BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

ORIGINAL ARTICLE

Phenolic compound, nutritional and antioxidant profile of pollen collected by the genus *melipona* in North Eastern Brazil

Perfil fenólico, nutricional e antioxidante do pólen coletado pelo gênero melipona *no Nordeste do Brasil*

Rosane Gomes de Oliveira[1]*, Sona Jain[2], Lisiane dos Santos Freitas[3], Edilson Divino de Araújo[1]

[1]Universidade Federal de Sergipe (UFS), Departamento de Biologia, Laboratório de Genética e Conservação dos Recursos Naturais, São Cristóvão/SE, Brasil
[2]Universidade Federal de Sergipe (UFS), Departamento de Morfologia, Laboratório de Parasitologia, São Cristóvão/SE, Brasil
[3]Universidade Federal de Sergipe (UFS), Departamento de Química, Laboratório de Química Analítica, São Cristóvão/SE, Brasil

***Corresponding author:** Rosane Gomes de Oliveira, Universidade Federal de Sergipe (UFS), Departamento de Biologia, Laboratório de Genética e Conservação dos Recursos Naturais, Av. Marechal Rondon, s/n, Jd. Rosa Else, CEP: 49100-000, São Cristóvão/SE, Brasil, e-mail: rosanevely@gmail.com

Cite as: Oliveira, R. G., Jain, S., Freitas, L. S. & Araújo, E. D. (2019). Phenolic compound, nutritional and antioxidant profile of pollen collected by the genus *melipona* in North Eastern Brazil. *Brazilian Journal of Food Technology, 22*, e2018079. https://doi.org/10.1590/1981-6723.07918

Abstract

The pollen collected by eusocial bees is often reported as being healthy food due to its important nutritional and therapeutic properties. However, studies reporting such properties are rare, especially for pollen collected by the genus *Melipona* in northeastern Brazil, which is the focus of this research. Pollen from seven species of stingless bees was analysed for its nutritional composition (sugar, lipid, protein and amino acids). The phenolic compound profile was described based on fourteen phenolic compounds (apigenin, kaempferol, luteolin, naringin, rutin, gallic acid, ferulic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid, abscisic acid, protocatechuic acid, vanillic acid and *trans*-cinnamic acid). The antioxidant property was analysed by quantifying of total phenolic compounds, total flavonoids and DPPH. Chromatographic methods were used to identify and quantify the phenolic compounds and amino acids. The pollen samples from the bees under study showed good concentrations of proteins and amino acids and good antioxidant potential. The phenolic compounds luteolin, *trans*-cinnamic acid and apigenin were identified and described in pollen for the first time. Of the amino acids analysed, asparigine, glutamic acid, leucine and proline showed the highest concentrations. The research related to the theme showed that this is one of the first studies to identify and quantify the phenolic compounds and amino acids in stingless bee pollen, reflecting its importance in therapeutic use and as a food supplement.

Keywords: Bee pollen; Carbohydrate; Protein; Phenolic compounds; Amino acids; chromatography.

Resumo

O pólen coletado pelas abelhas eussociais é frequentemente relatado como um alimento saudável devido às suas propriedades nutricionais e terapêuticas importantes. Entretanto, estudos relatando tais propriedades são raros, especialmente para o pólen coletado pelo gênero *Melipona*, no Nordeste brasileiro, que é o foco desta pesquisa. O pólen de sete espécies de abelhas sem ferrão foi analisado quanto à composição nutricional (açúcar, lipídios, proteínas e aminoácidos). O perfil fenólico foi descrito utilizando 14 fenóis (apigenina, kaempferol, luteolina, narigerina, rutina, ácido gálico, ácido ferúlico, ácido cafeico, ácido *p*-cumárico, ácido clorogênico, ácido abscísico, ácido protocatecuico, ácido vanílico e ácido *trans*-cinâmico). A propriedade antioxidante foi analisada pela quantificação de fenóis totais, flavonoides totais e DPPH. Para a identificação e quantificação de compostos fenólicos e aminoácidos, foram utilizados métodos cromatográficos. As amostras de pólen das abelhas em estudo apresentaram boas concentrações de proteínas e aminoácidos, e potencial antioxidante. Os compostos fenólicos luteolina, ácido *trans*-cinâmico e apigenina foram identificados e descritos pela primeira vez em pólen. Entre os aminoácidos analisados, asparagina, ácido glutâmico, leucina e prolina apresentaram maior concentração. A busca por pesquisas relacionadas ao tema mostrou que este é um dos primeiros estudos com identificação e quantificação de compostos fenólicos e aminoácidos do pólen de abelhas sem ferrão, refletindo sua importância no uso terapêutico e como suplemento alimentar.

Palavras-chave: Pólen de abelha; Carboidrato; Proteína; Compostos fenólicos; Aminoácidos; Cromatografia.

1 Introduction

Pollen is collected by worker bees during foraging, and later mixed with nectar and substances released from their hypo-pharyngeal glands. Bees of the genus *Apis* store pollen (also known as bee pollen) in the brood cells, which serve as a source of food for all the individuals (Almeida-Muradian et al., 2005; Sattler et al., 2015). Several studies have reported the nutritional and chemical composition of pollen from bees of the genus *Apis*, characterizing the product as high in protein, minerals, carbohydrates, lipids, vitamins A, B, C, D and E, and also rich in phenolic compounds, flavonoids and phytosterols (Bogdanov, 2014). Thus bee pollen possesses several beneficial properties such as its antioxidant potential, and its anti-inflammatory, antibiotic, immunomodulator and antimutagenic properties (Graikou et al., 2011; Pascoal et al., 2014).

Pollen collected by species of the genus *Melipona* is deposited and stored in beehive pots where it undergoes fermentation, resulting in decreases in the pH and oxygen tension, after which it becomes known as Samburá or Sabura in Brazil (Freitas, 2003). The characterization of this type of pollen is difficult, since there are large numbers of native stingless bee species and huge variations in the pollen types (Silva et al., 2006; Bogdanov, 2014). Also, studies evaluating the chemical and nutritional composition of the pollen from these native bee species of Brazil are only incipient. The few manuscripts published have reported the sugar, lipid, moisture and hydroxymethylfurfural contents, the hydrogen ion potential and some other physicochemical characteristics (Alves et al., 2005; Silva et al., 2006), properties that assess the quality and shelf life of the product. However, studies that deal specifically with the identification of the phenolic compounds and amino acid are rare (Silva et al., 2006; Silva et al., 2013).

This study presents the phenolic compound profile, nutritional values (sugars, lipids, proteins and amino acids) and the antioxidant potential (total phenolic compounds, total flavonoids and DPPH) of twenty one pollen samples from seven different stingless bee species from north eastern Brazil, which can contribute to several subsequent studies searching for new functional and therapeutic food products. To the authors' knowledge, this is the first study that identifies and quantifies phenolic compounds and amino acids (essential and non-essential) in the pollen of bees of the genus *Melipona*.

2 Material and methods

2.1 Samples

Pollen samples from *Melipona quadrifasciata* Lep (two subspecies: *Melipona q. quadrifasciata* and *Melipona q. anthidioides*), *Melipona asilvai*, *Melipona subnitida* and *Melipona scutellaris* were collected in triplicate from the meliponary of the Federal University of Sergipe, São Cristóvão, Brazil (11°00'54"S,37°12'21"W). Pollen samples from *Melipona compressipes fasciculata* were collected from a meliponary situated in the city of Anajatuba, Maranhão, Brazil (03º15'50"S, 44°37'17"W), and those from *Melipona mandacaia* were acquired from a meliponary located in the city of Irecê, Bahia, Brazil (11°18'14"S, 41°51'21"W).

The pollen samples were collected in 50 ml sterile falcon tubes and kept at -20 ° C for 96 hours. They were then freeze-dried for 24 hours using a CHRIST ALPHA-1-4LSC freeze dryer and stored in a desiccator to prevent moisture absorption by the samples.

2.2 Sugars and lipids

The sugars were broken down by acid hydrolysis and the reducing sugars determined using Fehling solution by the Lane and Eynon method (Association of Official Analytical Chemists,1984; Instituto Adolfo Lutz, 2008) and the lipids extracted and determined by the Soxhlet method (Instituto Adolfo Lutz, 2008).

2.3 Proteins and amino acids

The total protein content was quantified by the Kjeldahl method (American Association of Cereal Chemists, 1986; Yasuhara & Nokihara, 2001). To determine the total amino acid composition, 5-50 mg defatted solids were placed in 10 × 150 mm glass vials pre-pyrolyzed at 400 °C for 8 hours. 500 µl twice distilled 6N HCl was then added to each glass vial at 104 °C and the vials subjected to vacuum, sealed and maintained at 110 °C for 22 h. The HCl was then evaporated off in a rotary concentrator (SpeedVac AS160) and the hydrolysed samples resuspended in 1.0 ml MilliQ water. 5µL aliquots of the supernatant were transferred to another test tube, dried and subjected to pre-column derivatization with phenylisothiocyanate (PITC). The phenylthiocarbamide - amino acid derivative (PTC -aa) was separated on a C18 reverse phase column (Pico-Tag Waters, 3.9 × 150 mm) with monitoring at a wavelength of 254 nm. The amino acids in the sample were quantified based on their peak areas, comparing with those of fourteen amino acid standards (asparagine, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine and leucine) of known concentration (Bidlingmeyer et al., 1984).

2.4 Total phenolic compounds, total flavonoids and the DPPH analysis

The total phenolic compound content was estimated using the Folin-Ciocalteu spectrometric method (Beretta et al., 2005). The total flavonoid content was determined using a spectrophotometer according to the protocols described by Meda et al. (2005) and Ahn et al. (2007). The standard protocol for 2.2-diphenyl-2-picrylhydrazyl (DPPH) was used to determine the antioxidant activity (Tominaga et al., 2005; Brand-Williams et al., 1995). The DPPH radical was quantified by spectrophotometry with absorption at 517 nm (Kulisic et al., 2006).

2.5 Chemicals and reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), potassium persulfate, ascorbic acid, ethylene diamine tetra acetic acid (EDTA) and formic acid were acquired from Vetec (Sigma-Aldrich/Brazil), and methanol from Tedia, Brazil. The standards used for the liquid chromatography were: apigenin, kaempferol, luteolin, quercetin and

naringin, were all acquired from Sigma-Aldrich. The acids (ferulic, caffeic, *p*-coumaric, chlorogenic, abscisic, protocatechuic, vanillic, *trans*-cinnamic and gallic) and the Folin-Ciocalteu phenol reagent were also acquired from Sigma-Aldrich. All chemicals used were of analytical grade.

2.6 High Performance Liquid Chromatography (HPLC)

The phenolic compounds were extracted using 1g of pollen dissolved in 50 mL of methanol (Andrade et al., 1997). Due to the presence of sugars in the pollen, the samples were treated with Amberlite XAD-2 (Silva et al., 2013). The components in the samples were separated by HPLC using Prominence Shimadzu chromatograph (Kyoto, JAPAN) equipped with a diode array detector (SPDM2O), a Phenomenex Luna C18 reverse phase column (250 mm × 4.6 mm × 5 mm), a Phenomenex C18 pre-column (4 mm × 3 mm) and an oven (at 35 °C). The mobile phase consisted of a mixture of 1% aqueous formic acid (A) and methanol (B) with a flow rate of 1 mL/min and the following solvent gradient: 0-10 min 10% of B, 10-40 min 55% of B, 40-46 min 55% of B, 46-60 min 75% of B, 60-65 min 75% of B, 65-68 min 10% of B, and 68-70 min 10% of B. The injection volume was 20 µL. The phenolic compounds were identified based on their retention times and the UV/Vis spectra scan (245 nm to 370 nm) which was compared with analytical standards. Gallic, abscisic and *trans*-cinnamic acids were evaluated at 270 nm; protocatechuic acid, vanillic acid, luteolin, naringin and apigenin at 290 nm; chlorogenic, *p*-coumaric, and ferulic acids at 310nm; quercetin, caffeic acid, rutin and kaempferol at 370 nm.

2.7 Statistical analysis

All the analyses were carried out in triplicate. The means were calculated and the results presented as the mean ± standard deviation (SD). The differences between the compounds in each pollen sample were analysed using the analysis of variance followed by Tukey's test, with $\alpha = 0.05$, using the Assistat software (Statistical Assistance beta version 7.6) and the PAST software 3.4 (Paleontological statistics package for education and data analysis) to carry out the multivariate cluster analysis and Principal Component Analysis (Hammer et al., 2001).

3. Results and discussion

3.1 Sugars, lipids, proteins and amino acids

Normative instruction number 3 of January 19th, 2001 (Brasil, 2001), of the Brazilian Health Surveillance Agency (ANVISA) states that bee pollen should present 145 g to 555 g kg^{-1} of sugar on a dry weight basis. As shown in Table 1, the total sugar contents varied from 0 to 27.2 ± 0.15 g kg^{-1} and hence the total sugar contents found in the bee pollen in the present study were lower than those referenced above. The low sugar contents found in the pollen samples from *Melipona* could be due to their consumption by microorganisms during fermentation.

The lipid content varied between 18.8 ± 0.8 and 47.9 ± 1.0 g kg^{-1}, values higher than those established by Normative Instruction 3, of January 19th, 2001 of ANVISA (Brasil, 2001), which determined a maximum of 18 g kg^{-1} of lipids on a dry weight basis for bee pollen. The lipid contents found in the present study were lower than those found by Souza et al. (2004), which ranged from 70 to 300 g kg^{-1} in stingless bee pollen from the Amazon.

It should be mentioned that the amounts of sugar, lipid and many other nutritional compounds, established by Brazilian law, refer to bee pollen collected by the genus *Apis* and there are no nutritional values to use as a reference for *Melipona*, highlighting the need to establish a legal framework for products generated by this genus of stingless bees.

The reduced amount of sugar decreases the energetic value of *Melipona* pollen, but the value is compensated by an increased lipid concentration, which is an important source of nutritional energy, due to innumerable functions lipids carry out in the human organism (Philippi, 2008).

The total protein contents ranged from 199.2 ± 8.1 to 444.1 ± 9.1 g kg^{-1}, above those referenced by the Brazilian legislation (Normative Instruction 3, of January 19th, 2001 of ANVISA; Brasil, 2001), which determined a minimum of 80g kg^{-1} of protein on a dry weight basis. Since all the pollen samples analysed showed values above the minimum required by the legislation, it can be said that *Melipona* pollen is a protein-rich product. The pollen produced by *Melipona compressipes* showed the highest protein concentration, and should be further studied for its use as a food supplement. The variation amongst the pollen samples with respect to the concentrations of nutritional components can be explained by the seven different species of bees, the multiple floral origin, and other biological, ecological and geographical factors related to their collection, and also to the handling, storage and freeze-drying of the final product (Ratti, 2001; Orzáez Villanueva et al., 2002).

Table 1. Chemical characterization of the pollen produced by *Melipona* bees.

	M. asilvai	*M.q. anthidioides*	*M.q. quadrifasciata*	*M. mandacaia*	*M. scutellaris*	*M. compressipes*	*M. subnitida*
Sugar g/100 g	1.61 ± 0.01	0.43 ± 0.005	0	0	2.72 ± 0.015	0	0.35 ± 0.01
Lipid g/100 g	1.88 ± 0.08	1.48 ± 0.04	1.54 ± 0.02	4.56 ± 0.05	3.15 ± 0.01	4.79 ± 0.10	2.47 ± 0.15
Protein g/100 g	32.9 ± 0.47	39.1 ± 0.57	28.9 ± 0.77	33.3 ± 0.79	19.9 ± 0.81	44.41 ± 0.9	29.30 ± 0.86
Amino acids mg/100 g	1040.4 ± 2.9	750.1 ± 1.2	810.3 ± 5	890 ± 4	700.8 ± 3	660.1 ± 3. 5	861.1 ± 8
Total Phenols mg/100 g	899.05 ± 1.65	1715.20 ± 1.23	335.54 ± 2.9	845.18 ± 2.1	109.77 ± 1.25	227.35 ± 3.8	886.66 ± 1.56
Total Flavonoids mg/100 g	456.58 ± 2.68	337.53 ± 1.97	1469.57 ± 3.12	152.59 ± 1.07	491.4 ± 1.82	481.83 ± 1.79	227.57 ± 2.8
DPPH (EC 50 mg/100 ml)	50.08 ± 0.58	12. 38 ± 1.05	21.90 ± 1.16	49.50 ± 0.9	55.54 ± 0.85	39.79 ± 0.86	24. 57 ± 0.9

The amino acids (6.60 ± 0.03 and 10.40 ± 0.02 mg g^{-1} protein) identified and quantified by HPLC are shown in Tables 1 and 2. Asparigine was found in the highest concentration followed by glutamic acid, proline and leucine. Their concentrations are significant according to the Food and Agriculture Organization (1985). All the pollen samples analysed in this study could be used as an alternative source of amino acids for human consumption, however further studies are required with respect to the quantity of pollen produced, its economic viability and other quality parameters established for food.

3.2 Total phenolic compounds, total flavonoids and DPPH

The total phenolic compound contents found in the pollen samples varied between 109.77 ± 1.25 and 1715.20 ± 1.13 mg GAE g^{-1} (gallic acid equivalents), values much higher than those described for bee pollen (Morais et al., 2011). The total flavonoid contents varied between 152.59 ± 1.07 and 14.57 ± 3.12 mg QE g^{-1} (Quercetin equivalents) as shown in Table 1, values different from those described by Silva et al. (2006) in their study with *M. subnitida*. The DPPH values of the phenolic extracts varied between 12.38 ± 1.05 and 55.54 ± 0.85 µg mL^{-1}, as also shown in Table 1. The values obtained in the DPPH analysis and the total phenolic compound and flavonoid contents referenced in this study confirm the high antioxidant potential of all the pollen samples analysed. The antioxidant potential presented confirmed the beneficial health potential of all the pollen samples under study (Pérez et al., 2015; Silva et al., 2013; Silva et al., 2014).

According to Morais et al. (2011), the antioxidant potential can vary in some foods, including bee pollen, depending on the chemical structure of the compounds, the reactivity with other antioxidants, the chelation potential with Fe and Cu metals, and also their botanical origin (Lachman et al., 2010).

Table 2. Amino acids found in the pollen from bees of the genus *Melipona.*

Amino acids mg/g (protein)	*Melipona asilvai*	*Melipona q. anthidioides*	*Melipona q. Quadrifasciata*	*Melipona mandacaia*	*Melipona Scutellaris*	*Melipona Compressipes*	*Melipona subnitida*
Asparagine	177.75 ± 0.94	121.3 ± 8.6	117.71 ± 0.78	136.99 ± 3.86	109.79 ± 2.36	114.12 ± 2.4	107.27 ± 2.6
Glutamic acid	125.58 ± 1.25	122.19 ± 1.03	123.15 ± 3.54	112.86 ± 2.82	125.62 ± 2.76	110.98 ± 1.8	113.78 ± 0.1
Serine	58.82 ± 1.6	54.89 ± 1.14	59.06 ± 1.4	60.87 ± 2.71	59.28 ± 1.06	57.62 ± 1.05	57.05 ± 2.1a
Glycine	50.43 ± 3.42	50.83 ± 0.97	52.1 ± 1.2	54.79 ± 3.2	51.35 ± 2.26	48.19 ± 0.5	59.73 ± 1.17
Histidine	30.31 ± 2.4	26.14 ± 1.2	23.69 ± 0.5	32.52 ± 1.39	24.7 ± 0.9	26.87 ± 0.3	25.75 ± 2.36
Arginine	69.67 ± 5.44	68.94 ± 5.58	61.9 ± 1.49	61.34 ± 1.54	55.67 ± 1.32	63.99 ± 1.09	64.54 ± 0.36
Threonine	43.96 ± 0.76	45.18 ± 0.54	48.78 ± 2.53	43.55 ± 0.33	47.2 ± 0.3	43.34 ± 0.15	44.19 ± 1.74
Alanine	56.39 ± 0.17	69.55 ± 2.35	67.34 ± 0.13	60.44 ± 1.6	64.85 ± 0.93	60.74 ± 2.5	61.9 ± 1.76
Proline	122.34 ± 2.28	76.6 ± 4.24	78.37 ± 4.32	105.14 ± 3.25	108.73 ± 6.4	131.69 ± 2.8	131.18 ± 0.31
Tyrosine	34.79 ± 3.72	64.85 ± 2.35	42.57 ± 1.19	37.25 ± 0.37	32.4 ± 6.75	44.34 ± 1.8	35.01 ± 0.39
Valine	57.72 ± 0.01	36.85 ± 4.3	59.74 ± 0.03	56.56 ± 3.7	62.22 ± 2.51	57.87 ± 1.7	53.57 ± 2.12
Methionine	21.01± 1.3	23.04 ± 2	23.63 ± 0.6	23.63 ± 0.6	22.29 ± 0.74	21.82 ± 0.18	22.64 ± 0.8
Cysteine	0	0	0	0	0	0	0
Leucine	91.39 ± 0.3	93.94 ± 3.4	95.74 ± 1.74	95.74 ± 1.71	84.72 ± 2.4	90.26 ± 0.8	85.54 ± 0.57

3.3 Phenolic compound profile

Fourteen different phenolic compounds with known health benefits were identified and quantified, as shown in Table 3. Thirteen of these (except naringin) were identified and quantified for the first time in pollen samples. The flavonoids luteolin and apigenin were found in the highest concentrations, being distinguished from the other flavonoids found. The phenolic compounds *trans*-cinnamic acid, abscisic acid and *p*-coumaric acid, stood out in relation to the other phenolic compounds found, as shown in Table 3 and figure 1. Note that *trans*-cinnamic acid was the most prominent compound in the pollen samples from *Melipona q. anthidioides*, differentiating these samples from the others. It is also noteworthy that the pollen samples from *Melipona q. quadrifasciata* presented high concentrations of the flavonoid luteolin (Figure 1). The other phenolic compounds were common in one or other samples of the bee species, in varying concentrations.

Abscisic acid, apigenin and kaempferol were found as the main components in the pollen samples from *Melipona asilvai*; luteoline, gallic acid and *trans*-cinnamic acid in *M. q. anthidioides*; luteoline and abscisic acid in *M. q. quadrifasciata*; galic acid, *p*-coumaric acid, abscisic acid and *trans*-cinnamic acid in *Melipona mandacaia*; apigenin in *Melipona scutellaris* and *Melipona compressipes*; and *p*-coumaric acid and abscisic acid in *Melipona subnitida*. Table 3 also shows that each pollen sample belonging to a different *Melipona* species had its own phenolic compound profile, which was possibly related to the botanical origin of the samples.

The phenolic compounds identified and quantified determined that all the pollen samples from stingless bees studied here showed therapeutic and beneficial health properties, since these compounds are responsible for the antioxidant potential (independent of the compound found) (Basim et al., 2006; Abdella et al., 2009; Carvalho et al., 2009; Fatrcová-Šramková et al., 2013).

Table 3. Profile of the phenolic compounds found by HPLC-DAD.

	Melipona asilvai	*Melipona q. anthidioides*	*Melipona q. quadrifasciata*	*Melipona mandacaia*	*Melipona scutellaris*	*Melipona compressipes*	*Melipona subnitida*
Phenolic compounds (mg/100 g)							
Apigenin	130.01 ± 0.81	-	-	-	423.35 ± 1.67	262.73 ± 1.54	-
Kaempferol	101.65 ± 1.06	-	-	-	-	-	2.63 ± 0.41
Luteolin	-	-	1332.59 ± 2.69	-	4.32 ± 0.012	35.16 ± 0.76	-
Naringin	41.28 ± 0.7	203.42 ± 0.65	-	-	-	10.89 ± 0.91	-
Rutin	-	-	-	-	-	28.62 ± 0.86	5.01 ± 0.12
Gallic acid	36.03 ± 0.8	149.56 ± 1.59	30.46 ± 0.59	105.7 ± 1.11	10.41 ± 0.57	-	3.68 ± 0.08

Table 3. Continued…

	Melipona asilvai	*Melipona q. anthidioides*	*Melipona q. quadrifasciata*	*Melipona mandacaia*	*Melipona scutellaris*	*Melipona compressipes*	*Melipona subnitida*
Ferulic acid	-	-	25.6 ± 1.07		-	-	-
Caffeic acid	-	-	-	-	4.25 ± 0.10	-	-
p-coumaric acid	-	30.26 ± 0.82	0.96 ± 0.08	146.92 ± 1.86	11.74 ± 1.22	-	571.37 ± 1.86
Chlorogenic acid	-	-	-	33.022 ± 0.80	-	-	-
Abscisic acid	728.44 ± 1.4	-	160 ± 1.27	353.41 ± 1.84	-	-	274.44 ± 1.9
Protocatechuic acid	-	0.47 ± 0.02	-	0.84 ± 0.02		-	-
Vanillic acid	-	53.46 ± 0.63	3.15 ± 0.42	40.14 ± 0.52	-	-	-
trans-cinnamic	74.58 ± 1.59	1353.64 ± 2.57	19.53 ± 1.2	199.97± 1.04	17.98 ± 0.47	-	8.26 ± 0.54

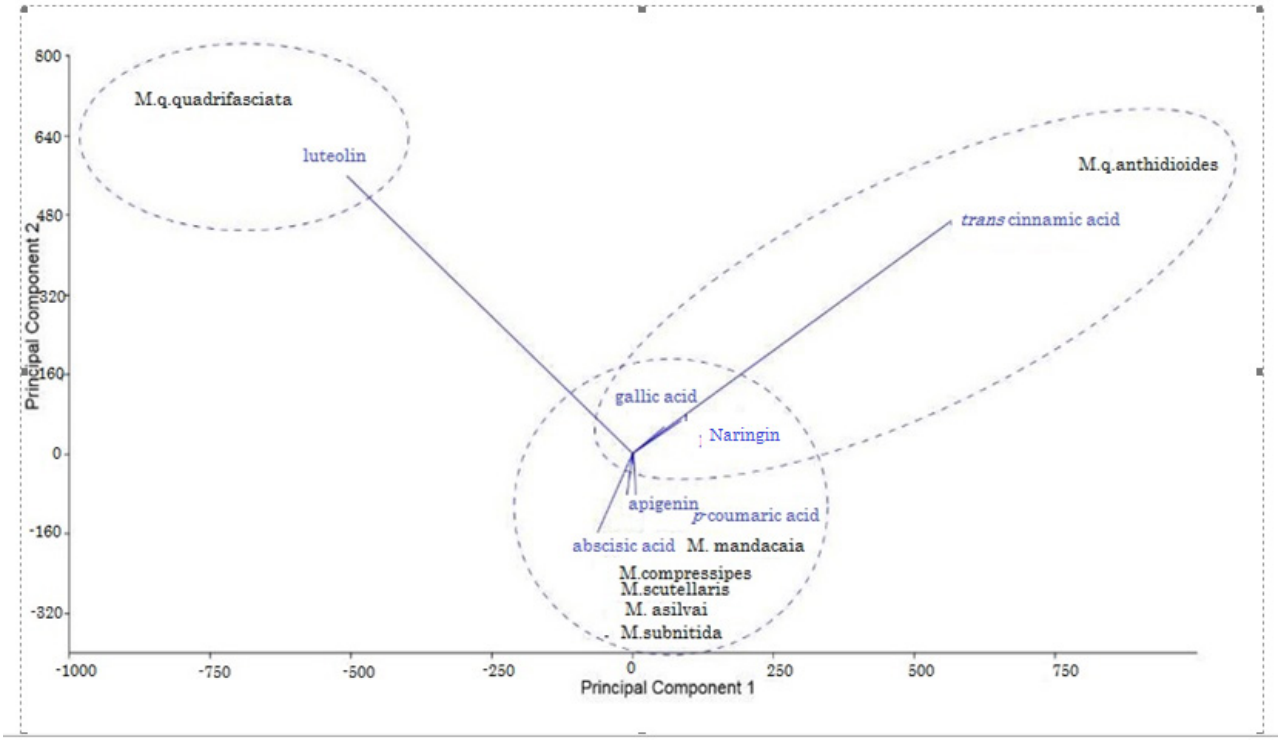

Figure 1. Principal components analysis of the phenolic compounds found in the different species of bees in this study. The trend lines represent the major phenolic compounds present in the pollen samples and the circles illustrate the species in which these compounds are most strongly associated.

4 Conclusions

All the pollen samples analysed in this study possessed significant quantities of sugars, lipids, proteins, amino acids, phenolic compounds and antioxidant activity. The pollen from *Melipona compressipes* showed the highest protein concentration and hence is most indicated for the production of food supplements. The pollens from *M.q.anthidioides* and *M.q.quadrifasciata* stood out mostly for their phenolic compound contents and antioxidant activity and are hence indicated for the development of therapeutic products. The analysis of pollens from *Melipona* is rare, especially with the above-mentioned properties, and there is no legislation in Brazil that can serve as a reference for the pollen produced by stingless bees, for its use as food or as a therapeutic agent. This study can serve as an important reference for future studies.

Acknowledgements

The authors are grateful to the "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)" and to the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)" for their financial support.

References

Abdella, E. M., Tohamy, A., & Ahmad, R. R. (2009). Antimutagenic activity of Egypt an propolis and bee pollen water extracts against cisplatin-induced chromosomal ab normalities in bone marrow cells of mice. *Iranian Journal of Cancer Prevention*, *2*, 175-181.

Ahn, M., Kumazawa, S., Usui, Y., Nakamura, J., Matsuka, M., Zhu, F., & Nakayama, T. (2007). Antioxidant activity and constituents of propolis collected in various areas of china. *Food Chemistry*, *101*(4), 1383-1392. http://dx.doi.org/10.1016/j.foodchem.2006.03.045

Almeida-Muradian, L. B., Pamplona, L. C., Coimbra, S., & Barth, O. M. (2005). Chemical composition and botanical evaluation of dried bee-pollen pellets. *Journal of Food Composition and Analysis*, *18*(1), 105-111. http://dx.doi.org/10.1016/j.jfca.2003.10.008

Alves, R. M. O., Carvalho, C. A. L., Souza, B. A., Sodré, G. S., & Marchini, L. C. (2005). Características físico-químicas de amostras de mel de *Melipona mandacaia* Smith (Hymenoptera: apidae). *Food Science and Technology (Campinas)*, *25*(4), 644-650. http://dx.doi.org/10.1590/S0101-20612005000400004

American Association of Cereal Chemists – AACC. (1986). *Approved Methods of the American Association of Cereal Chemists* (pp. 46-120). Nova York: AACC.

Andrade, P., Ferreres, F. E., & Amaral, M. T. (1997). Analysis of honey phenolic acids by HPLC its application to honey botanical characterization. *Journal of Liquid Chromatography & Related Technologies*, *20*(14), 2281-2288. http://dx.doi.org/10.1080/10826079708006563

Association of Official Analytical Chemists – AOAC. (1984). *Official Methods of Analysis*. Arlington: AOAC.

Basim, E., Basim, H., & Ozcan, M. (2006). Antibacterial activities of Turkish pollen and propolis extracts against plant bacterial pathogens. *Journal of Food Engineering*, *77*(4), 992-996. http://dx.doi.org/10.1016/j.jfoodeng.2005.08.027

Beretta, G., Granata, P., Ferrero, M., Orioli, M., & Maffei Facino, R. M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemo metrics. *Analytica Chimica Acta*, *553*(2), 185-191. http://dx.doi.org/10.1016/j.aca.2004.11.010

Bidlingmeyer, B. A., Cohen, S. A., & Tarvin, T. L. (1984). Rapid analysis of amino acids using pre-column derivatization. *Journal of chromatography*, *336*(1), 93-104. PMid:6396315. http://dx.doi.org/10.1016/S0378-4347(00)85133-6

Bogdanov, S. (2014). *Pollen: nutrition, functional properties, health: a review.* Bee Product Science.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, *28*(1), 25-30.

Brasil. Ministério de Agricultura e do Abastecimento. (2001, janeiro 23). Instrução Normativa n° 3, de 19 de Janeiro 2001. Regulamento Técnico de Identidade e Qualidade do Pólen Apícola. *Diário Oficial da União*, Brasília, Seção 16-I. pp.18-23.

Carvalho, C. A. L., Sodré, G. S., Fonseca, A. A. O., Alves, R. M. O., Souza, B. A., & Clarton, L. (2009). Phytochemical characteristics and sensory prolife of honey samples from stingless bees (Apidae: Meliponianae) submitted to a dehumidification process. *Anais da Academia Brasileira de Ciências*, *81*(1), 143-149. http://dx.doi.org/10.1590/S0001-37652009000100015

Fatrcová-Šramková, K., Nozkova, J., Kacaniova, M., Mariassyova, M., Rovna, K., & Stricik, M. (2013). Antioxidant and antimicrobial properties of monofloral bee pollen. *Journal of Environmental Science and Health*, *48*(2), 133-138. PMid:23305281. http://dx.doi.org/10.1080/03601234.2013.727664

Food and Agriculture Organization – FAO, World Health Organization – WHO, United Nations University – UNU. (1985). *Energy and protein requirements – Report of a joint FAO/WHO/UNU expert consultation* (Technical Report Series, 724). Geneva: WHO.

Freitas, B. M. (2003). *Meliponíneos - A vida das abelhas* (cd-rom). Fortaleza: Universidade Federal do Ceará.

Graikou, K., Kapeta, S., Aligiannis, N., Sotiroudis, G., Chondrogianni, N., Gonos, E., & Chinou, I. (2011). Chemical analysis of Greek pollen - Antioxidant, antimicrobial and proteasome activation properties. *Chemistry Central Journal*, *5*, 33. PMid:21699688. http://dx.doi.org/10.1186/1752-153X-5-33

Hammer, O., Harpe, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*, 1-9.

Instituto Adolfo Lutz – IAL. (2008). *Métodos físico-químicos para análise de alimentos* (4. ed., pp. 49-130). São Paulo: IAL.

Kulisic, T., Dragovic-Uzelac, V., & Milos, M. (2006). Antioxidant activity of aqueous tea infusions prepared from oregano, thyme and wild thyme. *Food Technology and Biotechnology*, *44*(4), 485-492.

Lachman, J., Orsak, M., Hejtmankova, A., & Kovarova, E. (2010). Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *Food Science and Technology*, *43*(1), 52-58. http://dx.doi.org/10.1016/j.lwt.2009.06.008.

Meda, A., Lamien, C. F., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and

proline contents in Burkina Fas an honey, as well as their radical scavenging activity. *Food Chemistry*, *91*(3), 571-577. http://dx.doi.org/10.1016/j.foodchem.2004.10.006

Morais, M., Moreira, L., Feás, X., & Estevinho, L. M. (2011). Honey bee-collected pollen from five Portuguese Natural Parks: palynological origin, phenolic content, antioxidants properties and antimicrobial activity. *Food and Chemical Toxicology*, *49*(5), 1096-1101. PMid:21291944. http://dx.doi.org/10.1016/j.fct.2011.01.020

Orzáez Villanueva, M. T., Díaz Marquina, A., Bravo Serrano, R., & Blazquez Abellán, G. (2002). The importance of bee collected pollen in the diet: a study of its composition. *International Journal of Food Sciences and Nutrition*, *53*(3), 217-224. PMid:11951585.

Pascoal, A., Rodrigues, S., Teixeira, A., Feás, X., & Estevinho, L. M. (2014). Biological activities of commercial bee pollens: antimicrobial, ant mutagenic, antioxidant and anti-inflammatory. *Food and Chemical Toxicology*, *63*, 233-239. PMid:24262487. http://dx.doi.org/10.1016/j.fct.2013.11.010

Pérez, M., Viejo, M., La Cuesta, M., Toorop, P., & Cañal, M. J. (2015). Epigenetic and hormonal profile during maturation of *Quercus Suber* L. somatic embryos. *Journal of Plant Physiology*, *173*, 51-61. PMid:25462078. http://dx.doi.org/10.1016/j.jplph.2014.07.028

Philippi, S. T. (2008). *Pirâmide dos alimentos: fundamentos básicos da nutrição.* Barueri: Manole.

Ratti, C. (2001). Hora ir and freeze-drying of high-value foods: a review. *Journal of Food Engineering*, *49*(4), 311-319. http://dx.doi.org/10.1016/S0260-8774(00)00228-4

Sattler, J. A. G., Melo, I. L. P., Granato, D., Araújo, E., Freitas, A. S., Barth, O. M., Sattler, A., & Almeida-Muradian, L. B. (2015). Impact of origin on bioactive compounds and nutritional composition of bee pollen from southern Brazil: A screening study. *Food Research International*, *77*, 82-91. http://dx.doi.org/10.1016/j.foodres.2015.09.013

Silva, G. R., Pereira, F. M., Souza, B. A., Lopes, M. T. R., Campelo, J. E. G., & Diniz, F. M. (2014). Aspectos bioecológicos e genético-comportamentais envolvidos na conservação da abelha Jandaíra: *melipona subnitida* Ducke (Apidae, Meliponini), e o uso de ferramentas moleculares nos estudos de diversidade. *Arquivos do Instituto Biológico*, *81*(3), 299-308. http://dx.doi.org/10.1590/1808-1657000812012

Silva, T. M. S., Camara, C. A., Lins, A. C. S., Barbosa, J. M., Silva, E. M. S., Freitas, B. M., & Santos, F. A. R. (2006). Chemical composition and free radical scavenging activity of pollen loads from stingless bee *Melipona subnitida* Ducke. *Journal of Food Composition and Analysis*, *19*(6-7), 507-511. http://dx.doi.org/10.1016/j.jfca.2005.12.011

Silva, T. M. S., Santos, F. S., Evangelista-Rodrigues, A., Silva, S. E. M., Silva, G. S., Novais, J. S., Santos, F. A., Camara, C. A. (2013). Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. *Journal of Food Composition and Analysis, 29*(1), 10-18. https://doi.org/10.1016/j.jfca.2012.08.010

Souza, R. C. S., Yuyama, L. K. O., Aguiar, J. P. L., & Oliveira, F. P. M. (2004). Valor nutricional do mel e pólen de abelhas sem ferrão da região amazônica. *Acta Amazonica*, *34*(2), 333-336. http://dx.doi.org/10.1590/S0044-59672004000200021

Tominaga, H., Kobayashi, Y., Goto, T., Kasemura, K., & Nomura, M. (2005). DPPH Radical–scavenging effect of several phenylpropanoid compounds and their glycoside derivatives. *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan*, *125*(4), 371-375. PMid:15802883. http://dx.doi.org/10.1248/yakushi.125.371

Yasuhara, T., & Nokihara, K. (2001). High-throughput analysis of total nitrogen content that replaces the classic Kjeldahl method. *Journal of Agricultural and Food Chemistry*, *49*(10), 4581-4583. PMid:11599991. http://dx.doi.org/10.1021/jf010448z

Funding: CNPq and CAPES.

Received: Apr. 12, 2018; Accepted: Apr. 05. 2019