

Original article

A comparative study on the antioxidative and anti-allergic activities of fresh and aged black garlic extracts

Joo Hee Kim¹, Seok Hyun Nam², Catherine W. Rico¹ & Mi Young Kang^{1*}

¹ Department of Food Science and Nutrition, Kyungpook National University, Daegu 702-701, Korea

² Department of Biological Science, Ajou University, Suwon, Korea

(Received 18 August 2011; Accepted in revised form 7 January 2012)

Summary The antioxidative and anti-allergic activities of fresh and aged black garlic extracts were investigated. The garlic samples were extracted with 70% ethanol (v/v) and the total phenolic content was measured. The antioxidant capacity of extracts was assessed by determining the scavenging activities on 1,1-diphenyl-2-picrylhydrazyl and hydroxyl radicals, ferricyanide reducing power, ferrous ion-chelating ability and inhibitory effect on linoleic acid peroxidation. The anti-allergic activity of extracts was analysed by measuring their inhibitory effects against β -hexosaminidase release. The aged black garlic exhibited significantly higher phenolic content and greater antioxidative activity than fresh garlic. Both garlic extracts showed strong antioxidant capacity in a dose-dependent manner. On the other hand, a considerably higher suppression of β -hexosaminidase release was found in fresh garlic extract at lower concentration compared with that of the black garlic. Results of this study illustrate that ageing of garlic could enhance its antioxidant capacity, but could decrease its anti-allergic activity.

Keywords Aged black garlic, anti-allergic, antioxidant, phenolics.

Introduction

For centuries, garlic (*Allium sativum*) has been widely cultivated and used not only as spice and condiment, but also as a traditional medicine against various diseases. It has been shown to possess a wide range of therapeutic effects, such as anti-cancer, hypolipidemic, antihypertensive, hepatoprotective and immunomodulatory, which are attributed to its bioactive organosulfur compounds (Banerjee *et al.*, 2002; Milner, 2005; Rahman, 2007). However, despite its numerous medicinal properties, garlic must be consumed in moderation because of its reported toxicity at high doses. Excessive consumption of garlic could cause damage in intestinal lining and stomach, anaemia, contact dermatitis and reduction in serum protein and calcium (Shashikanth *et al.*, 1986; Lembo *et al.*, 1991; Agusti, 1996; Kodera, 1997). Moreover, raw garlic produces a strong pungent odour that permeates the skin, resulting in an unpleasant body and breath odour in people.

With the recent development of aged black garlic, scientific research has focused on the potential health benefits of this new garlic product. Aged black garlic is prepared through natural fermentation of whole garlic

at controlled high temperature and humidity, a process that results in black cloves. Although the details of the process are a trade secret, some researchers reported that black garlic could be prepared at a temperature that ranges from 65° to 90 °C and a relative humidity of 60–80% (Jang *et al.*, 2008; Wang *et al.*, 2010; Lee *et al.*, 2011). This sweet fermented product can be eaten as a culinary snack or used as a food ingredient in Asian cuisines, particularly those from Japan, Korea and China. The aged black garlic does not have the strong odour of fresh garlic because the compound allicin responsible for the unique pungent odour (Milner, 2005; Rahman, 2007) is converted into water-soluble antioxidant compounds including S-allylcysteine and S-allylmercaptocysteine during the ageing process (Corso-Martinez *et al.*, 2007). A number of studies revealed that aged black garlic has powerful antioxidant and free radical scavenging properties (Jang *et al.*, 2008; Lee *et al.*, 2009; Wang *et al.*, 2010). Furthermore, extracts of this garlic product were found to have anti-tumour and anticancer activities, hypolipidemic action and anti-inflammatory property (Wang *et al.*, 2010, 2012; Kim *et al.*, 2011a; Lee *et al.*, 2011). In addition, dietary feeding of aged black garlic in laboratory animals showed hypoglycaemic, hypocholesterolaemic and hepatoprotective effects (Kang *et al.*, 2008; Seo *et al.*, 2009; Kim *et al.*, 2011b).

*Correspondent: E-mail: mykang@knu.ac.kr

While the pharmacological activities of both fresh and aged black garlic have been well-recognised, the issue of whether the aged black garlic has far better health benefits than fresh ones remains to be clarified. In addition, although their biological functions using animal models have been extensively studied, investigations on their comparative antioxidant and anti-allergic activities *in vitro* have been limited. Hence, this study was conducted to compare and evaluate the *in vitro* antioxidative and anti-allergic activities of fresh and aged black garlic extracts.

Materials and methods

Materials

Fresh and aged black garlic samples (5 kg each) were purchased from a local supermarket in Daegu, Korea. The 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), dimethyl sulfoxide (DMSO), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), Folin–Ciocalteu reagent, gallic acid and other chemicals were obtained from Sigma-Aldrich, Inc (Stenheim, Germany). All reagents were of analytical grade and used without further purification. The RAW 264.7 and RBL-2H3 cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA).

Preparation of ethanolic extracts from fresh and aged black garlic bulbs

Whole fresh and aged black garlic bulbs were soaked in 70:30 ethanol/water (v/v) solution for 24 h at 40 °C. The soaking process was repeated three times. The extract was then collected by filtration, concentrated in vacuum with rotary evaporation and lyophilised. The yield for fresh garlic and aged black garlic was 20.55% and 38.92% (w/w), respectively. The dried extract was dissolved in DMSO to a final concentration of 100 mg mL⁻¹ and kept at -20 °C until analysis.

Determination of total phenolic content

The total phenolic content of the garlic extracts was determined using Folin–Ciocalteu colorimetric method according to Singleton & Rossi (1965). Briefly, 0.1 mL of the extract was mixed with 0.5 mL distilled water and 0.5 mL of 20% Folin–Ciocalteu reagent. After 3 min, 1 mL of 20% sodium carbonate was added and the mixture was heated in a water bath at 100 °C for 1 min. The absorbance was measured at 685 nm after cooling in the dark. Gallic acid was used as standard, and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry material.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of the sample extracts was determined based on the method of Kato *et al.* (1988) with some modifications. Briefly, the garlic extract (0.2–2 mg mL⁻¹) was mixed with 1 mL of ethanolic solution containing DPPH radicals (0.5 mM) and shaken vigorously and left to stand for 30 min in the dark at room temperature. The absorbance of the mixture at 517 nm was measured, and the scavenging activity was calculated using the following equation:

$$\text{Electron-donating ability (\%)} = (A_0 - A_1)/A_0 \times 100$$

where A_0 was the absorbance of the control, and A_1 was the absorbance of the sample solution.

Measurement of reducing power

The reducing power of the garlic extracts was determined using the method developed by Oyaizu (1986). A 0.5 mL of the extract solution or distilled water (control) was spiked with 0.5 mL of sodium phosphate buffer (0.02 M, pH 7) and 0.5 mL of 1% potassium ferricyanide. The mixture was then kept in a water bath (50 °C) for 20 min. The resulting solution was cooled rapidly, spiked with 0.5 mL of 10% trichloroacetic acid and centrifuged at 800 g for 10 min. The supernatant (1.5 mL) was then mixed with 0.2 mL of 0.1% ferri-chloride. After 10 min, the absorbance at 700 nm was measured. A high absorbance was indicative of strong reducing power.

Determination of hydroxyl radical scavenging activity

The hydroxyl radical ($\cdot\text{OH}$) scavenging activity was determined by quantification of DMPO-OH spin trap adducts resulting from the reaction of DMPO with the radicals generated by the Fenton reaction (Rosen & Rauckman, 1981). The sample extract, in 50 μL of 0.1 M phosphate buffer solution (pH 7.4), was mixed with 50 μL of 0.3 mM DMPO, 50 μL of 10 mM FeSO_4 and 50 μL of 10 mM H_2O_2 . The ESR spectra were then recorded using an ESR spectrophotometer (TE-200; JEOL, Tokyo, Japan) under the following instrumental conditions: modulation amplitude, 0.1 mT; recording range, 4 mT; recording time, 1 min; time constant, 0.1 s; microwave power, 1.8 mW; and microwave frequency, 9.40432. The hydroxyl radical scavenging activity was expressed as the % reduction of the peak heights (representing the DMPO-OH spin trap adduct) as compared to the peak heights measured in the absence of the sample extracts.

Measurement of Fe^{2+} -chelating ability

The Fe^{2+} -chelating ability of the sample extracts was determined according to the method described by

Decker & Welch (1990). A 5 mL of the sample extract was spiked with 0.1 mL of 2 mM FeCl₂ and 0.2 mL of 5 mM ferrozine solutions. After reaction for 10 min, the absorbance at 562 nm of the resulting solution was recorded. A complex of Fe²⁺/ferrozine has a strong absorbance at 562 nm. A high ferrous ion-chelating ability in the test sample results in a low absorbance. The ferrous ion-chelating ability was calculated as follows:

$$\text{Chelating activity (\%)} = (A - B)/A \times 100$$

where *A* was the absorbance of the control group in reaction system, and *B* was the absorbance of the test group.

Evaluation of antioxidant activity in linoleic acid model system

The antioxidant activity was determined using the thiocyanate method described by Mathew & Abraham (2006). The reaction mixture consisted of 0.28 g linoleic acid, 0.28 g of Tween 20 and 50 mL of phosphate buffer (0.02 M, pH 7.0). A 2.5 mL of the above linoleic acid emulsion was added with 0.5 mL of test sample and 2.5 mL of phosphate buffer (0.2 M, pH 7.0) and incubated at 37 ± 0.5 °C for 5 days. The mixture (0.1 mL) was taken and mixed with 5.0 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature. Precisely 3 min after the addition of ferrous chloride to the reaction mixture, the absorbance at 500 nm was measured. The degree of oxidation was measured every 24 h, and the data are the average of triplicate analyses. The inhibition of lipid peroxidation (LPI) was calculated using the following equation:

$$\text{LPI (\%)} = (1 - A_s/A_c) \times 100$$

where *A_s* was the absorbance of the sample solution, and *A_c* was the absorbance of the control.

Cell viability assay

The cytotoxic effects of the fresh and black garlic extracts were examined using the MTT colorimetric assay employing intrinsic mitochondrial dehydrogenase activity as a parameter of cell viability (Mosmann, 1983). Briefly, the RAW 264.7 or RBL-2H3 cells were seeded in a 96-well plate at a density of 1 × 10⁵ per well. The fresh and black garlic extracts were added to the media at appropriate concentrations for the cultivation of cells at 37 °C for 48 h under humidified air containing 5% CO₂. Following the removal of the culture media, 0.1 mL of 5 mg mL⁻¹ MTT solution was added to each well. The incubation was continued for another 3 h. The DMSO was then added onto the supernatant-drained

cell layer to dissolve the intracellular chromogen by incubating for 30 min. The absorbance of the supernatant was then read in a microplate reader (Model-550; Bio-Rad Laboratories, Hercules, CA, USA) at 570 nm and a reference wavelength of 650 nm to eliminate the background absorbance contributed by the culture medium.

Determination of β-hexosaminidase inhibition

To evaluate the level of cell degranulation, β-hexosaminidase, a component of the basophilic secretory granule, was determined using the method of Jeong *et al.* (2002) with some modifications. The RBL-2H3 cells were seeded into 96-well plates at 1 × 10⁶ cells per well and incubated overnight. The cells were washed with Tyrode buffer (137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 11.9 mM NaHCO₃, 0.4 mM NaH₂PO₄, 5.6 mM glucose, pH 7.2). The test sample solution was added to each well and incubated for 15 min, followed by the addition of 20 μL of calcium ionophore (A23187, at a final concentration of 10 μM) at 37 °C for 20 min to stimulate cell degranulation. The recovered supernatant containing released β-hexosaminidase (50 μL) was added with the same volume of 1 mM p-nitrophenyl-*N*-acetyl-β-glucosaminid solution (pH 5.2). The sample was then incubated at room temperature for 1 h with continuous shaking. The reaction was terminated by the addition of 0.2 mL of 1 M sodium carbonate buffer (pH 10.2). The plate was read at 405 nm with a microplate reader (Model-550; Bio-Rad).

Statistical analysis

All experiments were performed in triplicate (*n* = 3) and the data are presented as mean ± SD. The results were evaluated by one-way ANOVA using a Statistical Package for Social Sciences software program version 18 (SPSS Inc., Chicago, IL, USA), and the differences between the means were assessed using Duncan's multiple range test and *T*-test. Statistical significance was considered at *P* < 0.05.

Results and discussion

Total phenolic content

The aged black garlic extract showed a markedly higher total phenolic content than fresh garlic (Table 1). This result is in accordance with the findings of Sato *et al.* (2006). In their study, there was a sevenfold increase in the polyphenol content of the ethanol extract from aged black garlic compared with that of the fresh ones. The increase in phenolic content of black garlic is likely associated with the chemical changes that occurred in raw garlic during the ageing process (Kwon *et al.*, 2006).

Table 1 Total phenolic content of fresh garlic and aged black garlic extracts

	Fresh garlic	Aged black garlic
Total phenolic content (mg GAE 100 g ⁻¹)	3.65 ± 0.17 ^a	22.17 ± 0.75 ^b

Values are means ± SD (*n* = 3). Means in the same row not sharing a common superscript are significantly different at *P* < 0.05.

When garlic undergoes fermentation, the irritating compounds from the raw garlic are converted into beneficial compounds through both enzymatic and natural chemical reactions. Kwon *et al.* (2006) reported that various compounds of garlic when exposed to high temperature and pressure changed into polyphenol compounds.

Antioxidant capacity

As the antioxidant capacity of a particular food differs with the different oxidants it is measured against, it is important to use a variety of assays to fully assess the antioxidant potential of a given sample (MacDonald-Wicks *et al.*, 2006). In the present study, the antioxidant capacity of the fresh and black garlic extracts was evaluated using four different approaches. Table 2 shows the electron-donating ability, ferricyanide reducing power and hydroxyl radical scavenging activity of the garlic extracts. Both garlic samples exhibited increased DPPH and hydroxyl radicals scavenging activities and reducing power with increased concentration of sample extracts.

The DPPH radical scavenging capacity assay is one of the most simple and accurate methods in the determination of antioxidant activity *in vitro* (MacDonald-Wicks *et al.*, 2006). It measures the ability of the extract to donate an electron or hydrogen to DPPH, a stable free radical, resulting in the discoloration of the DPPH solution. A significantly higher scavenging activity was observed in aged black garlic extract than that of the fresh garlic. At a concentration of 0.2 mg mL⁻¹, both extracts

showed relatively low scavenging activities compared with BHT, an antioxidant widely used as food additive. However, at 2 mg mL⁻¹, the aged black garlic extract exhibited a marked increase in the scavenging activity, indicating that the black garlic has a higher capacity to scavenge free radicals than the fresh garlic.

The ferricyanide reducing power assay measures the capacity of a given sample to donate electron to Fe³⁺, transforming it to Fe²⁺. The reducing capacity of the sample could serve as an indicator of its antioxidant potential, in which higher reducing power corresponds to greater antioxidant activity. In the present study, the black garlic extract at a concentration of 2 mg mL⁻¹ showed the highest reducing power. The fresh garlic at 2 mg mL⁻¹ concentration exhibited similar reducing power with the black garlic at a lower concentration of 0.2 mg mL⁻¹. These results suggest that the aged black garlic extract has greater antioxidant activity than the fresh garlic extract.

Among the reactive oxygen species, the hydroxyl radical is the most reactive, causing severe biological damage (Aurand *et al.*, 1977). The black garlic extract also showed significantly higher hydroxyl radical scavenging activity than the fresh garlic sample. At 2 mg mL⁻¹ concentration, the fresh garlic exhibited similar scavenging activity with the black garlic extract at 0.2 mg mL⁻¹. Highest scavenging activity was found in black garlic sample at a concentration of 2 mg mL⁻¹. Because the hydroxyl radicals are formed via the Fenton reaction (Fe²⁺ + H₂O₂ → Fe³⁺ + •OH + OH⁻), chelation of ferrous iron inhibits hydroxyl radical formation (Lopes *et al.*, 1999). Thus, the Fe²⁺-chelating ability of the garlic extracts was also measured in this study. Results showed that the garlic extracts have low-ferrous ion-binding capacity (Table 2). It was also noted that the Fe²⁺-chelating ability was considerably lower in black garlic than that of the fresh garlic extract and decreased with increased concentration of the extract. This indicates that the hydroxyl radical scavenging effects of the sample extracts may not be caused by the depletion of free Fe²⁺, but by direct quenching of the hydroxyl radicals.

Table 2 1,1-diphenyl-2-picrylhydrazyl and hydroxyl radicals scavenging activities, ferricyanide reducing power and ferrous ion-chelating ability of fresh garlic and aged black garlic extracts

	BHT	Fresh garlic		Aged black garlic	
	100 mM	0.2 mg mL ⁻¹	2 mg mL ⁻¹	0.2 mg mL ⁻¹	2 mg mL ⁻¹
Electron-donating ability (%)	85.90 ± 0.27 ^a	8.70 ± 0.34 ^a	35.10 ± 0.66 ^c	19.79 ± 0.22 ^b	82.53 ± 0.47 ^d
Reducing power (OD 700 nm)	–	0.06 ± 0.02 ^a	0.43 ± 0.01 ^b	0.42 ± 0.00 ^b	2.60 ± 0.01 ^c
(•OH) scavenging ability (%)	–	24.45 ± 0.22 ^a	60.72 ± 0.20 ^b	60.85 ± 0.36 ^b	74.95 ± 0.68 ^c
Fe ²⁺ -chelating ability (%)	–	46.04 ± 0.94 ^c	30.62 ± 1.35 ^b	29.54 ± 1.15 ^b	18.24 ± 0.86 ^a

BHT, butylated hydroxytoluene; OD, optical density.

Values are means ± SD (*n* = 3). Means in the same row not sharing a common superscript are significantly different at *P* < 0.05.

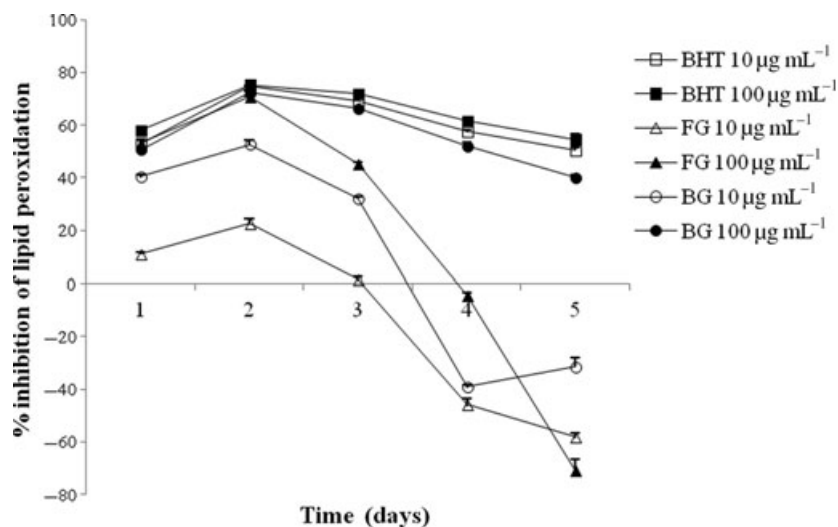


Figure 1 Inhibition of linoleic acid peroxidation of fresh garlic and aged black extracts.

The LPI is another widely used approach in the determination of antioxidant activity. Using a linoleic acid peroxidation model system, the antioxidant activity of the fresh and aged black garlic extracts was measured. Figure 1 shows the inhibitory effect of the sample extracts on lipid autoxidation. It was found that the inhibition of linoleic peroxidation increased with increased concentration of the extracts. All samples, including control BHT, exhibited increased rate of inhibition on the second day of incubation, but gradually decreased from the third day. At a concentration of $10 \mu\text{g mL}^{-1}$, the fresh garlic extract showed the lowest inhibitory effect. The black garlic extract, at similar concentration, also showed relatively low inhibitory activity compared with BHT. At higher concentration of $100 \mu\text{g mL}^{-1}$, the fresh garlic exhibited similar rate of inhibition with BHT on the second day only and then considerably decreased from the third up to fifth day of incubation. It appears that the antioxidants in fresh garlic extracts and black garlic extract at lower concentration were quickly oxidised and showed below 0% LPI at days 4 and 5, indicating that the extracts have pro-oxidant action. On the other hand, the black garlic at $100 \mu\text{g mL}^{-1}$ concentration showed similar inhibition rate of lipid peroxidation with BHT even after 5 days, suggesting that it has strong antioxidative effects. These findings further indicate that the black garlic has greater antioxidant potential than the fresh one.

The high total phenolic content of aged black garlic could be responsible for the stronger antioxidant capacity of black garlic compared with that of the fresh garlic. The results suggest that ageing of whole garlic can enhance its antioxidant activity. Studies have also shown that the ageing process of garlic could cause an increase in the activity of newer compounds, such as S-allylcysteine and S-allylmercaptocysteine, which are

stable and have high antioxidant effects (Lee *et al.*, 2009; Wang *et al.*, 2010). This high antioxidative activity of aged black garlic may be useful in the prevention of oxidant-induced disease conditions.

Cytotoxicity in RAW 264.7 and RBL-2H3 cells

Extracts from both fresh garlic and aged black garlic exhibited no cytotoxic effect in RAW 264.7 and RBL-2H3 cells, with 99% cell viability at concentrations of $< 10 \mu\text{g mL}^{-1}$ (Table 3). At a dose of $100 \mu\text{g mL}^{-1}$, the cell viability was still high with 97–98%, indicating that both garlic extracts were not cytotoxic in cells even at higher concentrations.

Inhibitory effect against β -hexosaminidase release

The RBL-2H3 cell line is commonly used as an *in vitro* model for allergy and immunological research (Passante

Table 3 Cytotoxicity of fresh garlic and aged black garlic extracts in RAW 264.7 and RBL-2H3 cells

Sample	Cell survival (%)	
	RAW 264.7	RBL-2H3
Vehicle	100.00 $\pm$ 2.62	100.00 $\pm$ 1.29
Fresh garlic ($\mu\text{g mL}^{-1}$)		
1	99.35 $\pm$ 1.94	99.06 $\pm$ 3.42
10	99.95 $\pm$ 1.57	99.22 $\pm$ 2.24
100	98.55 $\pm$ 0.76	97.75 $\pm$ 1.21
Aged black garlic ($\mu\text{g mL}^{-1}$)		
1	99.01 $\pm$ 1.07	99.90 $\pm$ 1.25
10	98.43 $\pm$ 1.13	99.40 $\pm$ 2.44
100	98.96 $\pm$ 3.40	98.30 $\pm$ 0.75

Values are means $\pm$ SD ($n = 3$).

Table 4 Inhibitory effect of fresh garlic and aged black garlic extracts on the β -hexosaminidase release from A23187-stimulated RBL-2H3 cells

Sample	% of inhibition
–	100.00 $\pm$ 1.84 ^d
A23187	0.00 $\pm$ 8.68 ^a
Curcumin ($\mu\text{g mL}^{-1}$)	
10	94.92 $\pm$ 2.04 ^d
100	99.79 $\pm$ 5.41 ^d
Fresh garlic ($\mu\text{g mL}^{-1}$)	
10	49.36 $\pm$ 6.26 ^c
100	34.96 $\pm$ 6.37 ^b
Aged black garlic ($\mu\text{g mL}^{-1}$)	
10	31.99 $\pm$ 2.94 ^b
100	30.51 $\pm$ 0.97 ^b

Values are means $\pm$ SD ($n = 3$). Means not sharing a common superscript are significantly different at $P < 0.05$.

& Frankish, 2009). In the present study, the capacity of extracts from fresh and black garlic to inhibit the release of β -hexosaminidase using this cell line was examined. The results showed that both garlic extracts have low inhibitory effect ($< 50\%$) at concentrations up to $100 \mu\text{g mL}^{-1}$ (Table 4). At a dose of $10 \mu\text{g mL}^{-1}$, the fresh garlic extract exhibited higher β -hexosaminidase inhibition compared with the aged black garlic extract. Fresh garlic was previously shown to inhibit β -hexosaminidase release in RBL-2H3 cells (Tanaka *et al.*, 1992). However, there were also reports that fresh garlic caused occasional allergic reactions because of the presence of allicin (Siegers, 1992). Hence, it may be possible that the decrease in the β -hexosaminidase release at higher concentration of fresh garlic extract may be due to the allergy-causing compounds in garlic, suggesting that its anti-allergic effect is not dose dependent. Likewise, the aged black garlic extract also showed a nondose-dependent anti-allergic activity. The garlic extract could have directly or indirectly modified the functions of mast cells and basophils, which are mainly involved in allergic reactions. To the best of our knowledge, this is the first report on the anti-allergic property of aged black garlic in comparison with that of the fresh garlic. Further investigations are needed to determine the changes in the composition responsible for the lower anti-allergic activity of aged black garlic to better understand its biological functions.

Conclusion

Results of the present study demonstrate that the antioxidative activities of the ethanolic extracts from both fresh garlic and aged black garlic were concentration dependent and that the latter has greater antioxidant effect. The ageing of whole garlic could increase its phenolic content, resulting in enhanced antioxidant

capacity, with no cytotoxic effect. However, the aged black garlic extract was found to have a relatively weak anti-allergic activity compared with that of the fresh garlic.

Acknowledgments

This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center No. PJ008176), Rural Development Administration, Korea.

References

- Agusti, K.T. (1996). Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). *Indian Journal of Experimental Biology*, **34**, 634–640.
- Aurand, L.W., Boone, N.H. & Giddings, G.G. (1977). Superoxide and singlet oxygen in milk lipid peroxidation. *Journal of Dairy Science*, **60**, 363–369.
- Banerjee, S.K., Mukherjee, P.K. & Maulik, S.K. (2002). Garlic as an antioxidant: the good, the bad and the ugly. *Phytotherapy Research*, **17**, 97–106.
- Corso-Martinez, M., Corso, N. & Villamiel, M. (2007). Biological properties of onions and garlic. *Trends in Food Science and Technology*, **18**, 609–625.
- Decker, E.A. & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry*, **38**, 674–677.
- Jang, E.K., Seo, J.H. & Lee, S.P. (2008). Physiological activity and antioxidative effects of aged black garlic (*Allium sativum* L.) extract. *Korean Journal of Food Science and Technology*, **40**, 443–448.
- Jeong, H.J., Koo, H.N., Na, H.J. *et al.* (2002). Inhibition of TNF- α and IL-6 production by aucubin through blockade of NF- κ B activation in RBL-23 mast cells. *Cytokine*, **18**, 252–259.
- Kang, M.J., Lee, S.J., Shin, J.H., Kang, S.K., Kim, J.G. & Sung, N.J. (2008). Effect of garlic with different processing on lipid metabolism in 1% cholesterol fed rats. *Journal of the Korean Society of Food Science and Nutrition*, **37**, 162–169.
- Kato, K., Terao, S., Shimamoto, N. & Hirata, M. (1988). Studies on scavengers of active oxygen species. 1. Synthesis and biological activity of 2-O-alkylascorbic acids. *Journal of Medicinal Chemistry*, **31**, 793–798.
- Kim, I., Kim, J.Y., Hwang, Y.J. *et al.* (2011a). The beneficial effects of aged black garlic extract on obesity and hyperlipidemia in rats fed a high-fat diet. *Journal of Medicinal Plants Research*, **5**, 3159–3168.
- Kim, M.H., Kim, M.J., Lee, J.H. *et al.* (2011b). Hepatoprotective effect of aged black garlic on chronic alcohol-induced liver injury in rats. *Journal of Medicinal Food*, **14**, 732–738.
- Kodera, Y. (1997). Dietary tolerance/absorption/metabolism of phytochemicals in garlic. In: *Nutraceuticals-Designer Foods III, Garlic, Soy and Licorice* (edited by P. Lanchance). Pp. 95–105. Trumbull, CT: Food and Nutrition Press.
- Kwon, O.C., Woo, K.S., Kim, T.M., Kim, D.J., Hong, J.T. & Jeong, H.S. (2006). Physicochemical characteristics of garlic (*Allium sativum*) on the high temperature and pressure treatment. *Korean Journal of Food Science and Technology*, **38**, 331–336.
- Lee, Y.M., Gweon, O.C., Seo, Y.J. *et al.* (2009). Antioxidant effect of garlic and aged black garlic in animal model of type 2 diabetes mellitus. *Nutrition Research and Practice*, **3**, 156–161.
- Lee, E.N., Choi, Y.W., Kim, H.K. *et al.* (2011). Chloroform extract of aged black garlic attenuates TNF- α -induced ROS generation, VCAM-1 expression, NF- κ B activation and adhesiveness for monocytes in human umbilical vein endothelial cells. *Phytotherapy Research*, **25**, 92–100.

- Lembo, G., Balato, N., Patruno, C., Auricchio, L. & Ayala, F. (1991). Allergic contact dermatitis due to garlic (*Allium sativum*). *Contact Dermatitis*, **25**, 330–331.
- Lopes, G.K., Schulman, H.M. & Hermes-Lima, M. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from the Fenton reaction by complexing ferrous ions. *Biochimica et Biophysica Acta*, **1472**, 142–152.
- MacDonald-Wicks, L.K., Wood, L.G. & Garg, M.L. (2006). Methodology for the determination of biological antioxidant capacity *in vitro*: a review. *Journal of the Science of Food and Agriculture*, **86**, 2046–2056.
- Mathew, S. & Abraham, T.E. (2006). Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various *in vitro* models. *Food Chemistry*, **94**, 520–528.
- Milner, J.A. (2005). Garlic (*Allium sativum*). In: *Encyclopedia of Dietary Supplements* (edited by P.M. Coates, M.R. Blackman, G.M. Cragg, M. Levine, J. Moss & J.D. White). Pp. 229–240. New York: Marcel Dekker.
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, **65**, 55–63.
- Oyaizu, M. (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, **44**, 307–315.
- Passante, E. & Frankish, N. (2009). The RBL-2H3 cell line: its provenance and suitability as a model for the mast cell. *Inflammation Research*, **58**, 737–745.
- Rahman, M.S. (2007). Allicin and other functional active components in garlic: health benefits and bioavailability. *International Journal of Food Properties*, **10**, 245–268.
- Rosen, G.M. & Rauckman, E.J. (1981). Spin trapping of free radicals during hepatic microsomal lipid peroxidation. *Proceedings of the National Academy of Sciences USA*, **78**, 7346–7349.
- Sato, E., Kohno, M., Hamano, H. & Niwano, Y. (2006). Increased anti-oxidative potency of garlic by spontaneous short-term fermentation. *Plant Foods for Human Nutrition*, **61**, 157–160.
- Seo, Y.J., Gweon, O.C., Im, J., Lee, Y.M., Kang, M.J. & Kim, J.I. (2009). Effect of garlic and aged black garlic on hyperglycemia and dyslipidemia in animal model of type 2 diabetes mellitus. *Journal of Food Science and Nutrition*, **14**, 1–7.
- Shashikanth, K.N., Basappa, S.C. & Murthy, V.S. (1986). Effect of feeding raw and boiled garlic (*Allium sativum* L.) extracts on the growth, caecal microflora and serum proteins of albino rats. *Nutrition Reports International*, **33**, 313–319.
- Siegers, C.P. (1992). *Allium sativum*. In: *Adverse Effects of Herbal Drugs* (edited by P.A.G.M. De Smet, K. Keller, R. Hansel & R.F. Chandler). Pp. 73–77. Berlin, Germany: Springer-Verlag.
- Singleton, V.L. & Rossi, J.A. Jr (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144–153.
- Tanaka, Y., Kataoka, M., Konishi, Y., Nishimune, T. & Takagaki, Y. (1992). Effects of vegetable foods on β -hexosaminidase release from rat basophilic leukemia cells (RBL-2H3). *Japanese Journal of Toxicology and Environmental Health*, **38**, 418–424.
- Wang, D., Feng, Y., Liu, J. *et al.* (2010). Black garlic (*Allium sativum*) extracts enhance the immune system. *Medicinal and Aromatic Plant Science and Biotechnology*, **4**, 37–40.
- Wang, X., Jiao, F., Wang, Q.W. *et al.* (2012). Aged black garlic extract induces inhibition of gastric cancer cell growth *in vitro* and *in vivo*. *Molecular Medicine Reports*, **5**, 66–72.