BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

ORIGINAL ARTICLE

Physicochemical composition and functional properties of bee pollen produced in different locations

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Cite as: Melo, B. K. C., Silva, J. A., Gomes, R. D. S., Custódio, P. P., Lira, G. A., Ramalho, A. M. Z., Gonçalves, M. C., Fonseca, S. B., Rangel, A. H. N., & Bezerra, M. F. (2023). Physicochemical composition and functional properties of bee pollen produced in different locations. *Brazilian Journal of Food Technology*, *26*, e2022006. https://doi.org/10.1590/1981-6723.00622

Abstract

This work aimed to investigate the physicochemical and functional composition of bee pollen produced in different municipalities in the state of Rio Grande do Norte/Brazil (RN) and commercial samples produced in other places. To perform these experiments, samples of dehydrated bee pollen were crushed and subjected to physicochemical evaluation, instrumental color, fatty acid profile, evaluation of the presence of bioactive compounds [Total Phenolic Compounds (TPC), carotenoids] and antioxidant activity (reduction of DPPH radical). The pH of the pollen samples varied from 4.55 to 5.08, the protein from 19.01% to 29.71%, and the ash from 2.44% to 4.27%. All pollen samples showed a yellowish-red hue (a* and b* positive), and the pollen produced in Touros/RN/BR stood out with a lighter color (L* = 32.91). The pollen produced in Touros/RN/BR showed a higher ($p < 0.05$) percentage of saturated fatty acids (41.60%), whereas the pollen collected in Taipu/RN/BR had a higher percentage of monounsaturated fatty acids (7.44%). Pollen samples showed a low concentration of Total Carotenoid Content (TCC) (0.81 to 22.82 µg/g), as well as a high content of TPC (1.79 to 2.28 mg GAE/g) and antioxidant activity (1.40 to 5.70 µmol TE/g) when compared with the consulted literature. According to the obtained results, we could infer that pollen samples from different locations showed variations in physicochemical composition, coloration, fatty acid profile, TPC and antioxidant activity which reflects the geographic diversity inherent to the production sites.

Keywords: Antioxidant activity; *Apis melifera*; Bioactive compounds; Carotenoids; Fatty acids; Phenolic compounds.

HIGHLIGHTS

- Pollens showed variations that reflect the diversity inherent to the production sites
- Pollens have a chemical composition in accordance with Brazilian legislation
- Aqueous extracts may have provided a low concentration of bioactive compounds

1 Introduction

The therapeutic potential of bee products has been recognized for many years by alternative medicine which uses them as complementary subsidies in some treatments due to their functional properties (Denisow & Denisow-Pietrzyk, 2016). Bee pollen is the main protein source of bees and stands out for its concentration of enzymes, amino acids, vitamins, minerals, polyphenols and carotenoids (Ares et al., 2018). Studies have shown that bee pollen has antioxidant, anti-inflammatory, anticarcinogenic, antibacterial and antifungal actions which provide health benefits to its consumer (Pascoal et al., 2014; Denisow & Denisow-Pietrzyk, 2016). However, the components in the pollen vary according to the pollen that the bees collect; it reflects its botanical origin, which, in turn, depends on the climatic conditions, soil type and time of year in which it was collected (Domínguez-Valhondo et al., 2011; Arruda et al., 2013; Ares et al., 2018; Mattos et al., 2018). In addition, commercial bee pollen undergoes a dehydration process to improve its conservation during storage, which also affects its characteristics (Domínguez-Valhondo et al., 2011; De-Melo et al., 2016).

Brazil is a continental country with a climate, vegetation and soils varying between different regions so these characteristics contribute to the diversity of pollens marketed in the country. In this sense, studies carried out in specific locations contribute to broadening knowledge of the characteristics of the various pollens produced. Although research has already been carried out addressing aspects such as vitamin stability (Melo & Almeida-Muradian, 2010; Arruda et al., 2013), the correlation between bioactive compounds and botanical origin (Sattler et al., 2015), physicochemical characteristics and antioxidant properties (Carpes et al., 2009; Vasconcelos et al., 2017) in some Brazilian states, there are still many Brazilian municipalities producing bee pollen which have not been contemplated by accurate studies regarding the properties present in the pollens which arrive to the consumer market.

Therefore, our research group opted to initiate an investigation of the physicochemical composition, fatty acid profile, presence of bioactive compounds and antioxidant activity of samples of dehydrated bee pollen produced in different municipalities in the state of Rio Grande do Norte and commercial samples found in the local market, however, these samples were from other Brazilian states.

2 Materials and methods

2.1 Bee pollen samples

The pollen samples produced by *Apis melifera* bees used in the study (Table 1) were acquired directly from producers in the state of Rio Grande do Norte (RN) in the first half of 2018 and also from local commerce. Commercial pollen samples produced in other Brazilian states of São Paulo (SP) and Minas Gerais (MG) in 2017 were also acquired.

Table 1. Identification of bee pollen samples.

Samples	Production location	Floral origin*
TAI	Taipu/RN	Predominance of coconut tree
TOU	Touros/RN	Multifloral
SJS	São João do Sabuji/RN	Multifloral
SMG	São Miguel do Gostoso	Predominance of coconut tree
ATI	Atibaia/SP	Multifloral
BAM	Bambuí/MG	Multifloral

*Information described on the commercial product's label or producer's report.

The dehydrated bee pollen samples were crushed and submitted to physicochemical evaluation (pH, aw, humidity, protein, fat and ash), instrumental color, fatty acid profile, evaluation of the presence of bioactive compounds [Total Phenolic Compounds (TPC), carotenoids] and antioxidant activity (reduction of the DPPH radical).

2.2 Physicochecmical characterization

Pollen samples were analyzed for pH [dilution of 1 g pollen to 10 mL of distilled water, TEC-7 model pH meter (Tecnal®, Brazil)] and A_w in a S3TE Water Activity Analyzer (Aqualab, Brazil). The methodology described by Instituto Adolfo Lutz (2008) was used to assess moisture, ash, protein and fat.

2.3 Instrumental color

The instrumental color was evaluated by spectrophotometry reflectance using a ACR-1023 model colorimeter (Instrutherm, Brazil) in the RGB system and converted to CIELab by the OpenRGB program. The L*, a*, and b* coordinates were read, which expresses the color luminosity (L* = 0, black; L* = 100, white), the degree of variation between green and red (a* negative = green; a* positive = red) and the hue between blue and yellow (b* negative = blue; b* positive = yellow), respectively. The C* (chroma) and h* (pitch angle) parameters were calculated according to equations described by Pathare et al. (2013): $h^* = tang^{-1}(b^*/a^*)$ and $C^* = (a^{*2} + b^{*2})^{1/2}$.

2.4 Fatty acid profile

The samples were prepared according to Bligh & Dyer (1959). First, the pollen was crushed in a blender and 5.0 g was weighed in Erlenmeyer flasks, in which 12.5 mL of chloroform, 25 mL of methanol and 9.5 mL of ultrapure water were added, then the mixture was placed on a shaking table for 20 minutes. After 16 h of rest, an additional 12.5 mL of chloroform and 12.5 mL of 2% sodium sulfate solution were added. In addition, the mixture was stirred and then left to stand for two hours to separate the lipid phase.

For the methylation of fatty acids, 40 mg of this lipid phase was added into a threaded tube, which was subjected to evaporation with the aid of nitrogen gas (Hartman & Lago, 1973). After evaporation, 2.5 mL of NaOH solution in methanol (0.5N) was added and then the samples were placed in a water bath for 15 minutes. After the tubes cooled to room temperature, 7.5 mL of esterification reagent was added and taken again to a water bath for 10 minutes. The tubes were subsequently cooled and 2 mL of Hexane grade HPLC and 5.0 mL of saturated NaCl solution (20%) were added; then, the samples were left to stand until biphasic separation occurred. Soon afterwards, the collected supernatant was stored in 1.5 mL amber vials. The fatty acid profile of the methylated samples was determined in a Trace 1310 model gas chromatograph (Thermo Scientific, Brazil) with FID detector (flame ionization detector) and silica capillary column of 30 m x 0.25 mm ID x 0.25 µm film (Thermo Scientific, Brazil).

2.5 Preparation of water extracts

An aqueous extract of the bee pollen samples was prepared as described by Nóbrega et al. (2015) to analyze the TPC and antioxidant activity. A concentration of 1 g per 50 mL of distilled water was chosen based on preliminary tests. Then, the mixture was homogenized for 1 hour using the magnetic stirrer. Soon after, the homogenized content was filtered using a funnel and hydrophilic cotton. The filtrate was centrifuged by a model Q-241 centrifuge (Quimis, Brazil) at 3500 rpm at a temperature of 5 °C for 10 min. The supernatant was placed in centrifuge tubes wrapped in aluminum foil and stored under refrigeration at 5 °C until use.

2.6 Total Phenolic Content (TPC)

The presence of TPC was evaluated as described by Azevêdo et al. (2014). First, a 1 mL aliquot of the extract was transferred to test tubes, in which the following sequence was added: 1 mL of 95% ethanol solution, 5 mL of distilled water and 0.5 mL of Folin-Ciocalteau 1 N reagent (Sigma-Aldrich, USA). The

solution was homogenized and then 1 mL of 5% sodium carbonate solution (w/v) was added after 5 minutes, followed by a new homogenization. The test tubes were kept in a dark room for 60 minutes, and the solution was subsequently homogenized again. The sample absorbance measurements were read at wavelength 725 nm against white (95% ethanol solution) in a UV 330G model spectrophotometer (Gehaka, Brazil).

2.7 Total Carotenoid Contents (TCC)

The method described by Lichtenthaler & Buschmann (2001) was used to assess the presence of carotenoids. First, 1 g of sample was weighed and placed in a test tube and then 9 mL of acetone PA were added. After constant stirring for one minute, the mixture was centrifuged with a Q-241 model centrifuge (Quimis, Brazil) at 3500 rpm for 5 min and the supernatant absorbance measurements were read at the following wavelengths: 470, 662 and 645 nm in a double-beam UV-330G model spectrophotometer (Gehaka, Brazil). From these readings, the chlorophyll a (Ca), chlorophyll b (Cb), and total C carotenoids (x + c) concentrations were calculated using the formulas proposed by Lichtenthaler & Buschmann (2001). The values found were expressed in μg/mL, taking into account the sample weight and converted into mg/100 g.

2.8 2.2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay (RSA)

The antioxidant activity was evaluated by reducing the DPPH radical (2,2-diphenyl-1-picrilhidrazil) according to Duarte-Almeida et al. (2006). To do so, DPPH 4 mg/100 mL methanolic solution was prepared and the determinations were performed in a 96-well polystyrene microplate (TPP, Switzerland). Then, 40 μL of extract and 200 μL of the DPPH solution were added into each well of the microplates. In addition, 200 μL of the DPPH solution and 40 μL of the solutions with known Trolox concentration in μM (10, 30, 50, 80, 100, 120, 150 and 200) were added to build the standard curve. The absorbance readings were performed after 25 minutes of reaction in a Thermo Plate Reader microplate spectrophotometer (Bio-Rad laboratories, Hercules, USA) at 25 °C. The antioxidant capacity of the sample was calculated in relation to the activity of the Trolox synthetic antioxidant (6-hydroxy-2,5,7,8-tetramethylchromo-2-acidocarboxylic; Sigma-Aldrich, USA) under the same conditions, and the results were expressed in Trolox equivalent micromoles per gram of sample on a dry basis (μmol TE/g BS).

2.9 Statistical analysis

The data were analyzed by calculating the mean and standard deviation and the statistical differences were evaluated through Analysis of Variance (ANOVA) complemented by Tukey's test at 5% significance in the Statistica 7.0 software program (StatSoft, Inc., USA).

3 Results and discussion

3.1 Physicochemical evaluation of the pollen samples

The physicochemical evaluation results can be seen in Table 2. The bee pollen samples showed average pH values between 4.55 and 5.09 with higher averages ($p < 0.05$) for the pollens produced in Touros/RN (TOU), Atibaia/SP (ATI) and Bambuí/MG (BAM), which were similar to each other, since the pollen collected in the municipality of São Miguel do Gostoso/RN (SMG) obtained the lowest pH value (i.e. it showed greater acidity when compared to the others).

The pollen samples analyzed showed pH results in accordance with the requirements in Brazilian legislation (Brasil, 2001) which recommends pH values between 4 and 6. The highest average pH reached was 5.09, demonstrating the acidity inherent to this bee product, noting that it can vary due to factors resulting from its dehydration process (Isidorov et al., 2009).

Table 2. Physicochemical characterization of dehydrated bee pollen samples.

Samples	pH	A_w*	Moisture (%)	Protein (%)**	Fat (%)**	Ash (%)*
SJS	4.89 ± 0.03^b	0.37 ± 0.00^c	11.92 ± 0.12^b	29.71 ± 2.24^a	1.02 ± 0.20^b	3.32 ± 0.30^b
TOU	5.07 ± 0.01^a	0.32 ± 0.00^e	9.12 ± 0.63^d	$21.45 \pm 1.83b^c$	1.51 ± 0.33^b	4.27 ± 0.20^a
TAI	4.74 ± 0.01^c	0.35 ± 0.00^d	11.87 ± 0.10^b	25.50 ± 0.85^b	1.39 ± 0.08^b	3.79 ± 0.13^b
SMG	4.55 ± 0.01^d	0.40 ± 0.00^a	13.30 ± 0.11^a	26.05 ± 0.24^b	1.44 ± 0.29^b	4.35 ± 0.06^a
ATI	5.09 ± 0.04^a	0.39 ± 0.00^b	5.12 ± 0.05^e	19.01 ± 0.22^c	4.50 ± 0.21^a	2.44 ± 0.19^c
BAM	5.08 ± 0.08^a	0.35 ± 0.01^d	10.31 ± 0.21^c	20.77 ± 0.48^c	1.65 ± 0.20^b	2.67 ± 0.04^c

Results presented as means ± standard deviation of three repetitions (N = 3). a, b, c, d, e: means in the same column with different letters represent a statistically significant difference according to the Tukey's test ($p < 0.05$). *A_w: activity water. **Results expressed on a dry basis.

It is observed that SMG showed greater water activity (0.40) when compared to the other samples ($p < 0.05$), followed by TAI and BAM which obtained A_w of 0.35. The lowest A_w ($p < 0.05$) was achieved by TOU (0.32). SMG also had the highest ($p < 0.05$) moisture (13.30%), whereas ATI had the lowest moisture (5.12%).

The results found for A_w (0.32 to 0.40) were higher than those reported by Domínguez-Valhondo et al. (2011) in pollen samples collected in Spain and dehydrated under similar conditions ($A_w < 0.17$), but similar to pollens collected in the South and Southeast of Brazil (Carpes et al., 2009). SMG stood out from the other samples in both A_w (0.40) and in the moisture percentage (13.30%). However, this relationship between moisture and A_w is not observed in the other samples; for example, ATI showed a lower mean for moisture (5.12%; A_w: 0.39), while a lower A_w was noted in TOU (0.32; moisture: 9.12%).

Pollen samples showed moisture above 4% (on a fresh basis), a percentage recommended by Brazilian legislation (Almeida-Muradian et al., 2005; Brasil, 2001). Although this legal requirement varies by country, for example, the limit in Argentina and Switzerland is 8%, while in Bulgaria is 10%, as reported by Ghosh & Jung (2017), it is interesting to note that values of humidity above 8%, as observed in most samples evaluated in the present work, can reduce the shelf life of the product (Campos et al., 2008).

The average values for protein ranged from 19.91% (ATI) to 29.71% (SJS). SJS pollen had a higher ($p < 0.05$) protein concentration compared to the others, followed by TAI, SMG and TOU samples, which were similar to each other and higher ($p < 0.05$) than ATI and BAM, with these two samples being statistically similar to TOU; in other words, the pollens collected in the cities of Rio Grande do Norte had a higher protein concentration than the commercial samples from São Paulo and Minas Gerais states, with the exception of TOU. The fat concentration in the pollens studied varied from 1.02% (SJS) to 1.65% (BAM), with the exception of the ATI sample, which obtained a fat content of 4.50%, thus constituting a statistically higher value than the pollens in other locations. A higher ash percentage ($p < 0.05$) was observed in TOU (4.27%) and SMG (4.35%), then TAI (3.79%) and SJS (3.32%), and a lower concentration for BAM (2.67%) and ATI (2.44%).

The average protein values achieved in the present study (19.91% to 29.71%) were higher than those found by De-Melo et al. (2016) in bee pollen samples from a predominance of eucalyptus (10.7%) collected in the state of São Paulo, and close to lyophilized pollen samples (23.2-26.5%) in studies conducted by Ghosh & Jung (2017). Protein is a major component of bee pollen (Ares et al., 2018), and the evaluated samples in the present study reached values comparable to traditional food sources of proteins such as grilled roast beef (26.4%) and higher than in cooked chicken eggs (13.3%) (Núcleo de Estudos e Pesquisas em Alimentação, 2011).

The results obtained for ashes (range from 2.44 to 4.35) are close to the findings of Arruda et al. (2013) in pollen samples from the state of São Paulo (2.98%). On the other hand, Ghosh & Jung (2017) analyzed freeze-dried pollens of oak and kiwi from the mountainous region of South Korea and were able to verify levels of 5.3% and 5.2%, which were higher than our results. Regarding the ash percentage, the results obtained showed that the samples produced in the state of Rio Grande do Norte (SJS, TOU, TAI and SMG) showed statistically higher concentrations than the pollens collected in Minas Gerais and São Paulo.

All of the dehydrated bee pollen samples had protein (minimum 8%) and ash (maximum 4%) concentrations according to the standards established by Brazilian legislation (Brasil, 2001), but only pollen from Atibaia/SP obtained fat percentage within the recommended range (minimum of 1.8%), while the others presented a lower lipid concentration.

The variation in the composition of bee pollen samples collected in different locations in the state of Rio Grande do Norte and in other Brazilian states reflects the floral diversity, soil, and the different climatic conditions, in addition to the possible variations in the processing and storage conditions employed (Kostić et al., 2015; Ares et al., 2018).

3.2 Colorimetric evaluation

Higher ($p < 0.05$) luminosity (L*) was presented by TOU pollen, demonstrating that it has a lighter color, whereas the results (Table 3) indicated a darker hue for SJS, SMG, ATI and BAM. The a* and b* values were positive for the evaluated pollens, indicating a yellow-red color. A reddish hue of the pollen samples was confirmed by the h* results, which reached values between 1.20 to 1.36. When comparing the instrumental color between pollens from different origins, it is possible to notice that ATI stood out with greater red color intensity, which reflects greater perception by human eyes as represented by C* with a higher average ($p < 0.05$); it should be noted that ATI presented statistically similar a* values to SMG and SJS.

The red-yellow color observed in the samples evaluated in the present study was also noticed by De-Melo et al. (2016) in pollens collected in the state of São Paulo/Brazil, with the authors reporting greater luminosity (L*: 49.9 – 51.1), which means lighter than our samples. However, different colors of bee pollen are possibly due to the floral diversity (Campos et al., 2008).

Table 3. Color parameters of bee pollen samples.

Samples	L*	a*	b*	h*	C*
SJS	20.36 ± 2.85^{c}	5.45 ± 0.82^{ab}	14.51 ± 2.50^{b}	1.20 ± 0.10^{b}	15.55 ± 2.25^{b}
TOU	32.91 ± 1.76^{a}	3.67 ± 2.36^{b}	16.35 ± 1.21^{ab}	1.36 ± 0.12^{a}	16.86 ± 1.68^{b}
TAI	27.54 ± 2.36^{b}	4.63 ± 0.63^{b}	16.24 ± 1.51^{b}	1.29 ± 0.04^{ab}	16.90 ± 1.47^{b}
SMG	20.28 ± 2.66^{c}	5.47 ± 0.45^{ab}	13.94 ± 1.58^{b}	1.19 ± 0.06^{b}	14.99 ± 1.43^{b}
ATI	22.36 ± 2.48^{c}	6.72 ± 1.87^{a}	19.35 ± 1.85^{a}	1.24 ± 0.09^{ab}	20.55 ± 186^{a}
BAM	18.58 ± 2.42^{c}	3.55 ± 0.98^{b}	15.64 ± 1.89^{b}	1.35 ± 0.05^{a}	16.06 ± 1.94^{b}

Results presented as means ± standard deviation of nine repetitions (N = 9). a, b, c: means in the same column with different letters represent a statistically significant difference according to the Tukey's test ($p < 0.05$).

3.3 Fatty acid profile

A total of 11 types of fatty acids were found, with emphasis on palmitic, linoleic and α-linolenic acids which appeared in greater concentration, as observed in Table 4. The pollen with the highest percentage ($p < 0.05$) of linoleic acid was found in the SJS sample (51.31%), whereas ATI had the lowest ($p < 0.05$) concentration (13.74%) when compared to the others. A-linolenic fatty acid was found in greater concentration ($p < 0.05$) in the ATI (47.03%) and BAM (47.83%) pollens; however, TOU (35.85%), SMG (33.14) and TAI pollens (32.83%), as well as presenting a greater predominance of palmitic acid with the latter, showed averages similar to BAM (31.52%) and ATI (30.79%). Miristic, pentadecyl, arachidic, behenic and lignoceric acids were detected in low concentration in only some of the analyzed bee pollen samples.

Table 4. Fatty acid composition (percentage by area of bee pollen).

	ATI	TOU	BAM	SJS	SMG	TAI
Miristic (C14:0)	0.54 ± 0.32^{a}	**	**	0.60 ± 0.02^{a}	**	**
Pentadecyl (C15:0)	**	**	**	1.46 ± 0.02	**	**
Palmitic (C16:0)	**30.79 ± 0.16^{b}**	**35.85 ± 0.11^{a}**	**31.52 ± 0.35^{b}**	**25.83 ± 0.10^{c}**	**33.14 ± 0.11^{ab}**	**32.83 ± 2.30^{ab}**
Palmitoleic (16:1)	**	0.49*	**	0.61*	**	0.49*
Stearic (C18:0)	1.35 ± 0.07^{c}	2.00 ± 0.02^{b}	1.51 ± 0.17^{c}	2.02 ± 0.01^{b}	2.33 ± 0.02^{a}	1.98*
Oleic (C18:1n9c)	4.81 ± 0.07^{c}	2.22 ± 0.03^{d}	4.30 ± 0.24^{c}	6.56 ± 0.18^{b}	6.01 ± 0.21^{b}	7.44*
Linoleic (C18:2n6c)	**13.74 ± 0.03^{d}**	**22.49 ± 0.18^{c}**	**14.21***	**51.31 ± 0.24^{a}**	**28.48 ± 0.11^{b}**	**32.82 ± 3.45^{b}**
α-Linolenic (C18:3n3) ALA	**47.03 ± 0.13^{a}**	**33.80 ± 0.33^{b}**	**47.83 ± 0.41^{a}**	**11.01 ± 0.11^{d}**	**27.80 ± 0.18^{c}**	**28.82 ± 3.45^{bc}**
Araquic (C20:0)	1.42*	**	0.87*	0.91 ± 0.02^{b}	**	0.36*
Behenic (C22:0)	**	2.06 ± 0.01^{b}	**	**	2.25*	1.30*
Lignoceric (C24:0)	1.65*	**	**	**	**	0.71*

Results presented as means ± standard deviation of two repetitions (N = 2). a, b, c, d, e: means on the same line with different letters represent a statistically significant difference according to Tukey's test ($p < 0.05$). *Detection in just one repetition. **Not detected. The main compounds are indicated in bold.

Palmitic, linoleic (ω-6) and α-linolenic acids (ω-3) stood out with greater concentration among the 11 types of fatty acids present in the evaluated pollen samples. This predominance was also noted by Gardana et al. (2018) in pollens collected in Colombia, Italy and Spain, and is also emphasized in a review work carried out by Thakur & Nanda (2020).

The presence of high percentages of A-linolenic fatty acid in the analyzed pollen is important due to the functional properties of this component for the human organism. The A-linolenic fatty acid, better known as omega 3, has anti-atherosclerotic effects, decreasing the percentage of LDL (Low Density Lipoproteins), which is the cholesterol that favors the production of atherosclerotic plaques. Thus, individuals can benefit from these properties by including bee pollen in their diet.

In relation to the total fatty acids (Table 5), polyunsaturated fatty acids showed a higher concentration in all pollens with percentages greater than 55%. The total saturated fatty acids took second place in showing a higher concentration in pollens, with a higher percentage ($p < 0.05$) in TOU (41.60%) and lower in SJS (29.35%). On the other hand, the total monounsaturated fatty acids had lower concentrations with values between 2.23% (TOU) and 7.44% (TAI).

Table 5. Total percentage of saturated, monounsaturated and polyunsaturated fatty acids from bee pollen.

	ATI	TOU	BAM	SJS	SMG	TAI
Total saturated fatty acids (SFA)	34.10 ± 0.42^{b}	41.60 ± 0.14^{a}	33.90 ± 0.14^{b}	29.35 ± 0.10^{c}	37.72 ± 0.14^{b}	37.18 ± 2.30^{b}
Total monounsaturated fatty acids (MUFA)	4.81 ± 0.07^{d}	2.23 ± 0.03^{e}	4.30 ± 0.24^{d}	6.56 ± 0.18^{b}	6.01 ± 0.21^{c}	7.44 ± 0.00^{a}
Total polyunsaturated fatty acids (PUFA)	60.78 ± 0.10^{a}	55.93 ± 0.51^{a}	62.05 ± 0.41^{a}	62.32 ± 0.13^{a}	56.27 ± 0.07^{a}	61.03 ± 6.38^{a}

Results presented as means ± standard deviation of two repetitions (N = 2). a, b, c, d, e: means on the same line with different letters represent a statistically significant difference according to Tukey's test ($p < 0.05$).

The human body is unable to synthesize some fatty acids, called essentials, such as omega 3, so their intake is necessary. Like us, bees need polyunsaturated fatty acids to maintain their hives and diet, both to produce royal jelly as an energy source, so it is to be expected that this type of fatty acid predominates (Belina-Aldemita et al., 2019).

3.4 Presence of total phenolic compounds, carotenoids and antioxidant activity

It is observed in Table 6 that SJS and ATI presented a higher ($p < 0.05$) concentration of TPC (2.27 and 2.22 GAE/g, respectively), whereas BAM reached a lower average (1.79 GAE/g), only being statistically similar to TAI, which, in turn, presented intermediate results and similar to SMG and TOU. It could be noted a statistical difference between the samples evaluated regarding the TCC. Higher means were achieved by BAM (22.82 μg/g) and ATI (14.38 μg/g) pollens, whereas TOU (0.81 μg/g) reached the lowest concentration in relation to the other experimental groups.

Table 6. Means and standard deviation of Total Phenolic Compounds (TPC), concentration of Total Carotenoid Content (TCC) and Antioxidant Activity (AA), expressed on a dry basis.

Samples	TPC mg GAE/g	TCC µg/g	AA µmol TE/g
SJS	2.28 ± 0.13^{a}	10.48 ± 0.08^{c}	5.70 ± 0.00^{a}
TOU	1.97 ± 0.01^{b}	0.81 ± 0.38^{f}	1.40 ± 0.44^{b}
TAI	1.87 ± 0.04^{bc}	3.55 ± 0.10^{d}	4.76 ± 0.32^{a}
SMG	1.92 ± 0.03^{b}	2.17 ± 0.39^{e}	5.51 ± 0.21^{a}
ATI	2.23 ± 0.13^{a}	14.38 ± 0.40^{b}	4.31 ± 0.35^{a}
BAM	1.79 ± 0.01^{c}	22.82 ± 0.23^{a}	5.16 ± 0.79^{a}

Results are presented as means ± standard deviation (TPC N = 6; TCC N = 3; AA N = 8). a, b, c, d, e, f: means in the same column with different letters represent a statistically significant difference according to Tukey's test ($p < 0.05$).

The evaluation of antioxidant activity (Table 6) by reducing the DPPH radical (2,2-diphenyl-1-picrilhidrazil) showed statistical similarity between the evaluated pollens, except for the TOU sample which presented a lower mean ($p < 0.05$) than the rest. Mean concentrations ranged from 1.39 µmol TE/g (TOU) to 5.70 µmol TE/g (SJS).

The results found for TPC were lower than those obtained from dehydrated pollen ethanol extracts collected in the state of Rio Grande do Sul/Brazil, in which the authors observed an average of 30.77 mg GAE/g (Carpes et al., 2009), and from São Paulo/Brazil, in which a variation of 9.2 to 19.7 mg GAE/g was observed according to the collection period (De-Melo et al., 2016). However, this low TPC concentration in the pollen samples in the present study may be due to the aqueous extraction used.

The disparity in the concentration of carotenoids between different bee pollen samples corroborates the results presented by Melo & Almeida-Muradian (2010) in dehydrated bee pollens marketed in the state of São Paulo (25.3 to 268.5 µg/g), and Sattler et al. (2015) for pollen samples collected in the states of the southern region of Brazil (5.3 to 1233.0 µg/g), in such a way which reflects the botanical diversity, in addition to factors such as climatic conditions, soil type, beekeeper activities and the different storage processes or treatments, as well as the conditioning carried out on fresh pollen before storage (Ares et al., 2018).

It is observed that the antioxidant activity (AA) of the analyzed pollens was inferior to the work done by De-Melo et al. (2016) with different types of dehydration, in which the mean values for lyophilized samples were 41.1 µmol TE/g, and 31.7 µmol TE/g for samples dehydrated by forced ventilation.

The presence of TPC and TCC may have influenced the antioxidant activity of the evaluated pollens, since the antioxidant capacity of the food is related to the bioactive compounds present. It should be noted that each of these groups is formed by several components which express greater or lesser AA (De-Melo et al., 2016).

We emphasize that in this work only the total compounds (phenolic and carotenoids) were evaluated. Therefore, there is a need to carry out a more detailed investigation with accurate analytical methods using chromatography to distinguish which components directly influence antioxidant activity. It should also be considered that aqueous extracts were also used for AA analysis, which may have contributed to lower values when compared to the literature. Finally, we emphasize that this was just an initial work and that more detailed assessments can be developed.

4 Conclusion

According to the results obtained, we could infer that bee pollen samples from different locations showed variations in physicochemical composition and functional properties that reflect the geographic diversity inherent to the different production sites. The pollens had a pH value, protein and ash concentration according to Brazilian legislation, in addition to a reddish-yellow color. The low concentration of phenolic compounds, carotenoids and antioxidant activities of the evaluated pollen samples may be due to the aqueous extracts used in the study.

Acknowledgements

Financial support from CAPES and CNPq (Brazil) is gratefully acknowledged.

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Funding: Ministério da Ciência, Tecnologia e Inovação/Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.

Received: Jan. 14, 2022; **Accepted:** Dec. 12, 2022

Associate Editor: Maria Teresa B. Pacheco.