Dehydrotomatine Content in Tomatoes†

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A variety of methods are used to analyze tomato glycoalkaloids. Because no single method has gained wide acceptance,the extraction and analysis of tomatine by HPLC with pulsed amperometric detection (PAD) was previously optimized for standard and transgenic tomatoes and processed tomato products. In the course of these studies it was discovered that commercial tomatine contained a second glycoalkaloid, which was named dehydrotomatine. This study demonstrates that the HPLC-PAD assay can be used to measure both the dehydrotomatine (DT) and α-tomatine (TMT) content of parts of the tomato plant and of low- and high-tomatine red and green tomatoes. Both the absolute concentration of dehydrotomatine and the percent dehydrotomatine in the mixture of the two extracted glycoalkaloids, defined as [DT/(DT + TMT) × 100], varied widely. The DT content of red tomatoes ranged from 0.05 to 0.42 mg/kg of fresh weight. The corresponding range for green tomatoes was from 1.7 to 45 mg/kg. The percent DT for the tomato plant parts was about 7 for fresh and senescent leaves and calyxes, 10 for green fruit, 14 for small stems and flowers, and 23 for roots and large stems. The corresponding values for 15 different tomato varieties ranged from ∼3 to 10%. This study demonstrates the advantages of the highly sensitive HPLC-PAD method for DT and TMT. The possible significance of these findings for plant and food sciences is discussed.

Keywords: *Dehydrotomatine analysis; HPLC-PAD; pulsed amperometric detection; α-tomatine analysis; tomatoes; tomato plants; transgenic tomatoes*

INTRODUCTION

Detection of tomato glycoalkaloids is of interest because they (a) are involved in host-plant resistance (Costa and Gaugler, 1989; Sandrock and Vanetten, 1998; Weissenberg et al., 1998), (b) disrupt cell membranes (Blankemeyer et al., 1997; Roddick and Drysdale, 1994), and (c) may have beneficial effects in the diet. For example, we recently discovered that feeding commercial tomatine to hamsters induced significant reduction in plasma low-density lipoprotein (LDL) cholesterol and that the reduction with high-tomatine green tomato diets was greater than with low-tomatine red tomato diets (Friedman et al., 1997a). The development of improved transgenic tomatoes has also stimulated interest in determining whether their changing glycoalkaloid content during different stages of fruit maturity differs significantly from that found in standard varieties.

Previously, we reported that commercial tomatine consists of a mixture of the known tomato glycoalkaloid, α-tomatine (TMT), and a new glycoalkaloid, which we named dehydrotomatine (DT) (Friedman et al., 1994, 1997b). Similar observations were made by Bushway and Perkins (1995). DT differs from TMT by having a double bond in ring B of the steroidal part of the molecule (Figure 1). We also showed that an improved HPLC pulse amperometric detection (PAD) analytical method can be used to measure the tomatine content of low- and high-tomatine tomatoes and tomato plant parts and for a variety of processed tomato products sold commercially or prepared in the kitchen (Friedman and Levin, 1995). Because the TMT content of red tomatoes and most tomato products is quite low and the DT content in tomatoes appears to be an order of magnitude lower than that of TMT, the highly sensitive HPLC-PAD method appeared to be promising for the analysis of DT in a variety of tomato substrates. The results of this study demonstrate that possibility.

MATERIALS AND METHODS

Materials. Commercial tomatine was obtained from two sources: Sigma (St. Louis, MO) and Research Plus, Inc. (Bayonne, NJ). Field-grown tomatoes were donated by DNA Plant Technology Corporation, Oakland, CA. Whole tomato plants, which provided a source of the individual parts of the tomato plant, were a gift of Dr. Merle L. Weaver of this laboratory.

Preparation of Dehydrotomatine and α-Tomatine. Commercial tomatine was resolved into DT and TMT by preparative HPLC using UV detection. Conditions were as follows: 3 mL of eluent/min was passed through a 25 cm × 10 mm, 5 μm particle, Supelco LC-ABZ column; the eluent consisted of 25% acetonitrile and 100 mM ammonium phosphate brought to pH 3 with phosphoric acid. Commercial tomatine (2 mg in 1 mL of 50% methanol and 0.1% acetic acid) was applied to the column; the two peaks were collected from the UV detector, which was monitored at 200 nm. The procedure was repeated 10 times with fresh samples of commercial tomatine. The fractions from different runs for each compound were pooled, and the acetonitrile was evaporated with the aid of a water aspirator. The solutions were made basic with NH_4OH and extracted into butanol, and the butanol was then evaporated. The structures of the DT and TMT were confirmed by mass spectrometry (Friedman et al., 1994).

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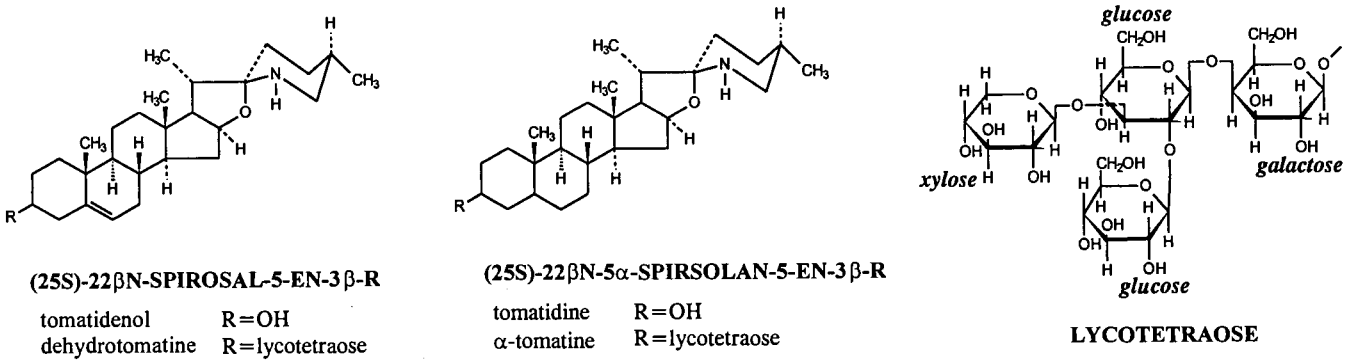

Figure 1. Structures of the aglycons tomatidenol and tomatidine and of the corresponding tetraglycosides DT and TMT.

HPLC Solvents. Solvents were of HPLC grade. Reagents were of ACS grade. The HPLC eluent for TMT analysis was prepared by combining 100 mL of concentrated buffer with 550 mL of polished water, 200 mL of acetonitrile, and 150 mL of methanol. Water was polished by passing it through a Supelclean Envi-18 solid-phase extraction (SPE) tube. The concentrated buffer was prepared by combining 28.97 g of disodium phosphate and 93.72 g of citric acid in 1 L of water. This buffer was filtered through a 0.45 μm nylon membrane from Schleicher and Schuell (Keene, NH), passed through a 3 × 1 cm bed of Chelex 100 (to remove any heavy metals), and then passed through a Supelclean Envi-18 SPE. We found that the PAD cell was less likely to foul, which can sometimes be a problem with electrochemical detection, with these cleanup precautions.

Instrumentation. UV detection was used for preparative collection and PAD was used for analysis. PAD was chosen for analysis to take advantage of its greater sensitivity. UV was chosen for separation because the following problems were encountered when PAD was used during collection. First, because the flow cell was not designed for high flow rates, peak shapes deteriorated and seal integrity was compromised. Second, poor recoveries and extra peaks lead us to believe that the applied current may have permanently changed the chemical structure of the molecules of interest. The sensitivity of the UV detector was adequate for preparative HPLC, and peaks appeared unchanged upon reinjection. We found that a combination of UV detection during preparative HPLC and PAD detection during analytical HPLC served our purposes.

HPLC-PAD. A Dionex system 500 was equipped with an ED40 electrochemical detector with a gold electrode installed. The reference electrode is a combination pH-Ag/AgCl. The mode of detection was integrated amperometry. The waveform was as follows:

step	time (s)	V	integrate
0	0	+0.5	
1	0.30	+0.5	begin
2	0.35	+1.0	
3	0.40	+0.5	
4	0.50	+0.5	end
5	0.51	+1.3	
6	0.70	+1.3	
7	0.71	−0.1	
8	2.00	−0.1	

The analogue output setup was as follows:

output	offset
zero position	1% full scale
volts full scale	1.0 V
rise time	0.5 s
polarity	+
range	1 μC full scale

The chromatography column for analysis was a 4.6 × 250 mm, 5 μm, Supelcosil LC-ABZ with a 2 cm guard of the same material (Supelco Inc., Bellefonte, PA). Flow rate was set to 1.0 mL/min, and eluent was recycled. We changed the eluent for a fresh solution after 2 months of use. The system was controlled and data were collected and analyzed by Dionex AL-450 system software release 3.32.

HPLC-UV. A Beckman 334 system had a variable-wavelength detector with a 10 mm light path. Wavelength was monitored at 200 nm. The chromatography column was a 10 × 250 mm, 5 μm, Supelcosil LC-ABZ. Flow rate was set to 3 mL/min. Fractions were collected with a Gilson (Middleton, WI) FC 203B fraction collector. The system was controlled and data were collected and analyzed by Thermo Separation Products PC1000 system software v.3.0.1.

Methods. Cubed fresh tomatoes and parts of the tomato plant were immediately frozen in liquid nitrogen. Samples were then lyophilized. Samples were weighed before and after lyophilization for moisture determination. The dried tomatoes were then ground in an Omnimixer (Ivan Sorvall Inc., Newtown, CT) so they passed through a 0.5 mm screen.

The samples were extracted by stirring 1 g in 20 mL of 1% acetic acid for 2 h. The suspension was then centrifuged for 10 min at 13 300 relative centrifugal force (RCF), and the supernatant was filtered through a Whatman GF/C filter. The pellet was resuspended in 10 mL of 1% acetic acid, centrifuged, and filtered, and the two extracts were combined. This extract was further purified using SPE. A C_{18} SPE tube, equipped with a 60 mL reservoir (Supelco), was conditioned with 5 mL of methanol followed by 5 mL of water. The aqueous extract (now ~30 mL) was applied and allowed to gravity drip. When the sample was fully absorbed onto the packing, the tube was washed with ~10 mL of water, followed by 5 mL of 30:70 acetonitrile/1% NH_4OH, and then 5 mL of water. The DT and TMT were eluted with 10 mL of 70:30 acetonitrile/1 mM HCl. This sample was then dried on a rotovapor. The residue was taken up in 1 mL of 50% methanol/0.1% acetic acid and filtered through a 0.45 μm HV membrane obtained from Millipore (Bedford, MA). This filtrate was ready for HPLC injection.

RESULTS AND DISCUSSION

A variety of methods are used to analyze tomato glycoalkaloids (Bajaj et al., 1988; Bushway et al., 1994; Courtney and Lambeth, 1977; Juvik et al., 1982; Kozukue et al., 1994; Pstrzycka, 1989; Roddick, 1976; Takagi et al., 1994; Tukalo and Ivanchenko, 1976; van Gelder et al., 1988; Voldrich et al., 1992). Because no single method has gained widespread acceptance, we initiated studies designed to optimize the extraction and analysis of tomato glycoalkaloids by HPLC with PAD. We found that the tomatine content of transgenic delayed-ripening varieties was not different from the range ordinarily seen in tomatoes. We also showed that the tomatine content of fresh and processed tomatoes determined by HPLC-PAD correlated with analyses performed on the same samples by an enzyme-sorbent immunoassay method (ELISA) (Stanker et al., 1994, 1996).

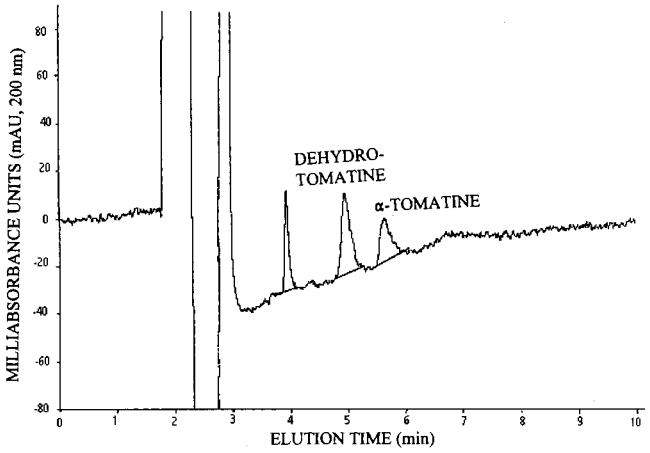

Figure 2. HPLC chromatogram with UV detection at 200 nm of 500 ppm commercial tomatine showing separation of DT and TMT. Conditions: 1 mL/min 100 mM ammonium phosphate, 35% acetonitrile, pH 3.5, through a 4.6 × 25 cm Supelcosil LC-ABZ 5 μm particle column; 25 μL injection.

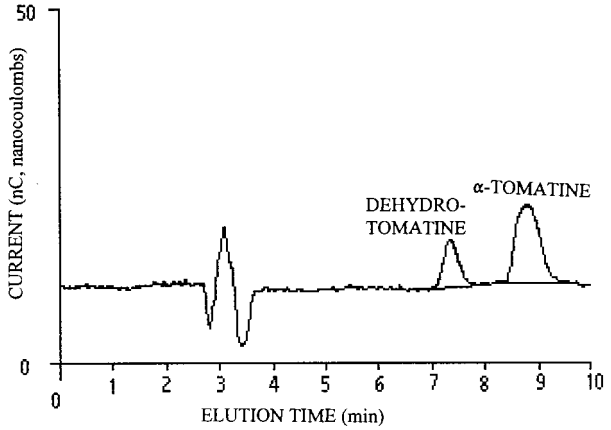

Figure 3. HPLC chromatogram with PAD detection of 50 ppm commercial tomatine showing separation of DT and TMT. Conditions: 1 mL/min 100 mM citrate/phosphate buffer, 20% acetonitrile, 15% methanol, pH 3.5, through a 4.6 mm × 25 cm Supelcosil LC-ABZ 5 μm particle column; 25 μL injection.

The objective of the present study was to measure the applicability of the HPLC-PAD method to the analysis of DT and TMT in parts of the tomato plant and in tomato fruit.

Concentration−Response Curves. Figures 2 and 3 show HPLC chromatograms with UV and PAD detection, respectively, for DT and TMT present in commercial tomatine. Although the structure of TMT differs from that of DT only by a single double bond, the two compounds separated well in our system. Note that the concentration of the UV sample is 10 times that of the PAD sample. Because TMT lacks a double bond, it is poorly UV active. The pure TMT peak is thus indistinguishable from baseline at concentrations below 50 ppm of commercial tomatine when using UV detection. Figures 4 and 5 show the concentration−response curves for purified DT and TMT prepared by preparative chromatography from the mixture present in commercial tomatine as described earlier for UV and PAD detection, respectively. Purified DT and TMT were found to have the same concentration−response by PAD detection (Figure 3) and very different responses by UV. Note that the scale on the *y*-axis for UV detection of DT is greater by approximately a factor of ~10 relative to that for TMT. Comparison of Figures 4 and 5 shows

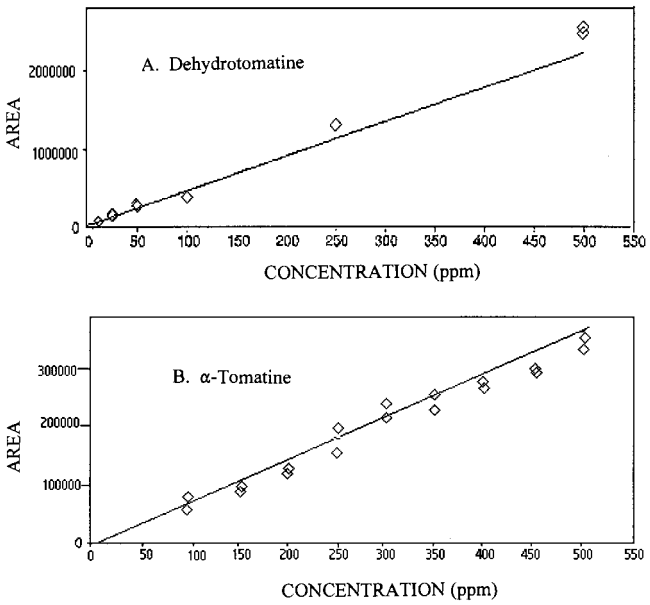

Figure 4. HPLC concentration−response curves with UV detection: (A) DT; (B) TMT. Twenty-five microliters of 100 ppm tomatine is at the limit of detection of 2.5 μg.

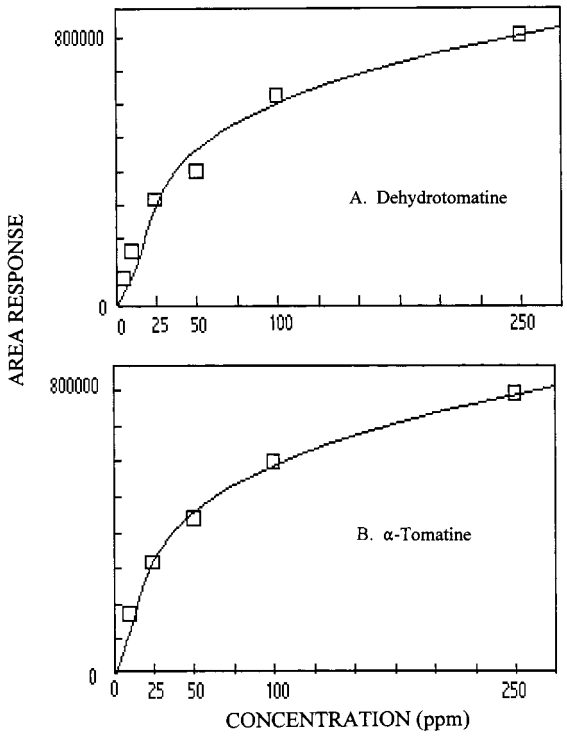

Figure 5. HPLC concentration−response curves with PAD detection: (A) DT; (B) TMT.

that the UV response is linear, whereas the PAD response is nonlinear. This is a common problem for PAD cells, which are easily overloaded by increasing concentrations. The response, however, was consistent and reproducible once the standard curve was constructed. We found it difficult to use UV detection for low-tomatine red tomatoes and tomato products. In our hands, UV detection is not sufficiently sensitive for very low levels of DT. In contrast, PAD analysis is quite sensitive. Thus, the estimated lower detection limit by UV is 2.5 μg and by PAD is 0.1 μg. Using the concentration−response curve, we estimated that both commercial samples of tomatine mentioned under Materials consisted of an 85:15 mixture of TMT and DT.

Table 1. TMT and DT in Various Parts of the Tomato Plant[a]

plant part	DT	TMT	%DT[b]
large immature green fruit	14, 14	148, 139	8.5, 9.1
roots	32, 35	113, 124	23, 23
small immature green fruit	55, 53	468, 462	10, 10
calyxes	65, 60	788, 802	7.6, 7.0
leaves	70, 72	978, 961	6.7, 7.0
small stems	139, 128	876, 910	14, 12
large stems	142, 142	462, 467	26, 23
flowers	190, 190	1090, 1110	15, 15
senescent leaves	330, 300	4780, 5010	6.5, 5.6

[a] Duplicate determinations (mg/kg) fresh weight. [b] [DT/(TMT + DT)] × 100.

DT Content of Parts of the Tomato Plant. Table 1 shows that the method was useful for the analysis of parts of the tomato plant including large immature green fruit (14 mg/kg of fresh weight), roots (34 mg/kg), small immature green fruit (54 mg/kg), calyxes (63 mg/kg), fresh leaves (71 mg/kg), small and large stems (140 mg/kg), and senescent leaves (315 mg/100 kg). Table 1 also shows that the percent DT [defined as the concentration of DT/(TMT + DT) × 100] for various parts of the tomato plant ranged from ~7 for leaves and calyxes to ~9−12 for green fruit and small stems, to ~15 for flowers, and to ~23−25 for large stems and roots. We have no obvious explanation for the apparent variation in the DT content of different parts of the tomato plant. Below we offer some possible reasons for these findings.

The observations with fresh and senescent leaves deserve further comment. Although the content of glycoalkaloids on a fresh weight basis in brown, senescent leaves was much higher than in fresh leaves that came from the same plant, on a dry basis the values for both glycoalkaloids were similar (DT, 465 mg/kg of dry weight for senescent leaves and 470 mg/kg for fresh leaves; TMT, 7300 mg/kg for senescent leaves and 6400 mg/kg for fresh leaves). These results demonstrate that drying of leaves on the plant does not degrade the tomato glycoalkaloids. In related studies, we describe procedures for optimizing the analysis of glycoalkaloids in leaves from potato plants (Brown et al., 1998; Dao and Friedman, 1996).

DT Content of Whole Tomatoes. Figure 6 shows the separation of the two peaks associated with DT and TMT extracted from red and green tomatoes using the HPLC-PAD method. Tables 2 lists the DT and TMT contents of a large number of red and green tomatoes obtained from various sources and purchased in a local market in terms of milligrams per kilogram of fresh weight. The values vary widely. The contribution of DT to the total for the two glycoalkaloids ranges from ~2.5% to ~9.5%. The corresponding value for red tomatoes is the range of ~6−16%.

The results show a wide scatter in the percent of DT content in the extracted mixture of the two glycoalkaloids. The data also suggest that for the same variety, there are apparent differences between the ratios of DT to TMT for green and red tomatoes. However, this difference does not appear to manifest itself in a systematic way because in some cases the ratio is higher for green tomatoes than for the red ones. The reverse is true in other cases. This is not surprising because, as discussed in detail elsewhere for potato glycoalkaloids (Friedman and McDonald, 1997), the glycoalkaloid content is strongly influenced by environmental factors as well as by the maturity and variety (cultivar) of the plant. Although similar factors could influence the trends in glycoalkaloid content of tomatoes, the situation with tomatoes is more complicated because tomatine is degraded as the tomato fruit matures, both during growth and after harvest (Eltayeb and Roddick, 1984a,b; Heftmann and Schwimmer, 1972). This event explains the high content of the glycoalkaloids in green tomatoes

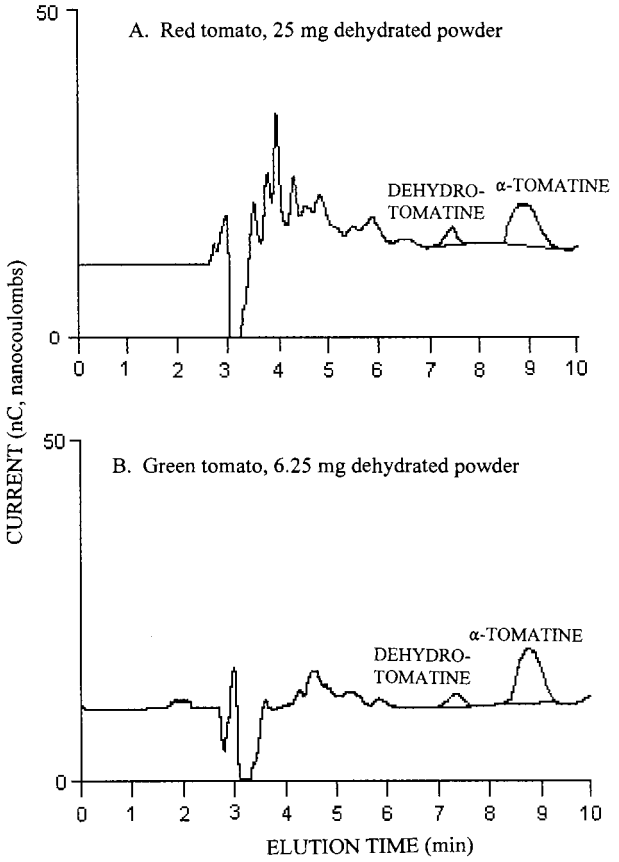

Figure 6. HPLC chromatograms with PAD detection of DT and TMT in fresh red and green tomato fruit: (A) red tomato sample contains the equivalent of 1 g of dehydrated tomato powder/mL; (B) green tomato sample contains the equivalent of 0.25 g of dehydrated tomato powder/mL. Conditions: 1 mL/min 100 mM citrate/phosphate buffer, 20% acetonitrile, 15% methanol, pH 3.5, through a 4.6 mm × 25 cm Supelcosil LC-ABZ 5 μm particle column; 25 μL injection.

Table 2. TMT and DT Content of Field-Grown Tomatoes[a]

tomato variety	DT		TMT		%DT[b]	
	green	red[c]	green	red	green	red
687	1.8, 1.6	0.05, nd[d]	32, 34	0.6, 0.5	5, 4	6, 0
1345-4	1.9, 2.1	0.10, 0.10	51, 54	1.4, 1.5	4, 4	8, 7
Shady Lady	2.6, 1.7	0.05, 0.05	41, 40	0.4, 0.3	6, 4	15, 18
C11103	2.7, 1.6	0.04, 0.04	45, 44	1.1, 1.0	6, 4	4, 4
422	3.3, 2.2	nd, 0.06	109, 101	0.4, 0.6	3, 2	0, 11
91127	3.5, 3.7	0.08, 0.12	68, 70	0.6, 1.0	5, 5	10, 9
692	5.3, 5.8	0.05, nd	137, 140	0.5, 0.5	4, 4	7, 0
Sunny	5.9, 5.9	0.05, 0.05	88, 89	0.3, 0.4	6, 6	11, 11
684	12, 12	0.05, 0.05	159, 157	0.6, 0.4	7, 7	11, 15
705	12, 14	0.13, 0.07	220, 221	0.8, 0.9	5, 6	16, 10
BEBC cherry (2nd batch)	20, 21	0.42, 0.42	309, 335	3.8, 3.9	6, 6	10, 10
B11009 cherry	21, 24	0.20, 0.20	489, 567	2.9, 3.1	4, 4	7, 6
B11009-7 cherry	25, 23	0.15, 0.20	305, 302	2.8, 2.7	8, 7	5, 7
438	25, 25	0.11, 0.05	361, 365	1.5, 1.4	7, 6	8, 5
BEBC cherry	44, 46	0.16, 0.21	431, 428	1.7, 1.7	9, 10	9, 12

[a] Duplicate determinations in mg/kg of fresh sample. [b] [DT/(TMT + DT) × 100]. [c] These peaks may be too small to accurately quantitate. [d] nd, not determined.

and the low content in red ones. An unanswered question is whether the rate degradation of DT differs from that of TMT.

OUTLOOK AND RESEARCH NEEDS

As far as we know, this is the first report on the quantification of the DT content in all parts of the tomato plant. Until the recent discovery of DT, it was thought that tomatoes contain only one glycoalkaloid, usually called α-tomatine or tomatine. The question arises why each of the major *Solanum* plants produced two glycoalkaloids [potatoes, α-chaconine and α-solanine (Friedman and McDonald, 1997); eggplants, solamargine and solasonine (Blankemeyer et al., 1998); tomatoes, DT and TMT]. As phytopathogens became adapted over time to resist the first glycoalkaloid's effects, to survive, the plant created a second, biologically more potent one. An alternative possibility is that both glycoalkaloids developed at the same time during evolution to exert the observed synergistic effect against phytopathogens. The second, evolutionary, approach is more efficient because it allows the plant to produce a smaller total amount of the two glycoalkaloids while maintaining resistance. These considerations suggest that the biological potency of one of the two glycoalkaloids should be greater than that of the other and that certain combinations of the two tomato glycoalkaloids may act synergistically both in the plant and in the diet (Rayburn et al., 1995). The validity of these hypotheses remains unresolved.

The biosynthesis of DT and TMT may arise by at least three different pathways. The first postulates that tomatidenol, the double-bond-containing aglycon of DT (Figure 1), arises from cholesterol, which also has a 5,6 double bond. The aglycon of TMT, tomatidine, which lacks a double bond, is derived from cholestanol, which also lacks a 5,6 double bond (Friedman and McDonald, 1997, 1999; Petersen et al., 1993). An alternative pathway postulates that the biosynthetic intermediate teneimine derived from cholesterol is partitioned; part of its double bond is hydrogenated by a hypothetical hydrogenase to tomatidine (Laurila et al., 1996), and the remainder forms tomatidenol. The third possibility is that a portion of the aglycon tomatidine is dehydrogenated to tomatidenol by a hypothetical dehydrogenase. Because the two glycoalkaloids have the same carbohydrate side chain, it is likely that tomatidine and tomatidenol are similarly glycosylated to the corresponding glycoalkaloids (Friedman et al., 1998; Moehs et al., 1997; Stapleton et al., 1991; Zimowski, 1994). If the first pathway is operative, differences in the availability of the cited precursors and/or enzymes catalyzing the transformations from cholestanol and cholesterol, respectively, in different parts of the plant would be expected to produce differing ratios of the two glycoalkaloids. If the second or third pathway is operative, then the concentration of the hydrogenase or dehydrogenase would be expected to be rate-controlling in the formation of different ratios of DT and TMT. Additional studies are needed to characterize the postulated enzymes to differentiate between these possibilities.

Glycoalkaloids may have evolved in nature to protect the plant against bacteria, fungi, insects, and animals. It is striking that both green tomatoes and tomato leaves have a very high glycoalkaloid content (Table 1), which makes them undesirable to eat because the green fruit and leaves not only taste bitter but may not be safe to the phytopathogen. An unanswered question is, what are the respective contributions of TMT and DT to host-plant resistance of tomato plants?

It is also worth noting that the fundamental molecular−cellular mechanisms by which glycoalkaloids resist pathogens—disruption of cell membranes and inhibition of cholinesterases—can also be used to explain the pharmacological effects of glycoalkaloids in animals and humans.

Because certain antibiotics exert their effect against human pathogens by similar cell-disruptive mechanisms, it would also be worthwhile to explore possible antimicrobial properties of DT and TMT.

LITERATURE CITED

Bajaj, K. L.; Kaur, P. P.; Sharma, O. N. Absorptiometric determination of α-tomatidine in tomato fruits. *Analusis* **1988**, *16*, 194−195.

Blankemeyer, J. T.; White, J. B.; Stringer, B. K.; Friedman, M. Effect of α-tomatine and tomatidine on membrane potential of frog embryos and active transport of ions in frog skin. *Food Chem. Toxicol.* **1997**, *35*, 639−646.

Blankemeyer, J. T.; McWilliams, M. L.; Rayburn, J. R.; Weissenberg, M.; Friedman, M. Developmental toxicology of solamargine and solasonine glycoalkaloids in frog embryos. *Food Chem. Toxicol.* **1998**, *36*, 383−398.

Brown, M. S.; McDonald, G. M.; Friedman, M. Sampling leaves of young potato plants for glycoalkaloid analysis. *J. Agric. Food Chem.* **1998**, submitted for publication.

Bushway, R. J.; Perkins, L. B. Identification of an impurity in commercial sources of the tomato glycoalkaloid tomatine. *J. AOAC Int.* **1995**, *78*, 691−693.

Bushway, R. J.; Perkins, L. B.; Paradis, L. R.; Vanderpan, S. High-performance liquid chromatographic determination of the tomato glycoalkaloid, tomatine, in green and red tomatoes. *J. Agric. Food Chem.* **1994**, *42*, 2824−28239.

Costa, S. D.; Gaugler, R. R. Sensitivity of *Beauveria bassiana* to solanine and tomatine: plant defensive chemicals inhibit an insect pathogen. *J. Chem. Ecol.* **1989**, *15*, 697−706.

Courtney, W. H., III; Lambeth, V. N. Glycoalkaloid content of mature green fruit of *Lycopersicon* species. *HortScience* **1977**, *12*, 550−551.

Dao. L.; Friedman, M. Comparison of glycoalkaloid content of fresh and freeze-dried potato leaves determined by HPLC and colorimetry. *J. Agric. Food Chem.* **1996**, *44*, 2287−2291.

Eltayeb, E. A.; Roddick, J. G. Changes in the alkaloid content of developing fruits of tomato (*Lycopersicon esculentum* Mill.). *J. Exp. Bot.* **1984a**, *35*, 252−260.

Eltayeb, E. A.; Roddick, J. G. Changes in alkaloid content of developing fruit of tomato (*Lycopersicon esculentum* Mill.). II. Effects of artificial acceleration and retardation of ripening. *J. Exp. Bot.* **1984b**, *35*, 261−267.

Friedman, M.; Levin, C. α-Tomatine content in tomato and tomato products determined by HPLC with pulsed amperometric detection. *J. Agric. Food Chem.* **1995**, *43*, 1507−1511.

Friedman, M.; McDonald, G. M. Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. *Crit. Rev. Plant Sci.* **1997**, *16*, 55−132.

Friedman, M.; McDonald, G. M. Steroidal glycoalkaloids. In *Naturally Occurring Glycosides*: *Chemistry, Distribution, and Biological Properties*; Ikan, R., Ed.; Wiley: Chichester, U.K., 1999; pp 311−342.

Friedman, M.; Levin, C. E.; McDonald, G. M. α-Tomatine determination in tomatoes by HPLC using pulsed amperometric detection. *J. Agric. Food Chem.* **1994**, *42*, 1959−1964.

Friedman, M.; Fitch, T. E.; Levin, C. E.; Yokoyama, W. H. Reduction of dietary cholesterol, plasma LDL cholesterol, and triglycerides in hamsters fed green and red tomatoes. Presented at the Division of Agricultural and Food Chemistry, National Meeting of the American Chemical Society, Las Vegas, NV, Sept 7−11, 1997a; Abstract AGFD 67.

Friedman, M.; Kozukue, N.; Harden, L. A. Structure of the tomato glycoalkaloid tomatidenol-3-*β*-lycotetraose (dehydrotomatine). *J. Agric. Food Chem*. **1997b**, *45*, 1541−1547.

Friedman, M.; Kozukue, N.; Harden, L. A Preparation and characterization of acid hydrolysis products of the tomato glycoalkaloid α-tomatine. *J. Agric. Food Chem*. **1998**, *46*, 2096−2101.

Heftmann, E.; Schwimmer, S. Degradation of tomatine to 3-*β*-hydroxy-5-α-pregn-16-en-20-one by ripe tomatoes. *Phytochemistry* **1972**, *11*, 2783−2787.

Juvik, J. A.; Stevens, M. A.; Rick, C. M. Survey of the Genus *Lycopersicon* for variability in α-tomatine content. *HortScience* **1982**, *17*, 764−766.

Kozukue, N.; Kozukue, E.; Yamashita, H.; Fujii, S. α-Tomatine purification and quantification in tomatoes by HPLC. *J. Food Sci*. **1994**, *59*, 1211−1212.

Laurila, J.; Laakso, I.; Valkonen, J. P. T.; Hiltunen, R.; Pehu, E. Formation of parental type and novel glycoalkaloids in somatic hybrids between *Solanum brevidens* and *S. tuberosum*. *Plant Sci*. **1996**, *118*, 145−155.

Moehs, C. P.; Allen, P. V.; Friedman, M.; Belknap, W. R. Cloning and expression of solanidine UDP-glucosyltransferase from potato. *Plant J*. **1997**, *11*, 227−236.

Petersen, H. W.; Molgaard, P.; Nyman, U.; Olsen, C. E. Chemotaxonomy of the tuber-bearing *Solanum* subspecies section Potato (Solanaceae). *Biochem. Syst. Ecol*. **1993**, *21*, 629−644.

Pstrzycka, J. Quantitative estimation of tomatine in the leaves and in the green fruits of tomato. *Herba Pol*. **1989**, *35*, 17−24.

Rayburn, J. R.; Friedman, M.; Bantle, J. A. Synergism of potato glycoalkaloids α-chaconine and α-solanine in the developmental toxicity of *Xenopus* embryos. *Food Chem. Toxicol*. **1995**, *33*, 1013−1019.

Roddick, J. G. Intracellular distribution of the steroidal glycoalkaloid α-tomatine in *Lycopersicon esculentum* fruit. *Phytochemistry* **1976**, *15*, 475−477.

Roddick, J. G.; Drysdale, R. B. Destabilization of liposome membranes by the steroidal glycoalkaloid α-tomatine. *Phytochemistry* **1994**, *23*, 543−547.

Sandrock, R. W.; Vanetten, H. D. Fungal sensitivity to an enzymatic degradation of the phytoanticipin α-tomatine. *Phytopathology* **1998**, *88*, 137−143.

Stanker, L. H.; Kamps-Holtzapple, C.; Friedman, M. Development and characterization of monoclonal antibodies that differentiate between potato and tomato glycoalkaloids and aglycones. *J. Agric. Food Chem*. **1994**, *42*, 2360−2366.

Stanker, L. H.; Kamps-Holtzapple, C.; Beier, R. C.; Levin, C. E.; Friedman, M. Comparison of ELISA and HPLC methods for detection and quantification of potato and tomato glycoalkaloids. *ACS Symp. Ser*. **1996**, *No. 621*, 243−255.

Stapleton, A.; Allen, P. V.; Friedman, M.; Belknap, W. R. Purification and characterization of solanidine glucosyltransferase from the potato (*Solanum tuberosum*). *J. Agric. Food Chem*. **1991**, *39*, 1187−1203.

Takagi, K.; Toyoda, M.; Shimizu, M.; Satoh, T.; Saito, Y. Determination of tomatine in foods by liquid chromatography after derivatization. *J. Chromatogr*. **1994**, *659*, 127−131.

Tukalo, E. A.; Ivanchenko, B. T. New procedure for determining tomatine content in plant raw material. *Rastit. Resur*. **1976**, *12*, 463−466 (in Russian).

van Gelder, W. M. J.; Jonker, H. H.; Huizing, H. J.; Scheffer, J. J. C. Capillary gas chromatography of steroidal alkaloids from *Solanaceae*. *J. Chromatogr*. **1988**, *442*, 133−145.

Voldrich, M.; Ondroušek, S.; Dobiáš, J. Steroid glycoalkaloids in fresh and processed tomatoes. *Potrav. Vedy* **1992**, *10*, 23−30.

Weissenberg, M.; Levy, A.; Svoboda, J. A.; Ishaaya, I. The effect of some *Solanum* steroidal alkaloids and glycoalkaloids on larvae of the red flour beetle: *Tribolium castaneum*, and tobacco hornworm, *Manduca sexta*. *Phytochemistry* **1998**, *47*, 203−209.

Zimowski, J. Characterization of UDP-galactose: tomatidine galactosyltransferase from tomato (*Lycopersicon esculentum*) leaves. *Acta Biochim. Pol*. **1994**, *41*, 202−204.

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