Effect of post-harvest temperature of tomato ‘Rinka 409’ on quality and autofluorescence during overripening

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Abstract. Japanese tomato cultivar ‘Rinka 409’ is suitable for harvesting at a fully ripe stage since it remains firm at the stage, resulting in longer shelf life. However, it is difficult to estimate the quality change because its color does not change after the fully ripe stage. For ‘Momotaro,’ we previously proposed a fluorescence-based indicator of overripening; however, its suitability for another cultivar, ‘Rinka 409’, and its relationship with temperature and quality were unclear. Therefore, in this study, we investigated the appropriateness of this fluorescence-based index for the above purposes. In 2021, 45 tomato seedlings planted in 2020 were harvested at the fully ripe stage and used for experiments. Weight loss and firmness were evaluated to determine the quality of the tomatoes. The post-harvest temperatures were set as 10, 17, and 25 °C. The results showed that the rate of weight loss increased linearly, whereas the firmness decreased exponentially. For non-destructive estimation of these properties, the excitation-emission matrix (EEM) was measured in the range of 250–460 nm and 280–750 nm for the excitation and emission, respectively. ‘Rinka 409’ showed a strong fluorescence emission at 400 nm/620 nm of the excitation/emission, respectively, regardless of days and temperature. The fluorescence intensity, as well as the firmness, decreased exponentially. Thus, a linear relationship was observed between them. The firmness of ‘Rinka 409’ can be estimated using a simpler linear regression model using this method than previous researches.

1. Introduction
Tomatoes are considered more colored when fully ripened on a tree than after ripening [1,2]. However, they soften during the ripening process, resulting in a trade-off with the shelf life [3]. In Japan, many new cultivars have emerged since the introduction of pinkish tomato cultivars in the 1980s, which has a certain degree of firmness even after ripening. For example, ‘Momotaro’ is relatively firm and can be harvested fully ripened. However, if the distribution or storage period is long, it will inevitably soften owing to overripening. ‘Rinka 409’ is considered as a cultivar that is firmer than Momotaro and has better fully-ripened-harvesting suitability [4]. Nevertheless, there is no guarantee that post-harvest problems will not occur due to overripening or low-temperature environments during distribution; moreover, appropriate temperature management is required during distribution. With the advancement of information technology, it will be possible to trace the distribution of individual fruits, and it will be necessary to enter the ripening information on their database.
Until now, a survey on the firmness of ‘Rinka 409’ at the time of ripening has been reported [5,6]; however, there is no study on its changes until it overripen. Moreover, there are very few reports on the quality after the red stage, although linear temperature equations [7] and exponential functions based on reaction kinetics [8] are well known before the red stage for quality prediction at different post-harvest temperatures. A method to non-destructively estimate the quality at that time together with a prediction model is also needed. Techniques for determining the progress of overripening based on appearance are based on slight changes in the surface color, calyx wilting and collapsing [9]. However, these indicators are still difficult for automation.

We focused on fruit autofluorescence to clearly identify the overripening of tomatoes and investigate its changes. Thus far, overripening indicators have been found as a 360-nm-excited blue fluorescence for ‘Momotaro 8’ [10] and a 280-nm-excited violet fluorescence and a 370-nm-excited yellow fluorescence for ‘Taian Kichijitsu’ [11].’ However, the effects of the post-harvest temperature of ‘Rinka 409,’ which is more suitable for ripe harvesting than other cultivars, on its quality and autofluorescence characteristics is not well understood and the scope of application of a fluorescence-based overripening index is also unclear.

Therefore, in this study, we examined the effect of temperature after a fully ripened harvest on the quality and the possibility of autofluorescence as an index of overripening for ‘Rinka 409’ regardless of the temperature.

2. Material and methods

2.1. Tomato samples
Tomato plant ‘Rinka 409’ was cultivated in an environmentally controlled high-wire system in high technology greenhouse at Ehime University. Tomato seedlings were planted in September 2020 and harvested on April 19, 2021, in long-term hydroponic cultivation. Forty-five fruits were harvested per experiment, and this was repeated twice. Please refer to our previous reports for detailed cultivation conditions [10]. Immediately after harvesting, the mass was measured using an electronic balance, and the dimensions (fruit diameter, fruit height) were measured using calipers 104–144 g, 62–71 mm, and 45–54 mm, respectively. Color was used as an index of the ripening extent at the time of harvest, and one point on the equator was measured with a colorimeter, TES135A Plus (TES Co., Taiwan). The brightness, hue, and saturation were approximately 20, 20, and 40, respectively.

Storage was conducted in a temperature-controlled incubator, MIR-254-PJ (PHC Co., Ltd.), for up to four weeks without packaging and without lighting. Temperatures of 10, 17, and 25 °C ± one °C were set, and the relative humidity ranged from 60–75%, 45–60%, and 30–35%. On the day of the experiment, three fruits under each condition were removed and washed with tap water for approximately 30 s to remove dust and chemicals before the experiment.

2.2. Quality
As a quality, weight loss and firmness were evaluated. Because tomatoes have a smaller weight loss than leafy vegetables, the weight loss is shown instead of their weight. The percentage of the weight loss was calculated using Equation (1) by measuring the fruit weights on the harvest and experimental days using an electronic balance.

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\text{Weight loss (\%)} = \frac{(\text{Weight \ Day of measurement} - \text{Weight \ Day of harvest})}{\text{Weight \ Day of harvest}}
\]

For firmness, Magness–Taylor firmness tester KM-05 (FUJIWARA SCIENTIFIC CO., LTD) was used at three locations (approximately 50° intervals) on the equator of the one side of the fruit, and the load (kgf) at 10% displacement from each location was recorded. The average of these three measurements was taken as the value of the fruit. The initial firmness was in the range of 0.73–1.03 kgf on the day of the harvest. After measuring the firmness, because the part where the scratch made by the firmness tester was on one side of the equator, the other side was used for fluorescence measurements. Specifically, one intact side was separated using a fruit knife on a flat surface by passing through the
apex of the fruit and the calyx, and the other half with the intact surface was kept cold until the fluorometer measurement.

2.3. Excitation-emission matrix
The pericarp was hollowed out with a 20-mm-diameter punch and placed in a sample holder with a quartz window to approximate it as non-destructive fluorescence. The excitation-emission matrix (EEM) of the fruit disk was measured using spectrofluorometer RF-6000 (SHIMADZU CORPORATION). The excitation and emission wavelengths were 250–460 nm (10 nm interval) and 280–750 nm (1 nm interval), respectively. Both bandwidths were 5 nm. It was confirmed that the time required for one measurement was around 3 min, and the appearance did not change before and after that.

3. Results and discussion

3.1. Quality of tomatoes
Figure 1 shows the rate of decrease in the fruit weight of tomato ‘Rinka 409.’ It is considered that the commercial value of the tomatoes decreases at a rate of approximately 2%, based on which the limit of product display is considered to be 5 days at 25 °C, 10 days at 17 °C, and 28 days at 10 °C.

Firmness is an indicator of the progress of internal overripening. It decreased significantly after the beginning of storage at any temperature, and after decreasing, it did not change much (Figure 2). The firmness at 17 and 25 °C became less than the half value of the initial firmness, 0.2 kgf and 0.44 kgf for each temperature, respectively, within a few days. Both values became less than 0.1 kgf after 21 days. Interestingly, at 10 °C, the firmness gradually decreased to 0.3 kgf until the 21st day, and subsequently hardly changed until the 28th day when the experiment was completed. Considering the kinetics, it can be understood that the decomposition of polysaccharides (i.e., polymers related with the firmness) was exponentially suppressed due to the Arrhenius Law of the reaction in the temperature drop from 25, 17 to 10 °C.

Figure 1. Changes in weight loss rate of tomato ‘Rinka 409’ at different temperatures.

Figure 2. Firmness changes of tomato ‘Rinka 409’ at different temperatures.
3.2. Excitation-emission characteristics of tomato

To non-destructively estimate the weight loss rate and firmness of tomatoes, it is possible to make on-site determinations without using chemicals if their autofluorescence can be utilized. Therefore, to examine whether fluorescence can be applied for non-destructive estimation of the weight loss rate and the firmness, the EEM on the outside of the pericarp was measured.

An exemplar EEM of the tomato measured at the day of harvest is shown in Figure 3. Unlike the 360-nm or 370-nm-excited blue fluorescence of ‘Momotaro’ and ‘Taian Kichijitsu’ already reported by our group [10,11], the excitation fluorescence characteristics of tomato ‘Rinka 409’ showed yellow fluorescence at the emission of 620 nm with the excitation of 400 nm.

To further investigate the 400-nm induced yellow fluorescence, the change in the fluorescence emission intensity under the spectral curve from 560 nm to 670 nm of the emission wavelength were analyzed (Figure 4). The fluorescence intensity, similar to firmness, first decreased sharply and subsequently decrease gradually. It was inversely correlated with the weight loss rate. From this result, the weight loss rate was expected to have a linear relationship with the 400-nm induced yellow fluorescence.

Figure 5 shows the relationship between the firmness and the fluorescence intensity of the 400-nm induced yellow fluorescence. It is found that their relationship is generally linear ($r = 0.67$), and the linear regression expressed in Equation (2) was obtained by the least-square method.

$$ Y = a (X + b) $$

where $a = 5111$, $b = 2.96$. Above, $X$ and $Y$ are the firmness (kgf) and the yellow fluorescence intensity (a.u.) excited by 400 nm, respectively. The coefficients $a$ and $b$ are multiplicative and additive factors of the relationship, respectively. The multiplicative factor $a$ includes the fluorometer set-up and the fluorescence quantum yield of the tomato surface. The $b$ represents the fluorescence intensity for the tomato with $X = 0$, meaning that the tomato was to soften fully. This indicates the value $b$ is related to biological factors such as cultivar, season, cultivation, etc.

It is noted that using such a simple regression equation, it may be possible to easily estimate the firmness even if the time of distribution, display at the store, storage at home are unknown.
Figure 4. Change in 400-nm-excited yellow fluorescence intensity under the curve from 560 to 670 nm of tomato ‘Rinka 409.’

Figure 5. Relationship between the firmness of tomato ‘Rinka 409’ and 400-nm excited yellow fluorescence intensity under the spectral curve from 560 to 670 nm.

4. Conclusions
In this study, we examined the effect of temperature after ripe harvest on the quality and the possibility of autofluorescence as an index of overripening, regardless of the temperature, for ‘Rinka 409.’ In 2021, 45 tomato seedlings planted in 2020 were harvested at full ripeness and used for experiments. The weight loss and the firmness were evaluated to determine the quality of the tomatoes. The post-harvest temperatures were set as 10, 17, and 25 °C. The weight loss rate increased linearly with the number of days, whereas the firmness decreased exponentially. To develop a simple estimation method for firmness, we used the fluorescence emission observed at an excitation of 400 nm and emission ranged from 560 to 670 nm (yellow fluorescence). The fluorescence emission intensity was found to decrease exponentially, similar to the firmness. Focusing on the similarity between the firmness and the fluorescence, the relationship between the two was further investigated, and an approximately linear relationship was obtained \((r = 0.69)\). The firmness of ‘Rinka 409’ tomato can be estimated by a simple linear regression model using the fluorescence intensity at the excitation of 400 nm and the emission ranging from 560 to 670 nm, irrespective of the temperature (10, 17, 25 °C).

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