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Variations in the sugar profiles of Brazilian stingless bee honeys as determined by ion chromatography: A preliminary study of the effect of bee species and botanical source

Jiali Zhang[a], Hans S.A. Yates[a,b], Cássia Regina de Avelar Gomes[c], Daniel Nicodemo[d], Mary T. Fletcher[a], Jean-Philippe Marelli[e], Natasha L. Hungerford[a,*]

[a] Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Health and Food Sciences Precinct, Coopers Plains, QLD 4108, Australia
[b] Queensland Health Coronial and Public Health Sciences (QHCPHS), Health and Food Sciences Precinct, Coopers Plains, QLD 4108, Australia
[c] Animal Science Graduate Program, School of Agriculture and Veterinary Sciences, São Paulo State University (Unesp), Jaboticabal, SP 14884-900, Brazil
[d] Department of Animal Science, School of Agriculture and Veterinary Sciences, São Paulo State University (Unesp), Jaboticabal, SP 14884-900, Brazil
[e] Arimubee, Fazenda Arimuguê, Santo André, Santa Cruz Cabralia, Bahía 45807-000, Brazil

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ABSTRACT

The honey of honeybees is the most used insect-produced food worldwide, and its physicochemical characteristics are well-known, facilitating quality control and commercialisation. However, stingless bee honey does not meet the established parameters for honeybee honey, and new research is needed to recognise the criteria for evaluation of these honeys. A distinguishing feature of stingless bee honey samples is the disaccharide trehalulose, a low glycemic index sugar that is becoming a biomarker for stingless bee honey authentication. This study assesses trehalulose levels and honey sugar content variation of fifteen different stingless bee species from Brazil, which has the greatest diversity of stingless bees in the world. The ion chromatography method achieved excellent separations of saccharides, including trehalulose, maltose and sucrose. Trehalulose was detected in honey samples from *Melipona* bees (below limit of quantitation to 2.79 %), *Scaptotrigona* bees (1.81 – 8.32 %), *Tetragonisca* bees (5.44 – 23.90 %), *Plebeia* bees (12.81 %), and *Tetragona* bees (24.68 – 39.03 %). Both bee species and local botanical sources potentially determine the trehalulose levels in stingless bee honey. In this sense, the definition of accepted limits for trehalulose in honey must be established per each species of stingless bee, based on a greater number of samples from different locations.

1. Introduction

Stingless bees belong to Meliponini tribe, a group of bees comprising more than 600 described species that occur naturally in tropical and subtropical regions (Engel et al., 2023). The social life of these bees is similar to that of honeybees, but with several peculiarities, with an emphasis on the morphology of the individuals, the architecture of the nests, the number of individuals per colony, and the interactions with the environment. In their relationships with angiosperms, field bees are able to obtain pollen and nectar, allowing all individuals in the colony to be fed (Couto and Couto, 2006). There are limited studies on why stingless bee species prefer to forage on certain flowers, with factors influencing this including nectar sugar concentrations, flower morphology and bee tongue morphology. These foraging preferences, along with the bee species involved, influence the characteristics of the honey produced. Stingless bee honey (SBH) has received considerable attention for its unique flavour and health benefits such as antioxidant and antimicrobial properties (Cabezas-Mera et al., 2024; Shamsudin

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Abbreviations: CE, capillary electrophoresis; FSANZ, Food Standards Australia and New Zealand; GI, glycaemic index; HPIC-PAD, high-performance ion chromatography - pulsed amperometric detection; HPLC, high-performance liquid chromatography; HPLC-RID, high-performance liquid chromatography-refractive index detector; IC, ion chromatography; Min, minutes; LC-MS/MS, liquid chromatography tandem mass spectrometry; ND, not detected; NMR, nuclear magnetic resonance; SBH, stingless bee honey.

* Corresponding author at: Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Health and Food Sciences Precinct, 39 Kessels Road, Coopers Plains, QLD 4108, Australia.
E-mail address: n.hungerford@uq.edu.au (N.L. Hungerford).

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et al., 2022). Owing to the distinct physicochemical properties and flavour profile of stingless bee honey (SBH), the Malaysian food standard MS2683 was established in 2017 to specifically regulate this product. Compared to the international standard set by the Codex Alimentarius Commission (CODEX) for honey, MS2683 permits higher moisture content and free acidity, as well as a lower sugar concentration, reflecting the unique characteristics of SBH (CODEX, 2001; Malaysian Standard, 2017). In the sugar profile of SBH, a rare low glycemic index (GI) sugar, trehalulose, has been identified as a major sugar in the honey of five species (*Tetragonula carbonaria*, *Tetragonula hockingsi* (both from Australia), *Geniotrigona thoracica*, *Heterotrigona itama* (both from Malaysia), and *Tetragonisca angustula* (from Brazil)) distinguishing SBH from honey produced by honeybees (Fletcher et al., 2020). Trehalulose is a sucrose isomer with a more stable α-1,1 glycosidic linkage between the glucose and fructose moieties. In trehalulose, the anomeric carbon of the glucose moiety is involved in the stable glycosidic bond linkage (an acetal). However, the anomeric carbon of the fructose moieties forms a hemiketal and remains free to ring open and able to react as a reducing agent. Therefore, trehalulose is classified as a reducing sugar just like the monosaccharides, glucose and fructose. The unique glycosidic bond of trehalulose is associated with its low glycemic index, acariogenic and potential antidiabetic properties, together with properties which highlight its promise as an emerging alternative sweetener (Zulkifli et al., 2023). In honeybees (*Apis mellifera*) honey, trehalulose was only reported in trace to low amounts (0.02 – 2.2 g/100 g honey) (de la Fuente et al., 2011; Nakajima et al., 1990). However, the trehalulose content of stingless bee honey of the Australian genus *Tetragonula* was reported up to 48.7 g/100 g honey with an average value of 18.5 g/ 100 g honey (n = 89) and was the dominant disaccharide content (Fletcher et al., 2021). SBH of Malay-Australian stingless bee species were examined and Zawawi et al. (2022) advocated for a new draft food standard to include trehalulose content. A recent approved stingless bee honey standard by Food Standards Australia and New Zealand (FSANZ) requires that honey produced by Australian stingless bees (in the genera *Tetragonula* and *Austroplebeia*) contain no less than 2 % trehalulose. This was the first time that trehalulose was specified in a stingless bee honey standard (Australia New Zealand Food Standards Code, 2024). In addition, the Malaysian food standard MS2683 was revised in draft form in 2024, with further trehalulose content requirement suggested as no less than 10 g/100 g honey (Department of Standards Malaysia, 2024).

With nectar sucrose acting as substrate, the stingless bee isomerisation to trehalulose occurs via a glucosyl-enzyme intermediate with a double-displacement mechanism resulting in retention of the α-anomeric configuration (Zhang et al., 2022). However, it is still unknown whether all stingless bee species have the ability to produce trehalulose and how the content differs between bee species and across regions. Hence, the sugar profile of more SBH samples from various stingless bee species across the world needs to be investigated. Bee species has been proven to affect the physicochemical profile of the produced honey (Mokaya et al., 2022). In addition, due to the limited foraging distance, the honey produced by stingless bees is associated with the available nectar botanical sources, geographical area and environmental conditions (Ávila et al., 2019; Smith et al., 2017).

Recently, there has been significant increased interest in compositional profile and chemical markers of stingless bee honey, as well as novel methods of analysis, with different techniques employed, including liquid chromatography-mass spectrometry (LC-MS) (dos Santos Diniz Freitas et al., 2025), nuclear magnetic resonance (NMR) (Gerginova et al., 2023; Kanazoe et al., 2024), high-performance thin-layer chromatography (HPTLC) (Mello dos Santos et al., 2023), and ion chromatography (IC) (Hungerford et al., 2023; Yates et al., 2025), dependent on the analytes examined. Honey is a complex natural product with significant compositional variation dependent on bee species, floral nectar collected and season, climatic conditions. The honey of many species is yet to be investigated for marker or bioactive compounds and for the shaping of global and country-based standards (Mokaya et al., 2022; Vit et al., 2024; Xu et al., 2025).

In Brazil, over 240 species of stingless bees have been recorded, predominantly belonging to the genera *Plebeia*, *Tetragona*, *Melipona*, *Scaptotrigona*, and *Tetragonisca* (De Menezes Pedro, 2014). Research emphasizes the high genetic diversity among Brazilian native bees in bee behaviours, honey production and nesting environment (Nogueira, 2023). Some Brazilian states such as Amazonas, Bahia, Paraná, Santa Catarina, Rio Grande do Norte and São Paulo have established their own quality standards for SBH, however, Brazil overall doesn't yet have the federal legislation that defines the physicochemical parameters for SBH (Sato et al., 2023). Recently, an extensive study of the chemical composition of stingless bee honeys from Brazil was published, including 55 samples from genera *Melipona*, *Scaptotrigona*, *Frieseomelitta*, *Tetragona* and *Tetragonisca* (Ressutte et al., 2025), however using the chosen chromatographic method (HPLC-RID), trehalulose could not be separated from maltose and was reported combined as trehalulose + maltose.

The aim of this current preliminary study was to investigate the trehalulose and other sugar levels in honey produced by different Brazilian stingless bee honey species and provide a basis for understanding the unique diversity of Brazilian SBH. Whilst the initial study by Fletcher et al. (2020) revealed trehalulose as the major disaccharide in a honey sample of *Tetragonisca angustula* from Brazil, there was no further knowledge of trehalulose in Brazilian SBH. A recent study reported trehalulose + maltose combined (Ressutte et al., 2025), but not trehalulose individually quantified. In our current study, SBH samples (n = 19) were collected from eight Brazilian states (Fig. 1) from five genera (15 species). Sugar content of SBH samples produced by different Brazilian stingless bee species were chromatographed and quantified by high-performance ion chromatography with electrochemical pulsed amperometric detection (HPIC-PAD). In the presence of the basic mobile phase, ion chromatography separates sugars on the column in their anionic forms, giving unrivalled separations and therefore sample differentiation and specificity. Distinct advantages of HPIC-PAD include excellent resolution, high sensitivity, reproducibility and minimal sample preparation (Zhu et al., 2019). Trehalulose levels are an important characteristic of SBH and this work describes the first step in assessing these levels in 15 species of Brazilian SBH.

2. Materials and methods

2.1. Stingless bee honey samples

Brazilian stingless bee honey samples (n = 19, approx. 30 g each) were freshly collected from managed hives by beekeepers in meliponaries. Holes were punched in the honey pots and honey allowed to drain into collection trays, before being transferred to glass jars and stored at 4 °C. The honey samples were delivered to Australia with gel ice pack and stored at 4 °C before analysis. The total collected samples belonged to five stingless bee genera: *Melipona*, *Plebeia*, *Tetragona*, *Tetragonisca* and *Scaptotrigona*, from eight Brazilian states: Amazonas, Bahia, Goiás, Maranhão, Paraná, Rio Grande do Norte, Santa Catarina and São Paulo (Table 1). Samples M5, M6, T2, T4 were collected from the hives at the same meliponary ~10 km NW of Santa Cruz Cabrália, in Bahia, and located in dense secondary forest of diverse flora. All other samples were collected from rural areas characterized by a mosaic of natural ecosystems and agroecosystems. All the samples were presumed multifloral.

2.2. Chemicals and reagents

Analytical sugar standards, D-glucose (>99.5 %), D-fructose (>99 %), D-galactose (>99 %), sucrose (100 %), palatinose (>99 %), lactose monohydrate (100 %) and maltose monohydrate (99 %) were purchased from Sigma-Aldrich (Castle Hill, Australia). Trehalulose (>94 %) was purchased from Alfa Chemistry (New York, United States). Erlose (98.4 %) was purchased from Nagase Viita Co. Ltd (formerly

Fig. 1. Locations for stingless bee honey samples collection, showing the Brazilian States where the honey samples were collected (created with mapchart.com).

Hayasgubara Co. Ltd, Okayama, Japan). Ultrapure water was obtained from a Milli-Q purification system.

2.3. *Ion chromatography quantification of Brazilian stingless bee honeys*

2.3.1. *Sample preparation*

All the analytical sugar standards were prepared with concentrations from 0.1 to 10 mg/L for calibration curves. SBH samples (0.25 g) were diluted with 25 mL ultrapure water. Samples were mixed on a Heathrow Scientific Vortexer for 30 sec and then centrifuged at 4 °C for 10 min at 9000 G. Sample solutions (10 μL) were further diluted with 9.99 mL (final dilution 1 in 100,000). Ultrapure water and filtered through a Phenomenex RC 0.22 μm syringe filter for analysis.

2.3.2. *Ion chromatography-based instrumental method for sugar analysis*

Sugars in SBH samples were separated and quantified by Thermo Fisher Interion Ion Chromatograph using a 2 μL injection loop, and a Dionex AS-AP autosampler with a tray cooler (20 °C). Separations were conducted on a Dionex CarboPac PA210G-Fast-4 μm analytical column (150 × 2 mm, P/N 088954) coupled with a Dionex CarboPac PA210-Fast-4 μm guard column (30 × 2 mm, P/N088956). Sugars were detected using a PAD detector with a gold electrode, a palladium hydride reference electrode, and the Gold, Carbohydrate, Quadrupole waveform. All compounds were eluted at 1.6 mL/min flow rate with 12 mM KOH for 40 min before cleaning program. The method referred to ThermoScientific Technical Note 73348 (Aggrawal and Rohrer, 2024). The sugar standard validation was described previously in a published paper of stingless bee sugar feeding experiments (Hungerford et al., 2021).

The sugar standards were eluted respectively with the retention times as mannitol (2.13 min), galactose (5.46 min), glucose (5.81 min), sucrose (6.78 min), fructose (7.70 min), lactose (11.60 min), trehalulose (17.00 min), palatinose (21.38 min), erlose (27.63 min) and maltose (33.9 min). Validation parameters are included in the Supplementary Material, Table S1.

2.3.3. *Statistical analysis*

One-way ANOVA was conducted using GraphPad Prism 10.2.2

(Graphpad Software, San Diego, CA, USA). Tukey's multiple comparisons test compared the mean of each group, correcting for multiple comparisons using statistical hypothesis testing, giving P values for trehalulose levels as multiplicity adjusted ($\alpha = 0.05$). For statistical purposes, the concentrations of trehalulose with levels < LOQ were taken as LOQ/2.

Table 1
Brazilian stingless bee honey samples by species, Brazilian federative states, and year of collection.

Genera	Species	Sample code	Brazilian State Origin	Collection Year
Melipona	*Melipona bicolor*	M1	Paraná	2022
	Melipona compressipes	M2	Amazonas	2022
	Melipona fasciculata	M3	Goiás	2022
	Melipona marginata	M4	Santa Catarina	2022
	Melipona mondury	M5	Bahia[*]	2023
	Melipona mondury	M6	Bahia[*]	2022
	Melipona quadrifasciata	M7	Bahia	2022
	Melipona seminigra	M8	Amazonas	2022
	Melipona subnitida	M9	Rio Grande do Norte	2022
Plebeia	*Plebeia emerina*	P1	Santa Catarina	2022
Tetragona	*Tetragona clavipes*	T1	Santa Catarina	2022
	Tetragona clavipes	T2	Bahia[*]	2023
Tetragonisca	*Tetragonisca angustula*	T3	São Paulo	2022
	Tetragonisca angustula	T4	Bahia[*]	2023
	Tetragonisca fiebrigi	T5	São Paulo	2023
Scaptotrigona	*Scaptotrigona bipunctata*	S1	Paraná	2021
	Scaptotrigona postica	S2	Santa Catarina	2021
	Scaptotrigona tubiba	S3	Maranhão	2022
	Scaptotrigona tubiba	S4	Maranhão	2022

[*] Samples M5, M6, T2, T4 were collected from the hives at the same location in Bahia (~10 km NW of Santa Cruz Cabrália, in Bahia).

3. Results and discussion

3.1. Trehalulose and other sugar levels in Brazilian stingless bee honeys

The sugar content of 19 Brazil SBH samples were quantified by HPIC-PAD (Table 2). Ion chromatography achieves an unrivalled degree of separation/resolution of a wide range of sugars, including mono-, di- and tri-saccharides, sugar alcohols and higher saccharides, with little sample preparation (dilution only, no derivatisation required) and can even be employed to collect sugar samples when combined with a suppressor (Yates et al., 2025). Limits of detection are comparable to sugar analysis by high performance liquid chromatography triple quadrupole/mass spectrometry and gas chromatography-mass spectrometry. The limit of detection (LOD) corresponds to the analyte amount for which the signal-to-noise ratio is equal to 3 (0.03 g/100 g honey), and limit of quantitation (LOQ) corresponds to the analyte amount for which the signal-to-noise ratio is equal to 10 (0.1 g/100 g honey). Repeatability (intraday, $n = 4$) and reproducibility (interday, $n = 5$) were evaluated by repeated analysis for each standard in a diluted honey sample.

Comparing trehalulose levels by stingless bee genera using one-way ANOVA, *Tetragona* honeys contained significantly higher trehalulose than *Melipona* ($P < 0.0001$), *Scaptotrigona* ($P = 0.0001$), *Tetragonisca* ($P = 0.0267$) and *Plebeia* ($P = 0.0421$) honeys. *Tetragonisca* honeys were also significantly higher in trehalulose content than *Melipona* ($P = 0.0026$) and *Scaptotrigona* ($P = 0.0478$) honeys.

Galactose, palatinose and lactose were below the limit of detection in all SBH samples. Maltose was detected in all samples, except one *Melipona mondury* sample (M6) and one *Tetragonisca angustula* sample (T4), and it was above the limit of quantitation in five samples (M2, P1, S3, T2 and T5, Table 2). Sucrose was not detected in most samples, being only detected, at levels less than the limit of quantitation (<LOQ) in three samples (M2, S1 and S2). Glucose, fructose and trehalulose were the three major sugars across the samples of each genera. The results indicated that *Melipona* samples (M1 – M9) were rich in glucose (24.77 – 37.92 g/100 g honey) and fructose (26.27 – 38.27 g/100 g honey), however, only a minor amount of trehalulose (< LOQ – 2.79 g/ 100 g honey) was detected.

Two *Tetragona clavipes* honey samples, (T1 and T2) contained the lowest glucose (12.00 – 19.49 g/100 g honey) and fructose (20.73 – 25.01 g/100 g honey), but, by contrast, the highest trehalulose (24.68 –

Table 2
Sugar content (g/100 g honey) in Brazilian stingless bee honey samples, as determined by ion chromatography-pulsed amperometric detection, with a method limit of detection (LOD) of 0.03 g /100 g honey and limit of quantitation (LOQ) of 0.1 g /100 g honey.

Species	Sample Code	Sugars									
		(g/100 g)									
		erlose	fructose	galactose	glucose	lactose	maltose	mannitol	palatinose	sucrose	trehalulose
Melipona bicolor	M1	<LOQ	32.16	<LOD	36.73	<LOD	<LOQ	3.00	<LOD	<LOD	2.21
Melipona compressipes	M2	<LOQ	26.27	<LOD	37.92	<LOD	1.08	<LOQ	<LOD	<LOQ	<LOQ
Melipona fasciculata	M3	<LOQ	36.53	<LOD	24.77	<LOD	<LOQ	<LOQ	<LOD	<LOD	1.35
Melipona marginata	M4	<LOQ	35.85	<LOD	32.17	<LOD	<LOQ	<LOQ	<LOD	<LOD	2.77
Melipona mondury	M5	<LOQ	33.11	<LOD	32.46	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOQ
Melipona mondury	M6	<LOQ	34.78	<LOD	37.50	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Melipona quadrifasciata	M7	<LOQ	28.77	<LOD	24.87	<LOD	<LOQ	2.30	<LOD	<LOD	2.79
Melipona seminigra	M8	<LOQ	38.27	<LOD	34.91	<LOD	<LOQ	<LOQ	<LOD	<LOD	2.55
Melipona subnitida	M9	<LOQ	35.43	<LOD	37.49	<LOD	<LOQ	<LOQ	<LOD	<LOD	0.41
Plebeia emerina	P1	<LOQ	33.13	<LOD	28.07	<LOD	1.78	7.20	<LOD	<LOD	12.81
Scaptotrigona bipunctata	S1	<LOQ	32.61	<LOD	32.79	<LOD	<LOQ	<LOQ	<LOD	<LOQ	4.85
Scaptotrigona postica	S2	1.09	43.57	<LOD	31.76	<LOD	<LOQ	<LOQ	<LOD	<LOQ	4.82
Scaptotrigona tubiba	S3	<LOQ	31.92	<LOD	26.14	<LOD	2.09	1.20	<LOD	<LOD	8.32
Scaptotrigona tubiba	S4	<LOQ	44.95	<LOD	36.06	<LOD	<LOQ	<LOQ	<LOD	<LOD	1.81
Tetragona clavipes	T1	<LOQ	25.01	<LOD	19.49	<LOD	<LOQ	<LOQ	<LOD	<LOD	24.68
Tetragona clavipes	T2	<LOQ	20.73	<LOD	12.00	<LOD	2.26	<LOQ	<LOD	<LOD	39.03
Tetragonisca angustula	T3	<LOQ	32.61	<LOD	30.88	<LOD	<LOQ	1.00	<LOD	<LOD	20.36
Tetragonisca angustula	T4	<LOD	39.95	<LOD	29.04	<LOD	<LOD	<LOQ	<LOD	<LOD	5.44
Tetragonisca fiebrigi	T5	<LOQ	27.03	<LOD	23.87	<LOD	1.39	1.30	<LOD	<LOD	23.90

39.03 g/100 g honey). Based on the sample collection information (Table 1), two *Tetragonisca* samples (T3 and T5) from São Paulo shared similar trehalulose content (20.36 and 23.90 g/100 g). In contrast, only 5.44 g trehalulose / 100 g honey was detected in *Tetragonisca* sample (T4) which was collected in Bahia State. The samples T3 (collected from São Paulo) and T4 (collected from Bahia) were from the same species of *Tetragonisca angustula* but displayed considerable variation in trehalulose content. Hence, the botanical recourse between São Paulo and Bahia was the potential factor for the different trehalulose levels in those *Tetragonisca* samples.

Our previous feeding trials demonstrated that stingless bees can form trehalulose from sucrose, but not from glucose and fructose, so the nectar sugar profile of differing botanical sources has the potential to have significant impact on the trehalulose content of the resultant honey (Hungerford et al., 2021). Our method has previously only detected sucrose in stingless bee honey of the *Austroplebeia* genus ranging from <LOQ to 10.1 g/100 g honey (Fletcher et al., 2021), although it has also been detected at low levels (<LOD – 2.47 g/100 g honey) in Malaysian SBH by Tiang et al. (2025) and similarly in Chinese SBH by (Zheng et al., 2025) (<LOD – 0.43 g /100 g honey).

For the SBH samples (M5, M6, T2, T4) collected from the same location in the state of Bahia, the trehalulose content varied from <LOQ (M5 and M6) to 39.03 g/100 g honey (T2). The stingless bee species evidently also heavily influences the trehalulose content in SBH. For *Scaptotrigona* samples (S1 – S4), the glucose levels ranged from 26.14 to 36.06 g/100 g honey, fructose levels were between 31.92 – 44.95 g/100 g honey and presented low to intermediate trehalulose levels (1.81 – 8.32 g/100 g). The single *Plebeia* sample contained intermediate levels of all three sugars, glucose (28.07 g/100 g), fructose (33.13 g/100 g) and trehalulose (12.81 g/100 g honey). All except two samples contained maltose (at low levels) as well as trehalulose. The ability to separate and quantitate both maltose and trehalulose, highlights the capability of the ion chromatography method. Previously disaccharides maltose and trehalulose were both identified and quantitated in Malaysian *Heterotrigona itama* honey (Ramlan et al., 2024) and by NMR in the honey from Tanzania of African stingless bee, *Meliponula ferruginea* (Popova et al., 2021). In both these studies, the reported maltose was minor compared to trehalulose, as in this study. Low levels of maltose were identified in Ecuadorian stingless bee honeys via ^{1}H NMR analysis, however, trehalulose was not included as part of the targeted honey profiling (Vit et al., 2023).

Mannitol was recently suggested as a marker in stingless bee honey (Shamsudin et al., 2022; Xu et al., 2025), ranging up to 13.49 g/100 g honey. It has also been identified in honeybee honey (Horváth and Molnár-Perl, 1998). In this study, mannitol was detected in all samples examined and was above the limit of quantitation in six samples (M1, M7, P1, S3, T3 and T5). The highest level of mannitol was 7.20 g/100 g honey in *Plebeia emerina* (P1) honey.

Trehalulose in SBH was previously considered to be maltose in the literature and this was clarified in 2020 (Chuttong et al., 2016; Fletcher et al., 2020; Souza et al., 2006; Zawawi et al., 2022). The reasons for misreporting trehalulose were the similar retention times of maltose/-trehalulose by high-performance liquid chromatography (HPLC), lack of trehalulose standard and only certain sugars routinely analysed through HPLC (Bogdanov et al., 1996; Fletcher et al., 2020; Oddo et al., 2008; Se et al., 2018). Interestingly, due to the lack of separation of trehalulose and maltose by HPLC-RID, these were recently reported together as trehalulose + maltose (Ressutte et al., 2025) in the sugar profiles of Brazilian SBH. However, generally, the results by Ressutte et al. (2025) reflected our current study, with lower trehalulose + maltose in *Melipona* and *Scaptotrigona* honeys and highest in *Tetragona clavipes* honeys, both consistent with the observed levels of trehalulose in Table 2.

Some methods also do not differentiate sucrose, maltose and/or trehalulose (Arif Zaidi et al., 2023; Fuenmayor et al., 2013). An examination of sugars of 10 samples of Brazilian stingless bee honey by capillary electrophoresis (CE) found a large variation of reducing sugar (glucose + fructose) levels by stingless bees species. The lowest levels were found in honey produced by *Tetragona clavipes* (48.6 g/100 g) and the highest levels in *Melipona marginata* honey (averaging 62.1 g/100 g) (Biluca et al., 2016). As the study only analysed glucose, fructose and sucrose (sucrose was not detected across all tested samples), the difference between reducing sugar content and the soluble solids (°Brix values) was speculated as due to the presence of “maltose”, as had been reported in SBH previously using HPLC-RID (Chuttong et al., 2016), but this could in fact be due to trehalulose.

From the record of what was believed to be “maltose” levels in the literature, it also confirmed the low levels or lack of this disaccharide (assumed now to be mainly trehalulose) in the honey from *Melipona* species (1.61 and 3.61 g/100 g honey for *Melipona compressipes*) and rich levels in *Tetragonisca* honeys (23.96 – 28.5 g/100 g honey for *Tetragonisca angustula*), although intermediate levels were observed in *Scaptotrigona* honeys (4.99 – 6.16 g/100 g honey) (Vit et al., 1998). The reducing sugar level of honey from *Plebeia* bees ranged from 42.0 – 71.61 g/100 g honey) (Andrade-Velasquez et al., 2023; de Araújo et al., 2023). As these studies of *Plebeia* honeys employed colorimetric/spectrophotometric methods, then the reported total reducing sugars would, in addition to glucose and fructose, include any trehalulose and maltose, both also reducing sugars. Otherwise, maltose in *Plebeia* bee honeys was not investigated in the literature. Interestingly, a maturation study of *Melipona mondury* honey using HPAEC-PAD analyzed glucose, fructose, maltose, and sucrose, but only glucose (22.8–25.5 g/100 g) and fructose (26.9–28.8 g/100 g) were detected. Trehalulose was not included in the analysis but is likely present at low levels, as suggested by the current study. The minimal maltose detection is also consistent with previous findings for *Melipona* bee honeys (Nordin et al., 2018).

Erlose was the only trisaccharide detected in the current set of 19 Brazilian stingless bee honeys. It was only above the limit of quantitation in one sample (S2) and was not detected in one sample (T4). Previously, small amounts of erlose were detected in Australian stingless bee honeys from the genera *Tetragonula* and *Austroplebeia*. (Fletcher et al., 2021) and in Malaysian and Australian honey samples (Zawawi et al., 2022), as confirmed by high-resolution accurate mass LC-MS/MS and NMR analysis (Hungerford et al., 2021). The trisaccharide raffinose was detected in Brazilian SBH by Ressutte et al. (2025) although erlose was not included as a standard in that study to assess if chromatographic separation of raffinose and erlose was achieved. Raffinose was also detected by ^{1}H NMR in Ecuadorian SBH, with the highest levels in the *Geotrigona* genus (5.0 ± 0.3 g / 100 g) (Vit et al., 2023). In the current study, no peak was observed at the retention time corresponding to raffinose, but minor saccharides including trisaccharides are likely present below the current limit of detection.

When confined colonies of the Australian stingless bee *Tetragonula carbonaria* were fed solutions of the sugars commonly found in flower nectar (sucrose, glucose and fructose), the stingless bees were able to convert sucrose to trehalulose (Hungerford et al., 2021). However, they were unable to convert glucose/fructose mixtures to trehalulose. This evidence indicated that trehalulose in honey produced by stingless bees originated from nectar sucrose. Further understanding of this process was achieved using bee part incubations and stable isotope labelling experiments (Zhang et al., 2022). Hence, the nectar sucrose content of the preferred floral sources of the different Brazilian stingless bee species could be a potential factor influencing the trehalulose levels in SBH. As yet, there has been no verification of whether a stingless bee enzyme or the intrinsic stingless bee microbiota are involved in the conversion of sucrose to trehalulose. Trehalulose is known to be produced by microorganisms, generally as a mixture with other sugars (Cookson et al., 1987). Trehalulose is also found in the excrement of whiteflies *Bemisia tabaci* (Bates et al., 1990), with the enzyme producing exclusively trehalulose (Salvucci, 2003).

Trehalulose levels have been reported for the honey samples produced by different stingless bee species from other countries, particularly Australia (Zawawi et al., 2022), China (Zheng et al., 2025), Malaysia (Ramlan et al., 2024) and Tanzania (Mduda et al., 2025). The highest trehalulose levels (43.1 – 57.0 g/100 g honey) have been reported for Malaysian species *Geniotrigona thoracica* (Zawawi et al., 2022). Variable levels were reported for honey of Chinese species of *Lepidotrigona flavibasis* (4.3 – 37.7 g/100 g) and *Lepidotrigona terminata* (4.3 – 35.9 g/100 g of SBH) (Zheng et al., 2025). In *Austroplebeia* species from Australia (Fletcher et al., 2021), the trehalulose levels were much lower (<0.1 – 15 g/100 g of SBH) than *Tetragonula* species. Hence, based on the observed lower trehalulose levels in honey from Brazilian *Melipona* species presented herein, there seems to be genus-specific variations in trehalulose levels.

3.2. Floral preferences of Brazilian stingless bees by species

Social bees are commonly generalists in their food source due to the perennial nature of their nests. According to the database on Brazilian Bee-Plant interactions, the most common plants visited by the stingless bee species are *Asteraceae, Fabaceae, Solanaceae, Ochnaceae* and *Melastomataceae* (Table 3). Both genera of *Melipona* (SBH low in trehalulose) and *Tetragona* (SBH high in trehalulose) share common floral preferences. *Melipona mondury* bees are observed additionally to forage on Anacardiacea, Arecaceae, Bixaceae, Myrtiaceae (Marelli, 2025).

Research on nectar sugar composition of *Asteraceae* (n = 35) states the majority of samples (77 %) detected a higher proportion of glucose/fructose rather than sucrose (Torres and Galetto, 2002). Sucrose is dominant in the nectar of *Fabaceae* (Małgorzata and Jacek, 2013). For *Scopolia carniolica* (*Solanaceae*), nectar contains exclusively fructose (32.21 – 34.45 %) and sucrose (65.55 – 67.79 %). As all the obtained SBH samples are presumed multifloral and the nectar sugar content, especially for sucrose content, of the native plants are varied, further research is needed to investigate the relationship between nectar sucrose content accessed and the corresponding trehalulose levels in the produced SBH.

SBH is a concentrated solution of sugars with a predominance of reducing sugars, which include glucose, fructose, maltose and trehalulose. Compared to honeybee honey, the higher moisture content of SBH means that food standards for SBH required slightly lower amounts of reducing sugars. The Argentine food standard for SBH of *Tetragonisca fiebrigi* states the minimum reducing sugar level is 40 g/100 g for SBH without preservation treatment and pasteurised honey products and 45 g/100 g of honey for dehumidified products (Argentina National Food Commission, 2019). For the national standard of Indonesia, the minimum level of reducing sugars is 55 g /100 g of honey for local SBH products (Badan Standardisasi Nasional, 2018).

All the analysed Brazilian SBH samples in this study comply with the requirement of the reducing sugar content in these food standards, despite the differences in species. However, trehalulose, the rare reducing sugar in SBH, has gained more and more attention and become an individual factor for evaluating the honey quality. Therefore, it is important to determine the concentration of trehalulose across different species to incorporate this data as part of total reducing sugars, to create appropriate SBH standards.

The department of food standards Malaysia has drafted a revised dehydrated SBH standard (major species are *Heterotrigona* and *Geniotrigona* species) for public comment that specifies not less than 10 g trehalulose/ 100 g honey (Department of Standards Malaysia, 2024). After looking at genera *Lepidotrigona*, *Tetragonula* and *Homotrigona*, as well as unidentified species, the new proposed food standard for Chinese SBH suggests a trehalulose level greater than 4 g/ 100 g honey for the standard grade of SBH (Zheng et al., 2025). For a higher-grade honey product, it was suggested trehalulose content of no less than 8 g/ 100 g honey in Chinese SBH. The previously developed Australian food standard of *Tetragonula* and *Austroplebeia* stingless bee honey also states the trehalulose content must be greater than 2 g due to observed variation in Australian samples (Australia New Zealand Food Standards Code, 2024).

Table 3
Plants visited by the stingless bee species examined in this work (Brazilian Bee Studies Association, 2010).

Bee genus	Species	Botanical family
Melipona	*Melipona mondury*	Asteraceae
	Melipona marginata	Anacardiaceae, Aquifoliaceae, Aaliaceae, Asclepiadaceae, Asteraceae, Begoniaceae, Clethraceae, Clusiaceae, Cunoniaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Lauraceae, Loranthaceae, Malvaceae, Melastomataceae, Moraceae, Myrsinaceae, Myrtaceae, Olacaceae, Polygalaceae, Polygonaceae, Rubiaceae, Sapindaceae, Solanaceae, Theaceae, Verbenaceae,
	Melipona bicolor	Aquifoliaceae, Asclepiadaceae, Asteraceae, Begoniaceae, Boraginaceae, Clethraceae, Cucurbitaceae, Ericaceae, Euphorbiaceae, Flacourtiaceae, Iridaceae, Lamiaceae, Lauraceae, Loranthaceae, Melastomataceae, Myrsinaceae, Myrtaceae, Ochnaceae, Rosaceae, Rubiaceae, Sapindaceae, Solanaceae, Theaceae Verbenaceae.
	Melipona subnitida	Convolvulaceae, Ochnaceae, Rubiaceae, Solanaceae.
	Melipona compressipes	Dilleniaceae, Fabaceae, Flacourtiaceae, Melastomataceae, Myrtaceae, Solanaceae.
	Melipona fasciculata	Asteraceae, Fabaceae, Ochnaceae, Verbenaceae.
	Melipona quadrifasciata	Acanthaceae, Amaryllidaceae, Araliaceae, Asclepiadaceae, Asteraceae, Bombacaceae, Boraginaceae, Chrysobalanaceae, Convolvulaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Liliaceae, Malpighiaceae, Melastomataceae, Myrtaceae, Ochnaceae, Polygonaceae, Portulacaceae, Rubiaceae, Rutaceae, Sapindaceae, Solanaceae, Styracaceae, Verbenaceae, Vochysiaceae.
	Melipona seminigra	Achariaceae, Anacardiaceae, Arecaceae, Bixaceae, Burseraceae, Clusiaceae, Dilleniaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Icacinaceae, Lythraceae, Malpighiaceae, Melastomataceae, Meliaceae, Myristicaceae, Myrtaceae, Proteaceae, Rutaceae, Sapindaceae, Solanaceae, Verbenaceae.
Plebeia	*Plebeia emerina*	Acanthaceae, Aizoaceae, Alismataceae, Amaryllidaceae, Anacardiaceae, Apiaceae, Araceae, Arecaceae, Asteraceae, Balsaminaceae, Bignoniaceae, Boraginaceae, Brassicaceae, Cactaceae, Campanulaceae, Caprifoliaceae, Clethraceae, Convolvulaceae, Cucurbitaceae, Cyperaceae Elaeocarpaceae, Ericaceae, Euphorbiaceae, Fabaceae, Gesneriaceae, Iridaceae, Lamiaceae, Liliaceae, Loganiaceae, Malpighiaceae, Malvaceae, Melastomataceae, Myrtaceae, Nyctaginaceae, Onagraceae, Oxalidaceae, Phytolaccaceae, Polygonaceae, Primulaceae, Proteaceae, Rosaceae, Rubiaceae, Solanaceae, Sterculiaceae, Styracaceae, Tiliaceae, Tropaeolaceae, Velloziaceae, Vitaceae.
Scaptotrigona	*Scaptotrigona postica*	Dilleniaceae, Myrtaceae.
	Scaptotrigona tubiba	Boraginaceae, Clusiaceae, Convolvulaceae, Dilleniaceae, Euphorbiaceae, Fabaceae, Lythraceae, Malpighiaceae, Myrtaceae, Polygonaceae, Sapindaceae.
	Scaptotrigona bipunctata	Amaranthaceae, Apiaceae, Araceae, Araliaceae, Asteraceae, Bombacaceae, Burseraceae, Campanulaceae, Caprifoliaceae, Clethraceae, Clusiaceae, Convolvulaceae, Cunoniaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Lauraceae, Loranthaceae, Lythraceae, Malphighiaceae,

(*continued on next page*)

Table 3 (*continued*)

Bee genus	Species	Botanical family
		Melastomataceae, Mutingiaceae, Myrtaceae, Ochnaceae, Olacaceae, Oleaceae, Piperaceae, Polygonaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Salicaceae, Sapindaceae, Solanaceae, Symplocaceae, Thymelaeaceae, Domke, Ulmaceae.
Tetragona	*Tetragona clavipes*	Acanthaceae, Anacardiaceae, Apiaceae, Apocynaceae, Araliaceae, Arecaceae, Asteraceae, Bignoniaceae, Bixaceae, Bombacaceae, Caryocaraceae, Chrysobalanaceae, Cucurbitaceae, Cyperaceae, Erythroxylaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Liliaceae, Loranthaceae, Lythraceae, Malpighiaceae, Meliaceae, Myrtaceae, Ochnaceae, Oleaceae, Oxalidaceae, Piperaceae, Plumbaginaceae, Poaceae, Proteaceae, Rubiaceae, Rutaceae, Sapindaceae, Solanaceae, Sterculiaceae, Styracaceae, Tiliaceae, Turneraceae, Ulmaceae, Verbenaceae, Vochysiaceae.
Tetragonisca	*Tetragonisca fiebrigi*	-
	Tetragonisca angustula	Acanthaceae, Aizoaceae, Amaranthaceae, Anacardiaceae, Apiaceae, Apocynaceae, Araceae, Araliaceae, Arecaceae, Asclepiadaceae, Asteraceae, Balsaminaceae, Bignoniaceae, Bixaceae, Bombacaceae, Boraginaceae, Burseraceae, Capparaceae, Caprifoliaceae, Caryocaraceae, Caryophyllaceae, Cecropiaceae, Chrysobalanaceae, Clusiaceae, Cochlospermaceae, Combretaceae, Commelinaceae, Connaraceae, Convolvulaceae, Cucurbitaceae, Cyperaceae, Dilleniaceae, Elaeocarpaceae, Ericaceae, Erythroxylaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Iridaceae, Labiatae, Lamiaceae, Lauraceae, Liliaceae, Loganiaceae, Loranthaceae, Lythraceae, Malphighiaceae, Malpighiaceae, Malvaceae, Melastomataceae, Meliaceae, Mimosoideae, Moraceae, Myrsinaceae, Myrtaceae, Nyctaginaceae, Ochnaceae, Olacaceae, Oleaceae, Onagraceae, Oxalidaceae, Piperaceae, Plumbaginaceae, Poaceae, Polygonaceae, Portulacaceae, Proteaceae, Ranunculaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Sapindaceae, Sapotaceae, Scrophulariaceae, Solanaceae, Sterculiaceae, Styracaceae, Theaceae, Tiliaceae, Turneraceae, Ulmaceae, Verbenaceae, Violaceae, Vochysiaceae.

Compared to the species described in Southeast Asia and Malaysia-Australia region, Brazil has greater numbers of reported stingless bee species with higher genetic diversity. As suggested in the current study, more sampling of the different species will be required for development of the local Brazilian stingless bee honey food standard.

4. Conclusions

The present study provides the first data on individually quantified trehalulose levels in Brazilian stingless bee honey from 15 species across five genera (*Melipona*, *Plebeia*, *Tetragona*, *Tetragonisca* and *Scaptotrigona*). The results indicate that trehalulose content in stingless bee honey varied according to bee species, hive location, and botanical origin.

The highest trehalulose levels were detected in *Tetragona* honey samples, while the lowest levels were found in *Melipona* honey samples, with concentrations ranging from below the limit of quantitation. (<LOQ) to 39.03 g/100 g honey. Trehalulose was undetectable in three samples and present at low levels (<3 g/100 g) in seven others, suggesting species- and genera-dependent variation that remains poorly understood. Further investigation is needed to characterize the sugar profile across the > 240 Brazilian stingless bee species.

Analysis of the honey of worldwide stingless bee species (over 600) would assist the development of country-specific, region-specific or worldwide stingless bee honey standards. The utilised ion chromatography method is a valuable technique for the resolution/separation of sugars and is recommended for honey samples with widely varying sugar composition.

CRediT authorship contribution statement

Hans S.A. Yates: Writing – review & editing, Formal analysis. **Jiali Zhang:** Writing – original draft, Visualization, Investigation, Data curation. **Natasha L. Hungerford:** Writing – review & editing, Supervision, Investigation, Data curation, Conceptualization. **Daniel Nicodemo:** Writing – review & editing, Resources. **Cássia Regina de Avelar Gomes:** Resources. **Jean-Philippe Marelli:** Writing – review & editing, Resources. **Mary T. Fletcher:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.108187.

Data availability

The data supporting this study is provided within this article.

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