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SHORT COMMUNICATION

Effect of pre-storage heat treatment on enzymological changes in peach

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Abstract Peach (*Prunus persica* (L.) Batsch) fruit was subjected to hot water and moist hot air treatment at varying temperatures. The activities of polyphenoloxidase (PPO) and polygalacturonase (PG) were monitored during storage for 0, 3 and 6 days. PPO activity decreased in all treatments during storage. This decrease was more in hot water treated fruits than in hot air. PPO activity decreased with the increase in treatment duration. However, the PG activity increased in heat treated fruits as well as control. This increase was more in mild heat treatments as compared to severe heat treatment. Both polyphenol and pectin contents decreased during storage in both heat treatments.

Keywords Peach · Heat treatment · Pectin · Polyphenol · Polyphenoloxidase · Polygalacturonase

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Introduction

Peach (*Prunus persica* L) has a limited post-harvest life because of its high moisture content and metabolic activities that take place during post-harvest phase. It has a shelf life of 3–5 days under ordinary conditions of storage (Tonini and Tura 1998). Though some post-harvest treatments have been recommended because of one or the other reason, these are not put to practical use (Anderson and Penney 1975, Phillips and Austin 1982). Post-harvest heat treatment of moist air at 52°C for 15, 30 and 45 min reduced softening as well as ethylene production of fruits (Anthony et al. 1989). A characteristic effect of heat treatment upon fruit is impairment or alteration of ripening process. The fruits subjected to hot water treatment showed more loss in weight as compared to those treated with moist hot air (Bakshi et al. 2006). Polyphenoloxidase (PPO) and polygalacturonase (PG) are the most important enzymes of peach fruit. Browning of fruit under the influence of PPO is a well known phenomenon caused by oxidation of phenolic compounds into quinines (Chambroy and Souty 1995). Polyphenolic compounds are also associated with the astringent flavour of fresh and frozen peaches. PG is responsible for pectin breakdown in fruits. Pectin, a cell wall polysaccharide, is responsible for fruit texture. A change in texture is an essential part of ripening in most fruits. In peach, PG activity was associated with an increase in water soluble pectin and fruit softening (Fischer and Bennett 1991). Downs and Brady (1990) described two forms of exo-PG in freestone and clingstone fruits and showed that the activity of each of the two forms was higher in ripe (soft) than in immature or mature firm fruit. Post-harvest heating of fruits resulted in lowering the activities of cell wall degrading enzymes. Thus, the present study was undertaken with an objective to monitor the activity of PPO and PG enzymes in peach after heat treatment.

Materials and methods

Fresh peach (*Prunus persica* L) fruits of uniform shape, size, maturity and colour of cv. 'Flordasun' were procured from the peach orchard of University located at Udheywalla, Jammu during 2003–04. Fruits were subjected to heat treatments consisting of hot water and moist hot air for 10, 20 and 30 min. Fifteen fruits were placed in each treatment and they were replicated 4 times. For each hot water treatment, peaches were placed in a plastic mesh bag and immersed in a stainless steel tank containing about 50 l of water at 40 ± 2°C. The contact of fruit with the internal surface of container was avoided to prevent the phytotoxic effect of hot surface on the fruit. The temperature of water during the treatment was maintained by placing it on heating source with a regulator. Cold water was also used to maintain temperature. The moist hot air treatment was given by erecting a brick chamber of 55 cm × 22 cm × 24 cm dimension with open top. A metallic mesh was placed on the top of the chamber. The mesh was covered with thick cotton cloth moistened in hot water. The fruit was spread evenly on the wet surface. A hot air convector (650 W) with regulators for temperature and fan was fitted inside the brick chamber. The moist hot air temperature was 52 ± 2°C. The temperature of the chamber was monitored using thermometer.

Pectin was estimated by precipitating it as Ca-pectate (Carre and Haynes 1922) and total phenol content by AOAC (1970) method. PPO activity was measured on pulp using 10 g tissue for extraction of chilling powder suspended 5 g of acetone powder in 200 ml of potassium phosphate (pH 6.8, 0.2M), stirred for 30 min at 2°C, centrifuged at 11,000×g for 20 min at 2°C, dialyzed the supernatant against phosphate buffer for 2 days with 2 changes of buffer and the dialyzate was used for assay. one ml of 0.05 M catechol + 3.5 ml of 0.2 M phosphate buffer (pH 6.8) were incubated at 30°C, the reaction was initiated by adding varying amounts of enzyme extracts in a final volume of 5 ml, the rate of increase in absorbance at 410 nm against blank (prepared in absence of enzyme) was measured at every 30 sec up to 3 min. The change in absorbance between 30 and 180 sec of incubation was plotted and the enzyme activity calculated from linear part of the curve. Enzyme activity was expressed as change in absorbance of 0.001/min and specific activity as units/min/mg protein (Thimmaiah 1999). For PG activity, 10 g of sample were homogenized in 13 ml of Tris-HCl buffer, centrifuged at 15,000 ×g for 15 min, the pellet incubated for 60 min in ~ 5 ml extraction buffer and centrifuged at 15,000 ×g for 30 min. The supernatant was used for assay. NH_4Cl (0.1 ml) + 1 ml polygalacturonic acid at 37°C was incubated, the reaction was initiated by adding 0.1 ml enzyme extract and incubated further for 30 min. The reaction was terminated by adding 0.3 ml of 5% TCA, centrifuged at 2000 ×g for 30 min and the supernatant was collected. The reducing sugars formed by DNS method was estimated from the standard curve of D-glucose as standard. The enzyme activity was expressed as moles of reducing sugars formed or katals and specific activity as μ moles/sec/mg protein (Thimmaiah 1999). The protein in enzyme extract was determined by method of Lowry et al. (1951). The fruits after heat treatment were stored at room temperature (24 ± 2°C) and the observations of enzyme activity were recorded at 3 days interval till the fruit remained acceptable. The data was analyzed statistically by using completely randomized design (Fisher 1950).

Results and discussion

Results are presented in Table 1. Pectin content decreased during storage in both hot water and moist hot air treated

Table 1 Effect of heat treatment on pectin (% Ca- pectate), polyphenols, polyphenoloxidase (PPO) and polygalacturonase (PG) of peach cv. 'Flordasun' during storage

Heating time (T), min	Storage (S) period, days		
	0	**3**	**6**
		Pectin, %	
		Hot water	
0	1.11	0.74	0.49
10	1.10	0.73	0.44
20	1.11	0.78	0.60
30	1.12	0.82	0.59
C.D. $_{0.05}$		T= 0.03 S= 0.02 T × S= 0.04	
		Moist hot air	
0	1.11	0.74	0.53
10	1.09	0.72	0.49
20	1.11	0.77	0.59
30	1.12	0.81	0.61
C.D. $_{0.05}$		T= 0.03 S= 0.03 T × S= 0.05	

	Polyphenols, mg/100 g		
	Hot water		
0	65.5	52.4	39.1
10	65.5	53.2	38.4
20	66.4	55.6	43.6
30	66.5	55.4	43.9
C.D. $_{0.05}$		T= 0.08 S= 0.07 T × S= 0.13	
	Moist hot air		
0	65.5	52.4	40.2
10	65.5	53.4	39.8
20	67.2	55.8	43.9
30	67.4	56.2	44.9
C.D. $_{0.05}$		T= 0.08 S= 0.07 T × S= 0.13	
	PPO activity, unit/mg protein/min		
	Hot water		
0	370.5	351.2	246.3
10	373.5	351.9	244.8
20	360.2	218.2	147.7
30	349.8	160.0	110.8
C.D. $_{0.05}$		T= 0.68 S= 0.59 T × S= 1.18	
	Moist hot air		
0	370.5	356.2	254.3
10	372.7	355.8	253.7
20	365.3	241.4	186.2
30	356.7	198.7	148.2
C.D. $_{0.05}$		T= 0.76 S= 0.65 T × S= 1.31	
	PG activity, μmole/mg protein/sec		
	Hot water		
0	0.024	0.041	0.050
10	0.026	0.044	0.052
20	0.025	0.036	0.043
30	0.023	0.033	0.041
C.D. $_{0.05}$		T= 0.0006 S= 0.0005 T × S= 0.0011	
	Moist hot air		
0	0.024	0.040	0.051
10	0.026	0.042	0.054
20	0.023	0.035	0.043
30	0.022	0.033	0.042
C.D. $_{0.05}$		T= 0.0007 S= 0.0006 T × S= 0.0012	

fruits. The decrease in pectin content was, however, faster in fruits treated with hot water as compared to hot air. Fischer and Bennett (1991) reported that solubilization of pectin is a fundamental and important aspect of fruit ripening. Garcia et al. (1996) reported that heat allows demethylation of pectin by pectin methyl esterase to form anionic COO^- groups with which Ca^{2+} ions can form salt bridge cross links. This may make the cell wall less accessible to the enzymes that cause softening. Therefore, the combination of both post-harvest dips and heat treatment may control ripening, softening and decay at the same time (Sams et al. 1993). Higher duration of heat treatment or higher temperatures resulted in lesser decline of pectin during storage due to inactivation of pectin degrading enzymes.

Total phenolic content decreased during storage in both treatments. The decline in total phenolic content was more in hot water treated fruits. Like pectin, polyphenol decline was more when treatment duration was less. Fruits typically show a decline in phenolic compounds with ripening and an increase in response to stresses like bruising and fungal infections (Haard and Chism 1996).

PPO activity decreased in all the treatments during storage. This decrease was more in hot water treated fruits than in hot air. PPO activity decreased with the increase in treatment duration. On the basis of their browning tendency, peach cultivars could be divided into two groups, (i) the strong browning tendency i.e., high substrate content in which chlorogenic acid constituted less than 50% of total phenols, and (ii) the slight browning tendency, i.e., low substrate content in which chlorogenic acid was practically the sole substrate (Vamos-Vigyazo 1981). The decrease in PPO activity was accompanied by a decrease in o-dihydroxy phenols and a marked increase in laccase activity (Harel et al. 1970). Joseph and Kristi (1974) also reported that PPO activity decreased at 37°C of storage temperature.

An increase in the activity of PG was observed in heat treated fruits as well as control. But the increase was more in mild heat treatments as compared to severe heat treatment i.e. more than 10 min heating duration. An increase in PG activity was also observed by Pollard (1975) in apple fruits during ripening. Chan et al. (1981) reported inhibition of cell wall degrading enzymes in papaya by heat treatment, which may also be involved in the slow softening of heat treated fruit.

Conclusion

Pectin content, polyphenols and PPO activity decreased during storage in both hot water and moist hot air treatments. This decrease was more in hot water treated fruits than in hot air. An increase in the activity of PG was observed in heat treated fruits as well as control. This increase was more in mild heat treatments as compared to severe heat treatment.

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