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Physicochemical and hygienic-sanitary quality of honey marketed in Chókwè city

Qualidade físico-química e higiénico-sanitária do mel comercializado na cidade de Chókwè

Calidad fisicoquímica e higiénico-sanitaria de la miel comercializada en la ciudad de Chókwè

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Abstract
The present study aimed to evaluate the hygienic-sanitary conditions and the physical-chemical parameters of honey bees with emphasis on understanding the risks to health. The experiment was conducted in a completely randomized design with 5 samples, 3 from artisanal production (A, B and C) and 2 from industrial production (Youngs honey (D) and Goldcrest (E)). Analyzes such as titratable acidity by titration with 0.1N sodium hydroxide solution (NaOH), soluble solids content, refractometry, pH, potentiometry, humidity, gravimetry and ash by incineration were carried out for the physical-chemical characterization. The hygienic-sanitary characterization was performed for molds and yeasts using the Spread Plate method. Data were evaluated using the statistical software RStudio 4.2.1 at 5% significance of the Tukey test. The pH was fixed in the range of 3.28 to 4.30, soluble solids from 76.36 to 77.10 ºBrix, titratable acidity at the level of 4.14 to 6.10 meq/mL, moisture content of 12.07 to 17.12% and ash from 0.29 to 4.34%. The microbiological characterization showed the presence of molds in the range of 1.5x10 to 4.9x10. On the other hand, an uncountable load was verified in the yeast count in samples A and B, exceeding the limit established by the Technical Regulation of Quality and Identity of Honey. In terms of physical-chemical properties, no significant differences were found between artisanal and industrialized honey. The level of mold in all samples met recommended standards for honey and there was evidence of compliance with good manufacturing and hygienic-sanitary practices.
Keywords: Honey; Physicochemical analyses; Quality control.

Resumo
O presente estudo teve como objectivo avaliar as condições higiênico-sanitárias e os parâmetros físico-químicos do mel de abelhas com ênfase a compreender os riscos à saúde. O experimento foi conduzido em Delineamento Inteiramente Casualizado com 5 amostras, sendo 3 de produção artesanal (A, B e C) e 2 de produção industrial (*Youngs honey* (D) e *Goldcrest* (E)). Foram realizadas análises como acidez titulável por titulação com solução de hidróxido de sódio (NaOH) 0,1N, teor de sólidos solúveis, refratometria, pH, potenciometria, humidade, gravimetria e cinzas por incineração para a caracterização físico-química. A caracterização higiênico-sanitária foi realizada para bolores e leveduras por meio do método *Spread Plate*. Os dados foram avaliados por meio do *software* estatístico RStudio 4.2.1 a 5% de significância do teste de Tukey. O pH foi fixado na faixa de 3,28 a 4,30, sólidos solúveis de 76,36 a 77,10 ºBrix, acidez titulável no nível de 4,14 a 6,10 meq/mL, teor de humidade de 12,07 a 17,12% e cinzas de 0,29 a 4,34%. A caracterização microbiológica mostrou a presença de bolores na faixa de 1,5x10 a 4,9x10. Por outro lado, foi verificada carga incontável na contagem de leveduras nas amostras A e B, ultrapassando o limite estabelecido pelo Regulamento Técnico de Qualidade e Identidade do Mel. Nas propriedades físico-químicas, não foram verificadas diferenças significativas entre o mel artesanal e o industrializado. O nível de bolores em todas as amostras atendeu aos padrões recomendados para o mel e houve indícios de conformidade com as boas práticas de fabricação e higiênico-sanitárias.
Palavras-chave: Mel; Análises físico-químicas; Controle de qualidade.

Resumen
El presente estudio tuvo como objetivo evaluar las condiciones higiénico-sanitarias y los parámetros físico-químicos de las abejas melíferas. El experimento se realizó en un diseño completamente al azar con 5 muestras, 3 de producción

artesanal (A, B y C) y 2 de producción industrial (Youngs honey (D) y Goldcrest (E)). Se realizaron análisis como acidez titulable por titulación con solución de hidróxido de sodio (NaOH) 0,1 N, contenido de sólidos solubles, refractometría, pH, potenciometría, humedad, gravimetría y cenizas por incineración para su caracterización físico-química. La caracterización higiénico-sanitaria se realizó para mohos y levaduras mediante el método Spread Plate. Los datos se evaluaron utilizando el software estadístico RStudio 4.2.1 al 5% de significación de prueba de Tukey. El pH se fijó en el rango de 3.28 a 4.30, sólidos solubles de 76.36 a 77.10 ºBrix, acidez titulable al nivel de 4.14 a 6.10 meq/mL, contenido de humedad de 12.07 a 17.12% y cenizas de 0.29 a 4.34%. La caracterización microbiológica mostró la presencia de mohos en el rango de 1.5x10 a 4.9x10. Por otro lado, se verificó una carga incontable en el conteo de levaduras en las muestras A y B, superando el límite establecido por el Reglamento Técnico de Calidad e Identidad de Miel. En cuanto a las propiedades físico-químicas, no se encontraron diferencias significativas entre la miel artesanal y la industrializada. El nivel de moho en todas las muestras cumplió con los estándares recomendados para la miel y se evidenció el cumplimiento de buenas prácticas de manufactura e higiénico-sanitarias.
Palabras clave: Miel; Análisis fisicoquímicos; Control de calidad.

1. Introduction

Honey is produced naturally by *honey bees* and is obtained from the nectar of flowers, secretions from living parts of plants or excretions of sucking insects. It is composed of a solution of concentrated sugars, mainly glucose and fructose, which directly influence its sweetness, in addition to having enzymes (invertase, amylase and glyco-oxidase), amino acids, organic acids, pigment minerals and pollen grains (Ribeiro & Starikoff 2018).

Honey is an organic food of great nutritional value. Its composition contains sugars, water, mineral salts, vitamins and other nutrients (Albuquerque, 2021). Honey is a liquid, viscous, aromatic and sweet food product (Silva *et al*., 2021).

Honey is appreciated for its characteristic flavor and considerable nutritional value, its price is relatively high and it is considered one of the purest foods in nature, which often encourages its adulteration (Souza, 2012).

Honey is a natural product of primary botanical origin, resulting from metabolic processes in the body of bees, which transform the nectar and sugary secretions present in parts of the plants into a viscous fluid with an aroma and variable flavors. It is a product that has been gaining more and more space in the consumer market, due to its therapeutic and functional properties (Freitas *et al*. 2022).

Honey has been used by man as food and enjoyed since ancient Greece, medicine and offering to the gods. There are reports of the use of honey as a medicine in Egyptian papyri from about 1500 BC, where honey was in the composition of hundreds of prescriptions for external and internal use. In ancient Greece and Babylon, honey was still employed to preserve the bodies of kings and generals killed in battle until they were transported to the funeral. In ancient Egypt, it was also used as an offering in religious ceremonies, with the Israelites earmarking the honey from their first harvests to give to God (Gois, 2013).

The standards of identity and quality of honey require, in terms of macroscopic and microscopic aspects, that the product be free from foreign substances of any nature, such as: insects, larvae, grains of sand and others. Because honey is a highly appreciated product and easy to adulterate, it becomes the target of actions that alter its quality. Therefore, it is necessary some analyses to determine its quality, so that it can be marketed (Lima *et al.*, 2020).

The quality control of honey production is crucial, making it essential to comply with good hygiene practices by producers, as well as the use of an appropriate place for the manufacturing and extraction of honey. Thus, it is important to diagnose the quality of honey, in order to direct support activities, and to assist in the development of small and large producers. These activities should prioritize the control of the entire honey production chain, from the field to its commercialization, in addition to guiding public managers for planning and actions that contribute to the monitoring of quality and guarantee of a safe product (Pires, 2011).

In Chókwè city it’s found that honey is packed in polyethylene terephtate (*PET*) and glass bottles to facilitate its commercialization. Taking into account that each trader distinguishes himself by presenting his sales procedures, without the knowledge of the microbiological and physicochemical quality of his product which has different physical and chemical properties. This research aimed to characterize the physicochemical and hygienic-sanitary qualities of honey marketed in the Chókwè city.

2. Methodology

2.1 Area of study

The present study was conducted in the laboratory of the Higher Polytechnic Institute of Gaza (ISPG), in Chókwè district. According to MAE (2014), this district is found in the south of the Gaza province, in the middle course of the Limpopo River, having as limits to the North the Limpopo River that separates it from the Massingir, Mabalane and Guijá districts, to the South the district of Bilene and the Mazimuchope river, Chibuto and Xai-Xai, to the East it borders the with Bilene and Chibuto districts and to the West with the districts of Magude and Massingir.

2.2 Sampling

3 samples of artisanal honey (A, B and C) and another 2 industrial production of *brand Youngs honey* (D) *and Goldcrest* (E), packed in glass bottles of 200 mL, were randomly acquired in the markets of the Chókwè city. The samples were coded according to the collection point and were taken to the hygiene and food quality laboratory of ISPG for microbiological and physicochemical analyses.

2.3 Physicochemical analysis

The potential of hydrogen (pH), total soluble solids (ºBrix), titratable acidity, moisture content and ash content were evaluated, following the methods described by IAL (2008) and AOAC (2016).

2.3.1 Determination of Hydrogen Potential (pH)

It was performed by the potentiometric method, where 5g of honey were weighed, diluted in 50mL of distilled water and homogenized manually until the sample was uniformly suspended. Finally, the pH value was read after the electrode of the pHmeter, model HANNA (*HI* 2212 pH/ORP Meter), previously calibrated by buffer solutions 7 and 10, had been emerged into of sample.

2.3.2 Determination of titratable acidity

It was done by titration. For this purpose, 5g of honey were homogenized in a 250mL beaker, containing 50mL of distilled water with subsequent addition of 3 drops of phenolphthalein solution. Titration was performed with 0.1N sodium hydroxide (NaOH) solution in a 50mL burette until the indicator turning point was reached. The titratable acidity was determined by equation 1.

$$\text{Acidity tiratable } \frac{VxFxM\,(0,9)}{P} \quad (1)$$

Where:

V - n° of mL of the sodium hydroxide solution expended in the titration;

F - Correction factor of the sodium hydroxide solution;

P - Mass of the sample in g or volume in mL;

M - Molarity of sodium hydroxide solution.

2.3.3 Determination of moisture content

The percentage of moisture was determined in triplicate by the gravimetric method by use of heat. In an analytical balance, 5g of honey was weighed in a petri dish and placed in an oven (EcoTherm brand) with circulating air, with a temperature of 105ºC for 2 hours after which, the plates were cooled to room temperature for 30 minutes and again weighed. Expression 2 was used to determine moisture content.

$$\text{Moisture content } (\%) = \frac{Pi - Pf}{Pi} * 100 \quad (2)$$

Where:

Pi - Initial weight of the wet sample;

Pf - Final weight of the dry sample.

2.3.4 Determination of soluble solids

A portable digital refractometer (ATAGO), previously calibrated with distilled water, was used. Then the sample was placed directly in the prism of the apparatus and the refractive index of the honey was obtained.

2.3.5 Ash determination

On an analytical balance, 5g of the sample was weighed in porcelain crucibles and placed in a muffle at 550°C until complete incineration of the organic matter into inorganic, shown by white powder, was verified. The crucibles were then transferred to an oven at 105ºC for 30 minutes with emphasis on lowering the temperature, followed by weighing them with the sample incinerated in inorganic matter. Equation 3 was used to determine the percentage of fixed mineral residue.

$$\text{Ash } (\%) = \frac{m2 - m}{m1 - m} * 100 \quad (3)$$

Where:

m - Weight of empty crucible;

m_1 - Weight of crucible with sample;

m_2 - Weight of crucible with ashes.

2.4 Microbiological analysis

Microbiological analyses were done in order to determine the hygienic-sanitary quality according to Silva *et al.,* (2007) and in triplicate mold and yeast

2.4.1 Sample preparation, culture media and dilutions

Honey samples were aseptically homogenized and left ready for microbiological analysis. For the present experiment, (i) peptone water was prepared by diluting 16.09 grams in 1000 mL of distilled water, and (ii) BHI agar obtained by diluting 52 grams in 1000 mL of distilled water. All the previous media were sterilized at a temperature of 100ºC for 20 minutes.

To obtain the different dilutions, the main dilution of each sample was prepared by homogenizing 6mL of honey in 56 mL of peptone water. From the mother solution, serial decimal dilutions were made to obtain concentrations 10^{-1} to 10^{-3} by adding 1 mL to each test tube containing 9 mL of peptone water.

2.4.2 Determination of molds and yeasts

The mold and yeast count was performed by the *Spread Plate* method, using dilutions of (10^{-1} to 10^{-3}). Then, aliquots of 1 mL of each dilution were inoculated into Petri dishes containing BHI agar, spreading the inoculum with a *Drigalski* loop, starting from the highest dilution plates to the lowest dilution until all excess liquid was absorbed. The plates were incubated in a digital oven at 25°C for 5 days, and then the existing colonies were counted by counting the colony-forming units per milliliter (CFU/mL). The results of molds and yeasts were calculated by expression 4:

$$N = \frac{\sum c}{Vx1,1xd} \quad (4)$$

Where:

$\sum C$ - Sum of the colonies counted in the two plates retained from two successive dilutions, at least one of them contains a minimum of 10 colonies;

V - Volume of inoculum placed in plate in milliliters;

d - Retained dilution [d=1 when the undiluted liquid product (test sample) is retained].

2.5 Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model (GLM) using the RStudio statistical package version 4.2.1. In the case of significant effects, the difference of the means of the experimental units was evaluated by Tukey's test at the level of 5%.

3. Results and Discussion

3.1 Physicochemical analysis

The physicochemical characterization of honey samples from bees is shown in Table 1.

Table 1 - Physicochemical characteristics of handmade and industrially produced honeys marketed in Chókwè.

Sample	Parameters				
	pH	**Total soluble solids (°Brix)**	**Titratable acidity (meq/mL)**	**Moisture (%)**	**Ash (%)**
A	3.28±0.03^{c}	76.40 ±0.10^{b}	6.10±0.17^{a}	17.12±1.59^{a}	1.33±0.09^{c}
B	3.73±0.01^{b}	76.36±0.11^{b}	4.14±0.54^{b}	12.07±0.20^{b}	4.34±0.48^{a}
C	4.30±0.04^{a}	76.46±0.30^{b}	4.92±0.37^{ab}	16.20±0.13^{a}	3.14±0.22^{b}
D	3.28±0.16^{c}	77.10±0.17^{a}	4.86±0.18^{b}	15.03±0.72^{ab}	0.53±0.30^{d}
E	3.54±0.31^{bc}	76.46±0.30^{b}	4.26±0.72^{b}	14.57±1.94^{ab}	0.29±0.05^{d}

Means ± standard deviation followed by the same letter in the same column do not show significant differences at Tukey's 5% level. A, sample acquired in Senta-baixo 1 market; B, sample acquired in Senta-baixo 2 market; C, sample acquired in central market; D, *Youngs* honey brand industrial production and E, Goldcrest brand industrial production honey. Source: Authors.

The results of the physicochemical characterization of honey marketed in Chókwè city are shown in Table 1, highlighting the differences between them, samples (A, B and C) of artisanal production and Youngs honey (D) and Goldcrest (E) industrially produced.

3.1.1 pH

The evaluated samples indicated pH values ranging from 3.28 to 4.32. A higher pH value (4.32) was observed in sample C, statistically different ($p < 0.05$) from the others. This significance is probably related to the conditions of storage and commercialization, concerning that it was exposed to atmospheric conditions, something not observed in the others. Another aspect may be related to the constitution of each sample, considering that Souza (2017) refers that the presence of organic acids, such as acetic, butyric, citric, formic, lactic, malic, pyroglutamic, succinic, but mainly the gluconic that represents 70 to 90%, influence the decrease in pH promoting the increase in acidity levels.

Results in agreement with those obtained in sample B and E of the present study were reported by Almeida (2002) in his research seeking to analyze honeys from remaining areas of cerrado where he obtained pH in the range of 3.7 to 4.45, and by Arruda (2003), when conducting his study on the physicochemical and pollen characteristics of honey samples from bees, when he obtained a pH value in the range of 3.58, and also by Barth *et al.* (2005) that obtained pH values in the range of 4.3, when determining physicochemical parameters and the botanical origin of honeys. Results close to those obtained in the present study were reported by Bendini & Souza (2008) when evaluating honeys from cashew blossoms, reporting pH in the range of 3.67, and raújo *et al*. (2006) in his study about the evaluation of the physicochemical quality of honey marketed in the city of Crato, indicating pH values around 3.45 to 3.70, and by Albuquerque *et al.* (2021) when studying about quality analysis of bee honey commercialized with and without inspection in Brasília, found pH around of 3.51 to 3.70, and also by Freitas *et al.* (2022), studying physicochemical characterization of honey produced in the municipality of Cachoeira do Arari Ilha de Marajó, Pará, found pH around of 3.54.

Lower results than those obtained in the present study were reported by Azeredo *et al.* (2003) when developing his study on protein content and physicochemical properties in bee honey samples of different origins when they obtained pH in the range of 3.10 to 4.05, and by Finco (2010) when studying physical and chemical properties of honey from bees when the values ranging from 3.40 to 4.20, and also by Castro *et al.* (2022), obteined 3.40, when Determination of the physicochemical properties and melissopalinological constitution of the honey from *Melipona (Michmelia) paraensis* Ducke (Jandaíra) from Mojuí dos Campos – PA, results below those obtained in sample A, D and C of the present study. Similarly, Marchini (2004), when carrying out his study on the physicochemical composition of honey samples from bees, obtained a pH of 3.26. This is possibly associated, Azeredo (2007), with the different concertations of acids and the production of hydroxymethylfurfural in honeys of floral origin.

Higher results to those obtained in the present study were reported by Vargas (2006) in his research on the quality of honey produced in the general fields region of Paraná, when it was obtained pH in the range of 5.35, and by Silva *et al.* (2008), when studying the determination of hydroxymethylfurfural in honeys, who obtained a pH of 4.70 and also by Sodré *et al.* (2007), studying the microbiological content of honeys from the states of Ceará and Piauí, when they obtained pH in the range of 3.78, and Schmitz et al. (2022), addressing the analysis of honey from different species of bees, obtained pH around 3.97 to 4.71. These differences may be correlated with the floristic composition in the honey collection areas, considering that Filho (2011) maintains that the pH of honey can be influenced by nectar.

3.1.2 Total soluble solids

The total soluble solids ranged from 76.36 to 77.10 ºBrix, and sample D was statistically different ($p < 0.05$) from the others. This range of values can be considered acceptable assuming that the high content of total soluble solids is directly proportional to the composition of the honey that according to Khan *et al.* (2007) has about 80% sugars.

In the evaluation made by Santos *et al.* (2010), on physicochemical and microbiological aspects of honey commercialized in the tabuleiro city, it was reported soluble solids content around 76.14 ºBrix, a result in agreement with those in samples A, B, C and E of the present study. Similarly, Barbosa *et al.* (2014), reported soluble solids content in the range of 77.50 °Brix when developing their research on biochemical study of quality of bee honey marketed in the municipality of Caraúbas - RN, results in line with those found in the present study.

Divergent results from those of this study were referenced by Sant'ana (2017), in his research on the physicochemical and microbiological characterization of the honeys of *melipona subnitida* and *melipon*a from the state of Piauí, whose values ranged from 67.20 to 78.80 ºBrix and by Oliveira and Santos (2011), in their study on the physical-chemical analysis of Africanized and native bee honeys, who found soluble solids content around 78.58 °Brix. Gois *et al.* (2015) highlighted soluble solids around 70.83 to 81.50 ºBrix in their research on physicochemical and microbiological study of *apis mellifera* honey marketed in the state of Paraíba. Damasceno (2012) when developing his research on physicochemical analysis of honey from bees marketed in the municipality of Ariquemes-RO, reported soluble solids content around 80 to 80.10 ºBrix, and Silva (2022) studying quality of honey marketed in the region of Barbacena – MG, found soluble solids around 78.6 to 87.4 ºBrix, results above those obtained in the present essay.

Lower results than those obtained in the present study were also reported by Souza *et al.* (2016), when studying honeys produced by the bees M. *subnitida and M. scutellaris* in the states of Rio Grande do Norte and Paraíba, indicating total soluble solids content around 71.1 to 74.7 ºBrix, and by Campos *et al.* (2010), who obtained 72 ºBrix when evaluating the physicochemical parameters of honey from *melipona scutellaris* bees produced in the state of Paraíba, and by Souza *et al.* (2006) when conducting his research on honey from stingless bees in South American countries, who obtained soluble solids contents ranging from 57.5 to 75 ºBrix, and by Schmitz *et al.* (2022), who found 71, 10 to 73,22ºBrix quando análisava mel de diferentes especies de abelhas.

3.1.3 Titratable acidity

A higher level of acidity was observed in sample A, around 6.10 meq/mL, and sample C with 4.92 meq/mL, which were statistically different ($p \leq 0.05$) from the others. These differences may be related to multifloral origin of nectar. Another directly associated factor may be the lack of nectar source control, which explains the variation in acidity values between samples.

With higher values than those found from the present study, Souza *et al.* (2012), evaluating the physicochemical parameters of honey samples from the middle Araguaia region, reported acidity around 21.2 to 35.6 meq/mL, Bertoldi *et al.* (2004), in their study about the physicochemical characteristics of honey from Africanized bees, found acidity levels around 31.60 meq/mL and Castro *et al.* (2022), obteined 27.80meq/mL, when Determination of the physicochemical properties and melissopalinological constitution of the honey from *Melipona (Michmelia) paraensis* Ducke, Albuquerque *et al.* (2021), reported 22.5 to 44.00meq/mL, researching quality analysis of bee honey commercialized with and without inspection in Brasília, found pH around of 3.51 to 3.70, Gois *et al.* (2015), in their research on physicochemical and microbiological study of honey, referenced acidity values from 15.0 to 85.33 meq/mL where 22 samples were considered outside the standards recommended for acidity by the legislation that regulates honey.

Thus, Chaves *et al.* (2015), evaluating the quality of honey sold in free markets, met acidity of 42 meq/mL, as well as Welke *et al.* (2008), in the physicochemical characterization of honeys, highlighted acidity of 30.1 meq/mL of flowering honeys. In turn, Ribeiro and Starikoff (2019), when evaluating the physicochemical and microbiological quality of honey, reported total acidity values ranging from 19.76 to 63.35 meq/mL, and 3 samples presented higher values, with averages of 54.17, 57.16 and 63.35 meq/mL, and Dias *et al.* (2009) when performing the physicochemical characterization of honey samples, obtained values around 17.69 to 52.57 meq/mL. These differences, according to Souza (2012), are possibly associated with factors such as the variation of organic acids caused by the different sources of nectar, enzymatic activity of glucose-oxidase that originates gluconic acid, action of bacteria during maturation and minerals present in its composition.

3.1.4 Moisture

All samples presented moisture contents considered acceptable by the Brazilian legislation, Brasil (2014), which imposes the maximum limit of 20% in honeys. Higher content (17.12%) was observed in sample A, statistically different ($p \leq$

0.05) from sample B. These differences may be allied with the environment in which the samples were produced, because according to Alvim (2004), the relative moisture of the air directly influences the honeys and the moisture content tends to absorb and, or lose water due to its hygroscopicity.

Similar results were referenced by Ribeiro & Starikoff (2019), 14.3%, when conducting their study on the physicochemical and microbiological quality of honey, Ritcher *et al.* (2011), 15.4%, in their study on the physicochemical quality of honey, Filho *et al.* (2011), highlighted moisture content around 15.92% in their study on physicochemical evaluation of honeys marketed in some cities of the state of Mato Grosso do Sul, Albuquerque et al. (2021), in his study about quality analysis of bee honey commercialized with and without inspection, obtained moisture around 15.83 to 17.24%, and by Vieira et al. (2023) when evaluating physicochemical and botanical characterization of honey from stingless bess (Meliponini), occurring in the Taquari Valley – RS, found moisture values around 16.37%, and also by Dantas et al. (2022) analyzing the physical-chemical characterization of bee honey sold in the municipality of Frei Martinho-PB, found a moisture content of around 17.2%, results in agreement with those obtained in the present study.

Higher results to those found in the present study were also reported by Gomes *et al.* (2015) in their research on physicochemical evaluation of honeys produced in the municipality of Soure-Marajó, having obtained moisture content of 18.26%, and by Oliveira & Santos (2011) in their study on the physicochemical analysis of honeys from bees, where they found moisture of 19.07%, and by Moraes *et al.* (2014), when they obtained around 19.22 and 19.53% in their research seeking to perform the physicochemical characterization of honey samples from two municipalities, and by Freitas *et al.* (2022), studying physicochemical characterization of honey produced in the municipality of Cachoeira, found 20.47% of moisture, and also by Silva (2022) found moisture around of 18 to 19%, when evaluating his study about the quality of honey marketed in the region of Barbacena - MG. These differences may be understood considering that Okaneku *et al*. (2020) say that relative moisture of the air at 60% directly influences honeys with 18% moisture tend to absorb water.

3.1.5 Ashes

Ash contents ranged from 0.29 to 4.30%. Samples A, B and C showed significant differences ($P \leq 0.05$) between them. On the other hand, samples D and E were not statistically different ($P > 0.05$). Farias *et al*. (2021) consider that honey ashes can vary depending on the botanical origin of the flower, geographical location, expressing the food richness in minerals.

A result in agreement (1.3%) with this study was referenced by Souza *et al.* (2012), when studying the honey of small producers of the middle Araguaia-Tocantins valley. Damasceno (2012) reported ash value of 0.27%, when developing his research on physicochemical analysis of honey from bees marketed and Souza *et al* (2021) who analysis of honey samples sold in open-air markets in the city of Barreiras-Bahia, found ashes around 0.27%, and also by Filho *et al.* (2014) in their study on physicochemical aspects and quality of bee honey marketed in the municipality of Pombal-PB found 1.41% of mineral residue.

Higher contents, 0.36 to 0.58%, of ashes were reported by Santos (2010), studying physicochemical and microbiological aspects of honey marketed in the Tabuleiro city, and also by Rodrigues *et al.* (2012), 1.36%, evaluating the physicochemical parameters of honey samples from the middle Araguaia region. On the other hand, lower results (0.01 to 0.48%) were mentioned by Flangini (2016) in his study on bromatology of honey marketed in the city of Rio Branco - Acre, and by Araújo *et al.* (2006), evaluating the physicochemical quality of honey marketed in Crato city, with ashes around 0.06 to 0.24%, and also by Alves *et al.* (2011), evaluating Africanized bee honey, reporting mineral residue content of 0.04 to 0.26%. Likewise, Souza (2017) reported results in the range of 0.0009 to 0.0053% when performing the physicochemical and microbiological characterization of honey produced in the rural territory of Emas-Goiás. Filho *et al.* (2011) highlighted ash contents ranging from 0.10 to 0.20%, studying the physicochemical properties of honeys commercialized in some cities of the state of Mato Grosso do Sul, Silva (2022) in his study about the quality of honey marketed in the region of Barbacena – MG, reported ashes content around 0.10 to 0.20%, results lower than those from the present study. Finco *et al.* (2010), evaluating the physical and chemical properties of honey from bees, obtained 0.14%, and also by Freitas *et al.* (2022), when analyzed physicochemical characterization of honey produced in the municipality of Cachoeira do Arari Ilha de Marajó, reported ashes around of 0.19%.

3.1.6 Microbiological Analysis

Table 2 shows the microbiological analyses results of the honey samples sold in Chókwè city. According to the acceptance criteria for microbiological analysis of fungi and yeasts contained in ADAB Ordinance nº. 207, Brazil (2014), the count should not exceed 1.0x10^2 colony-forming units per gram (CFU/g).

Table 2 - Microbiological count in artisanal and industrial samples of honeys commercialized in Chókwè city.

Sample	Parameters	
	Moulds (CFU/mL)	**Yeasts (CFU/mL)**
A	4.9x10	Countless
B	2.7x10	Countless
C	1.5x10	4.6x10
D	1.5x10	5.6x10
E	2.3x10	2.7x10

A, sample acquired in Senta-baixo 1 market; B, sample acquired in Senta-baixo 2 market; C, sample acquired in central market; D, Youngs honey brand industrial production and E, Goldcrest brand industrial production honey. Source: Authors.

Microbiological characterization of honey marketed in Chókwè city are shown in Table 2, highlighting the differences between them, (A, B and C) samples of artisanal production and and Youngs honey (D) and Goldcrest (E) industrially produced.

3.1.7 Moulds and Yeasts

The molds CFU/mL ranged from 1.5x10 to 4.9x10. This rang is acceptable according to the microbiological criteria regulated in the identity and quality of bee honey indicated by Brazil (2014). This microbial load, within the recommended, can be associated with good manufacturing practices.

On the other hand, yeasts ranged from uncountable to 5.6x10 CFU/mL, being above of 104 CFU/mL, recommended by the brazilian legislation (ANVISA, 2022) as maximum for those microbiological. This uncountable load may be related to the non-observance of good hygienic-sanitary practices at the time of production. This fact, as say Andrade *et al.* (2019), is related to hygienic-sanitary deficiencies during the manufacturing process.

Agreeing with the results of the present study, Almeida and Barion (2007), when evaluating the microbiological quality of honey samples produced by five species of stingless bees, reported mold and yeast counts between 1.50×10 and 1.58×10, respectively. Lower than 1.0×10 CFU/mL for molds and yeasts were found by Finola *et al*. (2007) in their related with chemical and microbiological characterization of honey. Denardi *et al.* (2005), when evaluating the water activity and the contamination of honey by molds and yeasts, verified less than 15 CFU/mL of molds and yeasts in 98.33% of the samples evaluated, and also by Damto *et al* (2022) studying physicochemical and microbiological characteristics of honey produced by bees, obtained 2x10CFU/mL of molds and 2.5x10 CFU/mL of yeasts.

In the evaluation made by Flangini (2016), in the research on bromatology of honey, referenced uncountable molds and yeasts, results similar to those obtained in samples A and B of the present study. Similarly, Wenzel (2012) reported above

100 CFU/mL for yeasts in his research evaluating the microbiological quality of uninspected honey. On the other hand, Matos *et al.* (2012) get 71.9x10^2 CFU/mL of yeasts researching the microbiological quality of *melipona sp.* honey, higher than those found in this present study.

Alves (2008), developing his work on flora identification and characterization of organic honey and Africanized bees, observed yeast 3.8x10^1 CFU/mL, close to those of this essay. However, this microbial load, considering Goias (2013), may be associated with the glucose-oxidase enzymatic reaction that infers in the production of gluconic acid and hydrogen peroxide being one of the main factors for microbial activity of honey.

4. Conclusion

No significant differences were observed between artisanal honey and industrial one in terms of physicochemical properties. All honey samples sold in Chókwè city met the microbiological standards recommended by the legislation regarding to molds. Artisanal samples have shown uncountable yeasts. There was sufficient evidence of compliance with good hygienic-sanitary practices in industrial samples.

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