ISSN 2349-0837

ChlorophyllaFluorescence Imaging Technique for Fresh Quality Assessment of Tomato and Pepper FruitsStoredUnder Different Conditions

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ABSTRACT

The objective of this study was to find a rapid determination of the freshness of tomato (Solanum lycopersicum L.) and pepper (Capsicum annuum L.)fruits using portable chlorophyll fluorescence imaging instrument. To assess the fresh quality of tomato and pepper fruits, an imaging anlaysis of the photochemical responses of pericarp or exocarp of tomato and pepper fruits 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 (25°C and 80% relative humidity), and chilling (4°C) conditions.

Chlorophyll fluorescence imaging (CFI) method showed that the decrease in F_v/F_mratios of pepper fruits was lower at room temperatureand under wet condition than the other conditions. Although F_v/F_mratios and ΦPSII values in tomato fruits showed low fluorescence responses, the changing patterns have permitted to determine th freshness. In heat condition, the photochemical parameters calculated from the images of F_v/F_m ratios, ΦPSII and non-photoquenching (NPQ) were also available to determine the freshness of fruits. In our study, it was clearly indicated that the chillingcondition was the suitable condition for thelong storage of tomato and pepper fruits. The CFI analysis is applicable as a rapid screening method for the determination of freshness of tomato and pepper fruits.

Keywords : Tomato, Pepper, Chlorophyll fluorescence imaging, Fruit freshness, Chilling, Heat, Wet, Storage

Council for Innovative Research

Peer Review Research Publishing System

Journal: JOURNAL OF ADVANCES IN AGRICULTURE

Vol. 3, No.3.

www.cirjaa.com, jaaeditor@gmail.com

ISSN 2349-0837

INTRODUCTION

Over the past decade, not only fruit producers but also consumers have needed to improve the non-destructive quality control technology. Fresh fruits and vegetables are two main sectors of agricultural markets, and fresh produce is important to the well-being of consumers. 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 usemany standards to evaluate the quality of fresh fruits and vegetables. Coolingtransport and chain systems are used to preserve the freshness of produce from harvesting through marketing and delivery to the consumer. transport and chain 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 product[1-3].

In tropical and subtropical countries, a lot of tomato and pepper 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 [4, 5]. The primary use of fluorescence has included the estimation of chlorophyll concentration and pigment–protein interactions and studies of the stability of thylakoid membranes. However, the relationship between chlorophyll and in vivo fluorescence varies widely over time and space. These processes include species changes, nutrient concentrations, and incident radiation[6]. The use of sun-stimulated fluorescence to estimate primary productivity is also possible.
Most studies analysing the effects of heat or chilling stress on OJIP transients have been conducted on plant leaves [7, 8] but not precisely in fruits. Even these studies have been limited to apple [9-11]. 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 [12]. 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[5]. 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 [13]. 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 [14].This study was performed to evaluate the validity of the fluorescence imaging technology to determine the freshness and apparent quality of tomato and pepper fruit.

MATERIALS AND METHODS

Fresh fruits of tomato (Solanum lycopersicum L.) and pepper (Capsicum annuum L.)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.

Storage Condition of Fruits

All of the treatments were measured just beforestorage treatment (control) prior to exposure to the desired temperature conditions 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 imposed under water 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.

Measurement of Chlorophyll FluorescenceImaging

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, tomato fruits were measured five times at 1, 2, 3, 5, and 6 days after treatment (DAT). Pepper fruits were measured six times at 1, 2, 3, 5, 6, and 13 DAT. Fruits in the chilling storage condition were measured 6 times at 1, 7, 14, 20, 23, and 30 DAT. Fruits in the wet condition treatment were measured six times at 3, 5, 7, 9, 13, and 16 DAT. Control fruits were measured five times, on days 5, 7, 9, 13, and 16. A chlorophyll fluorescence imaging (CFI) fluorcam (Handy FluorCam FC 1000-H,PS I, Cech Republic) was used to measure the fluorescence images of 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 [15-17]. The data were calculated according to the parameters of the CFI fluorCam, which measured quenching

ISSN 2349-0837

kinetics [15, 17]. Light conditions were: actinic light, red LED, 200 µmol m^{-2} s^{-1}; saturating light, moderate light, 1,250 µmol m^{-2} s^{-1}.

Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence parameters were defined as follows [18];

Table 1. Chlorophyll fluorescence parameters

$F_{0:}$ 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 state during 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

Data analysis

The measured data were analyzed with the CFI software (FluorCam Software 7.0, http://www.psi.cz/ products/fluorcams/). All statistical analysis were carried out in Microsoft Excel and SAS program (Version 9.02).

RESULTS

Chlorophyll fluorescenceresponse

In tomato, high fluorescence value (red color) at F_O was lower than in pepper. Fm value of 150 was 10 fold lower in tomato than in pepper of1500, respectively.The high fluorescence energy (red light at F_O) was twice higher in tomato than in pepper (800 versus 400) stored under chilling condition (4°C), but other values did not differ very much (Figure 1).

F_V/F_Mratios

In fruits stored at room temperature

In tomato, the maximum quantum yield (F_v/F_m)has started at 0.38 just before storage treatment. Thereafter, ithas decreased slowly and progressively until it reached to 0.06 at 16 DAT. In pepper, the F_v/F_mratio has started at 0.79 just before treatment, then decreased gradually and reached below 0.65 at 16 DAT. Thus, the value of pepper did not decrease very much during the 16days experimentcompared to tomato.

F_V/F_Mratios under chilling condition

In tomato, the F_v/F_mratiohas started at a value of 0.27 at 0 DAT, then decreased gradually and reached to 0.06 at 30 DAT. In pepper, the F_v/F_mratiohas begun at 0.76, decreased to 0.02 at 7 DAT, and then increased again to a value of 0.5 at 30 DAT. Thus, the F_v/F_mratioin tomato showed a four fold decrease over the 30-day experimentwhile the value of pepper has decreased only slightly.The absolute differences between initial and final values were similar (0.21 in tomato and 0.26 in pepper).

F_V/F_Mratios under heat condition

In tomato, the F_v/F_mratio of 0.5 at 0 DAThas decreased gradually to 0.053 at 5 DAT. In pepper, the F_v/F_mratio of 0.82 at 0 DAT has decreased slowly to 0.43 at 5 DAT. The F_v/F_mratioin tomato has alsoabout tenfolddecreased from 0.5 to 0.053 while those of pepper decreased only 2fold from 0.82 to 0.43 until 5 DAT.

F_V/F_Mratios under wet condition

In tomato, the F_v/F_mratio of 0.31 at 0 DAThas decreased to 0.1 at 7 DAT. In pepper, the F_v/F_mratio of 0.79 at0 DAThas decreased to 0.62 at 7 DAT. The F_v/F_mratio in tomato has also decreased about three fold (from 0.31 to 0.1) whereasthe F_v/F_mratio in pepper did only slightlyfrom 0.79 to 0.62.

ISSN 2349-0837

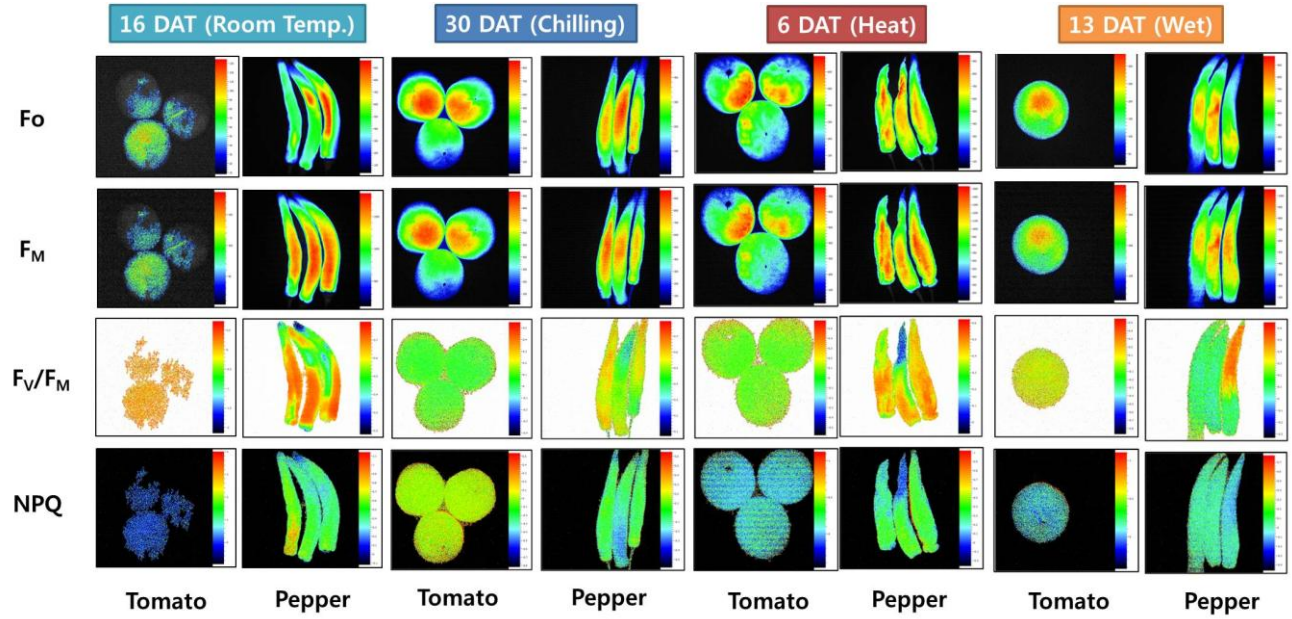

Fig 1: Imaging of chlorophyll fluorescence responses (Fo, Fm, Fv/Fm, NPQ) in fruits stored under control (room temperature, 22°C±2°C), chilling (4 °C) and heat (42°C) conditions. DAT means days after treatment.

Effective quantum yield of photochemical energy conversion (ΦPS II)

ΦPS II at room temperature

In tomato fruits, the ΦPS II was around 0.2 on starting day of storage at room temperature. Thereafter, the ΦPS IIhas slowly decreased to -0.9 at 9 DAT and finally reached to a value of –0.33 at 16 DAT. In pepper fruits, the the ΦPS II has started at 0.56 at 0 DAT, decreased slowly, and then finally reached to 0.34 at 16 DAT. The the ΦPS II in tomato fruitshas substantially decreased from 0.2 to -0.33.The ΦPS II of pepper fruits decreased slowly from 0.56 to 0.34 during 16daysof experiment(Figure 3).

ΦPS II under chilling condition

The ΦPS IIin tomatofruit stored underchilling conditionhas reached from 0.13 at 0 DAT to 0.03 at 16 DAT. In pepperfruits stored under chilling condition, the ΦPS II started relatively high around 0.43 at 1 DAT, decreased abruptly to 0.03 at 7 DAT. Ultimately the ΦPS II value has fluctuated again to a value of 0.21 at 30 DAT.

ΦPS II under heat condition

The ΦPS II in tomato fruits stored under heat (42°C) conditionhas started at 0.33 and then decreased slowly to 0.03 at 5 DAT. In pepper fruits stored under heat (42°C) condition, the ΦPS II value of 0.58 at 0 DAT has decreased slowly to 0.19 at 5 DAT.

ΦPS II under wet condition

In tomato fruits stored under wet condition, the The ΦPS II has decreased from 0.16 to 0.06 at 7 DAT. In pepper fruits stored under wet condition, the ΦPS II has decreased from 0.53 to 0.22 at 7 DAT.

Non-photochemical quenching (NPQ)

NPQ at room temperature

In tomato fruits stored at room temperature, the NPQ has increased from 0.02 to 0.67 at 16 DAT. In pepper, the NPQ values increased slowly from 0.2 to 0.5 at 16 DAT (Figure 4).

NPQ under chilling condition

In tomato fruits stored under 4°C chilling condition, the NPQ valueshave slowly increased from -0.05 to 0.04 at 23DAT, and then decreased finally to 0.02 at 30 DAT. In pepper stored under 4°C chilling condition, the NPQ valuehas started at 0.07 on 1 DAT and then steeply decreased to -0.21 at 14 DAT. The NPQ valuehas increased to a final value of -0.067 at 30 DAT(Figure 4).

ISSN 2349-0837

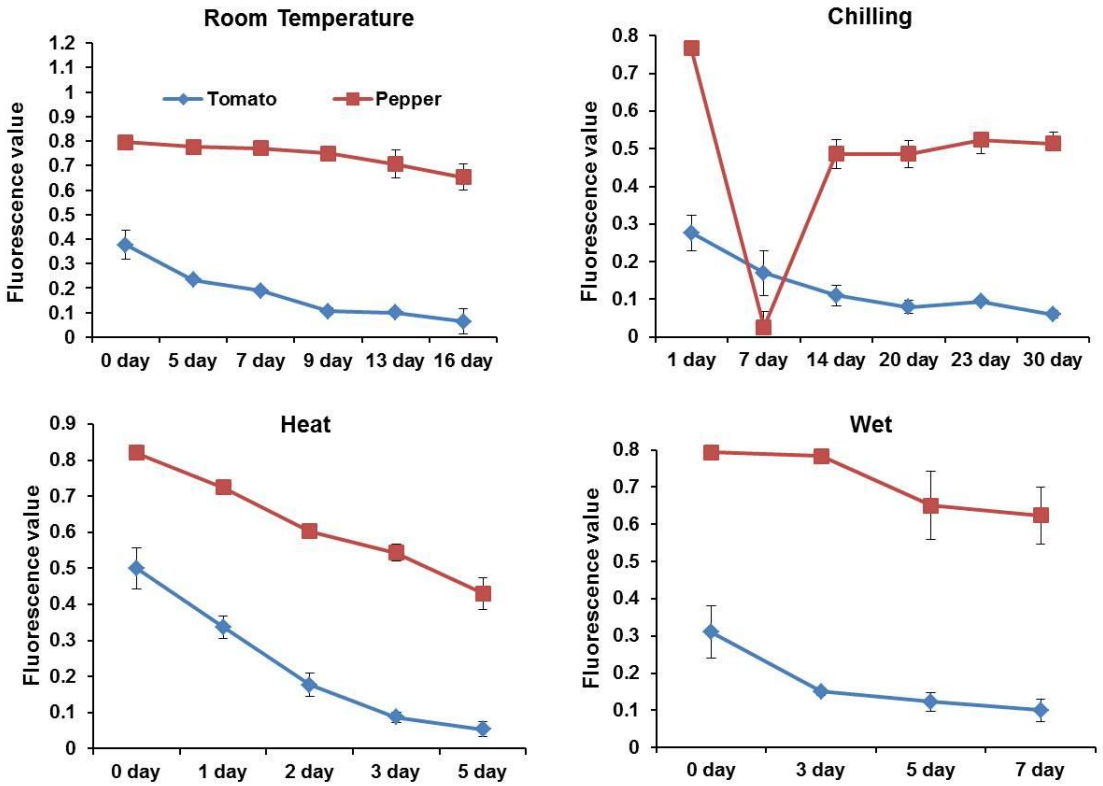

Fig 2: Changes in$F_V/F_M$of tomato and pepperfruits stored under control (room temperature, 22°C±2°C), chilling (4 °C), heat (42°C) and wet (80% relative humidity) conditions.

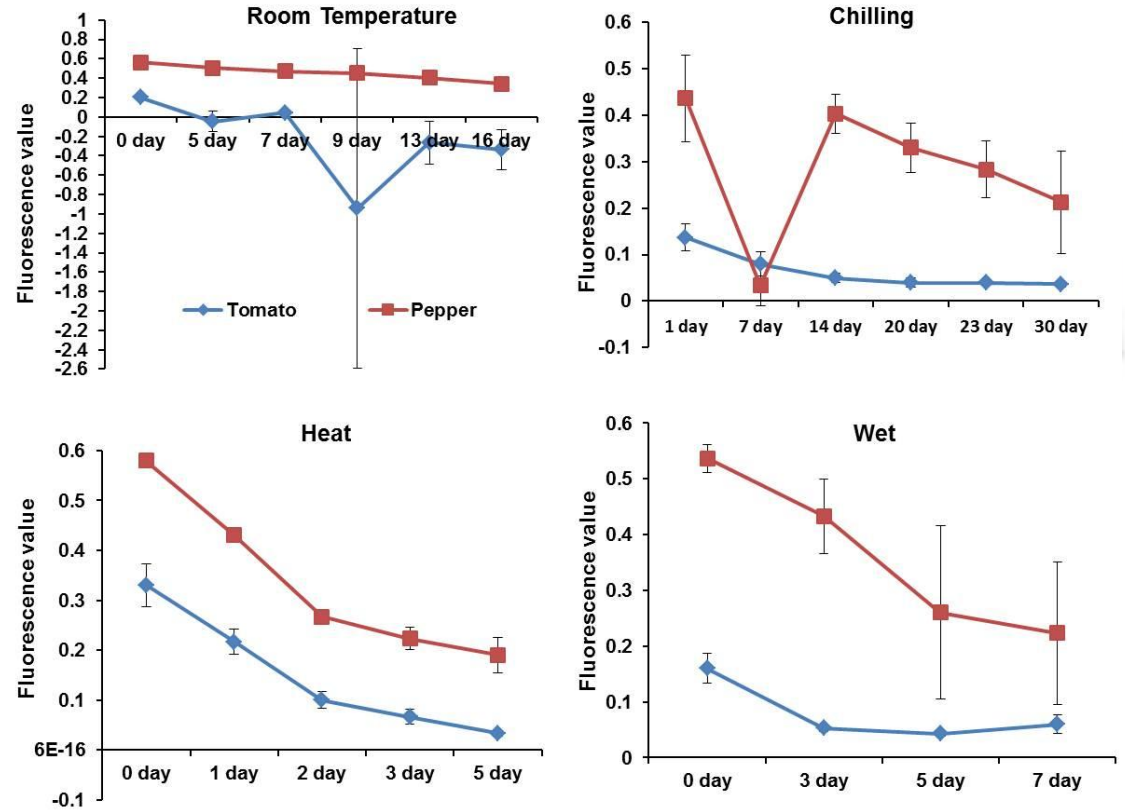

Fig 3: Changes inΦPS IIof tomato and pepperfruits stored under control (room temperature, 22°C±2°C), chilling (4 °C), heat (42°C) and wet (80% relative humidity) conditions.

ISSN 2349-0837

NPQ under heat condition

In tomato stored under heat (42°C) condition, the NPQvalues have slightly decreased from 0.12to 0.1at5 DAT whereas the NPQ valuesin pepper has steeply increased to 0.7 at 1 DAT. This NPQ values havethereafter decreased to 0.33 at 5 DAT with a bell-shaped curve.Compared to the initial value, the tomato values have retained from 0.115 to 0.1(Figure 4).

NPQ under wet condition

When tomato fruits were stored under wet condition with 80 % relative humidity, the NPQ values have showed a little change from 0.02 to 0.05 at 7 DAT. In pepper, however, the NPQ valueshas decreased from 0.29 to 0.01 at 7 DAT.It has implied that photosynthetic activityin tomato was lowered and energy dissipation increased (Figure 4).

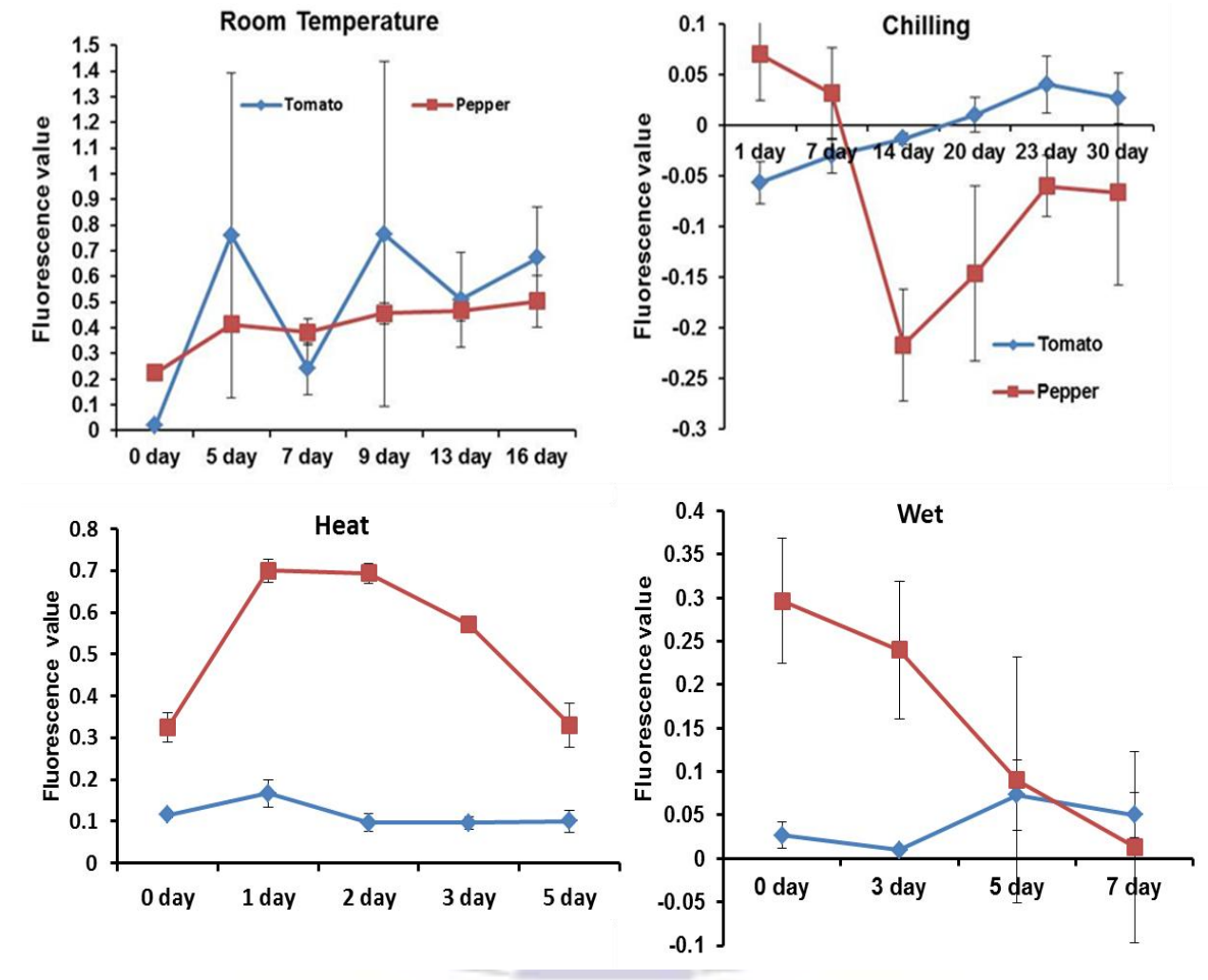

Fig 4: Changes inNPQof tomato and pepperfruits stored under control (room temperature, 22°C±2°C), chilling (4 °C), heat (42°C) and wet (80% relative humidity) conditions.

Discussion

CFI technique

CFI was initiated to investigate whether it can be used as a reliable indicator to evaluate the quality of tomato and pepper 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[10, 18], this study focused on the photochemical responses oftomato and pepperfruits to different temperature storage conditions.

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F_V/F_M ratios

In non-green tomato fruits, the low F_v/F_m may derive from chlorophyll deficiency in their exocarps.In general, the chlorophyll has degraded and then carotenoids are accumulated in the exocarp of tomato fruits during ripening stage [19]. At least photosynthetic bacteria, carotenoids has affect the fluorescence of chlorophyll [20, 21]. However, the fluorescence is still observed and used to measure the carotenoid content [22]. Therefore, the F_v/F_mratioin tomato fruits, maximum quantum yield, could be detected in low sensitivity (Figure 2).

In pepper fruits, $F_v/F_m$was slightly decreased from 0.79to0.65 under control condition. It may mean that the green pigmented pericarp of pepper is still photosynthetically active. The F_v/F_m ratios were greater than under chilling and heatconditions. It may mean that photosynthetic activity of pepper pericarps stroed at room temperature is theoretically higher than thosestored under chilling and heatconditions and still performed until 16 DAT. Therefore, this photochemical parameter can be good indicator for quality control. Under heat condition, the F_v/F_m was dramatically decreased already 1 days after storage. Under chilling stress, the F_v/F_m value decreased slightly gradually until the last day of the experiment for 30 dayswhen F_v/F_m was almost 0.5. In general, healthy plants have a very conservative F_v/F_m value of about 0.8 [2].

The F_v/F_m value decreased day by day, showing a large decrease after 2 days under heat condition. In the wet condition, F_v/F_mhas greatly retained compared to control. The F_v/F_m value under wet condition has decreased very slightly until the last day when F_v/F_m almost reached 0.7. It seemed to be still healthy plants. In this study, all values of F_v/F_m were lower than 0.8.Björkman and Demmig [23] and Johnson et al.[24] 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.However, it is clear that wet condition is a best storage method for pepper but not for tomato(Figure 2).

In pepper fruits, F_v/F_m values decreased very slowly under chilling condition except for an lier value at 7 DAT, and F_v/F_m remained over 0.5 for 30 days.Thus, for long storage, the chilling condition may be a best method.

ΦPSII

In tomato and pepper fruits, ΦPSII under heat condition was most rapidly lowered(Figure 3). Under room temperatureand chilling conditions, the ΦPSIIvalues of pepper have slowly decreasedthan under heat and wet condition. In the tomato fruits underallconditions, the ΦPSIIvalues have decreased under 0.1 at final days of experiments. It has implied that they were no more photosynthetic activity.The higher ΦPSII values in control tomato and pepper 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 [2, 3, 25]. Thus, we would like to recommend the measurement at room temperature after recovery to ambient temperature of other tomato and pepper fruits preserved under low temperature.

In earlier report in barley leaves [26], 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[5].

NPQ

NPQ values of tomato fruits under chilling condition hasincreased day by day until 23 DAT(Figure 4). The NPQ values in both fruitsstored at the room temperature has alsoincreased until 16 DAT. However, in tomato and pepper, NPQ under heat condition was highest already at 1 DAT but thereafter steeply decreased. It implied that the NPQ parameter ismost temperature sensitive. Because the higher value of NPQ indicates low possibility of photosynthetic electron transport resulting in an increase in inactive chlorophyll of pericarps of tomato and pepper fruits[16, 27]. Thus, this NPQ value of green pigmented fruit can be an available indicator parameter as recent study [5].

Comparison among photochemical parameters

The heat condition seemed to cause severe damage to the photosynthetic apparatus, resulting in changes in photosynthetic activity of pericarp of tomato and pepper fruits. The measurement of photochemical responses to storage conditionsmay be able to determine the storage periods for tomato and pepper 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 tomato and pepper and to recommend appropriate storage methods.

In tomato andpepper fruits stored under heat condition, NPQ values decreased gradually1 DAT, whileNPQ values atroom temperature were variable. NPQ values in steady state have nonphotoquenching characteristics in dark-adapted state [2]. Although changes in NPQ are nonlinearly related to higher values than ΦPSII in leaves as earlier suggestion [2, 28], the ΦPSII is also applicable to determine the freshness of tomato and pepper fruitsstored under heat and under wet storage conditions.In the chilling storage condition, the values of F_v/F_m were close to 0.5, indicating lower stress under chilling than inheat and wet conditions.

Different responses to temperature will result in different storage periods for tomato and pepper stored under various 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 tomato and pepper fruits and to recommend appropriate storage methods for tomato and pepperfruits.

ISSN 2349-0837

CONCLUSION

The fluorescence imaging and the numeric data of F_v/F_m, ΦPSII, and NPQ showed that different responses occurred under various storageconditions. This practical study of the CFI technique has shown that the numeric values in F_v/F_m, ΦPSII, and NPQ were rapidly decreased under heat and wet condition than under the other conditions intomato and pepper fruits. Room temperature (22±2 °C), chilling (4°C) and wetconditions are recommended as a suitable storage method foredible green pepper fruits, which retained F_v/F_m values of almost 0.7. On the basis of the results of this study, CFI is considered a reliable indicator to evaluate the fresh quality of tomato and pepperfruits. The CFI techniquecan be a rapid method for fruit freshness determination.

ACKNOWLEDGMENTS

The author (Mr. Soe Moe Win) is grateful to KOICA Master Program in Hankyong National University. This work was financially supported by RDAAgenda project No. PJ009243.This work was preliminary performed to assess for the selection of drought tolerant maize cultivars. We thanks deeply for the permission of project leaders Prof. Dr. Lee. Byung-Moo of Dongguk University and Dr. Shin, S. H. of RDA.

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Author' biography with Photo

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ISSN 2349-0837

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