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Chlorophyll fluorescence imaging analysis for fresh quality assessment of apple and kiwi fruits preserved under different storage conditions

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***Abstract*—The objective of this study was to find a rapid determination of the freshness of apple (*Malusdomestica*) and kiwi (*Actinidiadeliciosa*)fruits using portable chlorophyll fluorescence imaging instrument. An imaging analysis of the photochemical responses of apple and kiwi preserved under the different storage conditions were conducted to assess the fresh quality offruits on the basis of the photochemical chlorophyll fluorescence analysis. The experiments were executed on the fruits by chlorophyll imaging.**

The storage for the fruits were carried out under room temperature (control), heat (42°C), wet (25°C and 80% relative humidity), and chilling (4°C) conditions.

Chlorophyll fluorescence imaging (CFI) method showed that the decrease in $F_v/F_m$and ΦPSII were lower under the chilling stress than the other conditions in apple and kiwi. In heat condition of apple, the image of F_v/F_m ratios, ΦPSIIand non-photochemical quenching (NPQ) are effective photochemical parameters. It was clearly indicate that the chilling condition is the suitable storage method for fruits. The CFI method is applicable as a rapid screening method for thedetermination of fruit freshness.

Index terms -Chilling, Chlorophyll fluorescence imaging, Fruit freshness, Heat, Storage, Wet condition

I. INTRODUCTION

Fresh fruits andvegetables are two main sectors of agricultural markets, and fresh produce is important to the health and well-being of consumers. The term “quality” refers to the degree of excellence of a product or its suitability for a particular use. Quality of produce incorporates several properties, including sensory properties (appearance, texture, taste, and aroma), nutritive value, chemical constituents, mechanical properties, functional properties, and defects. To evaluate quality, producers and consumers use all of their senses, including sight, smell, taste, touch, and even hearing. The consumer integrates all of these sensory inputs (appearance, aroma, flavor, feel, texture, and sounds made while chewing) into a final judgment of the acceptability of that fruit or vegetable. Merchants, consumers, processors, and producers usemany standards to evaluate the quality of fresh fruits and vegetables. Cold chain systems are used to preserve the freshness of produce from harvesting through marketing and delivery to the consumer. Cold chain systems have had a tremendous impact on the marketing of fresh produce. For every 10°C temperature change, there is a corresponding two- to four-fold change in the respiratory activities of fresh produce[1]. Therefore, it is necessary and important to evaluate the degree of damage and change in physiology induced by the stress of exposure to different temperatures. Many fruits are stored under cold, heat or wet conditions. Therefore, a rapid and ease quality control technique during marketing and post harvesting is needed.

In the context of fruit quality control, chlorophyll a fluorescence transient analysis, so-called JIP-test, and chlorophyll fluorescence imaging (CFI) technique may be able to apply to investigate the energetic behavior of photosynthetic sensory systems. The JIP-test is a tool to analyze the polyphasic rise of the chlorophyll *a* (Chl *a*) fluorescence transients (phases labeled “OJIP”). Although it corresponds to only a very small fraction of the dissipated energy from the photosynthetic apparatus of fruit surface, Chl *a* fluorescence is widely accepted to provide a means to a better understanding of the structure and function of the photosynthetic apparatus. At room temperature, the Chl *a* fluorescence of plants, algae, and cyanobacteria, in the 680–740 nm spectral region, is emitted mainly by photosystem (PS) II, and thus it can serve as an intrinsic probe of the fate of its excitation energy. The spectra and the kinetics of Chl *a* fluorescence are powerful, non-invasive tools for such investigations [2].

Several studies investigating the stoichiometry of PS I and PS II have used Chl *a* fluorescence as a monitor of Q_A reduction and thus of PS II activity, and absorption changes at 820 nm have been used as a monitor of P700 oxidation and, hence, of PS I activity [3]. An early attempt to measure P700 absorption and Chl *a* fluorescence simultaneously at 77 K was made by Strasser and Butler [4] who studied migration of excitation energy from PS II to PS I. Schreiber et al. [5] introduced parallel measurements for quantum yields of PS II (using Chl *a* fluorescence) and PS I (using absorbance changes at 830 nm) in leaves, using a modulated instrument. This method for simultaneous measurements of PS I and PS II was further improved by Havaux et al. [6] and by Klughammer and Schreiber [7] and was recently exploited by Eichelmann and Laisk[8] to describe the cooperation between PS I and PS II in leaves.

Most studies analyzing the effects of heat or chilling stress on OJIP transients have been conducted on plant leaves [9, 10] but not precisely in fruits. Even these studies have been limited to apple [11-13]. Photosynthetic activities differ between leaves and fruits; for example, in the pericarp of cherry tomato, photosynthetic fixation of $^{14}CO_2$ has been shown to occur at higher rates than in the leaves [14]. Thus, under wet, heat, or chilling stress, changes in the photosynthetic apparatus of pericarp of fruits may differ for the storing of fruits. The effects of wet, heat, and chilling stresses on the photosynthetic apparatus in fruits surface have not been elucidated to determine the freshness.

The photosynthetic apparatus is the most sensitive component in evaluating the degree of temperature-related stress damage [15]. CFI technique has been mainly used as effective tools in order to study the damage and activity of the electron transport chain in the photosynthetic apparatus under various environmental stresses. CFI as a rapid and non-destructive technique has quickly progressed, and has been used successful in evaluating plant photosynthetic activity. CFI incorporates advancements in the technology of light emission, imaging detectors, and rapid data handling [16].

This study was performed to evaluate the validity of the fluorescence imaging information as a stress indication and to determine the freshness of apple and kiwi fruit.

II. Materials and Methods

Fresh fruits of apple (*Malusdomestica*) and kiwi (*Actinidiadeliciosa*)were purchased from a supermarket. For each crop, 15 fruits of similar appearance were selected and divided among four treatment groups of three fruits each. The four treatments were heat, chilling, wet, and room temperature as a control.

A. Storage condition of fruits

All of the treatments were measured on day 0 (control) prior to exposure to the respective temperature stress as described below. For the heat storage condition, the fruits were placed in a growth chamber at a temperature of 42°C. Fruits in the chilling storage condition were stored in a refrigerator at 4°C. For the wet condition treatment, the fruits were submerged under water in a bucket regulated with over 80 % relative humidity and kept at room temperature. Control fruitswere placed in a bucket and kept at room temperature. All treatments were performed in the dark, and each treatment was carried out in three replications.

B. Measurement of chlorophyll fluorescence imaging

The fruits were measured separately for each treatment after exposure to the respective stresses. Measurements were performed in a dark room, and fruits were measured until no further chlorophyll fluorescence was detected. For the heat storage condition, kiwifruits were measured five times,on days 1, 2, 3, 5,and 6, and apples were measured six times,on days 1, 2, 3, 5, 6,and 13. Fruits in the chilling storage condition were measured 6 times, on days 1, 7, 14, 20, 23, and 30. Fruits in the wet condition treatment were measured six times, on days 3, 5,7, 9, 13,and 16. Control fruits were measured five times, on days 5, 7, 9, 13, and 16. A CFI fluorcam (Handy FluorCam FC 1000-H,PS I, Czech Republic) was used to measure the fluorescence imagesof the fruits.

The source of actinic light was orange LED at an intensity of 200 µmol/m^2/s. The source of saturating light was a halogen lamp with an intensity of 2,500 µmol/m^2/s. The fluorescence parameters maximum quantum efficiency of PS II (F_v/F_m), PS II operating efficiency (ΦPS II = F'q/F'm), and non-photochemical quenching (NPQ) were monitored by quenching kinetics analysis [17-19].The data were calculated according to the parameters of the CFI fluorcam, which measured quenching kinetics [17, 19]. Light conditions were: actinic light, red LED, 200 µmol m^{-2} s^{-1}; saturating light, moderate light, 1,250 µmol m^{-2} s^{-1}.

C. Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were defined as follows [20];

F_0: Minimal chlorophyll fluorescence intensity measured in the dark-adapted state, when all PS II RCsare open
F_m: Maximal chlorophyll fluorescence intensity measured in the dark-adapted state during the application of asaturating pulse of light
F_v: Variable chlorophyll fluorescence ($F_m - F_0$) measured

in the dark-adapted state, when non-photochemicalprocesses are minimum

ΦPSII:Effective quantum yield of photochemical energy conversion in PS II (F'_q/F'_m)

F_v/F_m : Maximum quantum yield

NPQ: Non-photochemical quenching

D. Data analysis

The measured data were analyzed with the CFI software (FluorCam Software 7.0, http://www.psi.cz/products/fluorcams/). All statistical analyses were carried out in Microsoft Excel and SAS program (Version 10.02).

III. RESULTS AND DISCUSSION

A. Chlorophyll fluorescence imaging (CFI) analysis

In time course, Fig. 1 shows the photography of apple and kiwi fruits after the storage for designated periods under indicated conditions. Theappearing freshness seemed to be best in both fruits stored under chilling condition. The heat condition which occurs in tropical and subtropical climate is apparently worst (Fig. 1). In kiwi fruit, a shrinkage symptom occurred already by 5day after storage. Both fruits were darkly discolored.

B.CFI of the fruits stored under room temperature

Fig 2 shows the images of chlorophyll fluorescence response of the fruits stored under room temperature. Both the red and green color values were higher in apple than in kiwi (red, apple F_0: 660, F_m: 2200; red, kiwi F_0: 360, F_m: 370; green, apple F_0: 400, F_m: 1400; green, kiwi F_0: 170, F_m: 200), indicating higher fluorescence in apple than in kiwi (Fig.2).

On Day 0, the value of ΦPSII (effective quantum yield of photochemical energy conversion in PS II) was higher in kiwi (0.45) than in apple (0.2) whereas the values showed a higher decrease in kiwi (95%) than in apple (5%)(Fig. 3). On Day 0, F_v/F_m(maximum quantum yield) was the same (0.8) in both fruits.Thereafter, it decreased slowly in apple but quickly in kiwi after 5 days, indicating very strong stress in kiwi compared to apple. F_v/F_mratios showed higher decrease in kiwi (87.5%) than in apple (25%). On Day 0, the value of NPQ (non-photochemical quenching) was higher in kiwi (0.2) than in apple (0.10).Thereafter, NPQincreased in both fruits, and 3 days after storage treatment (DAT) it began to decrease (Fig.3).

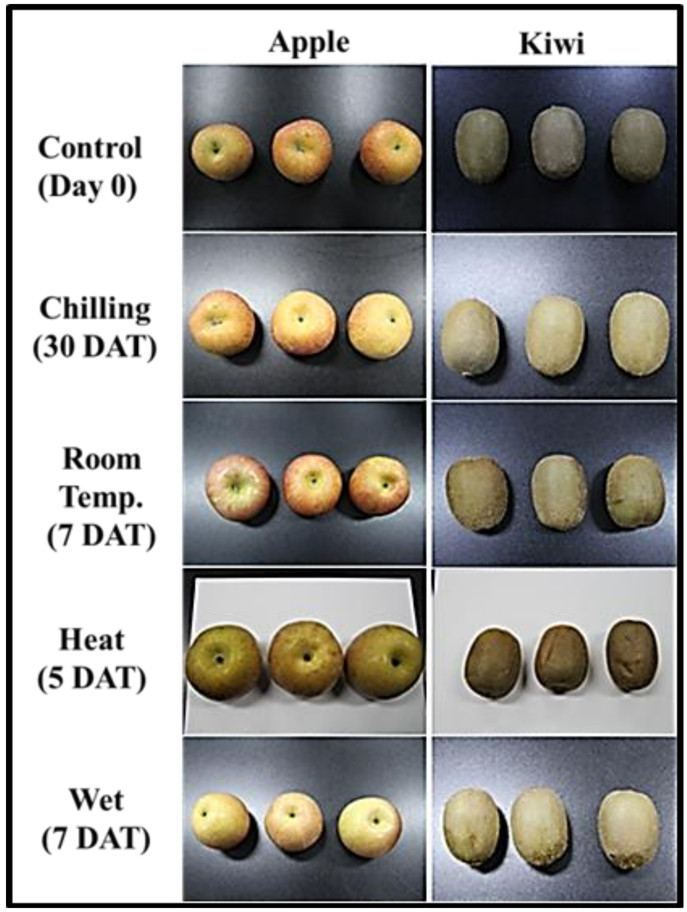

Figure 1. Apple and kiwi fruits were stored under chilling (4°C), room temperature (23±2°C), heat (42°C) and wet (80% relative humidity at room temperature) conditions. DAT means days after storage treatment. Control means the fruits just before storage treatment.

C. CFI of the fruits stored under heat condition.

Both the red and green color values were higher in apple than in kiwi (red, apple F_0: 1100, F_m: 1600; red, kiwi F_0: 250, F_m: 550; green, apple F_0: 700, F_m: 1000; green, kiwi F_0: 150, F_m: 290), indicating higher fluorescence in apple than in kiwi (Fig. 4).

On Day 0, the value of ΦPSII was higher in kiwi (0.55) than in apple (0.35 whereasthe values showed a higher decrease in kiwi (60%) than in apple (55%) (Fig. 5). On Day 0, $F_v/F_m$was the same (0.8) in both fruits, but thereafter, it decreased quickly in apple and slowly in kiwi. It decreased quickly by 2 DAT. These results indicate very high stress in kiwi and apple, with a greater decrease in apple (75%) than in kiwi (50%; Fig. 15). On Day 0, NPQ was higher in apple (0.4) than in kiwi (0.22); thereafter, it decreased slowly in apple, but increased two-fold in kiwi. NPQquickly decreased again, with a greater decrease in apple (75%) than in kiwi (60%; Fig. 5) at 1 DAT.

D.CFI of the fruits storedunder wet condition

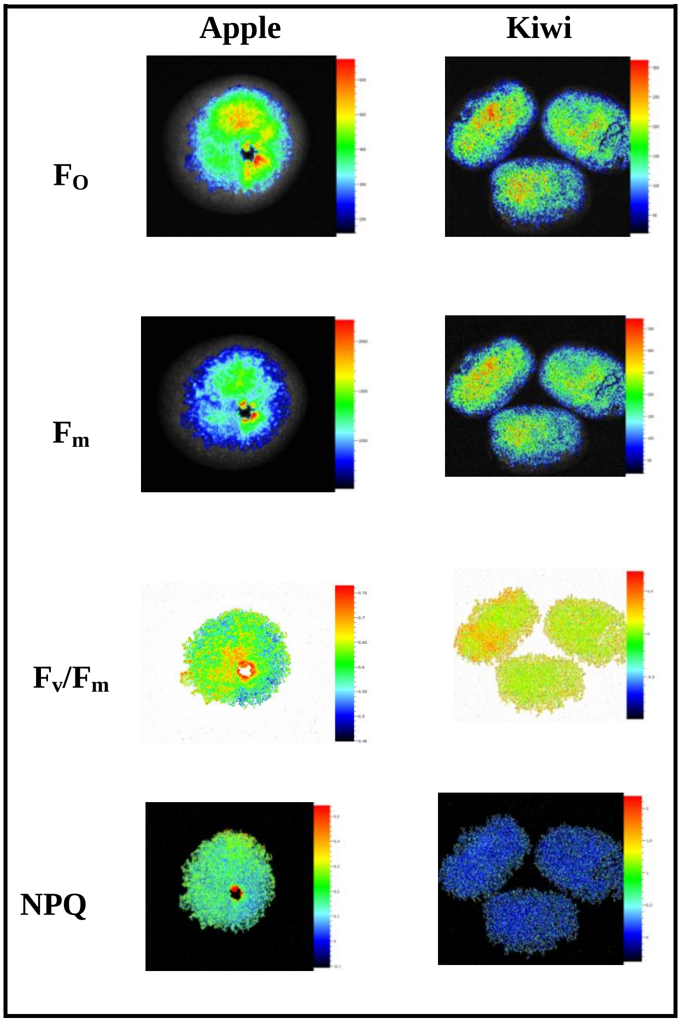

Figure 2. Changes in chlorophyll fluorescence response (F_o, F_m, F_v/F_m, NPQ) of apple and kiwi fruits stored under the room temperature for16day.

Both the red and green color values were higher in apple than in kiwi (red, apple F_v/F_m: 0.85, F_m: 2000; red, kiwi F_v/F_m: 0.45, F_m: 840; green, apple F_v/F_m: 0.7, F_m: 1000; green, kiwi F_v/F_m: 0.1, F_m: 500), indicating higher fluorescence in apple than in kiwi (Fig. 6).

On Day 0, the value of ΦPSII was higher in kiwi (0.45) than in apple (0.3) whereas the values showed a higher decrease in kiwi (90%) than in apple (30%) (Fig. 7). On Day 0, F_v/F_m (maximum quantum yield) was the same (0.8) for both fruits. Thereafter, it decreased slowly in apple and very quickly in kiwi, indicating very high stress in kiwi. Kiwi showed a greater decrease (87%) than apple (18%; Fig. 7).

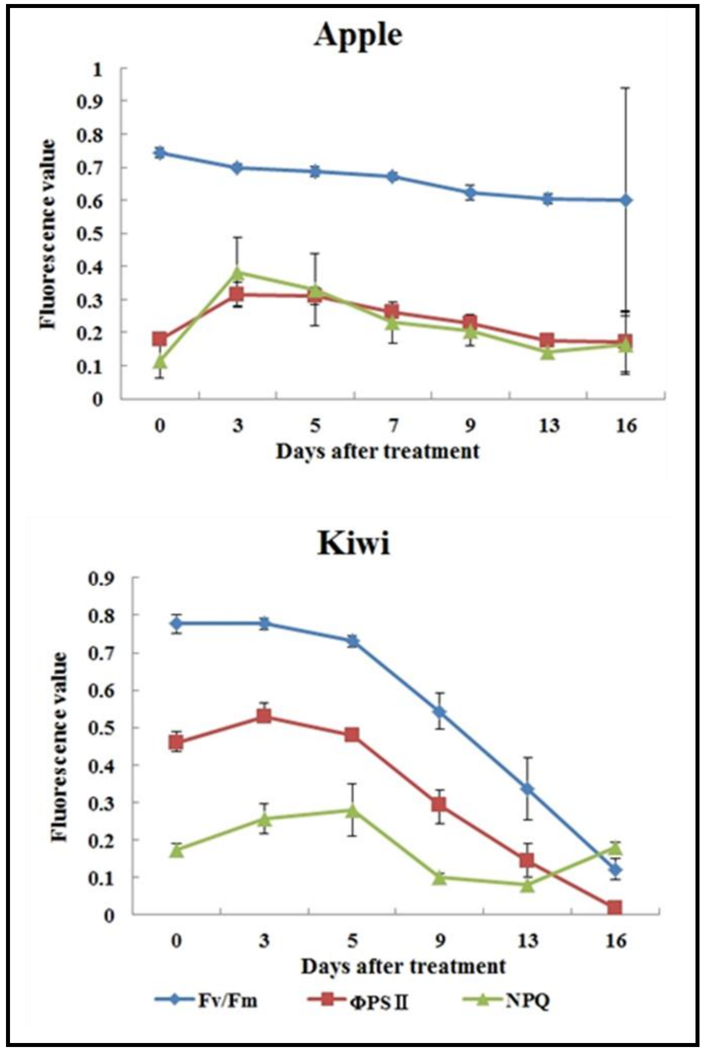

Figure 3. Changes in fluorescence parameters measured by CFI in control fruits preserved under room temperature condition.

On Day 0, NPQwas higher in apple (0.4) than in kiwi (0.2), and decreased thereafter in both fruits. NPQshowed fluctuations (increases and decreases) in both fruits, and ultimately decreased more in apple (87.5%) than in kiwi (75%) at 3 DAT (Fig. 7).

E. CFI of the fruits stored under chilling condition

Both the red and green color values were higher in apple than in kiwi (red, apple F_0: 520, F_m: 2400; red, kiwi F_0: 260, F_m: 860; green, apple F_0: 300, F_m: 1400; green, kiwi F_0: 130, F_m: 00), indicating higher fluorescence in apple than in kiwi (Fig. 8).

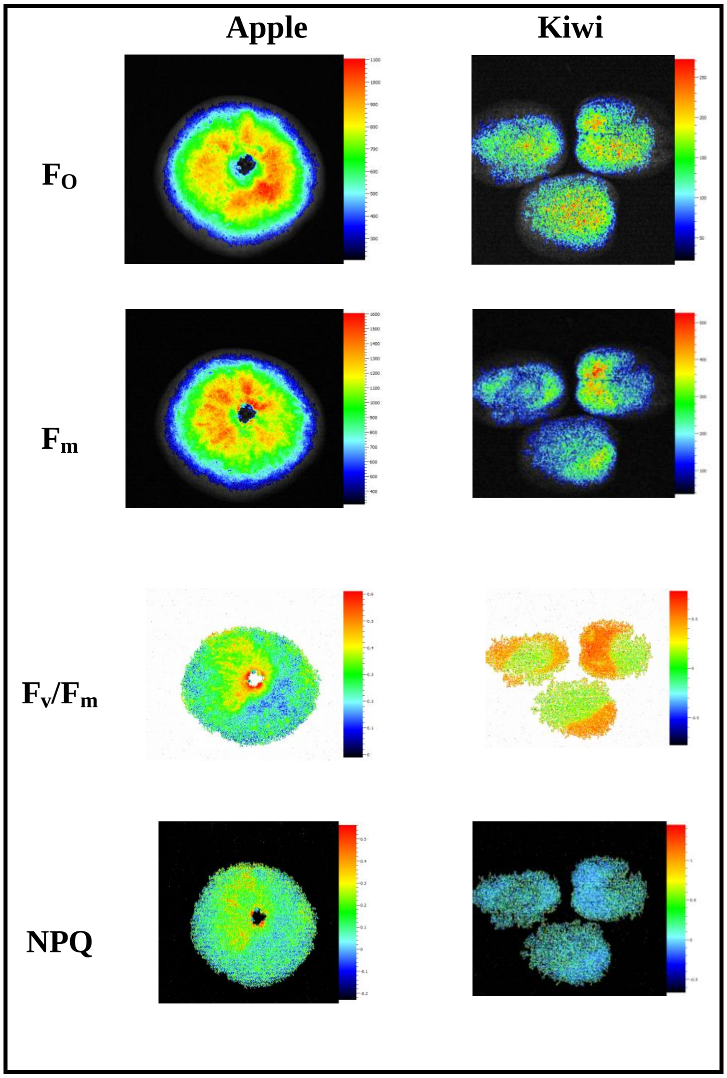

Figure 4.Changes in chlorophyll fluorescence response (F_o, F_m, F_v/F_m, NPQ) of apple and kiwi after 16 and 13 daysof exposure to heat conditions.

On Day 0, the value of ΦPSII (effective quantum yield of photochemical energy conversion in PS II) was higher in kiwi (0.22) than in apple (0.1) whereas the values showed a higher decrease in kiwi (100%) than in apple (16%) (Fig. 9). On Day 0, maximum quantum yield (F_v/F_m) was the same (0.8) for both apple and kiwi, and decreased very slowly thereafter (5%) in both fruits, suggesting very low stress (Fig. 9). On Day 0, non-photochemical quenching (NPQ) was the same for both fruits, followed by a very slight decrease (Fig. 9).

F. Discussion

This study was initiated to investigate whether CFI can be used as a reliable indicator to evaluate the quality of apple and kiwi fruit, and to study the stress-specific differences that may be involved in different stress responses of the photosynthetic apparatus in pericarp of apple and kiwi fruits.

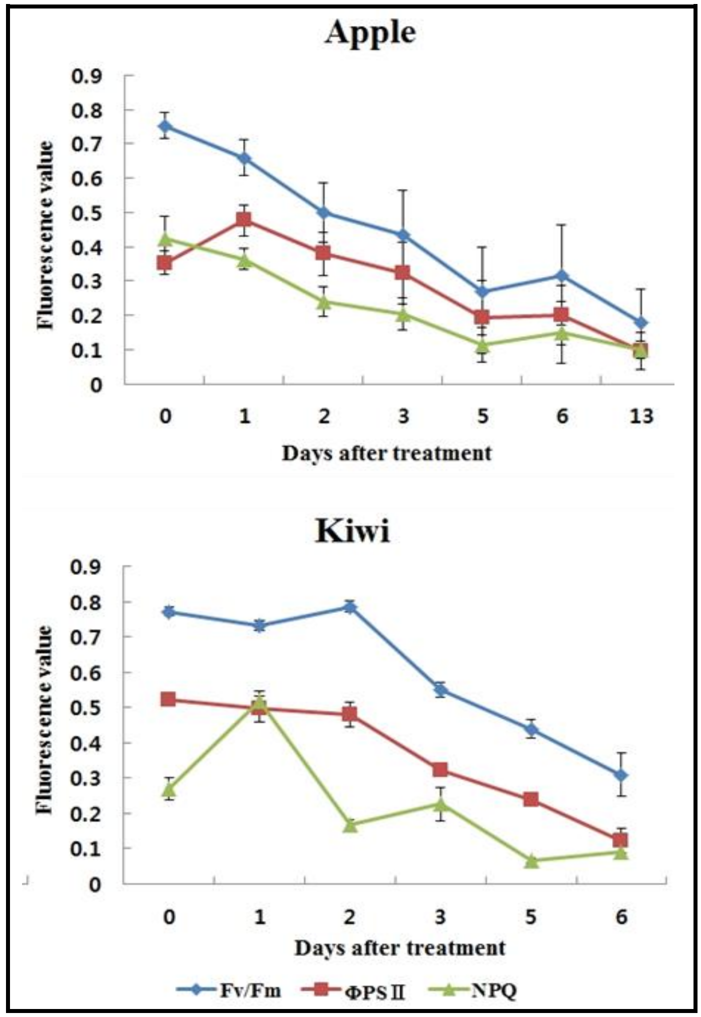

Figure 5. Changes in fluorescence parameters measured by CFI for fruits exposed to heat condition.

In apple, the F_v/F_m was almost the same in the wet condition and control treatments. A small decrease in the F_v/F_m value suggested only minimal stress. Under heat stress, the F_v/F_m was high and then decreased until Day 13, at which time apple had died, indicating high stress. Under chilling stress, the F_v/F_m value decreased slightly day by day until the last day of the experiment for 30 days, when F_v/F_m was almost 0.8.In general, healthy plants have a very conservative F_v/F_m value of about 0.8[21].

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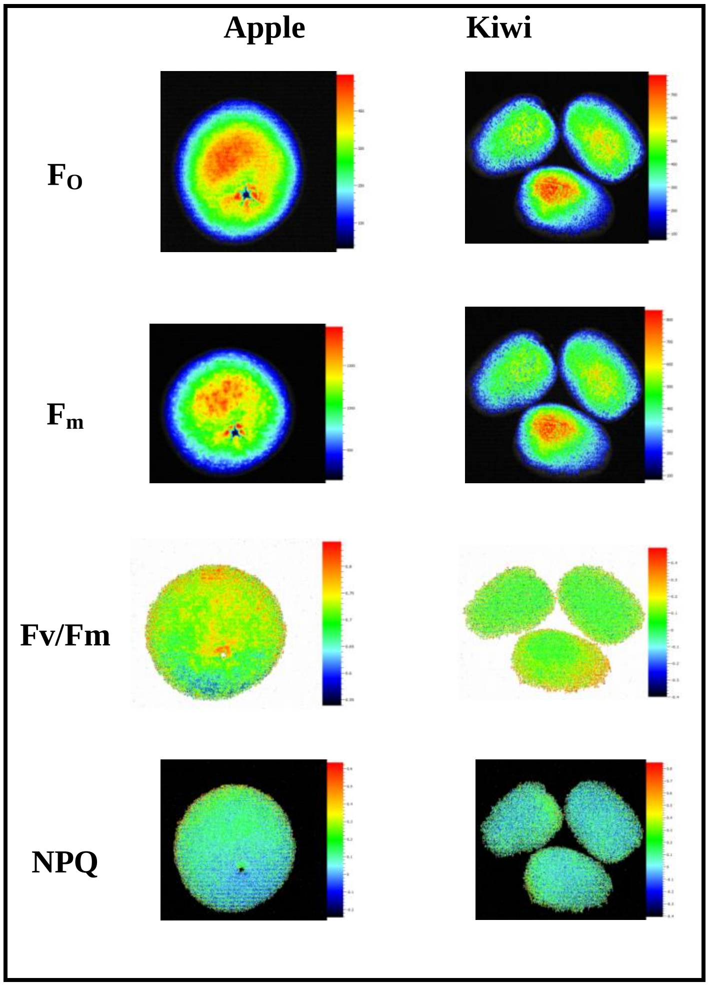

Figure 6. Changes in chlorophyll fluorescence response (F_o, F_m, F_v/F_m, NPQ) of apple and kiwi after 16 days of exposure to wet condition of 80 % relative humidity at room temperature.

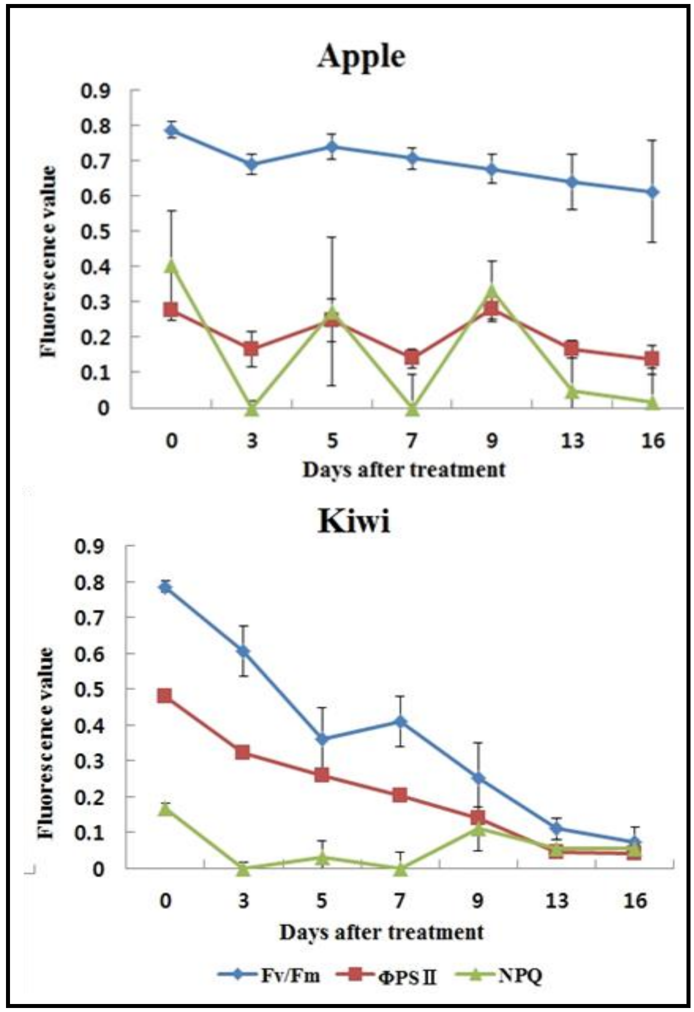

Figure 7. Changes in fluorescence parameters measured by CFI for fruits exposed to wet condition of 80 % relative humidity at room temperature.

In kiwi, the F_v/F_m value decreased day by day, showing a large decrease after 5 days in the control group at the room temperature and after 2 days under heat stress. In the wet condition treatment, F_v/F_m decreased greatly compared to Day 0, whereas the F_v/F_m value under chilling stress decreased very slightly until the last day. When F_v/F_m almost reached 0.8, it seemed to be again healthy plants. In this study, all values of F_v/F_m were lower than 0.8. Björkman and Demmig[22] and Johnson et al.[23] reported optimal values of F_v/F_m around 0.8 for most plant species, and values lower than this are observed in plants exposed to stress, indicating in particular the phenomenon of photoinhibition.

In apple and kiwi, F_v/F_m values decreased very slowly under chilling stress, and F_v/F_m remained near 0.8.In all storage conditions, both fruits were healthiest in the chilling storage condition.

Under heat stress, F_v/F_m values decreased steeply from Day 0 until Day 13 (apple) and Day 6 (kiwi).

In earlier report in barley leaves[24], it has been observed the inactive reaction centers were accumulated at 5°C.Under chilling condition, the photochemical efficiency of PS II in continuous steady states light (ΦPSII) was generally more depressed than the loss of Q_A protein at least in leaf. In fruits, these photochemical changes did not occurred indicating a difference between leaf and fruit.

Under chilling condition in this study, the ΦPSII in apple remained at almost the same value until the last day of the experiment, while the ΦPSII valuein kiwi remained almost the same until Day 14 and then decreased after Day 23. The ΦPSII values of both fruits decreased day by day in the control, heat, and wet conditions.

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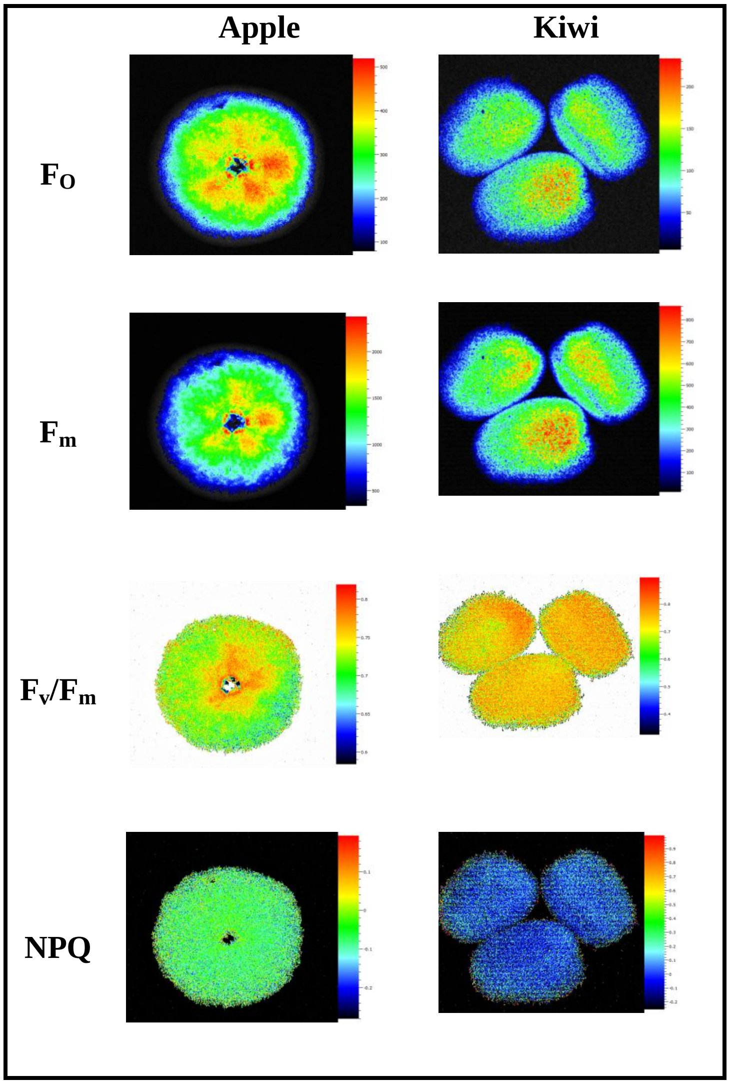

Figure 8. Changes in chlorophyll fluorescence response (F_o, F_m, F_v/F_m, NPQ) of apple and kiwi after 30 days of exposure to chilling condition (4°C).

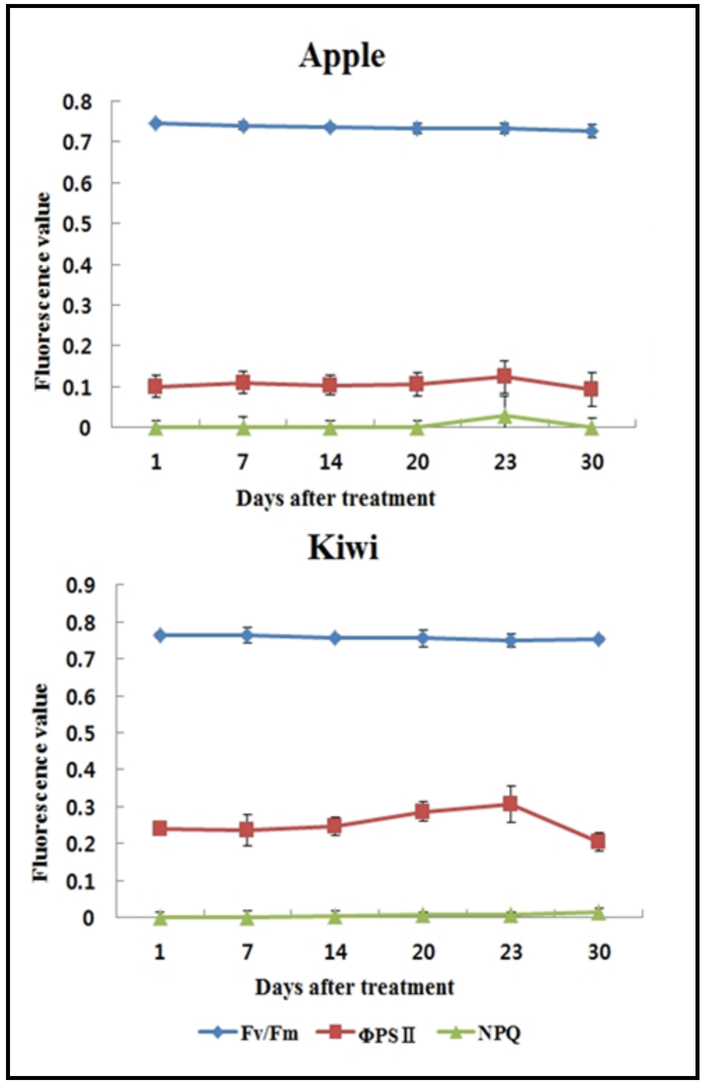

Figure 9. Changes in fluorescence parameters measured by CFI for fruits preserved under chilling condition (4°C).

NPQvalues of both fruits under chilling condition decreased marginally day by day until the last day, whereas at the room temperature, the NPQvalues increased until Day 5. Under heat stress, NPQvalues decreased day by day after Day 1, while under wet condition, NPQvalues were variable. NPQ values in steady state have similar nonphotoquenching characteristics in dark-adapted state [21]. Although changes in NPQ are nonlinearly related to higher values than ΦPSII in leaves as earlier suggestion [21, 25], ΦPSII is also applicable to determine the freshness of apple under heat storage condition and of kiwi fruits under wet storage condition.In the chilling storage condition, the F_v/F_m, ΦPSII, and NPQvalues decreased slightly gradually until the last day, and values of F_v/F_m were close to 0.8, indicating lower stress under chilling than in the other storage conditions.

Under chilling condition, both fruits were healthiest among all storage conditions, and apple was stronger than kiwi.Under heat condition, apple was affected more quickly compared to other storage conditions. Kiwi was rapidly affected under wet condition,at 20 DAT under heat condition, and at 5 DAT inroom temperature.

Different responses to temperature will result in different storage periods for apples and kiwi under different conditions. The different stresses cause severe damage to the photosynthetic apparatus, resulting in changes in appearingviability of fruits. This study has clearly shown that CFI can be used as a reliable tool to evaluate the quality of apple and kiwi fruits and to recommend appropriate storage methods for these fruits.

VI. Conclusion

The photos taken by the fluorescence imaging machine and the data of F_v/F_m, ΦPSII, and NPQshow that different responses occurred under different temperature stresses. This practical study of the CFI method has shown that the changes in F_v/F_m, ΦPSII, and NPQwere higher under heat stress than under the other stresses in both apple and kiwi fruit. Chilling (4°C) is recommended as a suitable storage method for apple and kiwi, which retained F_v/F_m values of almost 0.8. On the basis of the results of this study, CFI is considered a reliable indicator to evaluate the fresh quality of fruits.The CFI method is a rapid method for fruit freshness determination.

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