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Physicochemical and microbiological evaluation of honeys consumed by the elderly

Avaliação físico-química e microbiológica de méis consumidos por idosos

Evaluación fisicoquímica y microbiológica de mieles consumidas por ancianos

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ABSTRACT

Objective: to analyze physicochemical, microbiological and dirt parameters in marketed honeys, consumed by the elderly cared for at Integrated Health Center in Teresina, Piauí, Brazil. **Method**: the following analyses were performed: color, water activity, humidity, ash, pH, acidity, reducing sugars, total sugars, apparent sucrose and insoluble solids. Contamination indicator bacteria, mesophilic microorganisms, filamentous fungi and yeasts, as well as dirt and foreign matter, performed in the period from April to June 2016. **Results**: analyses of ash, pH, acidity and insoluble solids were outside current standards. Microbiological analyses did not present significant contamination. Also, analyses of dirt showed insect fragments, foreign matter in almost all the samples. **Conclusion**: parameters of ash, pH, acidity and insoluble solids, as well as dirt and foreign matter, indicated that the samples were not in accordance with current legislation.
Descriptors: Aged; Honey; Health; Microbiology.

RESUMO

Objetivo: analisar os parâmetros físico-químicos, microbiológicos e sujidades em méis comercializados, consumidos por idosos atendidos no Centro Integrado de Saúde em Teresina, Piauí, Brasil. **Método**: foram realizadas análises de cor, atividade de água, umidade, cinzas, pH, acidez, açúcares redutores, açúcares totais, sacarose aparente e sólidos insolúveis. Bactérias indicadoras de contaminação, micro-organismos mesófilos, fungos filamentosos e leveduras, além de sujidades e matérias estranhas, realizadas no período de abril a junho de 2016. **Resultados**: as análises de cinzas, pH, acidez e sólidos insolúveis estavam fora dos padrões vigentes. As análises microbiológicas não apresentaram contaminação significativa. Já as análises de sujidades mostraram fragmentos de insetos, matérias estranhas em quase todas as amostras. **Conclusão**: os parâmetros de cinzas, pH, acidez e sólidos insolúveis, além de sujidades e matérias estranhas, indicaram que as amostras não estavam em conformidade com a legislação vigente.
Descritores: Idoso; Mel; Saúde; Microbiologia.

RESUMÉN

Objetivo: analizar los parámetros fisicoquímicos, microbiológicos y de suciedad en mieles comercializadas, consumidas por ancianos atendidos en el Centro Integrado de Salud en Teresina, Piauí, Brasil. **Método**: se analizaron el color, la actividad del agua, la humedad, las cenizas, el pH, la acidez, los azúcares reductores, los azúcares totales, la sacarosa aparente y los sólidos insolubles. Bacterias que indican contaminación, microorganismos mesofílicos, hongos filamentosos y levaduras, así como suciedad y materias extrañas, llevadas a cabo de abril a junio de 2016. **Resultados**: los análisis de las cenizas, el pH, la acidez y los sólidos insolubles estuvieron fuera de los estándares actuales. Los análisis microbiológicos no mostraron contaminación significativa. El análisis de suciedad mostró fragmentos de insectos, materia extraña en casi todas las muestras. **Conclusión**: los parámetros de las cenizas, el pH, la acidez y los sólidos insolubles, así como la suciedad y la materia extraña, indicaron que las muestras no cumplían con la legislación vigente.
Descriptores: Anciano; Miel; Salud; Microbiología.

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INTRODUCTION

According to ordinance No. 9,013, from March 29, 2017, "honey is an alimentary product made by melliferous bees from flower nectar or secretions coming from living parts of plants or from excretions of plant-sucking insects that stay on living parts of plants that bees collect, transform, combine with own specific substances, store and let mature in honeycombs".[1]

Studies demonstrated that honey detains some functional and therapeutic applications, such as: curative, cicatrizing,[2] rehydrating, anti-inflammatory, energetic,[3] anticancer[4] and antibacterial[5] effect. In a study that analyzed the honey consumer profile by age group, authors identified that the elderly made use of this food seeking therapeutic purposes and also for following family tradition.[6]

Although honey contains structures that are benefic and have therapeutic purposes for health,[3] this food is not free from alterations in its structure and may suffer microbiological contaminations and physicochemical changes that must be evaluated to meet the quality standards required by legislation and consumer food safety.[7]

The Brazilian legislation declares that to honey, sugars and other elements that alter its original composition cannot be added.[8] However, the increase in product demand has led to adulteration problems, as addition of sucrose, such as corn syrups and inverted syrups. These habits generate an increase in studies for methods of detection in honey adulterations and quality control.[9]

Furthermore, honey may have its quality compromised due to failures regarding the extraction technology, handling, equipment to be utilized and mainly to the way of handling, processing, storage and conservation.[10] According to Normative Instruction No. 11 (IN-11), honey must be free from contamination by chemical, biological products and isolated particles transmitted through air, handlers or processing. Thus, this study is based upon investigation of genuineness of honeys sold in street markets and consumed by the elderly, since the preference of this public for this product is related to the belief that such food is pure when compared with industrial honey. This conviction is associated with traditions and customs perpetuated through generations.[8]

Concerning this matter, there is a continuous search for improvements in quality and purity of Brazilian honey as well as honeys sold in street markets. Although such mechanism is unusual, it is important to inform through analyses the whole creative process, from field to foundation. In this context, the present work had as objective to analyze the physicochemical, microbiological, dirt and foreign matter parameters in marketed honeys, consumed by the elderly cared for at Integrated Health Center in Teresina, Piauí, Brazil.

METHOD

It is a field, cross-sectional study with quantitative and descriptive approach. The study consisted of two fundamental variables: the participants, 44 elderly people cared for at Integrated Health Center – IHC, with 30 females and 14 males, and 44 samples of honeys consumed by them. The calculus of sample size was based on the number of elderly attendances provided in

IHC geriatrics sector, in which 11 elderly people are cared for per week. The sample was randomly defined through systematic sampling. Every two elderly people cared for at IHC, one was studied, totaling 44 elderly people in the period from April to June 2016. The calculus was performed with the following formula:

$$E = (1{,}64)^2 \times \frac{0{,}50 \times 0{,}50}{\sqrt{(44)}} = 0{,}1014 = 10{,}14\%$$

This sample size (n) has margin of error of 10.14% (E) with level of trust of 90% (E=1.64) and maximum variance P=0.50, Q=0.50. The statistical analysis of data was made using Chi-Square test and 5% of probability, with the results presented in mean, in minimum and maximum values.[11]

The 44 samples of honeys were directly acquired in participants' residences and consequently none had identification or labeling. The collected samples were placed in 240 mL plastic containers, previously sanitized with 70% alcohol, coded and conducted to Laboratory of Physicochemical Analysis of Food at Federal University of Piauí, Teresina, Piauí, Brazil.

The study had the consolidated opinion of Research Ethics Committee - REC: 1,476,832 and Certificate of Presentation for Ethical Consideration (CPEC): 53737116.9.0000.5210, approved on April 3, 2016, respecting the Resolution 466/12 from Brazilian National Health Council - NHC.

For honey color evaluation, a HANNA-branded colorimeter - model C221, Honey Color Analyzer, was used, with utilization of cuvettes, and the reading was directly obtained in the device display. Water activity (Aw) was determined utilizing the reading device (digital Decagon Pawkit), removing 10 g of each honey sample and placing them in containers coupled to the device, with direct reading in equipment panel.[12] Humidity analysis was determined by refractometric method, putting 2 honey drops on prism of bench refractometer model Biobrix; next, the reading was done. Honey refraction index (RI) value was converted in humidity (%), according to Chataway table.[13]

Ash content was performed in muffle (FANEM) model 412. 2 g of honey was weighed in a porcelain crucible and incinerated during 6 hours at 550°C. After this period, the ash content was weighed and calculated in percentage.[13] To measure the honey pH, a digital pH meter model Mpa-210P was utilized. Acidity determination was performed by titration with NaOH until it reaches pH 8.3 with use of digital pH meter Mpa-210P.[13]

Determination of reducing sugars was conducted weighing 2 g of honey and dissolving them in 30 mL of distillated water; next, the solution volume was completed for 100 mL in a volumetric flask. In each Erlenmeyer, the following were placed: 10 mL of water, 5 mL of Fehling A and 5 mL of Fehling B solution. The titration occurred under shaking for color changing (red brick).[14-15]

For analysis of total sugars, a 50 mL aliquot of honey remaining solution of analysis of reducing sugars was added in a 100 mL flask; next, 1 mL of concentrated HCl was added, and it was taken to water-bath for 15 minutes. After, the solution was kept at room temperature and neutralized with 5 M NaOH up to pH 7.0, under titration of Fehling A and B solution with boiling until red-brick color.[14-15]

For apparent sucrose determination, the subtraction of results of total sugars and reducing sugars was performed.[14-15]

Analysis of insoluble solids was performed with 20 g of honey diluted in 100 mL of distillated water, and heated at 60°C. Next, the solution was filtered in filter paper previously dried in chamber at 100°C, cooled in desiccator and weighed. Beaker and filter paper were washed with warm water until absence of sugars, they were taken to chamber, next to desiccator, and after they were weighed until reaching the constant weight. All the analyses were performed in triplicate.[13]

For microbiological analyses, sample weighing and dilution at 10^{-1} were performed, and after serial decimal dilutions up to 10^{-3} were prepared.

For analysis of contamination indicator bacteria, 1.0 mL of each dilution was inoculated in Lauryl Sulphate Tryptose (LST) broth as presumptive test at 37°C. The suspicious samples followed with the methodology for accurate results.[16]

In total count of aerobic mesophilic bacteria, 1.0 mL of each dilution was inoculated in Petri dishes, and Plate Count Agar (PCA) was added. After solidification of medium, the dishes were incubated in inverted position at 37°C for 24 to 48 hours. The counts were transformed into Colony-Forming Units (CFU/g) values.

In count of filamentous fungi and yeasts, 1.0 mL aliquots of each dilution were added in 20 mL of Potato Dextrose Agar (PDA) medium, incubated at 25°C for 5 days, for count.[17]

For study of dirt and foreign matter, 50 g of sample in beaker was weighed and diluted in 500 mL of water at 60°C, filtered in Büchner and read in magnifying glass (MICRONAL VM VMT), with 10 and 20 x magnification, of material trapped in filter paper, and slides were prepared by fixing coverslips using glycerin. The slide verification was made through microscope, and the identification of elements found in honey were registered through Scopelmage 9.0 software.[10]

RESULTS

The physicochemical results of honeys consumed by the elderly are presented in Tables 1 to 3 and in Figure 1.

Table 1: Physicochemical analysis of honeys consumed by the elderly cared for at Integrated Health Center. Teresina (PI), 2016. (n=44).

Parameters	Mean	Maximum	Minimum	Normative No. 11
Color (mm)	66.43	150.00	30.00	White to Dark amber
Water activity (Aw)	0.41	0.48	0.24	-
Humidity (%)	18.32	20.00	14.00	Max. 20%
Ash (%)	0.27	0.68	0.05	Max. 0.6%
pH	2.58	3.49	2.00	-
Acidity (mEq/kg)	70.28	105.93	33.17	Max. 50 mEq/kg
Reducing sugars (%)	71.50	77.27	68.19	Min. 65%
Total sugars (%)	76.44	80.32	71.77	-
Apparent sucrose (%)	5.00	9.57	1.07	Max. 6.0% (flower honey) and Max. 10% (honeydew)
Insoluble solids (%)	0.07	0.15	0.02	Max. 0.1%

Table 2: Microbiological analysis of honeys consumed by the elderly cared for at Integrated Health Center. Teresina (PI), 2016. (n=44).

Analyses	Mean	Maximum	Minimum
Coliforms at 35°C (MPN·g^{-1})	<3.0	<3.0	<3.0
Coliforms at 45°C (MPN·g^{-1})	<3.0	<3.0	<3.0
Mesophilic bacteria (CFU·g^{-1})	5.2×10^{2}	2.8×10^{3}	<10
Fungi and yeasts (CFU·g^{-1})	3.8×10^{2}	3.3×10^{3}	<10

Source: Direct research (2016).

Figure 1: Main images found: a) insect abdomen fragment; b), d), e) and h) insect leg fragment; c) insect thorax and abdomen fragment; f) insect fragment; g) piece of plastic; i) coal fragment; j) wood bark fragment.

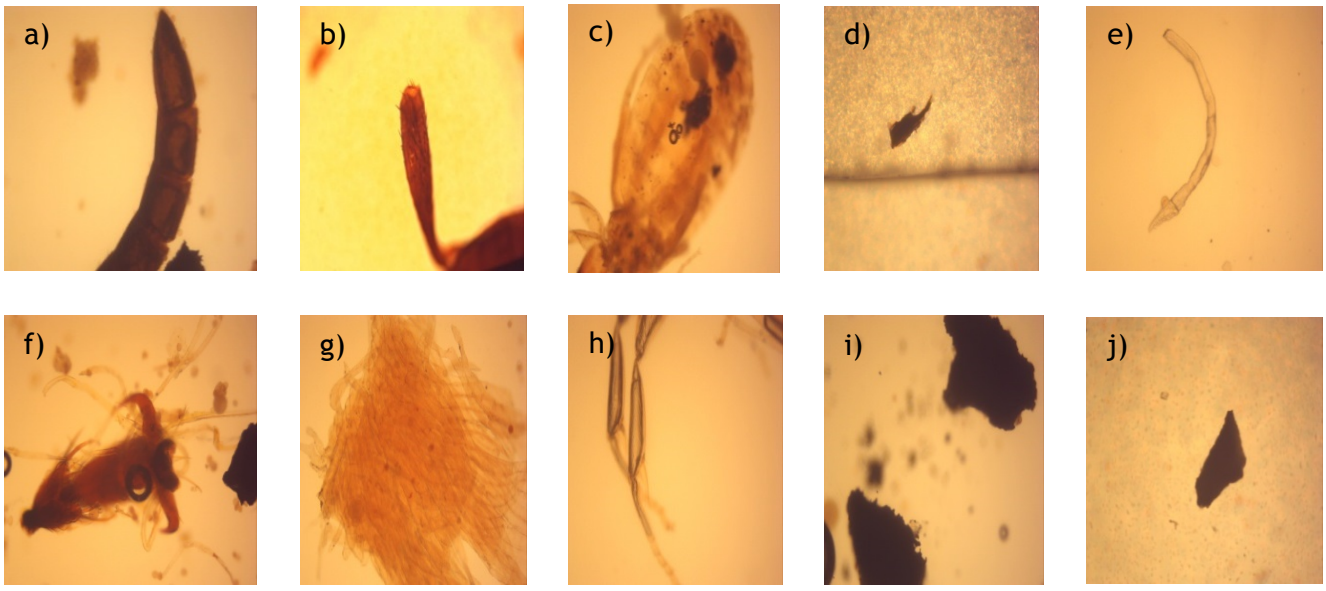

Table 3: Dirt and foreign matter analyses in honey consumed by the elderly cared for at Integrated Health Center. Teresina (PI), 2016. (n=44).

Dirt		No.	%
Foreign matter	Presence	40	90.91
	Absence	4	9.09
	Total	44	100.00

Source: Direct research (2016).

DISCUSSION

The honeys analyzed had light amber coloration, varying between 30 to 150 mm (Pfund scale), representing a 66.43 mean (Table 1), and it is classification parameter of sensorial characteristics.[8] The results found in this study were similar to a study of honeys in Piauí, also with predominance of light amber color.[18] The honey color reflects its composition, that is, the darker, the higher the content of mineral substances present.[19]

Honey color is associated with the origin in blossom, processing and storage, climatic factors and with ripening temperature of honey in hive. So, listing the coloration of honeys studied regarding the composition of minerals present, it is possible to realize that they have a reduced mineral content.[20-21]

The water activity (Aw) had 0.41 mean with a 0.24 to 0.48 variation (Table 1). Although it is a parameter not established by legislation, this variable is used to predict the microbial development as well as evaluate chemical reactions and shelf life of food. Authors found Aw values in honeys from semi-arid region of Piauí of 0.68 to 0.76 and 0.58 to 0.61, respectively.[18,21] The Aw critical value for microorganism multiplication is 0.60 for osmophilic yeasts and 0.65 for xerophilic filamentous fungi.[22] The Aw results of this study showed that honey does not favor microbial development.

As for the humidity content in honey consumed by the elderly, the mean found in this work was 18.32%, varying between 14.0 to 20.0% (Table 1). However, one sample reached the maximum limit of 20% established by legislation.[8] The ratios for humidity values above 20% may be related to improper storage conditions, resulting in humidity absorption of honey by environment.[19]

The mean of humidity of *Apis mellifera* honeys found by authors in State of Tocantins was 19.1% for wild honeys and 21.2% for eucalyptus honey.[19] By the results obtained in this study, the honey is in accordance with legislation.

About the ash result, three samples were not in accordance with legislation, presenting values above 0.6%.[8] The mean obtained was 0.270% (Table 1). With this result, it was possible to compare the dark amber color of some samples with the high ash content.

In a study evaluating the ash content in honeys of State of São Paulo, researchers found values of 0.072%, 0.125% and 0.279%, respectively.[19] Lower values of 0.01 and 0.03% were found in 10 samples from State of Paraíba.[23] Values above 0.6% of mineral residue show that there were irregularities in the process of obtaining honey, by beekeeper.[24]

The honey pH found in this study had 2.58 mean with variation between 2.00 and 3.49 (Table 1). This parameter shows the intensity of acidic or basic condition of a certain medium. The honeys should present values between 3.5 and 4.5 due to the presence of organic acids that contribute to form honey flavor and provide stability against microbial degradation.[25] Then, the honey analyzed showed values below the established one. This result shows that the honeys evaluated in this study may be adulterated, and it can cause health problems for consumer. pH is important for food quality assessment. On the other hand, in an experiment utilizing honeys in Botucatu/SP, 4.22 value was obtained.[26] Other researchers found pH values between 3.35 and 4.50.[27] In another study, 3.85 pH mean was found.[23]

The acidity values of honeys analyzed in this study presented a 70.28 mEq/kg mean with variation between 33.17 to 105.93 mEq/kg (Table 1). According to legislation, the maximum acidity limit for honey is 50 mEq/kg.[8] The high acidity may indicate the food deterioration due to fermentation by xerotolerant yeasts, which take advantage of favorable conditions of humidity and water activity to induce fermentation of honey sugars, causing a reduced pH.[18] The results of this study agree with possible deterioration or fermentation of sugars, since the pH acidity values were changed, and it can harm consumer's health. Also, climatic factors may be influenced in acidity of honey samples of this study, since Piauí has high temperatures. Another factor that may be related to the increased honey acidity is improper storage condition.[21] In acidity analyses of honeys evaluated in State of Rio de Janeiro, 30% of clandestine honeys were at odds with legislation and the values oscillated between 15 and 63 mEq/kg.[28] All values obtained in this work exceeded the maximum value (50 mEq/kg) allowed by current legislation for honeys.

The average value for reducing sugars found in this work was 71.50% (Table 1). According to legislation, the quantity of reducing sugars in honey must not be less than 65%.[8,20] *A. mellifera* honeys studied in State of Tocantins had 68.94 ± 3.65% mean of reducing sugars, with 62.70 to 76.20% variation.[27]

For the results of total sugars, this study presented a 76.43% mean (Table 1), with values that vary from 71.77 to 80.32%. For this parameter, there is no value established by Brazilian quality standards. As with these results, in a study, analyzing honeys in Londrina, 76.90 to 84.18% variation was obtained.[29]

The apparent sucrose of honeys analyzed in this study had a 5.00% mean (Table 1), with 1.07 to 9.57 variation. The maximum rates allowed by legislation are 5% of sucrose and 10% for mixture with flower honey. All values obtained in this study are in accordance with current legislation. These results corroborate those found by authors who analyzed honeys in Londrina, with obtained values of apparent sucrose varying between 2.76 to 9.78%.[29]

The average value for analysis of insoluble solids (IS) was 0.070 (Table 1). Nevertheless, 11 samples analyzed were above the limit established by legislation, which predicts the quantity of 0.1% for this parameter.[8] The sample values vary between 0.020 to 0.150%. IS in honeys indicate purity level and a possible inadequate processing of this product. The variation interval

proved to be similar to that observed by researches who found 0.013 to 0.192 values in honey samples in Paraíba/PB.[23] Other scholars also found high values in all the honey samples analyzed, 0.19 to 0.68%, and 0.10%, respectively.[30-31] The samples with results outside the limit established by legislation suggest that they have been improperly handled or adulterated.

In analyses of coliforms at 35°C and 45°C, values lower than 3.0 MPN·g^{-1} were observed in all the samples analyzed, demonstrating accordance with legislation.[8] Compared with results found by authors who analyzed bee honey (*A. mellifera*) produced in cooperatives in semi-arid region of Piauí, absence of coliforms at 35°C and 45°C was obtained in all the samples.[32] Similar results were obtained by other authors.[21,33] In this assessment, it was possible to conclude that all samples were with acceptable levels for these indicators.

In the analyses of count of aerobic mesophilic bacteria, the mean obtained was 5.2×10^2 CFU·g^{-1}. This assessment is utilized to estimate the sanitary quality of food even if no visible deterioration has occurred. The value found is quite low, possibly due to the product be considered antibacterial. Studies affirm that in most foods, counts greater than 10^6 CFU·g^{-1} are necessary to cause sensorial changes that are detectable in them.[22]

In fungi and yeasts count (Table 2), the average growth was 3.8×10^2 CFU·g^{-1}. These microorganisms can be found in honey through bees by production process, or also by environmental contamination. Similar values (2.6×10^2 CFU·g^{-1}) were found in a study that analyzed *A. mellifera* honeys. Authors reported that fungi and yeasts are commonly found in honey, due to the ability to endure low values of water activity and pH.[22,32]

Relating to the analyses of dirt and foreign matter in honey, it was possible to identify fragments of insects, wood bark, plastics and coal, presented in Figure 1.

These fragments were found in almost all the samples (90.91%), as presented in Table 3.

CONCLUSION

The verified nonconformities are derived from possible adulterations or contaminations during the process, since the honey processing, packaging and storage.

The microbiological analyses did not present contamination by coliforms at 35°C and 45°C, count of aerobic mesophilic bacteria and filamentous fungi and yeasts. The microscopic analysis showed insect fragments, foreign matter in almost all the samples, at odds with the current legislation, supposing thus that there was no proper production and handling process for the product.

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Does not apply.

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Does not apply.

CONFLICTS OF INTEREST
There are no conflicts of interest to declare.

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