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RIGHT-ANGLE FLUORESCENCE SPECTROSCOPY COUPLED WITH PLS-DA FOR DISCRIMINATION OF INDONESIAN PALM CIVET COFFEE

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ABSTRACT

Indonesian palm civet coffee is regarded as one the most expensive and rarest coffee in the world. Authentication of civet coffee is recently becoming important due to high potential of adulteration and mislabelling of Indonesian palm civet coffee with cheaper non-civet coffee. In the present study, right-angle fluorescence spectroscopy coupled with partial least squares discriminant analysis (PLS-DA) were used for discrimination of Indonesian palm civet coffee. Total 60 samples were used (30 samples for civet and non-civet coffee, respectively). All coffee samples were ground using a home-coffee-grinder. Since particle size in coffee powder has a significant influence on the spectra obtained, we sieved all coffee samples through a nest of U. S. standard sieves (mesh number of 40) on a Meinzer II sieve shaker for 10 minutes to obtain a particle size of 420 μm. The experiments were carried out at room temperature (around 27-29°C). All samples were extracted with distilled water and then filtered. For each samples, 3 mL of extracted sample then was pipetted into 10 mm cuvettes for spectral data acquisition. Excitation emission spectra were obtained for civet and non-civet coffee samples by acquiring emission spectra from 210 to 750 nm (interval 1 nm) with excitation in the range of 200-550 nm (interval 5 nm) using JASCO FP-8300 Fluorescence Spectrometer. The result showed that the discrimination model was successfully developed by PLS-DA and resulted in high performance of discrimination between civet and non-civet coffee with 100% of discrimination rate. The study demonstrated that the right-angle fluorescence spectroscopy technique combined with PLS-DA method has high potential in rapid and accurate qualitative analysis of Indonesian palm civet coffee discrimination

Keywords : Fluorescence spectroscopy; PLS-DA; Civet coffee; Discrimination; Authentication.

I. INTRODUCTION

Civet coffee is one of the most expensive coffees in the world due to its unique taste and rare production [1]. Most of the commercially available civet coffees are ground roasted civet coffee. On account of the high price of civet coffee as compared to non-civet coffee, undeclared additions of cheaper non-civet coffee (mislabelling) is commonly practiced for purposes of economy, which makes civet coffee fraud a real problem in Indonesia. For this reason, maintaining high production standards of civet coffee may enhance fair trading competition in the civet coffee industry as well as satisfaction of consumers. Therefore, there is need for developing analytical techniques to control authenticity of civet coffee samples.

Several analytical methods have been established to develop coffee authentication system and detect the level of adulteration. In general, the techniques most widely used are classified into two groups: based on spectroscopic and chromatographic methods: UV-Vis spectroscopy [1–4], NIR spectroscopy [5–7], gas chromatography (GC) [8], [9] and high performance of liquid chromatography (HPLC) [10]. Chromatographic methods are accurate but relatively expensive, time consuming and require highly skilled operators [11].

In the previous reports, several studies have been published on the authentication of civet coffees quantitatively and qualitatively using UV-vis spectroscopy and NIR spectroscopy [1], [12]. Recently, UV-Vis spectroscopy shows a great potential for civet authentication with acceptable result [1], [13], [14]. UV-Vis spectroscopy is simpler, less expensive and quicker than other widely used methods (NIR spectroscopy, HPLC and others). However, this method is less sensitive and its spectra are more overlapped compared to fluorescence spectroscopy.

So far the use of fluorescence spectroscopy for civet coffee authentication has not received sufficient attention. Therefore, the objective of this research was to investigate the application of fluorescence spectroscopy and partial least squares-discriminant analysis (PLS-DA) method for the discrimination between pure civet and pure non-civet coffees.

II. MATERIALS AND METHODS

A. Samples

Roasted coffee samples (civet and non-civet) were purchased at local farmers at Liwa, Lampung province, Indonesia. A number of 30 samples of civet coffee and 30 samples of non-civet coffee were used as samples. Each samples has 1 gram weight. An aqueous extraction procedure of the coffee samples was performed based on the previous reported studies [1], [2]. For PLS-DA, the samples were divided randomly into two groups: calibration sample set (50 samples) and prediction sample set (10 samples).

B. Fluorescence spectral data acquisition

The EEM (excitation-emission matrix) spectral data of coffee samples were obtained using a spectrofluorometer (JASCO model FP-8300, Tokyo, Japan) by dropping 3 mL of samples into 10 mm cuvettes in the excitation range from 200 nm to 500 nm (5 nm steps) and the emission range from 210 nm to 750 nm (1 nm steps). The excitation and emission bandwidth were both 5.0 nm. The software of Spectra Manager (JASCO Co., Tokyo, Japan) was performed to manage the EEM spectral data acquisition. This software was also used for spectral data pre-treatment such as fluorescence correction and finding peak for high intensity of EEM spectral data.

C. Data analysis using PLS-DA method

PLS-DA is working based on a PLS regression algorithm. PLS-DA attempts to build models that can maximize the separation among the classes of objects. For more detailed theory about the PLS-DA algorithm can be found in the literatures [15–17]. In this study, for developing a PLS-DA model, each sample in the calibration set was assigned a dummy variable as a reference value (variable *y*), which is an arbitrary number designating whether the sample belongs to a particular class or not [18] (1 = civet coffee; 2 = non-civet coffee). The PLS-DA calibration model was developed using the calibration sample set and the optimal number of PLS factors was determined using the lowest RMSECV value.

The PLS-DA calibration model was evaluated based on the following parameter: number of PLS factors, coefficient of determination (R^2_{cal}), and the RMSECV. The performance of the PLS-DA model was evaluated using the prediction sample set using ±0.5 as a threshold value to delimit the classes. In this study, a coffee sample was classified as civet coffee if its value was below 1.5 and classified as non-civet coffee if the value was above 1.5.

III. RESULTS AND DISCUSSION

A. Fluorescent spectra of civet and non-civet coffee.

The measurement of the excitation-emission matrix (EEM) for different coffee samples: civet and non-civet coffee, are shown in Fig. 1 and Fig. 2. It is seen that a peak at around excitation of 370 nm and emission of 453 was observed at both civet and non-civet coffee sample. This peak may be related to the typical emission spectra of flavonoid content in coffee.

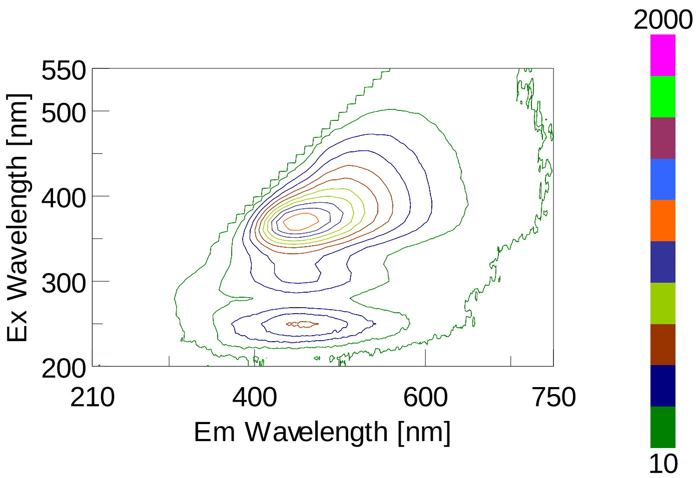

Fig. 1. Excitation-emission spectra of non-civet coffee

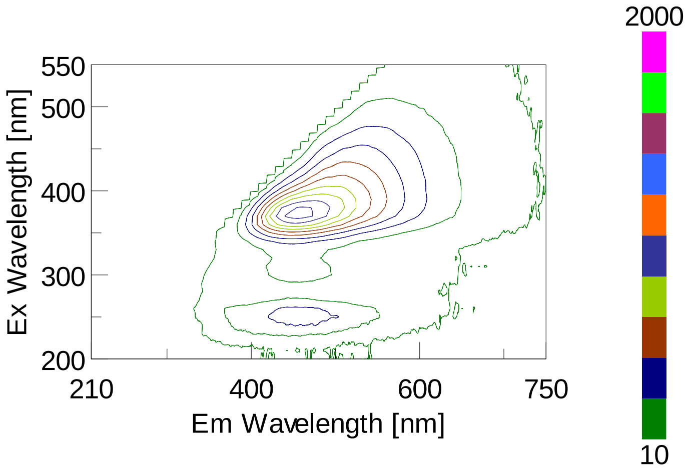

Fig. 2. Excitation-emission spectra of of civet coffee

B. Principal component analysis of all samples.

PCA is applied for all samples (30 civet and 30 non-civet) using emission spectra at 370 nm of excitation wavelength. Fig. 3 shows the score of PC1 and PC2 of the result of PCA on spectra dataset. Here, PC1 and PC2 in total could be able to explain 99% of the variation on the coffees spectral data. From Fig. 3, it can be seen that civet and non-civet coffee could be well separated. The civet coffee samples was located on the left quadrant and non-civet coffee samples was on the right one.

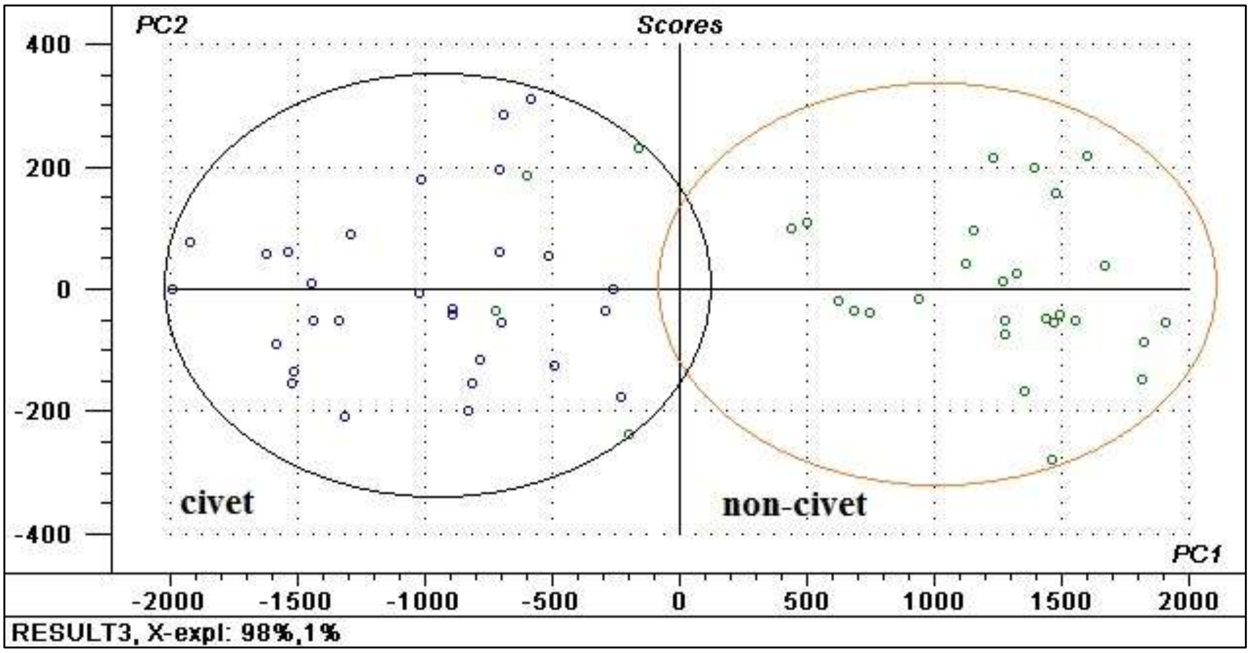

Fig. 3. PCA score plot for civet and non-civet coffees.

C. Developing PLS-DA calibration model

Using PLS regression algorithm, a PLS-DA model for discrimination between civet and non-civet coffee was developed on original spectra. The optimal number of PLS factors included in model was selected using the lowest root mean square error of cross validation (RMSCV). Fig. 4 shows the relationship between PLS factors and RMSECV. The lowest RMSECV was obtained at 9 PLS factors.

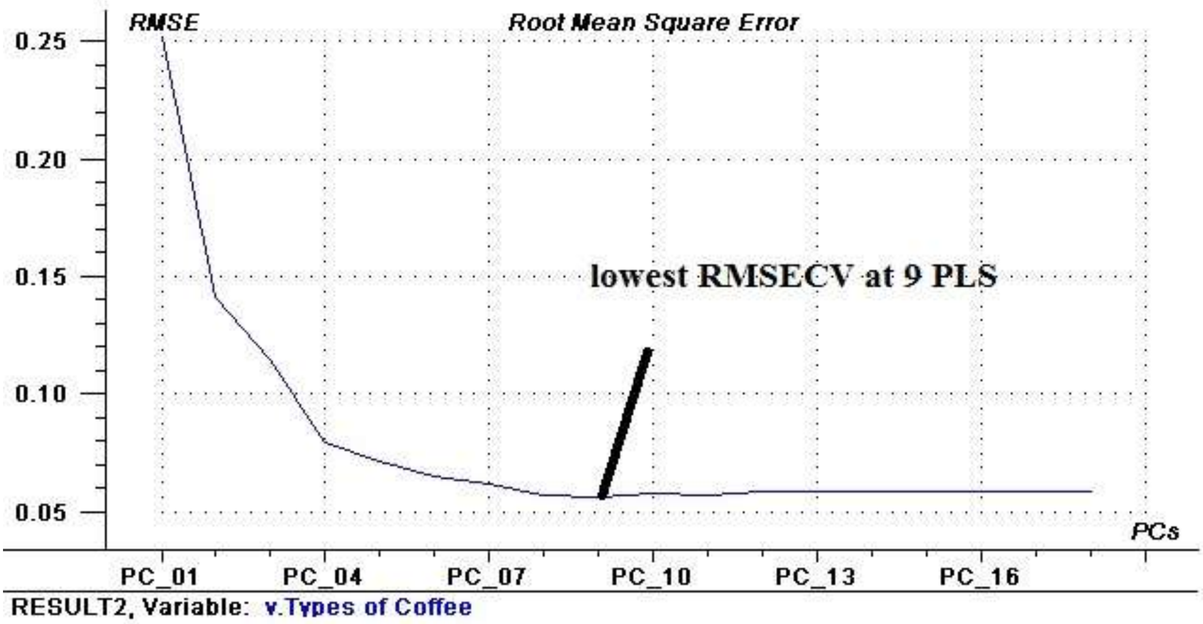

Fig. 4. Plot of PLS factors versus RMSECV for determination of type of coffee samples.

Fig. 5 shows the scatter plot of actual and predicted values of type of coffees using 9 PLS factor for calibration and validation, respectively. The calibration resulted in high coefficient of determination (R^2_{cal}=0.99). Low RMSECV could be obtained (RMSECV= 0.057). This PLS-DA model is used to predict the type of coffee (civet or non-civet coffee) in the prediction sample set.

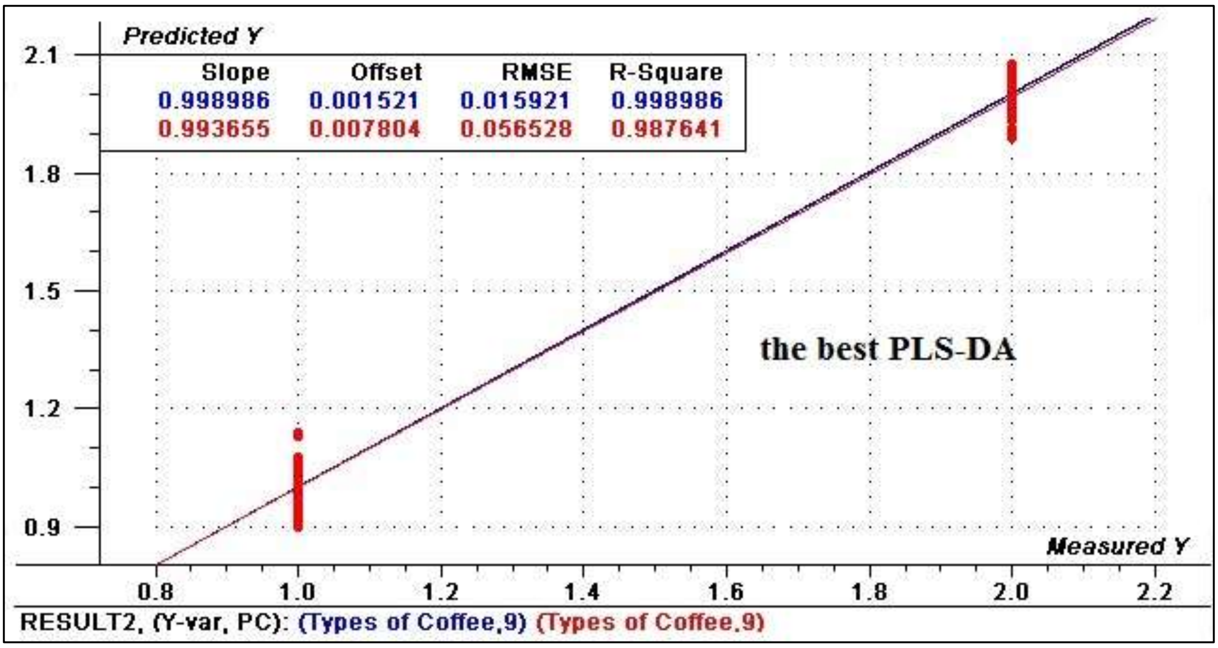

Fig. 5. Scatter plot between actual and predicted type of coffees for calibration and validation using PLS regression method

D. Prediction of type of coffee using PLS-DA model

Using the developed calibration model, a prediction for type of coffee was done using prediction sample set (10 samples). Tabel 1 shows the prediction result. We can see here that actual values are very close similar to that of predicted values.

Using student *paired t-test*, there is no any significant differences between the actual and predicted values. All prediction samples classify properly into its correspondence class (100% discrimination rate).

Table 1. The result of prediction using developed PLS-DA model for discrimination between civet and non-civet coffees.

Sample	Predicted values	Deviation	Reference/Actual values
Civet	1.065	0.071	1.000
Civet	1.029	0.071	1.000
Civet	1.053	0.063	1.000
Civet	1.042	0.066	1.000
Civet	1.026	0.076	1.000
Non-civet	2.072	0.091	2.000
Non-civet	2.025	0.063	2.000
Non-civet	2.100	0.076	2.000
Non-civet	2.073	0.067	2.000
Non-civet	2.057	0.075	2.000

IV. CONCLUSION

This study demonstrated the potential application of using fluorescence spectral data in the authentication of civet coffee samples. The right-angle fluorescence measurement was successfully applied to identify civet and non-civet coffee samples. In conclusion, this present study achieved the discrimination of civet and non-civet coffee. The combination of fluorescence spectroscopy and PLS-DA method for civet and non-civet coffee resulted in good prediction with 100% rate of discrimination. The right-angle fluorescence method can be used as a sensitive screening tool for the authentication of Indonesian civet coffee samples.

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REFERENCES

[1] D. Suhandy, and M. Yulia, "The use of partial least square (PLS) regression and spectral data in UV-visible region for quantification of adulteration in Indonesian palm civet coffee," *Int. J. Food Sci*., 2017 (*accepted manuscript*).

[2] D. Suhandy, and M. Yulia, "Peaberry coffee discrimination using UV-visible spectroscopy combined with SIMCA and PLS-DA. *Int. J. Food Prop*., 2017 (*accepted manuscript*).

[3] U.T.C.P. Souto, M.J.C. Pontes, E.C. Silva, R.K.H. Galvão, M.C.U. Araújo, F.A.C. Sanches, F.A.S. Cunha, and M.S.R. Oliveira, "UV-Vis spectrometric classification of coffees by SPA-LDA, " *Food Chem*., vol. 119, pp. 368–371, 2010.

[4] U.T.C.P. Souto, M.F. Barbosa, H.V. Dantas, A.S. Pontes, W. Lyra, P.H.G.D. Diniz, M.C.U. Araújo, and E.C. Silva, "Identification of adulteration in ground roasted coffees using UV-Vis spectroscopy and SPA-LDA", *Food Sci. Technol.,* vol. 63, pp. 1037–1041, 2015.

[5] C. Pizzaro, I. Esteban-Díez, and J.M. González-Sáiz, "Mixture resolution according to the percentage of robusta variety in order to detect adulteration in roasted coffee by near infrared spectroscopy", *Anal. Chim. Acta*, vol. 585, pp. 266–276, 2007.

[6] J.R. Santos, M. Lopo, A. Rangel, and J.A. Lopes, "Exploiting near infrared spectroscopy as an analytical tool for on-line monitoring of acidity during coffee roasting," *Food Control*, vol. 60, pp. 408–415, 2016.

[7] R. Calvini, A. Ulrici, and J.M. Amigo, "Practical comparison of sparse methods for classification of Arabica and Robusta coffee species using near infrared hyperspectral imaging, " *Chem. Intell. Lab. Syst*., vol. 146, pp. 503–511, 2015.

[8] F. Carrera, M. León-Camacho, F. Pablos, and A.G. González, "Authentication of green coffee varieties according to their sterolic profile," *Anal. Chim. Acta*, vol. 370, pp. 131–139, 1998.

[9] M.J. Martin, F. Pablos, A.G. González, M.S. Valdenebro, M.L. Carnacho, "Fatty acid profiles as discriminant parameters for coffee varieties differentiation, " *Talanta*, vol. 54, pp. 291–297, 2001.

[10] E.D. Pauli, F. Barbieri, P.S. Garcia, T.B. Madeira, V.R. Acquaro Junior, I.S. Scarminio, C.A.P. Camara, and S.L. Nixdorf, "Detection of ground roasted coffee adulteration with roasted soybean and wheat, " *Food Res. Int.*, vol. 61, pp. 112–119, 2014.

[11] P. Satora, M. Kostrz, P. Sroka, and T. Tarko, "Chemical profile of spirits obtained by spontaneous fermentation of different varieties of plum fruits, *"Eur. Food Res. Technol.*, vol. 243, pp. 489–499, 2017.

[12] D. Suhandy, and M. Yulia, "A method to compensate the influence of different particle size of coffee powder in NIR calibration model performance", In proceedings of AESAP, pp. 6-10, 2016.

[13] D. Suhandy, M. Yulia, S. Waluyo, N. Sugianti, R. Iriani, F.N. Handayani, and N. Apratiwi, "The Potential use of ultraviolet-visible spectroscopy and soft independent modelling of class analogies (SIMCA) for classification of Indonesian palm civet coffee (kopi luwak)", In proceeding of The USR International Seminar on Food Security (UISFS), pp.245-253, 2016.

[14] M. Yulia, D. Suhandy, S. Waluyo, and C. Sugianti, "Detection and quantification of adulteration in luwak coffee through ultraviolet-visible spectroscopy combined with chemometrics method", In proceeding of The USR International Seminar on Food Security (UISFS), pp.254-261, 2016.

[15] S. Wold, M. Sjostrom, and L. Eriksson, "PLS-regression: a basic tool of chemometrics", *Chem. Intell. Lab. Syst.*, vol. 58, pp. 109–130, 2001.

[16] M. Barker, and W.S. Rayens, "Partial least squares for discrimination", *J. Chemom.*, vol.17, pp. 166–173, 2003.

[17] L. Xie, Y. Ying, and T. Ying, "Combination and comparison of chemometrics methods for identification of transgenic tomatoes using visible and near-infrared diffuse transmittance technique", *J. Food Eng.*, vol. 82, pp. 395–401, 2007.

[18] D. Ballabio, and V. Consonni, "Classification tools in chemistry. Part 1: linear models. PLS-DA", *Anal. Methods*, vol. 5, pp. 3790–3798, 2013.