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Location of Sampling Points in Optical Reflectance Measurements of Chinese Cabbage and Kale Leaves

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Abstract

Purpose: A sampling scheme may significantly affect the accuracy of a sensor. This study was conducted to investigate the effects of sampling point locations on optical reflectance measurements of Chinese cabbage and kale plant leaves. **Methods:** Variability and similarity of multiple measurements for different parts of the leaves were compared. **Results:** The results indicate that the variability between the average and individual reflectance spectra was smaller for the blade part than for the vein part. Furthermore, the reflectance for the blade part over the upper leaf area was greater and more stable than those for the other parts for both the cabbage and kale leaf samples. **Conclusions:** The results provide guidelines for optical reflectance measurements of Chinese cabbage and kale plants. The effects of the number of sampling points, the number of leaves, and the relationships between optical reflectance and leaf components remain to be investigated in the future.

Keywords: Cross-correlation, Leafy vegetable, Optical reflectance, Reflectance difference, Sampling location

Introduction

Chinese cabbage (*Brassica rapa*, subspecies *pekinensis* and *chinensis*) and kale (*Brassica oleracea var. alboglabra Bailey*) are among the most important vegetables in many countries, including Korea. In Korea, the estimated production of cabbage in 2010 was 1.5 million tons (Kathryn and Smith, 2010). This vegetable is generally grown in early spring and fall in open fields, but has also recently been grown in greenhouses and plant factories, where the growth conditions can be controlled precisely. The yield, nutrition content, taste, disease resistance, and time to harvest depend on the variety of Chinese cabbage (Wu et al., 2008). Kale is a hardy, cool-season vegetable, similar in use and appearance to broccoli; however, it can be grown year-round in tropical regions because it tolerates summer heat (Noichinda et al., 2007). Epidemiological studies showed that there is a negative relationship between Brassicaceae vegetable intake and the risk of a number of cancers (La et al., 2009).

It is important to identify the growth and constitutional components of Chinese cabbage and kale. High-pressure liquid chromatography (HPLC) can yield accurate values of leaf components such as glucose, fructose, carotenoid, and glucosinolate. However, it is difficult to apply production line measurement (Suhandy et al., 2012), owing to labor-, cost-, and time-consuming procedures of plant sampling and subsequent destructive laboratory analyses. In-situ and rapid methods for evaluating the plants contents would be preferable for plant management in agriculture, horticulture, and forestry (Peňuelas and Inoue, 1999).

Visible and near-infrared (VIS-NIR) spectroscopy is a non-destructive and rapidly developing analysis technique with a promising potential applicability to agricultural and food industry. A sample can be analyzed in a fraction of a minute, by simultaneously collecting the data over multiple light frequencies that are transmitted to or reflected back from the sample (Luhimi et al., 2013). Extensive information on physical and chemical properties

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can be obtained based on the reflectance spectra at multiple wavelengths. The advantages of this approach are high efficiency, simplicity, low cost, good reproducibility, and fast and non-destructive measurement (Wu et al., 2008). Reflectance and absorption spectra of different plant species have been analyzed for determining spectral variability and related information. Leaf properties affecting the leaf optical properties are its internal and external structure, age, water status, mineral stress, and the leaf health. Three main factors affecting the leaf spectral features are the leaf pigments, cell structure, and water content (Zahran et al., 2009). Portable spectrometers have been used to measure the leaf reflectance (Andrew and Graeme, 2002; Lee and Kim, 2013), for replacement of conventional laboratory determination of the plant components.

Spectral measurements need to be accurate and precise representations of the target material, but various factors can affect the quality of spectral measurements. These factors include the optical propagation and environmental and experimental issues (Pfitzner et al., 2006). Factors affecting the accuracy of analysis using near-infrared reflectance have been discussed (Li et al., 2013). These factors include sampling issues (e.g., the number of sampling points, the locations of the sampling points, and the sampling time) and data processing methods (e.g., pre-processing for noise reduction and smoothing). Sampling location is one of the issues important for proper measurement of optical reflectance for obtaining highly accurate data and reducing the data collection time. Charles (1929) studied 23 species of wood, trees, and shrubs, and demonstrated the difference in reflectance between upper and lower surfaces of the leaves. He found that, in most cases, the lower surfaces reflected light stronger than the upper surfaces of the same leaves. Measurements of a chlorophyll meter were affected by the selection of a given leaf within the canopy and the measurement location on the leaf surface, for chlorophyll estimation at two transmittance wavelengths. To measure the wavelengths individually or simultaneously, optical meters were attached between the leaf mid-vein and edge parts, with the upper leaf pointing upward, as described by Davenport et al. (2005). Reflectance spectra were recorded for the upper surfaces of the leaves, inter-veinal areas, or middle parts of the uppermost fully expanded leaves (Wang et al., 2004; Follett et al., 1992; Gitelson et al., 1996; Liu et al., 2009). However, most sampling locations in spectral reflectance measurements described in the literature were selected based on the suggestions in previous works. Effects of sampling location need to be evaluated for obtaining high-quality spectral data of the crops under investigation.

Pre-processing of spectral data is often of vital importance if reasonable results are to be obtained, whether the subsequent analysis is concerned with missing exploratory data, data classification, or building a good and robust prediction model. Pre-processing techniques are designed to compensate for these deviations from linear relationships and to improve the linear relationship between the spectral signals and target material properties (Sun, 2009). Literature suggests that pre–processing of the collected reflectance data can affect the subsequent analysis. Suh et al. (2012) measured the reflectance in the 400~1,600 nm range, by using a miniature VIS/NIR spectrometer, at 6 points evenly distributed on melon surface. In establishing the calibration model for estimating the soluble solids content (SSC) and firmness, they compared the effects of 20 different pre–processing methods, including normalization, multiplicative scatter correction (MSC), standard normal variate (SNV), median filter, 1st and 2nd derivatives of Savitzky–Golay (S. Golay) and Norris gap. They reported that, for predicting the SSC, the performance of the models using the pre–processed data with range normalization and median filter with five smooth factors was better than that of the models using the original data. However, the pre–processing technique was not useful for predicting the firmness.

The present study is a part of the research effort to develop optical reflectance sensors for evaluation of internal components of Chinese cabbage and kale leaves. The objective of this study was to investigate the effects of the sampling point locations on measurements of Chinese cabbage and kale leaves.

Materials and Methods

Crop Growth and Sampling

Chinese cabbage and kale were cultivated at a plant factory with similar conditions of lighting, CO_2, temperature, humidity, pH, and electrical conductivity (EC), which were selected based on horticultural growth considerations. After transplanting the seedlings, a photoperiod of 16/8 (day/night) h was applied. The photosynthetic photon flux density (PPFD) was 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The air temperature and humidity were 20 ± 1°C and 65 ± 5%, respectively. The pH and electrical conductivity (EC) of the nutrients solution were 6.0 ± 0.5 and 1.2 ± 0.09 mS·cm^{-1}, respectively. A total of 6 plants (3 cabbage and 3 kale plants) were collected 4 weeks after transplanting the seedlings. For each plant, three healthy and normal-sized leaves were selected for spectral reflectance measurements.

Spectral measurement

Reflectance spectra of the sample leaves were obtained for wavelengths ranging from 190 to 1,130 nm by using a portable spectrometer (model: USB2000, Ocean Optics, FL, USA) in darkness. The spectrometer had two CCD detectors, and the wavelength ranges were 190 to 890 nm (UV/VIS) and 470 to 1,130 nm (NIR). Reflectance data from the two detectors were combined, centering at 720 nm. A deuterium–tungsten halogen (200 to 1,000 nm) lamp was used as the light source.

The collected leaf spectra for each sample consisted of 2,048 reflectance values for each detector, with the resolution of about 0.38 nm. For each leaf measurement, the scans were repeated 30 times (15 each for the blade and vein areas), evenly distributed over the entire leaf area (Figure 1), and the averaged spectrum for each part (upper, lower, blade, and vein parts) was recorded.

The measurement area was approximately 4.91 mm^2. However, the vein area was not sufficiently large for measuring the reflectance in it. Therefore, in measuring the vein part reflectance, the actual measurement area consisted partially of blade and vein parts. We tried to focus the measurement areas to cover the vein areas as much as possible (Figure 2). For the reflectance measurements, the leaves were not removed from the plants.

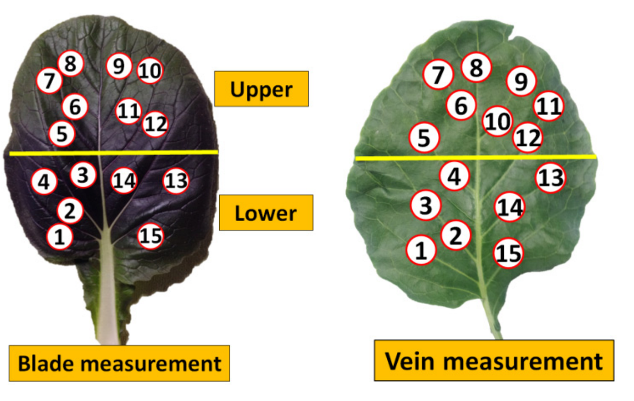

Figure 1. Locations of reflectance measurement points, for the cabbage (left) and kale (right) leaves.

Data analysis

Raw spectral reflectance data for the leaf samples were collected for wavelengths in the 190 to 1,130 nm range. Reflectance data from the two detectors were combined around 720 nm. Preprocessing was used to reduce the noise of the spectral data, while retaining the major variability. Baseline correction methods were not used because the main goal of these methods is to reduce the non-desired slow variations. Smoothing and derivative methods were used for the noise reduction and for retaining useful variability. Therefore, five methods were used to reduce the spectral noise: the median filter, the first and second derivatives introduced by S. Golay, and combinations of those that were suggested by Suh et al. (2012) and Rinnan et al. (2009). Only the wavelengths in the 250 to 1,100 nm range were used for analysis, and other data were removed due to the excessive noise at the detection range boundaries.

Reflectance difference (RD) and cross-correlation (CC) were used as the criteria for evaluating the measurements stability (or similarity). RD is a simple method for estimating the difference between two reflectance spectra (r_1 and r_2), which is computed by subtracting r_1 from r_2, as in Equation (1) (Hu et al., 2010). The RD method was initially developed as a reliable tool for monitoring the growth of cubic semiconductors: in this case, the response of the optically isotropic bulk cancels out, leaving only the contributions of the surface and interfaces with cubic symmetry. Since then, the RD technique has been successfully applied in many research fields, e.g., in the studies of semiconductor, metal, and organic surfaces and thin films. As a result, there is a strong demand to extend the capabilities of conventional RD spectrometers toward fast, multi-wavelength detection.

The basic problem is to describe and model the relationship between the two reflectance spectra. The CC method is helpful for identifying the x-variable lags that

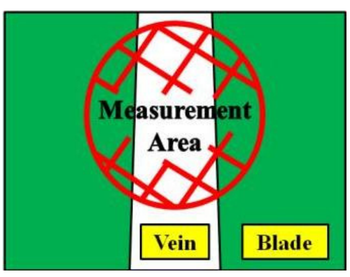

Figure 2. The measurement area of the reflectance measurement in the vein part.

might be useful for predicting the reflectance spectrum r_2. The coefficient, c, measures the size and direction of the linear relationship between the x and y variables in the two reflectance spectra r_1 and r_2. If these variables co-vary, increasing at the same rate, one obtains c = +1. If one of the variables does not vary, we obtain c = 0. If one of the variables exhibits the opposite trend at the same absolute rate, one obtains c = -1. If c is positive, the two variables are positively correlated. If c is negative, the two variables are negatively correlated (Lyon, 2010).

After the RD and CC methods were applied, their standard deviations were calculated. The standard deviation is the most common measure of variability, measuring the data spread and the relationship of the data mean to the rest of the data. The standard deviation is small when the data points are close to the mean, indicating that the responses are fairly similar. Conversely, the standard deviation is large when many data points are far from the mean, indicating a wide data spread. If all the data are equal, the standard deviation is zero. All data analysis, including the pre-processing of the raw reflectance data, the RD, the CC, and the standard deviation calculations, was conducted using the Matlab software package (v. 7.10, The Math Works, USA).

Results and Discussion

Leaf reflectance

Example reflectance spectra for one cabbage and one kale leaf, for wavelengths ranging from 250 to 1,100 nm, for the 15 blade parts and 15 vein parts, are shown in Figure 3. In general, the spectra of the cabbage and kale leaves differed by less than 10%. The maximal reflectance was observed at about 760 nm and was independent of the pigment concentration and the leaf developmental stage. The minimal reflectance was observed in the blue region of the spectrum (from 480 to 520 nm), which was strongly absorbed by chlorophyll and carotenoids. The reflectance of the cabbage leaf was slightly lower than that of the kale leaf in the 250 to 700 nm range. The reflectance of the cabbage leaf in the supra 700 nm region, however, was greater than that of the kale leaf. In the visible region, the highest reflectance for the blade part of the cabbage leaf was obtained around 550 nm, while for the vein part of the same leaf the highest reflectance was around 650 nm. For the blade and vein parts of the kale leaf, the lowest reflectance was observed near 670 nm, followed by the sharp increases of reflectance for longer wavelengths.

Near–infrared and mid–infrared characteristics are affected mainly by the leaf structure and water content, respectively. Water is a good absorber of near and middle infrared energy. As the moisture content of leaves decreased, their reflectance increased throughout the near and middle infrared (400 to 2500 nm) regions (Jensen, 2000). Water content of the kale leaves is likely to be greater than that of the cabbage leaves, although it appeared that the photosynthesis content of the kale leaves was greater than that of the cabbage leaves in sub 700-nm region. Veins carry water to the leaf cells. Furthermore, veins are used for nutrients and water transport throughout the leaf, for maintaining the leaf moisture content as well as for structural support. Therefore, the blade part reflectance would provide more stable reflectance values for estimating

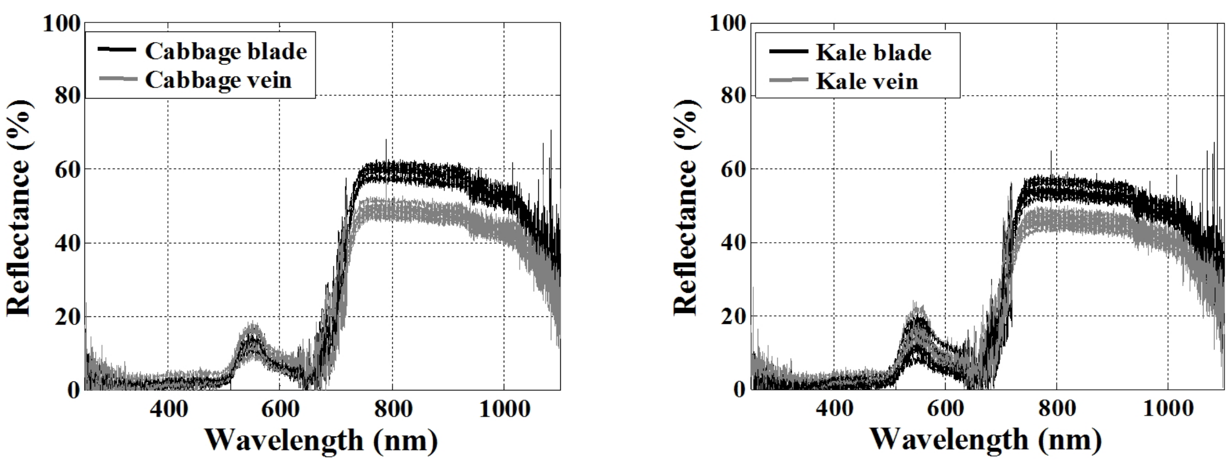

Figure 3. Reflectance of the cabbage (left) and kale (right) leaves.

the leaf component contents.

Figure 4 shows the reflectance of the cabbage and kale leaves (only the blade areas) in the lower and upper parts. The variability of the reflectance in the upper parts was smaller than that in the lower parts of the leaves. For the kale leaves, the difference between the upper and lower parts was not obvious, but it was somewhat larger for the cabbage leaves. The reflectance in the lower parts was slightly smaller than that in the upper parts of the cabbage leaves, whereas the reflectance values in the lower and upper parts were not considerably different for the kale leaves.

Combination processing using the median filter smoothing and S. Golay's derivative methods was also conducted

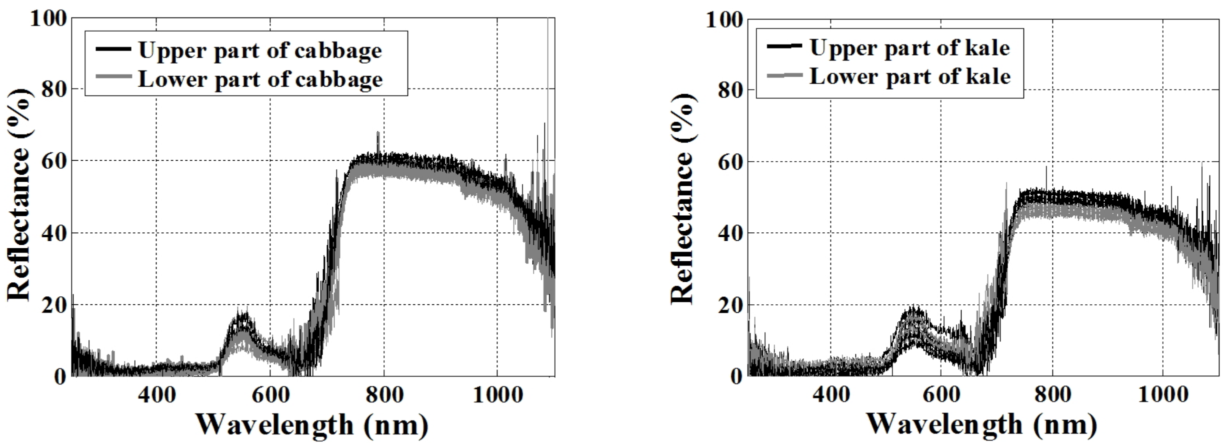

Figure 4. Reflectance of the cabbage and kale leaves (only the blade areas) in the lower and upper parts.

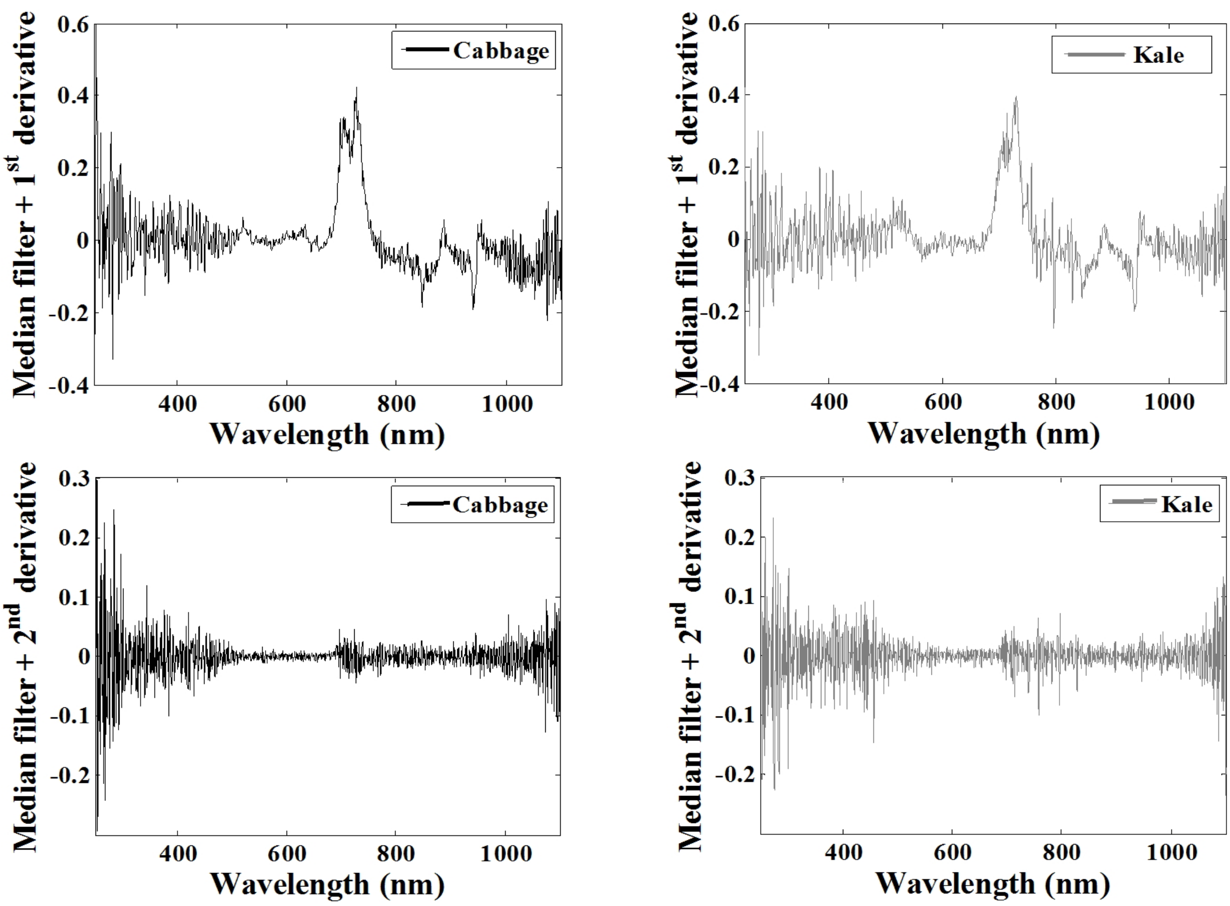

Figure 5. Results of the combination pre-processing of the median filter and S. Golay's pre-processing methods.

and the results are shown in Figure 5. The results of the combination pre-processing were less noisy compared with the results obtained after single processing using S. Golay's 1st or 2nd derivatives, or compared with the results obtained using the median filter smoothing method. The discrepancy around 720 nm was caused by combining the spectra from the two detectors. The discrepancy was clearer after the reflectance spectra were pre–processed using S. Golay's 1st derivative, but the difference between the cabbage and kale leaf spectra was not obvious after using the combination pre-processing, especially when S. Golay's 2nd derivative method was included.

Reflectance Difference

To determine the optimal sampling or measurement location, the reflectance spectra for the blade and vein parts were compared using the reflectance difference method. The RD standard deviation after pre–processing was calculated for evaluating the spectral variability. Figure 6 shows the RD for the raw data of the kale and cabbage leaves. In general, the RD values for the vein part were below the averaged spectrum whereas those for the blade part were above the averaged spectrum, for both the cabbage and kale leaves. The standard deviations for the cabbage and kale leaves were 0.58 and 0.67 for the vein parts, and 0.7 and 0.89 for the blade parts, respectively. When only S. Golay's 2nd derivative method was used for pre-processing the spectra, the standard deviations were 0.048 and 0.035 for the vein parts, and 0.047 and 0.034 for the blade parts of the cabbage and kale leaves, respectively. The RD values after the combined pre-processing were smaller for both the blade and vein areas than those after the pre-processing with S. Golay's 2nd derivative

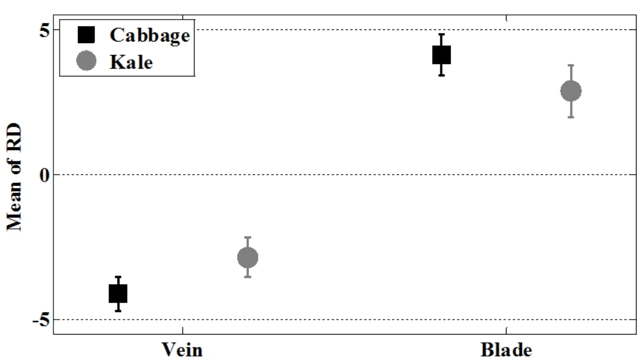

Figure 6. The mean reflectance difference, for the blade and vein parts of the cabbage and kale leaves.

Table 1. The mean reflectance difference between the blade and vein parts.

Species	Location	Mean of RD	$LSD_{0.01}$	CV%
Kale	Vein	2.86 ± 0.67^a	0.80	27.62
	Blade	2.86 ± 0.89^a		
Cabbage	Vein	4.12 ± 0.58^a	0.65	15.67
	Blade	4.12 ± 0.70^a		

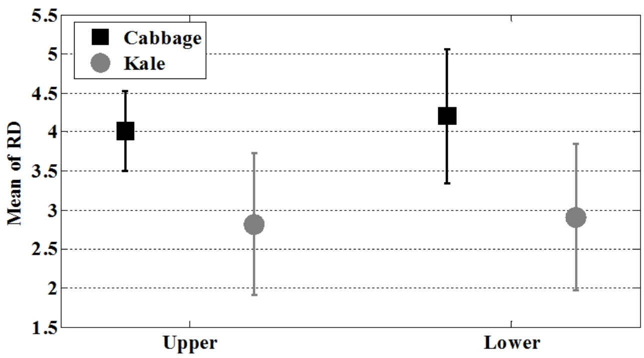

Figure 7. The mean reflectance difference, for the upper and lower parts of the leaves (blade areas).

method.

Table 1 shows the results of the least significant difference (LSD) test with the raw data, for the different locations. The p-value was equal to or smaller than 0.01 ($p \leq 0.01$). The LSDs for the cabbage and kale leaves were 0.65 and 0.80, respectively. The coefficients of variation were 15.67% and 27.62% for the cabbage and kale leaves, respectively. Based on these results, we concluded that there was no significant difference between the blade and vein parts.

Figure 7 shows the standard deviations for the upper and lower parts of the leaves (blade areas only), calculated from the raw data. The standard deviation for the lower parts of the cabbage leafs was greater than that for the upper parts, whereas it was smaller than that for the upper parts of the kale leafs, for most of the pre-processing methods. The standard deviations for the upper parts of the cabbage leaves were 0.063, 0.044, 0.059, and 0.021 for S. Golay's 1st derivative and 2nd derivative methods, and for the combination of media filter and S. Golay's 1st derivative, and for the combination of median filter and S. Golay's 2nd derivative, respectively. Using these methods, the standard deviations for the lower parts were slightly greater than those for the upper parts, taking the values of 0.069, 0.051, 0.060, and 0.022. For the kale leaves, the standard deviations were less than those for the cabbage

Table 2. The mean reflectance difference, for the upper and lower parts of the blade area.

Species	Location	Mean of RD	$LSD_{0.01}$	CV%
Kale	Upper vein	3.00 ± 0.64^{a}	1.07	23.87
	Lower vein	2.75 ± 0.72^{a}		
	Upper blade	2.82 ± 0.91^{a}	1.45	32.38
	Lower blade	2.91 ± 0.94^{a}		
Cabbage	Upper vein	4.02 ± 0.51^{a}	1.12	17.51
	Lower vein	4.20 ± 0.86^{a}		
	Upper blade	4.08 ± 0.71^{a}	0.94	14.68
	Lower blade	4.15 ± 0.5^{a}		

leaves (0.048, 0.034, 0.038, and 0.012 for the upper parts and 0.049, 0.037, 0.039, and 0.013 for the lower parts).

Table 2 shows that there is also no significant difference between the upper and lower parts, for both the cabbage and kale leaves. Therefore, the reflectance spectra measurements for the upper and lower parts were not different.

Cross-correlation

Similarity between the averaged spectrum and individual spectra was investigated by using the cross-correlation method. Figure 8 (top) shows the CC between the averaged spectrum and one individual spectrum, for the blade and vein parts of the cabbage leaves. The correlation coefficient, c, was greater for the reflectance spectrum from the blade part than from the vein part. Comparing between the upper and lower areas, the value of c was slightly higher for the reflectance spectrum from the upper area than from the lower area, as shown in Figure 8 (bottom). The standard deviation of CC was calculated to demonstrate the variability of the reflectance spectra from the different parts of the leaves. The CC standard deviations were similar for the raw data and for the data processed with median filter smoothing, but S. Golay's 1st and 2nd derivative methods and combination pre-processing resulted in the relatively lower values for the blade parts, for both the cabbage and kale leaves. For comparison between the blade and vein parts, the CC results for the combination of median filter and S. Golay's 1st derivative method are summarized in Table 3.

As can be deduced from Table 3, the reflectance spectrum measurements in the blade and vein areas were significantly different. In addition, the mean CC between the blade part and the averaged spectrum was greater than that between the vein part and the averaged spectrum.

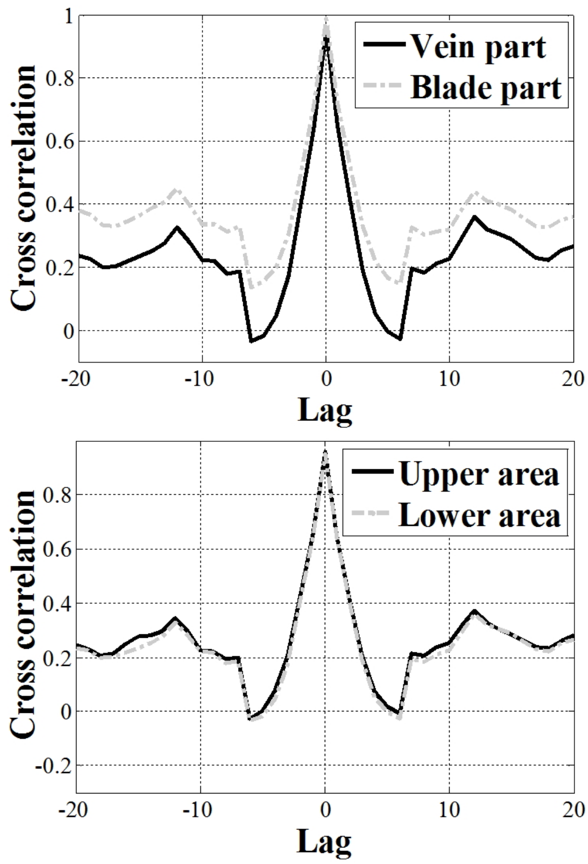

Figure 8. Cross-correlation between the averaged spectrum and an individual spectrum, for the blade and vein parts of the cabbage leaves (top), and for the upper and lower areas of the kale leaves.

Table 3. Cross-correlation for different sampling locations for the data processed by the combination of median filter and S. Golay's 1st derivative method.

Species	Location	Mean of CC	$LSD_{0.01}$	CV%
Kale	Vein	0.32 ± 0.01^{b}	0.01	3.13
	Blade	0.43 ± 0.01^{a}		
	Upper vein	0.32 ± 0.02^{a}	0.02	2.80
	Lower vein	0.32 ± 0.01^{a}		
	Upper blade	0.43 ± 0.01^{a}	0.02	3.84
	Lower blade	0.43 ± 0.01^{a}		
Cabbage	Vein	$0.32 \pm 0.0.1^{b}$	0.01	3.2
	Blade	0.39 ± 0.01^{a}		
	Upper vein	0.32 ± 0.01^{a}	0.0019	0.1262
	Lower vein	0.32 ± 0.01^{a}		
	Upper blade	0.40 ± 0.01^{a}	0.02	3.05
	Lower blade	0.39 ± 0.01^{a}		

However, the results show that there is no significant difference between the upper and lower parts.

Conclusions

This study investigated the effects of the sampling point locations on the quality of spectral measurements. Reflectance spectra were collected with a portable UV/VIS/NIR spectrometer in the 190 to 1,130 nm range of wavelengths. The variability between the averaged spectrum and individual reflectance spectra was similar for the blade and vein areas and for the upper and lower parts of the leaves. However, cross-correlation calculations yielded better results for the blade part than for the vein part for both the cabbage and kale leaves. These results provide guidelines for optical reflectance measurements of Chinese cabbage and kale plants. The effects of the number of sampling points, the number of leaves, and the relationships between optical reflectance and leaf components need to be investigated in the future.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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