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Determination of the sample number for optical reflectance measurement of vegetable leaf

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ABSTRACT

Visible and near-infrared spectroscopy provides a variety of information regarding leafy vegetables. However, various factors including optical propagation, environmental issues, and experimental issues affect the quality of spectral measurements. Therefore, stability and performance of optical reflectance sensors are affected significantly by sampling scheme. This study investigated the effects of the number of sampling on optical reflectance measurement of Chinese cabbage and kale plant leaves. For that purpose, variability and similarity of multiple measurements for different number of sampling of the leaves were evaluated. A combination of a median filter and the 2nd Savitzky-Golay (S. Golay) method was used to reduce the noise on the reflectance spectra introduced by background effects and other uncertainties. Reflectance difference and cross correlation were used as criteria to evaluate stability (or similarity) of the measurements. Results indicated that the standard deviation of reflectance difference was not considerably different for cabbage and kale leaves with 12 and 9 sampling points, respectively. For similarity of multiple measurements, results of cross correlation showed that the standard deviation of cross correlation was not greater than 5% with 12 and 9 sampling points for cabbage and kale leaves, respectively. Thus, this study concluded that 12 sampling points (cabbage) and 9 sampling points (kale) on a single leaf were the optimal number for spectral reflectance measurement. This study may provide guidelines on the number of sampling for optimum reflectance measurement of leafy vegetables.

1. Introduction

Precision agriculture has the potential to deliver higher economic returns without further degrading environmental quality. Visible (VIS) and near-infrared (NIR) spectroscopy, combined with some chemometrics regression approaches for monitoring process and detecting faults, has become an established method for rapid and nondestructive assessment of quality parameters in the food and agricultural sectors (Min et al., 2006; Park et al., 2013). When a light beam is illuminated to a sample, an incident light beam is reflected, transmitted, or absorbed. The characteristics of each phenomenon are different based on physical and chemical attributes of the sample. Thus, physical and chemical properties of a sample can be estimated by analyzing the reflected or transmitted spectra using multivariate statistics. The approach of spectroscopy has many advantages, such as high efficiency, simple use, low cost, good reproducibility, and fast and non-destructive measurement (Wu et al., 2008).

There has been some research conducted to determine the possible factors affecting spectral measurements to obtain better results. Some of these factors are specimen preparation, length of test specimen, the rate of loading, moisture content of the specimen, humidity and temperature of the testing environment, the previous history of the specimen, bundle thickness, fineness, maturity, convolution, and geometrical properties. Factors affecting the accuracy of measurement using VIS/NIR spectroscopy has also been discussed (Pfitzner et al., 2006). Most of those reports described the accuracy when VIS/NIR spectroscopy was used for laboratory analyses; however, there is little information on the accuracy of practical in-situ use of VIS/NIR spectroscopy. Sampling location is one of the important issues for proper measurement of optical reflectance to obtain reliable and representative measurement and to save time on data collection. According to a study of Ngo et al. (2013) on effects of location of sampling for Chinese cabbage and kale leaves, reflectance measurements were slightly more stable in the upper part of the blade area.

The number of sampling is also an important issue to consider for reliable and representative measurement of reflectance spectra. Gamon and Surfus (1999) reported that leaf reflectance values were measured at three to five locations for Douglas fir, coast live

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oak, and sunflower and expressed as a mean. They examined a novel optical instrument for assessing leaf pigment content and, by proxy, the changing physiology and structure of intact leaves. Specifically, they illustrated how three indices of chlorophyll, xanthophyll and anthocyanin pigment content could be used to assess pigment content change during development in leaves of contrasting structure and functional type, and how this status was influenced by growth light environment. The results indicated that rapid assessment of several pigments in the intact leaf was possible with spectral reflectance; therefore, it allowed repeated sampling changing optical properties during leaf development and senescence due to its non-destructive method. Keskin et al. (2004) collected reflectance data of turf grass clippings at three locations and averaged to obtain scientific information leading to the development of an inexpensive indoor reflectance-based sensor to assess the nitrogen content of fresh turf grass clippings. After analyzing 400 leaf samples including 53 species with three replicate measurements, Sims and Gamon (2002) reported that correction for leaf surface reflectance eliminated much of the variation in the relationships between leaf spectral reflectance indices and chlorophyll content across a wide range of species and leaf structures. Five spectra from one citrus sample were averaged to investigate relationship between nitrogen concentration and reflectance by Min and Lee (2005). Reflectance of eucalyptus leaves (208 leaves from 21 species) from five locations was measured, and the results were averaged over 400–2500 nm range to develop a new chlorophyll index that would be less sensitive to leaf scattering (Datt, 1999). NIR spectroscopy technique was also used to measure the spectral characteristics related to internal quality of the fruit. The quality attributes of muskmelons was predicted using a miniature spectrometer. Reflectance spectra were measured at evenly distributed six points around the equator of each fruit (Suh et al., 2012). Much previous research was carried out on 10 points for spruce, fir, birch and mountain-ash (60 leaves from 6 species; Richardson and Berlyn, 2002), winter wheat (Zhang et al., 2014), and 15 points for flowering cherries (Imanishi et al., 2010).

Originally cultivated in China, the Chinese cabbage (*Brassica rapa*, subspecies *pekinensis* and *chinensis*) and kale (*Brassica oleracea var. alboglabra Bailey*) are economically important plants among the Brassica crops of the *Cruciferae* family. In open fields, Chinese cabbage and kale are grown in early spring and fall. Recently, greenhouses and plant factories have been widely constructed over the world including Korea, Japan, and China. Environmental conditions for crops growth are controlled and monitored precisely in these facilities (Ryu et al., 2014a,b). Epidemiological studies have suggested that an inverse relationship exists between dietary intake of phytochemicals called glucosinolates in *Brassica* vegetables and induction of cancer (La et al., 2009). Chinese cabbage is a relatively rich source of a variety of nutrients, and phytochemicals, particularly carotenoids, gluconasturtiin (aromatic glucosinolate), glucobrassicin (indolyl glucosinolate), and progoitrin (aliphatic glucosinolate) (Kang et al., 2006). Chinese kale, an original Chinese vegetable, is distributed widely in South China and Korea, and it is also present in relatively small quantities in Japan, Europe and America (Sun et al., 2011). It has good flavor, and the texture of its leaves is tender and crisp.

Most of the spectral measurements were carried out as suggested by previous research, and the locations were selected randomly. Furthermore, researchers used different crop leaves of various numbers of sampling; however, criteria of the sample selections have not been reported. For successful and efficient utilization of optical reflectance, determination of the number of sampling would be very important to obtain stable and rapid measurement. Therefore, the objective of this research was to develop methodology for determination of optical reflectance measurement of vegetable leaf. More specifically, the effects of the number of sampling on the quality of spectral reflectance measurement of Chinese cabbage and kale leaves were investigated.

Table 1
Cultivating conditions of the vegetables.

Item	Growth condition
Light source (LED color ratio)	R:B:W = 11:4:3
PPFD (μmol m^{-2} s^{-1})	160
CO_2 (ppm)	1000 ± 100
Humidity (%)	65 ± 5
Temperature (°C)	20 ± 1
pH	6.0 ± 0.5
EC (μS/cm)	1200 ± 90

2. Materials and methods

2.1. Sample collection

Leaf samples used in this study were selected from a plant factory in Chungnam National University, Daejeon, Republic of Korea. The plant factory has been operated to evaluate the effects of environmental factors on plant growth and quality, and the environments were monitored and controlled through wireless sensor network (Ryu, 2014). Growth conditions such as humidity, temperature, pH, electrical conductivity (EC), photosynthetic photon flux density (PPFD), CO_2, and LED (Light-emitting diodes) color ratio are shown in Table 1. The cabbage and kale were harvested about 3–4 weeks after transplanting. A total of six plants for both cabbage and kale were selected at the harvesting time (i.e., four weeks after transplanting of seedlings). From each plant, three healthy and normal-sized leaves attached to the stem were selected for spectral reflectance measurements.

2.2. Spectral measurements

A spectral data acquisition system as depicted in Fig. 1 was composed of a UV/VIS/NIR spectrometer (model: USB2000[1]; Ocean Optics, FL, USA), an internal light source Deuterium–Tungsten Halogen (200–1000 nm) lamp, optical fibers (Model: R400-7-SR; Ocean Optics, FL, USA), and a computer. Light passed from the spectrometer to the leaf sample through an optical fiber which was designed to transmit light from one end of the fiber to the other with minimal loss of energy. Reflection probe arrangement had seven optical fibers – six illumination fibers around one read fiber – in a stainless steel ferrule. The reflected light was detected by the receiver in the spectrometer, and reflectance spectra were collected using manufacturer provided software with an integration time of 100 ms. Reflectance measurement was implemented in a dark condition to reduce the noise introduced by background effects and other uncertainties.

Leaf spectral reflectance data were collected at evenly distributed 15 points on the inter-veinal blade area of each sample (Fig. 2) as suggested by Ngo et al. (2013), and the reflectance spectra were obtained at wavelength bands from 190 to 1130 nm in a dark condition. The spectrometer was constructed by two detector systems: a CCD detector for 190–890 nm (UV/VIS) and a CCD detector for 470–1130 nm (NIR). For each detector, the collected spectral data were composed of 2048 values with a resolution of 0.38 nm. Only the wavelength range from 250 to 1100 nm was used to analyse, and the other data were removed due to excessive noise. Reflectance data from first and second detectors were

[1] Mention of trade names or commercial products in this paper is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the authors' organizations.