

## ALTERNATIVE OPTICAL METHODS FOR QUALITATIVE DETECTION OF VITAMIN B6 AND B12 OF BANANA

[Metode Optik Alternatif untuk Deteksi Vitamin B6 dan B12 secara Kualitatif pada Pisang]

Isnaeni\*, Baladika Sukma Zufara, and Ismira Wahyu Lestari Lewa

Research Center for Physics, Indonesian Institute of Science, Puspiptek, South Tangerang, Banten

Received February 3<sup>rd</sup> 2020 / Accepted December 10<sup>th</sup> 2020

### ABSTRACT

Bananas are known to contain fiber and vitamins essential for human body. Thus, the ability to detect these of vitamin in bananas is crucial. Information in the vitamin content of can affect procedures for harvest and post-harvest process. Methods to determine the nutrition content of foods are usually carried out using High Performance Liquid Chromatography (HPLC). However, this method requires complex sample preparation and chemical reaction processes. Due to this weakness, alternative techniques are needed to detect vitamin in simple ways. In this study, a simple, easy and fast methods to determine the vitamin content of banana was developed. Using reflectance and photoluminescence spectroscopy, the vitamin of bananas from five different species were able to be identified. From the reflectance spectra results, two peaks were observed, the first peak at a wavelength of 325 nm is the absorption peak of vitamin B6 and the second peak at 450 nm is the absorption peak of vitamin B12. From the photoluminescence spectra using excitation wavelength at 325 nm, an emission peak was found at wavelength 450 nm which is the peak emission from vitamin B6. These results proved that by using the methods proposed, the detection of vitamins in bananas can be done in an easy and simple ways.

**Keywords:** absorbance, banana, luminescence, reflectance, vitamin

### ABSTRAK

Pisang diketahui mengandung serat dan vitamin yang baik untuk tubuh manusia. Kondisi ini membuat deteksi vitamin dalam pisang menjadi penting untuk dilakukan. Informasi tentang kandungan vitamin pisang dapat memengaruhi cara panen dan proses pascapanen. Metode untuk menentukan nutrisi pada buah dan bahan makanan biasanya dilakukan dengan menggunakan High Performance Liquid Chromatography (HPLC). Kekurangan dari metode ini membutuhkan preparasi sampel yang kompleks dan melibatkan reaksi kimia yang kompleks. Kekurangan ini, teknik alternatif diperlukan untuk mendeteksi vitamin secara sederhana. Pada penelitian ini, metode sederhana, mudah, dan cepat untuk menentukan kandungan vitamin pisang untuk dilakukan. Kandungan vitamin dari lima spesies yang berbeda dapat diidentifikasi menggunakan spektroskopi reflektansi dan fotoluminensi. Hasil spektrum reflektansi, terdapat dua puncak yaitu puncak pertama pada panjang gelombang 325 nm yang merupakan puncak serapan vitamin B6 dan puncak kedua pada panjang gelombang 450 nm yang merupakan puncak serapan vitamin B12. Spektrum fotoluminensi menggunakan panjang gelombang 325 nm, puncak emisi ditemukan pada panjang gelombang 450 nm yang merupakan puncak emisi dari vitamin B6. Hasil ini membuktikan bahwa dengan menggunakan metode yang diusulkan deteksi vitamin dalam pisang dapat dilakukan dengan cara yang mudah dan sederhana.

**Kata kunci:** absorbansi, luminensi, pisang, reflektansi, vitamin

### INTRODUCTION

Indonesia is a tropical country that lies exactly on equator line. This location makes Indonesia is a great place to grow tropical fruits, such as guava,

papaya, star fruit, pineapples and bananas (Kumoro *et al.*, 2020; Bhat *et al.*, 2016). In Indonesia, there are a lot of species of bananas. Each of them has unique taste and shape. Bananas are well known as excellent nutrition source for human body, since bananas contain potassium, vitamin C, B6, fiber, amino acids, and tryptophan (Sampson *et al.*, 2014).

\*Corresponding Author:  
E-mail: [isnaeni@lipi.go.id](mailto:isnaeni@lipi.go.id)

Although bananas have good nutrition, nutrition level of each species of bananas might different.

Increasing the economic condition and people's living standards, fruit consumption increases. Banana is one of the most commonly eaten fruit in the world (Sampson *et al.*, 2014). According to this condition, it is important to determine the nutrients contained in bananas. Analysis of nutrition in Bananas is very important for consumers and producers. Nowadays, consumers pay attention to the nutritional factors contained in the food they eat (Spínola *et al.*, 2014). For producers, knowing the nutritional content in fruit is important for controlling the fruit processing post harvested. Not only external quality, but internal with determining the nutrition inside the fruit is also important to observed.

During the recent years, checking the quality of fruit is done in a traditional ways like observing the skin color changes using direct observation. However this methods is subjective and time consuming. Many researchers propose several methods used to determine nutrition of fruit and food. One of the methods is chromatography. Nowadays, in order to verify nutrition content of food, engineers use high performance liquid chromatography (HPLC) (Klimczak *et al.*, 2015). This measurement is quite complicated. Since, it uses a specific column and reference sample for every measurement. However, the vitamin content in the fruit can be quickly lost under certain condition such as heating process (Klimczak *et al.*, 2015). In addition, this method is only can be done in laboratory scale. Hence, it is difficult to detect the nutrition in fruit on a large scale.

To overcome the lack of mass quality detection, detection methods that can provide the internal content of fruits and non-destructive methods are needed (Zerbini *et al.*, 2015). There are several analytical methods such as acoustic technique, spectroscopic technique, machine vision, and electronic noses. The internal properties of the fruit cannot be detected using machine vision system techniques (Lu *et al.*, 2011). Spectroscopic method is a promising optical technique that can be used to measure directly. This method is simple, does not require pre-treatment of samples and chemical reactions. By using this method, detection process of nutrition in fruit can be done without damaging the surface of the fruit (Wang *et al.*, 2015).

The optical method studies the interaction between light and matter. From this interaction, the characteristics and properties of material can be studied. Study of the interaction between light and biological tissues has become an attraction in agriculture and biomedical fields because this phenomenon can provide indepth information about the chemical and physical properties of tissues through absorbance and scattering processes (Hu *et al.*, 2015). Several studies have succeeded in observing

quality of fruit using optical methods (Adebayo *et al.*, 2017; Hashim *et al.*, 2012). In addition, optical methods such as backscattering and Vis/NIR spectroscopy have also been used to observe the mechanical properties, firmness, pH, and the structure of the fruit (Cen *et al.*, 2013; Huang *et al.*, 2018; Rowe *et al.*, 2014; Zhu *et al.*, 2015). In this work, the simple and rapid methods for detecting vitamin in bananas were proposed. The purpose this study is to qualitatively measure vitamin content in five species of bananas using reflectance spectroscopy and photoluminescence spectroscopy. By using these techniques, we can get the information related to the fruit quality with simple, fast and no need a complex extraction process. Thus, these methods are possible for large scale detection in industry.

## MATERIALS AND METHODS

### Materials

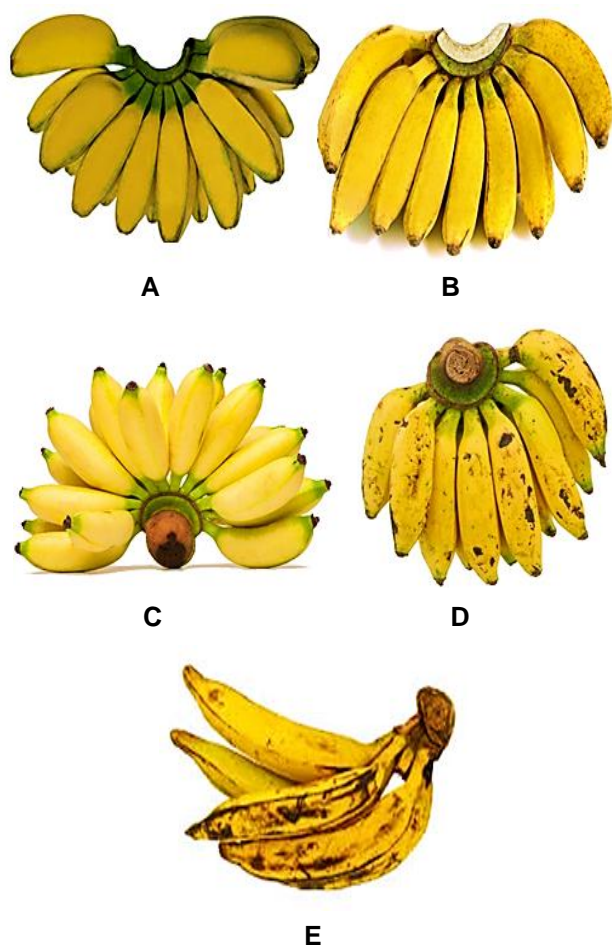
All the analysis and observations do not require chemical reactions. Therefore, banana is the only material used in this study. All bananas were obtained from local Serpong traditional market, South Tangerang, Banten, Indonesia. Information obtained from seller is that the origin of bananas was Bogor, West Java, Indonesia. Banana that used in this experiment was five species of local bananas in Indonesia. They are banana *kepok* (Figure 1A), banana *sereh* (Figure 1B), banana *susu* (Figure 1C), banana *uli* (Figure 1D) and banana *nangka* (Figure 1E). Bananas used in this study are bananas with perfect maturity which is around 110-150 days after flowering.

### Methods

The procedure for preparing samples is done by peeling bananas obtained from traditional markets. Then, banana sliced with diameter around 3cm. Furthermore, the sliced banana is placed on a glass preparation to be observed. Observations were made by repeated observations at 3 different points on the banana flesh.

Equipments used in this work are knife, object glass. For reflectance system, the equipments are the light source (deuterium and halogen lamp) from Ocean Optics USA Florida, fiber optic, and spectrometer (MAYA Pro2000, Ocean Optics). For photoluminescence system, the equipments are 325 nm laser source from Ocean Optics, fiber optic, and spectrometer (MAYA Pro2000, Ocean Optics from Florida USA). Optical fiber was used to capture the signal produced by the interaction between light and banana in the form of emission and reflectance spectra. The experiments are carried out without going through the extraction process, involving chemicals, or complex instruments. The experiments

only use the tools and materials mentioned in this manuscript.



Note: A= Banana *kepok*; B= Banana *sereh*; C= Banana *susu*; D= Banana *uli*; E= Banana *nangka*

Figure 1. Five different bananas used in this research

The observation method is carried out by using two optical measurement systems *i.e.* reflectance spectroscopy and photoluminescence spectroscopy. The method used in this study is a method that has been modified and simplified from several methods that have been carried out by several previous research (Valdivieso *et al.*, 2014; Jaiswal *et al.*, 2012). The system used in this study is very simple and does not require special pre-treatment actions.

Reflectance spectroscopy investigation utilized deuterium and halogen lamp as light source. The light was coupled into the fiber optic, directed to the banana flash. Furthermore, the reflectance signal from the banana is captured by the same optical fiber and recorded by a spectrometer. This spectrum is then transmitted to the computer for data analysis. Observation data is then compared with some

previous studies to find out what nutritional information that obtained in experiment.

For photoluminescence, laser ultraviolet at wavelength 325 nm was used to excited banana sample. The emission from banana was then collected by spectrometer probe. For both photoluminescence and reflectance spectroscopy, only used wavelength from 300 to 900 nm to analyze the vitamin content in Bananas. Each bananas were measured 5 times to get measurement consistency. The same spectra are obtained for every repeated measurement.

Furthermore, absorbance spectrum of bananas is observed. Therefore Kubelka-Munk (KM) formula to convert reflectance spectra into absorbance spectra as follow was used:

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \dots \dots \dots (1)$$

K/S is an absorbance over scattering and R is reflectance (Lana *et al.*, 2006).

## RESULTS AND DISCUSSION

### Optical characteristics of bananas

Optical characteristic studies the interaction between light and material to determine the properties of the material to be observed. When light hits a sample, there are three possible phenomenon that occur *i.e.* reflectance, transmittance and absorbance. From this optical phenomenon, the information about the properties of a material can be obtained (Wang *et al.*, 2015).

Material based on optical properties can be divided into three types *i.e.* transparent, semi-transparent and turbid. Biological objects such as banana tissue are classified into semi-transparent or turbid material (Mireei *et al.*, 2010). Optical characterization of biological tissue has several challenges, namely complex since the events of reflection, absorption, and scattering occur at the same time (Qin *et al.*, 2007). Light hits biological tissue, only about 4-5% of light can be reflected (Mireei *et al.*, 2010). The rest passes through the surface and interacts with the internal components of this biological tissue. This event is the basis for characterizing optical properties in bananas.

Observations using optical methods are simple and rapid. Since, it does not require complicated sample preparation like other methods such as HPLC. Research conducted by Koyuncu *et al.* (2010) uses the HPLC method to determine the vitamin C content of strawberries, the testing procedure requires extraction of strawberries with certain PH rules (Koyuncu *et al.*, 2010). Furthermore, Gundogdu *et al.* (2011) identified the vitamin content

of berries which required the samples to be extracted and supplemented with chemicals such as  $H_2SO_4$  which were harmful to the environment. In addition, using HPLC requires a standard solution so that it involves more chemical materials (Gundogdu *et al.*, 2011). By replacing these methods with optical spectroscopy method, observations can be simpler, faster, and safer for the environment.

### Reflectance spectra of bananas

Reflectance spectroscopy is the investigation of the spectral composition of surface-reflected radiation with respect to its angularly dependent intensity and the composition of the incident primary radiation. The reflectance spectroscopy measurement was done very quickly.

There are similar reflectance spectra for all bananas. The reflectance dips and peaks are almost identical (Figure 2). However, the reflectance intensities are different. Reflectance spectroscopy was done. Since, any sample preparations in this measurement do not want to be skipped. This procedure makes the measurement applicable in the field. Therefore edible banana part was chosen directly for measurement.

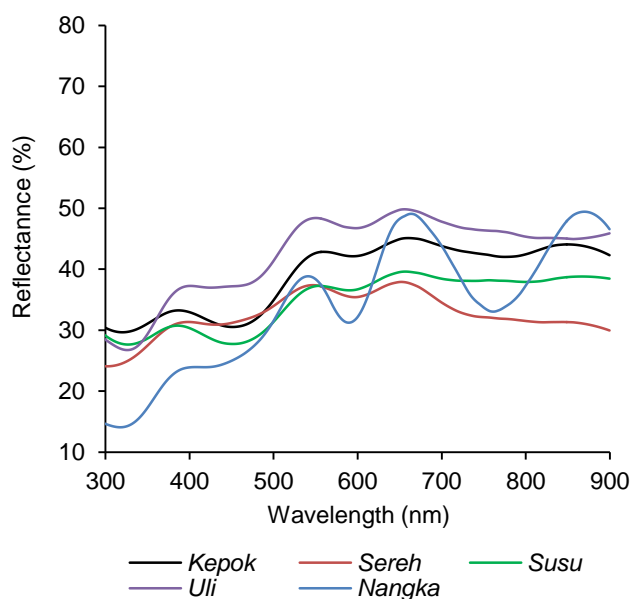


Figure 2. Reflectance spectra of five species of bananas

Figure 2 show that there is same peak of reflectance but different in intensities. The spectrum, there are four absorption regions at wavelengths of 300 to 320 nm. The peak at this wavelength is related to the uptake of Pyridoxine or better known as vitamin B6 (Bilski *et al.*, 2000). The second peak is at a wavelength of 450 which is the absorption of vitamin B12 (Wang *et al.*, 2015). While the third and

fourth peaks are predicted to be absorption from chlorophyll. To confirm this result, the reflectance spectrum was converted into absorbance. Observation of the structure of fruit and food using reflectance spectroscopy has been carried out by several previous studies (Davey *et al.*, 2009; Subedi *et al.*, 2011).

### Absorbance spectra of bananas

The KM methods is applicable to homogeneous tissues, and also can provide inhomogeneous and layered tissues with analytical expression of both transmittance or reflectance (Roy *et al.*, 2012). The KM model was known as the dual flux radiation transmission model. It has been used to study the optical properties of inhomogeneous media. The KM theory describes propagation of uniform, diffuse irradiance through one dimensional isotropic slab with no reflection at the boundaries. However, this model is empirical and has poor accuracy as a viable method for measurement of tissue optical properties. Therefore, it still needs to be studied and developed.

Absorbance spectra can be seen in Figure 3. Reflectance spectra of five banana species were converted into absorbance spectra. Basically, reflectance spectra and absorbance spectra consist of similar information; however, absorbance spectra are more familiar and easier to understand than reflectance spectra. The absorbance (K/S curve) spectra of bananas are shown in Figure 3.

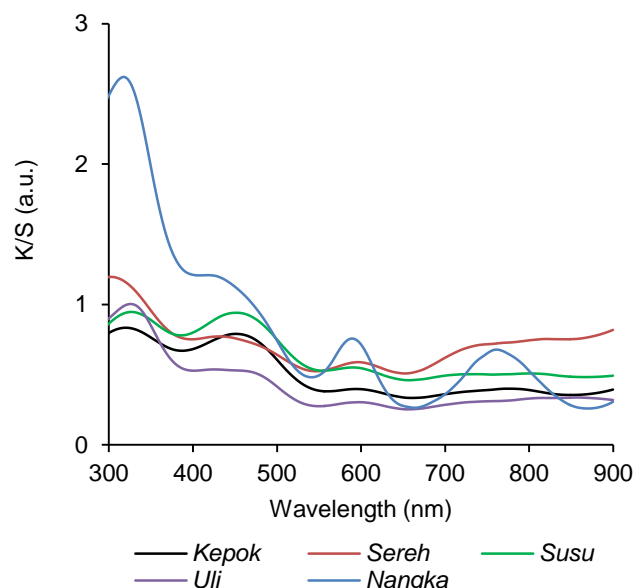


Figure 3. Absorbance spectra of five species of bananas

Similar to reflectance spectra, absorbance spectra of bananas are almost identical in term of

absorbance peak wavelength. However, the absorbance peak intensities are significantly different. In order to clarify this finding, multi-peak fitting (deconvolution) of each absorbance spectrum was performed. Gaussian fitting curves were used in this process, since most of organic spectra are Gaussian (Massicotte *et al.*, 2016). Gaussian fitting is an approach for removing frequency noise from a spectrum. The purpose of using this approach is to obtain an optimal value through the fitting or averaging of several points.

Figure 4A show the fitting results of absorbance spectrum of banana *kepok*. The black curve is the measured absorbance spectrum. The red line curve is our fitting result from multi-peak fitting. The fitting result is matched very well to measured absorbance. The fitting result indicates that there are at least 4 Gaussian deconvolution curves as shown in Figure 5B. These four curves build red fitting curve in Figure 4A. From Figure 4B, each peak wavelength can be investigated and normalized. Furthermore, the same fitting procedure was conducted for all absorbance spectra of remaining bananas. The full list of fitting parameters *i.e.*, peak wavelength and peak intensity of all Gaussians fitting curves for all bananas, can be seen in Table 1.

From Table 1, can be seen that curve 1 has dominant peak at every absorbance spectra. Curve 1 has peak wavelength at around 301 until 325 nm. This absorbance peak wavelength is predicted as absorbance of pyridoxine, which is known as (vitamin B6). Second most dominant curve is curve 2. banana *sereh* and banana *uli* has low peak intensity of curve 2. Other bananas show strong curve 2, which has absorbance at 432 until 453 nm. This peak is predicted as absorbance of vitamin B12. Furthermore, curve 3 and 4 are related to chlorophyll absorbance (Chen *et al.*, 2010; Hashim *et al.*, 2012).

### Photoluminescence spectra of bananas

In addition to reflectance spectroscopy, photoluminescence spectroscopy was used to observe the optical properties of bananas. Basically, bananas were excited using ultraviolet light to generate luminescent that comes out from bananas. Figure 5 shows luminescent spectra of five banana species upon laser 325 nm wavelength excitation.

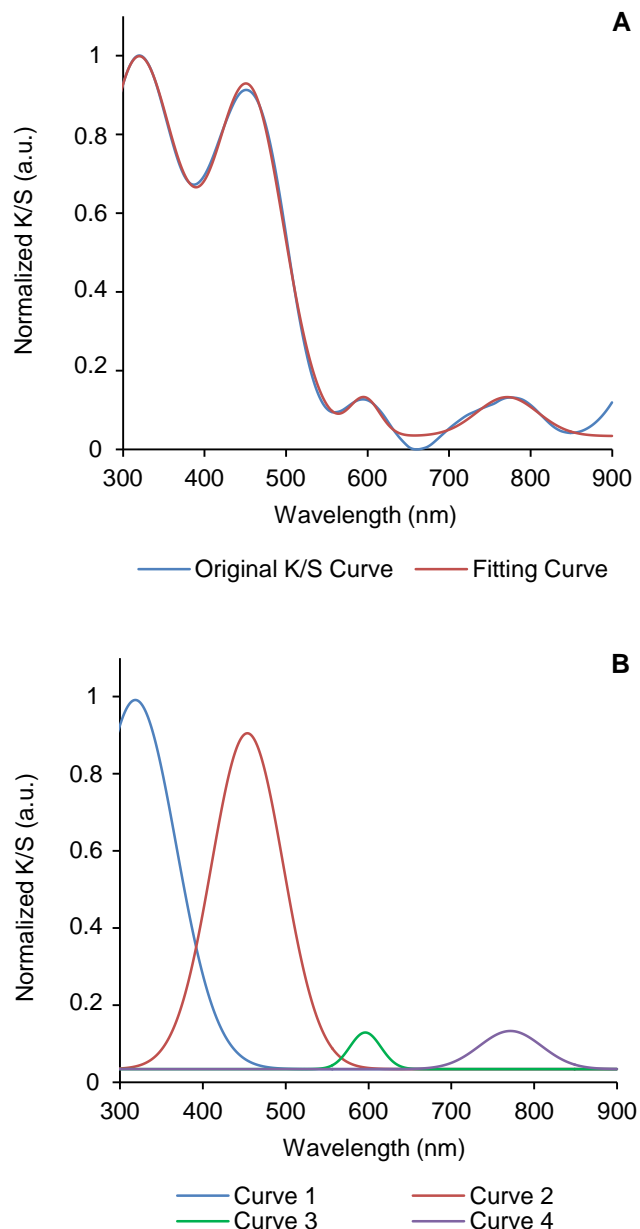


Figure 4. Fitting curve of K/S spectra (A) and deconvolution curves (B) of K/S spectra of banana *kepok*

Table 1. Absorbance peak wavelength (A) and normalized absorbance peak intensity (B) of four fitting curves of five bananas

Sample	Peak Wavelength (nm) of Curve (A)				Peak Intensity (a.u.) of Curve (B)			
	1	2	3	4	1	2	3	4
Banana <i>kepok</i>	318	453	596	768	1.00	0.91	0.13	0.13
Banana <i>sereh</i>	301	434	597	741	1.00	0.38	0.12	0.29
Banana <i>susu</i>	329	448	591	729	1.00	0.98	0.18	0.09
Banana <i>uli</i>	325	452	597	739	1.00	0.37	0.07	0.07
Banana <i>nangka</i>	315	432	591	763	1.00	0.39	0.21	0.18



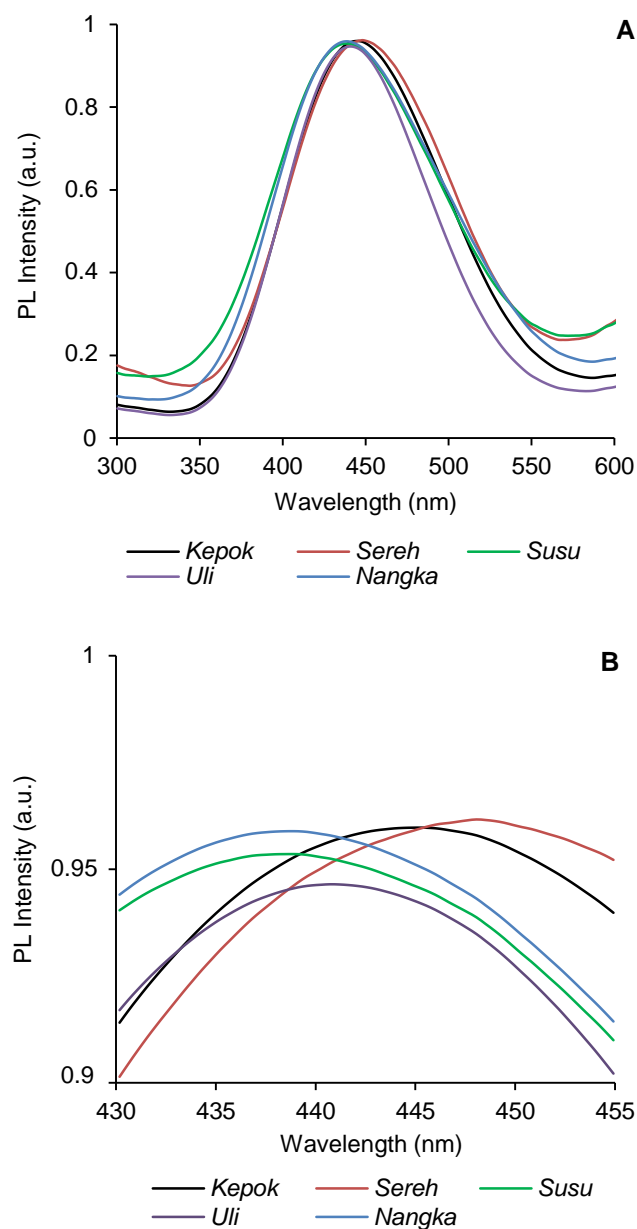


Figure 1. Photoluminescence spectra of five bananas excited by 325 nm laser (A) and magnification of peak photoluminescence spectra for five bananas (B)

## CONCLUSION

In this study, alternative methods for detecting nutrition of five local banana species using reflectance spectroscopy and photoluminescence was conducted successfully. The absorbance spectra, which were obtained from reflectance spectra, consist of four Gaussian fitting curves. The first and second curves indicate absorbance of vitamin B6 and B12, respectively. Furthermore, the wavelength

A of luminescence maximum peak slightly varies from 437 until 448 nm. By using these techniques, we can get the information related to the fruit quality with simple, fast and no need a complex extraction process. Thus, these methods are possible for large scale detection in industry.

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