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Glycosidase Activities and Development of Peach Fruit Mesocarp Tissues[1]

Eugene M. Kupferman[2] and Wayne H. Loescher[3]
Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164

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Abstract. **Mesocarp development of peach [*Prunus persica* (L.) Batsch cv. Redhaven] as measured by fresh weight and size increase, progressed along a double sigmoid curve which was reflected in the activity of extractable wall-associated α- and β-nitrophenylgalactosidases. Enzyme activities, both on protein and dry weight basis, rose rapidly during early fruit development, leveled off, then again rose rapidly at maturation. There was more α-nitrophenylgalactosidase activity than β-nitrophenylgalactosidase activity throughout development. Increases in both galactosidases followed rather than preceded increases in size. The final increases were, however, well correlated with fruit maturation.**

Plant cell growth is generally the result of biochemically mediated modifications of cell wall constituents. In fruit tissues these modifications should be considerable. Cell division in many fruits continues for only a short period after fertilization, and subsequent increases in fruit size require tremendous expansion and elongation of the original cells. Much work has been reported on wall-related enzymatic and polysaccharide changes in several fruits, including apple (2, 3, 8, 9, 17) avocado (12), pear (11, 20, 21, 22), tomato (6, 13, 18, 19), and strawberry (1). But most of this work, with some exceptions (20, 21), has been concerned with changes associated with fruit softening at maturity and not with changes associated with growth. This is especially true of the stone fruits where growth and softening appear to be distinct processes (4). Although changes in cellulase activity (5) and in polygalacturonase activities (14, 15) have been reported in ripening peaches, changes in activities of other enzymes that may be involved in growth and softening of these fruits are poorly documented (16).

Nitrophenylglycosidases have been investigated in apples (12, 17) and tomatoes (13, 19) with, in some cases, conflicting results as to the relationships between activity and wall modifications such as softening and growth. The following study investigates some relationships between nitrophenylglycosidases and development of Redhaven peach mesocarp tissues.

Materials and Methods

Peach fruit growth was measured by recording increases in size and fresh weight. Lengths and widths of tagged fruits growing in the Washington State University Horticulture orchard in Pullman were measured using a vernier caliper. Measurements began on June 6, 1977, 53 days after full bloom (AFB), and continued at weekly intervals until August 19, 118 days AFB. Samples of 20-30 fruits were harvested at biweekly intervals, weighed, and the mesocarp separated from the rest of the fruit. Mesocarp pieces were frozen at –40C for subsequent analyses. In the fall all samples were lyophilized and ground with a mortar and pestle.

Wall-associated glycosidases were extracted and assayed from the samples utilizing a modified Wallner and Walker (19) method. Samples (250 mg) of the dry, powdered tissues were homogenized with a Braun Polytron homogenizer in 20 ml of cold, 0°C, 50 mM Na-citrate buffer, pH 4.0, for 10 sec to solubilize non-cell-wall-bound enzymes. The extract was then centrifuged at 40,000 g for 15 min at 2°. The pellet was taken up in 6 ml of 500 mM Na-citrate buffer at pH 4.0, homogenized, and let stand for 30 min to solubilize cell-wall-bound enzymes. The extract was again centrifuged (40,000 g, 15 min) to bring down cellular debris, and dialyzed overnight against 10 volumes of cold water. Extracts were then brought up to 12 ml with cold 50 mM Na-citrate buffer, pH 4.0, centrifuged (40,000 g, 15 min), and the supernatant assayed.

The assay mixture consisted of 1 ml extract, 1 ml of substrate (ρ-nitrophenyl-α- or β-D-galactopyranoside or ρ-nitrophenyl-α- or β-D-glucoside, Sigma) at 6.5 mM in 50 mM Na-citrate buffer, pH 4.0, and 1 ml 50 mM Na-citrate buffer, pH 4.0. The mixture was incubated at 37°C for 1 hr in a shaking waterbath, and the reaction stopped by addition of 1 ml of 600 mM Na_2CO_3. Concentration of liberated ρ-nitrophenol was determined by measuring absorbance at 400 nm on a Gilford spectrophotometer. Blanks for each sample consisted of the assay mixture to which Na_2CO_3 was added before addition of the extract.

The pH optimum for the extraction procedure was determined by extracting the enzymes from a composite sample with both 50 and 500 mM Na-citrate buffers at various pH's between 3 and 7, and then analyzing with the assay mixture at pH 4.0. The pH optimum for the assay procedure was determined by extracting with both 50 and 500 mM Na-citrate at pH 4.0 and analyzing with all the components of the assay mixture at various pH between 3 and 7.

Protein content of the dialyzed extracts was determined with the Lowry method (10) using crystalline bovine serum albumin (Sigma) as a standard.

Results and Dicussion

In Pullman, in 1977, 'Redhaven' peach fruits grew rapidly during the first 65 AFB then the growth of the whole fruit slowed dramatically (Fig. 1). This slow growth continued until 90 days AFB. Subsequent growth was very rapid with the fruit gaining weight and increasing in size until abscission. These results are similar to the classical double sigmoid growth curves for peaches which are described as consisting of 3 stages, I, II, and III (16). Mesocarp growth is described as rapid in Stages I and III and less active during Stage II. The most rapid growth stage of the mesocarp is Stage III, often called the final swell.

Experiments to determine optimum extraction conditions indicated that the pH optimum for extraction of α-galactosidase from peach mesocarp tissue was at or below pH 3.0, while the

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[2]Current position and address: Area Extension Agent, WSU Cooperative Extension Service, N. 222 Havana, Spokane, Washington 99202.

[3]Address for Reprint Requests.

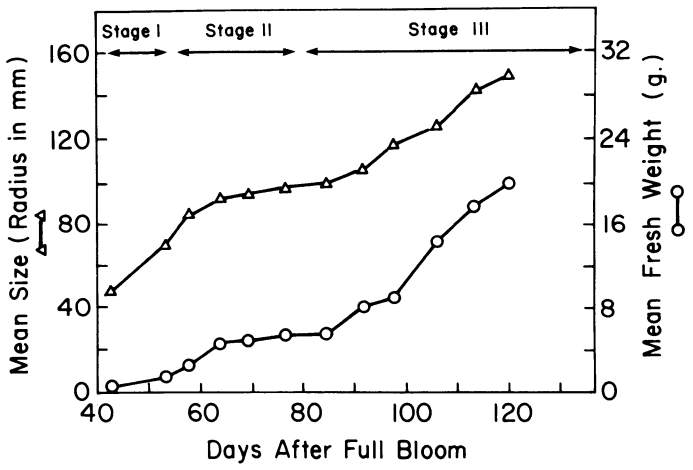

Fig. 1. Growth of 'Redhaven' peach fruits at Pullman, Washington in 1977.

optimum pH for assay was at pH 4.5. The pH optimum for both extraction and assay of β-galactosidase was pH 4.5. Consequently, all subsequent routine extractions and assays for both enzymes were performed at pH 4.0. The aqueous extraction procedure employed in this work was compared to a non-aqueous extraction procedure using a mixture of glycerol and ethylene glycol (13). No significant differences in specific activity of either α- or β- galactosidase were observed.

In an early study specific activities, on a protein basis, of both wall-associated α- and β-galactosidases (α- and β-nitrophenylgalactosidases) and α- and β-glucosidases (α- and β- nitrophenylglucosidases) in peach mesocarp tissues were compared at 3 stages of development (Table 1). This showed that α- and β-glucosidase activities were each much less than either galactosidase. Also, α-galactosidase activity was consistently greater than that of β-galactosidase.

Other comparisons of α- and β-galactosidase activities were made beginning 53 days AFB and at 2 week intervals until abscission, 118 days AFB. Both enzymes increased in activity, both on a unit protein as well as on a unit dry weight basis (Fig. 2). Only low levels of activity were detectable initially, but both enzymes increased activity, first slowly and then dramatically during Stage II and later in Stage III. Since timing of the large increases in activity of both the α- and β-galactosidases followed instead of preceded the size increases occurring in Stages I and III (Fig. 1), it seems unlikely that either enzyme is directly involved in growth of the tissue. Although other workers have shown that these and other wall-associated glycosidases are present in tomato fruit, the results are equivocal as to the relationships between these enzymes and growth (13, 19).

Table 1. Wall-associated nitrophenylglucosidase and nitrophenylgalactosidase activities in peach mesocarp tissue of fruits at 3 different stages of growth.

Days after full bloom	Activity[z]			
	Glucosidase		Galactosidase	
	α	β	α	β
70	0.015	0.009	0.269	0.208
85	0.019	0.011	0.497	0.366
120	0.024	0.014	1.685	0.978

[z]Activity in μmoles product/hr·mg protein.

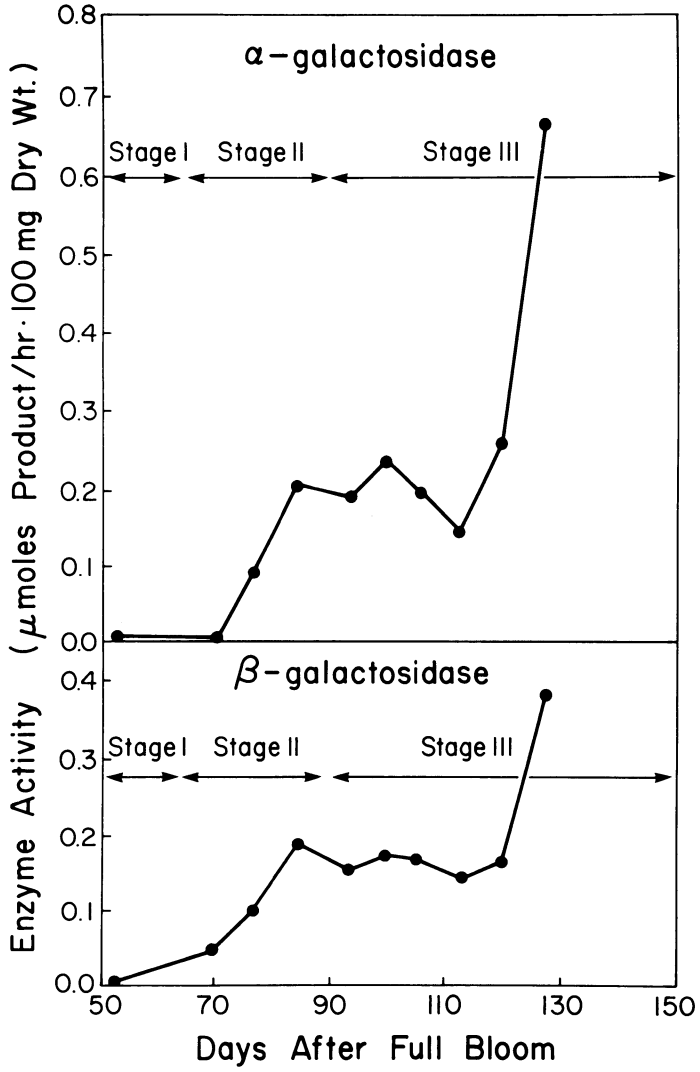

Fig. 2. Influence of developmental stage of peach mesocarp tissue on activity of α- and β-nitrophenylgalactosidase per 100 mg dry weight.

There are several alternative possibilities. Glycosidases like these may be involved in wall modifications that render wall components susceptible to attack by other hydrolytic enzymes at later stages of mesocarp development. There is a report that such enzymes exist (7). Also, although the changes reported here were not well correlated with growth, the second increase in activity which occurred just prior to fruit abscission did correspond well with the time when the fruit began softening. Firmness, measured weekly over a period beginning 2 weeks prior to the last sampling date, dropped from 9.1 to 6.1 to 4.0 to 1.0 kg. Whether this later increase was associated with that phenomenon and other increases in cellulase (5) and polygalacturonases (14) remains to be established. Finally, it may be that these changes in activity have no relationship to wall modifications, but instead represent ripening-associated or other modifications of phenolic glycosides present in the mesocarp tissue. This may be the more feasible explanation since nitrophenylglycosidic linkages do not, as far as we know, occur in plant cell walls. Whatever the explanation, it is clear that in peach mesocarp tissues levels of both nitrophenylgalactosidases, especially α-galactosidase, are good indicators of 'Redhaven' peach fruit maturity.

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Some Effects of Grapefruit Tree Canopy Position on Microclimate, Water Relations, Fruit Yield, and Juice Quality[1]

J. P. Syvertsen and L. G. Albrigo[2]

Department of Fruit Crops, University of Florida, IFAS, Agricultural Research and Education Center, P. O. Box 1088, Lake Alfred, FL 33850

Additional index words. Citrus paradisi, heat stress, leaf temperature, fruit temperature, water potential, stomatal conductance

Abstract. **Positional differences among leaf and fruit surface temperatures and water relations of 'Ruby' grapefruit (*Citrus paradisi* Macf.) were related to fruit load and juice quality. Southern top canopy positions experienced the highest temperatures and lower water potentials and yielded more fruit with more soluble solids than other canopy positions. Canopy depth was also an important determinant of fruit yield and early season juice quality. Based on data from 3 trees during 2 seasons, there were greater fruit loads with higher °Brix and lower acidity in the outside canopy positions than in the inside positions. Upper canopy positions tended to have lower acidity and consequently higher °Brix/acid ratios than the lower positions. Abaxial fruit hemispheres were smaller and had a lower percent juice than their paired adaxial fruit hemispheres. Grapefruit from sunlit canopy positions mature earlier than fruit from shaded positions. Since there were more fruit with higher soluble solids in the most exposed canopy positions, daily heat stress and leaf and fruit water stress were not limiting factors in grapefruit yield and juice quality with respect to different tree canopy positions.**

The quantity and quality of orange fruit are affected by the position of the fruit on the tree. In general, sun-exposed upper sectors of the canopy yield more fruit of higher quality than shaded lower or inside canopy sectors (11). Specifically, oranges from southern top canopy sectors tend to have higher concentrations of soluble solids and a higher juice content (15, 16, 17) than fruit from other sectors. Soluble solids in individual oranges and grapefruit increase from the stem region to the stylar end and several juice characteristics change from the periphery to the central core (18). Since there are reports

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[2]Assistant Professor of Plant Physiology and Professor of Horticulture. The authors gratefully acknowledge the technical assistance of M. L. Smith, Jr., C. Gardner, and many fruit pickers too numerous to cite individually.