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PROLONGATION OF STORAGE PERIOD OF PEAR FRUITS THROUGH INACTIVATION OF CERTAIN ENZYMES BY USING ETHYLENE-INHIBITING SOLUTIONS

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ABSTRACT

Ethylene-inhibiting substances such as n-propylagallate, silver nitrate and benzothiadiazole at different concentrations were sprayed on pear tree at 10, 15 days after full bloom. The fruits were harvested at maturity. Silver nitrate and benzothiadiazole at 100 mg L^{-1} concentration were found to be most effective in slowing down the polygalacturonase, cellulase and malic dehydrogenase enzyme activities of the fruit after harvest and during storage at 0±1 °C temperature and 90-95 percent relative humidity. These prolonged the shelf life of the fruits.

INTRODUCTION

Pear is one of the popular fruits. Though it has fairly good shelf-life but still it is desirable to further enhance the shelf-life, so that the fruits may be available for longer period for consumption and also can fetch better price. Several post-harvest treatments like use of calcium compounds, ethylene-inhibiting solutions, storage in perforated polyethylene bags and use of growth regulators are reported to extend the storage life of fruits (Ahlawat et al., 1984, Banik et al., 1987, 88; Singh, 1988, Kumar and Chauhan, 1990 and Chattopadhyay et al., 1992).

The fruits at maturity undergo an increase in respiration and ethylene production accompanied by marked changes in composition and texture such as enzyme synthesis, flesh softening, conversion of starch to sugars and synthesis of volatiles etc. The softening of fruit tissue during ripening is an important process affecting the edible quality of fruit as well as the length of time for which they can be stored (Knee and Bartley, 1981). Textural changes are result of the changes in the structure and composition of the cell wall. The enzymes involved in softenning of fruits are polygalacturonase (PGS) and cellulases (Knee, 1973; Bartley, 1978; Huber 1983). The respiratory enzyme, malic dehydrogenase is an important mitochondrial enzyme and its activity is reported to increase during ripening of apple (Hulme et al., 1964). Softening of the fruits also occurs during storage as a result of which the keeping quality of the fruit is reduced. In the present investigation, an attempt has been made to study the effect of preharvest application of ethylene inhibitors of the polygalaturonase, cellulase and malic dehydrogenase activities of delicious pear during cold storage with a view to reduce the softening and improve the keeping quality of the fruits, and to prolong their storage period.

MATERIALS AND METHODS

Pear trees (*Pyrus communis* L.) of uniform size and vigour were selected in Dana Farm, El-Nobbarrya, 151 Kilo Cairo-Alex. way. In each tree, 3 well spaced uniform branches with average to good crop load were selected for preharvest sprays. These branches constituted three replications for each treatment and the experiment was laid out in randomized block design. Two sprays of ethylene inhibitors viz. n-pro-pylgallate (100, 200

and 300 mg L^{-1}), $AgNO_3$ (25, 50 and 100 mg L^{-1}) and benzothiadiazole (25, 50 and 100 mg L^{-1}) were given to the pear fruits and surrounding foliage on the selected limbs at 10 and 15 days after full bloom. (These concentrations were used on apple fruits by Mahajand and Chopra, 1992). The control plants were sprayed with water.

Fruits were harvested at maturity and packed in standard size corrugated fibre board cartoon and stored in cold chamber maintained at 0± 1°C and 90-95 percent relative humidity. Ten fruits per replication from each treatment were drawn at 0, 90, 150, 180 and 210 days of storage and used to study the polygalacturonase (PG), cellulase and malic dehydrogenase enzyme activities.

PG and cellulase enzyme activities were determined according to the methods described by Abeles and Takeda (1990), and Mahadevan and Sridhar (1982). PG was extracted from pear fruits by homogenising 10 g of tissue with 50 ml of 0.15 M aqueous solution of sodium chloride. Then passed through four layers of cheese cloth and centrifuged at 10,000 g for 20 minutes at 4°C. The supernatant was used as source of enzyme. The enzyme reaction mixture consisted of 2 ml of enzyme extract, 4 ml of sodium polypectate in sodium acetate-acetic acid buffer (pH 5.2) and 1 ml of acetate buffer (pH 5.2). The contents were mixed thoroughly and incubated at 37°C for 16 hours. In a similar way the assay of cellulase was performed using carboxymethyl cellulose (CMC) as substrate. Rest of the procedudral steps are the same as described for PG activity.

Malic dehydrogenase activity was determined following the reduction of oxaloacetic acid with coupled oxidation of NADH to NAD^+ (Mallick and Singh, 1980). The enzyme activity was expressed as change in OD per minutes.

RESULTS AND DISCUSSION

A glimpse to the data on PG activity (Table 1) revealed that it was quite low at the time of sampling, increased with the storage upto 150 days and then declined towards the end of the sampling. The PG activity was the lowest in $AgNO_3$ (100 mg L^{-1}) and benzothiadiazole (100 mg L^{-1}). Control fruits on the other hand recorded the maximum activity upto 150 days and thereafter a sharp decline was observed.

The polygalacturonase due to their ubiquitous distribution and their temporal association with ripening are most often implicated in the wall metabolism responsible for softening (Huber, 1983). Softening of the fruit during ripening is normally accompained by an increase in the concentration of soluble pectic polysaccarides (Bartley and Knee, 1982; Huber, 1983). Hobsen et al. (1984) observed a decrease in PG activity of silver thiosulphate treated tomato fruits, which is also confirmed in the present study. They further suggested that silver may be involved in inhibition of PG activity by binding to the sites for ethylene action.

The cellulase activity at harvest was low in $AgNO_3$ (100 mg L^{-1}) treated fruits. The activity increased very slowly upto 150 days and then

declined gradually till the last sampling date. The control fruits on the other hand showed rapid decline in this regard.

Cellulases are responsible for the hydrolysis of cellulose to glucose. Knee (1973) has reported the loss of wall cellulose in some fruits during ripening. Abeles and Takeda (1990) have more recently reported that the slower steady loss of flesh firmness of apples may be caused by the continued action of cellulase already present in the fruit. However, Bartley (1976) suggested that pear has no cellulase activity. The lack of detailed information regarding the great numbers and specificity of fruit cellulases renders any attempt to assign a specific role to these enzymes in ripening, purely speculative (Huber, 1983).

The malic dehydrogenase activity was low in those fruits which were sprayed with $AgNO_3$ and benzothiadiazole (50 and 100 mg L^{-1}). These treatments slowed down the enzyme activity. The slowing down of malic dehydrogenase activity in some fruits may be attributed to the retardation of aerobic respiration and intermediatory metabolism in which the TCA cylce substrates are involved (Price and Thimann, 1954).

From the data it is obvious that silver nitrate and benzothiadiazole at 100 mg L^{-1} concentration were found to be most effective in slowing down the polygalacturonase, cellulase and malic dehydrogenase enzyme activities during storage and so these are the most effective in prolongation the storage period.

Table (1): Effect of preharvest application of ethylene inhibitors on polygalacturonase and cellulase enzyme activities of pear stored at 0±1°C and 90-95% relative humidity.

Treatments	Polygalacturonase					Cellulase				
	Days in storage					Days in storage				
	0	90	150	180	210	0	90	150	180	210
Control	16.5	24.8	31.2	21.8	17.4	11.2	12.2	13.2	9.4	8.0
n-Propylagallate										
100 mg L^{-1}	15.1	22.1	28.4	22.0	17.3	11.4	12.3	13.1	9.3	8.3
200 mg L^{-1}	14.8	21.6	25.6	22.7	17.6	10.5	11.2	11.8	9.5	8.4
300 mg L^{-1}	13.7	21.8	27.8	22.6	17.2	10.5	11.0	11.6	9.7	8.6
LSD 5%	1.004	1.239	1.132	0.213	0.067	0.137	0.081	0.066	0.068	0.038
1%	1.566	1.958	1.732	0.334	0.103	1.199	0.129	0.102	0.108	0.060
Control	16.4	24.9	31.5	21.4	17.9	11.4	12.1	13.5	9.5	8.1
$AgNO_3$										
25 mg L^{-1}	13.8	20.9	26.0	22.7	18.6	9.7	10.2	10.5	9.3	8.5
50 mg L^{-1}	11.9	18.3	24.8	22.9	18.5	9.0	9.5	10.2	9.6	8.3
100 mg L^{-1}	11.5	17.8	24.7	23.8	18.5	8.4	9.0	10.3	9.8	8.9
LSD 5%	0.817	0.284	0.731	0.275	0.374	0.284	0.417	0.352	0.831	0.113
1%	1.283	0.449	1.155	0.428	0.587	0.442	0.654	0.553	1.280	0.179
Control	16.5	24.9	31.2	21.7	17.8	11.3	12.3	13.3	9.3	8.0
Benzothiadiazole										
25 mg L^{-1}	13.6	19.8	25.6	23.2	17.7	9.6	10.2	11.4	9.2	8.8
50 mg L^{-1}	12.0	18.7	24.6	23.4	18.2	9.0	9.9	10.2	9.6	8.9
100 mg L^{-1}	11.5	18.1	24.8	23.7	18.4	8.9	9.3	10.1	9.9	9.0
L.S.D. 5%	1.063	0.980	1.097	0.993	0.984	1.132	0.976	0.966	1.365	0.783
%	1.648	1.548	1.711	1.559	1.525	1.766	1.533	1.507	2.1[illegible]	1.206

Table (2): Effect of preharvest application of ethylene inhibitors on malic dehydrogenase activity of pear stored at 0±1°C and 90-95% relative humidity.

Days of storage	Malic dehydrogenase				
Treatments	0	90	150	180	210
Control	0.079	0.135	0.190	0.065	0.020
n-Propylagallate					
100 mg L^{-1}	0.072	0.132	0.186	0.083	0.031
200 mg L^{-1}	0.068	0.130	0.184	0.086	0.028
300 mg L^{-1}	0.065	0.127	0.178	0.089	0.029
LSD 5%	0.012	0.021	0.009	0.004	0.019
1%	0.019	0.033	0.014	0.006	0.030
Control	0.079	0.135	0.190	0.065	0.020
$AgNO_3$					
25 mg L^{-1}	0.065	0.121	0.167	0.074	0.025
50 mg L^{-1}	0.062	0.109	0.158	0.081	0.029
100 mg L^{-1}	0.058	0.095	0.131	0.085	0.033
LSD 5%	0.010	0.018	0.011	0.006	0.014
1%	0.016	0.028	0.017	0.010	0.022
Control	0.079	0.135	0.190	0.065	0.020
Benzothiadiazole					
25 mg L^{-1}	0.061	0.107	0.155	0.080	0.028
50 mg L^{-1}	0.059	0.103	0.127	0.085	0.030
100 mg L^{-1}	0.055	0.075	0.119	0.087	0.036
L.S.D. 5%	0.002	0.019	0.018	0.003	0.016
1%	0.003	0.029	0.028	0.005	0.025