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Research Article

Quality of stingless bee mandaçaia (Melipona quadrifasciata) honey under different storage conditions

Qualidade do mel de abelha sem ferrão mandaçaia (Melipona quadrifasciata) em condições diversas de armazenamento

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ABSTRACT

The advancement in the commercialization of honey from native bees implies the need to develop applied research, for better conservation after its harvest. Therefore, the objective of the work was to analyze the quality of mandaçaia honey (*Melipona quadrifasciata*) under different storage conditions. The experimental design used was a 2x3 factorial, consisting of factor A "light" (with and without) and factor B "temperature" (5 °C, 20 °C and 35 °C), where a sample of 210 mL of honey was collected and subjected to analysis of physical-chemical-biological parameters (free acidity, ash, color, hydroxymethylfurfural (HMF), pH, humidity and CFU count of molds and yeasts). Then, the sample was divided into 6 other subsamples, which were subjected to treatments for 72 hours and then underwent analysis, generating seven treatments in total. The interaction of the results between the factors was tested at 5% probability, using the ANOVA analysis of variance, when no interaction was found, the light factor and the temperature factor were separated and analyzed by the Tukey test at 5% probability and an analysis of regression. The HMF levels observed were close to zero in all treatments, ash and moisture contents did not interact between the factors, and free acidity, pH, color and number of CFU of molds and yeasts showed an interaction between the factors. In all analyzes it was observed that temperature has greater influence and significance in maintaining honey parameters than light, demonstrating that for greater stability of honey parameters, it must be stored at 5 °C, regardless of the presence or absence of light.

KEYWORDS: Native Bees. Conservation. Commercialization. Products. Expiration Date.

RESUMO

O avanço da comercialização do mel de abelhas nativas implica na necessidade do desenvolvimento de pesquisas aplicadas, para melhor conservação após sua colheita. Com isso, o objetivo do trabalho foi analisar a qualidade do mel de mandaçaia (*Melipona quadrifasciata quadrifasciata*) em diferentes condições de armazenamento. O delineamento experimental utilizado foi fatorial 2x3, sendo composto pelo fator A "luz" (com e sem) e o fator B "temperatura" (5 °C, 20 °C e 35 °C), onde uma amostra de 210 mL de mel foi colhida e submetida a análises de parâmetros físico-químico-biológicos (acidez livre, cinzas, coloração, hidroximetilfurfural (HMF), pH, umidade e contagem de UFC de bolores e leveduras). Em seguida, a amostra foi fracionada em outras seis subamostras, às quais foram submetidas aos tratamentos por 72 horas e em seguida, passaram pelas análises, gerando sete tratamentos ao todo. Testou-se a interação dos resultados entre os fatores a 5% de probabilidade, pela análise de variância ANOVA, quando não constatada interação, o fator luz e o fator temperatura foram separados e analisados pelo teste Tukey a 5% de probabilidade e realizada análise de regressão. Os níveis de HMF observados foram próximos a zero em todos os tratamentos, teores de cinza e de umidade não interagiram entre os fatores, e teores de acidez livre, pH, coloração e número de UFC de bolores e leveduras apresentaram interação entre os fatores. Em todas as análises observou-se que que a temperatura tem maior influência e significância na manutenção

dos parâmetros do mel do que a luz, demonstrando que para maior estabilidade dos parâmetros dele, mesmo deve ser armazenado a 5 °C, independentemente da presença ou ausência de luz.
PALAVRAS-CHAVE: Abelhas Nativas. Conservação. Comercialização. Produtos. Validade.

INTRODUCTION

The stingless bees mandaçaia (*Melipona quadrifasciata quadrifasciata*), naturally found in the southern Brazilian region (BRAZIL 2021), but in danger of extinction in the wild (WITTER & BLOCHTEIN 2009), visit melliferous plant species originating from the Atlantic Forest, such as those from the botanical families Myrtaceae, Fabaceae, Solanaceae and Rhamnaceae, exemplified by: *Plinia cauliflora, Inga fagifolia, Solanum nicotiana* and *Colubrina glandulosa*, respectively (RADAESKI et al. 2022). Due to the plant diversity, this biome presents conditions that allow for the production of excellent quality stingless bee honey, with unique characteristics highly valued by consumers. This results in a high-value product that meets an important market niche within stingless beekeeping (KARPINSKI 2016).

However, in order to be marketed, honey must meet a series of chemical parameters (such as pH, concentrations of free acidity and hydroxymethylfurfural - HMF), physical parameters (such as moisture and ash content) and biological parameters (such as colony-forming units of molds and/or yeasts), which can be altered by several factors, such as: edaphoclimatic changes (relative humidity, temperature, among others), source materials (nectar and pollen) and harvesting methods (GOMES et al. 2017). Also, honey from native bees has a specific microbiota, which can be easily altered by the storage of the product, leading to degradation of the physicochemical and biological parameters, which can result in significant economic losses (MONTE et al. 2013). Therefore, the quality parameters for honey are established and monitored by competent municipal, state, or federal agencies.

The parameters of honey from a species of mandaçaia (*Melipona mandacaia*) were analyzed by ALVES et al. (2005), observing average values of: moisture content of 28.78%, with variations between 23.14 and 32.50%; pH of 3.27, with values ranging from 3.16 to 3.54; free acidity of 43.48, with values between 18.50 and 62.50; maximum HMF presence of 23.14, with a minimum value of 16.54; and color variation from white to light amber, demonstrating the existence of changes in the physicochemical parameters presented by different honey samples, even from the same species. ALVES et al. (2011), in their analyses of honeys from other species of native bees, observed that the colony-forming units of molds or yeasts ranged around $<1.0 \times 10^2$, demonstrating small variations in the values found.

Given these possibilities for variation in honey parameters, in addition to rigorous quality control, care is also necessary during harvesting and, especially, in the post-harvest storage and commercialization of the honey, as these are the main periods of its degradation. Therefore, this study aimed to analyze the quality of stingless bee honey under different storage conditions, with variations in light and temperature, in order to observe how these changes affect the final quality of the product, through physico-chemical and biological analyses.

MATERIALS AND METHODS

The experiment was conducted at the Federal Institute of Santa Catarina – Santa Rosa do Sul Campus, located at coordinates 29° 08' 15'' South and 49° 42' 45'' West. The region's climate is characterized, according to Köppen as Cfa, as subtropical, with uniform and well-distributed rainfall, without a dry season and average temperatures ranging from 18 to 22 °C. It is located within the Atlantic Forest biome (WREGE et al. 2012) and the soil of the region is classified as Gleysol (SILVA et al. 2018).

The quality of honey from the stingless bee mandaçaia (*Melipona quadrifasciata*) was evaluated using a 2x3 factorial experimental design. For this purpose, one mandaçaia colony from a group of 30 colonies at the Federal Institute of Santa Catarina – Santa Rosa do Sul Campus, was chosen by random selection, providing honey samples collected directly from mature honey pots, characterized as being completely sealed honey pots (PEREIRA et al. 2012), using sterile 50 ml needles and syringes. Needles were used to open the jars and syringes to collect 210 ml of honey, which was stored in a sterile jar, kept in a thermal box, in order to reduce the likelihood of changes in the characteristics of the samples until the application of the treatments and analyses. After collection, the material underwent triplicate testing for pH, free acidity, moisture, hydroxymethylfurfural, ash, color, and CFU count of molds and yeasts, following an adapted methodology described by EVANGELISTA-RODRIGUES et al. (2005), ALVES et al. (2005), ALVES et al. (2009) and LACERDA et al. (2010), aiming to observe whether the honey was within the parameters established by BRAZIL (2000) and CIDASC (SANTA CATARINA 2020) for consumption and also to generate data for later comparison, as shown in Table 01:

Table 1. Parameters established by Brazilian legislation for native bee honey.

Analysis Performed	Approval Parameter
Free Acidity	100 mEq/kg
Ashes	maximum 0.6 g/100 g
Coloring	Colorless to Dark Brown
CFU count	$1x10^4$ CFU/ml
Hydroxymethylfurfural	maximum 40 mg/kg
pH	2.8 to 4.8
Humidity	maximum 40 g/100 g

Source: CIDASC (SANTA CATARINA 2020).

Determination of free acidity and pH based on LACERDA et al. (2010):

A solution was prepared in a 1:5 ratio of honey and distilled water, using 5 ml of honey and 25 ml of distilled water, and its pH was measured using a pH meter. Next, 2 drops of phenolphthalein were added to the solution, and the free acidity was measured using the titrimetric neutralization method with 0.1 N NaOH. Subsequently, the data obtained were applied to the following formula:

Free acidity = (mL of 0.1 N NaOH used in the burette - 0.1 mL H2O) x 5

Determination of Ash/Mineral Content based on LACERDA et al. (2010):

For the mineral content, 10 g of the honey sample was weighed in a porcelain crucible, previously tared. The sample was heated in a Bunsen burner until it was carbonized, and then it was placed in a muffle furnace at 600 °C, where it remained for five hours. After this period, the sample remained in the muffle furnace cooling until

it reached 200 °C, then it was cooled to room temperature in a desiccator and weighed on a precision balance. The calculation was performed using the collected data and the following formula:

$$\%\ de\ minerais\ =\ (difference\ in\ crucible\ weight/sample\ weight)\ x\ 100$$

Determination of coloration based on ALVES et al. (2005):

For colorimetric analysis, the Bianchi method was used, which consisted of measuring the absorbance at 635 nm in a spectrophotometer of a 50% (m/v) solution of honey in distilled water. After dilution, the solution was left to stand for 15 minutes before determining the absorbance, while the equipment was calibrated with distilled water. The values obtained were used for classification on the Pfund scale.

Counting of mold/yeast CFU based on ALVES et al. (2009):

The biological analysis of the honey was performed using a 1 ml aliquot, which was diluted, inoculated, and cultured, following the protocol adapted from ALVES et al. (2009). The adaptations consist of: The honey was diluted in a 1:9 ratio of honey and 0.1% buffered peptone water, totaling two dilutions (10^{-1} and 10^{-2}). The sample diluted 10^{-2} of "raw" honey was plated in triplicate on potato dextrose agar (PDA) medium and incubated for 5 days in a bacteriological incubator at 25 °C, with three plates without inoculation for negative control of the medium, totaling six plates. After cultivation, the plates were analyzed to obtain quantitative parameters from the counting of colony-forming units of molds and/or yeasts. The values obtained were applied to the formula:

CFU/ml of honey = number of CFU counted x 100

Determination of Hydroxymethylfurfural (HMF) concentration based on EVANGELISTA-RODRIGUES et al. (2005):

For the hydroxymethylfurfural analysis, 5g of honey were weighed into an identified beaker. Subsequently, 25mL of distilled water were added and transferred to a 50mL volumetric flask. Next, 0.5 mL of Carrez solution 1 {15g of $K_4Fe(CN)6.3H_2O$ in 100mL of distilled water} and 0.5 mL of Carrez solution 2 {30g of $Zn(CH3COO)2.2H_2O$ in 100 mL of distilled water) were added, finally completing the volume with distilled water. The sample was filtered with filter paper, and 5 mL of the filtrate was pipetted into two test tubes. 5 mL of distilled water was added to the first tube, and 5 mL of a sodium bisulfite solution (0.2 g of $NaHSO_3$ in 100 ml of distilled water) was added to the second tube as a reference. The absorbance of the sample was measured using a spectrophotometer at wavelengths of 284 and 336 nm. The following formula was used to calculate the amount of HMF:

$$mg\ HMF\ of\ honey\ =\ (A284\ -\ A336)\ x\ 14.97\ x\ 5\ /\ sample\ weight$$

Where: F = 14.97 = (126/16.830) * (1000/10) * (100/5), where: 126 = Molecular weight of HMF; 16.830 = Molecular absorptivity of HMF at 284nm; 1000 = mg/g; 10 = centiliters/L; 100 = percentage of HMF; 5 = theoretical weight of the sample.

Determination of moisture content based on LACERDA et al. (2010):

For moisture determination, 5 ml of honey were placed in a benchtop refractometer with a Brix degree reading. The obtained value was recorded, and the amount of moisture was determined using the formula below:

$$\%\ humidity\ =\ 100\ -\ degrees\ Brix\ found$$

Following the initial physico-chemical and biological analyses, six subsamples of

honey were separated using sterile 50 ml syringes from the initial sample, each containing 30 ml of honey, and subjected to the following treatments: with and without light exposure, and at temperatures of 5 °C, 20 °C, and 35 °C, for 72 hours. The subsamples were kept in BOD incubators (at temperatures of 5 °C, 20 °C, and 35 °C), while receiving light from the equipment (with light) or were contained within Kraft paper packages, which inhibited light, without altering the temperature (without light).

After the period of submitting the subsamples to the treatments, they were again subjected to physico-chemical and biological analyses for pH, free acidity, hydroxymethylfurfural, moisture, ash, color, and CFU count of molds and/or yeasts. The results obtained were compared in a 2x3 factorial statistical analysis, where the first factor is light (in treatments with or without light) and the second factor is temperature (in treatments at 5°C, 20°C, and 35°C). In the absence of interaction, the "light" and "temperature" treatments were analyzed separately using Tukey's test at 5%. Regression analysis was applied when the analysis of variance was significant at the 5% level, accepting the curve with the highest coefficient of determination R^2.

RESULTS AND DISCUSSION

Chemical analyses of free acidity showed a significant difference ($p<0.0001$), with values ranging from 16.083 to 22.483 mEq/kg (Figure 1).

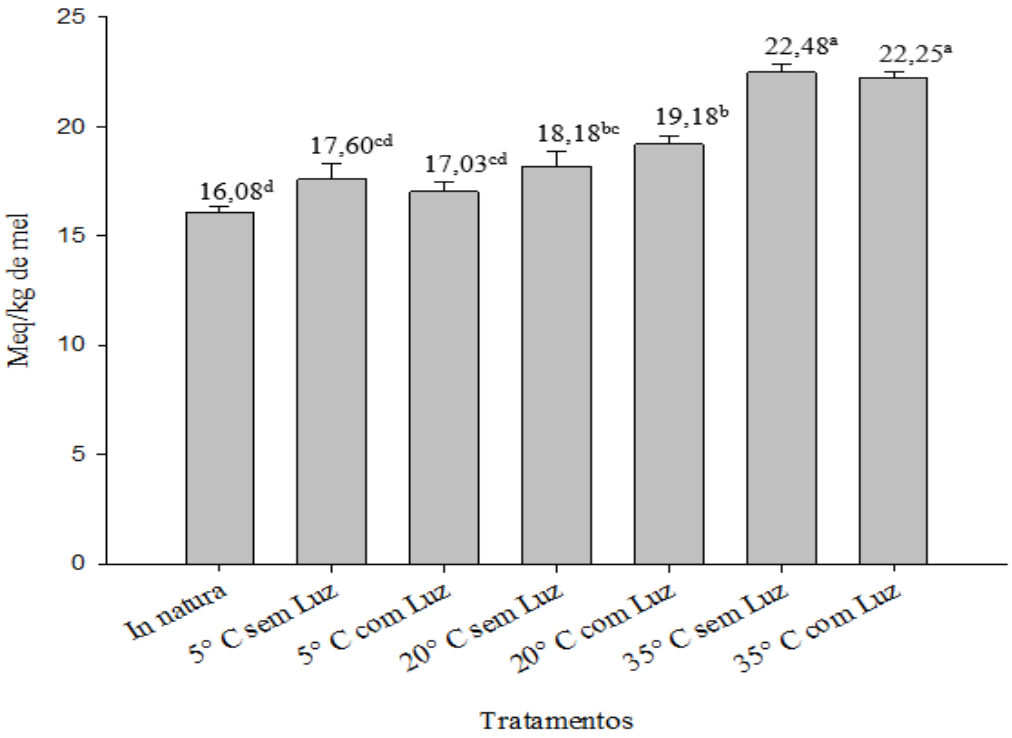
The "raw" honey showed the lowest free acidity value of 16.08 mEq/kg. The increase in temperature was the factor that influenced the free acidity, increasing by 0.1069 mEq/kg gradually as the temperature rose by one degree Celsius, according to the equation:

$$\text{Free acidity mEq/kg} = 16.098 + (0.1069 * T^{\circ}C) + ((0.1224 * (T^{\circ}C)^2);$$

$$(P<0.0001;\ R2 = 0.9092)$$

The influence of light intensity on free acidity was not significant ($P>0.05$) according to the Tukey test, demonstrating that the light passing through the container used for selling the honey will not influence the increase in free acidity.

The values observed are lower when compared to those observed by BILUCA et al. (2013) in mandaçaia bee honeys from different regions of Santa Catarina, in which the free acidity varied from 36.45 to 74.77 mEq/kg. According to the regulations governing honey quality parameters, the maximum amount of free acidity allowed is 100 mEq/kg of honey (SANTA CATARINA 2020). Therefore, all treatments were within the permitted limit, meaning the honey can be marketed based on the free acidity parameter without the need for refrigeration.



Letters that are the same do not differ according to the Tukey test at 5%.

Figure 1. Means and Standard Deviation of Free Acidity mEq/kg of honey.

According to FINCO et al. (2010), free acidity can be generated by factors prior to honey harvesting, such as the type of nectar or digestive enzymes of the bees themselves, or after harvesting, such as the microbiota present in the food during its maturation. The increase in temperatures during honey storage led to an increase in free acidity in the present study, and Alves et al. (2009) report that temperature facilitates changes in the microbiota present in honey, promoting the degradation of sugars and other compounds present in the food, releasing H+ ions, causing an increase in the free acidity of the medium.

During the analysis of ash content, one of the observed values from the 35 °C treatment with light was removed from the statistical analysis, as it was outside the normal distribution curve, resulting in Table 1, which presents the analysis of means and variance of the ash/mineral content of the honey samples:

There was no interaction between the factors "light" and "temperature" ($p>0.05$), therefore, the ash content in stingless bee mandaçaia honey will be discussed separately.

The observed values for the ash content of stingless bee honey showed significance ($p = 0.0027$) when the honey underwent heat treatment at 35°C, when compared to the treatments at 5°C and 20°C. However, the highest temperature did not differ significantly ($p>0.05$) from the "raw" honey, which was harvested and analyzed instantly.

Table 1. Means (g.100 g^{-1}), standard error, standard deviation and coefficient of variation of Ash content in relation to heat treatments.

Analyses	Treatments			
	"raw"	**5ºC**	**20 ºC**	**35ºC**
Mean, g/100 g	0.16^{ab}	0.14^{b}	0.15^{b}	0.18^{a}
Stand. Error	0.01	0.01	0.01	0.01
Stand. Dev.	0.01	0.02	0.01	0.01
Var. Coeff., %	6.00	12.00	7.00	5.00

Letters that are the same do not differ according to the Tukey test at 5%.

One hypothesis that may justify this fact, since there are few studies addressing this topic, is that the temperatures in question, along with other factors present in the honey, may lead to the formation of soluble chelates, that is, a bond between minerals and organic components forming molecules (OLIVEIRA 2018), which, during the muffle furnace burning process, may have removed these minerals from the analysis.

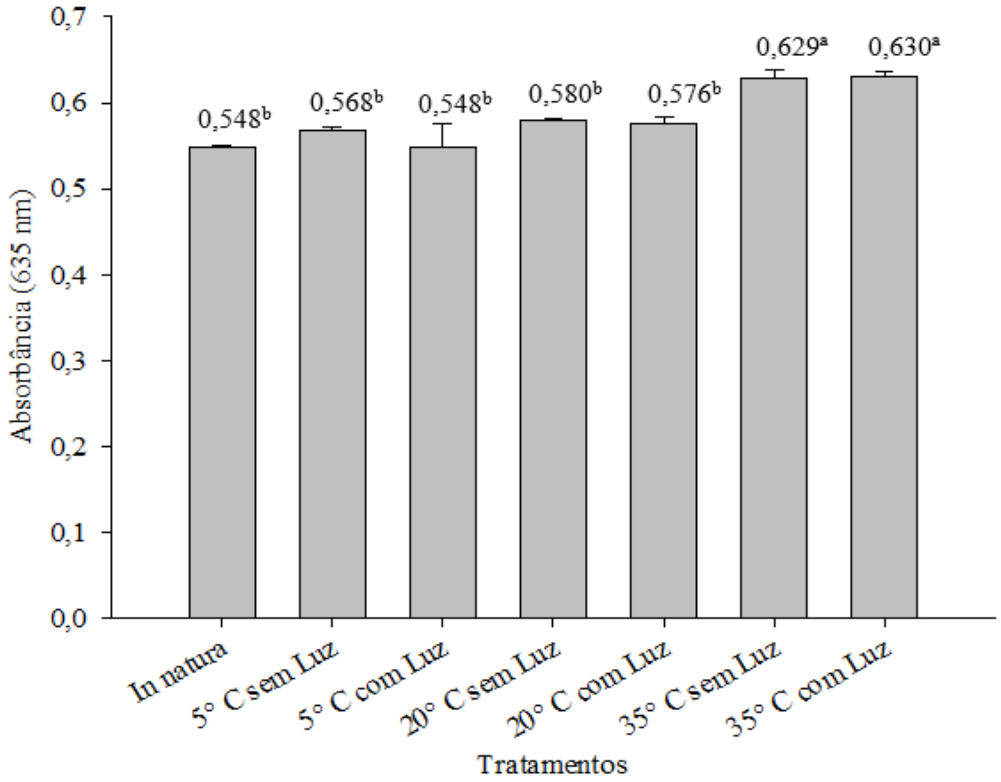
The observed values for the ash content of stingless bee honey were not affected by the presence of light ($p>0.05$), showing an average value of 0.15 g/100 g (Table 2).

Table 2. Means (g100 g^{-1}), standard error, standard deviation and coefficient of variation of Ash content in relation to luminosity treatment.

Analyses	Treatments		
	"raw"	**Without Light**	**With Light**
Mean, g/100 g	0.16	0.15	0.15
Stand. Error	0.01	0.01	0.01
Stand. Dev	0.01	0.01	0.02
Var. Coeff., %	5.64	9.03	15.11

Color tests of the honey samples (Figure 2), when analyzed in a spectrophotometer, showed absorbances between 0.548 and 0.630 L mol^{-1} cm^{-1}. Converting these absorbance values using the PFUND scale for determining honey color (VIDAL & FREGOSI 1984), the color of the honey in all samples was classified as amber, a class that presents an absorbance of 0.410 to 0.945 L mol^{-1} cm^{-1}.

A significant difference ($p<0.0001$) was observed between the treatments where the temperature was 35 ºC, compared to the other treatments which showed similarity ($p>0.05$). Despite the statistical difference when a temperature of 35 ºC was applied, it did not alter its classification in terms of color, and luminosity did not affect this parameter.



Letters that are the same do not differ according to the Tukey test at 5%.

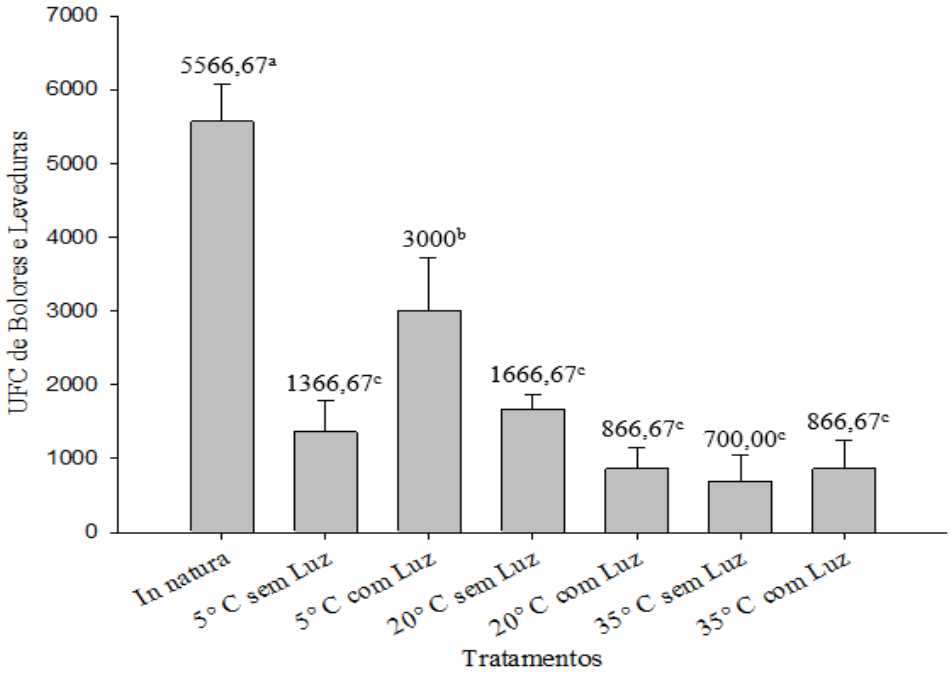
Figure 2. Means and Standard Deviation of Absorbance at 635 nm of honey.

Applying the regression curve, it showed the best quadratic fit, where: color = 0.5544 - (0.0047Tº) + (0.0024Tº²) (P=0.0001; R²=0.8597). The increase in temperature can cause honey to darken, according to MENDES et al. (2009), through structural changes in its components, such as sugars and proteins, by means of the caramelization process of these substances (Santos et al. 2018). Thus, although the color change was not pronounced, there was an increase in the absorbance of the samples from the treatments with higher temperatures, demonstrating a slight darkening of the honey.

The biological analyses, referring to the CFU count of molds and yeasts per ml of honey, are represented in Figure 3.

The treatment with "raw" honey showed higher quantities of CFU.ml^{-1} (P<0.001) compared to the other treatments. There was a sharp decrease in the number of colonies in the 5 ºC treatment without light, which was reflected in the other treatments (p>0.05), except for the 5 ºC treatment with light, where the number of CFU.ml^{-1} was higher than the 20 °C without light, 20 °C with light, 35 °C without light, and 35 °C with light treatments.

Therefore, it is observed that changes in the way honey is stored after harvesting reduce the populations of molds and yeasts, establishing an inversely proportional relationship between the number of CFU of molds and yeasts per ml of honey and the storage temperature, indicating that these populations are not directly responsible for the degradation of the honey, but rather for its preservation within the colony, warranting studies to identify the species of yeasts in raw honey to improve the quality of Mandaçaia bee honey.



Letters that are the same do not differ according to the Tukey test at 5%.

Figure 3. Means and Standard Deviation of CFU of Honey Molds and Yeasts.

The regression analysis that best represented the study was one in which the temperature was raised to the power of -0.947, generating the equation CFU.ml-1 = 4861.1*(TºC)-0.947 (P<0.0001; R2 =0.8131)

The culture medium used, known as Potato Dextrose Agar (PDA), has the capacity to select the molds and yeasts present in honey; therefore, studies with other culture media should be carried out to observe if there is an increase in CFU of other microorganisms that may be related to changes in honey parameters.

Based on the analyses performed and the comparison of the parameters obtained from the "raw" honey and the honey stored under different conditions, it was found that the HMF levels were within the normal range (SANTA CATARINA 2020) in all cases, showing concentrations close to zero, as observed by BILUCA et al. (2013), who consider that non-significant HMF values are predominant conditions for properly handled and stored honey.

According to FINCO et al. (2010), HMF production is directly related to the decomposition of fructose, one of the sugars that make up honey, in environments with high temperatures for extended periods of time.

Due to the floristic variability of the region, the honey used may have contained lower levels of fructose and higher levels of other sugars commonly found in plant nectars, such as sucrose and glucose (RECH et al. 2014).

Small changes were observed in the pH levels (Table 3), placing them within the parameter of 2.8 to 4.8 allowed by legislation, characterizing the honeys as naturally acidic. However, the treatment in which the honey was subjected to 35 ºC in the presence of light significantly reduced the pH ($p<0.05$) compared to the other treatments, as did the treatment at 35 ºC without the presence of light, which was similar to the value observed in the "raw" treatment.

The descriptive analysis of the average pH values is described in Table 3:

Table 3. Analysis of pH Means.

Analyses	Treatments						
	"raw"	**5 ºC without light**	**5 ºC with light**	**20 ºC without light**	**20ºC with light**	**35 ºC without light**	**35 ºC with light**
Mean	3.61^{ab}	3.64^{a}	3.62^{a}	3.60^{ab}	3.61^{a}	3.56^{b}	3.54^{c}
Stand. Error	0.01	0.02	0.01	0.01	0.01	0.01	0.02
Stand. Dev.	0.02	0.023	0.01	0.01	0.02	0.02	0.04
Var. Coeff.	0.42	0.69	0.28	0.28	0.58	0.43	1.14

Letters that are the same do not differ according to the Tukey test at 5%.

Inside the stingless bee mandaçaia colony (*Melipona quadrifasciata*), according to SILVA (2019), the temperature varies between 24 ºC and 29.5 ºC, and there is no light present. This condition may explain the similarities between the treatments with temperatures between 20 ºC and 35 ºC without the presence of light.

pH values ranging from 3.9 to 4.29 in stingless bee mandaçaia honey are reported by BILUCA et al. (2013), values that are higher than those observed in the present study, which showed a variation from 3.54 to 3.64..

Temperature (Table 4) and luminosity (Table 5) did not influence ($p>0.05$) the moisture content of stingless bee mandaçaia honey, presenting an average value of 36.64%, within the acceptable parameters for native bee honeys according to CIDASC, which can reach up to 40% (SANTA CATARINA 2020).

Table 4. Means, standard error, standard deviation and coefficient of variation of Mandaçaia honey Moisture, in relation to Temperature.

Analyses	Treatments			
	"raw"	**5ºC**	**20 ºC**	**35ºC**
Mean, %	36.63	36.72	36.65	36.58
Stand. Error	0.15	0.24	0.15	0.05
Stand. Dev.	0.25	0.58	0.36	0.12
Var. Coeff., %	1.57	0.97	0.32	0.68

Table 5. Means, standard error, standard deviation and coefficient of variation of Mandaçaia honey Moisture, in relation to Light.

Analyses	Treatments		
	"raw"	**Without Light**	**With Light**
Mean, %	36.63	36.66	36.64
Stand. Error	0.15	0.15	0.10
Stand. Dev.	0.25	0.45	0.31
Var. Coeff., %	0.69	1.24	0.85

The water content in the honey in all treatments was close to the maximum limit allowed by law. The water content in honey can be influenced by different factors, such

as the origin of the nectar and the climate (FINCO et al. 2010); however, the different effects of environmental storage factors did not alter the water content of the analyzed honey. The amount of water in the honey of native bees, according to ALVES et al. (2005), is one of the factors that most affect the preservation of this product. BILUCA et al. (2013) observed humidity values of 30.72% in wild honey from stingless bees mandaçaia collected in different regions of Santa Catarina.

CONCLUSION

Temperature was the most influential factor in maintaining the parameters of the honey; therefore, stingless bee mandaçaia honey should be stored under refrigeration, at approximately 5 °C, regardless of the presence or absence of light, for better stability of the parameters of color, pH, moisture, free acidity, ash, and CFU of molds and yeasts.

AUTHORS' CONTRIBUTIONS

Conceptualization, methodology and formal analysis, Pedro Henrique Peterle Bernhardt (ORCID 0000-0003-2955-0527), Miguelangelo Ziegler Arboitte (ORCID 0000-0002-9174-0017), Patrícia Castellen (ORCID 0000-0003-4718-740X); software and validation, Pedro Henrique Peterle Bernhardt (ORCID 0000-0003-2955-0527), Miguelangelo Ziegler Arboitte (ORCID 0000-0002-9174-0017), Patrícia Castellen (ORCID 0000-0003-4718-740X); investigation, Pedro Henrique Peterle Bernhardt (ORCID 0000-0003-2955-0527), Maisa Benedete Duarte (ORCID 0009-0001-0596-8403), Amanda Fonseca de Melo (ORCID 0000-0003-0594-0782); resources and data curation, Pedro Henrique Peterle Bernhardt (ORCID 0000-0003-2955-0527), Miguelangelo Ziegler Arboitte (ORCID 0000-0002-9174-0017); writing - original draft preparation, Pedro Henrique Peterle Bernhardt (ORCID 0000-0003-2955-0527); writing - review and editing, Pedro Henrique Peterle Bernhardt (ORCID 0000-0003-2955-0527); visualization, Pedro Henrique Peterle Bernhardt (ORCID 0000-0003-2955-0527); Supervision: Miguelangelo Ziegler Arboitte (ORCID 0000-0002-9174-0017), Patrícia Castellen (ORCID 0000-0003-4718-740X); project administration: Pedro Henrique Peterle Bernhardt (ORCID 0000-0003-2955-0527), Miguelangelo Ziegler Arboitte (ORCID 0000-0002-9174-0017), Patrícia Castellen (ORCID 0000-0003-4718-740X). All authors have read and agreed to the published version of the manuscript.

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STATEMENT OF THE INSTITUTIONAL REVIEW BOARD

Not applicable because this study did not involve humans or animals of the phylum Chordata.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available upon request.

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CONFLICTS OF INTEREST

There are no conflicts of interest in this work.

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