Advance Journal of Food Science and Technology 6(6): 755-760, 2014
DOI:10.19026/ajfst.6.106
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: March 13, 2014 Accepted: April 11, 2014 Published: June 10, 2014

Research Article

The Analysis of Saccharide in Black Garlic and its Antioxidant Activity

Mengmeng Lei, Mengying Xu, Zesheng Zhang, Min Zhang and Yunfeng Gao
Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology, Ministry of Education, Tianjin 300457, P.R. China

Abstract: Black garlic was created by keeping whole ordinary garlic in a humidity controlled room at 70-80°C for 10-15 days without any artificial treatments and additives. The black garlic was extracted with 80% ethanol and concentrated to obtain black garlic ethanol extracts. The saccharides of the extracts were analyzed according to the method of Phenol Sulphate colorimetry, DNS and High Performance Liquid Chromatography (HPLC). The antioxidant capacity *in vitro* of the extracts was assessed by measuring the scavenging activities on 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH). *Drosophila melanogaster* were employed to explore the effect of black garlic extract on lifespan and antioxidant index *in vivo*. The results showed that the total sugar content in the black garlic extracts was 55.5% including monosaccharide, disaccharide and polysaccharide, the content of reducing sugar was 25.22%. The results from this study demonstrated black garlic extracts possessed strong antioxidant capacity *in vitro* in a dose-dependent manner, the longevity of *Drosophila melanogaster* treated with black garlic extract was prolonged evidently and the content of MDA was decreased by improving SOD (including CuZn-SOD and Mn-SOD) and CAT activities.

Keywords: Antioxidant, black garlic, *Drosophila*, saccharides

INTRODUCTION

Garlic, which is the edible bulb of the *Liliaceae* family, has been used not only as flavoring agent and food ingredient but also as traditional medicine for treating and preventing several disorders for over 4000 years. A number of favorable therapeutic effects and biological activities of the garlic have been reported, however, garlic should be consumed in appropriate amount because cytotoxicity was reported at high doses (Kim *et al*., 2012). The volatility and permeability of the allicin resulted in irritating body and breathe odor. Thus, it is necessary to develop new type of the garlic product for unlimited consumption in people.

Recently, black garlic as a novel fastest-growing food emerged in Japan and Korea leading garlic product into a new age. Black garlic was produced through natural fermentation by aging whole ordinary garlic under controlled high temperature and high humidity condition for a several days without any artificial treatments and additives (Wang *et al*., 2010). During the aging production, the cloves of normal garlic change its color from white to brown and finally became black, caused by the Maillard Reaction. Black garlic has soft, sour and fruit-like sweetness, comestible just by peeling without any unpleasant smell in it (Wang *et al*., 2010). According to the previous report, compared with fresh garlic, the black one had a sevenfold increase in the polyphenol content (Sato *et al*., 2006), which indicated the increase in the antioxidant activity, the amino acid content increased 2.5-fold (Chao *et al*., 2012), carbohydrate content increased from 28.7 to 47.0%, the amount of SAC was almost 8 times (Sasaki *et al*., 2007). It is showed black garlic exhibited a wide range of biological activities, such as antioxidant (Kim *et al*., 2012), anticancer (Seo *et al*., 2009), hypoglycemic (Wang *et al*., 2012), hypolipidemic (Kim *et al*., 2011a), antiinflammatory property (Lee *et al*., 2011), hepatoprotective (Kim *et al*., 2011b) and immunostimulatory activities (Purev *et al*., 2012). Furthermore, black garlic showed stronger antioxidant activity *in vivo* (Zhu *et al*., 2008) and higher free radical scavenging properties *in vitro* (Kim *et al*., 2012) compared with fresh garlic.

Drosophila melanogaster is an excellent animal model to examine antiaging effect of compounds for it has a short lifespan and complete genome can be raised on simple diet (Boyd *et al*., 2011). Laboratory mice and cell lines were employed as model system to investigate antioxidant, anticancer and immunostimulatory activities of black garlic extracts were common (Sasaki *et al*., 2007; Seo *et al*., 2009; Zhu *et al*., 2008), but the antioxidant activity of black garlic extract in *Drosophila melanogaster* has never been reported yet.

Corresponding Author: Zesheng Zhang, Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology, Ministry of Education, Tianjin 300457, P.R. China, Tel.: 86-22-60601445; Fax: 86-22-60601445

Moreover, there were few reports about the content and distribution of carbohydrate in the black garlic and antioxidant activity of black garlic should be studied and utilized widely for its potential power to against various disorders. Therefore, the aim of the present study was to test the chemical composition of black garlic ethanol extract and evaluate antioxidant activity *in vitro*. And we designed studies to explore the effects of the black garlic extract on longevity in *Drosophila melanogaster* to confirm antioxidant property of black garlic extract *in vivo*.

MATERIALS AND METHODS

Materials: Garlic, corn meal and yeast powder were purchased from a local market in Tianjin, China. Black garlic was made in our laboratory according to the patent ZL 201110154928.1. Ethanol, phenol, glucosum anhydricum and agar were of analytical grade obtained from Jiangtian chemical reagents company of Tianjin, China. 1,1-Diphenyl-2-Picrilhydrazyl (DPPH), ascorbic acid (Vc) and T-series dextran standards were purchased from Sigma Reagents Company (St. Lous, MO, U.S.A.). Superoxide Dismutase (SOD) assay kit, Catalase (CAT) assay kit, Malondialdehyde (MDA) assay kit and Commassie Blue Staining Kit were purchased from Jiancheng Bioengineering institute of Nanjing, China. All other chemicals and reagents were of analytical grade and were purchased locally.

Preparation of black garlic extract: One hundred gram of peeled black garlic (moisture content: 52%) was chopped and mashed and mixed with 3 times dry matter volumes of 80% ethanol. The sample was extracted with ethanol for 24 h at room temperature for 3 times. The supernatant of extracted solution was collected by centrifugation and filtration and the solvents were removed by rotary evaporation to obtain black garlic ethanol extract.

Medium and experimental animals of *Drosophila melanogaster* survival: Wild type *Drosophila melanogaster* stain Orengon K was provided from our laboratory, all flies were free food intake and maintained on medium in (55±5)% relative humidity in the biochemical incubator at (25±0.5)°C on a 12-h light against 12-h dark cycle before being used.

Basic medium for flies contained 72 g corn meal, 72 g glucosum anhydricum, 6 g agar and 750 mL water, 40 mL of 1% ethyl p-hydroxybenzoate in 75% ethanol was added after the mixture was boiled, 10 g yeast powder should be mixed at last to keep yeast alive. Then medium was separately into culture tubes with approximately 8 mL of basic medium in it. The black garlic extract was added into medium to three final concentrations of 18.5, 37.5, 75 mg/mL as dose groups, respectively.

Determination of total sugar content: Total sugar content was determined according to the method of Phenol Sulphate colorimetry (Masukoa *et al*., 2005) with a slight modification. One milliliter of extract solution (1 mg/mL) was pipetted and 1 mL distilled water was added, then 1 mL of 6% phenol and 5 mL of concentrated sulfuric acid were added rapidly, the absorbance of the mixture was measured at 490 nm after shaken and stood for 20 min at room temperature. The blank should be prepared by substituting distilled water for the same volume of the extract solution. The total sugar content could be determined by referencing to a standard curve of fructose ($y = 0.080x - 0.063$, $R = 0.999$), which is drawn from various concentration of sugar solution by the same method as extract solution did.

Determination of reducing sugar content: Reducing sugar content was determined according to the DNS method (Zhao *et al*., 2008) with a slight modification by using fructose as standard. DNS reagent was added into serous concentration of fructose solution, the mixtures were shaken and heated for 7 min in the boiled water bath then cooled rapidly by running water and the standard curve ($y = 2.877x - 0.046$, $R = 0.999$) was completed after the absorbance at 540 nm of the resulting solutions was recorded. The ethanol extract and distilled water were executed the same operation as experimental and blank group, respectively.

Measurement of molecular weight distribution of carbohydrate: Samples were separately dissolved in triple-distilled water (10 mg/mL). The molecular weight distribution of carbohydrate was measured by Gel Permeation Chromatography (GPC) on a LC-20AT HPLC system (Shimadzu, Kyoto, Japan) with a RID-10A refractive index detector. The analytical data were evaluated by Shimadzu LC solution 1.26 SP1 processing systems. A Shodex OHpak SB-802.5 HQ (300×8 mm, 6 μm, Showa Denko, New York, U.S.A.) gel column was used under following conditions: mobile phase (distilled water), column temperature (30°C), column pressure (3.0 MPa), flow speed (0.8 mL/min), injection volume (20 μL). The column was precalibrated using T-series dextran standards under the same conditions. The molecular weight was determined using the calibration curve given by the following equation:

$$Y = -0.0723\ X^3 + 2.0859\ X^2 - 20.225\ X + 68.868 \quad (r = 0.9996)$$

where,

Y = The M_w of polysaccharide samples

X = The peak elution time of polysaccharide samples

Measurement of DPPH radical scavenging activity: The antioxidant activity of black garlic was evaluated by measuring DPPH radical scavenging activity based on the Blois's method (Yen and Hsieh, 1995) with a slight modification. The extract was dissolved in distilled water to a series of concentration with. (0.1 mL) of sample solution at different concentrations was mixed with 1.0 mL of DPPH ethanol solution and stand at 37°C for 60 min. The absorbance of the mixtures was read at 517 nm. The positive control and blank were prepared in the same manner except that Vc and distilled water instead of sample solutions, respectively. The DPPH radical scavenging activity was calculated by using following equation:

$$[DPPH\cdot] \text{ scavenging activity } (\%) = (A_{blank} - A_{sample}) /A_{blank} \times 100$$

Lifespan assay: Male flies of 2 days old were divided into four groups randomly, including control and three dose groups. Every tube contained 20 flies, 10 tubes formed one group. The number of dead flies was count when survival flies were transferred to fresh medium under light industrial nitrogen anesthesia every 3 days, till all the flies were extincted. The number of unnatural deaths did not take into account such as over anesthesia and stick to medium. The mean and maximum lifespan data and half death time of each group was recorded, respectively.

Determination of CAT, SOD activity and MDA content in *Drosophila*: Male flies of 2 days old were divided into control and three dose groups randomly. Every tube contained 25 flies, 10 tubes formed one group. All the flies from different group were fed on different mediums for 30 days. Then removed the mediums to keep them hungry for 2 h and recorded weighs of flies under narcosis. Flies were homogenized with sterile physiological saline on ice to obtain 2% dilution and the supernatant was collected after the homogenate was centrifuged at 3500 rpm for 10 min at 4°C. The determination of CAT, SOD activity and MDA content in *Drosophila* should be refer to kit introduction, respectively.

Statistical analysis: The student's test (SPSS) was employed to analyze the differences between control and experimental groups and statistical significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

Saccharide of black garlic extracts: The change of carbohydrate content in black garlic was previously reported which is increased from 28.7 to 47.0% during the aging process, which is probably resulted in the increase of sweetness in black garlic (Sasaki *et al*., 2007). We investigated the total sugar and reducing sugar content in black garlic ethanol extract. The results showed that the total sugar content in black garlic extract was 55.5%, which is similar with senior report, including sucrose, glucose and fructose, the content of reducing sugar was 25.22%, suggesting that reducing sugar is the main part of the total sugar in black garlic extract. The result from HPLC in the measurement of molecular weight distribution of carbohydrate is showed in Fig. 1, which is concordant with the discovery we found in the previous test: reducing sugar existed as the main part of total sugar in black garlic.

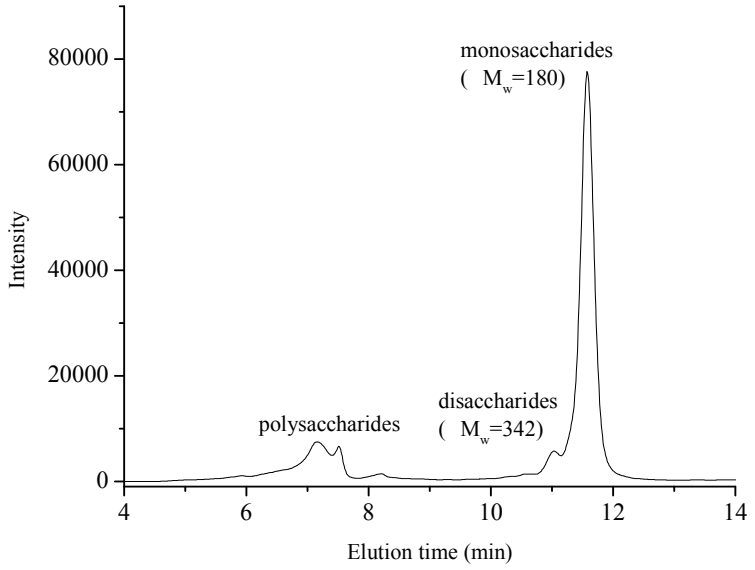

Fig. 1: HPLC chromatography of distribution of saccharides in black garlic ethanol extract with SB-802.5 gel column

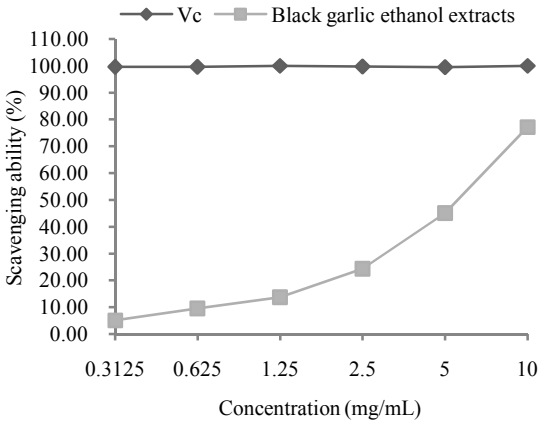

Fig. 2: The DPPH radical scavenging ability of black garlic ethanol extracts

Antiradical activity of black garlic extract *in vitro*: The DPPH radical scavenging activity of black garlic extract is shown in Fig. 2. The DPPH radical scavenging activity assay is one of the most simple and efficient manners to evaluate antioxidant activity *in vitro* (MacDonald-Wicks *et al*., 2006). In this experiment, it was electron-donating ability of black garlic we test, resulting in discoloration of DPPH solution, which could be revealed at 517 nm. The black garlic extract showed relatively lower scavenging activity at the concentration of 0.3125 mg/mL compared with V_C, however, at the concentration of 10 mg/mL, black garlic extract performed a marked increase in scavenging activity, which is close to V_C, suggesting that black garlic extract had a strong antiradical activity against DPPH in a dose-dependent manner.

The effect of black garlic extract on lifespan in *Drosophila*: Lifespan assay could investigate the regulation on life aging and clarify the effect of anti-aging manner through observation on half dead time, mean and maximum lifespan of organisms, which is one of most simple and efficient approach to evaluate anti-aging agent. *Drosophila melanogaster* is used as experimental subject commonly on genetics because it has a short lifespan and complete genome and easy to raise. In this study, we tested the effects of black garlic extract on lifespan in *Drosophila* and the data were shown in Table 1.

The results suggested the half dead time of black garlic dose groups were prolonged significantly compared with the control and the mean and maximum lifespan of flies treated with black garlic extract were extend markedly, significant increases at high concentration of black garlic extract were observed in three trials, which is in a dose-dependent manner.

The effect of black garlic extract on SOD activity in *Drosophila melanogaster*: Superoxide Dismutase (SOD) is an important antioxidant enzyme, which is widely distributed in various organisms. SOD activities play an important role in protecting and improving various diseases. There are two main forms of SOD in cells, including CuZn-SOD and Mn-SOD, contains CuZn and Mn, respectively (Sun *et al*., 1988). The effect of black garlic on SOD activity in *Drosophila melanogaster* is shown in Table 2.

Comparing with the control group, all the experimental groups performed higher activities in two forms of SOD. It appears that SOD activity in *Drosophila melanogaster* could be enhanced by black garlic. The T-SOD, CuZn-SOD and Mn-SOD activity were improved as the concentration of black garlic increased which is in a dose-dependent manner. At the higher concentration, the effects on SOD activity approach statistical significance at $p<0.05$.

Effect of black garlic on CAT activity and MDA content in *Drosophila melanogaster*: The distribution of Catalase (CAT) was screened in various organisms, Catalase as a essential enzyme in defense system, which is produced in the peroxisomes and necessary for catalyzing the decomposition of H_2O_2 into H_2O and O_2 (Noctor *et al*., 2000), could inhibit the generation of

Table 1: Effects of black garlic extract on lifespan of *Drosophila melanogaster* (x±s, n = 200)

Groups	Concentration of black garlic extract (mg/mL)	Half dead time/day	Mean lifespan/day	Max. lifespan/day
Control group	0	43.75±1.16	47.53±2.03	79.40±2.07
Experimental groups	18.75	44.82±1.55	49.06±2.82	80.85±1.91
	37.50	47.97±1.95**	51.16±2.57*	81.56±1.77
	75.00	49.66±1.45**	51.50±2.18*	82.02±1.92*

All data was expressed by mean±S.D.; *: Indicate significant difference at $p<0.05$ level to compare with the control group; **: Indicate significant difference at $p<0.01$ level to compare with the control group; Max.: Maximum

Table 2: Effect of black garlic on SOD activity in *Drosophila melanogaster* (x±s, n = 10)

Groups	Concentration of black garlic extract (mg/mL)	T-SOD activity (U/mg pro)	CuZn-SOD activity (U/mg pro)	Mn-SOD activity (U/mg pro)
Control group	0	95.16±2.28	56.33±1.75	38.83±2.12
Experimental groups	18.75	96.61±2.06	57.31±1.57	39.29±1.33
	37.50	98.16±1.99*	58.54±1.63*	39.62±2.13*
	75.00	99.16±1.95*	59.01±1.14*	40.14±1.15*

All data was expressed by mean±S.D.; *: Indicate significant difference at $p<0.05$ level to compare with the control group

Table 3: Effect of black garlic on CAT activity and MDA content in *Drosophila melanogaster* (x±s, n = 10)

Groups	Concentration of black garlic extract (mg/mL)	CAT activity (U/mg pro)	MDA content (nmol/mg prot)
Control group	0	28.50±1.90	2.43±0.23
Experimental groups	18.75	30.28±1.65	2.24±0.14
	37.50	31.71±2.34*	2.15±0.20*
	75	32.03±2.53*	2.08±0.14*

All data was expressed by mean±S.D.; *: Indicate significant difference at $p<0.05$ level to compare with the control group

harmful substance resulting from H_2O_2 react with iron chelate in order to protect cells from toxicity. Malonaldehyde as one of end-products in the cell membrane lipid peroxidation could reflect the level of cell membrane peroxidation indirectly (Zhu *et al*., 2008), the determination of MDA content was often accompanied with measurement of SOD activity, the level of MDA content and SOD activity reflects the level of cells attacked by free radicals and oxygen free radical scavenging ability in organism indirectly, respectively. The effect of black garlic on CAT activity and MDA content in *Drosophila melanogaster* was shown in Table 3.

The result illustrated the CAT activities of black garlic groups were increased evidently comparing with the control group. The MDA content in *Drosophila melanogaster* was decreasing obviously with the increasing concentration of black garlic. In both trials, a significant difference at $p<0.05$ level was detected at the higher black garlic concentration comparing with the control and in dose-dependent manner.

CONCLUSION

Results from the present study demonstrated the total sugar content in the black garlic extracts was 55.5%, reducing sugar as the main part of the total sugar, the content was 25.22%. In the antioxidant assays *in vitro* and *in vivo*, black garlic extract exhibited the free radical scavenging capacity in a dose-dependent manner, the longevity of *Drosophila melanogaster* was prolonged evidently, the SOD (including CuZn-SOD and Mn-SOD) and CAT activities were improved as the concentration of black garlic extract increased while the content of MDA was decreased, which indicated black garlic extract could extend the longevity of *Drosophila melanogaster* via SOD and CAT activation and inhibition of MDA production.

ACKNOWLEDGMENT

The project was supported by Project of National Key Technology Research and Development Program for the 12th Five-year Plan (NO. 2012BAD33B08) and the Ministry of education of the People's Republic of China's project (Grant no. IRT1166).

REFERENCES

Boyd, O., P. Weng, X. Sun, T. Alberico, M. Laslo *et al*., 2011. Nectarine promotes longevity in *Drosophila melanogaster*. Free Radical Bio Med., 50(11): 669-1678.

Chao, L.F., H. Guo and Z. Li, 2012. Research progress in the nutritional value and health effects of black garlic. Sci. Technol. Food Ind., 33(13): 429-432.

Kim, J.H., S.H. Nam, C.W. Rico and M.Y. Kang, 2012. A comparative study on the antioxidative and anti-allergic activities of fresh and aged black garlic extracts. Int. J. Food Sci. Tech., 47: 1176-1182.

Kim, I., J.Y. Kim, Y.J. Hwang, K.A. Hwang, A.S. Om *et al*., 2011a. The beneficial effects of aged black garlic extract on obesity and hyperlipidemia in rats fed a high-fat diet. J. Med. Plants Res., 5: 3159-3168.

Kim, M.H., M.J. Kim, J.H. Lee, J.I. Han, J.H. Kim *et al*., 2011b. Hepatoprotective effect of aged black garlic on chronic alcohol-induced liver injury in rats. J. Med. Food, 14(7-8): 732-738.

Lee, E.N., Y.W. Choi, H.K. Kim, J.K. Park, H.J. Kim, *et al*., 2011. Chloroform extract of aged black garlic attenuates TNF-α-induced ROS generation, VCAM-1 expression, NF-kB activation and adhesiveness for monocytes in human umbilical vein endothelial cells. Phytother. Res., 25: 92-100.

MacDonald-Wicks, L.K., L.G. Wood and M.L. Garg, 2006. Methodology for the determination of biological antioxidant capacity *in vitro*: A review. J. Sci. Food Agr., 86: 2046-2056.

Masukoa, T., A. Minamib, N. Iwasakib, T. Majima, S. Nishimura *et al*., 2005. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Anal. Biochem., 339: 69-72.

Noctor, G., S.V. Jovanovic and C.H. Foyer, 2000. Peroxide processing in photosynthesis: Antioxidant coupling and redox signalling. Philos. T. Roy. Soc. B., 355: 1465-1475.

Purev, U., M.J. Chung and D. Oh, 2012. Individual differences on immunostimulatory activity of raw and black garlic extract in human primary immune cells. Immunopharm. Immunot., 34(4): 651-660.

Sasaki, J., C. Lu, E. Machiya, M. Tanahashi and K. Hamada, 2007. Processed black garlic (*Allium sativum*) extracts enhance anti-tumor potency against mouse tumors. Med. Aromatic Plant Sci. Biotech., 1(2): 278-281.

Sato, E., M. Kohno, H. Hamano and Y. Niwano, 2006. Increased anti-oxidative potency of garlic by spontaneous short-term fermentation. Plant Food Hum. Nutr., 61: 157-160.

Seo, Y.J., O.C. Gweon, J.E. Im, Y.M. Lee, M.J. Kang *et al*., 2009. Effect of garlic and aged black garlic on hyperglycemia and dyslipidemia in animal model of type 2 diabetes mellitus. J. Food Sci. Nutr., 14(1): 1-7.

Sun, V.I., W.O. Larry and U. Ving, 1988. A simple method for clinical assay of superoxide dismutase. Clin. Chem., 34(3): 497-500.

Wang, D., Y. Feng, J. Liu, J. Yan, M. Wang *et al*., 2010. Black garlic (*Allium sativum*) extracts enhance the immune system. Med. Aromatic Plant Sci. Biotech., 4: 37-40.

Wang, X., F. Jiao, Q.W. Wang, J. Wang, K. Yang *et al*., 2012. Aged black garlic extract induces inhibition of gastric cancer cell growth *in vitro* and *in vivo*. Mol. Med. Rep., 5: 66-72.

Yen, G. C. and P.P. Hsieh, 1995. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. J. Sci. Food Agr., 67: 415-420.

Zhao, K., P. Xue and G. Gu, 2008. Study on determination of reducing sugar content using 3, 5-dinitrosalicylic acid method. Food Sci., 29(08): 534-536.

Zhu, B., H. Wu, Y. Liu and J. Tun and Z. Yao, 2008. The antioxidation of black garlic (In Chinese). Food Res. Dev., 29(10): 58-60.