JÉSSICA BARRIONUEVO RESSUTTE

MEL DE ABELHA-SEM-FERRÃO: ASPECTOS QUÍMICOS E APLICAÇÃO TECNOLÓGICA

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Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Londrina, como requisito parcial para obtenção do título de Doutora em Ciência de Alimentos.

Orientador: Profª. Drª Wilma Aparecida Spinosa

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RESSUTTE, Jéssica Barrionuevo. **Mel de abelha-sem-ferrão: Aspectos químicos e aplicação tecnológica**. 2024. 191 f. Tese (Doutorado em Ciência de Alimentos) — Universidade Estadual de Londrina, Londrina, 2024.

RESUMO GERAL

O mel das abelhas-sem-ferrão tem sido valorizado no mercado nacional e internacional devido às suas características apreciadas de sabor, cor, aroma e textura. Além disso, o mel e a própolis dos meliponíneos contêm compostos que conferem atividade bioativa, associados a diversos benefícios à saúde humana. A composição dos méis de abelha-sem-ferrão pode variar dependendo da origem geográfica e entomológica. Nesse sentido, o objetivo deste estudo foi investigar a influência da origem geográfica e entomológica nas características químicas dos méis por meio de análises quantitativas, FTIR-ATR e análise melissopalinológica. Além disso, realizou-se um estudo de revisão sobre os açúcares presentes no mel, provenientes de néctar floral e melato, avaliando os métodos diretos e indiretos para a análise de açúcares. Por fim, foram desenvolvidos compostos à base de mel e extrato de própolis nas concentrações de 0,25%, 0,50% e 0,75%, utilizando os produtos da abelha *Tetragonisca angustula*. Esses compostos foram avaliados em relação às suas propriedades físico-químicas, antioxidantes e sensoriais. Através de análise quimiométrica foi possível verificar uma separação entre grupos com base na origem entomológica, influenciada principalmente pela composição química do mel. Também foi possível observar uma separação entre dois grupos em termos de origem geográfica (Norte/Nordeste e Sul), influenciada principalmente pelos tipos polínicos. No estudo de revisão sobre açúcares, melezitose e trealulose foram apontados como biomarcadores de mel de melato e mel de abelha-sem-ferrão, respectivamente. Em termos de cromatografia líquida, a espectroscopia de massas e a detecção amperiométrica pulsada são citadas como os métodos mais adequados para separar e quantificar açúcares no mel; no entanto, o detector mais utilizado é o índice de refração. Métodos alternativos como a espectroscopia vibracional e a ressonância nuclear magnética, principalmente esta última, têm se mostrado eficazes na quantificação de açúcares no mel. Para os produtos de mel com extrato de própolis, foram observadas pequenas alterações nas características físico-químicas comparado ao mel sem adição de extrato de própolis. A adição do extrato de própolis potencializou a atividade bioativa do mel, sendo o aumento não proporcional e mais pronunciado no composto contendo 0,75% de extrato de própolis. O conteúdo de compostos fenólicos aumentou em 37%, o poder de redução férrica aumentou em 36%, e a atividade antioxidante medida através método de sequestro do radical ABTS•+ aumentou em aproximadamente três vezes. Na análise descritiva qualitativa, os atributos que melhor descreveram os compostos do mel com extrato de própolis foram sabor fermentado, sedimentos, pontos escuros, sabor ácido, aroma ácido, sabor de própolis e cor amarela.

Palavras-chave: Alimento funcional, Açúcares em mel, Mel de abelha-sem-ferrão, Própolis de abelha-sem-ferrão, Quimiometria

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ABSTRACT

The honey of stingless bees has been valued in the national and international market due to its appreciated characteristics of flavor, color, aroma, and texture. Additionally, the honey and propolis from meliponine bees contain compounds that confer bioactive activity, associated with various health benefits for humans. The composition of stingless bee honey can vary depending on its geographical and entomological origin. In this context, the objective of this study was to investigate the influence of geographical and entomological origin on the chemical characteristics of honey through quantitative analyses, FTIR-ATR, and melissopalynological analysis. Furthermore, a review study was conducted on the sugars present in honey, derived from floral nectar and honeydew, evaluating direct and indirect methods for sugar analysis. Finally, compounds based on honey and propolis extract were developed at concentrations of 0.25%, 0.50%, and 0.75%, using products from the *Tetragonisca angustula* bee. These compounds were evaluated in terms of their physicochemical, antioxidant, and sensory properties. Chemometric analysis revealed a separation between groups based on entomological origin, primarily influenced by the chemical composition of the honey. Additionally, a separation between two groups was observed in terms of geographical origin (North/Northeast and South), mainly influenced by pollen types. In the sugar review study, melezitose and trehalulose were identified as biomarkers for honeydew honey and stingless bee honey, respectively. Liquid chromatography, mass spectrometry, and pulsed amperometric detection were cited as the most suitable methods for separating and quantifying sugars in honey; however, the most commonly used detector is the refractive index. Alternative methods such as vibrational spectroscopy and nuclear resonance, especially the latter, have proven effective in quantifying sugars in honey. For honey products with propolis extract, there were slight changes in physicochemical characteristics compared to honey without propolis extract. The addition of propolis extract enhanced the bioactive activity of honey, with a non-proportional and more pronounced increase in the compound containing 0.75% propolis extract. The content of phenolic compounds increased by 37%, ferric reduction power by 36%, and antioxidant activity measured through the ABTS•+ radical scavenging method increased approximately threefold. In the qualitative descriptive analysis, the attributes that best described the compounds in honey with propolis extract were fermented flavor, sediments, dark spots, sour taste, sour aroma, propolis flavor, and yellow color.

Keywords: Functional food, Sugars in honey, Stingless bee honey, Stingless bee propolis, Chemometrics

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1. INTRODUÇÃO

O mel é um alimento natural de grande valor nutricional, amplamente consumido globalmente. Embora o mel de abelha *Apis mellifera* domine o mercado mundial, recentemente têm crescido o interesse pelo mel das abelhas-sem-ferrão. As abelhas indígenas, conhecidas como meliponíneos, englobam mais de 500 espécies distribuídas por regiões tropicais e subtropicais ao redor do mundo. Essas abelhas desempenham um papel crucial na polinização e na produção de mel, própolis, pólen e geoprópolis (BANA *et al.*, 2024; FERNANDES; ROSA; CONTI-SILVA, 2020; SATO *et al.*, 2023).

Na cadeia produtiva das abelhas-sem-ferrão, o mel é o principal produto comercializado. Em comparação ao mel de *Apis mellifera*, o mel de meliponíneo apresenta características distintas como cristalização lenta, sabor ácido e maior fluidez. A criação racional dessas abelhas não apenas gera renda e empregos, mas também oferece benefícios ecológicos significativos (ÁVILA *et al.*, 2018; RIBEIRO *et al.*, 2018). Contudo, apesar do crescente interesse por esse tipo de produto, a produção em larga escala do mel das abelhas-sem-ferrão apresenta algumas limitações, sendo as principais, a baixa produção, comparado a abelha *Apis mellifera*, e a falta de uma legislação abrangente (ÁVILA *et al.*, 2018; SATO *et al.*, 2023).

A dificuldade na criação de uma legislação nacional abrangente se deve às distintas características de méis de abelha-sem-ferrão, as quais são influências principalmente por fatores geográficos e entomológicos (ÁVILA *et al.*, 2019; SHAMSUDIN *et al.*, 2019; VIT *et al.*, 2023). Nesse sentido, as propriedades intrínsecas do mel de abelha sem ferrão poderiam ser usadas como potenciais biomarcadores para discriminar méis de diferentes gêneros (MDUDA; HUSSEIN; MURUKE, 2023).

O perfil de açúcar tem sido utilizado para diferenciar diferentes tipos de méis. A presença de trealulose e melezitose foram apontados, recentemente, como biomarcadores de amostras de mel de abelha-sem-ferrão e mel de melato, respectivamente (FLETCHER *et al.*, 2020; PITA-CALVO; VÁZQUEZ, 2017). Métodos diretos e indiretos têm sido aplicados para a quantificação de açúcares, como a cromatografia líquida de alta eficiência acoplada a diferentes detectores; espectroscopia vibracional e ressonância nuclear magnética (ANJOS *et al.*, 2015; CONSONNI; CAGLIANI; COGLIATI, 2012; GEANĂ *et al.*, 2020). Cada um dos métodos mencionados acima traz vantagens e desvantagens em termos de sensibilidade, adequação e custo-benefício.

A composição da própolis de abelha-sem-ferrão, assim como a composição do mel, pode variar de acordo com a espécie de abelha produtora (SUREK *et al.*, 2022). Os compostos

químicos predominantes na própolis incluem terpenoides, flavonoides e compostos fenólicos. Essas classes principais de substâncias têm sido associadas às propriedades antimicrobianas e antioxidantes da própolis (CISILOTTO *et al.*, 2018; OSÉS, S. M. *et al.*, 2016). A combinação do mel das abelhas nativas com extrato de própolis pode resultar em um produto com maior capacidade antioxidante. Até o momento, existem poucos estudos com a adição da própolis de abelha-sem-ferrão em produtos alimentícios (OSÉS *et al.*, 2015). Desse modo, a combinação do mel dos meliponíneos com própolis poderia oferecer claras vantagens para o consumidor do ponto de vista nutricional, além de agregar valor ao produto.

2. OBJETIVOS

2.1. OBJETIVO GERAL

- Investigar a influência dos fatores entomológicos e geográficos nas características dos méis de abelha-sem-ferrão brasileiros; entender quais são os açúcares apontados como biomarcadores de mel; trazer informações a respeito dos métodos de análise de açúcar e desenvolver um produto alimentício a base de mel e extrato de própolis, de modo a contribuir com a cadeia de produtos das abelhas-sem-ferrão.

2.2. OBJETIVOS ESPECÍFICOS

- Caracterizar 55 amostras de mel de abelhas-sem-ferrão, pertencentes aos gêneros *Tetragona*, *Tetragonisca*, *Melipona*, *Scaptotrigona* e *Frieseomelitta*, nativas dos estados do Pará, Maranhão, Amazonas, Paraná e Rio Grande do Sul, por meio das análises melissopalinológica e análise físico-química, perfil de açúcares, teor de compostos fenólicos e espectroscopia no infravermelho com transformada de Fourier – refletância total atenuada (FTIR–ATR) e aplicar análise multivariada para avaliar a influência da origem geográfica e entomológica nas características dos méis.

- Realizar um estudo de revisão sobre o perfil de açúcares em mel de abelha *Apis mellifera* e mel de abelha-sem-ferrão, derivados tanto de néctar floral quanto do melato; apresentar os açúcares apontados como biomarcadores e trazer informações sobre os métodos diretos e indiretos de análise de açúcar, bem como as vantagens e desvantagens de cada um.

- Desenvolver compostos a base de mel e extrato de própolis, usando os produtos da abelha *Tetragonisca angustula* nas concentrações de 0,25; 0,5 e 0,75% e caracterizá-los em relação as suas propriedades físico-químicas, antioxidantes e sensoriais.

3. REVISÃO BIBLIOGRÁFICA

3.1. ABELHA-SEM-FERRÃO

Os meliponíneos, conhecidos popularmente como abelhas indígenas sem ferrão, fazem parte de um grupo diversificado de abelhas amplamente distribuídas na Austrália, Américas, África e Ásia, em um total de mais de 600 espécies (ROUBIK, 2023, GRUTER, 2020). O Brasil possui uma grande diversidade de abelhas-sem-ferrão, com mais de 300 espécies distribuídas em todo o seu território. A predominância do clima tropical e vegetação diversificada, possibilita a criação racional dessas abelhas. A prática da meliponicultura não é apenas de importância econômica, mas também de importância socioambiental (LAVINAS *et al.*, 2019; SANT'ANA *et al.*, 2020). As abelhas são essenciais para manter o equilíbrio dos ecossistemas, garantindo a polinização de até 90% das plantações em qualquer bioma em que elas sejam encontradas. A presença desses insetos indica qualidade ambiental, sendo de extrema importância a sua preservação diante dos impactos ambientais que ameaçam a sua existência (REZENDE *et al.*, 2020).

As abelhas-sem-ferrão se diferem das abelhas africanizadas tanto em hábitos quanto em aspectos morfológicos. A principal diferença em relação aos aspectos morfológicos está relacionada a presença de um ferrão atrofiado nos meliponíneos, impossibilitando o seu uso. Com isso, algumas espécies utilizam algumas estratégias de defesa, como mordiscar a pele do agressor, enrolar-se nos cabelos e entrar em orifícios como ouvido e narinas, contudo, outras são incapazes de se proteger (BRAGHINI; BILUCA; GONZAGA; *et al.*, 2020; KOETHE *et al.*, 2018; LAVINAS *et al.*, 2019). Além disso, os meliponíneos apresentam maior diversidade comparados às abelhas *Apis mellifera* (por exemplo, tamanho do corpo, tamanho das colônias e estratégias de defesa). Em relação aos hábitos, as abelhas-sem-ferrão coletam preferencialmente o néctar de plantas rasteiras, localizadas a pequenas distâncias (HRNCIR *et al.*, 2019).

Em geral, as abelhas-sem-ferrão formam seus ninhos em troncos de árvores ocos, mas algumas espécies também podem utilizar cavidades presentes no solo, muros, paredes de casas e até mesmo em ninhos abandonados de cupins e formigas. Colmeias artificiais também podem ser utilizadas, como caixas de madeira. Os meliponíneos, diferentemente das abelhas africanizadas, constroem suas células na posição horizontal e armazenam seu alimento em potes, ao invés de favos, como é mostrado na Figura 1. O material de construção desses potes

é uma mistura de cera e própolis, provenientes de resinas vegetais, recolhidas de diversas plantas (ÁVILA *et al.*, 2018; RAMÍREZ; AYALA; GONZÁLEZ, 2018).

Figura 1 – Potes de alimento de *Tetragonisca angustula*

Fonte: De autoria própria

A população da colmeia das abelhas-sem-ferrão pode variar de 100 a 100.000 indivíduos, de acordo com a espécie. A colônia é constituída por uma rainha, pelas operárias e por alguns zangões. A rainha é responsável pela postura dos ovos no ninho. Enquanto as operárias são responsáveis por todo trabalho dentro da colônia: limpeza, coleta de néctar e resina, produção de cera, construção do ninho e potes de alimento. A função do macho é reprodutiva, contudo, em algumas espécies, os machos também podem regular a temperatura do ninho e produzir cera (RAMÍREZ; AYALA; GONZÁLEZ, 2018; GRUTER, 2020).

A prática da meliponicultura vem se expandindo em países tropicais e subtropicais como o Brasil, Austrália, Malásia, Venezuela, México e Tailândia. Devido ao manejo relativamente fácil, a criação dessas abelhas se popularizou entre os apicultores. Além disso, as abelhas-sem-ferrão se adaptam facilmente a colmeias artificiais, o que facilita a manipulação da colônia, aumentando consequentemente, a produção de mel (NORDIN *et al.*, 2018). A prática da criação racional de abelhas nativas é de fundamental interesse, não somente para a manutenção de um ecossistema equilibrado, mas também para a geração de renda e empregos. No Brasil, a diversidade de espécies de abelhas-sem-ferrão permite a seleção das espécies mais apropriadas para a polinização de culturas específicas (LIMA *et al.*, 2016; REZENDE *et al.*, 2020).

No Brasil, a resolução CONAMA nº 346 de 06 de julho de 2004 disciplina a utilização das abelhas silvestres nativas. Além disso, essa resolução reconhece a meliponicultura para fins de pesquisa, comércio, atividades de lazer e consumo artesanal dos seus produtos. Para fins comerciais, é necessário que os criadores estejam cadastrados no órgão ambiental competente. Ademais, os meliponicultores com mais de 50 colônias devem possuir autorização do IBAMA e registro no Cadastro Técnico Federal.

3.2. MEL DE ABELHA-SEM-FERRÃO

O mel produzido pelas abelhas-sem-ferrão pode ser obtido a partir do néctar das flores, das excreções de insetos e das secreções procedentes de plantas. Para produzir o mel, as abelhas recolhem, transformam e combinam esses materiais com substâncias específicas próprias, como enzimas das glândulas cefálicas e secreções salivares. Parte do mel produzido é utilizado para a própria alimentação das abelhas, e o restante é armazenado em potes para abastecimento do ninho em eventuais períodos de escassez (ÁVILA *et al.*, 2018).

O mel pode ser classificado em monofloral ou multifloral. O mel monofloral corresponde ao produto procedente de flores de uma mesma família, gênero ou espécie, enquanto o mel multifloral é procedente de diferentes origens botânicas (COSTA *et al.*, 2018). As características do mel estão intimamente relacionadas com a origem floral, as quais são influenciadas principalmente por fatores sazonais, geográficos e ambientais (SIDDIQUI *et al.*, 2017). Além disso, as características do mel podem variar de acordo com a espécie de abelha produtora, com o tipo de método de conservação empregado e com as condições de armazenamento do produto (BRAGHINI; BILUCA; OTTEQUIR; *et al.*, 2020; RIBEIRO *et al.*, 2018). A Figura 2 mostra méis de diferentes espécies de abelha-sem-ferrão.

Figura 2 - Méis de diferentes espécies de abelha-sem-ferrão

Fonte: EMBRAPA (2019).

Na meliponicultura, o mel destaca-se por ser o principal produto de exploração e comercialização (BARBOSA *et al.*, 2018; SATO *et al.*, 2023). Embora o mel de abelha *A. mellifera* tenha dominado o mercado mundial, nos últimos anos, o interesse pelo mel de abelha-sem-ferrão tem aumentado (BANA *et al.*, 2024; PIMENTEL *et al.*, 2022). O mel dos meliponíneos se difere do mel das abelhas africanizadas em termos de suas propriedades físico-químicas, microbiológicas e antioxidantes. De modo geral, o mel das abelhas nativas apresenta textura mais fluida, lenta cristalização e características sensoriais acentuadas de cor, sabor e acidez (SATO *et al.*, 2023).

O mel das abelhas-sem-ferrão é historicamente conhecido por proporcionar benefícios à saúde humana, os quais são atribuídos às suas propriedades antimicrobianas, anti-inflamatórias, antissépticas, anticancerígenas e antioxidantes. Além disso, o mel dos meliponíneos atua na inibição da atividade das enzimas α-glicosidase e α-amilase, controlando a glicemia do corpo humano (ALI *et al.*, 2020; HAU-YAMA *et al.*, 2020).

Apesar dos benefícios do mel das abelhas-sem-ferrão, e do crescente interesse do consumidor por esse tipo de produto, a produção em larga escala ainda possui algumas limitações, sendo a principal, a baixa produção (1-5 kg de mel por colônia/ ano) quando comparada à abelha *A. mellifera* (20 kg de mel por colônia/ ano). Essa diferença de produtividade é justificada devido ao número inferior de abelhas que ocupam a colmeia dos meliponíneos. Além disso, o Brasil não possui uma legislação nacional para esse tipo de produto, apenas alguns estados brasileiros possuem regulamentações próprias (SATO *et al.*, 2023).

A falta de legislação referente ao mel de abelha-sem-ferrão compromete a distribuição mundial desse produto. Logo, a competitividade com o mel das abelhas *A. mellifera* tem sido prejudicada em âmbito nacional e internacional. Do mesmo modo, a falta de dados abrangentes a respeito da composição físico-química e microbiológica dos méis provenientes de diferentes gêneros e espécies de abelha, torna difícil a detecção de possíveis adulterações. A aquisição de dados a respeito dos mais variados méis de meliponíneos é de extrema importância para que a qualidade do produto no mercado seja assegurada (ÁVILA et al., 2018; SCHVEZOV et al., 2020).

3.2.1. Composição físico-química

O mel das abelhas nativas apresenta algumas particularidades quando comparado aos padrões estabelecidos para o mel das abelhas *A. mellifera*. Em geral, o mel de abelha-sem-ferrão apresenta teores mais baixos de açúcar e sólidos solúveis totais e níveis mais elevados de atividade de água, umidade e acidez (BANA *et al.*, 2024). O teor de umidade e a atividade de água são os parâmetros mais críticos no mel, pois estes estão estritamente relacionados com as características finais do produto, como sabor, textura, peso e cristalização (SOUSA *et al.*, 2016).

Os teores mais elevados de umidade e atividade de água do mel das abelhas-sem-ferrão podem estar relacionados à diversos fatores como a alta umidade nas regiões tropicais e subtropicais e a preferência dessas abelhas por flores rasteiras e frutos maduros ricos em água (HRNCIR et al., 2019). No mel dos meliponíneos, o teor de umidade pode variar de 23 a 56%, enquanto a legislação estabelece que o mel de *A. mellifera* não deve conter mais do que 20% de umidade (ÁVILA *et al.*, 2018). Além disso, altos teores de umidade e valores de atividade de água superiores a 0,6 possibilitam a proliferação de leveduras osmofílicas e a ocorrência de processos fermentativos, devido a presença de microbiota nesse tipo de mel (NORDIN *et al.*, 2018; RIBEIRO *et al.*, 2018).

O valor da acidez corresponde ao conteúdo de ácidos orgânicos presentes no mel. A acidez mais elevada no mel de abelha-sem-ferrão está relacionada principalmente a fermentação dos açúcares por bactérias do ácido lático e acético, com consequente produção de ácidos. Da mesma forma, processos fermentativos reduzem a disponibilidade de açúcares no mel, uma vez que as bactérias utilizam esses açúcares como fonte de carbono, e com isso também ocorre redução dos sólidos solúveis totais. Desse modo, a fermentação influencia diretamente na acidez, no teor de açúcares redutores e sólidos solúveis totais do mel. Ainda,

esses parâmetros também podem variar de acordo com a espécie de abelha produtora e a origem floral (ÁVILA *et al.*, 2018; SOUSA *et al.*, 2016).

As vitaminas encontradas nos méis de abelhas-sem-ferrão incluem o ácido ascórbico (Vitamina C), tiamina (vitamina B1), riboflavina (vitamina B2), ácido nicotínico (vitamina B3), ácido pantotênico (vitamina B5), piridoxina (vitamina B6), biotina (Vitamina B8), ácido fólico (vitamina B9) e cianocobalamina (vitamina B12) (RAO *et al.*, 2016). Os minerais, assim como as vitaminas, desempenham um papel crítico no metabolismo biológico, sendo catalisadores de várias reações bioquímicas. Biluca *et al.* (2017) analisaram 13 diferentes méis de abelha-sem-ferrão e verificou que os minerais potássio, cálcio, sódio e magnésio estavam presentes em maiores concentrações. No entanto, estudos abrangentes sobre a biodisponibilidade de vitaminas e minerais em méis de abelha-sem-ferrão são escassos na literatura.

A medida de hidroximetilfurfural (HMF) é utilizada como parâmetro de frescor e qualidade dos méis. Essa substância é formada pela degradação dos açúcares redutores durante a reação de Maillard (escurecimento não enzimático). A produção desse composto está relacionada com processos de aquecimento, armazenamento por períodos prolongados e adulterações. No entanto, o mel de abelha-sem-ferrão tem demonstrado resistência a formação de HMF quando submetido a temperaturas elevadas (BRAGHINI; BILUCA; OTTEQUIR; *et al.*, 2020; RIBEIRO *et al.*, 2018).

Na literatura são reportados valores diversificados a respeito dos parâmetros físico-químicos de méis frescos de abelha-sem-ferrão. Sant'ana *et al.* (2020) realizaram um estudo comparativo entre os méis de duas espécies de abelhas (*Melipona subnitida* e *Melipona fasciculata*) coletados em diferentes regiões e períodos, no estado do Piauí, Brasil. A partir dos resultados, os autores constataram que 22% dos parâmetros analisados diferiram entre as espécies. Amostras coletadas em diferentes períodos também apresentaram diferença estatística entre si.

Em um outro estudo também realizado no Brasil, 33 amostras de méis de abelhas-sem-ferrão foram coletadas de diferentes regiões no estado de Santa Catarina. Para o estudo foram utilizados méis de 10 espécies de abelhas. Os resultados mostraram grande variabilidade na composição dos méis. Os valores variaram de 23,1 a 43,5% para a umidade, 3,33 a 6,56 para o pH, 55,2 a 76,1 °Brix para os sólidos solúveis totais e 48,6 a 70,5% para os açúcares redutores (BILUCA *et al.*, 2016). Esses resultados contraditórios confirmam a hipótese de que as características do mel de abelha-sem-ferrão podem variar de um gênero ou espécie de abelha para outra e de regiões e florações distintas (SE *et al.*, 2018; SOUSA *et al.*, 2016).

3.2.2. Capacidade redutora e antioxidante

O mel de abelha-sem-ferrão possui em sua composição, compostos que exibem capacidade redutora e antioxidante, como ácidos orgânicos, vitaminas, enzimas, proteínas, carotenoides, produtos da reação de Maillard, aminoácidos, minerais e polifenóis, especialmente compostos fenólicos e flavonoides (ÁVILA; HORNUNG; *et al.*, 2019; SHAMSUDIN *et al.*, 2019). A presença desses compostos reflete a qualidade do mel, sendo responsáveis, em parte, pelas características sensoriais e de cor. Diferentes concentrações de compostos bioativos podem ser encontradas em méis de diferentes espécies e origens botânicas (BILUCA *et al.*, 2017; TUKSITHA *et al.*, 2018).

Os antioxidantes, ou compostos com atividade bioativa, são as substâncias químicas que desempenham a função de inibir ou adiar a oxidação de outras moléculas. Essas substâncias atuam de duas formas, a primeira consiste na inibição das reações em cadeia, através da doação de um elétron ao elétron de valência não emparelhado do radical livre, e a segunda, consiste na remoção de células danificadas, seguida da sua reconstituição. Os antioxidantes também são capazes de interceptar os radicais livres e impedir o seu ataque aos aminoácidos, proteínas, lipídeos, bases do DNA e ácidos graxos poli-insaturados. Os radicais livres são formados devido a reações metabólicas celulares, incidência de luz solar, estresse oxidativo, processamento de alimentos, poluição, e presença de toxinas. Muitas condições patológicas, incluindo diabetes, doenças cardíacas, aterosclerose, distúrbios neurológicos, hipertensão, câncer e envelhecimento precoce, têm sido associadas aos níveis de radicais livres no corpo (RANNEH *et al.*, 2018; TUKSITHA *et al.*, 2018).

Diversos estudos têm investigado a capacidade antioxidante e redutora dos méis de abelhas nativas. Biluca *et al.* (2017) investigaram a composição fenólica de 13 amostras de mel de nove espécies de meliponíneos. Os autores encontraram 26 compostos fenólicos, sendo os principais o ácido salicílico, ácido p-cumarico, naringina e taxifolina. Os resultados também mostraram a presença de ácido cafeico, ácido mandélico, ácido rosmarínico, ácido clorogênico, isoquercetrina, carnosol, aromadendrina, umbeliferona, eriodictiol, vanilina e seringadeído. Em um outro estudo, foram avaliados três méis das espécies de abelhas localizadas na Amazônia, sendo elas a *Melipona mexicana*, *Melipona flavolineata* e *Apis mellifera*. Nas amostras foram identificados 14 compostos fenólicos, sendo os principais o ácido gálico e a quercetina (OLIVEIRA *et al.*, 2012).

Ávila *et al.* (2019) identificaram compostos bioativos como a quercetina, ácido p-cumarico e hesperetina em 32 amostras de méis de abelha-sem-ferrão, as quais foram

relacionadas com a capacidade antioxidante por meio dos ensaios de 2,2-difenil-1- picril-hidrazil (DPPH), capacidade de absorção do radical oxigênio (ORAC) e poder antioxidante férrico redutor (FRAP). Do mesmo modo, Ranneh *et al.* (2018) analisaram o perfil de fenólicos e as propriedades antioxidantes de mel de *Heterotrigona itama* da Malásia, pela análise de espectrometria de massas acoplado a cromatografia líquida, um total de 18 flavonoides e compostos fenólicos foram identificados, sendo também relacionados com a capacidade antioxidante e redutora através dos ensaios DPPH, ácido 2,2'-azino-bis (ácido 3-etilbenzotiazolina-6-sulfônico) (ABTS) e ORAC.

Biluca *et al.* (2020) avaliaram a composição fenólica, a capacidade redutora e antioxidante e o efeito anti-inflamatório contra macrófagos RAW264.7 estimulados por lipopolissacarídeos em oito méis de abelha-sem-ferrão. Os méis não demonstraram ser citotóxico nas concentrações analisadas (1-100 μM) e também foram capazes de reduzir o óxido nítrico e a secreção da citocina pró-inflamatória, enquanto duas das oito amostras aumentaram a secreção de citocinas anti-inflamatórias, indicando um efeito antioxidante e anti-inflamatório significativo.

3.2.3. Composto de mel com própolis

A própolis consiste em uma mistura de pólen, cera e resina vegetais, usado pelas abelhas para selar os buracos da colmeia e proteger o ninho (PRZYBYŁEK; KARPIŃSKI, 2019). A própolis das abelhas-sem-ferrão pode conter argila ou lama, motivo pelo qual, também pode ser chamado de geoprópolis (GEORGIEVA *et al.*, 2019; SALATINO; PEREIRA; SALATINO, 2019). Entre os compostos químicos presentes na própolis, os terpenoides, os flavonoides e os compostos fenólicos, representam as principais classes de substâncias, as quais têm sido relacionadas com as suas propriedades antimicrobianas e antioxidantes (CISILOTTO *et al.*, 2018; OSÉS, S. M. *et al.*, 2016; PRZYBYŁEK; KARPIŃSKI, 2019).

No Brasil, não existe uma legislação para controle e qualidade da própolis e extrato de própolis. A composição da própolis de abelha-sem-ferrão, assim como a composição do mel, pode variar de acordo o gênero/espécie de abelha produtora (SUREK *et al.*, 2022). A própolis *in natura*, sem tratamento prévio, não pode ser usada diretamente para a adição em produtos. Primeiro, ela necessita ser extraída de modo a se dissolver e liberar os compostos bioativos. Os solventes etanol, metanol, acetona, hexano, clorofórmio e diclorometano podem ser utilizados para a extração da própolis. No entanto, com exceção do etanol, os demais solventes apresentam toxicidade. Além disso, a escolha do solvente pode influenciar as concentrações de substâncias

como compostos fenólicos e flavonoides, que são importantes para as propriedades antioxidantes e antimicrobianas da própolis (PRZYBYŁEK; KARPIŃSKI, 2019).

O extrato de própolis, além de ser usado na medicina tradicional e popular, também tem sido comercializado como alimento funcional e complementar, podendo ser encontrado adicionado a uma ampla diversidade de produtos incluindo sprays, balas, tinturas, pomadas e cremes dentais, além disso, o extrato de própolis também pode ser encontrado misturado ao mel. Todavia, o extrato de própolis usado pelas indústrias é na sua maioria, proveniente da abelha *A. mellifera*, enquanto relativamente poucos produtos com adição de extrato de própolis ou geoprópolis das abelhas-sem-ferrão são encontrados no mercado (SALATINO; PEREIRA; SALATINO, 2019; OSÉS et al., 2016).

A combinação de extrato de própolis com mel de abelha-sem-ferrão pode resultar um produto com maior efeito antimicrobiano e maior capacidade antioxidante. No entanto, altas concentrações de extrato de própolis foram relacionadas com sabores adstringentes e amargos, portanto, a aceitação do produto depende da concentração do extrato de própolis que é adicionado, de modo a não afetar negativamente as características do produto final (OSÉS *et al.*, 2015).

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4. MATERIAIS E MÉTODOS

Materiais e métodos estão descritos em três artigos científicos apresentados no próximo item, **5. RESULTADOS E DISCUSSÃO**.

5. RESULTADOS E DISCUSSÃO

Os resultados e discussão foram redigidos na forma de três artigos científicos e estão apresentados nos subitens de 5.1 a 5.3

5.1. ARTIGO CIENTÍFICO 1[1]

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[1]Artigo a ser submetido na Revista Journal of Food Composition and Analysis

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Advanced classification of Brazilian stingless bee honey by genus using comprehensive analytical techniques and chemometrics

Abstract

This study aimed to investigate Brazilian stingless bee honey (SBH) samples to compare their chemical compositions. Fifty-five samples from five genera were evaluated by melissopalynological and physicochemical analysis, phenolic compounds, sugar profiling, and FTIR-ATR using chemometric approaches. Multiblock Analysis (MA) revealed a slight clustering of honeys from *Tetragonisca*, *Melipona*, and *Scaptotrigona*, along with a distinct cluster of *Tetragona* samples. Additionally, the honeys formed two groups based on their geographical origin. The Linear Discriminant Analysis (LDA) and Partial Least Squares Discriminant Analysis (PLS-DA) models effectively predicted samples from the *Melipona*, *Scaptotrigona*, and *Tetragonisca* genera using quantitative data and FTIR-ATR spectra, respectively. For the MA, the key parameters for separation were the sugar profile, the phenolic compounds and the FTIR-ATR spectrum comprised between 1800 and 750 cm^{-1}. The regions between 1610 – 1557; 1126 – 960 and 1170 – 915 cm^{-1} were important for classification using the PLS-DA model. Electrical conductivity, pH, color (parameter a*), raffinose content, reducing sugars and sum of trehalulose and maltose were crucial for classification by the LDA model. This study will enhance understanding of how entomological and geographic factors affect Brazilian SBH characteristics, aid in traceability for quality and authenticity, and contribute to the development of national regulations for SBH.

Keywords: Chemometric; Honey discrimination; Multiblock analysis; Sugar profile.

1. Introduction

Stingless bees or Meliponini constitute a diverse group of bees widely distributed in tropical and subtropical habitats such as Australia, Americas, Africa, and Asia. To date, more than 600 species have been cataloged (Roubik, 2023). These bees exhibit significant variation in size, color, and body shape (Grüter, 2020). Besides their taxonomic diversity, stingless bees also display various behaviors and lifestyles (Pedro, 2014; Sato et al., 2023). In Brazil, over 300 species of stingless bees have been cataloged and distributed across the entire national territory. These bees play a fundamental role in plant pollination and in maintaining the biodiversity of Brazilian biomes (Lima et al., 2016; Rezende et al., 2020; Sato et al., 2023).

In the stingless bees production chain, honey production and commercialization play a fundamental role (Ávila et al., 2018; Biluca et al., 2020). The stingless bees honey (SBH) is historically recognized for its beneficial properties to humans, attributed to its antioxidant, antimicrobial, anti-inflammatory and anticancer properties (Ali et al., 2020; Bana et al., 2024; Hau-Yama et al., 2020). When compared to *Apis mellifera* honey, the honey from stingless bees exhibits some differences, including higher acidity and a more fluid texture, as well as distinct sensory attributes in terms of flavor and aroma (Ávila et al., 2018; Bana et al., 2024; Sato et al., 2023).

The characteristics of SBH are influenced by geographic, seasonal, and environmental factors (Ávila et al., 2018; Siddiqui et al., 2017). The unique composition of the honey not only determines its quality but also imparts its distinctive characteristics, reflecting differences among varieties of SBH. Therefore, the intrinsic properties of SBH could be used as potential biomarkers to discriminate honey from different genera (Mduda et al., 2023). Several analyses have been conducted to identify the chemical constituents of SBH, associated with chemometric methods, aiming to elucidate the characteristics of

different types of honey (Ávila et al., 2019a). However, these analyses often rely on groups from a single geographical region.

Therefore, the objective of this study was (i) to characterize 55 samples of SBH belonging to five genera of bees native to five different Brazilian regions through melissopalynological, physicochemical, sugar content, phenolic compound content, and Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR–ATR) and (ii) to apply multivariate analysis to assess the influence of geographic and entomological origin (across five different genera) on the characteristics of Brazilian SBH.

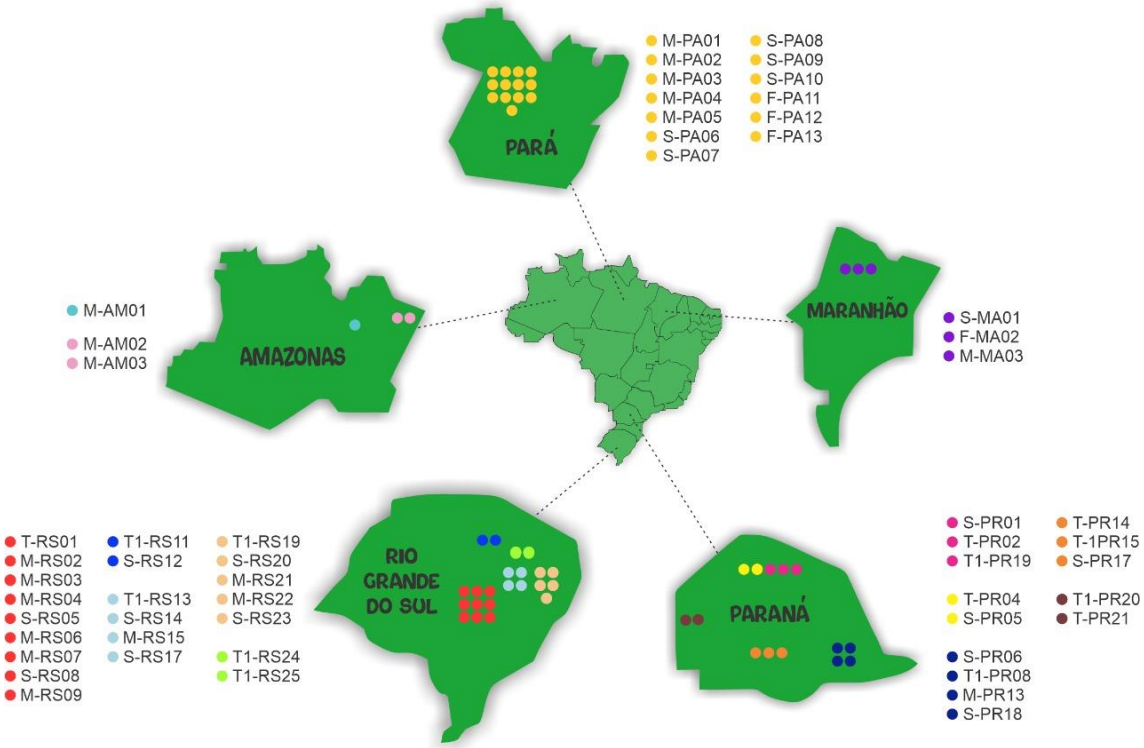
2. Materials and methods

2.1. Honey samples

For this study, 55 samples of SBH were collected in five states of Brazil (Pará, Maranhão, Amazonas, Rio Grande do Sul, and Paraná). The honeys were produced by five genera of stingless bee (*Melipona* (n=19), *Tetragonisca* (n=10), *Tetragona* (n=4), *Frieseomelitta* (n=4), and *Scaptotrigona* (n=18)), and different species (*Frieseomelitta doederleini* (n=4), *Melipona bicolor* (n=2), *Melipona compressipes* (n=4), *Melipona fasciculata* (n=2), *Melipona interrupta* (n=2), *Melipona marginata* (n=3), *Melipona mondury* (n=1), *Melipona quadrifasciata* (n=2), *Melipona scutellaris* (n=1), *Melipona seminigra* (n=1), *Melipona subnitida* (n=1), *Scaptotrigona aff. postiça* (n=1), *Scaptotrigona bipunctata* (n=8), *Scaptotrigona depilis* (n=1), *Scaptotrigona postiça* (n=3), *Scaptotrigona xanthotricha* (n=5), *Tetragona clavipes* (n=4), and *Tetragonisca angustula* (n=10)). The description of the geographical origin of the samples is shown in Figure 1. The honeys used in this study were produced by small Brazilian agricultural communities and

collected between November 2020 and October 2021. During the experimental period, the samples remained stored under refrigeration.

Figure 1. Locations where stingless bee honeys were collected in the states of Pará, Amazonas, Maranhão, Paraná, and Rio Grande do Sul in Brazil.



2.2. Melissopalynological analysis

The honey samples were prepared according to Luz et al. (2018), applying the European Classic method without acetolysis (Louveaux et al., 1978; Maurizio et al., 1965). Pollen grains were identified and counted using a BX50 light binocular microscope (Olympus, Japan). The identification of pollen grains was performed by comparison with the pollen reference palynotheca collection from the PALINO-IPA (Palynology Laboratory, from the Instituto de Pesquisas Ambientais, São Paulo) and with the floristic survey articles. Whenever possible, identification was at the genus and species level, using the term "pollen type", which refers to morphologically similar pollen grains which can be related

to a single plant species, several species of the same taxon, or several different taxa (Barth, 1989; Klerk and Joosten, 2007; Luz et al., 2018). The botanical origin of the samples was defined through the quantitative values of nectariferous pollen types (Barth et al., 2021). The estimation of the relative frequencies of pollen grains in each sample was categorized according to the proposal by Louveaux et al. (1978) as follows: Dominant pollen (>45%), accessory pollen (16 to 45%), important isolated pollen (3 to 15%), and occasional isolated pollen (<3%).

2.3. Sugar profile by high-performance liquid chromatography (HPLC-RID)

The honey samples were diluted in ultrapure water (1:20 m/v) and filtered with a 0.22 μm PVDF filter. For the determination of glucose, fructose, trehalulose + maltose, and raffinose concentrations, the Agilent chromatograph (model 1260 Infinity, USA) coupled to the refractive index detector (RID) and the Agilent Hi-Plex H analytical column (300× 7.7 mm i.d.; 8 μm) were used. The following running conditions were established: mobile phase composed of ultrapure water acidified with 5 mM sulfuric acid; isocratic mode; flow rate of 0.5 mL/min, column temperature of 50 ºC, detector temperature of 35 ºC, and injection volume of 10 μL. To determine the individual concentration of sugars, a standard curve was constructed, in the case of trehalulose + maltose, the average area obtained at each concentration was used to construct the curve. The analysis was performed in duplicate, and the results were expressed as percentages (g/100 g of honey). The content of reducing sugars was obtained by the sum of glucose, fructose, and trehalulose + maltose.

2.4. Phenolic compounds

The total phenolic compounds of the samples were determined according to the methodology proposed by Singleton et al. (1999) with minor modifications. One gram of honey was diluted in 10 milliliters of distilled water (1:10). Aliquots of 100 µL of the sample, 100 µL of Folin–Ciocalteu, 300 µL of 10% sodium carbonate solution, and 1500 µL of distilled water were transferred to a cuvette, shaken for 60 s, and left for 1 h in the dark, at room temperature. The absorbance was measured at a wavelength of 765 nm in a spectrophotometer (Jasco, model 7800, Japan). To determine the concentration of phenolic compounds of the samples, a gallic acid (GA) curve was constructed. The average of three repetitions was expressed in a mg GA/100 g sample. Distilled water was used in place of the sample for the blank reading.

2.5. Physicochemical analysis

The physicochemical analyses were carried out according to the methodologies proposed by AOAC (2016). The total soluble solids content was measured using a pocket digital refractometer at a temperature of 20 °C. The moisture content was determined by the difference of 100% and the total soluble solids content. For the analysis of pH and electrical conductivity, 10 g of sample was diluted in 75 mL of distilled water, the pH and the electrical conductivity of the solution were measured using a digital meter (Metler Toledo - SevenCompact™ Duo S213, Switzerland). The color parameters L*, a* and b*, given by the International Commission on Illumination (CIE), were determined using a colorimeter (Konica Minolta, Japan), where L* represents the variation from black (0) to white (100), a* represents the variation from green (-60) to red (+60), and b* represents the variation from blue (-60) to yellow (+60). The determination of the physicochemical parameters was carried out in triplicate.

2.6. Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR)

The FTIR-ATR spectra of the samples were acquired using a spectrometer (Alpha, Bruker, Germany). Thirty-two scans per spectrum were performed at a spectral resolution of 4 cm^{-1} in the wavenumber range from 4000 to 400 cm^{-1}. The average spectra of five replicates were used for analysis.

2.7. Multivariate analysis

All multivariate data analyses were performed on a user-friendly graphical interface, GAMMA-GUI (Food Matrix Multivariate Analysis Group) (Galvan and Bona, 2024), based on the Matlab 2023b® platform (The MathWorks Inc., Natick, USA), available for free download at: https://www.mathworks.com/matlabcentral/fileexchange/156857-gamma. For the analyses, the average of all parameters was considered.

2.7.1. Exploratory data analysis

In chemometrics, the first step in developing any supervised model is to conduct an exploratory analysis. This study used a Multiblock Analysis (MA) to explore the data from physicochemical parameters, sugar profiles, phenolic compounds, FTIR-ATR spectra, and melissopalynological data through the ComDim (Common Dimension) analysis. ComDim is a MA method that allows the simultaneous analysis of data from different sources for the same set of samples (Silva et al., 2024). The results are analogous to those of Principal Component Analysis (PCA). For each extracted CD (Common Dimensions), a set of scores, saliences, and weights is obtained. A detailed description of the ComDim method is given by Galvan et al. (2023). The FTIR-ATR spectra did not

undergo any processing, and the range from 1800 to 750 cm^{-1} was considered for analysis (Cherigui et al., 2024; Gok et al., 2015). For the melissopalynological data, the absolute count given by the natural logarithm was used, calculated as log (relative content + 1). Three data blocks were considered for this analysis: Block 1: Quantitative data (physicochemical parameters; total phenolic compounds and sugar profile), Block 2: FTIR-ATR spectra, and Block 3: melissopalynological data.

2.7.2. Supervised data analysis

Models were built for sample classification by genus using LDA (Linear Discriminant Analysis) and PLS-DA (Partial Least Squares Discriminant Analysis) applied to Blocks 1 and 2, respectively. Before developing the models, possible outliers were investigated through the Q residuals plot and Hotelling's T^2 (leverage test).

2.7.3. Construction of PLS-DA and LDA models

Each honey class was divided into calibration and prediction sets using the Kennard & Stone algorithm. For classification with LDA and PLS-DA, the data were split into two subsets: 67% of the samples from each class were used for calibration (training), and the remaining 33% were used for prediction (test). The leave-one-out cross-validation (LOOCV) method was employed for LDA and PLS-DA modeling. In PLS-DA, the number of latent variables (LVs) was chosen based on the lowest root mean square error for cross-validation (RMSECV).

2.7.4. Validation PLS-DA and LDA models

The models were evaluated using the following statistical parameters: accuracy (ACC), sensitivity (SEN), and specificity (SPE). These three parameters were calculated based on the rates of false positives (FP), true positives (TP), false negatives (FN), and true

negatives (TN). False positives refer to samples that belong to class 1 and are incorrectly classified as class 2. True positives refer to samples that belong to class 2 and are correctly classified as class 2. False negatives refer to samples that belong to class 2 and are incorrectly classified as class 1. Finally, true negatives are samples that belong to class 1 and are correctly classified as class 1. Thus, accuracy, sensitivity, and specificity can be defined as the ability of the LDA and PLS-DA models to correctly classify positive and negative samples, respectively. The closer the model is to 100%, the more accurate, sensitive, and specific it is (Bana et al., 2024).

3. Results and discussion

3.1. Melissopalynological analysis

The relative frequency of the pollen types from nectariferous plants (botanical origin) of the honey is shown in Table 1. The qualitative analysis of the 55 samples revealed a wide variety of pollen types. A total of 100 nectariferous pollen types belonging to 38 botanical families were identified. Among these, 34 pollen types were identified in Pará, 59 in Paraná, 33 in Rio Grande do Sul, 10 in Amazonas, and 16 in Maranhão. The wide variety of pollen types found in Brazil results from the rich diversity of vegetation across the country (Lavinas et al., 2019; Sant'ana et al., 2020). Figure 2 shows the nectariferous plants most frequently visited by Stingless bee.

Table 1. Relative content of different types of pollen present in stingless bee honeys.

Stingless bee species	Geographic origin	Sample	Relative content	Classification
Melipona compressipes	Pará	M-PA01	(PD) *Laetia procera* (84.5%); (PII) *Triplaris* (13.4%); (PIO) *Neptunia* (0.7%), *Phthirusa* (0.7%), *Pseudobombax* (0.7%).	Monofloral honey from *Laetia procera*.
	Pará	M-PA02	(PD) *Triplaris* (50.9%); (PA) *Protium* (20.6%); (PII) *Laetia procera* (12.3%), *Baccharis* (3.2%), *Banara* (3.2%), *Tapirira guianensis* (3.2%); (PIO) *Ocotea* (1.8%), *Derris* (1.4%), *Mimosa pudica* (1.1%), *Pseudobombax* (1.1%), *Turnera* (1.1%).	Monofloral honey from *Triplaris*.
	Pará	M-PA03	(PA) *Laetia procera* (42.0%), *Triplaris* (32.7%); (PII) *Derris* (11.3%), *Vismia* (9.3%), *Tapirira guianensis* (4.7%).	Multifloral honey with greater nectar contribution from *Laetia procera*.
	Pará	M-PA04	(PD) *Triplaris* (52.1%); (PA) *Tapirira guianensis* (26.3%); (PII) *Laetia procera* (7.9%), *Derris* (6.7%), *Pouteria* (3.3%); (PIO) *Protium* (2.9%), *Pseudobombax* (0.8%).	Monofloral honey from *Triplaris.*
Melipona subnitida	Pará	M-PA05	(PA) *Tapirira guianensis* (40.9%), *Pouteria* (17.2%), *Ocotea* (16.7%); (PII) *Mimosa pudica* (8.1%), *Zanthoxylum* (5.9%), *Copaifera* (5.4%), *Triplaris* (3.2%); (PIO) *Citrus* (2.7%).	Multifloral honey with greater nectar contribution from *Tapiria guianensis*.
Melipona bicolor	Paraná	M-PR13	(PA) *Scoparia dulcis* (32.4%), *Baccharis/Achillea* (21.6%); (PII) *Mimosa scabrella* (13.5%); *Attalea/Butia* (10.8%), *Eucalyptus* (10.8%), *Jacaratia spinosa* (10.8%).	Multifloral honey with greater nectar contribution from *Scoparia dulcis*.
	Rio Grande do Sul	M-RS06	(PD) *Eucalyptus* (68.4%); (PIO) *Allophylus edulis* (9.2%), *Mimosa scabrella* (7.9%), *Hovenia dulcis* (6.6%), *Attalea dubia*, *A. compta/Butia* (2.6%), *Melia azedarach* (2.6%), *Baccharis/Achillea* (1.3%), *Schinus* (1.3%).	Monofloral honey from *Eucalyptus*.
Melipona scutellaris	Rio Grande do Sul	M-RS02	(PD) *Hovenia dulcis* (83.7%); (PA) *Weinmannia* (16.3%).	Monofloral honey from *Hovenia dulcis*.
Melipona quadrifasciata	Rio Grande do Sul	M-RS03	(PD) *Hovenia dulcis* (100.0%).	Monofloral honey from *Hovenia dulcis*.

	Rio Grande do Sul	M-RS21	(PD) *Eucalyptus* (93.5%); (PIO) *Acacia/Senegalia* (2.3%), *Allophylus edulis* (2.3%), *Attalea dubia, A. compta/Butia* (1.8%).	Monofloral honey from *Eucalyptus*.
Melipona mondury	Rio Grande do Sul	M-RS04	(PD) *Hovenia dulcis* (97.9%); (PIO) *Weinmannia* (2.1%).	Monofloral honey from *Hovenia dulcis*.
Melipona fasciculata	Rio Grande do Sul	M-RS07	(PD) *Hovenia dulcis* (82.5%); (PII) *Eucalyptus* (8.2%), *Jacaranda* (5.2%), *Schizolobium parahyba* (4.1%).	Monofloral honey from *Hovenia dulcis*.
	Maranhão	M-MA03	(PA) *Zanthoxylum* (30.1%), *Sloanea* (26.9%); (PII) *Cocos nucifera* (9.7%), *Mimosa caesalpiniifolia* (9.7%), *Protium* (5.4%), *Xylosma* (5.4%), *Crotalaria* (4.3%), *Tilesia* (4.3%), *Vismia* (4.3%).	Multifloral honey with greater nectar contribution from *Zanthoxylum* and *Sloanea*.
Melipona marginata	Rio Grande do Sul	M-RS09	(PA) *Hovenia dulcis* (43.4%), *Attalea dubia, A. compta/Butia* (22.6%), *Euterpe edulis/Syagrus* (22.0%); (PII) *Allophylus edulis* (5.7%); (PIO) *Mimosa scabrella* (2.5%), *Schinus* (1.9%), *Weinmannia* (1.9%).	Multifloral honey with greater nectar contribution from *Hovenia dulcis*.
	Rio Grande do Sul	M-RS15	(PD) *Eucalyptus* (50.6%); (PA) *Casearia sylvestris* (30.7%); (PII) *Allophylus edulis* (7.7%), *Trichilia* (3.4%), *Astronium* (3.1%), *Mimosa scabrella* (3.1%); (PIO) *Attalea dubia, A. compta/Butia* (1.5%).	Monofloral honey from *Eucalyptus*.
	Rio Grande do Sul	M-RS22	(PD) *Eucalyptus* (65.6%); (PA) *Allophylus edulis* (18.8%); (PII) *Attalea dubia, A. compta/Butia* (9.4%), *Salix* (6.3%).	Monofloral honey from *Eucalyptus*.
Melipona interrupta	Amazonas	M-AM01	(PD) *Tapirira guianensis* (75.6%); (PII) *Triplaris* (12.2%), *Vismia* (9.8%); (PIO) *Casearia* (2.4%).	Monofloral honey from *Tapiria guianensis*.
	Amazonas	M-AM03	(PA) *Protium* (40.0%), *Tapirira guianensis* (34.5%), *Triplaris* (25.5%).	Multifloral honey with greater nectar contribution from *Protium*.
Melipona seminigra	Amazonas	M-AM02	(PA) *Tapirira guianensis* (43.7%), *Protium* (29.1%); (PII) *Triplaris* (10.3%), *Euterpe/Maximiliana maripa* (6.3%), *Mimosa pudica* (4.3%); (PIO) *Cupania* (2.0%), *Eichhornia* (1.7%), *Vismia* (1.7%), *Spondias* (0.9%).	Multifloral honey with greater nectar contribution from *Tapiria guianensis*.
Scaptotrigona xanthotricha	Pará	S-PA06	(PA) *Tapirira guianensis* (30.6%), *Warszewiczia coccinea* (30.6%), *Sclerolobium paniculatum* (16.9%); (PII) *Matayba* (10.2%), *Vismia* (9.4%); (PIO) *Protium* (2.4%).	Multifloral honey with greater nectar contribution from *Tapiria guianensis* and *Warszewiczia coccinea*.

	Pará	S-PA07	(PA) *Tapirira guianensis* (28.4%), *Warszewiczia coccinea* (28.0%); (PII) *Derris* (15.3%), *Acacia* (13.1%), *Ocotea* (5.6%); (PIO) *Neptunia* (2.2%), *Triplaris* (2.2%), *Mabea* (1.9%), *Zanthoxylum* (1.9%), *Paullinia* (1.5%).	Multifloral honey with greater nectar contribution from *Tapiria guianensis* and *Warszewiczia coccinea*.
	Pará	S-PA08	(PD) *Alibertia* (74.7%); (PA) *Tapirira guianensis* (18.7%); (PII) *Copaifera* (3.6%), *Matayba* (3.1%).	Monofloral honey from *Alibertia*.
	Pará	S-PA09	(PA) *Piptadenia* (36.1%), *Warszewiczia coccinea* (18.0%); (PII) *Casearia* (10.1%), *Matayba* (9.9%), *Zanthoxylum* (7.0%), *Tapirira guianensis* (6.8%), *Protium* (4.8%); (PIO) *Inga* (2.5%), *Derris* (2.3%), *Baccharis* (1.4%), *Pouteria* (1.1%).	Multifloral honey with greater nectar contribution from *Piptadenia*.
	Pará	S-PA10	(PA) *Tapirira guianensis* (40.9%), *Warszewiczia coccinea* (40.6%); (PII) *Zanthoxylum* (5.3%), *Muntingia calabura* (3.6%); (PIO) *Alibertia* (2.5%), *Sclerolobium paniculatum* (2.1%), *Casearia* (1.4%), *Derris* (1.4%), *Inga* (1.1%), *Piptadenia* (1.1%).	Bifloral honey from *Tapiria guianensis* and *Warszewiczia coccinea*.
Scaptotrigona postica	Paraná	S-PR01	(PA) *Eucalyptus* (27.4%); (PII) *Tropaeolum* (13.7%), *Holocalyx balansae* (11.7%), *Baccharis/Achillea* (10.7%), *Euterpe edulis/Syagrus romanzoffiana* (7.8%), *Mimosa bimucronata/Mimosa caesalpiniifolia* (7.8%), *Bauhinia forficata* (4.9%), *Jacaranda* (3.9%), *Peltophorum dubium* (3.9%), *Picramnia* (3.9%); (PIO) *Astronium* (2.0%), *Acacia/Senegalia* (1.0%), *Borreria/Spermacoce* (1.0%), *Scoparia dulcis* (0.3%).	Multifloral honey with greater nectar contribution from *Eucalyptus*.
	Rio Grande do Sul	S-RS05	(PA) *Eucalyptus* (40.6%), *Allophylus edulis* (22.6%); (PII) *Euterpe edulis/Syagrus* (9.8%), *Hovenia dulcis* (4.5%), *Ligustrum* (4.5%), *Matayba elaeagnoides* (4.5%), *Piptadenia gonoacantha/Parapiptadenia* (4.5%), *Protium* (4.5%), *Sebastiania* (4.5%).	Multifloral honey with greater nectar contribution from *Eucalyptus*.
	Rio Grande do Sul	S-RS23	(PA) *Eucalyptus* (30.0%), *Allophylus edulis* (29.7%), *Casearia sylvestris* (26.9%); (PII) *Salix* (8.5%); (PIO) *Attalea dubia, A. compta/Butia* (2.1%), *Cupania vernalis* (1.4%), *Serjania* (1.4%).	Multifloral honey with greater nectar contribution from *Eucalyptus* and *Allophylus edulis*.
Scaptotrigona bipunctata	Paraná	S-PR05	(PA) *Eucalyptus* (44.4%), *Astronium* (15.4%); (PII) *Peltophorum dubium* (11.2%), *Acacia/Senegalia* (4.7%), *Machaerium* (4.7%), *Anadenanthera* (2.9%), *Attalea/Butia* (2.9%), *Tropaeolum* (2.9%); (PIO) *Lobelia exaltata* (2.4%), *Jacaratia spinosa* (1.8%), *Oxalis* (1.8%), *Stemodia* (1.8%), *Picramnia* (1.2%), *Baccharis/Achillea* (0.6%), *Hovenia dulcis* (0.6%), *Zanthoxylum* (0.6%).	Multifloral honey with greater nectar contribution from *Eucalyptus*.
	Paraná	S-PR06	(PA) *Mimosa scabrella* (21.6%), *Allophylus edulis* (17.3%), *Cedrela* (16.2%), *Eucalyptus* (15.8%); (PII) *Casearia sylvestris* (14.4%), *Bougainvillea* (4.7%), *Holocalyx balansae* (4.3%); (PIO) *Astronium* (2.9%), *Actinostemon* (1.4%), *Schizolobium parahyba* (1.4%).	Multifloral honey with greater nectar contribution from *Mimosa scabrella*.

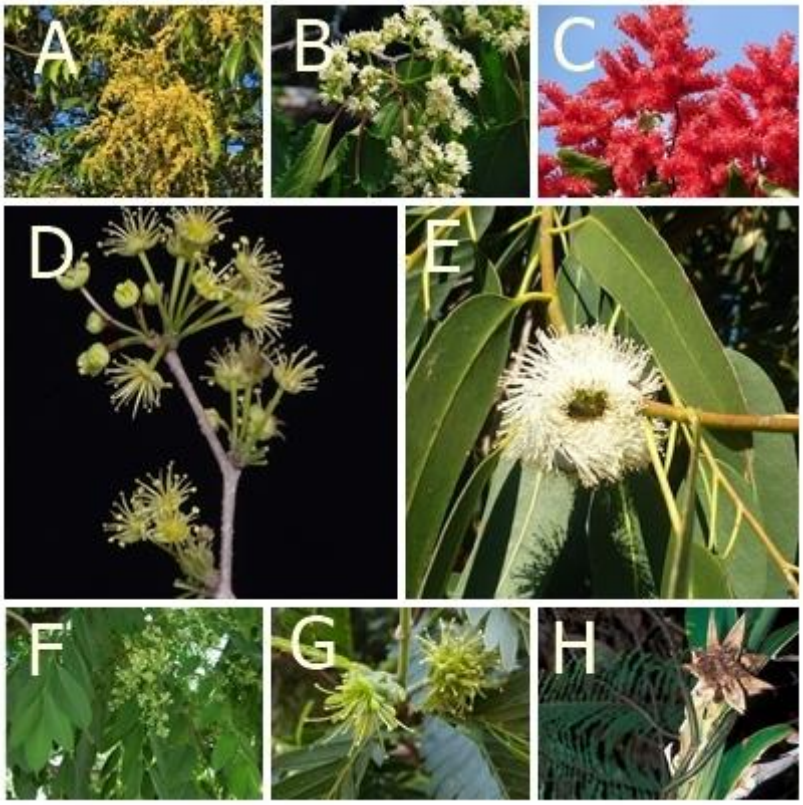
	Paraná	S-PR17	(PD) *Mimosa scabrella* (46.8%); (PA) *Allophylus edulis* (32.9%); (PII) *Zanthoxylum* (6.3%); (PIO) *Astronium* (4.0%), *Eucalyptus* (3.3%), *Casearia sylvestris* (3.0%), *Matayba elaeagnoides* (1.3%), *Scoparia dulcis* (1.3%), *Glycine max* (1.0%).	Monofloral honey from *Mimosa scabrella*.
	Paraná	S-PR18	(PA) *Prockia crucis* (25.3%), *Eucalyptus* (21.1%), *Bougainvillea* (16.3%); (PII) *Bidens* (8.1%), *Astronium* (7.2%), *Attalea/Butia* (6.3%), *Byrsonima* (6.3%), *Holocalyx balansae* (4.5%); (PIO) *Casearia sylvestris* (1.8%), *Jacaranda* (1.2%), *Matayba elaeagnoides* (0.9%), *Phoradendron/Struthanthus* (0.9%).	Multifloral honey with greater nectar contribution from *Prockia crucis*.
	Rio Grande do Sul	S-RS08	(PD) *Hovenia dulcis* (50.2%); (PA) *Attalea dubia*, *A. compta/Butia* (22.8%); (PII) *Eucalyptus* (13.7%), *Ligustrum* (13.3%).	Monofloral honey from *Hovenia dulcis*.
	Rio Grande do Sul	S-RS12	(PD) *Eucalyptus* (100.0%).	Monofloral honey from *Eucalyptus*.
	Rio Grande do Sul	S-RS14	(PD) *Eucalyptus* (60.8%); (PA) *Schinus* (21.5%); (PII) *Mimosa bimucronata/Mimosa caesalpiniifolia* (5.2%), *Attalea dubia*, *A. compta/Butia* (3.5%); (PIO) *Cupania vernalis* (2.4%), *Tripodanthus* (2.4%), *Hovenia dulcis* (2.1%); *Paullinia* (2.1%).	Monofloral honey from *Eucalyptus*.
	Rio Grande do Sul	S-RS20	(PA) *Salix* (35.3%), *Allophylus edulis* (17.0%); (PII) *Acacia/Senegalia* (15.0%), *Casearia sylvestris* (12.0%), *Eucalyptus* (10.3%); *Attalea dubia*, *A. compta/Butia* (8.3%); (PIO) *Weinmannia* (2.0%).	Multifloral honey with greater nectar contribution from *Salix*.
Scaptotrigona aff. postica	Maranhão	S-MA01	(PA) *Xylosma* (30.7%), *Orbignya* (28.0%); (PII) *Simarouba* (12.0%), *Anacardium* (10.7%), *Triplaris* (10.7%), *Protium* (5.3%); (PIO) *Piptadenia* (2.7%).	Multifloral honey with greater nectar contribution from *Xylosma* and *Orbignya*.
Scaptotrigona depilis	Rio Grande do Sul	S-RS17	(PA) *Eucalyptus* (32.7%), *Schinus* (18.0%); (PII) *Casearia sylvestris* (12.4%), *Allophylus edulis* (10.5%), *Sebastiania* (4.9%), *Attalea dubia*, *A. compta/Butia* (3.8%), *Euterpe edulis/Syagrus* (3.4%), *Salix* (3.4%); (PIO) *Ligustrum* (2.6%), *Mimosa bimucronata/Mimosa caesalpiniifolia* (2.6%), *Trichilia* (2.3%), *Apiaceae* (1.1%), *Baccharis/Achillea* (1.1%), *Mimosa scabrella* (1.1%).	Multifloral honey with greater nectar contribution from *Eucalyptus*.
Tetragona clavipes	Paraná	T-PR02	(PA) *Attalea/Butia* (32.3%), *Baccharis/Achillea* (24.6%), *Actinostemon* (16.9%); (PII) *Astronium* (9.2%), *Zanthoxylum* (9.2%), *Eucalyptus* (7.7%).	Multifloral honey with greater nectar contribution from *Attalea/Butia* and *Baccharis*.
	Paraná	T-PR04	(PA) *Jacaratia spinosa* (33.3%); (PII) *Attalea/Butia* (13.3%), *Amphilophium* (6.7%), *Arenaria* (6.7%), *Astronium* (6.7%), *Baccharis/Achillea* (6.7%), *Citrus* (6.7%), *Croton* (6.7%), *Ocotea* (6.7%), *Piptadenia gonoacantha* (6.7%).	Multifloral honey with greater nectar contribution from *Jacaratia spinosa*.

	Paraná	T-PR14	(PD) *Attalea/Butia* (46.5%); (PA) *Brassica napus/Raphanus sativus* (21.5%); (PII) *Citrus* (7.4%), *Bougainvillea* (6.3%), *Croton* (6.3%), *Scoparia dulcis* (5.6%); (PIO) *Actinostemon* (2.1%), *Jacaratia spinosa* (1.1%), *Mimosa scabrella* (1.1%), *Stemodia* (1.1%), *Vernonia* (1.1%).	Monofloral honey from *Attalea/Butia*.
	Paraná	T-PR21	(PD) *Piptadenia gonoacantha* (48.9%); *Schizolobium parahyba* (12.0%), *Baccharis/Achillea* (9.8%), *Holocalyx balansae* (9.4%), *Euterpe edulis/Syagrus romanzoffiana* (5.4%), *Citrus* (2.5%), *Allophylus edulis* (2.2%), *Jacaratia spinosa* (2.2%), *Machaerium* (2.2%), *Actinostemon* (1.8%), *Brassica napus/Raphanus sativus* (1.8%), *Zanthoxylum* (1.8%).	Monofloral honey from *Piptadenia gonoacantha*.
	Paraná	T1-PR08	(PA) *Allophylus edulis* (18.2%); (PII) *Actinostemon* (12.4%), *Casearia sylvestris* (12.1%), *Scoparia dulcis* (10.6%), *Eucalyptus* (9.1%), *Astronium* (6.1%), *Citrus* (6.1%), *Machaerium* (6.1%), *Brassica napus/Raphanus sativus* (5.2%), *Mimosa scabrella* (4.8%), *Baccharis/Achillea* (4.5%); (PIO) *Phoradendron/Struthanthus* (1.8%), *Holocalyx balansae* (1.5%), *Persea* (1.5%).	Multifloral honey with greater nectar contribution from *Allophylus edulis*.
	Paraná	T1-PR15	(PA) *Euterpe edulis/Syagrus romanzoffiana* (41.5%); (PII) *Actinostemon* (12.6%), *Piptadenia gonoacantha* (9.3%), *Astronium* (8.2%), *Brassica napus/Raphanus sativus* (6.4%), *Hovenia dulcis* (6.2%), *Allophylus edulis* (3.9%); (PIO) *Holocalyx balansae* (2.6%), *Glycine max* (1.8%), *Eucalyptus* (1.5%), *Scoparia dulcis* (1.5%), *Baccharis/Achillea* (1.0%); *Heliocarpus popayanensis* (0.8%), *Apiaceae* (0.5%), *Matayba elaeagnoides* (0.5%), *Phoradendron/Struthanthus* (0.5%), *Protium* (0.5%), *Stemodia* (0.5%).	Multifloral honey with greater nectar contribution from *Euterpe edulis/Syagrus romanzoffiana*.
Tetragonisca angustula	Paraná	T1-PR19	(PD) *Astronium* (60.2%); (PII) *Machaerium* (8.9%), *Bidens* (4.8%), *Brassica napus/Raphanus sativus* (4.5%), *Eucalyptus* (3.7%); (PIO) *Salix* (2.6%), *Serjania* (1.5%), *Anadenanthera* (1.9%), *Piptadenia gonoacantha* (1.9%), *Ocimun* (1.5%), *Apiaceae* (1.1%), *Aspidosperma* (1.1%), *Euterpe edulis/Syagrus romanzoffiana* (1.1%), *Holocalyx balansae* (1.1%), *Populus alba* (1.1%), *Schizolobium parahyba* (1.1%), *Scoparia dulcis* (1.1%), *Arenaria* (0.7%).	Monofloral honey from *Astronium*.
	Paraná	T1-PR20	(PD) *Holocalyx balansae* (48.1%); (PA) *Piptadenia gonoacantha* (23.5%), *Actinostemon* (12.6%), *Baccharis/Achillea* (7.4%), *Serjania* (1.8%), *Casearia sylvestris* (1.4%), *Jacaranda* (1.4%), *Peltophorum dubium* (1.4%), *Schizolobium parahyba* (1.4%), *Hovenia dulcis* (1.1%).	Monofloral honey from *Holocalyx balansae*.
	Rio Grande do Sul	T1-RS01	(PA) *Hovenia dulcis* (35.4%), *Euterpe edulis/Syagrus romanzoffiana* (28.3%); (PII) *Ligustrum* (14.6%), *Salix* (4.2%); (PIO) *Attalea/Butia* (2.8%), *Sebastiania* (2.8%), *Vicia* (2.8%), *Weinmannia* (2.8%), *Piptadenia gonoacantha/Parapiptadenia* (1.9%), *Apiaceae* (1.4%), *Bidens* (1.4%), *Schizolobium parahyba* (1.4%).	Multifloral honey with greater nectar contribution from *Hovenia dulcis* and *Euterpe edulis/Syagrus romanzoffiana*.

	Rio Grande do Sul	T1-RS11	(PD) *Eucalyptus* (69.6%); (PA) *Schinus* (18.0%); (PII) *Hovenia dulcis* (3.4%), *Weinmannia* (3.1%); (PIO) *Serjania* (1.9%), *Salix* (1.6%), *Apiaceae* (0.6%), *Attalea/Butia* (0.6%), *Protium* (0.6%), *Ligustrum* (0.6%).	Monofloral honey from *Eucalyptus*.
	Rio Grande do Sul	T1-RS13	(PD) *Brassica napus/Raphanus sativus* (46.2%), *Schinus* (45.1%); (PII) *Weinmannia* (3.3%); (PIO) *Baccharis/Achillea* (2.2%), *Euterpe edulis/Syagrus romanzoffiana* (1.1%), *Eucalyptus* (1.1%), *Attalea/Butia* (0.5%), *Hovenia dulcis* (0.5%).	Bifloral honey from *Brassica napus* and *Schinus*.
	Rio Grande do Sul	T1-RS19	(PD) *Hovenia dulcis* (60.5%); (PII) *Piptadenia gonoacantha/Parapiptadenia* (11.9%), *Attalea/Butia* (6.5%), *Weinmannia* (4.9%), *Schinus* (4.3%), *Baccharis/Achillea* (4.3%); (PIO) *Apiaceae* (2.7%), *Sebastiania* (2.7%), *Salix* (2.2%).	Monofloral honey from *Hovenia dulcis*.
	Rio Grande do Sul	T1-RS24	(PD) *Hovenia dulcis* (58.1%); (PA) *Brassica napus/Raphanus sativus* (17.2%); (PII) *Schinus* (13.5%), *Weinmannia* (6.9%); (PIO) *Salix* (1.7%), *Attalea/Butia* (1.3%), *Sebastiania* (1.3%).	Monofloral honey from *Hovenia dulcis*.
	Rio Grande do Sul	T1-RS25	(PD) *Astronium* (75.4%); (PA) *Eucalyptus* (15.3%); (PII) *Attalea/Butia* (6.0%); (PIO) *Serjania* (1.8%), *Ligustrum* (1.5%).	Monofloral honey from *Astronium*.
Frieseomelitta doederleini	Pará	F-PA11	(PD) *Alibertia* (72.1%); (PII) *Tapirira guianensis* (11.3%), *Derris* (5.0%), *Cocos nucifera* (3.8%); (PIO) *Triplaris* (2.5%), *Paullinia* (2.5%), *Tabebuia* (1.7%), *Peritassa laevigata* (1.3%).	Monofloral honey from *Alibertia*.
	Pará	F-PA12	(PD) *Alibertia* (84.3%); (PII) *Tapirira guianensis* (14.0%); (PIO) *Paullinia* (1.7%).	Monofloral honey from *Alibertia*.
	Pará	F-PA13	(PA) *Tapirira guianensis* (30.0%); (PII) *Derris* (10.8%), *Moronobea* (9.2%), *Casearia* (8.3%), *Neptunia* (8.3%), *Triplaris* (8.3%), *Alibertia* (7.5%), *Zanthoxylum* (6.7%), *Pouteria* (5.8%), *Maprounea* (5.0%).	Multifloral honey with greater nectar contribution from *Tapiria guianensis*.
	Maranhão	F-MA02	(PA) *Pontederia* (34.4%), *Mimosa caesalpiniifolia* (34.0%), *Mimosa pudica* (22.3%); (PII) *Anacardium* (3.6%); (PIO) *Simarouba* (1.6%), *Xylosma* (1.6%), *Triplaris* (1.2%), *Vismia* (1.2%).	Multifloral honey with greater nectar contribution from *Pontederia and Mimosa caesalpiniifolia*.

PD: Dominant pollen (>45%), PA: Accessory pollen (16-45%), PII: Important isolated pollen (3-15%), PIO: Occasional isolated pollen (<3%).

Figure 2. Nectariferous plants most frequently visited by stingless bees. A) *Tapirira guianensis*, B) *Hovenia dulcis*, C) *Triplaris*, D) *Xylosma*, E) *Eucalyptus*, F) *Astronium*, G) *Holocalyx balansae*, H) *Derris*.



The entirety of Pará is situated within the Amazon biome, with forests covering most of its territory (IBGE, 2012). In Pará, *Tapirira guianensis* (Capiúva), *Triplaris* (Pau-formiga), and *Derris* (Timbó) were the pollen types most frequently identified in honey samples. Among the 13 samples, six were classified as monofloral, with one being from *Laetia procera* (Periquiteira), two from *Triplaris*, and three from *Alibertia* (Puruí). *Tapirira guianensis* is an endemic tree throughout the Brazilian territory (Carvalho, 2006). *Triplaris* species are native to riparian forests and utilized in urban landscaping and restoring degraded areas (Brandbyge, 1986). Plants of the *Derris* genus are native to the Amazon region (Poletto et al., 2011).

Amazonas and Maranhão had the fewest honey samples (three samples each). Similar to Pará, Amazonas is entirely covered by the Amazon rainforest (IBGE, 2012). For this state, the pollen types most frequently identified in the samples were from *Tapirira guianensis* and *Triplaris*. One sample was classified as being monofloral (*Tapiria guianensis*). Maranhão comprises the following biomes: Cerrado (64% of the State), Amazon (35%), and Caatinga (1%) (IBGE, 2012). In this state, the most frequently identified pollen type in the samples was *Xylosma* (Assucará). No sample was classified as monofloral. Species of the genus *Xylosma* are small trees or shrubs found mainly in rainforests (Gillette, 2023).

Paraná is located within the Atlantic Forest (98%) and Cerrado (2%) biomes, presenting a great diversity of plants. The most frequently identified pollen types in the honey samples were: *Eucalyptus* (Eucalipto), *Astronium* (Guaritá), and *Holocalyx balansae* (Alecrim de Campinas). Five samples were classified as monofloral honey, these being from *Mimosa scabrella* (Bracatinga), *Attalea/Butia* (Palmeira-indaiá-açú or Butiá), *Piptadenia gonoacantha* (Pau-jacaré), *Astronium*, and *Holocalyx balansae*. Plants belonging to the *Eucalyptus* genus hold significant commercial value in the Brazilian economy, with Paraná ranking fifth in national production (Coutinho et al., 2017; Ignacio et al., 2019). The *Holocalyx balansae* species is used for urban tree planting and reforestation, while the *Astronium* tree is primarily used for wood sales (Carvalho, 2003). The state of Rio Grande do Sul comprises the Pampa (63%) and Atlantic Forest (37%) biomes (IBGE, 2012). In this state, the most frequently identified pollen types in the honey samples were from *Hovenia dulcis* (Uva-do-Japão) and *Eucalyptus*. Among the states, Rio Grande do Sul was the one that presented the most samples of monofloral honeys, with seven samples of *Eucalyptus*, seven samples of *Hovenia dulcis*, a bifloral sample of *Brassica napus* and *Schinus* (Canola and Aroeira), and one sample of

Astronium, totaling 16 monofloral samples out of a total of 22 samples. In Brazil, the *Hovenia dulcis* tree is widely cultivated in the South region for urban tree-planting purposes. Due to its abundant fruiting, *Hovenia dulcis* has been widely used to recover degraded areas, aiming to attract fauna (Schneider, 2007). The cultivation of *Eucalyptus* in Rio Grande do Sul, as well as in Paraná, significantly contributes to the local economy (Santos et al., 2016; Wink et al., 2015).

3.2 Sugar profile by high-performance liquid chromatography (HPLC-RID)

The contents of glucose, fructose, trehalulose + maltose, raffinose, and reducing sugars are shown in Table 2. Sugars are the main components of honey and are responsible for viscosity, hygroscopicity, granulation, and energy supply (Mădaş et al., 2020; Mduda et al., 2023). The representative sugar chromatograms of the honey samples from different genera of stingless bees and the sugar standards are shown in Figure S1 and S2 – Supplementary material. As can be observed, the samples from different genera present different profiles.

Table 2. Sugar profile and phenolic compounds of honeys produced by different stingless bees.

Stingless bee species	Sample	Fructose (g/100g)	Glucose (g/100g)	Trehalulose + maltose (g/100g)	Raffinose (g/100g)	Reducing sugars (%)	Phenolic compounds (mg/100g)
Melipona compressipes	M-PA01	37.31 ± 0.11	30.57 ± 0.04	4.74 ± 0.00	0.54 ± 0.02	73.81 ± 0.07	22.22 ± 0.19
	M-PA02	35.10 ± 0.05	28.60 ± 0.00	6.08 ± 0.06	1.13 ± 0.02	71.14 ± 0.03	28.09 ± 0.03
	M-PA03	33.96 ± 0.10	28.52 ± 0.08	7.57 ± 0.12	0.50 ± 0.00	71.57 ± 0.12	47.19 ± 0.01
	M-PA04	34.71 ± 0.09	27.70 ± 0.00	6.29 ± 0.14	1.30 ± 0.01	70.03 ± 0.25	21.84 ± 0.09
	M-PA05	31.08 ± 0.22	23.52 ± 0.07	10.09 ± 0.07	0.35 ± 0.05	66.61 ± 0.21	69.46 ± 0.18
Melipona bicolor	M-PR13	33.18 ± 0.12	28.64 ± 0.09	7.84 ± 0.04	1.02 ± 0.01	71.29 ± 0.25	53.09 ± 0.15
	M-RS06	28.23 ± 0.25	23.53 ± 0.23	8.18 ± 0.07	0.52 ± 0.00	61.57 ± 0.57	35.72 ± 0.12
Melipona scutellaris	M-RS02	34.90 ± 0.34	31.82 ± 0.29	5.27 ± 0.11	1.26 ± 0.01	73.21 ± 0.75	24.96 ± 0.21
Melipona quadrifasciata	M-RS03	31.58 ± 0.40	27.97 ± 0.22	7.60 ± 0.06	0.76 ± 0.01	68.72 ± 0.69	29.63 ± 0.11
	M-RS21	33.79 ± 0.05	30.54 ± 0.01	6.48 ± 0.03	0.88 ± 0.01	72.27 ± 0.03	36.48 ± 0.16
Melipona mondury	M-RS04	36.47 ± 0.01	26.30 ± 0.08	4.18 ± 0.01	0.76 ± 0.02	68.05 ± 0.10	21.98 ± 0.28
Melipona fasciculata	M-RS07	41.83 ± 0.10	22.00 ± 0.06	7.57 ± 0.10	2.03 ± 0.04	72.94 ± 0.28	28.91 ± 0.11
	M-MA03	31.69 ± 0.02	27.10 ± 0.12	12.69 ± 0.07	1.62 ± 0.04	73.77 ± 0.06	56.80 ± 0.02
Melipona marginata	M-RS09	34.20 ± 0.04	22.80 ± 0.02	8.53 ± 0.07	0.69 ± 0.01	67.23 ± 0.14	23.56 ± 0.02
	M-RS15	32.16 ± 0.02	26.73 ± 0.25	7.03 ± 0.02	0.57 ± 0.01	67.42 ± 0.29	50.38 ± 0.17
	M-RS22	32.52 ± 0.12	28.37 ± 0.12	6.85 ± 0.04	0.84 ± 0.07	69.22 ± 0.30	46.98 ± 0.07
Melipona interrupta	M-AM01	36.90 ± 0.56	33.08 ± 0.39	5.50 ± 0.04	1.15 ± 0.02	76.76 ± 0.90	18.80 ± 0.15
	M-AM03	38.85 ± 0.25	27.73 ± 0.10	5.09 ± 0.14	0.95 ± 0.01	72.89 ± 0.50	24.54 ± 0.13
Melipona seminigra	M-AM02	35.27 ± 0.05	28.33 ± 0.05	4.05 ± 0.05	0.27 ± 0.02	68.73 ± 0.15	23.27 ± 0.14
Scaptotrigona xanthotricha	S-PA06	31.40 ± 0.20	23.34 ± 0.05	9.03 ± 0.21	0.45 ± 0.03	65.58 ± 0.50	60.10 ± 0.02
	S-PA07	30.87 ± 0.01	23.60 ± 0.08	10.82 ± 0.01	0.62 ± 0.01	67.32 ± 0.08	66.54 ± 0.13
	S-PA08	28.31 ± 0.01	22.46 ± 0.06	15.33 ± 0.13	1.12 ± 0.01	68.76 ± 0.21	58.64 ± 0.22
	S-PA09	32.52 ± 0.03	23.64 ± 0.02	11.28 ± 0.05	0.54 ± 0.01	69.46 ± 0.05	57.25 ± 0.08
	S-PA10	32.42 ± 0.15	23.26 ± 0.19	4.68 ± 0.05	0.46 ± 0.03	61.52 ± 0.39	44.81 ± 0.05
Scaptotrigona postica	S-PR01	35.70 ± 0.11	28.15 ± 0.16	7.25 ± 0.16	0.31 ± 0.01	72.62 ± 0.44	73.88 ± 0.06
	S-RS05	30.30 ± 0.71	25.72 ± 0.59	14.19 ± 0.31	1.12 ± 0.04	72.69 ± 1.65	72.86 ± 0.08
	S-RS23	33.03 ± 0.04	27.24 ± 0.02	10.79 ± 0.05	0.75 ± 0.07	73.08 ± 0.04	48.65 ± 0.01
Scaptotrigona bipunctata	S-PR05	31.19 ± 0.49	32.42 ± 0.34	6.51 ± 0.14	0.32 ± 0.02	71.46 ± 0.98	56.89 ± 0.23
	S-PR06	33.25 ± 0.06	26.60 ± 0.08	7.84 ± 0.09	0.57 ± 0.02	69.32 ± 0.24	49.17 ± 0.04
	S-PR17	33.12 ± 0.19	29.59 ±0.72	6.84 ± 0.33	0.25 ± 0.06	71.00 ± 0.54	46.80 ± 0.12
	S-PR18	32.94 ± 0.02	27.38 ± 0.06	7.97 ± 0.06	0.49 ± 0.01	69.92 ± 0.11	41.16 ± 0.05
	S-RS08	31.06 ± 0.18	19.47 ± 0.06	17.10 ± 0.01	1.92 ± 0.03	70.50 ± 0.24	51.14 ± 0.16
	S-RS12	35.26 ± 0.07	25.40 ± 0.01	9.78 ± 0.00	0.56 ± 0.00	72.32 ± 0.06	56.08 ± 0.03
	S-RS14	31.07 ± 0.34	23.23 ± 0.28	14.48 ± 0.18	1.52 ± 0.05	71.31 ± 0.41	45.00 ± 0.23
	S-RS20	36.31 ± 0.38	28.69 ± 0.15	10.00 ± 0.09	0.65 ± 0.04	76.94 ± 0.63	47.16 ± 0.02
Scaptotrigona aff. postica	S-MA01	33.38 ± 0.09	25.69 ± 0.11	5.91 ± 0.04	0.59 ± 0.03	66.35 ± 0.24	59.88 ± 0.04
Scaptotrigona depilis	S-RS17	34.94 ± 0.33	28.12 ± 0.28	8.71 ± 0.07	0.52 ± 0.03	73.51 ± 0.69	59.96 ± 0.02
Tetragona clavipes	T-PR02	13.98 ± 0.07	6.81 ± 0.06	41.62 ± 0.04	5.34 ± 0.06	68.68 ± 0.18	93.22 ± 0.03
	T-PR04	15.43 ± 0.09	8.14 ± 0.14	38.63 ± 0.70	3.92 ± 0.08	68.06 ± 1.04	92.66 ± 0.01
	T-PR14	15.70 ± 0.01	10.01 ± 0.12	34.60 ± 0.11	2.73 ± 0.03	65.63 ± 0.26	87.90 ± 0.03
	T-PR21	15.10 ± 0.34	6.11 ± 0.18	37.54 ± 0.03	7.56 ± 0.22	64.47 ± 0.19	120.19 ± 0.09
Tetragonisca angustula	T1-PR08	31.74 ± 0.70	27.61 ± 1.25	12.12 ± 0.20	0.76 ± 0.01	73.64 ± 2.18	66.73 ± 0.29
	T1-PR15	29.23 ± 0.05	23.84 ± 0.02	18.00 ± 0.05	1.09 ± 0.00	74.09 ± 0.02	64.29 ± 0.21
	T1-PR19	28.04 ± 0.01	20.81 ± 0.04	22.83 ± 0.05	2.10 ± 0.03	75.39 ± 0.11	58.13 ± 0.09
	T1-PR20	24.78 ± 0.09	19.97 ± 0.07	26.23 ± 0.07	1.24 ± 0.01	75.14 ± 0.24	59.30 ± 0.20

	T1-RS01	25.80 ± 0.22	20.65 ± 0.21	22.97 ± 0.16	3.69 ± 0.09	73.14 ± 0.61	56.71 ± 0.06
	T1-RS11	30.22 ± 0.05	23.92 ± 0.07	17.09 ± 0.01	1.51 ± 0.03	74.12 ± 0.11	65.61 ± 0.23
	T1-RS13	27.55 ± 0.08	22.93 ± 0.06	20.77 ± 0.07	2.52 ± 0.04	74.64 ± 0.21	60.97 ± 0.20
	T1-RS19	26.65 ± 0.19	22.34 ± 0.20	23.93 ± 0.07	1.79 ± 0.03	76.76 ± 0.46	61.07 ± 0.19
	T1-RS24	28.95 ± 0.21	23.37 ±0.17	17.80 ± 0.08	2.10 ± 0.01	73.13 ± 0.46	47.05 ± 0.08
	T1-RS25	27.45 ± 0.11	22.73 ± 0.15	19.45 ± 0.13	2.07 ± 0.05	72.83 ± 0.41	75.73 ± 0.18
	F-PA11	23.70 ± 0.08	17.37 ± 0.01	29.33 ± 0.07	2.84 ± 0.06	75.03 ± 0.00	109.62 ± 0.16
Frieseomelitta	F-PA12	24.43 ± 0.05	15.85 ± 0.05	30.36 ± 0.08	3.14 ± 0.05	75.43 ± 0.10	135.83 ± 0.06
doederleini	F-PA13	23.73 ± 0.01	25.17 ± 0.02	21.60 ± 0.07	0.69 ± 0.02	74.02 ± 0.09	62.58 ± 0.17
	F-MA02	30.08 ± 0.09	28.06 ± 0.02	11.48 ± 0.04	0.74 ± 0.12	71.76 ± 0.16	68.95 ± 0.11

The results express the average ± standard deviation (replication number = 2).

The disaccharide trehalulose has recently been identified in SBH samples as one of the major sugars (Fletcher et al., 2020; Hungerford et al., 2021; Mohamat et al., 2021; Ramlan et al., 2023; Wu et al., 2022; Zawawi et al., 2022). Trehalulose is a disaccharide, an isomer of sucrose, formed by the α-(1→1) glycosidic bond between glucose and fructose. This sugar offers several benefits: it has a lower glycemic index than sucrose, exhibits anticariogenic properties, and is a powerful antioxidant. Therefore, trehalulose is an ideal sugar to replace commercial sucrose and synthetic sweeteners (Tian et al., 2019). According to Hungerford et al. (2021), trehalulose is directly related to honey production through bee pollen collection. In their study, stingless bees fed with glucose and fructose solutions did not produce trehalulose, suggesting that this sugar is derived from nectars with high concentrations of sucrose.

According to Fletcher et al. (2020), trehalulose could be mistakenly identified as maltose due to the close chromatographic retention times of these two sugars, a fact that was observed in this study. The standards for trehalulose and maltose showed similar retention times (maltose: 9.94 min, trehalulose: 10.09 min), while the honey samples from the five genera showed retention times close to the standards of the two sugars (*Melipona*: 9.89 min; *Scaptotrigona*: 9.93 min; *Tetragona*: 10.05 min; *Tetragonisca*: 10.06 min; *Frieseomelitta*: 10.05 min). Considering that it was not possible to adequately separate trehalulose and maltose using the chromatographic method adopted in this study, the chromatographic peak identified in the range of 9.87 to 10.05 min was considered as being the sum of both sugars.

The total variation of trehalulose + maltose in this study was 4.05-41.62%. A smaller amount of these sugars can be noted for the *Melipona* and *Scaptotrigona* groups. Fletcher et al. (2020) quantified trehalulose in honey from four stingless bee genera from Australia (*Tetragonula hockingsi*), Brazil (*Tetragonisca angustula*), and Malaysia (*Geniotrigona*

thoracica and *Heterotrigona itama*) with a total variation of 13 to 44%. Similarly, Zawawi et al. (2022) quantified trehalulose in honey from three genera in Malaysia and Australia (*Heterotrigona itama*, 28.38%), (*Geniotrigona thoracica*, 49.08%), (*Tetragonula carbonaria*, 23.18%), and (*Tetragonula hockingsi*, 24.90%). In another study, Ramlan et al. (2023) analyzed 43 samples of *Heterotrigona itama* honey from Malaysia and identified trehalulose in all samples with concentrations ranging from 35.29 to 45.16%. However, the authors also identified maltose as a minor sugar with a variation of 0.80 to 3.13%, proving that both sugars can be present in SBH. Popova et al. (2021) also identified both sugars present in *Meliponula ferruginea* honey from Tanzania, averaging 20.87% for trehalulose and 1.56% for maltose. In our study, high concentrations of trehalulose + maltose are observed for the genera *Tetragona*, *Tetragonisca*, and *Frieseomelitta*, especially in honey samples from *Tetragona clavipes* (34.60 to 41.62%). The chromatographic retention time of sucrose can also coincide with maltose and trehalulose, depending on the method used (Zaidi et al., 2023). However, sucrose was not detected in our study. In previous studies, when it was believed that only maltose/sucrose existed in SBH, high concentrations of what could in fact be trehalulose are observed, such as the values reported for maltose by Shamsudin et al. (2019) in samples from Malaysia (*Heterotrigona*, 22.56 to 37.35%) and (*Geniotrigona*, 28.96 to 43.15%) and reported by Chuttong et al. (2016) in samples from Thailand (*Tetragonula laeviceps*, 15 to 57%). Vit et al. (1998) also found high maltose values in honey from *Frieseomelitta aff. varia* from Venezuela (56.9%). In our study, honey from the same genus *Frieseomelitta* was analyzed trehalulose + maltose with a variation of 13.61 to 35.15%. Marcolin et al. (2021) reported similar values for sucrose content in samples from *Tetragonisca angustula* (15.5-25.4%) compared to what was found for trehalulose + maltose in our study for the same species (12.12-26.23%). High concentrations of maltose

or sucrose in SBH are also observed in other studies (Abdul Malik et al., 2019; Bana et al., 2024; Kek et al., 2017; Tuksitha et al., 2018; Wong et al., 2019).

Honey from the *Melipona* and *Scaptotrigona* genera have not yet had the presence of trehalulose confirmed, and the concentrations of maltose or sucrose reported so far are low compared to the high concentrations of trehalulose reported in honey from Australia and Malaysia. Therefore, the possibility of this disaccharide being absent in these genera cannot be ruled out. Considering samples of Brazilian SBH, only the species *Tetragonisca angustula* had confirmation of the presence of trehalulose (Fletcher et al., 2020). According to Zaidi et al. (2023), a more in-depth analysis should be carried out with honeys from other species and locations, given the numerous species of stingless bees spread worldwide.

Zaidi et al. (2023) state that analyses based on mass and NMR spectra are more reliable in distinguishing maltose, sucrose, and trehalulose. The methodology presented by Ramlan et al. (2023) reports using HPLC-ELSD to separate these sugars effectively. Zawawi et al. (2022) used HPAEC-PAD for the quantification of trehalulose, but this last study does not make clear whether there was co-elution of the maltose and trehalulose standards by this technique since the previous determination of trehalulose had been made by NMR. In our study, the use of HPLC-RID was not able to distinguish trehalulose and maltose.

All the methods previously presented have advantages and disadvantages in their application. While HPLC and HPAEC are more cost-effective in terms of maintenance, they only offer retention times as form of identification of compounds, necessitating the use of standards for comparison and quantification, in addition to requiring an appropriate detector and column for the analysis of sugars. In the case of trehalulose, many laboratories, may have difficulties acquiring the standard due to its relatively high cost

(Zaidi et al., 2023). Mass chromatography, on the other hand, eliminates the need for standards, however, an appropriate column for sugar separation is necessary (Zaidi et al., 2023). Regarding NMR, the equipment involves high costs both in acquisition and maintenance (Razali et al., 2018).

The glucose and fructose contents varied from 6.11 to 33.08% and 13.98 to 41.83%, respectively. Glucose and fructose are the major monosaccharides in honey samples and are positively correlated with each other by Pearson correlation (+0.85) in Table 4. The proportions of glucose and fructose are directly related to the crystallization process. Glucose is the first sugar to crystallize, while fructose remains in solution due to its higher solubility (4.4 times more soluble) (Amariei et al., 2020). The crystallization of honey can negatively affect consumer preference. According to Grand View Research (2023), consumers prefer to check the physical state of honey before buying (Honey Market Size, 2023).

Table 3. Pearson's correlation coefficients among physicochemical parameters, total phenolic, and sugar contents for stingless bee honeys.

	Moisture	Total soluble solids	Phenolic compounds	pH	Electrical conductivity	L	a*	b*	Reducing sugars	Trehalulose + Maltose	Glucose	Fructose	Raffinose
Moisture													
Total soluble solids	-1.00												
Phenolic compounds	-0.21	0.21											
pH	-0.67	0.67	0.24										
Electrical conductivity	0.07	-0.07	0.67	0.19									
L	-0.03	0.03	-0.64	-0.20	-0.70								
a*	0.00	0.00	0.50	0.20	0.42	-0.69							
b*	-0.08	0.08	0.44	-0.12	0.24	-0.11	-0.26						
Reducing sugars	-0.78	0.78	-0.27	0.53	-0.36	0.27	-0.28	-0.17					
Trehalulose + Maltose	-0.35	0.35	0.78	0.21	0.47	-0.37	0.35	0.38	-0.28				
Glucose	0.01	-0.01	-0.70	0.00	-0.50	0.41	-0.43	-0.31	0.59	-0.90			
Fructose	0.08	-0.08	-0.75	-0.01	-0.50	0.36	-0.33	-0.42	0.50	-0.93	0.85		
Raffinose	-0.31	0.31	0.61	0.20	0.33	-0.35	0.46	0.33	-0.32	0.83	-0.82	-0.75	

TSS: Total soluble solids, EC: Electric conductivity.

As shown in Table 2, the samples present higher fructose concentrations than glucose. These results are consistent with the data presented in other literature for SBH (Bana et al., 2024; Marcolin et al., 2021; Ramlan et al., 2023; Sousa et al., 2016; Villacrés-Granda et al., 2021). The lowest concentrations of fructose and glucose, as well as the sum of these two sugars, were found for the honey samples of *Tetragona clavipes,* with values ranging from 13.98 to 15.70% for fructose and 6.11 to 10.01% for glucose, which is associated with the high content of trehalulose + maltose in these samples. The Pearson correlation (Table 3) shows a strong negative correlation between glucose and fructose with the sum of trehalulose and maltose, that is, samples with high levels of trehalulose + maltose have lower concentrations of glucose and fructose. This fact had already been reported by Zawawi et al. (2022). The highest concentrations of glucose and fructose are observed for the samples of *Melipona* and *Scaptotrigona*, possibly due to these bees having more active enzymes to catabolize these sugars from nectar or as a result of the metabolism of symbiotic bacteria (Vit et al., 2023). Umaña et al. (2021) also reported significantly higher concentrations of fructose and glucose for *Melipona beecheii* honey compared to *Tetragonisca angustula* honey.

The total range of reducing sugars (sum of glucose, fructose, maltose and trehalulose) varied from 58.76% to 75.49%. Braghini et al. (2022) stated that the low content of reducing sugars in SBH samples is an intrinsic characteristic of this honey, however, the presence of trehalulose had not been considered previously. Raffinose content ranged from 0.25% to 7.56%, with the highest concentrations found in samples from *Tetragona clavipes*, *Tetragonisca angustula*, *Frieseomelitta doederleini*, and *Melipona fasciculata*. Except for *Melipona fasciculata*, all *Melipona* genus samples exhibited lower raffinose levels compared to honeys from the *Tetragona*, *Tetragonisca*, and *Frieseomelitta* genera. Similarly, honey samples from *Scaptotrigona* genus demonstrated lower raffinose

amounts. A strong positive correlation can be observed between raffinose and trehalulose + maltose (+0.83) (Table 3), while a negative correlation is observed between raffinose and the monosaccharides glucose (-0.82) and fructose (-0.75).

Raffinose is an α-galactoside composed of galactose, fructose, and glucose, not metabolized by the human body. This trisaccharide has notable potential as a prebiotic. It can inhibit the growth of pathogenic bacteria, stimulate the growth of lactic acid bacteria, reduce the risk of cardiovascular disease, relieve constipation, enhance the production of short-chain fatty acids, and prevent the formation of putrefactive compounds from proteins (Anggraeni, 2022). Raffinose was previously associated only with honeydew honey. It was later reported that both insect excretion and nectar could serve as sources of α-galactosides in honey (Moreira and Maria, 2001; Vit et al., 2023). Vit et al. (2023) also noted high concentrations of raffinose (5.6%) in honey samples from the *Geotrigona* genus. Other literature reports lower values of raffinose than those found in this study (Kamboj et al., 2020; Nayik et al., 2019; Umaña et al., 2021).

3.3 Phenolic compound content

The values found in the total phenolic compounds content in the SBH samples are shown in Table 2. The total phenolic compounds content found in the honey samples ranged from 18.80 to 135.83 mg GAE/100 g. Other studies had already reported significant variations in the phenolic compounds content of Brazilian SBH, with values ranging from 11.01 to 38.92 mg GAE/ 100 g (Biluca et al., 2021) and 22.04 to 70.81 mg GAE/ 100 g (Ávila et al., 2019a). It is widely known that SBH are a rich source of compounds with bioactive activity, such as phenolic compounds, which are largely responsible for the strong antioxidant effects *in vitro* and *in vivo* (Ávila et al., 2019a).

Biluca et al. (2017) investigated the phenolic composition of 13 honey samples from nine species of stingless bees. The authors found 26 phenolic compounds, with the main ones being salicylic acid, p-coumaric acid, naringin, and taxifolin. The results also showed the presence of caffeic acid, mandelic acid, rosmarinic acid, chlorogenic acid, isoquercitrin, carnosol, aromadendrin, umbelliferone, eriodictyol, vanillin, and seringa aldehyde. The bioactive properties of SBH can be explained by the fact that this type of honey is produced inside pots made of cerumen (wax and resin collected from plants). Therefore, the composition of the honeys in terms of bioactive compounds can be influenced by the presence of phytochemicals in the cerumen (Ávila et al., 2018).

The samples that showed the highest content of phenolic compounds were two samples from *Frieseomelitta doederleini* and all samples from *Tetragona clavipes*. These samples also exhibited high levels of trehalulose + maltose. A strong positive correlation can be observed between the total phenolic content and trehalulose (+0.78) (Table 3). Generally, samples from the *Melipona* and *Scaptotrigona* genera present lower levels of phenolic compounds than honey originating from the *Tetragona*, *Tetragonisca*, and *Frieseomelitta* genera. According to Ávila et al. (2019), in addition to the genus and species of the bee producer, the content and profile of phenolic compounds in SBH are intrinsically related to the floral origin. This is because, in the process of honey production from flower nectar, the bioactive compounds synthesized by the plants are transferred to the honey. However, this study could not establish this relationship since honey from the same species has different pollen predominance (Table 1).

3.4 Physicochemical parameters

In this study, five physicochemical parameters were determined: total soluble solids content, moisture content, pH, electrical conductivity, and color (Table 4).

Table 4. Physicochemical parameters of honeys produced by different stingless bees.

Stingless bee species	Sample	L*	a*	b*	TSS (ºBrix)	Moisture (%)	EC (mS/cm)	pH
Melipona compressipes	M-PA01	26.61 ± 0.19	-0.10 ± 0.02	5.83 ± 0.06	74.50 ± 0.26	25.50 ± 0.26	0.36 ± 0.00	3.48 ± 0.01
	M-PA02	30.5 ± 0.04	-0.4 ± 0.03	5.40 ± 0.01	72.90 ± 0.35	27.1 ± 0.35	0.28 ± 0.00	3.40 ± 0.01
	M-PA03	30.18 ± 0.16	-0.77 ± 0.02	7.15 ± 0.02	72.30 ± 0.72	27.70 ± 0.72	0.48 ± 0.00	3.52 ± 0.00
	M-PA04	29.89 ± 0.00	-0.34 ± 0.02	6.13 ± 0.02	71.57 ± 0.15	28.43 ± 0.15	0.23 ± 0.00	3.44 ± 0.01
	M-PA05	26.02 ± 0.13	-0.30 ± 0.01	9.97 ± 0.02	67.47 ± 0.06	32.53 ± 0.06	0.62 ± 0.00	3.53 ± 0.01
Melipona bicolor	M-PR13	29.34 ± 0.23	-2.82 ± 0.05	14.92 ± 0.04	72.70 ± 0.44	27.30 ± 0.44	0.34 ± 0.00	3.20 ± 0.00
	M-RS06	26.63 ± 0.01	0.03 ± 0.02	5.74 ± 0.02	62.47 ± 0.21	37.53 ± 0.21	0.41 ± 0.00	3.17 ± 0.01
Melipona scutellaris	M-RS02	31.55 ± 0.04	-0.41 ± 0.04	4.01 ± 0.04	75.33 ± 0.49	24.67 ± 0.49	0.11 ± 0.00	4.25 ± 0.00
Melipona quadrifasciata	M-RS03	27.30 ± 0.20	-0.27 ± 0.02	5.54 ± 0.03	70.03 ± 0.32	29.97 ± 0.32	0.60 ± 0.00	3.30 ± 0.01
	M-RS21	28.71 ± 0.08	-0.45 ± 0.03	6.92 ± 0.04	73.60 ± 0.26	26.40 ± 0.26	0.25 ± 0.00	3.83 ± 0.00
Melipona mondury	M-RS04	30.14 ± 0.02	-0.40 ± 0.02	3.87 ± 0.08	69.10 ± 0.10	30.90 ± 0.10	0.14 ± 0.00	3.90 ± 0.00
Melipona fasciculata	M-RS07	28.42 ± 0.07	-0.48 ± 0.02	6.47 ± 0.06	75.20 ± 0.10	24.80 ± 0.10	0.25 ± 0.00	4.19 ± 0.00
	M-MA03	27.26 ± 0.22	-0.33 ± 0.08	8.28 ± 0.05	76.33 ± 0.25	23.67 ± 0.25	0.63 ± 0.00	3.93 ± 0.01
Melipona marginata	M-RS09	30.81 ± 0.18	-0.15 ± 0.05	4.40 ± 0.05	68.23 ± 0.32	31.77 ± 0.32	0.25 ± 0.00	3.40 ± 0.01
	M-RS15	30.53 ± 0.22	-0.90 ± 0.03	5.87 ± 0.01	68.70 ± 0.46	31.30 ± 0.46	0.33 ± 0.00	3.65 ± 0.01
	M-RS22	29.19 ± 0.19	-0.22 ± 0.03	8.72 ± 0.23	70.93 ± 0.06	29.07 ± 0.06	0.30 ± 0.00	3.80 ± 0.00
Melipona interrupta	M-AM01	28.77 ± 0.06	-0.30 ± 0.04	4.72 ± 0.08	78.73 ± 0.21	21.27 ± 0.21	0.50 ± 0.00	4.99 ± 0.00
	M-AM03	29.72 ± 0.14	-0.48 ± 0.02	4.94 ± 0.03	74.37 ± 0.31	25.63 ± 0.31	0.34 ± 0.00	4.16 ± 0.00
Melipona seminigra	M-AM02	29.96 ± 0.04	-0.28 ± 0.02	5.48 ± 0.02	71.07 ± 0.40	28.93 ± 0.40	0.48 ± 0.00	3.74 ± 0.01
Scaptotrigona xanthotricha	S-PA06	28.67 ± 0.18	-0.56 ± 0.06	7.35 ± 0.03	66.93 ± 0.25	33.07 ± 0.25	0.69 ± 0.00	3.46 ± 0.00
	S-PA07	25.80 ± 0.20	-0.52 ± 0.03	8.96 ± 0.02	68.87 ± 0.38	31.13 ± 0.38	0.61 ± 0.00	3.48 ± 0.01
	S-PA08	28.09 ± 0.21	-0.83 ± 0.01	13.01 ± 0.10	70.73 ± 0.12	29.27 ± 0.12	0.57 ± 0.00	3.43 ± 0.00
	S-PA09	29.60 ± 0.21	-1.05 ± 0.05	7.93 ± 0.02	70.57 ± 0.12	29.43 ± 0.12	0.91 ± 0.00	3.74 ± 0.01
	S-PA10	26.28 ± 0.20	-0.14 ± 0.12	6.82 ± 0.02	62.90 ± 0.00	37.10 ± 0.00	0.77 ± 0.00	3.61 ± 0.01
Scaptotrigona postica	S-PR01	26.63 ± 0.03	0.77 ± 0.05	11.92 ± 0.01	73.17 ± 0.31	26.83 ± 0.31	0.30 ± 0.00	3.84 ± 0.01
	S-RS05	28.36 ± 0.09	-1.22 ± 0.03	10.29 ± 0.07	74.60 ± 0.10	25.40 ± 0.10	0.46 ± 0.00	3.97 ± 0.01
	S-RS23	31.28 ± 0.23	-1.01 ± 0.03	6.63 ± 0.06	74.20 ± 0.26	25.80 ± 0.26	0.40 ± 0.00	3.99 ± 0.01
Scaptotrigona bipunctata	S-PR05	27.24 ± 0.03	-0.42 ± 0.06	7.72 ± 0.02	72.10 ± 0.10	27.90 ± 0.10	0.35 ± 0.00	3.72 ± 0.00
	S-PR06	27.21 ± 0.04	-0.22 ± 0.03	2.93 ± 0.01	70.77 ± 0.47	29.23 ± 0.47	0.44 ± 0.00	3.75 ± 0.00
	S-PR17	28.04 ± 0.02	-0.67 ± 0.05	6.47 ± 0.02	72.03 ± 0.06	27.97 ± 0.06	0.59 ± 0.00	4.16 ± 0.01
	S-PR18	29.50 ± 0.04	-0.42 ± 0.05	9.20 ± 0.00	70.77 ± 0.06	29.23 ± 0.06	0.44 ± 0.00	3.79 ± 0.01
	S-RS08	27.32 ± 0.23	-0.74 ± 0.07	8.36 ± 0.10	72.90 ± 0.17	27.10 ± 0.17	0.44 ± 0.00	3.17 ± 0.01
	S-RS12	25.84 ± 0.10	-0.16 ± 0.03	7.90 ± 0.06	73.43 ± 0.15	26.57 ± 0.15	0.60 ± 0.00	4.02 ± 0.00
	S-RS14	29.25 ± 0.17	-1.66 ± 0.04	10.61 ± 0.04	73.77 ± 0.06	26.23 ± 0.06	0.41 ± 0.00	3.96 ± 0.01
	S-RS20	28.82 ± 0.13	-0.71 ± 0.03	4.81 ± 0.03	78.93 ± 0.32	21.07 ± 0.32	0.34 ± 0.00	3.98 ± 0.01
Scaptotrigona aff. postica	S-MA01	23.27 ± 0.01	0.15 ± 0.02	8.01 ± 0.02	67.17 ± 0.06	32.83 ± 0.06	0.86 ± 0.00	4.13 ± 0.01
Scaptotrigona depilis	S-RS17	30.36 ± 0.10	-0.77 ± 0.07	5.12 ± 0.03	74.73 ± 0.38	25.27 ± 0.38	0.40 ± 0.00	3.97 ± 0.01
Tetragona clavipes	T-PR02	25.72 ± 0.56	0.50 ± 0.02	10.05 ± 0.09	74.23 ± 0.15	25.77 ± 0.15	0.62 ± 0.00	3.86 ± 0.00
	T-PR04	27.41 ± 0.09	-0.21 ± 0.01	9.69 ± 0.03	72.13 ± 0.15	27.87 ± 0.15	0.70 ± 0.00	3.83 ± 0.01
	T-PR14	28.64 ± 0.06	-0.38 ± 0.02	9.58 ± 0.06	68.73 ± 0.15	31.27 ± 0.15	0.67 ± 0.00	3.58 ± 0.01
	T-PR21	24.18 ± 0.07	3.08 ± 0.06	12.34 ± 0.04	72.57 ± 0.38	27.43 ± 0.38	0.74 ± 0.00	3.68 ± 0.00
Tetragonisca angustula	T1-PR08	27.64 ± 0.07	-1.23 ± 0.01	9.07 ± 0.03	75.13 ± 0.32	24.87 ± 0.32	0.53 ± 0.00	4.21 ± 0.00
	T1-PR15	29.94 ± 0.03	-0.45 ± 0.02	5.63 ± 0.01	75.67 ± 0.15	24.33 ± 0.15	0.30 ± 0.00	3.92 ± 0.00
	T1-PR19	29.56 ± 0.09	-1.22 ± 0.06	10.99 ± 0.07	78.00 ± 0.30	22.00 ± 0.30	0.44 ± 0.00	4.02 ± 0.00
	T1-PR20	28.97 ± 0.27	-0.57 ± 0.02	6.98 ± 0.05	76.60 ± 0.53	23.40 ± 0.53	0.42 ± 0.00	4.02 ± 0.00
	T1-RS01	29.15 ± 0.23	-0.89 ± 0.05	9.90 ± 0.04	77.43 ± 0.35	22.57 ± 0.35	0.52 ± 0.00	4.13 ± 0.00
	T1-RS11	26.84 ± 0.18	-1.04 ± 0.07	8.16 ± 0.08	76.20 ± 0.36	23.80 ± 0.36	0.42 ± 0.00	4.38 ± 0.00
	T1-RS13	28.06 ± 0.03	-1.47 ± 0.04	13.82 ± 0.03	77.43 ± 0.21	22.57 ± 0.21	0.63 ± 0.00	4.26 ± 0.01

	T1-RS19	29.77 ± 0.10	-0.84 ± 0.09	7.63 ± 0.04	79.60 ± 0.72	20.40 ± 0.72	0.40 ± 0.00	4.09 ± 0.01
	T1-RS24	29.69 ± 0.09	-1.01 ± 0.07	7.33 ± 0.06	76.10 ± 0.10	23.90 ± 0.10	0.33 ± 0.00	3.98 ± 0.00
	T1-RS25	29.02 ± 0.10	-1.79 ± 0.04	14.61 ± 0.04	75.47 ± 0.25	24.53 ± 0.25	0.59 ± 0.00	4.21 ± 0.01
Frieseomelitta doederleini	F-PA11	21.13 ± 0.04	2.46 ± 0.04	4.74 ± 0.04	78.40 ± 0.61	21.60 ± 0.61	0.95 ± 0.00	4.60 ± 0.01
	F-PA12	21.44 ± 0.40	2.95 ± 0.03	6.03 ± 0.06	78.93 ± 0.58	21.07 ± 0.58	1.01 ± 0.00	4.71 ± 0.01
	F-PA13	28.20 ± 0.37	-0.50 ± 0.04	9.85 ± 0.06	75.07 ± 1.69	24.93 ± 1.69	0.60 ± 0.00	3.76 ± 0.01
	F-MA02	28.47 ± 0.18	-0.63 ± 0.01	11.57 ± 0.02	73.57 ± 0.50	26.43 ± 0.50	0.45 ± 0.00	3.76 ± 0.01

The results express the average ± standard deviation (replication number = 3). TSS: Total soluble solids, EC: Electric conductivity.

Regarding physicochemical parameters, SBH differs from the standards established for *Apis mellifera* honey. The main differences are related to higher moisture and water activity levels, lower pH, higher acidity, and lower levels of sugars and total soluble solids, which are directly influenced by geographic and entomological factors (Ávila et al., 2018). In Brazil, no federal regulations define SBH's physicochemical quality parameters. The existing federal legislation applies only to *Apis mellifera* honey, regulated by Instrução Normativa 11, de 20 de outubro de 2000 do Ministério da Agricultura, Pecuária e Abastecimento (MAPA) (Brasil, 2000; Sato et al., 2023).

Due to the physicochemical differences in the honey of *Apis mellifera* and stingless bees, some Brazilian states have created specific regulations. Currently, the Brazilian states that have state regulation are Paraná, Rio Grande do Norte, Santa Catarina, Bahia, São Paulo, and Amazonas (Amazonas, 2016; Bahia, 2014; Paraná, 2017; Rio Grande do Norte, 2019; Santa Catarina, 2020; São Paulo, 2017). A review study by Sato et al. (2023) showed that most of the physicochemical parameter data of Brazilian honey published between 2017 and 2022 followed local standards. Since the states of Pará, Maranhão, and Rio Grande do Sul do not have specific legislation, the data from this study were compared with the regulations of neighboring states in the same Brazilian region. The data from Pará were compared with the regulation of Amazonas, the data from Maranhão were compared with the regulation of Bahia, and the data from Rio Grande do Sul were compared with the regulation of Santa Catarina.

Regarding the moisture content, the states of Paraná, Bahia, and Amazonas establish a limit of 35%, and the states of Santa Catarina, São Paulo, and Rio Grande do Norte establish a limit of 40%. The total variation in moisture content in this study was from 20.40 - 37.53% (Table 4). In general, the lowest moisture contents are observed for the *Tetragonisca angustula* samples, while an inverse relationship can be observed for the

Tetragona clavipes samples. Biluca et al. (2021) also reported a lower moisture value for *Tetragonisca angustula* honey. Except for one sample (S-PA10), all data follow the guidelines established by the respective state regulations. All samples had a moisture content greater than 20%, meaning none followed the limit established for *Apis mellifera* honey. The higher levels of water activity and moisture in SBH are related to these bees' preference for ripe fruits and ground flowers, as well as the higher humidity in tropical and subtropical regions (Ávila et al., 2018; Ramlan et al., 2023).

The high moisture content of SBH (>20%) allows lactic and acetic acid bacteria to survive. These bacteria ferment the sugars and produce the organic acids responsible for the higher acidity of SBH (Ávila et al., 2018; Sousa et al., 2016). Regarding pH, the total pH variation obtained in this study was from 3.17-4.99% (Table 4). According to Biluca et al. (2021), the pH is mainly influenced by the floral origin, as well as honey with high pH is usually associated with a mixture of honey derived from honeydew. The enzymes secreted by bees, such as proteases, lactases, lipases, and proteins, can also affect the pH (Ávila et al., 2018). It is worth noting that although pH and acidity are proportional quantities, in the case of honey, these parameters may not always be directly proportional due to buffering reactions involving mineral salts and organic acids (Rodríguez et al., 2004).

The Brazilian legislation and the Codex Alimentarius do not establish a specific limit value for the pH of the honey of *Apis mellifera*. In the states of Bahia and Amazonas, there is also no definition for this parameter. However, it is important to highlight that foods with a pH higher than 4.5 are more susceptible to contamination by pathogenic bacteria (Sato et al., 2023). The state of Paraná stipulates a maximum value of 4.7 for the pH. For Santa Catarina, the pH values should be in the range of 2.8 to 4.8, for São Paulo from 2.9 to 4.5, and for Rio Grande do Norte from 2.9 to 4.5. According to existing state

regulations, all samples are within the stipulated values. The highest pH values are observed for the samples of *Tetragonisca angustula* (Table 4).

Total soluble solids include all organic and inorganic matter and are directly related to the total sugar content in honey (Ramlan et al., 2023). In this study, the total variation of total soluble solids was 62.47-79.6 ºBrix (Table 4). Moisture and total soluble solids are inversely proportional parameters. A higher content of total soluble solids was observed in the samples of *Tetragonisca angustula* (Ávila et al., 2018; Sousa et al., 2016). State regulations and Brazilian legislation do not stipulate specific limit values for this parameter.

The electrical conductivity of honey is associated with the mineral content (ash content), organic acids, ions, and proteins (Ávila et al., 2018). The total variation of electrical conductivity in this study was 0.11-1.01 mS/cm (Table 4). According to Alves et al. (2005), evaluating electrical conductivity can be an indirect alternative to determining the ash content. In addition, electrical conductivity is often used to distinguish between floral honey and honeydew honey. Brazilian state regulations for SBH do not stipulate limit values for this parameter. In contrast, Brazilian legislation for *Apis mellifera* honey and the Codex Alimentarius Commission (2001) determine that floral honey should have a maximum value of 0.80 mS/cm. In this study, four samples (S-PA09, F-PA11, F-PA12, and S-MA01) showed values higher than 0.80 mS/cm.

Color is one of the characteristics that most affect the consumer's purchasing decision (Pavlova et al., 2018). The color parameters represented by the letters L* (brightness), a* (green to red), and b* (blue to yellow) indicate that most of the samples tend to the colors green and yellow (Table 4). The total variation of a* was from -2.82 to 3.08, with only seven samples tending towards red. The total variation of b* was from 2.93 to 14.92, with all samples tending towards yellow. The brightness variation was from 21.13 to 31.55.

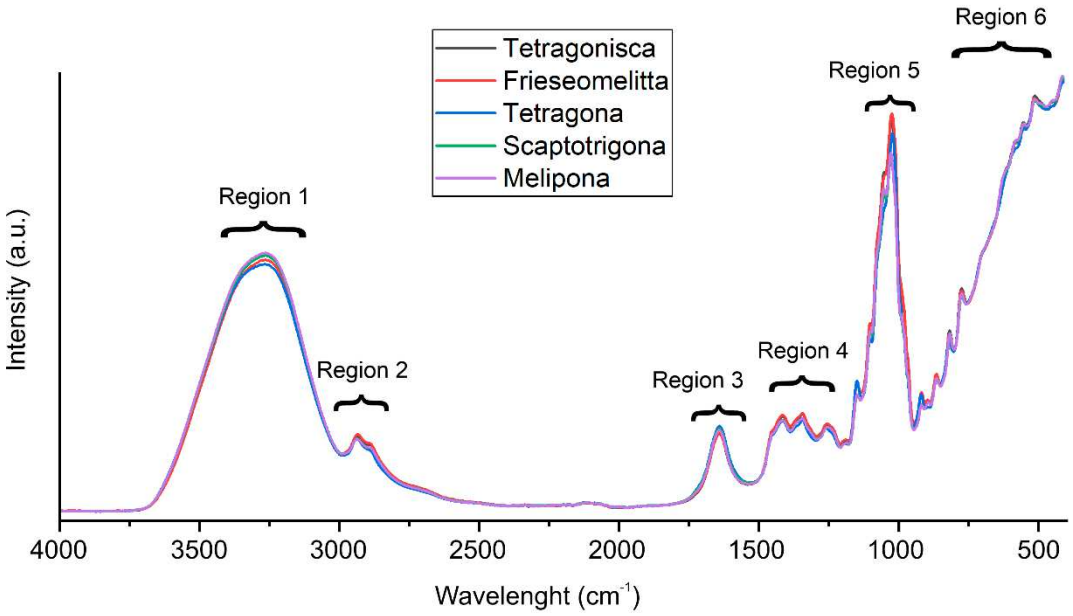
The color variations in honey are closely linked to its floral origin and chemical composition, which includes compounds like carotenoids, anthocyanins, and flavones. Over time and with storage, honey tends to darken due to non-enzymatic reactions known as Maillard reactions. Additionally, honey's transparency is affected by the quantity of suspended particles, such as pollen grains (Pavlova et al., 2018).

Strong and significant positive and negative Pearson correlations (above 0.7) were observed for some physicochemical parameters (Table 3). The moisture content showed a negative correlation with the total soluble solids content (-1.0) and reducing sugars (-0.94). The total soluble solids content showed a positive correlation with the content of reducing sugars (+0.94), and the L* color parameter showed a negative correlation (-0.70) with the electrical conductivity.

3.5 FTIR-ATR

The FTIR offers the benefit of being a rapid, non-destructive, and easy-to-operate technique. Moreover, it requires only a small sample volume for analysis, with minimal or null sample preparation (Anjos et al., 2018; Pita-Calvo and Vázquez, 2018). The FTIR spectra of the honey samples (Figure 3) were obtained from 4000 to 400 cm^{-1} and show six band regions.

Figure 3. Average FTIR-ATR spectra of honey samples from *Tetragona*, *Tetragonisca*, *Melipona*, *Scaptotrigona*, and *Frieseomelitta*.



The region 1 (3700-3000 cm^{-1}) is dominated by O–H stretching of water bands. In region 2 (3000–2800 cm^{-1}), the bands are attributed to the C–H stretching of carbohydrates, O–H stretching of carboxylic acids, and NH_3 stretching of free amino acids. In region 3 (1750–1540 cm^{-1}), the bands are attributed to O–H stretching/bending of water, C=O stretching of carbohydrates, phenolics, organic acids, and N–H bending of amide I (mainly proteins, presented in very low concentration in honey) (Anjos et al., 2015; Cherigui et al., 2024; Gok et al., 2015; Ioannou et al., 2022; Kasprzyk et al., 2018).

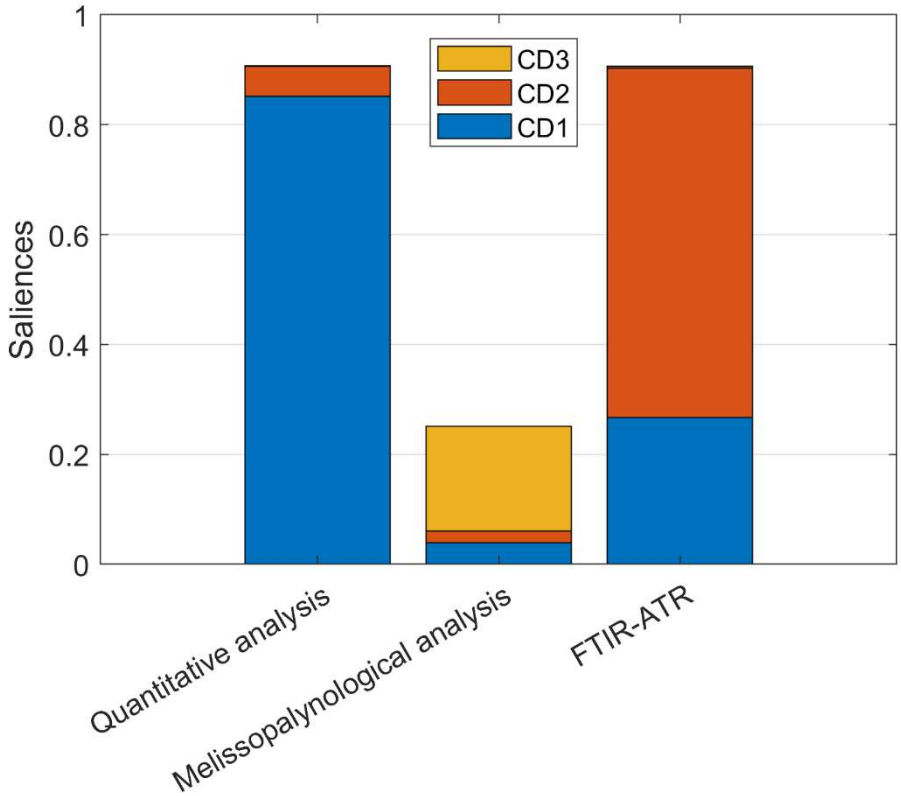
In region 4 (1540–1180 cm^{-1}), the bands are attributed to O–H stretching/bending, C–O stretching of carbohydrates, C–H stretching of carbohydrates, and C=O stretching of ketones. In region 5 (1180–946 cm^{-1}), the observed bands are attributed to the C–O/C–C stretching of carbohydrates and ring vibrations of carbohydrates. Region 6 (946–400 cm^{-1}) corresponds to the anomeric region of carbohydrates and is attributed to the C–H bending of carbohydrates and ring vibrations of carbohydrates (Anjos et al., 2015; Gok et al., 2015; Ioannou et al., 2022; Kasprzyk et al., 2018). Comparing the mean spectra of

honey from different genera of bees, it is possible to observe a slight difference in signal intensities in the six-band regions (Figure 3).

3.6 Multivariate *analysis*

Data analysis typically begins with unsupervised methods, such as Principal Component Analysis (PCA) or, in this case, MA. ComDim is particularly noteworthy for its ability to simultaneously examine multiple data sets, a task that traditional PCA cannot perform since it requires separate analysis of each data set. This comprehensive approach offers a broader and more cohesive understanding of the data, making it especially valuable in scenarios involving complex systems with interconnected aspects (Galvan et al., 2023; Silva et al., 2024). Figure 4 shows the predominance of data sets in each common dimension (CD).

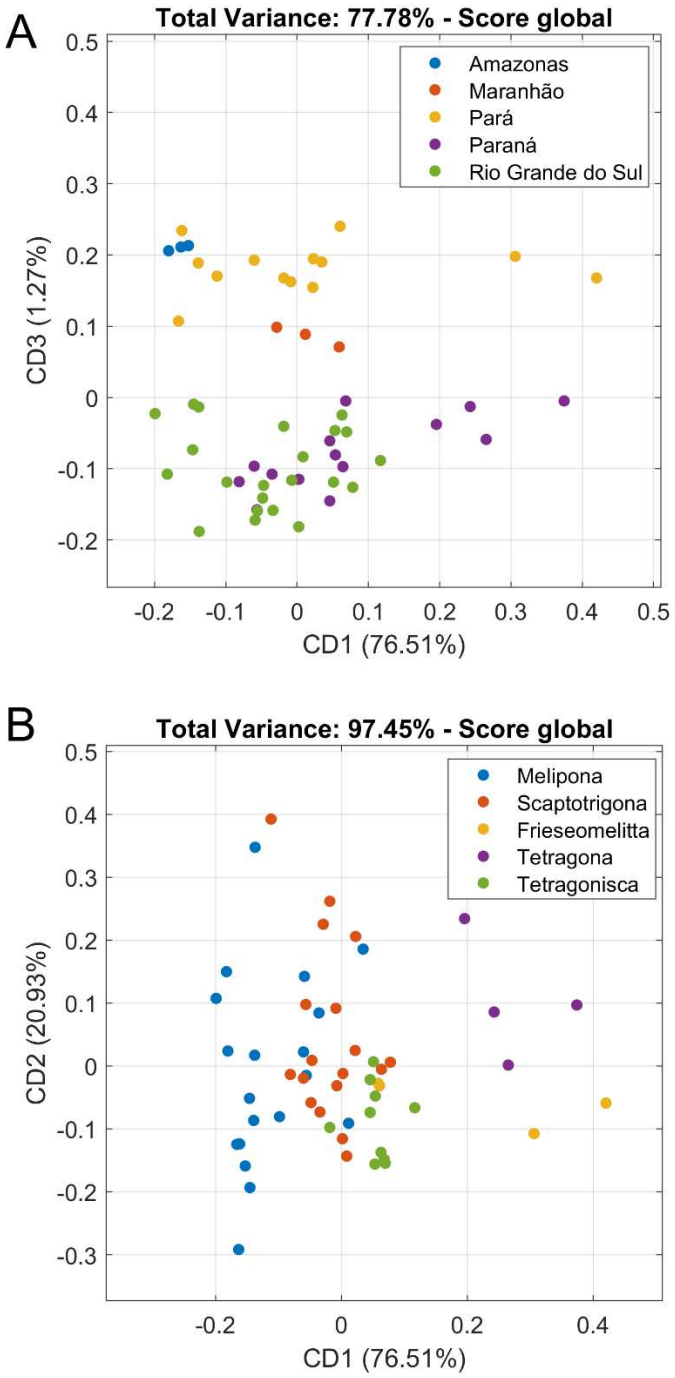
Figure 4. Saliences scores for the first three common dimensions.



CD1 is predominantly composed by Block 1 (physicochemical parameters, phenolic compounds, and sugars), CD2 is predominantly composed by Block 2 (FTIR-ATR), and CD3 is predominantly composed by Block 3 (pollen types). Three CDs were sufficient to represent 98.71% of the total variance, with 76.51% of the variance presented by CD1, 20.93% represented by CD2, and 1.27% represented by CD3. An initial analysis investigated geographical origin's influence on honey's characteristics. The combination between CD1 × CD2 does not bring us information about possible groupings (Figure S1 – Supplementary material); however, when combining CD1 × CD3 it is possible to observe two groupings, one group in the upper region with the samples from Pará, Amazonas and Maranhão (North and Northeast region) and another group in the lower region with the samples from Paraná and Rio Grande do Sul (South region) (Figure 5A). Block 3 mainly represented by CD3, was responsible for this grouping. The correlation between the melissopalynological analysis and the CDs is shown in Figure 6A. The most important pollen types for group separation were *Triplaris* and *Tapirira guianensis* (North and Northeast) and *Eucalyptus* (South).

In a second analysis, the influence of entomological origin (in terms of bee genera) was investigated (Figure 5B). A slight clustering can be observed among *Tetragonisca*, *Melipona*, and *Scaptotrigona* honey samples. Most of the *Melipona* samples are situated in the left quadrant. The majority of *Tetragonisca* samples are found in the lower right quadrant. *Scaptotrigona* samples are predominantly located on the left side, dispersed among the *Melipona* and *Tetragonisca* samples. *Tetragona* samples are well separated from other genera. *Frieseomelitta* samples are located on the right side, and a clear clustering of this genus is not observed.

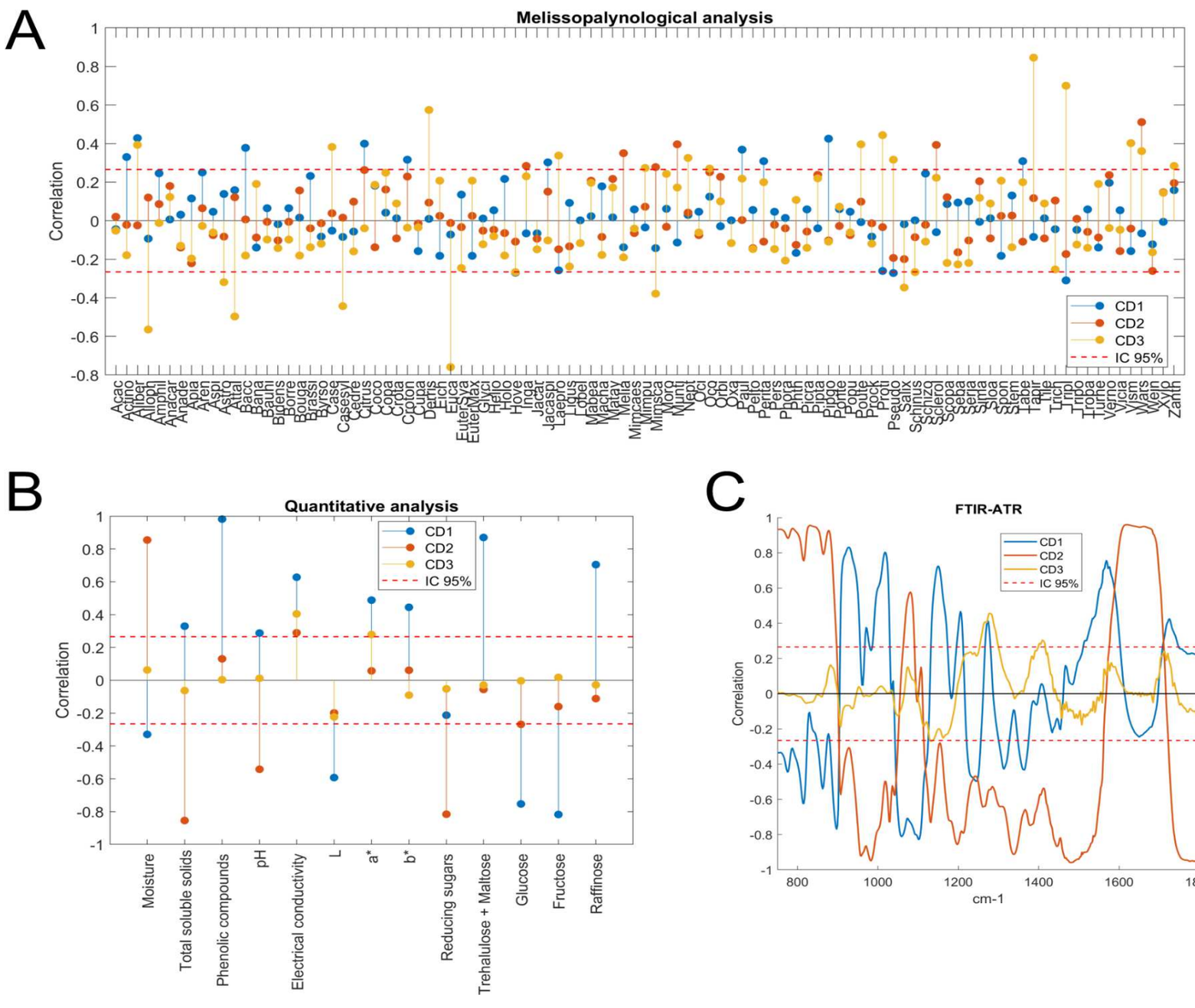
Figure 5. ComDim for discrimination of *Tetragona*, *Tetragonisca*, *Melipona*, *Scaptotrigona* and *Frieseomelitta* honeys using melissopalynological analysis data, quantitative data, and FTIR-ATR by (A) Geographic orign (CD1 × CD3) (B) entomological origin (CD1 × CD2).



The correlation between the quantitative analyses (Block 1) and the CDs is shown in Figure 6B. For CD1, the most important parameters for the separation of the samples were: phenolic compounds, electrical conductivity, a*, b*, L, trehalulose + maltose, raffinose, glucose, and fructose. The samples from *Tetragona*, *Tetragonisca*, and *Frieseomelitta* (positive scores) are characterized by the highest contents of phenolic compounds, trehalulose + maltose, raffinose, electrical conductivity, and by the greatest tendency towards the colors red and yellow given by the color parameters a* and b*. While the samples from *Melipona* and most samples from *Scaptotrigona* (negative scores) are characterized by the highest contents of glucose and fructose and by the greater brightness of the samples given by the parameter L. CD2 was important for the distinction of the samples from *Tetragonisca* and *Tetragona*. The separation between the two genera is mainly due to the higher values of pH, total soluble solids, reducing sugars (negative scores), and lower moisture content (positive scores) for the *Tetragonisca* samples.

The correlation between the FTIR-ATR spectra (Block 2) and the CDs is shown in Figure 6C. As discussed earlier, the FTIR-ATR data are essentially represented by CD2. As can be seen in Figure 6C, all bands between 1800 and 750 cm^{-1} were important for the separation of the samples.

Figure 6. Correlations between the first three common dimensions and (A) melissopalynological analysis data, (B) Quantitative data, (C) FTIR-ATR.



After exploratory analysis with ComDim, the supervised LDA and PLS-DA models were developed with the quantitative data (Block 1) and FTIR-ATR (Block 2), respectively. The genera classes included in the supervised analysis were *Tetragonisca*, *Scaptotrigona*, and *Melipona*. The samples from the genus *Tetragona* and *Frieseomelitta* were excluded from this analysis. In the case of the *Tetragona* samples, a clear separation from the other genera was observed through the exploratory analysis, and therefore, a supervised analysis would not be applicable. The *Frieseomelitta* class was not included due to the low number of representative samples (n=4).

The first step in developing a chemometric model for sample classification is the supervised detection of outliers (Bana et al., 2024). In this analysis, no outlier sample was identified. Eight LVs were selected to build the PLS-DA model. In general, the PLS-DA model showed superior performance compared to the LDA. Table 5 shows a performance comparison between the two models.

Table 5. Performance of the LDA and PLS-DA models.

Algorithm	Set	Class	FN	TP	FP	TN	ACC	SEN	SPE
LDA with quantitative data (Block 1)	Calibration	*Melipona*	2	11	3	16	84.4	84.6	84.2
		Scaptotrigona	3	9	3	17	81.3	75.0	85.0
		Tetragonisca	1	6	0	25	96.9	85.7	100.0
	Prediction	*Melipona*	0	6	1	8	93.3	100.0	88.9
		Scaptotrigona	2	4	0	9	86.7	66.7	100.0
		Tetragonisca	0	3	0	12	100.0	100.0	100.0
PLS-DA with FTIR-ATR (Block 2) plus MSC correction with range 750 to 1800 cm^{-1} (8 LVs)	Calibration	*Melipona*	0	13	0	19	100.0	100.0	100.0
		Scaptotrigona	0	12	0	20	100.0	100.0	100.0
		Tetragonisca	0	7	0	25	100.0	100.0	100.0
	Prediction	*Melipona*	1	5	1	8	86.7	83.3	88.9
		Scaptotrigona	0	6	0	9	100.0	100.0	100.0
		Tetragonisca	0	3	1	11	93.3	100.0	91.7

FN: False negative, TP: True positive, FP: False positive, TN: True negative, ACC: Accuracy, SEN: Sensitivity, SPE: Specificity.

Figure 7 summarizes the results of the LDA and PLS-DA models for the calibration and prediction set samples.

Figure 7. LDA and PLS-DA models for classification of *Tetragonisca*, *Melipona* and *Scaptotrigona* honey.

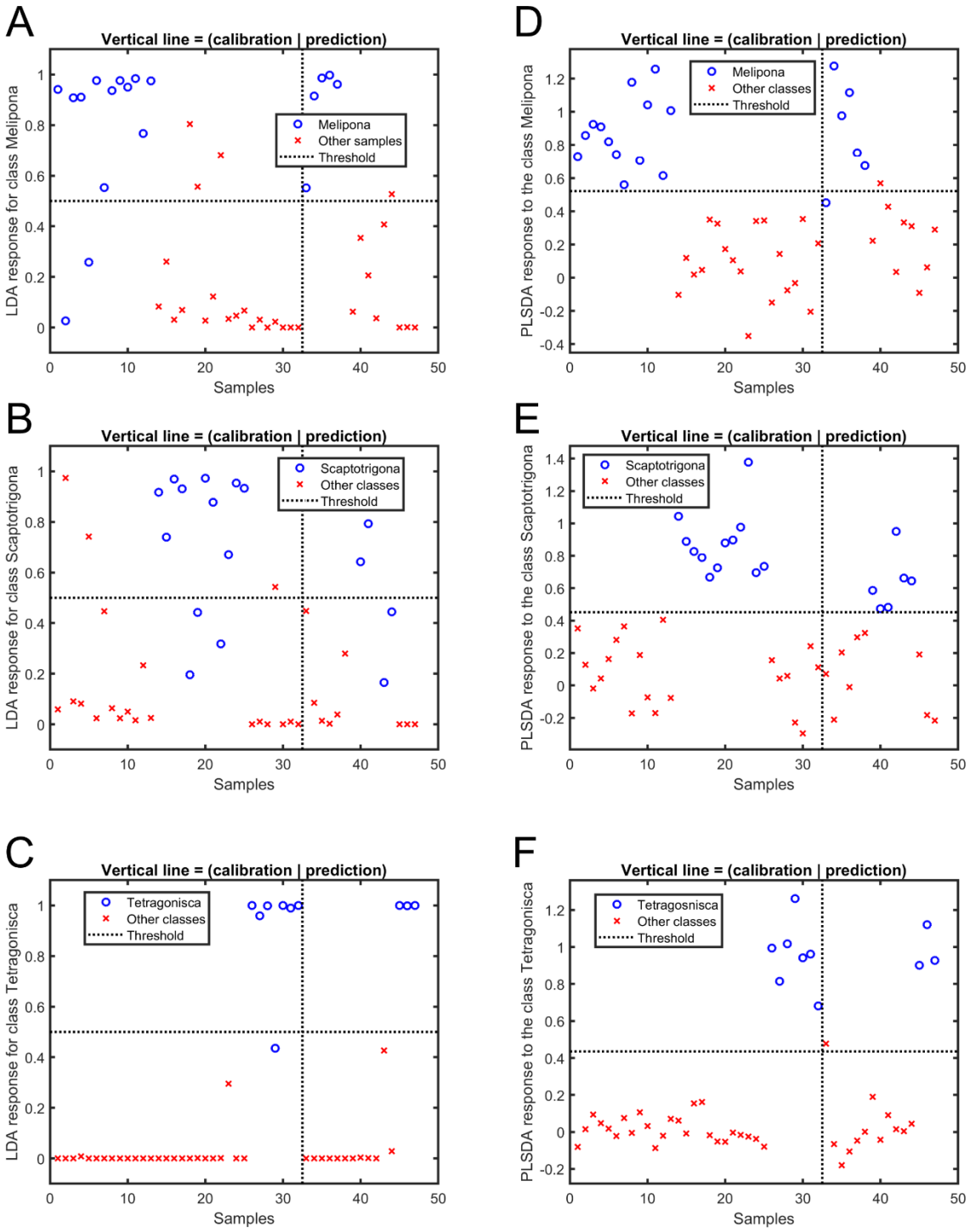
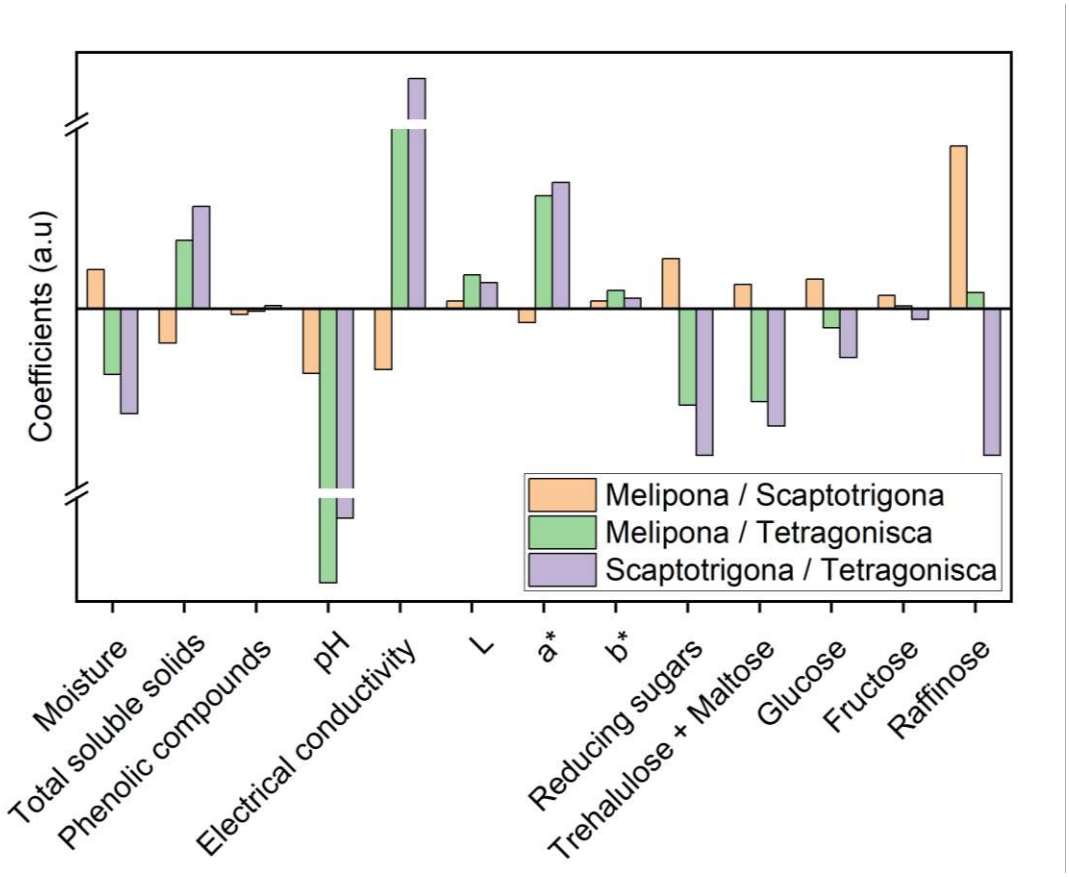


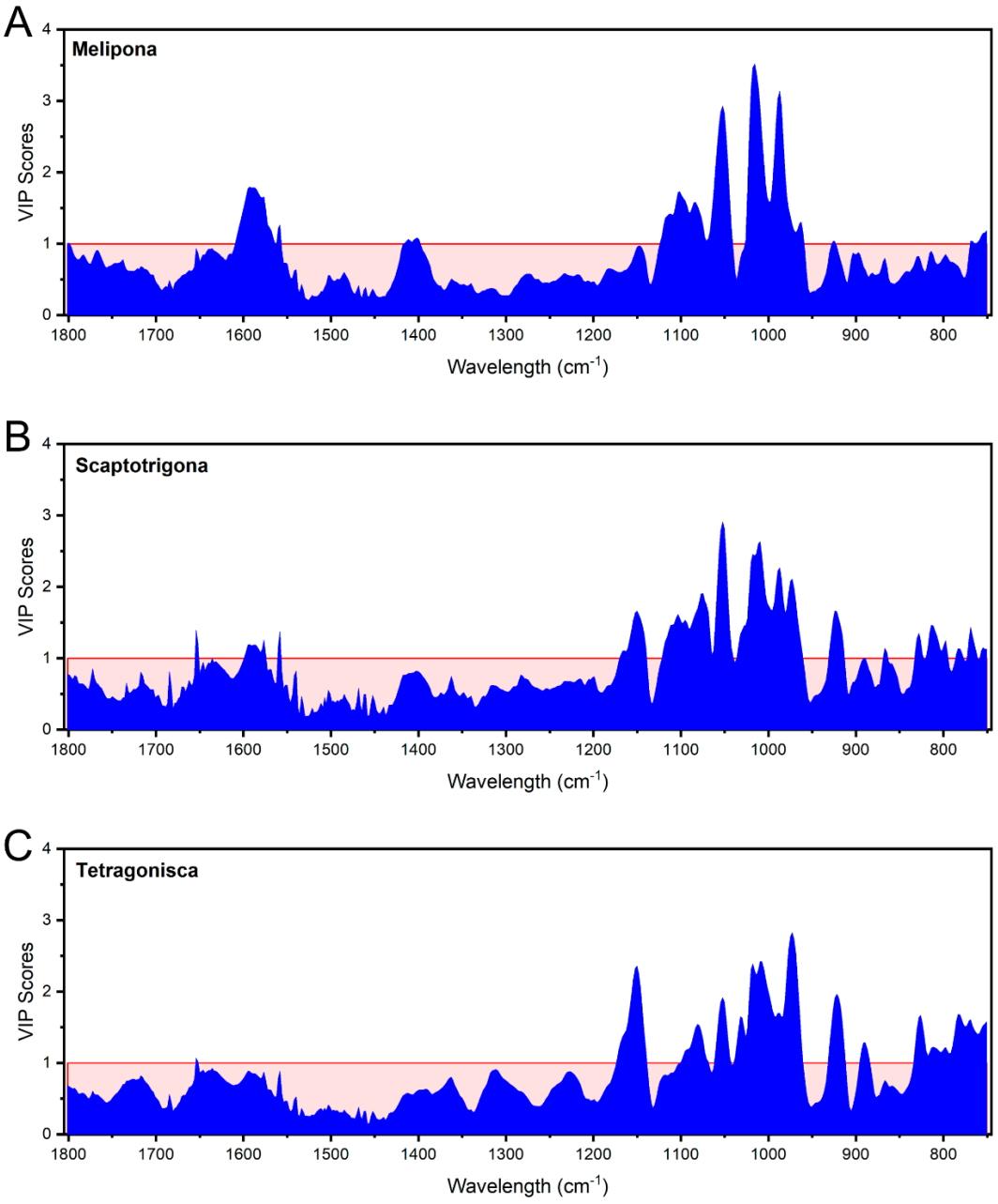
Figure 7A shows the LDA model for classifying *Melipona* honey, Figure 7B shows the LDA model for classifying *Scaptotrigona* honey, and Figure 7C shows the LDA model for classifying *Tetragonisca* honey. For FTIR-ATR data, Figure 7D shows the PLS-DA model for classifying *Melipona* honey, Figure 7E shows the PLS-DA model for classifying *Scaptotrigona* honey, and Figure 7F shows the PLS-DA model for classifying *Tetragonisca* honey. The dotted horizontal line represents the classification threshold between classes obtained according to Bayes' theorem, while the vertical line divides the samples between training and test sets obtained according to Kennard & Stone algorithm. Figure 8 shows the important parameters for distinguishing the groups based on the values of the LDA coefficients.

Figure 8. Parameters that were most important in distinguishing *Tetragonisca*, *Melipona* and *Scaptotrigona* honey based on the values of LDA coefficients using quantitative data.



The separation between the *Melipona* and *Scaptotrigona* classes was primarily influenced by the content of raffinose. The distinction between *Melipona* and *Tetragonisca* was defined by a combination of factors, including pH, electrical conductivity, reducing sugars, a*, and the content of maltose + trehalulose. Finally, the differentiation between *Scaptotrigona* and *Tetragonisca* samples was more influenced by the parameters of pH, electrical conductivity, a*, reducing sugars, maltose + trehalulose, and raffinose. Figure 9 presents the VIP score indicators for the bands that contributed the most to classify the samples by the PLS-DA model. VIP values greater than 1 were considered important (Bana et al., 2024).

Figure 9. Important variables responsible for the classification of (A) *Melipona*, (B) *Scaptotrigona* and (C) *Tetragonisca* honey by the PLS-DA model using FTIR-ATR data. The variables that contribute significantly to the classification of the classes of honey are those that are above the threshold line (VIP score > 1).



For the *Melipona* class, the regions that contributed the most to the separation were between 1610 - 1557 cm^{-1} and 1126 - 960 cm^{-1}. For the *Scaptotrigona* and *Tetragonisca* classes, the region that contributed the most to the separation was 1170 -915 cm^{-1}.

The findings of the multivariate analysis corroborate with other research that indicates that variations in the composition of SBH are predominantly influenced by bee genera (Ávila et al., 2019b; Mduda et al., 2023; Vit et al., 2023, 1998). Vit et al. (2023) used exploratory analyses with PCA and HCA (hierarchical cluster analysis) to group honeys from the genera *Geotrigona*, *Scaptotrigona*, and *Melipona* from Ecuador. The multivariate analysis of sugars and organic acids was performed independently, and *Geotrigona* samples were successfully separated from *Scaptotrigona-Melipona*. Similarly to our study, *Scaptotrigona* and *Melipona* samples could not be adequately distinguished by an exploratory method. Vit et al. (1998) reported that despite the honey of the genus *Scaptotrigona* belonging to the same group as Trigonoformes, the results of the multivariate analysis indicated that this genus was more similar to the *Melipona* samples. A high content of what was believed to be only maltose had been proposed as a separator of *Melipona-Scaptotrigona* honey samples from *Frieseomelitta-Nannotrigona-Tetragonisca* samples from Venezuela using HCA. Subsequently, an additional discriminant analysis (DA) was able to separate the samples into three groups: *Melipona*, *Scaptotrigona*, and *Frieseomelitta-Nannotrigona-Tetragonisca* (Vit et al., 1998).

In the study conducted by Ávila, Lazzarotto, et al. (2019), honey from the genera *Melipona* and *Scaptotrigona* from the state of Paraná-Brazil were distinguished through exploratory analysis (PCA and HCA) using physicochemical parameters and total mineral content. Cardona et al. (2019) distinguished samples of *Trigona nigra*, *Tetragonisca angustula*, *Scaptotrigona sp.*, *Nannotrigona sp.*, *Melipona compressipes*, *Melipona favosa*, and *Melipona fuscipes* from Colombia using multivariate analysis. In another

study, Umaña et al. (2021) analyzed honey samples from *Tetragonisca angustula* and *Melipona beecheii* from Costa Rica and found that the samples from these species were separated based on physicochemical parameters through PCA analysis.

The findings of this study suggest that the morphology of bees plays a crucial role in the characteristics of honey from different species. In addition to morphology, each species' distinct and specific behavioral patterns may justify the differences found. Although the constituents of honey mainly originate from floral sources, various species may seek out distinct plants to collect nectar, pollen, and plant resins. Furthermore, the bee's microbiota, enzymes, metabolites, and specific processes for transforming nectar into honey can also influence the observed variations (Mduda et al., 2023).

4. Conclusion

Our study demonstrated that entomological origin was the primary factor influencing the chemical profile of Brazilian SBH samples, as determined through a chemometric approach utilizing MA, LDA, and PLS-DA. We also observed that the separation of samples from the North and Northeast from samples from the South was primarily influenced by pollen types. The sugar profile was crucial for classifying the samples using supervised and unsupervised approaches. The physicochemical parameters of Brazilian SBH showed notable differences compared to the legislation for *Apis mellifera* honey; however, most samples follow the limits specified by state regulations. Our study indicates that FTIR-ATR spectra, physicochemical properties, sugar profile, and phenolic compound content, in combination with chemometric analysis, can be used to enhance the traceability and authenticity of this product. Further research is needed to clarify the entomological factors responsible for the distinct characteristics of honey produced by stingless bees.

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5.2. ARTIGO CIENTÍFICO 2[1]

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[1]Artigo a ser submetido na Revista Journal of the Science of Food and Agriculture

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Sugar detection and quantification in honeybee and stingless bee honey - A review

Abstract

Products derived from bees are renowned for their beneficial properties and nutritional value. This association has been substantiated by scientific investigations that delve into their composition and biological activities. Given the escalating interest in natural products and the prevalence of fraudulent practices, understanding the sugar profiles of honeys assumes paramount importance. In this review, we explore the sugars found in the honey of honeybee and stingless bee, sourced from both floral nectar and honeydew. Additionally, we discuss relevant sugar biomarkers. Our study comprehensively examines direct and indirect methods for sugar analysis, highlighting the advantages and disadvantages of each one. Direct and indirect methods for sugar analysis include liquid and gas chromatography, vibrational spectroscopy, and nuclear magnetic resonance. These techniques form the foundation for quality control of honey. Melezitose and trehalulose are identified as biomarkers of honeydew honey and stingless bee honey, respectively. In terms of liquid chromatography, mass spectrometry and pulsed amperometric detection are cited as the most suitable methods for quantifying sugars in honey; however, the most widely used detector is the refractive index. Alternative methods such as vibrational spectroscopy and nuclear magnetic resonance, especially the latter, have also proven effective in sugar quantification in honey.

Keywords: Honey, sugar analysis, sugars in honey, honey biomarkers, quality control.

1. Introduction

Since ancient times, honey has been consumed as a sweetener, in addition to being widely used in human nutrition and medicine by different cultures.[1–4] Honey is mainly composed of a sugar solution (which corresponds to approximately 95-99 g/100 g of honey's dry matter) and minor components such as vitamins, amino acids, phenolic compounds, organic acids, minerals, aromatic substances, pigments, pollen grains and enzymes.[1,5] To produce honey, bees collect and combine these substances with their own specific enzymes. Part of the honey produced is used to feed the bees themselves, and the rest is stored in combs or pots to supply the nest in periods of food shortage.[6]

Honeys can be classified based on their entomological origin (bee species) and food source (nectar or honeydew).[7] In terms of bee species, there are two types of beekeeping: apiculture and meliponiculture. Apiculture aims to breed *Apis mellifera* species from the Apini tribe, while meliponiculture refers to the breeding of stingless bee species from the Meliponini tribe.[8] The characteristics of honey are closely related to the bee species and food source, which are mainly influenced by seasonal, geographical and environmental factors.[9]

Research carried out, in 2022, by the Grand View Research, a market research company, revealed that the global honey market was valued at US$9.01 billion, with a projected annual growth (CAGR) of 5.3% in the period from 2023 to 2030. The increase in demand for natural and organic products, and the growing inclination towards healthy lifestyle habits have boosted the honey market.[10] Therefore, ways to ensure the quality and authenticity of honey have become the focus of several studies, with the aim of protecting consumers from consuming a product with inferior quality.[11]

Quality standards emphasize the importance of determining the product's origin to enable specific labeling, thereby necessitating the identification of biomarkers.[12] Sugar profiles

have been widely used to differentiate blossom honeys from honeydew honeys, due to the presence of higher levels of trisaccharides and lower levels of monosaccharides in honeydew honeys.[13] More recently, the presence of trehalulose, a previously unidentified disaccharide, has been identified as a biomarker in samples of stingless bee honey.[14] Furthermore, sugar profiling combined with chemometric tools has also been used to indicate botanical, geographical, and entomological origins.[15–18]

Determining the sugar profile of honey is also crucial for detecting fraud. Specifically, saccharides and mannose markers can reveal adulteration with sugar syrups.[13,19] Additionally, analyzing the sugar content helps elucidate honey's physical properties, such as hygroscopy, granulation, and viscosity. [12] Crystallization, influenced by the ratio of glucose to fructose, significantly impacts consumer preference. According to Grand View Research , consumers often prefer to physically inspect honey before purchasing, indicating that its physical characteristics play a key role in their buying decisions.[10]

Chromatographic methods have been used to detect and quantify sugars in honey. These methods include gas chromatography (GC), high-performance liquid chromatography (HPLC) and high-performance anion exchange chromatography (HPAEC). Different detectors can be coupled to the chromatograph: refractive index (RID), pulsed amperometric (PAD), mass spectrometer (MS), charged aerosol corona (C-CAD), evaporative light scattering (ELSD) and flame ionization (FID).[20] More recently, alternative methods have been used, such as Fourier transform infrared spectroscopy (FTIR), near-infrared spectroscopy (NIR), Raman spectroscopy (RAMAN) and nuclear magnetic resonance (NMR).[21–24]

Each method mentioned above has its own advantages and disadvantages regarding sensitivity, suitability, and cost-effectiveness. Therefore, the objective of this study was to review the sugar profiles of honeybee and stingless bee honey and present the direct

and indirect methods of sugar analysis, highlighting the pros and cons of each method. The findings in this review aim to elucidate a topic of national and international interest, assist the scientific community in selecting the most appropriate sugar analysis technique based on the characteristics of each method, and identify sugars that serve as quality biomarkers. This study utilized 128 articles published in the last decade from the IS Web of Science, Scopus, Google Scholar, and PubMed databases.

2. Honey classification

2.1. Honeybee honey

Honeybees refers to any of the members of the genus *Apis* (Tribe Apini, family Apidae, order Hymenoptera).[25] Honeybees are social and live in colonies with the presence of a queen, drones, and workers. The queen has a larger body than the workers and is the only one capable of reproduction. The drone has no sting, does not collect pollen, or defend the nest, its only function is to fertilize the queen. Worker bees are responsible for all the work in the hive, in the first phase of life, they are responsible for the internal cleaning of the hive, when they become older, they begin to dedicate themselves to defending the hive, collecting nectar, pollen, water, and resin.[25] There are several species of bees of the *Apis* genus, however, *Apis mellifera* honey is the most produced and traded worldwide. Honeybee honey can be classified according to its source: honeydew or flower nectar.[26–28] Various behaviors, some being unique to the *Apis* genus, have evolved and facilitated the transformation of nectar or honeydew into honey.[29]

According to FAO[30], China is the world's largest producer of honey from *Apis mellifera* and the largest exporter, while European Union countries occupy the second position. The main characteristics of *Apis mellifera* honeys refer to their moisture content, which is reduced to a final concentration of approximately 20 g/100 g. The high concentration of

sugars and moisture content lower than 20 g/100 g in *Apis mellifera* honeys makes the product microbiologically safe for commercialization at room temperature since pathogenic bacteria are not able to multiply in supersaturated sugar solution.[29]

Codex Alimentarius[31] has established identity and quality standards for *Apis mellifera* honey. These standards specify minimum or maximum limits for various parameters in honey, including moisture content, reducing sugar content, sucrose content, free acidity, diastase activity, hydroxymethylfurfural (HMF) content, electrical conductivity, and water-insoluble content. Honey should not exceed 20 g/100 g moisture content, must contain more than 60 g/100 g of reducing sugars, and should not exceed 5 g/100 g of sucrose. Additionally, it should have a free acidity of less than 50 milliequivalents acid/100g, diastase activity greater than 8 Schade units, HMF content lower than 40 mg per kg of honey, electrical conductivity less than 0.8 mS/cm, and water-insoluble content less than 0.1 g/100 g.

2.2. Stingless bee honey

Stingless bees, unlike honeybees, belong to the tribe Melipinini, within the same family Apidae. Stingless bees differ from honeybees both in habits and morphological aspects.[32] The main difference regarding morphological aspects is related to the presence of a stunted sting in stingless bees.[8,33,34] In addition, stingless bees have greater diversity than honeybees (body size, colony size and defense strategies). Stingless bees, unlike honeybees, build their cells in a horizontal position and store their food in pots instead of combs.[6,35] Regarding habits, stingless bees preferentially collect nectar from creeping flowers that are not far away.[36]

The honey from stingless bees differs from the honey from honeybee in terms of its physicochemical, microbiological and antioxidant properties. In general, honey from

stingless bees has a more fluid texture, slow crystallization and accentuated sensory characteristics of color, flavor and acidity.[37,38] This type of honey is historically known for providing benefits to human health, which are attributed to its antimicrobial, anti-inflammatory, antiseptic, anticancer and antioxidant properties.[39,40]

Despite the benefits of honey from stingless bees and the increasing consumer interest in this product, large-scale production faces several limitations. The primary constraint is the low yield, with stingless bees producing only 1-5 kg of honey per colony per year, compared to 20 kg per colony per year for *Apis mellifera*. This lower productivity is attributed to the smaller size of stingless bee colonies.[6,41,42] Furthermore, the lack of an international legislation regarding stingless bee honey compromises its global distribution. Therefore, competitiveness with honey from *Apis mellifera* has been harmed nationally and internationally.[6,38,43]

In general, stingless bee honey has lower sugar and total soluble solids and higher levels of water activity, moisture and acidity compared to *Apis mellifera* honey.[6,38,44] Moisture content and water activity are the most critical parameters in honey, as they are closely related to the final characteristics of the product, including flavor, texture, weight, and crystallization.[45] The higher moisture content and water activity of stingless bee honey can be related to several factors, such as the high humidity in tropical and subtropical regions and the preference of these bees for creeping flowers and ripe fruits. In stingless bee honeys, the moisture content can vary from 23 to 56 g/100 g, while legislation states that honey from *Apis mellifera* bees must contain no more than 20 g/100 g moisture.[6] In addition, high moisture contents and water activity values above 0.6 allow the proliferation of osmophilic yeasts and the occurrence of fermentation processes due to the survival of some microorganisms, such as lactic acid bacteria and acetic acid bacteria, which justifies higher acidity levels in stingless honey.[8,46]

2.3. Blossom honey

Blossom honey is derived from flower nectar and is the most common and commercialized type worldwide.[47] Monofloral honey corresponds to the product obtained from the nectar of flowers from the same family, genus, or species, while multifloral honey comes from various botanical sources.[4848] Monofloral honey possesses unique organoleptic qualities, such as specific fragrances and flavors derived from a single type of flower, making it a higher-value product. However, in the current market, multifloral honey and blended honey are more commonly available. These consist of mixtures of two or more types of honey with varying characteristics.[11]

The characteristics of monofloral honey are highly dependent on the type of vegetation and seasonal changes.[4] Plant diversity is generally proportional to the size of a country, meaning larger countries typically have a greater variety of monofloral honeys. While honey is a globally traded product, honey from certain types of flowers is often unique to the country where those flowers are found.[49]

2.4. Honeydew honey

In occasional periods of nectar shortage, honeybees and stingless bees collect honeydew to produce honey, which is known as honeydew honey or forest honey.[50] Honeydew is obtained from the secretion produced by some types of plants or by the excretion of insects, usually of the order Hemiptera.[51] These insects suck sap from the phloem of coniferous trees such as pine, spruce, oak and fir to produce honeydew.[5,51,52] Honeydew honeys are widely consumed and appreciated by Europeans due to their strong and striking flavor.[53,54] This type of honey typically has a darker color, higher viscosity and

less sweetness compared to blossom honey. Furthermore, honeydew honeys have a smaller market share and a higher sales price.[55]

Blossom honey and honeydew honey can differ greatly in terms of chemical composition.[26] The composition of honeydew honey can vary depending on the species of insects, their host plants, and the interactions between them. Seasonal changes also contribute to variations in the chemical and physical composition of honeys.[52] Honeydew honey commonly has a higher nitrogen concentration, higher electrical conductivity and pH, lower glucose and fructose concentrations and higher concentrations of trisaccharides.[13,56]

Some physicochemical parameters can indicate whether honey is derived from honeydew or nectar flowers. Honeydew honey must contain a minimum content of 45 g/100 g reducing sugars and a minimum of 0.8 mS/cm electrical conductivity.[31,53] Often, electrical conductivity and sugar profile are used to differentiate blossom and honeydew honey.[13,57] Some studies have also verified the presence of greater bioactive compounds in honeydew honey, which is related to its antioxidant activity.[5]

3. Sugars in honey

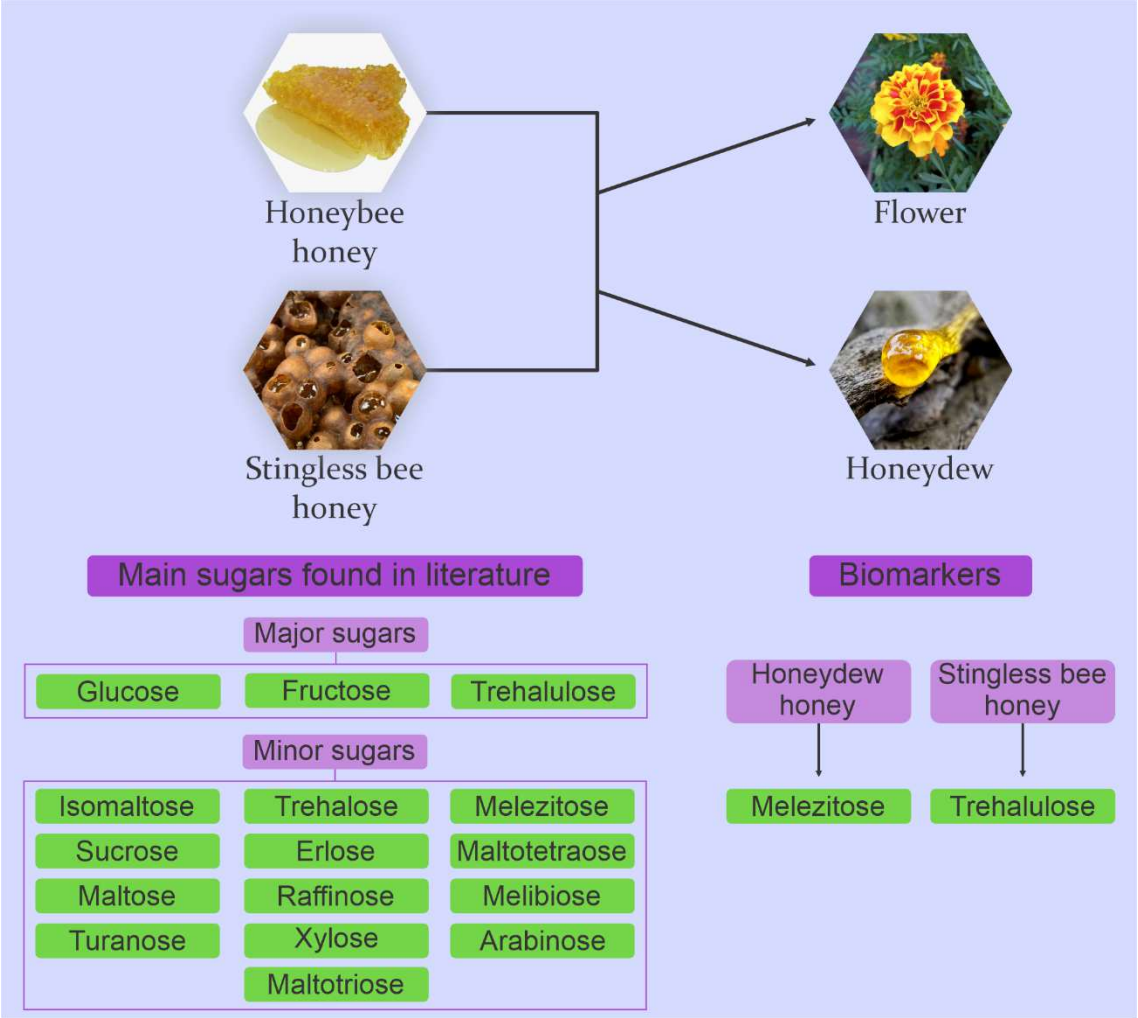
The sugars in honey are mainly formed by the action of enzymes secreted by the hypopharyngeal glands of bees on nectar or honeydew.[20,58] Honey production is only possible due to the temperature of the hive (30°C) and by inversion of sucrose.[58,59] The main enzymes are α- and β-amylase, α- and β-glucosidase, and β-fructosidase. Invertase (β-fructosidase) has transglucosylation activity, producing oligosaccharides from smaller sugars, as a result of its ability to transfer α-glucosyl residues to functional groups of other carbohydrates. Therefore, the disaccharides present in greater numbers in honey are α-glucosyl derived from monosaccharides. Other sugars present in honey can be formed by

microbial action and enzymatic reactions in the intestinal tract of insects that excrete honeydew.[58,59]

In honey, glucose and fructose are the main monosaccharides. The fructose/glucose ratio (F/G) and the glucose/water ratio (G/W) are parameters used to measure the ability of honey to crystallize. Typically, honey with low G/W and high F/G values do not crystallize easily.[60] Crystallization occurs because glucose has low solubility in water (909 g/L at 25°C), whereas fructose is significantly more soluble, being 4.4 times as soluble (4000 g/L at 25°C).[61] Over time, glucose tends to precipitate in the form of glucose monohydrate, and the solution changes to a more stable saturated state.[60]

Sucrose is also an important sugar found in honey, and high concentrations of this sugar usually indicate incomplete honey maturation or adulteration. For blossom and honeydew honeys, the maximum allowable sucrose concentration is 5 g/100 g of honey.[31,53] In addition to glucose, fructose and sucrose, more than 20 types of sugars, including di-, tri- and other oligosaccharides, have been identified in honeys, including maltose, turanose, maltulose, isomaltose, trehalose, erlose, raffinose, xylose, and rhamnose (Figure 1).[58,59,62]

Figure 1. Sugars reported in the literature for honeybee and stingless bee honey sourced from nectar flowers and honeydew.



The complete description of the results found in the literature, with the concentrations of sugars analyzed by chromatographic techniques, can be found in the supplementary material (Tables S1, S2 and S3). It can be observed that glucose, fructose, and maltose are the most analyzed sugars (Tables 1 and 2), and fructose content is higher than that of glucose for both honeybee honey and stingless bee honey (Tables S1 and S2). In honeybee honey, maltose is reported in low concentrations, with most honeys containing less than 2% of this sugar, which is considered a minor component for this type of honey.[3,21,22,27,50,63–66]

Table 1. Chromatographic techniques for sugar analysis in honeybee honey.

Geographical origin	Source	Botanical origin	Sugar	Equipment	Column	Detector	Operation conditions	Reference
Saudi Arabia	Nectar	*Ziziphus spina-christi, Acacia asak, Acacia tortilis, Acacia hamulosa, Lavandula dentata, Herbs and Hypoestes forskaolii, Herbs, Eucalyptus spp.*	Glucose, fructose, sucrose, and maltose	Agilent 1260 Infinity II LC System (Agilent Technologies)	ZORBAX Carbohydrate (Agilent Technologies)	HPLC-RI	Isocratic mobile phase Acetonitrile: water (75:25, v/v), flow rate: 1.0 mL/min, sample volume: 10 µL, column and detector temperature: 35 ºC	63
Ethiopia	Nectar	Acacia, *Becium grandiflorum, Croton macrostachyus, Eucalyptus globulus, Hypoestes, Leucas abyssinica, Schefflera abyssinica, Syzygium guineense*	Glucose, fructose, sucrose, maltose, turanose and isomaltose		ZORBAX Carbohydrate (Agilent Technologies)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (75:25, v/v); flow rate: 1.5 mL/min, sample volume: 20 µL, column and detector temperature: 30 °C	64
India	Nectar	Saffron, apple, cherry, *Plectranthus rugosus*	Glucose, fructose, sucrose, maltose, xylose, isomaltose, trehalose, raffinose, maltotriose, melezitose and maltotetraose	Waters isocratic (Waters™)	Waters X-bridge Amide (Waters™)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 0.6 mL/min, sample volume: 20 µL, column temperature: 85 °C	65
Poland and Slovaki	Nectar	Multifloral, tilia, forest, rape, acacia	Glucose, fructose, and sucrose	Dionex UltiMate 3000 (Thermo Fisher Scientific)	Asahipak NH2P-50 4E (Shodex™)	HPLC-CD	Isocratic mobile phase: Acetonitrile: water (78:22, v/v); flow rate: 1 mL/min, sample volume: 10 µL, column temperature: 55 °C	67
Romania	Nectar	Black locust, linden, rape, sunflower, multifloral	Glucose, fructose, sucrose, and maltose	Dionex ICS-3000 SP (Thermo Fisher Scientific)	CarboPac PA1 (Thermo Fisher Scientific)	HPAEC-PAD	Gradient mobile phase: ultra-pure water, 200 mM NaOH and 700 mM NaOH, flow rate: 1 mL/min, sample volume: 10 µL	3
India	Nectar	*Gossypium hirsutum, Coriandrum sativum, Dalbergia sissoo, Murraya koenigii*	Glucose, fructose, sucrose, maltose, turanose, isomaltose, trehalose, raffinose, maltotriose, melezitose, maltotetraose, melibiose, xylose and erlose	Agilent 1120 Compact LC (Agilent Technologies)	Hi-plex pb (Agilent Technologies)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 0.6 mL/min	66
Serbia	Nectar	Meadow, acacia, *Tilia tomentosa*, multifloral	Glucose, fructose, and sucrose	Dionex UltiMate 3000 (Thermo Fisher Scientific)	Hypersil GOLD™ Amino (Thermo Fisher Scientific)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 1 mL/min, sample volume: 5 µL, detector temperature: 35 ºC	60
Portugal	Nectar	Commercial honey - multifloral (year 2010), commercial honey - multifloral (year 2011), carob, eucalyptus, sunflower, orange, multifloral, arbutus, rosemary, rosemary + heather, thyme, thyme + rosemary, heather	Glucose, fructose, sucrose, maltose, turanose, melezitose and trehalose	Dionex ICS-3000 (Thermo Fisher Scientific)	CarboPacPA20 (Thermo Fisher Scientific)	HPAEC-PAD	Gradient mobile phase: 50 mM NaOH and 200 mM NaOH, flow rate: 0.5 mL/min, sample volume: 10 µL	21
Romania	Nectar	*Robinia pseudoacacia, Brassica napus oleifera*, polyfloral, *Tilia platyphyllos, Helianthus annus,* mixed, commercial honey	Glucose, fructose, sucrose, and maltose	Surveyor Plus (Thermo Fisher Scientific)	Hypersil™ APS-2 Amino (Thermo Fisher Scientific)	HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (85:15, v/v); flow rate: 0.8 mL/min, sample volume: 5 µL, column temperature: 30ºC, and detector temperature: 95 °C	22
Croatia	Honeydew	*Q. frainetto Ten* (Italian oak)	Glucose, fructose, sucrose, maltose, isomaltose, melezitose, raffinose and trehalose		Zorbax NH2 (Agilent Technologies)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (70:30, v/v); flow rate: 1.0 mL/min, sample volume: 10 µL	50

Romania and Poland	Honeydew		Glucose, fructose, and sucrose	Merck-Hitachi HPLC system (Merck)	Luna NH2 (Phenomenex)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 1.0 mL/min	54
Spain	Honeydew	Oak, evergreen oak	Glucose, fructose, sucrose, maltose, turanose, melezitose, and trehalose	Dionex ICS-3000 (Thermo Fisher Scientific)	CarboPac PA1 (Thermo Fisher Scientific)	HPAEC-PAD	Gradient mobile phase: ultrapure water and 200 mM NaOH	27
Romania	Honeydew		Glucose, fructose, sucrose, and maltose	Surveyor Plus system (Thermo Fischer Scientific)	Hypersil™ APS-2 Amino (Thermo Fischer Scientific)	HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (85:15, v/v); flow rate: 0.8 mL/min, sample volume: 5 µL, column temperature: 30ºC, and detector temperature: 95 °C	22
Spain	Honeydew		Glucose, fructose, sucrose, maltose, trehalose, turanose, isomaltose, and melezitose	Waters 2690 (Waters™)	Waters Carbohydrate Analysis (Waters™)	HPLC-DRI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 2.0 mL/min, sample volume: 25 µL, column temperature: 25ºC	57
Romania	Honeydew		Glucose, fructose, sucrose, maltose, turanose, trehalose, melezitose, erlose, and raffinose	Shimadzu HPLC system (Shimadzu)	Analytical column containing amino-modified silica gel	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (85:15, v/v); flow rate: 1.3 mL/min	68
Romania	Honeydew		Glucose, fructose, sucrose, maltose, melezitose, and trehalose	Dionex ICS-3000 (Thermo Fisher Scientific)	CarboPac PA1 (Thermo Fisher Scientific)	HPAEC-PAD	Gradient mobile phase: ultra-pure water, 200 mM NaOH and 700 mM NaOH, flow rate: 1 mL/min, sample volume: 10 µL	3

HPLC: high-performance liquid chromatography, HPAEC: high-performance anion exchange chromatography, RID: refractive index, PAD: pulsed amperometric, ELSD: evaporative light scattering, CD: corona discharge.

Table 2. Chromatographic techniques for sugar analysis in stingless bee honey.

Geographical origin	Bee species	Source	Botanical origin	Sugar	Equipment	Column	Detector	Operation conditions	Reference
Brazil	*Melipona subnida duke, Melipona subnida latrelle*	Nectar	*Ziziphus joazeiro Mart., Mimosa quadrivalvis L., Mimosa arenosa willd Poir, Croton heliotropiifolius Kunth*	Glucose, fructose, sucrose, maltose, and arabinose	Hewlett Packard 1100 (Hewlett Packard)	CARBOsep CHO 682 column (Transgenomics)	HPLC-RI	Isocratic mobile phase: Distilled water, flow rate: 0.4 mL/min, column temperature: 80ºC, detector temperature: 50 ºC	45
Thailand	*Homotrigona fimbriata, Lepidotrigona terminata, Lepidotrigona flavibasis, Lepidotrigona doipaensis, Lisotrigona furva, Tetragonilla collina, Tetragonula fuscobalteata, Tetragonula laeviceps-pagdeni complex, Tetragonula testaceitarsis, Tetrigona apicalis, Tetrigona melanoleuca*			Glucose, fructose, sucrose, and maltose	Shimadzu HPLC system (Shimadzu)	Inertsil NH2 (GL Science)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (75:25, v/v), flow rate: 1 mL/min, oven temperature: 40 ºC	69
Malaysia	*Heterotrigona itama sp*			Glucose, fructose, and sucrose	Agilent 1100 (Agilent Technologies)	Luna NH2 (Phenomenex)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (75:25, v/v); flow rate: 1.2 mL/min, sample volume: 20 µL, column temperature: 30 °C	70

Malaysia	*Heterotrigona itama, Geniotrigona thoracica*	Nectar	*Meleleuca cajaputi, Acacia mangium, Averrhoa carambola L*	Glucose, fructose, sucrose, and maltose		Luna NH2 (Phenomenex)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 1 mL/min, sample volume: 20 µL, column temperature: 40 °C	7
Ecuador	*Scaptotrigona polysticta, Melipona indecisa, Melipona cramptoni, Paratrigona sp., Melipona sp., Cephalotrigona sp., Melipona mimetica, Tetragonisca angustula, Trigona silvestriana, Oxytrigona mellaria, Nannotrigona chapadana, Melipona grandis*			Glucose, fructose, and sucrose	Agilent 1260 (Agilent Technologies)	Zorbax NH2 (Agilent Technologies)	HPLC-RI	Isocratic mobile phase: sulfuric acid (0.5 g/L); flow rate: 1 mL/55 min	71
Malaysia and Australia	*Heterotrigona itama, Geniotrigona thoracica, Tetragonula carbonaria, Tetragonula hockingsi*	Nectar	Multifloral	Glucose, fructose, sucrose, and trehalulose	ThermoFisher Interion Ion Chromatograph (ThermoFisher Scientific)	CarboPac PA210 (Thermo Fisher Scientific)	HPAEC-PAD	Isocratic mobile phase: 12 mM KOH; column temperature: 30 ºC	18
Malaysia	*Heterotrigona itama, Tetrigona binghami*	Nectar	*Acacia mangium*	Glucose, fructose, sucrose, and maltose	Agilent 1260 Infinity LC System (Agilent Technologies)	ZORBAX Carbohydate (Agilent Technologies)	HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (75:25, v/v); flow rate: 1.4 mL/min, oven temperature: 30 °C	72
Costa Rica	*Melipona beecheii, Tetragonisca angustula*			Glucose, fructose, sucrose, maltose, isomaltose, melezitose, raffinose, and trehalose	Shimadzu HPLC system (Shimadzu)	Econosphere amino (Grace/Alltech)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 1.3 mL/min, column temperature: 30 °C	73
Malaysia	*Heterotrigona itama*			Glucose, fructose, sucrose, maltose, and trehalulose	Waters e2695 (Waters™)		HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (88:12, v/v); flow rate: 1.0 mL/min, sample volume: 15 µL, detector temperature: 60 °C	74
Malaysia	*Heterotrigona itama*	Nectar	Mainly from *Acacia mangium* trees and other flowers	Glucose, fructose and sucrose		Prevail carbohydrate ES	HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (75:25, v/v); flow rate: 1.5 mL/min, sample volume: 20 µL.	75
Malaysia	*Geniotrigona thoracica Heterotrigona itama Heterotrigona erythrogastra*			Glucose, fructose and, maltose	Waters (WatersTM)	SupercosilTM LC-NH2	HPLC-RID	Isocratic mobile phase: acetonitrile: water (72:25), flow rate: 1 mL/min, oven temperature: 40 °C.	76
Tanzania	*Dactylurina schmidti Hypotrigona gribodoi Meliponula beccarii Meliponula ferruginea Meliponula togoensis Plebeina armata*			Glucose, fructose and sucrose	1260 Infinity II LC System (Agilent Technologies)	Carbohydrates column	HPLC-RID	Isocratic mobile phase: Acetonitrile: water (78:18, v/v); flow rate: 1.0 mL/min, sample volume: 10 µL, detector temperature: 35 °C, column temperature: 30 ºC.	77

HPLC: high-performance liquid chromatography, HPAEC: high-performance anion exchange chromatography, RID: refractive index, PAD: pulsed amperometric, ELSD: evaporative light scattering.

More recently, the disaccharide trehalulose was identified in stingless bee honey as a major sugar.[14,18,74,78] Trehalulose is an isomer of sucrose, formed by the monosaccharide's glucose and fructose joined by glycosidic linkages α-(1→1). This sugar is metabolized three times slower by enzymes in the small intestine compared to sucrose, which results in a more gradual release of glucose and fructose into the bloodstream, which can be beneficial for people with diabetes.[14] Research carried out by Hungerford et al.[79] showed that stingless bees fed with solutions of glucose and fructose do not produce the disaccharide trehalulose, suggesting that the formation of this sugar occurs through nectar rich in sucrose.

According to Fletcher et al.[14], trehalulose may be misidentified as maltose due to the similarity of chromatographic peaks between these two sugars; however, in the study of Ramlan et al.[74], both sugars: maltose and trehalulose, were detected in samples of stingless bee honey from Malaysia, although maltose appears as minor sugar. In the analyses conducted by Fletcher et al.[14], the authors used preparative HPLC to isolate trehalulose and performed UPLC–MS/MS and NMR analyses to elucidate the spectra corresponding to trehalulose. Five samples of honey were analyzed: *Tetragonula hockingsi* and *Tetragonula carbonaria* from Australia, *Geniotrigona thoracica* and *Heterotrigona itama* from Malaysia and *Tetragonisca angustula* from Brazil. The values found by the authors, using the UPLC–MS/MS technique, ranged from 13 to 44 g/100 g for each honey. *Geniotrigona thoracica* honey from Malaysia showed the highest proportion of trehalulose (84% of the total sugar content).

Zawawi et al.[18] quantified the sugars glucose, fructose, sucrose, and trehalulose in 36 honey samples from the species *Heterotrigona itama* and *Geniotrigona thoracica* from Malaysia, and *Tetragonula carbonaria* and *Tetragonula hockingsi* from Australia. The trehalulose content was measured as follows: *Heterotrigona itama*, 28.38 g/100 g,

Geniotrigona thoracica, 49.08 g/100 g, *Tetragonula carbonaria*, 23.18 g/100 g, and *Tetragonula hockingsi*, 24.90 g/100 g. High concentrations of trehalulose in stingless bee honey are also reported in other studies [74,78,80]. An inverse relationship with the content of fructose and glucose can be observed in honeys that have high levels of trehalulose; that is, the higher the trehalulose content, the lower the amount of glucose and fructose in the honey (Table S2).

In addition to maltose, sucrose can also co-elute with trehalulose, depending on the chromatographic method adopted. Prior to the discovery of trehalulose, high levels of maltose and sucrose, which could indeed be trehalulose, were reported in the literature for stingless bee honey. Examples include maltose values reported by Chuttong et al.[69] in samples from Thailand (*Tetragonula laeviceps*, 15 – 57 g/100 g), Shamsudin et al.[7] in samples from Malaysia (*Heterotrigona*, 22.56 - 37.35 g/100 g; *Geniotrigona*, 28.96 - 43.15 g/100 g), Vit et al.[81] in samples from Venezuela (*Frieseomelitta aff. varia*, 56.9 g/100 g), and sucrose values reported by Marcolim[82] in samples from Brazil (*Tetragonisca angustula*, 15.5 - 25.4 g/100 g). Other studies also report high values for sucrose or maltose in stingless bee honeys (Table S2). [44,72,75,76,83] However, despite Fletcher et al.[14] stating that trehalulose is a biomarker of stingless bee honey, relatively few samples have been analyzed for this sugar so far, indicating the need to analyze a larger number of honeys from different species and locations to determine whether trehalulose can indeed be considered a biomarker of stingless bee honey.[80]

Until now, few studies have quantified the sugars in honeydew honey, with these studies focused on a few origins and countries (Tables 1 and 2).[20] Some studies have shown that honeydew honey has a lower content of monosaccharides and a higher content of disaccharides and trisaccharides, such as melezitose, maltotriose and raffinose, compared to blossom honeys, which could be a good indicator of the type of honey.[13] According to

the research of Pascual-Maté et al.[17], honeydew honey samples showed higher disaccharide and trisaccharide contents (6.71 and 1.81 g/100 g, respectively) and lower monosaccharide contents (63.10 g/100 g) compared to heather honey.

Melezitose has been found mainly in honeydew, constituting approximately 70 g/100 g of the sugar content of honeydew excreted by insects and 40 g/100 g secreted by plants.[5,84] This sugar is formed by glycosidic linkages α-(1 → 3) between glucose and fructose and β-(2 → 1) between fructose and glucose. The chemical structure of melezitose is similar to that of sucrose, and the main difference is in optical rotation and crystalline form.[84] In the case of honeybees, the consumption of honeydew has been linked to the cause of the disease called 'honeydew flow disease'. Due to its high molecular weight, melezitose is not properly metabolized in the honeybee gut, resulting in malnutrition and hive death.[84] Seeburger et al.[51] carried out feeding experiments and found that honeybees fed with melezitose had high mortality compared to the control group. In addition, honeybees that were fed with melezitose showed severe symptoms of the disease, such as abdomen tipping, swollen abdomen, and impaired movement.

In addition to distinguishing between floral honeys and honeydew, and between honeybee honey and stingless bee honey, sugar concentrations combined with chemometric tools have also been used to indicate floral, geographic, and entomological origins. Vit et al.[15] analyzed samples from the genera *Geotrigona*, *Scaptotrigona*, and *Melipona* from Venezuela and demonstrated, through hierarchical cluster analysis (HCA), that the samples clustered into *Geotrigona* and *Scaptotrigona-Melipona* groups based on the raffinose content. *Geotrigona* honeys showed raffinose levels of 5.03 g/100 g, *Scaptotrigona* 1.24 g/100 g, and *Melipona* 0.45 g/100 g.

In Zawawi et al.[18] study, the sugar profiles showed that Malaysian samples from *Heterotrigona itama* and *Geniotrigona thoracica* had higher trehalulose content and

lower glucose and fructose levels compared to Australian honeys from *Tetragonula carbonaria* and *Tetragonula hockingsi*. Specifically, *Heterotrigona itama* exhibited trehalulose levels of 28.38 g/100 g, glucose 13.37 g/100 g, and fructose 16.23 g/100 g; *Geniotrigona thoracica* showed trehalulose 49.08 g/100 g, glucose 2.90 g/100 g, and fructose 4.11 g/100 g. *Tetragonula carbonaria* had trehalulose levels of 23.18 g/100 g, glucose 12.10 g/100 g, and fructose 17.43 g/100 g, and *Tetragonula hockingsi* had trehalulose levels of 24.90 g/100 g, glucose 12.71 g/100 g, and fructose 16.58 g/100 g.

Pascual-Maté et al.[17] investigated the differences among honeydew honeys, heather honey, chestnut honey, and lavender honey in terms of sugar profiles. Lavender samples showed the highest sucrose levels (0.05–5.18 g/100 g), while isomaltose levels were higher in chestnut (1.34–1.74 g/100 g) and honeydew samples (1.17–2.49 g/100 g), and lower in lavender honeys (0.6–1.16 g/100 g). Lavender honeys also exhibited lower raffinose levels (0.01–0.05 g/100 g). Other authors attribute sugar profiles as important for distinguishing groups through multivariate analysis in terms of botanical, geographical, and entomological origins. For example, Karabagias et al.[16] differentiated Greek honeydew honeys from pine and fir based on geographical, Cotte et al.[85] classified honey samples from France based on botanical origin, Vit et al.[81] clustered samples of stingless bee honey from Venezuela based on entomological origin. Sousa et al.[45] differentiated samples of Brazilian stingless bee honeys based on botanical origin and Mduda et al.[77] separated samples of stingless bee honey from Tanzania based on entomological origin.

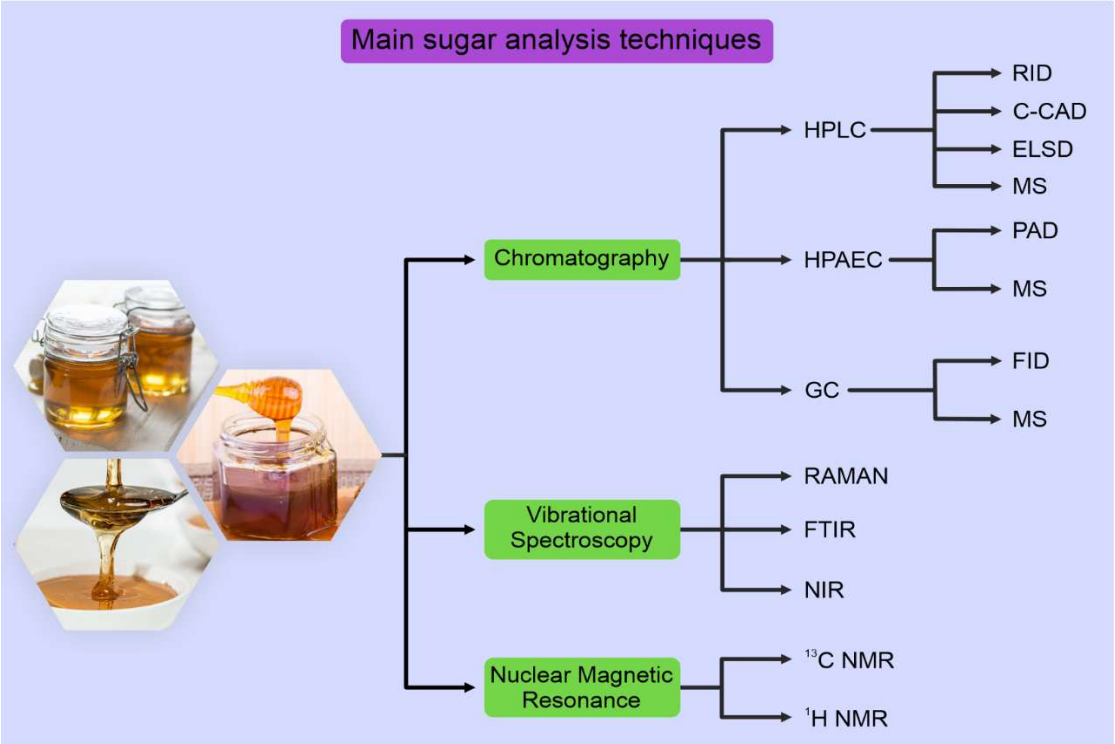
4. Sugar analysis

4.1. Chromatography techniques

Chromatography has been widely used to identify, separate, and quantify different sugars in honeys.[86] Considering the physical state of the mobile phase, chromatography can be divided into liquid (HPLC and HPAEC) and gas (GC), all of which employ the same basic principles (Figure 2). The fundamental principle is the separation of substances into two phases: a stationary phase and a mobile phase. Each analyte has a different interaction time between these two phases, which can be measured by the detector.[87]

The greatest difficulty in separating the sugars in honey samples is the structural similarity among them, which are formed mainly by different glycosidic bonds between glucose and fructose.[59] Different detectors coupled to chromatography can be used to analyze the sugars in honey, such as RID, PAD, MS, C-CAD and ELSD. For GC, FID can also be used.[17,20,67]

Figure 2. Main techniques for sugar analysis in honey.



4.1.1. High -performance liquid chromatography (HPLC)

HPLC is a versatile technique that allows the separation of a wide variety of compounds, including both ionic and non-ionic compounds. In HPLC, RID is the most commonly used detector for sugar analysis. Its principle is based on detecting variations in refractive indices between different analytes and the mobile phase. RID offers the advantage of universality, making it suitable for analyzing a wide variety of compounds, including sugars. Compared to other detectors, RID is more economical. However, it does have some drawbacks, such as the need for strict control of internal parameters, its lack of selectivity, and its incompatibility with gradient elution.[88–90]

Essentially, the mobile phase is chosen according to the column specifications. Sugar molecules have polar groups, and therefore, in terms of HPLC-RID, solvents with high polarity, such as acetonitrile, methanol, and water, are indicated. It has been proven that the use of a single solvent is not capable of promoting adequate separation of the sugars; because of this, a mobile phase consisting of a mixture of solvents is required.[91] For RID, a mixture of acetonitrile and water is the most commonly used mobile phase (Tables 1 and 2).[7,60,63–67,69–71] However, other mobile phases can be used, such as water acidified with sulfuric acid or other acids.[71]

Many columns are compatible for the separation of sugars in HPLC-RID. In recent studies (2016 – 2023), the following columns can be found: ZORBAX Carbohydrate (Agilent Technologies).[63,64], Waters X-bridge Amide (Waters Corporation)[65], Hi-plex pb (Agilent Technologies)[66], Hypersil GOLD Amino (Thermo Scientific)[60], Inertsil NH2 (GL Sciences)[69], Luna®NH2 100 Å (Phenomenex Inc.)[7,70], Zorbax NH2 (Agilent Technologies)[71] and Econosphere amino (Grace/Alltech).[73]

Ghramh et al.[63] and Belay et al.[64] used HPLC-RID and the Zorbax Carbohydrate column (Agilent Technologies) to quantify glucose, fructose, sucrose, and maltose in monofloral

honeys from Saudi Arabia and Ethiopia, respectively. The mobile phase used by the authors was a mixture of acetonitrile/water (75:25 v/v). In another study, Villacrés-Granda et al.[71] used a Zorbax NH2 column (Agilent Technologies) with sulfuric acid (0.5 g/ L) as the mobile phase to quantify glucose, fructose and sucrose in 26 samples of stingless bee honey from Ecuador.

ELSD and C-CAD detectors are recognized as universal detectors and are considered more suitable than RID due to their high detection sensitivity and compatibility with gradient elution, allowing for the use of multiple eluents. Additionally, these detectors maintain stable baselines during chromatographic runs. ELSD and C-CAD detectors can analyze a wide variety of compounds, making them regarded as comprehensive response instruments for HPLC.[86,92] The operation of ELSD and C-CAD is straightforward, requiring configuration of a few parameters such as pressure, gas input, signal output range, and temperature. The equipment operates in three main steps: effluent nebulization, mobile phase evaporation, and detection. In the ELSD detector, the aerosol generated during nebulization is transported to a heated region where the mobile phase vaporizes. The dried particles then enter a detection cell where they are exposed to a beam of light. The scattered light is detected by a photodiode or photomultiplier. In the case of C-CAD, the aerosol particles are directed to a corona discharge source and detected using a sensitive electrometer.[92,93]

The ELSD and C-CAD detectors are suitable for analyzing compounds with low volatility, such as sugars since the response generated does not depend on the chemical properties of the analyte. As a disadvantage, the analytes and the mobile phases need to be nonvolatile and volatile, respectively.[92,93] In theory, C-CAD was designed to improve the reproducibility, sensitivity and quantification of ELSD; however, a comparative study

carried out by Márquez-Sillero et al.[92] showed that the concentrations of glucose and fructose found in honey samples were similar using both detectors.

Wong et al.[72] used HPLC-ELSD to analyze fructose, glucose, maltose and sucrose from 96 monofloral (*Acacia mangium*) honey produced by *Heterotrigona itama* and *Tetrigona binghami* species. As the mobile phase, the authors used acetonitrile/water (75:25 v/v), and sugar separation was performed using a Zorbax Carbohydrate column (Agilent Technologies). HPLC-ELSD has been reported as one of the effective methods for separating maltose and trehalulose by Ramlan et al.[74] and Zaidi et al.[80] Zaidi et al.[80] used the mixture of acetonitrile and deionized water (85:15 v/v) and the Inertsil NH2 collum (GL Science). Tomczyk et al.[67] used HPLC coupled to a corona discharge detector to quantify glucose, fructose, and sucrose in honeys (n=60) from Poland and Slovakia. The column used was an Asahipak NH2P-504E (Shodex^{TM}), with a mixture of acetonitrile and water (78:22 v/v) as the mobile phase.

The MS detector is used to identify analytes of interest through their mass/charge (*m/z*) ratio. Molecules are ionized using an ionization source. Each ionized molecule forms a molecular ion, which is fragmented. Numerous ions are generated from a sample. These ions are accelerated in an electromagnetic field and detected based on their *m/z* ratio and relative abundance, resulting in a spectrum of the molecule.[94] Sugars are typically ionized by electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The MS detector offers the advantage of high sensitivity, selectivity, and specificity. It can analyze a wide range of compounds. However, executing the analysis and interpreting mass spectra require specialized knowledge, and a specific column for sugar analysis is also necessary.[80,94]

Zaidi (2023) analyzed glucose, fructose, sucrose, maltose, and trehalulose in samples from *Heterotrigona itama* and *Apis dorsata* from Malaysia. The authors used the same

HPLC-ELSD setup, replacing the detector with the Bruker Amazon SL ion trap mass spectrometer (ITMS) with electrospray ionization (ESI). According to the authors, despite HPLC-ELSD being effective in separating maltose and trehalulose, mass spectrum data is crucial to ensure correct identification of sugars, especially since the retention times of trehalulose, maltose, and sucrose are very close to each other.

4.1.2. High-performance anion-exchange chromatography (HPAEC)

Unlike conventional HPLC, HPAEC uses anion-exchange columns to separate ions based on their affinity for functional groups in the stationary phase of the column. The main advantages of this method are its sensitivity and specificity in separating different sugars.[89,95] The principle of amperometric detection involves applying a current to induce a chemical oxidation reaction based on the applied voltage. Sugars are detected by measuring the current produced during their oxidation on the surface of a gold electrode. The PAD detector is advantageous because it selectively detects compounds with oxidizable functional groups under the applied voltage.[95,96]

Most carbohydrates have pKa values in the range of 12 to 14. In the presence of an eluent with a high pH (>12), such as sodium hydroxide, the hydroxyl groups of the sugars are partially or completely transformed into oxyanions, which allows this class of compounds to be separated in a single chromatographic run.[97] Dionex Corporation (Thermo Fisher Scientific, Sunnyvale, USA) designed the CarboPac series of columns for the analysis of carbohydrates with anion exchange. These columns allow the separation of monosaccharides, oligosaccharides, and polysaccharides. Sodium hydroxide solutions are generally used as the mobile phase.[96,97]

Anjos et al.[21] used HPAEC-PAD to quantify glucose, fructose, sucrose, trehalose, melezitose, turanose and maltose in 63 honey samples from Portugal. The column chosen

was CarboPacPA20 (Dionex^{TM}), and the mobile phase adopted was a gradient of sodium hydroxide. Zawawi et al.[18] used HPLC-PAD to analyze 36 samples of stingless bee honey from Malaysia and Australia. The quantified sugars were glucose, fructose, sucrose and trehalulose. A CarboPacPA210 column (Dionex^{TM}) was used for sugar separation, and a gradient of potassium hydroxide was chosen as the mobile phase.

Vasić et al.[98] analyzed 64 honeydew honeys from Croatia, trehalose, arabinose, glucose, fructose, melibiose, isomaltose, sucrose, melezitose, turanose, raffinose, maltose, panose and maltotriose were quantified. As the mobile phase, the authors used three eluents, sodium hydroxide, sodium acetate and ultrapure water, with different gradients for the separation of the sugars, and a CarboPac PA10 column (Dionex^{TM}) was used.

4.1.3. Gas chromatography (GC)

The primary distinction between GC and traditional HPLC lies in the utilization of a gaseous mobile phase, such as nitrogen, hydrogen, and helium. For the separation of sugars in GC, detectors like the FID and MS are employed. The FID detector operates by measuring analytes within a gas stream. Organic compounds undergo combustion in a hydrogen flame, producing ions. These ions are proportionate to the organic compound content in the sample, increasing the flame's electrical conductivity. Consequently, the detector registers an electrical signal, reflecting the potential difference across two electrodes (positive and negative). Helium and nitrogen are commonly employed as carrier gases in this process.[99]

The main disadvantage of the GC method is the need for prior derivatization to obtain volatile carbohydrate compounds.[20] Although GC methods are quite selective, the effort and time to derive the sugar compounds make the analysis difficult. Therefore, few researchers choose to use this method in the analysis of sugar in honey.[89] Pascual-Maté

et al.[17] analyzed the sugar composition of 54 honeys from northern Iberian. The authors identified 14 sugars in the samples: fructose, glucose, sucrose, maltose, trehalose, isomaltose, gentiobiose, erlose, raffinose, maltotriose, melezitose, isomaltotriose, panose and maltotetraose. For analysis, samples were previously derivatized and analyzed by GC-FID using helium as the carrier gas.

Shamsudin et al.[100] used GC–MS to detect and quantify the metabolites present in monofloral honeys from stingless bees of the species *Heterotrigona Itama*, and samples of *Apis mellifera* honey were used as a positive control. Prior to analysis, the samples were subjected to derivatization. The authors used the HP 5973 mass selective detector (Agilent Technologies) and the DB-5MS 5% phenyl methyl siloxane column for sugar separation (Agilent Technologies). Helium was used as the carrier gas. Among the identified metabolites, glucose, fructose, and maltose were present.

In another study, Chessum et al.[101] analyzed turanose, erlose, palatinose and melezitose in honeydew honey samples from New Zealand. The samples were produced from the nectar excreted by the insect *Ultracoelostoma assimile*. This insect feeds on sap secreted by southern beech (*Nothofagus* spp.). The derivatized samples were analyzed in a GC coupled to the 5973 Network Mass Selective Detector with a quadrupole mass selective detector (Electron Ionization) (Agilent Technologies). The column used was the 122–5532 UI DB – 5MS UI (Agilent Technologies), and helium gas was used as the carrier gas.

4.2. Vibrational spectroscopy

Alternative analytical methods of analysis, such as vibrational spectroscopy, have been used to determine fraud in honeys, as well as to verify their chemical structure (Figure 2). Spectroscopy has the advantage of being a fast, nondestructive and easy-to-operate

method; in addition, the amount of sample necessary to perform the analysis is small, and sample preparation is minimal or null.[20,23] In general, electronic transitions are found in the visible or ultraviolet region, rotational transitions are found in the microwave region, and vibrational transitions are found in the infrared region. The infrared region is subdivided into near infrared (4000 – 12500 cm-1), medium (400 – 4000 cm-1) and far (10– 400 cm-1). The methods of vibrational spectroscopy most commonly used for honey sugar analysis are FTIR, NIR, and RAMAN.[22,102]

In a spectrum, signals are associated with functional groups such as -COOH and -OH; therefore, the spectrum corresponds to a simple sum of individual sample components, observed in specific bands, that are used to estimate the chemical structure of the molecule. In the past, samples were analyzed step by step, and with the arrival of Fourier transform (FT) spectroscopy, data collection started to be performed simultaneously in a wide spectral range.[102–104] To determine the sugar content in the sample, an initial calibration must be performed using known sugar standards at varying concentrations. The relationship between the absorption band intensities in the spectrum and the sugar concentrations in the sample is then analyzed using chemometric methods.[21] Examples of chemometric tools are principal component analysis (PCA), qualitative data analysis (QDA), partial least squares (PLS), principal components regression (PCR), linear discriminant analysis (LDA), soft independent modeling class analogy (SIMCA), artificial neural networks (ANNs) and data preprocessing methods such as normalization, spectral smoothing, noise removal and baseline correction.[105,106] Table 3 shows the complete results found in literature for vibrational spectroscopy.

Table 3. Vibrational spectroscopy techniques for sugar analysis in honey.

Geographical origin	Type of honey	Source	Botanical source	Sugar	Analytical technique	Chemometric tool	Reference
Portugal	Honeybee	Nectar	Commercial honey - multifloral (year 2010), commercial honey - multifloral (year 2011), carob, eucalyptus, sunflower, orange, multifloral, arbutus, rosemary, rosemary + heather, thyme, thyme + rosemary, heather	Sucrose, glucose, fructose, trehalose, turanose, maltose and melezitose	FTIR-ATR	PCA and PLS	21
Malaysia	Stingless bee *Heterotrigona itama*	Nectar		Glucose, fructose and maltose	FTIR-ATR	PCA and PLS	107
India	Honeybee	Nectar		Glucose, fructose, and sucrose	FTIR	PLS	108
Pennsylvania	Honeybee	Nectar		Sucrose, glucose, fructose, and maltose	FTIR-mATR	PCR and PLS	109
U.S.A, Thailand, China, Bhutan, U.K., Canada, Indonesia, Japan, New Zealand and Vietnam	Honeybee	Nectar		Maltose, sucrose, glucose, and fructose	FTIR–ATR	PCR and PLS	110
Turkey	Honeybee	Nectar		Fructose, glucose, sucrose, and maltose	Raman	PCA, PLS, ANN	105
Greece	Honeybee	Nectar		Fructose and glucose	FT-Raman	PLS	111
Italy	Honeybee	Nectar	Chestnut, citrus, and acacia	Fructose, glucose, maltose	Raman	PCA and PLS	112

Switzerland, German, Argentina, Chile, China, Cuba, France, Greece, Hungary, Italy, Mexico, Slovakia, Slovenia and Uruguay	Honeybee	Nectar and honeydew	*Castanea sp., Robinia sp., Tilia spp., Brassica spp., Taraxacum spp., Rhododendron sp., alpine polyfloral,* polyfloral*,* honeydew honey*, Robinia sp., Tilia spp., Taraxacum spp.*	Glucose, fructose, and sucrose	FT-NIR	PLS	113
Spain	Honeybee	Nectar		Fructose and glucose	NIR	MLR, PCR and MPLS	114

FTIR: fourier transform infrared spectroscopy, ATR: attenuated total reflectance, mATR: microattenuated total reflectance, NIR: near infrared spectroscopy, PCA: principal component analysis, PCR: principal components regression, PLS: partial least squares, ANN: artificial neural network, MLR: multiple linear regression, MPLS: modified partial least-square regression.

4.2.1. Fourier-transform infrared spectroscopy (FTIR)

The principle of infrared analysis is the application of infrared light with different wavelengths in the sample to cause rotational or vibrational changes in the molecules. Molecular changes can be classified into angular deformation (*bending*) and axial deformation (*stretching*). In this technique, the spectral acquisition region occurs in the range of 4000 cm-1 to 400 cm-1.[102] Attenuated total reflectance (ATR) combined with FTIR has been used to improve spectral collection power.[115] Anjos et al.[21] analyzed 63 honey samples from Portugal using FTIR-ATR, PCA and PLS. Calibration models of sugars (sucrose, glucose, fructose, trehalose, turanose, maltose and melezitose) with known concentrations were developed. According to the authors, the region of 1500-750 cm-1 corresponds to the most adequate range for sugar quantification and was the region chosen by the authors to develop the calibration models. For fructose, glucose, melezitose and turanose, good models were found.

Se et al.[107] analyzed maltose, glucose, and fructose in 62 honey samples from stingless bees of the species *Heterotrigona itama* from Malaysia using FTIR-ATR, PCA and PLS. HPLC was used as a reference technique. In another study, Rajalakshmi et al.[108] also quantified glucose, fructose, and sucrose in honey samples using FTIR and PLS. Tewari & Irudayaraj[109] used FTIR with microattenuated total reflectance sampling (FTIR-mATR) and chemometric tools (PCR and PLS) to quantify sucrose, glucose, fructose, and maltose in 45 samples of honey from different sources. The authors developed two calibration models that were validated and then verified by chromatographic measurements. Wang et al.[110] quantified maltose, sucrose, glucose, and fructose in 37 honey samples from different geographic origins using FTIR–ATR, PCR and PLS, and the calibration consisted of 45 standard mixtures composed of the four sugars. According

to the authors, the range 1500 and 800 cm−1 presented distinct peaks for all sugar mixtures used for calibration.

4.2.2. Raman spectroscopy (RAMAN)

Raman spectroscopy is based on the interaction of molecular vibrations with high-energy radiation emitted by a monochromatic beam of light. When the light beam interacts with a sample, it undergoes scattering, resulting in the Raman spectrum. This spectrum can be elastic, where the scattered light retains the same wavelength (Rayleigh scattering), or inelastic, where the scattered light has different wavelengths due to molecular vibrations (Raman scattering).[102,116,117] Özbalci et al.[105] quantified fructose, glucose, sucrose, and maltose in 22 samples of honey from Turkey using RAMAN. The authors used PCA, PLS and an artificial neural network (ANN) system to aid in sugar discrimination. The spectra were obtained in the range of 200-2000 cm^{-1}.

Batsoulis et al.[111] determined the concentrations of fructose and glucose in 21 monofloral honeys using FT-RAMAN. The quantitative determination was performed simultaneously in a spectral region in the range of 700-1700 cm^{-1}. The PLS chemometric tool was used for data analysis, and the values were compared to those obtained by HPLC. Both methods (HPLC and FT-RAMAN) showed similar results and scored equally in terms of reproducibility. In another study, Mignani et al.[112] used RAMAN spectroscopy with a dispersive detection scheme and excitation at 1064 nm to analyze fructose, glucose, maltose and the sum of other di- and trisaccharides in monofloral honeys from the Italian region of Calabria. The authors used HPLC as a comparative method and the PCA and PLS tools for predicting the sugar profile.

In the RAMAN technique, it is possible to analyze vibrations at very low wavelengths since the spectrum acquisition region occurs in the range of 4000 cm-1 to 50 cm-1[102];

however, according to Šugar and Bouř[103], when honey samples are irradiated with lower wavelengths, fluorescence can be considered a side effect, and nonsaccharide fluorescent components such as vitamins and aromatic amino acids can interfere with the correct interpretation of the sugar analysis. Based on this, the authors tested RAMAN optics activity (ROA) at a wavelength of 532 nm and used active carbon to prepurify the honey samples to reduce/eliminate the fluorescent components. This method was compared with RAMAN based on laser excitation at 1064 nm. The authors concluded that RAMAN provided greater accuracy than ROA. For this study, sucrose, D-fructose, D-glucose and D-maltose standards were used, and 12 honey samples were tested. Molnar et al.[118] proposed a simple method to analyze honey using RAMAN. According to the authors, a 1:1 w/v dilution of honey in distilled water produces reliable and reproducible spectra and overcomes the limitations of crystallization and fluorescence.

4.2.3. Near-infrared spectroscopy (NIR)

NIR spectroscopy uses the measurement of absorption of the chemical bonds of the molecules. In this technique, the spectral acquisition region is in the range of 12000 cm-1 to 4000 cm-1.[102] Relatively few studies have used NIR spectroscopy for sugar analysis in honey. Ruoff et al.[113] used FT-NIR to quantify glucose, fructose, and sucrose in 421 honeys of different botanical origins and countries. The chemometric tool PLS was used to develop the calibration models of sugars. In another study, NIR was used to analyze fructose and glucose in 161 honey samples collected over three subsequent years. Calibration models using MLR, PCR and MPLS were developed. For samples collected in the same year, good predictions were obtained.[114]

4.3. Nuclear magnetic resonance (NMR)

The principle of NMR involves the interaction of atomic nuclei, such as protons, with both static and oscillating magnetic fields. Initially, atomic nuclei align themselves with the static magnetic field. A radiofrequency pulse is then applied to disturb this alignment. When the pulse ends, the nuclei return to their equilibrium state, emitting detectable signals that provide insights into the molecular structure and chemical environment of the atoms. NMR spectra can offer information about various organic constituents in a sample, providing a comprehensive view of its chemical structure.[119]

The use of chemometric models for quantifying metabolites by NMR is often necessary due to signal intensity variations, signal overlap, and interferences from solvents or other substances present in the sample. In recent years, the use of NMR techniques combined with chemometric tools for the analysis of honey has aroused the interest of researchers, and NMR spectroscopy has been used to classify, characterize, and quantify the metabolites of a large number of honeys, including their main sugars.[119,120] The high efficiency of the ^{1}H NMR or ^{13}C NMR methods has been reported in several manuscripts (Table 4). The chemical shift range for ^{1}H NMR is between 0 and 12 ppm, while for ^{13}C NMR, it is between 0 and 220 ppm. Compared to traditional methods, NMR has the advantage of determining a wide range of analytes in a single run; in addition, NMR is a fast method.[16,121] As a drawback, NMR equipment has high acquisition and maintenance costs.

Table 4. Nuclear magnetic resonance techniques for sugar analysis in honey.

Geographical origin	Type of honey	Origin	Botanical source	Sugar	Analytical technique	Chemometric tool	Reference
Spain	Honeybee	Nectar		Glucose, fructose, maltose, sucrose and turanose	^{1}H NMR		120
Australia, Malasya and Brazil	Stingless bee: *Tetragonula hockingsi, Tetragonula carbonaria, Geniotrigona thoracica, Heterotrigona itama, Tetragonisca angustula*	Nectar		Trehalulose	^{1}H NMR		14
Argentina, Hungary, EC countries and Italy	Honeybee	Nectar	Acacia and polyfloral	Glucose, fructose, sucrose, turanose, raffinose,	^{1}H NMR and ^{13}C NMR	PCA and PLS-DA	122
Italy	Honeybee	*Nectar*	*Robinia pseudoacacia, Rhododendron ferrugineum,* polyfloral, *Castanea sativa,* and high mountain polyfloral	Fructose, glucose, sucrose, raffinose, kojibiose, isomaltose, maltose, gentiobiose, maltotriose, melibiose, maltulose, melezitose, palatinose, nigerose, kestose, erlose, turanose, isomaltotriose and maltotetraose	^{1}H NMR and ^{13}C NMR	PCA, OPLS-DA, PLS-DA	123

Italy, Hungary, South America, China	Honeybee	Nectar	Multifloral, *Rhododendron ferrugineum*, high mountain multifloral	Erlose, fructose, gentiobiose, glucose, isomaltose, isomaltotriose, kojibiose, kestose, maltose, maltotetraose, maltotriose, maltulose, melezitose, melibiose, nigerose, palatinose, raffinose, sucrose and turanose	^{1}H NMR and ^{13}C NMR	OPLS-DA, PLS-DA	124
Macedonia and Bulgaria	Honeybee	Nectar and honeydew	honeydew honey and polyfloral	Fructose, glucose, quinovose, sucrose, kojibiose, trehalose, trehalulose, isomaltose, nigerose, maltose, leucrose, maltulose, turanose, gentiobiose, isomaltulose, raffinose, melezitose, isokestose, panose and erlose	^{1}H NMR and ^{13}C NMR	PCA, PLS-DA, ANOVA	125
Italy	Honeybee	Nectar and honeydew	Multifloral, acacia, orange, citrus and honeydew honey	Sucrose, maltose, turanose, glucose, raffinose and isomaltose	^{1}H NMR	PCA, PLS-DA, OPLS-DA, ANOVA	126
Greece	Honeybee	Honeydew	Honeydew honeys produced from pine and fir	Fructose, glucose, sucrose, turanose and raffinose	^{1}H NMR	MANOVA, LDA	16
Greece	Honeybee	Nectar	Pine, chestnut, spruce, thyme, heather, citrus, and polyfloral	Glucose, fructose, maltose, isomaltose, nigerose, sucrose, turanose, maltulose, erlose, maltotriose,	^{1}H NMR and ^{13}C NMR		127

		isomaltotriose, panose, melezitose and 1-kestose			
Ecuador	Stingless bee: *Geotrigona*, *Scaptotrigona*, and *Melipona*	Fructose, glucose, sucrose, gentiobiose, maltose, maltotriose, mannose, melizitose, raffinose, and turanose	^{1}H NMR	PCA, HCA	15

NMR: Nuclear magnetic resonance, PCA: principal component analysis, PLS-DA: partial least-squares discriminant analysis, OPLS-DA: orthogonal projection to latent structures-discriminant analysis, ANOVA: analysis of variance, MANOVA: multivariate analysis of variance, LDA: linear discriminant analysis, SIMCA: soft independent modeling of class analogy, HCA: Hierarchical cluster analysis.

Del Campo et al.[120] used ^{1}H NMR to quantify 13 analytes in honey samples from Spain, including sugars, and the quantification was performed using external standards and considering the signal area of each analyte. According to the authors, the central region of sugars is in the range of 3.1 to 6.0 ppm. The strongest signals in this region correspond to glucose and fructose, which are superimposed with signals from malic and gluconic acids, acidic amino acids, and minor sugars (turanose, maltose, and sucrose). For the quantification of glucose, the authors selected two signals at 3.23 ppm and 5.21 ppm, and for fructose, the signals at 4.00 ppm and 4.10 ppm were assigned. For the minor sugars' maltose, sucrose and turanose, signals at 5.38, 5.42 and 5.28 ppm were assigned, respectively.

Fletcher et al.[14] analyzed the disaccharide trehalulose in five samples of stingless bee honeys from different species and countries using ^{1}H NMR. According to the authors, trehalulose exists in aqueous solution as a mixture of pyranose and furanose forms. Only the proton doublet of anomeric glucosyl (major fructopyranose conformator), present at 5.01 ppm and 3.7 Hz, could be clearly identified. The concentration of trehalulose varied according to the bee species and country of origin of the honey.

Consonni and Cagliani[122] analyzed 41 honey samples (*acacia* and multifloral) from different countries using ^{1}H NMR spectroscopy. Sugars were identified in the signal range between 3 and 5.5 ppm. Signals at 5.17 and 4.58 ppm were assigned to glucose (α and β forms, respectively), and signals at 4.04 and 3.97 ppm were assigned to α-fructose. Other minor sugars were identified by the addition of standard solution, with signals at 5.24 ppm for turanose, 5.35 ppm for sucrose and 5.37 and 4.94 ppm for raffinose. To evaluate the presence of different sugar isoforms, ^{13}C NMR spectroscopy was used. The signals showed dominance of the fructose and glucose present in different tautomeric isoforms. Fructose presented α,βFF and α,βFP forms, while glucose presented only α,βGP

forms. The same authors analyzed the sugar profile of 86 samples of Italian honey using ^{1}H NMR and ^{13}C NMR spectroscopy. The identified sugars were fructose, glucose, sucrose, raffinose, kojibiose, isomaltose, maltose, gentiobiose, maltotriose, melibiose, maltulose, melezitose, palatinose, nigerose, kestose, erlose, turanose, isomaltotriose and maltotetraose.[123]

Vit et al.[15] employed ^{1}H NMR spectroscopy to analyze the composition of stingless bee honey from Ecuador. The authors identified fructose, glucose, sucrose, gentiobiose, maltose, maltotriose, mannose, melizitose, raffinose, and turanose. In another study conducted by Consonni et al.[124], erlose, fructose, gentiobiose, glucose, isomaltose, isomaltotriose, kojibiose, kestose, maltose, maltotetraose, maltotriose, maltulose, melezitose, melibiose, nigerose, palatinose, raffinose, sucrose and turanose were identified through ^{1}H NMR and ^{13}C NMR spectroscopy. The authors analyzed 57 samples of honey from different floral origins and countries. For this study, the region between 4.4 and 5.7 ppm was assigned as the broadly informative range for identifying sugars, with the monosaccharide's glucose and fructose present in greater abundance.

Sixteen samples of honey from Macedonia and 22 from Bulgaria were analyzed by Gerginova et al.[125], the authors used ^{1}H NMR and ^{13}C NMR to evaluate honeydew honeys and blossom honeys. With the aid of chemometric tools, it was possible to notice that the honeydew honeys from North Macedonia had higher amounts of maltulose, kojibiose, trehalulose and nigerose, while the samples from Bulgaria had higher amounts of maltose and erlose. Melezitose was not identified in multifloral honeys and was found only in Bulgarian honeydew honeys. Quinovose was detected in some samples from North Macedonia, and this is the first report in the literature of this monosaccharide in honey. Other studies have also used ^{1}H and ^{13}C NMR to quantify sugars in honeys.[16,78,126–128]

5. Conclusion

The precise determination of honey origin is guided by quality standards, necessitating the identification of biomarkers for accurate labeling. Sugar profiles play a crucial role in distinguishing different types of honey, with honeydew honey typically exhibiting higher levels of trisaccharides, while stingless bee honey presents trehalulose as a novel biomarker. Sugar profiling, combined with chemometric tools, has also been used to trace the entomological, botanical, and geographical origins of both honeybee and stingless bee honeys. This study summarizes sugar profiles in both honeybee and stingless bee honeys, originating from nectar and honeydew sources, and evaluates direct and indirect methods of sugar analysis to assist researchers in selecting the most appropriate approach. Chromatographic techniques, such as GC, HPLC, or HPAEC, equipped with detectors like RID, PAD, MS, C-CAD, ELSD, and FID, offer advantages and disadvantages in terms of sensitivity, applicability, and cost-effectiveness. While HPAEC-PAD and mass detection are generally the most suitable methods for sugar separation, HPLC-RID remains the most used method. Notably, HPLC-ELSD has proven effective for separating maltose and trehalulose in stingless bee honeys. Recent advancements in spectroscopic methods, including FTIR, NIR, RAMAN, and NMR, provide alternative approaches for sugar quantification.

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5.3. ARTIGO CIENTÍFICO 3[1]

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[1]Artigo a ser submetido na Revista Food Science and Techonology Internacional

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Quality assessment and functional potential of honey with propolis extract from *Tetragonisca angustula*

Abstract

The propolis extract has been used to enhance the bioactive activity of various foods. However, the propolis extract from stingless bees has been relatively underutilized. Therefore, the objective of this study was to develop products based on honey and propolis from *Tetragonisca angustula* at concentrations of 0.25%, 0.5%, and 0.75%. These products were characterized in terms of their physicochemical, antioxidant, and sensory properties. Compared to honey without added propolis extract, no differences were observed in reducing sugars, sucrose, and hydroxymethylfurfural contents. There was a slight increase in total acidity and moisture content, along with a small reduction in pH and total soluble solids. The addition of propolis extract enhanced the antioxidant and reducing capacity of honey, with the increase being non-proportional and more pronounced in the compound containing 0.75% propolis extract. Phenolic compound content increased by 37%, ferric reducing power by 36%, and the antioxidant activity measured using the ABTS•+ radical scavenging by approximately threefold. In the qualitative descriptive analysis, attributes that best described the honey compounds with propolis extract included a fermented taste, sediment, dark spots, sour taste, sour aroma, propolis taste, and yellow color. Thus, it can be concluded that even at low concentrations, enriching honey with propolis extract can enhance its bioactive activity and provide an additional product option to increase meliponiculturists' profitability.

Keywords: Honey with propolis, Stingless bee honey, Propolis extract, Bioactive activity, *Tetragonisca angustula* honey.

1. Introduction

Honey, a natural food with added value, is widely consumed worldwide. Although *Apis mellifera* honey dominates the global market, interest in stingless bee honey has increased in recent years due to its beneficial health effects for humans. The stingless bee constitutes a diverse group found in tropical and subtropical countries, comprising over 500 species distributed globally (Ávila et al., 2018).

Propolis consists of a mixture of wax, plant resins, essential oils, balsam, and primary and secondary metabolites from plants, such as vitamins, amino acids, terpenoids, phenolic compounds, tannins, and alkaloids. The main function of propolis is to seal the beehive openings and protect the nest (Zulhendri et al., 2022). Propolis from stingless bees may contain clay or mud, which is why it is also called geopropolis (Georgieva et al., 2019; Salatino et al., 2019). The bee *Tetragonisca angustula* uses propolis without mixing it with clay or mud (Sanches et al., 2017).

Raw propolis, without prior treatment, cannot be directly added to products. First, it needs to undergo an extraction process to dissolve and release its bioactive compounds (Przybyłek & Karpiński, 2019). Propolis extract, in addition to its use in traditional and popular medicine, has also been marketed as a functional and complementary food. It can be found added to a wide variety of products, including sprays, lozenges, tinctures, ointments, and toothpaste. Furthermore, propolis extract can also be mixed with honey (Osés et al., 2015; Salatino et al., 2019). However, the propolis extract predominantly used by industries comes from the *Apis mellifera* bee, and relatively few products with added propolis extract from stingless bees are found in the market (Salatino et al., 2019). The combination of propolis extract with stingless bee honey can result in a product with enhanced antioxidant and reducing capacity. However, high concentrations of propolis extract have been associated with astringent and bitter flavors. Therefore, the acceptance

of the product depends on the concentration of added propolis extract, so as not to negatively affect the characteristics of the final product (Osés et al., 2016). Thus, the objective of this study was to develop compounds based on honey and propolis extract at concentrations of 0.25%, 0.5%, and 0.75%, using products from *Tetragonisca angustula*, and characterize them in terms of their physicochemical, antioxidant, and sensory properties.

2. Materials and methods

2.1. Samples of honey and propolis

For this research, honey and propolis from *Tetragonisca angustula* were used. These samples were collected in 2021 (23°11’04.9"S 51°12’07.6"W). The honey extraction was carried out in the food analysis laboratory at the State University of Londrina, Brazil. Honey pots were removed from the hives and then pressed and centrifuged (3000 g for 15 minutes) using an Eppendorf centrifuge 5804R (Germany). The propolis was collected directly by the producer under aseptic conditions. All utensils used in the extraction process were previously sterilized. After extraction, the honey was immediately pasteurized (63 °C/30 minutes) and kept refrigerated (4 °C) until the propolis extract was obtained.

2.2. Propolis extract

The propolis extract was prepared according to the method proposed by Surek et al. (2021), with modifications. Fifty grams of raw propolis were crushed and mixed with 500 mL of food-grade ethanol (95% purity). The mixture was periodically agitated for six days, followed by filtration through filter paper and subsequent centrifugation at 1800 g for 15 minutes using an Eppendorf centrifuge 5804R (Germany). After centrifugation,

the supernatant was collected and kept at -20 °C overnight to allow wax precipitation, followed by another centrifugation. The resulting supernatant from the second centrifugation was collected and rotary-evaporated at 40 °C to remove the ethanol (Tecnal - TE-210, Brazil). To complete ethanol evaporation, the concentrated propolis extract was placed in an oven at 30 °C for 24 hours.

2.3. Honey-propolis products

The preparation of honey-propolis compounds was carried out according to Osés et al. (2016), with modifications. The previously pasteurized honey was divided into three glass jars. The concentrated propolis extract was mixed with the honeys at concentrations of 0.25%, 0.5%, and 0.75%. The jars were homogenized by mechanical agitation. Next, a small amount of food-grade ethanol (95% purity) was added to each mixture at a final concentration of 0.4% to dissolve small granules of propolis extract that had settled on the surface of the honey and to suppress foam formation during agitation. The honey with propolis extract from each treatment was dispensed into seven glass jars with a capacity of 40 mL for physicochemical and antioxidant analyses. For sensory analysis, the samples were dispensed into glass jars with a capacity of 150 mL (two jars for each treatment). The jars were stored under refrigeration (4 °C) until analysis. The samples were labeled as follows: H1 = honey, H1EP-0.25 = honey with 0.25% propolis extract, H1EP-0.50 = honey with 0.50% propolis extract, H1EP-0.75 = honey with 0.75% propolis extract.

2.4. Physicochemical parameters

The total soluble solids (TSS) content and refractive index (RI) were determined using a digital refractometer (Metler Toledo - LiquiPhysics™ Excellence RM40, Switzerland) at a temperature of 20 °C. The moisture content was determined using a conversion table

based on the RI values (method 969.38, AOAC, 2012). For pH and total acidity analysis, 10 g of the sample were diluted in 75 mL of distilled water, and the pH of the solution was measured using a digital pH meter (Plox – P1000, Brazil). The acidity content was determined by the sum of lactonic and free acidity, following method 962.19, AOAC (2012). The hydroxymethylfurfural content was determined according to the method proposed by the International Honey Commission (Bogdanov, 2002). Water activity was measured directly using a digital meter (Aqualab – 4TEV, Brazil) at 20 °C. The reducing sugar content was determined using the complete reduction of cupric ions method (Lane & Eynon, 1923). The sucrose content was obtained after acid hydrolysis inversion of the solution used for reducing sugar analysis (Lane & Eynon, 1923).

2.5. Microbiological quality of raw honey

The microbiological quality of honey was assessed through analyses for *Salmonella spp.*, mold and yeast counts, total coliforms, and coagulase-positive S*taphylococcus aureus*. The analyses for *Salmonella spp*., mold and yeast counts, and coagulase-positive *Staphylococcus aureus* were conducted following the methods proposed by AOAC (2016). For the total coliform analysis, petrifilm plates (3M Company, St. Paul, MN, USA) were used.

2.6. Antioxidant and reducing capacity

2.6.1. Alcoholic extraction

Initially, 1.00 g of the sample was diluted in 80% ethanol (w/v) for the extraction of the compounds of interest.

2.6.2. Total phenolic compounds (TPC)

The total phenolic compounds were determined according to the methodology proposed by Singleton, Orthofer, & Lamuela-Raventós (1999), with adaptations from Bobo-García et al. (2015) for the microplate method. Aliquots of 20 µL of the sample, 100 µL of Folin–Ciocalteu reagent (1:4 v/v), and 75 µL of 10% sodium carbonate solution were transferred to the microplate, agitated for 60 seconds, and left in the dark at room temperature for 2 hours. The absorbance was measured using a microplate reader (Anthos Zenyth 200 RT, England) at a wavelength of 750 nm. For the blank, the sample was replaced by 80% ethanol (v/v). A gallic acid (GA) calibration curve (14–108 µg/mL) was constructed, with results expressed in mg GA per 100 g of sample.

2.6.3. ABTS•+ radical scavenging activity

The antioxidant activity of honey-propolis compounds was determined following the methodology proposed by Re et al. (1999). The free radical was prepared using a mixture of 5 mL of 7 mM ABTS•+ and 88 µL of 140 mM potassium persulfate. The mixture was left in the dark for 12-16 hours, and the absorbance was adjusted to approximately 0.70 using absolute ethanol (98%). For the analysis, aliquots of 10 µL of the sample and 200 µL of ABTS•+ radical were transferred to the microplate and mixed by agitation for 60 seconds. As a blank, 10 µL of the extraction solution (80% ethanol v/v) and 200 µL of absolute ethanol (98% v/v) were used. The microplate reading was performed at 734 nm (Anthos Zenyth 200 RT, England). A Trolox standard curve (50–600 µM/mL) was used for calibration, with results expressed in mM of Trolox per 100 g of sample (TEAC).

2.6.4. Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) was determined using the method proposed by Benzie and Strain (1996), with adaptations from Salvador et al. (2019) for

the microplate method. For the preparation of the FRAP reagent, 25 mL of 300 mM acetate buffer at pH 3.6, 2.5 mL of TPTZ solution (10 mM TPTZ diluted in 40 mM HCl), and 2.5 mL of 20 mM iron chloride were used. Aliquots of 20 µL of the sample, 30 µL of distilled water, and 200 µL of the FRAP reagent were transferred to the microplate, agitated for 60 seconds, and kept in the dark at room temperature for 30 minutes. As a blank, 80% ethanol (v/v) was used instead of the sample. The reading was performed using a microplate reader (Anthos Zenyth 200 RT, England) at 595 nm. A standard curve of ferrous sulfate (300–1500 µM/mL) was used for calibration, with results expressed in mM of Fe^{2+} per 100 g of sample.

2.7. Sensory analysis

2.7.1. Quantitative Descriptive Analysis (QDA)

The sensory evaluation was conducted with honey and honey-propolis products, following the Quantitative Descriptive Analysis (QDA) model. The project was approved by the Research Ethics Committee of the State University of Londrina, approval number 4948880 (CAAE: 50627521.2.0000.5231). For the test, the team previously trained for the research titled ‘Influence of freezing, pasteurization, and maturation on Tiúba honey quality’ (Ribeiro et al., 2018), approved under approval number 1124653 (CAAE: 46195515.9.0000.5231), was invited. Of the 13 judges considered trained, 10 accepted to participate in the new research. After signing the Informed Consent Form (ICF), the judges conducted a new term generation session for honey and honey-propolis products samples, where the team had the possibility to include or exclude any terms previously defined in the previous research.

In this session, the four samples were presented in pairs, and the judges indicated which terms would remain and which terms should be included regarding appearance, aroma,

flavor, and texture. No terms were excluded, and two new terms were added: “propolis taste” and “wildflower aroma” For these new terms, both quantitative and qualitative reference samples were suggested (Table 1).

Table 1. Terms descriptors and their definition and references.

Term Descriptor	Definition	Reference
Aroma		
Wildflower	Aroma derived from wildflowers	Weak: 5 ml orange blossom essence + 10 ml water Strong: 15 ml orange blossom essence
Flavor		
Propolis taste	Distinct sensation experienced when consuming products containing added propolis extract.	Weak: 5 ml propolis extract + 10 ml water Strong: 15 ml propolis extract

The remaining terms and their references can be found in Table 1 of the article by Ribeiro et al. (2018). After creating an evaluation sheet with an unstructured 9 cm scale anchored at 0.5 cm, a new training session was conducted with the judges regarding the new terms. Finally, the final session was held with the four samples and all the terms identified.

2.8. Statistical analysis

All analyses were conducted in triplicate. The data from the physicochemical and antioxidant analyses were treated using analysis of variance (ANOVA), and the difference between means was compared using the Tukey test at a significance level of 5%, employing Statistica 10.0 software (StatSoft, 2010). The sensory analysis data were processed using Principal Component Analysis (PCA) with the Past 4.10 software

(Oyvind Hammer, 2022), and the means of the evaluators were compared using the Duncan test at a significance level of 5% (StatSoft, 2010).

3. Results e discussion

3.1. Physicochemical parameters

The results of the physicochemical analyses for the honey sample and honey compounds with propolis are shown in Table 2.

Table 2. Physicochemical characterization of honey and honeys with propolis extract.

Parameters	H1	H1EP-0.25	H1EP-0.50	H1EP-0.75
pH	4.23±0.02a	4.08±0,01ab	4.03±0.01b	3.99±0.02c
Acidity (meq/kg)	90.33±0.99	96.16±0.01	96.96±1.78	101.11±1.14
TSS (ºBrix)	74.85±0.15a	74.23±0.02b	74.28±0.04b	74.20±0.04b
Moisture (%)	23.55±0.19a	24.20±0.00b	24.20±0.00b	24.25±0.10b
Water activity	0.71±0.00a	0.71±0.00a	0.71±0.00a	0.71±0.00a
Reducing sugar (%)	58.07±0.32a	58.08±0.45a	56.99±1.06a	56.83±0.75a
Sucrose (%)	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
HMF (mg/kg)	3.09±0.38a	3.23±0.39a	3.63±0.42a	3.42±0.48a

H1: Honey, H1EP-0.25: Honey with 0.25% propolis extract, H1EP-0.50: Honey with 0.50% propolis extract, H1EP-0.75: Honey with 0.75% propolis extract, SST: Total soluble solids, HMF: Hydroxymethylfurfural. Results are expressed as mean ± standard deviation (n=3). Means in the same row with the same lowercase letters do not differ statistically ($p<0.05$) by Tukey's test.

To date, few studies have evaluated the physicochemical parameters of honey with added propolis extract. Despite being a commercial product, there is no specific legislation in Brazil that defines its standard of identity and quality (PIQ) or the appropriate analytical methods for analyzing this type of product. For honey from the *Apis mellifera* bee, the current legislation is Instrução Normativa nº 11, de 20 de outubro de 2000, and for propolis and propolis extract, it is Instrução Normativa nº 3, de 19 de janeiro de 2001,

issued by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA). These products are marketed with authorization from the MAPA and the Serviço de Inspeção Federal (SIF) (Brazil, 2000; Brazil, 2001).

For stingless bee honey, there is no federal legislation that establishes identity and quality parameters. However, some states in Brazil, such as Paraná, have implemented specific state regulations. The difficulty in establishing federal legislation arises from the significant variations in this type of product, primarily due to the wide diversity of bee species and botanical origins. Additionally, there are no specific regulations for food products that include propolis or propolis extract from stingless bees. Therefore, the physicochemical parameters in this study were compared with the technical regulation for the identity and quality of stingless bee honey in the state of Paraná (Paraná, 2017).

For total acidity, measured as the sum of free acidity and lactonic acidity, a slight increase in concentration was observed (H1 = 90.33 meq/kg, H1EP-0.25 = 96.19 meq/kg, H1EP-0.50 = 96.96 meq/kg, H1EP-0.75 = 101.11 meq/kg). This increase was not statistically significant for the 0.25% and 0.50% concentrations. The elevation of acidity in honey compounds with propolis extract compared to honey can be attributed to the inherent acidity of the propolis extract. In comparison with state regulations, both the honey and honey compounds with propolis extract analyzed exceeded the stipulated total acidity, which should be less than 60 meq/kg. The acidity value corresponds to the content of organic acids present in honey. The higher acidity is primarily associated with sugar fermentation, resulting in acid production, along with phytogeographic factors. As this is a pasteurized product kept under refrigeration, the values found in this study may be related to fermentative processes within the hive prior to extraction (Ávila et al., 2018; Sousa et al., 2016).

There was a reduction in pH value, which was statistically significant for the samples with 0.5% and 0.75% added propolis extract compared to the honey, with values ranging between 4.23 and 3.99 (H1 = 4.23, H1EP-0.25 = 4.08, H1EP-0.50 = 4.03, H1EP-0.75 = 3.99). The values obtained in this study align with the state regulation in Paraná, where the pH should be below 4.7. Despite the strong relationship between pH and acidity, for honey samples, pH may not be directly related to acidity due to buffering reactions involving the mineral salts and acids present in honey (Rodríguez et al., 2004).

Regarding the total soluble solids, measured in ºBrix, there was a slight reduction for honey compounds with propolis extract compared to honey. This reduction can be considered practically negligible. The variation was from 74.20 to 74.85 °Brix. While the state regulation in Paraná does not establish specific limits for this parameter, the measurement of TSS is directly proportional to the sugar content of the sample. This is because honey is primarily composed of water and sugars (constituting 95-99% of the dry matter) (Nešović et al., 2020; Seraglio et al., 2019).

Considering reducing sugar concentration, there was no statistical difference among the samples, with values ranging between 56.83% and 58.08%. The major reducing sugars in honey are glucose, fructose and trehalulose (Fletcher et. al, 2020). Studies evaluating this parameter for propolis extract are scarce, as propolis is primarily composed of resins, wax, essential oils, and pollen grains (Zulhendri et al., 2022). Therefore, alterations in the reducing sugar content were not expected. In comparison with the state regulation in Paraná, the values are within the stipulated range (> 47%). No significant amounts of apparent sucrose were found in any sample, remaining within the maximum limit of 5% allowed by the state regulation in Paraná.

In relation to moisture content, a significant increase was observed in honey-propolis products compared to honey. Among the samples, H1EP-0.75 exhibited the highest value

(H1 = 23.55%, H1EP-0.25 = 24.20%, H1EP-0.50 = 24.20%, H1EP-0.75 = 24.25%). The rise in moisture content in honeys with propolis extract, compared to honey, could be attributed to the addition of ethanol to the mixture and the inherent moisture content of the propolis extract. Costa & Pereira (2002) also observed an increase in moisture content in honey-propolis blends, which was proportional to the added propolis concentration. Water activity and moisture content are critical parameters in stingless bee honey (Nordin et al., 2018; Ribeiro et al., 2018). According to Paraná state regulations, the moisture content in stingless bee honey should be below 35%. Therefore, both the honey and honeys with propolis extract fall within the stipulated range.

Regarding water activity, this parameter has recently been applied to stingless bee honey. It measures the available free water for chemical reactions. The regulations in Paraná for stingless bee honey do not establish specific limits for water activity. However, values exceeding 0.6 allow for the occurrence of fermentative processes due to the survival of lactic acid, acetic acid, lactic bacteria, and osmophilic yeasts (Nordin et al., 2018). The water activity for honey and honeys with propolis extract was 0.71. This value indicates that these products are susceptible to fermentation. However, considering that the honey was previously pasteurized, the shelf-life extension of this product can be ensured for 6 to 12 months (Ribeiro et al., 2018; Villas-Bôas, 2012).

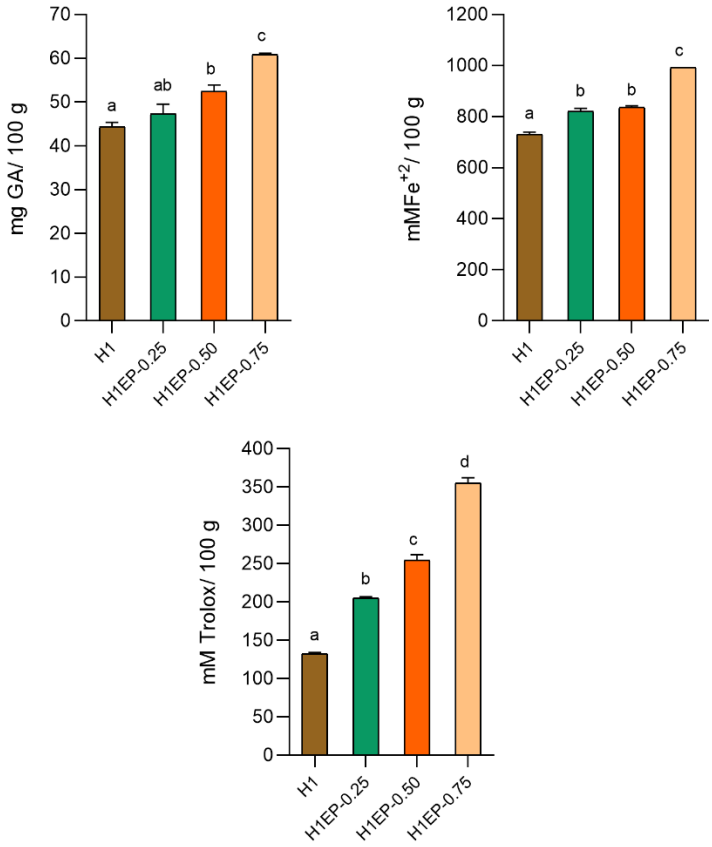
About the measurement of hydroxymethylfurfural (HMF), it is used as a parameter for the freshness and quality of honey. This substance is formed by the degradation of reducing sugars during the Maillard reaction (non-enzymatic browning). The production of this compound is related to processes involving heating above 70°C, prolonged storage, and adulteration. However, stingless bee honey has demonstrated resistance to HMF formation when subjected to elevated temperatures (Braghini et al., 2020). Propolis is not a source of sugars; therefore, non-enzymatic browning processes are not expected in

products with added propolis extract. The HMF values remained low, with no statistical difference among the samples, ranging from 3.09 to 3.63 mg/kg. According to Paraná state technical regulations, the HMF concentration should be below 40 mg/kg.

3.2. Antioxidant and reducing capacity

The results for the analyses of Total Phenolic Content (TPC), Ferric Reducing Antioxidant Power (FRAP), and ABTS•+ are shown in Figure 1.

Figure 1. Antioxidant and reducing capacity of honey and honeys with propolis extract. H1: Honey, H1EP-0.25: Honey with 0.25% propolis extract, H1EP-0.50: Honey with 0.50% propolis extract, H1EP-0.75: Honey with 0.75% propolis extract.



The addition of propolis extract to honey increased the TPC content of the samples. However, this increase was statistically significant only starting from the addition of 0.5% propolis extract. In other words, the addition of 0.25% was insufficient to raise the TPC content of honey-propolis products compared to honey (H1 = 44.37 mg GA/100 g, H1EP-0.25 = 47.26 mg GA/100 g, H1EP-0.50 = 52.42 mg GA/100 g, H1EP-0.75 = 60.79 mg GA/100 g). The best result obtained (adding 0.75% propolis extract to honey) represented an approximate 37% increase in TPC content.

Regarding the iron reduction analysis (FRAP), the results ranged from 730.80 to 992.80 mM Fe^{+2} / 100 g. The increase in reducing power was not proportional to the amount of propolis extract added to the mixture (H1 = 730.80 mMFe^{+2} / 100 g, H1EP-0.25 = 820.80 mM Fe^{+2} / 100 g, H1EP-0.50 = 836.80 mM Fe^{+2} / 100 g, H1EP-0.75 = 992.80 mM Fe^{+2} / 100 g). Interestingly, compounds with propolis extract concentrations of 0.25% and 0.50% showed no statistical difference. However, the addition of 0.75% propolis extract enhanced the reducing power of pure honey by approximately 36%, suggesting that the 0.75% concentration of propolis extract has a synergistic effect on enhancing the reducing power.

The TEAC content increased according to the concentration of propolis extract added to pure honey, with this increase being significant and non-proportional for all samples. The addition of 0.75% propolis extract enhanced the antioxidant activity of pure honey by approximately three times (H1 = 132.56 mM Trolox / 100 g, H1EP-0.25 = 205.24 mM Trolox / 100 g, H1EP-0.50 = 254.90 mM Trolox /100 g, H1EP-0.75 = 355.43 mM Trolox / 100 g). Similar to the observations in TPC and FRAP analyses, the 0.75% concentration of propolis extract exhibits the highest capacity to enhance the antioxidant and reducing activity of pure honey.

It is known that stingless bee honey is a source of bioactive compounds with antioxidant and reducing capacity, sometimes superior of *Apis mellifera* honey (Ávila et al., 2018). Ávila et al. (2019) identified compounds such as quercetin, *p*-coumaric acid, and hesperetin in 32 samples of stingless bee honey from Paraná. These compounds were associated with antioxidant capacity through assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorption capacity (ORAC), ferric reducing antioxidant power (FRAP), and total phenolic compound content (TPC).

The increase in antioxidant and reducing activity of honey-propolis products is related to the chemical compounds present in propolis, such as terpenoids, flavonoids, and phenolic compounds. These compounds have been associated with bioactive properties that surpass those of honey (Cisilotto et al., 2018; Osés et al., 2016). The bioactivity of propolis is not solely the sum of the reducing and antioxidant power of each individual compound. It also depends on the interaction of these compounds with substances present in the environment (food) where they are introduced. This interaction can lead to synergistic or inhibitory effects, which justifies the non-proportional results observed with increasing concentrations of propolis extract added to honey (Irigoiti et al., 2021).

Another interesting point to note is that, according to a study by Sawaya et al. (2006), the propolis from *Tetragonisca angustula* exhibits a similar composition regardless of the region in Brazil where it is collected. However, the same pattern is not observed for *Apis mellifera* propolis, where the composition is highly dependent on the collection region. According to the authors, this consistency in *Tetragonisca angustula* propolis composition can be attributed to the bee's preference for resins from *Schinus terebenthifolius*, whenever it is available.

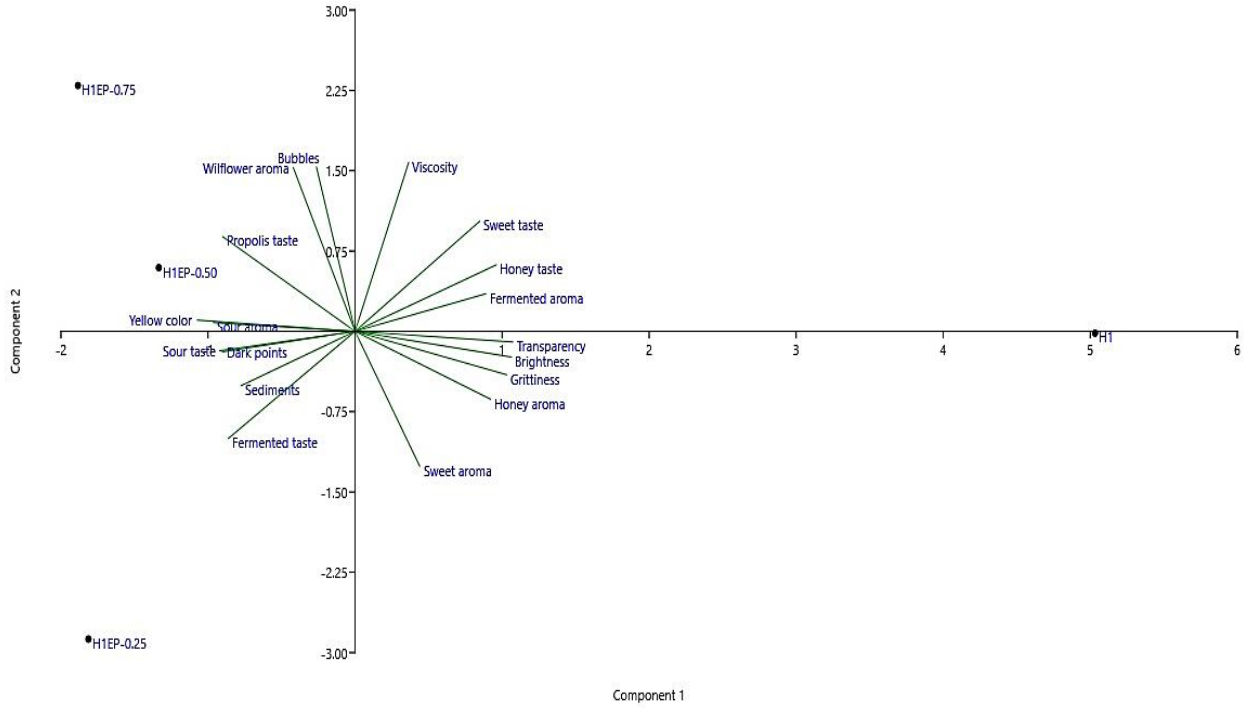
Osés et al. (2016) developed honey-based products with *Apis mellifera* propolis extract. The authors conducted the study using four different honey samples and propolis extract

in various combinations. The concentrated extracts were incorporated at concentrations of 0.1%, 0.3%, and 0.5% into honey. There was an increase in the TPC (total phenolic compound) content in all combinations, although in smaller proportions compared to the findings in this study. The authors observed the same pattern for antioxidant activity using the ABTS•+ assay, expressed in μmol Trolox/ g. On the other hand, in a previous study, Osés et al. (2015) analyzed the combination of honey with two samples of *Apis mellifera* propolis collected from different locations and periods. They did not find any statistical difference with the addition of 0.3% and 0.5% propolis extract to honey, demonstrating the strong dependence of *Apis mellifera* propolis composition on the type of floral source. In addition to honey, several products have been supplemented with propolis extract to enhance their antioxidant and reducing activity. Examples include beer (Galgowski et al., 2019; Ulloa et al., 2017), meat products (Ali et al., 2010; Gutiérrez-Cortés & Suarez Mahecha, 2014; Kročko et al., 2014; Kunrath et al., 2017), juices (Kahramanoglu & Usanmaz, 2017), dairy derivatives (Santos et al., 2020), and beverages (Vasilaki et al., 2019). Furthermore, propolis has been widely incorporated into edible films and coatings (Bertotto et al., 2022; Marangoni Júnior et al., 2022; Passos et al., 2016).

3.3. Sensory analysis

Figure 2 shows the PCA (Principal Component Analysis) of the descriptive analysis of the samples.

Figure 2. Projections of sensory attributes of honey and honeys with propolis extract in the factorial plane (PC I x PC II). H1: Honey, H1EP-0.25: Honey with 0.25% propolis extract, H1EP-0.50: Honey with 0.50% propolis extract, H1EP-0.75: Honey with 0.75% propolis extract.



A total of 3 principal components (PCs) were captured. According to Jolliffe (2002), the sum of the principal components should explain at least 75% of the existing variations. In this study, the primary component I (PC I) explained 62.88% of the variations, and the secondary component II (PC II) explained 25.7% of the variations, totaling 88.58%. Attributes with a correlation coefficient (r) greater than ±0.65 were considered important for the analysis. For PC I, the significant attributes were: yellow color (r = -0.99), brightness (r = +0.98), transparency (r = +0.99), dark spots (r = -0.83), sediment (r = -0.72), sour aroma (r = -0.89), fermented aroma (r = +0.82), honey aroma (r = +0.85), sour taste (r = -0.85), sweet taste (r = +0.78), fermented taste (r = -0.80), honey taste (r = +0.89), propolis taste (r = -0.83), and grittiness (r = +0.95). For PC II, the significant

attributes were air bubbles (r = +0.91), wildflower aroma (r = +0.90), sweet aroma (r = -0.74), and viscosity (r = +0.93).

In relation to PC I, honey is situated on the right side, characterized by its fermented aroma, honey aroma, grittiness, brightness, transparency, honey taste, and sweet taste. On the left side are the honey compounds with propolis extract, characterized by their fermented taste, sediment, dark spots, sour taste, sour aroma, propolis taste, and yellow color. Regarding PC II, the honeys with the addition of 0.50% and 0.75% propolis extract are positioned above, characterized by wildflower aroma, air bubbles, and viscosity. Below are honey and the compound with 0.25% propolis extract, characterized by sweet aroma.

The compounds with the addition of 0.50% and 0.75% propolis extract are closer together, demonstrating greater similarity between these samples. This similarity is particularly evident for attributes such as yellow color, propolis taste, wildflower aroma, air bubbles, and sour aroma. The perception of these attributes is less intense for the compound with 0.25% propolis extract. Statistical differences among the sensory panel were observed only in terms of appearance (Table 3).

Table 3. Intensity of sensory attributes of honey and honeys with propolis extract.

Attributes		H1	H1EP-0.25	H1EP-0.50	H1EP-0.75
Appearance	Yellow color	4.90±1.74a	7.30±1.33b	7.30±1.35 b	7.50±1.50b
	Brightness	6.40±1.66a	4.50±2.06ab	4.50±2.45ab	4.10±2.61b
	Transparency	6.30±2.25a	2.10±1.06b	2.40±1.97b	1.70±0.77 b
	Bubbles	1.30±1.18a	1.10±0.92a	1.60±1.75 a	1.60±1.38a
	Dark points	0.40±0.41a	1.40±0.88ab	1.80±2.10b	1.10±1.61ab
	Sediments	1.10±2.46a	2.60±2.32a	3.10±2.94a	1.70±2.46a
Aroma	Sour	3.10±2.58a	4.20±2.14a	3.70±2.33a	4.40±2.91a
	Sweet	5.20±2.51a	5.20±2.76a	5.10±2.44a	4.10±2.72a

	Fermented	5.30±3.19a	4.40±3.17a	5.00±2.95a	4.50±3.16a
	Honey	5.10±2.16a	4.80±2.45a	4.50±1.84a	4.60±2.69a
	Wildflower	3.90±2.30a	3.60±1.62a	4.70±2.32a	5.00±2.40a
Flavor	Sour	4.10±2.40a	4.60±2.32a	4.30±2.31a	4.60±2.50a
	Sweet	6.30±1.72a	5.10±3.09a	5.70±2.11a	5.80±1.97a
	Fermented	3.50±2.40a	4.50±2.70a	4.10±2.61a	3.90±2.40a
	Honey	6.10±1.69a	5.20±2.22a	5.30±1.63a	5.60±2.60a
	Propolis	2.30±2.04a	3.00±2.71a	3.30±2.38a	3.90±2.84a
Texture	Viscosity	3.90±2.81a	3.10±1.55a	3.80±2.18a	4.00±2.51a
	Grittiness	0.70±1.10a	0.40±0.71a	0.30±0.30a	0.30±0.40a

H1: Honey, H1EP-0.25: Honey with 0.25% propolis extract, H1EP-0.50: Honey with 0.50% propolis extract, H1EP-0.75: Honey with 0.75% propolis extract.

Samples with added propolis extract showed a stronger tendency toward yellow color, reduced transparency and brightness, and more dark spots. However, analyzing Figure 2, it can be inferred that the addition of propolis was perceptible to the sensory panel, even though the differences among the samples were minimal. On a scale of 100%, the sensory perception intensity of propolis taste was 25% for honey, 33% for honey with 0.25% propolis extract, 37% for honey with 0.50% propolis extract, and 43% for honey with 0.75% propolis extract. In other words, the difference in perceived intensity between honey and the product with the highest propolis addition (0.75%) was only 18%.

Table 4 displays the correlation coefficients among the sensory attributes.

Table 4. Pearson correlation coefficients among sensory attributes.

	Yellow color	Brightness	Transparency	Bubbles	Dark points	Sediments	Sour aroma	Sweet aroma	Fermented aroma	Honey aroma	Wilflower aroma	Sour taste	Sweet taste	Fermented taste	Honey taste	Propolis taste	Viscosity	Grittiness
Yellow color																		
Brightness	-0.99																	
Transparency	-1	1																
Bubbles	0.31	-0.36	-0.28															
Dark points	0.84	-0.79	-0.8	0.28														
Sediments	0.71	-0.64	-0.67	0.11	0.97													
Sour aroma	0.89	-0.91	-0.92	0.12	0.51	0.36												
Sweet aroma	-0.44	0.54	0.48	-0.61	0.02	0.26	-0.63											
Fermented aroma	-0.8	0.81	0.84	0.16	-0.44	-0.33	-0.96	0.45										
Honey aroma	-0.89	0.88	0.86	-0.67	-0.86	-0.72	-0.63	0.45	0.45									
Wilflower aroma	0.45	-0.51	-0.44	0.97	0.3	0.1	0.33	-0.77	-0.06	-0.73								
Sour taste	0.84	-0.86	-0.87	-0.06	0.46	0.33	0.99	-0.53	-0.99	-0.51	0.17							
Sweet taste	-0.75	0.7	0.75	0.39	-0.7	-0.71	-0.71	-0.1	0.81	0.42	0.26	-0.77						
Fermented taste	0.76	-0.7	-0.76	-0.33	0.77	0.8	0.66	0.15	-0.75	-0.48	-0.22	0.72	-0.99					
Honey taste	-0.87	0.82	0.86	0.03	-0.93	-0.92	-0.67	-0.05	0.68	0.72	-0.05	-0.67	0.91	-0.95				
Propolis taste	0.86	-0.91	-0.87	0.63	0.56	0.35	0.84	-0.81	-0.66	-0.86	0.78	0.74	-0.35	0.35	-0.51			
Viscosity	-0.28	0.21	0.29	0.8	-0.35	-0.47	-0.3	-0.52	0.52	-0.09	0.73	-0.43	0.85	-0.82	0.63	0.2		
Grittiness	-0.98	0.97	0.96	-0.5	-0.86	-0.71	-0.8	0.49	0.66	0.97	-0.62	-0.72	0.6	-0.63	0.81	-0.91	0.09	

The propolis taste attribute exhibited a positive and significant correlation with the yellow color, sour aroma, wildflower aroma, and sour taste. Conversely, propolis taste demonstrated a negative and significant correlation with brightness, transparency, sweet aroma, fermented aroma, and honey aroma.

4. Conclusion

The combination of propolis extract and *Tetragonisca angustula* honey resulted in a product with greater bioactivity, particularly observed for the addition of 0.75% propolis extract. Regarding the physicochemical parameters, changes were observed only in moisture content, total acidity, pH, and total soluble solids when compared to honey without the added. The results of the Quantitative Descriptive Analysis indicated that the propolis taste was perceived with low intensity compared to honey without added propolis extract. This suggests that the addition of propolis extract did not negatively affect the product characteristics. The descriptors for honeys with propolis extract include fermented flavor, sediment, dark spots, sour flavor, sour aroma, propolis taste, yellow color, wildflower aroma, air bubbles, and viscosity. Developing products based on stingless bee honey and propolis is essential for improving the distribution chain in this sector, increasing the profitability of beekeepers, and offering functional properties.

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6. CONCLUSÕES

- Através de análise não supervisionada (multibloco) foi possível obter uma separação parcial dos méis de abelha-sem-ferrão em termos de origem entomológica e geográfica.
- A origem entomológica foi o principal fator que influenciou o perfil químico de amostras de mel de abelha-sem-ferrão.
- Os tipos polínicos foram responsáveis pelos dois agrupamentos das amostras (Norte-Nordeste e Sul) em termos de origem geográfica.
- Os modelos supervisionados de Análise Discriminante Linear (LDA) e Análise Discriminante por Mínimos Quadrados Parciais (PLS-DA) previram efetivamente amostras dos gêneros *Melipona*, *Scaptotrigona* e *Tetragonisca* usando dados quantitativos e FTIR-ATR, respectivamente.
- O perfil de açúcares foi importante para o agrupamento das amostras em termos de origem entomológica pelos métodos não supervisionado e supervisionado.
- No estudo de revisão, melezitose e trealulose foram apontados como biomarcadores de mel de melato e mel de abelha-sem-ferrão, respectivamente.
- Em termos de cromatografia líquida, a espectroscopia de massas e a detecção amperiométrica pulsada são citadas como os métodos mais adequados para a separação e quantificação de açúcares no mel; entretanto, o detector mais utilizado é o detector de índice de refração.
- Métodos alternativos como a espectroscopia vibracional e ressonância nuclear magnética têm se mostrado eficazes na quantificação de açúcares em mel.
- A adição do extrato de própolis de *Tetragonisca angustula* ao mel, provocou um aumento da sua atividade bioativa, sendo esse aumento não proporcional mais pronunciado para o composto com adição de 0.75% de extrato de própolis.
- Na análise descritiva qualitativa, os atributos que melhor descreveram os compostos do mel com extrato de própolis foram: sabor fermentado, sedimentos, pontos escuros, sabor ácido, aroma ácido, sabor de própolis e cor amarela.
- O sabor de própolis foi percebido em baixa intensidade pelo painel sensorial, podendo-se concluir que a adição do extrato de própolis não afetou negativamente as características do produto.

- O enriquecimento do mel com extrato de própolis pode ser uma opção adicional de produto para aumentar a lucratividade dos meliponicultores.

ANEXOS

ARTIGO 1

Figure S1: Chromatograms of sugar standards at concentrations of 10 mg/mL for fructose, glucose, maltose and trehalulose and 1.0 mg/mL for raffinose.

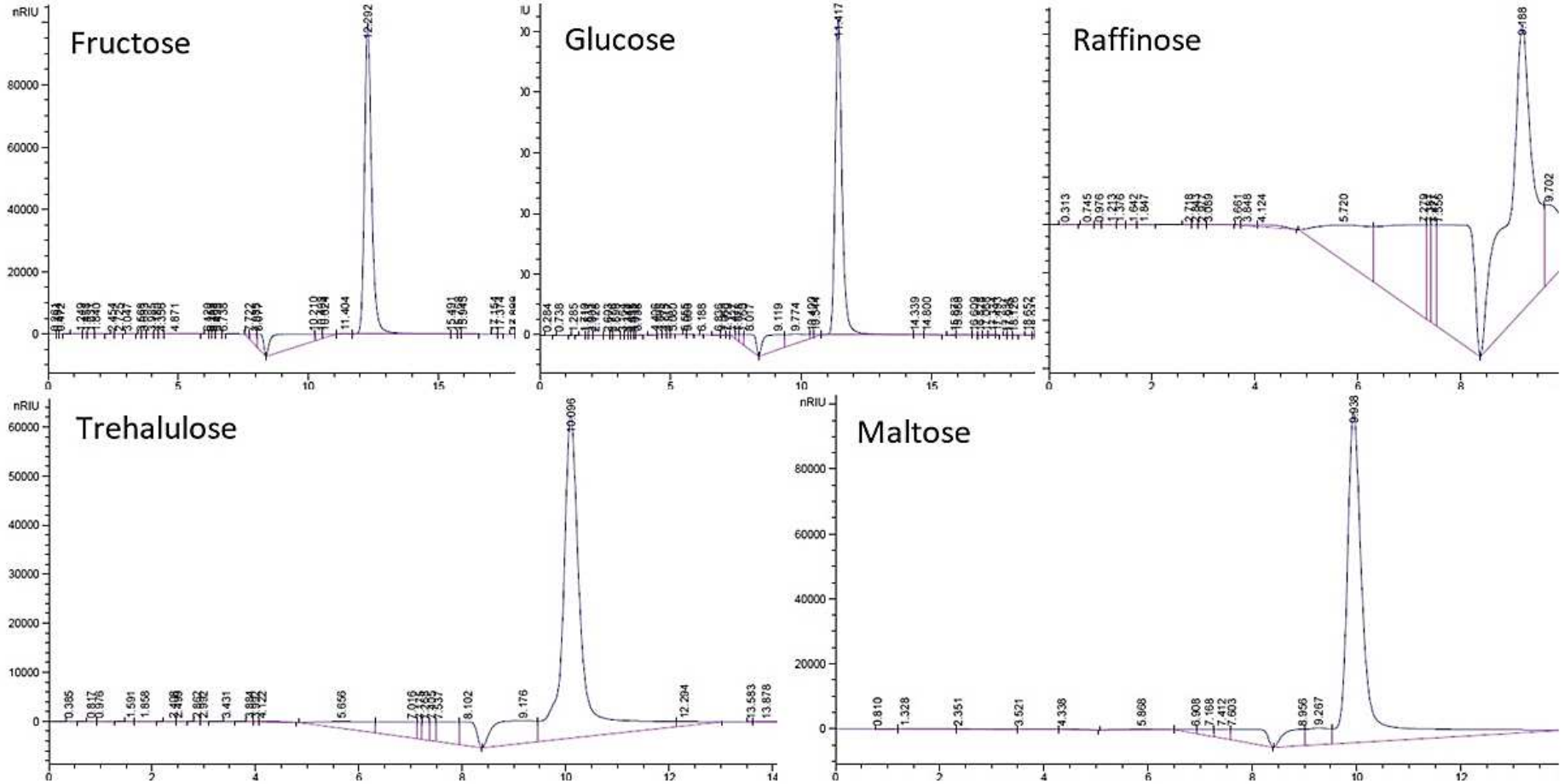


Figure S2: Representative chromatograms of honey samples from *Melipona*, *Scaptotrigona*, *Frieseomelitta*, *Tetragona* and *Tetragonisca*.

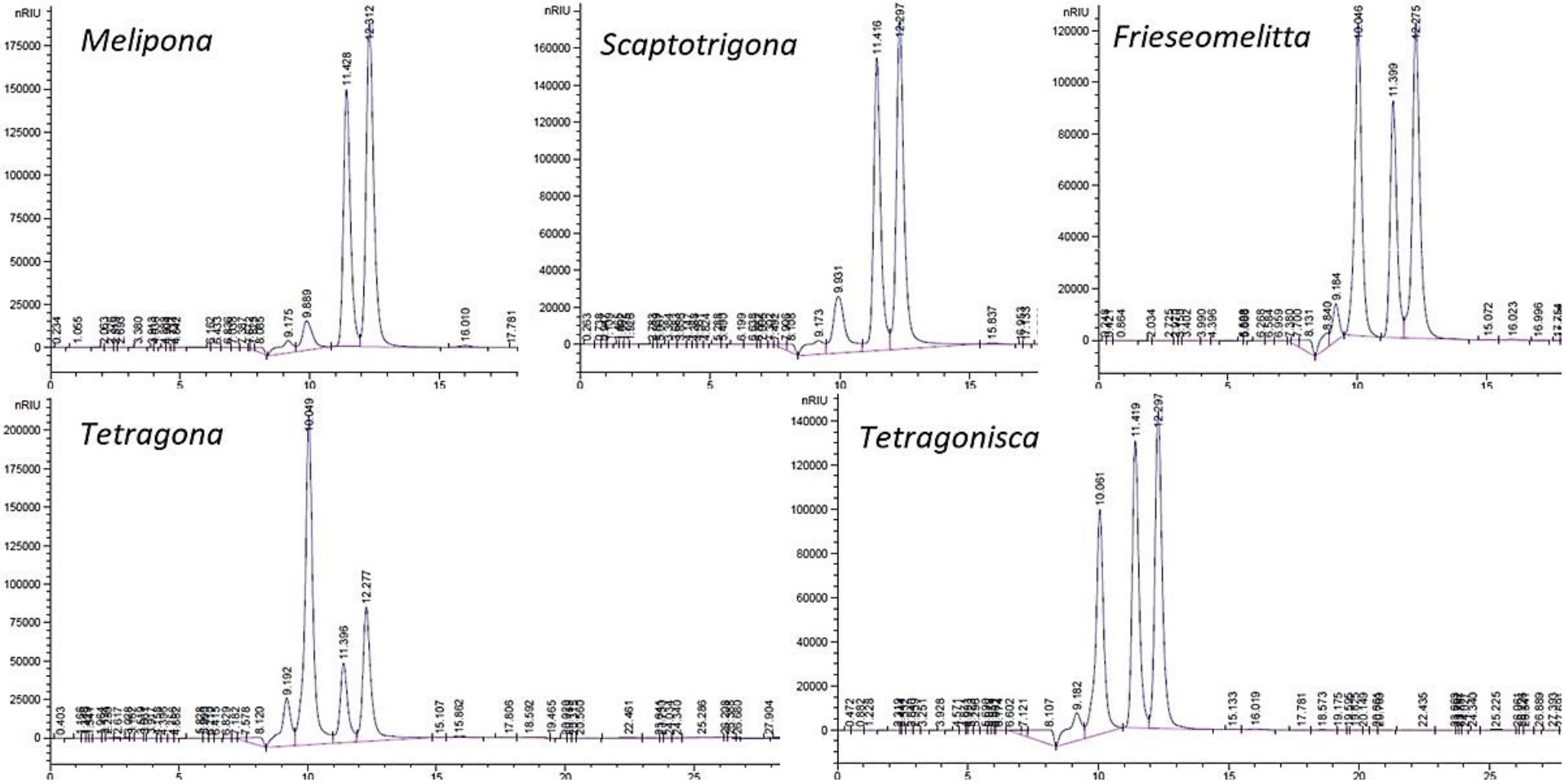
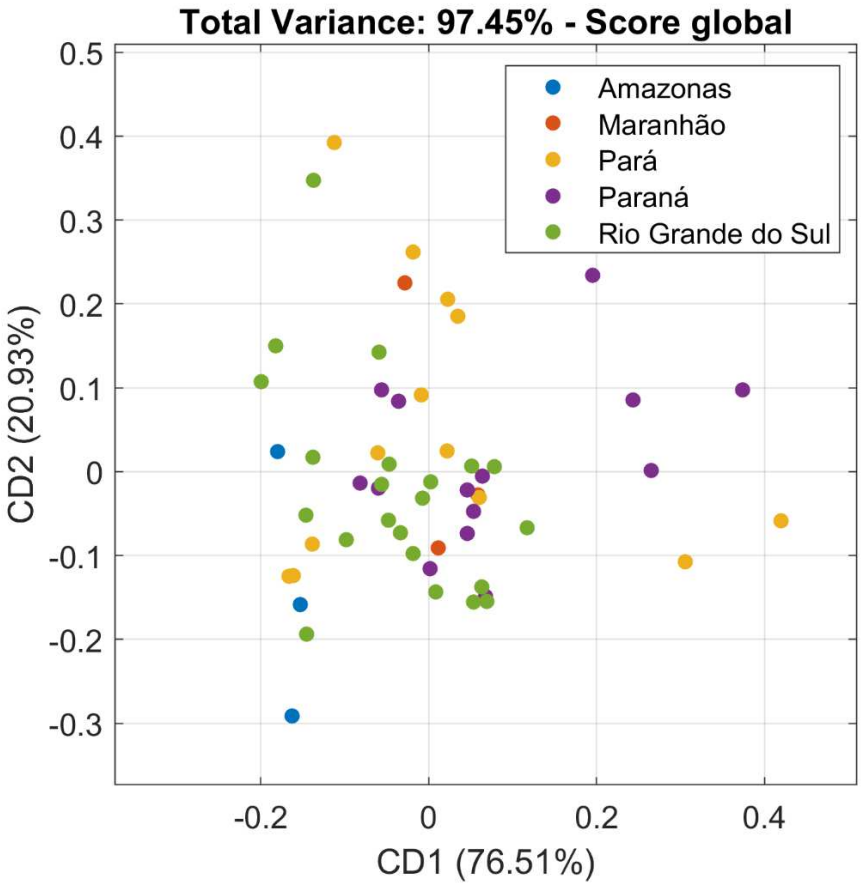


Figure S3: ComDim for discrimination of *Tetragona*, *Tetragonisca*, *Melipona*, *Scaptotrigona* and *Frieseomelitta* honeys using melissopalynological analysis data, quantitative data, and FTIR-ATR by geographic origin (CD1 × CD2).



ARTIGO 2

Table S1. Quantification of the sugar's glucose, fructose, sucrose and maltose in honeybee honeys by chromatography techniques.

Geographical origin	Location	Source	Botanical origin	Glucose (%)	Fructose (%)	Sucrose (%)	Maltose (%)	LOD and LOQ	Equipment	Column	Detector	Operation conditions	Reference
Saudi Arabia	Rijal Alma	Nectar	*Ziziphus spina-christi*	26.68 ± 0.07	33.10 ± 0.04	0.25 ± 0.11	1.04 ± 0.06	Sucrose: LOQ = 0.002% and LOD = 0.0006%	Agilent 1260 Infinity II LC System (Agilent Technologies)	ZORBAX Carbohydrate (Agilent Technologies)	HPLC-RI	Isocratic mobile phase Acetonitrile: water (75:25, v/v), flow rate: 1.0 mL/min, sample volume: 10 µL, column and detector temperature: 35 ºC	63
	Billahmar-Albatna		*Acacia asak*	36.38 ± 0.07	43.62 ± 0.07	<LOQ	0.46 ± 0.04						
	Bisha		*Acacia tortilis*	32.06 ± 0.05	37.98 ± 0.07	<LOQ	1.39 ± 0.04						
	Billahmar-Touma		*Acacia hamulosa*	36.49 ± 0.28	44.77 ± 0.19	<LOQ	1.05 ± 007						
	Billasmar-Alhijaz		*Lavandula dentata*	37.91 ± 0.21	38.77 ± 0.32	<LOQ	1.08 ± 0.05						
	Sarat Abeedah-Aljow		*Herbs and Hypoestes forskaolii*	37.20 ± 0.14	37.44 ± 0.23	<LOQ	0.98 ± 0.06						
	Al-Huridha		*Herbs*	29.51 ± 0.23	33.97 ± 0.05	3.25 ± 0.13	2.97 ± 0.09						
	Abha		*Eucalyptus spp.*	32.77 ± 0.13	35.15 ± 0.08	<LOQ	0.37 ± 0.10						
Ethiopia		Nectar	Acacia	33.28 ± 0.58	43.07 ± 0.37	1.22 ± 0.10	1.20 ± 0.49			ZORBAX Carbohydrate (Agilent Technologies)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (75:25, v/v); flow rate: 1.5 mL/min, sample volume: 20 µL, column and detector temperature: 30 °C	64
			Becium grandiflorum	29.34 ± 2.75	35.30 ± 3.53	2.75 ± 0.65	1.78 ± 0.16						
			Croton macrostachyus	31.65 ± 1.37	38.86 ± 0.82	2.57 ± 0.22	1.41 ± 0.96						
			Eucalyptus globulus	30.56 ± 0.32	40.26 ± 0.25	1.93 ± 0.14	2.04 ± 0.45						
			Hypoestes	36.17 ± 0.60	38.14 ± 0.53	1.85 ± 0.03	1.27 ± 0.08						
			Leucas abyssinica	37.20 ± 0.35	38.64 ± 0.23	1.33 ± 0.06	1.36 ± 0.29						
			Schefflera abyssinica	30.55 ± 2.69	38.81 ± 1.18	2.14 ± 0.16	0.55 ± 0.34						
			Syzygium guineense	34.33 ± 0.38	41.01 ± 0.30	1.13 ± 0.02	1.77 ± 0.33						
India	Kashmir valley	Nectar	Saffron	30.56 ± 0.35	37.56 ± 1.51	1.11 ± 0.05	1.28 ± 0.05	LOD = 0.05–0.19% and LOQ= 0.08–0.23% (total variation)	Waters (Waters™)	Waters X-bridge Amide (Waters™)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 0.6 mL/min, sample volume: 20 µL, column temperature: 85 °C	65
			Apple	32.44 ± 0.28	40.61 ± 1.48	1.40 ± 0.03	1.07 ± 0.02						
			Cherry	33.98 ±0.45	36.42 ±0.48	1.23 ± 0.04	0.95 ± 0.03						
			Plectranthus rugosus	31.21 ± 0.57	38.87 ± 0.17	1.05 ± 0.04	1.36 ± 0.09						
Poland and Slovaki	South-Eastern Poland and North-Eastern Slovaki	Nectar	Multifloral	31.82 ± 1.78	39.23 ± 3.08	4.40 ± 1.95			Dionex UltiMate 3000 (Thermo Fisher Scientific)	Asahipak NH2P-50 4E (Shodex™)	HPLC-CD	Isocratic mobile phase: Acetonitrile: water (78:22, v/v); flow rate: 1 mL/min, sample volume: 10 µL, column temperature: 55 °C	67
			Tilia	28.43 ± 1.37	36.89 ± 3.26	6.12 ± 1.32							
			Forest	29.10 ± 1.71	34.67 ± 0.31	6.29 ± 1.07							
			Rape	32.92 ± 2.80	36.35 ± 2.28	5.06 ± 0.54							
			Acacia	31.75 ± 1.77	37.64 ± 1.60	3.53 ± 0.76							
			Multifloral	27.78 ± 3.32	38.66 ± 2.52	4.47 ± 2.15							
			Tilia	28.21 ± 1.81	38.15 ± 2.35	4.68 ± 1.22							
			Forest	26.78 ± 1.83	37.83 ± 0.68	5.06 ± 0.61							
			Rape	29.69 ± 1.57	36.39 ± 1.44	4.61 ± 0.23							
			Acacia	26.14 ± 0.88	37.34 ± 1.66	5.27 ± 1.50							
Romania	Different regions of Romania	Nectar	Black locust	29.8 ± 1.8	42.3 ± 2.8	2.3 ± 1.5	1.9 ± 0.5		Dionex ICS-3000 SP (Thermo Fisher Scientific)	CarboPac PA1 (Thermo Fisher Scientific)	HPAEC-PAD	Gradient mobile phase: ultra-pure water, 200 mM NaOH and 700 mM NaOH, flow rate: 1 mL/min, sample volume: 10 µL	3
			Linden	33.3 ± 3.6	38.7 ± 3.1	1.5 ± 0.2	1.4 ± 0.5						
			Rape	37.2 ± 5.6	41.1 ± 1.8	1.46 ± 0.1	1.2 ± 0.4						
			Sun-flower	38.7 ± 2.1	39.5 ± 3.0	1.43 ± 0.01	1.3 ± 0.3						
			Multifloral	32.8 ± 2.2	41.6 ± 5.2	1.5 ± 0.2	1.6 ± 0.3						
India	Viz. Haryana, Himachal Pradesh, Punjab, and Rajasthan	Nectar	*Gossypium hirsutum*	33.91 ± 0.19	36.98 ± 0.58	3.54 ± 0.37	1.44 ± 0.21		Agilent 1120 Compact LC (Agilent Technologies)	Hi-plex pb (Agilent Technologies)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 0.6 mL/min	66
			Coriandrum sativum	33.94 ± 0.30	37.09 ± 0.47	3.11 ± 0.24	1.56 ± 0.22						
			Dalbergia sissoo	34.06 ± 1.04	41.15 ± 0.04	3.41 ± 0.43	1.86 ± 0.13						
			Murraya koenigii	33.40 ± 1.03	36.86 ± 0.10	3.59 ± 0.38	1.54 ± 0.28						
Serbia	Vojvodina region	Nectar	Meadow	34.21 ± 2.32	38.52 ± 3.46	0.29 ± 0.10		Sucrose: LOQ = 0.85% and LOD = 0.25%	Dionex UltiMate 3000 (Thermo Fisher Scientific)	Hypersil GOLD™ Amino (Thermo Fisher Scientific)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 1 mL/min, sample volume: 5 µL, detector temperature: 35 ºC	60
			Acacia	30.00 ± 3.83	41.64 ± 3.58	0.73 ± 0.85							
			Tilia tomentosa	32.62 ± 1.35	36.57 ± 2.81	<LOQ							
			Multifloral	34.53 ± 3.72	39.99 ± 1.42	0.28 ± 0.09							
Portugal	Different regions of Portugal	Nectar	Commercial honey - Multifloral (year 2010)	26.23 ± 1.55	37.52 ± 1.81	<LOQ	1.70 ± 0.46	Trehalose: LOQ = 0.7% Glucose: LOQ = 0.12 Fructose: LOQ = 0.17 Sucrose: LOQ = 0.08 Melezitose: LOQ = 0.13 Turanose: LOQ = 0.12 Maltose: LOQ = 0.09	Dionex ICS-3000 (Thermo Fisher Scientific)	CarboPacPA20 (Thermo Fisher Scientific)	HPAEC-PAD	Gradient mobile phase: 50 mM NaOH and 200 mM NaOH, flow rate: 0.5 mL/min, sample volume: 10 µL	21
			Commercial honey - Multifloral (year 2011)	25.83 ± 2.95	38.84 ± 3.16	<LOQ	1.77 ± 0.57						
			Carob	23.82 ± 4.96	34.69 ± 3.02	1.06 ± 0.51	1.38 ± 1.25						
			Eucalyptus	23.34 ± 1.58	35.82 ± 1.51	1.23 ± 0.47	1.30 ± 0.43						
			Sunflower	29.5	37.96	<LOQ	<LOQ						
			Orange	30.73 ± 0.32	37.57 ± 5.88	<LOQ	1.15 ± 1.04						
			Multifloral	23.21 ± 4.53	35.10 ± 4.15	1.06 ± 0.44	1.28 ± 0.69						
			Arbutus	22.5	37.49	0.99	0.89						
			Rosemary	25.27 ± 3.15	37.57 ± 3.19	1.05 ± 0.16	1.42 ± 0.94						
			Rosemary + heather	20.92	31.07	1.13	0.97						
			Thyme	19.98	31.87	1.47	1.14						
			Thyme + rosemary	24.83	41.02	0.91	2.14						
			Heather	23.15 ± 2.43	35.97 ± 1.17	1.04 ± 0.36	1.01 ± 0.48						
Romania	Different regions of Romania	Nectar	*Robinia pseudoacacia*	25.7 ± 3.0	41.9 ± 2.6	nd	2.5 ± 1.0		Surveyor Plus (Thermo Fisher Scientific)	Hypersil™ APS-2 Amino (Thermo Fisher Scientific)	HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (85:15, v/v); flow rate: 0.8 mL/min, sample volume: 5 µL, column	22
			Brassica napus oleifera	39.1 ± 3.8	35.0 ± 6.3	nd	1.3 ± 0.8						
			Polyfloral	27.5 ± 6.6	37.8 ± 6.5	nd	1.5 ± 0.3						
			Tilia platyphyllos	30.7 ± 0.2	34.5 ± 0.9	nd	1.3 ± 1.2						

			Helianthus annus	34.4 ± 1.3	37.1 ± 1.6	nd	1.0 ± 0.3					temperature: 30ºC, and detector temperature: 95 °C	
			Mixed	27.6 ± 6.4	35.5 ± 4.1	0.06 ± 0.10	1.4 ± 0.6						
			Commercial honey	26.9 ± 3.3	36.9 ± 4.5	nd	3.7 ± 1.9						
Croatia	Požega	Honeydew	Q. frainetto Ten (Italian oak)	28.7 ± 3.5	35.7 ± 1.2	1.7 ± 1.4	1.9 ± 1.8			Zorbax NH2 (Agilent Technologies)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (70:30, v/v); flow rate: 1.0 mL/min, sample volume: 10 µL	50
Romania and Poland	Western part of Romania	Honeydew		28.5 ± 2.6	36.4 ± 1.1	1.4 ± 0.1			Merck-Hitachi HPLC system (Merck)	Luna NH2 (Phenomenex)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 1.0 mL/min	54
	Different regions of Poland			25.6 ± 1.2	43.9 ± 1.7	1.9 ± 0.1							
Spain	Northwest and center-south area of Spain	Honeydew	Oak	27.2 ± 1.3	35.4 ± 1.8	0.2 ± 0.1		Fructose: LOD = 0.73 and LOQ= 2.42 Glucose: LOD = 0.67 and LOQ= 2.23 Sucrose: LOD = 0.13 and LOQ=0.44 Maltose: LOD = 0.28 and LOQ= 0.93 Melezitose: LOD= 0.02 and LOQ= 0.07 Trehalose: LOD = 0.11 and LOQ= 0.36	Dionex ICS-3000 (Thermo Fisher Scientific)	CarboPac PA1 (Thermo Fisher Scientific)	HPAEC-PAD	Gradient mobile phase: ultrapure water and 200 mM NaOH	27
			Evergreen Oak	24.1 ± 1.7	32.0 ± 3.9	0.2 ± 0.1	1.2 ± 1.0						
Romania	Different regions of Romania	Honeydew		27.6 ± 4.1	36.4 ± 7.9	nd	2.4 ± 1.1		Surveyor Plus system (Thermo Fischer Scientific)	Hypersil™ APS-2 Amino (Thermo Fischer Scientific)	HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (85:15, v/v); flow rate: 0.8 mL/min, sample volume: 5 µL, column temperature: 30ºC, and detector temperature: 95 °C	22
Spain		Honeydew		31.19 ± 2.33	39.64 ± 1.71	0.50 ± 0.56	5.52 ± 0.89		Waters 2690 (Waters™)	Waters Carbohydrate Analysis (Waters™)	HPLC-DRI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 2.0 mL/min, sample volume: 25 µL, column temperature: 25ºC	57
Romania		Honeydew		27.07	35.52	0.19	2.48		Shimadzu HPLC system (Shimadzu)	Analytical column containing amino-modified silica gel	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (85:15, v/v); flow rate: 1.3 mL/min	68
Romania	Different regions of Romania	Honeydew		28.2 6.9	37.7 5.4	1.82 0.7	1.5 0.8		Dionex ICS-3000 (Thermo Fisher Scientific)	CarboPac PA1 (Thermo Fisher Scientific)	HPAEC-PAD	Gradient mobile phase: ultra-pure water, 200 mM NaOH and 700 mM NaOH, flow rate: 1 mL/min, sample volume: 10 µL	3

Mean values ± standard deviation, LOD: limit of quantitation, LOQ: limit of quantitation, nd: not detected, HPLC: high-performance liquid chromatography, HPAEC: high-performance anion exchange chromatography, RID: refractive index, PAD: pulsed amperometric, ELSD: evaporative light scattering, CD: corona discharge, DRI: differential refractive index.

Table S2. Quantification of the sugar's glucose, fructose, sucrose, and maltose in stingless bee honeys by chromatography techniques.

Geographical origin	Location	Origin	Bee species	Botanical source	Glucose (%)	Fructose (%)	Sucrose (%)	Maltose (%)	LOD and LOQ	Equipment	Column	Detector	Operation conditions	Reference
Brazil	Seridó region, state of Rio Grande do Norte and Agreste region of State of Paraíba	Nectar	*Melipona subnida duke*	*Ziziphus joazeiro Mart.*	37.7 ± 0.4	59.2 ± 1.1	1.6 ± 0.2	nd		Hewlett Packard 1100 (Hewlett Packard)	CARBOsep CHO 682 column (Transgenomics)	HPLC-RI	Isocratic mobile phase: Distilled water, flow rate: 0.4 mL/min, column temperature: 80ºC, detector temperature: 50 ºC	45
				Mimosa quadrivalvis L.	45.4 ± 0.3	50.0 ± 0.9	3.9 ± 0.2	nd						
				Mimosa arenosa willd Poir	45.7 ± 0.4	52.6 ± 2.5	1.2 ± 0.2	nd						
				Croton heliotropiifolius Kunth	42.1 ± 0.6	55.7 ± 1.9	0.7 ± 0.0	nd						
			Melipona subnida latrelle	*Ziziphus joazeiro Mart.*	38.1 ± 0.3	57.6 ± 1.5	2.6 ± 0.5	nd						
				Mimosa quadrivalvis L.	42.6 ± 0.2	55.5 ± 1.0	1.9 ± 0.3	nd						
				Mimosa arenosa willd Poir	41.4 ± 0.5	53.6 ± 0.7	3.0 ± 0.5	nd						
				Croton heliotropiifolius Kunth	43.3 ± 0.1	53.8 ± 0.9	2.0 ± 0.3	nd						
Thailand	Chiang Mai	Nectar	*Homotrigona fimbriata*		15	7.4	nd	nd		Shimadzu HPLC system (Shimadzu)	Inertsil NH2 (GL Science)	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (75:25, v/v), flow rate: 1 mL/min, oven temperature: 40 ºC	69
	Chanthaburi		*Lepidotrigona terminata*		4.9	8.1	nd	53						
	Chiang Mai, Chanthaburi		*Lepidotrigona flavibasis*		13 ± 8.6	16 ± 8.8	nd	39 ± 15						
	Chiang Mai		*Lepidotrigona doipaensis*		14, 9.8	15, 9	nd	nd						

	Chanthaburi		*Lisotrigona furva*		25, 28	34, 33	nd, 6.0	nd						
	Chiang Mai		*Tetragonilla collina*		26	26	nd	nd						
	Chanthaburi		*Tetragonula fuscobalteata*		19, 4.4	23, 19	nd	nd						
	Chiang Mai, Chanthaburi, Trat		*Tetragonula laeviceps-pagdeni complex*		12 ± 7.7	17 ± 6.7	nd, 0.025 ± 0.019	37 ± 12						
	Chanthaburi		*Tetragonula testaceitarsis*		11, 27	15, 29	nd	nd						
	Chiang Mai		*Tetrigona apicalis*		7.7, 4.1	6.8, 6.7	nd	nd						
	Chiang Mai		*Tetrigona melanoleuca*		8.9	6	nd	nd						
Malaysia	Kuala Nerang	Nectar	*Heterotrigona itama sp*		18.89 ± 2.25	16.03 ± 1.64	6.72 ± 2.20		Agilent 1100 (Agilent Technologies)	Luna (Phenomenex)	NH2	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (75:25, v/v); flow rate: 1.2 mL/min, sample volume: 20 µL, column temperature: 30 °C	70
	Universiti Putra Malaysia				17.06 ± 1.85	28.91 ± 1.58	9.30 ± 0.93							
	Muar				20.36 ± 2.16	33.13 ± 1.76	25.63 ± 1.23							
	Kuala Terengganu				8.63 ± 2.30	16.19 ± 3.08	6.50 ± 0.56							
	Tumpat				16.51 ± 1.63	25.91 ± 1.49	7.77 ± 1.99							
	Sibu				17.01 ± 2.02	27.76 ± 2.13	6.91 ± 1.80							
	Kota Belud				16.18 ± 4.20	26.20 ± 0.64	2.68 ± 0.73							
	MARDI				20.72 ± 1.73	18.58 ± 2.04	14.48 ± 1.54							
Malaysia	Terengganu	Nectar	*Heterotrigona itama*	*Meleleuca cajaputi*	10.53 ± 0.22	13.58 ± 0.33	nd ± 0.12	31.50 ± 2.11		Luna (Phenomenex)	NH2	HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 1 mL/min, sample volume: 20 µL, column temperature: 40 °C	7
	Johor			*Acacia mangium*	11.69 ± 0.56	13.00 ± 0.21	nd ± 0.13	22.56 ± 1.22						
	Pahang			*Averrhoa carambola L*	8.14 ± 0.47	9.68 ± 0.72	nd ± 0.11	37.35 ± 0.58						
	Terengganu		*Geniotrigona thoracica*	*Meleleuca cajaputi*	6.95 ± 0.34	8.43 ± 0.11	nd	43.15 ± 2.36						
	Johor			*Acacia mangium*	8.12 ± 0.95	7.89 ± 0.36	nd	28.96 ± 1.17						
	Pahang			*Averrhoa carambola L*	11.21 ± 0.10	9.34 ± 0.06	nd	40.82 ± 0.79						
Ecuador	Andean, Amazonian and Coastal Pacific regions		*Scaptotrigona polysticta*		32.07 ± 0.40	40.54 ± 0.47	4.63 ± 0.11		Agilent 1260 (Agilent Technologies)	Zorbax NH2 (Agilent Technologies)		HPLC-RI	Isocratic mobile phase: sulfuric acid (0.5 g/L); flow rate: 1 mL/55 min	71
			Melipona indecisa		31.23 ± 0.14	41.34 ± 0.39	4.02 ± 0.14							
			Melipona cramptoni		29.54 ± 1.35	40.24 ± 0.75	5.00 ± 0.08							
			Paratrigona sp.		26.62 ± 0.10	44.57 ± 0.13	4.90 ± 0.02							
			Melipona sp.		38.26 ± 0.02	42.33 ± 0.13	5.14 ± 0.16							
			Cephalotrigona sp.		35.60 ± 0.11	43.42 ± 0.08	3.09 ± 0.07							
			Melipona mimetica		30.28 ± 3.14	34.77 ± 3.49	2.63 ± 0.33							
			Tetragonisca angustula		30.48 ± 2.99	41.86 ± 0.18	3.70 ± 0.26							
			Trigona silvestriana		34.38 ± 0.02	39.70 ± 0.02	3.11 ± 0.15							
			Oxytrigona mellaria		32.69 ± 0.02	42.09 ± 0.01	3.72 ± 0.02							
			Nannotrigona chapadana		33.03 ± 0.03	41.25 ± 0.42	4.75 ± 0.01							
			Melipona grandis		26.00 ± 0.40	39.74 ± 0.16	4.02 ± 0.30							
Malaysia and Australia	Malaysia - Sarawak, Selangor, Johor and Kedah		*Heterotrigona itama*	Multifloral	13.37 ± 7.22	16.23 ± 6.21	nd		ThermoFisher Interion Ion Chromatograph (ThermoFisher Scientific)	CarboPac PA210 (Thermo Fisher Scientific)		HPAEC-PAD	Isocratic mobile phase: 12 mM KOH; column temperature: 30 ºC	18
			Geniotrigona thoracica		2.90 ± 1.20	4.11 ± 2.61	nd							
	Australia - Brisbane and Bundaberg		*Tetragonula carbonaria*		12.10 ± 2.51	17.43 ± 2.87	nd							
			Tetragonula hockingsi		12.71 ± 2.91	16.58 ± 3.39	nd							
Malaysia	Sarawak		*Heterotrigona itama*	*Acacia mangium*	23.44 ± 3.23	22.00 ± 3.48	0.04 ± 0.24	27.41 ± 8.32	Agilent 1260 Infinity LC System (Agilent Technologies)	ZORBAX Carbohydrate (Agilent Technologies)		HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (75:25, v/v); flow rate: 1.4 mL/min, oven temperature: 30 °C	72
			Tetrigona binghami		19.84 ± 4.87	15.43 ± 3.97	nd	27.95 ± 3.47						
Costa Rica	Country's Pacific coast and Central Valley		*Melipona beecheii*		28.2 ± 4.0	32.7 ± 3.9	0.33 ± 1.32	1.54 ± 0.46	Shimadzu HPLC system (Shimadzu)	Econosphere amino (Grace/Alltech)		HPLC-RI	Isocratic mobile phase: Acetonitrile: water (80:20, v/v); flow rate: 1.3 mL/min, column temperature: 30 °C	73
			Tetragonisca angustula		22.3 ± 4.6	28.2 ± 4.5	0.17 ± 0.93	2.35 ± 2.79						
Malaysia	Distance less than 20 km from the West Coast		*Heterotrigona itama*		7.55 ± 3.21	13.25 ± 4.31	nd	3.13 ± 3.84	Waters e2695 (Waters™)			HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (88:12, v/v); flow rate: 1.0 mL/min, sample volume: 15 µL, detector temperature: 60 °C	74
	Distance less than 40 km from the West Coast				11.54 ± 4.73	18.86 ± 4.30	nd	0.96 ± 3.02						
	Inland (more than 40 km from each coast)				10.63 ± 4.17	17.80 ± 7.27	nd	1.13 ± 2.67						
	Distance less than 40 km from the East Coast				8.48 ± 3.06	14.97 ± 7.47	nd	1.17 ± 2.34						
	Distance less than 20 km from the East Coast				10.94 ± 5.82	17.70 ± 9.83	nd	0.80 ± 1.97						
Malaysia	Forests in Perak		*Heterotrigona itama*	Mainly from *Acacia mangium* trees and other flowers	9.22 ± 2.52	15.77 ± 2.47	32.30 ± 2.66			Prevail carbohydrate ES		HPLC-ELSD	Isocratic mobile phase: Acetonitrile: water (75:25, v/v); flow rate: 1.5 mL/min, sample volume: 20 µL.	75
Malaysia	Sibu, Sarawak		*Geniotrigona thoracica*		17.63 1.5	12.01 1.1	nd	35.3 1.4	Waters (Waters™)	Supercosil™ LC-NH2		HPLC-RID	Isocratic mobile phase: acetonitrile: water (72:25), flow rate: 1 mL/min, oven temperature: 40 °C.	76
			Heterotrigona itama		21.0 ± 1.7	19.5 ± 1.3	nd	33.7 ± 2.1						
			Heterotrigona erythrogastra		14.9 ± 0.7	12.3 ± 0.9	nd	45.2 ± 1.1						
Tanzania			*Dactylurina schmidti*		25.93 ± 3.31	24.53 ± 1.39	1.33 ± 0.64		1260 Infinity II LC System (Agilent	Carbohydrates column		HPLC-RID	Isocratic mobile phase: Acetonitrile: water (78:18, v/v); flow rate: 1.0	77
			Hypotrigona gribodoi		20.20 ± 3.76	30.64 ± 1.88	0.86 ± 0.27							

Meliponula beccarii	16.50 ± 2.12	28.30 ± 0.99	2.60 ± 2.97	Technologies)	mL/min, sample volume: 10 µL,
Meliponula ferruginea	14.28 ± 1.85	29.56 ± 2.73	0.51 ± 0.34		detector temperature: 35 °C, column
Meliponula togoensis	14.87 ± 1.71	28.91 ± 3.33	0.46 ± 0.37		temperature: 30 ºC.
Plebeina armata	16.28 ± 1.27	28.35 ± 1.24	0.51 ± 0.24		

Mean values ± standard deviation, LOD: limit of quantitation, LOQ: limit of quantitation, nd: not detected, HPLC: high-performance liquid chromatography, HPAEC: high-performance anion exchange chromatography, RID: refractive index, PAD: pulsed amperometric, ELSD: evaporative light scattering.

Table S3. Quantification of minor sugars in honeybee and stingless bee honeys by chromatography techniques.

Honeybee honey												
Botanical source	Origin	Isomaltose (%)	Xylose (%)	Melezitose (%)	Trehalose (%)	Raffinose (%)	Maltotriose (%)	Maltotetraose (%)	Melibiose (%)	Turanose (%)	Erlose (%)	Reference
Gossypium hirsutum	Nectar	1.66 ± 0.12	0.31 ± 0.10	0.36 ± 0.05	0.18 ± 0.07	0.08 ± 0.03	1.18 ± 0.04	0.89 ± 0.07	0.09 ± 0.03	0.03 ± 0.02	0.39 ± 0.16	66
Coriandrum sativum		1.96 ± 0.17	0.28 ± 0.03	0.39 ± 0.04	0.26 ± 0.08	0.09 ± 0. 03	1.29 ± 0.08	0.66 ± 0.15	0.12 ± 0.04	0.02 ± 0.01	0.54 ± 0.07	
Dalbergia sissoo		1.54 ± 0.12	0.14 ± 0.03	0.38 ± 0.04	0.16 ± 0.05	0.12 ± 0.04	1.26 ± 0.07	0.67 ± 0.05	0.18 ± 0.04	0.03 ± 0.01	0.69 ± 0.19	
Murraya koenigii		1.16 ± 0.13	0.31 ± 0.07	0.26 ± 0.05	0.11 ± 0.05	0.06 ± 0.02	1.15 ± 0.06	0.85 ± 0.07	0.12 ± 0.04	0.03 ± 0.03	0.45 ± 0.16	
Botanical source	Origin	Isomaltose (%)	Xylose (%)	Melezitose (%)	Trehalose (%)	Raffinose (%)	Maltotriose (%)	Maltotetraose (%)	Reference			
		0.92 ± 0.03	0.21 ± 0.02	0.46 ± 0.06	0.06 ± 0.01	0.08 ± 0.02	0.90 ± 0.06	0.75 ± 0.03	65			
		0.66 ± 0.12	0.29 ± 0.05	0.29 ± 0.03	0.17 ± 0.03	0.04 ± 0.02	0.85 ± 0.04	0.63 ± 0.06				
		0.81 ± 0.03	0.18 ± 0.06	0.33 ± 0.04	0.07 ± 0.03	0.10 ± 0.03	0.68 ± 0.03	0.86 ± 0.10				
		0.70 ± 0.03	0.16 ± 0.02	0.19 ± 0.03	0.02 ± 0.01	0.06 ± 0.01	0.48 ± 0.01	0.49 ± 0.04				
Botanical source	Origin	Turanose (%)	Melezitose (%)	Trehalose (%)	Reference							
Comercial honey - Multifloral (year 2010)	Nectar	2.37 ± 0.29	1.63 ± 0.70	<LOQ	21							
Comercial honey - Multifloral (year 2011)		2.42 ± 0.26	1.71 ± 0.58	<LOQ								
Carob		2.19 ± 0.78	2.34 ± 1.98	<LOQ								
Eucalyptus		2.98 ± 0.69	2.56 ± 0.78	<LOQ								
Sunflower		1.3	<LOQ	<LOQ								
Orange		2.38 ± 0.03	<LOQ	<LOQ								
Multifloral		3.05 ± 0.68	3.30 ± 1.92	<LOQ								
Arbutus		2.44	1.89	<LOQ								
Rosemary		2.85 ± 0.49	1.84 ± 0.71	<LOQ								
Rosemary + heather		2.34	5.61	<LOQ								
Thyme		3.65	5.2	<LOQ								
Thyme + rosemary		2.81	1.25	<LOQ								
Heather		2.74 ± 0.50	2.30 ± 0.74	<LOQ								
Botanical source	Origin	Turanose (%)	Isomaltose (%)	Reference								
Acacia	Nectar	0.43 ± 0.30	0.01 ± 0.01	64								
Becium grandiflorum		1.03 ± 0.25	1.52 ± 1.02									
Croton macrostachyus		0.82 ± 0.37	0.01 ± 0.03									
Eucalyptus globulus		1.73 ± 0.36	0.16 ± 0.09									
Hypoestes		0.87 ± 0.05	0.49 ± 0.04									
Leucas abyssinica		0.92 ± 0.24	0.19 ± 0.10									
Schefflera abyssinica		0.33 ± 0.36	0.00 ± 0.0									
Syzygium guineense		1.34 ± 0.11	0.13 ± 0.09									
Botanical source	Origin	Erlose + melezitose (%)	Raffinose (%)	Reference								
Q. frainetto Ten (Italian oak)	Honeydew	0.6 ± 0.5	0.1 ± 0.1	50								
Botanical source	Origin	Turanose (%)	Melezitose (%)	Trehalose (%)	Reference							
Oak	Honeydew	3.1 ± 0.8	0.1 ± 0.1	nd	27							
Evergreen Oak		2.4 ± 1.1	0.9 ± 2.2	0.1 ± 0.1								
Botanical source	Origin	Trehalose (%)	Turanose (%)	Isomaltose (%)	Melezitose (%)	Reference						
	Honeydew	1.89 ± 0.42	1.28 ± 0.25	1.11 ± 0.40	0.75 ± 0.39	57						
Botanical source	Origin	Turanose (%)	Trehalose (%)	Melezitose (%)	Erlose (%)	Raffinose (%)	Reference					
	Honeydew	2.1	0.74	4.23	0.53	0.35	68					
Botanical source	Origin	Melezitose (%)	Trehalose (%)	Reference								
	Honeydew	0.6 ± 0.6	0.3 ± 0.6	3								
Stingless bee honey												
Botanical source	Bee species	Isomaltose (%)	Melezitose (%)	Raffinose (%)	Trehalose (%)	Reference						
	Melipona beecheii	0.66 ± 0.64	0.16 ± 0.44	0.22 ± 0.51	0.91 ± 1.49	73						
	Tetragonisca angustula	1.27 ± 0.92	0.56 ± 0.84	0.45 ± 0.86	0.14 ± 0.56							
Botanical source	Bee species	Trehalulose (%)	Reference									
	Heterotrigona itama	28.38 ± 9.05	18									
	Geniotrigona thoracica	49.08 ± 5.84										
	Tetragonula carbonaria	23.18 ± 4.80										
	Tetragonula hockingsi	24.90 ± 6.49										
Botanical source	Bee species	Arabinose (%)	Reference									
Ziziphus joazeiro Mart.	*M. subnida duke*	nd	45									

Mimosa quadrivalvis L.		0.5 ± 0.0		
Mimosa arenosa willd Poir		0.4 ± 0.1		
Croton heliotropiifolius Kunth		1.0 ± 0.1		
Ziziphus joazeiro Mart.	*M. subnida latrelle*	0.3 ± 0.0		
Mimosa quadrivalvis L.		0.4 ± 0.1		
Mimosa arenosa willd Poir		0.2 ± 0.0		
Croton heliotropiifolius Kunth		0.7 ± 0.0		
Botanical source	Location	Bee species	Trehalulose	Reference
	Distance less than 20 km from the West Coast	*Heterotrigona itama*	45.16 ± 6.62	74
	Distance less than 40 km from the West Coast		35.29 ± 16.70	
	Inland (more than 40 km from each coast)		39.03 ± 13.61	
	Distance less than 40 km from the East Coast		44.83 ± 8.20	
	Distance less than 20 km from the East Coast		38.95 ± 12.53	

Mean values ± standard deviation. LOD: limit of quantitation, LOQ: limit of quantitation, nd: not detected.