Communications in Science and Technology 11(1) (2026) 272–280

COMMUNICATIONS IN SCIENCE AND TECHNOLOGY

Homepage: cst.kipmi.or.id

Sugar composition profiling of Indonesian stingless bee (Meliponini) honey from multiple geographical regions using HPLC-RID and multivariate analysis

Muhamad Saeful Anwar [a,b], Ekrima Astari[c], Nurlela[a,d], Muhamad Sahlan[b,e,f,*]

[a]*Department of Chemistry, Universitas Nusa Bangsa, Bogor 16166, Indonesia*

[b]*Department of Chemical Engineering, Universitas Indonesia, Depok 16424, Indonesia*

[c]*Department of Earth System Science and Technology, Kyushu University, Fukuoka 816-0811, Japan*

[d]*Research Center for Pharmaceutical Ingredient and Traditional Medicine, National Research and Innovation Agency (BRIN), Kawasan Sains dan Teknologi (KST) Soekarno, Cibinong 16911, Indonesia*

[e]*Research Centre for Biomedical Engineering, Universitas Indonesia, Depok 16424, Indonesia*

[f]*Department of Interdisciplinary Engineering, Universitas Indonesia, Depok 16424, Indonesia*

Article history:
Received: 18 May 2026 / Received in revised form: 23 June 2026 / Accepted: 23 June 2026

Abstract

Stingless bee honey (Meliponini) has attracted attention due to its unique sugar composition, particularly trehalulose, a low-glycemic disaccharide with potential health benefits. This study investigated the sugar profiles of 23 stingless bee honey samples collected from East Java, Central Java, Bali, Belitung, and Jambi using High-Performance Liquid Chromatography-Refractive Index Detection (HPLC-RID). Fructose and glucose were the dominant sugars, while trehalulose concentrations varied markedly among regions. The highest fructose content was observed in Bali honey (47.09 g/100 g), whereas Belitung honey exhibited the highest trehalulose concentration (43.22 g/100 g). Jambi samples contained relatively higher sucrose levels. Dehumidification treatment reduced trehalulose concentration while increasing fructose and glucose contents. Principal Component Analysis (PCA) revealed clear regional clustering patterns, explaining 83.3% of the total variance. Belitung honey samples were distinguished by exceptionally high trehalulose concentrations compared with samples from other regions. These findings provide a comparative overview of regional variation in sugar composition and trehalulose distribution in Indonesian stingless bee honey. The results contribute to the scientific database of Indonesian stingless bee honey and may support future efforts in quality control, honey characterization, and the development of high-value honey products.

Keywords: Geographical differentiation; HPLC-RID; stingless bee honey; sugar composition; trehalulose

1. Introduction

Stingless bee honey produced by bees belonging to the Meliponini tribe has attracted increasing scientific interest due to its distinctive physicochemical characteristics and potential functional properties. Compared with conventional honey produced by *Apis mellifera*, stingless bee honey generally contains higher moisture content, different sugar composition, and diverse bioactive compounds that may contribute to its nutritional and therapeutic value [1,2,3,4]. In Southeast Asia, stingless bee honey is widely consumed as a traditional functional food and is commonly known as “kelulut honey” in several regions, including Indonesia and Malaysia. Stingless bees produce various products with bioactive properties, including propolis, venom, and honey which contains the unique disaccharide trehalulose [5,6]. Among the sugars identified in stingless bee honey, trehalulose has gained considerable attention because of its potential health benefits.

Trehalulose is a naturally occurring sucrose isomer characterized by a low glycemic index, slow digestion rate, and non-cariogenic properties [7,8]. Previous studies have suggested that trehalulose may contribute to the functional value of stingless bee honey and may serve as a potential chemical marker for honey authentication [1]. The concentration of trehalulose has been reported to vary among stingless bee honey samples collected from different regions and production systems, although the relative contribution of geographical, botanical, biological, and post-harvest factors remains incompletely understood [9,10]. Although trehalulose has been widely reported as a characteristic sugar of stingless bee honey, comparative information regarding its distribution across geographically distinct Indonesian regions remains limited. Most previous studies have focused on honey collected

* Corresponding author.
Email: muhamad.sahlan@gmail.com
https://doi.org/10.21924/cst.11.1.2026.2001

from a single region, a limited number of colonies, or specific stingless bee species.

Consequently, regional variation in trehalulose abundance and its contribution to compositional differentiation of Indonesian stingless bee honey have not been comprehensively evaluated. The present study addresses this gap by combining chromatographic sugar profiling and multivariate statistical analysis to characterize honey samples collected from multiple Indonesian geographical regions. Indonesia possesses extensive biodiversity and abundant stingless bee populations distributed across various ecological regions. Several stingless bee species, such as Tetragonula laeviceps and Heterotrigona itama, are commonly cultivated by local farmers for honey production [1,11]. Previous studies in Indonesia have mainly focused on stingless bee diversity, habitat distribution, and physicochemical characterization of honey from specific regions.

However, information regarding regional variation in sugar composition among Indonesian stingless bee honeys remains limited. Sugar profiling using High-Performance Liquid Chromatography coupled with Refractive Index Detection (HPLC-RID) has been widely applied for the characterization and authentication of honey due to its reliability, sensitivity, and ability to separate mono and disaccharides effectively [12,13]. In addition, multivariate statistical approaches such as Principal Component Analysis (PCA) and hierarchical clustering analysis have demonstrated strong potential for evaluating compositional variability and classification patterns among honey samples from different geographical origins [14,15].

Therefore, this study aimed to: (i) determine the sugar composition of stingless bee honey collected from several geographical regions in Indonesia using validated HPLC-RID analysis; (ii) evaluate regional variations in fructose, glucose, sucrose, maltose, and trehalulose contents; and (iii) investigate compositional clustering patterns among honey samples collected from different Indonesian regions using multivariate statistical analysis. This study is expected to contribute to the chemical characterization and comparative evaluation of Indonesian stingless bee honey, particularly with respect to the distribution of trehalulose and other major sugars.

2. Materials and Methods

2.1. Materials

Analytical standards of fructose (purity: 99%), glucose (purity: 99%), and maltose (purity: 99%) were obtained from HiMedia Laboratories Pvt. Ltd. (Maharashtra, India). Sucrose analytical standard (purity: 99%) was purchased from PhytoTech Labs Inc. (Kansas, USA). Trehalulose analytical standard (purity: 91%) was obtained from Biosynth International, Inc. (Louisville, USA). Acetonitrile (pro analysis grade) for chromatographic analysis was obtained from Merck (Darmstadt, Germany). Ultrapure water was used throughout all analytical procedures. All chemicals and reagents used in this study were of analytical grade.

2.2. Collecting and preparation of honey sample

A total of 23 stingless bee honey samples were collected from five geographical regions in Indonesia, namely East Java, Central Java, Bali, Belitung, and Jambi. Honey samples were obtained directly from local stingless bee farmers and stored in airtight containers at room temperature prior to analysis. The collected samples represented different environmental conditions, floral sources, and geographical origins that may influence nectar composition and honey characteristics. Information regarding nectar sources and stingless bee species was obtained from local farmers whenever available. However, stingless bee species identification was not systematically confirmed for all samples and is therefore recognized as a limitation of the present study. In addition to raw honey samples, several Belitung honey samples were subjected to post-harvest dehumidification treatment by local producers prior to analysis. The dehumidification process was conducted at approximately 38°C to reduce moisture content and improve storage stability. Therefore, comparisons between raw and dehumidified honey were limited to selected Belitung samples included in this study.

2.3. Preparation of standard solutions

Individual stock solutions of fructose, glucose, sucrose, maltose, and trehalulose were prepared using ultrapure water. Calibration working solutions were subsequently prepared by serial dilution of the stock solutions to obtain final concentrations of 625, 1250, 2500, 5000, and 10,000 ppm for each sugar standard. Calibration curves were generated by plotting peak area against concentration for each standard compound. These calibration standards were used for the quantitative determination of sugar composition in stingless bee honey samples. Linearity of the analytical method was evaluated across the prepared concentration ranges.

2.4. Sample preparation

Prior to analysis, honey samples were homogenized thoroughly. Approximately 1 g of each honey sample was accurately weighed and dissolved in ultrapure water using a 10 mL volumetric flask. The solution was mixed until completely dissolved and subsequently filtered through a 0.45 µm membrane filter before HPLC-RID analysis.

2.5. High performance liquid chromatography-refractive index detector analysis

Sugar composition analysis was performed using an HPLC Shimadzu LC-20AD Black system equipped with a refractive index detector (RID-10A). Chromatographic separation was carried out using an Athena NH_2 HPLC column (4.6 × 150 mm, 3 µm particle size; CNW Technologies, China) maintained at 25 °C. The mobile phase consisted of acetonitrile and ultrapure water (75:25, v/v) delivered at a flow rate of 1.0 mL/min. The injection volume was 20 µL. Quantitative determination of fructose, glucose, sucrose, maltose, and trehalulose was conducted using external calibration curves generated from authentic analytical standards. Sugar concentrations were expressed as g/100 g honey.

2.6. Method validation

Method validation was evaluated based on linearity, limit of detection (LOD), and limit of quantification (LOQ). Calibration curves for all analyzed sugars demonstrated good linearity with correlation coefficients (R^2) greater than 0.999. LOD and LOQ values were determined based on the standard deviation of the analytical response and the slope of the calibration curves. The obtained values indicated adequate sensitivity for quantitative sugar determination in stingless bee honey samples. All analyses were performed in triplicate and expressed as mean ± standard deviation. Due to limitations in analytical standard availability and instrument access time, method validation in the present study was limited to linearity, LOD, and LOQ evaluation. Additional validation parameters such as recovery, repeatability, and inter-day precision were not investigated and should be considered in future studies.

2.7. Statistical analysis

Descriptive statistical analysis was performed for all quantified sugar components. Principal Component Analysis (PCA), hierarchical clustering analysis, heatmap visualization, and correlation analysis were conducted to evaluate clustering patterns and relationships among sugar variables and honey samples from different geographical regions. Multivariate statistical analyses and graphical visualizations were performed using Python-based statistical processing and visualization approaches. Differences in sugar composition among regions were interpreted descriptively based on clustering patterns and compositional variability observed in the dataset.

3. Results and Discussion

3.1. HPLC-RID performance for sugar analysis

The HPLC-RID method successfully separated fructose, glucose, sucrose, maltose, and trehalulose with satisfactory peak resolution and reproducible retention times (Fig. 1). Monosaccharides, including fructose and glucose, exhibited shorter retention times than disaccharides due to differences in molecular polarity and interaction with the stationary phase. The chromatograms obtained from stingless bee honey samples revealed distinct chromatographic sugar profiles and variations in peak intensity among geographical regions, indicating differences in sugar composition and concentration.

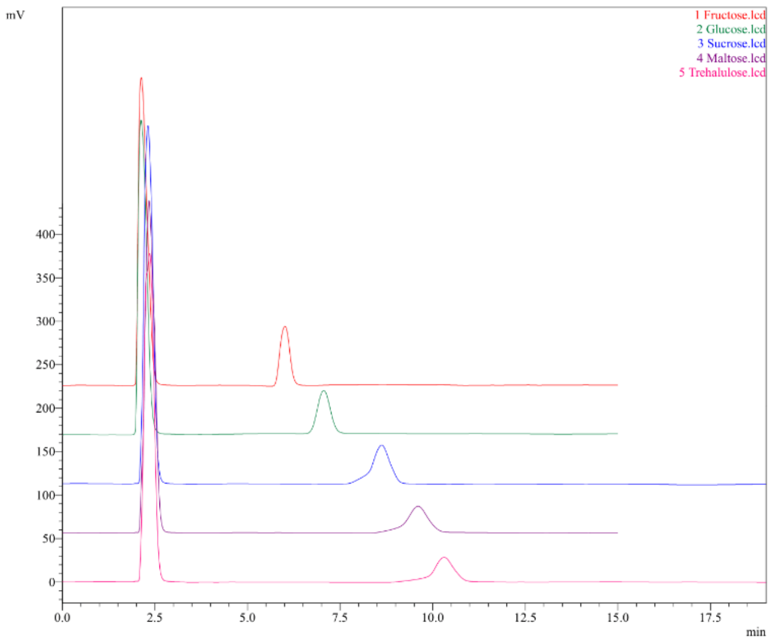

Fig. 1. Representative HPLC-RID chromatograms showing the separation of fructose, glucose, sucrose, maltose, and trehalulose in stingless bee honey samples

Table 1. Analytical performance parameters of the HPLC-RID method for sugar determination in stingless bee honey

Standard	LOD (g/100 g)	LOQ (g/100 g)	R^2
Fructose	0.0083	0.0252	0.9995
Glucose	0.0020	0.0062	0.9999
Sucrose	0.0310	0.0939	0.9998
Maltose	0.0087	0.0272	0.9996
Trehalulose	0.0047	0.0141	0.9993

The analytical performance of the HPLC-RID method demonstrated excellent linearity for all analyzed sugars, with correlation coefficient (R^2) values greater than 0.999 (Table 1). The obtained LOD and LOQ values indicated adequate sensitivity for quantitative determination of sugars in stingless bee honey samples. These findings are consistent with previous reports describing the suitability of HPLC-RID for sugar analysis in honey matrices [16]. The chromatographic profiles observed in this study were comparable with previous studies on stingless bee honey sugar characterization using HPLC-based methods. Clear baseline separation among fructose, glucose, sucrose, maltose, and trehalulose confirmed the reliability of the analytical method for evaluating sugar composition variability among Indonesian stingless bee honey sampel.

3.2. Sugar composition of Indonesian stingless bee honey

The sugar composition of stingless bee honey samples collected from different Indonesian regions is presented in Table 2. Fructose and glucose were identified as the dominant sugars in most samples, although considerable variability in sugar composition was observed among geographical regions.

Fructose concentrations ranged from 21.95 to 47.09 g/100 g honey, while glucose concentrations varied between 14.78 and 37.64 g/100 g honey. In general, fructose concentrations were higher than glucose concentrations in most samples, consistent with previous studies on stingless bee honey composition [1]. The predominance of fructose may contribute to the characteristic sweetness profile commonly reported for stingless bee honey.

The fructose-to-glucose (F/G) ratio is widely recognized as an important parameter influencing honey crystallization behavior (Alghamdi et al., 2020). Honey samples with relatively higher fructose proportions generally exhibit lower crystallization tendencies because fructose is more soluble than glucose [10,17,18]. Several samples from Bali and East Java exhibited relatively high fructose concentrations, indicating compositional characteristics that are generally associated with improved liquid stability.

Table 2. Analytical performance parameters of the HPLC-RID method for sugar determination in stingless bee honey

Sample Code	Region	Fructose (g/100 g)	Glucose (g/100 g)	Sucrose (g/100 g)	Maltose (g/100 g)	Trehalulose (g/100 g)
			East Java			
A1	Kediri (Gurah)	36.55	27.84	0.21	4.55	3.80
A2	Situbondo	38.03	26.71	0.00	6.48	4.32
A3	Gresik (Balong Panggang)	40.09	28.01	0.00	7.99	1.69
A4	Banyuwangi (Wongsorejo)	43.49	31.51	0.00	0.00	0.00
			Central Java			
B1	Pati (Gunung Wangkal)	41.78	24.49	0.00	2.30	0.15
B2	Kendal (Boja)	46.31	28.20	0.00	6.01	3.72
B3	Blora	34.00	32.53	0.00	3.37	2.33
B4	Temanggung (Candiroto)	33.01	37.64	0.00	2.50	1.71
B5	Semarang (Mijen)	38.21	28.80	0.00	4.78	0.88
B6	Blora (Tunjungan)	36.40	32.70	0.00	4.34	4.57
			Bali			
C1	Bali (Karang Asem)	47.09	27.17	0.00	2.68	2.24
			Belitung			
D1	Belitung 1	28.94	21.40	0.00	0.00	22.04
D2	Belitung 2	32.11	23.30	0.00	0.00	19.57
D3	Belitung 3	21.95	14.78	0.84	0.00	43.22
D4	Belitung 4	31.54	25.67	0.00	0.00	29.11
D5	Belitung 5	22.93	18.87	0.00	0.00	33.69
D6	Belitung 6	31.21	24.64	0.00	0.00	14.90
			Jambi			
E1	Pematang Tungkung (Teluk Nilau)	32.43	27.40	10.23	0.00	0.00
E2	Pematang Tungkung (Kelagian)	31.65	31.68	10.60	0.00	0.42
E3	Munim Alam Sakti 1	29.97	28.41	15.07	0.00	0.00
E4	Munim Alam Sakti 2	29.95	28.62	11.66	0.00	0.00
E5	Sungai Rantai (Usaha Mandiri)	28.78	32.59	7.10	1.92	0.00
E6	Kelagian (Usaha Mandiri)	31.53	35.12	8.76	0.00	0.00

Trehalulose was detected in several samples at markedly different concentrations. The highest trehalulose concentration was observed in sample D3 from Belitung (43.22 g/100 g), followed by D5 (33.69 g/100 g) and D4 (29.11 g/100 g). These findings support previous reports indicating that stingless bee honey may contain substantial amounts of trehalulose as a characteristic disaccharide [1,13].

Notably, trehalulose concentrations observed in several Belitung samples were within or above the upper range reported for many stingless bee honeys worldwide, highlighting the distinctive sugar profile of these Indonesian samples.

Belitung honey samples consistently exhibited substantially higher trehalulose concentrations than samples from other regions. Although the present study was not designed to investigate the underlying causes of this variation, previous studies have suggested that factors such as botanical origin, bee species, nectar composition, and honey maturation processes may influence trehalulose formation and accumulation in stingless bee honey [2,19,20]. Therefore, these factors may represent potential explanations that warrant further investigation.

In contrast, honey samples from Jambi were characterized by relatively elevated sucrose concentrations ranging from 7.10 to 15.07 g/100 g honey. The observed variability in sucrose content demonstrates substantial compositional differences among samples and may reflect differences in honey production systems or biological and environmental conditions. However, because botanical origin, bee species, and nectar composition were not systematically evaluated in this study, the specific factors responsible for these differences could not be determined.

An interesting anomaly was observed in sample A4 from Banyuwangi, East Java, which showed undetectable trehalulose and maltose concentrations. This sample differed markedly from other East Java samples that generally contained detectable amounts of these sugars. The reason for this unique profile remains unclear based on the available data. Further studies involving detailed botanical characterization, bee species identification, and physicochemical analyses are required to clarify this observation.

Compared with conventional Apis mellifera honey, stingless bee honey analyzed in this study exhibited greater compositional variability and the presence of trehalulose as a distinguishing disaccharide [1,21]. These characteristics support the uniqueness of stingless bee honey and its potential functional value.

3.3. Geographical variation in sugar composition

Honey samples collected from different geographical regions exhibited clear compositional differences in fructose, glucose, sucrose, maltose, and trehalulose contents. Samples from Bali generally showed higher fructose concentrations, whereas Belitung samples were characterized by elevated trehalulose levels. In contrast, samples from Jambi contained relatively higher sucrose concentrations compared with samples from other regions.

The observed geographical variation demonstrates that sugar composition differs substantially among regions. Indonesia possesses highly diverse ecosystems and vegetation types, which may contribute to regional variability in honey characteristics. Previous studies have also reported that geographical origin and botanical diversity can influence the physicochemical properties and sugar composition of stingless bee honey [2,3,11,22,23],. However, because environmental parameters, floral sources, and bee species were not comprehensively characterized in the present study, their specific contributions could not be evaluated directly.

Belitung honey samples demonstrated particularly distinctive characteristics due to their high trehalulose concentrations. This finding is consistent with previous reports describing trehalulose-rich stingless bee honey and reinforces the potential importance of trehalulose as a differentiating compositional marker [24,25,26]. The substantial variability observed among regions highlights the value of sugar composition profiling for honey characterization and suggests its potential utility for geographical differentiation and authenticity assessment of Indonesian stingless bee honey.

3.4 Dehumidification and crystallization behavior

Several Belitung honey samples were analyzed in both raw and dehumidified forms to evaluate compositional differences associated with post-harvest processing. The comparison indicated that dehumidified honey samples generally exhibited higher relative concentrations of fructose and glucose compared with their corresponding raw honey counterparts (Table 3) [27].

Table 3. Comparison of sugar composition between raw and dehumidified stingless bee honey samples from Belitung

Sugar Type	Belitung 1 (g/100 g)		Belitung 2 (g/100 g)		Belitung 3 (g/100 g)	
	Raw (D1)	Dehumidified (D2)	Raw (D3)	Dehumidified (D4)	Raw (D5)	Dehumidified (D6)
Fructose	28.94	32.11	21.95	31.54	22.93	31.21
Glucose	21.40	23.30	14.78	25.67	18.87	24.64
Sucrose	0.00	0.00	0.00	0.00	0.00	0.00
Maltose	0.00	0.00	0.00	0.00	0.00	0.00
Trehalulose	22.04	19.57	43.22	29.11	33.69	14.90

Table 3 presents the sugar composition of raw and dehumidified stingless bee honey samples from Belitung. Across all paired samples, fructose and glucose concentrations increased following dehumidification treatment, whereas sucrose and maltose were not detected in either condition. In contrast, trehalulose concentrations showed a consistent decrease after dehumidification across all sample pairs. For instance, trehalulose content in sample D3 decreased from 43.22 g/100 g in raw honey to 29.11 g/100 g after dehumidification, while sample D5 decreased from 33.69 g/100 g to 14.90 g/100 g after treatment.

To visualize these differences, a grouped bar chart was constructed based on the quantified sugar concentrations (Fig. 2). The graphical representation confirms consistent differences between raw and dehumidified samples, particularly the increase in fructose and glucose concentrations and the reduction of trehalulose across all analyzed sample pairs. The most pronounced reduction in trehalulose was observed in the D5–D6 pair. These compositional differences may reflect relative concentration effects between raw and processed honey samples. However, it is important to note that key processing variables, including moisture content, temperature conditions, and other operational parameters during dehumidification, were not measured or controlled in this study.

Therefore, the specific physicochemical mechanisms underlying the observed changes cannot be determined based on the available data.

The fructose-to-glucose (F/G) ratio and trehalulose content have been previously reported as important factors associated with honey crystallization behavior. In this study, samples with relatively higher fructose proportions and measurable trehalulose concentrations tended to show patterns consistent with lower crystallization tendency, in agreement with general solubility characteristics of these sugars reported in the literature [17,27,28].

Interestingly, Belitung honey samples consistently exhibited measurable trehalulose concentrations alongside variations in sugar distribution before and after dehumidification. While this observation does not allow causal interpretation, it highlights the compositional complexity of stingless bee honey and suggests that multiple sugar constituents may contribute to its physicochemical behavior.

Overall, the comparison between raw and dehumidified samples demonstrates measurable compositional differences in major sugars, particularly fructose, glucose, and trehalulose. These findings provide baseline information on the variability of stingless bee honey composition under different post-harvest conditions, while mechanistic explanations require further investigation involving controlled processing parameters and additional physicochemical measurements.

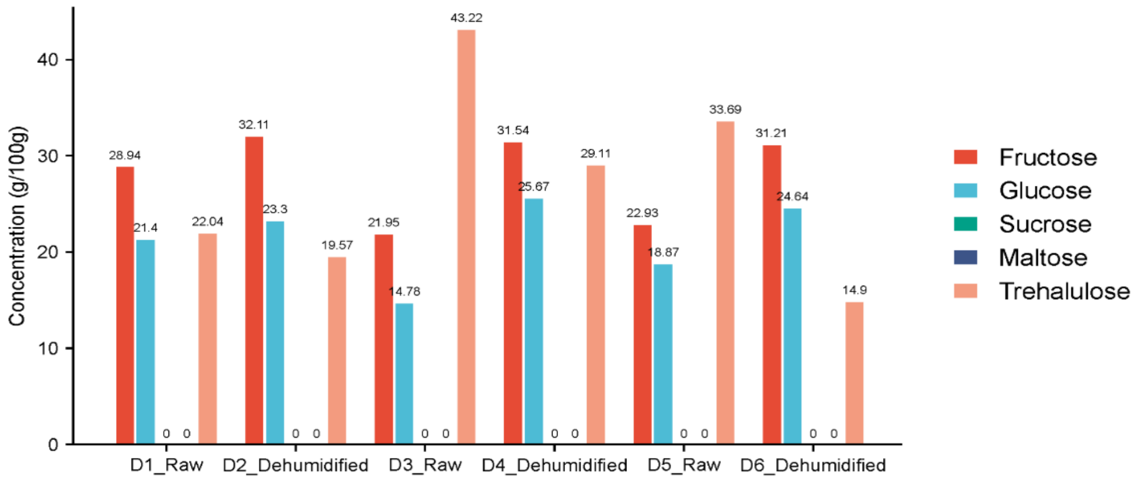

Fig. 2. Effect of dehumidification treatment on fructose, glucose, and trehalulose concentrations in Belitung stingless bee honey samples

Interestingly, Belitung honey samples consistently showed relatively high trehalulose concentrations together with lower crystallization tendencies, suggesting a possible contribution of trehalulose to maintaining the liquid stability of stingless bee honey. This observation may indicate that crystallization behavior in stingless bee honey is influenced not only by fructose-to-glucose ratio, but also by the presence of other dominant sugars such as trehalulose.

Table 4. Fructose-to-Glucose Ratio and Observed Physical State of Indonesian Stingless Bee Honey Samples

Region	F/G Ratio Range	Theoretical Crystallization Tendency	Observed Crystallization Behavior
East Java	1.31–1.43	Predominantly non-crystallized	Mostly crystallized
Central Java	0.88–1.71	Mixed tendency	Mostly crystallized
Bali	1.73	Non-crystallized	Crystallized
Belitung	1.22–1.49	Non-crystallized	Predominantly non-crystallized
Jambi	0.88–1.18	Mixed tendency	Mixed behavior

As summarized in Table 4, differences were observed between the fructose-to-glucose (F/G) ratios and the visual crystallization status of several honey samples. For example, some samples from East Java and Bali exhibited crystallization despite relatively high F/G ratios, which are generally associated with lower crystallization tendency.

Conversely, several Belitung samples remained in liquid form despite having F/G ratios comparable to those of samples from other regions. These observations indicate that the relationship between sugar composition and crystallization behavior in stingless bee honey may be complex and cannot be fully explained by F/G ratio alone. However, because crystallization parameters, moisture content, and storage conditions were not systematically evaluated in the present study, the factors contributing to the observed differences could not be conclusively determined. The regional differences and inconsistencies between F/G ratio and observed crystallization status are further visualized in Fig. 3.

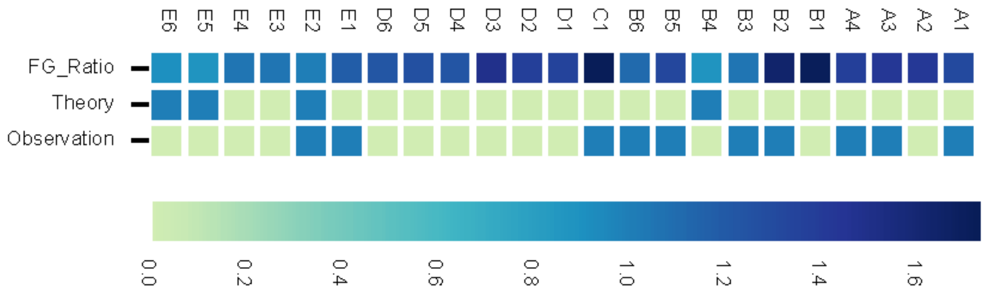

Fig. 3. Matrix heatmap of fructose-to-glucose ratios and crystallization behavior of Indonesian stingless bee honey samples

Therefore, the observed crystallization patterns should be interpreted as preliminary observations and warrant further investigation under controlled experimental conditions.

3.5. Principal component analysis (PCA)

Principal Component Analysis (PCA) was performed to evaluate clustering patterns among stingless bee honey samples based on sugar composition (Fig. 4). The PCA score plot demonstrated clear separation among samples originating from Bali, Belitung, Central Java, East Java, and Jambi. Principal Component 1 (PC1) explained 49.9% of the total variance, while Principal Component 2 (PC2) accounted for 33.4%, resulting in a cumulative explained variance of 83.3%.

Belitung honey samples were clearly separated on the positive side of PC1 and strongly associated with elevated trehalulose concentrations. This finding was consistent with the compositional data presented in Table 2 and indicates that trehalulose was the major variable contributing to the differentiation of Belitung honey samples.

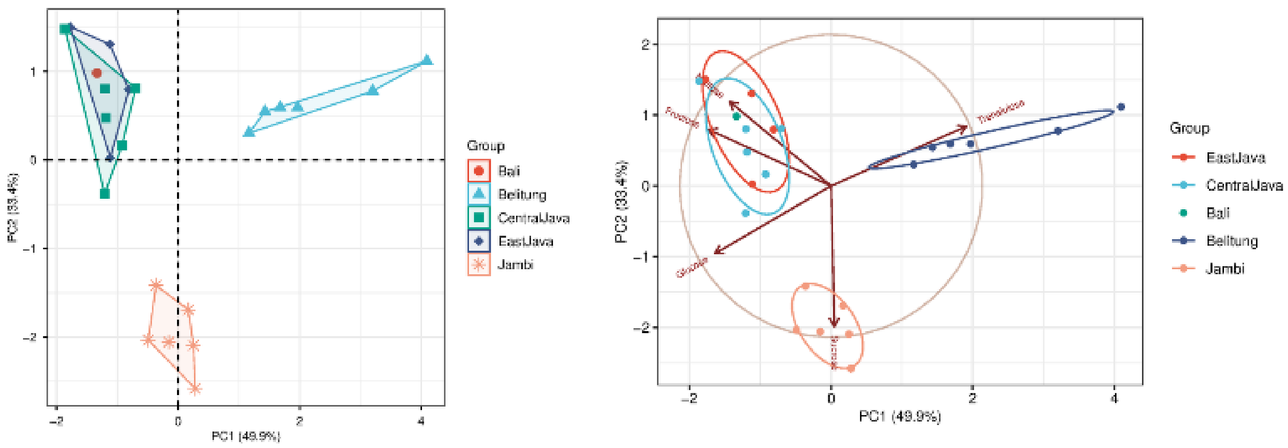

Fig. 4. Principal Component Analysis (PCA) score plot and biplot of stingless bee honey samples based on sugar composition

In contrast, samples from Bali, Central Java, and East Java clustered predominantly on the negative side of PC1 and were more closely associated with fructose and glucose variables. Meanwhile, Jambi samples were separated along the negative PC2 axis, likely due to their relatively higher sucrose concentrations.

The PCA biplot further confirmed that trehalulose contributed strongly to the differentiation of Belitung samples, whereas fructose and glucose were associated with samples from Bali and Java regions. These findings demonstrate that multivariate statistical analysis combined with sugar profiling can effectively differentiate stingless bee honey according to geographical origin.

3.6. Heatmap and correlation analysis

Hierarchical clustering analysis and heatmap visualization further demonstrated clear grouping patterns among honey samples based on sugar composition (Fig. 5). Fructose and glucose formed the closest cluster, indicating their strong compositional similarity and dominant contribution to stingless bee honey composition.

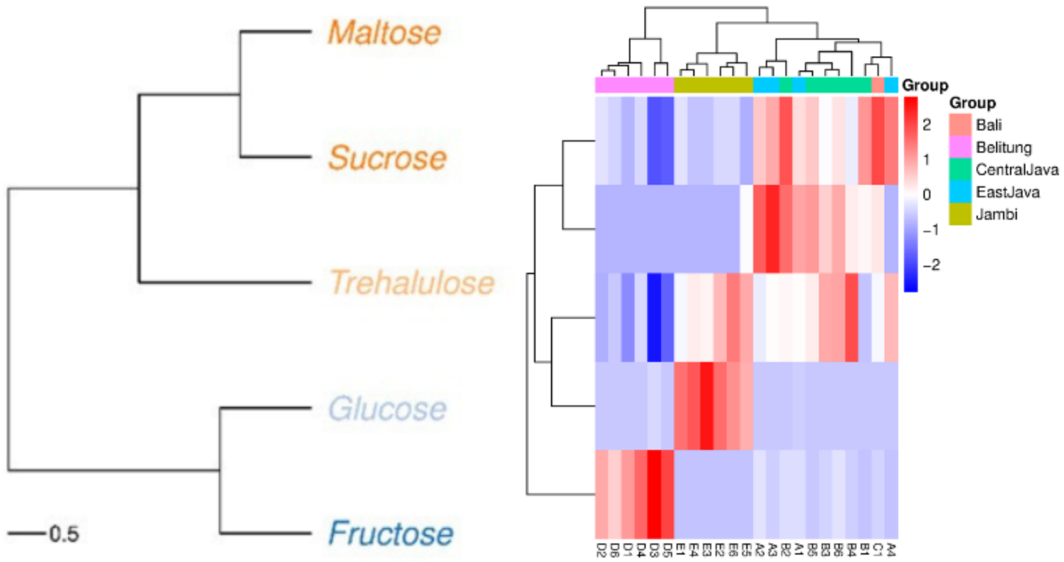

Fig. 5. Heatmap and hierarchical clustering analysis of sugar composition in Indonesian stingless bee honey

In contrast, trehalulose formed a separate cluster due to its unique distribution pattern among samples, particularly in Belitung honey. This distinct clustering suggests that trehalulose may serve as a useful chemical marker for the differentiation and authentication of stingless bee honey from specific geographical origins.

The heatmap visualization also revealed that Jambi samples were associated with relatively higher sucrose concentrations, while Belitung samples were characterized by high trehalulose levels. The observed clustering patterns support the hypothesis that geographical and environmental factors strongly influence the sugar composition of Indonesian stingless bee honey.

Overall, the integration of chromatographic sugar profiling with multivariate statistical analysis demonstrated strong potential for evaluating regional variability and supporting the authentication of stingless bee honey products.

4. Conclusion

This study demonstrated substantial geographical variation in the sugar composition of Indonesian stingless bee honey collected from East Java, Central Java, Bali, Belitung, and Jambi. Fructose and glucose were identified as the dominant sugars in most samples, whereas trehalulose distribution varied markedly among regions, with Belitung honey exhibiting considerably elevated trehalulose concentrations and Jambi samples showing relatively higher sucrose levels. The HPLC-RID method showed satisfactory analytical performance for the determination of fructose, glucose, sucrose, maltose, and trehalulose, while multivariate statistical analyses, including Principal Component Analysis (PCA) and hierarchical clustering analysis, successfully differentiated honey samples according to geographical origin based on their sugar composition profiles. Trehalulose was identified as the major variable contributing to the separation of Belitung honey samples, highlighting its potential as a chemical marker for geographical differentiation and honey authentication. The results also support the potential functional value of Indonesian stingless bee honey due to the presence of trehalulose as a naturally occurring low-glycemic disaccharide, while differences in fructose-to-glucose composition and trehalulose concentration may influence physicochemical stability and crystallization behavior. Nevertheless, limitations related to incomplete bee species identification, lack of detailed botanical origin analysis, and limited information regarding moisture content and standardized dehumidification treatment should be addressed in future studies integrating melissopalynological, physicochemical, and metabolomic approaches.

Acknowledgements

The authors gratefully acknowledge the financial support for the Article Processing Charge (APC) provided by the Unit of Research, Innovation, and Community Engagement, Faculty of Engineering, Universitas Indonesia, under Contract NKB-1776/UN2.F4.D/PPM.00.04/2026. The authors would like to express their sincere gratitude to Nurlela for her guidance, supervision, and valuable suggestions throughout this research. The authors also gratefully acknowledge Muhamad Sahlan for his support, insightful discussions, and assistance during the completion of this study. Appreciation is extended to the i-CELL Laboratory, Faculty of Engineering, Universitas Indonesia, for providing laboratory facilities and technical support. The authors further thank all individuals and collaborators involved in honey sample collection and analytical measurements.

References

1. M. T. Fletcher *et al.*, *Stingless bee honey, a novel source of trehalulose: a biologically active disaccharide with health benefits*, Sci. Rep. 10 (2020).
2. R. D. Rachmawati, A. Agus, N. Umami, Agussalim, dan H. Purwanto, *Diversity, distribution, and nest characteristics of stingless bees (Hymenoptera: Meliponini) in Baluran National Park, East Java, Indonesia*, Biodiversitas 23 (2022) 3890–3901.
3. Agussalim *et al.*, *Klanceng Honey (Tetragonula laeviceps): Its effect on fasting blood glucose, lipid and hematological profiles, and pancreatic*

histopathology of diabetic rats, Commun. Sci. Technol. 9 (2024) 331–342.

4. J. Thipraksa, P. Michu, A. Kongthong, dan P. Chaijak, *Exploring the impact of co-fermentation Saccharomyces cerevisiae and Lactobacillus sp. on stingless bee-honey cider fermentation*, Commun. Sci. Technol. 8 (2023) 93–99.
5. G. Sabrina, R. Adawiyah, A. Usman, S. C. Mayhana, D. I. Z. Sihotang, dan M. Sahlan, *Phytochemical Analysis and Antifungal Activity of Brunei Propolis Against Candida sp. and Cryptococcus sp.*, Int. J. Technol. 13 (2022) 1640–1650.
6. S. C. Asih *et al.*, *Evaluation of the Antiviral Characteristics of Tetragonula sapiens Propolis from Indonesia Using In Vitro and In Silico Methods*, Int. J. Technol. 16 (2025) 686–705.
7. M. F. Zulkifli, M. Sivakumar, M. Maulidiani, dan W. I. Wan Ismail, *Bibliometric approach to trehalulose research trends for its potential health benefits*, Trends Food Sci. Technol. 136 (2023) 1–10.
8. J. Waworuntu, *Sugar Content Composition of Various Types of Honey Produced by Apis mellifera L.: A Review*, J. Penelit. Pendidik. IPA 10 (2024) 98–106.
9. N. Lubis, S. Sofiyani, dan E. C. Junaedi, *Penentuan Kualitas Madu Ditinjau dari Kadar Sukrosa dengan Metode Luff Schoorl*, J. Sains Kesehat. 4 (2022) 290–297.
10. P. Ji *et al.*, *Natural crystallization properties of honey and seed crystals-induced crystallization process for honey performance enhancing*, Food Chem. 405 (2023).
11. N. Zawawi *et al.*, *Unique physicochemical properties and rare reducing sugar trehalulose mandate new international regulation for stingless bee honey*, Food Chem. 373 (2022).
12. I. Jalaludin dan J. Kim, *Comparison of ultraviolet and refractive index detections in the HPLC analysis of sugars*, Food Chem. 365 (2021).
13. A. Z. Jusoh, N. Zawawi, N. A. F. M. Ramlan, dan N. F. S., *Analysis of trehalulose in kelulut honey samples via HPLC-MS*, Food Res. 6 (2023) 278–283.
14. W. J. Ng, N. W. Sit, P. A. C. Ooi, K. Y. Ee, dan T. M. Lim, *Botanical origin differentiation of Malaysian stingless bee honey produced by Heterotrigona itama and Geniotrigona thoracica using chemometrics*, Molecules 26 (2021).
15. A. Gela, Z. A. Hora, D. Kebebe, dan A. Gebresilassie, *Physico-chemical characteristics of honey produced by stingless bees (Meliponula beccarii) from West Showa zone of Oromia Region, Ethiopia*, Heliyon 7 (2021).
16. M. Taleuzzaman, *Limit of Blank (LOB), Limit of Detection (LOD), and Limit of Quantification (LOQ)*, Org. Med. Chem. Int. J. 7 (2018).
17. B. A. Alghamdi *et al.*, *Analysis of sugar composition and pesticides using HPLC and GC–MS techniques in honey samples collected from Saudi Arabian markets*, Saudi J. Biol. Sci. 27 (2020) 3720–3726.
18. M. C. E. V. Schiassi *et al.*, *Effect of botanical origin on stability and crystallization of honey during storage*, Br. Food J. 124 (2022) 2689–2704.
19. M. A. I. Al-Hatamleh, J. C. Boer, K. L. Wilson, M. Plebanski, R. Mohamud, dan M. Z. Mustafa, *Antioxidant-based medicinal properties of stingless bee products: Recent progress and future directions*, Biomolecules 10 (2020) 1–28.
20. A. Setya Priambudi, R. Raffiudin, dan N. Ratna Djuita, *Identifikasi Tumbuhan Sumber Polen pada Madu Lebah Heterotrigona itama dan Tetragonula laeviceps di Belitung (Identification of Plants as Pollen Source in Honey of Stingless Bee Heterotrigona itama and Tetragonula laeviceps from Belitung)*, Berk. Penelit. Hayati 26 (2021).
21. N. L. Hungerford *et al.*, *Feeding Sugars to Stingless Bees: Identifying the Origin of Trehalulose-Rich Honey Composition*, J. Agric. Food Chem. 69 (2021) 10292–10300.
22. S. Shamsudin *et al.*, *Influence of origins and bee species on physicochemical, antioxidant properties and botanical discrimination of stingless bee honey*, Int. J. Food Prop. 22 (2019) 238–263.
23. Syafrizal *et al.*, *Diversity and honey properties of stingless bees from meliponiculture in East and North Kalimantan, Indonesia*, Biodiversitas 21 (2020) 4623–4630.
24. N. Agarwal dan S. P. Singh, *A Novel Trehalose Synthase for the Production of Trehalose and Trehalulose*, Appl. Biochem. Biotechnol. 193 (2021) 2512–2526.
25. J. Zhang, N. L. Hungerford, H. S. A. Yates, T. J. Smith, dan M. T. Fletcher, *How is Trehalulose Formed by Australian Stingless Bees?—An Intermolecular Displacement of Nectar Sucrose*, J. Agric. Food Chem. 70 (2022) 6530–6539.
26. S. Farooq dan Z. Ngaini, *The Enzymatic Role in Honey from Honey Bees and Stingless Bees*, Curr. Org. Chem. 27 (2023) 1215–1229.
27. M. A. Yegge *et al.*, *Reduction in moisture content of dehumidified and microwave-heated stingless bee (Kelulut) honey and its quality*, Mater. Today Proc. 17 (2019) 75–79.
28. A. Agus, Agussalim, M. Sahlan, dan A. Sabir, *Honey sugars profile of stingless bee Tetragonula laeviceps (Hymenoptera: Meliponinae)*, Biodiversitas 22 (2021) 5205–5210.