



Enzymological changes in peach cv. Flordasun during storage

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ABSTRACT

Peach was 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 upto 6 days under room temperature conditions where the fruit lost its acceptability after 12 days. Under refrigerated conditions the activity increased upto 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. The substrates of both the enzymes, pectin and polyphenol decreased during storage.

Key words: Peach, storage, polyphenoloxidase, polygalacturonase.

INTRODUCTION

Modified and controlled atmospheric storage plays a 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, 6). Injurious levels of CO_2 and O_2 under MAP conditions have been established as greater than 10 % for CO_2 and less than 1-2 % for O_2 particularly if these levels are reached with 14 days of cold storage. Macroperforated and unperforated polyethylene films maintain high quality of peaches stored at 0.5°C. Bakshi and Masoodi (2) stated that on the basis of quality characteristics, peach fruits stored in perforated bags remained acceptable upto 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 (Mayer and Harel, 21; Macheix *et al.*, 19; Lee, 16 and Nicolas, 22).

The quinines formed are highly reactive and polymerize giving rise to the brown discoloration and lead to quality deterioration of fruit during processing (Macheix *et al.*, 19; Fleuriet and Macheix, 11). Polyphenolic compounds are also associated with the astringent flavor of fresh and frozen peaches. Browning and off-flavors in fruits are related to the amount of oxidizable phenols present (Wankier *et al.*, 33). 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. In peach, PG activity was associated with an increase in water soluble pectin and fruit softening (Pressey *et al.*, 24). Downs and Brady (8) 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 fruit. 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 color of cv. Flordasun was procured from the peach orchard of University located at Udheywalla, Jammu during the year 2002-03. 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 x 14 cm dimension with 200 gauge thickness and having perforation coefficient (P_c) of 0, 251, 565 and 1006 mm holes/m² obtained by making 0, 8, 12 and 16 perforations of 5 mm diameter each. The P_c was calculated by following formula (Robertson, 26).

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.

$q = \pi (\frac{1}{2} d)^2 k 10^{-4} (\%)$

Thus, $P_c = \frac{1}{4} \pi d^3 k^2 10^{-4} (\text{mm holes/ m}^2)$.

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The bags were stored under both room temperature ($24 \pm 2^\circ\text{C}$) as well as refrigerated temperature ($3-7^\circ\text{C}$). There were 8 treatments with three replications. Pectin was estimated by precipitating it as Ca-pectate (Carre and Haynes, 5) whereas total phenol was determined by A.O.A.C. (1) 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. Used the dialyzate for assay. Incubated 1 ml of 0.05 M catechol (Central Drug House) and 3.5 ml of 0.2 M phosphate buffer (pH 6.8) at 30°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 seconds upto 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, 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 15,000g 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 by adding 0.1 ml enzyme extract and incubated further for 30 min. Terminated the reaction 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 katal and specific activity as m moles/sec/mg protein. The protein in enzyme extract was determined by method of Lowry *et al.* (18).

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 been reported by many earlier workers also (Doesburg, 7; Salunkhe *et al.*, 27; Sandhu *et al.*, 28 and Vidrih *et al.*, 31).

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/sec/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.*, 31). Huber (14) suggested that

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)										% decline
	0	3	6	9	12	15	18	21	24	27	
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 x 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 x Days = 0.03										

* Perforation coefficient (mm holes/m²); ** Fruit Not Acceptable

Table 2. Effect of storage temperature and package perforation on polygalacturonase (PG) activity (mmole mg protein⁻¹sec⁻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 x 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 x Days = 0.001									

* Perforation coefficient (mm holes/m²); ** Fruit Not Acceptable

PG acts by cleavage of covalent bonds in pectin backbones. PG has long been thought to contribute to fruit softening through its action on intercellular 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, 10). The initial 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 73 % of the total activity in ripe fruits (Downs and Brady, 8). Thus, the decrease at last is due to the reduction in the activity of post-climacteric stage (Tonutti *et al.*, 30). PG activity was generally observed to increase with 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 condition. The tannin content of peaches is known to vary with the variety (Blarke and Davidson, 4; Blarke, 3), locality (Guadagni and Nimmo, 13), season (Blarke, 3) and growth status of the tree (Nightingale *et al.*, 23). It was also reported to decrease in peach with maturity (Reeve, 25) and ripening (Lee *et al.*, 17). 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 statistically significant variation in enzyme activity. These results

are similar to those of Joseph and Kristi (15) 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, 9), whereas an increase in PPO activity in refrigerated 'Manila' mangoes as fruit stored at 6°C displayed greater activity than those stored at 12°C (Gilber *et al.*, 12). Several authors have found that exposing fruit to chilling stress at 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, 20; Shewfelt and Stanley, 29). Wang (32) 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.

Table 3. Effect of storage temperature and package perforation on total phenols (mg/100g) 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 x 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 x Days = 0.78									

* Perforation coefficient (mm holes/m²); ** Fruit Not Acceptable

Table 4. Effect of storage temperature and package perforation on polyphenol oxidase (PPO) activity (unit mg protein⁻¹min⁻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 x 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 x Days = 2.11									

* Perforation coefficient (mm holes/m²); ** Fruit Not Acceptable

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