Postharvest cold storage-induced oxidative stress in Japanese plums (*Prunus salicina* Lindl. cv. Amber Jewel) in relation to harvest maturity

Sukhvinder Pal Singh[†], Zora Singh*

Curtin Horticulture Research Laboratory, Department of Environment and Agriculture, School of Science and International Institute of Agri-Food Security (IIAFS), Curtin University, GPO Box U1987, Perth, 6845, WA, Australia

[*]**Corresponding author: E-mail: z.singh@curtin.edu.au**
[†]**Present address: National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, 160071 India. E-mail: spsingh@nabi.res.in**

Abstract

Cold storage-induced oxidative stress in relation to harvest maturity and storage duration together with its implications on fruit quality and storage potential of Japanese plums (*Prunus salicina* Lindl.) were investigated. 'Amber Jewel' plums harvested at commercial maturity and one week after commercial maturity (delayed harvest) were stored at 0 °C for 7 weeks. Oxidative stress related parameters were determined at weekly intervals. Similar to lipid peroxidation, the incidence and severity of chilling injury (CI) was higher in fruit from the delayed harvest compared to commercial harvest. The activities of primary antioxidant enzymes and ascorbate-glutathione cycle enzymes were determined in context to the levels of antioxidant pools of ascorbate and glutathione and the development of CI symptoms. The predominance of the oxidized state of the tissue as reflected by lower ratios of ascorbate (AA) to dehydroascorbate (DHA) and reduced glutathione (GSH) to oxidized glutathione (GSSG) was linked to the severity of CI symptoms during the last 3-4 weeks of storage. The status of enzymatic and non-enzymatic antioxidative system during cold storage of Japanese plums appeared to be more important in providing protection against oxidative injury expressed as CI than at-harvest antioxidant status. Delayed harvested fruit experienced more oxidative stress during cold storage compared to the fruit harvested at commercial maturity. In conclusion harvesting 'Amber Jewel' plums at commercial maturity is of paramount importance to ensure long-term cold storage with minimal adverse effects on fruit quality for better consumer experiences.

Keywords: Ascorbate-glutathione cycle; chilling injury; enzymatic antioxidants; lipid peroxidation; non-enzymatic antioxidants; phenolics.
Abbreviations: AA – ascorbic acid; APX – ascorbate peroxidase; CAT– catalase; CI – chilling injury; DHA – dehydroascorbic acid; DHAR – dehydroascorbate reductase; EL – electrolyte leakage; GR-glutathione reductase; GSH – glutathione (reduced); GSSG – glutathione (oxidised); GT – glutathione-S-transferase; LOX – lipoxygenase; MDHAR –monodehydroascorbate reductase; POD – peroxidase; ROS – reactive oxygen species; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances.

Introduction

Japanese plums (*Prunus salicina* Lindl.) have short postharvest life depending upon the cultivar and supply-chain conditions. The low-temperature storage at 0 °C is recommended for extending the shelf-life of plums facilitating prolonged marketability period and long-distance transport. The long-term cold storage of plums results in the development of chilling injury (CI) which appears in the form of flesh browning, mealiness and flesh translucency (Candan et al., 2008; Crisosto et al., 2004; Singh et al., 2009). Susceptibility of plums to CI depends upon several factors including cultivar, harvest maturity, and storage duration and conditions (Abdi et al., 1997; Crisosto et al., 2004; Taylor et al., 1995). Oxidative stress has been proposed to be an early response to development of CI during cold storage of many fruits (Mao et al., 2007; Sala, 1998; Zhao et al., 2009). The oxidative stress develops as a consequence of reactive oxygen species (ROS) generation exceeding the capability of the antioxidant system in the cell (Hodges et al., 2004). The chilling stress has been implicated in activation of the antioxidant defense system in response to increasing pro-oxidant levels (Sala, 1998). However, the reduction or failure of the enzymatic and non-enzymatic antioxidants to protect

against the ROS can cause oxidative damage leading to enhanced lipid peroxidation and loss of membrane integrity in the tissue (Hodges et al., 2004). The development of other postharvest physiological disorders including CI in fruits has been related to the antioxidant levels at harvest and the changes in their concentrations during cold storage. The accumulation of antioxidants in apple peel due to delayed harvest has been shown to decrease the incidence of superficial scald during cold storage (Diamantidis et al., 2002). On the other hand, the delayed harvesting of 'Conference' pears increased susceptibility to browning disorder due to decrease in the ability of antioxidant system to protect from ROS with the advanced maturation (Lentheric et al., 1999). The development of internal browning disorders in apples and pears has been linked to the decrease in concentrations of ascorbic acid (AA) during storage (Davey and Keulemans, 2004). Similarly, the decreased activity of superoxide dismutase (SOD) enzyme in flesh tissue increased the incidence of Braeburn browning disorder in late-harvested 'Braeburn' apples (Toivonen et al., 2003). It has been widely argued that the initiation or aggravation of various physiological disorders in fruit was due to an

inefficient antioxidant system in the skin or flesh tissue, but the cause-effect relationship is still unclear in many cases. In previous studies, the physiological and biochemical changes during cold storage of plums of different maturity groups have shown that the delayed harvesting increased the susceptibility to CI expressed as flesh translucency (Abdi et al., 1997; Taylor et al., 1995). However, no information is available on the oxidative behaviour of Japanese plums during prolonged cold storage in relation to harvest maturity. It was therefore hypothesized that the comprehensive analysis of the changes in enzymatic and non-enzymatic antioxidant components may explain the development of CI during cold storage of Japanese plums harvested at commercial and delayed maturities. Given the importance of harvest maturity in relation to antioxidative systems in fruits, in the present study, it was investigated if the differences in enzymatic and non-enzymatic antioxidants at harvest persisted or changed during the cold storage of Japanese plums and their relations to the development of CI symptoms.

Results and Discussion

Fruit quality

Harvest maturity significantly affected the fruit quality parameters that included flesh firmness, skin colour, soluble solids concentration (SSC), titratable acidity (TA) and SSC:TA ratio (data not shown). A significant reduction in the flesh firmness of fruit was observed during 7 weeks of cold storage; the extent of reduction was about 37 and 50 % in fruit harvested at commercial and delayed maturities, respectively. The decrease in L* and hue angle values was observed with the increase in storage duration and the fruit from delayed harvest showed changes in skin colour to a greater extent than those harvested at commercial maturity (data not shown). The results are consistent with the findings in other cultivars of the European and Japanese plums harvested at different maturities (Casquero and Guerra, 2009; Khan and Singh, 2008). The increased activities of polygalacturonase, pectin esterase, and endo-1,4-β-D-glucanase are reportedly responsible for fruit softening during cold storage in 'Tegan Blue' plums harvested at commercial maturity (Khan and Singh, 2008). In this experiment, delayed-harvesting might have triggered the activities of these enzymes before harvest which persisted through storage at higher levels causing more softening compared with commercial-harvest. Concurrent to fruit softening and skin colour development, the increase in SSC and decrease in TA in delayed-harvest indicates on-tree-advancement of fruit maturity (data not shown). The increase in SSC:TA ratio resulting from delayed harvest is generally favourable for improving fruit flavour and consumer acceptability of Japanese plums (Crisosto et al., 2004). The increase in SSC during the first 3-4 weeks of storage followed by decline is contrary to previous reports showing either no change (Casquero and Guerra, 2009) or decrease in SSC (Abdi et al., 1997) throughout the storage in plums. However, the decrease in TA in both harvests during storage agrees with earlier reports (Casquero and Guerra, 2009; Chen and Zhu, 2011). The decrease in TA during storage improved SSC:TA ratio as the storage progressed.

CI

The CI symptoms manifested as browning of the outer part of mesocarp were firstly observed after 3 weeks of storage in fruit from both harvests. As the duration of cold storage
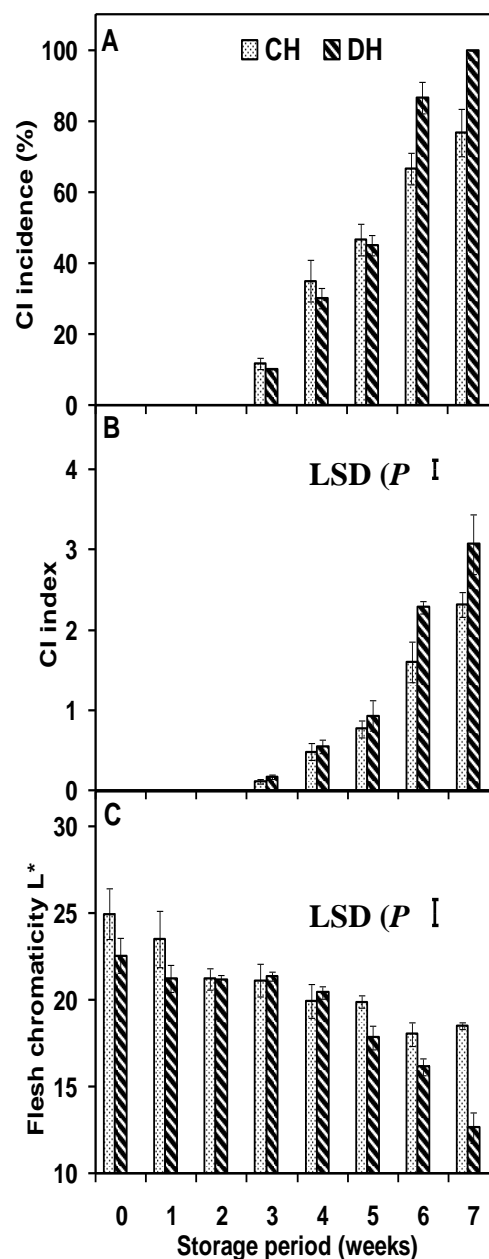

Fig 1. Effects of harvest maturity and storage duration on the CI incidence (A) and CI severity index (B) and flesh chromaticity L* (C) of 'Amber Jewel' plums as influenced by harvest maturity during cold storage at 0 ± 0.3 °C plus 4 h at 21 ± 1 °C. The legends, CH and DH, refer to the commercial and delayed harvests, respectively. Data on CI incidence were arcsine transformed, but back-transformed data are presented for simplifying comparisons. Vertical bars represent S.E. of means.

progressed, the severity and incidence of CI increased in fruit from both harvests (Fig. 1). The decrease in chromaticity values indicated the flesh browning (Fig. 1C). The incidence and severity of CI did not differ significantly during the first 5 weeks of storage, regardless of fruit maturity. The symptoms were mainly in the form of flesh browning in commercially-harvested fruit throughout the storage, whilst these symptoms further developed to form a gel-like texture in delayed-harvested fruit and predominated during the last 2 weeks of storage. Previous reports have also shown that early-harvested fruit have been reported to show CI mainly in

the form of flesh browning than the late-harvested fruit exhibiting flesh translucency (Crisoto et al., 2004; Taylor et al., 1995; Ward and Melvin-Carter, 2001). The flesh translucency is considered to be an advanced stage of flesh browning and predominates in late-harvested fruit. The loss of membrane permeability in delayed-harvested fruit might have increased the availability of cell fluids to form gel complexes around the stone earlier than the commercial-harvest. That is why the flesh translucency symptoms appeared earlier in delayed-harvested fruit compared with commercial-harvest as previously reported (Taylor et al., 1995). Even though, harvest maturity played a role in determining the severity of CI, but storage duration appears to be more critical factor in predisposing the fruit to CI. The data suggest that harvest maturity had a significant effect on the incidence and severity of CI only if the fruit were stored beyond 5 weeks at 0 °C.

Lipid peroxidation

The increased activity of lipoxygenase (LOX) during the initial stages of storage might be responsible for increase in concentration of thiobarbituric acid-reactive substances (TBARS) (Fig. 2). However, the increase in the TBARS concentration was observed to continue even after the decline in LOX activity. The peroxidative damage to plasma membrane to a greater extent during the late period of storage might have resulted in decreased concentrations of LOX substrate (Mao et al., 2007) which decreased the activity of membrane-bound LOX. The membrane disintegration due to lipid peroxidation can also continue even in the absence of high LOX activity because lipid peroxidation is a self-propagating process requiring LOX for initiation only (Song et al., 2009). Chilling-induced increase in LOX activity followed by a decline during cold storage has been reported in chilling-sensitive commodities such as cucumber (Mao et al., 2007), banana (Promyou et al., 2008) and mango (Ding et al., 2007). The increase in concentration of TBARS, which is often utilized as a suitable biomarker for lipid peroxidation and oxidative damage, has been found to increase in response to chilling stress and senescence in various fruits such as kiwifruit, banana, and mango (Ding et al., 2007; Promyou et al., 2008; Song et al., 2009). In response to chilling, ROS production may exceed the antioxidant potential of the system leading to an oxidative stress. The higher levels of TBARS in delayed-harvested fruit reflect the development of oxidative stress over the storage period to a greater degree than in commercial-harvest (Fig. 2B). The results demonstrate that fruit harvested at delayed maturity undergo lipid peroxidation to a greater extent than those harvested at commercial maturity.

Antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)]

SOD, CAT, and POD are part of the repertoire of enzymatic antioxidant defence system that provide protection against the ROS (Apel and Hirt, 2004). The data suggest that SOD activity increased significantly during the first two weeks of storage in commercial-harvest compared with a non-significant change in delayed-harvest. The increase in SOD could be to dismutate the superoxide ions (O_2^-) generated in response to chilling stress (Bowler et al., 1992). The enhanced SOD activity was also coupled with a significant increase in CAT activity, which is essential for the removal of hydrogen peroxide (H_2O_2) from the cell to avoid its accumulation to toxic levels (Fig. 3). As the storage duration
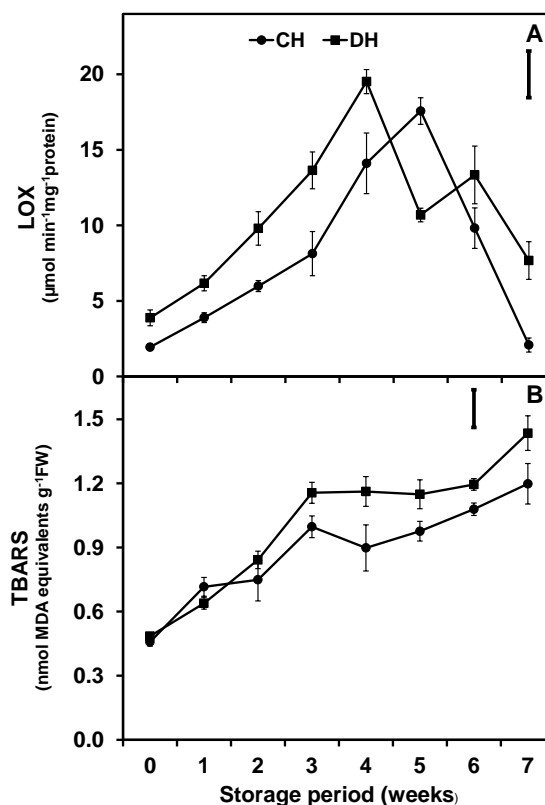

Fig 2. Changes in LOX activity (A) and TBARS content (B) in the flesh tissue of 'Amber Jewel' plums as influenced by harvest maturity during cold storage at 0 ± 0.3 °C plus 4 h at 21 ± 1 °C. The legends, CH and DH, refer to the commercial and delayed harvests, respectively. Vertical bars represent S.E. of means. All measurements were made in triplicate.

increased, decline in SOD activity might have contributed to the accumulation of O_2^- as reported during cold storage of kiwifruit (Song et al., 2009) and mango. The abundance of O_2^- due to lower SOD activity may increase the Haber-Weiss reaction rate which involves production of the most reactive and damaging hydroxyl radicals (Bowler et al., 1992). Therefore, SOD is likely to be an enzyme central to the antioxidant defense mechanism as its activity determines the levels of H_2O_2 and O_2^-, which are both substrates of the Haber-Weiss reaction. Another possible explanation for decrease in SOD activity is that in chilling-sensitive cultivars, such as 'Amber Jewel', increased H_2O_2 production may irreversibly inactivate SOD enzymes during prolonged chilling stress (Bowler et al., 1992). Despite a continuous decrease in SOD activity beyond 2 weeks of storage, CAT activity in commercial-harvest remained at significantly higher level than in delayed-harvest between 3 and 6 weeks of storage. The differences in CI incidence were not significant in both harvests during most of the storage time when CAT activity was elevated. It is evident that decline in SOD activity preceded the visibility of CI symptoms after 3 weeks of storage (Fig. 1). There was an overall decrease, except a few transient increases, in POD activity during cold storage in fruit of both harvests, but POD levels were higher in delayed-harvested fruit during most of the storage time compared with commercial-harvest. The decrease in POD has been associated with the reduction of antioxidant potential in some fruits such as kiwifruit (Song et al., 2009), mangoes (Ding et al., 2007), apples (Rao et al., 1998), and peaches (Wang et al., 2005). Increase in SOD and CAT activities in

response to low temperature stress has been associated with enhanced chilling tolerance in mangoes (Ding et al., 2007), and peaches (Wang et al., 2005), and resistance against browning in 'Conference' pears (Lentheric et al., 1999) and 'Braeburn' apples (Toivonen et al., 2003). Based on the previous studies in other fruits and our data, it is presumable that acquisition of chilling tolerance in Japanese plums may be possible with the concerted action of both SOD and CAT in the flesh tissue. The primary antioxidant system, in terms of activities of SOD and CAT, in delayed-harvested fruit was less efficient than in commercial-harvest. The data also suggest that the changes in primary antioxidant enzymes, not the actual levels at harvest, are likely to affect the response of tissue to oxidative stress developing during cold storage.

Ascorbate-glutathione cycle

Ascorbate (AA) and reduced glutathione (GSH)

The capacity of ascorbate-glutathione cycle to regenerate AA utilizing GSH is dependent on the concentrations of these antioxidants and the related enzyme activities, which indicates that these parameters would be suitable markers for the oxidative stress (Tausz et al., 2004). AA and GSH concentrations at harvest and during storage are important determinants of fruit's capability to withstand oxidative stress presumed to be developing due to suboptimal conditions prevailing postharvest (Davey and Keulemans, 2004). Compared to commercial harvest, the delay in harvesting by one week resulted in a significant increase in concentrations of AA and GSH without affecting their redox status as reflected by AA:DHA and GSH:GSSG ratios, at harvest (Figs. 4 and 5). AA and GSH concentrations decreased significantly during 7 weeks of cold storage in fruit from both harvests (Figs. 4 and 5) which could be attributed to their utilization as reductants to encounter the oxidative stress during cold storage. A significant decrease in AA and GSH levels has been reported to occur during long-term cold storage of other fruits, such as mangoes (Ding et al., 2007) and pawpaws (Galli et al., 2009). Contrarily, the GSH levels have been reported to increase in response to chilling in apples (Toivonen et al., 2003) and remain unaltered in peaches (Wang et al., 2006). GSH levels may or may not increase or may even decrease upon stress exposure (Tausz et al., 2004). GSH concentration was significantly higher after the 6th and 7th weeks of storage in commercial harvest than in delayed harvest, indicating a possible role of GSH as an antioxidant in reducing the CI symptoms in plums. Higher levels of GSH have been implicated in providing chilling tolerance in plants. The reduced levels of glutathione have been linked to the internal browning in methyl bromide-fumigated 'Thompson Seedless' grapes (Liyanage et al., 1993). However, the protective role of GSH as a cellular antioxidant in various postharvest disorders of fruits has not been conclusively studied. Apple cultivars with higher total AA have been reported to have higher GSH levels, and thus better storage characteristics (Davey and Keulemans, 2004). The concentrations of DHA and GSSG increased to a greater level towards the end of storage indicating a shift in the equilibrium towards more oxidized state in the tissue, which is an indication of the failure of the ascorbate-glutathione cycle (Figs. 4C and 5C). The redox status of fruit tissue, AA:DHA and GSH:GSSG ratios, was adversely affected after 3-4 weeks of storage, coinciding with greater incidence and severity of CI (Figs. 4D and 5D). The transient increases in AA:DHA redox state during the first 3-4 weeks of chilling exposure of fruit from both maturities suggest the onset of an
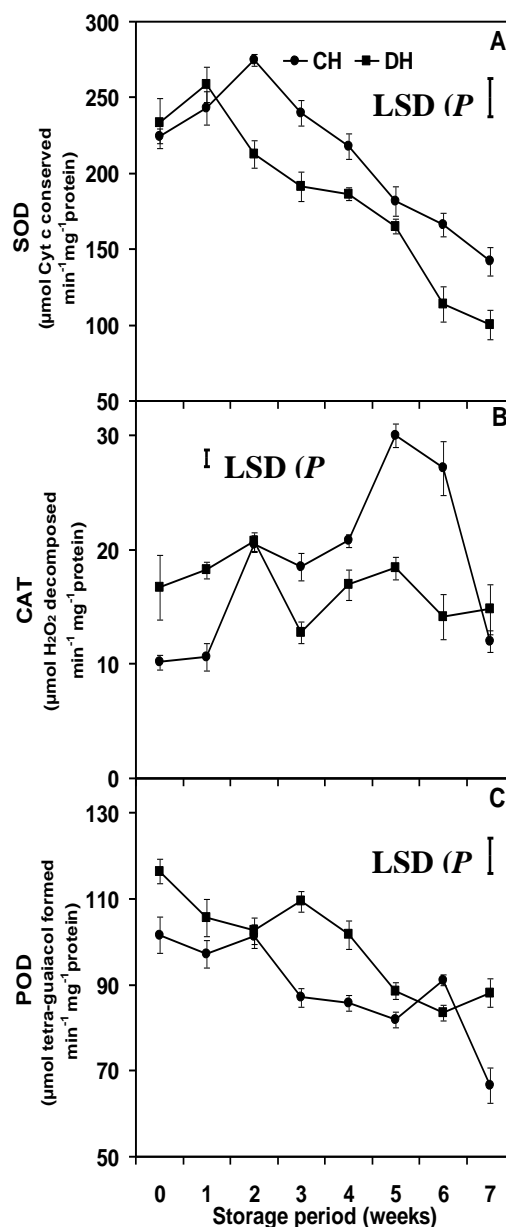

Fig 3. Changes in activities of SOD (A), CAT (B), and POD (C) in the flesh tissue of 'Amber Jewel' plums as influenced by harvest maturity during cold storage at 0 ± 0.3 °C plus 4 h at 21 ± 1 °C. The legends, CH and DH, refer to the commercial and delayed harvests, respectively. Vertical bars represent S.E. of means. All measurements were made in triplicate.

acclimation reaction to anticipated increasing load of ROS. However, a greater shift in AA:DHA and GSH:GSSG ratios towards more oxidized state during the late stages of storage reflects that acclamatory responses during the initial weeks of storage were too weak and slow to prevent the collapse of the antioxidant system during prolonged exposure to chilling stress (Galli et al., 2009). Though harvest maturity influenced the concentrations of both AA and GSH at harvest, but the decrease in their levels, particularly for AA, during storage appears to be independent of harvest maturity. The manifestation of CI as flesh browning and translucency in 'Amber Jewel' plums is, perhaps, due to decrease in AA and GSH concentrations below a certain level. The results
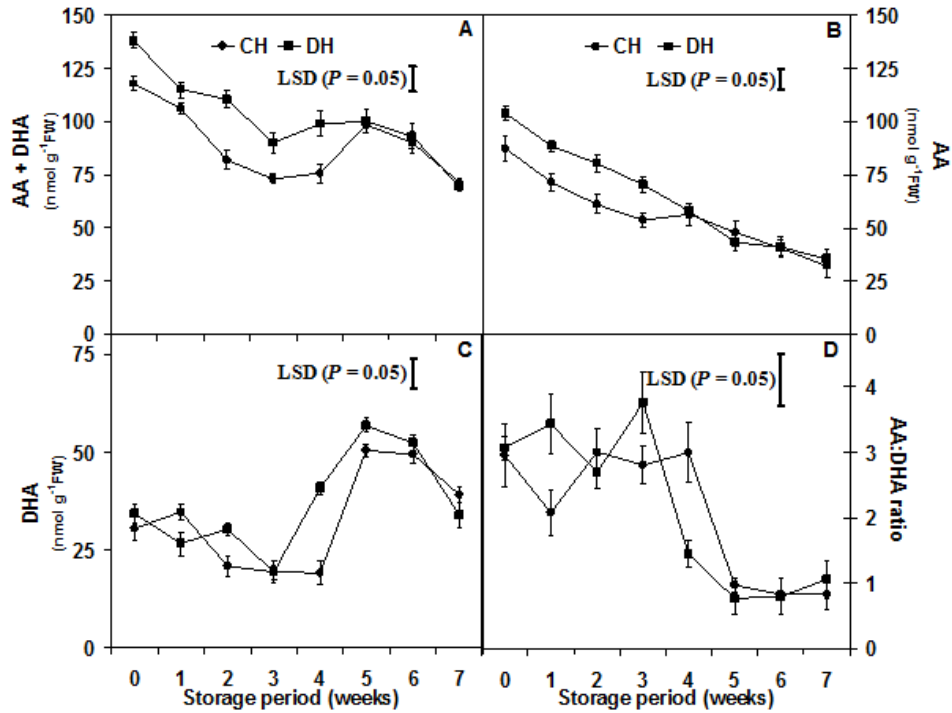
Fig 4. Changes in concentrations of total ascorbate (AA + DHA) (A), AA (B), DHA (C), and AA:DHA ratio (D) in the flesh tissue of 'Amber Jewel' plums as influenced by harvest maturity during cold storage at $0 \pm 0.3°C$ plus 4 h at 21 ± 1 °C. The legends, CH and DH, refer to the commercial and delayed harvests, respectively. Vertical bars represent S.E. of means. All measurements were made in triplicate.
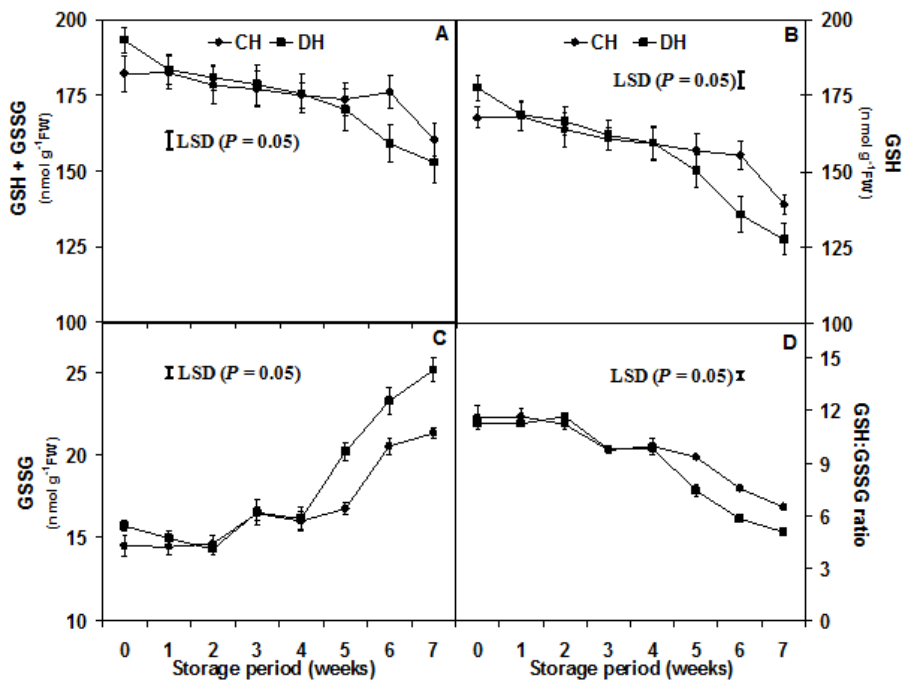

Fig 5. Changes in concentrations of total glutathione (GSH + GSSG) (A), GSH (B), GSSG (C), and GSH:GSSG ratio (D) in the flesh tissue of 'Amber Jewel' plums as influenced by harvest maturity during cold storage at 0 ± 0.3 °C plus 4 h at 21 ± 1 °C. The legends, CH and DH, refer to the commercial and delayed harvests, respectively. Vertical bars represent S.E. of means. All measurements were made in triplicate.

indicate that the response or maintenance of the AA and GSH pools during cold storage is more important than their levels at harvest.

Ascorbate-glutathione cycle enzymes

The activities of ascorbate-glutathione cycle enzymes, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR), which are responsible for the oxidation and recycling of AA and GSH pools in the flesh tissue are shown (Figs. 6 and 7). The increase in APX activity during the first two weeks of storage in commercially-harvested fruit was significantly higher than in delayed-harvested fruit, but a significant reduction in APX activity was noticed in the last 2-3 weeks of storage (Fig. 6A). The increase in APX activity, utilizing AA as a substrate in the oxidation reaction to prevent the accumulation of H_2O_2, has been involved in providing chilling tolerance in several fruits subjected to different postharvest treatments (Rao et al., 1998; Wang et al., 2006; Zhao et al., 2009). APX-catalysed oxidation of AA generates the unstable radical monodehydroascorbate (MDHA), which rapidly dissociates into DHA and AA. MDHAR and DHAR are two reductases catalysing the reduction of MDHA and DHA, respectively, to maintain the AA pool. In response to increased APX activity that might have generated MDHA and DHA, the activities of MDHAR and DHAR increased significantly in the initial weeks of storage followed by a great decline in last 2-3 weeks of storage (Figs. 6B and C). The sudden increase in DHA levels observed after 4 or 5 weeks of storage in fruit from both harvests coincided with a significant reduction in MDHAR and DHAR activities. Therefore, a role for MDHAR and DHAR is indicated in the maintenance of AA:DHA ratio at a higher level during chilling stress. The increase in MDHAR enzyme activity and transcript levels has been linked to increase in AA levels and chilling tolerance in tomatoes exposed to 4°C for 40 days (Stevens et al., 2008). The increase in activities of MDHAR and DHAR indicates an acclamatory response to chilling stress, while decrease in their activities is a reflection of the failure of the antioxidative system which resulted into increased concentrations of DHA and lower levels of AA in the tissue.

GR activity sustained for the first 4-5 weeks of storage without a significant change, but declined sharply after 6 and 7 weeks of storage and *vice-versa* in case of glutathione-S-transferase (GT) (Figs. 6 and 7). The down-regulation of GR and up-regulation of GT may explain the accumulation of GSSG in the final weeks of storage. GR is responsible for NADPH-dependent reduction of GSSG into GSH, whereas GT utilizes GSH as a substrate to detoxify lipid hydroperoxides accumulated as a result of increased lipid peroxidation (Apel and Hirt, 2004). The higher activity of GR can lead to a stronger pool of GSH in order to maintain the H_2O_2-scavenging ascorbate-glutathione cycle. With the increase in storage duration, GR activity has also been reported to decrease in response to chilling stress in peaches. The postharvest treatments that were reported to enhance chilling tolerance in peaches also showed higher levels of GR activity (Wang et al., 2006). Despite the increase in MDHAR and DHAR activities during initial stages of storage, GR activity either remained stable during the first 5 weeks of storage or decreased during the late stages. Consequently, the recovery of the antioxidant pools of AA and GSH was not observed in fruit from both harvests. These observations imply that ascorbate-glutathione cycle, in response to chilling
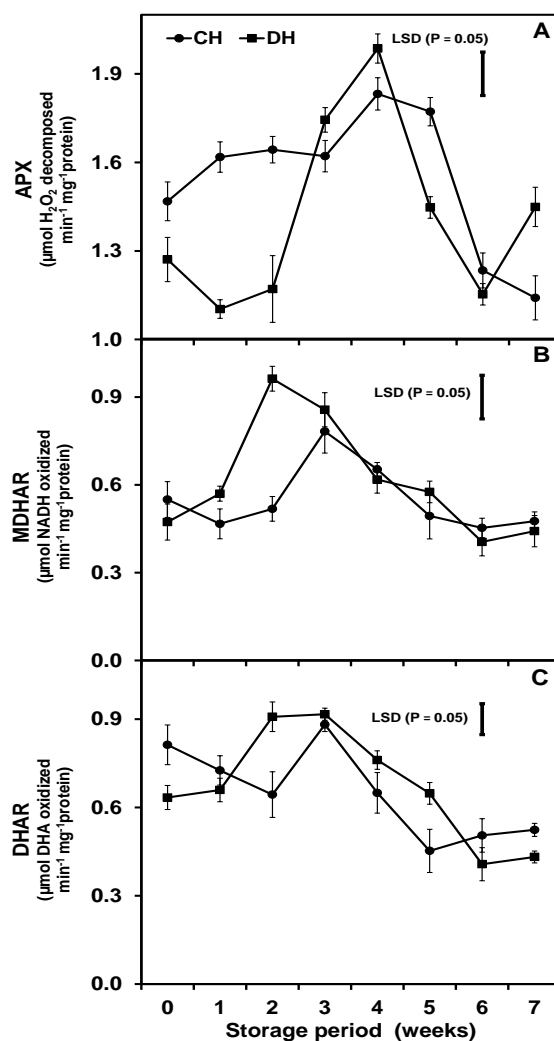

Fig 6. Changes in activities of APX (A), MDHAR (B), and DHAR (C) in the flesh tissue of 'Amber Jewel' plums as influenced by harvest maturity during cold storage at 0 ± 0.3°C plus 4 h at 21 ± 1 °C. The legends, CH and DH, refer to the commercial and delayed harvests, respectively. Vertical bars represent S.E. of means. All measurements were made in triplicate.

stress, did not operate efficiently to maintain antioxidants pools in more reductive state in flesh tissues of plum. Previous reports showed that the oxidation of AA and GSH did not always yield a proportionate increase in DHA and GSSG, respectively; the activities recycling enzymes did not correspond with the increase in the levels of their reductant forms, AA and GSH (Davey and Keulemans, 2004; Galli et al., 2009). It further suggests a complexity in the regulation of antioxidant mechanism in response to a variety of stresses in different plants. The results show that activities of the AA-GSH recycling enzymes, MDHAR, DHAR, and GR, could not consistently remain at higher levels which were required to provide adequate protection through maintenance of the redox status of plum flesh tissue in response to chilling stress imposed for 7 weeks.

Total phenolics and DPPH· radical scavenging activity

Storage duration had a significant impact on the concentrations of total phenolics as these increased during the

first 2-3 weeks of storage followed by a consistent decrease up to 7th week of storage in both maturity groups (Fig. 8A). The increase in concentration of total phenolics during initial storage period could be the result of acclimatization response of fruit to chilling conditions as previously reported in pawpaws (Galli et al., 2009) and plums (Díaz-Mula et al., 2009). Phenylalanine ammonia lyase (PAL) activity, which is the initial regulatory enzyme in biosynthesis of phenolics, has been reported to increase in response to CI and the same might have contributed to increase in phenolics concentrations in flesh tissue of plum during the first 2-3 weeks of storage. The increase in phenolics concentration is an indication of the activation of defense mechanism against chilling stress. The prolonged storage resulted in decrease in total phenolics concentration which could be due to their utilization as substrate of PPO in the browning reactions; flesh browning appeared after 3 weeks of storage and its severity increased as the storage progressed (Fig. 8A). Another possible explanation for decrease in phenols is that these may be oxidized by peroxidases in the presence of H_2O_2 because of the co-existence of phenolics and peroxidases in vacuoles. The decrease in phenolics has been reported to occur during 12 days of storage of mangoes at 2 °C, irrespective of harvest maturity (Ding et al., 2007). Unlike total phenolics, harvest maturity did not affect the DPPH· radical scavenging activity (Fig. 8B). Phenolic compounds are the major contributors to the total antioxidant capacity in plum flesh tissue, whereas a very little contribution by other compounds has been reported (Díaz-Mula et al., 2009). Apparently, the pattern of changes in DPPH· radical scavenging activity during cold storage of fruit from both harvests was almost similar with the changes in total phenolics (Fig. 8B). The data suggest that phenolic compounds in co-operation with other antioxidative components during initial stages of storage might have contributed to protection against oxidative injury to the tissue. The increase in storage duration accompanied by the decrease in total phenols and reduced efficiency of other antioxidative systems might have contributed to the build up of oxidative stress to potentially damaging levels.

Materials and methods

Fruit material and storage conditions

Japanese plums cv. 'Amber Jewel' fruit were harvested at commercial maturity (129 days after full bloom; firmness = 48.6 N, SSC = 16.4 °Brix, TA = 1.29 %) and one week after commercial maturity (delayed-harvest; firmness = 31.1 N, SSC = 17.9 °Brix, TA = 1.14 %) in the early morning hours from the Casuarina Valley Orchard, Karragullen, Perth Hills, Western Australia. Fruit of uniform size and maturity, free from visual blemishes and disease were harvested from the orchard, transported to the laboratory, and stored in plastic crates lined with 30 μm thick low density polyethylene film (AMCOR Packaging, Pvt. Ltd., Melbourne, Australia) at 0 ± 0.3 °C and 86.5±5.5 % RH for 7 weeks. Aliquots of 20 fruit from each of the three replicates from both maturity groups were transferred from cold store at weekly intervals, allowed to stay at 20 °C for about 4 h, and were analysed for changes in fruit quality. Fruit flesh tissue was cut into small cubes and immediately frozen with liquid nitrogen and stored at –80 °C until further analyses.
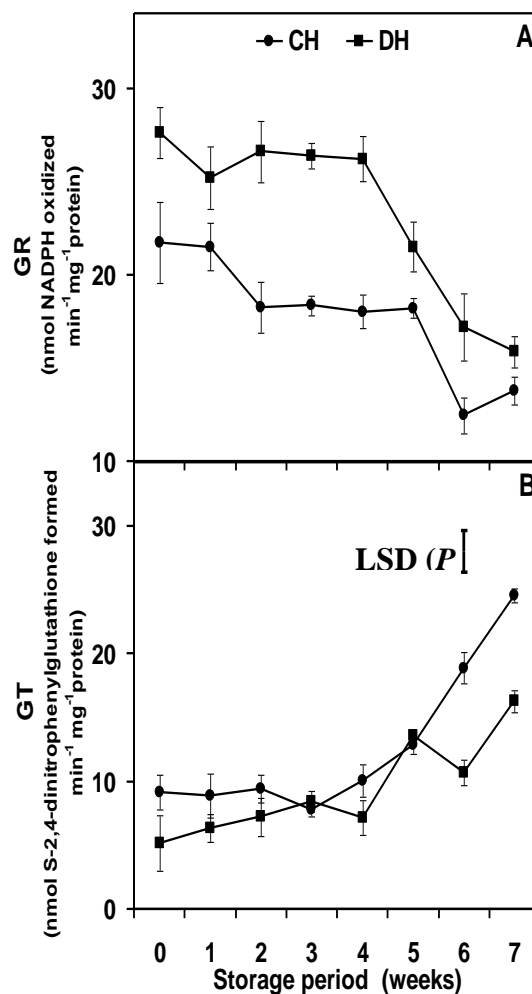

Fig 7. Changes in activities of GR (A) and GT (B) in the flesh tissue of 'Amber Jewel' plums as influenced by harvest maturity during cold storage at 0 ± 0.3 °C plus 4 h at 21 ± 1 °C. The legends, CH and DH, refer to the commercial and delayed harvests, respectively. Vertical bars represent S.E. of means. All measurements were made in triplicate.

Fruit quality evaluation

The changes in skin colour parameters including, CIE L^*, a^*, b^* were measured with a ColorFlex 45 ° / 0 ° Spectrophotometer (Hunter Associates Inc., Reston, VA, USA) using the 15 mm aperture. Flesh firmness was measured using an electronic pressure tester (Model: EPT-1, Lake City Technical Products, Kelowna, BC, Canada) fitted with an 8-mm spherical probe. Ten fruit per replication were subjected to firmness testing with each fruit punctured on both the sides at equatorial region. Juice obtained from 10 fruit was used for determination of SSC, titratable acidity and SSC: TA ratio as described by Singh et al. (2009). The incidence and severity of CI was 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 twisted 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).

Lipid peroxidation (TBARS concentration and LOX activity)

The TBARS concentration and LOX (EC 1.13.11.12) activity were determined as previously described (Singh et al., 2012). TBARS content was expressed as nmol of malondialdehyde equivalents per gram of fresh weight. The activity of LOX was determined by measuring the increase in absorbance at 234 nm due to formation of a conjugate diene from linoleic acid as previously mentioned. The LOX activity was expressed as µmol linoleic hydroperoxide formed min^{-1} mg^{-1} of protein.

Extraction and assays of antioxidant enzymes

Enzyme extracts of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), and POD (EC 1.11.1.7) were prepared as previously described (Singh et al., 2012). SOD activity was expressed as µmol Cyt c conserved min^{-1} mg^{-1} protein. CAT activity was assayed by measuring a decrease in the absorbance at 240 nm for 3 min due to decomposition of H_2O_2. CAT activity 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. POD activity was expressed as µmol H_2O_2 decomposed min^{-1} mg^{-1} protein.

Determination of AA, DHA and total ascorbate (AA + DHA)

Extraction procedure for AA and DHA was followed as described by Davey and Keulemans (2004) with some modifications as previously mentioned (Singh et al., 2012). The reverse phase-liquid chromatography was performed for determination of AA. Total ascorbate concentration was determined by the reduction of DHA into AA using dithiothreitol (DTT) as a reducing agent. 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).

Determination of GSH, 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. 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.

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 APX (EC 1.11.1.11) activity was expressed as µmol ascorbic acid oxidized min-1 mg-1 protein. The activities of MDHAR (EC 1.6.5.4), DHAR (EC 1.8.5.1), and GR (EC 1.6.4.2) were measured by the Hodges and Forney (2000) method from 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. The GT (EC 2.5.1.18) activity was determined
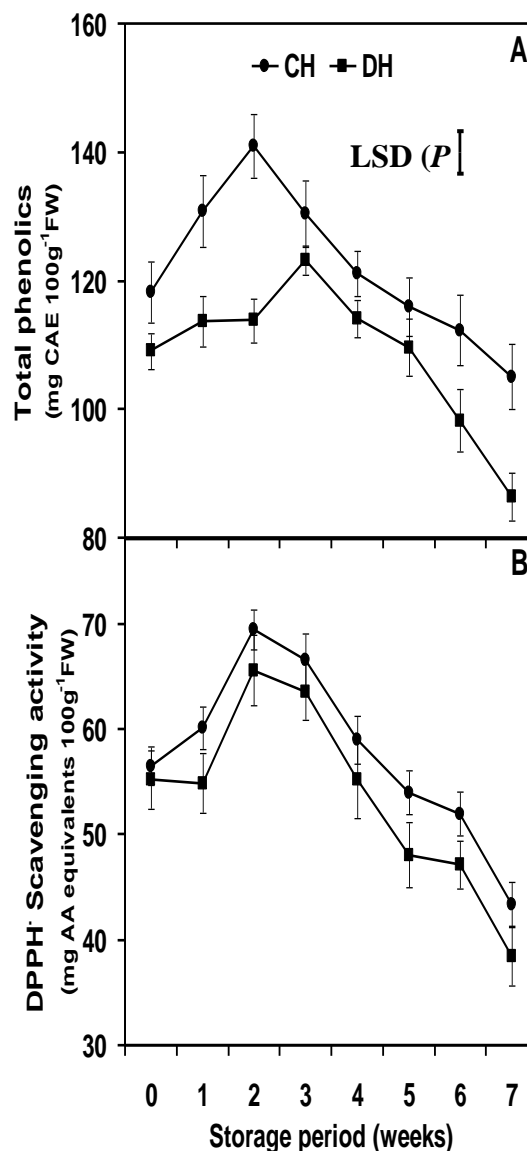

Fig 8. Changes in total phenolics concentration (A) and DPPH· scavenging activity (B) in the flesh tissue of 'Amber Jewel' plums as influenced by harvest maturity during cold storage at 0 ± 0.3°C plus 4 h at 21 ± 1 °C. The legends, CH and DH, refer to the commercial and delayed harvests, respectively. Vertical bars represent S.E. of means. All measurements were made in triplicate.

following the method described earlier (Singh et al., 2012) and was expressed as nmol of S-2,4-dinitrophenylglutathione formed min^{-1} mg^{-1} protein.

Determination of total phenolics and DPPH· radical scavenging activity

Total phenolic compounds were quantified by following the Folin-Ciocalteu reagent (FCR) based colorimetric method (Singh et al., 2012). Total phenolics were expressed in terms of mg chlorogenic acid equivalents (CAE) per 100 g of fresh weight. The DPPH· radical scavenging activity was determined using the method developed by Brand-Williams et al. (1995) and expressed in terms of mg ascorbic acid equivalents per 100 g of fresh weight.

Statistical analysis

The data were subjected to two-way analysis of variance (ANOVA) using GenStat Release 11.1 (VSN International Ltd., Hemel Hempstead, UK). Before statistical analysis, the data on CI incidence were subjected to arcsine transformation to reduce heteroscedasticity. The effects of harvest maturity and storage duration on different parameters were assessed within ANOVA and the least significant differences (LSD) were calculated following a significant F-test at $P \leq 0.05$.

Conclusion

In conclusion, the changes in antioxidant components during cold storage of Japanese plums seemed to be more important in providing protection against oxidative injury expressed as CI than their at-harvest antioxidant status. Delayed harvested fruit experienced more oxidative stress during cold storage compared to the fruit harvested at commercial maturity. Therefore, harvesting the fruit at commercial maturity is important to ensure the storability and maintain fruit quality. Cultivar-specific harvest maturity recommendations should be followed as the delayed harvest can improve the fruit flavour, but compromise the storage potential due to increased oxidative stress. The understanding of molecular basis of the oxidative stress in response to harvest maturity warrants further investigation.

Acknowledgements

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