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Amelioration of postharvest chilling stress by trehalose in pepper

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ABSTRACT

Currently, reduced shelf-life due to several issues including the elevated chance of chilling stress and the faster deterioration of freshly-cut versus whole fruits, long-term storage of freshly-cut pepper fruits at low temperature storage is still dangerous. In the current report, minimization of pepper chilling stress by the use of trehalose was investigated. Membrane permeability, as well as the concentrations of chlorophyll, vitamin C and malondialdehyde (MDA) were analyzed. Antioxidant peroxidase (POD) and catalase (CAT) enzyme activities were measured. Furtherly, the post-harvest cell ultrastructure of pepper was also observed. These results demonstrated that trehalose enhanced the chilling stress resistance in peppers by reducing cell membrane permeability and MDA content. Activities of POD and CAT in trehalose-treated-fruit were considerably higher than control fruit. Chloroplast membrane was relatively integrated, Osmiophilic globules were inhibited and mitochondria kept their characteristic shape and cristae. Cell walls were undisturbed and the layers of cell wall were clear. These results suggested that trehalose increased pepper-fruit cold tolerance by upregulating antioxidant enzymatic activity and reducing ultrastructural damage.

1. Introduction

Fresh-cut fruits and vegetables (FCFV) are fruits having undergone different modifications including but not limited to peeling, trimming, coring, slicing, shredding or dicing. Moreover, fresh-cut technology seeks to offer consumers an easy-to-obtain, fresh item that is able to last longer on the shelf, have better nutritional value and whose consumption is pleasant on the senses (Reyes, 1996). However, these processes procedures were shown to incur not only cell injuries to the plant, but also biochemical and, microbial variations, which were linked with quality loss for the product (Ayala-Zavala et al., 2009; González-Aguilar et al., 2010; Rico et al., 2007). Since then, several avenues have led to ways in which to preserve product quality such as, ultra-violet radiation, optimized storage air, comestible coverings, heat treatments, and the use of various organic and non-synthetic compounds (González-Aguilar et al., 2010).

There exists a paradox when it comes to maintaining the quality of pepper fruit. On one hand it must be cooled as quickly as possible to minimize fruit decay (González-Aguilar et al., 1999), but this rapid cooling leads to chilling injury (CI) when the fruit highly sensitive to chilling injury is stored colder than 7 °C. Thus, the normal benefits of refrigeration cannot be observed. CI leads to sunken lesions and fruit surface pitting (Fung et al., 2004) and these lead to severe limitations on shelf-life and loss of product quality. However, some avenues including optimized atmospheres, hot water treatments, UV and chemical treatments have led to the lessening of some of the chilling injury (Singh et al., 2014; Vicente et al., 2005; Gonzalez-Aguilar et al., 2000; Purvis, 2002; Korkmaz et al., 2010; Fung et al., 2004).

Many organisms combat drought, salinity and cold stress in similar fashions, they upregulate the production of carbohydrates and other such types of molecules to act as osmoprotectants to alleviate the stress on other biomolecules (Voit, 2003). One of these molecules is trehalose (Tre), a non-reducing glucose disaccharide (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) that a plethora of organisms stretching from bacteria to insects and other invertebrates as well as fungi and plants synthesize where it acts as an abiotic stress protectant (Benaroudj et al., 2001; Elbein et al., 2003). Nevertheless, little is known about the influence of trehalose on pepper chilling tolerance.

This study served to examine the impact of trehalose on green pepper fruit quality and, antioxidant enzymatic activities therein, more specifically peroxidase and, catalase. The post-harvest cell ultrastructure of pepper was also observed.

2. Materials and methods

2.1. Handling of plant tissue and trehalose

Peppers (*Capsicum annuum* L. cv. Luojiao NO.4) were acquired from

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a native Chinese farmer in October 2016. Pepper fruits showing even sizing free from any imperfections were cut in 2 cm small segments and indiscriminately separated into four groups. One of the groups was used as control and fruit were soaked in distilled water, while the other fruit were immersed in 5%, 10% or, 15% trehalose for 30 min at 20 °C.The fruits were then air-dried and stored at 4 °C.

2.2. Mass loss

Fruit weight was measured prior to the liquid bath and during storage. Mass loss was calculated as relative percentage of mass loss.

2.3. Chlorophyll and vitamin C measurements

The concentration of photosynthetic pigments were extracted in 95% alcohol using the soaking method, followed by spectrophotometry analysis at 665, 649 and 470 nm using a UV–vis spectrophotometer (UV-3900, Hitachi High-Technologies Corporation) (Xiao and Wang, 2005). The formulae used were as follows:

$C_a = 13.95A_{665}–6.88A_{649}$

$C_b = 24.96A_{649}–7.32A_{665}$

$C_T = C_a + C_b = 18.08A_{649} + 6.63A_{665}(mg/L)$

C_a,C_b respectively represent chlorophyll a and b concentrations, C_T represent total chlorophyll concentration.

Chlorophyll Content = C (Pigment Concentration) × V (Extraction Volume) × N (Dilution Multiple)/W (Fresh Weight)

The vitamin C content was determined by DCPIP (Dichlorophenoliodophenol) titration. Pepper fruit tissue (5 g) was ground in cold 4% oxalic acid and completed to 50 ml. Filter paper was then used to clear the homogenate. A standard titration was then performed whereby 5 ml of standard solution was mixed with 10 ml of 4% oxalic acid in a conical flask and titrated against the dye. A pink-colored end point appeared and persisted for 15s. The concentration of ascorbic acid was then calculated by measuring the amount of dye consumed during the titration. An identical test was performed using 5 ml of sample instead of a standard solution, this was titrated against the dye till the end point and the reading was noted.

2.4. Membrane permeability and MDA analysis

MDA content was analyzed by virtue of the methodology published by Wang et al. (2016). Homogenized plant samples (1.0 g) were mixed in 5 mL of 10% (w/v) Trichloroacetic acid (TCA). After a 4000 × *g*/10 min centrifugation, 2 mL of supernatant was added to 2.0 mL of 0.67% thiobarbituric acid (TBA). This solution was then boiled for 30 min at 100C, rapidly chilled, and then the OD = 532 nm, OD = 600 nm and OD = 450 nm of the collected supernatant, obtained by centrifugation at 4000 × *g* for 10mins, was measured using a spectrophotometer (UV-3900, Hitachi High-Technologies Corporation). MDA concentration was computed with the following equation, (MDA mass per sample mass, $mmol\,kg^{-1}$) = [6.45 × (OD_{532}-OD_{600}) − 0.56 × OD_{450}] × V_t × V_r/(V_s × m), where V_t, V_r and V_s and m are respectively the entire extract volume, the total mixture volume in which took place the reaction, the extract volume contained within the latter and the sample mass.

2.5. Antioxidant enzyme activity

To measure POD and CAT enzymatic activity, 3 g of fresh fruit tissue was crushed in 6 mL of 0.1 M ice-cold PBS (pH 7.8). POD enzymatic activity was calculated using the methodology published by Keren-Keiserman et al. (2004). POD activity was measured by mixing 1 mL of 0.1 M PBS (pH 7.8), 0.9 mL of 0.2% guaiacol, 0.5 mL of enzyme extract, and 0.6 mL of 0.3% H_2O_2. Absorbance measurements at 470 nm were taken every 5s. Using these measurements, one unit of enzyme is described as the enzyme concentration needed to increase absorbance by 1 per minute.

Catalase (CAT) unit content was calculated as shown by Ali et al. (2005). CAT activity was determined by mixing 1.9 mL of 0.1 M PBS (pH 7.8), 1 mL of 0.3% H2O2, and 0.1 mL of plant extract where the zero point corresponded to the instant the plant extract was added. Measurements were then taken every 5 s thereafter. Similarly to POD, one CAT unit was described as the amount of enzyme needed to lower absorbance by 0.01 after 1 min.

2.6. Ultrastructural investigations

Pepper tissue ultrastructure was observed using transmission electron microscopy (TEM) (JEOL-1200). Briefly, 1 × 3 mm pepper fruit pieces from fruit centers were fixed in 3.0% glutaraldehyde in 0.1 M PBS (pH 7.2) for 2 h and then thrice washed 15 min each using this same fixation buffer. 1% osmium tetroxide was then added to the fixation solution and samples were left for another 2 h. Next, samples were washed 3 times with PBS. Fixed tissues were then dehydrated using a standard ethanol gradient (35, 50, 70, 90, 95, and 100%), treated with propylene oxide, followed by propylene oxide/Spurr's resin mixture. Afterwards, dehydrated tissues were plunged into Spurr's resin till the next morning at 4 °C. A LKB-V ultramicrotome was used to make slices of each sample, which were gathered on copper grids, colored using uranyl acetate and lead citrate treatments and then inspected using TEM.

2.7. Statistical analysis

Statistics were done using one-way ANOVA accompanied by a Duncan's test at $P < 0.05$ (SPSS 10.0). Triplicates of each experiment were performed three times.

3. Results

3.1. Mass loss

Pepper fruit mass change that occurred during storage at 4 °C is shown in Fig. 1. After 14 days at 4 °C, control-treated fruit lossed approximately 4.41% of its mass while 10% trehalose-treated fruit lost 3.06%. However, no significant difference between control and 5% trehalose-treated fruit could be observed in the first 10 days.

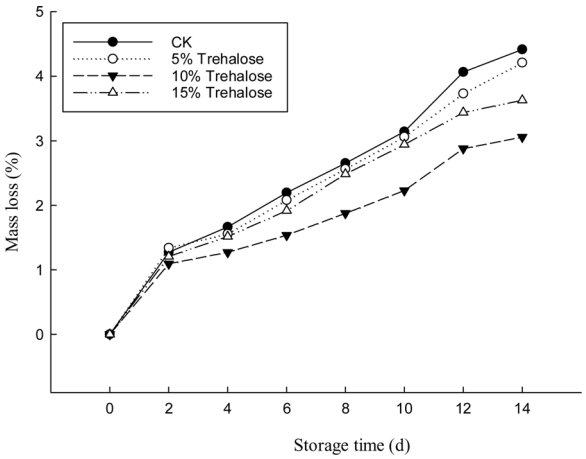

Fig. 1. Mass loss in trehalose-treated versus untreated pepper fruit stored at 4 °C for 14 days. Data shown are the means of 3 independent experiments in triplicates.

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