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Research Note

Physiological Activities of Thiacremonone Produced in High Temperature and High Pressure Treated Garlic

Koan Sik Woo[1], In Guk Hwang[2], Hyun Young Kim[1], Sang Hoon Lee[3], and Heon Sang Jeong[3]

[1]National Institute of Crop Science, Rural Development Administration, Gyeonggi 16616, Korea
[2]National Academy of Agricultural Science, Rural Development Administration, Jeonbuk 54875, Korea
[3]Department of Food Science and Technology, Chungbuk National University, Chungbuk 28644, Korea

ABSTRACT: To examine the possibility of using thiacremonone isolated from high-temperature-high-pressure treated garlic, this study investigated the physiological activities properties. The IC_{50} values of hydroxyl, superoxide, hydrogen peroxide, and nitric oxide radical scavenging activities of thiacremonone were 92.50, 65.05, 12.60, and 81.53 μg/mL, respectively. On the other hand, the activities of vitamin C were 104.93, 99.43, 42.42, and 122.64 μg/mL, and the activities of butylated hydroxyanisole were 37.22, 68.45, 22.47, and 40.54 μg/mL, respectively. The IC_{50} value of ACE inhibition activities of thiacremonone and captoprill were 0.265 and 0.036 μg/mL, respectively. The IC_{50} value of xanthine oxidase inhibition activities of thiacremonone and allopurinol were 39.430 and 9.346 μg/mL, respectively. The IC_{50} value of tyrosinase inhibition activities of thiacremonone and kojic acid were 101.931 and 65.648 μg/mL, respectively.

Keywords: thiacremonone, 2,4-dihydroxy-2,5-dimethyl-thiophene-3-one, garlic (*Allium sativum* L.), radical scavenging activity

INTRODUCTION

Compared to fresh foods, thermally processed foods, especially fruits and vegetables, have increased biological activity caused by chemical changes during heat treatment (1). Some studies have examined the chemical and physical properties of foods in response to high temperature and high pressure (HTHP) treatment. The polyphenol and flavonoid contents and antioxidant activities increase with HTHP treatment in foods (2-5).

Garlic (*Allium sativum* L.) is grown in many areas and has been used by many civilizations, including Greek, Egyptian, Asian, and Indian, since antiquity (6). The antioxidant activity of *Allium* plants has mainly been attributed to a variety of sulfur-containing compounds such as diallyl sulfide, diallyl trisulfide, allyl-cysteine, and selenium compounds (7). In addition to its antioxidant activity, garlic has antimicrobial, antibacterial, antiviral, antifungal, and antiprotozoal properties, as well as beneficial effects for the cardiovascular and the immune systems (8). Microwave heating of garlic cloves for 60 s reduces its anticancer properties (9). Interestingly, when microwave heating was applied 10 min after garlic crushing, the anticancer properties were preserved indicating that allinase activation is necessary to generate anticancer compounds, which are thermostable (10). In a similar way, the hydroxyl (OH) radical scavenging properties of garlic were essentially preserved when garlic extracts were heated at 100°C for 20, 40, or 60 min (11). An antioxidant is a substance that, when present at low concentrations compared to that of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (12). However, the safety and continued use of artificial antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in foods is being questioned. Therefore, a search for antioxidants of natural origin has attracted increasing attention.

Thiacremonone (2,4-dihydroxy-2,5-dimethyl-thiophene-3-one; Fig. 1) was the isolated active compound from heated garlic juice treated at 130°C for 2 h (13). This compound is the first report of the isolation of thiacremonone from heated garlic, although it has been isolated from the fungus *Acremonium* sp. strain HA33-95, an inducer of differentiation in mammalian cells (14). The latest studies have reported that thiacremonone had anti-cancer (15,16), anti-inflammatory, anti-arthritic (17), and anti-obesity effects (18).

Oxidation in foods produces peroxidation products,

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Correspondence to Koan Sik Woo, Tel: +82-31-695-4084, E-mail: wooks@korea.kr

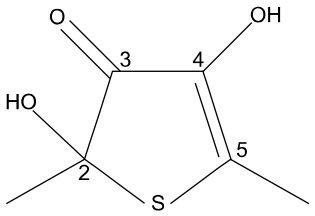

Fig. 1. Structure of thiacremonone (2,4-dihydroxy-2,5-dimethyl-thiophene-3-one).

toxic substances, and rancidity odors. *In vivo*, peroxidation by free radicals or molecular singlet oxygen causes damage to DNA, cancer, and aging (19). Accordingly, the development of new compounds to inhibit oxidation in foods and *in vivo* are very important. Antioxidants are generally used as protection materials of oxidation for storage and preservation of foods. In terms of stability of foods and human health, the development of highly effective antioxidants in nature is required. The overall objective of this study was to determine the physiological activities of thiacremonone isolated from HTHP treated garlic. Biological activity was used to monitor the functionality of thiacremonone as a radical scavenging, angiotensin converting enzyme (ACE), xanthine oxidase (XO), and tyrosinase inhibitor.

MATERIALS AND METHODS

Chemicals and sample preparation

Ascorbic acid, xanthine, XO grade I from buttermilk (EC 1.1.3.22), nitro blue tetrazolium (NBT), hydrogen peroxide (H_2O_2), 2-deoxyribose, ferrous sulfate, ACE, and Hip-His-Leu (HHL) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Water, dichloromethane, and methanol were purchased from J. T. Baker (Phillipsburg, NJ, USA). All other reagents were of analytical grade. Garlic was purchased from the Chungbuk Agriculture and Marine Products Market in Korea in June 2007 and was stored at −20°C. Heat treatment was performed using a temperature- and pressure-controlling apparatus (Jisico, Seoul, Korea). The samples were heated at temperatures of 130°C for 2 h. Thiacremonone was isolated according to the Hwang et al. method (13). Heated garlic juice was partitioned consecutively in a separating funnel using ethyl acetate. Isolation of thiacremonone from the ethyl acetate layer of heated garlic juice was subjected to column chromatography on silica gel. The fractions included thiacremonone were purified by preparative reverse phase-HPLC (Discovery® C18 column; 250×10 mm, i.d., 5 μm; Supelco, Bellefonte, PA, USA) on a SP930D solvent delivery pump (Younglin Instrument, Anyang, Korea) equipped with a UV detector, operating at 365 nm, at room temperature, and a flow rate of 3.5 mL/min. The pure thiacremonone was obtained after evaporating the solvents using a rotary evaporator.

Measurements of radical scavenging activities of thiacremonone

The scavenging activity for the OH radical was evaluated using the method of Lee et al. (5) at a wavelength of 520 nm with a UV-visible spectrophotometer. The scavenging activity for the superoxide (O_2) radical was evaluated using the following equation at a wavelength of 560 nm according to the xanthine-XO method (20). The scavenging activity of the H_2O_2 radical was evaluated using the method of Marklund and Marklund (21) at a wavelength of 405 nm with an enzyme-linked immunosorbent assay (Sunrise Tecan Co. Ltd., Vienna, Austria). The nitrite scavenging effect was evaluated using a UV-visible Spectrophotometer at a wavelength of 520 nm, according to the method reported by Gray and Dugan (22). Sample concentrations providing the 50% inhibition concentration (IC_{50}) were calculated from a graph of the inhibition percentage versus the sample concentration. All extracts were analyzed in triplicate.

Measurement of ACE, XO, and tyrosinase inhibitory activities

The ACE inhibitory effect was measured using the method of Maruyama et al. (23) with slight modifications. The thiacremonone solution (50 μL) and 50 μL of the ACE solution (0.2 units/mL) were pre-incubated at 37°C for 10 min. The mixture was then incubated with 150 μL of substrate (8.3 mM HHL in 50 mM sodium borate buffer containing 0.5 M NaCl, pH 8.3) for 30 min at the same temperature. The reaction was terminated by the addition of 250 μL of 1.0 M HCl. The resulting hippuric acid was extracted with 1.25 mL of ethyl acetate. After centrifugation at 1,200 *g* for 15 min, 1.0 mL of the upper layer was transferred to a test tube and heated at 120°C for 30 min. The hippuric acid was dissolved in 1.0 mL of distilled water, and the absorbance was read at 228 nm using a UV-spectrophotometer.

The XO inhibitory activity with xanthine as the substrate was measured spectrophotometrically based on the procedure reported by Owen and Johns with slight modifications (24). The assay mixture consisted of 0.1 mL of thiacremonone solution, 0.6 mL of 0.1 M potassium phosphate buffer (pH 7.5), and 0.1 mL of enzyme solution (0.2 units/mL in phosphate buffer, pH 7.5), which was prepared immediately before use. After mixing, the reaction was initiated by the addition of 0.2 mL of substrate solution (2 mM xanthine in the same buffer). The assay mixture was incubated at 37°C for 5 min. The reaction was then stopped by the addition of 1 mL of 1 N hydrochloric acid, and the absorbance was measured at 292 nm using a UV spectrophotometer. XO inhibitory

activity was expressed as the percentage inhibition. Allopurinol (1H-pyrazolo-[3,4-d]-pyrimidin-4-ol), a known inhibitor of XO, was used as a positive control.

Tyrosinase inhibitory activity was determined using the modified dihydroxyphenylalanine (DOPA)-chrome method with L-DOPA as a substrate (25). Samples were dissolved in 50% dimethyl sulfoxide. Each well contained 0.1 mL of thiacremonone solution with 0.5 mL of sodium phosphate buffer (1/15 M, pH 6.8), 0.2 mL of tyrosinase (110 units/mL) and 0.2 mL of L-DOPA (10 mM). After the assay mixture was incubated at 25°C for 2 min, the absorbance was measured at 475 nm using a UV spectrophotometer. Sample concentrations providing the IC_{50} were calculated from a graph of the inhibition percentage versus the sample concentration. All samples were analyzed in triplicate.

Statistical analysis

All data were expressed as means±standard deviation (SD). Analysis of variance (ANOVA) and Duncan's multiple range tests ($P<0.05$) were performed using the SAS program (version 9.1, SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Radical scavenging activities of thiacremonone

The hydroxyl radical has the capacity to break DNA strands, which contributes to carcinogenesis, mutagenesis, and cytotoxicity. In addition, hydroxyl radicals are thought to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (5). The OH, O_2, H_2O_2, and nitric oxide (NO) radical scavenging activities of thiacremonone are presented in Fig. 2. The IC_{50} of the OH radical scavenging activity of thiacremonone was 92.50±2.06 μg/mL, which was higher than that of the natural antioxidant ascorbic acid (104.93±2.09 μg/mL), while lower than that of the natural antioxidant α-tocopherol (12.93±1.64 μg/mL), and the synthetic antioxidant BHA (37.22±1.38 μg/mL). O_2 radicals indirectly initiate lipid oxidation because O_2 and H_2O_2 serve as precursors to singlet oxygen and OH radicals (5). The IC_{50} of the O_2 radical scavenging activity of thiacremonone was 65.05±3.14 μg/mL, which was higher than that of ascorbic acids (99.43±2.69 μg/mL) and α-tocopherol (84.92±2.34 μg/mL). Reactive oxygen species, including free radicals such as the O_2 radical and OH radical, and non-free-radical species such as H_2O_2 and singlet oxygen, play key roles in the oxidation process, which is considered one of the initial developmental steps of many chronic diseases (5). The IC_{50} of H_2O_2 radical scavenging activity of thiacremonone was 12.60±1.98 μg/mL, which was higher than that of ascorbic acid (42.42±1.09 μg/mL), α-tocopherol (39.40±0.77 μg/mL), and BHA (22.47±0.58 μg/mL). NO reactive nitrogen species are formed during reactions with oxygen or O_2, and NO_2, N_2O_4, N_3O_4, NO_3^-, and NO_2^- are very reactive. These compounds are responsible for altering the structures and functional behaviors of many cellular components (5). The IC_{50} of NO radical scavenging activity of thiacremonone was 81.53±0.34 μg/mL, which was higher than that of antioxidant ascorbic acid (122.64±2.84 μg/mL), while lower than that of α-tocopherol (15.72±1.22 μg/mL) and BHA (40.54±0.75 μg/mL).

The OH, O_2, H_2O_2, and NO radical scavenging abilities of thiacremonone shown in this study suggests that thiacremonone has beneficial effects for decreasing the toxicity of radicals. Therefore, thiacremonone isolated from HTHP treated garlic suggests that its use will be possible as a new antioxidant component.

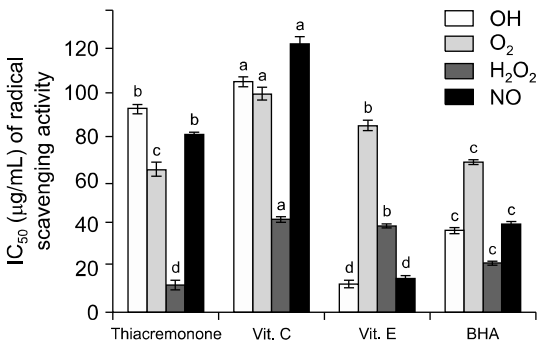

Fig. 2. OH, O_2, H_2O_2, and NO radical scavenging activities of thiacremonone isolated from garlic treated with high-temperature-high-pressure. Means within same radical by the same letter (a-d) are not significantly ($P<0.05$) different by Duncan's multiple range test. Ascorbic acid (Vit. C), a-tocopherol (Vit. E), and butylated hydroxyanisole (BHA) were used as positive control compounds.

ACE, XO, and tyrosinase inhibition activity of thiacremonone

The mechanism of the activity of thiacremonone involves the inhibition of ACE, the key enzyme responsible for the regulation of blood pressure by the rennin-angiotensin system (26). ACE inhibition activity of thiacremonone is presented in Table 1. The IC_{50} on ACE inhibition activity of thiacremonone was 0.265±0.041 μg/mL, while lower activity than that of the ACE inhibitor captoprill (0.036±0.007 μg/mL). Maruyama et al. (23) reported ACE inhibitory factors such as peptides, catechin, rutin, and phenolic compounds in *Ficus carica* extracts.

XO catalyses the metabolism of hypoxanthine and xanthine to uric acid. The overproduction and under excretion of this acid lead to the incidence of hyperuricemia, such as gout (24). Allopurinol has been the sole XO inhibitor drug on clinical applications in the past three decades (25). However, this drug gives inevitably rise to severe adverse effects such as hepatitis, nephropathy, al-

Table 1. Angiotensin-converting enzyme, xanthine oxidase, and tyrosinase inhibitory activities of thiacremonone isolated from high-temperature-high-pressure treated garlic

Compounds	IC_{50} (μg/mL)		
	Angiotensin-converting enzyme	Xanthine oxidase	Tyrosinase
Thiacremonone	0.265±0.041[a1)2)]	39.430±0.234[a]	101.931±2.601[a]
Captoprill	0.036±0.007[b]	–	–
Allopurinol	–	9.346±0.114[b]	–
Kojic acid	–	–	65.648±0.187[b]

Captoprill, allopurinol, and kojic acid are an angiotensin converting enzyme, xanthine oxidase, and tyrosinase inhibitor, respectively.
[1)]Values are each the mean±SD of three experiments, each performed in triplicate.
[2)]Means in the same column followed by the same letter (a,b) are not significantly ($P<0.05$) different by Duncan's multiple range test.

lergic reactions, and 6-mercaptopurine toxicity (26). Therefore, there is an urgent need to search for new XO inhibitors. The XO inhibitory activity of thiacremonone is presented in Table 1. The IC_{50} of XO inhibitory activity of thiacremonone was 39.430±0.234 μg/mL, while lower activity than that of the XO inhibitor allopurinol (9.346±0.114 μg/mL).

Tyrosinase is a copper-containing enzyme widely distributed in nature that catalyzes 2 distinct reactions of melanin biosynthesis, and also known as a polyphenol oxidase. The browning of some fruits, beverages, and vegetables are due to tyrosinase and can cause a significant decrease in their nutritional and aesthetic value (27). Therefore, the control of tyrosinase is important in relation to browning control of fresh materials. Additionally, tyrosinase inhibitors have become increasingly important in medicinal and constituents of cosmetic products in relation to hyperpigmentation (28). The tyrosinase inhibitory activity of thiacremonone is presented in Table 1. The IC_{50} of tyrosinase inhibitory activity of thiacremonone was 101.931±2.601 μg/mL, while lower activity than that of the tyrosinase inhibitor kojic acid (65.648±0.187 μg/mL). ACE, XO, and tyrosinase inhibitory activity of thiacremonone was lower than those of the inhibitor captoprill, allopurinol, and kojic acid; however, this study suggests that thiacremonone has beneficial effects for decreasing hypertension, hyperuricemia, and hyperpigmentation.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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