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L-Cysteine hydrochloride delays senescence of harvested longan fruit in relation to modification of redox status

Taotao Li[a,1], Qixian Wu[a,b,1], Yijie Zhou[a,b], Ze Yun[a], Xuewu Duan[a,*], Yueming Jiang[a]

[a] South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[b] University of Chinese Academy of Sciences, Beijing 100039, China

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ABSTRACT

Longan fruit has a limited postharvest life due to aril breakdown, a typical senescence characteristics. In this study, effects of L-cysteine hydrochloride (LCH) on senescence of harvested longan fruit associated with modification of redox status were investigated. Application of LCH delayed aril breakdown and maintained fruit quality, accompanied by reduced H_2O_2 accumulation and alleviated protein oxidation. Furthermore, expression of several genes related to elimination of H_2O_2 and peroxide, *APX* and *GST*, regeneration of glutathione and ascorbic acid, *GR* and *DHAR*, were up-regulated by LCH. Moreover, LCH treatment well maintained the expression level of three oxidized-protein repair-related genes, *Msr*, *MsrA5* and *MsrB2*. Additionally. LCH treatment inhibited expression of *EGase*, *PLD* and *LOX*. EGase was associated with degradation of cell wall, while PLD and LOX were associated with degradation of cell membrane. Overall, these findings suggested that LCH treatment enhanced anti-oxidant and oxidized-protein repair capacities, and maintained higher reducing state, which contributed to reducing oxidative damage and, thereby, delayed senescence of longan fruit.

1. Introduction

Longan (*Dimocarpus Longan* Lour.) is a typical non-climacteric subtropical fruit. Longan represents an important economic crop in some countries, including China, Thailand, Vietnam, India, Philippines, Indonesia and Australia (Jiang et al., 2002). However, the fruit deteriorates rapidly after harvest due to aril breakdown and decay development, which reduces market values (Duan et al., 2011a). Aril breakdown is a typical senescence characteristics for harvested longan fruit (Jiang et al., 2002). Postharvest technologies have been developed to control aril breakdown of longan fruit, such as sulfur fumigation (Jiang et al., 2002) and treatments with physiological active substances (Duan et al., 2007; Duan et al., 2011a; Li et al., 2015a; Chen et al., 2015). A better understanding of longan fruit senescence may help in developing strategies to improve nutritional and sensorial qualities and reduce postharvest loss of the fruit.

Fruit senescence is a developmentally programmed degeneration process and regulated by various internal and external factors such as genetic factors, developmental signal, hormones, light and temperature (Tian et al., 2013). According to ripening or senescence attributes, fruits are classified as climacteric or non-climacteric. Senescence mechanism of climacteric and non-climacteric fruits are rather different. Climacteric fruits, such as banana and mango, undergo ripening after harvest to develop edible quality. Ethylene plays a crucial role in regulating ripening and senescence of climacteric fruits (Lee et al., 2012). However, non-climacteric fruits, such as citrus and strawberry undergo no ripening process after harvest, which do not require increased ethylene biosynthesis during the ripening and senescence process (Alexander and Grierson 2002). Some studies have linked abscisic acid (ABA) to ripening and senescence of non-climacteric fruits such as strawberry (Ji et al., 2012) and citrus (Wu et al., 2014). However, the underlying regulatory mechanisms of non-climacteric fruit ripening or senescence are still inadequately understood.

Reactive oxygen species (ROS) are produced in living organisms by aerobic metabolism, which play important roles in regulating a broad range of processes. Under stress conditions or during aging, imbalance of ROS production and elimination results in excessive accumulation of ROS. ROS are highly reactive components that can cause oxidative damage to macromolecules such as proteins, DNA and lipids (Moller et al., 2007). Excessive oxidative damage can result in cell injury and accelerated senescence (Tian et al., 2013). Recent discoveries have related fruit senescence to ROS accumulation and oxidative damage of protein. Longan fruit is a typical non-climacteric fruit that is characterized by a vigorous aerobic metabolism and rapid senescence after

* Corresponding author.
E-mail addresses: wubangcai88@163.com (T. Li), wuqixian@scbg.ac.cn (Q. Wu), zhouyijie123@scib.ac.cn (Y. Zhou), xwduan@scbg.ac.cn (X. Duan), ymjiang@scbg.ac.cn (Y. Jiang).
[1] These authors contributed equally to this work.

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harvest (Jiang et al., 2002). It is speculated that regulation of redox status plays an important role in senescence of harvested longan fruit.

L-cysteine, one of the most effective anti-browning agent, is widely used in fresh cut fruits and vegetables either alone or in combination with different organic acids/edible coatings (Colantuono et al., 2015; Pace et al., 2015). L-cysteine acts as an inhibitor of PPO and PPO enzymes. During oxidation of phenolic compounds, L-cysteine traps o-quinones, developing colorless products 'cysteinyl-adducts' which are the competitive inhibitor of PPO enzyme (Richard et al., 1991). L-cysteine hydrochloride (LCH) is also a reactive oxygen species scavenger (Day et al., 2003). Our previous research showed that application of L-cysteine hydrochloride (LCH), a derivative of L-cysteine, delay aril breakdown of harvested longan fruit (Duan et al., 2011a). However, the underlying mechanism has not been elucidated. We hypothesize that modification of redox status by LCH is involved in the regulation of senescence in harvested longan fruit. The objective of this study was to evaluate the effect of L-cysteine hydrochloride (LCH) on quality and redox status of harvested longan fruit. In addition, expression of genes associated with ROS metabolism, repair of oxidized proteins, cell wall and lipid degradation were also investigated. The results will help to understand the role of redox regulation in senescence of harvested longan fruit.

2. Materials and methods

2.1. Plant materials and treatments

Commercial mature longan (*Dimocarpus longan* Lour.) cv. Shixia were obtained from an orchard in Conghua, Guangdong province of China. Fruit of uniform shape and color were disinfected with 0.1% Sportak solution for 3 min, air dried for 2 h, and then dipped for 5 min in 0.05% L-cysteine hydrochloride (LCH) solution. The concentration of LCH was decided according our previous research (Duan et al., 2011a). After dipping, the fruits were air-dried for 30 min, packed in 0.03 mm thick polyethylene bags (twenty fruit per bag) and then stored at 25 °C. The fruit dipped in water was used as control. Samples were taken initially and at 2 d intervals during storage for quality evaluation and following analyses. Aril tissue was immediately frozen in the liquid nitrogen, and then stored at −80 °C for RNA extraction. Thirty fruit were sampled for each replicate and three biological replicates were conducted independently.

2.2. Aril breakdown index

Aril breakdown index was measured as described by Duan et al. (2011a). A total of sixty individual fruit were scored on a scale from 0 (none), 1 (slight), 2 (moderate) to 3 (severe) in terms of firmness. The breakdown index was calculated using the following formula: Σ(aril breakdown scale × percentage of fruit within each scale).

2.3. Measurement of total soluble solids, sugar acid ratio and pH in longan aril

Aril tissue from thirty fruit was homogenized in a grinder and the supernatant was collected to analyze the total soluble solids (TSS). TSS was assayed using a hand refractometer (J1-3A, Guangdong Scientific Instruments). Sugar acid ratio was measured by Digital Brix/Acidity meter (PAL-BXIACID1, ATAGO, Japan). The pH value was measured using Digital pH Meter DPH-2 (ATAGO, Japan).

2.4. Measurement of amino acid content by HPLC

Samples obtained from 0 d, 4 d and 8 d were used for amino acid measurement. Amino acid composition was determined by high performance liquid chromatography (Sykam, Germany) equipped with a Cation Separation column (150 mm × 4.6 mm). Briefly, 1 g of the aril sample from thirty fruit was ground with liquid nitrogen for amino acid analysis. The sample was extracted using 50 mL 0.01 M HCl, then after filtrated, 2 mL of filtrate was mixed with ethanol (final ethanol content was 80%) and kept at still position for 30 min. The extract was then centrifuged and dried using SpeedVac and the residue was re-dissolved with 1 mL 0.01 M HCl. Then the solution was filtered through a 0.22 μm Millipore membrane filter prior to HPLC analysis. The amino acid standards included L-*O*-Phosphoserine (P-Ser), Taurine (Tau), phosphorylethanolamine (P-Eta), Urea, L-aspartic acid (Asp), L-threonine (Thr), L-serine (Ser), L-Asparagine (Asn), L-glutamic acid (Glu), L-α-aminoadipic acid (Ada), L-glycine (Gly), L-alanine (Ala), L-Citrulline (Cit), α-aminobutyric acid (Aba), L-valine (Val), L-cystine (Cys), L-methionine (Met), L-isoleucine (Ile), L-leucine (Leu), L-tyrosine (Tyr), L-phenylalanine (Phe), β-Alanine (β-Ala), β-aminoisobutyric acid (β-AiBA), γ-Aminobutyric acid (GABA), L-histidine (His), 3-methylhistidine (3-Mehis), 1-methylhistidine (1-Mehis), L-Carnosine (Car), L-tryptophan (Try), L-ornithine (Orn), L-lysine (Lys), L-arginine (Arg) and L-proline (Pro). The results of all amino acids were expressed in mg per kg of fruit fresh weight.

2.5. Respiration rate measurement

Respiration rate was measured according to the method of our previous research (Li et al., 2015b). Ten fruits were sealed inside a 4.2 L glass jar for 2 h at 20 °C. Then one mL aliquots of headspace gas which was withdrawn from the jars was injected into a gas chromatograph (GC-9A; Shimadzu, Kyoto, Japan) using a thermal conductivity detector (TCD) and a Poropak N column (Shimadzu). Respiration rate was expressed as increased CO_2 per second on a fresh weight basis.

2.6. Measurement of the contents of H_2O_2, protein carbonyl and total antioxidant capacity

The measurement of H_2O_2 content and total antioxidant capacity (T-AOC) were carried out using Hydrogen Peroxide Assay Kit and Total Antioxidant Capacity Assay Kit (Jiancheng Bioengineering, Nanjing, China) respectively, according to the manufacturer's instructions. H_2O_2 content and T-AOC were spectrophotometrically quantified with H_2O_2-specific reagent molybdic acid and Fe^{2+} (which was reduced from Fe^{3+})-specific reagent phenanthroline, respectively. H_2O_2 contents was expressed on a fresh weight basis, whereas T-AOC was expressed on a protein basis. Around 1 g of sample from thirty fruit was used for the protein carbonyl content analysis. Protein carbonyl content was determined a carbonyl-specific reagent 2, 4-dinitrophenylhydrazine according to Jiang et al. (2017). Protein carbonyl content was expressed on a fresh weight basis.

2.7. RNA isolation and real-time quantitative PCR

Total RNA of each sample (about 5 g) from thirty fruit was isolated according to the method described by Kuang et al. (2012). Real-time quantitative PCR was used to validate the gene expression level of the related genes. The first strand cDNA was synthesized using the PrimeScript™ RT Master Mix (TaKaRa-RR036A, Dalian, China). The specific primer pairs of the selected genes were designed using Primer Premier 6.0 software (Premier, Canada), which were listed in Supplementary Table S1. *18SrRNA* was used as the endogenous control to normalize the content of cDNA according to previous research (Wu et al., 2016). Our previous method was used for the detailed operation (Li et al., 2015b) and the comparative Ct ($^{\Delta\Delta}$CT) method was performed to determine the relative change in the gene expressions.

2.8. Statistical analysis

The results of physiological characteristics were expressed as the mean values of three biological replicates. SPSS version 7.5 was used to