Rapid High-Performance Liquid Chromatographic Method for the Quantification of α-Tomatine in Tomato

Erik A. J. Keukens,* Margareth E. C. M. Hop, and Wim M. F. Jongen†

Agrotechnological Research Institute (ATO-DLO), Bornsesteeg 59, P.O. Box 17, 6700 AA Wageningen, The Netherlands

A method for the determination of the glycoalkaloid α-tomatine in tomatoes and tomato plant material is described. The α-tomatine is extracted with methanol and subsequently prepared for analysis using a C_{18} cartridge column. The α-tomatine was applied on the cartridge in 40% methanol and eluted with 80% methanol. A Nucleosil 5-NH_2 column was employed for the high-performance liquid chromatography (HPLC) with acetonitrile–20 mM potassium dihydrogen phosphate (75:25, v/v, pH 6.1) as the mobile phase. Recoveries of spiked α-tomatine ranged from 91% to 98% for different amounts spiked. The calibration graph was linear in the range 5–250 μg/mL for α-tomatine. Survey data are presented for tomatoes and tomato plant material collected from commercial growers and from the Dutch auctions.

Keywords: *α-Tomatine; HPLC; tomato; glycoalkaloid*

INTRODUCTION

The steroidal glycoalkaloid α-tomatine is a natural toxin found in all tomato (*Lycopersicon*) species and in some potato species (*Solanum*). Because of both the host-plant resistance (Barbour and Kennedy, 1991; Dahlman and Hibbs, 1967; Roddick, 1974) and the reported toxicity to humans (Nishie et al., 1977), a lot of attention has been paid to α-tomatine (Roddick, 1974). α-Tomatine consists of the alkaloid (aglycon) tomatidine and a sugar moiety of four monosaccharides. In preliminary studies the α-tomatine content of tomatoes has been determined using several different methods ranging from bioassays (Fontaine et al., 1948; Ali and Schloesser, 1977) to colorimetric methods (Tukalo and Ivanchenko, 1976; Bajaj et al., 1988; Ostrzyka, 1989; Sosic, 1971) and modern techniques such as gas chromatography (Juvik et al., 1982) and fast bombardment mass spectroscopy (Price et al., 1986). However, most of these methods can suffer from low recoveries and high variation. Also, overestimation of the α-tomatine content might occur in colorimetric determinations and the bioassays mentioned.

For the glycoalkaloids from potato, α-solanine and α-chaconine, as well as for some other glycoalkaloids, several high-performance liquid chromatographic (HPLC) determinations have been developed (Saito et al., 1990; Carmen et al., 1986; Jonker et al., 1992; Friedman and Dao, 1992), which mostly include a simple and fast sample preparation resulting in high recoveries and detection limits as low as 1 μg/mL (Saito et al., 1990).

In this paper, a rapid, quantitative HPLC method, using Sep-Pak C_{18} environmental cartridges for extraction and a Nucleosil 5-NH_2 analytical column for α-tomatine separation from fruit and plant material of tomato, is presented. Furthermore, data on the α-tomatine content of several tomato cultivars in different stages of ripening are presented.

* Author to whom correspondence should be addressed (e-mail E.A.J.KEUKENS@ATO.AGRO.NL).

† Present address: Department of Food Science, Agricultural University, Bomenweg 2, 6703 HD, Wageningen, The Netherlands.

MATERIALS AND METHODS

HPLC Equipment. The HPLC system consisted of one Model 510 pump, a WISP 712 autosampler, a temperature control unit III, and a 990 photodiode array detector (all from Waters, Milford, MA). The separation was carried out with a Nucleosil 5-NH_2 (250 mm × 4.6 mm i.d.) obtained from Machery, Nagel & Co. (Düren, Switzerland).

Chemicals and Reagents. Methanol and acetonitrile were obtained from Merck (Darmstadt, Germany) and were of HPLC quality (Lichrosolv). Distilled water was withdrawn from a Millipore Milli-Q (Milford, MA), and buffers were filtered (0.45 μm, Millipore) before use. α-Tomatine was obtained from Sigma (St. Louis, MO) and dissolved in methanol.

The Sep-Pak C_{18} environmental cartridges (1 g of C_{18}) were used in a Sep-Pak cartridge rack, all supplied by Millipore. All other reagents were of analytical grade.

Sample Extraction. Fresh tomatoes were washed and, after removal of the peduncles, cut in slices, whereafter approximately 50 g was homogenized for 1 min with 40 mL of methanol in a Bühler HO 3 homogenizer (Tübingen, Germany). For determinations of the α-tomatine content in tomato leaves, 1–5 g of material was homogenized in 40 mL of methanol for 1 min. The homogenate was centrifuged (2400g for 10 min), and the supernatant was collected. The pellet was resuspended in methanol and centrifuged again. The combined supernatants were filtered through a suction filter 589/5 (Schleicher and Schüll, Dassel, Germany). The filtrate was adjusted to 40% methanol (v/v) with water. Subsequently, this solution was applied on the Sep-Pak C_{18} environmental cartridge which had been preconditioned with 10 mL of methanol followed by 10 mL of water. After application, the cartridge was washed with 10 mL of 40% methanol. This was followed by elution with 25 mL of 80% methanol. All steps were carried out at room temperature. The eluate was either directly injected on the NH_2 column or first concentrated by evaporation to dryness on a rotavapor (Büchi, Flawil, Switzerland) and subsequently redissolved in 3 mL of methanol. The eluate could be stored at 4 °C for more than a month without any breakdown of α-tomatine.

HPLC Method. The mobile phase was prepared by mixing a 20 mM potassium dihydrogen phosphate (KH_2PO_4) solution with acetonitrile (25:75 v/v, pH 6.1), followed by ultrasound degassing. The HPLC run was carried out isocratically at a flow rate of 1.0 mL/min. The column temperature was 40 °C, and the injection volume was 100 μL. α-Tomatine was detected at 200 nm, and the concentration was determined by calculating the peak area.

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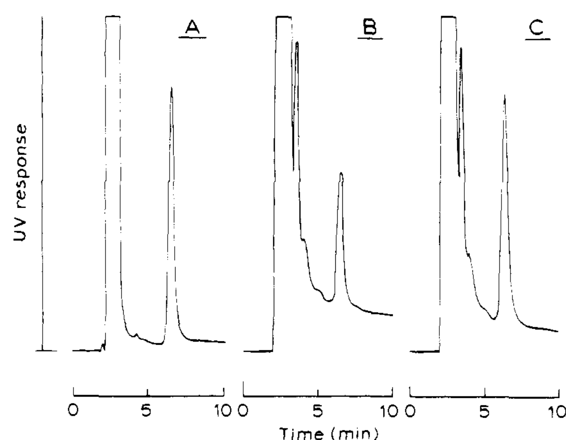

Figure 1. Chromatograms of 100 μL of (A) an α-tomatine standard (25 μg/mL, area 0.002050 AU/min), (B) an unknown tomato sample (area 0.001400 AU/min), and (C) the tomato sample spiked with 10 μg/mL α-tomatine (area 0.002220 AU/min). The column was a Nucleosil 5-NH_2 run isocratically with acetonitrile/20 mM KH_2PO_4 (75/25 v/v, pH 6.1) at 1 mL/min monitored at 200 nm.

Tomato Material. Fresh tomatoes and plant material were collected from commercial growers and from the Dutch auctions. Subsequently, the tomatoes were categorized in the 12 stages of ripening according to the scoring card used by the Dutch auctions (CBT). Only tomatoes of stage 3−4 (green), 6−7 (orange), or 9−10 (red) were selected and stored at −20 °C until analysis, which occurred within 30 days. From each stage, 10 tomatoes were cut into slices from which 4 subsamples were taken for extraction.

RESULTS AND DISCUSSION

Chromatographic Conditions. Considering the described methods for the separation and quantification of α-solanine and α-chaconine from potato, either a C_8 or a C_{18} column in reversed phase (Carmen et al., 1986; Friedman and Dao, 1992; Jonker et al.; 1992) or a NH_2 column in normal phase (Saito et al., 1990) were used. The latter was chosen for the separation of α-tomatine because for the analysis of potato glycoalkaloids it gave the highest resolution.

The mobile phase was run isocratically, which made it possible to measure with a constant background absorbance at 200 nm. The composition of the mobile phase turned out to be optimal at an acetonitrile/phosphate solution ratio of 75:25 (v/v) and a pH of 6.1. By increasing the acetonitrile content, the capacity factor of the column (k') increased compared to the optimal ratio, whereas this factor decreased when the KH_2PO_4 concentration was decreased. The column temperature used was 40 °C, which gave an increased k' value compared to the k' value at room temperature and eliminated any influences of environmental temperature fluctuations.

Figure 1 illustrates the results obtained for the injection of an α-tomatine standard (A), a tomato sample (B), and a spiked tomatine sample (C). The chromatograms were clean without any peaks interfering with the α-tomatine peak. In all cases, the standard deviation calculated for replicate assays of the same sample was less than 2%.

The calibration graph of α-tomatine was linear in the range 5−250 μg/mL, which means that the detection limit (signal/noise = 3) for this method was 500 ng at a sensitivity of 0.04 AUFS. Because of the low molar extinction coefficient ($\epsilon = 5000\ cm^{-1}\cdot M^{-1}$), the minimum

Table 1. Recoveries of Different Amounts of α-Tomatine Spiked to a Tomato Sample

spiked[a] (mg)	recovery of added α-tomatine[b] (%)	
	25 mL sample	3 mL sample[c]
0.125	97.6 ± 0.9	95.6 ± 0.6
0.625	97.5 ± 0.8	95.3 ± 0.5
1.250	95.6 ± 1.1	90.1 ± 0.3
2.500	95.6 ± 0.1	94.7 ± 0.2
5.000	91.1 ± 0.1	89.0 ± 0.2

[a] Added to a 50 mL subsample of tomato homogenate before sample preparation. [b] Each value represents the average ± SD of three experiments. [c] 25 mL eluents of Sep-Pak cartridge concentrated to 3 mL.

detectable quantity of α-tomatine was higher compared to that of α-solanine and α-chaconine, which were measured at 208 nm (Saito et al., 1990). This is due to the lack of a double bond at the 5-position of the α-tomatine aglycon, which results in a lower molar absorptivity.

Sample Preparation. Normal C_{18} cartridges (0.5 g of C_{18}), used for the sample preparation of potato glycoalkaloids (Saito et al., 1990), were already overloaded when a tomato sample spiked with 2.5 mg of α-tomatine was applied. This was probably due to another compound present in the tomato sample which also bound to the column. Therefore, environmental cartridges containing a double amount of C_{18} were used which were still not overloaded when tomato samples spiked with 12.5 mg had been applied.

To obtain a less contaminated sample, the α-tomatine was eluted with 80% methanol instead of 100% used for the elution of potato glycoalkaloids. The environmental cartridges contained twice as much C_{18} material than the normal cartridges and had to be eluted with 25 mL of 80% methanol.

Subsamples (50 mL) of homogenized red or green tomatoes were spiked with 0.125−5.0 mg of α-tomatine to determine the recovery of the added amounts after sample preparation. The samples obtained after elution from the Sep-Pak cartridge were either directly injected on the HPLC column or first evaporated to dryness in vacuo, after which the residue formed was dissolved in 3 mL of methanol. Table 1 shows that the recoveries of spiked α-tomatine were 91−97% for the 25 mL samples, whereas concentration of these samples to 3 mL resulted in decreased recoveries. Thus, it is recommended to concentrate samples only when necessary especially because of the lower recoveries.

Analysis of Tomato Cultivars. The α-tomatine content of 14 different tomato cultivars was determined in the green, orange, and red stages of ripeness and in leaf material. The results are shown in Table 2. The α-tomatine content of green tomatoes varied from 4.5 to 89.9 mg/kg, whereas for the red ones this ranged from 0 to 4.1 mg/kg. In all cultivars the α-tomatine content decreased during ripening, which is in agreement with previous results (Ali and Schloesser, 1977; Sander, 1956; Heftmann and Schwimmer, 1972). This biodegradation of α-tomatine during ripening varied for the different cultivars; the α-tomatine content of the cultivar Fortuna did not decrease very much during the color change from green to orange, whereas in cultivar San Marzano Baldoni (SMB) the biodegradation of α-tomatine during this change of color was almost complete. In all red tomatoes the α-tomatine content was very low and in many cases not detectable.

Table 2. Contents of α-Tomatine in Various Tomato Cultivars

cultivar	α-tomatine content[a] (mg/kg)			
	green	orange	red	leaves
Trend	4.5 ± 0.9	1.7 ± 1.1	nd[b]	399 ± 50
Samoa	4.5 ± 1.6	0.7 ± 0.7	nd	1593 ± 203
Forto	8.4 ± 1.5	nd	nd	764 ± 66
Tuckqueen	8.6 ± 2.2	3.1 ± 1.1	0.9 ± 0.7	2237 ± 220
Furon	8.9 ± 1.1	1.9 ± 1.2	0.5 ± 0.7	694 ± 103
Fortuna	11.7 ± 3.2	10.4 ± 0.9	nd	1905 ± 944
Renova	16.4 ± 2.2	13.0 ± 2.3	1.2 ± 0.8	2485 ± 210
Pronto	18.4 ± 3.0	1.9 ± 0.5	nd	1374 ± 240
Sonatine	25.4 ± 2.2	10.6 ± 1.6	nd	2018 ± 138
Dombito	26.4 ± 3.7	10.5 ± 2.8	4.1 ± 2.7	800 ± 65
Pipo	39.5 ± 7.1	2.6 ± 0.6	1.0 ± 0.7	1909 ± 332
Rondello	40.5 ± 2.4	3.4 ± 0.3	0.7 ± 0.5	1663 ± 137
Evita	59.6 ± 14.0	18.2 ± 7.1	nd	639 ± 103
SMB	89.9 ± 19.5	2.3 ± 2.3	nd	2156 ± 186

[a] Four subsamples were taken from 10 cut tomatoes. Each value represents the average ± SD of four experiments. [b] Not detectable.

It must be noted that the peduncles of the tomatoes have been removed prior to analysis. The content of α-tomatine in the peduncles of the cultivars Sonatine and Fortuna was about 1500 mg/kg, which means that the average α-tomatine content of those cultivars would be increased by approximately 6 mg/kg when the peduncles were still attached to the tomatoes. The content of α-tomatine in the leaves was much higher, and although α-tomatine is thought to be partly responsible for plant resistance, no relation could be found between α-tomatine content and any of the resistance factors of the used tomato cultivars. Also, no correlation ($R = 0.036$) between the α-tomatine content of leaves and tomatoes of the same cultivar existed.

Conclusions. In conclusion, the described method for the determination of the α-tomatine content in tomato and tomato plant material gave good results with excellent recoveries and a high reproducibility. During the sample preparation the α-tomatine is separated using reversed phase chromatography, whereas during the HPLC method a normal phase chromatography method is used in which the sugar groups of the α-tomatine interact with the column material.

The extraction is rather simple and fast compared to methods presently used. Those methods mostly involve precipitation of α-tomatine followed by hydrolysis to obtain the aglycon. Both steps are time-consuming and generally suffer from low recoveries. Furthermore, the α-tomatine content could be overestimated because of the presence of the original aglycon in the sample and because of other compounds having structures similar to that of the aglycon which will be detected because of the lower specificity of colorimetric methods.

Because of the fast sample extraction and an analysis time of only 10 min, this method is very suitable for the quantitative analysis and screening of α-tomatine in tomato and tomato plant material.

The survey data show that the relation between biodegradation of α-tomatine and the color stage of tomatoes during ripening differs for the various cultivars.

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