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Antioxidant Activities of Garlic (*Allum sativum* L.) with Growing Districts

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Abstract Hydrogen-donating capacity, scavenging activity of reactive oxygen including superoxide anion radical and hydrogen peroxide, metal-chelating activity, and reducing power of garlic extracts were investigated. All tested garlic extracts exhibited *in vitro* antioxidant activities, with Uiseong extract showing highest hydrogen-donating and hydrogen peroxide-scavenging activities, and reducing power, followed by Seosan and Samchek extracts, in proportion to total thiosulfinate contents. Higher scavenging activity of superoxide anion radical was observed in Uiseong than Seosan and Samchek extracts. Metal-chelating activity increased in order of Uiseong < Seosan < Samchek, showing inverse relations to total thiosulfinate content. Garlic extracts of Uiseong and Seosan showed weak prooxidant activities and that of Samchek showed strong antioxidant activity against Cu^{+2}-induced human LDL oxidation. Protective effects on peroxyl and hydroxyl radical-induced DNA strand damages were observed in all tested garlic cloves. These results indicate growing conditions of garlic cloves affect total thiosulfinate content and antioxidant activities.

Key words: garlic, antioxidant activity, LDL oxidation, DNA strand damage

Introduction

Reactive oxygen species (ROS) including superoxide anion radical (O_2^-), hydroxyl radical (OH^-), singlet oxygen (O_2^1), and hydrogen peroxide (H_2O_2) are generated as byproducts of normal cellular metabolism or are results of exogenous factors such as smoking and air pollution (1). Oxidative stress refers to the imbalance between the ROS generation and the antioxidant defense activity. Severe oxidative stress has been implicated in aging and such chronic diseases as cancer and coronary heart disease caused by damages to biological molecules such as lipids, proteins, carbohydrates, and DNA (2). In particular, chemical damages to DNA induced by ROS, leading to the modification of DNA bases and DNA strand breakage, may result in the development of cancer should the capacity to repair DNA be inefficient. A possible scavenger of these ROS may be used as a preventive tool to control oxidative stress-related diseases. Many polyphenolic and thiol compounds from plant materials including herb extracts have shown antioxidant activities against ROS (3, 4).

LDL is the major cholesterol carrier in the blood, and an elevation in the plasma level of LDL is correlated with an increased risk of atherosclerosis and cardiovascular disease. LDL does not cause atherosclerotic plaques in its native form; however, oxidative modification of LDL may contribute to the pathology of atherosclerosis, leading to the plaque buildup in arteries and the consequent coronary heart diseases (5). Several evidences support that oxidized LDL plays an important role in atherosclerosis (6). Thus, this has led to the increased interest in the role of natural compounds as antioxidants for the inhibition of LDL and membrane lipid oxidations. (7)

Garlic (*Allum sativum* L.) has been used as a valuable healing agent worldwide for thousands of years (8). Its consumption has become a widely accepted general dietary course for promoting overall human health in Eastern Europe and Asia. Many beneficial health-related biological properties are attributed to garlic, including anticarcinogenic (9, 10), antitumorigenic (11, 12), antimutagenic (13), cardiovascular-protective (14, 15), antimicrobial (16, 17), immunomodulatory (11), and antioxidant effects (18, 19), and much of the health-promoting and related biological activities of garlic have been ascribed to the organosulfur compounds (20).

Among the physiological functions of garlic tissue extracts, the antioxidant activities have been of particular interest due to the relationship between oxidative stress and pathologies such as atherosclelosis, cancer, and aging involving free radicals and reactive oxygen species (21-24). Recent studies on the antioxidant activities of garlic have used crude extracts or tissue derivatives, and several investigators have shown that thiosulfinates or related organosulfur compounds, particularly allicin (thio-2-propene-1-sulfinic acid S-allyl ester), the pungent-smelling compound, are primarily responsible for the observed antioxidant activities in garlic (17, 25-27), although many other endogenous compounds may also carry the antioxidant properties (19). During the crushing of garlic, allicin is produced through the interaction of alliin (S-allyl-L-cysteine sulfoxide), the non-protein amino acid, with the pyridoxal phosphate- containing enzyme, allinase (20).

In this study, *in vitro* oxidation assay was performed to investigate the effects of garlic clove growing district on total thiosulfinate concentrations, antioxidant and metal-chelating activities, reducing power, and inhibitions of copper-catalyzed oxidation of human LDL and oxidative DNA strand breakage.

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Materials and Methods

Preparation of garlic extracts Garlic cloves were purchased from local markets in Uiseong, Seosan, and Samcheok, Korea, and kept in a deep-freezer at -70°C until analysis. Twenty grams of garlic cloves were mashed with 50 mL of 50% ethanol in a hand mixer for 15 sec, stirred with a magnetic stirrer for 30 min (28), and centrifuged at 3,000 × g for 30 min at 4°C. Supernatant was then filtered through a filter paper (Whatman No 1) and kept in a deep freezer at -70°C until analysis.

Total thiosulfinates assay Concentrations of total thiosulfinates including allicin were estimated using 4-mercaptopyrimidine(4-MP), a thiol-containing chromophore, which specifically reacts with allicin (29). Garlic extract was mixed with 1 mL of 0.1 mM 4-MP solution in 50 mM Na-phosphate buffer (pH 7.2) containing 2 mM EDTA for 30 min at room temperature, and the absorbance was measured with a spectrophotometer (Shimadzu Inc., Kyoto, Japan) at 324 nm. Extinction coefficient (ε_M, 39,600 M^{-1} cm^{-1}) at 324 nm was used for the calculation of thiosulfinate concentrations of garlic samples.

DPPH radical-scavenging capacity DPPH radical-scavenging capacities of the garlic samples were analyzed using the slightly modified method of Chen *et al.* (30). Fifty microliters of test sample were mixed with 500 μL of 70 μM DPPH in ethanol, and centrifuged at 1,000 × g for 10 sec. Two hundred microliters of the supernatant was transferred into a microplate well and allowed to react for 40 min at 37°C. Absorbance of the resulting solution was measured at 525 nm using an ELISA reader (Tecan Austria, Salzburg, Austria). The DPPH scavenging percentage of the garlic extract was calculated as follows:

$$\text{DPPH radical-scavenging capacity } (\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of garlic extract.

Hydrogen peroxide-scavenging capacity The hydrogen peroxide-scavenging capacities of the test samples were determined spectrophotometrically (32). A solution (2 mM) of hydrogen peroxide was prepared in PBS (pH 7.4) at room temperature. Three hundred microliters of garlic extract diluted thirty times were mixed with 600 iL of 20 mM hydrogen peroxide solution and allowed to stand for 10 min at room temperature. The absorbance was determined using an ELISA reader at 230 nm against a blank solution containing the test sample in PBS without hydrogen peroxide. All tests were run in triplicates and averaged. The percentage of hydrogen peroxide-scavenging capacity of the garlic extract was calculated as follows:

$$\text{Scavenged } H_2O_2\ (\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of garlic extract.

Superoxide dismutase (SOD)-like activity Increase in absorbance of reduced cytochrome C produced by superoxide anion radicals resulting from the enzyme reaction system of xanthine-xanthine oxidase was measured at 550 nm (31). Xanthine (0.5 mM) was dissolved in 0.001 N NaOH with sonication. Solution A was made by mixing xanthine solution and 50 mM phosphate buffer (pH 7.8) containing 0.1 mM EDTA. Solution B was prepared by adding 12 μL xanthine oxidase (XOD) into 2 mL of 0.1 mM EDTA to make 0.2 unit XOD. Ten microliters of the test sample was mixed with 200 μL solution A for 10 sec and pre-incubated for 10 min at 37°C, followed by the addition of 10 μL solution B. The increase in absorbance at 550 nm was read for 2 min using an ELISA reader, and was compared with that of the control measured with deionized water instead of the test sample. One unit of SOD-like activity was defined as the amount of test sample resulting in 50% inhibition of cytochrome C reduction.

Metal-chelating activity The chelating activity of garlic extract was estimated by the method of Decker and Welch (33, 34). Two hundred microliters of each extract diluted five times were mixed with 20 μL $FeCl_2$ solution (2 mM in H_2O). The reaction was initiated by the addition of 20 μL of 2.4 mM ferrozine, and the mixture was shaken with a vortex mixer and stood at room temperature for 10 min. Absorbance of the solution was measured with an ELISA reader at 562 nm.

Reducing power The reducing power of garlic extract was determined according to the method of Aruoma *et al.* (7). Forty microliters of garlic extract in 50% ethanol were mixed with 160 μL of the mixture containing 0.5 mM $CuCl_2$ and 0.75 mM neocuproine in 10 mM phosphate buffer (pH 7.4). The absorbance was measured with an ELISA reader at 454 nm for 2 hr. Increased absorbance of the reaction mixture indicates increased reducing power.

Copper ion-induced human LDL oxidation EDTA-free human LDL was prepared by dialyzing LDL purchased from Sigma Co. (St. Louis, MO, USA) in 10 mM PBS (pH 7.4) at 4°C in the dark with a nitrogen infusion for 24 hr (35). The protein concentration of LDL was measured using the modified Lowry protein assay reagent kit (Pierce, Rockford, IL, USA) with bovine serum albumin as the standard. Nine hundred microliters of LDL (0.02 mg protein/ml) were mixed with 50 μL garlic extract diluted ten times. Reaction was started by adding 50 μL of 10 mM $CuSO_4$ at 37°C. The formation of conjugated dienes resulting from the oxidation of human LDL was measured at 234 nm using a spectrophotometer.

Supercoiled DNA strand scission assay Plasmid supercoiled strand DNA (pBR322 DNA) was dissolved in 10 mM PBS (pH 7.4). DNA (2 μg/mL) was mixed with different concentrations of garlic extract before the oxidation initiators were added. For generating the peroxyl radical, 2, 2'-azobis(2-amidinopropane)dihydrochloride (AAPH) in PBS, pH 7.4, was added to a final concentration of 5 mM, and reaction mixture was incubated at 37°C for 2 hr (36). The hydroxyl radicals were generated with UV photolysis of H_2O_2. In the UV photolysis system, the reaction mixture contained pBR322 plasmid DNA (2 μg/

mL) and 30 mM H_2O_2. Garlic extract dissolved in ethanol was added prior to H_2O_2 addition. Hydroxyl radicals were generated by irradiating the reaction mixture at a distance of 20 cm with a 10-W UV lamp for 20 min at room temperature (37).

After incubation, the loading dye (consisting of 0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol in H_2O) was added to the samples, and 15 μL sample was loaded onto 0.7% (w/v) agarose gel. Gel electrophoresis was performed in a Tris-acetic acid-EDTA buffer (40 mM Tris, 2 mM EDTA, pH 8.5) using a horizontal, submarine gel, electrophoresis apparatus (Amersham Pharmacia Biotech, Uppsala, Sweden) at 3 v/cm for 1.4 hr. DNA strands were stained with ethidium bromide (0.5 mg/mL deionized water), visualized under UV light, and photographed with a camera. Photograph negatives were scanned and analyzed by imaging densitometer (Vilber Lourmat Co., Marne-la-Vallee Cedex 1, France) using a molecular analysis software to quantitate DNA breakage in terms of the percentage of Form I (supercoiled: S), which was nicked by peroxyl or hydroxyl radicals. The protective effect of garlic extract was compared by the retention percentage, calculated by the following equation:

$$\text{Retention } (\%) = (A_{sample}/ A_{control}) \times 100$$

where A_{sample} and $A_{control}$ represent the concentrations of supercoiled DNA with and without oxidative radicals, respectively.

Statistical analysis All results were expressed in means ±SD. Statistical analyses were performed using the statistical package SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA) program, and significance of each group was verified through the analysis of one-way ANOVA, followed by the Duncan's test at $p<0.05$.

Results and Discussion

The content of total thiosulfinates Many beneficial health properties of garlic are attributed to the organosulfur compounds, particularly to thiosulfinates. Allicin (diallyl thiosulfinate) is the most abundant compound, representing about 70% of the overall thiosulfinates present or formed upon crushing, chewing or cutting (or dehydrating, thereby exposing pulverized garlic to water) the garlic cloves (38, 39). Because thiosulfinates react rapidly with free thiol groups, a colored thiol-containing chromophore, whose optical absorbance is shifted upon reaction with thiosulfinates, can be used for the direct determination of thiosulfinate concentration. In this assay, the amount of thiosulfinates in garlic was estimated using 4-MP, a commercially available thiol-containing chromophore (29).

Total thiosulfinates were extracted with 50% ethanol solution, known as an efficient solvent for the extraction of thiosulfinates from garlic cloves (28). The significant differences in the content of total thiosulfinates among garlic cloves cultivated in different districts are shown in Table 1 ($p<0.05$). Uisong garlic showed the highest amount of total thiosulfinates, followed by Seosan and Samchek garlic samples. In particular, total thiosulfinates of Uisong garlic was more than two-fold higher than that of Samchek, which indicates that the formation of thiosulfinate compounds of garlic cloves may be dependent on the conditions of cultivated land.

Table 1. The content of total thiosulfinates in garlic cloves

Growing district	Total thiosulfinates (mg/g garlic)
Uisong	28.3±1.4[a1)]
Seosan	22.2±0.4[b]
Samcheok	12.4±0.4[c]

[1)]Values represent mean±SD of three measurements. Different letters indicate significant differences at $p<0.05$.

Table 2. Antioxidant activities of garlic cloves

Growing district	Scavenging activity (%)		SOD-like activity (units/g garlic)
	DPPH	Hydrogen peroxide	
Uisong	85.1±0.1[a1)]	64.0±5.4[a]	1064±22[a]
Seosan	72.7±2.0[b]	44.5±2.7[b]	526±15[b]
Samcheok	45.3±1.1[c]	30.2±1.1[c]	543±20[c]
Trolox	66.8±0.8	-	-
Ascorbic acid	20.0±0.2	33.8±11.6	33.8±11.6

[1)]Values represent mean±SD of three measurements. Different letters indicate significant differences at $p<0.05$.

Antioxidant activities of garlic extracts Antioxidant activities of the garlic extracts are shown in Table 2. Hydrogen-donating capacity of garlic to DPPH radicals was in the order of Samchek < Seosan < Uiseong, consistent with results of total thiosulfinates. These results suggest that thiosulfinates compounds contribute to the scavenging capacity of garlic by donating hydrogen atoms or electrons to DPPH radicals. Xiao and Parkin (27) showed that several pure thiosulfinates reduced DPPH radicals in a concentration-dependent manner, and the allyl functional units of allyl-S(O)S-allyl provided for a more effective electron-donating ability than the saturated alkyl groups within the thiosulfinate structure. They showed that the relative effectiveness of thiosulfinate as a reducing agent on an equimolar basis was 2-3 orders of magnitude less than those of the more commonly used antioxidants such as ascorbic acid and Trolox.

The ability of garlic extract to scavenge superoxide anion radical was determined based on the inhibition of oxidized cytochrome C reduction. All tested garlic extracts showed SOD-like activities, which were not proportional to the amount of total thiosulfinates, by scavenging superoxide anion radicals. The SOD-like activity of Uiseong garlic (1,064 units/g garlic) was approximately twice that of Seosan (526 units/g garlic) and Samchek (543 units/garlic) ones. Halliwell *et al.* (2) reported, because many thiols can react with superoxide anion radical with a low rate constant, generally less than 10^3 M^{-1} sec^{-1}, very high thiol concentrations (often greater than 1 mM) would be required to achieve significant scavenging, which is consistent with our results. In our assay, the concentrations of total thiosulfinates were 3.49, 2.48, and 1.40 mM in Uiseong, Seosan, and Samchek garlics. However, the scavenging activity of superoxide anion radical was not

observed at levels up to 4.3 mM for three representative thiosulfinates, methyl-S(O)S-methyl, propyl-S(O)S-propyl, and allyl-S(O)S-allyl (27), which is in contrast to the results of Halliwell *et al.* (2).

The scavenging activity of hydrogen peroxide might be one of the important antioxidant activities for reactive oxygen species, because it enables the crossing of biological membranes (2). The abilities of the three garlic extracts to scavenge hydrogen peroxide were estimated by spectrophotometrically measuring the reduction in the concentration at 230 nm. The scavenging activities of the three garlic extracts for hydrogen peroxide were significantly different ($p<0.05$) and in the order of Samchek < Seosan < Uiseong, which were in proportion to their contents of total thiosulfinates. Xiao and Parkin (27) reported that the thiolsulfinate species, methyl-S(O)S-methyl, propyl-S(O)S-propyl, and allyl-S(O)S-allyl, at levels up to about 2 mM were not capable of scavenging hydrogen peroxide, suggesting the involvement of other thiosulfinate compounds other than these three.

Metal-chelating activity The chelating of ferrous ions by garlic extracts was estimated by the method of Decker and Welch (33). Ferrozine can quantitatively form complexes with Fe^{+2}. In the presence of other chelating agents, the complex formation is inhibited, resulting in decreased red color of the complex. Therefore, the measurement of the rate of color reduction allows the evaluation of the chelating activity of coexisting chelator.

Complete formation of the ferrozine-Fe^{+2} complex does not occur in the presence of garlic extracts, suggesting that garlic extracts chelate ferrous ions (Fig. 1). The absorbance of ferrozine-Fe^{+2} complexes decreased inversely to the amount of total thiosulfinates, resulting in the increased chelating activity of ferrous ions. Yin *et al.* (40) analyzed the metal-chelating activities of four major organosulfur compounds of garlic, diallyl sulfide, diallyl disulfide, *S*-ethyl cysteine, and *N*-acetyl cysteine, and found that the metal-chelating activities of diallyl sulfide and diallyl disulfide were undetectable and those of *S*-ethyl cysteine and *N*-acetyl cysteine were weak, indicating that specific thiosulfinate compounds including *S*-ethyl cysteine and *N*-acetyl cysteine, and not the content of total thiosulfinates, may contribute to the metal-chelating activity of the garlic extracts.

Metal-chelating agents have an important role as secondary antioxidants in the lipid peroxidation, because they can reduce the redox potential, thereby stabilizing the formation of the oxidized form of the metal ion (41). The results obtained from Fig. 1 confirm that garlic extracts are effective for the chelation of ferrous ion, and may be utilized as antioxidants for the protection of lipid by inhibiting the catalyzation of lipid peroxidation by the transition metal ion.

Reducing power Antioxidants can function through various mechanisms, among which are the prevention of chain initiation, binding of transition metal ions, breakage of lipid peroxides, radical-scavenging, prevention of hydrogen abstraction, and reducing capacity (42). However, certain antioxidants have both antioxidant and pro-oxidant activities depending on the samples tested, and their possibilities as pro-oxidants could be assessed by analyzing the reducing powers (43). The ability of garlic extracts to stimulate the reduction of copper ion was evaluated by measuring the absorbance of neocuproine-Cu^{+1} complex.

A significant difference in the reducing power of garlic extracts was observed among the three garlic extracts (Fig. 2) ($p<0.05$). The reducing power of garlic extract increased with increasing amount of total thiosulfinates, suggesting that total thiosulfinate content may contribute to both the reducing capacity and the hydrogen-donating capacity of garlic extracts. The potential reducing capacity of garlic extract suggests the possibility of the extract as a pro-oxidant by reducing the transition metal ions in some *in vitro* systems(which them? Meaning not clear). However, the actual physiological significance of pro-oxidant activity displayed by many antioxidants *in vitro* is uncertain, because the transition metal ions in a free state, which is a

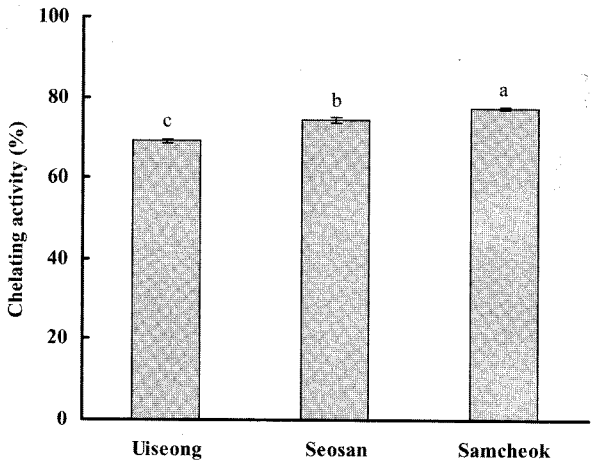

Fig. 1. Metal chelating activity of garlic extracts. Garlic extracts were diluted five times. Each bar represents mean±SD of three measurements. Different letters indicate significant differences at $p<0.05$.

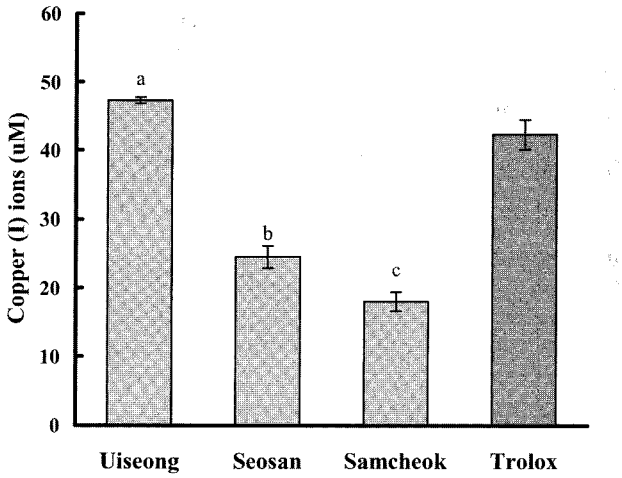

Fig. 2. Reducing power of garlic extracts. The concentration of Trolox as a positive control was 50 μM. Each bar represents mean ±SD of three measurements. Different letters indicate significant differences at $p<0.05$.

prerequisite for the pro-oxidant activity, can be sequestered due to their binding to the plasma proteins, except in pathological conditions (4, 44).

Protective effects of garlic extracts on human LDL The antioxidant activities of the garlic extracts were compared through EDTA-free human LDL *in vitro* assay with Cu^{+2}-catalyzed oxidation and spectroscopic monitoring of the development of conjugated diene hydroperoxides. In this assay, transition metal ions have been shown to be strong catalysts for LDL oxidation *in vitro* (45). Although the physiological significance of *in vitro* Cu^{+2}-induced LDL oxidation remains controversial, this method has been useful for evaluating naturally occurring antioxidant compounds (46).

The final concentrations of total thiosulfinates in garlic extracts were from 0.15 to 0.35 mM, and human LDL was oxidized at 37°C for 90 min with free air. Figure 3 shows the effects of garlic extracts on the inhibition pattern of Cu^{+2}-catalyzed human LDL oxidation. A weak pro-oxidant activity was observed in the Uiseong and Seosan garlic extracts by reducing the lag period of human LDL oxidation. On the other hand, the garlic extract of Samchek showed a potential antioxidant activity due to the increased lag period of human LDL oxidation. The observed antioxidant efficiencies of garlic extracts are probably related to their capacities to complex copper ions. Thus, their inhibitory activities in this LDL system could be due to the metal-chelating activity, rather than the amount of total thiosulfinates or DPPH radical-scavenging capacity, because the tested levels of total thiosulfinates in the three garlic extracts (0.15-0.35 mM) exceeded the concentration of copper ion (0.5 μM) used in copper ion-induced human LDL oxidation (47). According to the results of Highuchi *et al.* (48) and Lau *et al.* (49), the inhibitory activities of *S*-alk(en)yl-L-cysteines and their sulfoxides, volatile alk(en)yl disulfides and trisulfides, and vinyldithins in garlic against lipid peroxide formation in human LDL depend on the alk(en)yl substituents (methyl, propyl, and allyl) and the number of sulfur atoms in the thiosulfinate compounds. Therefore, further work is required to clarify the specific thiosulfinates, which are involved in the antioxidant activity against Cu^{+2}-catalyzed human LDL oxidation, in the tested garlic extracts.

Protective effects of garlic extracts on DNA strand damage Most oxidative damages in biological systems are due to the peroxyl radicals that have a comparatively long half-life and thus greater affinity to diffuse into the biological fluids in cells and tissues (35). While the reactions of peroxyl radicals are slower than those of hydroxyl radicals, they are more specific. This suggests that peroxyl radicals may be more efficient than hydroxyl radicals in causing damages to important cellular macromolecules such as DNA, proteins, and carbohydrates. Various compounds from plant products including polyphenolic substances and thiols have shown scavenging activity against 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH)-induced peroxyl radicals to protect the double strand plasmid DNA from oxidative stress (35, 50).

The results of peroxyl and hydroxyl radical/pBR 322 plasmid DNA experiments of garlic extracts are shown in Figs. 5 and 6, with lower and upper bands indicating supercoiled DNA (SC) and open-circular DNA (OC), respectively. Only the single strand scission caused the conversion of SC into OC. Second strand scission converts OC into linear DNA (Lin) that is located between SC and OC on the agarose gel electrophoresis (51). Thus, by measuring SC in the plasmid DNA treated with peroxyl or hydroxyl radicals, the effectiveness of the protective activity against oxidative stress of plasmid DNA could be determined.

Figure 4 represents the dose-dependent protective effects of garlic extracts on the oxidative DNA strand damage with peroxyl radicals. All garlic extracts from 1.6 to 16.0 mg/mL raw garlic increased the protective effects on the oxidative damage of plasmid DNA and after then up to 80.0 mg/mL decreased protective effects were

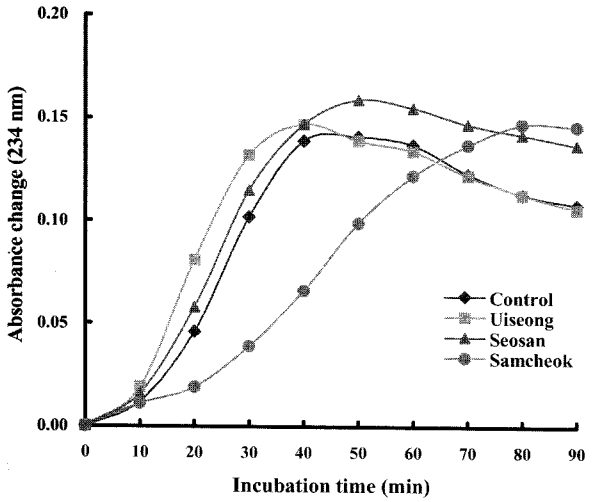

Fig. 3. Inhibitory effect of garlic extracts in Cu^{2+}-induced oxidation of human LDL at 37°C. The concentration of Cu^{2+} was 10 μM and that of protein in human LDL was 0.02 mg protein/mL.

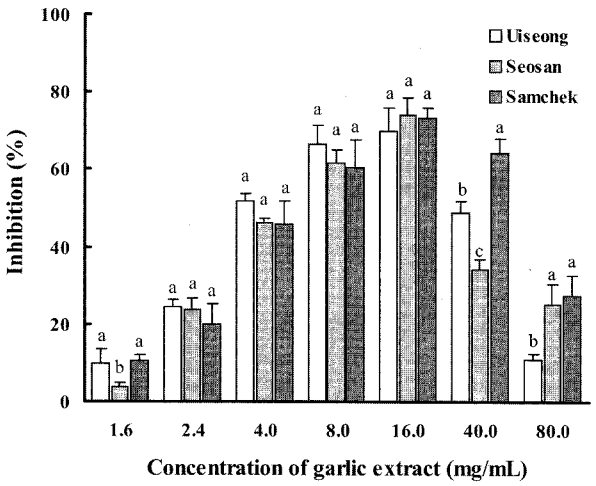

Fig. 4. Dose-dependent protective effect of garlic extracts on oxidative DNA strand damage at 37°C. Each bar represents mean±SD of three measurements. Different letters indicate significant differences at $p<0.05$.

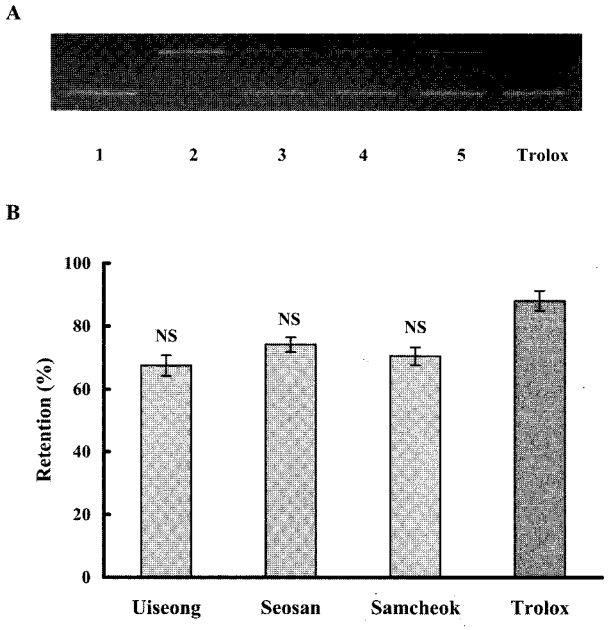

Fig. 5. Effects of garlic extracts in the prevention of peroxyl radical- induced DNA strand damage at 37°C. A. Electrodiagram of pBR322 DNA induced with AAPH as peroxyl radical. Lane 1 = DNA; lane 2 = DNA + peroxyl radical; lane 3 = garlic extract of Uiseong; lane 4 = garlic extract of Seosan; lane 5 = 5garlic extract of Samchek; lane 6 = 5 μg/mL Trolox. B. Effects of garlic extracts in peroxyl radical- induced DNA strand damage. Each bar represents mean±SD of three measurements. NS: not significantly different at $p<0.05$.

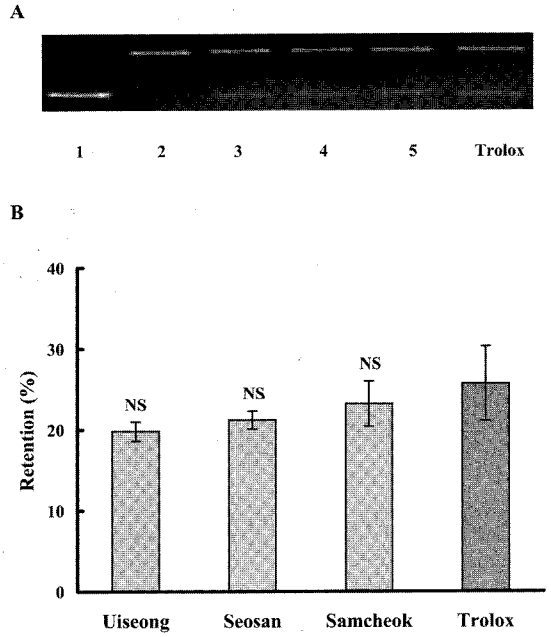

Fig. 6. Effects of garlic extracts in the prevention of DNA strand damage induced with hydroxyl radical from UV photolysis of hydrogen peroxide. A. Electrodiagram of pBR322 DNA induced with hydroxyl radical from UV photolysis of hydrogen peroxide. Lane 1 = DNA; lane 2 = DNA + hydroxyl radical; lane 3 = garlic extract of Uiseong; lane 4 = garlic extract of Seosan; lane 5 = garlic extract of Samchek; lane 6 = 50 μg/mL Trolox. B. Effects of garlic extracts in hydroxyl radical-DNA strand damage. Each bar represents mean±SD of three measurements. NS: not significantly different at $p<0.05$.

observed, suggesting that the maximum protection effects of the tested garlics on peroxyl radical-induced DNA strand damage occurred at 16.0 mg/mL raw garlic cloves, although no significant differences were observed among the extracts (Fig. 5, $p<0.05$). At 40.0 mg/mL, the protective effects of garlic samples were in the order of Seosan (34.2%) < Uiseong (48.8%) < Samchek (64.1%) garlics, in contrast with the results of DPPH radical-scavenging activity estimated at similar level of garlic concentration (36 mg/mL), in which the DPPH radical-scavenging capacities were in the order of Samchek (45.3%) < Seosan (72.7%) < Uiseong (85.1%) (Table 2). These results indicate that further study is necessary to explain the discrepancy observed between the protective effects on AAPH radicalinduced DNA strand damage and the DPPH radical-scavenging capacities in the tested garlic extracts.

Hydroxyl radical, which combines DNA molecules to produce adducts in biological systems, was generated via UV photolysis of hydrogen peroxide (37, 52). The protective effects of garlic extracts on the oxidative DNA damage by hydroxyl radicals are shown in Fig. 6. All garlic extracts efficiently suppressed the oxidative stress on plasmid DNA strand by hydroxyl radical. At 16 mg/mL, the protective efficiencies of garlic extracts on the hydroxyl radical-induced DNA strand damage were from 19.8% (Uiseong) to 23.2% (Samchek), showing no significant differences among the samples, while that of Trolox used as a positive control was 25.7% at 50 μg/mL.

In conclusion, the three garlic extracts exhibited different *in vitro* antioxidant activities, metal-chelating activities, and reducing powers depending on their growth districts. The Uiseong and Seosan garlic extracts showed weak pro-oxidant activities, whereas that of Samchek showed a strong antioxidant activity against Cu^{+2}-induced human LDL oxidation. The potential protective effects of the three garlic cloves on the peroxyl or hydroxyl radical-induced DNA strand damage were not significantly different. These results indicate that total thiosulfinate content and antioxidant activities of the garlic cloves may differ depending on the growing conditions.

Acknowledgments

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