The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2024), 48(1), 100-116

ORIGINAL RESEARCH PAPER

CHANGES OF ANTIOXIDANT PROPERTIES OF INDONESIAN HONEY UNDER HEAT TREATMENT

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Received on 20 May 2024
Revised on 1 July 2024

Abstract

Post-harvest heating on honey is crucial for moisture reduction, microbial inhibition, and crystallization prevention, yet it can diminish bioactive compounds such as antioxidants. This study aims to investigate the impact of heating on the antioxidant properties of Indonesian honey, encompassing three Apini and three Meliponini honey types. While all samples exhibited changes in antioxidant properties post-heating, Apini honey displayed a more consistent pattern compared to Meliponini honey. The DPPH assay, total phenolic content, and total flavonoid content consistently indicated a decline in antioxidants at lower temperatures and short durations of heating, followed by an increase at high temperatures and longer duration, suggesting a compensation mechanism through the generation of new antioxidants via Maillard's reaction. However, the ferric reducing power assay revealed a consistent decrease in antioxidants post-heating for Apini honey, indicating damage to natural antioxidants without compensation, whereas Meliponini honey displayed irregular dynamics, suggesting both damage and formation of antioxidants. The Apini honey showed the best antioxidant properties at high temperatures (100°C), whereas Meliponini honey showed an optimum at low temperature (50°C) for extended durations or medium temperature (75°C) for shorter durations.

Keywords: antioxidant, apini, heat treatment, Indonesian honey, meliponini

https://doi.org/10.35219/foodtechnology.2024.1.06

Introduction

Honey is naturally produced by bee species from nectar or extrafloral liquid. There are three groups of bees producing honey, those are Apini, Meliponini, and Bombini (Kahono *et al*., 2018). Honey is well-known to be a natural sweetener as well as a functional food, since it has a varied bioactivity, such as antioxidant, antimicrobial, anti-inflammatory and anticancer (Almasaudi, 2021; Weis *et al*., 2022; Zammit and Blundell, 2023). Honey also contains some important enzymes, such as glucose oxidase, catalase, acid phosphatase, invertase, and diastase which become key indicators of honey quality and authenticity (Siddiqui *et al*., 2017). Antioxidants are essential in protecting cells from oxidative stress, which has been related to aging and a variety of chronic illnesses. Moreover, the antioxidants in honey vary depending on factors such as floral sources, geographical locations, and processing methods (Becerril-Sanchez *et al*., 2021; Rachmawati *et al*., 2022).

Indonesia is known for its extraordinary floral biodiversity, varying climates, and the archipelago's geographical location. The flora is characterized by unique plant species, lush rainforests, and a wealth of botanical diversity (Kusmana and Hikmat, 2015). It is also home to a variety of honeybees producing many types of honey. Furthermore, Indonesia has three different groups of honey, which are formally stated in the Indonesian National Standard for Honey, namely honey produced from managed honey bees (*Apis mellifera* and *Apis cerana*), wild honey bees (*Apis dorsata*), and stingless bees (BSN, 2018).

In terms of managed honey bees, *A. mellifera* which is known as the Western honey bee, is positioned as the less-kept honey bee species in Indonesia and dominantly managed in Sumatera and Java Islands and a few in Kalimantan and Sulawesi Islands. Meanwhile, *A. cerana* which is commonly known as the Eastern honey bee or the Asian honey bee, is called the local honey bee in most Indonesian areas except Papua (Buchori *et al*., 2022; Aditya and Purwanto, 2023). The highest number of species kept by beekeepers is stingless bees and there are at least 19 species of stingless bees, mostly *Heterotrigona itama* and *Tetragonula laeviceps* (Buchori *et al*., 2022; Trianto and Purwanto, 2022). *A. dorsata*, which is popular as the Asian Giant Honey Bee, is distributed from the southeast to the southern part of Asia, including Indonesia (Kaligis and Mokosuli, 2022).

In the industry, a series of sequential chains of processing operations may involve raising the temperature of honey. These heating processes may be applied from initial extraction, dehumidification, liquefaction, pasteurization, crystallization, and final packaging (Baglio, 2018). The heating process is required so as to increase the viscosity, eliminate the spoilage, and prevent the crystallization (Baglio, 2018; Eshete and Eshete, 2019). Several alternatives can be applied to honey processing to replace conventional heating, including microwave, ultrasound, infrared, membrane processing, and micro fluidization (Leyva-Daniel *et al*., 2020). However, those technologies require specific technology which is not easy to apply in honey small-scale producers. Thus, defining the most optimum heating condition in conventional heat treatment is still preferred due to its simplicity. In addition, the optimum heating

condition relies on the geography and botanical origin of honey which varies depending on the biochemical composition of honey (Eshete and Eshete, 2019).

As the biochemical content of honey is diverse, depending on its bee species, source of nectar, and geographical origin, the optimum heating condition also differs in each type of honey (Zammit and Blundell, 2023; Eshete and Eshete, 2019; Handayani *et al*., 2022; Tkáč *et al.*, 2022). Therefore, this study aims to observe the dynamics of the antioxidant properties of Indonesian honey, including 3 Apini and 3 Meliponini, caused by several heating treatments.

Materials and methods

Materials

All of the reagents used in the present study were of the highest grade for analysis (pro-analytical). Folin-Ciocalteu reagent, trichloroacetic acid (TCA), aluminum trichloride ($AlCl_3$), potassium acetate (CH_3COOK), potassium ferricyanide ($K_3[Fe(CN)_6]$), sodium phosphate dibasic ($Na_2HPO_4 \cdot 2H_2O$), potassium phosphate monobasic (KH_2PO_4), ferric chloride ($FeCl_3$) and methanol were purchased from Merck. DPPH (2,2-diphenyl-2-picrylhydrazyl), gallic acid, and quercetin were purchased from Sigma Aldrich.

Sample collection

Honey used in the present study was collected from regions across Indonesia. All samples were gathered in the late 2022 and meticulously stored in a dry-sealed container within a room with a controlled air conditioning setting of 25°C. The provenance details of each sample were systematically delineated in Table 1.

Table 1. Sample collection

No.	Types of honey	Sample code	Origin		
			Bee species	Nectar or extrafloral liquid	Geographic
1.	Apini	A1	*Apis mellifera*	Acacia tree (*Acacia crassicarpa*)	Riau
2.		A2	*Apis cerana*	Calliandra tree (*Calliandra* sp.)	West Java
3.		A3	*Apis dorsata*	Multiflora, mostly coconut tree (*Cocos nucifera*)	North Maluku
4.	Meliponini	M1	*Heterotrigona itama*	Multiflora	Riau
5.		M2	*Heterotrigona itama*	Rubber tree (*Hevea* sp.)	East Kalimantan
6.		M3	*Tetragonula biroi*	Multiflora	South Sulawesi

Experimental design and heat treatment

Heat treatments were applied separately to each sample, including 3 variations of heat temperature and 3 variations of heat duration. The temperatures used in the present study were 50, 75, and 100°C, whereas the durations were 15, 30, and 45

minutes, respectively. Each temperature and duration of heating used in the present study was combined, so that nine types of heating treatment were designed. Therefore, there were two independent variables used in the present study, heat temperature and heat duration. The sample with no heating treatment (encoded as NT) was used as the baseline.

Moisture Content Analysis

The moisture content of honey was measured using a manual refractometer (BSN, 2018). The analysis was run in triplicate.

Determination of total phenolic content (TPC)

The quantification of TPC was conducted by Folin-Ciocalteu method, as described by Maeng *et al*. (2017). A total of 0.2 mL sample was mixed with 0.2 mL of Folin-Ciocalteu reagent and 1.8 mL of distilled water. Following a 6 minute incubation period, 2 mL of sodium carbonate 7% was introduced and the mixture was allowed to incubate for a period of 90 minutes. Absorbance measurements were taken at 750 nm. Gallic acid was used to create the standard curve with a range of concentration 0-200 µg/mL.

Determination of total flavonoid content (TFC)

The TFC was measured following the method described by Aryal *et al*. (2019). As much as 1 mL of sample is added into 0.2 mL of aluminum trichloride 10% in methanol and then mixed with 0.2 mL of potassium acetate 1 M, following with 5.6 mL of distilled water. The mixture was incubated at room temperature for 30 minutes, followed by measuring the absorbance at 415 nm. A standard curve of quercetin with a range of concentration 0-100 µg/mL was used to determine TFC.

Antioxidant-DPPH scavenging activity

Antioxidant DPPH scavenging activity was determined according to Boonsong *et al*. (2016). As much as 1 mL of each diluted honey sample was mixed with 3 mL of DPPH 0.1 mm solution in methanol. The mixture was then vigorously shaken and allowed to incubate in the dark, at room temperature, for 30 minutes, then measured at 517 nm using spectrophotometer UV-Vis (Genesys 10-S, Thermo Fisher Scientific Inc., Waltham, MA, USA). The IC_{50} values were determined from a regression curve of the serial concentrations of the sample with antioxidant scavenging activity.

Antioxidant-ferric reducing power (FRP)

The method of Fu *et al*. (2014) was used to assess the FRP. A total of 0.3 mL of honey sample was mixed with 0.6 mL of 0.2 M sodium phosphate buffer pH 6.6 and 0.6 mL of potassium ferricyanide 1%. The mixture was then incubated in a water bath at 50°C for 30 minutes. Subsequently, 0.6 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The supernatant (0.5 ml) was then mixed with 0.5 mL distilled water and 0.1 mL of $FeCl_3$ 0.1 %. The intensity of the blue-green color was measured at 700 nm. The absorbance was directly proportional to its reducing power.

Statistical analysis

The data for each type of honey, separately, was analyzed using two-way-analysis of variance (two way-ANOVA), since two independent variables were used in the present study, including heat temperature and heat duration. Tukey's post hoc test was also applied. The analysis was conducted using SPSS 25 software (IBM Corporation, Armonk, USA).

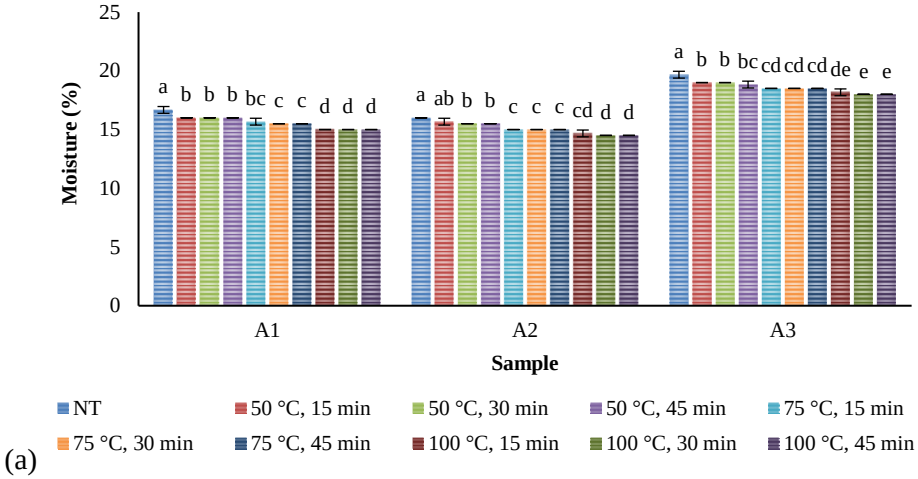
Results and discussion

Moisture

The two way-ANOVA test indicates that the moisture of samples A1, A2, A3, and M3 is significantly influenced only by the heating temperature, while the heating duration and the interaction between heating temperature and duration show insignificant contributions ($P<0.05$) (Figure 1). Heat temperature and heat duration significantly contributed to the moisture of M2 ($P<0.05$), while heat temperature, heat duration, and the interaction between heat temperature and heat duration significantly contributed to M1. The heating treatment applied to A1, A2, A3, and M3 samples slightly reduced the moisture content, since the maximum decreases of moisture compared to the corresponding control samples (no treatment) were less than 2%. However, the heating treatment resulted in a high reduction of water content in the case of M1 and M2, probably because of the relatively high moisture baseline of these samples compared to others.

Ensuring compliance with national and international standards is crucial for the trade and distribution of honey. Our study, centered on Indonesian honey, underscores the significance of adhering to the latest version of honey regulation documents, including Indonesian National Standard for Honey (BSN, 2018) and Codex Alimentarius Commission, Standard for Honey (CAC, 2022). While Indonesian National Standard sets moisture limits at 22% for Apini honey and 27.5% for Meliponini honey, the Codex mandates a general standard of less than 20%, except for heather honey (*Calluna vulgaris*) which can be less than 23%. This dichotomy poses challenges for honey producers, particularly in tropical climates like Indonesia with high humidity, often necessitating heat treatments to meet these standards. The potential impact on bioactivity, including antioxidant activity, must be considered, especially with prevalent industry practices such as open boiling.

High moisture content in honey can compromise its chemical stability during storage, leading to spoilage and crystallization (Chuttong *et al*., 2015; Braghini *et al*., 2020). Thermal treatments that can extend shelf life are employed to enhance the biological and physicochemical stability of honey. In our study, Apini honey met both national and international standards without requiring additional heat treatment, while Meliponini honey required heat treatment to align with these standards. The M3 sample required heating at 75°C for 15 minutes to meet both standards, while M1 and M2 necessitated higher temperature and longer duration to meet the standards. However, careful consideration of bioactivity and preservation of honey's unique characteristics is equally paramount.



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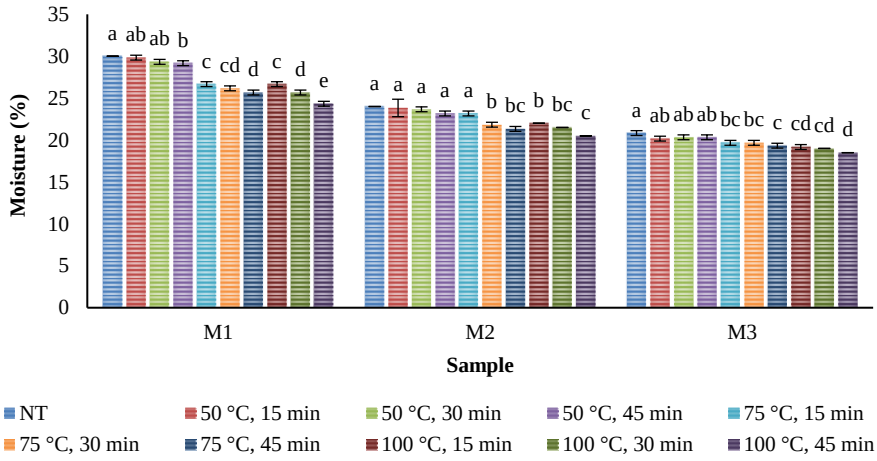

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Figure 1. Moisture changes of Indonesian (a) Apini and (b) Meliponini honey caused by heat treatments. In each type of honey, values with different letters indicate significant differences ($P < 0.05$) according to Tukey's post hoc test.

Total phenolic content

Two-way ANOVA analysis revealed a significant impact of heat temperature, heat duration, and their interaction on TPC for both honey types ($P<0.05$) (Figure 2). Apini honey exhibited a general increase in TPC values across three levels of temperature and duration, with the highest TPC observed at 100°C for 45 minutes. However, A1 and A2 showed a decrease in TPC at 50 and 75°C for 15 minutes, while A3 consistently increased. Notably, A1 experienced a significant TPC increase when heated for 30 minutes. In Meliponini, inconsistent results were observed with varying heating temperature and duration, but the combination of heating and duration significantly affected TPC ($P<0.05$). The best combination treatment varied

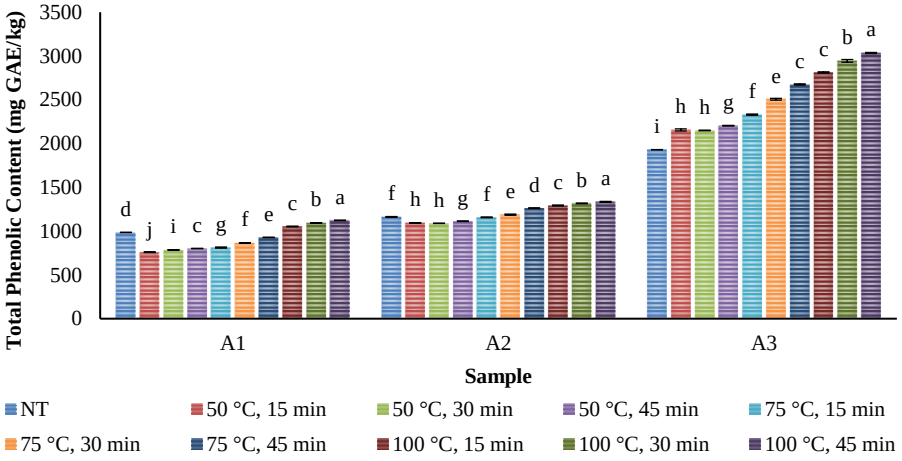
among Meliponini, with M1 reaching the highest TPC at 50°C for 15 minutes, and M2 and M3 achieving optimal TPC at 100°C for 15 minutes, respectively (Figure 2).

In recent times, there has been a growing focus on assessing the antioxidant capabilities of honey. The levels of the constituents responsible for honey's antioxidant properties exhibit significant variation based on the floral and geographical source of the honey. When honey is subjected to heat treatment, alterations in the presence of phenolic compounds occur (Cianciosi *et al*., 2018; Aydogan-Coskun *et al*., 2020). However, some studies mentioned that the impact of the heat process on the antioxidant activity of honey is minimal (Turkmen *et al*., 2006; Trinh *et al*., 2006). Moreover, Zarei *et al*. (2019) stated that there were no significant changes in the phenolic content of honey during the initial 20 minutes of thermal treatment at 63°C. Those studies, however, are not parallel to the result that revealed any significant changes even at the 50°C at 15 minutes except for M2 and M3.

The results of our study indicate that phenolic compounds in Apini honey continuously increased parallel to the incline of heat and duration. This result is similar to the studies conducted by Cianciosi *et al.* (2018), Aydogan-Coskun *et al*. (2020), and Šarić *et al*. (2020), that also used Apini honey. In this case, the existence of Maillard's reaction becomes the most plausible explanation. Sugary products undergo a Maillard's reaction at high heat levels, potentially increasing both intrinsic and certain non-nutritive antioxidant agent (Islam *et al*., 2012). Similarly, Jahan *et al*. (2015) proposed that both the total phenolic and flavonoid content tend to increase proportionally with increasing heat temperatures. It is suggested that the increase of phenolics and flavonoids content post-heat treatment might be attributed to enhanced bio-accessibility of specific heat-activated phenolics and flavonoids and the activation of certain thermostable compounds during thermal processing (Šarić *et al*., 2020).

On the contrary, Meliponini honey revealed an unclear trend between M1 and both M2 and M3. In M2 and M3, there was no significant difference between heat-treated and duration on TPC value at 50°C for 15 minutes. M1, on the contrary, showed a significant difference of TPC value when heated at 50°C for 15 minutes. The results of the TPC values in M2 and M3 suggested that the phenolic components are more stable and were not affected by heat than M1, when heated at 50°C for 15 minutes. In other words, the phenolic components available in M2 and M3 were more stable throughout that heat treatment. Nevertheless, all the TPC values from all Meliponini honey varieties changed when they were heated to more than 50°C for 15 minutes. In unprocessed Meliponini honey, a diverse range of phenolic compounds is present, with their concentration and composition primarily influenced by the floral source (Biluca *et al*., 2017; Ranneh *et al*., 2018; Braghini *et al*., 2020). Hence, concerning this study, the difference in Meliponini characteristics could be a plausible reason to explain the phenomena. However, similar to changes in antioxidant capabilities, the composition and concentration of these compounds can be altered through thermal processing and storage.

Moreover, Braghini *et al*. (2020) and Shahabuddin *et al*. (2022) mentioned that some phenolic compounds in honey are derived when heated at a range of temperatures of 100°C - 190°C, such as benzoic acid and quercetin. Phenolic compounds represent a category of aromatic secondary metabolites and have been reported to possess multiple biological effects, such as antioxidant capacity. There is a great interest in the food industry because they enhance the quality and nutritional value of foods.



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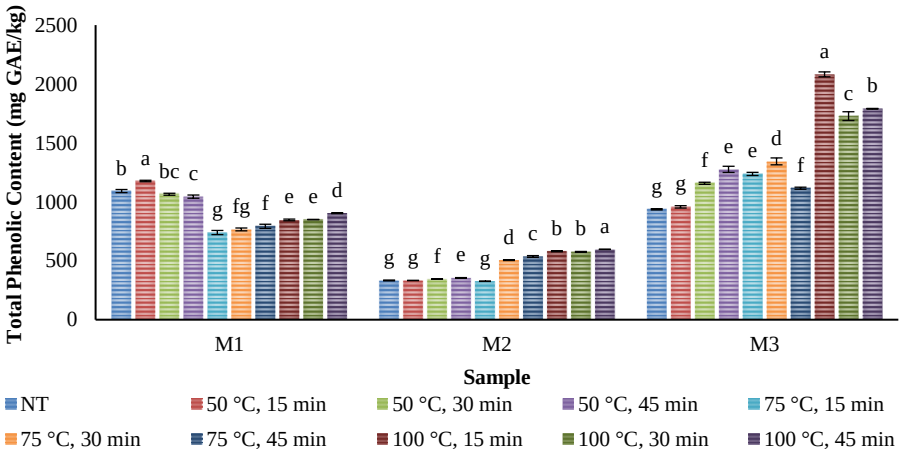

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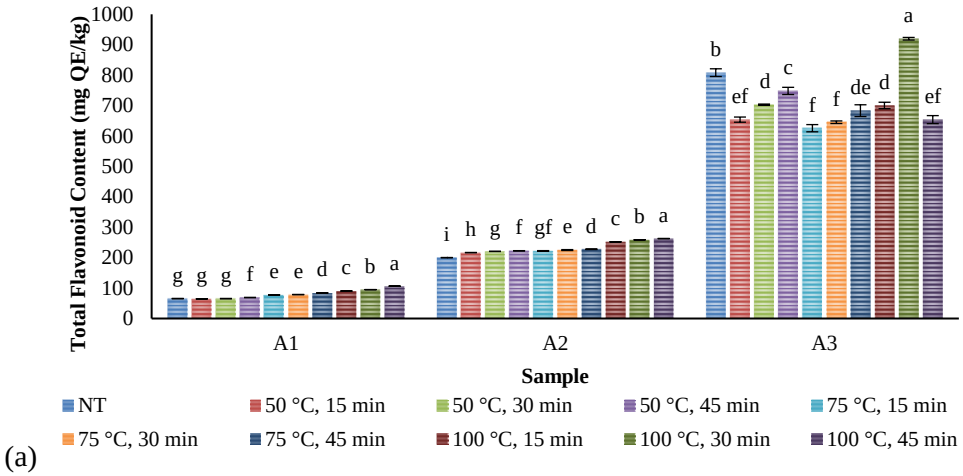
Figure 2. The changes on total phenolic content (TPC) of Indonesian (a) Apini and (b) Meliponini honey caused by heat treatments. In each type of honey, values with different letters indicate significant differences ($P < 0.05$) according to Tukey's post hoc test.

Total flavonoid content

Two-way ANOVA analysis demonstrated that temperature, duration, and their interaction significantly influenced the TFC in both Apini and Meliponini honey ($P<0.05$, Figure 3).



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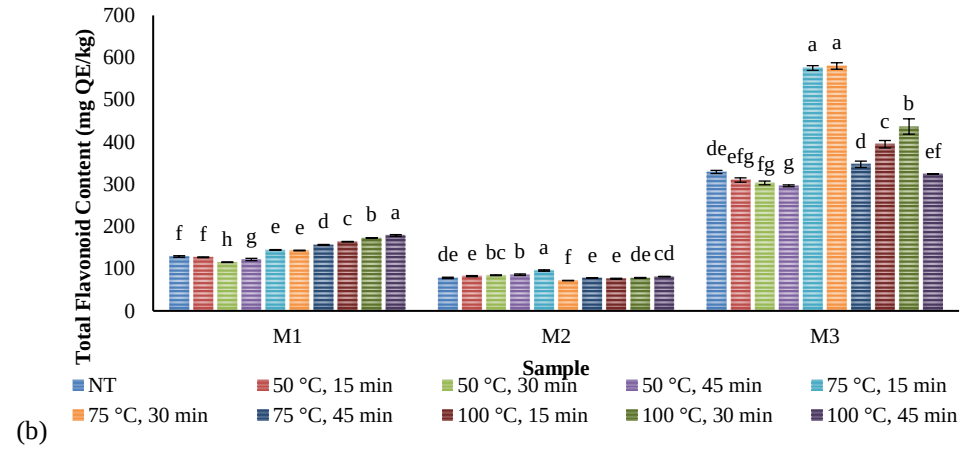

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Figure 3. The changes on total flavonoid content (TFC) of Indonesian (a) Apini and (b) Meliponini honey caused by heat treatments. In each type of honey, values with different letters indicate significant differences ($P < 0.05$) according to Tukey's post hoc test.

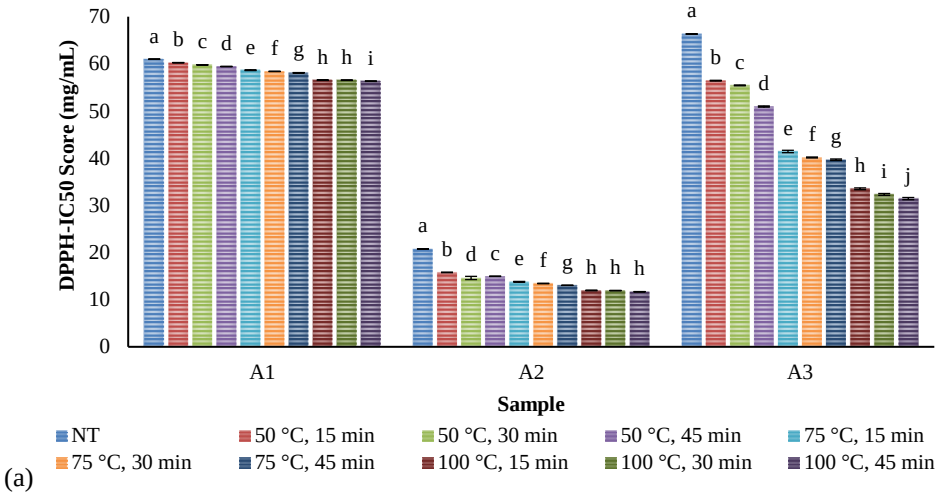
The Apini honey, especially A1 and A2, exhibited a consistent increase in TFC with rising temperature and duration of heating, while A3 showed an inconsistent trend with a slight decrease at 50°C and 75°C, and a slight increase only at 100 for 30 minutes. This indicated that the flavonoids in A3 were relatively heat-intolerant compared to A1 and A2. In Meliponini honey, the TFC pattern with heating treatment was irregular compared to Apini honey. M1 showed a decreasing trend at 50°C, and a gradual increase of TFC at 75°C and 100°C with the increase of durations. M2 displayed an increase at 50°C until 45 minutes and the maximum TFC was reached at 75°C for 15 minutes, while M3 showed a decreasing TFC trend at 50°C and instability at 100°C across durations, with the maximum increase at 75°C for 30 minutes.

The impact of heat on the flavonoid concentration in honey is intricate, influenced by factors like specific flavonoid type, heating temperature and heating duration (Trinh *et al*., 2006; Zarei *et al*., 2019). Notably, studies reveal an increase in flavonoid levels with heating, with specific flavonoids like quercetin and kaempferol showing elevated levels (Trinh *et al*., 2006; Zarei *et al*., 2019; Jan *et al*., 2022). Adebo *et al*. (2020) and Chen *et al*. (2022) attributed this rise to the breakdown of complex sugars, which liberates bound flavonoids and enhances their bioavailability, aiding efficient absorption. This enhancement is linked to heat disrupting cell walls in pollen grains, the primary source of flavonoids in honey (Martinello *et al*., 2021). This study supports the findings for all Apini honey types and one Meliponini honey type (M1), in which a tendency for TFC to increase parallel to heat treatment was observed.

Meanwhile, M2 and M3 had the highest TFC at 75°C and 15 minutes and the value declined significantly when heated to 100°C at any levels of duration. Some honey types, like M2 and M3, may experience a significant decline in flavonoid content due to heat sensitivity, as observed in other studies (Zarei *et al*., 2019). Specifically, rutin and chrysin, two honey flavonoids recognized for anti-inflammatory and antioxidant properties, are susceptible to degradation at temperatures exceeding 70°C (Šarić *et al*., 2020; Martinello *et al*., 2021; Masad *et al*., 2021). This degradation may impact the health-related benefits of honey, such as antioxidant activity. Mechanisms that contribute to flavonoid degradation during heat treatment include enzymatic oxidation, catalyzed by honey enzyme, and direct breakdown, triggered by elevated temperatures (Ayoub *et al*., 2023). It is suggested that variations in flavonoid compounds in M2 and M3, compared to all Apini and M1, may stem from distinct nectar sources and geographical origins, aligning with studies that emphasize the influence of melliferous plants and location on honey flavonoid compounds (Cianciosi *et al*., 2018).

Antioxidant-DPPH scavenging activity

The antioxidant activity based on DPPH scavenging activity is expressed in IC50 score (Figure 4). The IC50 score is inversely proportional to the antioxidant activity, the lower IC50, the higher antioxidant activity. The two-way ANOVA analysis revealed that heat temperature, heat duration, and their interaction significantly contribute to the IC50 score ($P<0.05$). The IC50 values represent the concentration of a sample required to neutralize 50% of DPPH free radicals upon exposure of free radicals. Overall, the data demonstrated that increasing heat temperature and duration correlated with elevated antioxidant activity, as reflected in a lower IC50 score. However, irregular trends were observed in M1 at 75°C for 30 minutes, and M2 at 50°C for 15 minutes and 30 minutes. Notably, A2 exhibited the highest antioxidant activity at 100°C for 45 minutes, thus reaching the lowest IC50 score in this study. By comparison, the application of heat treatment to Meliponini honey was found to enhance the antioxidant activity more than Apini honey, with the highest increase observed in M3.



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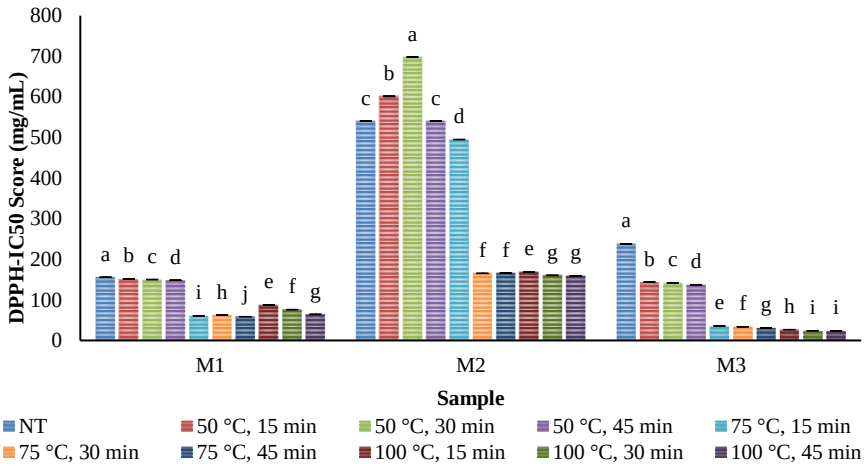

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Figure 4. The changes on DPPH-IC50 score of Indonesian (a) Apini and (b) Meliponini honey caused by heat treatments. In each type of honey, values with different letters indicate significant differences ($P < 0.05$) according to Tukey's post hoc test.

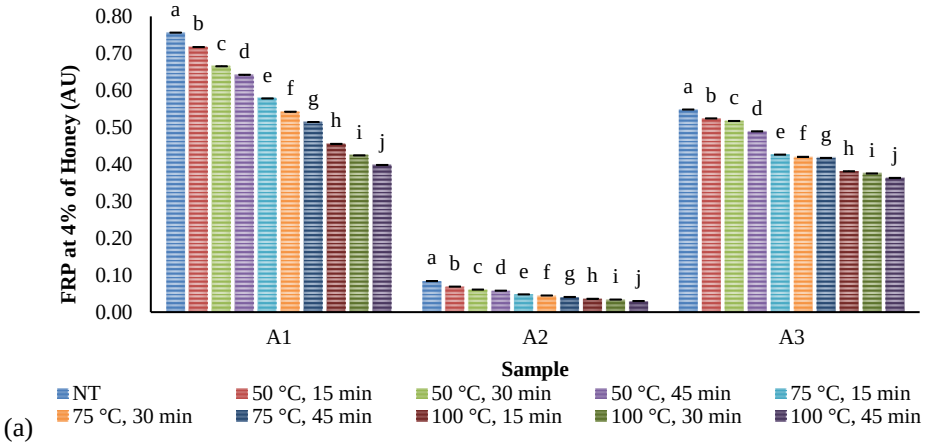
The efficacy of DPPH scavenging activity in honey extends beyond phenolics and flavonoids, encompassing factors such as pH and the presence of Al^{3+} ions that may interact with flavonoids (Pekal and Pyrzynsk, 2015). Their study on buffered tea suggests that antioxidant activity is heightened in less acidic environments, showcasing the influence of pH on DPPH scavenging activity (Pekal and Pyrzynsk, 2015). The alteration in hydrogen ion concentration influences the scavenging process of DPPH radicals by phenolic compounds, leading to a shift in the mechanism. As the pH decreases, the Proton-Coupled Electron Transfer (PC-ET) mechanism becomes dominant (Dawidowicz and Olszowy, 2012).

This mechanism explains the misalignment between the total phenolic and flavonoid content in honeys with their DPPH scavenging activity before heating treatment. In our study, heating treatment enhances DPPH scavenging activity, accompanied by elevated phenolics and flavonoids. The alteration of macromolecules, such as proteins, during heating, releases bonds with polyphenols and flavonoids, intensifying their interaction with DPPH radicals (Gulcin and Alwasel, 2023). Similar findings (Turkmen *et al*., 2006) highlight increased antioxidant activity after heating, attributing it to the Maillard's reaction and the formation of substances with varied antioxidant effectiveness. Contrarily, some studies reported by Šarić *et al*. (2020), Islam *et al*. (2012), and Jahan *et al*. (2015), suggested that heating may decrease the antioxidant activity of honey, revealing the complex interplay influenced by the botanical origin and unique chemical composition of honey.

Antioxidant-ferric reducing power

The FRP was employed as an indicator of antioxidant activity, with the reduction of Fe^{3+} to Fe^{2+}. The FRP value exhibited a linear proportionality to antioxidant activity, signifying that the higher absorbance corresponds to increased antioxidant activity based on the ferric reducing mechanism. The two-way ANOVA analysis of both independent variables, heat temperature and heat duration, and also their interaction significantly contributed to the FRP ($P<0.05$, Figure 5). The pattern of FRP score on Apini honey (A1, A2 and A3) decreased consistently with the increase of heating temperature and duration. However, irregular dynamics were observed in the Meliponini honey (M1, M2 and M3), where the Meliponini did not exhibit a consistent pattern when subjected to heating temperature and duration. In contrast with the Apini honey, heating treatment on Meliponini honey can relatively elevate the FRP, although the pattern showed irregular dynamics.

The FRP assay was used for evaluating the antioxidant content of the activity of water-soluble antioxidants. The results of the present study showed that the FRP value of Apini honey (A1, A1, A3) decreased after heat treatment, while Meliponini (M1, M2, M3) honey showed an irregular pattern. The results on Apini are in line with the findings of the study conducted by Zarei *et al*. (2019), which reported that the FRAP value decreased significantly in thyme and lotus honeys during the thermal treatment. Šarić *et al*. (2020), also reported that the antioxidant activity of acacia honey measured by FRAP decreased after thermal treatment with the average reduction in all samples being 31.4% (Šarić *et al*., 2013). The irregular pattern on Meliponini honey by FRP assay indicates that the damage and formation of antioxidant compounds occurred during heating. These results may stem from the breakdown of natural antioxidant compounds that reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). In contrast to DPPH assay, where the antioxidant activity generally increases with heat, compounds reducing ferric ions may not form after heating.



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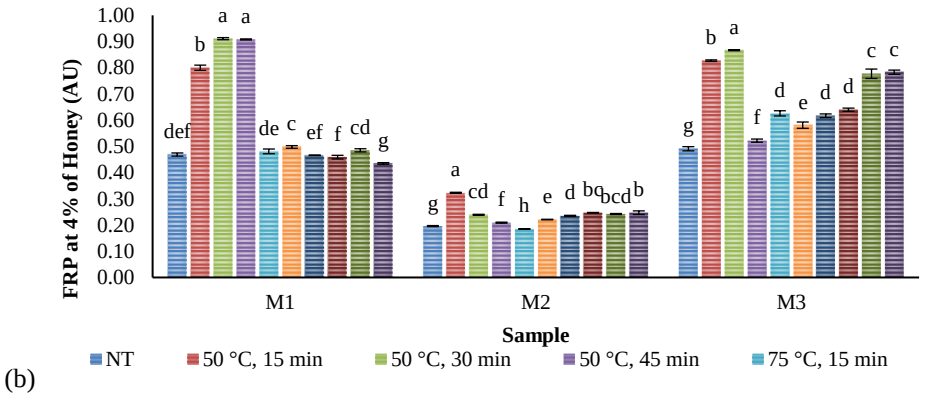

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Figure 5. The changes on ferric reducing power (FRP) of Indonesian (a) Apini and (b) Meliponini honey caused by heat treatments. In each type of honey, values with different letters indicate significant differences ($P < 0.05$) according to Tukey's post hoc test.

The high antioxidant activity detected by the DPPH assay post-heating could originate from the new antioxidant compounds formed through Maillard's reaction, potentially outweighing the decay of natural antioxidant compounds and resulting in heightened antioxidant activity after heating. We suggest that the type of Indonesian Apini honey used in the present study can be heated at high temperature (100°C) to enhance the antioxidant properties, while the Indonesian Meliponini honey, which was less tolerant to thermal treatment, can be heated at a low heat (50°C) for a long duration or at a medium heat (75°C) for a short duration so as to elevate the antioxidant activity.

Conclusion

All types of Indonesian honey used in the present study undergo changes of their antioxidant properties after heating, the Apini honeys having a less irregular pattern

than Meliponini ones. The effect of heat treatment was checked by DPPH-assay, TPC, and TFC. The results indicated a decrease of antioxidant properties at low temperature and short duration of heating, but a gradual increase of the antioxidant properties at higher temperatures and longer durations. It indicates that the heating breaks down the natural antioxidant compounds contained inside honey, but at the same time, the heating also generates a Maillard's reaction forming new antioxidant compounds. In contrast, the antioxidant activity of Indonesian Apini honey shown by FRP-assay, consistently decreased with the increase of heating temperature and duration, revealing that the natural antioxidant compounds which possess the ability to reduce the ferric ions were damaged permanently, without any new formation of this type of antioxidant compound. However, these changes found on Meliponini honey, based on the FRP assay, indicate that damage and formation of antioxidant compounds occurred. The application of heating on Indonesian honey induces dynamic alterations in its antioxidant properties, characterized by the concurrent degradation of natural antioxidant compounds and the concomitant generation of new antioxidant compounds.

Declaration of competing interest

The authors declare that there is no conflict of interest in writing this manuscript. All authors have contributed equally in the writing of this manuscript.

Acknowledgements

This project research was funded by Budget Implementation Check List of Research Organization for Life Sciences and Environment, National Research and Innovation Agency of Indonesia (BRIN) Fiscal Year 2023.

References

Adebo, A.O., Medina-Meza, I.G. 2020. Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: a mini review. *Molecules*, **25**, 297.

Aditya, I.R.A., Purwanto, H. 2023. Molecular detection of the pathogen of *Apis mellifera* (Hymenoptera: Apidae) in honey in Indonesia. *Biodiversitas Journal of Biological Diversity*, **24**, 2612-2622.

Almasaudi, S. 2021. The Antibacterial activities of honey. *Saudi Journal of Biological Sciences*, **28,** 2188-2196.

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R., Koirala, N. 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, **8**,1-12.

Aydogan-Coskun, B., Coklar, H., Akbulut, M. 2020. Effect of heat treatment for liquefaction and pasteurization on antioxidant activity anphenolic compounds of astragalus and sunflower-cornflower honeys. *Food Science and Technology*, **40**, 629–634.

Ayoub, W.S., Ritu, Z.I., Dar, A.H., Farooq, S., Mir, T.A., Ganaie, Srivastava, S., Pandey, V.K., Altaf, A. 2023. Exploiting the polyphenolic potential of honey in the prevention of chronic diseases. *Food Chemistry Advances*, **3**, 1-16.

Badan Standardisasi Nasional (BSN). 2018. *SNI 8664.2018 Madu*, Badan Standardisasi Nasional, Jakarta.

Baglio, E. 2018. Honey: processing techniques and treatments. In *Chemistry and technology of honey production*, SpringerBriefs in Molecular Science, Springer, Cham, pp 15-22.

Becerril-Sanchez, A.L., Quintero-salazar, B., Dublán-garcía, O., Escalona-Buendía, H.B. 2021. Phenolic compounds in honey and their relationship with antioxidant activity, botanical origin, and color. *Antioxidants*, **10** (11), 1-23.

Biluca, F.C., de Gois, J.S., Schulz, M., Braghini, F., Gonzaga, L.V., Maltez, H.F., Rodrigues, E., Vitali, L., Micke, G.A., Borges, D.L.G., Costa, A.C.O., Fett, R. 2017. Phenolic compounds, antioxidant capacity and bioaccessibility of minerals of stingless bee honey (Meliponinae). *Journal of Food Composition and Analysis*, **63**, 89–97.

Boonsong, S., Klaypradit, W., Wilaipun, P. 2016. Antioxidant activities of extracts from five edible mushrooms using different extractants. *Agriculture and Natural Resources*, **50**, 89–97.

Braghini, F., Biluca, F.C., Gonzaga, L.V. , Kracik, A.S., Vieira, C.R.W., Vitali, L., Micke, G.A., Costa, A.C.O., Roseane, O.F. 2020. Effect of different storage conditions on physicochemical and bioactive characteristics of thermally processed stingless bee honeys. *LWT*, **131**, 1-8.

Buchori, D., Rizali, A., Priawandiputra, W., Raffiudin, R., Sar-tiami, D., Pujiastuti, Y., Jauharlina, Pradana, M.G., Meilin, A., Leatemia, J.A., Sudiarta, I.P., Rustam, R., Nelly, N., Lestari, P., Syahputra, Hasriyanti, E., Watung, J.F., Daud, I.D.A., Hariani, N., Johannis, M. 2022. Beekeeping and managed bee diversity in Indonesia: perspective and prefer-ence of beekeepers. *Diversity*, **14**, 1-14.

Chen, L., Cao, H., Huang, Q., Xiao, J., Teng, H. 2022. Absorp-tion, metabolism and bioavailability of flavonoids: a review. *Critical Review in Food Science and Nutrition*, **62**, 7730–7742.

Chuttong, B., Chanbang, Y., Sringarm, K., Burgett, M. 2015. Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand). *Food Chemistry*, **192**, 149–155.

Cianciosi, D., Forbes-Hernández, Y.T., Afrin, S., Gas-parrini, M., Reboredo-Rodriguez, P., Manna, P.P., Zhang, J., Bravo-Lamas, L., Martínez-Flórez, S., Agudo-Toyos, P., Quiles, J.L., Giampieri, F., Battino, M. 2018. Phenolic compounds in honey and their associated health benefits: a review. *Molecules*, **23**(9), 2322.

Codex Alimentarius Commission (CAC). 2022. Standard for honey CXS 12-198, adopted in 1981, revised in 1987 and 2001, amended in 2019 and 2022; World Health Organization; Food and Agriculture Organization of the United Nations: Rome.

Dawidowicz, A.L., Olszowy, M. 2012. Mechanism change in estimating of antioxidant activity of phenolic compounds. *Talanta*, **97**, 312–317.

Eshete, Y., Eshete, T. 2019. A review on the effect of processing temperature and time duration on commercial honey quality. *Madridge Journal of Food Technolpgy*, **4**, 159-163.

Fu, C., Yan, F., Cao, Z., Xie, F., Lin, J. 2014. Antioxidant activities of kombucha prepared from three different substrates and changes in content of probiotics during storage. *Food Science and Technology*, **34**, 123–126.

Gulcin, I., Alwasel, S.H. 2023. DPPH radical scavenging assay. *Processes*, **11**, 1-20.

Handayani, T.H., Budiman, M.A., Amalia, R.L.R., Pribadi, A., Elfirta, R.R., Ferdian, P.R. 2022. Antioxidant activity, total phenolic and total flavonoid of Apis mellifera honey from Acacia crassicarpa forest-Riau, Indonesia with several drying treatments. *Jurnal Biologi Indonesia*, **18**, 231–243.

Islam, A., Khalil, I., Islam, N., Moniruzzaman, M., Mottalib, A., Sulaiman, S.A., Gan, S.H. 2012. Physicochemical and antioxidant properties of Bangladeshi honeys stored for more than one year. *BMC Complementary and Alternative Medicine*, **12**, 1–10.

Jahan, N., Islam, M.A., Alam, F., Gan, S.H., Khalil, M.I. 2015. Prolonged heating of honey increases its antioxidant potential but decreases its antimicrobial activity. *African Journal of Traditional, Complementary and Alternative Medicine*, **12**, 134–144.

Jan, R., Khan, M., Asaf, S., Lubna, Asif, S., Kim, K.M. 2022. Bioactivity and therapeutic potential of kaempferol and quercetin: new insights for plant and human health. *Plants*, **11**, 1-18.

Kahono, S., Chantawannakul, P., Enge, M. 2018. Social bees and the current status of beekeeping in Indonesia. In *Asian Beekeep 21st Century*, Chantawannakul, P., Williams, G., Neumann, P. (eds), Springer, Singapore, pp 287-306.

Kaligis, M., Mokosuli, Y. 2022. Characteristics and flavonoid content of honey Apis dorsata binghami from The Manembo Forest of South Minahasa. *Jurnal Biologi Tropis*, **22**, 1420–1430.

Kusmana, C., Hikmat, A., 2015. The Biodiversity of flora in Indonesia. *Jurnal Pengelolaan Sumberdaya Alam dan Lingkungan*, **5**(2), 187-198.

Leyva-Daniel, D.E., Alamilla-Beltrán, L., Villa-lobos-Castillejos, F., Monroy-Villagrana, A., Jimé-nez-Guzmán, J., Wel-ti-Chanes, J. 2020. Microfluidization as a honey processing proposal to improve its functional quality. *Journal of Food Engineering*, **31**, 17-36.

Maeng, J.H., Shahbaz, H.M., Ameer, K., Jo, Y., Kwon, J.H. 2017. Optimization of microwave-assisted extraction of bioactive compounds from Coriolus versicolor mushroom using response surface methodology. *Journal of Food Process Engineering*, **40**, 1-9.

Martinello, M., Mutinelli, F., Zooprofilattico, I., Delle-Venezie, S. 2021. Antioxidant activity in bee products: a review. *Antioxidants*, **10**, 1-42.

Masad, R.J., Haneefa, S.M., Mohamed, Y.A., Al-Sbiei, A., Bashir, G., Fernandez-Cabezudo, M. J., Al-Ramadi, B.K. 2021. The immunomodulatory effects of honey and associ-ated flavonoids in cancer. *Nutrients*, **13**(4), 1-15.

Pekal, A., Pyrzynska, K. 2015. Effect of pH and metal ions on DPPH radical scavenging activity of tea. *International Journal of Food Science and Nutrition*, **66**, 58–62.

Rachmawati, R.D., Agus, A., Umami, N., Agussalim., Purwanto, H. 2022. Diversity, distribution, and nest characteristics of stingless bees (Hymenoptera: Meliponini) in Baluran National Park, East Java, Indonesia. *Biodiversitas Journal of Biological Diversity*, **23**, 3890-3901.

Ranneh, Y., Ali, F., Zarei, M., Akim, A.M., Hamid, H.A., Khazaai, H. 2018. Malaysian stingless bee and Tualang honeys: a comparative characterization of total antioxidant capacity and phenolic profile using liquid chromatography-mass spectrometry. *LWT*, **89**, 1–9.

Šarić, G.,Vahčić, N., Kovačević, D.B., Putnik, P. 2020. The changes of flavonoids in honey during storage. *Processes*, **8**(934), 1-11.

Šarić, G., Marković, K., Vukičević, D., Lež, E., Hruškar, M., Vahčić, N. 2013. Changes of antioxidant activity in honey after heat treatment. *Czech Journal of Food Sciences*, **31**, 601–606.

Shahabuddin, M.M., Zailani, Z.A., Yusof, W.R.W., Ahmad, N.M. 2022. Effect of thermal treatment on kelulut honey towards the physicochemical, antioxidant and antimicrobial properties. *Borneo Journal of Resource Science and Technology*, **12**, 39–47.

Siddiqui, A.J., Musharraf, S.G., Choudhary, M.I., Rahman, A. 2017. Application of analytical methods in authentication and adulteration of honey. *Food Chemistry*, **15** (217), 687-698.

Tkáč, M., Vorlová, L., Borkovcová, I., Golian, J. 2022. Physicochemical and bioactive characterization of beekeeper and market honeys. *Emirates Journal of Food and Agriculture*, **34**, 268–278.

Trianto, M., Purwanto, H. 2022. Diversity, abundance, and distribution patterns of stingless bees (Hymenoptera: Meliponini) in Yogyakarta, Indonesia. *Biodiversitas Journal of Biological Diversity*, **23**, 695–702.

Trinh, V.K., Duyen, M., Nguyen, H.V. 2006. Effects of heating temperature, storage temperature, and storage time on total phenolic content and antioxidant capacity of Vietnamese honey. *7th Analytica Vietnam Conf*, 234–242.

Turkmen, N., Sari, F., Poyrazoglu, E.S., Velioglu, Y.S. 2006. Effects of prolonged heating on antioxidant activity and colour of honey. *Food Chemistry*, **95**, 653–657.

Weis, W.A., Ripari, N., Conte, F.L., Hanorio, M.S., Sartori, A.A., Matucci, R.H., Sforcin, J.M. 2022. An overview about apitherapy and its clinical applications. *Phytomedicine Plus*, **2**, 1-20.

Zammit, Y.G.W., Blundell, R. 2023. A review on the phytochemical composition and health applications of honey. *Heliyon*, **9**, 1-10.

Zarei, M., Fazlara, A., Tulabifard, N. 2019. Effect of thermal treatment on physicochemical and antioxidant properties of honey. *Heliyon*, **5**, 1-6.