Plant Physiol. (1983) 72, 382–385
0032-0889/83/72/0382/04/$00.50/0

Postharvest Variation in Cell Wall-Degrading Enzymes of Papaya (*Carica papaya* L.) during Fruit Ripening[1]

Received for publication August 16, 1982 and in revised form January 7, 1983

ROBERT E. PAULL AND NANCY JUNG CHEN
Department of Botany-HITAHR, University of Hawaii at Manoa, Honolulu, Hawaii 96822

ABSTRACT

Pectin methylesterase (PME), polygalacturonase (PG), xylanase, cellulase, and proteinase activity were determined and related to respiration, ethylene evolution, and changes in skin color of papaya (*Carica papaya* L.) fruit from harvest through to the start of fruit breakdown. PME gradually increased from the start of the climacteric rise reaching a peak 2 days after the respiratory peak. PG and xylanase were not detectable in the preclimacteric stage but increased during the climacteric: during the post climacteric stage, the PG declined to a level one-quarter of peak activity with xylanase activity returning to zero. Cellulase activity gradually increased 3-fold after harvest to peak at the same time as PME, 2 days after the edible stage. Proteinase declined throughout the climacteric and postclimacteric phases. A close relationship exists between PG and xylanase and the rise in respiration, ethylene evolution, and softening. Cultivar differences in postclimacteric levels of enzymic activity were not detected.

An inhibitor of cellulase activity was detected in preclimacteric fruit. The inhibitor was not benzyl isothiocyanate (BITC). BITC did inhibit PG activity, though no inhibitor of PG activity was detected in preclimacteric homogenates when BITC was highest. The results indicate that inhibitors did not play a direct role in controlling wall softening.

The postharvest papaya fruit ripening involves softening and the production of sugars and flavor constituents. There is a concomitant evolution of ethylene and an increased respiration rate (2, 17). The softening of the mesocarp and endocarp is due to the activity of cell wall-degrading enzymes, not starch degradation, as the fruit has no starch during ontogeny (8, 10).

Chan *et al.* (10) studied the relationship between fruit ripening, skin color, and the level of PG.[2] PG increased during fruit ripening and was greatest near the endocarp. The enzyme has both exo- and endopolygalacturonase activity (9). The role of other enzymes in wall degradation is unclear, and the relationship between wall-degrading enzymic activity, softening, respiration, and ethylene production has not been determined. There is variation between papaya cultivars in the rate of softening and particularly the rate at which the flesh loses all texture and becomes water soaked. Inasmuch as there is considerable variation in the time of ripening of individual fruit, we report here the changes in wall-degrading enzymes in relation to respiration and ethylene production in a single fruit. Three cultivars are compared as to the levels of postclimacteric wall-degrading enzymes. The information is crucial to the understanding of ripening and for selecting cultivars with desirable postharvest ripening characteristics.

MATERIALS AND METHODS

Papaya (*Carica papaya* L. cv Sunrise) grown at the Poamoho Experimental Station on the island of Oahu were used. Cultivar Kapoho Solo was obtained from the island of Hawaii and X-77 from the island of Oahu. Fruit was harvested at the mature green stage. Fruit was hot water-treated (49°C, 20 min), then dipped in 1% (w/v) Thiabendazole (Merck & Co., Inc.) for 1 min. Papayas were placed in glass jars at ambient temperatures (about 22°C) and flushed continuously with ethylene-free air. The CO_2 evolution was determined at 20-min intervals by means of an automatic gas sampling manifold connected to a Beckman IR gas analyzer (model 215A). The jars were sealed daily for 1 h and a 1-ml gas sample was taken from the head space for ethylene determination. Ethylene was determined with a gas chromatograph fitted with an alumina column and photoionization detector. Fruit skin color of the blossom end and the side of the blossom end was measured daily with a Hunter colorimeter.

To avoid the variability of fruits in their rate of ripening, samples for enzyme determinations were taken daily from around the circumference of each individual papaya with a sterile 5.5-mm cork borer. Four plugs were taken daily from each fruit. The holes were immediately closed with sterile cotton and warm lanolin, and the fruit was returned to the respiration jar. The plug minus the skin and placenta tissue was cut longitudinally in half. One half of the cylinder was used for the cellulase, proteinase, and PME assay and the other for determination of PG and xylanase. This method was modified from the procedure used by Awad and Young (5) for avocado. The sequential results presented in Figure 1 are from only one fruit. However, all determinations were carried out on 10 fruit, and all showed a similar pattern. The start of the climacteric respiratory rise was used to align the changes in enzyme activity to allow comparison.

Extraction and Assay of Cellulase, PME, and Proteinase. The pericarp tissue was homogenized at full speed in an Ultra-Turrax (Teckmar Instruments) at 1°C with 4 volumes of 0.5 M NaCl. After 15 min standing at 1°C (desorption time), the homogenate was centrifuged (10,000*g*, 10 min). Supernatant aliquots were removed for the cellulase, proteinase, and PME assays.

Cellulase assay mixture consisted of 0.2 ml of the enzyme suspension and 0.5 ml of 1% (w/v) carboxymethylcellulose (Nutritional Biochem.) in 40 mM sodium acetate buffer (pH 6.7). 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 (5). Drainage time was converted to relative units of cellulase activity (3). PME was determined by titrating the release of carboxyl groups by the action of PME on the substrate with 0.02 N NaOH to pH 7.5 for 5 min at 30°C. The assay mixture consisted of 0.2 ml of enzyme suspension and 10 ml 0.5% pectin (5). PME units were expressed as mol ester hydrolyzed/g fresh

[1] Hawaii Institute of Tropical Agriculture and Human Resources Journal Series No. 2699.

[2] Abbreviations: PG, polygalacturonase; PME, pectin methylesterase; BITC, benzyl isothiocyanate.

weight·min. Proteinase reaction mixture consisted of 2 ml 1% (w/v) casein (Nutritional Biochem.) in 0.1 M sodium phosphate buffer at pH 6.0 and 0.2 ml enzyme extract at 30°C for 30 min. Reaction was terminated by the addition of 1 ml 20% (w/v) TCA and centrifuged (3,000*g*, 10 min). The relative activity of proteinase was determined by the increase in absorbance of supernatant against reagent blank at 280 nm and expressed as absorbance changed per min (12).

Extraction Assay of PG and Xylanase. Pericarp tissue was ground with 4 volumes 7.5% (w/v) (NaCl + EDTA, 10:1) at pH 7.0 and 1°C. After standing at 1°C for 15 min (14), the mixture was centrifuged (10,000*g*, 10 min). The supernatant was collected and diluted with 2 volumes of extraction solution. The diluted supernatant was transferred to a Centriflo Membrane cone (Amicon Corp., type CF 25) and centrifuged (800*g*, 30 min, 1°C). The washing procedure was repeated twice to remove free reducing sugars present in the enzyme extract. The protein content of the final supernatant was measured (6), and aliquots were used for the PG and xylanase assays.

PG assay mixture consisted of 0.45% (w/v) purified polygalacturonic acid (Sigma Chemical Co.), twice precipitated from water with alcohol) and 0.1% (w/v) casein in 0.04 M Na-acetate buffer (pH 5.0). Enzyme extract (0.2 ml) was added to 2 ml of assay solution and incubated (4 h, 37°C). Reaction was terminated with 0.4 ml 2 M HCl (19); after centrifugation (10,000*g*, 10 min), the supernatant was removed for reducing sugar analysis (22). The activity of PG was expressed as mg glucose equivalent released/mg protein·h. Xylanase assay solution contained 0.1% (w/v) xylan (Sigma Chemical Co.), purified as described (27), and 0.1% (w/v) casein in 40 mM sodium acetate buffer (pH 5.0). The reaction mixture contained 0.2 ml enzyme suspension and 2 ml assay solution. The mixture was incubated for 4 h at 37°C and then terminated with 0.4 ml 2 M HCl. After centrifugation (10,000*g*, 10 min), the reducing sugars in the supernatant were determined (22). The xylanase activity was expressed as mg glucose equivalent released/mg protein·h.

RESULTS

Efficiency of Extraction of PG. Before comparing enzymic activity of PG contained by fruit showing the first sign of color change and ripe fruit, the efficiency of enzyme extraction was determined. A single extraction with NaCl and EDTA and 15 min standing at 1°C extracted at least 93 to 95% of the activity in ripe fruit just showing color break. The remainder of the enzymic activity (5–7%) was removed in the second extraction. Inasmuch as the amount extracted in a single extraction was constant, only one extraction was used routinely.

Change in Enzymes during Ripening. The variation in enzymic activity during papaya ripening (cv Sunrise) represents the common pattern of 10 individual fruits observed in four experiments. Removal of plugs from fruit accelerated ripening to some extent. The respiration curve was used as the time unit for comparing different fruit activities because mature green papaya harvested at the same time did not ripen simultaneously. The climacteric respiration rise was generally observed about 6 d after harvest (Fig. 1A). A low level of ethylene production occurred throughout the preclimacteric period with a surge of production at the same time as the respiratory climacteric (Fig. 1A). There was a concomitant increase in Hunter lightness value (Fig. 1B) as the skin became yellow. Mature green fruit had higher levels of proteinase activity, which declined as the fruit ripened (Fig. 1B).

PG activity was not measureable at harvest but increased after 4 d at the same time as the rise in respiration (Fig. 1C). PG activity peaked at the same time as the maximum increase in rate of respiration occurred, then declined to 20% of the peak PG activity. A second smaller peak in PG activity was frequently measured after the respiration rate had leveled off. Similarly, xylanase activity was very low in the pre- and postclimacteric period, and maximum activity was at the same time as the PG activity (Fig. 1D).

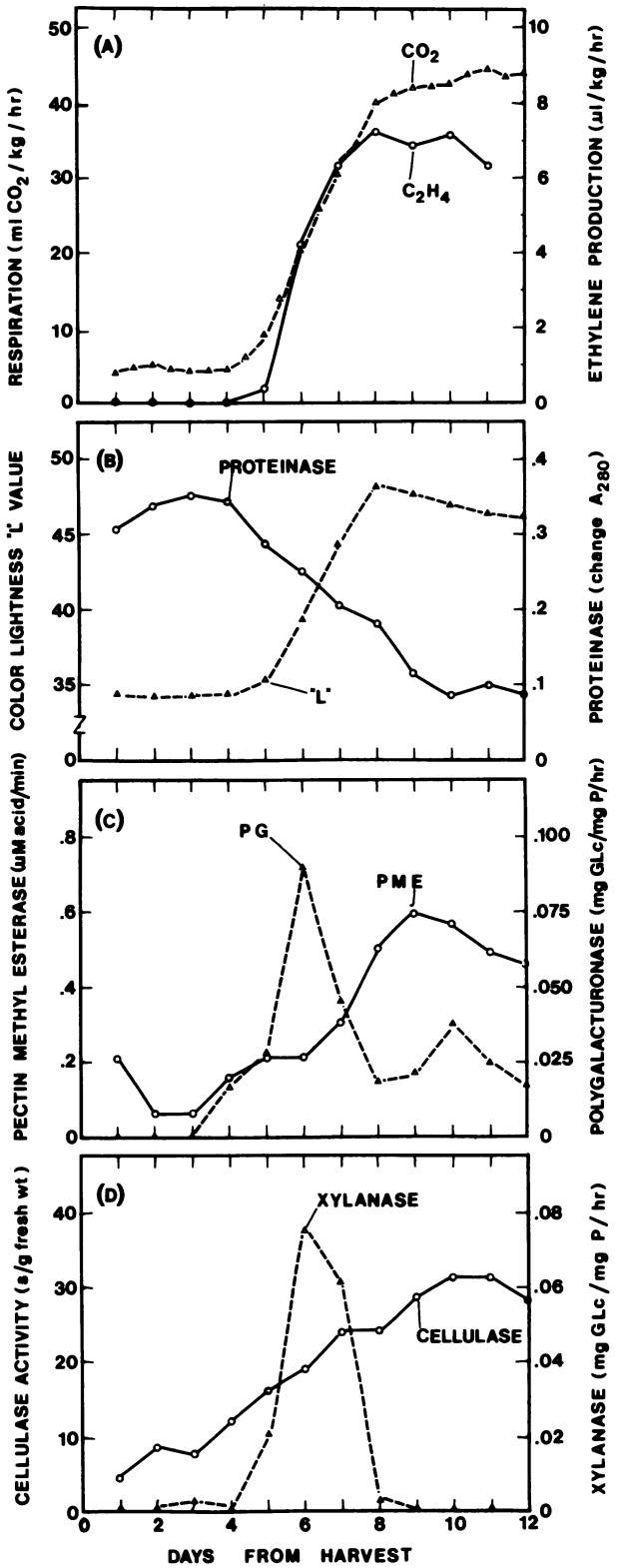

FIG. 1. Postharvest trends in: A, respiration and ethylene production; B, proteinase and Hunter "L" lightness value; C, PG and PME; and D, xylanase and cellulase in papaya fruit.

A different pattern of enzymic activity was observed for PME (Fig. 1C) and cellulase (Fig. 1D). A low level of activity was

Table I. *Effects of Ripeness on Cellulase Activity and Percentage Activity across the Pericarp Tissue from the Endocarp to the Exocarp*

Fruit Ripeness	Respiratory Stage	Cellulase Activity[a]						
		Endocarp		Mesocarp		Exocarp		Total
		s/g fresh wt	*%*	*s/g fresh wt*	*%*	*s/g fresh wt*	*%*	*s/g fresh wt*
Mature green		52.6 ± 17.6	96	2.3 ± 3.3	4	0	0	54.9
Turning	Preclimacteric	202.4 ± 54.9	61	88.9 ± 57.1	27	42.2 ± 19.9	12	333.5
10% yellow	Climacteric rise	335.5 ± 9.2	58	173.6 ± 40.0	30	71.6 ± 46.6	12	580.7
70% yellow	Climacteric peak	293.2 ± 40.9	48	168.5 ± 52.8	29	143.1 ± 34.5	23	604.8

[a] Mean ± SE.

Table II. *Cultivar Differences in Cell Wall-Degrading Enzymic Activity of Ripe Papaya*

Mean activity for the 6 d following the peak in the respiratory climacteric.

Enzyme	Cultivars		
	Sunrise[a]	Kapoho[a]	X-77[a]
PME (μmol acid/g fresh wt·h)	36.36 ± 2.59	20.11 ± 1.49	30.67 ± 1.760
PG (mg Glc/mg protein·h)	0.03 ± 0.02	0.03 ± 0.03	0.04 ± 0.03
Cellulase (s/g fresh wt)	28.00 ± 2.35	30.28 ± 0.94	25.06 ± 0.98

[a] Mean ± SE.

Table III. *Inhibition of Cellulase and PG Activity by Mixing of Enzyme Homogenates from Unripe and Ripe Fruit and by BITC*

Treatment	Cellulase Activity[a]	PG Activity[a]
	s/g fresh wt	*mg Glc/mg protein·h*
Homogenates		
Unripe fruit (A)	4.26 ± 0.86	0
Ripe fruit (B)	13.95 ± 0.27	0.041 ± 0.0021
Mix 0.5(A+B) = C	12.47 ± 0.70	0.041 ± 0.004
$\% \left(\frac{C}{A+B}\right)$	68	100
BITC		
Control (no BITC)	14.41 ± 1.17	0.041 ± 0.00
1×10^{-4} M BITC[b]		
Added before substrate (%)	16.76 ± 1.35 (112)	0.002 ± 0.003 (4)
Added after substrate (%)	12.79 ± 1.20 (89)	0 (0)

[a] Mean ± SE.

[b] $\% = \left(\frac{+\text{ BITC}}{\text{Control}}\right) \times 100.$

measured during the preclimacteric period which gradually increased throughout the postharvest ripening period. PME and cellulase increased for an additional 4 d after the ethylene peak. Cellulase activity began to increase 3 d before the ethylene production rise.

Changes in Cellulase Activity across the Mesocarp of Ripening Fruit. Cellulase activity was first detected near the endocarp of the fruit. As the fruit ripened, the activity progressively increased across the mesocarp tissue towards the exocarp (Table I). In mature green fruit, 96% of the total core activity was found at the endocarp with no activity detected near the exocarp. Fruit which were 70% yellow had 48% of the activity in the endocarp tissue and 23% near the exocarp. Higher cellulase activity was consistently measured near the endocarp of the fruit.

Postclimacteric Enzymic Differences between Cultivars. PME, PG, and cellulase activity for three cultivars are given in Table II. Cultivars Sunrise and X-77 did not vary appreciably in their postclimacteric enzymic activity; cv Kapoho showed a lower overall level of PME.

Inhibition of Enzymic Activity. No homogenate inhibitor or activator of PG activity was detected, but a 30% inhibition of cellulase activity was detected when homogenates from mature green and ripe fruit were mixed (Table III). BITC inhibited PG activity, but cellulase activity was not significantly inhibited (Table III).

DISCUSSION

There was close correlation, in a single fruit, between the trends in the wall-degrading enzymes and the ripening process. Daily enzymic determinations on a number of single fruit allow the variation found in ripening time of fruit to be avoided. There was a good relationship between measures of ripening, respiration, ethylene production, and skin color, and wall-degrading enzymic activity, particularly PG, xylanase, and cellulase (Fig. 1).

PME increases (13), decreases (5), or remains unchanged (7) during fruit ripening. PME is believed to have little effect on wall softening (7, 20) serving only to cause partial demethylation allowing PG activity. This deesterification has occurred with the loss of galactose and arabinose side branches from pectins during fruit ripening (1, 18, 24). Papaya PME activity increased gradually during fruit ripening (Fig. 1C) reaching a peak after the climacteric respiratory maximum. The importance of PME in the papaya fruit softening could be questioned because of its inhibition by sucrose (11), although it may be significant if the fruit is pulped. Low levels of PME were always detected in preclimacteric mature green papaya fruit. This potential activity might be sufficient for any required demethylation as is the case for tomatoes, bananas, and avocados (1, 7).

During papaya ripening, there is an increase in water-soluble pectin and a decrease in esterified protopectin (4, 25). Ethanol-insoluble material declines as the fruit ripens. These major changes have been ascribed to an increase in the levels of PG (10). This increase in PG occurred at the same time ethylene production rose, 4 d after harvest (Fig. 1C). The peak in PG activity correlated with maximum ethylene production. This peak was followed by a decline to about 20% of the peak values for the final senescent period. No PG or xylanase activity was detected prior to the rise in ethylene production indicating a close relationship between these events and respiratory climacteric. No inhibitors or activators were detected when preclimacteric and postclimacteric homogenates were mixed. Inhibition by BITC (Table III) may not be important in intact fruit at the climacteric stage, as the *in vivo* levels of BITC rapidly decline during ripening (28). This conclu-

sion is supported by an absence in unripe fruit of PG activity following purification which would remove free BITC. There is also the possibility of compartmentation of BITC within the cell. Softening of fruit was not detected prior to the rise in PG activity suggesting that PG and xylanase were early events in the ripening process.

Cellulase plays a minor role in softening of tomatoes (15), peaches (16), and pears (26) but appears crucial for avocado (5). Significant cellulase activity was detected at the time of papaya harvest, this increased gradually to peak 4 d after the ethylene production reached a maximum (Fig. 1D). The increase in cellulase activity began before the rise in ethylene production and therefore does not correlate with the increased respiration and fruit softening. A cellulase inhibitor was detected when preclimacteric and postclimacteric homogenates were mixed. BITC was not the cellulase inhibitor but seems to be an activator when added before the substrate, although it did inhibit PG activity (Table III) and does inhibit proteinase (29) and ethylene evolution (23). No inhibitors or activators of cellulase were detected in avocado using similar procedures (5). The presence of cellulase activity early in the ripening process may trigger some of the subsequent events.

Because of its gradual increase during papaya ripening, cellulase activity was utilized to show the ripening of the fruit from the endocarp placental area outward (Table I). This progression of activity agrees with the softening pattern of the fruit. A similar pattern was found with PG (9). This may indicate that the placenta and inner endocarp tissue are either more ready to ripen or are triggered to initiate ripening by the changes brought about by fruit detachment.

Acknowledgments—The authors express their appreciation for the technical assistance of Ted Goo and Shirley Lee.

LITERATURE CITED

1. AHMED AE, JM LABAVITCH 1980 Cell wall metabolism in ripening fruit. 1. Cell wall changes in ripening 'Bartlett pears'. Plant Physiol 65: 1009–1013
2. AKAMINE EK, T GOO 1979 Concentrations of carbon dioxide and ethylene in the cavity of attached papaya fruit. HortScience 14: 138–139
3. ALMIN KE, KE ERICKSSON, C JANSSON 1967 Enzymic degradation of polymers II. Viscometric determination of cellulase activity in absolute terms. Biochim Biophys Acta 139: 248–253
4. ARRIOLA MC, MC MARDRID, C ROLZ 1975 Some physical and chemical changes in papaya during its storage. Proc Trop Reg Am Soc Hort Sci 19: 97–109
5. AWAD M, RE YOUNG 1979 Postharvest variation in cellulase, polygalacturonase, and pectin methylesterase in avocado (*Persea americana* Mill, cv. Fuerte) fruit in relation to respiration and ethylene production. Plant Physiol 64: 306–308
6. BRADFORD MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilization the principles of protein dye-binding. Anal Biochem 72: 248–254
7. BRADY CJ 1976 The pectinesterase of pulp of banana fruit. Aust J Plant Physiol 3: 163–172
8. CHAN HT, KL HIBBARD, T GOO, EK AKAMINE 1979 Sugar composition of papayas during fruit development. HortScience 14: 140–141
9. CHAN HT, SYT TAM 1982 The characterization of endopolygalacturonase and exopolygalacturonase from papaya. J Food Sci 47: 78–1483
10. CHAN HT, SYT TAM, ST SEO 1981 Papaya polygalacturonase and its role in thermally injured ripening fruit. J Food Sci 46: 190–191, 197
11. CHANG LWS, LL MORITA, HY YAMAMOTO 1965 Papaya pectin methylesterase inhibition by sucrose. J Food Sci 30: 218–222
12. HANCOCK JG, RL MILLAR 1965 Association of cellulolytic, proteolytic, and xylolytic enzymes with southern anthracnose, spring black stem, and stemphylium leaf spot of alfalfa. Phytopathology 55: 356–360
13. HOBSON GE 1963 Pectinesterase in normal and abnormal tomato fruit. Biochem J 86: 358–365
14. HOBSON GE 1964 Polygalacturonase in normal and abnormal tomato fruit. Biochem J 92: 324–332
15. HOBSON GE 1968 Cellulase activity during maturation and ripening of tomato fruit. J Food Sci 33: 588–592
16. JERMYN MA, FA ISHERWOOD 1956 Changes in the cell wall of pears during ripening. Biochem J 64: 123–136
17. JONES WW, H KUBOTA 1940 Some chemical and respirational changes in the papaya fruit during ripening, and the effect of cold storage on these changes. Plant Physiol 15: 711–717
18. KNEE M 1973 Polysaccharide changes in cell walls of ripening apples. Phytochemistry 12: 1543–1549
19. LUI YK, B LUH 1978 Purification and characterization of endopolygalacturonase from *Rhizopus arrihizus*. J Food Sci 43: 721–726
20. MCCREADY RM, EA MCCOMB 1955 Pectin constituents in ripe and unripe fruit. Food Res 19: 530–535
21. Deleted in proof.
22. NELSON N 1944 A photometric adaptation of the Somogyi method for determination of glucose. J Biol Chem 153: 373–380
23. PATIL SS, CS TANG 1974 Inhibition of ethylene evolution in papaya pulp tissue by benzyl isothiocyanate. Plant Physiol 53: 585–588
24. REEVE RM 1959 Histological and histochemical changes in developing and ripening peaches. II. The cell wall and pectins. Am J Bot 46: 241–248
25. SHETTY SR, PJ DUBASH 1974 Relationship of pectin content of papaya fruit to its firmness and maturity. Indian Food Packer 28: 14–16
26. STERLING C 1961 Physical state of cellulase during ripening of peach. J Food Sci 26: 95–98
27. TAIZ L, WA HONIGMAN 1976 Production of cell wall hydrolyzing enzymes by barley aleurone layers in response to gibberellic acid. Plant Physiol 58: 380–386
28. TANG CS 1971 Benzyl isothiocyanate of papaya fruit. Phytochemistry 10: 117–121
29. TANG CS 1974 Benzyl isothiocyanate as a naturally occurring papain inhibitor. J Food Sci 39: 94–96