Advances in Agricultural Science

Volume 4 (2016), Issue 04, 64-71

Journal homepage: www.aaasjournal.org

ISSN: 2588-3801

Research Article Open Access

Characterization of the honey from *Myracrodruon urundeuva* (Anacardiceae - *Aroeira*) in the Dry Forest of northern of Minas Gerais/Brazil

Esther Margarida Alves Ferreira Bastos [1*], Paula de Souza São Thiago Calaça [2], Cláudia Marques Gonçalves Simeão [1], Mariem Rodrigues Ribeiro da Cunha [2]

[1] *Diretoria de Pesquisa e Desenvolvimento - Fundação Ezequiel Dias - Rua Conde Pereira Carneiro, 80, Gameleira, Belo Horizonte/MG, 30510-010, Brazil.*

[2] *Diretoria do Instituto Otávio Magalhães – Fundação Ezequiel Dias - Rua Conde Pereira Carneiro, 80, Gameleira, Belo Horizonte/MG, 30510-010. Brazil.*

ARTICLE INFO

Article history:
Received: February 22, 2016
Accepted: April 13, 2016
Available online: April 29, 2016

Keywords:
Dry Forest
Honey
Myracrodruon urundeuva
Apis mellifera
Melissopalynology

[*] Corresponding Author;
E. Mail:
embastos@funed.mg.gov.br

ABSTRACT

Brazilian Seasonally Dry Tropical Forests are deciduous and semi-deciduous forests conditioned by the prevailing semiarid climate and in Minas Gerais State, it is located in a region with one of the worsts social and economic parameters in the country and beekeeping represents a sustainable alternative income for inhabitants of this region. In this way, this study aimed to characterize a unique honey produced by *Apis mellifera* in a Brazilian Dry Forest, located in the northern of Minas Gerais State, Brazil. From 2010 to 2011 honey samples were collected, microscopic and physicochemical analyses were performed (color, invertase enzyme, electrical conductivity, ash, fructose, glucose, sucrose, erlose and melezitose content). Twenty pollen types were identified in honey samples but *Myracrodruon urundeuva* (Anacardiaceae - *aroeira*) was dominant, and was considered the most important source of pollen and nectar for honeybees during the studied period. This honey showed dark amber color, high invertase content (119.46 U/kg), electrical conductivity of 724,5 µS.cm-1, high acidity (50.11 meq.kg-1), high percentage of ash content (0.28%), presence of erlose (0.77%) and melezitose (0.08%), average values are given. This study contributes to the typification of the honey from *M. urundeuva* (Aroeira) in the Dry Forest of northern of Minas Gerais/Brazil.

Introduction

Seasonally dry tropical forests are deciduous and semi- deciduous forests distributed in regions characterized by mean annual precipitation below 1600 mm and a distinct seasonality in rainfall (Murphy & Lugo, 1995). In Brazil they are conditioned by the prevailing semiarid climate, with high potential evapotranspiration throughout the year (1500–2000 mm y^{-1}) and low rainfall (300–1000 mm y^{-1}), which is usually concentrated in 3–5 months and is very erratic (Reddy, 1983). Their inhabitant's main economic activities are cattle and subsistence agriculture, but land productivity is low and since resources are limited and birth rates have been high, the area has been a center of continuous migration, which increases during catastrophic drought periods. Social and economic parameters are the worst in the country, from

lowest per capita income to highest illiteracy (Murphy & Lugo, 1995). Besides that, this vegetation is threatened by economic activity (Miles *et al.*, 2006) and beekeeping represents a sustainable alternative income.

In this way, its needed to seek for strategies in the search for a balanced co-existence between farming families and the semi-arid environment such as recuperation and enrichment of depleted areas, besides increasing the biome's productive potential for beekeeping, and goat and sheep farming (Brasil, 2014).

Honey is a natural sweet substance produced by bees from the nectar (floral honey) and/or excretions from sap-sucking insects (Hemiptera) that is called honeydew honey. Bees collect and transform these substances combining them with enzymes, dehydrate and store the mixture in honeycombs to ripen (Codex Stan, 2001).

The aim of this study is characterize physicochemical and microscopically the honey originated from the Dry Forest from northern of Minas Gerais State in Brazil, in order to create its Geographical Indication (GI).

Materials and Methods

2.1 Obtaining samples of honey

To obtain the 26 honey samples, bimonthly collections were made in hyper dry season (Figure 1-A, N=18), from May to October 2010 (total rainfall=31.4mm; average maximum temperature=31.5ºC), and in the dry season (Figure 1-B, N=8) (total rainfall=885.2mm; average maximum temperature=31.9ºC) (INMET, 2014), from November 2010 to April 2011, in apiaries in the cities of Bocaiúva (43º 48'53"W 17º06 '29" S), Janaúba (43º 18' 31" W 15º 48 '09" S), Mato Verde (42º 51' 59" W 15º 23'50" S), district of Tocaias and Jenipapo de Minas (42º 15"29" W 17º 04'59" S). All these cities are located in areas bordering Dry Forest areas (Figure 2).

2.2 Microscopic analysis of honey samples

The sediment for microscopic analysis of honey was obtained from 10 g of honey thoroughly mixed, dissolved in 20 ml of distilled water and centrifuged for 3 to 5 minutes at 2500 rpm. After decanted, the sediment was washed with distilled water. After further centrifugation, the sediment was suspended in 5 ml of a mixture of equal parts of distilled water and glycerin and was centrifuged, decanted and mounted on microscope slides with glycerol-gelatin sealing with paraffin (Maurizio & Louveaux, 1965). For each sample two slides were made and analyzed. Pollen types were identified considering its morphology and comparing with reference pollen slide collection of Ezequiel Dias Foundation (Funed-Pol) made from plant species collected directly in the studied area. Quantitative analysis of the slides was made by counting the total pollen grains per sample and grouped by pollen types. Then, pollen types were grouped into four categories considering its relative frequency: Dominant Pollen (DP>45%); Accessory Pollen (AP; frequencies from15% to 45%); Minor Important Pollen (MIP; frequencies from 3% to 15%); Minor Occasional Pollen (MOP; <3%) according to Zander (*apud* Maurizio & Louveaux, 1965).

2.3 Physicochemical analyzes

The color of honey samples was determined by spectrophotometric method according to the Association of Official Analytical Chemists (AOAC, 1998); the activity of the enzyme invertase was determined according Bogdanov *et al.* (2002). Acidity were determined based on the 0,05 N NaOH by neutralization of gluconic acid (White, 1980; Moraes & Teixeira, 1998). The total

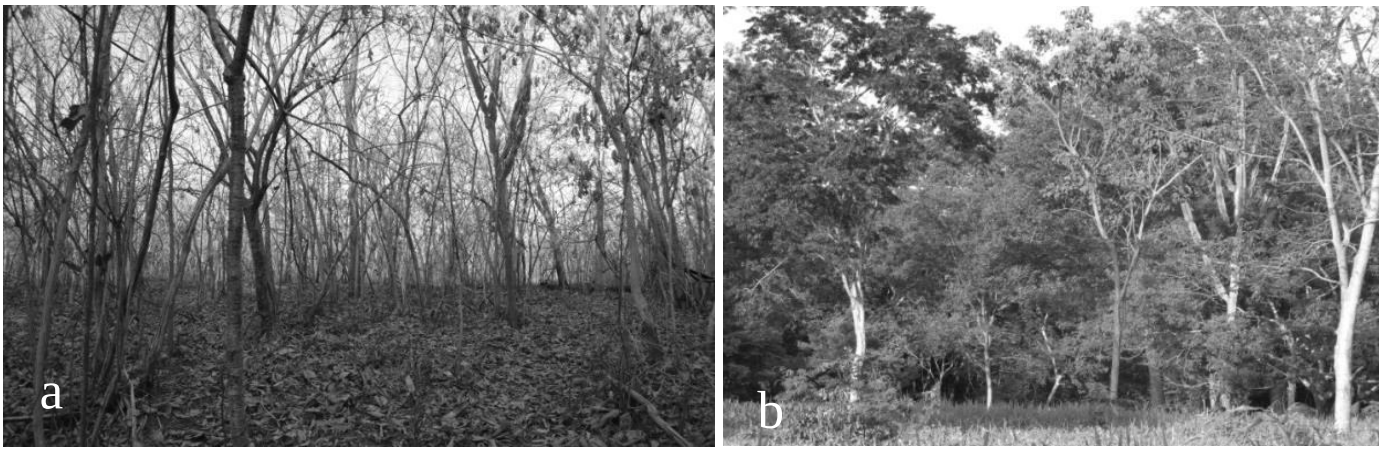

Figure 1. Dry Forest of Northern of Minas Gerais/Brazil. a – Tree canopies during the hyper dry season; b – Tree canopies during the dry season.

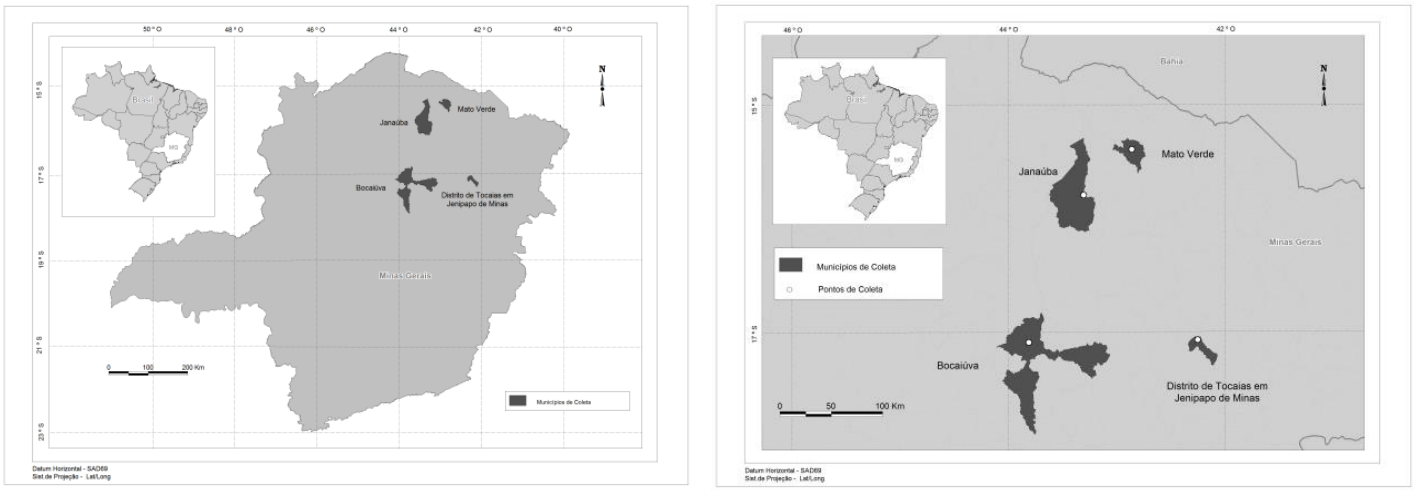

Figure 2. Map of Minas Gerais State/Brazil highlighting the studied areas.

acidity was determined by the sum of the free acid and lactone.

Ash content was determined by the method of porcelain crucibles using 5 to 10 g of honey (AOAC, 1998). The sample was initially heated on a Bunsen burner until carbonization and then heated in a muffle kiln at 600 °C for 12 hours (AOAC, 1998).

The sugars fructose, glucose, sucrose, erlose, melizitose and raffinose were determined by Reverser-Phase high performance liquid Chromatography (RP-HPLC). The studies were carried out using a Shimadzu (model CLASS-10AD), column Lichrospher NH2 (250 mm x 4.0 mm particle diameter of 5µm) (Merck, Darmstadt, Germany), mobile phase acetonitrile, methanol and water (78:5:17) with isocratic elution system; flow rate of mobile phase 1.0 ml/min; oven temperature 40 °C; a refractive index detector and an auto sampler with 20 µm of injection (Campos *et al.*, 1999; Sanz & Martínez-Castro, 2001). Approximately 2.5 g of sample was weighed, diluted in water: acetonitrile (1:1) to 25 mL and filtered through a PVDF membrane, 0.45 µm. Analyses were performed in triplicate and quantification were performed by external standard of fructose, glucose, sucrose, erlose, melezitose. All six standard sugars followed a

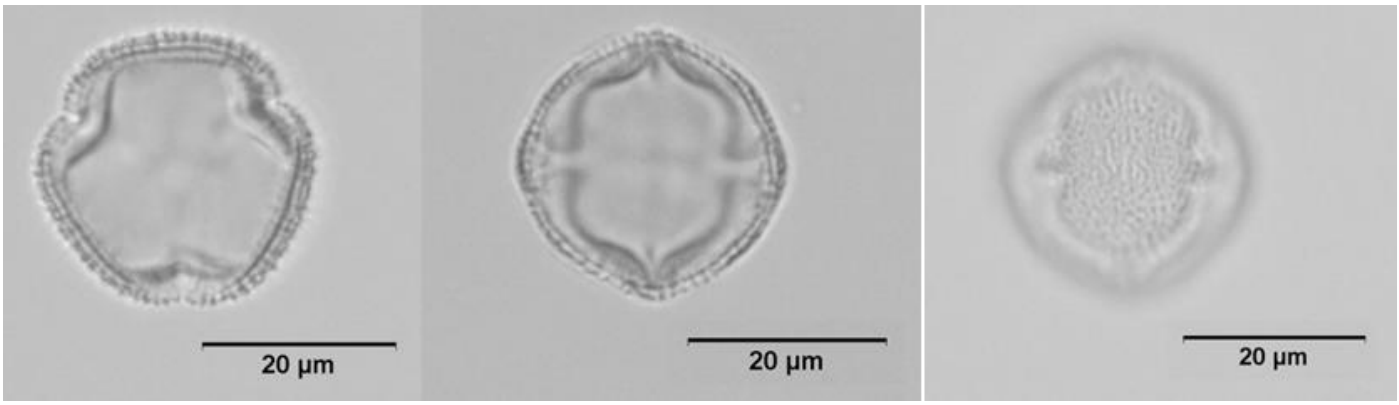

Figure 3. Pollen grain from *Myracrodruon urundeuva* (Anacardiaceae - Aroeira). a: Polar view. b: Equatorial view and c: Detailed of the ornamentation.

quadratic (2nd order) fit and had R2 coefficients > 0.98.

2.4 Statistical analyzes

To compare the physicochemical and microscopic parameters between the hyper dry and dry seasons, we used the Mann-Whitney test (Hollander, 1999). The significance level (alpha) considered was 10%. For these analyzes, we used the R software (R, 2012).

Results

3.1 Microscopic analyzes of honey

In honey samples were identified the total of 20 pollen types during the study (Table 1). During the hyper dry period, 16 pollen types were found, with a predominance of *Myracrodruon urundeuva* pollen grains (Anacardiaceae) (Figure 3), with 98.2% of the total grains (Table 1).

During the dry period 12 pollen types were identified, wherein the *Myracrodruon urundeuva* (Figure 3) contributed with 68% of total grains, and other plants like, *Eucalyptus* sp. with 19%, *Mimosa* sp. with 9,5% (Table 1).

3.2 Physicochemical analyzes

Physicochemical parameters of honey (invertase enzyme, electrical conductivity, acidity, ash, pH and sugar content) are reported in table 2. The color of 57.7% of honey samples was dark amber, followed by amber in 34.6% of the samples and clear amber in 7.7%.

Invertase was significantly higher in the hyper dry season (W = 105, p-valor=0.070), as well as fructose (W=59, p-valor=0.009) and glucose content (W=59, p-valor=0.009). On the other hand, honeys were significantly more acid during dry season (W=143.0, p-valor=0.0553).

Discussion

The predominance of *Myracrodruon urundeuva* pollen grains indicates that this specie is the main nectar source for *A. mellifera* in this Brazilian Dry Forest during the hyper dry period (May to October), which corresponds to the blooming period of this specie, from July to September (Andrade *et al.*, 2000) and field observations. It is known that plants of Anacardiaceae are an important nectar and pollen source to bees in Cerrado biome, represented by genera *Astronium*

Table 1. Pollen types identified in honey samples collected from May of 2010 to April of 2011 in the North of Minas Gerais State, Brazil. N=number of samples.

Pollen types	Hyper dry season (May to October / 2010) (N=18)			Dry season (November / 2010 to April / 2011) (N=8)		
	Numerical Frequency	Percentage %	Category*	Numerical Frequency	Percentage %	Category*
Acanthaceae						
Justicia clivalis	0.2	0.0				
Anacardiaceae						
Myracrodruon urundeuva	660.3	98.2	DP	218.9	68.0	DP
Spondias tuberosa				2.5	0.8	
Asteraceae						
Centratherum punctatum	0.2	0.0	MOP			
Tipo *Parthenium*				0.1	0.0	MOP
Tridax procumbens	2.2	0.3	MOP	0.1	0.0	MOP
Vernonia sp.	0.1	0.0	MOP	0.8	0.2	MOP
Cecropiaceae						
Cecropia sp.				0.4	0.1	MOP
Euphorbiaceae						
Astraea lobata	0.2	0.0	MOP	1.0	0.3	MOP
Fabaceae						
Acacia sp.	1.6	0.2	MOP	1.5	0.5	MOP
Bauhinia cf. *forficata*	0.2	0.0	MOP			
Macroptilium bracteatum	0.1	0.0	MOP			
Mimosa sp.	0.1	0.0	MOP	30.5	9.5	MIP
Pithecellobium sp.	0.2	0.0	MOP			
Malvaceae						
Wissadula hernandioides	0.1	0.0	MOP			
Myrtaceae						
Hyptis suaveolens	0.8	0.1	MOP	0.1	0.0	MOP
Eucalyptus sp.	1.2	0.2	MOP	61.3	19.0	AP
Poaceae						
Poaceae unidentified 1	4.8	0.7	MOP			
Rubiaceae						
Richardia scabra	0.6	0.1	MOP			
Sapindaceae						
Serjania sp.				4.6	1.4	MOP
Total	**672.8**	**100.0**	**-**	**321.8**	**100.0**	**-**

*DP: Dominant Pollen (DP>45%); AP: Acessory Pollen (15% to 45%); MIP: Minor Important Pollen (3% to 15%);MOP: Minor Ocasional Pollen (<3%) according to Zander (*apud* Maurizio & Louveaux, 1965).

Table 2. Physicochemical parameters of honey from the Dry Forest located in the northern of Minas Gerais State, Brazil. N = number of samples; s.d. = standard deviation;

Variables	**Hyper dry season (N=18)**		**Dry season (N=8)**	
	Mean	**s.d.**	**Mean**	**s.d.**
Invertase (U/kg)[1]	119.46	30.18	85.70	29.84
Electrical conductivity (µS/cm-1)	724,50	149,58	722,93	105,61
Total acidity (meq.kg^{-1})[2]	50.11	16.61	66.99	17.49
Ash (%)	0.28	0.11	0.24	0.15
pH[3]	4.35	0.33	3.90	0.41
Sugars (%)				
Fructose[4]	39.90	6.18	34.25	2.61
Glucose[5]	34.73	5.77	29.40	1.87
Sucrose	0.04	0.03	0.04	0.03
Erlose	0.77	0.56	0.63	0.38
Melezitose	0.08	0.04	0.13	0.10

[1]W = 105, p-value=0,070; [2]W=143,0, p-value=0,0553; [3]W=30,5, p-value=0,023; [4]W=59, p-value=0,009; [5]W=57, p-value=0,009.

and *Schinus* ("Author", 2003) and now in Dry Forest by *M. urundeuva* specie.

During the dry period (from November to April) *M. urundeuva* pollen grains were the most frequent in honey samples, besides this species was not blooming and the presence of pollen grains of *M. urundeuva* all over the year in the honey shows the great potential of this species as source of nectar and pollen. During the dry period bees find, also, flowers of other plant species, such as *Eucalyptus* sp. and *Mimosa* sp., that are known to be another nectar sources ("Author", 1995; "Author", 2015).

The color of most of analyzed honey samples was dark amber during the hyper dry season (61% of 18 samples) and dry period (50% of 8 samples) and this can be considered one of the distinctively characteristic of this honey when compared to multifloral honeys.

During the hyper dry season, the blooming period of *M. urundeuva,* pH was significantly higher (4.35) than in the dry season (3.90), showing a less acid honey during this period. Consequently, the total acidity was lower in the hyperdry season rather than in the dry season (50.11 and 66.99 meq/kg, respectively). According to Crane (1983) pH values can be influenced by nectar pH, soil or plant associations used by bees in order to elaborate honey.

The content of invertase enzyme in aroeira honey is known to be higher than other honeys. This enzyme plays a hole in the hydrolysis of sucrose on fructose and glucose, and it is directly related to the botanical origin of honey (Oddo *et al.*, 1999; Vorlova & Pridal, 2002). In this study averages of invertase content in hyper dry and dry period were 119.50 U/kg and 85.7 U / kg, respectively.

Honey is a mixture of glucose, fructose, maltose, sucrose and 22 di- and trisaccharides and higher sugars. Glucose and fructose are the major constituents, corresponding about 85% of the solid honey (Donner, 1977). Most of these sugars originate from the enzymatic action during ripening or storage. Some authors postulate that each type of honey has a distinct amount of various sugars, depending of its origin, from floral nectar or excretions of various aphids (Donner, 1977; White, 1980). The honey produced in of the Brazilian dry forest studied here had values of total

sugars ranging from 71.6% in hyper dry period and 64.5% in the dry season. The honey samples studied showed percentage of melezitose (hyper dry = 0.08%, dry = 0.13%) and erlose (hyper dry = 0.77%, dry = 0.63%).

The honey produced in this Brazilian Dry Forest is characterized as a *M. urundeuva* honey and in this biome, the scarcity of floral resources, associated with high temperatures and low humidity, induce bees to seek this massive food sources to ensure their food supply. This leads to the productions of a honey presenting unique characteristics: predominance of pollen grains of *M. urundeuva*, color dark amber, high electrical conductivity, high levels of ash content, less acid pH and acidity, high levels of invertase and the presence of melezitose and erlose. This study will contribute to the geographical indication (GI) for this honey, produced only in this Brazilian Region. Once this GI is established beekeepers will be able to export it and this product will have its market value increased.

Acknowledgement

To Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG) for financial support.

References

Andrade MW, Luz JMQ, Lacerda AS. & Melo PRA. (2000). Micropropagação da aroeira (*Myracrodruon urundeuva* Fr. All.). Ciência e Agrotecnologia. 24(1): 174-180.

AOAC - Association of Official Analytical Chemists (1998). Official methods of analysis of AOAC International: AOAC official method. Washington: AOAC.

"Author" (1995). Espectro polínico do mel produzido em algumas áreas antrópicas de Minas Gerais. Revista Brasileira de Biologia = Brazilian Journal of Biology. 55(4): 789-799. http://dx.doi.org/10.1590/S0006-87052000000100002

"Author" (2003). Pollen spectrum of honey produced in Cerrado areas of Minas Gerais State (Brazil). Brazilian Journal of Biology. 63(4): 599–615. http://dx.doi.org/10.1590/S1519-69842003000400007

Bogdanov S, Martin P & Lullmann C. (2002). Harmonised methods of the international honey commission. Swiss Bee Research Centre, FAM, Liebefeld.

Brasil. Ministério do Desenvolvimento Agrário. (2014). Final Report Project for Sustainable Land Management In The Northeastern Semi-Arid Region (Sertão). Site: http://www.projetodomhelder.gov.br/site/images/PDHC/rel_proj_sertao/Relatorio_Final_Projeto_Sertao_English_com_Anexos.pdf. Access in 03/15/2016.

Campos G, Nappi GU, Bastos M, Gomides MF, Cunha MRR, Baptista KES & Della-Modesta RC. (1999). Determinação de açúcares em mel floral e mel de melato de algumas regiões de Minas Gerais e de Santa Catarina. Revista do Instituto Adolfo Lutz. 58(2): 63–70.

Codex Stan. (2001). Revised Codex standard for honey. Codex Stan 12-1981, Rev.1(1987), Rev. 2 (2001). Italia: World Health Organization, Food and Agriculture Organization of United Nations.

Crane E. (1983) O livro do mel. Nobel.

Donner LW. (1977). The Sugars of Honey – A Review. Journal of the Science of Food and Agriculture. 28: 443-456.

Hollander M. & Wolfe DA. (1999). Nonparametric Statistical Methods. New York: John Wiley & Sons.

Inmet – Instituto Nacional de Meteorologia. Site: <www.inmet.gov.br>. Access in: 07/02/2014.

Maurizio A & Louveaux J. (1965). Pollens de plantes melliferes d'Europe. Paris, UGAF.

Miles L, Newton AC, Defries RS, Ravilious C, May I, Blyth S, Kapos V & Gordon JE. (2006). A global overview of the conservation status of tropical dry forests. Journal of Biogeography. 33(3): 491-505.

Moraes RM & Teixeira EW. (1998). Análise de mel (Manual técnico). Pindamonhangaba: Centro de Apicultura Tropical.

Murphy PG & Lugo AE. (1995). Dry forests of Central America and Caribbean islands. In: Bullock SH, Mooney HA & Medina E, editors. Seasonally dry tropical forests. Cambridge University Press, New York. p. 9–34.

Oddo LP, Piazza MG & Pulcini P. (1999). Invertase activity in honey. Apidologie. 30: 57-65. https://hal.archives-ouvertes.fr/hal-00891566

R Development Core Team. (2012). R: A language and environment for statistical computing: version 2.15.2.

Vienna, Austria: R Foundation for Statistical Computing. ISBN: 3-900051- 07-0.

Reddy SJ. (1983). Climatic classification: The Semiarid Tropics and its environment - a review. Pesquisa Agropecuária Brasileira. 18(8): 823–847.

Sanz ML & Martínez-Castro I. (2001). Recent developments in sample preparation for chromatographic analysis of carbohydrates. Journal of Chromatography A. 1153: 74-89. http://www.sciencedirect.com/science/article/pii/S0021967307000519

"Author" (2015). Pollen analysis of honey and pollen collected by *Apis Mellifera* Linnaeus, 1758 (Hymenoptera, Apidae), in a mixed environment of *Eucalyptus* plantation and native Cerrado in Southeastern Brazil. Brazilian Journal of Biology, (AHEAD), 0-0. http://dx.doi.org/10.1590/1519-6984.23513

Vorlova L & Pridal A. (2002). Invertase and diastase activity in honeys of Czech provenience. Acta Universitatis Agriculturae et Silviculturae Sbornik Mendelovy Zemedelske a Lesnicke Mendelianae Brunensis. 5: 57-66.

White Jr. JW. (1980). Detection of honey adulteration by carbohydrate analysis. Journal of the Association of Official Analytical Chemists. 63(1): 11-18.