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# Volatile Profile of Monofloral Honeys Produced by Stingless Bees from the Brazilian Semiarid Region

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***Abstract*—**In Brazil, there is a diverse fauna of social bees, known by *Meliponinae* or native stingless bees. These bees are important for providing a differentiated product, especially regarding unique sweetness, flavor, and aroma. However, information about the volatile fraction in honey produced by stingless native bees is still lacking. The aim of this work was to characterize the volatile compound profile of monofloral honey produced by jandaíra bees (*Melipona subnitida* Ducke) which used chanana (*Turnera ulmifolia* L.), malícia (*Mimosa quadrivalvis*) and algaroba (*Prosopis juliflora* (Sw.) DC) as their floral sources; and by uruçu bees (*Melipona scutellaris* Latrelle), which used chanana (*Turnera ulmifolia* L.), malícia (*Mimosa quadrivalvis*) and angico (*Anadenanthera colubrina*) as their floral sources. The volatiles were extracted using HS-SPME-GC-MS technique. The condition for the extraction was: equilibration time of 15 minutes, extraction time of 45 min and extraction temperature of 45°C. Through the results obtained, it was observed that the floral source had a strong influence on the aroma profile of the honey under evaluation, since the chemical profiles were marked primarily by the classes of terpenes, norisoprenoids, and benzene derivatives. Furthermore, the results obtained suggest the existence of differentiator compounds and potential markers for the botanical sources evaluated, such as linalool, D-sylvestrene, rose oxide and benzenethanol. These reports represent a valuable contribution to certifying the authenticity of those honey and provides for the first time, information intended for the construction of chemical knowledge of the aroma and flavor that characterize these honey produced in Brazil.

***Keywords*—**Aroma, honey, semiarid, stingless, volatiles.

## I. Introduction

HONEY is a complex mixture of carbohydrates produced by nature, being considered as a very important energetic food due to its nutritional properties as well as its aroma and flavor [1]. Traditionally, the botanical origin of honey can be determined through a series of techniques known as melissopalynology. However, this type of analysis is expensive, requires a large amount of time and depends a great deal on the qualification and judgment of the analyzer. Therefore, there is a tendency to replace pollinic analysis with analytic markers through the discrimination of volatile compounds and of other physicochemical parameters of honey [2]. The volatile compounds of bee honey can be derived from a variety of sources: From the plant or nectar; from the transformation of plant compounds by bee metabolism; from the heating or handling during processing and storage of honey, or even from microbial or environmental contamination.

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Brazil possesses the greatest vegetal genetic diversity in the world, which, combined with its vast territory and climatic variability, enables the production of honey throughout the year. The Brazilian semiarid region stands out for having a type of vegetation which is adapted to the typical climatic conditions of the rainy season and the dry season, thus providing a continuous flux of nectar and pollen during the whole year and favoring the production of different types of honey with singular properties [3].

This region is home to endemic species of stingless bees, also known as meliponas or Meliponini. Among these, two stand out: *Melipona subnitida* Ducke (jandaíra) and *M. scutellaris* Latrelle (uruçu) [4]. These bees produce honey which has been consumed since before the arrival of European colonizers in the American continent. Furthermore, some medicinal properties have also been accredited to this type of honey [3]. Nevertheless, there is almost no information regarding the profile of volatile compounds present in honey produced by stingless bees. Therefore, the aim of this work was to characterize the profile of volatile compounds found in monofloral honeys produced by stingless bees (jandaíra and uruçu) from specific botanical sources which are typically found in the Brazilian semiarid region. This type of information will shed more light on the chemistry of these natural resources, which are still relatively unknown, and will also help with the characterization of the floral and geographical identities of the honeys which are produced exclusively in this region.

## II. Material and Methods

### *A. Sample Acquisition*

The samples of honey obtained from stingless bees M. *subnitida* Ducke (jandaíra) were produced by the floral sources of chanana (*Turnera ulmifolia* L.), malícia (*Mimosa quadrivalvis* L.) e algaroba (*Prosopis juliflora* (Sw.) DC). Meanwhile, the samples of honey produced by stingless bees uruçu (Melipona *scutellaris* Latrelle) used the floral sources of chanana (*Turnera ulmifolia* L.), malícia (*Mimosa quadrivalvis* L.) and angico (*Anadenanthera colubrina*). The honey was obtained directly from meliponary located in the semiarid region of Northeastern Brazil (Seridó region, state of Rio Grande do Norte and Agreste region of state of Paraíba). The honey was collected directly from hives by suction with the aid of disposable syringes. After collection, the honey was stored for a period not exceeding 30 days in sterilized glass jars at 4 °C until analysis.

### *B. Melissopalynological Analysis*

For this analyses, 10 g of each honey sample were diluted in 20 mL of distilled water and then centrifuged at 4000 g for 20 min. The sediment was dried at 40 °C and then mounted with Entellan Rapid (Merck, 1.07961.0500). The honeydew elements and pollen grains (n ¼ 500) were counted and identified in 20 distinct optical areas using an optical microscope (Nikon Optiphot II microscopio; 400 and 1000) [5]. The pollen grains were compared to reference images of the University of São Paulo, São Paulo, Brazil. All samples contained more than 65% pollen grains of the same botanical origin.

### *C. Extraction and Analysis of Volatiles*

The volatiles compounds of honey from stingless bees were extracted by HS-SPME. The Fiber used was a PDMS / DVB, 65 μm (Supelco, Bellofonte, PA, USA). The extraction conditions of volatiles were: 10 g of honey in 10 ml of Milli-Q in 60 ml vials sealed with polytetrafluroethylene silicone septum (Supelco, Bellenfont, PA, USA); equilibrium time of 15 minutes, extraction time of 45 minutes and temperature of extraction of 45 °C.

For separation and identification of compounds extracted from honeys, Varian Saturn 3800 gas chromatograph coupled to Varian Saturn 2000R mass detector and VF-5MS (60 m x 0.25 mm x 0.25 μm) was used. The temperature programming of the gas chromatograph oven started at 40 °C for 2 min, followed by a 2 °C / min ramp up to 60 °C, 3 °C / min up to 90 °C and 4 °C / min up to 240 °C, maintaining this temperature for 10 minutes. The injector temperature was maintained at 250 °C and detector temperature at 250 °C. Helium was used as carrier gas at constant flow rate of 1.0 mL / min gas. Mass spectrometer was operated by electron impact at source temperature of 200 °C and ionization energy of 70 V and scan range from m/z 29 to m/z 400 at 3.33 scan/s. The identification of volatile compounds was based on the comparison of their mass spectra with spectra of compounds previously analyzed, with NIST / EPA / NIH Mass Spectral Database (Version 2008), or spectra published in journals. To confirm the identity of each component, the linear retention index (LRI) was calculated using the retention times of a homologous series of *n*-alkanes C8 - C25 and also by comparing the LRI of authentic compounds analyzed under similar conditions [1], [6]-[8].

### *D. Data Analysis*

The volatile data were analyzed by Principal Component Analysis (PCA) using the Multi-Variate Statistical Package (MVSP v.3.13).

## III. Results and Discussion

The use of gas chromatography associated with mass spectrometry (GC/ME) allowed the detection of 114 compounds in the headspace of honey from stingless bees. Terpenes were the main class of volatile compounds found in honey – a total of 48 were detected. The terpenes were accompanied by the following compounds: 14 esters, 11 norisoprenoids, 11 benzene compounds, 7 furans, 5 ketones, 4 hydrocarbons, 4 alcohols, 4 aromatic aldehydes, 3 aldehydes, 2 acids and 1 sulfur compound. There were terpenes in all of the honeys evaluated, while the acids and the sulfur compound were only found in the honey obtained from algaroba (Fig. 1).

The number of compounds was higher, for both bees, in chanana compared to the other floral species; meanwhile, angico honey presented the lowest number of volatiles in the honeys produced by stingless bees.

In the sample of honey produced by uruçu bees from malícia, a total of 33 compounds were identified. The volatile chemical profile of this samples is characterized primarily by the presence of terpenes (90.9 % of the total chromatogram area), followed by norisoprenoids (2.8 %), alcohols (2.2 %) and other minority compounds. Meanwhile, in the sample of honey produced by jandaíra bees, also from malícia sources, 35 compounds were identified. Once more, the predominant class of compounds were terpenes (85.5 % of the total area). The two different honeys produced using malícia (by jandaíra bees and by uruçu bees) had a total of 21 compounds in common.

As for the analysis of the honeys produced using chanana as the floral source, 41 compounds were detected in the honey produced by jandaíra bees, while the honey generated by uruçu presented 55 compounds in total. Those two honeys had 36 compounds in common. It can be observed that terpenes were once again predominant, occupying 54.5 % and 70.3 % of the total chromatogram area, in the uruçu and jandaíra honeys, respectively. The chemical profile of these samples was also characterized by the class of norisoprenoids, which represented 17.8 % of the total area in uruçu-made honey, and 7.5 % in jandaíra-made honey.

In the analysis of the honey sample produced by jandaíra bees from algaroba sources, 32 compounds were detected. Terpenes accounted for 42% of the total chromatogram, while norisoprenoids and alcohols occupied 16 % and 11 % respectively.

In the honey generated by uruçu bees from angico plants, 15 compounds were identified. They belonged to the following classes: benzene derivatives (41.4 %), terpenes (22.3 %), furans (18.9 %) and aromatic aldehydes (17.4 %).

The chemical classes present in the aroma of the honey samples analyzed were submitted to principal component analysis (PCA) (Fig. 2). The principal components (PC) I and II accumulated 71% of the variation which took place between the samples. In this figure, the chemical classes are represented by vectors whose size is associated with the importance of that class to the segmentation of samples. The direction of the vectors indicates in which samples that chemical class displays a greater influence [9]. The samples are represented by black circles.

Fig. 2 reveals the separation of honeys with different botanical origins. The honey produced by uruçu bees using angico, located in the negative axis of PC I, distanced itself from the other samples and was characterized by a higher concentration of furans and aromatic aldehydes. The sample of honey produced by jandaíra bees using algaroba as a floral source also distanced itself from the other honeys, being mostly influenced by the class of sulfur compounds and being located in the negative axis of PC I and II.

The honeys produced by the different bee species using chanana plants were located in the positive axis of PC I and II, drawing closer due to the influence of terpenes, norisoprenoids, esters, alcohols and hydrocarbons. However, the fact that the uruçu-made sample occupies the farthermost region to the right of axis 1 in Fig. 2 indicates that this sample displayed a higher concentration of the aforementioned compounds, when compared to the jandaíra-made honey.

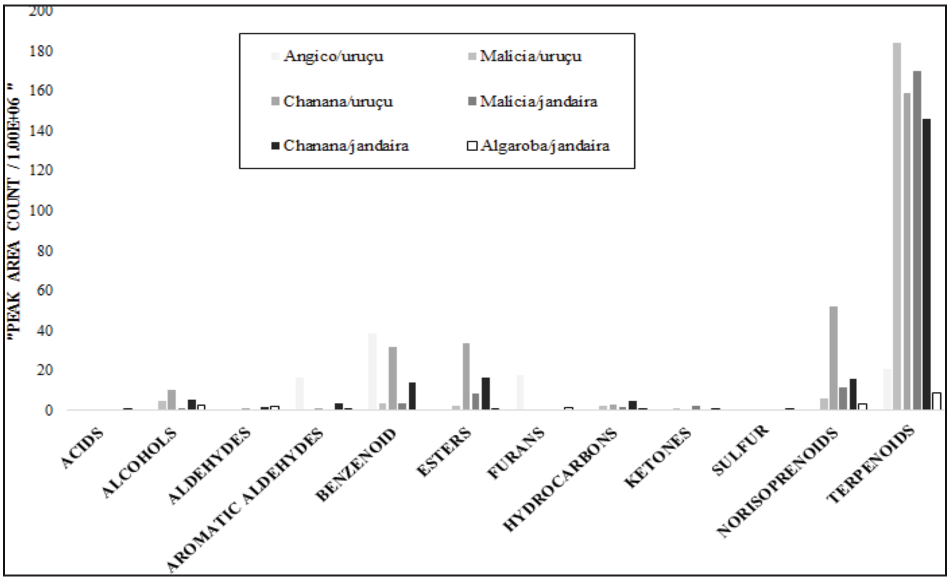


Fig. 1 Distribution of chemical classes of volatiles of monofloral honeys produced by different stingless bees (jandaíra and uruçu) in the semiarid region of Northeastern Brazil from different floral sources

The honeys obtained from malícia plants displayed very similar profiles to one another, which were distinct from the profiles of honeys produced using other botanical sources. The malícia honeys were characterized by the high influence exerted by the ketone class.

The volatile compounds found in honey belong to various classes, including hydrocarbons, aldehydes, alcohols, ketones, acids, esters, benzene and its derivatives, furan and pyran, norisoprenoids, terpenes and its derivatives, and cyclic compounds [10]. However, only compounds derived from plants and its metabolites (terpenes, norisoprenoids, benzenes and its derivatives) should be used to identify the floral origin of honey [2].

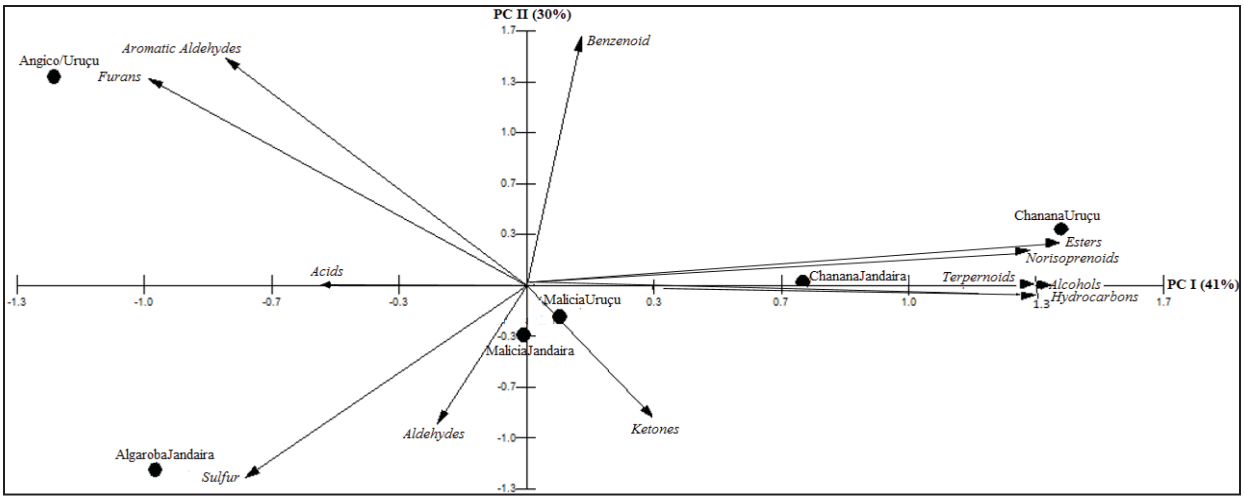


Fig. 2 Principal Component Analysis (PCA) of the chemical classes of volatiles of monofloral honeys produced by different stingless bees (jandaíra and uruçu) in the semiarid region of Northeastern Brazil from different floral sources

The honeys generated from malícia plants were marked by a number of different compounds; for uruçu-made honey, the most abundant compounds were linalool (53.1 %), cis-Linalool oxide (23.7 %) and hotrienol (4.3 %). Meanwhile, in the jandaíra-made honey, the most plentiful compounds were linalool (47.6 %), cis-Linalool oxide (16.4 %), nerol oxide (8.2 %) and hotrienol (5.2 %). These compounds have been frequently reported in studies as being the main constituents of the volatile profile of honey, regardless of the geographical origin [11]-[13]. Cis-linalool oxide (furanoid) detected in honeys from cashew and quince trees native to Northeastern Brazil [14]. Linalool and its derivatives, such as hotrienol, cis-linalool and trans-linalool oxides (piranoid), and lilac aldehydes were all mentioned [15] as volatile compounds which can mark the floral origin of citric honeys collected in Greece. In a similar study, [16] characterized 10 Spanish citric honeys based on their volatile profile, and suggested compounds such as linalool, linalool oxide, µ-terpineol, lilac aldehyde and lilac alcohol isomers, methyl anthranilate be considered floral markers for citric honeys. High levels of linalool oxides, linalool, hotrienol, epoxylinalool, and 2,6-dimethyl-3,7-octadiene-2,6-diol, whose concentrations exceed their detection threshold, are responsible for sweet and floral notes in honey [17].

The monoterpene D-Sylvestrene was the main compound in the two different honeys produced from chanana. Even though this floral origin monoterpene is not commonly found in honeys, its presence may not be a differentiator for honeys made from chanana because this terpene was also found in the honeys produced using the botanical source malícia.

Rose oxide (22.9 % of the total chromatogram) was the most abundant compound found in algaroba honey. This monoterpene is found in flowers, fruit and in essential oils from *Eucalyptus citriodora*, *Dracocephalum heterophyllum*, Damask rose, geranium, *Laggera* spp., and tropical fruit [18]-[20]. It contributes to the fruit and floral notes found in fruit such as lychee and grapes, and has also been synthesized from citronellol for industrial purposes, due to its characteristic rose aroma [21]. Cis-rose oxide has been proposed as a marker for honey from *Tilia cordata* [22]. This is the first time that rose oxide has been reported in Brazilian honey. Even though in literature no data were found regarding the volatile composition of algaroba honey, rose oxide can be considered a differentiator for this particular honey, since it is not commonly found in other honeys (being absent in the other samples analyzed) and it also comes from a botanical source and appears in high concentrations.

The main compound in the honey made from angico was benzenethanol, which reached 40% of the total chromatogram area. While studying tropical honeys produced by *Apis mellifera* bees using angico plants native to the Atlantic Forest, [23] detected benzenethanol as the second most abundant compound, contributing to floral and spicy notes. Benzenethanol has been considered an important odorant for the aroma of tília honey (*Tilia* sp.) and haze honey (*Corylus* sp.), both from Europe [22]; it also contributes to the aroma of honeys made from cashew trees found in the Caating biome [14] and to the aroma of honey made using morrão de cadeia (Atlantic Forest biome) and assa-peixe (Cerrado biome) [24].

The compounds 5-hydroxymethylfurfural, 2,5-dimethyl furan, furfural, 2-furanmetanol and furaneol were only present in the angico and/or algaroba honeys. These compounds are considered a negative indicator of the honey's quality, possibly having been generated by the excessive heating of the sample. However, some authors state that HMF can naturally occur in honey, especially in regions with a hot climate [25].

TABLE I
POTENTIAL VOLATILES MARKS OF MONOFLORAL HONEYS PRODUCED BY DIFFERENT STINGLESS BEES (JANDAÍRA AND URUÇU) IN THE SEMIARID REGION OF NORTHEASTERN BRAZIL FROM DIFFERENT FLORAL SOURCES

| Compounds[2] | LRI | Peak Area Count[1] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Uruçu | | | Jandaíra | | |
| | | Angico | Malícia | Chanana | Malícia | Chanana | Algaroba |
| BENZENOID COMPOUNDS | | | | | | | |
| Benzeneethanol | 1118 | 37.3 | nd[3] | nd | nd | nd | nd |
| FURANS | | | | | | | |
| 2.5-dimethyl furan | <800 | nd | nd | nd | nd | nd | 0.07 |
| Furfural | 836 | 8.3 | nd | nd | nd | nd | nd |
| 2-Furanmethanol | 860 | 0.4 | nd | nd | nd | nd | nd |
| Furaneol | 1052 | 2.3 | nd | nd | nd | nd | 0.1 |
| 5-Hydroxymethylfurfural | 1234 | 6.7 | nd | nd | nd | nd | nd |
| TERPENOIDS | | | | | | | |
| D-Sylvestrene | 1024 | nd | 6.5 | 58.9 | 7.1 | 46.8 | nd |
| Cis-Linalool oxide | 1076 | 10.6 | 47.9 | 5 | 32.5 | 1.7 | 0.5 |
| Linalool | 1091 | nd | 107 | nd | 94.3 | nd | nd |
| Hotrienol | 1092 | 1.4 | 8.8 | 3.7 | 10.4 | 12.1 | 0.3 |
| Rose oxide | 1116 | nd | nd | nd | nd | nd | 4.6 |
| Nerol oxide | 1143 | | 7.3 | 1.6 | 16.3 | 2.5 | nd |

[1] Valour of peak area count / $10^6$ average data from triplicate injections; [2] Compounds identified by MS and LRI; [3] nd = not detected

## IV. CONCLUSION

This study demonstrates that the botanical sources had a strong influence on the volatile profile of monofloral honeys produced by two stingless bees (jandaíra and uruçu) from the semiarid region of Northeastern Brazil. The chemical profiles were characterized primarily by the classes of terpenes, norisoprenoids and benzene derivatives. Furthermore, the results obtained indicate the existence of 'differentiator' compounds and potential markers for the botanical sources evaluated, such as linalool for malícia honeys; D-sylvestrene for chanana honeys; rose oxide for algaroba honeys; and benzenethanol for angico honeys. These reports represent a valuable contribution to certifying the authenticity of those honeys.

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