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ATR-FTIR Method for Detection of Cassava Impurities in Indonesian Commercial White Pepper (*Piper nigrum* L.) Based on Principal Component Analysis

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ABSTRACT

White pepper (*Piper nigrum* L.) is one of the most popular flavourings targeted for adulteration, i.e., cassava, resulting in quality loss and infringement on consumer rights and interests. Because it is easy and quick cleanup, minimal sample preparation, and excellent performance with strongly or thickly absorbed samples, the Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectrum signal is widely used to measure commercial herbal products. Manipulation of the spectrum signal can be used to quantify a sample as well as reduce its dimensionality. As a result, there is a lot of interest in developing methods for detecting cassava in commercial white pepper (*Piper nigrum* L.). The aim of the research was to get an ATR-FTIR spectroscopic technique combined with chemometrics to detect cassava as an adulterant in commercial white pepper (*Piper nigrum* L.). The chemical profiles of white pepper (*Piper nigrum* L.) and cassava were compared using principal component analysis (PCA). Bangka, Sudu, and Pontianak were used to collect white pepper (*Piper nigrum* L.) and cassava. White pepper (*Piper nigrum* L.) and cassava were macerated in 96 percent ethanol for 3×24 hours before being concentrated with a rotary evaporator until thick. Each extract's IR spectral was measured using ATR-FTIR spectroscopy in the 4000-650 cm^{-1} range. In addition, the IR spectral was clustered using PCA in the R program. To obtain the appropriate PCA model, we applied two IR spectral manipulation procedures (normal and derivative IR spectra) of the pure material. The PCA chosen was of the normal IR spectral type, with PC1 and PC2 values of 82.3 percent and 7.5 percent, respectively.. The projection of three commercial samples from the markets revealed that samples A and B were negative for cassava adulterant, while Sample C was suspected to be positive. Finally, the ATR-FTIR spectroscopic method tandem PCA was successful in clustering the presence or absence of cassava content in the commercial of Indonesian White Pepper.

Keywords: ATR, Cassava, FTIR, PCA, White pepper

INTRODUCTION

The white pepper from Indonesia has a strong, distinct aroma as well as a spicy, creamy flavor that is similar to black pepper.. The annual production of white pepper in Indonesia ranges between 20,000 and 25,000 metric tons. The most common and well-known white pepper variety comes from the Indonesian islands of Bangka and Belitung, as well as Kalimantan and Sulawesi (Supriana & Yanti, 2013). Vietnam's white pepper and black pepper are putting pressure on Indonesia's white pepper production and exports. The efforts to be required to maintain Indonesia's current market share, including preserving the quality of Indonesia's white pepper (Rahmah, 2017).

White pepper has a wide range of applications, including food flavoring, cosmetics, medicinal use, perfumery, and preservative properties (Lai et al., 2012; Sunila & Kuttan, 2004; Yin-Fu & Hong, 2000). There are numerous pepper varieties available on the market; however, adulteration of white pepper powder with some herbs is used as an adulterant. Papaya (Seed) (Bhattacharjee et al., 2003; Dhanya et al., 2009), chili (Seed) (Dissanayake et al., 2016), and cassava are some herbs that can be used as an adulterant for *Piper nigrum* L. Because of the similar color and small particle size, adulteration of white pepper powder with cassava may be difficult to detect.

The Fourier transform infrared spectroscopy (FTIR) method is used to identify various functional groups in organic and inorganic compounds in a sample. FTIR can be used to detect contaminants in a material, identify and characterize unknown materials, identify decomposition and oxidation, and detect adulterants (Yuliantini et al., 2020). Attenuated total reflection (ATR) is a popular sampling technique that, when combined with traditional infrared spectroscopy, qualifies samples in either solid or liquid form without the need for additional preparation. The IR radiation travels through the ATR crystal and interacts with the sample on the surface in ATR-FTIR spectroscopy. The advantages of ATR include the ability to analyze samples in their natural state, easy and quick cleanup, minimal sample preparation, and excellent performance with strongly or thickly absorbed samples (Glassford et al., 2013).

Principal component analysis (PCA) learns a new feature space that captures the original space's characteristics with minimal information loss. PCA uses an orthogonal linear transformation to transform the original space into a new coordinate system (Jolliffe, 2002). PCA is used to explain variances and covariances in a large data set. PCA is distinct from the various clusters that correspond to different lines of an individual's marker. PCA is a dimensionality reduction technique that is extremely useful in machine learning as well as in classifying big data and its relation to detecting impurities (Andrade et al., 2018). This approach, in addition to simplifying feature manipulation, also contributes to the classifier's performance improvement.

The objective of this study is to use an ATR-FTIR spectroscopic technique combined with PCA to detect cassava as an adulterant in commercial white pepper (*Piper nigrum* L.). Higher accumulation values of PC1 and PC2 were obtained by manipulating the spectrum signal, either by cutting off the wave number range or by taking the first derivative of the IR spectrum signal. Smoothing and the standard normal variate method were used to preprocess FTIR spectra. The chemometric method was carried out in R using PCA.

MATERIAL AND METHODS

Materials

White pepper (*Piper nigrum* L.) and cassava were collected from (Indonesia region): Bangka, Sudu, and Pontianak in 2019. These regions were expected to represent the source of white pepper (*Piper nigrum* L.) and cassava on the market. The plant in the SITH , Institute Technology of Bandung, Jatinangor, Indonesia Herbarium (Plant Identification Certificate, Number: 5251/II.CO2.2/PL/2019) was recognized by a botanist. The ethanol and other chemicals were purchased from Merck, and three commercial white pepper (*Piper nigrum* L.) samples were obtained from three different suppliers.

Extract preparation

White pepper (*Piper nigrum* L.) and cassava were washed with running water, cut into pieces, and dried in an open area with good air circulation but no direct sunlight. The dried pepper (*Piper nigrum* L.) and cassava were powdered and weighed 50 grams before being macerated in 500 mL of ethanol solvent overnight. The solvent replacement was collected, and the solvent was removed with a rotary evaporator until it became oily. The samples were extracted using the same method and under the same conditions.

FTIR spectral acquisition

A Cary 630 FTIR Spectrophotometer with an attenuated total reflectance (ATR) and a resolution of 4 cm^{-1} was used to obtain FTIR spectra. Spectra were scanned in absorbance mode from 4000 to 650 cm^{-1}, and data were processed using MicroLab Expert 1.0.0.7 software. The analyses were performed at a room temperature of 25 °C.

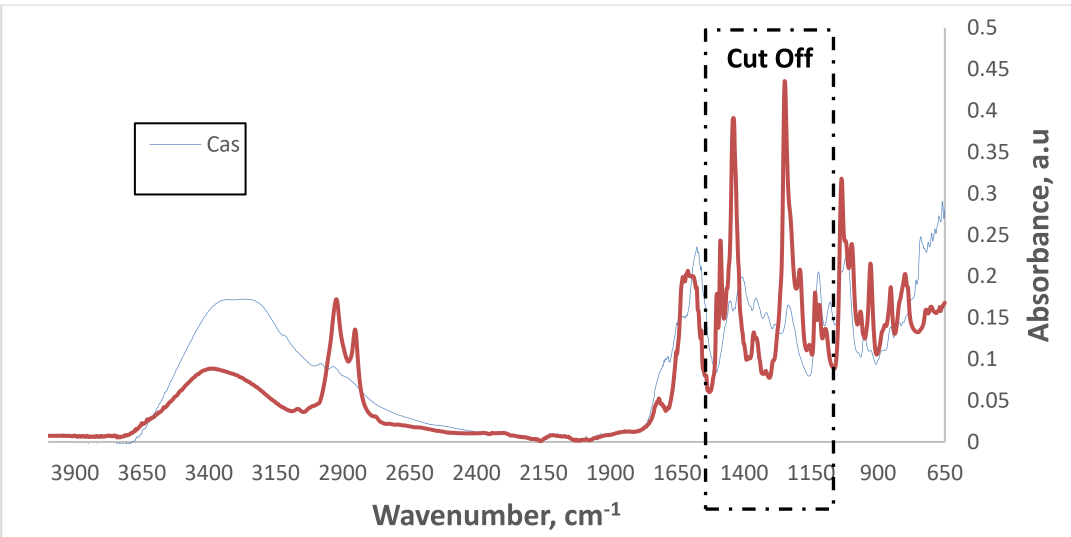
Multivariate analysis

The FTIR spectra were multivariately analyzed using PCA with the prcomp() function in R version 3.6.2 (Kassambara, 2017). The simulations were performed using a PC with an OS of Windows 10 64-bit. The PCA procedure consists of the following steps: standardizing the dataset, calculating the covariance matrix for the dataset's features, calculating the eigenvalues and eigenvectors for the covariance matrix, sorting the eigenvalues and their corresponding eigenvectors, selecting k eigenvalues and forming a matrix of eigenvectors, and finally, transforming the original matrix. In this study, each sample was divided into six replicates ($\boldsymbol{n}$ = 6).

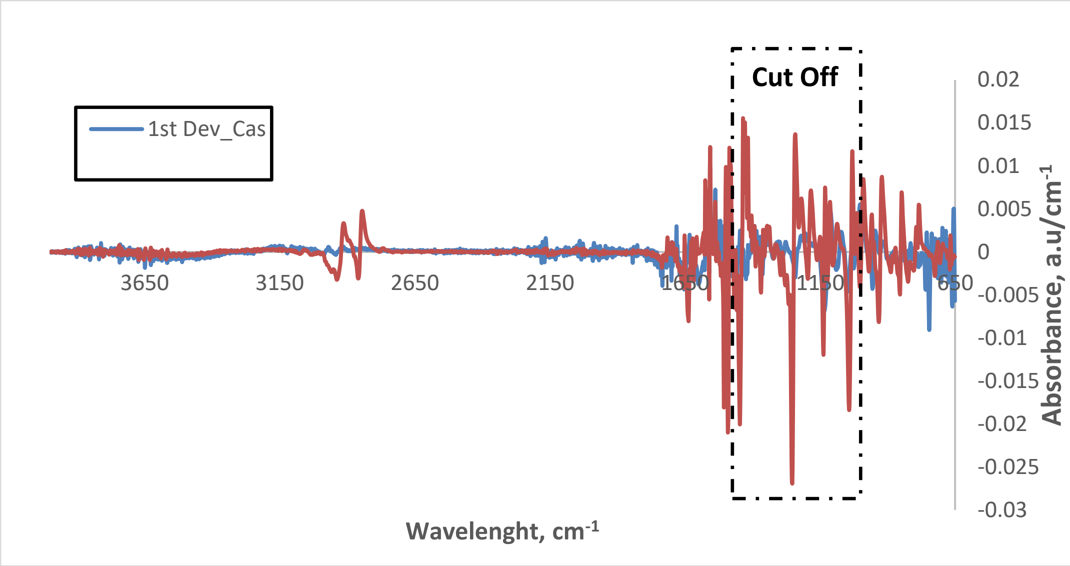
RESULTS AND DISCUSSION

FTIR spectral

In the spectral range of 650–4000 cm^{-1}, the FTIR spectra of white pepper (*Piper nigrum* L.) and cassava have numerous peaks that correspond to the different molecular bonds of these extracts interacting with IR radiation (Figure 1). The –CH groups are responsible for the spectral bands at 2918 cm^{-1} and 2849 cm^{-1}, while the hydroxyl and NH groups are responsible for the broadband at 3650-3150 cm^{-1}. The interaction of IR radiation with C=O groups of the ester functions, carboxylic acids, or proteins present dominates the spectral band at 1650 cm^{-1}. Furthermore, the spectral bands at 1427 and 1246 cm^{-1} correspond to C–H vibrations and –CO stretching vibrations, respectively. Because they do not attribute to a specific type of bond vibration, spectral ranges between 1050 and 750 cm^{-1} are more difficult to interpret. This region is famous for the abundance of infrared bands present. This region has a variety of distinct vibrations, including C-O, C-C, and C-N single bond stretches, C-H bending vibrations, and certain bands related to benzene rings. It appears in the FTIR spectra of white pepper (*Piper nigrum* L.) and cassava (Leite et al., 2017; Li et al., 2018). Furthermore, strong and distinct FTIR spectra for white pepper (*Piper nigrum* L.) were observed (Fig. 1a) with some strong-intensity peaks at the range spectral band of 2987-2800 cm^{-1}, 1498-1377 cm^{-1}, 1300-1144 cm^{-1}, and 1050-746 cm^{-1}.

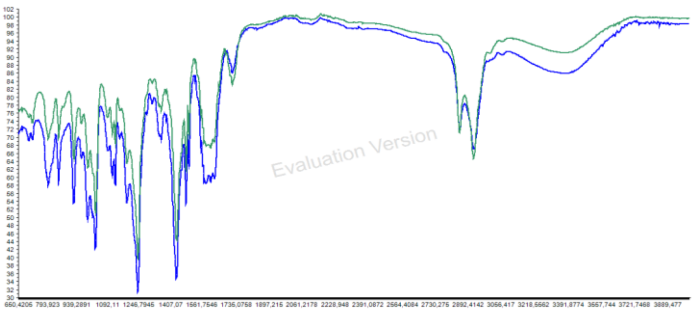


(A)

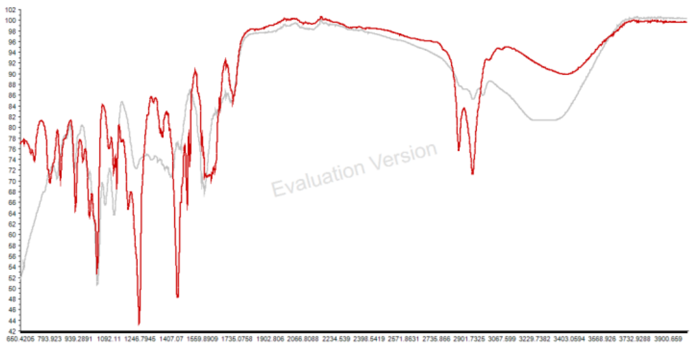


(B)

Figure 1. Overlapped normal (A) and 1st derivative (B) ATR–FTIR spectra of white pepper (*Piper nigrum* L.) (WhP) [color: brown] and cassava (Cas) [color: blue] in the spectral range of 650–4000 cm^{-1}.



(A)



(B)

Figure 2. A typical overlapped ATR–FTIR spectra of Sample A with white pepper (*Piper nigrum* L.) [color: blue] (A) and cassava [color: grey] (B) in the spectral range of 650–4000 cm^{-1}.

IR spectral clustering

PCA was used to cluster the FTIR spectra of white pepper and cassava from Indonesian regions (Bangka, Sudu, and Pontianak). These FTIR spectral sets were initially preprocessed by centering and autoscaling or unit variance (Husson et al., 2011). By reducing dimensionality, PCA summarizes the information content in big data. We only used the first and second principal components for clustering (PC1 and PC2). To obtain the 1st and 2nd Principal Components (PC1 and PC2), we transformed the IR spectrum using a wave number range cut off and the first derivative (Fig. 1). In the wave number range of 1000-1500 cm^{-1}, cut offs are performed for both the first and normal derivatives of the IR spectrum. Because there is a noticeable difference in the normal spectrum band signal within this range (1245 to 1145 cm^{-1}) it is used as a cutoff. At wave numbers 1040, 1135, 1245, 1250, 1430, 1445, and 1490 cm^{-1}, the absorption peak in the first derivative shows a strong signal.

The PCA score plots of white pepper (*Piper nigrum* L.) (A) and cassava (B) sets of 80.6 percent and 7.8 percent variance of PC1 and PC2, respectively (Table 1 and Fig. 3), achieved a well-defined sample separation by using a normal spectrum at a wavelength of 650-4000 cm^{-1}. Almost all of the data sets (88.5 percent) captured the original data set's characteristics. It indicates the consistency of sample measurements across data sets by employing overall normal spectral. It should be noted that the overall normal spectrum's accumulated PC1 and PC2 values (88.5 percent) had a higher rate than the normal cut-off spectrum, and the overall 1st derivative values were 85.4 percent and 63.2 percent, respectively. Meanwhile, when the accumulated values of PC1 and PC2 were compared based on the cut off range, there was no significant difference. This demonstrates that manipulating the spectrum signal using the first derivative and the cut-off wave number range for the FTIR spectrum group of white pepper and cassava does not significantly increase PC1 and PC2 accumulation. This is possible because the accumulated signals of IR groups in both white pepper and cassava have subtle differences, so when the signal is manipulated, the two signal groups differ slightly in terms of each feature. In particular, it has a significant effect on the overall features of PCA in the wave number range of 2970 to 2776 cm^{-1} (Fig. 1).

Table 1. Characteristics of PC1 and PC2 by IR spectrum mode

IR spectrum mode	Range of wavelength (cm^{-1})	PC1 (%)	PC2 (%)	Overall (%)
Normal (Overall)	650-4000	80.6	7.8	88.5
Normal (Cut Off)	1000-1500	70.9	14.5	85.4
1st Derivative (Overall)	650-4000	51.7	11.5	63.2
1st Derivative (Cut Off)	1000-1500	73.4	12.3	85.7

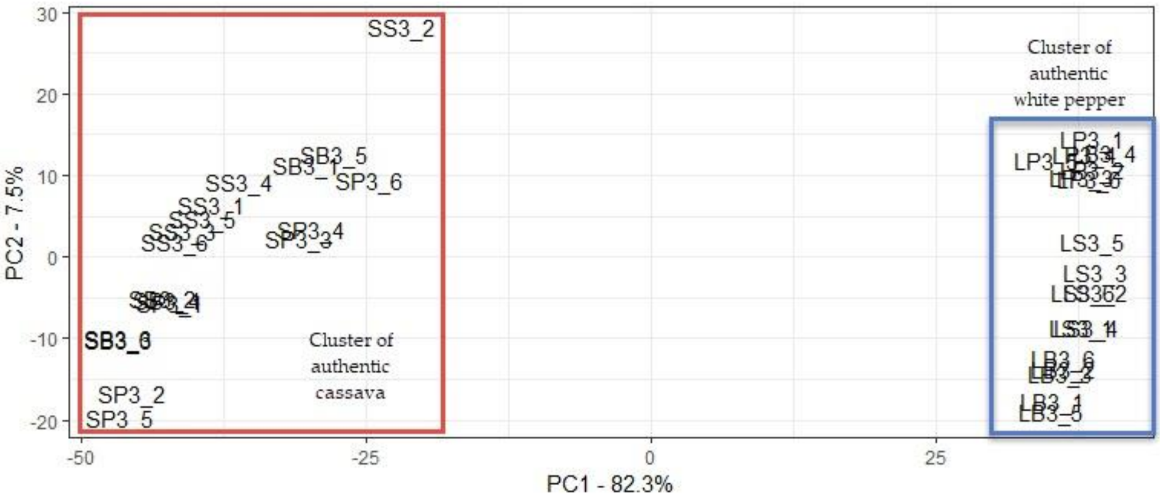
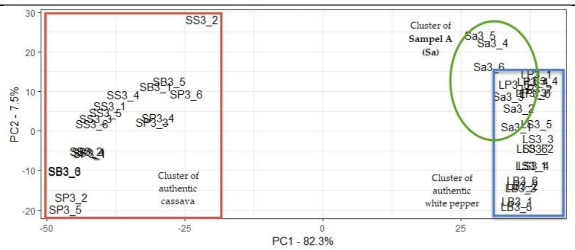


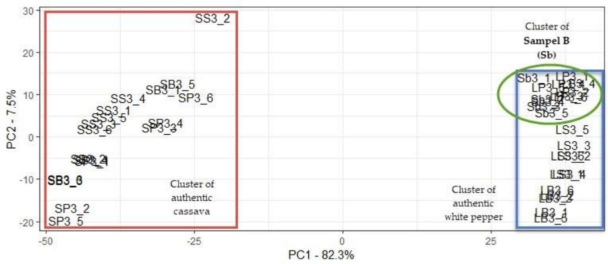
Figure 3. PCA score plot of normal spectral (overall) for pure cassava and pure white pepper (*Piper nigrum* L.). The LB, LP, and LS are pure white pepper (*Piper nigrum* L.) from Bangka, Sudu, and Pontianak, in that order. The SB, SP, and SS are pure cassava from Bangka, Sudu, and Pontianak, Indonesia, respectively.

Discrimination Samples

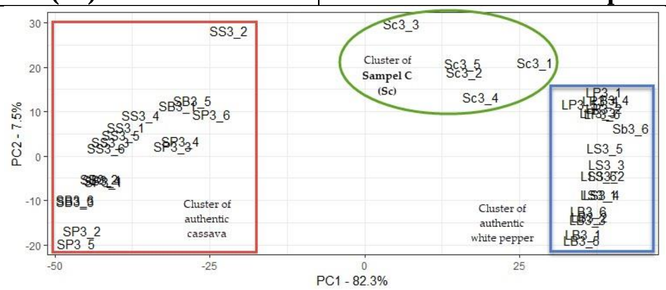
The cassava content of Indonesian commercial pepper samples was determined using a PCA score plot of normal spectral data (overall).



Sample A (Sa)



Sample B (Sb)



Sample C (Sc)

Figure 3. Project a sample of commercial white pepper (Sa, Sb, and Sc) onto the PCA score plot of pure white pepper (*Piper nigrum* L.) (colored in green) and pure cassava. The LB, LP, and LS are genuine white pepper (*Piper nigrum* L.) from Bangka, Sudu, and Pontianak, in that order (colored in blue). The SB, SP, and SS are genuine cassava from Bangka, Sudu, and Pontianak, respectively (colored in red).

Projections of three producers' commercial white pepper powder onto the PCA score plot of pure white pepper (*Piper nigrum* L.) and pure cassava (Fig. 4) revealed that Samples A and B were negative for cassava adulterant, while Sample C was suspected to be positive. Samples A and B were assigned to the white pepper group, whereas Sample C was assigned to the cassava and white pepper groups. Other than cassava, it was suspected that Sample C contained adulterants. More investigation is needed to discover adulterants in Sample C.

CONCLUSION

Finally, the ATR-FTIR technique based on PCA chemometrics techniques is an adequate method for cassava discrimination analysis in Indonesian commercial white pepper (*Piper nigrum* L.). The manipulation of IR spectra with the first derivative of white pepper and cassava IR spectra did not result in a better accumulation of PC1 and PC2.

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