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Comparison of Ripening Processes in Intact Tomato Fruit and Excised Pericarp Discs[1]

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ABSTRACT

Physiological processes characteristic of ripening in tissues of intact tomato fruit (*Lycopersicon esculentum* Mill.) were examined in excised pericarp discs. Pericarp discs were prepared from mature-green tomato fruit and stored in 24-well culture plates, in which individual discs could be monitored for color change, ethylene biosynthesis, and respiration, and selected for cell wall analysis. Within the context of these preparation and handling procedures, most whole fruit ripening processes were maintained in pericarp discs. Pericarp discs and matched intact fruit passed through the same skin color stages at similar rates, as expressed in the L*a*b* color space, changing from green (a* < −5) to red (a* > 15) in about 6 days. Individual tissues of the pericarp discs changed color in the same sequence seen in intact fruit (exocarp, endocarp, then vascular parenchyma). Discs from different areas changed in the same spatial sequence seen in intact fruit (bottom, middle, top). Pericarp discs exhibited climacteric increases in ethylene biosynthesis and CO_2 production comparable with those seen in intact fruit, but these were more tightly linked to rate of color change, reaching a peak around a* = 5. Tomato pericarp discs decreased in firmness as color changed. Cell wall carbohydrate composition changed with color as in intact fruit: the quantity of water-soluble pectin eluted from the starch-free alcohol insoluble substances steadily increased and more tightly bound, water-insoluble, pectin decreased in inverse relationship. The cell wall content of the neutral sugars arabinose, rhamnose, and galactose steadily decreased as color changed. The extractable activity of specific cell wall hydrolases changed as in intact fruit: polygalacturonase activity, not detectable in green discs (a* = −5), appeared as discs turned yellow-red (a* = 5), and increased another eight-fold as discs became full red (a* value +20). Carboxymethyl-cellulase activity, low in extracts from green discs, increased about six-fold as discs changed from yellow (a* = 0) to red.

Ripening in a climacteric fruit, such as tomato (*Lycopersicon esculentum* Mill.), is a complex phenomenon. It encompasses a diverse set of physiological processes progressing differentially through a number of anatomically distinct tissues. Progressive changes in color, texture, flavor, and aroma in each tissue appear to result from changing local patterns of gene expression and metabolic activity (4, 5, 13). These processes are induced and coordinated, in part, by the biosynthesis and diffusion of ethylene (5, 25, 26, 35), by local differences and changes in sensitivity to ethylene (5, 25), and, perhaps, by other regulatory signals (6).

Although ripening has been studied largely in intact fruit, excised tissues offer a number of advantages for the experimental analysis of ripening. Use of excised tissues allows separation of the various ripening processes by isolation of specific tissues, quantitative addition of metabolic intermediates or inhibitors to these tissues, and accurate measurement of local processes through nondestructive monitoring and destructive analysis of replicated samples. For these reasons, several laboratories have used discs cut from tomato pericarp for studies of ripening in tomato (10, 11, 23, 27, 29, 32).

A comparison of known ripening processes in intact and excised tissues is necessary if excised tissues are to be used with confidence in studies of ripening. Since ripening has been more thoroughly characterized in tomato pericarp than in any other fruit or tissue (4, 14), a detailed comparison is possible. Previous studies have shown that, during normal ripening of pericarp in intact tomato fruit, tissue color changes from green through orange to red (10, 18), ethylene biosynthesis and respiration undergo a climacteric rise (35), tissues decrease in firmness (21), extractable activities of specific hydrolytic enzymes increase (7, 17, 19, 28, 34), and various fractions of the cell wall change in composition (15, 16). In this article, these characteristics of ripening in whole fruit, as described here or by others, are compared with similar processes in excised pericarp discs.

Previous studies of excised pericarp tissue indicate that the details of disc preparation and handling are critical to the processes of color change, respiration, and ethylene biosynthesis (11, 23, 27, 32). An established procedure for disc preparation (9, 29) was combined with a novel handling system using 24-well tissue culture plates to order and protect the discs. The system permitted easy monitoring of color change, ethylene biosynthesis, and respiration in individual discs, application of treatments to tissues, and selection of discs at particular stages for destructive analyses.

Within the context of these preparation and handling procedures, we examine whether physiological processes characteristic of ripening in intact tomato fruit are duplicated in pericarp discs, to determine the utility of excised pericarp discs as a model system for the experimental analysis of ripening.

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MATERIALS AND METHODS

Tomato Fruit

Field-grown, mature-green tomato (*Lycopersicon esculentum* Mill.) fruit of varieties '674' or 'C-17' were obtained at the mature-green stage from Florida or California, respectively, through Bianchi and Sons Packing, Merced, CA. Unblemished fruit, 150 to 200 g in mass, were washed, placed in open 490-mL jars, and kept at 20°C at 60% ambient humidity. Rates of ethylene biosynthesis were measured daily to monitor intact fruit and to select fruit at the transition from MG3[3] to mature-green 4 maturity stages for preparation of discs. Transition from MG3 to mature-green 4 is characterized by the onset of internal color change and is usually accompanied by a rapid rise in ethylene biosynthesis (14). Jars were capped, and accumulated gases were sampled after 30 min. Ethylene was analyzed by gas chromatography at 80°C with an alumina column and quantified by the integration of the peak from a flame ionization detector. CO_2 was measured, as needed, by integration of the peak from an infrared gas analyzer.

Disc Preparation

Disc preparation (9) and subsequent manipulations were carried out with sterile technique in a sterile hood. The selected fruits were briefly sterilized in 1% sodium hypochlorite and rinsed thoroughly with water. Cylinders of pericarp were cut with a cork borer, then sliced by hand into discs with epidermis included. Discs were cut from over locules and septa in an equatorial band. For analysis of the spatial sequence of ripening processes, pericarp discs were also prepared from bands at 2 cm from the stem scar and 2 cm from the stylar scar. Discs of different sizes were used for specific studies, ranging from 10 to 15 mm in diameter, 1.5 to 6 mm in thickness, and 160 to 800 mg in weight. Up to 100 discs could be prepared from a single fruit. Cut discs were briefly rinsed twice with distilled water, drained, and then blotted, cut-surface-down, on sterile filter paper for about 60 s to remove absorbed water. Excess absorbed water can delay ripening (32), as can exposure to ethanol used for sterilization of tissue or equipment (29).

Tomato pericarp discs contain at least three distinct tissues: exocarp, endocarp, and vascular tissues. The exocarp lies immediately beneath the transparent epidermis, is 1 to 2 mm thick, and is composed of relatively small, densely packed cells, rich in pigments. The endocarp extends inward from the exocarp to the locule or septum, and is composed of larger, less densely packed, lightly colored cells. Vascular tissues lie within the endocarp.

Tissue Culture Plates

Blotted discs were immediately placed epidermis-side-down in individual wells of sterile, 24-well, clear plastic, tissue culture plates (Falcon 3047). Spaces between wells were partially filled with water. Plates of discs were stored in boxes flushed with 3 volumes of water-saturated air/h to keep peak ethylene concentrations in the boxes below 50 parts per billion, and were handled under isothermal conditions (20°C) when briefly removed for measurements in order to minimize tissue water loss. For analysis of weight loss, plates and individual discs were weighed after 1 and 2 weeks of storage. During other studies, plates were periodically weighed to monitor weight change. The design of the plates allowed handling of many discs and measurement of several disc parameters with minimal disturbance.

Color Measurement

The color of individual discs was measured through the clear plastic bottom of the tissue culture plate with a hand-held reflectance colorimeter (Minolta CR-200) and data were downloaded to a personal computer. Discs could be turned to measure the color of either the intact skin (exocarp) or cut flesh (endocarp) surface. The skin color of intact fruit was measured directly. Color was recorded using the L*a*b* uniform color space (CIELAB), where L* indicates lightness, a* indicates hue on a green (−) to red (+) axis, and b* indicates hue on a blue (−) to yellow (+) axis (20). The plastic plate altered color measurements in a linear manner; direct color measurements (d) were converted to through-plate measurements (p), for comparisons, by the following regression equations:

$$L(p) = 17.8 + 0.66\ L(d),\ r^2 = 0.98$$

$$a(p) = 0.31 + 0.69\ a(d),\ r^2 = 0.99$$

and

$$b(p) = -1.16 + 0.65\ b(d),\ r^2 = 0.84.$$

For comparison of skin color with reflectance spectra, pericarp discs 17 mm in diameter were prepared from intact fruit at progressive ripening stages, and through-plate a* was determined. The reflectance spectra of discs were measured directly with a hand-held reflectance spectrophotometer (Metron Colormet).

Ethylene and CO_2 Measurements

Rates of ethylene and CO_2 biosynthesis of individual discs were determined daily or as needed. In a sterile hood, the normal plate lid was replaced by a three-tiered assembly consisting of a sheet of fresh Parafilm, a soft neoprene-lined, rubber gasket, and a reinforced, perforated plastic lid. Hand clamps compressed the assembly against the raised rim of each well to produce a 3.5-mL, gas-tight chamber around each disc. After 15 to 20 min, a 1-mL sample of accumulated gases was drawn from each well, via a needle inserted through the gasket and Parafilm, and analyzed for ethylene and CO_2 as above. Ethylene measurements were made before those for CO_2 or color to minimize the impact of handling on ethylene measurement. Fruit was matched for initial color and ethylene biosynthetic rate before disc preparation for comparisons of color change, ethylene biosynthesis, and respiration between discs and intact fruit.

Determination of Tissue Firmness

Large pericarp discs, 16 mm in diameter and 6 mm in thickness, were selected at progressive skin color stages and

[3] Abbreviations: MG3, mature-green 3; AIS, alcohol-insoluble substances; PG, polygalacturonase; CM, carboxymethyl.

recut to 4 mm in thickness before measurement of flesh color and determination of firmness. Tissue firmness was measured using an Instron Universal Testing Instrument (Model 1122) fitted with a 2-kg load cell. A 2.3-mm-diameter, flat-tipped probe was driven 1.1 mm (27.5% of disc thickness) into the cut surface of the disc with a cross-head speed of 100 mm/min, and the peak force was recorded in newtons.

Analysis of Cell Wall Carbohydrates

Fresh or frozen pericarp discs at selected color stages were boiled in 95% ethanol (4 mL/g fresh weight) for 20 min, homogenized at top speed in a Polytron (Brinkman Instruments) for 60 s, then centrifuged at 1500*g* for 10 min, and the supernatant was decanted. The pellet was resuspended by homogenization in 80% ethanol in the Polytron, recentrifuged to a pellet, and the supernatant was again decanted; this washing cycle was repeated until the resulting supernatant was colorless. The crude cell wall pellet was dried under an air stream, then suspended in DMSO:water (9:1, v/v; 20 mL/g dry weight) and stirred at room temperature for 24 h to remove starch (30). The slurry was centrifuged at 1500*g* for 10 min and the DMSO was decanted, then the pellet was washed repeatedly in 95% ethanol to remove all traces of DMSO. The pellet was dried under air, then resuspended and washed once in acetone. The acetone-washed, starch-free material was air-dried to leave the AIS.

Samples of the AIS fraction were analyzed to determine the component carbohydrates of the starch-free cell wall. Noncellulosic neutral sugars were derivitized to alditol acetates by hydrolysis in 2 N TFA, reduction, and acetylation (2). The derivatives were identified by gas chromatography on a Perkin-Elmer chromatograph (Model 8290) fitted with a 30-m fused silica capillary column (DB-225; J&W Scientific). The chromatograph oven was held at 210°C and H_2 was used as carrier gas. Quantitation was based on integration of the peaks from the flame ionization detector with a Perkin-Elmer Sigma 10 chromatography data system. Cellulose was measured in the TFA-insoluble fraction by the anthrone colorimetric assay (8). Water-soluble uronides were extracted from the AIS by stirring a subsample in water for 3 h at room temperature, centrifuging at 1500*g* for 10 min, and washing the pellet with water. The combined water-soluble fraction and washings were lyophilized. Uronic acids were measured colorimetrically (1, 3).

Extraction and Analysis of 'Wall-Degrading' Enzymes

Nine frozen pericarp discs at selected color stages were added to 15 mL of cold (4°C) extraction medium containing 50 mM Na citrate buffer (pH 5.5), 1.7 M NaCl, 15 mM EDTA, and 5 mM 2-mercaptoethanol, and were homogenized with a Polytron at top speed for 30 s. The homogenate was centrifuged at 10,000*g* for 15 min at 4°C, and the supernatant was decanted. The pellet was again homogenized and centrifuged, the supernatant was decanted, then the combined supernatants were filtered through glass fiber paper (Whatman GF/C). The remaining pellet was resuspended in cold 50 mM Na citrate containing 3 M LiCl and stirred at 4°C for 1 h, and then the mixture was filtered through glass fiber paper. These NaCl and LiCl extracts were dialyzed for 3 d, with two buffer changes/d, against cold 10 mM Na citrate buffer (pH 5.5) and then concentrated by lyophilization to one-fifth of their original volumes. Polygalacturonase and carboxymethyl cellulase activities were assayed by measuring the generation of reducing groups following incubation of extracts with polygalacturonic acid or carboxymethyl cellulose (1 mg/mL reaction mixture; Sigma Chemical Co.) in 80 mM sodium acetate buffer (pH 5.0) at 37°C (24). Solutions of galacturonic acid or glucose were used as standards. Glycosidase activities were determined under the same conditions, with *p*-nitrophenyl-α-D-arabinofuranoside, and α-D- and β-D-galactopyranoside (200 μg/mL reaction mixture; Sigma) as substrates. Reactions were stopped by addition of 1 volume of 1 M NH_4OH containing 2 mM EDTA, A_{400} was determined, and activity was estimated relative to a *p*-nitrophenol standard.

RESULTS AND DISCUSSION

Weight Change

Maintenance of initial water status is critical for use of tissue discs as a model ripening system. Excess water uptake by tissues has been shown to delay normal ripening processes in tomato discs and to promote cell proliferation along the vascular bundles and at the disc surface (32), effects similar to those seen in intact fruit after water infiltration. Excessive water loss resulted in tissue browning along the vascular bundles and at the disc surface.

Tomato pericarp discs, prepared and stored as described, remained sterile and healthy for several weeks after excision. Extracellular water remaining after disc preparation was absorbed within several hours, leaving an outer layer of dry, broken cells. Discs developed a slightly frosted appearance over time. Subsequent cell proliferation was usually slight, but most apparent at the cut ends of vascular bundles and at the disc edges. Loss of disc fresh weight averaged less than 1%/week in the 4 × 6 well plates, with local loss rates of less than 2% for discs in corner wells, less than 1% for discs in edge wells, and no loss or slight weight gain for discs in center wells. Loss of weight from the free-standing water in the spaces between the wells was about four times the loss from the discs. Intact fruit decreased about 1% in fresh weight/week.

Color Change

Pericarp discs changed color in a pattern similar to that for intact fruit when compared temporally and spatially. Color change in ripening tomato fruit, from green through orange to red, has been shown to reflect changes in the two dominant pigments: rapid degradation of Chl and a progressive accumulation of lycopene in the chloroplast (10). Reflectance spectra of the skin surface of intact fruit at progressive ripening stages revealed an initial decline in Chl absorbance at a 665-nm peak and a subsequent rise in lycopene absorbance at a 565-nm peak (Fig. 1). This change in reflectance, when recorded on the L*a*b* axes, can be represented as a path through the a*b* color chart or as change along the a* axis (18).

When fruit was paired by initial skin color, both intact fruit and excised discs passed through the same skin color stages

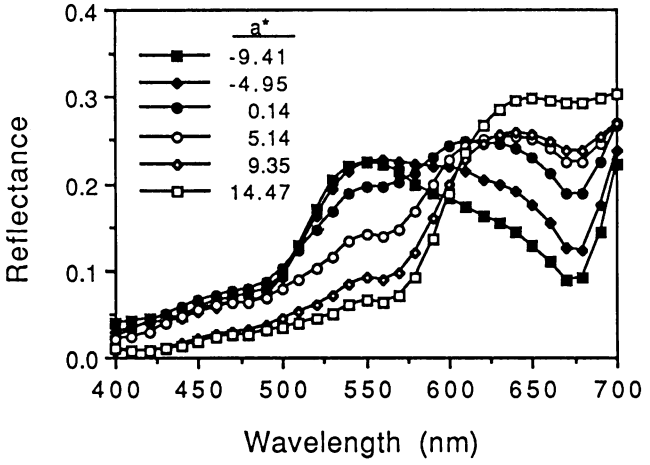

Figure 1. Reflectance spectra of tomato fruit at progressive skin color stages (a*). The a* shown are through-plate values.

as they ripened, as indicated by the path of movement through the a*b* color chart (Fig. 2). Thus, the processes of Chl degradation and lycopene synthesis appear to be similarly linked in pericarp discs and intact fruit. The temporal pattern of change in skin color, as indicated by movement along the a* axis, was similar in intact fruit and excised discs for 5 to 7 d following excision (Fig. 3). Once color change began, the rate of change rapidly increased and then declined in a consistent pattern, as both discs and fruit changed from an a* of −5 to +15 in about 6 d (Fig. 3). The rate of exocarp color change in discs was the same whether discs were 1.5, 3.0, or 5.0 mm in thickness, or excised from areas above locules or septa (data not shown). Thus, the regulatory mechanisms that determine the rates of pigment degradation and synthesis during ripening are apparently local and are maintained in pericarp discs. By 5 to 7 d after the onset of color change, however, the rate of reddening in discs sometimes lagged behind that of intact fruit (Fig. 2b).

During ripening of intact fruit, the exocarp is the first external tissue to change in color, endocarp color change begins later and continues after exocarp change, whereas vascular parenchyma is typically the last fruit tissue to redden (31). This tissue sequence of color change was maintained in pericarp discs (data not shown). The physiological processes responsible for this sequence apparently persist after disc excision; however, change in endocarp color appeared to decrease earlier in discs than in intact fruit.

In intact fruit, external color change typically begins at the distal (stylar or bottom) end, then progresses rapidly toward the proximal (stem or top) end (Fig. 3; refs. 14 and 31). With pericarp discs, change in skin color occurred first in discs cut from the distal (bottom) end of a tomato before beginning in discs from middle and top regions, even when discs were isolated several days before the onset of color change (Fig. 3). The persistence of this spatial pattern in excised discs suggests that the temporal sequence of events that leads to ripening is established locally in pericarp tissues at least several days before ripening becomes apparent, and is not disrupted by disc excision.

Wound-Induced Ethylene and CO_2

Ethylene biosynthesis in pericarp discs underwent two transient increases: one immediately after disc excision, and the second during the process of color change. Ethylene biosynthesis in pericarp discs increased sharply in response to disc excision, rising as much as 100-fold to a maximum approximately 2 h after the first cut, from below 1.0 nL/g fresh weight/h to over 20 nL/g fresh weight/h in a typical fruit and disc (Fig. 4; ref. 22). Ethylene biosynthesis in the discs then decreased slowly over the next 24 h to a steady rate below 5 nL/g fresh weight/h. This steady preclimacteric rate of ethylene biosynthesis in pericarp discs was approximately 10 times the rate measured in intact fruit.

Climacteric Ethylene and CO_2

During the process of skin color change, both tomato fruit and pericarp discs exhibited a climacteric increase in ethylene biosynthesis and CO_2 production. In pericarp discs, ethylene

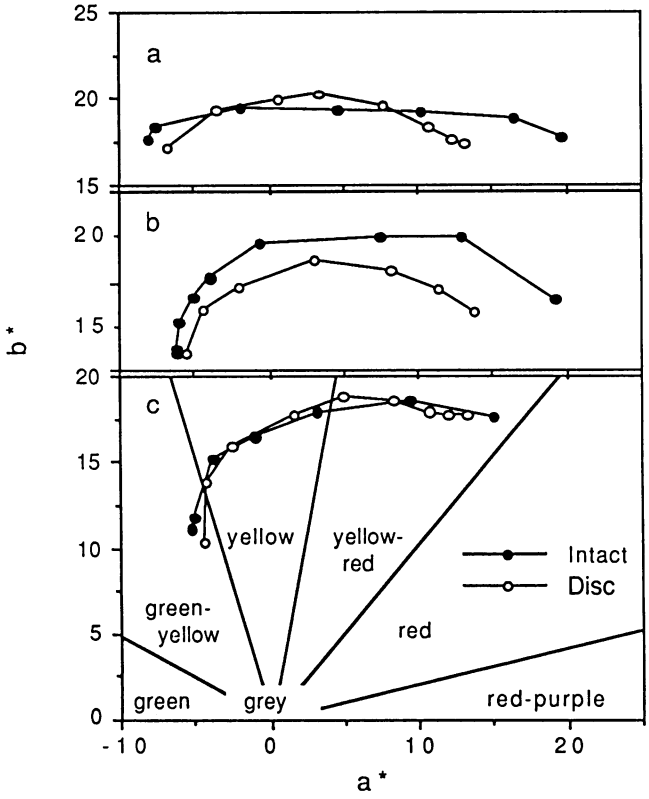

Figure 2. Comparison of skin color change in paired intact tomato fruit and excised pericarp discs matched for color at time of disc excision. Dark (a), medium (b), and light (c) mature-green tomato fruit were paired by color and rate of ethylene biosynthesis, and pericarp discs were prepared from one fruit of each pair. Disc color is an average of eight discs measured through the plastic bottom of their storage plate. Intact fruit color is an average of four equatorial readings measured directly, then mathematically converted to through-plate values. Color change is recorded as a path through the a*b* color chart in the L*a*b* uniform color space, where a* is the green-red axis and b* is the blue-yellow axis (20). Names for the sectors of the color chart are borrowed from the Munsel color system (20).

biosynthesis and CO_2 production increased with the onset of color change, reached a peak coincidental with the peak rate of color change, and then declined as color change ceased (Fig. 5). Typically, the rate of ethylene biosynthesis increased fivefold and CO_2 production increased twofold from preclimacteric levels.

The processes of ethylene biosynthesis, respiration, and color change were always tightly linked in pericarp discs, whether excised from bottom, middle, or top regions of a fruit (data not shown). This linkage was less apparent in intact fruit. In intact fruit, ethylene biosynthesis and respiration usually increased one to several days before the onset of external color change and continued to rise after external color change slowed (Fig. 5; refs. 14, 31, and 35). The rate of ethylene biosynthesis at first color change varied up to 10-fold (from 0.3 to 3.0 nL/g fresh weight/h), and the peak rate varied more than fourfold (from 1.4 to 7.0 nL/g fresh weight/h), among different intact fruit. Ethylene biosynthesis and CO_2 production typically increased five- to 20-fold and one-to threefold, respectively, from MG3 to peak rates in intact fruit.

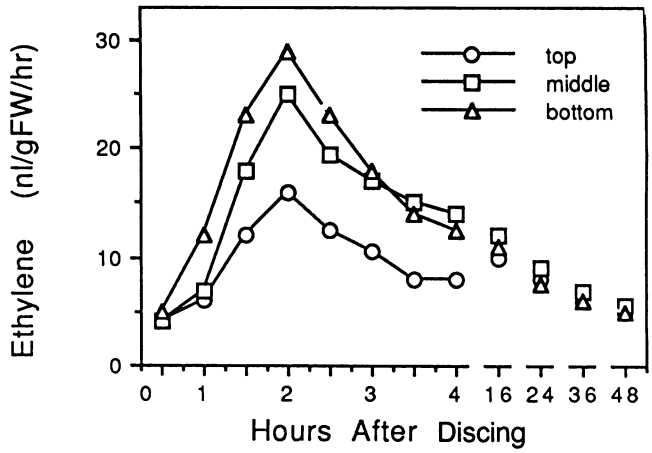

Figure 4. Wound-induced ethylene biosynthesis following excision of tomato pericarp discs. Pericarp discs were prepared from bands at 2 cm from the stem scar (top), at the equator (middle), or at 2 cm from the stylar scar (bottom) of one of the matched fruit. Values are an average for eight discs.

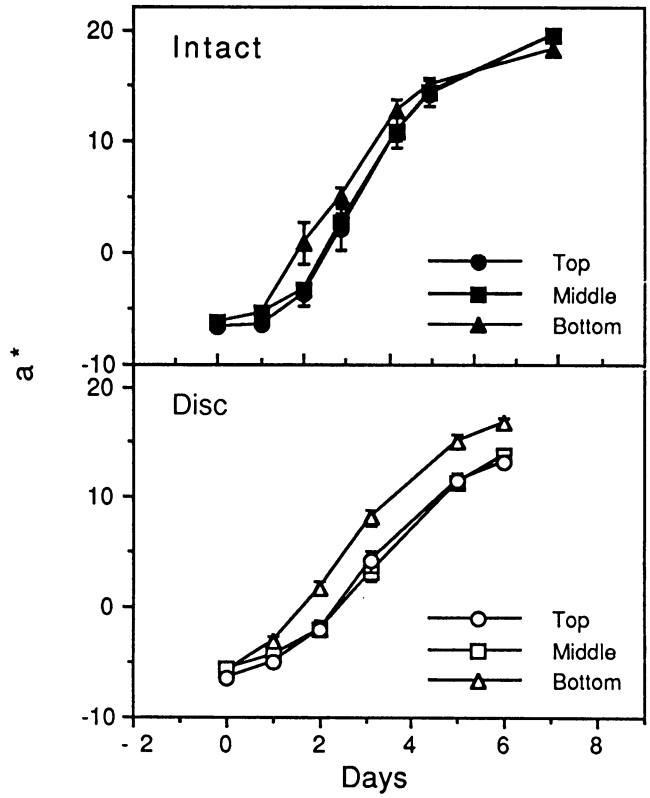

Figure 3. Change in skin color at the top, middle, and bottom of intact tomato fruit and in tomato discs isolated from the same regions. Mature-green tomato fruit were paired by initial color and rate of ethylene biosynthesis. Pericarp discs were prepared from bands at 2 cm from the stem scar (top), at the equator (middle), or at 2 cm from the stylar scar (bottom) of one of the matched fruit. Disc color is an average of 12 (top or bottom) or 24 (middle) discs. Intact fruit color is an average of four readings measured directly in corresponding bands on the intact fruit, then mathematically converted to through-plate values; a* is the green-red axis in the L*a*b* uniform color space. SE of the means are indicated.

The differences between discs and intact fruit in the linkage between ethylene evolution and color change suggest that in intact fruit local color change is associated with a local transient increase in the rate of ethylene biosynthesis and, further, that the pattern of ethylene production in whole fruit arises from the summation of transitory increases in local biosynthesis rates. Thus, in whole fruit, transient increases in ethylene biosynthesis may begin at the site of internal color change in the locular gel or columella, progress through the exocarp as external color change occurs, and then end in the extensive endocarp tissues, which are among the last to redden (5, 31). The severalfold difference in ethylene biosynthesis rates between intact fruit and pericarp discs (Fig. 5) might indicate that only a small and variable portion of intact fruit tissues is evolving ethylene at its peak rate at any given time.

Tissue Softening

A decrease in fruit firmness (tissue softening) is a consistent characteristic of ripening in tomatoes, and several studies of the ripening of intact fruits have reported significant correlations between decreased firmness and progressive change in fruit color (4, 14, 21). Tomato pericarp discs decreased in firmness during color change in a pattern comparable with that seen in intact fruit. The firmness of tomato pericarp discs was highly correlated to both skin and flesh color with r^2 values of 0.70 (data not shown) and 0.83, respectively (Fig. 6). The correlation of skin color with firmness may be lower than for flesh color due to the lag in endocarp color change and the presence of green strands of vascular tissue in the endocarp, which increase resistance to the probe but are detected only in measurements of the endocarp surface. The spread in firmness readings in discs at low a* values suggests that some softening occurs before skin color change begins,

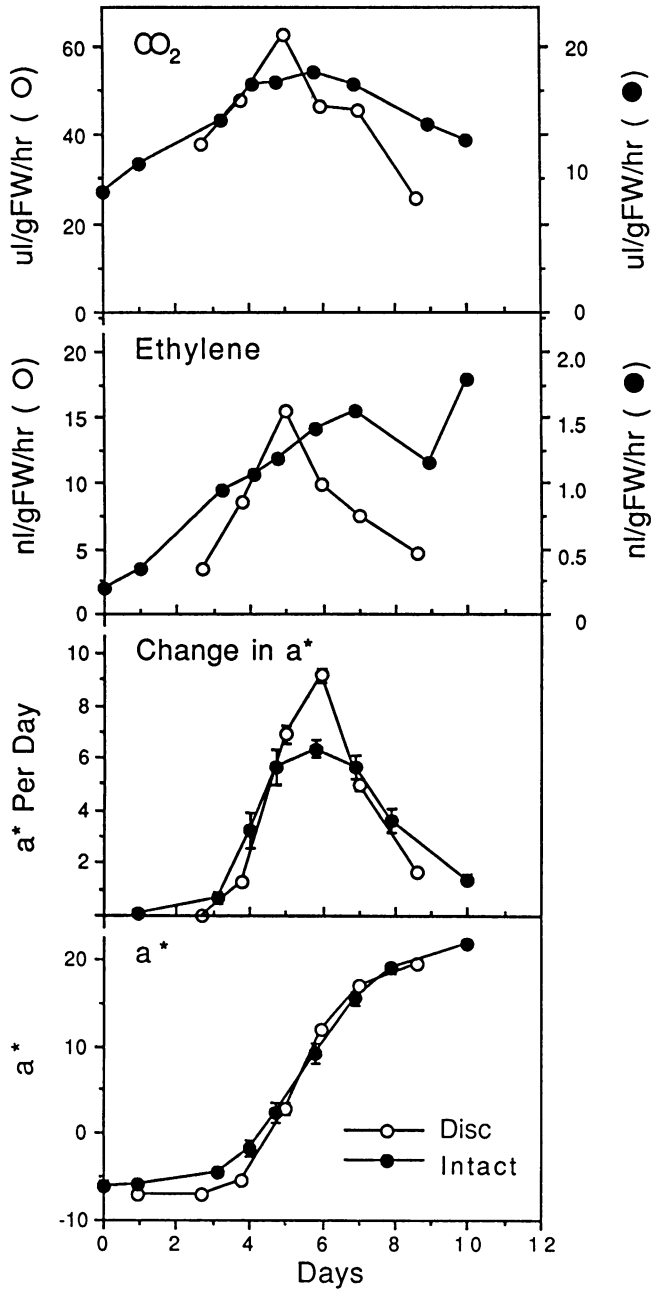

Figure 5. Comparison of skin color, change in skin color, ethylene biosynthesis, and CO_2 production during ripening of intact tomato fruit and excised pericarp discs. Mature-green tomato fruit were paired by initial color and rate of ethylene biosynthesis, and pericarp discs were prepared from the equatorial band of one fruit. All pericarp measurements are an average for 12 discs. Intact fruit gas measurements are from a single sample; intact fruit color measurements are an average of four equatorial readings mathematically converted to through-plate values. Change in a* is calculated as a* units/d since the preceding measurement. SE of the means are indicated.

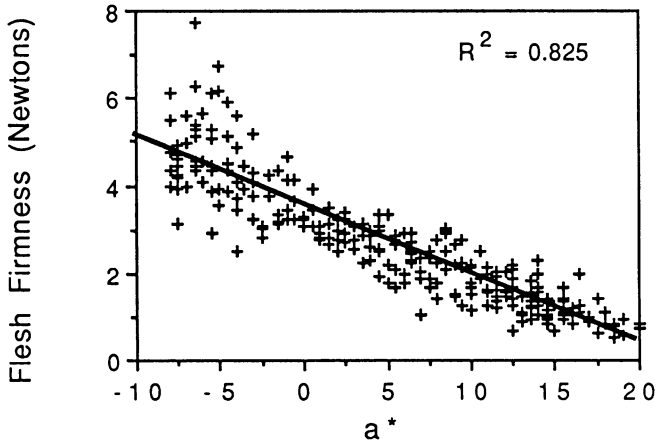

Figure 6. Correlation between flesh firmness and flesh color in tomato pericarp discs at progressive flesh color stages.

but firmness becomes linearly related to color at a* values greater than 0.

Cell Wall Composition

The softening of tissues during ripening of whole fruit is believed to result, in part, from progressive changes in cell wall composition (15, 16, 33). Several studies have described changes in the composition of pericarp cell walls during tomato fruit ripening (4, 15, 16, 33). Cell wall uronic acids, an important constituent of pectins, have been reported to decrease during ripening, and several neutral sugars have also been shown to decline. Cellulose, on the other hand, has not been reported to be lost from the wall as softening progresses.

Analysis of cell walls from pericarp discs at progressive color stages showed changes in cell wall carbohydrate constituents similar to those reported for intact fruit (Table I; refs. 15 and 16). With progression in disc color, the quantity of water-soluble pectin eluted from the starch-free AIS steadily increased. Since this increase in water-soluble pectin was matched by a decrease in more tightly bound, water-insoluble pectin, the total content of uronic acids in the pericarp cell walls did not change over time. The cell wall content of the neutral sugars arabinose, rhamnose, and galactose was found to steadily decrease as discs became red. With the exception of the decrease in rhamnose, the patterns of change in cell wall composition reported for ripening pericarp tissues in intact fruit (16, 33) are duplicated in excised pericarp discs.

Enzyme Activity

Ripening of intact tomato fruit is associated with changes in the presence and activity of a number of enzymes, which are presumed to regulate the alterations in cell wall composition (7, 17, 28, 34). PG has been shown to appear in pericarp tissue just as external color change begins in intact fruit, and to increase in activity throughout later ripening stages (4, 7). CM cellulase activity is present in whole tomato fruit at all ripening stages, but increases distinctly as ripening beings (4, 17, 34). While recent work has challenged the role of PG and

Table I. *Carbohydrate Composition of the Starch-Free Cell Walls of Tomato Pericarp Discs at Selected Color Stages*

Component	Color Stage (a*)					
	−5	0	5	10	15	20
	% AIS					
Uronic acids						
H_2O-soluble	4.5	5.9	7.2	7.1	9.4	10.7
H_2O-insoluble	23.3	23.0	21.3	20.4	19.7	18.2
Total	27.8	28.9	28.5	27.5	29.1	28.9
Cellulose	36.1	35.4	31.3	34.5	33.2	35.8
Neutral sugars						
Rhamnose	1.5	1.4	1.2	0.7	0.7	0.5
Fucose	0.5	0.3	0.4	0.4	0.5	0.5
Arabinose	2.2	2.0	1.9	1.7	1.6	1.6
Xylose	4.6	4.2	3.8	4.1	4.5	4.7
Mannose	1.5	1.8	1.6	1.7	1.8	2.0
Galactose	4.4	4.2	3.8	3.2	2.3	2.1
Glucose	1.3	1.4	1.6	1.4	1.4	1.6
Total	16.0	15.3	14.3	13.2	12.8	13.0

polyuronide degradation in the process of fruit softening (12), the appearance of PG activity and increases in both PG and CM cellulase have often been used as biochemical markers of the onset of ripening. We followed change in the activities of these and several other cell wall-degrading enzymes in extracts of homogenized discs.

Attempts to distinguish between apoplastic and symplastic enzyme activity by sequential extraction of homogenized preparations with low and high ionic strength buffers have been made by others. However, this strategy is confounded by the adsorption of intracellular enzymes on acidic cell wall polymers during extraction, and so was not adopted in this study. Enzyme activity in the 1.7 M NaCl extracts of fruit discs are assumed to represent the total activity in the tissue. Subsequent extraction of 1.7 M NaCl insoluble material with 3 M LiCl added less than 2 to 3% to this total.

PG activity was not detectable in extracts of green discs (a* value −5), but was present in extracts of turning discs and increased another eightfold as disc color went to full red (a* value +20; Table II). CM-cellulase activity was low in extracts from green discs and increased about sixfold during disc ripening (Table II). These observations are in full agreement with other reports of PG (4, 7, 14, 19, 34) and cellulase (4, 17, 34) activities in several cultivars of tomatoes.

Descriptions of changes in extractable glycosidase activities in ripening tomato fruits have not established a general pattern of change for these enzymes (*e.g.* refs. 28 and 34). This general absence of pattern was also seen in our measurements of α- and β-D-galactopyranosidase and α-L-arabinofuranosidase activities (Table II). We found more α- than β-galactosidase in our extracts, in contrast to Wallner and Walker (34), but this difference may be explained by the use of different cultivars of tomato in the two studies. Pressey (28) has suggested that one of the three β-galactosidase isozymes he detected in tomato fruit extracts is responsible for the substantial loss of wall galactose that occurs in ripening tomatoes (16; Table I). We have not attempted further analysis of the β-galactosidase(s) of tomato pericarp discs. It is not clear what, if any, substrates for α-galactosidase and α-arabinosidase are present in tomato walls.

SUMMARY

Excised tissues offer a number of potential advantages for the experimental analysis of ripening. These advantages include separation of the various ripening processes by isolation of specific tissues, quantitative addition of metabolic intermediates or inhibitors to these tissues, and accurate measurement of local processes through nondestructive monitoring and destructive analysis of replicated samples.

An established procedure for pericarp disc preparation was combined with a novel handling system to order and protect the discs. Tomato pericarp discs, prepared and stored as described, remained sterile and healthy for several weeks. The handling system permits easy monitoring of color change, ethylene biosynthesis, and respiration in individual discs, and selection of discs at particular stages.

We have compared several physiological processes characteristic of ripening in the individual tissues of intact tomato fruit with equivalent processes in excised pericarp discs. During normal ripening of pericarp in intact tomato fruit, tissue color changes from green through orange to red, ethylene biosynthesis and respiration undergo a climacteric rise, tissues decrease in firmness, specific hydrolytic enzymes increase in extractable activity, and various fractions of the cell wall change in composition. These processes characteristic of ripening in intact fruit were duplicated in excised pericarp discs, with few exceptions, within the context of our preparation and handling procedures. It appears that isolation of disc tissues from the context of the whole fruit does not disrupt the regulatory mechanisms that regulate and coordinate these diverse physiological processes. Tomato pericarp discs appear be a useful model of intact fruit tissues for the experimental analysis of tomato ripening.

Table II. *Activities of Several Potential Cell Wall Degrading Enzymes in 1.7 M NaCl Extracts of Tomato Pericarp Discs at Selected Color Stages*

Enzyme Activity	Color Stage (a*)					
	−5	0	5	10	15	20
	nmol or μmol·g fresh wt^{-1}·h^{-1}					
Polygalacturonase[a]	ND[d]	ND	0.6	1.14	1.92	5.22
CM-cellulase[b]		0.22		0.52	0.52	1.33
α-D-Galactopyranosidase[c]	8.9		7.7			7.6
β-D-Galactopyranosidase[c]	2.9		2.0			3.8
α-L-Arabinofuranosidase[c]	0.3		0.2			0.2

[a-c] Activity expressed as [a] nmol galacturonsyl reducing groups produced, [b] nmol glucosyl reducing groups produced, or [c] μmol *p*-nitrophenol produced. [d] Not detected.

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