PHYSICOCHEMICAL ANALYSIS OF HONEYS PURCHASED IN CUIABÁ, MATO GROSSO – BRAZIL

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The objective of this study was to determine the physicochemical characteristics of honeys sold in supermarkets of Cuiabá – MT. The physicochemical analysis were ashes, moisture, proteins, total sugars, reducing sugars, apparent sucrose, pH, acidity, electrical conductivity, water activity, soluble solids (°Brix), hydroxymethylfurfural (HMF) and color. The results were analyzed statistically using ANOVA and followed by a Tukey's test at 5% of significance. The results were compared with the values allowed by current Brazilian legislation and the literature that verified honeys in other regions of the country. All the analyzed samples were within the legal parameters for moisture, ashes and acidity, however in the case of apparent sucrose all presented results were out of recommended. In the analysis of proteins, total sugars, pH, electrical conductivity, Aw, soluble solids and HMF (qualitative) there are no Brazilian standards to determine the quality of honeys. Therefore, it was concluded that it is important to implement complete parameters to assist the PIQ (Identity and Quality Standard) and the characterization of honeys produced in the most diverse locations, biomes and environmental conditions in Brazil.

KEYWORDS: BEES, SUCROSE, ADULTERATION, QUALITY, SUGAR.

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1 INTRODUCTION

Brazil has a large beekeeping potential, due to its diversified flora, territorial extension, and climate variability. Honey is a substance naturally produced by bees from the nectar of flowers or saccharinic exudation of other parts of plants, which are collected and transformed by water evaporation and enzymes addition. This is a well-known bee product disseminated around the world, one of the first foods of humankind, which was also used as a medicinal resource (NEVES et al. 2015).

Bee honey is a food product with a great nutritional value and high acceptability by many consumers, mainly because it is considered a therapeutic product, beneficial to health (GOMES et al. 2017). It is a complex food both from a biological and analytical point of view, due to its composition varying according to the floral and geographical origin, as well as climate conditions (RACOWSKI et al. 2009).

It is known since ancient times, always attracted people’s attention, especially due to its sweetening properties, that led to develop increasingly advanced techniques to induce greater productivity in bees (BERA and ALMEIDA-MURADIAN, 2017). The key components of honey are sugars, which are monosaccharides fructose and glucose account for 70%; disaccharide, including sucrose, add around 10%; and water, between 17 and 20% (FERREIRA and SOUZA, 2015).

Bee honey is a very appreciated natural product; however, it can be easily adulterated with sugars or syrups. Thus, it is necessary to conduct an analysis to determine and control quality before the product can be marketed (GOMES et al. 2017).

Brazilian legislation, through the normative instruction n.11 from October 20, 2000 (BRAZIL, 2000) aims to establish identity and minimum requirements of quality and standards for honeys intended for human consumption; besides, the normative establishes parameters of acceptable qualities and presents the methods of analysis that must be carried out in order to assess bee honey quality.

The present study was to characterize the physicochemical properties of commercially available honeys from supermarkets of Cuiabá - MT.

2 MATERIAL AND METHODS

The analysis were carried out in the laboratories of the Federal Institute of Education, Science and Technology from Mato Grosso (IFMT), *campus* Bela Vista – Cuiabá/MT.

Physical and chemical analysis realized were ashes, moisture, proteins, total sugars and reducers, apparent sucrose, pH, acidity, electrical conductivity, water activity, soluble solids (°Brix), hydroxymethyfurfural and color, with the purpose of comparing parameters allowed by current legislation and with other scientific studies concerning to the honeys.

The honey samples chosen were 10 brands produced in Brazil, four of them being from Mato Grosso, five brands from São Paulo, and one from Paraná. All products were purchased in supermarkets in the city of Cuiabá – MT, classified as wild (multi-flower) varieties and codified according to table 1.

TABLE 1 – CODIFICATION AND PERIODS OF THE SAMPLES OF HONEYS COLLECTED IN SUPERMARKETS OF CUIABÁ - MT.

Code/Samples	Manufacturing Regions	Period of Manufacturing	Lot
A	Cuiabá – MT	December/15	Not Reported
B	Santo Antônio do Leverger – MT	December/14	52
C	Várzea Grande – MT	January/15	011/2015
D	Cáceres – MT	January/15	1
E	Itupeva – SP	February/15	A
F	Embu-guaçú – SP	January/17	96B
G	Barretos – SP	January/15	MB747
H	Bebedouro – SP	February/15	1502702556
I	São Paulo – SP	August/14	39
J	Maringá – PR	January/15	141

Ten treatments were carried out in triplicate, under room temperature and according to the following methodologies: moisture and hydroxymethyfurfural (HMF), carried out by the method of Association of Official Analytical Chemists – AOAC (2012); ashes, determination of proteins (Kjeldahl method), titrable acidity, total sugars, reducing sugars and apparent sucrose based on the Adolf Lutz Institute methodology (IAL, 2016). The pH testing used the pHmeter – Meat pH Meter HANNA HI 99163, according to the AOAC methods (2012). In order to assess objective color of the samples, the equipment spectrophotometer calorimeter Minolta was employed in the scale L*, a* and b*, in the scale of the system CIELab, calibrated by a white and illuminating standard D65. For water activity (Aw), the analyzer of water activity in dew point Aqualab model 4 TE was used, according to the method of AOAC (2012). Soluble solids (ºBrix) was determined through digital equipment bench refractometer RTD – 95, according to the AOAC method (2012). Finally, in the analysis of electrical conductivity, the equipment employed was the conductivimeter HydroSan. All data were analyzed on the statistical software ASSISTAT version 7.7 beta, employing analysis of variance (ANOVA); when differences were found, the Tukey test was applied at 5% significance (SILVA and AZEVEDO, 2016).

3 RESULTS AND DISCUSSIONS

It was verified that all of the samples analyzed were at the standard established by legislation for the moisture parameter, where a maximum of 20% is allowed (BRAZIL, 2000). The statistical data pointed that the averages of brands A and F did not show a significant difference (p>0,05) from each other, however they showed significant difference among the results of the other samples (table 2). Sample D was found to be far lower, with 9% of moisture, which can be explained by the high content of soluble solids (80 ºBrix) as shown on table 5. According to Mendes et al. (2009), the higher the sucrose concentration, the higher the solids gain and the greater the loss of water; in addition, moisture levels decrease with product maturation, causing hydrolysis of the sucrose and resulting in the formation of a mixture of glucose and fructose (SCHLABITZ et al. 2010).

TABLE 2 – AVERAGE OF MOISTURE, ASHES AND PROTEINS ANALYSIS OF HONEY PURCHASED IN CUIABÁ – MT (%).

Samples	Moisture (%)	Ashes (%)	Proteins (%)
A	20.070 a	0.073 cd	0.319 bc
B	15.170 c	0.133 cd	0.861 ab
C	15.166 c	0.230 c	0.523 abc
D	09.803 d	0.053 d	0.235 c
E	15.483 bc	0.143 cd	0.474 abc
F	19.650 a	0.180 cd	0.325 bc
G	16.593 b	0.177 cd	0.702 abc
H	15.416 bc	0.510 b	0.955 a
I	15.146 c	0.220 c	0.766 abc
J	16.043 bc	0.690 a	0.349 abc

Means with different letters differ statistically ($p < 0.05$) in Tukey's test.

Water is the second component in quantity in honey. Its concentration is a determining factor on parameters such as viscosity, specific gravity, crystallization and flavor, and is a key indicator of fermentation trend, which is directly related to the preservation of the product. This is because sugar-tolerant microorganisms present in bees, nectar, extraction places, soil, and storage can cause fermentation in honey when it has a high water concentration (NEVES et al. 2015).

Thus, the values of samples are similar with the studies of Schlabitz et al. (2010) and Filho et al. (2012); the latter analyzed honeys produced on Campo Grande – MS. According to the Santos and Oliveira (2013), honey moisture is influenced by its botanical origin, by weather conditions, by harvest time and by maturity degree, which is a parameter of great importance during product storage.

The results of ashes determination in the samples differ significantly from one another (table 2). They also show an average of 0.24%, with a variation from 0.05% to 0.69%. Current law establishes a maximum of 0.6g/100g (honey), with a 1.2g/100g tolerance for honeydew honey (BRAZIL, 2000). Therefore, of the results obtained only sample J (0.690%) is not in accordance with the legislation.

The ash content average in the present study was the same found by Santos and Oliveira (2013), which tested honey from Ceará (0.24%). However, Borges et al. (2017), when analyzing the quality of the honey sold in street markets of Salvador and Petrolina, found mineral values of 0.02% to 0.88%, and two samples presented contents of 0.86% and 0.88%, which indicates that these honeys with elevated ash concentration and very dark coloration could be honeydew honey.

Nevertheless, it is shown on the table 2 that honey J contained the highest value of ashes determination (0.69%). Quantities larger than 0.6% p/p indicate that there might have been adulteration on the honey, or that there were issues in the extraction process (BRAZIL, 2000; ANDRADE, 2006; SANTOS and OLIVEIRA, 2013). Ashes analysis allows us to verify hygiene conditions on which the honey was manipulated, as well as filtration and decantation efficiency and the degree of purity; this analysis is used as a quality criterion and is connected to botanical and geographical origins, such as differences on the soil, atmospheric conditions, and type and physiology of each plant (ANDRADE, 2006; SCHLABITZ et al. 2010; NEVES et al. 2015; BORGES et al. 2017).

For protein analysis, it can be observed that there was a significant difference between samples D and H (table 2), showing that the protein content of honey produced in Bebedouro - SP is higher than the protein content of honey produced in Cáceres - MT. However, there are no reference values for this parameter.

Current legislation requires only the pollen presence. Nonetheless, in the same study it is possible ascertain that there is a great variation in protein content in the honeys analyzed, independently of production region. It is observed that the protein content obtained in this search permeate the results found by Souza et al. (2009a), which varied between 0.04% and 1.21%. The same author mention that, despite there being little knowledge about characteristics of the proteinic material, the occurrence of the protein on honey is used to detect adulteration (SOUZA et al. 2009a).

The results of the analysis of total sugar in the samples varied between 70.030% and 88.355%, as shown on table 3, noting that there is no significant difference between the averages in samples coming from São Paulo (samples E, F, G, H, I), while samples A and D, both made in Mato Grosso, had significant differences between each other. However, this characteristic is not contemplated in the national legislation.

TABLE 3 – RESULTS OF THE ANALYSIS OF TOTAL SUGARS, REDUCING SUGARS AND SUCROSE APPARENT OF THE HONEY PURCHASED IN CUIABÁ – MT (%).

Samples	Total Sugars	Reducing Sugars	Apparent Sucrose
A	83.387 a	65.791 ab	20.886 a
B	79.545 ab	63.801 ab	18.933 ab
C	76.408 ab	62.088 ab	17.425 ab
D	70.030 b	57.716 b	15.200 b
E	86.171 a	68.498 a	21.098 a
F	88.355 a	71.287 a	20.632 a
G	82.653 ab	65.898 ab	20.050 a
H	85.128 a	70.440 a	18.209 ab
I	81.927 ab	66.338 ab	18.906 ab
J	83.741 a	66.598 ab	20.472 a

Means with different letters differ statistically ($p < 0.05$) in Tukey’s test.

The results of the reducing sugars content show a variation of 57.716% to 71.287% among the samples’ averages (table 3). The statistical test reveals that the sample D was the one that most significantly differs from the samples E, F and H, while 3 out of 4 samples from MT honeys (samples B, C and D) presented reducing sugars values of lower than 65% of the minimum content decreed by Brazilian legislation (BRAZIL, 2000), which marks them as deviant from the determined standard.

For the apparent sucrose, we observed a content interval from 15.200% to 21.098%, values that significantly single out samples D and E (table 3). The apparent sucrose quantity allowed by legislation for floral honeys is a maximum of 6%, and in honeydew honey and blends the maximum tolerated is 15% (BRAZIL, 2000). The results of the averages point that all samples are above the value established by current law. Sucrose concentration is a good criterion to differ monoflower honey from polyflower honey. A high content of sucrose can reveal a premature harvest, where the sucrose was not totally converted into glucose and fructose by the action of the invertase enzyme, or a possible adulteration of the product with commercial sugar (SCHLABITZ et al. 2010).

The levels obtained for pH and acidity of the samples varied from 2.79 to 4.82 and 15.90 to 32.71, respectively (table 4). It is noted that pH values in the samples of Mato Grosso are minor, whereas the three out of four MT samples – A, C and D – had pH levels below 4. While the others achieved pH values above 4.

TABLE 4 – RESULTS OF THE ANALYSIS OF PH ACIDITY ($MEQ.KG^{-1}$) AND ELECTRICAL CONDUCTIVITY ($MS.CM^{-1}$), OF THE SAMPLES OF THE HONEY PURCHASED IN CUIABÁ – MT.

Samples	pH	Acidity	EC[1]
A	2.79 i	15.900 e	262.433 b
B	4.19 e	27.383 abc	293.633 a
C	3.81 g	32.173 a	175.000 de
D	3.46 h	18.020 cd	99.176 g
E	4.03 f	20.846 bcd	136.533 f
F	4.82 a	17.490 cd	164.466 e
G	4.26 de	24.380abcd	202.400 c
H	4.34 cd	31.800 a	211.466 c
I	4.38 c	28.620 ab	189.600 cd
J	4.62 b	25.793abcd	285.833 ab

Means with different letters differ statistically ($p < 0.05$) in Tukey's test.
[1]Electrical Conductivity

Currently there are no indications of pH values in Brazilian legislation, therefore this is not a mandatory analysis for ascertaining honey quality. However, this measure is considered important to help assess quality, as it may indicate fraud and microbial contamination in the product, as it indicates state of conservation of honey, besides being a complementary parameter to verify total acidity (WELKE et al. 2008; FINCO et al. 2010).

The Codex Alimentarius (CODEX, 2001) determines the following pH values for honeys: 3.40 – 6.10, since honey acidity is interconnected with antimicrobial action, and large part of the microorganisms has great pH levels to aid growth in the range of 7.2 to 7.4. However, very low pH levels contribute to fungal growth and lower honey shelf life. Only sample A (2.79) showed pH levels below the minimum instituted by the Codex Alimentarius (CODEX, 2001), which can point to an occurrence of fermentation processes or adulteration in the product (GOMES et al. 2017).

Levels close to the ones found in this study were identified by Finco et al. (2010), when assessing values of pH between 3.35 and 4.50 in honeys from the southern region of Tocantins.

Besides temperature and storage time, pH levels are essential HMF forming speed and is directly connected to floristic composition in the collection points, as honey pH levels can be influenced by nectar pH levels, by different soil compositions, or by the association of vegetables species for the final honey composition (SILVA et al. 2009; FINCO et al., 2010).

Legislation allows for a maximum of 50 meq/kg^{-1}, in the acidity index; thus, we can see that all the acidity values obtained remain compliant with Brazilian legislation, making these products safe for consumption (table 4). According to Souza et al. (2009a), the pH and the acidity are important antimicrobial factors, providing not only stability to the product, but also development of microorganisms. Thus, acidity in honey is essential, because it noy only attributes chemicals and sensor characteristics, it also allows for stability in face of microbial growth (SILVA et al. 2009).

Borges et al. (2017) have found values of and meq.kg^{-1} in acidity analysis in honeys produced in Petrolina, and 30.1 to 69.9 meq.kg-1 in the honeys produced in Salvador. Two samples from the latter exceed the legal parameters, with 65.6 and 69.9 meq.kg^{-1} of acidity, respectively.

Honey is a food that cannot present signs of fermentation and acidity is a chemical characteristic that points to the deterioration process (SILVA et al. 2009). By means of gluconic acid, constituted through glucose by action of enzyme glucose oxidase, it tends to always increase, even during honey storage, because this enzyme remains in activity even after being processed. This way, acidity increases during storage and, consequently, the pH is reduced (FUJI et al. 2009).

As for electrical conductivity, the samples had a variation of 99.18 to 293.63 μS.cm^{-1}, having significant differences among them (table 4); however, all were within the limit of 800 μS.cm^{-1} established by the Codex Alimentarius (CODEX, 2001). This analysis is related to the ashes content, pH, acidity, protein, organic acids, mineral salts and other parameters, and is used to determinate the botanical origin of honeys. However, there are no values decreed by Brazilian legislation.

According to the study realized by Souza et al. (2009a), honeys from the same flower origin present similar conductivity, independently of geographical origin and weather conditions. Alves et al. (2005) evaluated 20 samples of honeys originated from São Gabriel – BA and obtained results between 267.5 and 462 μS.cm^{-1}, and an average of 352.25 μS.cm^{-1}.

Table 5 shows that the highest water activity was of 0.635 in sample A, being insufficient for the development of enteral pathogens, as bacteria need Aw > 0.91 to grow (BOBBIO and BOBBIO, 2001). Aw is not a parameter regulated by the Brazilian legislation, but it points at the possibility of microbial development. A high content of Aw in honey indicates shorter shelf life of the food and facilitates proliferation of bacteria, leading to a fermentation process that will render products unfit for consumption and commercialization.

However, when the samples present lower values, there can be xerophilic molds and osmotic yeasts (RIBEIRO et al. 2009). Besides, results close to the obtained in this study were found by Souza et al. (2009b) in trigonine honeys (Aw: 0.598 a 0.729), and in meliponine honeys evaluated by Lage et al. (2012), which had Aw values between 0.59 and 0.79.

TABLE 5 – RESULTS OF THE ANALYSIS OF THE WATER ACTIVITY, SOLUBLE SOLIDS (°BRIX) AND HYDROXYMETHYLFURFURAL (MG.KG^{-1}) OF THE SAMPLES OF HONEY PURCHASED IN CUIABÁ – MT.

Samples	AW[1]	°Brix	HMF[2]
A	0.635 a	75.266 c	Positive
B	0.575 c	75.966 bc	Negative
C	0.619 a	79.333 ab	Negative
D	0.609 ab	80.633 a	Negative
E	0.563 cd	75.600 c	Negative
F	0.629 a	75.966 bc	Negative
G	0.582 bc	78.666 abc	Negative
H	0.547 d	76.600 bc	Negative
I	0.579 c	75.433 c	Negative
J	0.578 c	78.200 abc	Negative

Means with different letters differ statistically ($p < 0.05$) in Tukey’s test.
[1]Water Activity
[2]Hydroxymethylfurfural

For soluble solids values, it can be noted in table 5 that there was a significant difference in brand D in relation to the others; in other words, product D presented a larger value of soluble solids (80.633 °Brix) than the other samples analyzed. Brix degree (ºBrix) points to the quantity, in grams, of solids that are dissolved in the water in a food. ºBrix analysis is very important for the agribusiness to control quality of the final product, process, ingredients and other items used in factories, such as: molasses, alcohol, sugar, liqueurs and beverages in general (CHITARRA and CHITARRA, 1990; CAMPOS et al. 2010).

Current legislation does not require ºBrix analysis in order to assess honey quality; however, this procedure was executed to add another comparison variable to the results. In the present study, we found contents oscillating between 75.26 and 80.63 ºBrix, and an average of 77.16 ºBrix. Authors Campos et al. (2010) found similar results for this parameter in samples of honey from Paraíba, ranging between 73.27 and 72.35 ºBrix for the city of Areia, and between 73.45 and 71.65 ºBrix for the city of Mamanguape.

The HMF test conducted was the qualitative type, highlighting positive results only for sample A (table 5). HMF is formed by the reaction of certain sugars with acids. Levels will increase with the increase of the temperature (overheating), longer storage, adulteration caused by addition of inverted sugar and corn and beet syrup, and is even influenced by honey acidity, pH, water and minerals. It is an indicator of honey quality, since its presence in high levels means less nutritional value through heating, which destroys vitamins and thermolabile enzymes (BARBOSA et al. 2014). Conversely, low HMF levels can be indicative of a recently picked honey, being also dependent on the species (NEVES et al. 2015; BORGES et al. 2017).

Qualitative HMF analysis is done through the Fiehe test and evince that, when the result is positive is due to the presence of HMF by Fiehe or Winkler reactions, showing adulteration in honeys by means of inclusion of syrups and commercial glucose, or overheating. A persistent red color marks a positive result or high quantities of HMF – possibly more than 200 mg/Kg (MENDES et al. 2009). Ribeiro et al. (2009), when researching honeys in Rio de Janeiro, reveal that 5 out of 10 clandestine samples analyzed indicated positive results, and 12% out of the 25 samples coming from inspected establishments presented faults; whereas in the study by Richter et al. (2011), only 2 out of 19 samples evaluated from the city of Pelotas had positive results for alteration/adulteration.

According to current Brazilian law, HMF analysis is mandatory in order to determine honey quality, allowing for a maximum of 60mg/Kg. Therefore, it would be interesting to conduct a quantitative analysis so as to measure HMF values in the samples of the present study, and compare them to the legislation (BRAZIL, 2000).

Color tests through the parameter CieL*a*b* presented results of luminosity (L*) with a variation from 3.03 to 5.93, a* from 0.39 to 10.52 and for b*, from 0.06 to 9.19 (table 6). This shows a great oscillation in the results between samples. Brand J has the highest value for L* (5.93), whereas brand A has the shortest result (3.03); similarly, in parameters a* and b*, since coordinate a*, concerning the color red (+), obtained the highest value in sample J (10.52), differently from sample A (0.34). Also, in the coordinate b*, corresponding to the yellow color, the same behavior was observed: brand J honey showed yellow coloration much more intensely (9.19) than brand A (0.06).

TABLE 6 – RESULTS OF COLOR PARAMETERS BY CIEL*A*B* SYSTEM OF THE SAMPLES OF THE HONEYS PURCHASED IN CUIABÁ – MT.

Samples	Color Parameters		
	L*	a*	b*
A	3.03 c	0.34 d	0.06 e
B	5.23 ab	2.00 cd	1.34 de
C	4.05 abc	3.98 bc	4.45 bc
D	5.49 a	8.31 a	8.28 a
E	4.71 abc	9.36 a	7.46 ab
F	5.63 a	2.34 bcd	3.09 cde
G	5.25 ab	9.24 a	7.87 a
H	3.27 bc	4.18 bc	4.20 cd
I	5.01 abc	4.97 b	4.03 cd
J	5.93 a	10.52 a	9.19 a

Means with different letters differ statistically ($p < 0.05$) in Tukey's test.

4 CONCLUSION

Physical and chemical analysis in honeys are necessary to secure reliability in quality of products sold in Brazil. This way, rigorous inspection and compliance to current legislation are fundamental criteria to avoid adulteration in these products and to protect the health of consumers. Due to the lack of diverse parameters in the determination of honey quality, it is important to create and implement rules to assist in IQS (Identity and Quality Standard) and in characterization of honeys produced in many different places, biomes and environmental conditions in Brazil.

RESUMO

O objetivo deste estudo foi determinar as características físico-químicas de méis vendidos em supermercados de Cuiabá – MT. As análises físico-químicas realizadas foram: cinzas, umidade, proteínas, açúcares totais, açúcares redutores, sacarose aparente, pH, acidez, condutividade elétrica, atividade de água, °Brix, hidroximetilfurfural e cor. Os resultados foram analisados estatisticamente utilizando a ANOVA e contrapostos entre si, através de teste de Tukey a 5% de confiabilidade. Comparou-se os resultados obtidos com os valores permitidos pela legislação brasileira vigente e com dados da literatura que verificaram méis em outras regiões do país. Todas as amostras analisadas estavam dentro dos parâmetros legais para umidade, cinzas e acidez, porém no quesito sacarose aparente todas se apresentaram fora do recomendado pelas normas. Já nas análises de proteínas, açúcares totais, pH, condutividade elétrica, A_w, ºBrix e HMF (qualitativo) não existem padrões brasileiros vigentes para determinar a qualidade dos méis. Portanto, conclui-se que é importante a implantação de parâmetros completos para auxiliar no PIQ (Padrão de Identidade e Qualidade) e na caracterização de méis produzidos nos mais diversos locais, biomas e condições ambientais do Brasil.

PALAVRAS-CHAVE: ABELHAS, AÇÚCAR, ADULTERAÇÃO, QUALIDADE, SACAROSE.

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