterization of microencapsulates and wall

Table 1
Physicochemical characterization, colour parameters, and bioactive compound content of camu-camu powders.

Powder Parameter	LCC	MDWP150	MDWP180	MD150	MD180	WP150	WP180
pH	2.62 ± 0.10^{a}	4.25 ± 0.01^{d}	4.47 ± 0.23^{e}	3.07 ± 0.08^{c}	2.86 ± 0.24^{b}	4.66 ± 0.36^{e}	4.39 ± 0.11^{e}
°Brix	6.5 ± 0.7^{b}	1.0 ± 0.0^{a}	1.0 ± 0.1^{a}	1.1 ± 0.0^{a}	1.5 ± 0.1^{a}	1.1 ± 0.1^{a}	1.2 ± 0.2^{a}
Moisture content (%)	6.82 ± 0.05^{d}	5.36 ± 0.49^{c}	4.10 ± 0.08^{a}	4.89 ± 0.33^{b}	3.86 ± 1.07^{a}	5.44 ± 0.06^{c}	4.37 ± 0.33^{a}
Aw	0.245 ± 0.009^{d}	0.150 ± 0.012^{d}	0.240 ± 0.008^{a}	0.191 ± 0.005^{c}	0.181 ± 0.003^{c}	0.232 ± 0.009^{d}	0.173 ± 0.005^{b}
L^*	47.86 ± 1.77^{a}	83.32 ± 3.89^{b}	82.36 ± 2.38^{b}	86.00 ± 2.47^{b}	88.29 ± 3.96^{b}	80.77 ± 0.67^{b}	80.11 ± 1.75^{b}
a^*	17.22 ± 1.01^{d}	8.57 ± 0.87^{b}	11.00 ± 1.03^{c}	19.12 ± 1.92^{e}	16.27 ± 0.92^{d}	4.29 ± 0.35^{a}	4.04 ± 0.32^{a}
b^*	9.31 ± 1.19^{c}	5.63 ± 0.52^{b}	5.85 ± 0.28^{b}	2.44 ± 0.04^{a}	2.27 ± 0.02^{a}	12.31 ± 0.01^{d}	13.07 ± 0.02^{d}
h_{ab}	28.32 ± 2.13^{c}	56.58 ± 2.13^{d}	61.99 ± 3.24^{e}	82.73 ± 0.27^{f}	82.07 ± 1.28^{f}	19.20 ± 0.84^{b}	17.18 ± 1.36^{a}
C^*_{ab}	19.57 ± 1.38^{d}	10.26 ± 0.80^{a}	12.46 ± 0.94^{b}	19.27 ± 0.82^{d}	16.43 ± 1.93^{c}	13.04 ± 0.89^{b}	13.68 ± 0.46^{b}
Anthocyanins content (mg of C3G/100 g of sample)							
D3G	2.51 ± 0.30^{e}	0.54 ± 0.56^{c}	0.51 ± 0.72^{c}	0.58 ± 0.33^{d}	0.51 ± 1.14^{c}	0.48 ± 0.14^{b}	0.42 ± 0.66^{a}
C3G	100.00 ± 5.42 ^{g}	3.53 ± 3.01^{d}	3.25 ± 0.11^{c}	5.42 ± 4.13^{f}	1.87 ± 0.43^{a}	4.69 ± 0.15^{e}	2.31 ± 0.89^{b}
Organic acids content (mg of acid/100 g of sample)							
Malic acid	7203.42 ± 2.61^{f}	2012.21 ± 2.83^{e}	2017.53 ± 2.82^{e}	1393.65 ± 3.31^{a}	1534.67 ± 3.21^{b}	1684.81 ± 3.04^{c}	1859.93 ± 2.94^{d}
Ascorbic acid	6306.53 ± 0.15^{f}	750.81 ± 8.73^{e}	708.23 ± 8.91^{d}	596.04 ± 7.83^{b}	548.47 ± 1.08^{a}	680.13 ± 0.70^{c}	578.58 ± 9.82^{b}

D3G = Delphinidin-3-glucoside; C3G = Cyanidin-3-glucoside. LCC = lyophilized camu-camu fruit. MD = 100 % maltodextrin, WP = 100 % whey protein, MDWP = mixture 50:50 of MD:WP, 150/180 °C = inlet temperatures of the process. According to Tukey test, different superscripts within the same row mean significant differences ($p < 0.05$) in ascending order (a-g). Data are expressed as mean ± SD, $n = 3$.

materials (Fig. 1) was performed by SEM. The encapsulating materials affected the outer morphology of microencapsulated camu-camu powders. The formation of microcapsules was confirmed in the samples MD150 and MD180 by the change from the irregular and non-defined shape of maltodextrin (Fig. 1A) to spherical-type shapes of the camu-camu powders (Fig. 1E and 1F); in contrast, microencapsulates obtained with whey protein showed cluster-irregular shapes (Fig. 1G and 1H) that slightly turned to spheroidal morphology when maltodextrin was present (Fig. 1C and 1D). There was no evident difference in the morphology related to the inlet temperature in each encapsulating agent.

Different particle sizes were obtained according to the encapsulating agent; for MD150 and MD180 90 % of particles were in the range of 1.5–12.5 μm, while for WP150 and WP180 most of the particles (>90 %) were between 5 and 45 μm, and for MDWP150 and MDWP180, the particles were distributed among 15–180 μm. These results are consistent with better spherical shapes found in the microencapsulates obtained with maltodextrin and the particle aggregation of WP microencapsulates. The hydrophobic whey globular protein structures affect the morphological and technological properties of microencapsulates (Feng et al., 2021). Furthermore, the formation of large aggregates could be attributed to the interaction between the polyphenols in the sample and whey proteins through covalent and electrostatic bonds (Manoj Kumar et al., 2022).

3.2. Bioactive compound content

The content of major bioactive compounds of camu-camu fruit (anthocyanins, ascorbic acid, and malic acid) was determined in FCC and the powders (Table 1).

Anthocyanins of camu-camu are mainly in the fruit peel. Previous studies reported that camu-camu fruit from Brazil contains C3G (89.5 ± 1.7 mg/100 g fruit) and D3G (4.2 ± 1.5 mg/100 g fruit) as the major anthocyanins (Zanatta et al., 2005). Fracassetti et al. (2013) reported only the presence of C3G on the spray-dried fruit (19.63 ± 0.60 mg/100 g powder), with higher values observed in this study when working with both the fruit pulp and peels. In this experiment, cyanidin-3-glucoside (C3G) was identified as the major anthocyanin, followed by delphinidin-3-glucoside (D3G). The drying process affected the initial content of anthocyanins (Table 1), reducing both C3G and D3G in final camu-camu microencapsulates, being more significant in C3G content (% reduction between 94.9 and 98.1 %) in comparison to D3G (% reduction between 83.3 and 76.9 %). The higher inlet temperature (180 °C) induced less content (8–65 %) of C3G compared to 150 °C when MD or WP was used; however, the mixture of MD and WP showed better protection of anthocyanins to the inlet temperature effect. Also, the type of encapsulating agent affected the anthocyanins content in the microencapsulates, for example, microencapsulates with MD presented the highest retention of C3G and D3G at 150 °C, compared to the other encapsulating agents. Prior findings revealed that the interaction of dextrins with the flavylium cation of anthocyanins prevents their conversion into less stable forms; thus, maltodextrin contributes to their stabilization by reducing the mobility of reactants (Estupiñan et al., 2011).

Malic and ascorbic acid contents were also assessed in camu-camu powders (Table 1). Malic acid (492.04 ± 0.14 mg/100 g fresh weight) is responsible for the antihyperglycemic activity of this fruit because it inhibits α-amylase (IC_{50} = 96.40 ppm) and α-glucosidase (IC_{50} = 58.15 ppm) (Alakolanga et al., 2015). In this work, it was found that malic acid content decreased in the camu-camu microencapsulates compared to the lyophilized fruit and fresh fruit (492.04 ± 0.14 mg/100 g fw, Table S1). However, it was observed that the highest amount of malic acid was found in samples with MD:WP showed a positive effect of WP in protecting this bioactive compound. Regarding the inlet temperature, and within every pair of camu-camu powders, samples obtained at 180 °C presented higher contents in comparison to those obtained at 150 °C, which can be explained because higher drying temperatures facilitate the spray-drying process, decreasing the moisture content of the final product, and transferring higher stability to malic acid over time (Ozkan et al., 2019). Igual et al. (2010) reported that low molecular weight organic acids such as malic acid are relativity stable with increasing temperature, whereby the spray-drying process could possibly improve the retention of malic acid in camu-camu.

Camu-camu is recognized to be one of the largest sources of ascorbic acid in nature, a potent antioxidant compound (Cunha-Santos et al., 2019). Ascorbic acid is a thermolabile and easily oxidable compound, so high temperatures, pH changes, and metal ions or enzymes can affect its content. In this study, all the camu-camu microencapsulates presented high ascorbic acid loss (*ca.* 88–91 %) related to the lyophilized fruit, which is in agreement with the literature (Fujita et al., 2017). Results showed that samples obtained at 180 °C had lower ascorbic acid values than those at 150 °C due to higher temperature; interestingly, WP presence favored ascorbic acid retention in powders. Vitamin C is essential for synthesizing different proteins such as collagen and *L*-carnitine (Bechara et al., 2022); it can interact with different enzymes by donating electrons. Consequently, the protein present in WP can electronically retain the ascorbic acid within the microcapsules.

3.3. Stability of colour and bioactive compounds during storage of powders

Changes in colour and concentration of anthocyanins (C3G and D3G), malic acid, and ascorbic acid of four powders (MDWP150, MDWP180, MD150, and LCC) were analyzed through storage time (31 days) at three relative humidity values (33 %, 75 %, and 95 %) in darkness. From data of Table 1, MDWP150 and MDWP180 microencapsulates were selected for the stability test and bioaccesibility evaluation, because they retained the highest amount of ascorbic and malic acids after spray-drying processes. Moreover, the MD150 powder was included as having the highest value of anthocyanins, and LCC was included because this powder preserved the highest content of bioactive compounds due to drying without thermal treatment. The results of the kinetic analysis of bioactive compound degradation are presented in Table 2, and the variation of different parameters through the storage time can be seen in Fig. S1 (supplementary material).

Regarding the colour evolution during storage, Fig. S1A shows the colour differences to the initial samples (t = 0 min). Colour is one of the attributes that allows for a quick assessment of whether the humidity has a significant impact on the microencapsulated products. In general, all samples underwent a colour change from reddish pink to dark yellowish pink hues over time. In the highest humidity (95 %) environment, more changes were detected, mainly in the MDWP180, MDWP150, and MD150 microencapsulated samples and in minor proportion in the LCC sample. These changes did not occur gradually, but a great quantum leap happened around day 10, followed by a plateau. In this leap and regarding each colour attribute, lightness decreased while chroma and hue increased. According to these results, the encapsulating material and relative humidity (RH) are critical parameters that directly affect the colour of camu-camu powders. This phenomenon could be associated with anthocyanin degradation (Figueiredo et al., 2020; Annunziata et al., 2020).

The relative humidity significantly affected the anthocyanin (C3G and D3G), ascorbic acid, and malic acid contents, being more stable at 33 % RH in each group. The content of bioactive compounds was higher in LCC compared to the microencapsulates; however, the stability of anthocyanins through time was higher in microencapsulates containing only maltodextrin because the variation in content is lower than in LCC samples (Table 2, and Fig. S1). For instance, final retention for D3G at the highest RH value (95 %) was 50 % for MD150 in comparison to 25 % for LCC, similarly, for C3G the final retention at 95 %RH was 12 % for MD150 and 8 % for LCC. These results probed the benefits of microencapsulation by spray-drying to protect some of the biofunctional

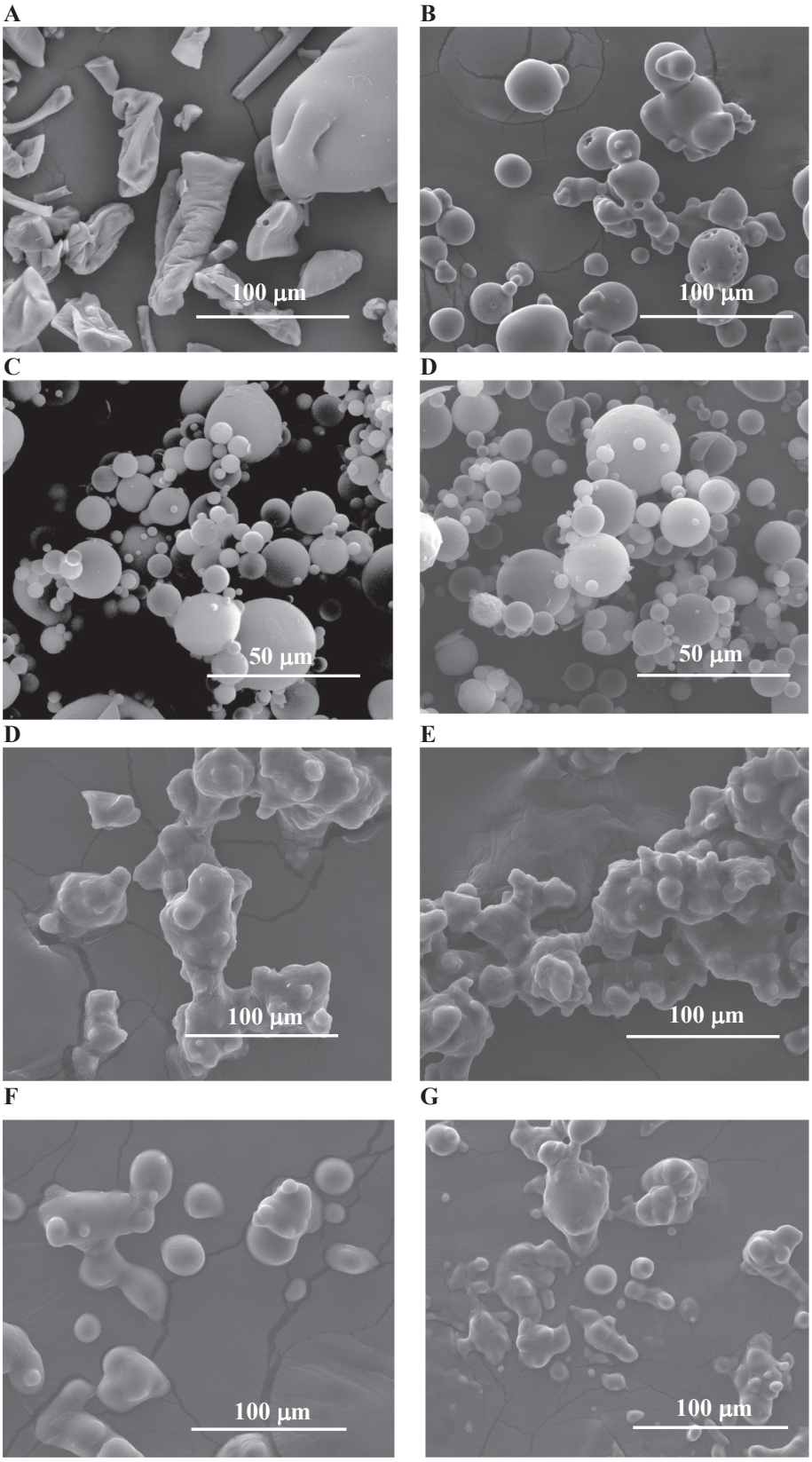

Fig. 1. Micrographs obtained by SEM of microencapsulates of camu-camu and the encapsulating agents. (A) MD, (B) WP, (C) MD150, (D) MD180, (E) WP150, (F) WP180, (G) MDWP150 and (H) MDWP180 (MD = Maltodextrin; WP = whey protein).

Table 2
Kinetic parameters of loss/degradation of bioactive compounds (anthocyanins, ascorbic acid, and malic acid) during camu-camu powders storage stability test.

Bioactive compound	Kinetic parameter				
	Relative humidity (%)	Camu-camu powder	k x 10^{-2} ($days^{-1}$)	Correlation coefficient (r^2)	$t_{1/2}$ (days)
Delphidine-3-glucoside	33 %	LCC	1.0^{b}	0.982	69.3^{b}
		MDWP 150	1.1^{c}	0.975	63.0^{a}
		MDWP 180	1.1^{c}	0.971	63.0^{a}
		MD 150	0.8^{a}	0.979	86.6^{c}
	75 %	LCC	3.4^{a}	0.964	20.4^{b}
		MDWP 150	3.9^{c}	0.977	17.8^{a}
		MDWP 180	3.6^{b}	0.973	19.3^{b}
		MD 150	3.2^{a}	0.970	21.7^{c}
	95 %	LCC	14.0^{d}	0.956	5.0^{a}
		MDWP 150	10.9^{b}	0.975	6.4^{c}
		MDWP 180	12.4^{c}	0.984	5.6^{b}
		MD 150	5.5^{a}	0.982	12.6^{d}
Cyanidin-3-glucoside	33 %	LCC	3.7^{a}	0.920	18.7^{c}
		MDWP 150	11.2^{c}	0.972	6.2^{a}
		MDWP 180	9.4^{b}	0.983	7.4^{b}
		MD 150	3.6^{a}	0.986	19.3^{c}
	75 %	LCC	22.0^{b}	0.974	3.2^{b}
		MDWP 150	42.6^{c}	0.981	1.6^{a}
		MDWP 180	45.8^{d}	0.980	1.5^{a}
		MD 150	16.5^{a}	0.990	4.2^{c}
	95 %	LCC	32.0^{a}	0.984	2.2^{d}
		MDWP 150	64.8^{c}	0.978	1.1^{b}
		MDWP 180	73.7^{d}	0.989	0.9^{a}
		MD 150	47.2^{b}	0.994	1.5^{c}
Ascorbic acid	33 %	LCC	20.1^{a}	0.954	3.4^{b}
		MDWP 150	26.4^{b}	0.971	2.6^{a}
		MDWP 180	26.4^{b}	0.971	2.6^{a}
		MD 150	27.5^{b}	0.957	2.5^{a}
	75 %	LCC	37.4^{a}	0.958	1.9^{c}
		MDWP 150	54.6^{c}	0.941	1.3^{b}
		MDWP 180	62.3^{d}	0.971	1.1^{a}
		MD 150	50.2^{b}	0.975	1.4^{b}
	95 %	LCC	50.4^{a}	0.956	1.4^{c}
		MDWP 150	69.1^{c}	0.973	1.0^{a}
		MDWP 180	62.3^{b}	0.956	1.1^{c}
		MD 150	68.1^{c}	0.969	1.0^{a}
Malic acid	33 %	LCC	5.0^{a}	0.960	13.9^{b}
		MDWP 150	5.0^{a}	0.985	13.9^{b}
		MDWP 180	5.0^{a}	0.986	13.9^{b}
		MD 150	9.5^{b}	0.971	7.3^{a}
	75 %	LCC	33.1^{b}	0.984	2.1^{a}
		MDWP 150	31.2^{a}	0.966	2.2^{a}
		MDWP 180	31.3^{a}	0.979	2.2^{b}
		MD 150	33.9^{b}	0.986	2.0^{a}
	95 %	LCC	35.0^{c}	0.981	2.0^{b}
		MDWP 150	32.1^{b}	0.981	2.2^{c}
		MDWP 180	32.4^{b}	0.985	2.1^{b}
		MD 150	48.4^{a}	0.968	1.4^{a}

k, First-order reaction rate constant; $t_{1/2}$, half-life time; data are expressed as mean, different superscripts in same columns and group are significantly different ($p < 0.05$) by Tukey test in ascending order (a–d).

compounds.

The kinetic parameters of Table 2 showed that D3G and C3G are better protected in the MD150 powder because higher half-life times were obtained at each relative humidity. In general, D3G was more stable than C3G, with a $t_{1/2}$ = 86.6 days for MD150 at 33 % RH. Anthocyanin stability depends on external factors such as pH, temperature, or oxygen. Structurally, the flavylium ion of anthocyanins is easily affected by pH changes and can be converted to carbinol pseudo-bases and chalcones as water content increases, converting colored flavylium cation into colorless compounds (Quatrin et al., 2020). Moreover, most anthocyanins are unstable at basic pH and tend to degrade to dark brown oxidized compounds (Tarone et al., 2020). Thus, MD150 powder (pH = 3.07 ± 0.08) presented higher stability and anthocyanin content than MDWP150 (pH = 4.25 ± 0.01) and MDWP180 (pH = 4.47 ± 0.23). As it can be seen in Table 1, the pH of samples containing whey protein exhibited higher pH values than those microencapsulated only with maltodextrin, due to the original pH value of encapsulating agents (pH of maltodextrin = 4.58 ± 0.04; and pH of whey protein = 6.23 ± 0.07).

In contrast, LCC samples showed higher protection for malic and ascorbic acids. And between the microencapsulates, there were no significant differences (Table 2). Ascorbic acid content ($t_{1/2}$ = 3.4 days for LCC at 33 %RH) is more affected by %RH than malic acid ($t_{1/2}$ = 13.9 days for LCC at 33 %RH) in camu-camu powders. But still, the amount of ascorbic acid in camu-camu microencapsulates (MDWP150 at 33 % RH, varies from 711.7 mg/100 g to 266.4 m/100 g after 31 days of storage) is higher than the reported for orange fruit (53.2 mg/100 g FW, USDA 2019), a reference in vitamin C content.

3.4. Sensory analysis of beverages

The four powders selected for the storage stability test (MDWP150, MDWP180, MD150, and LCC) were applied to commercial yogurt (Y) and white grape juice (G). The same amount of each powder was added to the beverages and then submitted to the assessment of colour, sweetness, acidity, and global acceptance for a trained sensory panel. The juice of fresh camu-camu fruit (FCC) was included in the sensory evaluation as a reference.

For the yogurt beverages, slightly significant differences were found in sweetness and acidity, finding a positive effect of whey protein enhancing the sweetness and decreasing the acidity in comparison to the YLCC sample (Table 3). Colour evaluation showed pink colour for YLCC and YMDWP150, while YMDWP180 and YMD150 were described as red by the sensory panel. Moreover, YMDWP180 showed better overall acceptance among judges. The yogurts that used powders containing whey protein got a better global acceptance; according to panelists comments, adding camu-camu powders in yogurt confers better sweetness-acidity balance and higher acceptance than FCC blank.

On the other hand, grape juice beverages differed widely in colour. GMD150 presented red colour, while GMDWP150 had a yellow one that resembled the grape juice blank. The highest sweetness value was for GMDWP180, and the highest acidity value was for GMD150 and GMDWP150. Commercial grape juice blank was described as sweet, and the camu-camu powder addition increased the acidity, thus reducing

Table 3
Sensory evaluation of yogurts and white grape must beverages with addition of selected camu-camu powders.

Beverage	Colour	Sweetness	Acidity	Global acceptance
Yogurt samples				
YLCC	4.5 ± 0.9^{b}	4.8 ± 1.8^{a}	4.1 ± 1.9^{a}	5.2 ± 1.6^{a}
YMDWP 150	4.1 ± 1.2^{a}	5.3 ± 2.1^{b}	4.5 ± 1.5^{b}	5.6 ± 1.3^{b}
YMDWP 180	5.8 ± 1.0^{c}	5.1 ± 1.5^{b}	4.6 ± 1.8^{b}	6.1 ± 1.9^{c}
YMD 150	7.0 ± 0.8^{d}	4.7 ± 2.3^{a}	5.1 ± 2.1^{c}	5.0 ± 2.8^{a}
Grape must samples				
GLCC	5.7 ± 1.3^{c}	5.9 ± 1.2^{c}	3.9 ± 1.5^{a}	6.3 ± 2.6^{c}
GMDWP 150	2.7 ± 1.0^{a}	4.5 ± 1.8^{a}	5.7 ± 1.3^{c}	4.1 ± 1.4^{a}
GMDWP 180	4.7 ± 1.3^{b}	6.3 ± 1.4^{d}	4.5 ± 1.6^{b}	4.9 ± 1.9^{b}
GMD 150	7.5 ± 1.4^{d}	4.9 ± 1.3^{b}	5.9 ± 1.4^{c}	7.1 ± 1.9^{d}

Yogurt with: 0.5 % addition of LCC (YLCC), 15 % addition of MDWP 150 (YMDWP 150), 15 % addition of MDWP 180 (YMDWP 180), or 15 % addition of MD 150 (YMD 150). Grape must with 0.5 % addition of LCC (GLCC), 15 % addition of MDWP 150 (GMDWP 150), 15 % addition of MDWP 180 (GMD 180), or 15 % addition of MD 150 (GMD 150). The results are expressed as mean ± SD ($n = 17$). Means with difference superscripts in the same column and group are significantly different ($p < 0.05$) by Tukey test in ascending order (a–d).

and balancing the sweetness perception. Only in the case of GMD150 and GLCC, a better freshness sensation was perceived, so these two beverages were better accepted. Likewise, the panellists noticed that the incorporation of camu-camu powders to the food matrices (both yogurt and grape juice) masked the high-acidity taste of FCC, enhancing its sensory properties and overall sensory quality (data not shown). The beverages with camu-camu powders that obtained the highest score in the global acceptance (YMDWP180 and GMD150) were selected by the bioaccessibility study as well as the samples containing LCC powder (LCC and GLCC) as references.

3.5. Bioaccessibility of bioactive compounds

The bioactive compounds are absorbed along the human digestive tract, transited in the circulatory system, and excreted in the urine and feces (Minekus et al., 2014). Hence, the bioaccessibility of compounds in the juice of fresh fruit (FCC) and the camu-camu powders (LCC, MDWP150, MDWP180, and MD150), and selected camu-camu beverages (YLCC, YMDWP180, GLCC, and GMD150) was measured to determine the influence of the gastric and intestinal phases on anthocyanins (C3G and D3G), ascorbic acid, and malic acid retention (Fig. 2). YLCC and GLCC samples were used as control because they contained the lyophilized fruit, and YMDWP180 and GMD150 were selected according to highest value of the global acceptance after sensory evaluation (Table 3). All data are found in Table S1 (supplementary material). It should be noted that the oral phase was not evaluated in the experiment due to the short mastication time of the samples under study, resulting in minimal to no interaction of oral enzymes with the food (Minekus et al., 2014).

Along the gastrointestinal tract, data showed higher recovery values of C3G than D3G in camu-camu powders (Fig. 2A), maybe due to a higher oxidizability of D3G (Tarone et al., 2021). Sample MD150 exhibited the highest anthocyanin bioaccessibility, with 50.0 % for D3G and 67.9 % for C3G, compared to fresh fruit (8.33 % for D3G and 42.79 % for C3G) and LCC (32.67 % for D3G and 46.63 % for C3G). These results are consistent with those of the stability test, where the protective role of MD in anthocyanins was evident. Better stability of C3G than D3G during the digestion process, was also observed in the digestion analysis of the *Myrciaria jaboticaba* peel, where a higher oxidizability of D3G was attributed to the presence of 1,2,3-trihydroxyphenyl moiety in its structure, in comparison to the 1,2-dihydroxyphenyl moiety of C3G (Tarone et al., 2021). Although total anthocyanins were stable under the *in vitro* gastric conditions, recovery values decreased during *in vitro* intestinal digestion, being more significant for D3G in the FCC reference sample. However, when comparing the results between FCC and LCC, it is observed that the lyophilization process increased the bioaccessibility of anthocyanins, with a more significant impact on D3G. The reduction of anthocyanin content in the small intestine could be attributed to their high instability in the less acid pH or the formation of anthocyanin-based complexes by proteins and bile salts bindings, forming precipitated and non-digestible compounds (Victoria-Campos et al., 2022). Furthermore, published data showed that recovery of anthocyanins during intestinal digestion did not exceed 5–10 % (Tarone et al., 2021); however, in camu-camu samples (FCC, LCC, and selected microencapsulates), C3G presented higher values (up to 46 %) possibly due to stability of cyanidin-3-glucoside in an alkaline environment despite of having just a single hydroxyl group in the B ring (Sengul et al., 2014). Indeed, anthocyanins stability could also decrease with the degree of hydroxylation of the B ring, and consequently, cyanidin glucosides are less susceptible to degradation than those of D3G.

Bioaccessibility of anthocyanins in camu-camu beverages is shown in Fig. 2B. No content of anthocyanins was detected, neither in commercial yogurt nor white grape juice (food matrices). It was observed that anthocyanins content in the yogurts (YLCC and YMDWP180) increased its bioaccessibility compared to the corresponding camu-camu powders (Fig. 2A). Both D3G and C3G presented higher recovery values during overall *in vitro* gastric digestion in yogurts. As raw materials, anthocyanins in yogurts showed the same digestion behavior, with high stability during the gastric phase and a content decrease in the intestinal phase. Previous studies indicated that the biological behavior of anthocyanins is also influenced by the food matrix, as it was observed between LCC and MDWP 180 applied in yogurt (Rodríguez-Roque et al., 2015). Likewise, the same behavior was observed in riceberry rice yogurts where C3G content remained constant through intestinal digestion (Anuyahong et al., 2020), and anthocyanins from pomegranate remained the same after *in vitro* digestion when pomegranate extract was applied to yogurt (Sengul et al., 2014). In contrast, the bioaccessibility of anthocyanins in grape juice (GLLC and GMD150) decreased after *in vitro* gastrointestinal digestion compared to raw materials, presenting values up to 5.0 % (D3G) and 10.4 % (C3G) for GLLC, and 14 % (D3G) and 7.75 % (C3G) for GMD150 (Fig. 2B). These results agree with anthocyanins bioaccessibility from grape juice sediments over *in vitro* digestion, where even no C3G or D3G were detected (da Silva Haas et al., 2018).

Bioaccessibility of ascorbic acid was calculated in camu-camu fruit juice (FCC) and powders (Fig. 2A). FCC is one of the main enriched natural sources of ascorbic acid (3813.5 ± 0.120 mg/100 g), however, dried camu-camu powders exhibited more ascorbic acid bioaccessibility (MD150 (63.55 %), MDWP180 (61.77 %), MDWP150 (54.34 %), and LCC (65.54 %)) than FCC (30.49 %) with significant differences ($p < 0.05$). The reason is related with food matrix, postharvest storage, type of encapsulating agent, and operating conditions as drying processes allowed a slow release of the acid in the stomach (De Ancos et al., 2017; Leyva-López et al., 2019). Additionally, acidic conditions of the stomach could protect ascorbic acid against chemical or enzymatic oxidation in the gastric digestion phase since, at low pH, fully protonated ascorbic acid is slowly attacked by oxygen (Rodríguez-Roque et al., 2015). In that sense, camu-camu dried samples presented ascorbic acid retention of up to 88 % under gastric conditions (Table S1, supplementary material). Nonetheless, ascorbic acid could be unstable under duodenal digestion because of conditions such as oxygen, temperature, enzyme activity, interaction with metal ions, and specifically alkaline pH that can affect its stability (De Ancos et al., 2017). Indeed, pH values above 4.0 could enhance ascorbic acid degradation to *L*-dehydroascorbic acid or non-reversible forms (2,3-diketogulonic acid) and ascorbic acid-complex formation because of the compound oxidation (Yaman et al., 2019). A reduction in ascorbic acid bioaccessibility was observed from camu-camu powders to yogurt samples (YLCC and YMDWP180) (Fig. 2B). The same phenomenon was observed in grape juice samples (GLCC and GMD150), where recovery values of ascorbic acid during digestion were low compared to LCC and MD 150, respectively. This phenomenon was

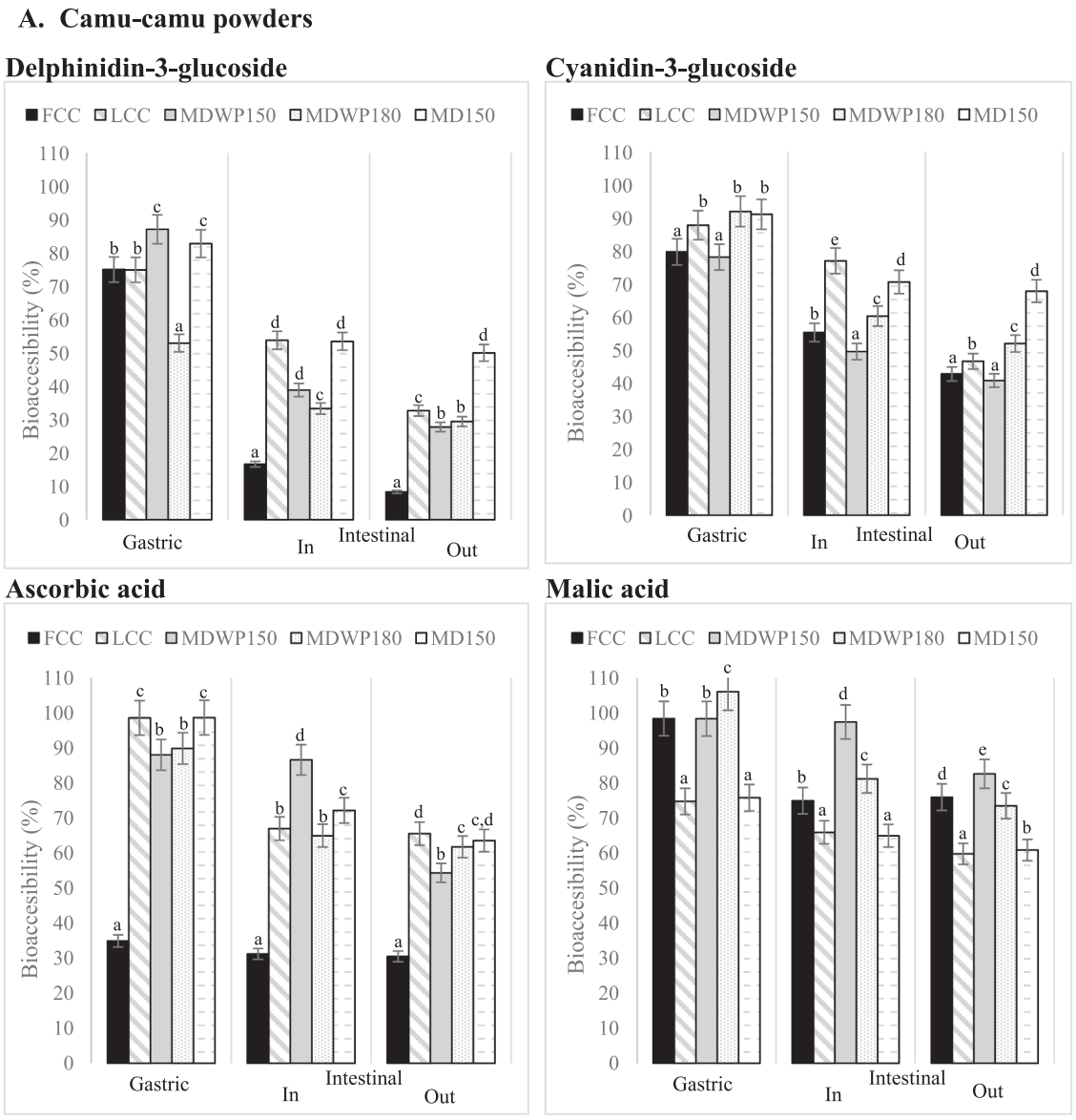

Fig. 2. Bioaccessibility (%) of delphinidin-3-glucoside, cyanidin-3-glucoside, ascorbic acid, and malic acid in (A) camu-camu powders and (B) camu-camu beverages. Yogurt with: 0.5 % addition of LCC (YLCC) and 15 % addition of MDWP 180 (YMDWP 180. Grape juice with 0.5 % addition of LCC (GLCC), and 15 % addition of MD 150 (GMD 150). Different letters indicate statistically significant differences ($p < 0.05$). Number of technical replicates, $n = 4$.
The bioaccessibility was calculated as a recovery percentage (%), considering the ratio between the concentration of the bioactive compound in the digest and in the initial sample (FCC). Data are summarized in Table S1 (supplementary material).

more significant in the GLCC sample, showing the protector effect of the encapsulating agent. Food matrix allows a higher release of this acid, even before digestion, promoting a higher contact with oxygen and, the degradation of ascorbic acid compared to the microencapsulates and LCC.

Previous studies have reported that malic acid exhibits bioactive properties, acting as a stimulant for metabolism and increasing energy production in cells (Santana Andrade et al., 2022). Although malic acid concentration decreased during the overall *in vitro* digestion process, it presented higher recovery values compared to total anthocyanins and ascorbic acid (Fig. 2). An increase of malic acid during the gastric phase is related to the transformation or generation of compounds, over-expressing organic acids as malic acid (surpassing the 100 % bio-accesibility level) (Santana Andrade et al., 2022). In fact, previous reports of blackberry showed a high release of malic acid during the whole *in vitro* digestion and exhibited a bioaccessibility of 68.90 % (Dou et al., 2022). The lowest value obtained for FCC and camu-camu powders in the gastric phase was near 61.0 % (Fig. 2A). Likewise, results determined that powders containing WP as wall material allowed the highest value of malic acid percentage of bioaccessibility during the digestion process (98.3–82.6 % for MDWP150, and 106.1 to 73.5 % for MDWP180, through three digestion phases). During the digestion process, whey protein also undergoes degradation due to the action of digestive enzymes. In the gastric phase, erosion of the encapsulated particles can occur, and under duodenal conditions, denaturation of whey proteins may take place, a characteristic that allows for greater release and hydrolysis of the compounds (Alvarado et al., 2019).

Malic acid was found in grape juice with an initial content of 1.05 ± 0.74 mg malic acid/ 100 g. Bioaccessibility of malic acid increased mainly in yogurts and grape juices with LCC powder after digestion. Indeed, this compound had high stability during the gastric phase and remained unchanged during the intestinal phase. The bioaccessibility of

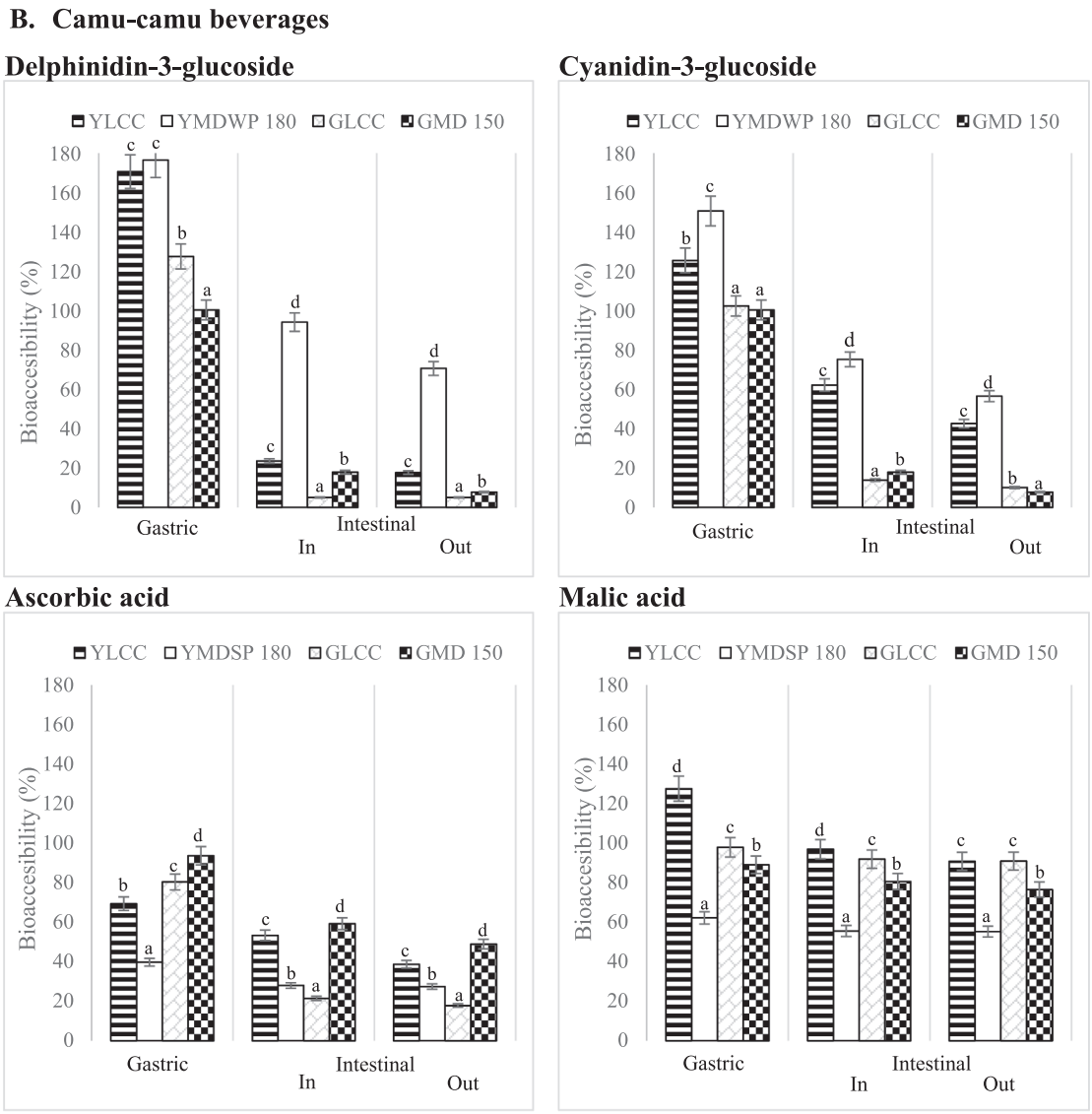

Fig. 2. (*continued*).

malic acid ranged between 50 and 90 % in yogurts and 76–91 % in grape juice (Fig. 2B). Similar results were found in on-milk products fortified with folic acid, where bioaccessibility of folic acid in milk products was lower than that in products which do not contain milk (Yaman et al., 2019). In Fig. 2B, YMDWP180 showed the lowest bioaccessibility value (55.3 ± 1.6 %), likely due to proteins interaction with the acid at gastric conditions. Subsequently, yogurt and grape juice, with the addition of camu-camu powders, increased the content of malic acid throughout the gastrointestinal tract, enhancing the bioaccessibility values and acid stability.

4. Conclusion

Dehydration processes (freeze-drying and spray-drying) improved the stability of bioactive compounds of camu-camu (*M. dubia*). Thus, microencapsulates obtained from *M. dubia* fruit exhibited Aw values lower than 0.250 and preserved anthocyanins (D3G and C3G) as well as ascorbic and malic acids from the fruit. Among encapsulating agents, maltodextrin was more suitable for protecting anthocyanins (C3G and D3G), while whey protein was more favorable for ascorbic and malic acids. Despite thermal treatment in spray-drying, ascorbic acid was present in camu-camu powders with a higher content than other known sources (for example orange fruit).

After incorporating these powders into different beverages, yogurt and white grape juice, a sensory study allowed the characterization of the most relevant sensory descriptors, resulting in a balanced sweetness-acidity profile. The processes of spray-drying and freeze-drying, and incorporation into two food matrices enhanced the recovery of the bioactive compounds mentioned after the *in vitro* gastrointestinal digestion, leading to higher bioaccessibility values and stability. Bioaccessibility values of anthocyanins (C3G and D3G), ascorbic acid, and malic acid were influenced by physiological conditions (gastric and duodenal), and food matrices (yogurt and grape juice). Notably, the mixture of encapsulating agents (MD:WP 50:50) exhibited a synergistic effect, reducing degradation rates of organic acids, mainly malic acid, which reached the highest levels of bioaccessibility after digestion, up to 76 %; while anthocyanins and ascorbic acid were more easily degraded. In conclusion, these promising results have the potential to lead to the development of stable and bioactive-enriched food products utilizing tropical and Amazonian fruits, providing an economical alternative for camu-camu consumption, and encouraging the cultivation of *M. dubia*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113820.

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