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Detoxification and function of immature tomato

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ABSTRACT

α-Tomatine and chlorophyll (*a* and *b*) decreased, and β-carotene and lycopene increased with ripening of tomatoes. α-Tomatine was localised in peel of immature green tomatoes. The dose–response curve of α-tomatine determined by WST-1 (water soluble tetrazolium) assay was the same as that by LDH (lactate dehydrogenase) assay, suggesting that the cytotoxicity of α-tomatine depends on the destruction of plasma membrane. Immature green tomatoes had little cytotoxic effect after one month-incubation with 25% ethanol or 4.5% acetate at 7 °C, and α-tomatine was decomposed by crude enzymes extracted from immature green tomatoes. Immature green tomatoes incubated with 4.5% acetic acid inhibited the accumulation of lipid in adipocytes. From the above facts the detoxification and the anti-obesity effect of immature green tomatoes are expected to be controlled by the removal of peel, the enzymatic decomposition or the incubation with 4.5% acetate or 25% ethanol.

1. Introduction

α-Tomatine of tomatoes is considered to be a harmless glycoalkaloid compared to α-solanine and α-chaconine because of its weak inhibitory effect on acetylcholinesterase (Roddick, 1989). Health hazards of α-tomatine have been studied by various animal tests, and no adverse reaction was observed in rats which were administered with 250 mg α-tomatine/kg body weight daily by stomach tube for 5 days (Wilson, Poly, & DeEds, 1961). However, α-tomatine appeared to be a potential teratogen when rats were treated with 250 mg α-tomatine/kg body weight for 9 days (Waalkens-Berendsen, Smits-van Prooije, Hoeter, & Leeman, 1992). The dose used in those tests would approximately correspond to a human consumption of 1500 kg ripe tomatoes a day on the basis of 1 mg of α-tomatine/100 g ripe tomatoes. Therefore, ripe tomatoes are considered to be a safe phytosanitary product.

Though immature green tomatoes are not generally eaten, some consumers and farm producers are interested in the taste, texture, and new nutritional functions of immature green tomatoes. For example, the extract from immature green tomatoes is expected to protect against obesity (Cayen, 1971; Choi et al., 2013; Friedman, Fitch, Levin, & Yokoyama, 2000). However, α-tomatine is concentrated in peel of immature green tomatoes, and the concentration is estimated to be several hundred mg/100 g fresh weight. Therefore, we should eat immature tomatoes with caution because they may cause vomiting and diarrhoea.

This study proposes a food processing step, to reduce the toxicity and maintain the antiobesity effect of immature green tomatoes.

2. Materials and methods

2.1. Organisms and growth conditions

Cells used for cytotoxicity tests were prepared according to our previous paper (Yamashoji & Matsuda, 2013). C6 rat glioma cells (C6 cells) and Hela cells were cultured in high glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Medium A). Mouse fibroblasts NIH/3T3 cells (NIH3T3 cells) were cultured in low glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Medium B). The cells were grown in a Falcon tissue flask in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Exponentially growing stock cells were trypsinised, and the cell density was diluted to approximately 100,000 cells/mL with the above corresponding medium. After 100 μL of the resulting cell suspension were added to each well of a 96-well plate, the cells were cultured for 2 days, and then were used for the following cytotoxicity tests.

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2.2. WST-1 assay

Water soluble tetrazolium-1 (WST-1) assay (Ishiyama et al., 1995) was modified according to our previous paper (Yamashoji & Matsuda, 2013). The adherent cells prepared under the above conditions were washed with Medium B and were incubated with 100 μL of Medium B containing

α-Tomatine or the extract of immature green tomatoes were added in each well of a 96-well plate for 4 h. The volume of α-tomatine solution or the extract of tomatoes was from 2 to 10 μL in 100 μL of Medium B. The dose of α-tomatine was from 0 to 40 μM. After incubation, the adherent cells in each well were washed twice with phenol red-free minimum essential medium containing glutamate and $NaHCO_3$ (Medium C), and 10 μL of Medium C containing 5 mM WST-1 and 0.2 mM 1-methoxyPMS (1-methoxy-5-methylphenazinium methylsulfate) were added to each well. The tissue culture plate was incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. After the desired incubation period, the absorbance in each well was determined at 450 nm. Viability was calculated by the following equation:

Viability (%) = (absorbance of the cells incubated with
× α-tomatine or the extract
/absorbance of the untreated cells) foll

2.3. LDH assay

Lactate dehydrogenase (LDH) assay (Koreniewski & Callewaert, 1983) was modified according to our previous paper (Yamashoji & Matsuda, 2013). C6 cells prepared under the above conditions were incubated with 100 μL of Medium A containing α-tomatine for 4 h. After the incubation, 50 μL of the supernatant in each well of a 96-well plate were transferred to each well of another plate, and were mixed with 50 μL of LDH assay reagent prepared with LDH-Cytotoxic Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The absorbance at 560 nm was determined after 10 min-incubation with the above reagent. The viability was calculated by the following equation.

Viability (%) = 100 − [(absorbance of the cells incubated with
× α-tomatine − absorbance of the untreated cells)
/(absorbance of the cells incubated with Tween 20
− absorbance of the untreated cells) ed wit.

2.4. Determination of pigments in tomato

The concentrations of chlorophylls, β-carotene and lycopene were determined by spectrophotometric analysis (Nagata & Yamashita, 1992).

2.5. Fractionation of tomatoes

Tomatoes were cut into quarters, and each piece was divided into peel, flesh and residue. Each fraction was homogenized and filtered with membrane filter (0.2 μm pore size). The filtrate was used as the extract of tomatoes, and the volume of the extract used for WST-1 assay was from 2 to 10 μL in 100 μL of Medium B.

2.6. Processing of immature green tomatoes with seasoning

Mature red tomatoes were involved in ripening degree 6, and immature tomatoes were involved in ripening degree from 1 to 5. In this study immature green tomatoes at ripening degree 1 were used as the most toxic tomatoes. Immature green tomatoes (about 60 g weight) corresponding to ripening degree 1 shown in Fig. 2 were cut into quarters, and each piece was incubated with 100 mL of various aqueous solution, oil or paste at 7 °C in the dark for a fixed period. Each piece was homogenized after the incubation and filtered with membrane filter (0.2 μm pore size). The filtrate was used as the extract of immature green tomatoes, and the volume of the extract used for WST-1 assay was from 2 to 10 μL in 100 μL of Medium B.

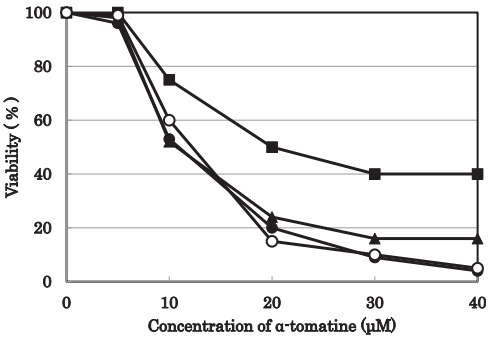

Fig. 1. Cytotoxicity of α-tomatine determined by WST-1 assay and LDH assay. ● C6 cells (WST-1 assay), ▲ HeLa cells (WST-1 assay), ■ NIH/3T3 cells (WST-1 assay), ○ C6cells (LDH assay). Each symbol represents the mean of four determinations, and the standard deviation was less than 5% of the mean.

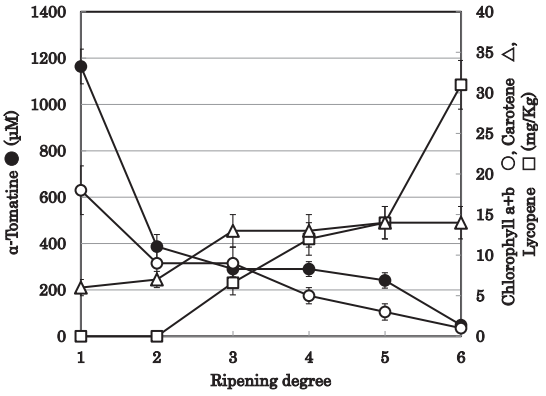

Fig. 2. Concentrations of α-tomatine, chlorophyll, β-carotene and lycopene in the whole fruit at different ripening degree. ● α-tomatine, ○ chlorophyll, △ β-carotene, □ lycopene. Each symbol represents the mean of four determinations, and each bar represents the standard deviation.

2.7. Differentiation of 3T3-L1 preadipocyte in the presence of α-tomatine

3T3-L1 preadipocyte cells were differentiated into adipocyte cells as already reported (Student, Hsu, & Lane, 1980). The induction medium containing α-tomatine or the extract of immature green tomatoes was added to the confluent cells and was incubated for 6 days. The total volume of medium was 500 μL in a 12-well plate, and the volume of α-tomatine or the extract of immature tomatoes was from 5 to 20 μL.

2.8. Determination of lipid content in adipocyte cells

Determination of lipid content (Reed & Lane, 1980) was modified as follows. Adipocyte cells grown on a 12-well plate were washed twice with 1 mL of PBS and were incubated with 1 mL of 10% formalin for 10 min. After the incubation, the cells were washed twice with 1 mL of PBS and were incubated with 1 mL of 60% isopropanol for 1 min. After the removal of 60% isopropanol, cells were stained with 1 mL 0.18% Oil Red O/60% isopropanol for