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# The authentication of peaberry and civet ground roasted robusta coffee using UV-visible spectroscopy and PLS-DA method with two different particle sizes

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**Abstract.** The objective of this research was to investigate the influence of particle size of ground roasted coffee samples in the authentication of peaberry and civet coffee using UV-visible spectroscopy combined with partial least squares-discriminant analysis (PLS-DA) method. For this purpose, we provide a total of 80 samples of peaberry and civet coffee with two different particle size of 1680 micrometer (mesh 12) and 297 micrometer (mesh 50). The all coffee samples were extracted using hot distilled water (90-98°C). UV-visible spectral data of the all coffee samples were acquired using a UV-visible spectrometer in transmittance mode. The result showed that the different absorbance spectra were observed for the different particle sizes of peaberry and civet coffee samples. The absorbance of samples having particle size of 297 micrometer (mesh 50) is higher than that of particle size of 1680 micrometer (mesh 12). The calibration model was developed using PLS-DA method for samples having same particle size and resulted in acceptable result with high coefficient of determination ( $R^2$ ) ( $R^2=0.99$  both for mesh 12 and 50, respectively), low root mean square error of calibration (RMSEC), and high residual prediction deviation (RPD). However, the performance of the prediction results was very low for samples having different particle size with high bias and high root mean square error of prediction (RMSEP).

## 1. Introduction

Peaberry coffee ('kopi lanang' in Indonesian language) and civet coffee ('kopi luwak' in Indonesian language) are one of the expensive specialty coffees in the world. A peaberry (also called caracol or snail in Spanish) is a natural mutation of the coffee bean inside the cherry. Normally two coffee beans grow in a fruit (dicotyledonous)—flat against each other like halves of a peanut; however, on rare occasions a single bean is produced (monocotyledon) [1]. Civet coffee is any coffee bean (arabica or robusta) which has been eaten and passed through the digestive tract of Asian palm civet (*Paradoxurus*



*hermaphroditus*), which uses its keen senses to select only the best and ripest berries [2-3]. The production of both peaberry and civet coffees are very limited. For this reason, peaberry and civet coffees have been a target of food adulteration.

Nowadays, the keyword of food authentication is becoming popular. This authentication system may give benefit to both producers and customers. For this reason, the area of research for food authentication is attracting many researchers. The development of new analytical method for food authentication system is one of the research focus in this area. No exception for coffee authentication system. Several analytical methods have been proposed for coffee authentication such as NIR and mid spectroscopy [4-9], fluorescence spectroscopy [10-11], image processing/computer vision [12], nuclear magnetic resonance (NMR) spectroscopy [13], and electronic nose [14]. However, most of these methods are expensive and not easy to follow.

Previously, Suhandy and coworkers has developed an authentication and quality evaluation of ground roasted coffee using UV-visible spectroscopy and chemometrics [1-2, 15-17]. This method is simpler and cheaper. However, all those reported studies used coffee samples with same particle size (same mesh) for developing calibration and validation model. Those researches involved procedure of sieving of ground roasted coffee that is laborious and time consuming. Therefore, for practical application, it is needed to remove the procedure of sieving and establish calibration model of coffee authentication which can be universally used for any particle sizes. However, there is lack of research regarding to relationship between particle sizes of ground roasted coffee and quality of spectral data in UV-visible region. In this research, an investigation on the influence of particle size of ground roasted coffee samples in the authentication of peaberry and civet coffee was conducted using UV-visible spectroscopy combined with partial least squares-discriminant analysis (PLS-DA) method.

## 2. Materials and Methods

### 2.1. Civet and peaberry coffee samples

In this research, 80 samples of peaberry and civet coffee with two different particle size of 1680 micrometer (mesh 12) and 297 micrometer (mesh 50) were prepared (1.0 gram weight for each samples). The samples were collected from coffee farmer in West Lampung, Lampung province of Indonesia. The extraction of coffee samples was performed by using hot distilled water. The samples were divided into two groups: calibration and validation set (30 samples for peaberry and civet, respectively) and prediction set (10 samples for peaberry and civet, respectively).

### 2.2. Acquisition of UV-visible spectral data

The spectral data of aqueous civet and peaberry coffee samples were acquired in the range of 190-1100 nm by using a UV-Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA). This spectrometer was equipped with a quartz cell with optical path of 10 mm. The spectral acquisition was done at spectral resolution of 1 nm at a room temperature (about 27-28°C). The original spectra were used for chemometric analysis.

### 2.3. Chemometric analysis

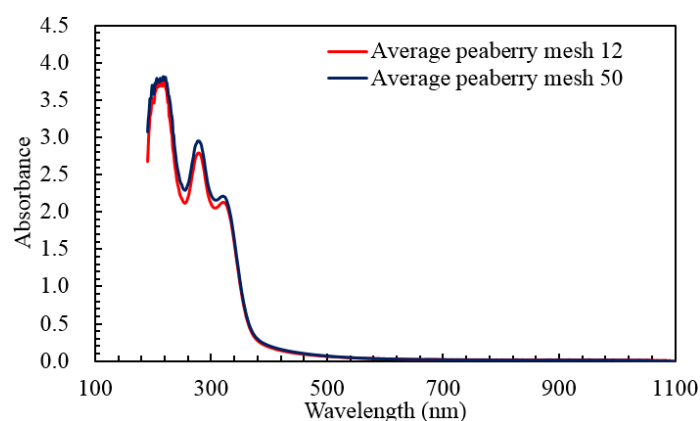
To study the influence of particle sizes of ground roasted coffee for authentication of peaberry and civet coffee, two methods were attempted. First is using principal component analysis or PCA (unsupervised chemometric analysis) method. The PCA was performed using all samples (80 samples) using original spectral data in the range of 250-350 nm. The second method is using PLS-DA or partial least squares-discriminant analysis. In this PLS-DA method, a PLS regression was employed with a dummy variable as a reference value (variable Y). In this research, the response variable Y is composed of 0's and 1's, where the value 1 is assigned to peaberry coffee samples and the value 0 to civet coffee samples. To evaluate the performance of PLS-DA model, the coefficient of determination ( $R^2$ ), root mean square error of calibration (RMSEC) and root mean square error of cross-validation (RMSECV) are used in this research. It is expected to have ideal models with lower RMSEC and RMSECV as well as higher

$R^2$ . In the prediction, two statistic parameters were used to assess the model: SEP (standard error of prediction) and bias. Those two parameters should be as low as possible. PCA and PLS-DA were performed using the multivariate software of the Unscrambler 9.7 (CAMO software AS, Oslo, Norway).

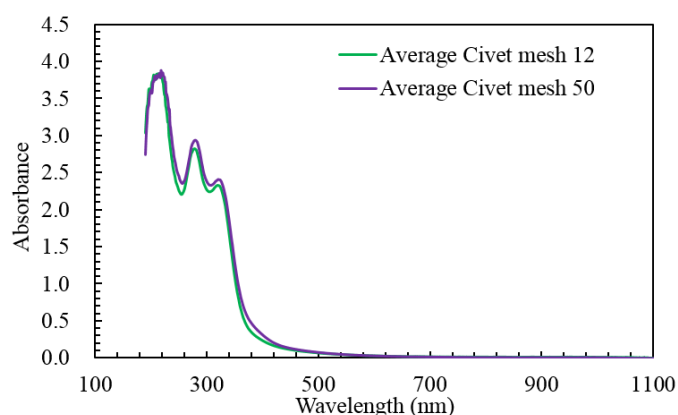
### 3. Results and Discussion

#### 3.1. UV-visible spectral data of civet and peaberry coffee with different particle sizes

UV-visible spectral data of peaberry coffee with different particle sizes was depicted in Figure 1. Each data (peaberry mesh 12 and 50) was averaging of 10 spectral data. From Figure 1, we can notice that there is a different of peaberry spectral data between mesh 12 and 50 especially in the wavelength of 250-350 nm. The intensity of absorbance of peaberry with mesh 50 is higher than that of mesh 12. The similar phenomenon was observed for civet coffee as seen in Figure 2. This result showed that the quality of spectral data in UV-visible spectroscopy of coffee samples is highly influenced by particle sizes of ground roasted coffee samples.



**Figure 1.** Average original spectra of peaberry coffee samples with different particle sizes (mesh 12 and 50) in the range of 190-1100 nm.

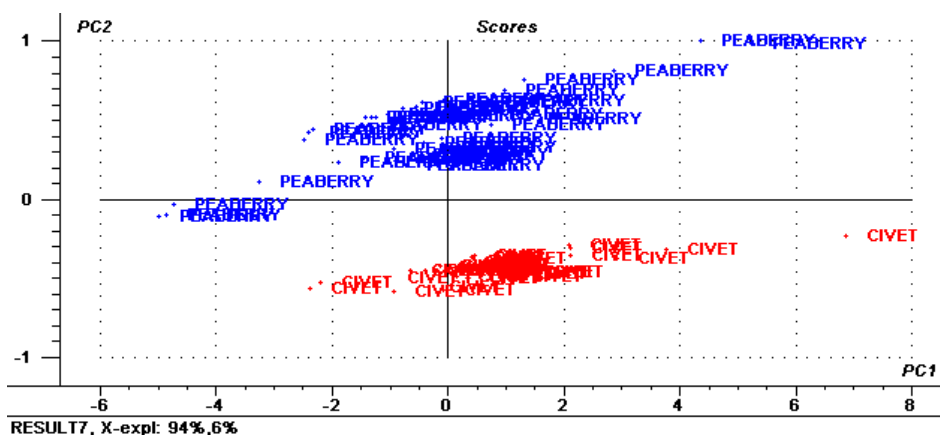


**Figure 2.** Average original spectra of civet coffee samples with different particle sizes (mesh 12 and 50) in the range of 190-1100 nm.

#### 3.2. Unsupervised method using PCA analysis

Figure 3 showed the result of PCA analysis of all samples (80 samples) using original spectra in the range of 250-350 nm. Using two PCs (PC1 and PC2), a clear separation between civet and peaberry coffee samples was observed. The total of PC1 and PC2 can explain 100% of the total variance of original data. From here, we cannot see clearly the influence of different particle sizes on separation of

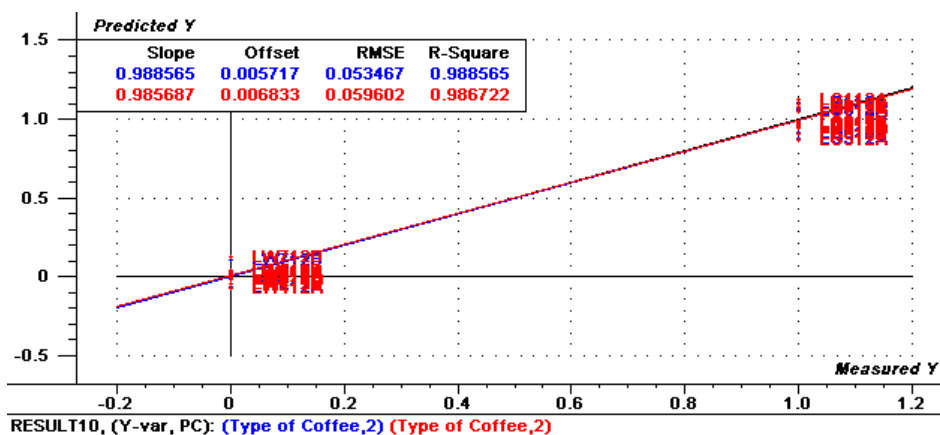
civet and peaberry. More quantitative analysis should be conducted using PLS-DA in order to see the influence of particle sizes on discrimination between civet and peaberry ground roasted coffee.



**Figure 3.** The result of PCA analysis of all samples using original spectra in the range of 250-350 nm.

3.3. *PLS-DA model using mesh 12*

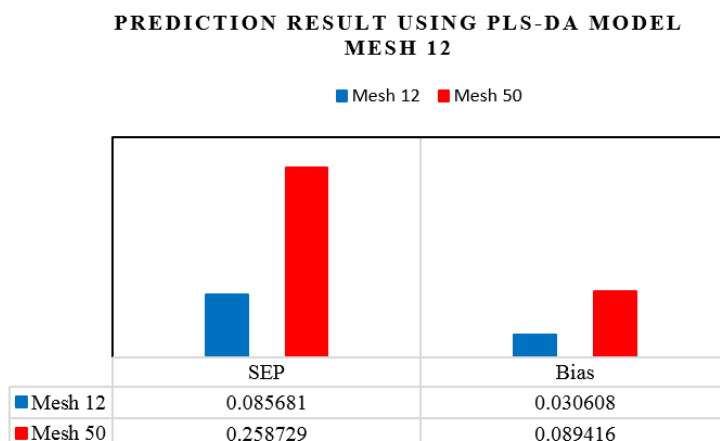
PLS-DA model was developed using calibration and validation samples with mesh 12. These samples consist of 15 samples of peaberry (mesh 12) and 15 samples of civet (mesh 12). The result was showed in Figure 4. The coefficient of determination was very high ( $R^2 = 0.99$ ) for both calibration and validation. The RMSEC = 0.053 and RMSECV = 0.060. It can be seen that all peaberry samples are very close to 1 and civet coffee samples are very close to 0.



**Figure 4.** PLS-DA model developed using mesh 12 of original spectra in the range of 250-350 nm.

3.4. *Prediction samples of mesh 12 and 50 using PLS-DA model mesh 12*

The performance of PLS-DA model mesh 12 was evaluated in two ways. First, the developed PLS-DA model mesh 12 was used to predict samples having mesh 12. Second, it was used to predict the samples having mesh 50. The result was showed in Figure 5. In term of SEP and bias, the prediction of samples having mesh 12 resulted in low SEP and bias (SEP = 0.085681 and bias = 0.030608). However, the result of the prediction of samples having mesh 50 became worst with increasing both the value of SEP and bias (SEP = 0.258729 and bias = 0.089416). This result has confirmed that the performance of PLS-DA model for civet and peaberry discrimination is highly influenced by particle sizes of the ground roasted coffee samples. For this, in the future for practical application, it is highly needed to develop PLS-DA model that can compensate the influence of particle sizes of coffee samples.



**Figure 5.** The result of prediction of mesh 12 and 50 using PLS-DA model mesh 12.

#### 4. Conclusion

In this research, we showed the influence of particle sizes of ground roasted coffee samples in the authentication of peaberry and civet coffee. The influence was not so clear based on the result of PCA. However, using PLS-DA method, the influence was seen clearly. It is confirmed that the performance of PLS-DA model for civet and peaberry discrimination is highly influenced by particle sizes of the ground roasted coffee samples. For this, in the future for practical application, it is highly needed to develop PLS-DA model that can compensate the influence of particle sizes of coffee samples. By doing this, a procedure of sieving may be removed. Finally, faster authentication process of coffee samples using UV-visible spectroscopy could be established.

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