Journal of Agricultural Science; Vol. 10, No. 7; 2018
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Physico-chemical Parameters of Honey From *Melipona mondury* Smith, 1863 (Hymenoptera: Apidae: Meliponini)

Rogério Marcos de Oliveira Alves[1,3], Jacqueline Lemos Viana[2], Henrique de Abreu Cerqueira Sousa[2] & Ana Maria Waldschmidt[2]

[1] Instituto Federal de Educação, Ciência e Tecnologia Baiano, Catu, Bahia, Brazil

[2] Universidade Estadual do Sudoeste da Bahia, Jequié, Bahia, Brazil

[3] Universidade Federal do Recôncavo Baiano, Cruz das Almas, Bahia, Brazil

Correspondence: Ana Maria Waldschmidt, Universidade Estadual do Sudoeste da Bahia, Rua José Moreira Sobrinho, s/n, CEP 45.200-000, Jequiezinho, Jequié, Bahia, Brazil. Tel: 55-73-3528-9725. E-mail: amwalds@uesb.edu.br

Received: March 15, 2018 Accepted: May 2, 2018 Online Published: June 15, 2018
doi:10.5539/jas.v10n7p196 URL: https://doi.org/10.5539/jas.v10n7p196

Abstract

The physico-chemical properties of honey produced by the stingless bee *Melipona mondury* from Atlantic Forest in the state of Bahia, northeastern Brazil were evaluated. The evaluated characteristics included: water content, electrical conductivity, pH, acidity, water activity, ashes, diastase, hydroxymethylfurfural (HMF), reducing sugars and saccharose values. The honey samples showed mean values of 29.18% for moisture; 391.5 μS for electrical conductivity; pH of 4.06; 34.3 meq kg^{-1} for acidity; 0.73 for water activity; 0.18% for ashes; 4.05 (Goethe unit) for diastase; 1.60% for HMF; 65.42% for reducing sugars; and 2.14% of saccharose. Only the diastase activity was above the limits accepted by the Technical Regulation for Identity and Quality of Honey (Brazilian Ministry of Agriculture and Supplies). Most parameters are according to those reported in honeys from other species of stingless bees in Brazil.

Keywords: Apidae, stingless bees, nutritional value, Atlantic Forest

1. Introduction

Bees' products such as honey, pollen and propolis are consumed by the world population mainly by items of nutritional and medicinal values (Rao et al., 2016: Yaacob et al., 2017). The production and consumption of honey from stingless bees has been increasing over the last decades in Brazil. Nonetheless, regulatory agencies prohibit their industrial commercialization because these products have not been properly classified yet. Therefore, studies about the physico-chemical properties of honeys produced by native species of stingless bees are essential to help establish the parameters required for approving their commercialization (Anacleto et al., 2009).

Indeed, some of the most important features to be considered in honey quality were established in previous reports (*e.g.* Vit et al., 1994; Carvalho et al., 2013). Recently, the Agency of Agropecuary Development and Protection (ADAB) from the state of Bahia in a partnership with the Universidade Federal do Recôncavo Baiano (UFRB) published the Technical Identity Regulation for the honey produced by *Melipona* stingless bees (ADAB, 2014). This initiative focused on the main parameters, including physico-chemical characteristics, to be evaluated to prevent frauds and to assure food safety of local population.

The composition of honey depends on the plant sources used by bees as well as other features, such as soil type, bee species, physiological state of colonies, maturation of honey, management conditions, and climate. In Brazil, 192 species of meliponines bees are described (Silveira et al., 2002), but the representatives of the genus Melipona stand out in relation to honey production. Hence, a national legislation for regulating the quality and characteristics of honeys derived from these native species is justified.

In the state of Bahia, northeastern Brazil, *Melipona scutellaris*, *M. quadrifasciata anthidioides*, and *M. mondury* are the main species exploited for honey. The latter is found in tropical forest areas and, even though, it is widely known by local population, the intensive culture of this species is still incipient and the characteristics of their honey are poorly known (Sousa et al., 2012).

The main parameters to be evaluated in honey for *Apis mellifera* quality in Brazil are: water content, color, acidity and values of hydroxymethylfurfural (HMF), reducing sugars and saccharose (Brazil, 2000). However, analyses of honey samples from several species of meliponines bees revealed that some features are not in agreement with the federal legislation for the identity of honey (Villas-Boas & Malaspina, 2005; Carvalho et al., 2013). These results point out that physical and chemical traits of honeys from other stingless bees should be investigated to establish their common parameters. Therefore, the goal of this study was to determine some physico-chemical characteristics of honey produced by *Melipona mondury* in order to increase our knowledge about the honey from stingless bees and to colaborate for the regulatory norms implementation of meliponine honey.

2. Material and Methods

2.1 Honey Samples

Twenty samples of honey were collected during August 2013 in nests of *M. mondury* raised in a stretch of Atlantic Forest from the municipality of Jequié (UTM 0407600/8474112). The main vegetation is Dense Ombrophilous Forest, integrating the "cabruca" (cocoa plantation) system from the Coast of Cocoa. The local climate ranges from humid to sub-humid, with a rainy season (starting in April) and a relatively dry season (January to March). The annual rainfall during the rainy season might reach up to 110 mm. The mean temperature during the year ranges from 17 °C to 30 °C (SEI, 2007). A volume of 20 mL of honey was collected from sealed pots using disposable syringes, stored in clean and sterilized glass recipients with hermetical lids, placed in coolers and transported to the laboratory INSECTA from the Center of Agricultural, Environmental and Biological Sciences (CCAAB) at Universidade Federal do Recôncavo da Bahia, in Cruz das Almas, Bahia. The analyzed physico-chemical parameters followed the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1990), the Harmonized Methods of the European Honey Commission (Bogdanov et al., 1997) and the Codex Alimentarius Commission (CAC, 2001). These methodologies are also recommended by the Brazilian legislation.

The evaluated parameters were:

2.2 Moisture (%)

The water content of honey samples was determined by using a digital refractometer ATAGO specific for analyzing honey (ATAGO Co. Ltd., 1988). This method is based on the refraction of light rays over honey solution, which contains soluble solids.

2.3 Electrical Conductivity

The electrical conductivity was determined using Tecnal conductivity meter (model R-TEC-04P-MP), according to manufacturer's instructions on a solution with 20% of honey dry matter. This analysis is based on the measurement of the electrical resistance, of which the electrical conductivity is the reciprocal.

2.4 Ash Values (%)

The ashes were also measured using Tecnal conductivity meter (model R-TEC-04P-MP) adjusted to read ash values. This ash values were established over a solution with 28% of honey dry matter.

2.5 pH and Acidity

The pH and acidity values were established based on the neutralization of an acid honey solution by adding sodium hydroxide (AOAC, 1990). Ten grams of honey were then dissolved in 75 mL of distilled water. The solution was titrated with 0.05 N NaOH up to reaching a pH of 8.5. The milli equivalents of acid per kg of honey were determined according to the following expression:

$$\text{Freed acidity (meq kg}^{-1}) = (\text{mL of 0.05 N NaOH} - \text{mL of negative control}) \times 5 \quad (1)$$

2.6 Water Activity

The water activity was determined by suing PAW KIT-Water activity meter, according to manufacturer's instructions.

2.7 Diastase Activity

Diastase activity was determined using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40 °C. The diastase value was calculated using the time taken for the absorbance to reach 0.235, and the results were expressed in Goethe units as the amount (mL) of 1% starch hydrolyzed by an enzyme in 1 g of honey per hour (CAC, 1990).

2.8 Hydroxymethylfurfural (HMF)

The HMF content was determined by UV absorbance at different scales. The absorbance was measured at 284 and 336 nm (AOAC, 1990).

2.9 Reducing Sugars and Saccharose (%) (CAC, 1990)

The amount of reducing sugars and saccharose (%) were determined by titration using Fehling's method modified by Soxhlet, against a solution of reducing sugar in honey.

2.10 Statistical Analysis

The honey samples were analyzed in triplicates (N = 3) over the same time period to provide reliable comparative values. The descriptive statistics (mean and standard deviation) of evaluated parameters was estimated using the software BioEstat version 5.0 (Ayres et al., 2007).

3. Results and Discussion

The physico-chemical values observed in 20 honey samples of *M. mondury* from the state of Bahia, northeastern Brazil, are shown in Table 1.

3.1 Moisture

In general, the water content is the most evaluated parameter in honey analysis, being considered a key feature to differentiate the honey of stingless bees (*Melipona*) from that produced by *Apis mellifera*. The mean moisture in honeys of *M. mondury* was 29.18±1%, ranging from 27.8 to 30.5%. These values are similar to those reported in other meliponines, particularly in *Melipona* species (Alves et al., 2005; Anacleto et al., 2009; Lage et al., 2012).

It should be pointed out that the water content in honeys of *Melipona* is similar even when species that inhabit rainforests are compared to those typical of caatinga (semiarid) regions (Souza et al., 2004; Alves et al., 2005; Lage et al., 2012). Nonetheless, a narrow variation in moisture values has been reported in meliponines honeys among Brazilian regions (Evangelista-Rodrigues et al., 2005; Campos et al., 2010; Alves et al., 2011).

The moisture affects several other parameters in honeys (acidity, viscosity, pH, conductivity, and concentration of sugars), but the main effect refers to proliferation of microorganisms in honey when water content is high (Costa et al., 1999; Massaguer, 2005). Moreover, the stability of honeys from stingless bees against fermentation might be influenced by the high antibacterial activity observed in honeys produced by *Melipona* (Cortopassi-Laurino & Gelli, 1991; Vit et al., 1994). This feature assures that these bees are able to produce and store honey without contamination of final product.

Indeed, the moisture has been regarded as the main issue to regulate the production and consumption of *Melipona* honeys since the Brazilian regulation (MAPA) establishes that the water content in honey from *Apis mellifera* should not exceed 20% (Brasil, 2000).

Table 1. Physical-chemical parameters in honey samples of *Melipona mondury* from the state of Bahia, Brazil

N	Moisture (%)	Conductivity	Ash (%)	Acidity	pH	Aw	Diastase	HMF	Reducing sugar (%)	Saccharose (%)
01	28.50	264	0.093	45.00	3.81	0.74	2.17	0.28	64.94	1.23
02	29.20	486	0.088	32.00	4.08	0.73	2.42	0.82	66.67	2.18
03	30.50	532	0.099	44.00	3.92	0.79	1.42	0.82	66.89	0.86
04	28.60	392	0.209	20.50	4.43	0.69	2.16	0.60	65.57	2.11
05	29.00	492	0.099	25.00	4.41	0.70	2.27	0.15	64.10	1.81
06	28.50	503	0.101	37.50	4.15	0.67	2.65	0.52	66.67	1.74
07	28.60	351	0.101	40.00	3.90	0.76	6.82	6.89	66.67	2.64
08	30.00	499	0.100	22.50	4.40	0.75	11.54	0.45	64.94	2.72
09	30.50	404	0.210	17.50	4.61	0.74	8.57	1.50	65.79	1.91
10	30.50	393	0.202	54.50	3.76	0.78	0.42	0.00	64.94	1.86
11	29.90	304	0.156	50.00	3.64	0.72	0.45	0.30	65.79	3.02
12	27.80	481	0.087	21.50	4.51	0.68	8.33	8.23	64.94	1.86
13	28.60	247	0.098	36.00	3.68	0.72	2.78	1.42	65.57	1.46
14	28.30	285	0.138	45.00	3.75	0.71	1.84	2.10	66.23	2.15
15	28.40	480	0.279	32.50	4 .17	0.75	6.00	2.69	64.25	3.12
16	28.50	354	0.098	22.00	4.37	0.72	6.98	0.22	70.18	3.70
17	29.80	444	0.966	41.00	3.92	0.76	4.48	0.67	63.49	1.98
18	28.70	235	0.091	28.50	3.79	0.76	3.30	1.65	65.36	1.45
19	29.30	243	0.258	32.00	4.16	0.75	2.59	0.30	64.31	1.82
20	30.50	441	0.232	39.00	3.91	0.82	4.00	2.40	61.16	3.19
X	29.18±1	391.5±88.5	0.18±0.069	34.3±3	4.06±0.05	0.73±0.04	4.05±0.915	1.60±1.06	65.42±1.89	2.14±0.98
A	27.8-30.5	235-532	0.087-0.966	17.5-54.5	3.64-4.61	0.67-0.82	0.415-11.54	1.06-8.23	61.16-70.18	0.86-3.7

Note. X = Mean; A = Amplitude.

In this sense, the studies carried out by Carvalho et al. (2013) supported the new regulation officially published by ADAB (2014) which established a limit of 20-30% of moisture in honey produced by *Melipona* for the state of Bahia inasmuch as the product is stored at cool temperature from collection to commercialization. Recently Resolution SAA-52/14, of 3-10-2017 of the Government of São Paulo approved the commercialization of matured honey with a maximum moisture content of 40 (Diario Oficial, 2017). This regulation favored the market of honeys from stingless bees and provided a new concept of honey identity.

3.2 Electrical Conductivity

This parameter is correlated to the ash values, pH, acidity, mineral salts, proteins and other substances found in honey (Bogdanov, 1999). The ashes increase the conductivity of honeys because of the presence of conducting salts (Alencar-Chaves et al., 2012). Most honey samples from *Melipona* have a pale color and high electrical conductivity.

Electrical conductivity in samples of honeys from meliponines ranged from 255.0 to 905.7 µS cm^{-1} (Souza et al., 2009). This feature is not referenced by the current Brazilian legislation but the highest value allowed by the international Codex Alimentarius (2001) equal 800.0 µS cm^{-1}. In honey samples of *M. scutellaris*, the electrical conductivity varied between 534.32 µS cm^{-1} and 586.20 µS cm^{-1} (Campos et al., 2010).

The conductivity reported in *Melipona* species by Alves et al. (2011) ranged from 120.0 to 449.5 µS cm^{-1}. In some cases, low electrical conductivity is reported as in honey samples of *M. fulva* (mean value of 34.453 µS cm^{-1}). Nonetheless, this finding can be affect by improper incineration of samples that eventually results in increases/decreases of water content or dry matter, influencing thereby the measurement of electrical conductivity (Alencar-Chaves et al., 2012).

3.3 Ash Values

Considered an important trait for the technical regulation of honey (Brasil, 2000), the ash values are directly related to the quality control of honeys (Carvalho et al., 2005). For meliponines, the maximum ash value allowed in honey equals 0.6 g/100 g of ashes (Brasil, 2000; ADAB, 2014).

In general, the ash values in honeys of *Melipona* seem to vary between 0.01 and 0.33% (Souza et al., 2009; Campos et al., 2010; Alves et al., 2011). These values are within the threshold established by the Brazilian legislation of MAPA (Brasil, 2000). In the case of *M. mondury*, the values of 0.185±0.069% in ashes are according to the quality parameters of honeys from *Apis mellifera*.

Several authors such as Almeida (2002), and Ortiz-Valbuena (1988) correlate the concentration of minerals in honeys to their color, *e.g.*, paler honeys usually present low ash values. Accordingly, Chaves et al. (2012) reported that honey samples of *Melipona* should have reduced amounts of ashes since they are distinguished from other honeys by their clear coloration.

3.4 pH and Acidity

This parameter has been under debate since the honey of meliponines presents higher acidity than those produced by *A. mellifera*, what affects directly the taste and thereby the preference of consumers for honeys from stingless bees (Evangelista-Rodrigues et al., 2003; Vit et al., 2004). The acidity is influenced by the variation in organic acids from distinct nectar sources as well as by the activity of glucose-oxidase enzyme that produces the gluconic acidor the bacterial activity during honey maturation and amount of minerals in honeys (Bogdanov et al., 1997; Vit et al., 2004). The acidity is also related to the level of honey maturation, being higher as fermentation takes place (Vit et al., 2004).

The acidity value observed in the honey of *M. mondury* was 34.3±3 meq kg^{-1} (Table 1), being within the limits (≤ 50 meq kg^{-1}) established by the legislation of MAPA (Brasil, 2000). For other three species of *Melipona*, Alves et al. (2011) reported honey acidity of 26.63%, 20.55%, and 145.28%. Similar values were reported for other authors in honeys of *Melipona* species (Bogdanov et al., 1996; Souza et al., 2004; Alves et al., 2005; Mesquita et al., 2007; Campos et al., 2010; Holanda et al., 2012; Lage et al., 2012).

The pH values are used to estimate or confirm the acidity in food (Freitas et al., 2010). In this sense, the pH of *Melipona* honeys is very similar to those produced by *A. mellifera* (Brasil, 2002). In general, all honey samples present low pH because of the presence of organic and inorganic acids (Simal & Huidobro, 1984). Other features are also important such as the amount of calcium, sodium, potassium and other compounds of ashes (Seeman and Neira, 1988), what might explain the high values observed in some honey samples.

The pH values in honeys of *Melipona* range from 3.15 to 4.61 (Souza et al., 2004; Alves et al., 2005; Mesquita et al., 2007; Dardón & Enríquez, 2008; Holanda et al., 2012; Lage et al., 2012; Souza et al., 2013). Therefore, the pH of 4.06±0.05 (Table 1) observed in the honey of *M. mondury* are compatible with the mean pH reported in honeys of congeneric species.

3.5 Water Activity (Wa)

The water is the main component of several food sources, can affect their biochemical stability (Gleiter et al., 2006). The analysis of water activity (Wa) is recommended to evaluate the safety of food products in relation to the growth of undesirable microorganisms, definition of risks in food intake, control of critical points and for the regulation of preserved food and labeling (Scott et al., 2001).

Water activity values of 0.61-0.62 hinder the growth of osmo tolerating yeasts in honey (Zamora & Chirife, 2006). In honey samples of meliponines, Wa values between 0.662 and 0.851 have been reported (Vit et al., 2006; Almeida-Muradian et al., 2007; Souza et al., 2009).

The Wa in honey samples of *M. mondury* presented a mean value of 0.737±0.04, being above the threshold established to avoid microorganism proliferation. In spite of these high values, several features in honeys are able to prevent contamination (low pH, high acidity, presence of glucose oxidase, among others) (Roubik, 1992). In the case of meliponines, the pots in which the bees store the honeys can provide additional support against microorganisms since they are composed of wax and propolis. The latter is recognized by the presence of antimicrobial agents that prevent fermentation (Michener, 1974; Roubik, 1983).

3.6 Diastase Activity

The diastase is an enzyme that digests starch. However, the origin of this enzyme is controversial and nowadays, the most accepted hypothesis is that diastase derives from salivary glands once diastase was found in honeys of bees fed with sugar and the diastase of bees was highly similar to that observed in honey. On the other hand, Gonnet et al. (1964) observed regular invertase but no diastase activity in honeys of *Melipona*.

Most reports available for diastase activity refer to honeys of *A. mellifera*, while the few studies in honey samples of *Melipona* have reported low activity of this enzyme (Vit et al., 1994), thus restraining the discussion about the origin of diastase. Despite of some divergence among authors, the reduced diastase activity should be considered a general rule in honeys of *Melipona* (Souza et al., 2009). Therefore, this parameter could rather be used as a marker for the identification of *Melipona* honeys instead of being considered a quality trait. To confirm this suggestion, further studies about diastase activity should be performed in honeys of stingless bees.

This enzyme is important to evaluate the quality of honeys because of their high sensitivity to heat (Carvalho et al., 2005). However, the remarkable variation in diastase activity reported by White Júnior (1979) in fresh honey samples of *A. mellifera* raised some questions if this enzyme would be useful to qualify honeys (Stefanini, 1984). The quantitative discrepancies in this enzyme of honeys obtained from distinct flowers suggest that substances present in nectar or honey might affect the diastase activity.

The mean diastase activity observed in honeys of *M. mondury* was 4.05±0.915 Goethe units (Table 1). Nonetheless, some samples presented high values as 11.54 and 8.33 when compared to honeys of other congeneric bees. Probably, these results are related to accidental feeding with honey of *A. mellifera* within some colonies of *M. mondury*, since they were near apiaries whose honeys are known to present higher diastase activity.

Honeys of *Melipona* usually present low contents of diastase. Studies indicate a variation from 0.60 to 3.01 for honey of *Melipona* (Vit et al., 1998; Dardón & Enríquez, 2008; Bogdanov, 2006; Guerrini et al., 2009; Carvalho et al., 2009; Holanda et al., 2012). Recently, mean values of 15.63 were obtained for Acre meliponas, considered non-standard for the genus (Do Vale et al., 2017).

3.7 Hydroxymethylfurfural (HMF)

Honeys of meliponines are characterized by lower content of sugar and by the prevalence of fructose (Oliveira et al., 2006). This sugar is the main responsible for the production of HMF in association with acids and high temperatures (White Junior, 1976). Acidity, pH, water content and amount of mineral also play a role in the production of HMF (White Junior, 1979). Usually, fresh honey samples exhibit low rates of HMF. Otherwise, high values of this parameter indicate poor nutritional value of honeys (Veríssimo, 1988). Under the same temperature conditions, the honeys of *A. mellifera* present higher amounts of HMF than honeys of stingless bees, what indicates that short heating treatments do not interfere in the HMF content of honey samples of meliponines (Biluca et al., 2014).

The average content of HMF in honeys of *M. mondury* was 1.60±1.06 mg kg^{-1}. High values in HMF have been reported for other *Melipona* species (Almeida, 2002; Dardón & Enríquez, 2008; Souza et al., 2009; Alves et al., 2011). One key feature that influences the variation in HMF values is the management (collection, storage, and transportation) of honeys up to laboratorial analysis. Prior to laboratorial analyses, the honey from Melipona should be removed from pots and kept immediately under refrigeration, once the association of high temperature and high humidity levels might result in their fermentation and to the formation og HMF, regarded as an important aspect to the evaluation of honey quality according to MAPA. Then, in tropical regions, the honey samples should be refrigerated right after their removal from nests up to analyses in order to avoid the increasing of HMF due to honey fermentation.

3.8 Reducing Sugars

Honey is a natural product characterized by variation in sugar composition, which includes more than 20 disaccharides and polysaccharides besides the monosaccharides glucose and fructose (Doner, 1977). The sugar profile of honeys produced by *Melipona* is similar to *A. mellifera*, *e.g.*, they are poor in oligosaccharides. Even though the sugar composition in honeys of *Melipona* and *Trigona* differ from each other, the content of reducing sugars is similar to both genera (Vit et al., 1994).

The sugar composition in honeys depends on the plant sources and the activity of enzymes produced by bees. In the case of honeys of meliponines, the sugar content is low and fructose is the main sugar component, being responsible for their sweetness and hygroscopicity (Bogdanov et al., 1997). The effectiveness of using the content of reducing sugars to determine the entomological origin of honeys by stingless bees was evaluated by Vit et al. (1998).

In the present study, the mean concentration of reducing sugar in honeys of *M. mondury* was 65.42±1.89%. The mean values of reducing sugars in honeys from other species range from de 50.13%, to 74.82% (Souza et al., 2004; Alves et al., 2005; Mesquita et al., 2007; Campos et al., 2010; Alves et al., 2011; Holanda et al., 2012; Souza et al., 2013).

3.9 Reducing Sugars and Saccharose

Usually, the saccharose content in honeys varies from 2 to 3% while this parameter ranges from 1.7 to 4.7% in honey samples of *Melipona* (Souza et al., 2006). Higher values of saccharose indicate immature or adulterated honeys (Carvalho et al., 2005). The adulteration of honeys can be caused by adding other substances or by improper management such as feeding the bees with commercial sugar and removal of honeys from nests.

The honey samples of *M. mondury* presented a mean value of 2.14±0.98% in saccharose (Table 1), being within the limits reported by Souza et al. (2006). Similar values were obtained by other authors (Bogdanov et al., 1996; Souza et al., 2004; Alves et al., 2005; Mesquita et al., 2007; Dardón & Enríquez, 2008; Alves et al., 2011; Holanda et al., 2012).

The content reported of saccharose in honeys of *Melipona* ranged from 0.60% to 5.6% (Vit et al., 1998). Nonetheless, higher values were reported by Souza et al. (2013) and Campos et al. (2010). These differences in saccharose content among honey samples and species are related to both intrinsic (*e.g.* flower sources) and extrinsic features of nectar, like location and climate.

4. Conclusions

The knowledge about the physico-chemical parameters of honey from stingless bees is still limited because of the high species richness and diversity of biomes in which they occur. This study is the first report about the honey from *Melipona mondury* and will contribute to the establishment of physico-chemical parameters in honey from bee species of the Atlantic Forest.

References

ADAB (Agência Estadual de Defesa Agropecuária da Bahia). (2014). *Regulamento Técnico de Identidade e Qualidade do Mel de Abelha social sem ferrão gênero Melipona* (Portaria ADAB Nº 207 DE 21/11/2014).

Alencar-Chaves, A. F., Gomes, J. E. H., & Costa, A. J. S. da. (2012). Caracterização físico-química do mel de *Melipona fulva* Lepeletier, 1836 (Hymenoptera: Apidae: Meliponinae) utilizada na meliponicultura por comunidades tradicionais do entorno da cidade de Macapá-AP. *Biota Amazônica, 2*(1), 1-9. https://doi.org/10.18561/2179-5746

Alves, R. M. O., Carvalho, C. A. L., Souza, B. A., Sodré, G. S., & Marchini, L. C. (2005). Características físico-químicas de amostras de mel de *Melipona mandacaia* Smith (Hymenoptera: Apidae). *Ciência e Tecnologia de Alimentos, 25*(4), 644-650. https://doi.org/10.1590/S0101-20612005000400004

Alves, T. T. L., Meneses, A. R. V., Silva, J. N., Parente, G. D. L., & Holanda Neto, J. P. (2011). Caracterização físico-química e avaliação microbiológica de méis de abelhas nativas do nordeste brasileiro. *Revista Verde, 6*(3), 91-97.

Anacleto, D. A., Souza, B. A., Marchini, L. C., & Moreti, A. C. C. C. (2009). Composição de amostras de mel de abelha Jataí (*Tetragonisca angustula* Latreille, 1811). *Ciência e Tecnologia de Alimentos, 29*, 535-541. https://doi.org/10.1590/S0101-20612009000300013

AOAC (Association of Official Analytical Council). (1990). *Official methods of Analysis* (15th ed., p. 1018). Arlington, VA, USA.

ATAGO Co. Ltd. (1988). Refratômetro para mel. *Abelhas, 31*(362/363), 9, 11-12, 41, 44.

Ayres, M., Ayres Júnior, M., Ayres, D. L., & Santos, A. A. (2007). *Bioestat—Aplicações estatísticas nas áreas das ciências biomédicas* (5th ed., p. 361). Belém-PA: Publicações Avulsas do Mamirauá.

Biluca, F. C., Della-Betta, F., De Oliveira, G. P., Pereira, L. M., Gonzaga, L. V., Oliveira Costa, A. C., & Fett, R. (2014). 5-HMF and carbohydrates content in stingless bee honey by CE before and after thermal treatment. *Food Chemestry, 159*, 244-249. https://doi.org/10.1016/j.foodchem.2014.03.016

Bogdanov, S. (1999). Honey quality and internacional regulary standards: Review by the international honey commission. *Bee World, 80*(2), 61-69. https://doi.org/10.1080/0005772X.1999.11099428

Bogdanov, S. (2006). Contaminants of bee products. *Apidologie, 37*(1), 1-18. https://doi.org/10.1051/apido:2005043

Bogdanov, S., Martin, P., & Lullman, C. (1997). Harmonised methods of the European Honey Commission. *Apidologie, Extra Issue*, 1-59.

Bogdanov, S., Vit, P., & Kilchenmann, V. (1996). Sugar profiles and conductivity of stingless bee honeys from Venezuela. *Apidologie, 27*, 445-450. https://doi.org/10.1051/apido:19960602

Brasil. (2000). *Regulamento Técnico de Identidade e Qualidade do Mel* (pp. 19696-19697). Leis, Decretos, etc. Instrução Normativa 11, Diário Oficial, 20 de Outubro de Seção 1.

CAC (Codex Alimentarius Commission). (1990). *Official methods of analysis* (V. 3, Supl. 2).

Campos, F. S., Gois, G. C., & Carneiro, G. G. (2010). Parâmetros físico-químicos do mel de abelhas *Melipona scutellaris* produzido no Estado da Paraíba. *FAZU em Revista, 1*(7), 186-190.

Carvalho, C. A. L., Sodré, G. S., Fonseca, A. A. O., Alves, R. M. O., Souza, B. A., & Clarton, L. (2009). Physicochemical characteristics and sensory profile of honey samples from stingless bees (Apidae: Meliponinae) submitted to a dehumidification process. *Anais da Academia Brasileira de Ciências, 81*, 143-149. https://doi.org/10.1590/S0001-37652009000100015

Carvalho, C. A. L., Souza, B. A., Sodré, G. S., Marchini, L. C., & Alves, R. M. O. (2005). *Mel de abelhas sem ferrão: contribuição para a caracterização físico-química*. Cruz das Almas: Universidade Federal da Bahia/SEAGRI-BA.

Carvalho, C. A., Alves, R. M. O., Souza, B. A., Véras, S. O, Alves, E. M., & Sodré, G. S. (2013). Proposta de regulamento técnico de qualidade físico-química do mel floral processado produzido por abelhas do gênero *Melipona*. In P. Vit, & D. W. Roubik (Eds.), *Stingless bees process honey and pollen in cerumen pots* (pp. 1-9). Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida, Venezuela.

Chaves, A. F. A., Gomes, J. E. H., & Costa, A. J. S. (2012). Caracterização físico-química do mel de *Melipona fulva* Lepeletier, 1836 (Hymenoptera: Apidae: Meliponinae) utilizada na meliponicultura por comunidades tradicionais do entorno da cidade de Macapá-AP. *Biota Amazônia,* 2(1), 1-9. https://doi.org/10.18561/2179-5746/biotaamazonia.v2n1p1-9

Cortopassi-Laurino, M., & Gelli, D. S. (1991). Analyse pollinique, propriétes physico-chimique setactiondes mieis d'abellies africanisées *Apis mellifera* et de Méliponinés du Brésil. *Apidologie, 22*, 61-73. https://doi.org/10.1051/apido:19910108

Costa, L., Albuquerque, M., Trugo, L., Quinteiro, L., Barth, O., Ribeiro, M., & de Maria, C. (1999). Determination of non-volatile compounds of different botanical origin Brazilian honeys. *Food Chemistry, 65*, 347-352. https://doi.org/10.1016/S0308-8146(98)00230-1

Crane, E. (1992). The past and present beekeeping with stingless bees. *Bee World, 73*, 29-42. https://doi.org/10.1080/0005772X.1992.11099110

Dardón, M. J., & Enríquez, E. (2008). Caracterización fisicoquímica y antimicrobiana de lamiel de nueveespecies de abejassinaguijón (Meliponini) de Guatemala. *Interciência* 13(22), 916-922.

Do Vale, M. A. D., Gomes, F. A., Santos, B. R. C dos, & Ferreira, J. B. (2017). Honey quality of *Melipona* sp. bees in Acre, Brazil. *Agroindustry and Food Sciences, 67*(2), 1-8. https://doi.org/10.15446/acag.v67n2.60836

Doner, L. W. (1977). The sugars of honey—A review. *Journal of the Science of Food and Agriculture, 28*, 443-455. https://doi.org/10.1002/jsfa.2740280508

Evangelista-Rodrigues, A., Silva, E. M. S., Beserra, E. M. F., & Rodrigues, M. L. (2005). Análise físico-química de méis das abelhas *Apis mellifera* e *Melipona scutellaris* produzidos em duas regiões no Estado da Paraíba. *Ciência Rural, 35*(5), 1166-1171. https://doi.org/10.1590/S0103-84782005000500028

Evangelista-Rodrigues, A., Silva, M. A. F., Dornellas, G. S., & Rodrigues, M. L. (2003). Estudo de plantas visitadas por abelhas *Melipona scutellaris* na microrregião do brejo no Estado da Paraíba. *Acta Scientiarum, 25*(2), 229-234.

Freitas, W. E. de S., Aroucha, E. M. M., Soares, K. M. de P., Mendes, F. I. de B., Oliveira, V. R., Lucas, C. R., & Santos, M. C. A. (2010). Parâmetros físico-químicos do mel de abelha sem ferrão (*Melipona subnitida*) após tratamento térmico. *Acta Veterinaria Brasilica, 4*(3), 153-157.

Gleiter, R. A., Horn, H., & Isengard, H. D. (2006). Influence of type and state of crystallisation on the water activity of honey. *Food Chemistry, 96*, 441-445. https://doi.org/10.1016/j.foodchem.2005.03.051

Gonnet, M., Lavie, P., & Nogueira-Neto, P. (1964). Étude de quelques caractéristiques des miels récoltés par certains Méliponines brésiliens. *Comptes Rendus de l'Académie des Sciences Paris, 258*, 3107-3109.

Guerrini, A., Bruni, R., Maietti, S., Poli, F., Rossi, D., Paganetto, G., … Sacchetti, G. (2009). Ecuadorian stingless bee (Meliponinae) honey: A chemical and functional profile of an ancient health product. *Food Chemistry, 114*, 1413. https://doi.org/10.1016/j.foodchem.2008.11.023

Holanda, C. A., Oliveira, A. R., Costa, M. C. P., Ribeiro, M. N. S., Souza, J. L., & Araújo, M. J. A. M. (2012). Qualidade dos méis produzidos por *Melipona fasciculata* Smith da região do cerrado maranhense. *Química Nova, 35*(1), 55-58. https://doi.org/10.1590/S0100-40422012000100011

Lage, L. G. A., Coelho, L. L., Resende, H. C., Tavares, M. G., Campos, L. A., & Fernandes-Salomão, T. M. (2012) Honey physico chemical properties of three species of the brazilian *Melipona*. *Anais Academia Brasileira de Ciência, 84*(3), 605-8. https://doi.org/10.1590/S0001-37652012005000051

Massaguer, P. R. (2005). *Microbiologia dos processos alimentares* (1st ed., p. 258). São Paulo: Varela.

Mesquita, L. X., Sakamoto, S. M., Maracaja, P. B., Pereira, D. S., & Medeiros, P. V. Q. (2007). Análises físico-químicas de amostras de mel de jandaira puro (*Melipona subnitida*) e com misturas. Mossoró. *Revista Verde, 2*, 65-68.

Michener, C. D. (1974). *The Social Behavior of the Bees: A Comparative Study*. Cambridge, MA: Harvard University Press.

Oliveira, E. G., Monteiro Neto, V., Silveira, L. M. S., Nascimento, A. R., Nahuz, M. S. R., Meneses, S. L., … Lima, J. C. (2006). Avaliação de parâmetros físico-químicos do mel de tiúba (*Melipona compressipes fasciculata* Smith), produzido no Estado do Maranhão. *Higiene Alimentar, 20*(146), 74-81.

Rao, P. V., Krishnana, K. T., Sallehb, N., & Gan, S. H. (2016). Biological and therapeutic effects of honey produced by honey bees and stingless bees: A comparative review. *Revista Brasileira de Farmacognosia, 26*, 657-664. https://doi.org/10.1016/j.bjp.2016.01.012

Roubik, D. W. (1983). Nest and colony characteristics of stingless bees from Panama (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society, 56*, 327-355.

Roubik, D. W. (1992). *Ecology and natural history of tropical bees* (p. 514). New York: Cambridge University Press.

Scott, V. N., Clavero, R. S., & Troller, J. A. (2001). Measurement of water activity (aw), acidity, and Brix. In F. P. Downes, & K. Ito (Eds.), *Compendium of Methods for the Microbiological Examination of Foods* (pp. 649-659). APHA, Washington, DC. https://doi.org/10.2105/9780875531755ch64

SEI (Superintendência de Estudos Econômicos e Sociais da Bahia). (2007). Retrieved January 12, 2015, from http://www.sei.ba.gov.br

Silveira, F. A., Melo, G. A. R., & Almeida, E. A. B. (2002). *Abelhas Brasileiras: Sistemática e Identificação* (p. 253). Belo Horizonte: Fundação Araucária.

Simal, J., & Huidobro, J. (1984). Parámetros de calidad de lamiel. III. Acidity (pH libre, lactónica& total) e índice de formol. *Offarm, 3*, 532.

Sousa, J. M. B., Aquino, I. S. A., Magnani, M., Albuquerque, J. R., Santos, G. G., & Souza, E. L. (2013). Aspectos físico-químicos e perfil sensorial de méis de abelhas sem ferrão da região do Seridó, Estado do Rio Grande do Norte, Brasil. *Semana: Ciências Agrárias, 34*(4), 1765-1774. https://doi.org/10.5433/1679-0359.2013v34n4p1765

Souza, B. A., Carvalho, C. A. L., Sodre, G. S., & Marchini, L. C. (2004). Características físico-químicas de amostras de mel de *Melipona asilvai* (Hymenoptera: Apidae). *Ciência Rural, 34*, 1623-1624. https://doi.org/10.1590/S0103-84782004000500048

Souza, B. A., Marchini, L. C., Oda-Souza, M., Carvalho, C. A. L., & Alves, R. M. O. (2009). Caracterização do mel produzido por espécies de *Melipona* illiger, 1806 (Apidae: Meliponini) da região nordeste do Brasil: 1. Características físico-químicas. *Química Nova, 32*(2), 303-308. https://doi.org/10.1590/S0100-40422009000200007

Souza, B. A., Roubik, D. W., Barth, O. M., Heard, T. A., Enríquez, E., Carvalho, C. A. L., … Vit, P. (2006). Composition of stingless bee honey: Setting quality standards. *Interciência, 31*, 867.

Stefanini, R. (1984). Variability and analysis of Italian honeys. *Apiacta, 19*(4), 109-114.

Veríssimo, M. T. L. (1988). Saiba o que é o HMF. *Apicultura no Brasil, 4*(24), 31.

Villas-Boas, J. K., & Malaspina, O. (2005). *Parâmetros fisico-químicos propostos para controle de qualidade do mel de abelhas indígenas sem ferrão no Brasil* (Vol. 82, pp. 6-16). Mensagem Doce, São Paulo.

Vit P., Persano Oddo, L., Marano, M. L., & Salas de Mejías, E. (1998). Venezuelan stingless bee honeys characterized by multivariate analysis of physicochemical properties. *Apidologie, 29*, 377-389. https://doi.org/10.1051/apido:19980501

Vit, P., Bogdanov, S., & Kilchemann, V. (1994). Composition of Venezuelan honeys from stingless bees (Apidae: Meliponinae) and *Apis mellifera* L. *Apidologie, 25*, 278-288. https://doi.org/10.1051/apido:19940302

Vit, P., Medina, M., & Enríquez, E. (2004). Quality standards for medicinal uses of Meliponinae honey in Guatemala, México and Venezuela. *Bee World, 85*(1), 2-5. https://doi.org/10.1080/0005772X.2004.11099603

White Junior, J. W. (1976). Composition of honey. In E. Crane (Ed.), *Honey a comprehensive survey* (pp. 157-206). London, Heinemann.

White Junior, J. W. (1979). Methods for determining carbohydrates, hydroxymethylfurfural and proline in honey; Collaborative study. *Journal of the Association of the Official Analytical Chemistry, 62*(3), 515-526.

Yaacob, M., Stanis, A. J., Rajab, N. F., Shahar, S., & Sharif, R. (2017). Current knowledge on honey and its derivatives with genomic stability: A mini review. *Journal of Agricultural Science, 9*(13), 145-155. https://doi.org/10.5539/jas.v9n13p145

Zamora, M. C., & Chirife, J. (2006). Determination of water activity change due to crystallization in honeys from Argentina [en línea]. *Food Control, 17*, 59-64. https://doi.org/10.1016/j.foodcont.2004.09.003