Fluorescence spectroscopy characteristics of Indonesian citrus

T H Siregar¹, U Ahmad², Sutrisno², and A Maddu³

¹Graduate School of Bogor Agricultural University, Bogor, Indonesia.
²Dept. of Mechanical and Biosystem Engineering, Fac. of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor, Indonesia.
³Dept. of Physics, Fac. of Natural Sciences and Mathematic, Bogor Agricultural University, Bogor, Indonesia.
E-mail: tikahafzara@gmail.com, uahmad2010@gmail.com, kensutrisno@yahoo.com, akhiruddin@apps.ipb.ac.id

Abstract. Citrus suffering physical damage to the peel would spread polymethoxylated flavone that can be detected by its spectroscopic characteristics. Fluorescence spectroscopy is expected to offer an important means for physical damage detection of Indonesian citrus. This study was conducted to determine the characteristics of fluorescence spectra such as absorbance and fluorescence spectra of four varieties of citrus from Indonesia and identified the fluorescence substance in citrus peels. From this study, the absorbance and fluorescence wavelengths of Indonesian citrus were found to vary from 315 nm to 340 nm and from 500 nm to 555 nm respectively. The fluorescence substances were identified as polymethoxylated flavone such as hesperidin, tangeretin, and nobiletin. The fluorescence spectroscopy could be used to detect defects in citrus peel.

Keywords: absorbance spectra, citrus, fluorescence spectra

1. Introduction

A study of spectroscopy characteristics is important to understand the material. Spectroscopy characteristic from fruit substances can be used to build a non-destructive evaluation system. Fluorescence spectroscopy has been used for oil classification [10,12], studying the chemical compounds [5,13], detecting fruit defects such as freeze damage and mold [11,3], detecting the disease stress in citrus plant [2], and quality evaluation of meat product [8,9]; also, fluorescence spectroscopy could be used to evaluate the quality of citrus fruit. Polymethoxylated flavone contained in citrus fruit was one of the fluorescence substances that glowed under UV light excitation. It is possible to detect defects in citrus peel using fluorescence spectroscopy.

Indonesian citrus is an exported commodity [1]. The citrus is subject to damage during handling and transportation. These damaged fruits should be separated as soon as possible to prevent spreading rot starting from the damaged ones. Separating them manually was difficult especially at early stage, because this citrus did not show any visual change before the damaged
part changed its color into brown as a browning reaction happened. Fluorescence spectroscopy could be one non-destructive method to detect the damage in citrus peel. The fluorescent substance can facilitate a different approach for early detection of the physically damaged citrus.

Many studies of fluorescence spectra from citrus fruit has been studied. The fluorescence substances in Mandarin citrus had been identified [4]. An investigation of wavelength excitation for fluorescence emission from orange peel exposed to UV light has been done [7]. From this research, it has been known to fluorescence wavelengths of 15 varieties of citrus grown in Japan were 490-540 nm. Fluorescence wave was derived from the substance available in citrus peel. To build up the image processing engine that can detect damage to the Indonesia citrus, data of citrus on fluorescence spectroscopy characteristics is required. Information of fluorescence and excitation wavelengths can be used to build the model to detect the defects of citrus fruit. The purposes of this research were to study the characteristic of fluorescence spectroscopy from Indonesian citrus and identify the fluorescent substance to support the development of a physical damage detection method of Indonesian citrus based on fluorescence spectroscopy.

2. Materials and methods

2.1. Sample preparation
This experiment was carried out with four varieties of citrus. Batu, Medan, Pulung, and Pontianak citrus were the samples as shown in Fig 1. Citrus was harvested from three different islands from Indonesia. These samples were the famous and favorite citrus in Indonesia. The samples were stored in a room with air conditioning set to 25˚C for one day. Some peel was taken from the samples for extraction. The procedure to prepare peel extract was as follows; citrus peel by weight of 3.1 g was mashed and mixed with 10 mL of pure n-hexane for spectroscopy as solvent. The extract was stirred and kept for one night at the same temperature as the fruits stored. Citrus peel extract was later used for data retrieval using spectrophotometer for absorbance and fluorescence wavelengths observation. First, the absorbance spectra were recorded. From spectra curve, peak of absorbance spectra were taken as excitation wavelength for measuring itself. After determining the best excitation wavelength, the fluorescence spectra were measured.

![Figure 1. Samples of Indonesian citrus; (a) Baru, (b) Medan, (c) Pulung, (d) Pontianak](image)

2.2. Spectroscopic measurement
Absorbance spectra was recorded on a UV-Vis Spectrophotometer (Ocean Optics, USA) with spectra bandwidth of 0.2 nm. Fluorescence spectra was recorded with a USB Fluorescence spectrophotometer (Ocean Optics, USA). Excitation light was provided by a UVGL-58 UV lamp with wavelength at 365 nm. Citrus peel extract was placed in quartz cell and spectra was determined.

2.3. Fluorescence substance identification
The fluorescence substance identification was done by testing the Indonesian citrus peel extract with standard solution of Polymethoxylated flavone (Kanglu Biotech) containing hesperidin,
tangeretin, and nobiletin using the High Pressure Liquid Chromatography (HPLC) fingerprint method. This analysis aimed to determine the chemical compound of fluorescence substance contained in citrus peel. For this analysis, citrus peel extract has been made. Indonesian citrus peel was dried with an oven at 45°C for 48 hours. 10 mg of mashed citrus peel was then dissolved with 100 mL of methanol and sonification for 20 minutes. The citrus peel solution was then filtered and the data was recorded using HPLC. HPLC used reverse phase colom (Zorbax sb C-18), solvent gradient system (water / acetonitrile, v / v) used in the mobile phase. The solvent gradient system was listed in Table 1. Then, the retention time from citrus peel solution and standard solution were measured. First, the standard solution and citrus peel solution (10µL each) were separately injected into the HPLC system and then the chromatograms recorded. Retention time of standard solution in chromatograms and the citrus peel solution in the chromatogram were measured. Finally, the hesperidin, nobiletin and tangeritin peaks in the chromatogram of citrus peel solution were identified by comparing the retention time with the standard solution’s chromatogram.

| Time (min) | Water (%v/v) | Acetonitrile (%v/v) | Elution         |
|-----------|--------------|---------------------|-----------------|
| 0         | 85           | 15                  | Linear gradient|
| 10        | 60           | 40                  | Linear gradient|
| 30        | 45           | 55                  | Linear gradient|
| 40        | 85           | 15                  | Linear gradient|

3. Results and discussion
3.1. Absorbance Spectra of Indonesian citrus
The primary peak of absorbance wavelengths from four citruses is almost the same. Fig 2. shows the absorbance spectra of Indonesian citrus. The primary peak appeared in 300 nm to 365 nm. The absorbance peak occurred in the UV region from 315 nm until 340 and then decreased as the wavelength become longer and the second smaller peak was found in 420 nm. From this information, the fluorescence emission would occur when excited by UV light. To measure the fluorescence spectra for each variety, maximum intensity of absorbance wavelength was used to determine the excitation wavelength. Most of these samples showed that the maximum value of absorbance was in 330 nm until 340 nm. In this study the proper lamp to excitate the sample was 365 nm. It was reported that the excitation wavelength of 365 nm provided better fluorescence spectra of eight varieties Japanese citrus [6]. The second peak appearing in 420 nm to 450 nm referred to another fluorescence substance. It meant that this citrus peel extract contained two kinds of fluorescence substances.

![Figure 2. Absorbance spectra of Indonesian citrus](image)

---

Table 1. Chromatographic system conditions

![Table 1](image)
3.2. Characteristic of fluorescence spectroscopy from Indonesian citrus

There were two primary peaks in fluorescence spectra. Fig 3. shows the fluorescence spectra of Indonesian citrus. This fluorescence spectra excited by 365 nm. Fluorescence spectra occurred in the visible region. The first fluorescence spectra peak occurred from 500 nm until 555 nm and the second occurred from 630 nm until 800 nm. This fluorescence spectra were almost the same with Japanese and Mandarin citrus. The fluorescence spectra peak from fifteen varieties of Japanese citrus appeared in 490 nm to 540 nm [7]. Mandarin citrus has fluorescence substance that excited with light from 320 nm to 390 nm and emitted the fluorescence of 520 to 570 nm [4]. The fluorescence substance in Mandarin citrus is Polymethoxylated flavone. From this literature, the fluorescence substance from Indonesia citrus is supposed to be the same as the mandarin citrus. The first peak should be the Polymethoxylated flavone. This substance would release in the citrus peel when the citrus experienced damage. From this study, the fluorescence method could be applied to be a detection method of Indonesian citrus. The second peak in 630 nm to 800 nm was chlorophyll substance. Chlorophyll’s fluorescence wavelength is 650 nm until 800 nm [2].

![Figure 3. Fluorescence spectra of Indonesian citrus](image)

3.3. Group of fluorescence intensity

It was important to know the energy level of fluorescence intensity from every variety of citrus to detect the defect of them. Different varieties had different fluorescence intensity levels. From Fig. 3, the samples could be divided into three groups based on the fluorescence intensity. Pontianak citrus was in the high fluorescence intensity group. Batu and Medan citrus were in the low fluorescence intensity group. Table 2 showed the group of citrus based on fluorescence intensity. This energy come from fluorescence substance contained in citrus peel. By acquiring knowledge of spectra characteristics from different varieties of citrus in the UV and VIS regions, classification based on the level of fluorescence emission could be used to detect the defect of citrus fruit.

| Group     | Name of Variety | Absorbance peak (nm) | Excitation (nm) | Fluorescence peak (nm) | Fluorescence Intensity |
|-----------|-----------------|-----------------------|----------------|------------------------|-----------------------|
| High FL   | Pontianak       | 315                   | 365            | 550                    | 4200                  |
| Medium FL | Pulung          | 335                   | 365            | 500                    | 2200                  |
| Low FL    | Batu            | 338                   | 365            | 530                    | 380                   |
|           | Medan           | 340                   | 365            | 555                    | 210                   |
3.4. Fluorescence substance of Indonesian citrus
The HPLC test has been conducted. The retention time from the citrus peel solution and standard solution were compared. Fig. 4 shows the fingerprint chromatograph. The retention time of three chemical compounds from the standard solution was the same as the citrus peel solution. The result showed that the fluorescence substance contained in Indonesian citrus was polymethoxylated flavone. Polymethoxylated flavone contained three main substances such as hesperidin (1), nobiletin (2), and tangeretin (3). The HPLC chromatograph fingerprint indicates that Indonesian citrus contained the same three substances of polymethoxylated flavone. Hesperidin was detected at 7.53 min, nobiletin was detected at 18.28 min, and tangeretin was detected at 21.8 min.

![HPLC chromatographic fingerprint from standard solution and citrus peel solution](image)

**Figure 4.** HPLC chromatographic fingerprint from standard solution and citrus peel solution

4. Conclusion
The absorbance and fluorescence wavelengths of Indonesian citrus were found to vary from 315 nm to 340 nm and from 500 nm to 555 nm respectively. The excitation wavelength was 365 nm. Pontianak citrus was the high fluorescence intensity and Pulung was the medium fluorescence intensity. Batu and Medan were the low fluorescence intensity. Fluorescence substances in Indonesian citrus were identified. Two fluorescence substances in citrus peel extract were Polymethoxylated flavone (hesperidin, nobiletin, and tangeritin) and chlorophyll. Fluorescence spectroscopy could be used to detect the physical damage in citrus.

5. References

[1] Agisimanto D C, Martasari A and Supriyanto 2007 Perbedaan primer RAPD dan ISSR dalam identifikasi hubungan kekerabatan genetik jeruk siam (Citrus suhuniensis L.Tan). Indonesia Jurnal Holtikultura. 17(2):101-110.

[2] Belasque J, Gasparoto M C G, Marcassa L G 2008 Detection of mechanical and disease stresses in citrus plants by fluorescence spectroscopy J. Applied Optics. 47:1922-1926.

[3] Blasco J, Aleixos N, Gomez J and Molto E 2007 Citrus sorting by identification of the most common defects using multispectral computer vision Journal of Food Engineering. 83 (2007) 384-393.

[4] Kondo N M, Kuramoto H, Shimizu Y, Ogawa M, Kurita T, Nishizu V K, Chong K and Yamamoto 2009. Identification of fluorescent substance in Mandarin orange skin for
machine vision system to detect rotten citrus fruits. *EAEF Research Paper*. 2(2): 54-59.

[5] Li Y, Gao E, Gao E, Shan E, Bian J, and Zhao C. 2009. Study on the Interaction between 3 Flavonoid Compounds and \( \alpha \)-Amylase by Fluorescence Spectroscopy and Enzymatic Kinetics. *Journal of Food Science*. Vol. 74 (199-203).

[6] Momin M A, Kondo N, Kuramoto M, Ogawa Y and Shiigi T 2015. Study on excitation and fluorescence spectrums of Japanese citruses to construct machine vision systems for acquiring fluorescent images *Proc. Of SPIE* Vol. 8027.

[7] Momin M A, Kondo N, Kuramoto M, Ogawa Y, Yamamoto K and Shiigi T 2012. Investigation of excitation wavelength for fluorescence emission of Citrus peels based on UV-VIS spectra. *EAEF Research Paper*. 5(4):126-132

[8] Oto N, Oshita S, Makino Y, Kawagoe Y, Sugiyama J, and Yoshimura M. 2013. Non-destructive evaluation of ATP content and plate count on pork meat surface by fluorescence spectroscopy. *Journal of Meat Science*. 93 (2013) 579-585.

[9] Sahar, Boubellouta T, and Dufour E. 2011. Synchronous front-face fluorescence spectroscopy as a promising tool for the rapid determination of spoilage bacteria on chicken breast fillet. *Food Research International*. 44 (2011) 471-480.

[10] Sikorska E, Gorecku T, Khmelinskii I V, Sikorski M, and Koziol J. 2005. Classification of edible oils using synchronous scanning fluorescence spectroscopy. *Journal of Food Chemistry*. 89 (2005) 217-225.

[11] Slaughter D C, Obenland D M, Thompson J F, Arpaia M L and Margosan D 2007. Non-destructive freeze damage detection in oranges using machine vision and ultraviolet fluorescence *Postharvest Biology and Technology*. ELSEVIER 48:341-346.

[12] Tan J, Li R, Jiang Z T, Tang S H , Wang Y, Shi M, Xiao Y Q, Jia B, Lu T X, and Wang H. 2017. Synchronous front-face fluorescence spectroscopy for authentication of the adulteration of edible vegetable oil with refined used frying oil. 2017. *Journal of Food Chemistry*. 217(2017) 274-280.

[13] Wang Z, Wu Z, and Tang S. 2009. Characterization of dissolved organic matter in a submerged membrane bioreactor by using three-dimensional excitation and emission matrix fluorescence spectroscopy. *Water Research*. 43 (2009) 1533-1540.