

# Identification of Sugar, Amino Acids and Minerals from the Pollen of Jandaíra Stingless Bees (*Melipona subnitida*)

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## Abstract

The aim of this investigation was to analyze two samples of pollen from jandaíra stingless bees (*Melipona subnitida*) in view of their mineral composition, free amino acids and sugars. Palynological analysis showed that the predominant pollen was monofloral from *Senna sp.* species (94.5%, pollen 2011) and the second pollen sample showed the presence of two primary species, *Chamaecrista sp.* (39.2% pollen 2009) and *Mimosa tenuiflora* (43.5%) (pollen 2009). The highest mineral content was potassium. The bee pollen contained 20.8% and 31.0% of mannitol in samples from 2011 and 2009, respectively. Proline and serine are the predominant amino acids. High content of essential amino acids, minerals and the sugar mannitol confirmed high nutritional value of pollen samples from jandaíra bees.

## Keywords

Jandaíra Bees, Mineral, Amino Acids, Sugar

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## 1. Introduction

Bee pollen has been used for many years in traditional medicine, supplementary nutrition and in alternative diets, primarily due to its nutritional properties and health benefits [1]. The major components of bee pollen are car-

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bohydrates, crude fibers, proteins and lipids. Other minor components are minerals and trace elements, vitamins and carotenoids, phenolic compounds, flavonoids, sterols and terpenes [1]-[4]. It is referred to as the “only perfectly complete food” as it contains all of the essential amino acids humans need. However, the composition of bee pollen depends strongly on the plant source and geographic origin, together with other factors such as climatic conditions, soil type and beekeeper activities [5].

Plant pollen is by far the most important source of protein and free amino acids for bees. Bee-collected pollen shows much higher protein content, but it varies greatly according to the plant source [1]. The amino-acid profile of bee-collected pollen is a potential tool for botanical or even geographical differentiation, and from a nutritional point of view it is a source of proteins or essential amino acids [1] [6] [7].

The exact nature of the carbohydrate content of pollen has not been specified, although it could be related to the sugars found in nectar [8]. The sugars of fructose, glucose and sucrose comprise approximately 90% of all low molecular weight sugars. Qian *et al.* [8] showed that the level of sugars present in five samples of bee pollen of these three main sugars are quite similar in all five samples despite geographical differences, suggesting that the samples are derived mainly from nectar. The presence of a high content (34.9% dry weight) of mannitol has been reported in bee pollen from the stingless bee *Melipona subnitida* [2].

In addition to containing essential compounds such as lipids, sugars, proteins, amino acids, vitamins and carotenoids that has led to its increasing use as a health food supplement, bee pollen has flavonoids that present antioxidant [4] and anti-inflammatory activities [9] [10].

In our continuing research for *Apis* and *Melipona* products [2]-[4] [11]-[13], we evaluated the minerals, sugars and free amino acids of two samples of bee pollen collected by jandaíra stingless bees. The formatter will need to create these components, incorporating the applicable criteria that follow.

## 2. Material and Methods

### 2.1. Pollen Samples and Palynological Identification

Analysis of the bee pollen profile allows us to determine its floral origin. Two samples of *M. subnitida* “jandaíra” bee pollen were collected in March 2009 and March 2011 at Sítio Riacho, which is in the municipality of Vieirópolis, Paraíba, Brazil. The pollen was collected in small storage bottles from hives and was refrigerated at 4°C until it was analyzed. Samples of pollen loads (2.0 g) were hydrated in water to free the pollen grains from the load mass. After being completely homogenized, they were dehydrated in glacial acetic acid, and the pollen sediments were prepared for palynological analysis using the acetolysis method [14]. Five slides with glycerin jelly (stained with safranin) were mounted from each sample. Pollen grains were counted (at least 500 per slide) to establish the frequency of the pollen types. Pollen types were identified by comparison with slides from the palynothesca of the Plant Micromorphology Laboratory (Univ. Est. Feira de Santana, Brazil).

### 2.2. HPLC Analysis of Sugars and Free Amino Acids

All chromatographic analyses were performed using a Shimadzu Prominence LC-20AT equipped with a SPD-M20A diode array detector (Shimadzu Corp. Kyoto, Japan). For amino acid analysis, the samples were injected into a Rheodyne 7125i injector with a 20 µl loop. Amino acid derivation with AccQ·Tag reagents was conducted according to the manufacturer’s protocol. Briefly, 10 and 20 µL of a standard amino acid mix solution or the extract of bee pollen (20 mg/mL), respectively, were mixed with 60 µL AccQ·Tag borate buffer and 20 µL AccQ·Tag reagent previously dissolved in 1.0 mL of AccQ·Tag reagent diluent. The reaction was allowed to proceed for 1 h at room temperature. The separation column was a Waters AccQ·Tag (3.9 mm i.d. × 150 mm, 4.0 µm particles). The column heater was set at 37°C, and the mobile phase flow rate was maintained at 1.0 mL/min. Eluent A was 1% AccQ·Tag solvent A, eluent B was acetonitrile and eluent C was Milli-Q water. The separation gradient was 0 - 0.5 min (100% - 99% A), 18 min (95.0% A), 19 min (91% A), 29.5 min (83% A), 33 min (60% A and 40% C), 36 min (100% A) 65 min (60% A and 40% C) and 100 min (60% A and 40% C). Ten microliters of the sample was injected for analysis. The PDA detector was set at 254 nm.

The sugars analysis was performed with arefractiveindex detector (RID-10A), Rezex<sup>TM</sup>RCM-Monosaccharide Ca<sup>+2</sup> (8%), a Phenomenex (300 × 7.8 mm) column at 85°C with water and MeOH (5%) as eluent. The volume injected was 20 µL. Analyses were performed by plotting a calibration curve. For each sample, the quantitative

analyses were performed in triplicate.

### 2.3. Metal Determination from Bee Pollen

Digestion of pollen was performed in a closed microwave acid digestion MARS 5 system (CEM corporation, USA). Samples (500 mg) were diluted in concentrated nitric acid. The program used had the following features: 800 W for 5 min at 120°C then 160°C for 20 min. Upon cooling, the solution was filtered to remove any remaining solid material. The solution was diluted with deionized water prior to analysis. Analysis of metals (Cu, Fe, K, Mn, Cd, Zn, Na and Ca) was performed with a Varian AA 240 (Victoria, Australia) by flame atomic absorption spectrometry (FASS). Under the optimum established parameters, standard calibration curves for metals were constructed by plotting absorbency against concentration in a definite range for each metal, and good linearity was observed. All analyses were made in triplicate, and the mean values were reported. All of the values obtained for metals contents in pollen samples were calculated as mg/kg pollen.

### 2.4. Statistical Analysis

All samples were analyzed in triplicate unless stated otherwise, and the results are expressed as the mean  $\pm$  standard deviation. All statistical analyses were carried out using the Microsoft Excel software package (Microsoft Corp., Redmond, WA, USA).

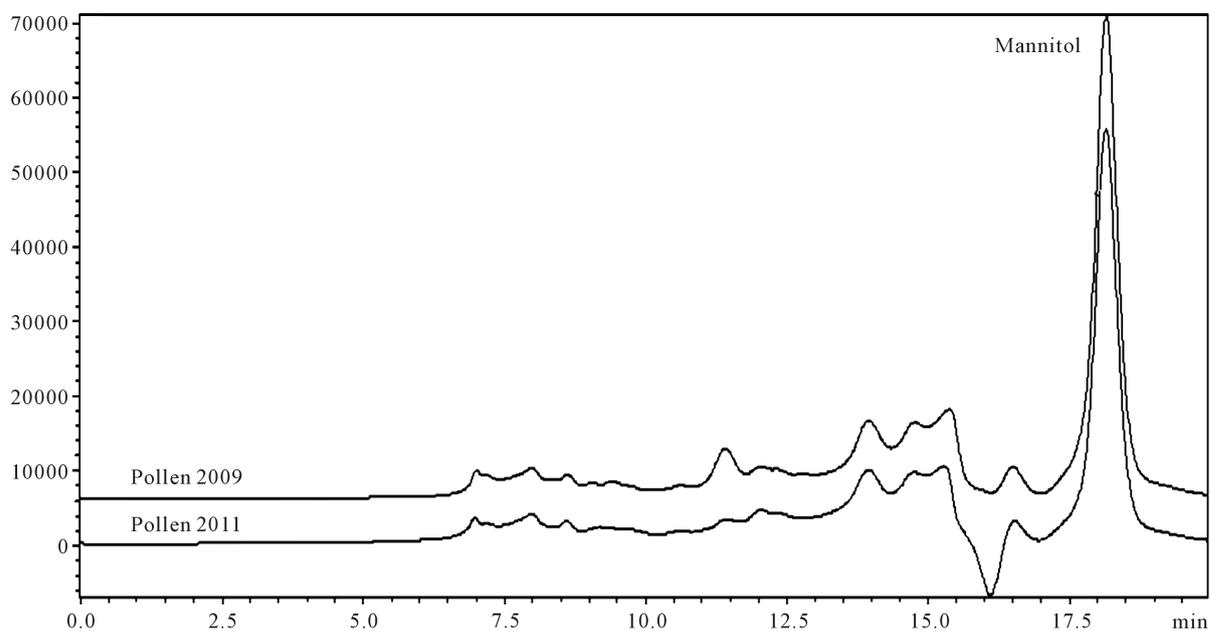
## 3. Results and Discussion

Palynological analysis showed that the predominant pollen was monofloral (pollen 2011) from *Senna sp.* species (94.5%). Species of *Senna* are commonly found near the jandaíra bee hives where the pollen was collected. Some of these species are popularly known as “mata-pasto” or “mata-pasto-liso”. The flowers are yellow, medium sized and have poricidal anthers. The pollen is the only floral resource, but it is produced in large quantities. Jandaíra bees collect pollen through vibrations. These plants are a source of pollen for native bees, especially during the rainy season and the transition from the rainy to the dry seasons in semiarid regions. To strengthen population of native bees, it is important to keep these shrubs in areas of conservation and beekeeping [15].

The pollen collected in 2009 showed the presence of two primary species, *Chamaecrista sp* (39.2%) and *Mimosa tenuiflora* (43.5%). The species *Mimosatenuiflora* is popularly known as jurema-preta. It is a small tree identified by presence of prickles in the branches. This species blooms for a long period of the year, but mostly during the dry season of the Caatinga. Its inflorescences are grouped in spikes formed by white, small flowers that are gently fragrant. The flowers are floral resources of pollen and nectar for many species of bees, wasps, flies and other insects. Jurema is very important for maintaining biodiversity and functioning ecosystems. Moreover, due to its rapid growth and its ability for regrowth, this species is very important for the restoration of degraded areas [15]. Species of the *Chamaecrista* genus are commonly found in Caatinga and are popularly known as palma-do-campo. It is a shrub, and the flowers have poricidal anthers. Pollen is the only available resource for flower visitors. Only a few species of bees adapted to using vibrations to collect pollen from the poricidal anthers. Its main pollinators are stingless bees from the *Melipona*, *Xylocopa* and *Bombus* genera. This plant is very important for the maintenance and conservation of bees and can be used in gardens for flora honey [15].

Regarding the chemical composition, the major nutrients in bee pollen (pollen collected by *Apis mellifera* bees) are sugars (13% - 55%) and protein (10% - 40%) [1]. Carbohydrates commonly found in bee pollen include sucrose, glucose and fructose. There are no studies that have quantified the sugars in the pollen from *Melipona* bees. Mannitol, a polyol, has been isolated and identified in pollen collected by jandaíra bees [2]. It is interesting to note that in the previous study, the bee pollen was monofloral from *Mimosa gemmulata*. This shows that the presence of this compound is independent of the floral source, so it can be speculated that the *Melipona subnitida* bee probably converts the sugars commonly found in flowers (glucose and fructose) in the polyol mannitol. This must be due to the presence of enzymes in the bees saliva. More detailed studies on this subject need to be performed. The chromatogram of samples (Figure 1) showed mannitol as the major component. The quantification of mannitol in pollen showed that 1 g of bee pollen contained 208.0 mg and 310.0 mg of mannitol in the samples from 2011 and 2009, respectively (Table 1).

Mannitol has multiple applications in the pharmaceutical, chemical and food industry. In the food industry, it is mainly used as a sweetener for low calorie sugars, and thus is used in food products for diabetics [16].



**Figure 1.** Chromatogram (HPLC-IR) of jandaíra bee pollen.

**Table 1.** Content (mg/g pollen) of free amino acids and sugar (mannitol) in jandaíra bee pollen.

| Amino acids   | Sample 2009   | Sample 2011 |
|---------------|---------------|-------------|
|               | (mg/g pollen) | (mg/pollen) |
| Asparagine    | 0.80          | 1.04        |
| Serine        | 3.72          | 4.27        |
| Glutamine     | 0.20          | 0.21        |
| Glycine       | 0.37          | 0.36        |
| Histidine     | 0.73          | 0.70        |
| Arginine      | 0.32          | 0.30        |
| Threonine     | 0.30          | 0.23        |
| Alanine       | 0.91          | 0.93        |
| Proline       | 11.79         | 9.54        |
| Cysteine      | -             | -           |
| Tyrosine      | 0.41          | 0.32        |
| Valine        | 0.41          | 0.29        |
| Methionine    | 0.17          | 0.14        |
| Lysine        | 0.53          | 0.32        |
| Isoleucine    | 0.21          | 0.14        |
| Leucine       | 0.61          | 0.61        |
| Phenylalanine | 0.41          | 0.36        |
| Mannitol      | 208.0 mg      | 310.0 mg    |

Because of the high protein content and rich amino acid composition, bee pollen has been the subject of numerous studies, mostly conducted by foreign researchers with the bee pollen from *Apis mellifera*. The samples of jandaíra-collected pollen contained 17 amino acids, including all of the essential amino acids except for tryptophan, which was not observed in the study. Of all of the identified amino acids, proline was found at the highest concentration (11.79 mg/g and 9.54 in samples from 2011 and 2009, respectively). Proline and serine were the predominant amino acids, constituting approximately 56% of total free amino acids (Table 1). One interesting finding was that serine was the second most predominant amino acid; other bee-collected pollen originating from Poland, South Korea and China showed a high content of glutamic acid, proline, aspartic acid, leucine and lysine [7]. Twelve common varieties of monofloral bee pollen collected from China's main producing regions were analyzed, and proline and glutamic acids were found to be the predominant amino acids in the form of both total and free amino acids [17].

Protein content and the free amino acids depend strongly on the botanical origin (flowers), while the qualitative pattern of the amino acids is generally similar in different types of pollen.

Interms of minerals, the highest content was determined for potassium, followed by calcium (Ca) and magnesium (Mg). Zinc (Zn), followed by Manganese (Mn), Iron (Fe) and Copper (Cu) were the predominant trace metals. Neither sample showed the presence of sodium (Table 2). The high K/Na ratio makes bee pollen potentially valuable for diets with a defined electrolytic balance. Selenium, Cu and Zn are considered antioxidant nutrients because they are structural components of the antioxidant enzymes. The metal contents of pollen are variable due to factors such as differences in plant species, geographical area and conditions of the drying process. Some mineral elements have already been quantified in bee pollen samples from several countries, including K, P, Mg, Ca, Na, S, Fe, Cu, Mn, Zn, Cr, Ni and Se [17]-[20]. In Brazil, the mineral composition of bee pollen was investigated in 154 samples from different regions. K, P, Ca and Mg were the major mineral elements present in the pollen. Northeastern Brazilian states seem to be promising for bee pollen production; samples from this region presented significantly higher amounts of minerals and showed constant production throughout the year [21]. Most studies use pollen collected by *Apis mellifera* bees. The study of the minerals in pollen collected by stingless bees is incomplete.

#### 4. Conclusion

The high content of essential amino acids, minerals and the sugar mannitol confirmed the high nutritional value of pollen samples of jandaíra bees. Mannitol is mainly used as a sweetener for low-calorie sugar and is used in food products for diabetics. This is an advantage when compared with the pollen collected by bee *Apismellifera*, which contains glucose and fructose as the primary sugars.

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**Table 2.** Mineral content (ppm) in jandaíra bee pollen.

| Mineral   | (ppm ± DP)     |                 |
|-----------|----------------|-----------------|
|           | 2011           | 2009            |
| Calcium   | 1864.1 ± 19.57 | 3424.9 ± 0.00   |
| Copper    | 0.8 ± 0.31     | 1.9 ± 0.13      |
| Iron      | 16.4 ± 0.56    | 33.5 ± 1.37     |
| Potassium | 5918.5 ± 98.81 | 13366.6 ± 0.00  |
| Magnesium | 975.4 ± 25.41  | 2166.1 ± 170.34 |
| Manganese | 35.1 ± 0.35    | 75.0 ± 1.09     |
| Sodium    | -              | -               |
| Zinc      | 36.4 ± 0.34    | 71.2 ± 7.06     |

PPBio 503285/2009-9) and CAPES.

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