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Dynamics of enzymatic and non-enzymatic antioxidants in Japanese plums during storage at safe and lethal temperatures

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ABSTRACT

We investigated the effect of storage temperature as a function of storage time on lipid peroxidation, enzymatic and non-enzymatic antioxidants of Japanese plums (*Prunus salicina* Lindl.). Commercially mature fruit of 'Amber Jewel' cultivar were stored at 0 °C (recommended temperature) and 5 °C (lethal temperature) for 6 weeks. Chilling injury (CI) symptoms in the form of flesh browning and flesh translucency were noticed after 4 weeks of storage at 0 °C and 5 °C, respectively. The lipid peroxidation as reflected by thiobarbituric acid-reactive substances concentration was significantly higher at 5 °C beyond 2 weeks of storage than at 0 °C. The activities of superoxide dismutase, catalase, and peroxidase were significantly higher during storage at 5 °C than at 0 °C. The activities of monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase, increased during the first 2 weeks of storage at 5 °C and helped maintaining ascorbate pool better than at 0 °C. Overall, the response of antioxidative system to chilling stress at 5 °C was comparatively faster and stronger than at 0 °C, but it could not sustain for 6 weeks of storage as the higher levels of oxidative stress associated with fruit ripening at 5 °C might have resulted in failure of the antioxidative protection system.

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1. Introduction

Optimum storage temperature is the basic requirement for postharvest handling and storage of Japanese plums. Japanese plums can be stored at 0 °C for 3–5 weeks depending upon the cultivar's susceptibility to chilling injury (CI) (Crisosto, Mitchell, & Ju, 1999). The cold storage of Japanese plums can cause CI in the form of flesh browning, mealiness, and flesh translucency (Singh & Khan, 2010; Singh & Singh, 2012; Singh, Singh, & Swinny, 2009). Japanese plum cultivars are susceptible to CI at 5 °C, but a few cultivars show susceptibility to CI at 0 °C and 5 °C (Crisosto et al., 1999). Some cultivars of peaches and nectarines also develop CI symptoms at both storage temperatures (Crisosto et al., 1999; Lurie & Crisosto, 2005). 'Amber Jewel' cultivar, a popular cultivar for its high demand in the South-East Asian export markets, showed more sensitivity to CI at 0 °C than at 5 °C, while over-ripeness was the limiting factor during storage at 5 °C (Khan, Ahmed, & Singh, 2011; Ward & Melvin-Carter, 2001). These studies showed that susceptibility to CI in stonefruits including Japanese plums is a phenomenon under the control of genetic factors.

Plants have evolved an efficient antioxidant system to encounter the increased reactive oxygen species (ROS) levels in response to a variety of environmental stresses such as extreme temperatures, salinity, drought, ozone exposure, and ultra-violet irradiation (Hodges, Lester, Munro, & Toivonen, 2004). In response to stress conditions, ROS are produced and rapidly removed or detoxified by various cellular enzymatic and non-enzymatic systems. The slow and weak response of the antioxidant protection system can cause accumulation of ROS to damaging levels leading to enhanced lipid peroxidation, loss of membrane integrity and oxidative injury to the tissue (Shewfelt & del Rosario, 2000; Wismer, 2003). Like other stresses, chilling conditions can alter the equilibrium between ROS production and removal, and can result in oxidatively induced CI in horticultural commodities (Hodges et al., 2004).

It is argued that chilling-tolerant species/cultivars are known to have a strong antioxidant system in response to stress and/or produce fewer ROS than chilling-susceptible counterparts (Sala, 1998; Wismer, 2003). The response to chilling conditions may vary depending upon the storage temperature and duration of exposure. The storage temperatures between 2.2 and 7.6 °C (killing temperature zone) have been considered to be responsible for development of CI in plums, peaches, and nectarines (Crisosto et al.,

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1999; Lurie & Crisosto, 2005), and may be favouring the development of oxidative stress in fruit. The stonefruit including plums are exposed to 'killing temperature zone' during the real-world supply chain conditions. The understanding of physiological and biochemical changes in response to these conditions can help devising postharvest strategies to minimise the detrimental effects on fruit quality and also be useful for informed decision making in the supply chain. No research work has been reported on the comparison of the enzymatic and non-enzymatic antioxidant components during storage at two contrasting temperatures (0 °C and 5 °C) which have been generally known to exert the opposite effects on CI incidence in stone fruits. We hypothesized that the oxidative behaviour of Japanese plums during storage at optimum temperature may differ from during storage in the 'killing/danger temperature zone'. Therefore, the effects of storage at two contrasting temperatures (0 °C and 5 °C) on the changes in enzymatic and non-enzymatic antioxidants in relation to CI and fruit quality were investigated in Japanese plums.

2. Materials and methods

2.1. Experimental material and design

The experiment was conducted on 'Amber Jewel' cultivar of Japanese plums. The reasons for selection of this cultivar included commercial importance for the south-east Asian markets, high susceptibility to CI, regular occurrence of CI, and light flesh colour facilitating visual observation of CI symptoms. 'Amber Jewel' fruit were harvested at commercial maturity (129 days after full bloom; soluble solids concentration (SSC) = 16.4 ± 0.3%; titratable acidity (TA) = 1.29 ± 0.01%; firmness = 48.6 ± 2.5 N) in the early morning hours from the Casuarina Valley Orchard, Karragullen, Perth Hills (latitude 31° 57′ S; longitude 115° 50′ E), Western Australia (WA). Fruit of uniform size and maturity, free from visual blemishes and disease were harvested from the orchard, and transported to the laboratory. The fruit were stored in plastic crates (~15 kg) lined with 30 μm thick low density polyethylene film (AMCOR Packaging, Pvt. Ltd., Melbourne, Australia) at 0 °C and 5 °C for 6 weeks. Three plastic crates represented three replicates for each storage temperature. Aliquots of 20 fruit from each of the three replicates from both storage temperatures were transferred from cold store at two-week intervals, allowed to stay at 20 °C for about 4 h, and were analysed for changes in fruit quality. Flesh tissue was frozen in liquid nitrogen and stored at −80 °C until further analyses. The experiment was laid out by following a completely randomised design with two factors including storage temperature and storage period.

2.2. Chilling injury (CI)

The incidence and severity of CI were evaluated 4 h after transfer from cold storage to 21 ± 1 °C. Twenty plums per replication were cut around the equatorial axis, the two halves of each fruit were separated by twisting in opposite directions, and the mesocarp was examined for symptoms such as flesh browning, mealiness, and translucency. The incidence and severity of CI were assessed as described previously (Singh et al., 2009).

2.3. Determination of lipoxygenase (LOX) activity, thiobarbituric acid-reactive substances (TBARS) concentration, and electrolyte leakage (EL)

The activity of LOX was determined by measuring the increase in absorbance at 234 nm due to formation of a diene conjugate from linoleic acid as previously mentioned (Singh, Singh, & Swinny, 2012). The LOX activity was expressed as μmol linoleic hydroperoxide formed min^{-1} mg^{-1} of protein. The TBARS concentration was determined as previously described (Singh et al., 2012) and was expressed as nmol of malondialdehyde equivalents per gram of fresh weight (FW). The EL as a measure of membrane integrity was determined as described in Singh et al. (2012). The data were expressed as percentage of the total EL.

2.4. Extraction and assays of antioxidant enzymes

Enzyme extracts of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were prepared and assays were conducted as previously described (Singh et al., 2012). The SOD activity was expressed as μmol Cyt c conserved min^{-1} mg^{-1} protein. The activity of CAT enzyme was assayed by measuring a decrease in the absorbance at 240 nm for 3 min due to decomposition of hydrogen peroxide (H_2O_2) and was expressed as μmol H_2O_2 decomposed min^{-1} mg^{-1} protein. For determination of POD activity, the increase in absorbance at 470 nm was monitored for 3 min with and without addition of enzyme extract. The POD activity was expressed as μmol H_2O_2 decomposed min^{-1} mg^{-1} protein.

2.5. Determination of ascorbic acid (AA), dehydroascorbic acid (DHA) and total ascorbate (AA + DHA)

Extraction procedure for AA and DHA was followed as described by Davey, Franck, and Keulemans (2004) with some modifications as previously described by Singh et al. (2012).

The reverse phase-liquid chromatography was performed for determination of AA as described in Singh et al. (2012). Total ascorbate concentration was determined by the reduction of DHA into AA using dithiothreitol (DTT) as a reducing agent (Davey et al., 2004). The reduced samples were then directly analysed for total ascorbate by HPLC under similar conditions as for AA. For each sample, the concentration of DHA was obtained by subtracting AA from the total ascorbate (AA + DHA) (Davey et al., 2004). The concentrations of AA, DHA and AA + DHA were expressed as nmol g^{-1} FW.

2.6. Determination of reduced glutathione (GSH), oxidised glutathione (GSSG) and total glutathione (GSH + GSSG)

Glutathione (Total and GSSG) was assayed spectrophotometrically by the 5,5′-dithio*bis*-(2-nitrobenzoic acid) (DTNB)-glutathione reductase (GR) recycling method as described by Hodges and Forney (2000). The extraction and assay procedures for GSH and GSSG have been mentioned elsewhere (Singh et al., 2012). The concentrations of glutathione (GSH, GSSG and GSH + GSSG) were expressed as nmol g^{-1} FW.

2.7. Extraction and assays of ascorbate–glutathione cycle enzymes

The extracts of enzymes involved in the ascorbate–glutathione cycle were prepared as previously described (Singh et al., 2012). The ascorbate peroxidase (APX) activity was assayed as described elsewhere (Singh et al., 2012) and was expressed as μmol ascorbic acid oxidized min^{-1} mg^{-1} protein. The activities of monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) were measured by the method adapted from Hodges and Forney (2000) with slight modifications (Singh et al., 2012) and were expressed as μmol NADH oxidized min^{-1} mg^{-1} protein, μmol DHA oxidized min^{-1} mg^{-1} protein and nmol NADPH oxidized min^{-1} mg^{-1} protein, respectively. Glutathione-S-transferase (GT) activity was determined as described earlier (Singh et al., 2012). The GT activity was expressed as nmol of S-2,4-dinitrophenylglutathione formed min^{-1} mg^{-1} protein.

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