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Determination of tomatine in foods by liquid chromatography after derivatization

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ABSTRACT

A liquid chromatographic method for measuring tomatine levels in tomatoes and tomato products was developed. Tomatine was extracted with 1% acetic acid and purified on a C_{18} cartridge. Tomatine in the eluate was acetylated with acetic anhydride and isolated on a C_{18} cartridge. The solvent in the eluate was evaporated and the residue was dissolved in acetonitrile. An aliquot was injected into an Inertsil ODS-2 HPLC column and the acetylated tomatine was measured at 205 nm using a UV detector. The limit of determination was 1 $\mu g\ g^{-1}$. Tomatine was detected in the green portions of tomatoes and in tomato ketchups and juices at levels below 7 $\mu g\ g^{-1}$.

INTRODUCTION

Tomatine is a steroidal glycoalkaloid which has anti-fungal activity [1] and appears to be restricted in its taxonomic distribution to the family Solanaceae and, in particular, to the genera *Solanum* and *Lycopersicon* [2].

The human toxicity of tomatine is twofold, that is, it inhibits cholinesterase [3] and cytostatic activities [3–5]. We previously showed the membrane-distruptive properties of tomatine, α-chaconine, α-solanine and solanidine, among which tomatine had the strongest effect [6].

Tomatine is contained in tomato leaves (2000–5000 $\mu g\ g^{-1}$) and in unripe fruits (300–900 $\mu g\ g^{-1}$) [2]. Food poisoning caused by tomatine however, has not been reported until recently, because tomatine disappears during the ripening process of tomatoes owing to enzymatic degradation [7].

Earlier methods used for determining tomatine levels were bioassays based on the degree of growth inhibition of cultured fungi. This was followed by spectrometric methods. However, as tomatine does not have any chromophoric groups, it has been determined photometrically after reaction with chromogenic reagents [8–11]. It can also be determined by thin-layer chromatography [12]. The separation and quantitation of *Solanum* steroidal alkaloids in potatoes by reversed-phase HPLC has been investigated [13]. However, these methods for measuring tomatine in foods were unsatisfactory

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as they were subject to interference from other tomato components.

In this paper, we describe a method for measuring residual tomatine levels in tomatoes and tomato products by extraction and high-performance liquid chromatography.

EXPERIMENTAL

Instrumentations

A mini-food processor (New Cooker SKC-03; Sogo-Giken, Tokyo, Japan) was used to homogenize the samples and a universal homogenizer (Nippon Seiki, Tokyo, Japan) was used for extraction. A Rheodyne Model 7125 injector, a Model 880-PU pump, a Model 870 UV detector (Japan Spectroscopic, Tokyo, Japan), an Inertsil ODS-2 column (25 cm × 4.6 mm I.D., particle size 5 μm) (GL Sciences, Tokyo, Japan) and a LiChrospher NH_2 column (25 cm × 4 mm I.D., particle size 5 μm) (Cica-Merck, Tokyo, Japan) constituted the HPLC system.

Mobile phase

Acetonitrile–water (90:10) was the solvent for Inertsil ODS column chromatography and acetonitrile–50 m*M* potassium dihydrogenphosphate (75:25) for LiChrospher NH_2 column chromatography.

Reagents

All chemicals were of special grade, with the exception of acetonitile, which was of analytical-reagent grade, and were obtained from Wako (Osaka, Japan). HiFlo Super-Cel, which is a kind of diatomaceous earth and helps rapid filtration, was also purchased from Wako. Sep-Pak C_{18} cartridges (volume 1 ml) were purchased from Waters (Milford, MA, USA). Tomatine was obtained from Sigma (St. Louis, MO, USA).

Materials

Commercial tomatoes and processed tomato foods were purchased in Tokyo and unripe tomatoes (*Lycopersicon esculentum*, Mill.) were supplied by the National Research Institute of Vegetables, Ornamental Plants and Tea (Aichi, Japan).

Extraction and purification of tomatine

One piece of tomato was homogenized using a mini-food processor. Five grams of the homogenate were mixed with 50 ml of 1% acetic acid and 2.5 g of HiFlo Super-Cel. The mixture was homogenized for 3 min using a universal homogenizer. The homogenate was filtered through Toyo filter-paper No. 5A (Toyo-roshi, Kyoto, Japan) and the residue was re-extracted with 40 ml of 1% acetic acid. The filtrates were combined and made up to 100 ml with 1% acetic acid. A 40-ml volume was applied to a Sep-Pak C_{18} cartridge previously washed with 10 ml of methanol and 10 ml of 1% acetic acid in that order. The sample on the column was washed with 5 ml of 1% acetic acid followed by 10 ml of 20% methanol, then tomatine was eluted with 5 ml of methanol. The methanol eluate was concentrated to dryness using a rotary evaporator in a 30-ml pear-shaped flask.

Acetylation of tomatine

To the residue, 0.2 ml of pyridine and 0.5 ml of acetic anhydride were added, then refluxed on a boiling water-bath for 1 h. After cooling to room temperature, 30 ml of 50% methanol were added to the flask. The mixture in the flask was applied to a Sep-Pak C_{18} cartridge that had previously been washed with 10 ml of methanol and 10 ml of 50% methanol, then the same flask was rinsed with 5 ml of 50% methanol, the rinsed solution was also applied to the cartridge and the cartridge was washed with 10 ml of 70% methanol. Acetylated tomatine was eluted with 5 ml of methanol. The methanol eluate was concentrated to dryness using a rotary evaporator, the residue was dissolved in 0.5 ml acetonitrile and the solution was analysed by HPLC.

HPLC conditions

Acetylated tomatine was separated on an Inertsil ODS-2 column at room temperature (25°C). The flow-rate was 1.0 ml min^{-1} and the eluate was monitored at 205 nm. The injection volume was 20 μl.

RESULTS AND DISCUSSION

Purification of tomatine on a Sep-Pak C_{18} cartridge

A 1-ml volume of tomatine standard (50 μg ml^{-1}) was applied to a Sep-Pak C_{18} cartridge and eluted with 5 ml each of 20, 30 and 100% methanol. Tomatine levels in the eluates were determined by HPLC with UV detection (205 nm) using an amino-bonded column. None of the applied tomatine was eluted with 5 ml of 20% methanol, 4.6% of it was eluted with 5 ml of 30% methanol and 100% of it was recovered with 5 ml of 100% methanol. We therefore decided to wash the column with 5 ml of 20% methanol before eluting tomatine with 5 ml of methanol.

Acetylation of tomatine

After passage through the C_{18} cartridge, numerous interfering compounds remained, as determined by HPLC (Fig. 1). To eliminate them, we derivatized tomatine with an acetylating agent and again passed it through the cartridge.

The methanol eluate from a Sep-Pak C_{18} cartridge was concentrated completely to dryness, then 0.2 ml of pyridine and 0.5 ml of acetic anhydride were added to the residue. The mixture was refluxed on a boiling water-bath for 120 min. Fig. 2 shows the time course of acetylation of tomatine. The maximum peak height was obtained after 60 min. Methanol (50%) was added to the solution, then the mixture was loaded on a Sep-Pak C_{18} cartridge. As shown in Fig. 3, 70–100% methanol was passed through the column, and 100% of the acetylated tomatine was recovered with 100% methanol.

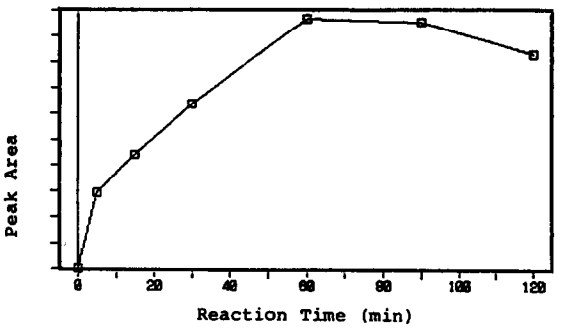

Fig. 2. Time course of tomatine acetylation. An 800-μg amount of tomatine was reacted with 0.2 ml of pyridine and 0.5 ml of acetic anhydride on a boiling water-bath for 5, 15, 30, 60, 90 or 120 min. The reaction mixtures were purified on a Sep-Pak C_{18} cartridge and analysed by HPLC.

Acetylation made it easy to purify tomatine in the methanol eluate and to separate it from interfering components in tomato, as shown in Fig. 4. Acetylated tomatine was determined by HPLC using an ODS column, and the calibration graph was linear in the range 10–800 μg ml^{-1} of tomatine.

One mol of α-tomatine, a steroidal glycoalkaloid, possesses 2 mol of D-glucose and 1 mol each of D-galactose and D-xylose. The removal of the sugars by partial hydrolysis in dilute acid

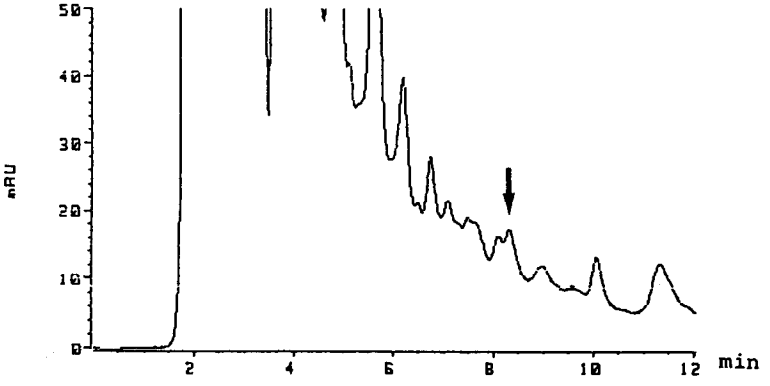

Fig. 1. HPLC of tomato extract after passage through a Sep-Pak C_{18} cartridge and before acetylation. Tomatine was added to the tomato homogenate at 50 μg g^{-1}. The arrow indicates the tomatine peak. HPLC conditions: column, LiChrospher NH_2 (250 mm × 4.6 mm I.D.); mobile phase, CH_3CN–50 m*M* KH_2PO_4 (75:25); detection wavelength, 205 nm.

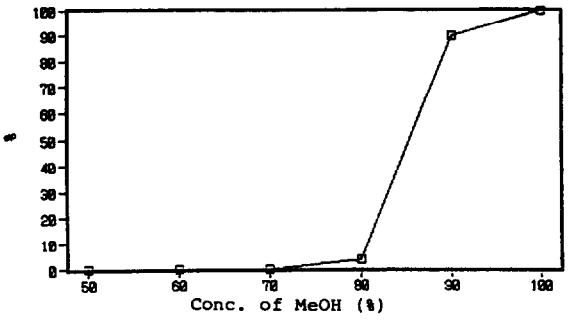

Fig. 3. Elution profile of acetylated tomatine from a Sep-Pak C_{18} cartridge using various concentrations of methanol. Acetylated tomatine (200 μg as tomatine) in 10 ml of 50% methanol was applied to a Sep-Pak C_{18} cartridge and eluted with 10 ml each of 50, 60, 70, 80, 90 and 100% methanol. Each eluate was concentrated to dryness, the residue dissolved in 0.5 ml of acetonitrile and the solution analysed by HPLC.

results in β_1-tomatine (minus D-xylose), β_2-tomatine (minus one D-glucose), γ-tomatine (minus D-xylose and one D-glucose) and the aglycone tomatidine [7]. We confirmed the presence of these sugar molecules in standard tomatine, which means that the main component of the tomatine used in this experiment was α-tomatine. Before and after derivatization the UV spectra did not change, but the polarities changed drastically. For this reason, the main peak on the chromatogram (Fig. 4) may correspond to acetylated α-tomatine, but elucidation of the structure is still under investigation.

Recovery of tomatine from ripe tomatoes

The recoveries of tomatine from commercial half-ripened fruit spiked at 200 and 20 μg g^{-1} were $89.3 \pm 1.4\%$ ($n = 3$) and $66.9 \pm 1.2\%$ ($n = 3$), respectively. The half-ripened fruit contained tomatine at 3.8 ± 0.4 μg g^{-1} ($n = 3$). Typical high-performance liquid chromatograms of a ripe fruit and a spiked extract are shown in Fig. 4. The limit of determination was 1.0 μg g^{-1} at a signal-to-noise ratio of 3 for raw samples. Fig. 5 shows a typical chromatogram of an extract from unripe tomato containing 3.0 μg ml^{-1} of tomatine.

Tomatine content in mini-tomato plants

Tomatine levels in leaves, green unripe fruit and red ripe fruits from two mini-tomato plants were analysed. Fairly large amounts of tomatine were detected in the leaves (493.4 and 616.3 μg g^{-1}), and green unripe fruits contained tomatine at levels of 154.4 and 196.7 μg g^{-1}, as reported previously [7]. On the other hand, all three red ripe fruits contained tomatine only at 1 μg g^{-1}.

Time course of disappearance of tomatine during storage of unripe tomatoes

Tomatoes were harvested before maturation, when their surfaces were almost all green. About twenty tomatoes were kept at 10°C in the dark and the tomatine levels in batches of three tomatoes were determined after 0, 4 and 9 days. On day 0, tomatine was detected at a level of 2.2–4.5 μg g^{-1} (average ± S.D. $= 3.0 \pm 1.3$ μg g^{-1}), on day 4 at 1.0–2.6 μg g^{-1} (average ±

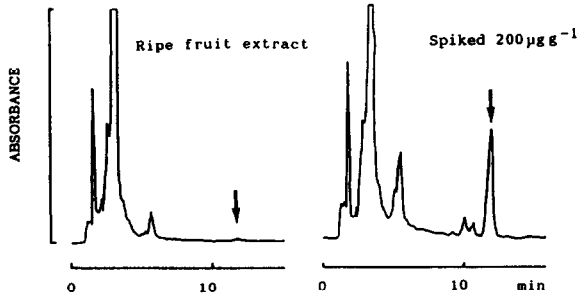

Fig. 4. Chromatograms of extracts from ripe tomatoes and from a spiked sample. Tomatine was added at 200 μg g^{-1} to the tomato homogenate. Arrows indicate acetylated tomatine peaks. The retention time was 12.3 min.

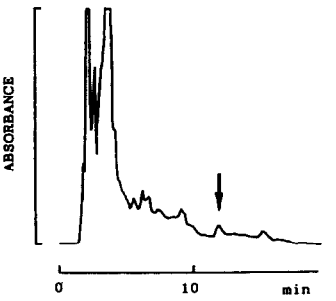

Fig. 5. Chromatogram of an extract from an unripe tomato containing 3.0 μg ml^{-1} of tomatine.

S.D. = 1.6 ± 0.7 μg g^{-1}) and on day 9 it was undetectable and all fruits were red ripe. On day 0, the green portion cut off from three tomatoes contained tomatine at an average level of 3.9 ± 1.6 μg g^{-1}. However, tomatine was not detected in the red portion removed from the same tomatoes. These results suggest that almost all the tomatine in the green portion of tomato disappears during ripening.

The tomatine contents in one commercial tomato purée, three ketchups and three juices were measured. Tomatine was detected in two of the ketchups at levels of 5.5 and 6.5 μg g^{-1} and in two of the juices at levels of 2.5 and 3.7 μg g^{-1}. The oral toxicity of α-tomatine has been examined only in mice and rats [7], and the lethal dose was 500 and 900–1000 mg kg^{-1}, respectively. The concentrations of tomatine detected in half-ripened fruits and tomato products were thus considerably lower than the lethal levels.

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