α-Tomatine Content in Tomato and Tomato Products Determined by HPLC with Pulsed Amperometric Detection

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Tomato plants (*Lycopersicon esculentum*) synthesize the glycoalkaloid α-tomatine, possibly as a defense against insects and other pests. As part of an effort to improve the safety of plant foods, the usefulness of a new HPLC pulsed amperometric detection (PAD) method for the direct analysis of α-tomatine in different parts of the tomato plant; in store-bought and field-grown, including transgenic, tomatoes; in a variety of commercial and home-processed tomato products; and in eggplant and tomatillos was evaluated. The method was found to be useful for analysis of a variety of products including high-tomatine calyxes, flowers, leaves, roots, and stems of the tomato plant (14–130 mg/100 g of fresh weight), low-tomatine red tomatoes (0.03–0.08 mg/100 g), intermediate-tomatine tomatoes (0.1–0.8 mg/100 g), and high-tomatine fresh and processed green, including pickled and fried, tomatoes (0.9–55 mg/100 g). No experimental difficulties were encountered with extraction and analysis of tomatine in complex foods such as tomato juice, ketchup, salsa, sauce, and sun-dried tomatoes. Microwaving and frying did not significantly affect tomatine levels of tomato foods. The tomatine content of fresh market and transgenic delayed-ripening varieties was not different from the range ordinarily seen in tomato. The possible usefulness of the findings to plant science, food safety, and human health is discussed.

Keywords: *Food safety; glycoalkaloids; HPLC-PAD; human health; processed tomatoes; α-tomatine analysis; tomato plants; pulsed amperometric detection*

INTRODUCTION

The glycoalkaloid α-tomatine was first isolated by U.S. Department of Agriculture (USDA) scientists from the wild tomato species *Lycopersicon pimpinelifolium* and the cultivated variety *Lycopersicon esculentum* (Fontaine et al., 1948, 1951). α-Tomatine disrupts cell membranes by lysing of liposomes. It is biosynthesized in the soluble phase of the cytoplasm of tomato fruit cells, not in the cell membranes as are other steroids (Roddick, 1976).

Scientists from this laboratory (Wilson et al., 1961) were apparently first to study the pharmacology and toxicology of α-tomatine. They reported that in mice α-tomatine (a) appears to be nontoxic following oral consumption except when fed in large doses, presumably because it is poorly absorbed from the digestive tract into the bloodstream; (b) irritated the eyes, skin, and stomach lining of the animals; and (c) caused rapid death of the animals when administered intravenously with an LD_{50} value equal to 18 mg/kg of body weight. The primary cause of death was a profound drop in blood pressure and hemolysis of red blood cells (Friedman, 1992; Keeler et al., 1991; Kyzlink et al., 1981; Nishie et al., 1975). α-Tomatine is also embryotoxic (Friedman et al., 1992) and has a strong affinity to cholesterol (Bloem et al., 1989; Micich, 1991; Heftmann and Schwimmer, 1972; Roddick, 1979).

α-Tomatine and other glycoalkaloids may be involved in the defenses of plants against phytopathogens (Ripperger and Schreiber, 1981; Roddick, 1974; Tingey, 1984). Specifically, the glycoalkaloid is reported to exert antifungal activity (Costa and Gaugler, 1989; Gallardo et al., 1990; Jiratko, 1993), to deter feeding of spruce budworms (Bentley et al., 1984), and to inhibit growth of the Mediterranean fruit fly (Chan and Tam, 1985), moths eggs (Chu and Lu, 1992), fruitworms (Elliger et al., 1981), spiny bollworm larvae (Weissenberg et al., 1986), and soybean looper larvae (Gallardo and Boethel, 1990).

These considerations show the need to accurately measure the tomatine content of plants, processed products, and body fluids and tissues. As part of a program to improve the safety of plant foods, we previously developed an HPLC method for α-tomatine using pulsed amperometric detection (PAD) (Friedman et al., 1994). α-Tomatine and a new glycoalkaloid (dehydrotomatine), which we discovered in commercial α-tomatine samples, separated well in our system.

In this study, we extend our previous findings by evaluating the applicability of the HPLC-PAD method to the analysis of α-tomatine in (a) different parts of the tomato plant; (b) different varieties of store-bought and field-grown, including transgenic, tomatoes; and (c) commercial and home-processed tomato products. Possible benefits of the findings and suggestions for future research are discussed.

MATERIALS AND METHODS

Materials. Solvents were of HPLC grade. Reagents were of ACS grade. α-Tomatine was obtained from Sigma (St. Louis, MO). Fresh and processed tomatoes, eggplants, and tomatillos were obtained from a local market. Field-grown control and transgenic tomatoes were donated by DNA Plant Technology Corp., Oakland, CA. Whole tomato plants and greenhouse-grown green tomatoes derived from tissue culture variants were a gift of Dr. Merle L. Weaver of this laboratory.

The HPLC eluent for α-tomatine analysis was prepared by combining 100 mL of concentrated buffer with 550 mL of polished water, 200 mL of acetonitrile, and 150 mL of

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methanol. Water was polished by passing it through a C_{18} solid phase extraction (SPE) device (Supelclean Envi 18 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 C_{18} SPE device.

Instrumentation. A Dionex Series 4500i gradient liquid chromatography system with a Dionex Ionchrom pulsed amperometric detector and a Spectra-Physics ChromJet integrator were used. The original cell was replaced with a newer cell designed for use with organic/aqueous eluents (Dionex part no. 42867). The working electrode was gold, the counter electrode was stainless steel, and the reference electrode was a silver/silver chloride combination. The cell design was thin layer. Applied potentials and their durations were as follows: $E_1 = 0.6$ V, $t_1 = 120$ ms; $E_2 = 1.0$ V, $t_2 = 420$ ms; $E_3 = -0.35$ V, $t_3 = 420$ ms. Response time was set to 1 s. The sampling period was 16.67 ms. The integrator attenuation was set to 1024. Sensitivity was controlled by the output range setting and was set at 100 or 300 nA.

The chromatography column for α-tomatine 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).

Chart speed was set to 0.5 cm/min. Flow rate was set to 1.0 mL/min, and eluent was recycled. The electrode was left on, and eluent was continuously recirculated from a 2 L vessel. We changed the eluent for a fresh solution after 2 months of use.

Methods. Cubed fresh tomatoes, eggplant, and tomatillos and processed tomatoes 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 a Omnimixer (Ivan Sorvall Inc., Newtown, CT) so they passed through a 0.5 mm screen. Ketchup was difficult to completely dry and grind. The product after drying was similar to tar. For this sample we started with 2.8 g of fresh material, which we calculated to be equivalent to 1 g of dried material, and extracted as usual.

Fried green tomatoes and microwaved tomatoes were prepared in the laboratory from green tomatoes obtained from Merle Weaver. The fried green tomatoes were prepared by following a cookbook recipe (Claiborne, 1992). Briefly, tomatoes were sliced 1/4 in. thick, breaded by tossing with equal parts flour and cornmeal, fried in 1/4 in. of oil in a skillet until golden brown, and drained on paper towels. The microwaved tomatoes were prepared by microwaving 400 g of fresh tomatoes on high for 5 min. Both samples were freeze-dried as usual.

Tomatoes 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 solid phase extraction (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 about 30 mL) was applied and allowed to gravity drip. When the sample was fully absorbed onto the packing, the tube was washed with about 10 mL of water, followed by 5 mL of 30:70 acetonitrile/1% NH_4OH and then 5 mL of water. The α-tomatine was eluted with 10 mL of 70:30 acetonitrile/pH 3 citric acid/disodium phosphate buffer (as used in the eluent). The organic solvent was then evaporated off. The aqueous residue was basified with ammonia water and extracted twice into water-saturated butanol, using a separatory funnel. 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.

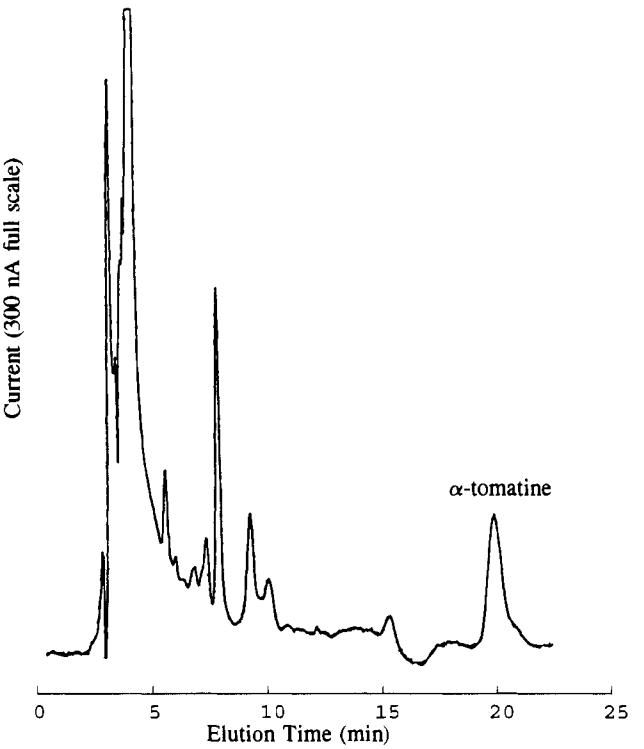

Figure 1. Chromatogram of tomato sauce, 25 mg of dried powder containing 1.6 μg of α-tomatine. Conditions: column, Supelcosil LC-ABZ, 5 μm, 4.6 × 250 mm; flow rate, 1 mL/min; eluent, 20% acetonitrile, 15% methanol, 100 mM disodium phosphate/citrate buffer, pH 3; detection by PAD.

RESULTS AND DISCUSSION

α-Tomatine Content of Parts of the Tomato Plant. Figure 1 shows a typical HPLC chromatogram of an extract of a tomato product, e.g. tomato sauce. Table 1 shows the α-tomatine levels in different parts of a tomato plant in terms of fresh and dry weight. From medium-small red fruit containing 0.19 mg of α-tomatine/100 g of fresh weight to flowers containing 130 mg/100 g, there was about a 700-fold variation. Ripe fruit contained the lowest levels of α-tomatine relative to any other part of the plant. As the green fruit matured from small (0.8–2.5 cm) to medium-small (2.5–4.0 cm), α-tomatine decreased by 65%. As it ripened to red, the level of the glycoalkaloid decreased by another 98.5%. These results strikingly demonstrate that the α-tomatine content decreases dramatically on maturity of the fruit, presumably due to enzymatic modification (Heftmann and Schwimmer, 1973). The high levels of α-tomatine in the other parts of the plant probably protect them against insects and fungal pathogens. Animals consuming tomato leaves would ingest large amounts of tomatine.

α-Tomatine Content of Whole Tomatoes. Tables 2 and 3 list the α-tomatine content of 12 tomato varieties, eggplant, and tomatillos purchased in local grocery stores. The values for fresh tomatoes ranged from 0.03 mg/100 g for the standard variety to 1.6 mg/100 g for unripe green tomatoes, about a 50-fold difference. Most of the standard-sized ripe varieties, including standard, Roma, Green Zebra, and Beefsteak tomatoes, fell in the lower range of values. Green Zebra is a variety that remains green even when ripe. Cherry tomatoes as a group had higher α-tomatine levels than the standard-sized varieties, but no other relation of α-tomatine content to size was evident (Table 3).

Table 1. α-Tomatine in Various Parts of the Tomato Plant

plant part	duplicate α-tomatine determinations, mg/100 g dry wt	fresh wt
small red fruit	2.2, 2.6	0.176, 0.207
roots	128, 141	13.3, 14.6
large immature green fruit	164, 154	17.4, 16.3
large stems	286, 289	54.3, 54.9
small immature green fruit	463, 457	55.1, 54.4
calyxes	562, 572	92.7, 94.4
leaves	645, 636	115, 113
small stems	677, 706	103, 107
flowers	738, 753	128, 131

The lack of such a correlation suggests multifactorial causes for the observed tomatine levels in plants. Thus, Eltayeb and Roddick (1984a) found that both growth and ripening processes may contribute to the decline in fruit tomatine. Other possibilities include nitrogen content of the soil, the presence of phytopathogens in the field, and the use or nonuse of insecticides to control plant pests. Environmental factors that stress the plant are expected to induce the biosynthesis of defensive allelochemicals including tomatine (Ames, 1983).

Our study also revealed that eggplants (*Solanum melongena*) contained no α-tomatine and that α-tomatine in tomatillos (*Physalis ixocarpa*) was low. It is interesting that tomatillos contain α-tomatine at all because they are not closely related to tomatoes, although they are in the same Solanaceae family.

Table 4 compares the values for α-tomatine of field-grown control and slow-ripening transgenic tomatoes at different stages of maturity (Eltayeb and Roddick, 1984a; Heftmann and Schwimmer, 1973). The data show that the α-tomatine decreases with increasing maturity of the fruit and that the values for the transgenic tomatoes did not differ from the parent control or commercial varieties grown under the same conditions. These findings suggest that suppression of the genes that control the biosynthesis of the plant hormone ethylene through anti-sense RNA or related techniques (Klee and Romano, 1994), resulting in transgenic tomatoes which take much longer to ripen on the vine, does not affect α-tomatine biosynthesis.

Initiation of fruit ripening induced by the compound Ethrel (ethephon), which releases ethylene, induced a lowering of tomatine levels in fruit compared to untreated controls (Eltayeb and Roddick, 1984b).

Previously, we reported that transgenic tomatoes with a different suppressed gene (Redenbaugh and Hiatt, 1993) also had normal tomatine levels (Friedman et al., 1994).

α-Tomatine Content of Processed Tomato Products. Table 5 lists the α-tomatine content of about a dozen commercial and home-processed tomato products. The values range from 0.11 mg/100 g of fresh weight for stewed red tomatoes to 7.1 mg/100 g of fresh weight for one brand of pickled green tomatoes. Results are reported per serving size as well as by weight. Although products such as ketchup and sun-dried tomatoes have a high α-tomatine content per weight, the level per serving size falls within a normal range.

Processing evidently has a minor effect on α-tomatine since the values per unit of weight (adjusted for moisture changes) are of the same order as in the fresh tomatoes from which the products were made. This is confirmed by our own studies of two processing techniques, microwaving and frying. Our results show that microwaving green tomatoes caused a 7% loss of α-tomatine and that preparation of fried green tomatoes caused a 22% loss (adjusting for added ingredients and moisture changes).

Table 2. α-Tomatine Content of Commercially Available Tomatoes, Eggplant, and Tomatillos

sample	duplicate α-tomatine determinations, mg/100 g of fruit dry wt A	dry wt B	fresh wt A	fresh wt B
eggplant	0	0	0	0
standard tomato	0.4	0.4	0.03	0.03
tomatillos	0.6	0.6	0.05	0.05
Roma	0.7	0.6	0.04	0.03
Green Zebra, ripe	0.8	0.8	0.06	0.06
Beefsteak	1.6	1.4	0.09	0.08
red pear cherry	1.6	1.7	0.13	0.13
yellow, large	2.0	1.9	0.11	0.10
yellow, medium	2.2	2.0	0.13	0.12
red cherry	4.0	4.0	0.27	0.27
yellow pear cherry	6.1	6.0	0.45	0.44
yellow cherry	6.9	7.4	0.9	1.0
red Sungold cherry	9.9	11.1	1.0	1.1
green, large, unripe	26.2	25.6	1.6	1.6

Table 3. Average α-Tomatine Content per Fruit

sample	av fruit size, g	α-tomatine fresh fruit, mg/100 g	α-tomatine fresh fruit, mg/fruit
ripe			
Sungold cherry	3.9	1.1	0.04
red pear cherry	6.5	0.13	0.01
yellow pear cherry	10.3	0.45	0.05
yellow cherry	11.4	0.97	0.11
tomatillos	34.0	0.05	0.02
Green Zebra	66.9	0.06	0.04
standard	123	0.03	0.04
large yellow	227	0.11	0.24
unripe			
small immature green	3.4	54.8	1.86
medium immature green	17.1	16.9	2.88
large immature green	37.9	1.0	0.39
pickled green	80.0	2.8	2.20
green	127	1.6	2.04

The USDA Nationwide Food Consumption Survey (USDA, 1993) reports that the average tomato consumption for those people that consume tomatoes was 45 g/day. This value includes tomatoes from mixed food sources, such as casseroles, as well as fresh and processed tomatoes. If we assume an α-tomatine value of 0.03 mg/100 g, the amount of tomatine in standard tomatoes, the average tomato eater consumes 0.0135 mg of tomatine/day. Although we do not know at this time what a safe level of consumption is, this seems to be relatively low. A greater concern is the level of α-tomatine intake in those individuals consuming products high in tomatine. For example, one serving of pickled green tomatoes, brand Y, contains 2.9 mg of α-tomatine, 212 times the average daily intake. One serving (one tablespoon) of green salsa made from green tomatoes, but not from tomatillos, contains 0.29 mg. If one were to consume the whole 8 oz (240 g) bottle, not an unreasonable amount, that individual would ingest 4.6 mg of α-tomatine, or about 340 times more than the consumer of red tomatoes.

These considerations suggest that the consumer of green tomato products needs to be aware of the high amounts of α-tomatine present in these products. Although green tomato products are not consumed as widely as those derived from red tomatoes, they appear

Table 4. α-Tomatine Content as a Function of Ripeness of Transgenic Tomatoes and Their Controls

variety	duplicate or quadruplicate α-tomatine determinations at different stages of ripeness, mg/100 g of fresh tomato fruit			
	immature green	mature green	breaker	red[a]
standard size				
LF (commercial)	4.0, 4.0	2.6, 2.5	0.67, 0.82, 0.73, 0.70	0.12, 0.12
91103−114 (parent)	3.4, 3.6	0.68, 0.68	0.36, 0.33	0.07, 0.07
1345 (transgenic)	1.2, 1.2, 1.4, 1.1	1.6, 1.5	0.60, 0.63, 0.62, 0.63	0.11, 0.11
cherry				
Baxter Early Bush (commercial)	21, 21	5.3, 5.8	1.4, 1.4	0.25, 0.24
B316 (transgenic)	29, 28	5.2, 5.2	2.7, 2.7	0.39, 0.38
B324 (transgenic)	19, 19	5.9, 5.7	2.1, 2.1	0.26, 0.25

[a] Standard variety 91103−114 and Baxters Early Bush cherry variety were allowed to ripen without ethylene treatment. Others were ripened with ethylene (DNA Plant Technology Corp., private communication, 1994).

Table 5. α-Tomatine Content of Processed Tomato Products

sample	duplicate α-tomatine determinations, mg/100 g		serving size[a]	α-tomatine per serving, mg
	dry sample	fresh sample		
condensed tomato soup, brand X	0.7, 0.7	0.16, 0.16	half cup (125 g)	0.20
condensed tomato soup, brand Y	0.8, 0.9	0.14, 0.15	half cup (125 g)	0.19
condensed tomato soup, brand Z	2.2, 2.6	0.32, 0.37	half cup (125 g)	0.43
stewed red	2.0, 1.9	0.11, 0.11	half cup (128 g)	0.14
sun-dried red	2.3, 2.3	2.1, 2.1	two half pieces (6 g)	0.13
ketchup	2.5, 2.4	0.88, 0.84	one tablespoon (15 g)	0.13
fried green[b]	4.3, 4.4	1.1, 1.1	one-fourth recipe (133 g)	1.5
juice	4.8, 4.9	0.27, 0.28	6 fl oz (183 g)	0.50
red sauce	6.4, 5.6	0.60, 0.53	one-fourth cup (62 g)	0.35
green salsa, brand X	0, 0	0, 0	one tablespoon (15 g)	0
microwaved green[b]	13.0, 13.8	1.1, 1.2	half cup (128 g)	1.5
green salsa, brand Y	20.9, 20.1	1.9, 1.9	one tablespoon (15 g)	0.29
pickled green, brand X	34.3, 36.3	2.7, 2.8	half fruit (40 g)	1.1
pickled green, brand Y	99.3, 98.4	7.2, 7.1	half fruit (40 g)	2.9

[a] Determined from information on the label or from reference amount (FDA, 1994). Serving size of fried green tomatoes was determined by splitting the recipe into four equal parts. [b] Prepared in the laboratory.

in numerous recipes including fried green tomatoes, pickled green tomatoes, cocktail sauces, tartar sauces, chutney, marmalades, and other products (Clairborne, 1992; Kibler et al., 1985; Šimeková and Horčin, 1980; Voldřich et al., 1992; White, 1989).

Conclusions. The results show that the improved extraction and HPLC-PAD assay for α-tomatine can be used for both low- and high-α-tomatine tomatoes and tomato plant parts and for a variety of processed tomato products sold commercially or prepared in the kitchen. This direct method avoids chemical modification of tomatine prior to analysis, such as hydrolysis to the aglycon tomatidine or acetylation of the carbohydrate groups (Juvik et al., 1982; Rick et al., 1994; Tagaki et al., 1994). It should therefore find application in plant-host resistance, plant breeding, plant molecular biology, and cell culture studies designed to control the biosynthesis of tomatine and related glycoalkaloids and to define the role of tomatine in nature. It should also be adopted by the food industry to measure the tomatine content during the preparation of commercial tomato products. Other uses that merit study are in pharmacology and toxicology, where a need exists for a reliable method to follow the distribution of tomatine in different organs and body fluids and its metabolism to new compounds.

Our survey of tomato products may also help dieticians and consumers design diets that minimize the α-tomatine content for individuals with compromised health who may be susceptible to α-tomatine's potent pharmacological and toxicological effects.

The results of the present study on the distribution of α-tomatine in foods complement previously described HPLC methods for the analysis of tropane alkaloids atropine and scopolamine (Friedman and Levin, 1989) and glycoalkaloids and metabolites in potatoes and potato products (Dao and Friedman, 1994; Friedman and Dao, 1992; Friedman and Levin, 1992; Friedman et al., 1993). The cited studies developed both theoretical and practical foundations to facilitate and optimize HPLC of closely related glycoalkaloids and aglycons. The HPLC data on α-tomatine and other glycoalkaloids should also facilitate development of new, improved immunoassays for glycoalkaloids. This effort, now in progress (Stanker et al., 1994), attempts to correlate data from immunoassays with those obtained by HPLC. Further improvements in the analysis of glycoalkaloids are expected.

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