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ORIGINAL RESEARCH ARTICLE

Effect of pasteurization, dehumidification, refrigeration and maturation on the composition, quality and acceptability of *Scaptotrigona depilis* honey

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ABSTRACT

Honey from stingless bees has parameters that are different from those produced by *Apis mellifera*. These differences have led to studies of honey conservation techniques. In this context, it is necessary to evaluate whether the use of different conservation processes impacts the quality and chemical composition of this type of honey. The aim of this study was to conduct sensory analysis and determine the chemical profile, quality, and antioxidant activity of *S. depilis* honey subjected to different conservation processes. The physicochemical characteristics were determined in the fresh sample (time-point zero sample) and after freezing, pasteurization, dehumidification and maturation for 180 days. The reducing sugar, apparent sucrose, total acidity, moisture, water-insoluble solids, minerals, diastatic activity, Brix, color and hydroxymethylfurfural (HMF) levels were determined. These samples were also evaluated for their volatile composition and sensory aspects. After 180 days, the processed honeys showed changes in the phenolic content, and dehumidification was the treatment that most interfered with the phenolic content of the samples. The physicochemical properties that presented significant differences were the acidity, moisture, diastatic activity and color for the matured honey and the HMF levels in the dehumidification and pasteurization treatments. The main volatile compounds differed in each treatment in relation to the untreated honey. The sensory acceptance test showed significant differences for some of the attributes evaluated and the preference of consumers was for the untreated honey.

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Volatiles; headspace; quality; phenolics; sensory

Introduction

Honey is a food that has been consumed for thousands of years; ancient rock paintings at the archaeological site of Cuevas de Las Arañas prove its consumption as far back as the Mesolithic period (Dams, 1978). The best-known honey and most marketed worldwide is produced by *Apis mellifera*, but the honey produced by stingless bees has aroused interest for its peculiarities. Its composition varies as a result of many factors, of which the bee species is one of the main ones (Taha et al., 2020).

The honey from honey bees, *Apis mellifera*, differs from stingless bees mainly in regards to its moisture content, which is the main challenge for its commercialization, since it is the expressive moisture content that causes instability in the product over time and makes this product susceptible to fermentation. In an attempt to overcome this adversity, good harvesting practices are necessary in order to reduce contamination by microorganisms, and processing should be carried out to aid the conservation of the product (Contrera et al., 2011; Venturieri et al., 2007). Nogueira-Neto (1997) describes pasteurization as a way of preserving meliponine honey. Dehumidification and maturation are described by Villas-Bôas (2012). Refrigeration is the simplest method, but it has limitations in relation to the commercialization of honey, since the supplier would have to refrigerate the honey shortly after it is harvested (Villas-Bôas, 2012).

Souza et al. (2021) published a review containing the physicochemical properties of honeys from different species of stingless bees from different countries. The data presented demonstrate that the physicochemical parameters of fresh honeys vary between different bee species and that treatments such as heating, pasteurization, dehumidification and maturation affect the physicochemical properties of the honey. The conservation techniques for honey of stingless bees can affect the chemical composition; however, in the literature, existing studies relate only to the effect of conservation methods on physicochemical properties and sensory analysis, because the main objective is to adapt the parameters and reduce the moisture content (Alves et al., 2012;

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Braghini et al., 2021; Ribeiro et al., 2018). In this context, the use of the methods aims to choose the technique that offers stability in conservation, as well as the preservation of therapeutic and organoleptic properties that promote the acceptability of the product.

Some studies have evaluated the stability of honey after the use of different processing methods and these have led to the discussion of possible alterations that may occur in the honey. In this perspective, the objective of this work is to evaluate the volatile profile, phenolic content and antioxidant activity, as well as the physicochemical properties and evaluate the sensorial analysis of *Scaptotrigona depilis* honey subjected to different conservation treatments.

Materials and methods

Collection

The honey was obtained from 50 colonies of *S. depilis* at the Embrapa Meio Ambiente bee farm in Jaguariúna, São Paulo in October 2020. The colonies are kept in modular hives and the honey was collected from the comb frames, upper compartments that are separated from the rest of the colony. It was collected by the use of a vacuum pump directly from the cells and then homogenized to create a single sample of 12 kg. The purpose of homogenization was to eliminate potential interference from the inherent natural variations observed between colonies. By ensuring uniformity across all the samples before processing, this step aimed to create a standardized baseline, where each sample was consistent with the others. This approach was instrumental in order to maintain the integrity of the results by minimizing the impact of colony-specific differences on the final outcome.

Honey processing

The honey collected in the colonies was divided into five samples:

Untreated

Samples of honey (3×250 g) freshly collected from the colonies was submitted to an analysis of the volatile compounds, the physicochemical characteristics, quantification of the phenolic content and the antioxidant activity. This sample was considered the control and will be known as the untreated sample throughout this work.

The other four samples of honey were processed in four different ways and then analyzed 180 days later.

Refrigeration

Samples of honey (3×250 g) were stored in a clear glass jar with a lid and kept under refrigeration in a household refrigerator.

Dehumidification

Samples of honey (3×250 g) were transferred to rectangular-shaped glass refractories (20×30 cm), evenly distributed in a thin layer (1 cm) and placed in a frost-free refrigerator. To aid the dehumidification, a container with silica gel (100 g) was placed in the refrigerator. The moisture was monitored with a refractometer until a Brix of 70% was obtained and then the sample was transferred to transparent glass bottles with lids and kept at room temperature (25 °C).

Pasteurization

In open transparent glass bottles, portions of honey (3×250 g) were subjected to heating in a water bath at 70 °C for 15 s. Then, the honey was refrigerated in an ice bath for 1 h and the bottle was closed and kept at room temperature (25 °C).

Maturation

Samples of honey (3×250 g) were transferred to a clear glass bottle with a cap (cotton and gauze and kept at room temperature (25 °C).

Quantification of phenolic content

The phenolic content was determined using the spectrophotometric method with Follin-Ciocalteu reagent and a plate reader (Synerg HT, BioTek) and by adapting the methodology described by Pontis et al. (2014). In 96-well plates, aliquots were pipetted for the calibration curve and, to each well, we transferred 100 μL of solutions in increasing concentrations of gallic acid (0.001, 0.002, 0.003, 0.004 and 0.005 $mg.mL^{-1}$), 20 μL of Follin-Ciocalteu and 120 μL of aqueous solution of sodium carbonate 5% (m/v). To complete the volume, 60 μL of distilled water was added. For the samples, 20 μL of each aqueous solution of the honeys (1 g/mL) were added to 20 μL of Follin-Ciocalteu and 120 μL of an aqueous solution of sodium carbonate 5% (m/v) and the volume completed with 140 μL of distilled water. The plate was exposed to light for 2 h, then analyzed at a wavelength of 798 nm. The analyses were performed in triplicate and the content determined by linear regression, with the values being expressed in milligrams of gallic acid equivalent per kilogram of honey (mg $GAE.kg^{-1}$).

Antioxidant activity

Antioxidant activity was determined by the 2,2-diphenyl picryl hydrazyl free-radical sequestration method using a plate reader (Synerg HT, BioTek) according to the methodology described by Mensor et al. (2001), with adaptations. In a 96-well plate, 100 μL of Trolox solutions were pipetted at concentrations of 0.002, 0.004, 0.006, 0.008 and 0.01 mg.mL^{-1}, and 150 μL of a 1 mM DPPH solution was added. From these aliquots, a calibration curve was constructed. For the samples, we added 150 μL of the DPPH radical solution to aliquots of 100 μL of the untreated honey solution and of each treatment in the concentration of 1 g.mL^{-1}. After 30 min of reaction, the plate was analyzed in the plate reader at a wavelength of 515 nm. From the equation of the line obtained from the calibration curve, the antioxidant activity was calculated, and expressed in mg of Trolox/kg of honey.

Physicochemical properties of honey

The determinations were performed in each sample, in triplicate, initially in fresh honey shortly after harvest and after a period of 180 days for the samples submitted to the conservation processes. The following were determined: reducing sugars (CAC/Vol. III, Supl.2, 1990, 7.1), moisture (A.O.A.C., 16th ed., 4th Rev., 1998-969.38B), apparent sucrose (CAC/Vol. III, Supl.2, 1990, 7.2), water-insoluble solids (CAC/Vol. III, Supl.2, 1990, 7.4), minerals (CAC/Vol. III, Supl.2, 1990, 7.5), acidity (A.O.A.C., 16th ed., 4th Rev., 1998-962.19), diastatic activity (CAC/Vol. III, Supl.2, 1990, 7.7), hydroxymethylfurfural (A.O.A.C., 16th ed., 4th Rev., 1998-980.23), Brix (A.O.A.C., 16th ed., 4th Rev., 1998-969.38B) and color (Brasil, 1981).

Extraction of volatile compounds using dynamic collection

For the extraction of volatile compounds using dynamic collection, a nitrogen (N_2) stream at a flow of 1 mL.min^{-1} and a magnetic stirrer were used. In a 100 mL two-neck round-bottom flask, we added 50 g of honey dissolved in 30 mL of 10% sodium chloride solution. Glass tubes (5 cm) packed with 50 mg of the adsorbent Porapak-Q were connected to the flask with the aid of reducing gaskets and hoses. The tube connected to the gas inlet was used as the blank and the other tube concentrated the volatile compounds of the sample. After 3 h, the adsorbed volatiles were extracted with twice-distilled dichloromethane (1 mL) and concentrated with nitrogen (N_2) and then analyzed *via* gas chromatography coupled to mass spectrometry.

Analysis by gas chromatography coupled to mass spectrometry

A gas chromatograph (Shimadzu, GC-2010) coupled to a mass spectrometer (Shimadzu, QP2010 Plus) was used for the analysis of the volatile compounds. Separation was performed using a fused silica capillary column (RTX-5MS, 30 $m \times 0.25$ mm $\times$ 0.25 μm). The injector temperature was 220 °C, the interface temperature was 280 °C and the column temperature was programmed to increase from 35 °C to 220 °C at 3 °C.min^{-1}. When this temperature was reached, the ramp rate was 20 °C.min^{-1} until 310 °C. Helium was used as the drag gas at a constant flow rate of 1.02 mL.min^{-1}. The mass spectra were acquired in the range of m/z 40–600 using electronic ionization with an ionization power of 70 eV and the ion source at 260 °C.

Determination of volatile compounds

The composition of the volatile compounds was determined by comparing the values of their retention indices obtained from a homologous series of *n*-alkanes (C_7-C_{30}), which were analyzed under the same conditions and calculated according to the Van den Dool and Kratz equation, in addition to comparing the mass spectra with data from the Wiley 8 and FFNSC 1.2 digital libraries, from the NIST database and with data from the literature (Adams, 2017).

Sensory analysis

The untreated and processed honeys were subjected to sensory analysis by a team of 20 untrained tasters who were recruited after completing the online interview form (https://forms.GLE/XCGSuY4n3hYuEgRz7). The tasters first signed the informed consent form to participate in the research, and then the order of preference and acceptability tests were applied using a hedonic scale. The order of preference test was used to determine the preferences between each sample. The adjudicator ordered the samples by establishing a descending scale from the most preferred to the least preferred samples (Minim, 2006). The 9-point structured hedonic scale, from I disliked it a lot (1) to I liked it very much (9), evaluated the parameters of color, aroma, flavor, acidity and overall appearance (Chaves, 2001) of the honey after being subjected to different treatments.

The testers received approximately 5 mL of each sample at a temperature of 25 °C on disposable spoons that were coded with random three-digit numbers (Minim, 2006). This study was approved by the Ethics Research Committee of the Federal University of Roraima (CAAE number: 52739921.0.0000.5302).

Statistical analysis

To analyze the results of the quantification of phenolic contents and antioxidant activity, as well as the physicochemical aspects, the data were submitted to one-way analysis of variance (ANOVA), and the means were evaluated using the Tukey test at 5%. The ordering test data were evaluated based on Friedman's order sum test, as described by Minim (2006). One-way ANOVA and Tukey's test at 5% significance were used to evaluate the data of the hedonic scale acceptability test. All data were evaluated using the program Sistema para Análise De Variância (SISVAR) (Ferreira, 2011).

Results

There were variations in honey properties over time, some properties were further modified while others suffered very subtle variations.

Quantification of phenolic contents and antioxidant activity

Scaptotrigona depilis honey submitted to different treatments had its phenolic content quantified, for which the values are listed in Table 1. The antioxidant activity values, determined by the free-radical sequestration method 2,2-diphenyl picryl hydrazyl, are also presented.

Statistical difference was observed among the treatments studied. The dehumidified honey presented a lower phenolic content and, consequently, lower antioxidant potential. For the other treatments, there were no significant differences in antioxidant activity. However, the phenolic content of the honeys subjected to the different treatments was very close to the values observed in the untreated honey, demonstrating that, in general, preservation of these important natural molecules occurred.

Physicochemical parameters

The physicochemical parameters (reducing sugars, free acidity, diastatic activity, ash, insoluble solids, sucrose, HMF, Brix, color and moisture) of the honey submitted to the conservation processes of pasteurization, dehumidification, refrigeration and maturation, in addition to untreated honey, are listed in Table 2. The conservation processes in the honey of stingless bees aim to confer greater food safety, preventing the proliferation of undesirable microorganisms, and making it more stable over time. In this sense, some important changes are observed when evaluating the quality of untreated honey and honey after the conservation treatments.

The free acidity in the matured honey was significantly higher than in the untreated honey; while, for the other treatments, this parameter presented lower values than those of the untreated honey, thus showing that maturation provides a more acidic honey. However, the honey of the other treatments has lower acidity compared to the untreated honey.

The diastatic activity of the matured honey was the lowest compared to the others, and its moisture content was the highest, in addition to the color, which differs from the other samples. This treatment was the one that presented the greatest alterations in the physicochemical properties. Pasteurization and dehumidification increased the hydroxymethylfurfural (HMF) content of the samples, with a statistical difference in relation to the other treatments.

Table 1. Quantification of phenolic contents and antioxidant activity in *S. depilis* honey submitted to different treatments.

	Phenolic content (mg GAE.Kg^{-1})	DPPH$^{\cdot}$ (mg TE.Kg^{-1})
Untreated	442.89 ± 0.01^{b}	23.53 ± 0.12^{a}
Refrigerated	480.88 ± 0.05^{a}	23.21 ± 0.11^{a}
Matured	445.81 ± 0.15^{b}	21.59 ± 0.08^{ab}
Pasteurized	479.90 ± 0.015^{a}	22.56 ± 0.07^{ab}
Dehumidified	397.11 ± 0.02^{c}	20.70 ± 0.04^{b}

Note: The values mean ± standard deviation. Different small letters on the same column indicate significant difference ($p \leq 0.05$) at 5% probability.

Volatile compounds in the honey

A total of 42 volatile compounds were identified in *S. depilis* honey, which is equivalent to more than 85% of the compounds detected, in which their contents varied among the samples processed after the 180-day period. Table 3 shows the compounds identified in the honeys.

As its main constituents, the honey in the untreated form presented 2-methyl heptan-3-one (28.09 ± 0.57%), ethyl phenylacetate (10.50 ± 0.29%), hotrienol (10.09 ± 0.09%) and 2,7-dimethyl-4,5-octandiol (7.34 ± 0.39%), while in the matured honey hotrienol (28.09 ± 36.94 ± 0.29%), 4-heptanone, 2,6-dimethyl (26.16 ± 0.81%), and phenethyl alcohol (5.42 ± 0.11%) were predominant. As its main compounds, refrigerated honey has 2,6-dimethyl heptan-4-one, (41,83 ± 0,28%), 2,7-dimethyl-octan-4,5-diol (28.43 ± 0.51%) and linalool (4.41 ± 0.09%). In the dehumidified honey, 2,7-dimethyloctan-4,5-diol (34.72 ± 0.08) and 2,6-dimethyl heptan-4-one, (33.12 ± 0.52) also predominate, in addition to phenethyl alcohol (7.65 ± 0.19%). The main volatile compounds of pasteurized honey were 2-methyl heptan-3-one (34.20 ± 0.20%), 2,7-dimethyl octan-4,5-diol (31.68 ± 0.13%) and hotrienol (13.04 ± 0.08%).

Table 2. Physicochemical parameters of untreated honey of *S. depilis* and honey submitted to different conservation treatments.

	Reducing sugar (g/100g)	Free acidity (Meq/Kg)	Diastatic activity (Göthe)	Minerals (ash) (g/100g)	Insoluble solids (g/100g)	Sucrose (g/100g)	HMF (mg/Kg)	Brix (%)	Humidity (g/100g)	Color
Untreated	66.65 ± 0.13^{a}	29.39 ± 0.11^{b}	9.71 ± 0.23^{a}	0.42 ± 0.04^{b}	0.06 ± 0.008^{a}	1.41 ± 0.46^{a}	1.20 ± 0.61^{b}	74.05 ± 0.041^{a}	24.35 ± 0.01^{b}	Amber
Refrigerated	68.97 ± 1.98^{a}	26.26 ± 0.56^{b}	8.90 ± 0.31^{a}	0.74 ± 0.07^{a}	0.08 ± 0.01^{a}	0.71 ± 0.17^{a}	1.02 ± 0.45^{b}	74.08 ± 0.03^{a}	23.98 ± 0.04^{b}	Amber
Mature	66.85 ± 0.63^{a}	117.02 ± 6.06	1.70 ± 0.74^{b}	0.74 ± 0.05^{a}	0.06 ± 0.01^{a}	0.43 ± 0.0001^{a}	1.61 ± 0.74^{b}	70.03 ± 0.34^{b}	28.26 ± 0.29^{a}	Dark Amber
Pasteurized	67.61 ± 2.98^{a}	24.21 ± 1.38^{b}	7.57 ± 0.87^{a}	0.48 ± 0.04^{ab}	0.06 ± 0.000^{a}	0.58 ± 0.17^{a}	10.17 ± 0.70^{a}	74.79 ± 0.01^{a}	23.58 ± 0.02^{b}	Amber
Dehumidified	71.34 ± 0.63^{a}	25.95 ± 2.94^{b}	5.13 ± 3.29^{ab}	0.56 ± 0.15^{ab}	0.06 ± 0.01^{a}	0.49 ± 0.01^{a}	10.81 ± 0.28^{a}	78.30 ± 0.43^{a}	20.06 ± 0.43^{c}	Amber

Note: The values mean ± standard deviation. Different small letters on the same column indicate significant difference ($p \leq 0.05$) at 5% probability.

Table 3. Volatile compounds extracted *via* the dynamic headspace of *S. depilis* honey subjected to different conservation processes.

	Compounds	RI_C	RI_L	Untreated	Matured	Refrigerated Area (%)	Dehumidified	Pasteurized
1	2-Hydroxy-3-pentanone[b]	828	821					0.29 ± 0.15
2	Ethyl 2-hydroxypropanoate[b]	833	836	4.12 ± 0.04	3.00 ± 0.25			
3	Heptane-2,3-dione[b,c]	847	–	0.47 ± 0.03	0.59 ± 0.01	0.92 ± 0.11	0.57 ± 0.02	0.42 ± 0.03
4	Heptan-4-one[b,c]	857	860	3.45 ± 0.13		3.28 ± 0.19	3.34 ± 0.08	2.89 ± 0.01
5	Ethyl isovalerate [b]	861	858	1.89 ± 0.16	0.72 ± 0.06			
6	2-Methyl hexane-3-ol[b]	865	858	2.88 ± 0.21		3.98 ± 0.23	5.84 ± 0.06	4.78 ± 0.11
7	3-Methyl butanoic acid[b,c]	868	875	0.26 ± 0.02	0.18 ± 0.07			
8	Hexan-1-ol[b,c]	873	867		0.19 ± 0.01	0.15 ± 0.01		0.14 ± 0.02
9	Heptan-2-one[b,c]	891	889			0.33 ± 0.02		0.09 ± 0.00
10	Heptan-4-ol[b,c]	893	889				0.08 ± 0.00	
11	Heptan-2-ol[b,c]	899	901		0.63 ± 0.03		0.10 ± 0.05	0.30 ± 0.10
12	2-Butoxy ethanol[b,c]	904	904			0.31 ± 0.09		
13	2,6-Dimethyl heptan-4-one[b,c]	940	943		26.16 ± 0.81	41.83 ± 0.28	33.12 ± 0.52	
14	2-Methyl heptan-3-one[b,c]	942	938	28.09 ± 0.57				34.20 ± 0.20
15	2,7-Dimethyl octan-4,5-diol[b,c]	943	944	7.34 ± 0.39	2.65 ± 0.17	28.43 ± 0.51	34.72 ± 0.08	31.68 ± 0.13
16	Benzaldehyde[a]	955	960	0.55 ± 0.39			0.47 ± 0.25	
17	2-Hydroxy-3-methyl butanoic acid ethyl ester[b,c]	962	968	2.55 ± 0.03	0.39 ± 0.04			
18	Mesitylene[c]	987	995	0.65 ± 0.02				
19	Benzyl alcohol[a,b]	1029	1026	0.95 ± 0.09				
20	2,6-Dimethylheptan-4-ol[b,c]	1031	–		2.68 ± 0.19			
21	Ethyl 2-hydroxycaproate[b,c]	1053	1061	1.02 ± 0.02				
22	*cis* Linalool oxide[a]	1066	1072	2.92 ± 0.18	3.04 ± 0.03	0.51 ± 0.04	0.31 ± 0.01	1.00 ± 0.01
23	Tetramethyl pyrazine[b,c]	1080	1086	0.34 ± 0.02	0.27 ± 0.04	0.45 ± 0.07	0.53 ± 0.08	0.32 ± 0.01
24	*trans* Linalool oxide[a]	1082	1086	1.23 ± 0.06	1.12 ± 0.08	0.51 ± 0.05	0.60 ± 0.05	0.51 ± 0.01
25	Linalool[a]	1094	1096	0.49 ± 0.10	1.45 ± 0.20	4.41 ± 0.09	0.36 ± 0.01	0.82 ± 0.01
26	Hotrienol[a,b,c]	1100	1103	10.09 ± 0.09	36.94 ± 0.29	1.09 ± 0.08	0.44 ± 0.02	13.04 ± 0.08
27	1,3-Dioxolane-2-methanol, 2,4-dimethyl[c]	1105	–				0.58 ± 0.15	
28	Phenethyl alcohol[b,c]	1108	1108	3.66 ± 0.2	5.42 ± 0.11	1.53 ± 0.06	7.65 ± 0.19	0.80 ± 0.01
29	Isophorone[b,c]	1113	1121	0.35 ± 0.04	0.35 ± 0.03	0.15 ± 0.04	0.13 ± 0.03	0.28 ± 0.01
30	Isophorone <4-keto->[b]	1137	1145	0.69 ± 0.05		1.49 ± 0.03	0.82 ± 0.07	1.58 ± 0.02
31	Lilac aldehyde B[b,c]	1145	1154				0.25 ± 0.02	
32	Isoneroloxide[b,c]	1149	1147		0.45 ± 0.02			
33	Dihydrooxophorone[b,c]	1161	1170		0.43 ± 0.03	0.43 ± 0.06	0.09 ± 0.01	0.14 ± 0.01
34	Ethyl benzoate[b,c]	1165	1169	3.16 ± 0.07	0.36 ± 0.04			
35	Nonan-1-ol[a]	1166	1169	0.31 ± 0.05	0.27 ± 0.02	0.26 ± 0.05	0.12 ± 0.01	0.25 ± 0.06
36	3,7-Dimethylocta-1,5-dien-3,7-diol[b,c]	1184	1176		0.86 ± 0.05			
37	Hex-3(Z)-enyl butyrate[b,c]	1185	1186	0.7 ± 0.04		0.69 ± 0.01	0.12 ± 0.02	0.25 ± 0.02
38	Verbenone[a,b]	1211	1205					0.45 ± 0.02
39	Ethyl-phenylacetate[b,c]	1240	1246	10.50 ± 0.29	4.02 ± 0.02			
40	Acetic acid, 2-phenylethyl ester	1251	1256		0.27 ± 0.08			
41	3-Hydroxy-4-phenyl-2-Butanone[b,c]	1337	1342	0.33 ± 0.02		1.36 ± 0.04	0.50 ± 0.05	0.99 ± 0.06
42	Benzenepropanoic acid, ethyl ester[b,c]	1342	1348	0.39 ± 0.01				
	Percentage of compounds identified			88.87	92.59	92.12	90.72	95.35

Note: RI_C: calculated retention index; RI_L: literature retention index; [a]identification *via* comparison with Adams (2017); [b]identification *via* comparison with the NIST library; [c]identification using the Willey library or FFNSC.

Sensory analysis

Table 4 shows the results of the ordering test used to evaluate the preference of the four conservation processes applied to *S. depilis* honey; the control sample was used to compare the preferences between treated honeys. Values followed by the same letter do not differ from each other at 5% significance in accordance with the results of the Friedman test.

Table 4. Sensory evaluation of processed samples of *S. depilis* honey showing preference by ordering.

	Treatment				
	DEH	PAS	UNT	REF	MAT
Total of orders	60^{b}	58^{b}	44^{b}	63^{b}	76^{a}
Difference vs. dehumidified	–	2^{ns}	16^{ns}	3^{ns}	16^{ns}
Pasteurized	–	–	14^{ns}	5^{ns}	18^{ns}
Untreated	–	–	–	19^{ns}	32^{*}
Refrigerated	–	–	–	–	13^{ns}

ns = not significant. Critical absolute value of minimum significant difference (MSD) $\alpha = 28$ (NEWELL; MACFARLANE, 1987. DEH – dehumidified; PAS – pasteurized; UNT – untreated; REF – refrigerated and MAT – matured.

In the evaluated samples, the moduli of the differences were lower than MSD = 28 (minimum significant difference) in the dehumidified, pasteurized, untreated and refrigerated processes; these being preferred to honey submitted to the maturation process. In the ordering test, the lowest totals indicate the most preferred formulations, while the highest totals indicate the least preference. This observation is possible from the analysis of the frequency of orders, in which it was observed that the untreated sample was in the first place more often, followed by the dehumidified and refrigerated samples and, finally, the pasteurized sample. In relation to the matured honey, this was significantly ordered as the sample most rejected by the tasters, i.e., the least accepted by the tasters.

The acceptability evaluated using the hedonic scale showed a significant difference in the attributes of color, flavor and overall appearance. However, there is no statistical difference for the aroma and acidity attributes according to the tasters' perception (Table 5), although the matured honey was more acidic than other samples according to chemical tests established in this study.

The averages of the scores given for the attributes color, flavor and overall appearance showed similar patterns of preference, in which untreated honey was classified as "I liked it very much" on the structured 9-point scale. The averages of the dehumidified, pasteurized and refrigerated treatment processes do not differ significantly from those of the untreated honey.

Discussion

Studies related to the determination of the phenolic content, volatile composition, physicochemical properties as well as the antioxidant potential of honey from stingless bees after thermal and physicochemical processing and shelf life were rare, and this evidences the importance of studies to evaluate the stability of these compounds after processing and storage.

Quantification of phenolic contents and antioxidant activity

From the data obtained, it can be said that the treatments and the storage period did not alter the antioxidant activity to a great extent, which is important data for the conservation of its biological properties. Silva et al. (2023) performed the maturation of *Melipona mondury* honey and verified that there was a decrease in the phenolic content during the maturation period. This did not occur with *S. depilis*, whose content of these compounds did not vary significantly in the matured sample (445.81 ± 0.15) compared to the untreated sample (442.89 ± 0.01). The antioxidant activity of *M. mondury* honey showed the same behavior as phenolic compounds with decreased potential throughout the analysis period. In the matured sample, the antioxidant activity was not significantly altered when compared to untreated honey.

Table 5. Average score for the attributes appearance, aroma, flavor, color and overall appearance of untreated *S. depilis* honey and after being submitted to refrigeration, dehumidification, pasteurization and maturation treatments.

	Color	Flavor	Aroma	Acidity	Overall appearance
Untreated	7.85^{a}	7.35^{a}	7.60^{a}	6.40^{a}	7.65^{a}
Refrigerated	7.00^{ab}	6.25^{ab}	7.00 ^{a}	5.85^{a}	6.65^{ab}
Matured	6.50^{b}	5.60^{b}	6.65 ^{a}	5.25^{a}	5.85^{b}
Dehumidified	7.55^{ab}	6.55^{ab}	6.85 ^{a}	6.75^{a}	6.90^{ab}
Pasteurized	7.30^{ab}	6.45^{ab}	7.15 ^{a}	6.35^{a}	6.75^{ab}

Note: The values mean ± standard deviation. Different small letters on the same column indicate significant difference ($p \leq 0.05$) at 5% probability.

Physicochemical parameters

The parameters reducing sugar, insoluble solids, and sucrose were not significantly altered in the storage process of *S. depilis* honeys; however, other parameters have been modified in some of the conservation methods.

HMF is an important indicator of honey quality and its content increases with the heating of the product or during its storage period (Silva et al., 2008). This compound is present in honey as a result of the Maillard reaction or dehydration of hexose in an acidic medium. The content of this substance has great importance in the quality control of honeys (Biluca et al., 2016). The honeys of *S. depilis* suffered changes in HMF content when comparing untreated honey (1.20 ± 0.61) with pasteurized (10.17 ± 0.70) and dehumidified (10.81 ± 0.28) honey.

In matured *S. depilis* honey, there was a change in color and sucrose levels, increased acidity and decreased diastatic activity. da Silva et al. (2022) performed treatments on *Melipona quadrifasciata* honey and found increased acidity, darkening and increased HMF content in matured honey samples. Matured honey from *S. depilis* did not show this increase in HMF content. Pasteurization and dehumidification of *Melipona quadrifasciata* honey increased HMF levels, as in the data obtained for *S. depilis*. The cooling of honey from *S. depilis* did not significantly alter the acidity of honey, which occurred in honey from *M. quadrifasciata*.

Compared to the results obtained by Menezes et al. (2018), who employed the pasteurization treatment for honey from *Melipona fasciculata* and *Melipona flavolineata*, the results showed that the process significantly influenced the HMF content (9.43 ± 0.09 and 43.10 ± 0.85, respectively). In addition, moisture and apparent sucrose also underwent significant changes when compared with unpasteurized honey, unlike the results found for pasteurized

honey from *S. depilis,* which did not present significant changes for these last properties.

Regarding dehumidified honey, the studies of Alves et al. (2012) showed that this process confers good stability for the properties moisture, reducing sugars, apparent sucrose, acidity and HMF during a storage period of 180 days for honey from *Tetragonisca angustula*. According to the studies conducted by Braghini et al. (2021), the thermal treatment used to preserve honey from *Tetragonisca angustula* minimally affected the physicochemical properties evaluated, and the absence of HMF in honey after heating can be highlighted.

Ribeiro et al. (2018) evaluated the influence of refrigeration, pasteurization and maturation on the physicochemical properties and the acceptability of honey from *M. fasciculata*. Honey matured at 30 °C did not cause significant alterations in its physicochemical properties, though its acidity content showed a slight increase from 23.87 ± 1.21 to 26.10 ± 1.20 when compared to untreated honey. This is different from what was observed for mature *S. depilis* honey, which had its acid content significantly elevated, from 29.39 ± 0.11 to 117.02 ± 6.06. In relation to refrigerated honey, the authors observed that this treatment affected the moisture levels and increased the content of reducing sugars. In the other treatments, there was a reduction in these properties, especially for maturation at 30 °C, though these changes were not observed in refrigerated *S. depilis* honey.

Determination of volatile compounds

In general, when comparing the untreated honey with the composition of honeys that were submitted to conservation processes, it is observed that 16 compounds are not present in untreated honey, and others had a significant increase in their concentrations. For example, we have hotrienol, which showed a considerable increase in the matured sample, but in the dehumidified and refrigerated samples its content was significantly decreased. The presence of this compound in *Apis mellifera* honeys has been associated with different botanical origins, and this is reported as something typical of citrus honey, in which hotrienol was found in a high proportion (Alissandrakis et al., 2007, 2009). However, other studies show that this compound may be a degradation product of honey that is produced by thermal variations. According to Jerković et al. (2010, 2014), hotrienol is the product of the dehydration of 3,7-dimethylocta-1,5-diene-3,7-diol (terpenediol I), and the hot and acidic conditions of the hive can promote dehydration of the diol, which is its precursor. In this perspective, it would be expected that the concentration of hotrienol in the pasteurized sample would be higher, considering that in this treatment the honey is subjected to heating; however, in this treatment the increase was small. On the other hand, in the matured honey, the increase was very significant, thus suggesting that the honey fermentation process was the determining factor in the production of this compound.

Ethylphenylacetate, which is one of the predominant constituents in the untreated honey, was only detected in matured honey, though in lower amounts. This compound is described as the odor associated to honey, and it is present in honey-flavored beers. In addition, ethylphenylacetate has been detected in high concentrations among the volatile compounds of wine samples, and it is responsible for giving wine the characteristic taste and odor of honey (Campos et al., 2012).

The results indicate that, after 180 days, the processed honeys have a composition that differs from the untreated honey, and exclusive compounds are observed mainly in the dehumidified honey. In all, four compounds present only in this sample were identified. In the matured honey, only three compounds were detected, while in refrigerated and pasteurized honeys, there was only one exclusive compound.

This is the first report of the volatile compounds of *S. depillis* honey after being submitted to pasteurization, dehumidification, refrigeration and maturation. The observed differences are an important contribution to the knowledge of the composition of the honey from this bee and show the need for further studies to understand the occurrence of certain compounds in different processes. Because it is a complex matrix, there are numerous possibilities for the origin of compounds in honey, given that it has a microbiota capable of promoting different types of reactions; in addition, storage conditions, as well as duration of storage are also factors that influence the occurrence of different compounds.

Sensory analysis

Regarding the sensory analysis of *S. depilis* honey samples, it can be verified that the lowest averages were related to the matured honey, which may be related to the higher acidity index, more intense aroma and darker color, which was observed in the physicochemical analysis and its volatile compound composition. Sensory analysis applied to honey from stingless bees plays an important role in the honey quality profile, as it determines consumer acceptance.

According to Deliza and Vit (2012), sensory attributes in terms of appearance, aroma, taste and texture vary from product to product, revealing the need to investigate each honey to better understand its characteristics. In studies conducted by Carvalho

et al. (2009) with honey from *Melipona scutellaris* and *Melipona quadrifasciata* submitted to the dehumidification process, sensory analysis indicated that the conservation process did not interfere with the acceptability of the product. In the studies of Ribeiro et al. (2018), the acceptance of samples of *Melipona fasciculata* honeys after the application of conservation, refrigeration, pasteurization and maturation methods at two temperatures, was good, with hedonic values equal to or greater than 6.0.

The sensorial analysis applied by Pires et al. (2020) to honey from two species of stingless bees, *Scaptotrigona* sp. and *Melipona interrupta,* showed a greater acceptance of *M. interrupta* honey when subjected to refrigeration and pasteurization. For *Scaptotrigona* sp., the preference was for pasteurized honey and had a positive correlation with the aroma of honey.

Conclusions

The study allowed us to determine that the acceptance profile, chemical profile, quality, and antioxidant activity are modified by the different conservation methods. The phenolic content and antioxidant activity showed little change as a result of the treatments used for honey conservation. The physicochemical properties suffered the greatest change in the free acidity and color of matured honey and hydroxymethylfurfural in pasteurized and dehumidified honeys. Regarding the profile of the volatile compounds, the study reveals the importance of a greater depth of study for determining the origin of the constituents, though it still presents relevant results in relation to the interference of treatments in the composition of the aroma of honey. The sensorial analysis points to a good acceptance of the samples from the pasteurized and dehumidified treatments; however, the judges' preference was for untreated honey.

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