

Maintenance of Fruit Firmness and Pectin Methyl Esterase Activity of Different Pear Cultivars through Post-Harvest Application of Diphenylamine

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Abstract

An experiment was conducted to find out the effect of diphenylamine on fruit firmness and pectin methyl esterase activity of hard, semi-soft and soft pear cultivars. Pear fruits of cultivars Patharnakh, Punjab Beauty and YaLi were harvested at proper maturity and treated with different concentrations of diphenylamine (DPA). The fruits were then stored for 45, 60, 75 and 90 days (Patharnakh), 45, 60 and 75 days (Punjab Beauty) and 30, 45 and 60 days (YaLi) in cold storage at 1—3°C temperature with 90—95% relative humidity (RH). After every storage interval, fruit firmness and pectin methyl esterase activity (PME activity) was analyzed in whole fruits. Fruit firmness decreased with increase in storage period and PME activity increased. Concentration of DPA (1500 ppm) was found to be most effective in maintaining higher fruit firmness by reducing the activity of PME throughout storage period as compared to other treatments.

Key words : Diphenylamine, Pear, Cultivars, Cold storage, PME activity.

In north Indian plains, low-chilling pear cultivars are gaining importance owing to their high productivity, good climatic and soil adaptability. Pear is one of the important fruit crop successfully thriving in Punjab in the districts of Amritsar, Gurdaspur, Jalandhar, Hoshiarpur and Patiala. It is delicious and juicy fruit and is consumed fresh, cooked, dried or preserved. It is a rich source of proteins and minerals like calcium, iron and vitamin A, B and C. A variety of products like jam, jelly, nectar, RTS, squash and murabha can be made from pear. Patharnakh is a common cultivar grown in Punjab since many years and is a hard pear variety. Punjab Beauty is a semi-soft and YaLi is a soft pear cultivar performing well in climatic conditions of Punjab. Area under low-chilling pears is increasing in this state, due to release of new soft pear cultivars. During harvest season there is glut in market leading to low rates and spoilage. Phased selling of produce is possible with aid of suitable storage treatments. It holds back the produce when there is glut and prices are unfavorable in the market. After harvesting, the mature fruits undergo a series of metabolic processes, which lead the fruit

towards ripening. The tissue continues to respire and transpire during these metabolic processes, and loses a significant quantity of moisture. A significant feature occurring during the storage of pear fruits is the softening of tissue, brought about by the changes in cell wall composition, which accompany the softening of fruit apparently results from the action of enzymes produced by the fruit. Prominent among the enzymes implicated are polygalactonase and pectin methyl esterase. These enzymes made striking changes in wall pectin content. Softening in fruits is caused either by breakdown of insoluble protopectin into soluble pectin (1). Post-harvest application of DPA at different concentrations yielded the fruits with high flesh firmness throughout the storage period as compared to control fruits, which might be due to reduction of PME activity. DPA at concentration of 3000 ppm most significantly retained fruit firmness in Granny Smith apple fruits (2). In Anjou Pear, Shield DPA 2000 ppm gave higher fruit firmness (3). Pre-storage treatment with dips in Ca and DPA solutions, reduced flesh browning also delays PME activity (4). Therefore an attempt is made to study the effect of

DPA on fruit firmness and PME activity of different pear cultivars.

Methods

Pear fruits of cultivars Patharnakh, Punjab Beauty and YaLi were harvested during 2006 and 2007 from New Orchard, Department of Horticulture, Punjab Agricultural University, Ludhiana. Fruits of uniform shape, size and disease free were sorted out and dipped in diphenylamine at three concentrations (500, 1000 and 1500 ppm) for 5 mins and water dip was given to control fruits. Treatments were replicated thrice employing randomized block design factorial design. Two kg of fruits for each replication were packed in corrugated fiber board (CFB) cartons and these cartons were placed in cold store at 1—3C and 90—95% relative humidity (RH) for 45, 60, 75 and 90 days (Patharnakh), 45, 60 and 75 days (Punjab Beauty) and 30, 45 and 60 days (YaLi).

At different storage interval fruits were taken out for estimation of fruit firmness and Pectin methyl esterase enzyme's activity (PME activity). Fruit firmness was recorded with the help of penetrometer. PME activity was recorded by measuring the increase in acidity after the hydrolysis of methyl esters of pectin by enzyme preparation (5). One gram of the fruit sample crushed with pestle mortar (which was previously cooled at 4C) by adding 10 ml of sodium-phosphate buffer solution and was centrifuged. Then one ml of centrifuge was taken and mixed with two ml of pectin (1%) and two ml of buffer. From this, one ml of solution was taken and titrated against 0.01 N NaOH by using phenolphthalein as an indicator. After one hour, again one ml of solution was taken from the same solution (incubated at 37 C) and titration was repeated. End point is colorless to light pink color.

$$\text{PME activity } (\mu\text{eq/ml of Enzyme}) = \frac{\text{Difference between reading at 0 and 1 hour} \times \text{Volume of 0.01 NaOH used} \times 5 \times 10^6}{1000}$$

Results and Discussion

Patharnakh Cultivar

The data with respect to fruit firmness of

Table 1. Effect of DPA on fruit firmness (kg/cm²) of Patharnakh pear fruits (pooled data of two years). Treatment : 0.52; Storage interval : 0.52; Interaction : NS.

Treatments (ppm)	Storage intervals (days)					Mean
	0	45	60	75	90	
Control	7.61	5.92	5.50	4.91	4.20	5.13
DPA 500	7.61	6.30	5.95	5.34	4.72	5.57
DPA 1,000	7.61	6.46	6.14	5.75	5.36	5.93
DPA 1,500	7.61	6.70	6.45	6.15	5.75	6.26
Mean	7.61	6.34	6.01	5.54	5.01	—

Patharnakh cultivar (Table1) revealed that fruits treated with DPA exhibited best results in delaying loss of proper firmness up to 75 days of storage when compared with fruits under control. After 90 days of storage, DPA (1,500 ppm) treatment recorded maximum firmness as compared to control fruits. Fruit firmness was high in DPA treated fruits might be due to reduced transpirational activity, which allowed the peel tissue to remain turgid in the treated fruits. Post-harvest application of DPA (1,500 ppm) yielded the fruits with highest flesh firmness throughout the storage period in apples as recorded earlier (6).

Pectin methyl esterase (PME) activity in Patharnakh cultivar increased with the advancement of storage interval irrespective of different treatments (Fig. 1a). Significantly higher PME activity was recorded after 45 days against minimum after 90 days of cold storage. Minimum, mean PME activity was recorded in DPA (1,500 ppm) treated fruits, followed by DPA (1,000 ppm). However the maximum mean PME activity, was observed in fruits under control. The amount of the pectin methyl esterase activity per gram fresh fruit weight and per gram protein, decreased in the pear fruit. There was a high correlation between PME activity and protein (7). Pre-storage treatment with dips in Ca and DPA solutions which reduced flesh browning also delays PME activity reported earlier (4).

Punjab Beauty Cultivar

In Punjab Beauty, fruits treated with diphenylamine were firm as compared to untreated fruits (Table 2). DPA (1,500 ppm) was found to be most effective for maintaining the fruit firmness. DPA at concentra-

Table 2. Effect of DPA on fruit firmness (kg/cm^2) of Punjab Beauty pear fruits (pooled data of two years). Treatment : NS; Storage interval : 0.6; Interaction : NS.

Treatments (ppm)	Storage intervals (days)				Mean
	0	45	60	75	
Control	6.20	4.50	4.05	3.42	3.99
DPA 500	6.20	4.80	4.37	3.73	4.30
DPA 1,000	6.20	4.92	4.44	4.04	4.46
DPA 1,500	6.20	5.13	4.76	4.26	4.71
Mean	6.20	4.84	4.40	3.86	—

Table 3. Effect of DPA on fruit firmness (kg/cm^2) of YaLi pear fruits (pooled data of two years). Treatment : 1.85; Storage interval : 1.17 ; Interaction : NS.

Treatments (ppm)	Storage intervals (days)				Mean
	0	30	45	60	
Control	5.3	4.45	4.04	3.36	3.95
DPA 500	5.3	4.64	4.10	3.69	4.14
DPA 1,000	5.3	4.73	4.19	3.86	4.26
DPA 1,500	5.3	5.08	4.75	4.19	4.67
Mean	5.3	4.72	4.27	3.77	—

tion of 3,000 ppm most significantly retained fruit firmness in Granny Smith apple fruits reported earlier (2). Results are similar as noted earlier (8) that post-harvest application of DPA in apples retained more firm fruits as compared to control.

The data concerning pectin methyl esterase (PME) activity in Punjab Beauty pear fruits recorded under various treatments and days of storage in pear fruits under cold storage conditions is presented in Figure 1b. There is steep increase in PME activity of fruits with the advancement of storage intervals. Minimum mean PME activity was recorded after 45 days and maximum after 75 days of cold storage. It was observed that higher PME activity was recorded in fruits under control. However, minimum enzymes activity was observed in DPA 1,500 ppm treated fruits. Results are in conformity that pre-storage treatment with dips in Ca and DPA solutions which reduced flesh browning also delays PME activity (4).

YaLi Cultivar

Amongst the post-harvest treatments YaLi fruits dipped in DPA, 1,000 ppm, exhibited best results in retaining firmness up to 60 days of cold storage as compared to fruits under control (Table 3). Fruit firmness was found to be more after 30 days of cold storage while lowest after 60 days. Similar results were reported earlier (6) in apples that post-harvest application of DPA (1,500 ppm) yielded the fruits with highest flesh firmness throughout the storage period. Results are similar as noted earlier (8) that post-harvest application of DPA in apples retained more firm fruits as compared to control.

The pectin methyl esterase (PME) activity showed an increasing trend with the advancement of storage intervals irrespective of different treatments (Fig. 1c). The minimum activity of PME was noticed after 30 and maximum after 60 days of cold storage.

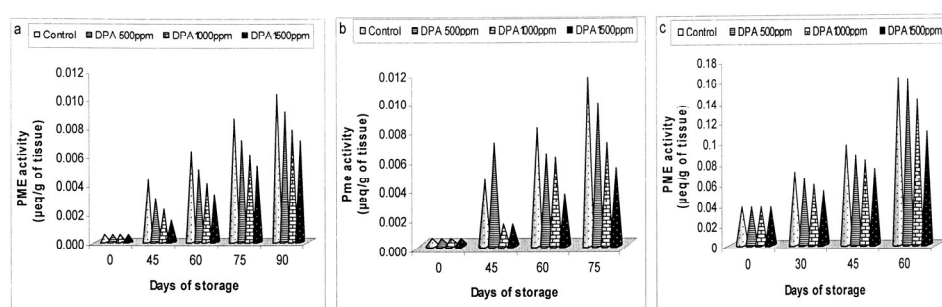


Figure 1. Effect of pre-storage treatments of DPA on pectin methyl esterase enzyme's activity ($\mu\text{eq}/\text{g}$ of fruit) in pear cv (a) Patharnakh, (b) Punjab Beauty and (c) YaLi (Pooled data of two years).

Higher DPA concentration was most effective to reduce the PME activity which was followed by DPA (1,000 and 500 ppm). There was a high correlation between PME activity and protein (7). The findings are similar as recorded earlier (4) that pre-storage dips in Ca and DPA solutions delayed the PME activity. Softening of tissue is the significant feature occurring during the storage of fruits, which is mainly brought about the acting of enzyme endopolygalacturonase.

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