

Published by

# Archives of Veterinary Science

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<http://dx.doi.org/10.5380/avs.v29i4.97032>



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# Physicochemical and microbiological profile of honey from *Melipona bicolor* (Lepeletier, 1836) from southern Brazil

Submitted: 26/09/2024

Accepted: 03/12/2024

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# ABSTRACT



Stingless bee honey has been attracting interest among consumers owing to its wide variety, sensorial characteristics, therapeutic properties, and high added value. Several studies have been carried out through native bee honey; however, studies around *Melipona bicolor* honey - a rare species remain scarce.



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# ABSTRACT



Accordingly, this research aimed to evaluate the physical-chemical and microbiological profile of *M. bicolor* honey based on the current stingless bee honey legislation of Parana. Hence, three honey samples (200 ml each) from the *M. bicolor* bee were collected by suction and stored in sterile bottles.



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# ABSTRACT



The collecting occurred in three different meliponaries in the Metropolitan region of Curitiba, state of Parana, directly from the bee boxes, it was collected from 4 to 6 boxes from each meliponarie, totalizing next to 2 kg from each farm. They were evaluated for moisture content, reducing sugars, total sugars, apparent sucrose, pH, water activity, hydroxymethylfurfural (HMF), aerobic mesophilic count, total and thermotolerant coliforms, yeast, and mold counts.



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# ABSTRACT



The average result for moisture content was 24.8%, reducing sugars 62.96%, total sugars 65.6%, and pH 2.95. No significant amounts of HMF were detected in the honey from the three properties analyzed. The results of counting aerobic mesophilic bacteria (2.6 to 4.08 log CFU/ ml), total and thermotolerant coliforms ( $\leq 3.0$  MPN/ml), and...



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...counting molds and yeasts ( $\leq 1.0$  to  $2.43$  log CFU/ml), together with the data of analyzing physical-chemical parameters, indicated that the *M. bicolor* honey analyzed in this study complies with the Identity Standards and Quality imposed by Parana State Legislation for Meliponiculture; therefore, it is suitable for consumption and ready for sale.

**Keywords:** Stingless bees; meliponine; Quality; Food Safety.



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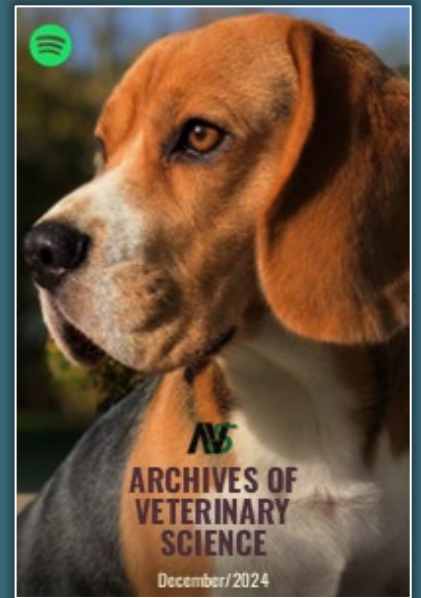
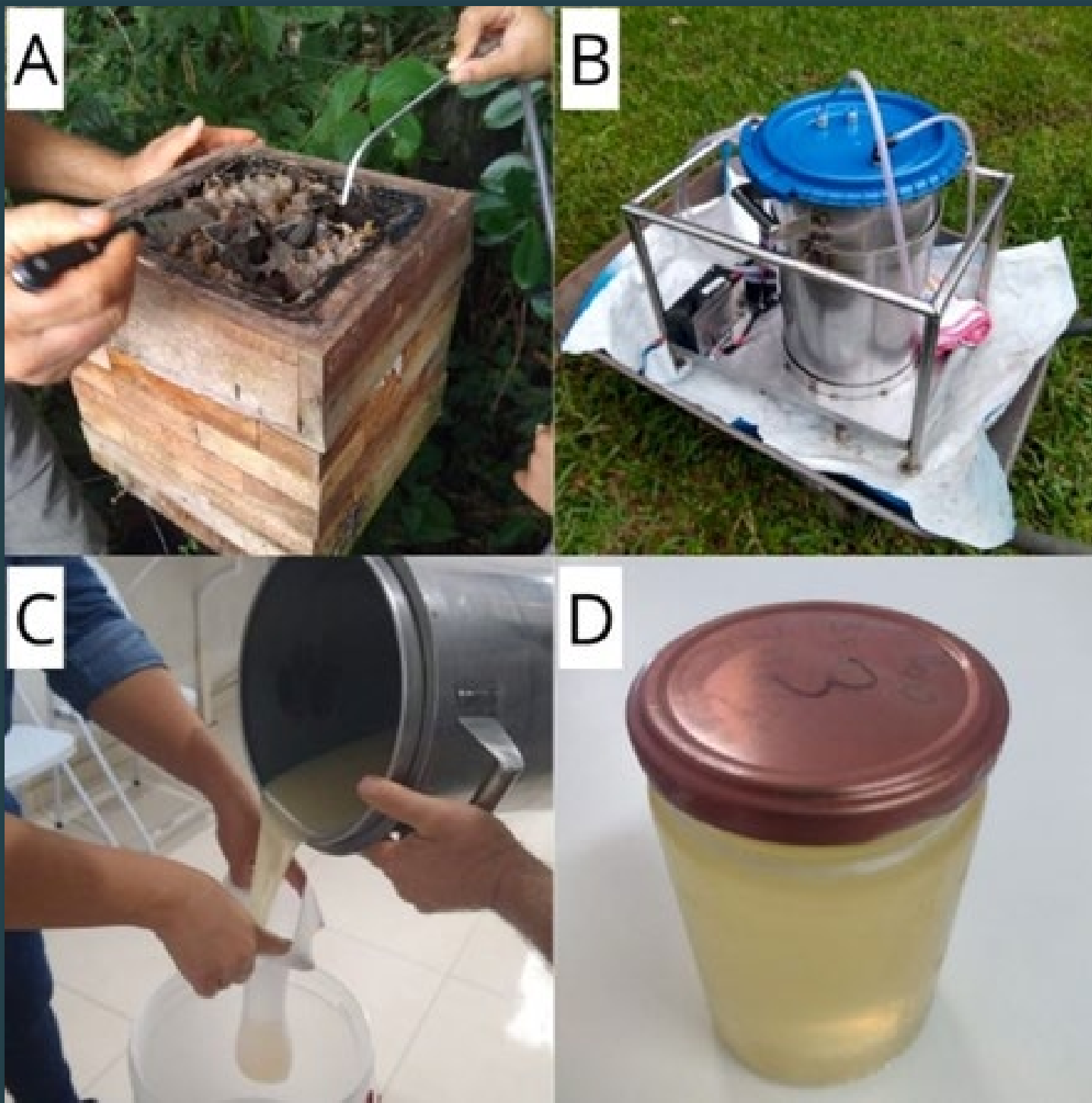
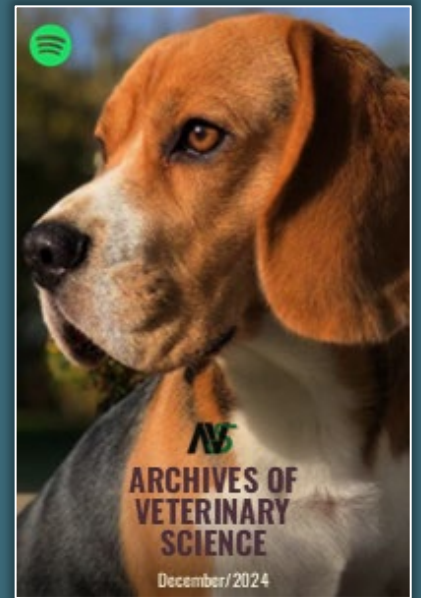


Figure 1 – Honey harvesting process (A) using a sucking machine (B) followed by filtration (C) and packaging in a previously sterilized glass container (D).

To carry out the hydrolysis of the samples, 50 ml of the clarified sample was pipetted into a 100 ml volumetric flask, followed by the addition of 1 ml of hydrochloric acid, and kept in a water bath at  $67^{\circ}\pm 3$  for 15 minutes. Then, after cooling the contents of the volumetric flask to room temperature, neutralization was carried out by adding 2.8 ml of 5 N sodium hydroxide with the aid of litmus paper as an indicator. After filling the volume of the flask with distilled water, titrations were carried out following the principle of reducing sugars; however, the inverted sugar solution was used.



The results were expressed in g/100 g using the following formulas:

$$ARG = \frac{(b - a)}{10 \cdot v} \cdot 5 \cdot f1 \cdot f2$$

$$ANR = \left[ \frac{(b - a) \cdot 5 \cdot (f1 \cdot 2)}{10} \cdot v - AR \right] \cdot f2$$



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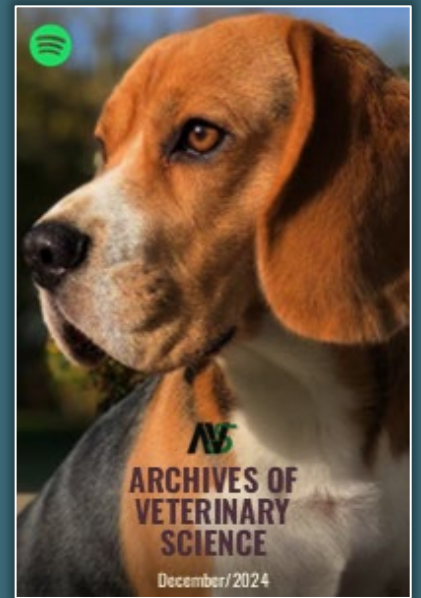
## Total Sugars

ARG is glucose-reducing sugars, ANR is non-reducing sugars, and AR is glucose-reducing sugars. f1 represents all dilutions and quantities of mass or volume used, f2 represents the conversion factor for expressing the results in glucose, a represents the number of ml of glucose solution used in titrating the sample, b is equivalent to the number of ml of the glucose solution used in the blank titration and v is the volume of the prepared sample used in the titration, in ml. To express total sugars, the sum of AR in glucose plus ANR in sucrose was performed. Sugars in sucrose were expressed using the formula: Total Sugars in Sucrose = (AR in glucose x 0.96) + ANR in sucrose. To express total sugars in glucose, the following formula was used: Total Sugars in Glucose = AR in glucose + (ANR in sucrose ÷ 0.96).



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## Hydroxymethylfurfural

Quantification of the HMF concentrations of the samples was done by comparing the heights of the sample peaks with the heights of the peaks corresponding to the HMF standard, then the following formula was used to obtain the results:

$$HMF \left( \frac{mg}{kg} \right) = \frac{C \cdot PH \cdot x}{PH'} \cdot W$$

Where C is the concentration of the standard mg/L, PH is the height of the unknown peak, PH' is the height of the standard peak, W is the weight of the sample in grams and the dilution factor.



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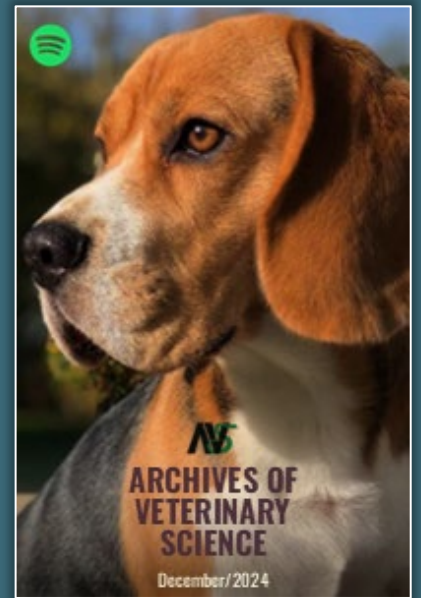
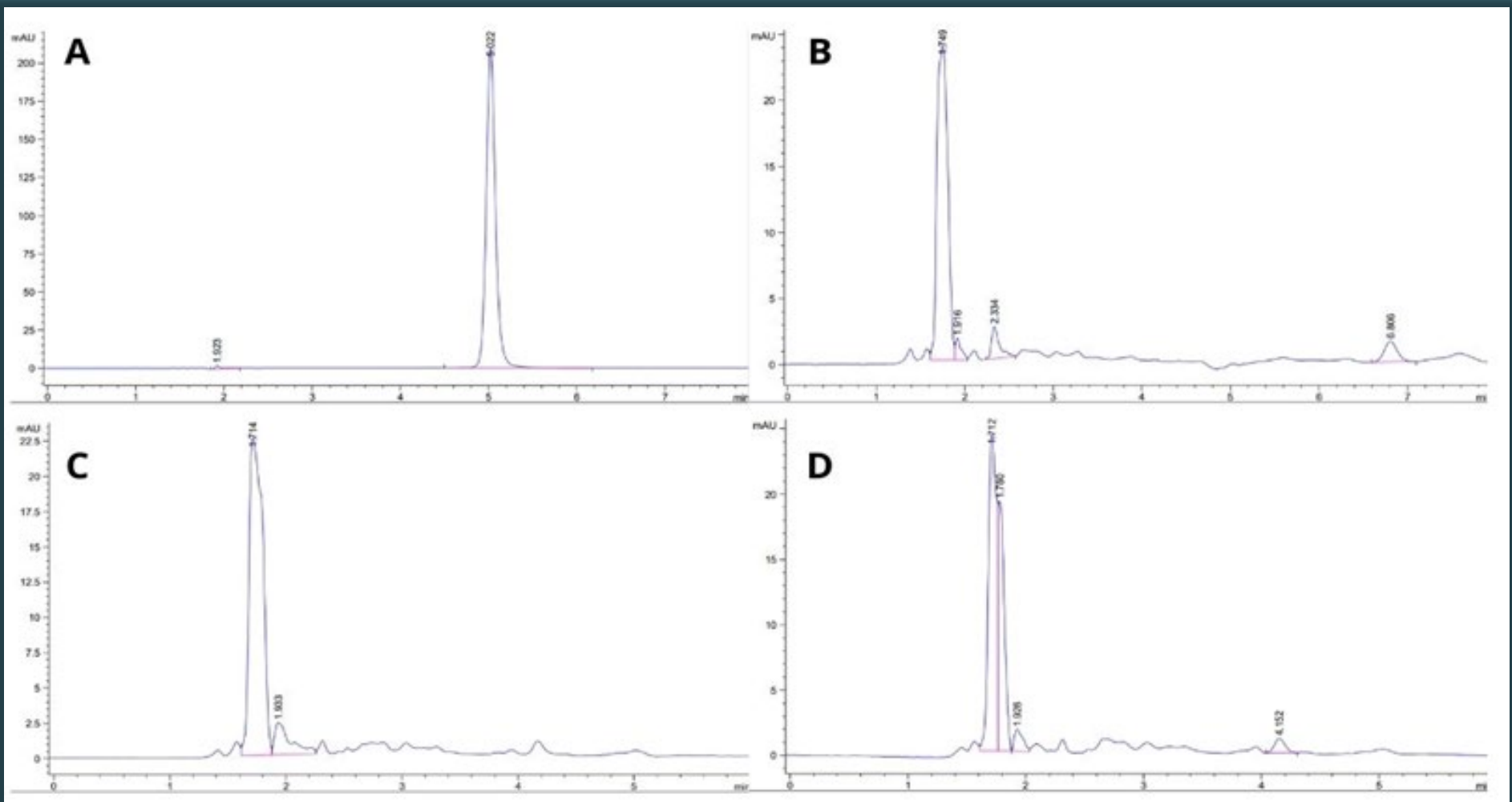


Figure 2 – Standard peak for HMF at concentration 0.01 mg/ml (A); HMF HPLC chromatography of *Melipona bicolor* honey collected in the metropolitan region of Curitiba-PR. A: -25.874460, -49.404861; B: -25.724054, -49.300099; and C: - 25.803695, -49.303595; and D: - 25.803695, -49.303595.



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# CONCLUSION



Physico-chemical and microbiological parameters of honey are fundamental to verify its quality and safety, enabling it to be sold by the standards required by legislation ensuring consumer health. The moisture content, sugars, pH, and HMF obtained in the present study indicate that the M. bicolor honey collected in three different properties located in the metropolitan region of Curitiba, PR, is aligned with the minimum requirements existing in the Legislation and Technical Regulations of Identity and Quality for Meliponiculture in Brazil.



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Therefore, they are suitable for consumption and commercialization. However, the lack of criteria for water activity and standard counts of mesophilic bacteria highlights the importance of determining parameters that represent the reality of stingless bee honey, both to help the producer market the product and to prevent fraud.



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# CONCLUSION



Furthermore, the distinctions between the physical-chemical and microbiological parameters of honey from bees native to different regions, even if it is produced by the same species, make it difficult to develop legislation that applies generally to the entire Brazilian territory. Therefore, each State must establish its parameters, having scales that enable compliance with the required standards, regardless of the species of bee used, or even classify the parameters according to the respective genera.



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