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Postharvest Variation in Cellulase, Polygalacturonase, and Pectinmethylesterase in Avocado (*Persea americana* Mill, cv. Fuerte) Fruits in Relation to Respiration and Ethylene Production

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ABSTRACT

Cellulase, polygalacturonase (PG), pectinmethylesterase (PME), respiration, and ethylene production were determined in single "Fuerte" avocado fruits from the day of harvest through the start of fruit breakdown. PME declined from its maximum value at the time of picking to a low level early in the climacteric. PG activity was not detectable in the preclimacteric stage, increased during the climacteric, and continued to increase during the postclimacteric phase to a level three times greater than when the fruit reached the edible soft stage. Cellulase activity was low in the preclimacteric fruit, started to increase just as respiration increased, and reached a level two times greater than at the edible soft stage. Cellulase activity started to increase 3 days before PG activity could be detected. Increased production of ethylene followed the increase in respiration and cellulase activity by about 1.5 days. These results indicate that a close relation exists between the rapid increase in the cell wall-depolymerizing enzymes and the rise in respiration and ethylene production and refocused attention on the role of the cell wall and the associated plasma membrane in the early events of fruit ripening.

The avocado fruit starts to ripen only after being detached from the tree. After harvest, the most obvious ripening change is the rapid transition of the mesocarp from a hard to a soft, butter-like consistency with an apparent total loss of structural integrity.

Several researchers have studied the relation between softening of the avocado fruit and the activity of cell wall-degrading enzymes. Lewis *et al.* (17) were the first to note that the hard avocado mesocarp presented barely detectable levels of cellulase activity whereas the activity in soft fruit was highest ever observed in plant tissues. Pesis *et al.* (20) found a direct correlation between cellulase activity, softening, respiration, and ethylene production. Awad (2) also found a close relation between the rapid increase in cellulase content after harvest, the climacteric rise in respiration, and softening of the fruit. He determined that edible softness occurred before maximum cellulase levels were reached.

Raymond and Phaff (21) first showed a positive relationship between PG activity and softening of the avocado fruit. Later, Barash and Khazzam (3) and Zauberman and Schiffmann-Nadel (24) found that PG[3] activity increased rapidly after harvest.

The postharvest decrease in PME activity in the avocado fruit has been demonstrated by Zauberman and Schiffmann-Nadel (24), Rouse and Barmore (23), Gertman and Fuchs (9), and Barmore and Rouse (4). We lack, however, precise information on the simultaneous variaton of these three enzymes as well as their relation to respiration, ethylene production, and softening of the fruit. Since there is considerable variation in the time of ripening of individual fruit, we show here the changes in three enzymes in relation to respiration and ethylene production in single fruit.

MATERIALS AND METHODS

Measurement of Respiration and Ethylene Production. "Fuerte" avocado fruits were placed in glass jars at 20 C immediately after harvest and flushed continuously with water-saturated, ethylene-free air (100 ml/min). At 4-h intervals, the outflow from each jar was passed through a Beckman 215-A IRGA for the determination of CO_2 evolution and then a 1-ml sample was injected automatically into a Varian Aerograph 1400 GC with a flame ionization detector for the determination of ethylene production.

Sample Method. To avoid the variability existing between fruits in their rate of ripening, samples for enzyme determinations were taken from single fruits each day, around the widest part of the fruit, using a 6-mm cork borer. The holes were immediately closed with warm lanolin and the fruit returned to the respiration jar. The plug obtained was cut in half longitudinally, one half of the cylinder being used for the cellulase and PG assays and the other for determination of PME activity. Respiration and ethylene production of control fruit were not significantly different from the sampled fruit, and enzyme assays of control fruit sampled on the last day of the experiment were not different from activities shown in the multiple sampled fruit. The control fruits were sampled once at the end of the experiment.

Extraction and Assay of Cellulase. After the experimental determination of the optimum conditions for the extraction and assay of avocado cellulase (article in preparation), the tissue was ground with 10 ml of 0.04 M Na-acetate buffer (pH 5.5) plus 0.2 M NaCl at 2 to 4 C, in a Polytron (Brinkmann Instruments) when the tissue was hard and with a glass mortar and pestle when the tissue was soft. After 15 min, the mixture was centrifuged at 6,000*g* and aliquots of the supernatant fraction (or its dilution) were taken for the cellulase assay. The assay mixture consisted of 200 μl of the enzyme suspension and 400 μl of 1.5% (w/v) CMC type 7H3SF (Hercules Incorporated) in 0.04 M Na-acetate buffer (pH 5.0). The change in drainage time of the mixture through a calibrated upper portion of a 0.1-ml pipette at 24 C was used as a measure of viscosity. The interval between the determination of the initial and the final drainage time was approximately 1 h. Drainage times were converted to relative units of cellulase activity (B) using the method of Almin *et al.* (1).

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[3] Abbreviations: PG: polygalacturonase; PME: pectinmethylesterase; IRGA: infrared gas analyzer; CMC: carboxymethylcellulose.

Extraction and Assay of PG. Samples from the same supernatant fraction used for the assay of cellulase were used for the assay of PG since the optimum conditions for the activity of PG determined by Raymond and Phaff (21) were very close to those obtained for cellulase. The assay mixture consisted of 200 μl of the enzyme suspension and 1 ml of 2.3% (w/v) Na-polypectate, Sunkist A-7598 (Sunkist Growers, Inc., Ontario, Calif.) in 0.04 M Na-acetate buffer (pH 5.5). A microviscosimeter, similar to the one used for the assay of cellulase, was used for the assay of PG. The method of Almin *et al.* (1) was used to convert drainage time to relative units of PG activity after verifying the applicability of the method for PG assays.

Extraction and Assay of PME. After the experimental determination of the optimum conditions for the extraction and assay of avocado PME (article in preparation), the tissue was ground in 10 ml of cold 0.4 M NaCl in a Polytron or a mortar and pestle as required. After 15 min (desorption interval), the mixture was centrifuged at 6,000*g* and PME activity measured by the procedure of Rouse and Atkins (22). Five ml of the supernatant fraction were added to 50 ml of a solution of 0.5% (w/v) pectin NF (purified polygalacturonic acid, methyl ester, Sunkist Growers, Inc.) in 0.1 M NaCl. The mixture was brought rapidly to pH 7.58 with NaOH and the release of carboxyl groups by the action of PME on the substrate was followed with an automatic titrator (Metrohm Herisau) with 0.095 N NaOH for 10 min at 24 C. PME units were expressed in meq of ester hydrolyzed/min·g fresh weight.

RESULTS

Trends in respiration and ethylene production (Fig. 1A) relate the changes in cell wall-degrading enzymes with ripening changes. A low level of ethylene production occurs throughout the preclimacteric period, but the first increase in rate was noted at the beginning of the 6th day just after respiration increased some 12 to 24 h earlier. Respiration and ethylene production were typical of earlier results (16). Ethylene production peaked 12 h before the peak in respiration. Softening commenced just as respiration increased and reached the best soft and edible stage about 12 h after the peak in respiration (Fig. 1A, 9.5 days after harvest).

The change in cellulase activity is shown in Figure 1B. Significant activity was measurable on the day after harvest and throughout the preclimacteric period; in fact this low level of activity is equivalent to the maximum level shown in some fruit (17). Simultaneous with the first increase in respiration, cellulase activity increased markedly. While the fruit was edibly soft 9.5 days after picking, cellulase activity continued to increase for 2 more days and attained a final activity of 55,000 units/min·g fresh weight. The increase in ethylene production was at least 1 day later than the first increase in cellulase activity and thus the enzyme does not appear to be activated by ethylene.

PG activity shown in Figure 1C could not be detected until the 8th day after picking which was the 3rd day of the climacteric and the 2nd day of increased ethylene production at which time the softening process had already started. PG activity continued to increase into the postclimacteric phase and reached a level of over 800 units which was 3-fold greater than when the fruit was edibly soft.

PME activity, also shown in Figure 1C, was highest at picking time and changed little until the 5th day after harvest when it decreased from 5.0 to 1.5 milliunits during the next 2 days. At the same time, respiration increased to only one-third of the climacteric peak. PME activity stabilized at a low level until the experiment was terminated on the 13th day.

Figure 1D shows respiration and ethylene production of a comparable fruit from which no tissue samples were taken throughout the climacteric cycle. Respiration and ethylene production were very similar to those shown in Figure 1A and demonstrated that the removal of the tissue plugs from the fruit of Figure 1A did not affect its pattern of respiration or ethylene production. Tissue plugs were removed from the fruit used for Figure 1D on the 12th day. The activities of the three enzymes were similar to those shown in Figure 1A for the same period. We believe that the removal of early samples has not affected the enzyme activity of samples taken later. Further, enzyme assays of fruit sampled only once at various stages of the climacteric yielded enzyme activities comparable to the same stage of the climacteric shown in Figure 1A. The data shown in Figure 1 represent four experiments.

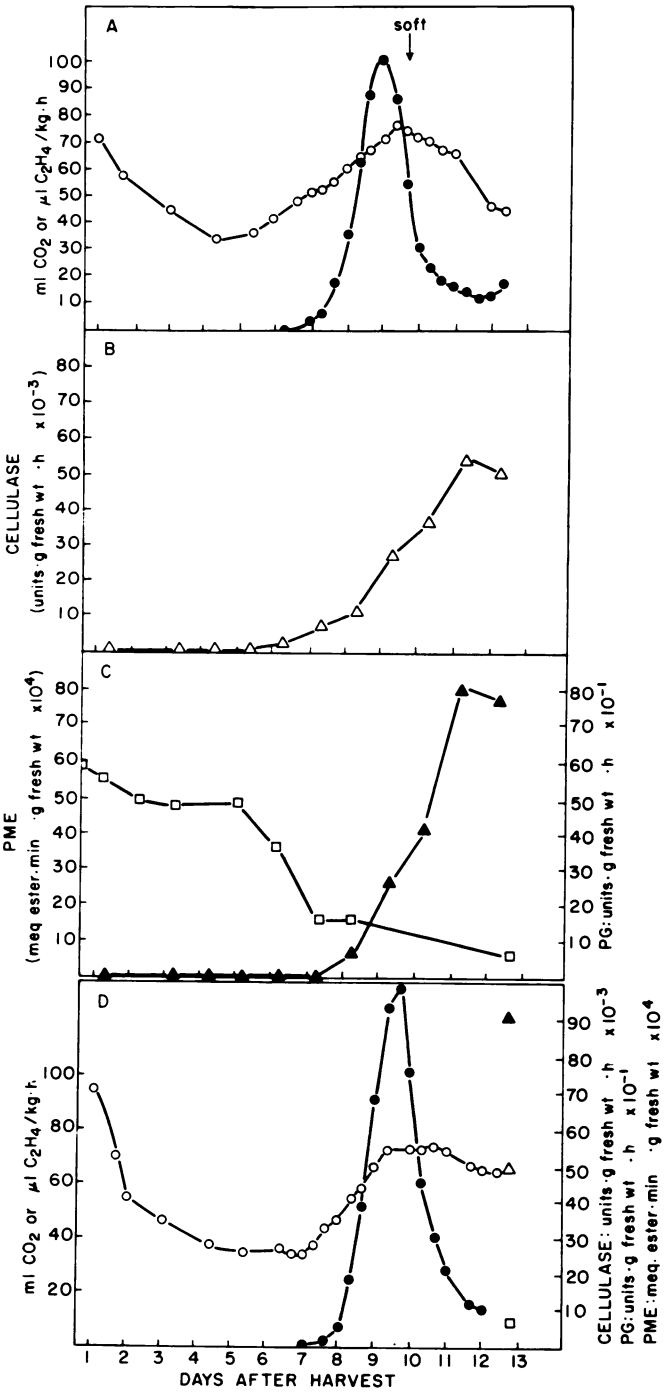

FIG. 1. Postharvest trends in cellulase (△——△), PG (▲——▲), PME (□——□) activity, and CO_2 (○——○) and C_2H_4 (●——●) production in an individual single "Fuerte" avocado fruit. Fruit in A was edibly soft after 9.5 days and in D after 10.5 days.

DISCUSSION

The analysis of trends in PME, PG, and cellulase activities from a single fruit allows close correlation of these activities with the ripening process. Ripening times of individual fruits vary from 5 to 15 days at 20 C which makes correlation of changes in enzyme activity with ripening difficult unless the same fruit can be sampled repeatedly. It was shown in Figure 1D that the multiple sampling procedure did not affect respiration or ethylene production and that the activity of the three enzymes in fully ripe fruit which had been sampled repeatedly was not different from that found in a fruit sampled only in the postclimacteric phase. Results reported are for a single fruit which was representative of four other fruit. Early in the climacteric cellulase and PG increased markedly while PME decreased.

PME activity in relation to ripening of various fruits has been shown to increase (11, 14), decrease (23), or remain unchanged (5,7). In avocado, we observed high PME activity for the first 5 days of the preclimacteric period followed by a sharp drop during the first 48 h of the climacteric while a low level of activity was measured during the main ripening period. These changes in activity of the extracted enzyme are not readily related to softening. Brady (5) has observed in banana and tomato (also true for avocado) that the amount of PME observed in salt extracts supplies a potential for demethylation which far exceeds the net change in methylation observed during ripening, yet little change in methoxyl content has been shown to occur while PME activity is highest (8). This may be explained by the observation of Jansen and Jang (15) who showed that PME may be completely inactive while bound to cell wall material. Further, the enzyme may be sequestered in a compartment separate from the substrate in the preclimateric period, or inhibited by phenolic acids (10), polyols (6), or by fatty acids (19) although none of these possible inhibitors are believed to increase at the beginning of the climacteric.

PME is believed to have little effect on changes in texture of ripening fruit (5, 15, 18) but these authors show that partial demethylation of pectin is necessary before PG can bring about any significant hydrolysis. Thus, PME may function to prepare the substrate for hydrolysis by PG.

We found no PG activity in our extracts for 7 days after picking. The first significant PG activity could be detected only on the 3rd day of the climacteric and 1 day after the increase in ethylene production. Softening was already apparent in the fruit when the first PG activity was detected, and it appears that activation or synthesis of PG is not an early event in the ripening process of avocado fruit. While a proteinaceous inhibitor of PG has been proposed by Raymond and Phaff (21), we have found no evidence for one by mixing homogenates of ripe and unripe fruit.

In tomato fruit, PG appears to be the main factor involved in fruit softening while cellulase plays a minor role (12). The opposite is the case with avocado fruit. Significant cellulase activity is measurable in avocado fruit at the time of picking. Precisely at the time of the climacteric rise, cellulase activity begins to increase which is also before the rise in ethylene production. The increase in cellulase activity was very closely correlated with increased respiration and softening of the fruit and continued to rise into the postclimacteric period. On the 12th day, activity was 2-fold higher than when the fruit was edibly soft.

We believe that cellulase is either synthesized or activated very early in the climacteric. Mixtures of preclimacteric and postclimacteric homogenates yielded only the sum of the activities of the two homogenates, giving evidence for neither inhibitors nor activators of cellulase.

Although in tomatoes, peaches, and pears (13) cellulase appears to play a minor role in softening, in avocados it appears that the initial phase of softening is most closely correlated with cellulase activity which is probably newly synthesized while PG is involved in the later stages of ripening.

The amount of cellulase activity present in ripe avocado fruit is prodigious. Lewis *et al.* (17) found 85 units/g fresh weight in abscission zones of kidney bean plants and 150 units in tomato fruit, whereas ripe avocado fruit yielded as much as 116,000 units of cellulase/g fresh weight.

LITERATURE CITED

1. ALMIN, KE, KE ERIKSSON, C JANSON 1967 Enzymic degradation of polymers. II. Viscometric determination of cellulase activity in absolute terms. Biochim Biophys Acta 139: 248–253
2. AWAD M 1977 Variation in cellulase content of Fuerte avocado fruit after harvest. HortScience 12: 406
3. BARASH I, S KHAZZAM 1970 The relationship and properties of pectic glycosidases produced by host and pathogen during anthracnose disease of avocado. Phytochemistry 9: 1189–1197
4. BARMORE CR, AH ROUSE 1976 Perctinesterase activity in controlled atmosphere stored avocados. J Am Soc Hort Sci 101: 294–296
5. BRADY CJ 1976 The pectinesterase of the pulp of the banana fruit. Aust J Plant Physiol 3: 163–172
6. CHANG LWS 1965 Papaya pectinmethylesterase inhibition by sucrose. J Food Sci 30: 218–222
7. DESWART GH, EC MAXIE 1967 Pectin methylesterase in the ripening banana. S Afr J Agric Sci 10: 501–506
8. DOLENDO AL, BS LUH, HK PRATT 1966 Relation of pectic and fatty aid changes to respiration rate during ripening of avocado fruits. J Food Sci 31: 332–336
9. GERTMAN E, Y FUCHS 1974 Changes in pectinmethylesterase (PME) activity caused by ethylene applied at different temperatures. Plant Cell Physiol 15: 501–505
10. HALL CB 1966 Inhibition of tomato pectinesterase by tannic acid. Nature 212: 717–718
11. HOBSON GE 1963 Pectinesterase in normal and abnormal tomato fruit. Biochem J 86:358–365
12. HOBSON GE 1964 Polygalacturonase in normal and abnormal tomato fruit. Biochem J 92: 324–332
13. HOBSON GE 1968 Cellulase activity during maturation and ripening of tomato fruit. J Food Sci 33: 588–592
14. HILTIN HO, AS LEVINE 1965 Pectin methylesterase in the ripening banana. J Food Sci 30: 917–921
15. JANSEN EF, R JANG 1960 Orange pectinesterase binding activity. Food Res 25: 64–72
16. KOSIYACHINDA S, RE YOUNG 1975 Ethylene production in relation to the initiation of respiratory climacteric in fruit. Plant Cell Physiol 16: 595–602
17. LEWIS LN, AE LINKINS, S O'SULLIVAN, PD REID 1974 Two forms of cellulase in bean plants. *In* 8th Intern Symp Plant Growth Regulators. Hirokawa Publishing Co, Tokyo, pp 708–718
18. MCCREADY RM, EA MCCOMB 1955 Pectin constituents in ripe and unripe fruit. Food Res 19: 530–535
19. MILLER GJ, RJ MCCOLLOCH 1959 Chain length specificity for pectinmethylesterase inhibition by anionic detergents. Biochem Biophys Res Commun 1: 91–93
20. PESIS E, Y FUCHS, G ZAUBERMAN 1978 Cellulase activity and fruit softening in avocado. Plant Physiol 61: 416–419
21. RAYMOND D, HJ PHAFF 1965 Purification and certain properties of avocado polygalacturonase. J Food Sci 30: 266–273
22. ROUSE AH, CD ATKINS 1955 Pectin esterase and pectin in commercial citrus juices as determined by methods used at the Citrus Experiment Station. Fla Agric Exp Sta Bull 570: 1–19
23. ROUSE AH, CR BARMORE 1974 Changes in pectic substances during ripening of avocados. HortScience 9: 36–37
24. ZAUBERMAN G, M SCHIFFMANN-NADEL 1972 Pectin methylesterase and polygalacturonase in avocado fruit at various stages of development. Plant Physiol 49: 864–865