Physio-biochemical Responses of Okra (*Abelmoschus esculentus*) to Oxidative Stress Under Low Temperature Storage

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Okra pods, commonly eaten at an immature stage, undergo quick postharvest deterioration due to high respiration, water loss, wilting, toughening and decay. As okra is a tropical crop, its pods are susceptible to chilling injury (CI) at low storage temperatures. The effects of low temperature storage on the physio-biochemical properties of okra pods were determined. Chilling injury symptoms were found only in pods stored at 4°C and were more apparent after transfer to 25°C. In seeds, the CI index was positively correlated with seed browning, H₂O₂, malondialdehyde (MDA) content, and catalase (CAT) activity. Chilling-injured seeds had lower total phenolic content (TPC), antioxidant activity (DPPH scavenging activity and FRAP assay), peroxidase (POD), and superoxide dismutase (SOD) activities than non-injured seeds. Additionally, the seed browning index was related to high polyphenol oxidase (PPO) activity. In the pericarp, the CI index was also positively correlated with the H₂O₂ and MDA contents. The POD and SOD activities in chilling-injured pericarp were significantly lower than in non-injured pericarp. Chilling injury resulted in an initial increase in DPPH scavenging and CAT activities which later decreased as CI became severe. These results indicate that CI in okra is due to accumulation of H₂O₂ and MDA, as well as its weak antioxidant defense mechanism. This resulted in development of CI symptoms, including seed browning.

**Key Words:** antioxidants, browning, chilling injury, hydrogen peroxide.

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**Introduction**

Okra (*Abelmoschus esculentus*) is a flowering plant of the mallow family, Malvaceae. Its pods are rich in minerals, vitamins, and antioxidant compounds (Liao et al., 2012; Lin et al., 2014; Sreeshma and Bindu, 2013). Fresh okra pods have long been used in traditional medicine to treat diabetes, gastric ulcer, jaundice, and hepatitis (Karim et al., 2014). Immature okra pods are usually consumed fresh or cooked. However, they have a short postharvest shelf life due to high respiration, especially when stored at an ambient temperature, and are susceptible to mechanical injury (bruising), desiccation, chlorophyll degradation and decay during postharvest handling (Babarinde and Fabunmi, 2009; Finger et al., 2008). Low temperature storage has been proposed to maintain the postharvest quality of okra pods, but prolonged storage at low temperature results in chilling injury (CI).

Chilling injury is known as a serious postharvest problem that affects the marketability and storability of most harvested agricultural produce of subtropical and tropical origin. Visible symptoms of CI in okra pods include pitting, surface browning, water-soaked lesions and decay (Huang et al., 2012). Chilling is known to stimulate physiological changes such as loss of firmness, fresh weight and color, and a high respiration rate (Carvajal et al., 2011; Wang et al., 2007). Chilling-induced browning due to oxidation of phenolics by the activity of PPO has been reported in basil (Wongseree et al., 2009) and eggplant (Gao et al., 2015).

Chilling stress induces the overproduction of reactive oxygen species (ROS) (Wismer, 2003), which structurally consist of one or more activated oxygen atoms such as superoxide radicals, hydrogen peroxide (H₂O₂), hy-
droxyl radicals and nitric oxide (Demidchik, 2015). The accumulation of ROS like H$_2$O$_2$ results in lipid peroxidation, and protein and DNA oxidation. Lipid peroxidation increases over a prolonged storage period and leads to cell degeneration and membrane damage (Shewfelt and Rosario, 2000). Antioxidants such as phenolics, ascorbic acid and antioxidant enzymes in chilling-tolerant species scavenge ROS, thereby shielding membranes from lipid peroxidation (Nukuntornprakit et al., 2015). Therefore, the inability of these antioxidants to cope with excessive formation of ROS is believed to be the cause of oxidative stress in chilled crops (Gill and Tuteja, 2010; Hodges et al., 2004).

To date, research is lacking on the physiological and biochemical impact of CI in okra. Therefore, the objectives of this study were to investigate the physiological and biochemical changes in okra pods during low temperature storage and to determine the mechanism (antioxidant defense system) underlying CI in okra.

Materials and Methods

Plant material and storage treatment

Okra (Abelmoschus esculentus ‘Shaan F1’) pods were harvested at the immature stage from a farm in Pathumthani province, Thailand. The pods were transported within 2 h of harvest at 24°C to the postharvest laboratory at King Mongkut’s University of Technology Thonburi. Uniformly sized pods (10–12 cm, harvested at 8 ± 2 d after flowering) that were free from mechanical injury and diseases were selected. The pods were washed with 150 mg·L$^{-1}$ sodium hypochlorite for 1 min, rinsed with water for 1 min and dried at 24°C. Then, approximately 0.1 kg of pods were weighed out per replicate, packed into perforated oriented polypropylene bags (15.9 cm × 11.4 cm with 1500 holes of 1 mm diameter) and stored at 4 and 13°C, 85 ± 5% relative humidity for 15 d. Four replications were randomly selected and used.

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CI index

Chilling injury was determined according to the method described by Huang et al. (2012) with modifications. The CI index was assessed based on the area of pitting, translucency or discoloration on the surface of the stored okra pods using a scale as follows: 0, 0% injury; 1, injury ≤ 20%; 2, 20% < injury ≤ 40%; 3, 40% < injury ≤ 60%; 4, 60% < injury ≤ 80% and 5, 80% < injury ≤ 100%. The CI index was calculated using the below formula:

CI index $= \sum \left( \frac{\text{chilling scale} \times \text{number of fruit in each scale}}{\text{number of total fruit} \times \text{highest chilling scale}} \right) \times 100$
for 1 h and left to stand at room temperature for another 15 min. Absorbance was read at 765 nm; the results were compared with the standard curve of gallic acid and expressed on a fresh weight basis (FW) as g·kg$^{-1}$·protein.

The antioxidant activity was determined following the 2,2-diphenyl-1-picyrylhydrazyl (DPPH) radical scavenging activity method by Rajurkar and Hande (2011) with some modifications. The DPPH scavenging activity was determined by mixing 150 μL of extract with 2.85 mL ethanolic DPPH (0.1 mM) and incubating the mixture in a dark at room temperature for 30 min. Absorbance was read at 515 nm and the antioxidant activity was calculated as follows:

$$\text{% inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

A ferric reducing antioxidant power (FRAP) assay was done by following Benzie and Strain (1996). Absorbance was read at 593 nm after the mixture was incubated in the dark for 30 min at 37°C. The results were expressed on a fresh weight basis (FW) as g·kg$^{-1}$ Trolox equivalent (TE).

**Enzyme extraction and antioxidant enzyme activities**

Two grams of the frozen pericarp was homogenized in 15 mL of 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetra acetic acid (EDTA) and 1% polyvinylpolypyrrolidone (PVPP) in ice water. For seeds, 1 g of frozen seeds was mixed with 7.5 mL 100 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 0.25 mM polyethylene glycol (PEG) and 1% PVPP. The homogenate was centrifuged at 12,000 × g for 20 min at 4°C. The supernatant was then filtered and used to analyze the activities of PPO, peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD).

The method of Benjamin and Montgomery (1973) with modification was applied to determine PPO activity. The reaction mixture contained 1.5 mL of 100 mM phosphate buffer (pH 7.0), 1 mL of 0.08 M 4-methyl catechol, and 0.5 mL of crude enzyme. One unit (U) of the enzyme activity was defined as 0.001 Δ410 min$^{-1}$·mg$^{-1}$ protein.

POD activity was estimated by the increase in absorbance at 470 nm, and guaiacol was used as a substrate (Wang et al., 2005). The final reaction mixture contained 1 mL 100 mM phosphate buffer, 1 mL 24 mM H$_2$O$_2$, 0.5 mL 8 mM guaiacol, and 0.5 mL crude enzyme. One unit (U) of enzyme activity was defined as 0.01 Δ440 min$^{-1}$·mg$^{-1}$ protein.

CAT activity was measured according to Aebi (1984) with some modifications by monitoring the decrease in absorbance at 240 nm. For the activity in the pericarp, the final reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.0), 40 mM H$_2$O$_2$, and 500 μL enzyme extract. The activity was calculated using an extinction coefficient of 40·M$^{-1}$·cm$^{-1}$ and expressed as mol H$_2$O$_2$·s$^{-1}$·kg$^{-1}$·protein.

SOD activity in the pericarp and seeds was determined by using a xanthine oxidase (XO)/nitroblue tetrazolium (NBT) system as described by Ukeda et al. (1997) with some modifications. The reaction mixture contained 50 mM sodium carbonate (pH 10.2), 4 mM EDTA, 3 mM xanthine, 1 mM NBT, 0.15% bovine serum albumin (BSA), and 100 μL crude enzyme extract or water. Then, XO (56 μL) was added to the reaction mixture and the absorbance change at 560 nm was checked after the mixture was incubated for 30 min at 25°C. One unit of the enzyme activity was defined as the amount of enzyme which caused a 50% decrease in formazan.

**Protein assay**

The total protein content of the crude enzymes was assayed following the method of Bradford (1976). The bovine serum albumin standard in a concentration range of 0 to 100 mg·L$^{-1}$ was plotted and used to calculate the protein content based on absorbance read at 595 nm.

**Statistical analysis**

The experiment was arranged in a completely randomized split plot design with five replications. The data were analyzed using analysis of variance (ANOVA). The means were compared using the least significant different (LSD) test. Additionally, simple correlation coefficients to determine the relationship among the studied parameters were also calculated with the significance level at the 5% (*) and 1% (**) level.

**Results**

**CI index, seed browning index and firmness**

Okra pods stored at 13°C and subsequently transferred to a warmer temperature (13 + WT) did not show any CI symptoms throughout the storage period (Fig. 1a). However, data on 13 + WT for day 15 was not available as the pods were badly damaged, i.e. they were fibrous and rotten. Okra pods stored at 4°C showed initial CI symptoms, slight pitting, on day 3. The CI index increased drastically from day 6 (17%) to day 15 (73%). When the pods were transferred to a warmer temperature (4 + WT), the symptoms were more apparent, and the CI index of the transferred pods was consistently higher than that of the pods stored at 4°C. No significant change in pod firmness was observed during storage at 4°C (Fig. 1b). On the contrary, a gradual increase in pod firmness was observed after 3 d of storage at 13°C, increasing by 36% on day 15 relative to 4°C. The seed browning index of the okra pods stored at 4°C increased gradually after day 3, and on day 15, the browning index was four times higher than at harvest and at 13°C (Fig. 1c). The browning index in okra pods stored at 13°C was always significantly lower than in those stored at 4°C.
Ultrastructure of the pericarp and seeds

Ultrastructural changes in the stored okra pericarps and seeds are shown in Figure 2 and 3. At harvest, the pericarp had a homogenous surface with almost perfectly shaped trichomes (Fig. 2A). After 12 d of storage, pods stored at 13°C maintained that quality with a slightly wilted surface (Fig. 2B). The chilling-injured okra pods had sunken, deep pitting, uneven surfaces with severely wilted, massively collapsed and void cells dislodged from trichomes, as well as mucilage bursting (Fig. 2C). A cross section of a non-chilled okra pericarp on day 12 showed slightly collapsed parenchymatous cells, possibly due to wilting and water loss (Fig. 2E). The epidermal cells of chilling-injured pericarps were badly damaged, and parenchymatous cells were badly collapsed (Fig. 2F). Seeds of pods stored at 13°C were slightly wilted, but had a normal homogenous shape (Fig. 3A, B), similar to seeds at harvest (not shown). Chilling injury resulted in seed wilting and loss of the outer peel layer (Fig. 3C). A seed cross section clearly revealed a collapsed endosperm and damaged seed coat (Fig. 3D).

Reactive oxygen species and lipid peroxidation

Cold storage at 4°C led to a drastic increase in the $\text{H}_2\text{O}_2$ content of pericarps after 9 d and on day 15 showed 71 and 87% higher $\text{H}_2\text{O}_2$ content than in those stored at 13°C and at harvest, respectively (Fig. 4a). However, the $\text{H}_2\text{O}_2$ content in seeds stored at 4°C and 13°C was not significantly different as the storage day progressed (Fig. 4c). After the pods were transferred to a warmer temperature, 4 + WT showed a considerable increase in $\text{H}_2\text{O}_2$ on days 6, 12, and 15, and this was two-fold, five-fold, and four-fold higher than at harvest time, respectively. The production of MDA in the pericarp and seeds was induced during storage at 4°C (Fig. 4b, d) and was significantly higher than at 13°C. A similar trend for MDA content was found after the pods were transferred to 25°C.
Total phenolic content and antioxidant activities

Overall, levels of TPC and antioxidant activity (FRAP assay) of pericarps during pod storage at 13°C and 4°C were not significantly different. However, on day 9 the pericarps of the pods stored at 13°C had 19% and 27% lower TPC and antioxidant activity, respectively, as assessed by FRAP assay (Fig. 5a, c). The DPPH scavenging activity of pericarps increased gradually throughout storage at 13°C (Fig. 5b). There were no consistent differences in antioxidant activity (DPPH scavenging and FRAP assay) of pericarps after the pods were transferred to a warmer temperature. The reduction in pericarp TPC was delayed after the pods stored at 4°C were transferred to a warmer temperature (4 + WT).

Seeds of pods stored at 13°C showed increased TPC and antioxidant activity (FRAP assay), both peaking on day 12 at which point the values were approximately two-fold higher than at harvest time (Fig. 5d, f). On day 15, TPC and antioxidant activity (FRAP assay) decreased, but remained significantly high. Storage at 4°C led to slight increases in TPC and antioxidant activity as assessed by FRAP assay. However, a significant drop in antioxidant activity (FRAP assay) during storage at 4°C was found on day 15, at which point the value was about four-fold and two-fold lower than in seeds of pods stored at 13°C and at harvest, respectively. Overall, seeds of pods stored at 13 + WT had higher TPC and antioxidant activity (FRAP assay) than those of 4 + WT. DPPH activity in seeds of pods stored at 13°C was maintained throughout storage, but the activity increased after the pods were transferred to a warmer temperature (13 + WT; Fig. 5e). In contrast, the antioxidant activity of seeds in pods stored at 4°C decreased sharply, reached a minimum value on day 6, increased drastically after 9 d and maintained a steady value afterwards. The obvious reduction in antioxidant activity shown by DPPH assay over the first 6 d could have been due to low temperature stress. Following the transfer of pods stored at 4°C to a warmer temperature (4 + WT), decreased antioxidant activity of seeds was found on days 3, 12, and 15.

PPO and antioxidant enzyme activities

The PPO activities in the okra pericarp and seeds are...
shown in Figure 6. The activity of PPO in the pericarp increased as the storage day progressed, but was not consistently different. For pods stored at 4 °C, the PPO activity in the pericarp increased after 12 d of storage and peaked on day 15, at which point it was 80% higher than at harvest. For pods stored at 13 °C, the activity in the pericarp remained low following transfer to a warmer temperature. Seeds of pods stored at 4°C and 4 °C + WT had consistently higher PPO activity than those stored at 13°C and 13 °C + WT.

Figure 7a, d show that POD activity increased in the pericarp and seeds of pods stored at 13°C and was significantly higher than at 4°C after 3 d (seeds) and 6 d (pericarp) until the end of the storage period. In contrast, following warming the POD activity in the pericarp and seeds of pods stored at 4 °C + WT increased more rapidly than at 13 °C + WT. Low storage temperatures (4°C and 13°C) activated CAT activity, but following prolonged storage (after day 6) of pods at 4 °C, the activity in the pericarp declined, reaching the lowest value on day 15 at which point it was 19% and 32% lower than at harvest and in pods stored at 13°C, respectively (Fig. 7b). For pods stored at 13°C and 13 °C + WT, the activity was significantly higher than at 4 °C + WT on day 12 onwards. CAT activity in seeds at 4°C and 4 °C + WT was significantly higher than at 13°C and 13 °C + WT, respectively (Fig. 7c). As shown in Figure 7c, f, SOD activity in the pericarp and seeds of pods stored at 13°C and 13 °C + WT was significantly higher than at 4°C and 4 °C + WT. On day 15, the activity in the pericarp of pods stored at 13°C was 74% higher than at 4°C. For seeds, SOD activity increased throughout storage at 13°C and was 45–156% higher than at 4°C.

**Discussion**

Okra pods are perishable and undergo fast deterioration when kept at ambient temperature. Considering their tropical origin, the adoption of cold storage to extend the storage life of okra pods is limited by the occurrence of CI (Raju et al., 2011). The findings here confirm that okra pods are susceptible to CI when stored below 13°C (Fig. 1a); this agrees with the report by Finger et al. (2008), who showed that okra pods suffer from CI when exposed to temperatures below 10°C. Following warming, the severity of the CI symptoms increased. The sunken pits dried up (Fig. 2C, F) and resulted in visible brown necrotic patches. These symptoms are consistent with previous reports of CI in okra pods including lesions, pitting and decay (Perkins-Veazie and Collins, 1992).

We also found that the CI index was positively correlated with the seed browning index ($r = 0.89**$ at 4°C and $r = 0.94**$ after transfer to 25°C), suggesting that CI is also visibly manifested as seed browning. Browning is one of the indicators of CI in horticultural commodities (Wang, 1982). The formation of browning pigmentation involves the oxidation of phenolics to o-quinone by the activity of the PPO enzyme, which then polymerizes into browning compounds (Degl’Innocenti et al., 2007). PPO activity was induced in chilling-
injured pods both in the pericarp and seeds (Fig. 6), and it was positively correlated with CI (pericarp, \( r = 0.81^{**} \) after transfer to 25°C; seeds, \( r = 0.86^{**} \) both at 4°C and after transfer to 25°C). The levels of phenolics increased and decreased as the chilling became intense in later storage days (Zhang et al., 2000). However, phenolics and browning in okra were not correlated. This is in agreement with the reports by Degl’Innocenti et al. (2007) and Ma et al. (2015) that the mechanism of browning in rocket salads and apples does not necessarily involve endogenous phenolics. Synthesis of phenolics could be stimulated by plant stress and phenylalanine ammonia lyase (PAL). After 12 d of storage at 4°C, ultrastructural changes including wounding and membrane damage of okra seeds were observed (Fig. 3c, d). The loss of tissue compartments as a result of chilling injury leads to the release of substrates which then come in contact with PPO to cause browning (Zhang et al., 2015).

In the present study, we detected higher \( \text{H}_2\text{O}_2 \) levels in the pericarp of chilling-injured pods than in the pericarp of non-chilling-injured ones (Fig. 4a). There was a significant positive correlation between \( \text{H}_2\text{O}_2 \) content and CI in the pericarp (\( r = 0.86^{**} \) at 4°C; \( r = 0.93^{**} \) at 4°C and WT). However, noticeable differences were only detected in the seeds after they were transferred to a warmer temperature, suggesting that pericarps are more susceptible to oxidative injury as they are exposed directly to cold. On the other hand, the low initial \( \text{H}_2\text{O}_2 \) level in the pericarp for the first 6 d suggests that \( \text{H}_2\text{O}_2 \) is not the only CI causative agent, but could be another ROS such as a superoxide anion. Reactive oxygen species are also known to be causal agents of lipid peroxidation and membrane damage (Shewfelt and Rosario, 2000). Malondialdehyde, a final product of lipid peroxidation, is a common indicator of membrane damage as a result of a reaction between radicals and fatty acids. The results in Figure 4b, d clearly show that CI induced high MDA synthesis in both pericarp and seeds throughout the storage period (pericarp: \( r = 0.81^{**} \) at 4°C and \( r = 0.93^{**} \) after transfer to 25°C; seed: \( r = 0.74^{**} \) at 4°C and \( r = 0.95^{**} \) after transfer to 25°C). Furthermore, a positive correlation between \( \text{H}_2\text{O}_2 \) and MDA content was also observed (pericarp, \( r = 0.87^{**} \) and seed, \( r = 0.72^{**} \)). These results support the CI mechanism whereby chilling stress stimulated high ROS production and caused oxidative damage to okra pods. These results support the findings that CI causes membrane degradation in hot pepper seeds (Boonsirir et al., 2007).

To further confirm that ROS was the causal agent of CI, non-enzymatic antioxidants such as TPC and antioxidant activity (DPPH scavenging activity and FRAP assay) were investigated. According to Shewfelt and Rosario (2000), the endogenous ROS level is controlled or limited by the presence of high antioxidants such as \( \alpha \)-tocopherol, carotenoids, flavonoid, and phenolics. Phenolics are secondary metabolites, structurally consisting of aromatic benzene ring compounds with one or more hydroxyl groups (Pereira et al., 2009). It is known that these phenolics possess antioxidant properties by quenching free radical reactions (Sakihama et al., 2002). Antioxidant activity shown by the DPPH assay is based on the antioxidant scavenging capacity of DPPH radicals (Kedare and Singh, 2011), while the FRAP assay reduces the potential of antioxidants by converting ferric tripyridyltriazine (Fe\(^{3+}\)-TPTZ) to colored ferrous tripyridyltriazine (Fe\(^{2+}\)-TPTZ) (Benzie and Strain, 1996). In seeds, TPC and antioxidant activity assessed by FRAP assay were stimulated at both storage temperatures (4°C and 13°C). However, the antioxidant activity at 13°C was higher than at 4°C (Fig. 5d, f). Furthermore, antioxidant activity assessed by the FRAP assay decreased significantly after 12 d, and in the DPPH assay at 4°C decreased until the end of storage after transfer to a warmer temperature. The elevation of antioxidants at 4°C is possibly insufficient to cope with high production of free radicals when exposed to chilling stress, eventually resulting in oxidative damage. The different tendencies in antioxidant activity between the DPPH and FRAP assays in seeds (Fig. 5e, f) could be attributable to structural differences in polyphenol compounds based on their substituents (Cseregi et al., 2016). Many studies have reported that the capacity of antioxidants to scavenge ROS is related to chilling tolerance (Nukuntoomprakit et al., 2015; Wongsheeree et al., 2009).

High levels of POD and SOD enzymes were found in the pericarp and seeds of pods stored at 13°C compared with those stored at 4°C (Fig. 7). An increase in SOD has been linked to the dismutation of superoxide anions which are then converted into \( \text{H}_2\text{O}_2 \) (Singh and Singh, 2013). This \( \text{H}_2\text{O}_2 \) was further eliminated by POD into water and oxygen. This explains the low \( \text{H}_2\text{O}_2 \) level in non-chilling injured okra pods stored at 13°C. The CI incidence in pericarps at 4°C can also be further explained by a decrease in SOD activity that led to the accumulation of superoxide anions, and in the presence of \( \text{H}_2\text{O}_2 \), resulted in formation of the most harmful radical, the hydroxyl radical, generated through the Haber-Weiss reaction (Pospisil et al., 2004). An early increase in CAT activity in the pericarp was triggered by the primary response to chilling stress. According to Lukatkin (2005), CI is initiated by the elevation of ROS and enzyme activities. At this stage, the cell works to reduce the chilling stress. However, extension of the chilling period, as well as an increase in lipid peroxidation, eventually led to membrane breakdown and disintegration in the secondary phase of chilling stress. In seeds, CAT activity was higher at 4°C than at 13°C, indicating that CAT could be a key enzyme that helps seeds to cope with stress. Additionally, CAT activity was correlated with water loss in chilling-injured seeds after transfer to a warmer temperature (\( r = 0.84^{**} \)). These re-
results support findings of elevated CAT activity in water-stressed sunflowers (Bailly et al., 2004) and chilling-sensitive immature cucumbers (Qian et al., 2013).

Similarly, high POD activity was found in the pericarp and seeds of chilling-injured pods after transfer to a warmer temperature. This could also be related to the water stress response as POD activity was positively correlated with water loss (data not shown) (pericarp: \( r = 0.84^{**} \); seed: \( r = 0.81^{**} \)). Nevertheless, increases in CAT and POD activities after warming were not sufficient to prevent oxidative damage, which already was severe and advanced. The POD enzyme not only plays a role as an antioxidant, but also causes browning in various crops (Cao et al., 2011; Zhang et al., 2015). In addition, the POD enzyme is also involved in lignification because a higher activity in pods stored at 13°C could have contributed to pod toughening (Fig. 1b).

Overall, the data suggest that the SOD and POD enzymes are likely key antioxidants that prevent oxidative damage to okra pods. These results are consistent with other studies on cucumber (Qian et al., 2013), hot peppers (Boonsiri et al., 2007), and kiwi (Yang et al., 2012).

In conclusion, okra pods were susceptible to CI only during storage at 4°C. The CI symptoms were more severe in pods that were transferred to a warmer temperature. The low storage temperature at 4°C resulted in increased H₂O₂ and MDA contents in okra pods. In addition, CI was also indicated by seed browning. Lower levels of enzymatic antioxidants (SOD and POD) and non-enzymatic antioxidants (phenolics, DPPH scavenging activity and FRAP assay) were correlated with CI on the pericarp of okra pods, as well as seed browning. This study did in-depth research on the CI mechanism in okra pods and provides insight into ways of improving the postharvest quality of okra.

Acknowledgements

Authors would like to acknowledge the financial support provided by Petch Pra Jom Klaos-Ph.D Research Scholarship (Agreement No. 22/2559), King Mongkut’s University of Technology Thonburi, Thailand.

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Physiol. 52: 542–546.


