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Comparative Analysis of *Coffea arabica* Honeys from Two Locations In Minas Gerais

Análise comparativa de méis de Coffea arabica *de duas localidades de Minas Gerais*

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ABSTRACT

Objective: To perform physicochemical analyses, pollen count, evaluation of antioxidant activity, and quantification of caffeine in honeys derived from the flowering of *Coffea arabica*, originating from two distinct locations: M1 (Santana da Vargem) and M2 (Felí-

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-cio dos Santos). **Methodology**: For the analyses, tests for botanical identification, Fiehe reaction, Lund test, diastase enzymatic test, dye test, presence of dextrins test, color classification, acidity determination, moisture determination, determination of hydroxymethylfurfural, determination of reducing sugars, determination of insoluble solids, ash content determination, apparent sucrose, total polyphenols, antioxidant activity analysis, and caffeine quantification were performed. **Results:** It was possible to observe that only the hydroxymethylfurfural content of sample M2 is above the allowed limit, the other analyses are within the limits established by normative instruction no. 11 of 10/20/2000, besides high levels of polyphenols, and similar caffeine dosage for both honeys. **Conclusion**: The differences observed between the honeys are likely related to the climate and region in which they were produced. Regarding antioxidant activity, the best result was found for M1; however, higher levels of polyphenols and caffeine were found in M2.

Keywords: antioxidant activity; caffeine; phenolics compounds; honey; physico-chemical analysis.

RESUMO

Objetivo: Realizar análises físico-químicas, contagem de pólen, avaliação da atividade antioxidante e quantificação de cafeína em méis provenientes da floração de *Coffea arabica*, provenientes de duas localidades distintas: M1 (Santana da Vargem) e M2 (Felício dos Santos). **Metodologia**: Para as análises foram realizados os testes de identificação botânica, reação de fiehe, teste de lund, teste enzimático de diástase, teste de corante, teste de presença de dextrinas, classificação de cores, determinação de acidez, determinação de umidade, determinação de hidroximetilfurfural (HMF), determinação de açúcares redutores, determinação de sólidos insolúveis , determinação do teor de cinzas, sacarose aparente, polifenóis totais, análise da atividade antioxidante e quantificação de cafeína. **Resultados:** Foi possível observar que somente o teor de HMF da amostra M2 está acima do permitido, as demais análises se encontram dentro dos limites estabelecidos pela instrução normativa nº 11 de 10/20/2000, além de altos teores de polifenóis, e dosagem de cafeína semelhante para ambos os méis. **Conclusão:** As diferenças observadas entre os méis provavelmente estão relacionadas ao clima e à região em que foram produzidos. Quanto à atividade antioxidante, o melhor resultado foi encontrado para M1; entretanto, níveis mais elevados de polifenóis e cafeína foram encontrados em M2.

Palavras-chave: atividade antioxidante; cafeína; compostos fenólicos; mel; análises físico-química.

INTRODUCTION

Brazil is the world's leading coffee exporter, contributing billions of dollars to the global food economy. Indonesia is the largest coffee producer in the world after Brazil, Vietnam and Colombia[1,2]. As it is cultural in Brazil, in addition to the wide territorial expansion, there is a vast production of grains, varying in different qualities of the drink, in Minas Gerais, the production has shown significant development, not only due to the growth of the cultivation area, but also to the improvement in the productivity index[3,4]. Studies have identified the sensory aspects and volatile and non-volatile compounds that characterize the flavor of different coffees[5].

Although coffee is considered self-fertile, studies show that the fruit can show a considerable increase in size with pollination carried out by bumble bees[6,7]. In an experiment carried out, the coffee pods that were visited by bumble bees are 1.22 times larger than unvisited pods[8]. In addition, other data indicate that the percentage of fruiting of the plant is higher when bees have access to the coffee branch, when access is not possible, the beans decrease by about 55.25% of their size[6,7]. During the pollination of coffee flowers, a byproduct of coffee culture is produced: honey.

Honey is a complex of substances in a viscous and aromatic solution, produced by bees from floral nectar, secretions from living parts of plants or sap-sucking insects[9,10]. Is a natural sweetener with a complex composition, whose characteristics vary depending on the botanical source, geographical origin, as well as climatic, processing and storage conditions[11]. It is a viscous and aromatic product made by using the nectar of flowers or honeydew[12]. Is composed of a complex mixture of carbohydrates and other substances such as organic acids, amino acids, proteins, minerals, vitamins, lipids, aroma compounds, flavonoids, pigments, waxes, pollen grains, several enzymes, and other phytochemicals[13]. In addition to also having antioxidant properties, due to the diverse composition that includes flavonoids and polyphenols[14]. Honey produced from a single botanical species has specific sensory characteristics, in relation to

color, texture, aroma and flavor, therefore, through testing it is possible to identify sources of fraud and quantify certain defects[15].

Due to the characteristics of coffee production, such as the aromatic arrangement of the flowers and fruits of this botanical flora, it is not uncommon for it to be attractive to honey bees, however the coffee honey by-product is a rare, expensive and particularly little explored product, and in a way, sustainable since the coffee tree provides resources for the bees, and the bees produce honey as a result of this interaction[16].

The physicochemical and sensory variations that can be found in the types of honeys bee are according to environmental factors, such as climate, flora, beekeeping practices used and their processing and storage[17]. According to the origin, honey can be considered monofloral or polyfloral. According to the International Commission for Bee Botany honeys can be categorized as monofloral depending on the pollen grain size. Thus, pollen grains < 20 µm need to be at least 96% for the sample to be considered monofloral, where as pollen grains > 85 µm need to be only 7% present J.[18,19]. It can also be polyfloral, coming from different floral origins, where it does not have dominant pollen of a specific species[20]. Honey is a highly consumed natural product, not only for its taste and nutritional value, but also for its health benefits. Consequently, this product produced by honeybee have been a target of adulteration through inappropriate/fraudulent production practices and mislabeling origin[21].

According to the State Secretariat for Agriculture (Seapa) in Minas Gerais, in addition to coffee, sugar, meat and soy, honey bee has been gaining ground in exports, and it also released it based on data from the Ministry of Development, Industry and Foreign Trade (MDIC) that honey bee exports have been gaining prominence in the state[22].

In Brazil, for honey to be marketed and to ensure quality, the Ministry of Agriculture, Livestock, and Supply (MAPA) establishes minimum quality requirements through Normative Instruction. This stipulates that honey intended for human consumption must not contain any foreign substances, such as pesticide residues, and the addition of any products or substances, like commercial sugars and syrups, is strictly prohibited due to the possibility of adulteration[23].

The objective of this work is to carry out physical-chemical, melisopalinological antioxidant analyses, quantification of phenolic compounds and caffeine from two samples of *Coffea arabica* honey produced in the state of Minas Gerais-Brazil and verify whether they are in accordance with Brazilian legislation.

MATERIALS AND METHODS

The honeys bee (*Apis mellifera*) analyzed were supplied and identified with the names *Coffea arabica* (M1 and M2), being from the cities of the state of Minas Gerais: Santana da Vargem (M1) (21W 14' 49", 45S 30' 22") and Felício dos Santos (18W 04' 46", 43S 14' 49"). The honeys were provided by the Beekeepers' Cooperative and Agricultores Familiares do Norte de MG-COOPEMAPI, in August 2019. All methods were based on specialized literature, including the Codex Alimentarius, AOAC, Normative Instruction nº 11 of 10/20/2000 and publications of the International Honey Commission and carried out in triplicate.

Botanical identification

The pollen count analysis was conducted using methodologies specifically tailored for melissopalynology[17,18], and the reference sheet was PROBEE Ltda.

Microscopic analysis

The preparation of the slide was performed with a drop of the sample and a drop of Lugol, the visualization and registration were made under a microscope with 40x magnification, to identify bumble bee parts, pollen grains, starch grains and sugar[24].

Fiehe reaction

Were added 10 drops of honey bee in a porcelain crucible, then extracted with 3 mL of ethyl ether and the ether layer was transferred to another porcelain crucible, after evaporation of the ether, at room temperature, 5 drops of 1% hydrochloric resorcinol, reading taken after 10 minutes. Visually observe if there has been a change in color to red [24].

Lund test

Were added 2 g of honey bee to 20 mL of water and transferred to a 50 mL graduated cylinder, then 5 mL of 5% tannic acid solution was mixed and the volume of the beaker was completed with distilled water up to the 40 mL mark, then gently stirred and the volume of precipitate observed after 24h [24].

Diastasis enzyme test

For the diastasis enzyme test 1g of honey bee is mixed in 20 mL of distilled water, the solution is brought to a boil and after cooled to 45°C. Then, 10 mL of honey solution was added

to a test tube and 1 mL of 1% soluble starch solution, freshly prepared and clear, was added. The same volume of 1% soluble starch solution was placed in another test tube and considered blank. The tubes were shaken and left in a water bath at 45°C for 1 h, after which a few drops of Lugol were added to both and the color developed was observed [24].

Dye test

For the research of dyes, 1 g of honey bee was mixed in 10 mL of distilled water, then 2 mL of 5% sulfuric acid solution were added, and the coloring was observed [24].

Test for the presence of dextrins

Were dissolved 5 g of honey in 10 mL of distilled water, then 0.5 mL of 5% tannic acid solution was added. After clarification, it was filtered and 5 mL of the filtrate portion were added to 10 drops of concentrated hydrochloric acid (HCl) and 10 mL of absolute ethanol. It was observed if there was turbidity in the sample [24].

Color classification

Readings were performed in a spectrophotometer at 560 nm and pure glycerin was used as a blank. The value found was then transformed into color according to the Pfund scale[25].

Determination of acidity

The total acidity of honeys bee are obtained through the determination of free and lactonic acidity and was determined according to method No. 962.19[26], in which the sample was titrated for free acidity with a solution of NaOH 0.05 mol/L, until reaching pH 8.5. For lactonic acidity, after the solution reached a pH of 8.5, 10 mL of 0.05 mol/L NaOH were pipetted and with 0.05 mol/L HCl, the back titration was performed until pH 8.3.

Determination of moisture

Moisture was determined by refractometry, according to method n° 969.38b[23]. The principle of this method is based on the determination of the refractive index of honey at 20°C, and for each degree above the temperature that the sample presented, 0.00023 was added. The corrected refractive index was converted to moisture percentage using a reference table.

Determination of Hydroxymethylfurfural (HMF)

The hydroxymethylfurfural (HMF) content was determined using the spectrophotometric method at 284 and 336 nm, according to method no. 980.23[26]. 5 g of honey bee was weighed in a beaker and transferred to a 50 mL flask. Then 25 mL of water was added; 0.5 mL of Carrez I solution and 0.5 mL of Carrez II solution, the resulting solution was homogenized and made up to 50 mL with deionized water. It was filtered through a quantitative filter paper, discarding the first 10 mL of the filtrate. Four test tubes were used to determine HMF: In the first tube, 5 mL of filtered solution and 5 mL of 0.2% sodium bisulfite solution were added, this tube being considered as a reference. In the others, 5 mL of the filtrate and 5 mL of deionized water were added, these are the test solutions. The test solutions were homogenized and measured in a UV-visible spectrophotometer at wavelengths 284 and 336nm. Prior to the reading that was taken, the device was calibrated with the corresponding reference solution.

$$(\text{HMF})\ \text{mg}/100\text{g honey} = (A284 - A336) \times 14.97 \times 5 / \text{g of sample}$$

Determination of reducing sugars

The determination of reducing sugars was carried out according to the Codex Alimentarius Commission (CAC)[27] from the modification of the Lane and Eynon procedure, involving the reduction of the Fehling solution, modified by Soxhlet, during the titration at boiling point with a solution of sugars honey bee reducers, using methylene blue as an indicator. The apparent sucrose content was determined after inversion by acid hydrolysis, according to the CAC method[27].

Determination of insoluble solids

The determination of the content of insoluble solids in water was done by gravimetry, according to the CAC method[27].

Determination of ash content

Samples (5 g of honey bee) were used, which were incinerated at a temperature of 600 °C in a muffle for 18 h. After cooling to room temperature, the ash obtained was weighed. All measurements were performed in triplicate[27].

Apparent sucrose

50 mL of the honey solution obtained in the determination of reducing sugars was pipetted into a 100 mL volumetric flask and 25 mL of water was added. Heating was carried out at 65 °C in a water bath. The flask was removed from the bath and 10 mL of hydrochloric acid solution was added and the solution was allowed to cool naturally to room temperature, then neutralized with sodium hydroxide solution[28].

$$\left[\frac{2X1000}{Px\ V_1} - C\right] X\ 0{,}95$$

P = sample mass in g

V_1 = number of mL of diluted sample solution spent in the titration

C = number of g of invert sugar percent, obtained before inversion, reducing sugars

Total polyphenols

For the determination of the total polyphenols content in the investigated honey samples, we used the Folin-Ciocalteu method, which is a colorimetric *in vitro* assay measuring the total reducing capacity of a sample[29]. An accurately weighed 1 g sample of each honey bee was put in a 10 mL volumetric flask, which was completed with water and filtered through with paper weight 80 g/m^2. 0.5 mL of this solution was then added with 2.5 mL Folin-Ciocalteu reagent (0.2 N), and mixed for 8 minutes followed by the addition of 2 mL of sodium carbonate (75 g/L). Then the mixture solution was allowed for incubation at room temperature for 2 h and the absorbance was measured at 760 nm, while methanol was used as blank.

Preparation of honey extracts

For the preparation of the extracts an aqueous solution of methanol 50% (v/v) was used. Then, the honey bee was diluted (8 mL of honey bee to 80 mL of 50% methanol solution). This solution was kept in a reflux device for two hours at 80°C. Dilution and extraction were performed on each honey bee sample separately, stored in sealed jars and kept in the freezer[30].

Analysis of the antioxidant activity

The DPPH radical scavenging activity of honey bee samples was determined as described by Brand-Williams et al. (1995)[31] with some modifications[32]. To carry out the test,

the extract, at concentrations between 50 and 100%, was used (500 µL). A DPPH stock solution of 40 µL/mL methanol was prepared. The sample was then added to 3000 µL of DPPH, shaken vigorously and kept in the dark for 25 minutes at 25°C. To obtain the standard curve of gallic acid, a stock solution at 80 µg/mL was prepared and concentrations between 30 and 80 µg/mL were used the absorbance of the solution was measured at 517 nm, using a spectrophotometer (SHIMADZU – UV-VIS 2550) against a methanol blank. All measurements were taken in triplicate. With the absorbance values, the percentage of antioxidant activity was calculated by the equation[33]:

{(AbsCont – AbsAmos) /AbsCont} x100, where:

AbsCont represents the absorbance value of the control;

AbsAmos represents the absorbance value of the sample.

Caffeine quantification by HPLC

The investigation of the chromatographic profile was performed using high performance liquid chromatography. The equipment used was a liquid chromatograph (Waters), equipped with a 1525 binary pump, 717 automatic injector, automatic fraction III collector, 2996 diode array detector and Empower Pro software.

Were diluted 2.5g of honey bee in 25 mL of 40% methanol solution were used. The caffeine standard was prepared at a concentration of 0.01 mg/mL, while the sample was prepared in triplicate, at a concentration of 0.1 g/mL. For the total quantification of caffeine, a C18 column, 250 x 4.6 mm and 5µm particle (Spherisorb, Waters) was used to separate the compounds. The mobile phase employed was HPLC grade methanol (J.T. Baker), pumped at a flow rate of 1.5 mL/min. and the reading was performed at the wavelength of 273 nm.

Statistical analysis

Analyzes were performed in triplicate and data are presented as simple arithmetic means.

RESULTS

Botanical identification

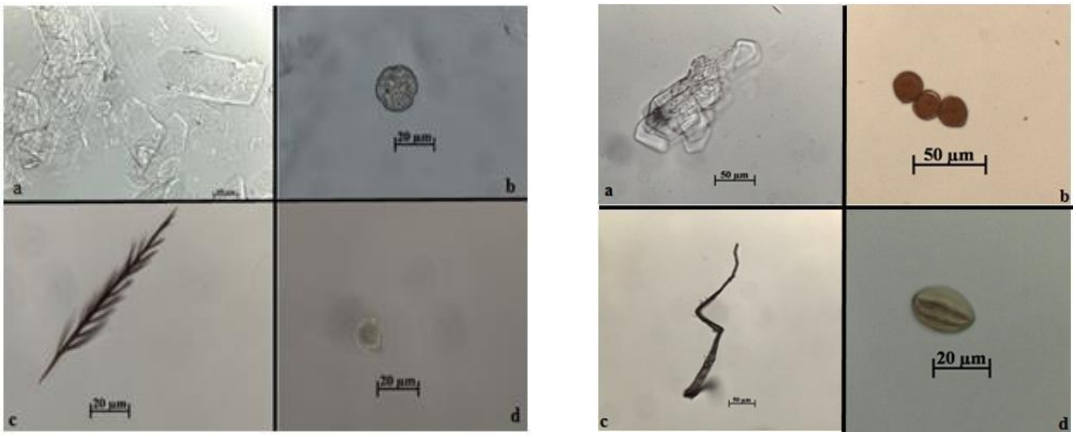
There was predominance of *Coffea arabica* pollen, classifying them as monofloral honeys bees (Table 1).

Table 1. Pollen analysis in honeys bees.

	Pollen type	Pollen count	Index %
M1	*Coffea arabica*	350	95.89
	Baccharis calvescens	7	1.92
	Eucalyptus robusta	4	1.09
	Croton urucurana	1	0.55
	Citrus sinensis	1	0.55
M2	*Coffea arabica*	500	99.60
	Eucalyptus robusta	2	0.4

Microscopic analysis

From microscopic analyses, it was possible to observe the presence of pollen grains, parts of bumble bees, sugar crystals, and starch grains (Figure 1).



M1 M2

Figure 1- Microscopy test of *Coffea arabica* honeys bee, M1: a) sugar crystals; b) pollen grain; c) bumble bee part; d) starch grain. M2: a) sugar crystals; b) pollen grain; c) bee part; d) starch grain.

Physicochemical analysis

Table 2- Results of analysis and parameter limits for *Coffea arabica* honeys bee (M1 and M2).

Analysis	M1	M2	Parameters
Fiehe reaction	Negative	Negative	Negative
Lund test	0.7	0.9	Between 0.6 and 3.0 mL of precipitate

Diastasis enzyme test	Violet	Violet	Violet
Dye test	Unchanged	Unchanged	Unchanged
Test for the presence of dextrins	No cloudiness	No cloudiness	No cloudiness
Color classification	Light amber	Dark amber	-
Acidity (meq/kg)	22.31	37.83	Maximum 50 eq/kg
Moisture	19%	20%	20g/100g (20%)
HMF (mg/kg)	40.35	64.53	Maximum 60 mg/kg
Insoluble solids (g/100g)	< 0.1	< 0.1	0.1g/100g
Ashes	0.3%	0.4%	1.2g/100g (1.2%)
Reducing sugars	64.5%	68.96%	Minimum 60g/100g
Apparent sucrose	4.46%	2.47%	Maximum 6g/100g
Phenols (mg/100g)	218.64	270.65	-
Antioxidant activity EC_{50} (mg/mL)	162.48	210.09	-
Caffeine quantification (mg/mL)	0.012	0.013	-

DISCUSSION

According to Normative Instruction No. 11 of October 20, 2000[20], honey bee intended for human consumption must be free of impurities, additives, and fermentation. It should not contain added sugars or other substances that alter its original composition, such as the presence of pesticides, inverted sugar syrups, and other sugary syrups used in honey adulteration.

For the Fiehe reaction, there was no change in color, with a negative result, meaning that none of the honeys bee underwent heating or addition of sugar[34]. The Fiehe test is a qualitative reaction to detect the presence of HMF (Hydroxymethylfurfural) in honey bee[28]. The colorimetric reaction occurs between the resorcin and HMF compounds. This experiment aims to determine the presence of substances produced in the overheating or addition of sugar syrups in honey bee. If the honey is overheated or has been tampered with by commercial glucose, the appearance of an intense red color will occur[35].

Lund's test it is based on the precipitation of natural honey bee proteins by tannic acid, which allows the identification of the presence of albuminoids[28]. In the presence of pure honey bee a solid deposit is formed in the range of 0.6 to 3.0 mL. In the presence of adulterated honey bee, there will be no deposit formation or it will be negligible. A deposit above 3.0 mL will indicate that this honey bee is a poor quality product [35,36] . The honeys presented deposit values

of 0.7 and 0.9 mL, which indicates authenticity of the coffee honey, within the parameters established for the Lund test. Lower values than those reported in research involving different honeys, with an average of 3.63 and minimum values of 2.00 and maximum values of 12.00 mL of deposit[36]. Disastase activity is often used to evaluate the freshness of honey, due to its ability to indicate possible heating of the sample, due to factors such as poor storage, or storage at high temperatures[37]. The diastase enzyme activity test has heat sensitivity, which checks if the honey bee has the invertase enzyme, responsible for transforming sucrose into fructose and glucose, thus evaluating the quality of the honey bee[38]. The violet color was observed in both, with M1 honey bee having a lighter color when compared to M2. The difference in the violet color variation may be due to the method of removing the honey bee present in the combs, which is done by means of centrifugation. Different factors can influence the enzymatic activity of honey bee, such as floral origin, environment of origin and bumble bee race, between others[39].

Color testing is a verification tool used to detect the presence of dyes that simulate colors close to natural honey, indicating artificial honey. In the dye test, the samples remained with unaltered color, indicating that there was no type of adulteration. The test result (Tab. 2) indicates that the color remained unchanged after the test, which confirms the authenticity of the honey. Since the presence of dyes would provoke noticeable color changes, the lack of such changes confirms the absence of artificial dyes. The color is related to the floral origin, process and storage, climate of the region where the nectar was collected and the temperature inside the hive, da quantidade e tipo de minerais presente[40,41]. Water, sucrose, inverted sugar, hydroxymethyl cellulose, dextrin and starch are adulterants which have been regularly identified by physicochemical analysis, if the sample shows turbidity, there is evidence of adulteration with commercial glucose[42].

According to the Pfund scale, the analyzed honeys were classified as light amber (M1) and dark amber (M2). This relationship may be related to the geographical and climatic variations involved in the honey production process. Some factors may be associated with honey color, such as the content of polyphenols[42], which is higher in sample M2 compared to sample M1, as well as other abiotic factors such as higher mineral content[43]. Color is the main characteristic that determines the choice of the consumer, who chooses the product only for its appearance, most of the time. In the world market, honey bee is evaluated by its color, light

colored honeys bee reach higher prices than dark colored honeys bee[42]. Despite studies indicating that darker honeys have higher levels of phenolic compounds and greater antioxidant activity, [44,45,46,47].

The observed limits for honey moisture content (M1=19.0% and M2=20.5%) (Table 2) are appropriate. These values are important, especially because natural fermentation processes, bacterial action during honey maturation, the activities of the glucose-oxidase enzyme, the sources of nectar that produce gluconic acid and other organic acids, and the mineral content all make it more difficult. Thus, there is no promotion of increased acidity, and consequently, these values contribute to maintaining stability regarding microbial development[48], and a greater difficulty in fermentation[49].

O 5-hydroxymethylfurfural (HMF) it's a substance formed from reducing sugars in honey bee by the Maillard reaction. Storage conditions also affect the formation of HMF and is an indicator of honey bee quality[50].

The results of HMF in M1 and M2 are 40.35 and 64.53 mg/kg, respectively. These results are already described in the literature for coffee honeys produced in Minas Gerais, with values of 55.70 mg/kg[51] and 16.04 mg/kg[52]. In Brazilian territory, honeys produced in São Paulo, Mato Grosso do Sul, and Espírito Santo had values ranging from 18.0 to 69.51 mg/kg[53]. Values between 0.048 and 2.933 mg/kg were reported for robusta coffee honeys produced in Vietnam[54], and 5.60 mg/kg for arabica coffee honey from Ethiopia[55]. Sample M1 has HMF levels within the limits established by Normative Instruction no. 11 of October 20, 2000[20], and close to coffee honey produced in the same region, as mentioned above. In contrast, sample M2 has levels higher than those allowed by legislation. Regarding the HMF levels of coffee honeys produced in Minas Gerais, higher values are observed compared to those produced in Ethiopia and Vietnam. However, the results observed for both M1 and M2 are within the standards of honeys bee from tropical climates[56].

From the analysis of insoluble solids in M1, a greater amount of sugar crystals was observed, in relation to M2, the sugar crystals are due to natural processes that occur and do not change the nature of the honey bees. As they are supersaturated glucose solutions, when stored at temperatures lower than the hive (37°C), they tend to crystallize[57,58]. There are several factors that can influence the formation of crystals, such as the concentration of sugars, the water

content in the natural composition, the floral origin of the nectar, handling during processing, as well as storage conditions [58,59].

According to Regulation No. 11 of October 20, 2000, in relation to the analysis of insoluble solids, the presence of pollen grains is considered desirable, whereas the presence of bumble bee parts and starch residues can present up to 0.1 g/100 g, the analyzed honeys are within the parameters considered normal.

The ash content determined for the honeys bee (M1=0.3% and M2=0.4%) is in accordance with the legislation, this result indicates the purity of the samples. Results close to the highest value (0.32% and 0.42) found for Baduy honey bee from Indonesia and honey produced in Benin, respectively[60,61]. An equal value to that of sample M1 was found in coffee honey from the Momma region in Ethiopia[62], and a similar value of 0.28% was also reported in Ethiopian honey[54]. The ash content is related to the amount of inorganic material present in a sample after the complete combustion of organic matter, leaving only the inorganic minerals.

Regarding the results observed for reducing sugars and apparent sucrose, the samples evaluated are also within the limits allowed in the legislation (M1=64.5% and M2=68.96%), which differs from a study carried out with honeys bee in the southern region of Brazil, where the results for reducing sugars and apparent sucrose did not meet the legislation[63]. Adulteration led to a significant increase in sucrose content as well as a decrease in reducing sugar content[64]. Analysis of coffee honeys from the São Lourenço[50] region and Bocaiúva[51] indicated reducing sugar contents of 66.70% and 70.42%, respectively, both in the state of Minas Gerais, which are similar to those reported in this research. This suggests that coffee honeys have a correlation in reducing sugar content, at least those produced in the state of Minas Gerais, with values close to each other.

The values of phenolic compounds found for M1=218.64 mg/100g and M2=270.65 mg/100g. Phenolic compound values for honeys from different regions of Ethiopia have been reported to range from 42.5 ± 0.94 mg/100g to 82.1 ± 6.48 mg/100g[61]. In Minas Gerais, values of 84.77 ± 0.05 mgGAE/100g[48] have been reported, while in Vietnam, values range from 0.519 ± 0.0083 to 0.863 ± 0.012 mg GAE/g[52]. The phenolic compound values found in the honeys analyzed in this study are higher than those found in the literature and superior to those found in soluble coffees, which ranged between 146 and 151 mg/100g[65].

To determine the antioxidant capacity, the equations of the straight line were used where the R^2 of gallic acid was 0.982, M1= 0.977 and M2= 0.979. The standard used was gallic acid (EC_{50}= 0.269 mg/mL) and for samples M1 (EC_{50} of 162.48 mg/mL) and M2 (EC_{50} of 210.09 mg/mL). It is important to note that the higher the EC_{50} value, the lower the efficiency of the sample in capturing the free radical. Thus, the result observed for M1 was better than for M2.

When comparing samples M1 and M2 with honey samples produced in the state of Minas Gerais, the EC_{50} values were higher, indicating lower antioxidant activity than the 7 investigated samples, which had EC_{50} values ranging from 51.48 ± 1.48 mg/mL to 150.71 ± 2.56 mg/mL. Specifically, the monofloral coffee honey had an EC_{50} value of 77.69 ± 3.55 mg/mL, being the most representative sample in terms of high polyphenol content [50].

Seasonal, environmental factors, honey processing, maturation time, producer species, and botanical origin diversity are factors that promote changes in honey composition and antioxidant effect[66]. Therefore, they may be related to the different results obtained among independent studies.

Coffee honey is attributed with antioxidant power associated with the presence of phenolic compounds that act by breaking the chain of free radicals through the donation of a hydrogen atom[62,67]. Honeys are natural sources of antioxidants, and study results confirm that they reduce inflammatory processes, heart diseases, and cancer[68,69].

Interest in antioxidant activity is due to the effects of compounds on free radicals and the benefits to the body. They play an important role in the prevention and treatment of chronic diseases caused by oxidative stress[70]. The effects result from the redox potential of some compounds; the competition of active and receptive sites in the various cellular structures; or even the ability to modulate the expression of genes that encode proteins that are involved in defense mechanisms against oxidative processes in cellular structures[71,72]. The main compounds with activity, which can be found naturally, are vitamins C and E, carotenoids and polyphenols[73,74,75]. These compounds can be found naturally in plants, animals and microorganisms or can be chemically synthesized. In foods, antioxidants act against spoilage, delay the production of toxic oxidation products, increase food durability, and maintain nutritional quality, which generates greater interest in foods that demonstrate this activity in a natural way[76,77].

In the test carried out in the sample in M1, 0.012 mg/mL of caffeine was determined, while in M2 the value was 0.013 mg/mL. Caffeine can be considered the main alkaloid present in coffee, it can be found in coffee seeds (*Coffea* sp.), green tea seeds (*Camilla sinensis*), cocoa (*Theobroma cocoa*) and others. Even being consumed in small amounts in pharmaceuticals, a large part is ingested in the drink[78]. According to the World Health Organization (WHO), the maximum recommended intake of caffeine per day is up to 200mg. The caffeine levels found in coffee honey are much lower than the maximum recommendation, as each kilogram of the analyzed honeys contains 12-13 mg of caffeine. The results obtained in this analysis are similar to those found for coffee honeys from the state of Espírito Santo, Brazil, where three samples analyzed had 12.40 ± 0.50, 12.5 ± 0.10, and 11.17 ± 0.80 mg/kg of caffeine[79]. Even with high consumption of coffee honey, caffeine levels are not capable of exceeding the WHO's daily recommendation nor causing an effective stimulating effect, as the minimum stimulating dose in humans ranges from 85 to 250 mg[80].

CONCLUSION

Despite the slightly higher level of hydroxymethylfurfural in sample Felicio dos Santos compared to the limit allowed by Brazilian legislation, the other tests conducted for both Santana da Vargen and Felicio dos Santos are within the limits set by Normative Instruction No. 11 of October 20, 2000. The observed differences between the honeys likely relate to the climate and region in which they were produced. Regarding antioxidant activity, the best result was found for Santana da Vargen; however, higher levels of polyphenols and caffeine were found in Felicio dos Santos. Although the data obtained provide guidance for new research involving coffee honey, the work is limited to standard tests for the analysis of honey, provided for in Brazilian normative instructions and the Codex Alimentarius Commission. Therefore, values for marketing products are limited.

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DISCLOSURE STATEMENT

No conflicts of interest.

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