Development of a Non-destructive Starch Concentration Measurement Technique in Saffron (Crocus sativus L.) Corms Using Light Scattering Image Analysis

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(Received January 29, 2020; Accepted April 11, 2020)

This research aimed to develop regression models that estimate starch concentration in saffron (Crocus sativus L.) corms from hyperspectral light scattering images. Light scattering images were captured from corms at wavelengths from 650 nm to 1,000 nm in 5 nm intervals. Light decay curves were measured, and the integrated value of the curve at each wavelength (S) was calculated by image processing algorithms. The starch concentration in each captured corm was also measured using the phenol sulfuric acid colorimetric method for validation. A principal component regression method was applied to develop regression models in order to estimate the starch concentration from the S spectra. The results indicated that the estimation accuracy was high, and this model had practical use based on the ratio of performance to deviation (RPD) criterion (RPD = 0.913, standard error of calibration (SEP) = 0.932, standard error of validation (SVP) = 1.54 w.b., RPD = 2.83). The S was found to be negatively correlated with starch concentration since light scattering increased considerably as starch concentration increased.

Keywords : chemometrics, decay curve, hyperspectral imaging, plant factory, saffron corn

INTRODUCTION

In recent years, the demand for acquiring information from agricultural products as part of quality inspection has greatly increased. In Japan, it is necessary to simultaneously realize high quality and produce high-value-added crops to secure a competitive advantage over foreign products (Cabinet Office, 2019). A plant factory is a food production system that enables the year-round production of crop plants under fully controlled environmental conditions (Takatsuji, 1997). While plant factories have contributed to a continuous supply of high-quality products, they have issues with profitability, which may arise from a lack of added-value. To develop value-added products for plant factories, this study focused on medicinal plants with high market value. Since medicinal plants usually require a long cultivation period and a large amount of labor, farmers have limited interest in cultivating these crops (Furumatsu and Inui, 2013). Furthermore, the quality of medicinal plants is strongly influenced by cultivation methods and the growing environment. Therefore, plant factories are well suited to the cultivation of medicinal plants due to their ability to produce high quality, value-added plants.

Saffron is a bulbous, medicinal plant in the genus Crocus of the family Iridaceae, and the stigma is harvested to produce one of the world’s most expensive spices (Shoyama, 2009). Moreover, it contains crocin, which has medicinal properties. To increase stigma production and enhance crocin content within a limited cultivation area, selecting corms that produce an abundance of flowers is necessary.

At present, corm weight is used as an indicator in selection due to previous reports that corm weight of > 20 g is highly correlated to desirable bloom capability (Pharmaceutical Affairs Bureau, 1995). However, corm weight is not a precise indicator since the number of flowers produced varies greatly, even when the weight of corms is the same. Therefore, the development of more accurate and precise selectable traits and techniques is necessary. Corms store a high concentration of carbohydrates needed for flowering (Ohyama et al., 1986). β-carotene, which is the initial material in the biosynthetic pathway of crocin, is composed of glucose (Bolhassani et al., 2014). Because starch is one of the storage forms of glucose, crocin content may be affected by starch content. Given these facts, this study also aimed to test the hypothesis that corm selection based on starch concentration prior to planting would increase the number of flowers harvested as well as the concentration of crocin within the stigma compared to selection by weight alone.

Starch granules in plant storage tissues, such as corms, are accumulated within amyloplasts in cells (Chino, 1991), which affects the physical cell structure within a corm. Detecting this accumulation is difficult using near-infrared spectroscopy to measure physical properties, but...
which has been commonly used as a non-destructive measurement of chemical ingredients (McGlone and Kawano, 1998; Asakuni et al., 2013).

Alternatively, light scattering image measurement is a method that utilizes physical phenomena depending on intercellular and extracellular structures (Qing et al., 2007). Light scattering image analysis has already been used successfully to non-destructively measure estimated soluble solids content and firmness in apple (Malus domestica L.) and peach (Prunus persica L.) ripeness (Tu et al., 2000); monitor tomato (Solanum lycopersicum L.) ripeness (Muhua et al., 2007; Qin et al., 2009); and monitor the change in moisture content of drying banana (Musa acuminata Colla) slices (Romano et al., 2008). In saffron, the accumulation of starch granules in amyloplasts would also likely change cell structure and induce variation in the light scattering properties within a corm, making corm starch content a suitable candidate for measurement by light scattering image analysis. Therefore, the objective of this research was to construct a regression model for estimating starch concentrations from the light scattering properties of saffron corms.

MATERIALS AND METHODS

Sample materials

Sample saffron (C. sativus) corms were grown at both Kobe University and in Takeda City, Oita Prefecture, Japan and harvested in 2018. The 78 saffron corms harvested in Takeda and 16 corms grown in the laboratory were examined. A total of 94 saffron corms were used in this research. The harvest date of the corms used in the experiment, the date of the experiment, and the number of the corms used in the experiment were shown in Table 1. To promote flower bud formation, all corms were placed in an incubator (MIR-154; Panasonic, Osaka, Japan) at 25°C under dark condition. The incubator was placed in a room, which had an air conditioner and a set value of air temperature was kept constant during this experiment. Experimental analysis was performed on randomly selected corms for 9 days. Light scattering images were captured during the flower bud formation period.

Light scattering imaging system

A light scattering imaging system (Fig. 1) was developed for this research, which mainly consisted of a light source unit (TII-EQ99FC-TUNABLE-SPOT-2; Tokyo Instruments, Inc., Tokyo, Japan) and a complementary metal oxide semiconductor (CMOS) camera unit. The light source unit included a high-intensity minute point light source, a dedicated monochromator unit, and a dedicated irradiation lens unit. The light source was a white laser spread over the wavelength range of 170-2,100 nm. The CMOS camera unit was composed of a CMOS camera (MF1-D1312IE-40-CL-12; Photonfocus AG., Switzerland) and camera lens (VIS-NIR lens Xenoplan 1/4/23; SCHNEIDER Inc., Germany). The effective wavelength range in the light scattering imaging system was set between 650 and 1,000 nm. A white laser beam was dispersed into specific wavelength light with a wavelength resolution of 5 nm by a monochromator. Focusing the light on the surface of the corm by the lens unit, the dispersed light was irradiated into a focal spot that was less than 1 mm in diameter at the equator of a corm that was placed on an X-Y-Z axis stage (X-Y-Z axis leadscrew drive met-
nic stages; Edmund Optics Inc., USA). The irradiated light was captured by the CMOS camera as a light scattering image and stored in a computer (CTO biz0H, Intel Core-i7-4771; 3.50 GHz; 4GB RAM, Win7Pro SP1, 32 bit; 1TB hard drive; UNITCOM, Japan). The same processing was repeated at each wavelength. All processes, such as the alternation of wavelength from 650 to 1,000 nm in 5 nm intervals and acquisition and saving of each image at each wavelength, were automated using LabVIEW (LabVIEW2012; National Instruments Inc., USA).

Image acquisition

The detection sensitivity of the CMOS camera and output of the light source varied. The imprecise information resulting from the device itself is referred to as a device function (Hasegawa, 2005). To extract only the accurate light scattering information, it was necessary to separately measure and subsequently remove the device function. To compensate for the imprecision, light scattering images of a standard reflector (Zenith Polymer® Dif- fuse Reflectance Standard ~2.5 %R; SphereOptics GmbH Inc., USA) and background images, which were captured in the dark, were acquired just before acquiring light scattering images of corms.

Once images for standardization were acquired, individual, peeled saffron corms were placed on the imaging stage. The top projected shape of corms is not a circle, but an ellipse. Using the shape as a guide, the longer axis of the corm was set at right angles to the optical axis direction of the CMOS camera. The height of the stage was adjusted so that the focal point of dispersed light was on the equator of the corm. The distance between the sample corm or the standard reflector and the CMOS camera was kept constant at 120 mm using the jig. Before acquiring a light scattering image of a corm, an appearance image of the corm was captured to grasp the shape of the corm and identify the maximum scattering region of the corm under LED lamp lighting (JANSJO/wall clip type spotlight; IKEA, Japan) and table lamp stand lighting (ODS-27; OHM ELECTRIC Inc., Tokyo, Japan). To minimize interference from ambient radiation, the light scattering image system was operated in a dark room (B-58; SCIENTEX Inc., Shizuoka, Japan).

Seventy-one light scattering images were acquired at wavelengths from 650 to 1,000 nm in 5 nm intervals. The average intensity value of each pixel over the background images was calculated (Fig. 2A). The intensity value of each pixel in the average background image was subtracted from that of the corresponding pixel in the original standard reflector image (Fig. 2B). Then, a differential image (Fig. 2C) was obtained. The spot region of the dispersed light in the differential image was specified by binarization and labeling processing. After the labeling processing, many labeled regions were detected because there were salt and pepper noises. The labeled region that showed the largest area was recognized as the light spot region (Fig. 2D). The binarization process was applied to the difference image only in the extracted rectangle by using the discriminant analysis binarization method (Mori and Sakakura, 1993). The binarized white region showed the light spot region (Fig. 2F). The average intensity value, \( I_\lambda \), was calculated over the pixels inside the binarized white region in the difference image. The binarized white region was defined as the region of interest (ROI). The intensity compensation of pixels in the light scattering images of corms was calculated using the following equation:

\[
I_\lambda(x, y) = \frac{I_\lambda(x, y) - D(x, y)}{k_\lambda} + k_0
\]

where \( I_\lambda \) and \( I_\lambda \) are intensity of pixel at coordinate \((x, y)\) in the light scattering images of corms before and after the compensation at wavelength \( \lambda \), \( D(x, y) \) is intensity of pixel at coordinate \((x, y)\) in the average background image, and \( k_\lambda \) and \( k_0 \) are the reference value for overall measurement day and each measurement day, which were the mean

### Table 2: Exposure time setting of CMOS camera

| Region of wavelength (nm) | Standard \(^a\) (ms) | Corm \(^a\) (ms) |
|---------------------------|---------------------|-----------------|
| 820–835, 873–900          | 1.40                | 5.00            |
| 650–815, 840–870, 905–930 | 2.24                | 8.00            |
| 935–1,000                 | 14.0                | 50.0            |

\(^a\) Standard: Exposure time to take standard reflector

\(^a\) Corm: Exposure time to capture light scattering images of corms

### Table 3: Image acquisition conditions

| Factors | Set value |
|---------|-----------|
| Distance from lens to sample (mm) | 120.0 |
| Incident angle (°) | 17.5 |
| Wavelength resolution (nm) | 5.0 |
| Image size (pixel) | 1024×1024 |
| Aperture value (°) | 2.8 |
| Image resolution (mm pixel\(^{-1}\)) | 0.047 |

\(^a\) The angle between the optical axis of incident light and the CMOS camera

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### Notes

- \( \lambda \): Wavelength

### References

- Hasegawa, 2005
- Mori and Sakakura, 1993
Intensity in the ROI of the standard reflector image at the wavelength \( \lambda \). The \( k_d \) obtained on the first measurement day was used as the reference value \( k_c \) for overall measurement days. Subsequently, light scattering images of corms were analyzed using the corrected intensity value.

Light decay curves were measured from the compensated light scattering images. A schematic diagram of analyzing procedure for the light decay curve is shown in Fig. 3. First, the center of gravity of the light scattering region was calculated. Light decay curves, which describe the relationship between the light intensity and distance from the incident point, were measured. The mean intensity over pixels on a circle of radius \( r' \) around the center of gravity was scanned. The increment of \( r' \) was 1 pixel. Accordingly, a light decay curve with a measurement interval of 1 pixel was obtained. However, the vicinity of the center of gravity was too close to the light incident point causing saturated pixels, which made it impossible to accurately measure intensity value. The minimum radius \( r'_{\text{min}} \), where no saturated pixel exists on the radius \( r' \), was measured for each wavelength in a corm. \( r'_{\text{max}} \), the maximum value of \( r'_{\text{min}} \) among all the examined corms, was redefined as the starting point of the light decay curve. Also, \( I_{\text{back}} \), the average intensity value of pixels outside of the corm region, was calculated. The scan of the mean intensity of pixels on a circle was terminated when the mean intensity went below \( (I_{\text{back}}/5.0) \). Because \( I_{\text{back}} \) was very small (1.0 or less), \( (I_{\text{back}}/5.0) \) was defined as the end point of the light decay curve to prevent scanning pixels outside of the corm region. Also, since the mean intensity value at the starting point of the light decay curve was more than 100, it was considered that the adding value 5.0 was relatively small and there was little effect of the adding operation on the following calculation. In this study, the light decay curves

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**Fig. 2** Procedures for the correction of definition and intensity of images. (A) Average background image, (B) original image of a standard reflector, (C) differential image of standard reflector, (D) labeling image, (E) extracted rectangle circumscribed to the light spot region, and (F) binarized image of the ROI.

**Fig. 3** Measurement method of the decay curve. (A) Relation between radius \( r' \) and intensity value \( I \) and (B) relation between radius \( r \) and intensity value \( I \).
Measurement of starch concentration

After acquiring the light scattering images, the starch concentration was measured destructively for model prediction validation. Each corm was sliced with a slicer (plastic slicer DH-2270; Kaishin Co., Tokyo, Japan), dried at 60°C for 24 hours, and ground using a mixer (TML161; TESCOM, Tokyo, Japan). Thereafter, the particle size was unified to 212 μm or less using a sieve (stainless steel sieve, φ75 × 20, 212 μm; ASONE, Japan). To completely remove the moisture, the ground samples were dried further at 60°C for an additional 24 hours.

The starch concentration was measured using the Suzuki method with modification (Suzuki et al., 2013). First, soluble sugar was removed from the dried sample using 80% (v/v) ethanol (Nacalai Tesque, Inc., Kyoto, Japan). Then starch was extracted from the residue using perchloric acid (Nacalai Tesque, Inc., Kyoto, Japan). Second, 100 μL of 5% phenol solution was added to 100 μL of starch extract solution diluted 50 times with distilled water. Thereafter, 500 μL of 95% (w/w) concentrated sulfuric acid was added and vortexed. After incubation for 30 minutes in an incubator (Ct-310; ADVANTECH Co., Ltd., Tokyo, Japan) set at 30°C, the absorbance at the wavelength of 490 nm was measured with an ultraviolet-visible spectrometer (V-530; JASCO Corporation, Tokyo, Japan). Soluble starch powder (Nacalai Tesque, Inc., Kyoto, Japan) was used as a standard solution to make a calibration curve. The starch concentration was calculated from the prepared calibration curve. Also, to improve the accuracy of the phenol sulfuric acid method, the absorbance was measured 5 times for each sample. Then, excluding outliers by Grubbs’ outlier test (Miller and Miller, 2011), the average value of absorbance was measured. Selection of the significant wavelength region was analyzed by the Martens uncertainty test (Martens and Martens, 2000).

Modeling and model evaluation

To establish a regression model to estimate the starch concentration of a saffron corm, the principal component regression (PCR) method was employed. The predictor variable was the S spectrum, and the predicted variable was the starch concentration. The first portion (calibration set) comprised approximately two-thirds of all samples, and the other portion (validation set) consisted of the remainder of samples to evaluate the performance of the developed models. Data sets were rearranged in ascending order according to the predicted variable, and the sorted data sets were partitioned into intervals of 3. In each interval, the first and second data were assigned to the calibration dataset, and the third data point was assigned to the validation dataset. To quantify the performance of regression models, the statistical parameters of the correlation coefficient (R), standard error of calibration set (SEC) and standard error of validation (SEP), a weighted average of SEC and SEP (WSE), were calculated using the following equations:

\[ S_{\lambda} = \int_{0}^{r_{u}} I(\lambda, r) \, dr = \int_{0}^{r_{u}} \frac{a_{\lambda}}{1 + (r/b_{\lambda})} \, dr + c_{\lambda} \]

\[ = a_{\lambda} h \left( \tan^{-1} \frac{r_{u}}{b_{\lambda}} - \tan^{-1} \frac{14}{b_{\lambda}} \right) + c_{\lambda} (r_{u} = 14) \]
RESULTS AND DISCUSSION

Starch concentration

The starch concentration measurements are shown in Table 4. Starch accounted for 10–27% of the fresh weight of a saffron corn. In the case of the nonlinear regression to approximate the light decay curve by Lorentzian function, the coefficient of determination was not appropriate as an index to evaluate the fitness of the Lorentzian function to the light decay curve (Spiess and Neumeyer, 2010). The RMSE of each light decay curve at wavelength \( \lambda \) was calculated as an indicator of fitness. The RMSE spectrum is shown in Fig. 5A. One outlier sample, which showed an extremely large RMSE and visually odd S spectrum, was excluded from the model construction. The RMSE values were low throughout all wavelengths, and the accuracy appeared sufficient for the regression of light decay curves.

Exposure time was set according to the wavelength regions in response to camera sensitivities. No unstable fluctuation in the measured decay curves around the starting point \( r'_{\lambda 0} \) occurred since \( r'_{\lambda 0} \) was fixed in order to avoid the saturation of pixel intensity for all the samples. Based on these settings, Lorentzian function accurately approximated the shape of the light decay curve of the corm tissue. Therefore, \( S_x \) which was calculated by integrating the Lorentzian function, effectively approximated the feature amount of the light decay curve.

Correlation between \( S_x \) and starch concentration

The measured S spectra for 71 wavelengths are shown in Fig. 5B. The correlation between \( S_x \) and starch concentration is shown in Fig. 6. \( S_x \) and starch concentration were negatively correlated at all wavelengths, which indicates that \( S_x \) decreased as the slope of the light decay curve increased.

Table 4 Statistics of starch concentration data.

| Sample | Mean (% w.b.) | Min (% w.b.) | Max (% w.b.) | SD (% w.b.) |
|--------|---------------|--------------|-------------|-------------|
| 93     | 14.30         | 9.684        | 27.30       | 4.118       |

* Sample number

* Standard deviation

Fig. 5 These figures show the spectra of the samples analyzed (excluding one sample that showed extremely large RMSE). (A) The RMSE spectra when regression analysis was performed using the Lorentzian function and (B) the measured \( S_x \) spectra as used for estimation of starch concentration in saffron corms (n = 93).
increased (Fig. 7), caused by an increase in starch concentration. In general, light attenuation is caused by scattering and absorption phenomena. Light absorption results from specific chemical components such as sugar, acid, and moisture. Conversely, scattering is known to result from physical structures, such as differing density and cell structure (Muhua et al., 2007). Starch accumulates as granules in the corms (Chino, 1991), and these are considered to be light scatterers. When the starch concentration is high, light scattering and absorption increase. Therefore, the slope of the light decay curve steepened and $S_l$ decreased notably at all wavelengths when corms had high starch concentrations.

**Constructing regression models to estimate starch concentration**

Table 5 shows the estimation accuracy of the best prediction model when PCR analysis was performed with the starch concentration as the predicted variable. A comparison between the measured and predicted starch concentrations is shown in Fig. 8. It has been reported that samples can be predicted with high accuracy when $RPD > 2.43$ or $RSD < 11.1$

![Fig. 6](image6.png)

*Fig. 6* The correlation coefficient between $S_l$ and measured starch concentration values at each wavelength.

![Fig. 7](image7.png)

*Fig. 7* The schematic diagram of the relationship between $S_l$ and the slope of the light decay curve: (A) $S_l$ decreased as the slope of the light decay curve increased. (B) $S_l$ increased as the slope of the light decay curve decreased.

![Fig. 8](image8.png)

*Fig. 8* Comparison of the measured and predicted starch concentrations obtained from the prediction model using light scattering images (A) calibration set, $n = 62$; (B) validation set, $n = 31$.

| Starch concentration | PCR | AM | NW | NL | SEC (‰ w.b.) | SEP (‰ w.b.) | RPD |
|----------------------|-----|----|----|----|-------------|-------------|-----|
| Predicted variable   | 30  | 12 | 0.913 | 1.330 | 0.932 | 1.544 | 2.807 |

$^*$ predicted variable

$^*$ analysis method

$^*$ number of wavelengths

$^*$ number of latent values

$^*$ coefficient of determination between measured and estimated calibration data

$^*$ correlation coefficient between measured and estimated validation data

Vol. 58, No. 4 (2020)
higher (Kovalenko et al., 2006). In this study, RPD showed 2.81, indicating that a highly accurate prediction model of starch concentration was constructed.

When a sample with high moisture is used as a prediction target, the incident light is greatly affected by the absorption of moisture. Given this phenomenon, a wavelength range of 700–1,100 nm is suitable for measuring trace components other than moisture with high sensitivity (Iwamoto, 1980). The measurement wavelength range set in the light scattering image measurement method was 650–1,000 nm, a wavelength range that was not readily affected by water absorption. Also, in this wavelength range, scattering rather than absorption strongly affects light attenuation characteristics (Tuchin, 2007). This wavelength range was selected understanding these different properties in order to best capture changes in starch concentration resulting from changes in physical structure.

In this study, the measured S spectra consisted of 71 wavelengths were used as the predictor variable. In order to suppress the decrease in estimation accuracy due to the multicollinearity between S, measured at each wavelength, the PCR method was employed to establish the regression model. Furthermore, the Martens uncertainty test was applied to eliminate unimportant wavelength to improve the prediction accuracy of the constructed model. Finally, the optimum prediction model was obtained (Table 5). Figure 9 shows the results of partial regression coefficients before and after the wavelength selection. The absorption wavelength of starch (990 nm) (Ikegaya et al., 1988) was not selected in the prediction model after wavelength selection. Therefore, the influence of the absorption on light attenuation was likely small in the wavelength region in the spectral range of 650–1,000 nm. It has been reported that the average particle size of starch granules in saffron corms is about 9.7 μm (Sugimoto et al., 1986). Mie scattering (Iwai et al., 1994) was applied to the scattering of light by spherical particles with a size larger than the wavelength of light. Mie scattering is only weakly dependent on wavelength. Based on these results, there was no consistent trend in the selected wavelengths.

CONCLUSIONS

The prediction model to estimate starch concentration was constructed using the light scattering image measurement method. Light decay curves were obtained from light scattering images at 71 wavelengths between 650 and 1,000 nm, which should minimize the impact of moisture on measurements. The feature amount S, of the light decay curve was measured by image processing. Based on the results and analyses, the regression accuracy of the decay curve was high, and S could be used to accurately represent the steepness of the light decay curve. Overall, the practical prediction model could be constructed (RSD = 0.913, SEC = 1.33% w.b., R = 0.932, SEP = 1.54% w.b., RPD = 2.81). Notably, S and starch concentration showed a negative correlation, indicating that the denser the starch granules stored in the corm, the more light was scattered, and the attenuation of light was abrupt in corms with high starch concentrations.

Saffron is a high-value crop with potential medicinal qualities. Since it can be grown hydroponically in plant factories, it is an ideal crop for dense, small area, urban agriculture as long as it can be produced consistently and efficiently. This research aimed to nondestructively measure and identify saffron corms most likely to produce high yields based on starch concentration. The predictive model and sampling method constructed achieved that goal. It also provides a tool that allows for the investigation of whether the selection of corms based on starch content contributes to the improvement of flowering ability and increase in stigma yield. In the future, this model and analysis may provide an efficient method for improving overall saffron selection and production.

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LIGHT SCATTERING MEASUREMENT

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