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REVIEW ARTICLE

Sugar detection and quantification in honey bee and stingless bee honey—A review

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ABSTRACT
Bee products are renowned for their beneficial properties and nutritional value. This association has been supported by scientific research that delves into their composition and biological activities. Given the increasing interest in natural products and the prevalence of fraudulent practices, understanding the sugar profiles of honeys is of paramount importance. In this review, we explore the sugars found in honey bee honey and stingless bee honey sourced from both floral nectar and honeydew. Additionally, our study provides a comprehensive review of direct and indirect methods of sugar analysis, highlighting the advantages and disadvantages of each method. Direct and indirect methods for sugar analysis include liquid and gas chromatography, vibrational spectroscopy, and nuclear magnetic resonance. These techniques form the foundation for the quality control of honey. High concentrations of trisaccharide and trehalulose have been 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 the quantification of 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 proved effective in quantifying sugar in honey.

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Introduction

Since ancient times, honey has been consumed as a sweetener and has been widely used in human nutrition and medicine by different cultures (Dobre et al., 2012; Mayer et al., 2014; Mohammed, 2022; Seraglio et al., 2019). Honey is mainly composed of a sugar solution (corresponding to approximately 95–99 g/100 g of honey dry matter) and minor components such as vitamins, amino acids, phenolic compounds, organic acids, minerals, aromatic substances, pigments, pollen grains, and enzymes (Nešović et al., 2020; Seraglio et al., 2019). To produce honey, bees collect these substances and combine them 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 during periods of food shortage (Ávila et al., 2018).

Honey can be classified based on its entomological origin (bee species) and food source (nectar or honeydew) (Shamsudin et al., 2019). In terms of bee types, there are two types of beekeeping: apiculture and meliponiculture. Apiculture aims to breed *Apis* species from the Apini tribe, while meliponiculture refers to the breeding of stingless bee species from the Meliponini tribe (Nordin et al., 2018). The characteristics of honey are closely related to bee species and food sources, which are mainly influenced mainly by seasonal, geographical, and environmental factors (Siddiqui et al., 2017).

Research carried out by Grand View Research in 2022 (a market research company), 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 increasing demand for natural and organic products and the growing trend toward healthy lifestyle habits have boosted the honey market (Honey Market Size, 2023). 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 of inferior quality (Machado et al., 2020).

Quality standards emphasise the importance of determining the product's origin to enable specific labelling, which requires the identification of

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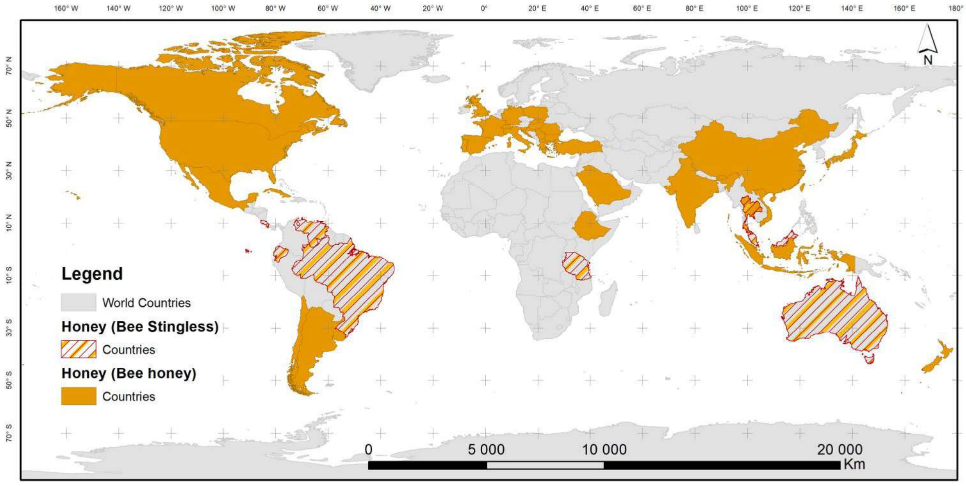

Figure 1. Geographical distribution of honey bee honeys and stingless bee honeys presented in this review.

biomarkers (Mădaş et al., 2020). Sugar profiles have been widely used to differentiate blossom honeys from honeydew honeys due to the presence of relatively high levels of trisaccharides and relatively low levels of monosaccharides in honeydew honeys (Pita-Calvo & Vázquez, 2017). More recently, the presence of trehalulose, a previously unidentified disaccharide, has been identified as a biomarker in samples of stingless bee honey (Fletcher et al., 2020). Furthermore, sugar profiling combined with chemometric tools has also been used to indicate botanical, geographical, and entomological origin (Karabagias et al., 2018; Pascual-Maté et al., 2018; Vit et al., 2023; Zawawi et al., 2022).

Determining the sugar profile of honey is also crucial for detecting fraud. Specifically, saccharides and mannose markers can reveal adulteration with sugar syrups (Egido et al., 2024; Pita-Calvo & Vázquez, 2017). Furthermore, the analysis of sugar content helps elucidate the physical properties of honey, such as hygroscopy, granulation, and viscosity (Mădaş et al., 2020). Crystallization, which is influenced by the ratio of fructose to glucose, significantly impacts consumer preference. According to Grand View Research, consumers often prefer to physically inspect honey before purchasing it, indicating that its physical characteristics play a key role in their buying decisions (Honey Market Size, 2023).

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 ionisation (FID) (Pita-Calvo & Vázquez, 2018). More recently, alternative methods, such as Fourier transform infrared spectroscopy (FTIR), near-infrared spectroscopy (NIR), Raman spectroscopy (RAMAN) and nuclear magnetic resonance (NMR), have been used (Anjos et al., 2015, 2018; Geană et al., 2020; Schievano et al., 2020).

Each of the above methods has its own advantages and disadvantages regarding sensitivity, suitability, and cost-effectiveness. Therefore, the aim of this study was to review the sugar profiles of honey bee honey and stingless bee honey and present direct and indirect methods of sugar analysis, highlighting the pros and cons of each method. The findings in this review aim to shed light on 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 articles published in the last decade from the IS Web of Science, Scopus, Google Scholar, and PubMed databases. Figure 1 presents the geographical distribution (by country) of honey bee honey and stingless bee honey included in this review.

Honey classification

Honey bee honey

Honey bees refer to any of the members of the genus *Apis* (Tribe Apini, family Apidae, order Hymenoptera) (Rangel & Fisher, 2019). Honey bees are social and live in colonies in the presence of a queen, drones, and workers. They are medium-sized insects, typically between 10 and 15 mm long, with a body divided into three main segments: the head, thorax, and abdomen. Honey bees have well-developed mandibles for manipulating materials, a long proboscis adapted for collecting nectar, and a functional sting for protection. Their wings are structured for efficient flight and foraging, while their compound eyes, complemented by three ocelli, enable them to detect light intensity and navigate their environment effectively (Michener, 2007).

The queen has a larger body than the workers and it 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. The worker bees are responsible for all the work in the hive. In the first phase of their life, they are responsible for the internal cleaning of the hive, when they become older, they begin to dedicate themselves to defending the hive, and collecting nectar, pollen, water, and resin (Rangel & Fisher, 2019).

There are several species of bees in the *Apis* genus, however, *Apis mellifera* honey is the most widely produced and traded honey worldwide. Honey bee honey can be classified according to its source: honeydew or flower nectar (Brugnerotto et al., 2019; Seijo et al., 2019; vanEngelsdorp & Meixner, 2010). Various behaviours, some of which are unique to the *Apis* genus, have evolved and facilitated the transformation of nectar or honeydew into honey (Berenbaum & Calla, 2021).

According to FAO (2021), China is the world's largest producer and exporter of honey from *Apis mellifera*, while European Union countries occupy the second position. The main characteristic of *Apis mellifera* honey refers to its moisture content, which is reduced to a final concentration of approximately 20 g/100 g. A high concentration of sugars and moisture content lower than 20 g/100 g in *Apis mellifera* honeys make the product microbiologically safe for commercialization at room temperature since pathogenic bacteria are not able to multiply in supersaturated sugar solution (Berenbaum & Calla, 2021).

Codex Alimentarius (Codex Alimentarius Commission, 2001) 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/100 g, a diastase activity greater than 8 Schade units, a HMF content of less than 40 mg per kg of honey, an electrical conductivity of less than 0.8 mS/cm, and a water-insoluble content of less than 0.1 g/100 g.

Stingless bee honey

Stingless bees, unlike honey bees, belong to the tribe Meliponini, within the same family Apidae. Stingless bees differ from honey bees in both their habits and morphology (Koethe et al., 2018; Lavinas et al., 2019; Nordin et al., 2018). Compared to honey bees, stingless bees exhibit greater diversity and are generally smaller, ranging from 2 to 10 mm in length, depending on the species. Like honey bees, they share the same basic body segmentation but possess a rudimentary or absent sting. Instead, they rely on their mandibular glands for defence, often using biting as their primary mechanism (Roubik, 2006).

The body shape of stingless bees can vary considerably, with some species exhibiting elongated forms and others appearing more compact. Their wings are adapted for shorter flights. They preferentially collect nectar from nearby creeping flowers and are more specialized in the pollination of local flora (Grüter, 2020; Heard, 1999; Hrncir et al., 2019). Unlike honey bees, stingless bees build their hives in a horizontal position and store their food in pots rather than combs (Ávila et al., 2018; Ramírez et al., 2018). Figure 2 shows an example of a stingless bee colony (*Tetragonisca angustula*).

Honey from stingless bees differs from honey from honey bees in terms of its physicochemical, microbiological, and antioxidant properties. In general, stingless bee honey has a more fluid texture, slower crystallisation and more pronounced sensory

Figure 2. Example of a colony of stingless bee (*Tetragonisca angustula*).

characteristics in terms of color, flavor, and acidity (Barbosa et al., 2018; Braghini et al., 2022). 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 (Ali et al., 2020; Hau-Yama et al., 2020).

Despite the benefits of honey from stingless bees and the increasing consumer interest in this product, there are several limitations to large-scale production. The primary constraint is the low yield, with stingless bees producing only 1–5 kg of honey per colony per year, compared with 20 kg per colony per year for *Apis mellifera*. This lower productivity has been attributed to the smaller size of stingless bee colonies (Ávila et al., 2018; Braghini et al., 2020a; Schvezov et al., 2020). Furthermore, the lack of international legislation regarding stingless bee honey limits its global distribution (Ávila et al., 2018; Braghini et al., 2020b, 2022).

Although there is no international legislation for stingless bee honey, Brazil and Malaysia have developed their own specific regulations for this type of product. In Malaysia, the MS 2683:2017 standard defines the quality requirements for stingless bee honey, including physicochemical and microbiological criteria, hygiene practices, testing methods, and requirements for packaging and labelling (Malaysian Standard, 2017). In December 2024, the standard was revised and released as DMS 2683, reflecting industry developments and further strengthening the safety and quality of the product.

In Brazil, regulations for physicochemical parameters have been established in the states of 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). Reference data for the physicochemical parameters of stingless bee honey from these states can be found in the article by Sato et al. (2023)—Table 4. A review by Sato et al. (2023) revealed that most of the physicochemical data on Brazilian honey published between 2017 and 2022 adhered to these regional standards.

In general, stingless bee honey has lower sugar and total soluble solids content and higher levels of water activity, moisture and acidity compared to *Apis mellifera* honey (Ávila et al., 2018; Bana et al., 2025; Braghini et al., 2022). 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 crystallisation (Sousa et al., 2016). The relatively high moisture content and water activity of stingless bee honey may 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 not contain more than 20 g/100 g moisture (Ávila et al., 2018). 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 (Nordin et al., 2018; Ribeiro et al., 2018).

Blossom honey

Blossom honey is derived from flower nectar and is the most common and commercialised type worldwide (Ng et al., 2020). 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 (Costa et al., 2018). Monofloral honey has unique organoleptic qualities, such as specific fragrances and flavors derived from a single type of flower, making it a high-value product. However, on the current market, multifloral honey and blended honey are more commonly available. Blended honeys consist of mixtures of two or more types of honey with different characteristics (Machado et al., 2020).

The characteristics of monofloral honey are highly dependent on the type of vegetation and seasonal changes (Mohammed, 2022). Plant diversity is generally proportional to the size of a country, meaning that larger countries typically have a greater variety of monofloral honeys. While honey is a globally traded product, honey from specific flowers is often unique to the country where those flowers are found (Mărgăoan et al., 2021).

Honeydew honey

During occasional periods of nectar shortage, honey bees and stingless bees collect honeydew to produce honey, which is known as honeydew honey or forest honey (Flanjak et al., 2021). Honeydew is obtained from the secretions produced by some plant species or from the excretions of insects, usually of the order Hemiptera (Seeburger et al., 2020). These insects suck sap from the phloem of coniferous trees such as pine, spruce, oak and fir to produce honeydew (Nešović et al., 2020; Seeburger et al., 2020; Shaaban et al., 2020).

Honeydew honeys are widely consumed and appreciated by Europeans due to their strong and distinctive flavour (Bergamo et al., 2019;

Purcărea et al., 2014). Compared with blossom honey, this type of honey typically has a darker colour, higher viscosity and less sweetness. Furthermore, honeydew honeys have a smaller market share and a higher sales price (Vasić et al., 2019).

Blossom honey and honeydew honey can differ greatly in terms of chemical composition (Brugnerotto et al., 2019). The composition of honeydew honey can vary depending on the insect species, their host plants, and the interactions between them. Seasonal changes also contribute to variations in the chemical and physical composition of honey (Shaaban et al., 2020). Honeydew honey commonly has a higher nitrogen concentration, higher electrical conductivity and pH, lower glucose and fructose concentrations and higher concentrations of trisaccharide (Pita-Calvo & Vázquez, 2017; Seraglio et al., 2021).

Some physicochemical parameters can indicate whether honey is derived from honeydew or flowers nectar. Honeydew honey must contain a minimum content of 45 g/100 g reducing sugars and a minimum electrical conductivity of 0.8 mS/cm (Bergamo et al., 2019; Codex Alimentarius Commission, 2001). The electrical conductivity and sugar profile are often used to distinguish blossom honey from honeydew honey (Manzanares et al., 2011; Pita-Calvo & Vázquez, 2017). Some studies have also demonstrated the presence of more bioactive compounds in honeydew honey, which is related to its antioxidant activity (Nešović et al., 2020).

Sugars in honey

The sugars in honey are mainly formed by the action of enzymes produced by honey bees on nectar or honeydew (De-Melo et al., 2017; Pita-Calvo & Vázquez, 2018). Honey production is only possible because of the temperature of the hive (30 °C) and the inversion of sucrose (Alvarez-Suarez, 2017; De-Melo et al., 2017). 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-glucosyls 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 (Alvarez-Suarez, 2017; De-Melo et al., 2017).

In honey, glucose and fructose are the main monosaccharides (Ressutte et al., 2025). Glucose is a monosaccharide classified as an aldohexose due to its composition of six carbon atoms and an aldehyde functional group. Fructose, while having the same molecular formula, differs structurally; it is a ketohexose, distinguished by six carbon atoms and a ketone group in place of the aldehyde (Berg et al., 2015) (Figure 3). The fructose/glucose ratio (F/G) and the glucose/water ratio (G/W) are parameters used to measure the ability of honey to crystallise. Typically, honey with low G/W and high F/G values does not crystallise easily (Baloš et al., 2020). Crystallisation occurs because glucose has low solubility in water (909 g/L at 25 °C), whereas fructose is significantly more soluble, being 4.4 times more soluble (4000 g/L at 25 °C) (Amariei et al., 2020). Over time, glucose tends to precipitate in the form of glucose monohydrate, and the solution changes to a more stable saturated state (Baloš et al., 2020).

Sucrose is also an important sugar found in honey and it is a disaccharide composed of glucose and fructose units linked by an α(1→2)β glycosidic bond (Berg et al., 2015) (Figure 3). 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 (Bergamo et al., 2019; Codex Alimentarius Commission, 2001). 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 (Alvarez-Suarez, 2017; De-Melo et al., 2017; Tedesco et al., 2020) (Figure 4). A complete description of the results reported in the literature, with the concentrations of sugars analysed by chromatographic techniques, can be found in the (supplementary material Tables S1–S3).

It can be observed that glucose, fructose, and maltose are the most analysed sugars (Tables 1 and 2), and the fructose content is higher than the glucose content in both honey bee honey and stingless bee honey (Tables S1 and S2). Maltose is a disaccharide composed of two glucose molecules linked by α-(1→4) glycosidic linkages (Berg et al., 2015) (Figure 3). In honey bee honey, maltose has been reported at low concentrations, with most honeys containing less than 2% of this sugar, which is considered a minor component of this type of honey (Anjos et al., 2015; Belay et al., 2017; Dobre et al., 2012; Flanjak et al., 2021; Geană et al., 2020; Ghramh et al., 2020; Kamboj et al., 2020; Nayik et al., 2019; Seijo et al., 2019).

More recently, the disaccharide trehalulose was identified as a major sugar in stingless bee honey (Fletcher et al., 2020; Popova et al., 2021; Ramlan et al., 2024; Zawawi et al., 2022). Trehalulose is an isomer of sucrose, formed by the monosaccharide's

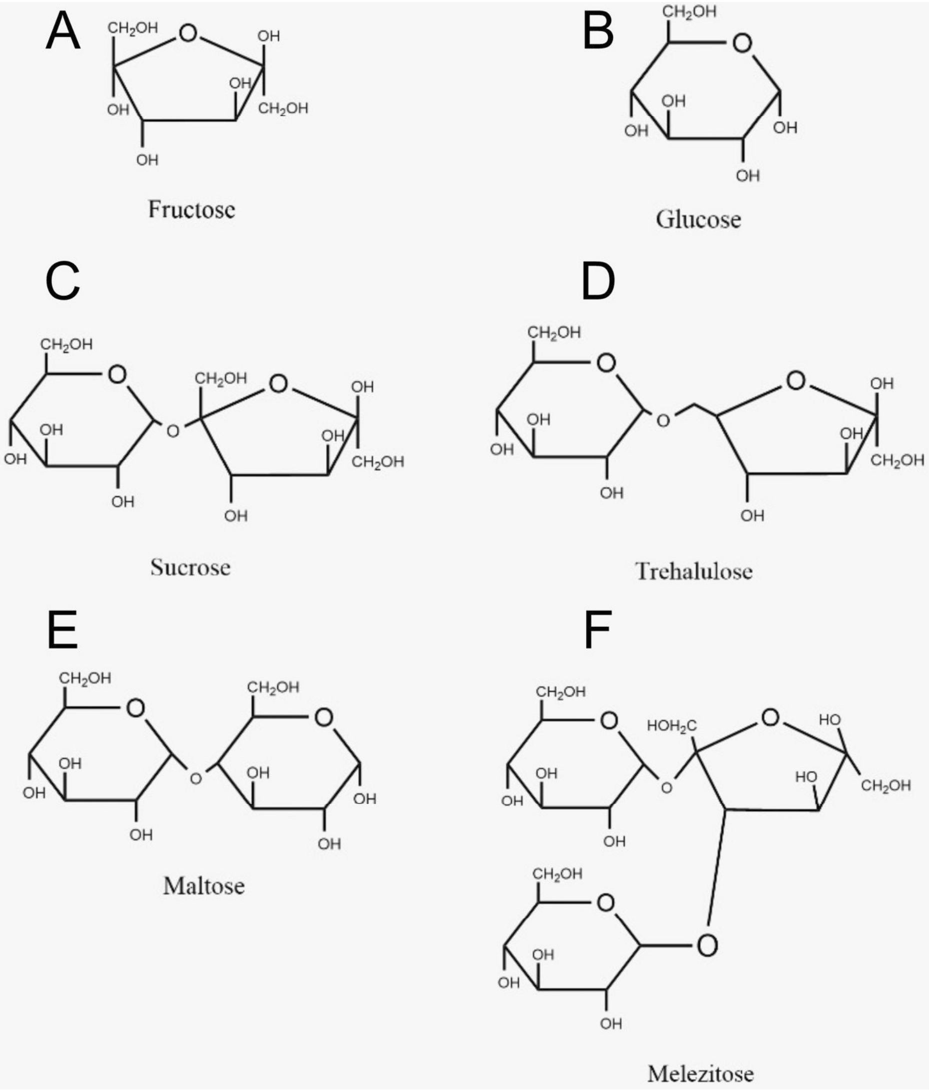

Figure 3. Chemical structure of A) Fructose, B) Glucose, C) Sucrose, D) Trehalulose, E) Maltose and F) Melezitose.

glucose and fructose joined by glycosidic linkages α-(1→1) (Figure 3). Compared with sucrose, this sugar is metabolised three times more slowly by enzymes in the small intestine, resulting in a more gradual release of glucose and fructose into the bloodstream, which may be beneficial for people with diabetes (Fletcher et al., 2020). Research carried out by Hungerford et al. (2021) showed that stingless bees fed with glucose and fructose solutions did not produce the disaccharide trehalulose, suggesting that the formation of this sugar occurs through nectar rich in sucrose.

According to Fletcher et al. (2020), trehalulose may be misidentified as maltose due to the similarity of chromatographic peaks between these two sugars; however, in the study by Ramlan et al. (2024), 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. (2020), the authors used preparative HPLC to isolate trehalulose and performed UPLC–MS/MS and NMR analyses to elucidate the spectra corresponding to this sugar. Five honey samples were analysed: *Tetragonula hockingsi* and *Tetragonula carbonaria* from Australia, *Geniotrigona thoracica* and *Heterotrigona itama* from Malaysia and *Tetragonisca angustula* from Brazil. The values obtained using the UPLC–MS/MS technique ranged from 13 to 44 g/100 g. *Geniotrigona thoracica* honey from Malaysia presented the highest content of trehalulose (84% of the total sugar content).

Zawawi et al. (2022) quantified glucose, fructose, sucrose and trehalulose in 36 honey samples of the species *Heterotrigona itama* and *Geniotrigona thoracica* from Malaysia, and from the species *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 have also been reported in other studies (Popova et al., 2021; Ramlan et al., 2024; Zaidi et al., 2023). An inverse relationship with the content of fructose and glucose can be observed in honeys with high levels of Trehalulose content; that is, the

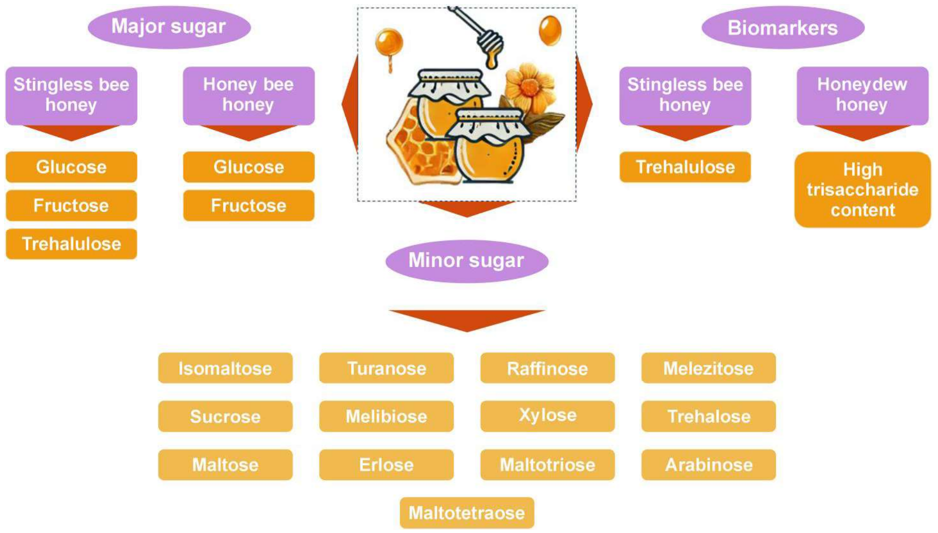

Figure 4. Types of honey and sugars found in literature.

higher the trehalulose content is, the lower the amount of glucose and fructose in the honey (Table S2).

In addition to maltose, sucrose can also coelute 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 the maltose values reported by Chuttong et al. (2016) in samples from Thailand (*Tetragonula laeviceps*, 15–57 g/100 g), Shamsudin et al. (2019) in samples from Malaysia (*Heterotrigona itama*, 22.56–37.35 g/100 g; *Geniotrigona thoracica*, 28.96–43.15 g/100 g), and Vit et al. (1998) in samples from Venezuela (*Frieseomelitta* aff. *varia*, 56.9 g/100 g), and sucrose values reported by Marcolin et al. (2021) in samples from Brazil (*Tetragonisca angustula*, 15.5–25.4 g/100 g). Other studies also reported high values for sucrose or maltose in stingless bee honeys (Table S2) (Abdul Malik et al., 2020; Bana et al., 2025; Kek et al., 2017; Tuksitha et al., 2018; Wong et al., 2019). Although Fletcher et al. (2020) stated that trehalulose is a biomarker of stingless bee honey, relatively few samples have been analysed for trehalulose so far, indicating the need to analyse a larger number of honeys from different species and locations to determine whether trehalulose can indeed be considered a biomarker of stingless bee honey (Zaidi et al., 2023; Ressutte et al., 2025).

To date, few studies have quantified the sugars in honeydew honeys, with these studies focused on a few origins and countries (Tables 1 and 2) (Pita-Calvo & Vázquez, 2018). 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, than blossom honey does, which could be good indicators of the type of honey (Pita-Calvo & Vázquez, 2017). According to the research of Pascual-Maté et al. (2018), honeydew honey samples presented higher disaccharide and trisaccharide contents (6.71 and 1.81 g/100 g, respectively) and lower monosaccharide contents (63.10 g/100 g) than heather honey.

Melezitose has been found in both blossom honey and honeydew honey, but it is generally present in higher concentrations in honeydew honeys (Pita-Calvo & Vázquez, 2017). This sugar constitutes approximately 70 g/100 g of the sugar content in honeydew excreted by insects and 40 g/100 g in honeydew secreted by plants (Behera & Balaji, 2021; Nešović et al., 2020). Melezitose 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 its optical rotation and crystalline form (Behera & Balaji, 2021) (Figure 3). In the case of honey bees, the consumption of honeydew has been linked to the cause of the disease called 'honeydew flow disease'. Owing to its high molecular weight, melezitose is not properly metabolized in the honey bee gut, resulting in malnutrition and hive death (Behera & Balaji, 2021). Seeburger et al. (2020) carried out feeding trials and reported that honey bees fed with melezitose showed severe disease symptoms such as abdomen tipping, swollen abdomen and impaired movement.

Sugar concentrations combined with chemometric tools have been used to indicate floral, geographic, and entomological origin. Vit et al. (2023) analysed samples of 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 raffinose content.

Table 1. Chromatographic techniques for sugar analysis in honey bee 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 °C	Ghramh et al. (2020)
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	Belay et al. (2017)
India	Nectar	Saffron, apple, cherry, *Plectranthus rugosus*	Glucose, fructose, sucrose, maltose, xylose, isomaltose, trehalose, raffinose, maltotriose, melezitose and maltotetraose	Waters isocratic (Waters^{TM})	Waters X-bridge Amide (Waters^{TM})	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	Nayik et al. (2019)
Poland and Slovaki	Nectar	Multifloral, tilia, forest, rape, acacia	Glucose, fructose, and sucrose	Dionex UltiMate 3000 (Thermo Fisher Scientific)	Asahipak NH2P-50 4E (Shodex^{TM})	HPLC-CD	Isocratic mobile phase: Acetonitrile: water (78:22, v/v); flow rate: 1 mL/min, sample volume: 10 μL, column temperature: 55 °C	Tomczyk et al. (2019)
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	Dobre et al. (2012)
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	Kamboj et al. (2020)
Serbia	Nectar	Meadow, acacia, *Tilia tomentosa*, multifloral	Glucose, fructose, and sucrose	Dionex UltiMate 3000 (Thermo Fisher Scientific)	Hypersil GOLD^{TM} 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 °C	Baloš et al. (2020)
Portugal	Nectar	Multifloral, carob, eucalyptus, sunflower, orange, multifloral, arbutus, rosemary, 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	Anjos et al. (2015)

(*continued*)

Table 1. Continued.

Geographical origin	Source	Botanical origin	Sugar	Equipment	Column	Detector	Operation conditions	Reference
		thyme, thyme + rosemary, heather						
Romania	Nectar	*Robinia pseudoacacia* (acacia), *Brassica napus oleifera* (rape), polyfloral, *Tilia platyphyllos* (linden), *Helianthus annus* (sunflower), mixed (acacia with some other floral impurities; polyfloral honey with small quantity of honeydew; acacia with floral sources; rape with linden floral sources; and rape with polyfloral floral sources), commercial honey (undeclared floral source)	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: 30 °C, and detector temperature: 95 °C	Geană et al. (2020)
Croatia	Honeydew	*Quercus 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	Flanjak et al. (2021)
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	Purcărea et al. (2014)
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	Seijo et al. (2019)
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: 30 °C, and detector temperature: 95 °C	Geană et al. (2020)
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: 25 °C	Manzanares et al. (2011)
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	Victorita et al. (2008)
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	Dobre et al. (2012)

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 and *Melipona scutellaris* Latrelle	Nectar	*Ziziphus joazeiro* Mart., *Mimosa quadrivalvis* L., *Mimosa arenosa* willd Poir and *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: 80 °C, detector temperature: 50 °C	Sousa et al. (2016)
Thailand	*Homotrigona fimbriata*, *Lepidotrigona terminata*, *Lepidotrigona flavibasis*, *Lepidotrigona doipaensis*, *Lisotrigona furva*, *Tetragonilla collina*, *Tetragonula fuscobalteata*, *Tetragonula laeviceps-pagdeni*, *Tetragonula testaceitarsis*, *Tetrigona apicalis* and *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 °C	Chuttong et al. (2016)
Malaysia	*Heterotrigona itama*			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	Cheng et al. (2019)
Malaysia	*Heterotrigona itama* and *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	Shamsudin et al. (2019)
Ecuador	*Scaptotrigona polysticta*, *Melipona indecisa*, *Melipona cramptoni*, *Paratrigona sp.*,			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	Villacrés-Granda et al. (2021)

(*continued*)

Table 2. Continued.

Geographical origin	Bee species	Source	Botanical origin	Sugar	Equipment	Column	Detector	Operation conditions	Reference
	Melipona sp., *Cephalotrigona* sp., *Melipona mimetica, Tetragonisca angustula, Trigona silvestriana, Oxytrigona mellaria, Nannotrigona chapadana,* and *Melipona grandis*								
Malaysia and Australia	*Heterotrigona itama, Geniotrigona thoracica, Tetragonula Carbonari,* and *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 °C	Zawawi et al. (2022)
Malaysia	*Heterotrigona itama* and *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	Wong et al. (2019)
Costa Rica	*Melipona beecheii* and 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	Umaña et al. (2023)
Malaysia	*Heterotrigona itama*			Glucose, fructose, sucrose, maltose, and trehalulose	Waters e2695 (Waters$^{\text{TM}}$)		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	Ramlan et al. (2024)
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.	Kek et al. (2017)
Malaysia	*Geniotrigona thoracica, Heterotrigona itama* and			Glucose, fructose, and, maltose	Waters (WatersTM)	SupercosilTM LC-NH2	HPLC-RID	Isocratic mobile phase: acetonitrile: water (72:25), flow rate: 1 mL/min, oven	Tuksitha et al. (2018)

(*continued*)

Table 2. Continued.

Geographical origin	Bee species	Source	Botanical origin	Sugar	Equipment	Column	Detector	Operation conditions	Reference
	Heterotrigona erythrogastra							temperature: 40 °C.	
Tanzania	*Dactylurina schmidti, Hypotrigona gribodoi, Meliponula beccarii, Meliponula ferruginea, Meliponula togoensis* and *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 °C.	Mduda et al. (2023)

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

Geotrigona honeys presented 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. (2022) study, the sugar profiles revealed that Malaysian samples of *Heterotrigona itama* and *Geniotrigona thoracica* had higher trehalulose content and lower glucose and fructose levels than Australian samples of *Tetragonula carbonaria* and *Tetragonula hockingsi*. Specifically, *Heterotrigona itama* presented 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* presented trehalulose levels of 23.18 g/100 g, glucose 12.10 g/100 g, and fructose 17.43 g/100 g, and *Tetragonula hockingsi* presented 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. (2018) investigated the differences among honeydew honeys, heather honey, chestnut honey, and lavender honey from the northern Iberian Plateau in terms of sugar profiles. Lavender samples showed the highest sucrose levels (0.05–5.18 g/100 g), whereas 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 have highlighted sugar profiles as being important for distinguishing groups through multivariate analysis in terms of botanical, geographical, and entomological origins. For example, Karabagias et al. (2018) differentiated Greek honeydew honeys from pine and fir based on geographical origin; Cotte et al. (2004) classified honey samples from France based on botanical origin, and Vit et al. (1998) clustered samples of stingless bee honey from Venezuela based on entomological origin. Sousa et al. (2016) differentiated samples of Brazilian stingless bee honeys based on their botanical origin and Mduda et al. (2023) separated samples of stingless bee honey from Tanzania based on entomological origin.

Sugar analysis

Chromatography techniques

Chromatography has been widely used to identify, separate, and quantify different sugars in honey (Zhou et al., 2014). Considering the physical state of the mobile phase, chromatography can be divided into liquid (HPLC and HPAEC) and gas (GC) methods, all of which are based on the same basic principles (Figure 5).

The fundamental principle is the separation of substances into two phases: a stationary phase and

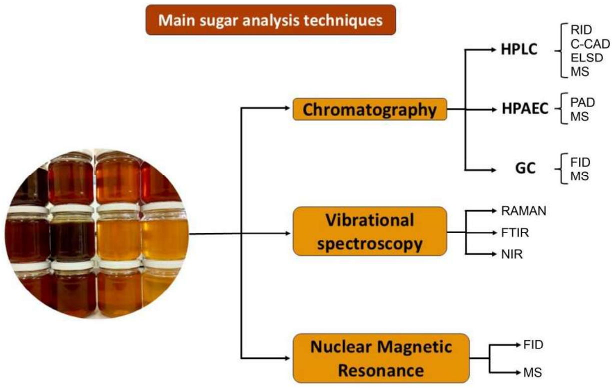

Figure 5. Main sugar analysis techniques in honeys.

a mobile phase. Each analyte has a different interaction time with these two phases, which can be measured by the detector (McNair et al., 2019).

The greatest difficulty in separating sugars in honey samples is the structural similarity among them, which are formed mainly by different glycosidic bonds between glucose and fructose (Alvarez-Suarez, 2017).

Various detectors coupled with chromatography, such as RID, PAD, MS, C-CAD, and ELSD, can be used to analyse sugars in honey. For GC, FID can also be used (Pascual-Maté et al., 2018; Pita-Calvo & Vázquez, 2018; Tomczyk et al., 2019).

High-performance liquid chromatography (HPLC)

HPLC is a versatile technique that allows the separation of a wide variety of compounds, including both ionic and nonionic compounds. In HPLC, Refractive Index Detector (RID) is the most commonly used detector for sugar analysis. Its principle is based on detecting variations in the refractive index 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 with other detectors, RID is more economical. However, it has some drawbacks, such as the need for strict control of internal parameters, its lack of selectivity, and its incompatibility with gradient elution (Condezo-Hoyos et al., 2015; Jalaludin & Kim, 2021; Vennard et al., 2020).

Essentially, the mobile phase is chosen according to the column specifications. Sugar molecules contain polar groups, therefore, in terms of HPLC-RID, solvents with high polarity, such as acetonitrile, methanol, and water, are recommended. It has been shown that the use of a single solvent is not capable of promoting adequate separation of sugars; because of this, a mobile phase consisting of a mixture of solvents is needed (Xu et al., 2015). For RID, a mixture of acetonitrile and water is the most commonly used mobile phase (Tables 1 and 2) (Baloš et al., 2020; Belay et al., 2017; Cheng et al., 2019; Chuttong et al., 2016; Ghramh et al., 2020; Kamboj et al., 2020; Nayik et al., 2019; Shamsudin et al., 2019; Tomczyk et al., 2019; Villacrés-Granda et al., 2021). However, other mobile phases can be used, such as water acidified with sulphuric acid or other acids (Villacrés-Granda et al., 2021).

Many columns are compatible with the separation of sugars *via* HPLC-RID. In recent studies (2016–2023), the following columns have been reported: ZORBAX Carbohydrate (Agilent Technologies) (Belay et al., 2017; Ghramh et al., 2020), Waters X-bridge Amide (Waters Corporation) (Nayik et al., 2019), Hi-Plex PB (Agilent Technologies) (Kamboj et al., 2020), Hypersil GOLD Amino (Thermo Scientific) (Baloš et al., 2020), Inertsil NH2 (GL Sciences) (Chuttong et al., 2016), Luna®NH2 100 Å (Phenomenex Inc.) (Cheng et al., 2019; Shamsudin et al., 2019), Zorbax NH2 (Agilent Technologies) (Villacrés-Granda et al., 2021) and Econosphere amino (Grace/Alltech) (Umaña et al., 2023).

Ghramh et al. (2020) and Belay et al. (2017) used HPLC-RID and a 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. (2021) used a Zorbax NH2 column (Agilent Technologies) with sulphuric 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 recognised as universal detectors and are considered more suitable than RID due to their high detection sensitivity and compatibility with gradient elution, which allows the use of multiple eluents. Additionally, these detectors maintain stable baselines during chromatographic runs. ELSD and C-CAD detectors can analyse a wide variety of compounds, and are regarded as comprehensive response instruments for HPLC (Márquez-Sillero et al., 2013; Zhou et al., 2014). The operation of ELSD and C-CAD is straightforward, requiring the configuration of a few parameters, such as pressure, gas input, signal output range, and temperature.

The equipment operates in three main steps: effluent nebulisation, mobile phase evaporation, and detection. In the ELSD detector, the aerosol generated during nebulisation is transported to a heated region where the mobile phase is vaporised. 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 *via* a sensitive electrometer (Costa & Conte-Junior, 2018; Márquez-Sillero et al., 2013).

ELSD and C-CAD detectors are suitable for analysing 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 mobile phases need to be non-volatile and volatile, respectively (Costa & Conte-Junior, 2018; Márquez-Sillero et al., 2013). 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. (2013) showed that the concentrations of glucose and fructose found in honey samples were similar when both detectors were used.

Wong et al. (2019) used HPLC-ELSD to analyse fructose, glucose, maltose and sucrose from 96 monofloral (*Acacia mangium*) honeys produced from *Heterotrigona itama* and *Tetrigona binghami* species. The authors used acetonitrile/water (75:25 v/v) as the mobile phase, and sugar separation was performed using a Zorbax Carbohydrate column (Agilent Technologies). HPLC-ELSD has been reported as an effective method for separating maltose and trehalulose by Ramlan et al. (2024) and Zaidi et al. (2023). Zaidi et al. (2023) used a mixture of acetonitrile and deionised water (85:15 v/v) and an Inertsil NH2 column (GL Science). Tomczyk et al. (2019) used HPLC coupled with 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 ionised *via* an ionisation source. Each ionized molecule forms a molecular ion, which is then 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 (Glish & Vachet, 2003). Sugars are typically ionised by electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI). The MS detector offers advantages of high sensitivity, selectivity, and specificity. It can analyse a wide range of compounds. However, executing the analysis and interpreting mass spectra requires specialized knowledge, and a specific column for sugar analysis is also necessary (Glish & Vachet, 2003; Zaidi et al., 2023).

Zaidi et al. (2023) analysed 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 a Bruker Amazon SL ion trap mass spectrometer (ITMS) with electrospray ionisation (ESI). According to the authors, despite HPLC-ELSD being effective in separating maltose and trehalulose, mass spectrum data are crucial for ensuring the correct identification of sugars, especially since the retention times of trehalulose, maltose, and sucrose are very close to each other.

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 (Kwon & Jeong, 2019; Vennard et al., 2020). 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 oxidisable functional groups under the applied voltage (Kwon & Jeong, 2019; Rohrer, 2013).

Most carbohydrates have pKa values in the range of 12–14. 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 (Corradini et al., 2012). 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 (Corradini et al., 2012; Rohrer, 2013).

Anjos et al. (2015) 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 used was a gradient of sodium hydroxide. Zawawi et al. (2022) used HPLC-PAD to analyse 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. (2020) analysed 64 honeydew honeys from Croatia and quantified trehalose, arabinose, glucose, fructose, melibiose, isomaltose, sucrose, melezitose, turanose, raffinose, maltose, panose, and maltotriose. 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.

Gas chromatography (GC)

The primary distinction between GC and traditional HPLC lies in the utilisation of a gaseous mobile phase, such as nitrogen, hydrogen, or helium. For the separation of sugars in GC, detectors such as FID and MS are used. The FID detector operates by measuring analytes within a gas stream. Organic compounds undergo combustion in a hydrogen flame, producing ions. These ions are proportional 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 used as carrier gases in this process (Holm, 1999).

The main disadvantage of the GC method is the need for prior derivatisation to obtain volatile carbohydrate compounds (Pita-Calvo & Vázquez, 2018). Although GC methods are quite selective, the effort and time required to derivatise sugar compounds make analysis difficult. Therefore, few researchers choose to use this method in the analysis of sugar in honey (Vennard et al., 2020). Pascual-Maté et al. (2018) analysed the sugar composition of 54 honeys from the northern Iberian Peninsula. 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 analysed by GC-FID using helium as the carrier gas.

Shamsudin et al. (2022) 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 derivatisation. The authors used an HP 5973 mass selective detector (Agilent Technologies) and a 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. (2022) analysed 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 analysed *via* a GC coupled to a 5973 Network Mass Selective Detector with a quadrupole mass selective detector (electron ionisation) (Agilent Technologies). The column used was a 122–5532 UI DB–5MS UI (Agilent Technologies), and helium gas was used as the carrier gas.

Vibrational spectroscopy

Alternative analytical methods, such as vibrational spectroscopy, have been used to detect fraud in honeys, and to verify their chemical structure (Figure 5). Spectroscopy has the advantages of being a fast, non-destructive and easy-to-operate method; in addition, the amount of sample necessary to perform the analysis is small, and sample preparation is minimal or negligible (Anjos et al., 2018; Pita-Calvo & Vázquez, 2018). 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}), mid-infrared (400–4000 cm^{-1}) and far (10–400 cm^{-1}) regions. The methods of vibrational spectroscopy most commonly used for honey sugar analysis are Fourier transform infrared spectroscopy (FTIR), near infrared spectroscopy (NIR) and Raman spectroscopy (Abbas et al., 2020; Geană et al., 2020).

In a spectrum, signals are associated with functional groups such as -COOH and -OH. Therefore, the spectrum represents a simple sum of individual sample components observed in specific bands that help estimate the chemical structure of a molecule. In the past, samples were analysed step by step, and with the arrival of Fourier transform (FT) spectroscopy, data collection started to be performed simultaneously in a wide spectral range (Abbas et al., 2020; Smith, 2011; Šugar & Bouř, 2016). To determine the sugar content in a 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 analysed *via* chemometric methods (Anjos et al., 2015). Examples of chemometric tools include 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 (Aliaño-González et al., 2019; Özbalci et al., 2013). Table 3 shows the complete results found in the literature for vibrational spectroscopy.

Fourier-transform infrared spectroscopy (FTIR)

The principle of infrared analysis involves the application of infrared light with different wavelengths to 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} (Abbas et al., 2020). Attenuated total reflectance (ATR) combined with FTIR has been used to improve the spectral collection power (Mellado-Mojica et al., 2016). Anjos et al. (2015) analysed 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 to develop calibration models. For fructose, glucose, melezitose and turanose, good models were found.

Se et al. (2018) analysed 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. (2021) also quantified glucose, fructose, and sucrose in honey samples using FTIR and PLS. Tewari and Irudayaraj (2004) used FTIR with micro-attenuated 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. (2010) 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 between 1500 and 800 cm^{-1} presented distinct peaks for all sugar mixtures used for calibration.

Raman spectroscopy (RAMAN)

RAMAN is based on the interaction of molecular vibrations with high-energy radiation emitted by a monochromatic beam of light. When a 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) (Abbas et al., 2020; Naila et al., 2018; Wu et al., 2017). Özbalci et al. (2013) quantified fructose, glucose, sucrose, and maltose in 22 samples of honey from Turkey *via* 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. (2005) determined the concentrations of fructose and glucose in 21 monofloral honeys *via* FT-RAMAN. The quantitative determination was performed simultaneously in the spectral region between 700 and 1700 cm^{-1}. The PLS chemometric tool was used for data analysis, and the values were compared with those obtained *via* HPLC. Both methods (HPLC and FT-RAMAN) showed similar results and scored equally in terms of reproducibility. In another study, Mignani et al. (2015) used RAMAN spectroscopy with a dispersive detection scheme and excitation at 1064 nm to analyse 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 applied PCA and PLS to predict the sugar profile. In the RAMAN technique, it is possible to analyse vibrations at very low wavelengths since the spectrum acquisition region occurs in the range of $4000-50\,cm^{-1}$ (Abbas et al., 2020); however, according to Šugar and Bouř (2016), when honey samples are irradiated with lower wavelengths, fluorescence can be considered a side effect, and non-saccharide 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 optical activity (ROA) at a wavelength of 532 nm and used activated carbon to prepare 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. (2020) proposed a simple method to analyze honey *via* 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.

Near infrared spectroscopy (NIR)

NIR measures the absorption of radiation by molecular chemical bonds. In this technique, the spectral acquisition region is in the range of 12,000 cm^{-1} to 4,000 cm^{-1} (Abbas et al., 2020). Relatively few studies have used NIR for sugar analysis in honey. Ruoff

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

Geographical origin	Bee type	Source	Botanical source	Sugar	Analytical technique	Chemometric tool	Reference
Portugal	Honey bee	Nectar	Multifloral, carob, eucalyptus, sunflower, orange, multifloral, arbutus, rosemary, rosemary + heather, thyme, thyme + rosemary and heather	Sucrose, glucose, fructose, trehalose, turanose, maltose and melezitose	FTIR-ATR	PCA and PLS	Anjos et al. (2015)
Malaysia	Stingless bee *Heterotrigona itama*	Nectar		Glucose, fructose and maltose	FTIR-ATR	PCA and PLS	Se et al. (2018)
India	Honey bee	Nectar		Glucose, fructose, and sucrose	FTIR	PLS	Rajalakshmi et al. (2021)
Pennsylvania	Honey bee	Nectar		Sucrose, glucose, fructose, and maltose	FTIR-mATR	PCR and PLS	Tewari and Irudayaraj (2004)
U.S.A., Thailand, China, Bhutan, U.K., Canada, Indonesia, Japan, New Zealand, and Vietnam	Honey bee	Nectar		Maltose, sucrose, glucose, and fructose	FTIR–ATR	PCR and PLS	Wang et al. (2010)
Turkey	Honey bee	Nectar		Fructose, glucose, sucrose, and maltose	Raman	PCA, PLS, ANN	Özbalci et al., 2013
Greece	Honey bee	Nectar		Fructose and glucose	FT-Raman	PLS	Batsoulis et al. (2005)
Italy	Honey bee	Nectar	Chestnut, citrus, and acacia	Fructose, glucose, maltose	Raman	PCA and PLS	Mignani et al. (2015)
Switzerland, German, Argentina, Chile, China, Cuba, France, Greece, Hungary, Italy, Mexico, Slovakia, Slovenia, and Uruguay	Honey bee	Nectar	*Castanea* sp., *Robinia* sp., *Tilia* spp., *Brassica* spp., *Taraxacum* spp., *Rhododendron* sp., alpine polyfloral and polyfloral	Glucose, fructose, and sucrose	FT-NIR	PLS	Ruoff et al. (2007)
Switzerland, German, Argentina, Chile, China, Cuba, France, Greece, Hungary, Italy, Mexico, Slovakia, Slovenia, and Uruguay	Honey bee	Honeydew		Glucose, fructose, and sucrose	FT-NIR	PLS	Ruoff et al. (2007)
Spain	Honey bee	Nectar		Fructose and glucose	NIR	MLR, PCR and MPLS	García-Alvarez et al. (2000)

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.

et al. (2007) used FT-NIR to quantify glucose, fructose, and sucrose in 421 honeys of different botanical origins and from different countries. The chemometric tool PLS was used to develop calibration models for sugars. In another study, NIR spectroscopy was used to analyse fructose and glucose in 161 honey samples collected over three consecutive years. Calibration models using MLR, PCR, and MPLS were developed. For samples collected in the same year, good predictions were obtained (García-Alvarez et al., 2000).

Nuclear magnetic resonance (NMR)

In recent years, the use of NMR techniques combined with chemometric tools for the analysis of honey has attracted 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 (Figure 5) (Del Campo et al., 2016; Razali et al., 2018). The principle of NMR involves the interaction of atomic nuclei, such as protons, with both static and oscillating magnetic fields. Initially, the 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 provide information about various organic constituents in a sample, offering a comprehensive view of its chemical structure (Razali et al., 2018).

The use of chemometric models for quantifying metabolites *via* NMR is often necessary due to signal intensity variations, signal overlap, and interference from solvents or other substances present in the sample. High efficiency ^{1}H NMR or ^{13}C NMR methods have been reported in several manuscripts (Table 4). The chemical shift range for ^{1}H NMR is between 0 and 12 ppm, whereas for ^{13}C NMR, it is between 0 and 220 ppm. Compared with traditional methods, NMR has the advantage of allowing the determination of a wide range of analytes in a single run; in addition, NMR is a fast method (Hatzakis, 2019; Karabagias et al., 2018). As a drawback, NMR equipment has high acquisition and maintenance costs.

Del Campo et al. (2016) used ^{1}H NMR to quantify 13 analytes, including sugars, in honey samples from Spain, with quantification based on external standards and considering the signal area of each analyte. According to the authors, the central region of sugars is in the range of 3.1–6.0 ppm. The strongest signals in this region correspond to glucose and fructose, overlapping 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 and 5.21 ppm, and for fructose, the signals at 4.00 and 4.10 ppm were assigned. For maltose, sucrose and turanose, signals at 5.38, 5.42 and 5.28 ppm were assigned, respectively.

Fletcher et al. (2020) analysed the disaccharide trehalulose in five samples of stingless bee honeys from different species and countries *via* ^{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 the country of origin of the honey.

Consonni and Cagliani (2008) analysed 41 honey samples (Acacia and multifloral) from different countries using ^{1}H NMR spectroscopy. Sugars were identified in the signal range between 3.0 and 5.5 ppm. The signals at 5.17 and 4.58 ppm were assigned to glucose (α and β forms, respectively), and the signals at 4.04 and 3.97 ppm were assigned to α-fructose. Other minor sugars were identified by the addition of a 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 indicated predominant fructose and glucose present in different tautomeric isoforms. Fructose presented α,βFF and α,βFP forms, whereas glucose presented only α,βGP forms. The same authors analysed the sugar profiles of 86 samples of Italian honey *via* ^{1}H NMR and ^{13}C NMR spectroscopy. In the aforementioned publication, the identified sugars were fructose, glucose, sucrose, raffinose, kojibiose, isomaltose, maltose, gentiobiose, maltotriose, melibiose, maltulose, melezitose, palatinose, nigerose, kestose, erlose, turanose, isomaltotriose, and maltotetraose (Consonni et al., 2012).

Vit et al. (2023) 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. (2013), 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 analysed 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

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

Geographical origin	Bee type	Origin	Botanical source	Sugar	Analytical technique	Chemometric tool	Reference
Spain	Honey bee	Nectar		Glucose, fructose, maltose, sucrose, and turanose	^{1}H NMR		Del Campo et al. (2016)
Australia, Malaysia, and Brazil	Stingless bee: *Tetragonula hockingsi, Tetragonula carbonaria, Geniotrigona thoracica, Heterotrigona itama* and *Tetragonisca angustula*	Nectar		Trehalulose	^{1}H NMR		Fletcher et al. (2020)
Argentina, Hungary, EC countries, and Italy	Honey bee	Nectar	Acacia and polyfloral	Glucose, fructose, sucrose, turanose, raffinose,	^{1}H NMR and ^{13}C NMR	PCA and PLS-DA	Consonni and Cagliani (2008)
Italy	Honey bee	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	Consonni et al. (2012).
Italy, Hungary, South America, China	Honey bee	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	Consonni et al. (2013)
Macedonia and Bulgaria	Honey bee	Nectar	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	Gerginova et al. (2020)
Macedonia and Bulgaria	Honey bee	Honeydew		Fructose, glucose, quinovose, sucrose, kojibiose, trehalose, trehalulose, isomaltose, nigerose, maltose, leucrose, maltulose,	^{1}H NMR and ^{13}C NMR	PCA, PLS-DA, ANOVA	Gerginova et al. (2020)

(*continued*)

Table 4. Continued.

Geographical origin	Bee type	Origin	Botanical source	Sugar	Analytical technique	Chemometric tool	Reference
				turanose, gentiobiose, isomaltulose, raffinose, melezitose, isokestose, panose, and erlose			
Italy	Honey bee	Nectar	Multifloral, acacia, orange, and citrus	Sucrose, maltose, turanose, glucose, raffinose, and isomaltose	^{1}H NMR	PCA, PLS-DA, OPLS-DA, ANOVA	Girelli et al. (2020)
Italy	Honey bee	Honeydew		Sucrose, maltose, turanose, glucose, raffinose, and isomaltose	^{1}H NMR	PCA, PLS-DA, OPLS-DA, ANOVA	Girelli et al. (2020)
Greece	Honey bee	Honeydew	Pine and fir	Fructose, glucose, sucrose, turanose, and raffinose	^{1}H NMR	MANOVA, LDA	Karabagias et al. (2018)
Greece	Honey bee	Nectar	Pine, chestnut, spruce, thyme, heather, citrus, and polyfloral	Glucose, fructose, maltose, isomaltose, nigerose, sucrose, turanose, maltulose, erlose, maltotriose, isomaltotriose, panose, melezitose and 1-kestose	^{1}H NMR and ^{13}C NMR		Kazalaki et al. (2015)
Ecuador	Stingless bee: *Geotrigona*, *Scaptotrigona* and *Melipona*			Fructose, glucose, sucrose, gentiobiose, maltose, maltotriose, mannose, melizitose, raffinose, and turanose	^{1}H NMR	PCA, HCA	Vit et al. (2023)
Malaysia	Stingless bee: *Geniotrigona thoracica* and *Heterotrigona itama*	Nectar	Multifloral	Glucose, fructose, and sucrose	^{13}C NMR	PCA	Ng et al. (2021)
Malaysia	Stingless bee: *Geniotrigona thoracica* and *Heterotrigona itama*	Honeydew	Acacia	Glucose, fructose, and sucrose	^{13}C NMR	PCA	Ng et al. (2021)

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.

identifying sugars, with the monosaccharides glucose and fructose present in greater abundance.

Ng et al. (2021) investigated the seven main sugar tautomers of glucose, fructose, and sucrose in 23 stingless bee honey samples from the species *Heterotrigona itama* and *Geniotrigona thoracica* in Malaysia, derived from both honeydew and floral nectar, using ^{13}C NMR spectroscopy. The compositions of the main sugars in both types of honey (honeydew and blossom) were not significantly different. The sucrose content found in the blossom honey in this study (0.40%) met the limit established by Malaysian regulations. Honeydew honey was found to have a very low sucrose content, which was practically undetectable by ^{13}C NMR. The glucose and fructose contents in the stingless bee honey samples (ranging from 73% to 73.61%) also complied with the limit set by the Department of Standards Malaysia, which is a maximum of 85.0%. The presence of trehalulose in the analysed stingless bee honey samples was not detected, contradicting previous findings by Fletcher et al. (2020) and Zawawi et al. (2022) for the same bee species. The authors also applied PCA to distinguish between blossom honey and honeydew honey according to their physicochemical properties and antioxidant activity.

Honeydew honeys and blossom honeys from Macedonia and Bulgaria were analysed by ^{1}H NMR and ^{13}C NMR. With the aid of chemometric tools, it was observed that the honeydew honeys from North Macedonia presented higher amounts of maltulose, kojibiose, Trehalulose and nigerose, while the samples from Bulgaria presented 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 to describe this monosaccharide in honey (Gerginova et al., 2020). Other studies have also used ^{1}H and ^{13}C NMR to quantify sugars in honeys (Girelli et al., 2020; Karabagias et al., 2018; Kazalaki et al., 2015; Ohmenhaeuser et al., 2013; Popova et al., 2021).

Conclusions

The precise determination of honey origin is guided by quality standards, necessitating the identification of biomarkers for accurate labelling. Sugar profiles play crucial roles in distinguishing different types of honey, with honeydew honey typically exhibiting relatively high levels of trisaccharides, whereas 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 honey bee honeys and stingless bee honeys. This study summarises the sugar profiles of both honey bee honeys 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 such as 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 commonly 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, have provided alternative approaches for sugar quantification.

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