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ANTIOXIDANT ACTIVITY AND PHYSICO-CHEMICAL PROPERTIES OF MEAD SUPPEMENTED WITH COFFE

ATIVIDADE ANTIOXIDANTE E PROPRIEDADES FÍSICO-QUÍMICAS DO HIDROMEL SUPLEMENTADO COM CAFÉ

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ABSTRACT: Mead is a fermented drink with honey as the main ingredient, being called melomel when fruits or grains are added. In this work, the objective was to evaluate the physical-chemical parameters and the antioxidant potential of mead produced with the addition of coffee. Meads were made in a process similar to wine making, involving the operations of preparing the pre-inoculum, the must, yeast inoculation, fermentation and packaging. The meads produced with and without the addition of coffee were subjected to physical-chemical analyzes (moisture, acidity, pH, alcoholic content, reducing sugars, soluble solids (ºBx), ash) and the antioxidant potential (phenolic compounds and total flavonoids, DPPH , ABTS and FRAP). It was observed that for the parameters of pH (3.41), ash (0.04%) and reducing sugar (107.71 g L^{-1}) the mead with coffee obtained higher values compared to the control drink without the addition of the adjunct coffee, which in turn demonstrated higher performance in the moisture (88.26%) and acidity (65.7 mEq L^{-1}, 4.9 g of tartaric acid L^{-1}) analyses. When related to the alcohol content of the drinks, both showed the same significantly value of 11.62% (without coffee) and 13.53% (with coffee). Regarding the properties of phenolic compounds and total flavonoids, the mead with coffee presented higher levels (305.85 mg GA100 mL^{-1}), flavonoids (33.47 mg Q 100 mL^{-1}), DPPH (153.01 mg T 100 mL^{-1}) and FRAP (1236.08 µmol Fe(II) 1000 mL^{-1}). The traditional drink presented ABTS as the best antioxidant activity with 245.67 mg T 100 mL^{-1}. Coffee additives significantly influenced the beverage's physicochemical and bioactive characteristics, promoting its antioxidant potential and functional performance.
Keywords: antioxidant activity; *Melipona subnitida*; Methglin; Methglin; phenolic compounds.

RESUMO: *O hidromel é uma bebida fermentada que tem o mel como ingrediente principal, sendo chamado de melomel quando são adicionadas frutas ou grãos. Neste trabalho o objetivo foi avaliar os parâmetros físico-químicos e o potencial antioxidante do hidromel produzido com adição de café. Os hidroméis eram elaborados num processo semelhante ao da vinificação, envolvendo as operações de preparação do pré-inóculo, do mosto, inoculação de leveduras, fermentação e acondicionamento. Os hidroméis produzidos com e sem adição de café foram submetidos a análises físico-químicas (umidade, acidez, pH, teor alcoólico, açúcares redutores, sólidos solúveis (ºBx), cinzas) e ao potencial antioxidante (compostos fenólicos e flavonóides totais, DPPH, ABTS*

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e FRAP). Observou-se que, para os parâmetros pH (3,41), cinzas (0,04%) e açúcar redutor (107,71 g L^{-1}), o hidromel com café obteve valores superiores em comparação à bebida controle sem adição do café, que demonstrou por sua vez valores superiores nas análises de umidade (88,26%) e acidez (65,7 mEq L^{-1}, 4,9 g de ácido tartárico L^{-1}). Quando relacionado ao teor alcoólico das bebidas, ambos apresentaram o mesmo valor significativo de 11,62% (sem café) e 13,53% (com café). Quanto às propriedades de compostos fenólicos e flavonóides totais, o hidromel com café apresentou maiores teores (305,85 mg GA100 mL^{-1}), flavonóides (33,47 mg Q 100 mL^{-1}), DPPH (153,01 mg T 100 mL^{-1}) e FRAP (1236,08 µmol Fe(II) 1000 mL^{-1}). A bebida tradicional apresentou ABTS como melhor atividade antioxidante com 245,67 mg T 100 mL^{-1}. Os aditivos do café influenciaram significativamente as características físico-químicas e bioativas da bebida, promovendo seu potencial antioxidante e desempenho funcional.
Palavras-chave: atividade antioxidante; Melipona subnitida; Methglin; compostos fenólicos.

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INTRODUCTION

Mead is an alcoholic drink with honey as one of its main ingredients, providing various nutritional and functional properties. The fermentation process, conducted by applying water and yeast to the must, contributes to its development (Isla *et al.*, 2011; Pereira *et al.*, 2017; Willey *et al.,* 2018). However, its chemical, physicochemical, and sensory characteristics can become specific with the addition of different adjuncts, such as fruits and grains (Araújo *et al.*, 2020; de Oliveira *et al.*, 2020).

The chemical properties of honey, together with the adjuncts used to prepare mead, can confer bioactive potential arising from its antioxidant activity from phenolic compounds (Czabaj *et al.*, 2017). The bioactive potential may vary depending on the type of honey and the addition of organic acids (Chen *et al.*, 2013; Starowicz and Granvogl, 2020). Finally, the technology involved in the process, such as heat treatment, storage, and fermentation, interferes with the phenolic profile found in the product (Kahoun *et al.*, 2008). The antioxidant activity of phenolic compounds can stabilize molecules (Socha *et al.*, 2015).

Furthermore, other factors also influence the quality of mead, such as the origin of the honey, the additives used (coffee, fruit juices, rose petals, among others), the yeast strains, and the fermentation parameters (Gaglio *et al.*, 2017). According to Decree No. 6871/2009, the alcoholic content of mead varies between 4% and 14% in its composition. The addition of adjuncts in its preparation imparts bioactive properties to the beverage, along with its core raw material (Brasil, 2009). Thus, when consumed appropriately, it can positively effect the human body, involving the processes of digestion and metabolic biochemistry (Gutiérrez-Grijalva *et al.*, 2016). Despite the inclusion of fruits and spices in its formulation, the main source of sugar for the fermentation process comes from honey (Nakada *et al.*, 2020). Mead can contain different chemical compounds beyond ethanol, such as organic acids, polyphenols, minerals, and vitamins (Estevinho et al., 2008; Švecová *et al.*, 2015).

Furthermore, other factors also influence the quality of the mead, such as the origin of the honey, the additives used (coffee, fruit juices, rose petals, among others), the yeast strains, and the fermentation parameters (Gaglio *et al.*, 2017). In general, the development of beverages with functional properties has gained prominence in research and innovation in the food industry, exploring ingredients with beneficial properties to create new drinks or enhance existing ones, as seen in the preparation of mead (Bambace *et al.*, 2021).

For this reason, coffee has been used as an ingredient in beverage preparation due to its biological properties such as antioxidants and sensory attributes (Andrade *et al.*, 2012; Castaldo *et al.*, 2018; Gemechu, 2020). Commonly found in its composition are sugars, volatile and phenolic compounds, fatty acids, caffeine, and proteins, which contribute to the distinctive flavor and aroma of coffee and are attributed to the products to which it is added (Esquivel and Jimenez, 2012; Corrêa *et al.*, 2015). Caffeine, chlorogenic acid, and their metabolites are the main antioxidant compounds found in coffee (Andrade *et al.*, 2012; Hu *et al.*, 2019). Chlorogenic acid and its byproducts are the main phenolic compounds, formed by ferulic, caffeic, fumaric, and quinic acids, belonging to the esters group - a subgroup of hydroxycinnamic acids. Within this group, the class of coffee chlorogenic acids forms the acid byproducts: caffeoylquinic acid (CQA), dicaffeoylquinic acid (4,5-diCQA), and 3-feruloylquinic acid (3-FQA),with a reducing capacity for reactive oxygen species (ROS) (Monteiro and Farah, 2012; Clifford *et al.*, 2017).

In this sense, the use of an ingredient like coffee in the crafting of mead becomes commendable, aiming to provide a product with bioactive potential and similar characteristics to the one without the addition of spices. Adding an ingredient widely consumed worldwide may contribute to expanding knowledge about mead, bringing visibility to the product in the market, and promoting its consumption.

MATERIALS AND METHODS

Samples

Stingless bee honey from the jandaíra species (*Melipona subnitida*) sourced from the Alenquer in the state of Pará, Brazil, was used, while the classic special coffee beans (500g) from the Octavio brand were obtained in São Paulo. The samples were taken to the Laboratory of Biochemical and Chemical Studies (LEBIQ) at the Technological Center for Bioactives of the Federal University of Western Pará (UFOPA), Campus Tapajós, where both the honey and the ground coffee were stored.

The fermentative process for producing mead

The production of mead followed procedures similar to the making of grape wine, involving operations such as must preparation and correction, starter culture preparation (50g for 1L of starter culture), yeast inoculation, fermentation, and bottling. Fifty grams of ground coffee beans were used to 10 L of mead (5 g L^{-1}), processed with a manual grinder.

Wort Preparation

To prepare 10 L (approximately 10kg) of mead, initially calculate the amount of honey and water that will be used to obtain a wort with an initial 30 ºBx. To do this, stipulate the amount of wort you wish to produce, medium or initial ºBx of honey, and research 1 was applied:

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$$M_h = (300kg\ ^oBx)/\ Brix_h \quad (1)$$

where: M_h = Mh = mass of honey used in the process, calculated from the equation; Brixh = initial ºBx of honey, determined using a refractometer.

After determining the amount of honey needed, the amount of water to be added to the must was calculated using equation 2:

$$M_{water} = M_w - M_h \quad (2)$$

With the amount of honey and water calculated, the must was prepared by mixing them in the fermentation tank, with the help of a funnel to avoid losses. About the amount of must prepared, 10% of the total volume was removed and separated for the preparation of the vat foot. The formulation proposed here produces a sweet mead, without the need to add any nutritional supplement to the must, nor to correct its acidity.

Fermentation process

A volume corresponding to 10% of the must (1.00 L) was separated to prepare the pre-inoculum. This aliquot was pasteurized at 100 ºC for 30 min and then cooled to room temperature (30 ºC) and then the yeast (1.0653 g of *Saccharomyces bayanus*, and 1.0070 g of *Saccharomyces cerevisiae*) was added. The tub foot was kept in a closed container for 24 hours. After this time, pasteurization was carried out on the remainder of the must at 100 ºC for 2 min. After the temperature was reduced to 80ºC the 50.0 g of roasted and ground coffee was added to the must. Finally, he allowed the worst to return to room temperature (25 ºC) and added the yeast.

During the fermentation process, sugar consumption was evaluated by measuring the total soluble solids content of the must, using a refractometer. The soluble solids content fell slowly, being measured over 25 consecutive days. After this process, the drink was left to mature for a total period of 90 days. Once fermentation and maturation were complete, the mead was transferred to another sanitized carboy, to prevent it from coming into contact with the sediments formed. Afterwards, the mead was transferred by siphoning. For decabonation, the mead was subjected to an ultrasonic bath for 15 minutes before being bottled.

Physicochemical analyzes and antioxidant activity of mead

The physicochemical characterization of the meads was carried out through analyzes of pH, final alcohol content (ºGL), total reducing sugars, acidity, soluble solids content (ºBx), and ash. In addition to checking total phenolic compounds and flavonoids, DPPH, ABTS, and FRAP antioxidant activity.

pH analysis

The reading was carried out directly in the liquid, with the aid of a properly calibrated pH meter (ION pHB 500).

Alcohol Content and Total Soluble Solids (TSS)

Brix degree readings were carried out by refractometry, using the refractometer of Abbé (FORMIS MODELO: FOR-1000S, Caieiras, São Paulo, Brasil), corrected to 20 ºC. The device was calibrated at room temperature with deionized water (Refractive index = 1.3330 and 0º Brix a 20 ºC) and the samples were read. Between the evaluations, a range of thirty minutes.

The alcohol content was determined by the principle of distillation of the drink, using the Clevenger distiller, and subsequent measurement of the alcohol by volume (°GL). To determine the alcohol content, the methodology of Santos and Reis (2013) was used, with adaptations. A 0.2 mol L^{-1} $K_2Cr_2O_7$ solution (Merck-Millipore, Darmstadt, Germany) was prepared in a 4.0 mol L^{-1} H_2SO_4 medium. The solution was then diluted with distilled water until the absorbance at 590 nm was around 0.850. For the analyses, 1.0 mL of the beverage and 2.5 mL of the $K_2Cr_2O_7$ solution were used. The blank solution was prepared by replacing the beverage with water. A calibration curve was prepared using absolute ethyl alcohol 99.8% (v/v) (Synth, Diadema, Brazil) with concentrations ranging from 0 to 15% ($y = 0.087329x + 0.031585$, $R^2 = 0.993972$) (Zimmerman, 1970; Santos Júnior, 2012).

Moisture Content (%)

Humidity was determined using 3,00 g of sample in an oven where it was stored for 48 hours at 94 ºC. After this period, the sample was weighed again, checking the moisture content evaporated in this period, as shown in Equation 3 (Instituto Adolfo Lutz, 2008).

$$\text{Moisture (\%)} = ((\text{crucible mass} + \text{sample massa}) - (\text{crucible mass}))/ (\text{crucible mass} + \text{sample})) \times 100 \quad (3)$$

Ash Content

The determination of ash content was carried out using samples previously dried in an oven for 48h at 94ºC. The dried samples were incinerated at 600 ºC in a muffle furnace (Jung, LF0612)

for 3 h. After returning to room temperature, the ash mass was measured about the wet mass and dry mass of the mead (Equation 4) (Instituto Adolfo Lutz, 2008).

$$\% \text{ Ash content} = 100 \times ((M3 - M1)/ (M2 - M1)) \quad (4)$$

where: M1 - mass of the crucible; M2 - mass of the crucible + dry mass and, M3 - mass of the crucible + mass of ash.

Total Titratable Acidity

10.0 mL of the sample were pipetted and subsequently transferred to a 100 mL Erlenmeyer flask containing 50 mL of water. Finally, 3 drops of 1% (m/v) alcoholic phenolphthalein solution were added, and titration was performed with 0.1 M sodium hydroxide solution until a pink coloration was obtained, following the methodology of Instituto Adolfo Lutz, (2008). For the calculation, the following formula was used:

$$\text{Total Titratable Acidity} = n * f * N * 1000 / V \quad (5)$$

where: n = number of mL of 0.0962 mol L^{-1} NaOH solution used in the titration; f = 0.1 mol L^{-1} NaOH solution factor; N = normality of NaOH; V = sample volume. The result was expressed in milliquivalents per liter (meq L^{-1}). To converter this measurement into g of tartaric acid per L, the following formula was used:

$$\text{mEq } L^{-1} \times 0.075 = \text{g of tartaric acid per L} \quad (6)$$

Total Reducing Sugars

The preparation of the 3,5-dinitrosalicylic acid (DNS) reagent was carried out using 1,0 g in 20 mL of a 2,0 mol L^{-1} NaOH solution (solution A). Concurrently, 30,0 g of sodium potassium tartrate ($KNaC_4H_4O_6$) was dissolved in 50,0 mL of distilled water (solution B) with constant heating and stirring. Solution A was then added to solution B until the DNS was completely dissolved. The resulting mixture was transferred to a volumetric flask and the volume was adjusted with distilled water to a total volume of 200 mL.

A standard glucose solution (2.00 g L^{-1}) to assemble the standard curve. The sample was diluted in a 1:50 ratio (mead:water, v/v), shaken vigorously, and incubated at 100 ºC for 5 minutes. The tubes were removed simultaneously and cooled for reading using a spectrophotometer at 540 nm. (Vasconcelos *et al.*, 2013).

Determination of total phenolic content (TPC)

It was determined using the Folin-Ciocalteu method, and the results were expressed in μg GA $100mL^{-1}$ (micrograms equivalent of gallic acid per 100 mL of mead). First, an aqueous mead solution (1:10 v/v) was prepared. Secondly, a 0.50 mL aliquot of this solution was mixed with 0.30 μL of Folin-Ciocalteu reagent (Sigma-Aldrich) and 2,00 mL of a 15% sodium carbonate solution. Subsequently, distilled water was added until the final volume was 5,00 mL. The mixture was left to rest at room temperature for 2 h, and then the absorbance was read at 798 nm. the total phenol content, expressed as milligrams of gallic acid equivalent per 100 mL of mead (mg GAE 100 mL^{-1}), was calculated using a calibration curve prepared with gallic acid standard solution (0-0.16 mg mL^{-1}; $y = 14.77626x + 0.069877$; $R^2 = 0.993116$), which was analyzed in the same way as the extracts. All measurements were performed in triplicate (Ferreira *et al.*, 2009).

Determination of total flavonoid content

The method was performed according to Salgueiro *et al.* (2014) using a 2% methanolic solution of $AlCl_3$. In a test tube, 2 mL of diluted mead sample (with and without coffee) (1:10) and 2 mL of 2% methanolic solution of $AlCl_3$ was added. For each sample, a blank sample was prepared using 2.00 mL of undiluted mead and 2.00 mL of distilled water. After 30 min of incubation, absorbance readings were taken at 415 nm using distilled water as a blank. The total flavonoid content, expressed as milligrams of quercetin equivalent per 100 mL of mead (mg QE 100 mL^{-1}), was calculated using a calibration curve prepared with quercetin (0 - 0.05 mg mL^{-1}; $y = 6.578157x + 0.001198$; $R^2 = 0.990827$) that was analyzed in the same manner as the extracts.

Determination of antioxidant activity (DPPH assay)

The antioxidant activity of the samples was measured using a DPPH assay using the method of Zhang and Hamauzu (2004) with some modification. The mead samples were diluted in deionized water (1:10, v/v); a 0.4 mL aliquot of this solution was mixed with 1.6 mL of ethanol and 0.2 mL of 1.2 mmol L^{-1} DPPH (2,2-diphenyl-1-picrylhydrazyl) solution. The mixtures were left for 30 min at room temperature. After 30 min of incubation at room temperature in a dark place, the absorbance values were measured at 517 nm in a UV/Vis spectrophotometer (NOVA3300) against the reference mixtures that were prepared similarly, replacing the DPPH solution with ethanol. As a standard, a calibration curve was prepared with

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α-Tocopherol (vitamin E) in an ethanolic solution of 0.2 mmol L^{-1}, with concentrations varying between 0 and 0.5 mg mL^{-1} ($y = -1.720335x + 1.206273$, $R^2 = 0.972433$). The DPPH activity was expressed as milligrams of α-Tocopherol equivalent per 100 mL of mead (mg T 100 mL^{-1}).

Determination of antioxidant activity (ABTS assay)

The method used to determine the antioxidant activity by the $ABTS^+$ ion followed the methodology described by Re *et al.* (1999). This methodology is based on the generation of $ABTS^+$, which has a blue-green color, through the reaction of $ABTS^+$ with potassium persulfate. With the addition of an antioxidant in the reaction, $ABTS^+$ is reduced to ABTS, thus causing the medium to lose color. After 6 min, absorbance readings were taken at 734 nm using absolute ethanol as a blank. A calibration curve was prepared with α-Tocopherol (vitamin E) solution (0.000–0.100 mg mL^{-1}; $Y = -4.55729x + 1.72879$; $R^2 = 0.98675$). The data were expressed as mg of α-Tocopherol equivalent per 100 mL of mead (mg T 100 mL^{-1}). All measurements were performed in triplicate.

Determination of antioxidant activity (FRAP assay)

The reduction of the ferric ion Fe^{3+} (FRAP) was determined according to the methodology of Rufino *et al.* (2006). The FRAP Reagent consisted of 250 mL of sodium acetate buffer and 0.3 M acetic acid pH 3.6; 4.5 mL of 10 mM TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine) solution; and 25 mL of 2.0 mM ferric chloride solution. The test was carried out in triplicate, subject to reactions in the proportion of 200 µL of the sample diluted 1:10. After 10 min of incubation at 37 °C, the absorbance was measured at 593 nm using 0.5 mL of methanol in 4.5 mL of the FRAP reagent as a blank. Quantitative analyses were performed using the external standard method using ferrous sulfate (0-120 µmol L^{-1}; $Y = 1.66429x + 0.51700$; $R^2 = 0.99059$) as the standard and correlating the absorbance with the concentration. The results were calculated and expressed as micromoles of Fe^{2+} equivalent per 1000 mL of mead (µmol Fe(II) 1000 mL^{-1}).

Statistical analysis

Data for descriptive statistics analyzes were plotted in Microsoft Office Excel software (2016 Microsoft Corporation) and results were expressed as mean ± standard deviation. To evaluate the statistical significance of the differences observed between the means, the SPSS program was used and the Student's t-test was applied, regardless of the significance level of 0.05 (95% confidence).

RESULTS AND DISCUSSION

Physicochemical analyzes

The initial soluble solids content in both meads (with and without the addition of coffee) was approximately 25.5 ºBx and over 90 days, it dropped to 8.2 and 7.1 ºBx for the without and with coffee, respectively. After this period, the alcoholic content was checked and it was confirmed that the control mead (without coffee) reached ºGL of 11.62 ± 0.51, and the mead with coffee reached 13.53 ± 0.97. When compared with current Brazilian legislation, both meads meet the legislative determination that establishes minimum values of 4 and maximum of 14 ºGL (Brasil, 2012). When making a comparison between mead with coffee and mead without coffee, it was possible to observe that the drinks produced achieved a progression of stability in their fermentation process after 25 days.

The fermentation time of meads developed with coffee compared to those without coffee may be related to the fact that the raw material, in addition to the ingredient used for its elaboration, contains non-fermentable carbohydrates such as cellulose and hemicellulose in the structure of its grain. The amount of total soluble solids establishes a fermentative parameter indicating the quantity of sugars present, such as fermentable carbohydrates that are consumed and transformed into ethanol by yeast during fermentation.

Santos *et al.* (2021) found total soluble solids values ranging from 22.6 to 8.6 over a 13-day mead fermentation. The pH ranges observed were from 3.39 to 3.14 and from 3.41 to 3.27 for mead without and with coffee, respectively. Monitoring pH is significant as it indicates optimal working conditions for yeast throughout the fermentation process.

Lower pH variations were observed in the mead sample developed without coffee compared to the control mead. Santos (2019) found similar pH values in the production of mead from different flowerings (high and low) with values ranging from 3.30 to 3.20, and values found by Brunelli (2015), ranging from 3.52 to 3.62. The mead pH is directly related to the honey's pH and the quality of the water used in alcoholic beverage production. Abrupt pH variations can hinder the product development process by interfering with the viability of yeast involved in fermentation (Finco *et al.*, 2015).

The observed physicochemical parameters for both mead samples (with and without coffee) can be seen in Table 2. It is noted that the profiles of moisture, ash, and reducing sugar in mead and mead with coffee showed no statistical difference ($p>0.05$), despite higher values for the sample developed with the adjunct, except for moisture. Thus, it can be said that the moisture value found in mead (88.26%) compared to mead with coffee (86.58%) is higher, resulting from the partial substitution

of water by the adjunct (coffee) in the honey:water (v/v) ratio established in the preparation of the beverage. Additionally, the ash content found in the coffee-added sample was 0.04 compared to conventional mead at 0.20 g L^{-1}, indicating the presence of more minerals in its composition. According to Brasil (2012), the ash content predicted in a mead-type beverage would be around 1.50 g L^{-1}, a value higher than those found in this study. No exact value related to moisture content during mead production was found. Regarding the presence of reducing sugars, mead with coffee obtained a value of 109.71 g L^{-1}, and mead had 95.28 g L^{-1}, indicating a higher presence of carbohydrates due to the adjunct addition. The consumption of reducing sugar by yeast can be influenced by the presence of hexamethylfurfural (HMF), prolonging its lag phase (Allen *et al.*, 2010; Biluca *et al.*, 2014). HMF interferes with enzymatic activity, causing damage to DNA and the synthesis of RNA and proteins, directly reducing microorganism growth (Allen *et al.*, 2010; Modig *et al.*, 2002). According to Wintgens (2009), the higher the amount of reducing sugars in the beverage, the greater the attribute of its sweet taste.

Table 1 – Physicochemical parameters of meads

Parameters	Hidromel without coffee	Hidromel with coffee	P value
Moisture (%)	88.26 ± 0.04	86.58 ± 0.04	0.777
Ash (%)	0.02 ± 0.01	0.04 ± 0.03	0.279
Reducing sugar (g L^{-1})	95.28 ± 0.83	109.71 ± 2.05	0.106
Acidity (mEq L^{-1})	65.71 ± 0.49	64.58 ± 0.48	0.975
Acidity (g of tartaric acid L^{-1})	4.93 ± 0.04	4.84 ± 0.04	0.975

p> 0.05 indicates no statistically significant difference. The t-test was used with a significance level of 0.05 (95% confidence).
Source: Research results.

However, authors point out that high humidity at the end of the fermentative process of the beverage becomes desirable. The high moisture content ensures that the corks do not dry out and serves as a mitigation for oxygen entry, thus preventing the oxidation of the beverage (Iglesias *et al.*, 2014; Katz, 2014). Another important factor to be observed, with significant influence when coffee is added to the product, is its acidity. The chosen coffee variety can vary in acidity, either increasing or decreasing it when added to the product (Perrois *et al.*, 2014). The acidity values presented here were also similar to those found by Bronzatto *et al.* (2014), with values of 69.1 mEq L^{-1} during the development of a long-fermentation mead. This acidity content complieswith Brazilian legislation which determines a maximum of 130 meq L^{-1} (Brasil, 2012).

Antioxidant activity

Table 2 presents the profile of total phenolics, flavonoids, and antioxidant activity by DPPH, FRAP, and ABTS. Within the observed profiles, it was noted that the content of total phenolics differed significantly at a 95% confidence level ($p > 0.05$), with values of 75.45 and 305.85 mg GA 100 mL^{-1}, for mead without and with coffee, respectively. The results of antioxidant activity by the ABTS method also differed statistically between the two samples, with values of 245.67 and 205.47 mg T 100 mL^{-1} mead without and with coffee, respectively. However, flavonoids, DPPH, and FRAP showed no significant differences ($p>0.05$).

ISSN 2238-5630
Brasília-DF, v. 13, n. 3, setembro-dezembro de 2024

Table 2 – Total phenolic compounds, total flavonoids, and antioxidant activity of meads

Parameters	Hidromel without coffee	Hidromel with coffee	P value
Total phenolic compounds (mg GA 100 mL^{-1})	75.45 ± 4.75	305.85 ± 14.35	0.000
Total flavonoids (mg Q 100 mL^{-1})	21.10 ± 1.49	33.47 ± 4.69	0.051
DPPH (mg T 100 mL^{-1})	70.31 ± 14.38	153.01 ± 9.00	0.515
FRAP (µmol L^{-1} de Fe (II) 1000 mL^{-1})	945.14 ± 123.99	1236.08 ± 3.30	0.058
ABTS (mg T 100 mL^{-1})	245.67 ± 2.35	205.47 ± 12.00	0.000

p> 0.05 does not show a statistically significant difference. The t-test was used with a significance level of 0.05 (95% confidence).
Source: Research results.

It can be observed that, except for the antioxidant activity by ABTS, the addition of coffee to mead showed an increase in bioactive compounds in the analyses performed. This results from the fact that coffee is a product rich in chemically active substances that provide flavor, aroma, and bioactive potential to the product when added (Medeiros *et al.*, 2006).

The total phenolic values found in mead with coffee are similar to those found in beverages developed by Chitarrini *et al.* (2020) with values of 304 mg GAE L^{-1}, using honey and honey added with blackcurrant. The presence of total phenolic compounds favored the understanding of the antioxidant activity of the studied mead samples. According to Souza *et al.* (2018), the consumption of beverages rich in bioactive compounds with antioxidant potential is directly related to health benefits for humans.

CONCLUSION

The mead with coffee showed a higher alcohol content compared to pure mead. The increase in ºBx in the samples of meads added with coffee was due to the addition of the adjunct, which also has soluble solids in its composition. The pH variation can also be explained by the presence of naturally occurring acidic compounds in coffee. Physicochemically, the greatest variation was observed in the content of reducing sugars, being higher for the mead with coffee. However, this led to an implication regarding the sweetness level of the beverage. The addition of coffee in the development of mead favored the antioxidant potential of the beverage. This is consistent due to the proven properties of coffee against oxidative stress. Furthermore, the inclusion of coffee in the beverage can enhance its flavor and aroma.

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