Research Paper

# Identification of UV-Fluorescence Components for Detecting Peel Defects of Lemon and Yuzu using Machine Vision

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## Abstract

Fluorescence imaging has been used to detect peel defects in many citrus varieties, but not in lemon and yuzu. In this study, we extract and identify fluorescent components from the peel of lemon and yuzu. The characteristics of excitation and fluorescence of these extracted components were determined, and their identity clarified using NMR spectroscopy. Extracted fluorescent components for both lemon and yuzu have a coumarin structure. Two coumarins (5-7-dimethoxycoumarin,5-geranyloxy-7-methoxycoumarin) were identified in lemon and one (5-geranyloxycoumarin) in yuzu. Their excitation and fluorescence wavelength were observed at 320 - 330 nm and 380 - 400 nm, respectively. Moreover, when a fluorescence imaging system was set-up with UV-A illumination, it was shown that this systemcan detect peel defects in lemon, and to a lesser extent in yuzu.

[Keywords] citrus, fluorescence, excitation, wavelength, defects, machine vision

## I Introduction

Visual fruit inspection of citrus for external quality attributes, such as fungal rots, mechanical damage, and injury, is still the most prevalent form of grading. This form of grading, however, is problematic due to variations in the pattern, size, position, and colour of defects. As this has important consequences for production and economic losses, researchers worldwide are searching for suitable alternative methods to detect peel defects and diseases in citrus.

Reports have noted that most organs in citrus accumulate significant quantities of flavonoids during their development, and that when these flavonoids are exited with ultraviolet (UV) light, a number of them fluorescence in the visible (VIS) region of the spectrum (Kondo *et al*., 2009; Benavente-Garcia, *et al*., 1993; Castillo, *et al*., 1992; Uozumi *et al*. 1987). It has been proposed that when the oil glands of citrus are ruptured, peel oil is released, migrates closer to the peel surface, allowing it to be visible when excited by UV light (Latz, and Ernes, 1978). Tangeretin, a polymethoxylated flavone, is one such fluorescing component of peel oil (Swift, 1967). This is also likely to be the source of yellow fluorescence visible in defect oranges in black light rooms.

A number of studies have based the detection of surface defects and disease, in a wide variety of citrus, on these fluorescent components. In a recent study, Blasco *et al*., (2007) examined the use of UV-induced fluorescence as a part of multispectral analysis to identify defects in citrus caused by green mould. In another study, Slaughter *et al*., (2008) evaluated the feasibility of using machine vision and long wave UV fluorescence to detect and separate freeze-damaged oranges.

Our lab has evaluated the ability of a machine vision system, based on UV-induced fluorescence imaging, to detect peel defects in citrus (Kurita *et al*., 2009, Kondo *et al*., 2009, Momin *et al*., 2010, Ogawa *et. al*., 2011). Kurita *et al*., (2009) proposed a machine vision system consisting of a pair of white and 365 nm UV LED lighting devices and a colour CCD camera for the task of grading orange fruit. Kondo *et al*., (2009) found that the fluorescent component extracted from mandarin orange peel had the basic structure of a flavone, and is quite possibly heptamethylflavone. This component was found to be excited at 360 to 375nm, and fluoresce at wavelengths between 530 to 550nm. Momin *et al*., (2010) proposed an algorithm for detecting the fluorescent area of citrus fruit when using fluorescence imaging. Ogawa *et al*., (2011) has demonstrated the

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detection of rotten areas using fluorescent images of mandarin oranges.

However, the extent of peel defect UV-fluorescence of fifteen common varieties of Japanese citrus (amanatsu, buntan, dekopon, harumi, hassaku, iyokan, kiyomi, lemon, navel, ponkan, sanpoukan, setoka, sweet spring, unshu, and yuzu) was found to vary substantially when using a LED-induced double image acquisition system, as proposed by Kurita *et al*. 2009. The system illuminates the citrus with white and UV LED (peak wavelength at 365 nm) lighting panels and develops colour and fluorescence images for visible and defect information, respectively. The same lighting system was applied to all fifteen varieties. Peel defects for some varieties had very strong green fluorescence, while others fluoresced moderately or only slightly, or not at all in the case of lemon and yuzu. For example, the observed greenish fluorescence of some varieties are shown in Fig. 1. Peel injuries in lemon and yuzu, however, did not show fluorescence under the existing UV excitation system. In this study, we attempt to identify which components of the peel in lemon and yuzu will fluorescence, and consequently can potentially be used for detection of surface defects. In addition, the proper excitation conditions for these components are determined, in order to reconfigure a detection system that can be used for a wide selection of Japanese citrus varieties, including lemon and yuzu.

Because the energy of a fluorescent light is small, it will be necessary to reconstruct a more efficient machine vision system which can capture higher grey level images after excitation at the appropriate wavelengths. Thus the objectives of this paper are to identify in vitro fluorescence components in peel of lemon and yuzu, as well determine the appropriate excitation wavelengths of these components. This will enable the construction of an efficient light excitation and fluorescence analysis system for potential detection of peel defects in lemon and yuzu. Once this analysis system has been constructed, the detection of surface peel defects in lemon and yuzu will be confirmed.

## II Materials and Methods

### 1. Sample preparation and spectra measurement

The lemon and yuzu used in this report were collected from a farmers market in Ehime Prefecture, Japan. Before measurement of the UV-VIS spectra took place, the fruit were stored at 25 ºC for one day, in order for them to reach an equilibrium temperature with laboratory

conditions. A one square centimeter area of peel (including flavedo and albedo part) was taken from both lemon and yuzu at three different locations; from the top, bottom and center of each fruit. The average weight and thickness of each peel sample of lemon and yuzu were 0.31 g and 0.356 g, and 3.96 mm and 4.5 mm respectively. Each peel sample was then mixed with 5 ml of chloroform, and crushed for extraction of the fluorescence components. This was then filtered, 4 ml of the extract placed directly into the spectrophotometer measuring cell (F11-UV-10 quartz type). This extraction procedure is hereafter referred to as "Extraction Method A". The sample was then illuminated for excitation and the resultant fluorescence spectra measured.

A fluoro-spectrophotometer (F-4500, Hitachi, Ltd., Tokyo, Japan) was used to record excitation and fluorescence spectra. The excitation and fluorescence spectra represent the relative photon intensity per wavelength interval. The excitation spectrum is a plot of the luminescence signal versus excitation wavelength with a constant emission wavelength. A plot of the luminescence signal versus emission wavelength with a constant excitation wavelength is denoted as an emission spectrum. Fluorescence spectral data are generally presented as an emission spectrum.
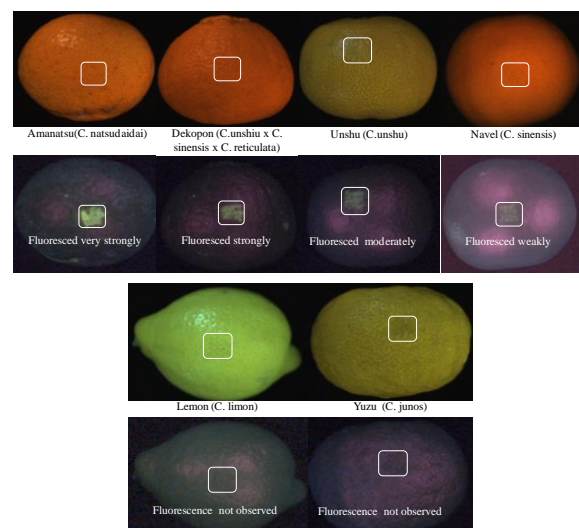


Fig. 1 Example of colour (1st and 3rd row) images captured by white LEDs and fluorescence (2nd and 4th row) images by UV LEDs

### 2. Further extraction of fluorescent components for identification

One kilogram of peel, including the flavedo layer for lemon, and 100 g for yuzu, were finely ground using a

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centrifugal mill, and were then soaked in chloroform for a day for extraction of components. The extract was filtered through cotton, the chloroform layer collected, and then concentrated with an evaporator (Rotary Vacuum Evaporator Type N-1, EYELA). This concentrate was then dissolved in 300 ml methanol, and then 200ml hexane, and then finally the hexane and methanol layers separated using a separation funnel (500 ml).

Both of these fractions were then illuminated with an UV-A (blacklight blue lamp). In the case of lemon peel, bluish fluorescence was observed in the methanol extract. In the case of yuzu, the hexane extract showed fluorescence. To further separate the active fraction of fluorescence components, the methanol extract for the lemon peel and the hexane extract for yuzu were run through a $SiO_2$ column on a chromatograph and on preparative thin layer chromatograph (PTLC). Nuclear magnetic resonance (NMR) analysis with a deuterated chloroform solvent was performed. The ^{1}H NMR spectra were recorded at 500 MHz frequency and a temperature of 23 ℃, using a Bruker AVANCE III NMR instrument. This extraction procedure is hereafter referred to as "Extraction Method B".

### 3. Fluorescence image acquisition

We also acquired fluorescence images of intact lemon and yuzu fruit to compare with the spectral information obtained during the identification procedure. The image acquisition device conisted of a VGA format camera (VCC-8350CLTS, CIS Corp., Japan) with 8-bit grey levels, and four sets of UV-A lamps (F6T5BLB, Sankyo Denki Co., Ltd., Kanagawa, Japan) for illuminating the sample at 315-400 nm, with a peak at 350 nm (Fig. 2).
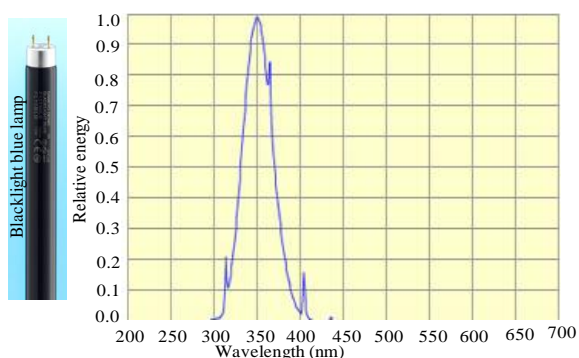


Fig. 2 Spectral energy distribution of black light blue lamp

This lighting panel was used for obtaining fluorescence images of surface peel defects and injuries. Fig. 3 represents the schematic layout of the fluorescence image

acquisition system. To detect the fluorescence of oil components on the surface of the peel, the surface of the peel was mechanically injured with a knife. The camera operating parameters were adjusted as shown in Table.1, the target object manually placed in the view of the camera, and fluorescence images with a resolution of 0.417 mm/pixel, captured using the camera and a capture board (MTPCI-TL2, Micro-Technica Co., Ltd.) connected to a computer. The processing of the resulting fluorescence image was done using programming software Visual C++ and OpenCV (Open Source Computer Vision) library functions under a Windows XP platform.
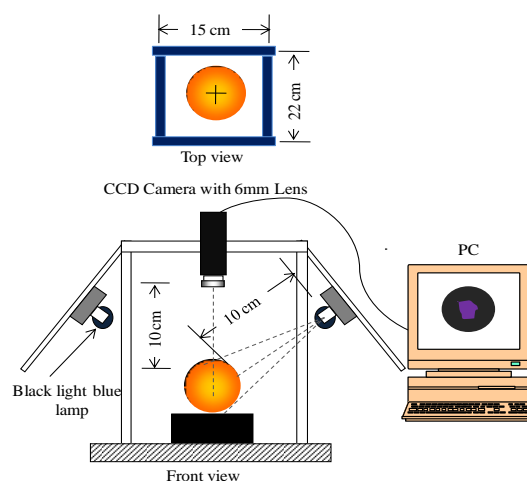


Fig. 3 Schematic layout of the image acquisition system

Table 1 Camera operation parameters

| | Lighting panel | UV-A |
|---|---|---|
| | Illumination (mW/cm$^2$) | 0.395 |
| Parameters | Shutter speed (sec) | 1/60 |
| | Iris | 1.4 |
| | Gain (dB) | 12 |
| | Gamma | 0.45 |
| | Color temp. (K) | 3200 |

## III Results and Discussion

### 1. Spectra of extracts from "Extraction Method A"

The measured spectra from the three locations on the fruit were almost identical. Thus only the spectrum from one location is shown here. Fluorescence and excitation spectra of lemon and yuzu peel components extracted using "Extraction Method A" are shown in Figs. 4 and 5, respectively. The fluorescence spectra of lemon and yuzu extracts (Fig. 4) reveal that both varieties have components that fluoresce in the range 350 - 450 nm, i.e., in the UV-blue region. Peak fluorescence for lemon and

yuzu was observed at 395 nm, and 390 nm, respectively. Peak relative fluorescence intensity for lemon was almost 70 % higher than that for yuzu. In both cases, the intensity of the fluorescence spectra decreased gradually after 400 nm, approaching zero after 500 nm. It is also apparent there are major differences in the lemon and yuzu fluorescence spectra from that of the mandarin orange cv. "Tokumori wase" described in Kondo *et al.*, (2009). According Kondo *et al.*, (2009) peak fluorescence both in normal and rotten mandarin orange peel was near 540 nm, i.e., it showed a greenish fluorescence under UV light.

For both lemon and yuzu, the excitation spectra increased linearly from 300 nm, peaked near 330 nm, and then decreased close to zero around 370 nm. This is in strong contrast to the excitation spectrum observed for mandarin orange (Kondo *et al.*, 2009), where peak excitation lies between 360 to 375 nm.
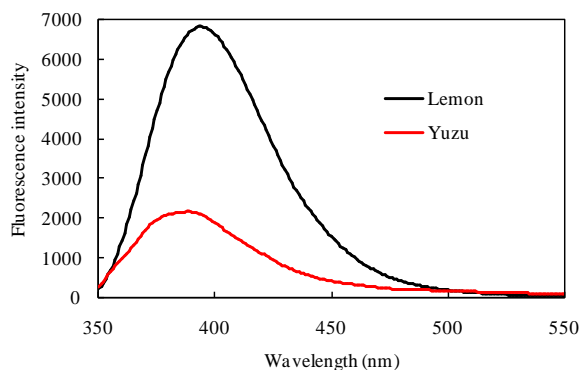


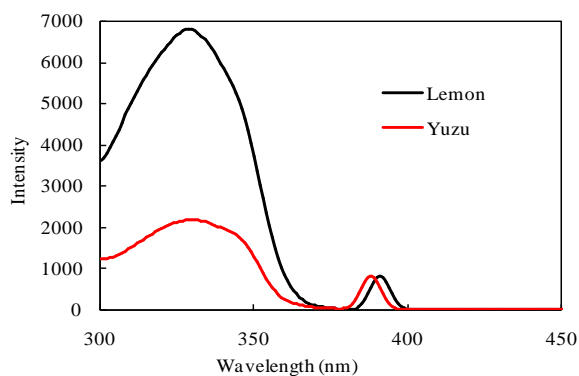Fig. 4   Fluorescence spectra of extracts of lemon and yuzupeel ("Extraction Method A")



Fig. 5   Excitation spectra of extracts of lemon and yuzu peel ("Extraction Method A")

## 2.   Spectra of extracts from "Extraction Method B"

Fluorescence and excitation spectra of the methanol and hexane extracts of lemon and yuzu obtained by "Extraction Method B" are shown in Fig. 6 and 7. It is clear that the components extracted by "Extraction Method B" (Fig. 6 and 7) and "Extraction Method A" (Fig. 4 and 5) are one in the same. The overall shape of each spectrum is almost identical.

Moreover, the observed excitation spectra of components in "Extraction Method B" rise rapidly in intensity from 300 nm, peaking near 330 nm, and then decreasing sharply after that for both varieties (Fig. 7). This is almost identical to that observed from extracts obtained using "Extraction Method A" (Fig. 5). That is, the fluorescence spectra are most intense in the range 350 to 450 nm, i.e., in the UV-blue region. For component A and B in lemon, peak fluorescence spectrum was observed at 393 nm and 396 nm respectively. For yuzu it was at 384 nm. The excitation peak for both components A and B in lemon was the same (327 nm). From Fig. 6 and 7, it can also be seen that the fluorescence and excitation spectrum of component B in lemon, and that of the component in yuzu peel are almost identical, suggesting they share similar structural properties.
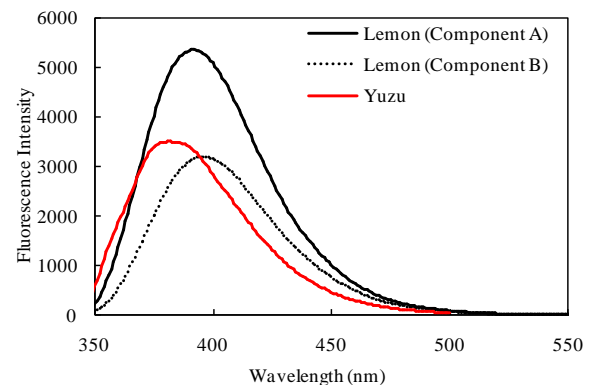


Fig. 6   Fluorescence spectra of extracts of lemon and yuzu peel ("Extraction Method B)
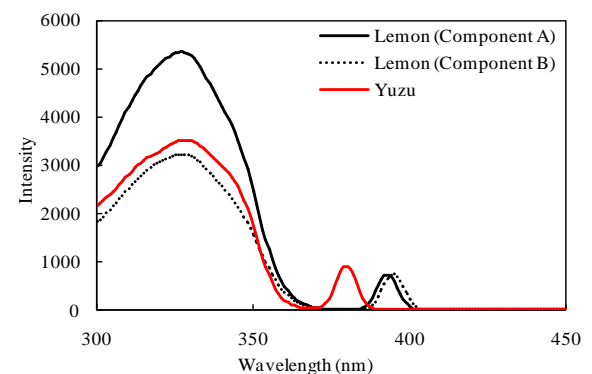


Fig. 7   Excitation spectra of extracts of lemon and yuzu peel ("Extraction Method B")

Detailed analyses of the extracted fluorescent components of lemon and yuzu were then conducted using nuclear magnetic resonance (NMR) spectra, in order to elucidate their structure. Results are presented in Figs. 8 to 10. As shown in Fig 8 and 9, the $^1$H NMR spectra of lemon component A and B are closely related. A signal characteristic of a 5,7-disubstituted coumarin ring is observed at 6.2 to 8 ppm for both compounds. Furthermore, component A of lemon has two methoxy groups that can be observed at 3.5 to 4 ppm, whereas component B has one methoxy group, and a geranyl group.
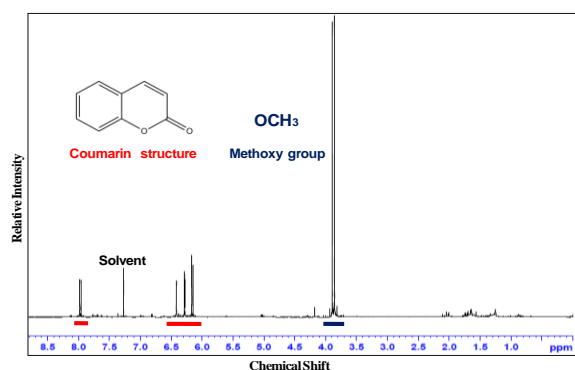


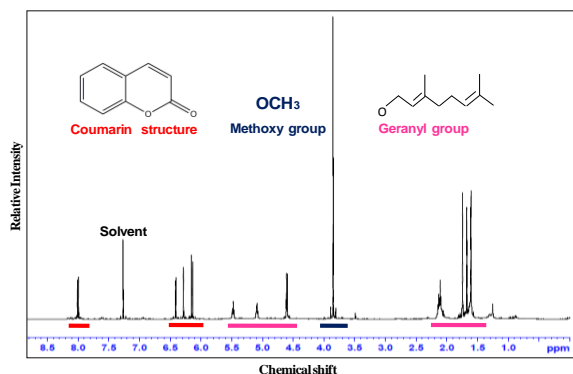Fig. 8    $^1$H NMR spectrum of extracted component from lemon peel (Component-A)



Fig. 9    $^1$H NMR spectrum of extracted component from lemon peel (Component-B)

Interestingly, the NMR spectral data of yuzu (Fig. 10) is almost identical to that of component B in lemon (Fig 9). Similar to that of component B in lemon, the component in yuzu has a geranyl group observed at 1 to 2.5 ppm and 4.5 to 5.5 ppm. Furthermore, the integration values and splitting pattern of the aromatic signals at 6.2 to 8 ppm, strongly suggest a mono substituted coumarin structure.

Thus, from the evidence gained from $^1$H NMR spectra of component A and B of lemon, and the component in

yuzu were identified as 5-7-dimethoxycoumarin, 5-geranyloxy-7-methoxycoumarin and 5-geranyloxy coumarin, respectively. This is in good agreement with components previously identified and reported in the literature (Miyake *et. al*., 1999, and Stanley and Jurd, 1971). The structures of these components are shown in Fig. 11.
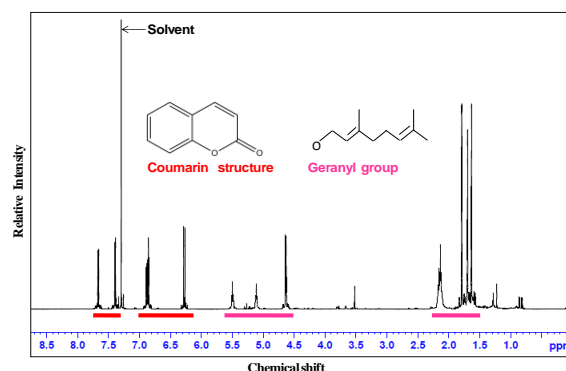


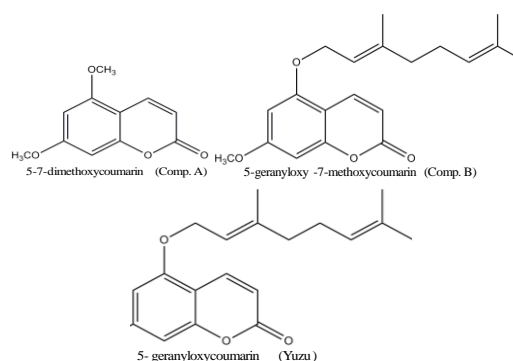Fig. 10    $^1$H NMR spectrum of the extracted component from yuzu peel



Fig. 11    Chemical structures of coumarin-related compounds extracted from lemon and yuzu peel

### 3.  Fluorescence image processing

Thus, in order to observe these fluorescent components in vivo, based on the observed fluorescence excitation spectra information (Fig. 5 and 7), the excitation illumination of lemon and yuzu peel needs to be carried out between 320 - 340 nm, or more appropriately at 330 nm. Since incidence light sources with an exact wavelength of 330 nm are currently still expensive, a UV-A lamp (blacklight blue lamps) was used, as it is thought there would be sufficient coverage at this wavelength (Fig. 2). However, in order to acquire the best fluorescence image, as well as an appropriate intensity level of fluorescence emmision, in the future it will better to illuminate samples with a light source that has an exact wavelength of 330 nm.

The captured fluorescence image under UV-A

illumination is shown in Fig. 12 (a) and (b). In the case of lemon, a bluish type fluorescence with strong emission from the injured area can be observed (highlighted by the white square). However, the appearance of fluorescence emission from yuzu is weak, and not as clearly visible as that for lemon. These fluorescence images are in good agreement with the spectral information obtained in section 3.1, and Fig. 4. To detect the fluorescence regions (injured area) in the images, the colour spaces, RGB (Red, Green and Blue; fundamental colour components) and HSI (Hue, Saturation and Intensity) of the images were analyzed. The RGB values of fluorescence (the bluish colour pixels), halation (purple colour pixels) and normal parts of the fruit surface were measured. Then the values of RGB were converted to HSI using colour space conversion equations described in Gonzalez and Woods, 1992.

The relationship of HSI colour values of lemon and yuzu are presented in Fig. 13. This reveals that the defective region can be distinguished based on HSI information for lemon, as partitioned by blue lines in Fig. 13, but for yuzu it is not as distinct due to overlapping of the colours. Hence, an excitation wavelength near 330 nm (e.g UV-A) using an incident light can be used to acquire fluorescence images of lemon, but not so effectively for yuzu.

Finally, the fluorescence image was converted to a binary image by using a multilevel global threshold (Gonzalez and Woods, 1992) segmentation method. The aim of this segmentation was to identify the defected areas which have a bluish colour in the original image. The threshold limit was chosen from the minimum and maximum HSI values that were found among several points in the defective region (marked by blue lines in Fig. 13). The resulting binary image is considered as a marker image. For example, in the case of lemon the minimum and maximum H, S and I threshold limit was 230 to 245, 30 to 50, and 80 to 150, respectively. The resulting processed image of lemon is shown in the Fig. 12(a1), where segmentation of fluorescence part, that appears as a green colour, represents the defect, while other parts in the image remain unchanged. It can be seen that the distinction between normal and fluorescence parts is very clear. The segmentation technique is able to detect the fluorescence region of lemon as shown in the Fig. 12(a1).

To segment the defect area of yuzu, the fluorescence image was converted to a binary image using HSI colour model and the H value between 230 and 280, the S value

between 20 and 40, and the I value between 55 and 85, was included. In the resulting binary image (12(b1)), there is some noise around the periphery of the fruit, and this noise may include the same HSI values as the defect area and be recognized as a defect, but actually there were no defects in this peripheral area. To erase this noise and identify only actual defects, median blur type smoothing (12(b2)) and size filtering (remove area below a given value, 12(b3)) operations were performed. Fig. 12(b3) represents the resulting defect segmentation on the yuzu peel.
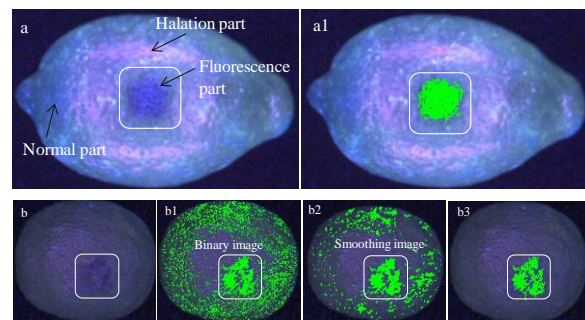


Fig. 12　Fluorescence image of the injured part excited by UV-A: a. lemon, b. yuzu; a1, b1, b2 & b3: processed images
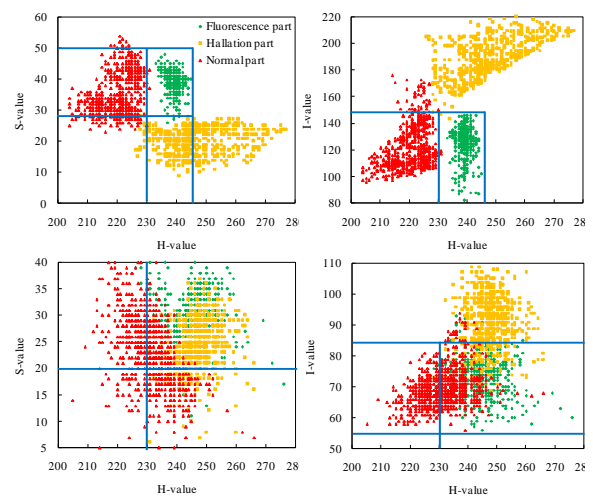


Fig. 13　The relationship of HSI colour spaces (top two figures for lemon, and bottom two for yuzu)

## IV　Summary and Conclusions

In conclusion, fluorescent components in the peel of lemon and yuzu were extracted and identified. The components extracted from these citrus varieties, have a basic coumarin structure. Two different coumarins, 5-7-dimethoxy and 5-geranyloxy-7-methoxy were isolated from lemon peel, and the one from yuzu was 5-geranyloxycoumarin. The excitation wavelengths of these fluorescence components were in the range 320 -

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340 nm, more specifically at 330 nm. Thus, if these fluorescent components are to be utilized for detection of surface peel defects in lemon and yuzu, when using a fluorescence imaging based machine vision system, we need to have an incident light source at 330 nm. This is different from the light source (360 - 375 nm) that is currently most often used for other citrus varieties in citrus grading facilities. When such a system was trialed, bluish type fluorescence was observed from injured areas on the surface of the peel; consistent with the in vitro fluorescence spectra results obtained. In addition, it was observed that the image acquisition system set-up with a UV-A lamp illumination can detect peel defects in lemon but not so effectively for yuzu.

### Acknowledgement

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