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Evaluation of freshness of lettuce using multi-spectroscopic sensing and machine learning

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Summary

We aimed to develop a method to evaluate lettuce freshness changes during storage using only the surface color. In the first experiment, the surface color of one lettuce were measured continuously for 6 days. At the same time, moisture contents, elemental composition and organic matter of lettuce leaves were measured by oven drying method, X-ray fluorescent analysis and Mid-infrared spectroscopy, respectively. Considering a combination of the surface color and moisture and elemental contents, it was found that there were several color change points before and after the time when the moisture contents and elemental balances in the lettuce changed. These results represented that the surface color could relate to the internal quality. Additionally, it is suggested that freshness of lettuce could be quantified and predicted using surface color information.

Furthermore, the data set and the method for freshness evaluation leading to machine learning were studied in the second experiment for the freshness judgement. In this experiment, 15 multispectral sensing data including lettuce color information were acquired, and the quality change point was determined using machine learning such as K-means and decision tree.

Keywords: Lettuce, Freshness, K-means, Decision tree, Color, X-ray fluorescence, FT-IR

Introduction

Vegetables contain important nutrients such as vitamins, minerals, and fibers that humans cannot synthesize and that are essential for maintaining human health. In recent years, lifestyle-related diseases caused by oily diets are global problems, a trend toward healthy eating and vegetarian are growing (Nicola et al., 2009; Zink, 1997).

However, the ratio of vegetable consumption in Japanese household has been declining from 1990 to 2000 (Kobayashi, 2003). On the other hand, there is an increasing demand for fresh-cut products that can be easily and quickly prepared and can be eaten as salad. This trend is considered to be due to the increase in working women, single and two person households, and senior citizens in recent years. (Kobayashi, 2018) The same trend of the increasing consumption of fresh-cut vegetables can also be seen in many European countries, the United States, Australia, and Asian countries (Nicola et al., 2006; Nicola et al., 2009).

Fresh-cut vegetables are very perishable and storing them in good quality for a long time is an important issue. The quality of fresh-cut products is, among other factors, determined by the quality of the raw material vegetables. Therefore, the preharvest environment such as soil conditions, climate conditions, agricultural practice, harvesting, etc. could affect the quality of the vegetables and their final products (Nicola et al., 2006; Nicola et al., 2009). Also, there is a concern that the quality will deteriorate due to global climate change in recent years (Bisbis et al., 2018).

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In addition, the way of handling the vegetables after harvesting affects the quality of the fresh-cut products. Many postharvest technologies such as proper storage conditions, packaging materials, modified atmosphere packaging (MAP), and other emerging technologies have been studied in order to maintain the quality of vegetables after harvesting and extend the shelf life (Nicola et al., 2009; Ma et al., 2017). The techniques are then needed to keep the freshness as much as possible from the stage of raw agricultural products before processing and to match the quality suitable for processing.

Although freshness is a commonly used word that expresses the quality of fresh fruits and vegetables, there is no clear definition. The freshness of vegetable could be subjectively evaluated by each individual from the viewpoint of the appearance such as color, sensory, firmness, texture, etc. Therefore, a quantitative evaluation method for fresh products is desirable.

So far, many studies have been conducted to relate the freshness of vegetables to the ratio of lipid peroxide to the total lipids contained in vegetables (Isamah et al., 2003; Nakano, 2010). Further on, studies to interpret freshness as decomposition of chlorophyll, and to relate chlorophyll change with change in temperature, humidity and sunshine conditions to freshness (Almeida et al., 2003; Hörtensteiner, 2013), a study on post-harvest changes in sugar content in tomato plants (Beckles, 2012), on post-harvest ascorbic acid content change in spinach (Cocetta et al., 2014), on post-harvest phenol and carotenoid content change in papaya (Rivera-Pastrana et al., 2010) have been published. In addition, there is a study focusing on plant hormones to link post-harvest deterioration of plants with general stress responses (Ludford, 1995). Furthermore, studies directed at quantifying post-harvest quality and freshness changes are conducted in multiple ways. As a research example of nondestructive quality analysis, Piazzolla et al. (2013) investigated the change of grape quality with harvest time using hyperspectral imaging. Amodio et al. (2013) defined quality using several parameters.

We have been testing optical sensing that can perform nondestructive measurement of appearance and internal quality for agricultural products and foods. Specifically, color image processing for appearance quality measurement (Motonaga et al., 1997), mid-infrared (MIR) spectroscopy measurement for organic matter measurement, and the like (Hashimoto et al., 2008). In recent years, the object of optical sensing was expanded to plant vigor diagnosis of rice, tomato, vine, orange etc. We have then established appearance measurement of leaves using color image processing, element measurement of leaves using X-ray fluorescent (XRF) spectroscopy, pigment measurement using fluorescence spectroscopy, and nitrogen analysis with different modes using MIR spectroscopy (Kameoka et al., 2015; Kameoka et al., 2017).

Since many of the conventional methods of measuring freshness and quality are separation analysis, a complex pretreatment such as chemical analysis is required. Therefore, execution on the site is difficult, and problems such as very long time for measurement exist. However, with the advancement of optical devices in recent years, miniaturization, and price reduction, optical sensing research (Young et al., 2016) for nondestructive measurement of appearance quality and internal quality of agricultural products and foods has been activated. Thus, the realization of nondestructive, chemical free, simple, and rapid measurement of freshness and quality of vegetables by multiband optical sensing is now expected.

In this research, we focused on the judgment and evaluation of freshness of lettuce using only the surface color information. In the first experiment "Evaluation of freshness of lettuce", we confirmed the continuous color change and water change of a single lettuce in order to clarify the color change of the lettuce during the storage. At the same time, focusing on the moisture, the macronutrient from the viewpoint of plant structure and the organic matter from the viewpoint of deterioration of the substrate of the plant, we have grasped the relationship between freshness (deterioration) evaluation by appearance quality performed by human being intuitively and objective evaluation obtained scientifically. Furthermore, in the second experiment, "freshness judgment experiment", we examined data sets and evaluation methods for evaluating the degree of freshness that will lead to machine learning in the future. Using the measured data, we tried to quantify the deterioration process of lettuce after examining the possibility of freshness judgment using multi-optical sensing and machine learning.

Materials and methods

Evaluation of freshness of lettuce

Test lettuce

24 heads of lettuce (cultivar: Verde-7) were harvested on Awajishima in Japan on December 5, 2017 were used. In this study, 8 heads of lettuce were used, and the others were used for preliminary experiments for determining the experimental conditions. The average weight of lettuce is 333.1 g with a standard deviation of 41.9 g. One head of lettuce was used for "Continuous storage experiment", 7 heads of lettuce were used for "Demolition experiment by cardboard box storage". By combining these two kinds of experimental results, it is possible to link the appearance characteristics and the moisture change characteristics of the lettuce head with the characteristics inside the lettuce head. The outline of two types of storage experiments using lettuce is shown in Fig. 1. In addition, since the experiment period becomes long when the temperature of 5 °C and the relative humidity of 96%, which are the optimum storage conditions of lettuce, are used, we carried out an accelerated experiment. As storage conditions, a temperature of 25 °C and a relative humidity of 33% were adopted.

Continuous storage experiment

A simple continuous storage experiment apparatus

In order to clarify the continuous color change and moisture change of the lettuce during the storage, a simple continuous storage experiment apparatus for imaging and measuring the weight of lettuce in a storage container was constructed as is shown in Fig. 2. The outer frame (length 450 mm, width 600 mm, height 600 mm) is made outside the storage container, and angles are bridged from the bottom to the positions 135 mm and 540 mm. High-performance LED lights (IMD-V201, IMMEDIA, Japan) for photography at each height and a single-lens reflex camera (EOS Kiss X3, Canon, Japan) were installed. A transparent glass is attached to the lid of the storage container so that the color image of the storage process of lettuce can be taken from the top, and Kent paper (Neutral white) was pasted. In order to continuously measure the weight change of lettuce in the storage process, an electronic balance (EK-1200i, A & D Co., Ltd., Japan) was placed in the storage container. In addition, the storage container was placed so that the LED light would strike the center of the lettuce on the balance.

The temperature and humidity in the container were controlled using a Peltier element and a saturated salt solution using $MgCl_2$. Temperature and humidity were set to 25 °C and 33%RH, and accelerated experiments of the degradation process were conducted. Furthermore, an electronic balance, a single-lens reflex camera,

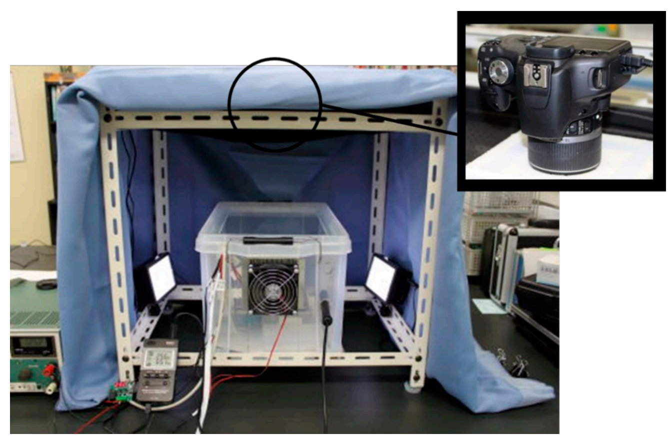


Fig. 2: Experimental equipment for continuous storage experiment.

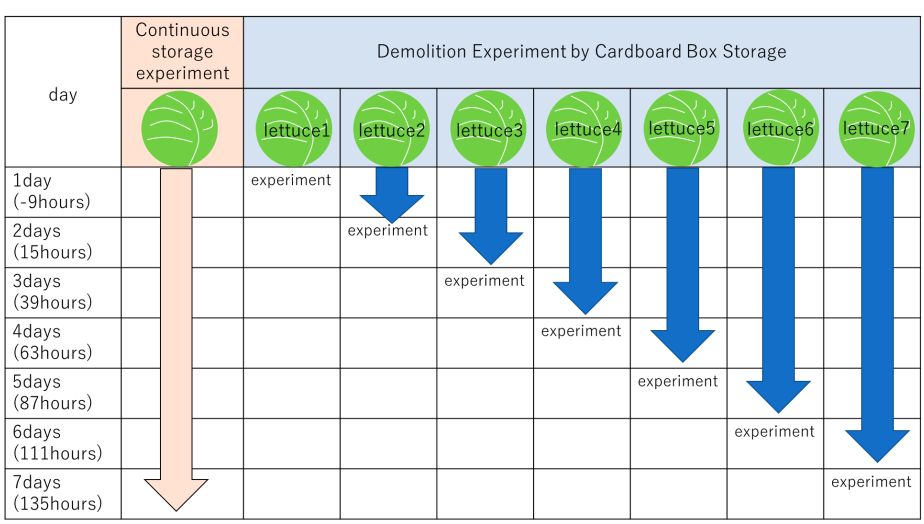


Fig. 1: Outline of experiments.
The continuous storage experiment was conducted 9 h after the demolition experiment started. Since the start time of the continuous storage experiment was set to 0 h, the start time of demolition experiment corresponds to -9 h.

and a thermoshygrometer (Weathercom II manufactured, EMPEX INSTRUMENTS, INC., Japan) were connected to the Raspberry PI, and the weight, images, temperature, and humidity were automatically measured using an acquisition program self-made with Python. Images were taken every hour to capture color changes of the lettuce surface. The digital timer was set to turn on the light 15 minutes before imaging and turn off after 15 minutes. The device was coverd with a dark screen to shield the light from the outside.

Continuous color image and weight acquisition

The following experiments were conducted to quantitatively evaluate the quality deterioration characteristics of lettuce from the appearance and moisture change of lettuce. Lettuce was stored for 143 h in the storage container, and the appearance image of the lettuce was automatically taken every hour, and the weight, temperature and humidity were automatically measured at intervals of 5 minutes.

Demolition experiment by cardboard box storage

Storage conditions and the workflow of the experiment

One lettuce was placed in each of 7 cardboard boxes whose relative humidity was controlled to 33% using the saturated salt method. In order to improve the air circulation, a fan was installed near the saturated salt solution. These boxes were stored in an incubator controlled at 25 °C, and one sample was taken out daily for 7 days and demolition experiment was performed. An actual and a schematic view of the cardboard box installed in the incubator are shown in Fig. 3 (a) and Fig. 3 (b) respectively. The demolition experiment was conducted according to the following procedure at different storage periods, 1, 2, 3, 4, 5, 6 and 7 days respectively.

(1) Take out the stored cardboard box and measure the weight of the lettuce.
(2) Strip lettuce leaves one by one and measure each element (from the outer leaf to the seventh sheet) by Handheld XRF analyzer (DELTA Premium, Olympus Corporation, Japan).
(3) Measure the organic matter of each leaf by MIR spectroscopy.
(4) Measure the weight of each leaf.
(5) Calculate the moisture content of each leaf by oven drying method, (100 °C 24 h).

The details of each measurement are described below.

Elements measurement

In order to evaluate the quality of lettuce from the viewpoint of the plant cell structure, elements of lettuce leaves were measured by XRF analyzer. The analyzer needs to be in close contact with the lettuce during the measurement time. However, since the lettuce is soft and spherical, the stand shown in Fig. 4 on which both the lettuce and the device can be fixed was prepared and measured. An XRF analyzer was vertically installed using DELTA Flex Stand (DELTA Flex Stand, Olympus Corporation, Japan). At this time, a platform for stabilizing lettuce spheres with expanded polystyrene was attached to the head of the detector, and elemental measurement was performed. When standard deviation of measurement was compared with the usual measurement method using the standard deviation of three measurements, the measurement error was improved nearly 10 times for any element. In this measurement, the macronutrients of lettuce, potassium K, Calcium Ca and phosphorous P, and Light Elements LE were focused. The data were taken as XRF spectral information and were then converted to weight percent concentrations by fundamental parameter method. We focused on relative changes in elements concentrations, not on absolute values. The detected value was used as an index of the change in the structure of the lettuce cells due to deterioration.

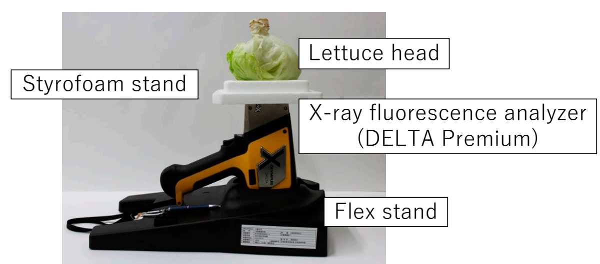
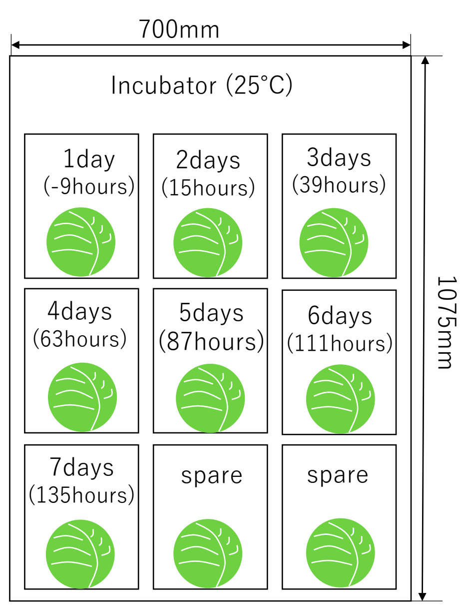


Fig. 4: Measurement method using XRF method



(a) Actual view (b) Schematic view

Fig. 3: The cardboard box installed in the incubator for demolition experiment.

Organic matter measurement

The lettuce continues to have metabolic activities after harvesting, and they decompose their substrates during storage. In order to quantitatively evaluate the quality of lettuce from the viewpoint of deterioration of the substrate, MIR spectral of each leaf was obtained by a FT-IR, Fourier transform infrared spectrophotometer (Spectrum Two, PerkinElmer, Inc., USA) with an ATR accessory (hereinafter referred to as FT-IR / ATR method). The measured spectral region was 4000 cm^{-1} to 400 cm^{-1}, where the peaks of organic matter such as sugar, protein, and lipid appear.

The number of measurements was 3 points per leaf. In order to obtain MIR spectra including information on the physical structure of lettuce leaves, we examined the optimal load that does not destroy the physical structure of lettuce leaves. Whether or not the physical structure was destroyed was visually judged whether there was water accompanying the collapse of lettuce leaf cells. The MIR spectra obtained by adjusting the pressure force gauge of FT-IR / ATR method to 7, 10, 13 and 15 steps are examined, and the leaf adheres to IRE without any destruction of the physical structure of the leaf. The force gauge 10 that gives the maximum pressure is adopted.

Moisture content measurement

Moisture content is one of the most important factors that affect the quality of lettuce. In this experiment, the moisture content of each leaf, M_{leaf} [% wet basis] was measured by oven drying method. The leaves were placed in an oven at 100 °C for 24 h. The value of moisture content was calculated using following equation (1).

$$M_{leaf} = \frac{W_0 - W_{dry}}{W_0} \times 100 \tag{1}$$

W_0 [g-fresh weight] is the original weight of the lettuce leaf and W_{dry} [g-dry matter] is the lettuce leaf weight after oven drying.

Freshness judgment experiment

Test lettuce

For the freshness judgment, 26 heads of lettuce (mild head glass, Tsuruta seedling) harvested at Furukawa City, Ibaraki Prefecture, Japan at 9:00 am on November 13, 2016 were used. Six heads of lettuce were used for the freshness judgment, and the remaining individuals were used in preliminary experiments for determining the experimental conditions. The average weight of 10 individuals was 496.5 g, and the maximum and minimum weights were 546.1g and 446.2 g respectively.

Storage condition and the workflow of the experiment

The humidity in the storage case was controlled using a saturated salt solution (JIS, 2000). In this experiment, 5 individuals were stored under the conditions of 75% relative humidity (sodium chloride) in order to examine the time-dependent change of the lettuce freshness decreasing process. Also, 1 individual was stored under the conditions of relative humidity 84% (potassium chloride). The 6 heads of lettuce were subjected to 15 multi-spectroscopic measurements twice a day at each storage period from 1st to 7th days. Color information was obtained using the color image processing system developed in our previous study, element information was obtained using an XRF analyzer described in the first experiment, and pigment information was obtained using a polyphenol measuring instrument (Force-A, Orsay, France).

Color image acquisition

The color image processing system consists of a single-lens reflex camera (D300, Nikon, Japan) and a twisted fluorescent lamp which has high color rendering (VITELITE, Light Sources, Inc., USA). By surrounding the photography table using the Cylindrical Kent paper (Neutral white), the sample could be illuminated by the diffusive light. Additionally, a black screen for shielding the light from the outside was covered the entire system. Furthermore, a monitor with high color reproducibility (SyncMasterXL24, Samsung, Korea) was used for image monitoring. The RGB values of each image were converted to the HSV ones. Additionally, the projected area (A) of each lettuce head was calculated based on the acquired image.

Elements measurement

The elements of the 6 heads of lettuce were measured by Handheld XRF analyzer as described for the first experiment, and the XRF spectra were measured at 3 points per each individual. As the first experiments, the macronutrients of lettuce, K, Ca, P, and LE were focused. The value of light LE was explained in the session of the result and discussion.

Pigment measurement

Four kinds of pigmentary values (chlorophyll value: Chl, flavonol value: Flav, anthocyanin value: Anth, and NBI value which means Chl/Flav) in lettuce leaves were measured by the polyphenol meter. In each leaf, the pigmentary information was measured at 5 points by pinching the leaf tip by the leaf-clip of the instrument.

Data set creation and machine learning

Basic statistical processing was performed on the obtained sensing data, and the data were organized for machine learning. Each parameter was standardized using the following equation (2).

$$X_n = \frac{X_i - \bar{X}}{\sigma} \tag{2}$$

Here, X_n is standardized value of each parameter, X_i is measured value, $\bar{X}$ is average value, and σ is standard deviation of each parameter. In machine learning, using the standardized data set, clustering was performed by the K-means method (Christopher, 2006), and those with good freshness and those with bad freshness were classified. Subsequently, the classification results were used as the correct answer, and discriminant analysis was performed using a decision tree (Guo et al., 2015; Christopher, 2006) capable of discrimination with raw data.

Results and discussion

Evaluation of freshness of lettuce

Continuous storage experiment

Continuous color image analysis

Color image analysis was performed on a total of 144 continuous images of the stored lettuce taken every hour during the storage period of 6 days, and the freshness of lettuce was quantitatively estimated from the color change. The image processing was performed according to the following procedure using OpenCV (Bradski et al., 2008).

(1) A mask of the background area where lettuce is not captured in the acquired image was created.

(2) This mask was put on the original image, histograms of hue H, saturation S and lightness L of pixels in the mask were created, and their average value and standard deviation were calculated.

In order to quantitatively evaluate the degree of deterioration of lettuce using color information, the color change of the lettuce surface images was acquired. The change of color is shown in Fig. 5. The hue H decreased from green to yellow, which was suggested to be

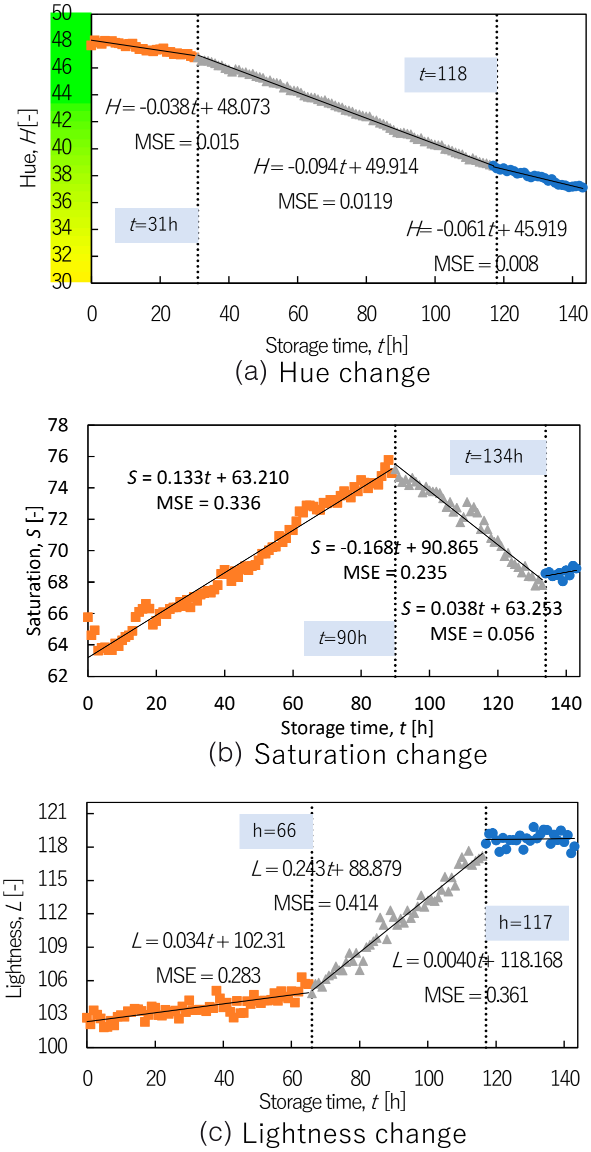


Fig. 5: The color change of the head lettuce surface.

caused by degradation of chlorophyll on the surface of the head (Xanthopoulos et al., 2016). The saturation S increased and then decreased, and the lightness L increased. In addition, the standard deviation of the color histogram at each time decreased as storage time passed and tended to converge to a constant value. The decrease in the standard deviation is considered to indicate that the substance in the early stage of lettuce storage is metabolized and the number of component substances in the lettuce is reduced and the substance is moving toward the unitization.

In consideration of the constant change and the decrease change, three stages of change were recognized from the change curves of hue, saturation and lightness respectively. Therefore, each color change curve was divided into three at the point where the sum of the mean square error was minimized shown in Fig. 5. In the case of hue value, the slope of the line changed in 31 h and 118 h from the start of storage. The saturation values increased up to 90 h, decreased at 91 to 133 h, and kept constant at 134 to 143 h. For the lightness value, the slope of the line changed between 66 h and 117 h. In each color parameter change, the approximate straight lines of the three stages were expressed by the following formulas: (3-1), (3-2), (3-3), (4-1), (4-2), (4-3), (5-1), (5-2) and (5-3).

$$H = \begin{cases} -0.038t + 48.073 & (0 \leqq t < 31) \quad (3-1) \\ -0.094t + 49.914 & (31 \leqq t < 118) \quad (3-2) \\ -0.061t + 45.919 & (118 \leqq t \leqq 143) \quad (3-3) \end{cases}$$

$$S = \begin{cases} 0.133t + 63.210 & (0 \leqq t < 90) \quad (4-1) \\ -0.168t + 90.865 & (90 \leqq t < 134) \quad (4-2) \\ 0.038t + 63.253 & (134 \leqq t \leqq 143) \quad (4-3) \end{cases}$$

$$L = \begin{cases} 0.034t + 102.310 & (0 \leqq t < 66) \quad (5-1) \\ -0.243t + 88.879 & (66 \leqq t < 117) \quad (5-2) \\ 0.004t + 118.168 & (117 \leqq t \leqq 143) \quad (5-3) \end{cases}$$

Here, H, S and L is the values of hue, saturation and lightness respectively and t [h] express the time from the start of storage. It was suggested that it might be possible to predict the degradation stage of lettuce by grasping the time when the color change tendency of lettuce surface changes.

In order to grasp the color distribution change of the lettuce surface color, each distribution for 143 h of hue, saturation and lightness was shown in Fig. 6. As a result, the hue distribution can be approximated by the normal distribution expressed by Gauss function, and the average value decreases while the maximum value of the distribution tends to increase. In addition, the saturation distribution was approximated by the Gamma distribution, and it was found that it

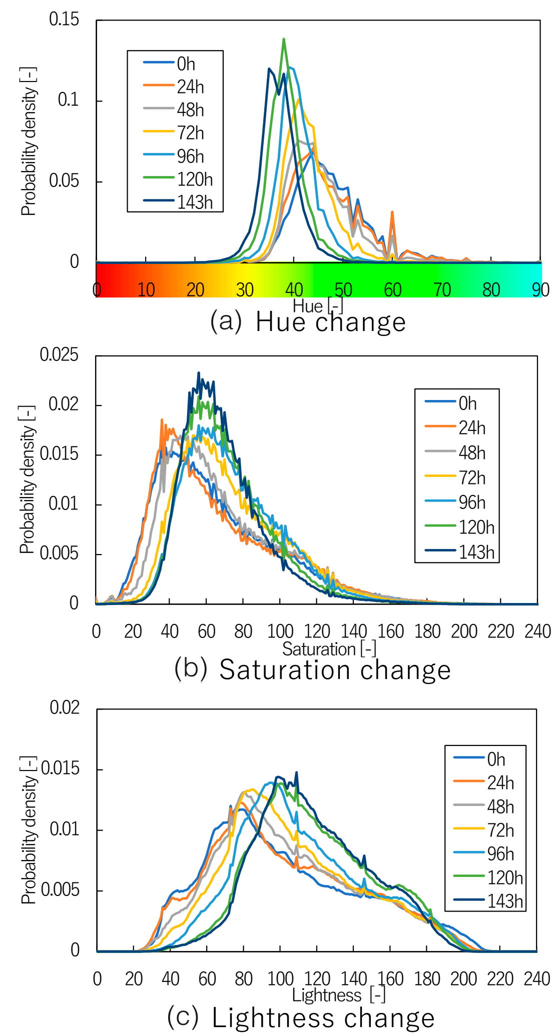


Fig. 6: The change of color distribution.

tended to decrease after the average value increased. From these results, the hue and saturation were respectively fitted with Gauss and Gamma function, and the change of each parameter was determined. It is suggested that it is possible to identify lettuce color distribution and estimate lettuce degradation stage by the parameter estimation of each distribution.

Continuous moisture change during storage

In order to quantitatively evaluate and consider freshness through the change in moisture content of lettuce, the 6-day change in weight of lettuce obtained in the experiment was converted to the change in water content and normalized, as shown in Fig. 7. The normalization was performed according to the following equation (6).

$$M_t = \frac{W_t}{W_i} \tag{6}$$

Here, M_t [-] is the normalized moisture content after t h from the storage. W_t [g-fresh weight] and W_i [g-fresh weight] are the weight of the moisture of the lettuce at the start of storage and after t h respectively.

This moisture change curve was divided into three at the point where the linear determinate coefficients of the two approximate line drawn from the both ends of the moisture curve were maximized respectively. It was shown that the moisture content decreased constantly until 4 h from the start of storage, decreased gradually between 4 and 108 h, and decreased again after 109 h. Therefore, we decided to treat this moisture change curve as the deterioration characteristic curve of lettuce.

Demolition experiment of lettuce by cardboard box storage

Deterioration characteristic curve of whole lettuce was obtained from water change of whole lettuce and color change of surface image in continuous storage experiment for 7 days of storage. The results are shown below in the order of water, element, and organic matter.

Moisture contents and, light element (LE) measured by XRF analysis

Fig. 8 shows the changes over time in the water and LE obtained by XRF analysis of the 1st to 7th outer leaves, and the correlation between water and LE. The value of LE was explained in the following passage.

Fig. 8 (a) shows that the water loss of lettuce mainly starts from the core and the outer leaf with 1st or 2nd leaves, and that the water inside the lettuce leaves hardly changes during storage. In the case of 1st and 2nd outer leaves, a rapid water loss occurs especially between the 3rd and 4th day. Such a drastic change in moisture is unusual.

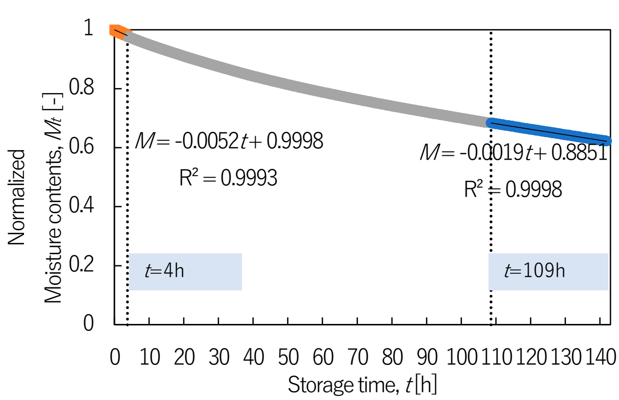


Fig. 7: The change of standardized moisture contents with two changing points.

The impression of a drastic decrease may be caused by the fact that the initial moisture of lettuce that had been disassembled on the 3rd day was very high, and the initial moisture of lettuce that had been disassembled on the 4th day was a little lower. As a matter of course, this rapid water loss also includes the effect of water change due to individual differences, so it was confirmed that the water decreased significantly between 2nd and 4th day of storage. However, the influence of individual differences was too large to be able to analyze quantitatively. Also, the water loss of 1st and 2nd leaves were almost finished until 4th day. The moisture content of the 3rd or more inner leaves are almost unchanged throughout the storage. However, the moisture content decreased by 0.70% during the 7-day storage period, so it was confirmed that the moisture content continued to decrease gradually over the storage period.

Fig. 8 (b) shows the change in LE of each leaf. LE refer to an element whose atomic number is sodium Na or less, which is undetectable by XRF analyzer. LE is calculated by subtracting the detected value of elements higher than magnesium Mg from the whole.

In addition, LE of lettuce contains water H_2O which accounts for 95% or more of the lettuce weight, a protein which constitutes a substrate of lettuce, and carbon C, hydrogen H, oxygen O, nitrogen N which constitute a carbohydrate. Therefore, it is considered that it is possible to obtain information of carbon dioxide and water loss due to respiration and transpiration from information of LE. As can

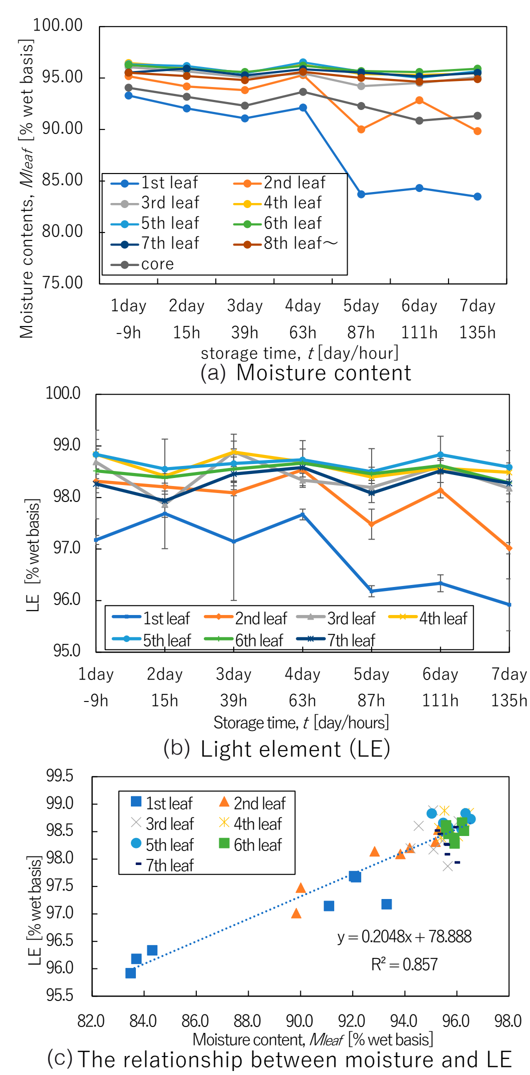
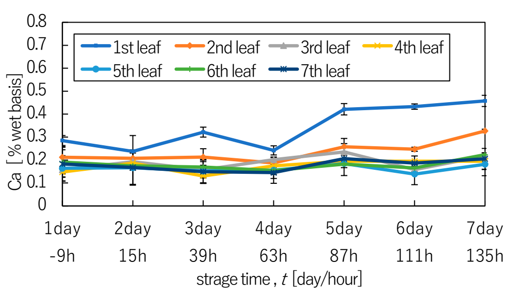


Fig. 8: Moisture contents and Light Elements (LE).

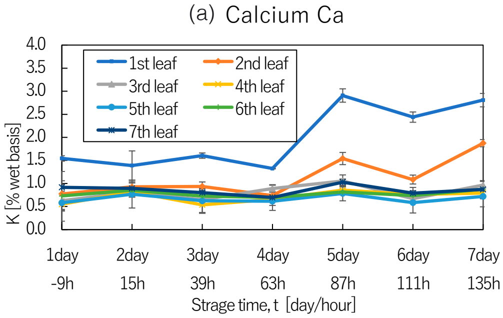
be seen from Fig. 8 (b), LE has a very similar tendency to moisture. When moisture and LE were plotted in the same graph shown in Fig. 8 (c), a high correlation with a coefficient of determination of 0.85 or more was observed. As a result, it is suggested that it is possible to estimate the water information of lettuce from the change of LE. Also, the results of LE reflect individual differences due to differences in initial moisture content.

Change of elements contents

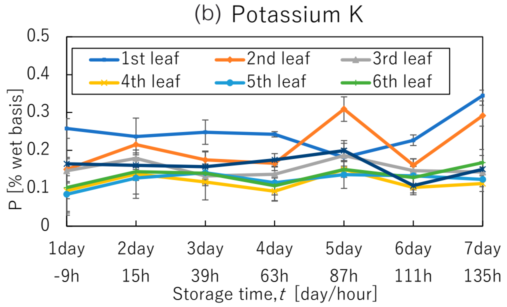
One of the causes for the apparent increase of each element during storage is the balance change of the elements accompanied by tissue deterioration, since generation and loss of elements do not occur in the lettuce. Another cause may be leaf shrinkage due to water loss results in concentration of elements and an increase in the number of measurable elements in the XRF field of view. Fig. 9 (a), (b) and (c) show the changes in the storage period of K, Ca, and P of the 1st to 7th leaves. In the 1st and 2nd outer leaves, K and Ca increased rapidly between the 3rd and 4th days when the moisture content changed greatly. The increasing tendency of these 2 elements is considered to be synchronized with the moisture change. In particular, the fact that the initial moisture of the lettuce demolished on the 3rd day was originally high is also reflected in the elemental information. It is considered that this data includes the effect of the localization of elements due to the deterioration of lettuce tissue or the increase of apparent element amount accompanying the decrease of moisture content. However, considering the effects of individual differences, it was difficult to determine the degree of lettuce degradation from this elemental information alone. P showed different behavior from K and Ca. If the lettuce tissue is not changed and the water is simply reduced, all elements are considered to show the same tendency to increase. However, each element showed different change tendency in this result. This is considered to be due to the fact that the balance of the macronutrients in the lettuce tissue is broken and the lettuce has some deterioration. This time, it was not possible to explain the deterioration phenomenon quantitatively only by the change result of the macronutrients. From this, it is necessary to construct a shrinkage model of lettuce accompanying water loss. Then, it is required to analyze the change tendency of the macronutrients using this model, and to associate the change tendency of the macronutrients with other quality evaluation methods such as images and viable counts. Through such analysis, it is thought that the deterioration of lettuce is captured quantitatively from the viewpoint of change tendency of macronutrients.



(a) Calcium Ca



(b) Potassium K



(c) Phosphorous P

Fig. 9: Changes in the amount of element per unit volume in lettuce leaves measured by XRF analysis.

Change of organic matter

MIR spectra were obtained using the FT-IR/ATR method to capture the degradation of saccharides, lipids and proteins that make up the cellular components of lettuce. In order to make the baselines clearer and to make it easy to understand each peak intensity, second-order differentiation was performed. All spectra were smoothed with the Savitzky-Golay 9-point algorithm (SAVITZKY et al., 1964) as shown in Fig. 10. Fig. 10 (a) shows the entire region 4000 to 452 cm^{-1}, Fig. 10 (b) the saccharides region 1800 to 900 cm^{-1}, and Fig. 10 (c) the lipid region 3000 to 2800 cm^{-1}. Fig. 10 shows that the peak positions are almost aligned in any leaf spectrum, and it is considered that the fingerprint spectrum of lettuce could be identified.

The leaf surface of plants is covered with a layer consisting of an oily compound to prevent water evaporation and invasion from pathogens. The covering components are, in order from the upper layer, 1 epidermal wax layer, 2 intermediate layer, 3 cuticular layer, and the cell wall. It is considered that this thickness is approximately several micrometers. Considering that the penetration depth by the ATR method is also several micrometers, it is considered that the spectrum information obtained in this experiment mainly includes these surface information (DA LUZ, 2006; HARDIN et al., 2011). Therefore, we focused on the 3 components of wax, cellulose and pectin and examined the change in peak intensity. Since wax appears at wave number 2924 cm^{-1}, we focused on the change tendency of the second derivative peak intensity of this wave number. This wave number can be taken as a change tendency derived from the CH_2 stretch of two components including cellulose information. In addition, information of pectin appears at 1736 cm^{-1}. This wave number is derived from the C = O stretch of the methyl ester of galacturonic acid which is uronic acid constituting pectin (KYOMUGASHO et al, 2015). Finally, for cellulose, we focused on 1636 cm^{-1}, which has the highest peak intensity except for 2924 cm^{-1}, which is overlapped with wax. Because the peaks of wax, cellulose and pectin for 7 days had a large error and the variation, deterioration was not confirmed. The spectrum obtained with the measurement pressure of 10 N by the ATR method, which was not stable, and no change in organic matter was observed with the deterioration of lettuce.

Relationship between external and internal quality of lettuce

In this section, by combining two kinds of experiments, “Evaluation of freshness of lettuce” and “Freshness judgement experiment”, the external quality and inside quality were linked.

In continuous storage experiments, the water change curve and the color change curve of the whole headed lettuce were obtained, and the stage of deterioration of lettuce was characterized from the analysis of each characteristic curve. By adding the daily internal

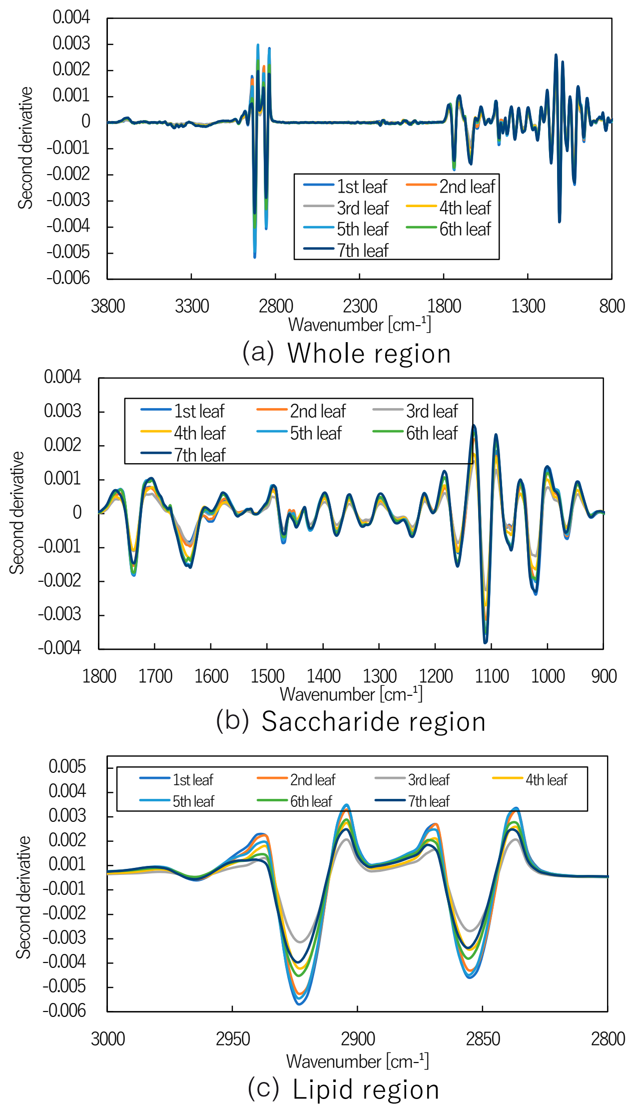


Fig. 10: FT-IR second derivative spectra of lettuce leaves.

information (elemental information and organic matter information) obtained in the demolition experiment with cardboard box storage to the deterioration characteristics of this lettuce, it is possible to clarify the overall external and internal deterioration characteristics of the lettuce.

The moisture change curve of lettuce obtained in the first experiment is considered as the basic characteristic of deterioration. Subsequently, color information, elemental information, and moisture information inside lettuce were added to the basic characteristics. Fig. 11 comprehensively represents the time when a large change was recognized in each kind of measurement information.

First, let us consider the moisture change curve of lettuce. At the first stage of moisture change up to 4 h after storage, i.e., at the initial stage of storage with the highest moisture content, the chemical potential gradient with the open air (solute potential + pressure potential) is the largest (Taiz et al., 2014). Therefore, since the surface moisture is most likely to decrease in this stage, it is thought that water loss is occurring constantly.

Furthermore, in the second stage up to 108 h after storage, the water loss from the inside of lettuce becomes steady after the water loss from 1st or 2nd outer leaves exposed to the lettuce surface is almost completed. Therefore, it is thought that the leaves near the surface keep constant moisture. This is also apparent from the fact that the moisture content of the 1st and 2nd leaves of the lettuce in Fig. 8.

In the third stage, after 109 h from the start of storage, the water loss of the outer leaves exposed to low relative humidity has ended and the ambient humidity inside the lettuce is kept high. This result is in good agreement with a small amount of constant rate moisture change from lettuce after 109 h.

Finally, in addition to the moisture change inside the lettuce at each stage, the quality change inside the lettuce caught from elements is considered in a combined manner. In this experiment, it was found that the macronutrients K and Ca tend to increase in the period of 3rd to 4th day of storage time, that is about 61 to 85 h. Therefore, it was speculated that the quality deterioration of lettuce occurred during this period.

In Fig. 11, firstly, the time axis of the moisture change curve and the surface color change curves were aligned. Next, 61 to 85 h of the quality degradation period captured from the elements were added to this time axis. As a result, it turns out that the change point of the hue value appears in 31 h before 61 h when the balance of macronutrients in the lettuce tissue was broken. In addition, the change point of the saturation value was recognized 90 h before 109 h when the moisture change rate of the outer leaf became constant. Thus, it was suggested that the change of the surface color could predict the deterioration and water loss of the lettuce. In addition, even after 109 h of the second stage change point of moisture, three change points were recognized in color information (hue, saturation, lightness). As a result, it was shown that it is possible to quantify or predict the degree of degradation inside lettuce using only the surface color of lettuce by clarifying the relationship between these change points and the internal quality. In order to clarify such relationships, it is urgently needed to improve the measurement and quantification methods of internal quality in the future. Furthermore, it is expected that it will be possible to connect the image information of lettuce and the internal information (elements and organic matter) of lettuce using machine learning and deep learning, and to establish a method of estimating the degree of deterioration (freshness).

Freshness judgment experiment -Storage characteristics

Color image analysis

The color image analysis was performed to acquire 6 items of hue H, saturation S, and lightness L of the leaf and core. In addition, the surface area of the sphere was determined by image processing. As a result, in these 7 items, a characteristic time-dependent change common to all individuals was observed. Color change of lettuce leaf was shown in Fig. 12. The hue H of the 6 heads of lettuce increased and then linearly decreased with the time, but some lettuce showed a completely different tendency as is shown in Fig. 12 (a). The saturation S in Fig. 12 (b) showed an increasing tendency after harvest, and the tendency changed between storage times of 91 to 115 h. The lightness L showed a decreasing tendency after harvest, and the decreasing tendency changed between storage time of 91-115 h as well as the saturation. The changing tendency was different for each lettuce individual as is shown in Fig. 12 (c). As a result, the time zone in which the change tendency of hue, saturation, and lightness changes is considered to be an important time zone in the determination of freshness.

In order to acquire the shrinkage information of the lettuce due to water loss, the projected areas of the lettuce heads were calculated from the acquired images. In each image, the contour extraction was performed, and the projected area was calculated in pixel unit. Fig. 13 shows the projected area change. As a result, the time-dependent change of surface area decreased linearly with a determination coefficient of 0.90 or more except for one individual. In addition,

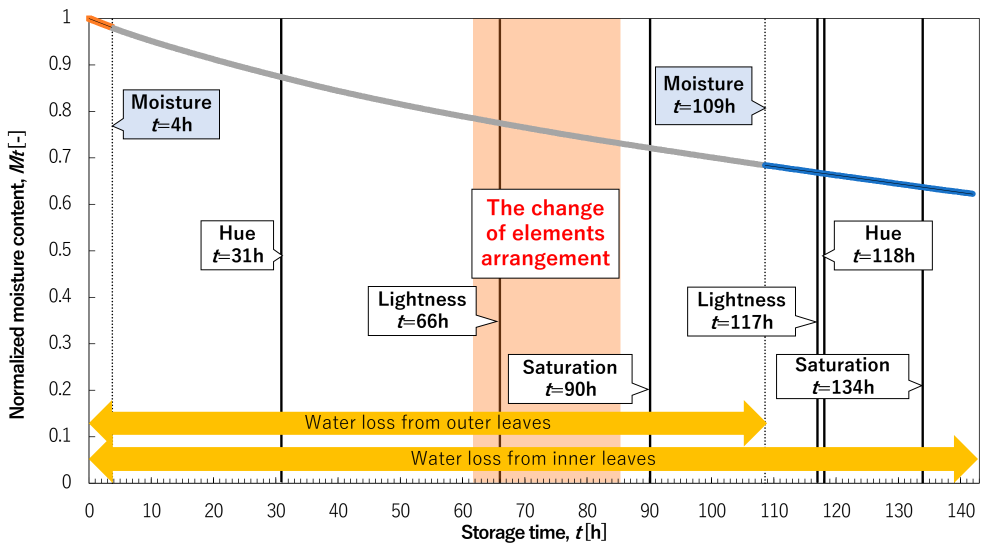


Fig. 11: The characteristic curb of the freshness of lettuce.

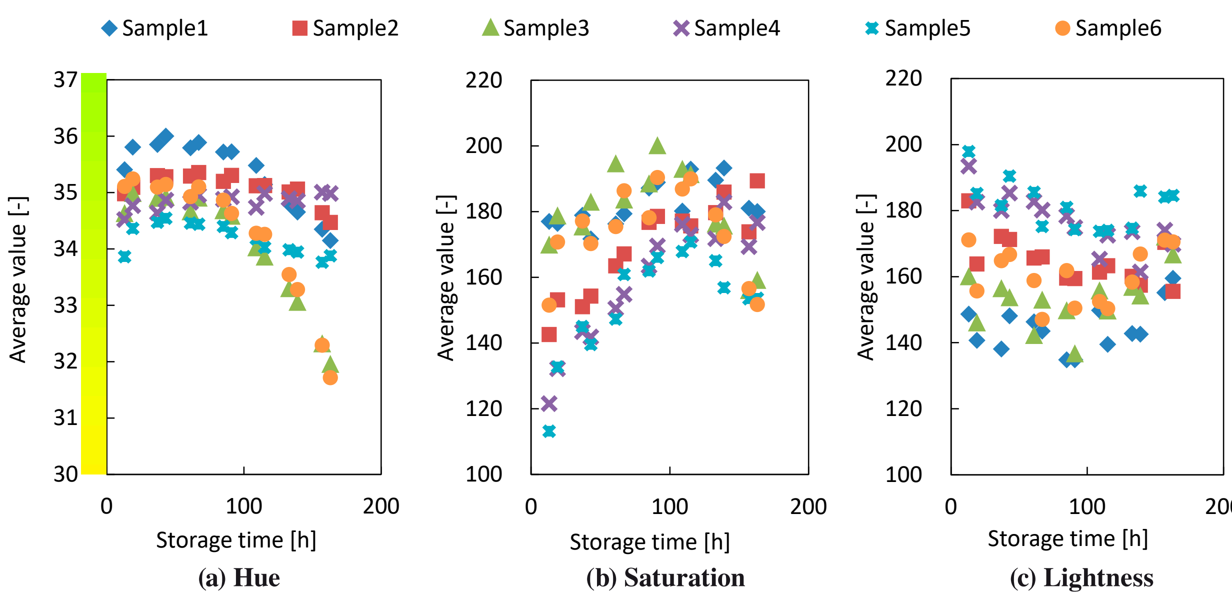


Fig. 12: Color change of lettuce leaves

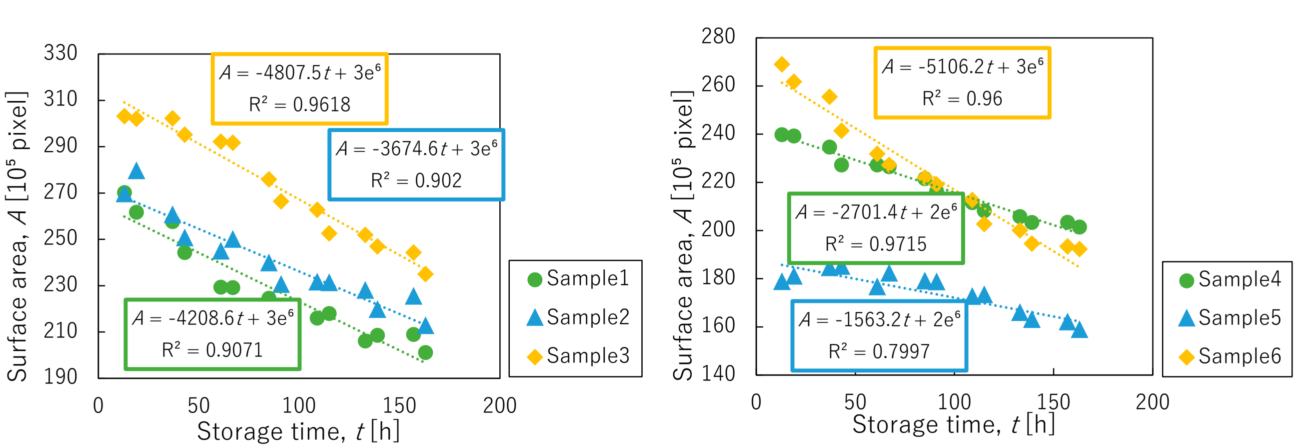


Fig. 13: Projected area (A) change of lettuce head

a positive correlation with a coefficient of determination of 0.90 or more was also found between the projected area and the water content except for one individual.

Change of elements contents

In the measurement of lettuce using an XRF analyzer, a total of 4 items of K, Ca, P and LE were measured. Fig. 14 shows the change of K, Ca, P and LE during storage.

Since information on a specific spot volume is acquired in XRF analysis, it is assumed that LE produces moisture information, and three other element information provides shrinkage information, assuming that no element outflow occurs in the cell. K in Fig. 14 (a) showed an almost linear increasing tendency in every individual. Ca, in Fig. 14(b), also showed a gradual upward trend. P in Fig. 14 (c) had a large variation in value and no characteristic tendency was observed. LE in Fig. 14 (d) showed a decreasing tendency, and a linear correlation with water showed a strong correlation with a coefficient of determination of 0.98.

Change of pigments contents

In pigments measurement, 4 items: chlorophyll value Chl, flavanol value Flav, anthocyanin value Anth, NBI value which means Chl/Flav were measured. Fig. 15 shows the change of these 4 values in the storage period. The Chl of almost all samples tended to decrease throughout the storage period. On the other hand, the Flav, the Anth, and the NBI value increased with time.

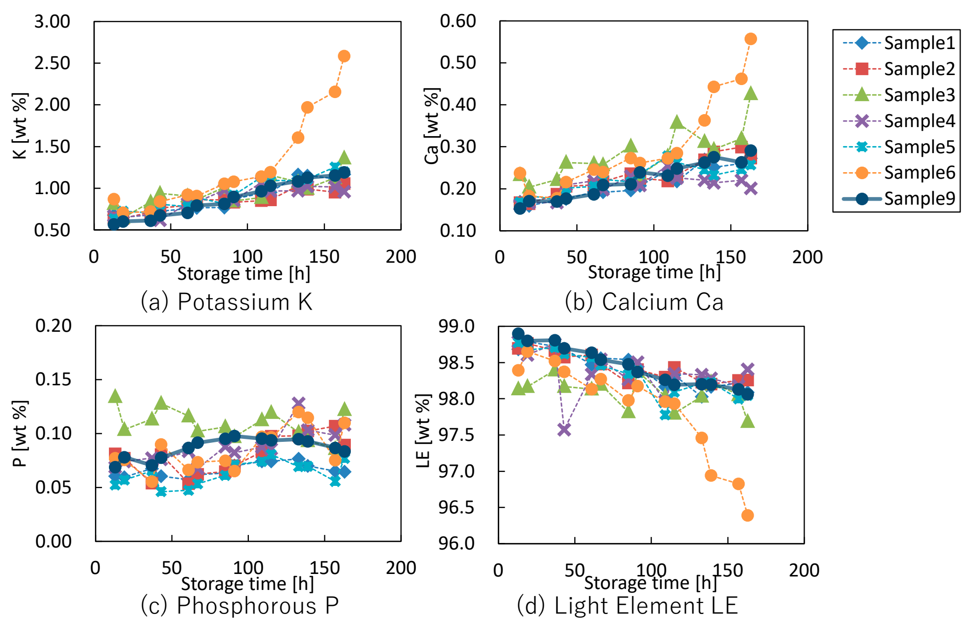


Fig. 14: Macronutrients (K, Ca, P) and light element (LE) change during storage

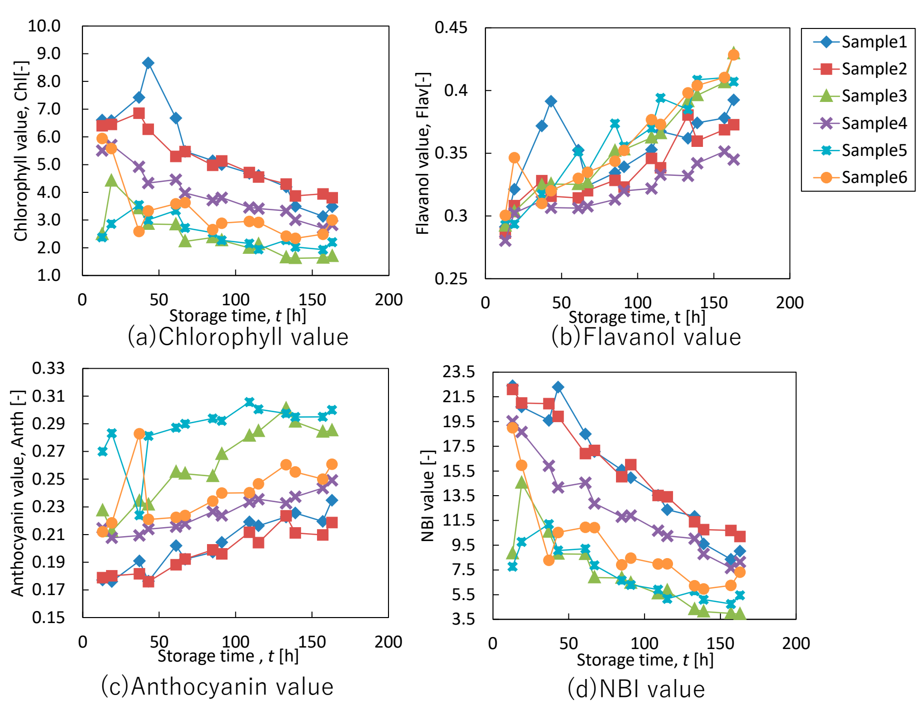


Fig. 15: Pigment (Chl, Flav, Anth, NBI) change

Data set creation and machine learning

The deterioration characteristic indices of all 15 items obtained by multi-optical measurement were used as a data set representing the freshness of lettuce. In order to find and classify patterns from a data set of 15 parameters for each measurement time of each individual, clustering by K-means was performed using 84 data sets of 15 measured items of 6 heads of lettuce in 7 days. The statistical package R (version 3.3.1) Cluster package was used for this analysis. Moreover, the measurement result of 15 parameters were standardized and used for analysis for each individual.

In order to visualize the results, the first principal component and second principal component scores of 15 parameters were plotted, and belonging clusters classified by the K-means method were color-coded. The results are shown in Fig. 16 (a). In addition, classification results for each sample are shown in Fig. 16 (b). As a result, the first principal component score decreased with the deterioration of lettuce in any sample. However, focusing on the score of the second principal component, it was found that the trend changed from increase to decrease between 85 h and 109 h after harvest, and that the group to which the sample belonged changed. The calculated cumulative contribution of the second principal component was about 77%. It can be read that the same classification is performed in the clustering result. Therefore, it was suggested that lettuce can be roughly classified at the degradation stage by using the data set by multi-optical measurement this time.

This classification result was used as the correct answer in discriminant analysis, and discriminant analysis was performed using decision trees. Subsequently, the data set was divided into 42 sets of training data and another 42 sets of test data, and a freshness judgment model was constructed. Then, its accuracy was verified. Python (version 2.7.10) and Scikit-learn (version 0.16.1), which is an open source machine learning library, were used to construct a decision tree classification model. Also, GraphViz (version 2.38.0) was used to visualize the graph. The model was created using entropy, a condition that attempts to maximize mutual information. In addition, the decision tree depth adopted 3 because it was confirmed in the previous examination that almost the same result was obtained at the tree depth 3 and 4. For evaluation of the model, k-fold cross validation was used, and the number of divisions specified was 10, which is generally used.

The internal information of lettuce is obtained only by XRF analysis. Therefore, the possibility of freshness judgment using only XRF analysis is considered. Decision tree modeling was performed with the detection values of K, Ca, P, and LE in the XRF analysis as feature amounts and the classification results obtained by the K-means method as dependent variables. The created model is shown in Fig. 17. The accuracy rate in cross validation was 83.3%, and the accuracy rate using the evaluation data set was 80.9%. It was confirmed that a model with relatively high accuracy can be created. In this study, only the classification of goodness and badness of freshness was performed, but it is necessary to construct a regression model with an increased number of samples.

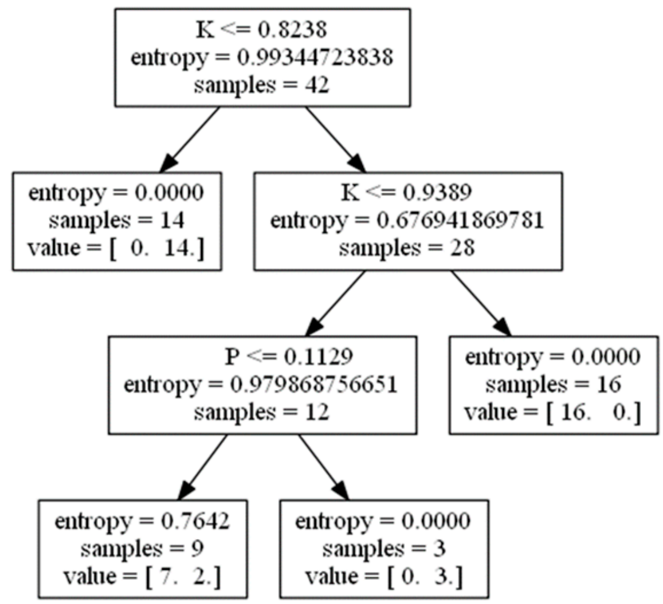
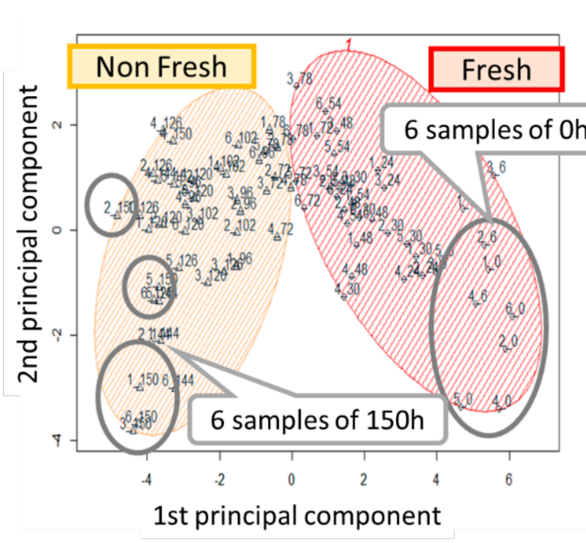


Fig. 17: Decision tree model using only XRF analysis data

Conclusions

This study showed the effectiveness of optical sensing for evaluating the freshness.

1. Water change and surface color change of lettuce individual
Water movement from inside to outside of one head of lettuce dur-



(a) Result of principal component analysis

Storage time [h]	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6
0h	Fresh	Fresh	Fresh	Fresh	Fresh	Fresh
6h	Fresh	Fresh	Fresh	Fresh	Fresh	Fresh
24h	Fresh	Fresh	Fresh	Fresh	Fresh	Fresh
30h	Fresh	Fresh	Fresh	Fresh	Fresh	Fresh
48h	Fresh	Fresh	Fresh	Fresh	Fresh	Fresh
54h	Fresh	Fresh	Fresh	Fresh	Fresh	Fresh
72h	Fresh	Non Fresh	Non Fresh	Non Fresh	Fresh	Fresh
78h	Non Fresh	Non Fresh	Fresh	Non Fresh	Non Fresh	Fresh
96h	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh
102h	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh
120h	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh
126h	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh
144h	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh
150h	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh	Non Fresh

(b) Detail of classification

Fig. 16: Classification results by K-means method

ing storage was qualitatively explained by combining the moisture change of the lettuce obtained in the continuous storage experiment with that of each leaf (1st to 7th leaves) obtained in the demolition experiment. Furthermore, it was suggested that the degradation stage of lettuce could be predicted by matching the lettuce color change point and water change curve by continuous image processing. In addition, since the distribution of the color (hue, saturation) on the lettuce surface could be fitted to the normal distribution and the Gamma distribution, it became possible to analyze the lettuce surface image using the parameters of the distribution function.

2. Machine learning of lettuce freshness
Focusing on the score of the second principal component, the quality changing trend changed from increase to decrease between 85 h and 109 h after harvest. This classification result was used as the correct answer in discriminant analysis, which was performed by means of decision trees using the data set obtained by XRF analysis. The accuracy rate in cross validation was 83.3%, and the accuracy rate using the evaluation data set was 80.9%, confirming that a model with relatively high accuracy can be created.

3. Elemental measurement by XRF
Since K, Ca, and P showed different behaviors in the intracellular balance of the multiple elements of lettuce, it is suggested that it is possible to correlate changes in the element balance inside lettuce with any deterioration during the lettuce storage process.
In addition, since the apparent increase in measurable macronutrients (Ca, K) in the XRF field was observed with the decrease in moisture content of lettuce leaves, it was suggested that shrinkage information associated with water loss in lettuce could be obtained through the change in the amount of elements.

4. Measurement of organic matter by MIR spectrum
From the MIR spectrum of lettuce leaves, peaks derived from wax, cellulose and pectin, which are surface components of the leaves, were identified. In addition, we could identify the fingerprint spectrum of lettuce, including other peaks.

5. Freshness determination using machine learning
It was shown that it is possible to quantify or predict the degree of degradation inside lettuce using only the surface color of lettuce individuals. In order to clarify such relationship, in the future, improvement of the measurement method and quantification method of internal quality is required. Furthermore, it is important to connect the image information of lettuce individual and the internal information (elements and organic matter) of lettuce using machine learning and deep learning, and to establish a method of estimating the degree of deterioration (freshness).

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Conflict of interest
No conflict of interest was declared by the authors.

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