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Effect of low temperatures on chilling injury in relation to energy status in papaya fruit during storage

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ABSTRACT

Papaya (*Carica papaya* L.), a typical tropical fruit, is susceptible to chilling injury (CI). In the present study, CI index, energy level, and energy metabolism-related enzymes activity in papaya fruit stored at 16, 11, 6, and 1 °C were investigated to evaluate the relationship between CI and energy metabolism of papaya fruit during cold storage. Results showed that there were no symptoms of CI in papaya fruit stored at 16 °C, while some typical CI symptoms including skin pitting, scald and flesh water soaking were appeared in papaya fruit stored 11 °C and 6 °C. Furthermore, we observed that papaya fruit did not appear obvious symptoms of CI during most duration of storage at 1 °C with the exception of slight CI at end of storage. ATP, ADP and the total content of AXP (=ATP + ADP + AMP) contents, EC and energy metabolism-related enzymes activity (H^+-ATPase, Ca^{2+}-ATPase, succinic dehydrogenase (SDH), and cytochrome c oxidase (CCO)) in papaya fruit stored at 1 °C were higher than those in fruit stored at 11 and 6 °C. The results suggest that higher energy status in papaya fruit during cold storage could contribute to the alleviation of CI.

1. Introduction

Papaya (*Carica papaya* L.) is a tropical fruit with important antioxidant properties and is in high demand in international markets (Ali et al., 2014). However, as a tropical and climacteric fruit, papaya fruit has a shorter shelf life due to the rapid pulp softening and peel yellowing in ambient air temperatures (da Silva et al., 2007; Fabi et al., 2007). Therefore, low-temperature storage has become effective method to slow these processes and prevent decay (Aghdam and Bodbodak, 2013). Unfortunately, papaya fruit, like other tropical fruits, are sensitive to cold temperatures (Emond and Brecht, 2005). Below 10 °C, papaya will usually show symptoms of chilling injury (CI), such as pitting of the skin, scald, hard lumps in the pulp around the vascular bundles, water soaking of the flesh, abnormal ripening, and high susceptibility to postharvest decay (Ali et al., 1992; Chan et al., 1985; Chen and Paull, 1986; El-Tomi et al., 1974; Emond and Brecht, 2005).

Energy is the basis of life activities, and energy status is closely related to biological characteristics, such as ripening, senescence, and various physiological disorders in postharvest fruits and vegetables (Saquet et al., 2000; Yang et al., 2009). Recently, several studies have shown that development of CI in fruit is partially dependent upon energy (Li et al., 2014). Longer cold storage led to a reduction in adenosine triphosphate (ATP) and adenosine diphosphate (ADP) contents in blueberries, and low ATP content and energy charge were associated with increased pitting incidence under chilling stress (Zhou et al., 2014). Peaches stored at 5 °C also showed lower contents of ATP, ADP, and energy charge, when compared to those stored at 0 or 10 °C, while the CI was much more severe in fruits stored at 5 °C (Chen et al., 2012). The maintenance of high levels of ATP and energy charge may mitigate CI. For example, pre-storage application of oxalic acid apparently inhibited the development of CI, and maintained high ATP levels and energy charge in mango fruit during cold storage (Li et al., 2014). Cucumbers treated with 6-Benzylaminopurine showed an increased ATP content and a higher level of energy charge, and prevented CI in fruit (Chen and Yang, 2013).

A change in energy level directly depends on energy metabolism. Adenosine triphosphatase (ATPase), succinic dehydrogenase (SDH), and cytochrome c oxidase (CCO) are key enzymes involved in energy metabolism that regulate ATP synthesis (Jin et al., 2013). H^+-ATPase, a proton pumping ATPase in plants, couples ATP hydrolysis with transport of protons out of the cell, establishing an electrochemical proton gradient used for nutrient transport (Sondergaard et al., 2004). Ca^{2+}-ATPase is the primary calcium transporter using energy from ATP hydrolysis. Ca^{2+}-ATPase

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maintains calcium homeostasis in cells and enhances chilling resistance of chilling-sensitive plants (Jian et al., 1999). SDH is responsible for the oxidation of succinate to fumarate in the tricarboxylic acid cycle (TCA) and the reduction of ubiquinone to ubiquinol in the aerobic respiratory pathway (Acevedo et al., 2013), while CCO is the terminal enzyme in the mitochondrial respiratory electron transport chain and plays a key role in aerobic metabolism and energy production during oxidative phosphorylation (Juan et al., 2011). Thus, the activity of CCO and SDH has been monitored as an indirect index of cell energy metabolism (Ekmekcioglu et al., 1999). Blueberries appeared the CI symptoms during storage at 0 °C, meanwhile there was a significant decline in the activity of the energy metabolism-related enzymes including H^+-ATPase, Ca^{2+}-ATPase, CCO, and SDH in the mitochondria of injured fruit (Zhou et al., 2014). A number of postharvest approaches such as nitric oxide (Wang et al., 2015), oxalic acid (Jin et al., 2014) and cryogenic (Jin et al., 2015) treatments have been reported to enhance cold tolerance in fruits, which were associated with improvements in energy metabolism-related enzymes activity in fruits.

Despite several studies indicate a close relationship between CI and energy in fruit, there have been few reports on changes in the energy status and energy metabolism related enzymes in cold-sensitive papaya fruit. Consequently, the objective of this study was to elucidate the relationship between CI and energy in papaya fruit through the study of changes in energy status and related enzymes in papaya fruit stored at a variety of low temperatures. Moreover, we noted that papaya fruit stored at 0 °C appeared to have no obvious CI symptoms such as pitting of the skin and water soaking of the flesh, it was also a point of concern in the present study, and we explicated this from the perspective of energy metabolism.

2. Materials and methods

2.1. Fruit materials

Papaya fruit (*Carica papaya* L. cv. Zhongbai) at the 'color break' stage of ripeness were collected from a commercial orchard in Chengmai county, Hainan province, China. Fruit were transported to the laboratory within 3 h of harvest. Fruit used in this study were of a uniform size and maturity, and were free of mechanical damage and disease.

2.2. Treatments and sampling

First of all, papaya fruit were randomly divided into four groups, each group contained 3 lots (each lot represents a replicate, with forty fruit from each lot), five fruit from each lot were packed into polyethylene bags (0.01 mm in thickness, 40 cm × 30 cm in size), and fruit of 4 groups were respectively stored at 1 ± 0.3 °C, 6 ± 0.3 °C, 11 ± 0.3 °C and 16 ± 0.3 °C for up to 48 d. To assay energy level and the activity of energy metabolism-related enzymes, pulp tissue samples were collected every 6 days during storage, rapidly frozen in liquid nitrogen, and kept at −80 °C until analyzed.

Three replicates of ten fruit each were used to measure the CI index and the yellowing index, and 3 fruit per replicate were used to analyze other physio-biochemical parameters.

2.3. Measurement of chilling injury index and yellowing index

The CI index for each group was visually assessed as described by Li et al. (Li et al., 2014) with minor modifications. Fruits were categorized into five scales of 0–4 according to chilling injury degree (0 = no injury; 1 ≤ 10% chilling spots; 2 = 10%–30% chilling spots; 3 = 30%–50% chilling spots; 4 > 50% chilling spots). The chilling injury index was calculated using the formula: CI index = ∑(chilling scale × number of fruit in each class)/(number of total fruit × highest chilling scale) × 100.

The yellowing index was evaluated based on the area of yellowing on the surface of the fruit using the following scale, with slight modifications: 0 = no yellowing; 1 = yellowing covering <1/5 of the surface; 2 = yellowing covering >1/5 but <2/5 of the surface; 3 = yellowing covering >2/5 but <1/2 of the surface; 4 = yellowing covering >1/2 but <3/5 of the surface; and 5 = yellowing covering >3/5. The yellowing index was calculated using the following formula: Yellowing index (%) = ∑(yellowing scale × number of papaya fruit with that yellowing level)/(total number of papaya fruit × highest yellowing scale) × 100.

2.4. Measurement of firmness

Fruit firmness was measured using a texture analyzer (TA. XTPlus, Stable Micro Systems, Haslemere, Surry, UK). Three fruits in each group were penetrated using an SMS P/2N needle probe with 2 mm diameter (Stable Micro Systems), at a speed of 2 mm/s and a penetration depth of 2 mm on three points in the equatorial region of the whole piece of fruit. Approximately 0.25 cm^2 of skin at each measurement point was removed from the lateral face of the fruit before these measurements were conducted. The compression force, measured at the maximum peak of the recorded force, was expressed in "*g*".

2.5. ATP, ADP and AMP content and energy charge measurements

The extraction and measurement of ATP, ADP, and AMP content were conducted using methods developed by Yi et al. (Yi et al., 2008) and Liu et al. (Liu et al., 2006). Approximately 2 g of fruit tissue were ground in liquid nitrogen and then homogenized with 6 mL of 0.6 M perchloric acid at 4 °C for 20 min. The extraction mixture was centrifuged for 15 min at 19,000 × *g* and at 4 °C, and the resulting supernatant was rapidly neutralized to a pH of 6.5 to 6.8 with 1 M KOH solution. The solution was then diluted to 5 mL with ultrapure water and filtered through a 0.45-mm hydrophilic membrane filter (Millipore Corp., Bedford, MA). ATP, ADP, and AMP measurements were analyzed using an Agilent 1260 Infinity HPLC system equipped with a reversed-phase Nova-Pak C18 column (5 μm, 250 mm × 4.6 mm, Agilent Corp.) and an ultraviolet detector at 254 nm. The mobile phase A consisted of 0.04 M potassium dihydrogen phosphate and 0.06 M dipotassium hydrogen phosphate dissolved in deionized water and was adjusted to a pH of 7.0 with 0.1 M potassium hydroxide, while mobile phase B consisted of 100% chromatographic grade methanol. HPLC separation was achieved using a continuous gradient elution. The elution program was as follows: 0 min 100% A, 0% B; 10 min 90% A, 10% B; 14 min 75% A, 25% B and 15 min 100% A. After this program, it took 2 min to return to the initial conditions and become stabilized. The flow rate of the mobile phase was 0.9 mL/min, while the injection volume was 20 μL. ATP, ADP, and AMP contents were expressed as nmol per gram of FW. Data were expressed as the means of six replicates. The energy charge (EC) was calculated using the formula: EC = ([ATP] + 1/2 [ADP])/([ATP] + [ADP] + [AMP]).

2.6. Energy metabolism-related enzymes activity assay

Mitochondria were crudely extracted from papaya fruit according to the methods Jin et al. (Jin et al., 2013), with some modifications. Twenty grams of frozen tissue were homogenized with 30 mL of 50 mM Tris-HCl buffer (pH 7.5) solution, containing 0.3 M mannitol, 1 mM EDTA, 0.25 M sucrose, 1 g L^{-1} BSA, 1 g L^{-1} cysteine and 5 g L^{-1} polyvinyl pyrrolidone at 4 °C. The extracts were then centrifuged at 4 °C for 20 min at 5000 × *g*. The