High-Performance Liquid Chromatographic Determination of the Tomato Glycoalkaloid, Tomatine, in Green and Red Tomatoes

Rodney J. Bushway,*,† Lewis B. Perkins,† Lance R. Paradis,† and Steve Vanderpan‡

Department of Food Science, 5736 Holmes Hall, University of Maine, Orono, Maine 04469-5736, and Calgene Inc., 1920 Fifth Street, Davis, California 95616

A high-performance liquid chromatographic method has been developed to quantify tomatine in green and red tomatoes using UV detection at 205 nm. Extraction was performed by blending tomatoes with tetrahydrofuran–water–acetonitrile–acetic acid (50:30:20:1). C_{18} Sep-Paks and alumina were employed for cleanup. Average percent recoveries of tomatine from mature green tomatoes ranged from 77 to 91, while the average percent recoveries from red tomatoes varied from 65 to 114. Tomatine in mature green tomatoes ranged from none detected to 8.79 mg/100 g of fresh weight, while tomatine in red tomatoes varied from none detected to 2.31 mg/100 g of fresh weight. Only 5 of 100 red tomatoes showed detectable levels of tomatine, but 61 of 80 mature green tomatoes had detectable amounts of tomatine. α-Solanine was not detected. Mass spectral and HPLC data indicate that there may be other glycoalkaloids in tomatoes besides tomatine, but they appear to be minor. No real differences in the glycoalkaloid levels between nontransgenic and transgenic tomatoes were observed.

Keywords: *HPLC; tomatine; tomato glycoalkaloids*

INTRODUCTION

Tomatoes are the third most popular vegetable consumed in the United States (Margen, 1992). To obtain sufficient shelf life for fresh tomatoes, they must be bred with certain characteristics that are not conducive to flavor and must be picked green. With the recent advances in biotechnology it is possible to maintain the flavor characteristics of fresh tomatoes without losing shelf life. However, tomatoes, like potatoes, belong to the Solanaceae family, meaning that they contain glycoalkaloids.

Steroidal glycoalkaloids have been shown to exhibit toxic properties by many (Bushway et al., 1987; Freidman, 1992; Jadhav et al., 1981; Keeler, 1986; Morris and Lee, 1984; Nischie et al., 1975; Roddick, 1974; Sharma and Salunkhe, 1985; Surak and Denning, 1978), in particular the potato glycoalkaloids (Bushway et al., 1987; Freidman, 1992; Jadhav et al., 1981; Keeler, 1986; Morris and Lee, 1984, Nischie et al., 1975; Sharma and Salunkhe, 1985) and to a lesser extent the tomato glycoalkaloid, tomatine (Bushway et al., 1987; Jadhav et al., 1981; Keeler, 1986; Nischie et al., 1975; Roddick, 1974; Surak and Denning, 1978). Because biotechnology techniques could conceivably increase the levels of glycoalkaloids and because glycoalkaloids are toxic, it is necessary to analyze transgenic Solanaceae vegetables for glycoalkaloids.

Most analytical methods for the determination of glycoalkaloids have focused on potato glycoalkaloids (Bushway et al., 1986; Carman et al., 1986; Freidman, 1992; Saito et al., 1990) since they are easier to analyze and since glycoalkaloids in red tomatoes usually undergo degradation (Eltayeb and Roddick, 1984, 1985; Heftmann and Schwimmer, 1972; Roddick, 1974). Therefore, methods for the analysis of tomatine in tomatoes

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* Author to whom correspondence should be addressed.
† University of Maine.
‡ Calgene, Inc.

are few and for the most part do not employ advanced chromatographic techniques (Bajaj et al., 1987; Heftmann and Schwimmer, 1973; Oleszek et al., 1986; Roddick and Butcher, 1972). Van Gelder et al. (Van Gelder and DePonti, 1987; Van Gelder et al., 1988) were the first to apply a modern chromatographic technique for the analysis of tomatine in tomatoes. They developed methods using capillary gas chromatography (GC) equipped with a nitrogen–phosphorus detector. However, there are two disadvantages of employing GC analysis for glycoalkaloids. One is that glycoalkaloids must be hydrolyzed to their corresponding alkaloids, and second, the GC temperatures needed for analysis are high, which can cause column deterioration and peak artifacts.

Recently two other methods were developed for the analysis of tomatine in tomatoes. The first was an HPLC method that uses a derivatization technique (Takagi et al., 1994), and the other was a MS/MS procedure (Chen et al., 1994).

This paper describes a high-performance liquid chromatographic (HPLC) method that was developed to analyze tomatine without derivatization in mature green and red tomatoes. Futhermore, the HPLC procedure was used to determine tomatine levels in nontransgenic vs transgenic green and red tomatoes.

MATERIALS AND METHODS

Samples. Tomato samples were obtained from Calgene Fresh Inc. (Davis, CA) and from local markets in the Bangor, ME, area. Calgene samples were picked fresh and shipped by overnight express. The tomatoes were refrigerated upon arrival, and samples were extracted immediately.

Reagents. All solvents were obtained from EM Science (Gibbstown, NJ) and were of HPLC grade except for the glacial acetic acid and phosphoric acid, which were of reagent grade. Tetrahydrofuran was nonstabilized UV grade. Glycoalkaloid standards, α-chaconine and tomatine, were purchased from Sigma Chemical Co. (St. Louis, MO), while α-solanine was isolated by using the procedure of Bushway (1983). The

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purities of the glycoalkaloids were as follows: α-chaconine, 95%; α-solanine, 96%; and tomatine, 80%. Although Sigma states that their tomatine is 95% pure, we found by LC/MS that the sample we received was actually 80% pure.

Standard Preparation. Tomatine (147 mg) was weighed into a 50 mL volumetric flask and diluted to volume with tetrahydrofuran—water—acetonitrile (50:30:20). This was the stock solution which was stable for 2 months at 4 °C. A working standard was prepared by diluting the stock standard 1/10 in tetrahydrofuran—water—acetonitrile (50:30:20). The α-chaconine and α-solanine stock standards were made by accurately weighing approximately 15 mg of each into separate 50 mL volumetric flasks. The flasks were brought to volume with the 50:30:20 solution of tetrahydrofuran—water—acetonitrile. Working standards were prepared by making 1/5 dilutions of the stock standards using the tetrahydrofuran mixture.

Sample Extraction. Tomatoes were homogenized in a Waring blender (1 qt glass container) to ensure that the samples were homogeneous. A 55 g subsample of the homogenate was weighed into another glass blender jar to which 50 mL of tetrahydrofuran—water—acetonitrile—acetic acid (50:30:20:1) was added. The mixture was blended for 3 min at medium speed and then filtered through rapid fluted filter paper (VWR No. 28331-048, Boston, MA). A 25 mL aliquot of the filtrate was transferred to a 250 mL round-bottom flask and evaporated on a Buchi rotary evaporator at 45 °C until approximately 10 mL was left. To this remaining 10 mL was added 25 mL of 0.02 M 1-heptanesulfonic acid containing 1% glacial acetic acid. This mixture was sonicated before being concentrated on tC$_{18}$ Sep-Paks (Waters Associates, Milford, MA).

Cleanup and Concentration. Sep-Pak tC$_{18}$s were employed for the concentration and initial cleanup steps. Each Sep-Pak was conditioned by passing 5 mL of methanol through it followed by 5 mL of 0.02 M 1-heptanesulfonic acid (1%) acetic acid. Once conditioned, the entire sample was passed through the Sep-Pak. Next, 5 mL of acetonitrile—water (20:80) was run through the C$_{18}$. The Sep-Pak was then dried for 2 min under vacuum before eluting with 4 mL of tetrahydrofuran—water—acetonitrile (50:30:20), but only the first 2 mL was collected. A 0.5 mL aliquot from the C$_{18}$ was passed through a Pasteur pipet packed with 1.5 cm of acid alumina type WA-4 (Sigma). A 5 μL aliquot from the alumina column was injected into the HPLC.

Apparatus. The HPLC system consisted of a Hewlett-Packard (HP, Avondale, PA) 1050 isocratic pump, a HP 1050 autosampler, and a HP 1040A photodiode array detector/intergrator system with an updated quartz flow cell, computer, and software.

Chromatography. An Ultremex C$_6$ 5 μm (stainless steel, 15 cm × 4.6 mm i.d.) (Phenomenex, Torrance, CA) column was employed for the separation along with a mobile phase of water—acetonitrile—methanol—0.1 M ammonium phosphate buffer, pH 3.5, using phosphoric acid (58:26:11:5) (use good analytical technique when preparing the mobile phases since a slight change in composition will cause problems in the separation) at a flow rate of 1.0 mL/min. The injection volume was 5 μL for both standards and samples. Detection was at 205 nm. Peak area was used for the quantitation.

Linearity Study. An 11-point standard curve was prepared by making serial dilutions of the tomatine working standard (1/10, 1/5, 1/2.5, 1/2, 1/1, and no dilution) which yielded the first 6 points, while the other 5 points were made by serially diluting the tomatine stock standard (1/5, 1/4, 1/3, 1/2, and no dilution). After injection, peak area was measured and the curve was made by plotting peak area vs tomatine concentration in micrograms per milliliter.

Spike Study. Tomatine was added at concentrations of 1.4, 7.2, 18, and 36 mg/100 g of fresh weight to green and red tomatoes. This was performed by adding 0.8, 4.0, 10, and 20 mg of tomatine/55 g of homogeneous sample. The 0.8 and 4.0 mg fortifications were made from the stock solution, while the 10 and 20 mg spikes were prepared by adding solid tomatine. There were four replications done at each fortification level.

Solanine was fortified into green and red tomatoes at levels of 0.8 and 1.42 mg/100 g of fresh weight, respectively. This was done by adding 0.439 and 0.793 mg/55 g of homogeneous sample. These fortifications were made by removing an aliquot from the stock standard. No replications of these spikes were performed. α-Chaconine was not used as a spike, but it should have a recovery similar to that of α-solanine since each compound is very similar in structure and properties. Also, if α-chaconine was present, it would elute from the HPLC system just before α-solanine.

Mass Spectroscopy: instrument, SCIEX API III biomolecular mass analyzer; interface, ion spray; mode, Q1 positive MS (150—1500 amu); parameters, ISV = 4800, OR = 35, MU = 4200, CGT = 0. HPLC condtions: column, Ultremex; mobile phase, 550 mL of water, 250 mL of acetonitrile, 100 mL of methanol, 50 mL of 0.1 M ammonium acetate, pH 3.5, with acetic acid; flow rate, 0.85 mL/min; split ratio, 950:50 μL; injection volume, 20 μL; run time, 16 min. Acetate buffer was employed in place of the phosphate buffer since phosphate buffer interferes with the mass spectrometer.
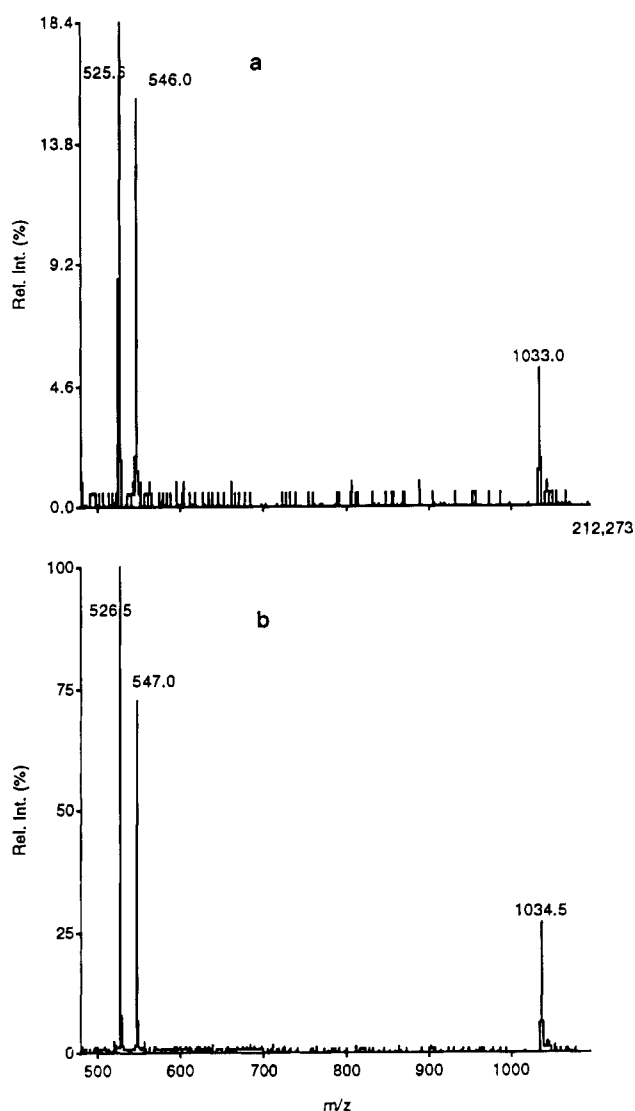
RESULTS AND DISCUSSION

Tomatine has a wide range of linearity (23.5—2352 μg/mL, which yielded a correlation coefficient of 0.999) at 205 nm. In fact, it covers a 100-fold concentration range. Thus, no dilutions would have to be performed on tomatoes containing up to 34 mg of tomatine/100 g of fresh weight of tomatoes. The lower limit of detection was ascertained to be 0.25 mg of tomatine/100 g of fresh weight of tomatoes, while the limit of quantitation was determined to be 0.5 mg/100 g of fresh weight (ACS, 1980), which is more than adequate for tomatine analysis of tomatoes.

Purity of the standard is an extremely important value in any analysis. For this study we purchased tomatine from Sigma Chemical Co. and the stated purity was 95%. However, when the standard was injected into our HPLC system, two peaks of approximately equal area were observed with very similar and typical glycoalkaloid UV spectra from 190 to 350 nm. (Glycoalkaloids' UV spectra are very simple in that they have very few chromophores in their molecules to create any spectral details or high extinction coefficients. The nitrogen, oxygen, and double bond, if present, are the only UV absorbing groups, and they absorb at very low UV wavelengths.) A LC/MS analysis was performed on the standard, and the tomatine was found to be the later eluting peak. From the mass spectroscopy work it was determined that the standard had 80% tomatine (*m/z* 1035 ion, which corresponds to M + H ion for tomatine since the MW of tomatine is 1034) (Figures 1 and 2) and 20% impurity (*m/z* 1033 ion, which corresponds to a double bond in the alkaloid portion of tomatine) (Figures 1 and 2). The HPLC results also indicate that this impurity may be tomatine with a double bond in the tomatidine portion of the molecule since double bonds cause an increase in UV absorption at 205 nm and this is what was seen in the HPLC chromatogram (Figure 3). Both peaks had similar areas, but the mass spectral results demonstrated the impurity was 4-fold less in concentration than tomatine. The impurity was linear from 5.9 to 556 μg at 205 nm with a correlation coefficient of 0.999.

Since tomatine belongs to the class of glycoalkaloids that do not have double bonds, analysis by HPLC/UV detection becomes difficult. Thus, it was imperative to do a concentration and cleanup procedure before tomatine could be quantified. Carman et al. (1986) developed for potato glycoalkaloids one of the easiest ways to
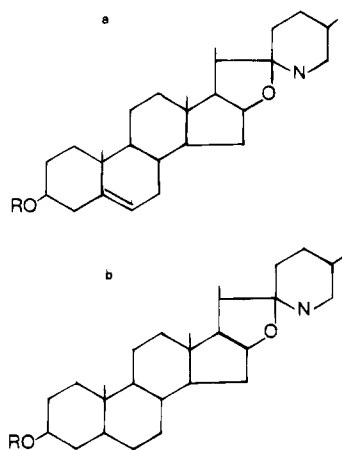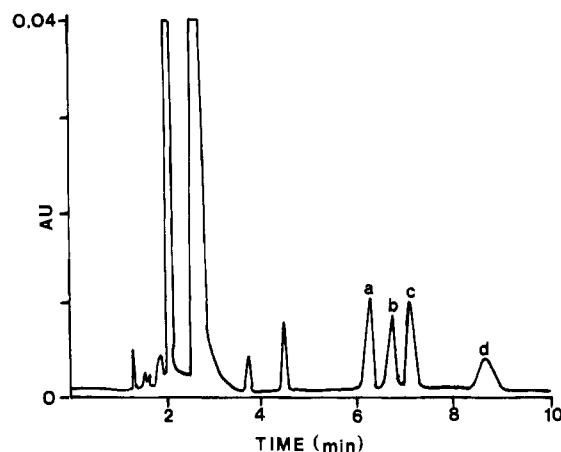
Figure 1. Structure a is the proposed tomatine-like glycoalkaloid in which tomatidine is replaced by tomatidenol. Structure b is tomatine. R is galactose-glucose-glucose-xylose for both structures.

Figure 3. HPLC chromatogram of tomatine (conditions given in text). Peak a is the tomatine-like glycoalkaloid, peak b is commersonine, peak c is tomatine, and peak d is α-solanine.

Table 1. Recovery of Tomatine Added to Green Tomatoes

tomatine added, mg/100 g	mean % recovery[a]	%CV[a]
1.4	85	22
7.2	91	17
18	84	14
36	87	16

[a] Means and percent coefficients of variation based on four determinations, except the 1.4 mg/100 g spike which was three because of sample loss. Overall mean percent recovery was 87%.

Table 2. Recovery of Tomatine Added to Red Tomatoes

tomatine added, mg/100 g	mean % recovery[a]	%CV[a]
1.4	65	17
7.2	104	19
18	114	11
36	112	11

[a] Means and percent coefficients of variation based on four determinations. Overall mean recovery was 99%.

Figure 2. Positive ion LC/MS spectra (M + H) of tomatine standard. Spectrum a (HPLC peak 1) is the tomatine-like compound with m/z ions at 526, 546, and 1033. Spectrum b (HPLC peak 2) is tomatine with m/z ions at 526, 547, and 1035.

concentrate and clean up glycoalkaloids from an aqueous system. They employed ion pairing in conjunction with C_{18}. This system was tried on the tomatine standard and it worked well, but with tomatoes there were interferences. Therefore, after the initial C_{18} cleanup, another cleanup step was added using acid alumina. The combination of both methods made it possible to analyze tomatine in tomatoes.

To test the efficiency of the entire tomatine analysis for tomatoes, a recovery study was performed on mature green and red tomatoes. The results are given in Tables 1 and 2. Both green and red tomatoes were fortified at four concentrations (1.4, 7.2, 18, and 36 mg of tomatine/100 g of fresh weight of tomatoes) with four determinations made for each concentration. Mean recoveries for the green tomatoes ranged from 84 to 91% with percent coefficients of variation varying from 14 to 22, while the mean recoveries for the red tomatoes ranged from 65 to 114% with percent coefficients of variation varying from 11 to 19. These results indicate that the method is sufficiently accurate and reproducible for the quantitation of tomatine in red and green tomatoes. A true blank was run with this method whereby only solvent was taken through the complete procedure. The only peaks present in the blank (none of which interfered with the analyses) were from the glycoalkaloid solvent system (tetrahydrofuran—water—acetonitrile—acetic acid), which was proven by injecting the mixture into the HPLC system.

Nonspike green and red tomatoes were also analyzed with this method (Tables 3 and 4). The red and green

Table 3. Amount of Tomatine Found in Green Tomatoes

| tomato variety	sample	tomatine, mg/100 g	tomato variety	sample	tomatine, mg/100 g
CR3-613-12[T] [a]	1	2.17	sunbelt[NT] [c]	1	ND
	2	1.90		2	ND
	3	1.57		3	ND
	4	2.67		4	1.35
	5	1.56		5	ND
	6	6.49		6	2.07
	7	3.92		7	1.33
	8	3.63		8	D[d]
	9	1.57		9	ND
	10	1.22		10	ND
	11	0.82		11	ND
	12	0.98		12	ND
	13	3.42		13	1.32
	14	ND[b]		14	0.60
	15	3.55		15	ND
	16	ND		16	1.60
	17	ND		17	D
	18	ND		18	ND
	19	ND		19	ND
	20	ND		20	1.00
CRC-623-19[T]	1	1.78	1011[NT]	1	1.78
	2	5.00		2	D
	3	2.77		3	1.44
	4	5.43		4	1.04
	5	7.90		5	1.40
	6	2.94		6	6.48
	7	2.89		7	1.88
	8	3.98		8	1.12
	9	3.76		9	2.05
	10	2.01		10	2.51
	11	1.61		11	2.25
	12	1.86		12	1.44
	13	1.82		13	ND
	14	3.04		14	2.11
	15	0.91		15	4.16
	16	8.79		16	1.03
	17	1.40		17	ND
	18	2.43		18	2.25
	19	3.42		19	1.25
	20	1.49		20	1.41

[a] T, transgenic variety. [b] ND, none detected at a detection limit of 0.25 mg/100 g of fresh weight. [c] NT, nontransgenic variety. [d] D, detected at the lower limit of detection and tomatine is between 0.25 and 0.49 mg/100 g of fresh weight.

Table 4. Amount of Tomatine Found in Red Tomatoes

tomato variety	sample	tomatine, mg/100 g
Mountain Springs[NT] [a]	1–10, 12–20	ND[b]
	11	0.88
CR3-613-12[T] [c]	1–19	ND
CR3-623-19[T]	1–9, 11–20	ND
	10	1.09
Sunbelt[NT]	1–10, 12–20	ND
	11	0.68
1011[NT]	1–6, 8–16, 18–20	ND
	7	1.71
	17	2.31

[a] NT, nontransgenic variety. [b] ND, none detected at a detection limit of 0.25 mg/100 g of fresh weight. [c] T, transgenic variety.

tomatoes were all approximately the same weight (90–110 g) and diameter (6–7 cm). Typical LC chromatograms of green and red tomatoes are shown in Figures 4 and 5. Tomatine levels were measured in 80 mature green tomatoes (20 tomatoes in each of four cultivars). Two of the cultivars (CR3-613-12 and CR3-623-19) were transgenic, while the other two (Sunbelt and 1011) were not. Overall, the tomatine content in green tomatoes ranged from none detected to 8.79 mg of tomatine/100 g of fresh weight of tomatoes. A further breakdown by cultivars demonstrates that CR3-613-12 had 6 samples containing no detectable levels of tomatine and 14
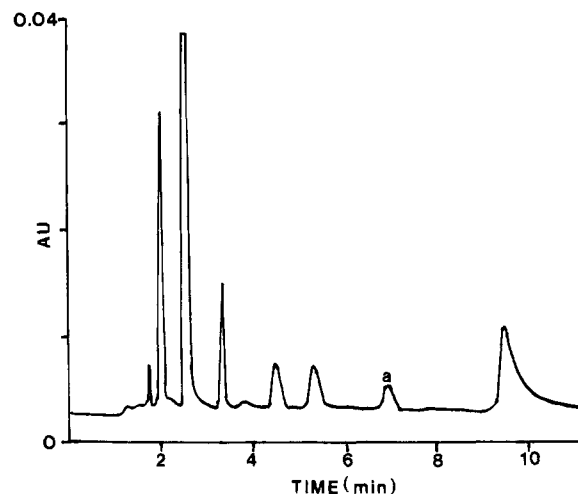
Figure 4. HPLC chromatogram of a green tomato extract from variety 1011. Peak a is tomatine.
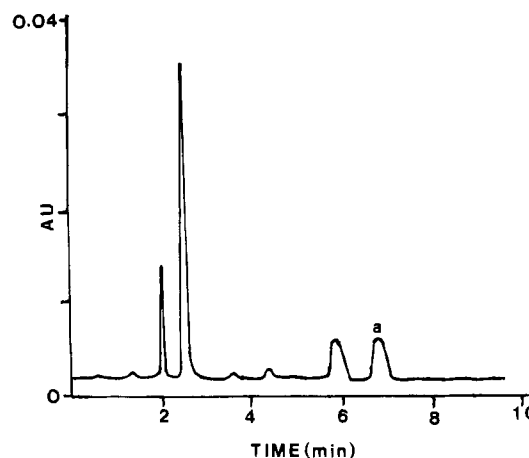
Figure 5. HPLC chromatogram of a red tomato extract from variety 1011. Peak a is tomatine.

positive tomatoes with a mean of 2.53 mg/100 g and a %CV of 61.1. All 20 CR3-623-19 green tomatoes were positive for tomatine. The average value was 3.26 mg/100 g with a %CV of 64.7. Of the 20 Sunbelt tomatoes, 9 had tomatine present and the mean tomatine amount was 1.10 mg/100 g with a %CV of 53.8. Of the 20 green 1011 tomatoes, 18 were tomatine positive with an average value of 2.1 mg/100 g and a %CV of 65. Even though the transgenic green tomatoes had slightly higher mean tomatine levels compared to the nontransgenic varieties, they were still extremely low. Bajaj et al. (1987) reported a range of 4.98–57.25 mg of tomatine/100 g of fresh weight of green tomatoes, while, according to Bajaj et al. (1987), Mikova et al. (1981) found 9 mg of tomatine/100 g of fresh weight of green tomatoes. This variation can be explained since the tomatine levels vary tremendously between varieties and developmental stages. These high %CVs demonstrate the biological variability of glycoalkaloid concentrations in tomatoes.

There were five different cultivars of red tomatoes analyzed. Four were the same as for the green tomatoes and the other was Mountain Springs. Like the green tomatoes, 20 samples of each red cultivar were analyzed for their tomatine content (Table 4). Of these 99 samples (1 was lost), only 5 had positive levels of tomatine. They were Mountain Springs (0.88 mg/100 g), CR3-623-19 (1.09 mg/100 g), Sunbelt (0.68 mg/100 g), and 1011 (1.71 and 2.31 mg/100 g). It is not surprising that tomatine was almost nonexistent in red

tomatoes because it has been widely demonstrated that tomatine is degraded in red tomatoes (Eltayeb and Roddick, 1984, 1985; Heftmann and Schwimmer, 1972; Roddick, 1974). Van Gelder et al. (1988) reported tomatine levels of 0.5 mg/100 g in red tomatoes. It should be pointed out that the transgenic tomatoes did not contain more tomatine than the nontransgenic cultivars.

Solanine has been reported in green tomatoes (Simekova and Horcin, 1980) at concentrations varying from 0.1 to 14.1 mg of solanine/100 g of fresh weight of green tomatoes. Because of these findings, solanine was analyzed in all of the above tomatoes, but none was found at a detection limit of 0.05 mg/100 g. To make sure solanine would be recoverable from our tomatine system, a minirecovery study was performed. It was demonstrated that solanine at concentrations of 0.8 and 1.42 mg/100 g could be recovered from the tomatine method at 100%. α-Solanine's retention time is a little more than 8 min, which means it elutes after tomatine. As discussed earlier, α-chaconine is so similar to α-solanine that one would expect to recover it from tomatoes. Futhermore, α-chaconine standard was injected, and it was found to elute just slightly before solanine.

Van Gelder and DePonti (1987) found other compounds in tomatoes that appear to be steroidal glycoalkaloids, but they could not identify them. A similar finding was observed in this study. Three tomato cultivars including red and green tomatoes of each cultivar were analyzed by LC/MS. The results indicate that there are three molecular ions (M + H), besides the one for tomatine, at m/z 1033, 1050, and 1092 present in both green and red tomatoes. From the molecular weight and from the fragment ions (m/z 526, 546, and 547) these three substances appear to be glycoalkaloids. Also, the UV spectra from the HPLC peaks further point to glycoalkaloids (all of these unknowns elute before tomatine). Thus, without further work, their identity cannot be finalized. However, one can speculate about two of these ions on the basis of their molecular weights. First, the m/z 1033 ion appears to be tomatine with a double bond in the tomatidenol portion, while the m/z 1050 ion may be commersonine. There are no ion differences in the nontransgenic vs the transgenic tomatoes analyzed.

CONCLUSION

The HPLC tomatine method was accurate and reproducible when used on red or green tomatoes. This procedure should be very useful for measuring tomatine levels in nontransgenic and transgenic tomatoes. No significant differences were observed between glycoalkaloids and their levels in nontransgenic vs transgenic tomatoes. Futher work needs to be done to identify the unknowns that appear to be other minor glycoalkaloids in both red and green tomatoes.

ACKNOWLEDGMENT

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