Extraction of antioxidant material from raw garlic by hydrothermal reaction method

（水熱反応法を用いた生ニンニクからの抗酸化物質の抽出）

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Contents

Chapter 1: Extraction method for increasing antioxidant activity of raw garlic using steam explosion

1.1. Introduction

Metabolic syndrome becomes a big social problem in Japan, and one of two people of man and one of five people of woman in old and middle ages are metabolic syndrome or spare groups [1]. It seems that the oxidation stress caused by harm of reactive oxygen species participates in mechanism of the metabolic syndrome deeply. When internal organs fat increases, the amount of adipocytokine secreted from cells is disturbed and not only hyperlipidemia, hyperglycosemia, and high blood pressure are caused but also the reactive oxygen species increases in this process and let their symptoms progress. There are two types of adipocytokines; good adipocytokines have antioxidant activities and bad adipocytokines has the inflammatory nature and increase the reactive oxygen species [2]. When the reactive oxygen species increases in the body, the synergetic effect of their oxidation stress and bad adipocytokines causes hyperlipidemia and high blood pressure with insulin resistance. Furthermore, the reactive oxygen species recommends the oxidation of blood LDL cholesterol and increases participation LDL as results in lets arteriosclerosis progress. For reducing internal reactive oxygen species, an intake of the food including the nourishment ingredient with the antioxidant activity is desired. A lot of plant food have an antioxidant activity and show a free radical scavenging ability, and they have been used as healthy food. Garlic, i.e. Allium sativum, is one of the highest antioxidant and hypoglycemic food and used for not only culinary but also medicinal purposes because it contains polyphenol-based antioxidant materials [3]. In recent years black garlic, i.e. aged old garlic, has been attracted as a higher antioxidant food compared to raw garlic [4,5]. However, in order to manufacture black garlic, it takes a long time for aging, i.e. 30–60 d, at high temperature, i.e. 55–80 ◦C,

and high humidity, i.e. 70–95% [6,7]. Therefore, it is desired to not only shorten the manufacturing time but also simplified the manufacturing process. In this study, the conversion method of raw garlic into garlic pieces with a higher antioxidant activity than black garlic was developed using a steam explosion. Steam explosion consisted of steam hydrolysis at high temperature and pressure followed by sudden reduction of the pressure for a mechanical treatment of the hydrolyzed product has been known as an effective treatment method for degrading and depolymerizing the components of biomass [8–10]. Therefore, this study examined the effect of steam explosion for increasing the antioxidant activity of product and compared the antioxidant activity of steam-exploded raw garlic with that of black garlic.

1.2. Materials and Methods

1.2.1. Sample

Raw garlic was purchased from a local market in Aomori Prefecture, Japan. Raw garlic was peeled and used as a sample in this experiment.

1.2.2. Steam explosion

For increasing an antioxidant activity of raw garlic, steam explosion was carried out in a batch apparatus equipped with a 2 l reactor (Steam explosion apparatus NK-2L; Japan Chemical Engineering and Machinery Co. Ltd, Osaka, Japan) [11]. One hundred grams of raw garlic was introduced into the reactor and exposed to saturated steam at a pressure of 10 (183 ◦C), 15 (200 ◦C), 20 (214 ◦C), 25 (225 ◦C), 30 (235 ◦C), and 45 atm (258 ◦C) for a steaming time of 1–10 min. The prescribed temperature was reached in a few seconds. After exposure to the saturated steam, a ball valve at the bottom of the reactor was suddenly opened to rapidly bring the reactor to atmospheric pressure. The liquid–solid reaction product, i.e. a steam-exploded raw garlic, was collected in the receiver. The severity factor of steam explosion treatment is expressed by a correlation between steam temperature and steaming time [12,13]. Since the volume of reactor (2 l) is small, the steam pressure may reach the target value in a very short time. In this case, the severity factor can be calculated by the following equation:

$$S = \mathrm{Log}\,[t \cdot \exp\{T - 100/14.75\}] \quad (1)$$

where S is the severity factor, T is the steam temperature (◦C), and t is the steaming time (min). 14.75 is the activation energy value under conditions where process is first order kinetics and obeys the Arrhenius law.

1.2.3. Extraction and separation method

After freeze-drying steam-exploded raw garlic, one gram of dry sample was extracted in a 300 ml Erlenmeyer flask with 100 ml distilled water and the extract was separated by a filtration used for determining radical scavenging activity and amount of total phenolic compounds.

1.2.4. Determination of radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl radical) is a stable nitrogen-centered free radical whose color changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation [14]. Radical scavenging activity was calculated based on the change of absorbance due to the decrease in DPPH in relation to the control value [15,16]. The extract (2 ml), ethanol (2 ml), and 0.5 mM DPPH in ethanol solution (1 ml) were mixed in the test tube, and the decrease in absorbance at 517 nm was measured after 30 min of reaction. Considering the color of extract, the ethanol solution (1 ml) instead of 0.5 mM DPPH in ethanol (1 ml) was used as a blank. As a control water (1 ml) was added instead of the extract. The radical scavenging activity can be calculated by the following equation:

$$\text{Radical scavenging activity (\%)} = [\, X0 - (X - Xa)/X0 \,] \times 100 \quad (2)$$

where X is the absorbance of the extract and DPPH at 517 nm after 30 min of reaction, X0 is the absorbance of DPPH at 517 nm as a control, and Xa is the absorbance of the extract at 517 nm as a blank.

1.2.5. Determination of total phenolic compounds

Amount of phenolic compounds in the extract was measured according to the Folin–

Ciocalteu method [17]. The extract (200 _l) was added to the test tube containing 4 ml of distilled water, followed by addition of 1 ml phenol reagent. The mixture was thoroughly stirred. In addition, 1 ml of 10%(w/v) sodium carbonate was added to this solution. The absorbance of reaction was measured at 760 nm after 1 h of reaction. Estimations were carried out in triplicate and calculated from a calibration curve obtained with catechin. The amount of phenolic compounds was expressed as catechin equivalent (mg-catechin equiv./g-dry raw garlic).

1.3. Results and discussion

Fig. 1 shows the relationship of radical scavenging activity and sample concentration of extract obtained from steam-exploded raw garlic at a steam pressure of 15 atm for a steaming time of 1, 3, and 5 min. The proportional correlation between radical scavenging activity and sample concentration was observed and EC50, a concentration at a radical scavenging activity of 50%, of each sample was obtained from the straight lines of Fig. 1. The EC50 values were 7.444, 0.538, and 0.202 g/l at steaming time of 1, 3, and 5 min, respectively. Since the EC50 value is a widely used parameter to measure the free radical scavenging activity and a lower value indicates a higher antioxidant activity [18,19], it seems that a longer steaming time increases the antioxidant activity of extract from raw garlic.

Fig. 2 shows the EC50 value of extract from the raw garlic treated under various steam pressures and steaming times. The EC50 values changed significantly with steam pressures and steaming times. At a steam pressure of 15 and 20 atm the EC50 value decreased rapidly with the increase of steaming time reaching about 0.5 g/l at 3 min and then decreased gradually, but at a steam pressure of 30 and 45 atm the EC50 value decreased very slowly from about 0.3 to 0.14 g/l at a steaming time of 1–10 min and no significant decrease was observed. It suggests that the part of polyphenols contained in raw garlic was low-molecularized and dissolved as low molecular phenolic compounds in water by steam explosion with a high temperature and pressure steam followed by the rapid decompression to the atmospheric pressure. Furthermore, it seems that the polysaccharides were also degraded and pyrolysis products like furfural and 5-HMF were produced at a steam pressure of 30 and 45 atm while protein, lipids, and other extractives suffered pyrolysis [20]. The high antioxidant activity of extract from the steam-exploded raw garlic radical is attributed to that such degraded products may contribute to the antioxidant activity in addition to the phenolic compounds resulting in

a lower EC50 value. Fig. 3 shows the amount of phenolic compounds in extract from the raw garlic treated under various steam pressures and steaming times. At a steam pressure of 15 and 20 atm the amount of phenolic compounds increased with the increase of steaming time, but at a steam pressure of 30 atm the amount of phenolic compounds for a steaming time of 10 min i.e. 70.9 mg/g, was lower than that for a steaming time of 5 min, i.e. 93.7 mg/g. Furthermore, the amount of phenolic compounds at 45 atm for 5 min, i.e. 80.5 mg/g, was also lower than that at 30 atm for 5 min. It seems that higher steam pressures and longer steaming times degraded phenolic compounds produced by steam explosion resulting in a little lower amount of phenolic compounds. Table 1 shows the chemical properties of extract obtained from steam-explosion raw garlic treated at various temperatures and steaming times. The chemical properties, i.e. severity factor, EC50 value, and amount of phenolic compounds, were changed significantly by steam explosional conditions. The lowest EC50 value, i.e. the highest antioxidant activity, was obtained at 45 atm for 5 min, but the highest amount of phenolic compounds i.e. 93.7 mgcatechin equiv./g-dry raw garlic, was obtained at 30 atm for 5 min. The reason why these optimal conditions differed is attributed that at a higher and longer steam explosion the degradation of phenolic compounds occurred and not only the phenolic compounds but also the degraded and pyrolysis products produced from protein, lipids, and other extractives contributed for increasing the antioxidant activity, described above. In Table 2, the chemical properties of extract obtained from raw garlic treated by other treatment methods such as milling, autoclaving, and aging are shown in comparison with the steam explosion treatment. For milling raw garlic Millser IFM-720G (Iwatani Co., Japan) was used. In case of using an extract from raw garlic treated by milling for 2 min, the EC50 value exceeded 100 g/l and the amount of phenolic compounds was 2.6 mg/g. Furthermore, in case of using an extract from raw garlic treating by not only autoclaving but also milling, the

EC50 values became lower than that obtained by only milling but they were still very higher than those obtained by steam explosion. Since little effect was observed even for a longer milling time beyond 2 min, the milling time was adjusted to 2 min in these experiments. From these results, the steam explosion treatment seems to be a very effective method for increasing the antioxidant activity of raw garlic. In case of using an extract from black garlic aged at 60 ◦C for 30 d and milling for 2 min, the EC50 value and the amount of phenolic compounds were 0.585 g/l and 13.7 mg/g, respectively. Since these values were almost the same as those, i.e. 0.538 g/l and 16.6 mg/g, obtained by steam explosion at 15 atm for 3 min as shown in Table 1, it was concluded that the steam explosion treatment was useful for increasing the antioxidant activity of raw garlic similarly to the aging method even if a comparatively low steam pressure and short steaming time steam explosion. Fig. 4 shows the relationship EC50 value of extract obtained from steam-explosion raw garlic and severity factor of steam explosion. Since both steam temperature, i.e. steam pressure, and steaming time are important variables in steam explosion, the severity factor consisted of steam temperature and steaming time is currently used to compare experimental results to facilitate process designand operation [21]. The EC50 decreased rapidly with the increase of the severity factor and then it decreased slowly beyond 3.5, reaching an almost constant value. The severity factor of 3.5 corresponded to the steam explosion at 15 atm for 3 min or 10 atm for 10 min. Since the steam explosion with a higher severity factor requires a large amount of heat energy, future studies will be focused on the determination of optimal steam explosional condition for increasing antioxidant activity of raw garlic with saving energy.

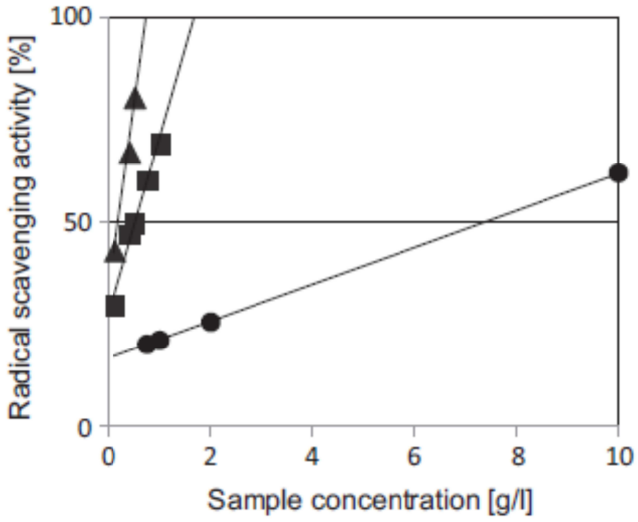

Fig. 1 Relationship of radical scavenging activity and sample concentration of extract obtained from steam-exploded raw garlic under various steaming times at a steam pressure of 15 atm. Symbols: ●, 1 min; ■, 3 min; ▲, 5 min

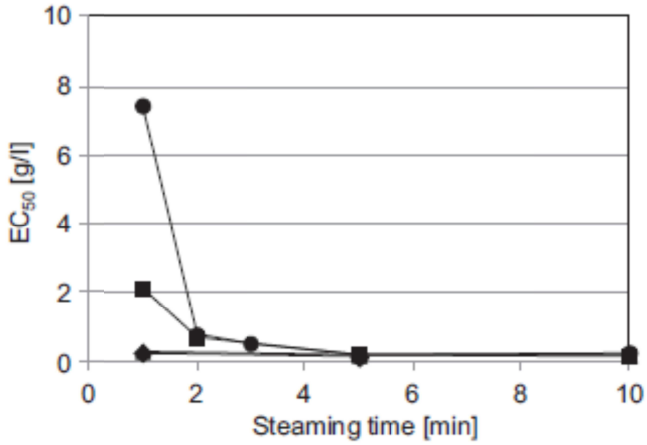

Fig. 2 EC50 value of extract from the raw garlic treated under various steam pressures and steaming times. Symbols: ●, 15 atm; ■, 20 atm; ▲, 30 atm; ♦, 45 atm.

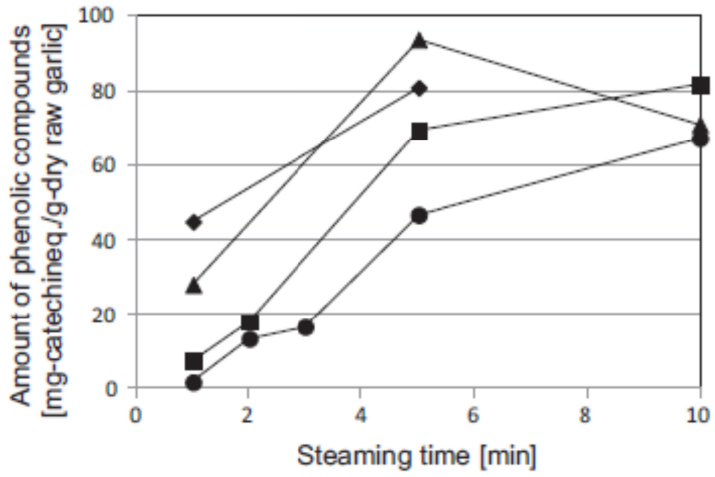

Fig. 3 Amount of phenolic compounds in extract from the raw garlic treated under various steam pressures and steaming times. Symbols: ●, 15 atm; ■, 20 atm; ▲, 30 atm; ♦, 45 atm.

Table 1
Chemical properties of extract obtained from steam-explosion raw garlic treated at various temperatures and steaming times.

Steam pressure [MPa]	10	15	15	15	15	15	20	20	20	25	25	30	30	30	45	45
Steaming time [min]	10	1	2	3	5	10	1	5	10	1	5	1	5	10	1	5
Severity factor	3,36	2,89	3,19	3,36	3,58	3,89	3,30	4,00	4,30	3,65	4,35	3,95	4,64	4,95	4,65	5,35
EC_{50} [g/l]	0,221	7,444	0,793	0,538	0,202	0,265	2,15	0,213	0,178	0,534	0,18	0,31	0,191	0,184	0,261	0,135
Phenolic compounds [mg/g]	53,2	1,7	13,4	16,6	46,6	67,1	7,3	69,3	81,7	20,1	85,9	28,0	93,7	70,9	44,8	80,8

Table 2
Chemical properties of extract obtained from raw garlic treated by various treatment methods.

Sample	EC_{50} [g/l]	Phenolic compounds [mg/g]
Raw garlic treated by milling for 2 min	>100	2.6
Raw garlic treated by autoclaving at 120 °C for 10 min and milling for 2 min	30.1	0.7
Raw garlic treated by autoclaving at 120 °C for 60 min and milling for 2 min	4.41	1.9
Black garlic aged at 60 °C for 30 d and milling for 2 min	0.585	13.7

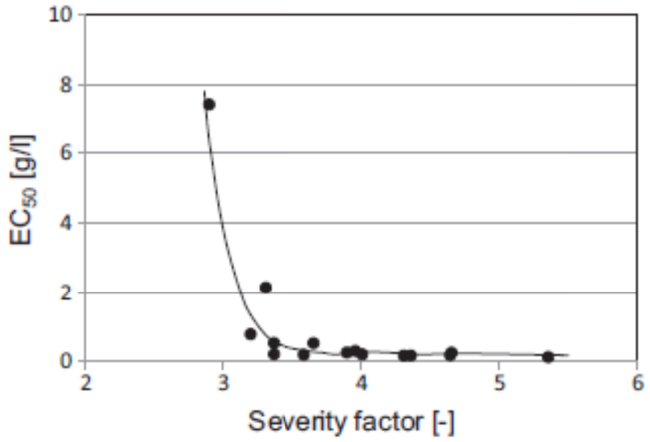

Fig. 4　Relationship EC50 value of extract obtained from steam-explosion raw garlic and severity factor of steam explosion.

1.4. Conclusion

A garlic extract was obtained from raw garlic by steam explosion. Its antioxidant activity was evaluated in this work. Garlic extract with a high free radical-scavenging capacity, exceeding that of black garlic significantly can be manufactured without passing through the time-consuming raw garlic maturing process. Moreover, since this manufacturing method requires only a short time, the garlic extract obtained by steam explosion may be preferably used as an antioxidant material blended with healthy food or medical supplements at low cost.

1.5. References

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Chapter 2: Effects of hydrothermal methods such as steam explosion and microwave irradiation on extraction of water soluble antioxidant materials from garlic husk

2.1. Introduction

Antioxidants are useful not only for food preservation but also for improving human health and disease [1,2]. The increasing of antioxidant demand lately forces the researcher to find another resource for natural antioxidant. Spices and herbs are the one of natural resource of antioxidant. Embuscado [3] reported that the antioxidant activity from spices and herbs are very effective. Availableness of spices and herbs in the market was growing significantly yearly, in line with sustained effort to make herbs and spices as a natural source of the antioxidant product. Antioxidant extraction from spices and herbs has been reported by various researchers. Zeng et al. [4] reported that Italian Oregano (*Origanum* × *majoricum*), Oregano (*O. vulgare ssp. Hirtum*), and Rosemary Mint (*Poliomintha longiflora*) has a high content of ORAC (Oxygen Radical Absorbance Capacity) and phenolic content. Qin et al. [5] reported about the enhancement of flavonoids extraction from fig leaf using steam explosion. Furthermore, black garlic, i.e. aged old garlic, has been attracted as a higher antioxidant food compared to raw garlic [6]. However, in order to manufacture black garlic, it takes a long time for aging at high temperature and high humidity [7]. Therefore, it is desired to not only shorten the manufacturing time but also simplify the manufacturing process. In a previous report [8], we undertook pretreatment of raw garlic (*Allium sativum*) by steam explosion for the extraction of antioxidants. Steam explosion is a hydrothermal pretreatment method for plant biomass (especially woods and herbaceous plants) that uses high pressure and high temperature steam without the addition of any chemicals to cause autohydrolysis, defibriation, and delignification. This method has several

advantages; it eliminates the use of toxic substances such as strong acids and alkalis, chemical-tolerant equipment, and waste processing systems etc. The steam-exploded garlic possessed antioxidant activity higher than black garlic. Garlic contain *alicin* as antioxidant which has potential mechanism for anti-cardiovascular disease i.e. to increasing antioxidant status, inhibit enzymes involved in lipid synthesis, decreased platelet aggregation, to prevent lipid peroxidation of oxidized erythrocytes, and inhibit angiotensin-converting enzyme [9-11]. However, the effective use of natural antioxidant sources is an important thing to do, to obtain the cost efficiency from each process to produce the natural antioxidant. Therefore, we continue to study the opportunity to use garlic husk (GH), i.e. a waste of peeled raw garlic, as a source of natural antioxidant for effective utilization of food waste. Though conventional solvent extraction is the most widespread technique for the extraction of antioxidant compounds from plant biomass, new hydrothermal methods have surfaced as environmentally friendly alternatives to the former method.

In this work, we used not only steam explosion (SE) but also microwave (MW) treatment as hydrothermal methods for the extraction of antioxidants from GH and compared their extraction effects. MW is an internal heating method that enables the rapid and homogeneous extraction of antioxidants compared with other processes with external heating method such as SE. MW has many advantages, i.e. fast heating rate and response, high heating efficiency, uniform/local heating and selective heating [12]. Thermal heating of MW treatment is the most effective and provides rapidly heating transfer, direct to the molecules that are present in the reactants, which is better than the conventional thermal heating that is slow and inefficient. Moreover, in the reaction on conventional heating, the surface of the material has higher temperatures than the reaction mixture [13]. MW treatment could be used to improve the process of increasing of antioxidant activity from GH. Furthermore, the MW treatment can modify by

selectivity conditions to conduct the reactions that do not occur under conventional thermal heating conditions. The aim of this work was to elucidate the effects of hydrothermal methods as SE and MW on the extraction of water soluble antioxidant materials from GH.

2.2. Materials and Methods

2.2.1. Sample

Raw garlic (*Allium sativum*) was purchased from a local market in Aomori Prefecture, Japan. Raw garlic was divided into flesh and husk, the husk was removed to a thickness of 0.02-0.17 mm manually. In case of addition of milling treatment, crush mill (D3V-10, Osaka Chemical Co. Ltd., Japan) was used for 1min (8.5 g per one time treatment).

2.2.2. Thermal treatment of garlic husk

We used three kinds of thermal treatment, heating by autoclave (HA), steam explosion (SE), and microwave irradiation treatment (MW) for the treatment of garlic husk (GH).

HA was carried out using an autoclave apparatus (high pressure steam sterilizer, LSX-500, TOMY SEIKO Co. Ltd.). 0.5 g of the GH was placed in 200 ml of beaker, heated at 121 °C for 10 min.

SE was carried out in a batch apparatus equipped with a 2L reactor (NK-2L, Japan Chemical Engineering and Machinery Co. Ltd., Osaka, Japan). Fifty grams of GH was introduced into the reactor and exposed to saturated steam at a temperature of 200 and 213 °C for a steaming time of 5min. After exposure to the saturated steam, a ball valve at the bottom of the reactor was suddenly opened to rapidly bring the reactor to atmospheric pressure. The liquid-solid reaction product was collected in the receiver.

MW was carried out using an initiator＋ instrument (Biotage Co. Ltd.) equipped with a 20 ml reaction tube, at a frequency of 2.45 GHz. The GH was ground by crush mill before the MW treatment. 0.5 g of the GH was suspended in 20 ml of distilled water, heated at 200 °C for 2 and 5 min.

2.2.3. Extraction and separation method

After the thermal treatment of GH, one gram of sample (dry matter) was extracted in a

300 ml of Erlenmeyer flask with 100 ml of distilled water and the extract (supernatant) was separated by a filtration followed by determining of radical scavenging activity and amount of total phenolic compounds.

2.2.4. Determination of radical scavenging activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) is a stable nitrogen-centered free radical whose color changes from violet to yellow upon reduction by either the process of hydrogen-or electron-donation [14]. Radical scavenging activity was calculated based on the change of absorbance due to the decrease in DPPH in relation to the control value [15, 16]. The extract (2 ml), ethanol (2 ml), and 0.5 mM DPPH in ethanol solution (1 ml) were mixed in the test tube, and the decrease in absorbance at 517 nm was measured after 30 min of reaction. Considering the color of extract, the ethanol solution (1 ml) instead of 0.5 mM DPPH in ethanol (1 ml) was used as a blank. As a control water (1 ml) was added instead of the extract. The radical scavenging activity can be calculated by the following equation (1):

$$\text{Radical scavenging activity (\%)} = [X_0 - (X - X_a)/X_0] \times 100 \qquad (1)$$

Where X is the absorbance of the extract and DPPH at 517 nm after 30 min of reaction, X_0 is the absorbance of DPPH at 517 nm as a control, and X_a is the absorbance of the extract at 517 nm as a blank. All determinations were performed in duplicate and the means were calculated.

2.2.5. Determination of total phenolic compounds

Amount of phenolic compounds in the extract was measured according to the Folin-Ciocalteu method [17]. The extract (200 μl) was added to the test tube containing

4 ml of distilled water, followed by addition of 1 ml of phenol reagent and the mixture was thoroughly stirred. In addition, 1 ml of 10%(w/v) sodium carbonate was added to this solution. The absorbance of reaction was measured at 760 nm after 1 h of reaction. Estimations were carried out in triplicate and calculated from a calibration curve obtained with catechin. The amount of phenolic compounds was expressed as catechin equivalent (mg-catechin eqiv./g-dry treated GH).

2.2.6. Severity factor

The severity factor of thermal treatment is expressed by a correlation between treatment temperature and treatment time [18-20]. The severity factor can be calculated by the following equation (2):

$$S = \mathrm{Log}\,[\, t \cdot \exp\{(T-100)/14.75\}] \qquad (2)$$

Where S is the severity factor, T is the treatment temperature (°C), and t is the treatment time (min). 14.75 is the activation energy value under conditions where process is first order kinetics and obeys the Arrhenius law.

2.2.7. ^{1}H NMR analysis

The ^{1}H NMR spectra of water extract from GH were obtained at 400 MHz using JEOL ECZ400S spectrometer and D_2O as the solvent.

2.3. Results and discussion

2.3.1 Extraction of antioxidant material from garlic husk

Noda et al. [8] reported that novel pretreatment method of extraction of the antioxidant material from raw garlic using steam explosion method, the antioxidant activity of raw garlic treated by steam explosion was higher than that of black garlic, i.e. aging garlic. Generally, the outside part of fruits or vegetables (garlic, in this study) is considered to be a richer resource of bioactive polyphenols than inside part because of its protective function [21]. Therefore, we used the GH as experimental material to extract antioxidant materials. At first, to extract the antioxidant materials from GH, we tested the simple three treatment method, i.e. milling, heating by autoclave apparatus (high pressure steam sterilizer, HA) at 121°C for 10 min, and milling combined with HA. EC_{50} value, a concentration at a radical scavenging activity of 50%, and amount of phenolic compounds obtained from untreated GH and treated GH by milling, heating by autoclave (HA), and milling combined with HA are summarized in Table 1. With untreated GH, the EC_{50} and amount of phenolic compounds were 29.73 g/l and 0.6 mg-catechin equiv./ g-dry treated GH, respectively. The EC_{50} values decreased (increased the antioxidant activity) and amount of phenolic compounds increased with milling treatment and HA, finally, with milling combined with HA method, the EC_{50} and amount of phenolic compounds were 12.73 g/l and 1.9 mg-catechin equiv./ g-dry treated GH, respectively. From this result, it was found that milling and heating the sample was effective to extract the antioxidant materials from the GH. Therefore, next, to obtain higher amount of phenolic compounds efficiently, two hydrothermal method, steam explosion (SE) and microwave irradiation method (MW) were investigated.

Table 1　EC_{50} value and amount of phenolic compounds of extract obtained from untreated GH and treated GH by milling, heating by autoclave (HA), and milling combined with HA.

	Untreated	Milling treatment	HA	Milling treatment ＋ HA
EC_{50} (g/l)	29.73	22.82	17.27	12.73
Phenolic compounds (mg-catechin equiv./ g-dry treated GH)	0.6	1.6	0.8	1.9

2.3.2. Extraction of antioxidant material from garlic husk using hydrothermal methods

Fig. 1 shows the relationship of radical scavenging activity and sample concentration of extract with water obtained from treated GH by SE (steam temperature of 200°C and 213°C for a steaming time of 5 min) and MW (treatment temperature of 180°C and 200°C for 2 min and 5 min). The proportional correlation between radical scavenging activity and sample concentration was observed and EC_{50} of each sample was obtained from the straight lines of Fig. 1. Fig. 2 shows the EC_{50} values determined from the straight lines of Fig. 1 and amounts of phenolic compounds at various treatment conditions, data obtained by milling combined with HA was used as a reference. The EC_{50} values obtained by SE and MW were quite lower than that obtained by milling combined with HA, the lowest EC_{50} value (the highest antioxidant activity) was 0.26 g/l obtained by MW at treatment temperature of 200°C for 5 min. Similar result, 0.202 g/l of EC_{50} value from steam exploded garlic (flesh, not husk) at steaming temperature of 200°C for steaming time of 5 min, was reported by Noda et al.[8]. Furthermore, the amounts of phenolic compounds obtained by SE and MW were higher than that obtained by milling combined with HA. The highest amount of phenolic compounds, 40.0 mg-catechin equiv./ g-dry treated GH, was obtained by MW at treatment temperature of 200°C for 5 min, which was 21 fold higher than that obtained by milling combined with HA (1.9 mg-catechin equiv./ g-dry treated GH). Ichikawa et al.[21] reported that some phenylpropanoids such as coumaric acid, ferulic acid, guaiacylglycerol-β-caffeic acid ether, and *N-trans*-coumaroyloctopamine could be extracted from garlic husk with 80% ethanol solution. These compounds have one or two phenolic structures in their chemicals structure, insoluble or less soluble in water. It suggests that the partial decomposition (low-molecularization) occurred by activated water generated by SE with a high temperature and pressure steam followed by the rapid decompression to the atmospheric pressure or MW with efficient heating (heat can

be generated throughout the volume of the material) by high temperature and pressure [23], water soluble phenolic compounds were generated.

Fig. 3 shows the relationship of EC_{50} value of water extractive obtained from treated GH by SE and MW method at various conditions and severity factor of each method. The EC_{50} value dramatically decreased with the increase of the severity factor and then it reached constant value (between 0.26 and 1.04 g/l) beyond 3.0. Similar result was observed about steam exploded garlic flesh [8], the EC_{50} decreased rapidly with the increase of the severity factor and then it decreased slowly beyond 3.5 (steam explosion at 15 atm for 3 min or 10 atm for 10 min), reaching an almost constant value. From these results, these relationships showed that appropriate condition to extract antioxidant material from garlic flesh [8] or GH (this study) using steam explosion or two hydrothermal methods, i.e. SE and MW (this study) could be determined.

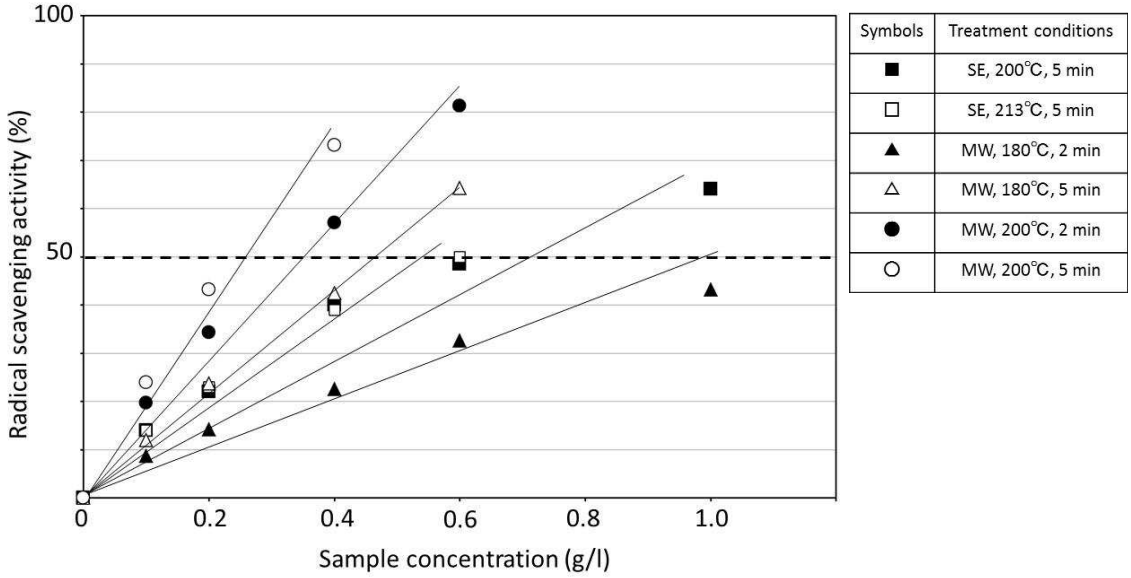

Fig. 1 Relationship of radical scavenging activity and sample concentration of extract obtained from treated GH by SE and MW under various conditions.

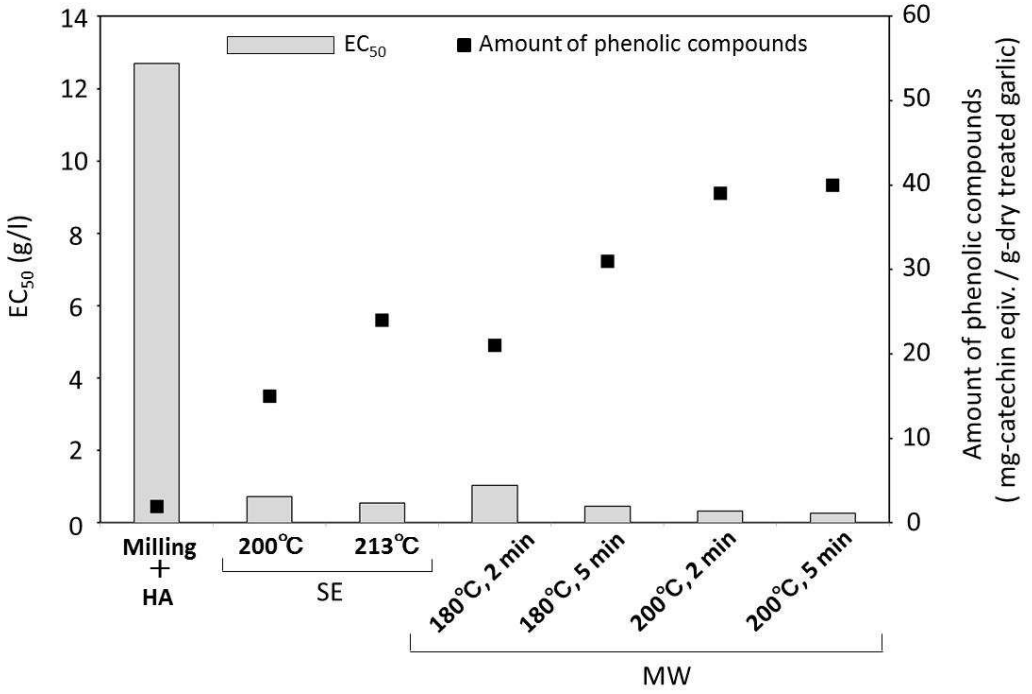

Fig. 2 EC_{50} value and amount of phenolic compounds of extract obtained from treated GH by SE and MW under various conditions.

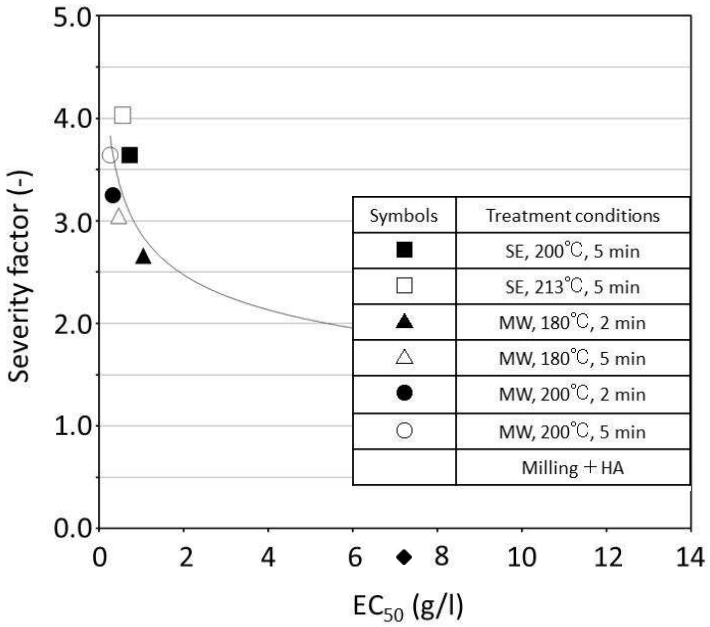

Fig. 3 Relationship of EC_{50} value of extract obtained from treated GH by SE and MW under various conditions and severity factor of each treatment condition.

2.3.3. ^{1}H NMR spectra of water extractive from garlic husk

To compare the contained chemical components in water extractives from untreated GH with that from microwave treated GH under 200 °C treatment temperature and treatment time of 5 min, we confirmed the spectroscopic data of water extractives using ^{1}H-NMR. Fig. 4 shows the ^{1}H-NMR spectra of the water extractive from untreated GH (a) and water extractive from microwave treated GH (b). With the water extractive from untreated GH, there were a few signals in high field (0.0-6.0 ppm) and low field (6.0-10.0 ppm) region of the spectrum, on the other hand, with the water extractive from microwave treated GH, many signals were observed. Especially, signals in low field region indicated that water extractive from microwave treated GH contained the constituent have aromatic ring such as water soluble phenolic compounds decomposed from phenylpropanoids mentioned above.

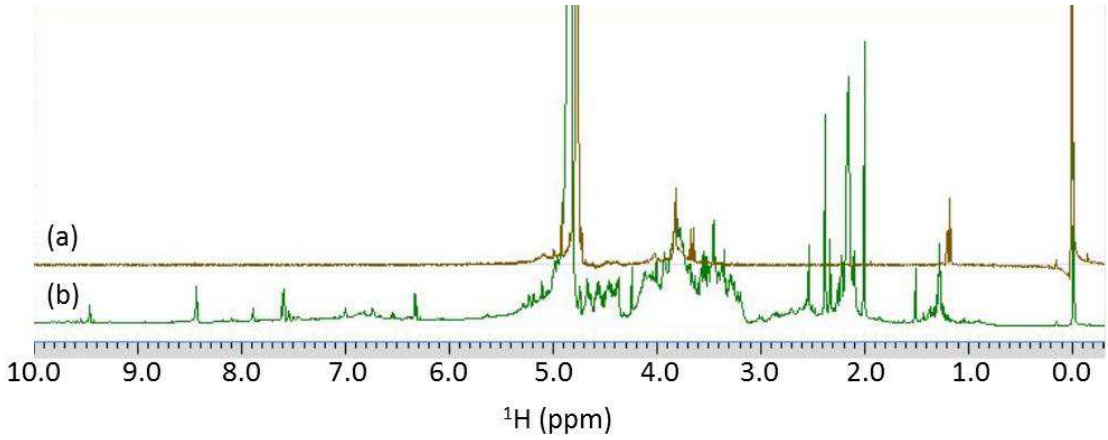

Fig. 4 ^{1}H NMR spectra of extract obtained from untreated GH (a) and treated GH by MW (200°C for 5 min)(b).

2.4. Conclusion

This is the first report of the extraction method of antioxidant material as water extractive from food waste GH using two environmental friendly hydrothermal methods, i.e. steam explosion and microwave irradiation method. From this study, it was cleared that water extractive from GH had a high free radical-scavenging capacity, therefore, GH could be a promising source to generate useful pharmaceutical products. Future study will be focused on the determination of optimal condition by SE and MW under various treatment temperatures and times.

2.5. References

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