Monitoring of fluorescence characteristic in tomato surface during over-ripening stage

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Abstract. After harvesting, the evaluation of tomato characteristics is essential to decide a correct postharvest handling process, maturity stages, and shelf-life prediction. Previous research shows that fluorescence imaging could be used to enhance the maturity level detection after the red stage. However, the correlation between external appearance and internal fluorescence change has not been studied well. In this research, tomato surface has been classified into two parts i.e. skin and flesh. The parts have been extracted, and the fluorescence characteristic has been monitored from red-stages to over-ripening. The fluorescence image changes are confirmed during the storages and correlated with the weight loss as a freshness index. The results show that the fluorescence characteristic of tomato skin and flesh is different. The highest fluorescence emission peak for excitation of 370 nm is 520 nm for the skin and 490 nm for the flesh. Both fluorescence intensity of skin and flesh changed during storage. Although both changes could affect the fluorescence images, confirming the previous result, the changes of the skin fluorescence are strong enough to be observed with an imaging method. Classification of fresh and spoilage tomato samples using weight loss and images were also conducted using a Partial Least Square - Discriminant Analysis (PLS-DA) model with 92% accuracy. These results demonstrate the potential of fluorescence imaging to monitor tomato freshness during storage.

1. Introduction
Tomato is one of the most popular fruit in the worldwide diet, reaching 38.3 million tonnes consumption globally [1]. For such a large production and consumption of a perishable product, quality assurance programs to fulfill consumer demands for fresh, safe, and tasty fruit are essential. Tomatoes are not usually stored for a long time for direct consumption. However, holding time is sometimes inevitable due to
transportation and display in the market. Some inspections related to tomato quality are often performed, including observation of time-dependent physical changes, non-uniformity of product, or contamination.

Present technologies to determine freshness are based on physical characteristics, such as acoustic signals [2] and thermal imaging [3]. However, such methods require time and expertise in operation. Among other ways to evaluate the organic components, fluorescence spectroscopy is well received for its high sensitivity and specificity. Since the pigment properties, especially carotenoid as the primary pigment in ripe tomatoes, still change during storage [4], fluorescence properties can potentially be applied for monitoring freshness.

Recently, fluorescence imaging has been proven to extend the monitoring system's sensitivity after red stages, which is difficult to solve with a conventional imaging system [5]. This sensitivity enhancement of the monitoring system was able to be achieved due to the auto-fluorescence changes of the tomato surface during storage after the red stages compared to normal color imaging [6]. Nevertheless, the auto-fluorescence change of the internal tomato part has not been discussed well in the previous report. On the other hand, comprehensive monitoring of fluorescence compound changes of tomato tissues from the green to the red stage has also been reported by Lai et al. [7]. However, no report has been provided on the fluorescence change of various tomato tissues after the red stages, which is important since this stage is the critical stage for storage related to quality and shelf life.

The overall objective of this research is to investigate the feasibility of fluorescence technology to monitor tomato freshness during storage, especially after reaching the red stage. Experiments were conducted firstly to observe excitation and emission matrix (EEM) characteristics of fluorescence substances in the surface of the tomato cultivar Taian kichijitsu and to investigate the time series characteristics of the fluorescence substances related to freshness in tomato harvested in winter and summer. Then by using such information, the machine vision system is designed to observe the application of UV fluorescence imaging of tomato during storage.

2. Materials and Methods

2.1. Materials

Thirty-six tomato fruits (Solanum lycopersicum, Taian kichijitsu var) grown in a greenhouse in Ehime prefecture were harvested at different growing seasons, 18 in winter (February 2016) and 18 in summer (June 2016) to investigate the effect of environmental conditions on the properties of tomato during storage (a time-series). Tomatoes were stored in a controlled room at 24°C (ambient humidity recorded at 30-40% in winter and 70-80% in summer) for 12 days. Three fruits from each storage condition were taken every two days, and spectra measurements were made over the 12 days. Then to validate the fluorescence analysis results, a machine vision experiment was then conducted. Twenty-two tomatoes from the same greenhouse were harvested in December 2016 and stored for 15 days.

2.2. Methods

2.2.1. Measurement of fluorescence compounds in tomato surface during storage

Extracts from the tomato were sampled to find the fluorescence bands responsible for the changes during storage [7]. Before measurement, tomatoes were cleaned by wiping them using ethanol and washed again with distilled water. Based on preliminary experiments, different parts of the tomato require a different dilution level to get detectable intensities. The tomato skin was carefully peeled off in a 2 x 2 cm square from the fruit's blossom end from four corners of the fruit. All four pieces of the skin were then diluted in 10 mL, while for the flesh part, 0.2 grams of the flesh were dissolved in 20 mL of ethanol. All solutions
were allowed to settle overnight and filtered through a cotton filter. For measurement, 4 mL of this sample was placed in a measuring cell (quartz fluorometer cell, path length 10 mm, four clear windows). All spectrofluorometric measures were acquired on a JASCO FP-8300 Spectrofluorophotometer (JASCO, Japan) using a 1 x 1 cm quartz fluorescence cell, xenon flash lamp, and SpectraManager software package to control the instrument, data acquisition, and data analysis. Intensity data were corrected by rhodamine B and halogen light source and then normalized by Raman scatter peak of water [8].

2.2.2. Image acquisition

![Figure 1. Layout of machine vision system.](image)

A machine vision system was used to acquire color and UV images. A camera (Canon EOS Kiss X7) equipped with a circular polarized (PL filter) 58 mm was set 26 cm above the surface of the tomato. Samples were laid on a black surface, with the calyx face down. For color image acquisition, Halogen lamps (4) (12V 50 W KLS, Japan) equipped with a PL 62 mm filter were used. For UV-fluorescence image acquisition, a UV-LED ring lamp was set vertically above the sample, 26 cm from the surface. The lamp has a spectral range of 340-390 nm. A long-pass filter (Y-49) was put in front of the camera to cut UV light emissions. Both types of images were processed using Open CV 3.0 in Windows XP 10 to extract RGB channels of fruits. Images of 22 fruit were taken during day 1. Then during storage images of 7 fruit were captured at days 3, 7, 9, 11, and 15.

2.2.3. Calculation of physiological weight loss (PWL)

Tomatoes were weighed at the start of storage and on the final day by a digital electronic balance (Shimadzu, Japan) and weight loss calculated as follows:

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PWL = \left(\frac{\text{initial weight} - \text{end weight}}{\text{initial weight}}\right) \times 100\%
\]  

(1)

Shelf life was determined at 5% of PWL, as this represents the loss of freshness, with the fruit having a wilted appearance [9].
3. Results and Discussion

3.1. Fluorescence EEM characteristics of tomato

Figure 2 shows the typical EEM spectrum of skin and flesh extracts of tomatoes. The EEM spectrum indicates excitation happens in the range from 200 to 450 nm, while the range of emission is much wider, between 300 to 710 nm. Area A had two small peaks with the highest excitation intensity at 230 and 280 nm, while for emission there was a peak at 336 nm. Area B has a wide excitation spectrum from 320 to 390 nm. Although these areas appear in all parts of the tomato, this peak has different characteristics for skin and flesh.

In the skin part, the emission band ranged from 400 to 650 nm with the highest intensity at 520 nm. However, the same area in the flesh part, the emission band was narrower, ranging from 420 to 600 nm with the highest intensity at 490 nm. Previous research [7] has identified these peaks as flavonoids (A), and carotenoids (B). Other secondary metabolites are mostly phenolic, such as flavonoids that are also constituents of the plant cuticle. Flavonols quercetin-rutinoside (rutin) and kaempferol rutinoside were found to accumulate to a lesser degree exclusively in the ripening tomato skin [10].

![Figure 2. Fluorescence EEM spectrum of tomato flesh and skin.](image_url)

During ripening, changes in the pigmentation of the tomato surface occur. The chloroplast of the mature green fruit changes into chromoplast, which accumulates lycopene in membrane-bound crystals [11]. Therefore in ripe and stored tomato, the chlorophyll peak is not observed in the outer parts (skin and flesh), but a carotenoid peak is clearly observed.

3.2. Fluorescence peak dynamics in the tomato surface during storage

The dynamic in the surface area shows that during storage, change in skin part is not clearly observed, yet carotenoids still accumulate in the flesh part. One way analysis of variance (ANOVA) showed that significant changes occur for peak A and B in the flesh part of the winter sample, while the others do not show significant changes and this result did not replicate for the next measurement in summer.
Figure 3. Fluorescence dynamic of peak A (280 nm) and peak B (370 nm) in skin and flesh.

3.3. Machine vision for monitoring tomatoes during storage
Fluorescence intensity monitoring results during storage suggest that two peaks in tomato change during storage, albeit the variation. Of those two peaks, the 370 nm peak was chosen as the basis for building a machine vision system because it has emission in the visible range. At beginning of the measurement, the fruit has already reached an initial red stage. As time passed, the red color began to spread more evenly, but it still seems complicated to distinguish the color after storage. Images obtained under UV light emission initially have a bluish color under excitation between 340-390 nm that change into yellow, as storage proceeded, getting stronger as time went by.

Figure 4. Color and UV image of tomato during storage.

One-way ANOVA analysis of the image using means of each color channel extraction shows that there are some significant changes of HSV value in the image properties during storage (p<0.05), as shown in Fig 5. The hue value decreases from 120 to 80, demonstrating the potential for using HSV properties to monitor changes during storage.
Figure 5. Hue, saturation and value of UV image during storage

Image acquisition result shows that although the measurement of intrinsic fluorescence in the surface by destructive analysis does not show significant changes, direct monitoring using a UV lamp can keep track of the changes. Since the filter attached is eliminating lamp reflection, the color change is suspected to be due to a fluorescence contribution, however, physical property changes might also play a role. This result is aligned with Konagaya's [6] findings that autofluorescence properties are observed in tomato cuticles and showing dynamics in excitation wavelength 360 nm during storage. Another possible reason for the fluorescence phenomena is the changes in wax properties. During storage, wax composition as well as fatty acid composition changes [12]. The intact cuticle of tomato has waxy nature, reducing light and UV absorbance above 350 nm, making the emission too weak to detect fluorescence [13]. As the wax properties changes, it reduces its ability in shielding and reflects the UV light. Thus the amount of UV light absorbed increases. As in the beginning, the amount of light absorbed only in a small amount, the light emitted in a small amount too. As the amount of light increased, the emitted light increased as well, hence affecting the absorption efficiency as the increasing factor of the absorption coefficient could shift the emission wavelength [14].

3.4. Classification based on physiological weight loss (PWL)

Figure 6. Physiological weight loss during storage.
During the image acquisition experiment, as storage progresses the fruit loses water and weight as shown in Figure 6. This weight loss is used as a basis for deciding whether a fruit is considered fresh or spoiled (PWL >5%), with all the fruits measured on the initial day being considered fresh (PWL=0).

After the samples were categorized as fresh and spoiled, each color channel's mean value was inputted into Unscrambler 9.8 version for Partial Least Square (PLS) analysis, to see if UV image color properties are able to classify the freshness. From 56 images, PLS could classify the samples with an accuracy of 92% with the highest contribution from saturation value. Despite the non-significant change of fluorescence peak dynamic after the red stage, fluorescence imaging displayed notable change, hence demonstrates the potential of UV imaging to monitor tomato freshness during storage.

4. Conclusions
In these experiments, we investigated fluorescence phenomena on the tomato surface. The skin and flesh of the tomato have fluorescence peaks at two wavelengths. Peak A, flavonoid-like compounds, has the highest intensity of excitation 280 nm and emission at 336 nm for both parts. Peak B, carotenoid-like compounds, have the highest intensity of excitation at 370 nm and emission at 520 nm for the skin, and at 490 nm for the flesh. Validation of a UV fluorescence machine vision system (365 nm UV LED light source) to monitor tomato freshness showed that tomato color under UV light changes significantly. Further analysis by PLS demonstrated that UV images can separate fresh from spoiled tomatoes with sufficient accuracy. In the future, this technology could be further optimized by manipulating variables to accelerate the deterioration speed.

Acknowledgement
We thank Ms. Wulandari for the help with the feature extraction program. We are also grateful to Ms. Noriko Takahashi for providing tomato samples for this experiment.

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