

Phomopsis longanae Chi causing the pulp breakdown of fresh longan fruit through affecting reactive oxygen species metabolism

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ABSTRACT

Phomopsis longanae Chi is a crucial pathogen causing fruit spoilage in postharvest fresh longan. The influence of *P. longanae* invasion with a suspension containing 1×10^4 *P. longanae* spores per mL on the breakdown occurrence and ROS metabolism in pulp of longan cv. Fuyan during storage at 28 °C was explicated. Compared to control group, more severe development of pulp breakdown (PB), higher PB index, O_2^- generation rate, H_2O_2 and MDA content, but lower SOD, APX and CAT activities, GSH, AsA, flavonoid and total phenolics amounts, ability of scavenging DPPH radical, and reducing power were displayed in the pulp of *P. longanae*-infected fruit during days 0–5. In this context, *P. longanae* induced breakdown of longan pulp by reducing the scavenging ability of ROS and increasing the cumulation of ROS, thereby enhancing the structural collapse and lipid peroxidation of cell membrane, which were responsible for the PB of harvested longans.

1. Introduction

Longan is planted in tropical and subtropical countries, especially in China, Vietnam and Thailand. The longan fruit is famous for its rich nutrition (Zhang, Guo, Ho, & Bai, 2020). After harvest, longan fruit is prone to spoilage such as fruit disease development, fruit decay, pulp breakdown (PB), and pericarp browning, which highly affects their storage period, transportation and economic value (Chen et al., 2022; L. J. Lin et al., 2019a; Y.F. Lin et al., 2019b; Y.Z. Lin et al., 2020b; Liu, Lin, Lin, Lin, & Fan, 2021). More importantly, PB caused by the pathogenic fungal infection in the harvested longans is regarded as the most important factor leading to a rapid spoilage of longan fruit. Thus, in order to control the occurrence of breakdown in longan pulp, the most critical imperative is to reveal the mechanism of pathogenic fungal infection induced-breakdown in longan pulp.

Phomopsis longanae Chi (*P. longanae*) is found to be a crucial pathogen fungus causing postharvest spoilage in longan (Chen et al., 2022; Wang et al., 2018). The appropriate temperatures for *P. longanae* mycelial

growth and spore germination are 28 °C (Chen et al., 2020; Zhang et al., 2013). Especially during the growth process of *P. longanae*, the color of its mycelia is white flocculent and the colony is round.

Recent studies have demonstrated that ROS (reactive oxygen species), e.g., O_2^- and H_2O_2 , may stimulate browning of harvested longan pericarp (Intarasit & Saengnil, 2021; Lin et al., 2014). Excessive ROS accumulation will further boost the membrane lipid peroxidation of cell and lead to the de-compartmentalization of cell, which can destroy cellular membrane structures (Ellouzi, Oueslati, Hessini, Rabhi, & Abdelly, 2021; Lin et al., 2016; Li et al., 2019). Additionally, the work of Lin et al. (2014) has reported that H_2O_2 could aggravate the pericarp browning of fresh longan through reducing the ability of scavenging ROS. On the contrary, the application of propyl gallate, a ROS exogenous scavenger that inhibits the accumulation of ROS, could effectively lengthen storage-life and inhibit pericarp browning of postharvest longans via increasing ROS scavenging ability (Lin et al., 2015). In addition, H_2O_2 could destroy cellular membrane structure and cause PB in longan fruit (Lin et al., 2019c). However, postharvest longans treated with

Abbreviations: AsA, ascorbic acid; APX, ascorbate peroxidase; CAT, catalase; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GSH, glutathione; H_2O_2 , hydrogen peroxide; MDA, malondialdehyde; O_2^- , superoxide anion; PB, pulp breakdown; *P. longanae*, *Phomopsis longanae* Chi; ROS, reactive oxygen species; SOD, superoxide dismutase.

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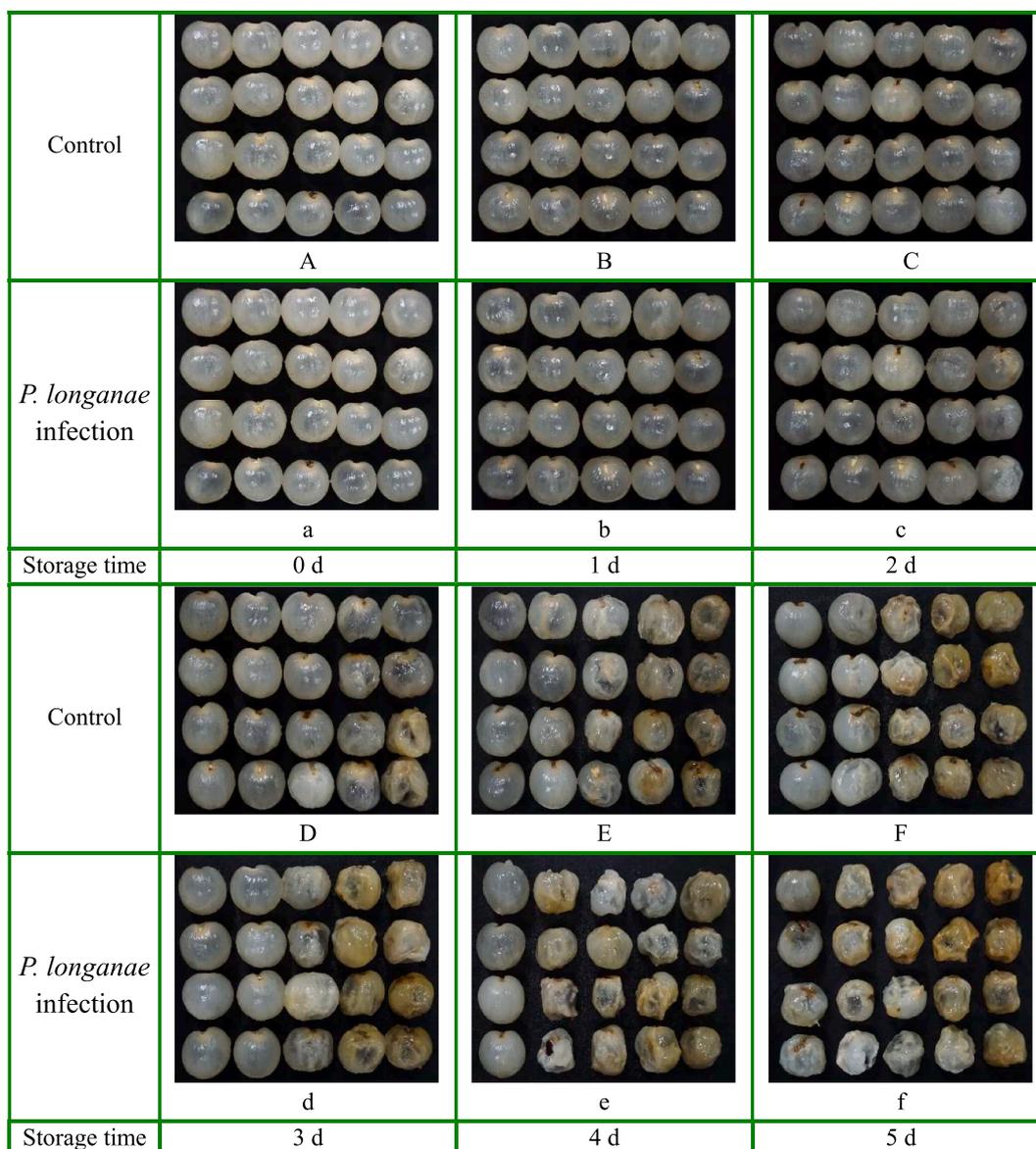


Fig. 1. Effect of *P. longanae* infection on pulp breakdown development of harvested longan fruit.

propyl gallate presented a slower PB development and better quality properties (Lin et al., 2020a). These findings indicate that ROS generation system is just as important as ROS scavenging system, and that it may be critical in PB, pericarp browning and fruit quality of fresh longans.

Additionally, pathogenic fungal infection may lead to the energy shortage, the disorder of ROS generation-scavenging system, the browning occurrence, and the disruption of cellular membranes structure in pericarp tissue of postharvest longans (Lin et al., 2017). However, the literatures involving *P. longanae* infection affecting breakdown occurrence and ROS metabolism in fresh longan pulp have not been reported. Therefore, this work aimed to illuminate how *P. longanae* infection affected PB index, O_2^- generation rate, H_2O_2 amount, amount of MDA (a metabolite in the lipid peroxidation of cell membrane), enzyme activities (APX, CAT, SOD) for scavenging ROS, amounts of non-enzyme antioxidant substances (GSH, AsA, flavonoid, total phenolics), level of reducing power and ability for scavenging DPPH radical in longan pulp. This study also aimed to elucidate the role of ROS metabolism in the PB of fresh longan fruit caused by *P. longanae*.

2. Materials and methods

2.1. Materials and treatments

P. longanae used this experiment was isolated from the rotten 'Fuyan' longans, which was isolated and identified in our laboratory in Fuzhou, China. Oat bran medium was applied to cultivate *P. longanae* at 28 °C for 15 d to obtain mature spores (Chen et al., 2014). The suspension with 1×10^4 *P. longanae* spores per mL was prepared as reported by Chen et al. (2014) and Chen et al. (2020) and used for the subsequent experiment.

The fresh 'Fuyan' longan, at commercial maturity stage with a light-yellow of longan peel and with 49.14 of chromaticity value L^* in fresh longan fruit appearance, were harvested from Fujian Nan'an orchard of China and immediately transported to the lab in Fuzhou, China. After removing the longan stalks, healthy fruit in homogeneous size, color, maturity, and without damage, disease spots or insect pests were selected for this experiment.

The selected 'Fuyan' longans were immersed in the solution of NaClO (0.5 %, v/v) about ten seconds for disinfection, then dipped in distilled water for 15 min, followed by air-drying. A total of 3150 disinfected 'Fuyan' longans were divided into two groups: one for *P. longanae*

inoculation (1500 fruit) and one served as the control (1500 fruit). The 150 'Fuyan' longans were selected to analyze the indices on day 0. The group of *P. longanae* inoculation was immersed in the suspension with 1×10^4 *P. longanae* spores per mL for five minutes, followed by air-drying, while the other group without *P. longanae* inoculation was taken as a control. After the postharvest handling, 'Fuyan' longans were packed in polyethylene film bags (fifty longans per bag), then stockpiled at 28 °C and 90% relative humidity. Three bags (150 'Fuyan' longans) of each group were collected on every day of storage to evaluate PB index, and to measure physio-biochemical parameters of ROS metabolism of longans.

2.2. Assessment of PB index

Fifty 'Fuyan' longans were applied to appraise PB index according to Lin et al. (2019a).

2.3. Determination of $O_2^{\cdot-}$ generation rate, MDA and H_2O_2 amounts

Pulp tissues (5 g) of longan fruit were gathered separately to determine the generation rate of $O_2^{\cdot-}$ (Lin et al., 2014; Lin et al., 2017), the amounts of MDA (Duan et al., 2011) and H_2O_2 (Chen et al., 2019). The unit of H_2O_2 , MDA amounts and $O_2^{\cdot-}$ generation rate were $mol\ kg^{-1}$, $\mu mol\ kg^{-1}$ and $mmol\ kg^{-1}\ min^{-1}$, separately.

2.4. Assessment of APX, CAT and SOD activities

Pulp tissues (5 g) of longans were used for APX, CAT and SOD activity assays following the protocol described by Duan et al. (2011). Protein content was determined by the method of Bradford (1976), using bovine serum as the standard. The units of the above enzyme activities were U kg^{-1} protein.

2.5. Assessment of the amounts of endogenous antioxidant substances

Pulp tissues (5 g) of longan fruit were applied to determine the GSH and AsA content in the light of the protocols of Jiang et al. (2018) and Lin et al. (2021). The GSH and AsA amounts were expressed in $g\ kg^{-1}$.

Five grams of pulp tissues were sampled to quantify the contents of total phenolics and flavonoid following the descriptions by Jiang et al. (2018) and Lin et al. (2020a); Lin et al. (2021). Catechin (CE) and gallic acid (GA) were the standards for flavonoid and total phenolics, respectively. The total phenolics and flavonoid amounts were expressed in $g\ GA\ kg^{-1}$ and $g\ CE\ kg^{-1}$, respectively.

2.6. Assay of the reducing power and ability of scavenging DPPH radical

Pulp tissues (5 g) of longans were collected to determine the scavenging ability of DPPH radical and reducing power with reference to the protocol of Lin et al. (2020a). The reducing power unit was displayed as $g\ kg^{-1}$, while scavenging ability of DPPH radical was %.

2.7. Statistical analysis

The evaluation of all indicators was executed in triplicate. The statistical analysis was accomplished by IBM SPSS Statistics.

3. Results

3.1. Pulp appearance

Longan pulp is the nutritious, succulent and edible aril of the longan fruit. As seen in Fig. 1A and a, longan pulp presented a white, semi-transparent and turgid appearance on day 0 of storage. However, as storage progressed, the breakdown of longan pulp occurred and worsened gradually, which was primarily manifested as opaqueness,

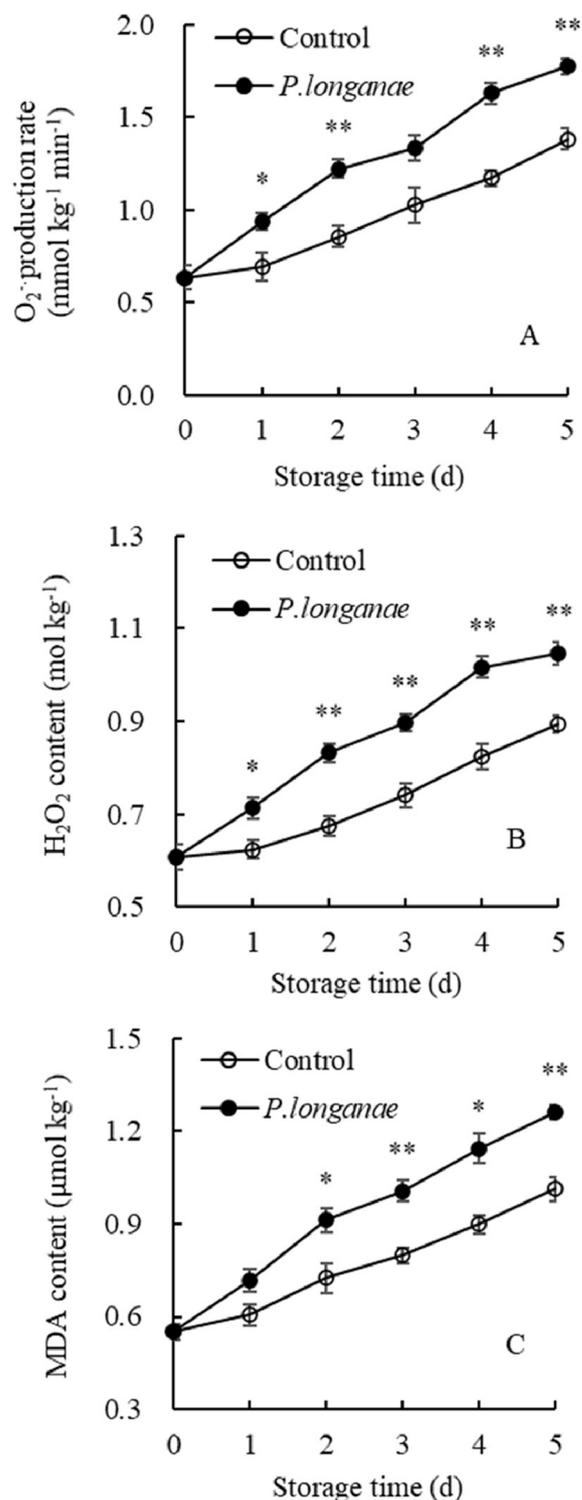


Fig. 2. Effects of *P. longanae* infection on the rate of $O_2^{\cdot-}$ production (A), the content of H_2O_2 (B) and MDA (C) in pulp of harvested longan fruit. Value presented in figure equals mean \pm standard error of triplicate analyses, vertical bars express the standard error of mean ($n = 3$). \circ , control; \bullet , *P. longanae*. The symbol (* and **) indicated the significantly difference ($P < 0.05$ and $P < 0.01$, separately) between the control longan fruit and the *P. longanae*-inoculated longan fruit on each storage day.

yellow–brown color and softening of the pulp. This phenomenon of PB in longan fruit started from the fleshy part near the pericarp and gradually developed inward (Fig. 1C–F). At the end of the storage, quite severe PB was observed in all pulp of the control group (Fig. 1F).

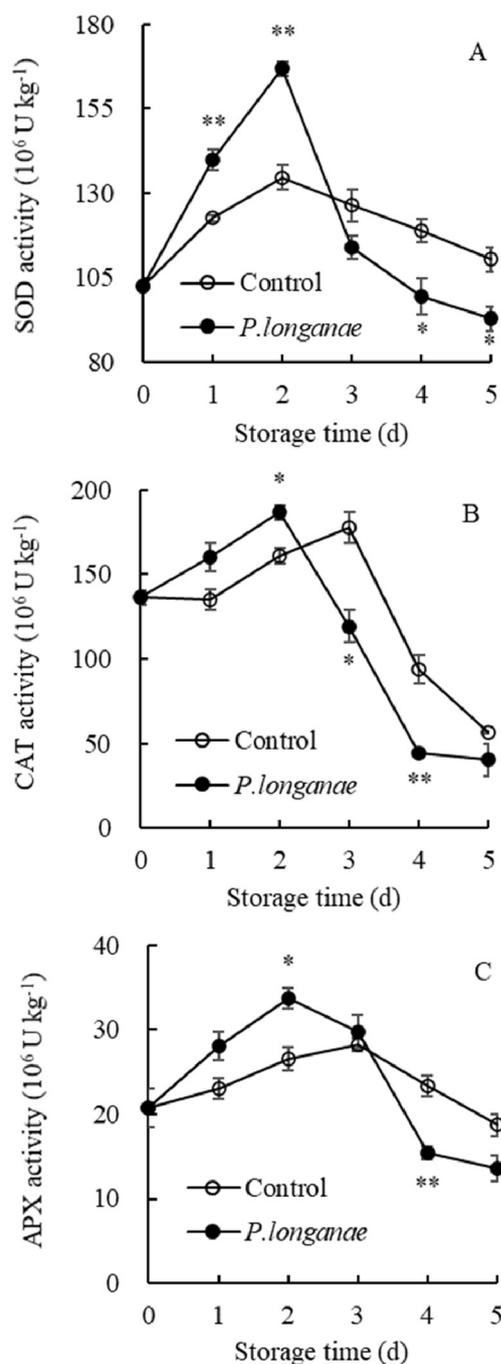


Fig. 3. Effects of *P. longanae* infection on the activities of SOD (A), CAT (B) and APX (C) in pulp of harvested longan fruit. Value presented in figure equals mean \pm standard error of triplicate analyses, vertical bars express the standard error of mean ($n = 3$). \circ , control; \bullet , *P. longanae*. The symbol (* and **) indicated the significantly difference ($P < 0.05$ and $P < 0.01$, separately) between the control longan fruit and the *P. longanae*-inoculated longan fruit on each storage day.

Correspondingly, the breakdown index of longan pulp went up with the extension of storage day (Fig. S1). Whereas, compared to control samples, PB occurred more rapidly and developed more severely after inoculation with *P. longanae* (Fig. 1c–f), and the PB index was clearly higher on days 2–5 than control longan ($P < 0.01$, Fig. S1). Therefore, *P. longanae* infection could significantly stimulate the occurrence of PB in longans, and consequently accelerate the quality deterioration of longans during postharvest storage.

3.2. O_2^- generation rate, H_2O_2 and MDA amount

The pulp O_2^- generation rate in postharvest longan fruit raised with the extension of storage day (Fig. 2A). The pulp O_2^- generation rate in the control group raised slowly throughout the entire storage, while the O_2^- generation rate in the pulp of *P. longanae*-infected group enhanced quickly from the start of storage to day 2, slowly from days 2–3, and quickly on days 3–5. The data analysis demonstrated that conspicuously higher ($P < 0.05$) O_2^- generation rate was shown in the pulp of *P. longanae*-infected fruit relative to the control group throughout the whole storage time, except for the day 3 (Fig. 2A).

The H_2O_2 content in longan pulp kept increasing over the entire storage (Fig. 2B). The pulp H_2O_2 content in the control group displayed a slight increment at storage day 0–1, then tardily went up until the end of storage. Whereas, H_2O_2 amount in the pulp of *P. longanae*-infected group enhanced quickly during 0–4 days, then enhanced slightly from days 4–5. Furthermore, trial analysis demonstrated that, compared to the control, conspicuously higher pulp H_2O_2 content was found in the *P. longanae*-infected fruit during 1–5 days ($P < 0.05$, Fig. 2B).

The MDA content of longan pulp, a substance from membrane lipid peroxidation, went up with the prolonged day of storage (Fig. 2C). A slow increase of MDA content was found in the pulp of the control group. Nevertheless, the pulp MDA content after infection with *P. longanae* raised sharply during 0–2 days, then raised slowly within the next three storage days. Significance analysis presented that, contrasted to the control group, conspicuously higher pulp MDA amount was appeared in the *P. longanae*-infected fruit within the storage days 2–5 ($P < 0.05$, Fig. 2C).

The above-mentioned data showed that *P. longanae* infection could raise pulp O_2^- production rate as well as increase the amounts of pulp H_2O_2 and MDA in postharvest longan.

3.3. ROS scavenging enzymes activities

The pulp SOD activity in postharvest longans manifested an increment trend from the beginning of storage to day 2, then a downward trend until the end (Fig. 3A). The activity of SOD in the control group raised progressively from days 0–2, and declined slowly during 2–5 days, while the SOD activity in the *P. longanae*-infected group exposed a rapid increment during 0–2 days, then a quick decrement until the final storage time (Fig. 3A). Significance analysis elucidated that notably higher ($P < 0.01$) pulp SOD activities were observed during the storage day 0 to 2 in *P. longanae*-infected fruit contrasted to the control, but conspicuously lower ($P < 0.05$) on the days 4 and 5 (Fig. 3A).

The CAT activity in longan pulp demonstrated an upward trend and then a decreasing trend with the increase of storage day (Fig. 3B). The pulp CAT activity in the control group manifested a slight change from the storage day 0 to 1, but a rapid increment in the next two storage days, then a sharp decrement during the storage days 3–5. The pulp CAT activity in the fruit infected with *P. longanae* enhanced quickly during 0–2 days, but quickly dropped in the next two days. It was also obtained that conspicuously higher ($P < 0.05$) pulp CAT activities on the second storage day, but conspicuously lower ($P < 0.05$) pulp CAT activities during the storage day 3 to 4 were found in the fruit infected with *P. longanae* in comparison to the control (Fig. 3B).

The pulp APX activity went up and then dropped as the storage time extended. The pulp APX activity in the control increased slowly on days 0–3, then dropped moderately on days 3–5 (Fig. 3C). Whereas, pulp APX activity in the group infected with *P. longanae* raised quickly in the first two days, but dropped sharply in the next two days, then dropped slowly until the end of storage. Data analysis demonstrated that, contrasted to the control group, conspicuously higher ($P < 0.05$) pulp APX activities on the second storage day, but conspicuously lower ($P < 0.01$) pulp APX activities on the fourth storage day were found in the group infected with *P. longanae* (Fig. 3C).

These data revealed that *P. longanae* infection could notably change

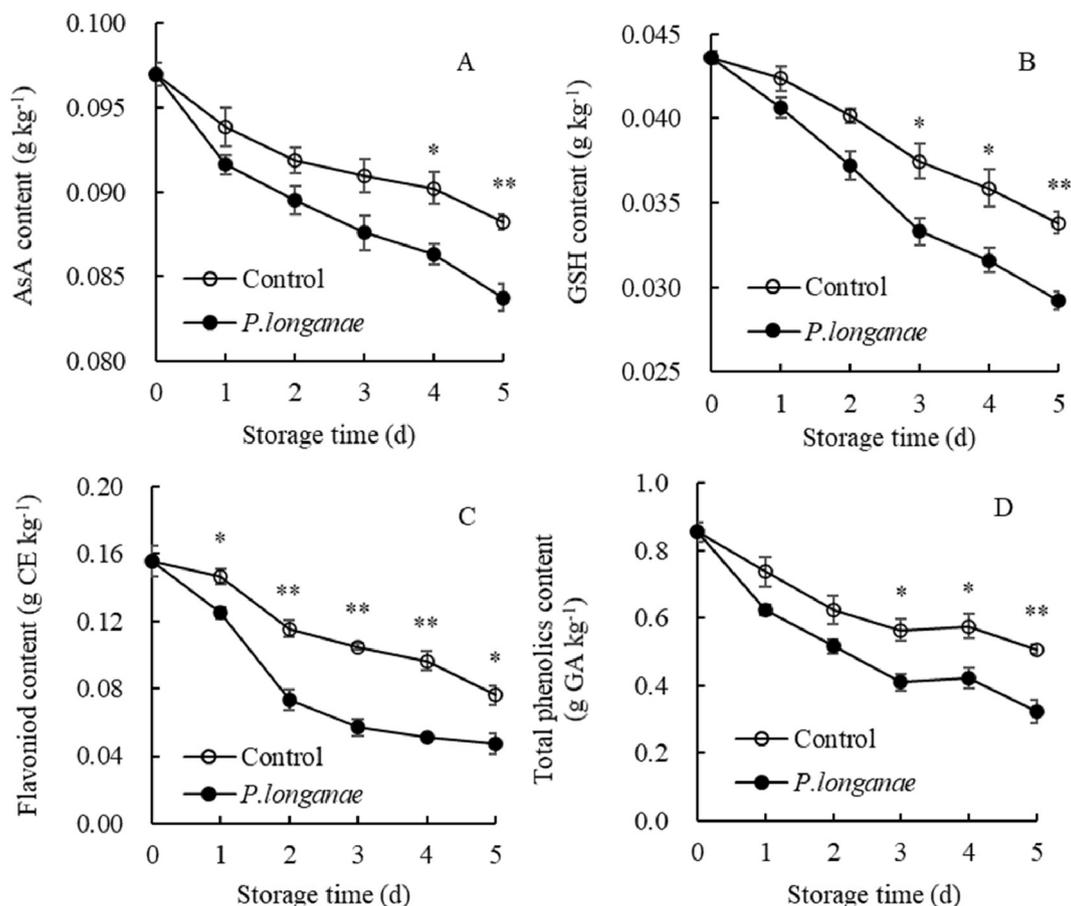


Fig. 4. Effects of *P. longanae* infection on the content of AsA (A), GSH (B), flavonoid (C) and total phenolics (D) in pulp of harvested longan fruit. Value presented in figure equals mean \pm standard error of triplicate analyses, vertical bars express the standard error of mean (n = 3). \circ , control; \bullet , *P. longanae*. The symbol (* and **) indicated the significantly difference ($P < 0.05$ and $P < 0.01$, separately) between the control longan fruit and the *P. longanae*-inoculated longan fruit on each storage day.

longan pulp ROS scavenging enzymes activities.

3.4. The levels of endogenous antioxidant substances

The longan pulp AsA amount displayed a decreasing trend with the extension of storage day (Fig. 4A). The pulp AsA amount in the control

samples reduced quickly within days 0–2, then dropped slowly during days 2–4, and reduced quickly during the storage day 4 to 5. Whereas the pulp AsA amount in the *P. longanae*-inoculated group declined quickly over the entire storage. Importantly, compared to control longan, fruit infected with *P. longanae* had lower pulp AsA content for the entire storage period, with a clearly ($P < 0.05$) difference on day 4 and

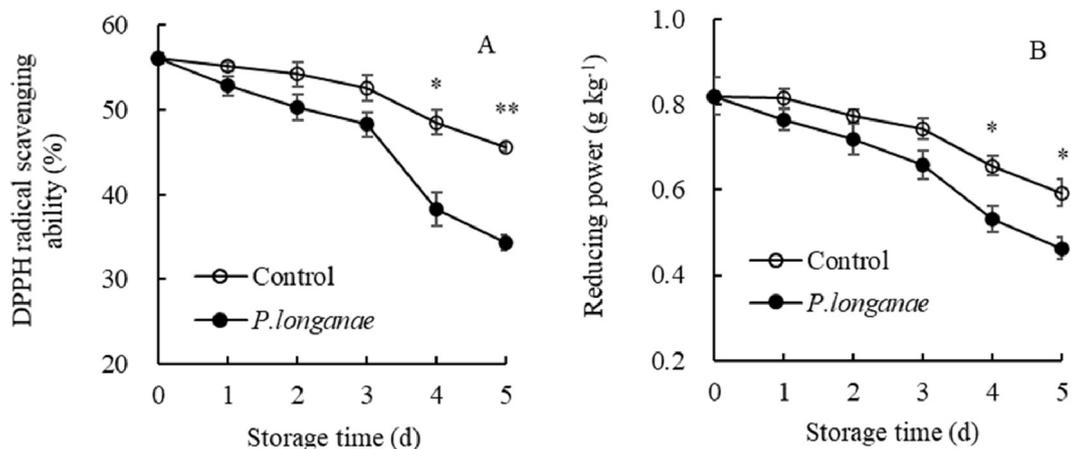


Fig. 5. Effects of *P. longanae* infection on the DPPH radical scavenging activity (A) and reducing power (B) in pulp of harvested longan fruit. Value presented in figure equals mean \pm standard error of triplicate analyses, vertical bars express the standard error of mean (n = 3). \circ , control; \bullet , *P. longanae*. The symbol (* and **) indicated the significantly difference ($P < 0.05$ and $P < 0.01$, separately) between the control longan fruit and the *P. longanae*-inoculated longan fruit on each storage day.

day 5 (Fig. 4A).

The GSH amount in longan pulp showed a dropping trend with the prolonged storage time (Fig. 4B). GSH content in the control longan pulp reduced slowly on the first day of storage, but decreased promptly until the end of storage. However, the pulp GSH amount in the *P. longanae*-inoculated group dropped quickly during entire storage period. Moreover, a lower GSH amount was shown in the pulp of *P. longanae*-infected fruit in contrast to the control during days 1–5 of storage, with a conspicuous discrepancy ($P < 0.05$) on days 3–5 (Fig. 4B).

The flavonoid content of longan pulp kept decreasing throughout the storage process (Fig. 4C). The pulp flavonoid content in control group exhibited a slight change on days 0–1, followed by a gradual decrease. In the group inoculated with *P. longanae*, rapid and gradual declines were observed on days 1–2 and 3–5, respectively. A marked decrease ($P < 0.05$) in flavonoid amount was found in pulp of *P. longanae*-inoculated group within days 1–5 in comparison to control longan (Fig. 4C).

The total phenolics content of longan pulp dropped with storage time (Fig. 4D). The total phenolics amount in the pulp of control longan declined promptly on days 0–3, then changed slightly on days 3–4, after that it declined again. In the group infected with *P. longanae*, the trend of total phenolics content changed similarly to the control group, but a clearly lower total phenolics content was observed on days 3–5 (Fig. 4D).

The above data confirmed that *P. longanae* infection could reduce the levels of longan pulp endogenous antioxidant substances.

3.5. DPPH radical scavenging ability and reducing power

As displayed in Fig. 5A, longan pulp DPPH radical scavenging ability dropped throughout the whole storage period. The pulp DPPH radical scavenging ability in control longan dropped gradually during days 0–3, and reduced rapidly in the next two storage days. The pulp DPPH radical scavenging ability in fresh longan inoculated by *P. longanae* declined mildly from the onset to day 3 of storage, and declined quickly in the following two days. Significant analysis revealed that, contrasted to the control group, a lower pulp DPPH radical scavenging ability was shown in *P. longanae*-inoculated group during days 1–5, with a conspicuous discrepancy ($P < 0.05$) on day 4 and day 5 (Fig. 5A).

The pulp reducing power in postharvest fruit showed a reducing trend during the period of storage (Fig. 5B). The pulp reducing power in the control group manifested a slow declination during the storage day 0 to 3, then a quick decrement from the storage day 3 to 5. Whereas, the reducing power of longan pulp after *P. longanae* inoculation dropped gradually from days 0–3, then declined sharply during days 3–5. The data analysis clearly showed that the reducing power of longan pulp infected with *P. longanae* was lower on days 1–5 relative to the control, with conspicuous discrepancy ($P < 0.05$) on day 4 and day 5 (Fig. 5B).

The above data illustrated that *P. longanae* infection could significantly lower the ability to scavenge DPPH radical and the value of the reducing power in pulp of fresh longan after harvest.

4. Discussion

4.1. Changes in ROS scavenging enzymes activities caused by *P. longanae* infection and their association with PB of harvested longans

Under normal conditions, there is a balance between the ROS generation and ROS scavenging activities to prevent plant tissue from the attack by excessive ROS. The ROS scavenging system contains two components: ROS scavenging enzymes and non-enzymatic endogenous antioxidant substances (Chen & Ko, 2021; Lin et al., 2021).

ROS scavenging enzymes include SOD, APX and CAT (Lin et al., 2021; Mandal, Mitra, & Mallick, 2008). O_2^- is transformed to H_2O_2 and O_2 by the action of SOD (Mandal et al., 2008). The other two functional enzymes, CAT and APX, stimulate the production of H_2O and O_2 from H_2O_2 (Zrig et al., 2021). Whereas, the stress conditions induce the

disorder in ROS scavenging and ROS generation, which may enhance the accumulation of ROS and lead to an imbalance of ROS generation-scavenging system. These activities can bring about an excessive accumulation of ROS, resulting in stimulated lipid peroxidation of cell membrane and causing accelerated cell de-compartmentalization and eventually quality deterioration and disease progress (Sun et al., 2018; Zrig et al., 2021). Evidence is found that pathogen invasion is capable of enhancing the SOD, APX and CAT activities at early stage of pathogen inoculation to remove excessive ROS in postharvest produces (Mandal et al., 2008). Whereas, SOD, APX and CAT activities decreased as the storage extended, which resulted in an increment of the ROS content and expedited lipid peroxidation, and consequently caused the quality deterioration and disease occurrence of fresh products (Jiang et al., 2018). Altogether, ROS scavenging enzymes may be crucial in the quality and storability of postharvest products.

In this work, PB development (Fig. 1), PB index (Fig. S1), generating rate of O_2^- (Fig. 2A), H_2O_2 amount (Fig. 2B) and MDA amount (Fig. 2C) in the pulp of control group manifested an increasing trend with the extension of storage days. It was further revealed that the raised PB index (Fig. S1) was positively related to the increased rate of O_2^- production (Fig. 2A), the increased content of H_2O_2 (Fig. 2B) and MDA (Fig. 2C) in the control group during storage, with correlation coefficient r of 0.899, 0.912 and 0.881, separately ($P < 0.05$). The results implied that PB in longan was closely related to an increment in O_2^- generating rate as well as an increase in H_2O_2 and MDA content. Additionally, contrasted to the control group, *P. longanae*-inoculated fruit manifested more severe PB (Fig. 1), higher index of PB (Fig. S1), rate of O_2^- production (Fig. 2A), and levels of pulp H_2O_2 (Fig. 2B) and MDA (Fig. 2C), implying that *P. longanae*-accelerated breakdown of longan pulp was highly linked to the increments of ROS production and ROS accumulation, and expedited lipid peroxidation of longan pulp.

In addition, the SOD, APX and CAT activities in the pulp of *P. longanae*-inoculated fruit manifested an upward trend within the early stage of storage (Fig. 3), while the production rate of O_2^- and H_2O_2 content increased rapidly (Fig. 2A, B). These data indicated that the oxidative burst activating-enzymes (APX, CAT, SOD) in the early stage of storage might be a defense response against the infection of *P. longanae*, which helped to scavenge ROS. Besides, the activities of enzymes (APX, CAT, SOD) in the group of *P. longanae* infection dropped rapidly at the late stage (Fig. 3), while the production rate of O_2^- and H_2O_2 content still increased (Fig. 2A, 2B). These findings demonstrated that, during the last stage of storage, the reduced enzyme activities for scavenging ROS in *P. longanae*-inoculated fruit could result in an imbalance between the generation of ROS and the scavenging of ROS, which led to an enhanced ROS generation and an accelerated ROS accumulation, expedited lipid peroxidation of cellular membrane, and consequently caused structural damaged of the membrane and the breakdown of the pulp of postharvest fresh longans.

The above data indicated that ROS-scavenging enzymes play a vital role in PB of longan. *P. longanae* infection could stimulate PB occurrence, accelerate O_2^- generating rate, and increase amounts of H_2O_2 and MDA in longan pulp tissue, which were resulted from decreased activities of ROS-scavenging enzymes (APX, CAT, SOD).

4.2. Changes in the content of endogenous antioxidant substances caused by *P. longanae* infection and their association with PB of harvested longans

Non-enzymatic endogenous antioxidant substances in postharvest fresh produces such as total phenolics, flavonoid, GSH and AsA are also essential for scavenging ROS and can effectively prevent the build-up of ROS (Xue et al., 2020). Previous investigation has demonstrated that a decrease in the content of endogenous antioxidant substances accelerates the dysfunction of ROS generation-scavenging system, which leads to disease occurrence and quality deterioration in fresh produces (Lin et al., 2017). Thus, keeping higher levels of endogenous antioxidant substances facilitates the maintenance of ROS production-scavenging

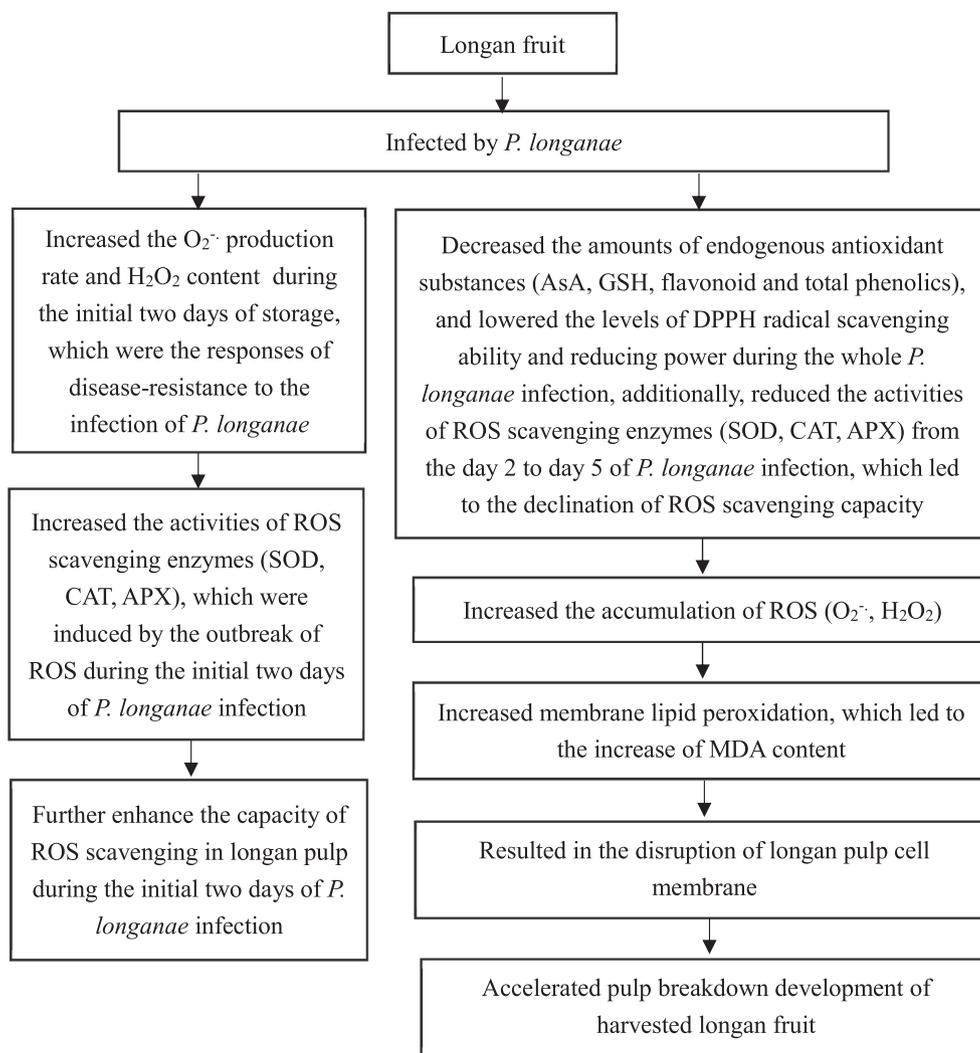


Fig. 6. The probable mechanism of *P. longanae*-induced pulp breakdown of postharvest longan fruit via acting on ROS metabolism.

system, thereby suppressing quality deterioration and disease development, and consequently enhances the shelf life and storability of postharvest produces (Xu et al., 2019).

In this work, during postharvest storage, the levels of pulp flavonoid, GSH, AsA, and total phenolics (Fig. 4) in control longan dropped with accelerated PB development (Fig. 1), increased PB index (Fig. S1), raised O_2^- generation rate (Fig. 2A) and enhanced content of H_2O_2 (Fig. 2B) and MDA (Fig. 2C). Further analyses demonstrated that the raised PB index (Fig. S1) during storage was negatively related to the reduced content of GSH (Fig. 4B) and flavonoid (Fig. 4C) in the control pulp, with r of -0.853 and -0.817 , separately ($P < 0.05$). Besides, the rise in pulp O_2^- generation rate (Fig. 2A) was negatively related to the declined content of pulp AsA (Fig. 4A), GSH (Fig. 4B), flavonoid (Fig. 4C) and total phenolics (Fig. 4D) in the control group, with r of -0.938 ($P < 0.01$), -0.995 ($P < 0.01$), -0.977 ($P < 0.01$) and -0.907 ($P < 0.05$), respectively. The enhanced level of H_2O_2 (Fig. 2B) was negatively linked to the reduced AsA (Fig. 4A), GSH (Fig. 4B), flavonoid (Fig. 4C) and total phenolics (Fig. 4D) content in the control pulp, with r of -0.914 ($P < 0.05$), -0.987 ($P < 0.01$), -0.957 ($P < 0.01$) and -0.873 ($P < 0.05$), respectively. Similar negative correlations were found between the elevated MDA content and the reduced amounts of endogenous antioxidant substances (-0.958 for ASA, -0.995 for GSH, -0.986 for flavonoid, -0.927 for total phenolics). Thus, the decrement of antioxidant substances in longan pulp contributed to an increased generating rate of O_2^- and a raised H_2O_2 amount, which finally led to lipid

peroxidation and PB.

Furthermore, more severe development of PB (Fig. 1), higher PB index (Fig. S1), higher generating rate of O_2^- (Fig. 2A), higher H_2O_2 (Fig. 2B) and MDA (Fig. 2C) content, and lower GSH, AsA, flavonoid and total phenolics content (Fig. 4) were observed in the *P. longanae*-inoculated group relative to the control during days 0–5. Those findings illustrated that *P. longanae* infection could reduce the amounts of longan pulp antioxidant substances (flavonoid, GSH, AsA, total phenolics), which led to an excessive accumulation of ROS, disrupted the membrane structure and boosted breakdown of longan pulp.

4.3. Changes in antioxidant activities caused by *P. longanae* infection and their association with PB of harvested longans

The evaluation parameters of antioxidant activities such as reducing power and ability to remove DPPH radical are usually used in postharvest fresh produces (Chen et al., 2018). Several investigations indicated that the decrement of antioxidant activity was associated with the accumulation of ROS, which resulted in the disease occurrence and the quality deterioration of fresh litchi (Yi et al., 2010) and longan (Sun et al., 2018). Whereas, an increment of antioxidant activity was highly associated with the lowered ROS cumulation, which was concomitant with the decreased disease incidence and the suppressed spoilage of fresh litchi (Jiang et al., 2018) and longan (Lin et al., 2018). Thus, the antioxidant activity may be closely connected with the quality and

storability of fresh products after harvest.

In the current observation, the reducing power and ability to scavenge DPPH radical (Fig. 5) in the control pulp manifested a decreasing trend with the aggravated PB (Fig. 1), the raised PB index (Fig. S1), the enhanced generating rate of O_2^- (Fig. 2A) and the raised H_2O_2 content (Fig. 2B) during storage. Further correlation analyses revealed that the raising PB index (Fig. S1) was negatively related to the declined ability to scavenge DPPH radical (Fig. 5A) and the decreased level of the reducing power (Fig. 5B) in control pulp during postharvest storage, with r of -0.949 and -0.942 , separately ($P < 0.01$). Besides, during postharvest storage, the enhanced generating rate of O_2^- (Fig. 2A) was negatively associated with the declined ability to scavenge DPPH radical (Fig. 5A) and the decreased value of the reducing power (Fig. 5B) in control longan, with r of -0.977 and -0.983 , separately ($P < 0.01$). The enhanced H_2O_2 content (Fig. 2B) was negatively associated with the declined ability to scavenge DPPH radical (Fig. 5A) and the decreased value of the reducing power (Fig. 5B) in the control pulp during postharvest storage, with r of -0.989 and -0.993 , respectively ($P < 0.01$). It was speculated that the decrease of antioxidant activity, including the declination in reducing power and the decrement in the ability to remove DPPH radical, led to the raised generating rate of O_2^- and H_2O_2 content, which was the primary cause of PB in longan.

In comparison with the control, more serious PB development (Fig. 1), higher PB index (Fig. S1), generating rate of O_2^- (Fig. 2A) and H_2O_2 content (Fig. 2B), but a lower ability of scavenging DPPH radical (Fig. 5A), and a lower level of the reducing power (Fig. 5B) were found in the pulp of *P. longanae*-infected fruit within days 0–5. It could be inferred that *P. longanae* infection was able to decrease the pulp antioxidant activity of postharvest longans, which suppressed the ability to scavenge free radical and resulted in over-accumulation of ROS, and ultimately boosted breakdown of its pulp.

Overall, the possible mechanism of *P. longanae*-stimulated longan PB involved the metabolism of ROS, which was elucidated in Fig. 6. Whereas, the probable molecular and biochemical mechanisms of *P. longanae*-accelerate longan PB remains unclear. Therefore, it is necessary to further explore the mechanism of *P. longanae*-accelerate longan PB, referring to the omics methods such as the analyses of metabolomics, transcriptomics and proteomics in the research of postharvest fresh produces (Chen et al., 2019; Li et al., 2021; Lin, Lin, Lin, Fan, & Lin, 2021; Wen et al., 2022).

5. Conclusion

In short, it could be confirmed that the PB in longan was resulted from a disorder of reactive oxygen generation-scavenging system. The increase of O_2^- production rate and the increments of H_2O_2 and MDA amounts in the longan pulp inoculated by *P. longanae* were closely relevant to the declined ability of enzymes to scavenge ROS, the declined content of non-enzymatic antioxidant substances and the decrement of antioxidant activity. This led to the reduced self-defensive ability against pathogen infection, and facilitated lipid peroxidation and structural collapse of cellular membranes, consequently accelerating PB in 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.2022.100301>.

References

- Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Chen, L., Tan, J. T. G., Zhao, X., Yang, D. Y., & Yang, H. S. (2019). Energy regulated enzyme and non-enzyme-based antioxidant properties of harvested organic mung bean sprouts (*Vigna radiata*). *LWT - Food Science and Technology*, 107, 228–235. <https://doi.org/10.1016/j.lwt.2019.03.023>
- Chen, L., Wu, J. E., Li, Z. M., Liu, Q., Zhao, X., & Yang, H. S. (2019). Metabolomic analysis of energy regulated germination and sprouting of organic mung bean (*Vigna radiata*) using NMR spectroscopy. *Food Chemistry*, 286, 87–97. <https://doi.org/10.1016/j.foodchem.2019.01.183>
- Chen, L. W., & Ko, Y. T. (2021). Exogenous hydrogen peroxide induces chilling tolerance in *Phalaenopsis* seedlings through glutathione-related antioxidant system. *Scientia Horticulturae*, 289, 110421. <https://doi.org/10.1016/j.scienta.2021.110421>
- Chen, L., Tan, G. J. T., Pang, X. Y., Yuan, W. Q., Lai, S. J., & Yang, H. S. (2018). Energy regulated nutritive and antioxidant properties during the germination and sprouting of broccoli sprouts (*Brassica oleracea* var. *italica*). *Journal of Agricultural and Food Chemistry*, 66, 6975–6985. <https://doi.org/10.1021/acs.jafc.8b00466>
- Chen, Y. H., Lin, H. T., Jiang, Y. M., Zhang, S., Lin, Y. F., & Wang, Z. H. (2014). *Phomopsis longanae* Chi-induced pericarp browning and disease development of harvested longan fruit in association with energy status. *Postharvest Biology and Technology*, 93, 24–28. <https://doi.org/10.1016/j.postharvbio.2014.02.003>
- Chen, Y. H., Sun, J. Z., Lin, H. T., Lin, M. S., Lin, Y. F., Wang, H., et al. (2020). Salicylic acid reduces the incidence of *Phomopsis longanae* Chi infection in harvested longan fruit by affecting the energy status and respiratory metabolism. *Postharvest Biology and Technology*, 160, 111035. <https://doi.org/10.1016/j.postharvbio.2019.111035>
- Chen, Y. Z., Yu, J., Lin, H. T., Zheng, Y., Fan, Z. Q., Wang, H., et al. (2022). *Phomopsis longanae* Chi-induced longan pulp breakdown and softening in relation to cell wall polysaccharides disassembly. *Postharvest Biology and Technology*, 186, 111837. <https://doi.org/10.1016/j.postharvbio.2022.111837>
- Duan, X. W., Liu, T., Zhang, D. D., Su, X. G., Lin, H. T., & Jiang, Y. M. (2011). Effect of pure oxygen atmosphere on antioxidant enzyme and antioxidant activity of harvested litchi fruit during storage. *Food Research International*, 44, 1905–1911. <https://doi.org/10.1016/j.foodres.2010.10.027>
- Ellouzi, H., Oueslati, S., Hessini, K., Rabhi, M., & Abdely, C. (2021). Seed-priming with H_2O_2 alleviates subsequent salt stress by preventing ROS production and amplifying antioxidant defense in cauliflower seeds and seedlings. *Scientia Horticulturae*, 288, 110360. <https://doi.org/10.1016/j.scienta.2021.110360>
- Intarasi, S., & Saengnil, K. (2021). Transient production of H_2O_2 and NO induced by ascorbic acid coincides with promotion of antioxidant enzyme activity and reduction of pericarp browning of harvested longan fruit. *Scientia Horticulturae*, 277, 109784. <https://doi.org/10.1016/j.scienta.2020.109784>
- Jiang, X. J., Lin, H. T., Lin, M. S., Chen, Y. H., Wang, H., Lin, Y. X., et al. (2018). A novel chitosan formulation treatment induces disease resistance of harvested litchi fruit to *Peronophythora litchii* in association with ROS metabolism. *Food Chemistry*, 266, 299–308. <https://doi.org/10.1016/j.foodchem.2018.06.010>
- Li, D., Zhang, X. C., Li, L., Aghdam, M. S., Wei, X. X., Liu, J. Q., et al. (2019). Elevated CO_2 delayed the chlorophyll degradation and anthocyanin accumulation in postharvest strawberry fruit. *Food Chemistry*, 285, 163–170. <https://doi.org/10.1016/j.foodchem.2019.01.150>
- Li, S. B., Tian, Y. F., Jiang, P., Lin, Y., Liu, X. L., & Yang, H. S. (2021). Recent advances in the application of metabolomics for food safety control and food quality analyses. *Critical Reviews in Food Science and Nutrition*, 61(9), 1448–1469. <https://doi.org/10.1080/10408398.2020.1761287>
- Lin, L. J., Lin, Y. X., Lin, H. T., Lin, M. S., Ritenour, M. A., Chen, Y. H., et al. (2019a). Comparison between 'Fuyan' and 'Dongbi' longans in aril breakdown and respiration metabolism. *Postharvest Biology and Technology*, 153, 176–182. <https://doi.org/10.1016/j.postharvbio.2019.04.008>
- Lin, Y. F., Chen, M. Y., Lin, H. T., Hung, Y. C., Lin, Y. X., Chen, Y. H., et al. (2017). DNP and ATP induced alteration in disease development of *Phomopsis longanae* Chi-inoculated longan fruit by acting on energy status and reactive oxygen species production-scavenging system. *Food Chemistry*, 228, 497–505. <https://doi.org/10.1016/j.foodchem.2017.02.045>
- Lin, Y. F., Chen, M. Y., Lin, H. T., Lin, M. S., Hung, Y. C., Lin, Y. X., et al. (2018). *Phomopsis longanae*-induced pericarp browning and disease development of longan fruit can be alleviated or aggravated by regulation of ATP-mediated membrane lipid metabolism. *Food Chemistry*, 231, 238–246. <https://doi.org/10.1016/j.foodchem.2018.07.060>
- Lin, Y. F., Lin, Y. Z., Lin, Y. X., Lin, M. S., Chen, Y. H., Wang, H., et al. (2019b). A novel chitosan alleviates pulp breakdown of harvested longan fruit by suppressing disassembly of cell wall polysaccharides. *Carbohydrate Polymers*, 217, 126–134. <https://doi.org/10.1016/j.carbpol.2019.04.053>
- Lin, Y. F., Lin, H. T., Lin, Y. X., Zhang, S., Chen, Y. H., & Jiang, X. J. (2016). The roles of metabolism of membrane lipids and phenolics in hydrogen peroxide-induced pericarp browning of harvested longan fruit. *Postharvest Biology and Technology*, 111, 53–61. <https://doi.org/10.1016/j.postharvbio.2015.07.030>

- Lin, Y. F., Lin, H. T., Zhang, S., Chen, Y. H., Chen, M. Y., & Lin, Y. X. (2014). The role of active oxygen metabolism in hydrogen peroxide-induced pericarp browning of harvested longan fruit. *Postharvest Biology and Technology*, *96*, 42–48. <https://doi.org/10.1016/j.postharvbio.2014.05.001>
- Lin, Y. F., Lin, Y. X., Lin, H. T., Zhang, S., Chen, Y. H., & Shi, J. (2015). Inhibitory effects of propyl gallate on browning and its relationship to active oxygen metabolism in pericarp of harvested longan fruit. *LWT - Food Science and Technology*, *60*, 1122–1128. <https://doi.org/10.1016/j.lwt.2014.10.008>
- Lin, Y. X., Lin, H. T., Chen, Y. H., Wang, H., Ritenour, M. A., & Lin, Y. F. (2019c). Hydrogen peroxide-induced changes in activities of membrane lipids-degrading enzymes and contents of membrane lipids composition in relation to pulp breakdown of longan fruit during storage. *Food Chemistry*, *297*, 124955. <https://doi.org/10.1016/j.foodchem.2019.124955>
- Lin, Y. X., Lin, H. T., Fan, Z. Q., Wang, H., Lin, M. S., Chen, Y. H., et al. (2020a). Inhibitory effect of propyl gallate on pulp breakdown of longan fruit and its relationship with ROS metabolism. *Postharvest Biology and Technology*, *168*, 111272. <https://doi.org/10.1016/j.postharvbio.2020.111272>
- Lin, Y. X., Lin, Y. F., Lin, M. S., Fan, Z. Q., & Lin, H. T. (2021). Influence of hydrogen peroxide on the ROS metabolism and its relationship to pulp breakdown of fresh longan during storage. *Food Chemistry: X*, *12*, 100159. <https://doi.org/10.1016/j.fochx.2021.100159>
- Lin, Y. Z., Li, N., Lin, H. T., Lin, M. S., Chen, Y. H., Wang, H., et al. (2020b). Effects of chitosan treatment on the storability and quality properties of longan fruit during storage. *Food Chemistry*, *306*, 125627. <https://doi.org/10.1016/j.foodchem.2019.125627>
- Liu, J. Y., Lin, Y. F., Lin, H. T., Lin, M. S., & Fan, Z. Q. (2021). Impacts of exogenous ROS scavenger ascorbic acid on the storability and quality attributes of fresh longan fruit. *Food Chemistry: X*, *12*, 100167. <https://doi.org/10.1016/j.fochx.2021.100167>
- Mandal, S., Mitra, A., & Mallick, N. (2008). Biochemical characterization of oxidative burst during interaction between *Solanum lycopersicum* and *Fusarium oxysporum* f. sp. *lycopersici*. *Physiological and Molecular Plant Pathology*, *72*, 56–61. <https://doi.org/10.1016/j.pmpp.2008.04.002>
- Sun, J. Z., Lin, H. T., Zhang, S., Lin, Y. F., Wang, H., Lin, M. S., et al. (2018). The roles of ROS production-scavenging system in *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl.-induced pericarp browning and disease development of harvested longan fruit. *Food Chemistry*, *247*, 16–22. <https://doi.org/10.1016/j.foodchem.2017.12.017>
- Wang, H., Chen, Y. H., Sun, J. Z., Lin, Y. F., Lin, Y. X., Lin, M. S., et al. (2018). The changes in metabolisms of membrane lipids and phenolics induced by *Phomopsis longanae* Chi infection in association with pericarp browning and disease occurrence of postharvest longan fruit. *Journal of Agricultural and Food Chemistry*, *66*, 12794–12804. <https://doi.org/10.1021/acs.jafc.8b04616>
- Wen, X. F., Geng, F., Xu, Y. S., Li, X., Liu, D. Y., Liu, Z. D., et al. (2022). Quantitative transcriptomic and metabolomic analyses reveal the changes in *Tricholoma matsutake* fruiting bodies during cold storage. *Food Chemistry*, *381*, 132292. <https://doi.org/10.1016/j.foodchem.2022.132292>
- Xu, Y. Q., Charles, M. T., Luo, Z. S., Mimeo, B., Chao, T. Z., Veronneau, P. Y., et al. (2019). Ultraviolet-C priming of strawberry leaves against subsequent *Mycosphaerella fragariae* infection involves the action of reactive oxygen species, plant hormones, and terpenes. *Plant Cell & Environment*, *42*, 815–831. <https://doi.org/10.1111/pce.13491>
- Xue, H. L., Sun, Y. X., Li, L., Bi, Y., Hussain, R., Zhang, R., et al. (2020). Acetylsalicylic acid (ASA) induced fusarium rot resistance and suppressed neosolanol production by elevation of ROS metabolism in muskmelon fruit. *Scientia Horticulturae*, *265*, 109264. <https://doi.org/10.1016/j.scienta.2020.109264>
- Yi, C., Jiang, Y. M., Shi, J., Qu, H. X., Xue, S., Duan, X. W., et al. (2010). ATP regulation of antioxidant properties and phenolics in litchi fruit during browning and pathogen infection process. *Food Chemistry*, *118*, 42–47. <https://doi.org/10.1016/j.foodchem.2009.04.074>
- Zhang, J. N., Chen, Y. H., Lin, Y. F., Chen, M. Y., Lin, H. T., & Wang, Z. H. (2013). Biological characteristics of *Phomopsis longanae* Chi. *Chinese Journal of Tropical Crops*, *34*, 695–699. <https://doi.org/10.3969/j.issn.1000-2561.2013.04.020>
- Zhang, X. F., Guo, S., Ho, C. T., & Bai, N. S. (2020). Phytochemical constituents and biological activities of longan (*Dimocarpus longan* Lour.) fruit: A review. *Food Science and Human Wellness*, *9*, 95–102. <https://doi.org/10.1016/j.fshw.2020.03.001>
- Zrig, A., AbdElgawad, H., Tounekti, T., Mohamed, H. B., Hamouda, F., & Khemira, H. (2021). Potassium and calcium improve salt tolerance of *Thymus vulgaris* by activating the antioxidant systems. *Scientia Horticulturae*, *277*, 109812. <https://doi.org/10.1016/j.scienta.2020.109812>

Further reading

- Lin, Q., Xie, Y. J., Guan, W. Q., Duan, Y. Q., Wang, Z. D., & Sun, C. D. (2019). Combined transcriptomic and proteomic analysis of cold stress induced sugar accumulation and heat shock proteins expression during postharvest potato tuber storage. *Food Chemistry*, *297*, 124991. <https://doi.org/10.1016/j.foodchem.2019.124991>