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Hydrogen peroxide reduced ATPase activity and the levels of ATP, ADP, and energy charge and its association with pulp breakdown occurrence of longan fruit during storage

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ABSTRACT

The effects of hydrogen peroxide (H_2O_2) on the contents of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP), the level of energy charge, and the activity of adenosine triphosphatase (ATPase) in pulp of harvested longan fruit, and its association with longan pulp breakdown occurrence were studied. The results showed that, compared to the control longans, H_2O_2-treated longans exhibited a higher index of pulp breakdown, a higher amount of AMP, but lower levels of ATP, ADP and energy charge. H_2O_2-treated longans also exhibited lower activities of Mg^{2+}-ATPase, Ca^{2+}-ATPase, and H^+-ATPase in mitochondrial membrane, vacuolar membrane, and plasma membrane as compared to the control longans. Above findings demonstrated that H_2O_2 caused longan pulp breakdown by depleting energy and lowering the ATPase activity, indicating H_2O_2-induced pulp breakdown in harvested longan fruit was due to energy deficit.

1. Introduction

Longan (*Dimocarpus longan* Lour.) is cultivated commercially in tropical and subtropical regions including China, Pakistan, India, Vietnam, Thailand, Australia, South Africa, and many other countries (Lin, Lin, Lin, Lin, Ritenour, et al., 2019). Longan is a medicine and food dual-purpose fruit containing different nutrients such as polysaccharides and vitamins (Lin, Chen, et al., 2018; Lin et al., 2013; Lin, Lin, Lin, et al., 2016; Lin, Lin, Lin, Lin, Chen, et al., 2019). However, longan is a highly perishable fruit that deteriorates easily. Quality deterioration of harvested longan fruit include pericarp browning and pulp breakdown, which can reduce appearance quality and edible quality, as well as decrease the commodity value of longan fruit (Wang et al., 2018; Zhang, Lin, et al., 2017). Typical symptoms of pulp breakdown in longans are rapid softening, erosion, poor aroma and flavor (Lin, Lin, Chen, et al., 2019; Lin, Lin, Lin, Lin, Chen, et al., 2019; Lin, Lin, Lin, Lin, Ritenour, et al., 2019). Thus, in order to find a good solution to preserve postharvest longans, it is of paramount importance to understand the mechanism of pulp breakdown of longans.

Energy metabolism is one of the principal metabolic activities in harvested crops (Jin, Zhu, Wang, Shan, & Zheng, 2014; Zhang et al., 2019). Energy metabolism intensity, evaluating by cell energy level and adenosine triphosphatase (ATPase) activity, is involved in the senescence and the storability of postharvest crops (Chen et al., 2014, 2020; Chen, Lin, et al., 2018; Li, Limwachiranon, Li, Du, & Luo, 2016; Zhang et al., 2019; Zhang, Lin, et al., 2017). Researcher illustrated that a low energy level and weak ATPase activity in plant cell could accelerate the senescence and the loss of storability of harvested crops such as bananas (Wang, Luo, Khan, Mao, & Ying, 2015), peaches (Jin et al., 2014; Zhao et al., 2019), litchis (Yi et al., 2008; Zhang, Hu, et al., 2017), and broccoli (Li et al., 2017). Moreover, increasing evidence revealed that the level of reactive oxygen species (ROS) in plant cell played a key role in energy metabolism, which influenced the cell energy level, and eventually effected the senescence and shelf life of postharvest crops (Lin et al., 2014, 2015; Lin, Chen, et al., 2017; Lin, Lin, et al., 2017; Liu, Luo, Zeng, Jiang, & Tang, 2016). Furthermore, previous studies found that the senescence and the loss of storability in postharvest fruit were associated with physiological disorder caused by multifarious adverse

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situation. It would result in the structural and functional damages of cell membrane and organelles (e.g., plasma, vacuolar, and mitochondrial), ultimately, led to an energy deficit in plant cell (Aghdam et al., 2019; Chen, Lin, et al., 2018; Lin, Lin, et al., 2018). During storage, the activities of Mg^{2+}-ATPase, Ca^{2+}-ATPase, H^+-ATPase and other energy metabolism enzymes can influence the level of cellular energy, resulting in a decrease of cellular energy level and the limitation of energy application, which are the major causes for the senescence and the loss storability of harvested crops (Chen, Lin, et al., 2018; Li et al., 2018; Lin, Lin, et al., 2018). Accordingly, it has practical and theoretical significance to study the mechanism of energy metabolism and its relation to postharvest longan pulp breakdown, and to explore available techniques to regulate pulp breakdown for extending the shelf life and increasing the storability of postharvest longans.

Accumulating studies have reported that ROS plays a significant role in regulating senescence and storability of harvested fruits (Lin et al., 2014, 2015; Lin, Chen, et al., 2017). Hydrogen peroxide (H_2O_2) is a type of ROS in the fruit tissue (Lin et al., 2014; Lin, Lin, Chen, et al., 2019; Lin et al., 2020; Liu et al., 2019). Low content of ROS, such as H_2O_2, can be employed as signal transduction molecule avoiding oxidative damage, cell procedural death, and the adjustment of growth and development in fruit tissue (Tian, Qin, & Li, 2013). Nevertheless, once ROS level outnumber a normal verge value, excessive ROS will promote the senescence and decrease the storability of postharvest fruit via causing membrane lipid oxidation, damaging cellular structure and mitochondrial dysfunction, and accelerating cell death (Tian et al., 2013). Moreover, our previous research indicated that the 1.96 mmol L^{-1} H_2O_2 postharvest treatment for longan fruit exhibited more serious pulp breakdown, the enhanced activities of enzymes related to degrading membrane lipids, as well as the accelerated degradation of membrane unsaturated fatty acids and membrane phospholipids in longan pulp (Lin, Lin, Chen, et al., 2019). Nevertheless, the role of energy metabolism in the occurrence of longan pulp breakdown induced by H_2O_2 is still unclear. Consequently, the purpose of this work were to evaluate the influences of exogenous H_2O_2 on adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP) amounts, energy charge level, and the activities of Mg^{2+}-ATPase, Ca^{2+}-ATPase, and H^+-ATPase in mitochondrial, plasma, and vacuolar membrane in the pulp of postharvest longans, and to elucidate the mechanism of ROS-stimulated pulp breakdown of postharvest longan in relation to energy metabolism.

2. Materials and methods

2.1. Fruits and treatments

Fruit of "Fuyan" longan (*Dimocarpus longan* Lour. cv. Fuyan) at commercial maturity (about 120 days after full bloom) were picked from a commercial orchard in Quanzhou, Fujian, China, and shipped to our laboratory. The longans were chosen in the light of the consistency of maturity, color, and shape. While the damaged longans or the samples with defects or diseases were discarded.

A batch of longans (n = 150) were chosen for assaying the indices of energy metabolism on the harvesting day. A second batch of longans (n = 2500) were immersed in 1.96 mmol L^{-1} H_2O_2 for 20 min. The selected conditions of H_2O_2 treatment in this experiment were based on our previous studies (Lin, Lin, Chen, et al., 2019; Lin et al., 2020). The third batch of longans (n = 2500) were used for the control group, and the longans were submerged 20 min with distilled water. Then the treated-longans were air-dried to eliminate the moisture on the fruit surface, afterward the fruit were packed in polyethylene bags (fifty fruit per bag), and stockpiled at 15 °C and relative humidity of 80% for 10 days. During postharvest storage, three bags with 150 longan fruit were sampled randomly at 2-day interval for measuring the following indices of energy metabolism.

2.2. Assay of AMP, ADP, ATP amounts and energy charge

Pulp tissue (ten grams) from thirty longans were applied to assay the amounts of AMP, ADP, ATP, and energy charge according to the methods of Chen, Lin, et al. (2018), Lin, Chen, et al. (2017) and Zhang et al. (2019). The amounts of AMP, ADP and ATP were displayed with the unit of mg kg^{-1}.

2.3. Assay of the ATPase activity

Five grams of pulp tissue from ten longans were sampled to measure the activities of Mg^{2+}-ATPase, Ca^{2+}-ATPase, and H^+-ATPase in plasma membrane, vacuolar membrane, and mitochondrial membrane based on the methods of our previous reports (Lin, Lin, et al., 2017; Zhang et al., 2019). The method of Bradford (1976) was applied to determine protein content. The activities of Mg^{2+}-ATPase, Ca^{2+}-ATPase, and H^+-ATPase were displayed with the unit of U mg^{-1} protein based on protein mass.

2.4. Statistical analysis

Measurements of all indexes in the experiment were carried out three times. Statistical analysis of the data was showed using the SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Influence of H_2O_2 on the pulp ATP, ADP, AMP amounts and energy charge in postharvest longans

In both the control and H_2O_2-treated longans, the ATP content (Fig. 1A), ADP content (Fig. 1B) and energy charge (Fig. 1D) in the pulp decreased during storage. Further comparison demonstrated that the lower amounts of ATP (Fig. 1A) and ADP (Fig. 1B), as well as the lower value of energy charge (Fig. 1D) were displayed in H_2O_2-treated longans than those in the control longans during storage, with significant ($P < 0.05$) difference from the storage day 4 till day 10, from day 2 till day 10, and day 4 till day 10, respectively, based on statistical analysis.

However, the AMP content in the pulp of control samples and H_2O_2-treated longans increased during storage (Fig. 1C). When compared to the control samples, a higher content of pulp AMP in H_2O_2-treated longans was displayed during storage with significant ($P < 0.05$) difference from storage day 4 till day 10.

3.2. Influence of H_2O_2 on the H^+-ATPase activities in pulp plasma, vacuolar and mitochondrial membrane of postharvest longans

Fig. 2A illustrated the change in the pulp plasma membrane H^+-ATPase activity of the control samples and H_2O_2-treated longans, in which both raised at the initial stage of storage (0–4 d), but decreased afterwards. When compared to the control longans, a lower activity of pulp plasma membrane H^+-ATPase in H_2O_2-treated longans was observed during storage with significant ($P < 0.01$) difference from storage day 4 till day 10.

Fig. 2B displayed that the vacuolar membrane H^+-ATPase activity in the pulp of control longans rose gradually at the initial stage of storage (0–4 d), but decreased slowly afterwards. While the vacuolar membrane H^+-ATPase activity in the pulp of H_2O_2-treated longans showed a slow increase at the initial stage of storage (0–4 d), then a quick reduction from storage day 4 till day 6, followed by a gradual decline. Statistical analysis displayed that, contrasted to the control sample, there was a lower activity of pulp vacuolar membrane H^+-ATPase in H_2O_2-treated longans during storage with significant ($P < 0.05$) difference on storage days 4, 6 and 10.

Fig. 2C illustrated that the mitochondrial membrane H^+-ATPase

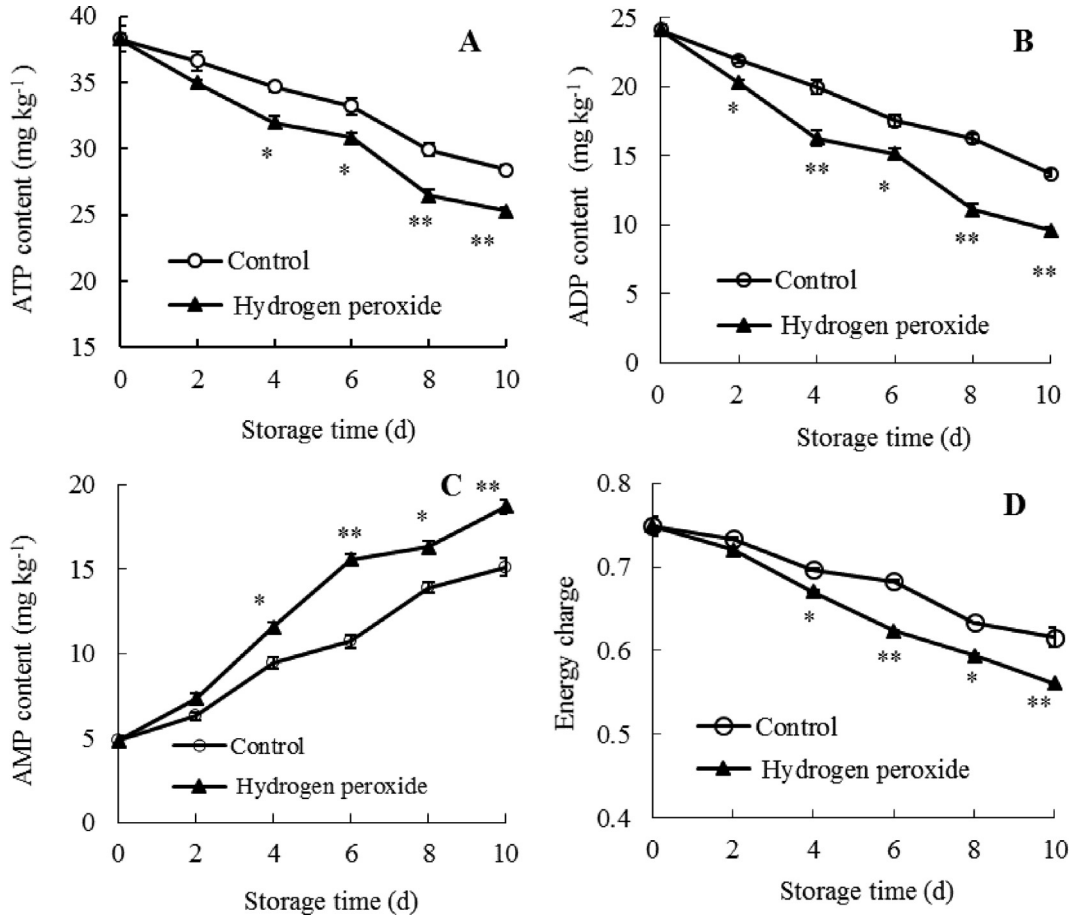

Fig. 1. Effects of hydrogen peroxide treatment on contents of ATP, ADP and AMP, and energy charge in pulp of harvested longan fruits. ▲, hydrogen peroxide treatment; ○, control. Each value is expressed as mean ± standard error (n = 3). The mark * and ** displayed significant ($P < 0.05$) difference and extremely significant ($P < 0.01$) difference, respectively, for each storage time point, according to the independent samples t-test.

activity in the pulp of control longans displayed a quick increase at the initial stage of storage (0–4 d), then a sharp reduction from day 4 till day 6, a slight increase from day 6 till day 8, followed by a gradual decrease. While for the H_2O_2-treated longans, the mitochondrial membrane H^+-ATPase activity exhibited a rapid increase at the initial stage of storage (0–2 d), then a slow increase from day 2 till day 4, a rapid decrease from day 4 till day 6, followed by a slow decrease. When compared to the control longans, a lower activity of pulp mitochondrial membrane H^+-ATPase in H_2O_2-treated longans was observed during storage with significant ($P < 0.05$) difference from day 2 till day 10.

3.3. Influence of H_2O_2 on the Ca^{2+}-ATPase activities in pulp plasma, vacuolar and mitochondrial membrane of postharvest longans

Fig. 3A illustrated that the pulp plasma membrane Ca^{2+}-ATPase activity of the control samples increased sharply at the initial stage of storage (0–4 d), but declined rapidly afterwards. While the pulp plasma membrane Ca^{2+}-ATPase activity of the H_2O_2-treated longans went up quickly during the initial two storage days, then changed slightly from day 2 untill day 4, and dropped rapidly from day 4 to day 6, followed by a gradual reduction. In addition, contrasted to the control longans, the obviously ($P < 0.05$) lower activities of plasma membrane Ca^{2+}-ATPase in pulp of H_2O_2-treated longans were observed from storage day 4 till day 10.

Fig. 3B showed that, for the control samples, the pulp vacuolar membrane Ca^{2+}-ATPase activity underwent a quick increase at the initial stage of storage (0–4 d), followed by a gradual decrease. Meanwhile, the pulp vacuolar membrane Ca^{2+}-ATPase activity of the H_2O_2-treated longans rose quickly from storage day 0 untill day 4, followed by a rapid decline. In addition, contrasted to the control longans, there were lower activities of pulp vacuolar membrane Ca^{2+}-ATPase in H_2O_2-treated longans during storage, with significant ($P < 0.05$) difference from the storage day 4 till day 10, based on statistical analysis.

Fig. 3C displayed that the pulp mitochondrial membrane Ca^{2+}-ATPase activity in the control samples went up rapidly at the initial stage of storage (0–4 d), and then experienced a speedy reduction from storage day 4 till day 6, followed by a slow decrease. Meanwhile, Fig. 3C showed that the mitochondrial membrane Ca^{2+}-ATPase activity in the pulp of H_2O_2-treated longans experienced a quick increase during the initial two storage days, a slight increase from storage day 2 till day 4, followed by a rapid decrease. In addition, contrasted to the control longans, the significantly ($P < 0.05$) lower activities of mitochondrial membrane Ca^{2+}-ATPase in pulp of H_2O_2-treated longans were observed from storage day 2 till day 10.

3.4. Influence of H_2O_2 on the Mg^{2+}-ATPase activities in pulp plasma, vacuolar and mitochondrial membrane of postharvest longans

As illustrated from Fig. 4A, for the control samples, the plasma membrane Mg^{2+}-ATPase activity experienced a speedy increase at the initial stage of storage (0–4 d), a quick decrease from day 4 till day 6, followed by a gradual reduction. Meanwhile, the activity of plasma membrane Mg^{2+}-ATPase in the pulp of H_2O_2-treated longans rose gradually at the initial stage of storage (0–4 d), then followed with a gradual decrease. Further statistical analysis displayed that H_2O_2-treated exhibited notable ($P < 0.05$) lower pulp plasma membrane

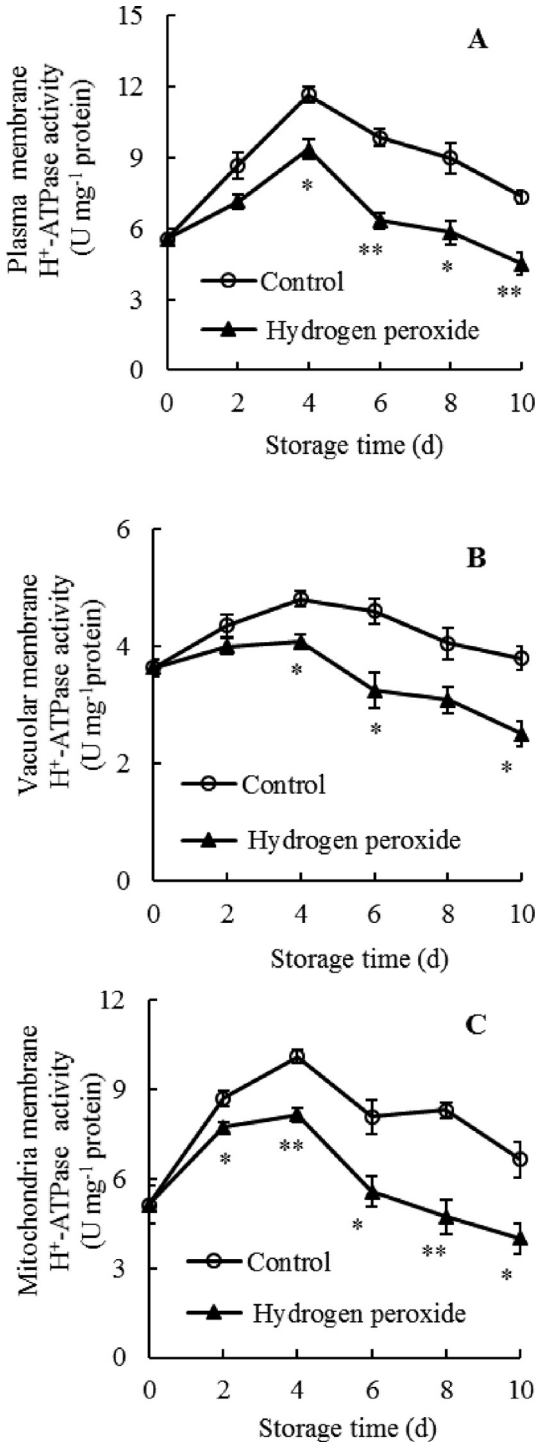

Fig. 2. Effects of hydrogen peroxide treatment on plasma, vacuolar and mitochondrial membrane H^+-ATPase activity in pulp of harvested longan fruits. ▲, hydrogen peroxide treatment; ○, control. Each value is expressed as mean ± standard error (n = 3). The mark * and ** displayed significant ($P < 0.05$) difference and extremely significant ($P < 0.01$) difference, respectively, for each storage time point, according to the independent samples t-test.

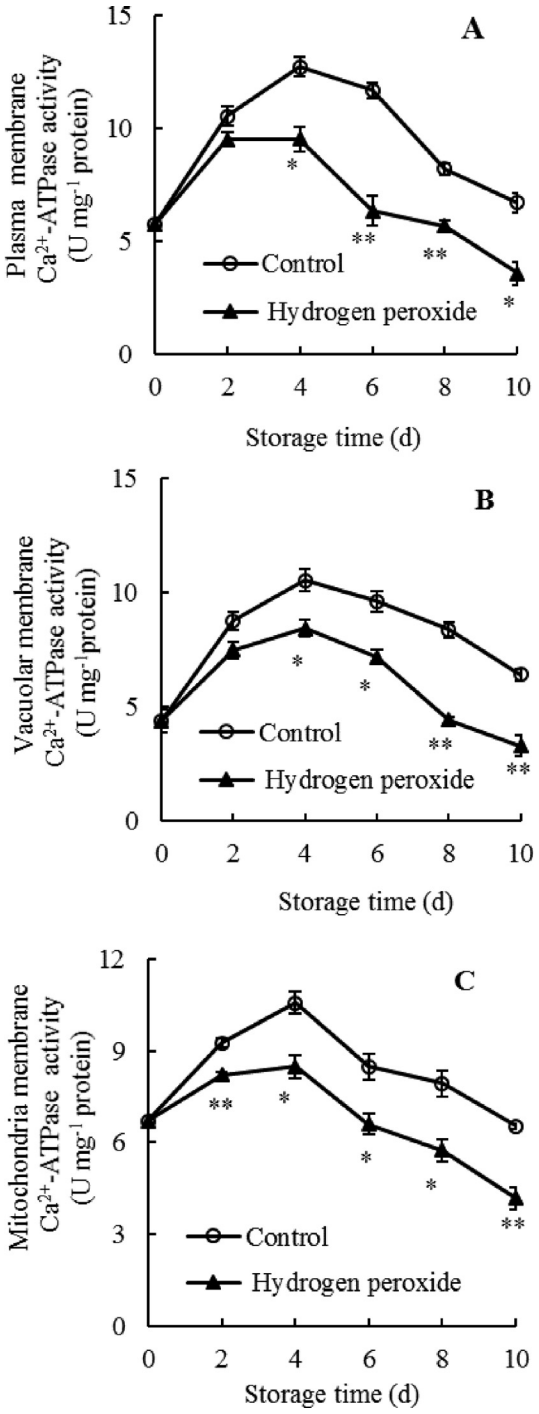

Fig. 3. Effects of hydrogen peroxide treatment on plasma, vacuolar and mitochondrial membrane Ca^{2+}-ATPase activity in pulp of harvested longan fruits. ▲, hydrogen peroxide treatment; ○, control. Each value is expressed as mean ± standard error (n = 3). The mark * and ** displayed significant ($P < 0.05$) difference and extremely significant ($P < 0.01$) difference, respectively, for each storage time point, according to the independent samples t-test.

Mg^{2+}-ATPase activity than that in control samples from storage day 2 till day 10.

Fig. 4B displayed that the activity of vacuolar membrane Mg^{2+}-ATPase in the pulp of control longans underwent a quick increase at the initial stage of storage (0–4 d), a slight increase from day 4 till day 6, followed by a sharp reduction. While the pulp vacuolar membrane Mg^{2+}-ATPase activity of H_2O_2-treated longans exhibited a rapid increase at the initial stage of storage (0–2 d), a slight increment from day 2 till day 4, a sharp reduction from day 4 till day 6, followed by a slow decline. In addition, contrasted to the control longans, there were lower activities of pulp vacuolar membrane Mg^{2+}-ATPase in H_2O_2-treated longans during storage, with significant ($P < 0.05$) difference from the storage day 4 till day 10, based on statistical analysis.

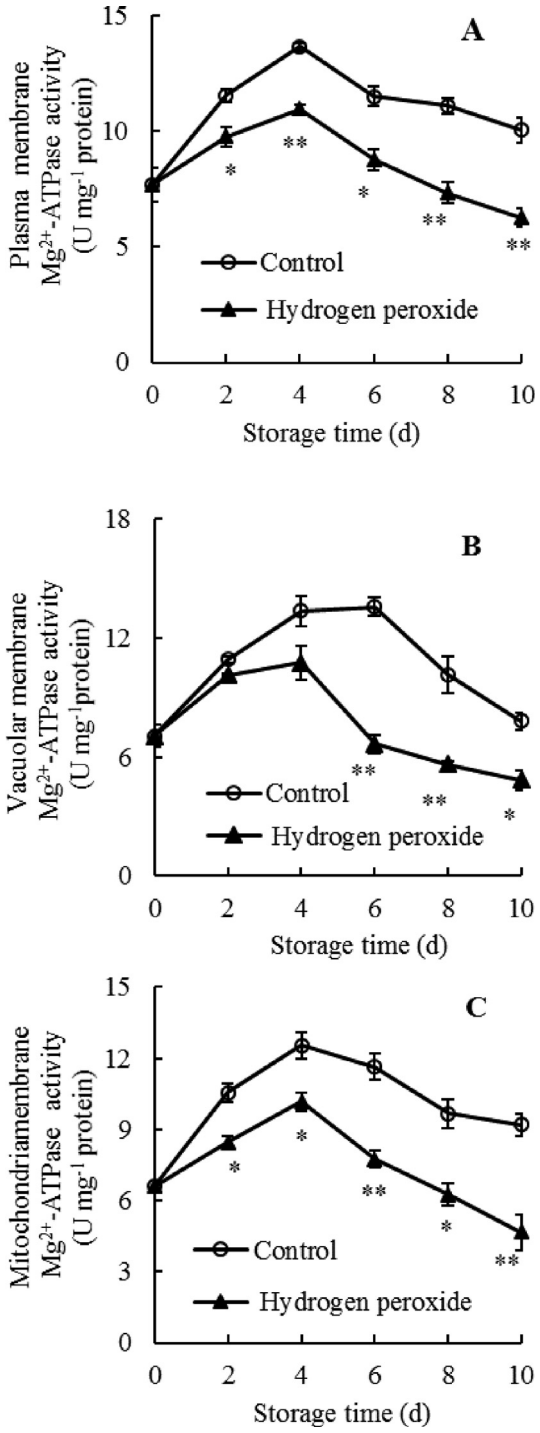

Fig. 4. Effects of hydrogen peroxide treatment on plasma, vacuolar and mitochondrial membrane Mg^{2+}-ATPase activity in pulp of harvested longan fruits. ▲, hydrogen peroxide treatment; ○, control. Each value is expressed as mean ± standard error (n = 3). The mark * and ** displayed significant ($P < 0.05$) difference and extremely significant ($P < 0.01$) difference, respectively, for each storage time point, according to the independent samples t-test.

Fig. 4C showed that the activity of pulp mitochondrial membrane Mg^{2+}-ATPase in the control longan fruit went up quickly from storage day 0 till day 4, then dropped gradually. While, the mitochondrial membrane Mg^{2+}-ATPase activity in the pulp of H_2O_2-treated longans rose rapidly during the initial four days of storage, followed by a sharp decrease. Additionally, contrasted to the control longans, the evidently ($P < 0.05$) lower activities of mitochondrial membrane Mg^{2+}-ATPase in pulp of H_2O_2-treated longans were observed from storage day 2 till day 10.

4. Discussion

4.1. H_2O_2 reduced-the level of energy status in relation to postharvest longan pulp breakdown

Cellular energy status is a crucial factor in regulating senescence, quality, and shelf life of postharvest fresh products (Chen et al., 2014, 2020; Chen, Tan, et al., 2018; Chen, Zhou, et al., 2018; Jiang et al., 2007; Li et al., 2017; Lin, Lin, Chen, et al., 2016). The attack of ROS, energy deficit, or the infection of pathogen, could weaken the function of mitochondria, reduce the biosynthesis ability of ATP, and decrease the cellular energy level, thereby accelerate the senescence progress, quality deterioration, and shorten the shelf life of postharvest fresh products (Chen et al., 2014; Chen, Lin, et al., 2018; Lin, Chen, et al., 2017; Yi et al., 2008; Zhang et al., 2019). Our previous work revealed that *Phomopsis longanae* or *Lasiodiplodia theobromae* stimulated-pericarp browning development and disease occurrence of postharvest longans were closely related to the decreases in the levels of energy charge, ATP, and ADP (Chen et al., 2014; Chen, Lin, et al., 2018; Lin, Chen, et al., 2017; Zhang et al., 2019). Whereas, the use of ATP or salicylic acid for alleviating postharvest pericarp browning development and disease occurrence of longans was due to keeping higher levels of energy charge, ATP, and ADP (Chen, et al., 2020; Lin, Chen, et al., 2017). Additionally, alleviated chilling injury, increased storability and prolonged shelf life of postharvest fresh products was benefited from higher level of energy charge, by applying treatment with ATP, nitric oxide, oxalic acid, or tea seed oil (Chen, Tan, et al., 2018; Chen, Zhou, et al., 2018; Jin et al., 2014; Wang et al., 2015; Zhang, Hu, et al., 2017). Therefore, the storability, quality property and shelf life of postharvest fresh products during storage may be related to the level of energy status.

This present study displayed that the pulp breakdown index (Supplementary Fig. S1) and the pulp cell membrane permeability (Supplementary Fig. S2) of the control longans increased with the extending of storage time. Meanwhile, during storage, the values of longan pulp energy charge, ATP and ADP reduced quickly, but the AMP content rose rapidly. As shown in Table 1, correlation analysis displayed that there were notable negative correlations between the pulp breakdown index (Supplementary Fig. S1) and the pulp ATP amount (Fig. 1 A) ($r = -0.976$, $P < 0.01$), between the pulp breakdown index (Supplementary Fig. S1) and the pulp ADP amount (Fig. 1 B) ($r = -0.972$, $P < 0.01$), and between the pulp breakdown index (Supplementary Fig. S1) and the pulp energy charge (Fig. 1 D) ($r = -0.961$, $P < 0.01$) in the control samples. Additionally, as shown in Table 1, there were notable negative correlations between the pulp cell membrane permeability (Supplementary Fig. S2) and the pulp ATP amount (Fig. 1A) ($r = -0.982$, $P < 0.01$), between the pulp cell membrane permeability (Supplementary Fig. S2) and the pulp ADP amount (Fig. 1B) ($r = -0.983$, $P < 0.01$), and between the pulp cell membrane permeability (Supplementary Fig. S2) and the pulp energy charge (Fig. 1D) ($r = -0.972$, $P < 0.01$) in the control samples.

Table 1
Correlation analysis between the pulp breakdown index (or the pulp cell membrane permeability) and the levels of pulp ATP, ADP, AMP, energy charge in the control longan fruit during storage.

Physiological and biochemical parameters	ATP	ADP	AMP	Energy charge
Pulp breakdown index	−0.976	− 0.972	0.950	−0.961
Pulp cell membrane permeability	−0.982	− 0.983	0.985	−0.972

Contrarily, as shown in Table 1, there were highly positive correlations between the pulp AMP content (Fig. 1C) and the pulp breakdown index (Supplementary Fig. S1) ($r = 0.950$, $P < 0.01$), as well as between the pulp AMP content (Fig. 1C) and the pulp cell membrane permeability (Supplementary Fig. S2) ($r = 0.985$, $P < 0.01$) in the control samples. The findings revealed that the decreased levels of ATP, ADP and energy charge were closely related to the breakage of longan pulp cell membrane integrity, and consequently accelerated postharvest longan pulp breakdown occurrence. This study agreed that the decreased storability of longan fruit featured by pericarp browning and rot development might be related to the decrease in cellular energy level during postharvest storage (Chen, Lin, et al., 2018; Lin, Chen, et al., 2017). Meanwhile, cellular energy level could directly affect the composition of membrane phospholipids, membrane fatty acids and regulate the function of cell membrane, thus regulating the storability of longan fruit (Lin, Chen, et al., 2018). Additionally, it had been reported that oxalic acid, nitric oxide, or tea seed oil treatment could increase the level of cellular energy, which alleviating senescence and prolonging shelf life of harvested peaches (Jin et al., 2014), bananas (Wang et al., 2015), or litchis (Zhang, Hu, et al., 2017), respectively.

Furthermore, the data from this work illustrated that, as contrasted to the control samples, the pulp breakdown index (Supplementary Fig. S1) and the permeability of pulp cell membrane (Supplementary Fig. S2) in H_2O_2-treated longans went up more rapidly and maintained at a higher level. In addition, the energy charge level, ATP and ADP contents in the pulp of H_2O_2-treated longans declined more rapidly and maintained at a lower value during postharvest storage (Fig. 1A, B, D). However, during storage, the content of pulp AMP in H_2O_2-treated longans maintained at higher value (Fig. 1C).

The above findings illustrated that, compared with the control samples, the treatment of H_2O_2 resulted in a lower level of pulp energy charge, lower amounts of pulp ATP and ADP, but a higher pulp breakdown index of longans during storage. The results indicated that H_2O_2-caused pulp breakdown occurrence of postharvest longans was caused by cellular energy shortage.

4.2. H_2O_2 reduced-ATPase activity in relation to postharvest longan pulp breakdown

The change of cellular energy is directly related to energy metabolism, which can lead to senescence, quality deterioration, and physiological disorder in postharvest fresh products (Li et al., 2017; Zhang et al., 2019; Zhao et al., 2019). Furthermore, ATPase is the crucial enzyme involving energy metabolism to regulate ATP synthesis, cellular homeostasis, and substance transport (Chen, Lin, et al., 2018; Lin, Lin, et al., 2017; Zhang et al., 2019). ATPase, such as Mg^{2+}-ATPase, Ca^{2+}-ATPase and H^+-ATPase, play important roles in the internal stable environment and normal energy metabolism of plant cell. These enzymes are located in the plasma, mitochondrial and vacuolar membranes (Chen, Lin, et al., 2018; Lin, Lin, et al., 2017; Zhang et al., 2019). The plasma membrane is responsible for regulating cellular homeostasis and substance transport (Olsen, Andersen, Lunding, Brasen, & Poulsen, 2009). Whereas, vacuolar membrane is the major site for regulating osmotic pressure and maintaining cell homeostasis (Anil, Rajkumar, Kumar, & Mathew, 2008). Additionally, mitochondrial membrane is the place for respiration metabolism and the main location for energy production. The enzymes in charge of electron transfer and energy synthesis are located inside of mitochondrial membrane (Chen, Lin, et al., 2018; Lin, Lin, et al., 2017; Zhang et al., 2019).

ATP can be catalyzed to generate ADP and free phosphate ions, and release energy via ATPase, particularly H^+-ATPase (Elmore and Coaker, 2011). In addition to construction of the transmembrane electrochemical gradient, it also provides the transmembrane electrochemical potential (Elmore and Coaker, 2011). Meanwhile, the imbalance of transmembrane proton electromotive force could influence physiological metabolism, such as the synthesis of ATP (Chen, Lin, et al., 2018). Also, Mg^{2+} plays a significant role in energy metabolism, but Mg^{2+} dysregulation may lead to oxidative stress and peroxidation (Nozadze et al., 2015). Ca^{2+} is a key cofactor in signal transmission, acting as the second messenger. If the balance of Ca^{2+} is destroyed, it may induce metabolic disorder and cell membrane damage (Anil et al., 2008; Chen, Lin, et al., 2018). Mg^{2+}-ATPase and Ca^{2+}-ATPase can use the energy, produced from the degradation of ATP, to remove Mg^{2+} and Ca^{2+} in plant cell. This helps to maintain osmotic pressure of normal plant cell, and to retain the integrity of cell structure (Anil et al., 2008; Chen, Lin, et al., 2018). However, the disorder of ATPase function might destroy the cellular homeostasis, then damage cell plasma and organelles like mitochondrial, vacuolar, resulting in the disorder of energy synthesis and the loss of membrane integrity, which might lead to energy deficiency and accelerate senescence of postharvest fruit (Chen, Lin, et al., 2018; Aghdam, Jannatizadeh, Luo, & Paliyath, 2018). Consequently, the activity of ATPase was involved in regulating the level of cellular energy, and then influenced the function of cell plasma, mitochondrial and vacuolar membrane, which was closely associated with the senescence and shelf life of postharvest crops. These results agreed with a previous report, for example, a reduction in the activities of H^+-ATPase, Ca^{2+}-ATPase and an imbalance in energy charge led to the pericarp browning and decreased quality of litchis stored at 25 °C without added tea seed oil treatment. Nevertheless, 0.1% tea seed oil treatment at 25 °C could enhance the activities of H^+-ATPase and Ca^{2+}-ATPase to alleviate pericarp browning and increase the quality of litchis (Zhang, Hu, et al., 2017). Moreover, the results of this research were in agreement with the finding of Zhao et al. (2019), in which displayed that a higher H^+-ATPase and Ca^{2+}-ATPase activity contributed to the prevented energy deficit and the enhanced storability of near-freezing temperature-treated nectarine fruit. Apart from these two reports, our previous works also could support these results. For instance, it reported that the expedited disease development and pericarp browning as well as the reduced storability of harvested longans-induced by *Phomopsis longanae* or *Lasiodiplodia theobromae* was owing to the reduction in H^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activity as well as the enhancement in reduction the contents of ATP, ADP and energy charge (Chen, Lin, et al., 2018; Zhang et al., 2019).

In this study, the data revealed that the accelerated pulp breakdown (Supplementary Fig. S1) of H_2O_2-treated longans could be ascribed to the decreased activities of plasma, vacuolar and mitochondrial membrane H^+-ATPase (Fig. 2A–C), which might lead to the energy deficit and unstable pH values in the plant cell, then reduce the proton electrochemical gradient and destroy the cellular homeostasis. Besides, the lower activity of H^+-ATPase cannot catalyze ATP to generate ADP, and release energy, thereby lead to decreased supply of energy in plant cell and caused the disorder of other physiological metabolisms. Consequently, the lower H^+-ATPase activity reduced the quality of harvested longan and aggravated the pulp breakdown occurrence (Supplementary Fig. S1) and shortened the shelf life of postharvest longans.

In addition, the current work showed that the accelerated pulp breakdown (Supplementary Fig. S1) of H_2O_2-treated longans could be ascribed to the declined activities of plasma, vacuolar and mitochondrial membrane Mg^{2+}-ATPase, Ca^{2+}-ATPase (Figs. 3, 4), and the decreased value of energy charge (Fig. 1D). These results demonstrated that the descended activities of Mg^{2+}-ATPase and Ca^{2+}-ATPase might induce the excessive accumulation of Mg^{2+} and Ca^{2+} in the organelles, and lead to ionic non-equalizing and damage the function of plasma, vacuolar and mitochondrial membrane, thereby resulting in the disorder of energy metabolism, and reducing the supply of energy in plant cell. Eventually, this led to pulp energy deficiency and accelerated the pulp breakdown of postharvest longan fruit. Consequently, the lower activities of Mg^{2+}-ATPase and Ca^{2+}-ATPase caused the deterioration of longan quality, aggravated pulp breakdown and shortened shelf life of postharvest longans.

The above results demonstrated that H_2O_2 treatment resulted in a higher pulp breakdown index, lower activities of Mg^{2+}-ATPase, Ca^{2+}-

ATPase, and H^+-ATPase in pulp mitochondrial, vacuolar, and plasma membrane of postharvest longans during storage, indicating that H_2O_2-induced pulp breakdown in postharvest longans resulted from lower activities of ATPase.

5. Conclusion

In brief, this work found that the occurrence of postharvest longan pulp breakdown was associated with energy metabolism. H_2O_2 treatment could obviously accelerate the longan pulp breakdown, which was owing to the fact that H_2O_2 reduced the level of longan pulp cellular energy and activities of Mg^{2+}-ATPase, Ca^{2+}-ATPase, and H^+-ATPase in longan pulp mitochondrial, vacuolar, and plasma membrane. These results proved that H_2O_2 induced the pulp breakdown of postharvest longan, which was related to the imbalance of osmotic pressure and internal cell environment, leading to the functional changes in longan pulp mitochondrial, vacuolar, and plasma membrane. The treatment also changed the energy status by the regulation of ATPase activity, and thus accelerated the pulp breakdown of harvested longan fruit. The possible mechanism of H_2O_2-caused postharvest pulp breakdown of longans via regulating the level of cellular energy and the activity of ATPase was summarized in Fig. 5.

Author Contribution

Hetong Lin and Yifen Lin designed the research program; Mark A. Ritenour supervised the research program; Yixiong Lin, Yihui Chen, Hui Wang, and Zhongqi Fan performed the experiments; Yixiong Lin analyzed the data and wrote the manuscript; Hetong Lin and Yifen Lin revised the manuscript; Mengshi Lin edited English language of the manuscript. All authors have approved the submission and publication of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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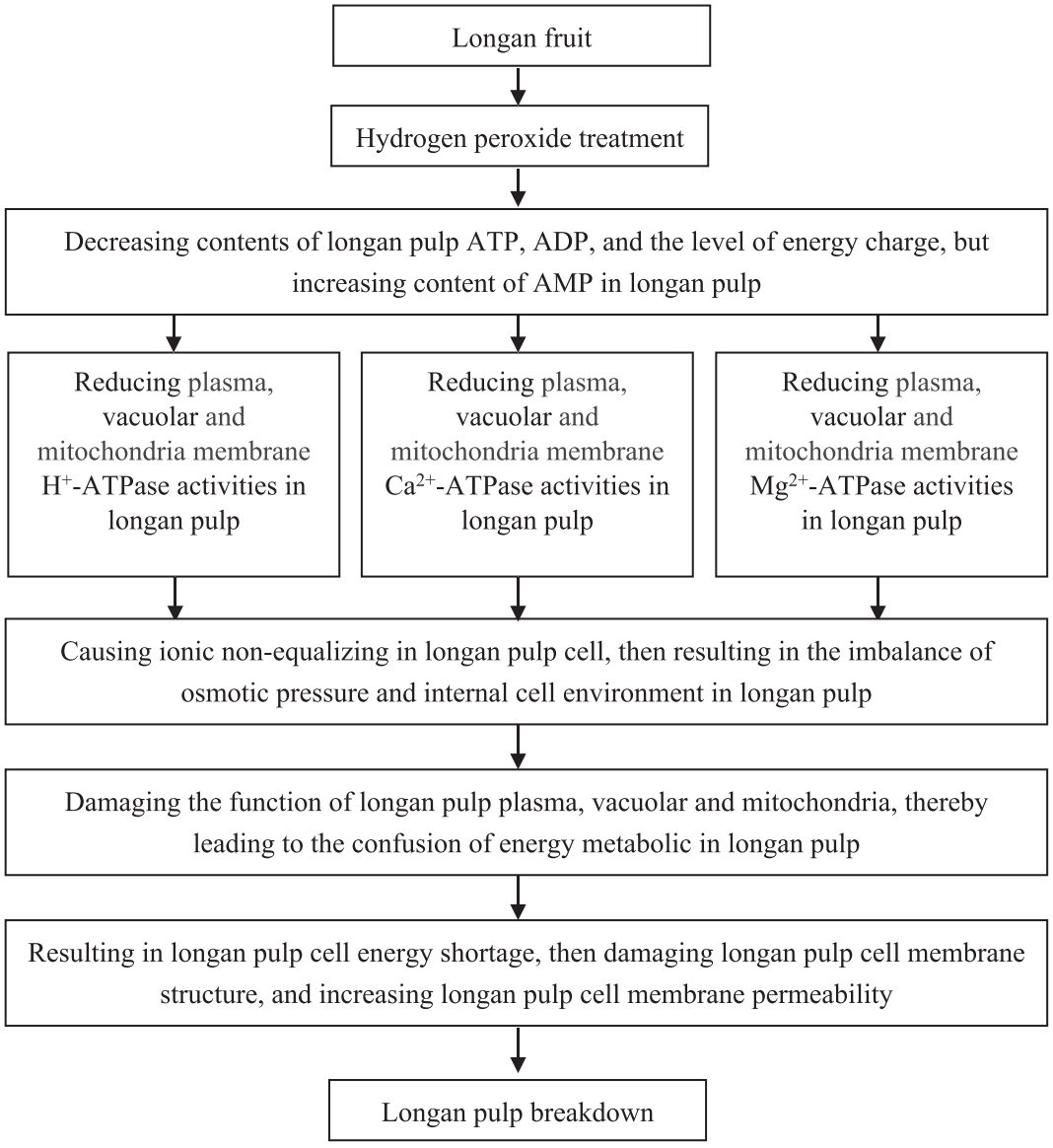

Fig. 5. The possible mechanism of hydron peroxide-caused pulp breakdown of postharvest longans by regulating cellular energy level and ATPase activity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.126008.

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