

Article

Simple Blue LED-Excited Fluorescence and Chromaticity Measurements as Screening Indices for Avocado Ripeness

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Abstract

In response to the need for a simple, non-destructive method for evaluating avocado ripeness, we measured chlorophyll-related fluorescence and chromaticity of the outer skin using simple optical equipment and evaluated their relationship with whole-fruit compression (wfc), which was used as a firmness-based ripeness index. A compact system consisting of a blue LED excitation source and a small spectrometer was used to measure fluorescence spectra, and a commercially available colorimeter was used to evaluate chromaticity. Hass avocado samples purchased from multiple retail stores in Japan and stored for different periods were examined. The combination of the fluorescence intensity ratio I_{740}/I_{685} and the lightness parameter L^* showed a moderate correlation with wfc, with $R^2 = 0.48$. The fluorescence ratio I_{740}/I_{685} was treated not as a direct measure of chlorophyll content, but as a spectral index associated with ripening-related changes in avocado skin, including chlorophyll-related fluorescence and skin optical properties. These results suggest that the combination of simple blue LED-excited fluorescence and chromaticity measurements may be useful as a practical screening approach for roughly estimating avocado ripeness in commercially available fruit.

Keywords: avocado ripeness; chlorophyll-related fluorescence; blue LED; chromaticity; CIE L^*C^*h color space; whole-fruit compression

1. Introduction

Avocados have attracted attention as an important food for health-conscious consumers because of their high nutritional value, including monounsaturated fatty acids, dietary fiber, and antioxidants. Avocados have also been reported to provide several health-related benefits, including support for cardiovascular health, digestive function, absorption of fat-soluble vitamins, inhibition of platelet aggregation-related ischemic diseases, and weight management [1–4]. Avocados are generally harvested at an unripe stage in production regions such as Central and South America and Asia, and are then distributed globally. However, their ripeness can change substantially between harvest and retail sale. This variation strongly affects commercial value and consumer acceptability because avocados continue to ripen after harvest through ethylene production, resulting in changes in taste, color, and particularly firmness, which is a critical indicator of eating quality [5,6].

At present, avocado ripeness is commonly assessed by sensory methods, such as gentle squeezing to evaluate firmness and visual observation of skin color. However, these



Academic Editor: Maurizio Cocucci

Received: 19 May 2026

Revised: 18 June 2026

Accepted: 22 June 2026

Published: 28 June 2026

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methods are subjective and inefficient, and repeated physical handling can damage the fruit, leading to spoilage and reduced quality. In addition, variability in human assessment can cause fruit at suitable ripeness stages to be discarded, resulting in economic losses and increased food waste. Therefore, there is a need for a simple, nondestructive inspection method that can roughly estimate or screen avocado ripeness using a compact and user-friendly device. Several nondestructive techniques, including near-infrared spectroscopy (NIR), acoustic response, and electrical impedance, have been investigated [7–10]. However, these methods can require relatively large equipment or complex data analysis, and their results may be affected by fruit size, shape, color, and surface condition. These difficulties are particularly important for avocados because their thick skin, large seed, and variable shape can affect nondestructive measurements.

Recent studies have explored the relationship between fluorescence spectral features of avocado skin and ripening. Kokawa et al. used fluorescence fingerprint data obtained from both the skin and flesh of Hass avocados and applied partial least-squares (PLS) regression analysis using flesh firmness as a ripeness index, resulting in a model with high predictive accuracy [11]. Lin et al. examined reflectance and fluorescence spectra obtained from avocado skin using white-light illumination and LED excitation at 365, 385, 395, and 405 nm. They reported that fluorescence intensity ratios, referred to as F1 (I_{740}/I_{685}) and F2 (I_{550}/I_{685}), changed consistently with storage time, suggesting their potential use as ripening-related indicators [12]. However, fluorescence fingerprint measurements require relatively large equipment and an analysis system, making them less suitable for simple market testing. In addition, although Lin et al. showed that fluorescence intensity ratios changed with storage time, their direct relationship with firmness-based ripeness indices was not fully clarified.

In this study, we developed a simple measurement system consisting of a blue LED excitation source and a small spectrometer for convenient nondestructive testing. We used this system to measure chlorophyll-related fluorescence spectra from the outer skin of Hass avocados at different ripeness levels. In addition, chromaticity was measured using a commercially available colorimeter as another simple nondestructive optical parameter. We then examined the relationship between these optical measurements and whole-fruit compression (wfc), a firmness-based index obtained by applying pressure to the intact fruit through the rind. Based on these results, we discuss the potential utility of simple fluorescence and chromaticity measurements as practical screening indices for avocado ripeness.

2. Materials and Methods

2.1. Avocado Samples and Measurement Positions

Thirteen commercially available avocados with different apparent skin colors were purchased from retail stores in Japan. All samples were Hass avocados. Table 1 shows the purchase date, storage period before measurement, and retail store for each avocado sample. The samples were stored in a temperature-controlled chamber at 4 °C until measurement. On the day of measurement, 14 April 2023, the samples were removed from the chamber and allowed to equilibrate to approximately room temperature before testing. We noted variations in size and shape among the sample lots; however, detailed information on production region, harvest date, and pre-retail handling history was not available.

Figure 1 shows schematic illustrations of the measurement positions used for hardness, fluorescence, and chromaticity measurements. The side including the stem was defined as the front side, and measurements were performed on the right, back, and left sides of each avocado sample.

Table 1. Purchase date, storage period, and retail store for the avocado samples.

Sample ID	Purchase Date	Storage Period (Days)	Retail Store
a, b	4 February 2023	69	Fuji Garden (Aoba-ku, Yokohama, Japan)
c, d, e, f	4 March 2023	41	Food Store Aoki (Toyosu, Tokyo, Japan)
g, h, i, j, k	1 April 2023	13	Oozeki (Togoshi-ginza, Tokyo, Japan)
l, m	14 April 2023	0	Bunkado (Toyosu, Tokyo, Japan)

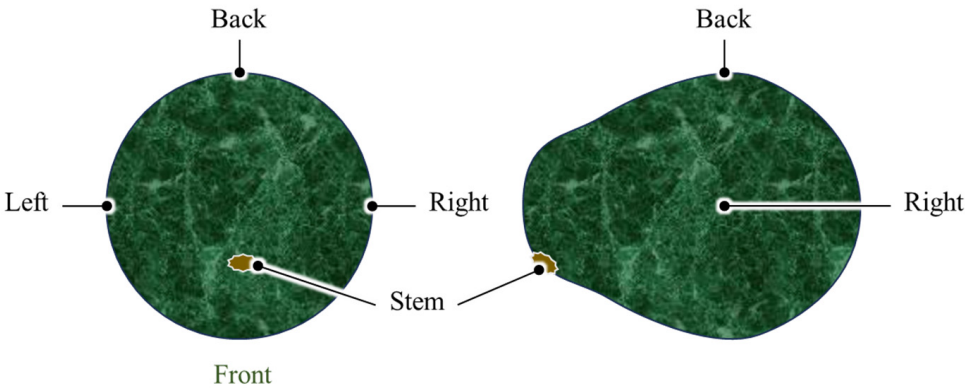


Figure 1. Measurement positions used for hardness, fluorescence, and chromaticity measurements.

2.2. Hardness Measurement

White et al. previously investigated the relationship between avocado hardness measured by several instrumental methods and sensory evaluation by manual soft squeezing [13]. In the present study, hardness was evaluated using a method based on the whole-fruit compression approach reported in Ref. [13]. Figure 2 shows a photograph of the hardness tester used in this study. The tester consisted of a force gauge (ZTA-5N, IMADA Co., Ltd., Toyohashi, Japan), a laser displacement sensor (ZX2-LD100, OMRON Corporation, Kyoto, Japan), an amplifier unit (ZX2-LDA41, OMRON), and a vertical drive handle. The force gauge was fitted with an A-2 attachment, which has a flat circular tip with a diameter of 13.3 mm. To approximate the hemispherical probe tip with a diameter of 8 mm described in Ref. [13], an 8-mm-diameter iron ball was fixed to the tip of the attachment using a doughnut-shaped silicone rubber support and adhesive.

The probe was moved vertically at approximately 20 mm/min using the vertical drive handle until a displacement of 1–2 mm was reached. Each avocado was placed directly under the probe, and the probe was aligned as vertically as possible with respect to the fruit surface. The output signal from the force gauge and the displacement signal from the laser displacement sensor were recorded using a data logger (VM02-A, NUBTECH, Yokohama, Japan) and its dedicated software. A linear fit was applied to the force–displacement curve, and the force required to produce a 2-mm deformation was defined as the whole-fruit compression value (wfc). When the measured force reached the upper measurable limit before the displacement reached 2 mm, the force at 2 mm was estimated by linear extrapolation from the measured linear region of the force–displacement curve. For each sample, measurements were performed at three positions: the central regions of the right, back, and left sides, as defined in Figure 1.

Before the avocado measurements, the contribution of instrument compliance to the measured displacement was evaluated using a rigid steel block. As a result, the force exceeded 2 N at a displacement of less than 0.1 mm on the rigid steel surface. Therefore, the measured displacement in the avocado tests was considered to primarily reflect deformation of the avocado, with only a negligible contribution from the measurement system itself.

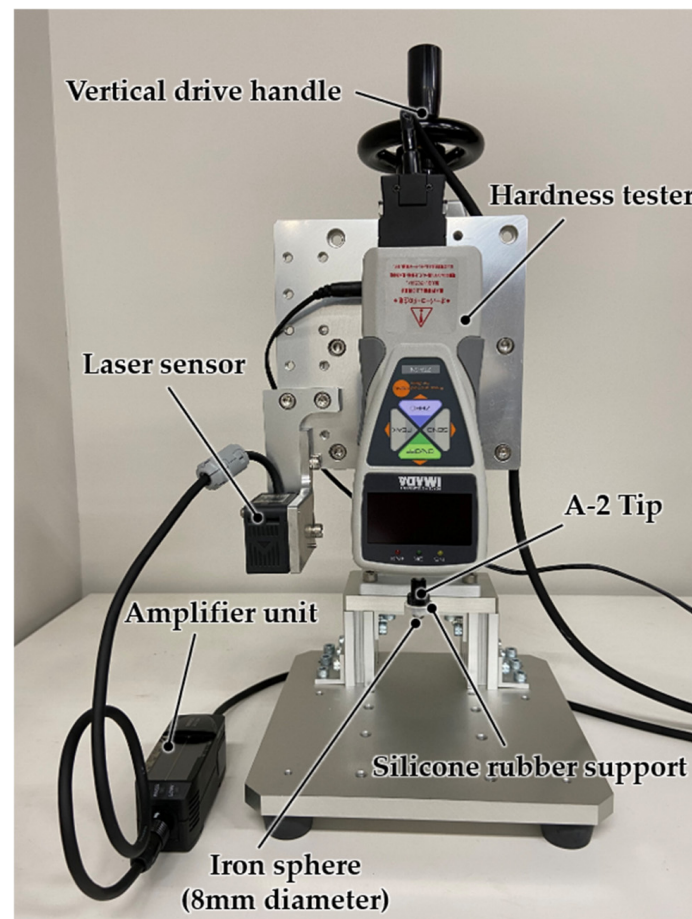


Figure 2. Overview of the hardness tester.

2.3. Fluorescence Measurement

Figure 3 shows a schematic diagram of the measurement system used for fluorescence evaluation of avocado skin. A blue LED (KD-JP3W-B-HS, 3 W, 30–40 lm) with a center wavelength of 450 nm was used as the excitation source. The emitted and reflected light from the avocado skin was detected using a mini-spectrometer (C12880MA, Hamamatsu Photonics, Hamamatsu, Japan), which was included in the Colorcompass MFA system (AT-System Corp., Hamamatsu, Japan). The detection plane of the spectrometer was positioned horizontally at a height of 90 mm on a metal stage. The LED was placed adjacent to the spectrometer at an angle of 28° from the horizontal. The avocado sample was placed directly below the optical components so that the upper surface of the sample was 25–35 mm below the spectrometer detection plane. During the measurement, the avocado sample and optical components were covered with a light-blocking box whose inner surface was covered with black cloth to reduce external light. Spectra were acquired using the software supplied with the Colorcompass system over a wavelength range of 340–850 nm, with a wavelength interval of 1 nm and an exposure time of 0.5 s. Each spectrum was obtained by averaging five spectral acquisitions. A five-point moving average, corresponding to five wavelength channels, was then applied to the acquired spectrum.

For each measurement point, a background spectrum (B) was first acquired with the LED turned off. A spectrum under LED illumination (S) was then acquired with the LED turned on. The background-corrected spectrum, obtained by subtracting B from S, was used to calculate the fluorescence intensity ratio. Because the measurement system did not include optical filters, the background-corrected spectrum contained both the reflected excitation component around 450 nm and the fluorescence components from the avocado

skin. Therefore, the fluorescence ratio was calculated using the spectral intensities at 740 and 685 nm, which are sufficiently separated from the excitation wavelength. The ratio I_{740}/I_{685} was used as a within-spectrum fluorescence index so that variations in absolute intensity caused by LED output, detector sensitivity, and measurement distance could be partly reduced. However, this ratio may still be affected by sample geometry, surface roughness, light scattering, reabsorption, and the signal-to-noise ratio of each fluorescence band. For each avocado sample, measurements were performed once at each of the right, back, and left positions defined in Figure 1.

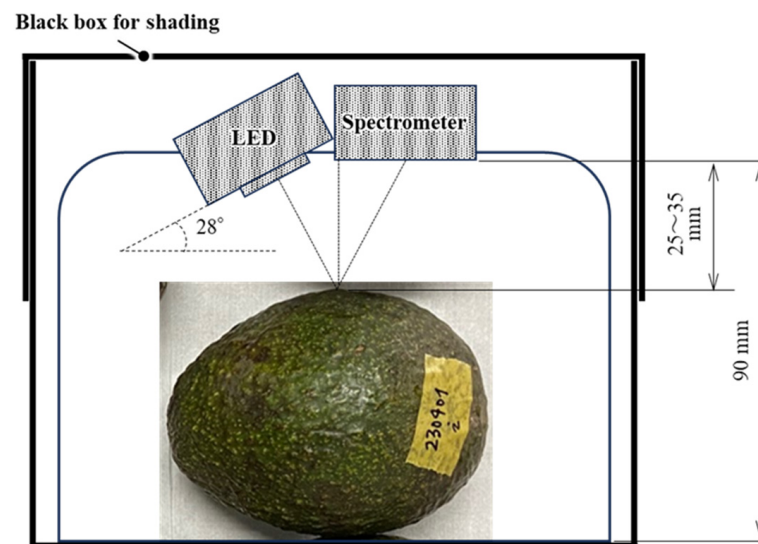


Figure 3. Schematic diagram of the simple spectrofluorometer. The numbers indicate the main dimensions and optical geometry used in the measurement.

To evaluate the reliability of the fluorescence intensity ratio, a post hoc signal-to-noise ratio (S/N) analysis was performed using the saved raw spectral data. For this analysis, the same background-corrected spectra used for calculating I_{740}/I_{685} were used. The signal intensities at 685 and 740 nm were obtained from the 5-point moving average spectrum. The noise level was estimated from the 600–650 nm region of the corrected spectrum, where no apparent chlorophyll fluorescence peak was observed. Specifically, a linear baseline was fitted to this region, and the standard deviation of the residuals was used as the noise level. The S/N values at 685 and 740 nm were calculated by dividing each signal intensity by this noise level. In this analysis, $S/N \geq 10$ was regarded as an approximate minimum level for quantitative comparison, whereas spectra with $S/N < 10$ were interpreted with caution.

2.4. Chromaticity Measurement

Chromaticity was measured on the outer skin of each avocado sample using a portable colorimeter (TES-3250, Sato Shouji Inc., Tokyo, Japan). Measurements were performed at three positions on each fruit: the right, back, and left sides, as defined in Figure 1. For the regression analysis, the chromaticity value obtained at each measurement position was paired with the wfc value and fluorescence ratio obtained at the corresponding position. Chromaticity is expressed using the CIE L^*C^*h color space [14,15]. In this color space, L^* , C^* , and h represent lightness, chroma, and hue angle, respectively. L^* ranges from 0 (black) to 100 (white). The parameters C^* and h were calculated from the a^* and b^* values in the CIE $L^*a^*b^*$ color space using Equations (1) and (2), respectively:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$h = \tan^{-1}(b^*/a^*) \quad (2)$$

where a^* represents the green–red axis and b^* represents the blue–yellow axis. The hue angle h was expressed in degrees.

3. Results

3.1. Hardness Measurement

Figure 4a,b show typical force–displacement curves obtained from the back side of samples a and h, respectively. In this measurement, probe displacement was controlled by the vertical drive mechanism, and the resulting force response was measured using the force gauge. Therefore, displacement was plotted on the x-axis and force on the y-axis. The point at which the spherical probe first contacted the avocado skin was defined as 0 mm displacement and 0 N force. A linear relationship was observed between displacement and force in the measured range. The average slope was calculated from three repeated measurements at each measurement point and multiplied by two to obtain the wfc value corresponding to a displacement of 2 mm. The coefficients of determination of the fitted linear lines were 0.975–0.996 for sample a and 0.968–0.992 for sample h. These results demonstrate that the force–displacement curves showed good linearity in the measured range. Similar linear relationships were also observed for the other samples.

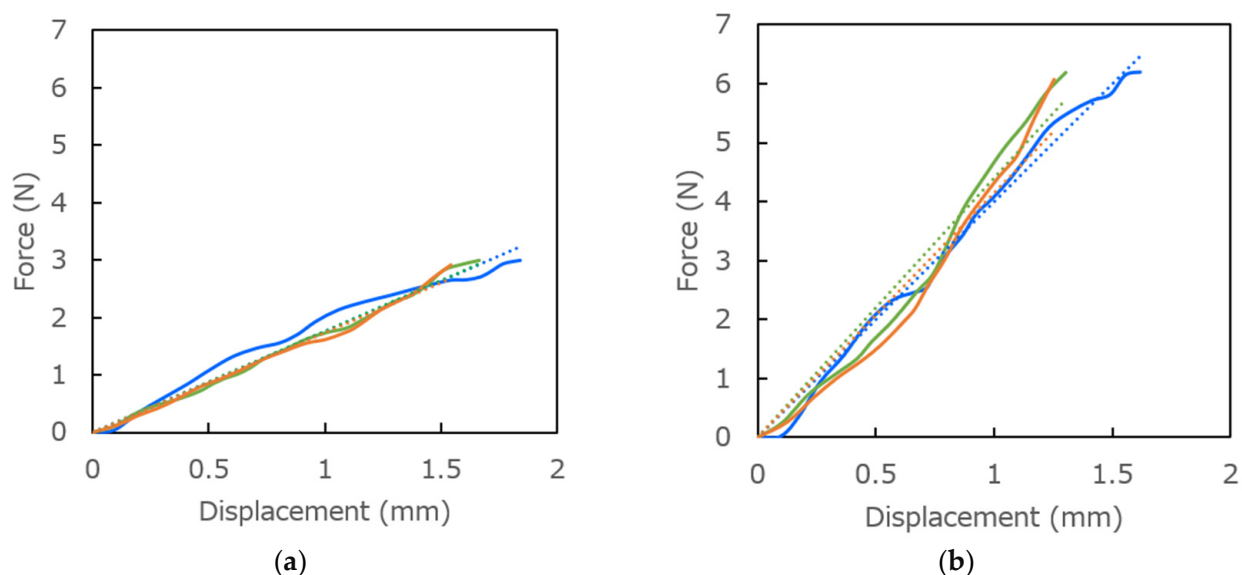


Figure 4. Typical force–displacement curves obtained during whole-fruit compression measurement. Three repeated force–displacement curves are shown for each sample at the back-side position: (a) sample a, and (b) sample h. Blue, orange, and green lines indicate the first, second, and third repeated measurements, respectively. Probe displacement was the controlled variable, and the resulting force response was measured using the force gauge. The linear region was used to estimate the force required for 2-mm deformation, defined as the whole-fruit compression value (wfc).

Figure 5 shows the average wfc values for all avocado samples. Samples a and b showed the lowest wfc values, which is consistent with their longer storage periods. For most samples, the variation among repeated measurements was comparable to the variation among the three measurement positions. In contrast, samples b and h showed relatively large differences in wfc among the measurement positions. This within-fruit variation may have been caused by local physical damage or heterogeneous softening in these samples.

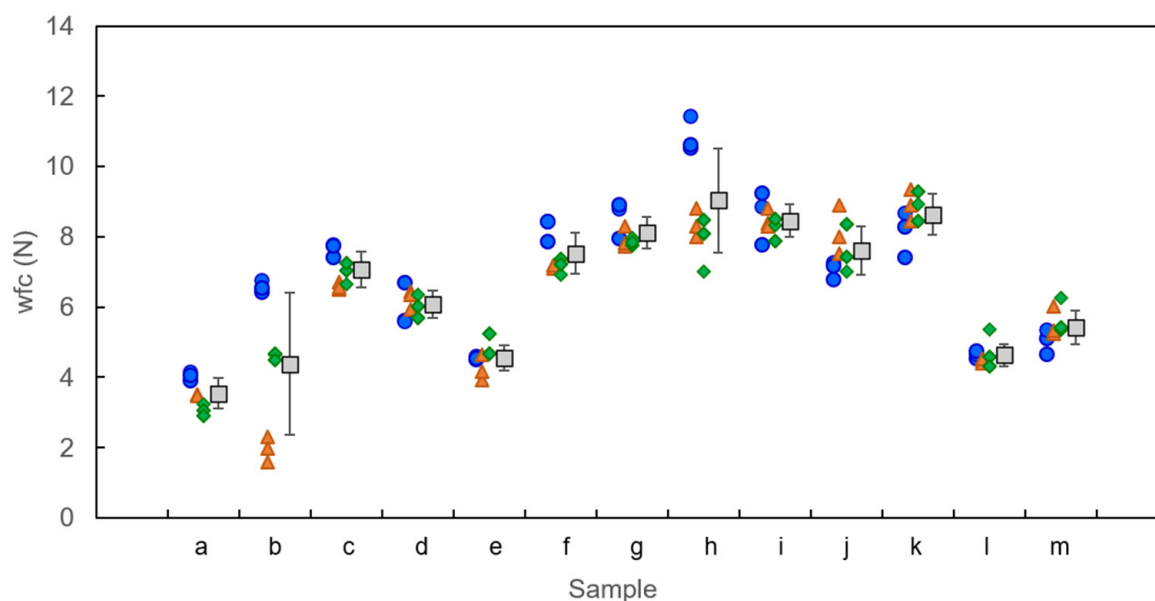


Figure 5. Whole-fruit compression values (wfc) for all avocado samples. Individual symbols indicate position-wise measurements obtained from the right (●), back (▲), and left (◆) sides of each fruit. Squares (◻) indicate the mean wfc value for each fruit, and error bars indicate the standard deviation among the three measurement positions.

3.2. Fluorescence Measurement

Figure 6 shows typical background-corrected spectra obtained from samples a, b, c, and h in the wavelength region of 550–850 nm, which includes the chlorophyll-related fluorescence bands used for calculating I_{740}/I_{685} . Peaks around 685 nm and 740 nm were observed in most spectra, although the 740 nm peak was very weak in sample b. These two bands are generally attributed to chlorophyll a fluorescence [16]. The 685 nm band is mainly associated with fluorescence from photosystem II, whereas the longer-wavelength band around 740 nm is considered to be influenced by chlorophyll fluorescence emitted from deeper tissue layers as well as by reabsorption and scattering within the tissue. In previous studies, the fluorescence intensity ratio I_{740}/I_{685} has been used as an index related to chlorophyll status in plant tissues [17]. Therefore, in this study, I_{740}/I_{685} was calculated as a practical fluorescence spectral index reflecting ripening-related changes in avocado skin, including chlorophyll-related fluorescence and skin optical properties, rather than as a direct indicator of chlorophyll content.

Figure 7 shows the fluorescence intensity ratio I_{740}/I_{685} for each sample. Samples a and b, which showed relatively low hardness values, also showed low I_{740}/I_{685} values. In particular, as shown in Figure 6, the 740 nm peak in the spectrum of sample b was very weak, resulting in the lowest I_{740}/I_{685} value. This result suggests a marked reduction in the chlorophyll-related fluorescence signal and/or a change in the optical properties of the skin in this sample. In contrast, samples i and j showed relatively large variations in I_{740}/I_{685} among the three measurement positions, indicating spatial heterogeneity in the fluorescence response of the skin.

Figure 8 shows I_{740}/I_{685} as a function of wfc. The relationship showed a moderate correlation, with $R^2 = 0.38$. Two data points, $(wfc, I_{740}/I_{685}) = (4.50, 0.13)$ and $(6.47, 0.28)$, deviated from the approximate linear trend. Both points were obtained from sample b, which was one of the samples stored for the longest period. This deviation may be related to the low signal-to-noise ratio of the 740 nm band caused by the weak chlorophyll-related fluorescence signal in this sample, as described above.

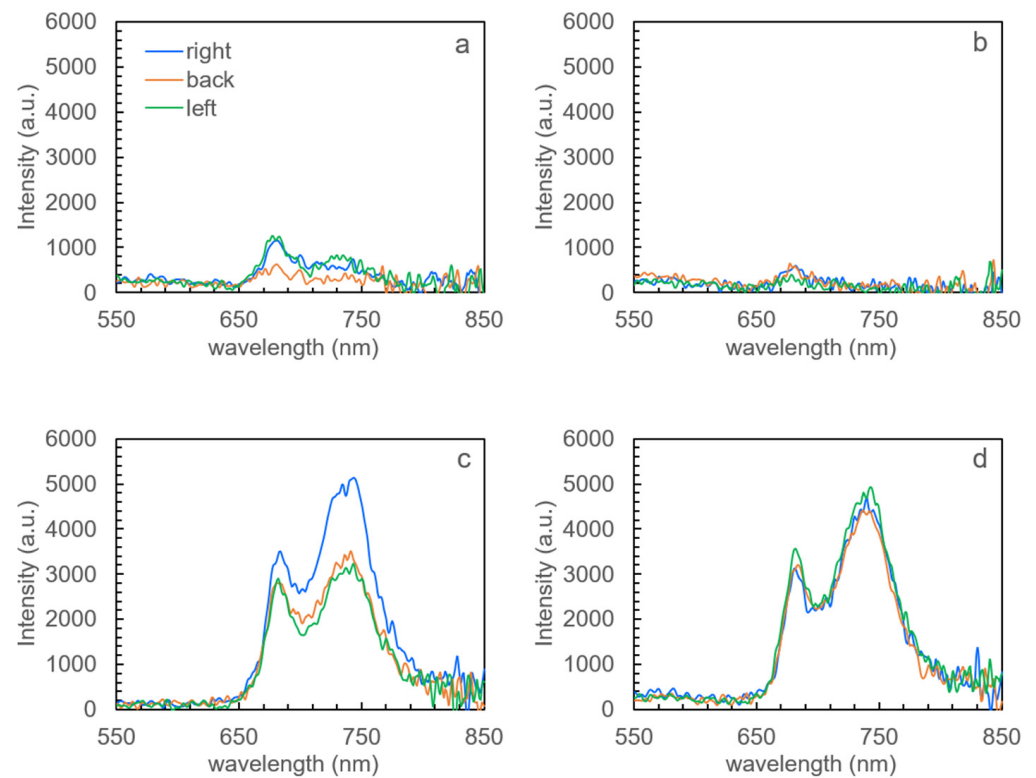


Figure 6. Typical background-corrected spectra of samples a, b, c, and h in the wavelength region of 550–850 nm, focusing on the chlorophyll-related fluorescence bands around 685 and 740 nm used for calculating I_{740}/I_{685} . Panels (a–d) show samples a, b, c, and h, respectively. Blue, orange, and green lines indicate spectra obtained from the right, back, and left sides, respectively.

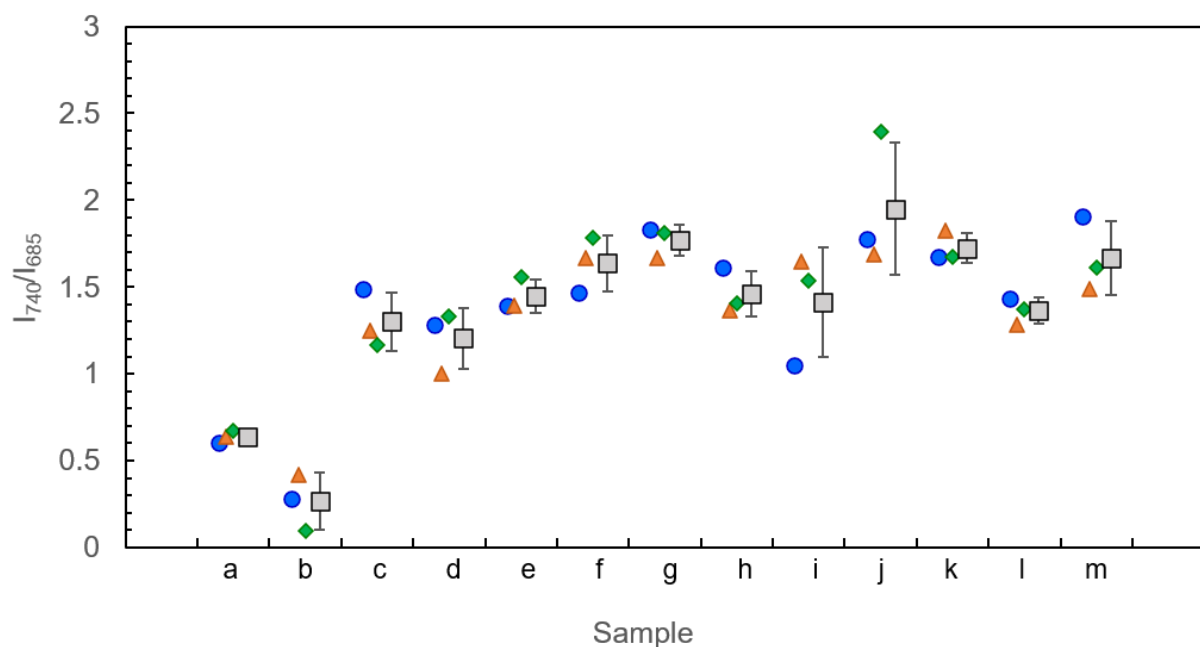


Figure 7. Fluorescence intensity ratio I_{740}/I_{685} for each avocado sample. Individual symbols indicate position-wise measurements obtained from the right (●), back (▲), and left (◆) sides of each fruit. Squares (◻) indicate the mean value for each fruit, and error bars indicate the standard deviation among the three measurement positions.

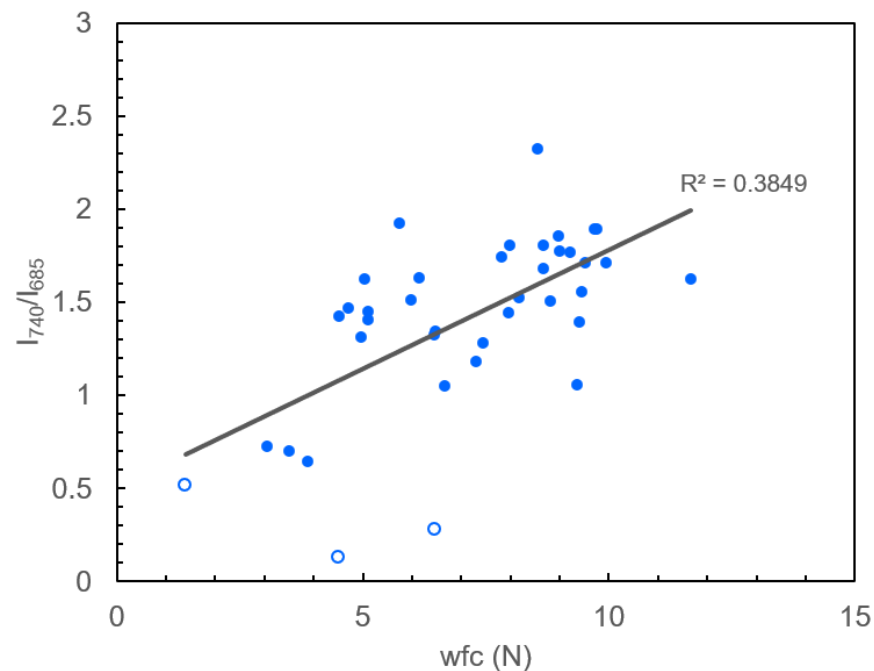


Figure 8. Relationship between the fluorescence intensity ratio I_{740}/I_{685} and whole-fruit compression value (wfc). Filled circles (●) represent position-wise measurements obtained from the right, back, or left side of avocado fruits. Open circles (○) indicate data points from sample b. The solid line indicates the linear regression line.

The additional S/N analysis indicated that most samples with clear chlorophyll-related fluorescence bands had sufficiently high S/N values at both 685 and 740 nm. In contrast, sample b showed very low S/N values at 740 nm, ranging from 0.4 to 2.7 depending on the measurement position. This result indicates that the 740 nm fluorescence signal of sample b was close to or below the reliable quantitation level. Therefore, the low I_{740}/I_{685} values and the deviation of sample b from the approximate linear trend may be partly attributable to the low S/N of the 740 nm band. Sample a also showed relatively low or borderline S/N values at 740 nm at some positions, suggesting that I_{740}/I_{685} should be interpreted with caution when the long-wavelength fluorescence band is weak. Because repeated independent spectral measurements were not obtained for each condition, the repeatability and day-to-day variability of the fluorescence measurement system could not be fully evaluated in the present study.

3.3. Chromaticity Measurement

Figure 9 shows the relationships between wfc and the chromaticity parameters of the avocado skin: (a) L^* , (b) C^* , and (c) hue angle h . The lightness parameter L^* and the chroma parameter C^* showed moderate correlations with wfc, with R^2 values of 0.37 and 0.33, respectively. In contrast, the hue angle h showed little correlation with wfc.

Figure 10 shows the relationship between a^* and b^* . A nearly linear relationship was observed between these parameters, suggesting that the hue angle h remained relatively consistent among the samples. This result is consistent with the weak correlation between h and wfc. In contrast, C^* , which is calculated from a^* and b^* , varied among the samples. The C^* value tended to decrease as wfc decreased, suggesting that the chroma of the avocado skin decreased with the progression of ripening.

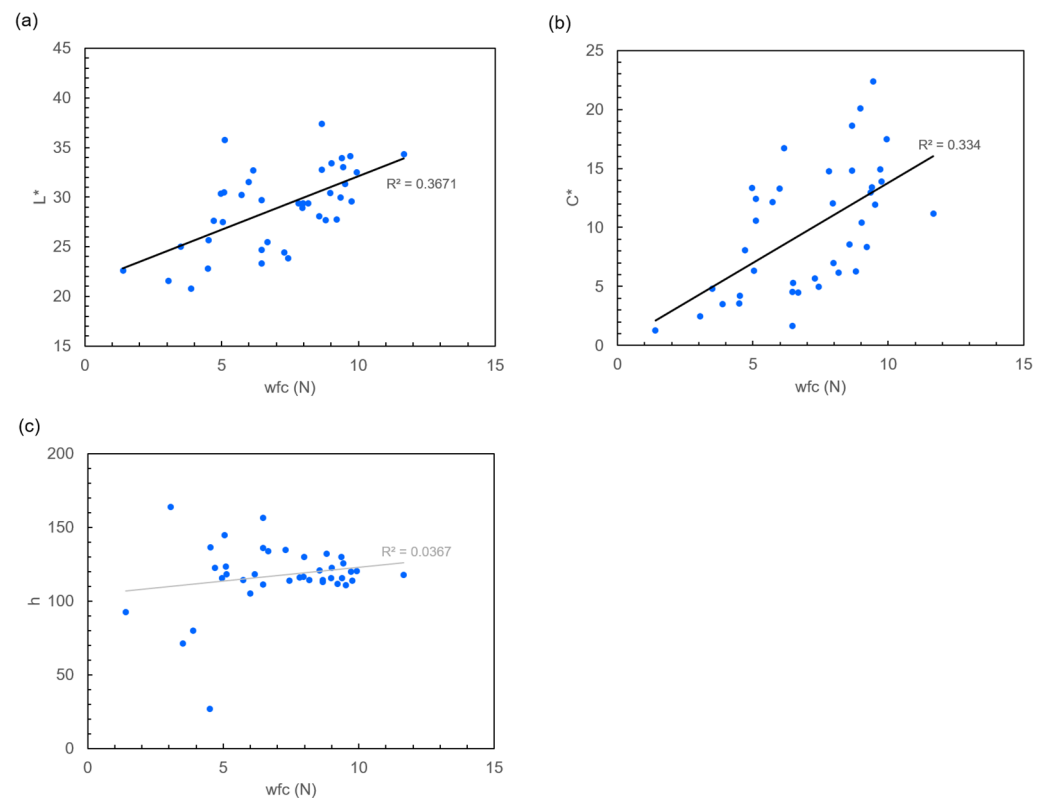


Figure 9. Relationships between whole-fruit compression value (wfc) and chromaticity parameters of avocado skin: (a) L^* , (b) C^* , and (c) hue angle h . Each point represents one position-wise measurement obtained from the right, back, or left side of each avocado fruit. Solid lines indicate linear regression lines where applicable.

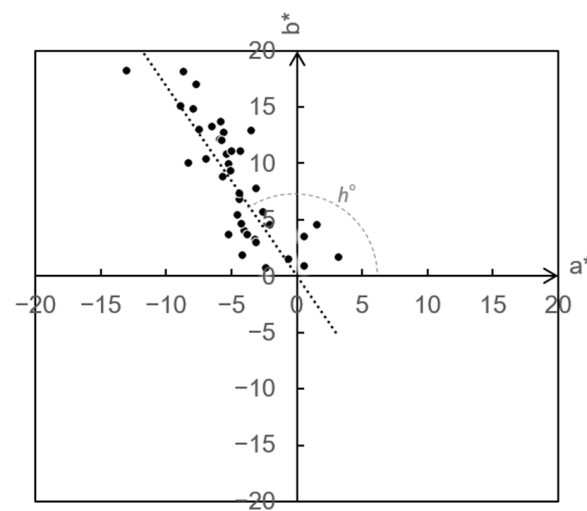


Figure 10. Relationship between a^* and b^* values of avocado skin. Each point represents one position-wise chromaticity measurement obtained from the right, back, or left side of an avocado fruit.

3.4. Correlation Analysis of Hardness with Fluorescence Intensity and Chromaticity

The relationships between wfc and the optical parameters were evaluated using single and multiple linear regression analyses. Position-wise paired data obtained from the right, back, and left sides of each avocado sample were used for this analysis. Thus, the regression analysis was performed using 39 observations from 13 fruits. Because the three position-wise observations obtained from the same fruit were not fully independent, the number of independent fruit samples was 13. Therefore, the regression results based on

position-wise observations were interpreted with caution, and a stricter leave-one-fruit-out cross-validation was also performed. For each measurement position, the wfc value and the corresponding optical parameters, including I_{740}/I_{685} , L^* , and C^* , were paired and used for single and multiple regression analyses. The hue angle h was excluded from the regression analysis because it showed little relationship with wfc, as shown in Figure 9.

To evaluate the robustness of the regression models, leave-one-out cross-validation (LOOCV) was additionally performed. In LOOCV, one observation was excluded from the dataset, the regression model was recalculated using the remaining observations, and the excluded observation was predicted. This procedure was repeated for all observations. The cross-validated coefficient of determination (Q^2) was calculated as $1 - \text{PRESS}/\text{TSS}$, where PRESS is the sum of squared prediction errors, and TSS is the total sum of squares of the observed wfc values. The root mean squared error of cross-validation (RMSECV) was also calculated.

The LOOCV results are summarized in Table 2 together with the full-data regression results. The combination of I_{740}/I_{685} and L^* showed the highest R^2 value among the two-parameter models, with $R^2 = 0.475$ and $\text{SE} = 1.720$ N. The LOOCV analysis gave $Q^2 = 0.380$ and $\text{RMSECV} = 1.795$ N, and this model showed the best cross-validated performance among the tested models. The combination of I_{740}/I_{685} and C^* also improved the relationship compared with the single-parameter models, but its LOOCV performance was slightly lower, with $Q^2 = 0.358$ and $\text{RMSECV} = 1.827$ N. The three-parameter model using I_{740}/I_{685} , L^* , and C^* gave a slightly higher full-data R^2 of 0.481, but its LOOCV performance did not improve, with $Q^2 = 0.355$ and $\text{RMSECV} = 1.832$ N. These results indicate that the combination of I_{740}/I_{685} and L^* provided the most balanced model among the tested parameter sets. However, because the regression and cross-validation performance remained moderate, the present model should be regarded as a practical screening approach rather than a highly accurate prediction model.

Table 2. Regression and cross-validation results for wfc using fluorescence and chromaticity parameters. The LOOCV values were calculated using position-wise observations. For the I_{740}/I_{685} and L^* model, the stricter leave-one-fruit-out cross-validation gave $Q^2 = 0.299$, and $\text{RMSECV} = 1.909$ N. Detailed regression coefficients, t values, and p values are provided in Supplementary Table S1.

Parameter Set	R^2	SE (N)	LOOCV Q^2	RMSECV (N)
I_{740}/I_{685}	0.385	1.836	0.306	1.900
L^*	0.367	1.863	0.288	1.924
C^*	0.334	1.911	0.268	1.952
I_{740}/I_{685} , L^*	0.475	1.720	0.380	1.795
I_{740}/I_{685} , C^*	0.446	1.766	0.358	1.827
L^* , C^*	0.400	1.839	0.298	1.911
I_{740}/I_{685} , L^* , C^*	0.481	1.735	0.355	1.832

4. Discussion

4.1. Relationship Between Hardness and Ripeness

The hardness index used in this study, wfc, can be related to the hand firmness scale reported by White et al. [13]. This scale ranges from 0, corresponding to very firm fruit with no deformation, to 7, corresponding to overripe fruit with almost liquid flesh. A hand firmness value of 5 is defined as “fully ripe” [13]. As shown in Figure 5, the lowest wfc value was 2.0 N for sample b, whereas the highest value was 10.9 N for sample h. According to the relationship between hand firmness and $\ln(\text{wfc})$ reported in Ref. [13], a wfc value of 10.9 N corresponds approximately to hand firmness values of 3–5, when the reported variation is considered. A hand firmness value of 5 corresponds to wfc values of

approximately 7.5–12 N [13]. By extrapolating this relationship to lower wfc values, wfc values of approximately 5.5 N and 3.5 N correspond roughly to hand firmness values of 6 and 7, respectively.

Based on this approximate conversion, the avocado samples used in this study were estimated to correspond to hand firmness values of approximately 3 to 7 or higher, as summarized in Table 3. Although the samples were selected to include fruit that appeared green and firm, most of them were classified as ripe, fully ripe, or overripe based on the literature indicators. This result is reasonable because commercially available avocados in the Japanese retail market have generally undergone transportation and storage after harvest. Therefore, although the degree of ripeness varied among samples, the samples used in this study were mainly distributed within the ripe to overripe range. The relatively narrow distribution of samples within the ripe to overripe range may have limited the range of wfc values and contributed to the moderate correlations observed in the optical measurements.

Table 3. Estimated ripeness classification based on hardness measurements and the hand firmness scale reported in Ref. [13].

Sample ID	wfc [N]	Hand Firmness	Ripeness Classification
a	3.1–4.1	approximately 7	overripe
b	2.0–6.6	5–8	fully ripe to overripe (depending on position)
c, f–k	6.6–10.9	4–5	ripe to fully ripe
d, e, l, m	4.2–6.2	approximately 6	fully ripe

4.2. Correlation of Ripeness with Optical Parameters

We investigated whether the fluorescence intensity ratio I_{740}/I_{685} and CIE chromaticity parameters were associated with wfc in commercially available avocado samples purchased from different retail stores and stored for different periods. As shown in Figures 8 and 9 and Table 2, I_{740}/I_{685} , L^* , and C^* each showed moderate relationships with wfc. Multiple regression analysis showed that the combination of I_{740}/I_{685} and L^* improved the relationship with wfc, giving $R^2 = 0.475$, whereas the LOOCV analysis gave $Q^2 = 0.380$ and $RMSECV = 1.795$ N. These results suggest that the combination of fluorescence and chromaticity measurements may provide a practical, non-destructive screening index for roughly estimating wfc in commercially available avocados. However, the correlation and cross-validated performance remained moderate, and the present results should not be interpreted as demonstrating a universally applicable prediction model.

The present study should be interpreted as a preliminary proof-of-concept study. The number of samples was limited, and the fruits were commercially available Hass avocados purchased from different retail stores. Therefore, information on production region, harvest maturity, transportation conditions, and storage history before purchase was not available. These factors may affect firmness, pigment composition, skin optical properties, and fluorescence signals. In addition, the storage periods before measurement differed substantially among samples. Therefore, the observed optical changes may reflect not only ripening-related changes but also the effects of prolonged storage and possible fruit deterioration.

Whole-fruit compression was used as a non-destructive firmness-based index because the objective of this study was to examine simple optical measurements applicable to intact commercial fruit. However, wfc alone does not fully characterize avocado ripeness. Future studies should include independent reference parameters such as flesh firmness, dry matter

content, and oil content, together with larger sample sets with controlled information on origin, harvest maturity, and postharvest conditions.

In the regression analysis, position-wise paired data from the right, back, and left sides of each fruit were used in order to evaluate the relationship between local firmness and local optical properties. However, these position-wise observations were obtained from the same 13 fruits and therefore were not fully independent. Within-fruit variability was observed in both wfc and optical parameters, particularly in some samples. For example, samples b and h showed relatively large differences in wfc among measurement positions, suggesting heterogeneous softening or local physical damage. Such spatial heterogeneity may have contributed to the moderate regression and cross-validation performance observed in this study.

To examine the influence of this data structure, leave-one-fruit-out cross-validation was also performed. In this analysis, all three position-wise observations from one fruit were excluded together. For the model using I_{740}/I_{685} and L^* , the leave-one-fruit-out validation gave $Q^2 = 0.299$ and $RMSECV = 1.909$ N. This performance was lower than that obtained by position-level LOOCV, indicating that the robustness of the model is partly limited by between-fruit variability and the small number of independent fruit samples. Therefore, further validation using a larger number of independent fruit samples is required.

The improvement obtained by combining I_{740}/I_{685} with L^* or C^* suggests that these optical parameters may reflect partially different aspects of ripening. The fluorescence ratio I_{740}/I_{685} is associated with chlorophyll-related fluorescence and skin optical properties, whereas L^* and C^* reflect visible color changes in the skin. Therefore, combining these parameters may provide more information on ripening-related changes than using either fluorescence or chromaticity alone.

Several factors may have contributed to the moderate correlation observed in this study. The samples were commercially available fruits, and detailed information on production region, harvest maturity, transportation, and storage history before purchase was not available. Previous studies have reported that fatty acid, oil, and dry matter contents can vary among Hass avocados depending on production region and ripening conditions [18]. In addition, mineral composition can vary among trees of the same cultivar grown under similar conditions [19]. Such variability may affect the relationship between firmness and skin optical properties, including pigment composition, light absorption, scattering, and reabsorption. Therefore, further studies using larger sample sets with controlled information on origin and postharvest history are needed to improve the robustness of the proposed screening approach.

4.3. Changes in Skin Chromaticity

As described in Section 4.2, the optical parameters I_{740}/I_{685} , L^* , and C^* showed moderate relationships with wfc. The R^2 values for the relationships of L^* and C^* with wfc were 0.37 and 0.33, respectively, which were comparable to the R^2 value of 0.38 obtained for the relationship between I_{740}/I_{685} and wfc (Table 2).

In the present spectra, the main fluorescence bands detected with sufficient signal-to-noise ratio were observed around 685 and 740 nm. These bands are generally assigned to chlorophyll-related fluorescence. Under the present blue LED excitation and detection conditions, no additional distinct fluorescence peaks attributable to non-chlorophyll fluorescent compounds were clearly detected. Therefore, the directly detected fluorescence signal in this study was considered to be dominated by chlorophyll-related fluorescence.

However, the fluorescence intensity ratio I_{740}/I_{685} should not be interpreted as a direct measure of chlorophyll content alone. During the ripening of the Hass avocado,

the skin color changes from green to purple/black. Previous studies have shown that this color change is associated with a decrease in chlorophyll and an increase in anthocyanins, particularly cyanidin 3-O-glucoside [14,20]. Although independent anthocyanin-derived fluorescence peaks were not detected in the present spectra, anthocyanins and other skin pigments may still influence the detected chlorophyll-related fluorescence indirectly through light absorption, screening, reabsorption, and changes in the optical path within the skin tissue [21–23]. Therefore, changes in pigment composition may affect both the absolute fluorescence intensities and the I_{740}/I_{685} ratio without appearing as independent fluorescence peaks.

In addition to pigment composition, changes in skin structure may also influence the optical response. Previous morpho-structural studies of avocado pericarp have reported differences between unripe and ripe tissues in exocarp, epidermal, sclerenchymal, and pigment-distribution features [24]. Although such observations were not obtained under the same cultivar and measurement conditions as those used in the present study, they suggest that ripening-related changes in pericarp structure and pigment distribution can occur. These structural changes may indirectly affect the detected fluorescence by modifying light scattering, penetration depth, and reabsorption within the skin.

Thus, I_{740}/I_{685} should be regarded as a practical spectral index mainly reflecting chlorophyll-related fluorescence, while also being affected by indirect optical effects associated with skin pigment composition and tissue structure. Further studies combining fluorescence spectroscopy with direct measurements of skin pigment composition and microscopic observation of skin structure are needed to clarify the relative contributions of these factors.

5. Conclusions

To develop a simple and non-destructive approach for screening avocado ripeness, we constructed a compact optical measurement system consisting of a blue LED with a central wavelength of 450 nm and a small spectrometer. Using commercially available Hass avocados purchased from retail stores in Japan, we evaluated the relationship between whole-fruit compression (wfc), used as a firmness-based ripeness index, and optical parameters obtained from intact skin, including the fluorescence intensity ratio I_{740}/I_{685} and CIE chromaticity parameters.

The fluorescence ratio I_{740}/I_{685} showed a moderate relationship with wfc, and similar levels of correlation were observed for L^* and C^* . Multiple regression analysis using I_{740}/I_{685} and L^* improved the relationship with wfc to $R^2 = 0.475$, while position-level LOOCV gave $Q^2 = 0.380$ and $RMSECV = 1.795$ N. A stricter leave-one-fruit-out validation further reduced the performance to $Q^2 = 0.299$ and $RMSECV = 1.909$ N, supporting the preliminary nature of the model. These results suggest that the combination of simple fluorescence and chromaticity measurements may be useful as a practical screening approach for roughly estimating avocado ripeness. However, the model should be regarded as a preliminary proof-of-concept and not as a validated prediction model. I_{740}/I_{685} should be interpreted as a fluorescence spectral index affected by chlorophyll-related fluorescence, skin optical properties, pigment composition, and tissue structure, rather than as a direct measure of chlorophyll content.

Because the present study used a limited number of commercially available fruits with different and partly unknown production, transportation, and storage histories, the obtained correlation and cross-validated performance remained moderate. In addition, wfc was used as the sole firmness-based ripeness index, and the position-wise observations from the same fruit were not fully independent. Further studies using larger sample sets with controlled information on origin, harvest maturity, storage temperature, ripening

stage, and independent reference parameters such as flesh firmness, dry matter content, and oil content are required to improve the robustness and general applicability of the proposed approach.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijpb17070051/s1>, Table S1: Detailed regression coefficients, t values, and p values for regression models of whole-fruit compression using fluorescence and chromaticity parameters; Table S2: Signal-to-noise ratio analysis of chlorophyll-related fluorescence signals at 685 and 740 nm.

Author Contributions: Conceptualization, I.T. and M.S.; methodology, I.T., F.T., and Y.B.; investigation, I.T.; formal analysis, I.T.; project administration, M.S.; equipment design and construction, F.T. and Y.B.; writing—original draft preparation, I.T.; writing—review and editing, H.I.; supervision, H.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available in the article and Supplementary Materials.

Conflicts of Interest: Author Makoto Saito was employed by the company DMR Corporation. Author Fujio Terai was employed by the company TRIF Limited Liability Company. Author Yoshiro Baba was employed by the company TWINKLE TEC. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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