

Tropical and Subtropical Fruits: Postharvest Biology and Storage

Lead Guest Editor: Shifeng Cao

Guest Editors: Zhenfeng Yang and Sunil Pareek





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Editorial

Tropical and Subtropical Fruits: Postharvest Biology and Storage

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This special issue is focused on the factors involved in ripening, senescence, and deterioration of harvested tropical and subtropical fruits and the postharvest technologies to restrict loss and ensure the maximum quality value.

Banana is one of the major commercial fruit crops grown in tropics and subtropics. It is very susceptible to mechanical damage during harvesting, handling, packaging, and transportation, which results in a substantial reduction in quality. Phospholipase D (PLD) is an important enzyme that initiates membrane phospholipid degradation during ripening, senescence, and signal transduction that takes place in response to hormones and environmental stresses. Li et al. clone one PLD α gene from banana fruit, which shows an increasing trend during fruit ripening, thereby leading to the damage of cell-membrane integrity and senescence of postharvest bananas. Then, they continue their PLD research and focus their paper on its response to mechanical wounding in postharvest banana fruit, which provides basic knowledge for further investigating the mechanism of postharvest banana adapting to environmental stresses.

Longan, litchi, and rambutan are typical subtropical fruit opulently cultivated in southern China, which are popular for customers to buy in summer. However, due to cell-membrane lipid peroxidation and polyphenol oxidation, the inherent perishability of these fruit has been considered as a serious problem, resulting in fruit browning or fungal infection in the first week after harvested from orchard, which greatly impairs economic chain of fruit agriculture. Li et al. report that both refrigeration and UV-C exposure can extend the shelf life of the three fruit.

Mangosteen is popularly grown in Southeast Asia. During on-tree maturation of mangosteen fruit, rainfall

frequently induces more translucent flesh disorder developing in ripe fruit. Translucent disorder, translucency, and a litter bit crispy texture, is an abnormal ripening process that occurs at the largest segment of aril. This disorder makes a serious problem for mangosteen grower from the past until now. Noichinda et al. present a research article on this disorder and find that the capillary water in fruit pericarp is one of the reasons in mangosteen aril.

Apricots are popular worldwide owing to their high nutritional value and delicious flavor, which not only can be used as fresh fruit but also has a high importance as processed product. Refrigeration is widely used to delay ripening and control fruit decay, but apricot is a cold-sensitive fruit and is subject to defects at low temperature. Jing et al. discuss the effects of different harvest maturity on the incidence of chilling injury and storage quality of apricots and provide a theoretical reference to the suitable harvest maturity of this fruit.

Each tropical and subtropical fruit possesses unique characteristics expressed in appearance and flavor and sometimes in its growth habit. Decay and quality deterioration are the major postharvest problems when the fruit are stored at ambient temperatures. Low temperature storage is an effective method to retard ripening and senescence; however, many tropical and subtropical fruit will develop chilling injury and be sensitive to fungal invasion. Therefore, better understanding of postharvest physiology of tropical and subtropical fruit and the development of adequate postharvest technologies are vital for successful handling and long distance transport. It is certainly worth investigating improved procedures for storage and ripening that would allow preconditioned, ripening-initiated,

ready-to-eat fruit to be offered to consumers. Genetic transformation to manipulate the progression and uniformity of ripening, disease resistance, and senescence may be also a helpful strategy.

Shifeng Cao
Zhenfeng Yang
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Research Article

Effects of Harvest Maturity on Chilling Injury and Storage Quality of Apricots

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Fresh apricots have high nutritional value and demand. Determination of the appropriate maturity is vital for fruit storage. The effects of harvest maturity on chilling injury and storage quality were investigated in this study. Xinjiang Saimaiti apricots were used as the material; the fruit was picked at three different maturity classes, maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) according to yellow conversion rate, and stored at 0°C and 90–95% RH. Chilling injury incidence, chilling index, and the physiological indicators were evaluated. The results showed that the incidence, index of chilling injury, and firmness in apricots of maturity class I were highest than other two groups, but maturity class I apricots did not ripe. Although the incidence and index of chilling injury in maturity class III were relatively low, fruit firmness decreased rapidly. The incidence and index of chilling injury of apricots in maturity class II were lower than those of fruits at maturity class I, whereas fruit firmness, soluble solid content, ascorbic acid level, and extractable juice quantity relatively were well-maintained. Therefore, maturity class II was considered the appropriate maturity stage at harvest for storage.

1. Introduction

Apricots (*Prunus armeniaca*) are popular worldwide owing to their high nutritional value and delicious flavor, which not only can be used as fresh fruit but also has a high importance as processed product [1]. However, fresh apricot production occurs mainly from June to July, when they become available in the market in large quantities for a short period. Fruits soften, and their quality and flavor reduce rapidly in a few days at room temperature after harvest, resulting in extensive fruit rotting [2]. Fruits are not always consumed immediately after harvest and are therefore held in cold storage, refrigeration is widely used to delay ripening and control fruit decay, but apricots is a cold-sensitive fruit and is subject to defects at low temperature [3]. Structural damage of cell membranes, the dysregulation of physiological processes, and metabolism occur in apricots during cold storage. In apricots, these symptoms, termed chilling injury, are manifested as fruit becomes of hard texture, coarse, less juicy and lacks the ability to ripe, which severely affects the sensory properties and commercial value of apricots [4].

Apricots are very perishable due to rapid ripening and softening after harvest, which confers sensitivity to mechanical injury and pathogen infection. Apricots are usually harvested at the preclimacteric stage to maintain sufficient fruit firmness to withstand postharvest handling, transportation, marketing, and consumption [5]. The classes of maturity at harvest are an important factor affecting postharvest fruit quality; consumer acceptability of apricot fruit was associated with sweetness, color, and juiciness, and the quality of fruits during the storage process is intimately associated with harvest maturity of fruits [6]. Therefore, the harvest maturity plays a vital role in determining the commodity values of apricots during the storage [7]. Over the past decade, great effort has been focused on mainly the effects of storage techniques on chilling injury and mechanisms of chilling injury in apricots [3, 4]. Studies have shown that treatments with polyamines and oxalic acid effectively inhibited chilling injury in apricots through different mechanisms [8, 9]. However, there are a few reports on the effects of harvest maturity on chilling injury in apricots. This study was aimed at investigating the effects of different harvest maturity on the

incidence of chilling injury and storage quality of apricots using apricots at different maturity classes and provides a theoretical reference to the suitable harvest maturity of apricots.

2. Materials and Methods

2.1. Fruit Material. Saimaiti apricots were harvested from an apricot orchard in Wuqia town (Kuche County, Xinjiang Province, China) and transported to the postharvest physiology laboratory of Xinjiang Agricultural University. The fruit was precooled for 24 hours and sorted for uniform size and maturity and any fruits with wounds or rots were removed. The apricots were classified into three different maturity classes as maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) according to the yellow conversion rate and were stored at 0°C and 90–95% RH. Each treatment was replicated three times with 10 kg of fruits for each maturity class per replication. The experiment was repeated three times.

2.2. Measurement of Chilling Injury Index and Chilling Injury Incidence. The chilling injury index for fruit was assessed by evaluating the extent of total chilling symptoms on each fruit surface using the following scale reported in [10] with minor modification: 0 = no visible chilling symptoms; 1 = <15% chilling spots; 2 = 15–25% chilling spots; 3 = 25–50% chilling spots; 4 = 50–75% chilling spots; 5 = >75% chilling spots. The chilling injury index was calculated using the following formula:

$$\frac{\sum (\text{chilling scale} \times \text{number of fruit in each class})}{(\text{number of total fruit} \times \text{highest chilling scale})} \times 100. \quad (1)$$

Fruits with a chilling scale of 2 and above were considered chill injured fruits and the percentage of injured fruits in the total number of fruits was calculated. 100 apricots were used for each maturity class. The incidence of chilling injury was calculated as follows:

$$\text{Incidence of chilling injury (\%)} = \left(\frac{\text{number of injured fruits}}{\text{total fruit number}} \right) \times 100. \quad (2)$$

2.3. Measurement of Fruit Firmness, Soluble Solid Content (SSC), Titratable Acid (TA), and Ascorbic Acid Levels. Fruit firmness was measured using a durometer (model CY-B Shanghai Lun Jie Instrument Co., Ltd.) on three paped sides of 10 fruits from each of the maturity classes fitted with a 1 cm diameter tip and data showed as kg/cm².

The SSC of the fruit juice obtained from 10 fruits on the longer transverse axis (about 2 mm deep under peel, two discs per fruit on opposite region) was determined using a WYT-J refractometer (Shenzhen Dingxin Yi Experimental Equipment Co., Ltd.).

Ten milliliters of extracted fruit juice (about 2 mm deep under peel) from 10 fruits on the longer transverse axis

(each fruit on opposite region) was homogenized with 100 ml distilled water and filtered. TA of the solution was determined by titration to pH 8.1 with 0.1 M NaOH [11]. TA was expressed as the percentage of malic acid per 100 g fresh mass.

Measurement of ascorbic acid levels was carried out as described of Famiani et al. [12].

2.4. Measurement of Fruit Extractable Juice and Weight Loss. The extractable juice (%) was estimated from the weight loss from the fruit pulp without apricot stone in response to centrifugation. A fruit pulp sample randomly of 8–10 g was weighed (W_1) and centrifuged for 10 min at 10,000 r/min. The supernatant was extracted and weighed (W_2). The calculation formula for extractable juice was as follows:

$$\text{Extractable juice (\%)} = \left(\frac{W_2}{W_1} \right) \times 100. \quad (3)$$

In order to calculate the weight loss, the weight of the fruit was measured every 7 days after harvest until at the end of the experiment (day 35) and data was expressed as a percentage, relative to initial value.

2.5. Measurement of Cell Membrane Permeability and Lipid Oxidation (Malondialdehyde (MDA) Content). Measurements of cell membrane permeability were carried out as described by Asrey et al. [13]. The cell membrane permeability expressed as the percentage of electrical conductivity.

The malondialdehyde (MDA) content analysis was carried out using 2 g of flesh tissue homogenized with 5 mL of 0.5% (w/v) trichloroacetic acid (TCA). The mixture was then centrifuged at 10,000g for 10 min at 4°C. The MDA levels were determined following the method of Karatas and Kanişlı [14]. The MDA content was expressed as nmol/g fresh weight (FW).

2.6. Statistical Analysis. All statistical analyses were performed with SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA). Data were analysed by one-way analysis of variance (ANOVA). Mean separations were performed by the least significant difference (LSD) test. The means were considered significantly different at $P < 0.05$. The means were considered extremely significant different at $P < 0.01$.

3. Results

3.1. Effects of Harvest Maturity on Chilling Injury Index of Apricots. The chilling injury index increased gradually in the three different maturity class apricots. As shown in Figure 1, the chilling injury index of maturity class I fruit was higher than maturity class II and III fruits. The chilling injury index of maturity class I fruits was 33.4% ($P < 0.05$) and 41.2% ($P < 0.05$) higher than that in maturity II and III fruits, respectively, on the 35th day.

3.2. Effects of Harvest Maturity on Incidence of Chilling Injury in Apricots. As shown in Figure 2, chilling injury occurred on the 14th, 21st, or 28th day in the apricots of maturity classes

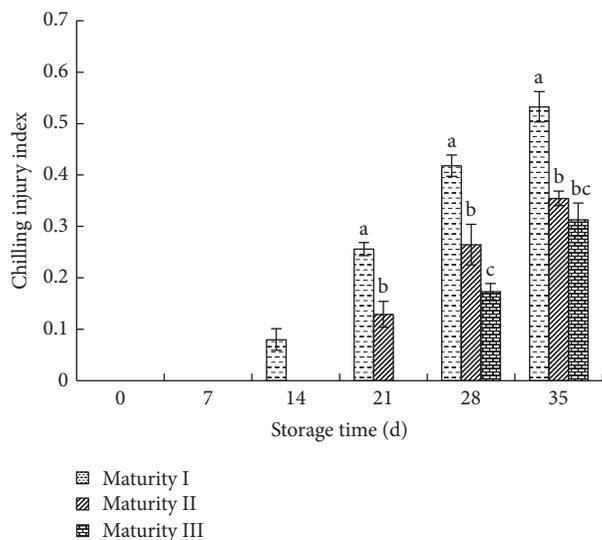


FIGURE 1: Effects of harvest maturity on chilling injury index in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to the yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates. a, b, c = bars at each storage time point have different letters which are significantly different at $P < 0.05$.

I, II, and III, respectively. The incidence of chilling injury in apricots of maturity class I was higher obviously than that of maturity classes II and III. The incidence of chilling injury in apricots of maturity classes I considerably exceeded that in the fruits of maturity classes II and III by 56.3% ($P < 0.05$) and 69.1% ($P < 0.05$), respectively, on the 35th day.

3.3. Effects of Harvest Maturity on Firmness in Apricots. As shown in Figure 3, apricot firmness increased initially and then decreased with increase in storage time. The firmness of the apricots of maturity class III was lower than that of the apricots of maturity classes I and II, and it declined rapidly. There was a slight increase in apricot firmness on the 21st day. The firmness of the fruits of class I was significantly higher than that of the fruits of maturity classes II and III. The firmness of apricots of maturity classes I, II, and III was 1.72 kg/cm², 1.35 kg/cm², and 1.01 kg/cm², respectively, on the 35th day.

3.4. Effects of Harvest Maturity on Soluble Solid Content in Apricots. The soluble solid content in the apricots of the three maturity classes was increased initially and, then, exhibited a downward trend, as shown in Figure 4. The soluble solid content in the apricots of maturity class III was higher than that of the apricots of maturity classes I and II throughout the storage. The soluble solid content in the apricots of maturity classes II and III increased gradually and, then, decreased gradually before 21 days of storage, whereas that of the apricots of maturity class I began to decrease on the 14th day of storage. The soluble solid contents in the apricots of

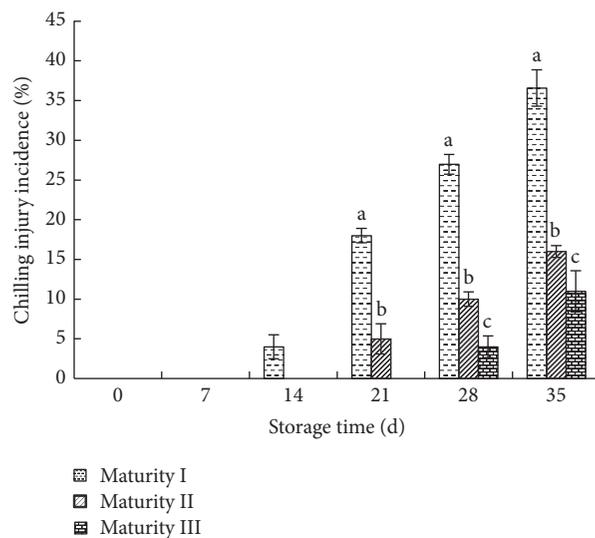


FIGURE 2: Effects of harvest maturity on the incidence of chilling injury in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to the yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates. a, b, c = bars at each storage time point have different letters which are significantly different at $P < 0.05$.

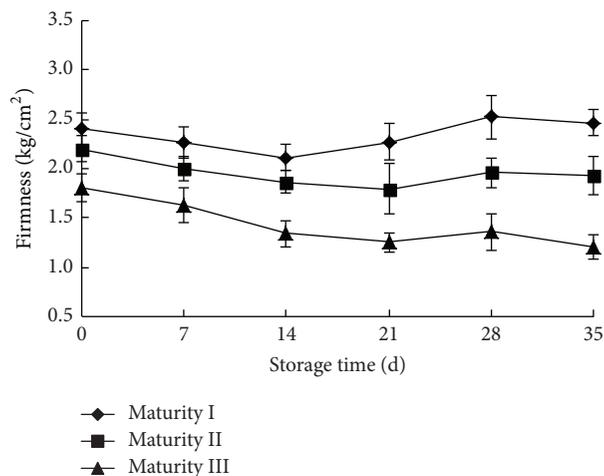


FIGURE 3: Effects of harvest maturity on firmness in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to the yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates.

maturity classes I, II, and III were 8.15%, 10.66%, and 10.77%, respectively, at the end of storage.

3.5. Effects of Harvest Maturity on TA Content in Apricots. Titratable acid is an important substance that affects fruit flavor. As shown in Figure 5, the TA level in the apricots of the three maturity classes decreased before increasing and, then, decreased slightly again during storage. The TA levels in the apricots of the three maturity classes were 1.18%,

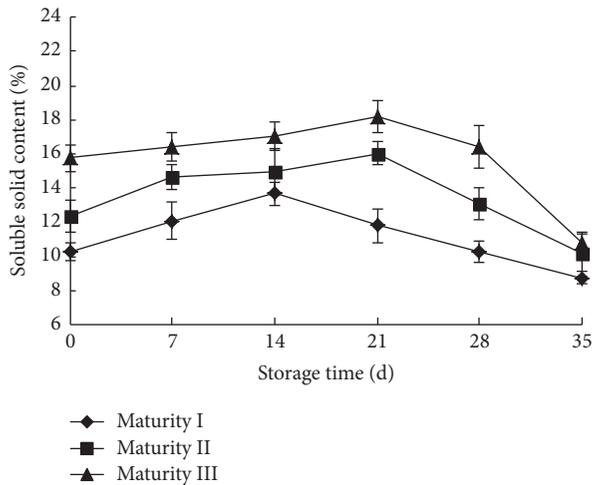


FIGURE 4: Effects of harvest maturity on soluble solid content in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates.

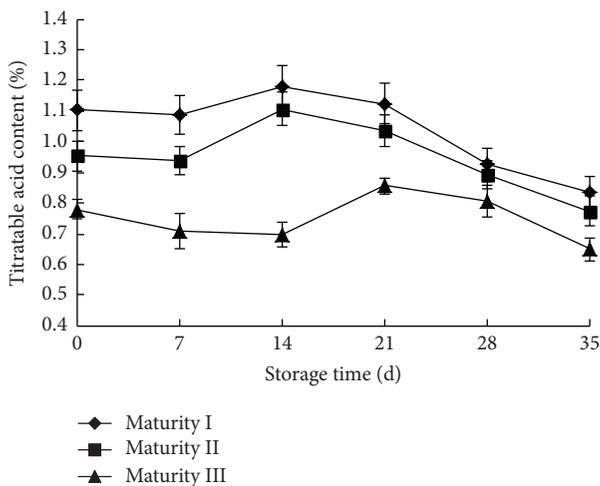


FIGURE 5: Effects of harvest maturity on TA content in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to the yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates.

1.10%, and 0.70% on the 14th day of storage, and these values were significant different ($P < 0.05$). Subsequently, TA level decreased gradually. The TA levels in the apricots of maturity classes I, II, and III were 0.84%, 0.77%, and 0.65%, respectively, at the end of storage.

3.6. Effects of Harvest Maturity on ASA Content in Apricots. As shown in Figure 6, the ASA level in the apricots showed an overall downward trend and, then, decreased rapidly on the 21st day of storage. At the end of storage, because of

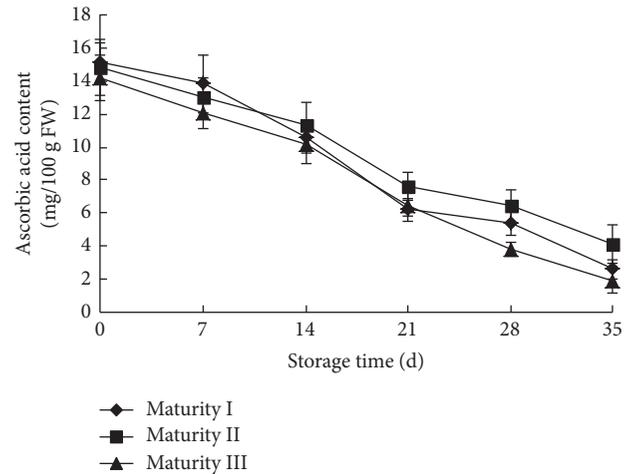


FIGURE 6: Effects of harvest maturity on ASA content in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to the yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates.

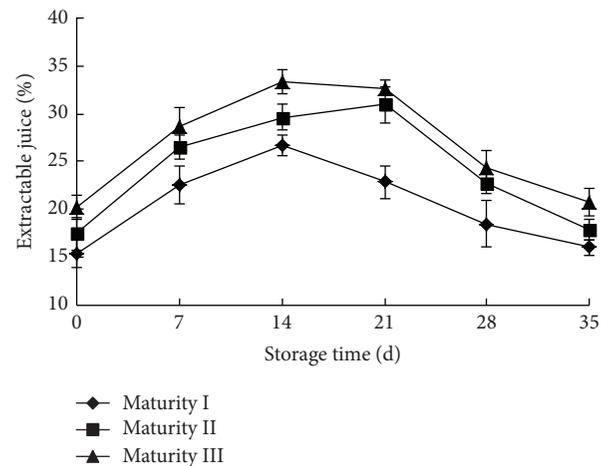


FIGURE 7: Effects of harvest maturity on extractable juice (%) in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to the yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates.

lignification, the ASA level in the apricots of maturity classes I, II, and III decreased to 2.62 mg/100 g, 4.13 mg/100 g, and 1.91 mg/100 g, respectively.

3.7. Effects of Harvest Maturity on Extractable Juice (%) in Apricots. As shown in Figure 7, the extractable juice quantity in the apricots of the three maturity classes increased continuously with no significant differences observed during the early storage period. The extractable juice quantity in the fruits of maturity classes I and III showed a peak value at day 14 of storage and, then, decreased gradually, whereas, in the

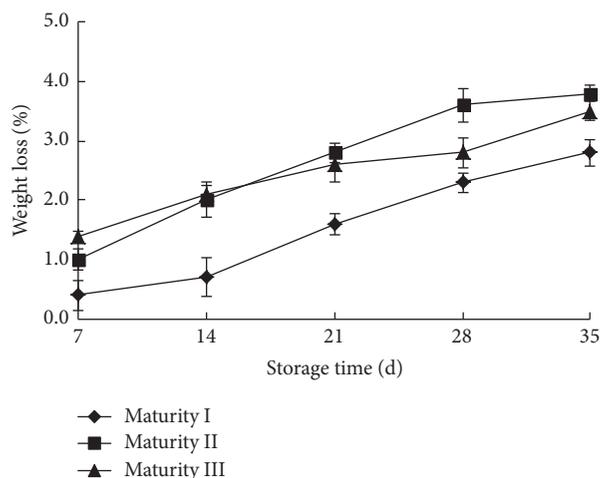


FIGURE 8: Effects of harvest maturity on weight loss rate in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to the yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates.

fruits of maturity class II, it reached the peak extractable juice value on day 21 and decreased subsequently. The extractable juice quantity in the apricots of maturity class III was 22.4% ($P < 0.05$) and 13.5% ($P < 0.05$) higher than that of the apricots of maturity classes I and II, respectively, on day 35 of storage.

3.8. Effects of Harvest Maturity on Weight Loss Rate in Apricots. The fresh weight of fruits is a key factor affecting their commodity value. Postharvest dehydration and respiration, leading to organic matter consumption, are the major causes of weight loss in fruits. As shown in Figure 8, the weight loss of apricots increased rapidly with increase in storage time. The weight loss of the apricots of maturity classes I, II, and III was 2.81%, 3.80%, and 3.51%, respectively, on day 35 of storage.

3.9. Effects of Harvest Maturity on Cell Membrane Permeability in Apricots. When fruits suffer adverse environmental stress, the integrity and function of the cell membrane are affected negatively at various degrees. Therefore, changes in membrane permeability are important indicators for chilling injury in fruits. As shown in Figure 9, cell membrane permeability of the apricots increased with storage time. Cell membrane permeability was higher in the fruits of maturity class III than in those of maturity classes I and II throughout storage. Cell membrane permeability increased rapidly during storage for 0–14 days and decreased subsequently. The cell membrane permeability of the apricots of maturity class III was 19.40% ($P < 0.05$) and 12.61% ($P < 0.05$) higher than that of the apricots of maturity classes I and II, respectively, on day 14 of storage. At the end of storage, the cell membrane permeability values of the apricots of maturity classes I, II, and III were 61.24%, 66.71%, and 75.37%, respectively.

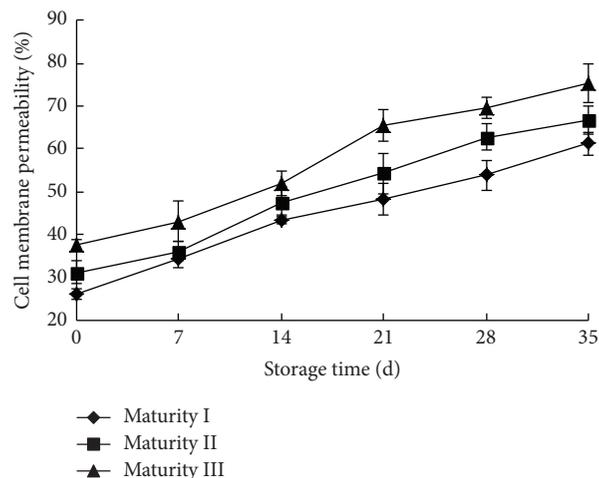


FIGURE 9: Effects of harvest maturity on cell membrane permeability in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to the yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates.

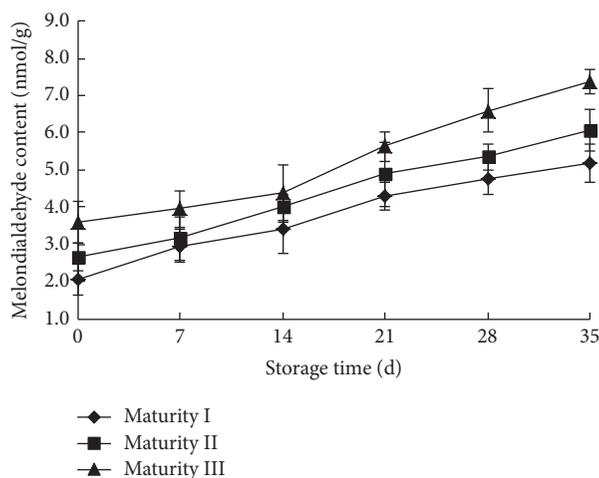


FIGURE 10: Effects of harvest maturity on malondialdehyde content in apricots; fruits with maturity class I (colored area < 50%), maturity class II (colored area 50–80%), and maturity class III (colored area > 80%) were categorized according to the yellow conversion rate. Vertical bars represent the standard errors of the means of triplicate replicates.

3.10. Effects of Harvest Maturity on Malondialdehyde Content in Apricots. MDA is a product of membrane lipid oxidation. Its level can reflect the severity of membrane injury, and it is an indicator of cellular senescence. As shown in Figure 10, the cumulative MDA level in the apricots of the three maturity classes increased gradually during storage. During the initial storage period, the MDA level in the apricots of all three maturity classes increased gradually until day 21 of storage, when MDA level increased dramatically. The MDA levels in the apricots of maturity classes I, II, and III were 5.18 $\mu\text{mol/g}$ FW, 6.08 $\mu\text{mol/g}$ FW, and 7.38 $\mu\text{mol/g}$ FW, respectively, on

day 35 of storage. The MDA level in the apricots of maturity class III was 42.47% ($P < 0.01$) and 21.38% ($P < 0.05$) higher than that in the apricots of maturity classes I and II, respectively.

4. Discussion

Because of chilling injury at unfavorable, low temperature, the cell membrane of cold-sensitive plants undergoes phase changes, and this affects its function and structural integrity [15]. Studies on loquats and peaches showed that the higher the unsaturated fatty acid level in the cell membrane, the higher the fluidity and stability of the cell membrane; this stability is beneficial and increases resistance of fruits to chilling injury. Saini et al. [16] observed that, with fruit maturation, the polyunsaturated fatty acid level increases gradually, and the unsaturated fatty acid level in fruits at a low maturity level is relatively low. Koushesh Saba et al. [8] observed that the higher the soluble solid content in fruits is, the higher their cold tolerance is. Qian et al. [17] reported the association between cucumber maturity and cold tolerance and its physiological mechanisms and showed that TA level in cucumbers at an early developmental stage was relatively high, whereas soluble sugar content was low, and these cucumbers were prone to chilling injury. The results of this study showed that the apricots of maturity classes I, II, and III began exhibiting symptoms of chilling injury on days 14, 21, and 28 of storage, respectively. The incidence and index of chilling injury in the apricots of maturity class I were significantly higher than those of the apricots of maturity classes II and III. This may be because the apricots of maturity class I were still at the developmental stage, and the levels of unsaturated fatty acids and soluble sugars in the fruits were relatively low, while TA level was high. These are the main causative factors of low cold tolerance and high incidence of chilling injury in the fruits. The chilling injury index and incidence of chilling injury in the apricots of maturity class III were relatively low, and this may be due to the high soluble solid content, which can increase intracellular solute concentration and decrease the freezing point of cells, thereby improving cold tolerance in apricots. However, because the maturity class III apricots at harvest were fairly mature, firmness decreased rapidly during storage, and this together with higher cell membrane permeability during the late period resulted in the accumulation of large MDA quantities. Hu et al. [18] observed that highly mature cantaloupes have a higher degree of membrane lipid peroxidation during storage, and significant MDA accumulation occurs, which is unfavorable for maintaining storage quality during the late storage period. The storage quality of the apricots of maturity class II could be maintained effectively, and they had lower incidence of chilling injury and chilling injury index.

The results of this study showed that the apricots of maturity class I began exhibiting chilling injury symptoms on day 14 of storage, with decrease in firmness and extractable juice quantity, increase in weight loss, decrease in vitamin C and TA levels, and other factors related to quality reduction. Because the apricots of maturity class III were highly mature,

postharvest respiratory metabolism could be active, resulting in the rapid TA and VC consumption and decrease in firmness, which were not conducive to the maintenance of storage quality. Mojević et al. [19, 20] studied the effects of different maturity stages on the storage quality of peaches and Jiashi melons and observed that the highly mature fruits were more prone to rotting and had lower firmness and reduced quality. Therefore, the selection of the appropriate harvest maturity can aid in effectively avoiding the occurrence of phenomena that reduce fruit quality, such as chilling injury, during storage.

5. Conclusions

The incidence of chilling injury and chilling injury index of the apricots of maturity class I were significantly higher than those of the apricots of maturity classes II and III, and the fact that these fruits cannot ripen during storage can be attributed to severe lignification. The maturity class III apricot exhibited rapid ripening and ageing and had a lower firmness. However, the storage quality can be better maintained in the apricots with maturity class II. It is suggested that maturity class II is the appropriate harvest maturity of apricots for storage.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Research Article

Capillary Water in Pericarp Enhances Hypoxic Condition during On-Tree Fruit Maturation That Induces Lignification and Triggers Translucent Flesh Disorder in Mangosteen (*Garcinia mangostana* L.)

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Translucent flesh of mangosteen normally occurs during fruit ripening. Rainfall, after water stress, enhanced on-tree mature green fruit to develop translucent flesh disorder more frequently. Thus, this research pursued the effect of applied water on translucent flesh disorder development. The on-tree mature green stage fruits were selected and wrapped with 3 layers of fabric sheet. After that, water was continuously dropped (flow rate of 0.6 ml/min) on the wrapped sheet for 0, 1, and 2 days before picking. The results showed that duration time of water applying enhanced the increasing of water absorption significantly in peel. All of water-treated fruits ripened within 2-3 days after harvest and obviously had high lignin in secondary cell wall. It was hypothesized that lignification played an important role in hypoxia defense mechanism since the Na_2CO_3 -SP fractionation extracted from alcohol insoluble residue (AIR) of translucent flesh aril was higher than those of normal aril. This Na_2CO_3 -SP reinforced the strength of cell wall complexity as well as displaying the translucency character. Hence, we concluded that the capillary water (took place in intercellular air space of fruit pericarp) induced hypoxia tolerance mechanism that triggered translucent flesh disorder in mangosteen aril.

1. Introduction

Mangosteen (*Garcinia mangostana*) belonging to Guttiferae family is popularly grown in Southeast Asia. This warming region has high volume of rainwater per year. During on-tree maturation of mangosteen fruit, rainfall (after water drought stress) frequently induced more translucent flesh disorder developing in ripe fruit. Translucent disorder, translucency and litter bit crispy texture, is an abnormal ripening process that occurs at the largest segment of aril. This disorder made a serious problem for mangosteen grower from the past until now. The recent advance elucidation for translucent flesh disorder mechanism and triggering factor in mangosteen was very scarce.

Fruit of mangosteen was classified as a berry type containing 7-8 aril segments whose fruit pericarp was developed

from ovary wall while the aril flesh was contributed from integument. The ripening behavior of this fruit started from changing in pericarp color along with soft structure and slight declining in aril firmness [1]. Normally, translucent flesh disorder is initiated from largest aril. This segment contained a healthy seed inside. Both aril flesh and seed had higher energetic activity than those of normal segment when testing with triphenyltetrazolium chloride [2]. Moreover, mangosteen fruit showed a fluctuation pattern of climacteric respiration during fruit ripening due to each segment ripened individually [3].

According to mangosteen grower's knowledge, in hot weather condition (sunny and moderately high level of temperature (30–40°C) and relative humidity (70–80%) in the daytime), rainwater or artificial rain from springer water over tree canopy frequently accelerated earlier ripening of on-tree



FIGURE 1: Water applying apparatus for on-tree mature green mangosteen fruit.

mature green fruit or translucent flesh disorder. Interestingly, the harvested fruit gained more fresh weight (calculated from the number of fruits, in terms of standard fruit size per kilogram) after rain. Besides, continuous rain for 1-2 days noticeably gave more serious development of translucent flesh disorder. Chuennakorn et al. [4] demonstrated that low or high levels of underground water had no relation with either fruit fresh weight or translucent flesh disorder in mangosteen. Possibly, the increasing weight of picked fruit might be contributed by the absorbed water in pericarp. Therefore, our research aimed to elucidate translucent flesh disorder mechanism and triggering factor of this incidence on the on-tree mature green mangosteen fruit.

2. Materials and Methods

The sixty off-season fruits in uniformity maturation, 12 weeks after fruit setting (mature green, pale green stage) of on-tree mangosteen from the commercial orchard in Trat Province (located at the eastern part of Thailand), were selected and either unwrapped or wrapped with fabric sheets (Figure 1). Instead of artificial rain, water was supplied from the on-tree hanging plastic container (2000 ml in capacity) at flow rate 0.6 ml/min for 0, 2, and 3 days and then fruits were picked and transported to KMUTNB laboratory within a day. Harvesting fruits were separated into two groups: the first group was investigated simultaneously while the second group was delayed until they completely ripened (peel color

changes to purple) at ambient room before inspecting quality parameters as follows.

2.1. Water Content and Translucent Flesh Disorder. 5 g of pericarp or aril flesh at the middle position between stem end and stylar end was placed into moisture can and dried in oven at 60°C for 4 days. Then, it was transferred into desiccator until cooling down. Moisture can weight was also measured for calculating the percentage of water loss. Furthermore, the percentage of translucent fruit was counted from number of fruits.

2.2. Firmness Measuring. Pericarp at the lateral side of fruit was removed from stylar end to stem end by knife and flesh firmness at center of the biggest segment was measured by texture analyzer TA-XT 21 with 2 mm spherical plunger in 5 mm distance depth and 1.0 mm/s test speed, expressed as Newton force.

2.3. Histological Structure Staining. Free-hand section of pericarp and aril flesh were stained in 10 times dilution of 0.25% Safranin O for 2 min and washed in distilled water followed by observation under light microscope.

2.4. Lignin Determination. Using the method of Bruce and West [5], 4 g of peel or aril was blended in 16 ml methanol by homogenizer for 1 min before being filtered through

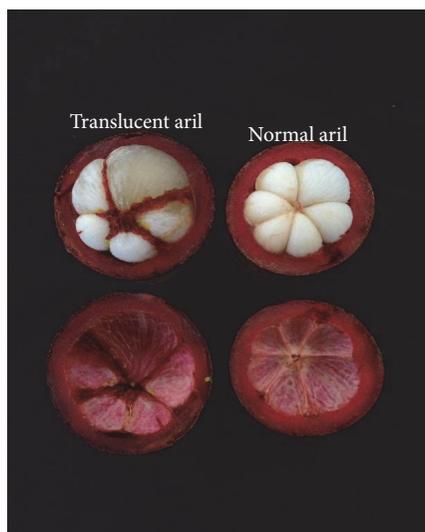


FIGURE 2: Character of normal ripe and translucent disorder arils in mangosteen fruits.

Whatman GF/A filter paper. The alcohol insoluble residue (AIR) was dried in oven at 60°C for 24 h. Then, 50 mg of AIR was dissolved in 5 ml of 2 N hydrochloric acid including 0.5 ml of 98% thioglycolic acid and placed in hot bath (100°C) for 4 h. The solution mixture was centrifuged at 12,000g for 30 min. Next, the pellet was washed in 5 ml distilled water and resuspended in 5 ml of 0.5 N sodium hydroxide for 18 h. The mixing solution was centrifuged at 12,000g for 30 min. Then, supernatant was mixed in 1 ml of conc. hydrochloric acid, waiting for thioglycolate, precipitated at 4°C for 4 h and centrifuged at 10,000g for 10 min. The orange-brown pellet was dissolved in 25 ml of 0.5 N NaOH and absorbance measured at 280 nm. The degree of lignification was expressed in absorbance unit at 280 nm/milligram/gram fresh weight.

2.5. Pectin Extraction and Determination. Prepared from alcohol insoluble residue (AIR), mangosteen aril flesh (5 g) was refluxed in 95% ethanol for 30 min and filtered with Whatman filter paper number 1; then, the residue was transferred to oven (50°C) for 12 hrs [6, 7]. The dissolved AIR (30 mg) in 20 ml water was shaken at 80 rpm for 2 hrs and then centrifuged at 13000 rpm for 30 min. The supernatant was used as water soluble pectin (WSP) fraction while the pellet was dissolved in 20 ml of 0.05 M sodium citrate buffer, pH 4.5, including 0.04 M EDTA, then shaken, and centrifuged same as the above method. The supernatant was used as EDTA soluble pectin (EDTA-SP) fraction while the pellet was dissolved in 20 ml of 0.05 mM Na₂CO₃ including 20 mM NaBH₄, then shaken, and centrifuged as mentioned above. The supernatant was used for sodium carbonate soluble pectin (Na₂CO₃-SP) fraction.

Pectin determination was performed using the method of Blumenkrantz and Asboe Hansen [8]: 0.5 ml pectin solution was mixed in 2.5 ml conc. H₂SO₄ including 0.0125 M sodium tetraborate and then boiled at 100°C for 10 min. After cooling down, 0.1 ml of 15% *m*-hydroxydiphenyl including 0.5%

NaOH was added. Instead of pectin content, the absorbance of mixing solution was measured at 520 nm in comparison with polygalacturonic acid standard.

2.6. Statistical Analysis. The descriptive statistics were firstly performed. Then, ANOVA from all data were carried out. If applicable, the mean values in water and lignin content were evaluated using Tukey's (HSD) test ($P < 0.05$).

3. Results and Discussion

3.1. Water Content and Translucent Flesh Disorder. When water was continuously applied on the surface of on-tree mature green fruit, translucent flesh disorder was the consequence (Figure 2). It was found that water content increased in peel of unripe fruits after day 2 of applying the water artificially when compared to day 0 (Table 1) which indicated that water passed through fruit peel surface and accumulated in intercellular air space as the same route of gases transportation [9]. These fruits ripened within 2-3 days after harvest with normal external quality appearance such as fresh green calyx and smooth purple pericarp color. However, after the peel of ripe fruit was removed by knife, about 30% and 60% of fruits showed translucent disorder at the largest segment of 2 and 3 days in water-treated type, respectively (Table 2).

3.2. Histological Structure Staining. Histological structure staining of abnormal flesh was practically observed under light microscope. The result indicated that lignin clearly accumulated in secondary cell wall (Figure 3). This synthesizing of lignin played a stress defense mechanism role which was found in several plant parts such postharvest bamboo shoot [10], cold stress in kiwifruit [11], chilling injury bamboo shoot [12], and bruised pericarp in mangosteen [13].

Normally, mangosteen bears different floral stages at the same time resulting in various maturity fruits within a crop

TABLE 1: Water content in peel and aril after water applying at fruit surface of on-tree mature green mangosteen fruit. The values were mean \pm SE ($n = 4$). Different letters in each column showed dissimilarity from Tukey's (HSD) test ($P < 0.05$).

| Water applying (days) | Water content (%) | | | |
|-----------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Unripe | | Ripe | |
| | Peel | Aril | Peel | Aril |
| 0 | 68.48 \pm 0.11 ^a | 83.83 \pm 0.13 ^a | 68.05 \pm 1.97 ^a | 84.97 \pm 1.43 ^a |
| 2 | 71.87 \pm 0.87 ^b | 84.16 \pm 0.91 ^a | 67.92 \pm 1.29 ^a | 83.43 \pm 0.96 ^a |
| 3 | 72.45 \pm 0.69 ^b | 85.62 \pm 0.96 ^a | 68.07 \pm 1.09 ^a | 84.57 \pm 0.46 ^a |

TABLE 2: The influence of applied water at fruit surface of on-tree mature green mangosteen fruit on lignin induction and triggering translucent flesh disorder. The values were mean \pm SE ($n = 4$). Different letters in each column showed dissimilarity from Tukey's (HSD) test ($P < 0.05$).

| Water applying (days) | Lignin content (Abs 280 nm/gFW) | | Translucent flesh disorder (%) |
|-----------------------|---------------------------------|---------------------------------|--------------------------------|
| | Ripe | | |
| | Peel | Aril | |
| 0 | 22.96 \pm 0.93 ^a | 251.88 \pm 1.59 ^a | 0 |
| 2 | 25.55 \pm 1.44 ^a | 278.59 \pm 8.00 ^{ab} | 30 |
| 3 | 73.82 \pm 8.91 ^b | 381.43 \pm 49.28 ^b | 60 |

season. Thus, Thai Ministry of Agriculture announced to mangosteen grower for early flower induction avoiding fruit maturation in rainy season; otherwise rainfall (>20 mm/day), after drought (lacking rain) for a week, could make serious translucent flesh disorder in on-tree fruits during their ripening. Noichinda [3] demonstrated that direct application of water to the peduncle of harvested mature green mangosteen fruit did not induce translucent disorder which suggested that only the mature green stage of on-tree fruit was suffering from over water.

In open orchard, after rainfall, water absorption volume in mangosteen fruit increased by water passing through the fruit peel resulting in fresh weight gain and translucent flesh development. This consequence was also found in sweet cherry [14] and tomato [15, 16]. The excess water from cell surface absorption (bound water) took the place of the air in intercellular air space by capillary force called "capillary water" that enhanced hypoxic condition. This hypoxia was influenced by energy crisis of waterlogging cells which utilized more soluble carbohydrate reserve while producing less energy. High concentration of releasing CO₂ from respiratory retarded succinate-oxidation process in mitochondria by depressing succinate dehydrogenase [17] causing more succinic acid accumulation. This agreed with Dangcham and Siriphanich [18] who reported that mangosteen translucent aril contained higher succinic acid content than normal aril.

Therefore, the adaptability of mangosteen fruit to hypoxic condition by inducing lignification in aril flesh was similar to the adaptive responses in wax apple root [19] or developmental cortical air space in maize under flooding condition [20]. Moreover, for endurance, plants developed their adaptive morphological traits, forming aerenchyma cell for availability of oxygen to continue aerobic respiratory pathway in particular. This phenomenon was related to many mechanisms, for example, phenylpropanoid and the related-enzymes such as phenylalanine ammonia lyase (PAL), cinnamyl alcohol

dehydrogenase (CAD), and peroxidase (POD) [21]. The hypoxia tolerance mechanism also induced higher amount of reactive oxygen species (ROS), especially superoxide anion (O₂⁻), and increased antioxidative enzymes activities such as superoxide dismutase (SOD) to scavenge O₂⁻ into H₂O₂ [22, 23]. Nevertheless, this H₂O₂ will serve as a substrate for lignin biosynthesis by peroxidase (POD).

3.3. Lignin Determination. Translucent flesh aril contained higher amount of lignin than normal aril (Figure 4(c)). Lignin is a complex phenolic polymer closely linked with cellulose and hemicellulose. It plays a crucial role in reinforcing the plant cell wall structure. Biosynthesis of lignin in plant begins with the oxidative coupling of three monolignols as a building block of coniferyl, sinapyl, and *p*-coumaryl alcohols via phenylpropanoid pathway and the last step of lignification is the polymerization of cinnamyl alcohols by POD and using hydrogen peroxide as a substrate [24, 25]. However, the expression of genes encoding plant lignification enzymes, including PAL, trans-cinnamate 4-hydroxylase, 4-coumarate CoA-ligase, ferulate 5-hydroxylase, COMT, and CCoAOMT, was repressed under flooding condition indicating that there was alternated lignin biosynthesis [26].

3.4. Pectin Composition. Plant cell wall can be categorized into 3 layers: layer one is adhesive between cell and another called "middle lamella" composed of calcium pectate (pectic acid binding Ca²⁺), layer two is a real cell wall synthesized during cell division called "primary cell wall" composed of cellulose, hemicellulose, and pectic substances, and layer three synthesized after primary cell wall or in nongrowing stage of cell called "secondary cell wall" composed of cellulose, hemicellulose, and lignin [27, 28].

Ordinarily, softening process of mangosteen fruit occurred during ripening influenced by the changes of pectic substances in middle lamella and primary cell wall. Pectin

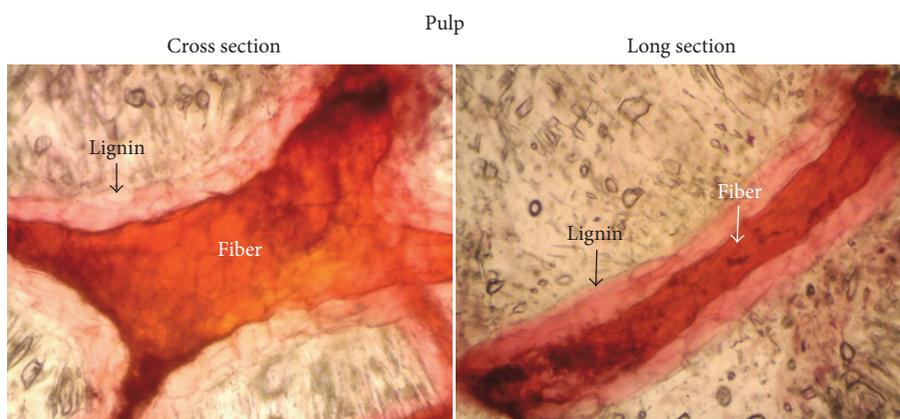
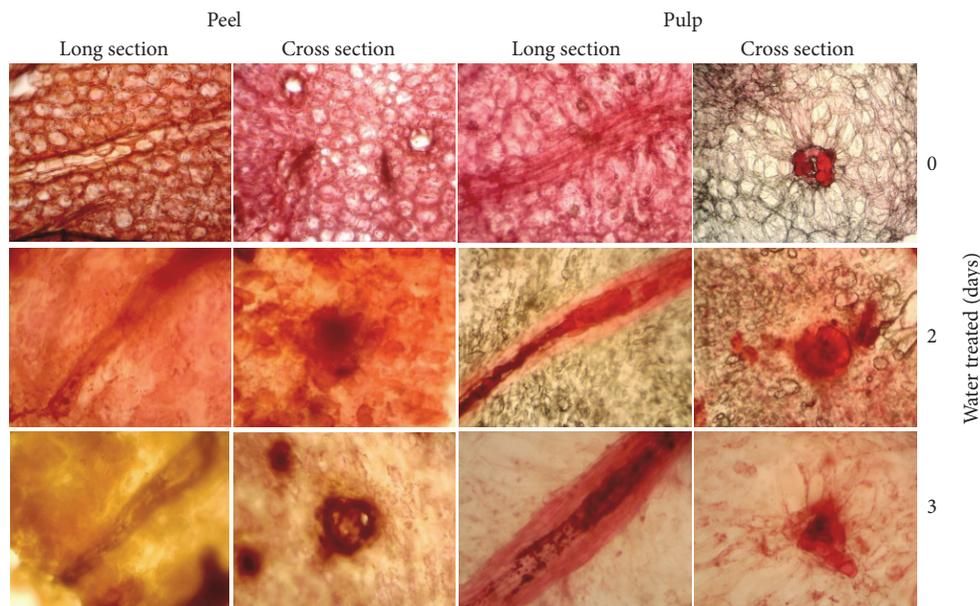


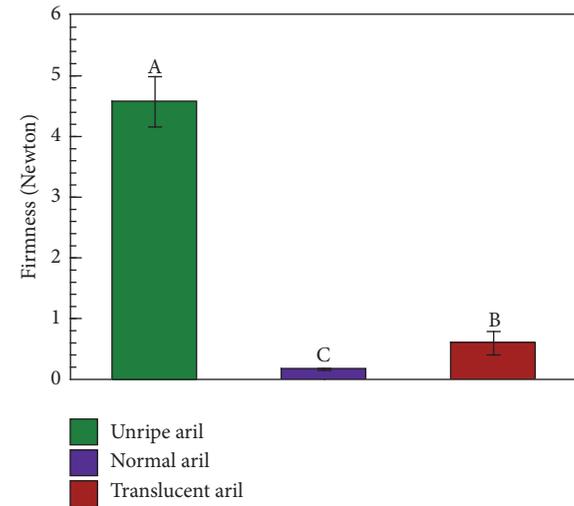
FIGURE 3: Histochemical structure staining in pericarp and aril of water-treated mangosteen fruits.

became more soluble by demethylation and deesterification with increasing in activities of pectin methyl-esterase (PME) and polygalacturonase (PG). In normal mangosteen fruit, the amount of water soluble pectin (WSP), galacturonic acid (demethyl-esterification) subunit, and chelating soluble pectin (EDTA-SP) increased while $\text{Na}_2\text{CO}_3\text{-SP}$ decreased with relation to the increasing in activities of PME and PG during fruit ripening [1]. This event rapidly enhanced the reduction of aril firmness. In contrast, translucent aril showed higher flesh firmness than normal aril (Figure 4(a)). From our preliminary observation, direct water infiltration into harvested mangosteen fruit showed translucency and low firmness in all aril segments (data not shown). It was also found that insoluble pectin content extracted by Na_2CO_3 was perceptibly accumulated in translucent flesh aril compared with other (Figure 4(b)). Typically, pectin is a group of partially methyl-esterified α -1, 4-linked galacturonan chain polysaccharides. The presence of high $\text{Na}_2\text{CO}_3\text{-SP}$ content accumulating in translucent flesh aril led to the speculation

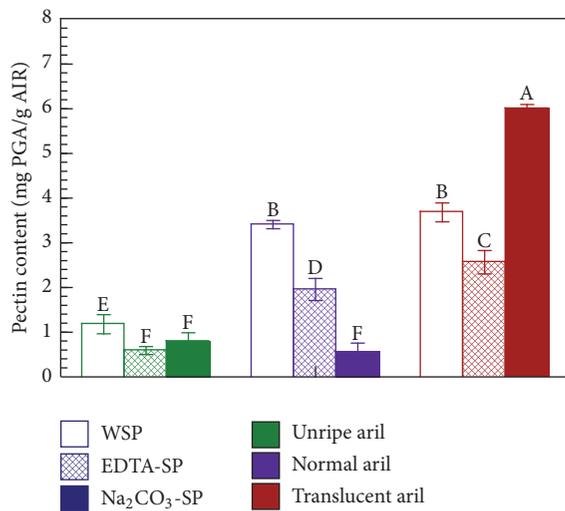
that there was reesterified process of pectin resulting in more covalent cross-links network and forming building blocks for cell to cell adhesion making stiff texture as insoluble jelly-like translucency pectin in middle lamella. This activity finally made aril flesh appearing in transparency which was a morphological disorder of mangosteen aril.

4. Conclusions

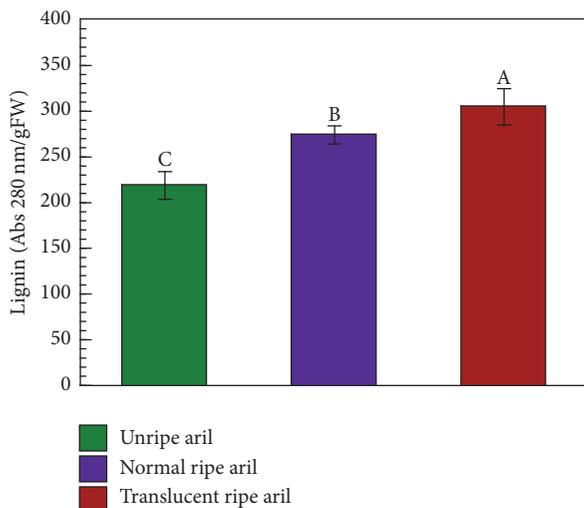
The disorder of mangosteen aril, especially translucency, had been explored in many ways. However, the supported evidences were inadequate hitherto. Most of the experiments indicated water supply as a source of this aberration but the explanation of its influence was still ambiguous. In this research, effect of capillary water on $\text{Na}_2\text{CO}_3\text{-SP}$ and lignin content in aril leading to translucent flesh was examined for the first time. Both physiological and morphological results from our experiments were evidently correlated. Lignin



(a)



(b)



(c)

FIGURE 4: Mangosteen firmness (a), pectin distributed fractions (b), and lignin content (c). Values were mean \pm SD ($n = 4$).

increased after water application to mangosteen fruit associated with the rising of translucent flesh disorder (Table 2 and Figure 3). In addition, Na₂CO₃-SP was distinctly elevated in translucent flesh, conceivably causing the translucency of mangosteen aril. Accordingly, it was realizable to conclude that the translucent flesh disorder of mangosteen fruit was a combination of Na₂CO₃-SP formed in middle lamella and lignification in cell wall triggered by capillary water in fruit pericarp as a hypoxia defense mechanism.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Effects of UV-C Light Exposure and Refrigeration on Phenolic and Antioxidant Profiles of Subtropical Fruits (Litchi, Longan, and Rambutan) in Different Fruit Forms

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The objectives of this study were to investigate how UV-C irradiation and refrigeration affect shelf-life and antioxidant level of litchi, longan, and rambutan. Three forms (whole, dehulled, and destoned) of fresh fruits were treated by refrigeration and UV-C irradiations. After processing, deterioration rate, phenolics compounds, and antioxidant capacity were quantified. The deterioration rate was recorded as decay index. The results showed that both refrigeration and UV-C exposure extended the shelf-life of the fruits. The refrigeration enriched antioxidant levels of litchi but caused nutritional degradation in longan and rambutan; UV-C radiation enriched litchi antioxidant contents but was related to reduction of antioxidant capacity in longan and rambutan. Removing hulls and stones was associated with the decrease of antioxidants in litchi. The effects on antioxidant levels varied from fruit to fruit, resulting from hormesis phenomenon. The change of phytochemical levels was hypothesized as an accumulative process. The effects of fruit forms were not consistent in different fruits, which could be multifactorially influenced.

1. Introduction

An epidemiological study revealed that human body can benefit from consumption of five servings (400 g in total) of fruits and vegetables per day, which potentially decreases the vulnerability to chronic noncommunicable diseases, like colon cancer, stroke, and arteriosclerosis [1]. To a great extent, merits brought by fruits can be attributed to phytochemical composition, especially antioxidants, including phenolic derivatives like polyphenols and flavonoids [2]. *L*-ascorbic acid, the heat-labile vitamin C with recommended dietary allowance of 90 mg/day for males and 75 mg/day for females [3], can be also largely obtained from fruits. Antioxidants can help to neutralize reactive oxygen species generated in human body, consequently reducing tissue damage and alleviating oxidative stress [4].

Longan (*Dimocarpus longan* Lour.), litchi (*Litchi chinensis* Sonn.), and rambutan (*Nephelium lappaceum* L.) are typical subtropical fruits opulently cultivated in southern China,

which are popular for customer to buy in summer (from June to August). However, due to cell-membrane lipid peroxidation and polyphenol oxidation [5], the inherent perishability of these fruits has been considered as a serious problem, leading to fruit browning or fungal infection in the first week after being harvested from orchard, which greatly impairs economic chain of fruit agriculture [6]. Consequently, perishable fruits are being investigated by researchers for extending their shelf-life [7]. Diluted chlorine is an approach for sanitization of fresh fruit in industry, which is commercially feasible considering cost-effectiveness ratio. However, chlorine has been hypothesized as a source of carcinogenic chlorinated chemicals [8], which thus urges industry to find other alternatives.

Ultraviolet radiation is commonly applied in food industry, including fruit postharvest processing, which barely has adverse impact on original flavor and texture, greatly ensuring the food quality compared with conventional blanching [9]. Ultraviolet (100 to 400 nm), the high-frequency electromagnetic waves, can extend the shelf-life by stress-induced

defense responses that reduce infection and cellular damage [10–12], and it has been the object of a number of research works. For instance, Erkan et al. [13] indicated that 5 and 10 min UV-C illumination (0.43, 2.15, and 4.30 kJ m⁻²) provided the best decay suppression on strawberries; Perkins-Veazie et al. [14] found 1–4 kJ m⁻² UV-C light exposure reduced 10% of decay incidence from ripe rot. Numerous studies showed UV treatment can stimulate synthesis of phytoalexin which are antimicrobial compounds contributing in disease resistance [15, 16]. More important, both UV-B (280–315 nm) and UV-C (100–280 nm) treatments have been reported that can enrich certain nutrients and nutraceutical compounds [12, 17]. Favory et al. [18] revealed that UV-B radiation stimulated COPI and UVR8 photoperception protein, enhancing accumulation of anti-ultraviolet molecules. Luthria et al. [19] pointed out that phenolic contents in tomato were enriched due to solar UV-B exposure (290 to 400 nm), especially caffeic acid. Castagna et al. [20] agreed that UV-B treatment (1 h, 6.08 kJ m⁻² d) enriched *L*-ascorbic acid as well as carotenoids in *Monkey marker* tomato. Allothman et al. [21] indicated that UV-C irradiation (2.158 kJ m⁻²) elevated levels of phenolic and flavonoid contents of guava and banana; *cis*- as well as *trans*-piceid in table grape was tripled after UV-C exposure [22].

Refrigeration is also an important postharvest procedure for fruits, which contributes to inactivation of polyphenol oxidase and inhibition of microbial growth, as well as decrease of membrane leakage, and thus can prolong shelf-life [5, 23]. Additionally, Crupi et al. [22] bridged UV-C exposure with storage time and investigated the effects of treatments on table grape and found that after being stored for 24 h, level of cyanidin-3-O-glucoside peaked. This phenomenon was attributed to defense response stimulated by UV-C light (0.8, 2.4, and 4.1 kJ m⁻²), which triggered transcription of mRNA afterward. It would also be meaningful to understand whether antioxidant levels of litchi, longan, and rambutan are altered immediately after UV treatment or, gradually, controlled by gene expression.

Litchi, longan, and rambutan have similar structure: outer hull (exocarp) protects edible pulp (mesocarp) containing internal core stone (endocarp and seed) [24]. In processing value-added product, like fruit beverage or dried fruit, industry always removes nonedible parts (hull and stone) in intermediate step. So far, no research was published in the effect of dehulling and destoning on fruit antioxidant profile during UV treatment. Understanding how fruit forms contribute to the changes of antioxidant components would be meaningful in value-added product processing.

This study aimed to investigate the effects of UV-C radiation and refrigeration on antioxidant profiles of litchi, longan, and rambutan, as well as the effects of fruit forms (dehulled and destoned), on the changes of nutritional level induced by UV-C treatment and refrigerated storage.

2. Materials and Methods

2.1. Fruit Samples. The experiments were conducted in summer in 2016: litchi in June, longan in July, and rambutan

in August. Litchi (*Litchi chinensis* Sonn. cv. Feizixiao) was freshly harvested from an orchard in Zhuhai, China. Longan (*Dimocarpus longan* Lour. cv. Shixia) was collected from farm in Guangzhou, China. Rambutan (*Nephelium lappaceum* L.) was collected from farm in Sanya, China. Before treatments, fruits were debranched and sorted to remove damaged samples. Sorted samples were divided into 300 g for each group. In each group, one-third fruits were dehulled; one-third fruits were destoned; the rest of one-third remained in whole fruit. Removing hulls and stones were carefully conducted to minimize juice leakage.

2.2. Chemicals and Reagents. (+)-Catechin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium hydroxide, citric acid, *L*-ascorbic acid, metaphosphoric acid, gallic acid, sodium nitrite, aluminum chloride, sodium acetate, and ferrous sulphate were purchased from Damao Chemical Co. (Tianjin, China). Folin-Ciocalteu working solution, 2,6-dichlorophenolindophenol (DCIP), 2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were provided by Yuanye Biotechnical Company (Shanghai, China). Acetic acid, hydrochloric acid, and acetone were obtained from Guangzhou Chemical Reagent Factory (Guangzhou, Guangdong, China). Ferric chloride was purchased from Sinopharm Chemical Reagent (Shanghai, China). Ethanol was supplied by Fuyu Fine Chemical Co. (Tianjin, China). All reagents used were of analytical grade.

2.3. UV-C Light Treatment and Refrigeration. Sample preparation of each group was described in Table 1. Group A samples were refrigerated at 4°C in dark (humidity: 45% ± 3%) for 24 h; Group B were stored in 4°C for 24 h and then treated by UV-C light (254 nm) using UV Crosslinker (CL-1000, Ultra-Violet Products Ltd., UK) with preset UV energy mode (dose: 3 kJ m⁻²); Group C were irradiated by UV-C light (3 kJ m⁻²) first and then stored at 4°C for 24 h; Group N, as control group, were not treated by refrigeration or UV-C irradiation. During storage, dehulled and destoned fruits were kept in clean glassware sealed by plastic film to reduce moisture loss. During UV-C exposure, 150 g of fruits (15 litchis, or 15 longans, or 10 rambutans) was treated as a batch; fruit stalk was horizontally oriented.

2.4. Determination of Moisture Content. After treatments, all treated samples were ground into homogenous pulp juice (without hull and stone). Grinder (Philips Co., HR2006, Zhuhai) was washed twice by approximately 50 mL distilled water for reducing sample loss. Homogenized samples were collected in clean, sealed glass bottles and stored in dark at 4°C for further using. Moisture of juice was measured according to National Standard of China [25]. Empty clean aluminum containers were preweighted, recorded as m_0 . Approximately 5 g of sample was transferred into each container, with exact weight record as m_1 . All open aluminum containers were placed in vacuum oven (Memmert Co., VO200, Shanghai) at $T = 70^\circ\text{C}$ and $P = 3\text{ kPa}$ for 4 h. Dry

TABLE 1: Group and subgroup codes with corresponding description.

| Code combinations | | Descriptions |
|-------------------|-------------|--|
| Treatments | Fruit forms | |
| N | 1 | Whole fruits in control group |
| | 2 | Dehulled fruits in control group |
| | 3 | Destoned fruits in control group |
| A | 1 | Whole fruits in solely refrigerated group |
| | 2 | Dehulled fruits in solely refrigerated group |
| | 3 | Destoned fruits in solely refrigerated group |
| B | 1 | Whole fruits refrigerated in dark and then irradiated by UV-C light |
| | 2 | Dehulled fruits refrigerated in dark and then irradiated by UV-C light |
| | 3 | Destoned fruits refrigerated in dark and then irradiated by UV-C light |
| C | 1 | Whole fruits irradiated by UV-C light and then refrigerated in dark |
| | 2 | Dehulled fruits irradiated by UV-C light and then refrigerated in dark |
| | 3 | Destoned fruits irradiated by UV-C light and then refrigerated in dark |

weight, when it is constant, was recorded as m_2 . The moisture content was calculated as $(m_1 - m_2)/(m_1 - m_0) \times 100\%$. Moisture determination was conducted in triplicate.

2.5. Extraction of Phenolic Compounds and Antioxidants from Fruit. Extractions for determination of TPC, TFC, DPPH scavenging capacity, ferric reducing antioxidant power, and ABTS radical scavenging capacity were conducted based on the established method [26]. Approximately 5 g of homogenized pulp (exact sample weight was recorded) was added in centrifugal tube with 5 mL of acetic acetone (acetone : water : acetic acid = 70 : 29.5 : 0.5, v/v/v). The mixture was shaken for 3 h on an orbital shaker and then was set stillly in dark for 12 h. Mixture was centrifuged at 5000 rpm for 10 min to collect extract. The above extraction step was performed twice and supernatant was combined with final volume recorded.

L-ascorbic acid was extracted based on the method previously reported by Reiss [27]. Approximately 25 g of homogenized pulp (exact sample weight was recorded) was added in grinder with 100 mL of 3% metaphosphoric acid. Mixture was homogenized at high speed for 2 min and then was filtered. The supernatant was collected and the volume was recorded. Extractions were conducted in triplicate for each combination between treatments and fruit forms.

2.6. Determination of *L*-Ascorbic Acid. The determination of *L*-ascorbic acid was based on DCIP colorimetric method [28]. The content of *L*-ascorbic acid was calculated through equation $y = -39.482x + 0.5054$ calibrated by *L*-ascorbic acid. Standard curve had a linear range from 0 to 10 mg L⁻¹ and correlation coefficient = 0.9996. Results were expressed in dry weight basis.

2.7. Determination of Total Phenolic Content (TPC). TPC was determined with Folin-Ciocalteu method reported by Singleton et al. [29]. The total phenolic content was calculated as gallic acid equivalents (g GAE kg⁻¹). Gallic acid calibration curve ($y = 0.0012x + 0.0068$) ranged from 10 to 500 mg L⁻¹ ($r^2 = 0.9998$). Results were recorded in dry weight basis.

2.8. Determination of Total Flavonoid Content (TFC). Determination of TFC was conducted using aluminum chloride colorimetric method which has been established previously [30]. The result of TFC was calculated by (+)-catechin-calibrated standard curve ranging from 10 to 1000 mg L⁻¹ with correlation coefficient = 0.9996 and was expressed as catechin equivalents (g CE kg⁻¹) in dry weight basis.

2.9. Determination of DPPH Free Radical Scavenging Capacity. DPPH free radical scavenging assay was conducted according to previous literature [31]. The scavenging rate was calculated as $(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100\%$. Scavenging capacity was calculated by applying sample scavenging rate to Trolox-calibrated curve: $y = 0.0012x - 0.0037$, $R^2 = 0.9905$ (0.1~1 mM). Results were expressed as Trolox equivalents (mol TE kg⁻¹) in dry weight basis.

2.10. Determination of Ferric Reducing Antioxidant Power. Ferric reducing antioxidant power was evaluated based on the method established previously [32]. FRAP was calculated by regression equation ($y = 0.639x - 0.0239$, $R^2 = 0.995$) calibrated by ferrous sulphate from 0.1 to 1 mM. Ferric reducing antioxidant power was expressed as Fe²⁺ equivalents (mmol FE kg⁻¹) in dry weight basis.

2.11. Determination of ABTS Free Radical Scavenging Capacity. ABTS radical scavenging assay was conducted based on the method established previously [33]. The inhibitory rate was calculated as $(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100\%$. Scavenging power was expressed as Trolox equivalents, computed through standard curve with range from 0 to 100 mM. Results were expressed as Trolox equivalents (g TE kg⁻¹) in dry weight basis.

2.12. Determination of Decay Index. The determination of decay index was illustrated previously [34]. Processed whole fruits were grouped (litchi and longan: 10 fruits for a batch; rambutan: 5 fruits for a batch). The decay areas (color change due to skin browning or fungal growth) were visually observed and recorded, which were categorized as follows:

level 0 (normal appearance), level 1 (decay proportion ≤ 0.25), level 2 ($0.25 < \text{decay proportion} \leq 0.5$), level 3 ($0.5 < \text{decay proportion} \leq 0.75$), level 4 ($0.75 < \text{decay proportion} \leq 1$), and level 5 (decay proportion = 1). The overall average decay index of each group was calculated with $\Sigma(\text{decay level} \times \text{numbers of fruits in this level})/\text{fruits number of each group}$. Determination of decay index was done in triplicate and results were averaged.

2.13. Statistical Analysis. All the above assays were conducted in triplicate from extraction step, and results were expressed as mean \pm standard deviation in dry weight basis. The data were analyzed by one-way ANOVA using SPSS (Version 19, IBM Co., USA). Duncan's multiple comparison was applied in order to figure out whether significant differences exist among phytochemical levels of different treatments and fruit forms with p value ≤ 0.05 . Two-way ANOVA was also applied to test the interaction between treatments and fruit forms at 0.05 significant levels. Correlation matrix of tested parameters was constructed in scatter plot with Pearson correlation coefficients noted.

3. Results

3.1. Effects of UV-C Radiation and Refrigeration on Decay Rate. The decay proliferations of litchi, longan, and rambutan, expressed as decay index, were illustrated in Figure 1. Basically, ordinary untreated litchi and longan started to brown in the second day after being harvested from tree, and rambutan even underwent darkening from the first day. In terms of fruit species, rambutan showed the fastest rate of decaying, which has been entirely darkened with decay index just slightly lower than 5.0 on day 7. On the contrary, litchi performs better in storage property, with fairly slow initial deterioration speed, reaching only 1.0 decay index in the 5th day.

Compared with control group (N), fruits either cold refrigerated or treated by UV-C light apparently had longer shelf-life, indicating that UV-C treatment and refrigeration both can delay deterioration rate. Additionally, compared with fruit treated by sole refrigeration (A), fruits irradiated by UV-C light (groups B and C) performed better in inhibiting decay rate. However, the results of fruits irradiated by UV-C light before and after 24-hour cold storage did not show significant gap in decay index: line chart of groups B and C cannot clearly be separated with each other.

3.2. Antioxidant Profiles of Fruits in Different Forms upon Different Treatments. Table 2 shows the results of antioxidant profile of litchi, longan, and rambutan treated differently. In each group, fruits were categorized by distinct fruit forms: whole fruits, dehulled fruits, and destoned fruits, respectively. Table 3 showed the result of two-way ANOVA ($p \leq 0.05$) for testing whether there was any interaction between treatments (UV-C radiation and refrigeration) and fruit forms (whole, dehulled, and destoned).

Generally, longan contained higher *L*-ascorbic acid content (ranging from 1.60 to 2.24 g kg⁻¹) than the counterpart of litchi (data of rambutan's *L*-ascorbic acid content was not

available). However, litchi performed stronger in TPC (ranging from 3.83 to 5.19 g kg⁻¹), TFC (from 1.23 to 1.68 g kg⁻¹), FRAP (from 31.61 to 38.27 mmol kg⁻¹), and ABTS free radical scavenging capacity (from 1.31 to 1.67 mol kg⁻¹). DPPH free radical scavenging capacity was higher in longan (from 8.80 to 12.26 mol kg⁻¹). Rambutan showed the weakest antioxidant capacity.

According to the correlation matrix in Figure 2, *L*-ascorbic acid did not show a clear relationship with most of tested parameters. On the contrary, the rest of paired scatter plots all showed statistically significant positive correlations except the pair between TFC and DPPH free radical scavenging capacity in litchi, and the pair between TPC and ABTS free radical scavenging capacity in longan. TPC strongly correlated with DPPH free radical scavenging capacity ($r^2 = 0.845$) as well as ABTS free radical scavenging capacity ($r^2 = 0.818$) in litchi and was closely associated with DPPH free radical scavenging capacity ($r^2 = 0.847$) in longan. It should be noticed that three assays of antioxidant capacity in rambutan closely related to each other with all correlation coefficients above 0.84 (0.843 between DPPH free radical scavenging capacity and FRAP, 0.928 between DPPH free radical and ABTS free radical scavenging capacity, and 0.850 between FRAP and ABTS free radical scavenging capacity).

3.2.1. Effects of UV-C Treatment and Refrigeration on Litchi. The change of levels of *L*-ascorbic acid, TPC, TFC, DPPH free radical scavenging capacity, FRAP, and ABTS free radical scavenging capacity in whole litchi fruit is illustrated in Figure 3(a). For each parameter, different subscripts note the existence of significant difference. Comparing solely refrigerated whole litchi fruit with control group, refrigeration enriched levels of TPC, TFC, DPPH free radical scavenging capacity, FRAP, and ABTS free radical scavenging capacity. Additionally, fruits which were irradiated by ultraviolet (Groups B1 and C1), compared with solely refrigerated whole fruits, had further increase in *L*-ascorbic acid, TFC, and DPPH free radical scavenging power. The values of TPC, FRAP, and ABTS free radical scavenging capacity were also higher after UV-C treatment, though not significant. The treatment sequence was also associated with the distinguishable disparities of phytochemical levels: litchis that experienced UV-C radiation before storage showed higher *L*-ascorbic acid content, whereas litchis treated by UV-C treatment after storage performed stronger in DPPH free radical scavenging capacity. The remaining parameters did not show significant gaps between fruits in these two treatments.

3.2.2. Effects of Dehulling and Destoning on Litchi's Antioxidant Profile. Levels of *L*-ascorbic acid, TPC, TFC, DPPH free radical scavenging capacity, FRAP, and ABTS free radical scavenging capacity in litchis with different fruit forms (whole, dehulled, and destoned) are illustrated in Table 2. In Group A (only refrigerated), compared with whole fruit, dehulled litchis contained higher level of *L*-ascorbic acid content, TFC, and DPPH free radical scavenging capacity; litchis without inner core stone performed stronger in *L*-ascorbic acid, TPC, TFC, and DPPH free radical and ABTS free

TABLE 2: *L*-ascorbic acid, TPC, TFC, FRAP, and DPPH free radical and ABTS free radical scavenging capacity in litchi, longan, and rambutan after different treatment with different fruit forms.

| Fruits | Treatments | Forms | <i>L</i> -ascorbic acid (g kg ⁻¹) | | TPC (g kg ⁻¹) | | TFC (g kg ⁻¹) | | DPPH (mol kg ⁻¹) | | FRAP (mmol kg ⁻¹) | | ABTS (mol kg ⁻¹) | |
|--|--|----------|---|----------------------------|----------------------------|---------------------------|-----------------------------|----------------------------|------------------------------|-----------|-------------------------------|-----------|------------------------------|--|
| | | | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | | |
| Litchi | Control | Whole | 0.32 ± 0.04 ^{de} | 3.83 ± 0.31 ^{de} | 1.34 ± 0.12 ^{cd} | 6.92 ± 0.67 ^c | 32.44 ± 2.99 ^{bc} | 1.31 ± 0.12 ^c | | | | | | |
| | | Whole | 0.34 ± 0.02 ^{de} | 4.23 ± 0.19 ^{cd} | 1.54 ± 0.07 ^{abc} | 7.87 ± 0.37 ^d | 36.42 ± 1.72 ^{abc} | 1.57 ± 0.13 ^{ab} | | | | | | |
| | Refrigeration | Dehulled | 0.43 ± 0.03 ^c | 4.14 ± 0.16 ^{cde} | 1.62 ± 0.09 ^{ab} | 8.06 ± 0.08 ^{cd} | 35.12 ± 2.04 ^{abc} | 1.57 ± 0.05 ^{ab} | | | | | | |
| | | Destoned | 0.58 ± 0.04 ^b | 4.54 ± 0.33 ^{bc} | 1.68 ± 0.14 ^a | 8.07 ± 1.03 ^{cd} | 33.29 ± 2.60 ^{bc} | 1.67 ± 0.16 ^a | | | | | | |
| | Refrigeration followed by UV radiation | Whole | 0.66 ± 0.01 ^a | 4.80 ± 0.40 ^{ab} | 1.63 ± 0.15 ^{ab} | 8.78 ± 0.20 ^{cd} | 39.37 ± 3.22 ^a | 1.64 ± 0.15 ^a | | | | | | |
| | | Dehulled | 0.40 ± 0.02 ^c | 4.58 ± 0.20 ^{bc} | 1.27 ± 0.03 ^d | 8.82 ± 0.60 ^{cd} | 31.61 ± 3.25 ^c | 1.50 ± 0.07 ^{abc} | | | | | | |
| Longan | Control | Destoned | 0.30 ± 0.03 ^e | 3.62 ± 0.36 ^f | 1.23 ± 0.13 ^d | 8.05 ± 0.02 ^{cd} | 32.56 ± 4.28 ^{bc} | 1.37 ± 0.16 ^{bc} | | | | | | |
| | | Whole | 0.32 ± 0.01 ^{de} | 5.19 ± 0.53 ^a | 1.61 ± 0.03 ^{ab} | 9.95 ± 0.83 ^a | 38.27 ± 4.78 ^{ab} | 1.60 ± 0.14 ^a | | | | | | |
| | Refrigeration followed by UV radiation | Dehulled | 0.35 ± 0.02 ^d | 5.07 ± 0.10 ^{ab} | 1.53 ± 0.14 ^{abc} | 9.80 ± 0.11 ^{ab} | 36.00 ± 1.02 ^{abc} | 1.67 ± 0.11 ^a | | | | | | |
| | | Destoned | 0.40 ± 0.01 ^c | 4.92 ± 0.24 ^{ab} | 1.47 ± 0.11 ^{bc} | 8.92 ± 0.29 ^{bc} | 36.34 ± 2.36 ^{abc} | 1.66 ± 0.03 ^a | | | | | | |
| | Refrigeration | Whole | 2.24 ± 0.22 ^a | 2.77 ± 0.26 ^{ab} | 0.41 ± 0.04 ^a | 11.89 ± 0.88 ^a | 23.60 ± 2.27 ^{ab} | 1.03 ± 0.10 ^{ab} | | | | | | |
| | | Whole | 2.04 ± 0.16 ^{ab} | 2.74 ± 0.26 ^{ab} | 0.27 ± 0.01 ^{bc} | 11.46 ± 0.96 ^a | 23.34 ± 0.44 ^{abc} | 1.10 ± 0.05 ^a | | | | | | |
| Rambutan | Control | Dehulled | 1.98 ± 0.02 ^b | 2.30 ± 0.05 ^c | 0.24 ± 0.01 ^{de} | 9.42 ± 0.44 ^b | 18.63 ± 0.69 ^d | 1.02 ± 0.02 ^{ab} | | | | | | |
| | | Destoned | 1.62 ± 0.06 ^d | 2.54 ± 0.13 ^{bc} | 0.25 ± 0.02 ^{cd} | 11.13 ± 0.46 ^a | 24.00 ± 2.01 ^{ab} | 0.95 ± 0.09 ^b | | | | | | |
| | Refrigeration followed by UV radiation | Whole | 2.05 ± 0.02 ^{ab} | 2.56 ± 0.33 ^{bc} | 0.23 ± 0.01 ^{de} | 9.90 ± 0.80 ^b | 20.89 ± 3.07 ^{bcd} | 0.77 ± 0.09 ^c | | | | | | |
| | | Dehulled | 1.96 ± 0.07 ^b | 2.46 ± 0.15 ^{bc} | 0.21 ± 0.01 ^{ef} | 9.87 ± 0.62 ^a | 20.97 ± 1.02 ^{bcd} | 0.81 ± 0.06 ^c | | | | | | |
| | Refrigeration followed by UV radiation | Destoned | 1.73 ± 0.11 ^{cd} | 2.95 ± 0.08 ^a | 0.28 ± 0.01 ^b | 12.26 ± 0.26 ^a | 24.94 ± 1.75 ^a | 1.09 ± 0.03 ^a | | | | | | |
| | | Whole | 1.94 ± 0.16 ^b | 2.55 ± 0.03 ^{bc} | 0.23 ± 0.01 ^{de} | 9.92 ± 0.12 ^b | 20.23 ± 0.57 ^{cd} | 0.81 ± 0.03 ^c | | | | | | |
| Rambutan | Control | Dehulled | 1.85 ± 0.09 ^{bc} | 2.30 ± 0.16 ^c | 0.20 ± 0.01 ^f | 8.80 ± 0.61 ^b | 18.17 ± 1.01 ^d | 0.55 ± 0.03 ^d | | | | | | |
| | | Destoned | 1.60 ± 0.04 ^d | 2.57 ± 0.08 ^{bc} | 0.24 ± 0.00 ^{de} | 11.46 ± 0.34 ^a | 22.08 ± 1.82 ^{abc} | 0.96 ± 0.06 ^b | | | | | | |
| | Refrigeration | Whole | NA | 2.86 ± 0.02 ^{bcd} | 0.25 ± 0.02 ^a | 5.23 ± 0.05 ^a | 17.62 ± 1.14 ^c | 0.96 ± 0.04 ^{ab} | | | | | | |
| | | Whole | NA | 3.13 ± 0.37 ^{ab} | 0.23 ± 0.01 ^b | 4.85 ± 0.21 ^b | 19.76 ± 0.28 ^b | 0.98 ± 0.01 ^a | | | | | | |
| | Refrigeration followed by UV radiation | Dehulled | NA | 2.67 ± 0.21 ^{cd} | 0.19 ± 0.01 ^c | 3.78 ± 0.14 ^{de} | 16.71 ± 0.33 ^{cd} | 0.76 ± 0.01 ^c | | | | | | |
| | | Destoned | NA | 2.70 ± 0.09 ^{cd} | 0.18 ± 0.01 ^c | 3.57 ± 0.02 ^e | 16.26 ± 0.26 ^d | 0.78 ± 0.02 ^c | | | | | | |
| Refrigeration followed by UV radiation | Whole | NA | 2.67 ± 0.02 ^{cd} | 0.18 ± 0.01 ^c | 3.90 ± 0.14 ^d | 16.16 ± 0.31 ^d | 0.79 ± 0.01 ^c | | | | | | | |
| | Dehulled | NA | 3.25 ± 0.08 ^a | 0.25 ± 0.02 ^{ab} | 5.18 ± 0.14 ^a | 21.03 ± 0.96 ^a | 0.97 ± 0.03 ^a | | | | | | | |
| UV radiation followed by refrigeration | Destoned | NA | 2.88 ± 0.08 ^{bcd} | 0.24 ± 0.02 ^{ab} | 3.55 ± 0.03 ^e | 14.68 ± 0.30 ^e | 0.80 ± 0.06 ^c | | | | | | | |
| | Whole | NA | 2.71 ± 0.03 ^{cd} | 0.19 ± 0.01 ^c | 4.26 ± 0.15 ^c | 16.33 ± 0.92 ^d | 0.81 ± 0.01 ^c | | | | | | | |
| UV radiation followed by refrigeration | Dehulled | NA | 2.96 ± 0.06 ^{bc} | 0.26 ± 0.01 ^a | 4.87 ± 0.06 ^b | 18.97 ± 0.87 ^b | 0.91 ± 0.01 ^b | | | | | | | |
| | Destoned | NA | 2.63 ± 0.15 ^d | 0.14 ± 0.01 ^d | 3.14 ± 0.29 ^e | 13.54 ± 0.70 ^e | 0.68 ± 0.03 ^d | | | | | | | |

Note. For one type of fruit, means within each column for each treatment with different superscripts are significantly ($p \leq 0.05$) different.

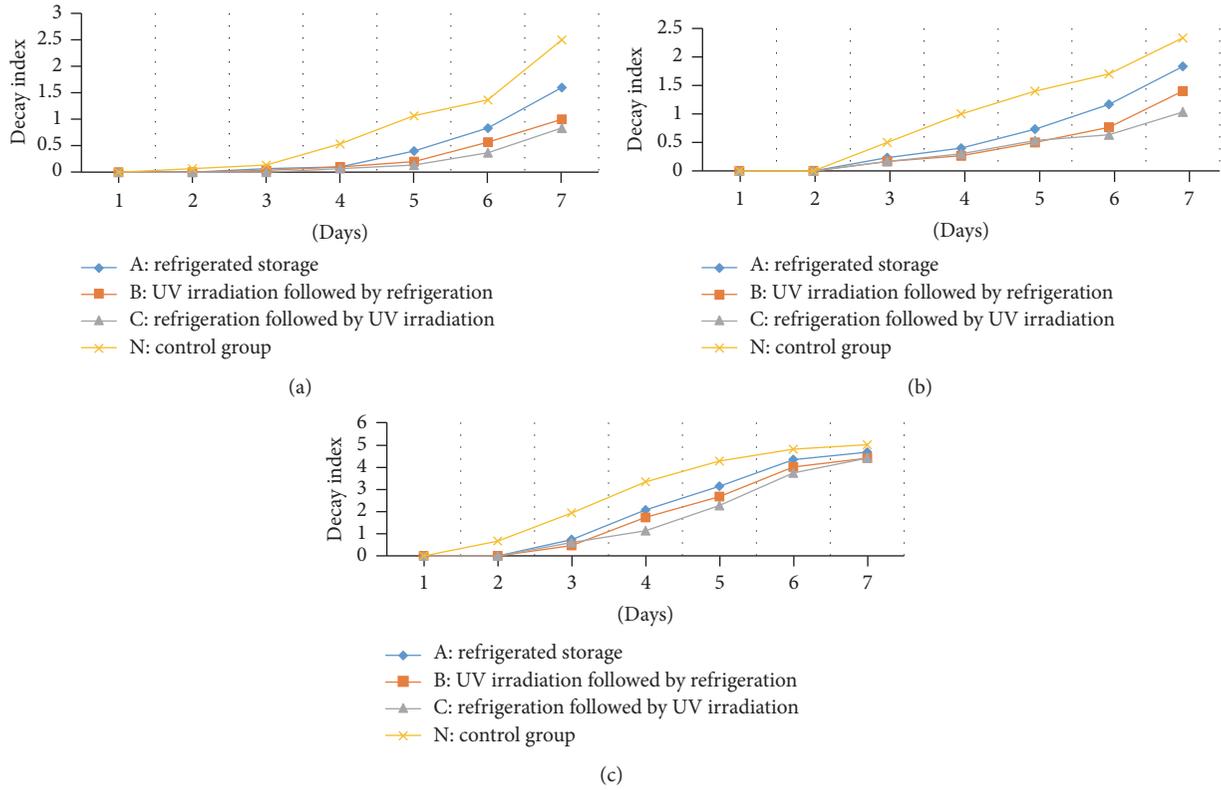
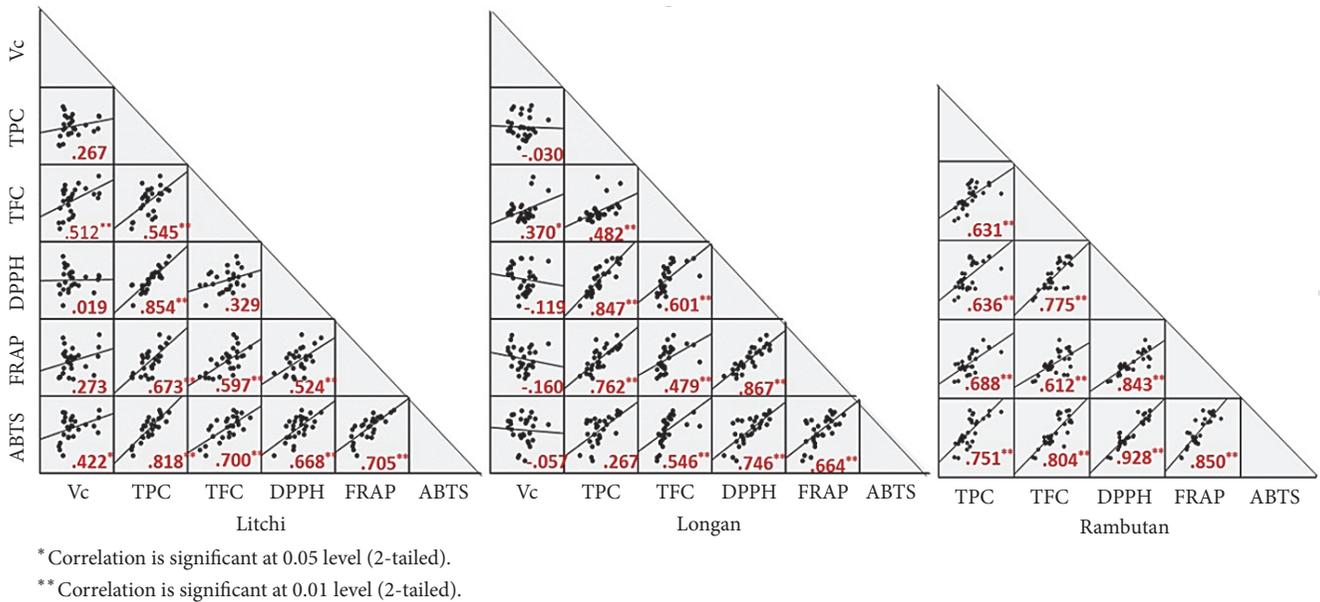


FIGURE 1: Decay proliferation of litchis (a), longans (b), and rambutans (c) during first week after treatments.



*Correlation is significant at 0.05 level (2-tailed).

**Correlation is significant at 0.01 level (2-tailed).

FIGURE 2: Correlation matrix of phytochemical parameters (*L*-ascorbic acid, TPC, TFC, FRAP, DPPH free radical, and ABTS free radical scavenging capacity) of litchi, longan, and rambutan.

radical scavenging capacities. According to results obtained from the litchi group irradiated by UV-C after cold storage, although whole litchis fruit in this group experienced sharp increases in all phytochemicals determined compared with control, peeling and destoning weakened this increase, and,

in particular, *L*-ascorbic acid, which was even lower than the level in whole litchi fruit, and these tendencies were not found in solely refrigerated group. Based on two-way ANOVA (Table 3), interaction between treatments and fruit forms was significant, showing *L*-ascorbic acid content, TPC,

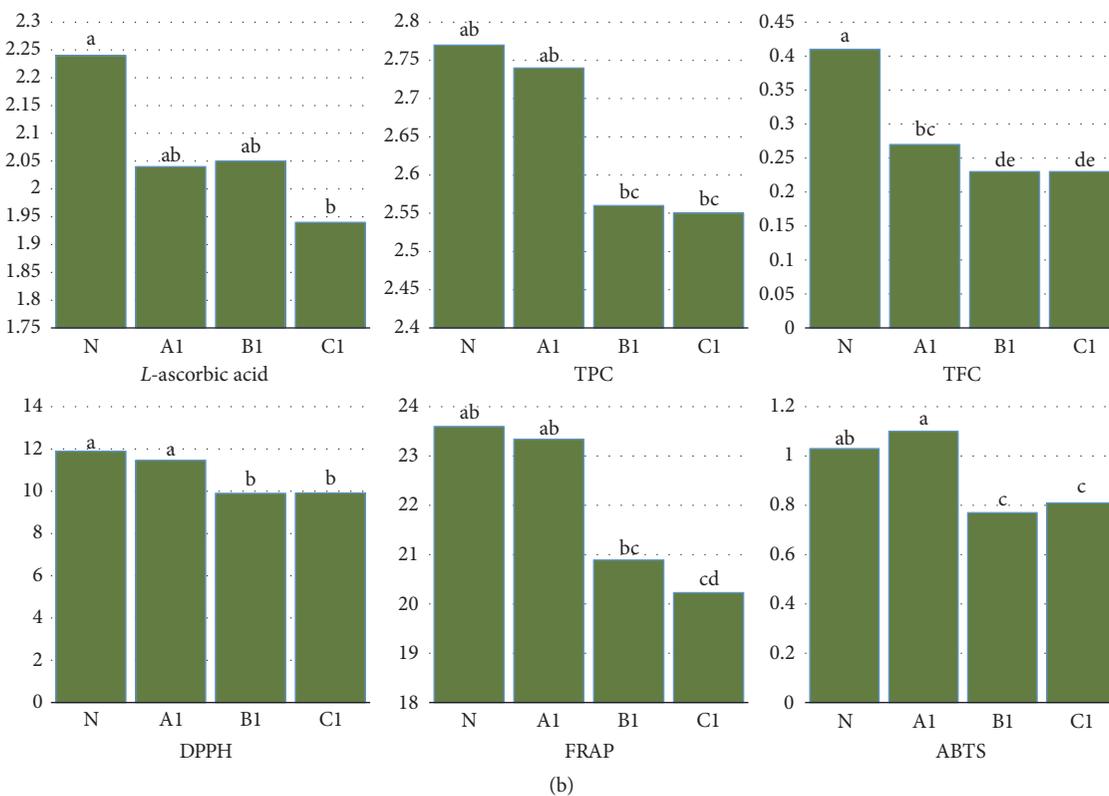
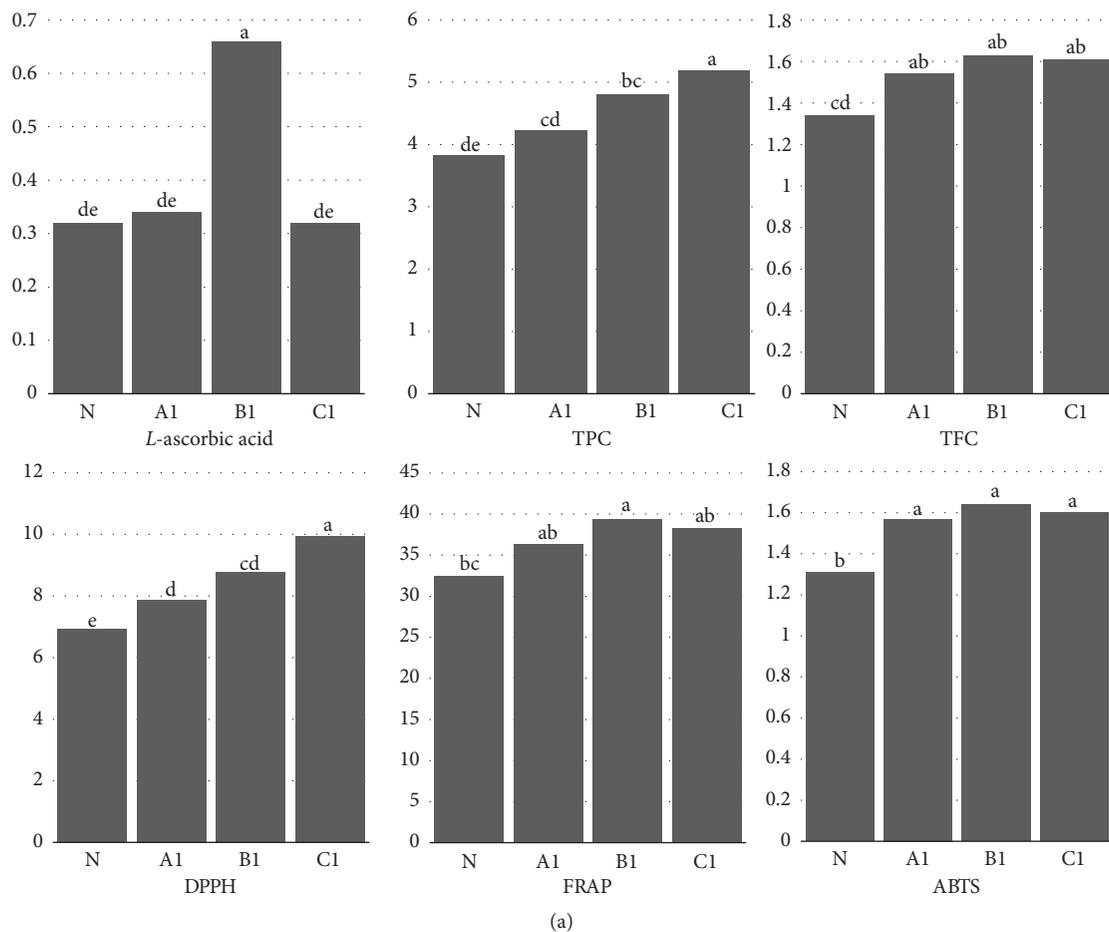


FIGURE 3: Continued.

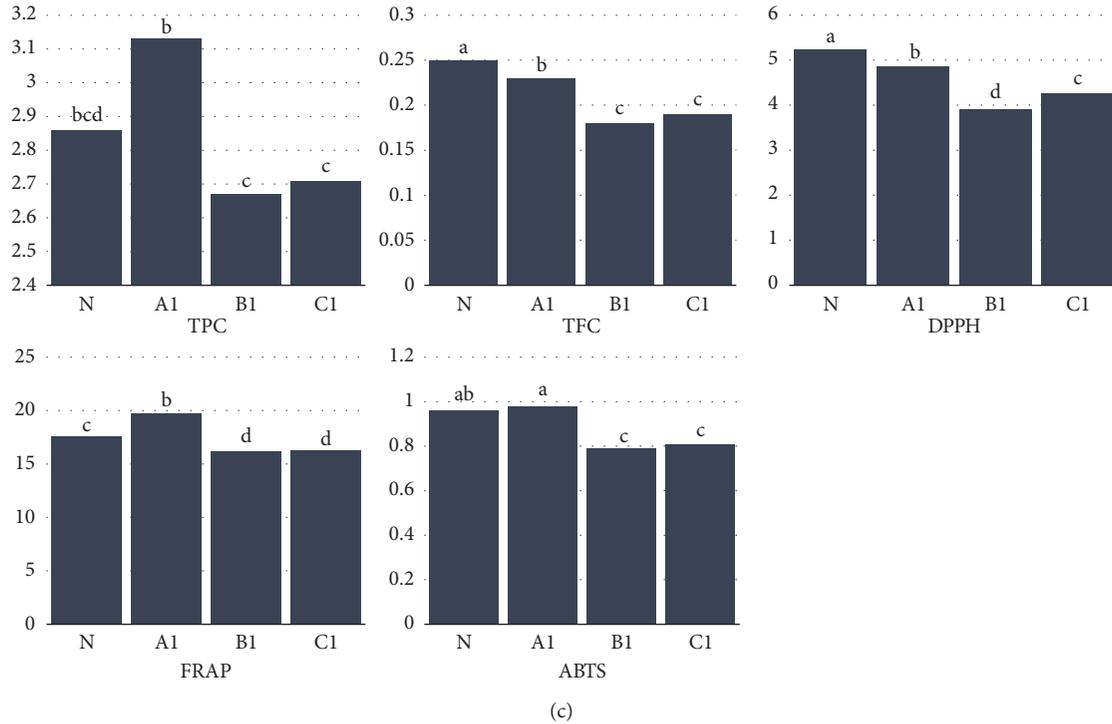


FIGURE 3: Levels of *L*-ascorbic acid, TPC, TFC, DPPH free radical scavenging capacity, FRAP, and ABTS free radical scavenging capacity in whole litchi fruit (a), longan (b), and rambutan (c) after different pretreatments: refrigeration (A), refrigeration-UV (B), UV-refrigeration, (C) and control (N).

TABLE 3: *p* value of interaction between treatments and fruit forms shown in examined parameters in litchi, longan, and rambutan tested by two-way ANOVA.

| | Fruits | <i>L</i> -ascorbic acid | TPC | TFC | DPPH | FRAP | ABTS |
|-------------------------------|----------|-------------------------|--------|--------|--------|--------|--------|
| <i>p</i> value of interaction | Litchi | 0.001* | 0.004* | 0.004* | 0.395 | 0.370 | 0.105 |
| | Longan | 0.845 | 0.127 | 0.006* | 0.011* | 0.195 | 0.001* |
| | Rambutan | NA | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |

*Interaction is significant at 0.05 level.

and TFC of litchi, whereas interaction between treatments and fruit forms was not strong in antioxidant assay.

3.2.3. Effects of UV-C Treatment and Refrigeration on Longan. Changes in *L*-ascorbic acid, TPC, TFC, DPPH free radical scavenging capacity, FRAP, and ABTS free radical scavenging capacity in whole longan fruit are illustrated in Figure 3(b). For each parameter, different subscripts denoted the existence of significant difference. Based on the comparison between fruits in control group (N) and fruits only undergoing refrigeration (A1), cold storage does not significantly affect TPC and antioxidant capacity, but it was associated with the decrease of *L*-ascorbic acid and TFC. When UV-C light was applied as well (Groups B and C), all tested variables showed significant reduction in contrast with UV-negative groups. Additionally, *L*-ascorbic acid content in longans that experienced UV-C irradiation before storage was lower than its counterpart exposed to UV-C light after storage.

3.2.4. Effects of Dehulling and Destoning on Longan's Antioxidant Profile. Levels of *L*-ascorbic acid, TPC, TFC, DPPH free radical scavenging capacity, FRAP, and ABTS free radical scavenging capacity in longans with different fruit forms (whole, dehulled, and destoned) are illustrated in Table 2. Dehulling and destoning were both associated with diminishing nutritional levels in solely refrigerated longans (Group A), where the effect of dehulling was weaker in changes of *L*-ascorbic acid and ABTS free radical scavenging capacity, but stronger in reduction of other parameters. In group undergoing UV irradiation after refrigeration, destoned longans were found with higher level of TPC, TFC, FRAP, and DPPH free radical and ABTS free radical scavenging capacities than the counterparts in whole longan fruit and dehulled longans. Although values of above parameters in destoned fruit were slightly lower than in control group N, removing core stone could help to reduce those antioxidant degradations. The similar tendency was observed in group refrigerated after UV-C

treatment as well. While UV-C treated longan fruits did not show any retention of *L*-ascorbic acid, its loss was exacerbated in the peeled and destoned fruits. Two-way ANOVA (Table 3) indicated interaction between treatments and fruit forms was not significant in phytochemicals of longan, except TPC and DPPH free radical and ABTS free radical scavenging capacities.

3.2.5. Effects of UV-C Treatment and Refrigeration on Rambutan. Changes of TPC, TFC, DPPH free radical scavenging capacity, FRAP, and ABTS free radical scavenging capacity in whole rambutan fruit are illustrated in Figure 3(c). Compared with control group, refrigeration enriched TPC, FRAP, and ABTS free radical scavenging capacity but reduced values of TFC and DPPH scavenging capacity. Additionally, fruits which were pretreated by ultraviolet (Groups B1 and C1), compared with control group, had lower phenolic contents. Levels of antioxidants were not significantly affected by the treatment order, except that rambutans irradiated by UV-C light before 24-hour storage performed higher DPPH scavenging rate.

3.2.6. Effects of Dehulling and Destoning on Rambutan's Antioxidant Profile. Levels of *L*-ascorbic acid, TPC, TFC, DPPH free radical scavenging capacity, FRAP, and ABTS free radical scavenging capacity in rambutan with different fruit forms (whole, dehulled, and destoned) are illustrated in Table 2. In the group of refrigeration, removing hull and core stone of rambutan were both associated with the decrease of phytochemical levels during refrigeration. However, for fruits treated by ultraviolet light, though phytochemical levels of TPC, TFC, FRAP, and DPPH free radical and ABTS free radical scavenging capacities diminished in whole fruit compared with in control, dehulling contributed to retention of above antioxidant parameters. TPC, FRAP, and ABTS free radical scavenging capacity in dehulled rambutans even rise. However, results indicated that if internal stones were removed from rambutan, levels of all examined phytochemicals lost more sharply. According to two-way ANOVA (Table 3), significant interactions between fruit forms and treatments were shown in all tested parameters of rambutan.

4. Discussions

According to Mercier et al. [35], low dose of UV-C exposure is not enough to kill microbes; therefore, the shelf-life extending effect of UV-C light is mainly attributed to the synthesis of antimicrobial phytoalexin. UV light exposure can be applied to fruits postharvest processing, which can extend 2-to-3-day selling time and thus reduce economic loss. Additionally, it was reported that UV irradiation can be potent when fruits were sprayed with chemicals, like ClO_2 and fumaric acid [36], or when fruits were irradiated in ozone atmosphere, which can achieve 6-log microbial reduction [37]. It should be noticed that cold storage can cause negative effect on rambutan quality: under 7°C, rambutan would suffer from chilling injury [38]. As rambutan spintern are dense with stomachs, moisture in spinterns can escape rapidly under 7°C and

anthocyanin can be converted to its colorless form, turning to maroon [39], which might impair consumer acceptance. In addition, the elevation of membrane permeability can lead to physiological dysfunction [40]. Therefore, refrigeration is not applicable to rambutan.

The significant linear relationship between TPC and antioxidant capacities indicated that phenolic compounds contribute to a majority of antioxidant phytochemicals, which agreed with viewpoint reported in previous literatures [26, 41, 42]. Strong relationship among assays of FRAP and DPPH free radical and ABTS free radical scavenging capacities can be attributed to the same mechanism underlying behind different chemical reactions: electron transfer [42]. Clarke et al. [41] indicated that conducting two or more assays to estimate antioxidant capacity seemed to be redundant considering high correlation coefficients. To improve this study, instead of spending too much effort on antioxidant capacities, malondialdehyde (MDA), the product of membrane lipid oxidation, can be taken into consideration for understanding whether treatments could suppress membrane structural deterioration [10]. The weak relationship between *L*-ascorbic acid and some other parameters seemed to be contradictory with the common sense that *L*-ascorbic acid is an important antioxidant. Possible reason was that *L*-ascorbic acid contributes little in scavenging capacity which was largely attributable to phenolic compounds. Sricharoen et al. [43] examined antioxidant capacity of tomato extract and found that radical scavenging ability of *L*-ascorbic acid was relatively low, inferior to polyphenols and flavonoids. Moreover, UV irradiation was associated with *L*-ascorbic acid degradation [44], but considering phenolic compounds are generally more stable, the overall changing trend of antioxidant capacity should be positively related with total phenolic contents.

Based on results, litchis which were only refrigerated had higher level of TPC and TFC as well as antioxidant capacity than control group. Enrichment of phytochemical was also observed in TPC, FRAP, and ABTS free radical scavenging capacities of rambutan. The similar phenomenon was previously reported by Kevers et al. [45], finding an increase of antioxidants in leek and asparagus after the first day in cold environment; Piljac-Žegarac et al. [46] also reported elevations of phenolic content and DPPH free radical scavenging capacity in a variety of berries in the first 48 h of refrigerated storage, though phenolic content started to drop afterward. Sanchez-Ballesta et al. [47] observed the increase of PAL and CHS transcripts and elevation of anthocyanin content in cold-stored grape; however, it is still unclear whether the cold environment activates the antioxidant defense in grapes or the low temperatures maintain the levels of antioxidants. After being harvested from farm, litchis can be recommended to be stored in cold environment to minimize textural change and meanwhile to enhance phytochemical values. However, on the contrary, in terms of longan, refrigeration caused dramatic decrease of *L*-ascorbic acid and TFC. Degradation was also shown in TFC of rambutan. Similar previous finding was observed on citrus juice [48]. Castro-López et al. [49] also found a slight degradation of *L*-ascorbic acid in eight fruits during storage and attributed the reason to heat- and light-sensitive property of *L*-ascorbic acid. The disparities that

different fruits responded differently under refrigeration can be related to postharvest metabolic rate: the original content could be oxidized and depleted if low metabolic rate cannot serve the adequate synthesis after stress response.

When ultraviolet was applied, result showed that TPC, TFC, and antioxidant capacity of litchi increased further, showing positive effects on varied phytochemicals. Likewise, Luthria et al. [19] observed the raise of phenolic content in tomato after UV exposure, especially caffeic acid, which was enriched to approximately 120% of its original level. Possible mechanism has been pinpointed by self-defense pattern of plant: Favory et al. [18] pointed out that UV radiation had triggered signaling pathway of COP1 and UVR8 photoperception protein to produce accumulation of anti-UV molecules, like tannins and flavonoids. Additionally, taking the order of treatments into consideration, litchi undergoing UV irradiation before storage contained higher level of TPC and DPPH free radical scavenging capacity, which indicated possible underlying accumulation pattern: UV treatment stimulates DNA expression, and time is needed to synthesize phytochemicals.

However, UV did not enrich phytochemicals levels in longan and rambutan; on the contrary, it caused diminution for all test parameters. Cote et al. [50] reported that strawberry fruit treated by high-intensity UV-C light (4 kJ m^{-2} , 33 W m^{-2}) showed a reduction in antioxidant capacity compared with control group. The possible reasons for the degradation could be attributable to the dose applied on longan and rambutan, though it is the same as the one applied on litchi, exceeding hormetic dose. Hormesis phenomenon is known as beneficial responses appear with low dose agent (hormetic dose) applied but harmful effect could be triggered by excessive amount [51, 52]. Although UV-C light gives benefits to fruit due to the stimulation of phytoalexin and phenolic compounds, it can also be harmful to plant cells [53]. Previous study indicated that ultraviolet could have adverse effect on plant tissues by altering epidermal cells' water permeability [54]. Besides gene of microbes, UV light has similar DNA damage on fruit, inducing pyrimidine dimers, which consequently lead to inhibition of production of secondary metabolites production. Contradictory with results of litchi, longan undergoing UV-C irradiation before refrigeration had lower level of *L*-ascorbic acid and FRAP, which could be explained as follows: due to hormesis effect, DNA of mesocarp cells in longan was impaired, and gene segments coding secondary metabolites were affected, which lead to suppression of phytochemical synthesis in the next storing day. Moreover, ultraviolet light was associated with the generation of free radicals in plant tissue [55], which can consequently neutralize antioxidants. Both inhibition of phytochemical synthesis and oxidation of phytochemicals contributed to the overall reduction of antioxidants.

So far, no research was conducted in study of how dehulling and destoning affect nutritional change induced by UV light, leading to insufficient previous research as references. In terms of litchi, removing external hull and removing core stone out both impede elevation of examined content induced by UV light, especially *L*-ascorbic acid, TPC, and TFC but did not affect litchis which were only refrigerated.

A possible reason was that bare pulp was exposed to the same dose of ultraviolet, which exceeded beneficial range and resulted into hormesis. The result of two-way ANOVA strengthened the interaction of fruit forms and UV treatment. However, it should be noticed that the effects of fruit forms on longan and rambutan's phytochemical levels did not corroborate with each other. For longans, compared with whole fruit, destoned longans generally retained nutritional levels, including TPC, TFC, and DPPH free radical and ABTS free radical scavenging capacities. This observation did not only appear in UV-treated groups, but also in UV-negative group, which means no matter what treatment was given, all destoned fruit can be subjected to nutritional lose. This was also corroborated by some large *p* values > 0.05 shown in Table 3. The reason could be hypothesized as that destoning triggered stronger defense mechanism of longan against wound-stress, enriching phytochemical levels. Nevertheless, according to data obtained from rambutan, fruits without hulls were observed having retaining effect on phytochemicals, and this effect did not appear in destoned rambutan. This phenomenon only arises in groups that experienced UV treatment, which indicated that the retention of nutrient was associated with ultraviolet irradiation; this was also supported by two-way ANOVA with small *p* values < 0.05 for all tested parameters of rambutan. The further losses of phytochemical level of dehulled rambutan and destoned longan could be associated with the exposure of mesocarp in air, thus boosting oxidation of antioxidant, because similar degradation was found in UV-negative groups. The effects of fruits forms on phytochemicals could be hypothesized as a multifactorial relationship of defense response against stress and contact surface with external atmosphere. The overall effect should be contingent on which factors take major accountability, which depends on fruit structure and pulp texture of varied fruit species.

A limitation of this study is that, instead of several different UV doses, only one UV-C dose was given to three different types of fruits. To figure out the most proper ultraviolet dose based on the hormesis phenomenon, further study might apply several different UV doses. Additionally, to understand whether phytochemical level change is an accumulative process or an immediate process, future study is suggested to perform molecular-level assays, like examination of mRNA coding phenylalanine ammonia lyase and photoperception protein, or to determine whether ultraviolet promotes free radical generation using electron paramagnetic resonance spin trapping.

5. Conclusions

In this study, UV-C light treatment (3 kJ m^{-2}) and refrigeration (24-hour) both can inhibit fruit decay rate and had different effects on phytochemical profiles of litchi, longan, and rambutan: cold storage enriched litchi's nutritional value but mainly adversely affected antioxidant level of longan and rambutan; ultraviolet exposure (3 kJ m^{-2}) led to the increases of phytochemical levels of litchi but exceeded hormetic dose of longan and rambutan and triggered adverse effect, causing degradation of antioxidants. Phytochemical levels in fruit

irradiated by UV before refrigeration and fruit treated by UV after refrigeration were not consistent in some parameters, which were associated with the theory that UV is an elicitor stimulating defense response against stress, initiating accumulative change of phytochemical contents. Taking fruit forms into consideration, destoning retained antioxidants of longan whereas dehulling retained antioxidants of rambutan, but both removing hull and removing stone were associated with suppression of phytochemical accumulation in litchi after UV irradiation. The effects of treatments and fruit forms on antioxidant profile could be different from fruit to fruit.

Abbreviations

ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
 DCIP: 2,6-Dichlorophenolindophenol
 DPPH: 2-Diphenyl-1-picrylhydrazyl
 FE: Ferric equivalents
 FRAP: Ferric reducing antioxidant power
 TFC: Total flavonoid content
 TPTZ: 2,4,6-Tri(2-pyridyl)-s-triazine
 TPC: Total phenolic content
 Trolox: 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
 UVR8: Ultraviolet response locus 8
 COPI: Constitutively photomorphogenic 1.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Cloning, Characterization, and Functional Expression of Phospholipase D α cDNA from Banana (*Musa acuminata* L.)

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Phospholipase D (PLD) plays a key role in adaptive responses of postharvest fruits. A cDNA clone of banana (*Musa acuminata* L.) *PLD α* (*MaPLD α*) was obtained by RT-PCR in this study. The *MaPLD α* gene contains a complete open reading frame (ORF) encoding a 92-kDa protein composed of 832 amino acid residues and possesses a characteristic C2 domain and two catalytic H \times K \times \times D (abbr. HKD) motifs. The two HKD motifs are separated by 341 amino acid residues in the primary structure. Relatively higher PLD activity and expression of *MaPLD α* mRNA were detected in developing tissues compared to senescent or mature tissues in individual leaves, flower, stem, and fruit organs, respectively. The expression profile of *PLD α* mRNA in postharvest banana fruits at different temperatures was determined, and the *MaPLD α* mRNA reached the highest expression peak on day 5 at 25°C and on day 7 at 12°C. The results provide useful information for maintaining postharvest quality and extending the storage life of banana fruit.

1. Introduction

Banana (*Musa acuminata* L.) is one of the major commercial fruit crops grown in tropics and subtropics and plays a key role in the economy of developing countries [1]. Banana fruits are widely consumed but have short shelf life (6 to 10 days) after harvest under tropical conditions because of their highly perishable nature related to membrane disruption in pericarp cells. Postharvest loss significantly reduces the commercial value of banana fruits [2].

Membrane deterioration during plant senescence is commonly associated with the progressive decrease in membrane phospholipid content. Phospholipase D (PLD) (EC 3.1.4.4) is an important enzyme that initiates membrane phospholipid degradation during ripening, senescence, and signal transduction that takes place in response to hormones and environmental stress [3, 4]. PLD in mammalian tissues hydrolyzes phospholipids, principally phosphatidylcholine (PC), to phosphatidic acid (PA) and choline. This enzyme has been implicated in a broad range of cellular processes

[5, 6]. PLDs in plants are classified into six gene families: *PLD α* , *PLD β* , *PLD γ* , *PLD δ* , *PLD ϵ* , and *PLD ζ* [7]. *PLD α* is the most active enzyme in the PLD family in plant tissues [8] and has been associated with the catabolism and turnover of membrane lipids [9]. *PLD α* enzymes from a number of species have been characterized, including strawberry, peach, tomato, castor, cabbage, grape, and oilseed rape [10–12].

Regulation of PLD activity has a major impact on ripening and senescence of some fruits in the *Sapindaceae* family [13]. Previously, we have provided molecular and physiological evidence that PLD is closely related to the senescence of longan and litchi fruits [14, 15]. We have cloned the full-length cDNA (registered in GenBank, accession number JF791814) and studied different expression patterns of longan *PLD* gene family members [16]. Nevertheless, there are no reports about the effects of PLD activity on banana senescence. There is also a lack of data on characterization and functional expression of *PLD* cDNA in banana. It is important to study the molecular mechanism of ripening and senescence in banana fruits. Therefore, the objective of

the present study was to determine the role of PLD α in the response to ripening and senescence-related signaling in banana. The *PLD α* gene was isolated and amplified by reverse-transcription polymerase chain reaction (RT-PCR); analyses of PLD α structure and nucleotide sequence of this gene were also conducted. Moreover, PLD expression and activities in different banana organs at several developmental stages and in the fruits during ripening and senescence are reported here for the first time. Our results will provide useful information for maintaining postharvest quality and extending storage life of banana fruits via specific regulation of PLD α expression.

2. Materials and Methods

2.1. Plant Materials. Banana (*Musa acuminata* L.) tissues (floral bud, flower, green and senescent leaves, pseudo stem, stem, developing and mature fruits, and fruits at different postharvest stages) were collected in a commercial orchard in Nanning of Guangxi province during July 2015 and transported to the laboratory immediately. Banana fruits of similar size and of the same maturity period, without infection and physical damage, were chosen and randomly subdivided into two groups (60 fruits in each group). One group was packed into polyethylene bags (0.03 mm thick) and stored at 25°C, and the other was packed into the polyethylene bags and stored at 12°C. Banana pericarp tissues were collected every 2 d, frozen in liquid nitrogen, and then stored at -80°C for further analysis. Three replicates per analysis were used in the subsequent measurements.

2.2. RNA Extraction. Total RNA samples were extracted from 100 mg of fresh banana pericarp tissues. The RNA Prep Pure Plant Kit (Tiangen Co., Beijing, China) was used, followed by RNase-free DNase treatment (TaKaRa, Dalian, China). Concentrations of total RNA were measured at 260 and 280 nm on a NanoDrop™ 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and the purity of the extracted total RNA was determined as the ratio of optical density at 260 nm to that at 280 nm (OD₂₆₀/OD₂₈₀). The integrity of total RNA was determined by electrophoresis in a 1% (w/v) agarose gel. Isolated RNA was dissolved in 50 μ L of RNase-free H₂O and stored at -80°C.

2.3. Cloning of *MaPLD α* cDNA. Sequences of the PLD α protein from the public database were aligned to identify regions of homology using the ClustalX v.2.0.5 software [17]. A PCR product was amplified with a forward degenerate primer and a specific reverse primer, both designed using highly conserved regions of PLD α peptides: 5'-GGCGNCCC-CGCGAACCGTGG-3' and 5'-TGGTTGGTAGGCGCC-CATTGC-3'. Both the 5' and 3' ends of the cDNA were identified using the Smart-RACE cDNA Amplification Kit (Clontech, CA, USA) and the internal oligonucleotides were 5'-GATTGATGGTGGGGCCGC-3', 5'-CCGATGTGGCCC-GAAGGG-3', 5'-GTCAGGCCGTTTCAGATGG-3', 5'-CCA-TGCGTACGAGCTTCC-3', and 5'-CGGTTTCGCGGG-TCCGCC-3'. The amplicons were cloned into the pMBL-T vector (MBL) and sequenced by Shanghai Sangon Biological

Engineering Technology Service Co., Ltd. (Shanghai, China). The identities were confirmed using software BLAST. Primers with internal *Sac*I and *Kpn*I restriction sites were designed to amplify the coding region of the mature protein by PCR: 5'-GCCGAGCTCGCTCAGAAGACACATCTCC-3' and 5'-GGCGGTACCCTATGAGGTAAGAATTGG-3'. The PCR product obtained was subcloned into the *Sac*I and *Kpn*I sites of pQE-80L (Qiagen, Hilden, Germany) to produce a fusion protein with a 6-His tag at the N terminus. Ligation into the correct reading frame was confirmed by sequencing. The resulting construct was designated as pQEPLD α [18].

2.4. Bioinformatic and Cladistic Analyses. Sequence alignments, open reading frame (ORF) translation, and molecular mass calculation of the predicted protein were performed in DNAMAN v.6.0.3.99. The putative domains of *MaPLD α* were predicted by means of Swiss-Model (<https://swissmodel.expasy.org/>). Swiss-Model was run in "first approach" and "project (optimize)" modes with default parameters. Structures were visualized using Swiss-PDBViewer [19]. The PLD gene family related by amino acid sequences was aligned in ClustalX v.1.81 (<http://www.clustal.org/>) at the default settings, and the alignment was further refined by visual inspection. A phylogenetic tree was constructed by the minimum evolution method in MEGA ver. 4.0 [20, 21]. The Poisson correction metric was used together with the pairwise deletion option. The confidence of the tree branches was checked by bootstrapping generated from 1,000 replicates.

2.5. PLD Activity. This activity was assayed by a highly specific and sensitive sandwich enzyme immunoassay technique (enzyme-linked immunosorbent assay; ELISA) [22]. Namely, 96-well ELISA microtiter plates (Shanghai, China) were coated with purified plant PLD to set up the solid-phase antibody (100 μ L per well, 1 mg/mL diluted 1:1000 in PBS, pH 7.2, 4°C). The plates were then blocked (2 h at room temperature) with 1% skimmed milk powder dissolved in PBS (pH 7.4). The wells were then washed twice with 200 μ L of wash buffer for 10 min. A series of PLD standards was prepared in the range 2–120 U/L in PBS (pH 7.4). Samples were also prepared in a series of dilutions from 1/5 to 1/50 in PBS. The standards and samples were added to wells (50 μ L per well) and incubated for 2 h at 37°C. The wells were then washed twice with 100 μ L of wash buffer. Conjugate binding was performed by adding a biotin-conjugated antibody specific for PLD (100 μ L of 0.1% antibody conjugate in PBS). After incubation for 1 h at 37°C, the plates were washed three times with wash buffer and three times with 100 μ L of carbonate buffer. Horseradish peroxidase (HRP; 50 μ L) was added and incubated for 15 min in the dark at 37°C. The absorbance values of the plates were then read at 450 nm. The activity of PLD in the samples was then determined by comparing the OD of the samples to the standard curve and expressed as U/mg.

2.6. Semiquantitative Reverse-Transcriptase (RT) PCR Analysis. Banana tissues of the floral bud, flower, green and senescent leaves, pseudo stem, stem, developing fruit, mature fruit, and postharvest fruit were collected. The PLD mRNA

expression patterns were determined by semiquantitative RT-PCR. Housekeeping gene actin served as an internal control (GenBank accession number AB046952). Protocols for total RNA extraction and synthesis of cDNA are described above. Gene-specific primers for *PLD* (*PLD*-S3: 5'-GAAATCGGGAGGTCAAGAAGAG-3'; *PLD*-A3: 5'-CTAAGTTGTGAGGATTGGAGG-3') and actin (forward: 5'-GATTCTGGTGTGGTGTGAGT-3'; reverse: 5'-GAC-AATTTCCCTTAGCAG-3') were employed in RT-PCR. PCR was carried out with an initial denaturation step at 94°C for 5 min, and amplification was achieved via 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR products (10 µL) were analyzed by electrophoresis in a 1% agarose gel.

2.7. Statistical Analysis. All the experiments were conducted in triplicate ($n = 3$) and were arranged in a completely randomized design. All statistical analyses were based on analysis of variance (ANOVA) in the SPSS 13.0 statistical software (SPSS Inc., Chicago, USA). Significance of differences between the means of parameters was determined by Fisher's least significant difference (LSD) test ($P < 0.05$). The results are presented as mean \pm standard error (SE) of three replicates.

3. Results

3.1. Isolation and Sequence Analysis of *MaPLD α* . In this study, the conserved regions from available *PLD α* sequences were used to design specific primers for *PLD α* . Using these primers, a 1246-bp fragment from banana pericarp cDNA was amplified by RT-PCR, which corresponded to the *PLD α* mRNA internal region. The 5' and 3' flanking sequences obtained by RACE were assembled with those of the consensus region to form the full-length cDNA sequence. The full-length cDNA of *MaPLD α* was found to be 2916 bp long, including a putative ORF of 2680 bp, a 3' untranslated region of 295 bp, and a poly-A tail. The translated protein encoded by *MaPLD α* contains 832 amino acid residues (aa) with a calculated molecular mass of 92 kDa and an isoelectric point (P_i) of 5.9.

On the basis of a BLASTp homology search, the deduced amino acid sequence of *MaPLD α* showed 87% identity to *PLD α* from *Zea mays* (GenBank accession number BAA11135.1), 86% identity to *PLD α* from *Oryza sativa* Japonica Group (GenBank accession number BAA11136.1), and 82% identity to *PLD α* from *Ricinus communis* (GenBank accession number AAB04095.1). The multiple sequence alignment of banana *PLD α* with other *PLD α* enzymes from higher plants was conducted in the ClustalX 1.81 software and the phylogenetic tree was generated in MEGA 4.0 (Figure 1). All these results suggested that *MaPLD α* shares high identity with other plant *PLD α* enzymes, indicating that it is a member of the *PLD α* superfamily.

3.2. Bioinformatic Analysis. The predicted *MaPLD α* protein contained three conserved domains: the C2 domain (protein kinase C conserved region 2) and two *PLDc* domains that possess duplicated H×K××D motifs (abbr. HKD motifs).

The C2 domain is present in all cloned plant *PLD*s, but not in yeast or animal *PLD*s. C2 is a Ca^{2+} -dependent phospholipid-binding structural fold, and this binding is coordinated by 4-5 aa present in two bipartite loops of the domain [23]. In *Arabidopsis*, Ca^{2+} binds to the C2 domain of *PLD α 1*; then a conformation change and an increase in C2's affinity for PC are induced by this binding [24].

All *PLD*s contain two HKD motifs and conserved amino acid residues (His, Lys, and Asp) form a catalytic triad responsible for the hydrolysis of phosphoester bonds [25]. Site-directed mutagenesis of *PLD* from several species has indicated that these amino acids are critical for catalysis *in vitro* and for *PLD* functions [23]. The first active site having the amino acid sequence HQKIVIVD was identified in the region 315–322 (amino acid positions) in banana *PLD α* , and the second active site was located further downstream comprising amino acid positions 663–670 with the sequence HTKMMIVD. The two HKD motifs of *MaPLD α* are separated at amino acid residue 341 in the primary structure. The characteristic HKD motif has been used to define the *PLD* family [26]. Immediately following the second HKD motif, a highly conserved sequence, IGSANINQR, contains an invariant serine residue that was proposed to be the nucleophile attacking the phosphorus atom of substrate phospholipids.

The tertiary structure of *MaPLD α* was predicted here on the basis of the segment crystal structure data on *PLD*s from other plants, such as *Zea mays*, whose structure fragments can be found in Swiss-Model. The tertiary structural model of *MaPLD α* was built using amino acid sequence with a deletion of the C terminus of 112 aa (positions 753–865), using protein modeling software according to the homology with proteins with known crystal structure (Swiss-Model and 3D-JIGSAW) [27, 28].

3.3. Expression Analysis of *MaPLD α* Gene in Different Organs. *PLD* activity variations were observed in different tissues of the same plant and in the same tissue at different developmental stages. As shown in Figure 2, *PLD* activity of green leaves was higher than that in senescent leaves, *PLD* activity of floral buds was higher than that in flowers, and *PLD* activity of stems was higher than that in pseudo stems. A relatively higher *PLD* activity was found in developing fruits compared to mature fruits. A similar pattern with respect to expression of *MaPLD α* was observed by semiquantitative RT-PCR using gene-specific primers. The highest mRNA expression of *MaPLD α* was found in both floral buds and flowers, and it was intermediate in fruits, low in leaves, and exceedingly low in stems (Figure 3). Relatively higher expression of *MaPLD α* mRNA was detected in developing tissues compared to senescent or mature tissues in individual leaves, flower, stem, and fruit organs, respectively. The expression of *MaPLD α* mRNA in green leaves was 1.73-fold higher than that in senescent leaves, the expression in floral buds was 1.1-fold higher than that in flowers, and the expression in stems was 1.3-fold higher than that in pseudo stems. Meanwhile the expression in developing fruits at different stages was also higher than that in mature fruits.

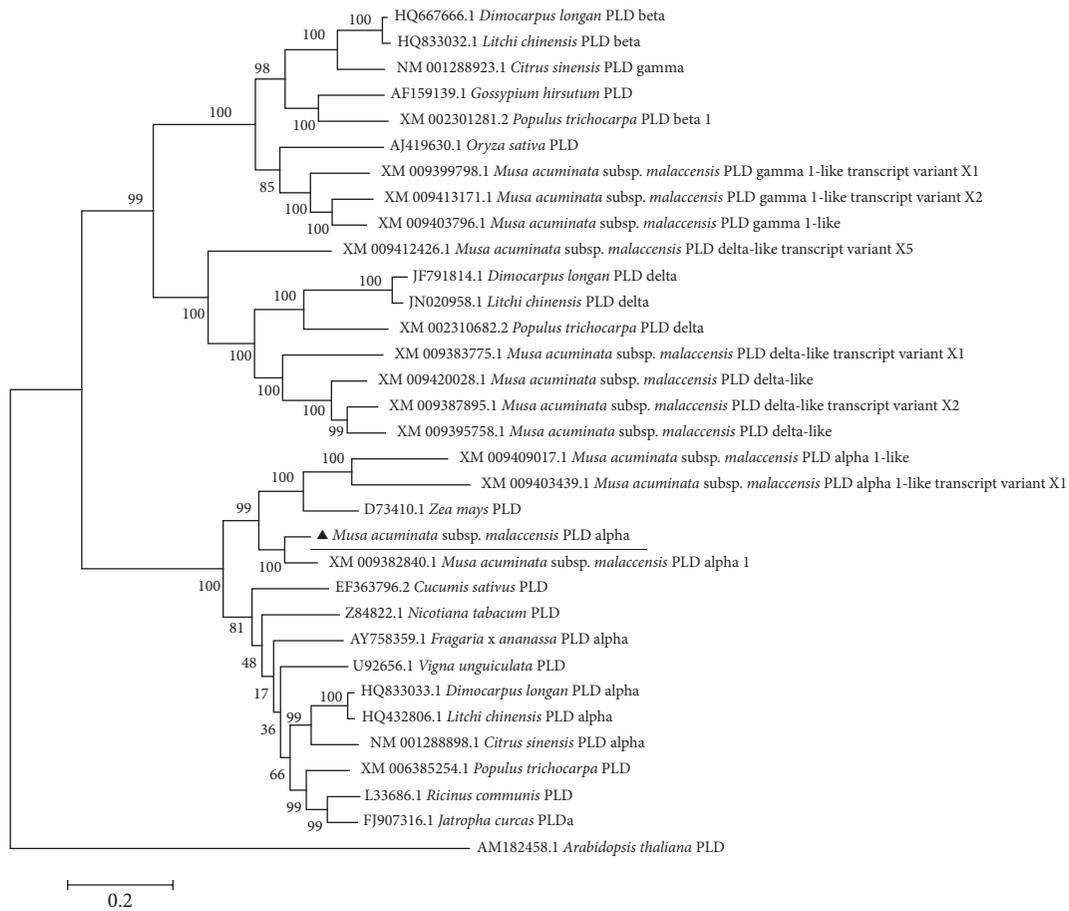


FIGURE 1: Phylogenetic analysis of MaPLD α and related PLD proteins (PLD α , PLD β , PLD γ , and PLD δ) from different plants based on alignments of amino acid sequences. The minimum evolution tree was constructed by means of MEGA 4.0. All the sequences are labeled with names and GenBank accession numbers.

3.4. Expression Analysis of the MaPLD α Gene at Different Postharvest Stages. As shown in Figure 4, PLD activities in banana fruits stored at 25 and 12°C attained a maximum on day 3 (126.71 ± 6.58 U/L) and day 25 (415.91 ± 3.47 U/L), respectively. Banana fruits stored at 12°C had relatively higher PLD activities than the fruits stored at 25°C, indicating that this enzyme was active at low temperature. The increased PLD activity might be involved in the loss of membrane function associated with ripening and senescence in banana fruits. The expression profiles of the *PLD α* gene in banana fruits stored at 25 and 12°C were investigated further by semiquantitative RT-PCR. The accumulation of *PLD α* mRNA in postharvest banana fruits at different temperatures was determined. The expression of the MaPLD α protein was found to be upregulated with the extended storage time at 25°C. From Figure 5, it reached the expression peak on day 5 (1.63-fold relative to the control sample on day 1) and then decreased on day 7 (0.51-fold relative to the highest expression on day 5). The expression of MaPLD α reached a maximum on day 7 at 12°C before decreasing to the control level (5.18-fold relative to the control sample on day 1).

4. Discussion

PLDs have been implicated in different cellular processes in plant growth, development, and stress responses. The subdivision of PLDs based on sequence alignment concomitantly produces groups of PLDs with common catalytic properties and gene structures. PLD α is the conventional plant phospholipase D, the characteristic feature of which is the necessity of millimolar Ca^{2+} for optimal activity *in vitro*. Some studies suggest that the amino acid and nucleotide sequences of *PLD β* and *PLD γ* are related more closely to each other than to *PLD α* [7]. The gene structures of *Arabidopsis PLDa*, castor bean *PLD*, and rice *PLD1* have been revealed and share the same gene architecture [29]. The MaPLD α protein is highly homologous to other known members of the PLD α family (Figure 1). This study showed that PLD α enzymes from different plant species share the same genetic lineage and may have the same catalytic and functional properties.

The predicted PLD α protein possesses three conserved domains, the C2 domain and two PLDc domains, which contain a duplicated HKD motif. The *PLD α* s cloned from eukaryotes all contain two HKD motifs [30], and they were found to be separated by approximately 321 aa in MaPLD α .

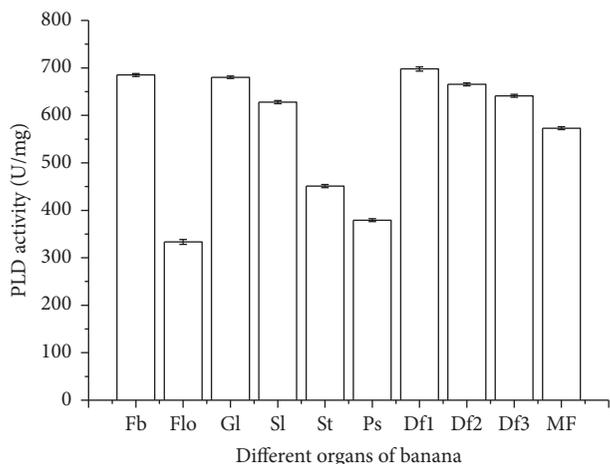


FIGURE 2: PLD activity of different banana organs, floral buds (Fb), flowers (Flo), green leaves (Gl), senescent leaves (Sl), pseudo stem (Ps), stem (St), developing fruits at the broken bud stage (Df1), 30 days after the broken bud stage (Df2), and 60 days after the broken bud stage (Df3), and mature fruits (MF).

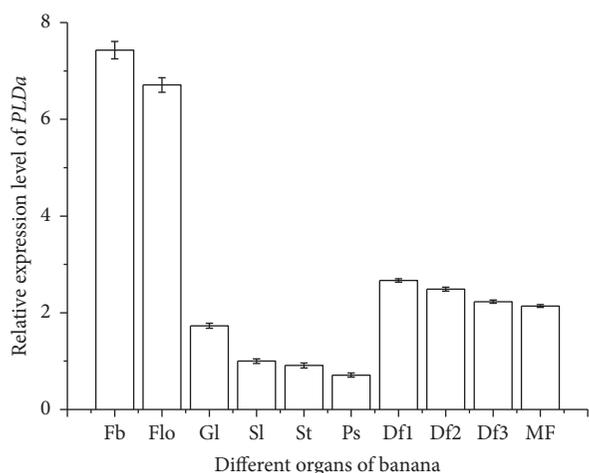


FIGURE 3: Expression of gene *MaPLDα* in different banana organs. Equivalent cDNA amounts from different banana organs, that is, floral buds (Fb), flowers (Flo), green leaves (Gl), senescent leaves (Sl), pseudo stem (Ps), stem (St), developing fruits at the broken bud stage (Df1), 30 days after the broken bud stage (Df2), and 60 days after the broken bud stage (Df3), and mature fruits (MF), were analyzed by semiquantitative PCR as described in Materials and Methods, using primers specific for *MaPLDα* and a housekeeping gene, actin, as an internal control. Expression levels are presented relative to that of senescent leaves, with a standard deviation ($n = 5$).

The absolute conservation of certain amino acid positions indicated that His, Lys, and Asp are active site residues. The necessity of these residues for PLD activity has been documented by site-specific mutagenesis in yeast PLD, and changes in one of the residues may lead to the loss of PLD activity [7]. The presence of the HKD motif is usually used to define members of the PLD superfamily. Immediately following the second HKD motif and in the middle of the highly conserved sequence IGSANINQR, there is an

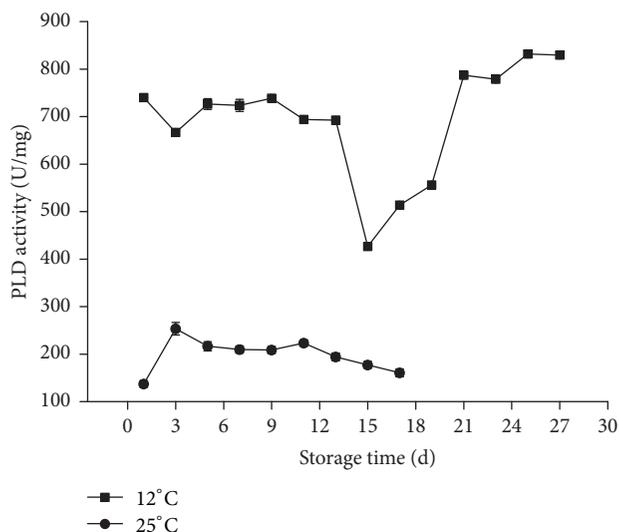


FIGURE 4: PLD activity stored for different periods at 25 or 12°C.

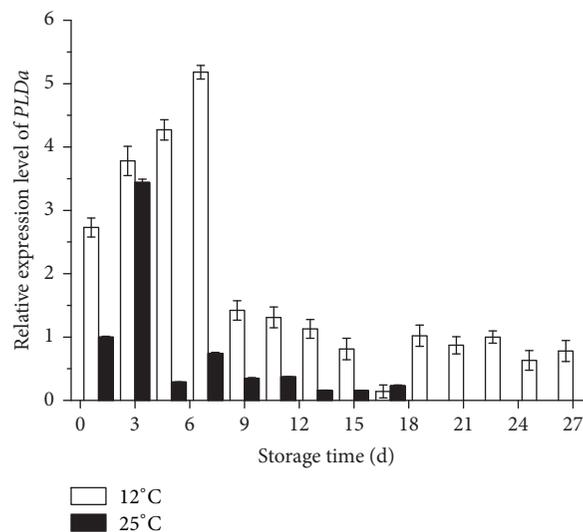


FIGURE 5: Expression of gene *MaPLDα* at different postharvest stages at 25 or 12°C. Actin (accession number AB046952) served as an internal control.

invariant serine residue. Recent structural research has led to expansion of the active site motif in the PLD family to H×K×××D×××××GS×N [31].

PLDα has long been known to be present in soluble and membrane-associated fractions, and its relative distribution between the two fractions varies depending on tissues and developmental stages [7, 13]. Centrifugal fractionation has revealed that most of PLDα in young castor bean leaves is soluble, whereas the bulk of PLDα in mature leaves is associated with microsomal membranes [32]. PLD promoter in vegetative tissues is highly active in the rapidly growing regions such as shoot apices and the secondary meristem producing axillary buds and vascular tissues of young leaves and stems [7]. In banana, the appearance of *PLDα* variants is associated with developmental stages and stress conditions

[16]. In this study, relatively higher PLD activity was detected in developing tissues compared to senescent or mature tissues in individual leaves, flower, stem, and fruit organs, respectively (Figure 2). A similar pattern with respect to expression of *MaPLD α* mRNA was observed by semi-quantitative RT-PCR. The level of *MaPLD α* mRNA expression was found to be higher in developing tissues like floral buds, young leaves, stem, and developing fruits than that in senescent tissues like senescent flowers, old leaves, pseudo stems, and mature fruits (Figure 3). Similar circumstance was found in other plants; for example, the expression level was higher in young leaves than that in old leaves in *Arabidopsis* [7]. PLD expression and activity are intimately linked to ripening and senescence. The activity PLD was high in expanding tissues with high biosynthetic activity, supporting its possible role in either lipid biosynthesis or the regulation of signals necessary for the formation of new tissues. In this study, PLD activity correlated well with gene expression of *MaPLD α* (Figures 2 and 3). Promoter and RNA analyses discussed earlier have indicated that gene expression performs an important function in regulating PLD activity [13].

The PLD-mediated lipid degradation has been proposed to play a role in membrane degradation in tissue senescence. Increased PLD activity and PA formation have been observed with several systems including tomato fruits, cabbage leaves, and ageing seeds [17]. The pattern of *PLD α* gene expression actually argues against a general role of the most common PLD as a promoter of natural plant senescence [7]. The *PLD α* promoter activity is higher in metabolically active tissues, such as meristematic and newly divided cells, than in mature and senescent ones. *PLD α* participates in the senescence of postharvest banana fruits, and PLD activity and its mRNA expression showed an increasing trend during the process, thereby leading to the damage of cell membrane integrity and postharvest fruit quality (Figures 4 and 5). PLD activity and expression of *MaPLD α* in banana fruits stored at 25°C were significantly higher than at 12°C, indicating that low temperature may somewhat activate the expression of *PLD α* of banana fruits. During earlier storage time, there is an increase in PLD expression, leading to the increased accumulation of transcripts, the increased PLD protein levels, and a higher PLD activity [33]. *PLD α* is inhibited by G_{α} proteins that produce important secondary messengers in plants: abscisic acid (ABA) involved in phytohormone signal transduction pathways [34]. Suppression of *PLD α* in *Arabidopsis* increases the period during which ABA and ethylene promote senescence [35], and similar studies have also revealed a role of this enzyme in plant responses to abiotic stress. The function of ethylene in banana ripening is well documented; thus, manipulation of *PLD α* may maintain postharvest quality and extend storage life. Further research is needed to genetically and physiologically characterize *PLD α* in banana and to gain a better understanding of its function and relation with environmental stress. Additionally, the application of molecular biology on inhibiting PLD activity and expression of *PLD α* in banana also needs further investigation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Responses of Phospholipase D and Antioxidant System to Mechanical Wounding in Postharvest Banana Fruits

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Banana fruits are susceptible to mechanical damage. The present study was to investigate the responses of phospholipase D (PLD) and antioxidant system to mechanical wounding in postharvest banana fruits. During 16 d storage at 25°C and 90% relative humidity, PLD activity in wounded fruits was significantly higher than that in control (without artificial wounding fruits). The higher value of *PLD* mRNA was found in wounded fruits than in control. *PLD* mRNA expression reached the highest peak on day 4 in both groups, but it was 2.67 times in wounded fruits compared to control at that time, indicating that *PLD* gene expression was activated in response to wounding stress. In response to wounding stress, the higher lipoxygenase (LOX) activity was observed and malondialdehyde (MDA) production was accelerated. The activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) in wounded fruits were significantly higher than those in control. The concentrations of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in fruits increased under mechanical wounding. The above results provided a basis for further investigating the mechanism of postharvest banana fruits adapting to environmental stress.

1. Introduction

Mechanical damage is the main cause for losses in postharvest horticultural products [1, 2]. Wounds represent vulnerable points that may lead to severe damage and compromise organ survival rate. Membrane deterioration is an early and characteristic feature in plant cells undergoing mechanical injuries [3]. Membrane integrity loss is often associated with lipid peroxidation or phospholipid degradation. The increased lipid peroxidation, mediated and sustained by phospholipid-degrading enzymes such as phospholipase D (PLD) and lipoxygenase (LOX), results in membrane integrity loss, which has been noted in senescing petal tissues [4]. PLD and LOX in plants play important roles in phospholipid catabolism, initiating lipolytic cascade in membrane deterioration during senescence and stress [5, 6]. LOX, encoding

a lipoxygenase involved in jasmonate (wounding-induced signaling molecule) synthesis, is transcriptionally upregulated in response to wounding in *Arabidopsis* [7]. It has been proposed that the liberated polyunsaturated fatty acids serve as substrates for LOX that produces activated oxygen and lipid peroxides leading to membrane damage [8]. PLD has been proposed to play several roles in wounding response. The increased PLD-mediated hydrolysis occurs in response to various stress conditions such as frost, senescence, and wounding [9]. Plants accumulate phosphatidic acid (PA) and unesterified fatty acids that are released from lipids, presumably by the action of wound-inducible phospholipases of types D after wounding [7, 10]. The wounding-induced activation of PLD may result from translocation of PLD to the membrane, which is mediated by an increase in cytoplasmic calcium and stimulated by low micromolar calcium levels

[11, 12]. In castor bean leaves, it has been reported that free fatty acid quantity and PLD activity increase with wounding. Increases in membrane-associated PLD and LOX have been observed in response to mechanical wounding in postharvest cucumber fruits [8].

A wound signal originates at injury site and propagates into adjacent tissue where it induces a number of physiological responses, including lipid degradation, peroxidation compound synthesis and accumulation, and subsequent tissue browning [13]. The oxylipin pathway is involved in wound responses in a number of plants. Antioxidant enzymes are critical in inhibiting oxidative stress. When reactive oxygen species (ROS) increases, chain reactions start in which superoxide dismutase (SOD) catalyzes the dismutation of superoxide radical ($O_2^{\bullet-}$) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). H_2O_2 is then detoxified by catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) [14]. CAT reduces H_2O_2 into water and O_2 , whereas POD decomposes H_2O_2 by oxidation of cosubstrate such as phenolic compounds [15].

Banana (*Musa acuminata* L.) is one of the major commercial fruit crops grown in tropics and subtropics. It is very important to the economy of developing countries [16]. Banana fruits are very susceptible to mechanical damage during harvesting, handling, packaging, and transportation, which results in a substantial reduction in quality. Mechanical wounding significantly reduces the commercial value of banana fruits [17]. Lipid degradation and peroxidation activities are directly involved in natural and induced senescence and mechanical wounding. With regard to harvested banana fruits, it is important to discern potential roles of lipid degradation and antioxidant enzymes in association with mechanical wounding. The aim of this research was to explore the role of PLD, LOX, and oxidative stress in banana fruits subjected to artificial wounding and senescence processes. Molecular characterization of PLD in response to wounds and senescence was also analyzed. These innovative results will provide a scientific basis for further investigating the mechanism of postharvest banana adapting to environmental stress.

2. Materials and Methods

2.1. Plant Materials and Postharvest Treatments. Banana fruits were collected from a commercial orchard in Nanning of Guangxi province during July 2016 and transported into a laboratory in Guangxi Academy of Agricultural Sciences immediately. The fruits without infection and physical injury were chosen as plant materials at similar size and same mature period. They were randomly divided into wounded and control groups (100 fruits in each group). In wounded group, banana fruits were punctured at 3 cm intervals from the calyx to the stalk end by a 10 mm diameter stainless steel puncher. The fruits which were not punctured were used as control group. Both groups were packed into polyethylene bag (0.03 mm thick) and stored at 25°C and 90% relative humidity (RH). Every 6 fruits were taken for the determination every 2 d after wounding.

2.2. PLD Activity. PLD activity was assayed by means of highly specific and sensitive sandwich enzyme immunoassay technique (ELISA) [18]. The 96-well ELISA plates (Nunc, Wiesbaden, Germany) were coated with the purified plant PLD antibody (100 μ L per well, 2 mg/mL diluted 1:1000 in PBS, 4°C, 8 h, pH 7.2–7.4). Plates were then blocked with 1% BSA in PBS for 16 h at 37°C. The wells were then washed twice with 200 μ L of wash buffer. A series of PLD standards were prepared in range 10–120 U/L in PBS/0.1% BSA. Samples were also prepared in a series of dilutions from 1/5 to 1/50 in PBS. A total of 50 μ L of standards and samples were added and incubated for 2 h at 37°C. The wells were then washed twice with 100 μ L of wash buffer. Conjugate binding was performed by adding a biotin-conjugated antibody specific for PLD (100 μ L of 0.1% conjugate antibody in 0.1% BSA/PBS). After being incubated for 60 min at 37°C, the plates were washed three times with wash buffer and three times with 100 μ L of carbonate buffer. Horseradish Peroxidase (HRP) (100 μ L of 15 mg HRP in 15 mL of carbonate buffer) was added and incubated for 10 min at 37°C. The absorbance of the plates was then read at 450 nm on an ultraviolet (UV) microplate reader (Bio-Rad Laboratories, Hercules, California, USA).

2.3. Semiquantitative RT-PCR Analysis. Total RNAs were extracted from 3 g of fresh banana pericarp tissues using CTAB method [19]. Total RNA samples were then dissolved in 50 μ L of RNase free H_2O and stored at 80°C prior to RT-PCR. Total RNA (5 μ g) was used for the cDNA synthesis with the Reverse Transcription System A3500 (Promega, Madison, WI, USA) according to the protocol provided by the manufacturer. The fragment of *PLD* cDNA was cloned by RT-PCR. PCR was carried out with an initial heat action step at 94°C for 6 min, and amplifications were achieved through 35 cycles at 94°C for 30 s, 53°C for 30 s, and 72°C for 90 s. A final extension reaction was carried out for 6 min at 72°C. The resulting PCR product was isolated, cloned, and sequenced (Invitrogen, Shanghai, China).

The mRNA expression patterns of *PLD* gene were examined by semiquantitative RT-PCR. The house-keeping gene *Actin* (GenBank accession number AB046952) was used as an internal control. Protocols for total RNA extraction and synthesis of cDNA were described as above. Gene specific primers for *PLD* (*PLD*-S3: 5'-GAAATCGGGAGGTCA-AGAAGAG-3'; *PLD*-A3: 5'-CTAAGTTGTGAGGATTGG-AGG-3') and *Actin* (forward: 5'-GATTCTGGTGATGGT-GTGAGT-3'; reverse: 5'-GACAATTTCCCTTAGCAG-3') were used in RT-PCR. PCR was carried out with an initial heat action step at 94°C for 5 min, and amplifications were achieved through 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. The amplified products (10 μ L) were analyzed by 1% agarose gel electrophoresis [19].

2.4. LOX Activity. The frozen banana pericarp tissues (5 g) were ground finely in liquid nitrogen and then homogenized in 15 mL of 50 mM phosphate buffer (pH 7). After centrifugation at 10,000 \times g for 20 min at 4°C, the supernatant was collected and then used as crude enzyme extract. LOX

activity was assayed by monitoring the formation of conjugated dienes from linoleic acid at 25°C and 234 nm [20]. A total of 3 mL of reaction mixture contained 50 mM sodium phosphate buffer (2.8 mL, pH 7), 10 mM sodium linoleic acid solution (0.1 mL), and crude enzyme solution (0.1 mL). One unit of LOX activity was defined as a change of 0.01 in absorbance per minute at 25°C.

2.5. Activities of Antioxidative Enzymes. CAT and POD were extracted and assayed according to the modified methods of Oracz et al. [21]. Banana pericarp tissues (0.5 g) were extracted for 10 min with 2.5 mL of 0.1 M sodium phosphate buffer (pH 7) at 4°C. The extract solution was centrifuged for 15 min at 12,000 ×g. The supernatant was collected as enzyme solution for the determinations of enzymatic activities. The assay mixture (3 mL) for determining CAT activity consisted of 2.8 mL of 15 mM H₂O₂ prepared by 0.05 M sodium phosphate buffer (pH 7.8) and 0.2 mL of enzyme solution. The increase in absorbance at 240 nm was recorded for 3 min at 25°C. The assay mixture (3 mL) for determining POD activity consisted of 2.5 mL of 0.1 M sodium phosphate buffer (pH 7), 0.2 mL of 0.46% (v/v) H₂O₂, 0.2 mL of 4% (v/v) guaiacol, and 0.1 mL of enzyme solution. The increase in absorbance at 470 nm was recorded for 3 min at 25°C.

SOD was extracted and assayed according to the modified methods of Sun et al. [22]. Banana pulp tissues (0.5 g) were extracted for 10 min at 4°C with 2.5 mL of 0.05 M sodium phosphate buffer (pH 7.8) containing 0.1% (w/v) polyvinyl pyrrolidone. The extract solution was centrifuged for 20 min at 12,000 ×g. The supernatant was collected for analyzing SOD activity. SOD activity was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). A total of 0.05 mL of enzyme solution was added to 3 mL of assay reagent consisting of 13 mM methionine, 63 μM NBT, 100 μM EDTA, and 1.3 μM riboflavin in 0.05 M sodium phosphate buffer (pH 7.8). The reaction solutions were incubated for 10 min under 4,000 lux illumination. The absorbance of sample was measured at 560 nm and 0.05 M sodium phosphate buffer (pH 7.8) was used as control.

The assay for APX activity was determined according to the modified method of Nakano and Asada [23]. APX activity consisted of 0.1 mM EDTA-Na₂, 0.5 mM ascorbic acid, and 0.1 mM H₂O₂ (above-mentioned reagents were prepared in 0.05 M sodium phosphate buffer, pH 7) and 0.1 mL of enzyme solution. The activity was recorded as the decrease in absorbance at 290 nm. One enzyme unit was defined as 1 mol of ascorbic acid oxidized per minute at 290 nm.

2.6. MDA Content. Malondialdehyde (MDA) content was measured according to the reported method of Sun et al. with a slight modification. Banana pulp tissues (3 g) were homogenized with 15 mL of 10% trichloroacetic acid and centrifuged at 15,000 ×g for 20 min [22]. One milliliter of supernatant was mixed with 3 mL of 0.5% 2-thiobarbituric acid, heated at 95°C for 20 min, and then immediately cooled in an ice-water bath. The absorbance was measured at 532 nm after centrifugation at 3,000 ×g for 10 min and the value for

nonspecific absorbance 600 nm was subtracted. The amount of MDA was estimated as follows: (μM/g FW) = [6.45 (OD₅₃₂ - OD₆₀₀) - 0.56 OD₄₅₀] × 5 mL/0.25 g.

2.7. Superoxide Anion Content. Superoxide radical (O₂^{•-}) was determined by the conversion of nitro blue tetrazolium (NBT) staining according to Dunand et al. [24]. The banana powder (1 g) was vacuum-infiltrated with 10 mM NaN₃ in 10 mM potassium phosphate buffer (pH 7.8) for 1 min and incubated in 1 mg/mL NBT (in 10 mM potassium phosphate buffer, pH 7.8) for 20 min in the dark at room temperature.

2.8. H₂O₂ Content. H₂O₂ levels were determined according to the modified method of Velikova et al. [25]. The banana powder (0.5 g) was homogenized for 10 min in 2.5 mL of cold acetone. The homogenate was centrifuged for 15 min at 12,000 ×g. A total of 1 mL of supernatant was added to 0.1 mL of 20% (v/v) titanium tetrachloride (TiCl₄) and 0.2 mL of ammonia water. After centrifuging for 15 min at 12,000 ×g, the precipitate was collected and dissolved in 3 mL of 1 M H₂SO₄ and centrifuged for 5 min at 6,000 ×g. The absorbance of the supernatant was measured at 412 nm. The content of H₂O₂ was calculated from a standard curve. The increasing rate of H₂O₂ content (%) = [(C_i - C₀)/C₀] × 100, where C_i indicated H₂O₂ content in banana fruits storing for different times, while C₀ was H₂O₂ content in banana fruits storing for 0 d.

2.9. Statistical Analysis. Experiments were arranged in a completely randomized design. All statistical analyses were performed by variance (ANOVA) analysis using Statistix version 8.0 (Analytical Software, Tallahassee, FL, USA). Treatment means were compared by Fisher's Least Significant Difference (LSD) test at a significance level of *P* < 0.05.

3. Results

3.1. Phospholipase D Activation and PLD Gene Expression in Postharvest Banana Fruits under Mechanical Wounding. PLD activity was correlated with mechanical wounding. From Figure 1, at 25°C and 90% RH, banana PLD activity demonstrated a rapid increase tendency in both control and wounded fruits with extended storage time and attained maximum on day 2 and day 4, respectively. PLD activity in wounded fruits was significantly higher (*P* < 0.05) than that in control throughout the entire storage period. After day 4, PLD activity increased 2-fold in wounded fruits while increasing only 1.6-fold in control. The accumulation of banana PLD mRNA in response to mechanical wounding was further investigated (Figure 2). Similar to PLD activity profile, PLD expression was found to be upregulated with the extended storage and reached the highest expression peak on day 4 in control (1.48 times the control on day 0) and then decreased on day 6. In wounded fruits, PLD expression also attained a maximum on day 4 (3.14 times to control on day 0). The higher value (3.95) of PLD mRNA was found in wounded fruits than that in control, and PLD mRNA expression in wounded fruits was 2.67 times that in control on day 4, indicating that PLD gene expression was

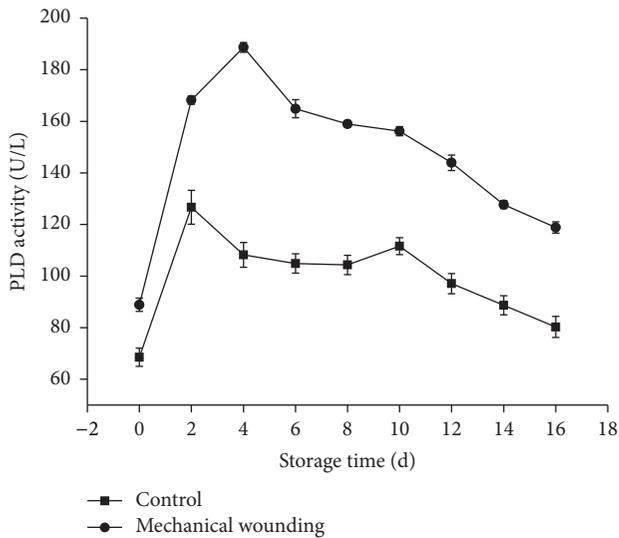


FIGURE 1: PLD activity in banana fruits dealing with mechanical wounding.

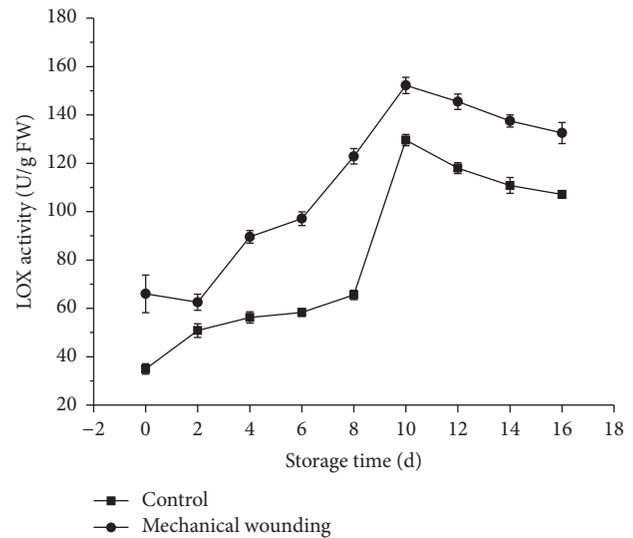


FIGURE 3: LOX activity in banana fruits dealing with mechanical wounding.

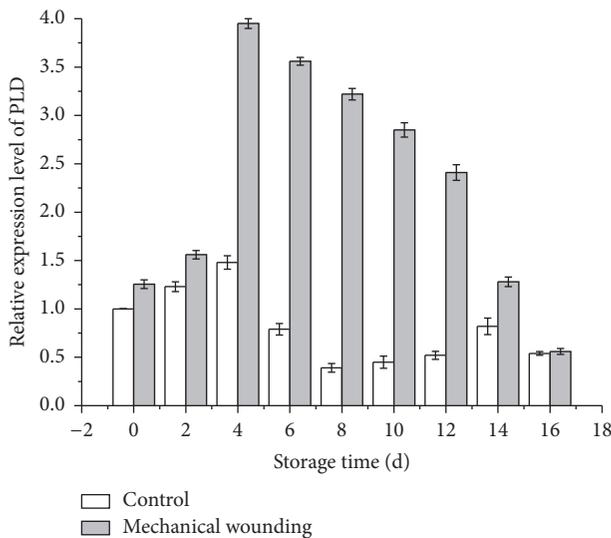


FIGURE 2: Expression analysis of *MaPLD* gene in banana fruits dealing with mechanical wounding.

activated by wounding. This was similar to the expression pattern found in lettuce [13]. These results suggested that PLD could be involved in producing wound signal and PLD activity in wounded fruits that were associated with the mRNA expression. PLD induced by wounding might stimulate corresponding physiological reactions related to fruit deterioration and senescence through increasing gene expression.

3.2. LOX Activity in Postharvest Banana Fruits under Mechanical Wounding. LOX is a key enzyme in phospholipid

catabolism of plants, initiating a lipolytic cascade in membrane deterioration during senescence and stress. From Figure 3, at 25°C and 90% RH, LOX activity from banana fruits increased first and then decreased during 16 d storage and attained maximum on day 10 in both control and wounded fruits. Much higher LOX activity was observed in wounded fruits than that in control throughout the entire storage period. Similar results were obtained by Zhao et al. who reported that LOX might be the main hydrolytic enzymes of phospholipids in response to mechanical wounding in postharvest cucumber fruits [8].

3.3. Activities of Antioxidative Enzymes in Postharvest Banana Fruits under Mechanical Wounding. The activities of antioxidative enzymes SOD, CAT, POD, and APX in postharvest banana fruits were shown in Figure 4. From Figure 4(a), at 25°C and 90% RH, SOD activity rose rapidly during 16 d storage. It was higher in wounded fruits than that in control, but there was no statistically significant difference ($P > 0.05$) on day 4. CAT activity in control showed a peak on day 14 and then declined (Figure 4(b)). However, CAT activity in wounded fruits demonstrated a rapid increasing tendency throughout the entire storage period and was significantly higher ($P < 0.05$) than that in control on days 2, 4, 6, 8, and 16. POD activity exhibited increasing trends in control and wounded fruits (Figure 4(c)). POD activity in wounded fruits was significantly higher ($P < 0.05$) than that in control during 16 d storage. APX activity was higher in wounded fruits than that in control during 16 d storage. This enzymatic activity presented a peak on day 12 in control. However, it still increased and significantly higher values were found in wounded fruits on days 14 and 16.

3.4. MDA Content in Postharvest Banana Fruits under Mechanical Wounding. MDA is an indicator to assess fruit

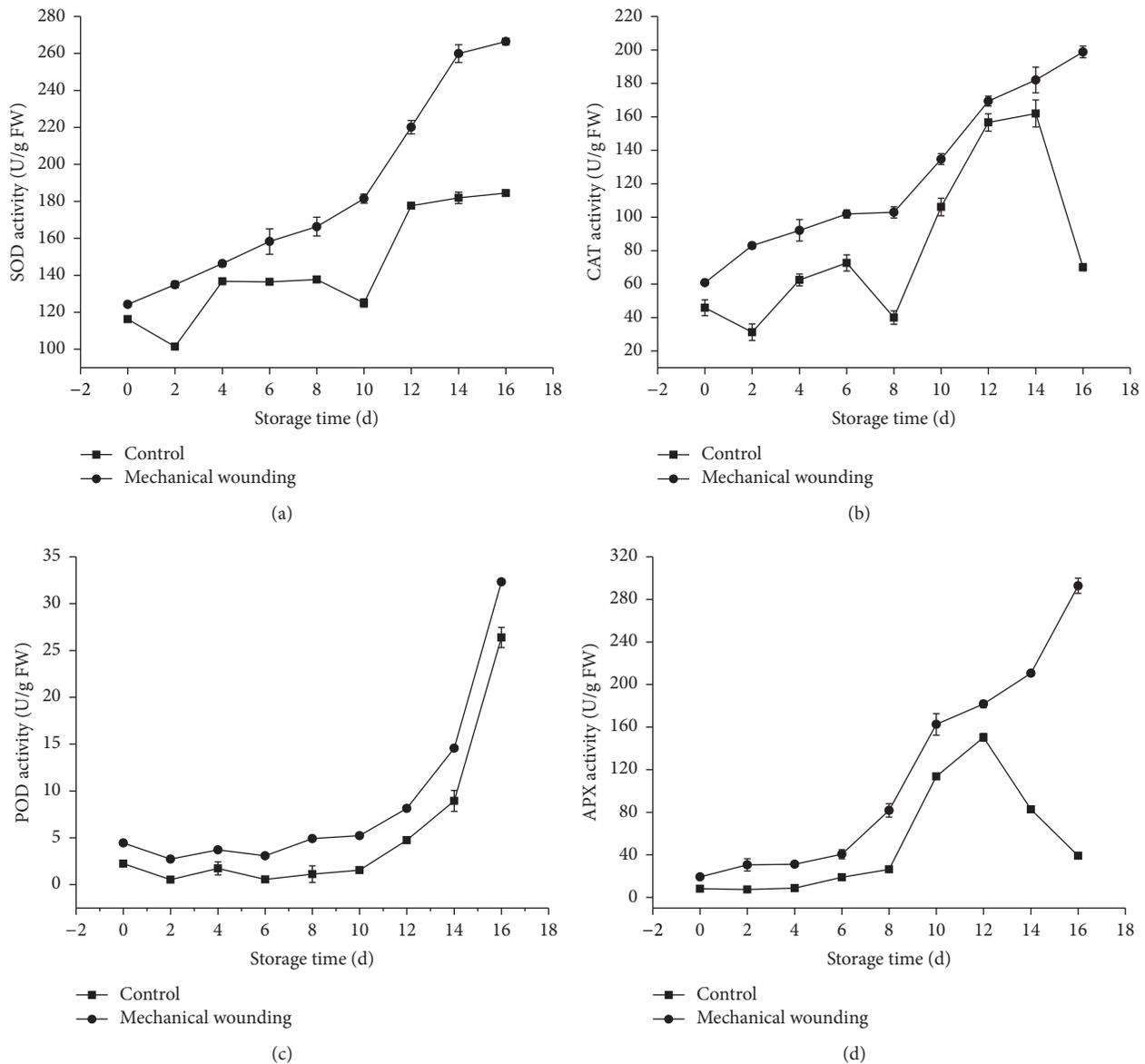


FIGURE 4: Activities of antioxidant enzymes SOD (a), CAT (b), POD (c), and APX (d) in banana fruits dealing with mechanical wounding.

damage progress, which represents the level of lipid peroxides and the structural integrity of cell membranes [26]. MDA level change is considered to be a marker for membrane lipid peroxidation of plants subjected to senescence or stress. From Figure 5, at 25°C and 90% RH, a continuous increase of MDA content was observed in both control and wounded fruits with extended storage time. MDA in wounded fruits was significantly higher ($P < 0.05$) than that in control during 16 d storage, indicating that mechanical wounding accelerated MDA accumulation in postharvest banana fruits.

3.5. Changes of $O_2^{\bullet-}$ and H_2O_2 Content in Postharvest Banana Fruits under Mechanical Wounding. Superoxide radical and H_2O_2 production in postharvest banana fruits response to

mechanical wounding were investigated. Generally speaking, the contents of $O_2^{\bullet-}$ and H_2O_2 in control and wounded fruits exhibited ascending first and then descending trends (Figure 6) when storing fruits for 16 days at 25°C and 90% RH. From Figure 6(a), $O_2^{\bullet-}$ content in wounded fruits was significantly higher ($P < 0.05$) than that in control. It reached the highest peak on day 4 in control but continually rose to maximums on day 6 in wounded fruits. $O_2^{\bullet-}$ level in wounded fruits was about 5-fold higher than that in control on day 16. From Figure 6(b), H_2O_2 content in wounded fruits showed significantly higher ($P < 0.05$) level than that in control throughout the entire storage period. It reached the highest peak on day 4 in both groups. The above results suggested that H_2O_2 could significantly increase the production rate of $O_2^{\bullet-}$ in response to wounding stress.

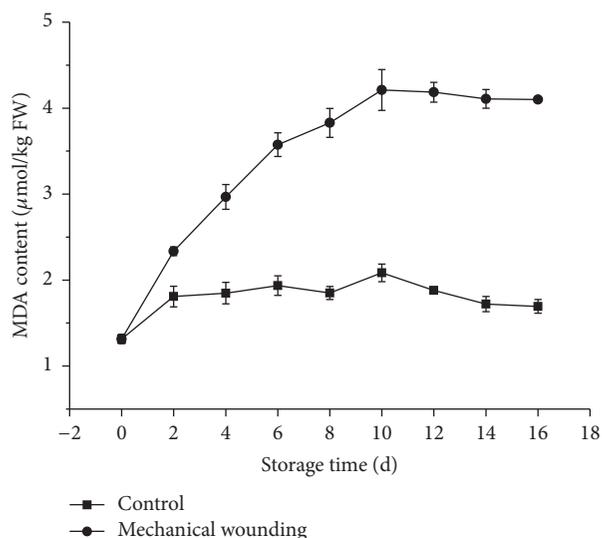


FIGURE 5: MDA content in banana fruits dealing with mechanical wounding.

4. Discussion

Lipase and PLD are very important enzymes involved in membrane phospholipid hydrolysis [27–29]. The activities of PLD and LOX not only have impacts on cellular membrane structure and stability but also play pivotal roles in regulating many critical cellular functions, including cellular signal transduction, vesicle trafficking, cell proliferation, hormone action, cellular backbone formation, seed germination, senescence, and defense responses [9, 30]. A direct correlation was found between lipid peroxidation and phospholipid hydrolysis [3]. PLD and LOX are proposed to initiate lipolytic cascades in membrane deterioration in response to senescence and environmental stress [11]. It is proposed that PLD initiates a lipolytic cascade in membrane deterioration [27]. The resultant phospholipid degradation products could be further catalyzed by LOX to produce activated oxygen and lipid peroxides, which lead to cellular compartmentalization and membrane damage [31]. In this study, the activities of PLD and LOX in wounded banana fruits were significantly higher ($P < 0.05$) than those in control throughout the whole storage period (Figures 1 and 3). The higher *PLD* gene expression accompanying higher PLD activity was observed in wounded fruits (Figure 2), and *PLD* gene is involved in the defense and repair process in the early damage and its effect reduced or is replaced after 4 days. Similar results were reported by Sang et al. [32], who found wounding increased the level of expression of the *PLDα* gene after stress in cucumbers. PLD and LOX induced by wounding might stimulate corresponding physiological reactions related to deterioration and senescence through increasing gene expression. Some evidences reported that the wounding-induced activation of PLD could result from PLD translocation to membrane, which is mediated by an increase in cytoplasmic calcium and stimulated by low micromolar calcium levels [10, 12]. In addition, PLD and PA promote

the production of superoxide [33, 34], a reactive oxygen species that is involved in various defense responses. PLD and its catalyzed products, phosphatidic acid and linolenic acid, initiate the oxylipin pathway and cellular signal transduction, which may be involved in producing the wound signal responsible for increased wound-induced activity, accumulation of phenolics, and enzymatic browning in plant tissues [6].

Membrane lipid degradation is an essential feature of signal transduction pathways that occur in response to wounding stress. Antioxidant defense systems can reduce membrane lipid peroxidation and protect plants from ROS damage. Among antioxidant systems, SOD can scavenge $O_2^{\bullet-}$ radicals into H_2O_2 , while H_2O_2 is further converted into water by CAT and APX [15]. In this study, the increasing rate of H_2O_2 and $O_2^{\bullet-}$ (major ROS) contents in wounded banana fruits exhibited ascending trend before day 4 (Figure 6), which suggested that the equilibrium between production and scavenging of H_2O_2 and $O_2^{\bullet-}$ was disturbed, and H_2O_2 and $O_2^{\bullet-}$ in banana fruits were largely produced during 4 d storage. Lipid peroxidation product MDA resulting from ROS activity was significantly higher ($P < 0.05$) than that in control (Figure 5). Although overproduced H_2O_2 was synchronously scavenged through chain reactions catalyzed by a series of antioxidant enzymes (such as SOD, CAT, POD, and APX) (Figure 4), H_2O_2 accumulation was more than its breakdown before 4 d storage. After day 4, the accumulation of H_2O_2 and $O_2^{\bullet-}$ slowed down because of the enhanced scavenging effects of antioxidant enzymes (Figure 6). The results indicated that antioxidant enzymes could scavenge overproduced ROS in banana fruits, retard peroxidation of membrane lipids, inhibit the loss of membrane function, and therefore control senescence of the fruits during storage. The overproduced ROS during storage of wounded banana fruits caused lipid peroxidation, induced membrane injury, destroyed membrane integrity, and resulted in cell senescence. The equilibrium between production and scavenging of ROS in banana fruits may be disturbed by mechanical wounding during storage and thereby it incurs fruit senescence.

Considering the above analysis, the biochemical characters (i.e., membrane-associated lipolytic enzymes such as PLD and LOX, ROS, and antioxidant enzymes) are related to the wounding stress. PLD plays a role in elicitor-induced production of ROS and is involved in signaling pathways in response to wounding stresses [35, 36]. PLD associated with membrane and soluble fractions had opposite behaviors in wound response. Wounding studies performed on *Arabidopsis* leaves showed that PLD was differently affected by wounding and transcript changes were observed after a few hours [37]. Further study is needed to genetically and physiologically characterize different PLD in banana fruits and the signaling pathway of the PLD so as to gain a better understanding on their function under environmental stress.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

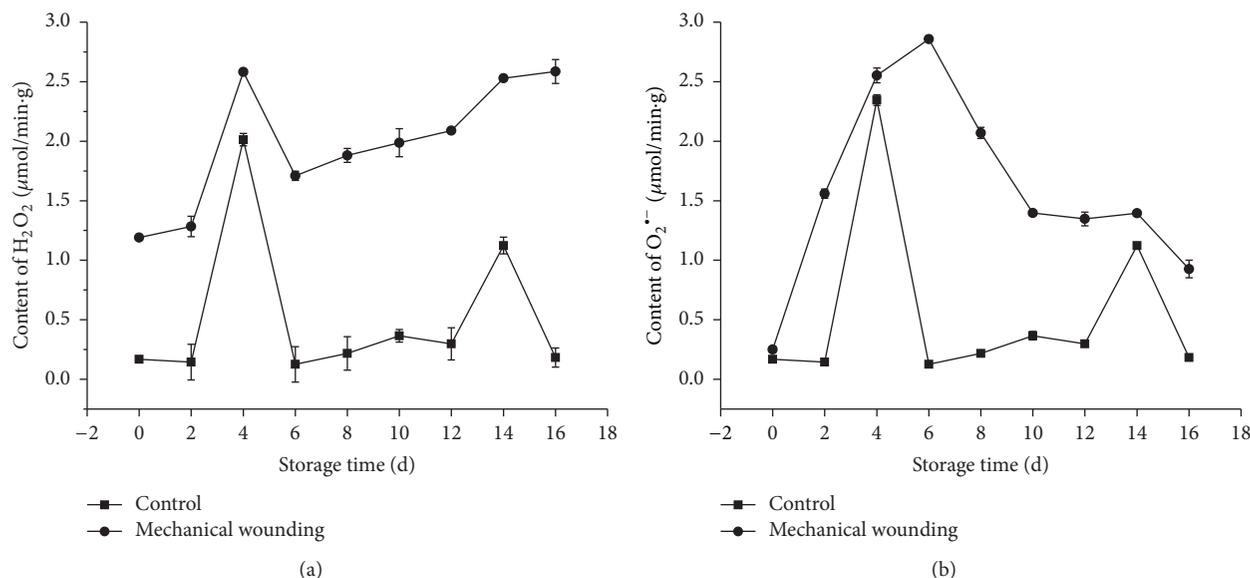


FIGURE 6: Changes of H₂O₂ (a) and O₂^{•-} (b) content in banana fruits dealing with mechanical wounding.

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