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REVIEW

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# Microbiological and physical-chemical characteristics of pollen and honey from stingless bees: a review

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**Abstract**

The physical, chemical and sensory characteristics of pollen and honey from stingless bees (SLB) are quite different when compared to those produced by *Apis mellifera*. Meliponine honey has a high moisture content (23–37.5%); reducing sugars (12.65–77.11%); and total acidity (11.23 to 124.2 mEq kg-1). Pollen also has fiber (0.87–13.65%); a high moisture content (23–53.93%); lipids (1.8–10.81%) and proteins (8–37.63%). The presence of yeasts, fungi and bacteria can be observed through fermentation (ethanolic and acid) that occurs during the maturation of pollen and honey from stingless bees. Among the microorganisms most associated with stingless bees are yeasts: *Pichia*, *Zygosaccharomyces*, *Starmerella*, *Metschnikowia*, *Candida*, *Debaryomyces*, *Dekkera* and *Kloeckera*; bacteria: *Streptomyces*, *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Neisseria*, *Bacillus*, *Ralstonia*, *Staphylococcus*, *Enterobacter*, *Lysinibacillus Pantoea*, *Fructobacillus Pseudomonas*, and *Clostridium*; and fungal filaments *Aspergillus*, *Talaromyces* and *Penicillium*. This review corroborates the differences that exist in the physicochemical and microbiological characteristics of stingless bee products and honeybee (*Apis melifera*) products. These differences not only challenge established standards of quality and identity in apicultural products but also pave the way for new perspectives in biotechnology and nutrition, as well as for new bioactive compounds. The unique properties of pollen and honey from Meliponini, such as high moisture content, acidity, and microbial diversity, can be a distinguishing factor that enhances their applications in various technological fields. The valorization of these unique characteristics may stimulate the creation of specific standards for these products and promote the sustainable use of stingless bee biodiversity.

**Keywords** *Meliponini*, Physicochemical parameters, Microbiota

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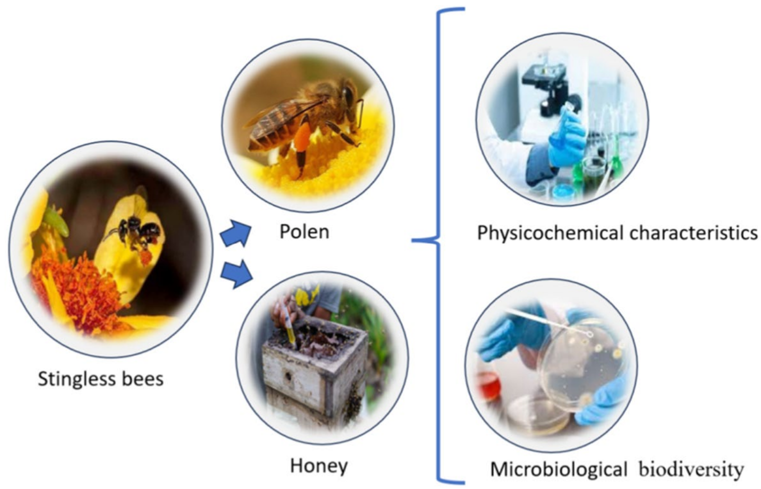
**Graphical Abstract**




## Introduction

Bees belonging to the order *Hymenoptera*, family *Apidae*, subfamily *Apinae* and tribe *Meliponini*, are popularly known as SLB because they have atrophied aculeus and, therefore, are unable to sting. The group comprises more than 500 species and 61 genera known worldwide, distributed across subtropical and tropical regions (Africa, tropical America, Southeast, Australia and Asia) (Hrncir et al., 2016; Souza et al., 2021).

Brazil is home to a great diversity of these species, with 244 identified, belonging to 29 different genera, 50% of these species are endemic to the Atlantic Forest, an area considered one of the largest tropical forest biomes in Brazil. SLBs have also been identified in Mexico and Costa Rica, with Mexico being one of the main countries to develop research on bee products (Cortopassi-Laurino et al., 2006; Pedro, 2014; Popova et al., 2021).

Australia has developed meliponiculture for a long time, but there are still few studies on the diversity of SLB species. Conducting research on this subject is important considering the pollination process of these bees in agricultural crops (Halcroft et al., 2013). In Africa, about 50 species of SLB have already been identified (Chidi and Odo 2017). The creation of SLB on the African continent is still very artisanal, some communities use trunks and clay pots as breeding grounds (Cortopassi-Laurino et al., 2006).

These insects, in addition to playing an important socioeconomic role, also contribute directly to the ecological structure of the ecosystems in which they are inserted, as they are the main pollinators of agricultural crops and natural environments (Koser et al., 2020; Pedro, 2014).

Meliponines store food (nectar and pollen) for long periods, aiming to ensure the survival of the colony during resource scarcity events, and transferring materials from the mother nest to the newly founded one (Michener, 2013).

Pollen and nectar are important resources for the colony, with pollen being the main source of vitamins, lipids and protein for bees, and nectar being the main source of carbohydrates. They are stored separately in pots of cerumen (a mixture of substances collected from plants: resin and wax) built by the bees themselves (Maia-Silva et al., 2015).

The pollen is stored in the colony inside the pots, being processed by the bees that deposit nectar and some secretions rich in enzymes, together with microorganisms. Afterwards, the pots are sealed, making the environment suitable for fermentation that transforms the collected pollen into a new product known as samburá (fermented pollen stored by bees), which is rich in essential nutrients for the survival of the colony (Cella et al., 2017; Nogueira-Neto, 1997).

The nectar of the flowers is transported through the melliferous vesicle (honey crop), a kind of bag where it also receives enzymatic secretions from the glands of the abdomen and the cephalic glands of the bees; this nectar begins to be processed, and later inside the colony this product is stored, matured, and transformed into honey. The most important enzymes for the transformation of honey and pollen from SLB are: invertase, amylase and glucose oxidase (Chuttong et al., 2016; Elias-Santos et al., 2013; Simone-Finstrom & Spivak, 2010).

Meliponines honey has very different chemical, physical, and sensorial characteristics when compared to those of honey by the Apis bee. For example, they differ in color, flavor, and viscosity, have a lower total carbohydrate and a high moisture content, are more acidic and their crystallization is slow; they also have microorganisms such as yeast and bacteria that induce their fermentation(Ávila et al., 2018; Biluca et al., 2014; De Almeida-Muradian et al., 2013).

The interior of these bees' pots becomes an anaerobic environment when completely filled and closed with cerumen. The composition of pollen and honey produced by bees, added to the high humidity of the environment, has favored microbial interactions and development. These interactions can be observed inside the hives, which harbor a diverse microbiota, composed of filamentous fungi, yeasts and bacteria (de Paula et al., 2021).

Microorganisms can interact with bees by symbiosis, contribute to bee nutrition, produce biomolecules that help transform products such as nectar and pollen, and even break down molecules that the bee is unable to digest. Some species can act by producing toxins and/or antimicrobial compounds that are intended to inhibit the growth of pathogens (de Paula et al., 2021; Douglas, 2015; Souza et al., 2021; Stefanini, 2018);.

The microbiota associated with pollen and honey from *Meliponini* bees is still poorly studied, as is information about the involvement of yeasts in the biochemical transformation of these products. However, the presence of these microorganisms can be perceived through the fermentation of these foods that are stored inside the colonies. The environment inside the nest works as a natural bioreactor, the composition of the substrate provides favorable conditions for the growth of yeasts and other microorganisms with biotechnological potential (da Silva et al., 2024; Kwong et al., 2017).

The pollen used by stingless bees has better nutritional composition and biological properties when compared to the pollen normally used by Apis bees, consequently, phenolic compounds, in their broad spectrum, can bring different health benefits. It is worth reiterating that the composition of honey depends on several geoclimatic factors (Komosinska-Vassev et al., 2015; Thakur & Nanda, 2020). This work presents condensed literature data related to the pollen used by SLB. Typically articles present data from some species and/or specific regions.

In view of the above, the aim of this literature review is to provide information on the physicochemical and microbiological characteristics of pollen and honey produced by SLB. Compiling this data into an article is extremely important, as studies on SLBs and their products are relatively recent. The results presented in the different articles evaluated at environmental, zoo technological, health benefits and as a source of nutraceutical food (bioactive compounds), etc., provide an overview of the different characteristics of pollen and honey from different species of SLB at a global level.

### Bibliographic research

The bibliographical research carried out focused on data referring to stingless bees, mainly with regard to the microbiological and physical-chemical characteristics of pollen and honey. To obtain this information, virtual data from different sources were accessed, including: Pubmed, theses, Science Direct, Scielo database, Medline, CAPES journal portal, e-books, Google Scholar and environmental/food virtual library.

The indexing terms used for individual searches were stingless bee, honey, pollen, physicochemical parameters of pollen and honey, microbiological quality of pollen and honey, yeasts, bacteria and filamentous fungi of pollen and honey. After evaluating the bibliographic sources, articles that did not correspond to the object of the study were excluded.

### Stingless bee

The SLB belong to the order *Hymenoptera*, family *Apidae*, subfamily *Apinae*, tribe *Meliponini*, and are popularly called "SLB" because they have an atrophied shape of the stinger, being unable to sting. They constitute a group formed by more than 500 species known worldwide, being found in the tropical and subtropical regions of South and Central America, Australia, Africa and Southeast Asia (Moure et al., 2007; Michener, 2013; Ribeiro et al., 2018).

SLBs live in colonies made up of many workers, which carry out cooperative work with a high degree of organization in the construction of the structure and functioning of the colonies. The workers collect and process the food, in addition to protecting the young (Hrncir et al., 2016; Silva et al., 2014); the queen is responsible for laying eggs, and for organizing the colony through communication based on pheromones produced in her mandibular glands (Ribeiro et al., 2018).

The colonies are made up of two main elements: the food pots (honey and pollen), the nest (brood discs), in addition to auxiliary structures, such as the casing, batume, entrance tunnel (Camargo & Pedro, 2003). To withstand long periods of adversity and protect their offspring, SLBs store food in pots located within the colony (Michener, 2013). Long-term storage and the conditions under which these foods are stored can favor numerous microbial interactions (de Paula et al., 2021).

The food pots are cerumen structures built by the bees themselves to store honey and pollen, which usually have a circular or oval shape, but can have different shapes depending on the species. Pollen and honey are stored separately in these pots, and thus, in a SLB colony, these two types of food pots can be found (Villas-Bôas, 2018).

The nest is built with various materials collected by the bees, such as mud, clay, resins, and others; wax, cerumen, and geopropolis are produced or processed within the colony (Nogueira-Neto, 1997).

SLBs, in addition to playing an important socioeconomic role, as the sale of their honey is considered a means of supplementary income for small and medium-sized beekeepers, also contribute directly to the ecological structuring of the ecosystems in which they operate. They are important propagators of the pollination of various agricultural crops, thus helping the sexual reproduction of plants and ensuring a wide genetic variety of vegetables and fruits (Chuttong et al., 2016; Costa et al., 2018; Pedro, 2014).

Some SLB species are described in Fig. 1 (A-G) (Chuttong et al., 2016; Costa et al., 2018; Pedro, 2014; Villas-Bôas, 2018). **A-*Melipona (Melikerria) compressipes*** is a stingless bee species that has a wide occurrence in forests and savannas (cerrado) environments in Brazil. **B-*Frieseomelitta longipes*** is a small bee, which has elongated hind tibiae. The entrance to the colony resembles a cone formed by layers of resins of different colors and transparency. **C-*Melipona (Michmelia) paraensis*** is a species of bee whose nests are easily found in trees in flooded forests such as floodplain forests and igapó (blackwater-flooded) forests. This bee seeks to nest in trees such as andiroba (Carapa guianensi), and its colonies quickly establish themselves in hives for management. **D-*Melipona (Michmelia) fulva*** has a wide occurrence in forest and savanna environments. In areas of savannas (cerrado) and in fragments of this type of vegetation, they are easily found nesting in mameira (*Vitex megapotamica*). **E-*Tetragonisca angustula***, commonly called yellow jataí, measures about 4–5 mm. This bee (Meliponini tribe) does not have a sting either, and has reduced leg bristles and wing venation. Nests of this bee are essential in the recovery of forest habitats, but they are also present in structured forests, in impoverished forests, and in urban areas. Like other SLBs, this bee uses pre-existing cavities, such as cavities in walls, holes in tree trunks or even abandoned termite or ant nests, as a hive. **F-*Scaptotrigona bipunctata***, also called Tubuna, is a social bee of the meliponine subfamily, present in several states of Brazil. It has a black head, thorax, and abdomen. Measures 7 millimeters long. It has a shiny-looking body and has two stripes at the end of its abdomen, one next to the other, which from a distance looks like a single white stripe. It builds nests in holes in larger trees in nature. **G-*Cephalotrigona capitata***, also called Mombucão is a shy and non-defensive bee, added to the fact that this type of bee do not make any type of entrance structure (except some resin and/or wax to reduce the diameter of entrance when it is larger than they like). For that reason, it becomes very difficult to discover their nests in nature, unlike other bees of the genus *Scaptotrigona* or *Trigonas* that tend to be, for the most part, defensive and have "entry pits", often ornamented or with different and/or exotic formats. They have been considered by beekeepers

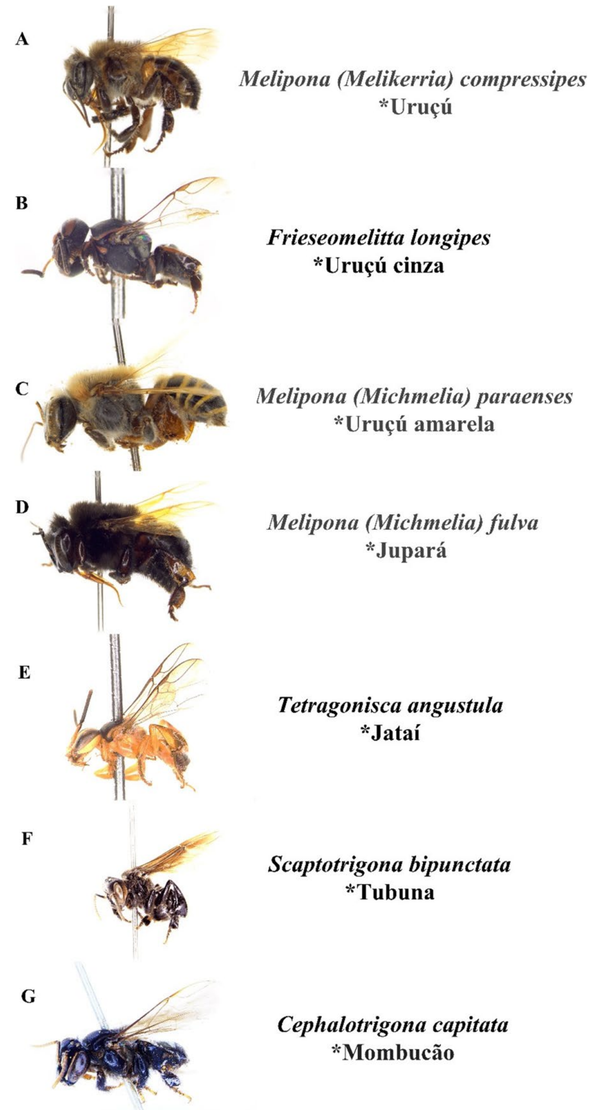



**Fig. 1** Species of stingless bees. * Popular Brazilian names. Figure created by the authors

**Table 1** Physicochemical characteristics of bee pollen established by Brazilian legislation, and results obtained from studies conducted with SLB pollen. Source of Brazilian legislation: Normative Instruction No. 03, of January 19, 2001 - Technical Regulation of Identity and Quality of Apicultural Pollen. Results obtained by Alves et al., (2018); Bárbara et al., (2018); Ferreira-Caliman et al., (2012); Oliveira et al., (2021); Rebelo et al., (2016) for pollen collected by different SLB species

| Specie | Moisture (%) | T.A (mEq kg$^{-1}$) | S.A (%) | *R.S* (%) | Ash (%) | D.A (ºGothe) | HMF (mg kg$^{-1}$) | Country | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *Cephalotrigona* sp. | 30.00 | 116.47 | - | 67.17 | 0.35 | 50.40 | 10.71 | Ecuador | (Villacrés-Granda et al., 2021) |
| *Nannotrigona chapadana* | 24.00 | 42.07 | | 77.11 | 0.40 | 12.00 | 10.98 | Ecuador | (Villacrés-Granda et al., 2021) |
| *Oxytrigona mellaria* | 30.00 | 58.84 | | 62.62 | 0.52 | 15.00 | 5.99 | Ecuador | (Villacrés-Granda et al., 2021) |
| *Paratrigona* sp. | 27.00 | 46.53 | - | 70.90 | 0.11 | 8.33 | 3.00 | Ecuador | (Villacrés-Granda et al., 2021) |
| *Melipona compressipes* | 22.47 | 21.52 | | | 0.11 | 3.45 | | Colombia | (Cardona et al., 2019) |
| *Nannotrigona* sp. | 30.19 | 25.36 | - | - | 0.24 | 3.77 | - | Colombia | (Cardona et al., 2019) |
| *Tetragonisca angustula* | 24.59 | 44.46 | - | - | 0.41 | 13.13 | - | Colombia | (Cardona et al., 2019) |
| *Tetragonisca angustula* | 17.45 | 17.39 | 4.83 | 65.78 | 0.35 | 12.27 | 0.20 | Guatemala | (Dardón & Eunice, 2008) |
| *Tetragonisca angustula* | 25.50 | 70.55 | - | 67.40 | 0.69 | 40.00 | 27.70 | Ecuador | (Villacrés-Granda et al., 2021) |
| *Melipona beechei* | 17.32 | 23.23 | 3.50 | 68.77 | 0.07 | 21.29 | 0.10 | Guatemala | (Dardón & Eunice, 2008) |
| *Melipona beechei* | 23.20 | 35.00 | | 67.70 | 0.16 | | 17.90 | Mexico | (Moo-Huchin et al., 2015) |
| *Melipona favosa* | 25.50 | 62.93 | 1.46 | 72.14 | 0.29 | | 1.20 | Venezuela | (Vit et al., 1994) |
| *Melipona* sp. | 26.50 | 34.60 | 1.60 | 65.30 | 0.16 | 2.90 | 11.10 | Venezuela | (Vit et al., 1998) |
| *Mlipona trinitatis* | 25.70 | 24.24 | 1.48 | 73,.66 | 0.12 | 10.00 | 1.30 | Venezuela | (Vit et al., 1994) |
| *Hypotrigona* sp. | 17.50 | 30.69 | 5.21 | 60.49 | - | - | 16.58 | Nigeria | (Nweze et al., 2017) |
| *Melipona* sp. | 13.86 | 11.23 | 5.06 | 75.64 | - | - | 5.50 | Nigeria | (Nweze et al., 2017) |
| *Meliponula beccarii* | 29.60 | - | - | - | 0.41 | - | 18.00 | Ethiopia | (Gela et al., 2021) |
| Tetragonilla collina | 28.00 | - | - | 52.00 | 0.24 | 0.34 | 5.90 | Thailand | (Chuttong et al., 2016) |
| *Tetragonula fuscobaltreata* | 26.00 | - | - | 32.70 | 0.67 | - | - | Thailand | (Chuttong et al., 2016) |
| *Tetragonula leaviceps* | 28.00 | - | - | 29.00 | 0.22 | 0.63 | 5.40 | Thailand | (Chuttong et al., 2016) |
| *Tetragonula leaviceps* | 26.98 | - | 19.15 | 47.87 | 0.26 | - | 1.07 | Thailand | (Suntiparapop et al., 2012) |
| *Tetragonula testaceitarsis* | 30.50 | - | - | 41.00 | 0.20 | - | 2.95 | Thailand | (Chuttong et al., 2016) |
| *Tetrigona apicalis* | 42.00 | | | 12.65 | 1.40 | | 0.25 | Thailand | (Chuttong et al., 2016) |
| *Trigona carbonaria* | 26.50 | 124.20 | 1.80 | 42.00 | 0.48 | 0.40 | 1.20 | Australia | (Oddo et al., 2008) |

as the "Ferrari of SLBs", for being tame and very productive, capable of producing 6 to 10 kilos of honey a year.

## Stingless bee pollen

Pollen is the main source of protein, lipids, and vitamins for bees (Brodschneider & Crailsheim, 2010). SLB pollen is a by-product made from pollen collected from flowers, which, after adding nectar and salivary enzymes (amylase and glycosidase) from the bee, undergoes a fermentation process in pots inside the colonies. SLB pollen can also be referred to as fermented pollen, pot pollen, stored pollen or samburá (Vit et al., 2016).

Pollen that has undergone fermentation has a varied chemical composition; in general, it consists of carbohydrates, proteins, and lipids, also containing other micronutrients, such as minerals (iodine, magnesium, calcium, chlorine, copper, molybdenum, iron, selenium), vitamins (A, B, C, D and E), phenolic compounds and essential amino acids. In addition to possessing anti-inflammatory, antioxidant, and antimicrobial properties. Due to the richness of nutrients, fermented pollen has been called a "perfectly complete food" (Kostić et al., 2015; Mc Fredrerik et al., 2012).

## Physicochemical parameters of stingless bee pollen

Fermented pollen has physicochemical characteristics that are different from bee pollen, as can be seen from the values established by Brazilian legislation, when compared to the results obtained by Alves et al., (2018), Rebelo et al., (2016), and Ferreira-Caliman et al., (2012) for physicochemical analysis of pollen collected by SLB (Table 1).

The results listed in Table 2 show that the SLB pollen samples have high moisture content, comparing with *Apis melliera* polen (28–53.93%), proteins (15.98–37.63%), lipids (2.5–10.81%), and for some species the amount of fibers is much higher (9.3–13.65%), when

**Table 2** Specifications of the Brazilian and international standards for the quality control of *Apis mellifera* honey. Source: Normative Instruction 11, of October 20, 2000 (Brasil 2000) and *Codex Alimentarius* (2020)

| Pollen | Moisture (%) | Protein (%) | Lipids (%) | Ahs (%) | Fiber (%) | Carbohydrates (%) | pH |
|---|---|---|---|---|---|---|---|
| *Apis mellifera* | 30,00 | 8,00 | 1,80 | 4,00 | 2,00 | 14,5 to 55 | 4,00 to 6,00 |
| *Melipona scutellaris* | 44,71 | 23,88 | 4,25 | 1,84 | 0,87 | 24,48 | 3,75 |
| *Melipona scutellaris* | 51,70 | 19,70 | 2,50 | 2,40 | 2,76 | - | 3,80 |
| *Melipona scutellaris* | 50,05 | 30,37 | 5,99 | 4,21 | 2,13 | - | 3,88 |
| *Melipona scutellaris* | 52,89 | 17,14 | 5,25 | 4,72 | - | - | 3,72 |
| *Melipona interrupta* | 37,12 | 24,00 | 6,47 | 2,74 | 13,65 | 44,27 | 3,34 |
| *Melipona seminigra* | 53,93 | 37,63 | 10,81 | 4,03 | 9,30 | 25,66 | 3,70 |
| *Scaptotrigona* sp. | 28,00 | 15,98 | 4,82 | 5,23 | 9,90 | - | 3,71 |
| *Melipona mandacaia* | 34,52 | 21,68 | 4,29 | 4,94 | 2,66 | - | 3,50 |
| *Melipona subnitida* | 30,44 | 23,19 | 4,21 | 5,54 | 3,56 | - | 3,51 |

compared to bee pollen. Physicochemical parameters for *Apis mellifera* pollen are determined because it is a product consumed in Brazil and in other countries such as Poland, Bulgaria, and Switzerland (Campos et al., 2010).

For meliponine pollen, standards of physicochemical parameters have not yet been established (Mohammad et al., 2021). SLB products have very peculiar physicochemical characteristics when compared to those of Apis bees, and it is important to carry out studies to obtain information on the physicochemical characteristics of products derived from SLB, in order to establish quality and identity standards, given the absence of specific legislation for these products and the great diversity of species that makes the composition of these products very variable (Villas-Bôas, 2018).

#### Microbiological quality of stingless bee pollen

The pollen microbiota favors the nutrition and development of the bees, assists in the digestive process of the bees and in the biochemical transformation of the product, in addition to protecting the colony against opportunistic microorganisms such as pathogenic ones (Engel et al., 2012; Roulston & Cane, 2000). Although records of the microbiota of *Meliponini* bee pollen are limited, it is possible to verify the presence of beneficial bacteria such as *Bifidobacterium*, *Lactobacillus*, *Bacillus* (Mohammad et al., 2021), yeasts such as *Zigosaccharomyces osmophilus* and *Starmerella* (Januário da Costa Neto & Benevides de Morais, 2020; Matos et al., 2020; Santos et al., 2018), and fungal species such as *Penicillium* and *Talaromyces* (Barbosa et al., 2018).

Lactic acid bacteria from fermented pollen can produce antimicrobial compounds, organic acids and enzymes that are industrially important for conferring health benefits, making fermented pollen a probiotic food (Mohammad et al., 2021; Ngalimat et al., 2019). Lactic bacteria with probiotic potential that were identified in fermented pollen suggests the application of these microorganisms in the food industry (Mohammad et al. 2020). Lactobacillus correspond to 83.9% of the species found in fermented pollen (Asama et al., 2015).

Bacteria such as *Bacillus* also participate in pollen fermentation, although to a lesser extent when compared to lactic acid bacteria (Gilliam et al. 2000). *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus licheniformis* were found in SLB pollen, and these exhibited the ability to ferment sugars such as glucose, fructose and sucrose as a substrate (Akcan, 2011; Aqeel & Umar, 2010; Ramos et al., 2020).

The yeast genera found in the pollen were *Hyphopichia*, *Zygosaccharomyces*, *Kodamaea*, *Candida*, *Pichia*, and *Wickerhamiella*, and the species *Starmerella meliponinorum*, *Wickerhamiella versalitis*, *Starmerella neotropicalis*, *Kodamaea ohmeri* and *Starmerella apicola* (Daniel et al., 2013; Rosa et al., 2003; Teixeira et al., 2003a). Yeasts play an important role in the processing and transformation of pollen within colonies, secreting enzymes that participate in the biochemical processes that contribute to the transformation of bee pollen into fermented pollen, making nutrients bioavailable, and improving nutritional quality of food (Meireles, 2018).

The association between yeast and SLB was first reported in the work by (Rosa et al., 2003). Yeasts associated with stingless bees can be found in plants and flowers, in pollen and nectar collected by them, in honey, in fermented pollen (samburá), in brood cells, in larvae, and in the digestive tract of bees (De Almeida Souza, 2009; Rosa et al., 2003).

The yeast species most associated with meliponines and/or substrates visited by these insects belong to the genus *Starmerella spp*. This group encompasses approximately 50 species (Gonçalves et al., 2020; Costa Neto &

de Morais, 2020; Santos et al., 2018). They are ascomycetic yeasts, and are restricted to using very few carbon sources. Many of these species have a preference for fructose as a source of carbon and energy (fructophilia), and production of sophorolipids (Gonçalves et al., 2020; Rosa et al., 2003).

Sophorolipids are compounds of microbial origin with industrial, cosmetic, and pharmaceutical applications because they have biosurfactant and emulsifying properties (Weber et al., 1992).

The species *Starmerella apicola*, *Starmerella meliponinorum*, *Starmerella etchellsii*, *Starmerella neotropicalis* are associated with bee substrates such as garbage pellets, nectar, pollen, mature honey and adult bees. Some yeast species, in association with bacteria, are able to ferment the nectar collected by bees (Daniel et al., 2013; de Paula et al., 2021; Costa Neto & de Morais, 2020; Santos et al., 2018).

Teixeira et al. (2003a) describes for the first time *Starmerella meliponinorum* as one of the most frequent species associated with different SLB. This species was found in the bee products (pollen, honey, and propolis) of different species of SLB, such as: *Melipona quadrifasciata*, *Tetragonisca angustula*, *Melipona rufiventris and Trigona fulviventris.*

Study carried out by Rosa et al., (2003) also identified a high prevalence of *Starmerella meliponinorum* in *Tetragonisca angustula* honey in *Tetragonisca angustula*, *Frieseomelitta* and *Melipona quadrifasciata* honey from the Brazilian state of Minas Gerais. The work by Daniel et al., (2013) shows the incidence of *Starmerella neotropicalis* in *Melipona quinquefasciata* bee pollen.

The second most prevalent species is the apicultural *Starmerella*, being identified in all honey samples from *Melipona fasciculata*, *Plebeia emerina*, *Nannotrigona testaceicornis*, *Melipona scutellaris*, *Plebeia saiqui* (Massaro et al., 2019). Genera such as *Candida*, *Starmerella and Metschnikowia* are responsible for producing and releasing enzymes that improve, protect and preserve the pollen, in addition to producing antimicrobial substances that can protect the colony from pathogens (Gilliam, 2006; Rosa et al., 2003). In addition to the genus *Metschnikowias*, *Debaryomyces* and *Zygosaccharomyces* have also been observed in SLB honey pots, in the digestive tract of bees, on the body surface of workers, and in the nectar of flowers visited by bees (Brysch-Herzberg, 2004; Carmen Seijo et al., 2011; Rosa et al., 2003; Saksinchai et al., 2012; Teixeira et al., 2003a). *Zygosaccharomyces* are osmophilic, and this characteristic helps in the safety of the product (honey) because this yeast even competes with pathogenic microorganisms (Villas-Bôas, 2018).

*Dekkera bruxellensis*, *Candida sp.*, *Debaryomyces hansenii*, *Kloeckera africana* and *Pichia anomala* species were identified in honey samples from SLB *Scaptotrigona sp.*, *Partamona sp.*, *Melipona asilvai*, and *Melipona mandacaia* that live in the Brazilian dry tropical forest (Barbosa et al., 2016). *Debaryomyces hansenii* as well as *Zygosaccharomyces sp.* were also found in association with adult bees of *Melipona quadrifasciata* (Rosa et al., 2003). *Wickerhamiella versalitis*, *Candida orthopsilosis*, *Debaryomyces sp.*, *Metschnikowia sp.*, *Candida sp.*, *Pichia sp.*, *Kodamaea ohmeri*, *Pichia kluyveri*, were associated with the nest of *Cephalotrigona femorata* and *Melipona interrupta* bees (Meireles, 2018).

The genus *Debaryomyces* encompasses fifteen species that can be found in different natural resourses such as pollen, soil, air, plants, insects and fruits(Kurtzman & Robnett, 1998; Suzuki, 2011). *Debaryomyces hansenii* is a non-pathogenic yeast often found in protein-rich products (Andrade et al., 2010; Encinas et al., 2000; Masoud & Jakobsen, 2005; Petersen et al., 2002). It has salt tolerance, is able to develop at low temperatures, low pH value, metabolizes amino acids and organic acids, regulates the acidity of fermented products, and has proteolytic and lipolytic activities that act as biocatalysts in the production of flavor in many products (Durá et al., 2002; Olesen & Stahnke, 2000; Sørensen & Samuelsen, 1996).

*Debaryomyces hansenii* is of biotechnological interest due to its fermentative potential, in addition to the production of proteases, α-galactosities, ethanol and xylitol from xylose (Bolumar et al., 2008; Menon et al., 2010; Prakash et al., 2011; Viana et al., 2007); *Dekkera bruxellensis* is capable of metabolizing various carbon sources such as glucose, fructose, sucrose, maltose, galactose; uses ammonia, proline, arginine, and nitrate as nitrogen sources; in addition, it is ethanol tolerant, has fermentative power at high glucose concentrations and is facultative anaerobic. This yeast produces metabolites such as acetic acid and ethylphenols (4-ethylphenol and 4-ethylguaiacol), substances that can impart unpleasant aromas to some alcoholic beverages (Malfeito-Ferreira, 2018).

*Pichia* species can be found in the environment, mainly in soil, plants and fruits (Kurtzman & Robnett, 1998). Studies have shown that *Pichia kluyveri* has aromatic and pectinolytic potential in the mixed fermentation of cocoa, in addition to improving the flavor of chocolate (Crafack et al., 2013). Yeasts of the genus *Saccharomyces* are commonly used in fermentation processes, with *Saccharomyces cerevisiae* being the most used species industrially. This is due to the ability of this species to have a high level of tolerance to ethanol, in addition to fermenting a wide range of sugars and adapting to a variable pH range (Lin et al., 2014; Mukherjee et al., 2014; Radecka et al., 2015).

Yeasts of the genus *Zigosaccharomyces* are associated with different aspects of SLB colonies (Chikano &

Takahashi, 2020; Rodríguez-Hernández et al., 2019). Due to its high substrate abundance, mainly sugars, honey is the habitat for these species (Matos et al., 2020). Studies show the interaction of SLB *Scaptotrigona depilis* with yeasts of the genus. Currently, it has been verified that yeasts of the genus *Zygosaccharomyces* produce precursor molecules for the synthesis of steroids that are important for the development of the larval stage of bees of the species *Scaptotrigona depilis* (Paludo et al., 2018). Some species of *Zigosaccharomyces* are associated with honey fermentation; however, other strains are involved in spoilage of products stored in the (Chikano & Takahashi, 2020). Species such as *Zigosaccharomyces bailii*, *Zigosaccharomyces rouxii* are recognized as relevant spoilage agents (Kurtzman; Fell; Boekhout, 2011).

Due to the diversity of yeasts and the number of SLB species, it is still necessary to study the association of these microorganisms with the products of these bees. These microorganisms play an important role in the nutrition of bees, in the production of biomolecules that act in the transformation of nectar and pollen, in the production of antimicrobial substances and have the purpose of inhibiting the growth of pathogens (de Paula et al., 2021; Douglas, 2015; Menezes et al., 2013). In addition, this interaction can influence yeast biodiversity, as these insects act in the dispersion of microorganisms in the environment (Stefanini, 2018).

### Stingless bee honey

The nectar from flowers and/or secretions from living parts of plants is collected by bees and receives some specific substances, such as enzymes present in the salivary glands of bees. This nectar is processed, and later stored and matured inside the pots in the colony, turning into honey (Chuttong et al., 2016; Elias-Santos et al., 2013). Figure 2 shows an SLB colony, showing the hive, pollen stored, and honey produced.

Nectar is also an important resource for bee nutrition, being the main source of carbohydrates (Maia Silva et al. 2015). Honey is a viscous solution composed basically of water (15–17%); 80–85% of sugars (about 38–40% fructose and 31–34% glucose, with smaller amounts of sucrose, usually between 1 and 3%.); minerals (0.2%) and smaller amounts of proteins and amino acids (0.1–0.4%), enzymes, lipids, minerals, aromatic components, vitamins, pigments (carotenoids), phenolic and photochemical compounds (Alqarni et al., 2014).

The botanical and geographic origin of the nectar is directly correlated with the chemical composition, color, aroma, and flavor of the honey. According to this origin, honey can be classified as floral (obtained through the nectars of flowers) and can be unifloral or monofloral (originating from flowers of the same family, genus or species) or multifloral (obtained through different floral origins). It can also be classified as nectar honey or honeydew honey. The first obtained from the nectar of plants and the second from secretions of plant-sucking insects or living parts of plants (Belay et al., 2017; Brasil, 2001).

The characteristics of honey produced by *meliponine* bees have different characteristics (chemical, sensory, and physical) from the ones of honey produced by *Apis mellifera*. Differing in color, flavor and viscosity, it is known for its high moisture content, peculiar sweetness, more acidic taste, and slower crystallization. It also has microorganisms (bacteria and yeasts) that induce its fermentation, in addition to having greater antioxidant activity (Ávila et al., 2018; Biluca et al., 2014; Braghini et al., 2022; de Almeida-Muradian et al., 2014).

Because honey contains a high moisture content, which can favor spontaneous fermentation, it becomes a challenge to preserve the characteristics of this melipona product. Some techniques such as pasteurization, refrigeration and dehumidification can be used to preserve the quality of this type of honey since it avoids the fermentation process. These techniques, however, can alter the sugar content, enzymatic activity and cause undesirable sensory changes (De Camargo et al., 2017; Ribeiro et al., 2018).

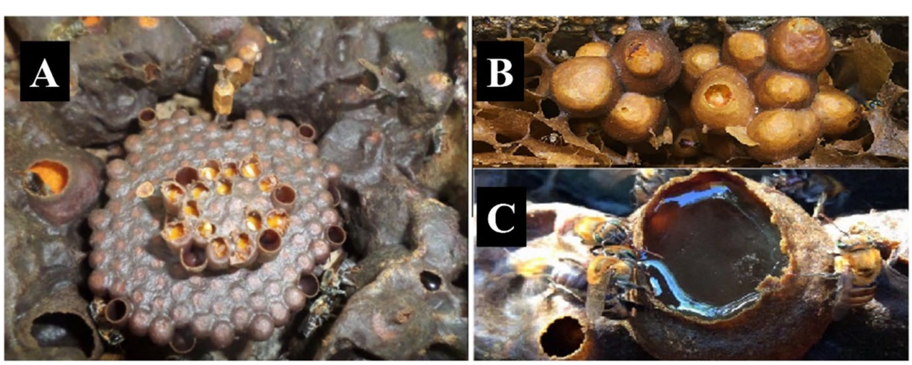



**Fig. 2** Stingless bee colony. A - Brood cells; B - Pollen pot; C - Pot of honey. Figure created by the authors

Alcoholic fermentation is usually carried out by yeasts (Saccharomyces and non-Saccharomyces) that transform carbohydrates into alcohol and carbon dioxide. Alcoholic fermentation under aerobic conditions can become acetic, as some strains of bacteria, those of the genus Bacillus, convert alcohol and oxygen into acetic acid and water. Lactic fermentation is usually provided by bacteria that convert carbohydrate molecules into lactic acid and other organic by-products (Gilliam, 1997; Rosa et al., 2003; Teixeira et al., 2003b).

Honey maturation is also a technique used by beekeepers to preserve the quality of SLB honey, but this method does not avoid the fermentation process. It consists of storing freshly harvested honey at a temperature of 30 °C so that it is naturally fermented by naturally present microorganisms, with three to eight months of fermentation being recommended to obtain a stable product with good sensory acceptance (De Camargo et al., 2017; Ribeiro et al., 2018).

## Physicochemical parameters of honey

In different countries, laws were created for the quality control of pure Apis mellifera honey. These laws are generally based on the following parameters: maturity (reducing sugars, humidity, apparent sucrose); purity (water-insoluble solids, minerals or ash, pollen); and deterioration (free acidity, diastase activity and hydroxymethylfurfural) (Brasil, 2001).

The physicochemical parameters established by the *Codex Alimentariu*s (General Principles of Food Hygiene, 2022) and by the Technical Regulation of Identity and Quality established by Normative Instruction No. 11/2000, refer only to honey from Africanized bees (*Apis mellifera*) (Table 2).

For Villas Bôas (2018), it is complex to establish, for an entire country, a single quality standard for these honeys, given the great diversity of bee species and vegetation. Also, the widespread use of physicochemical (Brazil, 2001)parameters established for honey from Apis bees can lead to problems for the commercialization of honey from stingless species, as they have very varied physicochemical characteristics (Alves et al., 2018). The physicochemical characteristics of honey can also vary between different species of SLBs. The data listed in Tables 3 and 4 show that meliponine honey has a different physicochemical composition than Apis mellifera honey.

## Sugars

The major components of honey are sugars. The total sugars in honey, the monosaccharides glucose and fructose, stand out, being 80% of the total, and the disaccharides sucrose and maltose represent only 10% of this

**Table 3** Specification of physicochemical characteristics for quality control and identity of different SLB honeys. *T.A* Total acidity, *A.S* Apparent sucrose, *R.S* Reducing Sugars, *D.A* Diastase Activity, *HMF* Hydroxymethylfurfural (-) absence of the parameter in the study

| Parameters | Brazilian regulations, Nº 11, (Brazil 2000) | Codex Alimentarius (2020) |
|---|---|---|
| Moisture (%) | Max. 20 | Max. 20 |
| Total acidity (mEq kg$^{-1}$) | Max. 50 | Max. 50 |
| Apparent sucrose (%) | Max. 6 | Max. 10 |
| Reducing sugars (%) | Min. 65 | Min. 60 |
| Ash (%) | Max. 0,6 | |
| Insoluble solids (%) | Max. 0,1 | Max. 0,1 |
| hydroxymethylfurfural (mg kg$^{-1}$) | Max. 60 | Max. 60 |
| Diastase activity (ºGothe) | Min. 8 | Min. 8 |

value (Silva et al., 2013). The presence of these carbohydrates can influence the density, viscosity, hygroscopicity, crystallization, energy value and antibacterial activity of honey (de Almeida-Muradian et al., 2014; Kuroishi et al., 2012).

Fructose is the monosaccharide present in greater quantity in stingless bee honey, it has high hygroscopicity (greater fluidity) and confers sweetness to honey; glucose is the second sugar in the highest concentration, has lower solubility in water and determines the tendency of honey to crystallize (Biluca et al., 2014, 2016; Escuredo et al., 2014). Although meliponine honey has a high level of fructose, it still has lower values of reducing sugars when compared to honey standards established for Apis mellifera (Biluca et al., 2014; Chuttong et al., 2016; de Sousa et al., 2016).

The presence of sucrose and maltose is often lower when compared with fructose and glucose, but they can also be found in SLB honeys (de Sousa et al., 2016). Sucrose values may vary, and high concentrations may be a consequence of the botanical origin, possible adulteration or an indication that the honey was extracted prematurely, consequently the invertase action was incomplete, failing to transform all sucrose into fructose and glucose (Chuttong et al., 2016).

The averages presented by the studies for reducing sugars, carried out in Brazil, ranged between 43% and 75.5%. This is due to the diversity of stingless bee species, in addition to the great botanical variety of the Brazilian regions (Table 1).

## Moisture

Water is the second component in quantity in the composition of honey, with moisture being one of the most

**Table 4** Specification of physicochemical characteristics for quality control and identity of different SLB honeys from Latin America, Africa, Asia, and Oceania.*T.A.* Total acidity, *A.S* Apparent sucrose, *R.S* Reducing Sugars, *D.A* Diastase Activity, *HMF* Hydroxymethylfurfural (-) absence of the parameter in the study

| Species | Moisture (%) | T.A (mEq kg$^{-1}$) | A.S (%) | R.S (%) | Ash (%) | D.A (ºGothe) | HMF (mg kg$^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|
| *Melipona scutellaris* | 23.00 | 26.93 | 3.51 | 51.23 | 0.03 | - | 38.08 | (Alves; et al., 2011) |
| *Melipona scutellaris* | 29.10 | 19.90 | 1.80 | 70.70 | 0.19 | < 3.00 | - | (Biluca et al., 2016) |
| *Melipona scutellaris* | 33.98 | 27.25 | 0.70 | 66.41 | 0.16 | 0.11 | 40.86 | (Nascimento et al., 2015) |
| *Melipona scutellaris* | 23.40 | 28.70 | - | 62.70 | - | < 3.00 | - | (de Sousa et al., 2016) |
| *Melipona scutellaris* | 30.00 | 37.00 | - | 59.00 | - | 2.00 | 21.00 | (Duarte et al., 2018) |
| *Tetragonisca angustula* | 24.00 | 79.00 | - | 43.00 | - | - | - | (Biluca et al., 2016) |
| *Tetragonisca angustula* | 24.30 | 39.20 | 4.20 | 53.60 | 0.21 | 16.70 | 1.30 | Chuttong et al., 2016 |
| *Tetragonisca angustula* | 23.75 | 41.15 | - | 63.75 | - | 49.60 | - | (Fuenmayor et al., 2012) |
| *Tetragonisca angustula* | 23.20 | 48.30 | 2.10 | 65.90 | 0.38 | 23.00 | 9.80 | (Nascimento et al., 2015) |
| *Melipona quadriasciata* | 32.47 | 42.52 | - | 61.77 | - | 11.25 | - | (Ávila et al., 2018) |
| *Melipona quadriasciata* | 31.40 | 37.70 | 2.90 | 75.50 | 0.09 | - | 30.90 | (Nascimento et al., 2015) |
| *Melipona quadriasciata* | 28.78 | 43.48 | 2.91 | 74.82 | - | - | 5.79 | (De Almeida Souza, 2009) |
| *Melipona quadriasciata* | 32.46 | 42.52 | - | 61.76 | - | - | - | (Biluca et al., 2016) |
| *Melipona quadriasciata* | 31.23 | 44.63 | - | - | 1.42 | - | - | Batiston et al. 2020 |
| *Melipona bicolor* | 34.68 | 91.62 | - | 60.14 | - | < 3.00 | - | (Biluca et al., 2016) |
| *Melipona bicolor* | 36.18 | 48.58 | 0.57 | 68.43 | 0.18 | 0.12 | 31.58 | (Nascimento et al., 2015) |
| *Melipona seminigra* | 28.85 | 30.44 | 1.61 | 69.12 | 0.22 | 0.20 | 29.50 | (Silva et al., 2013) |
| *Melipona seminigra* | 30.40 | 26.54 | 0.18 | 61.49 | - | - | - | (Alves; et al., 2011) |
| *Melipona compressipes* | 26.70 | 23.88 | 0.14 | 60.39 | - | - | - | (Almeida-Muradian et al., 2007) |
| *Melipona subnitida* | 27.00 | 20.55 | 0.78 | 61.17 | 0.03 | - | 8.64 | (Alves; et al., 2011) |
| *Melipona subnitida* | 23.17 | 41.58 | - | 57.67 | 0.07 | - | 13.60 | (de Sousa et al., 2016) |
| *Melipona fasciculata* | 25.45 | 29.05 | - | - | 0.32 | - | - | (Alves; et al., 2011) |
| *Scaptotrigona xanthotricha* | 29.84 | 28.78 | 1.22 | 66.32 | 0.21 | 0.62 | 58.27 | (Biluca et al., 2016) |
| *Melipona arufivestres mondiry* | 27.70 | 38.20 | - | 65.60 | - | < 3.00 | - | (Biluca et al., 2016) |
| *Melipona mandacaia* | 28.78 | 43.48 | 2.91 | 74,.82 | - | - | 5.79 | (Biluca et al., 2016) |
| *Melipona marginata* | 32.44 | 22.55 | 0.85 | 67.39 | 0.14 | 0.19 | 48.09 | (Biluca et al., 2016) |
| *Scaptotrigona bicunctata* | 23.95 | 48.95 | - | 62.95 | 4.34 | - | - | (Jimenez et al., 2016) |
| *Scaptotrigona depilis* | > 25.00 | 98.43 | - | 65.30 | 0.18 | - | 27.75 | (Lemos et al., 2018) |
| *Scaptotrigona* sp. | 30.22 | 60.98 | 4.83 | 62.34 | - | - | - | (Nascimento et al., 2015) |
| *Cephalotrigona capitata* | 32.10 | 34.33 | 0.36 | 75.21 | 0.19 | 0.18 | 35.40 | (Nascimento et al., 2015) |
| *Melipona marginata* | 32.65 | 79.82 | - | 63.50 | - | < 3.00 | - | (Nascimento et al., 2015) |
| *Melipona mondury* | 29.75 | 61.10 | 0.85 | 67.45 | - | < 3.00 | - | (Nascimento et al., 2015) |
| *Melipona mondury* | 29.18 | 39.43 | 2.14 | 65.42 | 0.18 | 4.05 | 1.60 | (Alves et al., 2018) |
| *Melipona asilvai* | 37.50 | 54.20 | 3.30 | 61.70 | 0.09 | - | 14.70 | (De Almeida Souza, 2009) |

important characteristics of honey, which can influence viscosity, maturity, flavor, crystallization, conservation, and stability (Al-Ghamdi et al., 2018; Biluca et al., 2016; Nascimento et al., 2015). The high moisture content in meliponine honey may be related to the origin of the flower nectar, ripe and water-rich fruits, different bee species, region, time of collection, and management of the meliponiniculturist (Biluca et al., 2016; Chuttong et al., 2016; de Sousa et al., 2016).

Moisture content can interfere with several honey characteristics such as viscosity, weight, maturity, flavor, and crystallization. Furthermore, water activity values greater than 0.6 allow the development of microorganisms (Nascimento et al., 2015; Zuccato et al., 2017).

The moisture content of honey from stingless bees is higher than the value established by the Brazilian and international standard for the quality control of honey from *Apis mellifera*, which is 20% (Ávila et al., 2018; Brazil, 2001; General Principles of Food Hygiene, 2022;

Souza et al., 2021). In studies carried out in Brazil, humidity values range from 23 to 37.5% (Table 3). Studies carried out in Guatemala and Nigeria with samples of honey from *Tetragonisca angustula* (17.45), *Melipona beechei* (17.5) and *Melipona sp.* (13.86) show that these studies presented values within established standards (Table 4).

### Acidity

The stability and conservation of honey depends on acidity, as this characteristic inhibits the action and development of microorganisms. If the acidity is high, it shows unfavorable storage conditions and the occurrence of fermentation processes, consequently affecting the shelf life of the product (Crane, 1996). The acidity of honey is related to the organic acids present in its composition, and these acids can come from the various sources of nectar collected, from the action of the glucose oxidase enzyme that gives rise to gluconic acid, in addition to the action of microorganisms that ferment the sugars in honey during its maturation (Finola et al., 2007; Villas-Bôas, 2018).

The acid present in greater quantity is gluconic, generated by the action of the glucose oxidase enzyme (produced by the hypopharyngeal glands of bees) on glucose. However, there are other acids that can be found in smaller quantities, such as acetic, formic, butyric, citric, pyruvic, malic, lactic, tartaric, fumaric, and oxalic acids. (de Almeida-Muradian et al., 2014; Olaitan et al., 2007; Sancho et al., 2013)

The mean acidity values presented in Brazilian studies for this review ranged from 19.9 to 98.43 mEq.kg-1. Studies carried out in Latin America, Africa, and Oceania present values between 11.23 and 124.2 mEq.kg-1 (Table 1). The lowest level recorded was in the study carried out in Nigeria (11.23 mEq kg-1) for honey from *Melipona sp*. The highest was 124.2 mEq kg-1 for honey from *Trigona carbonaria*, from Australia (Table 4). Samples with a limit greater than 50 mEq kg-11 would not be able to be commercialized at the federal level, as established by regulation (Brasil, 2001).

### Diastatic activity

Diastasis (α-amylase) is an enzyme present in honey that is formed in the hypopharyngeal glands of bees and can also be found in small proportions in pollen grains, and its function is to hydrolyze the starch molecule. Its relevance for honey is that this enzyme is more sensitive when subjected to temperatures above 40 °C for a prolonged period, being an important parameter to assess whether the product has undergone heating processes above 60 °C, adulterations due to the addition of invert sugar, or inadequate storage conditions (Gomes et al., 2010).

Diastatic activity for meliponine honey is relatively low or absent, with values of a maximum of 3 and a minimum of 0.3 on the Gothe scale. It is a lower value than that established by legislation for Apis, eve n in freshly collected and unheated honey samples, being an inherent characteristic of this type of bee (Biluca et al., 2016).

### Hydroxymethylfurfural (HMF)

HMF is one of the parameters used to indicate the freshness and quality of honey. Its presence may indicate that the honey was heated and/or stored for long periods, in addition to indicating adulteration caused by the addition of invert sugar. HMF is a compound formed by the degradation of sugars through the Maillard reaction or hexose dehydration in an acid medium (de Sousa et al., 2016).

In addition to heating and storage conditions, other factors such as hydrogenionic potential (pH), acidity, water activity, as well as the presence of simple sugars (glucose and fructose), acids, and minerals can promote the formation of HMF (Pasias et al., 2017).

### Microbiological quality of stingless bee honey

The microbiota associated with stingless bees is quite diverse, including bacteria, filamentous fungi, and yeasts (de Paula et al., 2021). In addition to contributing to the identification of the microbiological quality of the products and defining parameters to establish the shelf life and its safety, some of these microorganisms also act in the maturation process, being able to improve the organoleptic characteristics and the nutritional value of the products (Ribeiro et al., 2018; Snowdon & Cliver, 1996).

The chemical conditions of honey make the environment inhospitable for the development and permanence of most microorganisms, especially for pathogens, keeping the microbiota in a state of latency. However, many microorganisms can survive or proliferate under more severe conditions such as high concentrations of sugars, high osmolarity, low water content, low pH, and in the presence of antimicrobial compounds (Estevinho et al., 2012; Silva et al., 2017).

Normative Instruction No. 11/2000 (Brasil, 2001) which establishes quality standards for Apis mellifera honey does not require microbiological analysis; it only refers to the hygienic-sanitary standards and good manufacturing practices for establishments that prepare/manufacture food. In addition, the Resolution of the Collegiate Board of ANVISA No. 724, of July 1, 2022 (Brazil, 2001) which regulates the microbiological quality control of foods in force in Brazil and Normative Instruction No.

161, also of July 1, 2022 (Ministério da Saúde - MS Agência Nacional de Vigilância Sanitária – ANVISA, 2022) which establishes the microbiological standards of food, also do not require microbiological control of honey, including also excluding the need for analyzes of pathogens such as *Listeria monocytogenes*.

Microorganisms associated with SLB honey can be inherent from both primary and secondary sources. Primary microbial sources are introduced by bees through pollen, their digestive tract, soil, air, and nectar. Secondary sources include contamination from improper handling, processing, and storage. (Olaitan et al., 2007; Różańska & Osek, 2012)

The presence of coliforms, molds, and yeasts in different SLB honey samples may come from the microbiota of pollen, nectar, bee and/or inadequate hygienic sanitary conditions during product handling and processing (Fernandes et al. 2018).

Ávila et al. (2018) evaluated 32 samples of SLB honey. The results obtained for the presence of aerobic mesophiles varied between 2 and 4.77 CFU/g-1 for *Scaptotrigona bipuncata* and *Melipona marginata*, respectively. Regarding coliforms at 35 °C, 78% of the samples showed < 3 MPN/g-1 and only 6% of the *Scaptotrigona* samples showed coliforms at 45 °C. For molds and yeasts, counts were detected with an average value of 3.4 CFU/g-1. Rodrigues et al. (2018) observed the significant presence of molds and yeasts (9.4 × 104 to 1.3 × 105 CFU/g-1), microorganisms from the coliform group at 35 °C (> 2.3 × 101 MPN/g-1) and coliforms at 45 °C (4 and < 3 NMP/g-1) in honey samples of the species *Melipona quadrifasciata*, *Scaptotrigona depilis* and *Melipona quadrifasciata*. (David, 2017 identified the presence of molds and yeasts, finding tolerable values < 3 CFU/g-1 for samples of honey from the species *Tetragonisca angustula* from the State of Rondônia. These authors also observed that two samples showed contamination by coliforms at 35 °C (3 × 102 and 2.1 × 103 NMP/g-1) and one by coliforms at 45 °C (3 × 102 NMP/g-1).

Several bacterial and yeast species associated with SLB may also play an essential role in insect nutrition and development (Madden et al., 2018). These microorganisms can produce enzymes that may be involved in the breakdown of macronutrients present in nectar and pollen, in addition to fermenting sugars and producing organic acids, contributing to the transformation of products and to the improvement of their nutritional value (de Paula et al., 2021; Ngalimat et al., 2019). In addition, they can produce antimicrobial compounds capable of protecting the colony against pathogenic microorganisms (Menegatti et al., 2020; Rodríguez-Hernández et al., 2019).

The bacteria most associated with SLB are *Ralstonia*, *Fructobacillus*, *Pantoea*, *Lactobacillus*, *Enterobacter*, *Bacillus*, *Streptomyces*, *Staphylococcus*, *Streptococcus*, *Clostridium*, *Pseudomonas*, *Neisseria*, and *Lysinibacillus* (de Paula et al., 2021; Menegatti et al., 2020; Rodríguez-Hernández et al., 2019; Suphaphimol et al., 2021). Among these bacteria, the lactic acid bacteria stand out. Its presence can inhibit competing microbiota, such as spoilage and pathogenic bacteria. In addition to producing organic acids, such as lactic acid, resulting in a lower pH and obtaining antimicrobial compounds (Cintas et al. 2001). The results obtained by Ávila et al. (2018) for lactic acid bacteria in honey samples ranged from 1.24 CFU/g-1 for Scaptotrigona bipuncata to 5.82 CFU/g-1 for Melipona quadrifasciata.

Although honey has physicochemical properties that prevent the development and proliferation of microorganisms, such as low water activity, low pH, high concentration of sugars, high osmolarity, in addition to the presence of antimicrobial compounds; it is believed that the inoculation of yeasts in honey comes from the bees themselves (Estevinho et al., 2012; Różańska & Osek, 2012).

The most common yeast genera observed in SLB honey are *Debaryomyces*, *Candida*, *Metschnikowia*, *Dekkera*, *Pichia*, *Zygosaccharomyces*, *Kloeckera* and *Starmerella* (Barbosa et al., 2016; Carmen Seijo et al., 2011; Rosa et al., 2003; Saksinchai et al., 2012; Teixeira et al., 2003a), with *Starmerella meliponinorum*, *Metschnikowia sp.*, *Zygosaccharomyces rouxii*, *Zygosaccharomyces mellis*, *Zygosaccharomyces bailii*, *Saccharomyces cerevisiae*, *Saccharomyces mellis*, *Saccharomyces rosei*, *Lachancea fermentati*, *Pichia anomala*, *Pichia kudriavzevii*, *Wickerhamomyces anomalus*, *Dekkera bruxella*, and *Kloeckera africana* being the most common species (Carmen Seijo et al., 2011; Rosa et al., 2003; Sinacori et al., 2014).

Filamentous fungi such as *Aspergillus*, *Penicillium* and *Talaromyces* have been identified in honey, pollen, and inside *Melipona scutellaris* nests (Barbosa et al., 2018). Other species belonging to the genus *Monascus spp*. were also found in substrates of *Melipona scutellaris*. Both mycelia and fungal spores that develop in food stored in the nest are essential for the larval development of the bee, as they also serve as a food resource (Barbosa et al., 2017, 2018). Although there are several species of microorganisms associated with bees, nests and SLB products, the benefits related to their interactions with insects and the richness of existing species are still poorly studied(de Paula et al., 2021).

## Final considerations

The correlation between stingless bee species, the resulting products (such as honey, propolis, pollen, etc.), the botanical species involved, and the diversity of associated microbiota is extensively documented in the literature. Recent evidence of this correlation was provided by da Silva et al. (2024); Rocha et al. (2024) who investigated the composition of honey, pollen, and propolis from various stingless bee species in different Brazilian geographical regions. These studies revealed variations in identified yeasts, depending on the product (honey or pollen). Remarkably, concerning propolis, a significant distinction in chemical properties was observed, including phenolic compounds, flavonoids, antioxidant, and antimicrobial activity, among propolis produced by bees of the same species but in different geographical locations (distinct biomes). Additionally, bioactive compounds not previously described in the literature were identified, as well as chemical markers such as Formononetin, characteristic of red propolis produced by *Apis mellifera*.

## Conclusion

Pollen and honey produced by stingless bees have very different sensory, physical and chemical characteristics when compared to honey and pollen produced by *Apis mellifera* bees. The SLB pollen samples have a high moisture content ranging from 22.65 to 53.93%; proteins from 15.98 to 37.63%; lipids from 2.5 to 10.81%; some species have a considerable amount of fibers. The results presented in this work show that the moisture for SLB honey can vary from 23 to 37.5%; total acidity maximum value of 124.2 mEq kg-1; and reducing sugars of 77.11%. The values found for the physicochemical parameters of SLB honey are higher than those established by *Codex Alimentarius*/2020 and Normative Instruction No. 11/2000, which refer to honey from *Apis mellifera* bees and determines 20% moisture; 50 mEq kg-1 of total acidity; and 65% reducing sugars. The widespread use of physicochemical parameters established for honey and pollen from *Apis mellifera* bees can lead to problems for the commercialization of products from stingless species, since this honey has very different physicochemical characteristics.

The microbiota associated with pollen and honey of *Meliponini* bees is still little studied, however, microbial interaction can be observed through the fermentation of these foods that are stored inside the colonies. These microorganisms can contribute to bee nutrition, produce substances that can help transform products such as nectar and pollen; as well as they can produce antimicrobial compounds that have the purpose of inhibiting the growth of pathogens within the colony.

The observed data show the presence of coliforms, molds and yeasts in different SLB honey samples. The presence of these microorganisms may come from the microbiota of pollen, nectar, bee and/or inadequate hygienic sanitary conditions during product handling and processing.

The lactic acid bacteria found can produce antimicrobial compounds and enzymes that are industrially important for conferring health benefits. Yeasts secrete enzymes that participate in the biochemical processes that contribute to the transformation of bee pollen into fermented pollen, making nutrients bioavailable and improving the nutritional quality of food.

Few studies address the physicochemical characteristics and microbiological biodiversity of honey and SLB pollen. The development of new research depends on new knowledge. This knowledge can serve as a scientific basis for new legislation that will define the identity and quality standards of bee products. In addition, an opportunity to know, explore and apply this microbiological diversity biotechnologically.

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**Authors' contributions**
RNAS, KTM-G, COS, RMOA and MAUG performed the bibliographic revision. RNAS, KTM-G and MAUG wrote the manuscript. KTM-G, RNAS, and MAUG critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

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