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Meta-analysis of Physicochemical Characteristics in Beekeeping, Wild, and Stingless Bee Honey

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ABSTRACT

The two most extensively beekeeping honeybee species were *Apis mellifera* and *A. cerana*. Other species that produced honey, albeit with infrequent cultivation, include *A. dorsata*, *A. florea*, and some stingless bees, including *Trigona* and *Melipona*. Different types of honeybees were known to affect the quality of honey. Hence, this investigation aimed to conduct a meta-analysis to examine the similarities in honey quality between honey sourced from beekeeping and wild honeybees. Data analysis was performed using the OpenMEE software, facilitating the calculation of effect size and standard error. The effect size and common error data were subsequently organized into separate columns within a CSV file. This CSV file was then imported into the JASP 0.16.2 software to conduct heterogeneity and Egger tests to detect potential publication bias. The findings indicated significant disparities in the quality of honey produced by beekeeping, wild, and stingless bee honey, as determined by various parameters, including pH value, moisture, total sugar, acidity, HMF (hydroxymethylfurfural), and diastase enzyme levels ($p < 0.05$). According to the results of the meta-analysis, honey from beekeeping exhibited superior quality to that of wild and stingless bees. However, the average values of all parameters still adhered to the established honey quality standards set by the Standar Nasional Indonesia and the International Honey Standard (IHS).

Keywords: honey, honeybees, meta-analysis, physicochemical quality

INTRODUCTION

Honey is a naturally occurring substance synthesized by insects through the transformation of plant nectar, secretions from living plant parts, or excretions from plant-sucking insects on live plant tissues (SNI 2018). Rich in bioactive compounds, honey finds widespread applications across diverse industries, serving as a valuable resource in food, cosmetics, therapeutics, and pharmaceuticals (Gela *et al.* 2021). Essential enzymes, including diastase (amylase), invertase, glucose oxidase, catalase, and phosphatase, are essential in distinguishing authentic honey from adulterated counterparts (Pascual-Maté *et al.* 2018).

In particular, the quality and composition of honey are influenced by several factors, such as the source of nectar and the type of bees that produce honey (Da Silva *et al.* 2016). Honeybees are divided by type into beekeeping honeybees and wild honeybees. Honeybee species widely cultivated are *Apis mellifera* and *A. cerana* (Lamerkabel 2011). Other species that also produce honey but still need to be widely cultivated are *A. dorsata* and *A. Florea* (Kumar *et al.* 2012). Other types of bees can produce honey, such as *Trigona* and *Melipona* (Loh *et al.* 2018, Agussalim *et al.* 2021).

Honey quality assessment is determined based on evaluating the physicochemical characteristics of honey constituents. Indonesia itself has set honey quality standards based on SNI 8664-2018. In addition to Indonesia, the International Honey Standard (IHS) and Codex Alimentarius Commission have also set standards for the physicochemical quality of honey. Many studies have also compared the quality of honey from different types of bees, but these studies produce inconsistent conclusions. Therefore, it is necessary to conduct research that compares the quality of honey from different types of bees more systematically and quantitatively to summarize the various conclusions. Meta-analysis is one of the research methods that can summarize various conclusions from previous studies.

Meta-analysis is a rigorous research approach that systematically and quantitatively integrates multiple pre-existing studies to draw definitive conclusions (Borenstein *et al.* 2009). This study meticulously analyzed the disparities in the physicochemical attributes of honey sourced from beekeeping, wild, and stingless bees. The assessment encompassed various physicochemical parameters of honey, including pH value, total sugar content, moisture, acidity, Hydroxymethylfurfural (HMF) levels, and diastase enzyme activity.

MATERIALS AND METHODS

The study began with understanding meta-analytic techniques' theoretical foundations, concepts, and applications. A literature search for studies relevant to

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the research theme, data collection, and calculation of the data obtained followed.

Procedures

This study employed a meta-analysis approach to derive conclusive insights from data gathered from multiple journals. Journal selection was performed utilizing Harzing's Publish or Perish software version 8.8.4275.8412, incorporating the Scopus search option, with the specified search keywords being "Physicochemical properties of Honey from Different Honeybee." The search was limited to 1000 journals, and articles were considered without a publication year restriction. The resulting list of journals was subsequently imported into the Mendeley Reference Manager 2.70.0 software, applying specific inclusion criteria, i.e., international research journals published in English, facilitating subsequent analysis. The evaluation for suitability involved an in-depth assessment of each journal, gauging the relevance of the topic and the availability of pertinent data.

The chosen publications comprised journals containing pertinent information regarding comparing the physicochemical quality of honey sourced from cultivated, wild, and stingless bees. A meta-analysis was conducted to examine the physicochemical attributes of honey, encompassing pH value, total sugar, moisture, acidity, hydroxymethylfurfural (HMF) concentration, and diastase enzyme activity. A comprehensive database was constructed in Microsoft Excel 2019.

The dataset was divided into two categories: honey sourced from beekeeping bees, designated as the control group, and honey derived from wild and stingless bees, serving as the experimental group. The control group encompassed honey produced by beekeeping honeybees (*A. mellifera* and *A. cerana*), while the experimental group comprised honey from wild honey bees (*A. dorsata* and *A. florea)* and some type stingless bees *(Melipona fasciculata, M. flavoneata, M. beecheii, M. comprensipes manosenseis, M. subnitida, M. scutellaris, M. quadrifasciata, M. eburnea, M. mandacaia, Lepidotrigona flavibasis, Hypotrigona* sp.*, Scaptotrigona* sp.*, Tetragonisca angustula, Frisiomelitta longipes, F. varia, and Plebeia sp.*).

Data Analysis

Data processing commenced by importing the Comma Separated Value (CSV) file derived from Microsoft Excel 2019 into the OpenMEE software, facilitating the computation of Effect size and Standard error. Subsequently, the calculated Effect size and Standard error values were transferred to distinct columns within Microsoft Excel. The entire CSV dataset was then imported into the JASP 0.16.2 software to execute heterogeneity and Egger tests (for assessing publication bias).

RESULTS AND DISCUSSION

Selected Journal

A journal search in the Google Scholar database comparing honey's physicochemical properties from different honeybees resulted in 55 journals, which were then selected based on the homogeneity of the presented data, including all parameters required for the meta-analysis process. The collected journals fulfilled the criteria for meta-analysis, and there were only 15 of 55 journals. Detailed information about the selected journals for this meta-analysis study is presented in Table 1. The information presented includes the details of the selected journals, such as the source, country, journal's name, and bee species.

Physicochemical Qualities of Honey

The outcomes resulting from the meta-analysis in this investigation encompassed various essential parameters, including the number of samples (*N*), the summary effect size (SMD/*d*), the upper and lower bounds of the 95% confidence interval (RE 95% CI), the *p*-value of the summary effect size (*p*-SMD), the heterogeneity value (I^2), and the p-value of the Egger test (*p*-Egger) (Table 2). The number of samples contributing to each parameter falls within the range of 153 to 481 pieces. A positive effect size signified that honey produced by beekeeping honeybees exhibited a parameter value smaller than that of honey sourced from the wild and stingless bees. Conversely, a negative effect size indicated that honey produced by beekeeping honeybees has a parameter value greater than that of honey from wild and stingless bees. Significance in the differences can be identified when the confidence interval of the summary effect size did not include the value of zero (Figure 1) or when the *p*-value of the summary effect size was less than 0.05. Furthermore, potential indications of publication bias can be identified when the *p*-value of the Egger test is less than 0.05. The data from the meta-analysis study is presented in Table 2.

pH Value

Based on the results of the meta-analysis computation for the pH parameter, an effect size (SMD/*d*) of –1.26 was obtained with a 95% confidence interval (RE CI) of [–2.11; –0.41] (Figure 1). The confidence interval's exclusion of zero indicated a discernible difference between the experimental and control groups' treatment. The negative SMD/*d* value across all studies suggested that honey from wild and stingless bees (pH 3.89) has a lower pH level than honey from beekeeping bees (pH 4.00). The analysis demonstrated a statistically significant distinction in the pH value of honey derived from beekeeping, wild, and stingless bees ($p < 0.05$). The heterogeneity analysis revealed a notable variability in the pH of honey values across all studies, supported by the high I^2 value of 91.65%. Borenstein *et al.* (2009) provided I^2 benchmarks, designating 25% for low

Table 1 The selected journal or the meta-analysis study were subjected to detailed examination and analysis

Journals	Country	Publisher	Honeybees
Silva *et al*. (2013)	Brazil	*J. Braz. Chem. Soc.*	*A. mellifera; M. fasciculata; M. flavoneata*
Wu *et al*. (2020)	China	Elsevier	*A. mellifera; A. cerana; A. dorsata; Lepidotrigona flavibasis*
Nweze *et al*. (2017)	Nigeria	*BMC Research Notes*	*A. mellifera; Hypotrigona sp.; Melipona sp.*
Alvarez-Suarez *et al*. (2018)	Cuba	Elsevier	*A. mellifera; M. beecheii*
Joshi *et al*. (2000)	Nepal	HAL Open Science	*A. mellifera; A. dorsata; A. cerana*
Al-Ghamdi *et al*. (2019)	Saudi Arabia	SJBS	*A. mellifera; A. florea*
Gomes *et al*. (2022)	Brazil	*Food Sci.Technol*.	*A. mellifera; M. comprensipes manosenseis; Scaptotrigona sp.; Tetragonisca angustula; Frisiomelitta longipes; Melipona sp.*
Taha *et al*. (2020)	Saudi Arabia	*J. Apic. Res*.	*A. mellifera; A. florea*
Ismail *et al*. (2021)	Malaysia	MJAS	*A. mellifera; Trigona*
Moniruzzaman *et al*. (2013)	Malaysia	*Chem. Cent. J*.	*A. mellifera; A. dorsata*
Almeida-Muradian et al. (2013)	Brazil	IJFST	*A. mellifera; M. subnitida*
Duarte et al. (2012)	Brazil	*J. Apic. Res*.	*A. mellifera; M. scutellaris; M. quadrifasciata; M. subnitida; Plebeia sp.*
Isabel et al. (2023)	Colombia	*Foods*	*A. mellifera; M. eburnea.*
DeMera and Angert (2004)	Kosta Rika	*Apidologie*	*A. mellifera; Tetragonisca angustula.*
Araujo et al. (2023)	Brazil	*Acta Scientiarum*	*A. mellifera; M. subnitida; M. mandacaia; Plebela sp.; Frisiomelitta varia.*

Table 2 The results of a meta-analysis comparing honeybees to the physicochemical of honey

Parameter	N	SMD/d+(RE 95% CI)	p-*SMD*	I^2 (%)	z	p-*Egger*
pH	377	-1.26 (-2.11; -0.41)	0.004	91.65	-4.20	<0.001
Moisture	481	6.49 (4.88; 8.09)	<0.001	95.15	10.00	<0.001
Total sugar	399	-2.29 (-3.27; -1.31)	<0.001	93.06	-3.13	0.002
Acidity	379	1.92 (0.40; 3.44)	0.013	95.47	5.94	<0.001
HMF	332	-1.71 (-3.06; -0.36)	0.013	94.99	7.37	<0.001
Diastase	153	-6.47 (-9.62; -3.31)	<0.001	96.15	-13.18	<0.001

Description: N = Total sample, SMD = Standardized Mean Difference, RE 95% CI = Lower and Upper Limits of the 95% Confidence Interval, p-SMD: p-value Standardized Mean Difference, I^2 = Inconsistency, z = Egger's test value, p-Egger = p-value Egger's test.

heterogeneity, 50% for moderate heterogeneity, and 75% for high heterogeneity.

The higher pH value observed in honey from cultivated bees aligned with the findings of Wu *et al.* (2020), who also attended quality disparities among honey from bee species *A. mellifera*, *A. cerana*, *A. dorsata*, and *L. flavibasis*. Overall, honey from the wild, beekeeping, and stingless bees exhibited an acidic pH, falling within the general pH range of honey specified by the IHS, i.e., 3.4–6.1. The pH value of honey held significant relevance in the extraction process, as it affected the honey's texture, stability, and shelf life (Khalil *et al.* 2012). Disparities in honey pH values can arise due to various factors, including the botanical origin of the honey (nectar sources from bee feed plants), the secretion of bee saliva, enzymatic transformations, and fermentation processes occurring during the honey formation (Abselami *et al.* 2018).

Moisture

Based on the outcomes of the meta-analysis concerning the moisture parameter of honey, a notable SMD/*d* of 6.49 was obtained, accompanied by a 95% RE CI of [4.88; 8.09] (Figure 1). The exclusion of zero from the confidence interval implied a discernible distinction between the experimental and control groups' treatment. Specifically, the moisture of honey from wild and stingless bees (23.70% w/w) was higher than that of beekeeping bees (18.13% w/w), leading to a positive SMD/*d* value. The analysis demonstrated a statistically significant difference between honey moisture from beekeeping, wild, and stingless bees ($p < 0.05$). The heterogeneity analysis indicated variability in honey's moisture across all

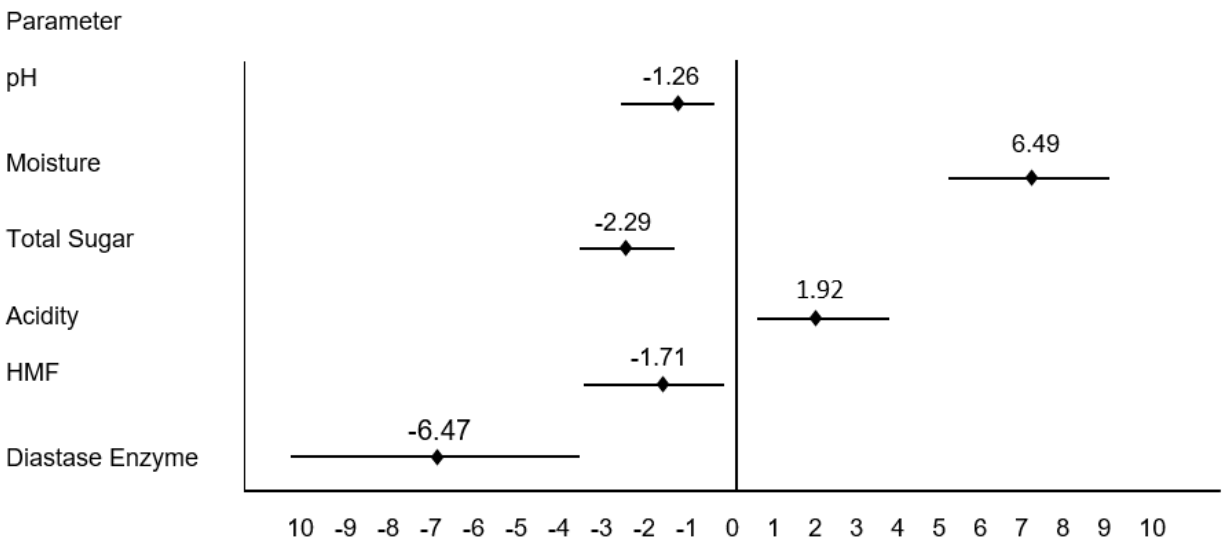

Figure 1 Cumulative forest plot comparison of physicochemical quality of honey from beekeeping, wild, and stingless bees.

studies, evident by the high I^2 value of 95.15%. The honey moisture from beekeeping bees adhered to the IHS, which stipulated a maximum limit of 20% w/w. In contrast, honey moisture from wild and stingless bees exceeded the IHS threshold.

The elevated moisture content observed in honey from stingless bees was consistent with the findings of Silva *et al.* (2013), who reported higher moisture values in *Melipona* bees compared to *A. mellifera* bees. The variation in the honey`s moisture was substantially affected by multiple factors, including geographical location, environmental conditions, harvest season, honey maturity, and the diversity of honeybee species (Ramón-Sierra *et al.* 2015). The moisture level in honey is a crucial determinant of honey quality and its shelf life (Wulandari 2017).

Total Sugar

The meta-analysis results for the total sugar parameter revealed an SMD/*d* of –2.29, accompanied by a 95% RE CI of [–3.27; –1.31] (Figure 1). The exclusion of zero from the confidence interval indicated a significant difference between the experimental and control groups' treatment. Specifically, the total sugar in honey sourced from wild and stingless bees (64.37% w/w) was lower than that found in honey derived from beekeeping bees (69.57% w/w), as evidenced by the negative SMD/*d* value. The analysis established a statistically significant distinction in the total sugar between wild, beekeeping, and stingless bees ($p < 0.05$). The heterogeneity analysis revealed variability in the total sugar of honey across all studies, demonstrated by the high I^2 of 93.06%.

The average total sugar observed in honey from wild, beekeeping, and stingless bees in this study aligned with the recommended threshold established by the IHS, which mandated that the total sugar in honey was prohibited below 60% w/w. The primary constituents of honey are sugars, predominantly fructose, and glucose (Solayman *et al.* 2016). The observed lower total sugar in honey from wild bees concurred with Araujo *et al.* (2023) findings. Reduced total sugar in honey arose due to the conversion of sugars into organic acids, leading to a slightly acidic taste (Wulandari 2017).

Acidity

The outcomes of the meta-analysis concerning the acidity parameter exhibited an SMD/*d* of 1.92, along with a 95% RE CI of [0.40; 3.44] (Figure 1). The exclusion of zero from the confidence interval indicated a notable distinction between the experimental and control group treatments. Specifically, the acidity level in honey from wild and stingless bees (70.91 mL NaOH/kg) exceeded that found in beekeeping bees (37.88 mL NaOH/kg), as indicated by the positive SMD/*d* value. The analysis confirmed a statistically significant disparity in acidity between honey sourced from wild, beekeeping, and stingless bee honey ($p < 0.05$). The heterogeneity analysis of honey acidity across all studies revealed variability, supported by the high I^2 value of 95.47%. The IHS has stipulated a maximum acidity limit of 40 mL NaOH/kg for honey. The average acidity value observed in all studies indicated that honey from beekeeping bees adhered to the IHS recommendation.

A study by Gomes *et al.* (2022) showed that honey from wild bees exhibited elevated acidity levels. The high acidity in honey can be attributed, in part, to the sugar fermentation process, which indicated increased acidity (Alvarez-Suarez *et al.* 2010). During the sugar fermentation process, acetic acid was generated, facilitated by enzymatic mechanisms, including converting glucose into gluconic acid by glucose oxidase (Avila *et al.* 2018). Additionally, the acidity value was interconnected with the moisture in honey. High moisture in honey makes it more susceptible to fermentation, resulting in elevated acidity levels (Shamsudin *et al.* 2019).

Hydroxymethylfurfural (HMF)

The results obtained from the meta-analysis concerning the HMF parameter revealed an effect size (SMD/*d*) of –1.71, with a 95% RE CI of [–3.06; –0.36] (Figure 1). The exclusion of zero from the confidence interval indicated a notable difference between the experimental and control groups' treatment. Specifically, honey from wild and stingless bees exhibited a lower HMF content (28.02 mL/kg) than beekeeping bees (37.38 mL/kg), as indicated by the negative SMD/*d* value. The analysis demonstrated a statistically significant divergence in HMF levels between honey derived from wild and stingless bees, and honey sourced from beekeeping bees ($p < 0.05$). The heterogeneity analysis indicated variability in HMF across all studies, supported by the high I^2 value of 94.99%.

The IHS has recommended a maximum HMF content of 40 mL/kg. Notably, the HMF values observed in beekeeping, wild, and stingless bee honey from this study were within the IHS's limits. HMF was a significant indicator of honey's freshness and purity (Da Silva *et al.* 2016). Elevated HMF levels can reduce the honey`s freshness and overall quality (Kowalski *et al.* 2013). The observed lower HMF content in honey from wild bees aligned with the findings of Alvarez-Suarez *et al.* (2018), who conducted a comparative study on HMF levels in *A. mellifera* and *M. beecheii* bees. The variation in HMF concentration, whether high or low, can likely be attributed to the sugar content and the fructose-to-glucose ratio present in the honey (Doner 1977).

Diastase

The outcomes derived from the meta-analysis concerning the diastase enzyme activity parameter demonstrated an SMD/*d* of –6.47, accompanied by a 95% RE CI of [–9.62; –3.31] (Figure 1). The exclusion of zero from the confidence interval indicated a notable difference between the experimental and control groups' treatment. Specifically, the diastase activity in honey from wild and stingless bees (8.26 IU) was lower than that observed in honey sourced from beekeeping bees (19.16 IU), as indicated by the negative SMD/*d* value. The analysis confirmed a statistically significant disparity in diastase activity between honey derived from wild and stingless bees, and honey obtained from beekeeping bees ($p < 0.05$). The heterogeneity analysis indicated variability in diastase activity across all studies, demonstrated by the high I^2 value of 96.15%. The IHS has stipulated a recommended diastase activity level of at least 8 units/g in honey. Notably, the average diastase activity values from all studies in this investigation aligned with the IHS recommendation.

The diastase enzyme is an enzyme honeybees add during honey maturation (Suranto 2005). The lower diastase activity observed in honey from wild bees followed the findings of Wu *et al.* (2020), who reported reduced diastase activity in honey from *A. dorsata* bees compared to honey from *A. mellifera* bees. Diastase activity can vary based on factors such as the age of the bees, the period of nectar collection, the physiological state of the colony, the quantity of nectar, and the sugar content. A more concentrated amount of nectar tends to lower diastase content (Da Silva *et al.* 2016).

Publication Bias

The assessment of publication bias was performed using the funnel plot test for all parameters, as depicted in Figure 2. The plot displayed a distinct asymmetrical pattern, and the statistical analysis employing Egger's trial resulted in significant findings ($p < 0.05$) (Table 2). These outcomes signified the

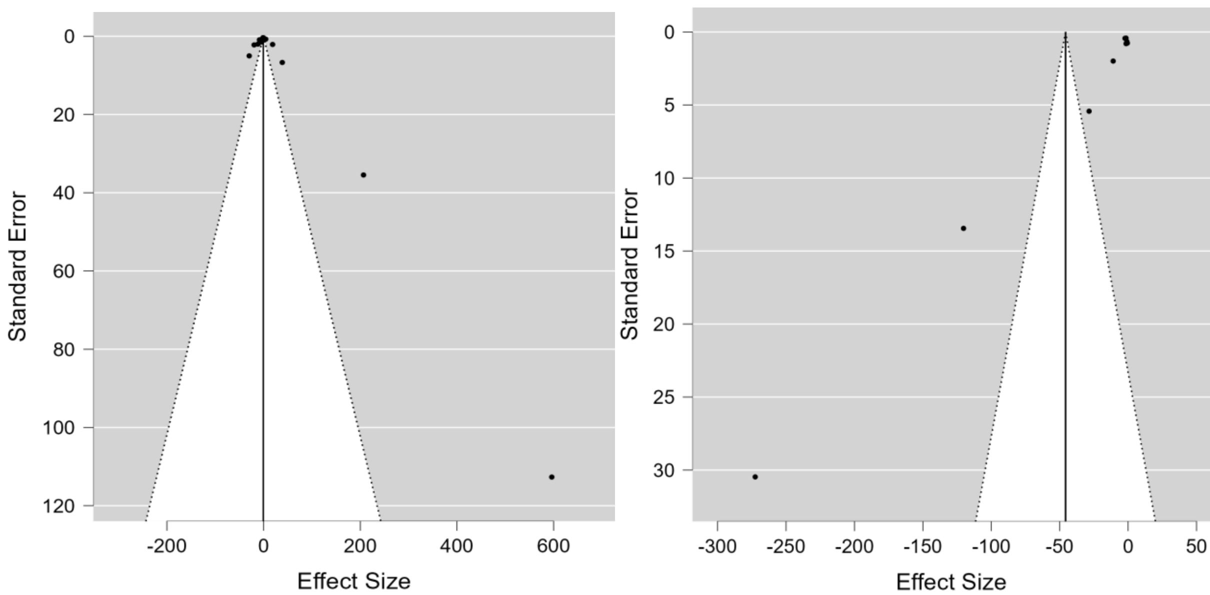

Figure 2 Cumulative funnel plot comparison of physicochemical quality of honey from beekeeping, wild, and stingless bees.

presence of publication bias within the context of the meta-analysis. This indication of publication bias can be attributed to the utilization of the random effect method in this study and the observed high heterogeneity across the analyzed studies. The random effects model assumes that the studies analyzed have a genuine effect size that varies from study to study (Retnawati *et al.* 2018).

CONCLUSION

Examining honey quality from beekeeping, wild, and stingless bees revealed discernible differences across each parameter, encompassing pH, moisture content, total sugar, acidity, HMF, and diastase enzyme. The data obtained from all studies exhibited heterogeneity, thereby indicating that the disparities between beekeeping, wild, and stingless bees significantly influenced honey quality. Based on the outcomes of the meta-analysis, honey from beekeeping honeybees demonstrated superior quality to honey from wild and stingless bees while still adhering to the established honey quality standards observed in the average values of all parameters.

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