

Sugars and °Brix in honey from *Apis mellifera*, *Melipona beecheii*, and commercial honey from a local market in Mexico

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Abstract

Fructose and glucose are the main sugars in honey, and their concentration is expected to correspond to the specifications of official standards. The study compared the composition of sugars and °Brix in honey from *Apis mellifera* and *Melipona beecheii* bees, and a product marketed as bee honey in a local market. The sugar content was determined by high-performance liquid chromatography (HPLC) with a refractive index detector, and °Brix was determined using a refractometer. None of the honey analyzed had detectable levels of sucrose. The average concentration of fructose and glucose in honey was 36.4 and 28.9 g 100 g⁻¹ for *Apis mellifera*, and 38.5 and 28.2 g 100 g⁻¹ for honey from *Melipona beecheii*. For honey from the local market, the respective concentrations of these sugars were 7.5 and 17.0 g 100 g⁻¹. The fructose:glucose ratio (F:G) was higher than one in *Apis mellifera* and *Melipona beecheii* honey, and 0.4 for honey from the local market. The three types of honey compared had similar values for °Brix. It is concluded that the honey under study by *Apis mellifera* and *Melipona beecheii* have similar qualities within international standards. In contrast, the product marketed as bee honey in the local market did not meet official regulations specifications and could not be considered authentic.

Keywords: Honey quality; Honey adulteration; Africanized bees; *Melipona* bees.

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Additional information and declarations
can be found on page 11

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Study contribution

Mexican beekeeping is seriously threatened by the presence of adulterated and fake honey in the market. At the field level, the determination of honey quality is only measured with °Brix. The sugar content in honey is a fundamental parameter that determines its authenticity and the degree of adulteration or falsification. Bee honey must contain ≥ 60 g 100 g⁻¹ of fructose + glucose, ≤ 5 g 100 g⁻¹ of sucrose and the Fructose/Glucose ratio must be greater than 1. With the results of this study, it was determined that the °Brix reading in honey is not enough to determine its authenticity. With the results of this study, it is possible to contribute both to the sustainability and profitability of beekeeping in Mexico and to the health care of honey consumers.

Introduction

Bee honey is a sweet natural substance produced by worker bees from flower nectar and other extra-floral secretions that bees suck, transport, transform, combine with other substances, dehydrate, concentrate and store in honeycombs.^(1,2) The characteristics of honey vary according to botanical and geographic origin, the climatic conditions where it is produced, and how it is processed and stored.⁽³⁾ The main components of honey are carbohydrates and water.⁽³⁾ Sugars such as fructose, glucose and sucrose are the main carbohydrates present in honey, and together they constitute 95 to 99% of honey's dry matter.⁽⁴⁾ Bee honey also contains other disaccharide carbohydrates such as maltose, isomaltose, oligosaccharides, and tetrasaccharides.⁽⁵⁾ Furthermore, bee honey contains various minor substances, including enzymes, amino acids, organic acids, antioxidants, vitamins, and minerals.⁽⁶⁾

Any product that does not comply with those mentioned above cannot be called honey, as with sugar syrups and plant syrup. Also, honey must not contain additives, organic and inorganic substances different from its composition.⁽⁷⁾ The total soluble solids content in honey is expressed in degrees Brix (°Brix), related to the sugar content. The °Brix value obtained using the refractometer represents the percentage of sugars in honey. This parameter is related to the moisture content since the second most abundant honey component is water, which is expressed as a percentage of moisture in honey.⁽⁸⁾

Based on this background, this research determined and compared the composition of sugars and degrees Brix (°Brix) in honey from *Apis mellifera* and *Melipona beecheii* bees from various Mexican states, in addition to a product commercialized as bee honey in the local market.

Materials and methods

Honey samples collection

The honey samples from *Apis mellifera* and *Melipona beecheii* were collected from different states of Mexico and from various botanical sources, depending on the site where they were collected (Table 1).

Table 1. The origin and distribution of the honey samples analyzed in this study

Region ¹	State ²	Bee species	n ³	Botanical source ⁴
Humid tropic	Campeche	<i>Apis mellifera</i>	2	Multifloral
	Quintana Roo	<i>Apis mellifera</i>	1	Multifloral
	Yucatán	<i>Apis mellifera</i>	1	Multifloral
	Yucatán	<i>Melipona beecheii</i>	2	Multifloral
	Chiapas	<i>Apis mellifera</i>	2	Multifloral
Dry tropic	Oaxaca	<i>Apis mellifera</i>	2	Chalahuite (<i>Inga vera</i>) and multifloral
	Guerrero	<i>Apis mellifera</i>	3	Coconut (<i>Cocos nucifera</i>) and multifloral
	Guerrero	<i>Melipona beecheii</i>	1	Multifloral
Temperate	Puebla	<i>Apis mellifera</i>	3	Mezquite (<i>Prosopis velutina</i>), acahual (<i>Tithonia tubiformis</i>) and palo dulce or palo azul (<i>Eysenhardtia polystachya</i>)
	Estado de México	Unknown	3	Unknown origin

¹Region: climatic region of Mexico, ²State: a state within Mexico, ³n: number of honey samples, ⁴Botanical source: the floral origin of honey samples.

Once the honey was extracted from the different hives, a sample of 250 g of mixed honey was taken. We had twenty samples of honey (14 from *Apis mellifera*, three from *Melipona beecheii*, and three from a product marketed locally as bee honey). To obtain the samples from the local market, three 250 g glass containers filled with honey from three different establishments known for offering honey were chosen.

Laboratory analysis

The determination of °Brix was made with decrystallized honey using a refractometer at a temperature of 22 °C. High-performance liquid chromatography (HPLC) was used to determine sugars with a refractive index detector. The samples were analyzed in triplicate for sugars and in quadruplicate for °Brix.

The reagents used were acetonitrile, methanol, water, and standards for glucose, fructose, and sucrose; all reagents were of HPLC grade. The materials and equipment used were 1.8 mL glass vials, 10 and 5 mL 'class A' volumetric flasks, 47 mm and 0.47 µm NYLON filtration membranes, SPE Chromabond C18 ec 3 mL 500 mg⁻¹ cleaning cartridges, acrodisk 0.47 µm, vacuum filtration system, analytical balance and 5 mL plastic syringe.

The procedure followed was the preparation of 40 mL methanol:water solution A (1:9). The mobile phase was acetonitrile:water (80:20). For the standard solution (DP), 0.1 g of glucose, fructose and sucrose standards were weighed; they were all placed together in a 10 mL flask, dissolved with solution A and brought to gauge, reaching the concentration of 10 mg mL⁻¹. To determine the retention time of each standard, 0.1 g of each was weighed individually. For the standard solution, 5 mL of DP was transferred to a 10 mL flask, and it was filled with the mobile phase until reaching the concentration of 5 mg mL⁻¹.

The three previous standards were injected into the HPLC to determine the retention time of fructose, glucose, and sucrose. A calibration curve of 0.3 to 5 mg mL⁻¹ was used to quantify sugars in the honey samples analyzed. The honey

samples' preparation was carried out using the procedure described by Karkacier et al.⁽⁹⁾ One gram of each sample was weighed on an analytical balance. The sample was diluted with HPLC grade water, transferred to a 10 mL volumetric flask, and gauged with the same water. Subsequently, 1 mL of the solution was taken, transferred to a 10 mL flask, and washed with HPLC water.

For the solid phase extraction, the Macherey-Nagel technique was used to clean the honey sample through cartridge filtration or a cleaning column placed on a universal support.⁽¹⁰⁾ The vacuum was generated with a 5 mL syringe's help, attached to a particular hose extension. The cleaning cartridge was conditioned with 6 mL of methanol and then 6 mL of HPLC water at the rate of one drop per second. The methanol was recovered and placed in a toxic waste container. Care was taken to ensure that the chromatographic bed did not dry at each stage.

Once the cleaning cartridge was conditioned, 1 mL of the honey samples' ultimate solution was deposited inside the cartridge. The sample was sucked without drying the chromatographic bed, and the eluate was recovered in a 5 mL flask; 3 mL of HPLC water was added to the column to recover the sugars and mixed with the above eluate. In this last step, the chromatographic bed was brought to dryness. Subsequently, the flask was washed with HPLC water. Finally, 1 mL of the solution resulting from the solid-phase extraction was taken, passed through an acrodisk, and placed in the HPLC's autosampler vial for analysis.

Statistical analyses

The MEANS procedure was used to obtain the descriptive statistics, and the SAS MIXED procedure was used to analyze variance and covariance.⁽¹¹⁾ For the variables of sugar concentration (fructose, glucose, fructose + glucose), the ratio of fructose:glucose (F:G) and °Brix, the adjusted statistical model was as follows:

$$y_{ijk} = \mu + S_i + EF_j + e_{ijk}$$

Where: y_{ijk} is the registered value for each one of the analyzed variables (fructose, glucose, fructose + glucose, F:G ratio, and ° Brix); μ is the overall mean; S_i is the effect of the i^{th} species of bee ($i = Apis mellifera, Melipona beecheii,$ and *unknown*); EF_j is the random effect of the j^{th} subclass, formed by the combination of the Mexican State where the honey samples were collected and the type of flowering origin of the honey analyzed; \sim NIID($0, \sigma_{EF}^2$); e_{ijk} is the random error \sim NIID($0, \sigma_e^2$). The least-square means for bee species were compared with the Tukey test.

The association between °Brix and the concentrations of fructose, glucose, fructose + glucose, and the F:G ratio was analyzed only for *Apis mellifera* and *Melipona beecheii* because they had more quality available data. For each sugar content variable in honey, the °Brix reading relationship was analyzed by fitting a mixed linear model without the overall mean, using the 'noint' option in the model statement. Likewise, the solution to the model's fixed effects was obtained by specifying the 'solution' option in the SAS MIXED procedure's model statement.⁽¹¹⁾ The fixed effect solutions corresponded to coefficients of °Brix regression on the concentration of each modeled sugar in honey. The adjusted statistical model is as follows:

$$y_{ijk} = \beta_{0i}S_i + \beta_{1i}S_iX_{ijk} + \beta_{2i}S_iX_{ijk}^2 + EF_j + e_{ijk}$$

Where: y_{ijk} is the k^{th} record of °Brix, from the i^{th} bee species, and the j^{th} subclass formed by the combination of Mexican State and the type of flowering origin of the honey; β_{0i} , β_{1i} and β_{2i} are, respectively, the regression coefficients for the intercept, and the linear and quadratic slopes of the regression of °Brix on each independent variable analyzed, for the i^{th} bee species; EF_j is the random effect of the j^{th} subclass formed by the combination of Mexican State and the type of flowering origin of the honey \sim NIID($0, \sigma_{EF}^2$); e_{ijk} is the random error \sim NIID($0, \sigma_e^2$).

Results and discussion

Table 2 shows the average sugar concentrations and °Brix readings for the honey analyzed. According to the Official Mexican Standard NMX-F-036- NORMEX-2006,⁽²⁾ *Apis mellifera*, and *Melipona beecheii* honey met the specifications of 63.88 g 100 g⁻¹ of honey as a minimum for fructose + glucose values and a maximum glucose concentration of 38 g 100 g⁻¹ of honey. However, the sugar concentration in the product marketed in the local market as honey was lower than that specified by the Mexican standard. For sucrose, no detectable levels were found in any sample analyzed. According to the *Codex Alimentarius*,⁽¹⁾ the honey analyzed from the two species of bees met the specifications regarding the concentration of sugars (fructose + glucose) greater than 60 g 100 g⁻¹ of honey and sucrose less than 5 g 100 g⁻¹ of honey.

The NMX-F-036-NORMEX-2006 and Codex Alimentarius standards are specific for *Apis mellifera* honey. There is no standard for honey from *Melipona beecheii*. Although the content of fructose and glucose in *Melipona beecheii* honey found in this study is within these standards' specifications, there could be one or more components in *Melipona beecheii* honey that do not comply with these standards. Thus, it is necessary to establish a standard that regulates the quality of honey from *Melipona beecheii*.

The fructose content in the honey of *Apis mellifera* and *Melipona beecheii* was not different ($P < 0.05$); however, the fructose content was 79 and 80 % higher than that found in the product marketed as bee honey in the local market. The glucose content was different ($P < 0.05$) for the three types of honey analyzed. Honey from *Melipona beecheii* had 9 % higher glucose content than *Apis mellifera*, and the latter had 42 % higher glucose concentration than the product marketed as honey in the local market (Figure 1).

The sugar content in the honey of *Apis mellifera* was slightly lower than that reported by Olaitan et al.⁽⁴⁾ (38.2 % fructose and 31.3 % glucose), which was lower than that reported by Belay et al.⁽¹²⁾ (39.2 and 32.9 % for fructose and glucose, respectively). Similarly, the glucose content was lower than that reported by Tigistu et al.⁽¹³⁾ (32.61 %). El Sohaimy et al.⁽¹⁴⁾ reported a wide range of fructose content (4.8 to 50.78 %) and glucose (10.63 to 26.54 %) in honey from *Apis mellifera* from different geographical locations.

On the other hand, de Almeida Muradian et al.⁽⁵⁾ reported higher fructose and glucose concentrations in *Apis mellifera* honey (38.78 and 23.50 %, respectively) than in *Melipona beecheii* honey (29.21 and 21.76 %), which was different

Table 2. Descriptive statistics for sugars and °Brix in honey from *Apis mellifera*, *Melipona beecheii*, and product marketed as honey in Mexico's local market

Bee species/Variable	n ¹	Min.	Max.	Mean	SD ²	CV ³ (%)
<i>Apis mellifera</i>						
Fructose (g 100 g ⁻¹ honey)	42	31.9	41.6	36.4	2.49	6.56
Glucose (g 100 g ⁻¹ honey)	42	21.4	38.7	28.9	4.36	15.08
Fructose + glucose (g 100 g ⁻¹ honey)	42	53.4	77.5	65.2	6.05	9.28
Fructose:glucose ratio	42	1.0	1.6	1.3	0.17	12.91
°Brix	56	76.7	81.5	79.3	1.30	1.64
<i>Melipona beecheii</i>						
Fructose (g 100 g ⁻¹ honey)	9	36.6	40.0	38.5	1.20	3.11
Glucose (g 100 g ⁻¹ honey)	9	25.1	32.1	28.2	2.69	9.51
Fructose + glucose (g 100 g ⁻¹ honey)	9	62.4	71.7	67.7	3.72	5.50
Fructose:glucose ratio	9	1.24	1.5	1.4	0.10	6.93
°Brix	12	72.5	80.4	76.0	3.03	3.99
<i>Unknown honey bee species</i> ⁴						
Fructose (g 100 g ⁻¹ honey)	3	7.0	7.9	7.5	0.46	6.21
Glucose (g 100 g ⁻¹ honey)	3	16.9	17.1	17.0	0.13	0.76
Fructose + glucose (g 100 g ⁻¹ honey)	3	24.1	25.0	24.5	0.43	1.76
Fructose:glucose ratio	3	0.4	0.5	0.4	0.03	6.52
°Brix	4	81.1	81.4	81.3	0.13	0.16

¹n: number of observations, ²SD: standard deviation, ³CV: the coefficient of variation, ⁴Unknown honey bee species: product sold as bee honey in the local market.

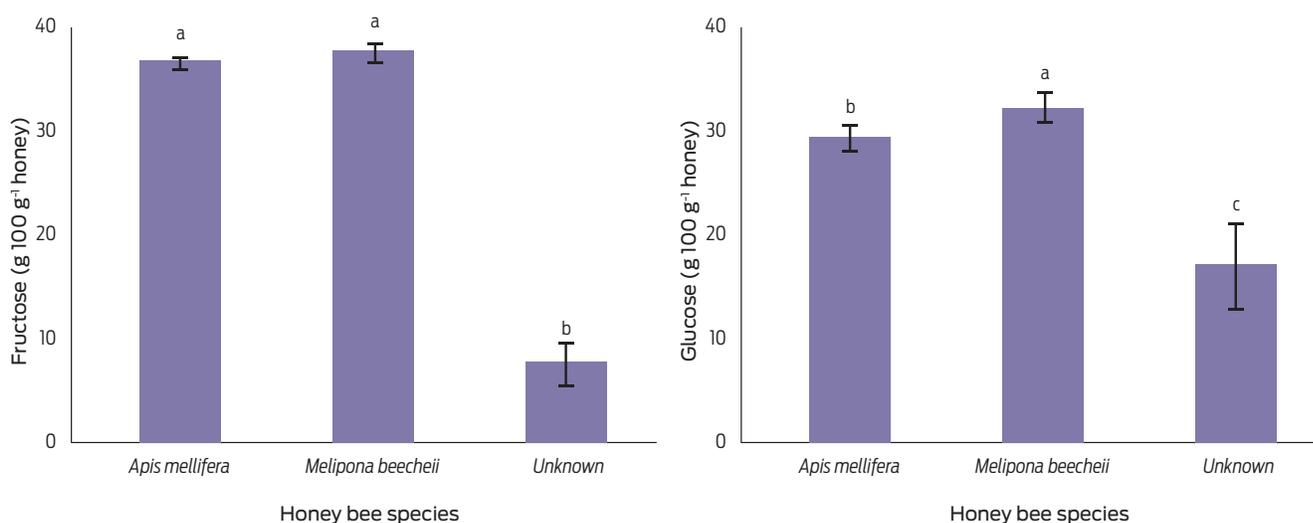


Figure 1. The concentration of fructose and glucose in honey from *Apis mellifera*, *Melipona beecheii*, and product marketing as honey in the local market. Different literals between bars indicate significant differences (P < 0.05).

from what was found in this study. In Brazil, de Almeida-Muradian et al.⁽¹⁵⁾ found concentrations of fructose and glucose in *Melipona beecheii* honey of 31.61 and 29.33 %, which were lower than what was found in this study. Similarly, Fonte et al.⁽¹⁶⁾ reported fructose and glucose concentrations of 34.11 and 29.30 in honey from *Melipona beecheii*.

The concentration of fructose + glucose was different ($P < 0.05$) in the honey analyzed, with honey from *Melipona beecheii* having the highest concentration. The lowest concentration of fructose + glucose was presented by the product marketed as honey in the local market. However, *Apis mellifera* honey presented the highest F:G ratio of the three compared honeys (Figure 2). The sum of fructose + glucose in *Melipona beecheii* honey found in this study coincides with that reported by MooHuchin et al.⁽¹⁷⁾ (67.7 g 100 g⁻¹ of honey).

In a study of *Apis mellifera* honey from different geographical origins, El Sohaimy et al.⁽¹⁴⁾ found a range of 64.21 to 72.36 g 100 g⁻¹ of honey for the sum of fructose + glucose. This result agrees with that found in this study. In contrast, Tigistu et al.⁽¹³⁾ reported fructose + glucose content of 66.83 g 100 g⁻¹ of honey, which was slightly higher than that found in this study for *Apis mellifera* honey.

According to international standards for *Apis mellifera* honey for export to the European Union, the F:G ratio's value must be greater than 1.⁽¹⁸⁾ In Brazil, de Almeida-Muradian et al.⁽¹⁵⁾ reported a value of 1.12 for the F: G ratio in *Melipona beecheii* honey; this value was lower than what was found in this study for both bee species. For *Apis mellifera* honey, Tigistu et al.⁽¹³⁾ found an F:G ratio of 1.05. For honey with different botanical origins, Belay et al.⁽¹²⁾ reported an average value for the F:G ratio of 1.19. Both values were lower than those observed in the study. For *Apis mellifera* honey, El Sohaimy et al.⁽¹⁴⁾ obtained values for the F:G ratio ranging from 0.42 to 2.35. The low end values were to those registered in this study for the product marketed as bee honey in the local mark.

According to *Apis mellifera* honey standards, regarding the content of the sugars analyzed in this study and the F:G ratio, the product marketed as honey on the local market does not meet the international standards for being considered authentic bee honey. More detailed studies must determine the composition and origin of this product. Figure 2 shows that the fructose + glucose concentration and the F:G ratio are 59 and 56 % lower than the minimum values specified in the standards.

For °Brix (Figure 3), *Apis mellifera* honey was not different ($P < 0.05$) from the product marketed as bee honey in the local market. *Melipona beecheii* honey was the one with the lowest °Brix reading. The soluble solids content (°Brix) in the analyzed honey was similar to that reported by López et al.⁽¹⁹⁾ in *Apis mellifera* honey (78.7 to 84.3 °Brix), and agrees with the range of 78.5 to 81.37 °Brix reported by Tapia-Campos et al.⁽²⁰⁾ Damasceno do Vale et al.⁽²¹⁾ found values of 67.5 °Brix in honey from *Melipona beecheii* bees, which was lower than what was found in this study; while, MooHuchin et al.⁽¹⁷⁾ reported for °Brix an average value of 75.1, similar to that found in this study.

The low °Brix value in honey from *Melipona beecheii* was possibly due to its higher moisture content than *Apis mellifera*. Damasceno do Vale et al.⁽²¹⁾ reported an average moisture percentage of 38.5 % in honey from *Melipona beecheii* bees, ranging from 27.7 to 45.8 %. In contrast, in a literature review, Machado de-Melo et al.⁽³⁾ found a range of 13 to 25 % for the moisture content in honey from

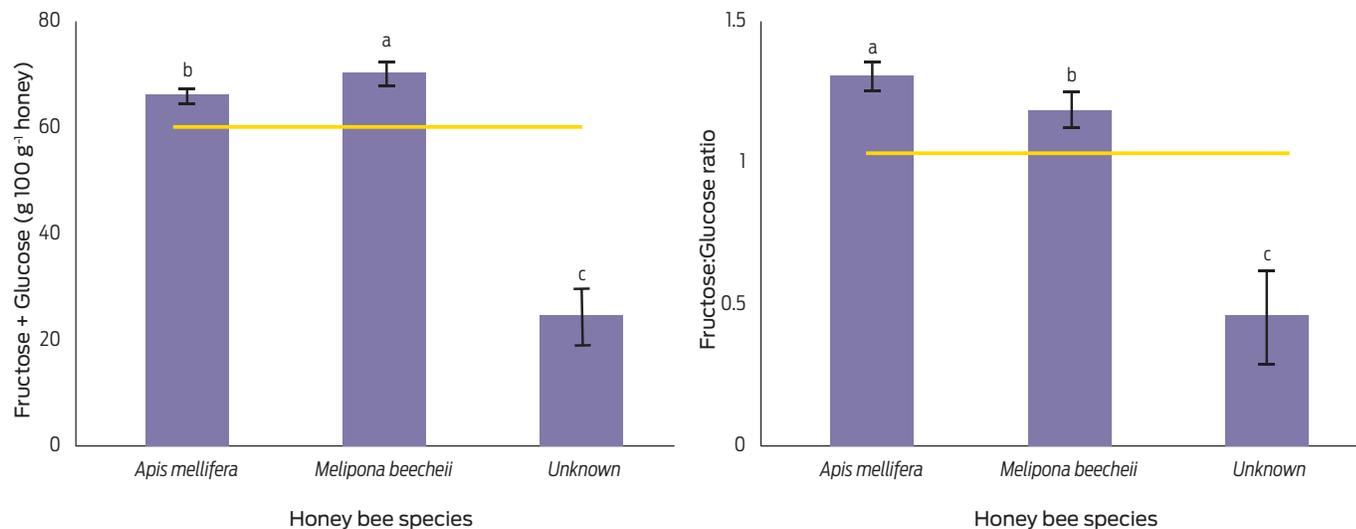


Figure 2. Fructose + glucose concentration and fructose:glucose ratio in honey from *Apis mellifera*, *Melipona beecheii*, and product marketed as bee honey in the local market. The horizontal line indicates the minimum value of fructose + glucose for *Apis mellifera* honey, specified by the Codex Alimentarius,⁽¹⁾ and the fructose:glucose ratio for *Apis mellifera* honey for export to the European Union.⁽¹⁸⁾ Different literals between bars indicate significant statistical differences (P < 0.05).

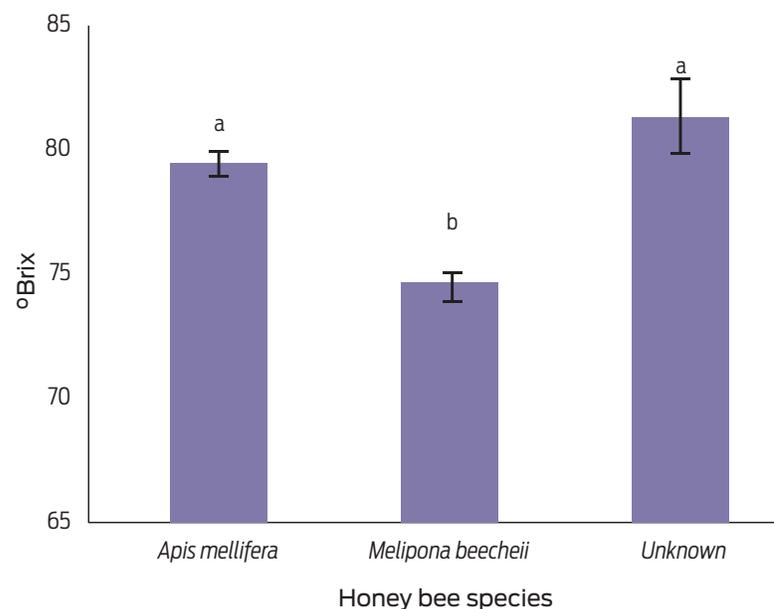


Figure 3. Degrees Brix in honey from *Apis mellifera*, *Melipona beecheii*, and product marketing as bee honey in the local market. Different literals between bars indicate significant statistical differences (P < 0.05).

Apis mellifera. For honey from *Melipona beecheii*, in other studies, Fonte et al.⁽¹⁶⁾ reported moisture values of 24 %, MooHuchin et al.⁽¹⁷⁾ of 23.2 %. Both studies conclude that, except for moisture content, for *Melipona beecheii* honey, the same compositional standards for *Apis mellifera* honey can be applied.

Based on the analyzed honey results, as there were no differences in °Brix between *Apis mellifera* honey and the product marketed as honey on the local market, it is not convenient to use this parameter to determine the authenticity of the honey from the local market.

Figure 4 shows the effect of fructose and glucose concentrations on the soluble solids content (°Brix) for the honey of *Apis mellifera* and *Melipona beecheii*. For *Apis mellifera* honey, as fructose increases, the °Brix value decreases linearly; however, the °Brix value displays a quadratic trend as glucose increases. In honey from *Melipona beecheii* bees, as both sugars increased in honey, the °Brix value decreased.

Figure 5 shows the effect of the fructose + glucose concentration and the F:G ratio on the soluble solids content (°Brix) for the honey of *Apis mellifera* and *Melipona beecheii*. In both bee species, the °Brix in honey decreased as the fructose + glucose concentration increased, contrary to the F:G ratio increases.

Conclusions

The concentrations of fructose, glucose, and the sum of both in the honey of *Apis mellifera* and *Melipona beecheii* complies with the specifications of the NMX-F-036-NORMEX-2006 and Codex Alimentarius standards, while the product marketed as bee honey in the local market does not meet these standards. For the fructose: glucose ratio, honey from *Apis mellifera* and *Melipona beecheii* meets the honey export standards for the European Union. According to the results of this study, honey from *Apis mellifera* and *Melipona beecheii* can be considered as authentic honey. In contrast, the product marketed as honey in the local market does not meet the standards for sugar concentration and the fructose:glucose ratio, and its authenticity cannot be guaranteed.

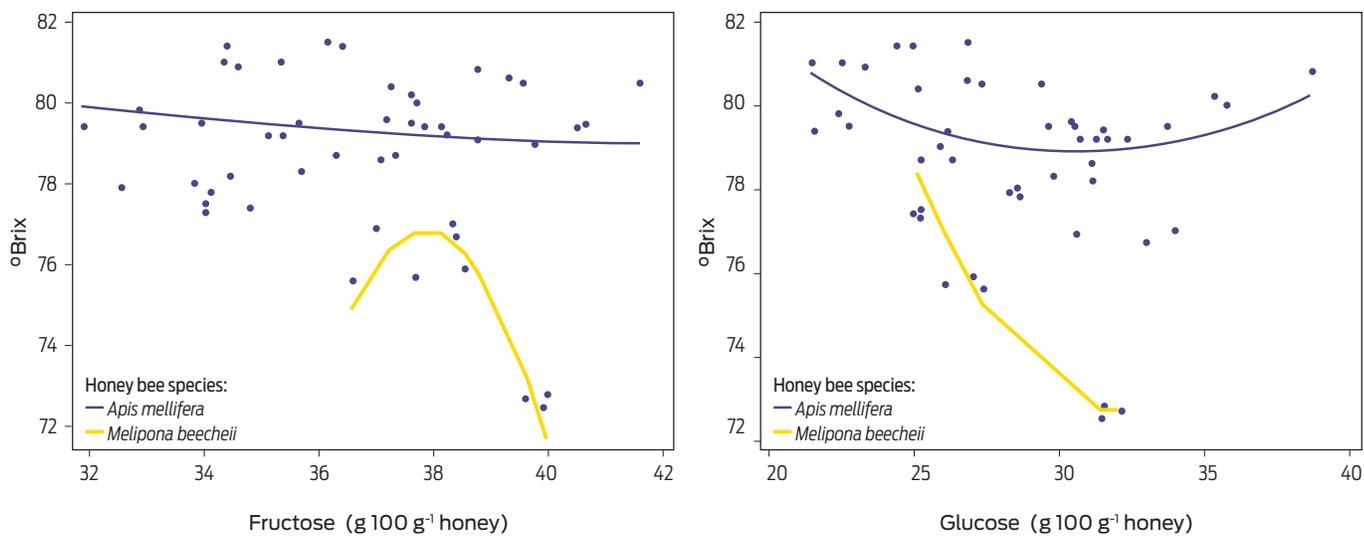


Figure 4. Solutions to the fitted regression lines of °Brix on the concentration of fructose (left panel) and glucose (right panel) in honey from *Apis mellifera* and *Melipona beecheii* bees.

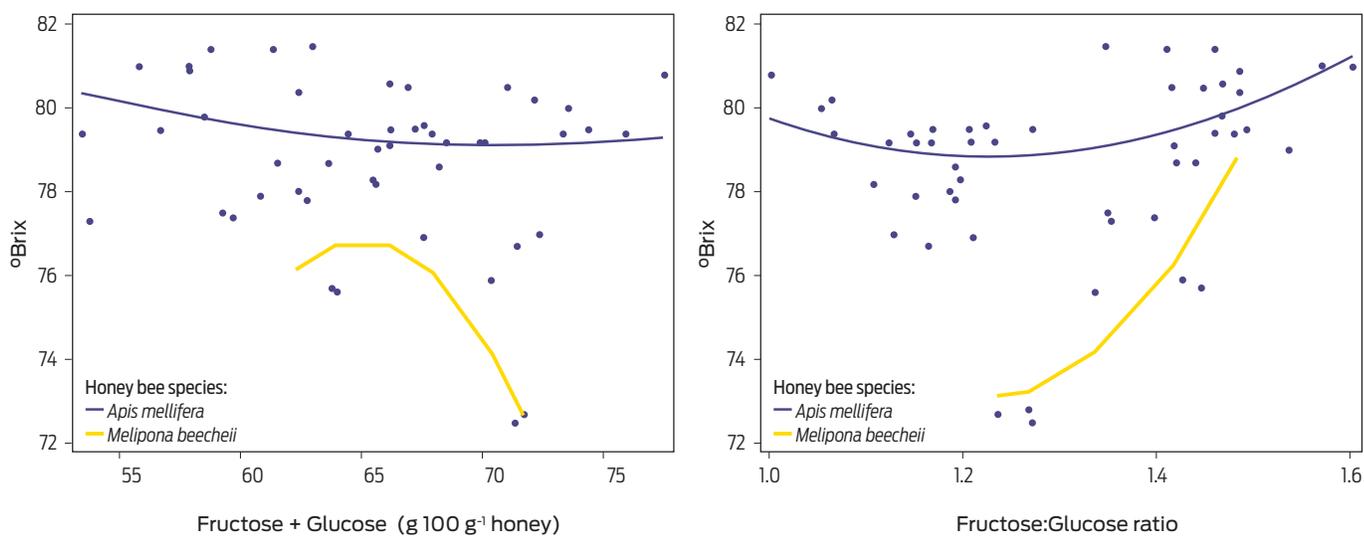


Figure 5. Solutions to the fitted regression lines of °Brix on the concentration of fructose + glucose and the fructose:glucose ratio in honey from *Apis mellifera* and *Melipona beecheii* bees.

Data availability

All relevant data are within the manuscript.

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Conflicts of interest

The authors have no conflict of interest to declare regarding this publication.

Author contributions

T Castillo: Conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, writing – original draft and writing- review and editing.

C García: Conceptualization, methodology and resources.

JG García: Conceptualization, formal analysis, methodology and writing- review and editing.

J Aguilar: Conceptualization and writing- review and editing.

R Ramírez: Conceptualization and writing- review and editing.

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