Application of Chlorophyll Fluorescence Imaging Technology for Fresh Quality Control of Grape Fruit 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 grape (*Vitis vinifera.* L.) fruits using portable chlorophyll fluorescence imaging instrument. To assess the fresh quality of grape fruits, an imaging technique of the photochemical responses of pericarp of grape fruit was performed with fruits preserved under the different storage conditions. The observed chlorophyll imaging photos were numerically transformed to the photochemical parameters on the basis of chlorophyll fluorescence. The storage conditions for fruits were regulated as follows; room temperature (control), heat (42°C), wet (22°C±2 and 80% relative humidity), and chilling (4°C) conditions.

Chlorophyll fluorescence imaging (CFI) technology showed that the decrease in F_v/F_m and ΦPSII were lower under the chilling stress than the other conditions in grape fruits. In heat condition, the photochemical parameters calculated from the images of F_v/F_m ratios, ΦPSII and non-photoquenching (NPQ) were available to determine the freshness of fruits. In our study, it was clearly indicated that the chilling condition was the suitable storage condition for grape fruits. The CFI technology is applicable as a rapid screening method for the determination of freshness of grape fruits.

Keyword- Chlorophyll fluorescence imaging, Chilling, Heat, Wet, Storage

I. Introduction

Over the past decade, not only red wine producers but also green grape consumers have needed to improve the non-destructive quality control technology. Fresh fruits and fresh vegetables are two main sectors of agricultural markets, and fresh produce is important to the well-being of consumers. The term "quality" refers to the degree of excellence of a product or its suitability for a particular use. The quality of products 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 of their senses, including sight, smell, taste, touch, and even hearing. The consumer integrates all of these sensory inputs into a final judgment of the acceptability of that fruit. Merchants, consumers, processors, and producers use many 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].

In tropical and subtropical countries, a lot of grape fruits are shortly transported to consuming place without a cooling system. 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 behaviour of photosynthetic sensory systems. The JIP-test is a tool to analyse the polyphasic rise of the chlorophyll *a* (Chl *a*) fluorescence transients (phases labelled "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, 3].

Most studies analysing the effects of heat or chilling stress on OJIP transients have been conducted on plant leaves [4, 5] but not precisely in fruits. Even these studies have been limited to apple [6-8]. 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 [9]. Thus, under wet, heat, or chilling stress, changes in the photosynthetic apparatus of pericarp of fruits may differ for the storing of apple and kiwi fruits [3]. 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 [10]. 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 [11].

This study was performed to evaluate the validity of the fluorescence imaging technology to determine the freshness and apparent quality of grape fruit.

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

II. MATERIALS AND METHODS

A. Storage conditions of fruits

All of the treatments were measured on day 0 (control) prior to exposure to the designed temperature conditions as described below. For the heat storage condition, the fruits were placed in a growth chamber at a temperature of 42°C. Grape fruits in the chilling storage condition were stored in a refrigerator at 4°C. For the wet condition treatment, the grape fruits were imposed under water vapor in a bucket regulated with over 80 % relative humidity and kept at room temperature. Control fruits were 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(CFI)

The green grape fruits were measured separately for each treatment after exposure to the designed storage conditions. Measurements were performed in a dark room. Grape fruits in different conditions were also measured in triplicate 1, 2, 3, and 7 days after treatments (DAT). A CFI fluorCam (Handy FluorCam FC 1000-H, PS I, Czech Republic) was used to measure the fluorescence images of the grape fruits.

The source of actinic light was orange LED at an intensity of 200 μmol m^{-2} s^{-1}. The source of saturating light was a halogen lamp with an intensity of 2,500 μmol m^{-2} s^{-1}. 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 [12-14]. The data were calculated according to the parameters of the CFI fluorCam, which measured quenching kinetics [12, 14]. 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 in Table 1 [15].

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 9.02).

TABLE I
Chlorophyll fluorescence parameters of chlorophyll fluorescence imaging analysis

Chlorophyll fluorescence parameters	Definition
F_0	Minimal chlorophyll fluorescence intensity measured in the dark-adapted state, when all PS II RCs (reaction centers) are open
F_m	Maximal chlorophyll fluorescence intensity measured in the dark-adapted stateduring the application of a saturating pulse of light
F_v	Variable chlorophyll fluorescence (F_m−F_0) measured in the dark- adapted state, when non-photochemical processes are minimum
ΦPSII	Effective quantum yield of photochemical energy conversion in PS II (Photosystem II)
F_v/F_m	Maximum quantum yield
NPQ	Non-photochemical quenching following Stern-Volmer coefficient

III. RESULTS

A. *Chlorophyll fluorescence imaging (CFI) analysis*

In time course, Fig. 1 shows the photography of grape fruits after the storage for designated periods under indicated conditions. The appearing 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). A shrinkage symptom occurred already by 1 day after storage under 1 hr heat condition at 42°C. Grape fruits were darkly discoloured.

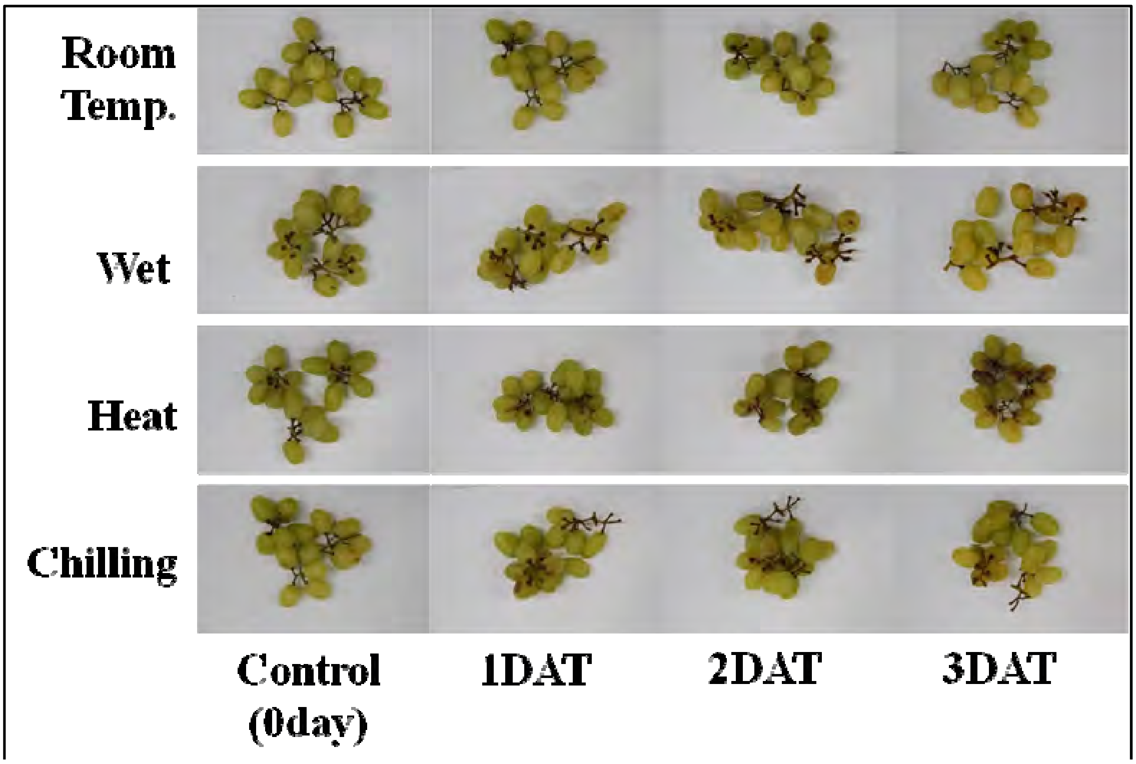

Fig. 1. Grape fruits were stored under chilling (4°C), room temperature (22±2°C), heat (42°C) and wet (80% relative humidity at room temperature) conditions. DAT means days after storage treatment. Control means the fruits just before storage treatment

B. CFI of the fruits stored under different conditions

The high fluorescence energy (red light at F_O) was lowest under chilling condition and highest under heat condition. The highest fluorescence at Fm was recorded in the control group and the lowest in chilling and wet stress treatments. The grape fruits preserved under chilling condition and room temperature showed the highest fluorescence energy in while the grape fruits stored under heat condition had the lowest. Non-photoquenching (NPQ) values under heat condition showed the highest image fluorescence and chilling and wet treatments the lowest (Fig. 2)

The maximum quantum yield began at almost the same value (~0.8) under chilling, control, and wet stress conditions at 1 day after treatment (DAT), and was higher than under heat stress (0.7). By 3 DAT, the control value did not change and remained the highest values whereas the values for the heat and wet conditions decreased almost 3-fold. The values for the chilling condition showed no change over 7 DAT. The grape fruits preserved under chilling condition and control at room temperature still showed almost the same values as non-treated control fruits and were higher than wet condition. No measurements were made for the heat stress condition at 7 DAT (Fig. 3). It means that the non-photosynthetic activity of pericarp of green grape fruits is stopped.

C. Effective quantum yield of photochemical energy conversion (ΦPSII)

The effective quantum yield of photochemical energy conversion in PS II (ΦPSII) was similar among the chilling, control, and wet stresses (~0.2) at 0 DAT, but was higher in the heat stress treatment (0.3). The F_v/F_m value of the control group increased to >0.3 at 3 DAT, whereas the value in the wet stress treatment decreased slightly. The F_v/F_m values of grape fruits stored under heat condition decreased two-fold. Fruits stored under the chilling stress condition were not measured at 3 DAT. The value of the control group at 7 DAT was almost the same as at 3 DAT while the value under the chilling condition slightly increased compared to 1 DAT. Also, the F_v/F_m ratios of fruits stored under wet condition slightly decreased (Fig. 4).

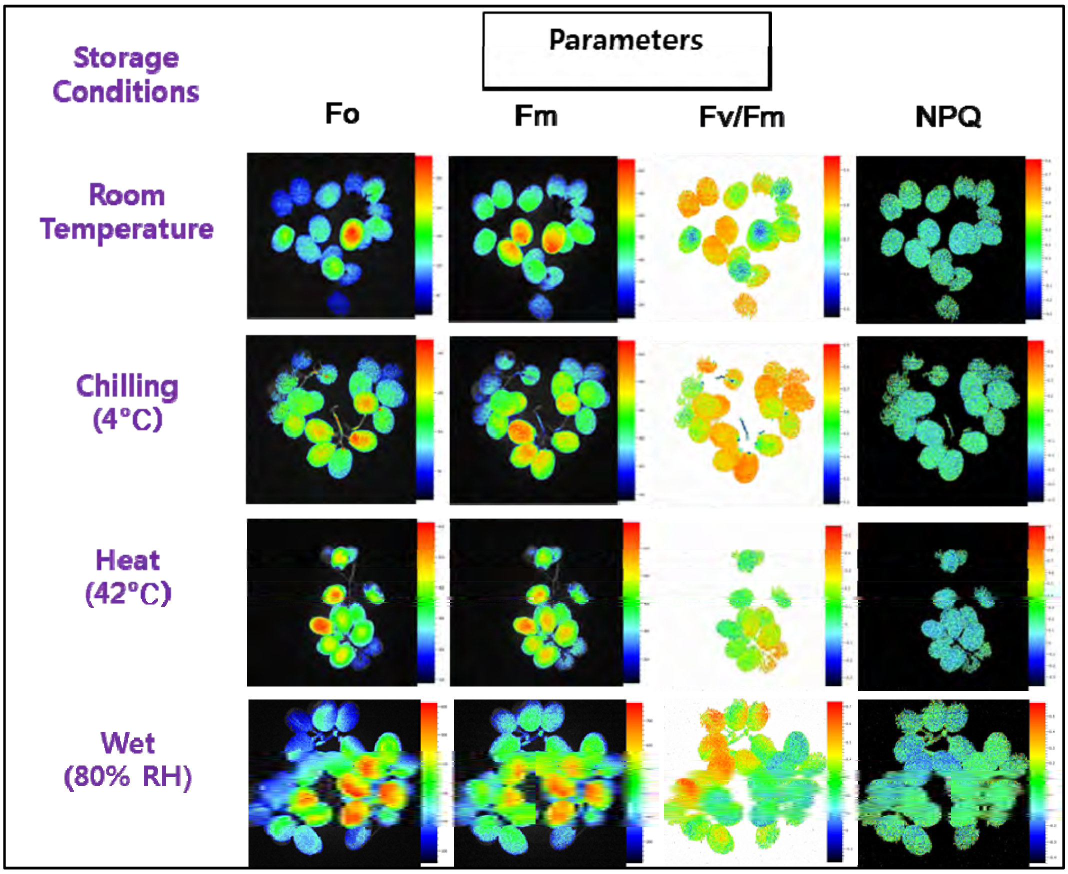

Fig. 2. Imaging of chlorophyll fluorescence responses (Fo, Fm, Fv/Fm, NPQ) in grape fruits stored under control (room temperature, 22°C±2°C), chilling (4°C), heat (42°C), and wet conditions (80% relative humidity at 22°C±2°C)

D. Non-photochemical quenching (NPQ)

The NPQ values of grape fruits had almost the same values under control and wet conditions less than 0.05 (Fig. 5), while the value of grape fruits stored under chilling condition was 0. The NPQ value under heat condition was much higher than that under other conditions (>0.2). The NPQ values under control and wet conditions slightly increased whereas that in the heat condition decreased up to 0.15 at 3 DAT. The NPQ value of the control group decreased slightly at 7 DAT. The values decreased to –0.04 under the chilling condition. The NPQ value under wet condition also decreased from positive values at 0 DAT (control) and then to a negative value at 7 DAT (Fig. 5). The negative NPQ values may be able to be a detectable limitation of instrument. However, the mean of these values indicated at least no consumable energy dissipation derived from non-photoquenching. Thus, it could conclude that both storage conditions are optimum on the basis of photosynthetic activity of pericarps of green grape fruits.

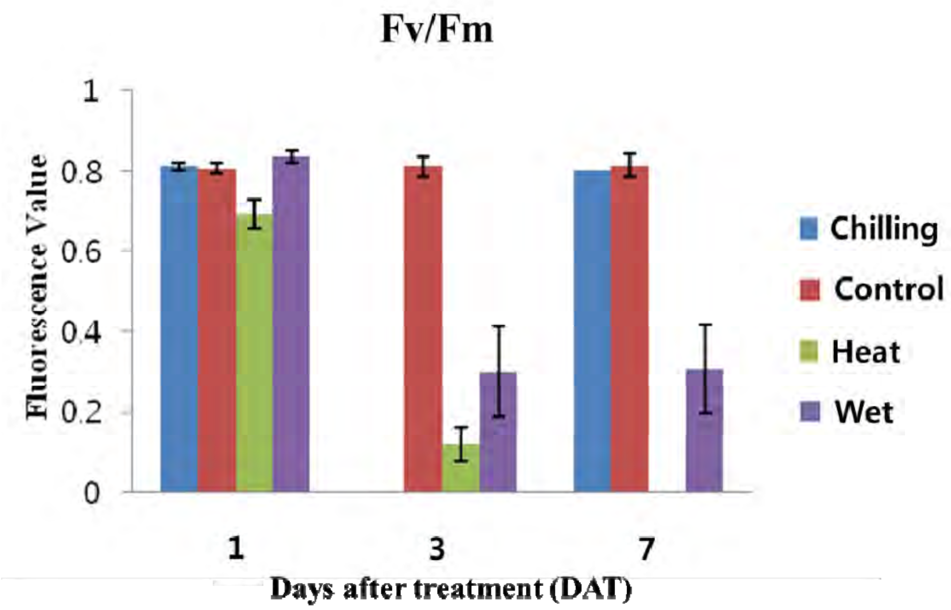

Fig. 3. Comparison of Fv/Fm ratios (maximum quantum yield) in grape fruits stored under control (room temperature 22°C±2°C), chilling (4°C), heat (42°C), and wet (80% relative humidity at 22°C±2°C) conditions

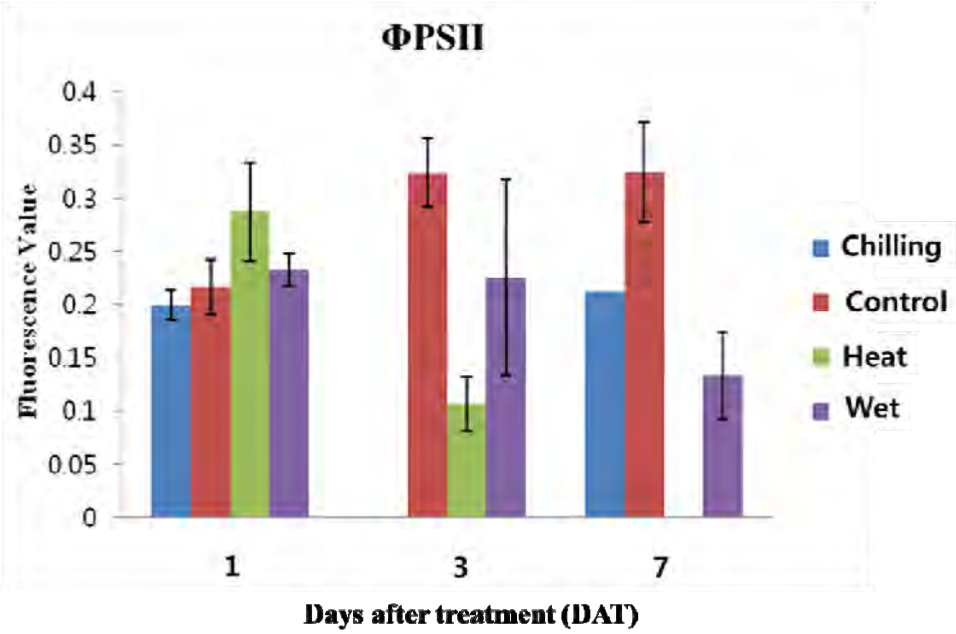

Fig. 4. Comparison of ΦPSII in grape fruits stored under control (room temperature 22°C±2°C), chilling (4°C), heat (42°C), and wet (80% relative humidity at 22°C±2°C) conditions

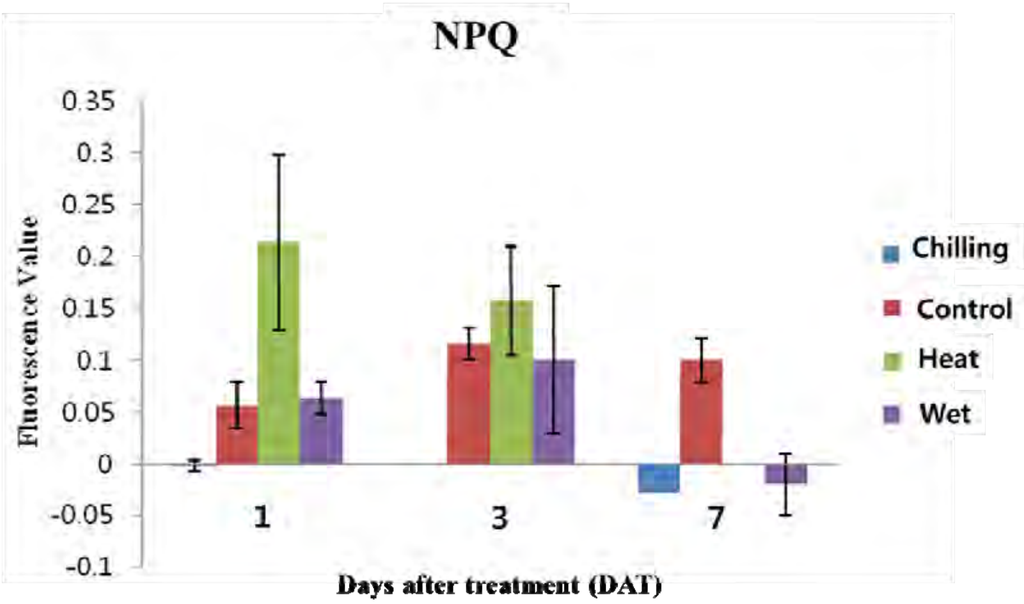

Fig. 5. Comparison of NPQ in grape fruits stored under control (room temperature 22°C±2°C), chilling (4°C), heat (42°C), and wet (80% relative humidity at 22°C±2°C) conditions

IV. DISCUSSION

A. CFI technique

CFI was initiated to investigate whether it can be used as a reliable indicator to evaluate the quality of grape in order to study the stress-specific differences that may be involved in different stress responses of the photosynthetic apparatus.

CFI has been applied for different purposes in the postharvest life of fruits, but the main focus has been on detecting factors that can increase or decrease product quality. Moreover, the technique has been used for various objectives both in pre- and postharvest conditions for the detection of biotic or abiotic stresses in plants and plant products. In comparison to the application of CFI for detection of abiotic stresses in different crops [7, 15], this study focused on the photochemical responses of green grape fruits to different temperature storage conditions.

B. Fv/Fm ratios

In grape, F_v/F_m was almost the same from Day 0 to Day 7 under chilling stress and control conditions. The F_v/F_m ratios were greater than under heat and wet conditions. It may mean that photosynthetic activity of green grape pericarps is theoretically higher than those stored under heat and wet conditions and still active until 7 DAT. Therefore, this photochemical parameter can be good indicator for quality control. Under heat condition, the F_v/F_m was dramatically decreased already 3 days after storage. Under chilling stress, the F_v/F_m value decreased slightly gradually until the last day of the experiment for 20 days (data not shown), 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 [16].

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, 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 [17] and Johnson et al.[18] 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 grape fruits, 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 condition, F_v/F_m values decreased steeply until 7 DAT.

C. ΦPSII

In grape fruits, φPSII under heat condition was lowest (Fig. 3). Under room temperature, chilling, and wet conditions, the values were similar to control but then decreased slightly under wet condition. In the grape fruits preserved under chilling condition, the values remained almost the same until 7 DAT. The higher ΦPSII values in control grape fruits than in chilling condition were assumed to be constant chlorophyll a florescence in steady state, i.e. under continuous light pulse. In general, the chlorophyll fluorescence near at 25°C is most sensitive

[16, 19, 20]. Thus, we would like to recommend the measurement at room temperature after recovery to ambient temperature of other grape fruits preserved under low temperature.

In earlier report in barley leaves [21] and kiwi fruits [3], it has been observed the inactive reaction centres 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.

D. NPQ

In grape, NPQ under heat condition was highest already at 1 DAT but thereafter decreased. It implied that the NPQ parameter is most temperature sensitive. Because the higher value of NPQ indicates low possibility of photosynthetic electron transport resulting in an increase in inactive chlorophyll of pericarp of green grape fruits. Thus, this NPQ value of green pigmented fruit is an available indicator parameter as recent study [3].

The different storage conditions cause severe damage to the photosynthetic apparatus, resulting in changes in photosynthetic activity of pericarp of grape fruits. The measurement of photochemical responses to storage conditions may be able to determine the storage periods for grape fruits under various conditions. This study has shown that CFI can be used as a reliable tool to evaluate the healthy or fresh quality of grape and to recommend appropriate storage methods.

NPQ values of grape fruits under chilling condition decreased marginally day by day until the last day, whereas at the room temperature, the NPQ values increased until 7 DAT. Under heat condition, NPQ values decreased gradually 1 DAT, while NPQ values under wet condition were variable. NPQ values in steady state have similar nonphotoquenching characteristics in dark-adapted state [16]. Although changes in NPQ are nonlinearly related to higher values than ΦPSII in leaves as earlier suggestion [16, 22], The ΦPSII is also applicable to determine the freshness of grape fruits stored under heat and under wet storage conditions. In the chilling storage condition, the values of F_v/F_m were close to 0.8, indicating lower stress under chilling than in other storage conditions.

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

V. CONCLUSION

The fluorescence imaging and the data of F_v/F_m, ΦPSII, and NPQ show that different responses occurred under various storage conditions. This practical study of the CFI technique has shown that the numeric changes in F_v/F_m, ΦPSII, and NPQ were higher under heat condition than under the other conditions in grape fruits. Chilling (4°C) and wet conditions are recommended as a suitable storage method for green grape fruits, 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 grape fruits. The CFI technique can be a rapid method for fruit freshness determination.

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