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Research Article

Different Chilling-Induced Symptoms and the Underlying Oxidative Stress and Antioxidative Defense in the Exocarp and Mesocarp of Immature Sponge Gourd (*Luffa cylindrica*) Fruit

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Abstract
Immature sponge gourd fruit is consumed as a vegetable with a limited shelf life. Although cold storage is a simple and powerful tool for maintaining postharvest fruit quality, storage at a low temperature may not be appropriate for vegetables as some chilling injury (CI) of the immature sponge gourd fruit may occur. Therefore, this research aimed to elucidate the relationship between CI, oxidative stress, and the antioxidative defense mechanisms in the exocarp and mesocarp of immature sponge gourd fruit. After storage at 5°C for 6 days, visual CI symptoms, including browning and surface pitting, were found in the peel (exocarp) but not in the mesocarp. There were, however, more dead cells (stained by Evans blue) in the mesocarp of the fruit stored at 5°C. There was a more considerable increase in the electrolyte leakage rate in both fruit tissues held at 5°C than 25°C. The CI was correlated with malondialdehyde (MDA) levels in the tissues. The MDA of fruit exocarp at 5°C was 1.6 fold higher than that at 25°C on day 6, while the lipoxygenase (LOX) activity in mesocarp was 50% higher in fruit stored at a lower temperature. The action of ascorbate peroxidase (APX) was high in the exocarp of the fruit stored at 5°C, but there appeared to be a continuous depletion of the co-substrate or ascorbic acid. In conclusion, the CI in the exocarp was mainly associated with a high level of reactive oxygen species (ROS). In contrast, the CI in the mesocarp appeared to be primarily associated with increased lipid peroxidation by the elevated LOX activity under cold stress compared to storage at 25°C.

Keywords: Ascorbic acid, Antioxidant, Chilling injury, Cold stress, Reactive oxygen species

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1 Introduction

In different Asian countries, immature fruit of sponge gourd (*Luffa cylindrica*) is generally consumed as a fresh vegetable after 12–15 days from full bloom. The fruit quality declines after this period of time as highly fibrous tissues would continue to appear in the mesocarp. This is an important consideration in any marketing plan of fresh sponge gourd to the consumer.

Under some extreme abiotic treatments and improper environments such as water, temperature, or chemical stress, affected plants or plant parts could be induced to accumulate some oxidative intermediates [1]–[4]. Cold storage of fresh produce is beneficial for extending the shelf life and the marketing window for the produce. The optimum temperature range for storing immature sponge gourd fruit is from 10 to 12°C [5]. At a lower storage temperature, although the rate of metabolism of fresh produce would be lowered, the lower temperature could induce an excess reactive oxygen species (ROS) leading to accelerated senescence of several plant parts during storage [6], [7]. Reactive oxygen species (ROS) may cause membrane malfunction mainly through lipid peroxidation if not counteracted by plants' antioxidative defense mechanisms including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) that scavenge ROS [6]. Nevertheless, long storage at an inappropriate low temperature, for example, 5°C, is known to cause chilling injury (CI) in several tropical commodities impacting negatively on the quality preference of products. The CI symptoms, including surface pitting, water soaking, discoloring and vulnerability to plant pathogenic inflection can be detected visually either from the outside (the peel), or from inside of the fruit (pulp) showing seed or flesh discoloration and water soaking [8]–[10]. Furthermore, inappropriately chilled fruits could exhibit additional major damages after transfer to room temperature conditions.

During postharvest storage of cucumber fruit at 2°C for 9 days, CI symptoms first appeared in the calyx end, then in the middle and the stalk end [11]. Recently, it has been shown that salicylic acid (SA) treatment could effectively alleviate CI and enhance chilling tolerance of whole immature sponge gourd fruit during storage at 9°C for 9 days [12]. There is, however, no report on the relationship between oxidative stress and CI in immature sponge gourd fruit. Moreover, it is unknown if the exocarp and mesocarp of immature sponge gourd fruit would also exhibit different CI susceptibility. Thus, in the present study, 2 different edible portions between the exocarp and mesocarp of immature sponge gourd fruit were investigated for the oxidative stress responses and CI defense under an inappropriate cold storage temperature (5°C) compared to storage at 25°C.

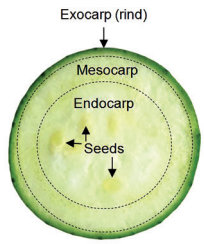

Figure 1: Internal structure of immature sponge gourd fruit.

2 Materials and Methods

2.1 *Plant materials and storage conditions*

Immature sponge gourd fruit cv. "Hom" at 15 days after full broom were collected from a commercial orchard at Pathum Thani province, western Thailand. Fruit were brought to the Laboratory within an hour. Fruit containing the pedicle were sorted for the uniformity of size (length 12–15 cm) and weight (1,500–2,000 g) and were then placed into plastic baskets (20 fruit/basket) and stored at 25 ± 2°C, 65–70% RH and 5 ± 2°C, 85–90% RH. The stored fruit were randomly chosen for investigation based on visual appearance and changes in the exocarp and mesocarp at 2 days interval (Figure 1).

2.2 *Evaluation of chilling stress and response*

2.2.1 External CI symptoms

The visual appearance and CI symptoms of the fruit during storage were recorded using a digital photo camera.

2.2.2 Tissue staining with Evans blue

For cell viability test, a freehand cross-section of

sponge gourd fruit was stained with an Evans blue dye solution (1%, w/v) for 2 min before washing with distilled water. After this, the sections were placed on a glass slide and observed under a light microscope [13].

2.2.3 Electrolyte leakage of tissues

To investigate electrolyte leakage (EL), 10 disks (8 mm in diameter and 2 mm in thickness) of sponge gourd flesh were prepared using a cork borer. The disks were washed twice with tap water and then incubated in 50 mL of distilled water for 30 min. The electrical conductivity (EC) of the water used for incubation was measured using an electrical conductivity meter (CyberScan PC 510, Singapore). These disks were boiled in distilled water for 10 min before EC was re-measured [14] and the percentage of electrolyte leakage EL was calculated using the following formula:

$$EL\ (\%) = (EC_{Soaking\ disk\ solution}/EC_{Boiling\ disk\ solution}) \times 100.$$

2.3 ***Free radical determination***

2.3.1 Superoxide anion content

The superoxide anion ($O2^{\bullet-}$) content in sponge gourd flesh was determined according to Krishan Chaitanya and Naithani [15]. Sponge gourd flesh (2 g) was homogenized in 2 mL of 0.05 M potassium phosphate buffer (pH 7.8) containing 1 mM diethyldithiocarbamate (a SOD inhibitor) using a homogenizer (IKA Ultrarax T 25, Germany) and centrifuged at 10,000 × g for 20 min. Supernatant (1 mL) was mixed with 3 mL of 0.1 M potassium phosphate buffer (pH 7.8) containing 1 mM diethyldithiocarbamate and 0.25 mM nitro blue tetrazolium (NBT). A change in the absorbance of the reaction mixture after 1 min was measured at 540 nm using a spectrophotometer (PG Instrument Ltd T80+ UV-Vis, United Kingdom).

2.3.2 Hydrogen peroxide content

The hydrogen peroxide (H_2O_2) content in sponge gourd flesh was determined according to the method of Zouari *et al.* [16]. One gram of sponge gourd flesh was ground in 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 15 min. One mL of the supernatant was mixed with 0.5 mL of 0.01 M potassium phosphate buffer (pH 7) containing 1 mL of 0.5 M potassium iodine and then the absorbance of the reaction mixture was measured at 390 nm using a spectrophotometer. This experiment was carried out at 4°C. A standard curve was constructed using different concentrations of H_2O_2.

2.4 ***Lipid peroxidation product and lipoxygenase activity***

2.4.1 Malondialdehyde content

Lipid peroxidation was determined in relation to the degradation of lipid by-product, malondialdehyde (MDA). Sponge gourd flesh (1 g) was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 15 min. The supernatant (1 mL) was mixed with 1 mL of 20% (w/v) TCA containing 0.5% thiobarbituric acid (TBA), and then incubated at 100°C for 10 min. After this, the mixture was placed on ice to cool down prior to centrifugation at 10,000 × g for 10 min. The supernatant was then used for reading absorbance at 532 and 600 nm (Extinction coefficient = 155 mM/cm) using a spectrophotometer. The MDA concentration (μM) was calculated using the following formula [17].

$$MDA\ (\mu mol \cdot g^{-1}FW) = (Abs_{532\ nm} - Abs_{600\ nm}/155\ mM/cm) \times 100$$

2.4.2 Assay of lipoxygenase activity

Lipoxygenase (LOX) extraction and assessment were carried out according to Mao *et al.* [11]. Sponge gourd flesh (3 g) was homogenized in 6 mL of 0.05 M potassium phosphate buffer (pH 7) and centrifuged at 10,000 × g for 15 min. For LOX activity assay, 20 μL of the enzyme extract was mixed with 3 mL of 0.2 M potassium phosphate buffer (pH 6.5) and 20 μL of linoleic acid (prepared from 0.5 g linoleic acid in 25 mL of de-ionized water containing 0.5 g Tween 20). The absorbance of the reaction mixture was then measured at 234 nm using a spectrophotometer. One unit of LOX activity was represented by an increase of 0.01 absorbance unit min^{-1}.

2.5 *Assays of enzymes involved in ROS defense mechanisms*

2.5.1 Enzyme extraction

ROS extraction was carried out according to the methods of Dhindsa *et al.* [18] and Jiménez *et al.* [19]. The sponge gourd flesh (1 g) was homogenized with 10 mL of 0.05 M potassium phosphate buffer (pH 7.8) containing 1% polyvinylpolypyrrolidone (PVPP) and 0.1% Triton X-100 and then the homogenate was centrifuged at 12,000 × g for 15 min. After centrifugation, the supernatant was used to determine SOD, CAT, APX and POD activities.

2.5.2 Assay of superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined according to the Dhindsa *et al.* method [18]. The enzyme extract (100 µL) was mixed with 3 mL of 0.05 M potassium phosphate buffer (pH 7.8) containing 13 mM methionine, 75 µM NBT, 0.1 mM EDTA and 4 µM riboflavin. After that, the reaction mixture was left under fluorescent light (15 Watt) for 30 min, before the absorbance was measured at 560 nm and compared to the reaction mixture without enzyme extract. One unit of SOD activity was represented by 50% of NBT inhibition calculated using the following formula.

$$SOD\ (unit \cdot g^{-1}FW) = (Abs_{Control} - Abs_{Sample}/Abs_{Control} \times 100)/2$$

2.5.3 Assay of catalase activity

The enzyme extract (100 µL) was mixed with 3 mL of potassium phosphate buffer (pH 7) containing 0.2 mM H_2O_2, and then the absorbance of the reaction mixture was measured at 240 nm using a spectrophotometer. One unit of CAT activity was represented by the decrease of 0.01 absorbance unit min^{-1} [20].

2.5.4 Assay of peroxidase activity

The enzyme extract (100 µL) was mixed with 3 mL of potassium phosphate buffer (pH 6) containing 25 mM guaiacol and 200 mM H_2O_2, and the absorbance of the reaction mixture was measured at 470 nm using a spectrophotometer. One unit of POD activity was represented by an increase of 0.01 absorbance unit min^{-1} [21].

2.5.5 Assay of ascorbate peroxidase activity

The enzyme extract (100 µL) was mixed with 3 mL of 0.05 M potassium phosphate buffer (pH 7.6) containing 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 0.1 mM EDTA. The absorbance of the reaction mixture was measured at 290 nm using a spectrophotometer. One unit of APX activity was represented by a reduction of 0.01 absorbance unit min^{-1} [22].

2.6 *Antioxidative defense substances*

2.6.1 Ascorbic acid content

Ascorbic acid (AsA) determination was carried out according to the Klein and Perry method [23]. Sponge gourd flesh (1 g) was homogenized with 10 mL of 0.5% metaphosphoric acid using a homogenizer and centrifuged at 12,000 × g for 10 min. The supernatant (0.5 mL) was mixed with 4.5 mL of 0.1 mM dichlorophenolindophenol (2,6-DCIP) and measured at 515 nm using a spectrophotometer. Ascorbic acid concentration was calculated using the ascorbic acid standard curve.

2.6.2 Determination of lignin content

Sponge gourd flesh (4 g) was homogenized in 16 mL of 100% methanol, filtered and dried at 60°C for 24 h. The dried powder (50 mg) was soaked in 5 mL of 2 N HCl containing 0.5 mL of 98% thioglycolic acid. The reaction mixture was then boiled at 100°C for 4 h. After cooling down, the mixture was centrifuged at 12,000 × g for 30 min. The pellet was washed with 1 mL of conc. HCl and was then centrifuged at 10,000 × g for 10 min to obtain the orange-red residue. Twenty five milliliters of 0.5 N NaOH was added into the residue before the absorbance of the mixture was measured at 280 nm using a spectrophotometer [24].

2.7 *Analysis of protein content*

Protein content was determined using the protein-dye binding method based on the Coomassie Brilliant Blue reagent and the absorbance of the protein-dye reaction

Figure 2: Visual appearance of immature sponge gourd fruit stored at 25 ± 2°C (a) and 5 ± 2°C (b).

was measured at 595 nm using a spectrophotometer. Bovine serum albumin (BSA) was used to construct a standard curve [25].

2.8 *Statistical analysis*

The experiment was managed as a completely randomized design with 4 replicates (one fruit/replicate) in each treatment. The variation of variance was analyzed as one-way analysis of variance using the SPSS statistical software version 26 (IBM, Chicago, IL, USA) ($p < 0.05$). Least Significant Difference was used for comparison of the means.

3 Results and Discussion

3.1 *CI sensitivity between two edible parts of immature sponge gourd fruit*

During cold storage, fresh produce could exhibit CI symptoms such as brown coloring, water soaking, and surface pitting (collapsed cells). Some parts of the produce might initially exhibit more resistance or susceptibility to CI, mainly on the associated CI defense mechanisms. Surface pitting and brown coloring were initially found on the exocarp part of immature sponge gourd fruit during storage at 5°C for 6 days [Figure 2(a)]. These were CI symptoms as they were not found on the fruit surface at 25°C [Figure 2(b)].

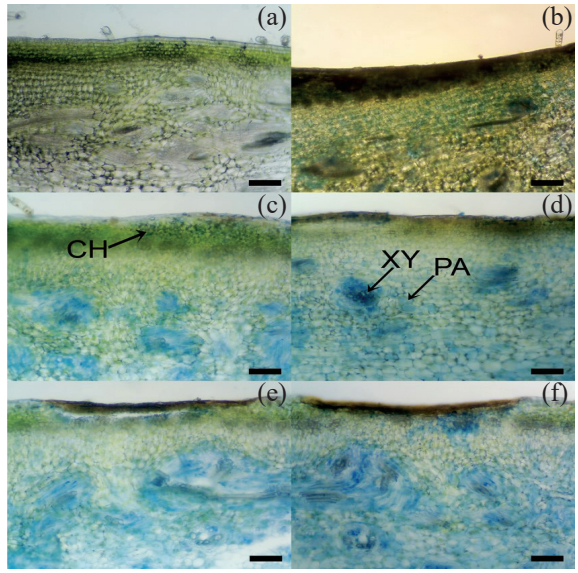

Figure 3: Photomicrographs of internal chilling injuries (CI) symptoms in immature sponge gourd tissue after Evans blue dye staining (a to f were 2, 4, 6, 8, 10, and 12 days during storage at 5 ± 2°C, respectively). Arrow pointed Evans blue dye staining area: CH=chlorenchyma, PA=parenchyma, and XY = xylem vessel. (scale bar = 100 µm).

Furthermore, following staining with Evans blue dye, a lot of dead cells taking up the blue dye presumably because of cold-induced membrane damages [26], [27] were revealed in the internal tissues of fruit stored at 5°C, whereas no dead cells were found in the fruit stored at 25°C. The dead cells in the exocarp tissues were mainly chlorenchyma cells, but in the mesocarp, most of the dead cells were both parenchyma cells and xylem vessels. More dead cells obviously appeared when the fruit were stored longer than 6 days (Figure 3). Based on the relative amount of living and dead cells remaining, the exocarp part of immature sponge gourd was presumably more susceptible to CI stress than the mesocarp which still had some living cells after 12 days of cold storage.

3.2 *Oxidative stress and the antioxidative defense mechanisms of the exocarp and mesocarp*

3.2.1 ROS induction by chilling stress

In this experiment, the exocarp contained a higher

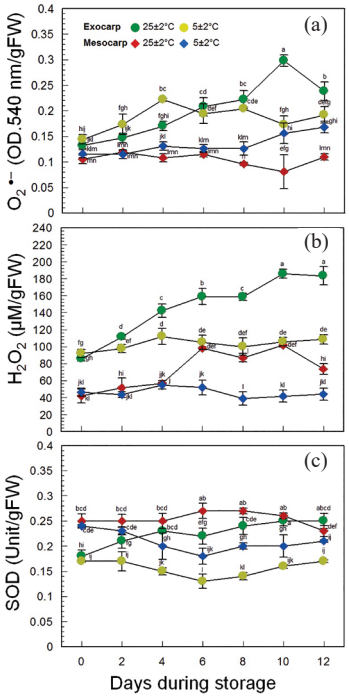

Figure 4: Changes in the $O2^{\bullet-}$ production rate (a), H_2O_2 content (b) and SOD activity (c) in the exocarp and mesocarp of the immature sponge gourd fruit during storage at 25 ± 2 and 5 ± 2°C. Vertical bars represent standard error (SE) of mean values. Different letters above symbols indicate significant differences ($p < 0.05$) of treatment combinations between tissues and storage temperatures according to least significant difference.

superoxide anion ($O2^{\bullet-}$) content than the mesocarp of immature sponge gourd stored at 25°C and 5°C [Figure 4(a)]. In the mesocarp of the fruit stored at 5°C, there was a higher level of superoxide than that stored at 25°C particularly after 6 days. In comparison, only at day 10 there was a particularly higher level of the superoxide content in the mesocarp of the fruit stored at 5°C than that stored at 25°C. Interestingly, at day 10, the superoxide content in the exocarp of the fruit stored at 25°C was higher than that stored at 5°C.

The hydrogen peroxide (H_2O_2) content in the exocarp of the fruit stored at 25°C was noticeably higher than that stored at 5°C [Figure 4(b)]. There was no change in the hydrogen peroxide level in the mesocarp of the fruit throughout storage at 5°C but it was increased in the mesocarp of the fruit stored at 25°C particularly after 6 days. Hydrogen peroxide (H_2O_2) is known as a non-active form of ROS but it will be changed to a strong-active hydroxyl ion (HO•) via Fenton-reaction in the presence of Fe^{2+} [28]. Normally, HO• is harmful to membrane lipid and this toxic free radical can activate lipid peroxidation as indicated by an increase in the MDA concentration, resulting in a high rate of electrolyte leakage. Eventually, peroxidation of membrane lipids can disturb the membrane integrity, which affects membrane fluidity and lipid-protein interaction, membrane permeability, and metabolic processes leading to cell death [29]. Living cells in all plant parts generally have superoxide dismutase (SOD) to defend against $O2^{\bullet-}$ by oxidizing it into H_2O_2. In both the exocarp and the mesocarp of the fruit stored at 25°C, there was a higher level of SOD activity than that stored at 5°C [Figure 4(c)].

3.2.2 Lipid peroxidation of the membranes

Lipoxygenase (LOX) activity in the exocarp and mesocarp of immature sponge gourd fruit stored at 5°C was higher than at 25°C throughout the experiment [Figure 5(a)]. LOX, an unsaturated fatty acid oxidizing enzyme, oxidizes polyunsaturated fatty acid (PUFA) into lipid hydroperoxide (LOOH) and an increase in the enzyme activity is associated with cell membrane injuries. Increased cellular electrolyte leakage, a major indicator for CI determination, would normally follow an increase in LOX activity [30], [31]. LOX seemed to play a role in PUFA oxidation in both the exocarp and mesocarp of immature sponge gourd fruit stored at 5°C which was also found in a similar study on cucumber fruit [32]. Malondialdehyde (MDA), a by-product of lipid degradation process, was higher in the exocarp and mesocarp of the immature sponge gourd fruit stored at 5°C than at 25°C, particularly from 6 days onwards [Figure 5(b)]. Electrolyte leakage (EL) in the exocarp and mesocarp of the immature sponge gourd fruit was found to increase during storage at both 5°C and 25°C, but the rate of increase was higher in the exocarp and mesocarp of the fruit at 5°C than at 25°C [Figure 5(c)]. The low temperature storage might trigger a higher rate of EL in the exocarp and mesocarp of the immature sponge gourd fruit, and this might be related to peroxidation of membrane lipid by either increased level of ROS or LOX activity [30],

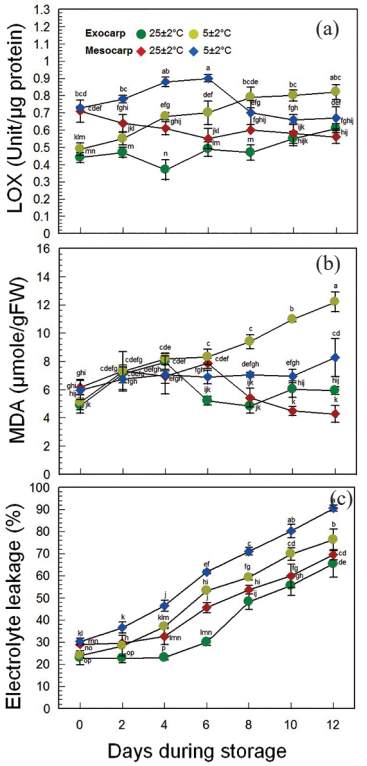

Figure 5: Changes in the LOX activity (a), MDA content (b) and Electrolyte leakage (c) in the exocarp and mesocarp of the immature sponge gourd fruit during storage at 25 ± 2 and 5 ± 2°C. Vertical bars represent standard error (SE) of mean values. Different letters above symbols indicate significant differences ($p < 0.05$) of treatment combinations between tissues and storage temperatures according to least significant difference

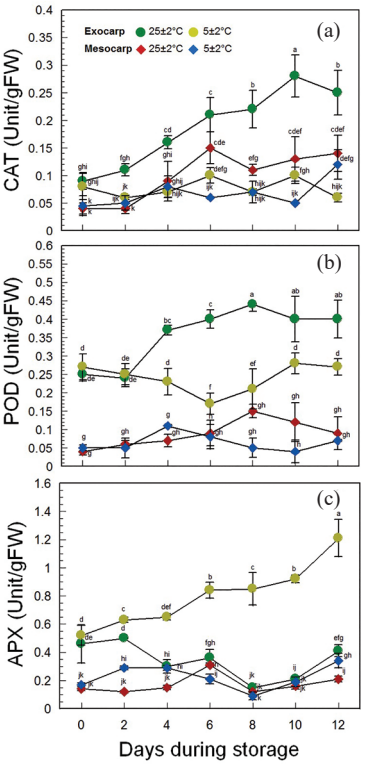

Figure 6: Changes in the CAT (a), POD (b) and APX activities (c) in the exocarp and mesocarp of the immature sponge gourd fruit during storage at 25 ± 2 and 5 ± 2°C. Vertical bars represent standard error (SE) of mean values. Different letters above symbols indicate significant differences ($p < 0.05$) of treatment combinations between tissues and storage temperatures according to least significant difference.

[31]. Similarly, in cucumber fruit, the MDA content was highly elevated following an increase in EL during storage at a low temperature [33].

3.2.3 CI defense mechanisms

Plant cells have antioxidative enzymes to counteract excessive ROS levels. For example, catalase (CAT), localized in peroxisomes catalyzes the conversion of H_2O_2 into water [34]. The catalase activity in the exocarp and the mesocarp of the immature sponge gourd fruit stored at 25°C was higher than at 5°C [Figure 6(a)], suggesting that CAT might not be involved in the defense against cold-induced damages in the immature sponge gourd fruit. Also, low temperature stress could possibly lower CAT activity. Peroxidase (POD) which may be involved in lignin production in the exocarp of immature sponge gourd fruit stored at 25°C was higher than at 5°C, particularly after 6 days of storage [Figure 6(b)]. The difference in the level of peroxidase activity in the mesocarp of the fruit stored at 25°C and 5°C relatively more minor [Figure 6(b)]. These results suggested that POD might not play a main role in the defense against ROS in the exocarp and mesocarp of immature sponge gourd during cold storage.

Ascorbate peroxidase (APX) catalyzes the conversion of ascorbate and H_2O_2 into water. The

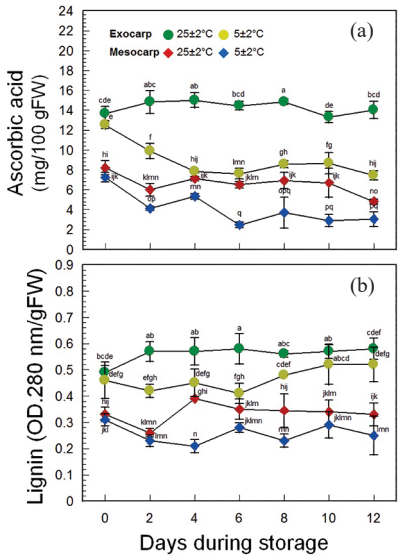

Figure 7: Changes in the ascorbic acid (a) and lignin content (b) in the exocarp and mesocarp of the immature sponge gourd fruit during storage at 25 ± 2 and 5 ± 2°C. Vertical bars represent standard error (SE) of mean values. Different letters above symbols indicate significant differences ($p < 0.05$) of treatment combinations between tissues and storage temperatures according to least significant difference.

activity of APX increased continuously in the exocarp of the immature sponge gourd fruit during storage at 5°C, but the APX activity was lower and started to decrease during storage at 25°C [Figure 6(c)]. This suggests that the increased APX activity in the fruit stored at the low temperature might play a role in the defense against excess H_2O_2. In contrast, the APX activity in the mesocarp was low and there was little difference when stored at 25°C or at 5°C. There was a higher level of ascorbic acid in the exocarp than the mesocarp of the immature sponge gourd fruit [Figure 7(a)]. There was little change in the ascorbic acid content in the exocarp of the fruit stored at 25°C, but that in the fruit stored at 5°C decreased continuously [Figure 7(a)], suggesting cold induced a loss of ascorbic acid which was an antioxidant for protection against excess damaging ROS. There was also little change in the ascorbic acid content in the mesocarp of the fruit during the first 10 days of storage at 25°C, while the ascorbic acid content in the mesocarp was lower at 5°C than at 25°C but also remained relatively the same during storage of the fruit at 5°C [Figure 7(a)]. Therefore, the ascorbic acid content was lowered in the exocarp and mesocarp of the immature sponge gourd fruit continuously during cold storage in the dark. This could occur because during storage there was no light source to produce NADPH by photosynthesis. Noichinda *et al.* [35] reported that exposure to dim light (21.8 μmol/m^2/s) during cold storage of Chinese kale could activate the production of sugar and ascorbic acid. Typically, the biosynthesis of ascorbic acid in plant cells occurred independently between the chloroplast and the mitochondria. This pathway required galactono-1, 4-lactone as a substrate for L-galactono-1, 4-lactone dehydrogenase in the mitochondrial electron transport chain and NADPH from photosynthesis under continuous light exposure [36].

In both the exocarp and mesocarp, there was a higher lignin content when the fruit was stored at 25°C than at 5°C [Figure 7(b)]. Lignin formation may be associated with abiotic stress in plant cells [37], but low temperature stress during storage of the immature sponge gourd fruit did not result in increased accumulation of lignin in the exocarp and the mesocarp compared to storage at a higher temperature.

3.3 *Putative model of CI in immature sponge gourd fruit*

Based on the present study results, we propose a putative model of the major chilling–induced biochemical change and ROS defense mechanisms in the exocarp and mesocarp of the immature sponge gourd fruit stored at 5°C (Figure 8). The major biochemical changes under CI injuries in immature sponge gourd at 5°C appeared to be the disruption in cell membrane functions, most notably related to the increased peroxidation of lipids by LOX and a higher rate of electrolyte leakage in the mesocarp and the increased level of ROS (particularly superoxide anion) [29] in the exocarp. The level of free radicle scavenging, both antioxidants by chemical and enzymatic mechanisms in the cells is crucial. As a result, the exocarp of the immature sponge gourd fruit was more susceptible to CI stress due to a declining level of ascorbic acid involved in antioxidative defense under cold storage. APX, seemed to play an important role in antioxidative defense in the immature sponge gourd fruit during storage at 5°C as same as detoxifying mechanisms in

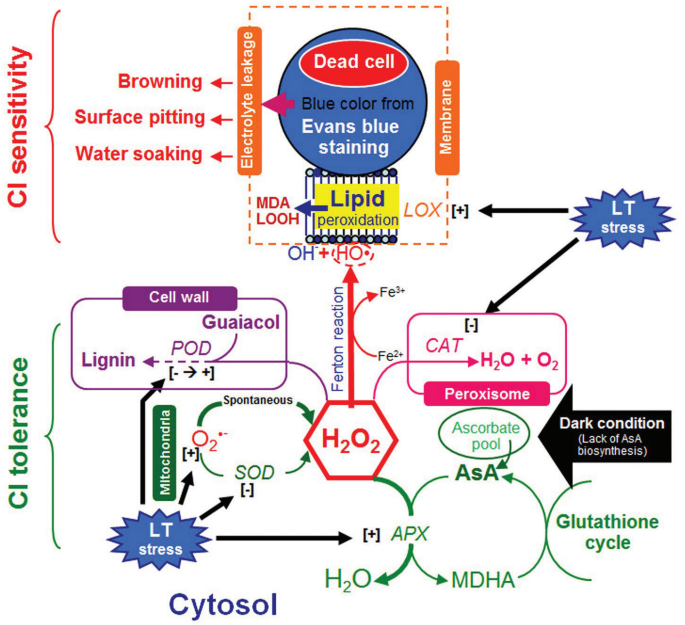

Figure 8: The proposed chilling injury (CI) sensitivity and tolerance in immature sponge gourd fruit during storage at low temperature (LT) condition: Superoxide anion ($O2^{\bullet-}$), hydrogen peroxide (H_2O_2), ascorbic acid (AsA), ascorbate peroxidase (APX), monodehydroascorbate (MDHA), superoxide dismutase (SOD), catalase (CAT), lipoxygenase (LOX), peroxidase (POD), hydroxyl radical (HO•), lipid hydroperoxide (LOOH) and malondialdehyde (MDA), induce [+] and inhibit [–].

the flesh of mangosteen fruit under hypoxic conditions [3]. Besides, the POD activity in the mesocarp of immature sponge gourd fruit stored at 5°C might also be involved in the defense against cold stress on the fruit during storage as found in some affected plants [3], [7].

4 Conclusions

When the immature sponge gourd fruits were stored at 5°C, 85–90% RH, different levels of CI damages were exhibited by the exocarp and mesocarp. Visual CI symptoms, including surface browning and pitting were found in the exocarp tissues but not in the mesocarp. Instead, there were more dead cells (stained by Evans dye) in the mesocarp. Increased lipid peroxidation in the exocarp of the fruit during storage at 5°C was mainly related to a higher level of ROS, whereas in the mesocarp it was mainly related to the increased LOX activity. Cold stress during storage of the fruit seemed to be able to lower the content of ascorbic acid for antioxidative defense, particularly in the exocarp.

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