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# The Potential of Fluorescence Technology for Quality Monitoring of Miyauchi Iyokan (*C. iyo* Hort. Ex Tanaka) during Post-harvest Treatment

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## ABSTRACT

The potential of fluorescence technology for monitoring Miyauchi Iyokan citrus during post-harvest treatment was investigated in this research. Citrus fruits were harvested when still a greenish-yellow color with a low soluble solids (SS)/acid ratio. Dark storage of fruits at 80-90% of RH and 6-10$^0$C for sixty days is expected to increase the quality of the fruits (becoming reddish orange in color and having a high SS/acid ratio). However, monitoring the quality during the treatment is substantial. In general, farmers monitored the quality by seeing the appearance, however, this way is subjective. Thus, the fluorescence technique was proposed since it was objective, relatively cheap and easy to apply. Two main fluorescence compounds from peel, polymethoxylated flavones (PMFs) (Ex. 370 nm/Em. 540 nm) and tryptophan-like (Ex. 260 nm/Em. 330 nm) compounds were found, and PMFs were used in this research since it is an essential constituent for the fruit quality. This work demonstrated that during treatment bright

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yellow color (suspected PMF compounds) had appeared when excited under 365 nm with UV-light from the beginning of storage period until day eighty, however, day fifty and sixty showed stronger intensity. Furthermore, SS/acid ratio reached the highest peak in day sixty. In addition, on day sixty an unpleasant odor appeared, which might be related to the off-flavor condition of metabolic excess. These results demonstrate the potential of fluorescence to monitor citrus fruit quality changes during postharvest treatment

*Keywords:* Acidity, iyokan, post-harvest treatment, polymethoxylated flavones, soluble solid content, tryptophan-like

## INTRODUCTION

Miyauchi Iyokan (*C. iyo* Hort. Ex Tanaka) is usually harvested in December in Japan when it is still greenish-yellow in color. Collection  is done preventing low-temperature injury to the fruit during the winter. However, at this stage the fruit still has a low Soluble Solid (SS)/acid ratio; thus an improvement in this ratio is required before fruit quality becomes acceptable for sales. To achieve this increased ratio, the Iyokan are stored for two months at 5-15$^0$C until they reach the desired criteria, such as color (reddish orange), a sweet and aromatic flavor, and a glossy surface (Kondo et al., 2000; Uchida et al., 1983). This storage period is referred to as the “post-harvest treatment” process.

In general, during the post-harvest storage period, fruit quality monitoring is not conducted by the farmer. Moreover, the main quality attribute which changes during this post-harvest period is the internal fruit quality characteristic of SS/acid ratio  (Marcilla et al., 2006). Techniques to determine the SS/acid ratio and color of fruit have been successfully applied to postharvest fruit sorting operations. In the case of citrus, many on a farm, as well as off-farm techniques, such as Brix and acid meters, Near Infrared Spectroscopy (NIR), and machine vision system have been utilized.

In the citrus industry brix and acid refractometers are widely used to measure SS and acidity (Olmo et al., 2000). While these methods are accurate, cheap, and fast, they are, unfortunately, destructive. On the other hand, near-infrared spectroscopy (NIR), which has also been widely used in the citrus grading industry for many years, is a robust and non-destructive method for quantifying SS content of citrus fruit (Ruslan et al., 2012; Zude et al., 2008). Its drawback is, however, the technology is relatively expensive and beyond the reach of many individual farmers. Another technique, a machine vision based system, quantifies citrus quality based on color (color component ratios, such as R/G (red/green), or the R/G value of the reddish fruit and the correlation of this to sweetness) (Iqbal et al., 2016; Kondo et al., 2000). The disadvantage of the methods based on this technology is the requirement for specific artificial lighting conditions in strictly controlled dark room situations.

Although it has not been applied to citrus, fluorescence spectroscopy is another possible technique without the above disadvantages, which has been widely used for assessing the maturity of mango by utilizing chlorophyll fluorescence (Lechaudel et al., 2010); the ripening of apples by utilizing anthocyanin, flavonol, and chlorophyll concentration (Betemps et al., 2012); the maturity of grapes by monitoring the anthocyanin accumulation (Agati et al., 2013; Ghozlen et al., 2010), and for the maturity classification for oil palm bunches using multiple spectral bands (Saeed et al., 2012). Fortunately, Iyokan is a fruit that is categorized as containing medium levels of fluorescence compounds that are relatively easy to detect. Thus, this study aims to explore the potential of fluorescence techniques to monitor citrus fruit quality during the post-harvest treatment process. To do this, fluorescence characteristics of the fruit were observed during the post-harvest period under 365 nm UV-light and compared to standard fruit quality measurements of SS/acid ratio.

## MATERIALS AND METHODS

### Tested Materials

In this experiment, 240 Miyauchi Iyokan (*C. iyo* Hort. Ex Tanaka) fruit were harvested in December 2016 (210 days after anthesis) from 20-year-old trees growing in the Ehime Research orchard, Ehime Prefecture, Japan.

### Methods

**Sample Preparation.** After harvest, the fruit was then sorted manually based on peel appearance, with only the whole fruit being selected as potential samples. Color images of the fruit were then captured using a color camera equipped with a polarized filter and illuminated by four halogen lamps. Subsequently, based on the color component ratio red/green (R/G) (using Python™ Version 2.7.12 with OpenCV Version 2.4.13 library algorithm, Delaware, USA), the potential samples were reduced to 135. Finally, 27 fruits which had the closest color component values were selected from this 135 fruit.

The 27 selected fruits were kept at ambient temperature and 80-90% RH. All fruits were kept in fruit baskets, covered with paper, and stored in a storage room for 80 days. Measurements were made at ten-day intervals for a total of nine times, with measurements replicated three times and data averaged (data reported in this article is the average value). At each measurement time, three fruit was extracted and measurements made. Time-lapse color and UV-images were taken under halogen lamps and a 365 nm UV-light. A cross-sectional peel image for observing the cuticle layer was also captured using a microscope (Keyence VH-Z250R microscope) coupled with image acquisition software.

**Standard Soluble Solid Content and Acidity Measurement.** Soluble solids content (SSC) and acidity were measured using a hand-held digital refractometer (Atago PAL-BX | ACID F5, Atago Co., Ltd., Tokyo, Japan). The flesh sample was squeezed manually to collect the juice and then filtered (Whatman No. 41 filter paper). A 0.2 mL clear liquid aliquot of the juice was directly pipetted (Pipetman P1000, Gilson Inc, USA) onto the refractometer and SSC content (% Brix) measured. Once the Brix value was recorded, the aliquot was diluted by adding distilled water up to 10 mL on the refractometer, and the acidity measured and recorded as a percentage of citric acid. Also in this paper, SSC/acid ratio was calculated as a fruit flavor determination.

## RESULT AND DISCUSSION

### Soluble Solids, Acidity during Storage

Citrus is considered to be a non-climacteric fruit, which means after harvesting the maturing process stops and the fruit enters a senescent period. Farmers generally perform postharvest treatment for sixty days without monitoring the process at all. Results show that during the experimental postharvest treatment, the SS/acid ratio of the Iyokan fruit juice remained relatively constant from day one to thirty, then significantly increased after that until day sixty (Figure 1) remaining stable until day eighty. The increasing SS/acid ratio from day thirty to sixty is thought to be due to the decreasing in acidity in citrus fruit, which is, at least in part, due to the breakdown of the organic acid for energy production and alcoholic fermentation (Echeverria & Valich, 1989; El-Otmani & Coggins, 1991).

Valencia oranges have been reported to develop a distinct flavor and high ethanol concentration after long storage (Marcilla et al., 2006; Shi et al., 2007). The main reason for these changes is caused by low concentrations of metabolic compounds in the juice and of off-flavour volatiles, such as ethanol and acetaldehyde (Shi et al., 2005). In our experiment, we observed from day fifty to eighty that the acid content declined to its lowest level and remained stable until the fruit began to decay. Besides, on day sixty we observed an unpleasant odor. This perceived odor give an ethanol-like aroma.

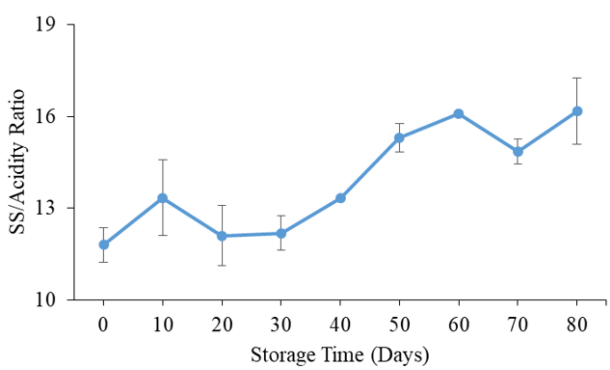


*Figure 1*. Soluble solids (SS) and acidity (as a % of citric acid) ratio during the post-harvest period under 80-90% RH.

## Fruit Condition during Postharvest Treatment

The appearance of Iyokan fruits under UV-light (UV-images) progressed as shown in Figure 2. Fruit observed under the halogen lamps showed little change in color (images are not shown). However, under UV-light, the differences can be easily found among the fruit. On the day sixty an intense bright yellow color could be observed, which we suspected to be related to the presence of polymethoxylated flavones (PMFs) (Dugo et al., 2005; Muharfiza et al., 2017). Since PMFs have a strong fluorescence when excited at 365 nm, this yellow color also has the potential to mark the deterioration of the fruit. Muharfiza et al. (2017) also found the main compounds in citrus Unshiu were suspected as tryptophan-like (Ex. 260 nm/Em. 330 nm) and polymethoxylated flavones (PMFs) (Ex. 370 nm/Em. 540 nm), based on their report PMFs has the strong fluorescence intensity. And machine vision based on PMFs fluorescence excitation wavelength was constructed as shown in Figure 3.

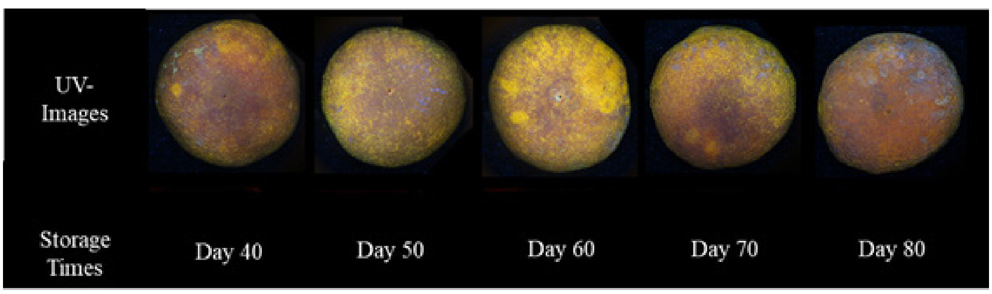


*Figure 2*. Iyokan appearance during postharvest treatment under 365 nm UV-light.

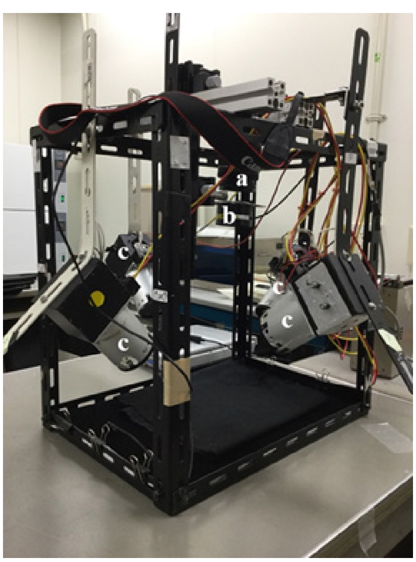


*Figure 3*. Machine vision system: *(a)* camera; *(b)* 365 nm UV-light; *(c)* halogen lamps

The bright yellow color became conspicuous from day fifty to sixty, this bright color might be a critical signal during the treatment period, and here, the internal fruit changes related to the quality are occurring. Figure 1 demonstrates that the peak ratio was reached on day sixty and remaining stable flavor afterward. Also, the abnormal odor perceived on day sixty reinforced the suspicion that during this period the metabolism of the organic acid for energy and the production of off-aromas from alcoholic fermentation marked the transition to over-maturity (Echeverria & Valich, 1989; Shi et al., 2005).

In fact, PMFs are suspected to be responsible for the bright yellow fluorescence that is visible on the peel surface because of the oil glands rupture and allowing oil diffusing until the peel surface (Slaughter et al., 2008). The literature would suggest that this bright yellow color is due to the breakdown of oil glands during postharvest treatment and the gradual movement of this oil to the peel surface, which is visible when illuminated under ultraviolet (Obenland et al., 2009). In addition, the degradation of the citrus cuticle layer at this time would enhance movement of the oil to the surface. This cuticle condition could play an important role as a sensing or an interaction with the surrounding environment (Domínguez et al., 2011; Lara et al., 2015).

The fresh peel has a thicker and smoother cuticle, and the epidermal cell beneath the cuticle was uniform (Figure 4). Several weeks after storage the cuticle became thinner, rougher and the epidermal cell became more difficult to observe, this phenomena caused by the climatic condition and might be affected the degradation of oil glands (Lara et al., 2014). This degradation (break-up) of the oil glands would enable the spread of the oil to the surface, making it visible under the UV-light. Thus, this signal could be an important signal for optimum storage time.

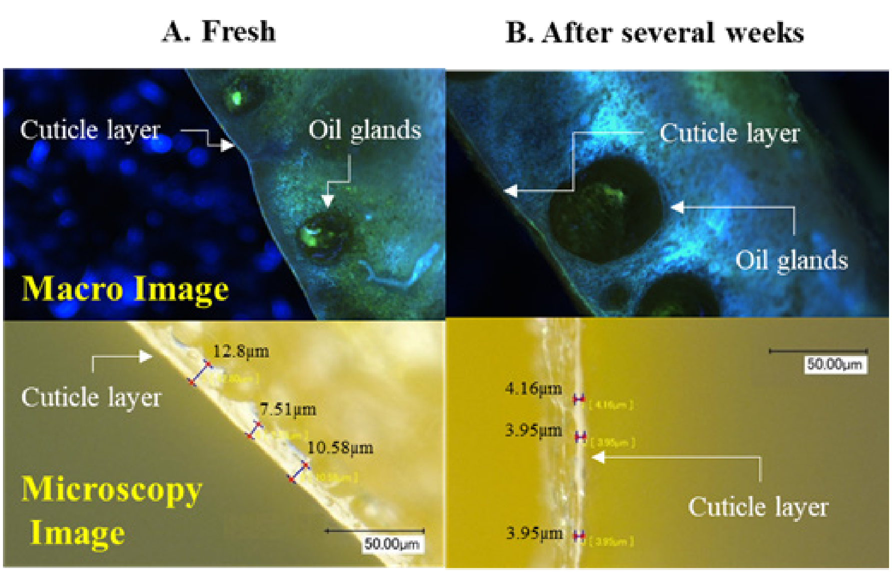


*Figure 4*. The cross-section macro and microscopy images of Iyokan peel: (A) fresh citrus; (B) stored after several weeks. Macro images were illuminated under 365 nm UV-light.

## CONCLUSION

Fluorescence measurements of Iyokan fruit made during the postharvest period identified PMFs as the main compounds observed. The presence of the compounds provides an opportunity to determine optimum storage time for high-quality fruit. Over the examined storage treatment period, from day fifty to sixty, a critical period was observed, where a bright yellow background color on the peel surface was apparent and was indicative of the beginning of fruit deterioration.

## RECOMMENDATION AND FUTURE WORKS

In this paper, fluorescence measurements were shown to have the potential to classify Miyauchi Iyokan quality in a sorting line application. This study provides basic research details on fluorescent spectroscopy of Miyauchi Iyokan using right angle measurement (extraction method), however, in industrial applications, a front-face measurement (solid method) is probably more applicable.

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