EFFECT OF ANTI-BROWNING AGENTS ON QUALITY CHANGES OF LOQUAT [*ERIOBOTRYA JAPONICA* (THUNB.) LINDLEY] FRUIT AFTER HARVEST

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Abstract

In loquat fruit, browning is a major problem that reduces the shelf life. Study was conducted to evaluate the potential of citric acid (CA) and ascorbic acid (AA) as anti- browning agents during storage of loquat fruit. Loquat fruit was immersed for 2 min in solutions of 250 mg/l, 500 mg/l, 750 mg/l ascorbic acid (AA) and citric acid (CA) then placed in corrugated soft board cartons and stored at 4°C for a period of 10 weeks. Changes in browning index (BI), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), total phenolic content (TP), polyphenoloxidase (PPO) and free radical scavenging activity (FRSA) as affected by different treatments were studied. Ascorbic acid at higher concentrations was useful in maintaining the fruit quality than citric acid, while AA and CA at higher concentrations significantly reduced the browning.

Introduction

Loquat [*Eriobotrya japonica* (Thunb.) Lindley] is an important fruit having good nutritional value, medicinal properties and economic importance (Hussain *et al*., 2011). In loquat fruit, browning is a major problem that reduces the shelf life and makes it perishable. A number of treatments are used to preserve foods by controlling the enzymatic browning (Abbasi *et al*., 2013). However, their use is restricted to the compounds, which are nontoxic and have no unpleasant effect on taste and flavor (Saper, 1993). In case of food items, safety has become major concern and modern research is more focused on use of Generally Regarded as Safe (GRAS) chemicals.

Anti browning activity of Citric acid (CA) and Ascorbic acid (AA) has been reported in minimally processed vegetables and fruits (Son *et al*., 2001). Vitamin C or Ascorbic acid (AA), an antioxidant is useful to inhibit the browning reactions (Davey *et al*., 2000). By the formation of ascorbyl, it directly scavenges the damaging radicals (Yamaguchi *et al.,* 1999) and reduces the *o*-quinones produced by PPO, back to phenolic substrates (Robert *et al*., 2003). Citric acid is on the list of GRAS compounds and is under common use as a preservative in food items (Pao & Petracek, 1997). Being an antioxidant, it controls the growth of micro-organisms, which cause food spoilage (Christopher *et al*., 2003). Due to its chelating property, it inhibits activity of polyphenoloxidase (Jiang *et al*., 1999).

During the postharvest storage, various physiological changes occur in loquat fruit. Among these changes, browning badly deteriorates its quality and is a cause of decay. Hence the search for appropriate and cost effective measures to maintain the postharvest quality and extend the shelf-life of this popular fruit has also gained importance. Present work was carried out to determine the usefulness of naturally occurring and readily available GRAS chemicals to extend the shelf life of loquat.

Materials and Methods

Fruit of "Sufaid" loquat, a local popular cultivar, was handpicked at mature stage and immediately transported to the Post Harvest Laboratory at Department of Horticulture, PMAS Arid Agriculture University Rawalpindi. After clipping and sorting, the fruit was washed with distilled water and air dried. Dipping treatments with ascorbic acid and citric acid were applied for two minutes at concentrations of 250, 500 and 750 mg l^{-1}. Fruit dipped in distilled water were kept as control. The fruit was packed in corrugated soft board cartons and stored at 4°C.

Ten fruits were randomly selected to prepare a sample on day of harvest and then at an interval of two weeks from each treatment during the storage. After determining the browning index, fruit was peeled, cut into pieces and a composite sample was frozen in liquid nitrogen which was stored at -80°C in order to record the data on different parameters.

Browning index (BI) was evaluated by the technique of Wang *et al*., (2005) at weekly interval. Enzyme extraction was carried out by the technique described by Abassi *et al*., (1998). SOD activity was recorded by the method of Dhindsa *et al*., (1981), Catalase, POD and PPO activity was determined according to Abassi *et al*., (1998). Total phenolic contents were determined with Folin-Ciocalteu reagent (Slinkard & Singleton, 1977) according to the method of Piga *et al*., (2003), with some modifications. A modified version of the method of Brand-Williams *et al*., (1995) was used to measure free radical scavenging activity. Data were analyzed using MSTAT-C software (Anon., 1991). Means were compared by Duncan's multiple range test at 5% level of significance.

Results and Discussion

Browning index: Browning index was highest in untreated fruit (control) followed by lower concentrations of both ascorbic acid (AA) and citric acid (CA). AA and CA at 750 mg l^{-1} were at par with each other and exhibited the lowest values of BI (Table 1). A simple explanation may be that these higher concentrations inhibited the activity of PPO which prevented the oxidation of phenolic compounds. Application of surface treatments is done to delay physiological decay in fruits. AA is a widely used natural inhibitor of PPO. It inhibits enzymatic browning very effectively, due to its ability to reduce *o*-quinones back to their native phenolics before they undergo further reaction to form pigments. Citric acid lowers the pH and chelates the copper at the active site of the enzyme (Robert *et al*., 2003). It prevents the browning of sliced apple and extends its shelf life (Santerre *et al*., 1988).

Table 1. Effect of anti browning agents on loquat fruit during storage

Treatments	BI	SOD (U/g)	CAT (U/g)	POD (U/g)	PPO (U/g)	TP (mg/100g)	RSA (%)	REC (%)
Water dip (control)	20.38A	33.33C	2.55CD	3.08B	39.86A	25.81B	42.53C	52.86A
AA 250 mg/l	10.99B	39.58A	2.77B	3.17A	35.10A	27.84AB	43.38C	38.83DE
AA 500 mg/l	9.88BC	40.51A	2.73BC	3.16A	18.49C	28.38AB	55.90A	41.51C
AA 750 mg/l	8.45CD	34.14C	2.61BCD	3.15A	13.71CD	29.51A	57.40A	37.99E
CA 250 mg/l	9.51BCD	37.49B	2.49D	3.06B	27.62B	27.07AB	43.13C	49.33B
CA 500 mg/l	9.72BCD	39.43A	2.97A	3.00C	12.50D	27.69AB	50.66B	42.3C
CA 750 mg/l	7.45D	34.09C	2.64BCD	2.98C	23.89B	28.66AB	49.85B	41.21CD
LSD	**2.24**	**1.73**	**0.17**	**0.05**	**5.34**	**3.26**	**3.28**	**2.40**

Effect on SOD and CAT activity: Both AA and CA at higher concentrations significantly reduced SOD activity and were statistically similar with control. Maximum activity (40.51, 39.58 and 39.43 U/g FW) was recorded in 250 mg l^{-1} AA, 500 mg/l AA, and 500 mg/l CA, while it was lowest (33.33 U/g) in control (Table 1). Overall activity gradually decreased in all treatments till the end of storage (Fig. 1). CAT activity was highest in case of CA 500 mg/l whereas, it was low in rest of the treatments with a significant difference (Table 1). It was also noted that CAT activity increased in all treatments during the first two weeks of storage and then gradually decreased (Fig. 1) The initial rise of the catalase activity during the earlier stage of storage may possibly be due to the burst in the level of free radicals (hydrogen peroxide, atomic oxygen and hydroxyl) as a result of low temperature stress which is used as signaling compound for the activity of catalase (Lafuente *et al.*, 2004). Earlier studied by Polidoros *et al.*, (2003) concluded that the induction of various stresses enhanced the activity of catalase enzyme as compared to control. Furthermore, the decrease in the activity onward was linked with the onset of senesce (Escuredo *et al.*, 1996; Gogorcena *et al.*, 1997).

Exposure of plants to unfavourable environmental conditions such as extreme temperature can increase the production of ROS e.g., O_2, OH^-, H_2O_2. Sustained accumulation of Reative oxygen species (ROS) could cause lipid peroxidation, aggravate oxidative damage hence accelerate senescence (Hariyadi & Parkin., 1991). To protect themselves against these toxic oxygen intermediates, plant cells and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defense system. A great deal of research has established that the induction of the cellular antioxidant machinery is important for protection against various stresses (Tuteja, 2007; Khan & Singh, 2008; Gill *et al.*, 2011; Singh *et al.*, 2008). Higher activity of CAT and SOD has been associated with stress tolerance in plants because it neutralizes the reactivity of the free radicals (Bowler *et al.*, 1992). In the present study lower concentration of both the antioxidants might be able to maintain the higher activity of CAT and SOD enzyme which could be due to lower level of generation of free radicals in the treated fruits. The lower activity in the higher doses of ascorbic and citric acid could be explained by the fact that the chemicals could be able to reduce the low temperature breakdown which is visible from the data of browning index in the Table 1, hence activity of CAT, SOD was also reduced. Therefore, it is conceivable that higher concentrations of AA and CA lowered the activity of SOD is due to its effect reduction of low temperature breakdown and

ultimately the ROS, however this assumption opens windows for future confirmation.

SOD is the first line of defense from damages caused by oxygen radicals (Mittler, 2002) and its activity has been linked to physiological stresses such as low temperature, high intensity light, water stress and oxidative stress (Bowler *et al.,* 1992). SOD in combination with catalase transforms superoxide radical and hydrogen peroxide into molecular oxygen and water, thus avoiding cellular damage (Scandalios, 1993). Ascorbic acid can donate electrons in many reactions making it the main ROS detoxifying compound (Blokhina *et al.*, 2003). Citric acid is an antioxidant synergist and is commonly used as an anti browning agent (Christopher *et al.*, 2003). Results of the study indicate that CA maintained CAT activity which shows it had a role in protection against oxidative damage as described by Ng *et al.*, (2005). It can be assumed that this effect was a consequence of the activity of some genes related to the cellular defense against stress (Ding *et al.*, 2002).

Effect on POD activity: All AA concentrations had significantly higher POD activity. Lowest activity was recorded in 500 mg/l CA and 750 mg/l CA (Table 1). Overall activity increased till the fourth week, decreased during the next two weeks and again increased by the end of tenth week. Ascorbic acid has a significant effect on POD activity as all concentrations resulted in higher POD activity, whereas higher concentrations of citric acid significantly lowered POD activity as compared to control.

Maturation (ripening) has been described as an oxidative process in climacteric fruits (Brennan & Frenkel, 1977; Rogiers *et al.*, 1998). Conceivably then, the antioxidant system may be involved in the control of fruit maturation.

Increasing ripening process results in abrupt increase of the ROS which alter membrane integrity and react with unsaturated fatty acids causing peroxidation (Lacan & Baccou, 1998; Jimenez *et al.*, 2002). However antioxidant enzymes such as SOD, POD and CAT play a crucial role in defense against ROS during ripening process of fruit, as SOD converts O_2^- to H_2O_2 while, H_2O_2 removal by POD, CAT and APX (Mondal *et al.*, 2004; Schanz *et al.*, 1995). In contrary to that POD is reported to oxidize polyphenol substances quickly in the presence of H_2O_2 and causes fruits or vegetables to brown (Zhang & Zhang, 2008). POD activity is known to increase with advancing senescence (Tian, *et al.*, 2004). In the present study peroxidase activity do not show any clear effect on browning of loquat fruit during storage.
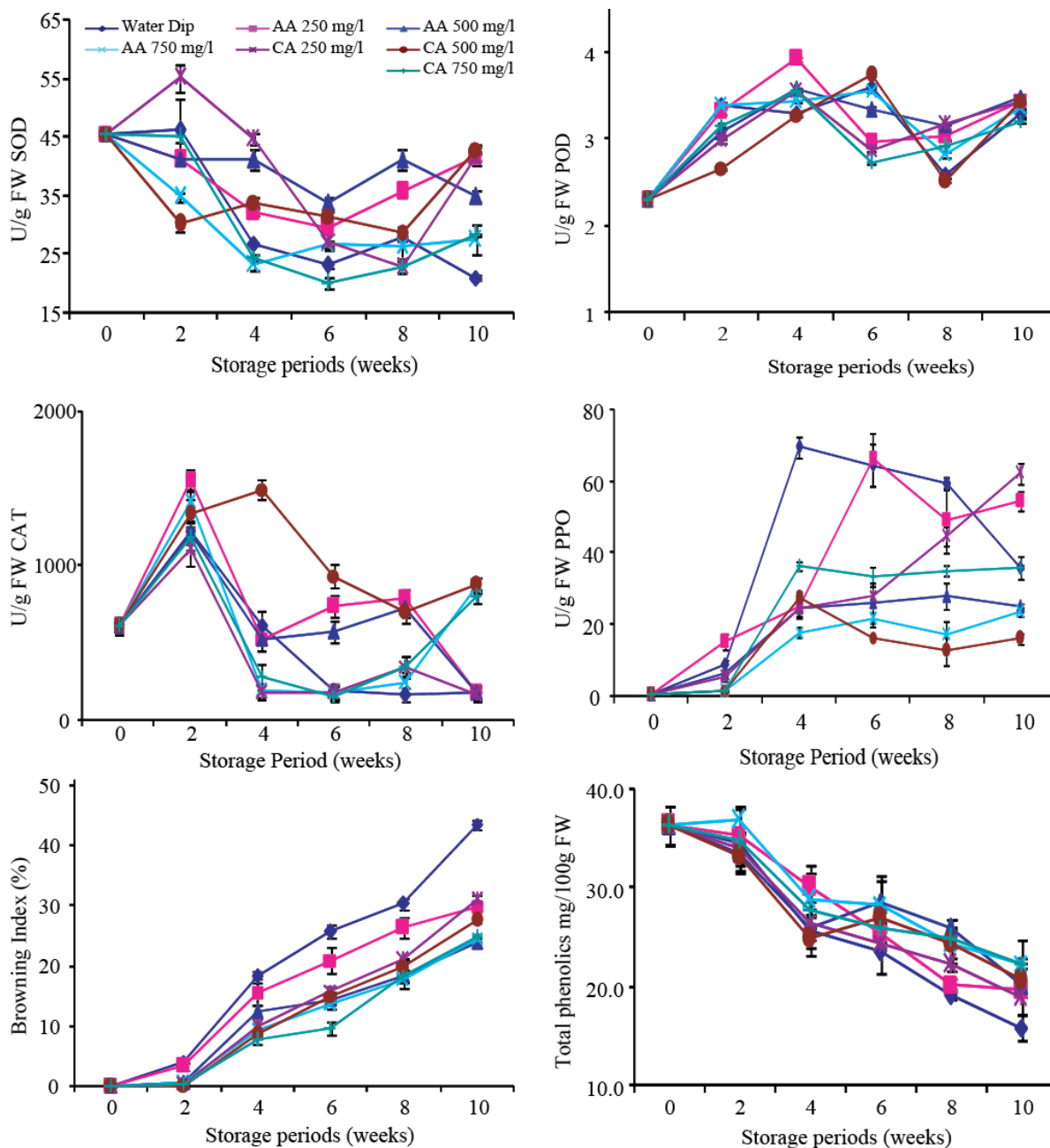
Fig. 1. Effect of anti browning agents on SOD, POD, CAT, PPO, BI and TP content of loquat fruit during ten week storage.

Effect on PPO activity: Lowest activity (12.5 and 13.71 U/g FW) was observed in 500 mg/l CA and 750 mg/l AA compared to control. Highest activity (39.86 and 35.10 U/g FW) was recorded in control and 250 mg/l AA. PPO is a key enzyme for enzymatic browning which is activated during ripening, senescence or stress condition when the membrane is damaged, resulting in increased PPO activity (Mayer, 1987). PPO activity has been known to increase during storage of loquat at low temperatures (Cai *et al.*, 2006a). Ascorbic acid inhibits browning reactions by reducing the *o*-quinones back to the phenolic substrates which are generated by the action of the PPO enzymes (Robert *et al.*, 2003); however, ascorbic acid is consumed

during the process and provides only temporary protection against discoloration unless very high concentrations are used (Gill *et al.*, 1998). Citric acid also inhibits PPO due to its chelating action (Jiang *et al.*, 1999). PPO activity has been reported to increase during storage of minimally processed 'Arkin' carambola stored at 4.4°C for 6 weeks (Weller *et al.*, 1997). According to Lattanzio *et al.,* (1989) both citric and ascorbic acid were effective in delaying browning reactions of the artichoke heads stored in closed polyethylene bags after 2 or 4 weeks.

Effect on total phenolic content: The treatments means show no significant difference of antibrowning agents

compared to control except for 750 mg/l AA which had a TP content of 29.51 compared to 25.81 of control (Table 1). At the end of tenth week, control had the lowest TP content (15.80) compared to other treatments while highest values of 22.37 and 22.33 was maintained by 750 mg/l AA and 750 mg/l CA (Fig. 1).

Browning changes have been correlated with a reduction in total phenolics and an increase in PPO activity (Cai *et al.*, 2006a). AA reduces the *o*-quinones generated by the action of the PPO enzymes, back to their phenolic substrates (Robert *et al*., 2003). Citric acid (CA) inhibits PPO due to its chelating action (Jiang *et al*., 1999). Both citric and ascorbic acid have proved to be effective in delaying of browning reactions (Lattanzio *et al.,* 1989). Lattanzio & Linsalata (1989) states that AA resulted in a stabilizing effect on the metabolism, however, at small concentrations, AA is quickly consumed in the reducing process therefore large AA concentrations may provide permanent protection against browning (Rocha & De Morais 2005).

The decrease in soluble phenolics observed toward the end of storage may be due to the breakdown of these cellular structure (Toor & Savage, 2006) leading to oxidation of TP (Cocci *et al*., 2006). Results show highly significant effects of high concentrations of AA and CA on PPO activity compared to control. It may be that AA inhibited browning by reducing the *o*-quinones whereas CA inhibited PPO due to its chelating action. Results show that both high concentrations of AA and CA effectively maintained the TP content compared to control. Gil *et al*., (1998) also reported that 2% AA effectively prevented decrease in TP content during storage of 'Fuji' apple slices.

Effect on free radical scavenging activity (RSA): Ascorbic acid 500 mg/l and 750 mg/l had the highest FRSA values (55.90% and 57.40%) whereas control had the lowest activity (42.53%). The next highest values (50.66 and 49.85%) were recorded in 500 mg/l CA and 750 mg/l CA (Table 1).

Losses of antioxidants during storage vary by the type of fruit, storage temperature and environment. Most studies of antioxidant losses have examined ascorbic acid as being the most reactive of the antioxidants (Shewfelt, 1990). During advanced tissue senescence, concentration of antioxidant substances increases (AA among them) to repair the effects of damage. Regeneration of AA helps to offset the growing production of ROS and other free radicals which lead to cellular injury or death (Sonia & Chaves, 2006). AA has the ability to donate electrons in a wide range of enzymatic and non-enzymatic reactions which makes it the main reactive oxygen species (ROS) detoxifying compound in aqueous phase (Blokhina *et al*., 2003), however, due to its irreversible oxidation its levels tend to decrease during storage and processing of fruits and vegetables and therefore its effect is only temporary (Rojas, *et al.* 2007). Kulkarni and Aradhya (2005) attributed low antioxidant activity in pomegranate arils during storage to a reduced concentration of total phenolics and ascorbic acid and whereas increase in FRSA could be due to higher total phenolic content on exposure to low temperature stress (Padda & Picha, 2008) which may explain the reduction in our results.

Several phytochemicals including AA contribute to the total antioxidant activity of fruits and vegetables (Chu *et al.*, 2000). Kulkarni & Aradhya (2005) attributed low RSA to a reduced concentration of total phenolics and ascorbic acid and a surge in antioxidant activity to an increased concentration of anthocyanin pigments whereas Padda and Picha (2008) attributed the increase in RSA to higher total phenolic content due to exposure to low temperature stress

Effect on relative electrical conductivity (REC): AA reduced REC more effectively than CA treatments. The lowest REC 37.99% was recorded in 750 mg/l AA followed by 250 mg/l AA (38.83%), whereas, it was highest (52.86%) in control (Table 1). Overall REC increased gradually during storage.

High conductivity is indicative of leakage of intracellular ions and, therefore, damage to membranes (Ade-Omowaye *et al.*, 2003). It is possible that during storage, plasma membrane of the cell would tend to be unstable and consequently lead to electrolyte leakage (Feng *et al*., 2005). Free radicals and lipid hydroperoxides lead to membrane degradation (Cai *et al*., 2006b). AA regeneration or synthesis helps to offset the growing production of ROS and other free radicals leading to cellular injury or death (Sonia & Chaves, 2006). From the results it is clear that the antioxidant properties of AA helped to scavenge the harmful free radicals which in turn reduced electrolyte leakage by keeping the membranes intact as compared to CA treatment.

The REC continually increased during storage suggesting a gradual loss of cell membrane integrity and increasing senescence of the tissue. During stress conditions, electrolytes leak into surrounding tissues therefore high conductivity indicates leakage of intracellular ions (Ade-Omowaye *et al.*, 2003). Results show that the antioxidant properties of AA helped to scavenge the harmful free radicals and keeping the membranes intact, which reduced electrolyte leakage compared to CA treatment.

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