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ORIGINAL RESEARCH ARTICLE

Botanical origin, microbiological quality and physicochemical composition of the *Melipona scutellaris* pot-pollen ("samburá") from Bahia (Brazil) Region

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Melipona scutellaris Latreille, 1811 is of economic importance for local beekeepers, besides its relevance in the pollination of native plant species of the Brazilian Atlantic forest. Currently, data on the use of floral resources by Meliponini colonies are scarce, particularly in urban environments. We evaluated the botanical origin, the microbiological and physicochemical characteristics of pollen stored by *M. scutellaris* in colonies in an urban environment. The samples (n = 44) were obtained from the metropolitan region of Salvador, Bahia, Brazil, a region of intense urban and industrial activities. We identified 52 pollen types belonging to 21 botanical families. The botanical families Fabaceae, Myrtaceae, and Anacardiaceae represented most pollen types. Aerobic psychrotrophic bacteria, *Bacillus* spp., molds and yeasts, fecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, sulfite-reducing *Clostridium*, and *Salmonella* spp. were not found in the samples. We identified and quantified fatty acids with carbon numbers varying from C4 to C20. For the physicochemical parameters, the following variations were verified: moisture (47.3% to 55.70%), ash (3.45% to 5.90%), protein (10.19 to 24.02%), pH (3.28 to 3.99), acidity (237.20 to 557.10 meq/kg), lipids (2.43 to 7.94%), carbohydrates (10.85 to 28.89%) and total energy value (170.60 to 216.99 kcal/100g). Pollen stored ("samburá") by bees is a complete food and a source of nutrients with therapeutic potential. Pollen stored by *M. scutellaris* consists of a heterofloral pollen with physicochemical and microbiological qualities, considered safe for human consumption. Moreover, it contains linoleic and linolenic essential fatty acids making it a potential nutraceutical product.

Keywords: pollen analysis; beekeeping microbiology; fatty acid; meliponiculture

Introduction

Melipona scutellaris Latreille, 1811, is a Meliponini species occurring in northeastern Brazil from Bahia to Rio Grande do Norte states, particularly in regions once occupied by the Atlantic Forest (Viana et al., 2013). *M. scutellaris* is easily domesticated (Rodrigues et al., 2008), this is the reason that the beehives are kept in areas where intense urban and industrial growth are overtaking the Atlantic forest (Oliveira et al., 2017). Besides its relevance in the pollination of native plant species of the Brazilian Atlantic forest, *M. scutellaris* is of great importance to local beekeepers that, in turn, help to maintain these pollinators. Currently, data on the use of floral resources by Meliponini colonies are needed, particularly in urban environments. Studies to better understand these bees, including the use of their floral resources in various environments and the effect on products is scarce.

Meliponiculture (stingless bee cultivation) has been prominent in the Brazilian Northeast, and honey is the main marketed product (Alves et al., 2018a; Villas-Bôas, 2018). However, pollen stored by stingless bees presents as an alternative for this beehive product, due to its bioactive compounds and complex composition (Duarte et al., 2018; Villas-Bôas, 2018). Information on quantification of pollen production of stingless bees is scarce in the literature; however, a recent study reported that the average weight of pollen pots of *M. scutellaris* was 13.96 grams, indicating the viability of cultivation of this bee for pollen production (Alves et al., 2018b).

Pot-pollen, or "samburá", or pollen stored by the stingless bee, is a result of floral pollen combination collected by worker bees with nectar and salivary enzymes from bees. It is stored and compacted in food pots (pollen-pots), different from bee bread, produced by *Apis mellifera* Linnaeus, 1758, which is stored in combs (Alves et al., 2018a; Dermarderosian & Beuther, 2005; Lima Neto et al., 2017).

According to Kroyer and Hegedus (2001), pollen stored by bees is considered a food supplement, highlighting the relevance of studies that characterize this

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Table 1. Origin of *Melipona scutellaris* pot-pollen ("samburá") samples from meliponaries in the metropolitan region of Salvador, Bahia, Brazil.

Sites	Municipalities/site	N*	Geographic Coordinates
A	Salvador/1	5	S 12°51′32.4″; W'038°27′9.9″
B	Salvador/2	7	S 12°51′28.3″; W 38°21′54.3″
C	Salvador/3	9	S 12°49′58.7″; W 38°22′27.4″
D	Lauro de Freitas	9	S 12°50′38.1″; W 38°21′12.1″
E	Simões Filho	6	S 12°43′55.5″; W 38°23′51.6″
F	Dias D'Ávila	8	S 12°32′28.0″; W 38°21′42.3″

*N = number of samples.

promising product for human food, and its consumption is stimulated by the market of natural products (Duarte et al., 2018).

Bee pollen is considered a complete food because it contains essential amino acids, carbohydrates, crude fibers, lipids, vitamins, and phenolic compounds (Feás et al., 2012). Pollen chemical composition depends mainly on bee species, botanical and geographical origin, climatic conditions, soil type, and agricultural practices (Pascoal et al., 2014). However, there is little information on the composition, origin, and microbiological quality of pollen stored by *M. scutellaris*. This is the first study that determines parameters of nutritional quality, microbiological, and botanical origin.

Therefore, this study aimed to analyze the botanical origin, and microbiological and physicochemical characteristics of *Melipona scutellaris* pot-pollen ("samburá") from Bahia region, Brazil.

Materials and methods

Sample collection

Sampling was carried monthly between August 2014 and August 2015 in four municipalities in the metropolitan region of Salvador, the capital city of Bahia state, Brazil, a region of the Atlantic forest with intense urban and industrial activities: A (Salvador/1, n = 5), B (Salvador/2, n = 7), C (Salvador/3, n = 9), D (Lauro de Freitas, n = 9), E (Simões Filho, n = 6) and F (Dias D'Ávila, n = 8) (Table 1). Every sampling month, 200 g of bee pollen was harvested from each melliponary. Samples of stored pollen (n = 44), known as "samburá", were obtained from six meliponaries. We used five colonies of *M. scutellaris* by meliponary (A-F), collected with disposable spatulas and nitrile gloves, placed in sterile plastic containers and properly identified, preserved in thermal bags with ice and sent to the laboratory for analysis. Upon receipt, none of the samples had signs of any visible contamination namely fermentation, spoilage or field residues.

Pollen analysis

To verify the botanical origin of stored pollen samples (n = 44), the samples were prepared according to the methods established by Jones and Bryant (2004). After samples were subsequently submitted to the acetolysis process according to Erdtman (1960) and up to 1000 pollen grains were consecutively counted per sample using optical microscopy as recommended by Louveaux et al. (1978). The pollen types were identified using a reference pollen collection and the specialized literature (Punt et al., 2007; Roubik & Moreno, 1991). Pollen types were categorized according to frequency class: predominant pollen - PP (>45% of total grains), secondary pollen - SP (16 to 45%), important minor pollen - IMP (3 to 15%) and minor pollen - MP (<3%) (Louveaux et al., 1978).

Microbiological determination

To evaluate the microbiological quality of the pollen sampled from beehives *M. scutellaris,* we checked for the presence of molds and yeasts, mesophilic, and psychrotrophic aerobic bacteria, *Bacillus* spp., total coliforms, *Escherichia coli, Staphylococcus aureus,* sulfite-reducing *Clostridium* spores, and *Salmonella* spp. All assays were performed in triplicate. The samples (n = 44) were prepared and analyzed according to the methodology described by the Association of Official Analytical Chemists (AOAC, 1989; Association of Official Analytical Chemists – 2005.03 (AOAC), 2005), (International Organization for Standardization (ISO), 2003) and Silva et al. (2010). Counts were expressed in colony forming units per gram (CFU/g) (International Organization for Standardization (ISO), 2006).

Sample preparation

Samples were prepared according to the American Public Health Association (APHA) method described in the international standards (Downes & Ito, 2001). Thus, 25 g of each sample was used to prepare the first dilution (10^{-1}) in 225 mL of 0.1%, buffered Peptone water (H_2Op), and the subsequent decimal dilutions (10^{-2} to 10^{-3}) were prepared in 9 ml aliquots of the same diluent.

Counting microorganisms

Growth and quantification of mesophilic and psychrotrophic aerobic bacteria were performed using standard plates count agar (PCA) (Himedia®). Plates were incubated at 37 °C for 48 hours for counting aerobic mesophilic bacteria and incubated at 7 °C for 10 days for counting aerobic psychrotrophic bacteria. Growth and counts of *Bacillus* spp. were performed using nutrient agar medium (Himedia®) with sodium chloride 1.5% and incubated at 30 °C for 48 h. To allow the growth and counts of molds and yeasts, we used the medium DG18 (Biolog) and samples were incubated at 25 °C for five days. We followed the manufacturer's instructions of CEC-CI SimPlate (BioControl® System, Bellevue, Washington, USA) to count total coliforms and *Escherichia coli*, according to AOAC (2005).

Table 2. Pollen types identified in the sample set of *Melipona scutellaris* pot-pollen ("samburá") from Bahia, Brazil.

Family	Pollen Type	Family	Pollen Type
Arecaceae	*Cocos nucifera*	Fabaceae	Fabaceae type
Anacardiaceae	Anacardiaceae type	Fabaceae	*Piptadenia*
Anacardiaceae	*Spondias*	Fabaceae	*Senna*
Anacardiaceae	*Tapirira*	Fabaceae	*Senna spectabilis*
Arecaceae	Arecaceae type	Gentianaceae	*Coutoubea*
Asteraceae	Asteraceae type	Lauraceae	*Persea americana*
Asteraceae	*Bidens*	Loranthaceae	*Struthanthus*
Asteraceae	*Vernonia*	Malpighiaceae	*Byrsonima*
Burseraceae	*Protium*	Malvaceae	Malvaceae type
Combretaceae	Combretaceae type	Melastomataceae	*Miconia*
Combretaceae	*Terminalia*	Melastomataceae	*Tibouchina*
Erythroxylaceae	*Erythroxylum*	Moraceae	Moraceae type
Euphorbiaceae	*Acalypha*	Moraceae	*Morus*
Euphorbiaceae	*Croton*	Myrtaceae	*Eucalyptus*
Euphorbiaceae	Euphorbiaceae type	Myrtaceae	*Eugenia*
Fabaceae	*Acacia*	Myrtaceae	*Myrcia*
Fabaceae	*Caesalpinia*	Myrtaceae	*Psidium*
Fabaceae	*Centrosema*	Rubiaceae	*Borreria*
Fabaceae	*Chamaecrista*	Rubiaceae	Rubiaceae type
Fabaceae	*Desmodium*	Sapindaceae	*Cupania*
Fabaceae	*Leucaena*	Sapindaceae	*Serjania*
Fabaceae	*Macroptilium*	Solanaceae	*Solanum*
Fabaceae	*Mimosa caesalpiniifolia*	Solanaceae	*Solanum paniculatum*
Fabaceae	*Mimosa pudica*	Urticaceae	*Cecropia*
Fabaceae	*Mimosa quadrivalvis*	Verbenaceae	*Lantana*
Fabaceae	*Mimosa tenuiflora*	Verbenaceae	*Lippia*

Sulfite-reducing *Clostridium* spores were detected and counted according to ISO (2003). The initial sample suspension (1 mL, 5 mL, and 10 mL) was added to pre-sterile test tubes (triplicate). The tubes were submerged in a water bath at 80 °C for 15 minutes to inactivate the sample. Subsequently, aliquots of 0.01 mL, 0.1 mL, and 1 mL of each sample were inoculated into Petri dishes, which were then topped with iron sulfite agar medium. The plates were packed in anaerobic jars and incubated at 37 °C ± 1 °C for 24 to 48 hours.

Search for Staphylococcus ssp.

Aliquots (0.1 mL) of each dilution were removed and inoculated on the surface of Petri dishes containing Baird-Parker medium (Himedia®) and the plates were incubated at 37 °C for 24 hours (Silva et al., 2010).

Search for Salmonella ssp.

The search for *Salmonella* spp. was carried out using immunodiffusion 1–2- test® following the manufacturer's recommendations (AOAC, 1989).

Physicochemical characterization

For the physicochemical characterization of *M. scutellaris* pollen samples (n = 44), the following parameters were evaluated: moisture content (AOAC, 1995), ash content, total lipids according to the Instituto Adolfo Lutz (IAL, 2008), protein content by the Kjeldahl method (Almeida-Muradian et al., 2012; AOAC, 1995; Kjeldahl, 1883), pH, total acidity (Association of Official Analytical Chemists (AOAC), 1990), total carbohydrates, and total energy value (Lima et al., 2011). All analyses were performed in triplicates.

Determination of fatty acids profile

To determine the fatty acid profile, 44 "samburá" samples were used, and the analysis was performed from a sample composed of a sampling site (A-F). The fatty acid profile of samples was determined by flame ionization (GC-FID)/capillary column detection, according to the method described by Human and Nicolson (2006). A soxhlet automatic device (FOSS, Soxtec ™ 2050, Höganäs, Sweden) was used for crude fat (CF) extraction (Association of Official Analytical Chemists (AOAC), 1995).

We used a Büchi fat determination system (Association of Official Analytical Chemists (AOAC), 2000). The extract was separated by gas chromatography using hydrogen as the transporter. Quantification of fatty acids was performed by the response factor using the mixture of fatty acids 37 (Supelco TM 37 Component FAME Mix) as standard. The levels of saturated, mono, and polyunsaturated fatty acids (SUFA, MUFA, and PUFA, respectively) and the content of each fatty acid were calculated automatically by the specific software Soxtec ™ 2050 Automatic System, using a pre-established factor. Three independent replicates were performed, and results were presented as mean and standard deviation.

Table 3. Frequency of predominant pollen (PP) and secondary pollen (SP) of the *Melipona scutellaris* pot-pollen ("samburá") from Bahia, Brazil.

Site	NPT	Pollen type (PP and SP)	Frequency class	FRS (%)
A (n= 5)	23	*Mimosa caesalpiniifolia* (Fabaceae)	PP	100
		Mimosa tenuiflora (Fabaceae)	PP	50
B (n= 7)	30	*Mimosa caesalpiniifolia* (Fabaceae)	PP	100
		Mimosa tenuiflora (Fabaceae)	SP	57
		Psidium (Myrtaceae)	SP	57
		Myrcia (Myrtaceae)	PP	43
		Mimosa quadrivalvis (Fabaceae)	SP	14
C (n= 9)	33	*Myrcia* (Myrtaceae)	SP	100
		Euphorbiaceae type (Euphorbiaceae)	SP	89
		Mimosa caesalpiniifolia (Fabaceae)	PP	89
		Tibouchina (Melastomataceae)	SP	89
		Cocos nucifera (Arecaceae)	SP	33
D (n= 9)	36	*Mimosa caesalpiniifolia* (Fabaceae)	PP	100
		Tibouchina (Melastomataceae)	SP	89
		Tapirira (Anacardiaceae)	PP	67
		Eugenia (Myrtaceae)	SP	44
		Psidium (Myrtaceae)	PP	44
		Solanum (Solanaceae)	SP	44
		Myrcia (Myrtaceae)	SP	33
		Cupania (Sapindaceae)	SP	22
E (n= 6)	26	*Mimosa caesalpiniifolia* (Fabaceae)	PP	100
		Myrcia (Myrtaceae)	SP	100
		Tapirira (Anacardiaceae)	SP	100
		Byrsonima (Malpighiaceae)	PP	33
F (n= 8)	30	*Miconia* (Melastomataceae)	SP	88
		Mimosa caesalpiniifolia (Fabaceae)	PP	88
		Myrcia (Myrtaceae)	SP	88
		Tibouchina (Melastomataceae)	SP	88
		Terminalia (Combretaceae)	SP	75
		Mimosa tenuiflora (Fabaceae)	SP	38
		Psidium (Myrtaceae)	SP	38

*NPT: Number of pollen types, Predominant pollen: > 45% of total pollen grain and Secondary pollen: 16–45% of total pollen grain. FRS: Relative frequency sample; Sites A, B and C (samples from Salvador 1–3), D (samples from Simões Filho), E (samples from Lauro de Freitas) and F (samples from Dias D'Ávila), as described in Table 1.

Statistical analyses

Mean, medians, and standard deviations were calculated using the physicochemical data were calculated. For the analysis of the botanical origin, we considered the predominant pollen (PP = >45% of total grains) and secondary pollen (SP = 16 to 45%) types. All variables were tested for normal distribution using the Shapiro-Wilk test. Likewise, the Levene test was applied to verify the homogeneity of variances. To assess significant differences between pollen collection sites, the one-way analysis of variance (ANOVA) was used. The Tukey test was performed as a post-hoc test when the effect was significant. Moreover, we used the canonical discriminant analysis (CDA) to verify the relationship between fatty acids and pollen collection sites. CDA is a dimension-reduction technique that explores linear combinations that provide maximum discrimination to the mean vectors of the treatment groups (Friendly, 2012). All 12 fatty acids were included in the CDA. The biplot was constructed for the first two canonical variables (Can1 and Can2), along with the 95% confidence ellipses, for each site. For all analyses, we adopted a significance level of 5% ($p < 0.05$). For the statistical analysis, the software "R" statistical and programming environment version 3.4.4 (R Development Core Team, 2018) was used.

Results

Pollen analyses

All 44 "samburá" samples collected at the six sites in an Atlantic rainforest region with intense urban and industrial activities revealed that *M. scutellaris* visited a wide diversity of botanical species. In the pollen spectrum of the sample, 52 pollen types were identified distributed into 21 families showing the heterofloral characteristic of "samburá" samples (Table 2).

We identified 15 pollen types as predominant (PP = >45% of total grains) and as secondary pollen (SP = 16 to 45% of total grains), representing 12 botanical families, with Fabaceae, Myrtaceae, and Anacardiaceae showing the greatest richness of pollen types (Table 3). *Mimosa caesalpiniifolia* pollen type was present at high frequency in all samples studied and was therefore characterized as PP (>45% of total pollen grain).

Table 4. Microbiological quality of the *Melipona scutellaris* pot-pollen ("samburá") from Bahia, Brazil.

	Sites (Means±SD)					
	A (n = 5)	B (n = 7)	C (n = 9)	D (n = 9)	E (n = 6)	F (n = 8)
M.A.*	$2.2 \times 10^3 \pm 1.0$	$17.0 \times 10 \pm 3.0$	$15.8 \times 10 \pm 21{,}9$	<10	<10	$20.9 \times 10 \pm 5.6$
P.A.	<10	<10	<10	<10	<10	<10
Bacillus spp.	<10	<10	<10	<10	<10	<10
M.Y. (CFU/g)	<10	<10	<10	<10	<10	<10
F coliformes	<1	<1	<1	<1	<1	<1
Escherichia coli	<1	<1	<1	<1	<1	<1
S. aureus	<10	<10	<10	<10	<10	<10
S.R.C.	<10	<10	<10	<10	<10	<10
Salmonella spp.	A	A	A	A	A	A

*A: absent; CFU/g: colony forming unit/gram; Site A, B and C (pollen samples from Salvador 1–3), D (pollen samples from Simões Filho), E (pollen samples from Lauro de Freitas) and F (pollen samples from Dias D'Ávila); M.A: Mesophilic aerobes (CFU/g); P.A.: Psychotropic aerobes (CFU/g); M.Y.: molds and yeasts (CFU/g); F coliforms: fecal coliforms; S.R.C.: sulfite-reducing clostridial spores in 0.01 g. SD: Standard deviation.

Microbiological quality

We did not detect the presence of *Salmonella* spp., spores of reducing sulfite clostridium, total coliforms, *Escherichia coli*, *Staphylococcus aureus*, aerobic psychrotrophic bacteria, *Bacillus* sp., molds, and yeasts (Table 4). However, we found mesophilic aerobic bacteria in 32% of the samples, with the highest average value (2.2×10^3 CFU/g) observed for the meliponary A (Salvador/1, environment with anthropic influence).

Physicochemical characteristics

The physicochemical parameters of the pollen stored by *M. scutellaris* are presented in Table 5. Data shows that the parameters did not differ significantly among all samples collected in six sites in a region of the Atlantic rainforest, but with intense urban and industrial activities ($p > 0.05$).

Fatty acid profile

We identified 12 fatty acids, nine saturated, and three unsaturated. The most abundant among the saturated fatty acids (SFA) was capric acid (C10: 0), ranging from 1.89 to 5.66 g/100 g of pollen. Oleic acid (C18: 1) was the most common monounsaturated fatty acid (MUFA). The most abundant polyunsaturated fatty acids (PUFAs) were the linoleic/omega-6 (C18: 2Δ6; 0.50 to 1.63 g/100 g) and linolenic acid/omega-3 (C18: 3Δ3; 0.30 to 0.86 g/100 g). There were significant differences ($p < 0.05$) in pollen fatty acid contents in terms of collection sites within the urban-industrial area addressed in this study (Figure 1).

Thus, we considered the significant fatty acid compositions indicated by ANOVA. The CDA and the Wilks Lambda multivariate statistical test revealed a significant difference regarding fatty acid composition between sites of sample collection ($p < 0.05$). Figure 2A shows the biplot of the two canonical discriminant functions revealing a clear separation between fatty acid pollen composition from different sites. The first canonical discriminant function (Can1) explained 35.3% of the total variance and the second canonical discriminant function (Can2) explained 30.3%. The first two canonical discriminant functions explained 65.9% of the total data variation, which may be considered a high percentage.

The pollen of D (samples from Simões Filho) and F sites (samples from Dias D'Ávila) were different from the other sites (Can1), presenting higher values for linoleic acid (C18: 2Δ6), linolenic acid (C18: 3Δ3), palmitic acid (C16: 0), stearic acid (C18: 0) and capric acid (C10: 0). These fatty acids contributed more to Can1 formation and were more important to discriminate pollen samples between the collection sites (Figure 2B). Sites B and C (pollen samples from different sites in Salvador) did not contribute to CDA since they are isolated from fatty acid composition vectors in the biplot, presenting negative values for C4: 0, C6: 0, C8: 0, C14: 0 and C20: 0. Using Can2, pollen of site E (samples from Lauro de Freitas) differs from sites B and C (pollen samples from different sites in Salvador) in terms of oleic acid (C18: 1). The ANOVA analysis confirmed the difference observed.

Discussion

Pollen analyses

The Fabaceae species, specifically *Mimosa* genus, have morphological features such as dense inflorescences with small and gently scented flowers that attract many species of stingless bees to collect trophic resources, such as pollen and nectar, highlighting the importance of this botanic family (Carneiro-Neto et al., 2017; Maia-Silva et al., 2012). In bee pollen samples evaluated by Almeida-Muradian et al. (2005) and Estevinho et al. (2012) pollen types of Fabaceae were also identified. In addition, the study by Di Marco et al. (2017) confirms the importance of species of this botanical family also as an important source of nectar for bees.

Mimosa caesalpiniifolia Benth. is a native botanical species of the Atlantic forest and is widely used as hedgerows (Santos et al., 2011; Souchie et al., 2005).

Few studies evaluate pollen stored by *M. scutellaris*. Alves et al. (2018a) sampled bee-derived products in an

Table 5. Physicochemical parameters of the *Melipona scutellaris* pot-pollen ("samburá") from Bahia, Brazil.

	Min.	Max.	Median	CI	Mean ± SD	SEM
Site A						
Moisture (%)	49.38	53.65	53.29	[50.33;54.80]	52.56 ± 1.80	0.80
Ash (%)	4.55	5.90	4.90	[4.34;5.66]	5.00 ± 0.54	0.24
Protein (%)	13.13	20.81	13.95	[11.46;20.89]	16.18 ± 3.80	1.70
pH	3.69	3.91	3.81	[3.68;3.90]	3.79 ± 0.09	0.04
T.A. (meq/kg)	237.20	474.20	448.20	[276.90;519.40]	398.18 ± 97.65	43.67
T.L. (%)	3.02	5.73	4.63	[3.16;5.72]	4.44 ± 1.03	0.46
T.C. (%)	12.48	28.19	21.88	[11.35;28.39]	19.87 ± 6.86	3.07
TEV (kcal/100g)	173.62	200.39	189.25	[176.30;200.00]	188.17 ± 9.56	4.27
Site B						
Moisture (%)	52.50	55.41	53.98	[53.09;54.94]	54.01 ± 1.00	0.38
Ash (%)	3.45	5.25	4.50	[3.99;5.01]	4.50 ± 0.55	0.21
Protein (%)	15.23	20.15	18.03	[16.47;19.53]	18.00 ± 1.66	0.63
pH	3.58	3.86	3.64	[3.59;3.77]	3.68 ± 0.10	0.04
T.A. (meq/kg)	370.80	525.40	487.80	[422.30;524.70]	473.49 ± 55.39	20.93
T.L. (%)	3.01	7.05	5.23	[3.61;6.55]	5.08 ± 1.59	0.60
T.C. (%)	12.48	21.61	13.74	[11.76;17.92]	14.84 ± 3.33	1.26
TEV (kcal/100g)	170.60	198.19	191.88	[178.50;196.50]	187.49 ± 9.69	3.66
Site C						
Moisture (%)	50.57	54.57	52.86	[52.10;54.00]	53.05 ± 1.24	0.41
Ash (%)	4.50	5.40	4.95	[4.73;5.15]	4.94 ± 0.27	0.09
Protein (%)	13.40	24.02	17.01	[15.13;20.97]	18.05 ± 3.80	1.27
pH	3.44	3.90	3.73	[3.60;3.83]	3.72 ± 0.15	0.05
T.A. (meq/kg)	433.00	512.40	460.80	[448.50;494.30]	471.43 ± 29.81	9.94
T.L. (%)	4.50	6.05	5.43	[5.02;5.74]	5.38 ± 0.47	0.16
T.C. (%)	10.85	24.00	16.44	[14.20;20.09]	17.15 ± 3.83	1.28
TEV (kcal/100g)	183.36	202.89	191.53	[186.90;196.00]	191.49 ± 5.91	1.97
Site D						
Moisture (%)	48.38	54.33	52.65	[50.86;53.91]	52.24 ± 1.79	0.60
Ash (%)	3.60	5.80	5.20	[4.40;5.56]	4.98 ± 0.76	0.25
Protein (%)	11.66	21.44	18.08	[14.94;19.60]	17.27 ± 3.03	1.01
pH	3.64	3.99	3.83	[3.72;3.91]	3.81 ± 0.13	0.04
T.A. (meq/kg)	342.00	51.40	450.60	[408.00;482.10]	445.08 ± 48.23	16.08
T.L. (%)	2.43	7.47	4.73	[3.60;5.80]	4.70 ± 1.43	0.48
T.C. (%)	15.33	28.19	17.01	[15.76;22.31]	19.04 ± 4.26	1.42
TEV (kcal/100g)	185.50	210.65	187.02	[184.70;198.60]	191.67 ± 9.05	3.02
Site E						
Moisture (%)	47.03	54.81	51.98	[48.13;54.58]	51.35 ± 3.07	1.25
Ash (%)	3.60	5.20	4.32	[3.72;5.06]	4.39 ± 0.64	0.26
Protein (%)	10.19	20.97	15.31	[11.36;20.42]	15.89 ± 4.32	1.76
pH	3.28	3.83	3.67	[3.39;3.83]	3.61 ± 0.21	0.08
T.A. (meq/kg)	342.70	513.80	435.65	[369.40;489.10]	429.25 ± 57.00	23.27
T.L. (%)	4.80	7.50	5.73	[5.03;6.90]	5.96 ± 0.89	0.36
T.C. (%)	12.03	28.89	20.55	[13.61;28.08]	20.85 ± 6.90	2.81
TEV (kcal/100g)	191.39	216.99	203.28	[192.60;214.30]	203.43 ± 10.36	4.23
Moisture (%)	51.59	55.70	53.35	[52.56;55.11]	53.83 ± 1.53	0.54
Ash (%)	3.55	5.70	4.50	[3.91;5.00]	4.46 ± 0.65	0.23
Protein (%)	11.76	22.95	17.46	[13.46;20.03]	16.75 ± 3.93	1.39
pH	3.52	3.93	3.74	[3.60;3.81]	3.70 ± 0.13	0.04
T.A. (meq/kg)	351.60	557.10	491.50	[423.00;527.00]	475.03 ± 62.19	21.99
T.L. (%)	4.77	7.94	5.50	[5.02;6.65]	5.84 ± 0.98	0.35
T.C. (%)	11.63	25.80	16.88	[13.33;21.54]	17.43 ± 4.91	1.74
TEV (kcal/100g)	181.35	205.84	191.00	[183.60;198.80]	191.19 ± 9.11	3.22
Global						
Moisture (%)	47.03	55.70	53.23	[52.31;53.47]	52.89 ± 1.90	0.29
Ash (%)	3.45	5.90	4.70	[4.54;4.91]	4.72 ± 0.61	0.09
Protein (%)	10.19	24.02	17.69	[16.11;18.17]	17.14 ± 3.38	0.51
pH	3.28	3.99	3.73	[3.68;3.77]	3.72 ± 0.15	0.02
T.A. (meq/kg)	237.20	557.10	456.50	[434.70;471.20]	452.95 ± 59.93	9.03
T.L. (%)	2.43	7.94	5.31	[4.89;5.61]	5.25 ± 1.18	0.18
T.C. (%)	10.85	28.89	16.82	[16.51;19.56]	18.03 ± 5.01	0.76
TEV (kcal/100g)	170.60	216.99	191.46	[189.20;195.00]	192.08 ± 9.83	1.45

(*Continued.*)

Table 5. Continued.

Legislation	Brazil	Argent.	France	Alves et al. (2018a)		
Moisture (%)	Max.30	Max. 8	Max. 6	44.71 ± 9.83	–	–
Ash (%)	Max. 4	Max. 4	2 à 6	1.84 ± 0.12	–	–
Protein (%)	Min. 8	15 à 28	10 à 41	23.88 ± 0.10	–	–
pH	4 à 6	4 à 6	–	3.75 ± 0.005	–	–
T.A. (meq/kg)	Max.300	–	–	150.57 ± 0.40	–	–
T.L. (%)	Min. 1.8	–	1 à 10	4.25 ± 0.10	–	–
T.C. (%)	–	–	–	24.48 ± 10.10	–	–
TEV (kcal/100g)	–	–	–	231.33 ± 37.83	–	–

*T.A.: total acidity; T.L.: total lipids; T.C.: total carbohydrates; TEV: total energy value; Max: maximum; Min: minimum; CI: 95% confidence interval; SD: standard deviation; SEM: standard error of the means.

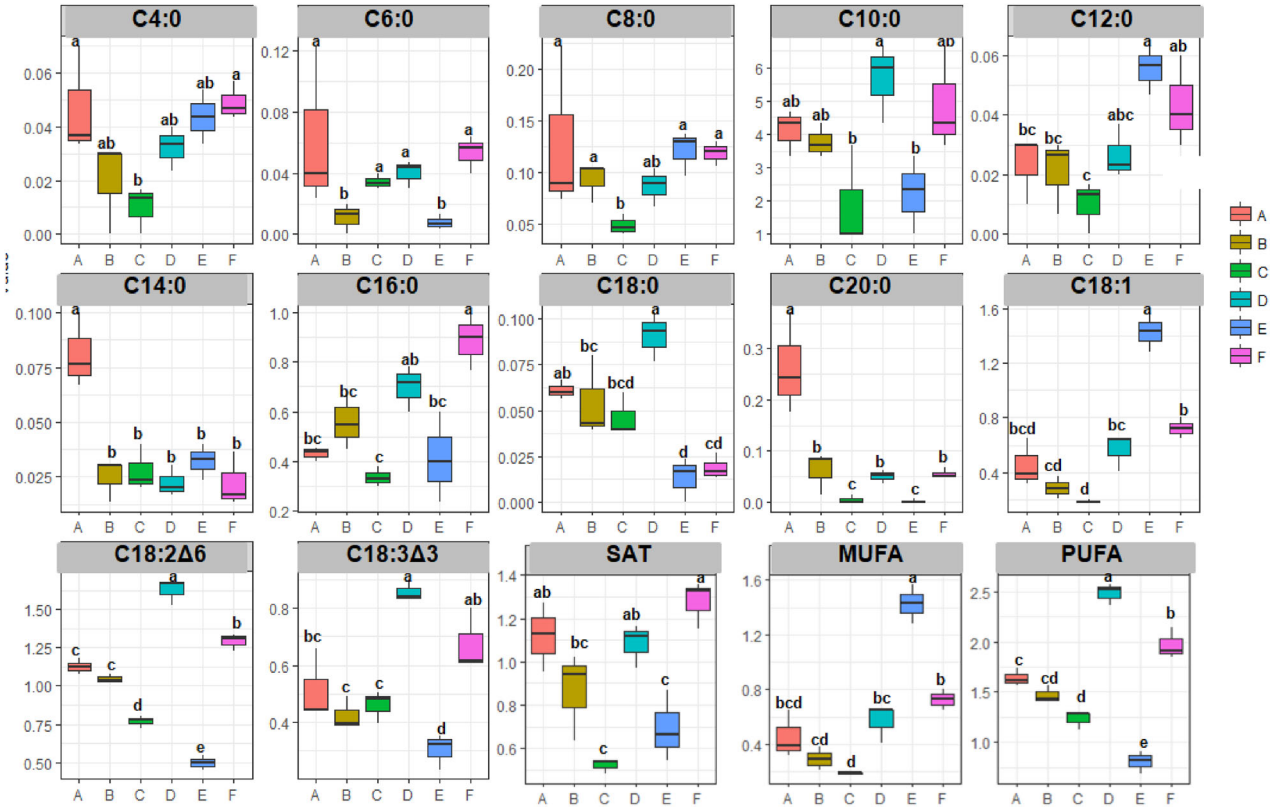

Figure 1. Fatty acid profile (g/100g) of the *Melipona scutellaris* pot-pollen (Samburá) from Bahia, Brazil. A, B, C (pollen samples from Salvador), D (pollen samples from Simões Filho), E (pollen samples from Lauro de Freitas) and F (pollen samples from Dias D'Ávila). For each fatty acid, different lowercase letters (ae) indicate significant differences ($p < 0.05$). Butyric acid (C4: 0); caproic acid (C6: 0); caprylic acid (C8: 0); capric acid (C10: 0); lauric acid (C12: 0); myristoleic acid (C14: 0); palmitic acid (C16: 0); stearic acid (C18: 0); arachidic acid (C20: 0); oleic acid (C18: 1); α-linolenic acid (C18: 3Δ3); linoleic acid (C18: 2Δ6); SAT: total saturated fatty acid; MUFA: total of monounsaturated fatty acid; PUFA: total of polyunsaturated fatty acid.

anthropic area close to sites (Table 1, Sites D and F) studied here and found seven pollen types in common with our samples *Eugenia*, *Miconia*, *M. caesalpiniifolia*, *Myrcia*, *Psidium*, *Solanum* and *Tapirira*. Oliveira et al. (2017) also studied an anthropic area (with transitional vegetation of a seasonal forest/Caatinga) and found three pollen types in common to those reported here, that is, *M. caesalpiniifolia, Psidium* and *Solanum*. The species *Byrsonima, Eugenia, Miconia, Mimosa tenuiflora, Myrcia, Psidium,* and *Solanum* were also found in pollen loads of *M. scutellaris* corbiculae in colonies established in a coffee plantation (Lucas et al., 2018). Previous studies on different products (geopropolis and honey) reported the presence of *M. caesalpiniifolia* pollen type, proving to be a source of the trophic resource of apicultural potential (Barros et al., 2013; Nascimento et al., 2015).

Similar pollen types were also found in studies on other *Melipona* species. Luz et al. (2011) studied *Melipona capixaba* and reported six pollen types (*Cupania*, *Eugenia*, *M. caesalpiniifolia*, *Myrcia*, *Solanum* and *Tapirira*) in common with our work. Pollen from *M. caesalpiniifolia* and *M. tenuiflora* were also found in samples of *Melipona mandacaia* (Carneiro-Neto et al., 2017).

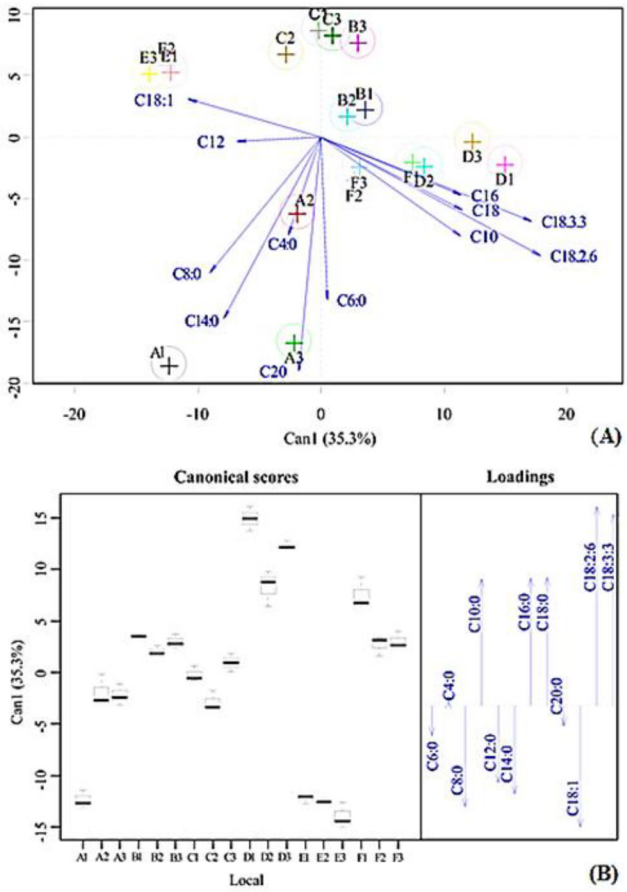

Figure 2. Canonical discriminant analysis of the relationship between the fatty acid composition and the different sites of an urbanized area where pot-pollen (samburá) by *Melipona scutellaris* was collected. **(A)** Biplot of the canonical variables (Can1, Can2). Butyric acid (C4: 0); caproic acid (C6: 0); caprylic acid (C8: 0); capric acid (C10: 0); lauric acid (C12: 0); myristoleic acid (C14: 0); palmitic acid (C16: 0); stearic acid (C18: 0); arachidic acid (C20: 0); oleic acid (C18: 1); α-linolenic acid (C18: 3Δ3); linoleic acid (C18: 2Δ6). **(B)** Boxplot of the scores of the canonical discriminant variable representing the origin of the pollen. Sites A, B and C (pollen samples from Salvador 1–3), D (pollen samples from Simões Filho), E (pollen samples from Lauro de Freitas) and F (pollen samples from Dias D'Ávila).

Thus, for *M. scutellaris*, the data available reveal pollen from *Psidium* and *Solanum* in all studies, while *M. caesalpiniifolia*, *Eugenia, Miconia,* and *Myrcia* are found in most, but not all samples. However, of all pollen types, *M. caesalpiniifolia* stands out, since pollen from this botanical species was found as PP in all samples of all six sites investigated in this study as well as in samples of other two different *Melipona* species. The pollen types found in the "samburá" samples suggest that these botanical species are a supply of protein resource for feeding and maintaining colonies of this genus of stingless bees.

The pollen spectrum of bee pollen samples from a region composed primarily of a range of tropical forest with mangroves evaluated by Alves and Santos (2014) revealed similar preferences of these social bees (*A. mellifera* and *M. scutellaris*) in the search of protein resources, being that pollen types *Cocos nucifera*, *Myrcia* and representatives of the *Mimosa* genus were the most frequent. In our study, these pollen types contributed to the composition of the samburá pollen spectrum (Tables 2 and 3).

Microbiological quality

Pollen is consumed *in natura* and, despite its benefits to human health, its consumption requires caution since it is susceptible to environmental contaminants and growth of microorganisms. Thus, safety is a relevant aspect to be considered in this food. This result was higher than that found by Alves et al. (2018a) when analyzing pollen from *M. scutellaris* from an Atlantic forest region and similar to those of Santa-Bárbara et al. (2015) studying pollen of *M. mandacaia* Smith, 1983.

Possibly, different sources of contamination available at the site and the management of the colonies favored contamination in samples of meliponary A (Salvador/1, environment with anthropic influence), since the occurrence of mesophilic bacteria is related to the conditions of food processing and storage (De-Melo et al., 2016; Feás et al., 2012). Brazilian legislation does not regulate the hygienic parameters for bee pollen (Brasil, 2001). However, the samples analyzed possibly do not pose a risk to human consumption, since the levels of mesophilic aerobic bacteria are below the recommended level indicated for food

safety by the Argentinian Food Code (below 1.5×10^5 CFU/g) (De Arruda et al., 2017).

Physicochemical characteristics

Pollen stored by stingless bees is agglomerated by the addition of saliva and honey, thereby acquiring pasty consistency and it is stored in wax pots that favor the maintenance of moisture (Alves et al., 2018a). Rebelo et al. (2016) found a mean value for the moisture of 53.39 ± 0.50% for *M. seminigra* Cockerell, 1919, similar to the result found in our study. Zuluaga et al. (2015) in a study of the physicochemical composition of bee bread (*A. mellifera*) recorded an average value of 15.6 ± 3.60% for this parameter. These results demonstrate that fermented pollen collected by stingless bees presents higher moisture when compared to bee bread. This difference is possibly related to environmental conditions of the site where the samples were collected since pollen can absorb the moisture of the environment, as it is a hygroscopic product (Marchini et al., 2006). High amounts of moisture contribute to pollen fermentation, a peculiar characteristic of "samburá" of stingless bees (Rebelo et al., 2016).

The pH values were similar to those observed by Alves et al. (2018a). Lower pH values were observed in pollen from *Melipona* (Santa-Bárbara et al., 2015) and *A. mellifera* (De-Melo et al., 2016). The range found for total acidity (237.20 to 557.10 meq/kg) was very different from that found in other studies with *M. scutellaris* and other Meliponini species (Almeida-Anacleto et al., 2009; Alves et al., 2018a). The lower pH and high acidity values found in this work may be related to the chemical reactions that occur during the pollen fermentation process, promoted by microorganisms (Ellis & Hayes, 2009; Kalaycıoğlu et al., 2017). Moreover, the botanical origin of this product may contribute to these results (Alves et al., 2018a).

The ash content of the samples evaluated presented an average value of 4.72%, which was higher than the ash content values reported in other studies (Alves et al., 2018a; Rebelo et al., 2016; Santa-Bárbara et al., 2015). The ash content expresses the minerals in pollen (Abadio Finco et al., 2010; Pita-Calvo & Vázquez, 2017) and ash values above the threshold established in Brasil (2001) may also be associated to inorganic contaminants in the environment (Kostić et al., 2015), which may show that our samples came from an urban and industrial area. Moreover, the relatively high ash content in the samples may be attributed to the different geographical conditions where the hives were located (Abadio Finco et al., 2010; Pita-Calvo & Vázquez, 2017).

The ash content is an important parameter, reflecting the abundance of minerals in the bee pollen sample, which indicates the need for monitoring the content of potentially toxic metals that may compromise the quality of this beehive product. Indeed, the presence of metals was verified in both honey and pollen in urban environments (Nascimento et al., 2018; Porrini et al., 2003).

The values of protein content in this work show an average of 17.14%, lower than that found by Alves et al. (2018a) (23.88%). In other studies on *Melipona,* Santa-Bárbara et al. (2015) and Rebelo et al. (2016) obtained higher values (21.00% and 24.00%), respectively. These differences may be related to different compositions of flora and edaphic conditions.

Lipids, such as fatty acids, sterols, and triglycerides, are of great importance in the food industry as they represent energy source in the diet and, consequently, directly affect the food nutritional values. Variation in the lipid content in pollen samples studied here (2.43 to 7.94%) was close to that obtained by Alves et al. (2018a) (3.97% to 4.46%). However, Souza et al. (2004) studied pollen stored by stingless bees from the Amazonian region *Melipona compressipes* Smith, 1854, *M. rufiventris* Lepeletier 1836, and *M. seminigra* Cockerell, 1919, and reported a large variation in lipid content (1.9 to 9.3%). This wide variation may be due to plant species available at the sites (Yang et al., 2013), which is likely to be more diverse than that found in the areas where our samples were collected due to the effect of urbanization and industrialization in these areas and, hence, the narrower variation observed here.

We observed that, although the pollen evaluated was obtained from an urban area (Table 1) with vegetation stratification, the results obtained for lipids and fatty acids in our study are similar to those recorded by other authors for *Melipona* (Alves et al., 2018a; Santa-Bárbara et al., 2015) and *A. mellifera* (Araújo et al., 2017), signaling the nutritional potential of pollen stored by *M. scutellaris*. As indicated by the bee behavior already described in literature that refers to the search of trophic resources in plant species with higher nutritional values (Filipiak et al., 2017; Newstrom et al., 1994; Silva et al., 2007).

Carbohydrates are important indicators of nutritional value and energy content, corresponding to sugars, starch, and fibers, and can comprise 2/3 of the total pollen constituents (Carpes et al., 2009). The mean value for total pollen carbohydrates of *M. scutellaris* was 18.03%, similar to that found in other studies on pollen of *M. scutellaris*, *M. seminigra* and *M. interrupta* Latreille, 1811 (Alves et al., 2018a; Rebelo et al., 2016). High values of carbohydrates in pollen are attributed to the presence of honey or nectar used by bees to compact the grains (Kostić et al., 2015).

The energy value is directly associated with the number of macronutrients accumulated in a given food (Souza et al., 2004). In this sense, the value obtained for total energy value (TEV) (192.08 ± 9.83 kcal/100g) in the pollen analyzed here indicates that this beehive product has the potential to be used as a food supplement because it is a source of macronutrients (carbohydrates, proteins and lipids) for humans. These results also

suggest that the flora used by *M. scutellaris* in urban areas has the essential nutritional and energetic requirements to ensure the health of these pollinators. TEV of the samples studied was lower than that found by Alves et al. (2018a) and Rebelo et al. (2016). For pollen *in natura* of *A. mellifera*, Barreto et al. (2012) found an average total energy value of 174.7 kcal/100g. This variation in TEV values reported by different studies may be related to the period of sample collection, bee species, edaphoclimatic conditions, as well as the geographic and botanical origin of stored pollen, which is an important factor considering that the composition of this product is directly related to its floral origin (Feás et al., 2012; Pascoal et al., 2014).

Possibly, the flora found at these collection sites does not change very much; however, a specific study is required to investigate that and it is beyond the scope of this study.

Fatty acid profile

Our study is the first to investigate the composition of fatty acids in pollen stored by *M. scutellaris*. For bees, fatty acids play an essential role in reproduction, development, and growth of these insects (Mărgăoan et al., 2014). Some fatty acids, such as linoleic, linolenic, myristic, and lauric have bactericidal action and antifungal properties that are important for colony hygiene (Yang et al., 2013).

There is variability in fatty acids of pollen samples stored by bees. Other authors evaluated the dehydrated pollen of *M. mandacaia* and commercial pollen of *A. mellifera* in an agro-industrial area and identified linoleic acid (C18:2Δ6) as the most abundant among the PUFAs (Dong et al., 2016; Santa-Bárbara et al., 2015).

Considering that the pollen samples evaluated here were heterofloral, with the contribution of several plant species (with 52 pollen types identified), this variability in the number of fatty acids identified (n = 12) is probably related to the place of origin, urban environment and, consequently, anthropogenic influence. Due to the lower abundance of individuals of the same plant species in urban areas, bees seek their trophic resources in different plants. The botanical origin affects composition as well as chemical and sensorial characteristics of the stored pollen (Agostini & Sazima, 2003; Aleixo et al., 2013; Faria et al., 2012).

The polyunsaturated fatty acids (PUFAs) of major relevance in pollen are linolenic, linoleic, and oleic acids (Kaplan et al., 2016). These acids have anti-atherogenic and anti-thrombogenic effects (Garaffo et al., 2011) and play an important role in human metabolism, such as decreasing the levels of triacylglycerol, blood pressure, insulin resistance, and cardiovascular problems (Ghaeni et al., 2013). PUFAs cannot be synthesized by the human body and, therefore, consumption of bee pollen can be considered a source of these acids for human diet (Kaplan et al., 2016).

A nutritional source rich in fatty acids is essential for bees because they are important for the structural integrity and functioning of the membranes of these insects (Somerville, 2005). Particularly, fatty acids are very important for bees, being metabolized mainly during the larval stages and are seen as a relevant source of energy, as well as precursors of other biosynthesis (Cantrill et al., 1981). Thus, it is suggested that the heterofloral pollen collected by *M. scutellaris* in an urban environment meets the nutritional requirements of this bee's species.

The diversity of floral species of the region is the most influential factor for the quality and chemical composition of "samburá". Sites D (samples from Simões Filho) and F (samples from Dias D'Ávila) presented the highest diversity of pollen species, including Fabaceae, Myrtaceae, and Anacardiaceae.

Innovation and relevance

This study is the first to determine the profile of fatty acids in pollen stored by the stingless bee *M. scutellaris* collected in six sites located in an urban-industrial region that was once occupied by the Atlantic forest in Brazil. Our data showed the predominance of linoleic acid (omega-6) in the samples. This fatty acid is considered important in the anti-atherogenic effect. Physicochemical, nutritional, and microbiological results presented here suggest that pollen stored by *M. scutellaris* may be consumed without risks to human health, revealing it as a promising nutraceutical product. Our data might be of interest to Brazilian food safety agencies, as they develop regulations for microbiological and physicochemical quality criteria for pollen stored by bees such as *M. scutellaris*.

The findings presented here might facilitate the activity of rearing stingless bees, making meliponiculture a potentially sustainable activity. The presence of colonies of *M. scutellaris* in urban-industrial areas may contribute to the ecosystem service in maintaining biodiversity and preserving the species as well as providing an income for beekeepers of stingless bees.

Conclusions

The bee pollen produced by *M. scutellaris* in the urban-industrial area is heterofloral, and *M. caesalpiniifolia* is the main plant resource used by these bees. "Samburá" presented good microbiological conditions and physicochemical quality in addition to essential fatty acids, such as linoleic and linolenic acid, making it a nutraceutical product.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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