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Characterization of Indonesia wild honey and its potential for authentication and origin distinction

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Abstract

Honey is a natural food derived from flowers nectar that has many health benefits. This reason made honey become one of category food product that has a risk to be adulterated because of economically motivation. This study was conducted for characterization and authentication of Indonesia wild honey (IWH) collected from seven geographical regions (Sumatra, Bangka Belitung, Java, Kalimantan, Sulawesi, West Nusa Tenggara, and East Nusa Tenggara) and harvested during 2016–2018 based on physicochemical parameters, sugar content, minerals, and antioxidant components. The study showed that the result differs widely among the type of honey. IWH has a moisture content between 16.52-33.41%, a pH value between 3.00 to 4.65 and color characteristic ranged from pale yellow to dark brown. All samples contain the highest amount in potassium, but several minerals found in the specific region. Evaluation of authenticity from sugar content data set by principal component analysis (PCA) and Linear Discriminant Analysis (LDA) revealed that the authentic and adulterated honey samples could be differentiated with a 95.5% accuracy. The honey samples were classified on their botanical and geographical origin using the antioxidant properties, and results of PCA and LDA demonstrated that the antioxidant parameters can provide adequate information to allow classification of the various types of IWH samples collected from different geographical regions with accuracy 80-100% for Bangka Belitung, Sulawesi, Kalimantan, West Nusa Tenggara, East Nusa Tenggara and Java island.

1. Introduction

Honey is a natural product obtained by honeybees from nectar or honeydew. It has been used as a valuable diet and medicine since ancient times. The medical and nutritional properties of honey have elicited increasing interest in extensive scientific research conducted in recent decades (Alvarez-Suarez *et al*., 2010; Horn, 2013; Al-Waili *et al*., 2013; Saranraj and Sivasakthi, 2018). In general, honey consists of sugar, water, and other substances such as proteins (enzymes), phenolic compounds, organic acids, pigments, vitamins, minerals, various volatile compounds, and solid particles (Pereira *et al.*, 2008; Alqarni *et al*., 2014; da Silva *et al*., 2016). The different properties of honey are associated with the various compounds derived from honey bees and plant sources, such as enzymes and phenolic compounds (Lachman *et al*., 2010; Kuś *et al*., 2014). Some of the properties of honey can confirm the quality and authenticity of honey and find similar characteristics in different types of honey using requirements such as purity, maturity, and deterioration (Silva *et al*, 2016). Honey can also be used as a bio monitor to provide information related to the environment where the bees live. Bees have contact not only with air but also with land and water, and the concentration of heavy metals such as lead and cadmium in honey could reflect the number of components present in the entire region (Ioannidou *et al*., 2005; Silici *et al*., 2008). Therefore, honey has been recognized as a biological indicator of environmental pollution.

The concentration of sugar in honey is generally approximately 83°Brix, or approximately 83% sugar (Ball, 2007). Depending on the source of honey, climatic conditions, and other factors, the moisture content of honey varies from 13.6% to as high as 23%. Fermentation is a natural process occurring in honey that

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can reduce its moisture content to <18%. Honey is acidic, but its high sugar content can suppress this taste; the average pH of honey is 3.9 (Ball, 2007). Another distinctive property of honey is its color. Mass-produced honey is a mixture prepared from several sources, so that it might a uniform color. However, individually harvested honey could have various colors depending on the season, the source of nectar, the age of honey (time between nectar collection and honey harvesting), and the product handling method after harvesting. Some researchers have argued that there are several possible factors responsible for the color variations of honey, such as carotene compounds, polyphenols, chemical caramelization of saccharide in the honey, and Maillard reaction between sugars and amino acids. However, the fact is whatever the source of the color, the darker the honey, the stronger the taste (Ball, 2007).

Limited availability and high price of a particular type of honey will generate economically motivated adulteration in honey products. Currently, the major concerns related to the authenticity of honey are focused on the geographic and botanical origin, but the addition of other substances that are generally not permitted, such as syrup and sugar, is also an important issue. Adulteration in terms of production includes the addition of sugar, thermal processing, and activities related to modifying its moisture content. The practice of counterfeiting such as feeding bees excessively with sucrose or other commercial sugars, harvesting before maturity, and the excessive use of veterinary drugs is still followed by some manufacturers around the world to meet the market demands (Bogdanov *et al*., 2004; Sahinler *et al*., 2004; Guler *et al*., 2007; Soares *at al*., 2017).

Previous studies have used existing information to develop authentication techniques combined with chemometrics due to certain positive aspects as follows: Minimal sample handling that would minimize the risk of contamination and problems associated with the viscosity of the sample. Rapid and simple. No need for a quantification process that involves creating a calibration curve. Determining the authentication of honey requires more than one variable such as the correlation among physicochemical properties (Corbella and Cozzolino, 2006; Yücel and Sultanoğlu, 2013), physicochemical properties and volatile components (Karabagias *et al*., 2014) and the antioxidant properties with mineral content (Alves *et al*., 2013), which requires performing multivariate analysis. In fact, an earlier study had used multivariate analysis in chemometrics to determine the variables having a high discriminant ability that contributed to the classification of honey (Nalda *et al.*, 2005).

Wild honey is produced in the forest by the bee *Apis dorsata* that feeds on the flowers of the forest trees and forms a nest on the branches of trees (Sarwono, 2003). *A. dorsata* is the largest among other honey bee species. This species is grown only in the subtropical and tropical Asian countries such as Indonesia, the Philippines, India, and Nepal and is not found out of Asia. This bee is the most productive in producing honey compared with other species, which constructs a single vertical comb that hangs on the branches and twigs of trees, open ceiling, and the cliff rocks. Indonesia wild honey (IWH) is generally known by names of a local origin, i.e., Sumbawa Honey, Pontianak Honey, Riau Honey, Flores Honey, Gunung Mutis Honey, Tessonilo Honey, and Lake National Park Sentarum. According to a study conducted by Sari and Bertoni (2014), some wild honey in Indonesia has various antioxidant and anticancer activities depending on the region. In the present study, we describe the results of the analysis of IWH and its properties and about the authentication process using the properties of honey. A number of previous studies have published multivariate techniques for honey authentication (Sivakesava and Irudayaraj, 2001; Nalda *et al*., 2005; Downey and Kelly, 2006; Gallardo-Velázquez *et al*., 2009; Siddiqui *et al*., 2017; Zhou *et al*., 2017; Boussaid *et al*., 2018; El-Haskoury *et al*., 2018; Ma *et al*., 2019). We believe that this study is important in identifying the differences in IWH and the previously investigated honey samples and in expanding the methods to tackle the problems of national-scale adulteration and mislabeling of honey in Indonesia.

2. Materials and methods

2.1 Samples and sampling

Honey sample was collected from 2017 to 2019 from 7 different forest areas, including Sumatra (Tesso Nilo, Riau and Gunung Kerinci National Park, Jambi), Bangka -Belitung (Pelawan Tourism Forest, Namang, Bangka Tengah, and Belitung Island), Java (Ujung Kulon National Park/Panaitan island, Banten), Kalimantan (Sentarum Lake National Park, Kapuas Hulu, West Kalimantan), West Nusa Tenggara (NTB; Sumbawa subdistrict, West Nusa Tenggara), East Nusa Tenggara (NTT; Muntis Mountain Conservation, South Central Timor and Maumere, Flores Timor), and Sulawesi (North Morowali, Central Sulawesi, Manado, North Sulawesi, and Tulak Tallu village, North Luwu, south Sulawesi (Figure 1)). Their authenticity and freshness were assured by collecting them directly from the forest areas, local beekeepers under the Indonesia Wild Honey Association Once received, honey samples were stored in clean, closed jars at room temperature and in the dark until use. Additionally, some honey samples were purchased from Indonesia Wild Honey Network, which

guarantees authenticity. The adulterated honey samples also prepared by adding of commercial sugars in Indonesia (aren, palm, and cane sugar solutions).

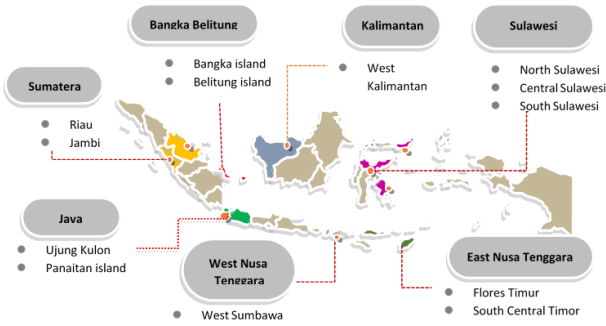

Figure 1. Indonesia wild honey source regions

2.2 Physicochemical analysis

2.1.1 Determination of color

The determination of color was carried out as described by Karabagians *et al*. (2014). Honey solution (10 g in 75 mL CO_2-free water) was taken in an optical cylindrical cell (base diameter 11.3 cm and height 2 cm). The parameters of color: L*, a*, b* (L* indicated Light/ bright, a* indicated the coordinates of the red/green, and b* indicated the coordinate of yellow/blue) measured by colorimeter Chroma Meter CR 410, Konica Minolta, Tokyo, Japan.

2.1.2 Determination of moisture content

The moisture content was determined using a refractometer (N3 Atago, Japan) at a temperature of 20° C on the basis refractive index using Chataway Charts through the formula:

$$W = \frac{1.73190\text{-}\log(\text{R.I-}1)}{0.002243}$$

W is the moisture content in g per 100 g honey and R.I. is the refractive index (Stefan, 2009)

2.1.3 Determination of pH

pH was measured at levels of 10% of honey solution in water using a pH meter (Mettler Toledo, Ohio, US) (Bogdanov, 2009).

2.2 Determination of the content of minerals and metals

The concentrations of various minerals and metals, lithium, beryllium, boron, potassium, magnesium, aluminum, potassium, calcium, chromium, manganese, iron, cobalt, nickel, copper, zinc, gallium, selenium, strontium, cadmium, tellurium, barium, mercury, thallium, lead, and bismuth were measured using inductively coupled plasma mass spectrometer (ICP-MS) *Agilent 7700* single quadrupole, with helium and no gas modes (Batista *et al*., 2012). Before analysis, the honey samples were homogenized by agitation and heating at a temperature of 65°C in a water bath for 30 mins to dissolve the sugar crystals contained in the honey. Then, 1 g of the homogenized honey was weighed accurately and 1 mL demineralized water was added and shaken (deionized water of 18 MΩ cm^{-1} resistivities, obtained from a Milli-Q system (Millipore), was used to prepare all solutions). Next, 0.4 mL of the honey solution was transferred to another container and 0.2 mL of 14 mol/L HNO_3 (Merck) was added and incubated for 20 mins. This was followed by adding 10 mL of demineralized water and measuring using the Agilent ICP-MS. Mineral concentration in the honey determined by comparing with the standard curve using a mix of minerals and metals (ICP *multi-element standard* solution *XXI* for MS Certipur®, MerckMilipore) (0.1–25 ng/mL).

2.3 Statistical and multivariate analysis

Data processing was performed using Minitab® 18.1 (Minitab Inc., State College, Pennsylvania, USA). The Pearson correlation was calculated to determine the relationship properties. The level of significance was set at $p<0.05$. Unsupervised and supervised pattern recognition techniques such as PCA and LDA were used to visualization and discrimination between authentic and adulterated honey or between honey samples of different floral origin.

3. Results and discussion

3.1 Physicochemical properties

3.1.1 Color

The results of color analysis of the wild honey samples collected from seven regions in Indonesia demonstrated high variability ranging from a light tone to an almost black yellow tone, with the L * value in the range of 43.65–59.77, the b * value in the range of 1.28–7.69, and the c * value in the range of 0.19–14.26 (Table 1). Color is one of the most varied parameters, and it is primarily determined by the floral origin. It also depends on the ash content, the temperature when honey stored in the honeycomb, and the storage time (Bertoncelj *et al*., 2007; Gámbaro *et al*, 2007). In this study, based on the region of origin, it was observed that the honey collected in Java had a degree of variation of L with the lowest value, and the honey in this area was brightly colored compared to some of the darker honey samples collected from areas such as Bangka Belitung and Sulawesi. Boussaid *et al.* (2018) evaluated six honey samples collected from different areas of Tunisia and found the values of light (L*) ranging from 36.64 to 51.37. Color is the first component of interest of honey and a very important parameter in terms of quality, perception, and preference of the consumer. Color measurement made by colorimetric technique using a standard CIE L * a * b *

Table 1. Physicochemical parameters of Indonesia wild honey

Parameters (Mean)	Sample Origin								
	Bangka-Belitung	Sulawesi	Kalimantan	Sumatera	West Nusa Tenggara	East Nusa Tenggara	Java	Average	Range
Color									
L*	54.47	53.31	55.46	50.8	52.56	53.62	58.5	54.10±2.25	43.65–59.77
a*	3.46	2.22	2.34	3.23	2.5	1.99	0.08	6.36±4.02	1.28–7.69
b*	3.46	8.82	7.44	13.74	2.98	7.24	0.82	2.57±0.51	0.19–14.26
Moisture content (%)	29.2	19.75	19.14	20.76	26.76	24.2	24.33	24.84±4.12	20.32–31.9
pH	3.96	3.89	3.92	3.75	4.42	4.01	3.86	3.75±0.71	3.08–4.65

or sometimes abbreviated simply as "Lab color space." In 1976, the International Commission on Illumination (CIE) had defined CIELAB color space expressing the following three values: L * for light from black (0) to white (100), a * for green (-) to red (+), and b * for blue (-) to yellow (+). In practice, space is generally mapped to the integer of three-dimensional space to a digital representation, and thus the values of L *, a *, and b * are generally absolute, with a range that has been predetermined. In several countries, the price of honey is associated with a color. In general, light-colored honey has a higher price, although dark honeys are appreciated in certain areas (Tuberoso *et al*., 2014). The dark color of honey might develop during storage and might also be associated with storage temperature and composition of the honey. In a correlation study of IWH, the color appeared to have a significant correlation with flavonoid content (Table 2), this might the reason why honey from Sumatera or Sulawesi are darker than Kalimantan or Java. IWH from Sumatera or Sulawesi has total phenolic content and total flavonoid content higher than IWH from Java or Kalimantan (Riswahyuli *et al*., 2019).

3.1.2 pH

Natural honey are acidic with a pH value in the range of 3.5–5.5, which is caused due to the presence of organic acids that contribute to the taste of honey and provide protection against microbes (Bogdanov *et al*., 2004). The pH of IWH in this study ranged from 3.00 to 4.65, with an average of 3.75±0.71. Due to the low pH variation between regions, this parameter could not be used for distinguishing the types of honey; however, this study showed that adulterated honey has a higher pH value than authentic honey (Table 1).

3.1.3 Moisture content

Moisture content is one of the most important characteristics that affect the physical properties of honey such as viscosity and crystallization and other parameters such as color, taste, specific gravity, and solubility (Escuredo *et al*., 2013). The moisture content of IWH ranged from 20.32 to 31.9, with an average value of 24.84±4.12 (Table 1), although it exceeded the requirements set by national and international standards, the result indicates the natural condition in a tropical forest with high humidity. Another factor is rainy harvesting season, low in the degree of maturity reached in the hive and climatic factors. In this study, there is a correlation between pH and moisture content (Table 3) where the low moisture content makes the tendency of pH to be maintained low. Similar result was showed in previous studies on Nigerian honey (James *et al*., 2009)

Table 2. Correlations between Color, TPC, TFC, RSA, and FRAP

	L*	a*	b*	Total Phenolic	Total Flavonoid	% inhibition of DPPH
a*	-0.304					
	0.015					
b*	-0.513	0.279				
	0	**0.025**				
Total Phenolic	-0.519	0.012	0.172			
	0	0.926	0.182			
Total Flavonoid	-0.577	0.127	0.587	0.474		
	0	0.32	**0**	0		
% inhibition of DPPH	-0.381	-0.097	0.439	0.434	0.567	
	0.002	0.451	**0**	**0**	**0**	
FRAP	-0.376	-0.016	0.178	0.368	0.391	0.084
	0.002	0.899	0.163	**0.002**	**0.001**	0.494

Table 3. Correlation between physicochemical parameters

	L*	a*	b*	Moisture content	pH
a*	-0.327				
	0.001				
b*	-0.582	0.386			
	0	**0**			
Moisture content	-0.079	0.052	-0.033		
	0.445	0.62	0.75		
pH	-0.024	0.134	-0.323	0.305	
	0.877	0.402	**0.039**	0.044	
EC	-0.304	-0.129	0.164	0.145	0.131
	0.004	0.238	0.133	0.159	0.401

3.2 Minerals

Various groups of chemicals were detected in the different types of IWH samples in this study. These chemical groups included macro minerals and micro minerals which were different compared to the results of a previous study reported by Alqarni *et al.* (2014). A total of 22 minerals and 3 heavy metals were analyzed to investigate the characteristics of honey in each region. Calcium was predominantly found in all regions, followed by Mg and Ca (Table 4). Fe and Sr were found in abundance in the Bangka Belitung honey compared with other honey samples. Mn was predominantly found in Sulawesi honey, whereas honey collected from West Nusa Tenggara and East Nusa Tenggara contained more Ba than in any other region. Cu was found in abundance in Sumatra honey than in any other region. The concentrations of heavy metals (Hg, Pb, and Cd) in IWH still met the requirements. Bangka Belitung and Sulawesi honey had higher levels of B than those from other areas, Mg was found in honey samples collected from Sulawesi, NTT and NTB areas, Al was highly found in Bangka Belitung honey, Ca showed the least abundance in Sumatran honey, Fe was predominantly found in Bangka Belitung honey, and Cu was found in abundance in Sumatran honey. In general, honey reflects the chemical components of the plant from which honey bees collect their food; hence, the content of trace elements present in the honey depends on the type of soil in which plants and nectar are found and could disclose the botanical origin of the particular honey sample (Escuredo *et al*., 2013; Alqarni *et al*., 2014; Priscila Misio da Silva *et al*., 2016). Analysis of Bangka Belitung honey showed that it had a higher mineral content than honey collected from other areas because of the rich mineral content in the groundwater in that area. Nalda *et al.* (2005) identified different minerals in honey from Spain and found a higher concentration of manganese in the honey of heather and ling. (Ajtony *et al*., 2007) evaluated the mineral content of honey samples from Hungary and found the least concentrations of cadmium, chromium, copper, and lead in linden honey, whereas other types of honey showed a predominance of potassium, contributing to one-third of the total of the minerals contained in honey (Yücel and Sultanoğlu, 2013; Alqarni *et al*., 2014). In this study, some minerals showed a correlation with other parameters. The minerals Mg, Cr, Ni, Cu, Se, and Al significantly correlated with the color of honey. Previous research also reported that honey collected from fruits (avocado, chestnut, and heather) contained a number of major and minor metals and certain higher trace elements (e.g., Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Ni, and Zn) than pale honey; interestingly, the color of dark honey was typically related to the concentrations of Cd, Fe, and Pb, whereas bright honey and brown-color honey were associated with the concentrations of Al and Mg (Pohl, 2009).

3.3 Sugar

The sugar content in honey is represented by monosaccharides glucose and fructose (Kamal and Klein, 2011). The sugar content in IWH was generally composed of fructose, followed by glucose and sucrose, except for Bangka Belitung and Sumatran honey, which contained slightly higher glucose levels than fructose. The concentration ratio between honey fructose: glucose: sucrose was approximately 1:1:0.1. Kaškoniene and Venskutonis (2010) reported that the ratio between fructose and glucose concentrations was a useful indicator for the classification of monofloral honey. Commercial sugar has a different sugar content compared with honey, in which the sucrose concentration is much higher than that in other sugars. Sucrose was found in small amounts, but this type of sugar is often added to honey and is commonly found in adulterated honey (Figure 2). In this study of honey collected from the seven regions in Indonesia, it was found that the honey could be well characterized compared with adulterated honey added with commercial sugars, as indicated by the relatively less concentration of sugar than that in the adulterated honey. However, it

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Table 4. Minerals and heavy metals in Indonesia wild honey

Concentration (ng/g)	Bangka Belitung		Sulawesi		Kalimantan		Sumatra		West Nusa Tenggara		East Nusa Tenggara		Java	
	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD
7 Li [He]	0.62	0.05	0.50	0.10	0.18	0.01	4.26	2.19	1.48	1.38	0.70	0.44	0.67	0.40
9 Be [He]	0.01	0.00	0.00	0.00	0.00	0.00	0.04	.0.02	0.01	0.01	0.09	0.35	0.01	0.01
11 B [He]	435.13	21.18	418.04	91.51	82.57	20.74	181.71	89.59	190.90	151.68	229.91	205.31	111.23	30.86
24 Mg [No Gas]	3960.29	1671.02	21906.77	15071.44	1227.80	133.61	1132.44	921.46	4844.80	8482.04	4575.27	5733.18	985.21	78.11
27 Al [No Gas]	103.55	26.73	3.67	13.93	42.86	3.35	4.63	6.41	13.76	21.59	7.95	8.82	13.52	2.49
39 K [He]	26959.72	31559.21	82352.20	6204.67	48909.30	3144.88	61808.74	53975.89	89219.00	24099.67	39482.28	42155.45	28251.79	9426.14
43 Ca [No Gas]	1841.24	933.65	912.58	523.96	212.27	13.05	48.14	84.17	318.52	419.60	848.27	1187.71	198.14	14.81
44 Ca [He]	754.81	367.48	391.16	229.98	80.94	1.01	57.86	21.30	160.96	155.70	338.51	438,.1	103.30	20.71
52 Cr [No Gas]	26.43	8.74	18.87	2.46	20.71	2.51	17.73	1.92	16.27	3.59	13.73	6.88	15.66	2.27
55 Mn [He]	88.39	75.34	751.30	816.75	86.42	4.97	57.91	46.59	132.72	259.09	261.65	222.27	46.19	41.66
56 Fe [He]	369.73	264.04	54.87	14.80	5.94	3.86	54.86	21.45	27.45	10.57	30.72	23.07	24.95	12.44
59 Co [He]	0.56	0.20	0.39	0.05	0.29	0.00	0.58	0.25	0.35	0.09	0.30	0.27	0.25	0.17
60 Ni [He]	2.78	2.89	3.21	3.37	0.45	0.09	5.96	3.83	2.30	1.90	2.28	1.81	1.66	1.49
63 Cu [He]	2.95	0,65	19.04	4.40	2.67	0.07	44.29	39.63	22.18	12.52	6.48	3.86	4.02	0.86
66 Zn [He]	9.14	5,71	5.99	0.85	6.93	3.89	19.24	16.46	9.56	7.06	15.00	13.98	9.31	5.00
71 Ga [He]	0.05	0,01	0.07	0.07	0.01	0.01	4.35	2.00	3,42	2.48	0.10	0.27	2.43	2.20
78 Se [No Gas]	0.88	0,15	0.72	0.27	0.03	0.03	0.57	0.21	0.59	0.57	0.75	0.50	0.47	0.02
88 Sr [He]	41.09	30,87	2.76	0.88	6.18	0.32	10.89	6.91	23.34	11.04	18.99	11.19	10.99	2.88
111 Cd [He]	0.08	0,03	0.09	0.06	0.04	0.05	0.06	0.04	0.05	0.03	0.03	0.20	0.03	0.01
125 Te [No Gas]	0.01	0.01	0..01	0.01	0.05	0.04	0.03	0.01	0.02	0.01	0.08	0.24	0.01	0.00
137 Ba [He]	10.38	6.75	9.29	9.92	7.06	0.53	23.32	11.99	35.64	19.98	29.58	20.19	18.42	7.27
201 Hg [No Gas]	0.38	0.02	0.67	0.33	0.89	0.01	0.05	0.04	0.43	0.84	0.41	0.96	0.38	0.48
205 Tl [No Gas]	0.06	0.00	0.04	0.00	0.04	0.00	0.04	0.02	0.03	0.02	0.10	0.21	0.02	0.01
208 Pb [No Gas]	0.90	1.00	0.38	0.78	3.54	4.60	0.38	0.25	0.48	0.56	2.41	3.06	1.28	0.23
209 Bi [No Gas]	0.09	0.01	0.14	0.09	0.12	0.01	0.05	0.04	0.06	0.07	0.36	0.68	0.08	0.15

Table 5. Discriminant analysis with cross-validation for sugar content properties of Indonesia wild honey

Put into Group	True Group							
	Adulterated	Bangka	Java	Kalimantan	West Nusa Tenggara	East Nusa Tenggara	Sulawesi	Sumatera
Adulterated	42	2	0	0	0	0	0	5
Bangka Belitung	0	1	1	0	0	2	4	0
Java	0	0	2	2	4	6	0	0
Kalimantan	1	1	7	4	2	5	0	1
West Nusa Tenggara	1	1	4	0	12	2	1	4
East Nusa Tenggara	0	3	3	0	0	11	0	0
Sulawesi	0	3	0	0	2	0	10	0
Sumatra	0	1	1	0	1	0	0	20
Total N	44	12	18	6	21	26	15	30
N correct	42	1	2	4	12	11	10	20
Proportion	0.955	0.083	0.111	0.667	0.571	0.423	0.667	0.667

was difficult to distinguish the honey samples according to the region on the basis of sugar attributes, wherein the accuracy values were <70% (Table 5).

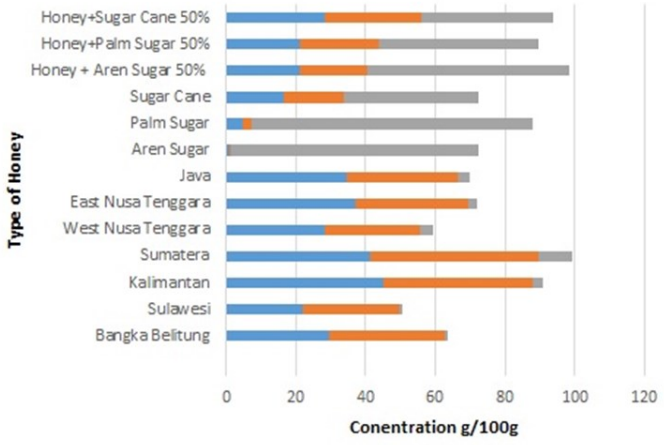

Figure 2. Sugar (Fructose, Glucose and Sucrose) in honey, adulterated honey, and commercial sugar.

3.4 Antioxidants

Parameters of total phenolic content (TPC), total flavonoid content (TFC), and radical scavenging activity in IWH is already determined in the previous study, with result range 188.03–467.84 mg of gallic acid equivalent (GAE) per kg for TPC, 0.81–3.09 mg quercetin (QEQ)/100 g for TFC, and 28.23–80.74% for inhibition of RSA, a positive correlation among the three parameters based on a Pearson's coefficient of <0.001% (Riswahyuli *et al*., 2019). In this study, FRAP testing was conducted based on the pH ability to reduce Fe^{3+} to Fe^{2+}. Results of the FRAP assay of IWH showed that Bangka Belitung and Sulawesi honey had a higher yield than honey collected from other regions. The values of the potential antioxidants obtained in the FRAP test positively correlated with the total phenolic and flavonoid contents, thereby indicating that the antioxidant potential was primarily due to phenolic compounds, in this case the flavonoids, which was in accordance with the results of other studies (Lachman *et al*., 2010; Kuś *et al*., 2014). Correlation with color demonstrated that the antioxidant activity of dark honey was higher than that of light honey (Socha *et al*., 2011).

3.5 Multivariate analysis

In this study, authenticity was evaluated by multivariate analysis of chemometrics data. Previous research on IWH demonstrated the capability of FTIR spectral data to distinguish genuine and adulterated honey samples. Meanwhile, in the present study, three types of sugars (fructose, glucose, and sucrose) were analyzed, which could also be used to distinguish between adulterated and authentic honey. The principal component analysis (PCA) were showed a grouping between authentic and adulterated honey (Figures 3a). It was found that three principal components (PC) explained 88.00% of the variation within the data. PC3 enabled the classification of honey into two major groups; the group shown on the right side is adulterated honey, whereas that on the left side is authentic honey. In PCA, data of the antioxidant properties were used to differentiate the honey samples according to the geographical origin (Figure 3b). LDA then was applied to explore the possibility of classifying honey samples according to the botanical origin. In LDA all variables and selected gradual variables were used to determine the accuracy of the classification into known groups, to evaluate how the predictor variables differentiated the groups, and to predict the observation of unknown groups. A summary of the classification using cross-validation for sugar revealed that Group 1 had the highest proportion of correct placement, with 95.5% of the observations being correctly placed. Group 1 was adulterated honey; meanwhile, the results of the discriminant analysis using the antioxidant data showed that the total proportion of correct placement was >80% among the different regions of origin except for Sumatera (Table 6). The results of this study indicate that a combination of antioxidant testing, comparative

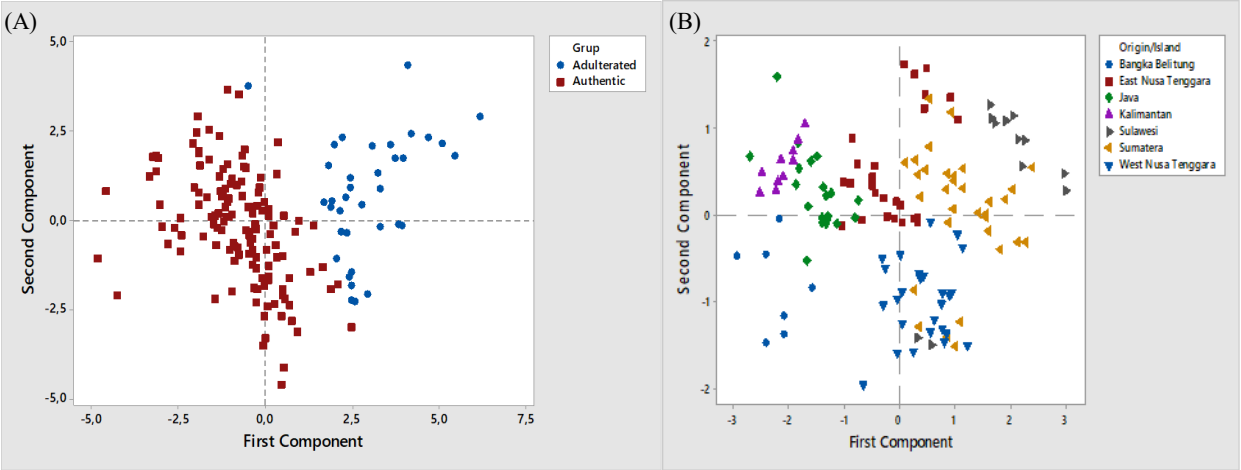

Figure 3. (A) Scatter plot of the PCA scores of authentic and adulterated Indonesia wild honey obtained from sugar content 2. (B) Scatter plot of the PCA scores of Indonesia wild honey based on geographical region obtained from antioxidant data analysis.

Table 6. Discriminant analysis with cross-validation for antioxidant properties of Indonesia wild honey

Put into Group	True Groups						
	Bangka Belitung	Kalimantan	Sulawesi	Sumatera	West Nusa Tenggara	Eat Nusa Tenggara	Java
Bangka Belitung	6	0	0	0	0	0	0
Java	0	0	0	0	0	4	16
Kalimantan	1	10	0	0	0	0	4
West Nusa Tenggara	0	0	2	5	25	0	0
East Nusa Tenggara	0	0	0	6	0	22	0
Sulawesi	0	0	10	8	0	0	0
Sumatera	0	0	0	14	2	0	0
Total N	7	10	12	33	27	26	20
N correct	6	10	10	14	25	22	16
Proportion	0.857	1	0.833	0.424	0.926	0.846	0.8

analysis, and chemometrics evaluation can provide basic information to the phytochemist before the identification and characterization of honey from various sources (Beretta *et al*., 2005).

5. Conclusion

This study evaluated IWH samples collected from seven different regions in Indonesia based on their physicochemical characteristics, sugar mineral content, and antioxidant properties. The results provide an overview of variations between the areas of origin. The moisture content ranged from 20.32% to 31%, pH ranged from 3.08 to 4.65, and the color ranged from yellow to amber tone. Some minerals were specific in several regions, but potassium was found and predominant in all honey types. Fructose and glucose were the more dominant sugars than sucrose, and these properties can be used to distinguish between authentic and adulterated honey samples by conducting multivariate analysis, PCA, and discriminant analysis. The antioxidant properties showed a correlation to the geographical origin, and grouping visualization can be made using PCA. Discrimination analysis using these parameters give accuracy >80% among regions, except Sumatera island.

Conflict of interest

The authors declare that no conflict of interest.

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