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Effect of relative humidity and light exposure on fluorescence compound dynamics, soluble solid and acidity of Japanese Citrus Iyokan during postharvest treatment

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KEYWORDS

Fluorescence
Light
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Relative humidity
SS/acid ratio

ABSTRACT

The Miyauchi iyokan (iyokan) citrus fruit is typically harvested in late December to prevent damage from the winter weather. At the time of harvest, the ratio of Soluble Solids (SS) to acid content is generally low, commonly used to assess the quality of the juice. Therefore, the goal during postharvest treatment is to decrease the acid content and improve the SS levels. The quality of citrus can be influenced by environmental factors such as relative humidity (RH) and exposure to light, so it is important to monitor their effects. Hence, this study aims to observe the changes in internal quality indicators, such as the SS/acid ratio and fluorescence compounds, under different RH and light conditions to understand how the citrus characteristics are affected. The postharvest treatment involved storing the citrus fruit at temperatures between 5-10°C for two months under various conditions i.e., in the dark and exposed to light, with high RH (80-90%) and low RH (40-50%). The SS/acid ratio did not show significant changes during the two months of storage under any treatments. However, the high RH condition resulted in a slightly higher SS/acid ratio. Similarly, the Tryptophan-like compound did not exhibit any significant response to the different treatments. However, the intensity of fluorescence from polymethoxylated flavones (PMFs) was higher in the dark treatment compared to the light treatment. PMFs play various roles in signaling and defense mechanisms in plants. Additionally, there was a notable increase in PMFs after thirty days of storage, indicating a response to light-induced stress.

Introduction

Citrus, a fruit famous worldwide, is represented by Miyauchi Iyokan (iyokan) in Japan. In general, farmers harvest the fruit in late December (winter season) to prevent winter injury. When harvested, it has a low Soluble Solids (SS)/acid ratio; and therefore, to improve quality to an acceptable level, postharvest treatment is necessary. Currently, farmers conduct postharvest treatment by keeping the humidity (RH) and light. Furthermore, research reports that RH and light can affect on fruit quality and fluorescence properties, such as for polymethoxylated flavones (PMFs) and tryptophan-like compounds (Henriod, 2006; Zoratti et al., 2014).

PMFs and tryptophan-like compounds, which exist in citrus fruit, are known to be linked to internal quality. PMFs, for example, are believed to play a role in plants's signaling and defense mechanisms. Tryptophan-like compounds, on the other hand, act as cell growth and development regulators in plants (Zhang et al., 2012; Zhao, 2012). Moreover, PMFs and tryptophan play an interesting role in citrus quality during postharvest treatment. The existence of these PMFs and tryptophan-like compounds could be a way of

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monitoring plant responses to the environment during postharvest treatment.

While many technologies have been used to monitor these compounds, such as reversed phase liquid chromatography (RPLC) (Swatsitang et al., 2000), high-performance liquid chromatography coupled to diode array detector in citrus (HPLC-DAD) and in mulberry (Thabti et al., 2012); and HPLC-coupled to electrospray ionization and quadruple-time of flight-mass spectrometry (HPLC-ESI-QTOF-MS) in mangoes (Dorta et al., 2014), these techniques involve complicated preparation and require skilled analysts. On the other hand, fluorescence spectroscopy, which has gained prominence in recent years, is rapid and does not require complex preparation. Since PMFs and tryptophan-like are fluorophores, they are potentially easy to monitor using fluorescence spectroscopy

Thus, the aims of this study were to observe internal fruit quality dynamics, such as SS/acid ratio and fluorescence compounds, and the effect of RH and fluorescent light exposure during postharvest treatment storage. By doing so, an understanding of citrus characteristics under different environmental conditions during postharvest treatment will be established, and important fluorescence wavelengths will be identified. In addition, while many reports have examined orchard light interactions with PMFs and tryptophan fruit in a cold storage room for several weeks without much environmental control, such as relative compound (Taulavuori et al., 2018; Zoratti et al., 2014), there have been no reports of postharvest (storage) effects on fruit quality. This study aims to establish a baseline to better understand internal fruit quality dynamics of SS/acidity ratio and fluorescence compounds in response to storage environment conditions.

Research Methods

Material and sample preparation

240 Miyauchi Iyokan fruit (C. iyo Hort. Ex Tanaka) were collected in December 2016 (around 210 days after anthesis) from 20-year-old trees grown in the Ehime Research Plant Orchard, Ehime Prefecture, Japan. When harvested, the fruit was immature (low in SS/acid ratio). From this fruit, 200 un-damaged fruit were manually selected and kept for 14 days at ambient temperature (in the winter season around 11-15ºC) and 80-90% RH. This period is "a pre-treatment" for peel color improvisation (Uchida et al., 1983).

After 14 days, all 200 fruit were graded based on color. Color images of each fruit were captured using a Canon X7 (Canon Inc, Japan) color camera, equipped with a polarizing filter (Kenko PRO1D wideband C-PL(W), 62 mm, Japan) and illuminated under four Ushio 150 W (JCR 15V 150W BAU, Co Ltd., Tokyo, Japan) halogen lamps. Then, based on the closest color component ratio red/green (R/G) (using Python™ Version 2.7.12 coupled with OpenCV Version 2.4.13 library algorithm, Delaware, USA), a total of 96 fruit were selected and divided into two main treatment groups for storage at 5 – 10ºC (Sanyo, MPR-20, Japan).

In the first treatment, the fruits were stored at high humidity (80-90% RH). In this treatment, there were two sub-treatments, which included 24 fruit exposed to artificial fluorescence lights using 8W SLG-10WN/14 (Global Co., Ltd. Japan). This light condition simulates standard storage conditions, using a commercial fluorescent lamps to illuminate the room. In this study, lighting was between 1600-1700 lux, and all samples had their positions rotated daily. Another 24 fruit were covered with paper and black cloth to avoid light.

In the second treatment, the fruits were stored at low humidity (40-50% RH), and like the first treatment, there were two sub-treatments. All fruit in both treatments were stored for eighty days and measured at ten days intervals. For each measurement, three fruit were selected from each treatment (a total of 12 fruit per measurement; three replicates per sub-treatment and results averaged).

For measurements that required extraction, the fruits were first cleaned using an ultrasonic cleaner (AS-One US-2A, As-One, Osaka, Japan) for about 5-10 minutes to clear away dust and dirt from the outer pericarp, then rubbed until dry and kept at room temperature (25ºC) overnight to reach an equilibrium temperature.

Citrus peel extraction, spectra measurements, and data processing

In this study, for each individual Iyokan, a section of peel (including albedo and flavedo) was taken from the bottom part as a sample for extraction. A total of three sections of the peel with an area of 100 square mm (10 mm × 10 mm) from the bottom part were used, weighed (Shimadzu AUW-220D, Shimadzu Co., Kyoto, Japan), and thickness measured (Mitutoyo PCX 15, Mitutoyo Co., Kanagawa, Japan).

Scissors were used to cut the peel samples into small pieces and mixed with chloroform (Wako Pure Chemical Industries, Osaka, Japan) with a ratio of 1:10 (1 section of peel to 10 mL chloroform liquid) into a 30 mL vial bottle. Mixed samples were

macerated in a dark room for six hours, filtered (Whatman No. 41 filter paper), all extracts placed directly into a 30 mL vial, and measured subsequently.

A right-angle fluorescence measurement of each extract was done the following day sequentially using a spectrofluorophotometer (Jasco FP-8300, Jasco Co., Tokyo, Japan). The fluorescence measurement chamber was dark during the measurement and at room temperature. The measurements were done using a 10 mm path-length glass quartz cuvette and ,3 mL of the sample was placed into a cuvette for scanning the excitation (Ex) and emission (Em) wavelength spectra, respectively.

The Ex was scanned from 230 ~ 450 nm at 10 nm increments and Em was scanned from 260 ~ 670 nm at 1 nm increments. The Ex and Em slit was maintained at 5,000 nm/min with a 50 nm response time throughout the measurements. The device was connected to a personal computer, with a special software application (SpectraManager™, Tokyo, Japan) for capturing spectral data, calibrating, and peak analysis using the "Peak Find" application.

Raw spectrum was saved in parallel after wavelength scanning; then the spectra were directly corrected using SpectraManager™ application software and calibrated using the Raman scatter peak of water from distilled water (Lawaetz and Stedmon, 2009). Lastly, the peak finder from the SpectraManager™ application software was used to observe desirable fluorescence intensity peaks. Statistical analyses were conducted and raw data were averaged using Microsoft Excel 2013, a *t-test* was applied with a 0.05 significance threshold. Principal component analysis (PCA) by using Unscrambler 9.7 was conducted to distinguish treatment effects on specific fluorescence compounds.

Citrus soluble solid (SS) and acidity measurement

Soluble solid content and acidity are important citrus quality constituents (flavor or sweetness); therefore, evaluating SS and acidity is necessary. In this study, SS and acidity were measured using a portable hand-held digital refractometer (Atago PAL-BX│ACID F5, Atago Co., Ltd., Tokyo, Japan). Citrus sample flesh was manually squeezed by hand to collect the juice, filtered (Whatman No. 41 filter paper), and then 0.2 mL (Pipetman P1000, Gilson Inc, USA) of clear aliquot juice was directly placed onto the refractometer window, and SSC (reflected as %Brix) measured. Later the SSC was recorded, an up to 10 mL of distilled water was added to the refractometer window for diluting the aliquot. The acidity was measured and represented as a percentage of citric acid.

Image acquisition system

A machine vision system was also prepared for monitoring sample quality during treatment. The images were taken just before extraction (Figure 1). Two types of images were captured; color and UV images. For the color image, a sample was illuminated with four 150 W halogen lamps equipped with a polarizing filter. A Canon X7 camera was used with a focal length set at 48 mm, shutter speed of 1/30 sec, F-number f/6.3, custom white balance, and ISO 100 for color images. ,Moreover for UV images, samples were illuminated at 365 nm UV-light (CCS Co., Tokyo, Japan). The images were captured using a Canon X7 camera with a focal length of 48 mm, shutter speed 4 sec, F-number f/6.3, shade white balance, and ISO 100. Color features were extracted using Python™ Version 2.7.12, coupled with OpenCV Version 2.4.13 library algorithm. The machine vision system is shown in Figure 1.

Results and Discussion

Suspected fluorescence compound in iyokan excitation emission matrices (EEM)

The spectra data of iyokan had three main peaks that were consistent throughout all measurements, as shown in Figure 2. Peak-A had two excitation wavelengths around 260 and 370 nm and one emission wavelength around 540 nm (A-1 and A-2) originating from the same compound. The compounds are suspected to be polymethoxylated flavones (PMFs) (Li et al., 2006; Muharfiza et al., 2017). These two compounds were calculated to be a single (A-1/A-2) value, and thenceforth discussed as the ratio of PMFs or PMFs ratio in this paper. PMFs are a group of phenolic compounds, also known as flavonoids, that have antioxidant properties and function as plant chemical defense compounds (Treutter, 2005). These compounds can be observed in almost all fruit parts, such as flavedo, albedo, membranes, juice, and seed. In particular, it is abundant in the flavedo of the peel (Gaydou et al., 1987; Ting et al., 1979).

Another fluorescence compound, labeled as "B", had an excitation wavelength of 260 nm and emission wavelength of 330 nm and is suspected to be tryptophan-like (Sun et al., 2010). Tryptophan-like is a type of amino acid that is converted to indole-3-acetic acid (IAA) in plant tissue by auxin biosynthesis (Zhao, 2012). The conversion plays an essential role in plant growth and development (Sheldrake, 1973).

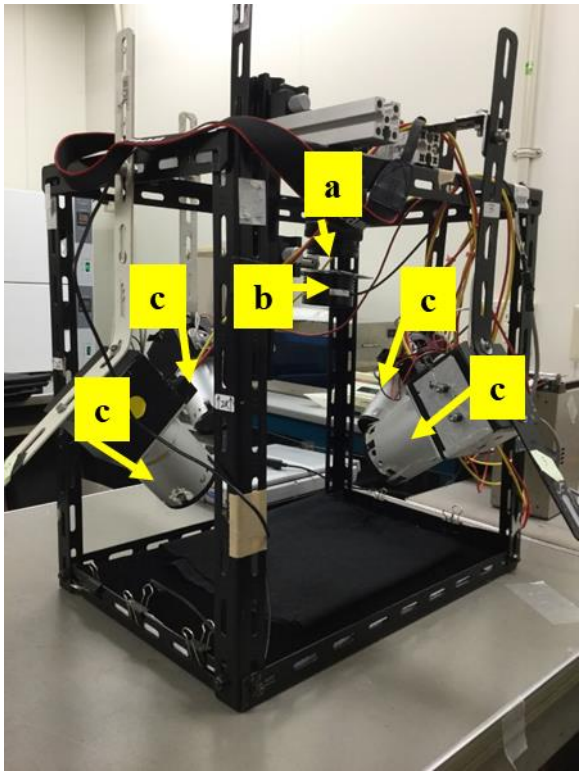

Figure 1. Image acquisition system in our experiment: a: camera with 18-55 mm, b: 365 nm UV-LED, c: halogen lamps.

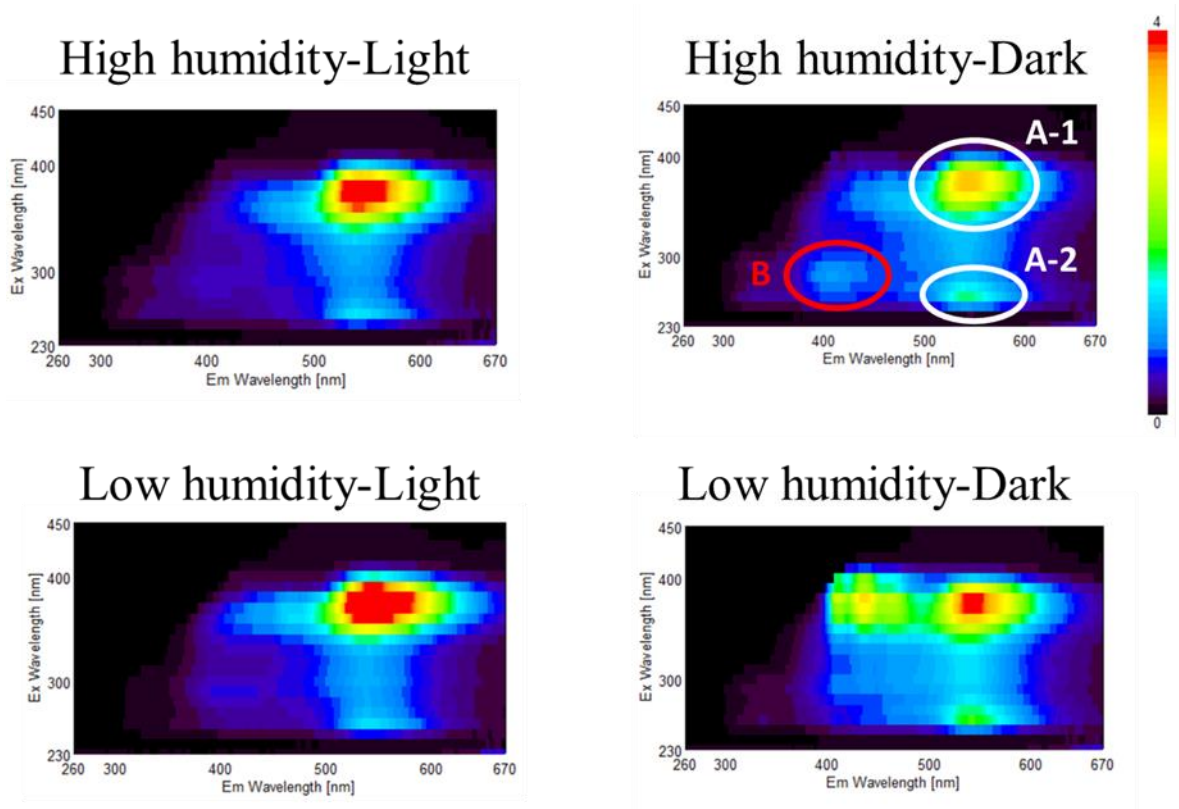

Figure 2. The Miyauchi Iyokan excitation and emission matrices (EEM) of the peel: The color map bars show the fluorescent intensity in Raman unit (r.u).

Soluble solid (SS) and acidity ratio during postharvest treatment

SS and acidity are well-established internal quality attributes that express flavor, where a high SS/acid ratio has a better flavor. The main chemical constituents of SS in citrus juice are sugar (around 80%); and acidity (organic acid) in citrus juice is citric acid (Albertini et al., 2006; Ladaniya, 2010).

After harvesting, Iyokan fruits have a low SS/acid ratio, making the fruit less marketable. During the postharvest storage treatments, the SS/acid ratio of iyokan stored under different relative humidity and lightning conditions showed a similar tendency to increase (Figure 3). This was due to slight increases in SS and decreases in acidity levels. Results indicate significant decreases in acidity between days 30 to 50, remaining relatively stable thereafter. Acid level decreases are thought to occur as organic acid is used to generate energy, which results in alcoholic fermentation (Davis et al., 1973; El-Otmani et al., 2011).

A result that is consistent with that seen in Hamlin oranges stored for 9 weeks; citric acid decreased significantly and remained relatively stable thereafter (Echeverria and Ismail, 1987). Furthermore, SS/acid ratios were slightly higher under high compared to low humidity storage conditions. Thus, the fruit stored under high humidity was sweeter in flavor (higher SS/acid ratio).

The time series dynamics of the ratio PMFs compound during postharvest treatment

Polymethoxylated flavones (PMFs) are one of the main components of Iyokan peel (Zhang et al., 2012). The ratio between two of these PMFs was used to minimize fluctuations and to quantify their presence in the sample. The accumulation of PMFs in plants has been documented to be affected by various environmental factors, such as light. Our data demonstrated that the PMFs increased during the treatment (Figure 4). However, there were differences between the light and dark storage conditions. There was a slight increment for the dark treatment condition over the entire storage period (days 0 to 70).

On the other hand, samples stored in light conditions rapidly increased PMFs from mid-way through the storage period (day thirty) until day fifty. Interestingly, the samples in this treatment also had a slightly higher intensity than dark storage. The response of PMFs under artificial light exposure is known to be sensitive (Koyama et al., 2012). This response is considered to be a defense mechanism of the plant tissue to certain stress conditions (light) (Arcas et al., 2000). Some researchers reported that blue light (400 -500 nm) could boost the phytochemical production in plant tissue, such as flavonoid, phenolic acid, anthocyanin, and carotenoids (Taulavuori et al., 2018).

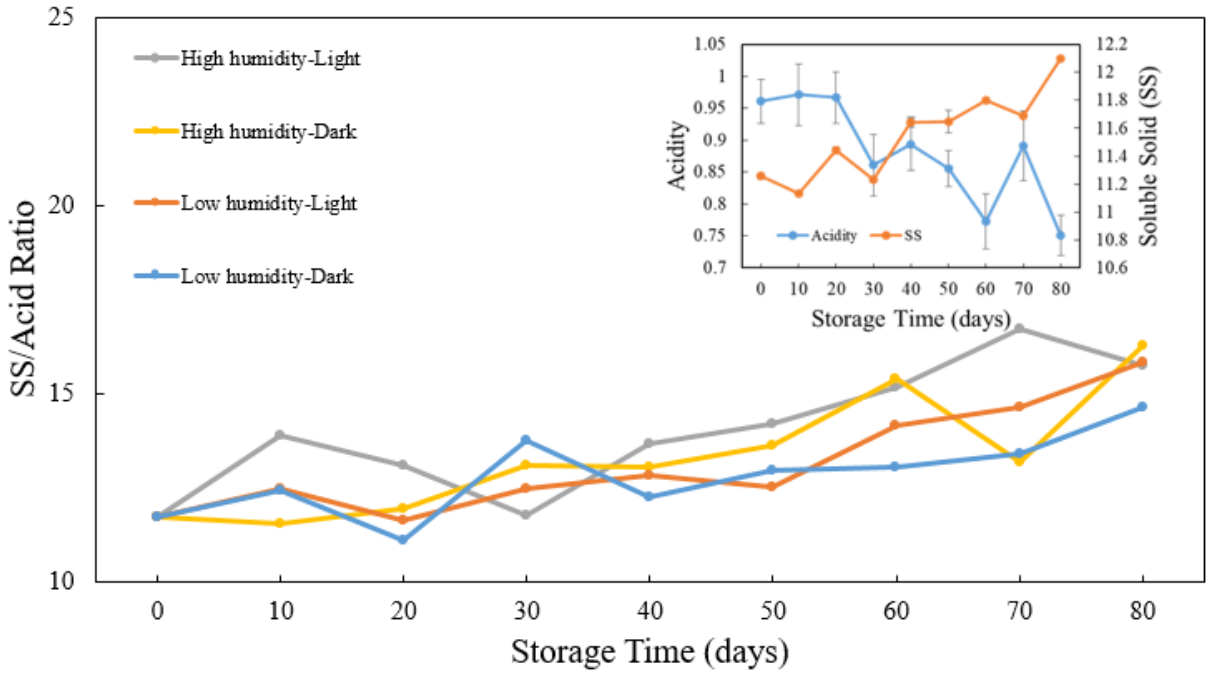

Figure 3. Soluble solid (SS) and acidity (reflected as a percentage of citric acid) ratio during postharvest treatment in different humidity and light condition.

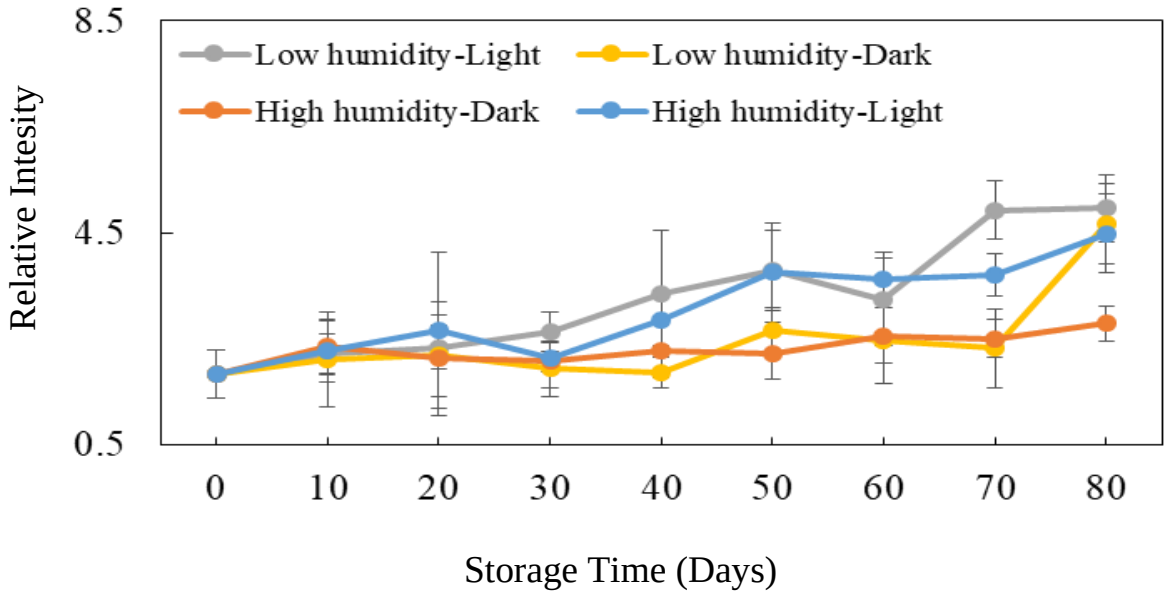

Figure. 4. PMFs ratio (A-1/A-2) compound dynamic in Iyokan during postharvest treatment in different humidity and light condition: Y-axis is the ratio between PMFs (A-1/A-2), expressed as relative instensity.

Light is an electromagnetic radiation wave with a specific energy; it is the reverse of wavelength. The fluorescent lamp used in the experiment, similar to the one used by farmers during storage, does not have a specific wavelength. It has peaks at 450, 550; and 600 nm; therefore, it is expected that fruit stored in lighted conditions will be stressed, and consequently, the plant tissue will have more PMFs (Huché-Thélier et al., 2016; Kotilainen et al., 2008; Li and Kubota, 2009).

We analyzed the effect of the treatment on PMF compounds. We divided PCA analysis into two groups, the first group is from day zero to thirty, and the other group is from day thirty to eighty. For the first group (day zero until day thirty), the resulting PCA1 and PC2 did not distinguish any effect of the treatment on fluorescence compounds and had low accuracy. On the other hand, differences were clearly observable from day thirty to eighty (Figure 5). The PC1 had a low accuracy, while the PC3 had a better accuracy. It seems that light has a significant impact (Huché-Thélier et al., 2016). In addition, we observed that wavelength has an effect on PCA results in the excitation range from 350-410 nm/emission ranging from 450-670 nm. This range may be related to our findings.

The time series dynamics of tryptophan-like compound during postharvest treatment

Tryptophan (an amino acid) exists in many plant cells, including citrus (Khalifah, 1967; Matsumoto and Ikoma, 2012). As explained above, tryptophan plays a role in cell development and growth. In this study, development and cell growth were captured when the fruits were harvested. Thereafter, fruit senescence with slow respiration and ethylene production (Iglesias et al., 2007; Kusunose and Sawamura, 1980). Tryptophan-like compound result (Figure 6) showed a slightly decreasing trend from the beginning of the treatment (day ten). This phenomenon commonly occurs with amino acid metabolism at low temperatures; however, at different temperatures, results will differ.

Some researchers have observed that five amino acids are responsive to heat stress, such as phenylalanine, valine, lysine, histidine, and tryptophan (Matsumoto and Ikoma, 2012). Their experiment showed that early-maturing citrus cultivars have larger amounts of tryptophan when stored at 20-30ºC. Tryptophan accumulates as a response that enhances a defense mechanism against pathogens or physical injury, which serve as precursors for various secondary metabolites (Kaplan et al., 2004). On the other hand, at a low temperature, tryptophan contents remain low (Matsumoto and Ikoma, 2012). Our results are consistent with this trend.

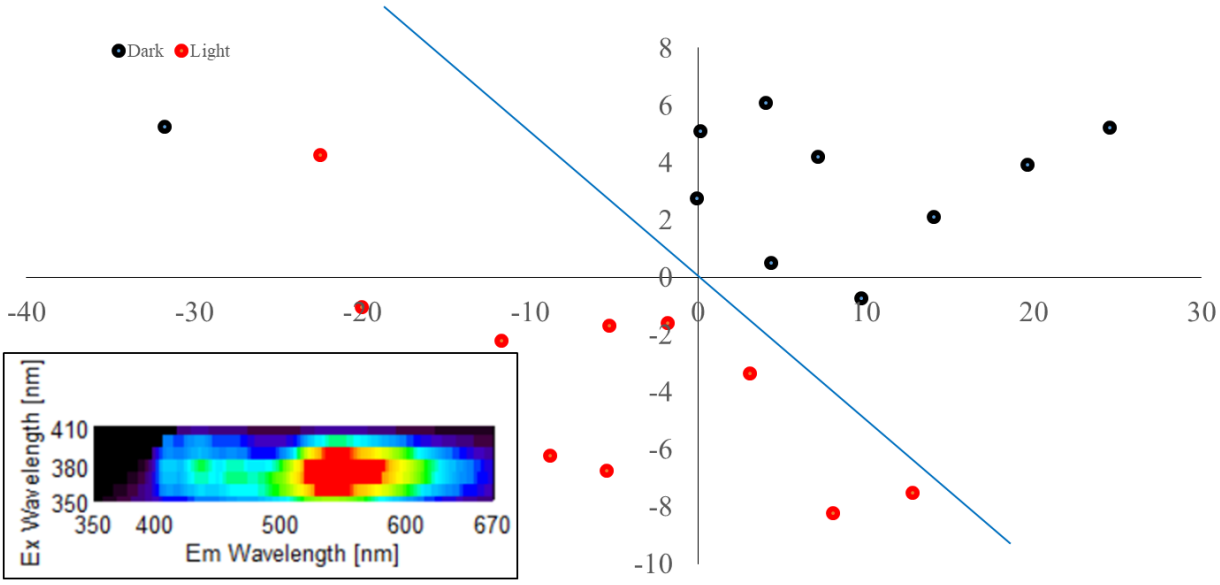

Figure 5. PC1 and PC3 result can distinguished treatment effect to some fluorescence compound and the fluorescence wavelength affected the PCA results.

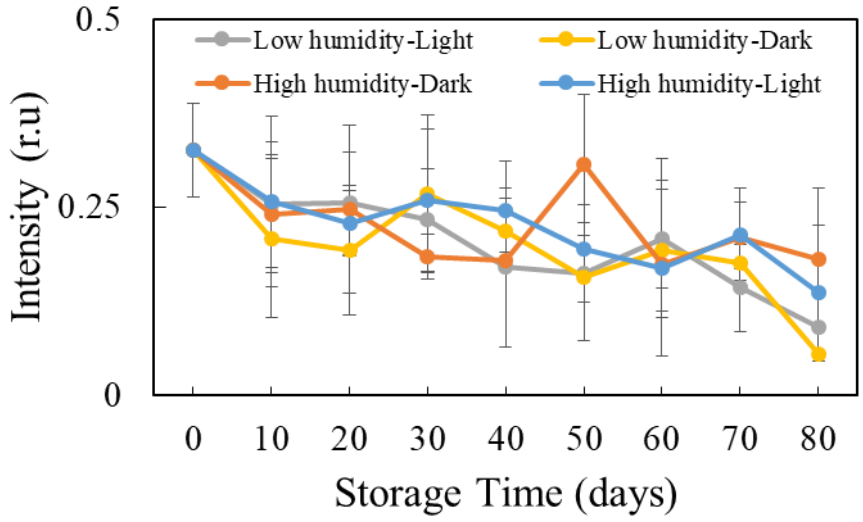

Figure 6. Tryptophan-like (B) compound dynamic in Iyokan during postharvest treatment: Y-axis is the intensity of tryptophan-like compound in raman unit (r.u)

Tryptophan is one of the primary metabolites and nitrogenous compounds in citrus juice (Ladaniya, 2010). It is also responsible for internal quality parameters, such as the taste and aroma of many horticultural crops. Tietel et al. (2010), explained that tryptophan is one of the precursors leading to off-flavors during storage, which is perceived to be an alcohol aroma at the end of all the treatments (day sixty to seventy). This alcohol-like aroma is taken to be a signal of citrus fruit entering the off-flavor stage of citrus (Shi et al., 2005).

Practical Application

Fluorescence testing has several advantages for the rapid assessment of citrus spoilage. First, fluorescence analysis is a non-destructive and non-invasive method that allows repeated measurements without compromising fruit integrity. This means multiple samples can be tested without compromising traditional chemical analysis. In addition, fluorescence measurements are highly sensitive and can detect subtle changes in fruit composition and quality, providing

valuable insight into the degradation process. The method is fast and efficient, allowing real-time monitoring and rapid decision-making regarding fruit quality and shelf life. In practice, fluorescence analysis can be incorporated into sorting and grading systems in citrus processing plants, enabling automated evaluation and separation of fruit based on the degree of deterioration. Additionally, the technology can be used at various supply chain stages, from post-harvest processing to distribution and retail, to ensure that high-quality citrus fruits reach consumers. Overall, fluorescence testing provides a convenient and effective tool for the rapid assessment of citrus spoilage, enabling improved quality control and optimization of storage and handling practices.

Conclusion

Storage experiments of fluorescence compound dynamics in Iyokan under different relative humidity, dark, and light storage conditions were conducted. Our results demonstrate that SS/acid ratio shows a general trend to increase, but they were no significant differences in the treatments. However, the postharvest treatment with high humidity had a slightly higher SS/acid ratio. Thus, together with a tryptophan-like compound, which had no significant difference for any treatments, this compound is responsive in higher temperatures. PMF compounds stored under artificial fluorescent light and dark conditions showed a different response from thirty days into storage; our PC1 and PC3 results could distinguish the effect of light. Further studies are needed to examine the observed trends and investigate the underlying mechanisms in preservation experiments of the dynamics of Iyokan fluorescent compounds. An important aspect to consider is the changes in fluorescent compounds over time and their responses to different storage conditions. Furthermore, it would be worthwhile to study the relationship between fluorescent compounds and other quality parameters (such as organoleptic properties and shelf life) to assess the practical impact of fluorescence analysis on citrus fruit quality assessment. Investigating the specific pathways and biochemical processes underlying PMF's response to the observed light conditions may provide insight into the mechanisms of its synthesis and degradation. Overall, further research is needed to understand better understanding the relationship between the dynamics of fluorescent compounds in citrus and storage conditions, ultimately contributing to improved post-harvest handling and quality control practices.

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Declarations

Conflict of interests The authors declare no competing interests.

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