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Investigation of Excitation Wavelength for Fluorescence Emission of Citrus Peels based on UV-VIS Spectra

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Abstract

A study was conducted to investigate the best wavelengths for fluorescence excitation and the resulting fluorescence wavelengths in the range of 300-700 nm with citrus peels taken from 15 varieties, which are concerned with detection of surface defects of citrus fruits. Characteristics of UV absorbance, excitation and fluorescence spectra were observed by variety, and principle components analysis (PCA) and discriminate analysis (DA) were used to categorize the citrus varieties by fluorescence intensity levels in order to give some tips for optimizing the fluorescence imaging based machine vision system. The observed proper excitation wavelength for best fluorescence emmision and resulting peak fluorescence wavelength varied variety to variety and ranged from 350 to 380 nm and 490 to 540 nm respectively. The selected varieties of citrus were categorized successfully into four groups of known fluorescence level, namely strong, medium, weak and no fluorescence groups.

[Keywords] citrus peels, excitation wavelength, fluorescence spectra, fluorescence intensity, surface defects

I Introduction

In Japan many commercial and cooperative grading systems have been developed by use of machine vision, NIR analysis, and other automation technologies and practically used for agricultural products. The citrus fruits are judged into quality categories according to parameters such as size, shape, colour, ripeness, sugar content, acidity, etc; by these systems. However, grading citrus according to the presence of defects is still very challenging for the packinghouse manager. Considering the importance of defect detection in the recent past, a number of techniques have been studied based on using machine vision systems (Aleixos et al., 2002; Blasco et al., 2007; Kurita et al., 2009; López-García et al., 2010; Slaughter et al., 2008) to detect various type of defects in citrus.

The fluorescent substances in citrus peel oils have been investigated extensively and are useful taxonomic markers (Tatum and Berry, 1979). The peel of citruses contains large amounts of essential oils that reside both within (Bosabalidis and Tsekos, 1982; Shomer, 1980) and outside (Obenland et al., 1997) of the oil glands in the flavedo. Latz and Ernes, (1978) reported that the fluorescence phenomenon is induced in the essential oil of the peel of the citrus fruits when it is released by some defects. Tangeretin, a polymethoxylated flavone that is a component of the peel oil (Swift, 1967), fluoresces under ultraviolet (UV) light and is a likely source of the yellow fluorescence visible from damaged or decayed oranges in dark rooms. The rotten or damaged parts of citrus fruit fluoresce when excited by UV (Uozumi, et al., 1987). Recently a study identified that heptamethoxy flavone is one of the major fluorescent substances in rotten citrus fruits and that the excitation and fluorescent wavelengths of one of the substances were between 360 to 375nm and between 530 to 550nm, respectively (Kondo et al., 2009). These studies have revealed that the fluorescence imaging technique has the potential to detect surface defects especially rot, injury, damage or decay, of a wide variety of citrus due to the presence of fluorescent substances in their skin.

The fluorescent compounds in citrus peel emit fluorescence in the visible region (VIS) of the spectrum when excited with UV radiation. The fluorescence emission from the object is a function of the angle and wavelength of the incident light and chemical and physical composition of the object. To acquire fluorescent image the importance of proper illumination for a machine vision system cannot be overstated (Chen et al., 2002). Recently, we were developing an algorithm for detecting the fluorescent area on defective citrus surfaces based on fluorescence imaging technique emitted by two different excitation wavelengths (Momin et al., 2010). The proposed system of fluorescence region detection was effective for some varieties of citrus but for other citruses they

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did not fluoresce properly as well as the intensity level of fluorescence emission was different from variety to variety. In addition, it is desirable to optimize machine vision system for detecting surface defects of citrus fruits.

Therefore, it is very important to know the spectral properties of citrus, at which excitation wavelength the peel of the citrus fluoresces properly to inspect the fluorescence regions as well as to classify citrus based on the levels of fluorescence emission. In addition for a lighting unit selection, and its configuration for constructing machine vision system, a spectral study is necessary. The investigation of the best wavelengths for fluorescence excitation and the resulting fluorescence wavelengths may be most practical for an automated sorting system using machine vision and UV illumination. However, no significant research has been done related to spectral investigation of citrus, especially with respect to excitation and fluorescence spectra. Therefore, the goal of this work was to determine the proper excitation wavelength for fluorescence emission by acquiring knowledge of spectra of different citrus varieties in the UV and VIS regions in order to develop a database and differentiate citruses for getting clear information.

II Materials and Methods

1. Citrus test sets

The citrus used in this project was collected from a farmers market in Ehime Prefecture, Japan during two harvest seasons in early February and April 2011. Before measurements of UV-VIS spectra the citrus were stored at 25°C for one day to reach equilibrium temperature with laboratory environment. The experiments were carried out with the fifteen common and popular varieties of Japanese citrus shown in Fig.1 and specific varieties are detailed in Table 1.

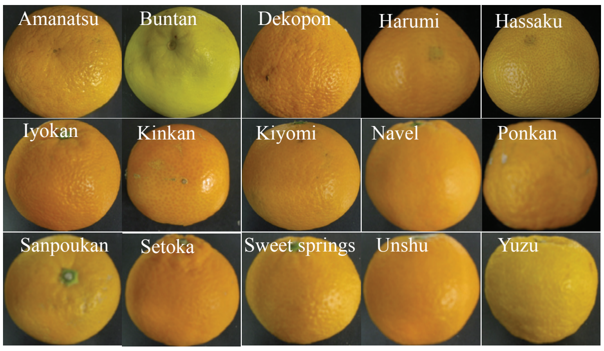

Fig. 1 Citruses used for experiment

2. Equipment used

A UV-VIS-NIR spectrophotometer (U-4000, Hitachi, Ltd., Tokyo, Japan) was used to measure the UV absorbance spectra and a fluoro-spectrophotometer (F-4500, Hitachi, Ltd., Tokyo, Japan) was used to measure excitation (EX) and fluorescence (FL) spectra of different citrus varieties. The spectrometers were connected via a PCI card to a PC, and specific software was used to modify spectrometer set-up and store acquired spectra. The spectrophotometers provide instant measurement and excellent performance over the entire wavelength ranges of 190-2650 nm and 200-900 nm respectively.

3. Sample preparation and spectra measurements

The citrus peels contain number of water-soluble and oil-soluble fluorescent compounds and these compounds are dissolved easily into lipophilic organic solvents (Frerot and Decorzant, 2004; Kurata et al., 2002). In this experiment, the fluorescent substance was extracted using chloroform as a solvent to prepare the sample for spectra analysis. One square centimeter area of peel from three different locations around top, bottom and center of each fruit was taken as sample. Each peel sample was mixed with 5 ml of chloroform and crushed together for extracting the fluorescence substances. Then after filtering 4 ml of the mashed sample mixture was directly placed into a quartz cell of F11-UV-10 type and the spectra were measured sequentially. First the UV absorbance spectra were determined and from this spectral curve the wavelength at absorbance peak was used for measuring the FL spectra. Then the peak FL wavelength obtained from FL spectra was used to measure the EX spectra for observing the proper EX wavelength of each variety. The measured spectra results from three preparations were found almost identical and thus the spectra of only one preparation are shown here.

4. Statistical analyses

The combination of spectroscopy and statistics method such as principal component analysis (PCA) and discrimination analysis (DA) was conducted using The Unscrambler software package (Version 9.7, CAMO, Norway) and TQ Analyst (Version 6, Thermo Electron Corp., USA) respectively to categorize selected citruses with different FL levels. All statistical analyses are based on the wavelengths of VIS spectral range obtained from FL spectra. Every spectrum has its own unique set of components or scores and hence a spectrum can be represented by its PCA scores in the factor space instead of intensities in the wavelength space (Park et al., 2003). The PCA transforms the original independent variables such as wavelengths into new axes, or PCs. These PCs are orthogonal, so that the data set presented on these axes are uncorrelated with each other (Martens and Naes, 1989). The second PC is orthogonal to the first PC expressing the largest amount of information of the variation in the data and followed by the second PC which conveys the second most important factor of the remaining analysis and so forth (Xie et al., 2007).

Table 1 Detailed information on the measured citrus varieties

Variety name	Species	Type	Wavelength (nm)			FL intensity	Possible FL substance type	FL group
			UV	FL	EX			
Amanatsu	C. natsudaidai	Daidai	330	535	378	2650	1	Strong
Dekopon	Kiyomi x C. reticulata	Hybrid	335	534	378	2570	1	
Buntan	C. grandis	Zabon	310	535	376	2165	1	
Sanpoukan	C. sulkata	Daidai	320	535	375	930	1	Medium
Kiyomi	C. unshiu x C. sinensis	Hybrid	325	533	373	850	1	
Sweet springs	C. unshiu x C. hassaku	Hybrid	320	538	376	810	1	
Unshu	C. unshiu	Mikan	335	535	375	810	1	
Iyokan	C. iyo	Daidai	310	538	375	755	1	
Navel	C. sinensis	Daidai	325	529	373	530	1	
Hassaku	C. hassaku	Zabon	315	533	373	475	1	
Setoka	Encore x Murcott	Hybrid	325	510	374	300	2	
Yuzu	C. junos	Yuzu	320	497	355	15	3	Weak
Harumi	Kiyomi x C. reticulata	Hybrid	310	500	355	12	3	
Ponkan	C. reticulata	Mikan	315	504	360	11	3	
Kinkan	Fortunella crassifolia	Magnoliophyta	No typical UV-VIS spectra					Zero

PC scores plot was performed to gain an overview of the similarities or differences among the fifteen samples. The closer the samples are within a score plot, the more similar they are with respect to the PC score evaluated (Al-Qadiri et al., 2006).

Though PCA allows the visualization of underlying structure in experimental data and provide information to distinguish samples, multivariate discriminate analysis was studied to understand more clearly about the similarities between data and samples as well as to determine the improved classification of the samples. DA was applied for the classification of citruses into groups based on known or predefined classes in terms of their nearness and in this study initially the samples were divided into three groups. Differentiation of the groups is based on computing the Mahalanobis distance (MD) of a sample from their centers of gravity of the considered groups; one can then clarify the properties that distinguish the different groups. If the individual sample is close to the center of gravity of its defined group, it is "correctly classified". In the case where the distance to the center of gravity of its group is superior to that to the center of gravity of another group, the individual is "poorly classified" and it will be reassigned to the other group (Xie et al., 2007).

III Results and Discussion

1. Spectral properties

The measured UV, EX and FL spectra using chloroform as reagent of fifteen varieties of citruses are shown in Figs. 2-4. From Fig. 2 it is seen that except one variety (kinkan) in the other fourteen varieties the absorbance peak occurred in the UV region and then decreased sharply as the wavelength became longer and then some smaller second peaks were found after 400 nm. As pointed out above the emission of FL of citrus peel is visible when excited by UV light and so those smaller peak absorbance wavelengths found after 400 nm were discarded in this experiment. Therefore to measure FL spectra for each variety we used the maximum absorbance wavelength value shown in Table 1. The UV spectrum of kinkan revealed no distinctive absorbance spectrum trend and then we tried to find FL spectrum excited by different wavelengths but did not find any typical FL spectrum curve (Fig. 3(c)) also. Therefore, though kinkan is citrus it may not have any FL substances in their peel or a very small amount, which is difficult to identify.

The FL spectra shown in Fig. 3 (a), (b) and (c), revealed that for all varieties the FL emission occurred in the VIS region of the spectrum as well as except three varieties (harumi, ponkan and yuzu) the other eleven varieties observed general or good trend of FL spectra with high and medium FL level even some varities has similar EX value. The FL spectral data were used for further statistical analyses to determine classification. The wavelength at which FL peak of citrus varieties observed are shown in the Table 1. From EX spectra shown in Fig. 4 it is observed that among fourteen varieties, twelve varieties have one peak of EX wavelength and two varieties (setoka and yuzu) have two peaks. Those varieties showed two EX peaks might be their collected peel sample contains two types of fluorescent substance. The proper EX spectra information provide to select the best wavelength of the incident light for inspecting the FL regions included in the citrus skin. Those variety had two EX values we excited successively by these values. Then checked which wavelength provided better FL spectrum trend compare to other one and considered that as proper EX value. For example in case of setoka when excited by 374 nm provided better spectrum and relatively high FL level compare to 356 nm.

Based on the EX and FL wavelength characteristics it was suggested that at least three different types of fluorescent substance possibly presented in the collected samples. For example EX wavelength over 365 nm and FL around 530 nm is one type, second type is EX over 365 nm and FL near 510 nm and another is EX between 355 and 360 nm and FL close to 500 nm (Table 1).

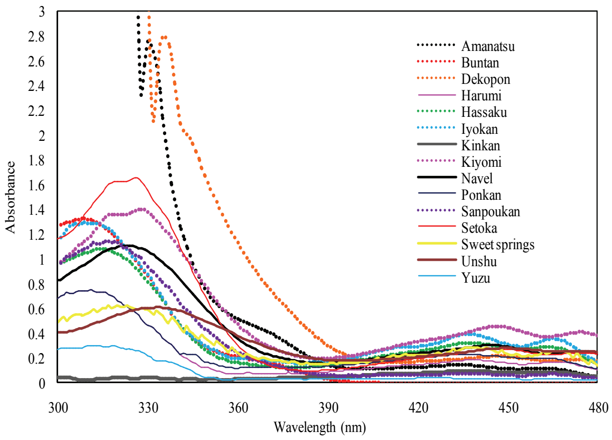

Fig. 2 Absorbance spectra

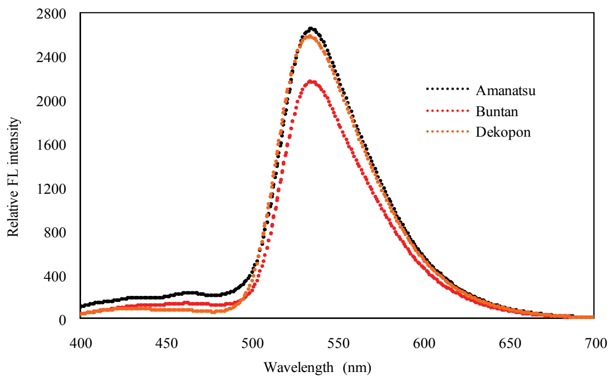

Fig. 3(a) Fluorescence spectra of strong group citruses

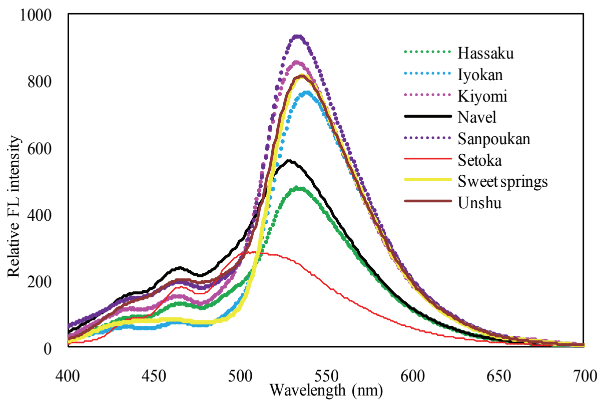

Fig. 3(b) Fluorescence spectra of medium group citruses

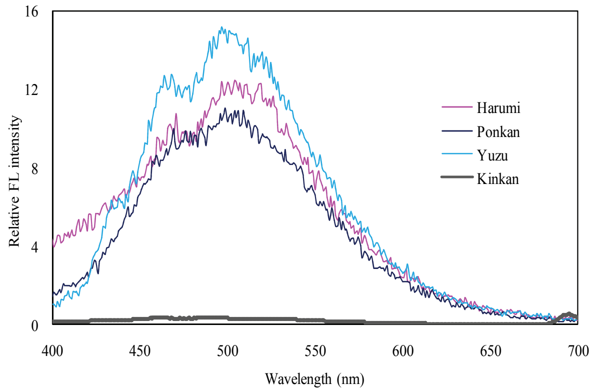

Fig. 3(c) Fluorescence spectra of weak and zero group citruses

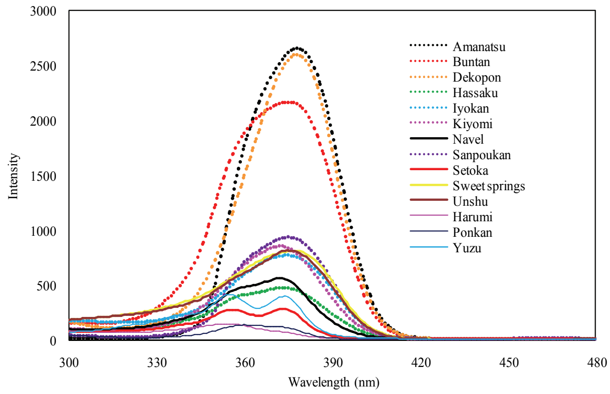

Fig. 4 Excitation spectra

2. Citrus classification

According to published reports citrus surfaces contain FL substances, so for every variety of citrus it is important to know the information on FL energy or intensity of these substances for various purposes like to design the image acquisition system for detecting surface defects. The FL spectral characteristics of the different varieties shown in Fig. 3(a), (b) and (c) were used for observing the level of fluoresce intensity for each variety and the result shows great variation and similarities within the different varieties. In terms on the intensity characteristics of FL substances, the citrus fell naturally into four FL groups namely strong, moderate, weak, and zero, from the measured fifteen varieties with details as shown in Table.

It is seen from Fig. 3(a) among all varieties that amanatsu, buntan and dekopon have shown almost similar characteristics of FL spectra and attained the highest intensity value at around FL wavelength 535 nm and this three varieties were selected in strong FL group. To detect the FL regions of strong group citruses UV LEDs (emitting rays 340-380nm) or UV-A lamps (emitting rays at 315-400 nm with peak at 368 nm) could be used as illumination system with combination of normal color camera (such as VGA format camera of 8 bit gray levels) for designing the image acquisition device. The characteristics of FL intensity ranged from 200 to 1000 is considered as medium group and Fig. 3 (b) shows that most of the varities are in this group. Excluding FL wavelength (510 nm) of setoka, EX and FL wavelength of the others almost over-lapped with strong group. Both groups may contain similar FL compounds but differ only in amount of compound. The same lighting device of strong group with high resolution camera (such as XGA or SXGA format of 8 or 10 bit gray levels) could be used for medium group citruses because of similar structure of FL compound. Harumi, ponkan and yuzu are selected in weak group as their FL intensity levels are very weak, as shown in Fig. 3 (c). The UV-A and UV-B lamps with high resolution camera (such as SXGA or UXGA format of 10 bit gray levels) could be suitable to identify the defective areas on their peel surface because of very low FL amount. From Fig. 3 (c) it is seen that kinkan did not show any specific FL spectrum due to absence of fluoresce components or containing very small amount of substances that are hard to measure. In addition, a study reported that no FL substance observed on the skin of kumquat (kinkan) when excited by UV LED with peak at 365 nm (Kondo et al., 2007). Therefore kinkan is considered in zero or no FL group.

The categorization result indicates that FL intensity characteristics will very likely to play an important role in the classification functions for citruses. However, the level of FL intensity characteristics cannot be the sole factor in classifying the citruses and to accomplish this a number of statistical analyses PCA and DA were used to examine the inherent structure of the data.

(1) Principal component analysis (PCA)

PCA is an effective mathematical tool which performs to reduce the multidimensionality of the data set while retaining as much information as possible between the spectroscopic data points. PCA was applied only the FL spectra (Fig.3) data in the range from 400 to 650 nm of 14 citrus varieties and spectral data of another variety (kinkan) did not analyse because no typical FL spectroscopic data observed from it. According PC scores derived from PCA, the PC1 explained 98.65% of the total variance in the data set while PC2, PC3, PC4, PC5 explained 1.09%, 0.21%, 0.03% 0.01% respectively. This mean PC1 and PC2 offer the main contribution to understand the relationship between the variables and the citrus groups. The result of PCA analysis of 14 varieties using PC1 and PC2 is presented in Fig. 5 along with possible grouping ellipses. From Fig. 5 it is apparent that if groupings are drawn in a logical manner the samples are separated very well into three groups. Firstly, all three varieties of week FL group are located on the bottom left-hand corner of the figure and overlapping each other, the medium FL group citruses are mostly scattered throughout the top left quadrant and rest two varieties observed on the lower left half close to the y axis of the graph, while the strong FL group citruses are found on the right half side of the plot, in contrast opposition to weak and medium groups. The score plots of PC1 and PC2 suggest that the differentiation between 14 citrus varieties is possible and the results are associated with classification based on characteristics of FL intensity of sample. Another useful discriminate analysis by inspecting the MD was used for an improved classification because PCA only indicates the visualizing dimension spaces.

(2) Discriminate analysis (DA)

In DA the samples are grouped by generating discriminate functions for the variables and then compute the MD. A graph of the data after obtained following MD of every sample to the three classes by DA is shown in Fig. 6. From this graph it is apparent that the selected 14 variety citruses can be well-differentiated into three groups using the FL spectral data range from 400 to 650 nm. The clusters of three different FL group can be clearly distinguished from each other with 100% accuracy, which demonstrated the quite useful discriminatory technique to classify the 14 varieties.

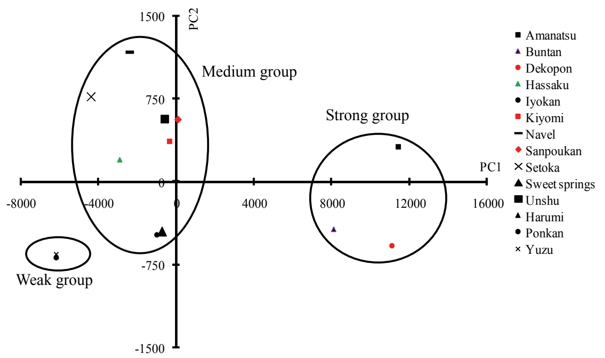

Fig. 5 Results of applying PC1 (98.65% variance) and PC2 (1.09%) score plots to the data (Suggested grouping ellipses are drawn.)

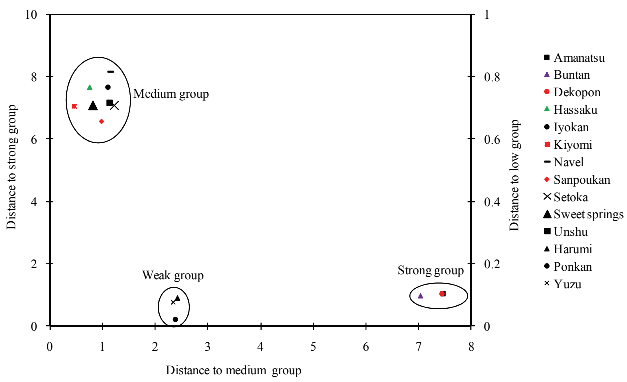

Fig. 6 Results of applying DA to the data (Suggested grouping ellipses are drawn.)

IV Conclusions

In this investigation, the absorbance, excitation and FL spectra of fifteen varieties of Japanese citruses were measured and analysed successfully in the wavelength range between 300 and 700 nm. The EX spectra results revealed that the informations of proper EX wavelength would be helpful to select the wavelength of the incident light for inspecting the fluorescence areas included in the citrus skin. By using spectral characteristics of FL intensity levels it is possible to classify selected citruses into four FL groups. In addition, the multivariate data analysis combined with VIS spectroscopy allows easy interpretation of similarities and differences between objects and this data analysis technique provided a perfect classification with high accuracy. The information from this study will be useful for citrus grading industry to design as well as optimize the fluorescence imaging based machine vision system in order to detect the defective citrus fruits.

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