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Pectinesterase Activity in Controlled Atmosphere Stored Avocados[1]

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Abstract. **Pectinesterase (PE) activity was determined in avocado fruit (*Persea americana* Mill) at various times during controlled atmosphere storage (CA) and subsequent softening. A reduction in the initial softening time as related to storage temperature or ethylene accumulation in the CA unit was associated with a decrease in the PE activity of firm fruit at time of removal from storage. It is suggested that PE activity in fruit could be used to monitor a change in softening time during CA storage.**

Extension of sotrage life of avocados by CA conditions was first reported by Overholser (5) in 1928. This was later confirmed by Young et al. (9) and by Hatton and Reeder (3) in the mid 1960's. The present recommended conditions for CA storage are 10% CO_2, 2% O_2, and 88% N_2 at 4.5 to 7.2°C (4). Recently, a commercial CA unit was built in Florida for storage of avocados (J. R. Brooks & Son, Inc., Homestead) 'Lula' avocados have been successfully stored under CA conditions for 60 days, as compared with a storage life in air under conventional refrigeration of 20 days (8). Development of anthracnose (*Collectotrichum gloeosporioides* Penz.) and chilling injury are the most often factors limiting storage time (1, 3, 8).

Softening time, as the sole criterion of ripening, is very important in the marketing of avocados. Under optimum CA conditions, decrease in softening time is often minimal. However, accumulation of ethylene or temp flucuation can reduce the effectiveness of CA conditions in retarding softening (2, 4). Thus, a rapid, accurate method for determining the status of a specific lot of fruit, with respect to softening time, would facilitate the use of CA storage of avocados. A decline in PE activity has been shown to occur prior to softening of avocados (7, 10). Our investigation was conducted to determine whether PE activity in fruit could be used to monitor, during CA storage, change in softening time.

Materials and Methods

Avocado fruit of several cultivars were harvested from the Agricultural Research and Education Center's (AREC) orchard at Homestead during the 1973 and 1974 seasons. Fruit were transported to the AREC at Lake Alfred and inspected. Sound fruit, free of blemishes, were randomly grouped into lots of 20 and cooled to their respective storage temp before being placed into the CA chambers.

CA chambers consisted of modified 208 liter (55 gal) steel drums. Each chamber was maintained at 10% CO_2, 2% O_2, and 88% N_2 with a flow-through system giving a complete gas exchange every 24 hr and a relative humidity of 95 to 98%. Storage temp used were 4.5, 7.2, and 10°C. A canister containing 300 g Purafil (H. E. Burroughs & Associated, Chamblee, Ga.) was placed in each chamber for ethylene removal. Oxygen and CO_2 concn were determined daily with a Fisher gas partitioner. Ethylene was checked periodically with a gas chromatograph equipped with a flame ionization detector.

PE activity and softening time at 21°C were examined following various periods of CA storage. Changes in both parameters were also examined for fruit stored in a commercial CA unit at 7.2°C under similar CA conditions.

PE activity was determined according to the procedure of Rouse and Atkins (6). Units of PE activity are expressed as milliequivalents (meq) of ester hydrolyzed/min/g fresh tissue. Avocados were prepared for analysis as described previously (7) except that the fruit were not peeled. Analyses were made on composite samples, each of 2 to 5 fruit. PE activity of fruit from the commercial CA storage unit was an average of 10 groups, each of 2 fruit. Sample preparation was done both immediately prior to and upon removal from storage. When the tissue was not analyzed immediately, it was frozen until used. Softening time was determined on an additional 10 to 15 fruit stored at 21°C. PE activity was also determined on a representative sample of unstored fruit when first considered soft.

Results

Change in PE activity during ripening at 21°C of CA stored and unstored fruit is shown in Fig. 1. There was no apprent difference in firmness, based on feel, at time of removal from CA storage regardless of their change in softening time. PE activity for "soft" fruit will differ for each cultivar and will vary several units within each cultivar for both firm and soft fruit depending on stage of maturity and softness selected, respectively.

CA storage at 4.5°C. Both softening time at 21°C and PE activity were unchanged for 'Hickson' and 'Booth 8' after 49 days storage (Table 1). A decrease in PE activity and in softening time of 1 to 1.5 days was observed for 'Taylor', 'Lula', and 'Booth 7' at the end of their respective storage periods. No visible internal or external injury was evident either during storage at 4.5° or softening.

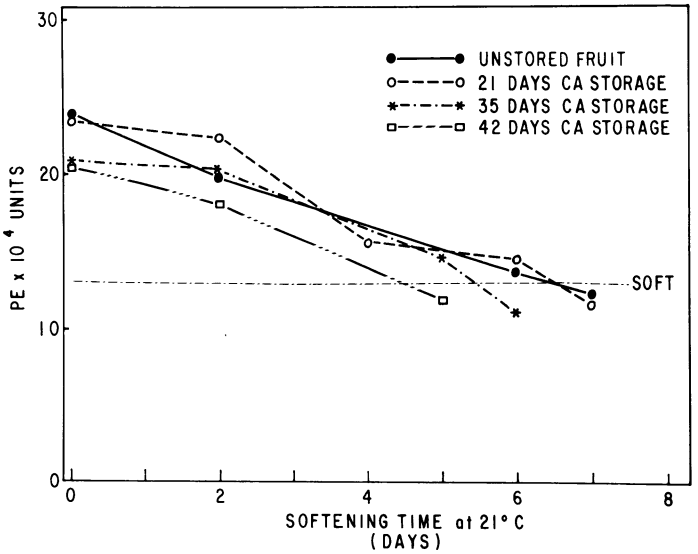

Fig. 1. Pectinesterase (PE) activity in unstored and CA stored avocado fruits ('Lula' cv.) at various stages of ripening at 21°C.

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Table 1. Pectinesterase (PE) activity and actual and estimated softening times at 21°C of several avocado cultivars following controlled atmosphere storage at 4.5, 7.2, and 10.0°C.

Cultivar / Storage time (days)	PE x 10^4 Firm	PE x 10^4 Soft[y]	Actual softening time[z] (days)	Estimated softening time (days)
Taylor		*Storage temp 4.5°*		
0	7.6[x]	0.5	4.5	–
14	7.6	–	4.5	4.5
28	7.6	–	4.0	4.3
49	5.9	–	3.5	3.5
Booth 8				
0	71.3	10.5	5.0	–
14	70.3	–	5.0	5.0
28	70.4	–	5.0	5.0
49	76.0	–	5.0	5.3
Hickson				
0	31.8	2.1	5.0	–
14	31.5	–	5.0	5.0
28	30.1	–	5.0	5.0
49	31.2	–	5.0	5.0
Lula				
0	23.9	13.0	6.5	–
21	24.0	–	6.5	6.5
35	20.8	–	5.5	5.5
42	20.8	–	5.0	5.5
Booth 7				
0	23.9	2.5	6.5	–
35	16.9	–	5.0	4.4
Waldin		*Storage temp 7.2°*		
0	86	7.5	7.0	–
42	54	–	4.0	4.0
Waldin		*Storage temp 10.0°*		
0	71	7.0	7.0	–
14	30	–	3.5	2.5
25	25	–	3.0	2.0
Unidentified				
0	112	6.9	6.0	–
10	81	–	4.0	4.0
20	69	–	3.0	3.0
30	69	–	3.0	3.0

[z]Ripening temp 21°.

[y]Unstored soft fruit.

[x]Meq of ester hydrolyzed/min/g fresh tissue.

CA storage at 7.2 and 10°C. After 42 days storage at 7.2°C, softening time of 'Waldin' avocados dropped from an initial 7 days to 4 days (Table 1). PE activity also decreased from 86 to 54 units. After 25 days CA storage at 10°C, softening time of 'Waldin' avocados dropped from an initial 7 days to 3 days, and PE activity decreased from 71 to 25 units. Purafil was not used in the latter test and 15 ppm ethylene was detected at 14 days of storage. Similar changes in PE activity and softening time were also observed for an unidentified cultivar stored under CA at 10°C.

A similar experiment was conducted with avocados held in the commercial CA unit. Examination of the fruit from each of the 3 rooms showed that both PE activity and softening time had decreased from the initial levels (Table 2). The greatest decrease in both parameters occurred in fruit stored in room 1. Fruit shipped from this room were reported as being slightly soft on arrival at the market. Ethylene accumulation had occurred in this room due to malfunction of the CO_2 generator.

Discussion

The relationship between PE activity and concurrent softening of avocados has been established by several workers (7, 10). A similar relationship was also found for fruit following CA storage. This work differs in that PE activity of firm fruit is shown to be a means of determining if, and how much, a decrease in softening time at a specific temp has occurred during extended storage under CA conditions. Softening time of avocados is an essential criterion of poststorage shelf life and subsequent marketing. The proposed procedure could, therefore, greatly facilitate the use of CA storage of avocados by making it possible to monitor any decrease in softening time. Under ideal CA storage conditions there should be little or no change in either PE or softening time.

The procedure requires measurement of PE activity of firm fruit sampled immediately prior to and during CA storage. An additional prestorage sample is placed at preferred ripening temp for determination of softening time and PE activity at a specific stage of softness. Using these 4 values [initial PE (PE_0), PE of unstored soft fruit (PE_1), PE of stored fruit (PE_2), and softening time (ST)] estimation of poststorage softening time at a specific temp would be as follows:

$$\text{Estimated days to soften} = \frac{PE_2 - PE_1}{PE_0 - PE_1}[ST]$$

An example of this method using data on 'Waldin' at 42 days CA storage is as follows:

$$\text{Estimated softening time} = \frac{54 - 7.5}{86 - 7.5}(7.0) = 4.0 \text{ days}$$

The actual softening time of 'Waldin' fruit at 42 days CA storage was 4.0 days. Generally, the estimate is within ± 1 day of the actual time. The estimated softening times of fruit following various storage times in CA for the cultivars test are given in Table 1. It is important that the fruit from CA storage be prepared immediately upon removal for freezing or analysis as PE activity decreases with time under ripening conditions (7). Commercially, a PE value would be selected at which fruit would be removed from storage to prevent premature softening during marketing since avocados are not stored continuously at 21°C.

We have observed that when the PE activity of CA stored fruit upon removal approaches that of soft fruit, even though they are still firm, softening does not occur within 1 day. This is apparently related to the role of polygalacturonase (PG)

Table 2. Pectinesterase (PE) activity and actual and estimated softening time of 'Lula' avocados during controlled atmosphere storage at 7.2°C.

Room no.	Storage time (days)	PE x 10^4 Firm	PE x 10^4 Soft[y]	Actual softening time[z] (days)	Estimated softening time (days)
1	0	26.2[x]	6.5	6.5	
	8	21.5	–	–	
	14	12.0	–	3.5	2.0
2	0	23.0	9.0	6.5	
	10	22.5	–	–	
	18	21.0	–	5.0	5.6
3	0	23.5	11.5	6.5	
	10	22.9	–	–	
	16	19.9	–	–	
	23	19.4	–	5.0	4.3

[z]Room temp (26.7–32.2°C).

[y]Unstored soft fruit.

[x]Meq of ester hydrolyzed/min/g fresh tissue.

in fruit softening. According to Zauberman and Schiffmann-Nadel (10), softening of avocado fruit occurred in both immature and mature fruit when PE activity was minimal and PG activity was maximal. Softening generally occurred within 2 to 3 days after PE reached its minimal activity and PG its maximum activity. This could account for the delay in softening, assuming that CA conditions inhibit gradual increase in PG activity.

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Influence of Temperature on Seed Development of *Allium cepa* L.[1]

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Abstract. **Male-sterile onion lines, M2399A and P54-306A were hand pollinated with pollen from 2 male-fertile lines M611B and P52-371B, in 3 Biotron chambers with maxima temperatures of 24°, 35°, and 43°C, respectively, and a minimum temperature of 18°C. There was no significant effect of temperature on magagmetophyte development. Percent abortion of young seeds was 21, 11, and 66% at 24°, 35°, and 43°, respectively; 35° was more favorable for ovule, seed and ovary growth than 24° and 43°. The endosperm nuclei divided soon after fertilization and continued normally the first 5 days after pollination at all 3 temperatures. Subsequent growth of endosperm nuclei was retarded at 43° and only 1 or 2 seeds per ovary continued to grow at a normal rate. The first embryo division was observed 7 to 8, 5 to 6, and 6 to 7 days after pollination at 24°, 35°, and 43°, respectively.**

Franklin (3) reported that onion capsules seemed to be forming normally with developing seed but later contained only seed coats devoid of embryo and endosperm. High temperature may adversely affect onion seed production since ovary wall temperatures as high as 60°C have been measured (6).

We studied onion ovary, ovule, endosperm and embryo development at 3 controlled temperatures after hand pollination.

Materials and Methods

Onion plants (bulbs supplied by Crookham Company, Caldwell, Idaho) of 2 male-fertile lines, M611B and P52-371B, and 2 male-sterile lines M2399A and P54-306A, were transferred from the greenhouse to 3 chambers in the Biotron prior to anthesis. Three Biotron chambers simulating a diurnal cycle with temp maxima of 24°, 35°, and 43°C, and a minimum of 18° were used. Plants were held at each temp maxima for 4 hr (2). The light intensity was 21.6 klx with 12 hr light from 6AM to 6PM.

Table 1. Percent fertilized and aborted ovules as affected by temp and time after hand pollination.

Days after pollination	24°C % ovules fertilized	24°C % aborted	35°C % ovules fertilized	35°C % aborted	43°C % ovules fertilized	43°C % aborted
1	17[z]	0[y]	50[z]	0[y]	9[z]	0[y]
2	50	0	66	0	33	0
3	75	0	100	0	50	0
4	80	0	91	0	66	0
5	50	0	84	0	66	0
6	91	9	100	0	33	0
7	100	36	70	11	17	59
8	82	0	91	34	13	57
9	84	40	84	0	18	71
10	91	8	100	9	17	77
Avg	72.0	21[x]	83.6	13.5[x]	32.2	66[x]

[z]Avg of 5 ovaries.

[y]% fertilized ovules aborting.

[x]Avg of aborted ovules 7 through 10 days after pollination.

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