論文の内容の要旨

生物・環境工学 専攻

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李玉（リ ユウ）

指導教員 牧野義雄

論文題目

Nondestructive detection of decay in green vegetables stored at different temperatures using chlorophyll fluorescence imaging

（各温度で貯蔵した緑色野菜のクロロフィル蛍光画像による非破壊鮮度評価）

1. Introduction

Vegetable soybeans (*Glycine max* (L.) Merr.) ("*Edamame*") are very popular because of their delicious taste, rich nutritional value, short crop cycle, and export value. However, in East Asia, which is the main region of vegetable soybean production, the harvest period is mainly during summer season. Because of the climatic heat and their active respiration, vegetable soybeans are very vulnerable to perishing after harvest (Sugimoto et al., 2010).

Broccoli (*Brassica oleracea* L. var. *italica*) is perishable and susceptible to senescence after harvest. Compared with other horticulture products, the respiration rate in broccoli heads is extremely high. The symptom of senescing broccoli is floret yellowing and freshness decreasing, resulting from chlorophyll losing (Aiamla-or, et al., 2014). Therefore, there is a need for research on how to evaluate the quality and predict the shelf-life in both vegetable soybeans and broccoli.

Chemical parameters, such as chlorophyll concentration, vitamin C, and enzymes (Wang et al., 2017) have been used to evaluate the quality of fruits and vegetables, but the process of obtaining these indicators is destructive. Hence, it is important to develop a non-invasive and chemical-free

method for estimating the quality of fruits and vegetables.

Chlorophyll fluorescence technology called as photosynthesis research probe having advantages of rapidity, no damage, objective and compact. In consideration of the chlorophyll fluorescence originates from light-excited chlorophyll *a* molecule associated with photosystem II (PSII) (DeEll et al., 1999; Henriques, 2009; Maxwell and Johnson, 2000), the ripening and senescence of fruits and vegetables may affect the chlorophyll fluorescence yield. Upon that, in recent decades, chlorophyll fluorescence technology, as a nondestructive detection, has also been used to investigate the decay and senescence of horticultural products (DeEll and Toivonen, 2003; Gorbe and Calatayud, 2012).

And for consumers, their primary demand for green vegetables is good appearance, the surface color in particular. In addition, if the nutritional content could be known, this will increase the possibility of purchase for consumers. Therefore, it's also meaningful to examination of the internal quality, such as L-ascorbic acid, by a non-invasive method of detection. However, there already have overwhelming publications of chlorophyll fluorescence imaging on photosynthesis, maturation and postharvest behavior of fruits and vegetables, but the research on exploring internal attribute L-ascorbic acid using this nondestructive method were rare.

The project of this study aimed at evaluating and estimating the freshness and senescence of vegetable soybeans and broccoli using the new approach that consists of 98 chlorophyll fluorescence parameters. Furthermore, the objective of this study was to investigate the velocity of decay by discussing the relationship between chlorophyll fluorescence parameters and external quality color, internal quality attribute L-ascorbic acid content in both vegetable soybeans and broccoli heads.

2. Materials and Methods

(1) Materials preparation: A. Vegetable soybeans (cv. Yuagarimusume) were harvested in Saitama and Ibaraki prefectures. They were stored at 10 °C (storage period: 14 d), 15 °C (storage period: 10 d), and 25 °C (storage period: 6 d), 90% relative humidity in a thermo-hygrosta. B. Broccoli heads (cv. "Pixel") were harvested at Saitama prefecture. The broccoli heads were stored at 10 °C (storage

period: 12 d) and 25 °C (storage period: 3 d), 90% relative humidity.

(2) Chlorophyll fluorescence imaging: The samples were dark-adapted for 20 min before measured the chlorophyll fluorescence applying FluorCam 800MF. A total of 44 fluorescence parameter, such as Fo, Fm, Fp and Ft, were recorded directly and the other 54 parameters, including Fv, qP_Lss, qL_Lss (steady state in light), NPQ_Lss and Rfd_Lss were calculated by FluorCam 7.

(3) Color determination: The value of h^{o} in vegetable soybeans and $-a^*/b^*$ in broccoli was employed to express the color. A digital camera was used to capture photographs, and the data were calculated using the MATLAB software.

(4) Measurement of chlorophyll content: Chlorophyll was extracted from samples using 80% acetone (v/v). The absorbance values were read at 663.2 nm and 646.8 nm for chlorophyll *a* and total chlorophyll content utilizing an UV-3600 spectrophotometer.

(5) Measurement of L-ascorbic acid content: The samples were mixed with 1 ml, 3% meta-phosphoric acid, and the centrifuged supernatant was measured with ascorbic acid test strip by reflectometer (RQflex 10).

(6) Partial least squares regression (PLSR): PLSR was used to determine the relationship between color, L-ascorbic acid, and 98 chlorophyll fluorescence parameters using the Unscrambler ver. X10.3.

3. Results and Discussion

This study not only investigated the relationship between the chlorophyll fluorescence and appearance color, but also rarely used this nondestructive method to explore interior quality L-ascorbic acid content.

(1) There is a strong relationship between color (h^{o}) and total of 98 chlorophyll fluorescence parameters. Furthermore, L-ascorbic acid (seeds) also exhibits high relationship with chlorophyll fluorescence parameters in seeds, with R^2 of 0.827 and RMSECV of 2.095 mg 100 g^{-1} obtained by PLSR. The more interesting thing is that we also achieved the well result in using the nondestructive (CF) parameters of the whole vegetable soybeans to estimate the internal L-ascorbic acid in seeds,

with an R^2 of 0.869 and RMSECV of 2.159 mg 100 g^{-1}. Accordingly, some parameters, such as Fm, Fv, Fq_Lss, QY_Lss, and qL_Lss, that make larger contributions to estimate color (h^o) were determined from a total of 98 chlorophyll fluorescence parameters. What's more, parameters Fv, Fq_Lss, QY_Lss, qP_Lss, and qL_Lss (seeds), which are important for predicting L-ascorbic acid were also selected. More importantly, parameters Fv_Lss, Ft_Lss (whole vegetable soybeans), may be possible to estimate vitamin C (seeds) by this nondestructive detection in vegetable soybeans.

(2) There also showed the well predictive ability of color (− a*/ b*) with total of 98 chlorophyll fluorescence parameters in broccoli conducted using PLSR. Some parameters, such as Fm, Fv, that has strong relationship with the color have also been selected in broccoli. And the changes in parameters NPQ_Lss and Rfd_Lss displayed more sensitive than color changes, particular from initial 0 d to the second storage period. That is to say, some chlorophyll fluorescence parameters could do earlier detection, which was not visible to the other nondestructive detection, particular at the beginning of the decay. Moreover, chlorophyll fluorescence presented good estimating ability in L-ascorbic acid of broccoli with the result of R^2 of 0.826 and RMSECV of 4.812 mg 100 g^{-1}.

In other words, the chlorophyll fluorescence technique, as a non-invasive, chemical-free, and simple screening method, has great potential in examining freshness and estimating the decay in green vegetables.

4. Reference

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