Heliyon 6 (2020) e03662

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Indonesian wild honey authenticity analysis using attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy combined with multivariate statistical techniques

Y. Riswahyuli [a,c], Abdul Rohman [b], Francis.M.C.S. Setyabudi [a], Sri Raharjo [a,*]

[a] Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora 1, Bulaksumur, Sleman, Yogyakarta, 55281, Indonesia
[b] Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia
[c] National Agency of Drug and Food Control (Badan PengawasObat dan Makanan), Jakarta, Indonesia

ARTICLE INFO

Keywords:
Food science
Food analysis
Wild honey
ATR-FTIR
Authenticity
Sugar
Chemometrics

ABSTRACT

Wild honeys in Indonesia are still widely believed to be good for health with high economic value. This honey is naturally produced by *Apisdorsata* bee. In this study, authentication analysis by classification and discrimination of attenuated total reflectance-fourier infrared spectroscopy (ATR-FTIR) spectra was conducted on several wild honeys from various places in Indonesia (n = 186) which then compared to adulterated honey contained commercial sugars of aren (*Arenga pinnata*), coconut, and cane sugar at 10–50% concentration (n = 57). Combination of spectra measurement at 4,000-650 cm^{-1} with Chemometric technique by several multivariate analyses resulted in visualization of honey grouping, classification, and regression model that differentiate these honeys, both partial and overall. Principle component analysis multivariate analysis was able to visualize the differentiation of adulterated honey from the authentic ones. Discriminant analysis, a supervised classification technique, was used to differentiate the fake from the authentic honey among those from various origins at wave number range of 4000–800 cm^{-1} with performance index of 91,8, 90.32–100% sensitivity, and 95. 70–100% specificity. Partial least-squares analysis was used to build a model provided quantitative results of commercial sugars content in honey allegedly added during adulteration. Authentic honeys had commercial sugars content less than 10% with R^2 of aren, coconut, and cane sugar of 0.9995, 0.9980 and 0.9998, respectively, with their predictive R^2 values of 0.9977, 0.9983 and 0.9946, respectively.

1. Introduction

Wild honey is specifically used to classify honeys naturally produced in the forest, and it is produced by wild honey bees, particularly by *Apisdorsata*. These honey bees suck nectar from forest flowers and store it in the beehive attached to trees. In Indonesia, wild honeys are named according to their origins, such as Sumbawa, Pontianak, Riau, Flores, Gunung Mutis, Tesso Nilo, and Sentarum Lake National Park honey; the tree that the beehive is attached to, such as Sialang, Pelawan or Tristania, white paperbark, and kapok tree honey; the local name of the bee, such as Odeng honey in West Java; and the honey farmer group's name (Sarwono, 2001). Sari and Bertoni (2014) reported that various Indonesian wild honeys have antioxidant and anticancer properties that vary between regions. Honey composition and properties are largely affected by factors such as the bee and tree species, nectar provider, geographical region, season, storage condition, and harvest method and condition (Colucci et al., 2016; Kaškoniene et al., 2010).

The price of honey is relatively high because of limited honey production such that manufacturers are unable to meet consumer demand. Therefore, honey is a potential target for product adulteration. Commercial sugars or syrups are common honey adulterants (Kelly et al., 2006) because they have a high similarity toits natural property (Soares et al., 2017). Honey comprises monosaccharides, mainly glucose or fructose (75%) and disaccharides (10%–15%) and a small portion of other sugars. The sugar composition of honey is mainly affected by the origin of the flower, geographical condition, climate, processing, and storage (Tornuk et al., 2013; Escuredo et al., 2014; Da Silva et al., 2016). The common commercial sugars used for honey adulteration are cane sugar, beet, maltose syrup, corn syrup (CS), high-fructose CS (HFCS), glucose syrup (GS), sucrose syrup, inverted syrup (IS), and high-fructose

* Corresponding author.
E-mail address: sraharjo@ugm.ac.id (S. Raharjo).

https://doi.org/10.1016/j.heliyon.2020.e03662
Received 10 September 2019; Received in revised form 4 December 2019; Accepted 20 March 2020

inulin syrup (HFIS) (Padovan et al., 2003; Zhu et al., 2010; Tosun, 2012; Ribeiro et al., 2014; Soares et al., 2017). Several analytical methods have been developed to assess honey authenticity based on the honey standard according to the regulation and requirements, both nationally, including the Indonesia National Standard, and internationally, including the Codex Alimentarius, European regulation, and US FDA regulation. These methods include analysis of physical properties (Nikolova et al., 2015), biological analysis, and analytical techniques such as chromatography (thin-layer chromatography, high-performance liquid chromatography, and gas chromatography) and polymerase chain reaction. However, these methods are complex and require a skillful analyst; therefore, a simple and fast method worth to be developed for routine analysis.

Vibrational spectroscopy techniques such as near-infrared (NIR), mid-infrared (MIR), Raman spectroscopy, and hyperspectral imaging (Lohumi et al., 2015) are among the most common methods for authentication analysis because of their low cost, rapid measurement, and nondestructive nature. Raman spectroscopy, NIR, and MIR are reliable, practical, and rapid and do not require sample preparation; these techniques were utilized in previous research to distinguish honey based on flower nectar. Fourier-transform mid-infrared (FTIR) technique was used in previous research, particularly for honey classification in the US (Tewari and Irudayaraj, 2005) and honey discrimination in Ireland (Kelly et al., 2006). With the development of the attenuated total reflectance (ATR) system as a sampling technique, ATR-FTIR has been used to analyze honey adulterated with three sugars (corn syrup, high fructose corn syrup, and inverted sugar) from four locations in Mexico (Gallardo--Velázquez et al., 2008), to predict sugar content in adulterated honey in Portugal (Anjos et al., 2014), to analyze rape honey authenticity in Poland (Kasprzyk and Depciuch, 2017), and to detect Campeche honey adulteration (Anguebes et al., 2016). Various studies have reported regarding the utilization of the multivariate method for honey authentication (Sivakesava and Irudayaraj, 2001; Nalda et al., 2005; Kelly et al., 2006; Gallardo-Velázquez et al., 2008; Zhou et al., 2014; Siddiqui et al., 2017). Andrade et al. (2019) was use application of Fourier-Transformed Infrared spectroscopy using ATR-FTIR to characterize and to detect adulteration of whey protein concentrate by principal component analysis (PCA) and partial least-square (PLS); however, no available publication regarding Indonesian honey authentication using a chemometric approach. Therefore, this study focused on group visualization, created a model for classification, and quantified adulterants of Indonesian wild honey using ATR-FTIR, a new, rapid, effective, non-destructive and cost-effective method, with a chemometric approach and multivariate analysis including PCA-DA, and PLS.

2. Material and methods

2.1. Sampling and sample preparation

Two series of samples were made. Total of 57 adulterated honey samples and 129 authentic honey samples were collected from 7 different forest areas: Sumatra (Tesso Nilo and Gunung Kerinci National Park, Jambi), Bangka-Belitung (Pelawan Tourism Forest, Namang, Bangka Tengah, and Belitung Island), Banten (Ujung Kulon/Pandeglang National Park, Banten), Kalimantan (Sentarum Lake National Park, Kapuas Hulu, Kalimantan Barat), West Nusa Tenggara (NTB; Sumbawa subdistrict, Nusa Tenggara Barat), East Nusa Tenggara (NTT; Muntis Mountain Conservation, Timor Tengah Selatan and Maumere, Flores Timor), and Sulawesi (Marisa, Gorontalo, Morowali Utara, Sulawesi Tengah, Manado, Sulawesi Utara, and Tulak Tallu village, Luwu Utara, Sulawesi Selatan; Figure 1). Approximately 300 g of each sample was collected directly from beekeepers during 2017–2018. Upon receipt, honey samples were stored in clean, closed jars at room temperature under dark conditions until use. Moreover, some honey samples were purchased from Indonesia Wild Honey Network, which guarantees authenticity. The adulterated honey samples were prepared by adding 10%–50% of aren (*Arenga pinnata*), palm, and cane sugar solutions. The authentic and adulterated honeys were standardized at 70° Brix of solid content.

2.2. Spectra measurement using FTIR

Direct measurement was conducted by placing the sample on the ATR surface in the MIR area with wave numbers of 4000–650 cm^{-1}(Nicolet 6700 FTIR spectrometer, Thermo Nicolet Corp, Madison, WI) at a controlled room temperature of 25 °C. The system was applied with 42 scanners at 4 cm^{-1} resolution. Generated spectra were automatically reduced or corrected by air background spectra that were previously measured, with duplicate measurement on different subsamples. Results were saved and analyzed using the OMNIC software (Version 8. 0, Thermo Nicolet, Madison, WI).

2.3. Chemometric analyses

Data analysis by PCA, DA and PLS was performed using TQ Analyst ™ Thermo Fisher Scientific software (version 9.7.0.27), a chemometric software package. PCA was performed to visualize honey grouping based on the latent variable, whereas DA was performed for discrimination and classification. To quantitatively predict the adulterants,a prediction model was determined using PLS. Data set was divided into the training and validation or prediction or test sets, containing about 75% and 25 % samples of every class (n = 139 for training set are authentic sample, n = 47 for tes set). A calibration model was calculated using the samples in the training set and subsequently used to predict the samples in the test set.

3. Results and discussions

Wild honey authenticity covers two main issues including its production, for example addition of sugars, and its origin. Common honey adulterants in various adulteration cases identified in Indonesia are palm, aren, and cane sugar which have composition mainly sucrose. Addition of this type of sugars does not change the appearance of honey. Chemical changes were analyzed using FTIR-ATR to distinguish between authentic and adulterated honey. FTIR-ATR which applied in collecting information from honey sample is an easy and versatile infrared spectroscopy technique. With ATR, the sample (liquid or solid) is easily placed in contact with the horizontal surface of the diamond crystal surface which has a high refractive index (Karoui et al., 2010). Each peak and shoulder on MIR spectra at wavenumbers of 4000–650 cm^{-1}can be classified into two regions, namely the functional group region (4000–1500 cm^{-1}) and fingerprint region (1500–650 cm^{-1}). Figure 1 reveals the FTIR spectra of 243 samples, comprising 129 authentic honey samples and 57 honey samples adulterated with 10%–50% of commercial sugars. The peak observed at 927 $cm^{-1}$is because of the C–H bending in the carbohydrate group; however, those observed at 991, 1042, 1106, and 1259 cm^{-1} may be because of the C–O stretching in the C–OH group and the C–C stretching in the carbohydrate structures. The peak observed at 1110 cm^{-1} could be associated with the stretching vibration of the C–O bond in the C–O–C linkage, which is present as a glycosidic bond in sucrose. The peak observed at approximately1327 cm^{-1} may be because of the O–H bending in the C–OH group, whereas the peak observed at 1419 cm^{-1} may be because of a combination of the O–H bending in the C–O–*H group* and the C–H bending in alkenes, 1650 cm^{-1} due to H-O-H stretching, and 2929 cm^{-1}due for C-H stretching (Gallardo-Velázquez et al., 2008; Hennessy et al., 2008; Tewari and Irudayaraj, 2005). More detailed observation indicated the difference between the functional group region and fingerprint region; thus, the two regions were used for further multivariate analysis. Prior to multivariate analysis, several FTIR spectra were subjected to Savitzky–Golay derivatization and smoothing pretreatment (see Figure 2).

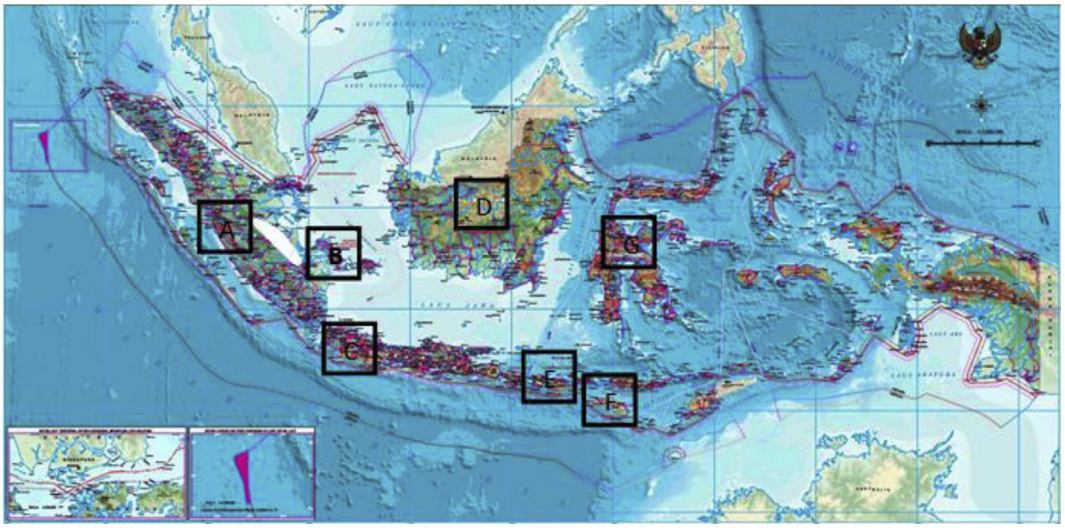


Figure 1. Sampling locations. (A = Sumatra; B = Bangka Belitung, C = Banten, D = Kalimantan, E = Nusa Tenggara Barat (NTB), F = Nusa Tenggara Timur (NTT), and G = Sulawesi).

3.1. Group visualization

The chemometrics of PCA was used to reduce the number of variable and early detection of sample grouping/differentiation. Figure 3a reveals PCA results that indicate the differentiation among adulterated and authentic honey samples and Figure 3b for honey grouping based on their origins, such as Riau and Bangka-Belitung honey. However, no grouping was observed in honey samples collected from other locations, such as NTT and NTB honey, the similar result was given by previous study, PCA analysis did not indicate any clustering between these New Zealand honey groups from different floral origin, but possible discrimination between manuka and clover (Jandrić et al., 2014).

3.2. Classification of IWH

PCA is preliminary evaluation for data structure, as the result did not showed a good clustering based on geographical origin, we should classify with Supervised pattern Recognition multivariate technique, DA that PCA-based alogarithm Model built on the basis of maximum 10 PCs, which forms clusters based on the "distance" between the cluster centers and that of the sample spectrum. This distance from each class center to the other class is known as the Mahalanobis distance. Classification of honey based on geographical region was conducted at a particular wave number (Table 1). The best classification was obtained at wave numbers of 800–4000 cm^{-1} with 91,8 performance index (PI; Table 1) which was

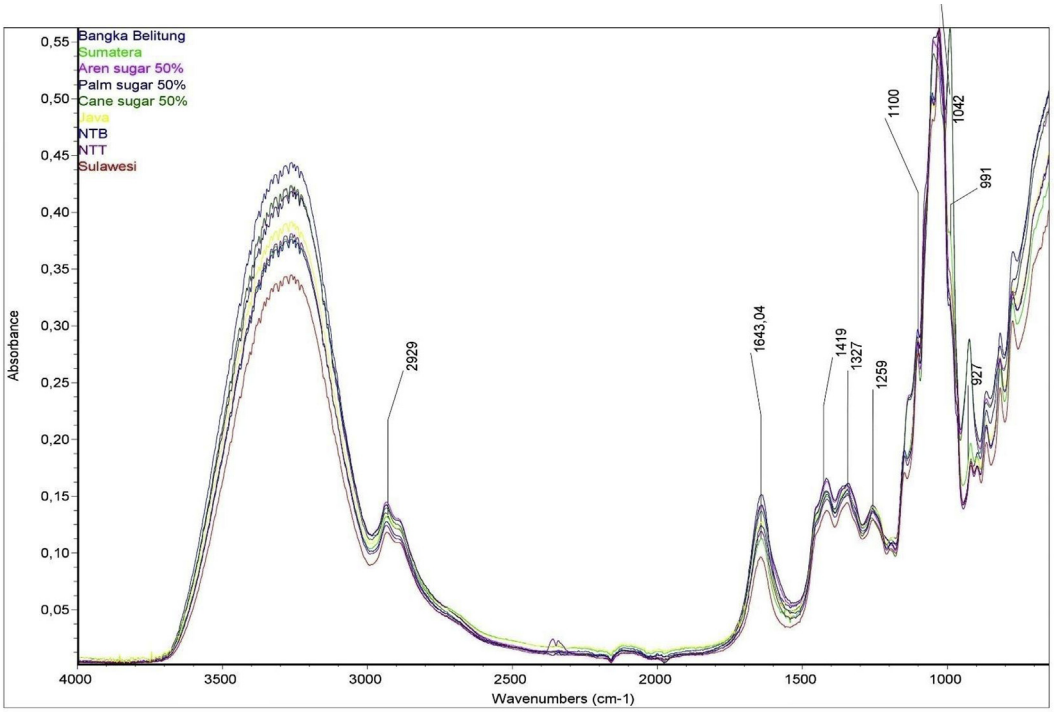


Figure 2. Fourier-transform infrared spectrum of Indonesia wild honey at mid-infrared wave numbers (650–4000 cm^{-1}).

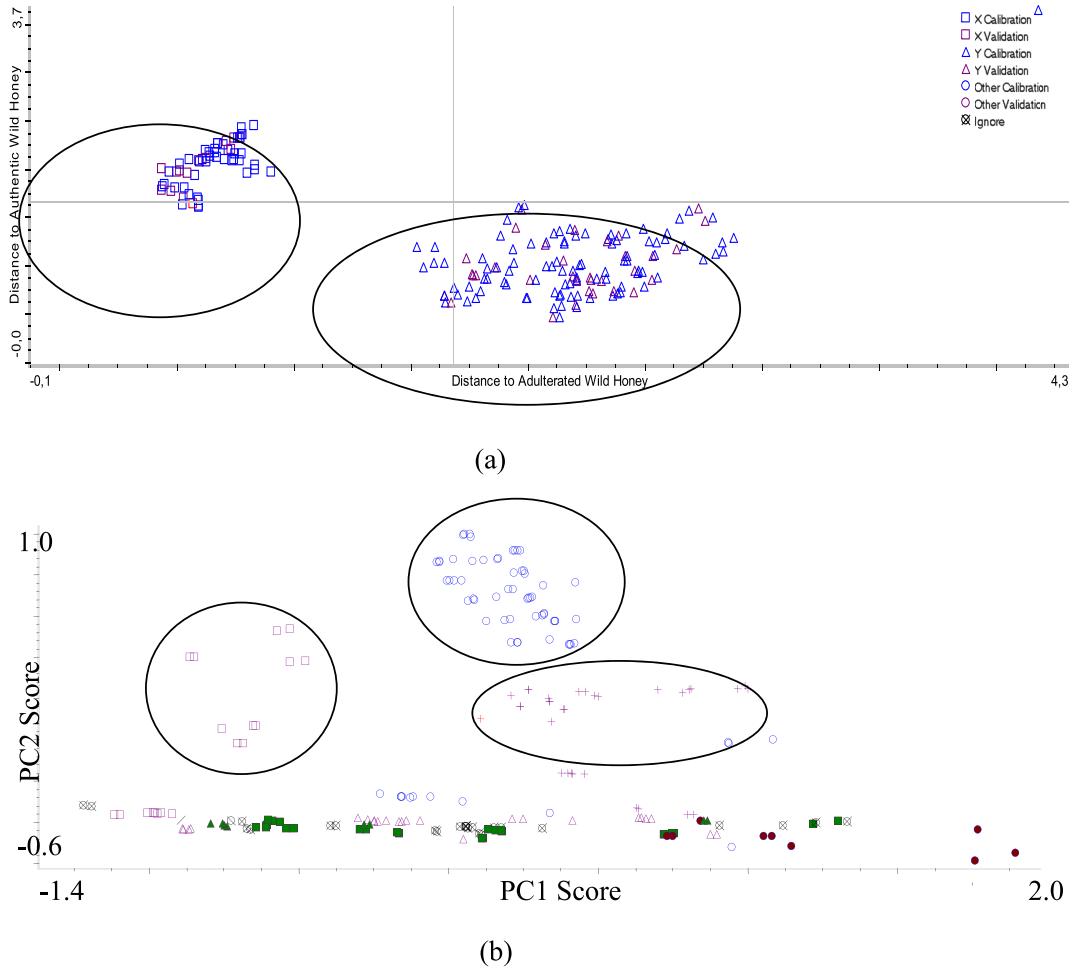


Figure 3. (a)Principle component analysis (PCA) for authentic wild honey (Δ) and adulterated wild honey (□).(b) PCA three-dimension for Indonesian wild honeys from different origin (+ Sumatra, Δ Java, □ BaBel, ● Kalimantan, Sulawesi, ■ NTT, and ⊗ NTB), and adulterated wild honey (○).

the same region chosen by Hennessy et al. (2008). Compared with a previous study of Anatolian Honey (Gok et al., 2015) which was achieved the best differentiation in the 1800–750 cm^{-1} region, for another study by Wang et al. (2010), 1500 - 800 cm would cover most of characteristic absorption bands relevant to major sugars. The different is specific wave number specifically contains information on sugar composition in region above 1800 cm^{-1}beside the anomeric region at 950–750 cm^{-1} which was frequently preferred for the spectral analysis of carbohydrates in IR spectroscopy. The evaluation of best region also was showed in PI value. The PI value represents the accuracy of the calibrated method that was used to classify the validated standard, The higher PI the closer the calculated concentration values are to the actual values. Average distance from the algorithm ratio was used to calculate PI using DA. The most significant wave numbers on variation in the discriminant model were 927, 1110, and 2933cm^{-1} (Figure 1).

Table 1. Range wave number of FTIR spectral and Performance Index region.

Wave number Range (cm^{-1})	Reference	Performance index (%)
4000–800	Hennessy et al. (2008)	91,8
1500–800	Wang et al., 2010	91,5
1800–750	Gok et al., 2015	91,5

Table 2. Evaluation of Performance PCA-DA class model based on the proportion authentic and adulterated honey.

Samples	N	FP(false positive)	FN(false negative)	TP (true positive)	TN (true negative)	Truepositive rate (sensitivity)	True negative rate (specificity)
Adulterated honeys	57	0,00	0,00	1,00	1,00	100,00	100,00
Authentic honeys	186	0,00	0,00	1,00	1,00	100,00	100,00
Sumatra	29	0,00	0,03	1,00	0,97	100,00	96,90
Bangka Belitung	18	0,00	0,02	1,00	0,98	100,00	98,45
Java	22	0,03	0,06	0,97	0,94	96,90	93,80
Kalimantan	5	0,00	0,03	1,00	0,97	100,00	96,90
NTB	22	0,02	0,05	0,98	0,95	98,45	95,35
NTT	25	0,09	0,08	0,91	0,92	91,47	92,25
Sulawesi	8	0,12	0,00	0,88	1,00	87,60	100,00

Sensitivity = TP/(TP + FN); specificity = TN/(TN + FP).

3.3. Evaluation of performance

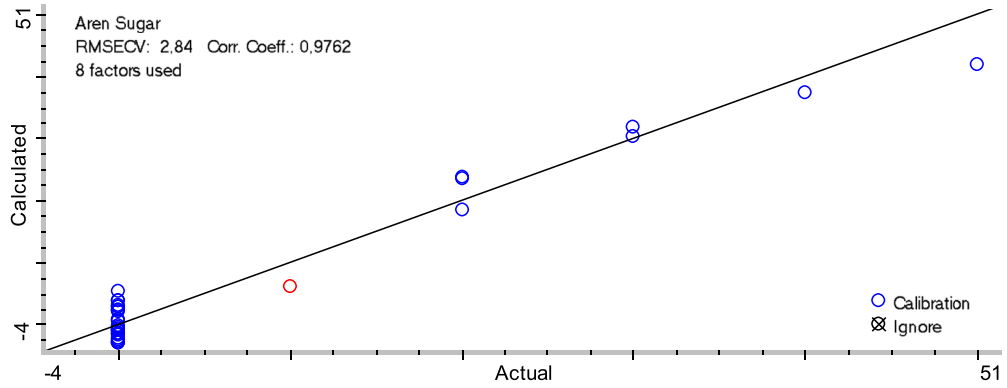
The model was evaluated in terms of sensitivity and specificity, the two statistical measurements that determine the performance of a binary classification system. The sensitivity of the group A model was calculated based on the proportion of authentic samples that can be identified as group A members or TP/(TP + FN), whereas specificity was calculated based on the proportion of non-group-A samples that can be rejected from group A or TN/(TN + FP),where TP, TN, FP, and FN are true positive, true negative, false positive, and false negative, respectively.

The samples belonging to the class being modeled and correctly found inside are called true positive (TP), false negative (FN) if they fall outside (Oliveri and Downey, 2012; Latorre et al., 2013). The authentic samples are the "positives" and the non-authentic samples the "negatives" under the hypothesis:

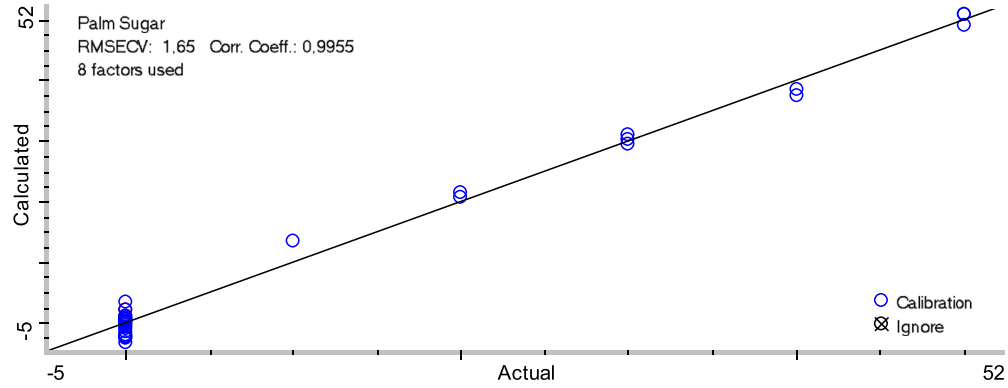
H0. The sample belongs the authentic population

H1. The sample does not belong to the authentic population (i.e. is not authentic).

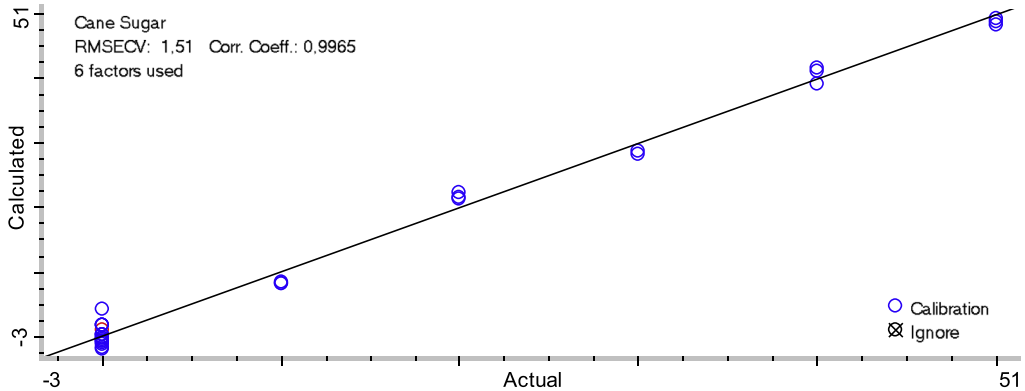
In a model with 100% sensitivity and specificity could identify authentic samples and reject adulterated ones (Latorre et al., 2013).



a



b



c

Figure 4. a. Linear regression curve of partial least-square partial least-square model of adulterated honey with10%–50% aren (*Arenga pinnata*) sugar. b. Linear regression curve of partial least-square partial least-square model of honey adulterated with 10%–50% palm sugar. c. Linear regression curve of partial least-square partial least-square model of honey adulterated with10%–50% cane sugar.

Table 3. Evaluation result of the partial least-square prediction model.

Adulterated honey sample with 10%–50% sugar	RC	RP	RCV	RMSEC (% v/v)	RMSEP (% v/v)	RMSECV (% v/v)
Arensugar	0.9988	0.9973	0.9762	0.185	1.54	2.84
Palm sugar	0.9997	0.9977	0.9965	0.442	0.940	1.65
Cane sugar	0.9988	0.9993	0.9968	0.626	0.666	1.51

RC: the calibration $R^{2;}$; RP: the predicted R^2; RCV: the cross validation R^2; RMSEC: a root mean square error calibration; RMSEP: a root mean square error of prediction; RMSECV: a root mean square error of cross validation. SavitzkyGolay filter, 15 point averaging, 3rd polynomial order.

Table 2 presents the sensitivity and specificity of authentic and adulterated honey samples with 100% accuracy; thus, the model could differentiate the samples. However, variation in classification based on sample origin were observed.

3.4. Quantitative analysis

The multivariate calibration model was developed using PLS, which was cross-validated, based on the partial least algorithm. This model was used to predict the commercial sugar content added into honey during adulteration. The frequency in the fingerprint region at 800–1700 cm^{-1} was then used to quantify the commercial sugar content in honey samples that were previously adulterated with 10%–50% of aren, palm, and cane sugar. For aren sugar, the analysis of the actual value on the*x*-axis and the FTIR prediction value (Figure 4a) resulted in a linear regression correlation with a measured R^2 (RC) of 0.9988 and a root mean square error calibration (RMSEC) of 0.185, whereas the predicted R^2 (RP) was 0.9973 with a root mean square error of prediction (RMSEP) of 1.54. For palm sugar, the actual value on the *x*-axis and the FTIR prediction value (Figure 4b) resulted in a linear regression correlation with an R Cof 0.9997, RMSEC of 0.442, RP of 0.9977, and RMSEP of 1.17. Furthermore, cane sugar (Figure 4c) showed a linear regression correlation, with an R Cof 0.99988, RMSEC of 0.626, RP of 0.9968, and RMSEP of 0.666. Here, R^2 represents the correlation level between actual and predicted values that were measured using FTIR. A higher R^2 indicated higher correlation; thus, R^2 value for the three calibration models of >0.99 was qualified according to the International Conference of Harmonization standard (ICH, 1994). RMSEC represents the uncertainty level of a model; thus a lower RMSEC is better. Based on the linear regression model obtained using PLS, commercial sugars added to honey can be predicted. Accordingly, authentic honey samples had a commercial sugar content of<10%; therefore, the model developed in this study could measure honey authenticity and detect added commercial sugars present in adulterated honey (Table 3).

The calibration model developed in this analysis was then cross-validated using the "leave-three-out" technique. This technique was selected based on Baumann's (2003) explanation to utilize the leave-multiple-out validation and avoid the leave-one-out cross-validation because of the over-fitting tendency and prediction errors. In the leave-three-out cross-validation technique, three samples were used interchangeably and calibrated into the PLS model and then cross-validated using the model. Cross-validation was applied to select the subset with the lowest root mean square error cross-validation (RMSECV) (Li et al., 2016). The results of cross-validation of aren sugar (RMSECV = 2.84; RCV = 0.9762), palm sugar (RMSECV = 1.65; RCV = 0.9965), and sugar cane (RMSECV = 1.51; RCV = 0.9968) were close to RMSEP, which indicates that the loss in the accuracy was very small when the calibration model was applied to new samples (Zhou et al., 2014).

4. Conclusions

Measurement of spectra using ATR-FTIR combined with the chemometric technique enabled the generation of grouping, classification, and a regression model that differentiated between authentic and adulterated IWH. Results confirmed that ATR-FTIR spectroscopy can be utilized as a rapid and nondestructive method for honey sample grouping, particularly to identify adulteration and measure added sugar concentrations. Utilization of PCA enabled the visualization of honey to differentiate between authentic and adulterated honey; however, it could not clearly differentiate wild honey based on origin, except differentiation between Sumatera and Bangka Belitung honey with the others. In general, PC-DA could classify authentic and adulterated honeys based on their geographical origins (Sumatera, Bangka Belitung, Java, Kalimantan, Sulawesi, NTB and NTT) with both sensitivity and specificity values of more than 87%. A linear regression model was developed using PLS to identify commercial sugar content (aren, palm, and cane sugar) in honey. The model enabled the determination of commercial sugar content added in IWH more than 10%.

Declarations

Author contribution statement

Y. Riswahyuli: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Abdul Rohman, Francis M.C.S Setyabudi: Analyzed and interpreted the data; Wrote the paper.

Sri Raharjo: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by an RTA Research grant from Universitas Gadjah Mada (2129/UN1/DITLIT/DIT-LIT/LT/2019). It was also supported by the National Agency of Drug and Food Control (NADFC) Republic of Indonesia/Badan PengawasObat dan Makanan (BPOM).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would like to thank Hermanto of Indonesia Wild Honey Network (IWHN)/Jaringan Madu Indonesia (JMHI), Budi Aulia and Afri Yondra of WWF Indonesia, Riau Program, Bahrizon of Riau beekeepers, Eman Sulaeman of Koperasi Hanjuang, Banten, Novemris Tefa, Asbanu Nicodemus and Manason Nubatonis of Gunung Mutis National Park, Anis Hayong of Rumadu, Flores, and Jalisman of Waslit, North Luwu, for supplying honey samples.

References

Andrade, J., Pereira, Cristina Guimarães, de Almeida Junior, José Carlos, Viana, Carolina Carvalho Ramos, de Oliveira Neves, Leandra Natália, da Silva, Paulo Henrique

Fonseca, Bell, Maria José Valenzuela, de Carvalho dos Anjos, Virgílio, 2019. FTIR-ATR determination of protein content to evaluate whey protein concentrate adulteration. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 99, 166–172.

Anguebes, F., Pat, L., Ali, B., Guerrero, A., Córdova, A.V., Abatal, M., Garduza, J.P., 2016. Application of multivariable analysis and FTIR-ATR spectroscopy to the prediction of properties in Campeche honey. J. Anal. Methods Chem. 2016.

Anjos, O., Campos, M.G., Ruiz, P.C., Antunes, P., 2014. Application of FTIR-ATR spectroscopy to the quantification of sugar in honey. Food Chem. 169, 218–223.

Baumann, K., 2003. Cross-validation as the objective function for variable-selection techniques. Trends Anal. Chem. 22, 395–406.

Colucci, G., De Vito, V., Varrichchio, E., De Cunzo, F., Coccia, E., Paolucci, M., Volpe, M., 2016. Identification of traceability markers in Italian unifloral honeys of different botanical origin. J. Nutr. Food Sci. 6 (1), 1–10.

Da Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C.O., Fett, R., 2016. Honey: chemical composition, stability, and authenticity. Food Chem. 196, 309–323.

Escuredo, O., Dobre, I., Fernández-González, M., Seijo, M.C., 2014. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. Food Chem. 149, 84–90.

Gallardo-Velázquez, T., Osorio-Revilla, G., de Loa, M.Z., Rivera-Espinoza, Y., 2008. Application of FTIR-HATR spectroscopy and multivariate analysis to the quantification of adulterants in Mexican honeys. Food Res. Int. 42 (3), 313–318.

Gok, S., Severcan, M., Goormaghtigh, E., Kandemir, I., Severcan, F., 2015. Differentiation of Anatolian honey samples from different botanical origins by ATR-FTIR spectroscopy using multivariate analysis. Food Chem. 170, 234–240.

Hennessy, S., Downey, G., O'Donnelll, C., 2008. Multivariate analysis of attenuated total reflection–fourier transform infrared spectroscopic data to confirm the origin of Honeys.pdf. Appl. Spectrosc. 62 (10), 1115–1123.

International Conference on Harmonisation (ICH), 1994. Validation of Analytical Procedures: Text and Methodology, 19/04/2011, Downloaded from: http://www.ich.org/fileadmin/Public/Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf.

Jandrić, Z., Haughey, S., Frew, R., McComb, K., Galvin-King, P., Elliott, C., Cannavan, A., 2014. Discrimination of honey of different floral origins by a combination of various chemical parameters. Food Chem. 2015.

Karoui, R., Downey, G., Blecker, C., 2010. Mid-infrared spectroscopy coupled with Chemometrics : a tool for the analysis of intact food systems and the exploration of their molecular structure - quality relationships - a review. Chem. Rev. 6144–6168.

Kaškoniene, V., Venskutonis, P.R., Ċeksteryte, V., 2010. Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 43 (5), 801–807.

Kasprzyk, I., Depciuch, J., 2017. FTIR-ATR spectroscopy of pollen and honey as a tool for unifloral honey authentication. The case study of rape honey. Food Contr. 84, 33–40.

Kelly, J.D.A.K., Etisco, C.R.P., Owney, G.E.D., 2006. Application of fourier transform midinfrared spectroscopy to the discrimination between Irish artisanal honey and such honey adulterated with various sugar syrups. J. Agric. Food Chem. 54 (17), 6166–6171.

Latorre, C.H., Peña Crecente, R.M., García Martín, S., Barciela García, J., 2013. A fast chemometric procedure based on NIR data for authentication of honey with protected geographical indication. Food Chem. 141 (4), 3559–3565.

Li, S., Zhang, X., Shan, Y., Su, D., Ma, Q., Wen, R., Li, J., 2016. Qualitative and quantitative detection of honey adulterated with high-fructose corn syrup and maltose syrup by using near-infrared spectroscopy. Food Chem. 218, 231–236.

Lohumi, S., Lee, S., Lee, H., Cho, B.K., 2015. A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. Trends Food Sci. Technol. 46 (1), 85–98.

Nalda, M.J.N., Yagüe, J.L.B., Diego Calva, J.C., Gómez, M.T.M., 2005. Classifying honeys from the Soria Province of Spain via multivariate analysis. Anal. Bioanal. Chem. 382 (2), 311–319.

Nikolova, K., Panchev, I., Sainov, S., Gentscheva, G., 2015. Selected physical properties of lime bee honey in order to discriminate between pure honey and honey adulterated with glucose. Int. J. Food Prop. 15, 1358–1368.

Oliveri, P., Downey, G., 2012. Multivariate class modeling for the verification of food-authenticity claims. Trac. Trends Anal. Chem. 35, 74–86.

Padovan, G.J., De Jong, D., Rodrigues, L.P., Marchini, J.S., 2003. Detection of adulteration of commercial honey samples by the 13 C/12 C isotopic ratio. Food Chem. 82, 633–636.

de Ribeiro, R.O.R., Mársico, E.T., da Carneiro, C.S., Monteiro, M.L.G., Júnior, C.C., de Jesus, E.F.O., 2014. Detection of honey adulteration of high fructose corn syrup by Low Field Nuclear Magnetic Resonance (LF 1H NMR). J. Food Eng. 135, 39–43.

Sari, R.K., Bertoni, R., 2014. Kajian manfaat Madu hutan anggota JMHI terhadap penyakit kanker dan anti aging. Jaringan Madu Hutan Indonesia Scientific Report.

Sarwono, B., 2001. Kiat Mengatasi Permasalahan Praktis Lebah Madu. Agro Media Pustaka, Jakarta.

Siddiqui, A.J., Musharraf, S.G., Choudhary, M.I., Rahman, A., 2017. Application of analytical methods in authentication and adulteration of honey. Food Chem. 217, 687–698.

Sivakesava, S., Irudayaraj, J., 2001. Prediction of inverted cane sugar adulteration of honey by fourier transform infrared spectroscopy. J. Food Sci. 66 (7), 972–978.

Soares, S., Amaral, J.S., Oliveira, M.B.P.P., Mafra, I., 2017. A comprehensive review on the main honey authentication Issues : production and origin. Compr. Rev. Food Sci. Food Saf. 16 (5), 1072–1100.

Tewari, J.C., Irudayaraj, J.M.K., 2005. Floral classification of honey using mid-infrared spectroscopy and surface acoustic wave based z-Nose sensor. J. Agric. Food Chem. 53 (18), 6955–6966.

Tornuk, F., Karaman, S., Ozturk, I., Toker, O.S., Tastemur, B., Sagdic, O., Kayacier, A., 2013. Quality characterization of artisanal and retail Turkish blossom honeys: determination of physicochemical, microbiological, bioactive properties and aroma profile. Ind. Crop. Prod. 46, 124–131.

Tosun, M., 2012. Detection of adulteration in mulberry pekmez samples added various sugar syrups with 13C/12C isotope ratio analysis method. Food Chem. 165 (2–3), 555–559.

Wang, J., Kliks, M.M., Jackson, M., Li, Q.X., 2010. Rapid analysis of glucose, fructose, sucrose, and maltose in honeys from different geographic regions using fourier transform infrared spectroscopy and multivariate analysis. J. Food Sci. 75 (2), 1–16.

Zhou, J., Yao, L., Li, Y., Chen, L., Wu, L., Zhao, J., 2014. Floral classification of honey using liquid chromatography-diode array detection-tandem mass spectrometry and chemometric analysis. Food Chem. 145, 941–949.

Zhu, X., Li, S., Shan, Y., Zhang, Z., Li, G., Su, D., Liu, F., 2010. Detection of adulterants such as sweeteners materials in honey using near-infrared spectroscopy and chemometrics. J. Food Eng. 101 (1), 92–97.