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PAPER

CRIMINALISTICS

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Provenance Establishment of Stingless Bee Honey Using Multi-element Analysis in Combination with Chemometrics Techniques*

ABSTRACT: As consumption of stingless bee honey has been gaining popularity in many countries including Malaysia, ability to identify accurately its geographical origin proves pertinent for investigating fraudulent activities for consumer protection. Because a chemical signature can be location-specific, multi-element distribution patterns may prove useful for provenancing such product. Using the inductively coupled-plasma optical emission spectrometer as well as principal component analysis (PCA) and linear discriminant analysis (LDA), the distributions of multi-elements in stingless bee honey collected at four different geographical locations (North, West, East, and South) in Johor, Malaysia, were investigated. While cross-validation using PCA demonstrated 87.0% correct classification rate, the same was improved (96.2%) with the use of LDA, indicating that discrimination was possible for the different geographical regions. Therefore, utilization of multi-element analysis coupled with chemometrics techniques for assigning the provenance of stingless bee honeys for forensic applications is supported.

KEYWORDS: forensic science, provenance, stingless bee honey, multi-elements, chemometrics techniques, Malaysia

Honey, a natural sweet substance produced by honeybees from nectar of flowers and fruits (1), has been consumed by people throughout the world for its medicinal and nutritional values (2). Honey has been reported to demonstrate numerous medicinal benefits such as antimicrobial, antioxidant, anti-inflammatory, anticancer, antihyperlipidemic, antidiabetic, and cardio-protective properties (3). Among the many different types of honeys, the one produced by stingless bee (*Trigona* species) remains one of the popular choices in Malaysia, costing about USD 100 per kilogram (4). The increasing demand for authentic and high-quality stingless bee honey has rendered significant increase in risks of fraud, in attempts to market the lower quality and/or adulterated honeys as the pure expensive ones. Due to its cheaper cost, mixtures of fructose and glucose are commonly used as adulterants for producing low-quality honeys (5), and such a practice may deteriorate its nutritional as well as medicinal benefits. It has been reported that differences in climate and environmental conditions within the vicinity of its foraging area, processing, and storage conditions (6) as well as the different species of bees (6,7) and geographical regions (3) would result in variations in the quality of honeys. Therefore, having the ability to accurately identify the geographical origin of stingless bee honey products becomes pertinent for unveiling fraudulent practices as well as promoting improvement of quality control and consumer protection.

Kreitals and Watling (8) indicated that chemical signatures resulted from climatological, geochemical, and anthropogenic influences “are incorporated in the region’s geology, soils, water and vegetation; making their way through the food chain to higher level organisms” and vary significantly among the different areas. In this context, identifying multi-element distribution patterns may prove useful for forensic provenancing. Although the use of genetic methods for population assignment has been suggested (9), and because such methods are “technique dependent and not all genetic tools provide the same resolution” (8), application of multi-element analysis for provenance establishment may greatly benefit ongoing forensic investigations. Coupled with chemometrics techniques, the multi-element analysis approach is especially useful when dealing with populations with low genetic divergence with no distinctive boundaries (9). In this context, the use of chemometrics techniques such as principal component analysis (PCA) and linear discriminant analysis (LDA) has been suggested. While applications of such integrated approach for provenance establishment of coffees (10), wines (11), pigs (8), beef (12), vegetables (13), and honey (6,14) have been indicated, specific studies focusing on provenancing stingless bee honeys have not been reported.

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Hence, this research that investigated the discriminatory power of interelement association pattern recognition in stingless bee honeys collected from the four geographical regions (five districts) of Johor state (the southernmost part of Peninsular Malaysia) for provenance establishment, acquires forensic significance.

Materials and Methods

Experimental Design and Sampling

This research involved sampling of pure stingless bee honeys harvested from the north (Segamat district), west (Kota Tinggi district), east (Muar and Batu Pahat districts), and south (Johor Bahru district) regions of Johor, Malaysia (Table 1). For each district, one honey beekeeper suggested by the Johor Entrepreneur of Stingless Bee Society was included in the sampling process. Considering the higher production of stingless bee honey during the less rainy season (February-July) in Johor (Johor Entrepreneur of Stingless Bee Society, personal communication), the sampling process was performed during May, June, and July 2016. The pure sample (10 g) was obtained directly from honey pots of stingless bees using separate needleless hypodermic syringes. The obtained pure sample was transferred into a new screw-capped universal container, secured with parafilm, and transported to the laboratory at ambient temperature. For investigating discriminatory power of the integrated multi-element analysis and chemometrics (PCA and LDA) techniques at establishing provenance of the pure stingless bee honeys, samples of the purportedly pure ones purchased from markets (Johor: Johor Bahru; Negeri Sembilan: Seremban) were also included. At the laboratory, all samples were kept at 4°C prior to conducting the multi-element analysis (in triplicates) using the inductively coupled-plasma optical emission spectrometry (ICP-OES).

Sample Preparation

For preserving the composition of trace elements, sample (1 g) of stingless bee honey was diluted with 10 mL of HNO_3 (65% v/v) (Merck, Germany), sonicated at 5000 rpm for 10 min followed by centrifugation (5111.496 G-Force) for 15 min. Whenever necessary, the pellet was filtered using a syringe filter for removing the suspension from pots during sampling. Upon completion, the cleaned pellet was transferred into a clean falcon tube, sealed with parafilm, and kept at room temperature prior to analysis. Using the same sample preparation procedure, a blank control sample that contained 10 mL of HNO_3 (65% v/v) alone was prepared.

TABLE 1—*Summary of stingless bee honey samples analyzed throughout the study.*

Geographical regions/state	Districts/ Markets	GPS Coordinates of Bee Keepers/Markets	Sample ID	Number of Replicates (*n*)
North of Johor	Segamat	2.5035°N, 102.8208°E	SG	9
East of Johor	Muar	2.0631°N, 102.5849°E	MR	9
East of Johor	Batu Pahat	1.8469°N, 102.9352°E	BP	9
West of Johor	Kota Tinggi	1.7294°N, 103.8992°E	KT	9
South of Johor	Johor Bahru	1.4927°N, 103.7414°E	JB	9
State of Johor	Johor Bahru market	1.4927°N, 103.7414°E	M	6
State of Negeri Sembilan	Seremban market	2.7259°N, 101.9378°E	S	3

Sampling of purely harvested stingless bee honey at each beekeeper (district) was performed in triplicates during three separate months, that is, May, June, and July 2016. While a single replicate of sampling for the purportedly pure stingless bee honey was performed (during May, June and July 2016) at Seremban market, the same was duplicate at the Johor Bahru market during the same sampling interval.

Analysis Using Inductively Coupled-Plasma Optical Emission Spectrometry (ICP-OES)

For quantitation, the standard solutions (0.5, 1.0, 5, 10 mg/L) of multi-element standard (1000 mg/L, Perkin Elmer, USA) were freshly prepared by diluting with suitable amounts of HNO_3 (65% v/v) *via* serial dilution. Considering the compositions of trace elements reported to occur in varying types of honeys in Malaysia (6,7), the multi-element analyzed here included Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sb, Se, Sn, and Zn. The analysis was performed using ICP-OES (PE 4300 DV, Perkin Elmer, USA) at the Department of Chemistry Malaysia Petaling Jaya Selangor. The analytical parameters standardized for ICP-OES were: radio frequency power (0.7–1.5 kW, 1.2–1.3 kW for axial); plasma gas flow rate (Ar) (radial: 10.5–15 L/min, axial:15 L/min); auxiliary gas flow rate (Ar: 1.5 L/min) and viewing height (5–12 mm).

Analytical Method Validation

Calculation of the linearity of the assay was made based on the regression line by the method of least square and the value was expressed as coefficient of determination (R^2). All the calibration curves were constructed using the obtained mean corrected intensity versus that of the concentrations of multi-element added. The calibration curves were accepted only when (a) $R^2 > 0.995$ and (b) the relative standard deviation (RSD) was <20% (15,16). Limit of detection (LOD) is the lowest concentration of an analyte determined with signal to noise ratio of at least 3:1, while limit of quantitation (LOQ) refers to the lowest concentration of an analyte in a calibration curve (10:1) (15,16). In this research, the LOD and LOQ for all the elements analyzed were mathematically determined from respective calibration curves following the formulae suggested by previous researchers (16,17).

Using quality control samples (0.5 or 5 mg/L), the intra- and interassay precision and accuracy were calculated. While the intra-assay precision and accuracy were determined within one run (six replicates for each concentration), the inter-assay precision and accuracy were calculated by comparing the data obtained among three separate runs. While the values of RSD were used for expressing precision, the percentage differences of the estimated concentrations from that of the nominal values were used for reporting the accuracy. The percent recovery was determined by comparing the mean corrected intensity of spiked elements in the randomly collected pure stingless bee honey with that of blank samples in HNO_3.

Statistical Analysis

The PCA and LDA were both performed using the Minitab version 16.2.3 (Minitab Inc. Minitab Incorporated, Pennsylvania, USA) statistical software. The dataset was prepared and preprocessed by means of standardization using Microsoft® Excel spreadsheet (Microsoft Corporation, Washington, USA) prior to

TABLE 2—*Analytical figures of merit (Calibration range,* R^2*, regression equation, LODs, and LOQs) for multi-element determination in stingless bee honey using ICP-OES.*

			Calibration Parameters				
Elements	Wavelength	Range of Calibration Curves (mg/L)	Coefficient of Determination (R^2)	Equation	Limit of Detection (LOD) (mg/L)	Limit of Quantitation (LOQ) (mg/L)	Relative Standard Deviation (RSD) (%)
Ag	328.068	0–1	0.999	$y = 140,398x + 45.173$	0.002	0.008	2.7
Al	394.401	0–10	0.999	$y = 200,690x + 330.17$	0.021	0.064	1.0
As	188.979	0–1	0.999	$y = 3078.1x - 32.375$	0.039	0.118	0.6
B	249.677	0–5	0.999	$y = 54,939x + 669.99$	0.512	1.537	0.4
Ba	233.527	0–1	0.999	$y = 7E + 06x - 3800.1$	0.009	0.028	3.0
Be	313.107	0–1	0.999	$y = 3E + 06x + 4767.9$	0.009	0.029	2.2
Cd	228.802	0–1	0.999	$y = 81,090x - 139.89$	0.005	0.017	3.1
Co	228.616	0–1	0.999	$y = 56,562x - 70.251$	0.006	0.020	3.3
Cr	267.716	0–1	0.999	$y = 77,520x - 163.48$	0.009	0.029	3.0
Cu	327.393	0–1	0.999	$y = 204,396x + 1216.6$	0.009	0.029	3.1
Fe	238.204	0–10	0.999	$y = 2E + 06x + 4474.5$	0.010	0.032	2.9
Mg	285.213	0–5	0.999	$y = 323,510x - 2884.2$	0.533	1.600	1.7
Mn	257.610	0–1	0.999	$y = 888,610x - 1746.6$	0.028	0.084	3.0
Mo	202.031	0–1	0.999	$y = 31,257x + 61.235$	0.010	0.030	3.3
Ni	231.604	0–1	0.999	$y = 42,242x - 105.46$	0.007	0.023	3.1
Pb	220.353	0–1	0.999	$y = 8741x + 37.398$	0.007	0.021	0.2
Sb	206.836	0–1	0.999	$y = 4595.1x + 18.021$	0.014	0.042	0.8
Se	196.026	0–1	0.999	$y = 2122.9x + 12.439$	0.013	0.040	0.4
Sn	189.927	0–5	0.999	$y = 7164.1x + 58.879$	0.518	1.556	0.4
Zn	206.200	0–10	0.999	$y = 809,552x + 2556$	0.013	0.039	0.9

TABLE 3—*The precision, accuracy, and recovery studies of multi-element in stingless bee honey using ICP-OES.*

		Within-assay (n = 6)			Between-assay (n = 18)				
Elements	Conc. (mg/L)	Observed conc. (mean ± SD) (mg/L)	Precision (%)	Accuracy (%)	Observed Conc. (mean ± SD) (mg/L)	Precision (%)	Accuracy (%)	Recovery (%) (n = 6)	Relative Standard Deviation (RSD) (%)
Ag	0.5	0.5058 ± 0.0080	1.6	1.2	0.4989 ± 0.0145	2.9	0.2	99.0	2.2
Al	5.0	5.0947 ± 0.1525	2.9	1.9	5.0060 ± 0.1725	3.4	0.1	104.2	1.2
As	0.5	0.5016 ± 0.0131	2.6	0.3	0.5118 ± 0.0193	3.8	2.4	101.6	4.0
B	5.0	5.1330 ± 0.0871	1.7	2.7	5.0545 ± 0.0882	1.7	1.1	101.5	1.9
Ba	0.5	0.5001 ± 0.0084	1.7	0.1	0.5005 ± 0.0178	3.6	0.1	98.1	1.6
Be	0.5	0.5053 ± 0.0061	1.2	1.1	0.5080 ± 0.0104	2.0	1.6	101.8	2.5
Cd	0.5	0.4980 ± 0.0114	2.3	0.4	0.4939 ± 0.0171	3.5	1.2	97.2	2.1
Co	0.5	0.4956 ± 0.0067	1.4	0.9	0.4993 ± 0.0111	2.2	0.1	98.7	2.0
Cr	0.5	0.4993 ± 0.0077	1.5	0.1	0.4987 ± 0.0130	2.6	0.3	98.1	2.0
Cu	0.5	0.5035 ± 0.0073	1.4	0.7	0.4890 ± 0.0293	5.9	2.2	99.0	1.8
Fe	5.0	5.1297 ± 0.1165	2.3	2.6	5.0732 ± 0.1339	2.6	1.5	104.9	1.7
Mg	0.5	0.5113 ± 0.0147	2.9	2.3	0.4991 ± 0.0108	2.2	0.2	103.2	1.6
Mn	0.5	0.4966 ± 0.0091	1.8	0.7	0.4926 ± 0.0120	2.4	1.5	98.2	1.6
Mo	0.5	0.5032 ± 0.0097	1.9	0.6	0.4952 ± 0.0091	1.8	0.9	97.7	2.0
Ni	0.5	0.4948 ± 0.0144	2.9	1.0	0.4977 ± 0.0152	3.1	0.5	98.3	1.8
Pb	0.5	0.5039 ± 0.0111	2.2	0.8	0.5150 ± 0.0174	3.4	2.9	101.0	2.7
Sb	0.5	0.4978 ± 0.0154	3.0	0.4	0.5018 ± 0.0118	2.4	0.3	101.1	3.1
Se	0.5	0.4994 ± 0.0127	2.5	0.1	0.5020 ± 0.0087	1.7	0.4	100.4	1.8
Sn	0.5	0.5111 ± 0.0055	1.1	2.2	0.5209 ± 0.0217	4.2	4.1	102.0	2.2
Zn	5.0	5.1665 ± 0.0833	1.6	3.3	5.1310 ± 0.0847	1.7	2.6	104.9	1.8

While six replicates of each of the quality control samples were analyzed for assessing the within-assay precision and accuracy, the same for between-assays was performed over three separate runs (n = 18). Precision of the assay was expressed as the percentage of RSD and the accuracy as the percentage different from the nominal values, respectively. The percent recovery was determined by comparing the mean corrected intensity of spiked heavy metals in the randomly collected pure stingless bee honey with that of blank samples in HNO_3.

transferring them into the Minitab environment. Following the use of PCA, LDA was performed for each dataset for enabling further visualization of stingless bee honey groupings. In contrast to PCA, LDA is a supervised statistical method that classifies objects into one of several user-defined groups. The LDA calculates linear discriminant function to create a model that would enable correct classification of objects within a given dataset. To ensure satisfactory and reliable classification, a developed model was validated using the cross-validation approach by means of "leave-one-out" technique. The percentage of correct classification from the cross-validation process would reflect the appropriateness of a model as a classifier for a given dataset.

Results and Discussion

The calibration parameters as well as LODs and LOQs for all the 20 elements (Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sb, Se, Sn, and Zn) analyzed here are presented in Table 2. Previous researchers have suggested that to accept a calibration curve, the R^2 must be higher than 0.995 with RSD < 20% (15,16). Because all the calibration curves included in this research (Table 2) were found to adhere to such prescribed criteria, responses obtained can be construed as linear, indicating the appropriateness of the analytical method for quantitating such elements in stingless bee honey samples. In this present research, the LODs and LOQs for all the elements were calculated using the data from accepted calibration curves, ranging between 0.002–0.533 mg/L and 0.008–1.600 mg/L, respectively (Table 2). During the entire 3 days of validation period, an average of $R^2 > 0.999$ was successfully achieved over a range of 0–10 mg/L for each of the element. As it was expected that the concentrations of multi-element in stingless bee honeys sampled from various locations in Johor would vary considerably, a wide linear range of analytical method was developed for enabling reliable assessment. For all the elements, the within-assay precision (1.1–3.0%) and accuracy (0.1–3.3%) were found to be lesser than 10%; the between-assay precision (1.7–5.9%) and accuracy (0.1–4.1%) of less than 10% were also observed (Table 3). In addition, the percentage of recoveries for all the elements ranged between 97.2 and 104.9% (RSD: 1.2–4.0%) (Table 3). As the precision and accuracy for both the within and between assays obtained were lesser than 10% and because the percentage recoveries for all the elements analyzed here fell well within the acceptable range (85–115%) (16), the multi-elements analysis *via* ICP-OES utilized in this present research appears reliable.

Taking into account that ascertaining the quality of honey would depend largely on its organoleptic characteristics that are dependent on botanical and geographical origins, and because honey "from one region may attain a surplus value than similar products from another area" (18), confirming its provenance following suitable chemical analysis may prove forensically important (19). In this context, it is pertinent to indicate that about 80% of the marketed honeys in Malaysia are fake or synthetically produced (20). The repercussions of such situation may include possible health threat as well as economic loss among consumers, considering its expensive value, especially for those produced by stingless bees. Therefore, providing an empirically robust forensic approach, in compliance with the prevailing Daubert standard (21), for separating the genuine and fake honeys, as well as differentiating the different types of honeys and their origins may prove useful whenever such aspects are questioned in court during criminal and civil proceedings. Considering that provenance establishment for honeys in Malaysia remains unreported, the specific attempt at provenancing stingless bee honeys from the different geographical regions of Johor reported here, merits forensic consideration.

By examining the entire dataset using PCA, generic separation of the stingless bee honeys from the different districts in Johor (Segamat, Muar, Batu Pahat, Kota Tinggi, and Johor Bahru), as well as the two different markets (Johor: Johor Bahru; Negeri Sembilan: Seremban) was attempted. It was observed that 91.2% of the variations in the dataset could be attributed to the first nine PCs. However, because the inclusion of a number of PCs that are accountable for 70–80% of variations has been suggested as adequate to represent the dataset in a much reduced dimension (22), the first three PCs that accounted for 70.2% of variations in the dataset (PC1 = 36.0%, PC2 = 28.8%, and PC3 = 5.4%) were used. This finding strongly justifies the representation of the PCs score plots in a three-dimensional (3D) form using the first three PCs as the axes.

The 3D score plots for stingless bee honeys (Fig. 1) revealed that while distinctive and neat grouping was only apparent for stingless bee honeys collected from Segamat, the remaining

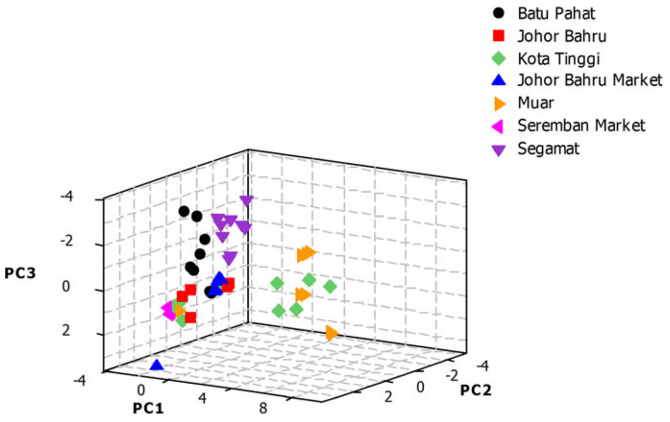

FIG. 1—*Three-dimensional principal component analysis score plot of the honey samples using the first three principal components (PC1, PC2, and PC3). [Color figure can be viewed at wileyonlinelibrary.com]*

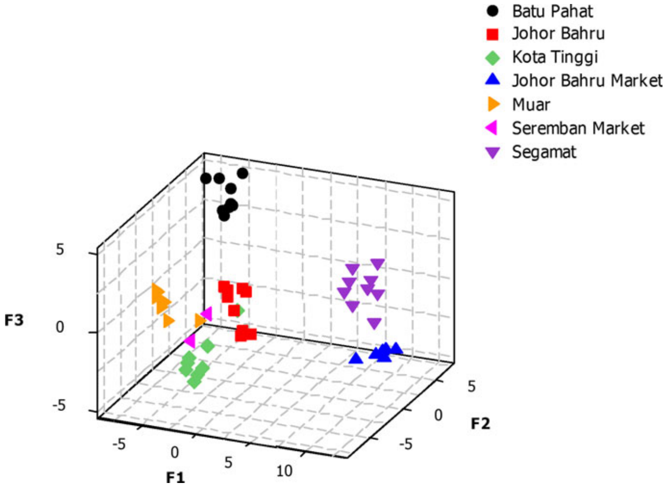

FIG. 2—*Three-dimensional linear discriminant analysis plot using three discriminant functions (F1, F2, and F3). [Color figure can be viewed at wileyonlinelibrary.com]*

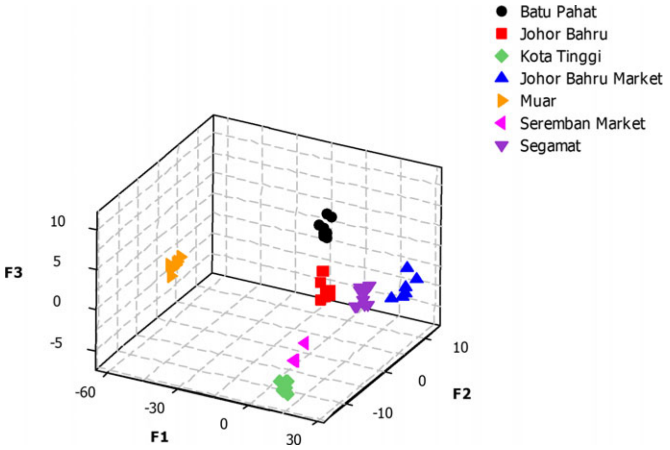

FIG. 3—*Three-dimensional linear discriminant analysis plot using three discriminant functions (F1, F2, and F3) after removal of suspected outliers. [Color figure can be viewed at wileyonlinelibrary.com]*

samples were convoluted into two separate groups. Although the purely harvested stingless bee honeys from Johor Bahru, Kota Tinggi, and Batu Pahat, as well as the purportedly pure ones purchased from the Johor Bahru and Seremban markets were grouped together, samples of purely harvested stingless bee honeys collected from Muar and Kota Tinggi were clustered in another group (Fig. 1). While based on the concentrations of 20 elements, PCA was able to reveal two natural groupings, the separation provided at this particular point may not be sufficient for forensic provenancing, considering the explanatory nature of the PCA (23). To resolve this issue, LDA was considered due to its classificatory nature (24). Similar observations have been reported by previous researchers, indicating better geographical discriminations for provenance establishment of food commodities, following the use of LDA (8,11) and PCA-LDA techniques (10) than that of PCA alone (6,7). Interestingly, while Chua et al. (6) and Moniruzzaman et al. (7) attempted to discriminate/classify the different types of honeys in Malaysia using PCA, aspect pertaining to provenance establishment of its geographical origins have not been reported. While both Chua et al. (6) and Moniruzzaman et al. (7) indicated that the highest positive score on the PC1 had been attributable to K, Mg was found to be the case in the purely harvested as well as purportedly pure stingless bee honeys analyzed in this present research.

The three-dimensional LDA plot using three discriminant functions (F1, F2 and F3) is presented in Fig. 2. Utilization of LDA had improved the groupings of stingless bee honeys when compared with that of PCA alone, with LDA cross-validation correct classifications rate of 87.0%. In general, the purely harvested stingless bee honeys collected from all the five districts (Segamat, Kota Tinggi, Batu Pahat, Muar, and Johor Bahru), as well as the purportedly pure ones purchased from Johor Bahru and Seremban markets were distinctively separated into separate groups (Fig. 2). However, two samples of purely harvested stingless bee honeys (i.e., one each for Kota Tinggi and Muar districts) were found to be distributed away from their corresponding groups; clustered into the purely harvested stingless bee honey group for Johor Bahru and the purportedly pure ones from Seremban market, respectively (Fig. 2). Considering them as outliers, the two samples were subsequently removed from the dataset for improving the groupings and cross-validation assessment of the overall samples. Figure 3 represent the three-dimensional LDA plot using three discriminant functions upon removing of the two suspected outliers. Such an approach resulted in substantial improvement (96.2% cross-validation correct classification) in the LDA groupings of stingless bee honeys (Fig. 3). Such a finding indicates the feasibility of using multi-element analysis that was coupled with chemometrics techniques (PCA and LDA) for differentiating purely harvested stingless bee honeys collected from the five districts in Johor, as well as those purchased from markets. However, due to the lack of information on provenance establishment of geographical origins for stingless bee honeys in the body of literature, suitable comparisons with the findings reported here could not be made. In agreement with Valentine and Watling (10), it was observed that the use of a single set of common elements may not be useful for providing regional discrimination; the use of combinations of recurring elements may prove useful for facilitating regional separation.

In conclusion, analyses of multi-element compositions coupled with chemometrics techniques have enabled sufficient discrimination (96.2% correct classification) of stingless bee honeys from the different geographical regions in Johor, Malaysia. Because in this research, samples were collected during the less rainy season alone, sampling process was only performed at five selected districts in Johor with only 20 elements analyzed; further studies focusing on the same aspect covering the different seasons of a year in Johor with a larger spectrum of elements may provide better insights into this matter.

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References

1. Kováčik J, Grúz J, Biba O, Hedbavny J. Content of metals and metabolites in honey originated from the vicinity of industrial town Košice (Eastern Slovakia). Environ Sci Pollut Res Int 2016;23(5):4531–40.
2. Cohen H, Rozen J, Kristal H, Laks Y, Berkovitch M, Uziel Y, et al. Effect of honey on nocturnal cough and sleep quality: a double-blind, randomized, placebo-controlled study. Pediatrics 2012;130(3):465–71.
3. Rao P, Krishnan K, Salleh N, Gan S. Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. Braz J Pharmacog 2016;26(5):657–64.
4. Kelly N, Farisya MSN, Kumara TK, Marcela P. Species diversity and external nest characteristics of stingless bee in meliponiculture. Pertanika J Trop Agric Sci 2014;37(3):293–8.
5. Zhu X, Li S, Shan Y, Zhang Z, Li G, Su D, et al. Detection of adulterants such as sweeteners materials in honey using near-infrared spectroscopy and chemometrics. J Food Eng 2010;101(1):92–7.
6. Chua L, Abdul-Rahaman N, Sarmidi M, Aziz R. Multi-elemental composition and physical properties of honey samples from Malaysia. Food Chem 2012;135(3):880–7.
7. Moniruzzaman M, Chowdhury M, Rahman M, Sulaiman S, Gan S. Determination of mineral, trace element, and pesticide levels in honey samples originating from different regions of malaysia compared to manuka honey. Biomed Res Int 2014;2014(2):1–10.
8. Kreitals N, Watling R. Multi-element analysis using inductively coupled plasma mass spectrometry and inductively coupled plasma atomic emission spectroscopy for provenancing of animals at the continental scale. Forensic Sci Int 2014;244:116–21.
9. Alacs E, Georges A, FitzSimmons N, Robertson J. DNA detective: a review of molecular approaches to wildlife forensics. Forensic Sci Med Pathol 2010;6(3):180–94.
10. Valentin J, Watling R. Provenance establishment of coffee using solution ICP-MS and ICP-AES. Food Chem 2013;141(1):98–104.
11. Martin A, Watling R, Lee G. The multi-element determination and regional discrimination of Australian wines. Food Chem 2012;133(3):1081–9.
12. Heaton K, Kelly S, Hoogewerff J, Woolfe M. Verifying the geographical origin of beef: the application of multi-element isotope and trace element analysis. Food Chem 2008;107(1):506–15.
13. Yan J, Liu J, Xiong Y, Qin W, Tang C. Identification of the geographical origins of pomelos using multielement fingerprinting. J Food Sci 2015;80(2):C228–33.
14. Baroni M, Arrua C, Nores M, Fayé P, Diaz M, Chiabrando G, et al. Composition of honey from Córdoba (Argentina): assessment of north/south provenance by chemometrics. Food Chem 2009;114(2):727–33.
15. Man C, Gam L, Ismail S, Lajis R, Awang R. Simple, rapid and sensitive assay method for simultaneous quantification of urinary nicotine and cotinine using gas chromatography-mass spectrometry. J Chromatogr 2006;844(2):322–7.
16. Ruggieri F, Alimonti A, Bocca B. Full validation and accreditation of a method to support human biomonitoring studies for trace and ultra-trace elements. Trends Anal Chem 2016;80:471–85.
17. Sanagi M, Ling S, Nasir Z, Hermawan D, Ibrahim W, Naim A. Comparison of signal-to-noise, blank determination, and linear regression

methods for the estimation of detection and quantification limits for volatile organic compounds by Gas Chromatography. J AOAC Int 2009;92 (6):1833–8.

18. Baroni M, Nores M, Díaz M, Chiabrando G, Fassano J, Costa C, et al. Determination of volatile organic compound patterns characteristic of five unifloral honey by solid-phase microextraction-gas chromatography-mass spectrometry coupled to chemometrics. J Agric Food Chem 2006;54(19):7235–41.
19. Woodcock T, Downey G, Kelly J, O'Donnell C. Geographical classification of honey samples by near-infrared spectroscopy: a feasibility study. J Agric Food Chem 2007;55(22):9128–34.
20. Ahmad M. Lambakan madu palsu, sintetik. Kosmo! Newspaper 2016 Aug 15; Sect. National, pg. 3.
21. Fradella H, Fogarty A, O'Neill L. The impact of Daubert on forensic science. Pepp L Rev 2004;30(2):403–44.
22. Suhr D. Principal component analysis vs. exploratory factor analysis. SAS Conference Proceedings of the SAS® Users Group International 30; 2005 April 10–13, Philadelphia, PA. Cary, NC: SAS® Institute Inc., 2005;203–30.
23. Osborne J, Costello A. Best practices in exploratory factor analysis: four recommendations for getting the most from your analysis. Pract Assess Res Eval 2005;10(7):1–9.
24. Martinez A, Kak A. PCA versus LDA. IEEE Trans Pattern Anal Mach Intell 2001;23(2):228–33.

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