Vol. 17(51), pp. 1422-1435, 19 December, 2018
DOI: 10.5897/AJB2018.16633
Article Number: B23C79059633
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Quali-quantitative characterization of the honey from *Myracrodruon urundeuva allemão* (Anacardiceae - Aroeira): macroscopic, microscopic, physico-chemical and microbiological parameters

Eliane Macedo Sobrinho Santos[1*], Hércules Otacílio Santos[1], Juliana Rezende Sá Miranda Gonçalves[1], Anna Christina Almeida[2], Igor Viana Brandi[2], Alexsander Rodrigues Cangussu[3], Janainne Nunes Alves[1], Ricardo Jardim Neiva[1], Grazielle Layanne Mendes Santos[2], Maria Izabel de Jesus Viana[2], Bruna Ruas Santos Araújo[2], Thalita Cordeiro Santos[2] and Kattyanne Souza Costa[4]

[1]Federal Institute of Northern Minas Gerais-Campus Araçuaí, Minas Gerais, Brazil.
[2]Institute of Agrarian Sciences of UFMG-Campus Montes Claros, Minas Gerais, Brazil.
[3]Federal University of Tocantins-Campus Gurupi, Tocantins, Brazil.
[4]MSD-Site Montes Claros, Minas Gerais, Brazil.

Received 30 August 2018; Accepted 31 October, 2018

The objective of the present study was to evaluate the external and organoleptic characters, microscopic, physico-chemical, chromatic and microbiological parameters of the honey from *Myracrodruon urundeuva allemão* (Anacardiceae - Aroeira) of the Jequitinhonha River Valley / Brazil, in order to create its geographical indication. In the first stage a data survey was carried out to characterize the production of honey in the Jequitinhonha Valley. In order to evaluate the use of land as well as the forms of agriculture in relation to beekeeping. For characterization of the aroeira honey of the area, the microscopic, physico-chemical and microbiological quality of the honey was evaluated in samples collected in AAPIVAJE. Samples were collected for convenience. Honey production did not correlate with the area occupied, in hectare, by none of the types of land use in the middle region of the Jequitinhonha River Valley. There was also no significant correlation between honey production and the form of agriculture. In general, all samples of honey analyzed had satisfactory physico-chemical characteristics in terms of parameters of sugars, moisture, ashes, pH, acidity, color, taste and consistency. The analyzed samples were free of commercial sugar and dyes and present diastase activity. In this study we can see that all honey samples had a low microbial count of coliforms at 30°C and coliforms at 45°C (thermotolerant), indicating good hygienic-sanitary quality of the honey extraction and processing procedures. In the same way, it was observed that the counts of fungi and yeasts of all the honey were within the recommended parameters in the legislation of Apis. The presence of *Salmonella* was not detected in any sample of aroeira honey. This study will contribute to the geographical indication for aroeira honey, produced only in the middle region of the Jequitinhonha River Valley.

Key words: *Apis mellífera*, honey quality, sugars, diastase enzyme, moisture, ashes, acidity, microbial.

INTRODUCTION

Beekeeping is a source of work and income, mainly in rural property formed basically by family farming, making it a sustainable strategy for the small farmer. This activity, in addition to being profitable, is capable of promoting regional development, reducing rural exodus, while preserving the environment (Freitas et al., 2004; Kizilaslan and Kizilaslan, 2007).

The main products derived from the apicultural practice are honey, beeswax, propolis, royal jelly, pollen and apitoxin. Honey, possessing unique nutritional qualities and a huge market, is the most important (Ozcan and Olmez, 2014). Honey is a complex natural product produced by bees from flower nectar, tree and plant secretions or exudates from plant-sucking insects (Pires et al., 2009; Feás et al., 2010; Ozcan and Olmez, 2014). This product is increasingly valued by consumers and the food industry because of its strong and characteristic taste (Castro-Vazquez et al., 2006; Jerkovic et al., 2010).

The growing demand for honey in many countries in the world requires its diversification of other types of honey as a response to the consumer request (Simova et al., 2012). Therefore, the determination of botanical essences of apicultural interest as an influencer of honey characteristics becomes essential, since the specific composition may depend on the diversity of flower sources (Anjos et al., 2015; Laallam et al., 2015).

In the Jequitinhonha River Valley, little attention has been focused on beekeeping and the various components of beekeeping efficiency despite the availability of a number of techniques to stimulate efficiency components in production units. In this region, the production of honey has presented very marked fluctuations over the decades. Factors related to climate, diseases of bees, incentives of prices paid in production and development of technologies associated with the production process are some causes that determine the success or failure of the harvest. Besides that, the vegetation this region is threatened by economic activity and therefore beekeeping represents a sustainable alternative income.

For beekeeping to develop in a sustainable way, it is essential, among other needs, to know the flora that provides resources to bees. Aroeira (*Miracrodruon urundeuva allemão*) is a native tree from the Jequitinhonha region and other regions of Brazil, which has excellent characteristics for apiculture pasture, releasing its flowering from May to July. Generally, at this time the aroeira is one of the few sources of nectar (Nunes et al., 2008). The predominance of *M. urundeuva* pollen grains indicates that this specie is the main nectar source for *Apis mellífera* in this Brazilian Dry Forest during the hyper dry period (Bastos et al., 2016).

Despite the empirical knowledge of beekeepers about the potential of Aroeira, there is no study that actually proves their real qualitative and quantitative importance for beekeeping activity. In view of the above, and knowing that the quality control analyses are extremely important in the evaluation of origin, quality, adulteration, storage conditions, and contamination of honey, the objective of the present study was to evaluate the external and organoleptic characters, microscopic, physico-chemical, chromatic and microbiological parameters of the honey from *M. urundeuva allemão* (Anacardiceae - Aroeira) of the Jequitinhonha River Valley/Brazil, in order to create its Geographical Indication.

MATERIALS AND METHODS

Data collection on honey productivity

In this stage a data survey was carried out to characterize the production of honey in the Jequitinhonha River Valley. The Jequitinhonha River Valley region is located northeast of Minas Gerais and is cut by the Jequitinhonha River and its main tributary is the Araçuaí River. The region is divided into Upper, Middle and Lower Jequitinhonha. The relief is formed by large flat plateaus and deep caves. The climate is semi-arid with predominance of the Caatinga, Cerrado and remnants of Atlantic Forest. The average temperature is between 21 and 24°C, with annual rainfall below 1,000 mm (Diniz et al., 2001).

The territory of the middle region of the Jequitinhonha River Valley covers 18 municipalities: Águas Vermelhas, Araçuaí, Berilo, Cachoeira do Pajeú, Chapada do Norte, Comercinho, Coronel Murta, Francisco Badaró, Itaobim, Itinga, Jenipapo de Minas, José Gonçalves de Minas, Medina, Padre Paraiso, Pedra Azul, Ponto dos Volantes and Virgem da Lapa. The population in this part of Jequitinhonha valley is 279,326 inhabitants (IBGE, 2010). The data collected at this stage of the work were related to the municipalities of Francisco Badaró, Jenipapo de Minas, Berilo, Araçuaí and Chapada do Norte. Information about the production of honey specific for aroeira flowers were extracted from the database of – the Association of Beekeepers of the Jequitinhonha Valley (AAPIVAJE), from March to September, 2017.

In order to evaluate the use of land as well as the forms of agriculture (family and non-family) in relation to beekeeping, the Brazilian Statistic and Geographic Institute (IBGE) secondary database was used for the agricultural sense of 2006. The area occupied by the landscape units: pasture, matas and tillage were quantified in hectare and in % and related to the honey production (kg) of each municipality in 2006.

Characterization of the aroeira honey of the Jequitinhonha River Valley

In this stage of the work the microscopic, physical-chemical and microbiological quality of the honey was evaluated in samples collected in AAPIVAJE, from the five municipalities that were part of

*Corresponding author. E-mail: elianemsobrinho@hotmail.com.

Table 1. Description of the samples analyzed in this study.

Sample	Flowering	Municipality
1	Aroeira	Araçuaí
2	Aroeira	Berilo
3	Aroeira	Berilo
4	Aroeira	Francisco Badaró
5	Aroeira	Francisco Badaró
6	Aroeira	Berilo
7	Aroeira	Francisco Badaró
8	Aroeira	Berilo
9	Aroeira	Jenipapo de Minas
10	Aroeira	Virgem da Lapa

the study. Samples were collected for convenience, depending on the supply availability of the combination. In this way we were able to collect the following samples in Table 1. The analyses performed in the present study are described as follows:

i) External and organoleptic characters and microscopic analyzes

The color classification of the honey was carried out in a spectrophotometer, which will consist of a reading at 560 nm (Abs560) using pure glycerine as white. The reading was later transformed into color expressed in millimeters (mm) by the Pfund scale (BRASIL, 1985). The color of the honey is variable, depending on its composition the darker (coffee color) richer in minerals and stronger is its flavor; the clearer, the poorer in minerals, and may even appear almost colorless, in which case the taste is mild (Crane, 1996). Thus, the taste of the samples was classified as strong or soft. The consistency of the honey was classified as liquid, liquid-crystallized, liquid-granulated, crystallized, granulated and creamy (Couto, 2002). Microscopically, the honey samples were analyzed under optical microscope at the Laboratory of Chemistry of the IFNMG - Campus Araçuaí. One drop of honey was deposited between the slide and the coverslip and observed in a 10x and 40x objective, to investigate dirt and foreign material.

ii) Pollen analysis

Samples of aroeira honey were sent to a reference laboratory for analysis of pollen that could prove the presence of the respective flowers in the product.

iii) Physical-chemical and chromatic analysis

The physico-chemical analyses included among the maturity indicators of honey: moisture; indicators of honey deterioration: pH and acidity; and sensory characteristics: color, taste and consistency. All analyzes were performed in triplicate, following the methods recommended by the Brazilian legislation (BRASIL, 2000). The procedures used were in accordance with the methodology of the Association of Official Analytical Chemists (AOAC, 1998). All the physical-chemical analyzes carried out are as follows:

(a) Moisture: The determination of the moisture of the samples was performed by the refractometric method. An Abbe benchtop refractometer was used. The measurement of the refractive index (IR) of the sample was converted to a percentage of moisture, based on the relationship between the refractive index and the moisture (%) of the honey.

(b) Determination of ashes: The ashes were determined by weighing about 10 g of honey in a tared porcelain capsule. The honey was carefully heated in flame until the swelling ceased, taking care to avoid projecting droplets. The sample was then incinerated at 450°C until white residue was obtained (about three hours) (UFPR - Laboratory of bromatology).

(c) pH and Acidity: A pre-calibrated digital pH (microprocessor / DLA-pH) pH was used for pH measurement. The method of measuring the acidity of honey was based on the determination of free, lactonic and total acidity, with the aid of pH meter. The free acidity was the measurement obtained from titration with sodium hydroxide (0.05 N) to the equivalence point (pH 8.5). The lactonic acidity was obtained by the addition of 10 ml of sodium hydroxide, later titrated with hydrochloric acid. Total acidity was the sum between free acidity and lactonic acidity. 10 ml of honey (beaker) were weighed and 75 ml of CO_2 free water was added, and the pH of this solution was checked. It was titrated with stirring of the solution with a magnetic stirrer and electrode dipped in the solution (honey + water), initially with sodium hydroxide (NaOH) at a rate of 5.0 ml per minute in the solution to pH 8.5 (free acidity). 10 ml of NaOH was quickly added to the solution (honey + water). The last titration was with hydrochloric acid (HCL) until it reached pH 8.3 (lactonic acidity). For the purposes of calculations and corrections it was necessary to prepare the blank, which consisted in measuring the pH of the distilled water and titrating with NaOH up to pH 8.5 (AOAC, 1998).

Calculation of acidity: Free acidity = (corrected NaOH volume corrected - white) × 50 × (correction factor/sample weight)
Lactone acidity = (10 - corrected HCl volume) × 50 ×(correction factor/sample weight)

(d) Sugar detection: Honey samples were sent to a reference laboratory for the analysis of non-reducing sugars (in sucrose) and reducing sugars (in glucose). The methodology used followed what is recommended by Ministry of Agriculture, Livestock and Food Supply (MAPA) - Administrative Rule No. 1 of 07/10/1981 (BRASIL, 1981).

In order to verify a possible addition of commercial sugar to honey, the Jagerschmidt reaction was performed. To begin this analysis, about 10 g of honey and 10 ml of acetone were crushed in porcelain grains. After decanting the solvent, about 2-3 ml was transferred into a test tube containing equal volume of concentrated HCl. And finally, the mixture was cooled in an ice bath or running water. The appearance of strong violet color indicates the presence of commercial sugar. If honey is natural product, a slight amber coloration may appear that turns to violet after some time

(Department of Health/SC, 1985).

(e) Research of diastase enzyme: Another way of verifying adulteration in honey was through the diastase enzyme research. In this analysis, 1 g of honey was dissolved in 20 ml of distilled water previously boiled and cooled to 45°C. In a pre-washed test tube with boiled water, 10 ml of the honey solution (unfiltered) was added and then 1 ml of freshly prepared, clear, 1% solution of starch. The remaining 10 ml were stored in another blank test tube to be done at the end of the experiment.

Thereafter, the tube containing the starch solution was well shaken, leaving in a water bath at exactly 45°C for 1 h. A few drops of lugol solution were then added to the two tubes (blank and assay) and the color the liquid developed was observed. The expected coloration should be around a greenish or yellowish brown, proving the presence of diastase enzymes, natural in honey. If it causes a violet color or lack of expected color, this will indicate poor extraction conditions or fraud, respectively (Department of Health/SC, 1985).

(f) Search for dyes: To verify the presence of dyes in honey, 1 g of honey was weighed, dissolving it in 10 ml of distilled water. Then, about 2 ml of 5% sulfuric acid solution was added. The honey should remain unchanged. If there are coloring substances added to honey, the color gradually changes from violet to pink (Department of Health/SC, 1985).

iv) Microbiological analyses

Microbiological analysis of the research focused on the occurrence of *Salmonella* spp., the most probable number of coliforms at 35 and 45°C, which were based on the methodologies described in Normative Instruction No. 62 (BRASIL, 2003). For standard counts on filamentous fungi and yeast plaques, and identification of fungal species, the serial decimal dilution methodology described by Pitt and Hocking (2009) was followed. From each sample 25 g of honey were collected and weighed aseptically and added to 225.0 mL of 0.1% peptone saline, thereby obtaining an initial dilution of 10^{-1} and from that dilution dilutions were prepared to 10^{-3}.

(a) *Samonella* spp search: For the research of *Salmonella* spp. the pre-enrichment was done by transferring 25 ml of honey to 225 ml in lactose broth incubating at 35°C for 24 h. For the selective enrichment, the Rappaport-Vassiliadis broth and the cystine broth selenite were used, transferring 0.1 and 1.0 ml, respectively, being incubated at 45 and 35°C respectively. In isolation, Hektoen Enteric agar (HB), bismuth sulphite agar (BS) and deoxycholate-lysine-xylose agar (XLD) were used and the inocula were incubated at 37°C for 24 h. Characteristic colonies were transferred to the medium sugar-iron triple agar and lysine-iron agar for preliminary biochemical characterization.

(b) Most probable number of coliforms at 35 and 45°C: The determination of total coliforms will be performed by the multi-tube fermentation method; using series of three tubes in the presumptive procedures by inoculating 1.0 ml of each dilution in the Lauryl Sulfate Triptose broth (LST) and the brilliant green bile lactose broth (BGBL) a for the confirmatory tests, with incubation at 36.0 ± 1°C for 24 to 48 h. Confirmation of the presence of coliforms at 45°C will be performed by inoculating the suspect colonies in EC broth and subsequent incubation at a selective temperature of 45 ± 0.2°C in a water bath with constant shaking for 24 h.

(c) Filamentous fungi and yeast counts: The count of filamentous fungi and yeasts was performed according to serial decimal dilution methodology described by Pitt and Hocking (2009). 25 g of the sample was homogenized in 225 ml of 0.1% peptone water. From this initial dilution (10^{-1}) serial decimal dilutions were prepared up to 10^{-3}. The inoculums were 0.1 ml aliquots per petri dish on the surface of the culture medium (in duplicate) potato dextrose agar (PDA), of each dilution, used for general counting (King et al., 1979). BDA plates were incubated at 25°C for seven days in the absence of light. All plaques were observed, being selected those that presented UFC.g-1 around 10 to 100 (Dalcero et al., 1997, 1998).

Statistical analyses

A preliminary exploratory and descriptive research was carried out to collect data on honey production in the middle region of the Jequitinhonha River Valley, as well as the microscopic, physico-chemical and microbiological characterization of honey produced in the region. The statistical analysis used to evaluate the relationship between honey production and land use and form of agriculture was the Pearson correlation, due to the data presented in the Normal distribution, in which each type of land use or form of agriculture was correlated with the production of honey. The significance level (alpha) considered was 5%. For these analyses, we used the program SPSS®, version 13.0, for Windows.

RESULTS

Honey productivity and relation to land use and forms of agriculture with beekeeping activity

It is verified that the pasture occupation was bigger in all municipalities (Figure 1A), pointing out the more recent tendencies of development and replacement of traditional activities by cattle raising with the use of native and artificial pastures. The occupation by forests and / or forests was lower in the municipality of Jenipapo de Minas, although this represents 21.10% of the total landscape, very similar to the municipality of Virgem da Lapa, which presented a greater occupation by forests and / or forests. Araçuaí presented the largest area occupied by forests and / or forests, represented by 32.99% of the total landscape. The occupation by crops and agroforestry varied among the municipalities, being the municipality Francisco Badaró with less occupation by agroforest, in absolute numbers and percentages, and the Araçuaí municipality with greater area occupied by agriculture, representing 10.33% of the total landscape (Figure 1A).

Honey production did not correlate with the area occupied, in hectare, by none of the types of land use in the middle region of the Jequitinhonha River Valley. The p values were greater than 0.05 under all conditions.

In the same way that the correlation of honey production with the occupation of the area in hectare occurred, also occurred with the occupation of the area in percentage. No significant correlation was observed ($p>0.05$).

Regarding the form of agriculture in the five municipalities contemplated in this study, family agriculture is predominant in all municipalities, with the largest area occupied by family agriculture located in the Araçuaí municipality and the smallest in the municipality

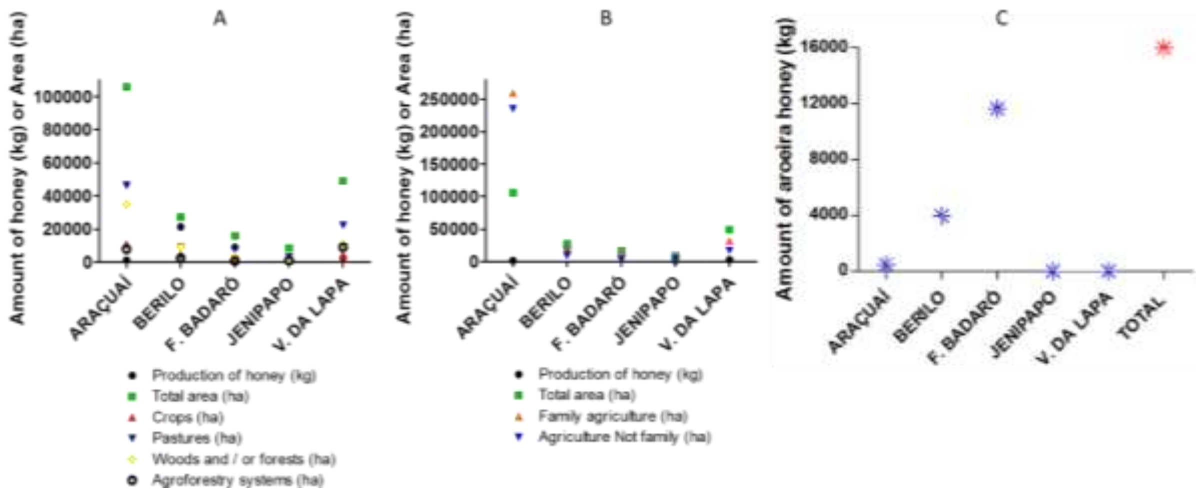

Figure 1. Forms of land use (ha) and honey production in 2006 in the five sampled municipalities of Jequitinhonha River Valley (A). Form of agriculture (ha) and honey production in 2006 in the five sampled municipalities of Jequitinhonha River Valley (B). Production of honey delivered to the association of beekeepers in the five sampled municipalities of the Jequitinhonha River Valley from March to September 2017 (C).

of Jenipapo de Minas. These being the municipalities that present the largest and smallest territorial extension, respectively. There was also no significant correlation between honey production and the form of agriculture ($p>0.05$) (Figure 1B). The production of honey delivered to the association of beekeepers was expressive in the five sampled municipalities of the middle region of the Jequitinhonha River Valley, from March to September 2017, with the production of honey of aroeira reaching 16 tons (Figure 1C).

Microscopic characterization and qualitative analysis of pollen

All samples were free from foreign matter as recommended by current legislation. The standards of honey identity and quality (BRASIL, 2000) require, for macroscopic and microscopic aspects, that the product be free of foreign substances of any nature, such as: insects, larvae, grains of sand and others. Samples of aroeira honey registered in the laboratory under the number of Reg. 17.5806 showed a positive pollen result for the aroeira flowering analyzed (Test code = 093).

External and organoleptic characterization of honey samples

As to the color of the honey samples, it was observed that the majority presented a dark amber coloration, with absorbance values at 560 nm ranging from 0.3876 to 1.8252, corresponding to light amber and dark amber, respectively. The flavors and consistencies of the honey samples were in accordance with the characteristics of honey from aroeira (Table 2).

Physico-chemical and chromatic characterization of aroeira honey

The results of the physical and chemical analyzes were expressed by means of the triplicates and compared to the values suggested by Normative Instruction No. 11 of the Ministry of Agriculture and Supply (BRASIL, 2000), when possible.

In general, all samples of honey analyzed had satisfactory physico-chemical and chromatic characteristics (Figures 2 to 4). As observed in Figure 2A, all samples of aroeira honey presented values of moisture within normality, that is, not exceed the limits recommended by the current legislation. The maximum moisture content for honey samples, considering the parameters established by the Brazilian Ministry of Agriculture and Supply is 20% (m/m). Implicating results within the law for samples of honey of aroeira, whose maximum value of moisture reached 16% (Figure 2A).

Soluble solids correspond to all substances that are dissolved in a given solvent. They are mainly composed of sugars, variable with the plant species and climate. They are designated as Brix and tend to increase with maturation. In honey, the soluble solids content is very close to the total sugars content, which makes this simple and economical technique very useful (Gois et al., 2013).

In this study the soluble solids content was between 80 and 90% (Figure 2B), values considered normal in accordance with regulation N° 11 of 2000 (Ministry of Agriculture, Livestock and Food Supply - Brazil), which

Table 2. Classification of color, taste and consistency of aroeira honey.

Sample	Absorbance at 560 nm	Color	Taste	Consistency
Aroeira 1	0.6669	Amber	Strong	Liquid
Aroeira 2	1.8025	Dark amber	Strong	Liquid
Aroeira 3	1.733	Dark amber	Strong	Liquid
Aroeira 4	1.4045	Dark amber	Strong	Liquid
Aroeira 5	1.6872	Dark amber	Strong	Liquid
Aroeira 6	1.6777	Dark amber	Strong	Liquid
Aroeira 7	1.8252	Dark amber	Strong	Liquid
Aroeira 8	1.7986	Dark amber	Strong	Liquid
Aroeira 9	1.7615	Dark amber	Strong	Liquid
Aroeira 10	0.3876	Light amber	Soft	Liquid

establishes a minimum of 65% for reducing sugars and 6% for sucrose (BRASIL, 2000).

The ash content indicates the amount of minerals found in the honey, determining its color. In this study the ash content varied from 0.21 to 0.37% (Figure 2C), which are values below the maximum limit (0.6%) determined by the Ministry of Agriculture, Livestock and Supply. The color of the honey was not altered when performing dye research, as shown in Figure 2D. This indicates that the honey samples were free of the addition of these compounds.

Although it is not mandatory, by official legislation, the pH analysis as indicative of the physico-chemical quality of the honey, it was performed as a complementary parameter for the evaluation of the total acidity. pH values ranged from 3.86 to 4.55 (Figure 2C). The value for free acidity varied from 17.32 to 30.64 Meq.kg $^{-1}$. Therefore, no sample was found to be in disagreement with the current legislation (Figure 2D). The Brazilian legislation accepts maximum acidity of 50 Meq.kg $^{-1}$ of honey (BRASIL, 2000). Codex and the European Union also determine a maximum acidity of 50 Meq.kg $^{-1}$, but specify that the parameter to be measured is free acidity (CAC, 2001; UNIÃO-EUROPÉIA, 2001). For MERCOSUL the maximum acidity limit is 40 Meq.kg $^{-1}$ (MERCOSUL, 1999). The content of individual sugars such as glucose, fructose and sucrose are important when evaluating the degree of sweetness of products, since the sweetness of these products is varied and increases in the glucose sequence: sucrose: fructose (Chitarra and Chitarra, 2005). In the present study, the mean values obtained for reducing sugars and non - reducing sugars were 66.44% (65.40 - 67.90%) and 2.99% (2.00 - 5.30%) respectively (Figure 4A – B).

In the Jagerschmidt reaction there was no evidence of strong violet color in any of the samples, which indicates the presence of commercial sugar (Figure 4C). Diastase enzyme research revealed violet staining for one sample, and in the other samples yellow staining. The result of the violet sample indicates a decrease in the activity of the diastase enzymes, probably due to the heating of the honey. The yellow staining of the other samples indicates the presence of the diastase enzymes (Figure 4D).

In the correlation analysis performed between the physical-chemical parameters, a negative correlation was observed between moisture and free acidity (Figure 3 and Supplementary Table 1). The acidity of the honey is due to the amount of minerals and the variations of the organic acids. With the increase of the water content in the honey, the minerals and organic acids become more diluted, and therefore diminished. The correlation between ash content and honey color was not significant ($p = 0.123$), based on absorbance values at 560 nm. Most of the samples showed dark amber coloration and for this color the ash contents ranged from 0.22 to 0.37%. It is important to note that the samples that presented lighter coloring also had lower gray levels.

Microbiological characterization of honey

Microbiological quality may be associated with the hygienic conditions of food production, processing and handling. In this work we can see that all honey samples had a low microbial count of coliforms at 30°C and coliforms at 45°C (thermotolerant), indicating good hygienic-sanitary quality conditions (Table 3). In the same way, it was observed that the counts of fungi and yeasts of all the honey presented within the recommended in the legislation of Apis (BRASIL, 1985, 1997, 2003). The presence of Salmonella was not detected in any sample of aroeira honey (Table 3).

DISCUSSION

Beekeeping is considered an important activity for the agricultural sector at the national level, and in the Jequitinhonha River Valley it has been highlighted in recent years, due to the income opportunity given to small producers. Thus, in the Jequitinhonha River Valley, beekeeping is among the most promising economic

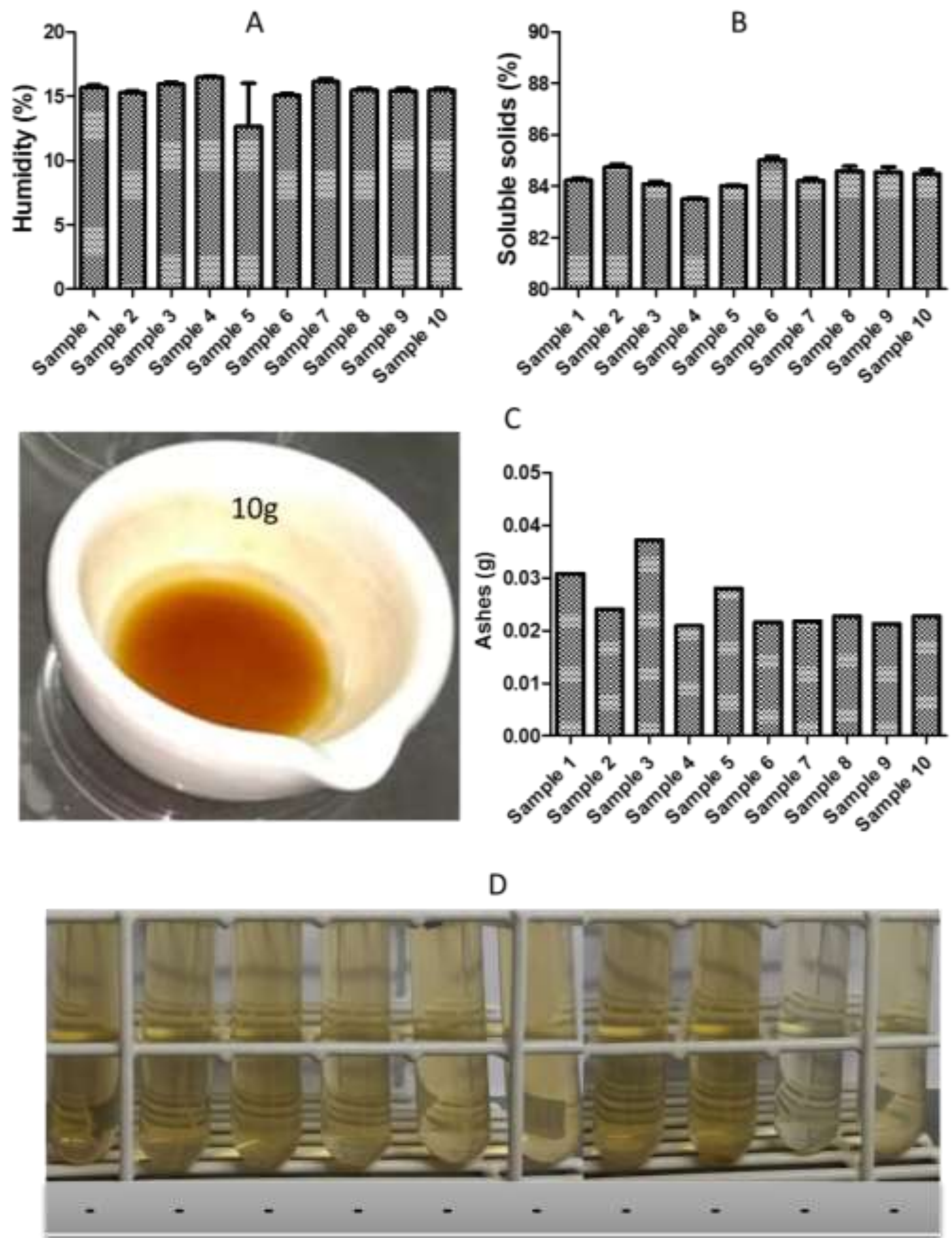

Figure 2. Physico-chemical parameters evaluated: Moisture (A), soluble solids (B) content of aroeira honey using the Chataway table. The values presented are triplicate averages. Ashes (C) and dye research (D) in aroeira honey.

activities in the region, especially due to favorable environmental conditions. However, the region has untapped beekeeping potential. In addition to the economic aspects, brazilian beekeeping, and thus the Jequitinhonha River Valley, meets some requirements that also credence as an activity with a high potential for social inclusion, considering the economic, social and environmental characteristics, which contributes to higher levels of profitability (Freitas et al., 2004).

In the present study we tried to investigate in which landscapes or in what form of agriculture the production of honey predominates. No significant correlation was found in the analyses. The absence of correlation between the production of honey and the landscape units may be due to the bees finding nectar resource in all landscape. In the same way, whether family farming or

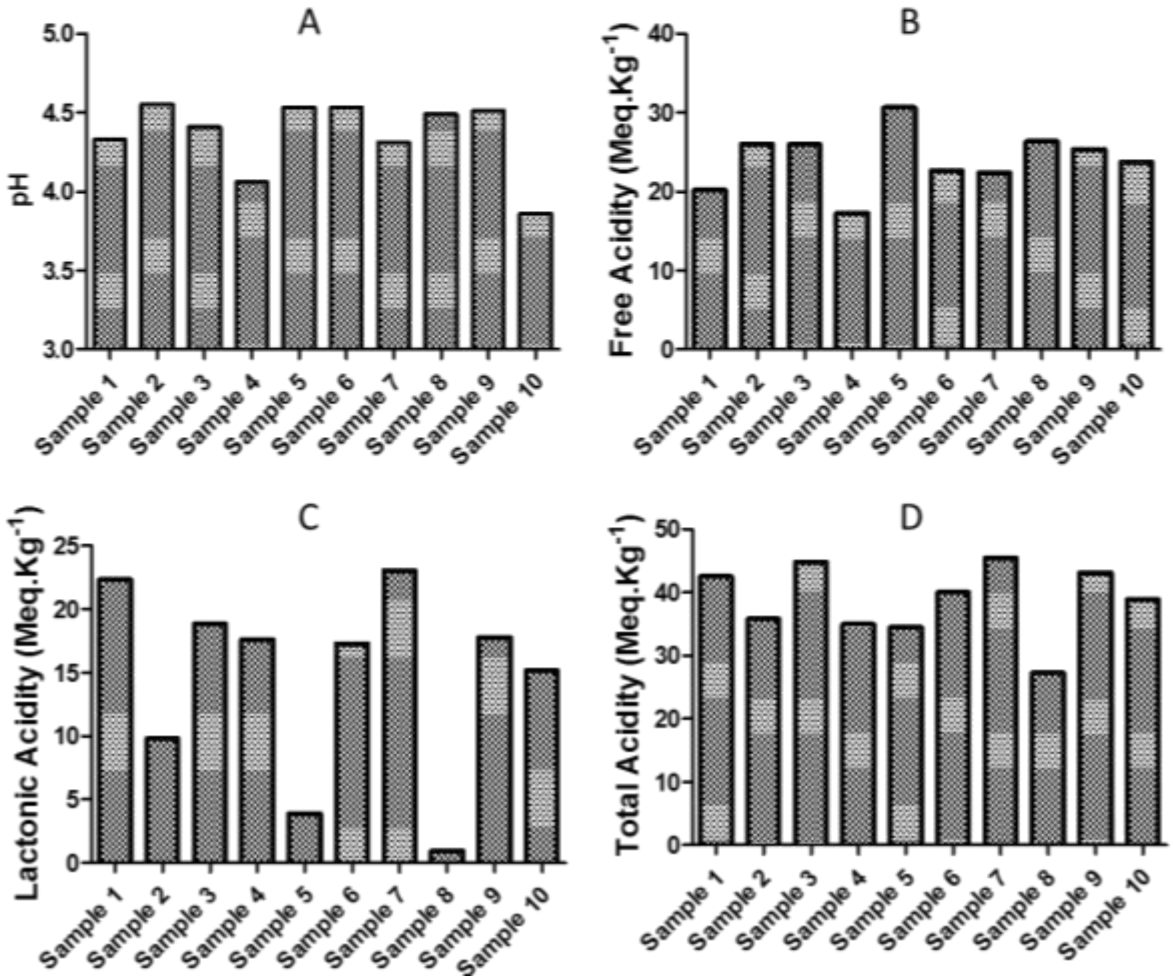

Figure 3. Physico-chemical parameters evaluated: pH (A), Free acidity (B), Lactonic acidity (C) and Total acidity (D) values of aroeira honey.

not, both are apt to the development of beekeeping fully. It is also worth mentioning that the honey of aroeira has found a prominent place in the production of honey in the region, due to the presence of the extensive forests of aroeira that beautify the region. In this sense, the quantity of producers, and consequently the production of aroeira honey has expanded in the region.

The honey produced in Brazilian Dry Forest, also present in the Jequitinhonha River Valley, is characterized as a *M. urundeuva* (aoriera) 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 (Bastos et al., 2016). As an animal product, all honey marketed is subject to the standards foreseen by the legislation. Therefore, make sure that aroeira honey meets the requirements of the current standards, can be an attraction to the marketing of honey. In addition, demonstrating the benefits of aroeira honey can add value to the product, enhancing production in the region and promoting expansion. So, providing support for the creation of a standard identity and specific quality for aroeira honey is crucially increases knowledge of the product, and thus allows supervision by food control authorities which, in turn, guarantees the quality of the honey for consumers.

The flavor and aroma are directly linked to the honey color. The darker the honey, the richer in minerals, consequently stronger flavor and aroma, which was the case of the majority of honey samples evaluated in this study. The aroma and flavor of the honey is that of the original flower, it goes from sweet to sweet and strong and may have acid or bitter taste (Ajlouni and Sujirapinyokul, 2010). The consistency of the honey can be liquid, liquid-crystallized, liquid-granulated, crystallized, granulated and creamy. In the Brazilian market there is a greater trend of consumption of liquid honey, while in Europe, the most sought-after honey is the creamy honey, there are equipment to beat the honey to make it creamy and consequently light in color (Couto, 2002).

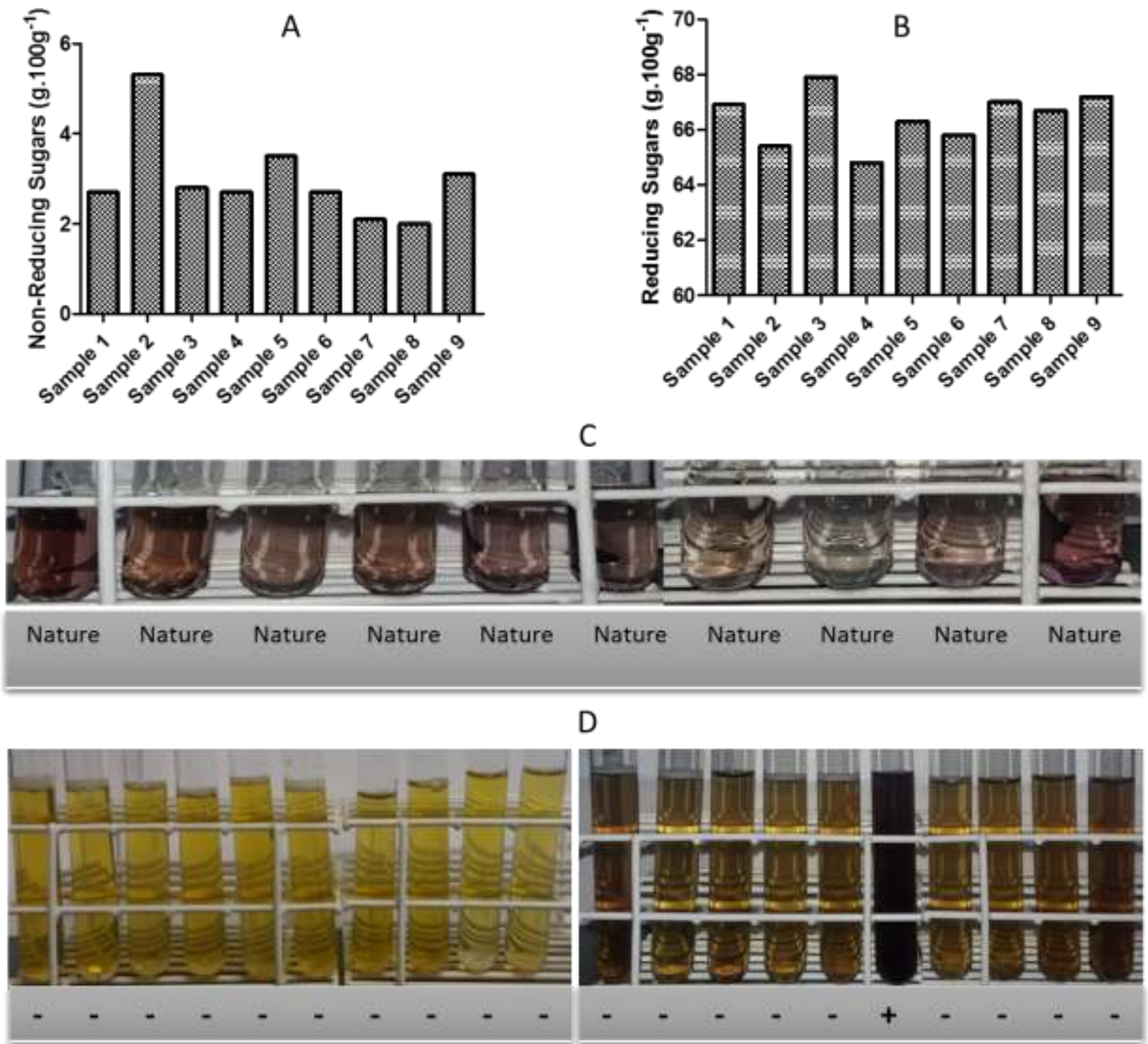

Figure 4. Physico-chemical and chromatic parameters evaluated: Detection of non-reducing sugars (in sucrose) (A), reducing sugars (in glucose) (B) in samples honey. Jagerschmidt reaction (C) and Diastase enzyme research (D) in aroeira honey.

Honey from aroeira usually presents in liquid form and is hardly presented in crystallized or granulated form.

In the physico-chemical analyzes of honey, it was verified that the moisture values of the samples, ranging from 15.07 to 16.47%, did not exceed the limits recommended by the current legislation (BRASIL, 2000). The water content in honey is one of the most important characteristics and constitutes the second component in quantity, varying according to the climate, floral origin and harvesting season. The moisture content is the main determinant of parameters such as viscosity, specific gravity, crystallization and flavor, besides being an important indicator of the fermentation tendency, mainly influencing the conservation of the product (Abramovic et al., 2008; Al-Ghamdi et al., 2017). In this way, it is evident that the conditions of processing and storage of the analyzed samples are adequate to guarantee this parameter.

Ash content provides important information about the quality of honey, as floral honey has lower ash content than honeydew honey (Almeida-Muradian et al., 2013). In the present study the ash content did not exceed 0.37%, indicating a good quality for the Jequitinhonha River Valley honey and also its floral origin. According to Brazilian legislation, the ash content in blossom honey should be at maximum 0.6%, and at maximum 1.2% for honeydew honey (BRASIL, 2000). The absence of significant correlation between the ash content and the

Table 3. Investigation of Total Coliforms, Thermotolerant Coliforms, *Salmonella*, Fungi and Yeasts in aroeira honey. The values presented are triplicate averages.

Sample	Total Coliformes MPN/ml	Thermotolerant Coliforms MPN/ml	*Salmonella* / 25 g	Fungi and Yeasts UFC/ml
1	< 3.0	< 1.0	Absence	40.0
2	< 3.0	< 1.0	Absence	1.5
3	< 3.0	< 1.0	Absence	< 1.0
4	< 3.0	< 1.0	Absence	< 1.0
5	< 3.0	< 1.0	Absence	2.6×10^2
6	< 3.0	< 1.0	Absence	< 1.0
7	< 3.0	< 1.0	Absence	< 1.0
8	< 3.0	< 1.0	Absence	< 1.0
9	< 3.0	< 1.0	Absence	< 1.0
10	< 3.0	< 1.0	Absence	< 1.0

The values presented are triplicate averages, MPN = Most Probable Number.

color of the honey can be attributed to the small number of samples evaluated in the present study, since it is known that the mineral content is also associated with sensorial properties as color and flavor, which are important for honey commercialization (Escuredo et al., 2013).

Regarding the pH parameter, there is no indication of pH analysis as mandatory for evaluation of honey quality; however, it was performed as a complementary parameter for the evaluation of total acidity. The pH ranged from 3.86 to 4.55. 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 (Bastos et al., 2016).

The pH varies very little between the samples, because of the presence of organic acids honey is naturally acidic (Welke et al., 2008). However, it has been identified that honey with a quantity of acidity above the permitted level have been identified (Araújo et al., 2006). Other studies indicated values within the parameters established by the legislation (Welke et al., 2008). Free acidity is characterized by the presence of organic acids in equilibrium with their respective lactones, esters, and inorganic ions (Moreira et al., 2007; Gomes et al., 2010). The aroeira honey of the Jequitinhonha River Valley presented values from 17.32 to 30.64 Meq.kg $^{-1}$, indicating a good state of conservation of the product, since this parameter used to evaluate honey deterioration, as fermentation of sugar into organic acids increases its value (Almeida-Muradian et al., 2013). Studies have shown an average value for total acidity of 30.21 Meq.kg $^{-1}$ in honey from the cashew tree bloom and attributed the physical and chemical characteristics found in the samples to the specificity of the flowering (Bendini and Souza, 2008). In the present study, the honey from aroeira presented average values of 27.27 and 45.45 Meq.kg^{-1}, indicating that the honey of the Jequitinhonha River Valley present important characteristics that help to preserve the product for long periods.

The negative correlation between moisture and free acidity suggests that increasing the water content dilutes the amount of acids present in honey. At least 18 organic acids have been reported, some of which are volatile and others are inorganic. Gluconic acid is the main one, which is formed by the action of the glucose oxidase enzyme produced by the hypopharyngeal glands of bees and by the action of bacteria during the process of honey maturation (De-Melo et al., 2017). The samples of honey analyzed are within the standards required by the legislation, which classifies honey from colorless to dark amber. The color of honey is associated with its floral origin, but the substances responsible for color are still unknown (Moraes et al., 2014). The predominance of dark colors in aroeira honey may result in a product of high acceptance in the national market, if it is reported that the dark color may be associated with the high concentration of minerals and other nutrients.

Honey is composed of different sugars, predominating the monosaccharides glucose and fructose. These attributes depend on climate, floral source and individual beekeeping practices (Racowski et al., 2007). Of the total samples analyzed in this study, 100% presented values below that allowed by the legislation for reducing sugars (minimum of 65%) (BRASIL, 2000). All samples were within the standard for non-reducing sugars according to the legislation.

Glucose is a sugar with little solubility, determines the tendency to crystallize and fructose by having high hygroscopicity, determines its sweetness. The mean proportion of fructose in honey is 38.5%, while glucose is 31.0%, and a honey with high fructose rates may continue to be liquid for a long period or never crystallize (Tette et al., 2016). However, the sucrose content is important to know if there was adulteration of the honey by the direct addition of sucrose or if the bees were fed at the beginning of the flowering with sugar (Puscas et al., 2013). The aroeira honey from the Jequitinhonha River Valley, presenting sugar levels below the limits established by current legislation and a negative Jagerschmidt reaction, shows the absence of adulteration of the product.

The measure of diastase activity is an indicative of honey's freshness and is useful to detect improper storage conditions (Almeida-Muradian et al., 2013) and it is may be also an indicative of honeybees fed artificially with glucose (Guler et al., 2014). Therefore, the presence of diastase activity in the honey samples evaluated in the present study points to an absence of adulteration of honey by addition of sugar and adequate conditions of manipulation and storage.

According to the literature, honey has low susceptibility to the proliferation of microorganisms due to its physicochemical characteristics, such as antimicrobial substances, low moisture content, low pH, and oxidation reduction potential, among others (Ananias et al., 2013). Therefore, the microbiological quality of honey is often related to the hygienic conditions of food production and handling. In this way, the results obtained in the present study, once again evidence the care given by beekeepers in the production and management of aroeira honey. In Brazil, the Ministry of Agriculture, Livestock, and Supply (MAPA) published the Technical Regulation of Identity and Quality of Honey (BRASIL, 2000). Regarding microbiological criteria, the MERCOSUL/GMC/RES document (n° 15/94) has the following technical specifications for honey: total coliforms/g: absence; *Salmonella* spp. and *Shigella* spp./25 g: absence; enumeration of molds and yeasts: maximum of 100 CFU/g (MERCOSUL, 1994).

The total coliform group includes four genera: *Escherichia, Klebsiella, Citrobacter*, and *Enterobacter*. Literature data confirm that the presence of these bacteria in food indicates that there was fecal contamination (Marquele-Oliveira et al., 2017). The analyzes of the present study showed that there was absence of coliforms in honey samples, that is, the harvesting, the management, and the processing of the samples were made as recommended in order to obtain a good quality of honey.

The genus *Salmonella* includes several pathogenic serotypes, which can cause from gastroenteritis to serious systemic infections, like *Salmonella typhi* that causes typhoid fever. However, gastroenteritis is the most common form of salmonellosis and the major mode of transmission is by means of contaminated food (Marquele-Oliveira et al., 2017). Some studies showed that there was an absence of Salmonella species in Brazilian honey samples from different regions of Brazil (Schlabitz et al., 2010; Tavares et al., 2015). Likewise, in the present study, all samples of aroeira honey from the Jequitinhonha River Valley were free these bacteria.

Presence of fungi and yeasts in the samples analyzed in this study were inexpressive and this is another positive point that makes the Jequitinhonha River Valley honey an attractive product. Brazilian honey samples produced in several cities of the state of Minas Gerais were according to Brazilian law, as regards the presence of fungi and yeasts (Tavares et al., 2015). Microbial measurements allow the hygienic evaluation of a product with regard to the application of hygiene practices throughout its production chain and exposure to consumption; however, the presence / absence or low numbers of these microorganisms is not sufficient and is not directly related to conclusions about consumer risk.

In view of the above, it was understood that the Jequitinhonha River Valley honey mostly met the basic requirements of quality control, that is, the set of inspection actions on the properties of a food, aiming to maintain these properties according to norms and standards. Aroeira honey of the Jequitinhonha River Valley was shown to be free from evidence of fermentation and was handled properly while maintaining the conditions for storage and provides high protection against contamination. Due to the concern of the consumers to acquire quality products, it is necessary that the honey meets the requirements demanded by the market and for this one must obtain a broad knowledge of its physicochemical and microbial characteristics.

Conclusion

By means of this study it was possible to characterize the honey of the region of the Jequitinhonha River Valley, proving through macroscopic, microscopic, physico-chemical and microbiological analyses the good practices of production and management adopted in the region. The honey of aroeira was characterized by the compliance with the current norms, presenting good quality and own characteristics, such as, color dark amber, moisture low, and less acid pH and acidity. All samples followed current Brazilian legislation, showing the good management practices adopted by the beekeepers of the Jequitinhonha River Valley. The honey produced in the Jequitinhonha River Valley is informally recognized for its quality, so the analysis of the macroscopic, microscopic, physico-chemical and microbiological identity was important, since it was possible to prove the quality of the honey, market and open up new markets. It is always important to note that to maintain the characteristics and quality, the local flora should be maintained. This study will contribute to the geographical indication for aroeira honey, produced only in Jequitinhonha River Valley. Once this geographical indication is established beekeepers will be able to export it and this product will have its market value increased.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors given special thanks to Instituto Federal do Norte de Minas Gerais - Campus Araçuaí, in Minas Gerais, Brazil; Instituto de Ciências Agrárias da UFMG – Campus Montes Claros, in Minas Gerais, Brazil; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Ministério da Agricultura Pecuária e Abastecimento (MAPA), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

REFERENCES

Abramovic H, Jamnik M, Burkan L, Kac M (2008). Water activity and water ontent in Slovenian honeys. Food Control 19(11):1086-90.

Ajlouni S, Sujirapinyokul P (2010). Hydroxymethylfurfuraldehyde and amylase contents in Australian honey. Food chemistry 119(3):1000-5.

Al-Ghamdi A, Mohammed SEA, Ansari MJ, Adgaba N (2017). Comparison of physicochemical properties and effects of heating regimes on stored *Apis mellifera* and *Apis florea* honey. Saudi Journal of Biological Sciences doi.org/10.1016/j.sjbs.2017.06.002

Almeida-Muradian LB, Stramm KM, Horita A, Barth OM, Silva de Freitas A, Estevinho LM (2013). Comparative study of the physicochemical and palynological characteristics of honey from Melipona subnitida and Apis mellifera. International Journal of Food Science & Technology 48(8):1698-706.

Ananias KR, Melo AAMd, Moura CJd (2013). Analysis of moisture content, acidity and contamination by yeast and molds in Apis mellifera L. honey from central Brazil. Brazilian Journal of Microbiology 44(3):679-83.

Anjos O, Iglesias C, Peres F, Martinez J, Garcia A, Taboada J (2015). Neural networks applied to discriminate botanical origin of honeys. Food chemistry 175:128-36.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. (1998). Official methods of analysis of AOAC International. Maryland, AOAC International.

Araújo DR, Silva RHD, Sousa JS (2006). Avaliação da qualidade físico - química do mel comercializado na cidade de Crato, CE. . Revista de Biologia e Ciências da Terra, Campina Grande 6(1):51-5.

Bastos MAF, Paula de Souza STC, Cláudia Marques GS, Ribeiro da Cunha MR (2016). Characterization of the honey from Myracrodruon urundeuva (Anacardiceae - Aroeira) in the Dry Forest of northern of Minas Gerais/Brazil. Advances in Agricultural Science 4(1):64-71.

Bendini JN, Souza DC (2008). Physicochemical characterization of the bee honey originating in cashew flowering. Ciência Rural 38(2)565-7

BRASIL (2000). Instrução Normativa n° 11, de 20 de outubro de 2000. Aprova o Regulamento Técnico de Identidade e Qualidade do Mel. . Ministério da Agricultura, Pecuária e Abastecimento: Diário Oficial da União.

BRASIL (2003). Instrução Normativa Nº 62, de 26 de agosto de 2003. Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água. . Ministério da Agricultura, Pecuária e Abastecimento Diário Oficial da União.

BRASIL (1997). Portaria n° 367, de 04 de setembro de 1997. Aprova o Regulamento Técnico sobre as condições Higiênico Sanitárias e de Boas Práticas de Fabricação para Estabelecimentos Elaboradores / Industrializadores de Alimentos. Ministério da Agricultura, Pecuária e Abastecimento Diário Oficial da União

BRASIL. Ministério da Agricultura. Secretaria Nacional de Defesa Animal. (1981). Portaria nº. 01, de 07 de outubro de 1981. Aprova os métodos analíticos para controle de produtos de origem animal e seus ingredientes.

BRASIL (1985). Secretaria de Inspeção de Produto Animal. Portaria n°6, de 25 de julho de 1985. Aprova as Normas Higiênico-Sanitárias e Tecnológicas para o Mel, cera de Abelhas e Derivados. Ministério da Agricultura, Pecuária e Abastecimento: Diário Oficial da União http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=7916

Codex Alimentarius Commission (CAC) (2001). Revised Codex standard for honey. . 2 ed ed. Roma: Codex stan 12 - 1981; http://www.fao.org/fao-who-codexalimentarius/en/

Castro-Vazquez L, Diaz-Maroto MC, Perez-Coello MS (2006). Volatile composition and contribution to the aroma of spanish honeydew honeys. Identification of a new chemical marker. Journal of agricultural and food chemistry. 54(13):4809-4813.

Chitarra MIF, Chitarra AB (2005). Pós-colheita de frutas e hortaliças: fisiologia e manuseio. Lavras: Editora UFLA; 783p. https://www.bdpa.cnptia.embrapa.br/consulta/busca?b=ad&id=505900&biblioteca=vazio&busca=autoria:%22CHITARRA,%20M.%20I.%20F.%22&qFacets=autoria:%22CHITARRA,%20M.%20I.%20F.%22&sort=&paginacao=t&paginaAtual=1

Couto RHN (2002). Apicultura: manejo e produtos. 2 ed. Jaboticabal: FUNEP 191 p https://www.bdpa.cnptia.embrapa.br/consulta/busca?b=ad&id=1074717&biblioteca=vazio&busca=autoria:%22COUTO,%20R.H.N.%22&qFacets=autoria:%22COUTO,%20R.H.N.%22&sort=&paginacao=t&paginaAtual=1

Crane E. Livro do Mel. São Paulo: Livraria e Editora Nobel S.A; (1996) 226p.https://www.bdpa.cnptia.embrapa.br/consulta/busca?b=ad&id=156840&biblioteca=vazio&busca=autoria:%22CRANE,%20E.%22&qFacets=autoria:%22CRANE,%20E.%22&sort=&paginacao=t&paginaAtual=1

Dalcero A, Magnoli C, Chiacchiera S, Palacios G, Reynoso M (1997). Mycoflora and incidence of aflatoxin B1, zearalenone and deoxynivalenol in poultry feeds in Argentina. Mycopathologia 137(3):179-184.

Dalcero A, Magnoli C, Luna M, Ancasi G, Reynoso MM, Chiacchiera S, Miazzo R, Palacio G (1998). Mycoflora and naturally occurring mycotoxins in poultry feeds in Argentina. Mycopathologia 141(1):37-43.

De-Melo AAM, Almeida-Muradian LBd, Sancho MT, Pascual-Maté A (2017). Composition and properties of Apis mellifera honey: A review. Journal of Apicultural Research 57(1):5-37.

Diniz HN, Pereira PRB, Gutjahr MR, Menegasse LN, Oliveira FR, Duarte U (2001). Geologia, climatologia e hidrologia da bacia do rio Araçuaí, estado de Minas Gerais - Brasil. In: Chile Sd, editor. ENCONTRO DE GEÓGRAFOS DA AMÉRICA LATINA; Santiago de Chile: Universidad de Chile. http://observatoriogeograficoamericalatina.org.mx/egal8/Procesosambientales/Hidrologia/03.pdf

Escuredo O, Miguez M, Fernandez-Gonzalez M, Carmen Seijo M (2013). Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. Food chemistry 138(2-3):851-6.

Feás X, Pires J, Estevinho ML, Iglesias A, De Araujo JPP (2010). Palynological and physicochemical data characterisation of honeys produced in the Entre-Douro e Minho region of Portugal. International Journal of Food Science & Technology 45(6):1255-1262.

Freitas DGF, Khan AS, Silva LMR (2004). Nível tecnológico e rentabilidade de produção de mel de abelha (Apis mellifera) no Ceará. . Revista de Economia e Sociologia Rural 42(1):171-188.

Góis GC, Rodrigues AE, de Lima CAB, and Silva LT (2013). Composição do mel de após melífera:Requisitos de qualidade. Acta Veterinária Brasilica 7(2):137-147

Gomes S, Dias LG, Moreira LL, Rodrigues P, Estevinho L (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food and Chemical Toxicology 48(2):544-548.

Guler A, Kocaokutgen H, Garipoglu AV, Onder H, Ekinci D, Biyik S (2014). Detection of adulterated honey produced by honeybee (*Apis mellifera* L.) colonies fed with different levels of commercial industrial sugar (C3 and C4 plants) syrups by the carbon isotope ratio analysis. Food Chemistry 155:155-160.

IBGE. Censo Populacional (2010). Instituto Brasileiro de Geografia e Estatística https://ww2.ibge.gov.br/home/estatistica/populacao/censo2010/

Jerkovic I, Marijanovic Z, Tuberoso CI, Bubalo D, Kezic N (2010). Molecular diversity of volatile compounds in rare willow (Salix spp.)

honeydew honey: identification of chemical biomarkers. Molecular Diversity 14(2):237-248.
King AD, Hocking AD, Pitt JI (1979). Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Applied and Environmental Microbiology 37(5):959-964.
Kizilaslan H, Kizilaslan N (2007). Factors Affecting Honey Production in Apiculture in Turkey. Journal of Applied Sciences Research 3:983-987
Laallam H, Boughediri L,Bissati S,Menasria T, Mouzaoui MS, Hadjadj S,Hammoudi R,Chenchouni H (2015) Modeling the synergistic antibacterial effects of honey characteristics of different botanical origins from the Sahara Desert of Algeria. Frontiers in Microbiology 6:1239.
Marquele-Oliveira F, Carrão DB, Souza RO de, Baptista NU, Nascimento AP, Torres EC, Moreno GP, Buszinski AFM, Miguel FG, Cuba GL, Reis TF dos, Lambertucci J, Redher C, Berretta AA (2017). In book: "Honey Analysis". Edition: 1. Chapter: Fundamentals of Brazilian Honey Analysis: An Overview. Publisher: InTech. Editors: Vagner de Alencar Arnaut de Toledo. DOI: 10.5772/67279
MERCOSUL (1999). Resolução nº 56, de 29 de setembro de 1999. Regulamento técnico "Identidade e Qualidade do Mel. Montevidéu. Mercado Comum do Sul MERCOSUL. http://www.inmetro.gov.br/barreirastecnicas/pdf/GMC_RES_1999-089.pdf
MERCOSUL (1994). Technical Regulation of Identity and Quality of honey. Buenos Aires: Grupo de Mercado Comum, 1994. Disponível em:http://www.inmetro.gov.br/barreirastecnicas/PdF/GMC_reS_1994-015.pdf
Moraes FJ, Garcia RC, Vasconcelos E, Camargo SC, Pires BG, Hartleben AM, et al (2014). Caracterização físico-química de 35 amostras de mel de abelha africanizada dos municípios de Santa Helena e Terra Roxa (PR). Arquivo Brasileiro de Medicina Veterinária e Zootecnia 66(4):1269-1275.
Moreira RFA, De Maria CAB, Pietroluongo M, Trugo LC (2007). Chemical changes in the non-volatile fraction of Brazilian honeys during storage under tropical conditions. Food Chemistry. 104(3):1236-1241.
Nunes YRF, Fagundes M, Almeida HS, Veloso MDM (2008). Aspectos ecológicos da aroeira (Myracrodruon urundeuva Allemão - Anacardiaceae): fenologia e germinação de sementes. Revista Árvore 32(2):248-252.
Ozcan MM, Olmez C (2014). Some qualitative properties of different monofloral honeys. Food Chemistry. 163:212-218.
Pires J, Estevinho ML, Feás X, Cantalapiedra J, Iglesias A (2009). Pollen spectrum and physico-chemical attributes of heather (Erica sp.) honeys of north Portugal. Journal of the Science of Food and Agriculture 89(11):1862-1870.
Pitt J, Hocking A (2009). Fungi and Food Spoilage. 3 ed ed. Heidelberg, London, New York: Springer Dordrecht 519 p.
Puscas A, Hosu A, Cimpoiu C (2013). Application of a newly developed and validated high-performance thin-layer chromatographic method to control honey adulteration. Journal of Chromatography A 1272:132-135.
Racowski I, Silvas FPC, Takushi DTT, Silva DWG, Miranda PS (2007). Ação antimicrobiana do mel em leite fermentado. Revista Analytica 30:115 -117.
Department of Health/SC (1985). Autonomous Department of Public Health. Central Laboratory of Public Health. Bromatological Analysis Standards. Analysis of honey and wax. v. II. Florianópolis: Division of Bromatology, 1985.
Schlabitz C, Aparecida S, Fernanda C, Souza VD (2010). Evaluation of physical-chemical and microbiological parameters in honey. Revista Brasileira de Tecnologia Agroindustrial 4:80-90.
Simova S, Atanassov A, Shishiniova M, Bankova V (2012) A rapid differentiation between oak honeydew honey and nectar and other honeydew honeys by NMR spectroscopy. Food Chemistry 134(3):1706-1710.
Tette PAS, da Silva Oliveira FA, Pereira ENC, Silva G, de Abreu Glória MB, Fernandes C (2016). Multiclass method for pesticides quantification in honey by means of modified QuEChERS and UHPLC–MS/MS. Food Chemistry 211:130-139.
UNIÃO-EUROPÉIA (EU) (2001). Diretiva CE nº 110, de 20 de dezembro de 2001. Define as normas comuns para o mel adaptando-as a legislação geral aplicada a produtos alimentícios. . Comissão do Conselho Europeu Bruxelas: EU 2001.
Welke JE, Reginatto S, Ferreira D, Vicenzi R, Soarea JM (2008). Caracterização físico-química de méis de *Apis mellifera* L. da região noroeste do Estado do Rio Grande do Sul. Ciência Rural, Santa Maria 38(6):1737-1741.

Supplementary Table 1. Correlation datas between the physical-chemical parameters evaluated in the aroeira honey samples.

		Moisture	Soluble_sólids	pH	Free_acidity	Lactonic_acidity	Total_acidity	Non_Reducing_Sugars	Reducing_Sugars
Moisture	Pearson Correlation	1	-0.135	-0.425	-0.761*	0.606	0.301	-0.328	0.037
	Sig. (2-tailed)		0.711	0.221	0.011	0.063	0.397	0.389	0.925
	N	10	10	10	10	10	10	9	9
Soluble_sólids	Pearson Correlation	-0.135	1	0.422	0.311	-0.165	-0.014	0.206	0.101
	Sig. (2-tailed)	0.711		0.224	0.381	0.649	0.969	0.594	0.796
	N	10	10	10	10	10	10	9	9
pH	Pearson Correlation	-0.425	0.422	1	0.551	-0.336	-0.083	0.396	0.274
	Sig. (2-tailed)	0.221	0.224		0.099	0.343	0.819	0.291	0.476
	N	10	10	10	10	10	10	9	9
Free_acidity	Pearson Correlation	-0.761*	0.311	0.551	1	-0.673*	-0.234	0.339	0.350
	Sig. (2-tailed)	0.011	0.381	0.099		0.033	0.515	0.372	0.355
	N	10	10	10	10	10	10	9	9
Lactonic_acidity	Pearson Correlation	0.606	-0.165	-0.336	-0.673*	1	0.877**	-0.241	0.215
	Sig. (2-tailed)	0.063	0.649	0.343	0.033		0.001	0.532	0.578
	N	10	10	10	10	10	10	9	9
Total_acidity	Pearson Correlation	0.301	-0.014	-0.083	-0.234	0.877**	1	-0.096	0.511
	Sig. (2-tailed)	0.397	0.969	0.819	0.515	0.001		0.806	0.160
	N	10	10	10	10	10	10	9	9
Non_Reducing_Sugars	Pearson Correlation	-0.328	0.206	0.396	0.339	-0.241	-0.096	1	-0.382
	Sig. (2-tailed)	0.389	0.594	0.291	0.372	0.532	0.806		0.311
	N	9	9	9	9	9	9	9	9
Reducing_Sugars	Pearson Correlation	0.037	0.101	0.274	0.350	0.215	0.511	-0.382	1
	Sig. (2-tailed)	0.925	0.796	0.476	0.355	0.578	0.160	0.311	
	N	9	9	9	9	9	9	9	9

*Correlation is significant at the 0.05 level (2-tailed).