Food Chemistry 240 (2018) 863–869

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Application of propyl gallate alleviates pericarp browning in harvested longan fruit by modulating metabolisms of respiration and energy

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ARTICLE INFO

Keywords:
Longan (*Dimocarpus longan* Lour.)
Pericarp browning
Energy metabolism
Respiration metabolism
Propyl gallate

ABSTRACT

Effects of propyl gallate on metabolisms of respiration and energy of harvested 'Fuyan' longans and its relationship to pericarp browning were investigated. Compared to control longans, propyl gallate could reduce ascorbic acid oxidase (AAO) activity, lower cytochrome *C* oxidase (CCO) activity during early-storage and mid-storage, increase NADK activity, elevate contents of NADP and NADPH, decrease contents of NAD and NADH, in addition, lower the decreases of ATP content and energy charge (E.C.), increase activities of mitochondrial H^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase during early-storage and mid-storage. Above results suggested that propyl gallate-retarded browning development in pericarp of harvested longans was resulted from decreases in activities of respiratory terminal oxidases like CCO and AAO, increase in proportion of pentose phosphate pathway (PPP) to Embden-Meyerhof pathway (EMP) and tricarboxylic acid (TCA) cycle, and maintenance of mitochondrial integrity via retaining higher levels of ATP content and energy charge, as well as higher activities of mitochondrial ATPase.

1. Introduction

Longan (*Dimocarpus longan* Lour.) is a characteristic fruit with high nutritional and medicinal value in southern China, also in many other countries in the world (Chen et al., 2014; Jiang et al., 2007; Lin, Chen, Chen, & Hong, 2001; Lin, Hu, et al., 2013; Lin et al., 2014; Lin, Lin, Lin, et al., 2016). However, it is vulnerable to pericarp browning, the main reason for restriction of transportation and loss of market value (Duan et al., 2007; Lin et al., 2001, 2014; Lin, Lin, Lin, et al., 2016; Lin, Lin, Lin, Ritenour et al., 2017; Su et al., 2005). Recently, there are mounting evidences showed that the disorder of energy metabolism might account for browning, chilling injury or other symptoms of senescence (Jin et al., 2015; Li, Yin, Song, & Zheng, 2016; Liu et al., 2007; Pan, Yuan, Zhang, & Zhang, 2017; Yang et al., 2009). Moreover, most energy (95%) is generated in the process of oxidative phosphorylation, the third stage of respiration metabolism conducted in the inner mitochondrial membrane (Qin, Wang, Liu, Li, & Tian, 2009). After several steps in the electron transport chain, oxygen is eventually reduced to hydrogen oxide, with the release of energy (Jin et al., 2013; Wang, Wang, Liang, & Zhao, 2008; Yang et al., 2009). The enzyme complexes like cytochrome *C* oxidase (CCO) on the inner mitochondrial membrane could use the released energy to generate ATP and to pump proton against electrochemical proton gradient into inter-membrane space of mitochondria (Jiang et al., 2007; Zhou et al., 2014). Although this process is efficient, there is still a small amount of electron may reduce oxygen prematurely to form superoxide reactive oxygen species (ROS) (Larsen, Schiffer, Weitzberg, & Lundberg, 2012). These substances can cause oxidative stress and mitochondrial recession, which, in turn, will block respiratory metabolism and energy production (Jiang et al., 2007; Li et al., 2016; Lin, Lin, Lin, Ritenour, et al., 2017).

Previous reports revealed that hydrogen peroxide (H_2O_2), the most consistent ROS, increased activities of CCO and ascorbic acid oxidase (AAO) with altered respiration metabolism pathway (Lin, Lin, Chen, et al., 2016). It also reduced ATPase activity and energy charge, and consequently aggravated longan pericarp browning (Lin, Lin, Lin, Ritenour, et al., 2017). In addition, propyl gallate was reported to decrease respiration rate, reduce generation of ROS, preserve unsaturated fatty acids of cell membrane lipid, and thus delay longan pericarp browning (Lin, Hu, et al., 2013; Lin, Lin, Chen, Chen, & Lin, 2013; Lin et al., 2015; Lin, Lin, Lin, Shi, et al., 2017). However, application of propyl gallate for alleviating pericarp browning of harvested longan fruit in association with the metabolisms of respiration and energy remains to be clarified. Therefore, effects of propyl gallate on activities of NAD kinase (NADK), CCO, AAO and adenosine triphosphatase (ATPase), contents of nicotinamide adenine dinucleotide phosphate (NADP), reduced nicotinamide adenine dinucleotide phosphate

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http://dx.doi.org/10.1016/j.foodchem.2017.07.118
Received 2 May 2017; Received in revised form 21 July 2017; Accepted 24 July 2017
Available online 25 July 2017
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(NADPH), nicotinamide adenine dinucleotide (NAD), reduced nicotinamide adenine dinucleotide (NADH), adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP), and energy charge were conducted in this work.

2. Materials and methods

2.1. Plant material and treatments

Mature longan (*Dimocarpus longan* Lour. cv. Fuyan) fruit were harvested from an orchard in Anxi County of Fujian province, China, and transported under ambient conditions within 3 h to the laboratory of Institute of Postharvest Technology of Agricultural Products, Fujian Agriculture and Forestry University in Fuzhou, Fujian province, China. Fruits were selected on the basis of the uniformity of maturity, color, shape and size. Any blemished or diseased fruits were excluded.

The selected fruits were separated into two groups. The fruits of group one were dipped in distilled water for 20 min and used as control. Another group of fruits were dipped with 0.5 mM propyl gallate solution for 20 min. The selection of suitable concentration of propyl gallate (0.5 mM) in this experiment was based on our previous published works (Lin, Hu, et al., 2013; Lin et al., 2015), which reported that 0.5 mM propyl gallate-treated longan fruit exhibited a significant ($P < 0.01$) lower pericarp browning index as compared to the control fruit (Appendix 1). The treated fruits were then dried under ambient condition for about one hour, packed in 0.015 mm thick polyethylene bags (50 fruits per bag), stored at (15 ± 1) °C and 80% humidity. Samples were taken initially and at 2-days interval during storage for determining the following physiological and biochemical indices in the process of browning development in pericarp of harvested longan fruit.

2.2. Measurement of activities of cytochrome C oxidase (CCO), ascorbic acid oxidase (AAO), NAD kinase (NADK) and mitochondrial ATPase activity

The methods of Pignocchi, Fletcher, Wilkinson, Barnes, and Foyer (2003), Qin et al. (2009), Jin et al. (2013), Lin, Lin, Chen, et al. (2016) and Zhang et al. (2017) were applied to extract and determine the activities of CCO, AAO and NADK. One unit of CCO, AAO and NADK activity was defined as the amount of enzyme that oxidized 1 μg of cytochrome *C*, ascorbic acid or catalyzed 1 μmol of NADP production in 1 min, respectively.

The method described in our previous study (Lin, Lin, Lin, Ritenour, et al., 2017) was applied to determinate the activity of H^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase in mitochondria. One unit of ATPase activity was defined as the amount of enzyme that catalyzed 1 μmol of inorganic phosphate (Pi) production by ATP decomposition per hour

The method of Bradford (1976) was used to determine protein content. The unit of $U \cdot mg^{-1}$ protein was used to express the activities of CCO, AAO, NADK and ATPase.

2.3. Assay of contents of NADP(H) and NAD(H)

The methods of Chen et al. (2015) and Zhang et al. (2017) were applied to determine the contents of NADP(H) and NAD(H). 1 g longan pericarp tissue from 10 fruit was homogenized with mortar and pestle at 4 °C in 5 mL of 0.1 M HCl for NAD or NADP determination or in 5 mL of 0.1 M NaOH for NADH or NADPH determination. The homogenates were then heated within boiling water bath for 5 min, cooled in an ice bath, and centrifuged for 10 min at 4 °C and 10,000 × *g*. Supernatants were neutralized with respectively 0.1 M NaOH or HCl and centrifuged for 10 min at 4 °C and 10,000 × *g*. Final supernatants were kept on ice for the coenzyme assays.

Because of the light sensitivity of phenazine ethosulfate (PES) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), manipulations were carried out in low light. Equal volumes of 1 M Tricine–NaOH buffer, 40 mM ethylene diamine tetraacetic acid (EDTA), 4.2 mM MTT, 16.6 mM PES, and 25 mM glucose-6-phosphate (for determination of NADP and NADPH) or 5 M ethanol (for determination of NAD and NADH) were mixed just before the assays, and 300 μL of this mixture was transferred to 1.5-mL microtubes. 50 μL supernatants were added to the mixture and the volume was brought to 800 μL with 0.1 M NaCl. Tubes containing the assay media were incubated for 5 min at 37 °C water bath. Enzyme cycling was initiated by adding either 50 μL glucose-6-phosphate dehydrogenase (G6PDH) solution [for NADP(H) determination] or alcohol dehydrogenase (ADH) solution [for NAD(H) determination]. ADH or G6PDH reactions were stopped by adding 600 μL 6 M NaCl stock solution after incubated for 40 min at 37 °C. After centrifugation at 10,000 × *g* for 10 min at 4 °C, absorbance of the precipitant dissolved by 4 mL 95% ethanol was measured. The result was expressed with the unit of $\mu mol \cdot g^{-1}$.

2.4. Measurement of contents of ATP, ADP and AMP, and energy charge

Contents of ATP, ADP and AMP, and energy charge were determined by applying the methods of Chen et al. (2014) and Lin, Chen, et al. (2017).

2.5. Statistical analyses

All assays were repeated in triplicate. Data were presented as means ± standard errors. Analytic variance was tested by SPSS version 17.0. Difference at $P < 0.05$ or $P < 0.01$ was considered significantly or extremely significantly, respectively.

3. Results and discussion

3.1. Effects of exogenous propyl gallate treatment on activities of CCO and AAO in pericarp of harvested longan fruit

Cytochrome *C* oxidase (CCO), also called as cytochrome oxidase or mitochondrial complex IV, is located in the end of the mitochondrial respiratory electron transport chain. It can bond with cytochrome *C* to transport electron, from mitochondrial complex I, II and III, to oxygen for forming H_2O (Larsen et al., 2012). When a couple of electrons are transported, two protons will be consumed in mitochondrial matrix. Meanwhile, two protons will be transported from mitochondrial matrix to inter-membrane space of mitochondria (Li et al., 2016). Alteration in CCO activity can reflect respiratory activity and functional characteristics of mitochondria (Zhou et al., 2014). It was presumed that restricted transfer of electrons in cytochrome pathway respiration in fresh-cut 'Hami' melon fruit treated by chlorine dioxide made a contribution to the regulation of respiration rate and the extended shelf life (Guo et al., 2013). Exposure to ultraviolet-C illumination could delay senescence of peach fruit via inhibiting respiratory activity due to reduced CCO activity (Yang, Cao, Su, & Jiang, 2014). The change of CCO activity was shown in Fig. 1A, which illustrated that, for propyl gallate-treated longan fruit, CCO activity decreased rapidly in the first 2 d of storage, increased rapidly during day 2 to the day 8 of storage, and then changed little. Meanwhile, propyl gallate-treated fruit showed significantly ($P < 0.05$) lower CCO activity than the control fruit on days 2, 4 and 6 of storage, coinciding with the lower respiratory rate (Lin, Lin, Chen, Chen, & Lin et al., 2013) and lower pericarp browning index published in our previous study (Lin et al., 2015, Appendix 1). These results together indicated that through the inactivation of CCO, propyl gallate treatment would inhibit the respiratory electron transport chain of mitochondria and reduce the transfer and leak of electron, which might preserve mitochondrial structure, to delay pericarp browning and senescence of longan fruit.

Ascorbic acid oxidase (AAO) containing copper is located in cytoplasm or cell wall. It couples with redox reaction to serve as respiratory terminal oxidase (Aloni, Karni, Deventurero, Turhan, & Aktas, 2008). It

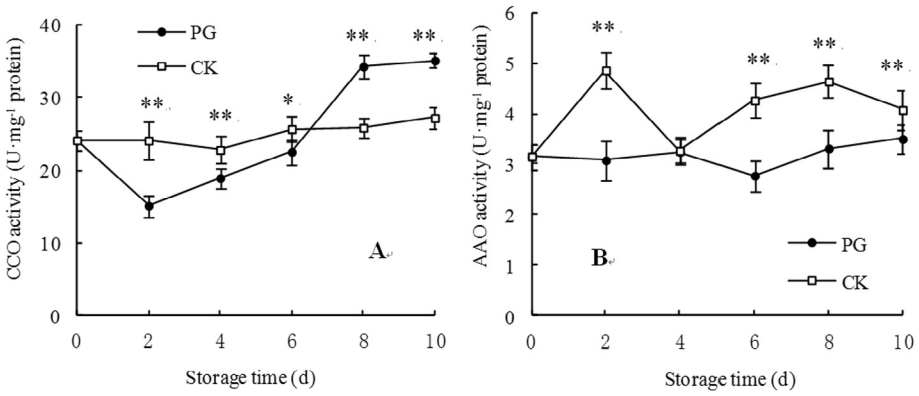
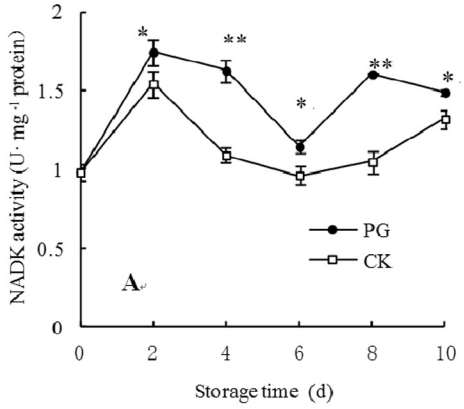


Fig. 1. Effect of propyl gallate treatment on activities of CCO (A) and AAO (B) in pericarp of harvested longan fruits. □, control, ●, propyl gallate (PG). The symbol (* and **) showed significantly difference according to the independent samples *t*-test ($P < 0.05$ and $P < 0.01$, respectively) for each time point.



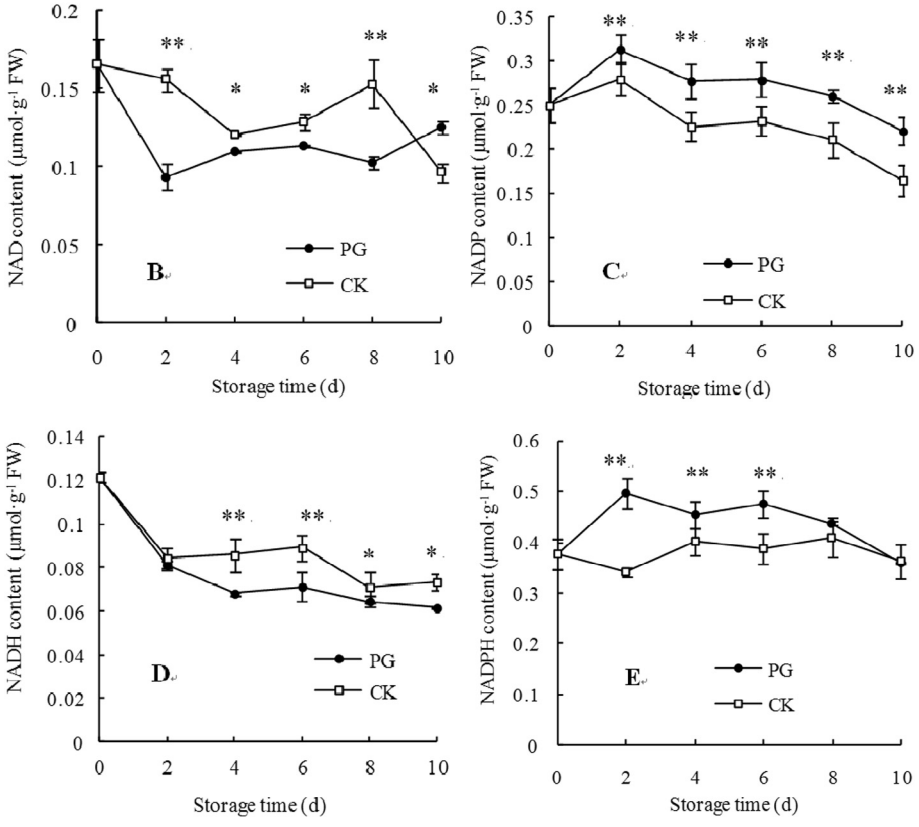


Fig. 2. Effects of propyl gallate treatment on NADK activity and contents of NAD(H) and NADP(H) in pericarp of harvested longan fruits. □, control, ●, propyl gallate (PG). The symbol (* and **) showed significantly difference according to the independent samples *t*-test ($P < 0.05$ and $P < 0.01$, respectively) for each time point.

can catalyze the oxidation of ascorbic acid (AsA) to dehydrogenation ascorbic acid, which may regulate the redox state of ascorbic acid library (Lin, Lin, Chen, et al., 2016). Moreover, AsA, the bioactive forms of ascorbate (ASC), plays crucial role in clearing ROS in plant (Lin et al., 2015; Yang et al., 2011). Yang et al. (2011) reported that higher content of ascorbate in pulp of orange (*Citrus sinensis* Osb.) than satsuma mandarin (*Citrus unshiu* Marc.) was due to lower activity of AAO in orange (*Citrus sinensis* Osb.). Increased concentrations of AsA as well as decreased activity of AAO were observed during pepper fruit ripening. Furthermore, pepper fruit with lower AsA concentration and higher AAO activity was especially prone to blossom-end rot (Aloni et al., 2008). In this work, except for the fourth day of storage, AAO activity in propyl gallate-treated longan fruit was extremely significantly ($P < 0.01$) lower than that in control fruit (Fig. 1B). These indicated that propyl gallate would reduce the oxidation of AsA to dehydrogenated ascorbic acid, which was in accordance with the previous published result that higher level of AsA and lower ROS was displayed in propyl gallate-treated longan fruit (Lin et al., 2015). These results together revealed that propyl gallate would maintain higher ROS scavenging activity and respiratory substrate through the inactivation of AAO, protecting mitochondrial function, and thus delaying pericarp browning and fruit senescence.

3.2. Effects of exogenous propyl gallate treatment on contents of NAD(H) and NADP(H) and NADK activity in pericarp of harvested longan fruit

The whole process of aerobic respiration metabolism can be divided into three stages including Embden – Meyerhof pathway (EMP), tricarboxylic acid (TCA) cycle and oxidative phosphorylation. They are conducted in the cytoplasm, mitochondrial matrix and mitochondrial inner membrane, respectively (Galeazzi et al., 2011; Lin, Lin, Chen, et al., 2016). In the process of EMP, NAD is reduced to NADH carrying electrons and proton, which will be transported to mitochondria with the help of mitochondrial shuttles. With proton-translocation across mitochondrial membrane, mitochondrial NAD transforms into NADH via TCA cycle (Gu, Zhu, & Li, 2007; Stenuit, Lamblin, Cornelis, & Agathos, 2012). NADH could be further oxidized to NAD by oxidative phosphorylation, accompany with the generation of ATP. Also, some NAD from cytoplasm would be converted to NADP by NADK, which consumes ATP as the source of the phosphate group (Chen et al., 2015; Galeazzi et al., 2011). Furthermore, NADPH can be generated by the reduction of NADP during the pentose phosphate pathway (PPP), a respiratory metabolism pathway parallel to EMP (Stenuit et al., 2012). Previous reports showed that the delayed-ripening of tomato (Zhu, Gu, Tao, & Huang, 2007) or strawberry fruit (Gu et al., 2007) stored at low temperature (4 °C) was due to the involvement of higher NADK, which regulated the proportion of NAD(H) to NADP(H), and influence the production rate of ROS. Exposure to H_2O_2 (Lin, Lin, Chen, et al., 2016; Lin, Lin, Lin, et al., 2016) or 2, 4-dinitropheno (Chen et al., 2015) could accelerate pericarp browning and disease development in harvested longan fruit via lowering NADK activity and NADP(H) content, and increasing the proportion of EMP and TCA to PPP. Therefore, different pathways of respiratory metabolism played crucial roles in browning, disease and senescence in harvested crops.

In this work, higher NADK activity (Fig. 2A) in propyl gallate-treated longans demonstrated that more NAD in cytoplasm might be converted to NADP, which was essential to generate NADPH during PPP. This result was in agreement with lower NAD content (Fig. 2B) as well as higher content of NADP (Fig. 2C) and NADPH (Fig. 2E) shown in propyl gallate-treated longans. These together revealed that propyl gallate could increase the proportion of PPP to EMP and TCA, reduce respiratory activity (Lin, Lin, et al., 2013), and thus delay pericarp browning (Lin et al., 2015, Appendix 1).

Furthermore, the rate of transferring electron by NADH-utilizing mitochondrial respiratory chain is much higher than NADPH-utilizing redox pathway, which is viewed as a high-flux circuit and a low-flux circuit, respectively (Stenuit et al., 2012). Meanwhile, ROS is generated in turn by the mitochondrial respiratory chain and redox pathway (Gu et al., 2007; Lin, Lin, Chen, et al., 2016; Zhu et al., 2007). Moreover, NADPH, as a hydrogen donor, is required for the hydrogenation synthesis of fatty acids, an essential component of cell membranes. It also serves as the coenzyme of glutathione reductase to maintain the normal level of reduced glutathione (GSH), an important antioxidant to clear ROS and to protect enzymes and thioredoxins as well as cell membrane from oxidant damage (Cai, Cao, Yang, & Zheng, 2011). The present work showed that propyl gallate-treated longans maintained higher NADPH content (Fig. 2E) as well as lower NADH content (Fig. 2D) than those in control fruit. The results, on one hand, illustrated that propyl gallate could lessen the leakage of electron and ROS. On the other hand, higher level of NADPH in propyl gallate-treated longans was beneficial to synthesize fatty acids for the consolidation of cell membranes and to remain enough GSH for the enhanced removal of ROS. This result supported similar results obtained in our previous experiments, which showed that propyl gallate treatment could increase GSH content, reduce ROS leakage and fatty acids disintegration of membrane lipids (Lin et al., 2015; Lin, Lin, Lin, Shi, et al., 2017).

3.3. Effects of exogenous propyl gallate treatment on contents of ATP, ADP and AMP, and the level of energy charge in pericarp of harvested longan fruit

Adenosine triphosphate (ATP), served as energy currency, is crucial for functional characteristics of cell such as vacuole and mitochondria (Jiang et al., 2007; Li et al., 2016). It involves in the threshold for the synthesis of fatty acid or phospholipid in cell membrane (Jin et al., 2013; Zhou et al., 2014). ATP shortage could cause lipid peroxidation, thereby launching more production of ROS, which, conversely, attack the cell membrane (Chen et al., 2014; Pan et al., 2017; Yang et al., 2014). Growing evidences suggested that damaged cellular membrane structure because of deficient energy or ATP would result in browning, chilling injury or senescence of various fruits such as longan, litchi, loquat, papaya, peach, tomato, and blueberry. Additionally, these symptoms could be alleviated or inhibited by the maintenance of higher levels of ATP and energy charge, resulted from application of low-temperature storage (Jin et al., 2015; Pan et al., 2017; Zhou et al., 2014), methyl jasmonate (Jin et al., 2013), oxalic acid (Jin, Zhu, Wang, Shan, & Zheng, 2014; Li et al., 2016), short-term anoxia (Liu et al., 2007), pure oxygen (Su et al., 2005) or adenosine triphosphate (Chen et al., 2015; Yi et al., 2010).

In this work, except for energy charge and AMP content on day 2 and day 4 of storage, as compared to the control fruit, there was higher level of ATP (Fig. 3A), ADP (Fig. 3B) and energy charge (Fig. 3D) as well as lower AMP content (Fig. 3C) in propyl gallate-treated longans. These suggested that propyl gallate treatment could supply longans more ATP to synthesize fatty acid in mitochondrial or the entire cellular membrane, to reduce lipid peroxidation and ROS accumulation. This would protect the integrity of mitochondrial or the entire cellular membrane to inhibit pericarp browning. These results were in agreement with lower ROS, MDA (one product of lipid peroxidation) and electronic leak of cell membranes (indicator of membrane integrity) in propyl gallate-treated longans (Lin, Hu, et al., 2013; Lin, Lin, et al., 2013; Lin et al., 2015; Lin, Lin, Lin, Shi, et al., 2017). In addition, higher ATP could provide sufficient phosphate group for the phosphorylation of NAD to NADP, according with higher NADP in propyl gallate-treated longans as shown in Fig. 2C.

3.4. Effects of exogenous propyl gallate treatment on mitochondrial ATPase activity in pericarp of harvested longan fruit

Mitochondrion is the site for energy production and the third stage of respiration metabolism, which is oxidative phosphorylation (Wang et al., 2008). It also combines with other organelles like vacuole,

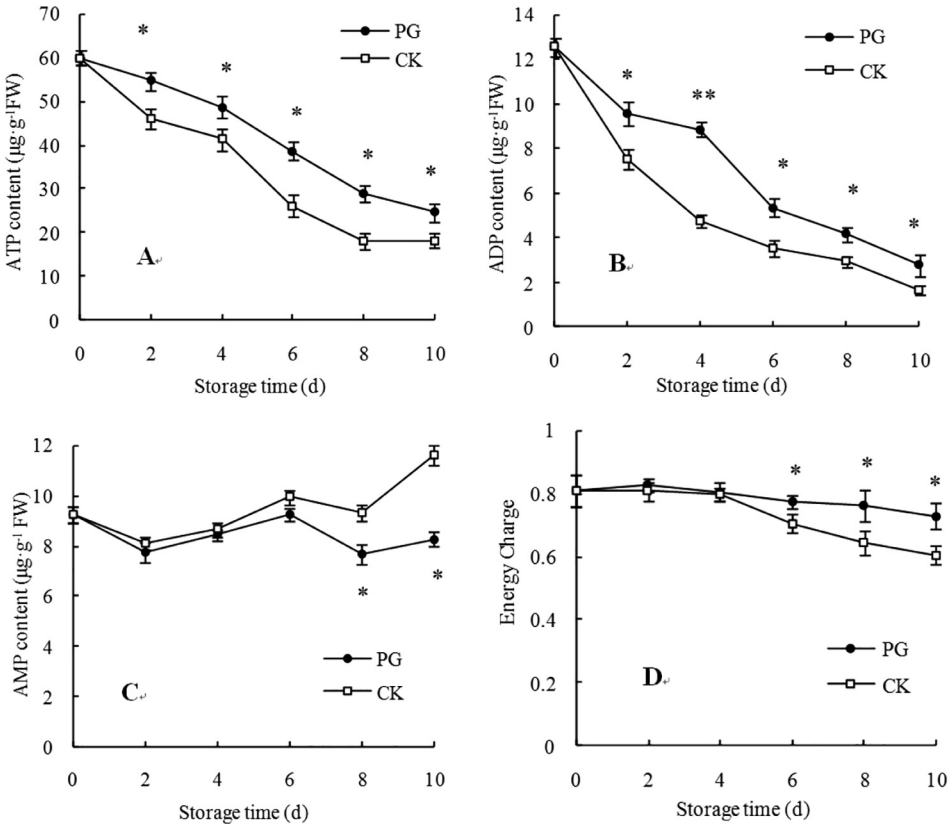


Fig. 3. Effects of propyl gallate treatment on contents of ATP (A), ADP (B) and AMP (C), and energy change (D) in pericarp of harvested longan fruits. □, control, ●, propyl gallate (PG). The symbol (* and **) showed significantly difference according to the independent samples *t*-test ($P < 0.05$ and $P < 0.01$, respectively) for each time point.

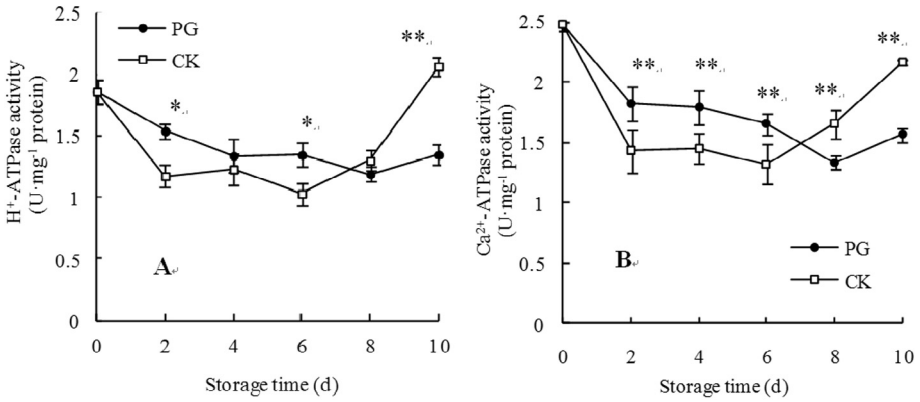
endoplasmic reticulum and extracellular matrix to serve as storage vault of ion such as Ca^{2+} and Mg^{2+} to keep ionic homeostasis (Olsen, Andersen, Lunding, Brasen, & Poulsen, 2009). H^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase, the key respiratory-related enzymes located on inner mitochondrial membrane, are responsible for the synthesis and supply of energy (Lin, Lin, Lin, Ritenour, et al., 2017; Yang et al., 2014). Mitochondrial H^+-ATPase, i.e. F_0F_1-ATP synthase or mitochondrial complex V, could transfer protons between inter-membrane space of mitochondria and the mitochondrial matrix, depending on the electrochemical proton gradient (Jin et al., 2013). When there is sufficient electrochemical proton gradient, the mitochondrial H^+-ATPase acts as an ATP-synthase to synthesis ATP and pumps protons from the inter-membrane space of mitochondria to the mitochondrial matrix. In contrast, it will couple with the energy derived from the hydrolysis of ATP to transport protons from the mitochondrial matrix to the mitochondrial inter-membrane space (Olsen et al., 2009). Calcium (Ca) and magnesium (Mg) are main compositions of phospholipid in mitochondrial membrane and cofactor of respiratory related-enzymes in mitochondrial electron transport chain, respectively (Lin, Lin, Lin, Ritenour, et al., 2017). Ca^{2+}-ATPase and Mg^{2+}-ATPase in mitochondria are crucial for the transport of Ca^{2+} and Mg^{2+} from cytoplasm to mitochondria, respectively. In addition, cells invest much energy to regulate changes in concentrations of Ca^{2+}, Mg^{2+} or other ions (Pan et al., 2017).

It was suggested that H_2O_2 treatment could promote longan pericarp browning via reducing activities of H^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase in mitochondria, and damaging mitochondrial structure (Lin, Lin, Lin, Ritenour, et al., 2017). In contrast, alleviation of skin pitting in papaya fruit stored at 1 °C than stored at 6 °C and 11 °C was resulted from higher activities of H^+-ATPase and Ca^{2+}-ATPase in mitochondria (Pan et al., 2017). In addition, oxalic acid or methyl jasmonate treatment benefited the control of pitting and browning of tomatoes (Li et al., 2016) and peaches (Jin et al., 2013, 2014) through the more active mitochondrial H^+-ATPase and Ca^{2+}-ATPase as well as the maintenance of mitochondrial integrity. It could be inferred that the levels of mitochondrial ATPase were closed to the integrity and the function of mitochondria, which was responsible for energy production and senescence development.

In this work, higher mitochondrial H^+-ATPase activity (Fig. 4A) was displayed in propyl gallate-treated longans during storage day 2, 4 and 6. These suggested that propyl gallate could enhance the ability to transport protons across the mitochondrial membrane and to establish proton electrochemical gradient, which was essential to synthesize ATP, supporting by Fig. 3A that higher ATP contained in propyl gallate-treated longans. Furthermore, from day 2 to day 6 of storage, propyl gallate also increased the activities of Ca^{2+}-ATPase and Mg^{2+}-ATPase (Fig. 4B, C). These demonstrated that free Ca^{2+} or Mg^{2+} could be promptly carried back to mitochondria and ionic homeostasis between mitochondrial matrix and the mitochondrial inter-membrane could be kept by propyl gallate. Also, it was beneficial for inhibiting the deterioration of phospholipid in mitochondrial membrane and supplying the enzymatic cofactor to maintain normal respiratory. These results coincided with lower relative permeability of cell membranes and delayed-metabolism of membrane lipids in propyl gallate-treated longans as shown in previous studies (Lin, Lin, et al., 2013; Lin, Lin, Lin, Shi, et al., 2017). These together contributed to the integrity of mitochondria or even the whole cell and alleviation of pericarp browning of longans. Moreover, as compared with propyl gallate-treated longans, higher activities of mitochondrial H^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase were observed in control longans from storage day 8 to day 10 which might be due to higher contractions of H^+, Ca^{2+} and Mg^{2+} accumulated from day 2 to day 6 of storage.

4. Conclusion

To sum up, the above data indicated that the reduction in pericarp browning of harvested longan fruit by propyl gallate treatment was associated with its inhibition of respiratory activity due to the



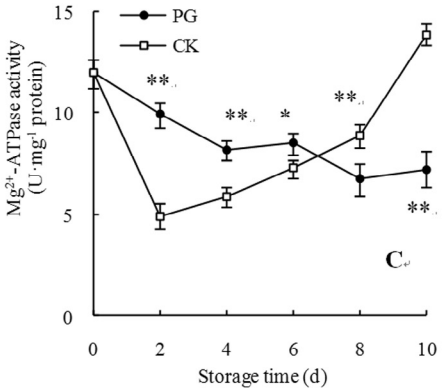


Fig. 4. Effects of propyl gallate treatment on mitochondrial H^+-ATPase (A), Ca^{2+}-ATPase (B) and Mg^{2+}-ATPase (C) in pericarp of harvested longan fruits. □, control, ●, propyl gallate (PG). The symbol (* and **) showed significantly difference according to the independent samples *t*-test ($P < 0.05$ and $P < 0.01$, respectively) for each time point.

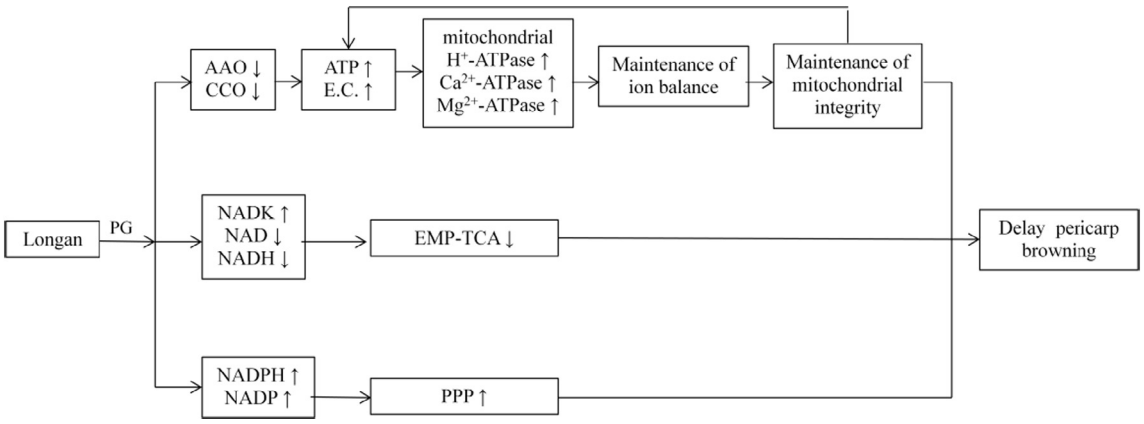


Fig. 5. The probable mechanism of propyl gallate-retarded browning development in pericarp of harvested longan fruit via modulating metabolisms of respiration and energy.

decreased activities of respiratory terminal oxidases like CCO and AAO, and the enhanced proportion of PPP to EMP and TCA. In addition, the maintenance of integrity of mitochondrial membrane in pericarp of propyl gallate-treated longan fruit was resulted from propyl gallate-retaining higher levels of ATP content and energy charge (E.C.), as well as higher activities of mitochondrial H^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase, which involved in retarding browning development in pericarp of propyl gallate-treated longan fruit. The probable mechanism of propyl gallate-retarded browning development in pericarp of harvested longan fruit via modulating metabolisms of respiration and energy was demonstrated in Fig. 5.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant Nos. 31671914, 31601801, 31171776, 30671464, 30972070 and 31201445), the Natural Science Foundation of Fujian Province of China (Grant No. 2015J01608), the Construction Projects of Top University at Fujian Agriculture and Forestry University of China (Grant No. 612014042) and the Science Fund for Distinguished Young Scholars at Fujian Agriculture and Forestry University of China (Grant No. xjq201619).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.07.118.

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