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Dehydrotomatine and α-Tomatine Content in Tomato Fruits and Vegetative Plant Tissues

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Tomato plants (*Lycopersicon esculentum*) synthesize the glycoalkaloids dehydrotomatine and α-tomatine, possibly as a defense against bacteria, fungi, viruses, and insects. We used a high-performance liquid chromatography method with UV detection at 208 nm for the analysis of these compounds in various tissues. An Inertsil ODS-2 column with a mobile phase of acetonitrile/20 mM KH_2PO_4 (24/76, v/v) afforded good separation of the two glycoalkaloids in mini-tomato extracts, fruit harvested at different stages of maturity, and calyxes, flowers, leaves, roots, and stems. The two peaks appeared at ~17 and ~21 min. Recoveries from tomato fruit extracts spiked with dehydrotomatine and α-tomatine were 87.7 ± 6.8 and 89.8 ± 3.4% ($n = 5$), respectively. The detection limit is estimated to be 0.39 μg for dehydrotomatine and 0.94 μg for α-tomatine. The dehydrotomatine and α-tomatine content of tomatoes varied from 42 to 1498 and 521 to 16 285 μg/g of fresh weight, respectively. The ratio of α-tomatine to dehydrotomatine ranged from 10.9 to 12.5 in tomatoes and from 2.3 to 7.8 in the other plant tissues. These results suggest that the biosynthesis of the glycoalkaloids is under separate genetic control in each plant part. Degradation of both glycoalkaloids occurred at approximately the same rate during maturation of the tomatoes on the vine. An Inertsil NH_2 column, with acetonitrile/1 mM KH_2PO_4 (96/4, v/v) as the eluent, enabled the fractionation of commercial tomatidine into tomatidenol and tomatidine, the aglycons of dehydrotomatine and α-tomatine, respectively. The information should be useful for evaluating tomatoes and vegetative tissues for dehydrotomatine/α-tomatine content during fruit development and their respective roles in host−plant resistance and the diet.

KEYWORDS: **α-Tomatine; dehydrotomatine; biosynthesis; degradation; tomato; HPLC**

INTRODUCTION

Tomatoes, a major food source for humans, accumulate a variety of secondary metabolites including glycoalkaloids, reviewed in ref *1*. These metabolites protect against adverse effects of pathogens and predators including fungi, bacteria, viruses, and insects. Because tomato glycoalkaloids are reported to be involved in both host−plant resistance and to have a variety of pharmacological and nutritional properties in animals and humans (*1*), a need exists to develop a better understanding of the individual roles of the two tomato glycoalkaloids both in the plant and in the diet. The discovery that tomatoes contain both dehydrotomatine and α-tomatine is significant because all previous studies with the so-called "α-tomatine" or "tomatine" are based not on the pure compound but rather on mixtures of two glycoalkaloids, whose biological potencies may differ, and which can act synergistically or antagonistically both in the plant and in the diet.

As part of an effort designed to define the dynamics of the biosynthesis of tomato glycoalkaloids, we previously defined the changes of tomatine (mixture of dehydrotomatine and α-tomatine) content as a function of growth and maturation of tomatoes (*2*, *3*) and inheritance in potatoes (*4*). The current study extends the previous study by defining changes in individual dehydrotomatine and α-tomatine levels of tomato fruit at different stages of maturity. As part of this effort, we also examined the content of the two glycoalkaloids in the vegetative parts of the tomato plant. To our knowledge, this is the first report of concurrent changes of both dehydrotomatine and α-tomatine in growing tomato fruit.

MATERIALS AND METHODS

Materials. High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from Kanto Chemical Company (Japan). The solvents were filtered through a 0.45 μM membrane filter (Millipore, U.S.A.) and degassed with an ultrasonic bath before use.

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Commercial tomatine and tomatidine were obtained from Sigma (St. Louis, MO). Pure dehydrotomatine and α-tomatine standards were obtained by chromatographic separation of commercial tomatine using conditions described elsewhere (*5*−*7*). Briefly, commercial tomatine was chromatographed on a preparative HPLC column. Each of the two peaks, corresponding to the two glycoalkaloids, were collected and characterized by mass spectrometry. The procedure was repeated several times to obtain sufficient material to be used as standards for the present study.

Growth and Harvesting of Tomatoes. Three Japanese tomato varieties (Momotaro, Momotaro-T93, and First Memory) were grown in a greenhouse in Himeji City, Japan, from seeds as described previously (*3*). Fruits were collected, weighed, and measured for size. Fruits were collected at 10, 20, 30, 40, and 50 days after flowering.

Mini-tomatoes (variety Senka) and the standard sweet tomatoes (variety Mini Kyaroru) were grown in a greenhouse at a field station in Gyonsan, Korea. The fruit of the Japanese tomatoes, all parts of the mini-tomato plants (calyxes, flowers, green and red fruit, leaves, roots, and stems) and the roots of the sweet tomatoes were analyzed for dehydrotomatine and tomatine content.

HPLC. The extraction method was adapted from previously described procedures for extraction of glycoalkaloids from tomatoes grown in a greenhouse (*2*, *3*). For each cultivar, pericarp sections (consisting of 91−97% of solid weight of whole tomatoes) from 4 to 5 tomatoes of approximately equal size at the given interval were used for the analyses. The weights and sizes of the pericarp sections analyzed were increased from one interval to the next as fruits grew.

Briefly, macerated samples (fresh tomato fruit, 20 g; calyxes, 2 g; flowers, 1 g; leaves, 5 g; roots, 5 g; and stems, 3 g) were extracted with a mixture of 100 mL of chloroform/methanol (2:1, v/v), concentrated to 2−3 mL with the aid of an aspirator, dissolved in 40 mL of 0.2 N HCl, and the tomatine precipitated with 2% $(NH)_4OH$. The alkaloid fraction was washed with 25 mL of 2% NH_4OH and centrifuged at 18100*g* for 10 min at 1 °C. Washing with 25 mL of 2% NH_4OH and centrifugation were repeated. The ammonia was then dissipated, and the pellet was dissolved in 2 mL of a mixture of tetrahydrofuran/acetonitrile/0.02 M KH_2PO_4 (50/30/20, v/v/v) and centrifuged at 18100*g* for 10 min at 1 °C. The supernatant (5−50 μL) was used for HPLC analysis.

HPLC analysis was carried out using a Hitachi liquid chromatograph (model 655A-11) with an Autosampler (model 655A-40). The stainless steel column (250 mm × 4.0 mm i.d.) was packed with Inertsil ODS-2 (5 μM particle diameter) (GL Science, Japan). The following conditions were used for analyses: temperature, 20 °C; mobile phase, acetonitrile/20 mM KH_2PO_4 (24/76, v/v); flow rate, 1 mL/min. The UV detector (Hitachi model 655A UV Monitor) was set at 208 nm. Three separate analyses were carried out with each sample.

Quantification. Quantification of the glycoalkaloid content of each sample was accomplished by comparing integrated chromatographic peak areas from the test samples to peak areas of known amounts of standard dehydrotomatine and α-tomatine by a Hitachi Chromato-integrator model D-2000.

Recovery Tests. Tomato fruit extracts were analyzed before and after addition of known amounts of dehydrotomatine and α-tomatine. Recovery (%) = (concentration of added glycoalkaloid in spiked sample)/(concentration of endogenous glycoalkaloid + spike) × 100.

Aglycons. Conditions were also devised to separate the aglycons tomatidenol and tomatidine in commercial tomatidine. The same chromatographic conditions as employed above for the glycoalkaloids were found to be effective except that the column was packed with Inertsil NH_2 and the mobile phase consisted of acetonitrile/1 mM KH_2PO_4 (96/4, v/v).

RESULTS AND DISCUSSION

HPLC Analysis of Dehydrotomatine and α-Tomatine. Figure 1 depicts the structures of glycoalkaloids and aglycons evaluated in this study. **Figures 2** and **3** demonstrate the excellent separation of the two peaks associated with these glycoalkaloids, which are present in commercial tomatine and in extracts of tomato fruits, flowers, calyxes, leaves, stems, and roots. The HPLC−UV detection method responds linearly over the concentration range of about 2−20 μg for dehydrotomatine and about 10−120 μg of α-tomatine, with detection limits of 0.39 μg for dehydrotomatine (eluting at ~17 min) and 0.94 μg for α-tomatine (~21 min). Recoveries from spiked tomato extracts were 87.7 ± 6.8 and 89.8 ± 3.4% (*n* = 5), respectively. The validity of the method is reinforced by the good separation of the two peaks, lack of baseline noise in the chromatograms, and the linear concentration response of integrated peak areas.

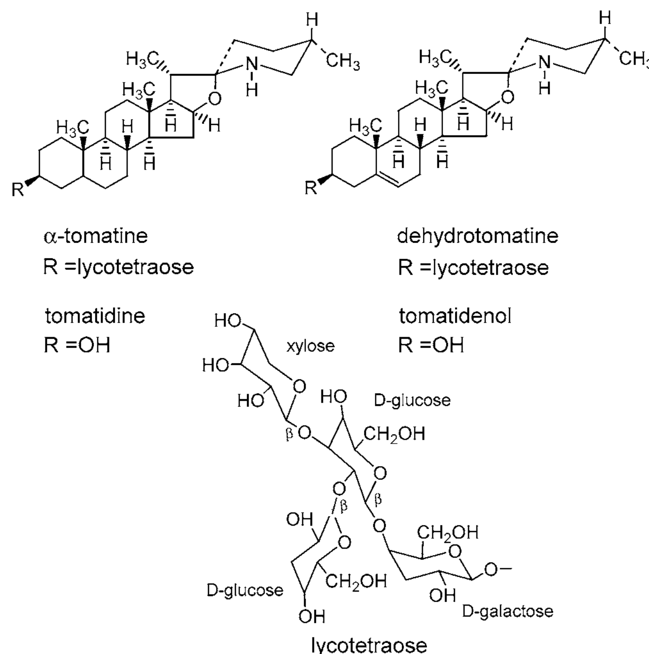

Figure 1. Chemical structures of glycoalkaloids dehydrotomatine and α-tomatine and of aglycons tomatidenol and tomatidine evaluated in this study.

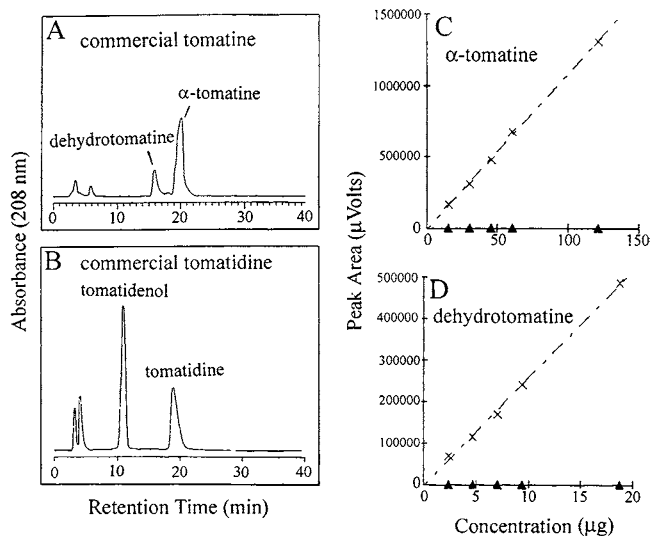

Figure 2. HPLC chromatograms of (**A**) dehydrotomatine and α-tomatine present in commercial tomatine and (**B**) tomatidenol and tomatidine present in commercial tomatidine. Linear relationships between their respective concentrations of standards and integrated peaks of (**C**) α-tomatine and (**D**) dehydrotomatine. Triangles are automatically placed on baselines of the figures by the TADKEN software program used to graph individual points on the curves.

On the basis of our previous time−temperature dependence studies of the acid-catalyzed hydrolysis of the carbohydrate side chains of glycoalkaloids (*8*, *9*), it is unlikely that the brief

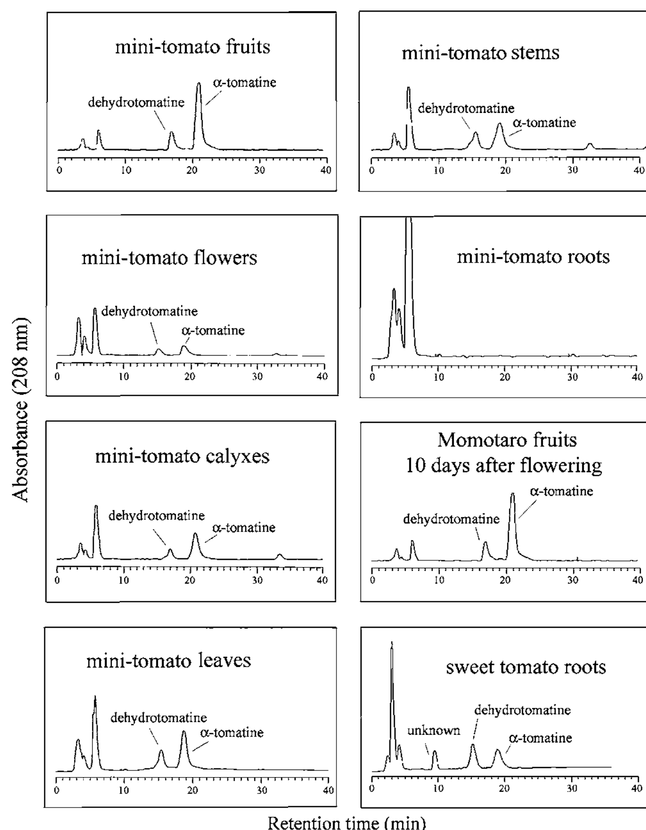

Figure 3. HPLC chromatograms of dehydrotomatine and α-tomatine isolated from different parts of the mini-tomato plant, the fruits of the Momotaro-T93 tomato plant, and the roots of sweet tomato. Absorbance values for the different chromatograms are not on the same scale.

exposure of the glycoalkaloids to HCl in the extraction process results in hydrolytic cleavage of sugar residues of the carbohydrate side chains.

Conditions were also devised to separate tomatidenol and tomatidine, the aglycons of dehydrotomatine and α-tomatine, respectively, present in commercial "tomatidine" (**Figure 2B**). Previously, we carried out HPLC, mass spectrometry, and hydrolysis studies, which unequivocally demonstrated that the mixture of aglycons present in commercial "tomatidine" consists of tomatidenol and tomatidine (*5*) and not of isomeric solasodine and tomatidine as listed on the label of the commercial material. This aspect is of analytical interest because it may be possible to devise a method for distinguishing dehydrotomatine and α-tomatine in extracts of tomatoes and tomato products based on HPLC analysis of tomatidenol and tomatidine, respectively, following hydrolytic removal of the identical carbohydrate side chain from the two glycoalkaloids.

Dehydrotomatine and α-Tomatine Content of Parts of Tomato Plant. Tomato glycoalkaloids are concurrently synthesized and then degraded during fruit maturation (*2*, *3*, *10*). We previously investigated the tomatine, β-carotene, lycopene, and chlorophyll contents at different stages of maturity for three widely consumed Japanese tomato varieties (*3*). The results showed that both tomatine and chlorophyll concentrations decreased rapidly during tomato fruit growth and maturation. Because the measured tomatine consisted of a mixture of dehydrotomatine and α-tomatine, it was of interest to find out whether the two glycoalkaloids are both degraded and if at similar rates. **Table 1** shows that the dehydrotomatine content of Momotaro-T93 tomatoes decreased from 68 μg/g of fresh weight 10 days after flowering to 2.6 μg/g after 20 days, a 96% decrease. Only trace amounts were present at 30 days, and none were detected after 40 or 50 days. The amount of α-tomatine decreased from 795 μg/g after 10 days to 49.4 μg/g after 20 days, a 94% decrease. These limited observations suggest that both glycoalkaloids appear to degrade at about the same rate during tomato plant growth until about 20 days after flowering. There appears to be a sharp transition in dehydrotomatine degradation during tomato fruit maturation at this time of flowering. The corresponding transition for α-tomatine appears to occur at a later stage, at around 30 days after flowering.

Comparing the levels of the two glycoalkaloids after 10 days after flowering (**Table 2**) shows a dehydrotomatine concentration of 41.6 ± 2.0, 55.2 ± 3.4, and 68.0 ± 8.2 μg/g of fresh weight for the Momotaro, First Memory, and Momotaro-T93 varieties, respectively. Their corresponding values for α-tomatine are 521 ± 3, 696 ± 48, and 795 ± 88 μg/g, respectively. These limited results show that these varieties differed in their two glycoalkaloid contents during early stages of tomato plant growth. However, this does not appear to be the case for the concentration ratios of α-tomatine to dehydrotomatine (B/A values in **Table 2**). For each variety, dehydrotomatine contributes about 7−8% to the sum of the two glycoalkaloids.

Table 3 and also **Figure 3** show that our method was also useful for the analysis of the two glycoalkaloids in different parts of the mini-tomato plant. The values for dehydrotomatine ranged as follows (in μg/g of fresh weight): green tomatoes, 1498; flowers, 1023; calyxes, 370; stems, 331; and leaves, 304. The concentrations of α-tomatine ranged as follows: green tomatoes, 16 285; flowers, 4825; calyxes, 3240; leaves, 2151; and stems, 1878. The ratio of α-tomatine to dehydrotomatine levels of 10.9 (B/A values in **Table 3**) for the green mini-tomatoes is similar to the ratios (11.7 to 12.5) of three varieties mentioned earlier (**Table 2**). However, the corresponding ratios in the other tomato plant parts were lower, ranging from 2.3 for roots, 4.7 for flowers and stems, 6.1 for leaves, and 7.8 for the calyxes (**Tables 3** and **4**). These results suggest that (i) the biosynthesis/degradation rates of the two glycoalkaloids in tomato fruit differ from those occurring in other the tomato plant parts and (ii) the biosynthesis in tomato fruit may be under separate genetic control than that in the vegetative parts of the plant.

Tables 3 and **4** and **Figure 3** and also show that the roots of the mini-tomato had only traces of dehydrotomatine and α-tomatine, whereas roots of sweet tomatoes contained significant amounts of both glycoalkaloids, 77 and 178 μg/g of fresh weight, respectively, corresponding to a ratio of α-tomatine to dehydrotomatine of 2.3 and to 30.2% of dehydrotomatine, the highest amount observed. We have no apparent explanation for this difference in content in the roots of the two tomato plants. The sweet tomato extract also contained a third, unknown compound, possibly a third glycoalkaloid.

A possible explanation or rationalization for the wide-ranging results is that dehydrotomatine and α-tomatine act synergistically in protecting the tomato plant against phytopathogens, as discussed in detail elsewhere (*1*). Different absolute amounts as well as different ratios of the two secondary metabolites may be needed for a synergistically optimal defense of the different tomato plant parts against different phytopathogens (bacteria, fungi, viruses, and nematodes). This suggestion is supported by the observations that dehydrotomatine and α-tomatine differ in their respective biological effects in cells (*11*, *12*) and that certain combinations of potato glycoalkaloids α-chaconine and α-solanine act synergistically in cells (*13*). Whether mixtures

Table 1. Effect of Maturation on Dehydrotomatine and α-Tomatine Content of Momotaro-T93 Tomato Fruit

days after flowering	dehydrotomatine (A) (μg/g of fresh weight)[a]	α-tomatine (B) (μg/g of fresh weight)	sum (A + B)	ratio (B/A)	% dehydrotomatine [(A/A + B) × 100]
10	68.0 ± 8.2	795 ± 88	863	11.7	7.9
20	2.6 ± 0.4	49.4 ± 2.3	52.0	19.1	5.0
30	trace	19.7 ± 3.1	19.7		
40	ND[b]	1.5 ± 0.6	1.5		
50	ND[b]	3.7 ± 1.5	3.7		

[a] Average ± SD; $n = 3$. [b] ND = not detected.

Table 2. Dehydrotomatine and α-Tomatine Content of the Fruit of Three Tomato Varieties Harvested 10 Days after Flowering of the Tomato Plants

tomato variety	dehydrotomatine (A) (μg/g of fresh weight)[a]	α-tomatine (B) (μg/g of fresh weight)[a]	total (A + B)	ratio (B/A)	% dehydrotomatine [(A/A + B) × 100]
Momotaro-T93	68.0 ± 8.2	795 ± 88	863	11.7	7.9
First Memory	55.2 ± 3.4	696 ± 48	751	12.5	7.3
Momotaro	41.6 ± 2.0	521 ± 3	563	12.5	7.4

[a] Average ± SD; $n = 3$.

Table 3. Dehydrotomatine and α-Tomatine Content of Parts of the Mini-Tomato Plant

tomato part	dehydrotomatine (A) (μg/g of fresh weight)[a]	α-tomatine (B) (μg/g of fresh weight)	sum (A + B)	ratio (B/A)	% dehydrotomatine [(A/A + B) × 100]
fruit (green)	1498 ± 49	16 285 ± 112	17 783	10.9	8.4
flowers	1023 ± 3	4825 ± 191	5848	4.7	17.5
calyxes	370 ± 17	2870 ± 129	3240	7.8	11.4
leaves	304 ± 15	1847 ± 112	2151	6.1	14.1
stems	331 ± 4	1547 ± 32	1878	4.7	17.6
roots	trace	trace	trace		
fruit (red)	ND[b]	ND[b]	ND[b]		

[a] Average ± SD; $n = 3$. [b] ND = not detected.

Table 4. Dehydrotomatine and α-Tomatine Content of "Sweet Tomato" Roots

glycoalkaloid	μg/g of fresh weight[a]
dehydrotomatine (A)	77 ± 7
α-tomatine (B)	178 ± 21
unknown (C)[b]	76 ± 1[b]
total (A + B)	255
ratio (B/A)	2.3
dehydrotomatine (%) [(A/A + B) × 100]	30.2

[a] Average ± SD; $n = 3$. [b] Expressed as α-tomatine.

of the two tomato glycoalkaloids can also exert synergistic biological effects merits study.

Related Studies. To place our findings in proper perspective, it is relevant to briefly examine previously reported analytical studies of dehydrotomatine and α-tomatine. The observation that commercial tomatine consists of a mixture of the known tomato glycoalkaloid α-tomatine and a new glycoalkaloid dehydrotomatine (*5*, *14*) stimulated interest in determing the distribution of these two glycoalkaloids in tomato fruit and other parts of the tomato plant. Friedman and Levin (*6*, *7*) appear to have been first to analyze the two glycoalkaloids in tomatoes, tomato plant parts, and processed tomato products by HPLC with pulse amperometric detection (PAD) or UV detection at 200 nm. Dehydrotomatine and α-tomatine were found to have the same concentration response by PAD and a very different response by UV detection. The lower detection limit by UV was ∼5 μg and that by PAD was ∼0.1 μg. Leonardi et al. (*15*) used our method to measure the dehydrotomatine and α-tomatine content of several Italian tomato varieties and of a tomato salad. Bacigalupo et al. (*16*) measured dehydrotomatine and α-tomatine in green tomato fruits and in tomato leaves by time-resolved fluorescence spectroscopy using a europium chelator entrapped in liposomes. The results obtained were comparable to those by HPLC using our method. Kuronen et al. (*17*) devised an HPLC method to separate commercial tomatine into dehydrotomatine and α-tomatine and commercial tomatidine into peaks associated with tomatidenol (dehydrotomatidine) and pure tomatidine. They did not study tomatoes. Väänänen et al. (*18*) used HPLC with UV detection to evaluate the recovery of dehydrotomatine and α-tomatine (added as commercial tomatine) to wild potato *Solanum brevidens* leaf material on different SPE sorbents.

To further improve the separation and analysis by HPLC of the two major potato glycoalkaloids present in potatoes, we recently reported a detailed study on the influence of the following parameters on retention times: composition and pH of mobile phase, concentration of buffer, capacity factors of column packing, and column temperature (*19*). All of the parameters except pH significantly influenced retention times. For the purpose of this study, we manipulated the cited parameters in order to further optimize the separation of dehdyrotomatine and α-tomatine by HPLC with UV detection at 208 nm. We discovered that (i) the column packing and mobile phase used in this study afforded better separation of the two glycoalkaloids than those obtained in previous studies mentioned above (∼4 vs ∼2 min) and (ii) the sensitivity (lowest concentration that can be measured) by the separation−detection method used in this study (0.39 μg for dehydrotomatine and 0.94 μg for α-tomatine) is severalfold lower than that cited above for the earlier HPLC−UV method.

CONCLUSIONS

The described method was useful for analysis of glycoalkaloids in immature green and red fruit, calyxes, leaves, roots, and stems. The observed distribution of the two glycoalkaloids in the various vegetative tomato plant parts should facilitate future studies designed to define their respective roles in host−plant resistance and during postharvest processing and storing of tomatoes, as well as their respective roles in animal and human nutrition and health.

The latter aspects deserve additional comment. Because high concentrations of tomatine in the diet may inhibit growth of hamsters (*20*, *21*), it is of interest to find out to what extent, if any, the glycoalkaloid content of tomato products and byproducts adversely affects their nutritional value. Reported compositional and nutritional studies of such products, which did not take into account possible effects of glycoalkaloids, include animal feeding studies of tomato cannery wastes (*22*), tomato peel (*23*), tomato pomace (*24*, *25*), tomato pulp (*26*), tomato seeds (*27*, *28*), and tomato vines (*29*).

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