Fundamentals of Brazilian Honey Analysis: An Overview

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Fundamentals of Brazilian Honey Analysis: An Overview

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Abstract

Brazilian honey possesses large floral sources with various colors and flavors due to botanical and geographical differences and the large extension of the country. The absence of antibiotics and pesticides contamination positively differentiates Brazilian honey in the international market. Thus, the present chapter presents an overview of regulatory aspects for identity and quality evaluation of honey produced and commercialized in Brazil and international markets, as well as, it compares the production and consumption of honey with other countries. In addition, the chapter presents physicochemical and microbiological analysis commonly used in honey, as fundamentals of the technics and literature results with different kinds of honey obtained in Brazil. Physicochemical quality control and microbiological analysis of honey samples is of fundamental importance for assessing their quality, possible adulteration and storage conditions. In the literature, several methodologies exist to be used in the performance of honey quality control and each one complements the results in order to have an idea about the quality of the product, the absence of adulteration, deterioration, and environmental pollution and geographical area. Finally, we will present the market scenario nowadays with future perspectives and some recognition obtained for Brazilian bee products in international events.

Keywords: Brazilian honey, regulation affairs, pollen analysis, physicochemical and microbiological analysis, contaminants, potential market, awards

1. Introduction

Honey is a natural product produced by bees from the nectar of flowers which can be modified by their digestive enzymes (floral honey) or from living plant fluids and/or excretions of plant-sucking insects (honeydew honey) [1].

Floral honey can be monofloral or polyfloral, depending on whether their production is derived from a single species or various species of plants, respectively. Polyfloral honey is universal, but monofloral honey can be produced by establishing hives where flowers of a particular plant species are dominant. Therefore, based on their peculiarity, unique flavors, and sometimes unique medicinal properties, monofloral honey has a higher commercial value. Manuka honey is an example of such type, which derives from two species of Leptospermum, and retail prices start at about $100/kg [2] due to its demonstrated health benefits [3]. In addition from the plant source, the commercial value and characteristics of the honey can also be based on insect source, as honey from stingless bees (e.g. Melipona beecheii) or honey from Apis mellifera, etc. exhibits different characteristics. Additionally, the absence of residues of contaminants may also play an important role in the international market, as in the case of Brazilian honey, which receives Organic Certification.

In this scenario, a variety of honey samples with different characteristics, biological effects, and commercial values are found worldwide. Because of the value of different types of honey could vary more than 100-fold, it is target for fraud. Reports have suggested the dilution of valued kinds of honey, such as from stingless bees, with low-value honey.

Biological honey activities are derived from compounds that are present in this natural food. In general, honey is composed of approximately 200 substances, particularly with those belonging to the classes of sugars, amino acids, proteins, organic acids, flavonoids, phenolic acids, vitamins, minerals, and volatile compounds. The chemical composition of honey is intrinsically related to factors such as the geographic region of origin, present flowers in this region, species of bee that produced it, climatic conditions, processing conditions, handling and storage, and the storage time [4]. Thus, honey chemical composition from different botanical areas can vary, also leading to differences to their biological properties.

Several efforts have been made worldwide to develop protocols aiming the identification and evaluation honey quality. The literature presents many methodologies that are used to determine honey identification and quality control, and they are complementary. Among them, it could be named ascertain the entomological sources of honey by pollen identification with checking of the morphological pollen of flowers present in each honey sample and quantification of the same [5] and physical and chemical tests, i.e. determination of 5-hydroxymethylfurfural (5-HMF), which aims to assess whether it has been stored properly and determine whether it is fresh, the determination of free acidity and pH, which can be used for checking the tampering and deterioration, respectively.

Additional or alternative methods to establish the plant source of an unknown honey have also been proposed through the genetic analysis of targeted gene regions isolated from honey. This technical approach was termed metabarcoding and it is gaining power because of increased access to high-throughput sequencing platforms [6].

According to the Technical Regulations for Honey identity and Quality of the Ministry of Agriculture, Livestock and Supply (MAPA) in Brazil [7], honey samples must be characterized by physical and chemical tests, such as moisture determination, minerals (ash), acidity, reducing sugars, apparent sucrose, insoluble solids in water, diastase activity, and hydroxymethyl furfural (HMF). These tests will be discussed deeply in the next sections, especially demonstrating the fundaments and importance of each one to guarantee honey quality in Brazil. Regarding the tests required worldwide, a comparison among the different regulations is also depicted. Additional assessments, which are not comprised in Brazilian Regulations, are also reported, as the determination of metals and pollen identification. Finally, honey market worldwide is exposed.

2. Regulation of honey in Brazil and in the world

The standard for honey was established in 1981 by CODEX Alimentarius organized by the World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) by delivering CODEX STAN 12-19811 to contribute to the safety, quality, and fairness of the international honey trade (see the parameters in **Table 1**) [8].

In general, each region of the world may also adopt regulations with parameters that will be committed to their market requirements, as well as, local environment. A summary of some important regulations with parameters and limits around the world is also demonstrated in **Table 1**.

Each parameter will be further explored in next section. In this section, however, it is important to demonstrate that Brazil is a country that has its own characteristics of climate, flora, and great biodiversity. This characteristic combined with the presence of Africanized bees allows the production of honey with its own characteristics of taste, purity, quality, and originality.

In Brazil, honey is a product regulated and supervised by the Ministry of Agriculture of Brazil, through the Federal Inspection Service (Serviço de Inspeção Federal—S.I.F) in accordance with Instruction No. 11, of October 20, 2000 [7, 9]. Because of the rustic characteristics of Africanized bees and richness of its flora, Brazilian honey has no residues of contaminants and is considered a high quality and pure honey which may be a product with Organic Certification.

Thus, one can observe that each country may establish its own quality parameters and there is still much to be aligned regarding the parameters and methodologies of analysis as they have many differences. With differences in parameters and methodologies, different results for the same honey sample may apply, leading to difficult negotiations between companies.

Parameter	CODEX Alimentarius [8]	Brazil (Instruction No. 11, of October 20, 2000) [7]	Europe (COUNCIL Directive 2001/110/EC of 20 December 2001) [10]	Japan [11]
Reducing sugars (fructose/glucose)	Not less than 60/100 g. Honeydew honey, blends of honeydew honey with blossom honey: not less than 45/100 g	Floral honey: minimum 65/100 g. Honeydew honey or mix of honeydew with floral honey: minimum 60/100 g	At least 60/100 g. Honeydew and honeydew mixes with nectar honey at least 45/100 g	Maximum 60/100 g
Moisture	Not more than 20%. Heather honey (*Calluna*)—not more than 23%	Maximum 20/100 g	Maximum 20/100 g	Maximum 20/100 g
Apparent sucrose	not more than 5 g/100 g. Alfalfa (*Medicago sativa*), *Citrus* spp., False *Acacia* (*Robinia pseudoacacia*), and others: not more than 10/100 g	floral honey: Maximum 6/100 g/ Honeydew or mix of honeydew with floral honey: maximum 15/100 g	Maximum 5/100 g. *Robinia pseudoacacia*, Medicago sativa, *Banksia menziesii*, *Hedysarum*, *Eucalyptus camaldulensis*, *Eucryphia lucida*, *Eucryphia milliganii*, *Citrus* spp.: Maximum 10/100 g *Lavandula* spp., *Borago officinalis*: Maximum 15/100 g	Maximum 5/100 g
Solid insoluble in water	Not more than 0.1/100 g Pressed honey —not more than 0.5/100 g	Maximum 0.1/100 g, except pressed honey, which is tolerated up to 0.5 g/100 g, only on products packaged for its direct sale to the public	Maximum 0.1/100 g	–
Electrical Conductivity	–	–	Maximum 0.8 mS/cm	Maximum 0.8 mS/cm
Minerals (ash):	Not mentioned	Maximum 0.6/100 g. Honeydew or mix of honeydew with floral honey is tolerated up to 1.2/100 g	Not declared	–
Pollen	Not mentioned	Honey must necessarily present pollen grain	Not declared	
Acidity	Not mentioned	Maximum of 50 mEq/kg	Maximum of 50 mEq/kg	Maximum of 50 mEq/kg

Parameter	CODEX Alimentarius [8]	Brazil (Instruction No. 11, of October 20, 2000) [7]	Europe (COUNCIL Directive 2001/110/EC of 20 December 2001) [10]	Japan [11]
Diastase activity	–	At least 8 on the scale of Göthe. Honey with a low-enzyme content should present at least 3 diastase activity on the scale of Göthe, where the content of hydroxymethylfurfural does not exceed 15 mg/kg	At least 8 on Schade scale. Honey with a low-enzyme content should present at least 3 on Schade scale, where the content of hydroxymethylfurfural does not exceed 15 mg/kg	–
Hydroxymethylfurfural	Not mentioned	Up to 60 mg/kg	Less than 40 mg/kg. But honey from tropical climate and blends of these honeys a maximum of 80 mg/kg is accepted	Maximum 50 mg/kg
Additives	Not mentioned	Absent	Not declared	Absent
Organic and inorganic contaminants	Honey shall be free from heavy metals in amounts which may represent a hazard to human health. The products covered by this Standard shall comply with those maximum levels for heavy metals established by the Codex Alimentarius Commission	Organic and inorganic contaminants and their limits are established by MERCOSUL Technical Regulation	Not declared	Dextrins: Absent Antibiotics: Absent High control regarding contaminants
Observations	The products covered by this standard shall comply with those maximum residue limits for honey established by the Codex Alimentarius Commission		In Germany, additional analysis of pyrrolizidine alkaloids (PA). It must be 50 μg/kg High control regarding genetically modified organisms—GMO	

Table 1. Parameters assessed in different regions of the world.

3. Honey market worldwide and the potential market for Brazilian honey

In order to study the potential of Brazilian honey market, it is interesting to evaluate the production/demand of the honey around the world, the characteristic of the business, as well as, the conditions of Brazilian honey, production, and circumstances that can influence in this scenario. It is important to remember that the market is something very flighty and then, problems in one country, which presents high involvement in this business, can directly affect and change all circumstances and perspectives.

In this turn, it has been possible to observe a decrease in honey production in the US in last decades. Beehives in this country decreased from around 6 million in 1947 to 2740 million in 2016. This internal reduction in the production, from 250 million pounds in the early 1990s to approximately 178 million pounds in 2016, increased the demand for honey importation from other countries [12]. Colony collapse disorder (CCD) can be one of the reasons for the decrease in honeybee populations in the USA [13]. Despite the production reduction, honey consumption in the US has increased from approximately 400 million pounds in 2000 to approximately 450 million pounds yearly in last years. To maintain internal honey consumption, importation has increased from near 200 million pounds (in 2000) to 300 million pounds [14].

Argentina honey production was around 21 thousand tons in 1969, 110 thousand tons in 2005, and 80 thousand tons in 2013 [15]. This increase was mainly attributed to the clover plantation, since although the clover was planted to feed the cattle it gave a lot of nectar for honeybees to produce honey. Now it seems that honey production in Argentina was reduced due to the reduction of pasture and increase of soybeans, corn, and wheat plantations [16]. Only 8% of honey produced in Argentina is consumed in the internal market, making this country one of the biggest exporters of honey [17].

Europe produced about 372 thousand tons of honey in 2013, but it used to produce 309 thousand tons of honey in 1993. It is a great increase. But we have to understand better this market, as all of its self-production honey is consumed in its internal market. Europe is also a big importer country, importing about 305 thousand tons of honey in 2013, but it also exports a lot, in the same year they exported 176 thousand tons of honey [15]. That means that they still consume a lot of imported honey, but they re-export more than half of what they import with aggregated value. Europe is an important destination of Brazilian honey, especially because organic honey production is a very important point to be considered for these countries when importing honey, besides the absence of OGM pollen.

The China honey production increased from 75 thousand tons in 1969 to 450 thousand tons in 2013, and in this meantime the exportation in this year reached 125 thousand tons. Therefore, the internal consumption was around 325 thousand tons [15]. This data demonstrate non only the high honey production, but also, the high honey consumption for this country. China is the biggest honey exporter (in quantity) in the exporting universe, ahead of Argentina, which is also an important exporter. However, Chinese honey suffered an EU embargo in 2003, because of residue and antibiotic contamination found in honey.

On the other hand, after this Chinese honey embargo, Brazil has increased exportation. As previously mentioned, honey in Brazil is produced by Africanized bees, which are very strong bees, requiring no treatments with antibiotics or medicines. Therefore, Brazil presents the best bees for honey production [17]. In addition, Brazil has a great extension of territory, as well as rich flora and all resources to develop the bees [18] and honey production, without using antibiotics and pesticides, offering consequently a honey without contaminant residues.

Moreover, Brazilian honey production is mostly made in native areas, which also do not requires pesticides. Nevertheless, in 2006 Brazilian honey was banned from EU markets due to a lack of governmental Plan for Residues in honey [19], situation that was normalized in March 2008.

Brazil has the biggest extension of natural forest in the world and tropical weather in most of its area. That is about 300 million hectares of reserves, Indian territories and other protection areas for the biodiversity and rainforest, according to the IBGE. This allows Brazil to have the biggest organic honey potential production in the world [20]. Added to this huge area of natural forest, Brazil presented 6.9 million sq. km of eucalyptus planted area in 2014 [20]. In all areas of Brazil (north, south, southeast, northeast, and central west), eucalyptus can be cultivated. It is well known that eucalyptus plantation is very interesting for producing honey. It can be planted without chemical treatments allowing honey being produced as an organic area, producing also organic certified honey. Summing the area planted only for soybeans, coffee, cotton, orange, and sunflower in 2013 we can achieve an area of 100 thousand hectares (ha) as per IBGE [21]. Added to eucalyptus honey production and the other planted areas, it is important to consider that pollination services are rarely used in Brazil, and then, a large potential for increasing honey production could use this technique. Brazil has about 2.5 million bee colonies. Most of them are involved in honey production. Pollination is rarely used yet [22]. Brazilian honey productivity per hive is about 15 kg/colony/year. Comparing to Argentina with 35 kg/colony/hive [23], Australia with 118 kg/hive/year in average [24] and China 100 kg/hive/year, Brazilian beekeeping has much to grow [23]. It gives Brazil a possibility to increase honey production by using with techniques. Beekeeping in Brazil is very unprofessional. That is good, for one side, because no medicine, no antibiotic, and no special food is given to bees, maintaining the honey very natural. But productivity is low since it is very unprofessional yet. In the average, Brazil has a production of 30–40 thousand tons of honey yearly, since 2003 (**Figures 1** and **2**).

Brazilian exports have started in 2003 with China's honey embargo in the EU. Average honey exports are between 15 and 20 thousand tons yearly (**Figures 2** and **3**).

From the total honey produced, in 2014, 66% of it was exported. Brazil still has a very strong internal market for honey, however, with the price increase in last years because of intense exportation, internal Brazilian consumer is being suffering and then, the consumption can be reduced to a premium market only, i.e., consumers with a high-quality life.

Data have shown that honey consumption was 81 grams per capita in Brazil in 2014, an average really low comparing with other countries. Many programs are being conducted to distribute honey to governmental schools for the snack, but in the regular markets as drugstores and supermarkets consume is lower because of high pricing (**Figure 4**).

In conclusion, Brazil has the biggest potential to produce organic honey in approximately 100 square ha and approx. 7 square km of eucalyptus area. The Africanized honey bee, the best bee, is very resistant and using few techniques we can double per hive productivity. We have a potential internal market that can absorb honey production in the case of international market unbalances. So, Brazil has the best potential to produce honey in the world with low risk. And can produce a very good and quality honey with organic certification.

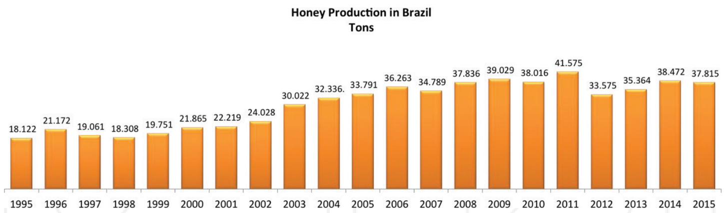

Figure 1. Brazilian honey production (tons). Data compiled for ABEMEL with information from: aliceweb.gov.br.

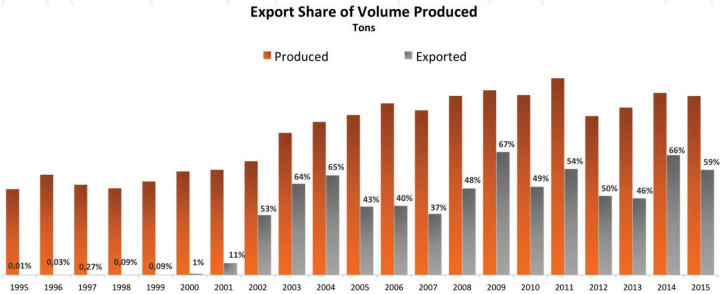

Figure 2. Brazilian honey production and exportation. Data compiled for ABEMEL with information from: aliceweb.gov.br.

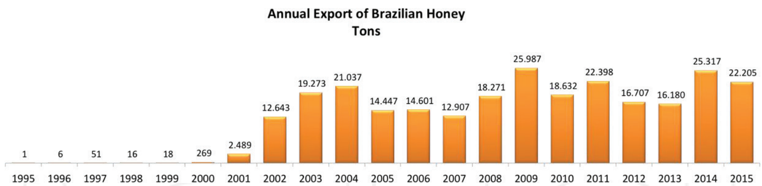

Figure 3. Brazilian honey exportation (tons). Data compiled for ABEMEL with information from: aliceweb.gov.br.

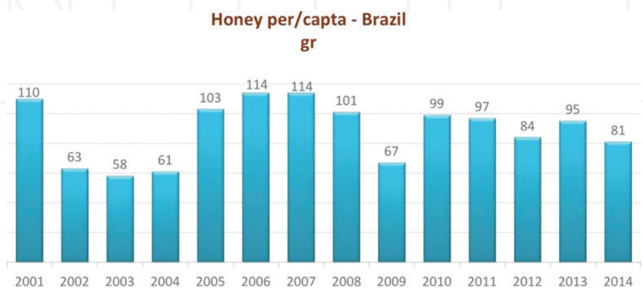

Figure 4. Brazilian honey per capita consumption (g)/year. Data compiled for ABEMEL with information from: aliceweb.gov.br.

4. Pollen microscopical analysis

Microscopical analysis of pollen from bee products can offer several interesting information, as geographical source of the material (honey and propolis), botanical origin [25] and also can help about investigations involving contamination, yeast content (fermentation), dust, microscopic particles and others. In this last case, i.e. when the analysis is more complex and involve contamination investigation, this analysis is called palynological analysis [26].

Geographical origin and botanical source usually can be determined when pollen has not been completely removed by a technological process by filtration. Besides, in several countries pollen determination is not a requirement of quality; in Brazil, this point is requested by Normative Instruction no. 11, 2000 [7], and European Community is using a lot of morphological or DNA analysis in order to validate botanical or geographical source, besides OGM material (DNA analysis for this last one). Although this point is not a quality requirement for several countries, it can be used to confirm the geographical and botanical source, especially when some doubts appear. The pollen identification can be carried out using very simple and classical methods as microscopical morphological identification or using more advanced technologies as "DNA barcoding" [27]. The micromorphological analysis is very useful and the analysis can involve identification, as far as possible, of all pollen grains in the sediment, after properly preparation of the sample. The results can be expressed as an (i) estimate value, (ii) determination of frequency classes, and the (iii) count expressed in percentage. For the first case, it is necessary to count around 100 grains and elements correspondent, in the second, around 200–300 pollen grains, in this case, if the pollen is of only a few species, around 200 pollen grains is enough, and finally, in the last case, the presentation of the frequencies as percentage is possible counting around 1200 pollen grains, with two slides counted [25].

When the honey is classified according to plant source, the common name or botanical name is written with word "honey" (CODEX STAN 12-1981) [8]. The MAPA use classical methods as the reference and the results are compared with the literature. The São Paulo's state government has a databank with more than 17,000 slides, but the access it is only in loco (http://botanica.sp.gov.br/palinologia/palinologia-colecao-cientifica-palinoteca/). Nevertheless, there is electronics databank available, as picture bank of Universidade de São Paulo (http://www.lea.esalq.usp.br/polen/) [28].

The pollen analysis also used to classify the honey as monofloral or unifloral, when the dominance of pollen of a single plant species, the bifloral dominance of pollen of only two plant species and plurifloral or heterofloral with no dominance of pollen of any plant species. Dominant pollen occurs in honey sediments above 45%, at least 300 pollen grains counted. This kind of classification is commercially important because monofloral honey is the most valued since it keeps the same physicochemical and organoleptic characteristics [26].

Despite the facility of preparation of slides in the traditional method, the interpretations of results and time involved with pollen grain counting sometimes is a challenge, in this way molecular tools could be applied. The "DNA barcoding" could be used to identify source plants in the honey. In this method, a short sequence of the DNA of the standardized portions of the genome is used and the results are compared with a reference database, as the GenBank [27]. DNA markers, such as nuclear 18S rDNA, the plastid trnL gene, plasmid coding regions rbcL and matK, trnH-psbA and ITS2, were used to test their ability to identify plant traces from different honey samples, and [27] suggested that the rbcL region and the trnH-psbA spacer could be considered to establish the origin, quality, and safety of honey with DNA barcoding, since besides more studies are necessary the stakeholder was established. In order to exemplify the microscopically analysis of pollen in Brazilian honey samples, our group evaluated five samples, including two samples of orange honey, one sample of plurifloral honey, one of "cipó-uva" honey, and a sample identified by beekeepers as "coffee" honey, that in fact is a plurifloral one, since only a very few amount of coffee pollen was found in the sample. **Figure 5** shows some pollen identified in the honey samples evaluated, and **Figure 6** shows the microscopical image of the pollen obtained in two increases 20 and 40×, usual way to count pollen grain on honey samples (for sample preparation, see [25]).

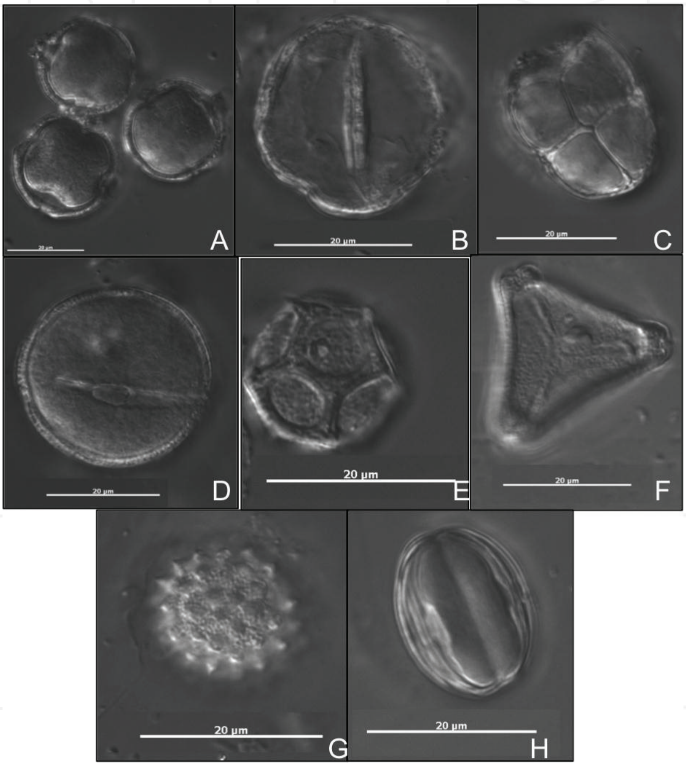

Figure 5. (A, B) Orange pollen, *Citrus* sp. (Rutaceae); (C) *Mimosa* sp. (Mimosaceae); (D) Coffee pollen, *Coffea arabica* (Rubiaceae); (E) *Alternanthera* sp. (Amaranthaceae); (F) "Cipó-uva" pollen, *Serjania* sp. (Sapindaceae); (G) "Vassourinha-do-campo pollen," *Baccharis* sp. (Asteraceae), and (H) Melastomataceae. All slides were viewed with a Carl Zeiss (Jena, Germany) microscope using the 100× magnification oil immersion objective. Phase contrast brightfield was taken with an AxioCam camera (Carl Zeiss). Images were processed using the AxioVision software version 3.1 and saved as TIFF files. Photographs were taken by Nathália U. Ferreira and Thaila F. dos Reis.

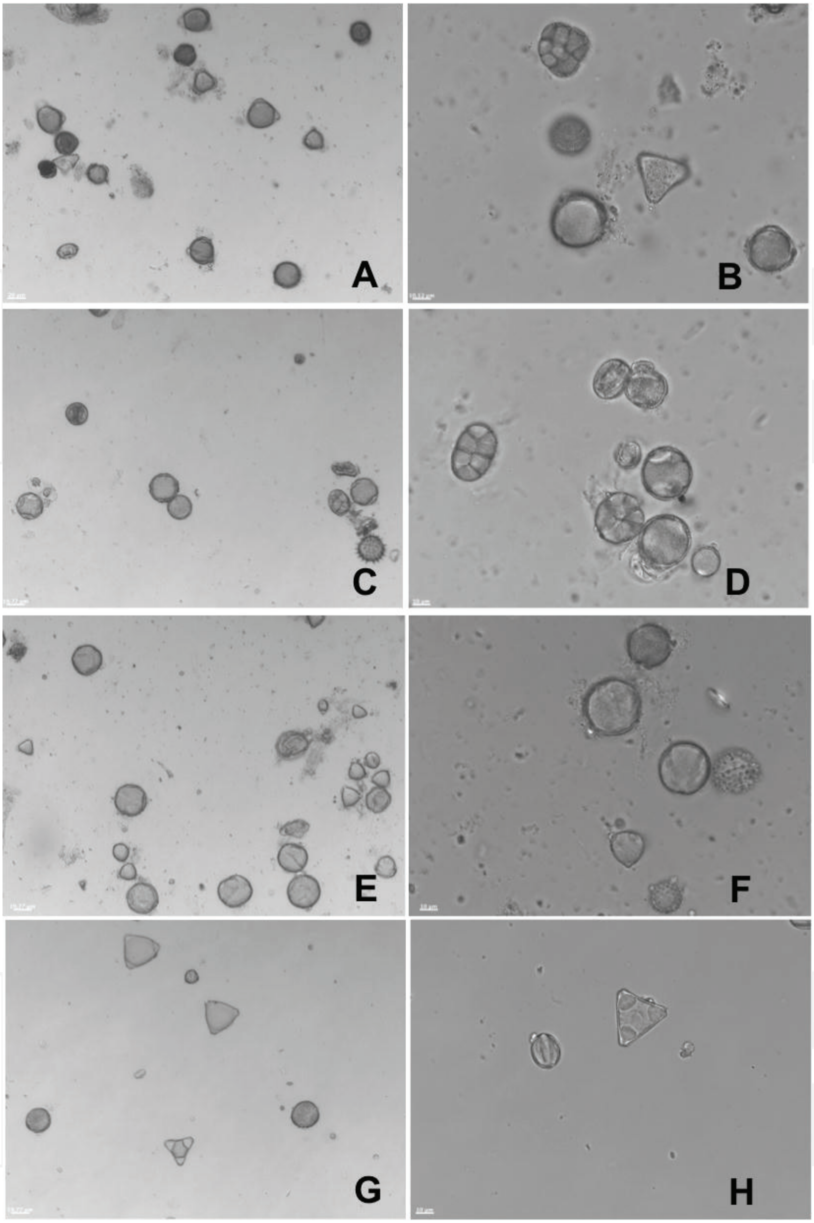

Figure 6. Microscopical analysis of honey samples obtained from different geographic and botanical areas. (A, B) Plurifloral honey, (C, D) orange (*Citrus* spp.) honey, (E, F) coffee (*Coffea arabica*) honey, and (G, H) "Cipó-uva" honey (*Serjania* spp.) (20 and 40× increase, respectively). All slides were viewed with a Carl Zeiss (Jena, Germany) microscope using the 40× magnification oil immersion objective or 20× lens. Phase contrast bright field was taken with an AxioCam camera (Carl Zeiss). Images were processed using the AxioVision software version 3.1 and saved as TIFF files. Photographs were taken by Thaila F. dos Reis.

5. Physicochemical analysis of honey: fundamentals and objectives of the analysis and results for some Brazilian samples

The honey chemical composition is intrinsically related to factors as bee species, geographical origin, flora, climate conditions, seasons, processing, manipulation, and storage conditions [4]. Brazil that presents a large biodiversity is able to offer several different types of honey, as shown in **Figure 7**, bees visiting "pau-Brasil" flowers and "cipó-uva" honey in the comb. As mentioned previously, in general, honey consists of approximately 200 substances including sugars, amino acids, proteins, organic acids, flavonoids, phenolic acids, volatile compounds, vitamins, minerals, pigments, wax, enzymes, pollen grains, and other phytochemicals [4, 29].

Figure 7. (A) Honeybees collecting nectar from "Pau-Brasil" (*Caesalpinia echinata*) flowers, showing the high biodiversity and infinite possibilities of floral honeys. (B) "Cipó-uva" (*Serjania* spp.) honey in honeycomb. Photography (A) was taken and gently donated by Mr. Antônio Carlos Meda, and photograph (B) was taken and gently donated by Lucas Eduardo Meda, both from Apis Flora Indl. Coml. Ltda, Ribeirão Preto, São Paulo, Brazil.

Quality control analyses are extremely important in the evaluation of origin, quality, adulteration, storage conditions, and contamination of honey. The physicochemical properties of a honey sample may provide important information about its biological and geographic origin [30]. Honey adulteration, mainly due to the addition of sugar derived from sugar cane, corn, and beet or even by providing sugar as a food source for bees, occurs due to its limited availability and high cost [31]. Suitable storage conditions are essential to ensure honey quality, as its chemical composition may change due to the thermal process, oxidation, and fermentation reactions [4]. Nowadays, the increasing use of pesticides in agriculture makes contamination of honey by its residues a public health issue [32].

Taking it into consideration, analytical methods are essential to provide reliable results. In the literature, there are several methodologies employed in honey quality control analyses, which are complementary for honey samples appreciation. In 1990, the International Honey Commission (IHC) was created with the goal to generate a new world honey standard. All employed honey analyses methods were then collaboratively tested and published as "Harmonised Methods of the European Honey Commission" [33]. Based on this fact, the EU Honey Directive and Codex Alimentarius Standards were revised for honey analyses. Since then, IHC continuously aims to improve and develop new analytical methods for honey analysis.

5.1. Color

The color of honey is an important quality parameter for commercialization as it is its first attractive attribute [4]. The color is directly related to its chemical composition, ash content, temperature of the hive, and it changes during storage time [34]. The main compounds related to the color of honey are phenolic compounds, pollen and mineral contents, which may vary widely according to its botanical and geographical origin [30]. During storage, the color of honey may change due to the fermentation process such as caramelization and Maillard reactions or due to the thermal process, which may change its chemical composition and consequently its color [35] or according the package used. To determine the color of honey, a photometer with direct readout in mm Pfund may be used. The Pfund scale compares an analytical standard scale of reference on the graduation of glycerin in order to provide repeatable and accurate results [30]. According to the Codex Alimentarius Committee on Sugars [8], color of honey may vary from nearly colorless to dark brown.

Regarding Brazilian honey color analysis, Sodré et al. [36] studying 36 honey samples from north coast of Bahia found predominance of the light amber color (75%) followed by amber color (16.6%) and in minor proportion, dark amber, extra light amber and extra white colors (with 2.8% each one). Moreti et al. [37] analyzed 52 samples of honey from several counties of Ceará state and found colors as water white (26.92%), white and extra white (17.31% each one), light amber (15.38%), extra light amber (11.54%), amber (9.61%), and dark amber (1.92%). **Figure 8** presents the different colors observed in only five samples studied here showing how different honey could be from Brazil especially because of the large biodiversity and extension of the country.

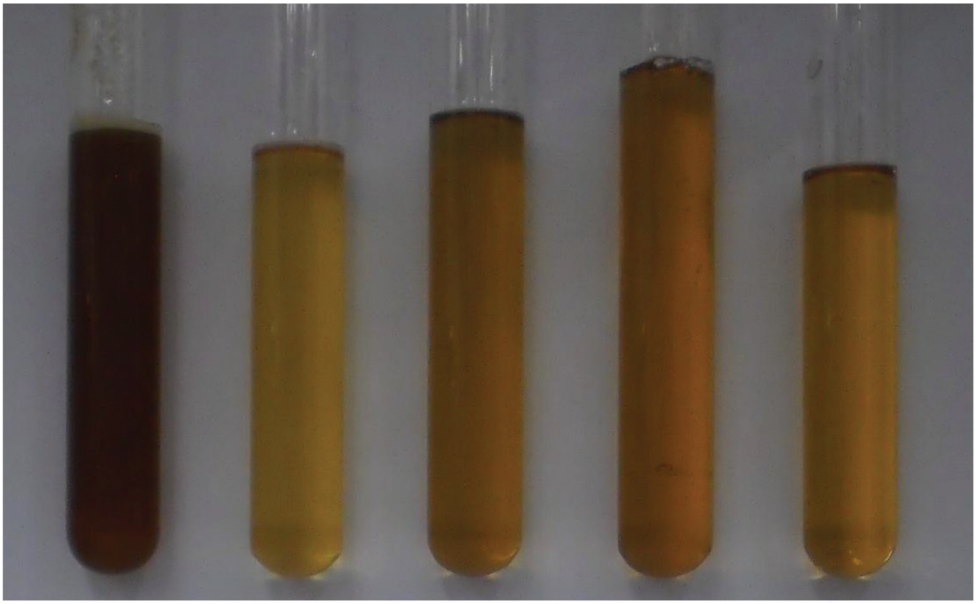

Figure 8. Color and botanical source according beekeepers about the samples used in this work, respectively, from left to right: Polifloral honey (Apiary Joel Souza, Altinópolis/São Paulo—batch 019400916) and orange (*Citrus sinensis*) honey (Apiary Hugo Charnet, Galvão Peixoto/São Paulo—batch 019300815), coffee (*Coffea Arabica*) honey (Apiary Roberto Quintino, Minas Gerais) samples gently donated by Apis Flora Indl. Coml. Ltda, Ribeirão Preto/São Paulo, Brazil. The second orange (*Citrus sinensis*) honey sample (Baldoni, batch 1607) followed by "Cipó-uva" (*Serjania* spp.) honey (Baldoni, batch 1484) were produced in Baldoni, Campinas/SP, Brazil and were acquired in Santa Terezinha Empório, Ribeirão Preto/São Paulo.

5.2. Moisture

The water content in honey samples varies according to botanical origin, climate conditions, processing techniques, and storage conditions [4]. Moisture influences honey's properties such as viscosity, crystallization, solubilization, color, and flavor [38]. The moisture may increase during processing and storage time and should be evaluated since its increase makes honey more susceptible to the fermentation process [39]. Determination of moisture in honey samples can be performed employing a refractometric method, which is based on the increases of refractive index related to solid content, and so it is possible to determine indirectly moisture of honey. According to the Codex Alimentarius Committee on Sugar [8], the moisture content in honey should not exceed 20% [8].

Several authors described the moisture content found in Brazilian honey samples. Périco et al. [40] analyzed 30 samples from Toledo, Paraná and found values ranging from 8.7 ± 0.3 to 17.6 ± 6.8/100 g. In the Rio Grande do Norte, Soares et al. [41] analyzed 24 samples from 12 commercial points of Apodi, RN, and found higher values of moisture, ranging from 16.5 to 21.5/100 g. In turn, Paulino et al. [42] found similar values of moisture (15.2–20.33/100 g) when analyzed 13 samples from various cities of Ceará state. Some examples of moisture and parameters described are shown in **Tables 2** and **3**.

Parameter	Rates	Local (state)	Reference
Color	Extra white to dark amber	Bahia Ceará	[36, 37]
Moisture (g/100 g)	15.2–20.33 16.5–21.5 8.7 ± 0.3–17.6 ± 6.8	Ceará Rio Grande do Norte Paraná	[40, 41, 42]
Ash content (%)	0.3 ± 0.10 0.17–0.20 0.01–0.41 0–1.34	Bahia Paraíba Ceará Ceará	[36, 42, 44, 46]
Electrical conductivity (μS/cm)	780.7 ± 302.70 192.00–798.67 179–198 120–750	Bahia Ceará Ceará Ceará	[36, 46] [45] [42]
pH	3.77 ± 0.25 2.90–5.10; 2.30–5.00; 2.70–4.60 3.53–4.60	Bahia São Paulo Paraná	[36] [49] [40]
Free acidity (mEq/kg)	29.10 ± 7.04 12.50–55.00; 14.00–75.50; 14.00–57.00 26.73–126.77	Bahia São Paulo Rio Grande do Norte	[36, 41, 49]
Sugars (%)	*Reducing sugars* / *Apparent sucrose* 69.20 ± 1.82 / 2.40 ± 1.42 78.84 ± 2.71 / 2.71 ± 2.40 62.89–86.93 / 1.13–10.12	Bahia Ceará Rio Grande do Norte	[36, 41, 46]
5-HMF (mg/kg)	20.70 and 23.90 7.00–355.50 70.62–150.27 31.28 ± 0.2–581.4 ± 4.2	Paraíba Ceará Rio Grande do Norte Paraná	[40, 41, 42, 44]
Diastase (Gothe scale)	34.11 ± 8,41 5.30–43.39 1.10–38.50	Bahia Ceará São Paulo	[36, 46, 49]

Table 2. Presentation of results obtained with different geographic and floral honey found in Brazil.

Honey Physicochemical Parameters	Polifloral*	Orange*	Coffee*	Orange#	"Cipó-uva"#
Aspect	Homogeneous, viscous liquid	Homogeneous, viscous liquid	Homogeneous, viscous liquid	Homogeneous, viscous liquid	Homogeneous, viscous liquid
Color	Dark amber	White, very clear yellow	Yellow, clear	White, very clear yellow	White, very clear yellow
Density (g/mL)	1.415 ± 0.00	1.420 ± 0.00	1.425 ± 0.00	1.426 ± 0.00	1.435 ± 0.00
Moisture (% w/w)	15.90 ± 0.00	15.80 ± 0.00	16.00 ± 0.00	16.10 ± 0.00	14.70 ± 0.00
Total ash (%w/w)	0.03 ± 0.03	0.03 ± 0.03	0.07 ± 0.03	0.08 ± 0.03	0.05 ± 0.01
pH determination	3.76 ± 0.01	3.78 ± 0.01	3.60 ± 0.01	3.51 ± 0.01	3.73 ± 0.01
Free acidity (%w/w)	0.19 ± 0.00	0.15 ± 0.00	0.19 ± 0.00	0.16 ± 0.00	0.18 ± 0.00
HMF determination** (mg/Kg)	55.2 ± 0.60	12.1 ± 0.20	16.5 ± 0.20	28.3 ± 0.10	32.2 ± 1.40

Honey Physicochemical Parameters	Polifloral*	Orange*	Coffee*	Orange#	"Cipó-uva"#
Insoluble material (%w/w)	0.05 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Reducing sugars (%w/w)	71.1 ± 0.60	70.2 ± 0.60	69.1 ± 0.60	70.9 ± 0.70	77.0 ± 1.80
Apparent sucrose (%w/w)	3.47 ± 0.43	2.80 ± 0.02	2.76 ± 0.02	3.48 ± 0.03	4.12 ± 0.37

*Gently donated by Apis Flora Company and #Baldoni Company.

**HMF was determined using spectrophotometry UV methodology.

Table 3. Physical-chemical analysis of different floral sources of Brazilian honeys (n = 3).

5.3. Ash content and electrical conductivity

Ash content and electrical conductivity are parameters mainly used to measure mineral content, which may be an indicative of environment pollution, the geographic and botanical origin of the honey [4, 39]. Mineral content is also associated with sensorial properties as color and flavor, which are important for honey commercialization [38]. Ash content provides important information about the quality of honey, as floral honey has lower ash content than honeydew honey [30]. Determination of ash content is performed by a gravimetric method [43]. The Codex Alimentarius Committee on Sugars [8] does not recommend a specific value for ash content. Electrical conductivity is related to the presence of ions, organic acids, and proteins in honey [4]. The determination of this parameter is based on the measure of the electrical resistance, which is reciprocal of the electrical conductivity [43]. According to the Codex Alimentarius Committee on Sugars [8], it is recommended a maximum value of 800 mS/cm for the electrical conductivity of honey samples.

Paulino et al. [42] found ash content in Brazilian honey ranging from 0 to 1.34%. According to Brazilian legislation, the ash content in blossom honey should be at maximum 0.6%, and at maximum 1.2% for honeydew honey [7]. Rodrigues-Evangelista et al. [44] found values from 0.17 to 0.20% of ash when analyzed honey samples from Paraíba state. Sodré et al. [36], in turn, found an average of 0.3 ± 0.10% of ash content in honey from Bahia state. In another study, the same group found values ranging from 0.01 to 0.41% of the total ash.

Bendini and Souza [45] analyzed 24 samples of blossom honey derived from cashew flowers from Ceará state and found electrical conductivity values from 179 a 198 μS/cm with an average of 187 ± 4.8 μS/cm. When 13 honey samples from Ceará state were analyzed by Paulino et al. [43], values ranging from 120 to 750 μS/cm were found. Sodré et al. [36] found an average of 780.7 ± 302.70 in 36 samples of bee honey from Bahia state and when honey samples from Ceará were analyzed by the same group, values between 192.00 and 798.67 μS/cm[1] were found [46].

5.4. pH and free acidity

The presence of organic acids in honey is responsible for its natural acid pH value. Determination of pH in honey samples is important to confirm its authenticity, as an addition of sugar in honey significantly increases pH values [47]. Free acidity is characterized by the presence of organic acids in equilibrium with their respective lactones, esters, and inorganic ions [29, 48]. It is a parameter used to evaluate honey deterioration, as fermentation of sugar into organic acids increases its value [30]. The determination of free acidity in honey is performed by a potentiometric titration method and the results are expressed in milliequivalents of acid per kg of honey [43]. The Codex Alimentarius Committee on Sugars [8] recommends a maximum value of 50 mEq/kg for free acidity in honey.

When 30 samples of honey from Paraná state were analyzed, Périco et al. [40] found pH values ranging from 3.53 to 4.60. Soares et al. [41] determined the acidity value in 24 bee honey samples from Apodi, Rio Grande do Norte and found results ranging from 26.73 to 126.77 mEq/kg. In turn, Sodré et al. [36] determined the pH and acidity value in 20 bee honey samples from Ceará state and their average were 3.77 ± 0.25 and 29.10 ± 7.04 mEq/kg, respectively. Marchini et al. [49] also analyzed the same parameters in 205 honey samples from different localities from São Paulo state and found pH values of 2.90–5.10 to eucalyptus honey, 2.30–5.00 to wild honey, 2.70–4.60 to orange honey, and acidity values of 12.5–55 mEq/kg of eucalyptus honey, 14–75.5 mEq/kg to wild honey, and 14–57 to orange honey.

5.5. Sugars

Sugars are intrinsically related to the flowers used by bees to produce honey, climate, and geographical conditions. Monosaccharides are the most common sugar in honey and fructose (38.5%) and glucose (31.0%) are the major sugars in honey [47]. The ratio of fructose and glucose in honey samples are used to evaluate the degree of crystallization of the honey sample [50]. Determinations of reducing sugars and apparent sucrose are based on a titrimetric method employing Fehling's reagent. The method is a titration of a Fehling's solution at boiling point by reducing sugars in honey using as indicator methylene blue [43]. Determination of the ratio of fructose and glucose may be performed by quantification of sugars in honey samples by GC methodology employing a sugar derivatization process or by HPLC methodology employing a refractive index detector or a pulsed amperometric detection [43, 51, 52]. The Codex Alimentarius on Sugars [8] stipulates that the minimum content of reducing sugars in floral honey is 60 /100 g.

In 2003, Sodré et al. [36] found an average of 69.20 ± 1.82% of reducing sugars and 2.40 ± 1.42% of apparent sucrose. The same group analyzed in 2006, 20 samples from different regions of Ceará state and found 78.84 ± 2.71% of reducing sugars and 2.71 ± 2.40% of apparent sucrose. Soares et al. [41] found a reducing sugar content of 62.89–86.93% and apparent sucrose from 1.13 to 10.12% in 24 samples of 12 providers from Apodi, the Rio Grande do Norte.

5.6. 5-HMF

Sugars present in honey may alter during storage time due to nonenzymatic reactions such as Maillard reaction, caramelization, and sugar degradation [47]. The compound 5-hydroxymethylfurfural (5-HMF) is a decomposition product of monosaccharides present in honey. Factors such as temperature, heating, floral origin, pH, and storage conditions may significantly influence in 5-HMF content [53]. Therefore, 5-HMF content is a parameter used to determine the freshness of honey, as it is absent in fresh honey and its concentration increases during storage time [30]. Furthermore, high 5-HMF content may indicate adulteration of honey by the addition of invert syrup [47]. Determination of 5-HMF content may be performed employing a spectrophotometric method [43], or a chromatographic method by HPLC using calibration curves of 5-HMF analytical standards to quantify this compound in honey [43, 53]. The Codex Alimentarius Committee on Sugars [8] stipulates 5-HMF content at the maximum value of 40.00 mg/kg and, if honey is from a tropical region, accepts a maximum value of 80.00 mg/kg.

The HMF values found in Brazilian honey are higher than those found in nontropical countries, as Paulino et al. [42] that encountered 7.00–355.50 mg/kg in 13 samples from several cities of Ceará. Périco et al. [40] also found high HMF values (31.28 ± 0.2 to 581.4 ± 4.2 mg/kg) when analyzed 30 samples from Toledo, Paraná, and Soares et al. [41] found values ranging from 70.62 to 150.27 mg/kg. When honey from two distinct regions (São João do Cariri and Areia, both in Paraíba state) were analyzed by Rodrigues-Evangelista et al. [44], the HMF content was between 20.70 and 23.90 mg/kg.

5.7. Diastase

Diastases are enzymes present in honey, which are sensitive to heat and consequently, may be used to evaluate honey overheating [47]. Therefore, the measure of diastase activity is an indicative of honey's freshness and is useful to detect improper storage conditions [30]. Diastase activity may be also an indicative of honeybees fed artificially with glucose, as a diastase enzyme deficiency is observed in this case [54]. The determination of diastase activity is based on a spectrophotometric kinetic method, which measures the activity of diastasis enzymes present in honey, in order to monitor adulteration by the addition of sugar and evaluate storage time and conditions [4, 43]. For that, under specific conditions, the activity of diastase enzymes of honey is measured in a standard solution of starch. The Gothe unit is used to express diastase activity and is defined as the amount of enzyme which will convert 0.01 g of starch in 1 hour at 40°C [43]. The Codex Alimentarius Committee on Sugars [8] stipulates a minimum value of 8.00 Gothe; however, a minimum value of 3.00 Gothe is accepted for honey with low diastase activity if the 5-HMF content is lower than 15 mg/kg.

The diastase activity was determined in 20 samples of honey from Ceará state by Sodré et al. [36] and found an average of 34.11 ± 8.41 (in Gothe scale). Sodré et al. [36] analyzed 36 honey samples from Bahia and found the value between 5.30 and 43.39. Marchini et al. [49] analyzed 205 honey samples from different localities of São Paulo state and found values ranging from 1.10 to 38.50, with an average of 8.14 for orange honey, 15.77 for eucalyptus honey, and 17.32 for wild honey. For different floral sources, the authors found values ranging from 7.80 to 19.00.

In complement to pollen microscopical analysis, physical-chemical results for these Brazilian honey samples were conducted and which is presented below, where it is possible to demonstrate the identity and quality of some floral sources of Brazilian samples studied here.

6. Microbiological analysis of honey: fundamentals and objectives of the analysis and results for Brazilian samples

Currently, safe food is a major global public health concern, since food may be contaminated by pathogenic microorganisms, which can cause severe diarrhea or debilitating infections. Furthermore, microorganisms can be responsible for the spoilage of food. Besides the contamination by microorganisms, food may be contaminated by chemical substances, such as toxins, environmental pollutants, and heavy metals [55].

Honey has low susceptibility to the proliferation of microorganisms due to its physicochemical characteristics, such as antimicrobial substances, low moisture content (low water activity), low pH, and oxidation reduction potential, among others [56]. Therefore, its antimicrobial properties discourage the growth or persistence of many microorganisms. Nevertheless, honey may be contaminated by primary and secondary sources of microbial contamination. Primary sources, including pollen, nectar, digestive tracts of bees, dust, air, and soil, are difficult to control. Secondary sources of contamination (after-harvest) include cross-contamination, equipment, food handlers, among others, and may be controlled by good manufacturing practices. Regarding the harvesting method, honey samples harvested using modern methods (colony established in man-made bees' accommodation called hives) have lower yeast and bacterial counts than samples harvested using traditional methods (honey hunting, which use flame to destroy the insects and are used in honey bee colony established in wood logs), that is, modern methods are more hygienic and produce the better quality of honey. Furthermore, exposure of colony to fire also kills bees and hampers the process of cross-pollination and may lead to consumption of the whole forest [57].

The honey samples should be subjected to quality control tests to evaluate their physicochemical and microbiological parameters. Thus, it is possible to assess whether the results are within specifications and detect if there was an adulteration of honey. In Brazil, the Ministry of Agriculture, Livestock, and Supply (MAPA) published the Technical Regulation of Identity and Quality of Honey (Brazil, 2000), which describes that the analysis of contaminants should follow the Technical Regulation of the Southern Common Market (MERCOSUL or MERCOSUR). Regarding microbiological criteria, the document "MERCOSUL/GMC/RES 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 [58].

According to MAPA, microbiological methods recommended by the International Organization for Standardization (ISO) should be used [59]. The enumeration of coliforms is performed using the colony-count technique (ISO 4832:2006) [60]. The total coliform group includes four genera: *Escherichia*, *Klebsiella*, *Citrobacter*, and *Enterobacter*. The presence of these bacteria in food indicates that there was fecal contamination. Therefore, they are commonly used as indicators of sanitary quality of honey. Some authors evaluated Brazilian honey samples from different regions of Brazil (states of Ceará, Bahia, Pernambuco, Piauí, Rio Grande do Norte, and the Rio Grande do Sul) and showed that there was absence of coliforms in these samples [9, 61–63], 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 detection of *Salmonella* spp. should be performed using the International Standard ISO 6579:2002 [64]. The genus *Salmonella* includes several pathogenic serotypes, which can cause from gastroenteritis (fever, diarrhea, and abdominal cramps) to serious systemic infections (enteric fevers), 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. Some studies showed that there was an absence of *Salmonella* species in Brazilian honey samples from different regions of Brazil [9, 61, 63, 65].

The enumeration of yeasts and molds, in its turn, is performed using the colony-count technique according to ISO 21527-2:2008, which specifies a method for the enumeration of viable xerophilic molds and osmophilic yeasts in products that have a water activity less than or equal to 0.95 [66]. Luiz et al. [65] evaluated Brazilian honey samples produced in several cities of the state of Minas Gerais (Southeast region), and the yeast and mold counts varied from <10.0 to 3.3×10^1 CFU/g, that is, all samples were according to Brazilian law. In another study by Schlabitz et al. [63] with honey samples from state of Rio Grande do Sul (South region of Brazil), the majority of samples (10 samples) were within specifications, since the enumeration of yeasts and molds varied from $<1.0 \times 10^1$ to 8.0×10^1 CFU/g. However, two samples had values above 100 CFU/g:1.3×10^2 and 6.1×10^2 CFU/g, respectively. Several honey samples produced in the state of Ceará (Northeast region of Brazil) were evaluated by Santos and Oliveira [61]. The authors showed that the majority of samples were within specifications, since yeast and mold counts varied from < 10.0 to 6.0×10^1 CFU/g. Only one sample had a count above 100 CFU/g, since it had 1.8×10^2 CFU/g.

Although not required by Brazilian law, the detection of *Clostridium* spp. (spore-forming bacteria) also is important, since honey samples may be contaminated with spores of *Clostridium botulinum*, the etiological agent of botulism (potentially fatal disease). While the ingestion of these spores is considered harmless to healthy adults, the spores may germinate in the gut of infants under 6 months of age, multiply and produce botulinum toxins. This would not occur in children older than about 6 months and adults due to natural defenses that develop over time [55]. Ragazani et al. [68] evaluated honey samples from different regions of Brazil (states of São Paulo, Minas Gerais, Goiás, Ceará, Mato Grosso, and Santa Catarina) and isolated *C. botulinum* from 7% of the samples. In other studies, Schlabitz et al. [63] and Luiz et al. [65] showed that there was an absence of sulfite-reducing clostridia in Brazilian honey samples from states of Rio Grande do Sul and Minas Gerais, respectively.

7. Contaminants analysis: metals, pesticides, analysis, and results

Honey is traditionally consumed by humans for being considered a product of natural origin and healthy. However, honey and other bee products can also be a source of toxic substances,

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such as antibiotics, pesticides (insecticides, fungicides, herbicides, and bactericides), heavy metals, bacteria, and radioactive materials due to environmental pollution and misuse of beekeeping practices, for example, when these substances overdose in beehive treatments. Honey bees collect pollen and nectar from the flowers and then they may return to hives collecting significant amounts of toxic contaminants, therefore their hives and products can result contaminated with many different kinds of pollutants [69, 70]. Thus, the monitoring of contaminants in honey is necessary to warrant consumers' safety.

7.1. Pesticides and antibiotics

The presence of contaminants in bee products decreases its quality and it may carry serious health hazards, consequently, being a public health problem. Widely used in agricultural practices, pesticide residues have been shown to cause genetic mutations and cellular degradation and the presence of antibiotics might increase resistant human or animal's pathogen [71].

The pesticide residues may originate from the treatment of beehives with acaricides and organophosphorus pesticides (OPPs) in the control of Varroa jacobsoni and Ascosphaera apis. Indirect honey contamination can occur during pesticide application in agriculture also for wax moth and small hive beetle control. Pesticide application in crops can contaminate soil, air, water, and the flowers from which bees collect nectar for honey production [72, 73].

Another source of contamination are the antibiotics such as tetracyclines, streptomycin, sulfonamides, and chloramphenicol used for the treatment of bee disease, migration from wax to honey, and also of some infestations such as Varroa destructor, Acarapis wood, and Paenibacillus larvae [69, 72].

The determination of pesticide in food due to the low concentration, the distinct chemical properties, and the matrices complexity, requires sample preparation, purification, identification, and quantification of compounds. Therefore, honey is a complex matrix and this implies the need for effective clean-up treatment before the analysis. Among the extraction methods commonly used in honey analysis are the typical clean-up/extraction procedures, such as liquid-liquid extraction (LLE) or solid-phase extraction (SPE); however, they have the disadvantages of being expensive and using large amounts of organic solvents, which are generally toxic for the technician and can contaminate the environment and usually enable the extraction of analytes belonging to only one chemical class [32, 70]. Additionally, there are other extraction techniques, which have been employed to reduce a number of reagents and time spends on sample preparation, for example, supercritical fluid extraction (SFE), matrix solid phase dispersion (MSPD), solid phase microextraction (SPME), and stir bar sorptive extraction (SBSE). Besides the extraction and purification procedures, the choice of the separation/detection approach is of fundamental importance. The step of identification and quantification of pesticide residues in honey is based mainly on gas chromatography (GC) or high-performance liquid chromatography (HPLC) techniques, both coupled with tandem mass spectrometric detection have shown great success in the multiresidue analysis of antibiotics and pesticides in honey [71].

Rissato et al. [74] confirmed 48 pesticides of different classes (organohalogen, organophosphorus, organonitrogen, and pyrethroids) in low levels in Brazilian honey samples (Bauru, São Paulo, Brazil) by gas chromatography-mass spectrometry (GC-MS/MS). Nevertheless, malathion residues were detected in all the samples, in a high concentration, and it was attributed to pesticide application for dengue vector control in the area. A study realized by De Pinho et al. [73] showed that of the 11 honey samples from eight regions of the state of Minas Gerais (Brazil) analyzed only two presented chlorpyrifos and k-cyhalothrin residues using liquid-liquid extraction with low-temperature purification for pesticide residue analysis by gas chromatography. However, the concentrations obtained were below the maximum residue levels (MRLs) established for pesticides in foods products. The presence of these compounds was confirmed by mass spectrometry (GC-MS).

Additionally, Orso et al. [75] developed and validated a method for the simultaneous determination of 79 pesticides and 13 antibiotics for 43 honey samples from different regions of Rio Grande do Sul State, Brazil, among them are monofloral and multifloral honey. The pesticides and antibiotic residues were extracted using a water-acetonitrile followed by a cleanup with dispersive solid phase (d-SPE) and analyzed by UHPLC-MS/MS. The results of the analysis demonstrated that 50% of the samples presented residues of one or more analytes in the samples. The maximum residue limit was not exceeded in any sample. Residues of insecticides and acaricides, fungicides, antimicrobials, and herbicide were found at concentrations below the MRLs, according to the limits established by National Program for Honey Residues Control established by the Brazilian Ministry of Agriculture (Brazil) for honey. Second, the authors, the residues found in honey samples are due to the proximity of the beehives with soybean, corn, or wheat crops, considering that bee realizes the pollination process, reaching large distances to collect nectar, water, and pollen of flowers.

7.2. Heavy metals

The bees are exposed to metals contained in pollen or nectar, it can to accumulate them and finally into the honey produced from it [76]. A number of different minerals and heavy metals in honey are largely dependent on the soil composition, as well as various types of floral plants [77]. Additionally, metal pollutants are discharged into the air, water, and soil through mining, agriculture practice, waste dump, coal burning, hydraulic fracturing to extract gas and oil, and industrial and municipal waste production. Agroecosystems fertilized with manures and biosolids can become contaminated with metals, and repeated fungicide application can cause the buildup of metals [78].

Trace metals such as sodium, potassium, calcium, iron, zinc, and copper can be considered essential for the biological metabolism of living organisms, when present in optimum concentrations are helpful. Other metals such as lead, cadmium, mercury, and aluminum are classified as microcontaminants of the environment, toxic or nonessential to living organisms, and at high concentrations can be even lethal, due to the inability of the heavy metal to be metabolized by the body, leading to accumulation in human or animal soft tissues without being fully inactivated or destroyed [77, 79]. In addition, the problems caused by heavy metals include headaches, metabolic abnormalities, respiratory disorders, nausea, vomiting, damage to the brain, kidney, nervous system, and red blood cells [77].

The methods used to determine the chemical elements in honey are based on spectroscopy or spectrometry techniques (including flame emission photometry or spectrometry (FES), inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ET-AAS), graphite furnace atomic absorption spectrometry (GF-AAS), hydride generation-atomic fluorescence spectrometry (HG-AAS), ion chromatography EDTA titration) [77].

De Andrade et al. [80] determined the trace elements, Pb, Cd, and Cr in 52 honey samples from eight different regions from the state of Paraná (Brazil), using slurry sampling and graphite furnace electrothermal atomic absorption spectrometry. The mean concentration of the elements followed the order Pb > Cr > Cd, but the study concluded that honey samples from Paraná have food security, as regular consumption of this product does not put risks to human health in terms of intake of this metallic species. Furthermore, Batista et al. [81] determined 42 chemical elements (toxic and essential elements) in Brazilian honey samples collected in different cities of Brazil (poli, orange, and sugarcane flowers) by the inductively coupled plasma mass spectrometry method. The authors observed that in general Brazilian honey presented higher mean concentrations for Ni, Mg, and Al and lower mean concentrations of Pb, Cd, and Cu. The mean values found for P, Zn, Mn, and Fe were very similar to those found in honey samples from other countries.

Thus, the presence of pesticides and antibiotic residues and trace metals in honey is of interest for quality control and also as a bioindicator of environmental contamination. Therefore, these analyses are important to determine the nutritional value and also the potential effect of honey on human well-being, and they can be called upon to ensure the general safety and purity of honey.

8. Brazilian honeybee products recognized around the world

Besides the quality observed in physicochemical and microbiological parameters, the absence of pesticides, antibiotics, and residues in general, it is important to recognize that several Brazilian honeybee products were awarded in important fairs and competitions around the world. The awards varied since color, taste, and flavor until high technology involved. Considering honey, several awards were attributed to Brazilian Companies (**Figure 9**). A Company situated in the State of Santa Catarina was awarded during several Apimondia Conferences. The dark honey received Gold Medal in Australia in 2007. The varieties of Dark honey were also awarded Gold Medal in Ukraine in 2013, followed by a Bronze Medal for clear honey. In 2015, this same Brazilian company was awarded one gold medal, two silver medals, and one bronze medal, for two varieties of honey and two creamy kinds of honey, during Apimondia that was held in South Korea. Another company focused on bee derivative products situated at Campinas, in the State of São Paulo. The gourmet honey of this company was awarded for superior taste in ITQI, International Taste and Quality Institute from Brussels in 2016, besides this important recognition, the

Figure 9. Photographs presenting some important awards for honey and Propolis from Brazil, as a demonstration of the international recognition of the quality and (A) Prodapys' representative, Mr. Célio Hercilio Marcos da Silva and Mr. Tarciano Santos da Silva, receiving four awards obtained for different types of Brazilian Honey in Apimondia 2015, Ukraine. (B) Baldoni's representative, Mr. Gustavo Delfino Calomeni and Mr. Daniel Augusto Cavalcante, receiving Gourmet's Honey award in Conbrapi conference that was held in Fortaleza, 2016. (C) Natucentro's representative, Mr. Cezar Ramos Júnior, receiving award for best photography of Green Propolis being produced by bees, Apimondia 2016, Ukraine. (D) Essenciale's owner, Nivia Alcici, receiving award for Gourmet Red Propolis wine, SIAL Innovation China, 2015; and finally (E) Dra. Andresa A. Berretta, from Apis Flora Indl. Coml. Ltda, receiving the second place for the development of a mucoadhesive gel containing propolis, Royal Academy of Engineering, Leaders in fellowship, London, 2015. All photographs were gently donated by the owners.

better taste was again attributed to this company in CONBRAPI, a Brazilian Conference, during the years 2012, 2014, and 2016, in Gramado/Rio Grande do Sul (RS), Belém/Pará (PA), and Fortaleza/Cerá (CE), respectively. These several awards can demonstrate the differences in taste, color, and flavor from Brazilian different geographical and botanical sources.

Brazilian propolis is also a very important honeybee product from Brazil, very recognized internationally, not only with several biological properties described but also considering the chemical differences of this type of propolis in comparison with the others found around the world, especially because of prenyl derivatives of p-coumaric acids, such as Artepellin C. Some companies received awards for propolis products as an award for Technological Innovation at China SIAL Fair in 2015, with two Gourmet line products, "Duo propolis green and red wine extract" and "premium red propolis extract wine." Another two medals were received with better photographs of bees collecting and producing green propolis, during Apimondia 2013 (Ukraine) and Apimondia 2015 (South Korea). And finally, a Brazilian Company situated at Ribeirão Preto, São Paulo state was selected for Royal Academy of Engineering Innovation Training because of the development of a mucoadhesive gel with propolis to treat vulvovaginal candidiasis. During the selection of the better project and presentation, Andresa A. Berretta was awarded the second place.

9. Conclusion

In conclusion, it is possible to show that some little differences in quality parameters exist between different countries/regulations because it is related to the floral sources. Several techniques are now available for the most of the analysis required and the most recent methodologies usually are more sensible than the oldest. Several different types of honey can be found in Brazil because of large extension of the country and the important biodiversity of each region. These differences directly affect the physical-chemical quality and also the presence contaminants. In general, it is possible to show that Brazilian beekeepers can improve techniques to increase honey production and Brazilian honey is very well recognized around the world especially because of the absence of residues, pesticides, and heavy metals, offering an Organic Certified honey and with very especial and nice taste.

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