Sensorial, physicist-chemistries and microbiological analyses of samples of honeys produced for Apis mellifera in the region of the Cocais Maranhenses, Maranhão State, Brazil

Análises sensoriais, físico-químicas e microbiológicas de amostras de méis produzidos por apis

mellifera na região dos cocais maranhenses

Análisis sensorial, físico-químico y microbiológico de muestras de productos de miel producidos por apis mellifera en la región de cocais maranhenses

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Abstract

This work had as objective to proceed sensorial analyses, microbiological physical-chemistries and, of comparative form, the honeys, collected for the producers and researcher, of mellifera Apis bee of the cities of Codó and Itapecuru-Mirim, pertaining to the region of the Cocais, Maranhao State. It observed in the first, collected in such a way for the producer how much for the researcher, presented a bigger water text (average humidity of 21,05%) when compared with the second (20.98%), making it difficult its storage, therefore the high water text of the product diminishes its useful life of shelf. For total acidity, the value highest was for the honey produced in Codó (79.19% to 91.80% b), when compared with the honey produced in Itapecuru-Mirim (37.78% to 48.36% b), what it can be explained by the temperature difference, being higher in Codó, For the values of pH the honey of Itapecuru_Mirim was gotten presenting lower value (3,2). For reducing sugars, the honey of Itapecuru - Mirim it presented average of next values (64.14% b - 64.20%) to the demanded minimum as standard. For the values of sacarose the samples of Codó, had presented minors average values of (23.59% b 24.29%); in the parameter you sweeten totals the samples of Itapecuru_Mirim presented greater average index (93,73). For the HMF analyses the samples of Codó had presented average values (67,77a the 76,33b). For the too much studied parameters it did not have significant difference between treatments.

Keywords: Honey; Sensorial analyse; Microbiological physical-chemistries; Apis mellifera.

Resumo

Este trabalho teve como objetivo realizar análises sensoriais, físico-químicas e microbiológicas, de forma comparativa de nove amostras de méis de *Apis mellifera*, coletadas pelos produtores e pela pesquisadora, nos municípios de Codó e Itapecuru-Mirim, pertencentes à região dos Cocais, Maranhão. O primeiro, coletado tanto pelo produtor quanto pela pesquisadora, apresentou um maior teor de água (umidade média de 21,05%) quando comparado ao segundo (20,98%). Para acidez total, o valor mais alto foi para o mel produzido em Codó (79,19^a - 91,80%^b), quando comparado com o mel produzido em Itapecuru-Mirim (37,78^a - 48,36%^b). Para os valores de pH, o mel de Itapecuru-Mirim apresentou o valor mais baixo (3,2). Para açúcares redutores, o mel de Itapecuru – Mirim apresentou valores

próximos $(64,14^{b} - 64,20\%^{a})$. Nas análises de sacarose, as amostras de Codó apresentaram menores valores $(23,59^{b} - 64,20\%^{a})$. 24,29%^a); no parâmetro açucares totais as amostras de Itapecuru-Mirim apresentaram maior índice médio (93,73). Para as análises de Hidroximetilfurfural (HMF) as amostras de Codó apresentaram valores médios de 67,77^a - 76,33^b. No parâmetro cor as amostras tiveram variância de extra âmbar claro à âmbar escuro. No parâmetro minerais e cinzas, apenas três amostras foram analisadas (A1 a A3) e apresentaram valor médio 0,35% a 0,73%. Nas análises microbiológicas realizadas também nas amostras (A1 a A3), a presença de bolores e leveduras e coliformes totais e fecais não foi significativa.

Palavras-chave: Mel; Análises sensoriais; Parâmetros físico-químicos; Apis mellifera.

Resumen

Este trabajo tuvo como objetivo realizar análisis sensoriales, físico-químicos y microbiológicos, de manera comparativa de nueve muestras de miel de Apis mellifera, recolectadas por los productores y el investigador, en los municipios de Codó e Itapecuru-Mirim, pertenecientes a la región. de Cocais, Maranhão. La primera, recolectada tanto por el productor como por el investigador, tuvo un mayor contenido de agua (humedad promedio de 21,05%) en comparación con el segundo (20,98%). Para la acidez total, el valor más alto fue para la miel producida en Codó (79.19a - 91.80% b), en comparación con la miel producida en Itapecuru-Mirim (37.78a - 48.36% b). Para los valores de pH, la miel de Itapecuru-Mirim presentó el valor más bajo (3,2). Para azúcares reductores, la miel de Itapecuru -Mirim mostró valores cercanos (64,14b - 64,20% a). En los análisis de sacarosa, las muestras de Codó presentaron valores más bajos (23.59b - 24.29% a); en el parámetro azúcares totales las muestras de Itapecuru-Mirim presentaron el índice promedio más alto (93,73). Para los análisis de Hidroximetilfurfural (HMF), las muestras de Codó presentaron valores promedio de 67,77a - 76,33 b. En el parámetro de color, las muestras tenían una variación de ámbar extra claro a ámbar oscuro. En el parámetro de minerales y cenizas, solo se analizaron tres muestras (A1 a A3) y presentaron un valor promedio de 0.35% a 0.73%. En los análisis microbiológicos realizados también en las muestras (A1 a A3), la presencia de mohos y levaduras y coliformes totales y fecales no fue significativa. Palabras clave: Miel: Análisis sensorial: Parámetros físico-químicos: Apis mellifera.

1. Introduction

Bees are considered the main pollinating agents of most species of wild plants and cultures pollinated by insects (Kevan & Phillips, 2001; Klein et al., 2006; Ollerton et al., 2011). About 75% of agricultural crops used directly for human consumption depend on pollination (Potts et al., 2016).

Rational beekeeping is an activity in which good economic, ecological and social results are obtained. This activity, developed over time by small and medium producers, has aroused the interest of many creators and institutions in Brazil.

Bees of the species Apis mellifera, are the most used worldwide for the pollination of cultivated plants. The preference for this species is justified by its easy management, the size of its colonies, its abundance in different ecosystems and its generalist profile in the search for resources (Potts et al., 2010). In addition, Apis mellifera plays an important role as a producer of honey and other bee products (Pires et al., 2016).

Honey has a great nutritional value, in addition to having a typical and sweet taste that pleases many palates. The properties present in it are related to the type of flower that was removed, the nectar, climate, and the species of bee, in addition to the handling of honey by the beekeeper. One of the main compounds in honey are carbohydrates, which serve as a source of nutritional energy (Alburquerque el al., 2021).

Much of the Brazilian population uses honey as a medicine, thus resulting in a low consumption of this product in the country, because of this, the product needs to be sold abroad. Honey consumption in Brazil is around 0.07 kg/person/year, being one of the least in the world, the United States is the largest importer of Brazilian honey and has one of the highest consumptions, at around 1kg/person /year. Another important point of honey consumption in Brazil is its high price and requirements regarding hygiene standards, nutritional values and practicality (Cabral & Oliveira, 2021).

Particularly in Maranhão, beekeeping has been placed as one of the strategic areas of interest to the State to promote the development of family farming in Maranhão (Beekeeping Development Program of the State of Maranhão, 2001). Currently, several beekeeping projects are being developed and supported in a planned manner by Nordeste Bank, in partnership with the State Government and Research Institutions.

One of the biggest challenges to the success of beekeeping in the State of Maranhão has been the lack of knowledge of the quality profile of the products produced, making it difficult to increase productivity and carry out the activity within the standards required by legislation. Little has been discussed about the quality of honey from the various wild flowers and the parameters involved in this characterization.

As the bee flora is very diverse and varies from place to place, it is essential to know the composition and qualities of the honeys obtained in each region to characterize them and establish standards (Carvalho, 2001), which justifies the present proposal of the study of honeys from the Cocais Maranhenses Region.

The physical, chemical and biological characteristics of the *Apis mellifera* honeys produced in the State of Maranhão are still poorly known, requiring characterization, mainly because the tropical regions are related to high rates of humidity and temperature (Marchini et al, 1998), the which favors the production of honeys that do not comply with the legislation standard.

The present work aimed to establish the profile of the honey quality of the Apis mellifera bee in the municipalities of Codó and Itapecuru-Mirim, located in the Cocais Maranhenses Region, which will contribute to the establishment of the profile of the honey produced in the State of Maranhão.

2. Methodology

The experiment was carried out at the Universidade Estadual do Maranhão, using the Honey Analysis laboratories of the Department of Chemistry and Biology and the Soil Analysis Laboratory of the Master of Agroecology, and at the Universidade Federal do Maranhão, using the Central Analytical laboratory. from the Chemistry Department of the Center for Technological Sciences. Nine samples of *Apis mellifera* honeys obtained in the Cocais Maranhenses region, in the municipalities of Codó (Codó microregion, height of 47 meters, geographically in the eastern portion of Maranhão, were coordinated latitude 04°27'19 "south and a longitude 43°53'08" west of equatorial climate) and Itapecuru-Mirim (microregion Itapecuru-Mirim, located in the eastern mesoregion of Maranhão, coordinates latitude 3 ° 23'33 "south and longitude 44 ° 21'31" west, equatorial climate).

The samples were obtained by two methods, the first from beekeepers and associations that packed the honeys in their own containers and the second directly from the hives collected by the researcher, where the collected samples went through a process of centrifugation, filtration, decanting and packaging in containers. and stored in a dark place for further analysis.

The honey from Codó (A1 and A5) was taken from Apis mellifera hives located in the Sítio dos Padres, belonging to the Santa Filomena Parish, with field vegetation and wild flowers, predominantly of assa-fish (Vernonia Polyanthes). The samples (A2, A3 and A4) were collected at Sítio 3 Irmãos, with a predominant cashew flowering. In the municipality of Itapecuru-Mirim, samples were collected in the Magnificat village (A6 and A7) and in the Carmo village (A8 and A9).

2.1 Evaluation of sensory, physical-chemical and microbiological characteristics

In all samples collected, physical-chemical analyses were performed to determine moisture, apparent sucrose, water insoluble solids, minerals, acidity, reducing sugars, diastase activity and hydroxymethylfurfural according to Codex Alimentarius commission (CAC) (1990) and Association of Official Analytical Chemists (AOAC) (1998), microbiological analysis determining molds, yeasts and coliforms (total and fecal) according to the American Public Health Association (APHA) (1984) and International Commission on Microbiological Specifications for Foods (ICMSF) (1978), respectively.

2.1.1 Color Analysis

To perform the color analysis of the samples it was used the spectrophotometric method using 560 nm wavelength, and as standard the Pfund scale that classifies the color from water white to dark amber (LANARA, 1981).

The color determinations were made with pure liquid honey sample in a UV-VIS Spectrophotometer Model: Cary 50 Brand: Varian at 560 nm in a 1 cm cell using pure glycerin as blank. The procedure is based on the different degrees of light absorption at different wavelengths, depending on the constituents present in the honey sample, which is determined by the color standard scale, Pfund scale.

2.1.2 Physicochemical Analysis

The preliminary identity research of honey from its physical-chemical characteristics was performed by determining the maturity and deterioration measures (Brasil, 2000).

In this work, to determine the honey maturity, the quantitative analysis of humidity, reducing sugars and apparent sucrose were performed. And for the deterioration determination, the acidity and hydroxymethylfurfural were performed; and in the purity determination: minerals.

2.1.2.1 Moisture

For the humidity identification it was used the refractometry method according to method no. 969.38b (AOAC, 1998), adopted as the official methodology by the Ministry of Agriculture and Supply (Brazil, 2000). The principle of this method consists in determining the refractive index of honey at 20 °C, which is converted to the moisture content using the Chataway table, used as a reference, which, in turn, provides the concentration as a function of the refractive index. In this method an Abbé table refractometer is used.

For the determination of the corrected refractive index at 20 °C the following equation is used:

$IR^{20} = IR^{t} + 0,00023 (T - 20)$

Where:

IR 20 = refractive index at 20 °C

IR ^t = refractive index read at laboratory temperature

T = temperature at the moment of reading

2.1.2.2 Acidity and pH

Based on the method No. 969.38b of AOAC (1998), the free acidity was determined by titration of the sample with 0.05N NaOH solution until it reached the pH 8.5, which method is recommended by the Technical Regulation of Identity and Quality of Honey (Brazil, 2000).

To calculate the free acidity, the blank volume is calculated, which consists in titrating with 0.05N NaOH solution 75ml of decarbonized water (used in the dilution of the samples) until the pH reaches 8.5; the blank volume is calculated from the product between the volume of NaOH and the NaOH factor.

For the determination of total acidity the volumetric method is used, adopting the methodology of the Ministry of Agriculture - National Laboratory of Animal Reference (1981) and CAC (1990).

For the determination of free, lactonic and total acidity the equations are applied:

Free acidity = $(mL \text{ de NaOH } 0,05 \text{ N used in the burette} - mL \text{ blank}) \times 50$ g of the sample

Lactonic acidity = $(10,0 - mL \text{ de HCl } 0,05 \text{ N used in the burette}) \times 50$

g of the sample

Total acidity = free acidity + lactonic acidity

For the pH, potentiometric readings were taken using a PHR - 725 model potentiometer, portable digital from INSTRUTHERM, according to the recommendations of the Adolfo Lutz Institute (1985).

2.1.2.3 Reducing sugars

They were determined according to the CAC (1990) method item 7.1, from the modification of Lane and Eynon's procedure, involving the reduction of Fehçing's solution, modified by Soxhlet by oxidimetry according to the CAC (1990), during titration at boiling point with a solution of reducing sugars from honey, using methylene blue as an indicator.

% Reducing sugar = <u>100 x 100 x fator (Fehling reagent)</u> Sample weight x Vg (mL)

Where:

100 = amount of water used in the dilution of the honey sample

100 = percentage

Factor = Fehling's reagent standardization

Vg = spent volume of honey solution in the titration

2.1.2.4 Apparent sucrose

The sucrose content was measured according to the method of item 7.2 of the CAC (1990), determining the apparent sucrose after inversion by acid hydrolysis, as recommended by the Normative Instruction of the Ministry of Agriculture and Supply (Brazil, 2000).

The apparent sucrose value is determined by:

Apparent sucrose = total sugars - reducing sugars

2.1.2.5 Total Sugars

The determination of total sugars was based on the CAC method (1990), being determined by the arithmetic sum of the values obtained for reducing sugars (glucose) and sucrose (non-reducing sugars).

2.1.2.6 Hydroxymethylfurfural

Determined using the quantitative method, which consists in the verification of HMF, using the spectrophotometric method at 284 and 336 nm, according to method number 980.23 (AOAC, 1998) recommended by the Normative Instruction of the Ministry of Agriculture and Supply (Brazil, 2000).

A UV-VIS Spectrophotometer Model: Cary 50 Brand: Varian was used at 560 nm to measure the absorbance of the sample.

To calculate the amount of HMF, the formula was used:

HMF (mg de HMF/ Kg honey) = $(A_{284} - A_{336}) \times 149,7 \times 5$

Sample weight

Onde:

HMF = mg de HMF/ Kg honey $A_{284} = absorbance at 284 nm$ $A_{336} = absorbance at 336 nm$ Factor = 149,7

2.1.2.7 Minerals and ashes

Based on the CAC method - Item 7.5, the amount of minerals and ashes was obtained by calcination of honey samples in a muffle furnace at 600°C, as recommended by the Ministry of Agriculture and Supply (Brazil, 2000) through which it is possible to determine some irregularities in honey, such as contamination caused by non-decantation and/or filtration at the end of the honey extraction process.

> % de minerals (honey ash) = <u>Crucible weight difference x 100</u> Total weight of sample used

2.1.3 Microbiological Analyses

2.1.3.1 Moulds and Yeasts

Based on the method (APHA, 1992) which consists in counting the formation of colonies in a Neubaner chamber after preparing dilutions obtained with 25g of honey and 225mL of 0.1% phosphate water, later placed in Petri dishes and exposed to room temperature for 48 hours.

2.1.3.2 Coliforms (Total and Fecal)

Based on the method (ICMSF, 1978), it consists in counting the formation of colonies by visualization of gas formation in tubes, from the preparation of dilutions obtained with 25g of honey and 225mL of 0.1% phosphate water; the tubes were placed in greenhouses at 25°C.

2.1.4 Statistical analysis of data

For statistical analysis of the data, as the number of determinations performed was in triplicate, the data was examined, comparing the two ways of honey collection, performed in both municipalities. From the average of the number of determinations, the population mean was defined, using the values of Student's t parameter, as a function of the number of determinations. This analysis consists of identifying the interval in which the population mean (μ) should be, with a certain probability, in this 90%, knowing the mean of determinations "X," the estimate "s" and the number of determinations "N".

The interval X ±ts \sqrt{N} is called the confidence interval (CI) of the mean, where X -ts \sqrt{N} and X +ts \sqrt{N} are the onfidence limits of the mean. The probability corresponding to the t-value from Student's table.(PUGH & WINSLOW, 1996).

For the comparison of data values, performed the F-test for a statistical evaluation of the results obtained, so it is possible to identify the existence of a significant difference in accuracy between this data set and another set, in this the CI. This test uses the ratio of the variances of the two data sets to establish whether there is indeed a significant difference in

accuracy, calculated by the expression TF, $F=s^2x/s^2y$. In this way, the F-value obtained is compared to calculated critical values, assuming that they will be exceeded purely on the basis of a 5% probability (BACCAN et.al 2001).

3. Results and Discussion

The analyses were performed in triplicate and the values obtained are expressed as means. With the data found for each parameter and after performing the statistical analysis, tables 1 to 4 were prepared with the mean values for the respective parameters analyzed.

 Table 1 - Average values of moisture, °Brix, acidity and pH of honey samples from the municipalities of Codó and Itapecuru

 Mirim, collected by the producer.

			Q-TEST (Q _{90%})					
	PRODUCER							
	Samples	Umidade	° BRIX	Acidity	рН			
	Default values	máx. 20%		máx. 50 meq/kg	3,3 a 4,6			
A1	Codó-St. dos Pe	21,07	75,75	71,07	2,5			
A2	Codó-St. 3 Irmãos	20,98	78,25	79,46	3,60			
A5	Codó-St. dos Pe	21,18*	72,50*	87,03	3,5			
A6	Itapecuru Mirim- Magníficat	20,98	78,25	35,53	3,4			
A8	Itapecuru Mirim- Carmo	20,96	78,75	40,03	3,2			
	Average	21,0339	76,7000	62,6200	3,2400			
	DP	0,0936	2,6244	23,4259	0,4393			
	IC	0,0821	2,3003	20,5334	0,3851			
	DP*DP	0,0088	6,8875	548,7742	0,1930			
(+)		21,1503	79,9628	91,7444	3,7862			
(-)		20,9175	73,4372	33,4956	2,6938			
		21,0339±0,12%	76,7000±0,12%	62,6200±0,12%	3,2400±0,12%			

* - Average values that statistically differed from each other. Source: Authors.

Q-TESTE (Q90%)							
RESEARCHER							
	Sample Humidity [°] BRIX Acidity pH						
D	efault Values	máx. 20%		máx. 50 meq/kg	3,3 a 4,6		
	dó-St. 3 Irmãos	21,03	76,50	110,57	4		
.4 Co	dó-St. 3 Irmãos	21,01	77,50	73,025	3,4		
A7 Itapecur A9	u Mirim-Magníficat	21,01	77,50	53,53	3,20		
Itapec	uru Mirim-Carmo	20,96	78,75	43,19	3,2		
	Average	21,0022	77,5625	70,0775	3,4500		
	DP	0,0292	0,9214	29,6943	0,3786		
	IC	0,0286	0,9029	29,0999	0,3710		
	DP*DP	0,0009	0,8490	881,7511	0,1433		
-)		21,0485	79,0275	117,2914	4,0520		
·)		20,95579376	76,0975	22,8636	2,8480		
		21,0022±0,05%	77,5625±0,05%	70,0775±0,05%	3,4500±0,05%		

Table 2 - Average values of moisture, °Brix, acidity and pH of honey samples from the municipalities of Codó and Itapecuru

 Mirim, collected by the researcher.

Source: Authors.

 Table 3 - Average values of reducing sugars, apparent sucrose, total sugars and hydroxymethylfural of honey samples from the

 Municipalities of Codó and Itapecuru-Mirim, collected by the producer.

	Q-TESTE (Q _{90%}) PRODUCER							
	Samples	Reducing sugars	Apparent Sucrose	Total Sugars	HMF			
	Default Values	mín. 65%	máx. 6,0%		máx. 60 mg/kg			
A1	Codó-St. dos Pe	55,56	23,81	79,37	83,70*			
A2	Codó-St. 3 Irmãos	55,56	22,73	78,28	56,78			
A5	Codó-St. dos Pe	59,06	26,32	85,38	62,84			
A6	Itapecuru Mirim- Magníficat	65,79	30,86*	96,65	44,96			
A8	Itapecuru Mirim- Carmo	64,10	25,51	89,61	42,52			
	Average	60,0126	25,8454	85,8580	58,1597			
	DP	4,7629	3,1381	7,5898	16,5441			
	IC	4,1747	2,7506	6,6526	14,5013			
	DP*DP	22,6848	9,8474	57,6049	273,7083			
(+)		65,9340	29,7468	95,2940	78,7283			
(-)		54,0911	21,9440	76,4219	37,5912			
		60,0126±0,12%	25,8454±0,12%	85,8580±0,12%	58,1597±0,12%			

* - Average values that statistically differed from each other. Source: Authors.

	Q-TEST (Q90%)							
	RESEARCHER							
	Samples	Reducing sugars	Apparent Sucrose	Total Sugars	HMF			
	Default Values	mín. 65%	máx. 6,0%		máx. 60 mg/kg			
A3	Codó-St. 3 Irmãos	47,17	20,38	67,55	81,41			
A4	Codó-St. 3 Irmãos	62,50	26,79	89,29	71,25			
A7	Itapecuru Mirim- Magníficat	65,79	31,65	97,44	66,90			
A9	-							
		62,50	28,74	91,24	24,56			
	Itapecuru Mirim-Carmo							
	Average	59,4898	26,8867	86,3765	61,0287			
	DP	8,3584	4,7752	13,0229	25,0620			
	IC	8,1911	4,6796	12,7622	24,5603			
	DP*DP	69,8635	22,8024	169,5970	628,1059			
(+)		72,7797	34,4793	107,0830	100,8774			
(-)		46,1999	19,2942	65,6701	21,1801			
		59,4898±0,05%	26,8867±0,05%	86,3765±0,05%	61,0287±0,05%			

Table 4 - Average values of reducing sugars, apparent sucrose, total sugars and hydroxymethylfural of honey samples from

 Codó and Itapecuru-Mirim, collected by the researcher.

Source: Authors.

During the humidity tests, the samples from Itapecuru-Mirim and Codó, respectively, had average values of 20.98% and 21.05%. These values are above the maximum limit allowed by current legislation, 20%, established by the Ministry of Agriculture and Supply (Brasil, 2000). Sample A5 differed statistically in this analysis because it already showed signs of fermentation. The high humidity value found in the samples is indicative of the honey being collected green, proved by the sample A3; as the honey comes from nectar, it can be suggested that the nectar collected by the bee may have in its composition a higher water content. The values obtained are close to that of Braghini *et al.* (2016) who analyzed honey samples from São Paulo, finding values for moisture ranging from 16.05% to 18.4%.

The °Brix (Table 2) determined in the samples had an average value of 77.13 °Brix, within a range of 76.50 to 78.75 °Brix. Sample A5 also differed statistically in this analysis. The results found were close to those of Borges et al. (2017) from 71.5% to 80.5%.

For the acidity values, with average value 66.35 meq/kg only three samples (A6, A8 and A9) showed results within the legislation (max 50 meq/kg), with average values of 35.53 meq/kg, 40.03 meq/kg and 43.19 meq/kg respectively, both correspond to the municipality of Itapecuru-Mirim and all were collected by the researcher.

The types of flowers in the regions can explain this variability, 43.04 meq/kg (Codó) and 84.23 meq/kg (Itapecuru-Mirim), since the acidity in honey comes from the various organic acids contained in the nectar collected by bees (Root, 1985), which by the action of glucose oxidase originates the gluconic acid (White Júnior, 1989) being also influenced by the amount of minerals present in nectar (Barth, 1989).

The pH (Table 2) of the samples analyzed ranged from 3.2 (A7 to A9) to 4 (A3) (Table 1), with average values of 3.24 to 3.45 (I.C \pm 0.05%). Of the samples analyzed 55.55% is within the current standard, which is 3.3 to 4.6. Terrab et al. (2004) state that pH influences the texture, stability and shelf life of honey, since altered pH values may indicate fermentation

or adulteration of honey, moreover Souza et al. (2009) reports that the acidity of honey is a chemical characteristic that in the same way as pH also gives evidence of fermentation, indicating the deterioration process.

The amount of total sugars found in the samples varied from 67.55 to 97.44% with a mean value of 85.86 \pm 0.12 (CI \pm 0.05%). For total sugars there is no value established in the current standards.

With regard to the content of reducing sugars, in the nine samples analyzed there was a variation of 55.56 to 65.79% with a mean value of $60.0126 \pm 0.12\%$ (CI $\pm 0.05\%$). Only 22.22% of the samples were within the standards of the current norm. The values are close to those of Braghini et al. (2016) which are between 63.94% and 78.22%.

For sucrose analysis, the values ranged from 20.38 to 31.65% with an average of $25.84 \pm 0.12\%$ (I.C $\pm 0.05\%$). Komatsu (1996), found average values of 0.1 to 27.4%. Sodré et al. (2001) stated that when the saccharose value is too high, it is an indication that the product is green or adulterated honey.

The amounts of hydroxymethylfurfural found in the nine samples varied from 24.56 to 81.41 mg/kg with an average of $58.16 \pm 0.12\%$ (I.C $\pm 0.05\%$). The analysis for sample A1 was not significant showing a value of 83.70 mg/kg. Braghini et al. found in their samples values between 19.17 to 21.10 mg/kg. High values of HMF in honey may be due to prolonged storage at high ambient temperatures and/or overheating, or even adulteration by addition of inverted sugar (Sodré et al., 2007). Dyrell & Vital (1991) analyzed samples of Brazilian honeys by the AOAC method and found values ranging from 1.1 to 248.2 mg/kg. The authors mention that honeys from tropical countries have a high HMF value, making it essential to quantify this component to verify the quality of the product.

High HMF content may indicate aging, prolonged heat treatment or fraudulent addition of commercial invert sugar according to Doner and White Jr. cited by STONOGA (1990). Prior to the formation of HMF during the acid hydrolysis of sucrose, the presence of high levels of these compounds indicates that the honey has been adulterated with invert sugar (SANZ *et al.*, 2003).

The colors of the nine samples are presented in Tables 5 and 6. It was observed for the analyzed samples, a slight predominance of the colors extra light amber (44.44%), amber (33.33%) being the distribution of the others very similar.

Table 5 - Identification of the samples and color analysis of the nine samples of Apis mellifera honeys from the municipalities
of Codó and Itapecuru-Mirim.

PRODUCER					
	Samples	Color			
	Water White to dark amber				
A1	Codó-St. dos Pe	0,595 nm – Amber			
A2	Codó-St. 3 Irmãos	0,563nm – Amber			
A5	Codó-St. dos Pe	2,295 – Dark amber			
A6	Itapecuru Mirim-Magníficat	0,1606 - Extra light amber			
A8	Itapecuru Mirim-Carmo	0,1470 - Extra light amber			

Source: Authors.

Table 6 - Identification of the samples and color analysis of the nine samples of Apis mellifera honeys from the municipalities	
of Codó and Itapecuru-Mirim.	

RESEARCHER					
Samples Color					
Amber Water White to dark ambe					
A3	Codó-St. 3 Irmãos	0,751 - Amber			
A4	Codó-St. 3 Irmãos	0,292 – Light amber			
A7	Itapecuru Mirim-Magníficat	0,1156 - Extra Light amber			
A9	Itapecuru Mirim-Carmo	0,1511 - Extra Light amber			

Source: Authors.

Depending on the coloration, the flavor and aroma suffer alterations, preserving the nutritional value. The darker the honey, the greater amount of minerals it has, but lower commercial value, because the light color is more accepted in the world market, being sold with higher price (Venturini; Sarcinelli; Silva, 2007).

With regard to minerals and ash (Table 7), only sample (A1) showed results compatible with the allowed by legislation, which is 0.6%, with this sample having only 0.35%, while the other samples had values higher than the mentioned, 0.68 to 0.73% (Table 3). Gomes et al. (2017), when analyzing honey samples from western Pará, found average values of 0.4 to 0.20%, values close to those analyzed.

	Samples	Minerals and Ash			
	Default Values	Máx 0,6%			
A1	Codó-St. dos Pe	0,35			
A2	Codó-St. 3 Irmãos	0,68			
A3	Codó-St. 3 Irmãos	0,73			
	Average	0,515			
	DP	0,2065			
	IC	0,2337			
	DP*DP	0,0426			

Table 7 - Average values of minerals and ash of honey samples from Codó.

Source: Authors.

In the parameter of microbiological analysis (Tables 8 and 9), there was no formation of gases inside the tubes proving the absence of total coliforms, consequently of fecal coliforms. In the analysis of mold and yeast, the maximum allowed by the legislation is 25 colonies; the samples did not present such formations, thus all the samples are within the standards of the Brazilian legislation for Apis mellifera.

		DILUTION				
Sample		N° OF COLONIES		ONIES	COUNTING (UFC/mL)	
		10-1		10-3		
A1	Codó-St. dos Pe		0	0		
A2	Codó-St. 3 Irmãos		0	0		
A3	Codó-St. 3 Irmãos		0	0		

Table 8 - Calculation of the number of CFU/mL in the total coliform plates.

Source: Authors.

Table 9 - Calculation of the number of CFU/mL on the mold and yeast plates.

		DI	LUTI	ON	
Sample		N° of COLONIES		ONIES	CONTING (UFC/mL)
		10-1		10-3	•
A1	Codó-St. dos Pe	0	0	0	
A2	Codó-St. 3 Irmãos	0	1	0	
A3	Codó-St. 3 Irmãos	9	7	0	

Source: Authors.

4. Conclusion

Considering the sensory analysis of the color parameter, all samples (A1 to A8) were within acceptable standards.

The physical-chemical analysis, regarding humidity and apparent sucrose, showed that all samples presented values out of the standards, which can be an indication of the type of flower of the region or this variability can explain the deterioration and/or adulteration of the samples. As for the parameters acidity, reducing sugars and hydroxymethylfurfural, only 33.33%, 22.22% and 44.44%, respectively, of the samples meet the standards.

Among the samples studied, those from the city of Codó presented a higher rate of rejection, since they are outside the standards, except for color, ash (one sample) and microbiological analysis. The sample (A5) that showed a higher index in the statistical differentiation, for the parameters moisture and °Brix is related to the form of collection and storage, since it in the period of analysis, proceeded in a short time, already showed signs of deterioration (fermentation). The high values of hydroxymethylfurfural, especially in the samples from Codó is due to the high average temperatures of the city, which will directly influence the quality of honey.

In the comparison between the samples collected by the producer and by the researcher, the parameter that most diverged was humidity and hydroxymethylfurfural, thus verifying that the two parameters can be a measurable indicative of honey quality.

The future perspective of honey analysis is very promising, considering that these analyzes prove the quality of honeys to be commercialized, making them quality products. In view of this, it is valid that after this study the community is benefited and informed, thus being a form of popularization of science.

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