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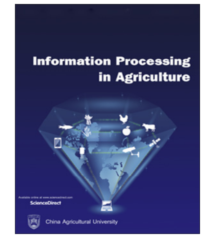

A statistical analysis of the freshness of postharvest leafy vegetables with application of water based on chlorophyll fluorescence measurement

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ABSTRACT

Vegetable freshness is very important for both restaurant and home consumers. In market, sellers frequently apply water to leafy vegetables to make them not lose weight and look fresh; however, these vegetables may not be stored for a long time as they appear. After a time limit, they may be quickly rotten. It is thus meaningful to investigate early and simple detection tools to measure leafy vegetable freshness while they are frequently applied water in selling. In this work, three types of newly harvested leafy vegetables were bought from a local farmer market and stored in the air with room temperature and roots submerging in water. Chlorophyll *a* fluorescence (ChlF) from the vegetables was measured each half a day for three days. The obtained ChlF data were analyzed statistically and the correlation of ChlF parameters and vegetable freshness/storage time was obtained. The k-mean classification was also performed. It is found that Fo, Fj, Fm/Fo, and Fv/Fm can be used as an early detection tool to differentiate the freshness of leafy vegetables on which water is constantly applied in storage without visible difference.

1. Introduction

Fresh food is very important [1]. Leafy vegetables are an important source of food and nutrition to human. As a counterbalance of many degenerative diseases including several bioactive compounds like vitamins, minerals, pigments, and antioxidants, consumption of green leafy vegetables has been increased [2,3]. Sometimes, they are consumed without cooking, for example, in salad and hamburger. Tourists are vulnerable to stomach problems because they are in transition of living environment such as water source and weather conditions. To them, serving non-fresh leaf vegetables may cause more serve problems. For home consumers, buying fresh vegetables is also very important because the vegetables are often stored in home for several days before they are consumed, during which, rotten may quickly happen after certain storage time.

Various methods have been proposed in literature to measure the freshness of leafy vegetables. Chlorophyll content of

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Abbreviations: Fo, minimum ChlF intensity in the dark-adapted state; Fj, ChlF intensity at the J step on the well known OJIP ChlF kinetics; Fi, ChlF intensity at the *I* step on the well known OJIP ChlF kinetics; Fm, maximum ChlF intensity in the dark-adapted state; Fv, maximum variable fluorescence, Fv = Fm – Fo; Vj, Vj = (Fj − Fo)/(Fm − Fo); Vi, Vi = (Fi − Fo)/(Fm – Fo)

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green leafy vegetables can be used as visible parameters for the quality of vegetables during storage since it will be degraded gradually along with post-harvest senescence [3]. However, extraction of the pigments using an organic solvent is time-consuming, destructive, and requires the use of flammable chemicals. Amin and Dey [4] studied the fruit and vegetable freshness through electrochemical analysis. Many factors affect postharvest leafy vegetable freshness, such as storage temperature, humidity, and packaging style. Many efforts were performed at different levels of details to study postharvest vegetable freshness even considering the influence of harvesting times of a day [5,6]. Spinardi et al. [7] studied quality changes of spinach and lettuce baby leaves in storage at the temperature of 4 °C and 10 °C. The obtained results showed that chlorophyll, carotenoids, and phenols did not change for the two types of vegetables after 6 days of storage at the two temperatures while ascorbic acid declined during the storage.

In photosynthesis, part of the absorbed light energy may be emitted through fluorescence from chlorophyll *a* molecules, which is the commonly known chlorophyll *a* fluorescence (ChlF). The ChlF emission is the competition of absorbed light energy with heat production and forward photosynthetic activities. When a dark-adapted plant leaf is excited by a constant light, the ChlF emission will go through multiple stages of initial increases followed by decreases. This is commonly referred to as the Kautsky effect. The initial increasing part itself of the Kautsky effect is a complex curve with the ChlF variations from the O step to the J step (commonly called the OJIP induction curve), reflecting high-order nonlinear dynamics of PSII resulting from light excitation, energy transfer, energy dissipation, and photochemical reactions [8–12].

Plant physiological status changes can lead to the forward photosynthetic activities changes and thus ChlF can be potentially used to indicate plant physiological conditions [13–16]. For vegetables on the market, removal from soil may lead to physiological changes because the roots are not able to interact with soil for nutrition although the vegetables are constantly applied water and look fresh in a few days. Therefore, ChlF can be used to detect leaf vegetable freshness before visible difference can be observed. ChlF is very attractive in horticulture because of its high sensitivity to the changes in plant metabolic status [17–20]. ChlF was found highly correlated with anaerobic volatile content in broccoli during modified atmosphere packages and after opening of the modified atmosphere packages [21]. Schofield et al. [22] studied the storage potential of iceberg lettuce through ChlF and found that at-harvest ChlF of a subset of individuals randomly selected could represent the postharvest quality of the entire group. Viacava et al. [23] studied the storability of mini head lettuces at optimal and abusive temperatures through ChlF measurement. Keutgen et al. [24] used ChlF to study possible changes of Spinach under simulated sale condition with the storage temperature of 16 h in a cooling room at 2-4 °C followed by 8 h at room temperature 22 °C. The results showed that the optimum quantum yield was linearly correlated with calcium content, which might indicate worse cell membrane integrity and reduction in cell wall stability. The decrease of Fv/Fm during storage time may indicate cold stress as well as senescence. However, they did not find a relationship between ChlF and color changes of leaves.

In markets, sellers constantly apply water to leaf vegetables to make them look fresh and not lose weight. Actually, they may have been harvested for several days. These vegetables may not be stored for as long as they appear because they may quickly become rotten after certain time. Unfortunately, there is no literature focusing on the changes of vegetable with constant application of water as simulated selling in farmer markets. It is thus meaningful to study sensing tools to measure leafy vegetable freshness while they are constantly applied water to make them appear fresh.

This work aims to find a potential early detection method to differentiate leafy vegetables with different harvesting time while they are constantly applied water and have no visible difference. ChlF was measurement from three types of leaf vegetables while they are stored in air and applied water constantly to keep them look fresh without visible difference. Various ChlF parameters were tested statistically to check if some ChlF parameters can be used to detect leaf vegetable freshness while they look fresh.

2. Materials and experiments

Fresh spinach, cabbage, and lettuce are all commonly consumed leafy vegetables. They were bought from a local farmer market in the early morning with environmental temperature around 20 °C. They were bought right after they were harvested and the seller did not apply water to the vegetables. The vegetables were all intact except for part of their roots were cut. They were transported to a laboratory with roots submerging in water to keep them fresh for all the time. The laboratory temperature was maintained at around 25 °C.

Dark-adaption clips were applied to the leaf 30 min before experiments were performed to reach dark adapted status as shown in Fig. 1. ChlF from the leaves were measured by FluorPen, PSI (Photon Systems Instruments) (Czech Republic) with the OJIP protocol as shown in Fig. 2. The ChlF was measured in each morning and afternoon for total five times (the 1st morning, the 1st afternoon, the 2nd morning, the 2nd afternoon, and the 3rd morning) in the 2.5-day time span. Because the measured ChlF parameters can show statistical difference between the measurements from different

Fig. 1 – Dark-adaption clips setup in the experiments.