Postharvest Biology and Technology 116 (2016) 26–35

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Effects of chlorine dioxide on mitochondrial energy levels and redox status of 'Daw' longan pericarp during storage

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ARTICLE INFO

Article history:
Received 31 August 2015
Received in revised form 3 January 2016
Accepted 4 January 2016
Available online 11 January 2016

Keywords:
Energy status
Redox status
Respiratory enzymes
Fruit senescence
Dimocarpus longan

ABSTRACT

Mitochondrial redox status and adenosine triphosphate (ATP) production play a central role in responding to physiological and environmental changes and metabolic control. Changes in mitochondrial redox potential are intimately associated with oxidative stress which could lead to plant senescence. This study aimed to demonstrate the involvement of an alteration of the redox status in energy production and fruit senescence of longan (*Dimocarpus longan* Lour. cv. Daw) during storage and to investigate the effects of gaseous chlorine dioxide (ClO_2) both on the redox and the energy status and on delaying senescence. Fresh longan fruit were fumigated with 5, 10 and 25 mg/L ClO_2 for 10 min and then stored in a cardboard box at 25 °C with 82% relative humidity for 7 days. ATP content and energy charge (EC), and activities of respiratory enzymes such as succinate dehydrogenase (SDH) and cytochrome *c* oxidase (CCO) were found to be steadily decreased in the untreated group during storage. These reductions coincided with a decrease in the redox status as determined by pyridine nucleotide (NAD/NADH) and ubiquinone (Q/QH_2) ratios and with fruit senescence including browning and the onset of disease symptoms. However, in the ClO_2 fumigated fruit, ATP content, EC, SDH and CCO activities as well as NAD/NADH ratio were immediately and markedly increased after treatment. The effects can be seen through the fourth day of storage. The Q/QH_2 ratio remained unchanged before rising after Day 4. Higher concentrations of ClO_2 (10 and 25 mg/L) were more effective than the lower one (5 mg/L) in altering the redox balance and increasing energy production. The altered redox state and increased energy by ClO_2 were found to be closely associated with a delay in fruit senescence during storage. These findings support the notion that energy production and redox balance involve directly in longan senescence and demonstrated that ClO_2 could restore ATP and the redox balance, leading to reduction and delay in fruit senescence.

1. Introduction

The oxidation–reduction (redox) status is an important regulator of various metabolic functions of cells. The cellular redox state is made tangible in terms of the redox state or redox status of the individual redox-active molecules in the cell (Potters et al., 2010). Cellular respiration, one of the major redox systems, produces energy needed for various activities of the cell including growth and survival. Electron transport chain (ETC) utilizes the potential energy harvested during the redox reaction steps to produce ATP. ETC enzymes such as reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase, succinate dehydrogenase (SDH), cytochrome *c* oxidase (CCO) and ATP synthase facilitate ATP synthesis from the inner mitochondrial membrane (Soole and Menz, 2013; Schertl and Braun, 2014). Redox status, in respiration, can be envisioned as the ratio of oxidized nicotinamide adenine dinucleotide (NAD) to NADH (NAD/NADH) and ubiquinone (Q) to ubiquinol (QH_2) (Q/QH_2). In a plant cell, both the redox and the energy status as well as respiratory enzyme activities have been shown to be affected by internal and external factors (Yang et al., 2009; Jin et al., 2013, 2014; Ostaszewska et al., 2014).

Considerable evidences suggested that the redox status is a key factor controlling senescence in some horticultural crops (Potters et al., 2010). Wang et al. (2013) reported that alteration in the redox potential and, consequently, the reduction in energy production during various stress conditions leads to senescence of fruits. Stress causes a reduction in the energy status of fruits during

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http://dx.doi.org/10.1016/j.postharvbio.2016.01.002
0925-5214/

storage. For example, reduction in ATP content and energy charge (EC) in pear fruit coincided with amplification of flesh browning when the fruit was stored at 0 °C (Saquet et al., 2003). During low temperature storage, it was found that ATP content, EC and SDH as well as CCO activities in pear fruit were significantly decreased while chilling injury was significantly increased (Jin et al., 2013, 2014). Similarly, in other fruits such as mango (Li et al., 2014), longan (Chen et al., 2014), litchi (Yang et al., 2009; Wang et al., 2013) and banana (Wang et al., 2015b), the energy status was found to be greatly reduced in conjunction with an increase in injury or other storage damages.

In addition to the energy status, stress conditions also caused changes in the cellular redox status. For example, an increase in QH_2/total ubiquinone (Qt) ratio of litchi fruit coincided with a decrease in ATP, while browning symptom increased under oxidative stress (Yang et al., 2009). The NAD/NADH ratio of bean seedlings and *Arabidopsis* was found to be significantly decreased in conjunction with a decrease in ATP, while growth was inhibited after sulphur-deficient stress (Juszczuk and Ostaszewska, 2011; Ostaszewska et al., 2014). These evidences suggested that senescence is closely related with the redox status and cellular energy supply. Maintaining a healthy redox status and energy efficiency in the cell could, therefore, delay or inhibit cellular deterioration to a certain extent.

In order to delay or reduce senescence via alteration of energy and redox status, various chemicals and physical methods have been employed. Prior treatment with chemical compounds such as methyl jasmonate (MJ), oxalic acid (OA) (Jin et al., 2013, 2014; Li et al., 2014), nitric oxide (NO) (Wang et al., 2015b) and pure oxygen (Su et al., 2005) had been shown to increase ATP content, EC and some respiratory enzyme activities and lessen purishment during storage of several fruits such as pear, mango, banana and longan. Peach fruit exposed to increased atmospheric pressure exhibited similar outcome during storage (Wang et al., 2015a).

Exogenously added ATP could also tip the cellular energy balance in favor of diminishing and controlling aging and senescence of fruits and vegetables. Yi et al. (2008) reported that application of exogenous ATP on litchi fruit before inoculation with *Peronophythora litchi* and storage at 25 °C for 6 days significantly induced the endogenous ATP level and EC while pericarp browning and disease symptom decreased. Litchi fruit immersed in ATP solution showed significant increase in their endogenous ATP and EC while pericarp browning decreased (Yang et al., 2009). Moreover, ATP-treated litchi fruit was found to have enhanced enzymatic antioxidant and antioxidant capacity while pericarp browning decreased (Yi et al., 2010). Similarly, Yao et al. (2014) reported that application of exogenous ATP on longan fruit before storage at 25 °C for 6 days significantly induced enzymatic antioxidant while pericarp browning decreased.

Chlorine dioxide (ClO_2) is a new and promising chemical found to be able to reduce and delay senescence including browning and disease in fruits during storage. Saengnil et al. (2014) reported that applications of gaseous ClO_2 on longan fruit before storage significantly reduced pericarp browning by reducing the oxidation of phenolic compound, delaying the occurrence of disease and maintaining higher fruit quality. Similarly, Chomkitichai et al. (2014a,b) reported that ClO_2 treatment of longan fruit reduced pericarp browning apparently by enhancing the antioxidant defense system which may involve the alteration of redox status in energy production.

Since overwhelming evidences have shown correlation between cellular energy and redox status with the quality of fruits during storage, it is imperative to explore the possibility that ClO_2 could alter energy producing capacities and cellular redox homeostasis such that senescence is delayed in longan.

2. Materials and methods

2.1. Plant materials

Mature longan fruit were harvested from a commercial orchard in Lamphun province, Thailand and transported to the Postharvest Physiology and Technology Research Laboratory at Chiang Mai University within 2 h. Fruit were individually selected for uniformity in shape, color, size and devoid of defect.

2.2. ATP treatments

Longan fruit were divided into 12 groups of 240 fruits each. A set of 3 groups were submerged in 0 (control), 0.5, 1 and 2 mM ATP solution for 10 min at 25 °C, followed by air drying for 30 min. The submerged fruit in each group were further divided into 8 subgroups of 30 fruits each which was stored in a cardboard box (25 cm (L) × 17 cm (W) × 9 cm (H)). The boxes were stored in a storage room at temperature of 25 ± 1 °C with a relative humidity of 82 ± 5% for 7 days. Every day from the beginning of the experiment, the fruit from each treatment were tested for storage fruit quality including browning index, color of pericarp, disease index and overall quality acceptance.

2.3. ClO_2 treatments

Freshly picked longan fruit were divided into 12 groups of 240 fruits each and exposed to ClO_2 (0, 5, 10 and 25 mg/L) in a fumigation chamber for 10 min. Each concentration was done in triplicate. After fumigation, the chamber was ventilated for 30 min to remove any residual ClO_2 Each group was collected and stored in a cardboard box as described in ATP treatment (2.2). From the beginning until the end of the experiment, samples from each replication of the treatments were taken every day to be quantified for the following parameters; energy status, energy producing enzyme activities, redox status and storage fruit quality including browning index, color of pericarp, disease index and overall quality acceptance.

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

ATP, ADP and AMP were extracted and evaluated as described by Liu et al. (2006) with some modifications. Longan pericarp (2 g) was sliced and homogenized at 4 °C for 1 min in 10 mL of 0.6 mM perchloric acid. The homogenate was centrifuged at 6000 × g for 10 min at 4 °C and 5 mL of the supernatant was taken and its pH immediately adjusted to 6.5–6.8 with 1 M KOH. The neutralized supernatant was let stand for 30 min in an ice bath before filtration with Whatman® No. 1 filter (Whatman, England) followed by 0.45 μm filter (MS® Nylon Syringe Filter, USA).

ATP, ADP and AMP were separated and measured using an Agilent 1200 high performance liquid chromatography (HPLC) system (Agilent Corporation, USA) using a reserved phase Eclipse XDB-C18 column (5 μm, 4.6 × 150 mm) and a model G1314B diode array detector at 254 nm. Mobile phase A consisted of 50 mM phosphate buffer (pH 7.0). Mobile phase B was pure acetonitrile. HPLC separation was achieved using continuous gradient elution as follows: 0 min 100% A, 0% B; 2 min 95% A, 5% B; 4 min 80% A, 20% B; 5.3 min 75% A, 25% B and 6 min 100% A, 0% B. The flow rate was 1.2 mL/min, while the injection volume was 20 μL. The total retention time was about 5 min and the gradient was run for 6 min to ensure full separation. ATP, ADP and AMP presence in the samples were identified by comparison with retention time of standards, while their concentrations were determined using the external standard method. Energy charge was calculated by [ATP + 0.5ADP]/[ATP + ADP + AMP].