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Enzymological changes in peach cv. Flordasun during storage

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ABSTRACT

Peaches were stored in polythene bags of varying perforation coefficient (P_C) under refrigerated as well as room temperature conditions. The activities of polygalacturonase (PG) and polyphenoloxidase (PPO) were monitored during storage at specific intervals. PG activity increased up to 6 days under room temperature conditions whereas the fruit lost its acceptability after 12 days. Under refrigerated conditions, the activity increased up to 18 days and decreased thereafter till the end of storage. The PPO activity, on the other hand, decreased under room temperature conditions but increased under refrigerated conditions.

Keywords: Peach, storage, pectin, enzymes, polyphenoloxidase, polygalacturonase.

INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is an important stone fruit grown under temperate climate. But with the better understanding of the physiology of the plant and advances in fruit breeding, such varieties have been evolved which have a chilling requirement of just 150-300 h and are suitable for sub-tropical climate. In India, the sub-tropical peach arrives in the market from last week of April to early May when there are only few fruits to compete with it and hence fetches good returns. The limited post-harvest life of fruit is one of the major problems. It has a shelf-life of 3-5 days under ambiant conditions (Tonini and Tura, 23). Both modified and controlled atmospheric storages play significant role in prolonging the storage life of fruits. There is a renewed interest in application of modified atmospheric packaging (MAP) for storage of stone fruits (Chambroy and Souty, 5; Meena *et al*., 18). However, perforated polythene bags played an important role in quality retention and reduction of peach spoilage during low temperature storage (Singh and Mandal, 21; Masoodi *et al*., 15). Bakshi and Masoodi (2) stated that on the basis of quality characteristics, peach fruits stored in perforated bags remained acceptable up to 12 days under room temperature and 18 days under refrigerated condition. Polyphenoloxidase (PPO) and polygalacturonase (PG) are the two most important enzymes from fruit quality point of view. Browning of fruit under the influence of PPO is a well known phenomenon caused by oxidation of phenolic compounds into quinines (Lee, 11; Macheix *et al*., 14; Mayer and Harel, 17; Nicolas *et al*., 19).

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The quinines formed are highly reactive and polymerize giving rise to the brown discolouration and lead to quality deterioration of fruit during processing (Macheix *et al*., 14). Polyphenolic compounds are also associated with the astringent flavour of fresh and frozen peaches. PG is the enzyme, which 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. Downs and Brady (6) described two forms of exo-PG in freestone and clingstone fruit and showed that the activity of each of the two forms was higher in ripe (soft) than in immature or mature firm fruits. The present investigation was undertaken with an objective of monitoring the activity of the above mentioned enzymes in peach during its modified atmospheric storage.

MATERIALS AND METHODS

The fruit of uniform shape, size, maturity and colour of cv. Flordasun was procured from the peach orchard of University located at Udheywalla, Jammu. Precisely 24 kg of fruit picked on 3rd of May, *i.e.* 48 days after full bloom. The fruits were stored in polythene bags of 18 × 14 cm dimension with 200 guage thickness and having perforation coefficient (P_C) of 0, 251, 565 and 1006 mm holes/m^2 obtained by making 0, 8, 12 and 16 perforations of 5 mm diameter each. The P_C was calculated by following formula (Robertson, 20).

Perforation coefficient (P_C) = d q k

Where q = % age of perforated area out of total film area.

d = perforation hole diameter.

k = number of perforations per sq. mt.

$q = p\ (½\ d)^2\ k\ 10^{-4}$ (%).

Thus, $P_C = ¼\ p\ d^3\ k^2\ 10^{-4}$ (mm holes/ m^2).

The bags were stored under both room temperature (24 ± 2°C) as well as refrigerated temperature (3-7 °C). There were 8 treatments with three replications. Pectin was estimated by precipitating it as Ca-pectate (Carre and Haynes, 4), whereas total phenols were determined by AOAC (1) method. The PPO activity was measured by Thimmaiah (22) method in pulp using 10 g tissue. Suspended 5 g of acetone powder in 200 ml of potassium phosphate (pH 6.8, 0.2 M). 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. Used the dialyzate for assay. Incubated 1 ml of 0.05 M catechol and 3.5 ml of 0.2 M phosphate buffer (pH 6.8) at 30 ^{0}C. Initiate the reaction by adding varying amounts of enzyme extracts in a final volume of 5 ml. Measured the rate of increase in absorbance at 410 nm against blank (prepared in absence of enzyme) at every 30 sec. up to 3 min. Plotted the change in absorbance between 30 to 180 sec of incubation and calculated the enzyme activity 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. For PG activity (Thimmaiah, 22), homogenized 10 g of sample in 13 ml of Tris-HCl buffer. Centrifuged at 15,000 g for 15 min. Incubated the pellet for 60 min. in about 5 ml extraction buffer and centrifuged at 15,000 g for 30 min. Used the supernatant for assay. Incubated 0.1 ml NH_4Cl and 1 ml polygalacturonic acid (Hi- Media) at 37 °C. Initiated The reaction was initiated by adding 0.1 ml enzyme extract and incubated further for 30 min. and terminated by adding 0.3 ml of 5 % TCA. Centrifuged at 2,000g for 30 min. and collect the supernatant. Estimated the reducing sugars formed by DNS method. Drew the standard curve D-glucose (Hi-Media) as a standard. The enzyme activity was expressed as moles of reducing sugars formed or katals and specific activity as m moles/mg protein. The protein in enzyme extract was determined by method of Lowry *et al*. (13).

RESULTS AND DISCUSSION

The pectin content of the fruit showed a continuous decline during storage (Table 1). The decrease in pectin content was, however, faster in fruits stored at room temperature as compared to those in refrigerated storage. The fruits stored in unperforated bags showed maximum pectin content under both the storage conditions. The decline in pectin content was also reflected as loss of fruit firmness during storage. Breakdown of pectin during storage of fruits has also been reported by Vidrih *et al*. (24).

PG activity showed an increase during first 6 and 18 days under room temperature and refrigerated conditions, respectively, and decreased thereafter with the increase in storage period (Table 2). Minimum enzyme activity of 0.046 and 0.040 m moles/mg protein was found in fruits at the end of storage in unperforated bags under room temperature and refrigerated conditions, respectively. The anaerobic conditions also inhibited PG activities resulting in slower softening (Vidrih *et al*., 24). PG acts by cleavage of covalent bonds in pectin backbones. It has long been thought to contribute to fruit softening through its action on inter-cellular and cell wall pectins, although some transformation experiments establish that in tomatoes, there is no simple relationship between endo-PG and fruit firmness (Fisher and Bennett, 8). The initial

Table 1. Effect of storage temperature and package perforations on pectin content (% Ca-pectate) of peach cv. Flordasun during storage.

P_C*	Storage interval (days)										Percent
	0	3	6	9	12	15	18	21	24	27	decline
					Room temperature						
0	1.11	0.97	0.84	0.70	0.58	FNA**					47.8
251	1.11	0.99	0.84	0.71	0.55	FNA					50.5
565	1.11	0.90	0.76	0.61	0.46	FNA					58.6
1006	1.11	0.93	0.77	0.63	0.49	FNA					55.9
$CD_{0.05}$	Treatment = 0.02			Days = 0.02		Treatment × Days = 0.05					
					Refrigerated temperature						
0	1.11	1.06	0.96	0.88	0.88	0.73	0.68	0.61	0.56	0.51	54.1
251	1.11	1.02	0.93	0.93	0.76	0.69	0.63	0.58	0.54	0.48	56.8
565	1.11	1.03	0.93	0.85	0.78	0.68	0.63	0.57	0.52	0.45	59.5
1006	1.11	1.05	0.87	0.87	0.80	0.72	0.65	0.58	0.52	0.46	58.6
$CD_{0.05}$	Treatment = 0.01			Days = 0.01		Treatment × Days = 0.03					

Table 2. Effect of storage temperature and package perforation on polygalacturonase (PG) activity (mmole mg protein^{-1}s^{-1}) of peach cv. Flordasun during storage.

P_C*	Storage interval (days)									
	0	3	6	9	12	15	18	21	24	27
					Room temperature					
0	0.024	0.047	0.133	0.095	0.046	FNA**				
251	0.024	0.052	0.141	0.106	0.051	FNA				
565	0.024	0.054	0.140	0.098	0.051	FNA				
1006	0.024	0.055	0.143	0.115	0.057	FNA				
$CD_{0.05}$	Treatment = 0.001			Days = 0.001		Treatment × Days = 0.001				
					Refrigerated temperature					
0	0.024	0.034	0.045	0.060	0.081	0.092	0.136	0.109	0.065	0.040
251	0.024	0.042	0.052	0.065	0.088	0.101	0.148	0.116	0.071	0.047
565	0.024	0.041	0.054	0.068	0.090	0.099	0.151	0.121	0.076	0.050
1006	0.024	0.044	0.055	0.067	0.091	0.103	0.152	0.119	0.077	0.049
$CD_{0.05}$	Treatment = 0.0003			Days = 0.001		Treatment × Days = 0.001				

* Perforation coefficient (mm holes/m^2).

** Fruit not acceptable.

increase in PG activity is due to the activity of 2 exo-PG enzymes in ripe fruits. Exo-PG1 increased 36-fold and other (exo-PG2) 90-fold but exo-PG2 accounted for 73% of the total activity in ripe fruits (Downs and Brady, 6). Thus, the decrease at last is due to the reduction in the activity of post-climacteric stage. PG activity was generally observed to increase with the increase in P_C.

The total phenolic content did not show any specific trend during storage (Table 3) but there was an overall decrease in phenols. The fruits stored in unperforated bags showed less phenolic content as compared to those stored in perforated bags under room temperature as well as refrigerated conditions. The tannin content of peaches is known to vary with the variety (Blarke and Davidson, 3), locality, season and growth status of the tree. It was also reported to decrease in peach with maturity and ripening (Lee *et al*., 12). PPO activities of fruit showed a gradual decrease during 12 days storage at room temperature (Table 4). The decrease was more in fruits stored in unperforated bags. Increase in P_C showed slight but

Table 3. Effect of storage temperature and package perforation on total phenols (mg/100 g) of peach cv. Flordasun during storage.

P_C	*Storage interval (days)									
	0	3	6	9	12	15	18	21	24	27
					Room temperature					
0	65.5	61.3	55.4	58.2	52.6	FNA**				
251	65.5	61.6	56.0	58.6	53.4	FNA				
565	65.5	61.4	55.7	58.4	53.1	FNA				
1006	65.5	61.9	56.3	58.8	53.9	FNA				
$CD_{0.05}$	Treatment = 0.13			Days = 0.14		Treatment × Days = 0.29				
					Refrigerated temperature					
0	65.5	63.2	64.1	61.2	58.3	60.2	56.5	52.4	50.1	43.4
251	65.5	63.5	64.5	61.8	58.8	60.5	57.1	53.1	51.1	46.7
565	65.5	63.3	64.3	61.5	58.5	60.3	56.8	52.9	50.8	44.2
1006	65.5	63.7	64.6	62.0	59.1	60.8	57.3	53.3	51.2	47.2
$CD_{0.05}$	Treatment = 0.25			Days = 0.39		Treatment × Days = 0.78				

Table 4. Effect of storage temperature and package perforation on polyphenol oxidase (PPO) activity (unit mg protein^{-1} min^{-1}) of peach cv. Flordasun during storage.

P_C*	Storage interval (days)									
	0	3	6	9	12	15	18	21	24	27
					Room temperature					
0	370.52	337.79	342.24	306.16	259.26	FNA**				
251	370.52	339.15	344.06	307.95	261.08	FNA				
565	370.52	340.54	342.08	307.24	261.98	FNA				
1006	370.52	341.48	340.98	305.60	260.34	FNA				
$CD_{0.05}$	Treatment = 0.96			Days = 1.07		Treatment × Days = 2.14				
					Refrigerated temperature					
0	370.52	384.33	472.06	496.12	564.13	651.07	646.84	702.33	751.17	803.09
251	370.52	382.68	495.04	494.02	568.33	654.39	672.24	710.14	757.07	809.95
565	370.52	386.35	489.19	480.15	566.06	652.98	670.31	711.66	758.13	808.35
1006	370.52	387.85	493.06	492.02	566.77	652.60	648.42	707.05	754.19	802.75
$CD_{0.05}$	Treatment = 0.67			Days = 1.06		Treatment × Days = 2.11				

*Perforation coefficient (mm holes/m^2).

**Fruit not acceptable.

statistically significant variation in enzyme activity. These results are similar to those of Joseph and Kristi (10) who reported that PPO activity declined in 'Redhaven' peaches at 37 °C storage temperature. The fruits stored under refrigerated conditions showed an increase in PPO activity with the increase in storage duration. These results are contradictory to the earlier reports that under refrigerated conditions, PPO activity declines (El-Sheikh and Habiba, 7), whereas an increase in PPO activity in refrigerated 'Manila' mangoes stored at 6 °C displayed greater activity than those stored at 12°C (Gilber *et al*., 9). Several authors have found that exposing fruit to chilling stress (6°C) caused a breakdown of cellular structure. Increased PPO solubilization facilitated its contact with phenolic substrates and thus resulted in more activity (Mayer and Harel, 16). Wang (25) reported that thylakoids from chloroplasts exhibit decompartmentation that could release PPO, which may interact freely with its substrates leading to browning. In early stages of ripening the presence of citric acid may prevent PPO activity. Under refrigerated conditions, PPO activity was slightly higher in perforated bags than in unperforated bags.

It is thus concluded that PPO activity increased under refrigerated conditions but decreased under ordinary storage. PG activity reaches its peak value within one week under ordinary storage conditions whereas under refrigerated conditions the activity reaches its peak after two weeks. Variation in P_C results in slight but statistically significant variation in the activities of both enzymes in peaches.

REFERENCES

1. A.O.A.C. 1970. *Official Methods of Analysis*. 11th edn., Association of Official Analytical Chemists, Washington, D.C., pp. 154.

2. Bakshi, P. and Masoodi, F.A. 2009. Effect of various storage conditions on processing and chemical characteristics of peach cv. Flordasun. *J. Food Sci. Technol*. **46**: 271-74.

3. Blarke, M.A. and Davidson, O.W. 1953. Some results of acidity and catechol tannin studies of peach fruits. *Proc. Amer. Soc. Hort. Sci*. **39**: 201-4.

4. Carre, M.H. and Haynes, D. 1922. The estimation of pectin as calcium pectate and the application of this method for determination of soluble pectin in apples. *Biochem J*. **16**: 60.

5. Chambroy, Y. and Souty, M. 1995. The role of CO_2 on stone-fruit performance. Utilization of modified atmospheres. **In**: *Post-harvest Quality and Derived Products in Stone Fruits* (Vendrell, M. and Audergon, J.M. Eds.), Ayutamiento de Lerida, Lerida, Spain, pp. 39-69.

6. Downs, G.S. and Brady, C.J. 1990. Two forms of exo-polygalacturonase increase as peach fruits ripen. *Plant Cell Environ*. **13**: 523-30.

7. El-Shiekh, A.F. and Habiba, R.A. 1996. Effect of storage time on the quality of peach fruit held in

cold storage in different types of packaging. *Gartenbauwissenschaft* **61**: 7-10.

8. Fischer, R.L. and Bennett, A.B. 1991. Role of cell wall hydrolysis in fruit ripening. *Ann. Rev. Pl. Physiol. Pl. Mol. Biol*. **42**: 675-703.

9. Gilber, V., Dinora, M.L., Hugo, S.G. and Javier, D.L.C. 2003. Polyphenoloxidase activity during ripening and chilling stress in 'Manila' mangoes. *J. Hort. Sci. Biotech*. **78**: 104-7.

10. Joseph, J.J. and Kristi, R.K. 1974. Characterization of polyphenoloxidase in peaches grown in the south-east. *HortSci*. **9**: 590-1.

11. Lee, C.Y. 1991. Enzymatic browning in relation to phenolic compounds and polyphenoloxidase activity among various peach cultivars. *J. Agri. Food Chem*. **38**: 99-104.

12. Lee, C.Y., Kagan, V., Jaworski, A.W. and Brown, S.K. 1990. Enzymatic browning in relation to phenolic compounds and polyphenoloxidase activity among various peach cultivars. *J. Agri. Food Chem*. **38**: 99-101.

13. Lowry, O.H., Rosebrough, N.J., Farr, A.I. and Randall, R.J. 1951. Protein analysis. *J. Biol. Chem*. **193**: 265.

14. Macheix, J.J., Sapis, J.C. and Fleuriet, A. 1991. Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. *CRC Crit. Rev. Food Sci. Nut*. **30**: 441-86.

15. Masoodi, F.A., Bakshi, P. and Kour, K. 2008. Effect of packaging and intermittent warming on the incidence of some post-harvest disorders of peach cv. Flordasun. *Env. Ecol*. **26**: 195-98.

16. Mayer, A.M. and Harel, E. 1979. Polyphenol oxidase in plants. *Phytochem*. **18**: 193-215.

17. Mayer, A.M. and Harel, E. 1981. Polyphenol oxidases in fruits: Changes during ripening. **In**: *Recent Advances in the Biochemistry of Fruits and Vegetables*. (Friend, J. and Rhodes, M.J.C., Eds.) Academic Press, London, pp. 161-80.

18. Meena, H.R., Kingsly, A.R.P. and Jain, R.K. 2009. Effect of post-harvest treatment on shelf-life of *ber* fruits. *Indian J. Hort*. **66**: 58-61.

19. Nicolas, J., Richard-Forget, F.C., Goupy, P.M., Amiot, M.J. and Aubert, S.Y. 1994. Enzymatic browning reactions in apple and apple products. *CRC Crit. Rev. Food Sci. Nut*. **34**: 109-57.

20. Robertson, G.L. 1993. Packaging of horticultural products. **In**: *Food Packaging: Principles and Practices*, Marcel Dekker, Inc., NewYork, USA, pp. 481-82.

21. Singh, D. and Mandal, G. 2006. Post-harvest quality and spoilage of peach fruits stored in perforated bags. *Indian J. Hort*. **63**: 390-92.

22. Thimmaiah, S.K. 1999. Enzymes. **In**: *Standard Methods of Bio-chemical Analysis*. Kalyani Publishers, New Delhi, pp. 244-45; 248-49.

23. Tonini, G. and Tura, E. 1998. Influence of storage and shelf-life time on rots of peaches and nectarines. *Acta Hort*. **464**: 364-67.

24. Vidrih, R., Tonutti, P. and Hribar, J. 1997. Biochemical changes during storage of peach in a very low oxygen atmosphere. *Sodobno-Kmetijstvo*, **30**: 423-30.

25. Wang, C.Y. 1982. Physiological and biochemical responses of plants to chilling stress. *HortSci*. **17**: 173-86.

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