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Visualization of Chlorophyll Change in Spinach by Hyperspectral Imaging

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ABSTRACT

Color, as an important characteristic of food quality, is evaluated first by consumers. The objective of this study is to develop and optimize an objective, fast and efficient hyperspectral imaging system to evaluate spinach exposed to three difference storage temperatures (0, 5 and 10 ℃) and the relative humidity of 90% during the 14 days storage period on the basis of chlorophyll deterioration, which is associated with the visual quality changes. Our results indicated that hyperspectral imaging can be applied to evaluate the change of chlorophyll in spinach throughout the storage period. Normalized Difference Vegetation Index at 705 nm ($NDVI_{705}$) has shown a steady decrease over the storage period because the reflectance at 705 nm is sensitive to the change of chlorophyll content but less sensitive to biophysical factors. Therefore, $NDVI_{705}$ can be applied to evaluate the chlorophyll change in spinach.

Keywords: hyperspectral imaging; chlorophyll; reflectance; NDVI; visual quality.

1. INTRODUCTION

Vegetables and Fruits, such as spinach (Spinacia oleracea L), provide essential micronutrients and vitamins to the human diet. Spinach is cultivated globally, and is an exceptional source of vitamins, notably high in vitamin K (270 μg/100g), vitamin B2 (200 μg/100g) and folate (210 μg/100g), as well as being a rich source of microelements, like potassium (690 mg/100g), iron (2.0 mg/100g) and magnesium (69 mg/100g)[1]. These phytonutrients are conducive to various physiological functions, like prevention of hemophilia, hypertension, and bone fracture[2]. Due to the characteristics mentioned above and the delicious flavor, they occupy an important position in the Japan's fruit markets. However, the highly perishable nature of these products results in a very short postharvest life that is a big challenge for the production, transportation and marketing chain. Therefore, the management and inspection of fresh commodities quality are of critical importance throughout the whole chain.

Accurate food quality classification is critical for grading to meet the needs of customers and setting prices. Visual quality rating, a conventional method of evaluating the quality of vegetables and fruits, is widely used in the agricultural and food sector owing to its ease of implementation. Nevertheless, this method is subjective, and the results would vary considerably among inspectors or even if by the same inspector over time[3]. There are four main characteristics that typically affect the purchasing decisions of consumers to a vegetable or fruit, and the color and appearance were thought to be the most important characteristics[4]. Prior to the purchase, consumers often evaluate fresh produces based mainly upon their color and appearance, for instance, discoloration of the leaves, browning at the petioles or objectionable softening in strawberries are causes that result in consumers refuse to purchase the produces.

The colors of vegetables and fruits are an indicator of their natural pigments and health. There are two main categories of color pigments in the leaves of plant: chlorophylls (appear green) and carotenoids (responsible for yellow, orange and red). The color of fresh green leafy vegetables, such as spinach only show the green color due to the bright colors of carotenoids are concealed by chlorophylls[5-9]. In addition to being responsible for leaf greenness, chlorophyll plays an important role in the process of photosynthesis and can be an estimator of plant physiological or nutrient status. Therefore, in food safety and quality evaluation of fruits and vegetables, the measurement and observation of chlorophyll contents and distributions in leaves are extensively applied for providing invaluable information about plant physiological activity such as leaf senescence and plant nutrient.

Hyperspectral imaging, which was originally developed for remote sensing[10], has been applied in the fields of food safety and quality control, such as contaminant detection[11], quality inspection[12-14], fungal infection[15,16], and disease analysis[17,18]. Hyperspectral imaging technique integrates imaging and spectroscopic techniques to capture both spatial and spectral information from sample radiative behavior. From the information, the internal characteristics and the distribution or change of pigment contents of sample can be derived. In the light of previous findings, hyperspectral imaging, which is considered as a powerful and nondestructive method, can be potentially applied to detect the visual quality change of fresh commodities in the whole production chain. Heretofore, there have been few studies contributed to the development of hyperspectral imaging system for the visual quality evaluation in fresh commodities. The aim of this study was to develop an alternative method for the visual quality evaluation of fresh products. Hyperspectral imaging system was used to investigate the change of chlorophyll content and to acquire deeper insight into the chlorophyll distribution in spinach leaves during the postharvest storage under different storage temperatures. The spectral curves obtained from hyperspectral images were studied and two types of NDVI based on the spectral reflectance that could offer effective information relevant to chlorophyll change were analyzed.

2. EXPERIMENTAL APPARATUS

2.1 Materials and methods

Spinaches were purchased from a supermarket located in Nagoya, Japan. 10 fresh unblemished spinach leaves, which free of decay, bruises and incurve, were selected respectively for hyperspectral imaging analysis and were placed on the transparent polypropylene boxes (331mm × 260mm × 48mm). Samples were then stored at 0, 5, and 10 ℃ and 90% relative humidity in a container in the dark.

2.2 Hyperspectral imaging system

The reflectance spectra of the samples were acquired through a lab-scale hyperspectral imaging system mainly consisting of a camera (Cosmos Eye HSC1702, Hokkaido Satellite Co., Ltd., Japan), a light source, and a computer. The camera creates a spectrum from 350 to 1050 nm with a spectral resolution under 10 nm and a sampling interval of 5 nm. One incandescent lamp (PS60, 110v, 60W, Toyo Trading Ltd., Japan) was used as light source, fixed at an angle of 75˚ to illuminate the sample stage. The hyperspectral images were acquired with scanner software HSCamSharp (Hokkaido Satellite Co., Ltd., Japan). HSDAnalyzer (Hokkaido Satellite Co., Ltd., Japan) was used to analyze the hyperspectral images by extracting an interested region (approximately 10000 data points) from each image of sample and the average value of the reflectance spectra was computed.

3. RESULTS AND DISCUSSION

3.1 Hyperspectral images investigation

The hyperspectral images together with the related digital images that obtained from spinach leaf samples at three different storage temperatures were illustrated in Figure 1. The hyperspectral images presented in this study were a combination of spectral images at two wavelength regions: the red (630 ~ 690 nm) and the near infrared (740 ~745 nm) regions. As illustrated in Figure 1, variations in color were detected among the different areas of a leaf sample, demonstrating that the chemical components in leaves are unevenly distributed. Consequently, the spectral characteristics of a leaf sample are not uniform. Inter-individual differences of chlorophyll distribution in samples were also found in the hyperspectral images, which may be caused by the various underlying factors such as the different degrees of change in the cell structures of the leaves or the difference in light conditions at the growing stage.

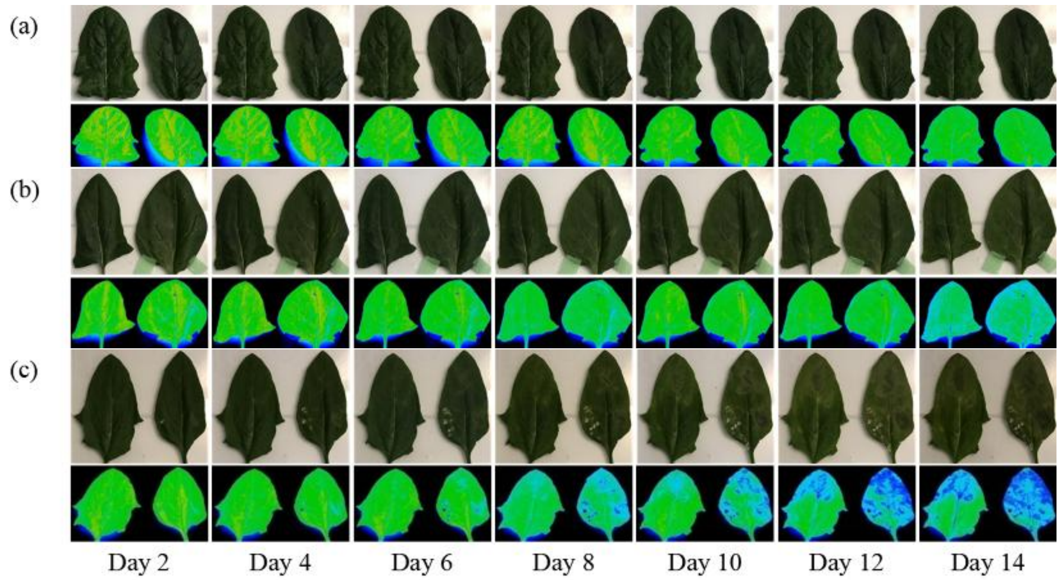

Figure 1. Hyperspectral images together with the related digital images of spinach over the storage period at various temperatures: (a) 0 °C; (b) 5 °C; (c) 10 °C.

The color intensity of the hyperspectral images became weaker across the storage period, and this phenomenon became even more apparent when the samples stored at the higher temperatures, as shown in Figure 1 (c). The color change in hyperspectral images can be ascribed to the chlorophyll degradation in leaves, as confirmed by the digital images, where discoloration of leaves can be observed over time, especially at 10 °C. In order to better identify the feasibility of using hyperspectral imaging for chlorophyll evaluation, we conducted a control experiment that samples of spinach were exposed 14 days to a 30 °C ambient temperature. As can be seen from Figure 2, remarkable discolorations were detected, the hyperspectral images turned dark which means there were no chlorophyll pigments in the leaves. Thus, we can infer that the distribution of chlorophyll could be identified in situ in sample leaves non-destructively by hyperspectral imaging.

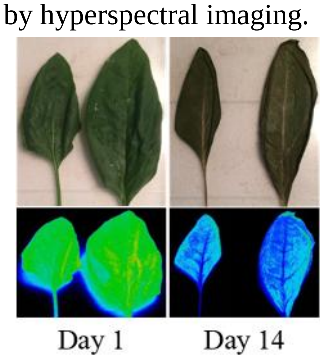

Figure 2. Hyperspectral images together with the related digital images of spinach at 30 °C

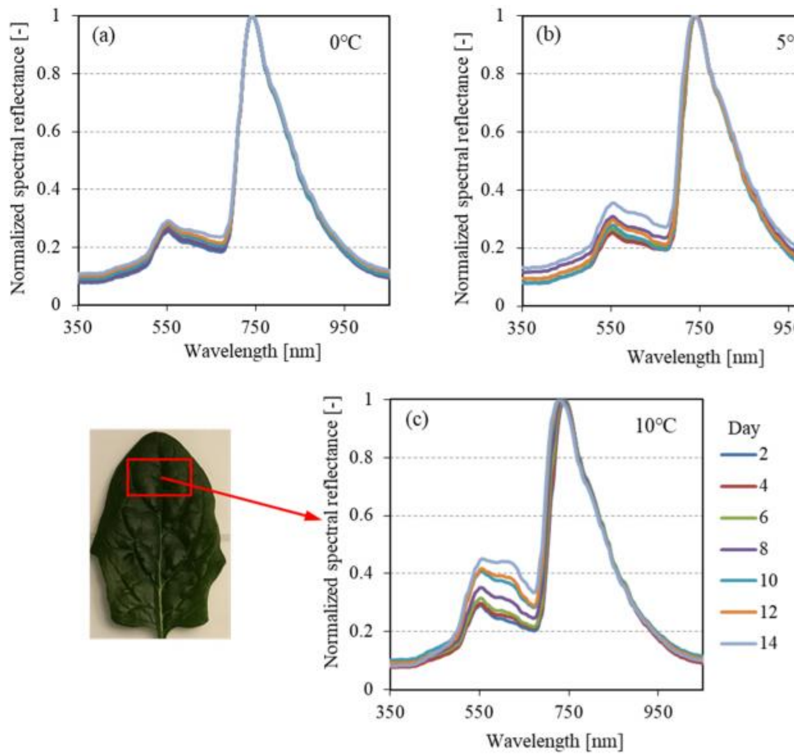

Figure 3. Spectral reflectance of spinach over the storage period at various temperatures: (a) 0 °C; (b) 5 °C; (c) 10 °C.

Moreover, due to the distal portion of the leaf exhibited a much more rapid appearance deterioration and noticeable color change than the other parts of leaf, this portion was defined as the interested region. As shown in Figure 3, the red rectangle part of a sample was selected as the interested region and its average normalized spectral reflectance (is calculated as the ratio of spectral reflectance at each wavelength to the maximum spectral reflectance which often appears at 740nm) are illustrated in the same figure.

As illustrated in Figure 3, the spectral reflectance in the wavelength region from 350 nm to 500 nm and near 675 nm was low. In the range between 350 nm and 480 nm, where the absorption by carotenoids and chlorophylls take place[19], the reflectance remained basically unchanged and with minimum values less than 0.2. There were two remarkable changes observed in the range of 500 nm to 550 nm which is called the green edge, and in the range of 690 nm to 730 nm which is called the red edge. The maximum value of reflectance was observed at around 740 nm which was irrelevant to the storage period due to the spectral normalization. Then a marked decline towards longer reflectance was measured. The reflectance in the green region increased and the green edge shifted towards shorter wavelengths over the storage period. The similar spectral character was identified in the red edge region where the reflectance moved towards shorter wavelengths over the storage period.

Higher storage temperatures would accelerate leaf senescence, facilitate plant transpiration and induce thermal stress those contribute to a considerable decrease in the chlorophyll content. The phenomenon of red edge shift was largely influenced by the storage temperatures, as can be seen from the spectral results. Thus, spectra extracted from the red and red edge regions by hyperspectral imaging may provide effective information to evaluate the chlorophyll change.

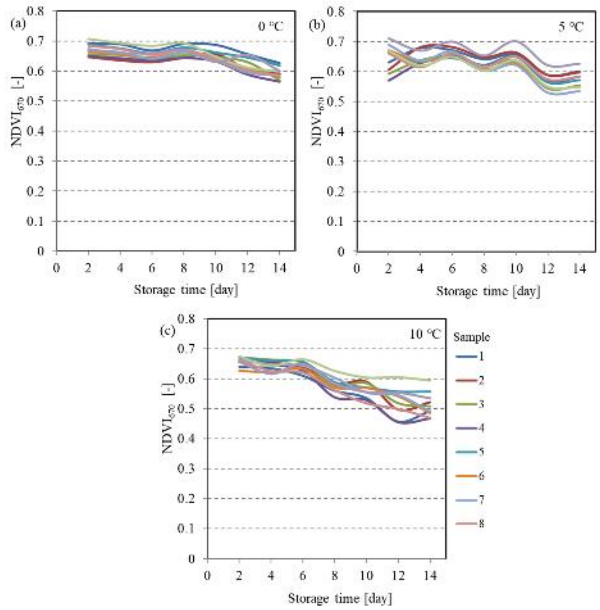

Figure 4. $NDVI_{670}$ of spinach over the storage period at various temperatures: (a) 0 °C; (b) 5 °C; (c) 10 °C.

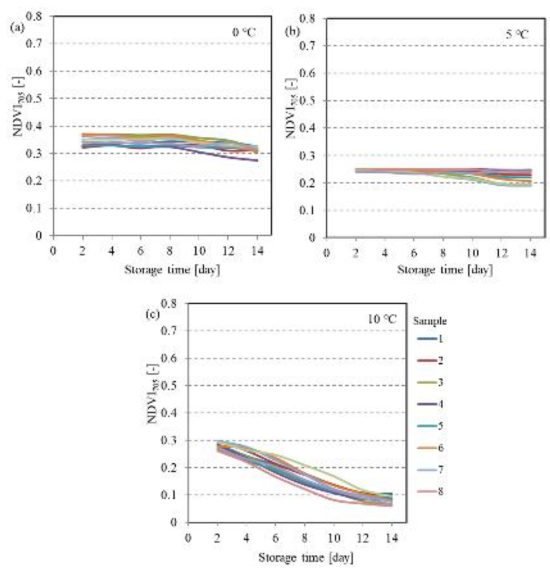

Figure 5. $NDVI_{705}$ of spinach over the storage period at various temperatures: (a) 0 °C; (b) 5 °C; (c) 10 °C.

Consequently, we investigated and compared two types of Normalized Difference Vegetation Index (NDVI) [20]: the first one ($NDVI_{670}$), a conventional index, was computed using spectral reflectance from near infrared (R_{740}) and red (R_{670}) regions; the second one ($NDVI_{705}$) was calculated by substituting the red edge reflectance (R_{705}) for the red reflectance (R_{670}), and the equation is given as follow.

$$NDVI_{670} = (R_{740} - R_{670})/(R_{740} + R_{670}) \quad \text{Equation (1)}$$

$$NDVI_{705} = (R_{740} - R_{705})/(R_{740} + R_{705}) \quad \text{Equation (2)}$$

In contrast to $NDVI_{670}$, $NDVI_{705}$ has shown a steady decrease over the storage period. This is consistent with the results of previous studies that the reflectance at 705 nm is sensitive to the change of chlorophyll content but less sensitive to biophysical factors, for instance, leaf orientation, status of the soil, optical properties and solar angle are comparatively poorer[21,22].

CONCLUSION

The laboratory-based hyperspectral imaging system is established to investigate the chlorophyll distribution in three species of plants for evaluating visual quality. The main conclusions are summarized as follows.

1. The results of our study indicate that hyperspectral imaging can identify and mapping chlorophyll pigments and their changes in samples non-destructively;
2. The spatially detailed information of chlorophyll distribution in different parts of the samples can be acquired from the hyperspectral images;
3. The reflectance at 705 nm is sensitive to the change of chlorophyll content but less sensitive to biophysical factors, resulting in a less variability of $NDVI_{705}$ over the storage period.

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