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DEVELOPMENT OF CACAO VARIETY IDENTIFICATION METHOD USING COMBINATION OF FTIR SPEKTROSCOPY AND MULTIVARIATE ANALYSIS

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Abstract

Indonesia is the third largest cocoa producer after Ivory Coast and Ghana. The low quality of cocoa beans caused by the wide variety of cocoa plants is one of the challenges that the Indonesian cocoa industry has not been able to overcome. Depending on the variety, cocoa contains varying amounts of phenolic chemicals. There are three types of cocoa plants with different qualities, namely Criollo (fine flavored cocoa), Forestero (bulk cocoa), and Trinitario (hybrid cocoa of Criollo and Forestero). This research aims to determine the quality of cocoa bean production quickly and accurately by identifying cocoa based on its variety. The method used is a combination of the FTIR (Fourier Transform Infrared) spectroscopy method and the multivariate analysis method. This combination can be used to identify closely related plant species. Based on FTIR spectroscopy analysis of 15 cocoa bean samples at a wavelength range of 4000-400 cm-1, relatively similar multicomponent spectra were obtained. The spectrum was then processed using PCA (Principle Component Analysis) multivariate analysis with a main component value (PC-1 and PC-2) of 92.03%.

Keywords: cocoa, multivariate analysis, FTIR spectroscopy, PCA

Abstrak

Indonesia merupakan produsen kakao terbesar ketiga setelah Pantai Gading dan Ghana. Rendahnya kualitas biji kakao yang disebabkan oleh berbagai macam variasi tanaman kakao merupakan salah satu tantangan yang belum dapat diatasi oleh industri kakao Indonesia. Tergantung pada varietas, kakao mengandung bahan kimia fenolik dalam jumlah yang bervariasi. Ada tiga jenis tanaman kakao:dengan kualitas yang berbeda-beda yaitu Criollo (kakao mulia), Forestero (kakao lindak), dan Trinitario (kakao hibrida Criollo dan Forestero). Penelitian ini bertujuan untuk mengetahui kualitas produksi biji kakao secara cepat dan akurat dengan mengidentifikasi kakao berdasarkan varietasnya. Metode yang digunakan merupakan kombinasi antara metode spektroskopi FTIR (Fourier Transform Infrared) dan metode analisis multivariat. Kombinasi ini dapat digunakan untuk identifikasi dan otentikasi spesies tumbuhan yang berkerabat dekat. Berdasarkan analisis spektroskopi FTIR terhadap 15 sampel biji kakao pada rentang panjang gelombang 4000-400 cm^{-1} diperoleh spektrum multikomponen yang realtif sama. Spektrum kemudian diolah dengan analisis multivariat PCA (Principle Component Analysis) dengan nilai komponen utama (PC-1 dan PC-2) sebesar 92,03%.

Kata Kunci: kakao, analisis multivariat, spektroskopi FTIR, PCA

1. Introduction

Plantation commodities are a mainstay for Indonesia's national income and foreign exchange. The contribution of the plantation sub-sector to the national economy is increasing and is expected to strengthen the overall plantation development. Indonesia is one of the most extensive cocoa cultivation countries in the world and is the third largest cocoa producing country after Ivory Coast and Ghana with a total production value of 777,500 tons. One of the cocoa-producing provinces in Indonesia is Banten Province with a total production of 1782 tons with a land area of 6171 Ha.

Cocoa-producing regions in Banten Province, namely Pandeglang, Serang, and Lebak Regencies (Dirjen Perkebunan Indonesia, 2015).

Indonesian cocoa agribusiness still faces various complex problems. One of the problems to date is the low quality of production (Towaha et al., 2012). The low productivity in general is influenced by factors such as: the use of unfavorable plant material, less optimal cultivation technology, age of plants and the problem of pest attack (Karmawati et al., 2010). In addition to the above factors, the type of cocoa plant variety also influences the determination of the quality of the cocoa beans, because the amount of phenolic compounds in cocoa depends on the variety (Mazor et al., 2011).

In general, cocoa plants are grouped into three types, namely Criollo, Forestero, and Trinitario which are the result of a cross between Forestero and Criollo. Criollo cocoa includes fine-flavored cocoa, while Forestero cocoa includes bulk cocoa (bulk cocoa) Hybrid cocoa varieties are Trinitario cocoa varieties that have higher production capabilities than Criollo and Forastero varieties (Surti, 2012). Criollo type cocoa has advantages compared to the types of forestero and Trinitario which are lower fat content in seeds, larger and rounder seed sizes and give a very good distinctive taste (Karmawati et al., 2010). Based on these advantages, Criollo type cocoa is valued more expensive and more desirable in national and international markets. Besides the type of cocoa, the application of cultivation technology and post-harvest handling are also important to note so that the selling value of cocoa can be maintained.

The quality control of cocoa beans as raw material for its processed products has only been done by sorting based on their physiological properties so that the authenticity of the type of seeds is still a problem. The method commonly used today in the identification and authentication in order to control the quality of raw materials or plant extracts is to show the levels of one or more active compounds known as the character compounds approach (Li et al., 2008). However, processed cocoa bean products are multicomponent so that the fingerprint spectrum pattern approach will be more accurately used for quality control.
Analytic methods for identification, discrimination and authentication of a plant in the context of quality assurance of raw materials are currently focused on the chemical components that cause certain activities (Purwakusuma et al., 2014). At present, identification of differences in varieties has been carried out with Liquid Chromatography, Gas Chromatography, Capillary Electrophoresis, Sensory Evaluation or Atomic Emission Plasma. However, these methods require relatively expensive costs, long enough time for destruction, and the use of many chemicals (Teye et al., 2016). Therefore, another method that is efficient, easy and inexpensive and the FTIR Spectroscopy method can be one of the options that can be used.

Complex IR spectra cause direct interpretation to be difficult, so to facilitate it an approach is needed using chemometric techniques such as multivariate analysis (Gad et al., 2012). The combination of FTIR fingerprint method and multivariate analysis has been widely carried out by researchers for the purpose of identification and authentication of plants that are closely related (Purwakusumah et al., 2014). Therefore, the combination of these two methods is believed to be able to identify the types of cocoa for quality control purposes.

This research is very necessary for the purpose of developing the cocoa quality control method in terms of determining the types of cocoa seed varieties quickly and accurately. This combination of methods will save time and cost of analysis because the samples analyzed in the form of rough extracts so that it does not require a long analysis phase.

2. Methodology

2.1. Materials

Cocoa beans (representing the three cocoa varieties), KBr (Sigma-Aldrich) and absolute ethanol (Merck).

2.2. Methods

Sample praparation

Representative samples of cocoa beans from each region in Banten Province (Table 1), taken 5 samples of cocoa beans representing the three varieties of cocoa (Criollo, Forestero and Trinitario). The sample is then fermented for 4-5 days and dried to obtain samples of dried cocoa beans. Samples of dried cocoa beans were refined to obtain sample powder. The powder is then sieved with a 100 mesh sieve. Extraction of sample powder was carried out by maceration using

absolute ethanol. The extract obtained was then dried so that a crude extract was obtained for analysis with FTIR.

Table 1. Sampling location

No	Varietas of cocoa	Sample code	Location source (District)
1.	Cocoa Criollo	KC-1	Menes, Pandeglang
2.		KC-2	Cikedal, Pandeglang
3.		KC-3	Pabuaran, Serang
4.		KC-4	Ciomas, Serang
5.		KC-5	Sajira, Lebak
6.	Cocoa	KF-1	Menes, Pandeglang
7.	Forestero	KF-2	Pabuaran, Serang
8.		KF-3	Ciomas, Serang
9.		KF-4	Sajira, Lebak
10.		KF-5	Warung Gunung, Lebak
11.	Cocoa	KT-1	Menes, Pandeglang
12.	Triniario	KT-2	Cikedal, Pandeglang
13.		KT-3	Ciomas, Serang
14.		KT-4	Sajira, Lebak
15.		KT-5	Warung Gunung, Lebak

Making FTIR Spectrum

A certain amount of dried extract is then mixed uniformly with KBr to form pellets using manual press equipment. FTIR analysis was carried out using FTIR Spectrophotometer (Bruker Optik GmbH) which was registered with DTGS detector (deuterated triglycine sulphate) in the middle infrared region (4000-400 cm^{-1}) at a resolution of 4 cm^{-1} with a total of 32 operated with OPUS software. FTIR spectrum in OPUS format is stored in the Data Point Table (DPT) format.

Data Analysis with Multivariate Analysis Method

In the resulting FTIR Spectrum, baseline correction is carried out. Then the model is made using multivariate analysis, Principal Component Analysis (PCA). The software used in making the model is XLSTAT 2014 version (Addinsoft, New York, United States).

3. Result and Discussion

FTIR Spectrum Data

Ethanol extracts from the three cocoa varieties were analyzed using FTIR Spectroscopy. This analytical method is considered very efficient because the sample preparation process is easy, fast and the entire data generated in the form of spectrum can describe the chemical properties of the samples analyzed. Figure 1 shows the results of the analysis of the three samples of cocoa varieties in a representative manner giving the FTIR spectrum pattern that is very similar to each other. The difference that appears is only in the value of the absorbance of each spectrum. This shows that qualitatively the chemical compounds in each sample are almost the same but quantitatively the levels vary.

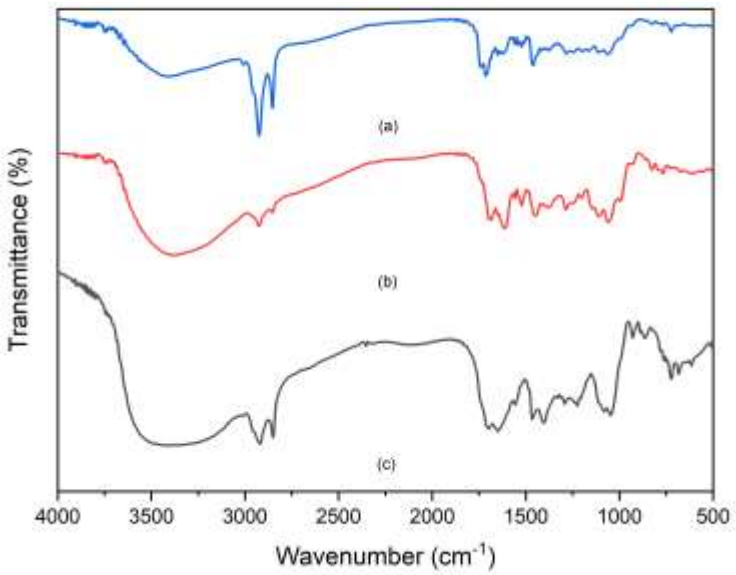

Figure 1. Spectrum pattern of FTIR extract of sample (a) Criollo Cocoa, (b) Forestero Cocoa, (c) Trinitario Cocoa

Identification of Cocoa Varieties

Determination of cocoa varieties is very important to be done in order to control the quality of cocoa production. Criollo cocoa (Noble Cocoa) has better quality and selling price compared to Forestero and Trinitario cacao types. However, from the results of the FTIR spectrum obtained, identification of cocoa varieties cannot be carried out so that it needs a combination with multivariate analysis methods. The combination of these two methods has been carried out on the identification of plants that are closely related, classification of plant origin and product quality control.

The initial stage before multivariate analysis on FTIR data is obtained, it is necessary to normalize the baseline. The treatment aims to minimize basic line shifts and improve the data spectrum overlapping. Improving information on spectrum data can cause specificities of the spectrum more clearly visible.

FTIR Spectrum Data Analysis

Principle Component Analysis (PCA) is one of the chemometric techniques that can be used to extract information from the spectrum data obtained. PCA is done to reduce data by grouping data with similar properties into groups that are not correlated with each other or in other words the large data is reduced to the main component or PC Component which can represent the structure and variance in the data (Miller & Miller, 2000).

In this study, factor analysis using PCA method was carried out using XLSTAT software. The results of the analysis with PCA can be shown by the proportion of PC variance as shown in Figure 2. PCA aims to project the data in the direction that has the greatest variation, which is shown by the eigenvector corresponding to the largest eigenvalue of the covariance matrix and reduce dimensions by doing linear transformations from a high-dimensional space into a low-dimensional space.

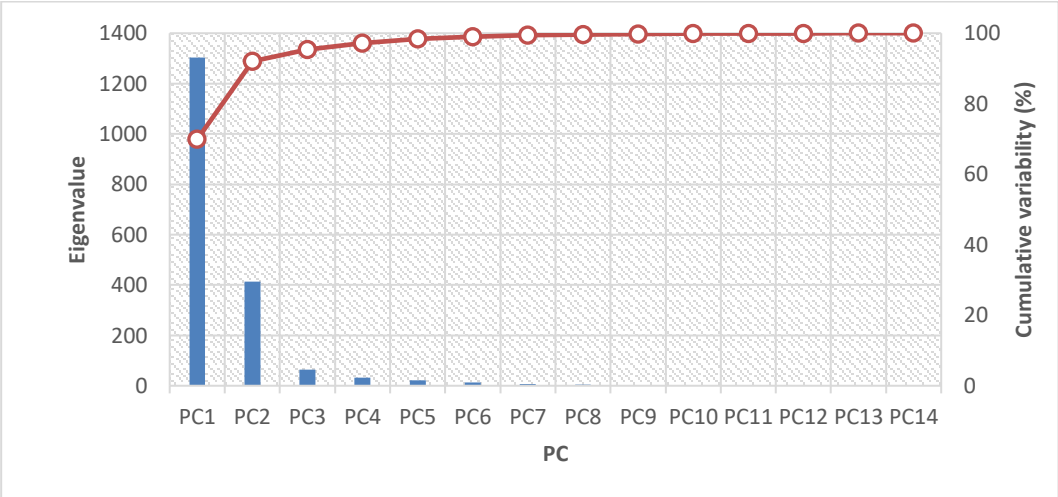

Figure 2. Proportion of Variance and Eigenvalues

The proportional plot of variance provides information about the patterns found in the sample spectrum. Plots for two initial PC values are usually most useful in analysis because these two PCs contain the most variety in the data. The proportion of PC variance shows that PC-1 has the greatest variance value of 69.90% followed by PC-2 with a variance value of 22.13% while PC-3 and so on have a small data variance value. PC-1 has the greatest variability value because PC-1 is made by maximizing the variance in the data. Whereas the PC is then made by maximizing the residual or variance left in the data after calculating PC-1 so that the entire PC can explain the variance of the data with a total of 100%.

Based on the proportion value of the variance in Figure 2, the score plot is made using the PC-1 value and PC-2 value because the two PCs can represent the overall variance of the data. This also indicates that only with the first two PCs can a good PCA model be obtained. The score plot can be seen in Figure 3.

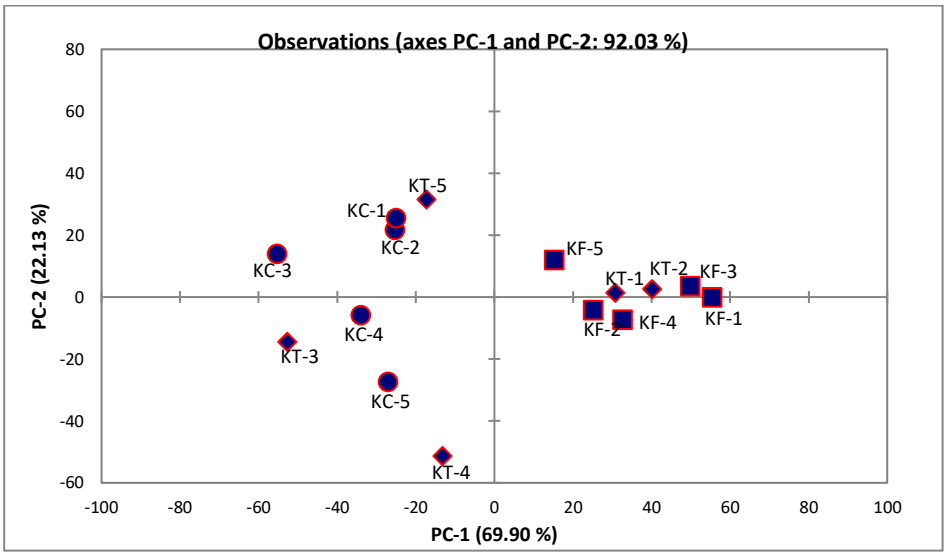

Figure 3. Score of Criollo Cocoa Plots (KC), (KF) Forestero Cocoa and (KT) Trinitario Cocoa

Based on the score obtained from the plot, grouping of cocoa based on the variety has been seen as 92.03%. Criollo type cocoa is only in quadrants II and III and Forestero type cocoa is only in quadrants I and IV. However, for the type of Trinitario cocoa is scattered in every consciousness, in groups with other varieties. This may be because Trinitario cocoa is a hybrid of Criollo and Forestero cocoa so that physically and chemically (the amount of phenolic compounds) of this variety is a combination of criollo and forestero varieties.

4. Conclusion

FTIR analysis method combined with multivariate analysis can be used to identify varieties of cocoa effectively and efficiently with the percentage of Principal Component (PC) amounted to 92.03%.

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