Maret 2026, Vol. 23, No. 1, 83–90
https://doi.org/10.5994/jei.23.1.83

JURNAL ENTOMOLOGI INDONESIA

Short communication

Physicochemical comparison of *Wallacetrigona incisa* (Sakagami & Inoue) and *Tetragonula sapiens* (Cockerell) honey from West Sulawesi

Perbandingan fisikokimia madu *Wallacetrigona incisa* (Sakagami & Inoue) dan *Tetragonula sapiens* (Cockerell) asal Sulawesi Barat

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Diterima: Juli 2025
Disetujui: Maret 2026

Sitasi:
Hasan PA, Purwanto H. 2026. Physicochemical comparison of *Wallacetrigona incisa* (Sakagami & Inoue) and *Tetragonula sapiens* (Cockerell) honey from West Sulawesi. *Jurnal Entomologi Indonesia.* 23(1):83–90 DOI: https://doi.org/10.5994/jei.23.1.83

ABSTRACT

Wallacetrigona incisa (Sakagami & Inoue) and *Tetragonula sapiens* (Cockerell) are two stingless bee species widely cultivated for honey production in West Sulawesi, Indonesia. Yet, no comparative physicochemical data on their honey from this region exist. This study compared the physicochemical properties of *W. incisa* honey from a highland meliponary (1340 m a.s.l) and *T. sapiens* honey from a lowland meliponary (9 m a.s.l), and documented the flowering plant assemblages and abiotic conditions at each site. Ten parameters were assessed, including water content, ash, glucose, reducing sugar, fat, vitamins A and C, and antioxidant activity (IC_{50}). *W. incisa* honey had higher water content (28.61%), glucose (61.18%), and vitamin C (374.61 µg/g) than *T. sapiens* (22.45%, 44.86%, 270.77 µg/g, respectively). *T. sapiens* honey had higher ash content (0.91% vs. 0.18%) and a higher IC_{50} value (562.81 vs. 423.40 ppm), indicating weaker antioxidant activity. These differences may be attributed to the contrasting altitudinal environments, including ambient humidity at the highland site (77.5% vs. 69.5% RH) and differences in surrounding vegetation (24 vs. 12 flowering plant species). *W. incisa* honey complied more closely with the Indonesian National Standards (SNI 8664:2024) threshold than *T. sapiens* honey. Further studies with replicated sampling are needed to confirm these preliminary findings.

Keywords: environmental bioindicators, floral nectar, honey quality, stingless bees, Wallacea

ABSTRAK

Wallacetrigona incisa (Sakagami & Inoue) dan *Tetragonula sapiens* (Cockerell) adalah dua spesies lebah tanpa sengat yang banyak dibudidayakan karena produksi madunya yang melimpah di Sulawesi Barat, Indonesia. Namun, belum ada data perbandingan sifat fisikokimia madu *W. incisa* dan *T. sapiens* dari wilayah ini. Studi ini membandingkan sifat fisikokimia madu *W. incisa* dari lokasi budi daya di dataran tinggi (1340 m dpl) dan madu *T. sapiens* dari lokasi budi daya di dataran rendah (9 m dpl), serta mendokumentasikan jenis tumbuhan berbunga dan kondisi abiotik di setiap lokasi. Sepuluh parameter dinilai, termasuk kadar air, abu, glukosa, gula pereduksi, lemak, vitamin A dan C, serta aktivitas antioksidan (IC_{50}). Madu *W. incisa* memiliki kadar air (28,61%), glukosa (61,18%), dan vitamin C (374,61 µg/g) yang lebih tinggi daripada *T. sapiens* (22,45%, 44,86%, 270,77 µg/g, masing-masing). Madu *T. sapiens* memiliki kadar abu yang lebih tinggi (0,91% vs. 0,18%) dan nilai IC_{50} yang lebih tinggi (562,81 vs. 423,40 ppm), yang menunjukkan aktivitas antioksidan yang lebih lemah. Perbedaan ini mungkin disebabkan oleh perbedaan ketinggian, termasuk kelembapan lingkungan di lokasi dataran tinggi (77,5% vs. 69,5% RH) dan perbedaan vegetasi di sekitarnya (24 vs. 12 spesies tumbuhan berbunga). Madu *W. incisa* lebih sesuai dengan ambang batas Standar Nasional Indonesia (SNI 8664:2024) dibandingkan dengan madu *T. sapiens*. Studi lebih lanjut dengan pengambilan sampel berulang diperlukan untuk mengonfirmasi temuan awal ini.

Kata kunci: bioindikator lingkungan, kualitas madu, lebah tidak bersengat, nektar floral, Wallacea

INTRODUCTION

Honey is a natural, sweet liquid produced by bees from floral and extrafloral nectaries. Beyond stinging bees (*Apis* spp.), stingless bees are increasingly recognized as commercially and ecologically important honey producers. Sujanto et al. (2021) reported that stingless bee honey contains flavonoids and phenolic compounds as well as enzymes that are useful with antioxidants, antimicrobials, anticancer, and antidiabetic properties. Stingless bee honey typically has higher water content and a more sour taste than *Apis* honey (Vit et al. 2023). Consequently, honey's physicochemical properties vary considerably among bee species. However, the differences in honey quality between *Wallacetrigona* and *Tetragonula* in West Sulawesi have not been investigated.

Wallacetrigona incisa (Sakagami & Inoue) and *Tetragonula sapiens* (Cockerell) are ecologically and economically important stingless bee species in West Sulawesi. These two stingless bee species have been recorded on Sulawesi Island (Sayusti et al. 2021; Trianto et al. 2024; Hasan et al. 2024) and widely cultivated for honey production; *W. incisa* colonies reportedly yield approximately 5 liter per three-month harvest period (Suhri et al. 2025), compared with approximately 500 ml for *T. sapiens* (interview with a beekeeper named Haris in 2024). Based on their natural habitat, these two species are ecologically distinctive: *W. incisa* has been recorded exclusively in the highlands of Sulawesi (>800 m a.s.l.), while *T. sapiens* is reported in the lowlands of Sulawesi (130 m a.s.l.) (Rasmussen et al. 2017; Suriawanto et al. 2017). Such altitudinal differences are associated with variation in multiple biological traits: highland stingless bee individuals exhibit larger body sizes than lowland in *W. incisa* (Pongbulaan 2010), and honey quality may also differ accordingly.

The physicochemical properties and antioxidant activity of *W. incisa* honey were documented by Gunawan et al. (2018). Budiaman et al. (2019) subsequently assessed water content, ash content, acidity, water-insoluble solids, diastase enzyme activity, hydroxymethylfurfural (HMF), reducing sugars, sucrose, and harmful metal contaminants. Octaviani et al. (2020) compared honey quality between *W. incisa* and *Tetragonula biroi* (Friese), while Rosmarlinasiah et al. (2023) conducted a similar comparison between *T. sapiens* and *T. biroi*. The *W. incisa* honey analyzed by Gunawan et al. (2018) was sourced from Samarinda Botanical Gardens, East Kalimantan, and that analyzed by Octaviani et al. (2020) was sourced from Mappedeceng District, South Sulawesi. *T. sapiens* honey examined by Rosmarlinasiah et al. (2023) was collected from the West Wawonii District, Southeast Sulawesi. There have been no reports on the quality of *W. incisa* and *T. sapiens* honey from West Sulawesi Province, Indonesia. Furthermore, no reports have compared the quality of *W. incisa* and *T. sapiens* honey in relation to habitat differences. This knowledge gap is noteworthy given the numerous biotic and abiotic factors known to influence honey quality.

The quality of honey, both physically and chemically, is largely determined by the interaction between internal and external factors (Adityarini et al. 2020; Hasan et al. 2020). The larger body size of *W. incisa* relative to *T. sapiens* likely influences its foraging range and ability to access a broader range of floral resources. Therefore, the diversity and composition of flowering plants surrounding each hive may partly explain interspecific differences in honey quality (Hasan et al. 2020). Currently, many studies focus solely on laboratory testing of honey quality, without documenting vegetation in the field. Therefore, this study aims to examine the physical and chemical characteristics of honey produced by *W. incisa* and *T. sapiens*. Additionally, this study documents the flowering plant assemblages and abiotic conditions surrounding each beehive, which are discussed in relation to observed differences in the physicochemical properties of honey between the two species.

METHODOLOGY

Study location

Honey samples were collected from managed meliponiculture hives at two sites in West Sulawesi Province, Indonesia. *W. incisa* honey was collected at Taupe village in the Mamasa District of Mamasa Regency, and *T. sapiens* honey was from Lombong Timur village in the Malunda District of Majene Regency (Figure 1). The *W. insica* Meliponary in Taupe village was located at the foot of Mount Mambulilling, whereas the *T. sapiens* meliponary in Lombong Timur village was located within a residential settlement. Taupe village lies at 1340 m a.s.l. (Hasan et al. 2024), whereas Lombong Timur village lies at 9 m a.s.l. The physicochemical analysis of honey was carried out at the Makassar Health Laboratory Centre in Indonesia. This laboratory is accredited for testing and calibration competence (accreditation no. LP-400-IDN SNI ISO/IEC 17025:2017).

Collection of *W. incisa* and *T. sapiens* honey

Stingless bees store their hive products in discrete wax structures known as pots. Honey was stored in honey pots, while pollen was stored in pollen pots. Mature honey pots are sealed by the bees, whereas immature pots remain open and contain honey of lower ripeness.

W. incisa and *T. sapiens* honey were collected from both sealed and unsealed honey pots using a tool aspirator (Figure 2). Honey was collected from five hives of each species in July 2023, yielding a total volume of 250 ml per species for physicochemical analysis. 250 ml was obtained from taking honey from several honey pots in five hives. Samples were stored in sealed glass containers at room temperature before physicochemical analysis.

Physiochemical analysis of *W. incisa* and *T. sapiens* honey

The following parameters were measured to assess honey quality: odor, taste, water content, ash content, fat content, glucose content, reducing sugar content, vitamin A content, vitamin C content, and antioxidant IC_{50}. Analyses employed spectrophotometric, organoleptic, gravimetric, and titrimetric methods, as described below.

Odor and taste tests are conducted by competent panelists for the organoleptic method (SNI 8664:2024). Honey temperature was measured using a calibrated digital thermometer (electrometric method) (SNI 8664:2024). The water content is calculated by reading the refractive index on a refractometer (SNI 8664:2024). Ash and fat content are calculated using the gravimetric method, as described in AOAC Official Method 920.181

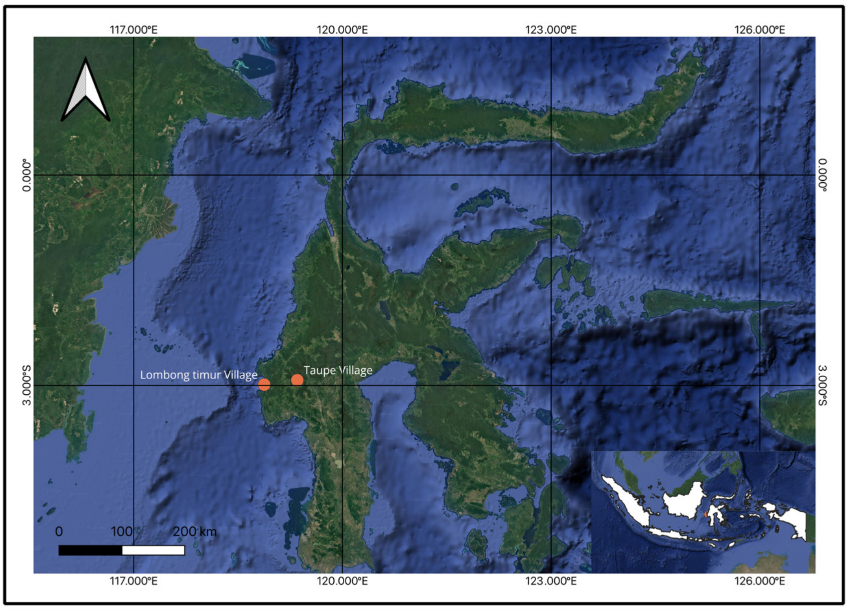

Figure 1. Map of the two study sites in West Sulawesi Province, Indonesia. The *Wallacetrigona incisa* meliponary was located in Taupe village, Mamasa District (1,340 m a.s.l), and the *Tetragonula sapiens* meliponary in Lombong Timur village, Malunda District (9 m a.s.l.).

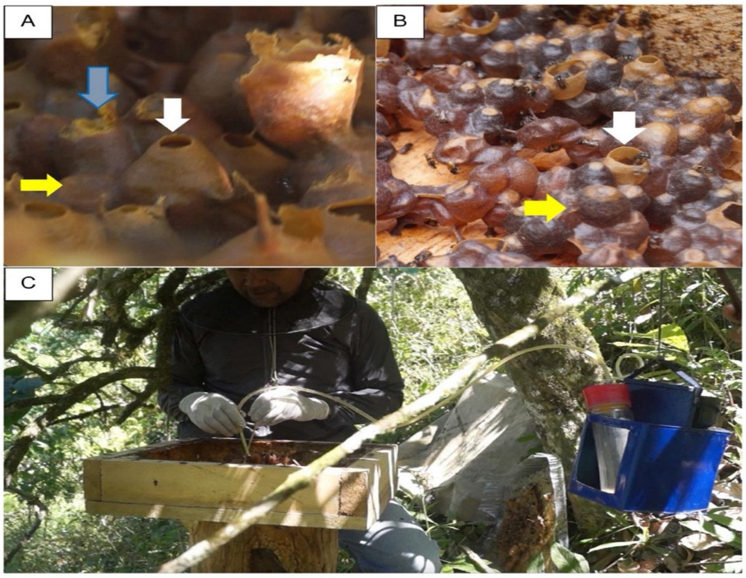

Figure 2. Pollen pots (blue arrow), closed honey pots (yellow arrow), and open honey pots (white arrow) in *Wallacetrigona incisa* (A) and *Tetragonula sapiens* nests (B), and a way to collect honey with an aspirator tool (C).

(AOAC 2016). Ash content was determined by heating the honey in an oven at 100 °C and in a furnace at 600 °C, and weighing the residual ash. Fat content was determined by solvent extraction, followed by drying and weighing the extracted fat. Glucose and reducing sugar contents were determined by the Lane-Eynon titrimetric method, as described in AOAC Official Method 920.183 (AOAC 2016), in which honey was reacted with Fehling solution, and the sugar content was calculated based on the volume of titrant used. Vitamins A and C were quantified spectrophotometrically at 325 and 265 nm. Antioxidant activity was expressed as IC_{50} (Inhibitory Concentration 50%) and determined using the DPPH (diphenylpicrylhydrazyl) radical scavenging assay (Molyneux 2004) by measuring absorbance at 517 nm.

Identification of flowering plant species

Flowering plant species were identified through systematic field observation and voucher documentation. Observations were conducted within a 200–300 m radius of each meliponiculture site. Unidentified plant species were collected and prepared as herbarium voucher specimens for subsequent identification. Plants were identified using the mountain flora of Java (Van Steenis 2006) and the Pl@ntNet image recognition application (Pl@ntNet 2024). A plant was classified as a nectar source when bees were observed landing on it and extending their proboscis to collect the nectar. Furthermore, a plant was classified as a pollen source when bees were observed landing on it and loading their corbiculae with pollen. A plant was classified as a resin source when bees were observed on stems or leaves, working their mandibles and packing material into their corbiculae.

Environmental parameters (abiotic factors)

Abiotic factors were measured at both collection sites, including air temperature and relative humidity using a thermo-hygrometer, light intensity using a luxmeter, and wind speed using an anemometer. Measurements were recorded twice daily – at 7:00 and 13:00 h – over seven consecutive days to capture diurnal variation in microclimate conditions.

Data analysis

Descriptive statistics were used to summarize the physicochemical parameters of honey produced by *W. incisa* and *T. sapiens*. No inferential statistics were applied, as each species was represented by a single composite sample per site. Results were compared against the Indonesian National Standard for honey (SNI 8664:2024) as local market standards, the Codex Alimentarius (2022) as a global food safety standard, and Brazil Instruction No. 11 of October 20, 2000 (Marquele-Oliveira et al. 2017) as a general reference for stingless bees. Moreover, these data were examined in connection with flowering plants and abiotic factors.

RESULTS AND DISCUSSION

The physicochemical characteristics of *W. incisa* and *T. sapiens* honey are presented in Table 1. Honey quality is influenced not only by bee species (Rosmarlinasiah et al. 2023; Apriantini et al. 2022) but also by surrounding biotic (flowering plant assemblage) and abiotic conditions. *W. incisa* honey had higher water content, glucose, and vitamin C than *T. sapiens* honey (Table 1). Ash content, vitamin A, and antioxidant IC_{50} values were notably higher in *T. sapiens* honey than in *W. incisa* honey. The higher IC_{50} value in *T. sapiens* honey (562.81 ppm) compared with *W. insica* honey (423.40 ppm) indicates weaker radical scavenging activity in *T. sapiens* honey. Fat and reducing sugar content were marginally higher in *T. sapiens* honey (0.20% and 6.82%, respectively) than in *W. incisa* honey (0.19% and 6.63%); however, these differences are too small to be considered meaningful given that each species was represented by a single composite sample. The high glucose content in *W. incisa* honey may reflect differences in the botanical origin of nectar sources available at the highland site, where mountain flora such as *Castanopsis* and *Rhodondendron* predominated (Table 2). Nectar sugar composition is strongly influenced by plant species (Apriantini et al. 2022), and the greater diversity of flowering plants surrounding *W. incisa* (24 species vs 12 species for *T. sapiens*) may have contributed to the distinct sugar profile observed.

The water content of *W. incisa* honey in this study (28.61%) exceeded both the SNI 8664:2024 maximum threshold (27.5%) and the values reported for *W. incisa* honey from South Sulawesi, which ranged from 20% to 22.41% (Budiaman et al. 2019; Octaviani et al. 2020). The elevated water content of *W. incisa* honey may reflect the higher ambient humidity of the mountainous environment (77.5% RH, data from the current study), which could reduce evaporative concentration of nectar within the hive. This, combined with the hygroscopic character of honey (Sarwono 2007), likely contributed to the higher water content observed. In contrast, the water content of *T. sapiens* honey (22.45%) was lower than the 25.5–26.5% reported by Rosmarlinasiah et al. (2023) for *T. sapiens* from Konawe Islands Regency, Southeast Sulawesi Province, and this fell within the SNI 86664:2024 maximum threshold (27.5%). The honey examined by Rosmarlinasiah et al. (2023) was harvested during the rainy season (December 2021 to January 2022), whereas the *T. sapiens* honey in this study was harvested during the dry season (July 2023). This difference supports the view that honey's water content is influenced by season (Suhri & Bahar 2023) and geographical location (Adityarini et al. 2020).

The ash content of *T. sapiens* honey (0.91%) was substantially higher than that of *W. incisa* honey (0.18%) (Table 1), suggesting a higher mineral content in *T. sapiens* honey. Notably, the ash content of *T. sapiens* honey exceeded the SNI 8664:2024 maximum threshold (0.5%). The ash content of *W. incisa* honey in this study was lower than the 0.42% reported for *W. incisa* honey from South Sulawesi (Octaviani et al. 2020). High ash content in cultivated honey== has also been reported by Apriantini et al. (2022). Furthermore, high ash content may correlate with elevated amino acid concentrations in honey (Agussalim et al. 2021), although the mechanistic basis for this association requires further investigation.

This study identified 14 flowering plant species during the field observations. Combined with records from Hasan et al. (2024) and Suhri et al. (2023), a total of 31 flowering plant species were documented across both sites (Table 2). The *W. incisa* meliponary had a greater number of associated flowering plant species (24 species) than the *T. sapiens* meliponary (12 species). Of the 31 species recorded, 31 served as pollen sources, 9 as nectar sources, and 3 as resin sources, with many species providing multiple resources (Table 2). Of the total 31 flowering plant species, 18 were trees (58.06%), 10 were shrubs (32.26%), and 3 were bushs (9.68%) (Figure 3). The flowering plants surrounding *W. incisa* hives were dominated by montane taxa (e.g., *Quercus, Castanopsis, Rhododendron,* and *Litsea*). In contrast, those surrounding *T. sapiens* hives were dominated by cultivated yard plants (e.g., *Lansium*, *Nephelium*, and *Cocos*) (Table 2). Tree species were also more numerous around the *W. incisa* meliponary than around the meliponary *T. sapiens* (Figure 3).

Abiotic conditions differed between the two collection sites. Air temperature, light intensity, and wind speed at Lombong Timur village (*T. sapiens*) were higher (29.05 °C, 247.5 × 10 lux, 3.27 m/s) than in Taupe village (*W. incisa*) (22.3 °C, 77.5 × 10 lux, 2.30 m/s). Only relative humidity was higher at Taupe village (77.5%) than at Lombong Timur village (69.5%). These differences in vegetation composition and abiotic conditions between the highland and lowland sites likely contributed to the observed differences in the physicochemical properties of honey from the two species.

The odor and taste of both *W. incisa* and *T. sapiens* honey were assessed as characteristic and met the SNI 8664:2024 standard (Table 1). A comparison of the remaining physicochemical properties with regulatory standards revealed that several parameters did not meet the SNI 8664:2024 thresholds. For *W. incisa* honey, four parameters (odor, taste, glucose, and ash content) complied with the SNI standard, while water content (28.61%) exceeded the maximum threshold (27.5%). For *T. sapiens*, three parameters (odor, taste, and water content) met the SNI standard, whereas ash content (0.91%) exceeded the maximum (0.5%) and glucose (44.86%) fell below the minimum (55%). These results differ from the findings of Budiaman et al. (2019), who reported that the ash and glucose content of *W. incisa* honey from South Sulawesi did not comply with SNI standards. Rosmarlinasiah et al. (2023) reported that

Table 1. Physicochemical characteristics of *Wallacetrigona incisa* and *Tetragonula sapiens* honey from West Sulawesi

Parameter	Unit	Result		SNI 8664: 2024
		W. incisa	*T. sapiens*	
Odor	-	Normal	Normal	Normal
Taste	-	Normal	Normal	Normal
Water content	%	28.61	22.45	Max 27.5
Ash content	%	0.18	0.91	Max 0.5
Fat	%	0.19	0.20	Not specified
Glucose	%	61.18	44.86	Min 55
Reducing sugar	%	6.63	6.82	Not specified
Vitamin A	mg/g	65.06	88.36	Not specified
Vitamin C	mg/g	374.61	270.77	Not specified
Antioxidant IC_{50}	ppm	423.40	562.81	Not specified

SNI, Indonesian National Standard for honey (SNI 8664:2024); Not specified, no threshold specified; IC_{50} concentration required to inhibit 50% of radical activity (lower values indicate stronger antioxidant activity); Reducing sugars are the total of all sugars, while glucose is measured as a single parameter.

Table 2. Flowering plant species and their resource utilization by *Wallacetrigona incisa* and *Tetragonula sapiens* at two meliponary sites in West Sulawesi

Flowering plant species	Location		Source		
	A	B	N	P	R
Alpinia sp.[1,3]	√	√		√	
Ageratum conyzoides L.[2]	√	√		√	
Ardisia sp.[3]	√			√	
Barringtonia asiatica (L.) Kurz[3]	√			√	
Caldcluvia celebica (Blume) Hoogland[3]	√			√	
Castanopsis acuminatissima (Blume)A.DC.[3]	√			√	
Cocos nucifera L.[1]		√	√	√	
Cuphea ignea A.DC[1,3]	√	√	√	√	
Cyrtandra tenuicarpa H.J.Aktins[3]	√			√	
Diplycosia aperta J.J.Sm[3]	√			√	
Eupatorium sp.[1]	√			√	
Hibiscus rosa-sinensis L.[1]		√		√	
Lansium domesticum Corrêa[1]		√	√	√	
Lantana camara L.[1]		√		√	
Litsea ochracea (Blume) Boerl.[3]	√			√	
Mangifera sp. L.[1,2]		√	√	√	√
Muntingia calabura L.[1]		√	√	√	
Musa sp.[1]	√			√	
Medinilla crassifolia Blume[1,3]	√		√	√	
Nephelium lappaceum L.[1]		√	√	√	
Pimenta racemosa (Mill.) JW Moore[3]	√			√	
Pinus merkusii Jungh. & de Vriese[3]	√			√	√
Pigafetta elata (Mart.) H.Wendl.[3]	√	√	√	√	
Quercus spp.[3]	√			√	
Rhodondendron spp.[3]	√			√	
Rubus fraxinifolus Poir.[1,3]	√		√	√	
Syzgium sp.[3]	√	√		√	
Symplocos sp.[3]	√			√	
Saurauia trystila Burkill.[3]	√			√	
Vaccinium latissimum J.J.Sm[1,3]	√			√	√
Weinmannia blumei Planch.[3]	√			√	
Total	24	12	9	31	3

A: *W. incisa* at Taupe village; B: *T. sapiens* at Lombong Timur village; N: nectar; P: pollen; R: resin, indicates observed utilization; Superscripts indice data source: [1]: This study, [2]: Hasan et al. (2024); [3]: Suhri et al. (2023).

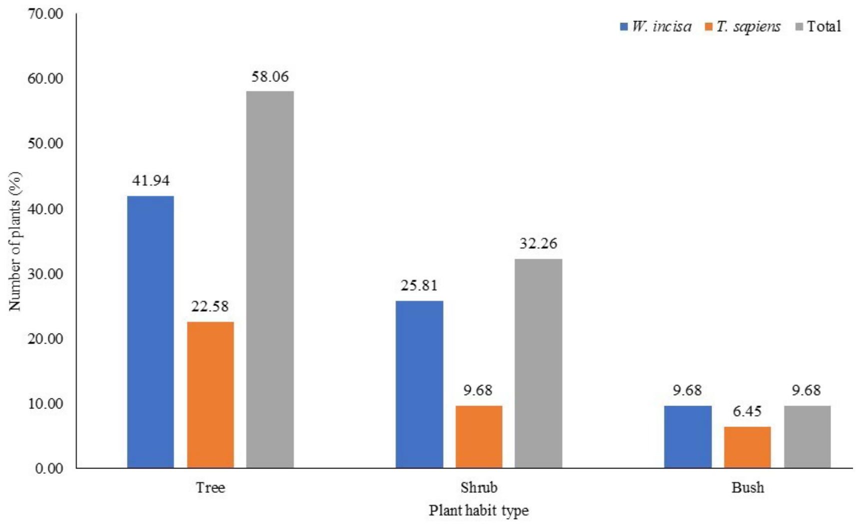

Figure 3. Percentage of flowering plant species by growth habit (tree, shrub, bush) associated with *Wallacetrigona incisa* and *Tetragonula sapiens* meliponaries in West Sulawesi, based on data from the current study and Suhri et al. (2023).

both water and ash content of *T. sapiens* honey from Southeast Sulawesi complied with SNI standards. The glucose content of *W. incisa* honey (61.18%) met the minimum standard of the CODEX Alimentarius (60 g/100 g) but not the Brazilian Normative Instruction No.11/2000 (65 g/100 g), whereas *T. sapiens* honey (44.86%) did not meet either standard (Vit et al. 2025). It should be noted, however, that both the CODEX Alimentarius and Brazilian standards were developed for *Apis* honey and may not be directly applicable to stingless bee honey.

This study provides the first physicochemical comparison of honey from *W. incisa* and *T. sapiens* in West Sulawesi and highlights how altitude and environmental conditions influence honey quality. Further studies with replicated sampling across multiple harvest seasons are needed to confirm these preliminary findings.

ACKNOWLEDGMENTS

This research was funded by HIBAH DIKTI through the Penelitian Dosen Pemula (PDP) scheme (grant number 247/UN55.C/PT.01.03/2023). The authors are grateful to Mr. Markus and Mr. Mufradat, owners of the meliponaries, for providing access to their hives and honey samples. The authors also thank Dr. Sih Kahono for assistance with the taxonomic identification of *W. incisa*.

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