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High frequency of fraud in honey without sanitary inspection illegally sold in São Paulo State

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Abstract

The aim of this study was to analyze honey from São Paulo State according to its physical and chemical properties. Determination of free acidity, moisture, hydroxymethylfurfural, Lund test, diastase test, and Fiehe reaction were performed in 73 samples (32 illegal honey, without sanitary inspection, and 41 legal honey, with sanitary inspection). An ANOVA test was applied to evaluate differences in the samples. Three samples had humidity greater than 20%, and one sample had a positive profile in the qualitative analysis of HMF (Fiehe) and quantitative analysis above 60 mg/kg. A total of 21 (65%) illegal honeys had at least one parameter outside the legislation. The irregularities presented were in the acidity, followed by the increase of HMF, negative Lund, high acidity, and high moisture. Significant differences were observed when comparing illegal and legal honey for acidity and hydroxymethylfurfural analysis ($p < 0.05$). The Fiehe test was positive in 2.44% of legal honeys and 64.52% of illegal honeys. No legal honey was negative for the Lund test, and 22.58% of illegal honeys had a negative profile. The results showed that illegal honey is not suitable for consumption, being outside the standard of identity and quality determined by the Codex Alimentarius Commission and the legislation.

Keywords: adulteration; analysis; physicochemical; quality.

Practical Application: Honeys without inspection have a great chance of being defrauded.

1 INTRODUCTION

Honey is a viscous fluid produced by bees from the nectar of plants and the excreta of insects sucking the juice of living parts of plants or the secretions of living parts of plants. Bees collect, transform, combine with specific substances of their own, store, and allow mature in the honeycomb of the hive (Brasil, 2000; 2017).

The physical and chemical compositions of honey can be influenced by the intrinsic and extrinsic factors, such as the variety of honey, species of producing bees, nature of the soil, climatic conditions, floral species, harvesting conditions, period of maturation, storage methods, and others. In this way, the product becomes susceptible to changes in its aroma, color, viscosity, medicinal properties, and taste (Chien et al., 2019; Machado De-Melo et al., 2018; Seraglio et al., 2021). Natural changes in honey are due to environmental factors such as fluctuations in humidity, high temperatures, and natural aging, favoring the degradation of its components (Azeredo et al., 1999; Buligon et al., 2015).

Sanitary inspection and certification of the quality and origin of honey are made by governmental inspection agents and the productive sector, taking into account public health, economic, and legal aspects, in order to avoid foodborne diseases and frauds (Espíndola et al., 2021; Paes et al., 2013).

Fraudster aims to increase profits with the sale of adulterated honey since the supply is lower than the demand, making it a product with a relatively high price and encouraging its adulteration (Buligon et al., 2015; Vicinieski et al., 2018). According to Anklam (1998), the frauds adopted in honey are mainly the incorporation of sugar syrups, sale of honey with a fraudulent name of origin, excessive heating of honey, false information regarding the floral or geographical origin, and presence of antibiotics to treat hive diseases.

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The quality parameters of honey are considered useful for detecting possible adulterations and also for confirming the hygiene conditions in its manipulation and storage (Puscas et al., 2013). Physical and chemical analyses applied to honey can evaluate its quality and detect frauds such as increased humidity and acidity, detection (Fiehe test) and quantification of hydroxymethylfurfural (HMF), detection of albuminoid substances (Lund test), and verification of diastatic activity (ABNT, 2009a; 2009b; 2016b; 2016a; Brasil, 2000; Instituto Adolfo Lutz, 2008).

The aim of this study was to analyze honey sold without inspection in São Paulo State, Brazil, seeking changes in legal standard, identifying possible fraud, and verifying the safety of products illegally traded.

2 MATERIAL AND METHODS

2.1 Honey samples

A total of 73 honey samples were collected, of which 32 samples were classified as "illegal honey" since it was not possible to determine if they were subjected to official sanitary inspection and lacked legal information (package and label with production date, batch, inspection seal, nutrition values, origin, etc.). The other 41 samples were considered "legal honey" because they met the legal recommendation. All samples of legal honey were "wild honey" because they had no definite botanical origin.

The samples were sent to the Food Inspection Laboratory of the Department of Animal Production and Preventive Veterinary Medicine, Universidade Estadual Paulista "Júlio de Mesquita Filho", School of Veterinary Medicine and Animal Science, Botucatu.

The preparation of samples for the physicochemical analysis of honey followed the procedures described by ABNT NBR 15714-1 (ABNT, 2009a).

2.2 Determination of free acidity

The determination of the free acidity of the honey followed the procedures described by ABNT NBR 15714-6 (ABNT, 2016a). A quantity of 10 g of honey was weighed into a 250-mL beaker, and 75 mL of pure water and 10 drops of 1% phenolphthalein alcoholic solution were added. It was titrated with 0.1 N sodium hydroxide solution until the color changed to pink. It was applied in the Equation 1:

$$mEq/kg = V \times fc \times 10 \quad (1)$$

Where:

V: volume spent on the titration;

fc: 0.1 N NaOH solution correction factor;

10: mass (g) used in the titration.

2.3 Moisture content by the refractometric method

The determination of the honey moisture using the refractometric method followed the procedures described by ABNT NBR 15714-2 (ABNT, 2009b). A drop of honey was used, and the point of intersection of the lines in the field of observation was adjusted. The refractive index correction was performed according to the ambient temperature by subtracting or adding 0.00023 to each degree below 20°C. The tabletop Abbé refractometer manufactured by Bausch & Lomb® (USA) was used to measure the moisture content.

2.4 Fiehe test

An amount of 5 g of sample was weighed into a 50-mL glass beaker, 5 mL of ethyl ether was added. The beaker was shaken vigorously with a glass rod and the ethereal layer was transferred into a porcelain dish and allowed to evaporate. A volume of 0.5 mL of 1% resorcinol hydrochloric solution was added. An intense red color appeared in the presence of commercial glucose or honey overheated (Instituto Adolfo Lutz, 2008).

2.5 Lund test

An amount of 2 g of sample was weighed into a 50-mL beaker and transferred to a 50-mL graduated conical tube with the aid of 20 mL of pure water. A volume of 5 mL of 0.5% tannic acid solution was added. Pure water was added until the volume of 40 mL was attained. The conical tube was capped and shaken and allowed to stand for 24 h. In the presence of pure honey, a precipitate was formed in the bottom of the tube in the range of 0.6–3 mL. In the presence of adulterated honey, neither a precipitate was formed nor did it exceed the maximum volume in the specified interval (Instituto Adolfo Lutz, 2008).

2.6 Hydroxymethylfurfural spectrophotometric method (Winkler)

An amount of 10 g of honey was dissolved in 20 mL of water and transferred to a 50-mL volumetric flask. A volume of 2 mL of the solution and 5.0 mL of *p*-toluidine solution were taken in two different test tubes, and 1 mL of distilled water (reference solution) was added to the first tube and 1 mL of 0.5% barbituric acid solution (sample) was added to the second tube. The absorbance of the solutions at 550 nm was determined using the Equation 2:

$$HMF\ (mg/kg) = 190 \times Absorbance/Cuvette\ thickness\ (1\ cm) \quad (2)$$

Where:

The number 190: the dilution factor and extinction coefficient (Zappalà et al., 2005).

2.7 Diastatic yeasts research

An amount of 10 g of sample was weighed in a 50-mL test tube. A volume of 20 mL of pure water was added and homogenized. A volume of 10 mL of this solution was transferred to a tube of rehearsal and 1 mL solution of 1% soluble starch was added. The solution was shaken and the tube was placed in a water bath at 45°C ± 2°C for 1 h. The tube was taken out of

the bath and 1 mL of iodine solution was added. A blank test was carried out without heating. Colors were compared and obtained. In the presence of diastase enzyme, an olive green or brown color appeared. A blue color appeared in the absence of these ferments (ABNT, 2016b; Instituto Adolfo Lutz, 2008).

2.8 Statistical analysis

Statistical analysis of quantitative variables (acidity, moisture, and HMF) was performed using ANOVA and Tukey's multiple comparison test to determine the difference between treatment means (illegal honey vs. legal honey). Results were expressed as mean ± standard error of mean with 5% significance. Qualitative variables were mathematically evaluated using absolute frequencies.

3 RESULTS

Of the 32 illegally traded honeys, 21 had at least one parameter outside the legislation. The highest irregularities presented were in the acidity analysis, followed by the increase in HMF, negative Lund, high acidity, and high moisture. Of the 41 samples of honey commercialized legally, four samples did not comply with the legislation. Three samples had humidity greater than 20% (20.4, 20.6, and 20.72%), and one sample had a positive profile in the qualitative analysis of HMF (Fiehe) and quantitative analysis above 60 mg/kg (141.34 mg/kg) (Figure 1).

According to the qualitative analyses of research on diastatic yeasts, Fiehe and Lund reactions showed 25% absence, 64.52% positive, and 22.58% negative for illegal honey. For legal honey, Fiehe and Lund reactions showed 0% absence, 2.44% positive, and 0% negative, respectively. Fiehe test was positive on 67.52% of the illegal honey, and only one illegal sample was out of standard.

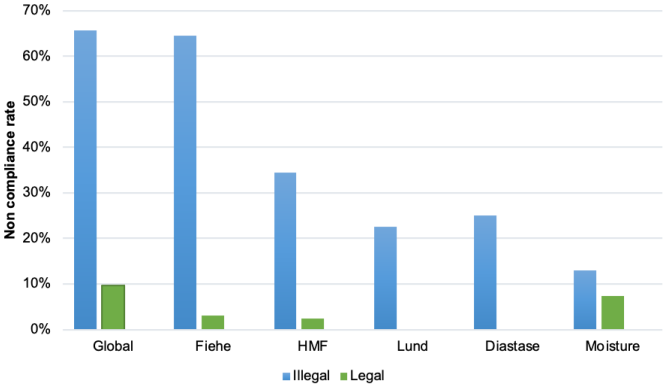

Figure 1. Percentage of non-standard samples of illegally and legally traded honey in cities in the state of São Paulo, Brazil.

The acidity (33.80 mEq/kg ± 10.47 mEq/kg) and HMF (143.13 mg/kg ± 22.18 mg/kg) of illegal honey were significantly ($p < 0.05$ for acidity and HMF) greater than acidity (8.56 mEq/kg ± 6.98 mEq/kg) and HMF (143.13 mg/kg ± 280.02 mg/kg) of legal honey. The acidity of illegal honey was 10.34% higher than the recommended maximum of 50 mEq/kg. Regarding values greater than 60 mg/kg of HMF, legal honey and illegal honey presented 2.44% and 34.38%, respectively. The acidity and HMF of the honey (legal and illegal) showed a high coefficient of variation ($CV = 45.17\%$ for acidity and $CV = 27.83\%$ for HMF). The high values of CVs demonstrated the instability and heterogeneity of the analyzed values of the acidity and HMF tests of the honey. The moisture values of legal honey (18.37% ± 1.54%) and illegal honey (18.30% ± 1.63%) did not show significant differences ($p = 0.8538$). Values greater than 20% moisture were 7.32% for legal honey and 12.90% for illegal honey. The coefficient of variation of the moisture values ($CV = 8.60\%$) was stable and homogeneous (Table 1).

4 DISCUSSION

One legal honey that had a positive HMF profile in the qualitative analysis, was above the standard in the quantitative analysis, and had no other irregularities can demonstrate the probability of being a honey with irregularities in its storage such as prolonged time and high temperature (Instituto Adolfo Lutz, 2008; Zappalà et al., 2005).

Three samples of legal honey (7.32%) with humidity above the maximum standard of 20% showed the contents of 20.40%, 20.60%, and 20.72%. This may be related to the fact that honey was collected before maturing in the hive combs or because honey is a product with hygroscopic characteristics, which can absorb moisture from the environment during its storage irregularly (Marchini et al., 2005).

Of the 21 non-standard illegal honeys, the enzyme diastase was absent in 8 (25%) samples. The absence of this enzyme, which is naturally present in the product, may indicate its falsification, or the hydrolysis of starch by the action of amylases may have suffered interference from the pH and irregular temperature of product storage (Brasil, 1981; Instituto Adolfo Lutz, 2008). Diastase is an amylase used as a freshness indicator due

Table 1. Mean ± standard deviation (SD) and coefficient of variation (CV) of physicochemical parameters of legal and illegal honeys from São Paulo State.

Test	Sampling	N	Mean ± SD	CV	P-value
Acidity (mEq/kg)	Legal honey	41	8.56 ± 6.98a[1]	45.17	$p < 0.05$ ($p = 0$)
	Illegal honey	29	33.8 ± 10.47b		
Moisture (g/100 g)	Legal honey	41	18.37 ± 1.54a	8.6	$p = 0.85$
	Illegal honey	31	18.3 ± 1.63a		
Hydroxymethylfurfural (mg/kg)	Legal honey	41	7.15 ± 22.18a	27.83	$p < 0.05$
	Illegal honey	32	143.13 ± 280.02b		

[1]Different lowercase letters in the same column indicate a statistically significant difference ($p < 0.05$) when comparing different types of honeys (legal and illegal).

to its thermal resistance and can also be used as an adulteration indicator (Pasias et al., 2017; Seraglio et al., 2021). Honey must present a minimum of eight on the Goethe scale and may be three on the Goethe scale when HMF does not exceed 15 mg/kg. In the Holanda et al.'s (2012) study, the diastase indices found in the samples ranged from 0.60 to 2.93 Goethe.

The moisture content of the legal and illegal honeys (7.32 and 12.9%) shows variation from the legislation standard, indicating that the analyzed legal honey may also have a shorter shelf life because high moisture content facilitates the honey fermentation process (Czipa et al., 2019; Khansaritoreh et al., 2021). Out-of-compliance samples for moisture content were also reported by other studies. Ito et al. (2018) obtained a variation of 21.44 to 27.51% in 14 analyzed samples, similar to the pattern found in samples of green honey analyzed by Rajindran et al. (2022), where a high moisture content is expected due to its early collection.

In the experiment, increased free acidity was frequent in the illegal honey samples. The acidity in honey is the result of organic acids derived from nectar by the action of glucose oxidase, which forms gluconic acid through bacteria during the honey maturation process, in addition to the number of minerals present in honey. A high acid value indicates the fermentation of sugars into organic acids and significantly interferes with the sensory characteristics of the product, as high values can cause a decrease in the existing microbial control (Imtara et al., 2018; Rajindran et al., 2022; Seraglio et al., 2021).

Rajindran et al. (2022) obtained values above 50 mEq/kg in unripe honey with a high moisture content, which enables even more deterioration, corroborating this study that 12.9% of illegal honey had a high moisture content. However, studies show that honey with an inspection indication was outside the standard of legislation (Holanda et al., 2012; Ito et al., 2018; Sakač et al., 2019).

HMF is formed through reducing sugars when honey is heated and/or stored for a long time. In fresh honey, HMF is usually found in low concentrations (Seraglio et al., 2021). Thus, this analysis is used as a quality parameter to evaluate the freshness and/or overheating of the honey (Seraglio et al., 2021). The high HMF may also be an indication of falsification by adding inverted syrup because HMF can be produced by heating sugars in the presence of an acid to the inversion of sucrose (Capuano & Fogliano, 2011; Yücel & Sultanoğlu, 2013).

The Fiehe reaction is a qualitative analysis that detects the presence of hydroxymethylfurfural in honey (Brito et al., 2020; Instituto Adolfo Lutz, 2008; Ito et al., 2018; Vicinieski et al., 2018). The high positivity of the analyzed illegal samples (64.52%) may be an indication that the honey has been defrauded to be sold. This implies a significant reduction in the nutritional properties of honey, which promotes the destruction of vitamins and enzymes in the honey (Ito et al., 2018; Vicinieski et al., 2018).

In this study, 34.38% of the illegal honey had HMF over 60 mg/kg, and only one sample with an inspection indication presented a value above the legislation. Rajindran et al. (2022) presented the physical and chemical results, where HMF values were below 60 mg/kg. However, in a study with samples collected from markets, the HMF ranged from 15.0 to 95.6 mg/kg, which is not within the standard range (Iftikhar et al., 2014).

Lund reaction is a qualitative test that indicates the presence of albuminoids, a natural protein present only in bee honey, so the absence of albuminoids indicates fraud (Brito et al., 2020; Instituto Adolfo Lutz, 2008; Ito et al., 2018). All honey samples analyzed by Ito et al. (2018) and Vicinieski et al. (2018) were positive for the reaction. This study demonstrated that 22.58% of the analyzed samples of illegal honey are known not to be honey by Lund reaction. Nonetheless, according to Santos et al. (2011), the established value of 0.6–3 mL of positive precipitation does not allow accuse adulterations considered gross and should be used jointly with tests having greater specificity, inducing the interpretation that, according to the other analyses and the low test specificity, more analyzed samples could not be honey.

5 CONCLUSION

In this study, several physical and chemical parameters were determined in honey samples from different cities in the state of São Paulo. The legally traded honey did not show significant changes that were justified by storage failures. However, the analysis showed that the existence of illegally marketed products can harm consumers due to their irregularity with legal standard and their falsification. The physical and chemical tests carried out are not able to identify all types of fraud, but they can help in detection, collaborating with the diagnosis of outbreaks of fraudulent actions. Based on the data presented, illegal honey is not suitable for consumption and disagrees with the Codex Alimentarius Commission and legislation on the identity and quality of honey.

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