Nondestructive Detection of Decay in Vegetable Soybeans Stored at Different Temperatures Using Chlorophyll Fluorescence Imaging

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(Rceived October 31, 2019; Accepted January 8, 2020)

To achieve nondestructive evaluation of the quality of vegetable soybeans, we have applied chlorophyll fluorescence imaging, which has been used as a non-invasive technology to investigate the ripening and senescence of horticultural products. This study measured changes in chlorophyll content, color (h), and chlorophyll fluorescence of vegetable soybean pods and seeds and rarely used a nondestructive approach to explore interior quality L-ascorbic acid content. The relationship between chlorophyll fluorescence parameters and visual quality attribute h, and that between chlorophyll fluorescence parameters and L-ascorbic acid content were performed using partial least square regression (PLSR). Thus, the fluorescence parameters Fm, Fv, Fq_Lss, QY_Lss, and qL_Lss, which make larger contributions to evaluating h in both whole vegetable soybeans and seeds were selected from 98 chlorophyll fluorescence parameters. Moreover, parameters Fv, Fq_Lss, QY_Lss, qP_Lss, and qL_Lss in seeds, which are important for estimating L-ascorbic acid of seeds were also determined. Most importantly, parameters Fv_Lss and Ft_Lss in whole vegetable soybeans, which may be possible to predict L-ascorbic acid content of seeds by a nondestructive detection. The chlorophyll fluorescence technique presents great potential in examining freshness and estimating the decay in vegetable soybeans.

Keywords: ascorbic acid, chlorophyll fluorescence, color, nondestructive detection, quality, vegetable soybeans

INTRODUCTION

Vegetable soybeans (Glycine max (L.) Merr.) (“Edamame”) have emerged as one of the most commercially important foods, and they are very popular because of their delicious taste, rich nutritional value, short crop cycle, and export value. Vegetable soybeans are a good source of protein, monounsaturated fatty acids (with no cholesterol), anti-carcinogenic isoflavones, dietary fiber, vitamin C (L-ascorbic acid), vitamin E (tocopherols), and phytoestrogens (Johnson et al., 1999; Miles et al., 2000). From this, vegetable soybeans have high commercial value as an agricultural product that is healthy for consumers. 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).

Therefore, with the rapid worldwide increase in demand for high-quality vegetable soybeans, there is a need for research on how to evaluate the quality and predict the shelf-life of vegetable soybeans. 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, which is related strongly to the photosynthesis reactions, has the advantages of causing no damage and being both objective and compact. It has been widely applied to studies of plant photosynthesis (Baker, 2008), plant adversity and stress (Omasa, 1990; Calatayud et al., 2006), and plant pathology (Meyer et al., 2001).

Consumers’ primary demand for vegetable soybeans is good pod appearance, the surface color in particular, and the pods color should be bright green with no sign of yellowing (Konovsky et al., 1994). The loss of green color is indicative of declining freshness and chlorophyll degradation. The decrease in chlorophyll content is due to the decomposition of chloroplast. This will lead to the reduction of photosynthetic activity. In consideration of the chlorophyll fluorescence originates from light-excited chlorophyll a molecule associated with photosystem II (PSII) (DeEll et al., 1999; Maxwell and Johnson, 2000; Henriques, 2009), the ripening and senescence of fruits and vegetables may affect the chlorophyll fluorescence yield. Upon that, in recent decades, chlorophyll fluorescence technology has also been used to investigate the ripening and senescence of horticultural products (DeEll et al., 1999; DeEll and Toivonen, 2003; Gorbe and Calatayud, 2012), including apple (DeEll, 1999), banana (Smilie et al., 2000), and mango (DeEll et al., 2000).
and Ibaraki prefectures, Japan. They were stored at 10°C for several days and tested early in the morning from local farms in Saitama Prefecture, Japan. Vegetable soybeans were harvested in different years: 2017, 2018, and 2019. The experiments were conducted using fresh vegetable soybeans from the field and from the market. The vegetable soybeans were stored at 10°C for several days and tested early in the morning from local farms in Saitama Prefecture, Japan.

Data were collected using a non-destructive method of chlorophyll fluorescence imaging on photosynthesis, maturation, and postharvest behavior of fruits and vegetables. The purpose of this study was to evaluate and predict the freshness and senescence of vegetable soybeans using the new method that consists of 98 chlorophyll fluorescence parameters. Some of these parameters, including Fv, Fv/Lss, Fq/Lss, QY/Lss, qP/Lss, and qL/Lss, were calculated using FluorCam 7 (Table 1).

### MATERIALS AND METHODS

#### Materials preparation

Vegetable soybeans (cv. Yuagarimusume) were harvested early in the morning from local farms in Saitama and Ibaraki prefectures, Japan. They were stored at 10°C (storage period: 0 day, 10 days, 14 days), 15°C (storage period: 0 day, 15 days, 20 days, 30 days), and 25°C (storage period: 0 day, 3 days, 5 days, 6 days), at 90% relative humidity in a thermo-hygrostat (FLI-301NH, EYELA, Tokyo, Japan). Twelve vegetable soybeans and ten seeds were used for non-destructive detection (such as color determination and chlorophyll fluorescence imaging). Some other samples were frozen by liquid nitrogen and stored at −80°C. Seeds and pods were collected separately. That's for the chemical measurement of chlorophyll content and L-ascorbic acid content.

#### Chlorophyll fluorescence imaging

Twelve vegetable soybeans and ten seeds were dark-adapted for 20 minutes after each storage period and the chlorophyll fluorescence was then measured using FluorCam 800MF (Photon Systems Instruments (PSI), spol. s r. o., Brno, Czech Republic). Operation of both the camera and the light sources was controlled according to a protocol that was designed in the FluorCam software, and the fluorescence data were also analyzed using FluorCam 7 (PSI). A total of 44 fluorescence parameter, such as Fv, Fm, and Ft/Lss, were recorded directly and the other 54 parameters, including Fv, Fv/Lss, Fq/Lss, QY/Lss, qP/Lss, and qL/Lss, were calculated using FluorCam 7 (Table 1).

#### Color determination

External color was determined using the Commission Internationale de l’Éclairage (CIE) color system: the a* axis extends from green (−a*) to red (+a*), and the b* axis extends from blue (−b*) to yellow (+b*). The brightness (L*) increases from the bottom (black) to the top (white) in three dimensions. The value of the h' with red at 0°, yellow at 60°, and green at 120° was used to express the color of the vegetable soybeans according to the method published by Makino et al. (2016). A computer vision system (FMVU-1352C-CS, Point Grey Research Inc., Richmond, British Columbia, Canada) was used to capture photographs for the vegetable soybeans, and the data were calculated using the MATLAB (2018a) (Mathworks, Inc., Natick, MA, U.S.A.) software (12 samples for vegetable soybeans and 10 for seeds). The h' was calculated as: h' = tan⁻¹(b*/a*). The absorbance values were read at 663.2 nm and 646.8 nm for chlorophyll a and total chlorophyll content using an UV-3600 spectrophotometer (Shimadzu Corp., Kyoto, Japan).
ble soybeans by this nondestructive detection.

ACKNOWLEDGEMENTS

This study was supported by JSPS Grant-in-Aid for Scientific Research (A) (25252045), JSPS Grant-in-Aid for Scientific Research (B) (15K00626), and the Toyojo-Iijima Foundation for Food Science and Technology (2018 Research Fund). The authors also feel like appreciating the anonymous reviewers for their constructive suggestions and comments.

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