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From Blossom to Bottle: Investigating the Distinctive Qualities of Coffee Honey in Southern Minas Gerais, Brazil

Da Flor à Garrafa: Investigando as Qualidades Distintivas do Mel de Café do Sul de Minas Gerais, Brasil

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Abstract

Honey is a beekeeping product that varies according to the region, season and origin of the flowers and must comply with legally established the quality standards. In this study, honey derived from a monoculture of *Coffea arabica* L. in southern Minas Gerais, Brazil, was characterized for its microbiological quality, physicochemical properties, pollen spectrum, and caffeine content. All samples showed the absence of total coliforms, thermotolerant coliforms, and *Salmonella* spp.; however, they exhibited the presence of molds and yeasts, ranging from 10 to 500 CFU/g, and aerobic mesophilic bacteria, ranging from 40 to 3,000 CFU/g. The moisture content was 17.30%, conductivity 368.92 µS/cm, pH 4.17, free acidity 16.94 mEq/kg, lactic acidity 10.59 mEq/kg, total acidity 27.57 mEq/kg, insoluble solids 0.0278 g/100g, ash 0.2021 g/100g, HMF 1.6155 mg/kg, color ranging from extra light amber to amber, fructose content 43.27 g/100g, glucose 45.10 g/100g, and sucrose 1.45 g/100g. The frequency of coffee pollen grains ranged from 63 to 94%, indicating a monofloral origin. The average caffeine concentration was 63.30 mg/kg. Caffeine and the frequency of coffee pollen grains can be considered important biomarkers for the characterization of this honey, in addition to its microbiological quality. The results contribute to the added value of this natural bee product produced in the southern region of Minas Gerais.
Keywords: Microbiological Quality. Caffeine. *Coffea Arabica*. Physicochemical Analyses. Pollen.

Resumo

O mel é um produto apícola que varia conforme a região, época do ano e floradas, devendo atender aos padrões de qualidade estabelecidos pela legislação. Neste estudo, o mel proveniente de uma monocultura de Coffea arabica L. no sul de Minas Gerais, Brasil, foi caracterizado quanto à sua qualidade microbiológica, propriedades físico-químicas, espectro polínico e teor de cafeína. Todas as amostras apresentaram ausência de coliformes totais, coliformes termotolerantes e Salmonella spp.; contudo, apresentaram presença de bolores e leveduras, de 10 a 500 UFC/g e bactérias mesófilas aeróbias, 40 a 3.000 UFC/g. O teor de umidade foi de 17,30%, a condutividade de 368,92 µS/cm, o pH de 4,17, a acidez livre de 16,94 mEq/kg, a acidez lática de 10,59 mEq/kg, a acidez total de 27,57 mEq/kg, os sólidos insolúveis de 0,0278 g/100g, as cinzas de 0,2021 g/100g, HMF de 1,6155 mg/kg, cor variando de âmbar claro extra a âmbar, teor de frutose de 43,27 g/100g, glicose de 45,10 g/100g e sacarose de 1,45 g/100g. A frequência dos grãos de pólen de café variou de 63 a 94%, indicando origem monofloral. A concentração média de cafeína foi de 63,30 mg/kg. A cafeína e a frequência dos grãos de pólen de café podem ser consideradas biomarcadores importantes para a caracterização deste mel, além de sua qualidade microbiológica. Os resultados contribuem para o valor agregado deste produto natural de abelhas produzido na região sul de Minas Gerais.
Palavras-chave*: Qualidade Microbiológica. Cafeína. Coffea Arabica. Análise Físico-Química. Pólen.*

1 Introduction

Coffee is one of the most consumed and valued products in the world and is an extremely important economic factor, especially in Brazil. The Minas Gerais state is considered one of the largest coffee producers in southeastern Brazil and is characterized by extensive monocultures that cover an important area in national territory (Barbosa; Aguilar; Maciel, 2021). During the flowering period, which can occur up to three times a year depending on climatic conditions (Soares *et al.*, 2005), *Coffea arabica* L. produces aromatic flowers that attract pollinators such as bees and thus promote the production of coffee beans, although it is a self-pollinating species (Oliveira, 2015).

In this context, the production of honey from these flowers is a rare product, as it depends on seasonal climatic conditions and agricultural logistics (Schievano *et al.*, 2015). Honey is a natural product of bees obtained from flower nectar and is the most utilizable bee product. About the nectar extracted from the one or different of species of flowers, there are different kinds of honey divided into two categories: monofloral honey when the bees predominantly visit a single plant family, genus or species; and flower honey when there is no dominant flower group that is visited (Brasil, 2000).

Therefore, it is important that the products derived from animals comply with the sanitary conditions and the legislation in force, which establishes identity and quality standards for products consumed in the national territory (Brasil, 2022). However, the adulteration rates of this product,

through dilution with corn syrup, feeding the hives during honey production and the use of resins to remove residues and lighten the color of the honey (García, 2018), are still significant. Consequently, quality analyses of honey bring commercial benefits by identifying certain characteristic properties of the product to be sold, thus increasing its value in the national sector.

In the international market, honey consumption is increasing every year in various countries, especially in Europe, which emphasizes the importance of research and investment in the quality of this product (Vidal, 2021). Thrasyvoulou *et al.* (2018) emphasized the importance of the legislation enacted by the European Union (EU) regarding the requirements for information on commercially traded honey in member countries, especially regarding the botanical origin of the product.

Considering this scenario, it is of utmost importance to study and understand the properties of honey because its microbiological, physicochemical and microscopic aspects depend on its botanical origin (Hempattarasuwan; Settachaimongkon; Duangmal, 2019). Therefore, honey derived from a monoculture of *Coffea arabica* L. in southern Minas Gerais, Brazil, was characterized for its microbiological quality, physicochemical properties, pollen spectrum, and caffeine content.

2 Material and Methods

2.1 Study area

The honey samples were produced by *Apis mellifera* bees in a forest next to a *Coffea arabica* plantation in the municipality of Coqueiral in the state of Minas Gerais, Brazil. The samples came from two different hives (10 hives per hive): apiary 1 (21º08'25"S, 45°26'07"W - 874 m) and apiary 2 (21º08'32"S, 45°25'57"W - 873 m). The climate can be categorized as Cwb, or mesothermal, according to the Köppen classification. It is distinguished by moderately warm summers and dry winters.

2.2 Honey samples

Twenty samples were collected between August 21-31, 2022, and October 15-25, 2022. Additionally, the hives were placed during the corresponding coffee flowering periods in the region and removed shortly after these periods ended.

2.3 Microbiological analyses

The microbiological analyses were used for the detection of total and thermotolerant coliforms, the detection of *Salmonella spp*., the quantification of aerobic mesophilic bacteria and the quantification of fungi and yeasts according to the official methods for microbiological analyses of animal products regulated by Ministério da Agricultura e Pecuária (Brasil, 2022). Therefore, 25 g of each honey sample was added under aseptic conditions to 225 mL of 0.1% sterilized buffered peptone water, giving a dilution of 10^{-1}. The mixture was homogenized, and decimal dilutions were made up to 10^{-3} (Soares *et al.*, 2021; Souza *et al.*, 2009). The detection of coliforms was assessed by the most probable number (MPN) by inoculating 1.0 mL of each dilution into 9.0 mL lactose broth tubes in triplicate. The tubes were incubated at 35 ºC. Gas formation was observed after 24 and 48 h. Positive samples were confirmed by inoculation into 9.0 mL tubes containing 2% brilliant green bile lactose incubated at 35 ºC for 24–48 h. MPN tables were then consulted to determine the MPN/g.

For the detection of *Salmonella* spp., a pre-enrichment was performed by incubating the 10^{-1} dilution at 36 ± 1.0 ºC for 16-20 h. The selective enrichment step consisted of inoculating 1.0 mL of the pre-enriched samples into 9 mL of Rappaport-Vassiliadis broth and 1.0 mL into selenite-cystine broth, incubating at 41 ± 0.5 ºC for 24-30 h. A volume of 0.1 mL of the cultures obtained was streaked onto the selective media Xylose-Lysine Deoxycholate Agar (XLD4), *Salmonella-Shigella* Agar (SS) and Brilliant Green Phenol Red Lactose Sucrose Agar (BPLS). All isolation plates were incubated at 37 ºC for 18-24 h (Soares *et al.*, 2021; Instituto Adolfo Lutz, 2011). The results were expressed as presence or absence of *Salmonella*/25 g.

Aerobic mesophilic bacteria were quantified by inoculating 1.0 mL of each dilution into 15-20 mL of sterilized Plate Count Agar (PCA) using the pour plate technique. The plates were incubated at 35 ºC for 48 h (Soares *et al.*, 2021; Souza *et al.*, 2009). The results were expressed as CFU/g.

For the quantification of molds and yeasts, 0.1 mL of each dilution was inoculated onto Potato Dextrose Agar (PDA) acidified with 10 % tartaric acid to inhibit bacterial growth. The plates were incubated at 25 ºC for 5 days (Gois *et al.*, 2015; Souza *et al.*, 2009). The results were expressed as CFU/g.

2.4 Physicochemical analyses

The following physicochemical analyses, as prescribed by the technical regulation for the identity and quality of honey (Brasil, 2000), were conducted: moisture content (Mettler Toledo refractometer), free acidity, lactonic acidity, total acidity, pH (Digimed pH meter), and ash content (Lavoisier muffle furnace). These analyses were performed in triplicate according to AOAC International methods (2016), with the ash procedure adapted to 550 ºC for 3 hours in a muffle furnace.

The electrical conductivity was evaluated by dissolving 10 g of honey in 30 mL of ultrapure water (Milli-Q) and measuring it in triplicate using a digital conductivimeter (Mettler Toledo) at 20 ºC (Water Environment Federation, 2017).

The insoluble solids were determined by gravimetric analyses in triplicate using a sintered crucible (16-40 microns) placed in an oven at 135 ºC for 1 h and then weighed. The

honey was diluted in ultrapure water (Milli-Q) at 80 ºC and then filtered through a vacuum filtration system using the sintered crucible as a filter element. After filtration, the sample was placed in the oven at 135 ºC and weighed until a constant weight was reached (World Health Organization, 2019; Bogdanov, 1999).

For the analysis of honey colour, triplicate aliquots were filled into 10 mm plastic cuvettes. Measurements were performed with a UV-VIS spectrophotometer (Shimadzu UV 1700) at a wavelength of 560 nm. Colour classifications were based on the conversion of absorbance results using the Pfund scale (Vidal; Fregosi, 1984).

Hydroxymethylfurfural (HMF) was analysed in triplicate using high performance liquid chromatography (HPLC) with a Shimadzu SPD-M20A diode array detector at an absorbance of 285 nm according to the method of Truzzi *et al.* (2012). The sample was prepared by diluting 1 g of honey in 10 mL of mobile phase consisting of ultrapure water and methanol (90:10), filtered through a 0.22 µm filter and stored in vials. The analysis was performed with a C18 column (Supelco, 250 mm × 4.6 mm, 5 µm particles) with an injection volume of 25 µL and an isocratic flow rate of 1.0 mL/min at 35 ºC, using reference standards and the 5-(hydroxymethyl)furfural standard (Sigma-Aldrich).

The determination of sugar was performed according to the procedures of AOAC International (2016) with adjustments using high performance liquid chromatography (HPLC) with a Shimadzu Refractive Index Detector-20A. The sample was prepared by diluting 1 g of honey in 10 mL of a solution of ultrapure water and acetonitrile (50:50), filtered through a 0.45 µm nylon filter and stored in vials. The analysis was performed using a C18 NH2 column (Supelco, 250 mm × 4.6 mm, 5 µm particles) with an injection volume of 20 µL, an isocratic flow rate of 1.0 mL/min at 30 ºC, with the identification of sugars based on the curve of fructose, glucose and sucrose standards from Sigma-Aldrich. The mobile phase used in the procedure consisted of (83:2:15) acetonitrile, methanol and ultrapure water, respectively.

2.5 Caffeine analyses

The quantitative analysis of the caffeine content was carried out using High-Performance Liquid Chromatography (HPLC) according to the method of Kadri *et al.* (2016). A total of 4.5 g of honey per sample was taken in triplicate and diluted in 25 mL of ultrapure water (Milli-Q). Then, 25 mL of chloroform was added for liquid-liquid extraction and three fractions were collected and placed in Falcon tubes. The fractions were evaporated using a rotary evaporator (2550 rpm) under vacuum at 40 ºC until complete evaporation of the chloroform. The extracts were reconstituted in 2 mL of mobile phase consisting of 50 % methanol and ultrapure water with 0.5 % acetic acid, filtered through a 0.22 µm membrane and stored in vials. The separation of caffeine was performed using a C18 column (Varian, dimensions 4.6 mm × 250 mm, particle size 5 µm) with an isocratic flow rate of 0.8 mL/min. To identify the caffeine peak, caffeine was detected with a UV detector (Waters 2996 photodiode array detector) at 272 nm using pure caffeine standard (CPAchem). The area of this peak was calculated for the quantitative determination of caffeine in honey.

2.6 Melissopalinological analyses

The honey pollen sediment from the samples was subjected to an acetolysis procedure (Erdtman, 1960). The pollen slides were examined under a light microscope (Olympus BX50) and identified by comparison with reference slides prepared from the anthers of coffee plants in the study area. Reference slides from Funed and specific literature were also used for comparative analyses of non-coffee pollen species found in honey sediment. The relative abundance of pollen grains was determined by counting 1000 grains per sample, as established by Louveaux, Maurizio and Vorwohl, (1978). Photomicrographs were taken using a Motic 3.0MP camera and Image-Pro software (version 10).

2.7 Statistical analysis

For the quantification of HMF, fructose, glucose, sucrose and caffeine content in honey samples, a standard curve analysis was performed to establish the relationship between the absorption values and the corresponding concentration values. Outliers, Gaussian error distribution, autocorrelation, variance homogeneity and linearity deviation were tested to ensure the accuracy and reliability of the standard curve. The Spearman correlation coefficient was tested with all physicochemical, microbiological and pollen variables to assess the significant correlations between them. The Spearman correlation coefficient was calculated using R software (R Core Team, 2022). The assumed significance level was 5 %.

3 Results and Discussion

3.1 Microbiological quality

The results of the microbiological analysis show that all samples are free of coliforms, thermotolerant coliforms and *Salmonella* spp. The absence of these contaminants, which are commonly found in foodstuffs, testifies to the hygienic quality of the honey during production. In addition, other physicochemical factors naturally present in honey - including high sugar concentration, high pH and low moisture content - contribute to its microbiological resistance (Lima *et al.*, 2011).

The samples tested had a mold and yeast count of between 1.0×10^1 and 5.0×10^2 CFU/g (Table 1). When evaluating the quality of commercial Brazilian honeys from Manaus, Soares *et al.* (2021) linked the high incidence of molds and yeasts to the production methods and humidity. Parpinelli *et al.* (2021) in their evaluation of the microbiological characteristics of stingless bee honey in Paraná, Brazil, emphasized that

despite the presence of molds and yeasts in the honey, the levels of over 100 CFU/g observed in the study indicate possible external contamination. The presence of fungi in honey indicates a link between bees and yeasts related to the increased attractiveness of nectar to bees, as emphasized by Scoaris *et al.* (2021). However, Brazilian legislation does not establish specific limits for mold and yeast levels, mainly because honey is not a sterile product and naturally contains different amounts of bacteria and fungi. Therefore, based on the analysis, the coffee honeys were considered suitable for consumption according to microbiological standards.

Table 1. Growth of aerobic mesophilic bacteria and molds and yeasts in *Coffea arabica* honey samples, expressed in colony-forming units per gram (CFU/g), to estimate the microbiological quality of the samples.

Samples	Mesophilic aerobic bacteria (CFU/g)	Molds and yeasts (CFU/g)
1135	3.00E+03	8.50E+01
1136	5.50E+02	7.50E+01
1137	8.50E+02	5.00E+01
1138	6.50E+02	3.00E+01
1139	2.60E+02	8.00E+01
1140	3.00E+02	6.00E+01
1141	1.40E+03	7.00E+01
1142	8.50E+02	1.40E+02
1143	4.50E+02	6.00E+01
1144	6.70E+02	5.00E+01
1191	6.50E+01	1.00E+02
1192	4.00E+01	1.00E+02
1193	7.00E+01	5.00E+01
1194	4.00E+01	1.00E+02
1195	6.50E+01	1.00E+02
1196	1.60E+02	5.00E+02
1197	9.50E+01	6.00E+01
1198	6.00E+01	6.00E+01
1199	8.00E+01	2.00E+01
1200	9.00E+01	4.00E+02

Source: research data.

Aerobic mesophilic microorganisms are characterized by their growth at temperatures between 20 and 45 ºC. In the present study, values between 4.0 x 10^1 and 3.0 x 10^3 CFU/g were found (Table 1). It is interesting to note the importance of identifying the species of these microorganisms in honey, as many species can occur naturally in the product and are associated with bees and flower nectar as non-pathogenic microorganisms (Wen *et al.*, 2017; Anderson *et al.*, 2013). In addition, the presence of certain bacterial groups also underlines the co-evolutionary interaction with *Apis mellifera* bees, which contributes to bee immunity (Scoaris *et al.,* 2021).

3.2 Physicochemical parameters

The moisture content in the honey samples of this study ranged between 16.87 and 17.60, with an average of 17.30 % (Table 2). This average value was also observed by Machado *et al.* (2022) in monofloral honeys in Portugal and by Luiz *et al.* (2015), who obtained samples with moisture contents between 16.2 and 17.9 %. Another aspect that should be analyzed to determine quality is honey adulteration, as the addition of sugar and other syrups can make the product less resistant to the proliferation of microorganisms. This is due to the increase in water content in the product, which favors the growth of bacteria and fungi, as shown by Rolim *et al.* (2016), in which adulterated honey showed contamination levels of 76 to 100 % by molds and yeasts, highlighting the significant correlation between these parameters, even considering the establishment of legally prescribed maximum levels.

Table 2 - The physicochemical, caffeine, and pollen grain analyses conducted on 20 samples of *Coffea arabica* honey

Parameters	Samples of Coffe Honey*
Moisture (%)	17.3 ± 0.21
Electrical Conductivity (µS/cm)	368.92 ± 53.13
Free acidity (mEq/kg)	16.94 ± 2.44
Lactonic acidity (mEq/kg)	10.59 ± 1.99
Total acidity (mEq/kg)	27.54 ± 4.05
pH	4.17 ± 0.15
Insoluble solids (g /100 g)	0.0278 ± 0.0136
Ashes (g /100 g)	0.2021 ± 0.0623
HMF (mg/kg)	1.6155 ± 0.2424
Fructose (g/100g)	43.27 ± 4.19
Glucose (g/100g)	45.11 ± 7.11
Sucrose (g/100g)	1.45 ± 0.66
Caffeine (mg/kg)	63.30 ± 16.87
Relative pollen distribution (%)	77.38 ± 10.07

Mean, three replicates and standard deviation.
Source: research data.

The color of honey is a characteristic that can vary according to botanical origin (Terrab *et al.*, 2004) and is related to consumer acceptance (Haidamus *et al.*, 2019). The samples showed color variations from “extra light amber” to “amber”,” like the results of González-Miret *et al.* (2005) in monofloral honeys and honeydew honeys, which correlate with the mineral composition and floral origin of the samples.

Although Brazilian legislation is based on the Codex Alimentarius and other regulations, the analysis of the electrical conductivity of honey is not mandatory. This parameter can be used to differentiate between blossom honey and honeydew honey with a maximum value of 800.00 µS/cm for blossom honey, according to the international standard (World Health Organization, 2019). The average value found in the samples was 368.92 µS/cm (Table 2), thus complying with international regulations for blossom honeys. Rodopoulou *et al.* (2017) and Belay *et al.* (2013) discussed the correlation between color, pH and ash with electrical conductivity, as high values of these attributes were associated with honeydew honeys, while lower values below 800 µS/cm - were consistent with floral honeys. Thrasyvoulou *et al.* (2018) confirmed this result and found similar values in blossom honeys below 800 µS/cm.

The total acidity is directly related to the content of organic

acids in honey and is influenced by the botanical origin and the time of harvest (Machado *et al.*, 2022; Alves *et al.*, 2013; Kahraman *et al.*, 2010). The normative instruction (Brasil, 2000) establishes a limit of 50 mEq/kg, and the samples in this study showed an average free acidity of 16.94 mEq/kg (Table 2). These values correspond to those of Pauliuc *et al.* (2022), who found values between 3.86 and 33.17mEq/kg in monofloral honeys from Romania. Lactonic acid showed an average amount of 10.59 mEq/kg (Table 2), like the commercial honeys analyzed by Aazza *et al.* (2013), with values between 8.17 and 26.83 mEq/kg in monofloral honeys from Portugal. The total acidity averaged 27.54 mEq/kg (Table 2), which correlates with some values found by Özcan and Ölmez (2014) 17.5 to 62.5 mEq/kg in monofloral honey.

The pH is not specified in the Brazilian regulations and can have values between 3.2 and 4.5 according to Karabagias *et al.* (2014). It is worth mentioning the importance of pH, as it can vary according to the botanical origin of the honey and can affect other properties of the food (Silva *et al.*, 2021), as well as the shelf life of the product in the points of sale (Gois *et al.*, 2015). In this study, the samples had an average pH value of 4.17 (Table 2), which corresponds to the values determined by Hailu and Belay (2020) of 3.8 on average for monofloral honeys.

The insoluble solids in the samples averaged 0.0278 g/100 g (Table 2). The honeys analyzed complied with Brazilian regulations (Brasil, 2000), which allow a maximum insoluble solids content of 0.1 g/100 g. Almeida *et al.* (2018) observed similar values in the characterization of flower honeys from the northeast of Brazil, with values between 0.003 and 0.067 g/100g of insoluble solids.

The ash content in honey depends on the amount of minerals present in the samples, so that the mineral content in the nectar influences the ash content. Electrical conductivity is another aspect that can be related to the ion concentrations present in the ash (Oroian; Sorina, 2017). Brazilian regulations (Brasil, 2000) limit the maximum ash concentration to 0.6 g/100 g, and the samples in this study had an average of 0.2021 g/100 g (Table 2). These values are in line with Brazilian regulations and the results of Ferrauto and Pavone (2013), who found values between 0.03 and 0.54 g/100 g in monofloral carob honey from Sicily (Italy).

Hydroxymethylfurfural (HMF) is a compound that occurs in various types of honey and is formed when the sugar in honey is dehydrated during processing. As the processing temperature and storage time of the honey increases, the HMF content increases significantly. It is worth noting that there are other factors that can influence its concentration, such as humidity, pH, origin of the flowers and others (Silva *et al.*, 2016). In this study, the samples had an average value of 1.6155 mg/kg (Table 2), indicating low values compared to the 60 mg/kg established in the Brazilian quality regulations for honey (Brasil, 2000). Godoy *et al.* (2022) found values of less than 36.92 mg/kg in commercial honeys from different regions of Brazil. Schievano *et al.* (2015) observed results of 23.7 mg/kg and 26.2 mg/kg in *Coffea* spp. honeys in Italy and emphasized that aspects such as floral and geographical origin may influence the concentration of HMF.

Honey contains considerable amounts of sugars, with a higher concentration of fructose and glucose, which is due to the transformation of sucrose during the ripening process (Pauliuc *et al*., 2022). The normative instruction (Brasil, 2000) establishes a minimum limit of 65 g/100g of reducing sugars, the sum of the values for fructose and glucose, and the samples in the present study had an average value of 88.38 ± 11.3 g/100g (Table 2). Aazza *et al.* (2013) highlighted sunflower honey with 79.34 g/100g in studies on monofloral honeys, while Kadri *et al.* (2016) found 79.33 g/100g when analyzing coffee honey. Sucrose, a compound that is consumed during the conversion process into fructose and glucose, has lower concentrations in honey, with a limit of 5% in international legislation (World health organization, 2019). In the coffee honey samples, sucrose averaged 1.45 g/100g (Table 2), which is consistent with the studies of Manzanares *et al.* (2017), who found an average value of 1.16 g/100g in monofloral honeys from Spain. The low sucrose value also indicates the maturity of the honey and underlines the good product quality at harvest. The values determined indicate a higher sugar concentration in coffee honey from the southern region of Minas Gerais compared to other regions of Brazil and other monofloral honeys.

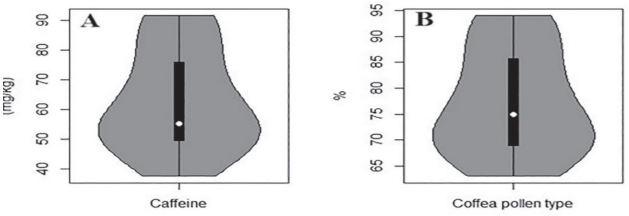
3.3 Caffeine

Caffeine is an alkaloid contained in various foods and medicines that humans consume. It has a stimulating effect on the central nervous system, and its consumption can lead to dependence (Meredith *et al.*, 2013). Trinh *et al.* (2022) highlights the relationship between caffeine and coffee honey and identifies caffeine as a chemical marker for the characterization of coffee honey, which varies quantitatively depending on the geographical and botanical origin of the product.

All honey samples had a caffeine content averaging 63.30 mg/kg (Table 2) and a more homogeneous distribution of coffee pollen (Figure 1A). The presence of caffeine at such a high level is a useful biomarker for the botanical origin of this honey, as this substance is present in the nectar of *Coffea arabica* flowers (Kadri *et al.*, 2016). In a study to characterize *Coffea arabica* honey in Espírito Santo, Brazil, the researchers found the alkaloid in all samples with values of 12.40, 12.5 and 11.17 mg/kg. They found that the coffee honey had a higher concentration of caffeine than the nectar of *Coffea arabica*, suggesting that bees can concentrate the caffeine extracted from coffee nectar in honey (Kadri *et al.*, 2016). Debela and Belay (2021) studied the caffeine content in coffee honey and found an average value of 96.11 mg/kg,

emphasizing that the alkaloid present in honey is related to the low moisture content of the nectar, which leads to a higher concentration of caffeine. Schievano *et al.* (2015) observed a caffeine content of 15.0 mg/kg in Honduran coffee honey, 52.0 mg/kg and 97.8 mg/kg in Colombian coffee honey.

Figure 1 A - Violin plot of caffeine concentration in mg/kg of honey; **B**: Violin plot of percentage amount of coffea pollen type

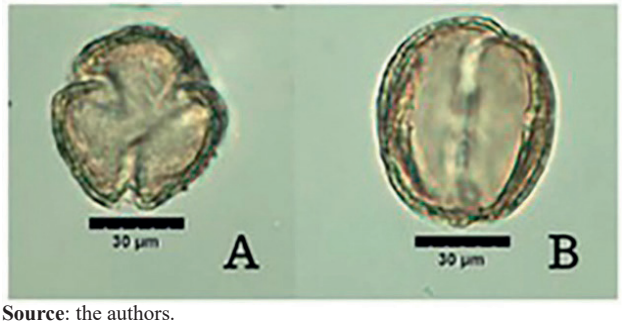


Source: research data.

3.4 Melissopalinology

The samples showed a according to Brazilian legislation (Brasil, 2023), the indication of the floral source in the labeling of honey must be supported by laboratory-based botanical identification methods. In the melissopalinological analysis, the samples showed a pollen grain frequency (Figure 2) that ranged between 63 and 94 % and a more homogeneous distribution of coffee pollen (Figure 1B) with a higher frequency close to 77,38 % (Table 2). This result could qualify this honey as a monofloral honey of *Coffea arabica*. Coffee honey samples from Espírito Santo, Brazil, showed values between 73 and 78 % according to Kadri *et al.* (2016). In Ethiopia, coffee pollen grain values between 54 and 94 % were found in *Coffea arabica* honey (Debela; Belay, 2021).

Figure 2 - *Coffea arabica* pollen grains on an acetolyzed slide. A: Polar view of the pollen grain with three apertures; B: Equatorial view of the pollen grain



Source: the authors.

3.5 Correlation between honey variables

A significant inverse correlation between the caffeine content in honey samples and the presence of molds and yeasts (r = -0.58; p-value = 0.01) and mesophilic aerobic bacteria (r = -0.67; p-value < 0.001) was observed. Exposure of bees to caffeinated nectar from coffee flowers may influence the foraging behavior of honeybees. Consumption of nectar from these flowers, which contains low concentrations of caffeine, appears to improve memory performance and may provide protection against viruses and fungal parasites. This synergy between caffeine and the bees' natural gut microbiota could promote protection against bacterial pathogens (Motta *et al.*, 2023). The correlation between the percentage of coffee pollen was significant with electrical conductivity (r = 0.74; p-value < 0.01), pH (r = 0.83; p-value <(r = 0.83; p-value < 0.01), insoluble solids (r = 0.78; p-value < 0.01), ash content (r = 0.38; p-value = 0.1) and glucose content (r = 0.44; p-value = 0.05).

The glucose and fructose content were also strongly and significantly correlated (r = 0.59; p-value = 0.01). In coffee honey, the average glucose content was higher than that of fructose (Table 2). The composition of carbohydrates in honey and the ratio between them can serve as a biomarker for certain types of honey (Kaškonienė; Venskutonis, 2010). In coffee honey from southern Minas Gerais, the fructose to glucose (F/G) ratio was 0.95. Honeys with a higher glucose than fructose content, i.e. with an F/G ratio below 1, are not common (Moreira; Maria, 2001), and this characteristic may be another differentiating biomarker for coffee honey from southern Minas Gerais.

The microorganisms (mesophilic bacteria with molds and yeasts) were significantly correlated with each other (r = 0.53, p-value = 0.02). This indicates that the more bacteria present, the more fungi and yeasts are present in this honey. In addition, the presence of mesophilic bacteria was positively and significantly correlated with the presence of coffee pollen (r = 0.64, p-value < 0.01). The higher the proportion of coffee pollen grains in the honey, the greater the contribution of the nectar of this plant, as the origin of the microorganisms present in the honey is the nectar of the flowers.

The electrical conductivity correlated directly and significantly with the pH value (r=0.67, p-value <0.01), the insoluble solids (r=0.75, p-value <0.01), the ash content (r=0.6, p-value=0.01), glucose (r=0.61, p-value <0.01), mesophilic bacteria (r=0.84, p-value <0.01), molds and yeasts (r=0.45, p-value=0.05) and the presence of coffee pollen in honey (r=0.74, p-value <0.01). The electrical conductivity of honey is directly influenced by organic acids, proteins and minerals, the concentration of which varies depending on the predominant pollen. It is often used as an indicator of honey quality (Lazarević *et al.*, 2012). In addition, the correlation of electrical conductivity with other physicochemical parameters can be used to distinguish blossom honeys from other sources (Mateo; Bosch-Reig, 1998).

The pH of the honey plays a crucial role in determining the electrical conductivity, as more acidic environments characterized by lower pH values tend to have higher electrical conductivity due to the higher concentration of hydrogen ions (H+) in the solution (Acquarone; Buera; Elizalde, 2004). The composition of the ash in honey, which contains minerals with different electrical conductivities, can directly influence the electrical conductivity. The concentration of glucose, one of the main components of honey, is directly related to the electrical conductivity, as glucose in solution is a

conductive substance. Microorganisms present in honey, such as mesophilic bacteria, molds and yeasts, can influence the electrical conductivity, although their influence is generally limited and depends on their quantity and their effect on the chemical composition of the honey. Therefore, the presence of pollen in honey, although it is a solid substance, plays a minor role in the determination of electrical conductivity, as its proportion is relatively small compared to the liquid matrix of honey.

4 Conclusion

Caffeine and caffeine-containing pollen can be regarded as biomarkers of this honey, given the consistently high levels found in all samples. Our study indicates that the microbiological quality of the honey meets the appropriate hygienic and sanitary standards, making it safe for consumption. Caffeine emerged as a significant chemical marker for the characterization of coffee honey, supported by the high percentage of corresponding pollen grains identified. Moreover, all honey samples complied with the physicochemical requirements and standards set by Brazilian legislation.

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