

## Effects of Harvest Time and *Acacia crassicarpa* Age on the Physicochemical Characteristics of *Apis mellifera* L. Honey in Tropical Indonesian Forests

Eni Suhesti<sup>1,2</sup>, Lili Zalizar<sup>1</sup>, Joko Triwanto<sup>1</sup>, Ervayenri Ervayenri<sup>2</sup> and Indra Purnama<sup>3,4\*</sup>

<sup>1</sup>Department of Postgraduate, University of Muhammadiyah Malang, Malang 65144, East Java, Indonesia

<sup>2</sup>Department of Forestry, Faculty of Forestry and Sciences, Universitas Lancang Kuning, Pekanbaru 28261, Riau, Indonesia

<sup>3</sup>Graduate School of Agricultural Sciences, Universitas Lancang Kuning, Pekanbaru 28261, Riau, Indonesia

<sup>4</sup>Center for Sustainable Tropical Agricultural Research, Universitas Lancang Kuning, Pekanbaru 28261, Riau, Indonesia

### ABSTRACT

The comprehensive understanding of the physicochemical profile of monofloral honey derived from *Acacia crassicarpa*, specifically in the Indonesian tropical forest ecosystem, has not been fully explored. The physicochemical characteristics of honey significantly influence its quality and consumer acceptance. Harvest time and the age of *Acacia* plants, which are suspected to affect honey's physicochemical properties, are this study's focal points. Our objective is to analyze the impact of harvest time and *Acacia* age on the physicochemical characteristics of honey. Using a complete randomized block design, treatments were administered at 14, 21, and 30 days of harvest within three *Acacia* age groups: 3, 8, and 18 months. The honey composition was assessed following the Indonesian National Standard 8664:2018 procedure. The statistical analysis determined the optimal harvest period for honey by assessing its physicochemical properties and comparing

them to the Indonesian National Standard 8664:2018 procedure (SNI 8664:2018 standards). One-way analysis of variance evaluated the effects of harvest time and plant age on composition, followed by a least significant difference tests to identify significant differences between harvest times. Results indicate a significant influence of harvest time and *Acacia* age on all honey composition variables, including diastase

### ARTICLE INFO

#### Article history:

Received: 19 February 2024

Accepted: 18 April 2024

Published: 25 October 2024

DOI: <https://doi.org/10.47836/pjtas.47.4.10>

#### E-mail addresses:

suhestieni@unilak.ac.id (Eni Suhesti)

lily@umm.ac.id (Lili Zalizar)

joko.fpumm@gmail.com (Joko Triwanto)

erva@unilak.ac.id (Ervayenri)

indra.purnama@unilak.ac.id (Indra Purnama)

\*Corresponding author

enzyme activity, hydroxymethylfurfural content, moisture level, sugar content, and acidity ( $P < 0.01$ ). Our findings suggest optimal honey harvest at 30 days, aligning with the 8<sup>th</sup> and 18<sup>th</sup> months of *A. crassicarpa*. Most variables met SNI 8664:2018 standards, except acidity levels. Further investigation is needed to discern the causes of acidity in *Apis mellifera* honey from Indonesian peat swamp forests.

*Keywords:* *Acacia crassicarpa*, *Apis mellifera*, monofloral honey, peat swamp forest, tropical forest honey

## INTRODUCTION

Honey is a sweet liquid with a complex composition comprising carbohydrates and other compounds (De-Melo et al., 2018; Hailu & Belay, 2020). The composition of honey is influenced by the type of plant forage, bee species, environmental conditions, geographical location, handling procedures, and storage methods (Alvarez-Suarez et al., 2018; Baroni et al., 2015; Viteri et al., 2021). Based on the diversity of bee forage plant types, honey is classified into monofloral honey and polyfloral honey (Hailu & Belay, 2020). Monofloral honey is produced by honeybees with forage from a single plant species. Generally, monofloral honey is preferred by consumers over multifloral honey due to reasons related to taste, aroma, and health attributes (Ghramh et al., 2023; Taha et al., 2021).

Studies on the physicochemical properties of monofloral honey from various forage plant species have been extensively

conducted previously in countries such as Romania, Brazil, and Hungary (Czipa et al., 2019; do Nascimento et al., 2018; Oroian & Sorina, 2017). Information regarding the physicochemical characteristics of honey is crucial as it significantly influences honey's nutritional quality, taste, texture, and health value (Oroian & Sorina, 2017; Siddiqui et al., 2017). *Acacia crassicarpa* honey in Riau has a rich history, with beekeeping practices becoming popular in recent years, particularly during the COVID-19 pandemic due to increased demand (Purwanto et al., 2024). Meanwhile, the production of *A. crassicarpa* honey is declining due to reduced natural forest area (Pribadi & Wiratmoko, 2023).

In Indonesia, monofloral honey derived from the extrafloral nectar of *A. crassicarpa* is predominantly produced by local beekeepers utilizing *A. mellifera* within acacia plantation forest areas, including Siak Regency, Riau Province, Indonesia. This region is characterized by peat swamp forests with distinctive acidic soil properties. Local honey is widely marketed within Riau Province and other provinces in Indonesia. Indonesian beekeepers face challenges in consistently producing honey that adheres to the quality benchmarks established by the Indonesian National Standard (Standard Nasional Indonesia, SNI) 8664-2018. This issue is particularly concerning for industrial consumers and international buyers, who require honey that meets specific quality criteria. Therefore, a comprehensive investigation into the factors causing the honey quality from these

beekeeping practices to fall short of the standards is imperative.

The honey harvesting practices among *A. mellifera* beekeepers in Siak Regency, Indonesia, exhibited variability attributed to several factors, including meteorological conditions, workforce availability, and fluctuations in consumer demand. Based on observation, the interval between harvests typically ranged from 14 to 30 days. Additionally, *A. crassiparva* plantations aged between 3 and 18 months were predominantly selected as apiary locations. Hence, it can be hypothesized that these combinations influence the honey's physicochemical properties. According to research by Lewkowski et al. (2019), the maturity level of honey within the hive and the age of the plants serving as the primary forage source for bees are believed to influence honey composition. The timing of honey harvest demonstrably influences its maturity (Wu et al., 2022). Wu et al. (2022) employed a research design to investigate the impact of harvest time on the physicochemical properties of honey produced by two stingless bee species, *Heterotrigona itama* and *Tetrigona binghami*, and observed significant variations. Observations and interviews with local beekeepers suggest that different ages of *A. crassiparva* tend to yield varying amounts of nectar and may exhibit different characteristics. Nonetheless, a comprehensive understanding of how harvest timing and the age of *A. crassiparva* influence the composition of *A. mellifera* honey in tropical forests remains elusive.

This knowledge gap necessitates further rigorous investigation.

This study addresses this knowledge gap by specifically investigating the influence of harvest time and the age of *A. crassiparva* on the physicochemical characteristics of *A. mellifera* honey. *Acacia crassiparva*, chosen for its ecological significance and potential impact on honey composition, is a prominent nectar source in the tropical forests of Indonesia. Understanding the intricate relationship between harvesting period, plant maturity, and honey composition is crucial for optimizing apicultural practices, particularly within peat swamp forests, and ensuring the production of high-quality honey. Explaining the complicated relationship between harvesting period, plant maturity, and honey composition is principal to optimizing apicultural practices, especially within peat swamp forests, and guaranteeing the production of honey with superior quality. The objectives of this study are: 1) to analyze the influence of harvest timing, *A. crassiparva* age, and the interaction between harvest timing and *A. crassiparva* age on physicochemical characteristics of honey, and 2) to determine the optimal harvest timing and plant age for *A. mellifera* honey produced by communities in Siak Regency, Riau.

## MATERIALS AND METHODS

### Experimental Design

The experimental design employed a factorial design. The honey samples were categorized based on three distinct harvest times (14, 21, and 30 days) and

further stratified into three age groups of *A. crassicarpa* (3, 8, and 18 months). All treatments were replicated three times, resulting in 27 experimental units. This study employed a research design that specifically accounted for the variation

introduced by harvest timing, plant age, and interaction between harvest time and plant age. This comprehensive approach allowed for a robust assessment of how these factors influence honey physiochemical composition.

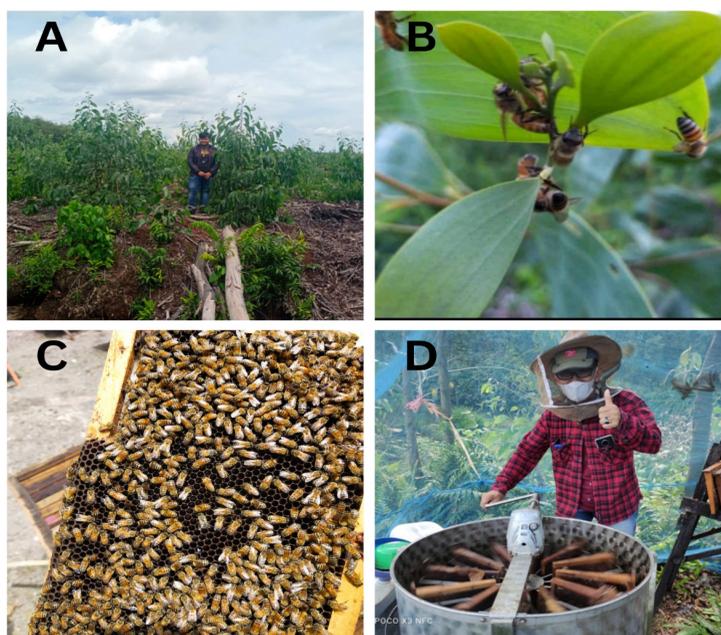


Figure 1. Overview of the study with image descriptions: (A) *Acacia crassicarpa* tree at 3 months old; (B) Bees are collecting extrafloral nectar of *A. crassicarpa*; (C) Honeycomb being lifted from the beehive box; and (D) A honey extractor functions to mechanically separate honey from its frames, facilitating the collection of fresh honey

### Honey Samples Preparation

The honey samples used in this study originated from bee yards located within *A. crassicarpa* plantations managed by Arara Abadi Co., Ltd., Siak Regency, Riau, Indonesia ( $0^{\circ}48'32.21057''N$   $101^{\circ}36'22.85467''E$ ). The honey sampling was conducted in May 2023, during the dry season, with an average temperature of  $32.6^{\circ}C$  prevailing throughout the research

period. Honey samples were collected from bee yards placed within three age groups (3, 8, and 18 months) of *A. crassicarpa* plantations. Each age group of plantations encompassed a minimum area ranging from 100 to 200 ha. Therefore, it was assumed that *A. mellifera* colonies placed within one age group of *A. crassicarpa* plantations would not fly to other age groups of plantations. This assumption is based on

the factors that influence bee flight distance, including nectar's adequacy and quality (Tong et al., 2019). *Acacia crassicarpa* plants produce a considerable amount of nectar, averaging 42,774 ml/ha per day for 12-month-old plants and 73,766 ml/ha per day for 50-month-old plants (Pribadi & Purnomo, 2013).

Harvesting took place at 8 a.m. to ensure consistency and account for diurnal variations following the previous study (Pasias et al., 2018). The process of fresh honey retrieval involved meticulous combing of the hives and extraction of honeycombs using a mechanical honey extractor for efficient honey extraction. The freshly extracted honey, collected through this process, was promptly transferred to sterile glass vessels. These vessels were sealed and shielded from light to prevent alterations in honey composition during transportation to the laboratory. Honey samples were stored at room temperature in dark conditions, as suggested by Radtke and Lichtenberg-Kraag (2018). This controlled environment guaranteed the stability of the honey samples until the initiation of detailed physicochemical analysis. Figure 1 illustrates an overview of the research in this study.

### Physicochemical Analysis

All honey samples were handled with precision, and the utmost care was taken to maintain their purity. Notably, analytical-grade chemicals were exclusively used throughout the entire process to ensure

accuracy and reliability in the analysis. The physicochemical analysis procedures, diastase activity, determination of hydroxymethylfurfural (HMF), moisture content, sucrose content, reducing sugar content, and acidity for honey composition followed established protocols as outlined in Berhe et al. (2018) and Suhesti, Zalizar, et al. (2023). Determining diastase enzyme activity and HMF content utilized spectrophotometry methods, while refractometry methods measured moisture levels. The analysis of reducing sugar (glucose) and sucrose content employed the Luff-Schoorl method, and acidity was assessed using the neutralization method (Suhesti, et al., 2023).

### Data Analysis

The influence of harvest time, the age of *A. crassicarpa* plants, and the interaction between these two factors (age and harvest time) on honey physicochemical properties was analyzed using the one-way analysis of variance (ANOVA) and least significant difference (LSD) post-hoc test in SPSS (version 25). This analysis determines the optimal harvest time and plant age for each aspect of honey composition. Additionally, one sample *t*-test was employed to compare the mean values of honey composition variables with the quality standards specified by SNI 8664-2018. Quantitative descriptive analysis was conducted to illustrate the mean values, standard deviations, and comparisons with the quality standards.

## RESULTS AND DISCUSSION

### Diastase Activity and HMF Determination

Enzyme activity, specifically diastase and HMF levels, indicates honey freshness (Al-Ghamdi et al., 2019; Bell & Grainger, 2023). The diastase enzyme activity value is crucial in assessing honey quality and is closely associated with its nutritional quality and freshness (Can et al., 2015; Erban et al., 2021). Elevated levels of HMF in honey may indicate adulteration, such as adding inverted sugar syrup (Pasiyas et al., 2018; Yücel & Sultanoglu, 2013).

The one-way ANOVA revealed a significant influence ( $P < 0.01$ ) of harvest time, the age of *A. crassiparva* plants, and the interaction between harvest time and age of plants on diastase enzyme activity and HMF level (Table 1). According to the LSD test, diastase enzyme activity exhibited significant differences among harvest times of 14, 21, and 30 days across all plant ages ( $P < 0.01$ ) (Table 1). Conversely, harvest time had a significant effect on the HMF values of *A. crassiparva* at 3 and 8 months of age ( $P < 0.01$ ). However, this effect was not significant at 18 months of age ( $P > 0.05$ ) (Table 1).

The analysis of diastase enzyme activity in honey revealed an interaction between harvest time and the age of *A. crassiparva*, with the most optimal combination being 30 days of harvest time and 3 months of plant age, yielding a result of  $11.21 \pm 0.02$  diastase number (DN). The one sample *t*-test results indicated a significant difference between this value and the standard specified in

SNI 8664-2018 ( $P < 0.01$ ), which requires a minimum of 3 DN, meeting export requirements set at above 8 DN (Bell & Grainger, 2023). Furthermore, this value aligns with the diastase enzyme activity range reported by Sajid et al. (2019) in their study on fresh honey from various regions in Pakistan, ranging from 10.70 to 23.00 DN.

The tendency for higher diastase enzyme activity in honey harvested over a longer period is likely due to the extended honey ripening process, leading to increased secretion of enzymes from the bee's stomach. It aligns with the statement by Eyer et al. (2016) that honey bees release  $\alpha$ -amylase enzymes during nectar collection and ripening into honey. Diastase enzyme activity is a combination of  $\alpha$ -amylase and  $\beta$ -amylase activities secreted from the bee's saliva, playing a crucial role in honey production by aiding in the conversion of starch into maltose (Chua & Adnan, 2014; Sajid et al., 2019).

The one-way ANOVA revealed a significant influence ( $P < 0.01$ ) of harvest time, the age of *A. crassiparva* plants, and the interaction between harvest time and age of plants on HMF value (Table 1). Based on the results of the one-sample *t*-test, all honey samples from every harvest time and plant age group exhibited significant differences compared to the SNI 8664-2018 standard ( $P < 0.01$ ), meeting the established criteria. Interestingly, honey derived from *A. mellifera* foraging for three months *A. crassiparva* exhibited an increase of HMF value when harvest time was extended. It aligns with the findings of research

Table 1  
Physicochemical data of fresh honey samples from various acacia plant ages and harvest times

Parameters	Honey samples								
	3 months			8 months			30 days (n = 3)		
	14 days (n = 3)	21 days (n = 3)	30 days (n = 3)	14 days (n = 3)	21 days (n = 3)	30 days (n = 3)	14 days (n = 3)	21 days (n = 3)	30 days (n = 3)
Diastase activity (DN)	1.95 ± 0.03a**	2.54 ± 0.04b**	11.21 ± 0.02c	2.55 ± 0.05b**	6.55 ± 0.10c	1.52 ± 0.02a**			
HMF-content (mg/kg)	0.00 ± 0.00a	0.00 ± 0.00a	1.87 ± 0.09b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00a			
Moisture (%)	26.40 ± 0.40c**	23.87 ± 0.30b**	20.25 ± 0.20a	23.87 ± 0.35b**	23.20 ± 0.10b**	22.20 ± 0.10a**			
Sucrose content (w/w)	12.05 ± 0.50a**	7.76 ± 0.01b**	19.55 ± 0.40a**	14.19 ± 0.40b**	14.91 ± 0.01c**	1.50 ± 0.30a			
Glucose content (w/w)	27.21 ± 0.20a**	27.80 ± 0.10a**	61.15 ± 0.05b**	58.13 ± 0.20b**	52.10 ± 0.10a**	66.56 ± 0.04c			
Acidity (NaOH/kg)	92.15 ± 0.15**	70.01 ± 0.07**	59.41 ± 0.10**	114.76 ± 0.02**	131.97 ± 0.05**	113.05 ± 0.05**			
Parameters	honey sample								
	18 months			30 days (n = 3)			57.73 ± 0.05b**		
Diastase activity (DN)	3.91 ± 0.01c	2.80 ± 0.05b**	1.23 ± 0.05a**						
HMF-content (mg/kg)	0.00 ± 0.00a	0.00 ± 0.00a	0.000.00a						
Moisture (%)	22.60 ± 0.25b**	21.80 ± 0.2b	21.00 ± 0.10a						
Sucrose content (w/w)	19.55 ± 0.40c**	14.08 ± 0.01b**	5.00 ± 0.30a						
Glucose content (w/w)	53.28 ± 0.30a**	52.75 ± 0.20a**	57.73 ± 0.05b**						
Acidity (NaOH/kg)	142.61 ± 0.03**	140.63 ± 0.10**	119.45 ± 0.20**						

Note. Data = Means ± Standard deviations; Symbol \*\* = The observed values do not meet the Indonesian National Standard; Different letters indicate significant differences in values ( $P < 0.01$ ) as determined by the least significant difference test

conducted by Al-Ghamdi et al. (2019) and Pasiadis et al. (2018), indicating that hot tropical weather can elevate HMF content in honey within the hive. Despite these findings, the HMF concentration detected in the investigated honey is demonstrably lower when compared to honey produced in other tropical regions, such as Brazil that range from 2.61 to 3.81 mg/kg (dos Santos Scholz et al., 2020) and from 2.0 to 4.4 mg/kg (da S. Sant'ana et al., 2020).

The presence of HMF in honey can have negative and positive effects on human health. Negative effects include genotoxic, mutagenic, organotoxic, and enzyme-inhibiting properties, while positive effects encompass antioxidant, anti-allergic, anti-inflammatory, anti-hypoxic, and anti-hyperuricemia effects (Shapla et al., 2018). Analysis of HMF content in all honey samples employed within this investigation suggests the honey to be fresh and suitable for consumption.

### Sugar Content

The sugars found in honey are monosaccharides and disaccharides (Chua & Adnan, 2014). This investigation focuses on quantifying monosaccharides within the sample, specifically those identified as reducing sugars. Quantification is performed by converting them to a glucose equivalent. Additionally, the analysis encompasses the disaccharide sugar sucrose.

The one-way ANOVA conducted in this study demonstrated a statistically significant effect ( $P < 0.01$ ) of both honey harvest time and the age of *A. crassiparva*,

the primary bee forage source, as well as their interaction, on the glucose content within the honey samples. LSD tests reveal a significant difference in glucose content between honey harvested at 14 and 21 days compared to honey harvested at 30 days ( $P < 0.01$ ) (Table 1). Honey samples collected from hives in the 8-month age group exhibited the highest glucose concentration (66.56% w/w). This value satisfies the established honey quality criterion for minimum reducing sugar content, set at 65% w/w.

The one-way ANOVA for sucrose content in honey also indicates a significant influence of harvest time, the age of *A. crassiparva*, and their interaction. The lowest sucrose concentrations were observed in all honey samples harvested at 30 days. The optimal combination for sucrose content is a harvest time of 30 days with *A. crassiparva* age of 8 months. The LSD test results indicate a significant difference between the 30-day harvest time and 8-month-old age combination compared to other combinations ( $P < 0.01$ ). Honey harvested 30 days from both the 8- and 18-month-old complied with the SNI 8664-2018 standard for sucrose content, which specifies a maximum level of 5% w/w sucrose in honey. Sucrose is a crucial parameter in testing honey's authenticity and maturity level. A high sucrose content in honey may indicate adulteration by adding sugarcane or beet sugar or prolonged feeding of honey bees with artificial substances such as syrup (Escuredo et al., 2013; Puscas et al., 2013).

The glucose and sucrose levels in honey samples display an opposing pattern, as the average glucose concentrations during the 30-day harvest surpass those during the 14 and 21-day harvests across all age groups of plants. In contrast, sucrose values are lower during the 30-day harvest. The extended duration of honey harvest is hypothesized to contribute to the observed transformation of sucrose into glucose. This proposition is in line with Boussaid et al. (2018) assertion that prolonged harvest times lead to heightened diastase enzyme activity, facilitating the conversion of disaccharides into monosaccharides. The composition of glucose and sucrose in honey is not solely influenced by harvest time but also by climatic conditions and nectar sources (Chua & Adnan, 2014; Escuredo et al., 2014; Juan-Borrás et al., 2014; Tornuk et al., 2013; Vranić et al., 2017). Monofloral honey derived from acacia plant nectar sources generally exhibits elevated sucrose levels compared to other nectar sources (Can et al., 2015; Juan-Borrás et al., 2014). The varying ages of *A. crassiparva* plants lead to fluctuations in glucose and sucrose concentrations in the honey produced at that location, likely attributed to the distinct sugar compositions of nectar produced by these plants. However, currently, no research is available addressing the nectar composition produced by *A. crassiparva* plants.

### Moisture Content

Moisture content is a crucial quality parameter of honey, as it influences viscosity,

specific gravity, taste, fermentation rate, and crystallization rate (Escuredo et al., 2013; Pasiás et al., 2018; Wu et al., 2022). Excessively low moisture content can lead to caramelization in honey. In contrast, excessively high moisture content can result in fermentation and the formation of acetic acid, thereby increasing the likelihood of honey spoilage during storage (Boussaid et al., 2018; Kek et al., 2018).

This study revealed that the harvest time, age, and their interaction significantly influenced honey's moisture content ( $P < 0.01$ ). This study revealed that age significantly influenced honey's moisture content ( $P < 0.01$ ). LSD analysis further indicated a significant difference in honey moisture content between 30-day and 14- and 21-day harvests. However, there was no significant difference between the 14- and 21-day harvests ( $P > 0.05$ ) across all age groups of *A. crassiparva*. Regarding the comparison of moisture content between the ages of *A. crassiparva* for all harvest times, differences are only detected between the 3- and 18-month ages.

The moisture content in honey samples decreases with the increasing harvest time. The lowest moisture content is observed in honey harvested at 30 days across all age groups of plants. However, based on the results of the one-sample *t*-test, the honey harvested at 30 days from *A. crassiparva* plants aged 3 and 18 months is the one that meets the Indonesian national honey quality standards, specifically below 22% w/w. Prolonged harvest time provides bees with additional time to decrease the water

content in the hive, resulting in more mature honey. This observation is consistent with the findings of do Nascimento et al. (2018) as well as Taha and AL-Kahtani (2020), who reported that honey moisture content is affected by harvest time and honey maturity level. The process of water content reduction within the hive occurs through active evaporation by worker bees, achieved by vibrating their wings (Abou-Shaara et al., 2017; Eyer et al., 2016).

The moisture content of honey harvested at 14 and 21 days is still relatively high, especially in the 3- and 8-month age groups, ranging from 23.4 to 26.4% w/w. These values are nearly identical to the findings of Suhesti, Roni, et al. (2023) for the moisture content of *A. mellifera* honey harvested at 14 days in a different location in Riau Province, Indonesia, measuring 26.73% w/w.

### Acidity Level

An analysis of the acidity levels in all honey revealed values exceeding the maximum permissible limits established by SNI 8664:2018 (Badan Standardisasi Nasional, 2018). According to SNI 8664:2018, the maximum allowable acidity in honey is 50 milliequivalents sodium hydroxide (NaOH)/kg. This finding suggests that the investigated honey may not comply with current quality regulations. One-way ANOVA followed by LSD analysis indicates that harvest time, the age of *A. crassiparva*, and their interactions significantly affect the acidity of honey, and acidity values differ among all harvest times and ages of *A. crassiparva* in honey ( $P < 0.01$ ) (Table 1).

There is a tendency for the acidity of honey to decrease with a longer harvest time, while an increase in the age of the plant as a nectar source tends to result in higher honey acidity.

The influence of harvest time on honey acidity is presumed to be due to the honey's maturation level. Da Silva et al. (2016) stated that an extended harvest time increases honey maturation. The dominant acid in honey is gluconic acid, formed through the oxidation of glucose during honey maturation by bees (Karabagias et al., 2014). This study investigates the potential correlation between the age of *A. crassiparva* and the resulting honey acidity produced by *A. mellifera* in the local region. It hypothesizes that as *A. crassiparva* matures, the extrafloral nectar secreted from its leaf bases exhibits an increase in acidity, which may, in turn, influence the honey produced by *A. mellifera*. While the correlation between nectar sugar composition and honey acidity is a well-established concept, further study is necessary to explore the potential influence of the peatland environment on honey acidity. Since the acidic nature of peat swamp environments, a characteristic habitat for *A. crassiparva* may contribute to the observed acidity levels in the honey produced from its nectar (Suhesti, Zalizar, et al., 2023). Studies by Erniaty et al. (2023) support this hypothesis, demonstrating the low pH and high acidity typical of peat swamp ecosystems.

Acidity in honey imparts chemical and sensory characteristics, influencing honey's taste and consumer preferences (Al-Ghamdi

et al., 2019; dos Santos Scholz et al., 2020; Suhesti, Roni, et al., 2023). Furthermore, the acidity level in honey can serve as an indicator that the honey has undergone fermentation (Boussaid et al., 2018), which can occur rapidly in conditions with high moisture content. However, all honey samples in this study are freshly harvested, so that the elevated acidity levels can be attributed to factors other than fermentation. Ananias et al. (2013) stated that honey with high acidity values but without signs of fermentation cannot be considered of lower quality, as factors influencing acidity include environmental conditions, harvest time, nectar source plants, and climate (da Silva et al., 2016; dos Santos Scholz et al., 2020). The acidity in honey may also indicate the presence of antioxidants often associated with ascorbic acid or vitamin C (Pribadi & Wiratmoko, 2023). A study by Handayani et al. (2022) investigated *A. mellifera* honey produced from *A. crassiparva* nectar in Siak Regency, Indonesia. Their research demonstrated that the honey possessed antioxidant activity, with a measured value of 21,103.74 µg/ml.

Additionally, the study identified the presence of various secondary metabolites within the honey, including alkaloids, phenolics, flavonoids, terpenoids, saponins, and tannins. These compounds suggest that honey has the potential to be developed into a new functional beverage when combined with other ingredients, offering enhanced health benefits through their antioxidant, anti-inflammatory, and antimicrobial properties (Maulida et al., 2024).

## CONCLUSION

The results indicated significant influences of harvest time, *A. crassiparva* age, and the interaction between harvest time and *A. crassiparva* age on various parameters, such as enzymatic activity, HMF content, sugar composition, moisture content, and acidity. Based on the analysis, the research findings indicate a significant interaction between the harvest time of 30 days and the age of *A. crassiparva* plants, particularly those aged 8 and 18 months. This interaction resulted in the production of honey with the highest levels of HMF and glucose, as well as the lowest moisture and sucrose content. Importantly, these parameters met the specified national quality standards. Future research should focus on elucidating the chemical composition of *A. crassiparva* nectar and exploring the broader implications of honey composition for various honeybee species. The challenging aspects of this study involve addressing the complex interplay between environmental factors, harvest time, and plant age, which may contribute to the variability in honey composition, especially for acidity level.

## ACKNOWLEDGMENTS

We are grateful to the Ministry of Education, Culture, Research, and Technology, Directorate General of Higher Education of the Republic of Indonesia, for funding this research under the Doctoral Dissertation Research Grant scheme, grant number 183/E5/PG.02.00.PL/2023; 013/SP2H/PT/LL7/2023.

## REFERENCES

- Abou-Shaara, H. F., Owayss, A. A., Ibrahim, Y. Y., & Basuny, N. K. (2017). A review of impacts of temperature and relative humidity on various activities of honey bees. *Insectes Sociaux*, *64*, 455–463. <https://doi.org/10.1007/s00040-017-0573-8>
- Al-Ghamdi, A., Mohammed, S. E. A., Ansari, M. J., & Adgaba, N. (2019). Comparison of physicochemical properties and effects of heating regimes on stored *Apis mellifera* and *Apis florea* honey. *Saudi Journal of Biological Sciences*, *26*(4), 845–848. <https://doi.org/10.1016/j.sjbs.2017.06.002>
- Alvarez-Suarez, J. M., Giampieri, F., Brenciani, A., Mazzoni, L., Gasparrini, M., González-Paramás, A. M., Santos-Buelga, C., Morroni, G., Simoni, S., Forbes-Hernández, T. Y., Afrin, S., Giovanetti, E., & Battino, M. (2018). *Apis mellifera* vs *Melipona beecheii* Cuban polyfloral honeys: A comparison based on their physicochemical parameters, chemical composition and biological properties. *LWT*, *87*, 272–279. <https://doi.org/10.1016/j.lwt.2017.08.079>
- Ananias, K. R., de Melo, A. A. M., & de Moura, C. J. (2013). Analysis of moisture content, acidity and contamination by yeast and molds in *Apis mellifera* L. honey from central Brazil. *Brazilian Journal of Microbiology*, *44*(3), 679–683. <https://doi.org/10.1590/S1517-83822013000300003>
- Badan Standardisasi Nasional. (2018). *SNI 8664-2018: Madu* [SNI 8664-2018: Honey]. <https://pesta.bsn.go.id/produk/detail/13137-sni86642018>
- Baroni, M. V., Podio, N. S., Badini, R. G., Inga, M., Ostera, H. A., Cagnoni, M., Gautier, E. A., García, P. P., Hoogewerff, J., & Wunderlin, D. A. (2015). Linking soil, water, and honey composition to assess the geographical origin of Argentinean honey by multielemental and isotopic analyses. *Journal of Agricultural and Food Chemistry*, *63*(18), 4638–4645. <https://doi.org/10.1021/jf5060112>
- Bell, A. R., & Grainger, M. N. C. (2023). Accelerated loss of diastase in mānuka honey: Investigation of mānuka specific compounds. *Food Chemistry*, *426*, 136614. <https://doi.org/10.1016/j.foodchem.2023.136614>
- Berhe, A., Tesfaye, E., & Terle, D. (2018). Evaluation of physicochemical properties of honey bees (*Apis mellifera*) in Godere woreda, Gambella, Ethiopia. *American Journal of Food Science and Technology*, *6*(1), 50–56. <https://doi.org/10.12691/ajfst-6-1-8>
- Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsi, F., Ferrari, G., & Hamdi, S. (2018). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arabian Journal of Chemistry*, *11*(2), 265–274. <https://doi.org/10.1016/j.arabjc.2014.08.011>
- Can, Z., Yildiz, O., Sahin, H., Turumtay, E. A., Silici, S., & Kolayli, S. (2015). An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry*, *180*, 133–141. <https://doi.org/10.1016/j.foodchem.2015.02.024>
- Chua, L. S., & Adnan, N. A. (2014). Biochemical and nutritional component of selected honey samples. *Acta Scientiarum Polonorum, Technologia Alimentaria*, *13*(2), 169–179.
- Czipa, N., Phillips, C. J. C., & Kovács, B. (2019). Composition of acacia honeys following processing, storage and adulteration. *Journal of Food Science and Technology*, *56*(3), 1245–1255. <https://doi.org/10.1007/s13197-019-03587-y>
- da S. Sant'ana, R., de Carvalho, C. A. L., Oda-Souza, M., de A. Souza, B., & de S. Dias, F. (2020). Characterization of honey of stingless bees from the Brazilian semi-arid region. *Food Chemistry*, *327*, 127041. <https://doi.org/10.1016/j.foodchem.2020.127041>
- da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry*, *196*, 309–323. <https://doi.org/10.1016/j.foodchem.2015.09.051>
- De-Melo, A. A. M., de Almeida-Muradian, L. B., Sancho, M. T., & Pascual-Maté, A. (2018).

- Composición y propiedades de la miel de *Apis mellifera*: Una revisión [Composition and properties of *Apis mellifera* honey: A review]. *Journal of Apicultural Research*, 57(1), 5–37. <https://doi.org/10.1080/00218839.2017.1338444>
- do Nascimento, K. S., Sattler, J. A. G., Macedo, L. F. L., González, C. V. S., de Melo, I. L. P., da Silva Araújo, E., Granato, D., Sattler, A., & de Almeida-Muradian, L. B. (2018). Phenolic compounds, antioxidant capacity and physicochemical properties of Brazilian *Apis mellifera* honeys. *LWT*, 91, 85–94. <https://doi.org/10.1016/j.lwt.2018.01.016>
- dos Santos Scholz, M. B., Júnior, A. Q., Delamuta, B. H., Nakamura, J. M., Baudraz, M. C., Reis, M. O., Kato, T., Pedrão, M. R., Dias, L. F., dos Santos, D. T. R., Kitzberger, C. S. G., & Bianchini, F. P. (2020). Indication of the geographical origin of honey using its physicochemical characteristics and multivariate analysis. *Journal of Food Science and Technology*, 57, 1896–1903. <https://doi.org/10.1007/s13197-019-04225-3>
- Erban, T., Shcherbachenko, E., Talacko, P., & Harant, K. (2021). A single honey proteome dataset for identifying adulteration by foreign amylases and mining various protein markers natural to honey. *Journal of Proteomics*, 239, 104157. <https://doi.org/https://doi.org/10.1016/j.jprot.2021.104157>
- Erniaty, E., Gumiri, S., Ardianor, A., Haryono, A., Yulintine, Y., & Masliani, M. (2023). Analysis of water physical, chemical and biological properties in different peat swamp forests converted to rice fields. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1153, No. 1, p. 012026). IOP Publishing. <https://doi.org/10.1088/1755-1315/1153/1/012026>
- Escuredo, O., Dobre, I., Fernández-González, M., & Seijo, M. C. (2014). Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry*, 149, 84–90. <https://doi.org/10.1016/j.foodchem.2013.10.097>
- Escuredo, O., Míguez, M., Fernández-González, M., & Seijo, M. C. (2013). Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry*, 138(2–3), 851–856. <https://doi.org/10.1016/j.foodchem.2012.11.015>
- Eyer, M., Neumann, P., & Dietemann, V. (2016). A look into the cell: Honey storage in honey bees, *Apis mellifera*. *PLOS One*, 11(8), e0161059. <https://doi.org/10.1371/journal.pone.0161059>
- Ghramh, H. A., Alrumman, S. A., Ahmad, I., Kalam, A., Elbehairi, S. E. I., Alfaify, A. M., Mohammed, M. E. A., Al-Sehemi, A. G., Alfaifi, M., Al-Shehri, B. M., Alshareef, R. M. H., Alaerjani, W. M. A., & Khan, K. A. (2023). Chemical characterization of honey and its effect (alone as well as with synthesized silver nanoparticles) on microbial pathogens' and human cancer cell lines' growth. *Nutrients*, 15(3), 684. <https://doi.org/10.3390/nu15030684>
- Hailu, D., & Belay, A. (2020). Melissopalynology and antioxidant properties used to differentiate *Schefflera abyssinica* and polyfloral honey. *PLOS One*, 15(10), e0240868. <https://doi.org/10.1371/journal.pone.0240868>
- Handayani, T. H., Budiman, M. A., Amalia, R. L. R., Pribadi, A., Elfitra, R. R., & Ferdian, P. R. (2022). Aktivitas antioksidan, total fenolik, dan total flavonoid madu *Apis mellifera* dari hutan akasia (*Accacia crassicarpa*) Riau, Indonesia dengan beberapa perlakuan pengeringan [Antioxidant activity, total phenolics, and total flavonoids of *Apis mellifera* honey from acacia (*Accacia crassicarpa*) forests in Riau, Indonesia with several drying treatments]. *Jurnal Biologi Indonesia*, 18(2), 231–243. <https://doi.org/10.47349/jbi/18022022/231>
- Juan-Borrás, M., Domenech, E., Hellebrandova, M., & Escriche, I. (2014). Effect of country origin on physicochemical, sugar and volatile composition of acacia, sunflower and tilia honeys. *Food Research International*, 60, 86–94. <https://doi.org/10.1016/j.foodres.2013.11.045>
- Karabagias, I. K., Badeka, A., Kontakos, S., Karabournioti, S., & Kontominas, M. G. (2014). Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical

- parameters and chemometrics. *Food Chemistry*, 146, 548–557. <https://doi.org/10.1016/j.foodchem.2013.09.105>
- Kek, S. P., Chin, N. L., Yusof, Y. A., Tan, S. W., & Chua, L. S. (2018). Classification of entomological origin of honey based on its physicochemical and antioxidant properties. *International Journal of Food Properties*, 20(sup3), S2723–S2738. <https://doi.org/10.1080/10942912.2017.1359185>
- Lewkowsky, O., Mureşan, C. I., Dobritzsch, D., Fuszard, M., & Erler, S. (2019). The effect of diet on the composition and stability of proteins secreted by honey bees in honey. *Insects*, 10(9), 282. <https://doi.org/10.3390/insects10090282>
- Maulida, I. D., Al Marsam, M. R., Purnama, I., & Mutamima, A. (2024). A novel beverage with functional potential incorporating cascara (*Coffea arabica*), roselle (*Hibiscus sabdariffa*), and red ginger (*Zingiber officinale* Rosc. var. *rubrum*) extracts: Chemical properties and sensory evaluation. *Discover Food*, 4, 94. <https://doi.org/10.1007/s44187-024-00180-x>
- Oroian, M., & Sorina, R. (2017). Honey authentication based on physicochemical parameters and phenolic compounds. *Computers and Electronics in Agriculture*, 138, 148–156. <https://doi.org/10.1016/j.compag.2017.04.020>
- Pasias, I. N., Kiriakou, I. K., Kaitatzis, A., Koutelidakis, A. E., & Proestos, C. (2018). Effect of late harvest and floral origin on honey antibacterial properties and quality parameters. *Food Chemistry*, 242, 513–518. <https://doi.org/10.1016/j.foodchem.2017.09.083>
- Pribadi, A., & Purnomo. (2013). *Agroforestry sorghum (Sorghum spp.) pada HTI Acacia crassicarpa sebagai sumber pakan lebah Apis cerana di Provinsi Riau untuk mendukung budidaya lebah madu* [Agroforestry sorghum (*Sorghum* spp.) on IPF *Acacia crassicarpa* as a source of feed for *Apis cerana* bees in Riau Province to support honey bee cultivation]. In D. P. Kuswanto, T. S. Widyaningsih, E. Fauziyah, & R. Rachmawati (Eds.), *Prosiding Seminar Nasional Agroforestri 2013: Agroforestri untuk Pangan dan Lingkungan yang Lebih Baik* (pp. 36–41). Balai Penelitian Teknologi Agroforestry, Fakultas Pertanian Universitas Brawijaya, World Agroforestry Centre (ICRAF), and Masyarakat Agroforestri Indonesia.
- Pribadi, A., & Wiratmoko, M. D. E. (2023). Karakteristik fisikokimia madu *Heterotrigona itama* asal Provinsi Riau [Physicochemistry characteristics of *Heterotrigona itama* honey from Riau Province]. *Wahana Forestra: Jurnal Kehutanan*, 18(2), 105–120. <https://doi.org/10.31849/forestra.v18i2.11107>
- Purwanto, D. B., Kuntadi., Adalina, Y., Meilin, A., Raffiudin, R., & Sahlan, M. (2024). Evaluation of honey production for industry purposes, the case of beekeeping with *Apis mellifera* in Indonesia after the COVID-19 pandemic. *International Journal of Social Service and Research*, 4(3), 702–715. <https://doi.org/10.46799/ijssr.v4i03.719>
- Puscas, A., Hosu, A., & Cimpoiu, C. (2013). Application of a newly developed and validated high-performance thin-layer chromatographic method to control honey adulteration. *Journal of Chromatography A*, 1272, 132–135. <https://doi.org/10.1016/j.chroma.2012.11.064>
- Radtke, J., & Lichtenberg-Kraag, B. (2018). Long-term changes in naturally produced honey depending on processing and temperature. *Journal of Apicultural Research*, 57(5), 615–626. <https://doi.org/10.1080/00218839.2018.1494893>
- Sajid, M., Yasmin, T., Asad, F., & Qamer, S. (2019). Changes in HMF content and diastase activity in honey after heating treatment. *Pure and Applied Biology*, 8(2), 1668–1674. <https://doi.org/10.19045/BSPAB.2019.80109>
- Shapla, U. M., Solayman, M., Alam, N., Khalil, M. I., & Gan, S. H. (2018). 5-hydroxymethylfurfural (HMF) levels in honey and other food products: Effects on bees and human health. *Chemistry Central Journal*, 12, 35. <https://doi.org/10.1186/s13065-018-0408-3>
- Siddiqui, A. J., Musharraf, S. G., Choudhary, M. I., & Atta-ur-Rahman. (2017). Application of analytical methods in authentication and adulteration of honey. *Food Chemistry*,

- 217, 687–698. <https://doi.org/10.1016/j.foodchem.2016.09.001>
- Suhesti, E., Roni, Y., Yanti, R. N., Ningsih, A. T., & Hadinoto. (2023). Kualitas dan preferensi konsumen terhadap madu lebah *Apis mellifera* L. dan *Apis dorsata* F. [Quality and consumer preferences for *Apis mellifera* L. and *Apis dorsata* F. honey]. *Jurnal Penelitian Hasil Hutan*, 41(2), 87–96. <https://doi.org/10.55981/jphh.2023.766>
- Suhesti, E., Zalizar, L., Triwanto, J., Ervayendri, E., Setyobudi, R. H., Waskitho, N. T., Ibrahim, J. T., Maftuchah, M., Hadinoto, H., Vincēviča-Gaile, Z., Tonda, R., Angara, A., Hartono, R., Lestari, S. U., & Pakarti, T. A. (2023). Quality assessment on honey produced from six months old *Acacia crassicarpa*. In *E3S Web of Conferences: The 3<sup>rd</sup> International Conference on Natural Resources and Life Sciences 2020* (Vol. 374, p. 00012). *EDP Sciences*. <https://doi.org/10.1051/e3sconf/202337400012>
- Taha, E.-K. A., & AL-Kahtani, S. N. (2020). The relationship between comb age and performance of honey bee (*Apis mellifera*) colonies. *Saudi Journal of Biological Sciences*, 27(1), 30–34. <https://doi.org/10.1016/j.sjbs.2019.04.005>
- Taha, E.-K. A., Al-Kahtani, S., & Taha, R. (2021). Comparison of the physicochemical characteristics of sidr (*Ziziphus* spp.) honey produced by *Apis florea* F. and *Apis mellifera* L. *Journal of Apicultural Research*, 60(3), 470–477. <https://doi.org/10.1080/00218839.2020.1746036>
- Tong, L., Nieh, J. C., & Tosi, S. (2019). Combined nutritional stress and a new systemic pesticide (flupyradifurone, Sivanto®) reduce bee survival, food consumption, flight success, and thermoregulation. *Chemosphere*, 237, 124408. <https://doi.org/10.1016/j.chemosphere.2019.124408>
- Tornuk, F., Karaman, S., Ozturk, I., Toker, O. S., Tastemur, B., Sagdic, O., Dogan, M., & Kayacier, A. (2013). Quality characterization of artisanal and retail Turkish blossom honeys: Determination of physicochemical, microbiological, bioactive properties and aroma profile. *Industrial Crops and Products*, 46, 124–131. <https://doi.org/10.1016/j.indcrop.2012.12.042>
- Viteri, R., Zacconi, F., Montenegro, G., & Giordano, A. (2021). Bioactive compounds in *Apis mellifera* monofloral honeys. *Journal of Food Science*, 86(5), 1552–1582. <https://doi.org/10.1111/1750-3841.15706>
- Vranić, D., Petronijević, R., Stojanović, J. Đ., Korićanac, V., Milijašević, J. B., & Milijašević, M. (2017). Physicochemical properties of honey from Serbia in the period 2014–2016. In *IOP Conference Series: Earth and Environmental Science* (Vol. 85, No. 1, p. 012058). IOP Publishing. <https://doi.org/10.1088/1755-1315/85/1/012058>
- Wu, M.-C., Wu, C.-Y., Klaithin, K., Tiong, K. K., & Peng, C.-C. (2022). Effect of harvest time span on physicochemical properties, antioxidant, antimicrobial, and anti-inflammatory activities of Meliponinae honey. *Journal of the Science of Food and Agriculture*, 102(13), 5750–5758. <https://doi.org/10.1002/jsfa.11924>