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Polyphenoloxidase Activity, Polyphenol and Ascorbic Acid Concentrations and Internal Browning in Asian Pear (*Pyrus serotina* Rehd.) Fruit during Storage in Relation to Time of Harvest

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Summary

One of the major disorders that develop during storage in Asian pears is fruit internal browning (IB) of the core and/or flesh. The relationship between IB, ascorbic acid (AA) concentrations, total phenolic content (TPC) or polyphenoloxidase (PPO) activity during five months storage was investigated in Asian pear fruit. Mature Asian pear (*Pyrus serotina* Rehd.) trees cultivar 'KS'$_{13}$ grown on European pear seedling rootstocks were used during the 2006 growing season. Fruit were harvested on August 1 (H1) and August 15 (H2), representing early and late harvest for this cultivar. Fruit were picked according to the maturity standard based on soluble solid content (SSC), titrable acidity (TA), firmness and background colour and stored at 1 °C and 80–85 % relative humidity (RH). Results indicated that IB incidence was higher in fruit from H2 than H1. The activity of PPO in disordered fruit varied with the intensity of the disorder. Ascorbic acid decreased during long-term storage and was lower in H2 fruit compared with H1 fruit. There was a trend for IB to develop when the AA concentration dropped below a threshold. This threshold value depended on picking date and time elapsed from storage and varied between about 3.3 to 9.4 mg 100 g^{-1} FW. The amount of TPC declined during storage in both H1 and H2 fruit as PPO activity and intensity of IB damage increased. It is suggested that the lower incidence of IB in earlier harvested fruit was a result of the presence of a higher level of AA and a lower level of PPO activity than in later harvested fruit.

Key words. ascorbic acid – Asian pear – total phenolic content (TPC) – IB incidence – date of harvest – soluble solid concentration (SSC) – titrable acidity (TA) – enzyme activity

Introduction

Internal browning (IB) of the core and/or flesh usually occurs during handling, processing and storage in apple (Jang et al. 2002) and pear (Crisosto et al. 1994). The IB phenomenon usually impairs the sensory properties of products because of the associated changes in colour, flavour and softening (Kim et al. 2005). Browning in fruit is caused by the enzymatic oxidation of phenolic compounds by polyphenoloxidase (PPO) (Martinez and Whitaker 1995). The degree of browning depends on the nature and amount of endogenous phenolic compounds and the activity of PPO (Goupy et al. 1995). Plant phenolics are readily oxidized by PPO, most often following tissue damage since PPO is suggested to act as a defensive enzyme (Mayer and Harel 1990). Although PPO has generally been recognized to be largely responsible for enzymatic browning of fruit, it has not been clearly established (Gauillard and Richard-Forget 1997). Relationship between PPO activity and phenolic level are variable depending on fruit type, maturity stage and postharvest treatment (Nicolas et al. 1994). Ascorbic acid (AA) is an antioxidant, which acts against reactive O_2 (Jimenez et al. 1997). In European pears, a relationship was found between AA content, harvest date and the susceptibility to browning during the storage period (Veltman et al. 2003). It has been reported that in 'Conference' pear tend to develop IB when AA levels drop below a certain value. Asian pear is new fruit crops in Iran with characteristics such as a crisp texture, very good taste, flavour and slight aroma, different colour and shape, long shelf life and high productivity (Arzani 2002; Arzani 2005). Asian pear cultivar 'KS'$_{13}$ is a mid-season and productive cultivar with brown fruit skin colour, but damaged with IB disorder during storage (Arzani 2004). In this study, we investigated the changes of PPO activity, total phenolic content (TPC) and AA concentrations and their relationships to IB incidence in early and late harvested Asian 'KS'$_{13}$ pear fruit during five months storage. AA and IB are discussed in relation to TPC, PPO activity and time of fruit harvest.

Materials and Methods

Plant material

Twenty mature five years old Asian pear (*Pyrus serotina* Rehd.) trees of the cultivar 'KS'$_{13}$ grown on European

pear seedling rootstocks at Tarbiat Modares University (TMU) research orchard were used during the 2006 growing season. The trees selected for this study were similar in size and crop load and were growing with normal orchard practices. Fruit were picked (5 kg from each tree) on August 1 (H1) and 15 (H2) from the same trees at each harvest. At H1, fruit had soluble solid content (SSC) of 12.2 to 13 °Brix, titrable acidity (TA) of 0.25 to 0.24 %, firmness of 29.5 to 28.5 N and Hunter's a* 55, b* 17.5 and L* 53 values. At H2, fruit had SSC of 14.5 to 15.2 °Brix, TA of 0.22 to 0.21 %, firmness of 24.5 to 23.5 N and Hunter's a* 51, b* 19.2 and L* 58 values. Harvested fruit were stored at 1 °C and 80–85 % relative humidity (RH) for five months. Measurements and fruit chemical analysis were performed on 4 to 5 fruit taken randomly at harvest and after storage for 1, 2, 3, 4 and 5 months.

Sample preparation and PPO assay

Asian pear fruit (100 g) (cortical flesh with skin and core tissues without seeds) was homogenized using a blender with 200 ml of a 50 mM phosphate buffer at pH 6.8 for 3 min, then centrifuged at 15,000 g for 20 min. The supernatant was used for PPO activity assay. All steps were carried out at 4 °C. The PPO activity was assayed with 0.2 M catechol as a substrate by a spectrophotometric procedure (Zauberman et al. 1991). Absorbance, at 420 nm, was monitored continuously at 25 °C for 1 min.

Determination of total phenolics

The concentration of phenolic compounds were determined according to the Folin-Ciocalteu method with a spectrophotometer (at 765 nm using UNICAM 8620 UV-Vis spectrophotometer) (Singh et al. 2002) and results were expressed as mg tannic acid per 100 g fresh weight of samples (mg TAE 100 g^{-1} FW).

Ascorbic acid measurements

Samples were prepared from fruit of Asian pear. The fruit (5 g) was milled, filtered through filter paper and then centrifuged at 11000×g at 4 °C for 20 min. The pear fruit juice (5 ml of supernatant) was diluted to 20 ml with $K_2HPO_4 \cdot 3H_2O$ (0.01 M, pH = 2.6), and then filtered through a 0.45 µl Millipore organic filter. An aliquot of 20 µl was injected into the HPLC system (Waters, USA), equipped with a column compartment model Waters, 2478 UV detector (Waters, USA) with wavelength set to 210 nm (Chen et al. 2007), and a reversed phase PRONTOSIL 120-10-C_{18}H (250 × 4.6 mm i.d., d.p.10.0 µm) column, (Waters, USA). The mobile phase consisted of an aqueous solution of $K_2HPO_4 \cdot 3H_2O$ 50 mM (pH = 2.6) at a flow rate of 0.7 ml min^{-1} at 30 °C.

IB evaluation

Fruit flesh and core browning (IB) was assessed in fruit that was cut longitudinally into four equal parts and rated as percentage of incidence and intensity using the scale of 1 = 1–10 %, 2 = 11–30 %, 3 = 31–55 %, 4 = 56–75 % and 5 = 76–100 % of the cut surface affected. Note that IB assessment was performed on 5 replications (10 fruits per sub-replicate, a total of 50 fruits per replicate).

Statistical analysis

The results were statistically evaluated by analysis of variance (ANOVA) and were expressed as mean ± standard error (SE). The differences at $P \leq 0.05$ were considered statistically significant. In addition, comparison of the means of 5 fruits per replicate (50 fruits for IB evaluation) and 5 replicates per assessment was made using Duncan's Multiple Range Test (DMRT).

Results

PPO activity, AA, TPC and IB incidence and severity in relation to date of harvest

Changes during storage in AA, TPC, PPO activity and IB incidence and severity for the early (1st August; H1) and late (15th August; H2) harvested 'KS'$_{13}$ Asian pear fruit is given in Table 1, Fig. 1 and Fig. 2. The IB incidence and severity varied with harvest dates. H2 fruit had a 19, 37 or 50 % higher incidence of IB than H1 fruit after 1, 3 or 5 months of storage, respectively. Ascorbic acid concentrations were higher in H1 fruit than H2 fruit after 2, 3, and 5 months of storage because of a greater decrease in AA in H2 fruit after the first month of storage. The PPO activity increased significantly during the first month of storage and then peaked after 3 months before declining slightly. The PPO activity was higher and increased sharply during storage in the later harvested fruit compared with earlier harvested fruit. The TPC declined slowly during storage in H1 and H2 fruit, but there was no significant difference in TPC between the two harvest dates (Table 1).

Relationships between IB, PPO activity and TPC content

PPO activity in disordered fruit varied with the intensity of IB. Slightly affected fruit (index 0 to 2) showed a significant increase in PPO activity (Fig. 3). Later, as the IB damage progressed (index 3 to 5), PPO activity decreased. In this study, the PPO activity was depended on the duration of fruit storage. In contrast to IB, PPO activity increased steadily with an increase of time of storage in the H1 and the H2 fruit harvest until after 3 months of storage (Table 1). The PPO activity was higher at harvest and increased more sharply during storage in H2 fruit than H1 fruit. A relationship between polyphenol content and PPO activity was observed in the early and late harvested fruit during the storage period. The TPC decreased as the PPO activity and the IB incidence and severity increased (Fig. 4).

Relationships between AA content and PPO activity and IB value

The data was examined for relationships between AA content and the susceptibility to browning during storage. 'KS'$_{13}$ fruit tended to develop IB disorders as the AA content decreased during storage, and between the harvests there was a clear difference in the level of AA between sound and disordered pears (Fig. 5). Fruit developed IB symptoms when AA had dropped below a certain level and these thresholds varied between 3.3 and 11 mg 100 g^{-1} FW depending on harvest date and storage

Table 1. The changes of polyphenoloxidase (PPO) activity, total phenolic content (TPC) and ascorbic acid (AA) concentration of Asian pear fruit cultivar 'KS'$_{13}$ during storage at 1 °C for 5 months. Fruit were harvested on 1st August (H1) or 15th August (H2), 2006. Values are the means ± standard error of the mean based on 5 fruits (50 fruits for IB evaluation) per replicate and 5 replicates per assessment.

Harvest date	Storage* (months)	PPO activity (min^{-1} 100 g^{-1} FW)	TPC (mg TAE 100 g^{-1} FW)	AA (mg 100 g^{-1} FW)	IB (%)	IB (IB score)
H1	0	221 ± 21.4 c	51.2 ± 1.1 a	13.2 ± 0.7 a	2 ± 0.3 d	0.0 ± 0.4 b
	1	281 ± 31.1 bc	46.1 ± 0.9 b	12.1 ± 0.9 ab	4 ± 0.8 c	1.0 ± 0.3 b
	3	397 ± 41.7 a	32.3 ± 0.8 c	9.9 ± 0.4 b	18 ± 0.7 b	2.8 ± 0.7 a
	5	341 ± 52.8 b	21.0 ± 0.8 e	6.6 ± 0.2 d	29 ± 0.6 a	3.2 ± 0.4 a
H2	0	491 ± 44.2 b	55.3 ± 1.3 a	13.5 ± 0.3 a	18 ± 0.3 e	1.0 ± 0.2 c
	1	515 ± 62.1 b	48.2 ± 0.8 ab	12.7 ± 0.5 ab	23 ± 0.2 d	1.8 ± 0.3 c
	3	930 ± 59.3 a	34.5 ± 0.9 c	6.1 ± 0.3 c	55 ± 0.1 c	3.1 ± 0.5 bc
	5	850 ± 71.1 a	22.1 ± 1.1 e	3.7 ± 0.2 e	79 ± 0.7 a	4.1 ± 0.7 a

Mean values ± SE. Within a column, for each harvest, values followed by different letters are significantly different: Mean separation by Duncan multiple range tests at P≤0.05.

time. After 1 month of storage, the AA concentration in sound pears was always higher than 9.4 (H1 fruit) and 11 mg 100 g^{-1} FW (H2 fruit), and after 5 months was higher than 3.3 (H1 fruit) and 4.2 mg 100 g^{-1} FW (H2 fruit) (Fig. 5).

Discussion and Conclusion

In general, the browning tendency of European and Asian pear fruit increases with advancing maturity (Veltman et al. 2003; Lin et al. 2007). IB can be avoided by early harvesting of Asian pears. Crisosto et al. (1994) reported Asian pears grown under Californian conditions and picked later than 180 days after full bloom are likely to develop IB during storage. Browning capabilities during storage, increased sharply in the more mature fruit (Veltman et al. 2000). Those reported changes in IB incidence and severity during storage are in agreement with our results. In addition, PPO activity increased steadily after storage in a similar manner for 'KS'$_{13}$ fruit. In this study, a relationship between PPO activity and IB incidence and severity were observed in the early and late harvested 'KS'$_{13}$ fruit after storage. A correlation between PPO activity and IB was previously reported for peach fruit (Cheng and Crisosto 1995). It has been suggested that PPO activity was not a limiting factor in the enzymatic browning and that some other factor such as decline in the concentration of ATP, sugars, fatty acids and organic acids increased the tendency for browning in pear (Veltman et al. 2003). As shown in Table 1, Fig. 1 and Fig. 3, PPO activity declined during cold storage, and after 4 month of storage fruit had a high severity of IB. This reduction in PPO activity is probably due to a process of degradation of the enzyme which occurs in senescening fruit.

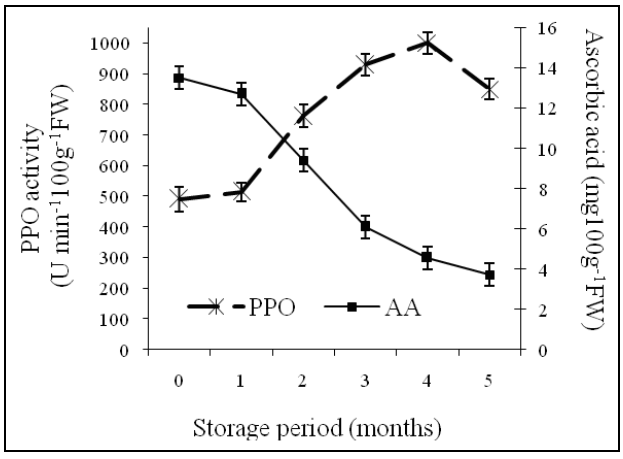


Fig. 1. Changes in ascorbic acid (AA) concentration and polyphenoloxidase (PPO) activity during five months storage of Asian pear fruit cultivar 'KS'$_{13}$ after harvested on 15th August. n=5 fruits per replicate and 5 replicates per assessment.

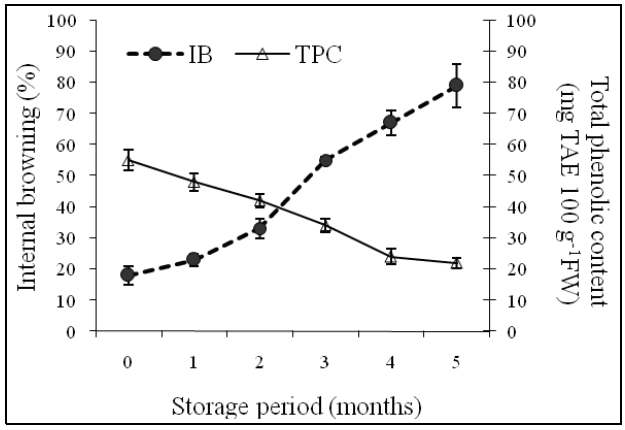


Fig. 2. Changes in degree of internal browning (IB) and total phenolic content (TPC) of Asian pear fruit cultivar 'KS'$_{13}$ harvested on 15th August during five months storage. n=5 fruits (for TPC assay) and 50 fruits (for IB evaluation) per replicate and 5 replicates per assessment.

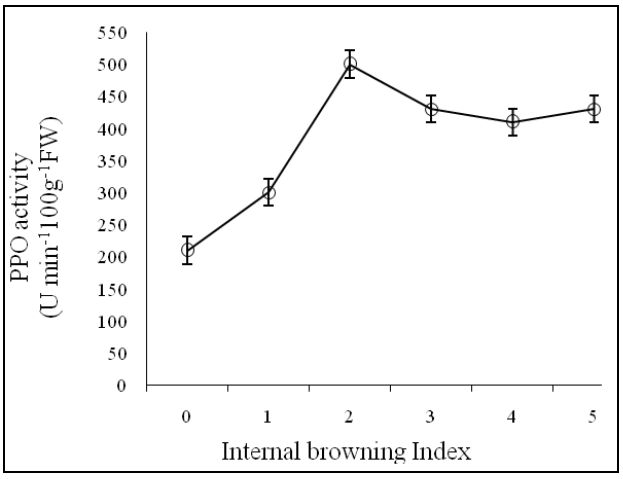


Fig. 3. The relationship between polyphenoloxidase (PPO) activity and the intensity of the damage by internal browning (IB) in damaged fruit in 'KS'$_{13}$ Asian pear. Index of IB, 0 = no damage, 1 = 1–10 %, 2 = 11–30 %, 3 = 31–55 %, 4 = 56–75 % and 5 = 76–100 % of damage. Each value is the mean of 5 fruits per replicate and 5 replicates per assessment in each month of storage.

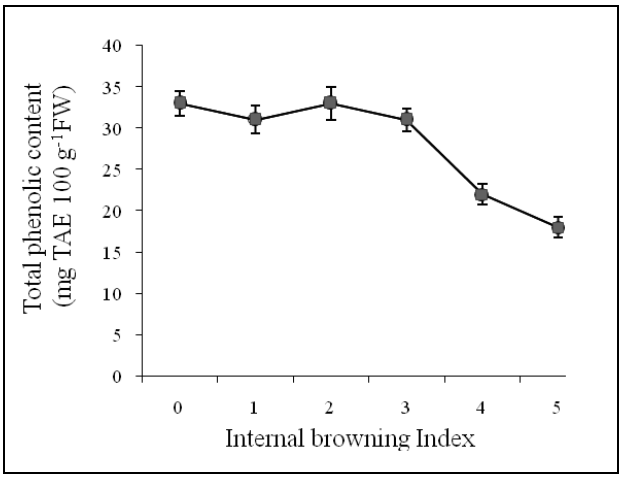


Fig. 4. The relationship between total phenolic content (TPC) and the intensity of the damage by internal browning (IB) in damaged fruit in 'KS'$_{13}$ Asian pear. Index of IB, 0 = no damage, 1 = 1–10%, 2 = 11–30%, 3 = 31–55%, 4 = 56–75% and 5 = 76–100% of damage. Each value is the mean of 5 fruits per replicate and 5 replicates per assessment in each month of storage.

A decrease in TPC of early and late harvested fruit was observed during storage. Phenolics may be degraded during storage and their decline is higher in the more mature fruit. Both TPC and activity of PPO have been found to be influenced by maturity and postharvest storage of most fruit, as well as by several external and internal factors affecting phenolic metabolism (Daniel et al. 1999). Total phenolic acid contents decreased and the composition of phenolic acids in 'Yali' pear fruit changed during storage, especially the content of chlorogenic acid, from time of harvest (148 mg l^{-1}) to 5 months after harvest (128.5 mg l^{-1}) (Chen et al. 2006). In this study, TPC decreased during storage in early and late harvested fruit,

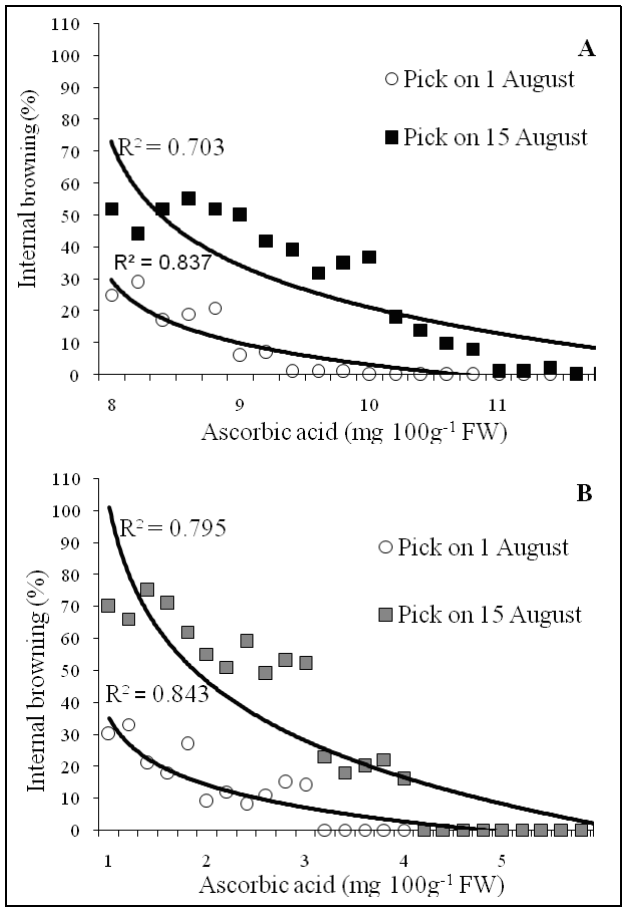


Fig. 5. The relationship between internal browning (IB) and ascorbic acid (AA) in Asian pear fruit cultivar 'KS'$_{13}$ recorded after 1 month (A) and 5 months (B) after storage. Every data point is an average of a mixed sample of five fruit harvested on the 1st and 15th August.

and a decrease in TPC was observed with increase in PPO activity. It has been reported that during ripening of 'Yali' pear, the phenolic acids content dropped (Ju 1991; Lin et al. 2007). This can be explained by the concurrent increase in polyphenoloxidase (PPO) activity. An association between TPC and PPO activity in pear fruit has previously been reported (Carbonaro and Mattera 2001). Ascorbic acid (AA) is an antioxidant, which acts against PPO activity (Jimenez et al. 1997). AA concentration of pear fruit decreased during storage and a hypothesis has been developed (Veltman et al. 2000), which states that a higher AA concentration leads to the less susceptibility of fruit to IB, and that pears will be affected when AA drops below a certain value (AA threshold hypothesis). Following this hypothesis, a threshold value of 1.3 mg 100 g^{-1} FW, defined as the AA concentration of cortex tissue at which the brown index exceeded 0.35 was found (Veltman et al. 2003). Zerbini et al. (2002) determined a critical AA concentration of 0.2 mg 100 g^{-1} FW based on analysis of core tissue of sound pears. The AA threshold value in this study was defined as the concentration at which the first appearance of browning was noticed and appeared (about 5 % IB damage) after 1 month of fruit storage. In addition, in this study, the thresholds AA was

correlated with picking date and storage time, varying between about 3 mg 100 g^{-1} FW (early harvest and stored for 1 month) and 11 mg 100 g^{-1} FW (late harvested) (Fig. 5). VELTMAN et al. (2000) reported that the thresholds AA depend on cultivar and picking date, varies between 2 and 6 mg 100 g^{-1} FW.

In the present research, incidence of IB was related to harvest date, the later picking date (H2), having the higher browning than early picking date (H1). PPO activity was higher and increased sharply during storage in late harvested fruit. PPO activity in damaged fruit varied with the intensity of the IB damage. AA was lost mainly during long-term storage and more AA was lost in fruit that were harvested late than fruit that were harvested earlier. LIN et al. (2007) found that the core browning index (CBI) was reduced by 10.4, 24.5 or 40.0 % in 'Yali' pears treated with 0.1, 1.0 or 10.0 mM AA, respectively, after 180 days of storage. In our research a relationship was found between AA content and the susceptibility to browning during the storage period. The Asian pear cultivar 'KS'$_{13}$ tended to develop IB disorders when AA content decreased during storage. It is suggested that the PPO activity was inhibited by the presence of AA in fruit. The 'KS'$_{13}$ pears tended to develop IB when AA levels had dropped below a threshold value which was depended on picking date and storage duration and varied between 3.3 to 9.4 mg 100 g^{-1} FW. Total phenolic content (TPC) declined during storage in early and late harvested fruit as PPO activity and the incidence and severity of IB increased. Hence, fruit picked earlier at the earlier maturity level had a lower incidence of IB.

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