Endogenous Levels of Phenolics in Tomato Fruit during Growth and Maturation

J. G. Buta* and D. W. Spaulding

Horticultural Crops Quality Laboratory, Agricultural Research Service, USDA, Beltsville, Maryland 20705, USA

Abstract. Changes in the metabolism of several types of phenolics in the pulp and pericarp of tomato (*Lycopersicon esculentum*) fruit var. Ailsa Craig and Pik-Red were related to the stage of development. The highest levels of chlorogenic acid were found in the pulp and pericarp at the earliest stage of fruit development, and quantities declined rapidly during fruit ripening. Levels of rutin, found only in the pericarp, followed a similar pattern of change. The *p*-coumaric acid conjugate of rutin was found in low levels through fruit growth and ripening. High levels of *p*-coumaric acid glucoside were detected in the pulp only as the fruit matured with no rapid decline in levels during ripening. The decline of chlorogenic acid and rutin levels during fruit ripening paralleled the decline in indole-3-acetic acid levels measured previously in the pericarp tissues of these two varieties of tomato fruit during maturation. These phenolics are among those that have been suggested as regulants of auxin metabolism.

Physiologic studies have suggested that several phenolic compounds regulate auxin, indole-3-acetic acid (IAA), metabolism, and others affect polar transport of auxin. The dihydroxyphenolic, chlorogenic acid, was shown to be a protector of auxin against oxidation in sunflower leaves (Stonier et al. 1979). Dihydroxyphenolic compounds have been considered inhibitors of IAA oxidation, whereas monohydroxyphenolic derivatives stimulated IAA oxidation (Volpert et al. 1995). Certain flavonols, such as quercetin, were found to inhibit polar transport of IAA (Jacobs and Rubery 1988). Changes in the levels of IAA as well as abscisic acid had been measured during growth and ripening in the pericarp tissue of two cultivars of tomato fruit, Ailsa Craig and Pik-Red (Buta and Spaulding 1994). The metabolic changes in various phenolics in tomato fruit have not been correlated with changes in IAA levels occurring during the same stages of growth and ripening. Usually only the phenolic contents of ripe fruits have been analyzed (Schuster and Herrmann 1985). Compartmentation of phenolics within the fruit has not been investigated intensively. Changes in the metabolism of several of these phenolics in the pulp and pericarp tissue of tomato fruit during growth and maturation were investigated more than 10 years ago (Fleuriet and Macheix 1985). Using the chromatographic methods then available, the metabolic changes of hydroxycinnamic derivatives were studied in one cultivar of cherry tomato. In this study the changes in levels of several phenolics, including the hydroxycinnamic acid conjugates, in the pulp and pericarp tissues of the above mentioned two varieties of tomato fruit have been investigated during growth and ripening.

Materials and Methods

Plants

Tomato fruit (*L. esculentum* Mill var. Ailsa Craig and Pik-Red) were grown in cold frames and selected by color. Five developmental stages were studied: growth stages of immature green (IG) and mature green V (MG) followed by ripening stages of breaker (B), pink (P), and red (R). Replicate samples (3 g) of pericarp and pulp (including seeds) tissue were taken from individual fruit, frozen in liquid nitrogen, and stored at −80°C.

High Performance Liquid Chromatography

Frozen tissue samples were homogenized in 80% MeOH after the addition of sesamol (3,4-methylenedioxyphenol) as an internal standard and filtered before analysis. After rotary evaporation the sample residue was redissolved in MeOH, and HPLC was done using a Waters 600 instrument with a Waters 990 diode array detection system. Separation was accomplished with a PLRP-S 5-μm column (150 × 4.6 mm inner diameter, Polymer Laboratories, Amherst, MA) using a linear gradient elution with solvent at t_0 of CH_3CH:aq 1% HOAc (1:9) to 100% CH_3CN at 30 min with a flow rate of 0.8 mL/min. Elution of the phenolics with the gradient system was: *trans-p*-coumaric acid glucoside, 5.4 min; *cis-p*-coumaric acid glucoside, 7.3; chlorogenic acid, 9.3; *p*-coumaric acid conjugate of rutin, 11.6; and rutin (quercetin-3-rutinoside), 12.3. Rutin and chlorogenic acid were identified by UV and HPLC comparison with standards. Triplicate determinations were made.

Identification of *p*-Coumaric Acid Glucoside and *p*-Coumaric Acid Rutin Conjugate

The compounds were isolated by collection from HPLC using the same chromatographic conditions as above. Characterization of *trans-p*-coumaric acid glucoside was done by UV, CIMS, EIMS and NMR. The m/z 326 was observed by use of NH_3-CIMS, and the glucoside linkage was confirmed by ^{1}H NMR (Schuster et al. 1986). The *cis*-isomer of *p*-coumaric acid glucoside was converted to the *trans* form by warming with dilute HCl. The identification of the *p*-coumaric acid rutin conjugate was achieved by partial acid hydrolysis, which yielded rutin, *p*-coumaric acid glucoside, and *p*-coumaric acid (Harborne 1965).

Results and Discussion

Differences in levels of the predominant compounds of three types of phenolics were found, and these differences were associated with successive stages of fruit growth and maturation through ripening for both tomato varieties. The three compounds were: chlorogenic acid (5′-caffeoylquinic acid; CaQ), a dihydroxycinnamic acid ester conjugate; *p*-coumaric acid glucoside (CouGlu), a monohydroxycinnamic acid derivative; and rutin, a flavonol glycoside.

CaQ was the major dihydroxycinnamic acid conjugate found in both the pulp and pericarp tissues of the two tomato varieties through all stages of fruit growth and maturation. Higher levels of CaQ (85 μg/g, fw) were found in the pulp of the IG fruit than in the pericarp tissue (Fig. 1). Decreased levels of CaQ were found in the pulp of both varieties of the MG stage. However, with the onset of ripening in the B stage of development, increased levels of CaQ were measured in the pulp. Lower CaQ levels were found in the pericarp of both varieties compared with the pulp through the B stage of maturation. As ripening progressed through the P to the R stage, CaQ levels declined to a small fraction of the levels found in the IG fruit with no varietal difference. The decline in CaQ levels with maturation resembled the pattern of change of CaQ found in cherry tomato (Fleu-
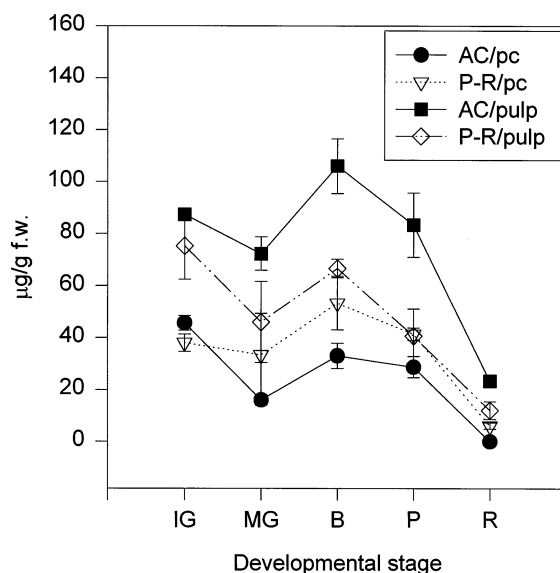

Fig. 1. Changes in CaQ in pericarp and pulp tissue of tomato fruit cultivars Ailsa Craig (AC) and Pik-Red (P-R) during different stages of development. The standard error shown is greater than the size of the marked point ($n = 3$).

riet and Macheix 1981) and apple fruit (Mayr et al. 1995). CaQ levels in the fruit of other tomato varieties could be investigated to determine the significance of the large differences in CaQ at the B stage of maturity of the Ailsa Craig and Pik-Red varieties.

The pattern of change in CaQ levels of the fruit of these varieties during growth and maturation paralleled the changes in IAA levels measured in these varieties during growth and ripening (Buta and Spaulding 1994). The CaQ levels were 1,000-fold greater than the IAA levels in the tomato pericarp, which ranged from 20 ng/g fw in IG fruit to a minimum of 2 ng/g fw in MG stage V to a small increase to 5 ng/g fw as ripening progressed. These changes in CaQ levels (Buta and Spaulding 1994) should be sufficient to function in inhibiting the degradation of IAA. Similar IAA levels were found in the placenta and mesocarp tissues of the variety Vilmorin 686, and a decline of the hormone levels in these tissues during ripening was observed also (Hocher et al. 1992).

Trace levels of another dihydroxycinnamic acid conjugate, caffeic acid glucoside, were found in the pulp tissues of both varieties at the P and R stages of ripeness. These quantities were lower than those found in fruit of other tomato varieties analyzed at IG and R stages of maturation (Winter and Herrmann 1986).

CouGlu was not detected in IG Pik-Red tomato fruit pericarp and only in trace quantities in the IG pulp (Fig. 2). However, levels comparable to CaQ levels (40 μg/g fw) were found in the pulp of MG V fruit (Fig. 2). A large increase in CouGlu levels to 160 μg/g fw was found in the B stage Pik-Red pulp followed by a decline
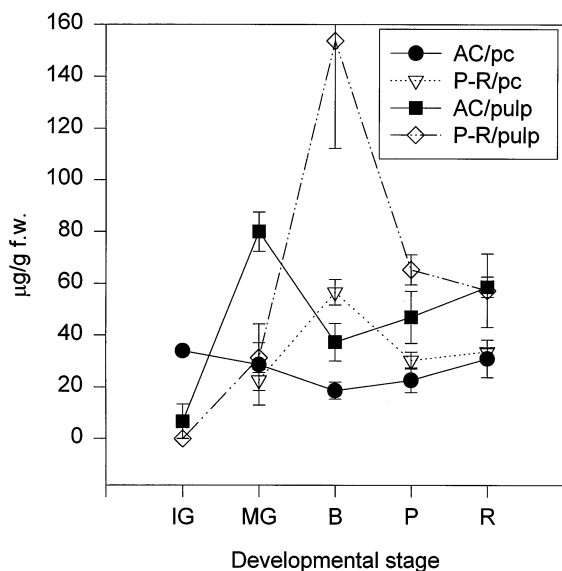
Fig. 2. Changes in CouGlu levels in pericarp and pulp tissue of tomato fruit cultivars Ailsa Craig (AC) and Pik-Red (P-R) during different stages of development. The standard error shown is greater than the size of the marked point ($n = 3$).
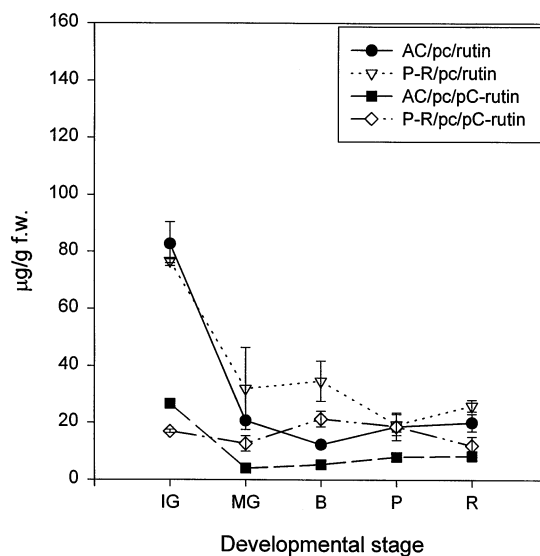

Fig. 3. Changes in rutin and pC-rutin levels in pericarp tissue of tomato fruit cultivars Ailsa Craig (AC) and Pik-Red (P-R) during different stages of development. The standard error shown is greater than the size of the marked point ($n = 3$).

to 60 μg/g fw as ripening proceeded. In contrast, no large fluctuation in CouGlu levels occurred in the Pik-Red pericarp tissues during growth and ripening, although approximately equal quantities of the *cis-* and *trans*-isomers of CouGlu were found in the pericarp. A somewhat different metabolism of CouGlu was found in the Ailsa Craig fruit. Low levels of the *cis*-glucoside were found in IG fruit pulp, and the major increase in *trans*-CouGlu levels to 80 μg/g fw occurred in the MG stage of the pulp followed by a 50% decline as ripening progressed (Fig. 2). In contrast in the Ailsa Craig pericarp the highest levels of only *cis*-CouGlu (40 μg/g fw) were found in the IG pericarp, and then lower levels of approximately equal amounts of *cis-* and *trans*-CouGlu were measured throughout ripening. Previous analyses of several varieties of tomato fruit at an unspecified green stage of maturity and the fully ripe red stage indicated the presence of high levels of the *p*-coumaric acid 4-*O*-β-D-glucoside in the red fruit and highly variable quantities of the CouGlu in green fruit (Winter and Herrmann 1986). This variability of CouGlu levels in green fruit could have been the result of the degree of maturity of the fruit when collected. Earlier analyses of cherry tomato fruit at MG and R stages had also found high levels of mono- and dihydroxycinnamic acid glucosides, and the accumulation of the glucose derivatives was suggested as a biochemical marker for maturation (Fleuriet and Macheix 1985). The data presented for the appearance of CouGlu in the maturing Ailsa Craig and Pik-Red fruit support the suggestion of a shift in hydroxycin-

namic acid conjugate metabolism with maturation and the onset of ripening.

Rutin, identified earlier in whole fruit (Fleuriet 1976), was detected in the pericarp of both varieties only during the successive stages of development (Fig. 3). The highest levels of rutin (80 μg/g fw) were measured in this tissue of the IG fruit at 14 days after anthesis, which was about the earliest sampling time that it was possible to distinguish pulp and pericarp tissues. Levels of rutin then decreased rapidly as the fruit matured, and the lower levels were maintained through ripening with not much difference in flavonol glycoside levels for the two varieties. Another quercetin derivative, identified as the *p*-coumaric acid conjugate of rutin (pC-rutin), was also found only in the pericarp tissue of both tomato varieties. The levels of pC-rutin found were 4–27 μg/g fw, and the fluctuations in levels did not appear to be associated with stages of fruit maturation (Fig. 3). The localization of the quercetin glycosides in the tomato pericarp is somewhat similar to the gradient of concentration of quercetin glycosides found in maturing apple fruit (Mayr et al. 1995). The highest concentrations of those quercetin compounds were found in the apple peel and lowest quantities in the core. In apple fruit, however, the relative quantities of the various glycosides changed with the stage of maturation. That pattern of change was found in the maturing tomato fruit only for the simpler glycoside, rutin.

Flavonoid aglycones have been suggested as natural regulators of polar auxin transport and auxin efflux from cells in plants (Jacobs and Rubery 1988). Flavonol glycosides were reported to be inactive in the assays of polar

auxin transport, and the presence of micromolar concentrations of the free flavonols necessary for inhibition of polar auxin transport was suggested to occur in only a few cell layers. In this study no quercetin was detected in any of the pulp or pericarp tissues analyzed, and only decreasing levels of quercetin glycosides could be found in tomato pericarp tissue throughout the successive stages of fruit growth and ripening. Therefore the data obtained do not support the above mentioned suggestions, at least with the analytical procedures used.

The changes in levels of two of the predominant phenolics (the decline in CaQ levels during ripening, which would result in less protection of endogenous IAA; and the decline in rutin, possibly associated with decreased control of IAA transport by the quercetin aglycone) parallel the decline in IAA levels during the later stages of growth and during ripening of the two varieties of tomato studied earlier. Recently it was shown that supplying exogenous IAA to tomato fruit in culture delayed the ripening of Ailsa Craig fruit (Cohen 1996). It is possible that the maintenance of endogenous IAA levels in tomato fruit by some phenolics (CaQ) could be a portion of the metabolic control of the ripening process in the fruit.

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