

## DETECTION AND QUANTIFICATION OF ADULTERATION IN LUWAK COFFEE THROUGH ULTRAVIOLET-VISIBLE SPECTROSCOPY COMBINED WITH CHEMOMETRICS-METHOD

MEINILWITA YULIA<sup>1</sup>, DIDING SUHANDY<sup>2\*</sup>, SRI WALUYO<sup>2</sup> AND  
CICIH SUGIANTI<sup>2</sup>

<sup>1</sup>) Department of Agricultural Technology, Lampung State Polytechnic, Jl. Soekarno Hatta  
No. 10, Rajabasa Bandar Lampung, Lampung, Indonesia.

<sup>2</sup>) Department of Agricultural Engineering, The University of Lampung, Jl. Soemantri  
Brojonegoro No. 1 Gedong Meneng Bandar Lampung, Lampung, Indonesia 35145.

\*corresponding author: [diding.sughandy@fp.unila.ac.id](mailto:diding.sughandy@fp.unila.ac.id)

### ABSTRACT

Luwak coffee is the most expensive coffee in the world and currently, the authentication of Luwak coffee has become very important due to the possible adulteration of Luwak coffee with non-Luwak coffee. In this research, we investigated the potential application of using ultraviolet-visible (UV-Vis) spectroscopy combined with chemometric techniques (partial least square/PLS1) for quantification of adulteration in Luwak coffee. The adulterant was Arabica coffee which was added into Luwak coffee with degree of adulteration in the range of 10-50%. A number of 30 samples were used. All samples were extracted with distilled water and filtered. The spectral acquisition was performed with 10 mm of cuvette cell using a UV - Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA) in the range of 200-500 nm. PLS1 model correlates the actual and UV-Vis estimated values of adulterants (concentration of Arabica coffee in Luwak coffee) with coefficients of correlation ( $r$ ) of 0.99 and 0.97 for calibration and validation, respectively. The low RMSECV values of 0.044 gram could be obtained. The method, therefore, is potential to be used as a rapid method for quantification of adulterant in Luwak coffee.

**Keywords:** Luwak coffee, chemometrics, PLS1 regression, calibration, UV-Vis spectroscopy

### 1. INTRODUCTION

Coffee is one of the most popular beverages in the world (Duarte *et al.*, 2005) with high consumption level in developed countries; 4 kg per capita in the US and 5 kg per capita in Europe

([http://www.worldmapper.org/posters/worldmapper\\_1038\\_coffee\\_consumption\\_ver2.pdf](http://www.worldmapper.org/posters/worldmapper_1038_coffee_consumption_ver2.pdf)). On other hand, coffee is mostly produced in several developing countries. Four countries account for more than half of the world's production: Brazil, Vietnam, Colombia and Indonesia. ([http://www.worldmapper.org/posters/worldmapper\\_1037\\_coffee\\_production\\_ver5.pdf](http://www.worldmapper.org/posters/worldmapper_1037_coffee_production_ver5.pdf)).

Luwak coffee is a name for Arabica or Robusta coffee which has been eaten by Asian palm civet (*Paradoxurus hermaphroditus*). The coffee bean which is eaten by Asian palm civet (*Paradoxurus hermaphroditus*) is the best and ripest berries. After several hours inside the civet animal, the best coffee berries passed through the digestive tract of civet animal resulted in a unique flavour of Luwak coffee. This kind of unique production is a reason why the production of Luwak coffee is very limited and worldwide it is approximately only 250-500 kg per year (<http://www.most-expensive.coffee>).

Luwak coffee is one of the most expensive and the rarest coffee in the world. Due to its commercial importance, detection of adulterated matters has been a constant concern in fraud verification, especially when it is difficult to percept adulterations with the naked eye in samples of Luwak roasted coffee ground. The inspection of adulteration materials becomes more difficult in samples of Luwak powder coffee. In Indonesia, Luwak coffee is adulterated with other cheaper non-Luwak coffee. Around 70% of Luwak coffee or civet coffee available at coffee stores and the internet (online store) is not 100% pure Luwak coffee and sometimes it does not contain anything of the genuine coffee (<http://www.most-expensive.coffee>).

In order to protect the authenticity of Luwak coffee, it is important to develop a simple method which can be used to detect and quantify the degree of adulteration. Recently, there is no internationally accepted method of verifying whether a bean is civet coffee or Luwak coffee. Traditionally, coffee aroma has been used to characterize coffee quality. Sensory panel evaluation is commonly used to assess the aroma profile of coffee. However, this technique has some limitations. For example, it is quite difficult to train the panel effectively in order to limit subjectivity of human response to flavour and the variability between individuals (Shilbayeh and Iskandarani, 2004). Human sensory method is also difficult to verify the authenticity of Luwak coffee when a small amount of adulterated materials such as cheaper non-Luwak coffee is added. Indonesia, as one of important player in Luwak coffee production, is now just starting to develop an advanced technology for coffee processing. It is including a search for a novel inspection system for Luwak coffees characterization.

This technology is very important for coffee industry to protect high expensive Luwak coffees from any adulteration. In the previous study, Souto *et al.* (2010) reported the use of UV-Visible spectroscopy as an analytical method for the identification of adulterations in ground roasted coffees (due to the presence of husks and sticks). This analytical method is one of the most common and inexpensive techniques used in routine analysis and it will be compatible with situation in Indonesia for further technology development. For this reason, in this research,

we attempt to use UV-Visible spectra combined with PLS regression method to detect and quantify content of adulterant in Luwak- Arabica blend coffee samples.

## **2. MATERIALS AND METHODS**

### **2.1. Sample preparation**

A number of 1 kg ground roasted Luwak coffee (Indonesian palm civet coffee) samples were collected directly from coffee farmers at Liwa, Lampung, Indonesia (Hasti coffee Lampung). Another 1 kg ground roasted Arabica coffee samples were also provided for making Luwak coffee adulteration. All coffee samples were ground using a home-coffee-grinder (Sayota). Since that particle sizes in coffee powder has significant influence to spectral analysis, it is important to use same particle size in coffee powder samples (Suhandy *et al.*, 2016). In this research we use particle size of 420  $\mu\text{m}$  by sieving through a nest of U. S. standard sieves (mesh number of 40) on a Meinzer II sieve shaker (CSC Scientific Company, Inc. USA) for 10 minutes. The experiments were performed at room temperature (around 27-29°C). In this research we prepare 30 samples of Luwak-Arabica blend coffee samples which different content of adulteration. The adulteration content range is 0-50% by adding Arabica coffee into Luwak coffee samples.

The spectral acquisition of Luwak-Arabica blends coffee samples were done in solution samples. For this purpose, an aqueous extraction procedure of the coffee samples was performed as described by Souto *et al.* (2010). First, 1.0 g of each sample was weighed and placed in a glass beaker. Then, adding 10 mL of distilled water at 90-98°C then mixed with magnetic stirring (Cimarec™ Stirrers, model S130810-33, Barnstead International, USA) at 350 rpm for 5 min. Then the samples were filtered using a 25 mm pore-sized quantitative filter paper coupled with an erlenmeyer. After cooling process to room temperature (for 20 min), all extracts were then diluted in the proportion of 1:20 (mL: mL) with distilled water. UV-Vis-NIR spectra from the aqueous extracts were acquired using a UV-Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA).

### **2.2. Spectral acquisition**

The spectral data of coffee blend (Luwak-Arabica) were acquired using UV-Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA) in the range of 200-450 nm. This spectrometer is a dual-beam spectrometer equipped with 5 cell sample holder and 1 cell for blank or reference holder. The wavelength accuracy is 1 nm with dual silicon photodiodes as detector and Xenon flash as illumination source.

The absorbance data of Luwak-Arabica coffee blend in solution samples were acquired in the range of 200-450 nm at room temperature. For this, we put 2 mL of solution samples into cuvettes. Before the sample measurements step, blank (the same distilled water used in extraction process) was placed inside reference cell holder to adjust the 100% transmittance signal.

### 2.3. PLS regression

The correlation between spectral data and content of adulteration (content of Arabica) was investigated using partial least squares (PLS) regression. The spectral data has many overlapped information. Some information is important and it has strong relation to the target response (content of adulteration). However, some information is not related to the target response. So, the general idea of PLS is to try to extract those information. PLS find several latent factors which account for most of the variation in the response. For this reason, the acronym PLS has also been taken to mean “projection to latent structure.” It should be noted, however, that the term “latent” does not have the same technical meaning in the context of PLS as it does for other multivariate techniques. In particular, PLS does not yield consistent estimates of what are called “latent variables” in formal structural equation modelling (Dykstra, 1983). PLS regression model has been used widely for multivariate data analysis including spectral data for qualitative and quantitative analysis from UV to terahertz region (Suhandy *et al.*, 2012; Shan *et al.*, 2014)

In this study, PLS regression model was developed using The Unscrambler® version 9.7 (CAMO, Oslo, Norway), statistical software for multivariate calibration. A student’s t-test was performed using Statistical Package for the Social Science (SPSS) version 11.0 for Windows in order to evaluate the significance level of the model.

## 3. RESULTS AND DISCUSSION

### 3.1. Spectral data of Luwak-Arabica coffee blend

In Fig. 1 we can see the original spectra of coffee blend (Luwak-Arabica) in the range of 200-450 nm. We notice the difference of absorbance intensity due to difference of content of adulterant (content of Arabica). However, the spectral difference is not consistent and it may be due to baseline differences. In order to remove the influence of baseline effect, we processed the spectra using smoothing and derivation algorithm (Savitzky-Golay 1<sup>st</sup> derivative with polynomial order 2 and number of segments 11) as shown in Fig. 2. Here we can see that it is not easy to see the spectral differences among the samples having different content of adulterant. So it is really necessary to use multivariate analysis to extract such kind of spectral information. From Fig. 1 and 2 we can see that there are several peaks which may

be related to the information of content of adulteration. The peak at 280 nm can be found both in original and processed spectra. This wavelength is close to the wavelength related to caffeine absorbance at 272 nm in the previous study (Belay *et al.*, 2008). The shift of caffeine absorbance peak (from 272 nm to 280 nm), it might be happen due to the presence of other component in coffee solution (not pure distilled water).

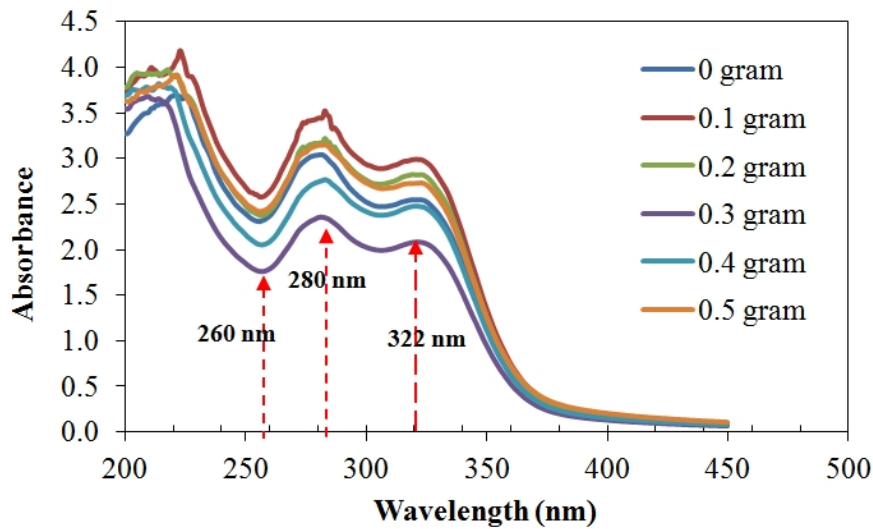


Figure 1. Original spectra of coffee blend (Luwak-Arabica) with different content of adulterant (Arabica) in the range of 200-450 nm

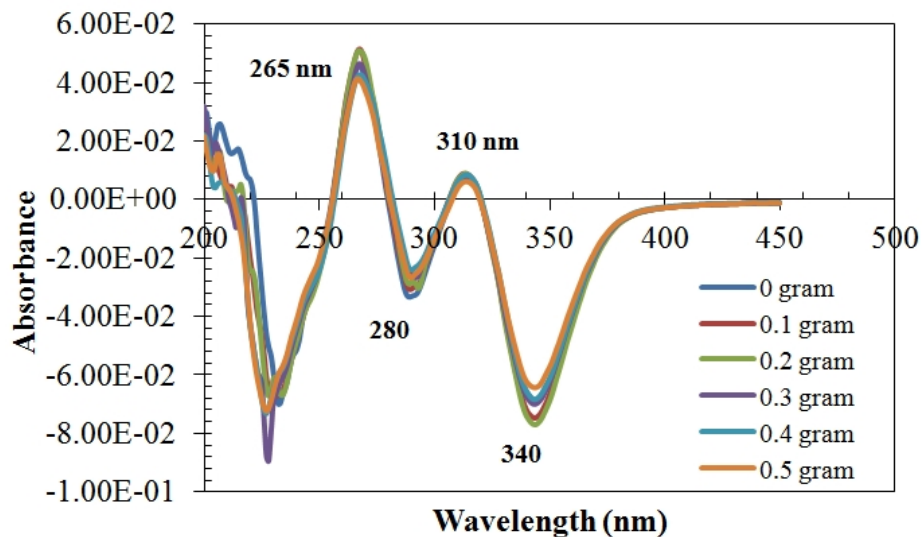


Figure 2. Processed spectra (smoothing +Savitzky-Golay derivation) of coffee blend (Luwak- Arabica) with different content of adulterant (Arabica) in the range of 200-450 nm.

### 3.2. Developing a PLS regression model for prediction content of adulteration

Here we perform a quantitative study for prediction content of adulteration in Luwak-Arabica coffee blend. For this purpose, we develop a PLS regression model using all wavelength in the range of 200-450 nm as predictor ( $x$  variables) and content of adulteration

or content of Arabica coffee added into Luwak-Arabica coffee blend as target response ( $y$  variable). Fig. 3 shows the result of PLS regression model in calibration step. It can be seen that there is a strong correlation between actual content of adulteration and predicted one using UV-Vis spectra with high coefficient of determination ( $R^2$ ) = 0.99 and low RMSEC = 0.013884 gram. The developed PLS model was well validated as shown in Fig. 4. The cross-validation resulted in low RMSECV = 0.044242 gram and low bias = 0.003850 gram.

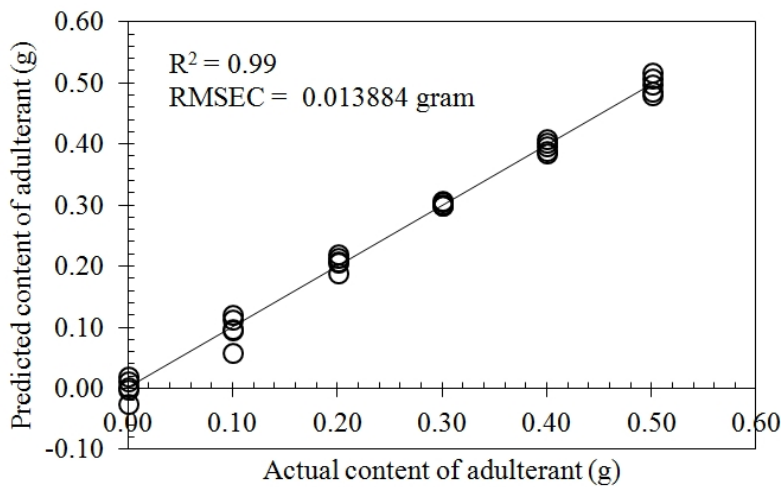


Figure 3. Scatter plot between actual and predicted content of adulteration in calibration step in the range 200-450 nm.

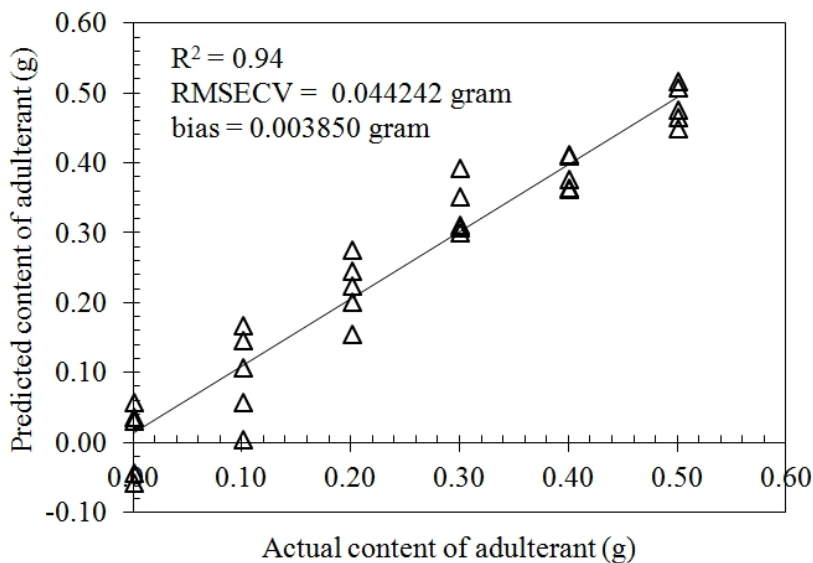


Figure 4. Scatter plot between actual and predicted content of adulteration in cross-validation step in the range 200-450 nm.

By a 95% confidence pair t-test, there were no significant differences between the actual content of adulteration and that predicted by UV-Visible spectroscopy. This result



showed that a calibration model for determination content of adulteration in Luwak-Arabica blend using UV-Visible spectroscopy could be well developed.

In order to understand the structure of the developed PLS model, we plot a relationship between the wavelength and regression coefficient as shown in Fig. 5. It can be noticed several wavelengths have significant value of regression coefficient. Those wavelengths are 275 nm, 300 nm, 342 nm and 378 nm. The wavelength at 275 nm is related to absorption of caffeine. The wavelength at 300 nm may be related to absorption of caffeic acid (Souto *et al.*, 2010).

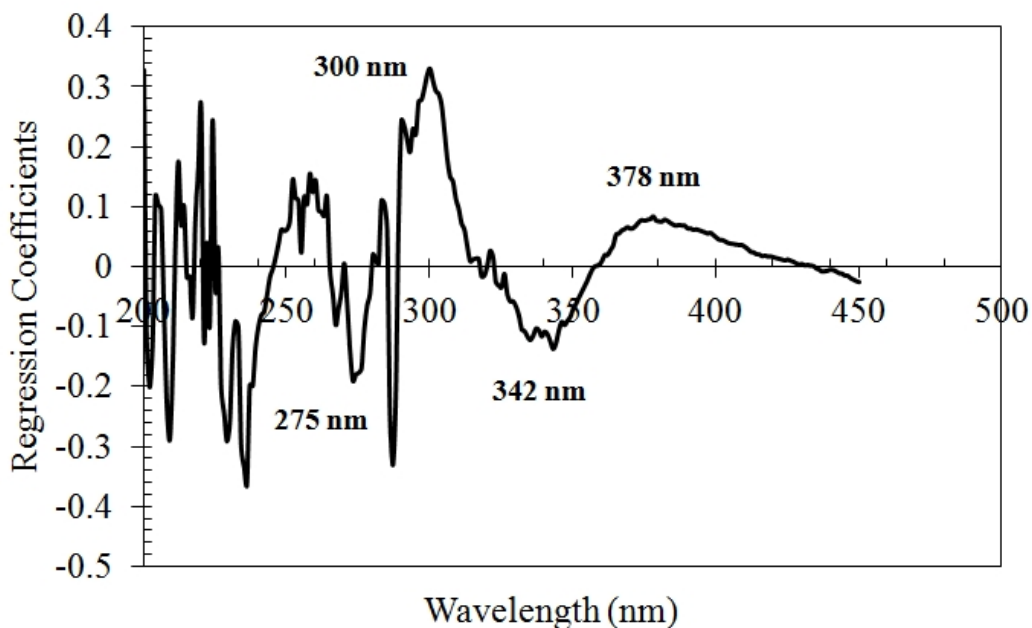


Figure 5. The regression coefficient versus wavelength plot of PLS model determination for prediction content of adulteration in Luwak-Arabica coffee blend.

This study has demonstrated the promising application of using PLS regression model for prediction the content of adulteration in Luwak-Arabica blend coffee samples. The developed PLS model resulted in a strong correlation between actual and predicted content of adulteration with  $R^2 = 0.99$ . The cross-validation resulted in low bias. By a 95% confidence pair t-test, there were no significant differences between the actual content of adulteration and that predicted by UV-Visible spectroscopy. This result may open a development of simple and fast method to detect and quantify the content of adulteration in Luwak-Arabica blend coffee samples.

## ACKNOWLEDGEMENT

This research is part of Penelitian Strategis Nasional (STRANAS) 2016 which is funded by Ministry of Research, Technology and Higher Education (KEMENRISTEKDIKTI) Indonesia (Nomor: 419/UN26/8/LPPM/2016). We acknowledge this support. We thank also Hasti coffee Lampung for providing us the samples.

## REFERENCES

- Belay, A., Ture, K., Redi, M., and Asfaw, A. 2008. Measurement of caffeine in coffee beans with UV/vis spectrometer. *Food Chemistry* **108**: 310–315.
- Dijkstra, T. 1983. Some comments on maximum likelihood and partial least squares methods. *Journal of Econometrics*, **22**: 67–90.
- Duarte, S.M.d.S., Abreu, C.M.P.d., Menezes, H.C.d., Santos, M.H.d., Gouvea, C.M.C.P. 2005. Effect of processing and roasting on the antioxidant activity of coffee brews. *Food Sci. Technol. (Campinas)* **25**:387–393.
- Shan, J., Suzuki, T., Suhandy, D., Ogawa, Y., and Kondo, N. 2014. Chlorogenic acid (CGA) determination in roasted coffee beans by Near Infrared (NIR) spectroscopy. *Engineering in Agriculture, Environment and Food*, **7**(4): 139–142.
- Shilbayeh, N.F. and Iskandarani, M.Z. 2004. Quality control of coffee using an electronic nose system. *American Journal of Applied Sciences* **1**(2): 129–135.
- Souto, U.T.C.P., Pontes, M.J.C., Silva, E.C., Galvão, R.K.H., Araújo, M.C.U., Sanches, F.A.C., Cunha, F.A.S., and Oliveira, M.S.R. 2010. UV–Vis spectrometric classification of coffees by SPA–LDA. *Food Chemistry* **119**: 368–371.
- Suhandy, D., Suzuki, T., Ogawa, Y., Kondo, N., Naito, H., Ishihara, T., and Liu, W. 2012. A Quantitative study for determination of glucose concentration using attenuated total reflectance terahertz (ATR-THz) spectroscopy. *Engineering in Agriculture, Environment and Food*, **5**(3): 90–95.
- Suhandy, D., Waluyo, S., Sugianti, C., Yulia, M., Iriani, R., Handayani, F.N., and Apratiwi, N. 2016. The Use of UV-Vis-NIR Spectroscopy and Chemometrics for Identification of Adulteration in Ground Roasted Arabica Coffees -Investigation on the Influence of Particle Size on Spectral Analysis-. *Proceeding of Seminar Nasional Tempe. Bandar Lampung, 28 Mei 2016.*