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# QUINYL ESTERS AND GLUCOSE DERIVATIVES OF HYDROXYCINNAMIC ACIDS DURING GROWTH AND RIPENING OF TOMATO FRUIT*

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**Key Word Index**—*Lycopersicum esculentum* var. *cerasiforme*; Solanaceae; cherry tomato; growth and maturation; quinic and glucose derivatives; hydroxycinnamic acids; hydroxycinnamate:CoA ligase.

**Abstract**—Quinyl esters of hydroxycinnamic acids usually occur in greater abundance than their corresponding glucose esters in tomato fruits. During fruit growth and ripening, the predominant derivatives of hydroxycinnamic acids were found to be chlorogenic acid (76%) and the glucosides (84%) respectively. The variations in the ratio of Benedict-reactive (chlorogenic acid) and non-reactive compounds (mainly caffeic acid glucoside) are discussed in relation to their possible role in the regulation of fruit growth and maturation.

## INTRODUCTION

Tomato fruits contain, in addition to the glucosides and glucose esters of hydroxycinnamic acids [1], a number of quinyl esters, mainly chlorogenic acid [2]. The quantitative significance of this compound [3] has been the subject of many studies, since 1959 [4], concerning its metabolism and its possible role in plant physiology [5].

Although available evidence indicates that caffeoyl-CoA is an intermediate in the biosynthesis of chlorogenic acid [6–8], two other pathways exist that may be involved in its formation: (a) direct hydroxylation of *p*-coumaroylquinic [9] and (b) involvement of hydroxycinnamoyl glucose esters [10], which have recently been reported to have a high turnover [11]. Both monohydroxy- and dihydroxycinnamic acids appear to influence plant physiology either at the level of auxin metabolism as it affects growth [12–14], rhizogenesis [15, 16] and dormancy [17], or in the biosynthesis of ethylene [18, 19] during fruit maturation [20].

We wish to report in this paper the changes that occur in the amounts of hydroxycinnamic acid derivatives during growth and maturation of 'cherry' tomato fruits, with particular reference to monohydroxy- and dihydroxyphenols.

## RESULTS

The quinyl esters of three hydroxycinnamic acids were identified as feruloylquinic (FQ), *p*-coumaroylquinic (pCQ) and 3-*O*-caffeoylquinic or chlorogenic (CQ) acids. The two latter compounds have already been identified in tomato fruits [2]. At no time in fruit growth were we able to detect sinapoylquinic acid.

### *Evolution of quinyl esters*

The changes in the amounts of quinyl esters during growth and maturation of tomato fruits are shown in Fig. 1. CQ occurred in abundance in young fruits, but it rapidly declined towards the end of growth and during maturation. In growing fruits, FQ and pCQ occurred in smaller amounts which increased during ripening, though still lower in amount than CQ (21 nmol for FQ and pCQ compared with 188 nmol for CQ in red fruits).

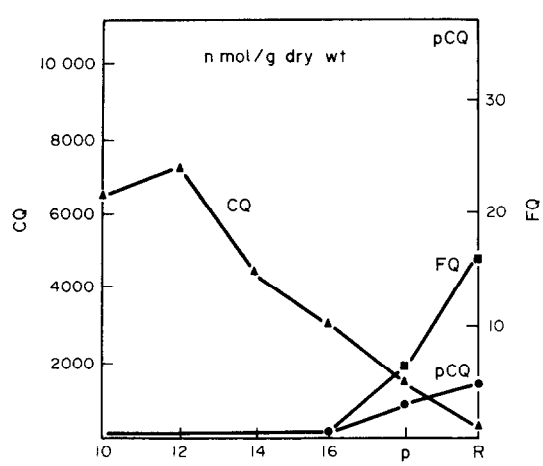


Fig. 1. Changes in quinyl esters of caffeic (CQ = chlorogenic acid), *p*-coumaric (pCQ) and ferulic (FQ) acids during the growth and ripening of tomato fruit. 10, 12, 14 and 16 = stages of growth (diameter in mm). p and R = stages of ripening (pink and red).

* Part 2 in the series "Metabolism of Hydroxycinnamic Acid Derivatives in the Tomato Cv Cherry". For Part 1 see Fleuriet, A. and Macheix, J.-J. (1980) *Phytochemistry* **19**, 1955.

*Changes in hydroxycinnamate:CoA ligase activity*

The changes in the activities of CoA ligases during growth and maturation of tomato fruits were studied using the four common hydroxycinnamic acids as substrates. Fig. 2 shows that during fruit growth caffeoyl-CoA ligase activity was approximately two-fold higher than that of either *p*-coumaric or ferulic acids. The maximal enzyme activity against the three substrates was found in green fruits (*ca* 12–14 mm diameter) and declined rapidly thereafter. There was no detectable activity of sinapoyl-CoA ligase at any time during fruit growth or maturation.

Although the addition of quinic acid to each of the hydroxycinnamoyl-CoA esters should give rise to the corresponding quinyl ester, no quantitative assay of hydroxycinnamoyl:CoA-quinate hydroxycinnamoyl transferase (HQT) activity was made. In any case, the activation of hydroxycinnamic acids is probably the rate-limiting step in the formation of their respective quinyl esters.

*Comparative evolution of hydroxycinnamic derivatives*

*Quinyl and glucose esters.* The changes in total accumulation of quinyl esters (pCQ, CQ and FQ) and of glucose esters (*p*-coumaroylglucose, $P_1$ and feruloyl glucose, $F_1$) were found to be similar; they reached their maximum in young fruits and declined to a minimum in red fruits (Fig. 3). Their concentrations, on the other hand, were quite different, the quinyl esters being 150 times higher than the glucose esters in the 12 mm diameter fruits. This considerable difference was mainly due to the high accumulation of CQ.

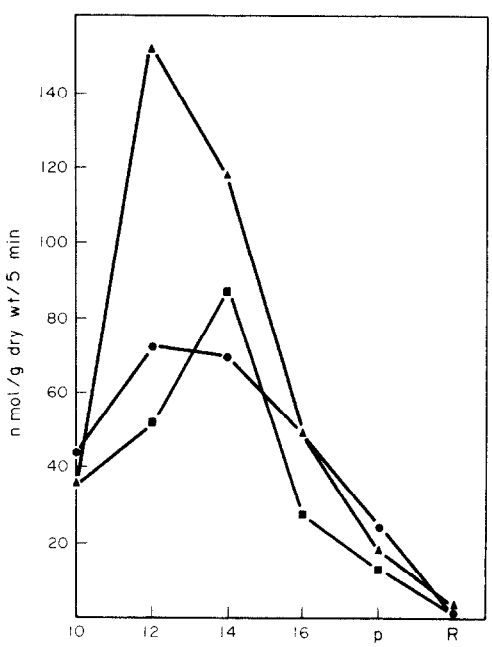


Fig. 2. Changes in hydroxycinnamate:CoA ligase activity during fruit growth and ripening (nmol of CoA thioesters formed per g of dry matter and per 5 min). ▲—▲, Caffeoyl-CoA; ●—●, *p*-coumaroyl-CoA; ■—■, feruloyl-CoA.

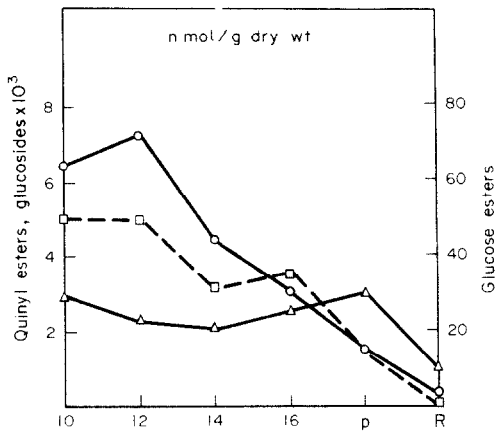


Fig. 3. Changes in quinyl or glucose esters and in glucosides during fruit growth and ripening. ○—○, Quinyl esters; □---□, glucose esters; △—△, glucosides.

*Quinyl esters and glucose derivatives.* In contrast with the pattern described above, the glucose derivatives (esters and glucosides) occurred in higher amounts than the quinyl esters during maturation of the fruit (Fig. 3). It is evident, therefore, that the ratio of $(pCQ + CQ + FQ)/(P_1 + P_2 + C_2 + F_1 + F_2)$ was $>1$ during fruit growth and declined to $<1$ in red fruits (Fig. 4). In the latter case, the glucosides of *p*-coumaric ($P_2$), caffeic ($C_2$) and ferulic ($F_2$) acids became 5 times more abundant than the quinyl esters, with a ratio of 1 in 16 mm diameter fruits.

*Mono- and o-diphenolic derivatives.* Among the derivatives of hydroxycinnamic acids of 'cherry' tomato, only chlorogenic acid has a free *o*-dihydroxy grouping. On the other hand, there are five derivatives that contain one free OH group: two quinyl derivatives (pCQ and FQ) and three glucose derivatives ($P_1$, $F_1$ and $C_2$), of which $C_2$

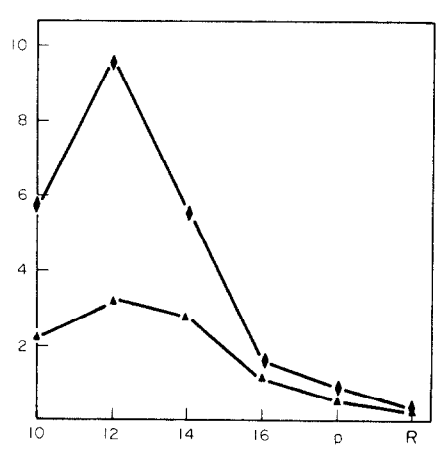


Fig. 4. Relative changes in the ratio of chlorogenic acid (CQ) to glucoside of caffeic acid ($C_2$) and quinyl esters (pCQ + CQ + FQ) to glucose derivatives ($P_1 + P_2 + C_2 + F_1 + F_2$) during fruit maturation. ♦—♦, $CQ/C_2$. ▲—▲, $(pCQ + CQ + FQ)/(P_1 + P_2 + C_2 + F_1 + F_2)$.

is the most abundant. The latter five are referred to as monophenols. Therefore, the ratio of *o*-diphenols (Benedict-reactive) to monophenols (Benedict non-reactive), which is equivalent to $CQ/C_2$, was found to increase in young fruits, reaching a maximum at 12 mm diameter. This ratio rapidly declined with fruit growth, reaching unity in pink fruits and $<1$ in red fruits (Fig. 4).

## DISCUSSION

*p*-Coumaric, caffeic and ferulic acids of 'cherry' tomato fruits occur in a bound form with quinic acid or glucose as esters and glucosides in a manner similar to other fruits [21–23]. Other phenolic constituents were partially identified as sinapic acid derivatives and as possibly the amines of hydroxycinnamic acids [24].

Considerable variations were observed in the concentration of the different hydroxycinnamic acid derivatives (Fig. 1) that were characteristic of the different stages of fruit growth and maturation (Table 1). Whereas other quinyl esters were absent in the young green fruits, CQ reached 76% of the total hydroxycinnamate content. Furthermore, with the onset of maturation, both FQ and pCQ began to appear whereas $C_2$ and $P_2$ amounted to 66% of total hydroxycinnamate. Both glucosides increased in concentration, reaching 84% in red fruits. The latter were further characterized by the almost complete disappearance of the glucose esters, $P_1$ and $F_1$, and the high concentration of the quinyl esters, pCQ and FQ. Therefore, CQ, $P_1$ and $F_1$ were substituted by the glucosides ($P_2$ and $C_2$) and the quinyl esters (pCQ and FQ) at the end of fruit growth and during maturation.

Although we have never detected any of the free hydroxycinnamic acids in healthy fruit, except under special experimental conditions [25], the accumulation of glucosides at the end of growth may be interpreted as a classic pathway of detoxification [26–28]. In the special case of caffeic acid, the accumulation of $C_2$ (379 nmol/fruit between green 12 mm and pink stages) may have resulted, in part, from the disappearance of CQ (239 nmol/fruit between the same stages) despite the existing quantitative difference.

The variation of PAL and hydroxycinnamate:CoA ligases may explain, in part, the evolution of hydroxycinnamic derivatives. Indeed, although the concentration of phenylalanine may be a limiting factor [29], the decline in PAL activity observed during fruit ripening [30] may account for the decrease in the amount of hydroxycinnamic acid derivatives, a situation often described in other fruits [31–33]. Whereas the formation of caffeoyl-CoA appears to be the limiting step in CQ accumulation (Figs. 1 and 2), no similar relation was observed with pCQ and FQ and associated CoA ligases during maturation; however, the ratio of the activities of caffeoyl-CoA ligase to *p*-coumaroyl- and feruloyl-CoA ligases was near 1 in young fruits while it

Table 1. Hydroxycinnamic acid derivatives of 'cherry' tomato at two stages of fruit growth and maturation

| Hydroxycinnamic derivatives | Growth (green fruits) 12 mm diameter | Ripening | |
|---|---|---|---|
| | | Pink fruits | Red fruits |
| Quinyl esters | | | |
| CQ | 7258 | 1532 | 188 |
| pCQ | t | 3 | 5 |
| FQ | t | 6 | 16 |
| Glucose esters | | | |
| $P_1$ | 39 | 15 | t |
| $F_1$ | 11 | t | t |
| Glucosides | | | |
| $P_2$ | 700 | 1260 | 538 |
| $C_2$ | 1120 | 1720 | 540 |
| $F_2$ | 420 | 18 | t |
| Total | 9548 | 4554 | 1287 |
| % chlorogenic acid | 76 | 33.6 | 15 |
| % Glucosides | 23 | 66 | 84 |
| % (pCG + FG) | 1 | 0.3 | 0 |
| % (pCQ + FQ) | 0 | 0.2 | 1 |

CQ: 3-*O*-caffeoylquinic (chorogenic acid); pCQ and $P_1$: *p*-coumaroylquinic and glucose; FQ and $F_1$ = feruloylquinic and glucose; $P_2$, $C_2$ and $F_2$: glucosides of *p*-coumaric, caffeic and ferulic acids. Results are in nmol/g dry wt; t = trace.

decreased to 0.5 in pink fruits: thus, during maturation, *p*-coumaroyl-CoA and feruloyl-CoA esters appear to be formed in preference to caffeoyl-CoA. These results are in agreement with those obtained with another variety of tomato in the same physiological stage and indicate that CoA ligase is more active with *p*-coumaric acid than with caffeic acid as substrate [34]. The absence of sinapoylquinic acid in tomato fruit is in agreement with the observed lack of activity of the corresponding CoA ligase at any stage of fruit growth; a form of CoA ligase which does not react with sinapic acid has already been reported [35, 36].

The changes observed for the different hydroxycinnamic derivatives during fruit growth and maturation seem to imply a possible role for these compounds in fruit physiology. The relative ratio of *o*-diphenols to monophenols may play a role in regulation of growth [37] and ripening fruits [20, 38]. During growth, phenols are implicated in the regulation of IAA-oxidase activity which controls the endogenous concentration of IAA [12, 13]; thus, rapid growth of tomato fruit is associated with high chlorogenic acid concentration. During ripening, phenolic compounds may be implicated in ethylene biosynthesis: monophenols are co-factors while *o*-diphenols are inhibitors [18, 19]. The variation in the ratio $CQ/C_2$ at the ripening stage clearly suggests that it could be one of the factors involved in ethylene biosynthesis which control ripening. Similar data have been reported for wounded fruits [38] and a variety of tomato in which naringenin is the main monophenol [20]. We have not taken into consideration rutin concentration, one of the *o*-diphenolic flavonol glycosides, but its variations are similar to that of chlorogenic acid [2].

## EXPERIMENTAL

*Material.* The chromatographic separation of quinyl esters and the determination of hydroxycinnamate:CoA ligase activities were carried out on the same material (lyophilized powder) which was used for the estimation of glucose derivatives [1]. The stages of fruit growth and ripening were defined as follows: green fruits of diameter 10, 12, 14 and 16 mm; pink; red fruits.

*The extraction and separation of quinyl esters* and the estimation of chlorogenic acid are previously described [2]. Quantitative analysis of *p*-coumaroylquinic and feruloylquinic acids was made by a UV spectrophotometric method after elution of spots separated by 2D chromatography [2]. The results are expressed in nmol/g dry wt using the extinction coefficients of *p*-coumaric and ferulic acids. The coefficient of variation of all determinations was within 8%.

*Assay of hydroxycinnamate: CoA ligase activity.* The enzymatic extracts were prepared by the method previously described [30], applied to a column of Sephadex G 25, then eluted with 0.1 M Pi buffer, pH 7.5. The enzyme activity was determined by the method of ref. [7] with some modification. The assay mixture contained 5.5 ml enzyme extract, $MnCl_2$ (50 μmol), 25 μl mercaptoethanol (10%) 5 μmol substrate and 5 μmol ATP. The assay mixture was set up in two 1 cm path cuvettes of the spectrophotometer and was allowed to equilibrate at 30° for 5 min. Then, 125 nmol of CoA SH was added to one cell and the increase in absorbance at 360 (caffeic acid as substrate), 333 (*p*-coumaric), 345 (ferulic) and 355 nm (sinapic) was followed for 5 min at 30°. The rate of increase in absorbance was linear with time over this period and was used to calculate the rate of formation of CoA esters using the extinction coefficients reported by Gross and Zenk [39]. The coefficient of variation was approximately 10%.

*Quinyl ester formation* was determined by the addition to the same assay mixture described above of 5 μmol quinic acid and 5 μmol EDTA. After 4 hr incubation at 30°, the reaction was stopped and quinyl esters formed were extracted and separated by chromatography.

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