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SEASONAL VARIABILITY IN POLLEN COLORING OF *APIS MELLIFERA* AND STINGLESS BEES

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Abstract: Currently, bee pollen has stood out due to its nutritional characteristics and more recently due to its functional properties. Bee pollen has a variable composition and color, usually associated with its botanical or geographic origin. Therefore, it is essential to standardize its production and processing to improve its use in industrialized products or to simply offer the consumer a more uniformly colored product. In order to assess how the foraging behavior of different bee species and seasonality may affect bee pollen color, a nested factor ANOVA was applied to instrumental color data of 243 pollen samples collected by Apis bee species. mellifera and stingless bees (*Melipona marginata, Melipona quadrifasciata, Scaptotrigona bipunctata and Tetragona clavipes*) over four seasons from June 2018 to May 2019. The results showed that the coloration has a greater contribution from the bee species, but can also be influenced by the seasonality and characteristic flora of the producing region. Furthermore, significant correlations were observed between the L*, a* and b* color coordinates of the bee pollen samples. Correlations may be associated with the predominant contribution of certain plant species due to the availability of flora in the producing region and the foraging habit of bee species. These results are important for the development of national beekeeping and meliponiculture, since they provide subsidies for the production of bee pollen with a more uniform color in the industry of this sector.
Keywords: bee pollen, coloring, ANOVA, season, bee species.

INTRODUCTION

Bee pollen is produced as a result of the collection of plant pollen grains by bees that agglutinate them using salivary secretions, nectar or honey in acorns that are stored in the hive alveoli until consumption and serves as a source of nutrients for the colony, thus ensuring its maintenance and development (Giampieri et al., 2022; Ares et al, 2022).

Recently, this bee product has aroused the interest of the world scientific community due to numerous studies that point to its beneficial effects on health. Bee pollen is potentially being used as active ingredients in pharmaceuticals, as a food supplement or as an ingredient in processed foods due to its functional character (Giampieri et al, 2022; Laaroussi et al., 2023). These effects are associated with its composition, which includes significant amounts of carbohydrates, proteins, lipids and important amounts of micronutrients such as minerals, vitamins, phenolic compounds and essential amino acids. In view of this, bee pollen has therapeutic properties such as anti-inflammatory, antioxidant, antimicrobial, antitumor and antihypertensive activities, among others (Lu et al., 2022; Ares et al., 2022; Kieliszek et al, 2018; Li et al., 2018; Thakur and Nanda, 2020).

The biggest current challenge for the pharmaceutical and food industries is to use bee pollen with standardized color as raw material, since it presents high variability in its chemical, nutritional and phytochemical composition. These variations in its composition and consequently in its color depend on the bee species, as well as on the plant species, geographic area, seasons, soil type, beekeeping practices, among other factors (Giampieri et al, 2022; Rozman et al., 2022; Kieliszek et al., 2018; Li et al., 2018; Thakur and Nanda, 2020). Therefore, studies that, although still scarce, demonstrate how these factors affect the chemical, nutraceutical and phytochemical composition and consequently the color of bee pollen are essential for the development of national beekeeping and meliponiculture, (Negrão and Orsi, 2018). Due to the wide diversity of flora available in Brazil, as well as native bee species ('stingless bees') and honey bees (Apis

mellifera) for the production of bee pollen, it is essential to develop research that can to assist beekeepers and meliponiculturists in choosing the most suitable places and species of bees according to the intended use of this bee product (drug, food supplement or ingredient in food formulations).

Stingless bees are widely distributed in countries in South America, Africa, Southeast Asia and Australia, bringing development to meliponiculture in these places and promoting the conservation of biodiversity in different biomes (Rozman et al, 2022; Silva et al., 2006).

In Brazil, the 'stingless bees' are indigenous and are present throughout the country, although the species differ from region to region. In addition to their importance as major pollinators of most wild plants and some monocultures, honey and pollen from meliponids are also sources of food, medicine (geopropolis) and income for rural populations. There is a strong culture of using meliponiculture products, especially honey and propolis and more recently pollen, in Brazil and in other parts of the world where these species of bees are native (Silva et al., 2006). Despite this, while numerous publications are reported on the composition, coloring and nutritional properties of pollen from *Apis mellifera* (De Souza et al., 2018; Negrão e Orsi, 2018; Negrão, Barreto e Orsi, 2014; De Melo et al., 2018), the chemical composition, phytochemistry and color of pollen collected by 'stingless bees' remains unexplored and demands further research on the factors that lead to the great variability observed in this bee product (Rebelo et al., 2021; Silva et al., 2006; Duarte et al, 2018; Barth, Freitas and Vanderborght, 2020; Vit et al., 2016).

In view of this, it is our knowledge that no effort has been made to determine the influence on pollen color of *Apis mellifera* and stingless bees (*Melipona marginata, Melipona quadrifasciata, Scaptotrigona bipunctata and Tetragona clavipes*) collected in different seasons of the year, in a delimited region, in Maringá in the State of Paraná, Brazil. Therefore, a nested factor ANOVA was applied to instrumental color data from the CIELAB system (L*, a* and b* coordinates) to assess the influence of bee species and seasonality on the color of pollen samples collected by Apis mellifera and by stingless bees (*Melipona marginata, Melipona quadrifasciata, Scaptotrigona bipunctata and Tetragona clavipes*).

MATERIAL AND METHODS

SAMPLE

Apis mellifera and stingless bee pollen samples (*Melipona quadrifasciata*, *Melipona marginata*, *Scaptotrigona bipunctata* and *Tetragona clavipes*) were collected from June 2018 to May 2019, in the Beekeeping and Meliponiculture sector of the Experimental Farm of ``Universidade Estadual de Maringá``, in Maringá, PR (coordinates 23º15´15" and 23º33´27" S and 51º50´05" and 52º05´59" W).

Pollen samples of the Apis mellifera species were produced in Langstroth hives and collected in five colonies. Every two weeks, a pollen collector was inserted at the entrance of the hives, retaining the pollen for two days and, at the end of this period, the pollen of the bees was collected. The two bee pollen samples collected throughout the month were homogenized after cleaning, in order to obtain a single monthly sample during the collection period. Pollen samples from stingless bees were produced in Inpa hives and collected monthly from five colonies of each stingless bee species. With the aid of a spatula, about 10 g of pollen (when available) were removed from each bee colony, directly from the pollen pots inserted in the nest. Subsequently, fresh bee pollen samples were cleaned, removing

impurities inherent to the collection process (dead bees, bee larvae and propolis). Each bee pollen sample was packed in Falcon tubes, identified by bee species, colony and month of collection, and stored at -20 °C until analysis.

Pollen samples from *Apis mellifera* and stingless bee species were collected according to the procedure described above in the Beekeeping and Meliponiculture sector of the Experimental Farm of ``Universidade Estadual de Maringá``, Paraná, Brazil (coordinates 23º15'15" and 23º33' 27" S and 51º50'05" and 52º05'59" W. The collection period covered the period between June 2018 and May 2019, totaling 243 pollen samples that were distributed in 53 samples of the species: *Apis mellifera*, 39 samples of the species: *Melipona marginata*, 48 samples of the species: *Melipona quadrifasciata*, 57 specimens of the species: *Scaptotrigona bipunctata* and 46 samples of the species: *Tetragona clavipes*.

POLLEN ANALYSIS

In order to identify the botanical origin (vegetable species) of bee pollen, pollen analysis of the samples was performed. Initially, the bee pollen samples were thawed and placed in an oven with forced air circulation at 55°C for 72 hours. After that time, they were ground in a mortar and sieved through a 1 mm sieve.

For pollen analysis, a mass of about 2.0 g of each bee pollen sample was kept in 70% alcohol for 24 hours. After this period, the suspension was centrifuged and the alcohol discarded. 4.0 mL of glacial acetic acid were added to the pollen material and the suspension was left to rest for 24 hours (Silva et al. 2014). The pollen material was subsequently acetolyzed following the method proposed by Erdtman (1960). After acetolysis, the pollen material was kept in 50% glycerin. Slides were prepared for each bee pollen sample using Kisser gelatin and sealed with clear varnish. In the qualitative analyses, the types of pollen found on the slides were identified by comparison with the types of pollen on the reference slides of the study area, deposited at the Bee Laboratory of ``Universidade Estadual de Maringá``-PR, Brazil, and by comparison with the literature specialized for the identification of pollens collected by bees (Miranda and Andrade 1990; Silva et al. 2010-2014). In the quantitative analyses, the first 400 pollen grains found in each bee pollen sample were counted (Montero and Tormo, 1990). Then, the percentage and classes of occurrence were determined according to the classification proposed by Barth (1970), Louveaux et al. 1970 and Louveaux et al. 1978: dominant pollen (> 45.0% of the total pollen grains present on the slide), accessory pollen (from 15.0% to 45.0%), important isolated pollen (from 3.0% to 15.0%) and occasional isolated pollen (< 3.0%).

INSTRUMENTAL COLOR ANALYSIS

Instrumental color measurement in bee pollen samples was performed with a Konica Minolta CR-400 chroma meter, a color measuring device equipped with six highly sensitive silicon photodiodes with a measurement range from 0.01 to 160%. Prior to measuring the samples, the device was calibrated using a white standard (C: Y - 87.0; x - 0.3169; y - 0.3239 and D: Y - 87.0; x - 0.3193; y - 0.3367). To measure the instrumental color, a mass of around 3.0 to 8.0 g of the bee pollen sample, previously ground and homogenized, was placed on white bond paper and compacted with a Petri dish, forming a uniform layer of approximately 0.3 cm thick. Thus, the pollen sample completely covered the surface of the sensor, with no white spaces occupying an approximate space of 9.0 cm^2, where three readings were taken in different positions. The measuring device was lowered over the pollen sample, placing the

reader completely under the sample, before the start of the measurement. The L*, a* and b* color coordinate values were obtained after a few seconds of sample measurement. Parallel measurements were performed in triplicate for each pollen sample (243 samples x 3 replicates = 729 color coordinate records) and color coordinate results were expressed as mean values.

STATISTICAL ANALYSIS

To evaluate the influence of the factors 'species of bee' and 'season of the year' on the instrumental color of the bee pollen samples, an ANOVA was performed with nested factors of the Generalized Linear Models (GML) at the 95% confidence level, in which the 'season of the year' factor was nested within the 'bee species' factor. For the factor 'species of bee' (i = 5) five levels were adopted (*Apis mellifera, Melipona marginata, Melipona quadrifasciata, Scaptotrigona bipunctata and Tetragona clavipes*) and for the 'season of the year' factor (j = 4) four levels were adopted (winter, spring, summer and fall). To evaluate the experimental error, different samples of bee pollen collected in the four seasons of the year by each bee species from June 2018 to May 2019 were used. For *Apis mellifera*, 14 samples were collected in spring, 13 samples in winter, 15 samples in the summer and 13 samples in the Fall.

For *Melipona marginata*, 11 samples were collected in spring, 07 samples in winter, 14 samples in summer and 09 samples in fall. To the *Melipona quadrifasciata*, 12 samples were collected in spring, 08 samples in winter, 12 samples in summer and 12 samples in fall.

For *Scaptotrigona bipunctata* 14 samples were collected in spring, 14 samples in winter, 15 samples in summer and 14 samples in fall. For Tetragona clavipes, 12 samples were collected in spring, 11 samples in winter, 12 samples in summer and 11 samples in fall.

The *Tukey* test for multiple comparison of means was applied at the 95% confidence level to the instrumental color data of the bee pollen samples in order to identify significant differences between the means of the color coordinates obtained for the bee species and between the averages determined for the seasons within each bee species. *Pearson*'s correlation analyzes were also performed for all data at the same 95% confidence level to verify existing correlations between the evaluated instrumental color coordinates. All statistical analysis was performed using Minitab for Windows, version 16.2.2 and Statistica, version 8.0.

RESULTS AND DISCUSSION

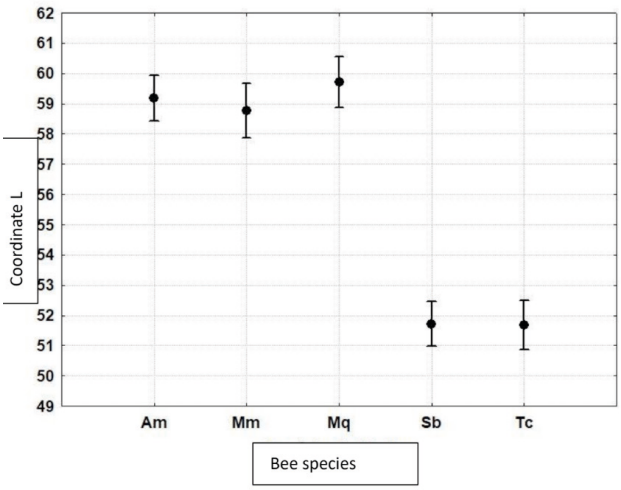
Bee pollen has a variable color with shades that can vary from grayish white to dark brown, with a predominance of yellow, orange and red, and this variability can be attributed to differences in the composition of plant pollens, or in their chemical composition or processes. oxidation arising from production and processing processes (De-Melo et al., 2018; Sipos et al., 2020 Straumite et al., 2022; Castiglioni et al., 2022; Thakur and Nanda, 2020; Yang et al., 2013; De Melo et al, 2016).

The instrumental color values of the CIELAB system (Table 1) indicated variations in the coordinates L* (45.32 - 63.01), a* (7.65 - 16.17) and b* (17.48 - 31.93) for bee pollen samples collected in different seasons of the year by *Apis mellifera* and stingless bees. To assess whether there were significant differences in the instrumental color of bee pollen collected by different bee species and in different seasons, a nested factor ANOVA was performed.

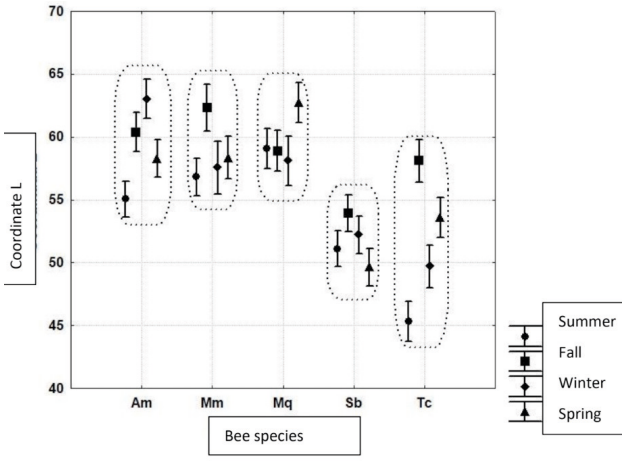
The bee species factor was significant for the coordinates: L^*, a^* and b^* (F_{obs} = 26,49; $p = 0,000$ *for* L^*; $F_{obs} = 18,50$; $p = 0,000$ *for**; $F_{obs} = 22,94$; $p = 0,000$ *for* b^*). The season factor also significantly influenced these color

coordinates (L^*, a^* and b^*) (F_{obs} = 4,32; p = *0,000 for* L^*; F_{obs} = *10,41;* p = *0,000 for**; F_{obs} = *7,85;* p = *0,000 para* b^*) responsible for the luminosity and characteristic color (red and yellow tones) of bee pollen.

In the CIELAB system, the L* parameter expresses the luminosity of the object being evaluated in the gray scale, ranging from absolute black to absolute white (Sipos et al., 2020). In bee pollen samples, L* values were between 45.32 – 63.01 and were similar to those reported in the literature for ground and homogenized bee pollen (Castiglioni et al., 2022) and for bee pollen loads from the state of Paraná, Brazil (De-Melo et al., 2018). Furthermore, it can be attributed that (+) and higher values for L* indicate samples of bee pollen with lighter shades (Sipos et al., 2020). When comparing the L* values for samples from different species of bees, it is observed that the pollen collected by the bees: *Apis mellifera* (59,02 ± 6,77), *Melipona marginata* (58,57± 6,88) and *Melipona quadrifasciata* (59,85 ± 4,52) showed lighter shades and significantly different from those found in bee pollen samples: *Scaptotrigona bipunctata* (51,75 ± 5,52) and *Tetragona clavipes* (51,58 ± 6,53) (Figure 1-a).



(a)



(b)

Figure 1. Influence of factors (a) bee species and (b) season of the year on the color coordinate (L*) of bee pollen samples. Am = *Apis mellifera*; Mm = *Melipona marginata*; Mq = *Melipona quadrifasciata*; Sb = *Scaptotrigona bipunctata*; Tc = *Tetragona clavipes*

For the pollen collected by the bee: *Apis melífera*, it was observed that the luminosity values (L*) were higher for the samples collected in winter (63.01 ± 7.78) and significantly different ($p < 0.05$) from the values determined in the other seasons of the year (Figure 1 -B). In winter, bee pollen samples were characterized by high pollen frequency rates of plant species: *Mimosa pigra* (76,9 %), *Raphanus raphanistrum* (76,3 %) and *Eugenia uniflora* (37,1 %). However, for bee pollen samples from bee species: *Scaptotrigona bipunctata* (53,91 ± 3,76) and *Tetragona clavipes* (58,10 ± 4,21) a different behavior is observed in which the samples collected in the autumn presented higher luminosity values (L*) when compared to those obtained in the other seasons of the year (Figure 1-b). In autumn, bee pollen samples from *Scaptotrigona bipunctata* were characterized by the predominance of *Eucalyptus robusta* (88,3 %; dominant pollen) and the samples of *Tetragona clavipes* by *Eriobotrya japonica* (48,7 %; dominant pollen) and important contributions from *Eucalyptus robusta* (33,0

Bee species	Seasons	L*	a*	b*
Apis mellifera	Winter	63,01 ± 7,78^{a}	10,37 ± 3,15^{c,d,e,f}	31,27 ± 4,24^{a}
	Fall	60,38 ± 3,86^{a,b,c}	8,69 ± 1,05^{d,e,f}	23,51 ± 1,78 ^{b,c}
	Spring	58,29 ± 8,11^{a,b,c,d,e}	10,47 ± 2,08^{c,d,e,f}	24,75 ± 5,04^{b}
	Summer	55,05 ± 4,14^{b,c,d,e,f,g}	11,28 ± 2,10^{b,c}	22,12 ± 3,07^{b,c,d}
	Annual average	59,02 ± 6,77^{A}	10,25 ± 2,35^{A}	25,28 ± 5,05^{A}
Melipona marginata	Winter	57,56 ± 7,76^{a,b,c,d,e,f,g}	11,65 ± 1,88^{b,c,d}	22,17 ± 1,64 ^{b,c,d}
	Fall	62,31 ± 7,21^{a,b,c}	7,65 ± 1,01^{f}	20,09 ± 2,53 ^{b,c,d}
	Spring	58,35 ± 7,58^{a,b,c,d,e}	11,63 ± 1,41^{b,c}	23,13 ± 3,89^{b,c}
	Summer	56,83 ± 5,29 ^{a,b,c,d,e,f,g}	11,99 ± 2,50 ^{b,c}	22,23 ± 4,02^{b,c,d}
	Annual average	58,57± 6,88^{A}	10,88 ± 2,51^{A}	21,99 ± 3,45^{C}
Melipona quadrifasciata	Winter	58,11 ± 5,00^{a,b,c,d,e,f,g}	11,39 ± 1,42^{b,c,d}	22,03 ± 1,35^{b,c,d}
	Fall	58,90 ± 3,08^{a,b,c,d}	8,06 ± 0,66^{e,f}	21,08 ± 1,88^{b,c,d}
	Spring	62,74 ± 5,97^{a,b}	10,77 ± 1,83^{b,c,d,e}	23,57 ± 2,05^{b,c}
	Summer	59,08 ± 2,42^{a,b,c,d}	11,45 ± 2,68^{b,c}	23,07 ± 3,61^{b,c}
	Annual average	59,85 ± 4,52^{A}	10,33 ± 2,28^{A}	22,48 ± 2,57^{B,C}
Scaptotrigona bipunctata	Winter	52,22 ± 7,21^{d,e,f,g,h}	10,19 ± 1,52^{c,d,e,f}	19,48 ± 3,48^{b,c,d}
	Fall	53,91 ± 3,76^{c,d,e,f,g}	8,26 ± 0,58^{e,f}	17,48 ± 1,34^{d}
	Spring	49,64 ± 5,95^{g,h}	11,67 ± 1,10^{b,c}	18,97 ± 3,62 ^{c,d}
	Summer	51,11 ± 4,34^{e,f,g,h}	10,81 ± 1,71 ^{b,c,d}	19,24 ± 3,92^{c,d}
	Annual average	51,75 ± 5,52^{B}	10,21 ± 1,79^{A}	18,78 ± 3,26^{D}
Tetragona clavipes	Winter	49,71 ± 4,64^{d,g,h}	12,19 ± 2,26^{b,c}	22,70 ± 7,00^{b,c,d}
	Fall	58,10 ± 4,21^{a,b,c,d,e,f}	10,32 ± 1,08^{c,d,e,f}	23,76 ± 2,74 ^{b,c}
	Spring	53,58 ± 3,66^{c,d,e,f,g}	16,17 ± 1,83^{a}	31,93 ± 4,78 ^{a}
	Summer	45,32 ± 5,68 ^{h}	13,25 ± 2,77^{b}	19,35 ± 7,71^{b,c,d}
	Annual average	51,58 ± 6,53^{B}	13,06 ± 2,95^{B}	24,49 ± 7,44^{A,B}

Table 1: Mean values and standard deviations of color parameters (L*, a*, b*) of the CIELAB scale of pollen samples collected by different species of bees and in different seasons.

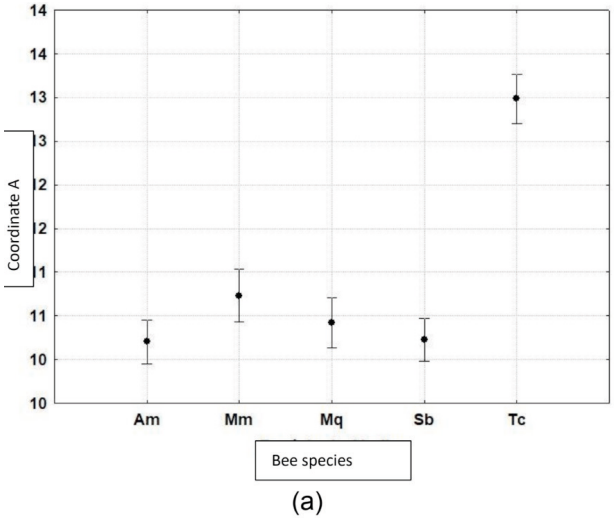
%; accessory pollen), *Macherium stipitatum* (22,0 %; accessory pollen) and *Baccharis dracunculifolia* (16,0 %; accessory pollen).

On the other hand, bee pollen samples from *Melipona marginata* and of *Melipona quadrifasciata* did not show the luminosity values influenced by the seasons in which the collection was performed (Figure 1-b). This behavior can be associated with the fact that the samples of these stingless bee species show predominance in one or more seasons of the plant species.: *Eucalyptus robusta*, *Psidium guajava* and *Alchornea triplinervia*. Thus, it can be inferred that among the bee species and season of the year factors, the bee species has a greater influence on the luminosity values (L*) of the bee pollen samples, which was confirmed by the results of the ANOVA analysis. of nested factors.

The color coordinate in the CIELAB system, called a*, can assume values (+) for the red color and values (-) for the green color (Sipos et al., 2020). In this study, for the pollen collected by different species of bees and in different seasons of the year, positive values for a* were always observed, with a range between 7.65 - 16.17, indicating a greater contribution of red in relation to green in the sample staining. These intervals for the a* coordinate was similar to those observed in

the literature by De-Melo et al. (2018) (4.9 – 11.7), Sipos et al. (2020) (0.9 – 15.6) and by Castiglioni et al. (2022) (5.1 - 14.8).

For the a* coordinate values, a certain homogeneity is observed, without significant differences ($p > 0.05$) for the pollen samples of the species: *Apis mellifera* (10,25 ± 2,35), *Melipona marginata* (10,88 ± 2,51), *Melipona quadrifasciata* (10,33 ± 2,28) and *Scaptotrigona bipunctata* (10,21 ± 1,79) (Figure 2-a). Bee pollen samples from *Tetragona clavipes*, in turn, presented higher a* values (13.06 ± 2.95) and significantly differed from those found for the pollen samples of the other investigated bee species, indicating that these samples present a color that tends towards red (Figure 2 -The). For pollen samples from *Tetragona clavipes*, the relevant contribution of plant species that were not present in the pollen samples of other bee species was observed. For example, the presence of *Eriobotrya japonica*, *Ceiba speciosa*, *Alternanthera tenella*, *Piper amalago* and *Murraya paniculata*. Considering that the pollen of plant species can vary in color, the presence of a diversity of plants in its composition may interfere and contribute to the final color of the bee pollen and, therefore, to the values of the color coordinates of the CIELAB system.



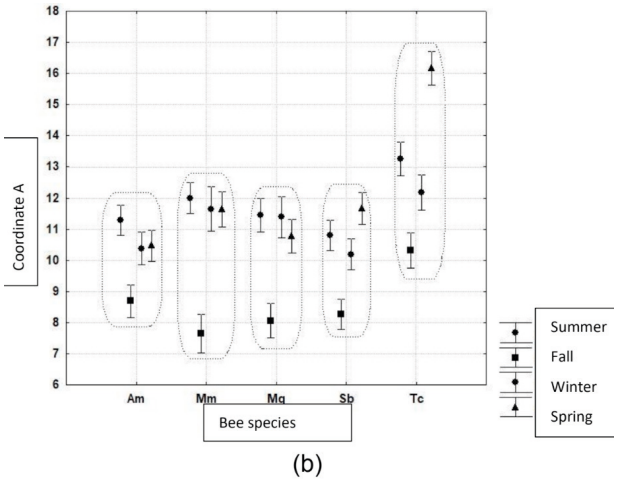


Figure 2. Influence of factors (a) bee species and (b) season of the year on the color coordinate (a*) of bee pollen samples. Am = *Apis mellifera*; Mm = *Melipona marginata*; Mq = *Melipona quadrifasciata*; Sb = *Scaptotrigona bipunctata*; Tc = *Tetragona clavipes*

Regarding seasons, the lowest values of a* were detected in bee pollen samples collected in autumn by bees: *Apis mellifera* (8,69 ± 1,05), *Melipona Marginata* (7,65 ± 1,01), *Melipona quadrifasciata* (8,06 ± 0,66) and *Scaptotrigona bipunctata* (8,26 ± 0,58), while similar values were obtained for samples collected in other seasons of the year (Figure 2-b). The pollen samples collected in autumn showed a predominance of *Eucalyptus robusta* to *Melipona marginata* (98,1 %; dominant pollen), to *Melipona quadrifasciata* (94,6 %; dominant pollen), to *Scaptotrigona bipunctata* (88,3 %; dominant pollen) and important contributions from *Mimosa pigra* (65,0 %; dominant pollen) and *Eucalyptus robusta* (44,0 %; accessory pollen) to *Apis mellifera*.

Again, the a* values of the pollen samples collected by the bee: *Tetragona clavipes* stand out, showing the highest values for this coordinate in spring (16.17 ± 1.83) and the lowest values in autumn (10.32 ± 1.08). Pollen samples collected in spring showed contributions from different plant species such as: *Raphanus raphanistrum* (25,5 %; accessory pollen), *Eucalyptus robusta* (33,0 %;

accessory pollen), *Alchornea triplinervia* (33,0 %; accessory pollen), and *Croton floribundus* (33,0 %; accessory pollen), while the samples collected in autumn were characterized by the marked presence of *Eriobotrya japonica* (48,7 %; dominant pollen), *Macherium stipitatum* (22,0 %; accessory pollen), *Eucalyptus robusta* (33,0 %; accessory pollen), and *Baccharis dracunculifolia* (16,0 %; accessory pollen). The pollen samples collected by this stingless bee species were also the ones that presented the most differentiated behavior between the seasons of the year in relation to this color coordinate. This behavior can be associated with a generalist habit of collecting plant pollen by the bee: *Tetragona clavipes*, which leads to the relevant contribution of different plant species to the pollen collected in each season of the year, thus interfering with the color of the samples obtained in each season of the year.

The color coordinate in the CIELAB system, called b*, can assume values (+) for yellow coloring and values (-) for blue coloring (Sipos et al., 2020). In this study, for the bee pollen collected by different species of bees and in different seasons of the year, positive values for b* (17.48 – 31.93) were always observed, indicating a greater contribution of yellow compared to blue in coloration of the samples. However, in the literature, higher values were observed for this coordinate by other authors ((De-Melo et al. (2018) (50.0 – 63.0), Sipos et al. (2020) (17.1 – 64, 5), Castiglioni et al. (2022) (41.6 – 68.1)). These differences may be related to the richness of pigments with antioxidant activity and the differences in the concentration of minerals in bee pollen samples, which interfere in the values of the a* and b* color coordinates (Castiglioni et al., 2022).

For the b* coordinate, significant differences ($p < 0.05$) were found between the pollen samples collected by: *Apis mellifera* and by stingless bees (Figure 3-a). For pollen samples collected by *Scaptotrigona bipunctata,* b* values were the lowest (18.78 ± 3.26) and differed significantly ($p < 0.05$) from those found in samples collected by other bee species.

The b* values for pollen samples from *Apis mellifera* (25,28 ± 5,05) also showed significant differences ($p < 0.05$) in relation to the values observed for the samples of *Melipona marginata* (21,99 ± 3,45) and of *Melipona quadrifasciata* (22,48 ± 2,57), but they were similar to the values found for the Tetragona clavipes samples (24.49 ± 7.44) (Figure 3-a).

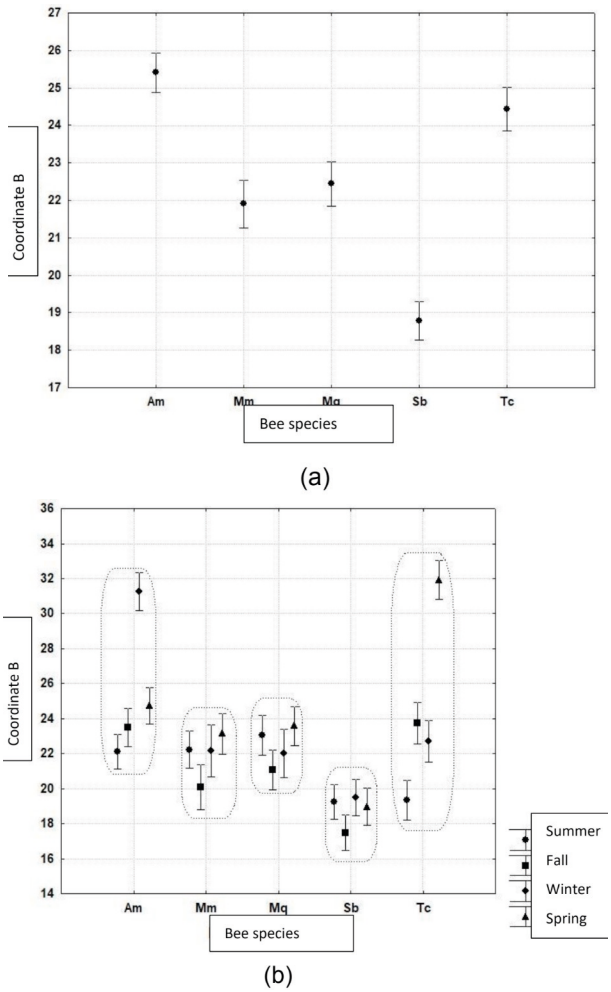


Figure 3. Influence of factors (a) bee species and (b) season of the year on the color coordinate (b*) of bee pollen samples. Am = *Apis mellifera*; Mm = *Melipona marginata*; Mq = *Melipona quadrifasciata*; Sb = *Scaptotrigona bipunctata*; Tc = *Tetragona clavipes*.

For pollen samples from bee species: *Melipona marginata* (21,99 ± 3,45) and *Melipona quadrifasciata* (22,48 ± 2,57) no significant differences ($p > 0.05$) were observed in the b* values between the seasons of the year, while for samples from *Apis mellifera* higher values were obtained when collection was carried out in winter (31.27 ± 4.24) and for samples of *Tetragona clavipes* when they were collected in the spring (31.93 ± 4.78) (Figure 3-b). As previously described, pollen samples collected in winter by: *Apis mellifera* were characterized by high frequency rates of plant pollens from *Mimosa pigra* (76,9 %; dominant pollen) and *Raphanus raphanistrum* (76,3 %; dominant pollen), while the pollen samples collected by the *Tetragona clavipes* in spring were characterized by relevant contributions from different plant species (*Raphanus raphanistrum* (25,5 %; accessory pollen), *Eucalyptus robusta* (33,0 % ; accessory pollen), *Alchornea triplinervia* (33,0 %; accessory pollen), and *Croton floribundus* (33,0 %; accessory pollen). To better understand how the chemical composition can influence the variability of bee pollen color, linear correlation analyzes were performed between the color coordinates (L*, a* and b*) of the CIELAB system in the 95% confidence interval. Significant linear correlations were observed between color coordinates L* and b* ($r = 0.617$; $p = 0.000$) and between a* and b* ($r = - 0.357$, $p = 0.000$), which may be associated with the type and amount of phytochemicals present (pigments, carotenoids and phenolic compounds) or other components such as mineral salts. Correlations between instrumental color coordinates and the chemical composition of bee pollen samples have been observed by other authors (Thakur and Nanda, 2020; Yang et al., 2013; De Melo et al, 2016), and in particular, correlations with the mineral contents (Ca, Mg and Fe) or with the contents of total phenolic compounds and with antioxidant and antimicrobial potential. According to Thakur and Nanda (2020), the type of processing adopted, such as milling and drying, can result in lighter samples with a predominance of yellow (positive and higher b* values), such as those observed in this study, which can come from oxidation reactions of some compounds such as polyphenols during the drying process.

CONCLUSIONS

An intrinsic variability in the instrumental color (coordinates L*, a* and b*) of bee pollen was observed, being mainly attributed to the species of bee that collects plant pollen. However, seasonality, another factor as important as the bee species, can produce changes in the plant sources available for bee nutrition in the producing region. Thus, the foraging habit of different bee species associated with flora and plant climatic conditions in each season of the year in the producing region may explain, in part, the variability in the color of this bee product.

Bee pollen samples classified as monofloral in which there is a predominance of certain plant species such as: *Eucalyptus robusta*, *Psidium guajava, Mimosa pigra, Raphanus raphanistrum and Alzibia niopoides* showed characteristic values for color coordinates (L*, a* and b*) that differentiated them from samples classified as heterofloral, regardless of the bee species responsible for collecting bee pollen.

Important correlations between the color coordinates (L*, a* and b*) demonstrated that the search for the supply of plant pollen for the maintenance and reproduction of hives by bee species: *Apis mellifera* and stingless bees (*Melipona marginata, Melipona quadrifasciata, Scaptotrigona bipunctata* and *Tetragona clavipes*) has great influence on the final color of the produced bee pollen.

Information about the foraging behavior

(collection of plant pollen) of each bee species, as well as the availability of flora in the producing region can help beekeepers and meliponiculturists in setting up hives close to certain predominant plant species around the apiary, thus aiming, to obtain bee products with certain coloring characteristics that can be used in industries of the sector.

Knowledge of how the plant composition and bee species determine the color of bee pollen produced in a given region can be decisive in its use in an industrial context, as food, cosmetics or medicine, especially for pollen from stingless bees which has its coloring little studied so far. In addition, this information can contribute to the development of Brazilian beekeeping and meliponiculture, since monofloral bee pollen samples can be sold more easily and with greater added value, ensuring the consumer a product with a more uniform and standardized color.

RECOGNITION

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REFERENCES

ARES, A.M.; TORIBIO, L.; TAPIA, J.A.; GONZÁLEZ-PORTO, A.V.; HIGES, M.; MARTÍN-HERNÁNDEZ, R.; BERNAL, J. **Differentiation of bee pollen samples according to the apiary of origin and harvesting period based on their amino acid content.** Food Bioscience, 50, 8, 102092, 2022.

BARTH, O.M. **Análise microscópica de algumas amostras de mel. 1. Dominant pollen.** Anais da Academia Brasileira de Ciências. 42: 351-366, 1970.

BARTH, O.M.; DE FREITAS, A.S.; VANDERBORGHT, B. **Pollen preference of stingless bees (*Melipona rufiventris* and *M. quadrifasciata anthidioides*) inside an urban tropical forest at Rio de Janeiro city.** Journal of Apicultural Research, 59, 5, 1005-1010, 2020.

CASTIGLIONI, S.; STEFANO, M.; ASTOLFI, P.; PISANI, M.; CARLONI, P. **Characterisation of bee pollen from Marche Region (Italy) according to the botanical and geographical origin with analysis of antioxidant activity and colour, using a chemometric approach.** Molecules, 27, 7996, 2022. https://doi.org/10.3390/molecules27227996.

DE-MELO, A.A.M.; ESTEVINHO, L.M.; MOREIRA, M.M.; DELERUE-MATOS, C.; DE FREITAS, A.S.; BARTH, O.M.; ALMEIDA-MURADIAN, L.B. **A Multivariate approach based on physicochemical parameters and biological potential for the botanical and geographic discrimination of Brazilian bee pollen.** Food Bioscience, 25, 91-110, 2018.

DE MELO, A.A.M.; ESTEVINHO, M.L.F.; SATTLER, J.A.G.; SOUZA, B.R.; DA SILVA FREITAS, A.; BARTH, O.M. et al. **Effect of processing conditions on characteristics of dehydrated bee-pollen and correlation between quality parameters**. LWT-Food Science and Technology, 65, 808-815, 2016. https://doi.org/10.1016/j.lwt.2015.09.014.

DE-SOUZA, B.R.; SATTLER, J.G.; DE-MELO, A.A.M.; GRANATO, D.; DA SILVA, A.E.; DA SILVA, F.A.; BARTH, M.O.; SATTLER, A.; ALMEIDA-MURADIAN, L.B. **Physicochemical parameters and content of B-complex vitamins: na exploratory study of bee pollen from Southern Brazilian states**. Revista Chilena de Nutricion, v. 45, n. 3, p. 232-242, 2018.

DUARTE, A.W.F.; VASCONCELLOS, M.R. dos S.; ODA-SOUZA, M.; OLIVEIRA, F.F. de; LÓPEZ, A.M.Q. **Honey and bee pollen produced by Meliponini (Apidae) in Alagoas, Brazil: Multivariate analysis of physicochemical and antioxidant profiles**. Food Science and Technology, 38, 3, 493-503, 2018.

ERDTMAN, G. **The acetolysis method. A revised description**. Svensk Botanic Tidsk 54: 561-564, 1960.

GIAMPIERI, F.; QUILES, J.L.; CIANCIOSI, D.; FORBES-HERNÁNDEZ, T.Y.; ORANTES-BERMEJO, F.J.; ALVAREZ-SUAREZ, J.M.; BATTINO, M. **Bee products: an emblematic example of underutilized sources of bioactive compounds**. Journal of Agricultural and Food Chemistry, 70, 6833-6848, 2022.

KIELISZEK, M.; PIWOWAREK, K.; KOT, A. M.; BŁAŻEJAK, S.; CHLEBOWSKA-ŚMIGIEL, A.; WOLSKA, I. **Pollen and bee bread as new health-oriented products: A review.** Trends Food Science and Technology, 71, 170, 2018. https://doi.org/10.1016/j.tifs.2017.10.02.

LAAROUSSI, H.; FERREIRA-SANTOS, P.; GENISHEVA, Z.; BAKOUR, M.; OUSAAID, D.; GHOUZI, A.E.; TEIXEIRA, J.A.; LYOUSSI, B. **Unveiling the techno-functional and bioactive properties of bee pollen as an added-value food ingredient.** Food Chemistry, 405, 134958, 2023.

LI, Q.; WANG, K.; MARCUCCI, M.C.; SAWAYA, A.C.H.F.; HU, L.; XUE, X.; WU, L.; HU, F. **Nutrient-rich bee pollen: a treasure trove of active natural metabolites.** Journal of Functional Foods, 49, 472-484, 2018.

LOUVEAUX, J., MAURIZIO, A. & VORWOHL, G. **Methods of Melissopalynology.** Bee World, 59:4, 139-157, 1978. DOI: 10.1080/0005772X.1978.11097714.

LOUVEAUX, J., MAURIZIO, A. & VORWOHL, G. **Methods of Melissopalynology.** Bee World, 51:3, 125-138, (1970). DOI: 10.1080/0005772X.1970.11097312.

LU, P.; TAKIGUCHI, S.; HONDA, Y.; LU, Y.; MITSUI, T.; KATO, S.; KODERA, R.; FURIHATA, K.; ZHANG, M.; OKAMOTO, K.; ITOH, H.; SUZUKI, M.; KONO, H.; NAGATA, K. **NMR and HPLC profiling of bee pollen products from different countries.** Food Chemistry: Molecular Sciences, 5, 100119, 2022.

MIRANDA, M.M.B. & ANDRADE, T.A.P. **Fundamentos de palinologia.** Fortaleza, Imprensa Universitária da UFC. 99, 1990.

MONTERO, I. & TORMO, R. **Análisis polínico de mieles de cuatro zonas montañosas de Extremadura.** Annais Asociación de Palinólogos de Lengua Española 5: 71-78, 1990.

NEGRÃO, A.F.; BARRETO, L.M.R.C.; ORSI, R.O. **Influence of the collection season on production, size, and Chemical composition of bee pollen produced by *Apis mellifera* L.** Journal of Apicultural Sciences, v. 58, n.2, 2014.

NEGRÃO, A.; ORSI, R.O. **Harvesting season and botanical origin interferes in production and nutritional composition of bee pollen.** Anais da Academia Brasileira de Ciências, 90, 1, 325-332, 2018.

REBELO, K.S.; CAZARIN, C.B.B.; IGLESIAS, A.H.; STAHL, M.A.; KRISTIANSEN, K.; CARVALHO-ZILSE, G.A.; GRIMALDI, R.; REYES, F.G.R.; DANNESKIOLD-SAMSOE, N.B.; JUNIOR, M.R.M. **Nutritional composition and bioactive compounds of *Melipona seminigra* pot-pollen from Alagoas, Brazil.** Journal of the Science Food and Agriculture, v. 101, n. 12, p. 4907–4915, 2021. DOI 10.1002/jsfa.11134.

ROZMAN, A.S.; HASHIM, N.; MARINGGAL, B.; ABDAN, K. **A comprehensive review of stingless bee products: phytochemical composition and beneficial Properties of honey, própolis, and pollen.** Applied Sciences, 12, 6370, 2022. https://doi.org/10.3390/app12136370.

SILVA, C.I., ALEIXO, K.P., NUNES-SILVA, B., FREITAS, B.M. & IMPERATRIZ-FONSECA, V.L. **Guia ilustrado de abelhas polinizadoras no Brasil.** Inst. Avançado da Univ. de SP 1: 01-50, 2014.

SILVA, C.I., BALLESTEROS, P.L.O., PALMERO, M.A., BAUERMANN, S.G., EVALDT, A.C.P. & OLIVEIRA, P.E. **Catálogo polínico: Palinologia aplicada em estudos de conservação de abelhas do gênero Xylocopa no Triângulo Mineiro.** Edufu, Uberlândia 1: 154, 2010. doi: 10.14393/EDUFU-978-85-7078-239-7.

SILVA, T.M.S.; CAMARA, C.A.; LINS, A.C. da S.; BARBOSA-FILHO, J.M.; DA SILVA, E.M.S.; FREITAS, B.M.; DOS SANTOS, F.A.R. **Chemical composition and free radical scavenging activity of pollen loads from stingless bee *Melipona subnitida* Ducke.** Journal of Food Composition and Analysis, 19, 507-511, 2006.

SIPOS, L.; VEGH, R.; BODOR, Z.; ZAUKUU, J-L. Z.; HITKA, G.; BÁZÁR, G.; KOVACS, Z. **Classification of bee pollen and prediction of sensory and colorimetric attributes – a sensometric fusion approach by e-nose, e-tongue and NIR.** Sensors, 20, 6768, 2020. https://doi:10,3390/s20236768.

STRAUMITE, E.; BARTULE, M.; VALDOVSKA, A.; KRUMA, Z.; GALOBURDA, R. **Physical and microbiological characteristics and antioxidant activity of honey bee pollen.** Applied Sciences, 12, 3039, 2022. https://doi.org/10.3390/app12063039.

THAKUR, M.; NANDA, V. **Composition and functionality of bee pollen: a review.** Trends in Food Science & Technology, v. 98, p. 82-106, 2020.

VIT, P.; BERTHA, S.; PEDRO SILVIA, R.M.; RUÍZ, J.; MAZA, F.; PEÑA-VERA, M. PÉREZ-PÉREZ, E. **Chemical and bioactive characterization of pot-pollen produced by Melipone and Scaptotrigona stingless bees from Paria Grande, Amazonas state, Venezuela.** Emirates Journal of Food and Agriculture, 28, 2, 78-84, 2016.

YANG, K; WU, D.; YE, X.; LIU, D.; CHEN, J.; SUN, P. **Characterization of chemical composition of bee pollen in China.** Journal of Agricultural and Food Chemistry, 61, 3, 708-718, 2013. https://doi.org/10.1021/jf304056b.