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Viscosity of *Apis mellifera* honey from different floral origins

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Abstract

Viscosity is a critical parameter that quantifies the flow rate of a liquid under an applied force, influenced by various factors including chemical composition, temperature, and humidity. This rheological property is particularly significant in the honey industry as it plays a crucial role in the design and optimization of processing equipment. The primary aim of this study was to characterize the viscosity of honey produced by *Apis mellifera*, sourced from various botanical origins, including organic varieties. The floral sources of the honey samples analyzed comprised eucalyptus (n = 8), wildflower (n = 8), orange blossom (n = 7), and organic production (n = 8). The results revealed that honey derived from orange blossoms exhibited the lowest viscosity, measured at 1,385.07 mm²/s, while organic honey displayed the highest viscosity, recorded at 4,344.78 mm²/s. Furthermore, organic honey demonstrated the highest concentration of hydroxymethylfurfural (33.59 mg/kg) alongside the lowest moisture content (18.20%). These findings suggest that the lower moisture content and varied chemical composition of honey contribute to enhanced crystallization processes, resulting in increased viscosity.

Keywords: composition; crystallization; properties; quality.

Practical Application: This study identifies key factors influencing honey viscosity, aiding in processing optimization and quality assessment.

1 INTRODUCTION

Floral honey is a naturally sweet, high-viscosity substance produced by bees through the collection, transformation, and maturation of flower nectar within their hives (Se et al., 2019; Zhang et al., 2021). This complex matrix primarily consists of simple sugars, chiefly fructose, and glucose, alongside amino acids, minerals, and vitamins, which confer significant nutritional and therapeutic benefits (Pasupuleti et al., 2017).

The sensory attributes of honey are profoundly influenced by the species of pollen-producing flowers, the specific bee species involved, and the climatic conditions and geographical context of production (da Silva et al., 2016). Honey is classified as monofloral when it predominantly originates from the nectar of a single plant species, thereby imparting unique aroma and flavor profiles associated with particular bloom periods, such as those of orange blossom and eucalyptus honey. Conversely, honey is categorized as multifloral or wildflower when it includes pollen from multiple plant species, resulting in diverse flavor characteristics reflective of the local flora (Kadri et al., 2016).

Viscosity, a fundamental rheological property, quantifies a liquid's resistance to flow under applied force; higher viscosity indicates greater flow resistance (Lahoud & Campos, 2010). This property is influenced by various factors, including chemical composition, temperature, and humidity (Oroiam, 2015). In the honey industry, viscosity is critical throughout all stages of production, including extraction, filtration, pumping, processing, and packaging. It significantly affects the design and selection of equipment utilized across the production chain (Anupama et al., 2003; Nayik et al., 2016).

The Botucatu region is recognized as one of the primary honey-producing areas in São Paulo, Brazil, particularly noted for its eucalyptus monofloral honey. The municipality is characterized by extensive apicultural pastures that ensure a rich availability of flowering plants throughout the year (dos Reis & De Paula Aragão, 2015), supported by a humid subtropical climate featuring dry winters and rainy summers (Franco et al., 2023).

The composition of honey is crucial in determining its final characteristics, with viscosity being a key rheological parameter of substantial importance to the honey industry. Given the significant role of Botucatu in the state's apicultural landscape, this study aims to characterize the viscosity of honey from *Apis mellifera* with various botanical origins, including organic varieties, produced in the Botucatu region of São Paulo, Brazil.

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2 MATERIAL AND METHODS

2.1 Samples

A total of 31 honey samples from four distinct floral origins in the Botucatu region, São Paulo, Brazil, were analyzed: eucalyptus ($n = 8$), wildflower ($n = 8$), orange blossom ($n = 7$), and organic ($n = 8$). These samples were submitted to the Food Physicochemical Laboratory of the Public Food Guidance Service (SOAP), within the Department of Preventive Veterinary Medicine at the School of Veterinary Medicine and Animal Science at Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), Botucatu Campus, São Paulo, Brazil. Each quantitative analytical assay was performed in triplicate ($n = 93$), and the results were presented as the mean ± standard deviation.

2.2 Determination of viscosity

The viscosity of the honey samples was determined using a Ford cup viscometer (Gehaka®), in accordance with the standards established by ABNT (1986) and ASTM International (2023). Calibration of the equipment and testing procedures were conducted as per the manufacturer's recommendations.

To select the appropriate size of the Ford cup orifice, three aliquots of honey samples were utilized. The flow time of these aliquots through each numbered orifice (numbers 2, 3, 4, 5, 6, 7, and 8) was recorded, and mean values along with standard deviations were calculated for each orifice. The evaluation criteria for orifice selection were based on the following parameters (Gehaka, 2000): (a) a flow time between 20 and 100 s; (b) a standard deviation of less than 3% of the mean, indicating that the orifice could be used without correction; (c) a standard deviation between 3% and 10% of the mean, necessitating correction via a calibration curve; and (d) a standard deviation exceeding 10% of the mean, recommending replacement of the orifice and recalibration of the Ford cup. The selected orifice met the criteria of (a) a flow time within the range of 20–100 s and (b) a standard deviation of less than 3% of the mean (Gehaka, 2000).

To validate the viscosity determination method for honey from various floral origins, several parameters were assessed, including linearity via a standard curve, detection limit, quantification limit, and repeatability (Brasil, 2011, 2014, 2017; SBM, 2022).

Following the selection of the appropriate orifice, the honey samples were heated in a water bath to a maximum temperature of 40°C to facilitate decrystallization and homogenization. The samples were then cooled to 25°C, the temperature at which the viscosity tests were performed.

The Ford cup was leveled, and the orifice was sealed with a finger while the sample was poured in until it reached the highest level, ensuring that air bubbles were not formed. The excess sample was removed using a flat glass plate. Afterward, the finger was removed from the orifice, and a stopwatch was started. The timing was stopped upon the first interruption of the flow. The recorded time (in seconds) was applied to the formula provided by the manufacturer to calculate the viscosity (mm^2/s) based on the selected orifice,

Here, *viscosity* = mm^2/s, t = seconds, and 1 mm^2/s = 1 cST; thus, 1 cST = 0.000001 m^2/s = 1 mm^2/s (Gehaka, 2000).

2.3 Determination of acidity

An amount of 10 g of the honey sample was weighed in a 250-mL glass beaker, to which 75 mL of pure water was added. The sample was dissolved using a glass stirring rod. Approximately 10 drops of 1% alcoholic phenolphthalein solution were added, and the mixture was titrated with a 0.1 N sodium hydroxide solution until a faint pink color persisted. The volume of NaOH consumed was recorded and used in Equation 1:

$$mEq/Kg = V \times cf \times 10 \quad (1)$$

where:

V: volume (mL) of the sodium hydroxide solution used in the titration;

cf: correction factor for the 0.1 N NaOH solution;

10: mass of the sample (Brasil, 1981; Codex, 2022; IHC, 2009).

2.4 Determination of hydroxymethylfurfural

An amount of 5 g of the honey sample was weighed in a 50-mL beaker. Then, 20 mL of pure water was added to dissolve the honey. The resulting solution was transferred to a 50-mL volumetric flask, and the volume was adjusted to the mark with pure water, sealed, and homogenized (Brasil, 1981; Codex, 2022; IHC, 2009). The prepared honey solution was analyzed immediately for the quantification of hydroxymethylfurfural (HMF) using the Reflectoquant® method, in accordance with the manufacturer's instructions (Supelco et al., 2021). The concentration of HMF was reported in mg/kg.

2.5 Determination of moisture

The method utilized for measuring moisture content was based on Abbé refractometry at a controlled temperature of 20°C, with the refractive index interpreted using the Chataway table (Brasil, 1981; IAL, 2008). The temperature was stabilized, and the Abbé refractometer was calibrated at 20°C prior to measurement. A drop of honey was placed on the prism, and the instrument was focused for precise measurement.

The reading obtained from the refractometer scale was converted to moisture content (g/100 g) according to the Chataway table. Temperature corrections were applied: For each degree Celsius above 20°C, a value of 0.00023 was added, while for each degree below 20 °C, 0.00023 was subtracted (Brasil, 1981; Codex, 2022; IAL, 2008; IHC, 2009).

2.6 Determination of color

Approximately 2 mL of the sample was transferred to a 10-mm path-length quartz cuvette. Absorbance was

measured at 560 nm using a spectrophotometer, with pure glycerin as the blank. The absorbance of the sample was analyzed using the Pfund scale (Table 1) to determine the honey color (Brasil, 1981; IAL, 2008; Sechrist, 1925; USDA, 1985).

2.7 Investigation of diastatic

An amount of 5 g of the sample was weighed into a 50-mL clear polyethylene flask. A volume of 10 mL of pure water was added, and the flask was shaken to completely dissolve the honey. A volume of 1 mL of 1% starch solution was added, and the mixture was heated in a water bath at 45°C for 1 h. Subsequently, 1 mL of Lugol's solution was added, and the color change was observed.

Here, the presence of diastatic yeasts in honey was indicated by a color change ranging from olive green to brown, while their absence was indicated by blue.

2.8 Fiehe reaction

An amount of 5 g of the sample was weighed into a 50-mL glass beaker. A volume of 5 mL of ethyl ether P.A. was added, and the mixture was vigorously homogenized using a glass rod. The ether supernatant was transferred to a porcelain dish. After complete evaporation of the ethyl ether, 0.5 mL of 1% resorcinol hydrochloric solution was added. After waiting for 5–10 min, the color at the bottom of the porcelain dish was observed.

Here, a red color indicates a positive reaction, while a yellow or orange color indicates a negative result.

Table 1. Classification of honey color according to the Pfund scale.

Color	Pfund scale (mm)	Absorbance
Water White	1–8	Up to 0.030
Extra White	> 8–17	> 0.030–0.060
White	> 17–34	> 0.060–0.120
Extra Light Amber	> 34–50	> 0.120–0.188
Light Amber	> 50–85	> 0.188–0.440
Amber	> 85–114	> 0.440–0.945
Dark Amber	> 114	> 0.945

Source: Brasil (1981); IAL (2008), Sechrist (1925); USDA (1985).

2.9 Lund test

An amount of 2 g of the sample was weighed into a 50-mL beaker, followed by the addition of 20 mL of pure water to dissolve the sample using a glass rod. The honey solution was transferred to a 50-mL graduated conical glass tube with a cap. A volume of 5 mL of 0.5% tannic acid solution was added, and the volume was brought up to 40 mL with pure water. The tube was capped, homogenized, and then left to rest for 24 h to check for the presence or absence of albuminoid deposits.

Here, in the presence of natural honey, a deposit ranging from 0.6 to 3 mL was formed; no deposit was observed in artificial honey, while in adulterated honey, the deposit volume was less than 0.6 mL (IAL, 2008).

2.10 Statistical analysis

The quantitative values obtained from the samples, which were analyzed in triplicate, were statistically evaluated using analysis of variance (ANOVA) with a completely randomized design, supplemented by Tukey's test for mean comparisons, considering a 5% significance level (Montgomery, 2020). For the qualitative assays, data analyses were performed using absolute and relative frequencies, as well as percentages (%).

3 RESULTS

The best orifice sizes to use with the Ford cup for measuring the viscosities of honey from *Apis mellifera* bees of various floral origins (eucalyptus, wildflower, orange blossom, and organic) are numbers 6, 7, and 8 (Table 2). Orifice number 6 was employed, using the viscosity formula from the manufacturer's manual: viscosity (mm^2/s) = 14.92t – 15.56. The statistical analysis of the orifice data showed a coefficient of variation (CV) of 13.48% and p-value < 0.0001.

The validation of the viscosity method through the parameters of linearity, limit of detection (LD), limit of quantification (LQ), and repeatability is presented in Tables 3–5. For linearity, the correlation coefficient (r), linear coefficient (a), and angular coefficient (b) were 0.9989, -12.0076, and 13.8685, respectively (Table 3). The LD and LQ were 1,231.14 and 3,917.10 mm^2/s, respectively (Table 4). In terms of repeatability, three CVs were obtained: 2.89%, 2.57%, and 2.12% (Table 5).

Table 2. Mean ± standard deviation of flow time (seconds) for three replicates of honey from *Apis mellifera* through orifices numbered 2, 3, 4, 5, 6, 7, and 8 of the Ford cup for standardization of viscosity analysis of honey samples from four different floral origins in the region of Botucatu, São Paulo, Brazil.

Ford cup orifice number	Observation 1	Observation 2	Observation 3	Mean ± standard deviation ($\bar{x} \pm s$)
2	825	917	1020	920.67 ± 97.55^{1} d*
3	565	733	739	679.00 ± 98.77^{1} c
4	469	474	470	471.00 ± 2.65^{2} b
5	388	401	395	394.67 ± 6.51^{2} b
6	93	93	93	93.00 ± 0.00^{2} a
7	99	97	95	97.00 ± 2.00^{2} a
8	81	89	78	82.67 ± 5.67^{3} a

Statistical analysis was complemented by Tukey's test at a 5% significance level; A standard deviation greater than 10% of the mean indicates the need to change the orifice and perform calibration of the Ford cup (Gehaka, 2000); A standard deviation of less than 3% of the mean demonstrates that the orifice can be used without correction (Gehaka, 2000); A standard deviation between 3% and 10% of the mean should be corrected using the calibration curve (Gehaka, 2000); *CV = 13.48%; $p < 0.0001$.

The average viscosity values (mm²/s) for honey from *Apis mellifera* sourced from eucalyptus (2,809.00 mm²/s ± 1,929.44 mm²/s) and organic origins (4,344.78 mm²/s ± 3,269.78 mm²/s) were significantly higher (p = 0.0008) than those from wildflower (2,274.74 mm²/s ± 1,159.67 mm²/s) and orange floral origins (1,385.07 mm²/s ± 1,352.42 mm²/s). Additionally, the honey from *Apis mellifera* originating from orange flowers exhibited the lowest viscosity value when compared statistically to the other honey types (Table 6).

Table 3. Linearity of the standard curve for the determination of viscosity (mm²/s) of honey samples from four different floral origins in the region of Botucatu, São Paulo, Brazil.

Order	Honey concentration (g/100 g)	Mean ± standard deviation (mm²/s)[1]
1	25	342.52 ± 1.81
2	40	551.40 ± 2.42
3	55	715.52 ± 4.67
4	70	969.16 ± 7.01
5	85	1.178.04 ± 32.04
6	100	1.372.00 ± 11.27
Linear coefficient (a)		-12.0076
Angular coefficient (b)		13.8685
Correlation coefficient (r)		0.9989
Equation of the line		Y = 13.8685X - 12.0076

[1]The x-axis represents the concentration (g/100 g) of the aqueous honey solution, and the y-axis represents the viscosity (mm²/s) of the solutions. Measurements were taken using orifice number 6 of the Ford cup, with viscosity calculated using the formula: *viscosity* (mm²/s) = 14.92t – 15.56 (Gehaka, 2000; Vision, 2024).

Table 4. Limits of detection (LD) and quantification (LQ) for the determination of viscosity (mm²/s) of honey from four different floral origins in the region of Botucatu, São Paulo, Brazil.

Observation	mm²/s
1	3,194.06
2	3,727.86
3	2,942.86
4	3,068.46
5	3,225.46
6	3,413.86
7	2,471.86
Mean ± standard deviation	3,149.20 ± 391.71
t (unilateral with 99% confidence)	3.143
Limit of detection (LD) (mm²/s)[1]	1,231.14
Limit of quantification (LQ) (mm²/s)[2]	3,917.10

[1]LD: $t_{(n-1;\ 1-\alpha)}$. s; [2]LQ: 10 . s; The orifice used in the Ford cup was number 6, with the following formula: viscosity (mm²/s) = 14.92t - 15.56 (Gehaka, 2000; Vision, 2024).

Table 5. Repeatability for the determination of viscosity (mm²/s) of honey from four different floral origins in the region of Botucatu, São Paulo, Brazil.

Repetition	Analysis 1 (mm²/s)	Analysis 2 (mm²/s)	Analysis 3 (mm²/s)
1	2,992.06	3,194.06	2,928.86
2	2,828.86	3,127.86	3,068.46
3	2,938.06	2,992.86	3,099.86
4	2,991.66	3,068.46	3,056.86
5	3,099.86	3,125.46	2,991.66
6	2,996.06	3,113.86	2,960.26
7	2,899.46	2,971.86	2,971.86
Mean	2,963.72	3,084.92	3,011.12
Desvio padrão	± 85.77	± 79.34	± 63.94
CV[1]	2.89%[2]	2.57%[2]	2.12%[2]

[1]CV (%) = [Standard Deviation/Mean] x 100; [2]Ideal = CV < 10%; The orifice used in the Ford cup was number 6, with the following formula: viscosity (mm²/s) = 14.92t – 15.56 (Gehaka, 2000; Vision, 2024).

The acidity levels (mEq/kg) of honey from eucalyptus (44.82 mEq/kg ± 11.34 mEq/kg), wildflower (35.90 mEq/kg ± 5.92 mEq/kg), and organic origins (43.05 mEq/kg ± 8.43 mEq/kg) were significantly higher (p = 0.0035) than those from orange floral origins (28.96 mEq/kg ± 5.67 mEq/kg). Notably, the honey from orange flowers exhibited the lowest acidity value (mEq/kg) when statistically compared to the other samples. The CV for acidity determinations was 21.43% (Table 7). The relative frequency of samples exceeding 50 mEq/kg acidity was 0.25 (25%) for honey from both eucalyptus and organic origins (Table 8).

The average HMF content in organic honey (33.59 ± 22.40 mg/kg) was significantly higher (p = 0.0049) compared to eucalyptus honey (3.12 ± 2.88 mg/kg), wildflower honey (18.43 ± 21.56 mg/kg), and orange blossom honey (7.09 ± 9.59 mg/kg). The lowest HMF value was observed in orange blossom honey. The CV for the HMF determinations was 104.43% (Table 7). Table 8 demonstrates that 13% (0.13) and 25% (0.25) of the wildflower and organic honey samples exhibited HMF levels exceeding 60 mg/kg.

Table 6. Means ± standard deviations of viscosity analysis (mm²/s) of honey from four different floral origins in the region of Botucatu, São Paulo, Brazil.

Floral origin of honey	Mean ± Standard Deviation
Eucalyptus	2,809.00 mm²/s ± 1,929.44 mm²/s ab[1]
Wildflower	2,274.74 mm²/s ± 1,159.67 mm²/s a
Orange Blossom	1,385.07 mm²/s ± 1,352.42 mm²/s a
Organic	4,344.78 mm²/s ± 3,269.30 mm²/s b

[1]p = 0.0008 and CV = 77.26%; Values followed by different letters in the same column differ significantly (p<0.05). Statistical analysis was complemented by Tukey's test at a 5% significance level. The Ford cup used had an orifice number 6, and the formula for viscosity (mm²/s) is as follows: viscosity = 14.92t - 15.56 (Gehaka, 2000; Vision, 2024).

Table 7. Means ± standard deviations of acidity (mEq/kg), HMF (mg/kg), and moisture (g/100 g) analyses of honey from four different floral origins in the region of Botucatu, São Paulo, Brazil.

Test	Floral origin of honey	Mean ± standard deviation
Acidity (mEq/kg)	Eucalyptus	44.82 ± 11.34 b[1]
	Wildflower	35.90 ± 5.92 b
	Orange Blossom	28.96 ± 5.67 a
	Organic	43.05 ± 8.43 b
HMF (mg/kg)	Eucalyptus	3.12 ± 2.88 a[2]
	Wildflower	18.43 ± 21.56 ab
	Orange Blossom	7.09 ± 9.59 a
	Organic	33.59 ± 22.40 b
Moisture (g/100 g)	Eucalyptus	18.81 ± 1.07 a[3]
	Wildflower	18.24 ± 0.91 a
	Orange Blossom	17.68 ± 0.38 a
	Organic	18.20 ± 1.70 a

[1]p = 0.0035 and CV = 21.43%; [2]p = 0.0049 and CV = 104.43%; [3]p = 0.3186 and CV = 6.22%; Statistical analysis was complemented by Tukey's test at a 5% significance level. Values followed by different letters in the same column differ significantly (p < 0.05).

The average moisture content of eucalyptus honey (18.81 g/100 g ± 1.07 g/100 g), wildflower honey (18.24 g/100 g ± 0.91 g/100 g), orange blossom honey (17.68 g/100 g ± 0.38 g/100 g), and organic honey (18.20 g/100 g ± 1.70 g/100 g) did not show significant differences (p = 0.3186). The CV for the moisture determinations was 6.22% (Table 7). Additionally, 13% (0.13) and 25% (0.25) of the eucalyptus and organic honey samples had moisture levels greater than 20 g/100 g (Table 8).

The absence of diastatic enzymes was noted in 13% (0.13) of the wildflower honey samples. The Fiehe reaction was present in both wildflower honey (13%) and organic honey (25%). The Lund test was positive in 100% of all honey samples evaluated (Table 9).

In the study of dominant color, the mean absorbance values showed no significant differences (p = 0.2574) among the samples from eucalyptus (1.3156 ± 0.9870), wildflower (1.2473 ± 0.8373), orange blossom (1.0267 ± 0.6543), and organic type (1.6131 ± 0.8710). The CV was 65.37% (Table 10). According to the Pfund scale (Table 1), the mean absorbance values of the honey samples indicated that the dominant color was dark amber (> 114 mm). Supporting this statistical result, Table 11 shows that 63% (0.63), 50% (0.50), 71% (0.71), and 75% (0.75) of the honey samples from eucalyptus, wildflower, orange blossom, and organic type exhibited the dominant color of dark amber.

4 DISCUSSION

Organic honey samples exhibited the highest viscosities, while the orange blossom honey samples had the lowest. Filtration and/or centrifugation with heat are methods commonly employed during the industrial processing of honey to reduce the concentration of impurities that may be present after harvest. These procedures are often associated with the application of heat, for both pasteurization purposes and melting microcrystals of glucose, which are responsible for the natural crystallization of honey (Soares et al., 2017). The crystallization was one of the factors associated with the higher viscosity of the organic honey samples, which was observed to be intense, in addition to the fact that pasteurization of this type of honey is rarely practiced in Brazil.

Orange blossom honey samples have intermediate fructose levels compared to glucose (Mateo & Bosch-Reig, 1997). This monosaccharide is more soluble in water, favoring the formation of a more liquid honey with a lower tendency to crystallize, resulting in lower viscosity (Moreira & De Maria, 2001).

The acidity of honey is influenced by the presence of organic acids, particularly gluconic acid, and their corresponding lactones (Truzzi et al., 2014). The obtained data indicate that acidity varies according to the botanical origin of the honey: Orange blossom honey statistically exhibited the lowest acidity (28.96 ± 5.67 mEq/kg) compared to other origins, including wildflower

Table 8. Absolute frequencies (AF), relative frequencies (RF), and relative frequencies expressed as percentages (RF%) for the determination of acidity, hydroxymethylfurfural (HMF), and moisture content in honey samples from four distinct floral origins in the Botucatu region, São Paulo, Brazil.

Test	Floral origin of honey	Parameters*	AF	RF	RF (%)
Acidez	Eucalyptus	> 50 mEq/kg	2	0.25 (2/8)	25
		≤ 50 mEq/kg	6	0.75 (6/8)	75
	Wildflower	> 50 mEq/kg	0	0.00 (0/8)	0
		≤ 50 mEq/kg	8	1.00 (8/8)	100
	Orange Blossom	> 50 mEq/kg	0	0.00 (0/7)	0
		≤ 50 mEq/kg	7	1.00 (7/7)	100
	Organic	> 50 mEq/kg	2	0.25 (2/8)	25
		≤ 50 mEq/kg	6	0.75 (6/8)	75
HMF	Eucalyptus	> 60 mg/kg	0	0.00 (0/8)	0
		≤ 60 mg/kg	8	1.00 (8/8)	100
	Wildflower	> 60 mg/kg	1	0.13 (1/8)	13
		≤ 60 mg/kg	7	0.87 (7/8)	87
	Orange Blossom	> 60 mg/kg	0	0.00 (0/7)	0
		≤ 60 mg/kg	7	1.00 (7/7)	100
	Organic	> 60 mg/kg	2	0.25 (2/8)	25
		≤ 60 mg/kg	6	0.75 (6/8)	75
Moisture	Eucalyptus	> 20 g/100 g	1	0.13 (1/8)	13
		≤ 20 g/100 g	7	0.87 (7/8)	87
	Wildflower	> 20 g/100 g	0	0.00 (0/8)	0
		≤ 20 g/100 g	8	1.00 (8/8)	100
	Orange Blossom	> 20 g/100 g	0	0.00 (0/7)	0
		≤ 20 g/100 g	7	1.00 (7/7)	100
	Organic	> 20 g/100 g	2	0.25 (2/8)	25
		≤ 20 g/100 g	6	0.75 (6/8)	75

*Codex (2022); IHC (2009); Usda (1985).

Table 9. Absolute frequency (AF), relative frequency (RF), and relative frequency in percentage (RF%) for the determination of diastase enzyme activity (PFD), Fiehe reaction, and Lund test in honey from four different floral sources in the region of Botucatu, São Paulo, Brazil.

Test	Floral source	Parameter*	AF	RF	RF (%)
PFD	Eucalyptus	Present	8	1.00 (8/8)	100
		Absent	0	0.00 (0/8)	0
	Wildflower	Present	7	0.87 (7/8)	87
		Absent	1	0.13 (1/8)	13
	Orange blossom	Present	7	1.00 (7/7)	100
		Absent	0	0.00 (0/7)	0
	Organic	Present	8	1.00 (8/8)	100
		Absent	0	0.00 (0/8)	0
Fiehe reaction	Eucalyptus	Present	0	0.00 (0/8)	0
		Absent	8	1.00 (8/8)	100
	Wildflower	Present	1	0.13 (1/8)	13
		Absent	7	0.87 (7/8)	87
	Orange blossom	Present	0	0.00 (0/7)	0
		Absent	7	1.00 (7/7)	100
	Organic	Present	2	0.25 (0/8)	25
		Absent	6	0.75 (8/8)	75
Lund test	Eucalyptus	Present	8	1.00 (8/8)	100
		Absent	0	0.00 (0/8)	0
	Wildflower	Present	8	1.00 (8/8)	100
		Absent	0	0.00 (0/8)	0
	Orange blossom	Present	7	1.00 (7/7)	100
		Absent	0	0.00 (0/7)	0
	Organic	Present	8	1.00 (8/8)	100
		Absent	0	0.00 (0/8)	0

*Codex (2022); IHC (2009); Usda (1985).

Table 10. Mean ± standard deviation of absorbance in the analysis of honey from four different floral origins in the region of Botucatu, São Paulo, Brazil.

Floral origin of honey	Mean ± standard deviation*
Eucalyptus	1.3156 ± 0.9870 a[1]
Wildflower	1.2473 ± 0.8373 a
Orange blossom	1.0267 ± 0.6543 a
Organic	1.6131 ± 0.8710 a

[1]p = 0.2574 and CV = 65.37%; *560 nm in a 10-mm path length quartz cuvette. Statistical analysis complemented by Tukey's test at 5% significance.

(35.90 mEq/kg ± 5.92), organic (43.05 ± 8.43), and eucalyptus (44.82 mEq/kg ± 11.34). A study conducted in the same region demonstrated that acidity in honey of different origins was lower in orange blossom honey compared to wildflower and eucalyptus honey (Komatsu et al., 2002).

Acidity should not exceed 50 mEq/kg of honey (Codex, 2022; USDA, 1985; IHC, 2009). Thirteen percent (4/31) of the samples had results outside the current standards (Table 8): 25% (2/8) from eucalyptus and 25% (2/8) from organic honey. Values exceeding this indicate possible deterioration of the product due to the proliferation of fungi and yeasts associated with high moisture content. This can result in fermentation and promote bacterial growth, potentially compromising honey quality (Salgado et al., 2008).

The measurement of HMF is used to assess the freshness and/or overheating of honey or to indicate if it has been adulterated with inverted sugar. This is a furano compound found in small amounts or even absent in fresh, unprocessed foods. Honey stored for long periods or overheated presents higher levels of HMF (Soares et al., 2017). Orange blossom honey statistically exhibited the lowest HMF levels (7.09 g/100 g ± 9.59), indicating greater freshness compared to other floral types. Orange blossom honey is the most commercially available due to its sensory characteristics of color, aroma, and flavor, which may contribute to higher commercial turnover. 9.68% of the honey samples exhibited HMF levels exceeding 60 mg/kg (Table 8), with 25% (2/8) of organic honey and 13% (1/8) of wildflower honey falling outside the standards set by legislation, indicating that they were subjected to excessive heating and/or prolonged storage or were adulterated (Codex, 2022; IHC, 2009; Pasias et al., 2017; USDA, 1985).

Moisture is an important parameter for determining honey quality as it influences preservation, crystallization, stability, maturity, flavor, and viscosity (Singh & Singh, 2018). It depends on climatic conditions, storage conditions, and harvesting conditions (Soares et al., 2017). No significant differences in moisture levels among the different types of honey evaluated were found (Table 7). The lowest moisture content was found in organic honey (18.20%).

The crystallization of honey and its subsequent reduction in flow may be linked to a lower water content within the product. According to legislation, the moisture levels in honey must not exceed 20% (Codex, 2022; USDA, 1985; IHC, 2009). Alarmingly, 9.68% (3/31) of the samples evaluated failed to meet these regulatory standards (Table 8). This increase in moisture content can be influenced by various factors, including the management

Table 11. Absolute frequency (AF), relative frequency (RF), and relative frequency as a percentage (RF %) for the classification of honey color (light amber, amber, and dark amber) from four different floral origins in the region of Botucatu, São Paulo, Brazil.

Floral origin	Color*	Pfund scale (mm)	Absorbance range	AF	RF	RF (%)
Eucalyptus (*n* = 8)	Light Amber	> 50 to 85	> 0.188 to 0.440	3	0.37 (3/8)	37
	Amber	> 85 to 114	> 0.440 to 0.945	0	0.00 (0/8)	0
	Dark Amber	> 114	> 0.945	5	0.63 (5/8)	63
Wildflower (*n* = 8)	Light Amber	> 50 to 85	> 0.188 to 0.440	2	0.25 (2/8)	25
	Amber	> 85 to 114	> 0.440 to 0.945	2	0.25 (2/8)	25
	Dark Amber	> 114	> 0.945	4	0.50 (4/8)	50
Orange Blossom (*n* = 7)	Light Amber	> 50 to 85	> 0.188 to 0.440	2	0.29 (2/7)	29
	Amber	> 85 to 114	> 0.440 to 0.945	0	0.00 (0/7)	0
	Dark Amber	> 114	> 0.945	5	0.71 (5/7)	71
Organic (*n* = 8)	Light Amber	> 50 to 85	> 0.188 to 0.440	0	0.00 (0/8)	0
	Amber	> 85 to 114	> 0.440 to 0.945	2	0.25 (2/8)	25
	Dark Amber	> 114	> 0.945	6	0.75 (6/8)	75

*According to the criteria of the Pfund scale (mm) and its corresponding absorbance range (Brasil, 1981; Sechrist, 1925; USDA, 1985).

practices employed by the meliponist, the specific geographical region, and the timing of the harvest (Sousa et al., 2016).

Of the analyses conducted, 13% (1/8) of wildflower honey samples were negative for the diastatic yeast test (Table 9). The enzyme diastase functions in the hydrolysis of starch and is produced by the hypopharyngeal glands of bees, and it can also be found in smaller quantities in pollen grains. It is sensitive to elevated temperatures and, for this reason, is important for assessing whether honey has been overheated (above 60 °C), improperly stored, or adulterated with inverted sugar (Holanda et al., 2015).

The Fiehe reaction is the qualitative analysis of HMF. Thirteen percent (1/8) of wildflower honey samples and 25% (2/8) of organic honey samples showed positive results (Table 9). The color change indicates overheating or prolonged storage time, resulting in the breakdown of sucrose, producing HMF (Salgado et al., 2008).

In the Lund test, all evaluated samples exhibited sediment of albuminoids, rendering them positive (Table 9). This qualitative test is one of those performed to certify the quality of marketed honey, and the results obtained in this study align with those required by legislation (Codex, 2022).

The color of honey is one of the primary attributes influencing consumer purchase decisions. Variations in honey color relate to different factors: floral origin, processing, storage, climatic conditions during nectar collection, and maturation temperature in the hive (Abadio Finco et al., 2010). Commercially, lighter honey is often more valued compared to darker ones (Ito et al., 2018). No significant differences were observed among the evaluated honey colors (Table 10). Dark amber coloration was predominant among all evaluated floral origin groups (Table 11): 63% (5/8), 50% (4/8), 71% (5/7), and 75% (6/8) of eucalyptus, wildflower, orange blossom, and organic honey samples, respectively. Divergent results were found in different studies, with light amber coloration predominating (Ito et al., 2018), which may relate to the region where the honey was produced.

5 CONCLUSION

The organic honey samples exhibited the highest viscosity, while the honey derived from orange blossoms demonstrated the lowest viscosity. Organic honey tends to crystallize more readily due to its sugar composition and lower moisture content, which contributes to its increased viscosity.

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