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Identification of Primary and Secondary Metabolites of *Apis cerana* Honey using FTIR-ATR Diamond Spectroscopy and Their Botanical Origin

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Abstract

Apis cerana Fab. is one of the popular honeybees species among beekeepers in Indonesia. This species is easy to care for and produces valuable honey products. Honey from *A. cerana* is abundantly available in traditional and modern markets in Indonesia. The purpose of this study was to identify the primary and secondary metabolites in the honey produced by *A. cerana* using FTIR-ATR Diamond spectroscopy. Twelve samples of honey from three provinces in Java Island were used in this study. In general, all honey samples contained protein, carbohydrate, water, alcohol, cellulose, alkaloid, tannin, and flavonoid. Variation on primary and secondary metabolites in honey samples was strongly affected by the botanical origin, geographical origin, and the local condition around beekeeping areas where the honeycombs were placed.

Keywords: *Apis cerana*; biochemical characterization; honey; spectroscopy analysis.

INTRODUCTION

Honey is one of the foodstuffs containing many nutrients, such as carbohydrates, proteins, fats, and vitamins (Kasprzyk et al., 2018). Honey has been produced, marketed, and sold as healthy food and for traditional medicine around the world (Formosa et al., 2020). Honey is nutritious food safe for consumption by various age groups, ranging from one year old babies to the elderly. In addition, the antioxidant and antibacterial properties of honey make it a great food supplement for human health. Honey also contains vitamins, enzyme, and secondary metabolites such as flavonoid, phenolic, and tannin (de Almeida-Muradian, 2014). In fact, these chemical components affect its medicinal properties as well as the quality, granulation, texture, and shelf life of honey (Adeniyi et al., 2014; Joshi, 2008).

The chemical composition of honey as source of natural products is determined by various variables, such as the floral origin, the climate, environmental and seasonal conditions during the period of beekeeping, and the processing methods from the time of harvesting until its storage (Silva et al., 2009). Indeed, honey produced by various honeybees species differs not only in their chemical properties, physical properties, and the taste, but also in their biological activities (Cimpoiu et al., 2012). Honey that has many health benefits is produced by bees who take the best natural ingredients in the form of nectar and pollen from various flowers. Honey found on the market in Indonesia comes from various species of bees, including *Apis cerana*, *A. mellifera*, *A. dorsata*, and *Trigona* spp.. Some of these honey bees species are kept on farms, but some live in the wild and their honey is harvested from the forest. In this work, we focused our study on the honey produced by *Apis cerana.*

A. cerana is one of the most widely bred honey bees species in Indonesia because it is suitable for living in humid rain forests and dry grasslands in the tropics (Koetz 2013; Radloff et al., 2010). *A. cerana* does not require a large area for its maintenance and does not need to be fed outside the nest like *A. mellifera*. This species is also known as a non-aggressive bee species compared to *A. mellifera* and *A. dorsata*. Although the honey produced by *A. cerana* is not as much as the other two *Apis* species, this species has good potential to be developed in smallholder beekeeping and large scale farms (Theisen-Jones and Bienefeld, 2016).

The criteria to assess the quality of pure honey determined by the Indonesian Standardization Agency are mainly based on physical characteristics. The agency does not include nutritional content as standards for honey marketed in Indonesia. Considering that honey is generally consumed as a health supplement, its

biochemical characterization including the content of primary and secondary metabolites is important to study.

Currently a more detailed analysis of honey quality has been carried out by researchers around the world (Kasprzyk et al., 2018). The analysis includes biological testing, such as identifying the type and amount of pollen contained in honey which become important factor in determining the quality of honey. This is because pollen contains more complex nutrients than nectar as the major plant-based substances to produce honey. Currently the testing for honey quality is gaining attention by the community. For example, in Poland, honey quality testing was carried out by calculating the amount of pollen and comparing the chemical compounds of the original pollen with the pollen present in honey using the Fourier Transform Infra-Red (FTIR) analysis using attenuated total reflection (ATR) diamond method (Kasprzyk et al., 2018). A similar method has also been used in Croatia, where the quality of honey is determined from the comparison of chemical compounds of honey with chemical compounds of pollen and nectar using FTIR-ATR (Svečnjak et al., 2017).

The FTIR-ATR diamond is a type of spectroscopy method that uses infrared waves to identify a compound or molecule present in living or dead samples (Barth 2007). This type of spectroscopy is very sensitive to the chemical composition and arrangement of a molecule, both proteins and molecular structures that are larger than proteins. The current use of FTIR-ATR diamond includes the analysis of the chemical structure of living things and the compounds contained in them. One of the application of this method is the qualitative and quantitative analysis of chemical components in honey for determination of botanical or geographical origin, and detection of adulteration (Saksangawong et al., 2021). This method is a new, rapid, effective, non-destructive, and cost-effective for analysis honey components (Riswahyuli et al., 2020), and therefore suitable to be applied for quality assessment of Indonesian honey.

This study aims to determine the primary and secondary metabolites in A. *cerana* honey samples from Java Island, Indonesia. Results of this study provide important information on the biochemical content of *A. cerana* honey in Indonesian market, and thus bring beneficial impact for the community as well as for marketing purposes.

MATERIALS AND METHODS

Fresh honey samples were collected from 12 *A.cerana* farming locations covering nine districts in three provinces of Java Island (Figure 1) namely, Kulonprogo and Gunung Kidul (Yogyakarta Special Province); Cilacap, Solo, Magelang, Pati, Boyolali, and Karanganyar (Central Java Province); and Depok (West Java Province). During the visit for collecting honey samples, supporting information from the farmers was noted including the beekeeping management, methods of honey harvesting, and the blooming flowers during the recent seasons. Honey samples were stored in a refrigerator at 4^{o}C to maintain their quality prior to FTIR-ATR analysis.

The primary and secondary metabolites content of honey samples was analyzed using FTIR-ATR diamond (Alpha Bruker). For the analysis, 1 mL of honey was put in the sample holder. The screening was carried out from the wave number of 4000 to 400 cm^{-1}. The results of the analysis are peak graphs which were then validated according to the provisions as presented in Table 1.

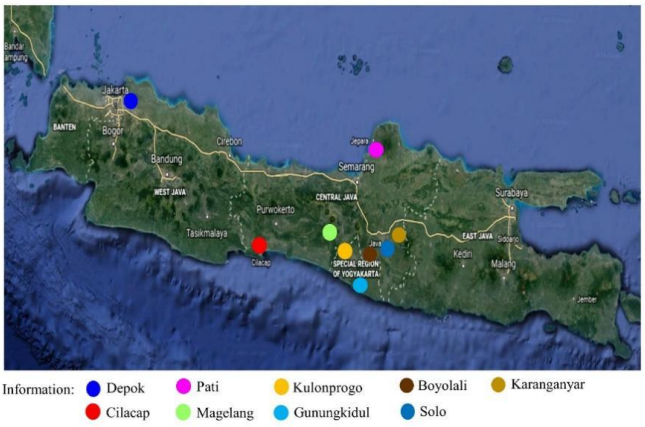

Figure 1. Map of honey sampling locations (Source: Google Earth).

Table 1. Interpretation of the results of FTIR-ATR diamond analysis (Kasprzyk et al., 2018).

Wave number (cm^{-1})	Vibrations
1000-1200	C–O and C–C stretching vibrations
1200-1350	N–H deformation and C–N stretching vibrations from amide III
1370-1420	C–H deformation vibrations of lipids and cellulose
1540	N–H deformation and C–N stretching vibrations from amide II
1650	C=O stretching vibrations from amide I
1740	C=O stretching vibrations from lipids
2850-3000	C–H stretching vibrations from cellulose and lipids
3250	O–H stretching vibrations from water
3300-3500	N–H stretching vibrations from protein

RESULTS AND DISCUSSION

Visual observations on honey samples and vegetation surrounding *A. cerana* nests

Honey samples from nine districts in three provinces in Java Island were all classified as multifloral honey based on the composition of pollen types identified using melissopalynological analysis (Lestari, 2019). Honey

samples showed variation in color as shown in Table 2. Differences in the color might be attributed to the differences in pollen and nectar sources.

Table 2. Honey samples from *A. cerana* used in this study.

Sample code	Color	Honey category	Origin of sample
AH-01	Yellow	Multifloral	Pati, Central Java
AH-02	Orange-brown	Multifloral	Boyolali, Central Java
AH-03	Orange	Multifloral	Karanganyar, Central Java
AH-04	Orange	Multifloral	Kulonprogo, Special Region of Yogyakarta
AH-05	Yellow	Multifloral	Karanganyar, Central Java
AH-06	Yellow	Multifloral	Gunungkidul, Special Region of Yogyakarta
AH-07	Yellow	Multifloral	Magelang, Central Java
AH-08	Brown-red	Multifloral	Solo, Central Java
AH-09	Dark brown	Multifloral	Cilacap, Central Java
AH-10	Light orange	Multifloral	Solo, Central Java
AH-11	Yellow	Multifloral	Magelang, Central Java
AH-12	Orange-brown	Multifloral	Depok, West Java

General features of vegetation in each location from which honey samples were collected is shortly described as follows. Sample AH-01 was labelled as Randu Tanjung Sari Honey from Pati, Central Java. The location of the *A. cerana* nests were in the field near the beekeeper's house with a white silk-cotton tree (*Ceiba pentandra*) grows nearby. Sample AH-02 was known as Dasa Dharma Honey from Boyolali, Central Java. Based on direct observation, the honeybee nests were placed in a garden area of about 1 hectare next to the house. Various plants species were found in the garden such as longan tree (*Dimocarpus longan*), mango tree (*Mangifera indica*), and bitter bean tree (*Parkia speciosa*). In addition, around the farmer's house there is an area of maize (*Zea mays*) and rice (*Oryza sativa*) fields as well as residential areas with small garden commonly found in front of the houses.

Sample AH-03 was collected from Matesih, Karanganyar, Central Java. The honeybee nests were placed in the house yard, where the farmer's house is bordered by residential areas and plantations of woody plants such as Chinese albizia (*Albizia chinensis*) and teak (*Tectona grandis*). Sample AH-04 was originated from Jatimulyo Village, Kulonprogo, Yogyakarta. The honey was produced by honey bees whose nests were placed in the farmer's house yard adjacent to the community forest with various plants species such as *Albizzia procera* and *Dioscorea japonica.* Meanwhile, sample AH-05 was called as Kaliandra honey obtained from Jumapolo, Karanganyar, Central Java. Honeybee nests were placed in the house yard surrounded by residential areas (villages), rice fields, gardens, and cemeteries. Sample AH-06 was commercially labelled as Sari Alami Honey collected from Kedung Poh village, one of the ecotourism villages in Gunungkidul, Yogyakarta. This village is located adjacent to the community forest, and is a well-known beekeeping ecotourism area for educational purposes in Yogyakarta. Honeybee nests were placed around the residents' yards. Major plant species found in the community forest are *Acer serrulatum, T. grandis*, and *C. pentandra.*

Sample AH-07 labelled as Kaliandra Tanjung Sari Honey was collected from Magelang, Central Java. The honeybees producing this honey were reared and fed on *Calliandra* plantation in Menoreh Hill. Sample AH-08 was collected from Jebres, Solo, Central Java. The honeybee's nests were placed in farmer's house yard, which is in a densely populated residential area near Jebres Railway Station where various flowering plants in grow in the area, including frangipani (*Plumeria alba*), guava (*Psidium guajava*), *Acacia glomerosa*, and Jamaica cherry tree (*Muntingia calabura*). Meanwhile, the honey sample AH-09 was obtained from Cilacap, Central Java. The honeybee's nests were placed in the garden of the farmer's house which is close to urban area. The plants found around the nest were *Desmodium paniculatum, Panicum grande,* dan *Cassia obtusifolia*.

Sample AH-10 is honey marketed with a label of Sekarpace Honey from Solo, Central Java. The nests of *A. cerana* were placed in the house yard where *M. calabura,* mango (*M. indica*) and queen's jewels vine (*Antigonon leptopus*) were planted. The beekeeping area is densely populated settlements in the city and close to public cemetery where frangipani (*P. alba*) dominated the area. Based on information from the farmers, various flowering and fruit plants such as Jamaica cherry (*M. calabura*) and common ornamental vines such as queen's jewels (*A. leptopus*) are deliberately planted to improve the quality of honey. Sample AH-11 from Magelang, Central Java was named as Insulin Honey. Based on observations during honey collection period, the honeybees producing insulin honey were reared in nests located in farmer's house yard close to Menoreh Hill, Magelang. The location of this bee farming is in a rural area close to community forests, rice fields, and vegetable gardens. In this area, Mexican sunflower

(*Tithonia diversifolia)* and red calliandra (*Calliandra calothyrsus*) are abundant. The honey sample AH-12 is marketed under the name of Organic Honey from Depok, Central Java. The location of honeybee's nests in this farm is in the gardens close to residential areas. Residents' gardens are planted with various plants such as cottonwood, bananas, and some woody plants such as *Acacia* sp., parasol leaf tree (*Macaranga tanarius*), and African tulip tree (*Spathodea campanulata*).

The FTIR-ATR profiles of *A. cerana* honey

The results of honey analysis with FTIR-ATR diamond in the form of infrared absorbance spectrum are presented in Figure 2 and summarized in Table 3 and 4. In the spectrum, the wavenumber indicates the number of infrared waves (the inverse of frequency) emitted to the sample. A chemical bond is detected as a band at a certain position that can be used to identify the type of chemical compound content, especially primary metabolites in honey. The intensity of the band represents the amount of absorbance that occurs after infrared passes through the sample. Band intensities greater than or equal to 0.1 will be read and represent a chemical compound content as listed in Table 1. In addition, the band intensities also show the quantity of chemical compounds in each honey sample.

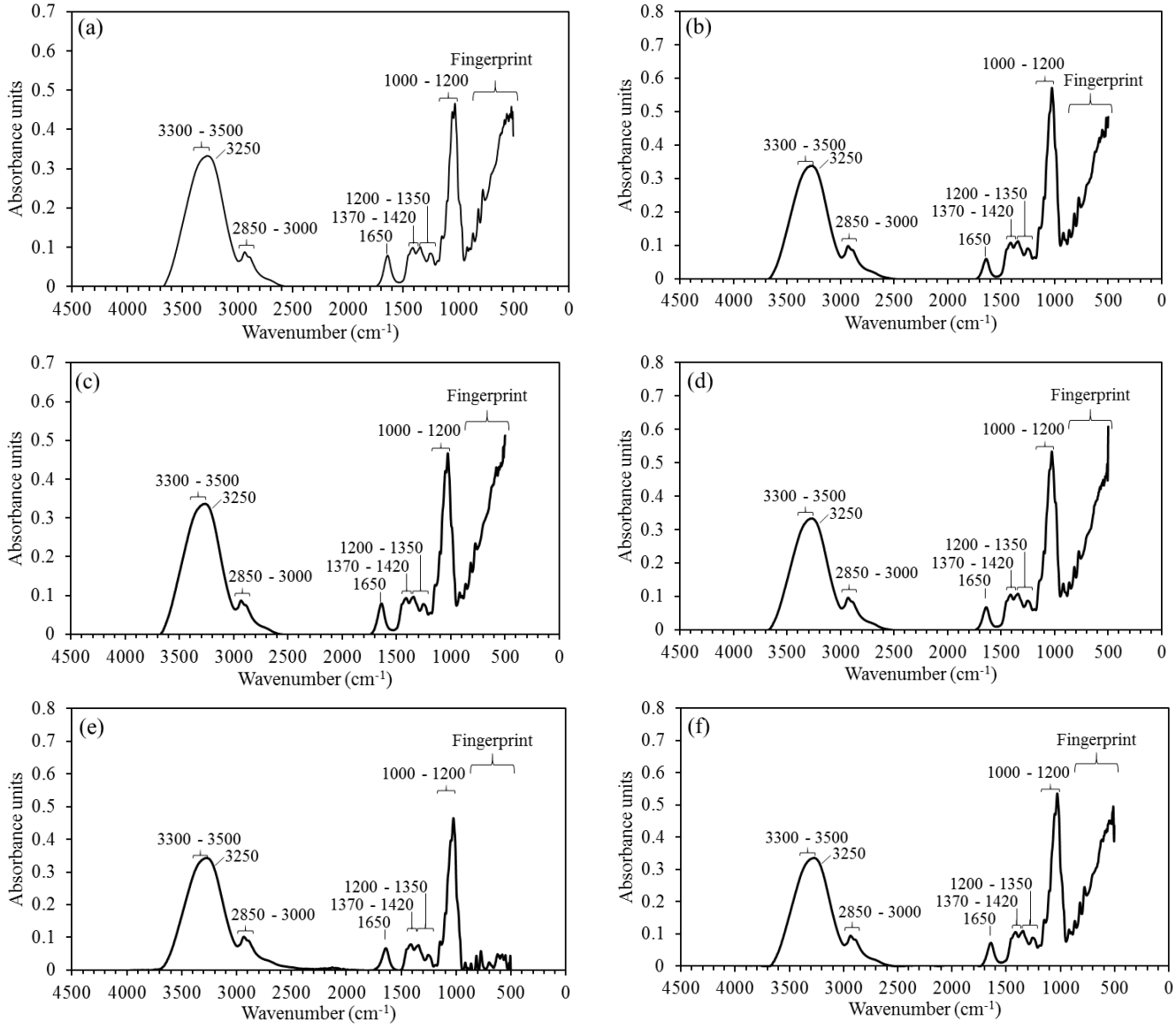

Figure 2. Results of FTIR-ATR diamond analysis.

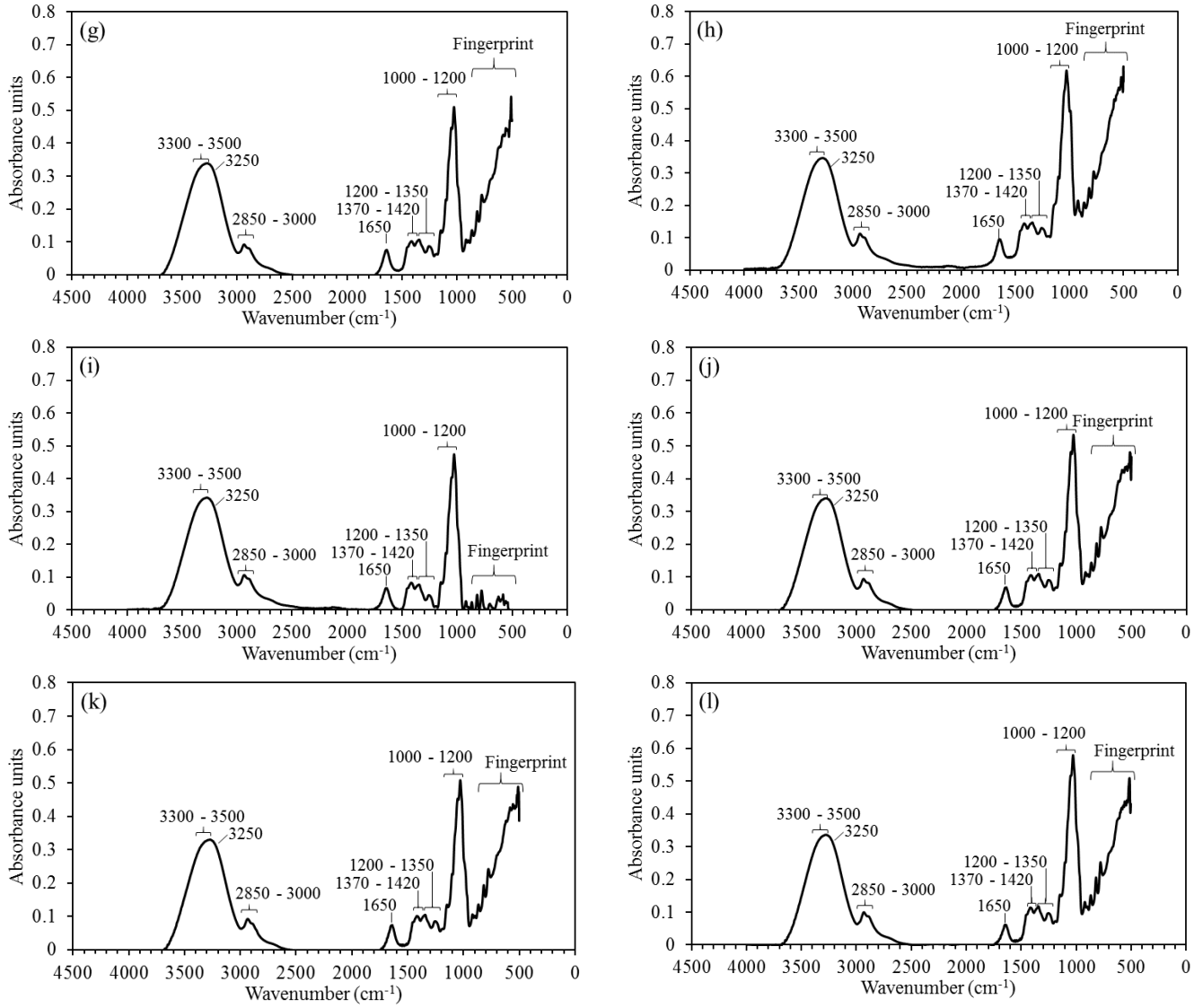

Figure 2. *Cont*.

Tabel 3. Summary of results of the FTIR-ATR diamond analysis for primary metabolites.

Wavenumber (cm^{-1})	Honey samples											
	AH-01	**AH-02**	**AH-03**	**AH-04**	**AH-05**	**AH-06**	**AH-07**	**AH-08**	**AH-09**	**AH-10**	**AH-11**	**AH-12**
1000-1200	125.93	**132.81**	127.77	133.37	15.36	129.32	128.81	175.27	14.31	129.51	127.49	132.37
1200-1350	48.03	56.06	47.04	53.51	41.07	53.13	50.71	**65.50**	41.28	53.41	50.89	57.56
1370-1420	11.66	**12.67**	10.97	12.42	6.48	12.35	12.01	18.81	6.28	12.46	12.06	13.51
1540	4.81	5.22	4.57	5.16	3.73	5.15	4.95	**7.16**	3.86	5.11	5.01	5.56
1650	×	×	×	×	×	×	×	×	×	×	×	×
1740	0.10	0.08	0.11	0.09	0.09	0.10	0.10	**0.13**	0.09	0.09	0.10	0.08
2850-3000	×	×	×	×	×	×	×	×	×	×	×	×
3250	10.92	11.85	10.86	11.82	**12.94**	11.65	11.35	14.14	13.43	11.48	11.43	12.07
3300-3500	0.47	0.47	0.47	0.47	0.48	0.47	0.48	**0.49**	0.48	0.48	0.46	0.47

Notes: the × sign: there is no FTIR-ATR diamond spectra in this wave.

Tabel 4. Secondary metabolites on honey samples detected by FTIR-ATR diamond and their comparison to results from previous studies.

Secondary metabolites	Honey samples												References
	AH-01	AH-02	AH-03	AH-04	AH-05	AH-06	AH-07	AH-08	AH-09	AH-10	AH-11	AH-12	
Alkaloid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Kavanagh *et. al*., 2019
Flavonoid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Li et al., 2018
Tannin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Malacarne et al., 2018
Terpenoid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Mashwani et al., 2016

The FTIR-ATR diamond has been widely used in the analysis of chemical compounds in honey. Several studies showed that FTIR-ATR was able to analyze secondary metabolite compounds in food products, such as honey (Tahir et al. 2017; Malacarne et al. 2018). Secondary metabolite compounds such as phenolics, flavonoids, tannins, alkaloids, and terpenoids can also be identified by representing the bands that appear on certain wavenumbers.

Bands at 1000–1200 cm^{-1} indicate that the honey sample contains C–O and C–C bonds. In infrared (IR) based analysis, the C–O bond is a representation of the alcohol group, so it is likely that in result of honey analysis the band at 1000–1200 cm^{-1} indicated the presence of alcohol group. The alcohol groups can cause bitter taste in honey with different levels of bitterness. In honey, the alcohol group most likely comes from phenolic compounds which have antibacterial properties (Kavanagh et. al., 2019). The band at 1200-1350 cm^{-1} contains deformed N–H bonds and C–N bonds of amide III compounds. According to Singh et al. (1993), amide III compounds detected using FTIR were used to determine the presence of secondary protein structures.

The band at 1370-1420 cm^{-1} represents the presence of deformed C–H bonds originating from lipids and cellulose. In honey, lipid commonly found at very low level, and this might be because honey contains antioxidants that has beneficial effect of lowering cholesterol level (Münstedt et al., 2009). In addition, it is most likely the band at 1370-1420 cm^{-1} also comes from cellulose as the main component of plant cell walls such as pollen or any plant tissue accidentally carried by the bees during their foraging activity. The presence of cellulose in honey samples might indicate the presence of pollen and thus indicates the authenticity of the honey. The band at 1540 cm^{-1} contains deformed N–H bonds and C–N bonds of amide II. In IR analysis, amide II compounds are used to determine whether a protein structure is unfolding. The unfolding condition of the protein is caused by heating. In this study, honey samples were not heated, so amide II compounds were not detected in the FTIR-ATR diamond for all honey samples.

The band at 1650 cm^{-1} represents the C=O bond of amide I. According to Singh et al. (1993), amide I compounds in FTIR analysis were used to determine the presence of secondary protein structures. However, amide I compounds are more complex than amide III, so the determination of secondary protein structure is more complicated. Figure 2.a shows that the absorbance value of amide I is lower than that of amide III, and these two compounds indicate the presence of secondary proteins in honey. The band at 1740 cm^{-1} indicates the presence of C=O bonds derived from lipids. High lipid levels in any food ingredients can cause health problems (Majid, et al., 2013). Interestingly, the results of the FTIR-ATR analysis of diamonds did not detect any lipid content or only a small amount of lipid content. This result indicated that all honey samples are of good quality and safe for health. The band at 2850-3000 cm^{-1} is a representation of the stretching C–H bonds derived from lipids and cellulose. This band is the same as the band at 1370-1420 cm^{-1} which indicates the presence of cellulose. Cellulose is a polysaccharide that has an asymmetrical and symmetrical structure (Dassanayake, et al., 2018). These two cellulose structures can be detected by FTIR-ATR diamond but based on the absorbance value, the band at 2850-3000 cm^{-1} is lower than the band at 1370-1420 cm^{-1}.

The band at 3250 cm^{-1} indicates the presence of O–H bonds from water. Moisture content is one of the indicator to determine honey quality because it is related to honey's resistance to decay, viscosity, and increased microbial contaminants (Prica, et al., 2014). In terms of microbial contamination, the presence of polluting microbes most likely comes from the equipment used for harvesting the honey. The microbial contamination is known to affect the hygiene of honey. The water content in honey might indicate the level of rainfall during honey production, because each harvest season may have different rainfall. Low water content generally makes honey become semi-solid or has relatively medium viscosity. Meanwhile, the band at 3300-3500 cm^{-1} represents the presence of N–H bonds of the protein. In this case, protein is known to be a primary metabolite that is important for the growth and development of body organs.

Compared to other studies on metabolite analysis of honey, the presence of band at 750-1800 cm^{-1} was reported by Devi et al. (2018), Anjos et al. (2015), and Se et al. (2018) which represent the presence of carbohydrates. The band at this spectrum could be used to identify different groups of carbohydrates, such as glucose, fructose, and sucrose. According to Se et al.

(2018), the band at 800-1500 cm^{-1} indicates the presence of monosaccharides and disaccharides. This is due to the presence of C–O and C–C bonds which are characteristic of carbohydrate structures (bands at 950-1200 cm^{-1}). Moreover, Se et al. (2018) noted that the bands at 1054 (C–OH: carbohydrate structure), 867, 822, and 779 cm^{-1} represent the presence of fructose; the bands at 1022, 991, and 898 cm^{-1} represent the presence of glucose; and the bands at 991 and 921 cm^{-1} represent the presence of glucose.

Based on the research of Tahir et al. (2017), the bands at 2800-3000 cm^{-1} represent C–H, O–H, and NH3 bonds indicating carbohydrates, carboxylic acids, free amino acids, and phenolics; the band at 1600-1700 cm^{-1} contains stretching bands of carbonyl groups, namely C=O and C=C which represent phenolic molecules; while the band at 1175-1540 cm^{-1} contains deformation bonds of O–H, C–O, C–H, and C=C representing flavonoids and phenolics; and the band at 940-1175 cm^{-1} contains C–OH groups, C–C and C–O represent carbohydrate structures, and C–O represents phenolics. Tannin compounds were also reported to be detectable in the band at 926–2955 cm^{-1} (Malacarne et al., 2018). Alkaloid compounds were detected by FTIR-ATR in the bands at 2300-3040 and 500-1750 cm^{-1} (Monfreda et al., 2015). Terpenoid compounds can also be detected with the presence of bands at 1591, 1390, and 1116 cm^{-1} which represent C═C and C═O bonds as functional bonds of terpenoids (Mashwani et al., 2016). Flavonoid compounds were detected by FTIR-ATR in the bands at 1734, 1627, 1522, 1440, 1410, 1367, 1315, and 1255 cm^{-1} (Li et al., 2018). Overall, based on the results of the analysis using FTIR-ATR diamond in this study, all honey samples contain chemical compounds that have bands at wavenumbers of 1000–1200, 1200–1350, 1370–1420, 1650, 2850–3000, 3250, and 3300–3500 cm^{-1}.

Concerning on differences of metabolites content in honey samples, the AH-08 honey has the highest alcohol group (band at 1000-1200 cm^{-1}) compared to other honey samples (Figure 2.h), while AH-01 (Figure 2.a); AH-03 (Figure 2.c); and AH-11 (Figure 2.k) had the lowest alcohol groups. This might be because the botanical source of AH-8 honey is dominated by Poaceae at the proportion 45% as showed from the pollen analysis reported by Lestari (2019). This indication was supported by the fact that location of the honey bee nests were close to the place where Poaceae plants grow. The presence of pollen of particular plant species might affect the content of chemical compounds in honey such as alcohol groups (Kasprzyk et al., 2018).

The honey samples AH-01 (Figure 2.a) and AH-08 (Figure 2.h) had the largest absorbance values in the bands at 1200-1350, 1370-1420, and 1650 cm^{-1} compared to other samples. This indicates that in both samples the content of amide III, cellulose, and amide I was higher than the other samples. Meanwhile, the amide II content in the band at 1540 cm^{-1} was not detected in most of honey samples. In AH-01 (Figure 2.a) and AH-08 (Figure 2.h), the peak appears with an absorbance value of 0.02, but this data did not indicate heating at the time of honey harvesting process. Instead, this is most likely due to the effect of light during storage. Honey sample AH-01 was stored in the front window of the house, so that it was exposed to sunlight, while honey sample AH-10 was stored inside the house in a densely populated residential area with open spaces that allowing unexpected heating occurred from direct sunlight.

The absence of amide II in all honey samples indicated that there was no heating process to reduce water content in honey. This result showed that all honey samples are of good quality because after the harvesting process the honey is immediately packaged in bottles, making it more natural and healthier. The absence of amide II compounds in honey is known to be affected by the water content in honey (Barth, 2007). Environmental changes, such as irregular rainfall intensity might cause the quality of honey to decline. This is due to the high water content, especially during high rainfall in particular times of honey harvesting seasons. The problem of high water content can be modified using unacceptable practices in honey processing by heating the newly harvested honey to reduce the water content. Heating process can damage the protein content in honey which affects the enzymes, and thus affect the quality of honey. The lipid content as indicated by the band at 1740 cm^{-1} was not detected in most honey samples except for AH-01(Figure 2.a) and AH-10 (Figure 2.h). In these honey samples, the band was detected with a fairly small intensity, namely 0.01. This result indicated that all honey samples have no or only a very small amount of lipid content.

Based on the results of this study and comparison to the honey analysis reported by Tahir et al. (2017), all honey samples contain phenolic compounds due to the appearance of bands at 2800-3000, 1600-1700, 1175-1540, 940-1175 cm^{-1}. In addition, all honey samples also contain tannin compounds, as indicated by the band at 926–2955 cm^{-1} which was in accordance to Malacarne et al., (2018). The presence of alkaloid compounds detected by the bands at 2300-3040 and 500-1750 cm^{-1} was in line with the study of Monfreda et al., (2015), while the presence of terpenoid compounds in the bands at 1591, 1390, and 1116 cm^{-1} was reported by Mashwani et al., (2016). The occurrence of flavonoid compounds as indicated by the bands at 1734, 1627, 1522, 1440, 1410, 1367, 1315, and 1255 cm^{-1} was in agreement with the study of Li et al. (2018). Results of FTIR-ATR diamond form honey samples in this study showed similar patterns to the analysis of pollen and honey using the same method as reported by Kasprzyk et al. (2018). Therefore, FTIR-ATR can be used as standard method in determining the quality of *A. cerana* honey.

Results of this study showed that all samples are pure honey without any additional ingredients, and showed good practices of honey production process since there are no indications of adding sugar in honey or feeding

the bees with corn flour. There re also no indication of heating the honey to produce a nice color and adding the water content to increase honey volume. The results of FTIR-ATR diamond analysis clearly showed that this spectroscopy method is useful in identifying primary and secondary metabolites as one of the quality indicators for *A. cerana* honey from Indonesia. The primary and secondary metabolites analysis using the FTIR-ATR diamond method is thus can be proposed as standard analysis in determining the quality of honey in Indonesia because this method is easy, cheap, and fast to get the results.

CONCLUSIONS

Honey produced by *A. cerana* contains various primary and secondary metabolites which include carbohydrates, proteins, cellulose, alcohol, alkaloid, tannin, flavonoid, and terpenoid. The results of FTIR-ATR diamond analysis indicated that 12 honey samples in this study showed variation in the quantitatively the amount of primary metabolites. Finally, given that the FTIR-ATR diamond is a simple method to practically evaluate the quality of the honey, it is suggested that the improvement of the FTIR-ATR diamond library of honey is important for quality assessment of honey.

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***Authors' Contribution*:** Tiffany Hanik Lestari collected honey samples, carried out laboratory work, and wrote the manuscript. Ratna Susandarini designed the study, interpreted results of FTIR-ATR spectroscopy, and wrote the manuscript. All authors read and approved the final version of the manuscript.

***Competing Interests*:** The authors declare that there are no competing interests in this study and publication.

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