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Using UV-Visible Spectroscopy Coupled with Linear Discrimination Analysis to Discriminate between Monofloral and Multifloral Honey from Indonesia

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Abstract. In the market, two types of honey are available: monofloral and multifloral honeys. Monofloral honey has been regarded having higher market values than multifloral one. In order to protect a fraud trading between monofloral and multifloral honeys, it is very important to develop an analytical method which can be used to discriminate the two type of honeys. We utilized spectral data in the UV-visible region (250-450 nm) coupled with linear discrimination analysis (LDA) to classify monofloral and multifloral honeys from Indonesia. Total 400 samples of monofloral and multifloral honeys were used as samples. The spectral data were recorded using UV-vis spectrometer in the wavelength of 190-1100 nm with 1 nm of resolution. Several preprocessing methods was applied to improve the quality of spectral data. Principal component analysis (PCA) was calculated to map the samples and the PCA scores were used as input for classification task using linear discriminant analysis (LDA). The result suggested that UV-Visible spectroscopy is a powerful tool for quality evaluation of Indonesian honeys.

INTRODUCTION

Recently, there is an increasing consumer demand for premium quality of agricultural products. Honey is no exception. Two important factor that significantly influence the price of honey product is differentiated by "flavor," which generally indicates floral source [1] and geographical (region or territorial) origin of the honey [2]. In term of floral source, generally in the market, two types of honey are available: monofloral and multifloral honeys. As defined by Lenhardt *et al.* [2] monofloral honey is honey that contains with more than 45% of pollen concentration from a single species while multifloral honey contains nectar or honeydew from different plant species. In the market, monofloral honey has higher market values than multifloral one, especially from unusual monofloral sources (e.g., the acacia tree) [1].

In Indonesia, there are several types of monofloral honeys such as durian honey, longan honey, sun flower honey and etc. The big disparity in term of price between authentic monofloral honey with multifloral honey has promoted the occurrence of honey adulteration. Two common type of adulteration in honey is falsely-labelled Indonesian monofloral honey and the addition of sugar and cheaper multifloral honey [3]. For this reason, it is important to precisely identify the pollen type of marketed honeys.

For many years, the melissopalynological technique (pollen analysis by microscope) has been used for the determination of floral origin for honey [4]. Several analytical methods also have been developed to determine the botanical origins and to verify the authenticity of the honey samples by its chemical and sensory properties such as high performance liquid chromatography (HPLC) [5], gas chromatography mass-spectrometry (GC–MS) [6-7], ion-

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chromatography (IC) [8], near infrared spectroscopy [9-10], fluorescence spectroscopy [11] and mid-infrared spectroscopy [12].

UV-visible spectroscopy has been popular and widely used especially attractive for its simplicity and low cost for authentication of several high quality of agricultural products such as specialty coffee [13-17], tea [18-19], wine [20] and olive oil [21]. A simpler and cheaper analytical method for honey authentication based on UV-visible spectroscopy also has been reported [22]. However, the previous works utilized ethanol solution for sample extraction. To our best knowledge, the dilution of honey sample using distilled water for UV-visible measurement has not been reported yet. For this reason, in this present research, UV-visible spectroscopy with water dilution coupled with linear discriminant analysis (LDA) was proposed to discriminate between monofloral and multifloral Indonesian honey.

MATERIALS AND METHODS

Honey Samples and Sample Preparation

Total 400 samples of *Apis dorsata* monofloral of durian and multifloral Indonesian honeys were used as samples. All honey samples were purchased directly from beekeepers. Prior to spectral measurement, samples were heated using a water batch at 60°C for 30 minutes in order to melt the crystals of honey and then keep at room temperature [23]. Each honey sample was diluted using a distilled water in a proportion of 1:20 (mL: mL).

Data Acquisition and Spectral Transformation

The spectral data were recorded in transmittance mode using a benchtop UV-Vis spectrometer in the wavelength of 190-1100 nm with 1 nm of resolution (Genesys™ 10S UV-Vis, Thermo Scientific, USA). Two types of spectral data were used: original and transformed spectral data. Original spectral data was directly obtained from spectral data measurement without any spectral preprocessing. Transformed spectral data was obtained by applying three different spectral preprocessing simultaneously: multiplicative scatter correction (MSC), moving average 3 segments (MA 3s) and Savitzky–Golay first derivative with 3 segments and ordo 2 (SG 1d 3s).

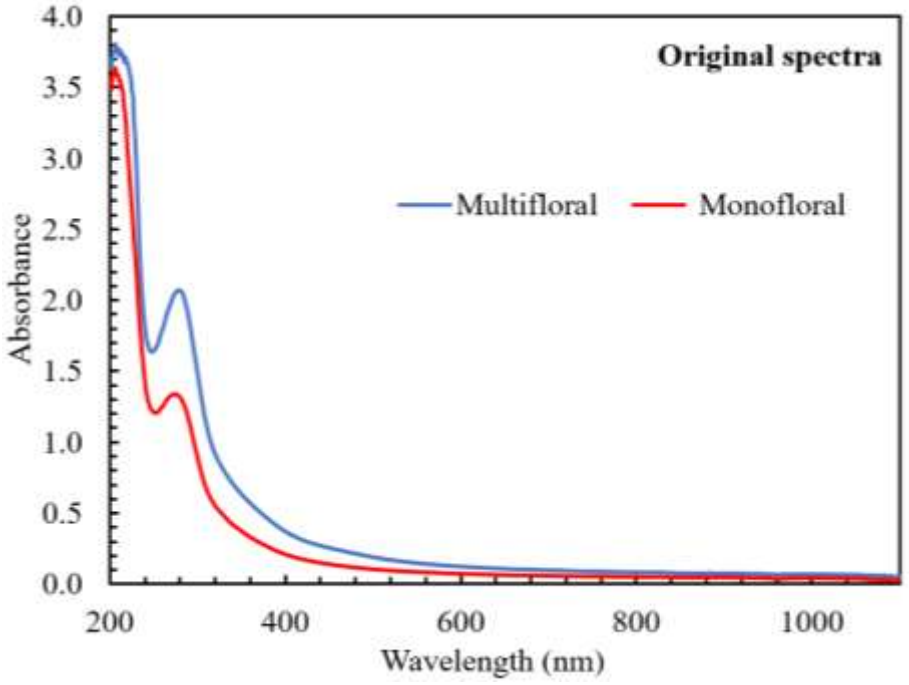
Development of Linear Discriminant Analysis (LDA)

Principal component analysis (PCA) was employed on both original and transformed spectra in order to perform unsupervised classification. To develop a supervised classification, the samples were randomly divided into groups: a calibration set (334 samples) used for developing LDA model and prediction set (66 samples) used for evaluating the performance of the LDA model. The LDA model was developed for original and transformed spectral data based on the first five principal components (PCs) [24]. The percentage of correct classification (%CC) was calculated to assess the performance of LDA model. The Unscrambler X version 10.4 (64-bit) (Camo Software AS, Oslo, Norway) was used to perform spectral preprocessing, PCA and LDA calculation.

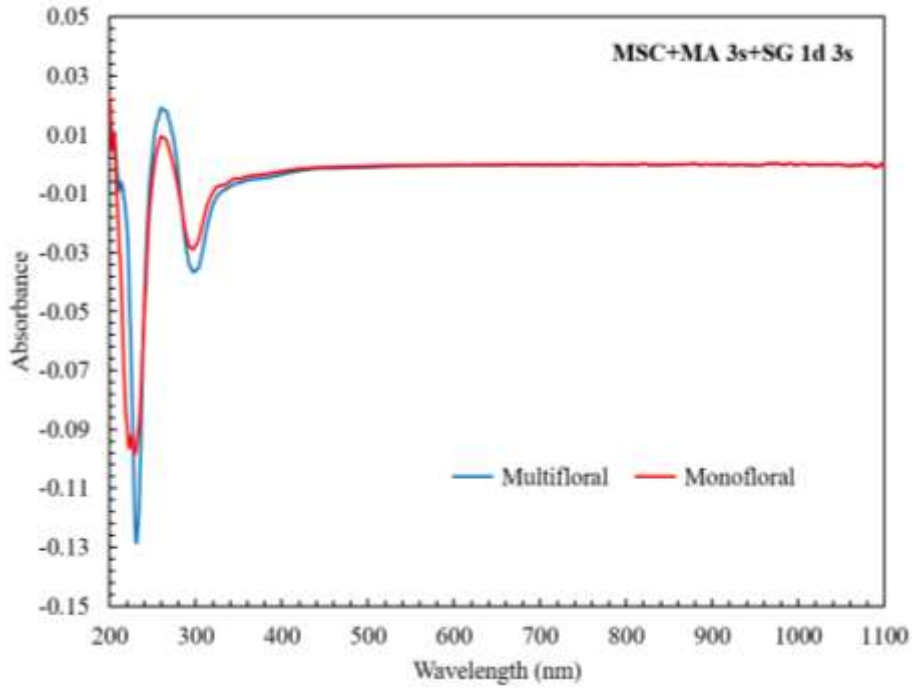
RESULTS AND DISCUSSION

Spectral Data of Monofloral and Multifloral Honey Samples

The average original spectra (a) and transformed spectra (b) of monofloral and multifloral honey samples were demonstrated in Figure 1. The intensity of absorbance of multifloral honey was higher than that of multifloral honey both in original and transformed spectral data. One important finding from Figure 1 is about informative and sensitive spectral region. It was concluded that spectral region of 250-450 nm contained several important peaks related to absorbance of honey chemical composition. Two observed peaks at 260 nm and 300 nm was related to the absorbance of benzoic, salicylic and aryl-alyphatic acids [25]. Based on this result, the calculation of PCA and LDA was performed on selected spectral region of 250-450 nm.



(a)

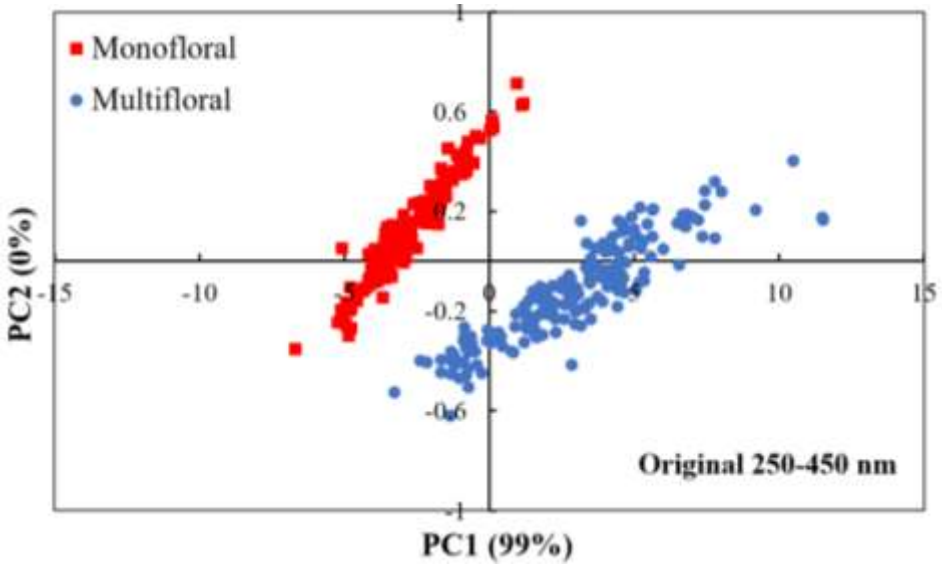


(b)

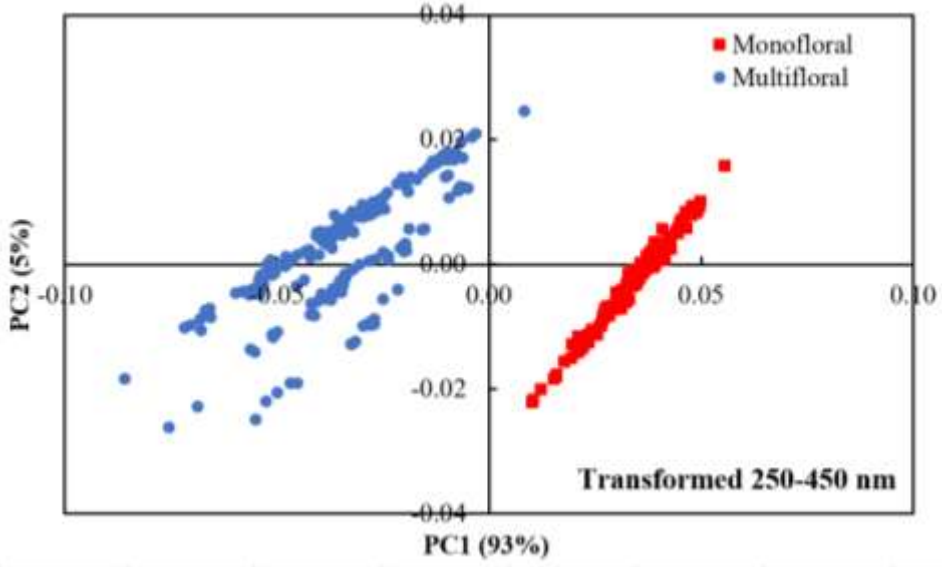
FIGURE 1. The average original (a) and transformed spectral data (b) of monofloral and multifloral honey samples in the wavelength of 190-1100 nm.

The Result of Principal Component Analysis

PCA was performed both for original and transformed spectral data in the range of 250-450 nm. The result was plotted in Figure 2. The accumulative explained variance for the PC1 and PC2 was 99% and 98% for original and transformed spectral data which meets the general requirements of CPV more than 70%–85% for PCA analysis [17]. Using original spectra, monofloral and multifloral were clustered in different part of PC1. However, some of monofloral and multifloral samples were overlapped located with similar value of PC1. Better separation was achieved using transformed spectra. All monofloral samples was situated at positive PC1. Only one multifloral sample was located at positive PC1 while most of the multifloral samples was located at negative PC1. Calculation of LDA was performed using PCA score for original and transformed spectra.



(a)



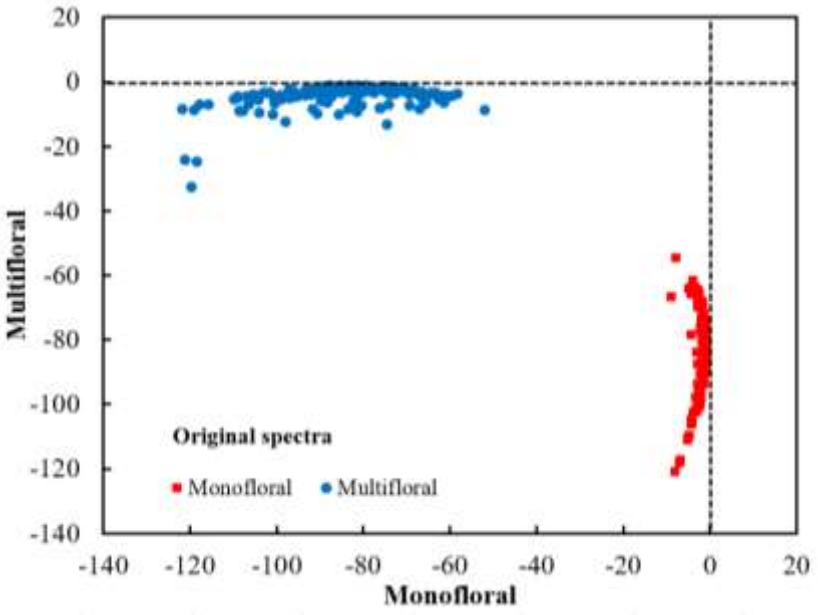
(b)

FIGURE 2. The PCA score plots of monofloral and multifloral honey samples based original spectral data (a) and transformed spectral data (MSC+MA 3s+SG 1d 3s) (b).

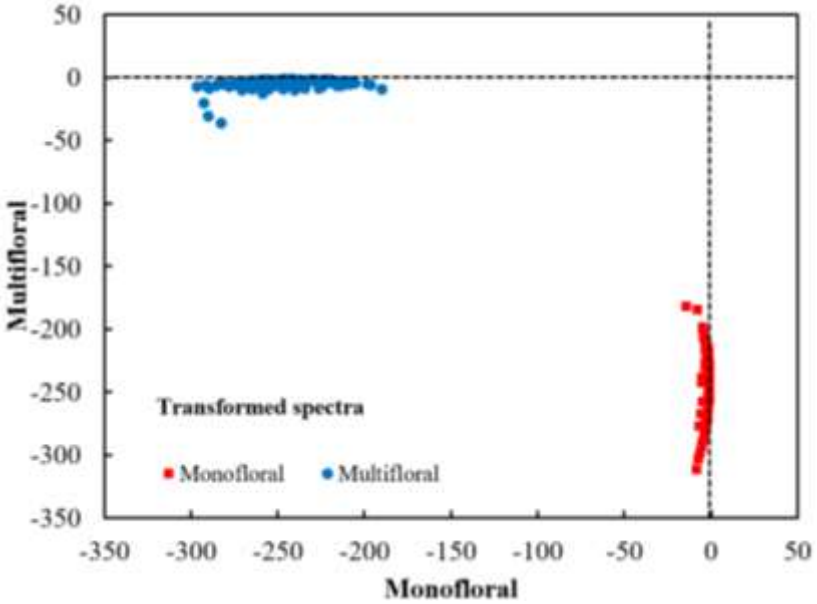
The Result of Classification using LDA Method

Using calibration sample set (334 samples), LDA classification model was developed for original and transformed spectra in the wavelength of 250-450 nm. The result was depicted in Figure 3. Samples lying close to 0 for a class are associated with the class. It can be seen that the honey samples in original and transformed model were situated very close to 0 for monofloral and multifloral class.

Evaluation of the performance of the LDA classification model was performed. For this purpose, a class prediction was done using 66 samples which is not included in the calibration step. The result was summarized in Table 1. All samples were properly predicted into monofloral and multifloral honey resulted in 100% of correct classification (%CC) for both original and transformed spectra.



(a) LDA classification model using original spectra.



(b) LDA classification model using transformed spectra.

FIGURE 3. LDA classification model. (a) developed using original spectra and (b) developed using transformed spectra.

TABLE 1. Classification result for honey class determination using original and transformed spectra in the range of 250-450 nm using developed LDA model with 5 factors.

Spectral data pre-processing method	Class		Actual	
			Monofloral	Multifloral
Original spectra	Predicted	Monofloral	33	0
		Multifloral	0	33
Transformed (MSC+MA 3s+SG 1d 3s)	Predicted	Monofloral	33	0
		Multifloral	0	33

SUMMARY

In this present research, a discrimination between monofloral and multifloral *Apis dorsata* Indonesian honey was demonstrated using UV-visible spectroscopy and LDA method. PCA result showed a clear separation between monofloral and multifloral honey both in original and transformed spectral data. The prediction result was acceptable with all samples were properly predicted into monofloral and multifloral honey resulted in 100% of correct classification (%CC) for both original and transformed spectra. This work presented that UV-Visible spectroscopy is a potential method for authentication of Indonesian honey with simple sample preparation, shorter analysis time, easy to use and simple measurement method.

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