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ORIGINAL PAPER

Seasonality, composition and storage of aroeira bee pollen

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Abstract

Bee pollen from aroeira (*Astronium urundeuva*) is rich in nutrients and possesses medicinal properties. This tree species is native to the semi-arid regions of Brazil and shows notable potential for beekeeping. This study aimed to characterize the structure and chemical composition of aroeira bee pollen, considering the effects of seasonality and storage. Samples were collected in the central region of Brazil in May/June over two consecutive years and stored at -21ºC for up to 240 days. Structural and biochemical analyses showed that the May/June samples were monofloral (>70% aroeira pollen), exhibiting a brown color and 37.5% higher protein content compared to samples without aroeira pollen. The sample with over 84% aroeira pollen grains exhibited markedly higher levels of phenolic compounds (57.5%), yellow flavonoids (20%), and carotenoids (1000%) compared to the sample lacking aroeira pollen. Dehydration after storage led to a significant decrease in these bioactive compounds, with reductions of 32% in carotenoids, 500% in total phenolics, and 250% in yellow flavonoids; however, antioxidant activity did not change. Phenolic acids identified include salicylic, vanillic, syringic, gallic, ferulic, and caffeic. Aroeira bee pollen can be considered a functional food and superfood with high nutritional value. However, dehydration and long-term storage reduce its nutritional quality.

Highlights

- Aroeira monofloral bee pollen (AMBP) contains over 70% Aroeira pollen grains.
- AMBP has brownish coloration and production concentrated in May and June.
- AMBP is a functional food, rich in protein, phenolics, flavonoids, and carotenoids.
- Dehydration and storage (-21 ºC / 240 days) reduce AMBP nutritional quality.

Keywords *Astronium urundeuva* · Bee product · Bioactive compounds · Functional food · Superfood

Introduction

Bee pollen, the result of the agglutination of pollen grains by bees through the addition of nectar and salivary substances, is considered a superfood, serving as a source of proteins, lipids, minerals, and bioactive compounds [1]. This product also possesses remarkable medicinal value, as it contains compounds with antioxidant, antimicrobial, anti-inflammatory, and anticancer properties [2, 3]. Due to the growing demand for natural and healthy products, the number of studies on the physicochemical and functional properties of bee pollen has increased considerably in recent years [4]. However, information about bee pollen obtained from neotropical tree species, particularly in semi-arid environments, remains scarce.

Bee pollen exhibits structural variation, which is influenced by its botanical composition; it can be monofloral,

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with a predominance of pollen grains from a single species, or multifloral, when no single pollen type predominates [5, 6]. Color can be the primary characteristic for identifying the type of bee pollen, as it is determined by the diversity of chemical compounds present in the pollen grains. Microscopic analyses are essential for determining the botanical origin and quantifying the pollen grains, which define the morphological characteristics of this product [3, 7, 8].

The characterization of the physicochemical aspects of bee pollen is essential for establishing quality standards for its application in the food and medicinal sectors. This has been demonstrated in studies where nutrients such as proteins, minerals, vitamins, and the antioxidant potential of monofloral and multifloral bee pollen samples were quantified [9–11]. Apart from its botanical origin, the chemical profile of bee pollen can be influenced by environmental factors such as climate, soil, temperature, seasonality, and radiation [3, 6, 12]. Additionally, management practices can affect the quality of bee pollen during the collection, cleaning, dehydration, packaging, and storage phases of the fresh product [13–16].

Astronium urundeuva (M. Allemão) Engl., aroeira, is a tree abundant in Brazilian deciduous seasonal forests, of great economic importance due to the use of its wood and its pharmacological properties [17]. The apicultural use of *A. urundeuva* has proven to be important in semi-arid regions of Brazil, as the species blooms during the dry season, a time of floral resource scarcity, and the honey obtained is distinctive, with medicinal properties [18, 19]. Apicultural studies on aroeira are focused on determining the quality of honey, with a significant knowledge gap regarding the bee pollen from this species [18, 20].

Detailed information regarding the quality of bee pollen is necessary to encourage both public and private entities to subsidize its collection by beekeepers, thereby providing a highly nutritious food product [4]. Furthermore, the production of bee pollen can contribute to the generation of work and income for beekeepers in vast semi-arid regions of Brazil and, indirectly, support the conservation of *A. urundeuva*, either by promoting pollination or by preserving the environment, which is inherent to the activity.

The objective was to characterize the structure and chemical composition of bee pollen from *A. urundeuva,* to address the following questions: i) What aspect can be used as a morphological marker for identifying monofloral bee pollen? ii) What is the chemical composition of the bee pollen? iii) How does seasonality influence the structure and composition of bee pollen? iv) What changes occur in the quality of chemical compounds with storage?

Materials and methods

Study area and material collection

The fresh bee pollen from *A. urundeuva* was collected from an apiary located in the rural area of the municipality of Januária, Minas Gerais, Brazil (15°29′52.5094"S; 44°30′13.4180"W) (Fig. S1A-D). The samples were collected, weekly, from May to August 2022 and in March, May, and June 2023, and stored in Falcon tubes under refrigeration, at −21 (±2) ºC, until the time of analysis.

The study area is located in a semi-arid region with the presence of species from the Cerrado, Caatinga, and Seasonally Dry Tropical Forest (SDTF) biomes [18, 21]. Aroeira is a deciduous, heliophytic, and selectively xerophytic species that occurs in dense clusters [19]. The species exhibits intense blooming during the dry season (Fig. S1B, D) and is among the most frequent and widely distributed species in the SDTF region [18]. The climate is classified as Aw type, with well-defined rainy and dry seasons, dry winters and rainy summers. Maximum temperatures in the region ranged from 29.7 °C to 37.6 °C, while minimum temperatures ranged from 12.5 °C to 23.1 °C (Fig. S1E). There was no recorded precipitation from May to August 2022, as well as in June and July 2023 (Fig. S1E) (Instituto Nacional de Meteorologia – INMET, station nº 83386).

Pollen analysis and morphology

The bee pollen samples, collected weekly, were grouped by month. From each batch, 4 g were weighed using an analytical balance (Shimadzu, ATX224), divided into four subsamples, the pellets were classified by color (yellow, white, and brown) to determine the percentage of each in the bee pollen. For each subsample, the length and width of 50 pellets were measured using ImageJ software. For pollen analysis, 2 g of bee pollen from each batch were weighed and grounded using a mortar and pestle. Then, 13 mL of 70% alcohol were added, and the mixture was left to rest for 30 min. A drop of this solution was used to prepare the slide, which was mounted with glycerinated gelatin. For identification and counting, a sample of at least 350 pollen grains was analyzed. Photographic documentation was carried out using an AxioCam MRC camera attached to an AxioVision LE microscope (Zeiss, Oberkochen, Germany). The percentages of each pollen type in the sample were classified according to their frequency as follows: dominant pollen (DP > 45%), accessory pollen (AP between 15 and 45%), and isolated pollen (IP < 15%) [22].

The dry mass and moisture content were determined by drying four replicates of 2.0 g of bee pollen from each batch in an oven (Fanen, 315SE), at 105 ± 5 ºC until a constant weight was reached [23].

Micromorphology

For the micromorphological analysis, fresh pellets of different colors (white, yellow, and brown) were directly sputter coated with a 10 nm layer of gold and examined using a DSM 940A scanning electron microscope (Zeiss, Cambridge, United Kingdom) at 15–20 kV [24] (Fig. S2).

Anatomy and histochemistry

For structural analysis, pellets containing more than 90% aroeira pollen grains were fixed in Karnovsky's solution for 12h [25], dehydrated in an ethanol series (5%−95%) [26], and embedded in hydroxyethyl methacrylate resin (Leica Microsystems, Heidelberg, Germany) [27]. Cross sections, with thicknesses of 3 and 7 µm, were obtained using a rotary microtome (Leica, Autocut, Germany) and stained with toluidine blue at pH 4.7 [28] (Fig. S2).

Histochemical tests were performed using Lugol's reagente for starch detection [29], Xylidine Ponceau (XP) for proteins [30], Sudan Black for total lipids [31], Nile blue sulfate for neutral and acidic lipids [32], ruthenium red for pectins [29], coriphosphine under UV light for pectins [33], ferric chloride for phenolic compounds [29], α-naphthol and NADI reagent for terpenes [34], vanillin hydrochloride for tannins [35], Dittmar's reagent for alkaloids [36], and p-dimethylaminocinnamaldehyde (DMACA) for flavonoids [37]. Unstained sections were used as controls. Photographic documentation was carried out using an AxioCam MRc camera attached to an AxioVision LE microscope (Zeiss, Oberkochen, Germany) (Fig. S2).

Chemicals and reagents

Ascorbic acid (CAS number: 50–81-7; molecular weight: 176.12), gallic acid (CAS number: 5995–86-8; molecular weight: 188,13), ethanol (CAS number: 64–17-5; molecular weight: 46.07) sulfuric acid (CAS number: 7664–93-9; molecular weight: 98.08), sodium hydroxide (CAS number: 1310–73-2; molecular weight: 40), sodium carbonate (CAS number: 497–19-8; molecular weight: 105.99), chloride acid (CAS number: 7647–01-0; molecular weight: 36.46), boric acid (CAS number: 10043–35-3; molecular weight: 61.83), methyl red (CAS number: 493–52-7; molecular weight: 269.3), ethyl ether (CAS number: 60–29-7; molecular weight: 74.12), hexane (CAS number: 110–54-3; molecular weight: 86.18), acetone (CAS number: 67–64-1; molecular weight: 58.08), ammonium molybdate (CAS number: 12054–85-2; molecular weight: 1235.86), were purchased from Neon (Brazil). Sodium phosphate (CAS number: 10101–89-0; molecular weight: 141.96), Folin-Ciocalteu phenol reagent (CAS number: 12111–13-6; molecular weight: 94.11), 2,2-diphenyl-1-picrylhydrazyl (DPPH; CAS number: 1898–66-4; molecular weight: 394.32), and BSTFA (CAS number:25561–30-2; molecular weight: 2547.40) were purchased from Alphatec (Brazil), Dinâmica (Brazil), and Sigma-Aldrich (USA), respectively. All the chemicals and reagents used in this experiment are of analytical grade.

Physical and chemical analyses

The methodological procedure for the chemical analyses is summarized in the supplementary material (see Fig. S3). Physical and chemical analyses were conducted on the following treatments: I) fresh bee pollen stored under refrigeration at −21 ± 2 °C for 30 days; II) fresh bee pollen stored under refrigeration at −21 ± 2 °C for 240 days; and III) dehydrated bee pollen stored at room temperature (25 ºC) and protected from light for 90 days.

A 30 g sample of bee pollen from June 2023 was weighed using an analytical balance (Shimadzu, ATX224) and placed in an oven (Fanem, 315 SE) at 40 °C until a constant weight was achieved to obtain the dehydrated sample [6].

The color analysis was determined for each batch by measuring ten different points on the sample using the Konica Minolta CR-400 colorimeter. The parameters analyzed were L* (lightness coefficient), which ranges from 0 (completely black) to 100 (completely white); a*, which ranges from −80 (green) to + 100 (red); and b*, which ranges from −50 (blue) to + 70 (yellow). The numerical values of a* and b* were converted into C* (Chroma) and Hue Angle (ºH) using the following equations: $C^* = \sqrt{a2 + b2}$ and ºH = tg (a/b) [38], where C* is the quantitative parameter of color intensity and ºH is the qualitative parameter of color [39], with the angle 0º indicating red, 90º yellow, 180º green, and 270º blue [38].

To determine the ash content, incineration was performed in a muffle furnace (Analog, AN1221), at 550 ºC [40], using four replicates of 2.0 g of bee pollen. To determine the lipid content, 2.0 g of bee pollen was weighed and transferred into a pre-prepared filter paper cartridge. The cartridges were then placed in a Soxhlet extractor (Marconi, MA044/8/50) and pre-weighed reboilers, maintained at 105 °C, and containing 70 mL of (AR) ethyl ether, were attached to the extractor. The cartridges were immersed in the solvent at 100 °C for 1 h. The cartridge containing the sample was raised to the intermediate position for 1 h at 150 °C. After this period, the intermediate connection was replaced, and solvent recovery was initiated for 1 h. The reboilers, now containing the extracted lipids, were disconnected, and the residual ethyl ether was evaporated in an oven (Fanem, 315 SE) at 105 ºC. The reboilers were then cooled in a desiccator to room temperature (25 ºC) and weighed [40]. The analysis was performed in triplicate.

The protein percentage was analyzed using the Micro Kjeldhal method [23]. A 0.3 g sample of bee pollen was weighed and placed in a digestion tube along with 2.0 g of digestion mixture and 20 mL of 0.2 N sulfuric acid. The analysis was performed in quadruplicate. Digestion was carried out at a moderately low initial temperature until reaching 350 °C, followed by continued heating until the solution was clarified. After cooling, 20 mL of distilled water was added to the solution under agitation. The digestion tubes containing the digested samples were then attached to the distillation unit, and 20 mL of 50% sodium hydroxide was added. In an Erlenmeyer flask, 20 mL of 4% boric acid and two drops of methyl red solution were added. The flask was then connected to the distillation unit to receive the released ammonia. Steam distillation was performed, keeping the condenser outlet submerged in the receiving solution until the complete release of ammonia, with a total distilled volume of 100 mL. The Erlenmeyer flask was then removed and titrated with 0.1 N chloride acid until the indicator changed color from green to pink. The volumes used were recorded. Carbohydrates were quantified by calculating the difference between the average percentages of moisture, proteins, lipids, and ash content [41].

To determine the total phenolic content, antioxidant capacity using the phosphomolybdenum complex and the DPPH methods. Bee pollen samples (1 g each) were ground using a mortar and pestle, extracted with 70% ethanol, and homogenized. After 30 min, the extract was filtered and stored under refrigeration (7 ºC) until analysis. Extractions and analyses were performed in triplicate.

The total phenolic content was determined using the spectrophotometric method described by [42]. An aliquot of 0.5 mL of the ethanolic extract of bee pollen was added to test tubes, followed by the addition of 2.5 mL of 10% (v/v) FolinCiocalteau solution and 2 mL of 4% (p/v) sodium carbonate solution. The tubes were shaken on a vortex mixer and left to rest in the dark for 2 h. After this period, readings were taken using a spectrophotometer (Shimadzu—UV 1280) at 720 nm. The analytical curve was prepared using a gallic acid solution (20–200 µg/mL), and the results were expressed as g GAE/100 g of sample (GAE = gallic acid equivalent).

The antioxidant activity, using the phosphomolybdenum complex method, was described by [43]. An aliquot of 0.1 mL of the ethanolic extract of bee pollen was placed in test tubes, and 3 mL of reagent solution (0.6 M acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added. The tubes were sealed and incubated in a water bath at 95 °C for 90 min. After cooling, the absorbance readings were measured using a spectrophotometer (Shimadzu – UV 1280) at a wavelength of 695 nm. The analytical curve was prepared using an ascorbic acid solution (1.95 to 500 µg), and the results were expressed as g AAE/100 g of sample (AAE = ascorbic acid equivalent).

The antioxidant activity, performed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, followed the methodology developed by [44, 45]. In test tubes, 0.5 mL of ethanolic bee pollen extract and 0.3 mL of 0.3 mM ethanolic DPPH solution were added. The blank was prepared with 3.3 mL of absolute ethanol and 0.5 mL of ethanolic pollen extract, while the negative control was prepared with 3.5 mL of ethanol and 0.3 mL of 0.3 mM ethanolic DPPH solution. The test tubes were homogenized using a vortex mixer and stored at room temperature and in the absence of light for 30 min. After this period, the absorbance of the samples was measured using a spectrophotometer (Shimadzu – UV 1280) at a wavelength of 517 nm. The antioxidant activity values were calculated as the average percentage of activity using the formula from [46]:

$$\%\mathrm{AA} = 100 - \frac{[(ABSsample - ABSblank) * 100]}{\mathrm{ABS\ control}}$$

where: %AA = antioxidant activity; ABS = absorbance reading.

The determination of yellow flavonoids and anthocyanins was performed according to the methodology of [47]. For each bee pollen sample, 1 g was weighed, and 20 mL of extracting solution was added. (ethanol 95%: HCl 1,5 N – 85:15 v/v). The samples were homogenized and stored under refrigeration (7 °C) and protected from light for 16 h. After this period, the extract was filtered, and the absorbances at 535 nm (anthocyanins) and 374 nm (yellow flavonoids) were measured. The content of yellow flavonoids and anthocyanins was calculated according to the equation below.

$$\text{yellow flavonoids/anthocyanins}\,(\mathrm{g}/100\mathrm{g}) = \frac{(\text{ABS x final volume}) * 10^4}{(sample\ weight\ x \varepsilon^{1\%}_{1cm,535ou374})}$$

where: ABS = measured absorbance; $\varepsilon^{1\%}_{1cm,535}$ = 98,2 (mol/cm) (molar absorptivity coefficient of anthocyanins); $\varepsilon^{1\%}_{1cm,374}$ = 76,6 (mol/cm) (molar absorptivity coefficient of yellow flavonoids).

For the determination of total carotenoids, the methodology of [48] was used. From each bee pollen sample, 1 g was weighed, ground using a mortar and pestle, and mixed with 5 mL of extraction solvent (hexane/acetone/ethanol, 50:25:25, v/v/v). The mixture was then centrifuged at 5,000 × g for 10 min at 4 °C (Thermo Scientific

– ST 16R). The hexane supernatant containing the pigment was collected and transferred to a 25 mL volumetric flask, and the volume was adjusted with PA-grade hexane. Subsequently, absorbance was measured using a spectrophotometer (Shimadzu – UV 1280) at a wavelength of 450 nm. The total carotenoid content was determined using the equation below.

$$\text{Total carotenoid (mg/100g)} = \frac{(\text{ABS x final volume}) * 1000}{(sampleweightx\varepsilon^{1\%}_{1cm,450})}$$

where: ABS = absorbance reading; $\varepsilon^{1\%}_{1cm,450}$ (molar absorptivity coefficient) = 2505.

The chemical composition of phenolic acids obtained from bee pollen was determined according to [49]. Before performing the main gas chromatography coupled to mass spectrometry (GC–MS) analysis, a derivatization step was required. The derivatization of the extracts involved mixing 600 µL of the extract, 100 µL of BSTFA, and 60 µL of anhydrous pyridine in a conical flask, followed by shaking and heating to 50 °C for 30 min. Subsequently, the reaction mixture was transferred to an injection vial with an insert and subjected to GC–MS analysis. Chromatographic analysis was performed on an Agilent Technologies gas chromatography system (GC 7890 A) coupled to a mass detector (CG-EM), using Helium as carrier gas at a rate of 1.0 mL min^{-1}. The sample (1.0 µL) was injected into the chromatography column using an auto-injector (CTC combiPaL) in splitless mode. The injector was maintained at 280 °C. The capillary column used was SLB5 MS (30 m × 0.25 mm × 0.25 µm; Supelco). Initially, the GC oven was at 100 °C, after which it was heated at the rate of 10 °C/minute, gradually heated up to 150 °C. The temperature was increased to 225 °C at a rate of 5 °C/minute, followed by further heating to 300 °C with an increment of 20° C/minute. The column temperature was maintained at 300 °C for 2 min. The interface temperature was maintained at 280 °C and the electron impact ionization at 70 eV. The phenolic compounds in the extracts were identified by comparing the standard mass spectra with the apparatus library (NIST 2.0).

Statistical analysis

Quantitative data were subjected to the Shapiro–Wilk normality test and Levene's test for homogeneity of variances. An analysis of variance (ANOVA) or, alternatively, the Kruskal–Wallis's test was performed. When significant differences among treatments were detected, means were compared using Tukey's test for parametric data or Dunn's test for non-parametric data, both at $P < 0.05$.

Results

Pollen analysis and morphology

Bee pollen samples with a higher percentage of aroeira pollen grains exhibited a dark coloration (Fig. 1A) and were composed of pellets in three colors: brown (Fig. 1B), yellow (Fig. 1C), and white (Fig. 1D). The percentage of pellets coloration varied over time; the batch from June 2022 had the highest proportion of brown pellets (83.9%), while August 2022 (1.4%) and March 2023 (5.2%) showed the lowest percentages (Fig. 1E).

Based on the pollen spectrum, it was possible to determine the botanical origin of the bee pollen (Table 1; Fig. 2). Aroeira pollen grains were present in almost all samples, ranging from 36.5% to 93.4%. Only the March 2023 sample did not contain aroeira pollen grains.

Micromorphology

Through scanning electron microscopy (SEM) analysis, it was identified that the pellets are composed of numerous pollen grains and that their color is related to the variety of constituent species (Fig. 3). The brown pellets (Fig. 3A-C) were predominantly composed of aroeira pollen grains. The pollen grains were dehydrated, exhibiting reticulate ornamentation and colpus-type apertures (Fig. 3B-C), with the occurrence of a figured element (Fig. 3B). The yellow pellet (Fig. 3D-F) contained pollen grains of *A. urundeuva* and Poaceae, both in a dehydrated form. The white pellet (Fig. 3G-I) was the most heterogeneous, containing pollen grains of *A. urundeuva*, *Richardia*, *Bauhinia*, and *Alternanthera*.

Pollen grains of aroeira were agglutined (Fig. 4A), had an oblate-spheroidal shape, with three colpi (Fig. 4B-C), and a reticulated exine with small lumens (Fig. 4D). The pollen of *Alternanthera* (Fig. 4E) had 12 poros and exine with tiny spicules (Fig. 4F). The pollen of *Bauhinia* (Fig. 4G) showed triangular shape and an exine with ornamentation kind of clava (Fig. 4H). In *Richardia* (Fig. 4I), the exine had sharp spicules of two sizes (Fig. 4J). The *Poaceae* type exhibited a circular pore (Fig. 4K) and exine with granulate ornamentation (Fig. 4L).

Anatomy and histochemistry

The pollen grain of aroeira exhibited a variety of chemical compounds, as evidenced by histochemical tests. The exine is lignified (Fig. 5A) and impregnated with phenolic compounds (Fig. 5C, H), acidic lipids (Fig. 5D-E) and terpenes (Fig. 5F). The intine has a pectic composition (Fig. 5A, I, J) and is associated with neutral lipids (Fig. 5D-E), phenolic compounds (Fig. 5C, H), flavonoids (Fig. 5G), and terpenes

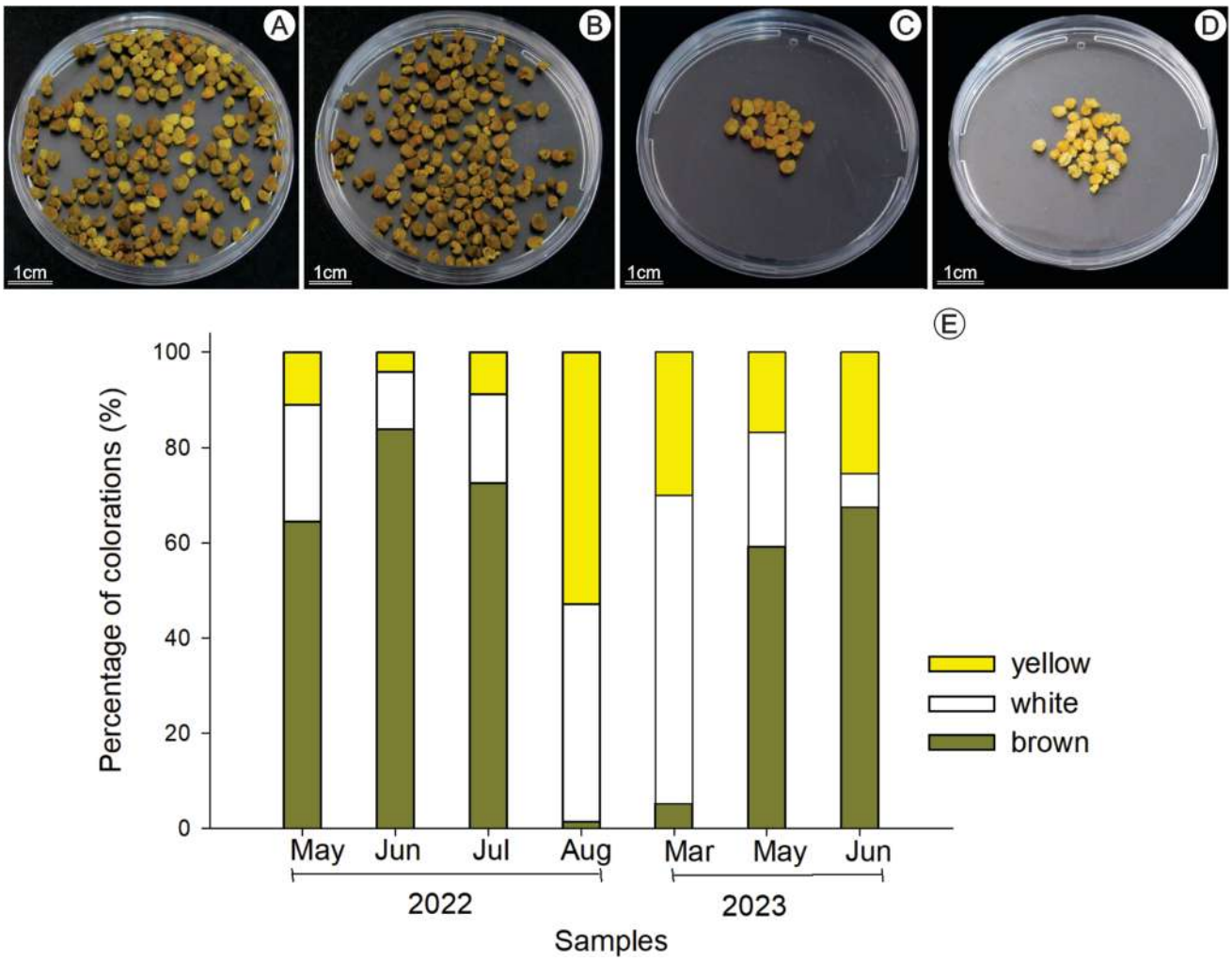

Fig. 1 Variation in pellet coloration in the bee pollen sample. **A** Pellets with various colors. **B** Brown pellets. **C** Yellow pellets. **D** White pellets. **E** Percentage of pellets coloration in bee pollen samples of *Astronium urundeuva*

(Fig. 5F). The vegetative cell protoplast contains mucilage (Fig. 5A), proteins (Fig. 5B), starch (Fig. 5C), lipids (Fig. 5D-E), terpenes (Fig. 5F), flavonoids (Fig. 5G), phenolic compounds (Fig. 5H), and pectin (Fig. 5I-J). The aroeira pollen grain does not contain tannins or alkaloids.

Biometric, physical, and chemical analyses

The pellets ranged in length from 2.75 to 3.23 mm (Fig. 6A) and in width from 2.18 to 2.45 mm (Fig. 6B). The sample with the lowest percentage of aroeira pollen grains had the largest pellet dimensions (Fig. 6A-B). The average dry mass ranged from 78.52% to 86.0% (Fig. 6C), while the moisture content varied between 14.00% to 21.48% (Fig. 6D).

The coloration varied among the samples. The L* parameter (lightness coefficient) was higher in samples with 0% and 36.5% of aroeira pollen grains and lower in samples with 73.3% and 93.4% of aroeira pollen grains (Fig. 6E). The a* parameter (variation from green to red) was lower in samples with 73.3%, 79.8%, and 93.4% of aroeira pollen grains (Fig. 6F). The b* (variation from blue to yellow), C* (Chroma, color intensity), and hº (Hue angle) parameters were lower in the sample with 0% aroeira pollen grains and higher in samples with 73.3% and 93.4% aroeira pollen grains (Fig. 6G-I). The samples exhibited ash content ranging from 2.93% to 4.65% (Fig. 6J), lipids ranging from 2.82% to 5.03% (Fig. 6K), and proteins varying from 17.90% to 25.54% (Fig. 6L), with significant differences between them. Carbohydrates showed no significant difference, with an average of 52.66% ($P=0.121$).

The storage altered some color parameters. Treatment III showed an increase in the L* parameter (Fig. 7A) and a decrease in the a* parameter (Fig. 7B), while treatment II showed a decrease in the a* parameter (Fig. 7B). The hº parameter showed an increase in both treatments II and III (Fig. 7C). The b* and C* parameters did not exhibit

Table 1 Pollen spectrum and percentagem of pollen grains of bee pollen samples. Dominante pollen (DP > 45%); acessory pollen (AP from 15 to 45%), isolated pollen (IP < 15%)

Image (Fig. 2)	Pollen types	Family	Collection Period						
			2022				2023		
			mai	Jun	jul	Ago	mar	mai	jun
D	*Alternanthera*	Amaranthaceae	17.54	0.10	3.23	0.28	0.50	3.27	4.46
A-C	*Astronium urundeuva*	Anacardiaceae	66.15	93.39	73.29	36.36		79.81	84.16
-	*Schinopsis brasiliensis*	Anacardiaceae		0.72	1.08				
-	*Bidens*	Asteraceae						0.19	0.12
S	*Vernonia*	Asteraceae				2.27	4.98		
O	*Ipomea*	Convolvulaceae					2.99		
-	*Convulvulaceae*	Convolvulaceae	0.20				0.25		
-	*Merremia*	Convolvulaceae					14.18		
-	*Croton*	Euphorbiaceae	0.85						
-	*Ricinus*	Euphorbiaceae				0.57			
-	*Euphorbiaceae sp.*	Euphorbiaceae		1.10	6.25	44.32		1.35	
H-I	*Bauhinia*	Fabaceae	1.06	2.00	4.96	7.53			0.50
-	*Bauhinia forficata*	Fabaceae					35.82		
K-L	*Ceiba*	Fabaceae		0.20	6.68	3.98		1.92	8.79
J	Tipo *Mimosa*	Fabaceae					7.46		
-	*Senegalia*	Fabaceae		0.10		0.71		0.96	
-	Fabaceae	Fabaceae						0.77	
P	*Hyptis*	Lamiaceae					0.75		
M	*Malvastrum*	Malvaceae		0.10			0.50		
-	*Melochia*	Malvaceae							
-	*Sida*	Malvaceae					0.50		
Q-R	*Pseudobombax*	Malvaceae	0.88			2.27			
-	Melastomataceae	Melastomataceae				2.13			
N	*Eucalyptus*	Myrtaceae				0.28			
-	*Passiflora*	Passifloraceae					0.50		
G	*Poaceae*	Poaceae	6.83				15.42	5.96	0.87
-	*Zea mays*	Poaceae					3.73		
E–F	*Richardia*	Rubiaceae	6.21	1.00			12.44	5.77	0.62
T	*Serjania*	Sapindaceae				0.28			
-	*Dilodendron bipinnatum*	Sapindaceae	0.27	1.30	1.29				0.50

significant differences between the samples, according to the Tukey test, with means of 30.01 ($P = 0.538$) and 30.30 ($P = 0.677$), respectively.

The chemical analysis of bee pollen, considering seasonality, showed variation in the composition of total phenolics (Fig. 7D), yellow flavonoids (Fig. 7E), anthocyanins (Fig. 7F), carotenoids (Fig. 7G), and antioxidant activity by the phosphomolybdenum complex method-PCM (Fig. 7H). The sample without aroeira pollen grains had the lowest amounts of total phenolics, anthocyanins, and carotenoids (Fig. 7D, F and G) and the highest antioxidant activity (Fig. 7H). The sample with 84.2% aroeira pollen grains had the highest concentration of total phenolics (Fig. 7D), yellow flavonoids (Fig. 7E), and carotenoids (Fig. 7G). Antioxidant activity, by the DPPH method, showed no significant difference, with an average of 91.2% ($P = 0.132$).

The sample with 84.2% aroeira pollen grains was tested for storage. The sample stored under refrigeration for 240 days (treatment II) and the dehydrated sample stored for 90 days (treatment III) showed a reduction in the values of total phenolics (Fig. 7I), yellow flavonoids (Fig. 7J), anthocyanins (Fig. 7K), and carotenoids (Fig. 7L), compared to the sample stored under refrigeration for 30 days (treatment I). The antioxidant activity, measured by the PCM and the DPPH methods, showed no significant differences between the samples, with averages of 29.54 g/100 g AAEs ($P = 0.05$) and 92.80% ($P = 0.44$), respectively.

The chromatograms for the analyses of phenolic acids (salicylic, vanillic, syringic, gallic, ferulic, and caffeic) in the samples are shown in Figure S4, and the values obtained are presented in Table 2. The sample without aroeira pollen grains exhibited the highest amounts of vanillic, syringic, gallic, ferulic, and caffeic acids, while the sample with 79.8% aroeira pollen had the highest concentration of salicylic acid. The dehydration of the 84.2% aroeira pollen sample increased the concentration of salicylic, vanillic, syringic, gallic, and ferulic acids compared to the frozen sample.

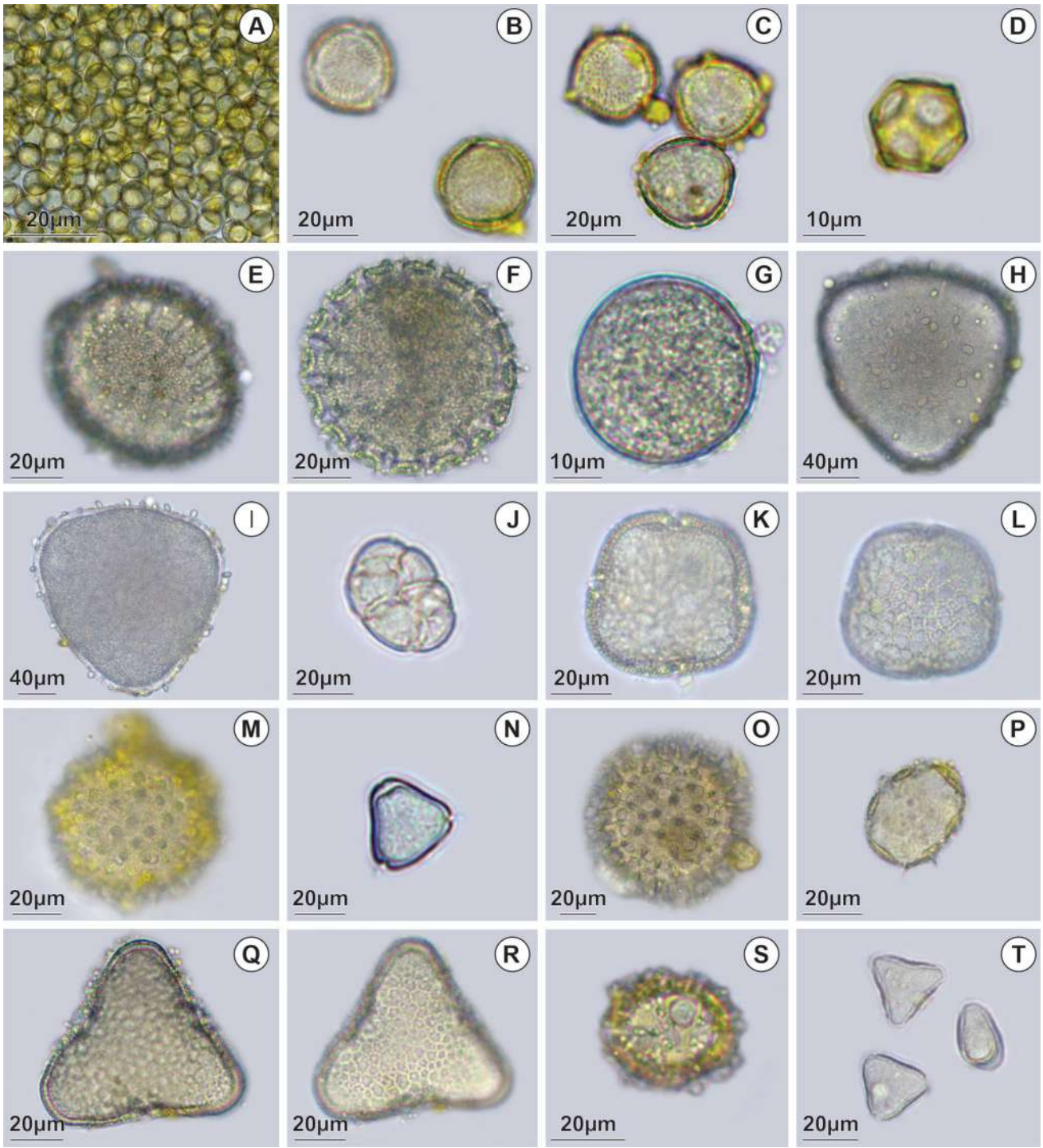

Fig. 2 Pollen types presents in bee pollen samples. **A-C***Astroniun urundeuva* (aroeira). **D***Alternanthera*. **E**–**F***Richardia*. **G** Poacaeae. **H-I***Bauhnia*. **J** Tipo *Mimosa*. **K-L***Ceiba*. (M) *Malvastrum*. **N***Eucalyptus*. **O***Ipomea*. **P***Hyptis*. **Q-R***Pseudobombax*.**S** Vernonia. **T***Serjania*

Discussion

Unprecedented characteristics have been reported in this study regarding an important beekeeping product, the bee pollen of *A. urundeuva*, a species widely found in the Cerrado and Caatinga biomes. Our data indicate that monofloral bee pollen consists of more than 70% of pollen grains from a single species. The product exhibits morphological characteristics that may be associated with the phenology and seasonality of the species in its area of

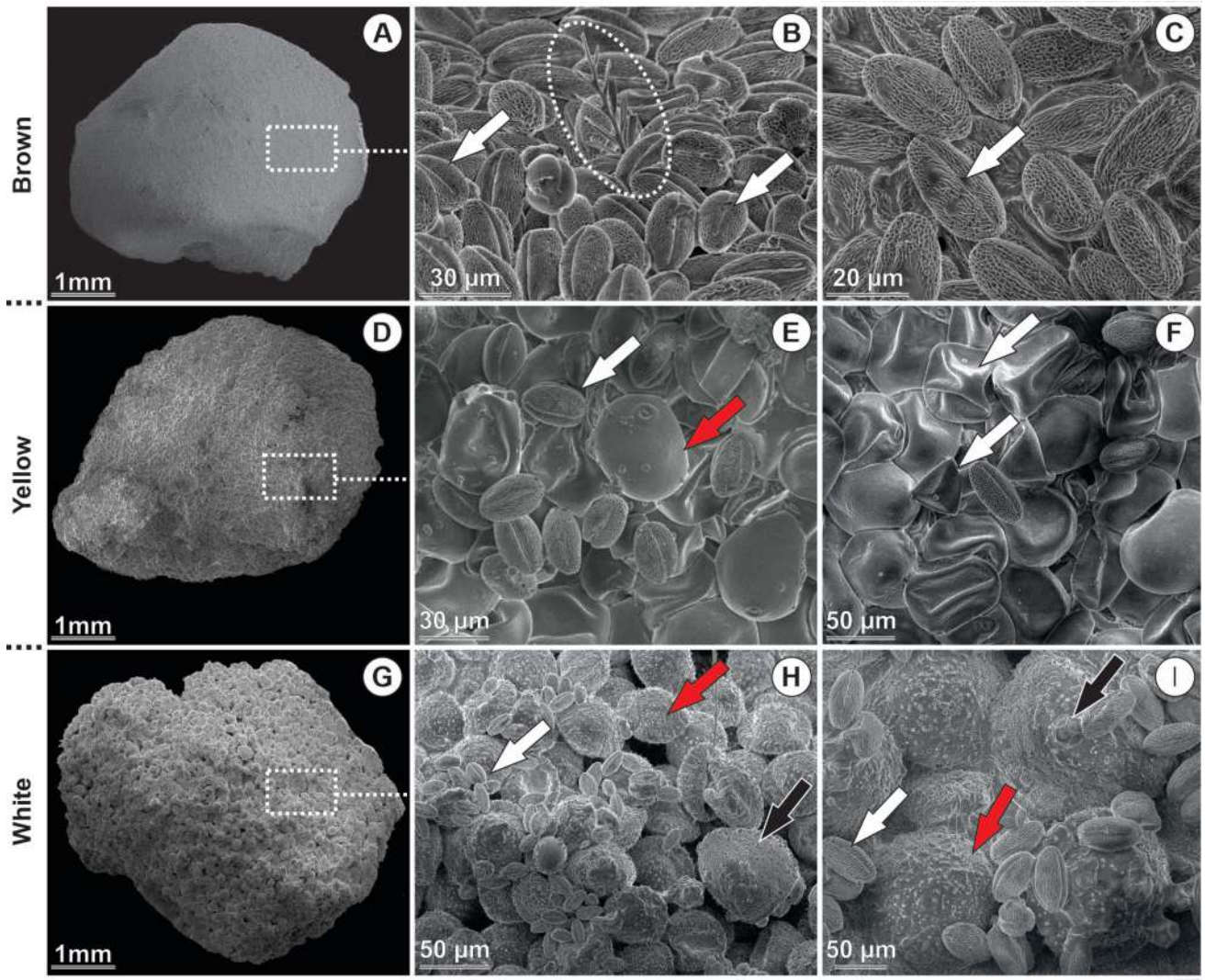

Fig. 3 Scanning electron micrographs of brown, yellow, and white pellets from the bee pollen sample. **A** General view of the brown pellet with a slightly smooth surface. **B** Pollen grains of *A. urundeuva* (white arrows); focus on the figurative element – bee hair (dashed). **C** View of the reticulate ornamentation of the *A. urundeuva* pollen grain with elongated colpi (white arrows) in the dehydrated form. **D** General view of the yellow pellet with an irregular surface. **E** Presence of various pollen types, *A. urundeuva* (white arrow) and Poaceae (red arrow). **F** Poaceae pollen grain (arrows) dehydrated. **G** General view of the white pellet with an irregular surface. **H** Presence of various pollen types, *A. urundeuva* (white arrow), *Richardia* (red arrow), and *Bauhinia* (black arrow). **I***A. urundeuva* pollen grain (white arrow), *Richardia* (red arrow), and *Alternanthera* (black arrow)

occurrence. Bee pollen is rich in essential nutrients, and combined with its antioxidant activity, this suggests that it is a potential superfood. We found that the processing method and storage period are factors that compromise the quality of bee pollen.

Morphological marker and identification of monofloral bee pollen

Color and pellet quantity can be considered a morphological marker for identifying monofloral bee pollen of *A. urundeuva*. Bee pollen consists of pellets in various colors, reflecting its botanical diversity. The highest percentage of brown pellets was observed in the sample with the highest concentration of aroeira pollen grains. Samples with few or no pollen grains of this species also contained brown pellets but in smaller quantities. Bee pollen from the same botanical species may exhibit different colors, while pollen from different species may share the same coloration [4]. In the study area, a higher number of brown pellets corresponded to a greater occurrence of *A. urundeuva* pollen grains in the bee pollen.

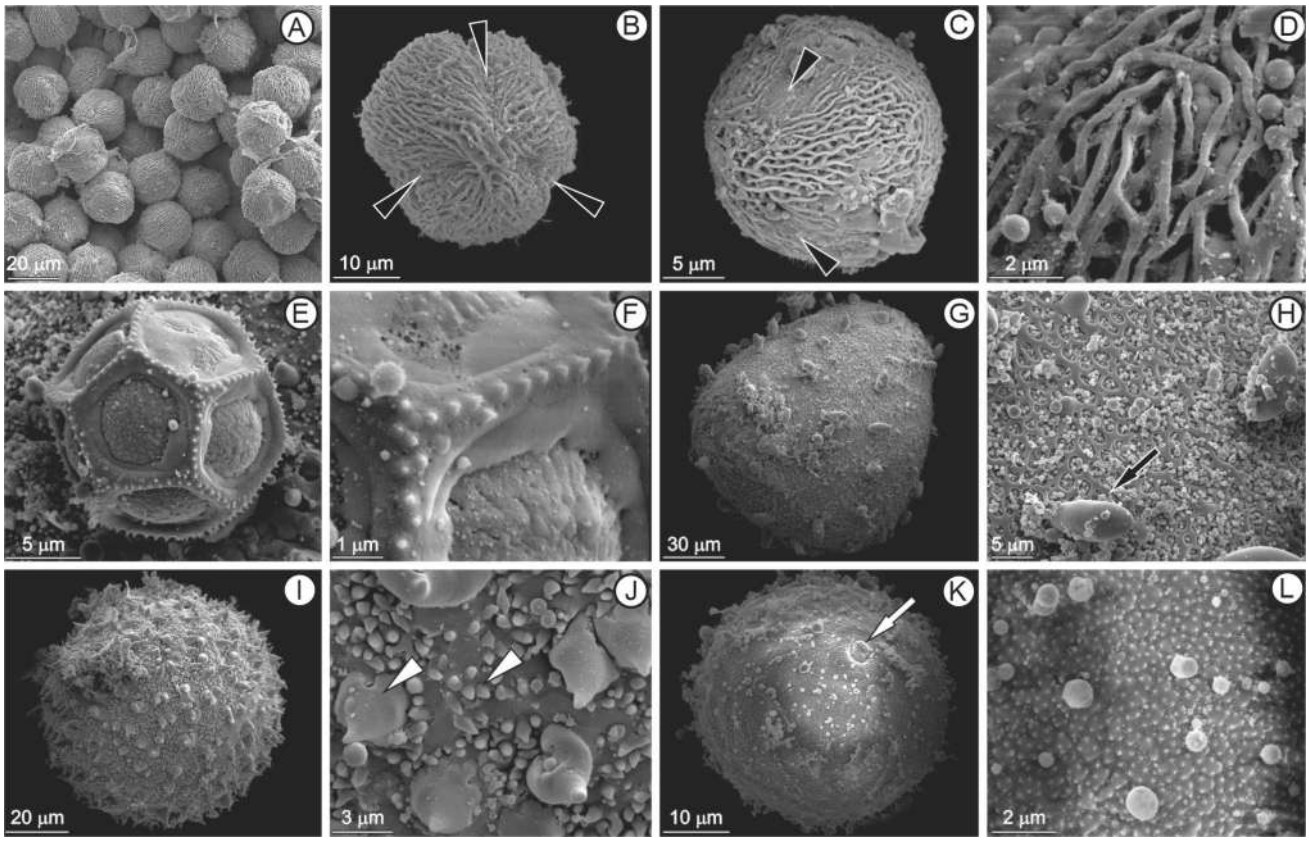

Fig. 4 Scanning electron micrographs of pollen grains present in bee pollen samples. **A**-**D***A. urundeuva* pollen grains. **A** Agglutinated pollen grains. **B**-**C** Pollen grains with different levels of hydration. The tips of the arrows point to the elongated colpi. **D** Detail of the reticulate ornamentation of the exine with small, deep lumens. **E**–**F***Alternanthera* pollen type, showing 12 pores. **F** Detail of the small spicules present in the exine. **G**-**H***Bauhinia* pollen type. **H** Detail showing the presence of clavae on the surface (black arrow). **I**-**J***Richardia* pollen type. **J** Detail of the ornamentation in the exine, with the presence of spicules (arrow tips). **K**-**L** Poaceae pollen type, with a circular pore (white arrow). **L** Detail of the granular exine

The length and width measurements of bee pollen pellets can be influenced by the collection process carried out by the bees and by the retention grid in the pollen traps, which have 4.3 to 5 mm openings. These openings allow the bees to pass through while ensuring the separation of the pellets attached to their legs [50]. A study conducted on a bee pollen sample from the Czech Republic found average length values of 2.7–3.5 mm and width values of 3.3–3.8 mm [3], which are similar to the results found in the present study.

Samples without aroeira pollen grains had a higher water content, possibly due to the diversity of pollen types with different water retention capacities [51]. Additionally, this sample was collected during a month with precipitation, suggesting a potential correlation. Aroeira pollen is abundant during dry periods, typical of this region with defined seasonality, indicating its resistance to water deficit, which may be related to its chemical composition.

Chemical composition of bee pollen concerning seasonality

The bee pollen samples showed variations in chemical composition due to the seasonality typical of the study area. Botanical origin is an influencing factor in the concentrations of chemical compounds, as the samples studied displayed differences in their pollen spectrum. The color of bee pollen is determined by the chemical composition of the pollen grains from the different species it contains [4]. The sample with the highest percentage of aroeira pollen grains appeared darker, with a tendency toward red and yellow, and one of the highest color intensity values. The concentration of chemical compounds was correlated with the color of bee pollen in the study conducted by [52].

The samples obtained in May/23, June/22 and 23, and July/22 were considered monofloral, as they contained more than 70% (or > 80%) of aroeira pollen grains with

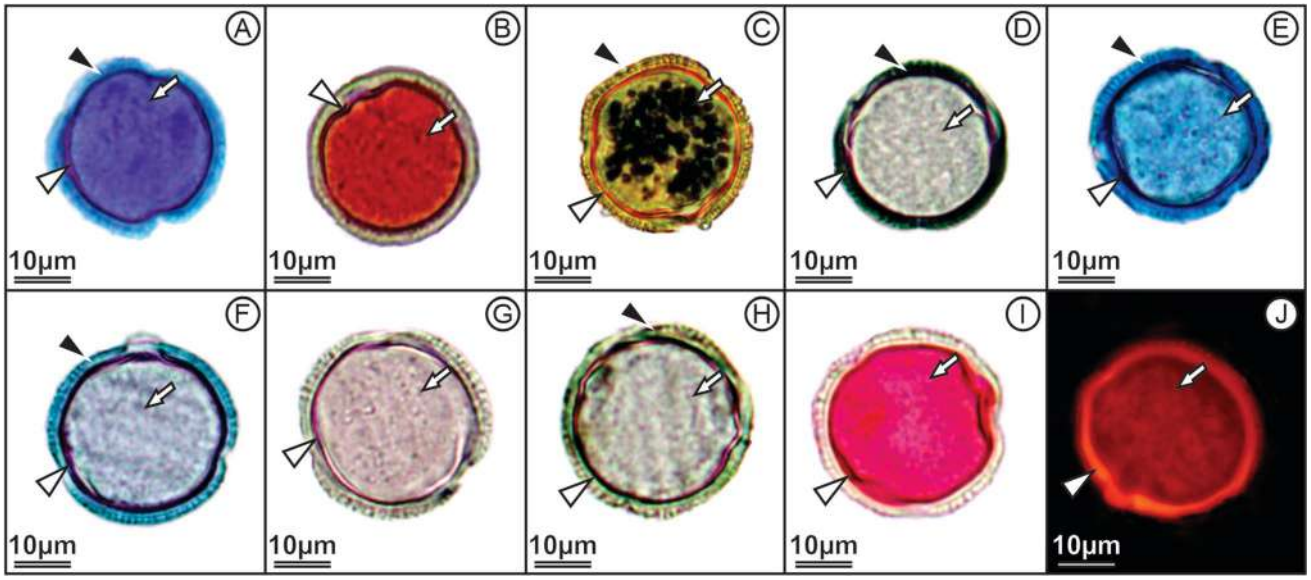

Fig. 5 Histochemical tests on *Astronium urundeuva* pollen grains. **A** Pollen grain showing lignified exine, pectic intine, and a protoplast rich in mucilage. **B** Proteins in the intine and protoplast. **C** Phenolic compounds in the exine and intine and starch in the protoplast. **D** Total lipids in the exine, intine, and protoplast. **E** Neutral lipids in the intine and acids in the exine and protoplast. **F** Terpenes in the exine, intine, and protoplast. **G** Flavonoids in the intine and protoplast. **H** Phenolic compounds in the exine na exina, intine, and protoplast. **I**-**J** Pectin in the intine and protoplast. Black arrow tip: exine; white arrow tip: intine; white arrow: protoplast

no occurrence of accessory pollen. This criterion has also been used by other researchers to classify monofloral pollen [5, 22, 53]. The samples from May/22, August/2022, and March/2023 were considered multifloral.

The samples containing aroeira pollen grains exhibited protein values ranging from 22–26%. A similar result was found in a study on bee pollen samples with 84% *Eucalyptus* pollen grains from southern Brazil, which had a protein content of 25% [11]. In monofloral bee pollen samples from *Cocos nucifera* in southern Bahia, protein values ranged from 13–15% [54]. The protein content found in aroeira bee pollen is higher than that found in other foods, such as eggs (12.8%) or pork meat (14.2%) [10].

Brazilian legislation establishes that raw bee pollen must have a maximum of 30% moisture, 4% ash, and at least 1.8% lipids and 8% protein [1]. The results found in monofloral bee pollen are in accordance with the values set by the legislation. However, samples containing 36.5% and 66.2% aroeira pollen grains showed ash content above the permitted limit. Therefore, it is important to select the period when the species' flowering peak occurs in the area to ensure the collection of monofloral bee pollen and avoid the presence of pollen from other species, which could reduce the product's quality.

The bioactive compounds analyzed in this study showed significant differences in relation to the concentration of aroeira pollen grains. The sample with the highest amount of pollen grains from this species exhibited the highest concentration of total phenolics, yellow flavonoids, and carotenoids, indicating the great nutritional potential of monofloral bee pollen. The bioactive compounds found in foods are the result of secondary metabolism in plants and play an important role in defense responses against pathogens and in attracting pollinators. These compounds have a variety of beneficial effects for humans and attract the interest of the food, pharmaceutical, and cosmetics industries. They are the subject of research in numerous studies [5, 51, 55–58].

Antioxidant activity was assessed using two distinct methods, both of which yielded satisfactory results. The sample that did not contain *A. urundeuva* pollen grains exhibited higher antioxidant activity using the phosphomolybdenum complex method, which may be related to a higher concentration of caffeic acid compared to the other samples. Using the DPPH method, the samples did not show significant differences. The results found in this study are consistent with studies conducted on multifloral samples from southern Brazil and monofloral samples from the Czech Republic, where values of 94% and 25 to 93%, respectively, were reported [3, 5]. Bee pollen samples, both monofloral and multifloral, from Tukey showed contrasting data, with values ranging from 4 to 23% [53].

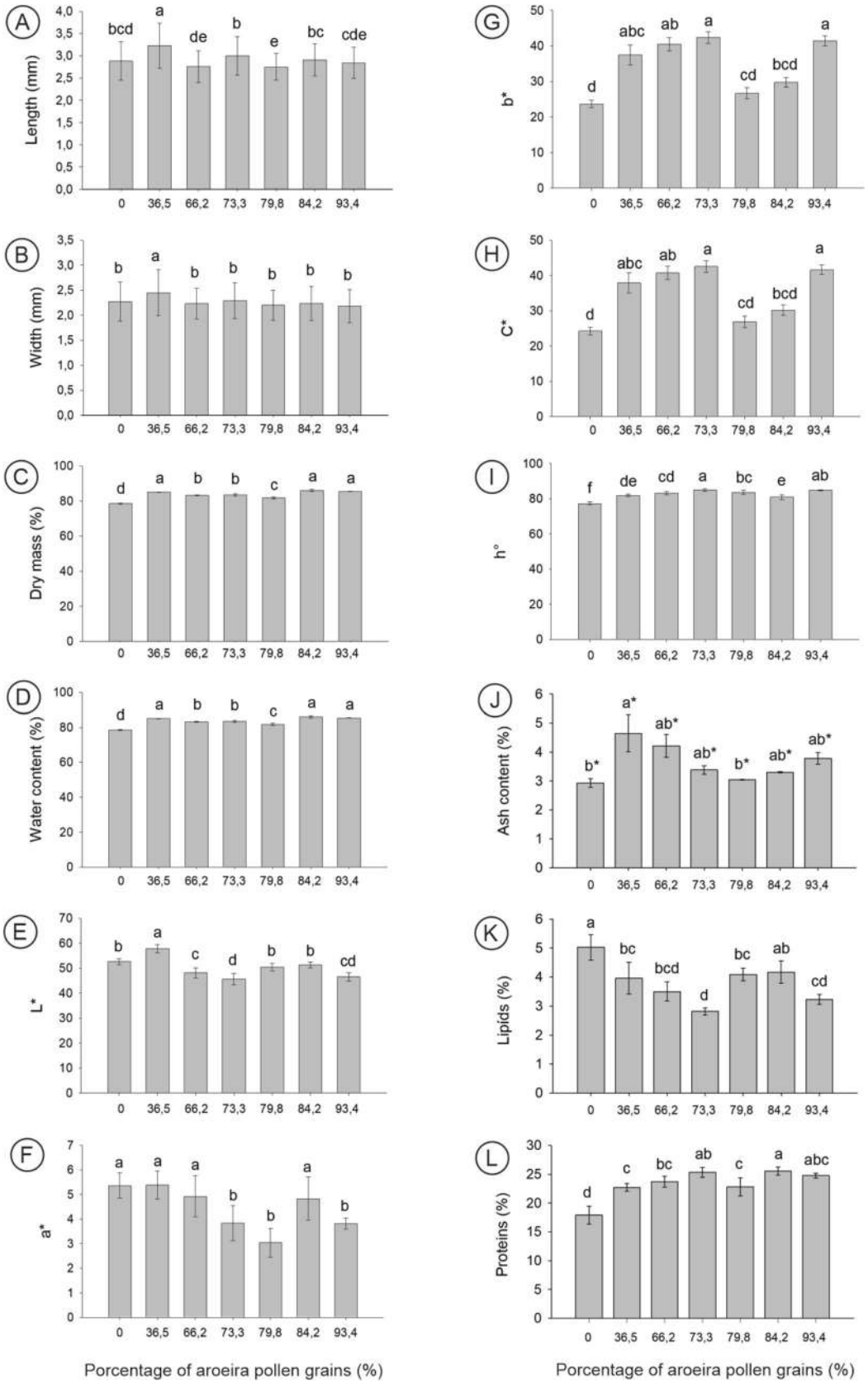
A
Length (mm)
bcd a de b e bc cde
B
Width (mm)
b a b b b b b
C
Dry mass (%)
d a b b c a a
D
Water content (%)
d a b b c a a
E
L*
b a c d b b cd
F
a*
a a a b b a b
G
b*
d abc ab a cd bcd a
H
C*
d abc ab a cd bcd a
I
h°
f de cd a bc e ab
J
Ash content (%)
b* a* ab* ab* b* ab* ab*
K
Lipids (%)
a bc bcd d bc ab cd
L
Proteins (%)
d c bc ab c a abc
0 36,5 66,2 73,3 79,8 84,2 93,4
Porcentage of aroeira pollen grains (%)
Porcentage of aroeira pollen grains (%)

Fig. 6 Average values of **A** length, **B** width, **C** dry mass, and **D** water content in bee pollen samples. Vertical bars represent the standard deviation of the means; different letters indicate significant differences by the Dunn's test (A-B) and Tukey's test (C-D) ($P<0.05$). (E-I) Color parameters values L*, a*, b*, C* and hº and of bee pollen samples with different percentages of *Astronium urundeuva* pollen grains in relation to seasonality. **E** L* (Light coefficient, ranging from 0 [completely black]) to 100 [completely white]. **F** a* (ranging from −80 [green] to+100 [red]). **G** b* (ranging from −50 [blue] to+70 [yellow]). **H** C* (Chroma, color intensity parameter). **I** hº (Hue angle, a qualitative color parameter, ranging from 0º [red], 90º [yellow], 180º [green] and 270º [blue]). Vertical bars represent the standard deviation of the means; different letters indicate significant differences by the Dunn's test (G-H) ($P<0.05$) and by the Tukey's test ($P<0.05$) (E, F and I). **J**-**L** Chemical composition of bee pollen samples with different percentages of pollen grains from *A. urundeuva*. Vertical bars represent the standard deviation of the means, and different letters indicate a significant difference according to the (J) Dunn's test ($P<0.05$) and the (K-L) Tukey's test ($P<0.05$)

In the study area, monofloral bee pollen from *A. urundeuva* was found during the dry season, in May, June, and July, which coincide with the species' flowering period. The peak of flowering occurred in June, with the samples from this month having the highest percentage of pollen grains from the species. We highlight the need for further studies to characterize *A. urundeuva* bee pollen in other regions, in order to assess its structure and composition under the influence of adverse abiotic conditions.

Quality of chemical compounds and storage

The storage methods commonly implemented by beekeepers alter the quality of the chemical compounds in bee pollen. After dehydration, we observed that the sample became darker, and the storage methods evaluated in this study caused a significant reduction in the levels of total phenolics, yellow flavonoids, anthocyanins, and carotenoids. These changes may be related to oxidation reactions of the chemical compounds, particularly phenolic compounds [4]. However, antioxidant activity, as assessed by both methods, did not show differences between the values. A similar result was found by [15], who quantified total phenolics and total flavonoids in both monofloral and multifloral bee pollen samples that were either frozen or dehydrated. [9] evaluated the levels of total carotenoids and other bioactive compounds in fresh and dehydrated pollen, and all the samples showed a reduction in the levels of these compounds. [59] quantified B vitamins in relation to processing and storage time, finding varying results, some levels increased, while others decreased over time. The dehydration temperature must be controlled and monitored. [60] evaluated the formation of compounds generated in heat-processed foods that are harmful to health, across various food products, and found that foods prepared under high-temperature conditions form a greater amount of these compounds.

Aroeira bee pollen is rich in polyphenols, such as flavonoids and phenolic acids, with their concentration depending on the botanical origin. Polyphenols provide health benefits, exhibiting antioxidant, antimicrobial, and anti-inflammatory properties [61]. Chromatographic analyses revealed several compounds in the bee pollen samples, as well as their variation over the collection period and dehydration process. Six types of phenolic acids were analyzed; the sample that did not contain Aroeira pollen grains showed the highest concentration of caffeic acid (1133.00 µg/kg), while the sample with 79.8% Aroeira pollen grains presented the highest concentration of salicylic acid (273.20 µg/kg). In addition to the identified compounds, the chromatogram indicated the presence of a variety of other compounds that require further investigation to determine their chemical classes and functional roles. These findings suggest a potential enhancement in the nutritional quality of bee pollen derived from this species, which could, in turn, increase its appeal for consumption and add value to the product.

This study demonstrated that monofloral bee pollen from *A. urundeuva* had the highest number of brown pellets, with more than 70% of the pollen grains from the species. The ideal period for obtaining monofloral bee pollen from aroeira, considering the study area, is from May to July, when the species' flowering peak occurs. The bee pollen samples exhibited a diverse chemical composition in relation to seasonality. The monofloral samples from aroeira showed the highest fractions of proteins and bioactive compounds, such as total phenolics, yellow flavonoids, anthocyanins, and carotenoids, and also exhibited antioxidant activity. The storage methods (freezing and dehydration) resulted in a decrease in the levels of total phenolics, yellow flavonoids, anthocyanins, and carotenoids; however, antioxidant activity did not change. Based on all the results, bee pollen, especially the monofloral pollen from *A. urundeuva*, can be considered a superfood or functional food.

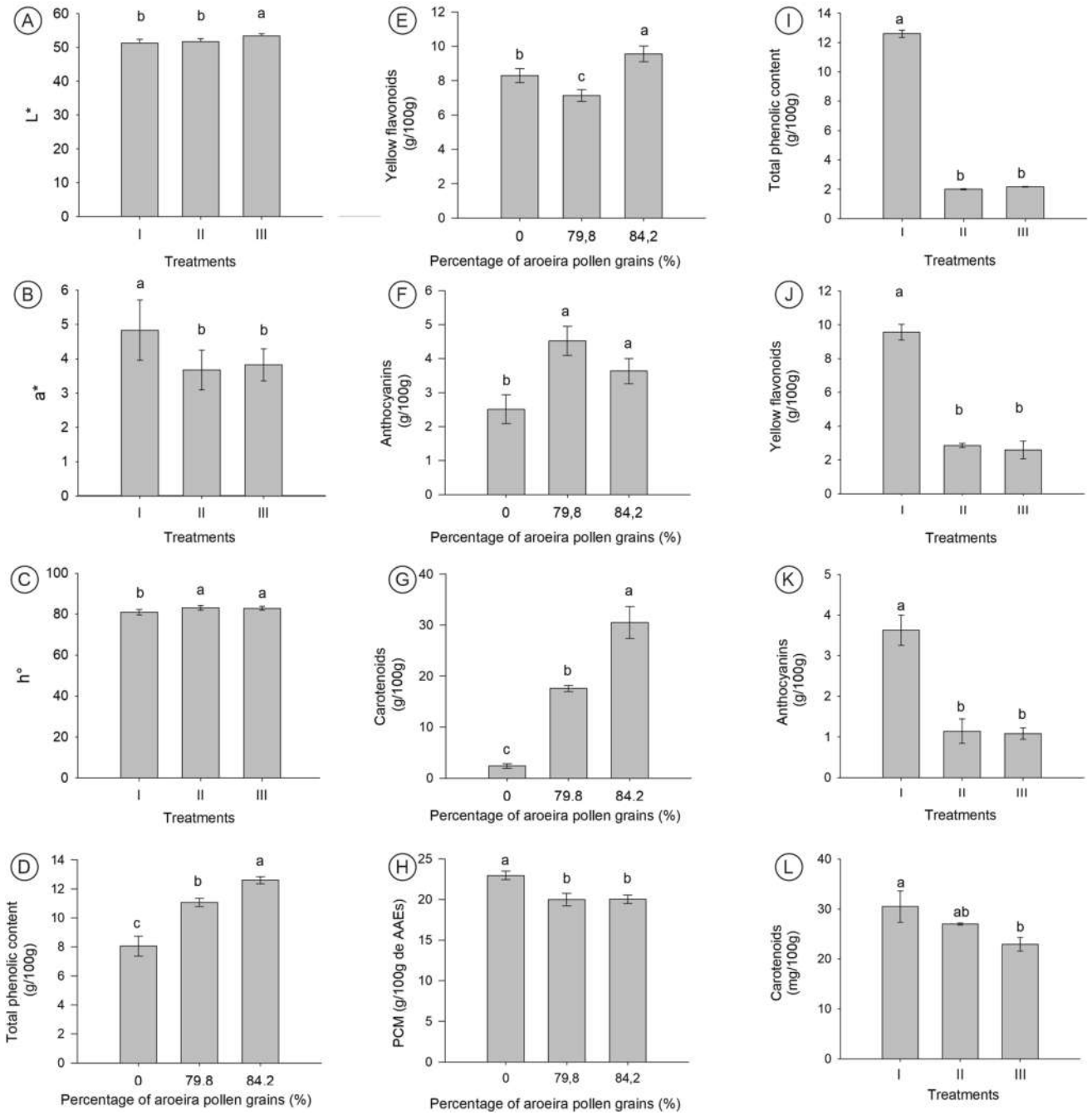

Fig. 7 (A-C) Values of the color parameters: L*, a* and hº of the sample with 84.2% of *Astronium urundeuva* pollen grains in relation to storage. **A** L* (Light coefficient, ranging from 0 [completely black]) to 100 [completely white]). **B** a* (ranging from −80 [green] to + 100 [red]); b* (ranging from −50 [blue] to + 70 [yellow]). **C** hº (Hue angle, a qualitative color parameter ranging from 0º [red], 90º [yellow], 180º [green] e 270º [blue]). Vertical bars represent the standard deviation of the means, and different letters indicate a significant difference according to the Tukey's test ($P < 0.05$). Treatments: I) raw bee pollen kept refrigerated at −21ºC for 30 days; II) raw bee pollen kept refrigerated at −21ºC for 240 days; and III) dehydrated bee pollen stored away from light for 90 days. **D-G** Bioactive compounds present in bee pollen samples with different percentages of pollen grains from *A. urundeuva* in relation to seasonality. **H** Antioxidant activity by the phosphomolybdenum complex method (PCM). Vertical bars represent the standard deviation of the means, and different letters indicate a significant difference according to the Tukey's test ($P < 0.05$). **I-L** Bioactive compounds in the bee pollen sample with 84.2% *A. urundeuva* pollen grains in relation to storage

Table 2 Phenolic acids content of the bee pollen samples, in µg/Kg

% aroeira pollen grains	Phenolic acids					
	Salicylic	Vanillic	Syringic	Gallic	Ferulic	Caffeic
0 (II)	83.21	161.70	19.64	340.20	361.10	1133.00
79.81 (II)	273.20	91.29	8.76	68.40	32.26	63.55
84.16 (II)	78.98	22.24	3.57	12.83	11.87	29.45
84.16 (III)	126.4	23.09	6.99	19.82	13.73	13.62

Sample treatment: II) raw bee pollen stored under refrigeration at −21 °C for 240 days; and III) dehydrated bee pollen stored away from light for 90 days

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11694-025-03757-x.

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Author contributions HCMS and LMR conducted the design of the work. HCMS and EAMM performed the structural and chemical analyses. LMR, HCF, JPL logistical and development support. HCMS, EAMM and LMR conducted the first draft of the manuscript. HCMS, LMR, EAMM, HCF, and JPL read and approved the final manuscript.

Data Availability Data from this work are available from the corresponding author upon request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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