ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 11, pp. 41815-41818, November, 2020
https://doi.org/10.37118/ijdr.20405.11.2020

RESEARCH ARTICLE OPEN ACCESS

MICROBIOLOGICAL QUALITY OF TIÚBA HONEY (*MELIPONA FASCICULATA* SMITH) PRODUCED IN A MINERATOR

Claudio A. de J. Nascimento[1*], Jonas A. Mesquita[1,2], Bruna F. S. de Sousa[1,3], Gabriel G. Santos[4], Thailson de J. S. Silva[4], André de S. Rodrigues[4], Edyane M. dos Santos[1,5], Camila M. Silva[6], and José de R. S. Barros[1,6]

[1]Master's student in Agronomy, Soil Science, at the Federal Rural University of Rio de Janeiro (UFRRJ); [1,2]Master's student in Agronomy, Genetics and Plant Breeding, at Universidade Estadual Paulista (UNESP); [1,3]Agronomist Engineer at the Universidad Politécnica de Madrid/ Centre for Plant Biotechnology and Genomics (CBGP); [4]Graduating in Agricultural Engineering from the State University of Maranhão (UEMA); [5]PhD student in Evolutionary Genetics and Molecular Biology at the Federal University of São Carlos (UFSCAR); [6]PhD in Fisheries Engineering and Adjunct Professor I at the State University of Maranhão; [1,6]PhD in Genetics, Conservation and Evolutionary Biology (INPA), Prof. of Genetics at the State University of Maranhão (UEMA), São Luís / MA, Brazil

ARTICLE INFO

Article History:
Received 14th August, 2020
Received in revised form
20th September, 2020
Accepted 22nd October, 2020
Published online 24th November, 2020

Key Words:
Contaminant; Meliponario; Safety.

****Corresponding author:***
Claudio A. de J. Nascimento

ABSTRACT

The objective of this work was to evaluate the microbiological quality of honey from Tíuba, Melipona fasciculata, produced in a mining company, where the air particulate index is high, in the city of São Luís, Maranhão. Seventy honey samples from 15 M. fasciculata bee hives from the mining company Meliponário were analyzed during the period from 2017 to 2018. Of the analyzed samples, 14% had contamination by Molds and Yeasts, while Aerophilic Mesophilic Bacteria were detected in 13% of the samples. All the honey samples analyzed did not present microbiological contaminants from the group of Coliforms and Salmonella sp. The moisture content was slightly higher (21.1%) than established by law, but within the expected for native bee honeys. The content of total soluble solids found was 79.36% and specific weight of 1.40 kg.L-1. The analyzes showed that before the relocation of the meliponary, the honeys presented microbiological contaminants such as molds and yeasts and aerobic mesophilic bacteria. Analyzes that study the physical-chemical profile and the presence of trace elements should be performed to attest the safety of the honey produced.

Citation: ***Claudio A. de J. Nascimento, Jonas A. Mesquita, Bruna F. S. de Sousa, Gabriel G. Santos, Thailson de J. S. Silva, André de S. Rodrigues, Edyane M. dos Santos, Camila M. Silva and José de R. S. Barros, 2020.*** "Microbiological quality of tiúba honey (melipona fasciculata smith) produced in a minerator", *International Journal of Development Research*, 10, (11), 41815-41818.

INTRODUCTION

The knowledge about stingless bees and meliponiculture is very old in the American continent. Before the arrival of the *Apis mellifera* bee on the continent, or the exploration of cane for the manufacture of sugar, honey from native bees, or stingless bees, was used as the main natural sweetener, and an indispensable energy source in long journeys (Villas-Bôas, 2012). Among the species of stingless bees, the species known as Tiúba, *Melipona fasciculata*, has an important insertion in the regional market, producing quality honey and with wide possibilities for exploration on an economic scale. The honey from Tiúba, as well as from other native bees, because it is more liquid, acidic and soft in color than honey from bees of the genus *Apis*, reaches much higher prices (Holanda *et al*., 2015). Honey is the main product made by bees, standing out in quality as food, this unique product has numerous therapeutic properties, being used by popular medicine in various forms and associations with herbal medicines, with emphasis on native bee honeys (Ávila *et al*., 2019; Tavares *et al*., 2006). The honey of stingless bees has a higher moisture content, which increases its water activity, thus making it more susceptible to microbial growth, mainly due to the presence of yeasts in their original composition. This moisture content results in a less viscous honey and a slower crystallization when compared to that of A. mellifera honey (Silva *et al*., 2010). Alves *et al*. (2009), affirm that the non application of Good Manufacturing Practices during the

handling, harvesting and processing of honey of Melíponas bees, associated with its high humidity (21% to 45%) and the presence of microorganisms, can cause problems conservation of the product, reducing its shelf life, which may make it unsuitable for human consumption. In addition, the proximity to sources of contamination, such as streams, animal husbandry and others, can impair the microbiological quality of honey (Oliveira*et al*., 2015). In this context, the work aimed to evaluate the microbiological quality of honey from Tíuba, *M. fasciculata*, produced in a mining company, where the index of air particulate matter is high, in the city of São Luís, Maranhão.

MATERIAL AND METHODS

The collection of the material was carried out in two moments: (i) old location of the meliponary; (ii) after the transfer of the meliponary (Figure 1). The change was the result of observations on the structure of hives in the meliponary.

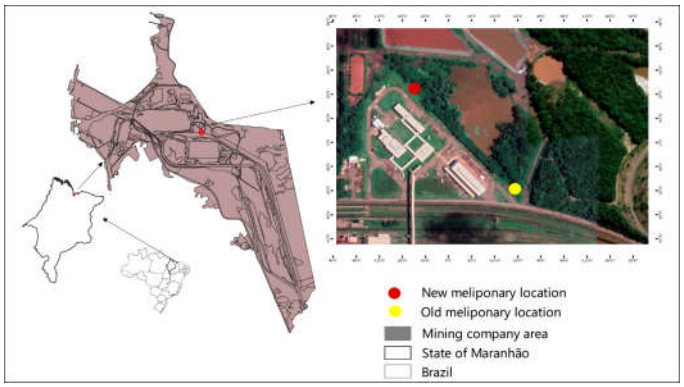

Figure 1. Location of the mining company's meliponary in São Luís, Maranhão, before and after its new installation

Nine monthly collections were carried out between 2017 and 2018, where 70 honey samples were collected from 15 hives of *M. fasciculata* located in the company's meliponary (Table 1). The samples were collected with the aid of kits containing sterile materials (sterile plastic tubes, gloves, syringes, syringe elongation devices and identification labels), in order to reduce possible contamination in the honey packaging. Then they were stored at a temperature of 25ºC in a room with controlled temperature, without exposure to direct light and analyzed at the Laboratory of Genetics and Molecular Biology - LabWick (State University of Maranhão).

Table 1. Number of samples of honey Tiúba (*M. fasciculata*) collected by hive located in the meliponary of the mining company in São Luís, Maranhão

Amostra	Nº de amostras
Colmeia 01	07
Colmeia 02	05
Colmeia 03	04
Colmeia 04	04
Colmeia 05	06
Colmeia 06	03
Colmeia 07	08
Colmeia 08	05
Colmeia 09	07
Colmeia 10	06
Colmeia 11	05
Colmeia 12	03
Colmeia 13	01
Colmeia 14	03
Colmeia 15	03
Total	70

The analyzes were carried out in accordance with Normative Instruction No. 11, of October 20, 2000, of the Ministry of Agriculture and Supply of Brazil, (Brazil, 2001). The instruction establishes the regulation for the analysis of *A. mellifera* honey at an acceptable value of 1.0 x 10^2 UFC.g^{-1} for molds and yeasts and absence (<3.0 NMP.g^{-1}) for total and thermotolerant coliforms. Since there is no standard count in Brazilian legislation, the reference number of molds and yeasts was used for the analysis of aerobic mesophilic bacteria (Brazil, 2000). The methodology used was recommended according to the Manual of Official Analytical Methods for Microbiological Analysis for Control of Animal and Water Products (Brazil, 2003).

Sample preparation: 25g were weighed in aseptic conditions for each honey sample and diluted in 225 mL of peptone water, corresponding to the 10-1 dilution and from which the other dilutions (10^{-2} and 10^{-3}) were made.

Quantification of thermotolerant coliforms (CT): The determination of the Most Probable Number (NMP) of TC was made using the fermentation technique in multiple tubes, using the Tryptose Lauryl Sulfate broth (35ºC/48h) for the presumptive test and the EC Broth (45ºC/48h) for the CT test. The calculation of the NMP was made using the Hoskins table, cited by Bloggett (2006) and expressed in NMP.g^{-1}.

Mold and Yeast Count: For standard mold and yeast counts, the pour-plate technique was used, where 1 mL of the selected dilutions (10^{-1} to 10^{-3}) was transferred to duplicate Petri dishes containing potato dextrose agar (BDA). The plates were incubated for five days in BOD chambers (temperature adjusted to 25 °C) and after that, plates containing 30 to 300 colonies were selected. Colonies were counted with the help of colony counters, then determining the number of colony-forming units (CFU). The standard counting of Mesophilic Aerobic Bacteria plates was performed using the deep sowing technique, where 1 mL of the selected dilutions (10^{-1} to 10^{-3}) were transferred to Petri dishes containing the standard counting agar (PCA). The plates were incubated in an oven at 35 ºC for 48 hours and after that period they were selected for counting, determining the number of colony forming units (CFU).

Detection of Salmonella sp.: The detection of the absence/presence of *Salmonella* was made according to the recommendations of Andrews and Hammack (2007). Aseptically, 25 g of each sample was taken and inoculated in Lactated Broth for 24 hours at 35 °C. After the incubation period, selective enrichment in Tetrathionate Broth (Difco) and Rappaport (Difco) followed and selective plating in Hektoen Agar (Difco) and MacConkey (Difco). Colonies with morphological characteristics compatible with *Salmonella* were screened on Triple Sugar Agar Agar (TSI-Difco), Lysine Iron Agar (LIA-Difco) and Indol Motility Sulfide Agar (SIM-Difco), in addition to serological characterization with polyvalent somatic antiserum (poly O:H).

Humidity, Total Solids and Specific Weight: For the determination of the honey moisture, the Chataway refractometric method was used, which has values on the specific weight and total soluble solids, going through a correction according to the temperature, following the physicochemical methods for food analysis of Instituto Adolfo Lutz (2008).

RESULTS AND DISCUSSION

During the collection of the samples and before the change of location, it was possible to observe problems in the structure of the meliponary and in the hives, such as, for example, the lack of orientation (direct incidence of the setting sun) and absence of fluid to control ants, presence of ants in the structure of the meliponary and hives, in addition to the presence of eggs from other insects. The results of the microbiological analysis of the 70 samples from 15 hives of *M. fasciculata*, collected in the mining company, during the years 2017 and 2018, are shown in Table 2, showing that 73% of the samples did not present contamination. All samples analyzed did not show total coliforms and thermotolerant coliforms. Although not necessarily disease-causing, bacteria in the coliform group are microorganisms considered indicators (Matos*et al*., 2011). The same results were observed in the studies by Fernandes *et al*. (2020), working with Tíuba in Baixada Maranhense and by Holanda *et al*. (2015), with Tíuba in the Cerrado Maranhense. This indicates that species in question has hygienic habits that favor non-contamination by this group of microorganisms. According to Barros and Batista (2008), coliforms are only found in honey of this species when failures in hygiene or lack of good practices on the part of the producer, during the collection and storage of the product.

Table 2. Percentage of samples free of contamination, thermotolerant coliforms, molds and yeasts and aerobic mesophilic bacteria in different locations in the Baixada Maranhense and average values of humidity, total soluble solids and specific weight

Parâmetros	Antiga localização	Nova localização
Coliformes Totais (NMP.g-1)	100%	100%
Bolores e Leveduras (UFC.g^{-1})	86%	100%
Bactérias Mesófilas (UFC.g-1)	87%	100%
Salmonella sp. (ausência/presença)	Ausência	Ausência
Umidade (%)	21,10	-
Peso específico (kg.L^{-1})	1,40	-
Sólidos Solúveis Totais (°Brix)	79,36	-

The tolerable levels of fungi in food are standardized by the Brazilian health surveillance agency and also by the Ministry of Agriculture and Supply, which advocates in the technical regulation for fixing the identity and quality of honey, establishing a tolerable value of 1.0x10² UFC.g- 1, for molds and yeasts (Brazil, 2001). Before the change of the meliponary and the adoption of measures of good honey collection practices, 14% of the analyzed samples presented values above the maximum established by the technical regulation for food, RDC 012 (Brazil, 2001), being considered, therefore, inappropriate for the direct human consumption (Table 2). Contamination may be due to pollen, nectar, bee microbiota and/or inadequate conditions for handling and processing honey (Fernandes*et al*., 2018). In addition, according to Gois et al. (2013), osmophilic microorganisms, such as molds and yeasts, represent the greatest risk to the quality of honey, because they can survive in acidic conditions and are not inhibited by sugar. Thus showing that molds and yeasts are naturally found in honey and are associated with the physical and chemical characteristics of the product (Sousa *et al*., 2012). The types of molds most found and considered dangerous because they produce toxic metabolites are *Penicillium*, Mucor and *Aspergillus* (Nishio*et al*., 2016). Although they do not reproduce in honey, they survive in this environment and have high counts indicating recent contamination by the bee collection environment, hive or processing equipment (Pereira, 2008). After the fourth month of collection and establishment in its new location, it was possible to find results <30 UFC.g^{-1} and reach a considerable acceptable standard for consumption of the honey produced in the mining company, in relation to the microbiological characteristics. Although Brazilian legislation does not determine the pattern of aerobic mesophilic bacteria for honey, they can indicate unsatisfactory health quality. In the analysis, 13% of the honey samples analyzed showed values above 100 UFC.g^{-1} (Table 2) in their former location. This can be explained by the fact that the meliponary was initially implanted in an improper location, with deficiencies in the installation project, such as the direct incidence of the setting sun, in addition to the action of factors such as wind, and susceptibility to the invasion of insects such as ants in the interior. of the hive. Another factor that was taken into account was the presence of lakes containing contaminants, preventing the search for drinking water by bees (Figure 01). Grosso*et al*. (2002), state that one of the factors responsible for the presence of these microorganisms is the implantation of the meliponaries in inappropriate places due to the presence of winds and insects. These authors emphasize that honey is not a sterile product, being susceptible to contamination when handled without the adoption of adequate hygienic measures.

In all samples analyzed, the presence of *Salmonella* sp. (Table 2), which characterizes these honeys, according to RDC nº 12, of January 2, 2001 (Brazil, 2001), which advocates the absence of *Salmonell*a sp. in 25 grams of honey. These results corroborate those obtained by Fernandes*et al*. (2018), who assessed the quality of honey from *M. fasciculata* bees in four municipalities in the Munim watershed and six municipalities in the Pericumã watershed in the state of Maranhão. When observing the humidity values in Table 2, it is noticed that the humidity is slightly above that established by the legislation (maximum of 18%), however when compared with the proposal Camargo *et al.* (2017), for the regulation of honey from meliponines, which proposes up to 40g/100g of moisture, or 40% moisture in fresh honey, the honeys are within the acceptable range. Moisture is an extremely important factor in food because it is related to stability, quality and composition, and can affect product characteristics such as storage, packaging, processing, being also the main factor for microbiological processes, such as the development of fungi , yeasts, bacteria and for the development of insects (Park and Antônio, 2006). The content of total soluble solids found was 79.36%, a result greater than that found for *Melipona scutellaris* (72%) by Campos *et al*. (2010), and very close to that of *A. mellifera* (Oliveira*et al*., 2015).

Conclusions

The analysis showed that before the relocation of the meliponary, the honeys presented microbiological contaminants such as molds and yeasts and aerobic mesophilic bacteria. After changing the location of the meliponary site and applying good collection and handling practices, the honey samples were characterized as within the microbiological standards established by Normative Instruction No. 11, of October 20, 2000, of the Ministry of Agriculture and Supply Brazil and therefore considered fit for human consumption. However, studies that assess the

physical-chemical profile and the presence of trace elements must be carried out to attest the safety of the honey produced.

Acknowledgments

The authors would like to thank the mining company located in Maranhão for funding the research and for the scholarship granted, the Foundation for Supporting Research and Scientific and Technological Development of Maranhão (FAPEMA) (Process: AGRIF-04921/15) for structuring the microbiology sector LabWick (UEMA).

REFERENCES

Alves, E. M., Toledo, V. A. A., Marchini, L. C., Sereia, M. J., Moreti, A. C. C. C., Neves, C. A.; Santos, A. A. (2009) Presença de coliformes, bolores e leveduras em amostras de mel orgânico de abelhas africanizadas das ilhas do alto rio Paraná. *Ciência Rural*, pp. 2222-2224.

Ávila, S., Hornunga, P.S., Teixeiraa, G.L., Malungab, L.N., Apea-Bah, F.B., Beuxa, M.R., Betab, T., Ribani, R.H. (2019) Bioactive compounds and biological properties of Brazilian stingless bee honey have a strong relationship with the pollen floral origin. *Food Research International,* 123, pp. 1-10.

Barros, H. D., Batista, E. (2008) Avaliação físico-química e microbiológica de diferentes marcas de mel. *Higiene Alimentar*, 22(166/167), p. 76-79.

Brasil, Ministério da Agricultura, Pecuária e Abastecimento. (2000) Defesa Animal. Legislações. Legislação por Assunto. Legislação de Produtos Apícolas e Derivados. Instrução Normativa n. 11, de 20 de outubro de 2000. *Regulamento técnico de identidade e qualidade do mel*.

Brasil, Ministério da Saúde, Agência Nacional de Vigilância Sanitária. (2001) *Resolução RDC 12*, de 02 de janeiro de 2001. Regulamento técnico sobre padrões microbiológicos para alimentos.

Brasil, Ministério de Agricultura e do Abastecimento (MAPA). (2003) Manual de Métodos de Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água – *Instrução Normativa SDA Nº 62*, 26 de agosto.

Camargo, R. C. R. et al. (2017) Stingless bee honey: technical regulation proposal. *Brazilian Journal of Food Technology*, 20(20), pp. 1-6.

Campos, F. S., Gois, G. C., Carneiro, G. G. (2010) Parâmetros físico-químicos do mel de abelhas *Melipona scutellaris* produzido no Estado da Paraíba. *Zootecnia/Zootechny*, Uberaba, 7, pp. 186-190.

Fernandes, R. T., Silva, A. C. C., Rosa, I. G. (2020) Características de qualidade do mel de abelha sem ferrão (*Melipona fasciculata*) produzidos na baixada maranhense. *Brazilian Journal of Development*, 6(6), pp. 41268-41275.

Fernandes, R.T. et al. (2018) Microbiological and physicalchemical characteristics of honeys from the bee *Melipona fasciculata* produced in two regions of Brazil. *Ciência Rural*, 48(5), 2018.

Grosso, G.S. et al. (2002) Características microbiológicas de las mieles tropicales de *Apis mellifera***.** *Apicultura*, Tolima-Espanã, pp. 1-7.

Holanda, C.A., Brandão, C.M., Souza, J.L., Ribeiro, M.N.S., Alves, L.M.C., Costa, M.C.P. (2015) Qualidade e estimativa do tempo de consumo do Mel de Tiúba (*Melipona fasciculata* SMITH) produzido na região do cerrado maranhense. *Brazilian Journal of Food Research*, pp. 53-64.

Ial (Instituto Adolfo Lutz). (2008) *Normas analíticas do Instituto Adolfo Lutz*. 4 ed: IAL, São Paulo, 2008. pp. 1018.

Matos, I. T. S. R. et al. (2011) Qualidade microbiológica do mel de Meliponas produzido na Amazônia Central (Parintis-AM-Brasil). *Revista Verde, Mossoró*, 6(4), pp. 91-95.

Nishio, E.K. et al. (2016) Antibacterial synergic effect of honey from two stingless bees: *Scaptotrigona bipunctata*Lepeletier, 1836, and *S. postica* Latreille, 1807. *Scientific Reports*, 6, pp .21641.

Oliveira, M. E. J., Vieira, R. P., Gherardi, S. E. M. (2015) Qualidade de mel produzido sob a influência do agronegócio convencional e agroecológico. *Revista Brasileira de Produtos Agroindustriais*, Campina Grande, 17(4), pp.433-439.

Park, K. J., Antonio, G. C. (2006) *Análises de materiais biológicos*. Campinas: Universidade Estadual de Campinas, Faculdade de Engenharia Agrícola.

Pereira, A.P. (2008) *Caracterização do mel com vista à produção de Hidromel***.** Dissertação, Escola Superior Agrária de Bragança.

Silva, N., Junqueira, C. A. V., Silveira, F. A. N., T, H. M., Santos, F. S. R., Gomes, A. R. R. (2010) *Manual de Métodos de Análise Microbiológica de Alimentos e Água*, 4ª edição, pp. 95-164.

Sousa, A.A., Azevedo, E., Lima, E.E., Silva, A.P.F. (2012) Alimentos orgânicos e saúde humana: estudo sobre as controvérsias.*Revista Panamericana de Salud Publica*, Washington, 31(6), p. 513-517.

Tavares, J.P., Martins, I. L., Vieira, A. S., Lima, F. A. V., Fernando A. F. B., Moraes, M. O., Moraes, M. E. A. (2006) Estudo de toxicologia clínica de um fitoterápico a base de associações de plantas, mel e própolis. *Revista Brasileira de Farmacognosia*, 16(3), pp. 350-356.

Villas-Bôas, J. (2012) *Manual Tecnológico: Mel de abelhas sem ferrão*.

****