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Influence of hydrogen peroxide on the ROS metabolism and its relationship to pulp breakdown of fresh longan during storage

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ABSTRACT

The influence of hydrogen peroxide (H_2O_2) on the ROS metabolism and its relationship to pulp breakdown of fresh longan cv. Fuyan during storage was evaluated. Contrasted to control fruit, H_2O_2-treated samples manifested a higher index of pulp breakdown, an enhanced rate of $O_2^{\cdot-}$ generation, and an increased amount of MDA, but lower APX, CAT and SOD activities, reduced expressions of *DlAPX, DlCAT* and *DlSOD*, and lower concentrations of total phenolics, flavonoid, AsA, and GSH as well as lower levels of free radicals scavenging capacity. These data revealed that H_2O_2-induced pulp breakdown of longan was because H_2O_2 reduced ability of removing ROS but increased ROS generation and accumulation, which promoted peroxidation of cell membrane lipid, and subsequently led to damaging cell membrane structure and breakdown occurrence in pulp of postharvest fresh longan.

1. Introduction

Longan is an important tropical and subtropical fruit in some countries and districts of the world based on both economic value and production annually (Chen et al., 2021; Luo et al., 2021). Longan fruit is rich in nutritive phytochemicals such as polysaccharide, flavonoids, polyphenols, carbohydrate, vitamin C, protein, which has many kinds of bioactivities including antitumor, immunomodulatory, antioxidant effects (Lin et al., 2020a; Zhang, Guo, Ho, & Bai, 2020). Thus, longan fruit is very popular for consumers. However, since longan fruit ripen in hot season, the quality of postharvest longans deteriorates quickly due to pericarp browning, pulp breakdown or fruit diseases (Chumyam, Shank, Uthaibutra, & Saengnil, 2016; Intarasit & Saengnil, 2021; Li et al., 2018, 2020; Lin et al., 2019a; Tang et al., 2021; Vichaiya, Uthaibutra, & Saengnil, 2020; Wu et al., 2021). In particular, pulp breakdown, manifested as juice extravasation and erosion in longan pulp, is a key factor limits its commercial value and storage life (Lin et al., 2019b) . It is a conundrum for longan industry to inhibit postharvest longan pulp breakdown, which can be solved by elucidating the metabolism of pulp breakdown of postharvest longans and using proper preservation methods.

ROS (reactive oxygen species) metabolism is an important physiological metabolic activity in postharvest fresh crops (Lin et al., 2015; Tian, Qin, & Li, 2013). ROS level in plant cell can be regulated by ROS production-scavenging system (Sun et al., 2018; Xu et al., 2019). It is associated with the levels of antioxidant ingredients such as ascorbic acid (AsA), flavonoid, total phenolics, and glutathione (GSH) (Lin et al., 2020a). Also, it is linked to ROS removing enzymes activities, for example, catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) (Tang et al., 2021). Relevant literatures indicated that dysregulation of ROS production-scavenging system could enhance the accumulation and generation of ROS, which might lead to damaging cell membrane structure, and consequently result in quality deterioration of postharvest fresh fruits and vegetables such as bananas (Hao, Li, Xu, Huo, & Yang, 2019), blueberries (Chen, Hung, Chen, Lin, & Lin, 2019), grapes (Zhang, Xu, Chen, Jia, & Wu, 2019), litchis (Zhang et al., 2018), broccoli (Xu et al., 2016), and mung bean sprouts (Chen et al., 2018). Moreover, the infection of pathogens like *Peronophythora litchii* (Jiang et al., 2018), *Phomopsis longanae* Chi (Wang, Chen, Lin, Sun, & Lin, 2018) or *Lasiodiplodia theobromae* (Sun et al., 2018) could decrease the ability of removing ROS, enhance the accumulation and generation of superoxide anion ($O_2^{\cdot-}$, a kind of ROS), and accelerate membrane lipid

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Table 1
The sequences of specific primers used for qRT-PCR analysis.

Gene	Accession number	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Product (bp)
DlSOD	HM773388.2	GCGATCAGCGGCGAAATCAT	CGCCGTTGAACTTGATGGCA	157
DlCAT	FJ460504.2	AGCTCCGGTCTGGAATGACG	TGCATGGACAACACGTTCTGG	133
DlAPX	EU000322.1	GCATGGCACTCAGCTGGAAC	GATGGGCTCCAACAGCCTCA	135
DlACTIN	AB650589.1	TGGTGGTTCAACTATGTTCCCTG	ATGGACCAGACTCGTCATACTCAC	203

peroxidation, which might result in destroying cell membrane structure, consequently lead to the reduced storability and stimulate quality deterioration of postharvest crops. However, little literatures involved in ROS metabolism related to breakdown of longan pulp. Therefore, understanding the role of ROS metabolism in longan pulp breakdown is very important.

Hydrogen peroxide (H_2O_2) is the main ROS in plant cell (Lin et al., 2020b). Our previous studies found that H_2O_2-expedited pulp breakdown of longans was because H_2O_2 induced energy deficit, the accelerated metabolisms of respiratory and membrane lipid, which resulted in stimulating the consumption of pulp nutrient substances as well as accelerating the disruption of cellular structure in pulp, thereby induced longan pulp breakdown (Lin et al., 2019b). Nevertheless, no studies have been conducted about the role of exogenous H_2O_2 on the ROS metabolism during longan pulp breakdown. Accordingly, the purpose of present investigation was to assess the influence of H_2O_2 on activities of ROS removing enzymes, the levels of antioxidant ingredients, and expression of ROS scavenging-related genes during H_2O_2-expedited longan pulp breakdown. This work also explicated the principle of ROS metabolism in ROS-expedited longan pulp breakdown.

2. Materials and methods

2.1. Molecular biology reagents and chemical reagents

RNA extraction kit was obtained from Beijing TransGen Co., Ltd., China, while qRT-PCR kit and reverse transcription kit were purchased from Beijing Takara Co., Ltd., China.

Chemical reagents (analytical grade), such as dibasic sodium phosphate (Na_2HPO_4), ferric chloride, potassium ferricyanide, ascorbic acid (AsA), hydrogen peroxide (H_2O_2), sodium diethyl dithiocarbamate trihydrate, sodium dihydrogen phosphate (NaH_2PO_4), polyvinyl pyrrolidone (PVP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxylamine hydrochloride, *α*-naphthyl amine, *p*-aminobenzenesulfonic acid, sodium nitrite, hydroxylamine, trichloroacetic acid, thibabituric acid, dithiothreitol, nitro blue tetrazolium, riboflavin, ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2), bovine serum albumin, catechin, gallic acid, methionine, dithio nitrobenzoic acid (DTNB), glutathione (GSH), methanol, were obtained from Shanghai Sinopharm Co. Ltd., China.

2.2. Longan fruit material and treatment

Fruit of longan cv. Fuyan, at commercial maturity stage with the value of chromaticity *b** 53.73 and light yellow in longan pericarp appearance, was gathered from longan orchard at Nan'an, Fujian, China, and delivered to our laboratory at Fuzhou (Fujian, China) with three hours, then selected for the following experimentation to guarantee longans with uniform colour, maturity, shape and size, and free of disease or blemishes.

The selected 150 longans were employed to assess the relevant indicators on the 0^{th} day. The more selected 5000 longans were assigned into two groups (2500 longans per group) for the treatment of the distilled water (the control group) or 1.96 mmol L^{-1} H_2O_2, the selected concentration of 1.96 mmol L^{-1} H_2O_2 in the current work was based on our former works of Lin et al. (2019; 2020). Then the fruit were packed into the bag of polyethylene film (50 longans per bag), 50 bags per group were stockpiled for 10 d at the storage conditions of 80 % relative humidity and 15 °C. During the storage and the breakdown process in pulp of longan (Fig. S1, Fig. S2; (Lin et al., 2019b) (Lin et al., 2020b)), three bags per group (150 longans in total) were taken at two days interval to determine the following parameters of ROS metabolism. Brief information of measuring the parameters was provided below, and the detail methodologies of assaying the parameters of ROS metabolism in longan pulp were described in our former work and could be found in reference of (Lin et al., 2020a).

2.3. Measurement of malondialdehyde (MDA) amount and $O_2^{-}\cdot$ production rate

Longan pulp tissue (5 g) was sampled to assess MDA amount and $O_2^{-}\cdot$ production rate referring to our former detail methods of Lin et al. (2014; 2020), μmol kg^{-1} was expressed the unit of MDA amount, and mmol kg^{-1} min^{-1} was applied to represent the unit of $O_2^{-}\cdot$ production rate.

2.4. Assay of ROS scavenging enzymes activities

Longan pulp tissue (5 g) was sampled to assess APX, CAT, and SOD activities based on our former detail protocols of Lin et al. (2014; 2020). The protocol of Bradford (1976) was used to estimate longan pulp protein amount. U kg^{-1} protein was expressed the unit of enzyme activity.

2.5. RNA isolation and detecting ROS scavenging-related genes expression

Total RNA isolation and detecting gene expression related to scavenging ROS including *DlAPX, DlCAT,* and *DlSOD* in longan pulp were performed referring to detail methodology of our prior investigation (Lin et al., 2020a). Table 1 displayed the specific primers of gene which used in present work. The expression levels of *DlAPX, DlCAT,* and *DlSOD* were estimated referring to the protocol of Livak & Schmittgen, 2001.

2.6. Evaluation of the levels antioxidant ingredients

GSH and AsA amounts in longan pulp were measured using 5 g pulp from ten longans referring to protocols of (Lin et al., 2020a), g kg^{-1} was represented the unit of GSH and AsA amounts.

The amounts of longan pulp total phenolics and flavonoid were determined using 5 g pulp from ten longans referring to detail methodology of our prior investigation (Lin et al., 2020a), g gallic acid equivalent (GAE) kg^{-1} and g catechin equivalent (CE) kg^{-1} were used to express the unit of total phenolics and flavonoid, respectively.

2.7. Measurements of the scavenging ability of free radicals

Reducing power and DPPH radical scavenging ability were assayed using 5 g of pulp from ten longans referring to method of (Lin et al., 2020a), g kg^{-1} and % was applied to represent the unit of above-mentioned two indicators, separately.

2.8. Statistical analysis

Assessments of all above-mentioned indicators were carried out three times. SPSS version 22.0 was applied to analyze the experiment

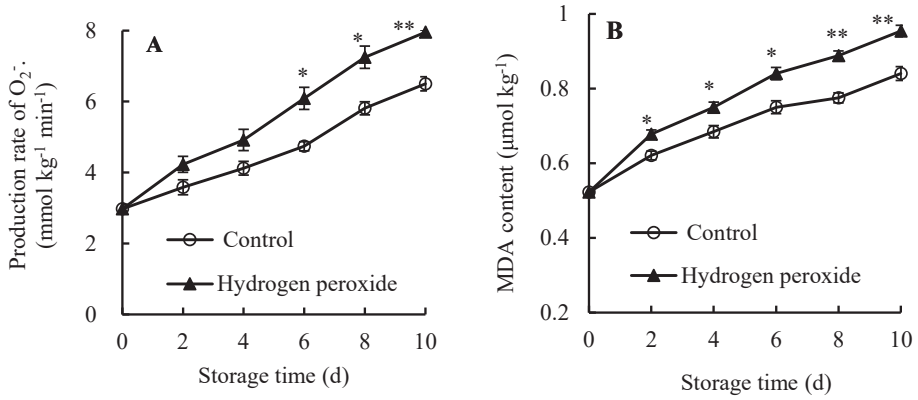

Fig. 1. Effects of hydrogen peroxide treatment on $O_2^{\cdot-}$ production rate (A) and MDA amount (B) in pulp of harvested longan fruit. Each value is expressed as mean ± standard error (n = 3), vertical bar denotes standard error. The mark * and ** displayed significant ($P < 0.05$) difference and extremely significant ($P < 0.01$) difference between the control longans and the hydrogen peroxide treated-longans at the same storage days according to the independent samples *t*-test, respectively.

data.

3. Results

3.1. $O_2^{\cdot-}$ Production rate and MDA amount

Figure 1A illustrated that pulp $O_2^{\cdot-}$ production rate in control fruit increased progressively with the extending days of storage. Whereas, for the H_2O_2-treated samples, the rate of $O_2^{\cdot-}$ production increased quickly during storage. In comparison with control group, H_2O_2-treated samples displayed a higher pulp $O_2^{\cdot-}$ production rate, with a clear ($P < 0.05$) discrepancy within day 6 to 10.

MDA amount in control and H_2O_2-treated longan pulp gradually raised throughout the whole storage period (Fig. 1B). Compared to control fruit, H_2O_2-treated longan displayed a manifestly ($P < 0.05$) higher MDA content during storage day 2 to 10.

3.2. ROS scavenging enzymes activities

Figure 2A displayed that pulp SOD activity in control fruit enhanced slowly within storage day 0 to 4, but decreased rapidly afterwards. While pulp SOD activity in H_2O_2-treated fruit manifested a gradual increment during storage 0–2 d, and then a gradual reduction. In comparison with control fruit, an obviously ($P < 0.05$) lower SOD activity was observed in pulp of H_2O_2-treated fruit during storage 4–8 d.

Figure 2B displayed that pulp CAT activity in control fruit enhanced slightly within storage 0–2 d, but dropped gradually afterwards. Whereas CAT activity in pulp of H_2O_2-treated longan displayed a slight increase within storage 0–2 d, then declined slowly. In comparison with control fruit, a conspicuously ($P < 0.05$) lower CAT activity was found in pulp of H_2O_2-treated fruit within storage 4–8 d.

Figure 2C illustrated that pulp APX activity in control fruit displayed a slight increase within storage 0–2 d, but reduced rapidly afterwards. While pulp APX activity in H_2O_2-treated samples showed a rapid decrease during storage. Compared with control longan, an obviously ($P < 0.05$) lower APX activity was observed in pulp of H_2O_2-treated samples within storage 4–10 d.

3.3. Expression levels of ROS scavenging-related genes

As shown in Fig. 2D-F, during storage, the alterations in expression levels of longan pulp *DlAPX, DlCAT* and *DlSOD* showed similar trend as the changes of APX, CAT and SOD activities (Fig. 2A-C). The expression levels of pulp *DlAPX, DlCAT* and *DlSOD* in control samples showed a rapid increment within storage 0–2 d, then dropped quickly during storage day 2 to 10. While the expression levels of pulp *DlAPX, DlCAT* and *DlSOD* in H_2O_2-treated fruit displayed a gradual enhancement within storage 0–2 d, but dropped quickly afterwards (Fig. 2D-F). Compared to control fruit, lower expression levels of pulp *DlAPX, DlCAT* and *DlSOD* were observed in H_2O_2-treated fruit, with an obvious ($P < 0.05$) difference on days 4, 8, 10, days 2–10, and on days 2, 6, 10, respectively (Fig. 2D-F).

3.4. Amounts of antioxidant substances

The amounts of pulp AsA (Fig. 3A), GSH (Fig. 3B), flavonoid (Fig. 3C) and total phenolics (Fig. 3D) in control and H_2O_2-treated samples exhibited a slow reduction during storage. Compared to control fruit, lower amounts of pulp AsA (Fig. 3A), GSH (Fig. 3B), flavonoid (Fig. 3C) and total phenolics (Fig. 3D) were displayed in H_2O_2-treated fruit, with an apparent ($P < 0.05$) difference within storage 2–10 d, 4–10 d, 6–10 d, and from day 2 till day 10 except day 6, separately (Fig. 3).

3.5. Scavenging ability of DPPH radical and reducing power

Figure 4A showed that, during storage, the control longan displayed a gradually decreasing ability to scavenge DPPH radical. Compared to control longan, a lower level and a quicker declination of ability to scavenge DPPH radical were displayed in H_2O_2-treated fruit, with an obvious ($P < 0.05$) difference at storage 4–10 d.

The pulp reducing power in control longan displayed a slight increment within storage 0–2 d, then a rapid decline from day 2 to 4, and a gradual declination afterwards (Fig. 4B). Whereas pulp reducing power in H_2O_2-treated fruit displayed a slight reduction within storage 0–2 d, and a rapid declination from day 2 to 4, but dropped gradually afterwards. Compared with control longan, a remarkably ($P < 0.05$) lower reducing power was observed in pulp of H_2O_2-treated fruit within storage 4–10 d (Fig. 4B).

4. Discussion

4.1. H_2O_2-induced alterations of ROS level and MDA amount and its relationship to longan pulp breakdown

The regulation of ROS production-scavenging system, to maintain normal ROS level, plays a crucial role in modulating membrane lipid peroxidation and storability of postharvest fresh fruit (Lin et al., 2017). Additionally, $O_2^{\cdot-}$ production rate is a major index for evaluating the level of ROS in plant cell (Lin et al., 2014). Meanwhile, MDA amount is a critical parameter to assess the degree of lipid peroxidation of cell membranes in plant tissues (Li, Limwachiranon, Li, Du, & Luo, 2016; Tang et al., 2021). Previous works showed that the infection of

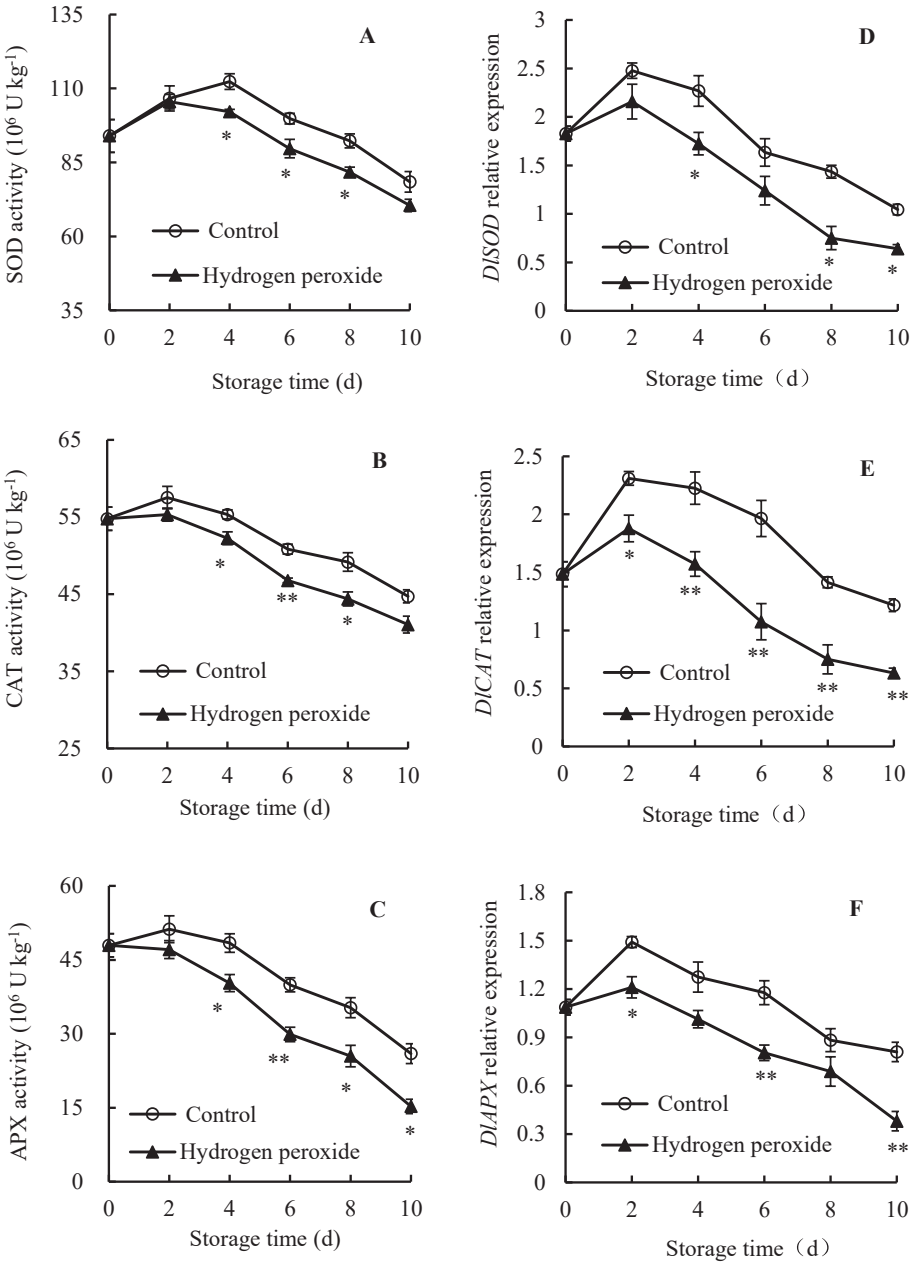

Fig. 2. Effects of hydrogen peroxide treatment on the activities of SOD (A), CAT (B) and APX (C) and the gene expression of *DlSOD* (D), *DlCAT* (E), and *DlAPX* (F) in pulp of harvested longan fruit. Each value is expressed as mean ± standard error (n = 3), vertical bar denotes standard error. The mark * and ** displayed significant ($P < 0.05$) difference and extremely significant ($P < 0.01$) difference between the control longans and the hydrogen peroxide treated-longans at the same storage days according to the independent samples *t*-test, respectively.

pathogens, like *P. longanae* (Lin et al., 2017) or *L. theobromae* (Sun et al., 2018), could stimulate quality deterioration of fresh fruit, which was associated with the raised $O_2^{\cdot-}$ generating rate and MDA amount. Whereas, the uses of propyl gallate (Lin et al., 2015), adenosine triphosphate (ATP) (Lin et al., 2017), acidic electrolyzed water (AWE) (Tang et al., 2021), nitric oxide (Zhang et al., 2019), or chitosan (Jiang et al., 2018) for improving quality maintenance of fresh fruit and lengthening its shelf-life might be due to the dropped $O_2^{\cdot-}$ generating rate and MDA amount. Thus, production rate of $O_2^{\cdot-}$ and MDA amount can, directly or indirectly, reflect the ROS level, and reflect the postharvest quality and storability of fruit.

The present study illustrated that, compared to control fruit, during postharvest storage, H_2O_2-treated samples showed an accelerated development of pulp breakdown (Fig. S1; Lin et al., 2020b), a higher pulp breakdown index (Fig. S2; Lin et al., 2019b), higher generation rate of $O_2^{\cdot-}$ in pulp (Fig. 1A), and higher content of pulp MDA (Fig. 1B), indicating that H_2O_2 stimulated ROS accumulation and production, which promoted lipid peroxidation of cell membranes, and subsequently resulted in damaging cell membrane structure and breakdown development in pulp of fresh longan during storage.

4.2. H_2O_2-induced alterations of ROS scavenging enzymes activities and its relationship to longan pulp breakdown

Under normal situations, plant tissues can keep the equilibrium of ROS removal and ROS production (Lin et al., 2014). APX, CAT and SOD are primary enzymes for scavenging ROS to remove ROS and reduce ROS accumulation (Lin et al., 2020c). SOD can catalyze the dismutation of $O_2^{\cdot-}$, which generated in mitochondria, to O_2 and H_2O_2 (Mandal, Mitra, & Mallick, 2008). Meanwhile, H_2O_2 is further catalyzed into H_2O and O_2 by APX and CAT (Lin et al., 2014). Relevant literatures showed that the declined storability of fresh fruit was owing to the decreased ROS scavenging enzymes activities (Chen et al., 2019; Duan et al., 2011). Lin et al. (2017) demonstrated that 2, 4-dinitrophenol (DNP) treatment for postharvest longans inoculated with *P. longanae* could reduce activities of longan pericarp APX, CAT and SOD, which

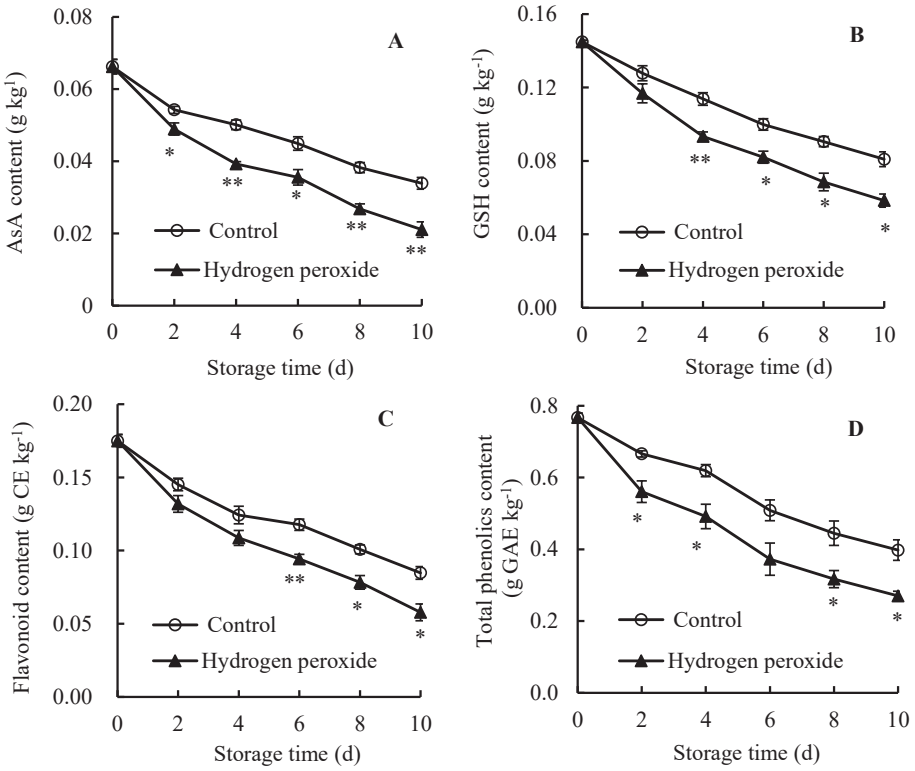

Fig. 3. Effects of hydrogen peroxide treatment on amounts of AsA (A), GSH (B), flavonoid (C), and total phenolics (D) in pulp of harvested longan fruit. Each value is expressed as mean ± standard error (n = 3), vertical bar denotes standard error. The mark * and ** displayed significant ($P < 0.05$) difference and extremely significant ($P < 0.01$) difference between the control longans and the hydrogen peroxide treated-longans at the same storage days according to the independent samples *t*-test, respectively.

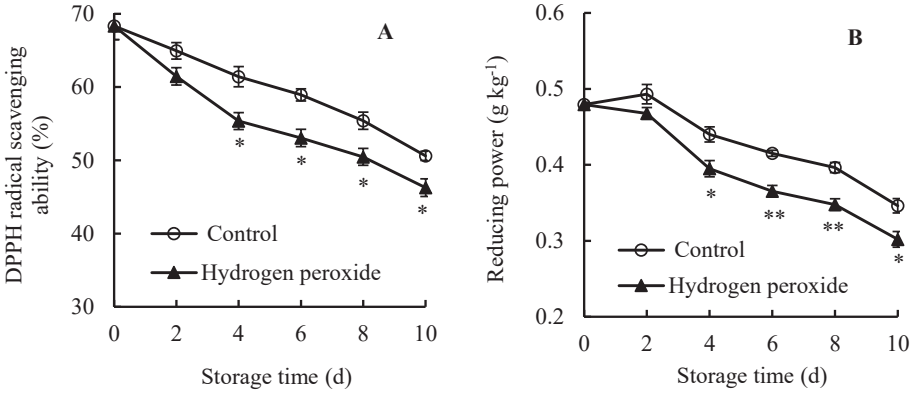

Fig. 4. Effects of hydrogen peroxide treatment on DPPH radical scavenging ability (A) and reducing power (B) in pulp of harvested longan fruit. Each value is expressed as mean ± standard error (n = 3), vertical bar denotes standard error. The mark * and ** displayed significant ($P < 0.05$) difference and extremely significant ($P < 0.01$) difference between the control longans and the hydrogen peroxide treated-longans at the same storage days according to the independent samples *t*-test, respectively.

subsequently reduced ROS scavenging ability and increased ROS generation, consequently stimulated longan pericarp membrane lipid metabolism, accelerated fruit disease occurrence and pericarp browning of fresh longan. In contrast, the treatment of pure oxygen (Duan et al., 2011), AEW (Chen et al., 2019), or glycine betaine (Sun et al., 2020) could raise activities of enzymes related to scavenging ROS, which could help to alleviate cell membrane lipid peroxidation and structural disruption, and consequently delay quality decline and lengthen shelf life of fresh fruit including litchis (Duan et al., 2011), blueberries (Chen et al., 2019), and 'Nanguo' pears (Sun et al., 2020). Thus, the enzymes of ROS scavenging might play a pivotal role in quality maintenance and storability of fresh fruit.

Current work manifested that, compared to control group, during storage, H_2O_2-treated group showed an accelerated development of pulp breakdown (Fig. S1; Lin et al., 2020b), a higher pulp breakdown index (Fig. S2; Lin et al., 2019b), higher generation rate of $O_2^{\cdot-}$ (Fig. 1A) and higher MDA amount in pulp (Fig. 1B), but the lower pulp APX, CAT and SOD activities (Fig. 2A-C). These data suggested that H_2O_2 reduced APX, CAT and SOD activities, and induced an excessive ROS accumulation in pulp of postharvest longans, which could expedite cell membrane lipid peroxidation and subsequently stimulate cell membrane structure disruption and, in turn, accelerated pulp breakdown of longan.

4.3. *H_2O_2-induced alterations in the expression levels of ROS scavenging-related genes and its relationship to longan pulp breakdown*

The gene expression levels of *APX, CAT* and *SOD* are highly considerable for regulating activities of corresponding enzymes and scavenging ROS (Hao et al., 2019; Zhang, Xu, Chen, Jia, & Wu, 2019). Relevant literatures indicated that the up-regulated expression levels of *APX, CAT* and *SOD* were beneficial for increasing activities of their corresponding enzymes and alleviating the quality deterioration of harvested banana fruit (Hao et al., 2019). While the down-regulated expression levels of *SOD* and *CAT* could lead to ROS accumulation and aggravate quality deterioration in harvested fresh grapes (Zhang et al., 2019). Therefore, the higher expression levels of ROS scavenging-related genes could help to keep a lower ROS level, and eventually mitigate the quality deterioration of harvested fruit.

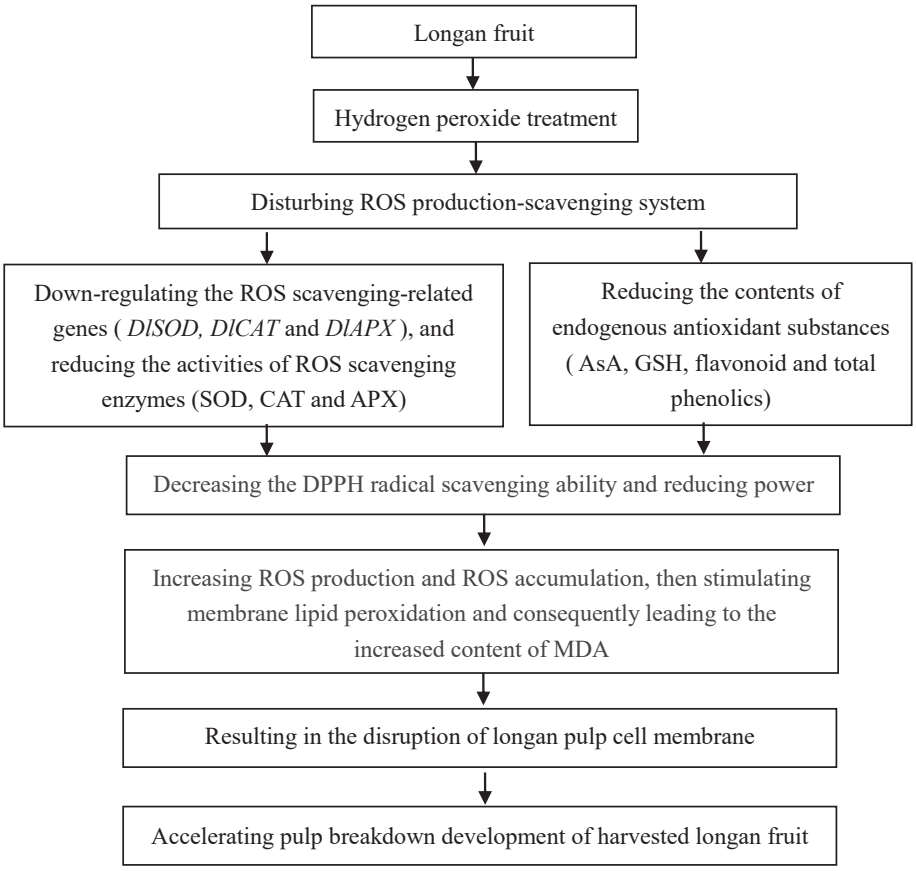

Fig. 5. The probable mechanism of hydrogen peroxide-induced pulp breakdown occurrence of longan fruit via modulating ROS production-scavenging system.

The present work indicated that, in comparison with control longan, during storage, H_2O_2-treated samples showed the down-regulated expression levels of *DlAPX, DlCAT* and *DlSOD* (Fig. 2D-F), the lower APX, CAT and SOD activities (Fig. 2A-C), but the higher production rate of $O_2^{-\cdot}$ (Fig. 1A), the accelerated development of pulp breakdown (Fig. S1; Lin et al., 2020b), and higher pulp breakdown index (Fig. S2; Lin et al., 2019b). These findings revealed that H_2O_2 induced the down-regulated expression of *DlAPX, DlCAT* and *DlSOD* in pulp of harvested longans, which could decrease the APX, CAT and SOD activities, and reduce ROS scavenging capacity, in turn, resulted in the excessive accumulation of ROS, and consequently expedited longan pulp breakdown occurrence.

4.4. H_2O_2-induced alterations in the levels of antioxidant ingredients and its relationship to longan pulp breakdown

Apart from scavenging ROS-related gene expression levels and enzyme activities, endogenous antioxidant ingredients in plant tissues such as AsA, GSH, total phenolics, and flavonoid, also play important roles in removing ROS, which can effectively eliminate ROS and prevent ROS accumulation (Lin et al., 2016; 2020c). AsA acts as reduction substance (hydrogen donor) in the conversion of H_2O_2 to H_2O, in which AsA is oxidized into dehydroascorbic acid (Lin et al., 2014). GSH is an important substance to revert dehydroascorbic acid to AsA (Sun et al., 2018). The reduced levels of endogenous antioxidant ingredients could accelerate the functional loss of ROS production-scavenging systems and reduce quality attributes of fresh fruits including litchis (Duan et al., 2011; Jiang et al., 2018), blueberries (Chen et al., 2019), and grapes (Zhang et al., 2019). However, the application of chitosan, AEW, or nitric oxide could extend storage life and improve quality maintenance of postharvest fresh produces like litchis (Jiang et al., 2018), blueberries (Chen et al., 2019), and grapes (Zhang et al., 2019), which were highly related to keeping higher contents of antioxidant ingredients. Therefore, the levels of antioxidant ingredients were association with ROS production-scavenging systems' function and the storability of post-harvest fresh crops.

This work illustrated that, compared to control fruit, during post-harvest storage, H_2O_2-treated samples displayed an accelerated pulp breakdown development (Fig. S1; Lin et al., 2020b), a higher pulp breakdown index (Fig. S2; Lin et al., 2019b), higher generation rate of $O_2^{-\cdot}$ (Fig. 1A) and higher amount of MDA (Fig. 1B), but much lower contents of total phenolics, flavonoid, AsA, and GSH (Fig. 3), and lower activities APX, CAT and SOD (Fig. 2A-C). These data revealed that H_2O_2 could decrease the contents of longan pulp antioxidant substances (total phenolics, flavonoid, AsA, and GSH) and reduce ROS scavenging-related enzymes activities, which could reduce ROS scavenging capacity, resulted in the raised production of ROS and an excessive accumulation of ROS, and consequently accelerated cell membrane lipid peroxidation and structure disruption and, in turn, expedited breakdown occurrence of longan pulp.

4.5. H_2O_2-induced alterations in reducing power and scavenging capacity of DPPH radical and its relationship to longan pulp breakdown

The free radicals are unstable in cell tissue of fresh crops (Jiang et al., 2018). In general, the reducing power and scavenging capacity of DPPH radical are the two major indicators to appraise scavenging ability and various dynamic process of free radicals in postharvest fresh crops, which can evaluate the ROS production-scavenging system (Lin et al., 2020a). Prior works indicated that the lower scavenging ability of free radical could lead to the raised accumulation of ROS and the increased metabolism of membrane lipids, and consequently shortened storage life and accelerated quality deterioration of harvested fruit (Jiang et al., 2018; Lin et al., 2017; Sun et al., 2018). Whereas, applications of

β-aminobutyric acid (Wang et al., 2016), ATP (Lin et al., 2017), or chitosan (Jiang et al., 2018) for lengthening shelf life and retarding quality deterioration of fresh produces were due to the higher ability of removing free radical, which helped to remove ROS and enhance the storability of postharvest fresh crops. Therefore, scavenging ability of free radical might be closely associated with storability of harvested fruit.

This work indicated that, in comparison with control longan, during storage, there were an accelerated development of pulp breakdown (Fig. S1; Lin et al., 2020b), higher pulp breakdown index (Fig. S2, Lin et al., 2019b), higher production rate of $O_2^{\cdot-}$ (Fig. 1A), but lower reducing power (Fig. 4B) and lower ability of scavenging DPPH radical (Fig. 4A) in H_2O_2-treated longan pulp. These findings implied that H_2O_2 could decrease reducing power and reduce ability of scavenging DPPH radical, which led to the reduced ability of scavenging free radical and the raised accumulation of ROS in longan pulp, and thus accelerated breakdown occurrence in longan pulp.

From the above-mentioned results, the probable mechanism, which involved in modulating ROS production-scavenging system, of H_2O_2-accelerated longan pulp breakdown was shown in Fig. 5. Whereas, the molecular mechanism of H_2O_2-accelerated longan pulp breakdown is still unclear. Therefore, the underlying molecular mechanism of H_2O_2-accelerated longan pulp breakdown should be further clarified through transcriptomic and metabolomics analyses.

In the practice of fresh longan preservation, the application of ROS scavenger, such as propyl gallate, L-cysteine hydrochloride or ascorbic acid, could help reduce ROS production and accumulation in fresh longan during storage, and thereby, repress longan pulp breakdown and improve storability of fresh longan. But, the probable mechanism of ROS scavenger to improve storability and inhibit pulp breakdown of fresh longan needs further elucidation.

5. Conclusions

In summary, ROS production-scavenging system was an important factor affecting the storability and pulp breakdown occurrence of postharvest longans·H_2O_2 could reduce storability and expedite pulp breakdown of harvested longans, which were resulted from the lower APX, CAT and SOD activities, the lower expression levels of *DlAPX, DlCAT* and *DlSOD*, the lower contents of antioxidant substances including total phenolics, flavonoid, AsA and GSH, the lower reducing power, and the lower capacity of scavenging DPPH radical. Additionally, H_2O_2 could reduce the capacity of scavenging ROS, enhance ROS generation and increase ROS accumulation, and subsequently stimulate lipid peroxidation in the longan pulp cell membrane and, thus, leading to cell membrane structure disruption and pulp breakdown of longan.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100159.

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